RECENT ADVANCES IN THE CHEMISTRY OF TERPENOID TUMOR INHIBITORS*

S. MORRIS KUPCHAN

University of Wisconsin, Madison, Wisconsin, U.S.A.†

ABSTRACT

A continuing search for tumor inhibitors from plant sources has yielded over two hundred extracts with reproducible growth-inhibitory activity. Systematic fractionation, guided by assay in cell culture and animal tumor systems, has led to the isolation of the active principles of more than sixty plants. Chemical studies of novel sesquiterpene lactones, diterpenoid quinone methides, steriod lactones and other terpenoid tumour inhibitors are discussed.

INTRODUCTION

The past two decades have witnessed the synthesis of many hundreds of chemical variants of known classes of cancer chemotherapeutic agents. Synthesis of modifications of presently-known drugs does (and should) continue. However, some pessimism is evident among workers in the field, because of the relatively small improvements over the prototype drugs which have resulted from the extensive synthetic efforts to date. There exists a need for new prototypes, or templates, for the synthetic organic chemist to use in the design of potentially superior chemotherapeutic agents. As a corollary statement, there exists a need for elucidation of new biochemical mechanisms of growth regulation which may be more amenable to selective regulation. Recent studies in the isolation and structural elucidation of tumor inhibitors are yielding a fascinating array of novel types of growth-inhibitory compounds. There appears to be reason for confidence that this approach may point the way to useful templates for new synthetic approaches to cancer chemotherapy.

Studies of plant-derived tumor inhibitors are proceeding in many laboratories of wide geographic distribution. However, to limit the scope of the present discussion, the author will review only recent studies of terpenoid tumor inhibitors from his own laboratory at the University of Wisconsin.

The programme at the University of Wisconsin started modestly, in 1959, with a screening study of crude extracts of a limited number of accessible plants for inhibitory activity against animal-tumor systems. Some plants were procured by summer collections in Wisconsin, others by cooperative arrangements with botanists in India, Costa Rica, and other countries. The results of testing of the first plant extracts prepared in our laboratory

*Part XLIX in the series 'Tumor Inhibitors.' For preceding article *see* S. M. Kupchan, A. Karim, and C. Marcks. *J. Org. Chem.* **34**, 3912 (1969).

†Present address: Department of Chemistry, University of Virginia, Charlottesville, Virginia 22901, U.S.A.

and elsewhere revealed that a small but significant number of the extracts showed reproducible tumor-inhibitory activity. Encouraged by these results, the Cancer Chemotherapy National Service Center of the National Institutes of Health arranged with the U.S. Department of Agriculture to procure several thousand plant samples per year for evaluation. Shortly thereafter, the CCNSC arranged a contract with the Wisconsin Alumni Research Foundation, in Madison, to execute the initial extraction and screening studies. From that point onward, the University of Wisconsin programme concentrated on the isolation and structural elucidation of new tumor inhibitors. To date, the active principles of more than sixty active plants have been isolated in the Wisconsin programme, and the chemical studies of some of the most interesting compounds constitute the focus of this review.

ISOLATION OF TUMOR-INHIBITORY PRINCIPLES FROM PLANTS

One aspect of the approach of our programme differs significantly from the classical, and most widely practiced, approach to the biological study of plant constituents. In the classical phytochemical approach, those compounds are studied which are most easily separated from a plant extract and most easily crystallized. In our programme, however, the fractionation and isolation studies are guided at every stage by biological assays. The systematic fractionation, guided by biological assays, has made possible the isolation of important minor constituents which would most probably have been missed in the classical approach.

Solanum dulcamara L.

Solanum dulcamara L., collected near Madison, Wisconsin, was one of the first plants found active in the Wisconsin programme. *Figure 1* summarizes the fractionation procedure which led to isolation of the tumor-inhibitory principle, the steroid alkaloid glycoside β-solamarine, whose structure is shown in *Figure 2*. It is noteworthy that *S. dulcamara* L. has been used to treat cancers, tumors, and warts from the time of Galen (*c.* A.D. 180), and references to its use have appeared in the literature of many countries[1].

Marah oreganus and *Brandegea bigelovii* Cogn.

Systematic fractionation of an exceedingly cytotoxic extract from *Marah oreganus* (Cucurbitaceae), from California, led to isolation of the four previously-known cucurbitacins shown in *Figure 3*[2]. Similar fractionation of a cytotoxic extract of *Brandegea bigelovii* Cogn. (Cucurbitaceae) gave three new cucurbitacins, *O*, *P*, and *Q*. The structures were deduced from their formulae and spectra. Interrelation was accomplished by conversion of both cucurbitacins *O* and *Q* to cucurbitacin *P*, which was also prepared from cucurbitacin *B*. Conversion of cucurbitacin *P* to a 2,3-acetonide showed that it was the 3β-hydroxy-epimer of dihydrocucurbitacin *F*, thus confirming its absolute configuration. The interrelationships showed that cucurbitacin *O* was 3-epi-cucurbitacin *F* and cucurbitacin *Q* its 25-acetate derivative (*Figure 4*)[3]. Although some of the cucurbitacins (e.g. *B* and *E*) number among the

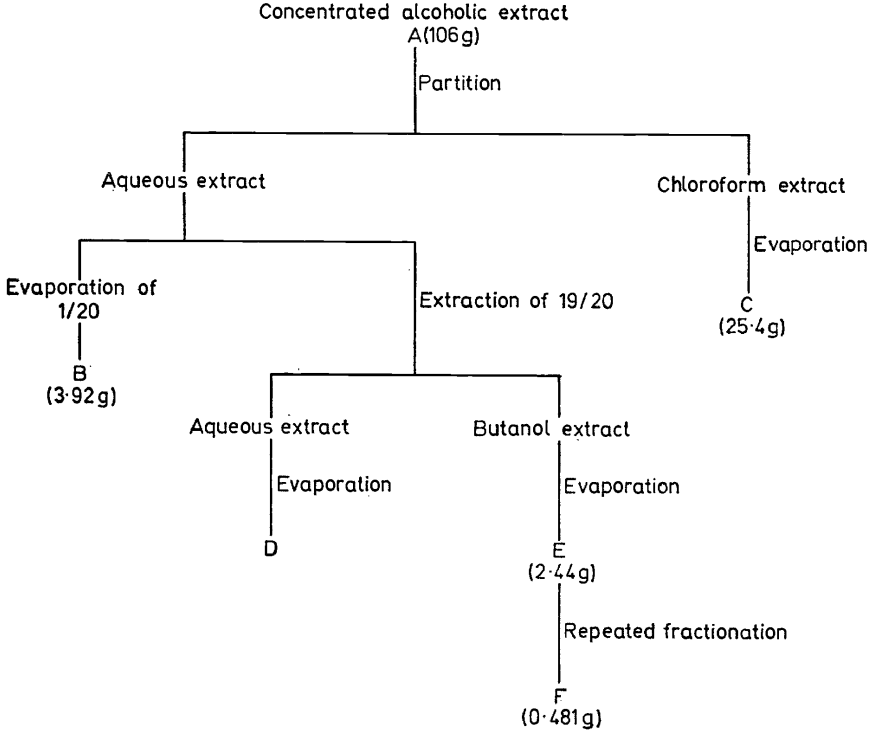
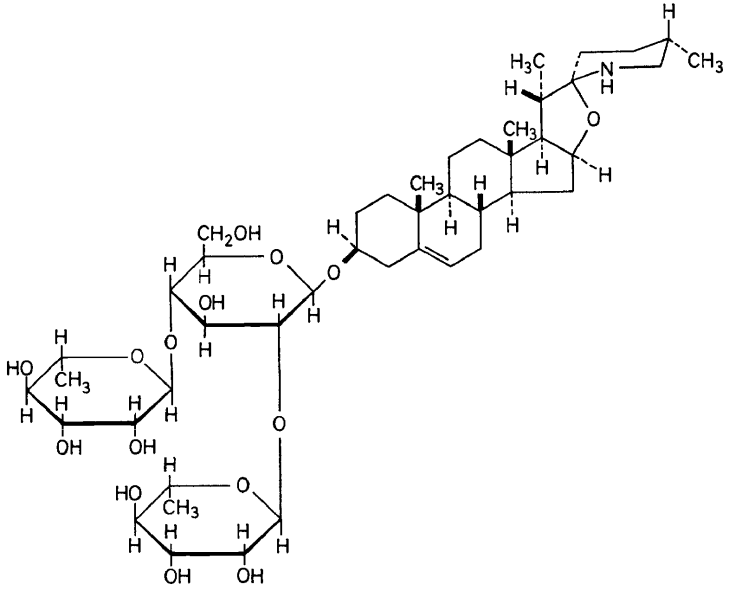


Figure 1. Fractionation of tumor-inhibitory extract from *Solanum dulcamara* L.



β-Solamarine

Figure 2. SA-active principle of *Solanum dulcamara* L.

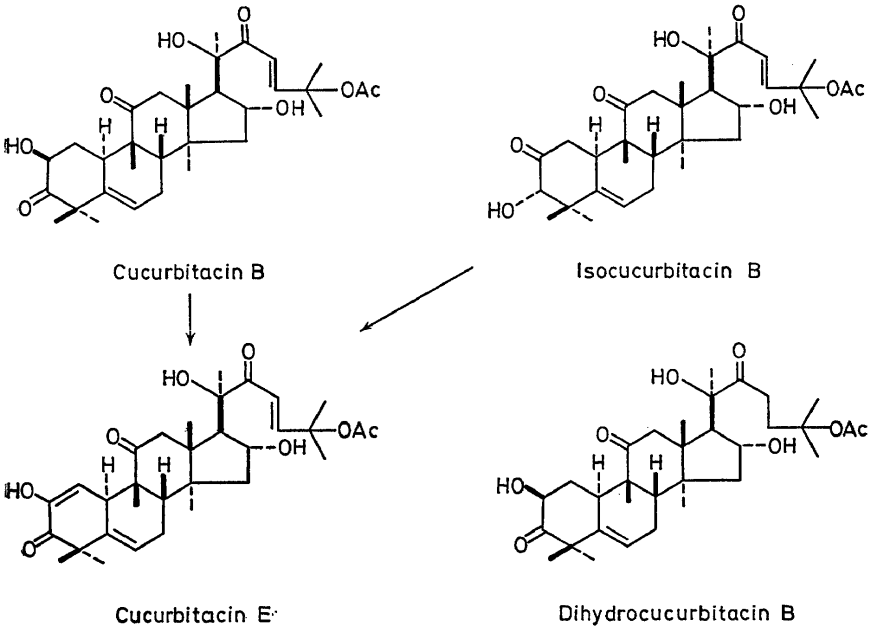


Figure 3. KB-active principles of *Marah oreganus*

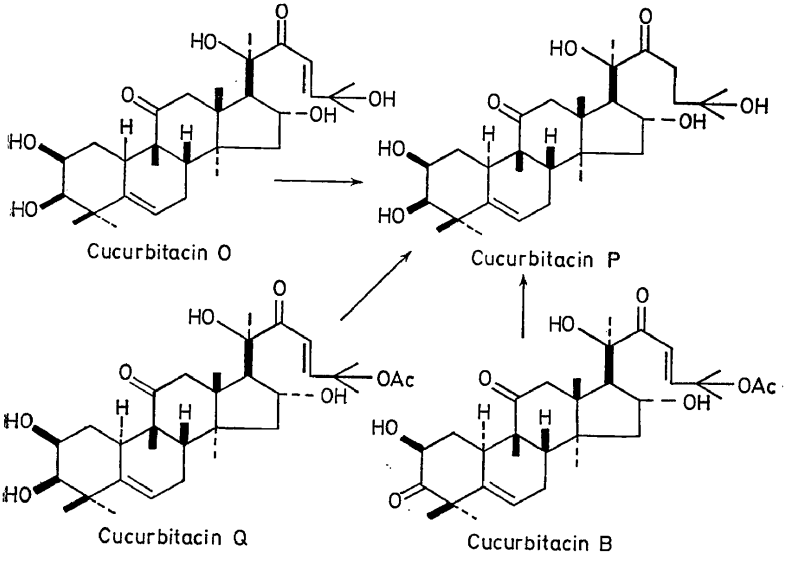


Figure 4. KB-active principles of *Brandegea bigelovii*

most cytotoxic compounds known[2], their relatively narrow therapeutic index render the materials unpromising as therapeutic agents.

Taxodium distichum Rich.

Systematic studies of a tumor-inhibitory extract from *Taxodium distichum* Rich led to isolation and structural elucidation of two novel diterpenoid quinone methides, taxodione (I) and taxodone (V) (*Figure 5*)[4]. The structures were deduced from their formulae and spectra, and confirmed by inter-

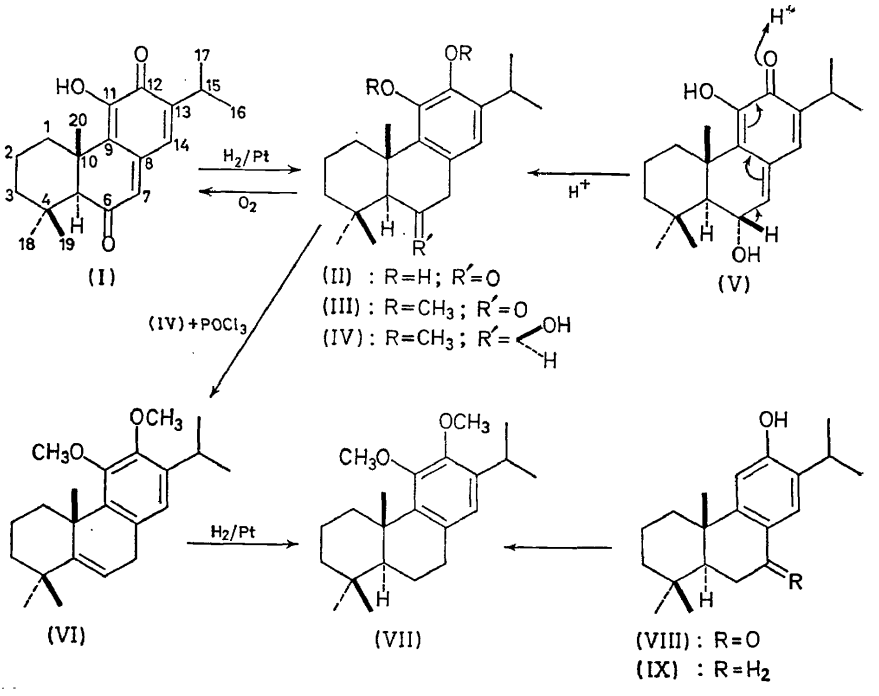


Figure 5. Taxodione and taxodone, WA-inhibitory principles of *Taxodium distichum*

relation with a known diterpene. Both quinone methides were converted to II, which was methylated to III. Reduction of III with lithium aluminium hydride gave the 6β-alcohol IV, which was dehydrated to VI. Catalytic hydrogenation of VI gave VII, characterized by direct comparison with a sample prepared from sugiol (VIII). Among the six diterpenoid derivatives isolated from *Taxodium distichum* only the quinone methide derivatives taxodione and taxodone showed significant inhibitory activity *in vivo* against the Walker carcinosarcoma 256 in the rat and *in vitro* against cells derived from human carcinoma of the nasopharynx (KB). This fact and the known sensitivity of quinone methides to nucleophilic attack[5] suggested that taxodione and taxodone might exert their biological effect by interaction with a biological nucleophile at C-7. The plausibility of the

suggestion was supported by the recent finding that both diterpenoid quinone methides are powerful inhibitors of the sulphydryl enzyme, phosphofructokinase[6].

Acnistus arborescens L. Schlecht

The leaves of *Acnistus arborescens* (L.) Schlecht have been used for many years to treat cancerous growths, and an extract of the leaves was forwarded to us by Professor J. A. Saenz Renauld, of the University of Costa Rica. Fractionation by the procedure outlined in *Figure 6* led to isolation of the tumor-inhibitory principle, withaferin A. A combination of degradative,

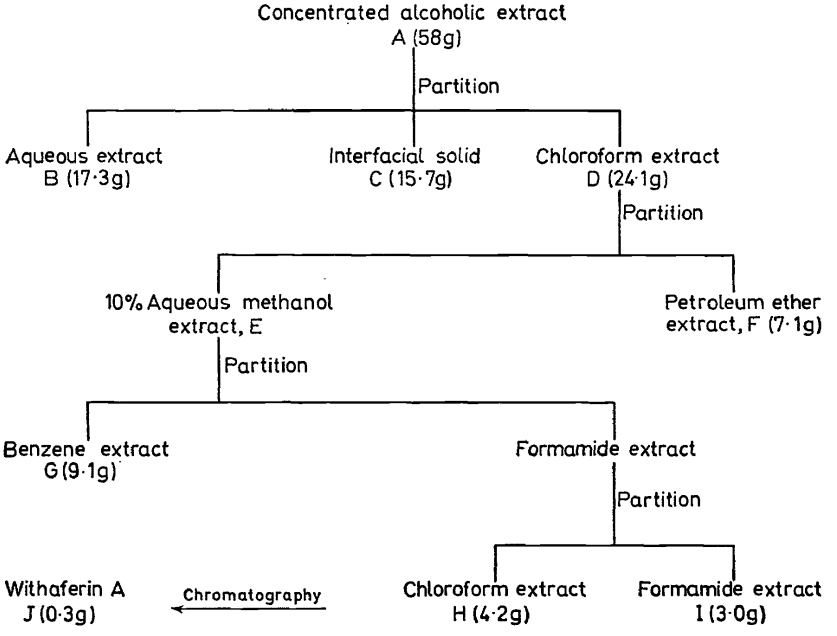
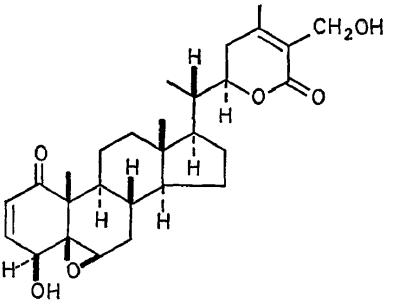


Figure 6. Fractionation of tumor-inhibitory extract from *Acnistus arborescens*

spectral, and x-ray crystallographic studies resulted in elucidation of the structure shown in *Figure 7* for withaferin A[7]. While our work was in progress, Professor David Lavie and his coworkers independently reported the isolation and structural elucidation of withaferin A from *Withania somnifera* L.[8]. Withaferin A was the prototype of a novel class of polyfunctional steroid lactones, the withanolides. Further chemical and biological studies are under way.

Elephantopus elatus Bertol.

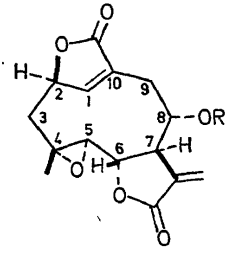
A systematic study of the cytotoxic principles of *Elephantopus elatus* Bertol. led to the isolation of two novel tumor-inhibitory germacranolide dilactones, elephantin and elephantopin[9]. Although the compounds were concentrated



Withaferin-A

Figure 7. SA-active principle of *Acnistus arborescens*

and isolated solely on the basis of *in vitro* cytotoxicity, elephantopin has subsequently been found to show significant inhibitory activity against the P-388 lymphocytic leukemia in the mouse and Walker carcinosarcoma 256 in the rat. (The KB cytotoxicity assay has been invaluable in many other of isolation studies as well. Significant results were frequently obtained from the bioassay of 2 to 10 mg samples of materials which could be evaluated in *in vivo* animal tumor systems only at the cost of 0·5 to 1 g samples.) A combination of degradative, spectral, and x-ray crystallographic studies resulted in elucidation of the structures of elephantin and elephantopin shown in *Figure 8* [9].



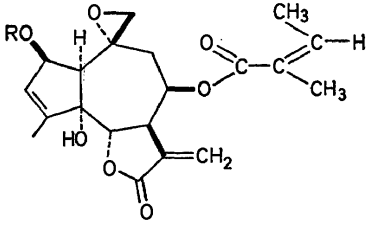
Elephantin : $R = (CH_3)_2C{=}CHCO-$

Elephantopin: $R = CH_2{=}C(CH_3)CO-$

Figure 8. Active principles of *Elephantopus elatus*

Eupatorium rotundifolium L.

Eupatorium rotundifolium L. initially yielded the new and novel guaianolide tumor inhibitors euparotin and euparotin acetate. Preliminary chemical and spectral characterization revealed that euparotin could be readily acetylated to euparotin acetate without structural rearrangement. Consequently, euparotin was acylated with bromoacetic anhydride to yield the nicely crystalline derivative, euparotin bromoacetate. X-ray crystallographic analysis of the bromoacetate by Professor G. A. Sim and Dr. A. T. McPhail established (some three and one-half weeks later) that the bromoacetate has the structure depicted in *Figure 9* (with R = $COCH_2$ Br), and it follows



Euparotin: R=H

Euparotin acetate: R=CH_3CO

Figure 9. Structures of cytotoxic principles from *Eupatorium rotundifolium* L.

therefore, that euparotin and euparotin acetate have the indicated structures[10]. Further studies of the extract from *Eupatorium rotundifolium* L. led to the isolation of six additional cytotoxic sesquiterpene lactones, and the structures of these compounds (depicted on the periphery of *Figure 10*) were

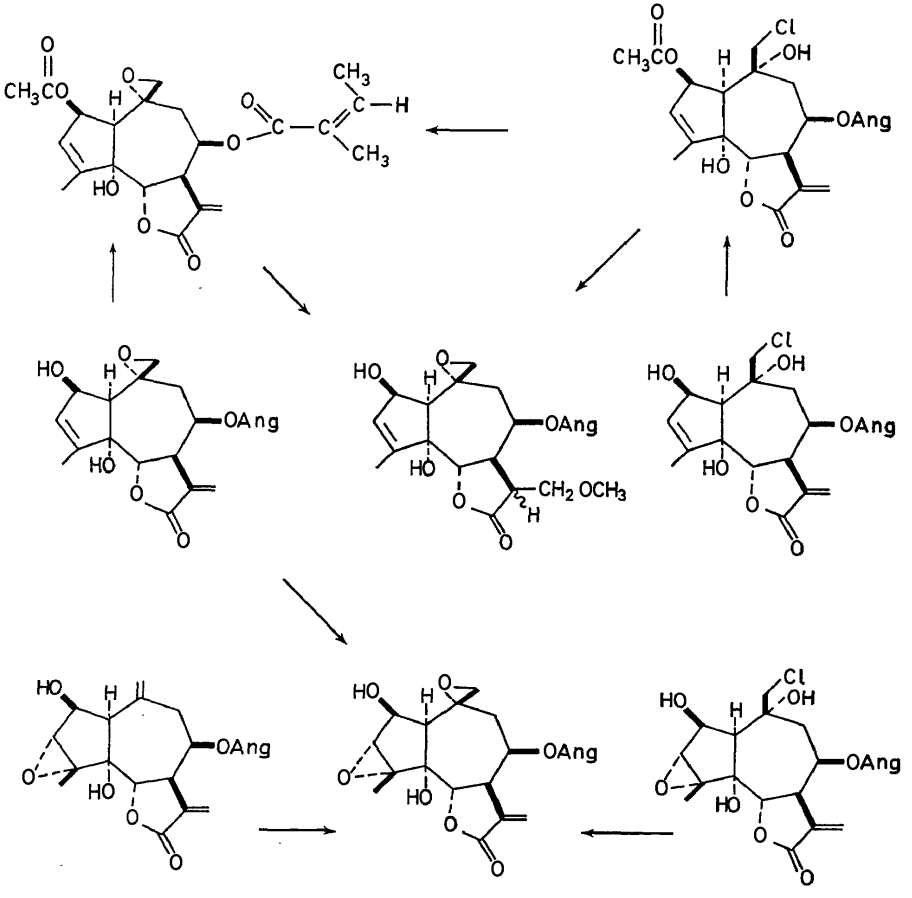


Figure 10. Cytotoxic principles from *Eupatorium rotundifolium* L.

determined by interrelation with the x-ray defined structure of euparotin acetate[11, 12]. For example, eupachlorin acetate, $C_{22}H_{27}ClO_8$, showed spectral characteristics indicative of the presence of an angelate ester, an α,β-unsaturated lactone with an exocyclic methylene group, an allylic acetate, and the absence of an exocyclic-epoxide-methylene grouping. Location of the chlorine atom on the C-14 methylene group was supported by the downfield shift of the signal assigned to the C-14 methylene protons (τ 6·47) relative to the signal for the C-14 methylene protons (τ 7·32) in the n.m.r. spectrum of euparotin acetate. Two D_2O-exchangeable hydroxyl proton signals were detected in contrast to one in euparotin acetate's spectrum. The tertiary nature of these hydroxyl functions was indicated by their resistance to acetylation upon treatment with acetic anhydride and pyridine. These data were consistent with the structural formula depicted in the upper right corner of *Figure 10*. Chromatograpny of eupachlorin acetate upon acid-washed alumina resulted in transformation to euparotin acetate in 65 per cent yield. This interrelation proved the functional group pattern, stereochemistry, and absolute configuration of eupachlorin acetate. Incidentally, eupachlorin acetate and its companions, eupachlorin and eupachloroxin, are the first reported chlorine-containing sesquiterpenes.

Vernonia hymenolepis A. Rich

Recent studies of *Vernonia hymenolepis* A. Rich led to isolation and structural elucidation of two novel elemanolide dilactones, vernolepin and vernomenin. Vernolepin showed significant *in vitro* cytotoxicity and *in vivo* tumor inhibitory activity against the Walker 256 carcinosarcoma in the rat. Elemental analysis and mass spectrometry indicated a $C_{15}H_{16}O_5$ molecular formula for vernolepin. Chemical and spectral evidence indicated the presence of two α,β-unsaturated lactone functions, a secondary alcohol, an additional double bond, and, therefore, a monocarbocyclic ring skeleton. The structure and stereochemistry for vernolepin (R = H) depicted in the upper left of *Figure 11* were established by x-ray crystallographic analysis of the *p*-bromobenzenesulphonate (R = $SO_2C_6H_4Br$). Vernomenin showed similar chemical properties to vernolepin, and its structure was proven by (a) its conversion to the same methanol adduct as that obtained from vernolepin (lower left of *Figure 11*), and (b) a comparison of the n.m.r. spectra of the respective acetate esters (R = $COCH_3$). In vernomenin acetate, the triplet centred at τ 4·78 could be assigned to the proton at acetate-bearing C-6, while the multiplet centred at τ 5·90 corresponded to the proton (spin coupled to three protons) at lactone-bearing C-8. In contrast, the spectrum of vernolepin acetate showed a multiplet at τ 4·95, assigned to the proton at acetate-bearing C-8, while the lactone proton signal appeared as a triplet centred at τ 5·96, indicative of attachment to C-6[13].

Vernonia amygdalina Del.

Vernonia amygdalina Del. has yielded a new cytotoxic sesquiterpene lactone, vernodalin. Vernodalin has been shown to be vernolepin mono-hydroxymethacrylate (*Figure 12*). Mass spectrometry indicated a $C_{19}H_{20}O_7$ molecular formula for vernodalin. Acidic hydrolysis in methanol gave the same methanol adduct as that obtained from vernolepin. An additional interre-

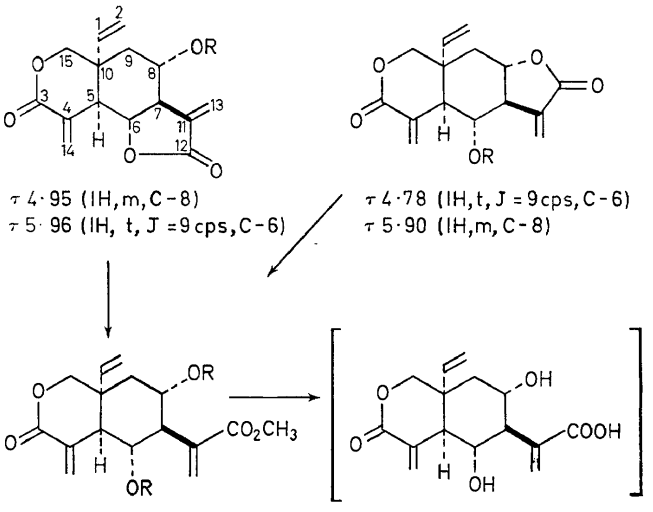


Figure 11. Structures of vernolepin and vernomenin

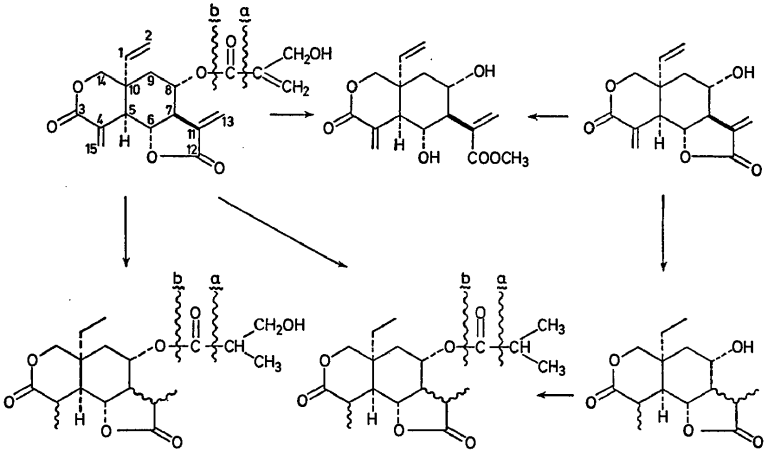


Figure 12. Vernodalin, cytotoxic principle of *Vernonia amygdalina*

lation with vernolepin was effected by conversion of vernodalin to hexahydrovernolepin isobuty rate[14].

BIOLOGICAL ACTIVITY OF TUMOR-INHIBITORY PRINCIPLES

In an attempt to elucidate a possible function of a series of new plant-derived tumor inhibitors, we enlisted the collaboration of Professor Luis Sequeira in an evaluation of the effects of these compounds on plant-growth. Several of the sesquiterpene dilactones, specifically elephantin, elephantopin, and vernolepin were found to be strong inhibitors of extension growth of wheat coleoptile sections[15]. Vernolepin inhibits extension growth in concentrations of 5 to 50 micrograms per ml (from 20 to 80 per cent, *Figure 13*).

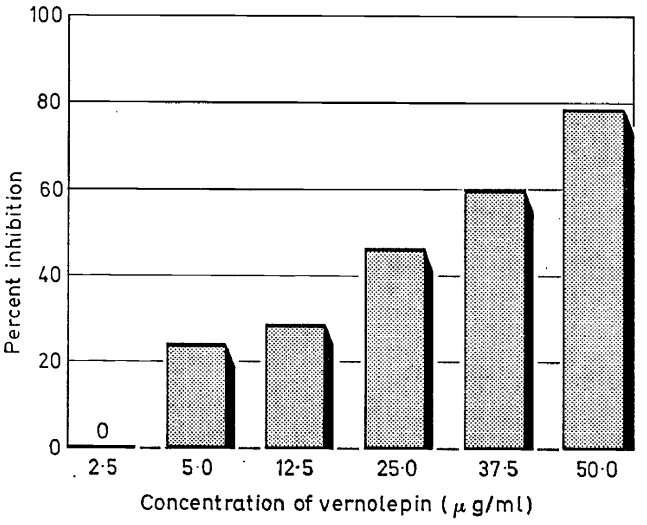


Figure 13. Wheat coleoptile bio-assays

If the inhibited sections are washed and subsequently treated with indole-3-acetic acid, the tissues respond to the auxin, but the degree of elongation is determined by the length of prior treatment with vernolepin (e.g., *Figure 14*). The fact that vernolepin's plant growth inhibitory activity is reversible suggests that the compound may have a natural function in the regulation of plant growth.

Several recent observations have focused attention on the importance of the conjugated α-methylene-lactone function for the biological activity of the sesquiterpene lactones. For instance, the plant-growth inhibitory effect of vernolepin is completely blocked by addition of sulphydryl compounds such as mercaptoethanol to the medium. Second, vernolepin, and other sesquiterpene lactones are potent inhibitors of the sulphydryl-bearing enzyme, phosphofructokinase[6]. Third, as shown in *Figure 15*, the cytotoxicity of vernolepin derivatives appears to be directly related to the presence of free conjugated α-methylene-lactone functions. Thus, selective reduction of the ethylidene double bond (*vide infra*) does not appear to affect the cytotoxicity. However, modification of the α-methylene-γ-lactone (by transesterification to the methanol adduct or by hydrogenation) results in a 10-fold diminution in cytotoxicity. Modification of both α-methylene-lactone systems, as in hexahydrovernolepin, leads to a derivative which is essentially inactive.

The synthesis of dihydrovernolepin exemplifies a new blocking sequence for the protection of the highly reactive conjugated α-methylene groups of lactones. Vernolepin was treated with an excess of *n*-propyl thiol at pH 9·2 to give a *bis*-thiol-adduct. Hydrogenation of the *bis*-thiol-adduct (with one mole-equivalent of hydrogen) followed by methyl iodide methylation and sodium bicarbonate-catalysed elimination, gave dihydrovernolepin[16].

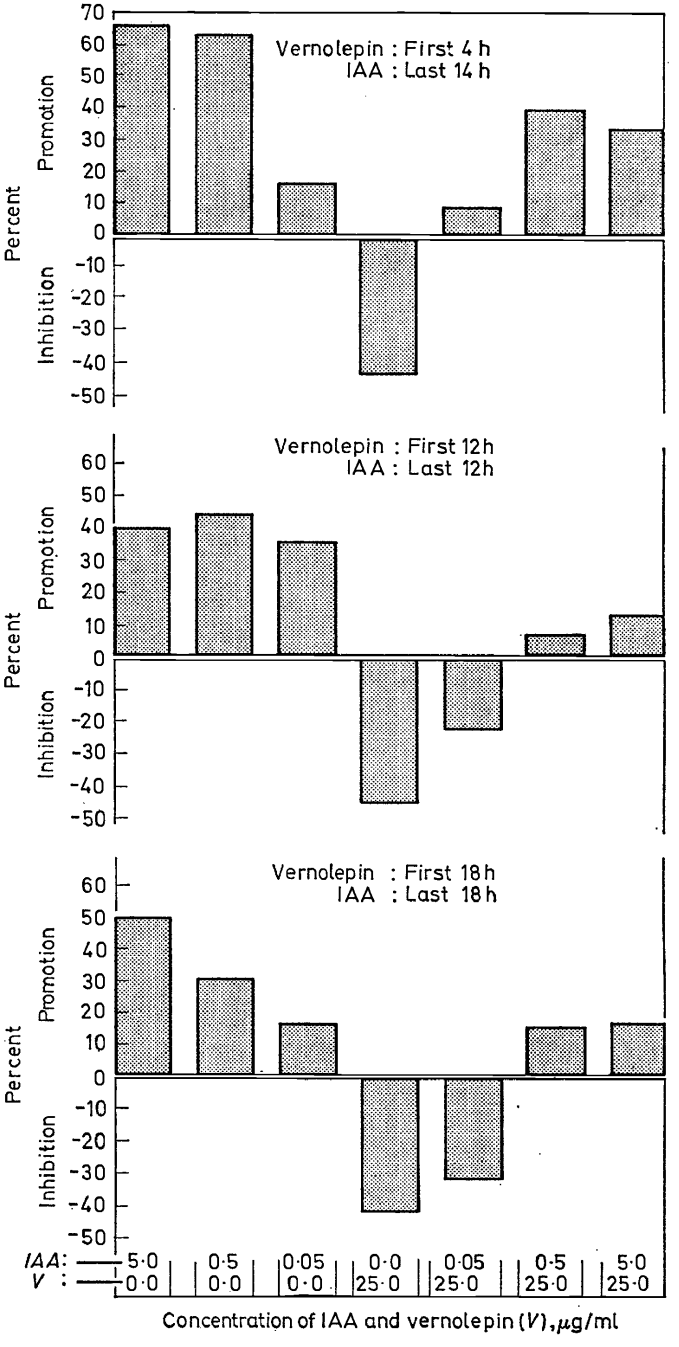
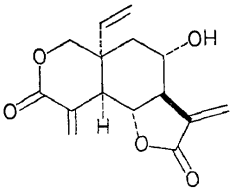
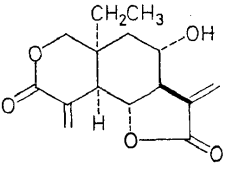


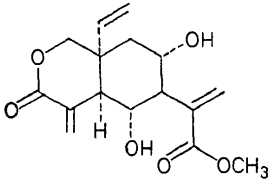
Figure 14. Degree of elongation after prior treatment with different concentrations of vernolepin



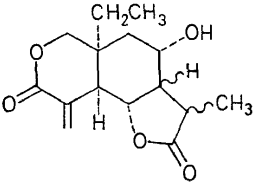
Vernolepin, 2·0



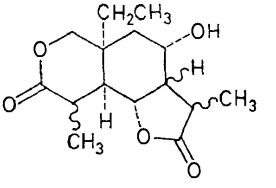
Dihydro-
vernolepin, 2·0



Vernolepin
methanol adduct, 26



Tetrahydro-
vernolepin, 19



Hexahydro-
vernolepin, >100

Figure 15. Cytotoxicity of vernolepin derivatives (ED_{50}, μg/ml)

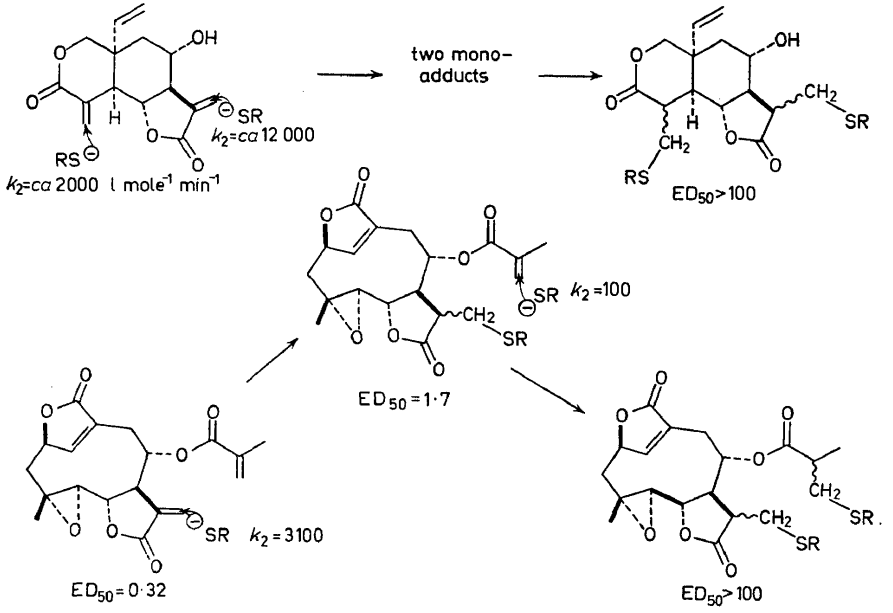


Figure 16. Rate of formation of L-cysteine Michael adducts at 25°C and pH 7·4

We have recently studied the reactions of several conjugated α-methylene-lactones with model biological nucleophiles. For instance, the rates of the reactions of vernolepin and elephantopin with cysteine at pH 7·4 were determined spectrophotometrically (*Figure 16*). The most reactive function in each case was the conjugated α-methylene-γ-lactone, and the second order rate constants for the 'Michael-type' addition of cysteine showed the same order of reactivity towards cysteine as iodoacetate, a commonly-used sulphydryl reagent. In contrast to the reactivity of the lactones towards sulphydryl groups, their reaction with amino groups appeared to be very slow. When a solution equimolar in lysine and vernolepin at pH 7·4 was allowed to stand for 6 days at 25°, 75 per cent of the original lactone could be recovered. Similarly, guanine proved unreactive toward either vernolepin or elephantopin. The *bis*-cysteine-adducts, in accord with expectation, were essentially inactive[17].

A search for the cytotoxic principle of *Asclepias curassavica* L. led to isolation and characterization of calotropin (*Figure 17*)[18].

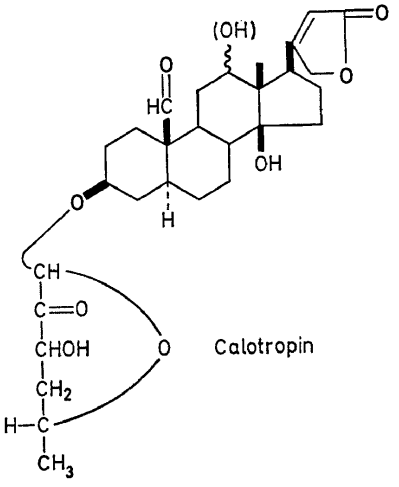


Figure 17. KB-active principle of *Asclepias curassavica* L.

In a parallel study, the cardenolide glycosides apocannoside and cymarin (*Figure 18*) were identified as the cytotoxic principles of *Apocynum cannabinum* L.[19]. At this point, Professor L. E. Hokin, of the University of Wisconsin Medical School, enlisted our collaboration in a search for a selective irreversible inhibitor of transport ATPase. Ouabain and strophanthin had long been known to effect powerful and highly specific reversible inhibition of the ATPase. We undertook to modify the strophanthin molecule in the search for an irreversible inhibitor. Our first studies of the chemistry *vs.* the biological activity of cardenolides as cytotoxic agents, cardiotonic agents, and ATPase inhibitors revealed that only those compounds modified solely in Ring A retained the major proportion of biological activity[20]. We found that the

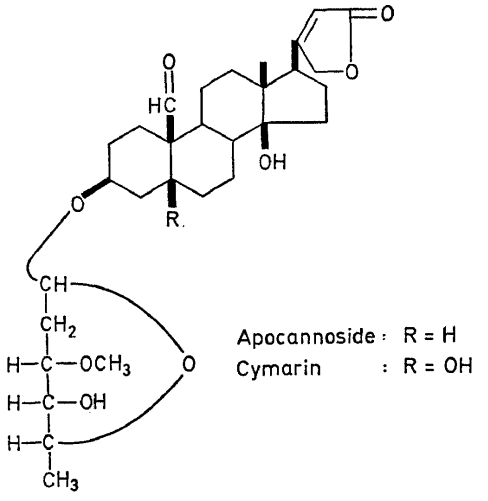


Figure 18. KB-active principle of *Apocynum cannabinum* L.

aglycone, strophanthidin, retained most of the ATPase-inhibitory activity, and that the activity was not lessened by acetylation to strophanthidin 3-acetate (SA, *Figure 19*). We next prepared strophanthidin 3-bromoacetate (SBA) and strophanthidin 3-iodoacetate (SIA), and these were found to be

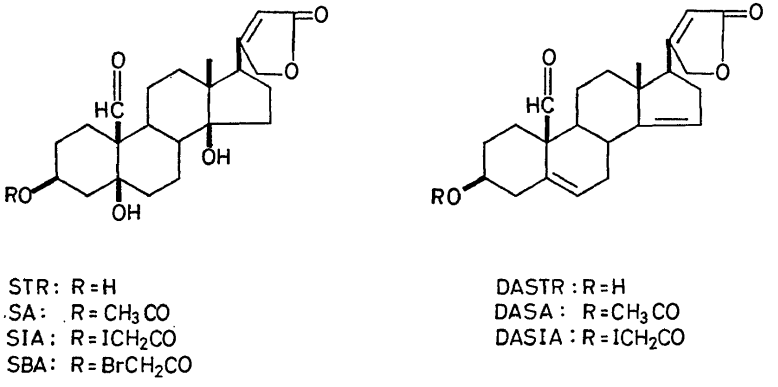


Figure 19. Strophanthidin and derivatives

powerful and *irreversible* inhibitors of the transport ATPase (see *Figure 20*, which indicates transport ATPase activity before and after washing)[21]. Strophanthidin 3-[1-^{14}C]-bromoacetate was synthesized for alkylation of the ATPase for purification of the labelled protein. Unfortunately, at concentrations required to alkylate a fair percentage of the enzyme (10^{-4} M), much more membrane protein was labelled than could be accounted for by specific labelling of the enzyme. A haloacetate derivative of a cardiotonic

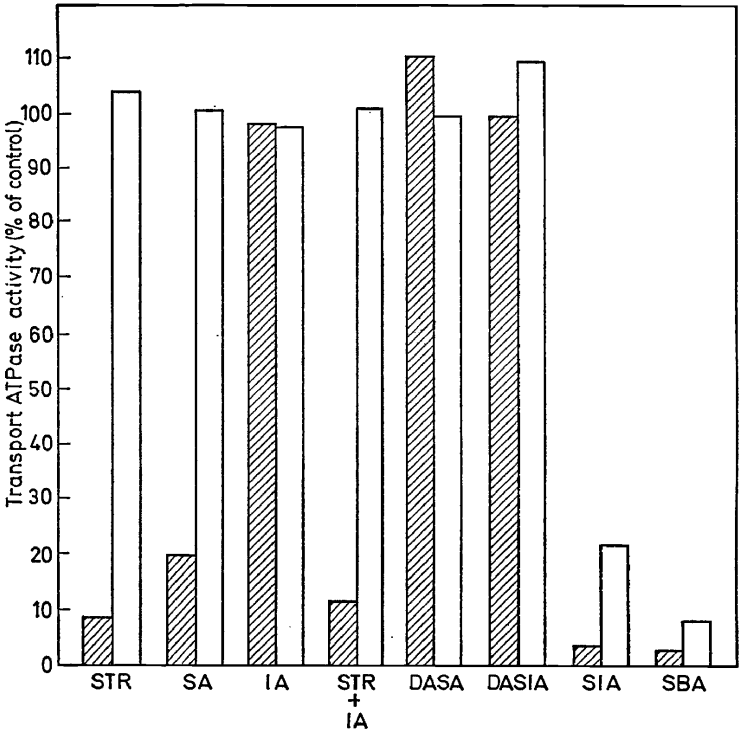
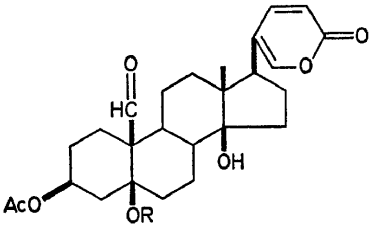


Figure 20. Irreversible inhibition of the transport ATPase by various agents (shaded parts show transport ATPase activity before washing out inhibitor)

steroid with much higher affinity for the cardiotonic steroid site of the enzyme appeared desirable.

In a parallel study, an investigation of *Bersama abyssinica* Fresen. from Ethiopia led to isolation and characterization of hellebrigenin 3-acetate and hellebrigenin 3,5-diacetate as the powerfully cytotoxic principles (*Figure 21*)[22].



Hellebrigenin acetate : R = H
Hellebrigenin diacetate: R = Ac

Figure 21. WA-active principles of *Bersama abyssinica*

When it was found that hellebrigenin has 30 times the affinity of strophanthidin for transport ATPase, a series of hellebrigenin 3-haloacetates was synthesized. It was found that hellebrigenin 3-iodoacetate is 100 times more potent as an irreversible inhibitor of the enzyme than strophanthidin 3-bromoacetate[23].

Further study of the cytotoxic principles of *Bersama abyssinica* Fresen. has yielded a series of new bufadienolides. We recently reported the isolation and structural elucidation of bersaldegenin 1,3,5-orthoacetate, which appears to be the first recognized naturally occurring orthoacetate (*Figure 22*)[24].

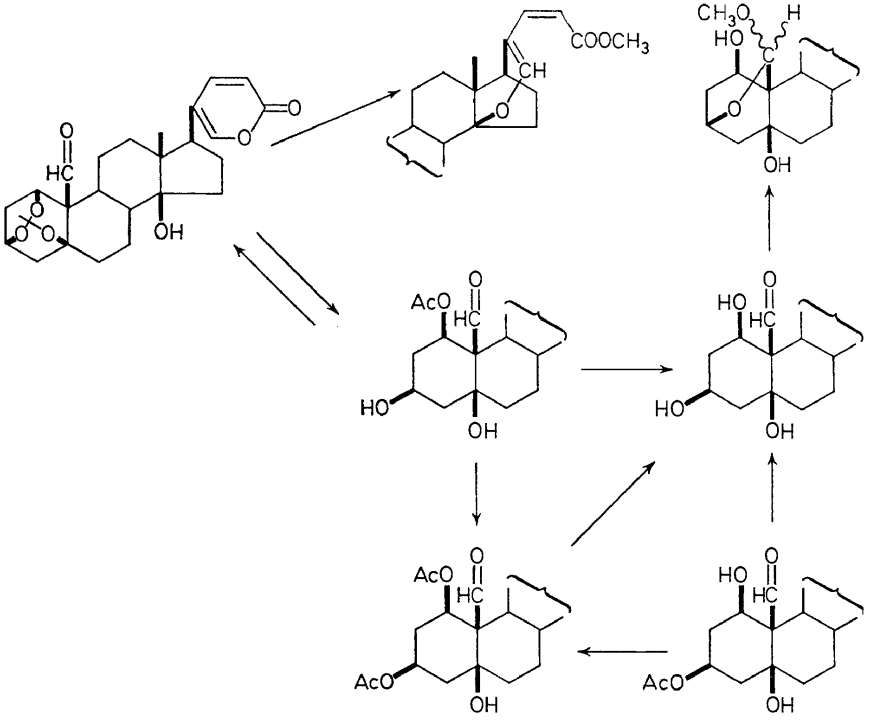


Figure 22. Bersaldegenin 1,3,5-orthoacetate

Treatment of bersaldegenin 1,3,5-orthoacetate with 80 per cent aqueous acetic acid for 3 hours at 90–100° yielded a 1:1 mixture of the starting material and bersaldegenin 1-acetate. When bersaldegenin 1-acetate was treated with 0·5 per cent hydrogen chloride in absolute methanol, a quantitative yield of the orthoacetate was obtained. A co-occurring bufadienolide, bersamagenin 1,3,5-orthoacetate, has been characterized as the C-10 methyl member of the bersaldegenin 1,3,5-orthoacetate series. Bersamagenin 1,3,5-orthoacetate is quite stable towards 80 per cent aqueous acetic acid. We believe that the equilibration of bersaldegenin 1,3,5-orthoacetate and bersaldegenin 1-acetate is facilitated by participation of the aldehyde group (presumably *via* the intramolecularly-solvated cation depicted in *Figure 23*),

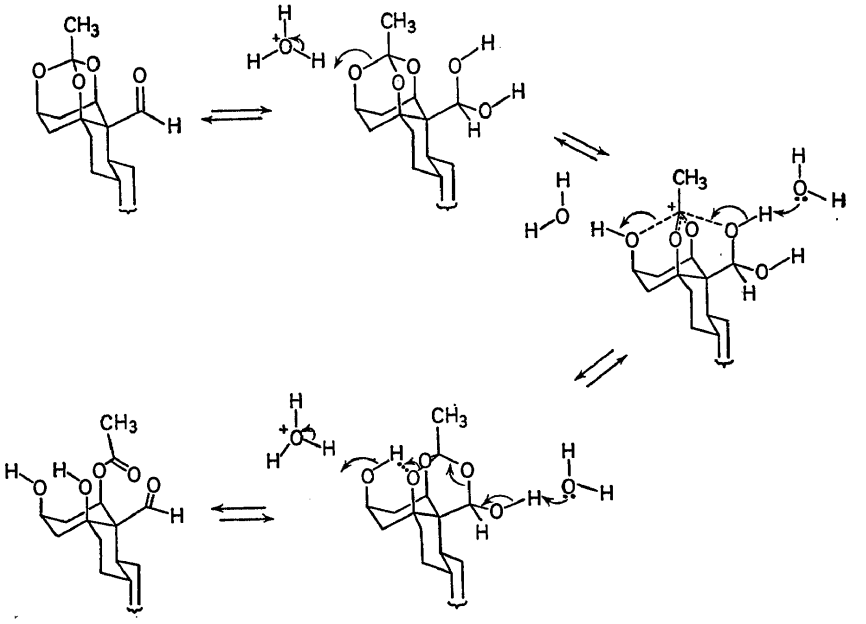


Figure 23. Equilibration of bersaldegenin 1,3,5-orthoacetate and bersaldegenin 1-acetate

which allows facile interconversion of the two isomeric orthoacetates. In the orthoacetate formation under anhydrous conditions, participation of the aldehyde again leads to facile interconversion of the isomeric orthoacetates (*Figure 24*). However, the irreversible loss of water under these conditions drives the orthoacetate formation to completion.

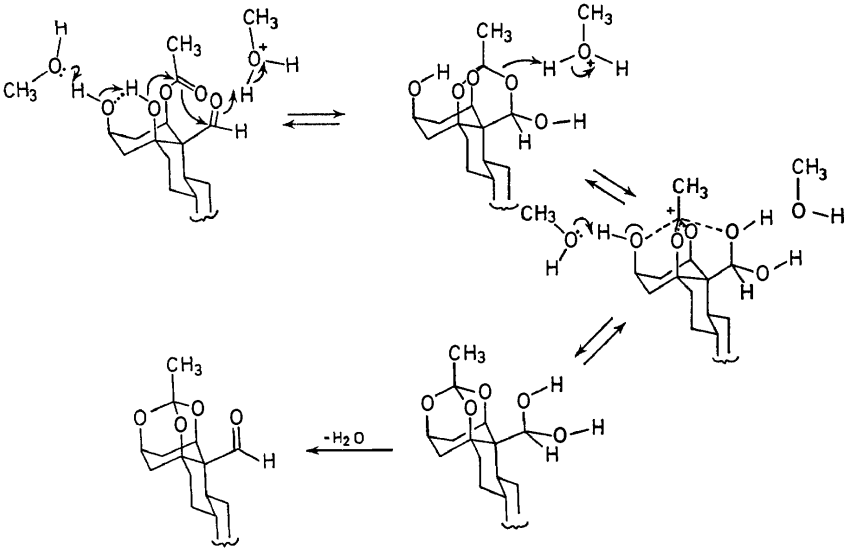


Figure 24. Orthoacetate formation in methanolic hydrogen chloride

CONCLUSION

The programme to date has demonstrated that several new types of compounds show significant growth-inhibitory activity against standard tumor systems in the National Cancer Institute's screen. Thus, for instance, steroid lactones, sesquiterpene lactones, and diterpenoid quinone methides represent chemical types not recognized previously as growth inhibitors.

We are optimistic about the future of our approach, from several points of view. First, some of the new natural products are showing sufficient promise in the advanced preclinical animal studies now in progress to become candidates for clinical trial in the near future. Secondly, we are encouraged by the fact that several of the new and remarkably cytotoxic compounds are showing usefulness as tools for studying biochemical phenomena. Finally, from a long-range point of view, we are hopeful that some of the unusual types of biologically-active compounds may serve significant roles as novel chemical templates for new synthetic approaches to cancer chemotherapy.

Acknowledgement

I would like to pay tribute to the skill and devotion of my collaborators and students whose names are given in the various references. I cannot adequately express my indebtedness to these colleagues. Our work has been generously supported by the National Cancer Institute and the American Cancer Society.

References

[1] S. M. Kupchan, S. J. Barboutis, J. R. Knox, and C. A. Lau Cam. *Science* **150,** 1827 (1965).
[2] S. M. Kupchan, A. H. Gray, and M. D. Grove. *J. Med. Chem.* **10,** 337 (1967).
[3] S. M. Kupchan, R. M. Smith, Y. Aynehchi, and M. Maruyama. *J. Org. Chem.* In press.
[4] S. M. Kupchan, A. Karim, and C. Marcks. *J. Amer. Chem. Soc.* **90,** 5923 (1968); *J. Org. Chem.* **34,** 3912 (1969).
[5] A. B. Turner. *Progr. Chem. Org. Nat. Prod.* **24,** 288 (1966).
[6] R. L. Hanson, H. A. Lardy, and S. M. Kupchan. *Science.* In press.
[7] S. M. Kupchan, R. W. Doskotch, P. Bollinger, A. T. McPhail, G. A. Sim, and J. A. Saenz Renauld. *J. Amer. Chem. Soc.* **87,** 5805 (1965);
A. T. McPhail and G. A. Sim. *J. Chem. Soc., (B),* 962 (1968);
S. M. Kupchan, W. K. Anderson, P. Bollinger, R. W. Doskotch. R. M. Smith, J. A. Saenz Renauld, H. K. Schnoes, A. L. Burlingame, and D. H. Smith. *J. Org. Chem.* **34,** 3858 (1969).
[8] D. Lavie, E. Glotter, and Y. Shvo. *J. Chem. Soc.* 7517 (1965); D. Lavie, S. Greenfield, and E. Glotter. *J. Chem. Soc., (C)* 1753 (1966).
[9] S. M. Kupchan, Y. Aynehchi, J. M. Cassady, A. T. McPhail, G. A. Sim, H. K. Schnoes, and A. L. Burlingame. *J. Amer. Chem. Soc.* **88,** 3674 (1966);
S. M. Kupchan, Y. Aynehchi, J. M. Cassady, H. K. Schnoes, and A. L. Burlingame. *J. Org. Chem.* **34** 3867 (1969).
[10] S. M. Kupchan, J. C. Hemingway, J. M. Cassady, J. R. Knox, A. T. McPhail, and G. A. Sim. *J. Amer. Chem. Soc.* **89,** 465 (1967).
[11] S. M. Kupchan, J. E. Kelsey, M. Maruyama, and J. M. Cassady. *Tetrahedron Letters* 3517 (1968).
[12] S. M. Kupchan, J. E. Kelsey, M. Maruyama, J. M. Cassady, J. C. Hemingway, and J. R. Knox. *J. Org. Chem.* **34,** 3876 (1969).
[13] S. M. Kupchan, R. J. Hemingway, D. Werner, A. Karim, A. T. McPhail and G. A. Sim. *J. Amer. Chem. Soc.* **90,** 3596 (1968);
S. M. Kupchan, R. J. Hemingway, D. Werner, and A. Karim. *J. Org. Chem.* **34,** 3903 (1969).
[14] S. M. Kupchan, R. J. Hemingway, A. Karim and D. Werner. *J. Org. Chem.* **34,** 3908 (1969).
[15] L. Sequeira, R. J. Hemingway, and S. M. Kupchan. *Science* **161,** 789 (1968).

[16] S. M. Kupchan, T. J. Giacobbe, and I. S. Krull. In preparation.
[17] S. M. Kupchan, D. C. Fessler, M. A. Eakin, and T. J. Giacobbe. *Science*. In press.
[18] S. M. Kupchan, J. R. Knox, J. E. Kelsey, and J. A. Saenz Renauld. *Science* **146,** 1685 (1964).
[19] S. M. Kupchan, R. J. Hemingway, and R. W. Doskotch. *J. Med. Chem.* **7,** 803 (1964).
[20] S. M. Kupchan, M. Mokotoff, R. S. Sandhu, and L. E. Hokin. *J. Med. Chem.* **10,** 1025 (1967).
[21] L. E. Hokin, M. Mokotoff, and S. M. Kupchan. *Proc. Nat. Acad. Sci.* **55,** 797 (1966).
[22] S. M. Kupchan, R. J. Hemingway, and J. C. Hemingway. *Tetrahedron Letters* 149 (1968); *J. Org. Chem.* **34,** 3894 (1969).
[23] A. E. Ruoho, L. E. Hokin, R. J. Hemingway, and S. M. Kupchan. *Science* **159,** 1354 (1968).
[24] S. M. Kupchan and I. Ognyanov. *Tetrahedron Letters* 1709 (1969).