temperature overnight. The resulting suspension was filtered and an amorphous precipitate which formed was discarded. The filtrate was evaporated to dryness *in vacuo*, and the residue was suspended in cold water (5 ml.) and filtered to give 2.1 g. (93%) of a crystalline product, m.p. 165–170°. Upon repeated recrystallization from ethyl acetate colorless crystals shaped like arrow heads were obtained, m.p. 175–176°.

Anal. Calcd. for $C_7H_8N_4S$: C, 46.64; H, 4.47; N, 31.09; S, 17.79. Found: C, 46.45; H, 4.98; N, 31.14; S, 17.74.

The same material was obtained by reaction of an ethanolic solution of III with methyl mercaptan in a sealed tube at 100°, in lower yield.

6-Ethylthiomethylpurine (XIV).—6-Chloromethylpurine (III, 3.4 g., 0.020 mole) was dissolved in 70% aqueous ethanol (30 ml.), ethyl mercaptan (6 ml.) was added, and the solution was heated to 50°. After 1 hr., sodium acetate (2 g.) was added, and the mixture was heated at the same temperature for an additional 5 hr. The resulting solution was evaporated to dryness *in vacuo*, and the residue was recrystallized from ethyl acetate to give a colorless crystalline product (0.95 g., 24%), m.p. 144°. Upon three recrystallizations from ethyl acetate, short needles were obtained, m.p. 154°.

Anal. Calcd. for $C_7H_{10}N_4S$: C, 49.46; H, 5.19; N, 28.84; S, 16.51. Found: C, 49.39; H, 5.20; N, 28.80; S, 16.45.

6-Phenylthiomethylpurine (XV).—Benzenethiol (1.25 ml., 12 mmoles) was added to a stirred solution of 6-chloromethylpurine (III) (1.68 g., 10 mmoles) and sodium acetate (2 g.) in 70% aqueous ethanol (15 ml.). The mixture was heated at 80° for 16 hr. and a small precipitate of amorphous material was filtered off and discarded. The filtrate was concentrated to dryness *in vacuo* and taken up in water (10 ml.). The resulting crystalline material was washed with a little cold water and then thoroughly with ether, to yield 1.55 g. (64%) of a yellow product, m.p. 148°. Repeated recrystallizations from ethyl acetate gave thin, light yellow needles, m.p. 150°.

Anal. Calcd. for $C_{12}H_{10}N_4S$: C, 59.48; H, 4.16; N, 23.12; S, 13.24. Found: C, 59.54; H, 4.09; N, 23.28; S, 13.18.

6-Benzylthiomethylpurine (XVI).—6-Chloromethylpurine (III, 2.50 g., 0.0148 mole) was dissolved in a solution of anhydrous sodium acetate (2.50 g.) in 70% aqueous ethanol (25 ml.). Benzyl mercaptan (5.5 ml., 0.042 mole) was added and the mixture refluxed for 6 hr. The resulting solution was evaporated to dryness *in vacuo*, washed with cold water, and recrystallized from water to yield 1.87 g. (49%) of colorless crystals, m.p. 94°. Further recrystallization from water afforded rectangular plates, m.p. 96°.

Anal. Calcd. for $C_{13}H_{12}N_4S$: C, 60.91; H, 4.72; N, 21.86; S, 12.51. Found: C, 61.12; H, 4.71; N, 22.10; S, 12.83.

The same product was obtained by treatment of 6-acetylthiomethylpurine (IV) with benzyl chloride in similar conditions to those described above.

6-S-Purinyl(6′-thiomethylpurine) (XVII).—A solution of 6-chloromethylpurine (III, 0.340 g., 2 mmoles) in 5 ml. of ethanol was mixed with 6-mercaptopurine (0.320 g., 2 mmoles), and water (30 ml.) was added. After a few minutes of boiling, the pH dropped to 4. Anhydrous sodium acetate (0.5 g.) was added, and the solution refluxed for 1 hr. The precipitate which appeared on cooling was collected, washed with cold water, and dried to yield 0.27 g. (43%) of brown-yellow needles, m.p. 290–292°. Upon recrystallization from water, thin yellow needles, m.p. >300°, were obtained.

Anal. Calcd. for $C_{11}H_8N_8S$: C, 46.47; H, 2.84; N, 39.42; S, 11.28. Found: C, 46.44; H, 3.03; N, 39.01; S, 11.58.

XVII could also be obtained in similar yield from 6-chloropurine and 6-acetylthiomethylpurine (IV) using the conditions described above.

Reaction of 6-Substituted Purines with Thioacetamide.—To a solution of 6-chloropurine[17] (0.65 g., 4 mmoles) in ethanol (25 ml.) thioacetamide (0.90 g., 12 mmoles) was added and the mixture refluxed for 3 hr. On cooling, a precipitate of 6-mercaptopurine (0.60 g., quantitative yield) was obtained. A yield of 32% was obtained when 6-iodopurine was used.

Similar treatment of 6-N-hydroxylaminopurine[19] and 6-hydrazinopurine[20] did not lead to 6-mercaptopurine, and the starting materials were recovered unaltered.

Acknowledgment.—The authors wish to thank Dr. George B. Brown, Dr. J. J. Fox, and Dr. W. Berkowitz for their interest and valuable discussions. They wish to express their gratitude to Dr. Sugiura of the Division of Experimental Chemotherapy for the data on the screening tests, and to Miss Lillian Medrek and Mr. Stevan Adler for technical assistance. One of us (A. G.-S.) would like to express his gratitude to Professor A. R. Todd and Dr. D. M. Brown for the hospitality of their laboratory where a portion of these studies was conducted.

Structural Studies of an Active Principle from *Croton tiglium* L.

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Received February 18, 1965

The isolation, semisynthesis, and structure elucidation of a pure crystalline highly active tumor-enhancing principle from the seed of *Croton tiglium* L. is described. The alkaline hydrolysis of the pure crystalline cocarcinogen called C-3 (I) yielded myristic and acetic acids and a polyhydroxy compound, $C_{20}H_{28}O_6$. Crystalline derivatives of the active compound were prepared. The chemistry of the active material, the parent alcohol, and their crystalline derivatives is discussed.

In a recent communication[2] we reported the isolation and partial structure for an active cocarcinogen from *Croton tiglium* L. Additional n.m.r. studies with decoupling suggested a revision of several discrepancies in the proposed structure.

We now present the results of further work on the active principle from *Croton tiglium* L. In earlier communications[3,4] Hecker, *et al.*, reported the isolation of three cocarcinogens A_1, B_1, and B_2 having the same parent alcohol, but B_1 and B_2 differing in the acids forming the two ester functions. However, these workers failed in their attempts to obtain a crystalline compound, indicating that cocarcinogens, A_1, B_1, and B_2 are not pure chemical entities but amorphous chromatographic fractions.

In 1941 Berenblum[5] showed that croton oil is a potent tumor-enhancing agent, *i.e.*, it stimulates the appearance and rapid growth of tumors on mouse skin after application of a minute dose of a carcinogenic

(1) To whom inquiries should be addressed.

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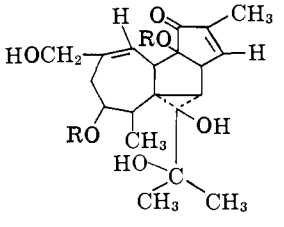
hydrocarbon. At the low concentration used the hydrocarbon, when applied alone, is inactive and croton oil itself is not carcinogenic. This unique activity of croton oil has important implications in studies of cancer causation and many attempts have been made to isolate and determine the structure of the active components.[5-9]

The seed of *Croton tiglium* L. was extracted with methyl alcohol to given an oily extract. This extract has vesicant, toxic, irritant, and tumor-enhancing activities. The separation and isolation of the crystalline active principle from all the other components from croton resin (about eight noncrystalline fractions, all with similar infrared spectra) was hampered by the fact that these compounds were held together by strong polar interactions which were not broken by solvent partition and column chromatography. The complete separation and isolation of the crystalline, active, principle compound C-3 (I) was finally accomplished



I

R = $COCH_3$ or $CO(CH_2)_{12}CH_3$

through the combination of extraction methods, column chromatography, countercurrent distribution, and thick layer chromatography. Compound I is unsaturated and can be hydrogenated with palladium black catalyst in methyl alcohol.

The ultraviolet and infrared absorption of I shows an α,β-unsaturated ketone carbonyl group. A carbonyl derivative of I could not be obtained with 2,4-dinitrophenylhydrazine. Previous workers[4] also were not able to prepare a carbonyl derivative. There are three free hydroxyl and two esterified hydroxyl groups. The latter, on alkaline hydrolysis, gives acetic and myristic acids. One of the free hydroxyl groups was esterified with 4′-nitroazobenzene-4-carbonyl chloride and gave II. The two other hydroxyl groups resisted esterification. The esterifiable hydroxyl group is therefore primary, the two other remaining free hydroxyl groups are tertiary. The presence of an allyl alcohol group was established by n.m.r. studies and oxidation of I with activated manganese dioxide.[10] The product was converted to its 2,4-dinitrophenylhydrazone (III).

Catalytic hydrogenation of I with palladium black in methyl alcohol gives mixtures of products. Chromatography on thick layer plates (silica gel G) gave the hydrogenated derivative IV. An acetate derivative of IV could not be obtained. Attempts to esterify IV with other acetylating agents failed. Quantitative hydrogenation indicates the presence of two double bonds and four rings. The hydrogenated derivative (IV) was not oxidized by chromic acid in pyridine. Accordingly, IV must contain two tertiary hydroxyl groups. Active hydrogen determination of IV showed that only two of the oxygen atoms are present in hydroxyl groups. Infrared absorption and n.m.r. spectrum of IV showed complete saturation of the double bonds. Integrated n.m.r. shows three free hydroxyls for I and two free hydroxyl groups for the hydrogenated derivative IV. The n.m.r. spectrum of I in dimethyl sulfoxide-d_6 contains a peak for the primary and allylic alcohol (2.91 p.p.m.) and a singlet for the tertiary alcohol groups at (5.40 p.p.m.). The n.m.r. spectrum for the hydrogenated derivative (IV) gives only a singlet for the two tertiary alcohol groups present at 5.40 p.p.m. Accordingly there are three free hydroxyl groups in I to two free hydroxyl groups in IV, indicating that the primary and allylic hydroxyl group was lost on hydrogenolysis of IV. The allyl alcohol system is known to be susceptible to hydrogenolysis.[11] N.m.r. absorption at 2.34 p.p.m. for CH_3C of IV confirmed that the allylic primary hydroxyl group was lost during hydrogenation and a methyl group attached to a ring was formed.

Alkaline Hydrolysis of I.—Cocarcinogen C-3 (I) was treated with 5% $Ba(OH)_2$ in methyl alcohol to give a crystalline parent polyhydric alcohol V. The mass spectrum, elemental analysis, and active hydrogen determination showed that V possessed the formula $C_{20}H_{28}O_6$. The n.m.r. spectrum in dimethyl sulfoxide-d_6 shows peaks for one isopropyl group at 1.25 p.p.m. (6 protons), for the primary and allylic hydroxyl group (1 proton) at 2.91 p.p.m., a doublet at 4.17 p.p.m. is ascribed to one secondary hydroxyl proton, a singlet at 5.52 p.p.m. is ascribed to three tertiary hydroxyl protons, and peaks at 5.44 and 7.55 p.p.m. are ascribed to two olefinic protons. The proton at 7.55 p.p.m. is on an unsaturated carbon conjugated with a keto group. An integrated spectrum indicates five OH protons: one proton for one primary and allylic hydroxyl group, one secondary hydroxyl group, and three tertiary hydroxyl groups. Infrared and ultraviolet absorption indicates an α,β-unsaturated cyclic ketone on a five-membered ring.

Two acids were obtained in equal molar ratios from the hydrolysate of I and were identified by gas–liquid chromatography and a mass spectrum of the methyl esters of acetic and myristic acids. This is consistent with the experimentally determined saponification equivalent for the two ester functions.

The parent polyhydric alcohol V obtained from I was converted by treatment with acetic anhydride and myristoyl chloride (1 mole of each) in pyridine to an acetyl myristoyl diester cocarcinogen (VI). The mass spectrum, n.m.r., infrared absorption spectrum, and elemental analysis showed that VI is identical with I. The compound is optically active, $[\alpha]^{24}D$ +48° (dioxane); ultraviolet absorption maxima of 232 and 333 mμ (ϵ_{max} 5500, 77) indicate the presence of an α,β-unsaturated cyclic ketone. Melting and mixture melting points of I and VI indicate that both compounds are the same. Biological assay of I and VI shows that

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they are highly active tumor-enhancing cocarcinogens. Compound C-3 was tested on 10 female Swiss Millerton mice initiated with 300 γ of 7,12-dimethylbenz[*a*]-anthracene. The promoter C-3 was applied three times weekly, 5 γ/application. First tumors were observed 40 days after initiation, and after 62 days 8 animals bore tumors. Compounds V, VI, and VII were tested in the same manner as I. The first tumors were observed 68 days after initiation with promoter VI, and 90 days after initiation two animals bore tumors. Compound VII showed slight activity, and V (the parent alcohol) showed no activity after 16 weeks.

Treatment of V with 3 moles of acetic anhydride in pyridine gave VII triacetate. The mass spectrum shows a parent mass of 490, which agrees with the formula $C_{26}H_{34}O_9$. Active hydrogen determinations show that only two oxygen atoms are present in hydroxyl groups.

An integrated n.m.r. spectrum of VII shows two methyl groups for one isopropyl group (1.20–1.22 p.p.m.), two hydroxyl groups (2 protons, 5.48 p.p.m. for 2 tertiary hydroxyl groups), a methylene group attached to an acetate group and to a ring (2 protons, 4.30 p.p.m.), three acetate groups (9 protons, 2.05, 2.08, and 2.11 p.p.m.), two uncoupled hydrogens (2 protons, 2.28–2.41 p.p.m.), and two olefinic protons (5.35 and 6.82 p.p.m.). The proton at 6.82 p.p.m. is on an α,β-carbonyl-conjugated carbon atom, C=C-(CH_3) 1.80 p.p.m. (3 protons), C(CH_3) 1.04–1.06 p.p.m. (3 protons).

Further acetylation of the triacetate VII led to the tetraacetate VIII. Its n.m.r. spectrum shows peaks at 2.04, 2.06, 2.08, and 2.12 p.p.m. for four acetate groups, two peaks at 1.20–1.25 p.p.m. for two methyl groups for one isopropyl group, two olefinic protons at 5.37 and 6.85 p.p.m., and one hydroxyl proton at 5.45 p.p.m.

From the infrared, n.m.r., ultraviolet, and other evidence on hand, a partial structure is presented for the active principle C-3 from *Croton tiglium* L.

Experimental[12]

Extraction of the Seed of *Croton tiglium* L.—Shelled croton seeds (*Croton tiglium* L.) (3.0 kg.) were crushed in a Waring Blendor, 200 g. at a time. The oily crushed seeds were packed in chromatographic columns (12-cm. diameter) with alternate layers of glass wool, the columns were filled with methyl alcohol, and the mixture was allowed to extract at room temperature. The extracts were run at intervals varying between 3–16 hr. and the columns were refilled each time with methyl alcohol. The material was extracted until the methyl alcohol extract left a negligible residue; the extraction required 100 l. of methyl alcohol. The solvent was distilled *in vacuo* in a nitrogen atmosphere at 30°. The extracts were dark brown in color and, on removal of solvent, solid material precipitated. The concentrate was flushed with nitrogen and stored at 4°. An oil and a crystalline precipitate separated. This mixture was filtered through glass wool and traces of solvent were removed from the extract in high vacuum under nitrogen at 50°. The total weight of extract (including the precipitate) was 1030 g. (41%). The weight of the insoluble precipitate was 65 g. This material is crude crotonoside.

Fractionation of the Croton Seed Extract. A. Solvent Fractionation.—The dark brown extract obtained after removal of solid and solvent (965 g.) was a mobile liquid which separated in two layers on standing. This material was dissolved in 2.3 l. of hexane and extracted with 100-ml. portions of methyl alcohol–water (9:1). In the beginning the extract was dark brown in color but after 5–6 extractions became light yellow in color. During the fractionation a straw-colored amorphous solid separated out. This was combined with the methyl alcohol layer. The hexane layer was extracted 20 times with methyl alcohol–water (9:1). By the fifteenth extraction the extract was almost colorless. The methyl alcohol–water extract was then re-extracted with petroleum ether (b.p. 30–60°) (ten 100-ml. portions), and this extract was extracted once with 200 ml. of methyl alcohol–water (9:1). All methyl alcohol–water extracts were combined and all hexane and petroleum ether extracts were combined. No further work was done on the hexane–petroleum ether extract. The methyl alcohol–water layer was filtered to remove an amorphous solid which was probably more crotonoside.[9] The filtrate was evaporated to dryness *in vacuo* at 50° under nitrogen, and the residue was extracted several times with ether; the ether-soluble portion (114 g.) was a dark brown resin. This material was dissolved in ether and washed with five 50-ml. portions of water. A water- and ether-insoluble black gum separated between the ether and water layers and was combined with the water layer and discarded. The water layer was back-extracted twice with ether. The combined ether solutions were dried (Na_2SO_4), filtered, and taken to dryness as before; 71.7 g. of a viscous brown resin was obtained. This material was called croton resin.[9]

B. Florisil Chromatography.—Croton resin (45 g.), dissolved in 50 ml. of ether, was chromatographed on acid-washed Florisil[13] (1500 g.) wetted with hexane. Fractions were eluted with ether and since their thin layer chromatograms were identical and indicated mixtures of at least 4 components, all ether eluates were combined. The elution of this material required 20 l. of ether; 33.1 g. of a viscous resin was obtained. A benzene–methanol (1:1) eluate gave a dark brown residue (10.6 g.) which was not combined with the ether eluate. This material was not used in subsequent work.

C. Countercurrent Distribution.—A portion of the residue from the ether eluate (5.2 g.) was subjected to countercurrent distribution in a 200-tube (20 ml./tube) machine. The solvent for the lower phase was methyl alcohol–water–acetic acid (94.5:5.0:0.5); the upper phase was hexane. The progress of the distribution was followed by thin layer chromatography on silica gel G (Brinkman Instrument Co.) plates using ether as solvent; spots were visualized by spraying with 50% H_2SO_4 and brief heating with an infrared lamp. Fractionation was allowed to proceed for 1000 transfers. At this stage, tubes 0–16 still contained three components; tubes 17–55 contained a mixture which gave only two spots on a thin layer chromatogram; these two materials were labeled A and C with R_f 0.44 and 0.25, respectively. The contents of tubes 17–55 were combined and taken to dryness; 4.10 g. of a white amorphous solid was obtained.

D. Separation of A and C.—A and C were separated by preparative t.l.c. on silica gel G using spray technique.[14] The layer thickness was 2 mm. (about 15 g. of silica gel/20 × 20 cm. plate) with ether as solvent. Seventy milligrams of material was applied per plate as 6-cm. bands. The mixture (4.42 g.) yielded 2.30 g. of A and 2.12 g. of C (yield 65%). Both A and C were colorless amorphous materials which resisted all attempts at crystallization and, although they showed single spots by t.l.c., were subsequently shown to be still mixtures of closely related materials.

Isolation of Compound C-3.—Fraction C (2.0 g.) was further fractionated by countercurrent distribution into the nonactive component C-1 (R_f 0.27) and the active component C-2 (R_f 0.30, 70% yield) (lower phase: nitromethane–nitroethane (22:78); upper phase: 1:1 mixture of pentane–isohexane; 2000 transfers). Tubes 40–95 gave 1.4 g. of C-2. Tubes 96–125 gave 0.5 g. of C-1. C-2 was purified further by preparative t.l.c. on silica gel G plates sprayed with 2% boric acid solution: 60 mg. of sample was applied per plate (2 mm. thickness) and it was developed with 0.5:4:4:1.5 formic acid–pentane–hexane–chloro-

(12) All melting points are uncorrected and measured with a Thomas–Hoover silicone bath. N.m.r. spectra were run in deuteriochloroform using a Varian Associates, HA-100 100-Mc. spectrometer. Spectra were calibrated by the side-band technique.

(13) Acid-washed Florisil: 1500 g. of Florisil was mixed with 20 l. of 2% acetic acid–water. The mixture was stirred for 10 min., then filtered, washed with 20 l. of distilled water, and finally with 15 l. of methanol. After being aerated for 20 min. the dried Florisil was put into an oven at 200°. The activation was completed in 16 hr. It could be kept for several months in a well-stoppered bottle without loss in activity.

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form to yield 0.60 g. of C-3 (I), R_f 0.26; crystallized from ethanol–water; m.p. 73–74°; $[\alpha]^{24}D$ +48°; λ_{max} 233 and 333 mμ (ϵ_{max} 5500 and 75) in ethanol; infrared, 2.95 (OH), 5.76 (ester carbonyl), 5.8–5.85 (cyclic ketone in a five-membered ring), 6.10 μ (C=C double bonds). The n.m.r. spectrum showed a series of peaks from 0.9–1.3 p.p.m. which are characteristic for methyl or methylene protons next to an oxygen-bearing carbon: 2.1, 2.2, 2.3, 2.51 p.p.m., CH_2 α to CO; 5.4 and 7.5 p.p.m. ascribed to two olefinic double bond protons.

Anal. Calcd. for $C_{56}H_{56}O_8$: C, 69.96; H, 9.13; active H (3), 0.57; mol. wt., 616. Found: C, 69.74; H, 9.10; active H (3), 0.56 (Grignard); mol. wt. (mass spectrum), 616.

4′-Nitroazobenzene-4-carboxylic Acid Ester of I.—To 200 mg. of I and 140 mg. of 4′-nitroazobenzene-4-carbonyl chloride[15] were added 10 ml. of dry benzene and 0.1 ml. of pyridine. The mixture was heated to reflux for 1.5 hr., cooled, and extracted with 25 ml. of 0.1 *N* H_2SO_4, 25 ml. of 1% Na_2CO_3 solution, and finally with 25 ml. of water; the organic solvent layer was dried (Na_2SO_4) and the solvent was removed under nitrogen. The red residue was chromatographed on t.l.c. silica gel G plates, and developed with 1:1 ether–hexane. After crystallization from ether–hexane solution, a deep red crystalline ester (II), m.p. 89–90°, was obtained; infrared absorption spectrum in KBr, 2.95 (OH), 5.8–5.85 (CO), 6.1 μ (C=C); λ_{max} (230), 331 (344), (460) mμ [ϵ_{max} in ethanol (15,000), 30,900, (26,800), (634)].

Anal. Calcd. for $C_{49}H_{63}N_3O_{11}$: C, 67.72; H, 7.34; N, 4.83; mol. wt., 869; Found: C, 67.77; H, 7.26; N, 4.89; mol. wt., 872 (osmetric).

Manganese Dioxide Oxidation of I.—The diester I (100 mg.) in $CHCl_3$ (10 ml.) was shaken with activated MnO_2 (200 mg.) for 2 days. The mixture was filtered and the solvent was removed. The residue (75 mg.) was dissolved in ether and chromatographed on Florisil. Elution with hexane–ether (1:1) gave, after evaporation, 62 mg. of a resin. The product was converted into its 2,4-dinitrophenylhydrazone in the usual manner. Chromatography of the crude product on Florisil from hexane–ether (1:1) with elution with the same solvent mixture afforded, after crystallization from ethanol, red needles (45 mg.) of III: m.p. 109–110°; infrared spectrum in a KBr pellet, 5.83 μ (saturated C=C).

Anal. Calcd. for $C_{42}H_{58}N_4O_{11}$: C, 63.05; H, 7.69; N, 7.00; mol. wt., 800. Found: C, 63.12; H, 7.60; N, 7.06; mol. wt., 810 (osmetric).

Catalytic Hydrogenation of I.—From the hydrogenation of 600 mg. of I using palladium black as a catalyst in methyl alcohol, chromatography, using thick layer silica gel G plates developed with (1:1) ether–hexane, and crystallization from ethanol–water yielded 85 mg. of a hydrogenated derivative (IV): m.p. 101–102°; $[\alpha]^{21}D$ +126° in $CHCl_3$; ultraviolet absorption spectrum in cyclohexane, λ_{max} 237, 302 mμ (ϵ_{max} 5000, 49); infrared absorption, 2.9 (OH), 3.37, 3.51 (methyl and methylene), 5.74 (ester CO), 5.81–5.85 μ (ketone CO group); C=C absorption does not appear in the infrared spectrum of IV.

Anal. Calcd. for $C_{36}H_{62}O_7$: C, 71.58; H, 10.01; active H (2), 0.19; mol. wt., 604. Found: C, 71.30; H, 10.09; active H (2), 0.32; mol. wt., 604 (mass spectrum).

Alkaline Hydrolysis of I.—Ten grams of I was dissolved in 15 ml. of absolute methyl alcohol, and the solution was neutralized with $Ba(OH)_2$ in MeOH precipitating a barium soap. The methanol solution was filtered, and further treatment with $Ba(OH)_2$ produced a precipitate of barium soap and alcohol layer containing the parent alcohol. The soap produced was extracted with absolute ethanol. The residue after distilling the alcohol was dissolved in 10 ml. of water. The aqueous layer was extracted with ether and petroleum ether to extract any decomposition products. The aqueous layer was lyophilized, and the residue was dissolved in absolute ethanol; after crystallization from ethanol 0.5 g. of V, m.p. 238–240°, was obtained; recrystallization from ethanol raised the melting point to 240–241°. The barium soap was acidified with dilute H_2SO_4 and the acids were extracted with ether. Esterification of the acids with a freshly prepared solution of diazomethane gave the methyl esters of acetic and myristic acids. They were isolated by vapor phase chromatography and characterized by mass spectrometry[16]; $[\alpha]^{29}D$ +118° (dioxane); λ_{max} 234 and 335 mμ (ϵ_{max} 5100 and 75 in ethanol); infrared spectrum in KBr, 2.86 and 3.05 (hydroxyl), 5.86 (carbonyl ketone), 6.10 μ (C=C); n.m.r. spectrum, 0.52, 0.88, 1.10, 1.67, 2.32, 2.91, 3.77, 4.17, 5.44, 7.42 p.p.m. (in deuterated dimethyl sulfoxide; internal standard, tetramethylsilane).

Anal. Calcd. for the parent alcohol (V) isolated from I, $C_{20}H_{28}O_6$: C, 65.99; H, 7.75; mol. wt., 364. Found: C, 66.05; H, 7.60; mol. wt., 364 (mass spectrum).

Partial Synthesis of the Active Principle C-3.—To 350 mg. of the polyhydroxy cyclic ketone, $C_{20}H_{28}O_6$ (V), in 10 ml. of dry pyridine was added 90 mg. of acetic anhydride. The solution was stirred at room temperature for 24 hr. The pyridine was removed under reduced pressure and gave 300 mg. of an oil; infrared absorption spectrum showed a strong hydroxyl band at 2.97 μ, ester CO at 5.76 μ, ketone CO at 5.82 μ, C=C at 6.10 μ. The oil was dissolved in 10 ml. of dry pyridine, 245 mg. of myristoyl chloride was added, and the mixture was stirred at room temperature for 24 hr. Pyridine was removed under reduced pressure and the oily residue was taken up in ether, washed with water, cool 2% $NaHCO_3$ solution, then with water, and dried (Na_2SO_4). Removal of the solvent yielded 500 mg. of an oil, which showed four components on silica gel G thin layer plates. The component having an R_f value, identical with the naturally occurring I from *Croton tiglium* L., was subject to countercurrent distribution and preparative thin layer chromatography. Isolation of VI was accomplished by procedures essentially the same as those used for isolation of C-3 (I). After crystallization from ethanol–water 150 mg. of VI, m.p. 73–74°, was obtained. A mixture melting point with a sample of I from *C. tiglium* L. gave no depression. The infrared and n.m.r. spectra, optical activity, and melting point of the active principle from *C. tiglium* L. and the partially synthesized active material VI were identical.

Preparation of the Polyhydroxy Cyclic Ketone Triacetate.—Crystalline V from I (300 mg.) was acetylated with acetic anhydride–acetic acid in 10 ml. of dry pyridine and chromatographed on 20 g. of acid-washed Florisil, wetted with hexane. The 350 mg. of the crude acetate was dissolved in 5 ml. of ether. Fractions were eluted with ether–hexane (9:1), then ether. The ether eluate yielded an oil which was purified by countercurrent distribution using as solvent: lower phase, methanol; upper phase, heptane (500 transfers). Tubes 40–60 contained 200 mg. of the diol cyclic ketone triacetate (VII), which readily crystallized from ether; m.p. 118–119°. This product gave a single spot on a thin layer silica gel G chromatogram, ether–hexane (9:1) as solvent, R_f 0.50, $[\alpha]^{20}D$ +66° (ethanol); ultraviolet absorption in ethanol, λ_{max} 233 and 330 mμ (ϵ_{max} 4500 and 51). The infrared absorption spectrum showed a sharp OH band at 2.92 μ, ester CO at 5.75 μ, ketone CO at 5.85 μ, C=C at 6.10 μ.

Anal. Calcd. for $C_{26}H_{34}O_9$: C, 63.73; H, 6.85; mol. wt., 490. Found: C, 63.74; H, 6.79; mol. wt., 490 (mass spectrum).

Preparation of the Tetraacetate (VIII).—To 300 mg. of the triacetate (VII) in 1.0 ml. of dry pyridine was added 5 ml. of acetic anhydride. The solution was refluxed for 3 hr. and cooled. The pyridine was removed under reduced pressure and the oily residue taken up in ether, washed with water, cool 2% $NaHCO_3$ solution, then with water, and dried (Na_2SO_4). After crystallization from ether–petroleum ether 250 mg. of VIII, m.p. 175–176°, was obtained. This product gave a single spot on t.l.c. [silica gel G, ether–hexane (8:2) solvent], R_f 0.52; $[\alpha]^{20}D$ −138° (1% ethanol); ultraviolet absorption in ethanol, λ_{max} 233 and 324 mμ (ϵ_{max} 8000 and 53). The infrared absorption spectrum showed a sharp OH peak at 2.96 μ, ester CO at 5.75 μ, ketone CO at 5.76–5.81 μ, C=C at 6.10 μ.

Anal. Calcd. for $C_{28}H_{36}O_{10}$: C, 63.58; H, 6.96; mol. wt., 532. Found: C, 63.30; H, 6.86; mol. wt., 539 (osmetric).

Acknowledgment.—The authors are grateful to Dr. P. de Mayo, Professor of Chemistry, The University of Western Ontario, London, Canada, for constructive criticism and helpful suggestions. We also thank Dr. C. J. Umberger, Director of Toxicological Laboratories of New York City, for stimulating interest and encouragement throughout the work. We are indebted to Miss Florence Stefano for infrared spectra. Part of this work was initiated at New York University Medical Center. This research was partially supported by a grant from the National Cancer Institute of the U. S. Public Health Service.

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