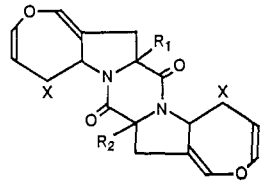
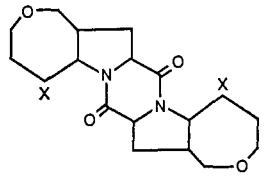
and 310 mμ (ε 2200); nmr[17] τ 2.92 (d, 2, J = 7.5, OC*H*=CHCO) and 4.02 (d, 2, J = 7.5 Hz, OCH=C*H*CO).





5a R_1=H, R_2=SCH_3, X=<H, OAc

5b R_1=R_2=H_G, X=<H, OAc

5c R_1=R_2=H, X=<H, OH

5d R_1=R_2=H, X=O

6a X=<H_C, OAc

6b X=<H, OH

6c X=O

Four moles of hydrogen was consumed when bisdethioacetylaranotin was hydrogenated; the reaction product was octahydrobisdethioacetylaranotin[18] (**6a**); mass spectrum, M^+ 450; nmr τ 4.63 (sextet, 2, C*H*OAc), between 5.5 and 6.5 (m, 12, four CH_2O and four C*H*NCO groups), and 7.95 (s, 6, two OCOCH_3). Spin decoupling showed that H_C was coupled to one proton at τ 5.65 and two protons at τ 7.74 and 8.09, consistent with structure **6a**. Deacetylation of **6a** afforded the diol **6b**, $C_{18}H_{26}O_6N_2$; mp 230–232°; ir (Nujol) 3350, 3200 (OH) and 1635 cm^{-1} (amide); CD (H_2O), negative Cotton effect at 222 mμ. Oxidation of **6b** with acetic anhydride and dimethyl sulfoxide[19] afforded the ketone **6c**, $C_{18}H_{22}O_6N_2$; mp 229–230°; ir (Nujol) 1720 (ketone) and 1655 cm^{-1} (amide); nmr τ 5.07 (d, 2, CONC*H*CH).

Acknowledgments. We thank Mr. W. Jankowski of Varian Associates for 100-MHz and double-irradiation experiments.

(17) The nmr spectrum was taken in CF_3COOH. All other nmr spectra were taken in $CDCl_3$ with TMS as internal standard.

(18) This compound was chromatographically homogeneous, but resisted crystallization. Upon deacetylation it afforded crystalline diol **6b**. All compounds gave satisfactory elemental analyses except for **6a**. All compounds gave the expected molecular ions in the mass spectra.

(19) J. D. Albright and L. Goldman, *J. Am. Chem. Soc.*, 87, 4214 (1965).

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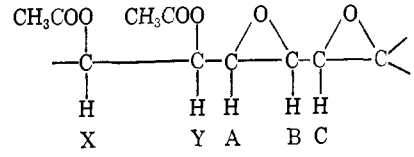
Crotepoxide, a Novel Cyclohexane Diepoxide Tumor Inhibitor from *Croton macrostachys*[1,2]

Sir:

In the course of a continuing search for tumor inhibitors of plant origin, alcoholic extracts of the fruits of *Croton macrostachys* Hochst. *ex* A. Rich. (Euphorbiaceae)[3] showed significant inhibitory activity in Lewis lung carcinoma in mice (LL).[4] We report herein the isolation and structural elucidation of crotepoxide, a novel tumor-inhibitory cyclohexane diepoxide derivative from *C. macrostachys*.

Fractionation of the ethanol extract, guided by assay against LL, revealed that an active principle was concentrated, successively, in the methanol layer of a 10% aqueous methanol–Skellysolve B partition and in the 1-butanol layer of a 1-butanol–water partition. Further fractionation involving silicic acid chromatography yielded crotepoxide (II),[5] $C_{18}H_{18}O_8$; mp 150–151°; $[\alpha]^{25}D$ +74° (*c* 1.70, $CHCl_3$); λ_{max}^{MeOH} 274 mμ (ε 1050) and 281 mμ (ε 860); $\lambda_{max}^{CHCl_3}$ 3.35, 5.71, 5.78, 6.24, 6.31, 6.89, 7.29, 7.87, 8.20, 9.00, 9.60, 10.24, and 11.12 μ; nmr signals (in $CDCl_3$) at τ 2.28 (5 H, m, aromatic), 4.27 (1 H, d, J_{XY} = 9.5 cps, >CHOAc), 5.02 (1 H, d, d, J_{XY} = 9.5 and J_{AY} = 1.5 cps, >CHOAc), 5.42 and 5.75 (2 H, doublets, J = 12.0 cps, CH_2OCOPh), 6.32 (1 H, d, J_{BC} = 2.5 cps), 6.56 (1 H, d, d, J_{BC} = 2.5 and J_{AB} = 4.0 cps), 6.90 (1 H, d, d, J_{AB} = 4.0 and J_{AY} = 1.5 cps), 7.88 (3 H, s, acetate), and 7.95 (3 H, s, acetate).



I

Crotepoxide was converted into several crystalline derivatives. Hydrogenation using platinum oxide catalyst yielded the hexahydro derivative ($C_{18}H_{24}O_8$; mp 121–122°; $[\alpha]^{30}D$ +59° (*c* 1.35, $CHCl_3$)) which exhibited no signals for aromatic protons in the nmr spectrum. Treatment with aqueous methanolic potassium hydroxide yielded a triol, V ($C_7H_{10}O_5$; mp 101–102°; $[\alpha]^{27}D$ +30° (*c* 1.06, CH_3OH)), and benzoic acid. Treatment with aqueous methanolic hydrochloric acid for 30 min yielded the monochlorohydrin III ($C_{18}H_{19}ClO_8$; mp 170–171°; $[\alpha]^{29}D$ −4° (*c* 1.35, $CHCl_3$)), while prolonged treatment yielded the deacetyldichlorohydrin IV ($C_{14}H_{16}Cl_2O_6$; mp 241–242°, $[\alpha]^{25}D$ −10° (*c* 0.71, CH_3OH)). IV was readily converted to a triacetate, $C_{20}H_{22}Cl_2O_9$, mp 217–218°, and under more drastic conditions to a tetraacetate, $C_{22}H_{24}Cl_2O_{10}$, mp 153–154°. The triacetate was unreactive toward Jones reagent, and its nmr spectrum in acetone or in DMSO showed a sharp singlet for the hydroxyl proton, indicative of the direction of opening of the second epoxide to yield IV.

Treatment of crotepoxide with aqueous methanolic hydriodic acid yielded a monoiodohydrin, VI ($C_{18}H_{19}IO_8$; mp 143–144°; $[\alpha]^{28}D$ −46° (*c* 1.40, $CHCl_3$)), and an ene diol derivative, VII ($C_{18}H_{20}O_8$; mp 145–146°; $[\alpha]^{28}D$ +127° (*c* 1.51, $CHCl_3$)). The ene diol was converted into the triacetate ($C_{20}H_{22}O_9$; mp 141–142°; $[\alpha]^{28}D$ +151° (*c* 0.91, $CHCl_3$)) and, under more drastic conditions, into an oily product, the spectral characteristics of which were in agreement with the tetraacetate structure. Oxidation of the ene diol with Jones

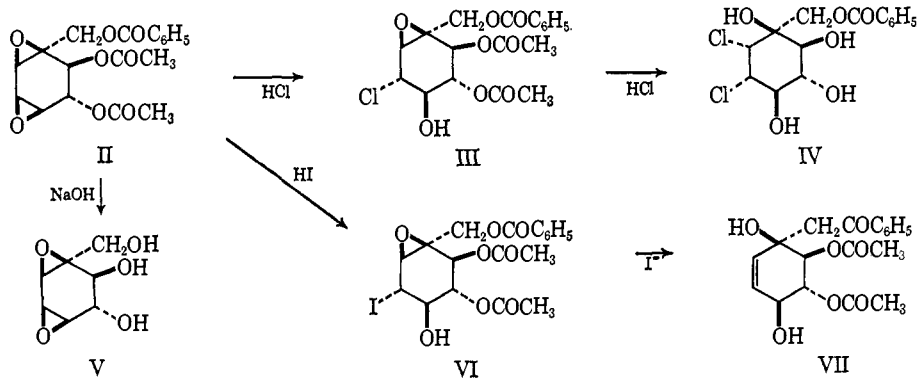
(1) Tumor Inhibitors. XXIX. Part XXVIII: S. M. Kupchan, A. P. Davies, S. J. Barboutis, H. K. Schnoes, and A. L. Burlingame, *J. Am. Chem. Soc.*, 89, 5718 (1967).

(2) Supported by grants from the National Cancer Institute (CA-04500) and the American Cancer Society (T-275), and a contract with the Cancer Chemotherapy National Service Center (CCNSC), National Cancer Institute, National Institutes of Health (PH-43-64-551).

(3) Fruits were gathered in Ethiopia in March 1965. The authors acknowledge with thanks receipt of the dried plant material from Dr. Robert E. Perdue, Jr., U. S. Department of Agriculture, Beltsville, Md., in accordance with the program developed with the U. S. Department of Agriculture by the CCNSC.

(4) The *in vivo* inhibitory activity was assayed under the auspices of the Cancer Chemotherapy National Service Center by the procedures described in *Cancer Chemotherapy Rept.*, 25, 1 (1962).

(5) Crotepoxide showed significant inhibitory activity against Walker carcinosarcoma 256 in rats at 300 mg/kg and Lewis lung carcinoma in mice at 200 mg/kg.



reagent yielded an oil, with spectral characteristics of an α,β-unsaturated ketone, and hydrogenation of the oil yielded the saturated ketone ($C_{18}H_{20}O_8$; mp 117–118°; $[\alpha]^{24}D$ −3° (*c* 1.00, $CHCl_3$).

Crotepoxide iodohydrin crystallized from ether in the monoclinic system, space group $P2_1$, with two molecules of $C_{18}H_{19}IO_8$ in a cell of dimensions a = 5.21, b = 15.85, c = 12.18 Å; β = 100° 42′. The X-ray reflections were recorded on equiinclination Weissenberg photographs of the 0*kl* through 4*kl* layers, and visual estimation of the intensities gave a total of 1150 $|F_o$ values. The coordinates of the iodine atom were derived from a Patterson synthesis, and the carbon and oxygen atoms were located in three-dimensional electron-density distributions calculated with weighted Fourier coefficients.[6] The initial maps were complicated by the pseudo-symmetry associated with the space group $P2_1$. The approximate atomic coordinates are now being refined by least-squares calculations, and the present value of R is 16%. The absolute configuration of the molecule was determined by Bijvoet's anomalous-dispersion method.[7]

The results of the X-ray analysis establish that the iodohydrin has the constitution and absolute stereochemistry VI. The ring adopts a half-chair conformation in the crystal, with the neighboring hydroxyl and acetyl substituents oriented equatorially. That the compound is in this stable conformation in solution as well is indicated by the magnitude of the coupling constants for the protons on the carbons bearing the relevant functional groups. It may be concluded that the epoxide opening proceeded in a *trans*-diaxial fashion and that crotepoxide has structure II. The coupling constants derived from the nmr spectra are in good accord with structure II. It is apparent that initial *trans*-diaxial opening of the epoxide, to an intermediate in an unfavorable boat conformation, is followed by inversion to the half-chair form with the neighboring iodine and hydroxyl group in equatorial conformations.[8]

Crotepoxide belongs to a small group of naturally occurring highly oxygenated cyclohexane derivatives, other members of which are terreic acid,[9] epoxydone,[10] senepoxyde and seneol,[11] and shikimic acid. However, crotepoxide is the only member of this group which possesses the diepoxide functionality. This function has been shown earlier to confer tumor-inhibitory activity on other classes of synthetic compounds.[12]

Investigations are in progress to determine the significance of various structural features in relation to the tumor-inhibitory activity of crotepoxide.

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A Randomly Labeled Tropylium Ion in the Mass Spectrum of Toluene-α,1-$^{13}C_2$[1]

Sir:

Toluene has been shown to fragment under electron impact by the sequence shown in eq 1.[2,3] Considerable evidence has accumulated[4,5] which demonstrates that the $C_7H_7^+$ ion is not a simple benzyl ion (I) but that its fragmentations can be explained by a tropylium ion structure (II).[4] Other isomeric hydrocarbons and α-substituted toluenes fragment *via* a $C_7H_7^+$ ion of similar

$$\begin{array}{lccccccc} & C_7H_8^+ & \xrightarrow{-H\cdot} & C_7H_7^+ & \xrightarrow{-C_2H_2} & C_5H_5^+ & \xrightarrow{-C_2H_2} & C_3H_3^+ \\ m/e & 92 & & 91 & & 65 & & 39 \end{array} \qquad (1)$$

properties. Much of the earlier evidence for a tropylium ion structure rests on isotope-labeling data, perhaps the most significant being that derived from the spectrum of toluene-α-^{13}C,[3] in which nearly random loss of ^{13}C occurred in the $C_7H_7^+ \rightarrow C_5H_5^+$ transition. However, the earlier data do not allow one to distinguish between a tropylium ion arising from simple 1,2 insertion of the methyl (α) carbon (path a) and a tropylium ion formed in some way by random insertion of the α carbon between any carbon–carbon bond in the benzene ring (path b). We present here evidence which indicates

(1) Presented in part at the 154th National Meeting of the American Chemical Society, Chicago, Ill., Sept 1967, Abstracts, S85.
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