The *ortho*-methyl groups in lidocaine should force the amido plane to be orthogonal to the benzene ring as in the similar compounds 2,6-diiodoacetanilide (2) and 2,6-dimethylformanilide (5). In this conformation the amide carbonyl group in lidocaine would be "exo" to the phenyl ring whereas in the above compounds (2, 5) the carbonyl group has the "endo" conformation.

The PMR chemical shift assignments (Varian A-60 A) are for lidocaine with the *cis* amide configuration (δ, p.p.m., in CCl_4 relative to TMS internal reference): 1.09, (6H, t), CH_3 (Et); 2.15, (6H, s), CH_3 (Ar); 2.61, (4H, q), CH_2 (Et); 2.99, (2H, s), $CO \cdot CH_2N$; 6.93, (3H, s), Ar ring H; and 8.48, (1H, s), $CO \cdot NH$.

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Tumor Inhibitors: Liriodenine, a Cytotoxic Alkaloid from *Annona glabra*

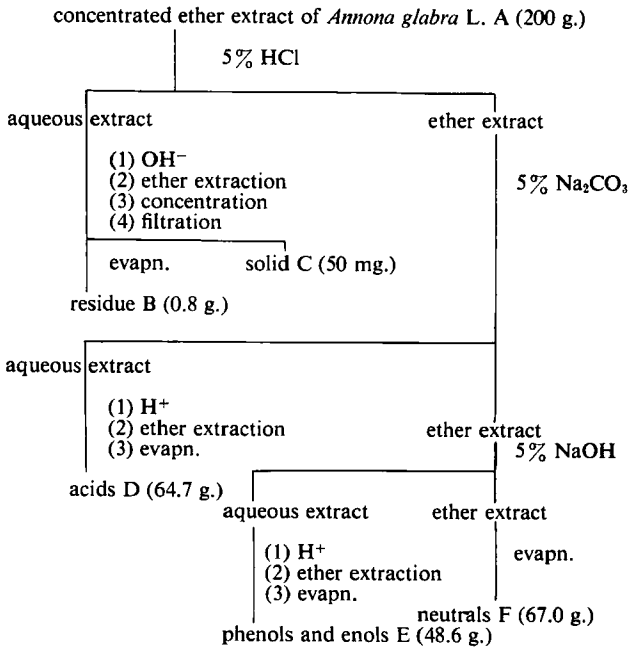
Keyphrases ☐ Cytotoxic alkaloid—*Annona glabra* ☐ Liriodenine—isolation, identification ☐ TLC—separation, identity ☐ UV spectrophotometry—identity ☐ Visible spectrophotometry—identity ☐ NMR spectroscopy—identity, structure ☐ IR spectrophotometry—identity ☐ Mass spectroscopy—identity, structure

Sir:

During our search for tumor inhibitors from plant and insect sources, ether extracts of the dried wood and stem bark of *Annona glabra* L. (*Annonaceae*) from Florida showed significant inhibitory activity when tested *in vitro* against cells derived from human carcinoma of the nasopharynx (1).[1] We have succeeded in isolating and characterizing a cytotoxic principle, which is identical with liriodenine.

The concentrated ether extract (200 g. A in Fig. 1), obtained from 8.21 kg. of wood and stem bark of *A. glabra*, was treated with 5% hydrochloric acid and the mixture was extracted with ether. The ether extract was further fractionated into acids (D), phenols and enols (E), and neutrals (F). The acidic aqueous extract was made alkaline with 10% sodium hydroxide and extracted with ether. Upon concentration, a yellow solid (C) precipitated and was removed by filtration. Evaporation of the filtrate left a residue (B). These fractionations resulted in a concentration of activity in Fraction C. TLC of this fraction (developing with 20% methanol in benzene on Kieselgel DF-5[2]) revealed only one spot: R_f of 0.25 with respect to an R_f of 0.79 for Sudan III. Likewise, only one spot was obtained with other developers (methanol–chloroform mixtures). Fraction C melted at 268–270°[3] and showed UV absorption maxima at 248 mμ (log ε 4.18), 267 mμ (log ε 4.05), and 305 mμ (log ε 3.59) in ethanol solution.

Fraction C was crystallized from chloroform as yellow needles, m.p. 275–277°. The mass spectrum of the crystalline material showed a very large parent ion (*m/e* 275) compared with the rest of the ions, indicating a very stable conjugated system not subject to extensive fragmentation. The NMR spectrum (see Fig. 2) showed



fraction	ED_{50} (mcg./ml.)
A	1.5
B	15.0
C	1.6
D	9.0
E	17.0
F	18.0

Figure 1—*Fractionation of a cytotoxic principle of* Annona glabra *L. and cytotoxicity of the fractions A through F.*

[1] Cytotoxicity was assayed, under the auspices of the Cancer Chemotherapy National Service Center, against Eagle's KB strain of human epidermoid carcinoma; H. Eagle, and G. E. Foley, *Am. J. Med.*, **21**, 739(1956); *Cancer Res.*, **18**, 1017(1958).

[2] Company and trade names are given for identification purposes only and do not constitute endorsement by the U. S. Department of Agriculture.

[3] Melting points are uncorrected and were taken on a Thomas-Hoover capillary melting point apparatus.

signals (chemical shift in δ) due to a methylenedioxy group (6.65 δ, 2 protons, singlet) and 7 aromatic protons (7.53 δ, 1 proton, singlet; 7.73, 1 proton, triplet; 8.01, 1 proton, triplet; 8.44, 2 protons, triplet; and 8.73, 2 protons, triplet).

The foregoing properties indicated that the active compound was an aporphine alkaloid closely related to or identical with liriodenine (2,3) (see Fig. 2), m.p. 282°, $\lambda_{max.}^{EtOH}$ 247.4, 268.2, 309.2, 413 mμ (log ϵ 4.22, 4.13, 3.62, and 3.82). There was no melting point depression on admixture with authentic liriodenine and their IR and mass spectra were essentially superimposable.

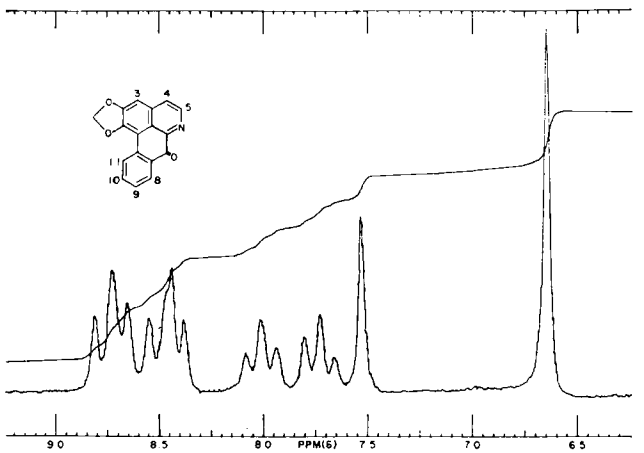


Figure 2—*NMR spectrum[4] and structure of liriodenine. The sample (3 mg. dissolved in trifluoroacetic acid in a 30-µl. sample bulb (Kontes) surrounded by carbon tetrachloride with a small amount of tetramethylsilane) was scanned 215 times. The tetramethylsilane shifts by +0.24 δ when used as a reference in this manner (benzene in trifluoroacetic acid for a signal lock at 7.3 δ shows this shift of tetramethylsilane; the position at which benzene normally appears in trifluoroacetic acid with tetramethylsilane as an internal standard is 7.3 δ). Therefore, the chemical shifts in Fig. 2 are corrected by + 0.24 δ.*

A small peak at (*m/e* 305) in the spectrum of the isolated compound indicated the presence of a methoxyliriodenine impurity (4), most probably atherospermidine (3-methoxyliriodenine) (3). Further investigation of this minor impurity, 1.1% of the height of the parent ion, or of other possible alkaloids in fraction B was not pursued.

The 1-proton singlet at 7.53 δ in the NMR spectrum of the isolated compound is attributed to the C-3 position due to its absence in the spectrum of atherospermidine. The protons at C-4 and C-5 positions can probably be assigned to the triplets at 7.73 and 8.73 δ, respectively, by comparison with the protons of *O*-methylatheroline (1,2,9,10-tetramethoxydibenzo[*de*,*g*]-quinolin-7-one) (7.63 and 8.76 δ) (5). Assignments for the 4 remaining aromatic protons would be difficult without more evidence. The chemical shifts for the sample are essentially those reported by Bick and Douglas for liriodenine (3).

Liriodenine was initially isolated from the heartwood of the yellow poplar, *Lirodendron tulipifera* L. (*Magnoliaceae*) (2) and assigned the structure in Fig. 2 by Taylor (6). The alkaloid has also been isolated from other magnoliaceous plants: *Michella compressa* (Maxim) Sarg. (7), *M. alba* DC., *M. champaca* L., *Magnolia coco* DC. (8), and *M. grandiflora* L. (9). More recently it has been found in the family *Monimiaceae*, *Atherosperma moschatum* Labill (3) and *Doryphora sassafras* Endl. (10); the family *Araceae*, *Lysichitum camtschatcense* Schott var. *japonicum* Makino (11); and the family *Papaveraceae*, *Roemaria refracta* DC. (12). Other oxoaporphines have been found in *Cassytha americana* Nees. (*C. filiformis* L.) (*Lauraceae*) (4). The presence of liriodenine in *A. glabra* was not surprising, since atherospermidine and liriodenine have been found in *Guatteria psilopus* Mart. (13) and *Asimina triloba* (L.) Dunal (14), respectively, which are also members of the family *Annonaceae*.

Although the cytotoxicity of liriodenine was of a borderline magnitude for a synthetic (4), liriodenine was synthesized for *in vivo* antitumor tests.

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[4] The NMR spectrum was taken on a Varian HA-100 spectrometer with a C-1024 time averaging computer.