Withanolides and Related Steroids

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A.D. Kinghorn et al. (eds.), *Progress in the Chemistry of Organic Natural Products, Vol. 94*, DOI 10.1007/978-3-7091-0748-5_3,

1. Introduction

Since the isolation of the first withanolides in the mid 1960s (*1*, *2*), *ca*. 650 members of this family of compounds have been described, with most of these from genera of the Solanaceae. The basic structure of withaferin A (**1**), a C_{28} ergostane with a modified side chain forming a δ-lactone between carbons 22 and 26, was considered for many years the basic template for withanolides (Fig. 1). Nowadays this has given place to a considerable number of related structures that are considered part of the withanolide family. Withanolides have shown to possess many biological activities that include anti-inflammatory, antitumor, cytotoxic, immunomodulating, and cancer chemopreventive as well as antibacterial and antifungal properties. Extracts from *Withania somnifera*, known by its Sanskrit name "Ashwagandha" or "Indian ginseng", have been used for over 3,000 years in traditional medicine in India (Ayurvedic system). Many of the curative properties of this extract may be associated with the presence of withanolides. Several withanolides also exhibit insecticidal activities, mostly as feedant deterrents, and selective phytotoxicity. A number of review articles have dealt with structural aspects of this group of compounds (*3*–*5*) as well as with their biological activities (*6*, *7*). The present chapter covers developments and findings in the chemistry and bioactivity of withanolides and related compounds since 1994.

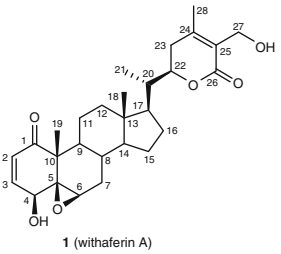


Fig. 1. Structure of withaferin A (**1**) and numbering scheme

2. Withanolides in the Plant Kingdom

2.1. Solanaceous Genera Containing Withanolides

The Solanaceae, also known as the nightshade or potato family, is one of the largest flowering plant families in containing nearly 100 genera and *ca*. 2,500 species, with a worldwide distribution in temperate and tropical continents (*8*–*10*). However, they are much more diversified in the Andean/Amazonian regions of South America in habitats that vary dramatically. The Solanaceae is also the third most important plant taxon economically and the most valuable in terms of vegetable crops, including the tuber-bearing potato, a number of fruit-bearing vegetables (tomato, eggplant, peppers), ornamental plants (petunias, *Nicotiana*, *Nierembergia*), plants with edible leaves (*Solanum aethiopicum*, *S. macrocarpon*), and medicinal plants (*e.g*. *Atropa*, *Capsicum*, *Datura*).

A modern traditional classification of the Solanaceae, predominantly based on morphological evidence with a minor emphasis on chemistry, recognizes six subfamilies (*9*). However, a recently proposed phylogenetic classification of the Solanaceae provides a different framework to the morphological system, with the inclusion of genera traditionally excluded from the family and with a greater resolution among lineages within the subfamily Solanoideae (*10*).

Among the nearly 100 genera included in Solanaceae (*10*), the occurrence of withanolides is restricted to the subfamily Solanoideae. Table 1 summarizes all genera and species known to contain withanolides to date. The four major contributors of withanolide structures are the genera *Jaborosa* Juss., *Datura* L., and *Physalis* L. from the North and South American continents, and *Withania* Pauq., native to the Old World. Up to the present, *ca*. 50% of the species in these genera have been investigated. *Jaborosa* is an interesting South American genus with a much varied corolla odor, color, and morphology as an adaptation to different pollinators growing from southern Peru to Argentina in very diverse habitats. *Datura* comprises annual or short-lived perennial herbs, mostly with erect large flowers, common in semiarid habitats in Mexico and the southwest of the United States, but introduced in many countries. *Physalis* includes mostly American herbs (Central America, Mexico and United States, except for the Euroasian *P. alkekengi*), with solitary pendant yellow or white flowers, and fruiting calyxes that become enlarged and inflated. Finally, *Withania* is a well-known genus

Table 1. Genera and species of the Solanaceae containing withanolides

Genera[a]	Species	Previous names[b]
Acnistus (1/1)	*arborescens* (L.) Schltdl.[c]	*Acnistus ramiflorus* Miers
Brachistus (2/4)	*stramoniifolius* (Kunth) Miers[c]	
	hunzikeri (D'Arcy) Sousa-Peña[c]	*Witheringia hunzikeri*
Datura (6/*ca*. 11)	*ferox* L.[c]	
	inoxia Mill.[c]	
	metel L.[c]	
	metel var. *fastuosa* (L.) Saff.[c]	*Datura fastuosa* L.
	quercifolia Kunth[c]	
	stramonium L.	
	stramonium var. *tatula* (L.) Torr.[c]	*Datura tatula* L.
Deprea (1/7)	*orinocensis* (Kunth) Raf.[c]	
Discopodium (1/1)	*penninervium* Hochst.[c]	
Dunalia (2/5)	*brachyacantha* Miers[c]	
	solanacea Kunth[c]	
Eriolarynx (1/3)	*lorentzii* (Dammer) Hunz.[c]	*Vassobia lorentzii* Dammer; *Acnistus lorentzii* (Dammer) Hunz.
Exodeconus (1/6)	*maritimus* (Benth.) D'Arcy[c]	
Hyoscyamus (1/17)	*niger* L.[c]	
Iochroma (4/*ca*. 25)	*australe* Griseb.[c]	*Acnistus australis* (Griseb.) Griseb.; *Dunalia australis* (Griseb.) Sleumer
	coccineum Scheid.	
	fuchsioides Miers	
	gesneroides Miers[c]	
Jaborosa (13/23)	*araucana* Phil.[c]	
	bergii Hieron.[c]	
	caulescens var. *bipinnatifida* (Dunal) Reiche[c]	
	caulescens Gillies & Hook. var. *caulescens*[c]	
	integrifolia Lam.	
	kurtzii Hunz. & Barboza[c]	
	laciniata (Miers) Hunz. & Barboza[c]	*Trechonaetes laciniata* Miers
	lanigera (Phil.) Hunz. & Barboza[c]	
	leucotricha (Speg.) Hunz. [c]	
	magellanica (Griseb.) Dusén	
	odonelliana Hunz.[c]	
	rotacea (Lillo) Hunz. & Barboza[c]	
	runcinata Lam.[c]	
	sativa (Miers) Hunz. & Barboza[c]	
Larnax (2/*ca*. 30)	*glabra* (Standl.) Sawyer[c]	
	subtriflora (Ruiz & Pav.) Miers[c]	*Deprea subtriflora* Ruiz & Pav.
Lycium (2/*ca*. 80)	*chinense* Mill.	
	barbarum L.	*Lycium halimifolium* Mill.

(continued)

Table 1. (continued)

Genera[a]	Species	Previous names[b]
Nicandra (1/2)	*physalodes* (L.) Gaertn.	
Margaranthus (1/1)	*solanaceous* Schltdl.[c]	*Physalis solanaceous* (Schltdl.) Axelius
Physalis (15/*ca*. 90)	*alkenkegi* L.[c]	
	alkenkegi var. *franchetii* (Mast.) Makino[c]	
	angulata L.[c]	
	chenopodifolia Lam.[c]	
	cinerascens (Dunal) Hitchc.[c]	
	coztomatl Dunal[c]	
	divaricata D. Don[c]	
	lanceifolia Nees	
	minima L.[c]	
	peruviana L.[c]	
	philadelphica Lam.[c]	*Physalis ixocarpa* Brot. ex Hornem.
	pruinosa L.	
	pubescens L.	
	virginiana Mill.[c]	
	viscosa L.	*Physalis curassavica* L.
Salpichroa (1/16)	*origanifolia* (Lam.) Thell.[c]	
Schraderanthus (1/1)	*viscosus* (Schrad.) Averett[c]	*Saracha viscosa* Schrad. *Leucophysalis viscosa* (Schrad.) Hunz.
Solanum (2/*ca*. 1,500)	*ciliatum* Lam.[c]	*Solanum cilistum* Lam.
	sisymbriifolium Lam.[c]	
Tubocapsicum (1/2)	*anomalum* (Franch. & Sav.) Makino[c]	
Vassobia (1/2)	*breviflora* (Sendtn.) Hunz.[c]	*Acnistus breviflorus* Sendtn.
Withania (6/20)	*adpressa* Cors.[c]	
	aristata (Aiton) Pauq.[c]	
	coagulans (Stocks) Dunal[c]	*Withania coagulance*
	frutescens (L.) Pauq.	
	obtusifolia V. Tackh.	
	somnifera (L.) Dunal[c]	
Witheringia (2/12)	*coccoloboides* (Dammer) Hunz.[c]	
	solanacea L´Hér.[c]	

[a]In parenthesis are the number of species with withanolides/number of total species. [b]Previous names cited in literature. [c]Species included in this chapter

of perennial herbs or shrubs with flowers that are perfect or functionally imperfect, occurring in Europe and Asia.

Table 1 also includes four monotypic genera, *Acnistus* Schott (Southern Mexico to Eastern Brazil and Paraguay), *Margaranthus* Schltdl. (Southern United States to the Antilles), *Schraderanthus* Averett (Mexico to Guatemala), and *Discopodium* Hochst. (tropical Africa). In the remaining genera containing withanolides, only isolated species have been characterized phytochemically.

2.2. Non-Solanaceous Genera Containing Withanolides

Withanolides have been detected in six species belonging to different families (Table 2), including the rhizomes of species of *Tacca* J.R. Forst. & G. Forst. (family Dioscoreaceae, formerly the family Taccaceae), in the aerial parts of *Senna siamea* (family Leguminosae), in the bark of *Eucalyptus globulus* (family Myrtaceae), and in species of *Ajuga* L. (family Lamiaceae).

3. Classification of Withanolides

The withanolides are polyoxygenated steroids with a C_{28} ergostane skeleton. A common feature is the presence of oxygen atoms at C-1, C-22, and C-26 although a few exceptions with a non-functionalized C22 are included. They may be classified into two major groups depending on the arrangement of the side chain, those with a δ-lactone or δ-lactol comprising C-22 and C-26 and those with a γ-lactone usually involving C-23 and C-26.

3.1. Withanolides with a δ-Lactone or δ-Lactol Side Chain

Most of the known withanolides belong to this group, which may be further divided into 13 subgroups: withanolides with the parent skeleton of withaferin A (**1**), withaphysalins, physalins, acnistins, withajardins, withametelins, sativolides, subtriflora-δ-lactones, spiranoid-δ-lactones, norbornane-type withanolides, ring-D aromatic withanolides, ring-A aromatic withanolides, and taccalonolide-δ-lactones (Fig. 2). The δ-lactone formed between a carboxyl group at C-26 and a hydroxy at

Table 2. Genera containing withanolides from outside the Solanaceae

Family and genera	Species	Name cited[a]
Monocotyledoneae	*chantrieri* André[b]	
Fam. Dioscoreaceae		
Tacca	*paxiana* H. Limpr.[b]	
	plantaginea (Hance) Drenth[b]	
	subflabellata P.P. Ling & C.T. Ting[b]	*Tacca subflaellata*
Dicotyledoneae	*siamea* (Lam.) Irwin & Barneby	*Cassia siamea* Lam.
Fam. Leguminosae		
Senna		
Fam. Myrtaceae	*globulus* Labill.[b]	
Eucalyptus		
Fam. Lamiaceae	*bracteosa* Wall. ex Benth.[b]	
Ajuga	*parviflora* Benth.[b]	

[a]Names cited in literature. [b]Species included in this chapter

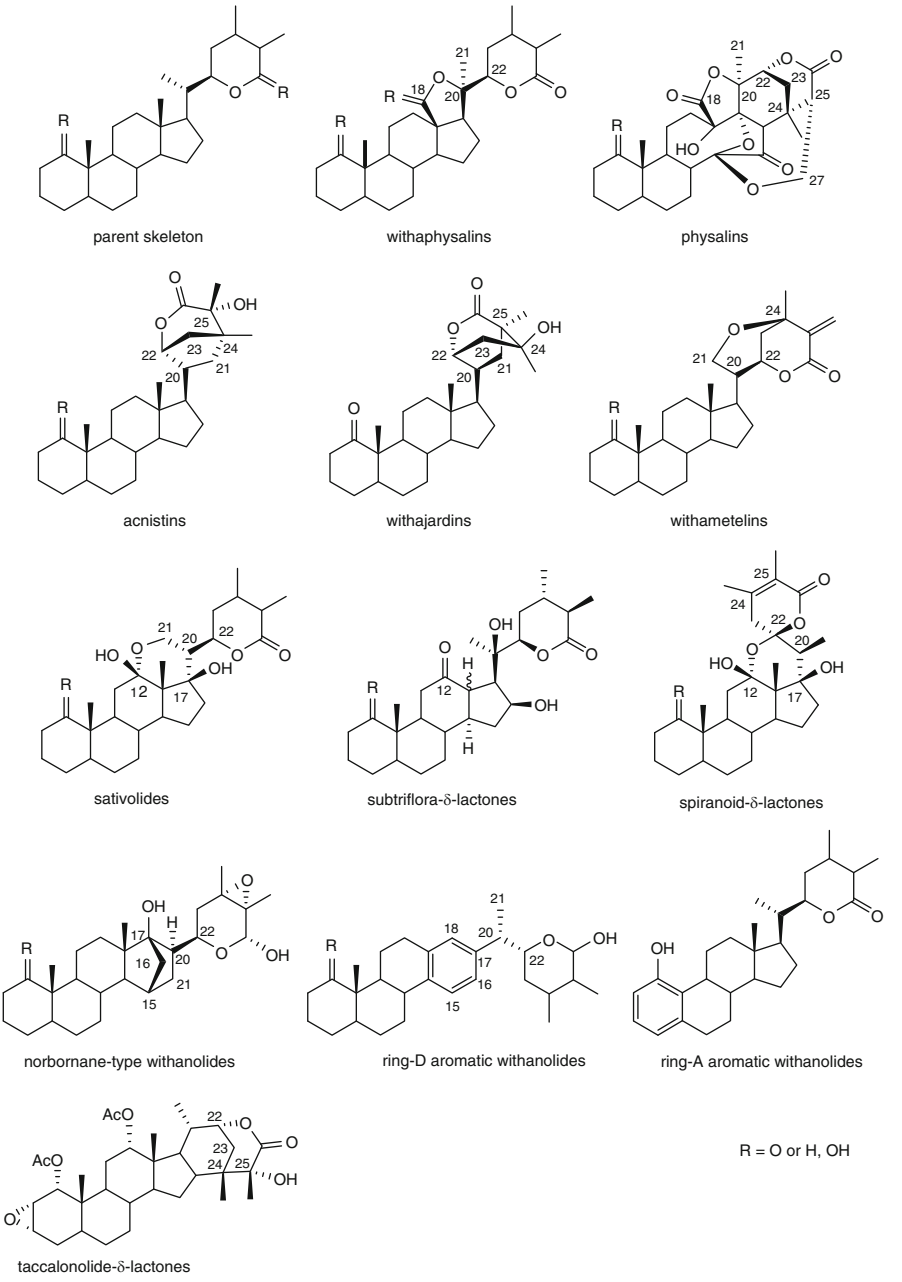


Fig. 2. General structures of withanolides with a δ-lactone or δ-lactol side chain. Numbering of relevant positions has been added for clarity in some structures

C-22 is the most common arrangement; other types include lactone formation with a hydrated carbonyl at C-22 and δ-lactols between an aldehyde at C-26 and a hydroxy at C-22. All known withanolides have the same stereochemistry at C-22, which corresponds to (22*R*) except when substituents at C-23 or C-22 change the relative priorities of groups around the asymmetric center.

Withanolides with an unmodified skeleton are the most abundant and are regarded as possible precursors of most of the other compounds in this group. A further subdivision of the withanolides is usually made according to the orientation of the side chain; those with the "normal" 17β-oriented side chain as well as those with the less usual 17α-oriented side chain are known. In the latter case a 17β-hydroxy (either free or involved in a cyclic ether) is generally present although several exceptions are known.

3.2. Withanolides with a γ-Lactone Side Chain

The presence of an oxygenated function at C-23 allows the formation of a γ-lactone with a carboxyl group at C-26; these withanolides may be divided in five subgroups, spiranoid withanolides, trechonolides, subtriflora-γ-lactones, ixocarpalactones, and taccalonolide-γ-lactones. A sixth subgroup with a γ-lactone side chain involving C-26 and C-28 corresponds to the perulactones (Fig. 3).

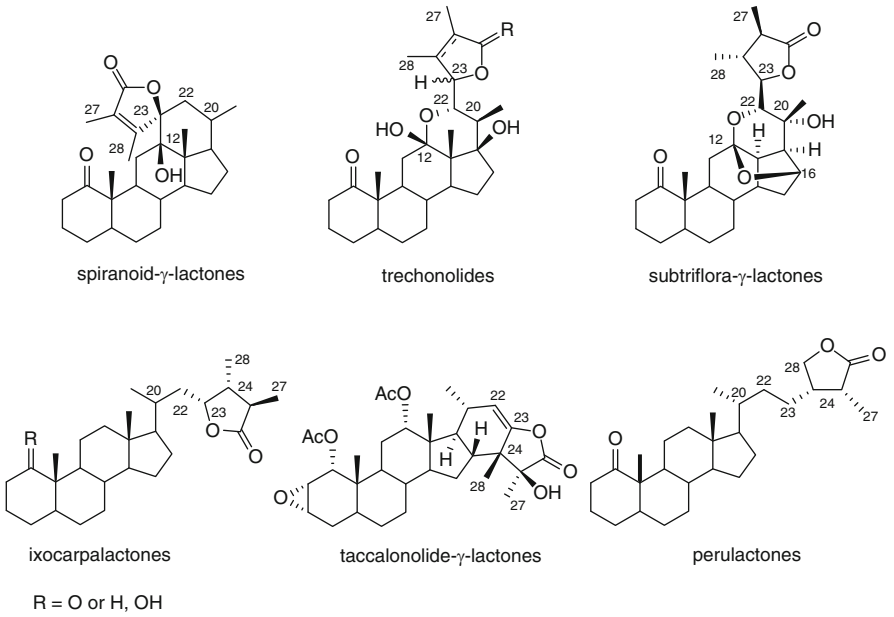


Fig. 3. General structures of withanolides with a γ-lactone side chain. Numbering of relevant positions has been added for clarity

4. Withanolides with an Unmodified Skeleton

Despite the large number of withanolides with the parent skeleton of withaferin A (**1**) that are already known, many new entities have been described with minor variations. These correspond mostly to different combinations of hydroxylated substituents and the occurrence of glycosidated derivatives. Among the many structures that fall within this group, the *Withania* withanolides comprise a major subgroup and are presented separately. Then, other representative substitutions on the parent skeleton will be described.

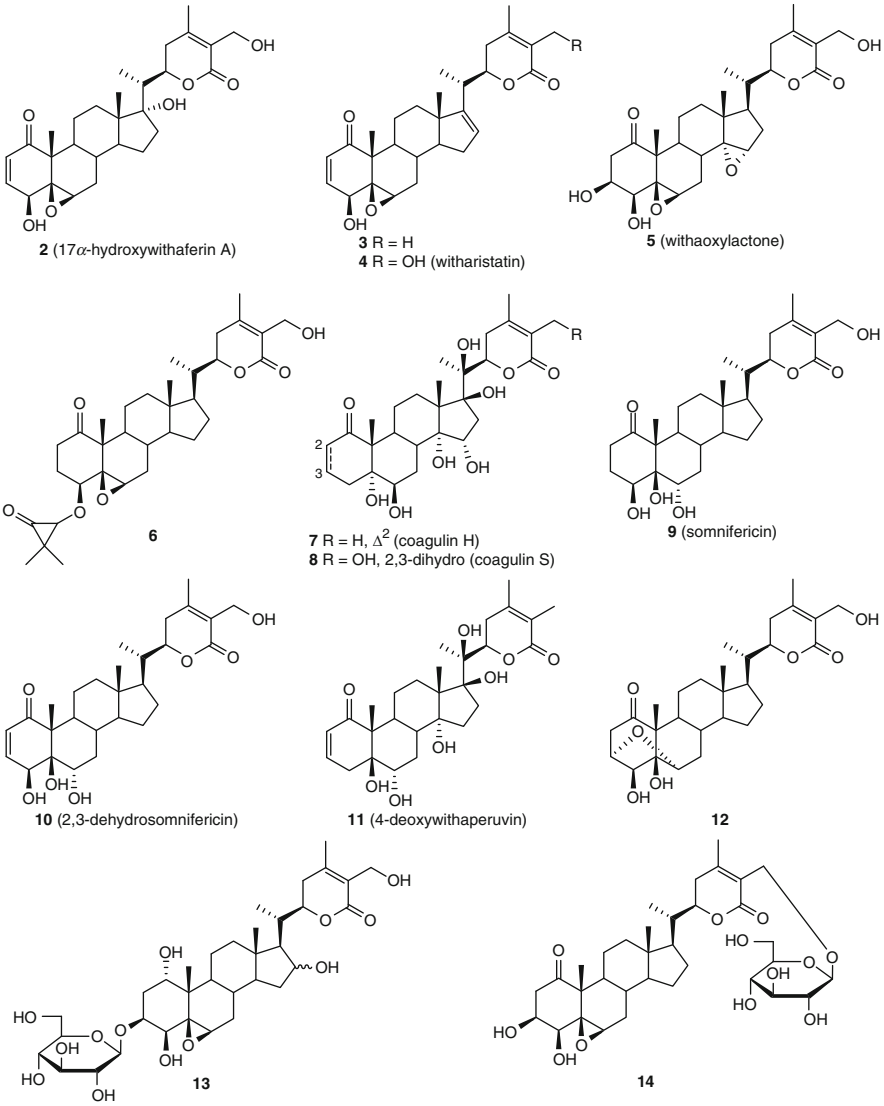
4.1. The Withania *Withanolides*

The *Withania* genus, although studied extensively in the past, has continued to provide new withanolide structures. Leaves, roots, and fruits of *W. somnifera* and *W. coagulans* have been investigated, with a total of 45 and 29 new structures reported, respectively. New withanolides have also been isolated from *W. adpressa* and *W. aristata*. Unfortunately, with several groups working simultaneously on the same plant, some structures have been reported as new more than once. As mentioned above, all of them conform to the classical withanolide skeleton with few unusual features.

4.1.1. 5*β*,6*β*-Epoxywithanolides and Related Compounds

The basic structure of withaferin A (**1**) may be found in several new structures isolated from *W. somnifera*, and simple variations are the 17α-hydroxy derivative **2** (*11*) and the Δ^{16} analogues **3** (*12*) and witharistatin (**4**) (*13*), with the latter isolated from *W. aristata*. The same basic structure may be found in **5** where the 2,3-double bond has been hydrated (*14*) and in **6** in which the double bond is reduced (*15*). The latter compound also has an unusual fragment etherifying the 4*β*-hydroxy group. One of the most common variations in the withaferin A substitution pattern is the hydrolytic cleavage of the 5,6-epoxide according to the *Fürst-Plattner* rule (*16*), to give the *trans*-diaxial 5α,6*β*-diol as in coagulin H (**7**) and coagulin S (**8**) isolated from *W. coagulans* (*17, 18*). A less common cleavage is that occurring in a *trans*-diequatorial manner, as found in **9** and **10** (*14*), later reported as new by *Kuroyanagi et al.* (*19*), and in 4-deoxywithaperuvin (**11**) (*20*). Compound **12** represents an unusual variant of the above, with a 3α,6α-epoxy bridge (*21*), which could derive from the cyclization of **10** also present in the same extract. While *Michael*-type addition of alcohols to the Δ^2-1-keto system of withanolides is well known, intramolecular addition is rare. Nevertheless, the same arrangement has been found previously in withaperuvins D (*22*) and F (*23*), although the authors did not directly compare the spectroscopic data of these substances.

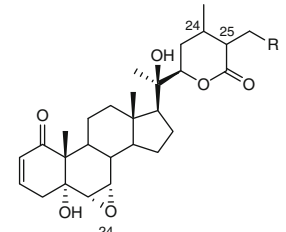
Compounds **13** and **14** are two glycosides closely related to withaferin A (**1**). In the case of **13** the configuration of the hydroxy group at C-16 was not determined (*15*).



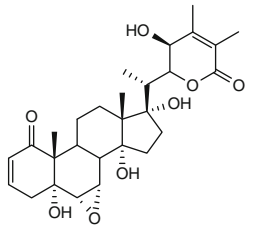
4.1.2. 5α-Hydroxy-6α,7α-Epoxywithanolides and Related Compounds

The 5α-hydroxy-6α,7α-epoxy substitution pattern is also a common arrangement found in *W. somnifera* withanolides. Simple variations arising from the

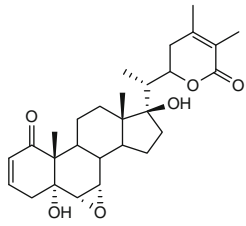
combination of hydroxy substituents at both typical positions (C-14, C-17, C-20) and some less common ones such as C-16 or C-23 are observed in withasomniferols A (**15**) and B (**16**) (*24*), 14α,17α-dihydroxywithanolide R (**17**) (*20*), iso-withanone (**18**) (*25*), and the 16*β*-acetate **19** (*26*). Withasomniferol B (**16**) is a stereoisomer of the known ixocarpanolide, although the configurations at positions 24 and 25 were not specified. Hydration of the 2,3-double bond was found in compounds **20** (*11*), **21**, and **22** (*12*), and the latter was isolated as the 3-sulfate, an unusual feature in withanolides (see Sect. 4.2.5.). Other variations found were the reduced 1α-alcohol **23** (*25*) and the related glycosides withanoside II (**24**), withanoside I (**25**), and withanoside III (**26**) (*27*), with the 1α,3*β*-dihydroxy arrangement being fairly common among withanolides (see next section). Also, the chlorinated withanolide Z (**27**) (*28*), arising from *trans*-diaxial cleavage of the 6,7-epoxide and the Δ^7-withanolide **28** (*24*) are known. Compound **29** contains an unusual 5α,7α-epoxy bridge that would result from rearrangement of the 5α-hydroxy-6α,7α-epoxide (*26*).



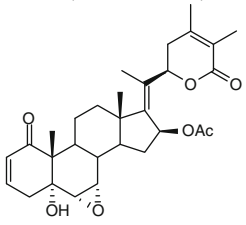
15 R = OH, Δ^{24} (withasomniferol A)
16 R = H (withasomniferol B)



17 (14*α*,17*α*-dihydroxywithanolide R)



18 (iso-withanone)



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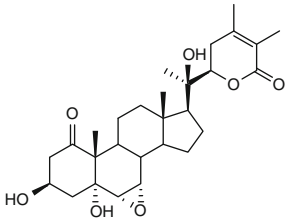

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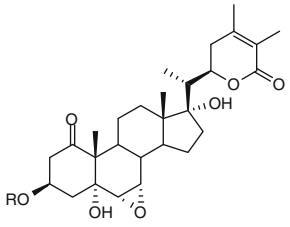
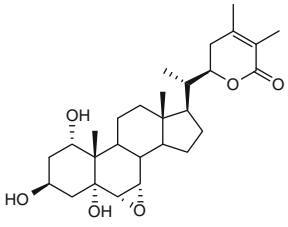


21 R = H
22 R = SO_3H



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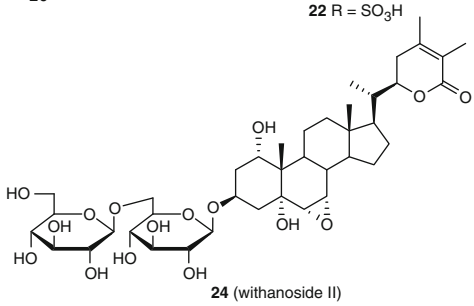
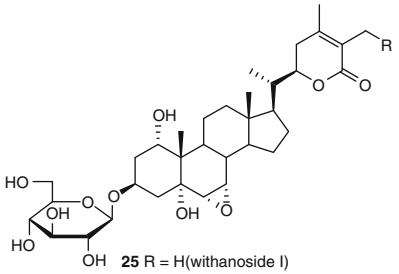
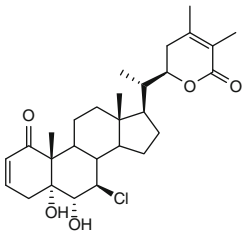


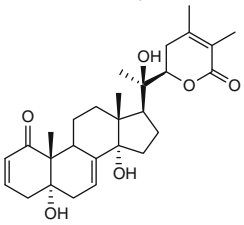
24 (withanoside II)



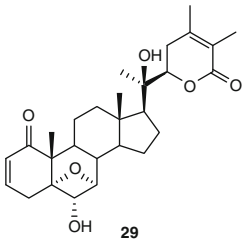
25 R = H(withanoside I)
26 R = OH (withanoside III)



27 (withanolide Z)



28 (withasomniferol C)



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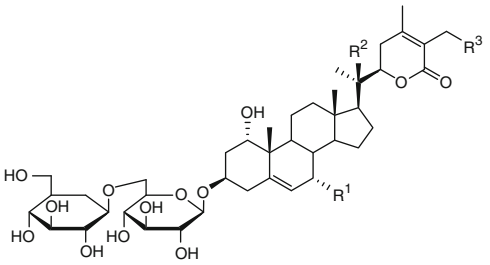
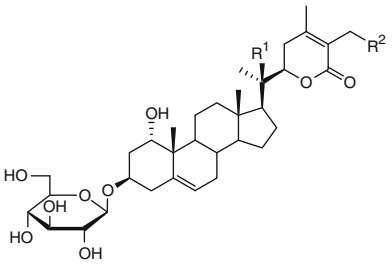
4.1.3. 1α,3β-Dihydroxy-Δ^5-Withanolides and Related Compounds

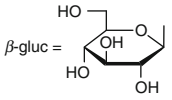
As mentioned above, the 1α,3β-dihydroxy arrangement is a well-known structural variation among the withanolides and several examples have been shown in combination with 5β,6β- or 6α,7α-epoxides. Eleven 3β-*O*-glycosides isolated from *W. somnifera* contained the 1α,3β-dihydroxy arrangement combined with a 5,6-double bond in ring B. These include compound **30**, later reported as withanoside V (*19, 27*), withanosides IV (**31**), VI (**32**) and VII (**33**) (*27*), and 24,25-dihydrowithanoside VI (**34**) containing a disacharide at position 3 (*29*). The configurations at positions 24 and 25 of the latter compound were not elucidated. Also within this group are three withanolides with an additional sugar moiety at C-27 (*21*), withanosides VIII (**35**), IX (**36**), and X (**37**) (this structure was reported again as new, one year later (*15*)), an analogue of withanoside VI with a monosacharide at C-3 named withanoside XI (**38**) (*21*), and an analogue of withanoside IV with a trisacharide moiety at C-3 (**39**) (*15*). Coagulin Q (**40**) isolated from *W. coagulans* (*30*), has the same aglycone as withanoside VI (**32**), but with a monosacharide unit at C-3. In all cases the carbohydrate units are β-D-glucose.

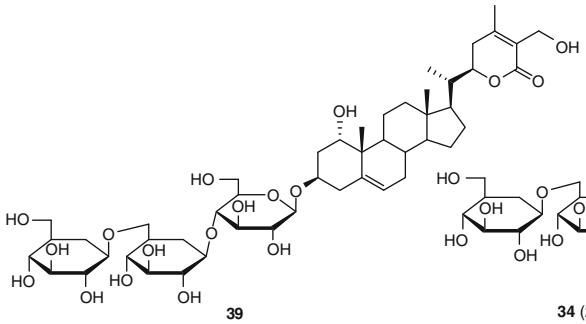


30 $R^1 = R^2 = R^3 = H$ (withanoside V)
31 $R^1 = R^2 = H$, $R^3 = OH$ (withanoside IV)
32 $R^1 = R^3 = H$, $R^2 = OH$ (withanoside VI)
33 $R^1 = OH$, $R^2 = R^3 = H$ (withanoside VII)
35 $R^1 = R^2 = H$, $R^3 = O$-β-gluc (withanoside VIII)
36 $R^1 = R^2 = H$, $R^3 = O$-β-gluc(1-6)-β-gluc (withanoside IX)

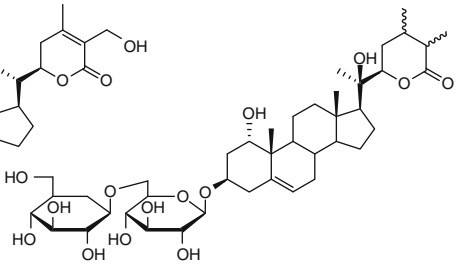


37 $R^1 = H$, $R^2 = O$-β-gluc (withanoside X)
38 $R^1 = R^2 = OH$ (withanoside XI)
40 $R^1 = OH$, $R^2 = H$ (coagulin Q)





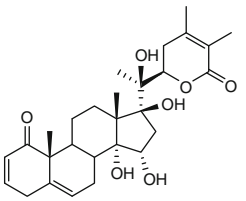
39



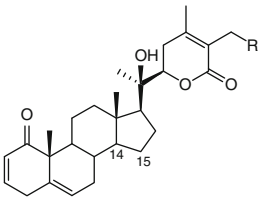
34 (24,25-dihydrowithanoside VI)

4.1.4. Other Δ^5-Withanolides

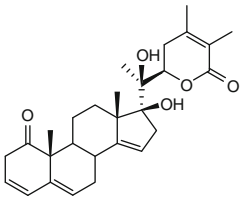
Withanolides containing a 5,6-double bond in combination with either a Δ^2- or Δ^3-1-ketone or a 3β-hydroxy-1-ketone are quite common and are the biosynthetic precursors of the 5β,6β-epoxywithanolides. Several new withanolides with these arrangements have been isolated from *Withania* species, mostly from *W. coagulans*. Compound **41** was isolated originally from *W. adpressa* (*31*) and later reported as new from *W. coagulans* (*32*). Compound **42** had been synthesized by *Lavie* and coworkers (*33*) but was isolated for the first time as a natural product by *Atta-ur-Rahman et al.* from *W. coagulans* (*34*). Ten withanolides with closely related structures were also isolated from this plant, withacoagulin (**43**) (*34*), withacoagulins A–F (**44**–**49**) (*35*), the 14β,15β-epoxide **50**, the 14α-alcohol **51** (*36*), and the 17-epimer of withacoagulin D, coagulansin A (**52**) (*37*). The 3β-hydroxy-Δ^5 arrangement is present in coagulansin B (**53**) (*37*) and in the three 3β-*O*-glycosides, coagulin L (**54**) (*17*), coagulin O (**55**) (*38*), and coagulin P (**56**) (*30*).



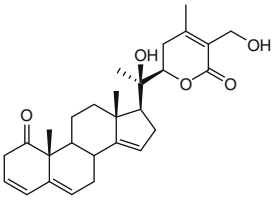
41



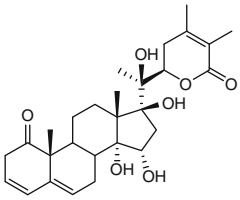
42 R = H
43 R = OH, Δ^{14} (withacoagulin)



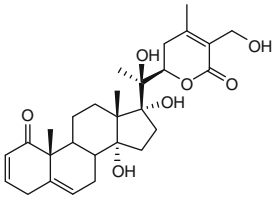
44 (withacoagulin A)



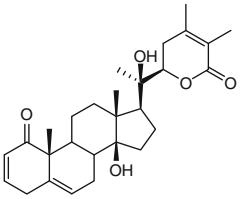
45 (withacoagulin B)



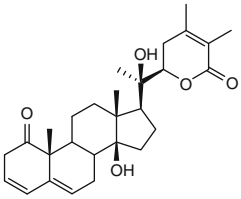
46 (withacoagulin C)



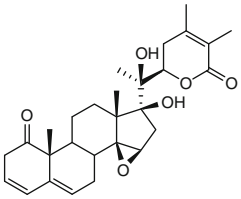
47 (withacoagulin D)



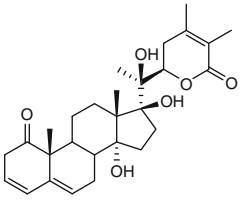
48 (withacoagulin E)



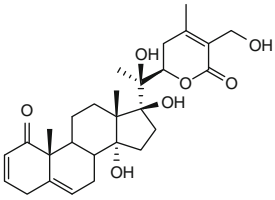
49 (withacoagulin F)



50 (14,15β-epoxywithanolide I)

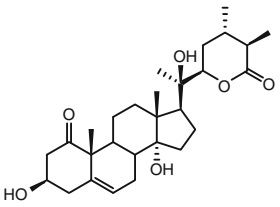


51 (17β-hydroxywithanolide K)

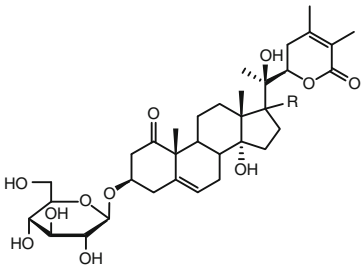


52 (coagulansin A)

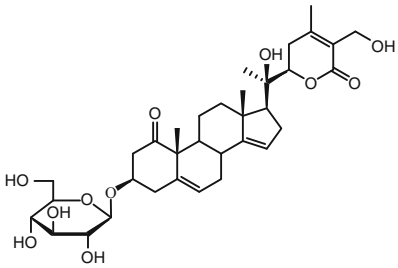
Glucosomniferanolide (**57**), isolated from *W. somnifera*, contains a glucose unit at the tertiary hydroxy group at C-20 (*39*). This compound was described as having a (22*S*) configuration, opposite to that found in all withanolides with a δ-lactone side chain. However, spectroscopic observations supporting this assumption only indicate that H-22 is equatorial instead of axial (broad signal with $W_{1/2} = 5$ Hz), an orientation that can result from a simple conformational inversion of the lactone ring half chair, probably due to the presence of the bulky substituent at C-20. Thus, this structure should be revised.



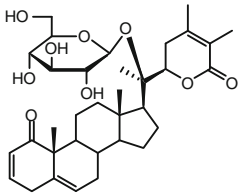
53 (coagulansin B)



54 R = β-OH (coagulin L)
55 R = α-H (coagulin O)

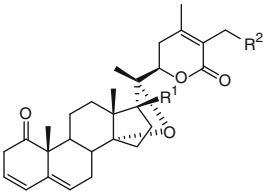


56 (coagulin P)

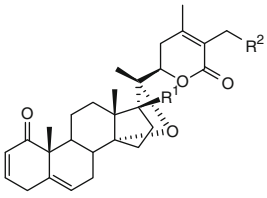


57 (glucosomniferanolide)

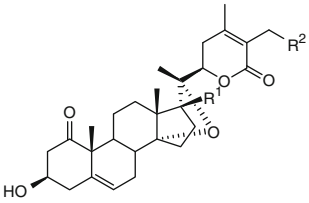
Several 14α,20-epoxywithanolides closely related to the known coagulin (**58**) (*40*), were isolated from *W. coagulans*. These included coagulins B–E (**59**–**62**) (*41*), coagulins F and G (**63**, **64**) (*42*), coagulin R (**65**) (*30*), the diol coagulin M (**66**) and the glycoside coagulin N (**67**) (*38*), coagulin J (**68**), the diol coagulin I (**69**) and the glycoside coagulin K (**70**) (*17*), and compound **71** (*34*). *Malik* and coworkers had previously reported the isolation of **71** and the 3-*O*-glycoside of coagulin R from *Physalis peruviana* (*43*, *44*) and of ajugin, identical to coagulin R from *Ajuga parviflora* (see Sect. 4.2.) (*45*).



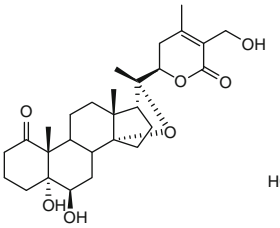
58 R^1 = OH, R^2= OH (coagulin)
62 R^1 = R^2 = H (coagulin E)
63 R^1 = H, R^2 = OH (coagulin F)
71 R^1 = OH, R^2 = H



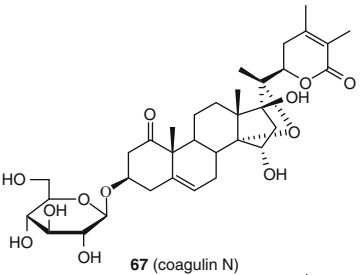
59 R^1 = H, R^2 = OH (coagulin B)
60 R^1 = OH R^2 = H (coagulin C)
61 R^1 = R^2 = H (coagulin D)
64 R^1 = R^2 = OH (coagulin G)



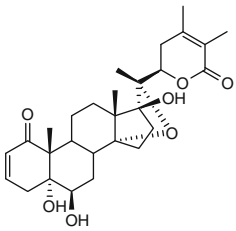
65 R^1 = OH R^2 = H (coagulin R)
68 R^1 = H R^2 = OH (coagulin J)



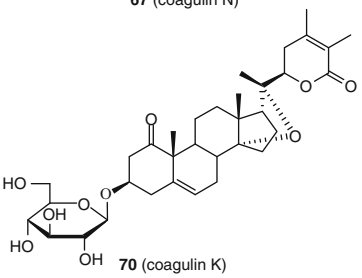
66 (coagulin M)



67 (coagulin N)



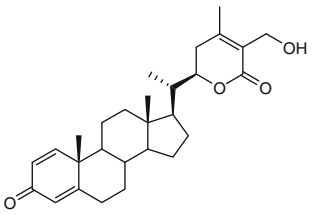
69 (coagulin I)



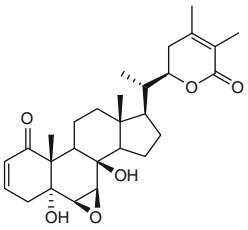
70 (coagulin K)

4.1.5. Other Substitution Patterns

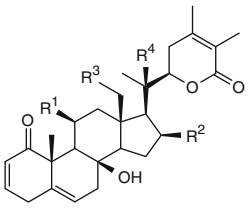
Some less common substitution patterns isolated from *W. somnifera* include a $\Delta^{1,4}$-3-keto withanolide (**72**) (*12*), a series of four 8β-hydroxywithanolides (**73**–**76**), a 7β-hydroxywithanolide with a $\Delta^{2,4}$-1-keto arrangement in ring A (**77**) (*46*), and two dimeric withanolides bound by a thioether linkage (**78**) (*47*) or a sulfoxide (**79**) (*48*). Other less common features found in these compounds are the presence of an 11β-hydroxy (**74**, **76**) or an 18-hydroxy group (**75**, **77**) (*46*). The 3β-*O*-sulfate of 2,3-dihydrowithaferin A (**80**) was isolated from aeroponically grown *W. somnifera* plants (*49*).



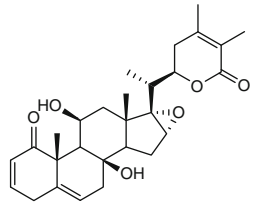
72



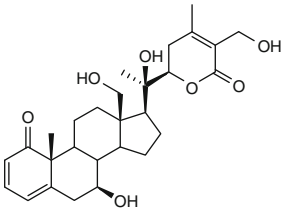
73 (withasomnilide)



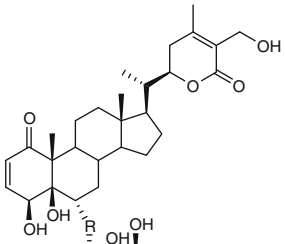
74 $R^1 = R^2 = OH$, $R^3 = R^4 = H$ (withasomniferanolide)
75 $R^1 = R^2 = H$, $R^3 = R^4 = OH$ (somniferawithanolide)

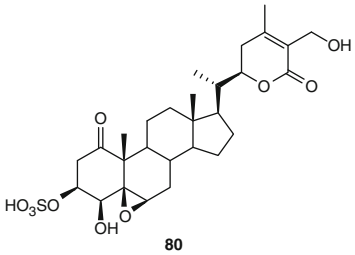


76 (somniferanolide)

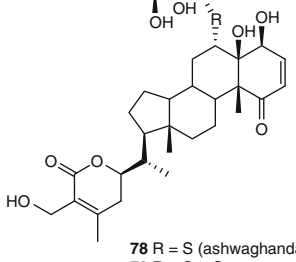


77 (somniwithanolide)





80



78 R = S (ashwaghandanolide)
79 R = S = O

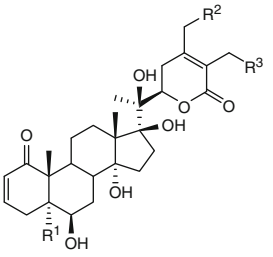
4.2. Other Withanolides with an Unmodified Skeleton

As mentioned above, a considerable number of withanolides with the parent skeleton of withaferin A (**1**) have been isolated. With few exceptions, in new withanolides the substitution patterns of rings A and B correspond to those described in the previous section for the *Withania* withanolides. Structural variations consist mainly of combinations of oxygenated functions (hydroxy or carbonyl groups) at different positions of the steroid nucleus (most commonly at positions 12, 14, 16, 17, and 18) and the side chain (mostly at C-20, C-21, and C-27). These functionalities may also be involved in cyclic entities as lactones, lactols, or cyclic ethers. These withanolides are presented in Sect. 5., with the exception of 14α,20-epoxywithanolides that are included in Sect. 4.2.1.

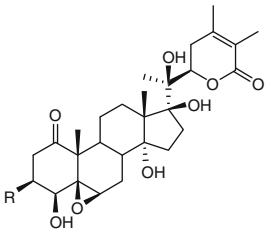
4.2.1. C-14, C-17, and C-20 Hydroxylated Withanolides and Related Compounds

Hydroxylation at C-14, C-17 and C-20 is common in many withanolides. Usually the 14-hydroxy group has the α-orientation (see Sect. 4.1.), although there is a growing number of 14β-hydroxywithanolides. As already mentioned, hydroxy substitution at C-17 occurs with either the α− or β−orientation, of which the former is more common. New structures with different combinations of hydroxy groups at the above-mentioned positions have been reported, occasionally combined with hydroxy groups at positions 15, 16, and 18. The genus *Physalis* is particularly rich in 14-hydroxywithanolides, with these probably being the biosynthetic precursors

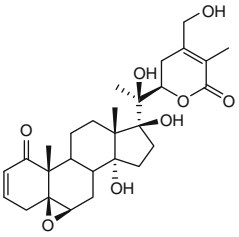
of polyoxyfunctional structures such as the physalins, present in many *Physalis* species (see Sect. 5.2.). Both 14α- and 14*β*-hydroxywithanolides are present in *Physalis* plants. A series of 14α,17*β*,20-trihydroxywithanolides was isolated from *P. peruviana* (**81**–**85**) (*50*). Phyperunolides B (**81**) and C (**82**) have the unusual feature of a free hydroxy group at C-28; another 28-hydroxywithanolide, **86**, was reported by *Dinan et al.* from the same plant (*51*). Hydroxylation at C-28 is a prerequisite in the formation of perulactones (Fig. 3), common components of *P. peruviana*. The 3-ethoxy withanolide **85** is probably an artifact formed during isolation. Also from *P. peruviana*, *Ahmad* and coworkers isolated the closely related **87** (*52*), the glycosides **88**–**91** (*53*), and a glycoside of coagulin R (**92**) (*44*). As already mentioned (Sect. 4.1.4.), another withanolide with a 14α,20-ether bridge (**71**), was also isolated from this plant (*43*). From *P. cinerascens* collected in Mexico, *Maldonado et al.* isolated 24,25-dihydrowithanolide S (**93**) with a saturated lactone side chain, together with the known withanolide S (*54*).



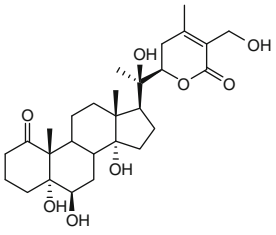
81 $R^1 = R^2 = OH$, $R^3 = H$ (phyperunolide B)
82 $R^1 = Cl$, $R^2 = OH$, $R^3 = H$ (phyperunolide C)
83 $R^1 = R^3 = OH$, $R^2 = H$ (phyperunolide D)



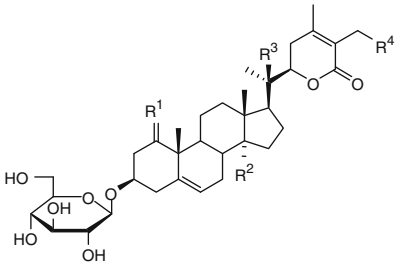
84 R = OH (phyperunolide E)
85 R = OCH_2CH_3 (phyperunolide F)



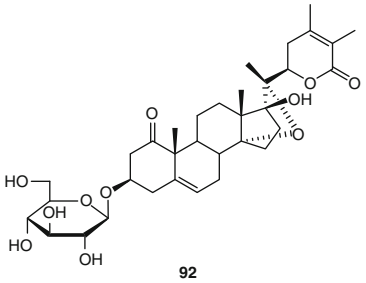
86 (28-hydroxywithanolide E)



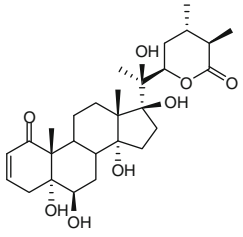
87



88 $R^1 = H$, α-OAc, $R^2 = R^3 = OH$, $R^4 = H$
89 $R^1 = H$, α-OAc, $R^2 = R^3 = H$, $R^4 = OH$
90 $R^1 = O$, $R^2 = H$, $R^3 = R^4 = OH$
91 $R^1 = O$, $R^2 = R^3 = R^4 = OH$

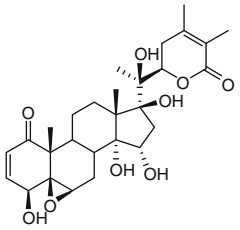


92

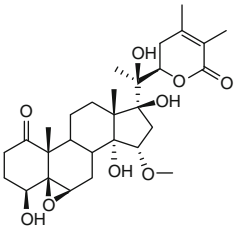


93 (24,25-dihydrowithanolide S)

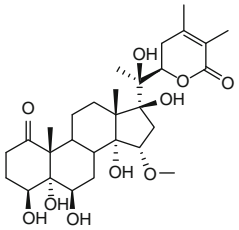
An investigation of *P. angulata* growing in Taiwan gave the 15-oxygenated withangulatins B–D (**94–96**) and the 16-hydroxylated withangulatins G (**97**) and H (**98**) together with withangulatin E (**99**) and a Δ^{16} 14α-hydroxywithanolide, withangulatin F (**100**) (*55*). Several other Δ^{16} 14α-hydroxy withanolides have been reported from *Physalis* species. These include phyperunolide A (**101**) isolated from *P. peruviana* (*50*) and four 15-acetyloxy withanolides isolated from *P. angulata*, withangulatin I (**102**) (*56*), physagulin M (**103**) with the unusual feature of a free hydroxy group at C-23 (*57*), physagulin O (**104**), and compound **105** (*58*). The latter withanolide was incorrectly named physagulin L, as this name had already been assigned (see below). Physagulin N (**106**), the methanol addition product of physagulin A, was probably formed during its isolation (*57*). Δ^{16}-Withanolides with oxygen substituents at C-14 and C-15 have the appropriate functionalities for cleavage of the 13,14-bond, and the occurrence of such withanolides in physalin-rich plants strongly suggests that they are either precursors or shunt products in the biosynthesis of physalins (*59*). A Δ^{14}-withanolide, **107**, was also reported from *P. minima* collected in Pakistan (*60*).



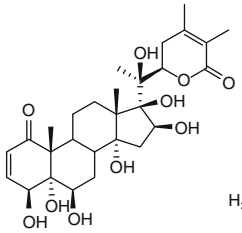
94 (withangulatin B)



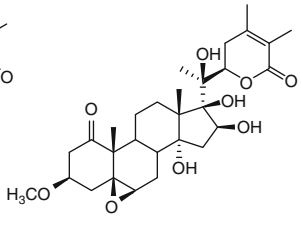
95 (withangulatin C)



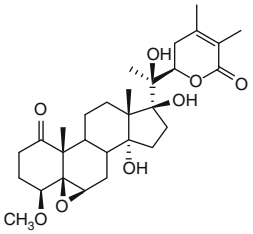
96 (withangulatin D)



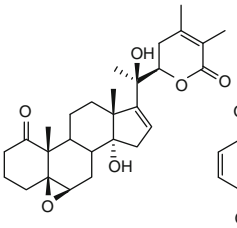
97 (withangulatin G)



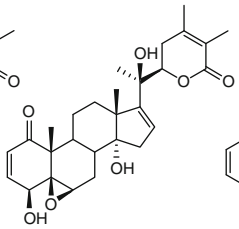
98 (withangulatin H)



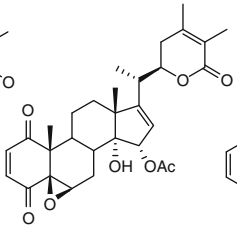
99 (withangulatin E)



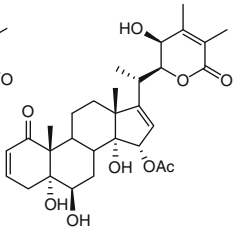
100 (withangulatin F)



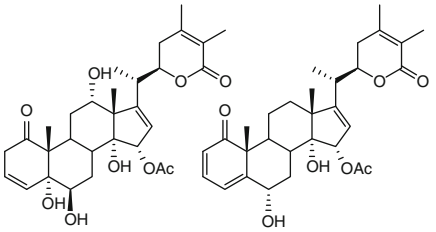
101 (phyperunolide A)



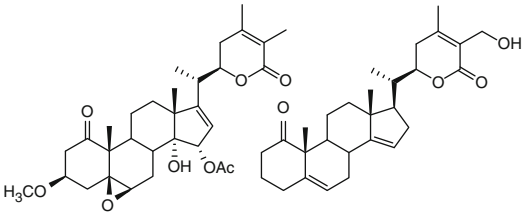
102 (withangulatin I)



103 (physagulin M)



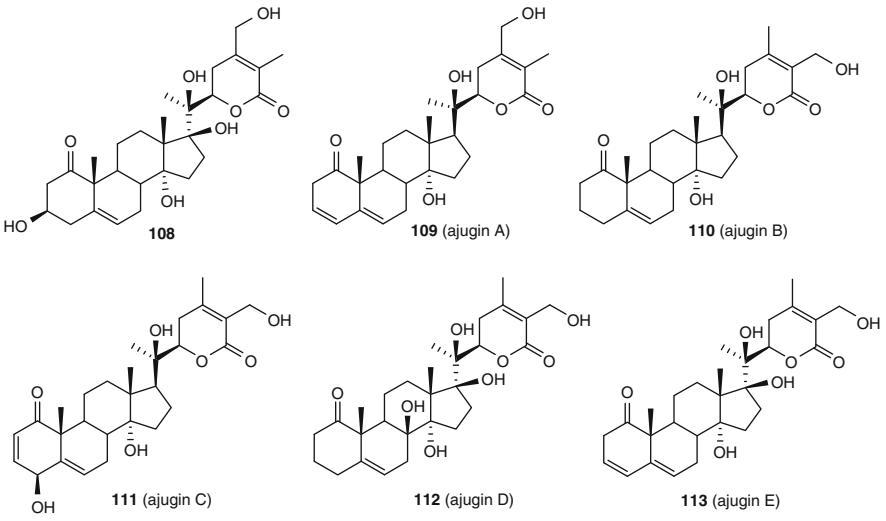
104 (physagulin O) **105**



106 (physagulin N) **107**

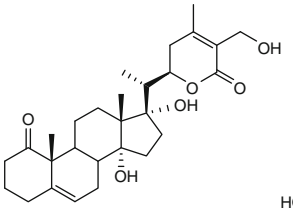
Ajuga (Lamiaceae) is one of the few genera outside the Solanaceae that contains withanolides, most of which are closely related to the coagulins (Sect. 4.1.). From *A. parviflora*, *Malik* and coworkers have reported seven 14α-hydroxywithanolides, compound **108** (isolated together with coagulin J) (*61*), ajugins A (**109**), B (**110**) (*62*), C (**111**), D (**112**) (*63*), E (**113**), and F (**114**) (*64*), a Δ^{14} withanolide (**115**), and a 14α,20-epoxywithanolide (**116**) (*65*). As already mentioned, ajugin, identical to coagulin R (**65**), was first isolated from this plant (*45*).

Several 14β-hydroxywithanolides have been isolated from *Physalis* species, and all of them also have an α−oxygenated function (hydroxy or acetate) at position 15. These include physagulins H–K (**117–120**) (*66*), physagulin L (**121**) (*57*), and compounds **122** and **123** (*58*) from *P. angulata*. The latter two compounds were

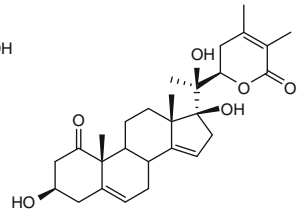


108 **109** (ajugin A) **110** (ajugin B)

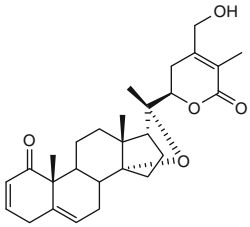
111 (ajugin C) **112** (ajugin D) **113** (ajugin E)



114 (ajugin F)

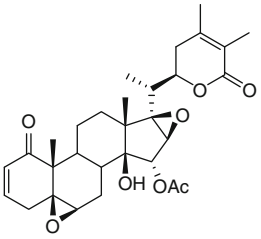


115

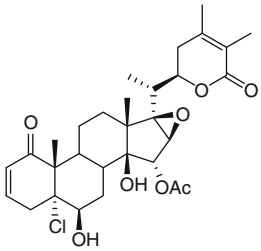


116

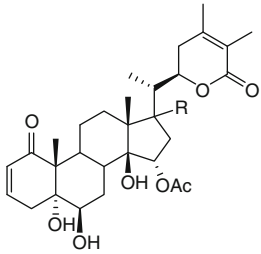
incorrectly named physagulins M and N, as these names had already been assigned (see above). The chlorohydrin **124** was reported from *P. alkekengi* var. *franchetii* (*67*) and the deacetylated analogue of physapubenolide (**125**) from *P. peruviana* (*52*). Outside the *Physalis* genus, new 14*β*-hydroxywithanolides have been isolated from *Jaborosa leucotricha* (jaborosalactone 8 (**126**)) (*68*) and *J. bergii* (jaborosalactol 23 (**127**)) (*69*).



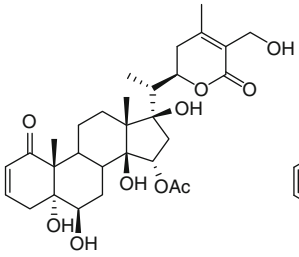
117 (physagulin H)



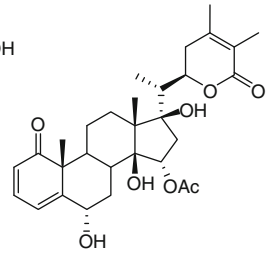
118 (physagulin I)



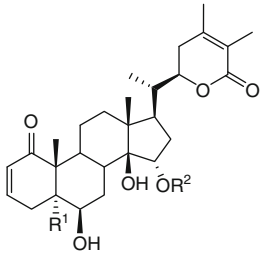
119 R = *α*-H (physagulin J)
120 R = *β*-OH (physagulin K)



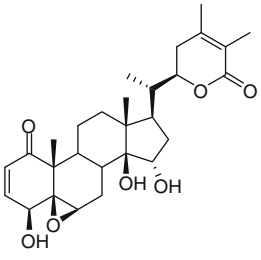
121 (physagulin L)



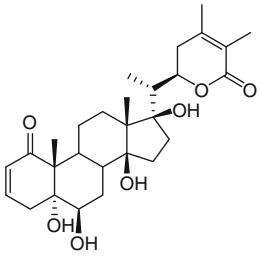
122



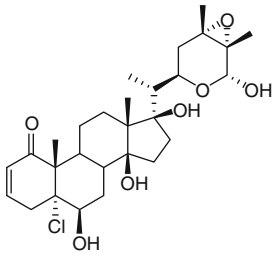
123 R^1 = OH, R^2 = H
124 R^1 = Cl, R^2 = Ac



125 (deacetylphysapubenolide)



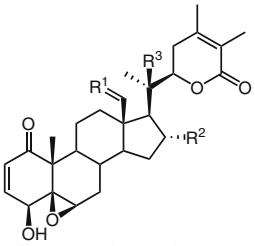
126 (jaborosalactone 8)



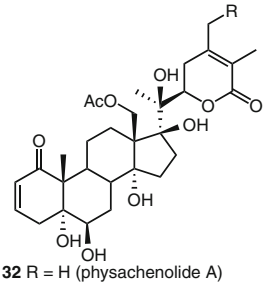
127 (jaborosalactone 23)

4.2.2. C-18 Hydroxylated Withanolides

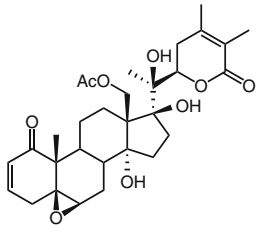
Withanolides with a functionalized C-18 at various oxidation levels (alcohol, aldehyde, and lactone carbonyl) have been isolated from plants of the genera *Withania* (see Sect. 4.1.5.), *Acnistus, Dunalia*, *Eriolarynx*, *Iochroma*, and *Physalis* (*4*, *5*). Hydroxylation at C-18 is usually combined with hydroxy groups occurring at positions 14, 17, or 20. Thus, 18-hydroxywithanolide D (**128**) was isolated from *Eriolarynx lorentzii* (synonym *Vassobia lorentzii*) (*70*), and the related 18-hydroxywithanolide **129** and the corresponding 18-aldehyde **130** were isolated from *Dunalia brachyacantha* (*71*) (both plants collected in Argentina), while the 18-acetoxywithanolide **131** was reported from *Iochroma gesneroides* together with several 3-methoxylated derivatives formed during the extraction procedure (*72*). As part of a systematic study of Mexican *Physalis* species, five 18-acetoxywithanolides, the physachenolides A–E (**132–136**) were isolated from the leaves, flowers, and stems of *Physalis chenopodifolia* (*73*). Physachenolide B (**133**) has a 28-hydroxy group while physachenolide E (**136**) is a Δ^{14}-withanolide. The aerial parts of *Physalis coztomatl*, also collected in Mexico,



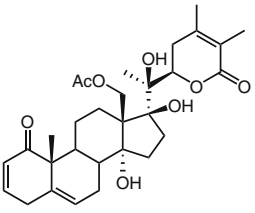
128 R^1 = H,OH, R^2 = H, R^3 = OH (18-hydroxywithanolide D)
129 R^1 = H,OH, R^2 = R^3 = H
130 R^1= O, R^2 = OH
131 R^1 = H,OAc, R^2 = OAc, R^3 = H



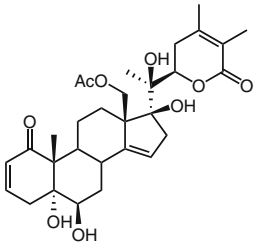
132 R = H (physachenolide A)
133 R = OH (physachenolide B)



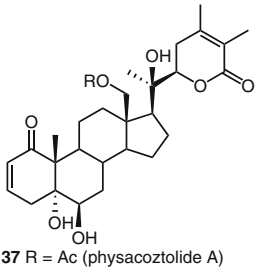
134 (physachenolide C)



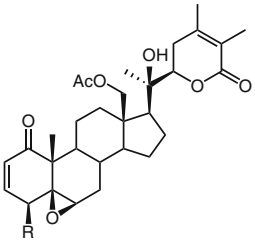
135 (physachenolide D)



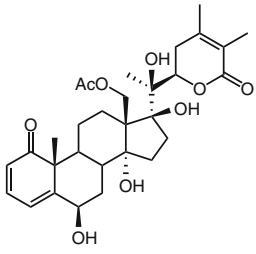
136 (physachenolide E)



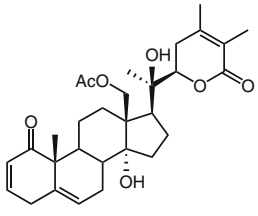
137 R = Ac (physacoztolide A)
138 R = H (physacoztolide B)



139 R = H (physacoztolide C)
142 R = OH (18-acetoxywithanolide D)



140 (physacoztolide D)

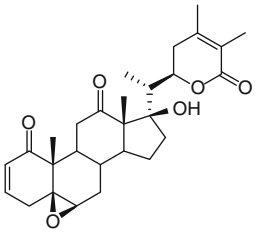


141 (physacoztolide E)

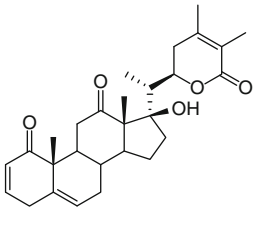
rendered five new withanolides functionalized at C-18, the physacoztolides A–E (**137**–**141**), together with physachenolide C (**134**), 18-acetoxywithanolide D (**142**), and 18-hydroxywithanolide D (**128**) (*74*).

4.2.3. C-12 and C-21 Oxygenated Withanolides

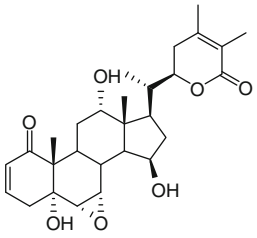
Withanolides with a free hydroxy or keto group at C-12 are mostly restricted to the *Datura* genus. In the genus *Jaborosa*, 12-ketowithanolides are most probably involved as precursors in the formation of additional rings with the side chain, giving rise to several modified skeletons (see Sect. 5.9.). However, only two withanolides with unmodified skeletons containing a free ketone at C-12 have been reported from these plants, (−)-jaboromagellonine (**143**) from *J. magellanica* (*75*) and jaborosalactone 44 (**144**) from *J. kurtzii* (*76*). Four new 12-oxygenated withanolides were isolated from plants of *Datura ferox* collected in Argentina, together with other known daturalactones. 15*β*-Hydroxynicandrin B (**145**) was found to have the common 5α-hydroxy-6α,7α-epoxy substitution pattern in ring B (see Sect. 4.1.2.) (*77*), while daturalactones 5–7 (**146**–**148**) exhibit related arrangements resulting from hydrolytic cleavage of the epoxide or rearrangement of the epoxyalcohol (*78*). The 12*β*-epimer of **145**, baimantuololine A (**149**) (*79*) and the closely related glycosides baimantuoluosides A–C (**150**–**152**) (*80*), were isolated from the dry flowers of *Datura metel* (used in Chinese medicine). The 1*β*-alcohol **153** from *Datura quercifolia* collected in India is closely related to the compounds mentioned above (*81*). 12-Oxygenated withanolides have also been reported from the stem bark of the Ethiopian shrub *Discopodium penninervium* (**154**) (*82*), from *Iochroma gesneroides* (**155**) (*72*), and from *Acnistus arborescens* (**156**) (*83*), with the latter two compounds bearing a 12*β*-acetoxy group.



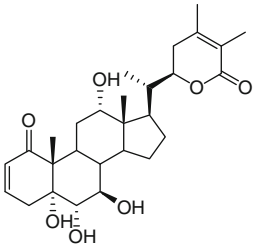
143 ((-)jaboromagellonine)



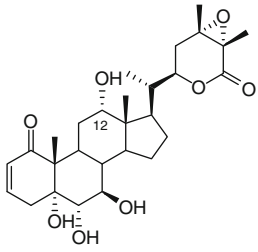
144 (jaborosalactone 44)



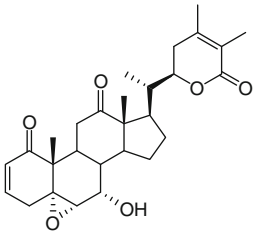
145 (15*β*-hydroxynicandrin B)



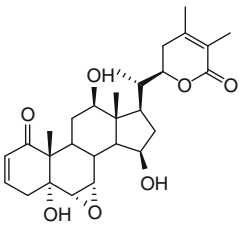
146 (daturolactone 5)



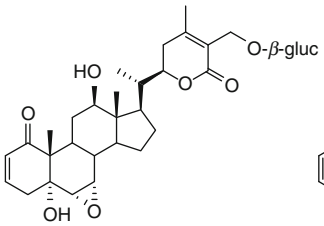
147 (daturolactone 6)



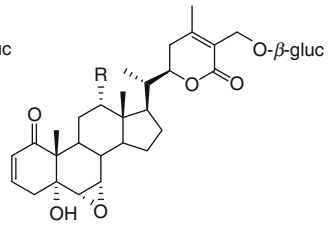
148 (daturolactone 7)



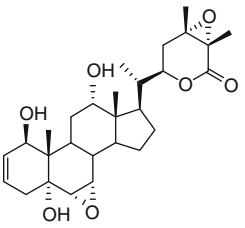
149 (baimantuoluoline A)



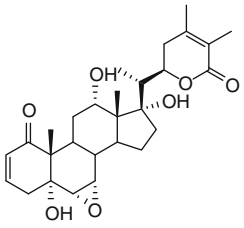
150 (baimantuoluoside A)



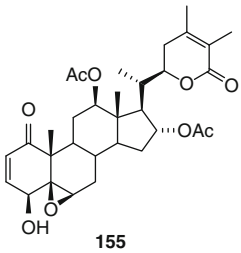
151 R = OH (baimantuoluoside B)
152 R = H (baimantuoluoside C)



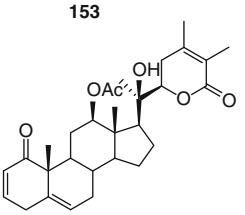
153



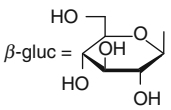
154



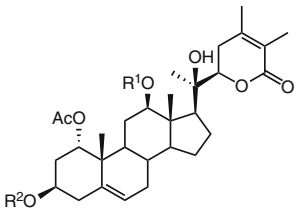
155

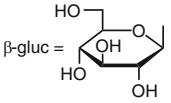


156

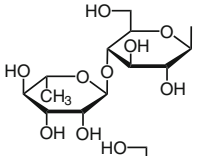


From *Dunalia brachyacantha* collected in Bolivia, *Bravo et al*. isolated the glycosides dunawithanine G (**157**) and dunawithanine H (**158**) (*84*), closely related to the known dunawithanine F (**159**) (*85*).

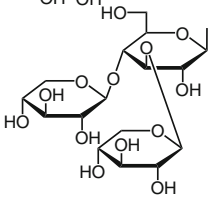




157 R^1 = Ac, R^2 = (dunawithanine G)

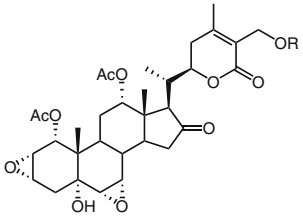


158 R^1 = H, R^2 = (dunawithanine H)

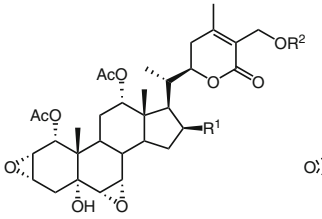


159 R^1 = H, R^2 = β-gluc(1-3)-[β-gluc(1-4)]-β-gluc- (dunawithanine F)

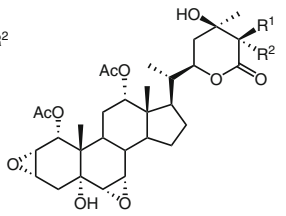
Outside the Solanaceae, most withanolides from *Tacca* species bear a 12α-acetoxy group. *Tacca* species originate in the tropical and subtropical regions, mostly in Asia and Africa. They contain highly oxygenated withanolides, usually with modified skeletons (see Sect. 5.4.4.) characterized by a 1α-acetoxy group and a 2α,3α-epoxide in ring A, although several have been reported with the unmodified parent skeleton. From the rhizomes of *Tacca chantrieri*, *Yokosuka et al*. isolated two glycosides, chantriolides A (**160**) and B (**161**), with a glucose unit at C-27 (*86*). From *Tacca plantaginea*, *Liu et al*. isolated plantagiolides A–E (**162**–**166**) (*87*). *Tacca* species are also rich in glycosidated sterols not included in this review; the withanolide **167** with a steroid nucleus that resembles a sterol was also isolated from the rhizomes of *T. chantrieri* (*88*).



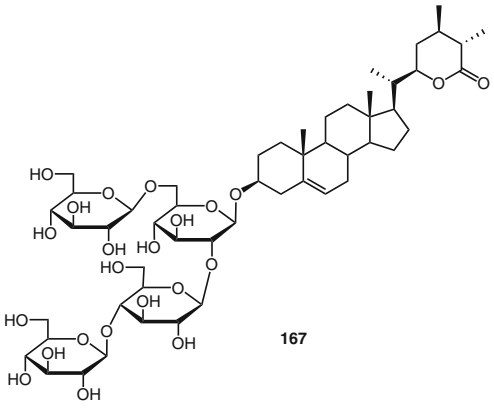
160 R = β-gluc (chantriolide A)
162 R = H (plantagiolide A)



161 R^1 = OH, R^2 = β-gluc (chantriolide B)
163 R^1 = OH, R^2 = H (plantagiolide B)
164 R^1 = R^2 = H (plantagiolide C)



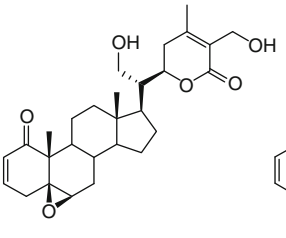
165 R^1 = CH_3, R^2 = H (plantagiolide D)
166 R^1 = OH, R^2 = CH_3 (plantagiolide E)



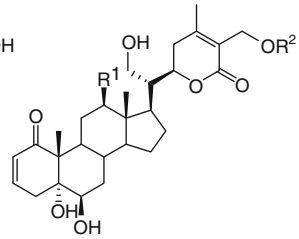
167

Although 21-oxygenated withanolides are present in *Datura*, *Jaborosa*, and *Tacca* species, 21-hydroxywithanolides with an unmodified skeleton have only been reported in the genus *Datura*, in many cases combined with a 12β-hydroxy group. Withafastuosin D (**168**) is the major withanolide of *D. metel* var. *fastuosa* (synonym *D. fastuosa*), and was isolated from the leaves together with withafastuosin E (**169**) (*89*); withafastuosin F (**170**) was isolated from the flowers of the plant (*90*). Withametelin H (**171**) isolated from the leaves of *D. metel* has the unusual feature of a methoxy group at C-27 (*91*). From flowers of *D. metel*, *Pan et al*. isolated the 27-glycoside withametelin P (**172**) (*92*) and *Yang et al*.

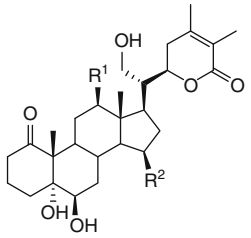
isolated baimantuoluolines C, B, and F (**173–175**), with the former also having a 27-methoxy group (*79*, *93*). It should be noted that the name "withametelin" is usually reserved for withanolides possessing an oxygen bridge between C-21 and C-24 (see Sect. 5.1.1.), and 21-hydroxywithanolides are the most probable biosynthetic precursors of these compounds. Another five 21-hydroxywithanolides, withatatulin (**176**) (*94*) and withatulins B–E (**177–180**), were isolated by *Ray* and coworkers from *D. stramonium* var. *tatula* (synonym *D. tatula*), which grows in the sub-Himalayan tracts of India and is often cultivated as an ornamental plant (*95*, *96*).



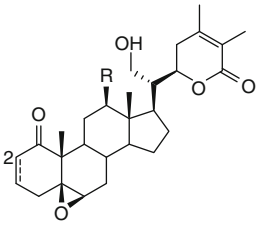
168 (withafastuosin D)



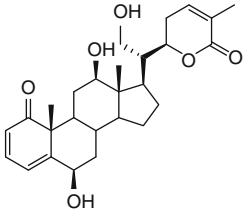
169 R^1 = H, R^2 = H (withafastuosin E)
170 R^1 = OH, R^2 = H (withafastuosin F)
171 R^1 = H, R^2 = CH_3 (withametelin H)
172 R^1 = H, R^2 = β-gluc (withametelin P)
173 R^1 = OH, R^2 = CH_3 (baimantuoluoline C)



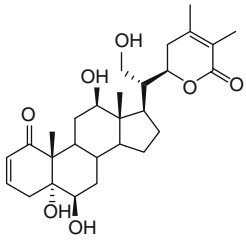
174 R^1 = H, R^2 = OH (baimantuoluoline B)
175 R^1 = OH, R^2 = H (baimantuoluoline F)



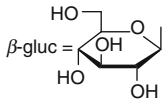
176 R = H (withatatulin)
177 R = OH, Δ^2 (withatatulin B)
180 R = H, Δ^2 (withatatulin E)



178 (withatatulin C)



179 (withatatulin D)

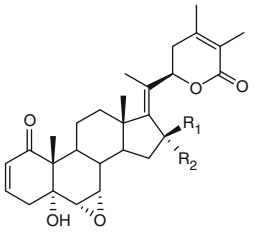


β-gluc =

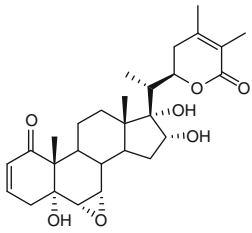
4.2.4. Other Hydroxylated Withanolides

Besides those already mentioned above, several other 16-oxygenated withanolides have been reported. Exodeconolides A–C (**181–183**) were isolated from *Exodeconus maritimus* collected in Peru (*97*), the 16-acetates **184** and **185** were reported from *Acnistus arborescens* collected in Brazil (*98*), 16α-acetoxyhyoscyamilactol (**186**) from the seeds of *Hyoscyamus niger* used in

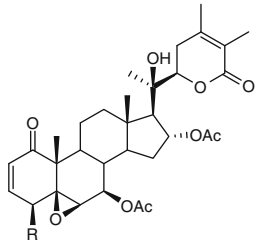
Chinese medicine (*99*), **187** from *Dunalia brachyacantha* collected in Argentina (*71*), **188–190** from the leaves of *Discopodium penninervium* (*100*), and virginols A (**191**) and C (**192**) from *Physalis virginiana* (*101*). Virginol B (**193**) was also reported from *P. virginiana* (*101*) and **194** was isolated from the roots of *D. penninervium* (*102*). The known withaphysacarpin (**195**) and its 3-methoxy derivative, **196**, with a 16*β*-hydroxy group, were isolated from the fruits of *Physalis philadelphica* and the configuration of the lactone methyl groups was established as shown, for both compounds (*103*). LC-MS analysis of an ethyl acetate extract of the plant suggested that **196** may occur naturally although in minor amounts, being generated to a larger extent as a result of the extraction procedure. From leaves and stems of *P. philadelphica*, *Kinghorn* and coworkers isolated philadelphicalactones A and B (**197**, **198**) and compound **199**, all of them with a saturated δ-lactone side chain (*104*, *105*).



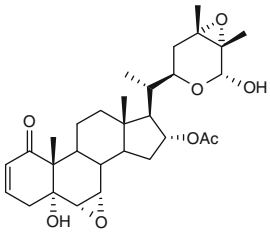
181 R^1 = OH, R^2 = H (exodeconolide A)
182 R^1 = H; R^2 = OH (exodeconolide B)



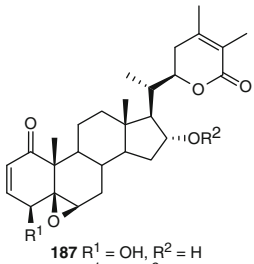
183 (exodeconolide C)



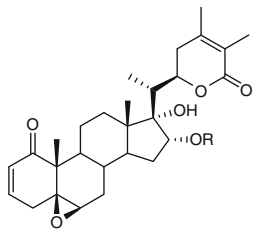
184 R = OH
185 R = H



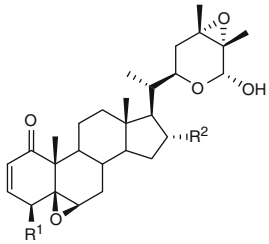
186 (16*α*-acetoxyhyoscyamilactol)



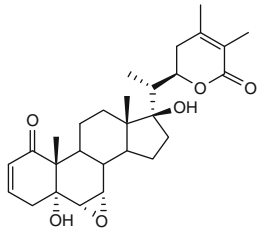
187 R^1 = OH, R^2 = H
188 R^1 = H, R^2 = Ac



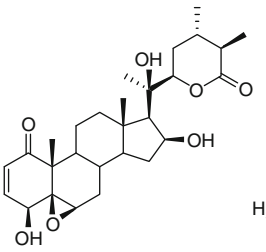
189 R = Ac
190 R = H



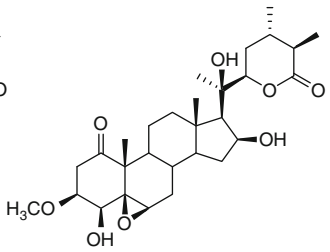
191 R^1 = OH, R^2 = OAc (virginol A)
192 R^1 = H, R^2 = OAc (virginol C)
193 R^1 = OH, R^2 = H (virginol B)



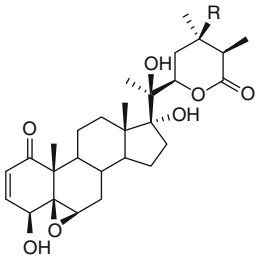
194



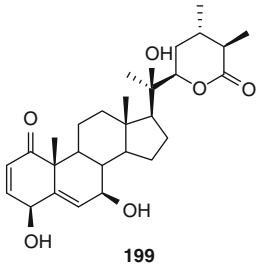
195 (withaphysacarpin)



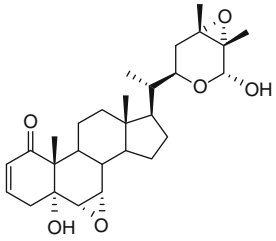
196



197 R = H (philadelphicalactone A)
198 R = OH (philadelphicalactone B)



199

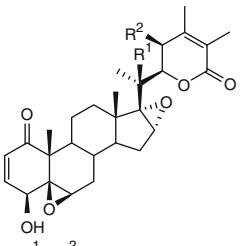


200 (Nic-3)

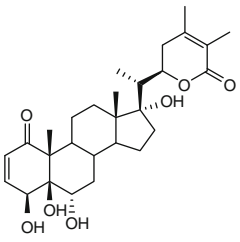
A 24,25-diol related to Nic-3 was reported from *Larnax glabra* (*106*), but, however, comparison of the NMR data indicates that this compound (larnaxolida A) is actually Nic-3 (**200**) (*3*). An epimer at C-5 was also reported (larnaxolida B), but the spectroscopic data do not correspond to the proposed structure.

Tubocapsanolides A (**201**), D (**202**), and F (**203**) and the 20-hydroxy and the 23-hydroxy derivatives of tubocapsanolide A **204** and **205** were isolated from *Tubocapsicum anomalum* (*107*). From *Ajuga bracteosa* collected in the north of Pakistan, *Malik* and coworkers isolated two 28-hydroxywithanolides, bracteosins A (**206**) and B (**207**), with C-19 in the latter compound oxidized to a carboxylic acid, and bracteosin C (**208**) (*108*). As in previous cases, these compounds probably derive from the corresponding Δ^2-1-ketones, upon reaction with methanol during their isolation.

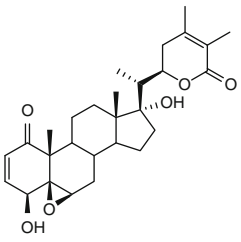
From the aerial parts of *Datura metel* collected in China, *Ma et al*. isolated three new 27-glycosides, daturametelins H, I, and J (**209–211**), together with the known daturataturin A (**212**) and the aglycone **213** (*109*). The closely related 7-acetate **214** was isolated from *Iochroma gesneroides* (*72*).



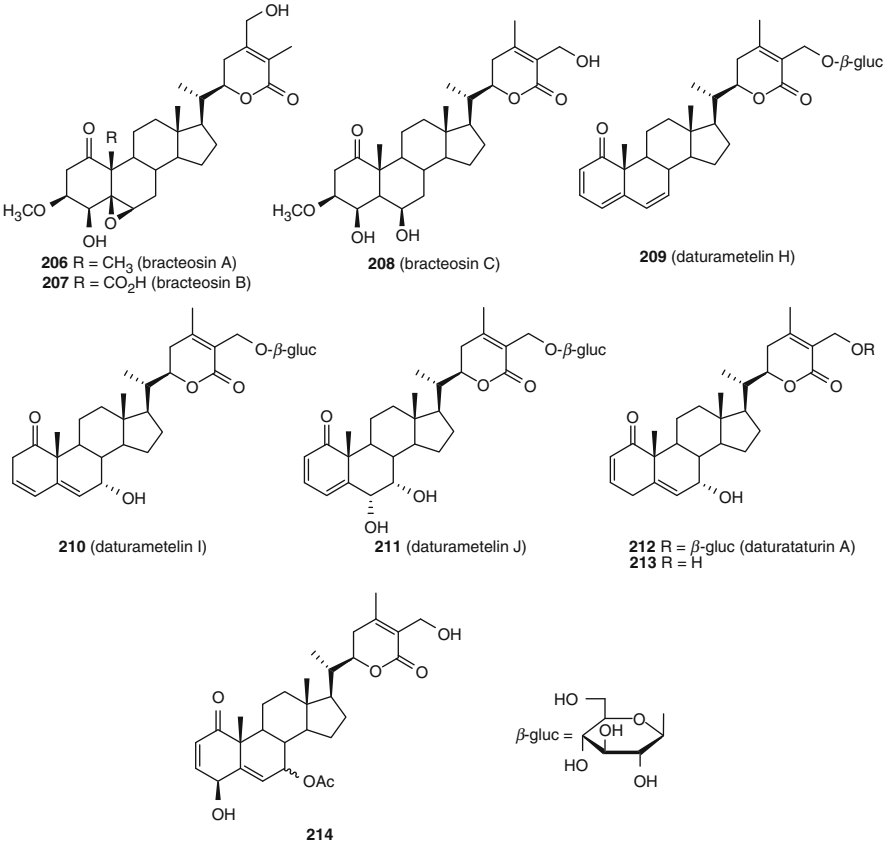
201 $R^1 = R^2 = H$ (tubocapsanolide A)
204 $R^1 = OH$, $R^2 = H$
205 $R^1 = H$, $R^2 = OH$



202 (tubocapsanolide D)



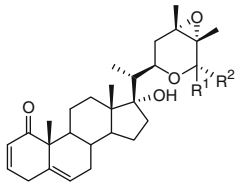
203 (tubocapsanolide F)



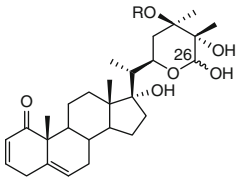
4.2.5. Cilistols and Related Withanolides

Nohara and coworkers investigated *Solanum ciliatum* (synonym *Solanum cilistum*) and isolated a series of withanolides with a δ-lactol side chain, which included several Δ^5-3β-*O*-sulfates and 6-substituted 3,5-cyclowithanolides. Variations at the side chain comprise a 24,25-epoxide or the corresponding 24,25-diol from hydrolytic cleavage, 26-*O*-glycosides, or reaction products of the epoxide or the hemiketal with methanol. Cilistols a, b, d, q, g, and f (**215–220**) contain a $\Delta^{2,5}$-1-ketone in rings A/B, and the latter two compounds (with the cleaved epoxide) exist as equilibrium mixtures of the epimeric C-26 hemiketals (*110*). The C-26 glycosides, cilistols t, I, and j (**221–223**) present a Δ^5-1α,3β-diol arrangement in rings A/B and cilistols y (**224**) and w (**225**) have the unusual feature of a 3β-*O*-sulfate group (*111*). The 3,5-cyclosteroid moiety found in cilistols u, p, pm, and p1 (**226–229**) (*112*) has no precedent among natural products but is easily formed from Δ^5-steroids with a good leaving group at C-3

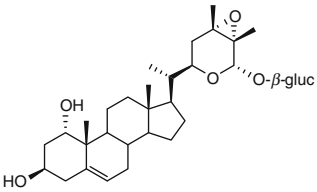
(*e.g.* a sulfate); this rearrangement occurs even under very mild conditions (traces of methanol or water in the extraction or purification solvents may be enough), thus the assumption that these compounds are natural products should be taken with caution. The ergostane glycoside, cilistol v (**230**), or a closely related sterol, is probably the biosynthetic precursor of cilistols in this plant (*111*). Cilistepoxide (**231**) and cilistediol (**232**) are two closely related withanolides isolated from *Solanum sisymbriifolium* collected in Brazil (*113*).



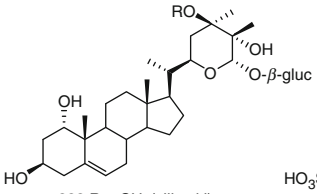
215 R^1 = H, R^2 = OH (cilistol a)
216 R^1 = OCH_3, R^2 = H (cilistol b)
217 R^1 = H, R^2 = OCH_3 (cilistol d)
218 R^1 = H, R^2 = O-*β*-gluc (cilistol q)



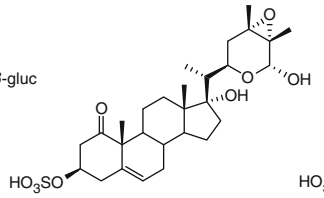
219 R = H, (26*R*/*S*) (cilistol g)
220 R = CH_3, (26*R*/*S*) (cilistol f)



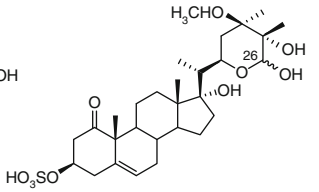
221 (cilistol t)



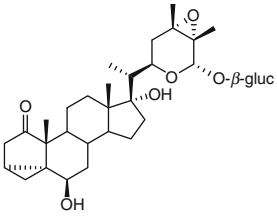
222 R = CH_3(cilistol i)
223 R = H (cilistol j)



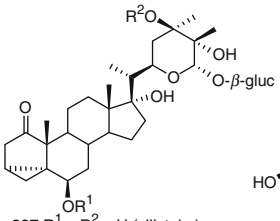
224 (cilistol y)



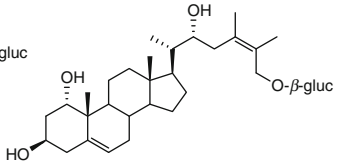
225 (26*R*/*S*) (cilistol w)



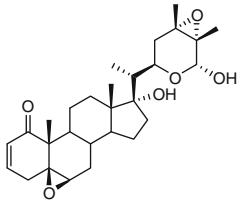
226 (cilistol u)



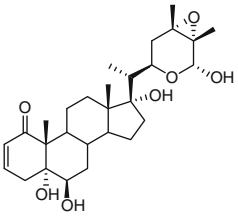
227 R^1 = R^2 = H (cilistol p)
228 R^1 = CH_3, R^2 = H (cilistol pm)
229 R^1 = R^2 = CH_3 (cilistol p1)



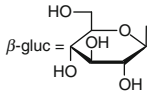
230 (cilistol v)



231(cilistepoxide)

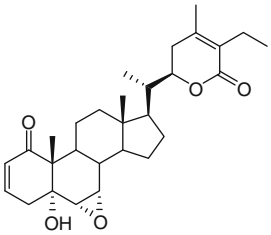


232 (cilistediol)



5. Withanolides with Modified Skeletons

A large number of withanolides with structures that depart from the classical withaferin A (**1**) parent structure are known nowadays. Modifications usually found are additional rings formed by direct C–C bonds, cyclic ethers and hemiketals, or lactones that may involve carbons from the steroid nucleus or from the side chain. Other modifications include formation of *seco*-steroids (as in physalins), a rearranged steroid nucleus, aromatic rings, and lack of an angular methyl as among the most important. One particularly interesting example is that present in a C_{29} withanolide from the bark of *Eucalyptus globulus* (**233**) that has an ethyl substituent at C-25 instead of the usual methyl group (*114*).



233

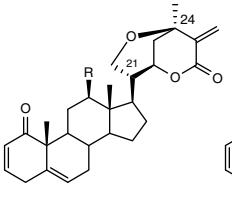
5.1. Withanolides with Additional Rings Involving C-21

Carbon-21 may participate in additional rings by forming either direct C–C bonds or ether bridges with other carbons in the side chain or in the steroid nucleus. Several new withanolide types with these arrangements have been reported.

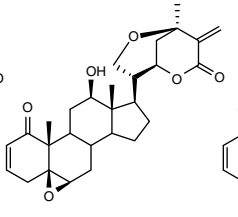
5.1.1. Withametelins and Sativolides

Withametelin (**234**) exhibits an oxygen bridge between C-21 and C-24 resulting in a bicyclic side chain; it was isolated by *Ray* and coworkers from the dried leaves of *Datura metel* (*3*). Several withanolides with this functionality were subsequently isolated; some have also been named as withafastuosins, daturametelins, and baimantuoluolines on different occasions. The flowers of *D. metel*, used for centuries in traditional Chinese medicine, have been shown to contain several withametelins. *Pan et al*. isolated the 12β-hydroxylated derivatives withametelins I–M (**235**–**239**), together with withametelins N (**240**) and O (**241**), and the *seco*-withametelins **242** and **243** (*92*). Also from the dry flowers, *Yang et al*. isolated baimantuoluolines D (**244**) and E (**245**) (*93*). The closely related structures withametelinone (**246**), withametelinol (**247**), withametelinols A (**248**) and B (**249**), witharifeen (**250**), and daturalicin (**251**) were isolated by *Siddiqui et al*.

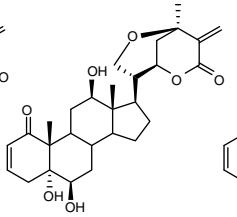
from the aerial parts of *Datura inoxia* collected in Pakistan (*115*–*117*). Daturacin (**252**) was reported also from *D. inoxia* as the first withanolide with a (22*S*) configuration based on a negative *Cotton* effect at 249.4 nm (*118*). The proposed configuration inversion at C-22 and C-24 compared to other withametelins (*92*), would require H-20 to occupy an *axial* instead of *equatorial* position. However, the almost perfect coincidence of the ^{1}H and ^{13}C NMR data of the side chain with those of other "normal" withametelins and the assignment of the configuration at C-5 based exclusively on CD data, suggest that this structure should be extensively revised.



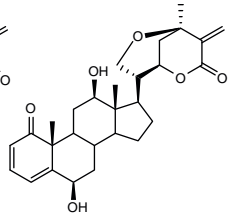
234 R = H (withametelin)
238 R = OH (withametelin L)



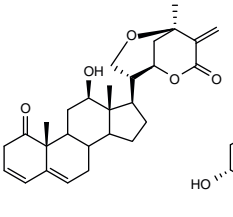
235 (withametelin I)



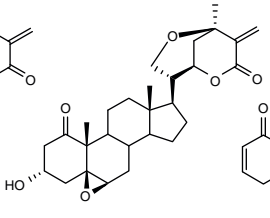
236 (withametelin J)



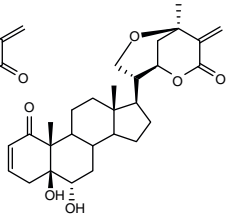
237 (withametelin K)



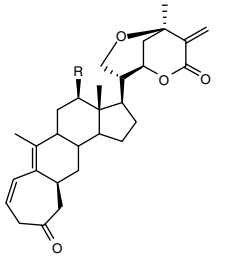
239 (withametelin M)



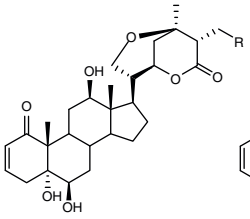
240 (withametelin N)



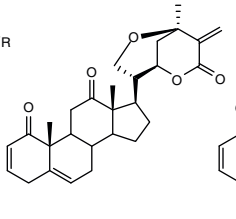
241 (withametelin O)



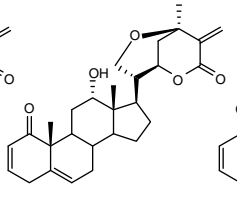
242 R = H
243 R = OH



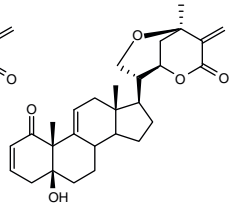
244 R = OCH_3 (baimantuoluoline D)
245 R = OH (baimantuoluoline E)



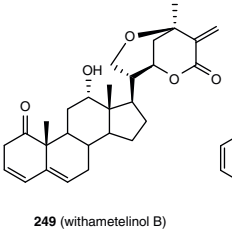
246 (withametelinone)



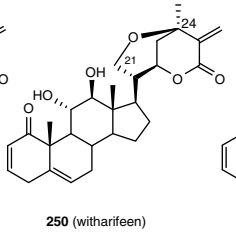
247 (withametelinol)



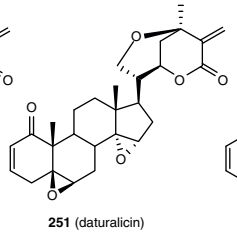
248 (withametelinol A)



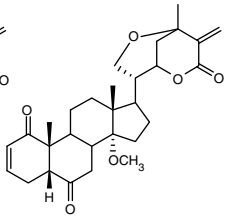
249 (withametelinol B)



250 (witharifeen)

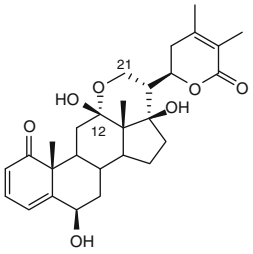


251 (daturalicin)

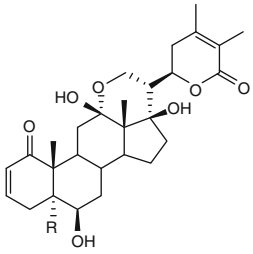


252 (daturacin)

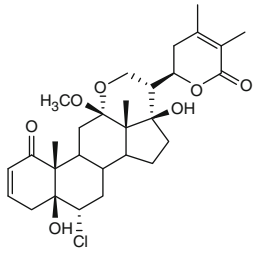
At variance with the above, sativolides have an oxygen bridge between C-21 and C-12 in the steroid nucleus. The additional six-membered hemiketal (or ketal) ring, results from what must have been originally a C-12 ketone and a C-21 hydroxy group. Jaborosalactones R (**253**), S (**254**), and T (**255**), were isolated from *Jaborosa sativa* (synonym *Trechonaetes sativa*) collected in Argentina (*119*). C-12-Hemiketals are highly reactive towards simple alcohols (see Sect. 5.4.2.), thus the methyl ketal **255** is probably formed during isolation. *Nicotra et al.* reported the isomeric clorohydrin, jaborosalactone 37 (**256**), from *Jaborosa rotacea* (*120*). Interestingly, the Δ^2-withanolide **257** was present in *Jaborosa caulescens* var. *caulescens* (isolated together with the 12-*O*-methyl derivative) while its 2,3-dihydro derivative **258** was present in *J. caulescens* var. *bipinnatifida* (*121*).



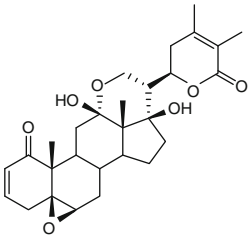
253 (jaborosalactone R)



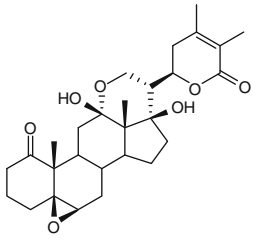
254 R = OH (jaborosalactone S)
256 R = Cl (jaborosalactone 37)



255 (jaborosalactone T)



257 (jaborosalactone 38)

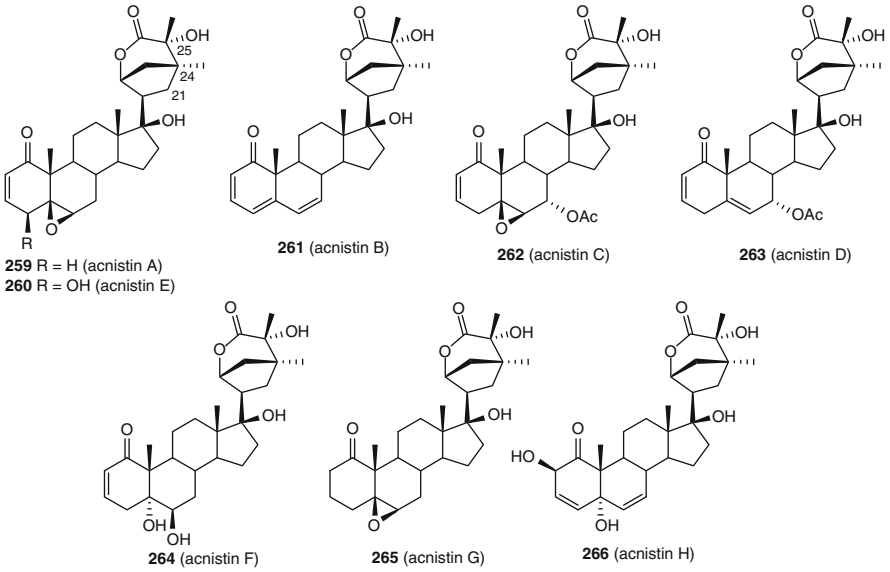


258 (jaborosalactone 39)

5.1.2. Acnistins

The acnistins also exhibit a bicyclic side chain involving C-21 and the lactone ring but, at variance with withametelins, C-21 is directly bonded to C-24 via a C–C bond instead of an ether bond (*3*, *4*). It has been proposed that the 21,24 bond is probably formed via a SN_2 type reaction in withanolides having a good leaving group at C-21. The first examples of this family, acnistins A (**259**) and E (**260**), were isolated by *Usubillaga et al.* from plants of *Acnistus arborescens* (synonym *Acnistus ramiflorus*) collected in Venezuela, with their stereochemical and spectroscopic assignments later revised by *Luis et al.* (*122*, *123*). Withanolides with this bicyclic side chain have been reported also from *Tubocapsicum anomalum* (*3*). *Luis* and coworkers isolated acnistins A and E, together with the new acnistins B (**261**),

C (**262**), D (**263**), F (**264**), G (**265**), and H (**266**), from the leaves of *Dunalia solanacea* collected in Medellin (Colombia) (*124–126*).



259 R = H (acnistin A)
260 R = OH (acnistin E)

261 (acnistin B)

262 (acnistin C)

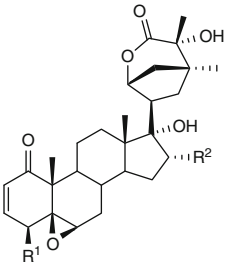
263 (acnistin D)

264 (acnistin F)

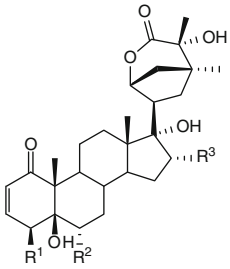
265 (acnistin G)

266 (acnistin H)

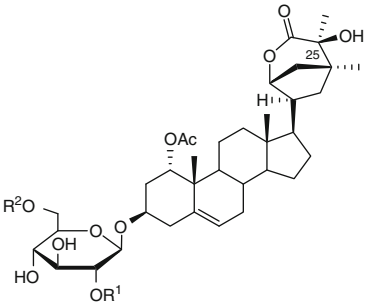
An epimer of acnistin A, 17-epiacnistin A (**267**) was isolated from *Discopodium penninervium* collected in Ethiopia (*127*). Recently, using bioassay-directed fractionation, six new 17-epiacnistins were isolated from *Tubocapsicum anomalum* collected in Taiwan (*107*). Anomanolides A (**268**) and B (**269**) were identified as the 17-epimer of acnistin E and the ring B diol resulting from diequatorial cleavage of the epoxide. Anomanolides C–F (**270**–**273**) had an additional 16α-hydroxy substituent. From fruits of *T. anomalum* collected in Japan, *Kiyota et al.* isolated three acnistin glycosides, isotubocaposides A–C (**274–276**) with a 1α,3β-dihydroxy substitution pattern in ring A (*128*). The distinctive feature of these acnistins was an inverted configuration at C-25 as determined by X-ray crystallography.

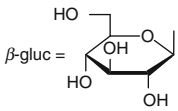


267 $R^1 = R^2 = H$ (17-epiacnistin A)
268 $R^1 = OH$, $R^2 = H$ (anomanolide A)
270 $R^1 = R^2 = OH$ (anomanolide C)



269 $R^1 = R^2 = OH$, $R^3 = H$ (anomanolide B)
271 $R^1 = R^3 = OH$, $R^2 = Cl$ (anomanolide D)
272 $R^1 = H$, $R^2 = R^3 = OH$ (anomanolide E)
273 $R^1 = R^3 = OH$, $R^2 = H$ (anomanolide F)

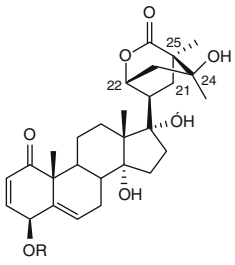




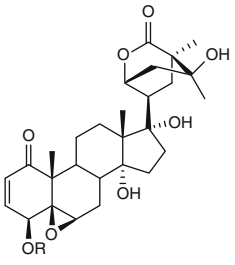
274 $R^1 = R^2 = \beta$–gluc (isotubocaposide A)
275 R^1 = H, $R^2 = \beta$–gluc (isotubocaposide B)
276 $R^1 = \beta$–gluc, R^2 = H (isotubocaposide C)

5.1.3. Withajardins

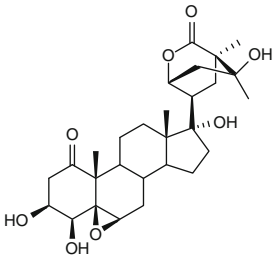
In the withajardins, C-21 is directly bonded to C-25 resulting in a bicyclic lactone side chain with a six-membered homocycle. Withajardins A–E (**277**–**281**) were isolated from plants of *Deprea orinocensis* collected in Colombia (*129*, *130*). A 16,17-dihydroxylated withajardin, tubonolide A (**282**), was isolated from the stems and leaves of *T. anomalum* (*107*) and the glycosides tuboanosides A and B (**283** and **284**) from the fruits of this same plant (*131*). The latter three compounds have an inverted configuration at C-24 compared to the other withajardins, and this was confirmed by X-ray diffraction in the case of tuboanosides A and B (Fig. 4). (It should be noted that in the original publication, the configuration at C-20 in the structure drawing of these compounds is incorrect; the structure shown here was taken from the X-ray data deposited at the Cambridge Crystallographic Data Centre, CCDC). A common precursor has been proposed in the biogenetic routes to acnistins, withajardins, and withametelins; the simultaneous finding of acnistins and withajardins in *T. anomalum* supports this proposal.



277 R = H (withajardin A)
280 R = Ac (withajardin D)



278 R = H (withajardin B)
279 R = Ac (withajardin C)



281 (withajardin E)

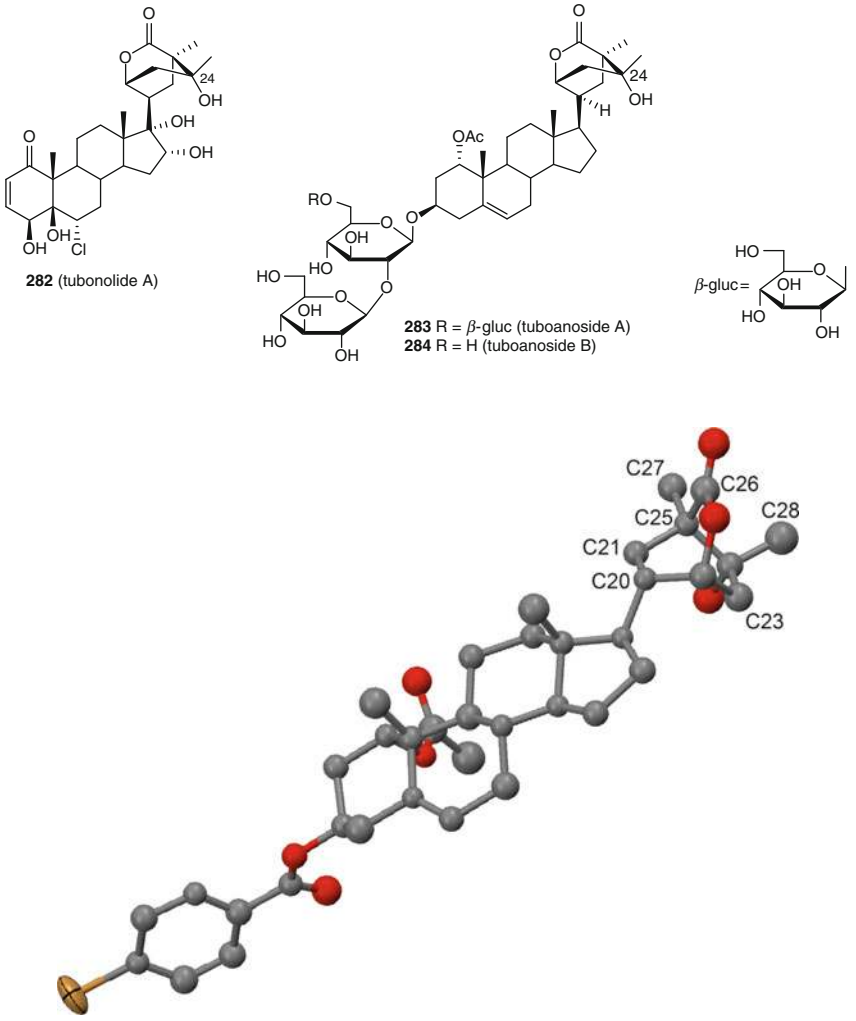
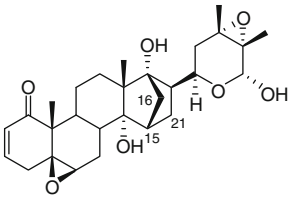


Fig. 4. X-ray crystal structure of the *p*-bromobenzoate of tuboanosigenin (CCDC 680092), aglycone of tuboanosides A (**283**) and B (**284**) (*131*). Structure drawing generated with Mercury 2.3

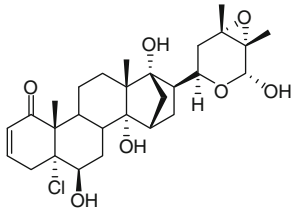
5.1.4. 15,21-Cyclowithanolides (Norbornane Type)

Nicotra et al. reinvestigated *Jaborosa bergii* and isolated five new withanolides with a carbon-carbon bond between C-15 and C-21, resulting in a novel norbornane-type structure in ring D (*69*). Jaborosalactols 18 (**285**) and 22 (**286**) have a 14α-hydroxy group while jaborosalactols 19–21 (**287–289**) contain a 8,14 double bond. 14α-Hydroxywithanolides are known to dehydrate easily giving a mixture of $\Delta^{8,14}$ and Δ^{14} unsaturated derivatives (*132*), and the finding

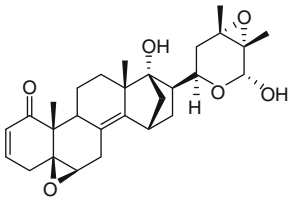
that jaborosalactone 22 (**286**) spontaneously gave **289** strongly suggests that, in this case, the $\Delta^{8,14}$ unsaturated compounds are artifacts formed during isolation. The bridgehead nature of C-15 would prevent the formation of Δ^{14} derivatives (*69*).



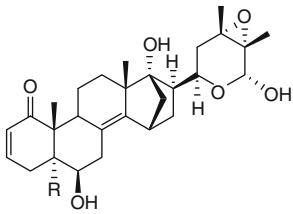
285 (jaborosalactol 18)



286 (jaborosalactol 22)



287 (jaborosalactol 19)



288 R = OH (jaborosalactol 20)
289 R = Cl (jaborosalactol 21)

5.2. Physalins and Withaphysalins

The physalins are a group of 13,14-*seco*-16,24-cycloergostane constituents of certain *Physalis* species (*3*, *133*). In recent years, some known physalins have been found in *Brachistus stramoniifolius* (physalins B, F, and H) (*134*), *B. hunzikeri* (*sub nom. Witheringia hunzikeri*) (physalin B) (*135*), *Margaranthus solanaceous* (*sub nom. Physalis solanaceous*) (physalins A, B, D, and F) (*136*), *Schraderanthus viscosus* (*sub nom. Saracha viscosa*) (physalins D, F, and H) (*137*), and *Witheringia solanacea* (physalins B, D, and F) (*138*). A total of 22 new physalins have been reported from *Physalis* species and several structures described previously have been revised. Withaphysalins, with an oxidized C-18 involved in a lactone or lactol ring with C-20 (see Fig. 2), are believed to be the biosynthetic precursors of physalins.

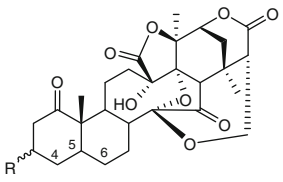
5.2.1. Normal Physalins

Fifteen new withanolides with the normal physalin skeleton were isolated from *Physalis* species, differing in the substitution pattern of rings A and B. From the

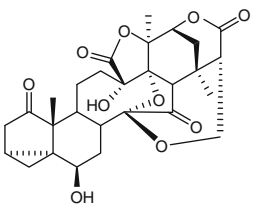
calyces of *P. alkekengi* var. *franchetii*, *Qiu et al.* isolated three 3-hydroxy-1-ketophysalins (*139*), the new physalins Y (**290**) and Z (**291**) with a 3α-oriented hydroxy group, and compound **292** that had NMR data coincident with isophysalin G previously isolated from the same plant by *Sunayama et al.*, but was not fully characterized (*140*). N*O*E correlations of **292** established the *β*-orientation for the 3-hydroxy group of isophysalin G. The 3-methoxy analogues **293** and **294** had been isolated previously by the same authors (*141*). Physalin S (**295**) isolated from the same plant, had a 6*β*-hydroxy-3,5-cyclo arrangement, a common acid rearrangement product of 3-hydroxy-Δ^5 steroids (see Sect. 4.2.5.) (*142*).

Two other physalins from *P. alkekengi* var. *franchetii* corresponded to the 5α,6*β*-diol, physalin T (**296**) (as already mentioned derived from hydrolytic opening of a 5,6-epoxide) (*143*), and the less common 2,5-endoperoxy-Δ^3 arrangement of physalin Q (**297**) (*144*). *Choudhary et al.* isolated the four physalins **298**, **299** (*60*), **300**, and **301** (*145*) from *P. minima*, the latter three containing a 11*β*-hydroxy group. A reduced derivative at C-1, physalin V (**302**), was also isolated from *P. angulata* (*146*).

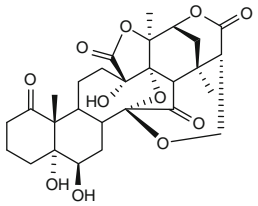
Several 3-alkoxy derivatives besides those mentioned above have been reported, but most probably all of these are artifacts formed by reaction of a Δ^2-1-ketone with ethanol or methanol during isolation. Thus, the 3-ethoxy derivative **303** isolated from *P. alkekengi*, was found only in trace amounts in the original extract when analyzed by HPLC (*67*). The 3-methoxy derivative **304** (physalin U) was initially isolated from *P. minima* (*60*) and later from *P. angulata* (*146*), while the 3-methoxy derivative **305** was isolated from *P. angulata* (*55*). The latter compound was named physalin W although this name had already been assigned to another physalin (see below).



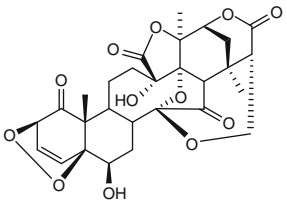
290 R = α-OH, Δ^5 (physalin Y)
291 R = α-OH, $\Delta^{4,6}$ (physalin Z)
292 R = β-OH, $\Delta^{4,6}$ (isophysalin G)
293 R = α-OCH_3, $\Delta^{4,6}$
294 R = β-OCH_3,$\Delta^{4,6}$



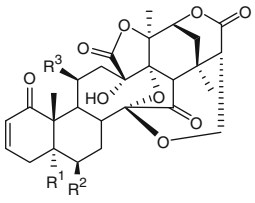
295 (physalin S)



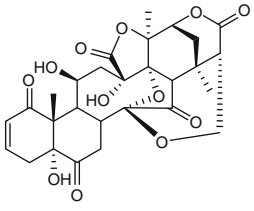
296 (physalin T)



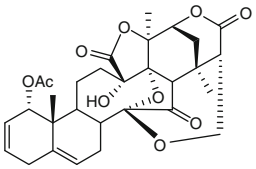
297 physalin Q



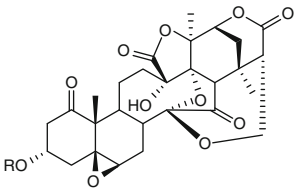
298 R^1 = OH, R^2 = R^3 = H
299 R^1 = R^2 = R^3 = OH
300 R^1 = OCH_3, R^2 = H, R^3 = OH



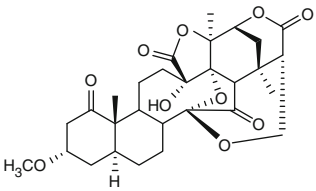
301



302 (physalin V)



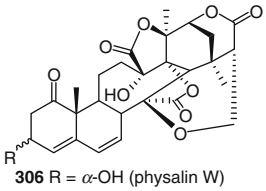
303 R = CH_2CH_3
304 R = CH_3 (physalin U)



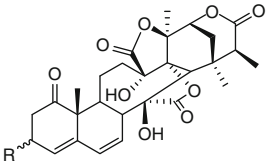
305

5.2.2. Neophysalins and Cyclophysalins

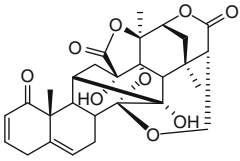
Neophysalins have a rearranged skeleton in which C-14 is directly bound to C-16 and the C-15 carbonyl forms a lactone with the oxygen atom at C-17. Four new neophysalins and a 11,16-cyclophysalin were isolated from *P. alkekengi* var. *franchetii*. Physalins W (**306**) and X (**307**) were initially isolated by *Chen et al.* (*147*) and their structures revised by *Qiu* and coworkers (*139*). The latter authors also reported the isolation of the 3-methoxy analogues physalin I (**308**) and physalin II (**309**). Physalin R (**310**) is a normal physalin with an additional bond between C-11 and C-16 (*142*). The authors showed that this cyclophysalin skeleton could be obtained from normal physalins upon irradiation with an halogen-tungsten lamp under argon.



306 R = α-OH (physalin W)
307 R = β-OH (physalin X)



308 R = α-OCH_3 (physalin I)
309 R = β-OCH_3 (physalin II)



310 (physalin R)

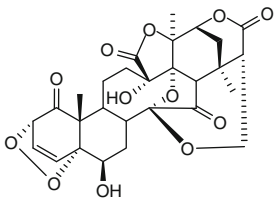
5.2.3. Revised Physalin Structures

The structures of several physalins have been revised. Spectroscopic studies and chemical correlations revealed that the reported structure of physalin K containing a 4α,5α-epoxy-6α-hydroxy-2-en-1-one arrangement in rings A/B was incorrect, the revised structure corresponds to a 2α,5α-epidioxy-6β-hydroxy-3-en-1-one (**311**), an isomer of physalin Q (**297**) (*144*).

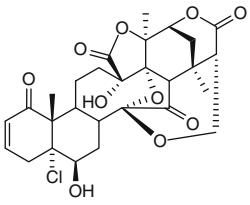
Makino et al. have revised the structure of physalin H, originally reported as having a Δ^5-7β-hydroxy arrangement in ring B. The correct structure corresponded to the clorohydrin **312** (*148*). The authors also demonstrated that physalin E originally reported as a 5α,7α-dihydroxy-2-en-1-one is identical to the 5,6-diol

physalin D (**313**). The acetate of physalin E also corresponds to the 6-acetate of physalin D. *Chen et al.* revised the structure of physalin G (**314**), and the NMR resonances were assigned using 2D NMR and the configuration at C-6 established as (*R*) based on N*O*E data and the coupling constants of H-6 with H-7α and H-7β (*149*).

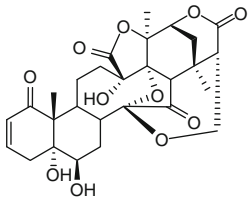
Sen and *Pathak* reported a constituent of *P. minima* as "physalin L" but the proposed structure **315** (*150*) was different from that reported previously for this compound and was also inconsistent with the spectroscopic data given as shown by *Kawai et al.*, who synthesized compound **315** (*151*). The true structure of the compound isolated by *Sen* and *Pathak* has not been established although its ^{1}H NMR spectrum was comparable to the 2,3-dihydro derivative of **315**.



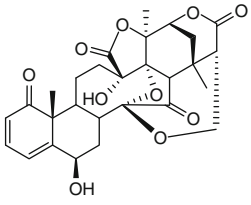
311 (physalin K)



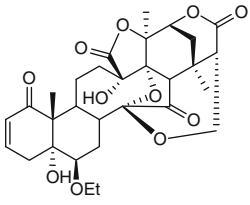
312 (physalin H)



313 (physalin D and E)



314 (physalin G)



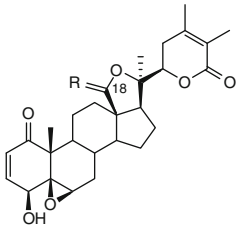
315

5.2.4. Withaphysalins and Related Withanolides

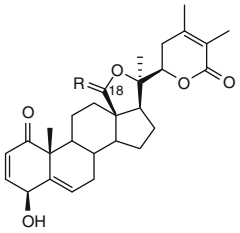
Withaphysalins comprise a group presenting an oxygen bridge between C-18 and C-20; depending on the oxidation state of C-18, a lactol or lactone ring may result (see Fig. 2). Hemiketals at C-18 are highly reactive towards simple alcohols and usually the methyl ketals are formed when methanol is used for extraction or purification. When the free hemiketals are isolated, they exist as an equilibrium mixture of both epimers at C-18; epimeric methyl ketals on the other hand may be separated.

Withaphysalins F–L (**316–322**) were isolated from *Eriolarynx lorentzii* (*sub nom. Vassobia lorentzii*) collected in Argentina (*70*). Compounds **318** and **319**

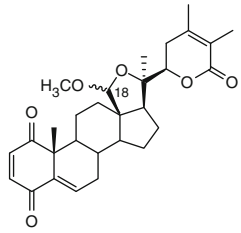
are the corresponding methyl ketals of withaphysalin G (**317**) and most probably artifacts. The hemiketal corresponding to withaphysalins K and L was not isolated. *Veras et al*. isolated withaphysalins M (**323**), N (**324**), O (**325**), 2,3-dihydrowithaphysalin F (**326**), and withaphysalin F (**316**) from *Acnistus arborescens* collected in northeastern Brazil (*152*, *153*). The ethyl ketal **325** is most probably an artifact from reaction of **316** with ethanol used for extraction. The 4-acetate of withaphysalin F (**327**) and the saturated lactone derivative **328** were isolated from *Dunalia brachyacantha* (*71*).



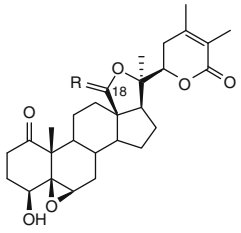
316 R = H,OH, (18 *R*/ *S*) (withaphysalin F)
323 R = O (withaphysalin M)
325 R = H,OEt (withaphysalin O)



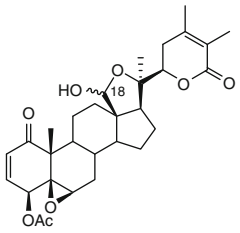
317 R = H,OH, (18 *R*/ *S*) (withaphysalin G)
318 R = H,OCH_3,(18 *R*)(withaphysalin H)
319 R= H,OCH_3, (18 *S*) (withaphysalin I)
320 R = O (withaphysalin J)



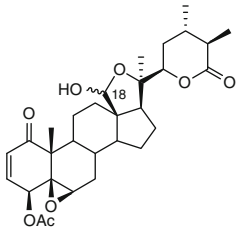
321 (18*R*) (withaphysalin K)
322 (18*S*) (withaphysalin L)



324 R = O (withaphysalin N)
326 R = H,OH, (18*R*/*S*)

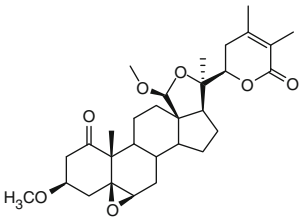


327 (18*R*/*S*)

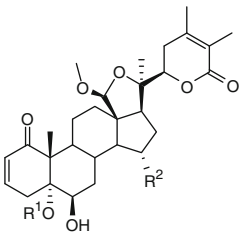


328 (18*R*/*S*)

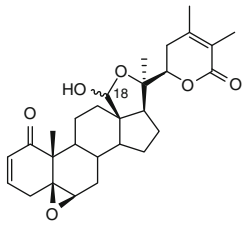
From *Physalis minima*, *Ma et al*. reported seven new withaphysalins (*154*). Withaphysalins Q–S (**329**–**331**) and the 5-*O*-methyl derivative **332** were isolated as the methyl ketals at C-18; as hemiketals are highly reactive and methanol was extensively used during isolation and purification, it is possible that the actual natural products are the free hemiketals. The 3-methoxy group in **329** probably derives from the Δ^2-1-ketone and the 5-methoxy group in **332** could result from addition of methanol to a 5β,6β-epoxide, thus both compounds might be artifacts derived from the known withaphysalin B (**333**). Withaphysalin P (**334**) and the acetylated derivatives of the known withaphysalin C, **335** and **336**, appear to be biosynthetic intermediates in the conversion of withaphysalins to physalins. The authors also mention the isolation of the known 5α,6α-epoxywithaphysalin A (**337**), but this compound has been described only as a synthetic product (*155*). 5α,6α-Withanolides are rare and this would be the first report of **337** as a natural product.



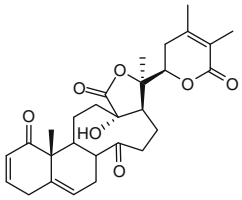
329 (withaphysalin Q)



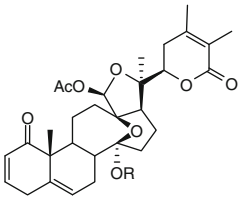
330 R^1 = H, R^2 = H (withaphysalin R)
331 R^1 = H, R^2 = OH (withaphysalin S)
332 R^1 = CH_3, R^2 = H



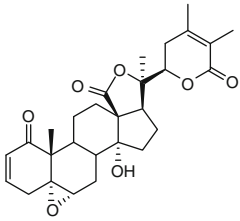
333 (18*R/S*) (withaphysalin B)



334 (withaphysalin P)



335 R = H
336 R = Ac



337 (5α,6α-epoxywithaphysalin A)

5.3. Withanolides Containing an Aromatic Ring and Related Steroids

Two distinct groups of withanolides containing aromatic rings in the steroid nucleus have been found. One of them corresponds to an *abeo*-ergostane skeleton with an expanded 6-member ring D that incorporates C-18 (see Fig. 2) (*3*). The other group presents an aromatic A ring with loss of the angular methyl at C-10. In the discussion that follows, closely related withanolides from a biosynthesis standpoint are also included.

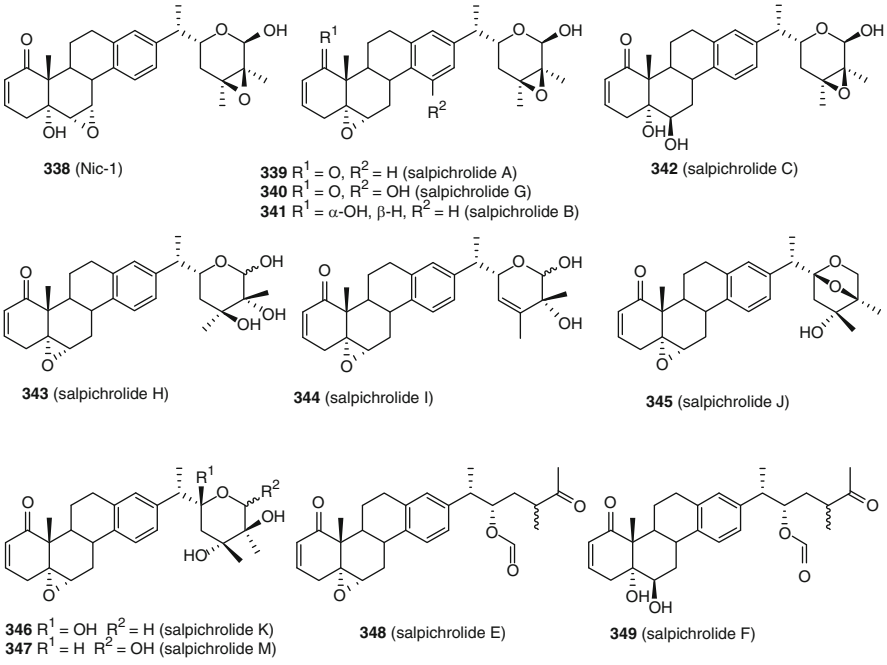
5.3.1. Aromatic Ring-D Withanolides and Related Steroids

A small group of withanolides and related steroids with a six-membered aromatic ring D, the nicandrenoids, were isolated in the early 1970s from the Peruvian "shoofly" plant *Nicandra physalodes* (*e.g.* Nic-1, **338**) (*59*). These compounds remained a curiosity within the withanolides for almost 20 years, until *Veleiro et al.* isolated salpichrolide A (**339**) from *Salpichroa origanifolia* (*156*). Compound **339** was also the first withanolide having a 5,6-epoxide with α-configuration, a feature that proved to be characteristic of several salpichrolides. Further studies on this plant showed that the withanolides present and the relative amounts were strongly dependent on the time of the year in which plants were collected and

also on their geographical origin. The major components in *S. origanifolia* plants collected in Buenos Aires and Córdoba provinces (Argentina), were salpichrolides A (**339**) and G (**340**), with salpichrolides B (**341**) and C (**342**) being isolated as minor components (*157*, *158*).

At variance with other withanolide families, salpichrolides present limited modifications in the substitution pattern of rings A and B. On the other hand, a higher variability was observed for the side chain. Salpichrolides H (**343**) and I (**344**) were isolated from plants collected in Buenos Aires in the winter (*158*), and salpichrolides J (**345**), K (**346**), and M (**347**) from plants collected in Salta province (Argentina) in the summer (*159*). Salpichrolides H (**343**) and M (**347**) correspond to the two possible products resulting from hydrolytic (*trans*) cleavage of the side-chain epoxide. Salpichrolides J (**345**) and K (**346**) are the first examples of withanolides with a side chain in which the oxidation levels at C-22 and C-26 are reversed; salpichrolide K (**346**) slowly cyclized to salpichrolide J (**345**) in solution. Figure 5 shows a possible biosynthesis pathway for these compounds.

Plants collected in Buenos Aires in winter also contained two ergostane derivatives, salpichrolides E (**348**) and F (**349**), probably resulting from degradation of the lactone side chain of salpichrolides A and C (*160*). The configuration at C-22 was assumed to be the same as that in the salpichrolides with an intact side chain (*i.e.* (22*R*)), but the orientation of the C-24 methyl could not be determined. It is noteworthy that similar degradation products are present in *N. physalodes*, the other plant known to contain withanolides with an aromatic ring D (*59*).



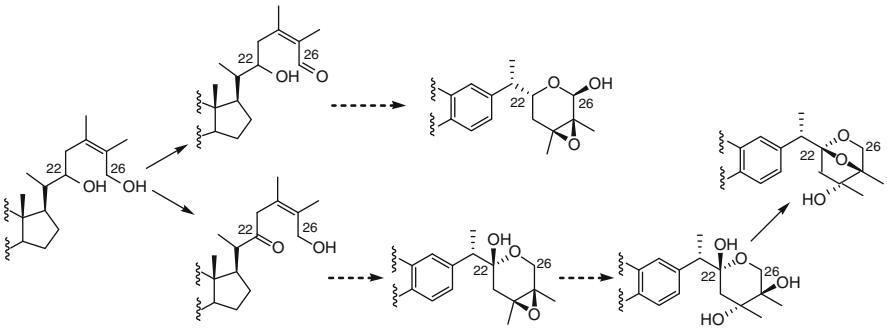


Fig. 5. Proposed biosynthetic pathway for the formation of the side chain in normal salpichrolides and in salpichrolides J (**345**) and K (**346**)

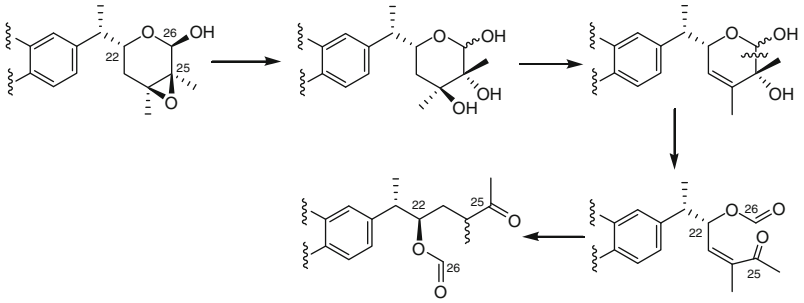


Fig. 6. Proposed degradative pathway for the formation of the side chain in salpichrolides E (**348**) and F (**349**). Starting from salpichrolide A (**339**) the first two intermediates correspond to compounds **343** and **344**

Salpichrolides H (**343**) and I (**344**) could be intermediates in the degradation pathway leading from salpichrolide A (**339**) to salpichrolide E (**348**). Oxidative cleavage of the C-25–C-26 bond would give rise to the formyloxy group (C-26) and the methyl ketone (Fig. 6).

Besides the withanolides with an aromatic D ring, salpichrolides D (**350**), (*157*) L (**351**), and N (**352**) (*159*), with a normal (5-membered) D ring were isolated from *S. origanifolia*. All of these have a characteristic 5α,6α-epoxide moiety, unique to *S. origanifolia*. A possible pathway for ring D aromatization proposed by *Whiting* involves the oxidation of C-18 followed by a 1,2-shift of C-17 to form a new six-membered ring via a cyclopropyl fused intermediate (*161*). Salpichrolide L (**351**) may be the precursor of the putative 14,16-diene intermediate; cleavage of the C-13–C-17 bond would lead to salpichrolide A and related compounds (Fig. 7, route *a*). An alternative cleavage of the cyclopropyl intermediate through the C-13–C-18 bond would result in migration of the angular methyl to give salpichrolide N (**352**) (Fig. 7, route *b*).

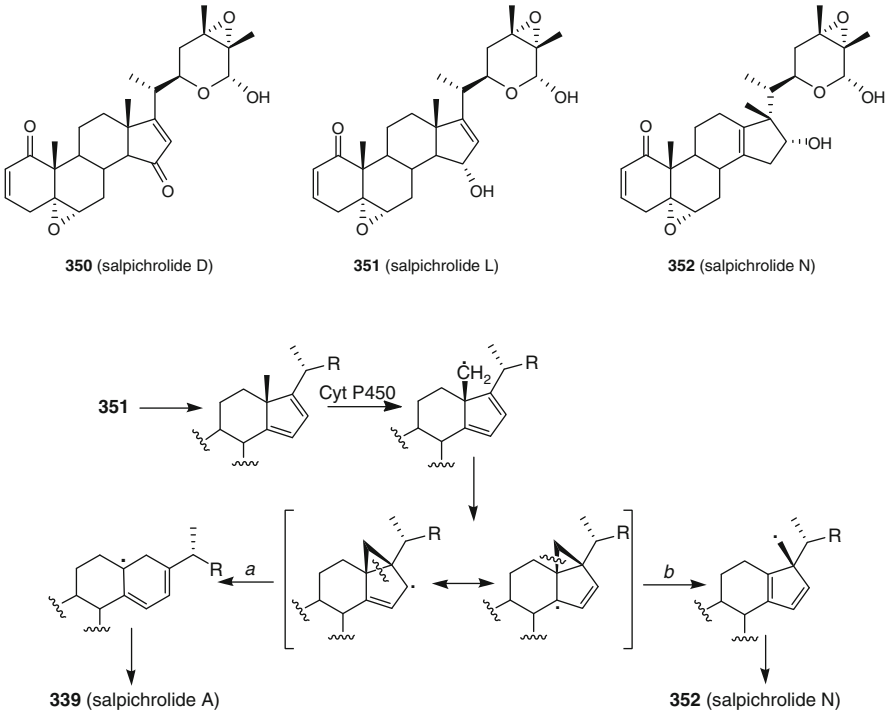
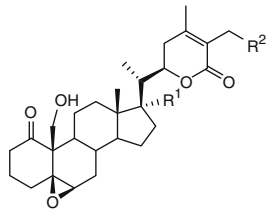


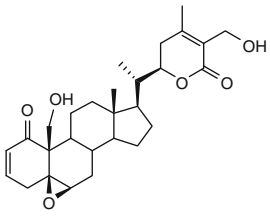
Fig. 7. Proposed biosynthesis pathways for the formation of withanolides with an aromatic D ring (*e.g.* salpichrolide A (**339**), pathway *a*) and for the rearranged skeleton in salpichrolide N (**352**) (pathway *b*)

5.3.2. Aromatic Ring-A Withanolides and 19-Hydroxywithanolides

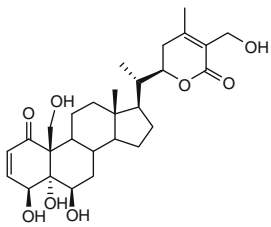
The first 19-hydroxylated withanolide, jaborosalactone O (**353**), was isolated from *Jaborosa leucotricha* collected in late spring in Argentina (*162*). Another three 19-hydroxywithanolides, jaborosalactones V (**354**), W (**355**), and X (**356**) were isolated from plants collected in the autumn together with jaborosalactone Q (**357**) (*163*) and jaborosalactone 7 (**358**) (*68*), with the latter two containing an aromatic A ring. Compound **357** had been previously found in plants of *J. leucotricha* collected at a different location (*164*). Cinerolide (**359**), isolated from *Physalis cinerascens* collected in Mexico is the only 19-hydroxywithanolide outside the *Jaborosa* genus (*54*). The coexistence of 19-hydroxywithanolides and A-ring aromatic 19-norwithanolides in *J. leucotricha* is indicative of an oxidative degradation pathway for the loss of C-19 and aromatization. (+)-Jaborol and jaborosalactone 45 (see Sect. 5.4.2.) are the only other known withanolides with an aromatic A ring.



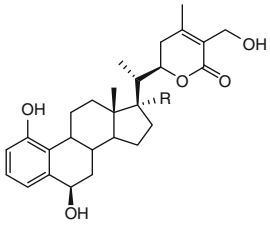
353 R^1 = OH, R^2 = H (jaborosalactone O)
355 R^1 = H, R^2 = OH (jaborosalactone W)



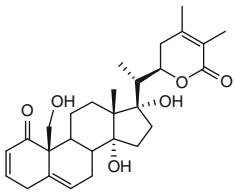
354 (jaborosalactone V)



356 (jaborosalactone X)



357 R = H (jaborosalactone Q)
358 R = OH (jaborosalactone 7)



359 (cinerolide)

5.4. Withanolides with a γ-Lactone Side Chain

Ixocarpalactone A (**360**) was the first withanolide isolated with a γ-lactone side chain. It is the major withanolide of *Physalis philadelphica* (*sub nom. Physalis ixocarpa*), a plant with edible fruits (tomatillos) cultivated in Mexico and Guatemala (*165*). Nowadays several groups of withanolides containing variations of this γ-lactone moiety are known (see Fig. 3).

5.4.1. Ixocarpalactones and Perulactones

Kinghorn and coworkers reinvestigated *Physalis philadelphica* and isolated two new withanolides with a γ-lactone side chain of the ixocarpalactone type (**361**, **362**), also the configuration of ixocarpalactone A was confirmed by X-ray crystallography (*104*, *105*) (Fig. 8). The 3-methoxy derivatives of ixocarpalactones A and B, resulting from the addition of methanol were also isolated and shown to be artifacts of the isolation procedure. The 4-acetate of **361** had been reported previously from fruits of the same plant (*165*). *Huang et al*. isolated two perulactones, taccalonolides O (**363**) and P (**364**), from rhizomes and tubers of *Tacca*

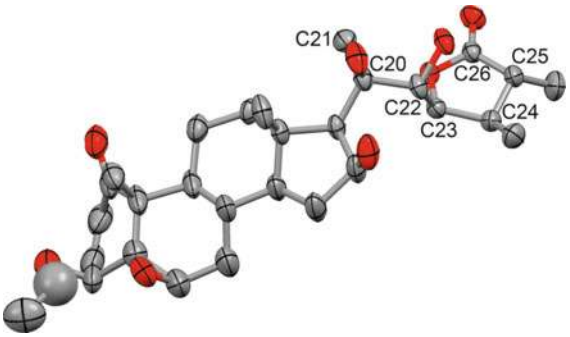
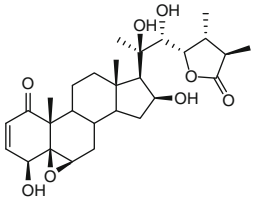


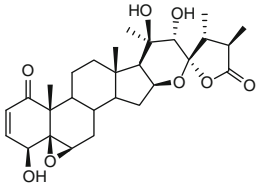
Fig. 8. X-ray crystal structure of ixocarpalactone A (**360**, CCDC 171420) (*104*). The two atoms close to the 4-hydroxy group probably correspond to a solvent molecule. Structure drawing generated with Mercury 2.3

subflabellata (*166*, *167*). Recently, perulactones C (**365**) and D (**366**) were reported from *Physalis peruviana* (*168*).

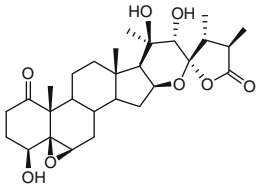
Physanolide A (**367**) was isolated from *Physalis angulata* by *Kuo et al.* (*146*). This withanolide has a novel skeleton related to the perulactones (γ-lactone between C-26 and C-28) with a carbon-carbon bond between C-16 and C-25 that results in a seven-membered ring.



360 (ixocarpalactone A)



361 (ixocarpalactone B)



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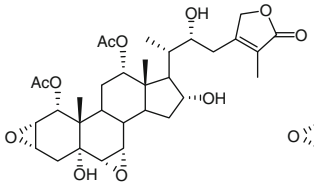
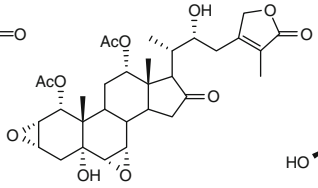
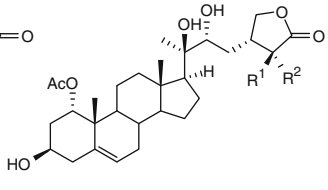


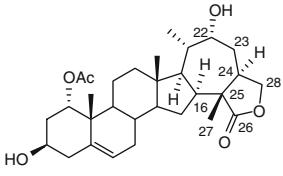
363 (taccalonolide O)



364 (taccalonolide P)



365 $R^1 = CH_3$, $R^2 = OH$ (perulactone C)
366 $R^1 = OH$, $R^2 = CH_3$ (perulactone D)



367 (physanolide A)

5.4.2. Trechonolides

The first member of this group of withanolides was trechonolide A (**368**), isolated by *Lavie et al*. from *Jaborosa laciniata* (*sub nom. Trechonaetes laciniata*) collected in Argentina (*169*). Closely related to the ixocarpalactones, an unusual characteristic feature of this compound was a hemiketal bridge formed by the 22-hydroxy group and a ketone at C-12, resulting in a six-membered ring with a β-oriented hydroxy at C-12. The same compound was later isolated by *Parvez et al*. from the Chilean *J. magellanica* (*170*). Curiously, although in both cases the structure was elucidated by X-ray crystallography, the configuration at C-23 was incorrectly assigned as (*R*). In 2006 *Nicotra et al*. reported the isolation of the C-23 epimer of trechonolide A (jaborosalactone 32, **369**) from *J. rotacea* (*120*). The configuration at C-23 was established by X-ray crystallography and shown to be (*R*) (Fig. 9). Careful inspection of the original X-ray data for trechonolide A (*169*, *170*) confirmed the (23*S*) configuration. The C-23 epimers of jaborotetrol and jaborochlorotriol (**370** and **371**) were also isolated from *J. rotacea* and shown to be (23*R*) (*120*). The chemical shift of C-23 and the sign of the *Cotton* effect at 218 nm may be used as direct indicators of the configuration at this position of trechonolides, thus the (23*S*) epimers have a negative *Cotton* effect and the C-23 resonance at δ 82.0–82.5 ppm, while the (23*R*) epimers exhibited a positive *Cotton* effect and a downfield shift for C-23 to δ 85.5–86.0 ppm. According to this, the structures of all previously known trechonolides that have been assigned the (23*R*) configuration upon comparison with trechonolide A should now be revised. For the above structures and in those that follow, the configuration at C-23 has been corrected according to *Nicotra et al*. when appropriate.

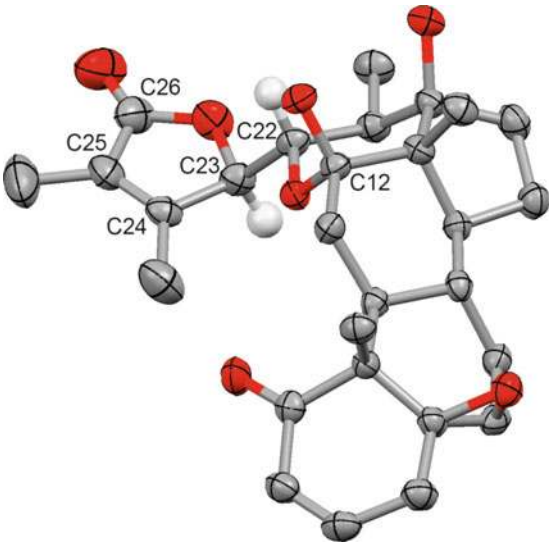
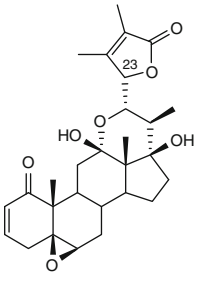
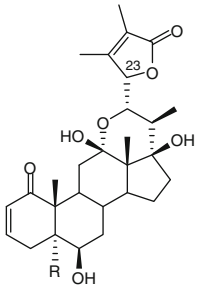


Fig. 9. X-ray crystal structure of jaborosalactone 32 (**369**, CCDC 255337), the C-23 epimer of trechonolide A showing the (23*R*) configuration (*120*). Hydrogens at positions 22 and 23 are included for clarity. Structure drawing generated with Mercury 2.3

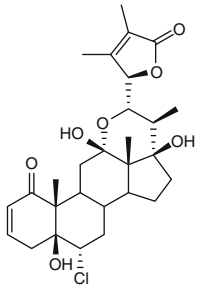
Several withanolides structurally related to trechonolide A with the classical variations in the substitution pattern of rings A and B, have been subsequently isolated from different species of *Jaborosa*. Jaborotetrol (**372**), previously isolated from *J. magellanica*, and trechonolide A (**368**) are the most commonly found. As already observed with the sativolides, the C-12 hemiketal of the trechonolides is highly reactive towards alcohols and even small amounts of methanol or ethanol used during the isolation procedure will give the 12-*O*-methyl or ethyl derivatives. For example, when **370** was dissolved in deuterochloroform containing a few drops of deuteromethanol (to enhance solubility), the 12-*O*-trideuteromethyl derivative was formed (*120*). The clorohydrins jaborosalactone 42 (**373**) and jaborosalactone 49 (**374**) were isolated from *J. caulescens* var. *bipinnatifida* (*121*) and *J. laciniata* (*171*). Also from *J. caulescens* var. *bipinnatifida* were isolated two 21-hydroxytrechonolides epimeric at C-23, **375** and **376** (*121*). The 19-oxygenated trechonolides **377**–**379** were isolated from *J. laciniata* together with **380** (and its 12-*O*-methyl derivative) containing an aromatic ring A (*171*). Again, the 19-hydroxy withanolides appear as intermediates in an oxidative degradation pathway leading to the loss of the C-10 methyl and aromatization of ring A (see Sect. 5.3.2.). As already mentioned, the 12-*O*-methyl derivatives are most probably formed during the isolation procedure, with the 12-hydroxy compounds being the actual natural



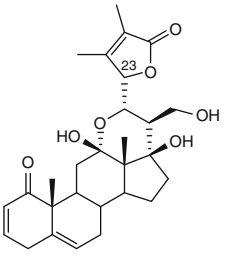
368 (23*S*) (trechonolide A)
369 (23*R*) (jaborosalactone 32)



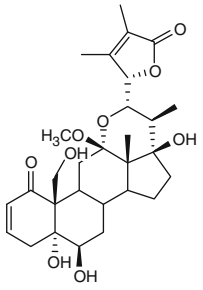
370 (23*R*), R = OH (jaborosalactone 33)
372 (23*S*), R = OH (jaborotetrol)
373 (23*R*), R = Cl (jaborosalactone 42)
374 (23*S*), R = Cl (jaborosalactone 49)



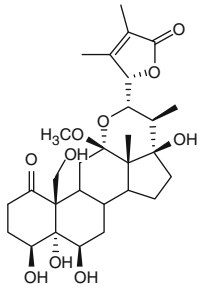
371 (jaborosalactone 34)



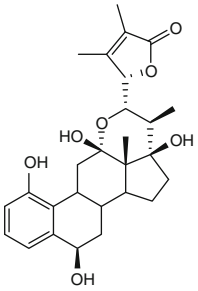
375 (23*R*) (jaborosalactone 40)
376 (23*S*) (jaborosalactone 41)



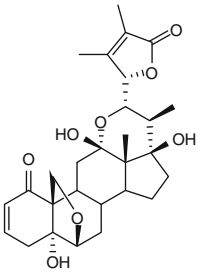
377 (jaborosalactone 46)



378 (jaborosalactone 47)



380 (jaborosalactone 45)



379 (jaborosalactone 48)

products. The 6,19-oxygen bridge present in **379** is an unusual functionality for a natural product. Interestingly, synthetic steroids with this moiety exhibit remarkable biological properties as selective glucocorticoid receptor modulators (*172*).

Although epoxy-δ-lactones and lactols are quite common among the withanolides, this did not appear to be the case for the γ-lactone side chains. Jaborosalactone U (**381**) isolated from *J. sativa* (Argentina), is the only known example of a 24,25-epoxy-γ-lactone (*119*). The (23*R*) configuration was originally proposed for this compound based on N*O*E data, however, more recently X-ray crystallography showed that the configuration is (23*S*) (Fig. 10) (*173*). Recently, several 24,25-epoxy-γ-lactols were isolated from plants of *J. parviflora* (**382**–**385**); some 12-*O*-ethyl derivatives were also reported (*174*). The authors used powder

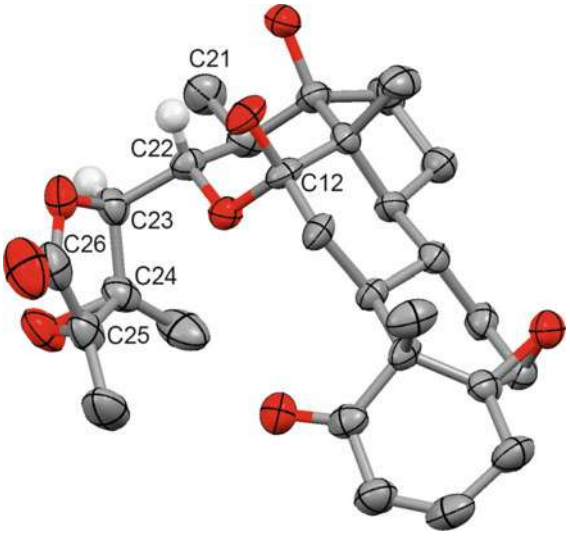
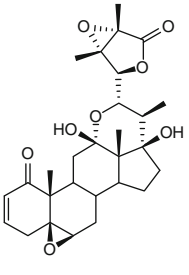


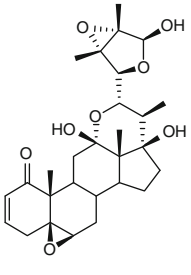
Fig. 10. X-ray crystal structure of jaborosalactone U (**381**) showing the configuration of the side chain epoxylactone (*173*). Hydrogens at positions 22 and 23 are included for clarity. Structure drawing generated with Mercury 2.3

X-ray diffraction analysis and NMR spectroscopy residual dipolar couplings to establish the absolute configuration of the epoxy-lactol side chain of **382**, confirming it was (23*S*). Both methodologies proved to be valid alternatives to single crystal X-ray diffraction. For the other compounds the same configuration was established by comparison of their ^{13}C NMR spectra.

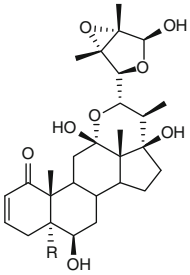
Tettamanzi et al. had reported the isolation of a closely related epoxy-γ-lactol from *J. lanigera* for which the (23*R*) configuration was proposed (*175*); this compound had ^{13}C NMR data identical to **382** for C-12–C-18 and C-20–C-28 indicating that both compounds should have the same configuration in the side chain, hence the revised structure **386** is proposed. Thus so far, all known epoxy-γ-lactones and lactols have the same configuration at C-23, opposite to that of trechonolide A. Jaborosalactones 35 (**387**) and 36 (**388**) isolated from *J. rotacea* would result from cyclization of a 21-hydroxy-epoxy-γ-lactone (*120*). It should be noted that to date, trechonolides have been reported in nine of the thirteen *Jaborosa* species studied. A group of closely related γ-lactones isolated from *Larnax subtriflora* (*sub nom. Deprea subtriflora*) is discussed in Sect. 5.5.2.



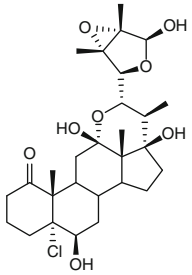
381 (jaborosalactone U)



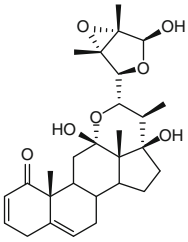
382 (jaborosalactol 24)



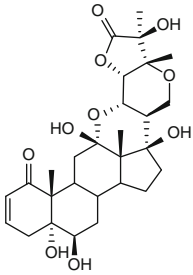
383 R = Cl (jaborosalactol 25)
385 R = OH (jaborosalactol 27)



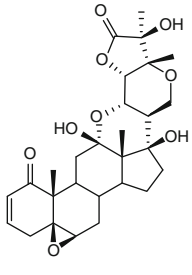
384 (jaborosalactol 26)



386



387 (jaborosalactone 35)



388 (jaborosalactone 36)

5.4.3. Spiranoid-γ-Lactones

The first withanolide with a spiranoid γ-lactone side chain, jaborosalactone P (**389**), was isolated by *Monteagudo et al.* from plants of *Jaborosa odonelliana* collected in Argentina (*176*). *Cirigliano et al.* reinvestigated this plant collected at different times of the year. Jaborosalactones 10 (**390**), 14 (**391**), and 15 (**392**) were found in plants collected in the summer while jaborosalactones 11 (**393**), 12 (**394**), 13 (**395**), and 14 (**391**) were present in plants collected in the autumn (*177*). Jaborosalactone P was the major component in both cases. The C-23 epimer of jaborosalactone P, jaborosalactone 24 (**396**), was isolated as a minor component from plants collected in April and December (*178*). This is the only spiranoid withanolide with a (23*R*) configuration isolated so far.

A group of six spiranoid withanolides with a 17(*20*)-ene-22-keto system, jaborosalactones 1–6 (**397**–**402**) was isolated from *Jaborosa runcinata* collected in Argentina. Jaborosalactone 2 (**398**) was also isolated from *Jaborosa araucana* (*179*). More recently, jaborosalactone 25 (**403**) was isolated as a minor component of *J. runcinata* (*178*).

The structural similarity of jaborosalactone 2 (**398**) and trechonolide A (**368**), both present in *J. araucana*, suggests that these compounds may have a common biosynthetic precursor. In trechonolide A, ring closure on C-12 has occurred with a C-22 hydroxy group as shown in Fig. 11 pathway *a*. Oxidation of the intermediate or a related compound to the 22-ketone would allow cyclization between C-23 and the C-12 ketone to give the 22-keto-spiranoid withanolides (Fig. 11 pathway *b*).

Jaborosalactone 31 (**404**), isolated from *J. rotacea,* is closely related to the spiranoid withanolides isolated from *J. odonelliana*, *J. runcinata*, and

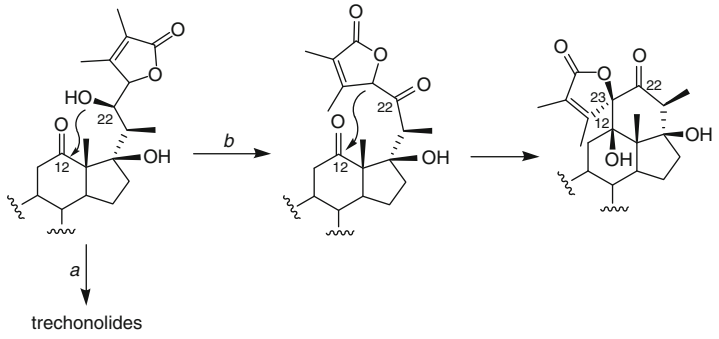
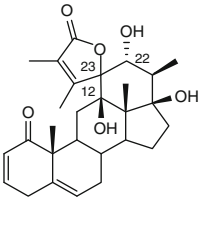
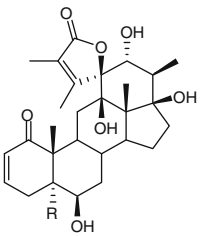


Fig. 11. Proposed biosynthetic routes to trechonolides (pathway *a*) and spiranoid withanolides (pathway *b*) via a common precursor

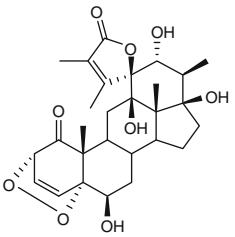
J. araucana. In this case, the C-12–C-23 bond is still present but instead of the spiranoid-γ-lactone arrangement (there is no oxygenated function at C-23), a δ-lactone is formed between the C-26 carboxyl and the C-12 hydroxy group (*120*).



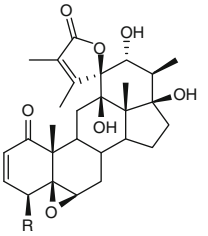
389 (23*S*) (jaborosalactone P)
396 (23*R*) (jaborosalactone 24)



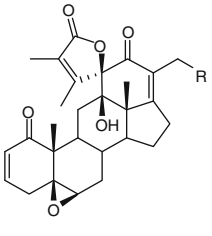
390 R = Cl (jaborosalactone 10)
391 R = OCH_3 (jaborosalactone 14)
395 R = OH (jaborosalactone 13)



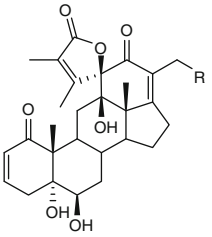
392 (jaborosalactone 15)



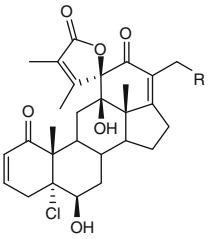
393 R = H (jaborosalactone 11)
394 R = OH (jaborosalactone 12)



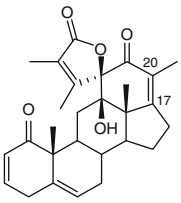
397 R =H (jaborosalactone 1)
400 R = OH (jaborosalactone 4)



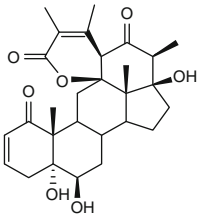
398 R = H (jaborosalactone 2)
403 R = OH (jaborosalactone 25)



399 R = H (jaborosalactone 3)
402 R = OH (jaborosalactone 6)



401 (jaborosalactone 5)

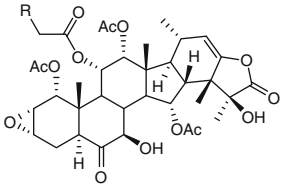


404 (jaborosalactone 31)

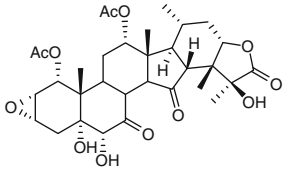
5.4.4. Taccalonolides

Tacca species contain highly oxygenated ixocarpalactone-type withanolides with an additional ring formed by a carbon-carbon bond between C-16 and C-24, taccalonolide A (**405**) being the first example of these compounds (*3*). Eight

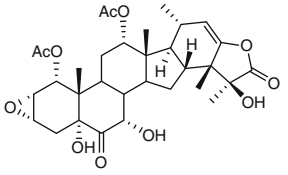
new withanolides related to taccalonolide A were isolated from *Tacca plantaginea*, taccalonolides L (**406**), M (**407**) (*180*), G, H, I, J, K (**408–412**) (*181*), and W (**413**) (*182*). Taccalonolides R, S, T, U, and V (**414–418**) were isolated from the Vietnamese plant *Tacca paxiana* together with the known taccalonolides A (**405**), K (**412**), B, E, and N (**419–421**) (*183*). A distinctive feature of most taccalonolides is the absence of a hydroxy group at C-22. Taccalonolide Q (**422**) (*167*) and Y (**423**) (*182*) containing a δ-lactone side chain with a C–C bond between C-16 and C-24 were isolated from *T. subflabellata* and *T. plantaginea*.



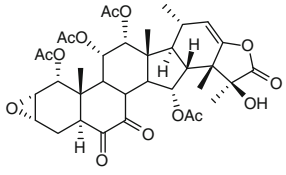
405 R = H (taccalonolide A)
406 R = OH (taccalonolide L)



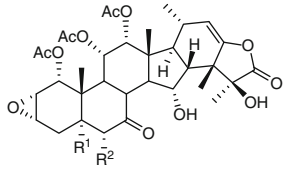
407 (taccalonolide M)



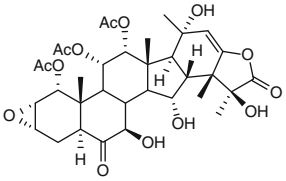
408 (taccalonolide G)



409 (taccalonolide H)

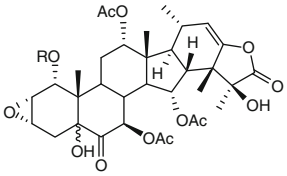


410 R^1 = H, R^2 = OH (taccalonolide I)
411 R^1 = H, R^2 = OAc (taccalonolide J)
412 R^1 = R^2 = OH (taccalonolide K)

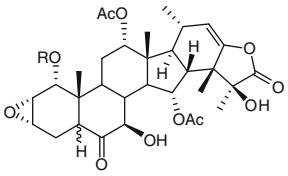


413 (taccalonolide W)

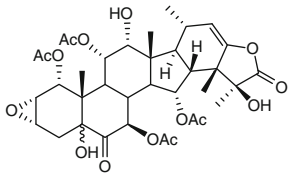
A sizeable number of steroids partially resembling withanolides have been isolated from *Tacca* species and some also dubbed taccalonolides, *e.g.* taccalonolide X (**424**) (*182*), but they are not included in this chapter. Other taccalonolides structurally related to the perulactones have been presented previously (see Sect. 5.4.1.).



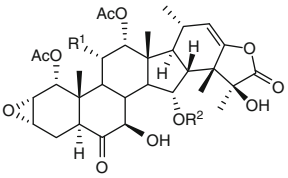
414 R = Ac (taccalonolide R)
416 R = isovaleryloxy (taccalonolide T)
417 R = H (taccalonolide U)



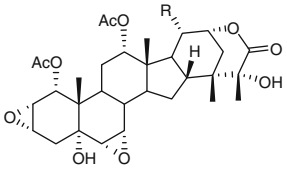
415 R = isobutyryloxy (taccalonolide S)



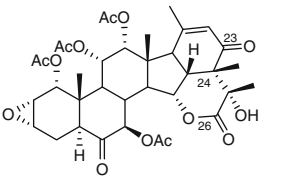
418 (taccalonolide V)



419 R^1 = OAc, R^2 = H (taccalonolide B)
420 R^1 = H, R^2 = Ac (taccalonolide E)
421 R^1 = R^2 = H (taccalonolide N)



422 R = CO_2H (taccalonolide Q)
423 R = CH_2OH (taccalonolide Y)



424 (taccalonolide X)

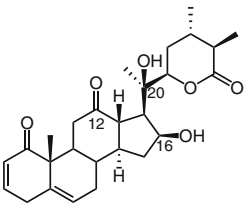
5.5. 18-Norwithanolides

Kinghorn and coworkers used a quinone reductase induction assay for the activity-monitored fractionation of an extract of *Larnax subtriflora* (*sub nom. Deprea subtriflora*) collected in Peru. This led to the isolation of a novel group of highly oxygenated C_{27} 18-norwithanolides, dubbed subtrifloralactones. All subtrifloralactones found so far, have oxygenated functions at positions 12, 16, and 20 and a saturated lactone (γ or δ) side chain (see Figs. 2 and 3) (*184*, *185*). Another C_{27} 18-norwithanolide related to the physalins, has been reported recently from a *Physalis* species. A small group of C_{28} 17-methyl-18-norwithanolides is also included in this section.

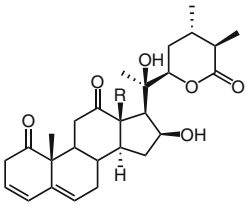
5.5.1. Subtriflora-δ-Lactones and Related Withanolides

Subtrifloralactones D (**425**) and E (**426**) resemble the classic withanolide structure except for the lack of C-18. On the other hand, subtrifloralactones H, I, and J

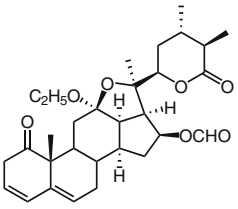
(**427–429**) have rings C and D *cis* fused, due to epimerization at position 13 (probably favored by the presence of the neighboring 12-ketone), allowing formation of a ketal bridge between a 20-hydroxy group and a 12-ketone (*184*). Due to the high reactivity of C-12 hemiketals towards even traces of small alcohols (see Sect. 5.4.2.), the formation of the methyl and ethyl ketals in the latter compounds probably took place during isolation. Another distinctive feature of subtrifloralactones H, I, and J is the presence of a formate group esterifying the 16-hydroxy group. The isolation of 13β-hydroxymethylsubtrifloralactone E (**430**) from the same plant (*185*) indicates an oxidative pathway for the loss of C-18 that could end as the 16-formate group via rearrangement of a 16,18-hemiketal (Fig. 12).



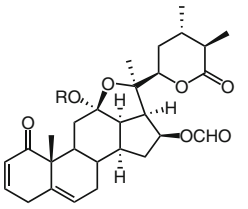
425 (subtrifloralactone D)



426 R = H (subtrifloralactone E)
430 R = CH_2OH



427 (subtrifloralactone H)



428 R = C_2H_5 (subtrifloralactone I)
429 R = CH_3 (subtrifloralactone J)

5.5.2. Subtriflora-γ-Lactones

Subtrifloralactones A (**431**), B (**432**), C (**433**), K (**434**), and L (**435**) present a side chain arrangement closely related to that observed in the trechonolides, with a γ-lactone between C-26 and C-23 and a ketal bridge between a 22-hydroxy and a 12-ketone (*184*, *185*). However, the *cis* fusion of rings C and D and the presence of a 16-hydroxy group allow an additional ketal bridge involving this hydroxy and C-12. In subtrifloralactones F (**436**) and G (**437**) the *trans* fusion of rings C and D results in an ixocarpalactone-type structure (*184*).

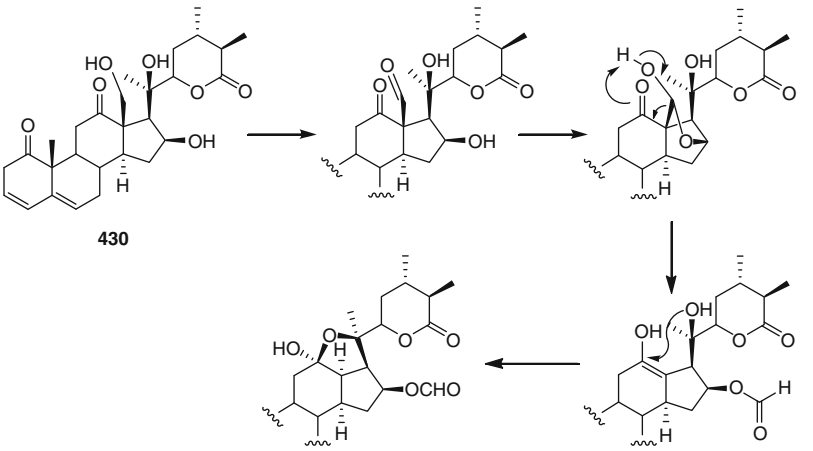
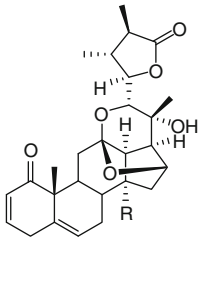
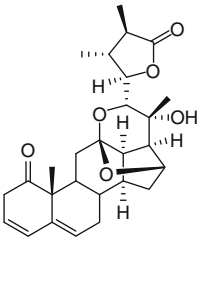


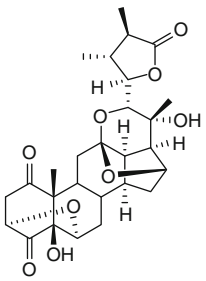
Fig. 12. Proposed biosynthetic pathway for the conversion of 13β-hydroxymethylsubtriflor-alactone E (**430**) to subtriflora-δ-lactones



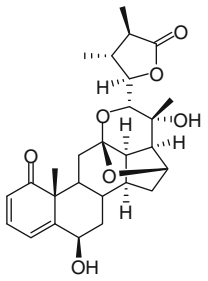
431 R = H (subtrifloralactone A)
433 R = OH (subtrifloractone C)



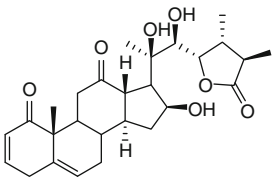
432 (subtrifloralactone B)



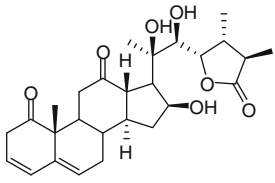
434 (subtrifloralactone K)



435 (subtrifloralactone L)



436 (subtrifloralactone F)

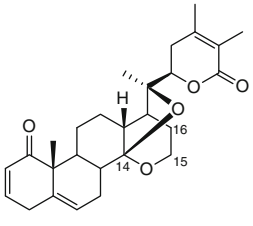


437 (subtrifloralactone G)

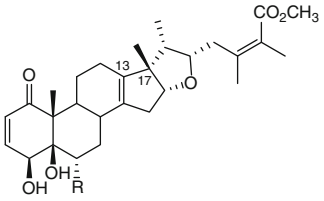
5.5.3. Other 18-Norwithanolides

Recently, *Ma et al*. reported the isolation of another 18-norwithanolide structurally related to the physalins, withaphysanolide A (**438**), together with several known physalins and withaphysalins from *Physalis divaricata* collected in Pakistan (*186*). The structure and configuration of withaphysanolide A was confirmed by X-ray crystallography.

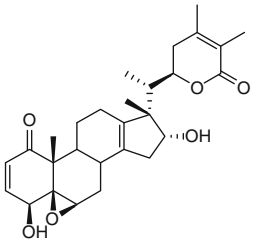
TH-6 (**439**) and TH-12 (**440**) are two 17-methyl-18-nor-ergostanes isolated in 1990 by *Shingu et al*. from the acid hydrolysate of a methanolic extract of *Tubocapsicum anomalum* (*187*). The authors related these compounds to a putative precursor with a withanolide side chain, that would rearrange in acid media. Recently *Hsieh et al*. isolated from the same plant three withanolides with the rearranged skeleton of TH-6 and TH-12 named tubocapsenolides A, F, and G (**441–443**) (*107*). Salpichrolide N (**352**) isolated from *Salpichroa origanifolia* (see Sect. 5.3.1.), was the first withanolide reported with this rearranged skeleton (*159*).



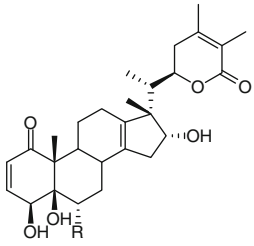
438 (withaphysanolide A)



439 R = Cl (TH-6)
440 R = OH (TH-12)



441 (tubocapsenolide A)

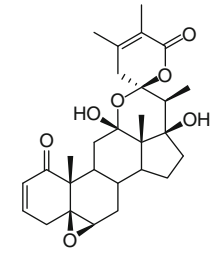


442 R = OH (tubocapsenolide F)
443 R = Cl (tubocapsenolide G)

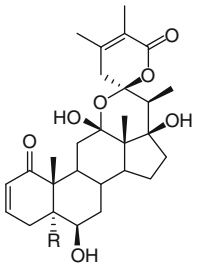
5.6. *Spiranoid Withanolides at C-22*

This group of withanolides has a hemiketal bridge between what must have originally been ketone functions at C-12 and C-22. This gives rise to a new

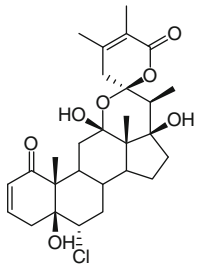
six-membered ring with a β-oriented hydroxy group at C-12 and a spiroketal at C-22 upon formation of the δ-lactone. Jaborosalactones 26–30 (**444–448**) were isolated from *Jaborosa rotacea* (*120*) and jaborosalactone 43 (**449**) was isolated from *J. kurtzii* (*76*) both collected in Mendoza, Argentina. The 12-ketowithanolide jaborosalactone 44 (**144**) also present in *J. kurtzii* (see Sect. 4.2.3.) has been proposed as the biogenetic precursor of **449**.



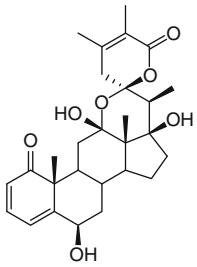
444 (jaborosalactone 26)



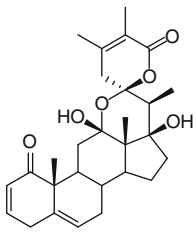
445 R = OH (jaborosalactone 27)
446 R = Cl (jaborosalactone 28)



447 (jaborosalactone 29)



448 (jaborosalactone 30)



449 (jaborosalactone 43)

6. Chemical and Bio-transformations of Withanolides

The early synthesis work carried out on withanolides has been reviewed by *Glotter* (*59*), *Ray* and *Gupta* (*3*), and more recently by *Kovganko* and *Kashkan* (*188*). No new attempts on total or partial syntheses of withanolides have been reported in the last two decades. On the other hand, transformations usually involving interconversions of functional groups, of one withanolide into another or synthesis of simple derivatives, are fairly common and mostly used for structure confirmation or to study biological activities.

6.1. Chemical Transformations

Reactivity of the Δ^2-1-keto system of withanolides towards *Michael* addition (mostly of simple alcohols) has been discussed in the previous sections and several examples shown. As already mentioned, 5*β*,6*β*-epoxides are fairly common among the withanolides and also constitute a highly reactive center. Many chemical transformations involve the cleavage of the epoxide moiety to give the corresponding diols, chlorohydrins or alcohol addition products, with their configuration according to the *Fürst-Plattner* rule (*16*).

For the structure assignment of physalin H (**312**), *Makino et al*. treated physalin F (**450**) with aqueous hydrochloric acid to give a mixture of the diol **313** (physalin D) and the 5α,6*β*-clorohydrin **312** (physalin H) (Chart 1). When concentrated hydrobromic acid in THF was used the brominated analog of physalin H (**451**) was obtained. Reaction of physalin D (**313**) with phosphorous oxychloride in dry pyridine gave the isomeric chlorohydrin **452** (*148*). Cleavage of the epoxide of physalin F (**450**) with concentrated hydrochloric acid in ethanol gave the 5α-ethoxy-6*β*-hydroxy withanolide **453** (*151*).

The 5α,6α-epoxide present in the salpichrolides and a few other withanolides, reacts in a similar way. Thus, treatment of salpichrolide A (**339**) with THF containing 0.75% of 1.5 *N* sulphuric acid gave the corresponding diol **342** (salpichrolide C)

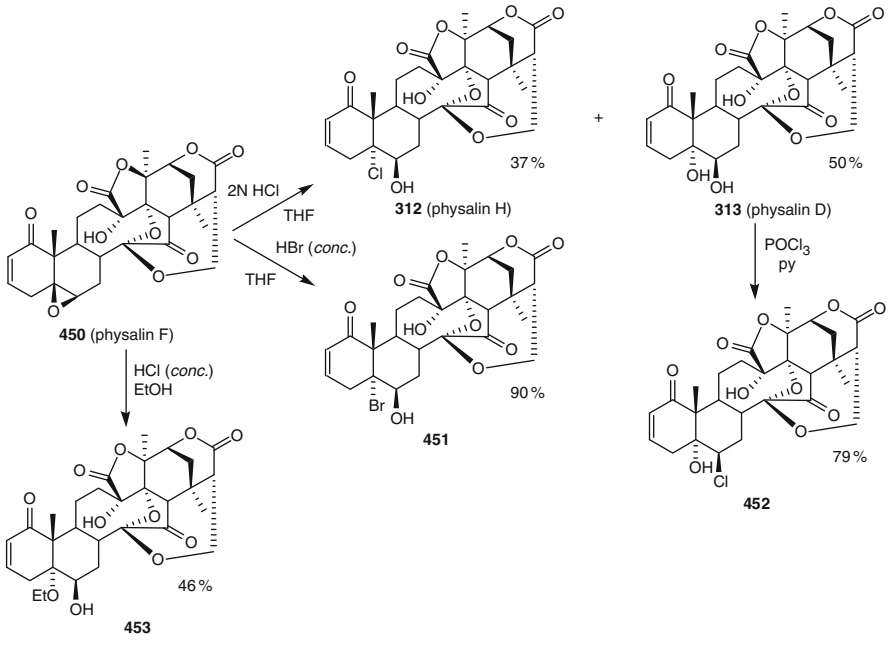


Chart 1 Cleavage reactions of the 5*β*,6*β*-epoxide of physalin F (**450**)

(*157*); increasing the amount of acid 10-fold, resulted in the concomitant cleavage of the 24,25-epoxide (compound **454**) (*159*). Reaction of salpichrolide A (**339**) with *Jones* reagent in acetone for 3 h gave the hydroxyketone **455** where oxidation of the side chain lactol to the lactone also occurred (*189*). On the other hand, cleavage of the epoxide with potassium bicarbonate in methanol gave the $\Delta^{2,4}$-6α-hydroxy withanolide **456** (Chart 2) (*190*). Reaction of physalin J (**457**) with sulfuric acid in ethanol gave the 5α-hydroxy-6*β*-ethoxy physalin **315** (*151*).

Misra et al. studied the reaction of 2-mercaptoethanol with several withanolides from *W. somnifera*. Withaferin A (**1**) and other 5*β*,6*β*-epoxywithanolides reacted readily under mild acid catalysis to give the 5*β*,6α-oxyethylenethio derivatives (Chart 3) in 47–60% yield. The additional ring would result from nucleophilic attack of the thiol at position 6, followed by acid-catalyzed condensation of the 6α-hydroxyethylthio intermediate. Withanolides with a 6α,7α-epoxide did not react

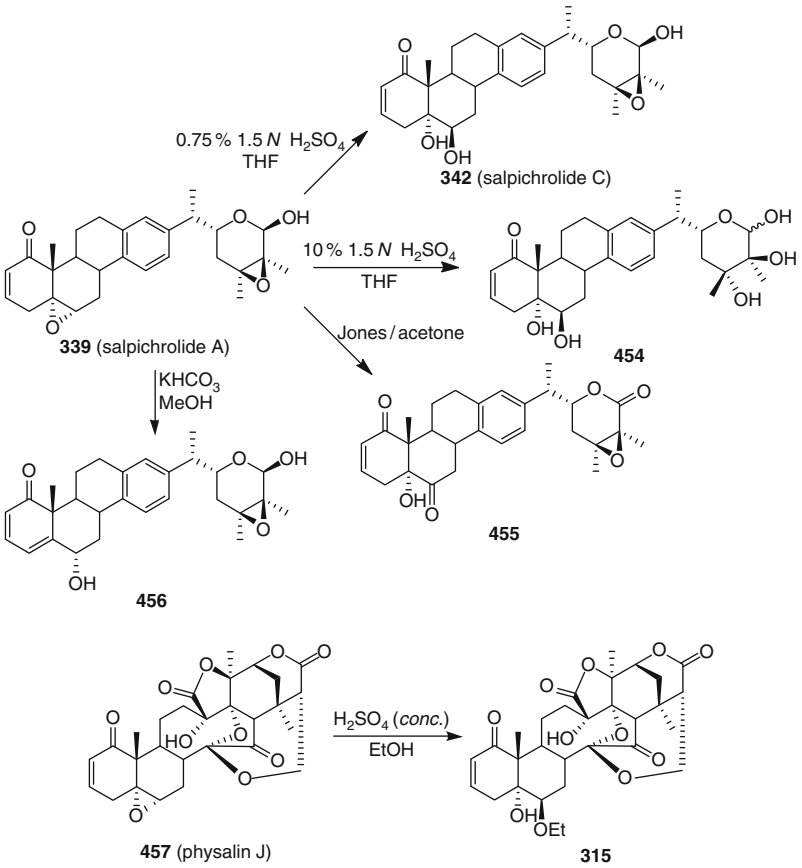


Chart 2 Cleavage reactions of 5α,6α-epoxy withanolides

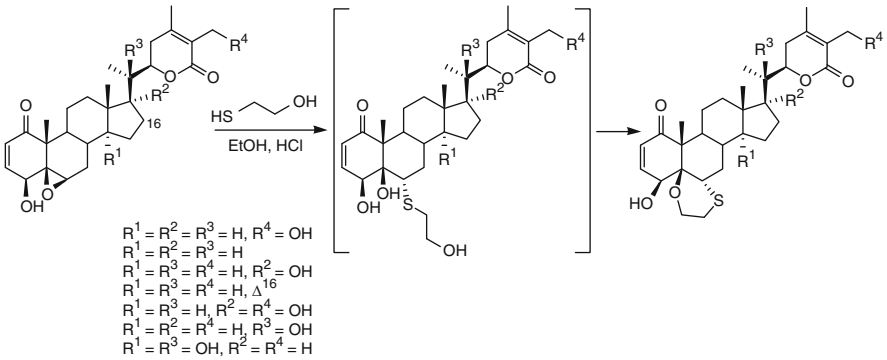


Chart 3 Reaction of withaferin A ($R^1 = R^2 = R^3 = H$, $R^4 = OH$) and related 5β,6β-epoxy withanolides with mercaptoethanol

under the same reaction conditions; attempts to force the reaction using a higher temperature and a lower pH resulted in complex mixtures (*191*).

Salpichrolide B (**341**), a minor constituent of *S. origanifolia* (see Sect. 5.3.1.), was obtained from the abundant salpichrolide A (**339**) by acetylation of the side chain hemiketal followed by stereoselective reduction of the C-1 ketone with sodium borohydride; deacetylation of the side chain occurred during workup. Following a similar protection/deprotection sequence, salpichrolide C (**342**) was oxidized to the 6-ketone **458** (Chart 4) (*190*).

Modifications at the side chain are less common, and one interesting example by *Mohan* and coworkers is the synthesis of a biotinylated analog of withaferin A (**459**) (Chart 5) for use as a probe to study angiogenesis (see Sect. 7.6.3.) (*192*).

6.2. *Photochemical Transformations*

Physalins containing an endoperoxy moiety in ring A were prepared from the corresponding $\Delta^{2,4}$-6-hydroxy physalin by photosensitized oxygenation. Thus, a *ca.* 1:1 mixture of physalins K (**311**) and Q (**297**) was obtained in 65% yield, by bubbling oxygen through a solution of physalin G (**314**) containing Rose Bengal and irradiated at 480 nm (Chart 6) (*144*). The same reaction carried out on 6-epiphysalin G (**460**) gave the 2β,5β-endoperoxide **461** as the major product (76% yield) and a trace amount (2%) of the 2α,5α-isomer **462**. (Note that names of physalin G and its 6-epimer have been assigned according to the revised structure of the former; see Sect. 5.2.3.). Photosensitized oxygenation of physalin B (**463**) gave a mixture of physalin K (**311**), the isomeric endoperoxide **461**, the 5α-hydroperoxide **464** and the corresponding alcohol **465** (Chart 6) (*144*).

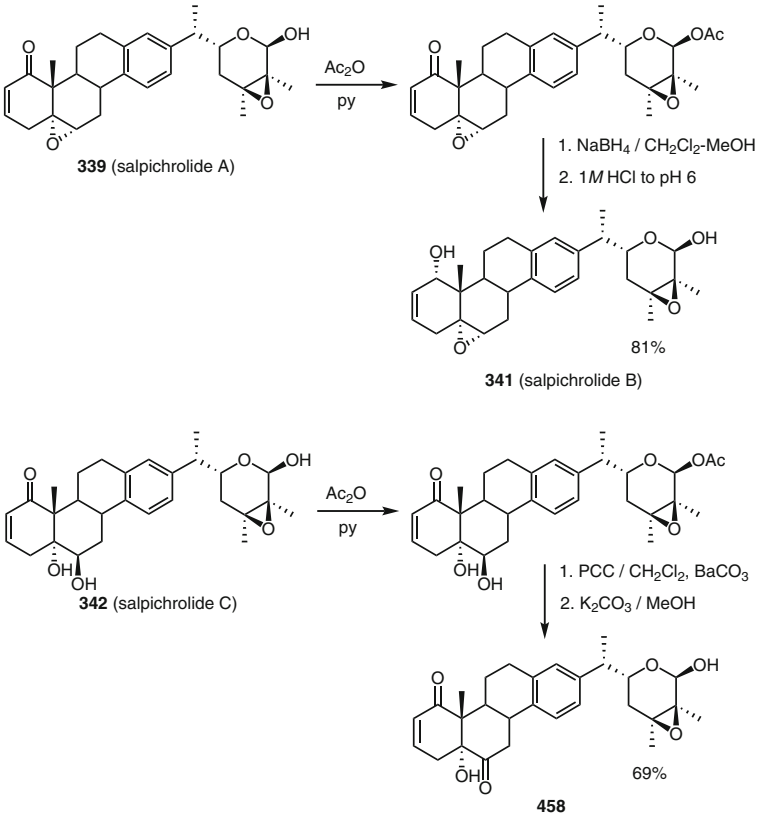


Chart 4 Chemical modification of salpichrolides A (**339**) and C (**342**)

Irradiation of an acetone solution of physalin B (**463**) with a tungsten-halogen lamp under argon, gave physalin R (**310**) in 49% yield. Irradiation of physalin F (**450**) under similar conditions gave the corresponding cyclophysalin (**466**). The 7α-hydroxy physalins, physalin N (**467**), A (**468**), and O (**469**), the latter two lacking the C-14–C-27 oxygen bridge, also gave the cyclophysalins (**470**–**472**) but required irradiation with a high-pressure mercury lamp (Chart 7) (*142*). The Δ^2-1-ketone system of the physalins would be involved in a self-sensitizing mechanism, as 2,3-dihydrophysalins failed to give the cyclized product.

6.3. Biotransformations

The modification of natural products by microorganisms may lead to new structures with potential biological activities. Application of this strategy to withanolides has been reviewed by *Anjaneyulu et al.* (*4*). Biotransformation of physalin H (**312**) by the fungus *Rhizopus stolonifer* gave the elimination product **473** in 2.1%

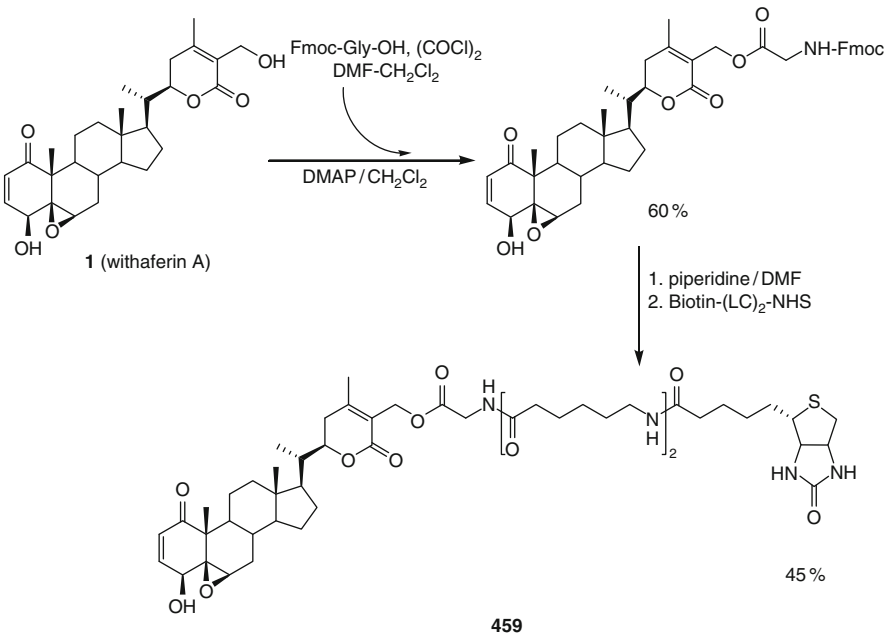


Chart 5 Synthesis of a biotinylated analog of withaferin A (**1**)

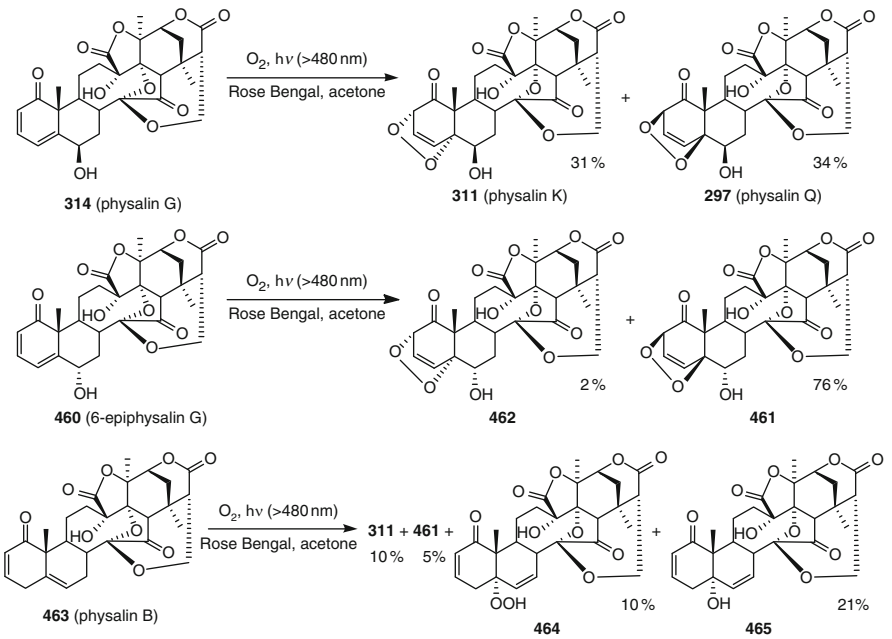


Chart 6 Photooxygenation of physalin G (**314**), 6-epiphysalin G (**460**) and physalin B (**463**)

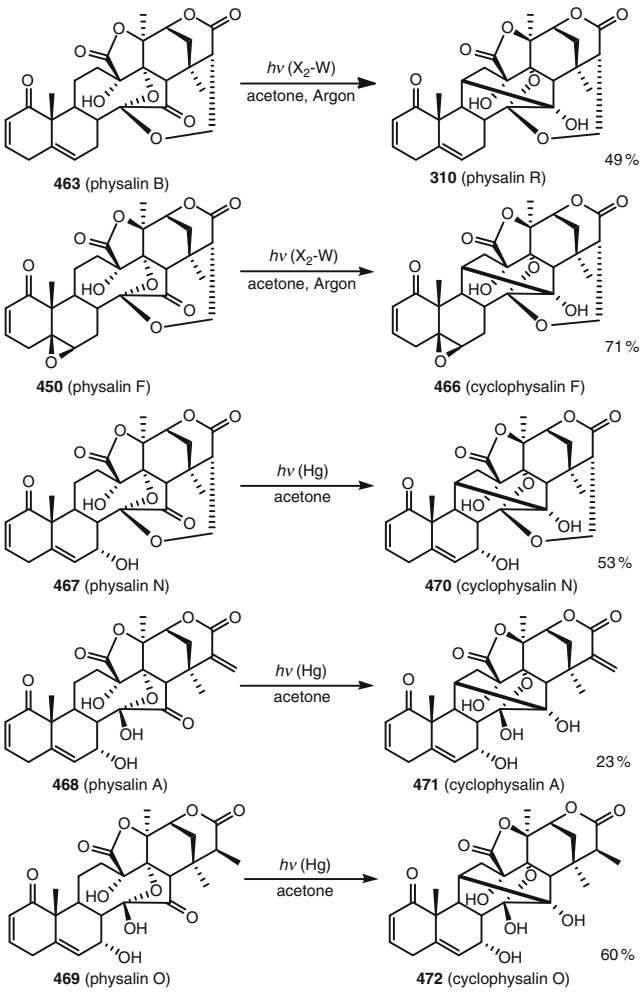


Chart 7 Photocyclization of physalins to cyclophysalins. *X_2-W* halogen-tungsten lamp; *Hg* high pressure mercury lamp

yield, while incubation with *Cunninghamella elegans* gave isophysalin B (**474**) and 6-deoxyphysalin H (**475**) in 9.4% overall yield (Chart 8) (*193*). Both **473** and **475** are new compounds. Incubation of withaferin A (**1**) with *Cunninghamella echinulata* gave 12β-hydroxywithaferin A (**476**) and 15β-hydroxywithaferin A (**477**) (*194*); the same hydroxylated products have been obtained upon incubation with *Cunninghamella elegans* (*4*). Several reports also describe the production of withanolides (mostly withaferin A) by shoot cultures of *Withania somnifera* (*195–198*).

Tuli and coworkers isolated two specific glucosyltransferases from *W. somnifera*. The cytosolic 3β-hydroxy sterol glucosyltransferase was most active on 24-methylene-cholesterol, and showed moderate activity for the 3β-*O*-monoglucosylation of the

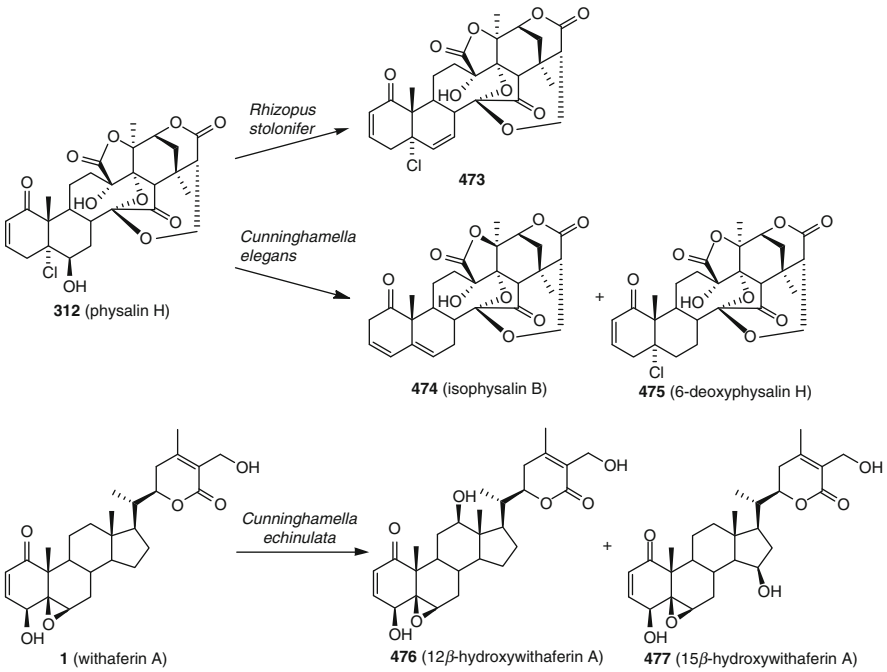


Chart 8 Biotransformations of physalin H (**312**) and withaferin A (**1**) with microorganisms

aglycone of withanoside V (**478**) (*199*). On the other hand the 27-hydroxy glucosyltransferase monoglucosylated several 27-hydroxywithanolides, provided a hydroxy group was also present at C-17 (Chart 9) (*200*).

7. Biological Activities of the Withanolides

As already mentioned, several withanolide-containing plants are used in traditional folk medicine throughout the world and many of the verified activities can be traced to their withanolide constituents. *Withania somnifera* used in Ayurvedic medicine in India since ancient times is the prototypical example, but similar uses have been accorded to *Datura metel* in traditional Chinese medicine and to several *Physalis* species in Asia and the Americas. Withanolides have proven active not only in a wide variety of assays related to human ailments, but also in potential applications as natural agrochemicals for pest and weed control. Despite the structural variety of withanolides and the many different activities they present, it is noteworthy, as will become evident in the following sections, that some structural characteristics are usually associated with biological activity (or the lack of it). One such feature is, with few exceptions, the lack of activity of 5α,6β-diols as compared to the corresponding epoxides (or the Δ^5 analogues) that are usually active.

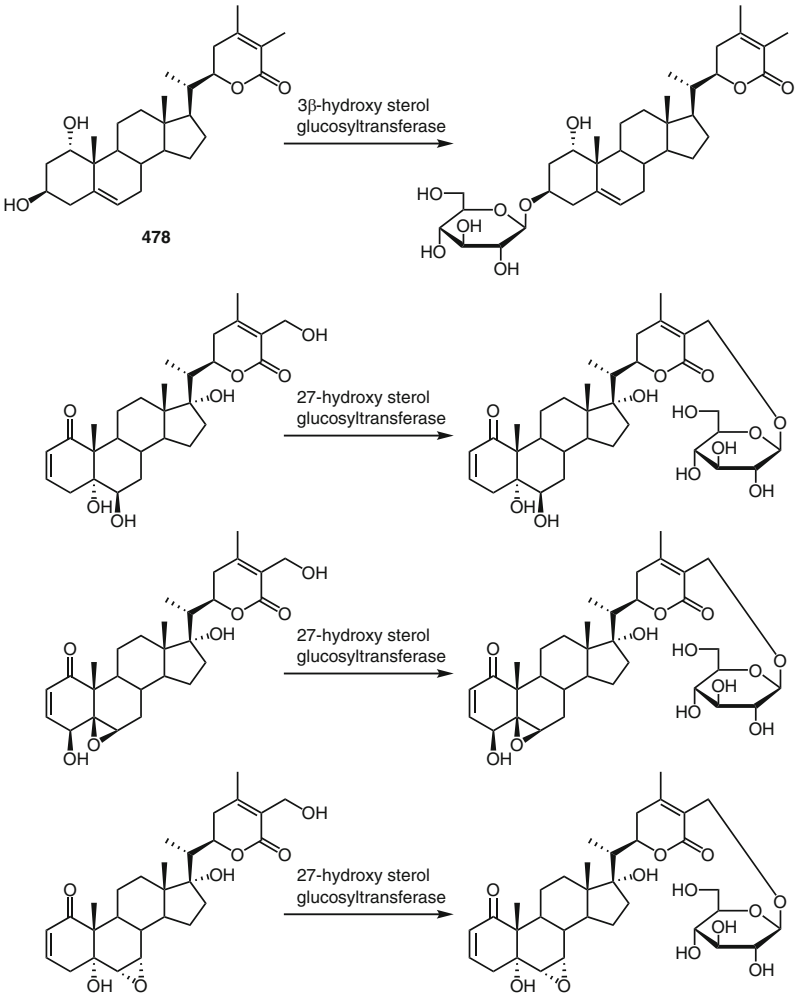


Chart 9 Enzymatic glucosylation of 3β- and 27-hydroxy withanolides

7.1. Insecticidal Activities

Insecticidal properties of withanolides were first noted for components isolated from the Peruvian plant *Nicandra physalodes* in the early 1960s. The major component of this plant, nicandrenone or Nic-1 (**338**), was later shown to be responsible for the insecticidal properties (*59*). Since then, several withanolides have been shown to exhibit insecticidal activity.

7.1.1. Antifeedant and Growth Inhibition

Antifeedant effects and species-specific activity were initially shown for the cotton leafworm *Spodoptera littoralis* (Boisd.) (Lepidoptera), the Mexican bean beetle, *Epilachna varivestis* Muls. (Coleoptera), and the red flour beetle, *Tribolium castaneum* (Herbst) (*59*). *Elliger* and coworkers found that some chromatographic fractions of an extract of *Physalis peruviana* leaves had a strong inhibitory effect on the development of larvae of the corn earworm *Helicoverpa zea*, an economic pest of numerous crops including tobacco and tomato (*201*). Bioassay-directed fractionation led to the isolation of a series of saccharide esters structurally related to the withanolides, with the δ-lactone side chain open and the carboxyl group esterified by mono-, di-, or trisacharides (*202*, *203*). The most active compound in inhibiting larval growth was the 11β-hydroxy diglucoside ester **479** (ED_{50} 5.4 ppm) and the least active was the closely related monoglucoside ester **480** (ED_{50} 110 ppm). No clear structure-activity relationships could be established, but the lack of toxicity of the compounds led the authors to conclude that growth inhibition was a consequence of feeding deterrence resulting in semi-starvation of the animals. Previously, the feeding deterrent withanolides 4β-hydroxywithanolide E (**481**) and withanolide E (**482**) had been found in very high concentrations in the leaves and berries of *P. peruviana* (*204*).

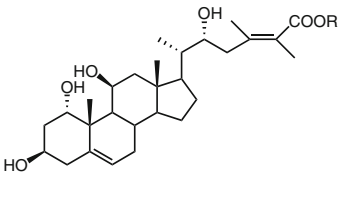
The antifeedant activities of the major withanolides of *Salpichroa origanifolia*, salpichrolides A (**339**), C (**342**), and G (**340**), and some synthetic derivatives, were investigated on larvae of the common fly *Musca domestica* (*189*), the stored grain pest *Tribolium castaneum* (*205*), and the Mediterranean fly *Ceratitis capitata* (*190*). Salpichrolide A (**339**) was the most active in all cases producing a significant development delay in surviving larvae, when added to the diet at 500 ppm concentration. Similar delays were observed when medium and low nutrition diets (without withanolides) were offered as food, supporting the idea that these compounds act as feeding deterrents. The effects of salpichrolides C (**342**) and G (**340**) differed from one insect species to the other, thus both compounds produced development delays only at high concentrations (2,000 ppm) in *M. domestica* and *C. capitata*, but salpichrolide G was almost as effective as salpichrolide A in *T. castaneum*. Regarding toxicity, the three salpichrolides produced significant mortality for *M. domestica* larvae (EC_{50} 200–300 ppm), but only salpichrolides A and G had this effect on *C. capitata*. The authors proposed that differences could be related to different detoxifying mechanisms.

Oxidation of the hemiketal in the side chain to give the δ-lactone **483** eliminated the activity both for *M. domestica* and *T. castaneum*. However, acetylation to give **484** drastically reduced the activity only for the latter insect. Reduction of the Δ^2 double bond had only a minor negative effect on activity (*189*, *205*). The effect of functional group modifications in rings A and B on the resultant activity was evaluated on *C. capitata* larvae (*190*). Salpichrolide B (**341**), a minor component of *S. origanifolia*, was prepared from salpichrolide A in sufficient amounts for testing (see Sect. 6.1.). This compound was the most active of all salpichrolides tested, producing significant mortality when incorporated to the diet even at low doses (EC_{50} 83 ppm). It also produced clearly observed development delays in surviving

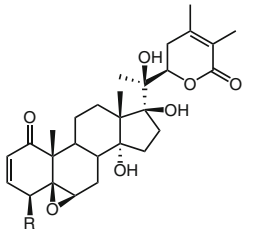
larvae at 25 ppm. Reduction of the Δ^2 double bond (**485** and **486**) produced a significant decrease in activity. Oxidation of salpichrolide C to the 6-ketone (**458**) or rearrangement of the 5,6-epoxide in salpichrolide A to the $\Delta^{2,4}$-6α-alcohol **456** resulted in inactive compounds.

The content of the salpichrolides in *S. origanifolia* was monitored during plant development, reaching a maximum during summer when insect populations are higher. These results, in conjunction with the observed toxic and feeding deterrent activities, suggest that these compounds may act as a chemical defense providing protection to the plant against phytophagous insects (*189*).

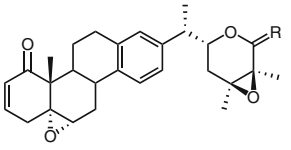
Feeding-deterrent activity of the major components of *Jaborosa odonelliana*, the spiranoid withanolides jaborosalactone P (**389**) and jaborosalactone 10 (**390**), was studied against *T. castaneum* (*177*). In this case, only jaborosalactone P (**389**) produced a significant delay in the development of neonate larvae. In the case of *J. integrifolia*, only jaborosalactone A (**487**) exhibited antifeedant activity on larvae of *Spodoptera littoralis* (*206*). Rearrangement of the epoxide to the allylic alcohol as in jaborosalactone B (**488**) or cleavage to the diol as in jaborosalactone D (**489**) resulted in complete loss of the activity. Jaborosalactone S (**254**) from *J. sativa* was a feeding deterrent for *Tenebrio molitor* (*207*) and the trechonolide-type withanolide **386** isolated from *J. lanigera* produced significant development delays in *C. capitata* larvae (*175*).



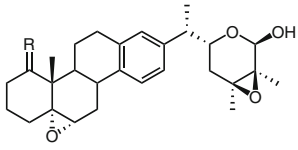
479 R = gluc-(1-2)-gluc-6'-Ac
480 R = gluc



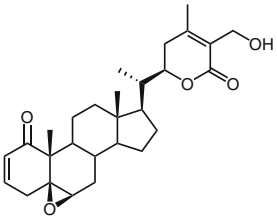
481 R = OH (4β-hydroxywithanolide E)
482 R = H (withanolide E)



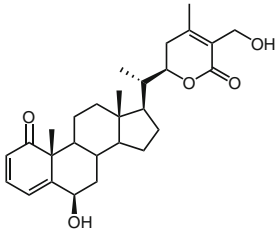
483 R = O
484 R = α-H, β-OAc



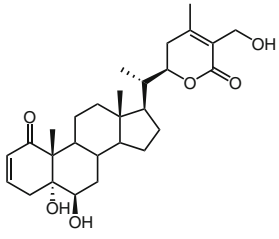
485 R = O
486 R = α-OH, β-H



487 (jaborosalactone A)



488 (jaborosalactone B)

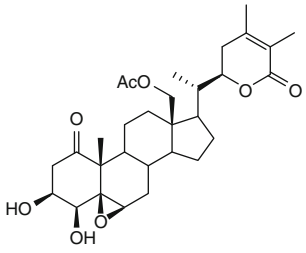


489 (jaborosalactone D)

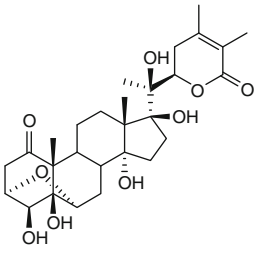
Azambuja and coworkers studied the effect of several physalins from *Physalis angulata* on the blood-sucking insect *Rhodnius prolixus*, vector of *Trypanosoma rangeli*. Physalins B (**463**), D (**313**), F (**450**), and G (**314**) produced immune depression in *R. prolixus*, although apparently by different mechanisms. Without their defense system, insects infected with *T. rangeli* die (*208–210*).

7.1.2. Ecdysteroid Agonists and Antagonists

The ecdysteroid endocrine system is vital for insect development and a variety of secondary metabolites from plants have been shown to interfere with ecdysteroids, probably as a chemical defense mechanism. *Dinan* and coworkers developed a microplate-based bioassay with the ecdysteroid-responsive *Drosophila melanogaster* B_{11} cell line and used it to evaluate 16 withanolides isolated from *Iochroma gesneriodes*, for agonistic/antagonistic activity (*211*). Only withanolides containing an oxygenated functionality at C-3 (hydroxy or methoxy) and an α,β-unsaturated lactone in the side chain showed antagonistic activity, with 2,3-dihydro-3*β*-hydroxywithacnistine (**490**) being the most active (ED_{50} 2.5 μ*M* versus 0.05 μ*M* for 20-hydroxyecdysone). In a systematic study of 128 species of solanaceous plants including those known to contain high levels of withanolides, only a few of the methanolic extracts showed weak ecdysteroid antagonist activity (*212*). The high reactivity of the Δ^2-1-keto system present in many withanolides, raises the possibility that, even though inactive *in vitro*, they could be activated by conversion to the 2,3-dihydro-3*β*-hydroxywithanolides upon ingestion by insects (*213*). Further studies on 21 withanolides from different sources showed that withaperuvin D (**491**), with a C-3, C-6 oxygen bridge, had moderate agonistic activity (EC_{50} 25 μ*M*) (*214*).



490 (2,3-dihydro-3*β*-hydroxywithacnistine)



491 (withaperuvin D)

7.2. *Phytotoxic Activities*

Selective phytotoxicity has been reported for several withanolides. Three 7-oxygenated withanolides, **492–494**, isolated from *Iochroma australe*, reduced

radicle growth of the weeds *Sorghum halepense* (L.) Pers. (Monocot.) and *Chenopodium album* L. (Dicot.). Compound **493** inhibited radicle growth of *Lactuca sativa* L. (lettuce) but only at high concentration (1,000 ppm) (*215*). Jaborosalactol 18 (**285**) a major constituent of *J. bergii* (see Sect. 5.1.4.) showed significant inhibition of radicle growth at 2 m*M* on the dicotyledoneous species *C. album*, *Ipomoea purpurea* (L.) Roth and *L. sativa* (phytogrowth inhibitory activity $> 49\%$). On the other hand, **285** had a strong stimulatory effect in the monocotyledoneous species tested (*Zea mays* L. and *Sorghum halepense*) (*69*). Several withanolides isolated from *J. rotacea* also exhibited different activities towards mono- and dicotyledoneous species. Thus, jaborosalactones 29 (**447**), 30 (**448**), 31 (**404**), and 33 (**370**) had opposite effects on the dicotyledon *L. sativa* and the monocotyledon *Phalaris canariensis* L. Jaborosalactone 29 (**447**) was the most active, selectively inhibiting radicle growth, germination and the emergence rate index of *L. sativa* but with no effect on *P. canariensis*. Jaborosalactones 30 and 31 (**448**, **404**) had a strong stimulatory effect on radicle growth of *P. canariensis*. The chlorohydrin jaborosalactone 28 (**446**) was the only withanolide that inhibited the radicle growth of *P. canariensis* (*120*). A similar selectivity was exhibited by the major component of *J. kurtzii*, jaborosalactone 43 (**449**) (spiranoid type), and by withanolides of *J. caulescens*, the 12-*O*-ethyl derivative of jaborosalactone 42 (12-*O*-ethyl-**373**) (trechonolide type) and the sativolides **258**, and 12-*O*-methyl-**258**. All these compounds strongly inhibited radicle growth of *L. sativa* but exhibited a marginal effect on the monocotyledoneous *Avena sativa* L. (*76*, *121*).

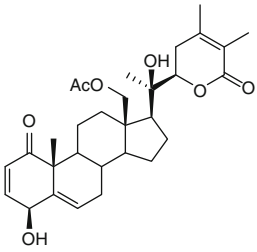
7.3. Antiparasitic Activities

The first withanolides with antileishmanial and antitrypanosomial activities were isolated from *Dunalia brachyacantha* when screening extracts of Bolivian plants against *Trypanosoma cruzi* (Chagas disease), and several *Leishmania* species. Bioassay-guided fractionation of the leaf extract gave the known 18-acetoxywithanolide D (**142**) and its Δ^5 analogue **495**, with the latter compound being the most active against epimastigote forms of *T. cruzi* and promastigote cultures of *L. amazonensis*, *L. braziliensis*, and *L. donovani* (*84*).

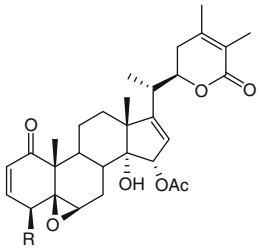
7.3.1. Trypanocidal Activity

In a systematic study of the trypanocidal activity of secondary metabolites isolated from plants from northeastern Brazil, *Vieira et al.* found withaphysalins M and O (**323**, **325**) from *Acnistus arborescens* (see Sect. 5.2.4.) to be the most active compounds against epimastigotes of *T. cruzi*, with IC_{50} values of 100-fold less than the reference compound benznidazole (*216*). Reduction of the 2,3 double bond as in withaphysalin N (**324**) reduced the activity by an order of magnitude. Most

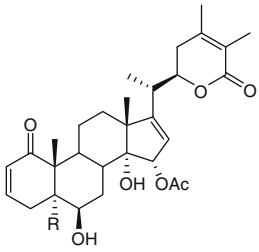
interestingly, these withaphysalins had no cytotoxic activity against dividing normal cells. Physalin F (**450**) from *Physalis angulata* was shown to be moderately active. *Abe* and coworkers investigated the trypanocidal activity of ten withanolides from *P. angulata* growing in Japan, against *T. cruzi* epimastigotes and trypomastigotes (the infectious form of the parasite) (*57*, *66*). Physagulins A–C (**496–498**), H (**117**) and I (**118**), and withangulatin A (**499**) had activity against both forms of *T. cruzi* similar to their cytotoxicity; activity against trypomastigotes was higher for these withanolides. Physagulins F (**500**), J (**119**), K (**120**), L (**121**), and M (**103**) and withaminimin (**501**) containing the 5α,6β-diol moiety were weakly active against epimastigotes, but the first two of these compounds were moderately active against the infectious trypomastigote form. Physagulin N (**106**), the methoxy derivative of physagulin A, was also marginally active confirming that the A ring enone is required for activity.



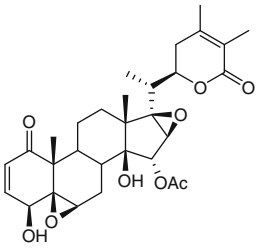
495



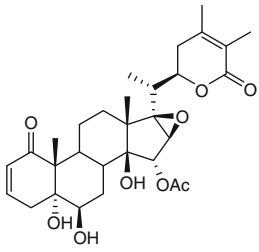
496 R = H (physagulin A)
499 R = OH (withangulatin A)



497 R = Cl (physagulin B)
501 R = OH (withaminimin)



498 (physagulin C)

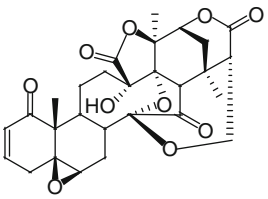


500 (physagulin F)

7.3.2. Leishmanicidal Activity

Atta-ur-Rahman and coworkers have reviewed the antileishmanial activity of withanolides from *Physalis minima* as well as some of their biotransformation products (*217*). Physalin **299** was the most active substance against *Leishmania major*

promastigotes, (see Sect. 5.2.1.) while physalins **298**, **300**, **301**, physalin H (**312**), isophysalin B (**474**), and 5β,6β-epoxyphysalin B (**502**) also had significant activity. The 3-methoxy derivative physalin U (**304**) and withanolide **107** with an unmodified skeleton were only marginally active (*60*, *145*). Removal of the C-6 hydroxy group of physalin H (**312**) to give either the dehydration product **473** or the deoxygenated physalin **475** (see Sect. 6.3.) did not affect the antileishmanial activity (*193*).



502 (5β,6β-epoxyphysalin B)

Echeverri and coworkers investigated the leishmanicidal activity of several acnistins (A, B, C, E, F, and G) and withajardins (A, B, and C) isolated from *Dunalia solanacea* and *Deprea orinocensis*, respectively (see Sects. 5.1.2. and 5.1.3.). Efficacy was evaluated using intracellular amastigotes of *Leishmania (V) panamensis* (*218*). Withajardin B (**278**) and acnistins A (**259**), C (**262**), and E (**260**) were the most active; withajardin A (**277**), and acnistins B (**261**), and F (**264**) were the least active. The 3-methoxy derivative of withajardin A was inactive. All compounds had poor selectivity, with antileishmanial activity closely paralleling cytotoxicity in all cases. The authors did a 3D-QSAR study and concluded that differences in bioactivity could be explained by the influence of steric and electrostatic fields in the vicinity of ring A. According to the model, bending of the steroid nucleus at the A/B ring junction, an increase of positive charge near positions 2, 3, and 4, or of a negative change near positions 5 and 6, increased the bioactivity.

Withaferin A (**1**) inhibited growth of *L. donovani* promastigotes. *In vitro* studies showed that withaferin A inhibits protein kinase C in the parasite, leading to apoptosis (*219*). Withanolide Z (**27**), but not withaferin A, partially inhibited *L. donovani* topoisomerase I (*28*).

7.4. Antimicrobial Activities

The antibacterial and antifungal properties of withaferin A (**1**) have been known for a long time and many other withanolides are known to display these activities (*3*, *4*). However, data are scattered throughout the literature involving the action of different compounds on different microorganisms thus making it difficult to rationalize the results. Withaferin A has been shown to be strongly active against *Bacillus subtilis* and moderately active for *Escherichia coli* and *Staphylococcus aureus*, but inactive against *Pseudomonas aeruginosa*. As an antifungal it exhibited

strong activity against *Aspergillus niger*, but was inactive against *Rhizopus oryzae* and *Candida albicans* (*220*). 4-Deoxywithaperuvin (**11**) was moderately active against several *Gram*-positive (*B. cereus*, *B. subtilis*, *Streptomyces* spp.), and *Gram*-negative (*Pseudomonas fluorescens*, *Serratia marcescens*) bacteria, but inactive against *Micrococcus luteus*, *M. roseus* and *S. aureus*; antifungal activity was poor (*20*). On the other hand, the 18-oxygenated withanolides 18-acetoxywithanolide D (**142**) and its Δ^5 analogue **495** from *D. brachyacantha* were active against *S. aureus* and *B. subtilis* and inactive against *E. coli* and *Shigella flexneri* (*84*).

17*β*-Hydroxywithanolide K (**51**) and the closely related 14α,20-epoxywithanolide, **71** (see Sect. 4.1.4.) showed antifungal activity against the human pathogens (*MIC* 300 μg/cm^3) *Aspergillus niger*, *Stachybotrys atra*, *Allescheria boydii*, *Drechslera rostrata*, *Microsporum canis*, and *Curvularia lunata* (*36*, *43*).

Among the physalins, physalin B (**463**) was active against *S. aureus* (several strains) and *N. gonorrhoeae* but not active against *E. coli* and *P. aeruginosa* (*221*). Physalin D (**313**), but not physalin B, was moderately active against *Mycobacterium tuberculosis* (*222*).

7.5. *Anti-inflammatory and Glucocorticoid Related Activities*

7.5.1. Anti-inflammatory Activity

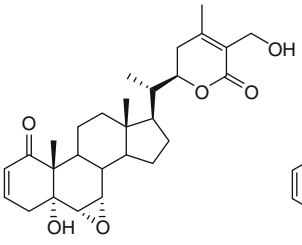
The antiinflammatory properties of several withanolides are well known (*3*). *Souza* and coworkers have shown that in the case of physalins B (**463**) and F (**450**), the anti-inflammatory activity parallels that of the synthetic glucocorticoid dexamethasone in preventing inflammatory injury and lethality after intestinal ischemia and reperfusion in mice (*223*). Furthermore, they found that the effect could be reversed by pretreatment with the glucocorticoid antagonist RU-486, indicating that the *in vivo* activity displayed by the physalins is mostly due to activation of the glucocorticoid receptor. As observed with dexamethasone, physalins also decreased TNF-α concentration and enhanced the anti-inflammatory interleukin IL-10 concentration in tissues. Physalins B (**463**) and F (**450**) have been shown to inhibit TNF-α induced activation of NF-κB and either a 5,6 double bond or a 5*β*,6*β*-epoxide are required for activity; the 5α,6*β*-diol physalin D (**313**) is inactive (*138*).

An ethanol extract of *W. somnifera* significantly suppressed lipopolysaccharide (LPS)-induced production of the pro-inflammatory cytokines TNF-α, IL-1β, and IL-12p40 in peripheral blood mononuclear cells of normal individuals and reumathoid arthritis patients, and inhibited nuclear translocation of the transcription factors NF-κB and AP-1 and phosphorylation of IκBα. The major component, withaferin A (**1**), inhibited NF-κB translocation and was associated with these effects (*224*). Withaferin A inhibited NF-κB activation by preventing the TNF-induced activation of IκB kinase via a thioalkylation-sensitive redox mechanism

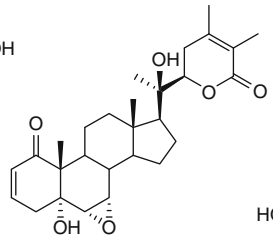
(*225*). 12-Deoxywithastramonolide (**503**) and withanolide A (**504**) were much less effective. A more detailed study on the inhibition of NF-κB activation by various agents was conducted by *Ichikawa et al*. (*226*). The authors isolated a series of withanolides from a *W*. *somnifera* leaf extract, which included withaferin A (**1**), viscosalactone B (**505**), withanosides IV (**31**) and X (**37**), and related compounds. The 1α,3β-dihydroxy-Δ^5-withanolides and their glycosides did not inhibit NF-κB activation while withaferin A and its diacetate were strong inhibitors. Reduction of the 2,3-double bond of withaferin A rendered the compound inactive but addition of a 3-hydroxy substituent (as in viscosalactone B) restored activity. Acetylation of the hydroxy groups of the latter compound did not affect activity, but glycosylation at C-27 gave an inactive compound.

Nair and coworkers reported the selective cyclooxygenase-2 (COX-2) inhibitory activity of leaf extracts of *W*. *somnifera* and related such information to the use of this plant as an antiinflammatory. From the methanolic extract they isolated 12 withanolides and evaluated their abilities to inhibit COX-1 and -2. None of the withanolides inhibited COX-1 even at high doses but most of them exhibited some inhibitory activity on COX-2, with withaferin A (**1**) and viscosalactone B (**505**) being the most active (*15*). Molecular docking studies showed that most of the withanolides had more favorable binding to COX-2 than to COX-1 (*227*).

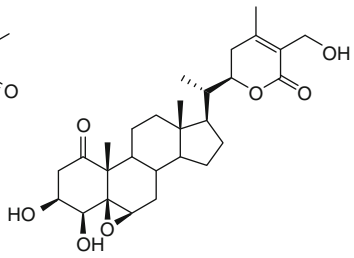
The 12-oxygenated withanolide **154** from *Discopodium penninervium* (see Sect. 4.2.3.) was a selective inhibitor of cyclooxygenase-2 and also of leukotriene formation; as both these pathways are involved in cell proliferation and angioneogenesis, the dual inhibition of COX-2 and leukotriene formation by **154** has been proposed as a starting point for the development of anti-inflammatory and cancer chemopreventive agents (*82*).



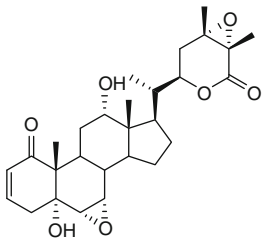
503 (12-deoxywithastramonolide)



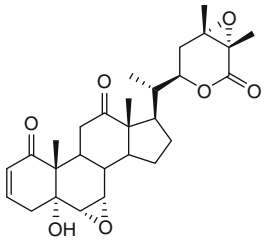
504 (withanolide A)



505 (viscosalactone B)



506 (daturalactone 1)



507 (daturalactone 2)

7.5.2. Antistress Activity

Withafastuosin D (**168**), the major withanolide in *Datura fastuosa* leaves, maintained corticosterone levels in male albino rats during experimental stress and exhibited antistress activity evidenced as an anxiolytic effect (*228*). Withafastuosin E (**169**) has also been found to increase the release of prostaglandins, which play an important role in the resistance to gastroduodenal mucosa to ulceration (*229*, *230*). On the other hand, the antistress activity of *W. somnifera* glycowithanolides (*e.g.* sitoindosides) has been linked to their antioxidant activity (*231*, *232*).

7.5.3. Immunosuppressive and Immunomodulatory Activity

The 16-oxygenated withanolides **188–190** isolated from *Discopodium penninervium* (see Sect. 4.2.4.) exhibited potent immunosuppresive activity; they inhibited the incorporation of [^{3}H]-thymidine in cultured rat spleen cells without being overtly toxic to the cells (*233*). Acnistins A (**259**), B (**261**), and E (**260**) from *Dunalia solanacea* significantly inhibited the incorporation of [^{3}H]-thymidine in human lymphocytes at doses as low as 0.1 μg/cm^3 (*124*). Coagulin H (**7**) isolated from *W. coagulans* also was a strong inhibitor of incorporation of [^{3}H]-thymidine in stimulated human mononuclear cells. It inhibited T-cell proliferation with an activity similar to that of the synthetic glucocorticoid, prednisolone. Like prednisolone, the T-cell suppression effect was correlated with a decrease in production of the cytokine IL-2. However, at variance with the glucocorticoid, coagulin H did not have any damaging effects on the cells (*234*).

Immunomodulatory effects of three daturalactones from *Datura quercifolia* were evaluated by observing their effects on antibody production, T-cell and B-cell activation, and cytokine production from splenocytes. The 1β-alcohol **153** was immunosuppressive at lower doses while daturalactones 1 (**506**) and 2 (**507**) were immunostimulators (*81*).

Physalins B (**463**), F (**450**), and G (**314**), but not D (**313**), inhibited nitric oxide production by activated macrophages. Addition of physalin B to lipopolysaccaride-stimulated peritoneal macrophage cultures induced decreases of TNF-α IL-6, and IL-12 production. Physalins B, F, and G also protected mice against administration of a lethal dose of lipopolysaccaride (*235*). The effects of the above-mentioned physalins were not blocked by the antiglucocorticoid, RU-486, suggesting that they act by a mechanism different from that of the glucocorticoids. Physalins B, F, and G also have potent suppressive activities *in vitro* on splenocyte cultures and *in vivo* on allogeneic transplants (*236*). The effects of physalins on transplant rejection could be explained by a direct effect of these withanolides on lymphocytes.

Several physalins isolated from *Physalis alkekengi* were found to be strong inhibitors of nitric oxide production induced by lipopolysaccaride, including the above-mentioned physalins B and F and also physalins A (**468**) and O (**469**). Compounds of the neophysalins class (see Sect. 5.2.2.) were inactive (*139*).

7.6. Cancer-Related Activities

Many studies have dealt with the potential antitumor activity of withanolides, and large amounts of data are available, mostly their cytotoxicity to cancer cell lines. However, other cancer-related activities specific to certain withanolide structural types have also been investigated in the last decade. Particularly interesting are those related to cancer chemoprevention, inhibition of angiogenesis, and microtubule stabilization.

7.6.1. Cytotoxicity

Most withanolides exhibit some level of cytotoxicity against different tumor cell lines, but usually this is non-selective and in the micromolar concentration range. Withaferin A (**1**) has been investigated extensively in this respect, with several recent studies also addressing its mechanism of action (*191*, *237–239*).

Nair and coworkers assayed several withanolides from *W. somnifera* against human lung, breast, CNS, and colon cancer cell lines. Withaferin A (**1**), its diacetate, viscosalactone B (**505**), compound **6**, and ashwagandhanolide (**78**) were the most active (IC_{50} range 0.5–1 μM) and comparable in potency to a reference compound, adriamycin (*47*, *240*). Compound **78** also inhibited lipid peroxidation and the activity of the enzyme cyclooxygenase-2 *in vitro*. Several withanolides from *Acnistus arborescens* were evaluated against panels of human cancer lines, including some 16-acetoxywithanolides and withaphysalins F (**316**), M (**323**), N (**324**), and O (**325**), with IC_{50} values in the 0.2–2 μM range (*98*, *152*, *153*, *241*). The 16-oxygenated withanolides **188–190** isolated from *Discopodium penninervium* (see Sect. 4.2.4.) exhibited significant cytotoxicity only against murine RAW 264.7 carcinoma cell lines (*233*). The 12β-acetoxywithanolide **156** did not show any cytotoxic activity up to 10 μM (*83*).

Bioassay of seventeen withanolides from *Tubocapsicum anomalum* showed significant cytotoxic activity (comparable to that of doxorubicin) against a panel of human cancer cell lines for eight of the compounds (*107*). From the 18-nor withanolides (see Sect. 5.5.3.), only tubocapsenolide A (**441**) exhibited potent cytotoxicity; this compound was shown to inhibit proliferation and induce apoptosis in MDA-MB-231 cells by thiol oxidation of heat shock proteins (*242*). Potent cytotoxicity was also exhibited by tubocapsanolide A (**201**) and

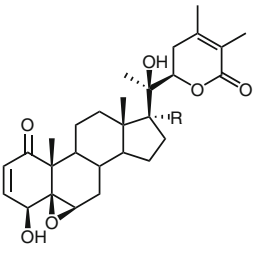
its 20-hydroxy (**204**) and 23-hydroxy (**205**) derivatives, tubocapsanolide F (**203**), and anomanolide B (**269**) (*107*, *243*). The other active withanolides isolated were the known withanolide D (**508**) and its 17α-hydroxy derivative (**509**). Withaferin A (**1**), also used as a reference compound, was *ca*. 10 times more active.

Withametelins I (**235**), K (**237**), L (**238**), and N (**240**), isolated from *Datura metel*, exhibited cytotoxicity against selected human cancer cell lines, namely, A549 (lung), BGC-823 (gastric), and K562 (leukemia), with IC_{50} values of 0.05–3.5 μ*M*. Withametelin J (**236**) was only moderately active for the last two cell lines (*92*).

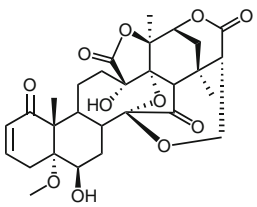
Withanolides from *Physalis* species have also been studied extensively in terms of their cytotoxicity against human and murine cancer cells. Among the physalins, physalins B (**463**), D (**313**), F (**450**), and H (**312**) showed strong cytotoxicity against multiple tumor cell lines, while physalins G (**314**), I (**510**), and physanolide A (**367**) were inactive. Physalins J (**457**) and U (**304**) were marginally active (*55*, *134*, *146*, *244*, *245*). The 18-*nor*-physalin, withaphysanolide A (**438**), also was weakly active (*186*). For physalins B and D, the antineoplastic activity was confirmed *in vivo* by inhibition of tumor proliferation in mice bearing sarcoma 180 tumor cells (*245*). Both physalins B and F have been shown to inhibit hedgehog (Hh)/GLI-mediated transcriptional activation, which is involved directly in tumor formation and progression (*246*). Recently, *Magalhães et al*. reported different activities for physalins D and E isolated from *P*. *angulata*. However, as already mentioned in Sect. 5.2.3., the structure of physalin E was incorrectly assigned and later shown to be identical to physalin D (**313**) (*244*). Accordingly, these bioactivity results should be considered with caution. Among the *Physalis* withanolides with an unmodified skeleton, potent cytotoxic activity has been reported for withangulatins A (**511**), B (**94**), and I (**102**), physangulin B (**512**), phyperunolide A (**101**), withaphysacarpin (**195**), philadelphicalactones A (**197**) and B (**198**), 18-hydroxywithanolide D (**128**), and withanone (**513**) (*50*, *55*, *56*, *58*, *104*, *247*).

Ixocarpolactones A (**360**) and B (**361**), isolated from *P*. *philadelphica*, exhibited significant inhibition of murine epidermal JB6 cell transformation (*104*) and ixocarpolactone A (**360**) present in the edible fruit of the plant showed potent antiproliferative and apoptotic activity in SW480 human colon cancer cells (*247*). Evaluation of 12 withaphysalins from *P*. *minima* and *P*. *divaricata*, including **331–334**, showed only moderate cytotoxicity against the HCT-116 and H460 human cancer cell lines (*154*, *186*).

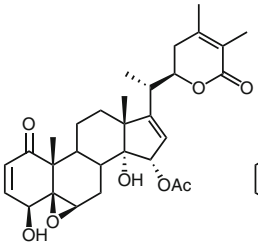
The potential antitumor activities of an alcoholic extract of *W*. *somnifera* roots and of withaferin A (**1**) have also been studied in conjunction with radiation therapy in experimental tumors *in vivo*. Both exhibited significant antitumor and radiosensitizing effects without systemic toxicity (*248–250*).



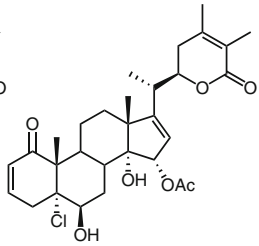
508 R = H (withanolide D)
509 R = OH



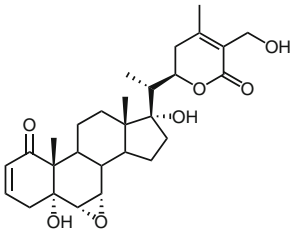
510 (physalin I)



511 (withangulatin A)



512 (physagulin B)



513 (withanone)

7.6.2. Cancer Chemopreventive Activity

Cancer chemoprevention by the ingestion of modulators of carcinogenesis from synthetic or natural origin has been proposed as a strategy to reduce cancer mortality in apparently healthy persons at risk of cancer (*6*, *251*). *Pezzuto*, *Kinghorn* and collaborators have used a battery of *in vitro* bioassays to monitor inhibition of tumorigenesis at the stages of initiation, promotion, and progression by plant extracts and pure compounds. One key bioassay used, is based on the induction of the phase II drug-metabolizing enzyme NAD(P)H:quinone reductase (QR), on Hepa 1c1c7 hepatoma cells; QR induction is indicative of potential cancer prevention at the initiation phase (*252*). A large number of compounds of plant origin have been evaluated as QR inducers, including many withanolides (*6*). Of particular interest are QR inducers that exhibit low cytotoxicity and thus a high selectivity measured as the chemopreventive index, the ratio between the concentration needed to double QR activity (CD) and the concentration that inhibits cell growth by 50% (IC_{50}).

Kinghorn and coworkers investigated QR induction by the withanolides in fruits and aerial parts of *Physalis philadelphica* (see Sect. 4.2.4.) (*103*–*105*). With the exception of withanone (**513**), all the withanolides were potent QR inducers with activity comparable to or better than the reference compound, sulforaphane, a known chemopreventive agent. Ixocarpalactone A (**360**), philadelphicalactone A (**197**), withaphysacarpin (**195**), and 4β,7β,(20*R*)-trihydroxy-1-oxowitha-2,5-dien-22,26-olide (**199**) exhibited the highest chemopreventive indexes. The fact

that ixocarpolactone A (**360**), is present in the edible fresh fruit of *P. philadelphica* (tomatillo) at a concentration level of 143 ppb (*105*), make these findings especially important.

In a broader study comprising withanolides from 13 Solanaceae species, 37 withanolides representative of a variety of structural types were evaluated for their potential to induce quinone reductase (*253*). Jaborosalactone 1 (**397**), jaborosalactone O (**353**), jaborosalactone P (**389**), trechonolide A (**368**), and withaphysalin J (**320**), were significant QR inducers with CD values in the range of 0.27–1.52 μ*M*. Results indicated that a functionalized methyl-18 plays an important role in improving QR induction while the presence of 5α-substituents is deleterious for the activity. Overall, spiranoid- and trechonolide-type withanolides exhibited good QR induction. Some of the active withanolides had low cytotoxicity, with chemopreventive indexes that compared favorably with the reference compound sulforaphane. One such compound, the spiranoid, jaborosalactone P (**389**), was selected to test its capacity to induce steady-state levels of quinone reductase in multiple organ sites of BALB/c mice. With jaborosalactone P-treated mice, a significant induction was observed in liver and colon, but not in lung, stomach, or mammary gland (*253*). This *in vivo* study confirmed the *in vitro* results, indicating that withanolides may function as potent phase II enzyme inducers.

Withanolides **184** and **185** from *A. arborescens*, bearing acetates at positions 7*β* and 16α, were also very potent QR inducers, but exhibited high cytotoxicity resulting in poor selectivity (*98*). From *Larnax subtriflora*, subtrifloralactones A (**431**), C (**433**), D (**425**), F (**436**), I (**428**), and J (**429**) containing an α,β-unsaturated ketone functionality in ring A (see Sect. 5.5.) had significant QR induction activity (*184*). Both δ- and γ-lactones were active, indicating that this part of the molecule was not critical for activity. Subtrifloralactone D was the most active, but subtrifloralactones A and F had the highest chemopreventive index (*ca*. 3 times that of sulforaphane). Subtrifloralactone L (**435**) with a doubly unsaturated ring A ketone, was inactive in the QR assay, while subtrifloralactone K (**434**) with the less common 3,6-epoxy arrangement, was active (*185*). The above data although limited, suggest that even though the presence of an α,β-unsaturated ketone unit in ring A of withanolides appears to be important for inducing QR, other structural features may compensate the lack of this functionality or block its beneficial effects.

Panjamurthy et al. have reported that pretreatment with withaferin A (**1**) significantly reduced *in vivo* 7,12-dimethylbenz[*a*]anthracene (DMBA)-induced genotoxicity, in the bone marrow of golden Syrian hamsters (*254*).This effect could also be related to the induction of phase II detoxifying enzymes.

7.6.3. Antiangiogenic Activity

Formation of new blood vessels from existing vasculature or “angiogenesis” is characteristic of all solid tumors allowing for nutrition, oxygenation, and

metastasis. Thus, angiogenesis inhibitors provide an alternative way of controlling the growth of tumor cells in both pre-invasive and invasive stages. Angiogenesis is also involved in the pathogenesis of several non-malignant inflammatory diseases (age-related macular degeneration, arthritis, endometriosis, *etc*.). *Mohan et al*. reported the antiangiogenic activity of extracts of *W. somnifera* containing non-cytotoxic levels of withanolides and also of withaferin A (**1**) (*255*). Thus, withaferin A inhibited cell proliferation in human umbilical vein endothelial cells (HUVECs) (IC_{50} 12 n*M*) through a process associated with inhibition of cyclin D1 expression. A potent antiangiogenic effect was also observed *in vivo*, at doses that are 500-fold lower than those previously reported to exert antitumor activity. The authors proposed that the inhibition of NF-κB by withaferin A in HUVECs occurs by interference with the ubiquitin-mediated proteasome pathway as suggested by the increased levels of poly-ubiquitinated proteins. The biotinylated derivative **459** was used to identify protein targets of withaferin A in HUVECs (see Sect. 6.1.). Preliminary results showed that withaferin A binds irreversibly with a 56 kDa protein target and a less abundant 180 kDa species but interacts reversibly with a 70 kDa protein species (*192*). Withaferin A and withanolide D (**508**) also displayed antiangiogenic activity in human choroidal endothelial cells (HCECs). These results may lead to novel treatments of choroidal neovascularization, the major contributor to age-related macular degeneration and one of the leading causes of irreversible blindness in the Western hemisphere (*256*). Physalin B (**463**) has been identified recently as an inhibitor of the ubiquitin-mediated proteasome pathway in the DLD-1 human colon cancer cell line, producing an accumulation of ubiquitinated proteins and inhibiting TNFα-induced NF-κB activation (*257*). The antiangiogenic activity of this compound remains to be tested.

7.6.4. Microtubule Stabilizing Activity

The microtubule stabilizing activity of taccalonolides A (**405**) and E (**420**) was reported by *Mooberry* and coworkers after bioassay-directed fractionation of an extract of *Tacca chantrieri* (*258*). Microtubule stabilizers are highly effective drugs used in the treatment of many types of cancers. The taccalonolides are particularly interesting as they appear to have a unique mechanism of action, which does not involve direct binding to tubulin. Special attention has been drawn to these compounds and also to their closely related analogues, taccalonolides B (**419**) and N (**421**), as they retain efficacy in taxane- and epothilone-resistant models (*259*). Although these taccalanolides are less potent than other microtubule stabilizers in drug-sensitive cell lines, they are effective at similar concentrations against taxane-resistant cell lines. The microtubule stabilizing activity of taccalonolides has been reviewed recently by *Risinger* and *Mooberry* (*260*).

7.7. *CNS-Related Activities*

Reports on activities affecting the CNS by withanolides are mostly based on properties attributed to "Ashwagandha" in ayurvedic medicine and are restricted to withanolides from *W. somnifera*.

7.7.1. Synaptogenesis and Neuritic Outgrowth

Facilitating synaptogenesis and reconstructing neuronal networks in the damaged brain is required for the therapeutic treatment of neurodegenerative diseases that produce neuronal degeneration and atrophy. Several withanolides isolated from the methanolic extract of *W. somnifera* roots as well as the extract itself, have been shown to possess neurite outgrowth activity (*21*, *261*). Withanoside IV (**31**) and VI (**32**) predominantly induced dendritic outgrowth in normal cortical neurons, while withanolide A (**504**) predominantly induced axon outgrowth (*262*). These withanolides also showed neuritic regeneration and synaptic reconstruction of damaged cortical neurons and prevented both dendritic and axonal atrophy induced by Aβ(25–35) (*261*, *263*). Oral administration of withanoside IV significantly improved memory deficit in Aβ(25–35) treated mice, and sominone, the aglycone of withanoside IV, was shown to be the major metabolite after administration. Sominone was more active than withanoside IV (*264*).

7.7.2. Cholinesterase Inhibition

Natural cholinesterase inhibitors are of special interest in drug development due to the involvement of cholinesterases in *Alzheimer*'s disease and other related dementias. Acetylcholinesterase (AChE) inhibitors activate central cholinergic function by increasing the acetylcholine levels in the brain. Bracteosins A (**206**), B (**207**), and C (**208**), isolated from *Ajuga bracteosa* (*108*), and withanolide A (**504**) and withaferin A (**1**) from *W. somnifera*, were moderate inhibitors of AChE and BChE (butyrylcholinesterase) (*11*). Molecular docking studies indicated that all compounds are imbedded in the aromatic gorge of AChE. All these withanolides also showed dose-dependent spasmolytic and Ca^{2+} antagonistic activities that may help in prolonging neuron survival and function (*265*). Withanoside VI (**32**) and withaferin A attenuated the desensitization to clonidine of smooth muscle, and this effect was related to the effect of *W. somnifera* on morphine tolerance and dependence (*27*). It should be noted that clonidine inhibits the release of acetylcholine by acting on α_2-adrenoceptors in these tissues.

8. Chemotaxonomic Considerations

As already mentioned, *ca*. 650 withanolides have been described, most of them from genera of the Solanaceae, subfamily Solanoideae. The absence so far of withanolides in members of the other subfamilies is curious. In Table 3, the genera of subfamily Solanoideae containing withanolides are arranged according to the most recent phylogenetic classification (*10*). As different groups of withanolides have been reported in 23 genera and *ca*. 70 species of the Solanoideae, some chemotaxonomic considerations can be made.

Withanolides with an unmodified skeleton (Fig. 2) are the most common, occurring in 14 genera of the different tribes (Physaleae, Datureae, Hyoscyameae, Lycieae, and Solaneae). Among the six genera still not assigned phylogenetically to

Table 3. Genera of the subfamily Solanoideae containing withanolides arranged according to a established phylogenetic system

Tribe (subtribe)	Genus (section)	Main withanolide types
Physaleae (Physalinae)	*Physalis*	Physalins, neophysalins, cyclophysalins, withaphysalins, unmodified skeleton
	Margaranthus	Physalins, unmodified skeleton
	Witheringia	Physalins
	Brachistus	Physalins
Physaleae (Iochrominae)	*Acnistus*	Acnistins, withaphysalins, unmodified skeleton
	Iochroma	Unmodified skeleton
	Eriolarynx	Withaphysalins, unmodified skeleton
	Vassobia	Unmodified skeleton
	Dunalia	Acnistins, withaphysalins, unmodified skeleton
Physaleae (Withaninae)	*Withania*	Unmodified skeleton
	Tubocapsicum	Acnistins
	Discopodium	Acnistins, unmodified skeleton
Physaleae[a]	*Larnax*	Subtrifloralactones, unmodified skeleton
Datureae	*Datura (Datura)*	Unmodified skeleton
	Datura (Dutra)	Withametelins, unmodified skeleton
Hyoscyameae	*Hyoscyamus*	Unmodified skeleton
Lycieae	*Lycium*	Unmodified skeleton
Solaneae	*Solanum*	Unmodified skeleton
Genera not assigned to a more inclusive clade	*Deprea*	Withajardins
	Exodeconus	Unmodified skeleton
	Jaborosa (Jaborosa)	Aromatic ring A, spiranoid-γ-lactones, unmodified skeleton
	Jaborosa (Lonchestigma)	15,21-cyclowithanolides, sativolides, spiranoid-γ-lactones, spiranoid at C-22, trechonolides, unmodified skeleton
	Nicandra	Aromatic ring D, unmodified skeleton
	Salpichroa	Aromatic ring D, unmodified skeleton
	Schraderanthus	Physalins

[a]Genus not assigned to a subtribe

a more inclusive clade but within the Solanoideae (Table 3), withanolides with an unmodified skeleton have now been reported only in the *Larnax* (*Deprea*) and *Schraderanthus* genera.

8.1. Tribe Physaleae

Different withanolides with modified skeletons are present within this tribe (Plates 1 and 2). Physalins and related withanolides (Fig. 2) are frequent in the subtribe Physalinae (Plate 1, a–f), particularly in *Physalis*, *Margaranthus*, *Witheringia*, and *Brachistus*. The occurrence of physalins and the non-occurrence of the unmodified skeletons in *Witheringia* and *Brachistus* support the close phylogenetic relationship between both genera, which appear together in a small clade and are sisters to one another (*10*). In addition, the presence of physalins in *Schraderanthus* is noteworthy, which is a monotypic genus recently segregated from *Leucophysalis* (tribe Physaleae, subtribe Physalinae). *Schraderanthus* still remains unassigned to any tribe due to the lack of molecular analysis; the chemical information would support the inclusion of *Schraderanthus* in the subtribe Physalinae, the only subtribe where physalins have been found.

In the subtribe Physalinae, withaphysalins (Fig. 2), ixocarpalactones, and perulactones (Fig. 3) have been reported in certain *Physalis* species (*P. minima, P. philadelphica*, and *P. peruviana*); since this genus comprises *ca.* 90 species, these metabolites may probably appear in other species. As more information emerges from research conducted in other *Physalis* species, it will be able to evaluate the potential chemotaxonomical value of these compounds, either as chemical generic markers or as an exception in the genus.

Withaphysalins are more frequent in the subtribe Iochrominae (Plate 2), a well-supported clade but with the relationships within this clade poorly resolved (*10*). The chemical data presented in this chapter, support some taxonomic changes proposed by morphological data (*9*) and confirmed by molecular evidence (*10*), as is the case for the segregation of *Eriolarynx* containing withaphysalins F-L (see Sect. 5.2.4.) from *Vassobia* (no withaphysalins).

Acnistins (Fig. 2) appear in the subtribes Iochrominae and Withaninae and are absent in all the remaining genera investigated so far. Within the latter subtribe, three small clades have been identified with unresolved relationships between them (*10*). One clade includes *Aureliana* and *Athenaea,* two South American genera not investigated phytochemically up to now. The second clade comprises a monotypic Asiatic genus (*Mellisia*) and *Withania*, the "parent genus" of the withanolides (Plate 1, g–j); finally, *Nothocestrum*, *Tubocapsicum* and *Discopodium* are included in the third clade. The chemical evidence is consistent with this proposal since *Withania* is characterized by the presence of a large number of withanolides with the unmodified parent skeleton of withaferin A (**1**). In contrast, *Discopodium* and *Tubocapsicum* produce withanolides with structural variations. The close affinity

Plate 1. Solanaceae species of Tribe *Physaleae*. Subtribe *Physalinae*: (**a**) *Physalis viscosa*, branch with fruit; (**b**) *P. viscosa*, flower (photographs: J. Toledo and M.T. Cosa). (**c**) *P. pubescens*, plant with flower and fruits (photograph: G. Barboza); (**d**) *P. peruviana*, detail of corolla (photograph: J. Toledo); (**e**) *Witheringia solanacea*, stem with fruits; (**f**) *W. solanacea*, flower and immature fruits (photograph: G. Beltrán). Subtribe *Withaninae* (photographs with permission of the Experimental Garden and Genebank of the Radboud University of Nijmegen, The Netherlands): (**g**) *Withania somnifera*, flowering branch; (**h**) *W. somnifera*, fruits; (**i**) *W. adpressa*, flowers; (**j**) *W. aristata*, flowering branch. Genus not assigned to a subtribe: (**k**) *Larnax* sp., branch with flowers and fruits; (**l**) *Larnax* sp., flowering branch (photographs: S. Leiva)

Plate 2. Solanaceae species of Tribe *Physaleae* subtribe *Iochrominae*. (**a**) *Eriolarynx lorentzii*, flower; (**b**) *Vassobia breviflora*, branch with flowers and fruits; (**c**): *Acnistus arborescens*, flowering branch (photographs: F. Chiarini); (**d**) *Iochroma australe*, flowering branch; (**e**) *I. australe*, fruit (photographs: M. T. Cosa and G. Barboza); (**f**) *I. fuchsioides*, flowering branch and (**g**) *I. gesneroides*, flowering branch (photographs with permission of Experimental Garden and Genebank of the Radboud University of Nijmegen, The Netherlands)

between the latter two genera is evidenced by them both producing the 17-epiacnistins, which, are absent in *Withania*.

8.2. Tribes Hyoscyameae, Lycieae, and Solaneae

In these tribes (Plate 3, d–i), only five species have been investigated (Tables 1 and 3), thus, only preliminary chemotaxonomic suggestions may be proposed. These species are consistent in always containing withanolides with an unmodified skeleton. Worth mentioning is the fact that in the largest genus of the family, *Solanum* (with *ca*. 1,500 species), withanolides have been reported in only two species (*S*. *ciliatum* and *S*. *sisymbriifolium*) (See Sect. 4.2.5.).

8.3. Tribe Datureae

The tribe comprises two genera, *Brugmansia* and *Datura*, but, however, no information is available on *Brugmansia*. In the conventional classification, the 14 species of *Datura* are included in three sections: sect. *Datura* (*D*. *quercifolia* and *D*. *stramonium*), sect. *Ceratocaula* (*D*. *ceratocaula*), and sect. *Dutra* (the remaining species) (Plate 3, a–c). Withanolides from seven taxa have been investigated extensively, and more than 70 different compounds have been reported. The withametelin skeleton (Fig. 2), occurring in *ca*. 30 compounds, is exclusive to *Datura*. Withametelins were reported in *D*. *inoxia* and *D*. *metel*. Genetic similarity and phylogenetic analysis both suggest that *D*. *metel* is related more closely to *D*. *inoxia* than to the other taxa of section *Dutra*, based upon the small genetic distance between them (*266*, *267*), which is supported by the available chemical information. Other chemical coincidence with conventional and phylogenetic proposals is represented by the close relationship between the two members of the section *Datura* (*266*), *i.e*. *D*. *quercifolia* and *D*. *stramonium*, which share the occurrence of 12α-hydroxylated unmodified withanolides.

8.4. Genera with Uncertain Positions in the Solanaceae Taxonomic System

Some genera (*Jaborosa*, *Nicandra*, *Salpichroa*) contain withanolides with exclusive arrangements, which can be considered as chemotaxonomic markers at the generic level. *Jaborosa* is a good example since more than 50% of its species have been studied (Plate 4). In this genus, several peculiar modified skeletons of the

Plate 3. Solanaceae species of Tribe *Datureae*: (**a**) *Datura ferox*, plant; (**b**) *D. ferox*, fruit (photograph: F. Chiarini); (**c**) *D. inoxia*, plant (photograph: M.T. Cosa). Species of Tribe *Lycieae*: (**d**) *Lycium chinense*, flower; (**e**) *L. chinense*, fruits (photograph: B. Liu); (**f**) *L. barbarum*, branches with fruits (photograph: M. Li). Species of Tribe *Solaneae*: (**g**) *Solanum sisymbriifolium*, fruits; (**h**) *S. sisymbriifolium*, flowers (photographs: M.T. Cosa). Species of Tribe *Hyoscyameae*: (**i**) *Hyoscyamus niger*, flowering branch (photograph: B. Liu). Species not assigned to a tribe: (**j**) *Deprea* sp., Flower (photograph: S. Leiva); (**k**) *Exodeconus maritimus*, plant (photograph: E. Rodríguez R.); (**l**) *Salpichroa origanifolia*, flowering branch (photograph: G. Barboza)

Plate 4. *Jaborosa* spp.: (**a**) *J. rotacea*, plant; (**b**) *J. rotacea*, flower (photographs: F. Chiarini); (**c**) *J. leucotricha*, plant; (**d**) *J. leucotricha*, flowers (photographs: G. Barboza). (**e**) *J. sativa*, flowering branch (photograph: F. Chiarini); (**f**) *J. laciniata*, plant and flowers in detail; (**g**) *J. odonelliana*, flower (photographs: G. Barboza); (**h**) *J. caulescens*, flower (photograph: A.A. Cocucci); (**i**) *J. kurtzii*, flowers (photograph: G. Barboza); (**j**) *J. reflexa*, plant; (**k**) *J. reflexa*, flowers (photographs: G. Barboza); (**l**) *J. bergii*, fruits; (**m**) *J. bergii*, flower (photographs: A.A. Cocucci); (**n**) *J. magellanica*, plant (photograph: G. Barboza)

withanolides appear repeatedly in different species, such as the sativolides, trechonolides, spiranoid withanolides at C-22, and spiranoid-γ-lactones (Figs. 2 and 3).

The trechonolides, sativolides, and spiranoid withanolides at C-22 are exclusive to species in *Jaborosa* section *Lonchestigma*. The trechonolides are the most widespread in the genus, being present in nine species (*J. araucana*, *J. caulescens*, *J. laciniata*, *J. lanigera*, *J. leucotricha*, *J. magellanica*, *J. parviflora*, *J. rotacea*, and *J. sativa*); sativolides appear in *J. caulescens*, *J. rotacea*, and *J. sativa*, while the spiranoid withanolides at C-22 are only present in *J. kurtzii* and *J. rotacea*.

Spiranoid-γ-lactones have been isolated from *J. odonelliana*, *J. runcinata* and *J.integrifolia* (unpublished data), all belonging to *Jaborosa* sect. *Jaborosa*. Only one spiranoid-γ-lactone, jaborosalactone 2, was obtained from *J. araucana* (section *Lonchestigma*). As was stated in Sect. 5.4.3., the structural similarity of jaborosalactone 2 with trechonolide A, both present in *J. araucana*, suggests that these compounds may have a common biosynthetic precursor, and probably this species could be a link between section *Jaborosa* and section *Lonchestigma*.

Other distinctive variations with modified skeletons are the withanolides and related steroids with an aromatic ring D, the nicandrenoids isolated from *Nicandra*, and the salpichrolides from *Salpichroa* (Plate 3, l). Moreover, both genera exhibit the same δ-lactol side chain at C-17. In spite of this chemical similarity, *Nicandra* and *Salpichroa* are not phylogenetically close (*10*).

Nicandra has been suggested as being close to *Exodeconus* (*10*) (Plate 3, k). Although the withanolides isolated from *Exodeconus* have an unmodified skeleton and the major components of *Nicandra* have an aromatic ring D, all the withanolides found in both genera share the same 1-oxo-2-ene-5α-hydroxy-6α,7α-epoxy substitution pattern in rings A and B. In this case, the phytochemical evidence coincides with the phylogenetic proposal. *Salpichroa* and *Jaborosa* have traditionally been placed in tribe Jaboroseae (*9*). However, the phylogenetic evidence proposes *Salpichroa* in a clade informally named "Salpichroina", from which *Jaborosa* is excluded. Again, chemical information supports this proposal.

Despite the fragmentary chemical contribution described in this section of the chapter, it is evident that a large and species-rich study in Solanaceae in both molecular and phytochemical aspects would provide a better comprehension of the relationships among the species contained therein.

Acknowledgments We thank CONICET, Universidad de Buenos Aires and Universidad de Córdoba for financial support.

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