cooled to −12° was added dropwise with magnetic stirring and over a period of 0.5 hr. a solution of 0.54 ml. (7.4 mmoles) of methanesulfonyl chloride in 3 ml. of chloroform. The reaction mixture was held overnight (16 hr.) at −5°. Methanol (5 ml.) was then added and the solution evaporated to dryness *in vacuo*. This procedure was repeated twice with ethanol after which the residue crystallized from the same solvent as colorless irregular prisms, 1.45 g. (73% yield), m.p. 142–145° dec. Two recrystallizations from ethanol provided analytical material, m.p. 148–149°, $[\alpha]^{26}\mathrm{D}$ +10° (*c* 0.5, ethanol); $\lambda^{\mathrm{EtOH}}_{\mathrm{max,\,min}}$ 266 mμ (ε 9700), 234 (2230).

Anal. Calcd. for $C_{11}H_{16}N_2O_7S$: C, 41.24; H, 5.04; N, 8.75. Found: C, 41.47; H, 5.00; N, 8.73.

3′-Azido-3′-deoxythymidine (**7**).—A solution of 3.27 g. (6.7 mmoles) of **2** in 20 ml. of DMF containing 1.0 g. of lithium azide[16] was stirred for 3 hr. at 100° under an atmosphere of nitrogen. The cooled reaction mixture was poured, with stirring, into 600 ml. of ice–water and the product (**6**) was collected. The air-dried, off-white solid (3.4 g.), which showed a prominent azide peak ($\lambda^{\mathrm{KBr}}_{\mathrm{max}}$ 4.76 μ), was dried (P_2O_5) for 4 hr. at 50° (0.1 mm.) and used without further purification.

To a solution of 3.4 g. (6.7 mmoles) of **6** in 5 ml. of chloroform cooled to 0° was added 36 ml. of a cold chloroform solution containing 8.6 mmoles of hydrogen chloride. The cloudy reaction mixture was refrigerated for 1 hr. and then poured into 5 ml. of a saturated solution of sodium bicarbonate. The two-phase mixture was evaporated to dryness in a stream of air and the residue triturated with four 10-ml. portions of acetone. The acetone extract was treated with Norit and concentrated to *ca.* one-half of the original volume. The addition of petroleum ether (b.p. 30–60°) caused the slow deposition of the product in the form of a mat of colorless needles, 1.34 g. (62% yield based on **2**), m.p. 106–112°. Recrystallization of the product from ether did not change the melting point. However, material of m.p. 119–121° was obtained after drying at 100° (1×10^{-2} mm.) for 16 hr., $[\alpha]^{25}\mathrm{D}$ +99° (*c* 0.5, water); $\lambda^{\mathrm{KBr}}_{\mathrm{max}}$ 4.76 μ (azide); $\lambda^{\mathrm{H_2O}}_{\mathrm{max,\,min}}$ 266.5 mμ (ε 11,650), 234.5 (2610).

Anal. Calcd. for $C_{10}H_{13}N_5O_4$: C, 44.94; H, 4.90; N, 26.21. Found: C, 44.98; H, 4.74; N, 26.22.

3′-Amino-3′-deoxythymidine Hydrochloride (**8**).—A solution of 0.6 g. (1.9 mmoles) of **7** in 25 ml. of ethanol containing 0.25 g. of platinum oxide was shaken under 3 atm. of hydrogen for 2.5 hr. The catalyst was removed by filtration; the filtrate was evaporated to dryness *in vacuo*. The residue was dissolved in 5 ml. of 2-propanol and to this solution was added an equal volume of cold, saturated 2-propanolic hydrogen chloride. The salt that was deposited was collected, 0.35 g. (57% yield), m.p. 240–245° dec. Two recrystallizations from methanol–2-propanol provided an analytical sample, m.p. 248–249° dec., $[\alpha]^{25}\mathrm{D}$ +11° (c 0.58, H_2O); $\lambda^{\mathrm{H_2O}}_{\mathrm{max,\,min}}$ 265.5 mμ (ε 10,015), 234.5 (2320).

Anal. Calcd. for $C_{10}H_{16}N_8O_4Cl$: C, 43.24; H, 5.80; N, 15.13. Found: C, 43.07; H, 6.12; N, 14.83.

3′-Deoxy-3′-iodo-5′-*O*-tritylthymidine (**9**) **from 2**.—A solution of 0.335 g. of **2** and 0.2 g. (1.33 mmoles) of anhydrous sodium iodide in 15 ml. of dry acetone was heated at 100° in a pressure bottle for 15 hr. The inorganic salts were removed and the filtrate was evaporated to dryness *in vacuo*. The residue was triturated with water and the yellow amorphous solid was collected. The product crystallized from acetone–methanol in the form of colorless cubes, 0.13 g. (37% yield), m.p. 145–148° (lit.[13] m.p. 147°). Recrystallization from the same solvent system raised the melting point to 148–150°, $[\alpha]^{25}\mathrm{D}$ +59° (*c* 0.42, acetone); $\lambda^{\mathrm{EtOH}}_{\mathrm{max,\,min}}$ 266 mμ (ε 10,110), 244 (4650).

From 3′-*O*-Mesyl-5′-*O*-tritylthymidine (**11**).—A solution of 0.335 g. (0.6 mmoles) of **11** and 0.2 g. (1.3 mmoles) of anhydrous sodium iodide in 15 ml. of acetone was treated in a pressure bottle at 100° for 18 hr.[17] The work-up and recrystallization of the product was identical with that described above, 0.15 g. (44% yield), m.p. and m.m.p. 148–150°, $[\alpha]^{24}\mathrm{D}$ +59° (*c* 0.51, acetone); $\lambda^{\mathrm{EtOH}}_{\mathrm{max,\,min}}$ 266 mμ (ε 10,500), 244 (4900).

Samples of **9** obtained by the two routes gave 3′-deoxy-3′-iodothymidine (**10**) as a colorless crystalline solid on detritylation with 80% acetic acid, m.p. 166–167° dec. (lit.[13] m.p. 166–167° dec.).

1-(2′,5′-Dideoxy-5′-iodo-β-D-lyxosyl)thymine (**13**) **from 5**.—A solution of 0.32 g. (1 mmole) of **5** and 0.3 g. (2 mmoles) of anhydrous sodium iodide in 15 ml. of butanone was refluxed for 17 hr. under an atmosphere of nitrogen. The inorganic salts were removed by filtration and the solution was evaporated to dryness *in vacuo*. The residue was triturated with *ca.* 5 ml. of ice–water; the solid was collected and then recrystallized from ethanol, 0.20 g. in two crops (57% yield), m.p. 154–157° dec. A second recrystallization from ethanol provided an analytical sample, m.p. 156–157° dec., $[\alpha]^{25}\mathrm{D}$ −38° (*c* 0.53, ethanol); $\lambda^{\mathrm{EtOH}}_{\mathrm{max,\,min}}$ 266 mμ (ε 10,930), 234 (3160).

Anal. Calcd. for $C_{10}H_{13}N_2O_4I$: C, 34.11; H, 3.72; N, 7.96. Found: C, 34.11; H, 3.78; N, 7.81.

From 12.—A solution of 1.0 g. (4.5 mmoles) of **12**[4] and 2.0 g. (13.3 mmoles) of sodium iodide in 25 ml. of butanone containing 0.4 ml. of glacial acetic acid was refluxed under an atmosphere of nitrogen for 16 hr. The inorganic salts were removed; the filtrate was evaporated to dryness *in vacuo*. The gummy residue solidified on trituration with *ca.* 5 ml. of ice–water. The solid was collected, washed with two 5-ml. portions of water, and sucked dry, 1.04 g. (66% yield), m.p. 156–157° dec. The product obtained by this procedure gave spectrophotometric and polarimetric data identical with that obtained above.

[16] J. P. Horwitz, A. J. Tomson, J. A. Urbanski, and J. Chua, *J. Org. Chem.*, **27**, 3045 (1962).

[17] A reaction period of 2 hr. as employed by the English workers (see ref. 13) gave **9** in only 24% yield.

Monoglucose Derivatives of Gentisic Acid

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A glucoside of gentisic acid (2,5-dihydroxybenzoic acid) has been found to accumulate in sunflower leaves that have become deficient in boron.[1] As the position of attachment of the glucose unit in the unknown glucoside was not determined, synthesis of the hitherto unknown monoglucosides of gentisic acid for use as reference standards was undertaken. Gentisic acid-5-β-D-glucopyranoside (I), gentisic acid-2-β-D-glucopyranoside (II), and 1-*O*-gentisoyl-β-D-glucopyranose (III) have been prepared.

Glucosides I and II were obtained from their known methyl esters[2] by de-esterification with barium hydroxide. Compound III was produced by the condensation of 2,5-dibenzyloxybenzoyl chloride with the sodium salt of 4,6-*O*-benzylidene-α-D-glucopyranose in chloroform, followed by the catalytic reduction of the product. It was then purified on a polyamide column. Acetylation of III produced the hexaacetate, 1-*O*-(2′,5′-diacetylgentisoyl)-β-D-glucopyranose tetraacetate. This derivative was identical with that obtained by the condensation of gentisic acid diacetate with tetra-*O*-acetyl-α-D-glucopyranosyl bromide in the presence of silver oxide and quinoline. Steps in the preparation of monoglucosides of gentisic acid are summarized in Fig. 1.

Experimental

Melting points are not corrected and were taken in open capillary tubes. No efforts were made to obtain maximum possible yields.

2,5-Dibenzyloxybenzoic Acid.—To a solution of 61.6 g. (0.4 mole) of gentisic acid in 800 ml. of absolute ethyl alcohol was added 228 ml. of freshly distilled benzyl chloride, followed by 332 g. of anhydrous potassium carbonate. The mixture was refluxed for 20 hr. under anhydrous conditions and cooled to room

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[1] R. Watanabe, W. Chorney, J. Skok, and S. Wender, *Phytochemistry*, in press.

[2] G. Wagner, *Arch. Pharm.*, **291**, 278 (1958).

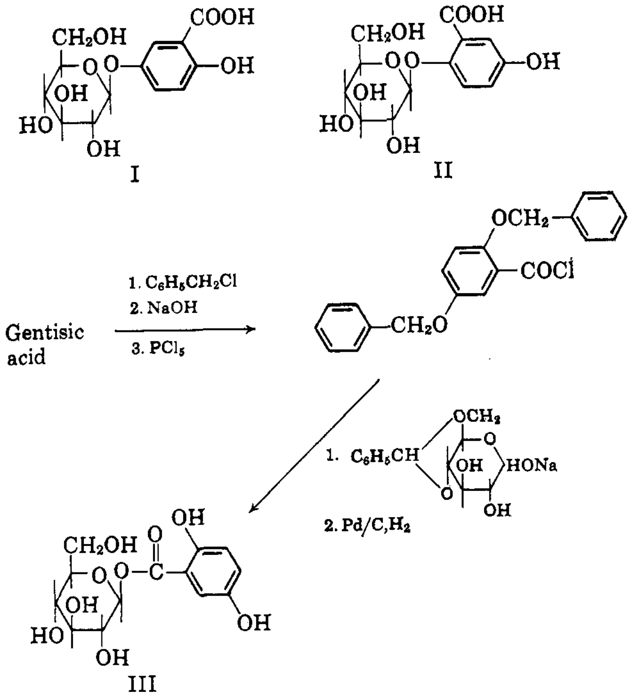

Fig. 1.—Summary of steps in synthesis of monoglucose derivatives of gentisic acid.

temperature; 900 ml. of cold water was added. Three phases were formed. After separation, the middle yellow layer was refluxed for 45 min. in 680 ml. of ethyl alcohol-water (100:70 v./v.) containing 60 g. of sodium hydroxide. After cooling the reaction mixture to room temperature, 800 ml. of cold water was added. Neutralization with concentrated hydrochloric acid produced a pale yellow product which was filtered off, washed with water, and air-dried to yield 92 g. (69%) of a pale yellow powder. Crystallization from benzene–isooctane gave a white crystalline product (82 g., 61%), m.p. 108–109°.

Anal. Calcd. for $C_{21}H_{18}O_4$ (334.37): C, 75.43; H, 5.43. Found: C, 75.61; H, 5.45.

2,5-Dibenzyloxybenzoyl Chloride.—To a solution of 40 g. (0.12 mole) of 2,5-dibenzyloxybenzoic acid in 160 ml. of anhydrous benzene–isooctane (1:1 v./v.) was added 29 g. (0.14 mole) of phosphorus pentachloride. When the initial reaction had subsided, the solution was refluxed for 1 hr. and then left overnight at 5°. Filtration yielded 30 g. (71%) of a pale yellow crystalline product, m.p. 83–86°. Crystallization from hexane produced granular white crystals (24 g., 57%), m.p. 86–88°.

Anal. Calcd. for $C_{21}H_{17}ClO_3$ (352.81): C, 71.49; H, 4.86; Cl, 10.05. Found: C, 71.64; H, 4.98; Cl, 9.88.

Sodium Salt of 4,6 *O*-Benzylidene-α-D-glucopyranose.—The 4,6-*O*-benzylidene-α-D-glucopyranose was prepared by the method of Zervas.[3] The crude product was crystallized twice from hot water and once from ethyl acetate to yield white crystals, m.p. 184–185°, lit. m.p. 188°. The sodium salt was obtained by the Zervas method.

1-*O*-Gentisoyl-β-D-glucopyranose (III).—To a solution of 10 g. (0.0286 mole) of 2,5-dibenzyloxybenzoyl chloride in 35 ml. of anhydrous chloroform was added 8.3 g. (0.0286 mole) of freshly prepared sodium salt of 4,6-*O*-benzylidene-α-D-glucopyranose. The suspension was shaken for 2 days at room temperature and then concentrated under reduced pressure to a thick sirup. Ethyl acetate (800 ml.) was added, and the cloudy solution then was washed three times with distilled water. The organic layer was dried over anhydrous sodium sulfate and then hydrogenated at room temperature and atmospheric pressure in the presence of 2 g. of 10% palladium on charcoal until absorption had substantially ceased (3 hr.). The catalyst was removed by filtration, and the filtrate was concentrated *in vacuo* to a small volume. The addition of excess benzene produced a white precipitate which was filtered off, washed with benzene, and air-dried to give a white powder (5.7 g., 63%). Purification of III was achieved by chromatography on a column packed with "Ultramidpulver" (Badische-Anilin and Soda-Fabrick AG, Ludwigshafen am Rhein) in water, under a pressure of 2 p.s.i. The absorbent was washed with five column lengths of dimethylformamide–acetic acid–water–ethyl alcohol (1:2:6:4, v./v./v./v.) under gravity, followed by distilled water. Impure III (1 g.), dissolved in 50 ml. of distilled water, was added onto the polyamide. On development of the chromatogram with distilled water, two zones formed which fluoresced blue under ultraviolet light (3660 Å.). The major zone was eluted in 1300 ml. of colorless solution. On "freeze-drying," a white product (0.7 g.) resulted. Paper chromatography showed the presence of only one compound. Using descending chromatography and Whatman No. 1 chromatography paper, R_f values for the glucoside were 0.62 in *n*-butyl alcohol–acetic acid–water (6:1:2 v./v./v., called BAW), 0.83 in 2% acetic acid–water, and 0.38 in isobutyl methyl ketone–formic acid–water (3:1:2 v./v./v., called IBFW). The glucoside III showed $[\alpha]^{25}\mathrm{D}$ −17.3° (distilled water).

Anal. Calcd. for $C_{13}H_{16}O_9$ (316.27): C, 49.37; H, 5.10. Found: C, 49.16; H, 5.19.

After spraying III on paper chromatograms with a 0.2% aqueous solution of β-glucosidase (Calbiochem, Los Angeles, Calif.) and leaving for 1 hr. in a moist chamber, gentisic acid was obtained.

1-*O*-(2′,5′-Diacetylgentisoyl)-β-D-glucopyranose Tetraacetate.—To a suspension of 0.5 g. of 1-*O*-gentisoyl-β-D-glucopyranose in 5 ml. acetic anhydride was added 1 drop of concentrated sulfuric acid. The solid dissolved rapidly, and the solution was kept at 50–60° for 30 min., and then quenched in 100 ml. of ice-water. Filtration yielded a white product (0.7 g.). Two crystallizations from hot methyl alcohol yielded a white crystalline product (0.2 g.), m.p. 158–159°.

In another preparation, gentisic acid diacetate[2] (19 g., 0.08 mole) and tetra-*O*-acetyl-α-D-glucopyranosyl bromide (32.8 g., 0.08 mole) were dissolved in warm quinoline (55 ml.) The addition of silver oxide (10 g.) with stirring produced heating and thickening of the reaction mixture. After 2 hr., the dark viscous material was extracted with 200 ml. of hot acetic acid and filtered. Quenching in 2.5 l. of ice-water produced a brown precipitate. After filtration and air-drying, the brown material (28.1 g.) was crystallized from methyl alcohol and charcoal to produce 20 g. (44%) of white crystals, m.p. 156–157°. Recrystallization from methyl alcohol gave a product melting at 158–159°.

Anal. Calcd. for $C_{25}H_{28}O_{15}$ (568.50): C, 52.82; H, 4.96. Found: C, 52.99; H, 4.97.

A mixture melting point determination of the 1-*O*-(2′,5′-diacetylgentisoyl)-β-D-glucopyranose tetraacetate samples prepared by the two different pathways showed no depression of melting point of either sample.

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[3] L. Zervas, *Ber.*, **64**, 2289 (1931).

Isolation of Gentiobiose from Gentian Root

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The trisaccharide, gentianose, was isolated in 1882 from roots of *Gentiana lutea*[2] and shown later to give rise when treated with invertase to a disaccharide, gentiohexobiose, now called gentiobiose, and fructose.[3] Later work confirmed this, a yield of 1.2 g. of gentiobiose octaacetate being obtained from a kilogram of the root.[4]

The relative inaccessibility of the gentian plant and the uncertainty of the presence of gentianose in the

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[1] Paper No. 5286, Minnesota Agriculture Experiment Station.

[2] A. Meyer, *Z. physiol. Chem.*, **6**, 135 (1882).

[3] E. Bourquelot and H. Hérissey, *Compt. rend.*, **132**, 571 (1901).

[4] G. Zemplén, *Z. physiol. Chem.*, **85**, 402 (1913); *Ber.*, **48**, 233 (1915).