The Isolation and Structural Elucidation of Liatrin, a Novel Antileukemic Sesquiterpene Lactone from *Liatris chapmanii*[1]

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The isolation and structural elucidation of liatrin, a novel sesquiterpene lactone from *Liatris chapmanii*, are reported. Liatrin has significant antileukemic activity in mice and possesses the unusual germacranolide *cis,cis*-diene structure **1**. Reduction of liatrin with sodium borohydride gave the diol **5**, which was converted to a crystalline mono-*o*-bromobenzoate (**6**). X-Ray crystallographic analysis of **6** established the structure of diol **5**. In the light of the structure of **5**, chemical and spectral evidence was adduced in support of structure **1** for liatrin.

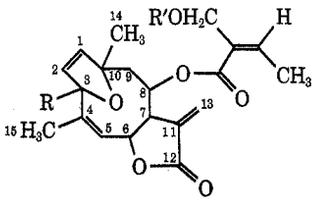
In the course of a continuing search for tumor inhibitors of plant origin, we found that chloroform extracts of *Liatris chapmanii* (Compositae)[2] showed significant inhibitory activity *in vitro* against cells derived from human carcinoma of the nasopharynx (KB).[3] A preliminary communication[4] outlined the structural elucidation of liatrin. It is the purpose of this paper to present in detail the isolation and the structural elucidation of the active constituent, liatrin (**1**).

Fractionation of the chloroform extract (Chart I) was guided by assay against KB to give the most active fraction.[5] Activity was concentrated in the benzene-soluble fraction upon trituration with benzene. Partition of this fraction between acetonitrile–hexane concentrated the activity in the acetonitrile fraction. Rapid elution chromatography of the acetonitrile-soluble material on a deactivated alumina column with benzene–ethyl acetate (3:1, saturated with water) yielded two active fractions (G and H) which were combined and crystallized from cyclohexane–methylene chloride to afford liatrin (**1**). While the isolation of the active principle was guided by assay against KB, liatrin also showed significant *in vivo* antileukemic activity.[3,6]

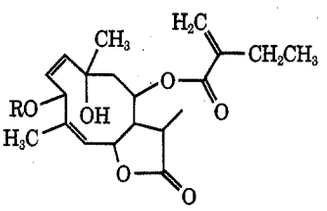
Liatrin (**1**) was assigned the molecular formula $C_{22}H_{26}O_8$ on the basis of elemental analysis and high-resolution mass spectrometry (Table I). The presence of high-intensity end absorption in the ultraviolet spectrum, bands at 5.67 and 6.03 μ in the infrared spectrum, and signals at τ 4.31 (d, J = 2.3 Hz) and 3.70 (d, J = 2.3 Hz) in the nmr spectrum of **1** suggested the presence of an α-methylene-γ-lactone group. The infrared spectrum also indicated the presence of free hydroxyl (2.92 μ), acetate (5.76 and 7.86 μ), and α,β-unsaturated ester (5.84 μ) groupings. Furthermore, the nmr spectrum showed a sharp one-proton singlet at τ 7.24 (exchangeable with D_2O) and a three-proton singlet at τ 7.94 corresponding to the hydroxyl and acetate methyl protons, respectively. The failure of **1** to undergo acetylation on treatment with acetic anhydride–pyridine suggested that the hydroxyl group was tertiary, and the one remaining oxygen atom in the molecular formula was assumed to be involved in an ether linkage. Further examination of the chemistry and spectra of liatrin (**1**) indicated that the tertiary hydroxyl and ethereal oxygen functions were present in the form of a cyclic hemiketal (see below).

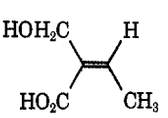
TABLE I

Compd	Ion	Calcd mass	Found mass
1	$C_{22}H_{26}O_8$	418.162	418.164
	$C_{15}H_{16}O_4$	260.104	260.104
	$C_7H_9O_3$	141.055	141.057
5	$C_{15}H_{19}O_4$	263.128	263.128
	C_5H_7O	83.049	83.049



1, R = OH; R′ = Ac
2, R = OH; R′ = H
3, R = OH; R′ = $COCH_2Br$
4, R = H; R′ = Ac





5, R = H
6, R = *o*-bromobenzoyl
7, R = *p*-bromobenzoyl

8

Reduction of liatrin with sodium borohydride gave the crystalline diol **5**, which on treatment with *o*-bromobenzoyl chloride gave the crystalline mono-*o*-bromobenzoate derivative **6**. The structure, stereochemistry, and absolute configuration of **6**, and therefore of

(1) (a) Tumor Inhibitors. LXXXVI. Part LXXXV: S. M. Kupchan, M. Maruyama, R. J. Hemingway, J. C. Hemingway, S. Shibuya, and T. Fujita, *J. Org. Chem.*, in press. (b) This investigation was supported by grants from the National Cancer Institute (CA-11718 and CA-11760) and the American Cancer Society (IC-57), and a contract with the Division of Cancer Treatment, National Cancer Institute (NIH-NCI-C-71-2099).

(2) Whole plant was gathered in Florida in September 1964. The authors acknowledge with thanks 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 National Cancer Institute.

(3) Cytotoxicity and *in vivo* inhibitory activity were assayed under the auspices of the National Cancer Institute by the procedures described in *Cancer Chemother. Rep.*, **25**, 1 (1962).

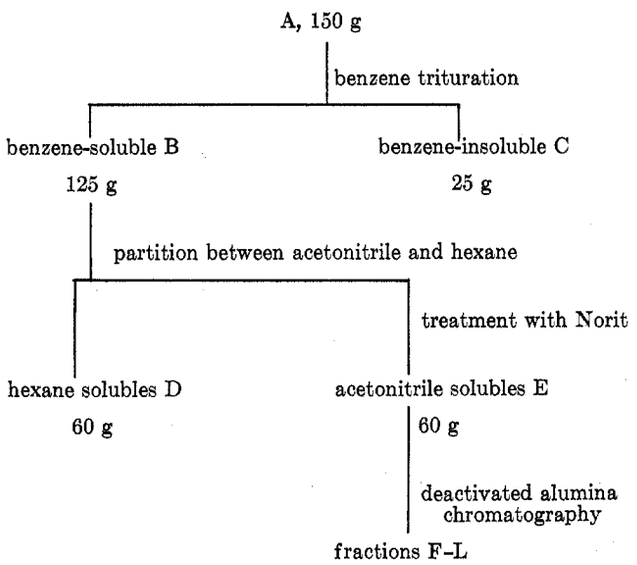
(4) S. M. Kupchan, V. H. Davies, T. Fujita, M. R. Cox, and R. F. Bryan, *J. Amer. Chem. Soc.*, **93**, 4916 (1971).

(5) Cytotoxicity was assayed by differential agar diffusion by Professor D. Perlman, University of Wisconsin; *cf. J. Pharm. Sci.*, **58**, 633 (1969).

(6) Liatrin showed significant antileukemic activity against the P-388 lymphocytic leukemia in mice, and cytotoxicity (ED_{50}) against KB cell culture at 1.5 μg/ml.

CHART I

FRACTIONATION OF THE CYTOTOXIC EXTRACT FROM *L. chapmanii*



5, were determined unequivocally by X-ray crystallographic analysis.[7]

A view of the molecular structure found in the crystal is shown in Figure 1. Bond lengths and bond angles in the molecule are shown in Figure 2, as are the torsion angles defining the conformations of the five- and ten-membered rings.

The estimated standard deviations in the parameters, calculated from the elements of the least-squares matrices by the method of Hodgson and Rollett,[8] lead to estimated standard deviations in bond lengths of C–C 0.035, C–O 0.025, and C–Br 0.016 Å, and in bond angles of not more than 2°, except for quantities involving the poorly defined terminal atoms C-33 and C-34, where the uncertainty is greater by a factor of at least 2.5. C-33 and C-34 appear quite clearly, but with reduced peak height, in normal electron-density maps, and in difference electron-density maps calculated with phases from which the contribution of the two atoms has been excluded the only structurally significant peaks are at the locations of the atoms. However, their thermal parameters, $B \cong 12.0$ Å,^{2} are much higher than those of the other atoms, and imply a substantial amplitude of vibration for each atom about its mean position. Because of this, C-33–C-34 is anomalously short, and the assumption of single-bond character rests also on chemical evidence.

Within these limits, the observed bond lengths have normal values. The valence angles within the ring of of the sp^3-hybridized carbon atoms of the ten-membered ring show the expected increase over the regular tetrahedral angle,[9] the mean value being 114.8°. The mean

(7) Positional parameters defining the crystal structure, the anisotropic thermal parameters of the atoms, and the observed and calculated structure amplitudes will appear following these pages in the microfilm edition of this volume of the journal. Single copies may be obtained from the Business Operations Office, Books and Journals Division, American Chemical Society, 1155 Sixteenth St., N.W., Washington, D. C. 20036. Remit check or money order for $3.00 for photocopy or $2.00 for microfiche, referring to code number JOC-73-1853.

(8) L. I. Hodgson and J. S. Rollett, *Acta Crystallogr.*, **16**, 329 (1963).

(9) R. F. Bryan and J. D. Dunitz, *Helv. Chim. Acta*, **43**, 3 (1960).

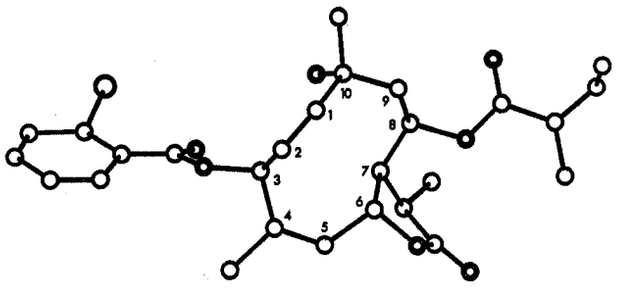


Figure 1.—Molecular structure of the *o*-bromobenzoate derivative 6 as found in the crystal. The central ring is numbered to correspond to the structural formula given in the text. Oxygen atoms are represented by double circles, and the bromine atom by the larger circle.

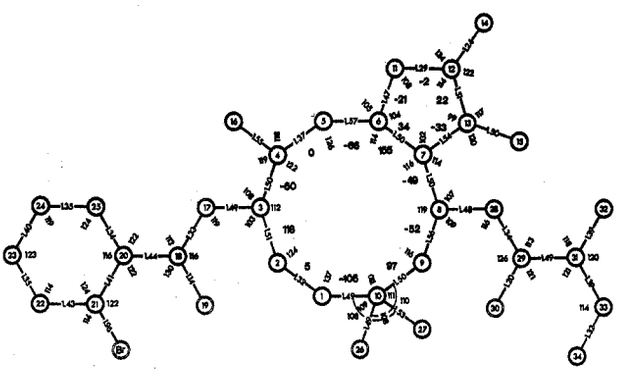


Figure 2.—Bond lengths and angles and torsion angles in the *o*-bromobenzoate 6.

of the observable extra-ring sp^3 angles is correspondingly reduced to 107.7°. The angle C-2–C-1–C-10 is greatly increased over the expected value for an sp^2 valence angle, presumably to relieve stress from the close approach of the hydroxyl group at C-10 to the hydrogen atoms at C-3 and C-7. Tables II and III show intra- and intermolecular contacts in the crystal.

TABLE II

SELECTED INTRAMOLECULAR CONTACTS (Å) WITHIN THE TEN-MEMBERED RING

C-1···C-6	3.28	C-3···C-10	3.28
C-2···C-5	3.07	C-3···O-26	2.97
C-2···C-6	3.16	C-4···C-7	3.27
C-3···C-6	3.08	C-6···C-9	3.12
C-3···C-7	3.33	C-7···C-10	3.17

TABLE III

SHORTER INTERMOLECULAR APPROACH DISTANCES (Å)[a]

Br···C-9^{I}	3.92	C-9···O-19^{II}	3.47
Br···O-28^{I}	3.59	O-14···O-26^{IV}	2.80
Br···C-31^{I}	3.98	C-15···O-17^{I}	3.43
C-1···O-19^{II}	3.47	C-22···O-30^{V}	3.40
C-2···C-15^{II}	3.71	C-23···O-30^{V}	3.48
C-2···O-26^{II}	3.50	C-27···O-32^{VI}	3.79
C-5···C-15^{III}	3.73		

[a] Contacts are between the first atom at x, y, z and the second atom in the symmetry-related position denoted by the Roman superscript: I = $x - 0.5, 1.5 - y, 1 - z$; II = $0.5 + x, 1.5 - y, 1 - z$; III = $0.5 + x, 1.5 - y, -z$; IV = $x, y, z - 1$; V = $1 - x, y - 0.5, 1.5 - z$; VI = $x, y, 1 + z$.

With the X-ray-defined structure of 6 (and hence of 5) in hand, liatrin could now be shown to have the unusual germacranolide *cis,cis*-diene structure 1 by

TABLE IV

NUCLEAR MAGNETIC RESONANCE DATA FOR *Liatris chapmanii* DERIVATIVES[a]

Compd	C-1	C-2	C-3	C-5	C-6	C-7	C-8	C-9	C-11	C-13	C-14	C-15	Side chain	Other
Liatrin (1)	3.64 d (5.6)	4.21 d (5.6)		4.35 dd (1.5, 6.5)	4.12 m	6.53 m	4.78 t (3.5)	7.63 d (3.5)		4.31 d (2.3)	8.61 s	8.09 m	3.51 q (7) 5.36 AB q (12)	7.24 s C-3 OH
										3.70 d (2.3)			7.94 s OAc 7.97 d (7)	
Deacetyl-liatrin (2)	3.57 d (5.4)	4.21 d (5.4)		4.34 dd[b]	4.03 m	6.50 m	4.79 t (3.6)	7.59 d (3.6)		4.27 d (2.0)	8.54 s	8.05 br s	3.60 q (7) 5.81 AB q (12)	
										3.69 d (2.0)			7.97 d (7)	
Bromo-acetate 3	3.65 d (5.6)	4.20 d (5.6)		4.38 dd[b]	4.15 m	6.55 m	4.80 t (3.5)	7.65 d (3.5)		4.31 d (2.0)	8.62 s	8.11 br s	3.44 q (7) 5.28 AB q (12)	7.37 C-3 OH
										3.71 d (2.0)			6.09 s (CH_2Br) 7.94 d (7)	
Deoxyliatrin (4)	4.26 dd (1.5, 6)	3.92 dd (2.5, 6)	4.89 dd (1.5, 2.5)	4.36 m	3.90 m	6.40 dt (2.4, 4.4)	4.76 t (4.0)	7.64 d (4.0)		4.32 d (2.4)	8.71 s	8.10 br s	3.51 q (7) 5.34 AB q (12)	No exchange-able signal
										3.72 d (2.4)			7.90 s (OAc) 7.90 d (7)	
Deoxyliatrin (4)[c]	4.73 dd (1.5, 6)	4.18 dd (2.5, 6)	5.20 m	4.52 br d (6.5)	3.78[b] m	6.58 m	4.8[b]	7.90 m		3.79 d (2.5)	8.50 s	8.14 s	3.94 q (7) 5.34 AB q (12)	
										4.81 d (2.5)			8.02 d (7)	
Diol 5									7.37 quintet (7.5)	8.91 d (7.5)	8.50 s	8.14 s	3.94 br s 4.45 br s	
													7.75 q (7.5)	
													8.95 t (7.5)	

[a] 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; q, quartet; m, multiplet; br, broad. Numbers in parentheses denote coupling constants in hertz. [b] Pattern obscured by other signals. [c] Measured in deuteriobenzene.

careful analysis of its chemical and spectral properties as described below. Liatrin (1) gave on acid hydrolysis an amorphous deacetylated product 2 which was reconverted to 1 on acetylation and could also be converted to a crystalline bromoacetate 3. Alkaline hydrolysis of 1 gave sarracinic acid (8),[10,11] identified by comparison of infrared spectra, melting point, and mixture melting point with those of an authentic sample.[12]

Closer examination of the nmr (Table IV) and mass spectra of liatrin (1), the deacetylation product 2, and the bromoacetate 3 indicated that 1 contained an acetylsarracinate ester moiety. For example, decoupling experiments on 1 revealed that a methyl proton doublet at τ 7.97 (J = 7 Hz) was coupled to a one-proton quartet at τ 3.51 (J = 7 Hz). In addition, the chemical shift of the methylene group centered at τ 5.36, 5.81, and 5.28 in the nmr spectra of 1, 2, and 3, respectively, showed that the acetyl group in 1 was attached to the hydroxyl group in the sarracinate ester. Additional corroboration of these results was found in the mass spectrum of 1, which contained a large peak at m/e 141, shown by high-resolution mass spectrometry to be due to the presence of the $C_7H_9O_3$ ion, whereas the mass spectrum of the bromoacetate 3 displayed a large double peak at m/e 221 and 219 ($C_7H_8O_3{}^{81}Br$, $C_7H_8O_3{}^{79}Br$). Finally, the presence of a peak in the high-resolution mass spectrum of 1 at m/e 260 corresponds to loss of acetylsarracinic acid from the molecular ion at m/e 418 and thus is representative of the sesquiterpene nucleus of 1.

The high-resolution mass spectrum of the borohydride reduction product 5 displayed peaks at m/e 263 and 83 due to the $C_{15}H_{19}O_4$ (sesquiterpene nucleus) and C_5H_7O (α-ethylacrylium) ions, respectively. This indicated that the acetylsarracinate side chain of 1 had undergone reductive elimination to give the α-ethyl acrylate group of the diol 5. Accordingly, the nmr spectrum of 5 revealed signals attributable to an ethyl group at τ 8.95 (3 H, t, J = 7.5 Hz) and 7.75 (2 H, q, J = 7.5 Hz), and a terminal methylene group at τ 3.94 and 4.45.

Treatment of liatrin (1) with dimethylamine borane afforded an oily deoxy compound 4, whose molecular formula, $C_{22}H_{26}O_7$, was assigned on the basis of mass spectrometry. The nmr spectrum of 4 was similar to that of liatrin (1), except that an AB type pattern at τ 3.64 (d, J = 5.6 Hz) and 4.21 (d, J = 5.6 Hz) in the spectrum of 1 was replaced by an ABX pattern at τ 3.92 (d, d, J = 2.5, 6 Hz), 4.26 (d, d, J = 1.5, 6 Hz), and 4.89 (d, d, J = 1.5, 2.5 Hz) in the spectrum of 4. Accordingly, the signals at τ 3.92 and 4.26, on irradiation at τ 4.89, collapsed to a pair of doublets (J = 6 Hz). In addition, 4 contained no D_2O-exchangeable protons. These facts suggested that the tertiary hydroxyl group of 1 had been replaced by a methine hydrogen in 4. The low chemical shift (τ 4.89) indicated that the methine hydrogen was probably attached to an ethereal carbon atom. The ease of replacement of the tertiary hydroxyl group of 1 by a hydrogen on treatment with dimethylamine borane, and the formation of the diol 5 on treatment of 1 with sodium borohydride,

(10) C. C. J. Culvenor and T. A. Geissman, *J. Org. Chem.*, **26**, 3045 (1961).

(11) J. D. Edwards, Jr., T. Matsumoto, and T. Hase, *J. Org. Chem.*, **32**, 244 (1967).

(12) The authors are indebted to Dr. J. D. Edwards, Jr., for a generous sample of authentic sarracinic acid.

are indicative of the presence of a hemiketal function in **1**.

Further evidence for the structure of **1** could be gained from the nmr spectrum of **4** in benzene-d_6 solution. Irradiation at τ 5.20, corresponding to the chemical shift of the methine hydrogen, collapsed a doublet of multiplets centered at τ 4.52 (J = 6.5 Hz) to a broad doublet, which could thus be assigned to the C-5 vinyl hydrogen. Consequently, the tertiary hydroxyl group in **1** and the new methine hydrogen in **4** could now be located at C-3 between the two olefinic bonds.

The crystalline diol **5** obtained from liatrin (**1**) on treatment with sodium borohydride in ethanol requires further comment. The presence of two hydroxyl groups in **5** was evident from the ready formation of both the mono-*o*-bromobenzoate **6** and the mono-*p*-bromobenzoate **7** derivatives, which, from their spectral characteristics, clearly contained one tertiary hydroxyl group. Three-proton singlets at τ 8.14 and 8.50 in the nmr spectrum of **5** indicated the presence of a vinyl methyl and a methyl attached to a carbon bearing oxygen, respectively. Consequently, one of the two tertiary methyl groups could be located in the sesquiterpene skeleton of **5**, geminal with a tertiary hydroxyl group. The appearance of a doublet at τ 8.91 (3 H, J = 7.5 Hz) and an apparent quintet at τ 7.37 (1 H, J = 7.5 Hz) in the nmr spectrum of **5**, which were shown to be coupled, indicated that reduction of the α-methylene-γ-lactone had occurred.

Examination of the high-resolution nmr spectrum of liatrin made possible elaboration of complete structure **1**. An AB quartet signal at τ 3.64 (d, J = 5.6 Hz) and 4.21 (d, J = 5.6 Hz) indicated the existence of an isolated cis double bond. Since this system was not coupled to any other protons, and appeared as part of an ABX system in the deoxy derivative **4**, it could be assigned to the C-1 and C-2 vinyl hydrogens, respectively. Chemical evidence had suggested the presence of a cyclic hemiketal, which on reduction with sodium borohydride gave the diol **5**. Clearly, from the X-ray derived structure of **5** the hemiketal in **1** must be in the form of a dihydrofuran. This was supported by the coupling constant for the C-1, C-2 vinyl hydrogens (5.6 Hz), which was in close agreement with that of unsaturated pentafuranosides described by Lemieux, *et al.*[13] A three-proton singlet at τ 8.61, indicative of a methyl group on carbon bearing oxygen, was then assigned to the C-10 methyl group.

Irradiation at τ 6.53 (1 H, m) caused the pair of doublets at τ 4.31 and 3.70 (each 1 H), previously assigned to the C-13 protons, to collapse to singlets.[14] The τ 6.53 multiplet could then be assigned to the C-7 proton. In addition, irradiation at τ 6.53 caused the multiplet at τ 4.12 (1 H) to collapse to a broad doublet. The same multiplet at τ 4.12 was collapsed to a doublet of doublets upon irradiation at τ 8.09 (3 H, br s). Thus the τ 4.12 signal could be assigned to the C-6 proton and the τ 8.09 signal to the C-4 methyl protons. Furthermore, irradiation of the C-4 methyl group caused a doublet of doublets at τ 4.35 (1 H, J = 6.5, 1.5 Hz) to collapse to a doublet (J = 6.5 Hz), which could thus be assigned to the C-5 vinyl proton.

(13) R. M. Lemieux, K. A. Watanake, and A. A. Pavia, *Can. J. Chem.*, **47**, 4413 (1969).

(14) Z. Samek, *Tetrahedron Lett.*, 671 (1970).

To complete the nmr assignments in **1** it was found that a two-proton doublet at τ 7.63 (J = 3.5 Hz) was coupled to a one-proton triplet at τ 4.78 (J = 3.5 Hz), allowing assignment of these signals to the C-9 methylene and C-8 methine protons, respectively. The formation of the diol **5** and the deoxy compound **4** from **1** on treatment with sodium borohydride and dimethylamine borane, respectively, can be explained as having involved reduction of the hemiketal in **1**.

The X-ray analysis of the *o*-bromobenzoate **6** established the stereochemistry as C-1, C-2, cis; C-3, *S*; C-4, C-5, cis; C-8, *R*; and C-10, *S*; the γ-lactone ring was found to be trans-fused to C-6, C-7.

Experimental Section

Melting points were determined on a Kofler block or a Mettler Model FP2 hot stage, and are corrected. Ultraviolet absorption spectra were determined on Beckman Model DK-2 and Coleman Hitachi Model EPS-3T recording spectrophotometers. Infrared spectra were determined on Beckman Model IR-9, Perkin-Elmer Model 257, and Perkin-Elmer Model 337 recording spectrophotometers. Nuclear magnetic resonance spectra were determined on a Perkin-Elmer Model R-20 spectrometer at 60 Mc/sec and on a Varian HA-100 spectrometer in deuteriochloroform solution with tetramethylsilane as an internal standard. Mass spectra were obtained from Hitachi Perkin-Elmer Model RMU-6A (RMU-6E) and AEI Model MS-902 spectrometers. Values of $[\alpha]_D$ were determined on a Perkin-Elmer Model 141 automatic polarimeter. Microanalyses were carried out by Spang Microanalytical Laboratory, Ann Arbor, Mich. Thin layer chromatography (tlc) was carried out on precoated plates supplied by E. Merck. The tlc-solvent system most commonly used was benzene–ethyl acetate–isopropyl alcohol (85:10:5, system A). Tlc plates were visualized with a mixture of concentrated sulfuric acid–25% vanillin in absolute ethanol (5:1). Evaporations were carried out at reduced pressure at less than 40°.

Isolation Procedure.—The dried ground roots, stems, leaves, and flowers (1.15 kg) of *L. chapmanii* were continuously extracted with chloroform in a Soxhlet apparatus for 48 hr and the chloroform extract was evaporated under reduced pressure to yield a dark green residue (A, 150 g). Fraction A was triturated with benzene (2.5 l.) for 8 hr and filtered, and the residue was again triturated with benzene (1 l.). The combined benzene extracts were evaporated to yield a dark brown gum (B, 125 g), which contained most of the KB activity present in the chloroform extract. Fraction B was partitioned between acetonitrile (1 l.) and hexane (1 l.). The acetonitrile layer was again extracted with hexane (4 × 750 ml) and the combined hexane layers were evaporated to give a green oil (D, 60 g). The acetonitrile layer was treated with Norit (20 g), and filtered to give a brown solution which was evaporated to yield a gum (E, 60 g). Fraction E showed significant KB activity. The acetonitrile solubles (E, 60 g) were chromatographed on 3 kg of Woelm neutral alumina (activity I) deactivated by the addition of 60 ml of distilled water. The column was packed in benzene saturated with water and eluted with benzene–ethyl acetate (3:1, saturated with water). A very fast flow rate (3.5–4 l./hr) was employed. Fractions were collected as follows: F (6 l., 1.4 g), G (4 l., 2.9 g), H (4 l., 2.6 g), I (3 l., 0.9 g), J (6 l., 1.6 g), K (7 l., 6.7 g), and a methanol wash L (8 l., 20.9 g). Tlc examination of these fractions on a silica gel plate showed that liatrin (1) (which gave an orange spot with the spray reagent described) was concentrated in fractions G and H. These fractions were combined and crystallized from methylene chloride–cyclohexane to give liatrin (1, 1.8 g) as colorless needles, mp 129–131°. Recrystallization from the same solvent gave material with mp 130–132°; $[\alpha]^{24.5}D$ −142.0° (*c* 1.93, chloroform); uv λ^{EtOH} end absorption, 220 nm (ϵ 19,420); ir λ_{max}^{KBr} 2.92, 3.37, 5.67, 5.76, 5.84, 6.03, 7.81, 8.67, and 9.71 μ; mass spectrum *m/e* (rel intensity) 418 (<1), 260 (6), 259 (12), 141 (30), 99 (20), 79 (15), 81 (70), 69 (15), 53 (16), and 43 (100).

Anal. Calcd for $C_{22}H_{23}O_8$: C, 63.15; H, 6.26. Found: C, 63.21; H, 6.22.

Sodium Borohydride Reduction of Liatrin (1).—Sodium borohydride (1.30 g) was gradually added over a period of 1 hr to a stirred solution of 1 (403 mg) in ethanol at −60 to −50°, and

then the bath temperature was kept at −20 to −5° for 3 hr. The mixture was poured into ice cold 5% hydrochloric acid (200 ml) to decompose excess reagent, then extracted with methylene chloride (3 × 50 ml). The combined organic layers were washed with water, dried over sodium sulfate, and evaporated to give a gum (386 mg). The gum was crystallized from methylene chloride–cyclohexane to give 284 mg of diol **5**, which was purified by preparative thin layer chromatography and recrystallization from methylene chloride–cyclohexane to give colorless needles of **5**: mp 89.2–90.9°; uv λ^{EtOH} end absorption, 210 nm (ε 14,700); ir λ_{max}^{KBr} 2.92, 5.72, 5.83, and 6.15 μ; mass spectrum *m/e* (rel intensity) 346 ($M^+ - H_2O$, 4), 263 (20), 246 (32), 83 (100).

***o*-Bromobenzoylation of Diol 5.**—A solution of the diol **5** (80 mg) in anhydrous pyridine (8 ml) was treated with *o*-bromobenzoyl chloride (599 mg) at 0° for 30 min. The reaction mixture was diluted with methylene chloride and ice–water and then stirred for 30 min. The layers were separated and the water layer was extracted with methylene chloride. The combined organic layers were washed with 10% aqueous sodium carbonate solution, 1 *N* hydrochloric acid, and water, then dried over sodium sulfate and evaporated to dryness at reduced pressure. The solid residue obtained was crystallized from methylene chloride–cyclohexane to give the mono-*o*-bromobenzoate **6** (50 mg): mp 181.5–182.3°; ir λ_{max}^{KBr} 2.93, 5.69, 5.79, 5.88, 6.16, and 6.31 μ; mass spectrum *m/e* (rel intensity) 530, 528 ($M^+ - H_2O$, <1), 263 (12), 246 (10), 185, 183 (100), 83 (95).

Anal. Calcd for $C_{27}H_{31}BrO_7$: C, 59.24; H, 5.71; Br, 14.60. Found: C, 59.28; H, 5.76; Br, 14.68.

Deacetylliatrin (2).—A solution of liatrin (**1**, 203 mg) in water–dioxane (40:60, 15 ml) was treated with 6 *N* sulfuric acid (1 ml) at 5° for 25 days. Excess sodium bicarbonate solution was added and the reaction mixture was diluted with water (100 ml) and extracted with methylene chloride (3 × 100 ml). The combined organic layers were washed with water (200 ml), dried over sodium sulfate, and evaporated to yield a foam (185 mg) which was purified by preparative tlc on eight Silica Gel F254 plates using solvent system A. The medium R_f material was combined and extracted with methanol–methylene chloride (1:9). Evaporation of the solvents gave deacetylliatrin (**2**) as a gum: ir $\lambda_{max}^{CHCl_3}$ 2.76, 3.33, 3.39, 5.65, 5.80, 5.99, 6.20, 7.80, 8.70, and 9.80 μ; mass spectrum *m/e* (rel intensity) 260 (22), 259 (37), 191 (20), 149 (20), 99 (100), 97 (24), 81 (33), 69 (22), 53 (38), 43 (75), and 41 (55).

Bromoacetylation of Deacetylliatrin (2).—Deacetylliatrin (**2**, 60 mg) in anhydrous benzene (3 ml) was treated with anhydrous potassium carbonate (315 mg) and bromoacetic anhydride (10 drops) under nitrogen. The reaction mixture was intermittently stirred at 24° for 48 hr, then water (40 ml) was added and after 15 min the mixture was extracted with methylene chloride (3 × 33 ml). The combined organic layers were washed with water (50 ml), dried, and evaporated to dryness. The oily residue was purified by preparative tlc on four Silica Gel F254 plates (0.25 mm) using solvent system A. The high R_f band containing the desired bromoacetate was removed and extracted with methanol–methylene chloride (1:9). The solvent was evaporated and the residue was crystallized from methylene chloride–cyclohexane to give the bromoacetate **3** (15 mg) as colorless needles: mp 135–137°; ir λ_{max}^{KBr} 2.87, 3.37, 3.41, 5.69, 5.76 (sh), 5.80, 6.05, 7.85, 8.70, and 9.79 μ; mass spectrum *m/e* (rel intensity) 498, 496 (1), 260 (44), 259 (60), 242 (20), 221, 219 (32), 163 (22), 123, 121 (20), 99 (40), 97 (20), 81 (10), 53 (24), and 43 (32).

High resolution mass spectrum: calcd for $C_{22}H_{25}O_8{}^{79}Br$, 496.0733; found, 496.0721.

Acetylation of Deacetylliatrin (2).—Deacetylliatrin (**2**, 32 mg) in anhydrous benzene (2 ml) was treated with anhydrous potassium carbonate (170 mg) and acetic anhydride (5 drops) under nitrogen. The reaction mixture was stirred for 24 hr at 24°, after which time an additional quantity of acetic anhydride was added. After a further 24 hr, the reaction mixture was poured into water (30 ml). After 15 min the aqueous solution was extracted with methylene chloride (3 × 33 ml), and the combined organic layers were washed with water (50 ml), dried, and evaporated. The residue was dissolved in benzene and the benzene solution was evaporated, thereby removing the last traces of acetic acid. The crude product (41 mg) was chromatographed on two Silica Gel F254 plates (0.25-mm thickness) using solvent system A. The band corresponding to liatrin (**1**) was removed and extracted in the usual manner. Evaporation of the solvent and recrystallization of the residue from methylene chloride–cyclohexane gave liatrin (**1**, 17 mg), mp 132°, which was shown to be identical (tlc, ir, mass spectrum, mixture melting point) with authentic liatrin (**1**) described above.

Sarracinic Acid (8).—A solution of liatrin (**1**, 299 mg) in 5 *N* sodium hydroxide (25 ml), dioxane (35 ml), and water (45 ml) was heated at 60° for 30 min. After cooling, the reaction mixture was adjusted to pH 8.5 with hydrochloric acid, concentrated to approximately 40 ml, and basified with 10% sodium carbonate. The aqueous layer was extracted with chloroform to remove any neutral material, then acidified with hydrochloric acid and saturated by the addition of solid sodium chloride. Extraction with ether gave an acidic fraction which, after removal of solvent, was chromatographed on Cellulose F precoated tlc plates (0.1-mm thickness) using *sec*-butyl alcohol–2 *N* ammonium hydroxide (4:1) as development solvent. The acidic band (visualized by bromphenol blue spray reagent) was removed and extracted with methanol, and the solvent was evaporated. The residue was dissolved in ether and washed with 5% aqueous hydrochloric acid. The ether layer was washed with saturated sodium chloride solution, dried over sodium sulfate, and evaporated to a solid (25 mg). Recrystallization from ether–petroleum ether (bp 60–68°) gave sarracinic acid (**8**), mp 53.4–54.5°, which was shown to be identical (ir and mixture melting point) with an authentic sample of sarracinic acid.[12]

Deoxyliatrin (4).—To a solution of liatrin (**1**, 84 mg) in acetic acid (0.5 ml) was added dropwise a solution of dimethylamine borane (15 mg) in acetic acid (0.5 ml). The solution was heated over a steam bath for 5 min, cooled, neutralized with saturated potassium carbonate solution, and extracted with methylene chloride (3 × 10 ml). The combined organic layers were washed with water, dried over sodium sulfate, and evaporated to dryness. The residue was chromatographed on four Silica Gel F254 plates (0.5 mm thickness) using solvent system A. The major high R_f band was removed and extracted with methanol–methylene chloride (1:9) to afford, after evaporation of the solvent, pure deoxyliatrin (**4**) as an amorphous solid which failed to crystallize from several solvents. This material was homogeneous by tlc and displayed the following spectral data: ir $\lambda_{max}^{CHCl_3}$ 5.71, 5.82, 6.04, and 6.08 μ; mass spectrum *m/e* (rel intensity) 402 (4), 387 (20), 261 (14), 245 (55), 244 (50), 141 (70), 81 (70), 43 (100).

***p*-Bromobenzoylation of Diol 5.**—A solution of the diol **5** (47 mg) in anhydrous pyridine (1 ml) was treated with *p*-bromobenzoyl chloride (200 mg) at 25° under nitrogen in the dark for 24 hr. The reaction mixture was diluted with methylene chloride (10 ml) and added slowly to ice-cold 0.6 *N* sulfuric acid (50 ml). The aqueous solution was extracted with methylene chloride (2 × 100 ml). The combined organic layers were washed with 0.6 *N* sulfuric acid (2 × 50 ml) and with 10% aqueous sodium carbonate solution (2 × 50 ml), then dried over sodium sulfate. Removal of the solvent gave a gummy solid (100 mg) which was chromatographed on four Silica Gel F254 plates (2 mm) using solvent system A. The high R_f band, containing the desired *p*-bromobenzoate derivative, was removed, extracted with methanol–methylene chloride (1:3, 500 ml), and evaporated to give a gum (55 mg) which was further purified by preparative tlc on two alumina type T, F254 plates using chloroform as solvent. Extraction of the high R_f, uv-visible band and work-up in the usual manner gave a solid residue (27 mg). Crystallization from methylene chloride–Skelly B gave the *p*-bromobenzoate derivative **7** as needles: mp 156–158°; uv λ_{max}^{EtOH} 212 nm (ε 6230) and 245 (18,460); ir λ_{max}^{KBr} 2.90, 3.38, 5.63, 5.84, 6.14, 6.30, 7.82, 8.92, and 10.12 μ; mass spectrum *m/e* (rel intensity) 530, 528 ($M^+ - 18$, 2), 448, 446 (1), 430, 428 (1), 346 (2), 328 (2), 264 (5), 263 (18), 246 (21), 228 (11), 202, 200 (14), 185, 183 (100), 85 (15), 83 (60), 57 (37), and 55 (78).

Anal. Calcd for $C_{27}H_{31}BrO_7$: C, 59.24; H, 5.71; Br, 14.60. Found: C, 58.97; H, 5.83; Br, 14.64.

High resolution mass spectrum: calcd for M − 18, $C_{27}H_{29}{}^{81}BrO_6$, 530.1128; found, 530.1166.

X-Ray Crystallographic Structural Data.—Crystals of the *o*-bromobenzoate are colorless, transparent lathes elongated along the *c* axis. Intensity measurements were made, at room temperature, from a crystal 0.35 × 0.25 × 0.05 mm[3] mounted with *b* parallel to the φ axis of a Picker four-circle diffractometer controlled by an XDS Sigma 2 computer.[15] Monochromatic molybdenum K_α radiation was used with scintillation counting.

Within a single octant, 1530 independent reciprocal lattice points were surveyed out to $2\theta = 42°$, and scattered intensity significantly above background was found at 1150 of them. No

(15) R. C. Haltiwanger and R. F. Bryan, *J. Chem. Soc. B*, 1598 (1971).

loss of intensity was observed during the experiment as judged by regular monitoring of three reference reflections so that a single scale factor could be used. No corrections for absorption were deemed necessary.

Crystal Data for *o*-Bromobenzoate 6.—$C_{27}H_{31}BrO_7$ had formula weight 547.4, orthorhombic, a = 11.25 (2), b = 26.63 (4), c = 8.96 (2) Å, U = 2685 Å^3, D_m = 1.34 (by pycnometry with an aqueous ZnI solution), Z = 4, D_c = 1.35, F (000) = 1136. Space group $P2_12_12_1$. Precession photography, Mo K_α radiation, λ 0.7107 Å, μ 17 cm^{-1}.

Structure Determination and Refinement.—The structure was solved by the heavy-atom method in the usual way, and five cycles of Fourier refinement with a single, overall, isotropic thermal parameter, B = 4.0 Å^2, gave R = 0.22.

Further refinement of the structural parameters was by block-diagonal least-squares methods. With anisotropic thermal parameters assumed only for the bromine atom, R was 0.118 at convergence. Inclusion of the anomalous dispersion terms[16] for bromine in the structure factor calculations gave R = 0.123 and 0.116 for the two possible enantiomeric structures. By Hamilton's R-factor ratio test[17] a significant distinction is implied between the two absolute configurations at the 99.5% confidence level. The crystal was accidentally dislodged and lost during the measurement of intensity differences in Friedel pairs of reflections. The very few measurements made confirmed the correctness of the choice indicated by the ratio test.[18]

The least-squares refinement was continued for the favored enantiomorph, and with anisotropic thermal parameters assumed for all atoms R was 0.079 at convergence.

The scattering functions used were those for the neutral atoms.[19] The weighting scheme used was based on counting statistics with some allowance for errors of a nonstatistical nature in the stronger intensities.[20] All calculations were performed on an XDS Sigma 2 computer with programs written in this laboratory.

Registry No.—**1**, 34175-79-6; **2**, 38821-16-8; **3**, 38821-17-9; **4**, 38821-18-0; **5**, 34160-71-9; **6**, 34160-72-0; **7**, 34160-73-1; *o*-bromobenzoyl chloride, 7154-66-7; *p*-bromobenzoyl chloride, 586-75-4.

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Synthesis and Spectral Characterization of Some *C*-Alkylphospholes and Phospholecarboxylates[1]

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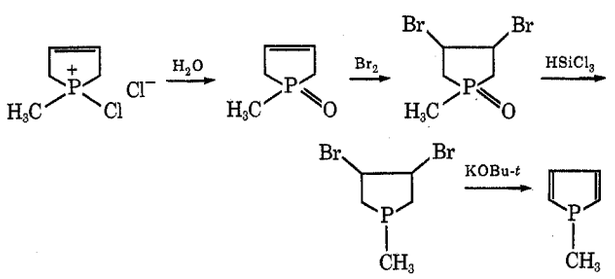
Ten phospholes, bearing either methyl, benzyl, or phenethyl substituents on phosphorus and methyl or carbomethoxy on carbon, have been synthesized by dehydrohalogenation of 3,4-dibromophospholanes or of 1-halophospholenium halides. The two esters prepared are the first phospholes with a reactive functional group. Significant differences were noted in the rates of reaction of phospholes to quaternization with alkyl halides; the fastest reacting phospholes (3,4-dimethyl derivatives) exhibited other differences, including (1) a small blue shift in the characteristic uv maximum of phospholes, (2) diminished allylic coupling between β CH_3 and an α proton, (3) a slight upfield shift of the α proton, and (4) a pronounced upfield shift of the ^{31}P signal. Steric or electronic effects, or a combination of these, are apparently leading to a diminution of electron delocalization from phosphorus in these derivatives. Some of the *P*-methyl phospholes had readily interpreted P–H coupling patterns, permitting experimental verification of computed values made earlier. It was possible to consider the ^{31}P value of the phosphole as derived from definite contributions for the ring fragment and for the P substituent. The phosphorus in phospholes is more strongly deshielded than in 2-phospholenes and in these more than in phospholanes. The carbomethoxy substituent caused quite strong additional deshielding, moderated, however, by steric interaction with a methyl substituent adjacent to it. The sensitivity of the ^{31}P value to conjugative effects is revealed by these observations.

In 1967, we announced[2] the synthesis of 1-methylphosphole, the first phosphole of sufficient structural simplicity to allow a meaningful evaluation[3] of properties of this ring system relative to those of the heteroaromatics thiophene, pyrrole, and furan. This study, as well as subsequent work of others,[4,5] has revealed that the phosphole ring has some of the properties associated with systems partaking of electron delocalization through p_π–p_π bonding. Following our initial work, we proceeded to pursue a synthetic program designed to provide appropriate phospholes for exploring further some unique features present in this system. Some of the results of this study are described in this paper.

Synthesis.—The phospholene ring system, available from the cycloaddition of dienes and trivalent phosphorus halides,[6] serves as a useful starting point for construction of the phosphole system. Thus, 1-methylphosphole was prepared in our earlier work[2,3] by the following sequence.



(1) Taken from the Ph.D. dissertations of J. F. E. (1971) and S. G. B. (1972). Supported in part by Public Health Service Research Grant CA-05507 from the National Cancer Institute. The National Science Foundation provided funds toward the purchase of the Bruker spectrometer (Grant No. GP 10301), and the AEI spectrometer is sponsored by Special Facilities Grant No. FR-0330-01, National Institutes of Health.
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