acetate gave β-apoPP (II, R = CH_3), m.p. and mixed m.p. 218.5–219.5°.

The composition of the reaction product was studied further by preparing solutions in chloroform, diluting with ethanol and measuring ultraviolet absorbances at various wave lengths. The proportions of α-apoPT (or α-apoPP), β-apoPP and dehydroanhydroPP were determined by three-component analysis.[8,14] Fair agreement was obtained between the values calculated from the absorbances at 350,[13] 311[4] and 290[4] mμ, and the ones obtained by the method of least squares from absorbances at ten different wave lengths. Thus the "crude solid" in one experiment appeared to contain 14% of α-apoPT (or α-apoPP), 65% of β-apoPP and 7% of dehydroanhydroPP.[15,16] In another run, the reaction mixture was directly diluted with chloroform, then with ethanol, without isolation of the product. About 16% of dehydroanhydroPP and 7 to 10% of α-apoPT (or α-apoPP)[15] were found to be present, correction being made for the absorption of pinene. After chromatography on acid-washed alumina, the following percentage yields of α-apoPT (or α-apoPP), β-apoPP and dehydroanhydroPP, respectively, were calculated: 0.8, 64, 7 (chloroform solution of "crude solid" chromatographed); 2.4, 61, 16 (reaction mixture diluted with chloroform, then chromatographed). Isomerization of α-apoPT to β-apoPP thus seems to have taken place during chromatography.

4′-Demethyl-β-apopicropodophyllin (II, R = H).—PT bromide (33.85 g.) was heated at 165° for 30 minutes. The red-brown plastic mass was triturated with 400 ml. of boiling ethyl acetate; the pink powder (6.68 g.) was collected after cooling and washed with ethyl acetate; m.p. 266–282° (immersed at 260°). The brown mother liquor, when treated with charcoal and concentrated with addition of ethanol, gave another 1.35 g. of tan-colored solid, m.p. 265–279° (immersed at 260°), bringing the total yield to 8.03 g. (30%). Crystallization from chloroform–ethanol provided 6.6 g. of light-colored crystals, m.p. 272–282° (darkening, immersed at 260°). Further recrystallization gave small colorless rectangular plates without any improvement in the melting point, which varied with the temperature of immersion; $[\alpha]^{20}D$ +106° (*c* 0.31, chloroform), λ_{max}^{EtOH} 287.5 mμ (log ε 3.74), λ_{min}^{EtOH} 261.5 mμ (log ε 3.54). The compound gave a red-brown color with concd. sulfuric acid and its chloroform solution did not absorb bromine rapidly.[4] It was soluble in aqueous sodium hydroxide.

Anal. Calcd. for $C_{21}H_{18}O_7$: C, 65.96; H, 4.75; OCH_3, 16.23. Found: C, 65.67; H, 5.04; OCH_3, 16.48.

In a similar run, which yielded 25% of this compound, impure β-apoPP (II, R = CH_3), m.p. 208–214°, was isolated from the mother liquors in 7% yield. Recrystallization from chloroform–ethanol, then from ethyl acetate, gave colorless needles, m.p. and mixed m.p. 217–218°.

4′-Ethyldemethyl-α-apopodophyllic Acid (III).—A refluxing solution of 1.56 g. of II (R = H) in 27 ml. of 80% ethanol and 2.6 ml. of 5 *N* sodium hydroxide was treated at 30-minute intervals with three 1.6-ml. portions of ethyl sulfate. Alkalinity was maintained by the gradual addition of 6.5 ml. of 5 *N* sodium hydroxide. The solution was finally refluxed for 30 minutes, treated with another 6.6 ml. of 5 *N* sodium hydroxide, concentrated to remove ethanol and filtered from a small amount of tar (charcoal). It was then chilled, after adding 25 ml. of chloroform, and acidified gradually with 2 *N* hydrochloric acid. Separation of layers and further extraction with chloroform gave a yellow solution, which was washed twice with water, dried over sodium sulfate and evaporated. The remaining oil was dissolved in 15 ml. of benzene and crystallization induced by scratching. The yellowish solid (0.92 g., 53%) melted at 152° (effervescence). Two further recrystallizations from benzene produced soft, colorless, tiny needles, m.p. 153° (effervescence, immersed at 130°), 155–156° (immersed at 150°); $[\alpha]^{21}D$ −159° (*c* 1.0, chloroform). The compound was soluble in warm aqueous sodium bicarbonate.

Anal. Calcd. for $C_{23}H_{24}O_8$: C, 64.48; H, 5.65; alkoxyl (as OCH_3), 21.73. Found: C, 64.50; H, 5.88; OCH_3, 21.81.

Oxidation of 4′-Ethyldemethyl-α-apopodophyllic Acid to Syringic Acid Ethyl Ether (IV).—One gram of III was oxidized with potassium permanganate by the method previously described.[11] The yield was 137 mg. (26%), m.p. 114–120.5°. Recrystallization from benzene–hexane, followed by vacuum-sublimation, gave material melting at 123.0–123.6°; no depression with an authentic sample[11] (m.p. 123–124°).

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(14) The ultraviolet spectrum of α-apoPT was assumed to be identical with that[4] of α-apoPP.

(15) Percentages calculated as yields obtained from starting material.

(16) The large amounts of dehydroanhydroPP that were isolated when the "crude solid" was boiled with ethanol were, therefore, not present in the original material. The absorbances at 350 and 342 mμ did not increase when a chloroform–ethanol solution of this material was heated. No further study of this discrepancy was made.

Components of Podophyllin. XVI.[1] Podophyllotoxin Haloacetates and Quaternary Derivatives

BY ANTHONY W. SCHRECKER, GERTRUDE Y. GREENBERG AND JONATHAN L. HARTWELL

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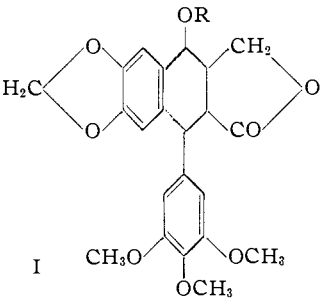
Because of its tumor-damaging property,[2] podophyllotoxin[3] (I, R = H) has been employed in pharmacological[4] and biochemical[5] studies on the mechanisms of its actions. Its low solubility in aqueous vehicles, however, constitutes an obstacle to its use. Therefore, attempts were made to prepare active water-soluble derivatives. The present communication describes results obtained so far in this direction.



I

Reaction of podophyllotoxin with chloroacetic anhydride in pyridine furnished the chloroacetate (I, R = $COCH_2Cl$). The yield of the corresponding bromoacetate (R = $COCH_2Br$), obtained with bromoacetyl bromide, was much lower because of formation of tarry by-products. The iodoacetate (R = $COCH_2I$) was prepared from the chloroacetate by metathetical reaction with sodium iodide in acetone.[6]

The quaternary pyridinium iodide I (R = $CO\text{-}CH_2NC_5H_5{}^+I^-$) was obtained in good yield from the iodoacetate by treatment with pyridine. This compound did, however, not prove satisfactory since its solubility in cold water was low, while

(1) Paper XV, A. W. Schrecker, G. Y. Greenberg and J. L. Hartwell, THIS JOURNAL, **76**, 1182 (1954).

(2) J. Leiter, V. Downing, J. L. Hartwell and M. J. Shear, *J. Natl. Cancer Inst.*, **10**, 1273 (1950).

(3) (a) J. L. Hartwell and A. W. Schrecker, THIS JOURNAL, **73**, 2909 (1951); (b) A. W. Schrecker and J. L. Hartwell, *ibid.*, **75**, 5916 (1953).

(4) M. G. Kelly, J. Leiter, O. Ghosh and P. K. Smith, *J. Natl. Cancer Inst.*, **12**, 1177 (1952).

(5) V. S. Waravdekar, A. Domingue and J. Leiter, *ibid.*, **13**, 393 (1952).

(6) The three haloacetates have been submitted to Dr. Ray Pepinsky of Pennsylvania State College for X-ray diffraction analysis.

hot water caused decomposition. The corresponding chloride (R = $COCH_2NC_5H_5^+Cl^-$) was very soluble in cold water. Preparing it in relatively good yield was difficult because its rate of formation was lower, as expected, than that of the iodide and because part of the product was decomposed as the reaction proceeded, apparently by transesterification with ethanol employed as a solvent.[7] The chloride was hydrolyzed in aqueous solution to N-carboxymethylpyridinium chloride (presumably) and what appeared to be a mixture of epipodophyllotoxin[3] and podophyllotoxin. In the presence of a buffer (*p*H 7), podophyllotoxin free of its epimer was isolated.[8]

Experimental[9,10]

Podophyllotoxin Chloroacetate (I, R = $COCH_2Cl$).—To an ice-cooled solution of 9.6 g. of anhydrous podophyllotoxin[3a] (I, R = H) in 30 ml. of dry pyridine was added with swirling 9 ml. of chloroacetic anhydride. The mixture was kept at room temperature for 15 minutes, then poured with stirring into 500 ml. of ice and water. The yellow solid was collected after an hour, washed thoroughly with water and dissolved in chloroform. The solution was washed with water, dried over magnesium sulfate, concentrated and chromatographed on alumina. Elution with chloroform and concentration with addition of ethanol yielded 8.95 g. (79%) of colorless needles, m.p. 207–207.5° (immersed at 195°). Recrystallization from chloroform–ethanol provided material, m.p. 208–209° (immersed at 150°), 209–210° (immersed at 195°), $[\alpha]^{22}D$ −140° (*c* 1.05, chloroform).

Anal. Calcd. for $C_{24}H_{23}O_9Cl$: C, 58.72; H, 4.72; Cl, 7.22. Found: C, 58.64; H, 4.86; Cl, 7.22.

Podophyllotoxin Bromoacetate (I, R = $COCH_2Br$).—An ice-cooled mixture of 1.3 ml. of bromoacetyl bromide and 5 ml. of chloroform was treated with 1.4 ml. of dry pyridine, then with 2.07 g. of I (R = H) in 5 ml. of chloroform. The dark brown solution, after 10 minutes at room temperature, was poured into 100 ml. of ice-water. The combined chloroform solutions, obtained by separating layers and extracting the aqueous phase with additional solvent, were washed with 0.2 *N* hydrochloric acid, sodium bicarbonate solution and water, then dried, concentrated and chromatographed. Concentration with addition of ethanol gave 0.91 g. (34%) of colorless needles, m.p. 192° (immersed at 150°), $[\alpha]^{22}D$ −133° (*c* 1.03, chloroform).

Anal. Calcd. for $C_{24}H_{23}O_9Br$: C, 53.84; H, 4.35; Br, 14.93. Found: C, 53.79; H, 4.63; Br, 14.82.

Podophyllotoxin Iodoacetate (I, R = $COCH_2I$).—A solution of 7.36 g. of the chloroacetate I (R = $COCH_2Cl$) in 75 ml. of acetone was refluxed with 25 g. of sodium iodide for four hours, then poured into a stirred mixture of 600 ml. of water and 75 ml. of chloroform. The aqueous layer was extracted with additional chloroform and the combined chloroform solutions washed with very dilute aqueous sulfur dioxide, then with water and dried. Concentrating and adding an equal volume of hexane yielded 8.23 g. (94%) of colorless needles, melting at 190° with effervescence, darkening and liberation of iodine (immersed at 150°); m.p. 192° (dec.) after recrystallization from chloroform–hexane; $[\alpha]^{22}D$ −128° (*c* 1.01, chloroform).

Anal. Calcd. for $C_{24}H_{23}O_9I$: C, 49.50; H, 3.98; I, 21.79. Found: C, 49.32; H, 3.90; I, 21.72.

Picropodophyllin Chloroacetate (I, R = $COCH_2Cl$).—The compound was obtained in 74% yield from picropodophyllin[3a] (I, R = H) by the procedure used for the epimeric[3] podophyllotoxin chloroacetate. It formed colorless cottony needles, which melted at 154–155° (immersed at room temperature), resolidified at 156° and remelted at 178–191°; $[\alpha]^{21}D$ +47° (*c* 1.01, chloroform).

Anal. Calcd. for $C_{24}H_{23}O_9Cl$: C, 58.72; H, 4.72; Cl, 7.22. Found: C, 58.76; H, 4.72; Cl, 7.36.

Acetylpodophyllotoxin-ω-pyridinium Chloride (I, R = $COCH_2NC_5H_5^+Cl^-$).—A solution of 10 g. of the chloroacetate I (R = $COCH_2Cl$) in 50 ml. of chloroform and 50 ml. of ethanol was refluxed with 2.5 ml. of dry pyridine with exclusion of moisture for seven hours, then evaporated under reduced pressure. The solid was dissolved in 50 ml. of hot chloroform. Adding 75 ml. of hot ethyl acetate precipitated pale crystalline material, which was collected after standing at room temperature; yield 2.56 g. (21.4%), m.p. 153–154° (effervescence and darkening, immersed at room temperature). Recrystallization from methanol–ethyl acetate provided leaflets, m.p. 158–159° (dec.), $[\alpha]^{20}D$ −102° (*c* 0.57, water), $[\alpha]^{21}D$ −97° (*c* 0.54, methanol). The product was faintly greenish when still moist with solvent, but took on a brilliant pink color upon drying in air. It then contained one mole of water of crystallization, which could not be removed completely without partial decomposition. The compound was very soluble in water and methanol, less soluble in chloroform and nearly insoluble in ethyl acetate.

Anal. Calcd. for $C_{29}H_{28}O_9NCl \cdot H_2O$: C, 59.23; H, 5.14; N, 2.38; Cl, 6.03; H_2O, 3.06. Found: C, 58.99; H, 5.30; N, 2.56; Cl, 6.03; wt. loss (vac., 100°), 2.89.

A solution of 588 mg. of the compound in 30 ml. of 0.2 *M* sodium phosphate buffer (*p*H 7.3) became milky within five minutes, while prismatic needles began to separate after 10 minutes. The mixture was kept at 37° for 64 hours and filtered. The *p*H of the filtrate was 7.05; the solid (330 mg., 80%) melted at 183–185° (after drying at 110° *in vacuo*)[3a] and gave the infrared spectrum of podophyllotoxin. When 500 mg. of the pyridinium chloride in 10 ml. of water was kept at 60° for 17 hours, a plastic mass separated, which crystallized gradually; yield 327 mg. (93%), m.p. 139–141° (dec.). The infrared spectrum appeared to indicate that the product was a mixture of epipodophyllotoxin[3] with a lesser amount of podophyllotoxin. The filtrate was acid to congo red.

Acetylpodophyllotoxin-ω-pyridinium Iodide (I, R = $COCH_2NC_5H_5^+I^-$).—A solution of 1.16 g. of the iodoacetate I (R = $COCH_2I$) in 5 ml. of chloroform, 5 ml. of ethanol and 0.24 ml. of pyridine was refluxed for 30 minutes, at which time a yellow solid separated. This was removed and the mother liquor concentrated with addition of chloroform to obtain further solid. The combined crops (1.06 g., 78%) were washed with chloroform; m.p. 155–156° (foaming, immersed at room temperature). The product was purified by dissolving it in methanol, concentrating and adding chloroform. It then formed faintly yellowish needles, m.p. 156–157° (dec.), much less soluble in water, methanol and chloroform than the corresponding chloride.

Anal. Calcd. for $C_{29}H_{28}O_9NI \cdot H_2O$: C, 51.26; H, 4.45; I, 18.68. Found: C, 51.39; H, 4.43; I, 18.41.

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(7) The product could not be obtained in the absence of ethanol; this is in line with the experience that polar solvents strongly accelerate the rate of analogous quaternizations; *cf.* E. D. Hughes, *Trans. Faraday Soc.*, **37**, 603 (1941).

(8) V. S. Waravdekar, A. D. Paradis and J. Leiter, *J. Natl. Cancer Inst.*, **14**, 585 (1953), ascribe the tumor-damaging action of the quaternary chloride to the liberation of podophyllotoxin *in vivo*.

(9) Melting points varied with the temperature of immersion and rate of heating. The values reported are corrected and were obtained with the Hershberg apparatus by heating rapidly to within 5° of the melting point, then raising the temperature gradually at the rate of 2°/min.

(10) We are indebted to Dr. W. C. Alford and co-workers for the microanalyses.

Synthesis of Monochloroacetone

BY ROBERT E. VAN ATTA, HARRY D. ZOOK AND PHILIP J. ELVING

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In the course of an investigation of halogenated ketones, monochloroacetone uncontaminated by *sym*- or *unsym*-dichloroacetone was required. Owing to the very slight difference in boiling points of chloroacetone and *unsym*-dichloroacetone,[1] it is practically impossible to separate them completely

(1) E. R. Buchman and H. Sargent, THIS JOURNAL, **67**, 401 (1945).