Tumor Inhibitors. XLVI.[1a] Vernolepin, a Novel Sesquiterpene Dilactone Tumor Inhibitor from *Vernonia hymenolepis* A. Rich.[1b]

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An alcoholic extract of *Vernonia hymenolepis* A. Rich. was found to show significant inhibitory activity *in vitro* against cells derived from human carcinoma of the nasopharynx (KB) carried in tissue culture. The isolation and structural elucidation are reported of vernolepin (**1**) and vernomenin (**5**), two novel elemanolide dilactones from *V. hymenolepis*. Vernolepin showed significant *in vitro* cytotoxicity (KB) and *in vivo* tumor inhibitory activity against Walker intramuscular carcinosarcoma in rats. 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 of **1** were established by X-ray crystallographic examination of the *p*-bromobenzenesulfonate, **3**. Vernomenin (**5**) showed similar chemical properties to vernolepin, and its structure was proven by its conversion to **7**, one of the transformation products of vernolepin, and by comparison of the nmr spectra of **2** and **6**, the respective acetates of **1** and **5**. Certain aspects of the structure of the lactones in relation to the tumor-inhibitory properties are discussed.

In the course of a continuing search for tumor inhibitors from plant sources, an alcoholic extract of *Vernonia hymenolepis* A. Rich. (Compositae)[3] was found to show significant inhibitory activity *in vitro* against cells derived from human carcinoma of the nasopharynx (KB) carried in tissue culture.[4] Consequently, a systematic study aimed at the isolation of the KB-inhibitory principles was undertaken.

A preliminary communication[5] has outlined the isolation and structural elucidation of vernolepin and vernomenin from an alcoholic extract of *V. hymenolepis*. Further studies have led to an improvement in the yields of the two lactones and to a simplification of the technique of isolation. Consequently, we report herein the systematic fractionation of an active chloroform extract of *V. hymenolepis* and the isolation and structural elucidation of vernolepin (**1**), the major active principle, together with vernomenin (**5**), a related lactone.

The dried ground leaves were extracted twice continuously with chloroform for 16 hr to afford, after evaporation of the solvent, active extract A (see Chart I and Tables I and II). The marc was subsequently extracted continuously with methanol for 16 hr to afford the inactive extract B, which was not investigated further. Partition of the concentrated chloroform extract (A) between 10% aqueous methanol and petroleum ether resulted in the concentration of the activity in the aqueous methanol phase. The material (E) remaining after evaporation of the aqueous methanol was fractionated by silicic acid chromatography. The major active fractions all contained vernolepin; the richest of these fractions was crystallized to yield vernolepin, a novel elemanolide dilactone which showed significant inhibitory activity against the Walker intramuscular carcinosarcoma 256[6] and cytotoxicity against KB cell culture.[4] Examination of the less active fractions from the silicic acid chromatography showed that a major component was eluted before vernolepin. Rechromatography, on silica gel, of a fraction rich in this second component yielded a new dilactone, vernomenin, closely related to vernolepin. Vernomenin was obtained as a colorless brittle foam, which resisted attempts at crystallization.

The molecular formula of vernolepin, $C_{15}H_{16}O_5$, was assigned on the basis of elemental analysis and the molecular weight determination by mass spectrometry.[7] The ultraviolet high end absorption, infrared bands at 5.65 and 6.16 μ, and nmr signals at τ 3.77 and 3.97 (a pair of doublets, J = 3 Hz) (Table III) suggested the presence of an α,β-unsaturated γ-lactone. The presence in the infrared spectrum of a peak at 2.95 μ and in the nmr spectrum of one D_2O-exchangeable proton signal at τ 7.87 indicated that vernolepin possessed a single hydroxy group. The nature of the remaining two oxygen atoms was revealed by determination of the equivalent weight of vernolepin by lactone titration.[8] An equivalent weight of 143 indicated that vernolepin consumed 2 mol equiv of base, indicative of two lactone functions. Support was derived from the infrared spectrum of the neutralized residue obtained from the titration, which showed no strong absorption bands in the carbonyl region other than a broad band in the 6.4-μ region corresponding to carboxylate anion. These results indicated that vernolepin was a sesquiterpene dilactone with eight double-bond equivalents. The nature of the remaining functions was evident from the nmr spectrum, which showed five olefinic proton signals at τ 4.6 (3 H, m), 3.28 (1 H, d, J = 1 Hz), and 4.07 (1 H, d, J = 1 Hz). The pair of doublets indicated

(1) (a) Part XLV: S. M. Kupchan, R. J. Hemingway, and R. M. Smith, *J. Org. Chem.*, **34**, 3898 (1969). (b) This investigation was supported by grants from the National Cancer Institute (CA-04500) and the American Cancer Society (T-275), and a contract with Chemotherapy, National Cancer Institute, National Institutes of Health (PH 43-64-551).

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(3) Leaves gathered in Ethiopia in 1965. The authors acknowledge receipt of the dried plant material from Dr. Robert E. Perdue, Jr., U. S. Department of Agriculture (USDA), Beltsville, Md., in accordance with the program developed with the USDA by the Cancer Chemotherapy National Service Center (CCNSC).

(4) Assays were performed under the auspices of the CCNSC. The procedures were those described in *Cancer Chemotherapy Rept.*, **25**, 1 (1962).

(5) S. M. Kupchan, R. J. Hemingway, D. Werner, A. Karim, A. T. McPhail, and G. A. Sim, *J. Amer. Chem. Soc.*, **90**, 3596 (1968).

(6) The evaluation of *in vivo* assay results by the CCNSC on a statistical basis in sequential testing is such that a material is considered active if it causes reduction of tumor weight to 42% or less. For further details, compare protocols described in the reference cited in footnote 4.

(7) For the mass spectral data, we thank Dr. G. Van Lear and Dr. F. W. McLafferty of the Purdue Mass Spectrometry Center and Dr. H. K. Schnoes and Dr. A. L. Burlingame, University of California, Berkeley.

(8) M. Maruyama, A. Terahara, Y. Itagaki, and K. Nakanishi, *Tetrahedron Lett.*, 299 (1967).

Chart I

Fractionation of the Cytotoxic Extract of *V. hymenolepis*

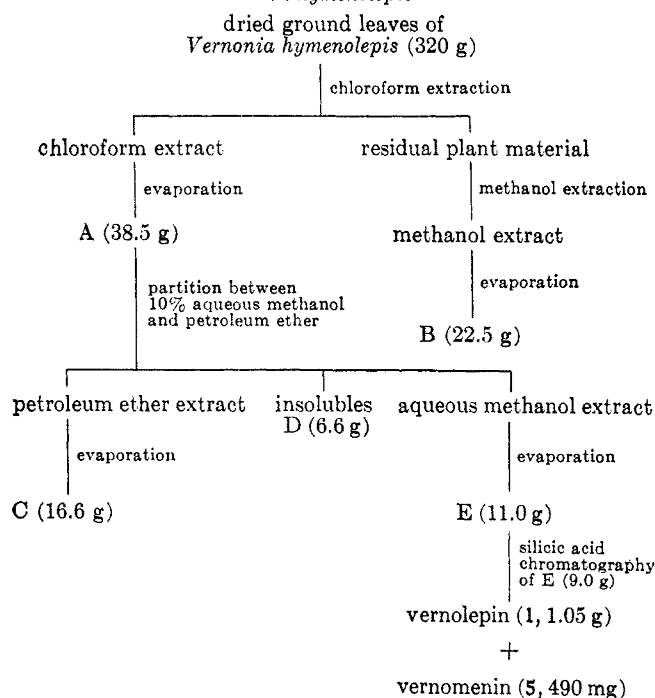


Table I

Assay of Activity of Fractions from *V. hymenolepis* against KB Cell Culture

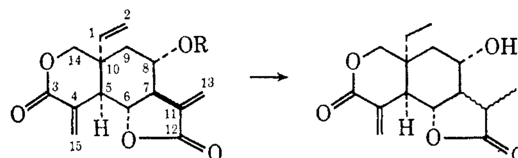
Fraction	ED_{50}, μg/ml
A	17
B	>100
C	36
D	17
E	1.7
1	2.0
5	20
7	26

Table II

Assay of Activity of Compounds from *V. hymenolepis* against Walker Intramuscular Carcinosarcoma 256[a]

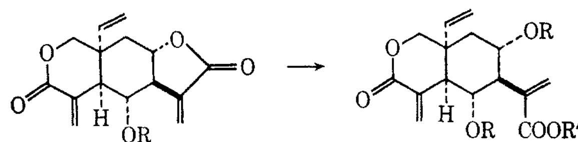
Compd	Dose, mg/kg	Survivors	Animal wt change difference, g T − C	Tumor wt, mg, T/C	T/C × 100
1	14	2/6	−14	700/5200	. . .
	12	5/6	−10	1700/5200	32
	10	6/6	−12	2400/5200	46
5	10	2/4	−9	2800/6500	. . .
	7	4/4	−5	4100/6500	63
	5	4/4	−7	4100/6500	63
7	100	4/4	+1	6300/6500	96
	50	4/4	+0	6100/6500	93
	25	3/4	+3	6400/6500	98

[a] T, treated animals; C, control animals.



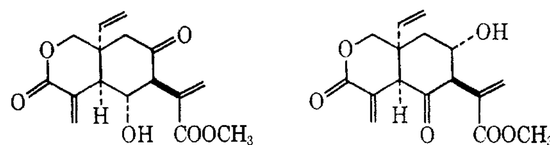
1, R = H
2, R = $COCH_3$
3, R = $SO_2C_6H_4Br$

4



5, R = H
6, R = $COCH_3$

7, R = H; R′ = CH_3
8, R = H; R′ = C_2H_5
9, R = $COCH_3$; R′ = CH_3



10

11

the presence of an exocyclic double bond conjugated to the second lactone function.

Vernolepin readily gave the monoacetate **2**, the nmr spectrum of which was similar to that of vernolepin. The significant difference, aside from the acetate methyl signal at τ 7.88, was that the region of τ 5.50–6.00 now contained signals for three protons (compared with four in vernolepin) and there was a new multiplet at τ 4.95 for a proton on an acetate-bearing carbon. This downfield shift of a multiplet indicated that the hydroxyl group of vernolepin is adjacent to complex proton systems, a point of particular significance later in assigning a structure to vernomenin (**5**). The spectrum of vernolepin acetate showed no D_2O-exchangeable signals, and hence vernolepin has only one hydroxyl.

Hydrogenation of vernolepin, using 10% palladium-on-charcoal catalyst, resulted in the rapid uptake of 2 mol equiv of hydrogen, and the reaction was terminated at this stage to yield tetrahydrovernolepin (**4**). The nmr spectrum of **4** showed signals in the vinyl region for only two protons, as doublets (J = 1 Hz) at τ 3.30 and 4.10. The ultraviolet absorption of **4** was also less intense than that of vernolepin, with only weak end absorption. These data indicated that **4** contained only the double bond exocyclic to the second lactone. The absence of the signals for five vinylic protons present in the nmr spectrum of vernolepin from the nmr spectrum of **4** was accompanied by the appearance of new high-field signals. The disappearance of the doublets corresponding to the protons of the methylene conjugated to γ-lactone was accompanied by the appearance of a three-proton doublet (J = 7 Hz) at τ 8.61 for the newly formed methyl group. The loss of the τ 4.6 complex three-proton multiplet, unusual in the sesquiterpene lactone series, was interpreted as indicative of the presence in vernolepin of a monosubstituted double bond. This was further supported by the presence in the spectrum of **4** of a distorted three-proton triplet (J = 8 Hz) at τ 9.08, indicative of an ethyl group. The presence of an ethyl group in **4**, and therefore of a monosubstituted ethylene in vernolepin, excluded the more common sesquiterpenoid ring systems and led to consideration of an elemane skeleton.

Difficulties were initially encountered in the isolation of vernolepin due to the presence of a companion compound of similar polarity. It was shown later that

TABLE III[a]

NUCLEAR MAGNETIC RESONANCE DATA FOR VERNOLEPIN AND VERNOMENIN AND THEIR DERIVATIVES

Compd	C-1 and C-2 (H_2)	C-5	C-6	C-7	C-8	C-9 (H_2)	C-13 (H_2)	C-14[b] (H_2)	C-15[b] (H_2)	OH or OR
1	4.6 m	7.03 m	5.75 m	7.34 tt (3, 10.5)	5.75 m	8.20 m	3.77 d (3) 3.97 d (3)	5.75 m	3.28 d (1) 4.07 d (1)	7.87 s (OH)
1[c]	4.5 m	6.87 m	5.75 t (10)	7.12 tt (3, 10)	5.75 m	7.80 dd[d] 8.11 dd	3.74 d (3, 2 H)	5.25 d (12) 5.74 dd (12, 1.5)	3.25 d (1) 4.13 d (1)	3.25 s (OH)
2	4.6 m	7.00 m	5.96 t (11)	7.10 tt (3, 10)	4.95 m	8.15 m	3.79 d (3) 4.38 d (3)	5.51 d (12) 5.80 dd (12, 1.5)	3.28 d (1) 4.06 d (1)	7.88 s (OAc)
3	4.6 m	7.01 m	5.99 t (11)	7.09 tt (3, 11)	4.95 m	8.00 m	3.80 d (3) 4.30 d (3)	5.55 d (12) 5.82 dd (12, 1)	3.28 d (1) 4.05 d (1)	2.20 s[e]
4[f]	(C-1) 8.30 m (C-2) 9.08 t (8) C_2H_5	7.35 m	6.02 t (11)		5.90 m	8.30 m	8.61 d (7, CH_3)	5.65 d (12) 5.91 d (12)	3.30 d (1) 4.10 d (1)	7.58 s (OH)
5[c]	4.5 m	7.16 m	5.72 t (10)	7.08 tt (3, 10)	5.66 m	8.00 m	3.70 d (3)	5.16 d (11) 5.61 dd (11, 2)	3.13 d (2) 3.70 (2)	4.10 br s (OH)
6	4.6 m	7.26 m	4.78 t (10)	7.19 tt (3, 10)	5.93 m	7.95 m	3.86 d (3) 4.58 d (3)	5.39 d (12) 5.70 dd (12, 2)	3.47 d (2) 4.40 d (2)	7.88 s (OAc)
7	4.6 m	7.58 m	6.05 t (10.5)	7.59 tt (10.5, 2)	6.00 m	8.30 m	3.46 d (2) 4.30 d (2)	5.44 d (11) 5.72 dd (11, 2)	3.61 s 4.23 s	6.24 s (OCH_3) 7.65 s (2 OH)
8	4.6 m	7.51 m	5.97 t (10.5)	7.55 t (10.5)	5.90 m	8.21 m	3.42 d (2) 4.37 d (2)	5.48 d (12) 5.68 dd (12, 2)	3.57 d (1) 4.20 d (1)	5.72 q[g] (7, 2 H) 8.65 t (7, 3 H) 7.62 s (2 OH)
9[h]	4.6 m	7.13 d (10.5)	4.75 t (10.5)	7.04 t (10.5)	4.79 m	8.25 m	3.74 d (2) 4.38 d (2)	5.16 d (12) 5.60 dd (12, 2)	3.82 s 4.05 s	6.31 s (OCH_3) 8.13 (2 OAc)
10	4.5 m	6.69 d (10.5)	5.77 t (10.5)	7.17 dd (10.5, 2)		7.43 d (16) 7.58 d (16)	3.45 d (2) 4.20 d (2)	5.53 d (12) 5.76 dd (12, 2)	3.53 d (1) 4.24 d (1)	6.23 s (OCH_3) 7.26 s (OH)
11	4.5 m	6.49 s		6.58 d (10)	5.65 m	7.80 m	3.25 d (2) 4.41 d (2)	5.70 m	3.54 d (1) 4.26 d (1)	6.23 (OCH_3) 7.72 (OH)

[a] Spectra were determined on a Varian A-60A spectrometer in deuteriochloroform solutions unless otherwise indicated. Values are given in τ units relative to tetramethylsilane as internal standard. Multiplicity of signals is designated as follows: s, singlet; d, doublet; dd, doublet of doublets; t, triplet; tt, triplet of triplets; m, multiplet. Numbers in parentheses denote coupling constants in hertz. [b] The designations of C-14 and C-15 have been changed from those used in ref 5, to conform with the current numbering convention for sesquiterpene lactones; *cf.* F. Šorm and L. Dolejš, "Guaianolides and Germacranolides," Editions Scientifiques Hermann, Paris, 1966. [c] Pyridine-d_5, 100 MHz. [d] ABX pattern; AB portion, J_{AB} = 14 Hz, J_{AX} = 4.5 Hz, and J_{BX} = 10 Hz (obtained from decoupled HA-100 spectrum). [e] p-$SO_2C_6H_4Br$. [f] Assignment of C-7 H and C-11 H cannot be accurately made; one proton resonates at *ca.* τ 7.5, the other at τ 8.1. [g] Signals for ethoxy group. [h] DMSO-d_6.

vernolepin readily undergoes reaction with methanol in the presence of a trace of acid to afford the methanol adduct **7**, $C_{16}H_{20}O_6$. During the original chromatography with methanol in the solvent system, vernolepin was partially converted to **7**. This conversion was avoided in subsequent isolations by the use of chloroform–acetone mixtures for chromatography. The ultraviolet spectrum of **7** was similar to that of vernolepin, and hence the α,β-unsaturated carbonyl chromophore had not been destroyed. The course of addition of methanol under acidic conditions differs from that under basic conditions, which normally affords C-13 methoxy adducts. That **7** was not the C-13 methoxy adduct was further supported by its infrared spectrum, which showed carbonyl peaks at 5.82 and 5.91 μ, and the absence of the normal γ-lactone peak. In addition, a stronger hydroxyl peak was present at 2.94 μ. The nmr spectra of vernolepin and its meth-

anol adduct were similar in most respects. However, the spectrum of 7 exhibited a three-proton singlet at τ 6.24 for the methoxyl group and two D_2O-exchangeable protons. The presence of a new hydroxyl group was confirmed by the acetylation of 7 to yield a diacetate, **9**. The infrared spectrum of 7 resembled that of vernolepin, and the only significant change was in the bathochromic shift of the carbonyl band. These observations and the mildness of conditions required to bring about the conversion of vernolepin to its methanol adduct supported the view that the change involved a transesterification, and the methanol adduct was assigned a methyl ester structure as in **7**. This type of conversion of the γ-lactone function of a sesquiterpene lactone to a methyl ester does not appear to be general, and the ease with which the reaction proceeds in this series may be due to the strain imposed on the lactone ring. The facile transesterification also occurred readily with ethanol and acid to yield the corresponding ethanol adduct **8**.

Oxidation of **7** with Jones reagent yielded two ketonic products, one crystalline and the other an oil. The crystalline product (**10**) was assigned the formula $C_{16}H_{18}O_6$ on the basis of elemental analysis. The vinylic region of the nmr spectrum of **10** was similar to that of the spectrum of **7**, the changes being in the remainder of the spectrum. In addition to the methoxyl peak, signals were present for only three protons on carbon bearing oxygen substituents and only one D_2O-exchangeable proton. It was apparent, therefore, that one of the hydroxyl groups had been oxidized to a ketone. The signal for the proton on the carbon which carried the lactonic hydroxyl appeared as a triplet at about τ 6 in **1**, and this peak was also present in both the ring-opened lactone **7** and the oxidation product **10**. Thus, the hydroxyl group which was free in **1** must have been oxidized, and the absence of a multiplet at τ 5.7 (corresponding to the proton on the same carbon) supported this view. This proton must be part of the ABX system observed at about τ 8.2, which now is present as a simple AB system at τ 7.43 and 7.58 (J = 16 Hz). The second oily product, **11**, was clearly the ketone formed by the oxidation of the lactonic hydroxyl, as its spectrum lacked the triplet but still contained the ABX system and a multiplet at τ 5.65.

The interrelationship of the functional groups and the structure and configuration of vernolepin were established by the X-ray crystallographic examination of the *p*-bromobenzenesulfonate ester **3**, which showed that vernolepin has structure **1**.[5,9]

Vernomenin (**5**) was assigned the molecular formula $C_{15}H_{16}O_5$ on the basis of elemental analysis and mass spectrometry. Ths similarity between the spectra of vernolepin and vernomenin suggested that the two compounds were closely related. Treatment of vernomenin with acidic methanol afforded **7**, the methanol adduct previously obtained from vernolepin. This interrelationship indicated that **1** and vernomenin differed only in the attachment of the γ-lactone. That the lactone of vernomenin was closed to C-8 was revealed by comparison of the nmr spectrum of vernomenin acetate (**6**) with that of vernolepin acetate (**2**).

(9) The full X-ray crystallographic data will be published elsewhere by Professor G. A. Sim and coworkers.

The spectrum of vernolepin acetate showed a multiplet at τ 4.95 assigned, by comparison with the spectrum of **1**, to the proton at acetate-bearing C-8. The C-6 lactone proton appeared as a triplet at τ 5.96 (J = 11 Hz). In the spectrum of vernomenin acetate, the proton on the acetate-bearing carbon appeared as a triplet (J = 10 Hz) at τ 4.78. In contrast, the lactone proton appeared as a multiplet at τ 5.93 corresponding to the C-8 proton of vernolepin. It follows, therefore, that vernomenin has a lactone closed to C-8 and a C-6 hydroxyl, as in **5**.

It is interesting to compare the biological activities of vernolepin, vernomenin, and the methanol adduct **7** (see Tables I and II). Only vernolepin showed significant cytotoxicity and *in vivo* tumor-inhibitory activity. Similarly, when these compounds (and others) were evaluated for possible effects on the growth of wheat coleoptile sections, vernolepin was found to be the most potent plant-growth inhibitor.[10]

Vernolepin and vernomenin appear to be the first recognized elemanolide dilactones, and, in fact, may be the first reported *naturally occurring* lactones which possess the elemane skeleton. A few elemanolides, such as saussurea lactone[11] and the lactones from *Lindera* spp.[12,13] have been reported. It has been shown that saussurea lactone can be prepared by heating dihydrocostunolide,[11] and isolinderalactone has been shown to arise from linderalactone.[12] A distillate of an extract of *Lindera strychnifolia* has also yielded several germacrane and elemane lactones.[13] In view of the demonstrated transformation of germacranolide precursors to elemanolides by heating, the question has been raised as to whether some of the elemanolides may be artifacts. This was of additional interest, since a sample of *Vernonia colorata* has recently been reported to yield the germacrane monolactone, vernolide.[14] During our early studies, vernolepin was noted to have the unusual property of significant solubility both in water and in many organic solvents. Consequently, to evaluate whether vernolepin was an artifact formed during the hot extraction process, a sample of plant material was extracted by cold aqueous maceration. The resulting aqueous solution was extracted with ethyl acetate and the organic phase was evaporated to dryness at room temperature under reduced pressure. The residue, after chromatography on silicic acid and silica gel, afforded a sample of vernolepin in comparable yield with that obtained from the hot chloroform extract. These observations support the view that vernolepin is indeed a naturally occurring compound.

Several sesquiterpene lactones have previously been reported to exhibit significant tumor-inhibitory properties.[15-17] It has been noted earlier that all of the

(10) L. Sequeira, R. J. Hemingway, and S. M. Kupchan, *Science*, **161**, 789 (1968).

(11) A. S. Rao, A. Paul, Sadgopal, and S. C. Bhattacharyya, *Tetrahedron*, **13**, 319 (1961).

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(13) K. Takeda, I. Horibe, and H. Minato, *ibid.*, 569 (1968).

(14) R. Toubiana and A. Gaudemer, *Tetrahedron Lett.*, 1333 (1967).

(15) 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); *J. Org. Chem.* in press.

(16) 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); *J. Org. Chem.*, in press.

(17) S. M. Kupchan, J. E. Kelsey, M. Maruyama, and J. M. Cassady, *Tetrahedron Lett.*, 3517 (1968).

unsaturated lactones which show significant *in vivo* tumor-inhibitory activity possess at least two "alkylating" functions, *i.e.*, structural moieties readily sensitive to attack by nucleophiles.[16] Vernolepin, with two α,β-unsaturated lactone functions, clearly extends the generalization noted earlier. Investigations are in progress which are aimed at evaluation of the significance of the α,β-unsaturated lactone, and of other structural features, in relation to tumor-inhibitory activity.

Experimental Section

Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are corrected. Infrared spectra were determined on Beckman Model IR-5A and IR-9 recording spectrophotometers. Ultraviolet spectra were determined on a Beckman Model DK-2A recording spectrophotometer. Nuclear magnetic resonance spectra were determined on a Varian A-60A spectrometer using tetramethylsilane as internal standard. Specific rotations were determined on a Zeiss-Winkel polarimeter and are approximated to the nearest degree. Petroleum ether refers to the fraction with bp 60–68°. Evaporations were carried out at temperatures less than 40° under reduced pressure. Thin layer chromatography was carried out on silica gel (E. Merck) plates and chromatograms were visualized by spraying with a 3% $Ce(SO_4)_2$-3 *N* H_2SO_4 solution followed by heating. Microanalyses were performed by Spang Microanalytical Laboratory, Ann Arbor, Mich.

Isolation of Vernolepin (1) and Vernomenin (5).—The dried ground leaves of *Vernonia hymenolepis* (320 g) were extracted twice continuously for 16 hr with chloroform and the combined extracts were concentrated under reduced pressure to a thick oil (A, 38.5 g). The marc after chloroform extraction was extracted continuously with methanol for 16 hr and the extract was evaporated under reduced pressure to yield a thick oil (B, 22.5 g). Fraction A was partitioned between petroleum ether (300 ml) and 10% aqueous methanol (250 ml). The extracts were evaporated to yield petroleum ether solubles (C, 16.6 g) and 10% aqueous methanol solubles (E, 11.0 g). The material insoluble in either phase was dried separately to yield a sticky solid (D, 6.6 g). A portion of the aqueous methanol solubles (9.0 g) in a mixture of equal parts of acetone and chloroform (25 ml) was treated with silicAR CC-7 (20 g) and the suspension was evaporated to dryness. The residue was transferred to a column of silicAR CC-7 (630 g) packed in chloroform and the column was eluted with 15% acetone in chloroform. Fractions (90 ml each) were collected and analyzed by tlc on silica gel using a mixture of methanol–acetone–chloroform (5:15:80). Flasks 1–7, on tlc, showed several similar high R_f materials and were combined and evaporated to afford fraction F (453 mg). Flasks 8–11 contained little residue and were combined to afford fraction G (88 mg). Flasks 12–18, on tlc, showed mainly two-spot material and were combined and evaporated to yield fraction H(211 mg). Flasks 19–31 and 32–46 showed no significant spots on tlc and were combined and evaporated to afford fractions I (309 mg) and J (133 mg), respectively. Flasks 47–54 contained a mixture of spots including some vernomenin and were combined and evaporated to yield fraction K (1.07 g). Flasks 55–62, which contained significant quantities of vernomenin, were combined and evaporated to afford fraction L (1.03 g). Flasks 63–65, which contained a mixture of vernomenin and vernolepin, were combined and evaporated to afford fraction M (374 mg). Flasks 66–82, which contained significant quantities of vernolepin, were combined and evaporated to afford fraction N (1.46 g). When the fractions contained little additional vernolepin, the column was washed with 25% acetone in chloroform (2 l.) and the eluate was evaporated to afford fraction O (291 mg). The residual material was removed from the column by washing with acetone. Evaporation of the acetone afforded fraction P (3.08 g).

A larger batch of fraction N (5.0 g) was crystallized from chloroform–petroleum ether to give colorless prisms (3.5 g) of vernolepin (1): mp 179–180°; $[\alpha]^{28}D$ +72° (*c* 1.04, acetone); uv λ^{MeOH} end absorption 207 mμ (ϵ 20,300); ir $\lambda_{max}^{CHCl_3}$ 2.95, 3.44, 5.65, 5.79, 6.16, 7.12, 8.60, 8.90, 9.52, and 10.20 μ; mass spectrum *m/e* 276 (M^+).

Anal. Calcd for $C_{15}H_{16}O_5$: C, 65.21; H, 5.84. Found: C, 64.31; H, 5.92.

A larger batch of fraction L (6.5 g) was rechromatographed on a column of silica gel (200 g) packed in chloroform. Elution with 15% acetone in chloroform gave the major component, vernomenin (5), as a white brittle foam (1.2 g): $[\alpha]^{27}D$ −62° (*c* 1.44, acetone); uv λ^{MeOH} end absorption 210 mμ (ϵ 20,000); ir $\lambda_{max}^{CHCl_3}$ 2.78, 2.91, 3.28, 3.41, 5.63, 5.79, 5.97 and 6.15 μ; mass spectrum *m/e* 276 (M^+).

Anal. Calcd for $C_{15}H_{16}O_5$: mol wt, 276.09978. Found: mol wt, 276.10056 (mass spectrum).

The column also afforded a sample (4.2 g) of less pure vernomenin.

Vernolepin Acetate (2).—A solution of vernolepin (70 mg) in pyridine (0.3 ml) was treated with acetic anhydride (70 mg) and allowed to stand at room temperature for 6 hr. The solvent was evaporated and the residue was dissolved in chloroform and chromatographed on silicic acid (7 g). Using chloroform as solvent, a fraction (71 mg) was eluted and crystallized from methanol–ether to give colorless prisms of **2**: mp 146–147°; $[\alpha]^{25}D$ +134° (*c* 0.89, $CHCl_3$); uv λ^{MeOH} 208 mμ (ϵ 18,300); ir λ_{max}^{Nujol} 5.62, 5.72, 5.78, 6.16, 6.82, 7.25, 8.05, and 8.65 μ.

Anal. Calcd for $C_{17}H_{18}O_6$: C, 64.14; H, 5.70. Found: C, 63.91; H, 5.83.

Vernolepin Brosylate (3).—A solution of vernolepin (100 mg) and *p*-bromobenzenesulfonyl chloride (100 mg) in pyridine (0.5 ml) was allowed to stand for 8 hr at room temperature. The solvent was evaporated and the residue was chromatographed on silicic acid (7 g) with chloroform, and a fraction (50 mg) was eluted and crystallized from methanol as colorless prisms of **3**: mp 178–179° dec; $[\alpha]^{25}D$ −16° (*c* 0.59, acetone); uv λ_{max}^{MeOH} end absorption 208 (ϵ 19,800), 235 (ϵ 13,800), and 265 mμ (ϵ 600); ir $\lambda_{max}^{CHCl_3}$ 3.42, 5.63, 5.79, 6.16, 6.35, 7.18, 7.30, 7.77, 7.96, 8.50, 8.92, 9.12, 9.32, and 9.85 μ.

Anal. Calcd for $C_{21}H_{19}BrO_7S$: C, 50.71; H, 3.87; Br, 16.13. Found: C, 50.62; H, 3.72; Br, 16.28.

Tetrahydrovernolepin (4).—A solution of vernolepin (100 mg) in ethyl acetate (15 ml) was hydrogenated with 10% Pd–C (60 mg) as catalyst. After 6 min, 2 mol equiv of hydrogen had been absorbed. The catalyst was removed by filtration and the solvent was evaporated to give an oil which crystallized after standing for 10 days. The crystalline material was recrystallized from acetone–ether to give colorless crystals (**4**, 65 mg): mp 145–146°; $[\alpha]^{28}D$ +55° (*c* 1.00, acetone); uv λ^{MeOH} end absorption 214 mμ (ϵ 5200); ir λ_{max}^{film} 2.90, 5.68, 5.75, 6.10, 7.80, 8.56, 8.72, 9.50, and 10.40 μ.

Anal. Calcd for $C_{15}H_{20}O_5$: C, 64.27; H, 7.19. Found: C, 64.47; H, 7.42.

Vernolepin–Methanol Adduct (7). A. From Vernolepin.—A solution of vernolepin (60 mg) in methanol (5 ml) containing hydrochloric acid (1.5%) was refluxed for 5 hr. The solvent was evaporated to give an oil which was crystallized from methanol–ether to give colorless needles (47 mg) of **7**: mp 174–175°; $[\alpha]^{28}D$ +47° (*c* 1.09, acetone); uv λ^{MeOH} end absorption 208 mμ (ϵ 20,400); ir λ_{max}^{Nujol} 2.94, 5.82, 5.91, 6.15, 7.68, 8.42, 8.64, 9.10, 9.60, 9.70, 9.92, 10.10, 10.32, 10.50, and 10.75 μ.

Anal. Calcd for $C_{16}H_{20}O_6$: C, 62.32; H, 6.54. Found: C, 62.38; H, 6.59.

B. From Vernomenin.—A solution of vernomenin (50 mg) in methanol (20 ml) containing hydrochloric acid (1.5%) was refluxed for 1 hr. The solvent was evaporated to afford a crystalline residue which was recrystallized from acetone–hexane to give colorless plates (32 mg) of **7**, mp 174–175°. This material was identical by mixture melting point, tlc, and ir and nmr spectroscopy with the sample from vernolepin.

Vernolepin–Ethanol Adduct (8).—A solution of vernolepin (100 mg) in ethanol (5 ml) containing concentrated hydrochloric acid (0.5%) was refluxed for 9 hr. The solvent was evaporated and the oily residue was crystallized from acetone–petroleum ether to give colorless prisms (**8**, 62 mg): mp 140–141°; $[\alpha]^{28}D$ +90° (*c* 0.50, acetone); uv λ^{MeOH} end absorption 208 mμ (ϵ 15,100); ir λ_{max}^{Nujol} 2.96, 5.84, 5.91, 6.15, 7.70, 7.90, and 8.52 μ.

Anal. Calcd for $C_{17}H_{22}O_6$: C, 63.34; H, 6.88. Found: C, 63.37; H, 7.02.

Vernolepin–Methanol Adduct Diacetate (9).—A solution of vernolepin–methanol adduct (**7**, 55 mg) in pyridine (0.5 ml) was treated with acetic anhydride (0.3 ml) and warmed to 60° for 15 min. The solvent was evaporated and the residue was chromatographed on silicic acid (5 g) with chloroform as solvent. The major fraction (40 mg) was crystallized from acetone–

petroleum ether as colorless crystals of **9**: mp 148–149°; $[\alpha]^{27}D$ +8° (c 1.17, acetone); uv λ^{MeOH} end absorption 208 mμ (ε 17,300); ir λ_{max}^{Nujol} 5.75, 5.82, 6.14, 8.10, 8.58, 9.72, and 10.28 μ.

Anal. Calcd for $C_{20}H_{24}O_8$: C, 61.21; H, 6.17. Found: C, 61.26; H, 6.20.

8-Dehydrovernolepin–Methanol Adduct (10) and 6-Dehydrovernolepin–Methanol Adduct (11).—A solution of vernolepin–methanol adduct (**7**, 305 mg) in acetone (10 ml) was treated with Jones reagent (0.5 ml) at 0°. After 15 min, water (30 ml) was added and the solution was extracted with chloroform (70 ml). The chloroform extract was dried (Na_2SO_4) and evaporated. The residue (315 mg) was chromatographed on silicic acid (20 g), and two discrete fractions were eluted with 0.5% methanol in chloroform. The less polar material was a colorless oil (150 mg), the spectra of which were in accord with the C-8 ketone structure, **10**.

The more polar material was crystallized from acetone–petroleum ether to give colorless plates (26 mg) of **11**: mp 190–193° dec; uv λ^{MeOH} end absorption 211 mμ (ε 11,800); ir λ_{max}^{KBr} 2.84, 3.39, 5.82, 5.89, 6.12, 7.64, 8.45, 9.67, 10.40, and 10.73 μ.

Anal. Calcd for $C_{16}H_{18}O_6$: C, 62.74; H, 5.92. Found: C, 62.78; H, 5.94.

Lactone Titration of Vernolepin (1).—Vernolepin (26.0 mg) was dissolved in 0.0947 *N* aqueous sodium hydroxide (5 ml), the solution was evaporated to dryness at 40°, and the residue was dissolved in water (1 ml). The solution was heated on a steam bath for 10 min, diluted with water (4 ml), and titrated with 0.121 *N* sulfuric acid using phenolphthalein as indicator; 2.36 ml of acid was required to neutralize the excess of base. On this basis, **1** was found to have equiv wt 138. When the titration was repeated using 29.2 mg of **1** and sodium hydroxide solution (4 ml), the volume of acid required was 1.51 ml, corresponding to equiv wt 149 for **1**.

Vernomenin Acetate (6).—A solution of vernomenin (100 mg) in pyridine (10 ml) and acetic anhydride (5 ml) was left overnight at room temperature, cooled to 0–2°, and diluted with water (50 ml). The mixture was extracted with chloroform and the chloroform extract was washed with diluted hydrochloric acid followed by saturated sodium bicarbonate solution. Evaporation of the dried chloroform extract (Na_2SO_4) gave a crystalline residue (94 mg), which was recrystallized from chloroform–ether to afford colorless plates of **6**: softens at 210–212° but does not melt completely below 300°; $[\alpha]^{26}D$ −135° (c 2.00, $CHCl_3$); uv λ^{MeOH} end absorption 210 mμ (ε 17,500); ir $\lambda_{max}^{CHCl_3}$ 3.28, 3.38, 5.63, 5.71, 5.77, 5.96, 6.14, 7.94, 8.06, and 8.58 μ.

Anal. Calcd for $C_{17}H_{18}O_6$: C, 64.14; H, 5.70. Found: C, 63.91; H, 5.59.

Registry No.—**1**, 21887-20-7; **2**, 20107-21-5; **3**, 20107-25-9; **4**, 20107-22-6; **5**, 20107-26-0; **6**, 20071-38-9; **7**, 20107-23-7; **8**, 21887-26-3; **9**, 20107-24-8; **10**, 21887-28-5; **11**, 21887-29-6.

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Tumor Inhibitors. XLVII.[1a] Vernodalin and Vernomygdin, Two New Cytotoxic Sesquiterpene Lactones from *Vernonia amygdalina* Del.[1b]

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A chloroform extract of *Vernonia amygdalina* Del. was found to show significant inhibitory activity *in vitro* against cells derived from human carcinoma of the nasopharynx (KB) carried in tissue culture. The isolation and structural elucidation are reported of vernodalin (**1**) and vernomygdin (**8**), two new cytotoxic sesquiterpene lactones. Mass spectrometry indicated a $C_{19}H_{20}O_7$ molecular formula for vernodalin. Acidic hydrolysis in methanol gave methanol adduct **2**, identical with the methanol adduct of vernolepin. An additional interrelation with vernolepin (**5**) was effected by conversion of vernodalin to hexahydrovernolepin isobutyrate (**4**). Elemental analysis and mass spectrometry indicated a $C_{19}H_{25}O_7$ molecular formula for vernomygdin (**8**). Its structure and relationship to vernolide (**7**) was established by catalytic hydrogenation to tetrahydrovernolide (**9**).

In the course of a continuing search for tumor inhibitors from plant sources, a chloroform extract of *Vernonia amygdalina* Del. (Compositae)[3] was found to show significant inhibitory activity *in vitro* against cells derived from human carcinoma of the nasopharynx (KB) carried in tissue culture.[4] Consequently, a systematic study aimed at isolation of the KB inhibitory principles was undertaken. The dried ground leaves were extracted continuously with chloroform to afford active fraction A (see Chart I and Table I). The marc was extracted continuously with methanol to give an inactive fraction (B), which was not investigated further. Partition of fraction A between 10% aqueous methanol and petroleum ether resulted in concentration of the activity in the aqueous methanol fraction (D). This material was fractionated by silicic acid chromatography to give two main cytotoxic fractions, F and H.

Further chromatography of fraction H yielded vernodalin (**1**) as a colorless oil. The molecular formula $C_{19}H_{20}O_7$ was assigned on the basis of mass spectrometry. The ultraviolet spectrum exhibited a high end absorption and the infrared spectrum showed bands at 5.64 and 6.14 μ, characteristic of an α,β-unsaturated γ-lactone. The nmr spectrum exhibited two doublets (J = 3 Hz) at τ 3.77 and 4.30, indicative of the protons of a methylene exocyclic to a γ-lactone. The infrared spectrum also showed a second carbonyl band at 5.81 μ, and the general features of the spectrum were very similar to those of vernolepin (**5**) and vernomenin, previously isolated from *V. hymenolepis*.[1a] Comparison of the nmr spectra of vernodalin and vernolepin suggested that both compounds had a similar sesquiterpene lactone skeleton and that vernodalin might be an ester of vernolepin or vernomenin. The additional signals exhibited by vernodalin, namely, one-proton broad singlets at τ 3.67 and 4.00, a two-

(1) (a) Part XLVI: S. M. Kupchan, R. J. Hemingway, D. Werner, and A. Karim, *J. Org. Chem.*, **34**, 3903 (1969); (b) This investigation was supported by grants from the National Cancer Institute (CA-04500) and the American Cancer Society (T-275), and a contract with Chemotherapy, National Cancer Institute, National Institutes of Health (PH 43-64-551).

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(3) Leaves gathered in Ethiopia in 1965. The authors acknowledge receipt of the dried plant material from Dr. Robert E. Perdue, Jr., U. S. Department of Agriculture (USDA), Beltsville, Md., in accordance with the program developed with the USDA by the Cancer Chemotherapy National Service Center (CCNSC).

(4) Assays were performed under the auspices of the CCNSC. The procedures were those described in *Cancer Chemotherapy Rept.*, **25**, 1 (1962).