ture of 1.5 g. of ethyl acetamido-2-(benzylveratryl)-malonate and 40 ml. of 47% hydriodic acid, stabilized with hypophosphite, was refluxed under a slow stream of nitrogen for 9 hr. Excess hydriodic acid was distilled off *in vacuo* at below 95°. The yellow viscous oily residue was dissolved in a minimum of water, and the amino acid was liberated by adjusting to pH 6 with a saturated potassium carbonate solution. The colorless solid was recrystallized from dimethylformamide, m.p. 246–248° dec. The yield was 0.70 g. (71.5%).

Anal. Calcd. for $C_{19}H_{21}NO_4$: C, 66.88; H, 5.96. Found: C, 66.79; H, 6.22.

Extensive decomposition occurred when freshly distilled and hypophosphite-stabilized hydrobromic acid was substituted for hydriodic acid in this experiment.

Communications to the Editor

The Tumor-Enhancing and Irritant Principles from *Croton tiglium L.*

Sir:

Croton oil is obtained from *Croton tiglium L.* (Euphorbiaceae) by pressing of the seed and has long been known for its vesicant, toxic, and purgative activity.[1] In 1941 Berenblum[2] showed that this material is also a potent tumor-enhancing agent, *i.e.*, it stimulates the appearance and rapid growth of tumors on mouse epidermis pretreated with a minute dose of a carcinogenic hydrocarbon. At the low concentration used the hydrocarbon, when applied alone, is inactive and croton oil itself is not carcinogenic. This unique activity of croton oil has important implications in studies of cancer causation and many attempts have been made to isolate and determine the structure of the active components.[1,3–5] Tumor-enhancing agents of known chemical constitution are all much less active than croton oil.[6]

The present report deals with the isolation of two active materials and the structure elucidation of a pure crystalline material obtained by catalytic hydrogenation of a biologically active fraction of croton seed extract.

The shelled seed of *Croton tiglium L.* was extracted with methyl alcohol to give a 41% yield of an oily extract. The vesicant fraction of the extract, croton resin (I), was prepared by solvent partition between aqueous methyl alcohol (1:9) and hexane.[1] This polar mixture of compounds (3% of whole extract) has shown potent tumor-enhancing activity[2] and it was therefore chosen for further chemical studies and biological assay. Thin layer chromatography of I on silica gel showed the presence of at least 14 components. Column chromatography of I on acid-washed florisil gave an amorphous solid (II, 1.0% of whole extract). Both I and II showed a pronounced increase in tumor-enhancing activity compared to that of the whole croton seed extract or commercial croton oil.[7] Thin layer chromatography of II showed the presence of four components (A, B, C, and D). This material was further fractionated by countercurrent distribution and thin layer chromatography to give two biologically active amorphous materials, A and C, which showed single spots on thin layer chromatograms. Both A and C are irritant and show tumor-enhancing activity. Compound C was tested on 20 female Swiss Millerton mice initiated with 300 γ of 7,12-dimethylbenz[*a*]anthracene. The promotor (C) was applied three times weekly, 5 γ per application. First tumors were observed at 44 days after initiation and at 68 days 17 animals bore tumors. Tumors were not observed in appropriate control groups.

All the components of II are unsaturated and can be hydrogenated with palladium black as catalyst in methyl alcohol solution. Catalytic hydrogenation of II followed by countercurrent distribution (lower phase: aqueous methyl alcohol, 0.5:9.5, containing 0.4% glacial acetic acid; upper phase: hexane; 200 tubes, 10 ml. per phase, 400 transfers) gave a 50% yield of hydrogenation product which was purified by chromatography on silica gel plates (2 mm. thickness.)[8] The band with the highest R_f was eluted with ether and gave a crystalline product from ethyl alcohol–water; colorless needles, m.p. 96°, R_f 0.25 (hexane–ether–acetic acid, 9:1.5:0.5, silica gel). The analytical data agree with a molecular formula, $C_{36}H_{60}O_8$, molecular weight 620, with three ester functions, two free hydroxyl groups, and six carbon-methyl groups. The compound is optically active, $[\alpha]D^{21}$ +124.6° (l = 1 dm., c 0.77, chloroform).

The ultraviolet absorption spectrum showed two low-intensity maxima in cyclohexane and in methyl alcohol (cyclohexane: λ_{max} 248 mμ; ϵ_{max} 410; λ_{max} 302 mμ; ϵ_{max} 56) which suggested the presence of a polysubstituted benzene ring.

The infrared absorption spectrum was examined in various media and revealed the following features: intramolecularly bonded hydroxyl (0.001 M in carbon tetrachloride: a weak band at 3570 cm.$^{-1}$ and a strong sharp band at 3440 cm.$^{-1}$); intense aliphatic stretching and bending absorptions (KBr pellet, methyl and methylene at 2965, 2935, 2860, 1465, and 1370 cm.$^{-1}$; tertiary –CH at 2885 cm.$^{-1}$); intense ester carbonyl absorption (KBr pellet, 1742, 1720 cm.$^{-1}$; 0.001 M in carbon tetrachloride, 1735 cm.$^{-1}$); weak aromatic C=C absorption (0.05 M in chloroform, 1600 cm.$^{-1}$). Aliphatic C=C absorption is present in II, A, and C at 1650 and 1630 cm.$^{-1}$ but does not appear in the infrared spectrum of the crystalline hydrogenated compound. However, the other bands in the infrared spectra of A and C are very similar to that of the hydrogenated compound.

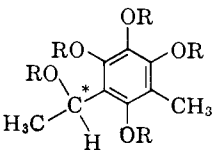
The n.m.r. spectrum showed a series of four peaks from 0.9 to 1.25 p.p.m. ascribed to aliphatic CH, CH_2,

(1) E. Cherbuliez, E. Ehninger, and K. Bernhard, *Helv. Chim. Acta*, **15**, 658 (1932).
(2) I. Berenblum, *Brit. J. Cancer*, **1**, 44 (1941).
(3) N. L. Drake and J. R. Spies, *J. Am. Chem. Soc.*, **57**, 184 (1935).
(4) W. S. Lijinsky, *Biochem. J.*, **70**, 5P (1958).
(5) E. Hecker, *Angew. Chem. Intern. Ed. Engl.*, **1**, 602 (1962).
(6) R. K. Boutwell and D. K. Bosch, *Cancer Res.*, **19**, 413 (1959).
(7) Biological tests were carried out using established test procedures: I. Berenblum and P. Shubik, *Brit. J. Cancer*, **1**, 379 (1947).
(8) I. Bekersky, *Anal. Chem.*, **35**, 261 (1963).

and CH_3 protons (type 1). Four weaker peaks at 2.0, 2.15, 2.25, and 2.34 p.p.m. are characteristic for methyl or methylene protons next to oxygen-bearing carbon and/or aromatic ring (type 2). A peak at 4.65 p.p.m. is ascribed to a hydroxyl proton. Integration indicates 46 protons of type 1, 12 of type 2, and 2 hydroxyl groups.

The compound does not react with diazomethane and does not give a coloration with ferric chloride. Hydrolysis with 10% aqueous ethanolic sodium hydroxide (1:1) resulted in darkening of the solution. The acidified hydrolysate gave a persistent purple color with ferric chloride. Three acids were obtained in equal molar ratios from the hydrolysate and were identified by gas–liquid chromatography of the methyl esters: 1-methylbutyric, capric, and lauric acids. This is consistent with the experimentally determined saponification equivalent for three ester functions. Subtracting the various functions indicated, a C_7H residue is left. This residue can be accommodated reasonably as a fully substituted benzene ring, and this is in agreement with the ultraviolet absorption and infrared spectra. The CH residue left constitutes then the center for optical activity. The high specific rotation suggests also that this optically active center contains a strongly hydrogen-bonded function.[9] The instability of the water-soluble phenolic residue indicated either a pyrogallol or a 1,2,3,5-tetrahydroxybenzene nucleus.

From the n.m.r. data the two remaining carbon–methyl groups must be attached either to a carbon atom which also carries oxygen or directly to an aromatic ring. The available information leads to a partial structure



where each R is one of the following: $-COCH(CH_3)-CH_2CH_3$; $CO(CH_2)_8CH_3$; $CO(CH_2)_{10}CH_3$; H(two).

Other closely related hydrogenation products have been isolated in crystalline form and these differ from the compound described in the nature of the acids and possibly also their relative positions.

Compounds B and D and the catalytic hydrogenation product (m.p. 96°) described here have not yet been tested for cocarcinogenic activity. However, the close similarity in infrared absorption spectra and chemical properties of A, B, C, D, and the hydrogenated compound leaves little doubt that they are all chemically closely related. The exact nature of the structural features required for activity are currently under examination.

It is expected that the biologically active material and the hydrogenation product described will exhibit both hydrophilic and lipophilic behavior which may play an important role in the biological activity of the material. It is of interest also that the partial structure proposed is similar to the long chain olefinic catechols which constitute the vesicant principles of poison ivy.[10]

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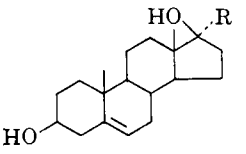
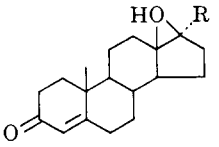
Aldosterone Antagonists. 2′3′α-Tetrahydrofuran-2′-spiro-17-(4-androsten-3-one) and Related Compounds

Sir:

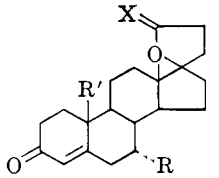
The spirolactones, *e.g.*, XVII, prepared by Cella and associates,[1] are effective aldosterone antagonists in animals[2] and in humans.[3] One series of spirolactone



I, R = $-C\equiv CCH_2OTHP$
II, R = $-CH_2CH_2CH_2OTHP$
XVIII, R = $CH_2CH_2CH_2OH$



III, R = $-CH_2CH_2CH_2OTHP$
IV, R = $-CH_2CH_2CH_2OH$
V, R = $-CH_2CH_2CH_2OTS$



VI, R′ = CH_3, R = H, X = H_2
VII, R′ = CH_3, R = $SCOCH_3$, X = H_2
XVI, R′ = H, R = H, X = H_2
XVII, R′ = CH_3, R = H, X = O
XXIX, R′ = CH_3, R = $SCOCH_3$, X = O

analogs, the spirolactams,[4] have marginal activity. Cella and co-workers[1b] showed that 3-keto-Δ^4 system in the A-ring of spirolactones was an activity-enhancing, although not an essential, function. Introduction of methyl groups [1d] and increased unsaturation,[1b] effective activity-enhancing devices in the antiinflammatory steroid series, have had relatively little effect on spirolactone activity. The 9-fluoro-11-oxygenated derivatives,[1c] however, are more active.

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