[CONTRIBUTION FROM THE LABORATORY OF CHEMICAL PHARMACOLOGY, NATIONAL CANCER INSTITUTE[1]]

Components of Podophyllin. XVII.[2] Ionic Derivatives of Podophyllotoxin and of the Peltatins[3]

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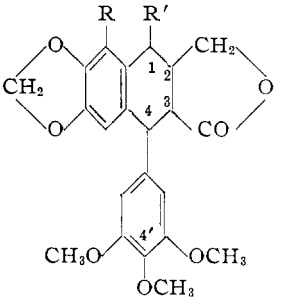
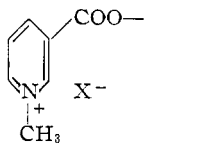
Several water-soluble derivatives of podophyllotoxin and of the peltatins have been prepared for pharmacological studies. They include cationic and anionic compounds.

In the preceding paper of this series,[2] the preparation of acetylpodophyllotoxin-ω-pyridinium chloride, a water-soluble derivative of podophyllotoxin (I, R = H, R′ = OH),[4] was described. This derivative, which has a tumor-damaging potency similar in magnitude to that of the water-insoluble parent compound,[5] has been found useful in pharmacological and biochemical studies of drug action[6] because of the ease with which it can be administered. This has prompted us to synthesize additional water-soluble derivatives of podophyllotoxin and of the peltatins[7] for pharmacological and clinical study in the program of Shear and his associates.[8] Two main types have been prepared: one in which the residue I is part of a cation, the other in which it forms part of an anion.[9]

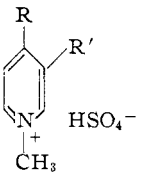
Cationic Derivatives.—Podophyllotoxin nicotinate (I, R = H, R′ = -O-nicotinoyl), obtained from podophyllotoxin with nicotinic anhydride in pyridine, was quaternized in good yield to the methiodide (I, R = H, R′ = IIa), which was rather sparingly soluble in cold water, and to the methosulfate (I, R = H, R′ = IIb), which was quite soluble. Similarly, a methiodide (I, R = IIa, R′ = H) was obtained readily from β-peltatin nicotinate (I, R = -O-nicotinoyl, R′ = H). These quaternary salts proved to be rather unstable and were rapidly decomposed when warmed in aqueous solution. An attempt to quaternize the nicotinate of picropodophyllin, the C_3-epimer of podophyllotoxin,[4] with methyl sulfate produced ester cleavage, with essentially quantitative isolation of trigonelline hydrogen sulfate (IIIa). Similarly, treatment of podophyllotoxin isonicotinate with methyl sulfate and with methyl iodide led, respectively, to N-methylisonicotinic acid hydrogen sulfate (IIIb) and to "basic N-methylisonicotinic acid iodide" (IV).[10] This is consistent with the well-known fact that substituents in the 4-position of the pyridine nucleus are rendered more reactive by a positive charge on the nitrogen atom than those in the 3-position.



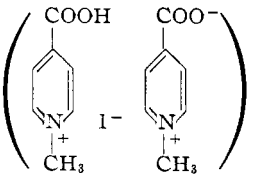
I



IIa, X = I^-
IIb, X = $CH_3SO_4^-$



IIIa, R = H, R′ = CO_2H
IIIb, R = CO_2H, R′ = H



IV

Reaction of podophyllotoxin bromide[4] (I, R = H, R′ = Br) with thiourea yielded an isothiuronium bromide (I, R = H, R′ = $-SC(NH_2)_2^+Br^-$). Since epipodophyllotoxin and its ethyl ether, rather than the corresponding podophyllotoxin derivatives, are obtained by treatment of the bromide with water and ethanol, respectively,[4] it appears likely by analogy that the isothiuronium salt also belongs to the epipodophyllotoxin series (inversion at C_1[4]).

Anionic Derivatives.—Treatment of β-peltatin[7] with chlorosulfonic acid and pyridine gave the sulfate ester, which was isolated as the potassium salt (I, R = $-OSO_3K$, R′ = H). Analogously, the two free phenolic hydroxyl groups of α-peltatin[7] were esterified, leading to the dipotassium disulfate. That inversion at C_3 had not occurred during the isolation was proved by recovery of the levorotatory starting materials, free of the epimeric "B" compounds,[7] after acid hydrolysis of the ester salts.

Amorphous succinic and glutaric acid half-esters of podophyllotoxin (I, R = H, R′ = $-OCO(CH_2)_2CO_2H$ and $-OCO(CH_2)_3CO_2H$, respectively) and a crystalline acid phthalate were prepared by reaction with the appropriate anhydrides in pyri-

(1) National Institutes of Health, Public Health Service, U. S. Department of Health, Education, and Welfare.

(2) Paper XVI, A. W. Schrecker, G. Y. Greenberg and J. L. Hartwell, THIS JOURNAL, **76**, 1184 (1954).

(3) Presented at the 3rd International Congress of Biochemistry in Brussels, August 3, 1955.

(4) J. L. Hartwell and A. W. Schrecker, *ibid.*, **73**, 2909 (1951); A. W. Schrecker and J. L. Hartwell, *ibid.*, **75**, 5916 (1953).

(5) V. S. Waravdekar, A. D. Paradis and J. Leiter, *J. Natl. Cancer Inst.*, **14**, 585 (1953).

(6) G. H. Algire, F. Y. Legallais and B. F. Anderson, *ibid.*, **14**, 879 (1954); V. S. Waravdekar, A. D. Paradis and J. Leiter, *ibid.*, **16**, 99 (1955); V. S. Waravdekar, O. Powers and J. Leiter, *Proc. Am. Assoc. Cancer Research*, **2**, (No. 1) 52 (1955); M. Woods and D. Burk, *ibid.*, **2**, (No. 1) 54 (1955); M. V. Freeman, L. D. Saslaw and J. Leiter, *J. Pharmacol. Exptl. Therap.*, **113**, 22 (1955).

(7) J. L. Hartwell and W. Detty, THIS JOURNAL, **72**, 246 (1950); J. L. Hartwell, A. W. Schrecker and G. Y. Greenberg, *ibid.*, **74**, 6285 (1952); A. W. Schrecker and J. L. Hartwell, *ibid.*, **75**, 5924 (1953).

(8) M. J. Shear, *J. Natl. Cancer Inst.*, **12**, 569 (1951); E. M. Greenspan, J. Colsky, E. B. Schoenbach and M. J. Shear, *ibid*, **14**, 1257 (1954).

(9) Water-soluble, but non-ionic, derivatives, *i.e.*, glucosides, of some of these compounds have been isolated from *Podophyllum* species by A. Stoll, J. Renz, E. Angliker and A. von Wartburg, THIS JOURNAL, **76**, 3103, 5004, 6413 (1954); **77**, 1710 (1955); *Helv. Chim. Acta*, **37**, 1747 (1954).

(10) R. Turnau, *Monatsh.*, **26**, 537 (1905).

dine. These compounds formed water-soluble crystalline isopropylamine salts.

All of these compounds, as well as some of Stoll's glucosides,[9] have been examined for activity against Sarcoma 37 in mice. The biological results will be presented in a separate report.

Experimental[11]

Podophyllotoxin Nicotinate (I, R = H, R′ = -O-Nicotinoyl).—A mixture of 4.14 g. of solvent-free podophyllotoxin,[4] 6.9 g. of nicotinic anhydride,[12] and 20 ml. of anhydrous pyridine was warmed gently to effect solution, kept at room temperature for 24 hours, then diluted with 500 ml. of water. The precipitate was collected after standing in the refrigerator, washed well with water and dried. Chromatography on alumina, using chloroform, followed by evaporation and crystallization from ethanol provided 4.73 g. of colorless needles, m.p. 176.5–177.5°. Another 0.25 g., m.p. 172–175°, was recovered from the alcoholic mother liquor, raising the yield to 96%. Recrystallized from chloroform–ethanol, the compound had m.p. 177–178° and $[\alpha]^{21}D$ −112° (*c* 0.63, chloroform).

Anal. Calcd. for $C_{28}H_{25}NO_9$: C, 64.73; H, 4.85; N, 2.70. Found: C, 64.81; H, 4.94; N, 2.63.

The following compounds were prepared analogously (all rotations in chloroform): **picropodophyllin[4] nicotinate**, 100% yield (mixture heated on the steam-bath one hour and poured into aqueous sodium carbonate), thin needles (from chloroform–ethanol), m.p.[11b] 200.5–201.5°, $[\alpha]^{22}D$ +30° (*c* 1.00) (found: C, 64.53; H, 4.85; N, 2.65); **β-peltatin nicotinate** (I, R = -O-nicotinoyl, R′ = H), 94% yield (24-hour heating), amorphous solid (from ethanol–water), m.p. 124–136°, $[\alpha]^{19}D$ −140° (*c* 0.56) (found: C, 64.51; H, 4.78; N, 2.80); **β-peltatin-B[7] nicotinate**, 74% yield, prismatic needles (from ethanol), m.p. 202.3–203.6°, $[\alpha]^{21}D$ −21° (*c* 1.00) (found: C, 64.99; H, 4.97; N, 2.63); **podophyllotoxin isonicotinate** (I, R = H, R′ = -O-isonicotinoyl), 94% yield (with isonicotinic anhydride[12]), glistening rectangular plates (from ethanol), m.p. 187.2–187.6°, $[\alpha]^{21}D$ −123° (*c* 0.75) (found: C, 64.73; H, 4.85; N, 2.68); **β-peltatin isonicotinate** (I, R = -O-isonicotinoyl, R′ = H), 97% yield (20-hour heating), amorphous solid (from ethanol), m.p. 134–145° (softening 130°), $[\alpha]^{20}D$ −134° (*c* 0.86) (found: C, 64.53; H, 4.91; N, 2.86).

Podophyllotoxin Nicotinate Methiodide (I, R = H, R′ = IIa).—A solution of 2.32 g. of podophyllotoxin nicotinate and 0.5 ml. of methyl iodide in 10 ml. of chloroform was allowed to stand in the dark for five days, then diluted with ethyl acetate to yield 2.25 g. (76%) of yellow solid, which melted at 138° (foaming), resolidified and remelted[11b] at 209–214°. Crystallization from chloroform, followed by recrystallization from methanol–ethyl acetate, provided yellow prismatic needles, m.p. 142–144° (slight foaming), remelting[11b] after resolidification at 208–210°; $[\alpha]^{21}D$ −55° (*c* 0.10, methanol). The compound was rather sparingly soluble in cold water. At 50° it was soluble to the extent of *ca.* 1%, but was rapidly decomposed.

Anal. Calcd. for $C_{29}H_{28}INO_9$: C, 52.66; H, 4.27; N, 2.12; I, 19.19. Found: C, 52.64; H, 4.37; N, 1.88; I, 19.03.

The following methiodides were prepared similarly: **β-peltatin nicotinate methiodide** (I, R = IIa, R′ = H), 81% yield, small yellow prisms (from methanol–chloroform–ethyl acetate), m.p. 230–233° (darkening), $[\alpha]^{19}D$ −84° (*c* 0.51, methanol) (found: C, 52.85; H, 4.45; N, 1.92; I, 19.00), more than 1% soluble in water at 40°, solution decomposing on standing; **β-peltatin-B nicotinate methiodide**, 96% yield, amorphous yellow powder (from chloroform–ethyl acetate), m.p. 165–180° (found: C, 51.18, 51.09; H, 4.76, 4.58; I, 17.53).

Podophyllotoxin Nicotinate Methosulfate (I, R = H, R′ = IIb).—Reaction of 2.50 g. of podophyllotoxin nicotinate with 2.5 ml. of methyl sulfate in 10 ml. of chloroform during four days, followed by dilution with ethyl acetate, gave viscous material, which was dissolved in hot methanol. Addition of ethyl acetate to incipient cloudiness and scratching provided 2.09 g. (67%) of pale yellowish small prisms, m.p.[11b] 164–166°. Recrystallization afforded colorless prisms, m.p. [11b] 171–172° (208–211° after resolidification), $[\alpha]^{21}D$ −94° (*c* 1.00, methanol). This compound was quite soluble even in cold water. On warming, decomposition took place, with separation of a solid, m.p. 122–133°, which apparently was a mixture. In the acid filtrate, trigonelline was identified as the chloroaurate,[13] m.p. 195–198° (lit.[13] 198°). Decomposition in phosphate buffer, *pH* 7.3, also yielded an impure solid, m.p. 122–132°.

Anal. Calcd. for $C_{30}H_{31}NO_{13}S$: C, 55.81; H, 4.84; S, 4.97. Found: C, 55.56; H, 4.99; S, 4.59.

An attempt to prepare β-peltatin nicotinate methosulfate (I, R = IIb, R′ = H) yielded an amorphous product, which could not be obtained analytically pure.

Trigonelline Hydrogen Sulfate (IIIa). (a) **From Picropodophyllin Nicotinate with Methyl Sulfate.**—A solution of 5.0 g. of picropodophyllin nicotinate and 5 ml. of methyl sulfate in 70 ml. of chloroform was allowed to stand at room temperature for four days, during which time colorless prisms separated gradually; yield 2.20 g. (97%); m.p. 185–195°. Recrystallization from methanol–ethyl acetate afforded prisms, m.p. 201.5–203.5°, undepressed by admixture of an authentic sample (b), and further identified by conversion to the picrate and chloroaurate (see below).

Anal. Calcd. for $C_7H_8NO_2{}^+HSO_4{}^-$: C, 35.74; H, 3.86; S, 13.63. Found: C, 35.82; H, 4.18; S, 13.45.

(b) **From Trigonelline.**—A solution of 300 mg. of trigonelline[14] in 2 ml. of methanol was treated with 2 ml. of 2 *N* methanolic sulfuric acid, then with hot ethyl acetate to incipient cloudiness; yield 378 mg. (83%); m.p. 195–202.5°. Two recrystallizations from methanol–ethyl acetate provided colorless prisms, m.p. 202.5–203.5°.

Anal. Found: C, 35.58; H, 3.78; S, 13.47.

Trigonelline Sulfate.—Prepared from 388 mg. of trigonelline with 1.25 ml. of 2 *N* methanolic sulfuric acid in 97% yield, this compound crystallized from methanol–ethyl acetate in colorless prisms, m.p. 202–204° (foaming), which depressed the melting point of trigonelline hydrogen sulfate.

Anal. Calcd. for $(C_7H_8NO_2{}^+)_2SO_4{}^{--}$: C, 45.16; H, 4.33; N, 7.52; S, 8.61. Found: C, 44.61; H, 4.40; N, 7.39; S, 8.47.

Trigonelline Picrate.—This was prepared either from trigonelline hydrogen sulfate with sodium picrate in water or picric acid in 80% ethanol, or from trigonelline with picric acid in absolute ethanol. Recrystallized from water, it formed yellow needle-shaped prisms, m.p. 204–205° (lit.[15] 198–200°).

Anal. Calcd. for $C_7H_7NO_2{\cdot}C_6H_3N_3O_7$: C, 42.63; H, 2.75; N, 15.30. Found: C, 43.00; H, 2.85; N, 14.82.

Recrystallization of this picrate from 95% ethanol produced the "basic" picrate, yellow transparent prisms, m.p. 243–244° dec. In turn, the "basic" picrate, when recrystallized from water, afforded the "normal" picrate, m.p. 204–205°.

Anal. Calcd. for $2C_7H_7NO_2{\cdot}C_6H_3N_3O_7$: C, 47.72; H, 3.40; N, 13.91. Found: C, 47.79; H, 3.41; N, 13.55.

Trigonelline Chloroaurate.—Prepared from trigonelline hydrogen sulfate and recrystallized from 2 *N* hydrochloric acid, it formed deep yellow transparent prisms, m.p. 202–204° (lit.[13] 198°).

Anal. Calcd. for $C_7H_8NO_2AuCl_4$: C, 17.62; H, 1.69; Au, 41.33. Found: C, 17.52; H, 1.39; Au, 41.18.

N-Methylisonicotinic Acid Hydrogen Sulfate (IIIb).—A solution of 2.20 g. of podophyllotoxin isonicotinate and 2.2 ml. of methyl sulfate in 10 ml. of acetone was kept for six days. Addition of ethyl acetate precipitated an oil, which began to crystallize after another three weeks and became completely solid after six weeks; yield 0.72 g. (72%); m.p. 168–169°. The compound was recrystallized readily from methanol–acetone to afford colorless leaflets, m.p.

(11) (a) Melting points are corrected and were determined with the Hershberg apparatus; (b) the melting point, which varied with the heating rate, was determined by immersing the sample at room temperature, heating rapidly to within 10° of the m.p., then at the rate of 3°/min.

(12) A. W. Schrecker and P. B. Maury, THIS JOURNAL, **76**, 5803 (1954).

(13) E. Jahns, *Ber.*, **18**, 2518 (1885); **20**, 2840 (1887).

(14) Nutritional Biochemicals Co., Cleveland, Ohio.

(15) K. Yoshimura and G. Trier, *Z. physiol. Chem.*, **77**, 290 (1912); K. Yoshimura, *ibid.*, **88**, 334 (1913).

168.3–169.5°. It was identified by conversion to the known picrate and chloroaurate.

Anal. Calcd. for $C_7H_8NO_2^+HSO_4^-$: C, 35.74; H, 3.86; S, 13.63. Found: C, 35.94; H, 3.68; S, 13.55.

N-Methylisonicotinic acid picrate crystallized from 95% ethanol in yellow hexagonal prisms, m.p. 217–219° (lit.[16] 215–217°).

Anal. Calcd. for $C_7H_7NO_2 \cdot C_6H_3N_3O_7$: C, 42.63; H, 2.75; N, 15.30. Found: C, 42.55; H, 2.72; N, 15.02.

N-Methylisonicotinic acid chloroaurate crystallized from 2 *N* hydrochloric acid in deep yellow transparent prisms, m.p. 237–238° (lit.[16] 233–234°).

Anal. Calcd. for $C_7H_8NO_2AuCl_4$: C, 17.62; H, 1.69; Au, 41.33. Found: C, 17.57; H, 1.60; Au, 41.52.

"Basic N-Methylisonicotinic Acid Iodide" (IV).—Reaction of 2.08 g. of podophyllotoxin isonicotinate with 0.45 ml. of methyl iodide in 10 ml. of chloroform for five days, followed by dilution with ethyl acetate yielded 1.93 g. of orange-colored solid, melting gradually between 133 and 224°. Crystallization from acetone–ethyl acetate, then from methanol–ethyl acetate provided small yellow prisms, m.p. 253–257° dec. (lit.[10] 247°). Analogous results were obtained by carrying out the reaction in acetone for 24 hours.

Anal. Calcd. for $2C_7H_7NO_2 \cdot HI$: C, 41.81; H, 3.76; N, 6.97; I, 31.56. Found: C, 42.00; H, 3.78; N, 7.12; I, 31.73.

Epi(?)podophyllotoxin Isothiuronium Bromide (I, R = H, R′ = $-SC(NH_2)_2^+Br^-$).—A solution of 0.41 g. of thiourea in 10 ml. of absolute ethanol was refluxed with 2.40 g. of podophyllotoxin bromide (I, R = H, R′ = Br)[4] for ten minutes. The colorless needles, which separated shortly, were collected after standing in the refrigerator overnight and washed with ice-cold ethanol; yield 1.50 g. (54%); m.p.[11b] 150° (foaming), unchanged after recrystallization from ethanol; $[\alpha]^{20}D$ −172° (*c* 0.50, ethanol), −168° (*c* 0.50, water).

Anal. Calcd. for $C_{23}H_{25}BrN_2O_7S$: C, 49.91; H, 4.55; Br, 14.44; N, 5.06; S, 5.59. Found (sample dried at 110° *in vacuo*): C, 49.83; H, 4.88; Br, 14.18; N, 4.80; S, 5.30.

Potassium β-Peltatin Sulfate (I, R = $-OSO_3K$, R′ = H).—Following in part the procedure of Fieser and Fry,[17] 2.0 g. of β-peltatin was stirred and boiled for ten minutes with a mixture of 4 ml. of anhydrous pyridine, 10 ml. of absolute chloroform and 1.2 ml. of chlorosulfonic acid. Addition of dry ether precipitated a heavy oil, which was washed repeatedly with dry ether and treated with 30 ml. of cold water, then with potassium carbonate to *p*H 8. The solution obtained on slight warming was saturated with potassium chloride, which again precipitated an oil. The supernatant was decanted, and the oil washed with saturated potassium chloride solution. It was then triturated with ether, which caused it to solidify, redissolved in water and reprecipitated with potassium chloride. The crude product was extracted with boiling 95% ethanol, and the solution evaporated to yield 2.46 g. of colorless solid. Crystallization from 85% ethanol, then from 95% ethanol gave colorless prismatic needles, which were dried at 110° *in vacuo*; $[\alpha]^{21}D$ −99° (*c* 1.00, water).

Anal. Calcd. for $C_{22}H_{21}O_{11}SK$: C, 49.61; H, 3.97; K, 7.34. Found: C, 49.83; H, 4.04; K, 7.16.

When 200 mg. of this ester salt was heated with 8 ml. of 2 *N* hydrochloric acid at 100° for seven minutes, it was hydrolyzed to β-peltatin, which separated as colorless crystals (recovery 146 mg., 94%), m.p. 231.5–236° (darkening), $[\alpha]^{20}D$ −100° (*c* 1.00, ethanol) (reported[7] m.p. 231–238° and $[\alpha]^{20}D$ −115° in ethanol for β-peltatin, m.p. 208–210° and $[\alpha]^{21}D$ +40° in acetone for β-peltatin-B).

Dipotassium α-Peltatin Disulfate (I, R = $-OSO_3K$, R′ = H, $-SO_3K$ **instead of** $-CH_3$ **at 4′**).—α-Peltatin[7] (1.0 g.) was stirred and boiled for ten minutes with a mixture of 4 ml. of pyridine, 10 ml. of carbon tetrachloride and 1.2 ml. of chlorosulfonic acid. The oil, which solidified on cooling, was washed with ether. Treatment with potassium carbonate and potassium chloride provided colorless needles, which were recrystallized from 75% ethanol and dried at 110°; yield 0.38 g.; $[\alpha]^{20}D$ −88° (*c* 1.00, water).

Anal. Calcd. for $C_{21}H_{18}O_{14}S_2K_2$: C, 39.61; H, 2.85; K, 12.28. Found: C, 39.32; H, 3.21; K, 11.96.

Hydrolysis of the disulfate in 2 *N* hydrochloric acid yielded α-peltatin, m.p. 236–239°, $[\alpha]^{20}D$ −113° (*c* 1.00, ethanol) (reported[7] m.p. 230.5–232.5° and $[\alpha]^{22}D$ −111° in ethanol for α-peltatin, m.p. 275–276.5° and $[\alpha]^{20}D$ +39° in acetone for α-peltatin-B).

Podophyllotoxin Hydrogen Succinate (I, R = H, R′ = $-OCO(CH_2)_2CO_2H$).—A solution of 5.0 g. of podophyllotoxin and 12 g. of succinic anhydride in 50 ml. of dry pyridine was heated on the steam-bath for one hour, cooled and stirred into 500 ml. of ice-water containing 70 ml. of concd. hydrochloric acid. The colorless amorphous solid was collected, washed thoroughly with water, dissolved in methanol and reprecipitated with water; yield 5.50 g. (89%). The material, which melted gradually between 104 and 135°, could not be obtained analytically pure.

The **isopropylamine salt** was obtained by dissolving 5.0 g. of the half-ester in 30 ml. of absolute ethanol and adding 0.83 ml. of isopropylamine to the chilled solution. Diluting with 70 ml. of ether and scratching precipitated 4.42 g. (70% yield from podophyllotoxin) of crystalline solid, m.p. 140–143° dec. Recrystallization by dissolving the product in cold ethanol and adding ether afforded colorless prisms, m.p. 141–143° dec., $[\alpha]^{19}D$ −103° (*c* 1.02, water), very soluble in water.

Anal. Calcd. for $C_{29}H_{35}NO_{11}$: C, 60.72; H, 6.15; N, 2.44. Found: C, 60.34; H, 6.18; N, 2.25.

Picropodophyllin hydrogen succinate was prepared similarly from picropodophyllin, except that the reaction mixture was heated for two hours. Crystallization of the crude product from chloroform–ethanol provided 95% of small colorless needles, m.p. 201–201.5°. Another recrystallization gave material, m.p. 201.5–202°, $[\alpha]^{20}D$ +25° (*c* 1.00, pyridine).

Anal. Calcd. for $C_{26}H_{26}O_{11}$: C, 60.70; H, 5.09. Found: C, 60.52; H, 5.23.

The **isopropylamine salt**, which was much less soluble than the corresponding podophyllotoxin derivative, was prepared by dissolving the half-ester in chloroform–ethanol, adding isopropylamine and diluting with hexane; yield 97%; m.p. 185–187° dec. Recrystallization from methanol–ether–pentane provided felt-like needles, m.p. 192–196° dec.,[11b] $[\alpha]^{20}D$ +20° (*c* 0.99, pyridine).

Anal. Calcd. for $C_{29}H_{35}NO_{11}$: C, 60.72; H, 6.15; N, 2.44. Found: C, 60.83; H, 6.12; N, 2.40.

Podophyllotoxin Hydrogen Glutarate (I, R = H, R′ = $-OCO(CH_2)_3CO_2H$).—Heating 5.0 g. of podophyllotoxin with 8.5 g. of glutaric anhydride in 25 ml. of pyridine for two hours gave, after dilution with acid, 6.07 g. (95%) of colorless amorphous solid, melting between 90 and 120°.

The **isopropylamine salt** was prepared from 4.50 g. of the half-ester in 25 ml. of acetone with 0.75 ml. of isopropylamine. Addition of 50 ml. of ether precipitated 4.30 g. (82% yield from podophyllotoxin) of crystalline solid, m.p. 142–146° dec. Recrystallization from acetone–ether provided colorless prisms, m.p. 145–148° dec., $[\alpha]^{20}D$ −99° (*c* 1.01, water), very soluble in water.

Anal. Calcd. for $C_{30}H_{37}NO_{11}$: C, 61.32; H, 6.35; N, 2.38. Found: C, 61.38; H, 6.30; N, 2.15.

Podophyllotoxin Hydrogen Phthalate.—The optimum yield was obtained with a slight excess[18] only of phthalic anhydride, in contrast to the preparations of the succinate and glutarate, in which a large excess of anhydride was found to be desirable. Heating 4.14 g. (10 mmoles) of podophyllotoxin with 1.55 g. (10.5 mmoles) of phthalic anhydride in 10 ml. of pyridine for two hours, followed by dilution with cold acid, washing and drying, gave 5.48 g. of colorless solid, melting between 130 and 160° (sintering 107°). The oil, which remained after dissolving the product in chloroform and evaporating the solution in an air-stream, was dissolved in 40 ml. of methanol. Scratching and chilling caused separation of 4.16 g. (73%) of colorless prismatic needles, m.p. 141–145°. The analytical sample was prepared by recrystallizing the product similarly at low temperature; m.p. 143–145°; $[\alpha]^{19}D$ −157° (*c* 0.98, chloroform).

Anal. Calcd. for $C_{30}H_{26}O_{11} \cdot {}^1/_2H_2O$: C, 63.04; H, 4.76; H_2O, 1.58. Found: C, 62.77; H, 5.04; wt. loss (79°, vac.), 1.51.

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(17) L. F. Fieser and E. M. Fry, THIS JOURNAL, **62**, 228 (1940).

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The **isopropylamine salt** was obtained by adding at 0° 0.64 ml. of isopropylamine, then 70 ml. of ether to a solution of 4.16 g. of the half-ester in 17 ml. of chloroform. The gummy solid crystallized while standing in the refrigerator; yield 4.47 g. (97%); m.p. 134–137°. Recrystallization from cold ethanol-ether afforded colorless felt-like needles, m.p. 137–139°, $[\alpha]^{20}D$ −91° (*c* 0.61, water), more than 2% soluble in cold water.

Anal. Calcd. for $C_{38}H_{35}NO_{11}\cdot{}^1/_2H_2O$: C, 62.85; H, 5.75; N, 2.22. Found: C, 62.86; H, 5.81; N, 2.16.

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[CONTRIBUTION FROM THE CEREAL CROPS SECTION, NORTHERN UTILIZATION RESEARCH BRANCH[1]]

Interpretation of Periodate Oxidation Data on Degraded Dextran

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The amount of formic acid produced from reducing end group units of dextrans on oxidation by periodate is different from the amount produced from the other units, and also varies with the position through which the reducing end group is linked to the remainder of the chain. Consideration of this fact, together with the possible structures of dextran and several assumed modes of hydrolytic cleavage, has enabled a more complete interpretation of periodate oxidation analyses of partially degraded dextrans. There appears to be an increase in the proportion of 1,6′-linkages in partially hydrolyzed dextran due to the greater ease of hydrolysis of other linkage types present. Periodate oxidation data on dextran derived from *Leuconostoc mesenteroides* NRRL B-512 are consistent with the simplified modes of degradation depicted while those from the NRRL B-1254 and B-742 dextrans are not.

Measurement of the amount of formic acid produced after oxidation of dextrans with sodium metaperiodate has been used as an index of the proportion of 1,6′-linked anhydroglucose residues.[2,3] In view of current interest in partially acid-hydrolyzed dextran as a blood volume expander[4] the use of periodate oxidation studies for the characterization and/or detection of differences between products will undoubtedly increase. In anticipation of such use the significance of the method, as applied to degraded dextrans, will be discussed in this paper. Emphasis will be placed on the change in periodate oxidation data as a function of the types of linkages broken and the nature of the breakdown (homogeneous *vs.* discard of small fragments resulting from cleavage of a particular type of linkage), on the correction of the periodate oxidation data for the reducing end groups present in the molecule, and on the magnitude of change in periodate oxidation values which may be expected in the degradation of a native dextran to a size suitable for use as a blood-plasma volume expander.

Introduction

Dextran is an anhydroglucose polymer in which most of the glucosidic linkages are α-1,6′. In addition, 1,3′- and 1,4′-linkages are present in at least certain ones of the known dextrans[5] and these apparently are generally but not necessarily at branch points.[6] The various possible types of structures for dextran, involving 1,3′(x)- or 1,4′(x)- and 1,6′-(o)-linkages are shown diagrammatically in Fig. 1. In addition, various combinations of these might be encountered. In the following discussion emphasis generally will be placed on the 1,3′-linkage because it occurs[5e] at the branch point in dextran NRRL B-512 which is of greatest importance at present as a commercial source of blood plasma volume expander.[4]

Structures A, C and D will be recognized as analogous to structures which have been proposed for starch.[7] Thus the "backbone"- or "comb"-type structure (A) was suggested by Staudinger for starch. Structure C is the laminated-type structure used by Haworth. Structure D is the randomly branched or bush-like structure, containing more than one branch on some branches, as proposed by Meyer. For dextran, structure B is a limiting case of the backbone structure A, in which side chains of only one glucose unit are joined to the main chain by 1,3′- or 1,4′-linkages. Similarly, F is a limiting case of structure C.

In these various structures for dextran the main types of chain linkages which would be involved are shown in Fig. 2. In addition, structure B would provide a special type, in which the chain at Y in (b) or (b′) of Fig. 2 is replaced by a hydrogen atom, as shown in (e) and (e′) of Fig. 2.

Inspection shows that hydrolytic cleavage of the linkage between the two units will give from (a) of Fig. 2 a 6-linked reducing end group plus a non-reducing terminal group, (b) and (b′) a 6-linked reducing end group plus a 1,6-linked unit of a chain, (c) a 6-linked reducing end group plus a 1,3-unit of a chain, (c′) a 6-linked reducing end group plus a 1,4-unit of a chain, (d) and (d′) same as (a), and (e) and (e′) D-glucose and a non-reducing end group. The same products as from (e) or (e′) also will be obtained by cleavage of the non-reducing end group if linked 1,6′. Periodate oxidation of the molecular fragments resulting from one of these

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