Pure & Appl. Chem., Vol. 70, No. 2, pp. 385–389, 1998.
Printed in Great Britain.

New natural products from medicinal plants of Pakistan

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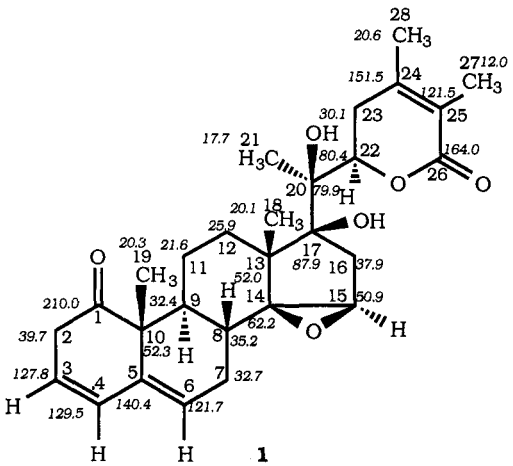
Abstract: A number of new natural products have been isolated from medicinal plants of Pakistan. Some of them have exhibited antifungal activities. Structure of those compounds were determined with the help of spectroscopic studies.

1. ANTIFUNGAL STEROIDAL LACTONES FROM *WITHANIA COAGULANCE*

Withanolides (C_{28} steroidal lactones) have been isolated from many plants of the family Solanaceae such as *Withania somnifera, W. coagulance, Acnistus australis* and *Datura metel.* Plants of genus *Withania* are known to exhibit a variety of pharmacological activities mainly due to the presence of withanolides (1-3).



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The two new withanolides were isolated from the EtOH extract of the whole plant of *W.coagulance* by CC and TLC techniques. The EIMS of **1** showed the M^+-18 ion at *m/z* 450.2465 analysing for $C_{28}H_{34}O_5$ Hence, compound **1**, possesses 11 degrees of unsaturation. Seven of these were accounted for by the pentacyclic α,β-unsaturated steroidal lactone skeleton, two were due to the double bonds, one due to the ketonic carbonyl and one due to the epoxide. The ion at *m/z* 125.0666 ($C_7H_9O_2$) further confirmed the presence of a six-membered lactone moiety. The ion at *m/z* 169.0902 ($C_9H_{13}O_3$) could arise by the cleavage of the C-17/C-20 bond. The overall mass fragmentation pattern of **1** was characteristic of withanolides (4).

The UV spectrum of **1** showed an absorption at 227 nm, characteristic of an α,β-unsaturated lactone chromophore while the IR spectrum indicated the presence of a hydroxyl group, a six membered cyclic ketone and an α,β-unsaturated lactone .

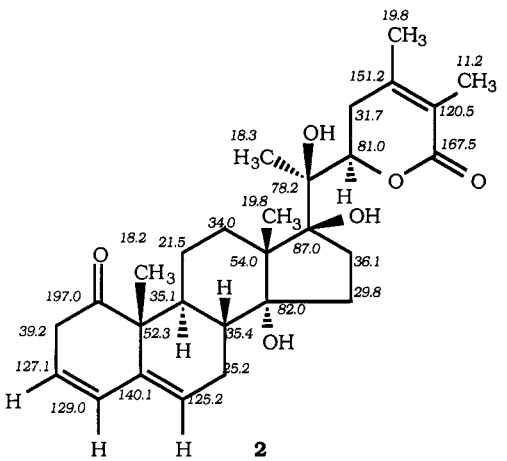
The ^{1}H-NMR spectrum of **1** exhibited signals for five tertiary methyl groups at δ 1.40, 1.42, 1.70, 1.78 and 1.93. Two mutually coupled olefinic signals resonating at δ 5.55 (multiplet) and 6.08 (*dd*, $J_{4,3}$ = 9.7 Hz, $J_{4,2}$ = 2.0 Hz) were assigned to the C-3 and C-4 vinylic protons, respectively. Another downfield proton resonating at δ 5.81 (*dd*, $J_{6,7a}$ = 5.1 Hz, $J_{6,7b}$ = 2.4 Hz) was only to coupled two protons of a methylene group (δ 2.62 and 2.10). These observations indicated a trisubstituted conjugated diene with a quaternary olefinic carbon (C-5) which lies between the olefinic bonds. This arrangement of double bonds is only possible in rings A and B. A 1H doublet of doublet centred at δ 3.10 ($J_{15,16a}$ = 3.2 Hz, $J_{15,16b}$ = 1.6 Hz) was characteristic of a proton geminal to the epoxide function and it was assigned

to the C-15 methine proton. A downfield double doublet at δ 5.21 ($J_{22,23a}$ = 13.7 Hz, $J_{22,23b}$ = 3.5 Hz) was assigned to the C-22 methine proton of the lactone moiety. The C-3 vinylic proton (δ 5.55) showed vicinal couplings in the COSY-45° spectrum with H-2α (δ 2.70) and H-2 β (δ 3.32) as well as with the C-4 vinylic proton (δ 6.08). H-4 also exhibited allylic couplings with H-2α (δ 2.70) and H-2β (δ 3.32) protons. The C-15 oxirane proton (δ 3.10) exhibited couplings with H_2-16 (δ 1.11 and 1.32). The β-stereochemistry of the C-14/C-15 epoxide was deduced on the basis of ^{1}H NMR chemical shift comparison with other compounds (5,6) and on the basis of coupling constants ($J_{15,16a}$ = 3.2, $J_{15,16b}$ = 1.6 Hz). H-22 showed coupling with H_2-23 resonating at δ 2.75 and 3.08, respectively. H_2-23 showed weak homoallylic coupling with the C-27 methyl protons.

The ^{13}C NMR spectra of **1** showed 28 signals. A notable feature was the appearance of downfield signals for the quaternary and tertiary carbons at δ 62.2 and 50.9, respectively, which were assigned to the epoxy bearing C-14 and C-15. The chemical shifts of the various carbons are presented on structure **1**. One-bond ^{1}H-^{13}C correlations were determined by the HMQC (7) technique and the HMBC data was used to connect different structural fragments as well as to confirm the above chemical shift assignments.

The EI mass spectrum of **2** showed the M^+ at *m/z* 470.2677 corresponding to the formula $C_{28}H_{38}O_6$, and indicated 10 degrees of unsaturation. The presence of a hydroxyl, ketonic carbonyl and α,β-unsaturated lactone was indicated by the IR absorptions at 3420, 1690 and 1675 cm^{-1}. The presence of an α,β-unsaturated lactone was also indicated by the UV absorption at 223 nm.

The ^{1}H NMR spectrum of **2** showed distinct resemblance to that of **1**. The only notable difference was the lack of a doublet of doublet centred at δ 3.10, indicating that **2** did not contain a C-14/C-15 epoxide. Similarly, the ^{13}C NMR spectrum showed an additional downfield signal at δ 82.0, which may be assigned to an oxygen-bearing carbon.



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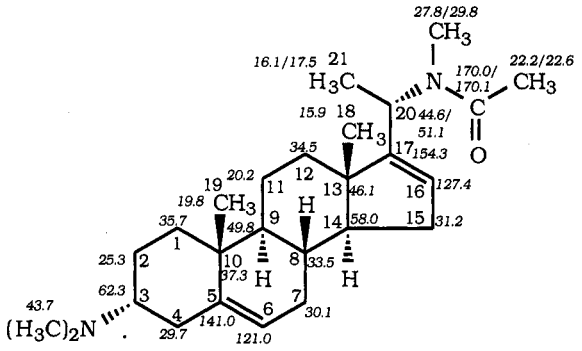
Compound **2** was obtained earlier by partial derivatization of withanolide F by Vande Velde *et al.* [8], but has not been isolated from natural sources. Compound **2** exhibited antifungal activity against the human pathogens *Nigrospora oryzae, Aspergillus niger, Curvularia lunata, Stachybotrys atra, Allescheria boydii, Drechslera rostrata, Microsporum canis* and *Epidermophyton floccosum* and the plant pathogen *Pleurotus ostreatus* (MIC 300 μg /ml) (9). It also showed activity against gram positive *Staphylococcus aureus* (10).

2. STEROIDAL ALKALOID FROM *SARCOCOCCA SALIGNA*

S. saligna Muel (syn. *S. pruniformis* Lindl.) is an evergreen shrub found widely distributed in the northwest region of Pakistan (11). The leaves of the herb enjoy considerable reputation as a remedy for different diseases and for the treatment of fever and rheumatism in the indigenous system of medicine. Our studies on the crude ethanolic extract of the aerial parts of *S. saligna* also showed good antibacterial activity against *Pseudomonas pseudomalliae, Shigella boydii* and *Carnebacterium diphtheria.*

The present investigation on the plant has resulted in the isolation of a new alkaloid, saracocinaene (**3**). The HREI mass spectrum of **3** exhibited the M^+ at *m/z* 393.3260 analysing for $C_{26}H_{42}N_2O$ (calc. 398.3296). Hence, **3** possessed 7 degrees of unsaturation. Four of these were accounted for by the tetracyclic structure of a pregnane type steroid, two were due to endocyclic double bonds and one due to a carbonyl function. The UV spectrum showed only terminal absorption. The IR spectrum showed an intense absorption at 1620 cm^{-1} characteristic of an amide function.

The ^{1}H-NMR spectrum of **3** showed a doubling of signals for various protons due to the hindered rotation of the C-20 amide function. The methyl signals at δ 0.71/0.77 and 1.04/1.06 were due to the quaternary methyl groups (12). Another 3H doublet at δ 1.14/1.26 was due to the secondary C-21 methyl group. A 3H singlet at δ 2.66/2.69 was due to *N*-methyl group and another 1H singlet at δ 5.27 was characteristic of a Δ^5-double bond. A 1H triplet at δ 5.66 was assigned to the C-16 olefinic proton. The small coupling constant indicated a *gauche* conformation of the olefinic proton with respect to the C-15 methylenic protons. Similar doubling of the *N*-acetyl (δ 2.05/2.17) signal and various other neighbouring proton signals were also observed due to the restricted rotation. A 1H quartet at δ 4.40/5.40 showed COSY 45° interaction with the C-21 methyl group at δ 1.14/1.26, confirming it to be H-20. Extensive 2D-NMR experiment (COSY 45°, NOESY, HOHAHA, HMQC and HMBC) (7) further confirmed the above mentioned assignments. The ^{13}C-NMR assignments are presented on structure **3**.



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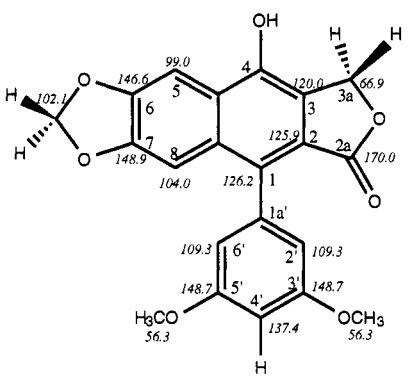
3. ANTIFUNGAL ARYLTETRALIN LIGNANS FROM LEAVES OF *PODOPHYLLUM HEXANDRUM*

The genus *Podophyllum* (Podophyllaceae) has been the subject of vigorous phytochemical investigations in the early 1960's after the isolation of several antitumor lignans such as podophyllotoxin, etc. A number of lignans isolated from *Podophyllum* species have shown a wide range of biological activities such as antitumor, antimitotic and antiviral activities. Some of them have also shown toxicity to fungi, insects and vertebrates (13,14). The successful chemical conversion of the major constituent podophyllotoxin into the clinically useful anticancer drug Etoposide® and Teniposide® has also triggered further researches in this area (15).



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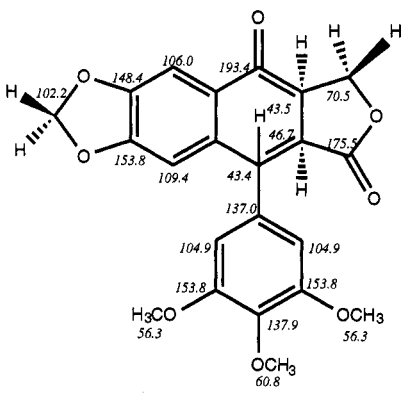

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Our investigation on *P. hexandrum* has resulted in the isolation of two aryltetralin lignans, 4'-*O*-demethyldehydropodophyllotoxin (**4**) and picropodophyllone (**5**) earlier reported as semisynthetic products.

Compound **4** was isolated as an amorphous powder. The molecular formula $C_{21}H_{16}O_8$ indicated 14 double bond equivalents in the molecule. The M$^+$was further confirmed by +ve FABMS. The overall spectral behaviour of **4** was identical to that of the semisynthetic product 4'-*O*-demethyldehydropodophyllotoxin (16). The UV spectrum exhibited absorption bands at 205, 229,

264, 314 and 357 nm, characteristic of a tetradehydropodophyllotoxin lignan nucleus (17) The absorption bands in the IR spectrum at 3437, 2900, 1742, 1609 and 1448 and 1029 cm^{-1} indicated the presence of phenolic OH, C-H, lactone carbonyl, aromatic C=C and C-O functionalities, respectively.

The ^{13}C-NMR spectra (7) showed 16 signals representing 19 carbons. An examination of the structure shows that the aromatic ring substituted at C-1 contains three pairs of identical carbons (the two -OCH_3 carbons appearing at δ 56.3, the two carbons at which the -OCH_3 groups are attached resonating at δ148.7 and the two carbon *ortho* to the methoxy group resonating at δ 109.3). The signals for the C-1' and C-4' quaternary carbons were too weak to be detected in the broad-band decoupled ^{13}C NMR spectrum. An ester carbonyl carbon resonated at δ 170.0, a methylenedioxy carbon appeared at δ 102.1 and a downfield methylene carbon resonated at δ 66.9, while the remaining 12 signals appeared between δ 100.0-150.0 again confirming a functionalized aromatic system.

In the ^{1}H-NMR spectrum of **4**, a 6H singlet resonating at δ 3.81 represented two -OCH_3 groups in identical magnetic environment. A downfield methylene singlet at δ 6.11 was characteristic of a methylenedioxy group and its appearance as a singlet was indicative of the lack of chirality in the molecule. Another 2H downfield singlet at δ 5.50 was due to the methylene protons (C-3H) sandwiched between an oxygen function and the quaternary carbon. A 2H singlet in the aromatic region of the spectrum at δ 6.97 was assigned to the two aromatic protons in an identical magnetic environment. *i.e.* C-2' and C-6' protons. Two more downfield signals appearing as broad singlets at δ 8.14 and 7.46 were ascribed to the two remaining aromatic protons of the skeleton. The absence of coupling interactions between these aromatic protons indicated their *para* disposition and they were therefore assigned to the C- 5 and C-8 protons, respectively.

An HMQC experiment (7) was performed to establish connectivities between the protons and their respective carbons. Hence, the C-5 and C-8 carbons (δ 99.0 and 104.0) in the aromatic moiety displayed one-bond interactions with the protons resonating at δ 8.14 and 7.46, respectively, while another set of carbons resonating at δ 109.3 (C-2' and C-6') in ring D showed correlation with the C-2' and C-6' protons (δ 6.97). The C-3a methyleneic protons exhibited heteronuclear couplings with the C-3a carbon (δ 66.9).

The HMBC experiment (7) was used for the unambiguous chemical shift assignments. Thus, the long-range interactions between the protons at δ 6.97 (C-2'H and C-6'H) with the carbons resonating at δ 148.7(C-5') and δ 137.4 C-4') suggested that they are part of the ring D. The aromatic C-5 proton resonating at δ 8.14 exhibited long-range interactions with the carbons at δ 148.9 (C-7) and 132.5 (C-4a). The C-3a methylene protons displayed HMBC interaction with C-3 (δ 120.0), while C-8H showed interactions with C-7, C-8a and C-1.

The mass spectrum included peaks at *m/z* 396 M^+, 353, 334, 281 and 139. This spectroscopic data unambiguously established that **4** is the naturally occurring 4'-demethyl derivative of dehydropodophyllotoxin.

Picropodophyllone (**5**), a 1*R*, 2*S*, 3*R* isomer of podophyllotoxone, has been known synthetically for several years, but has not previously been reported as a natural product (18). The possibility of its formation by heat or by base catalysed isomerization did not exit since no base was employed during the isolation process. The molecular formula $C_{22}H_{20}O_8$ of **5** was again determined by HREIMS (*m/z* 412.1138). The UV spectrum of **5** displayed absorption bands at 206, 240, 280 and 324 nm characteristic of a podophyllotoxone skeleton. The IR spectrum of **5** contained bands at 2839 (C-H), 1772 (lactone carbonyl), 1661 (ketone carbonyl), 1584 (C=C) and 1125 (C-O) cm^{-1}.

The ^{13}C-NMR spectra contained a lactone carbonyl signal at δ 175.5 and an α, β-unsaturated ketonic resonance at δ 193.4. The three methoxy carbons appeared at δ 56.3 (2 x OCH_3) and δ 60.8 (-OCH_3). The methylenedioxy carbon yielded a characteristic signal at δ 102.2. A downfield methylene signal at δ 70.5 was assigned to the methylene carbon containing an oxygen function. The methine carbons resonated at δ 43.5, 46.7 and 43.4 represented the carbon atoms of ring B. The aromatic carbons appeared in two groups. The signals between δ 104-107 were due to unsubstituted aromatic carbons while the signals resonating between δ 127.0-154.0 represented either oxygen-bearing or quaternary aromatic carbons. Only 17 signals were visible in the ^{13}C-NMR spectrum representing 22 carbons.

The ^{1}H-NMR spectrum of **5** contained one 6H and one 3H singlets at δ 3.73 and 3.77, which could be assigned to three methoxy groups, two of which have identical magnetic environment. The methylenedioxy protons appeared as two AB doublets at δ 6.02 and 6.01 indicating the presence of chirality in the molecule. Similarly, a set of geminally coupled protons resonating at δ 4.73 as doublet and δ 4.32 as multiplet represented a methylene *i.e.* C-3a protons sandwiched between an oxygen and methine. The COSY 45° (36-40) spectrum displayed cross-peaks between signals at δ 4.73 and 4.32 due to their geminal disposition, while the cross-peak between δ 4.32 and 3.28 represented vicinal coupling between one of the methylenic proton with the neighbouring methine proton (C-3H).

The EI mass spectrum of **5** displayed M^+ peak at *m/z* 412.1138 ($C_{22}H_{20}O_8$). The ion at *m/z* 367 (M^+-HCO_2) represented the fragment $C_{21}H_{19}O_6$. The peaks at *m/z* 167 (M^+-245) and 200 (M^+-212) were due to fragments $C_9H_{12}O_3$ and $C_{12}H_8O_{37}$, respectively. Other peaks at *m/z* 353, 337, 297, 227, 139, etc. were also in complete agreement with the structure **5**.

These two new liganans showed antifungal activity against *Epidermophyton Floccosum, Curvularia lunata, Nigrospora oryzae, Microsporus canis, allescheria boyudii* and and *Pleurotus ostreatus* (19).

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