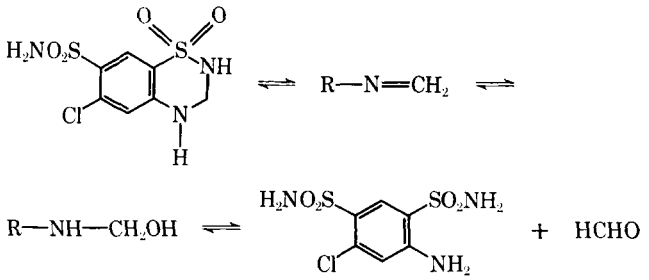
chlorothiazide (9); and analogous compounds have been reported from the reaction of *o*-aminosulfonamides with various aldehydes, formic acid, and orthoesters (10–12).

The hydrolytic reaction therefore most likely proceeds with ring opening to form an imine which undergoes attack by water or hydroxide ion to yield a carbinolamine (13); decomposition of this aminoalcohol then yields formaldehyde and 4-amino-6-chloro-*m*-benzene disulfonamide (Scheme II).



Scheme II

Other mechanisms could be postulated. We are presently extending this study to consider the susceptibility to general acid-base catalysis and to consider the effects of substituents in the 2, 3, and 4 positions on the hydrolytic reaction.

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Received December 12, 1968.
Accepted for publication January 24, 1969.

Lidocaine—An Unusual Incidence of an Acyclic *Cis* Amide Configuration

Keyphrases ☐ Lidocaine configuration—acyclic *cis* amide ☐ IR spectrophotometry—structure ☐ PMR spectroscopy—structure

Sir:

Very little spectroscopic data [infrared (IR) or proton magnetic resonance (PMR)] have been published for lidocaine (2-diethylamino-2′,6′-acetoxylidide) or for molecules closely related to lidocaine. By analogy with various ring-substituted anilides, lidocaine might be expected to have the *trans* amide configuration (1, 2). We have found, surprisingly, that the IR spectra of the free base in the solid state (flurolube/mineral oil) and in solution (CCl_4) indicated the existence of the *cis* amide configuration, in contrast to salts of the base where the *trans* configuration has been deduced (3).

The following evidence supports our assignment of the *cis* structure (Fig. 1). In the solid state the IR spectrum of lidocaine showed only one symmetrical broad band at 3,235 cm.$^{-1}$ due to the NH stretching vibration, a strong amide I band at 1,662 cm.$^{-1}$ (with a weak shoulder at 1,685 cm.$^{-1}$), and a strong amide II band at 1,490 cm.$^{-1}$. On deuterium substitution these bands were replaced by bands at 2,385, 1,655, and 1,407 cm.$^{-1}$, respectively. The IR spectrum of a CCl_4 solution (0.26 *M*) of lidocaine gave only one symmetrical band at 3,312 cm.$^{-1}$ attributed to NH stretching, and strong bands at 1,690 and 1,494 cm.$^{-1}$ for the amide I and II bands, respectively. On deuteration these bands were shifted to 2,460, and 1,694 and 1,393 cm.$^{-1}$, respectively. Since the amide II frequency [a mixed vibration involving NH in-plane bending and C-N stretching (4)] is quite characteristic of *trans* amides at ~1,550 cm.$^{-1}$, and of *cis* amides at ~1,485 cm.$^{-1}$, it is inferred that the free base has the *cis* amide configuration. Moreover, the fact that the amide NH stretching band at 3,312 cm.$^{-1}$ in CCl_4 shows no shift on dilution to 0.003 moles/l. confirms the absence of polymeric *trans* forms and supports the existence of dimeric *cis* amide forms (4).

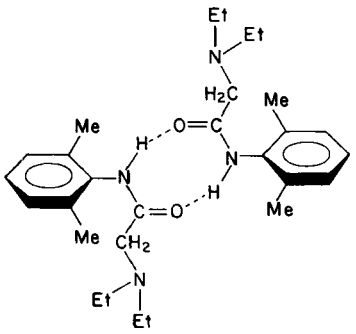

Figure 1—*Lidocaine in the* cis *amide configuration.*

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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Received January 2, 1969.
Accepted for publication February 24, 1969.

The authors are indebted to Astra Pharmaceuticals (Canada) Ltd., Cooksville, Ontario, for a gift of lidocaine, to Mr. H. W. Avdovich for the PMR spectrum, and to Mr. Z. R. Regnier for the IR spectra.

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

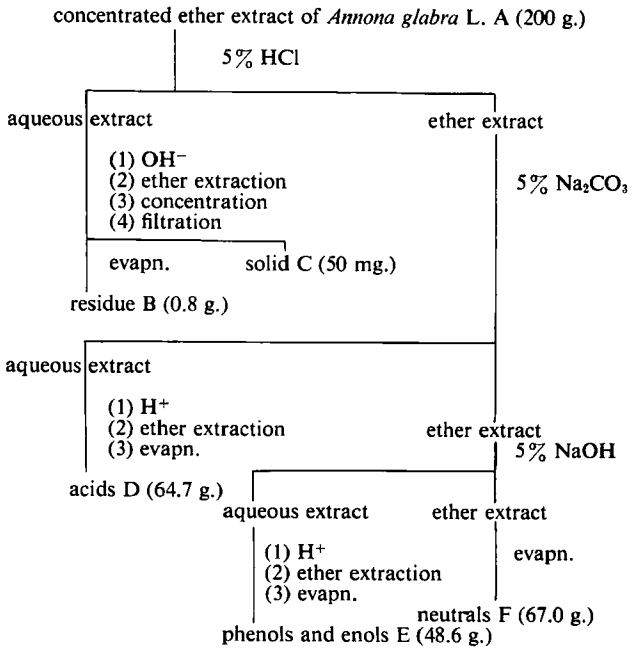
Keyphrases ☐ Cytotoxic alkaloid—*Annona glabra* ☐ Liriodenine—isolation, identification ☐ TLC—separation, identity ☐ UV spectrophotometry—identity ☐ Visible spectrophotometry—identity ☐ NMR spectroscopy—identity, structure ☐ IR spectrophotometry—identity ☐ Mass spectroscopy—identity, structure

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.