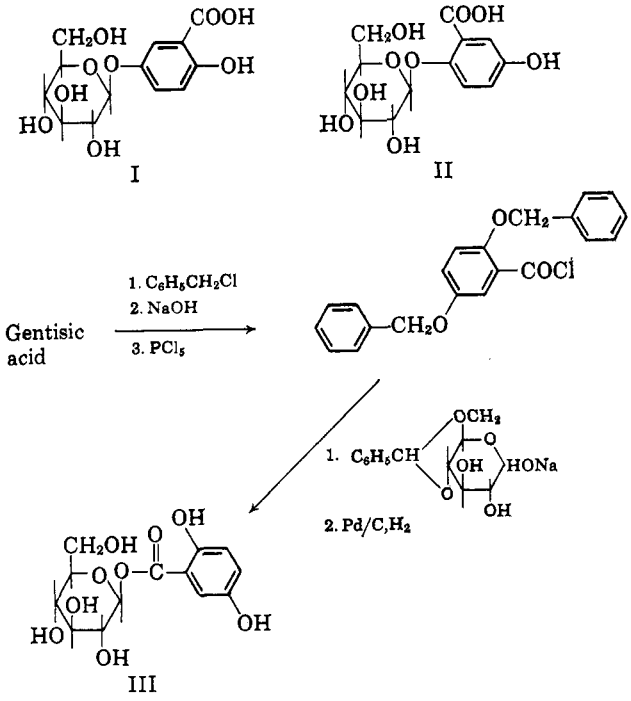

Fig. 1.—Summary of steps in synthesis of monoglucose derivatives of gentisic acid.

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 $[\alpha]^{25}D$ −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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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

(1) Paper No. 5286, Minnesota Agriculture Experiment Station.
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(3) E. Bourquelot and H. Hérissey, *Compt. rend.*, **132**, 571 (1901).
(4) G. Zemplén, *Z. physiol. Chem.*, **85**, 402 (1913); *Ber.*, **48**, 233 (1915).

powdered gentian root of commerce,[5] which may depend upon the stage of development of the plant, has led to the suggestion that the best way to prepare gentiobiose is to synthesize it by treating 2,3,4,6-tetra-*O*-acetyl-α-glucosyl bromide with 1,2,3,4-tetra-*O*-acetyl-D-glucose. This provides the crystalline octaacetate[6,7] from which the free disaccharide may be obtained by deacetylation. A recent modification of this synthetic approach involving the interaction of 2,3,4,6-tetra-*O*-acetyl-α-D-glucosyl bromide with 1,2,3,4-tetra-*O*-acetyl-6-*O*-trityl-β-D-glucose in the presence of silver perchlorate is worthy of note.[7] A biochemical synthesis effected by the action of almond emulsin (β-D-glucosidase) on D-glucose has also been recommended,[8] and recently controlled hydrolysis of yeast glucan[9] and of the β 1 → 6 linked D-glucan (pustulan) from *Umbilicaria pustulata*[10] has been shown to give gentiobiose.

During the summer of 1961, roots of the yellow gentian (*Gentiana lutea*) were collected in the area of Lausanne, Switzerland, and shown to contain gentiobiose in such amounts that acetylation of the 50% aqueous ethanol extract readily afforded crystalline gentiobiose octaacetate, the yield amounting to 23 g./kg. of dried roots.

Roots of a second species of gentian (*Gentiana andrewsii*) collected in September, 1963, in New Hampshire (U. S. A.) have also been found to be a good source of gentiobiose. In this case, the roots were extracted with water and the extract was treated with ethanol to precipitate a polysaccharide which was composed of arabinose, galactose, glucose, and traces of rhamnose. Acetylation of the mixture of sugars recovered from the aqueous ethanolic solution readily afforded gentiobiose β-octaacetate, the yield of the latter corresponding to 26 g./kg. of dried roots. Thus, treatment of the material with yeast invertase as formerly recommended[3-5] is unnecessary.

Experimental

All evaporations were carried out under reduced pressure at about 40°.

Isolation of Gentiobiose from Gentian Roots.—A. From *Gentiana lutea*. Roots were collected from flowering plants of the yellow gentian found in the vicinity of Lausanne, Switzerland, during the first week of July, 1961. The partially dried roots (150 g.) were cut into small pieces and extracted with 50% aqueous ethanol (900 ml.) at room temperature during 12 hr. The extract was decanted and the residue was ground in a mortar and a second extraction was carried out in the same manner. After three extractions had been made, the combined solutions were concentrated *in vacuo* at 40° to a volume of about 200 ml. This solution was treated with ethanol (400 ml.) and, after adding charcoal, the solution was filtered and concentrated to 100 ml. Paper chromatography showed that this solution contained glucose, sucrose, and gentiobiose, and smaller porportions of other slow moving components. The solution (100 ml.) was treated with water (100 ml.) and invertase (10 mg.) was added. Addition of invertase is believed to be unnecessary (see B below). After keeping overnight, the solution was concentrated to dryness and the residue was dissolved in pyridine (30 ml.) and treated with acetic anhydride (20 ml.) at room temperature for 12 hr. The reaction mixture was poured with stirring into water and after 2 hr. the product was extracted with chloroform (200 ml.) The chloroform solution was washed with water (three times), dried ($MgSO_4$), and concentrated to a sirup. This sirupy product was dissolved in warm methanol and after nucleating with β-gentiobiose octaacetate, the solution was allowed to crystallize. After keeping for 12 hr., filtration and washing gave a crude product (3.5 g.) which when recrystallized from methanol gave β-gentiobiose octaacetate, m.p. 192.5–194°, $[\alpha]^{25}D$ −5.6° (*c* 1.3, chloroform); lit.[5] (β-gentiobiose octaacetate) m.p. 193°, $[\alpha]D$ −5.3° (chloroform); yield 2.5 g. of octaacetate from 250 g. of gentian root of unspecified origin; lit.[5,7] (α-octaacetate) m.p. 188–189°, $[\alpha]D$ +52.4° (chloroform).

B. From *Gentiana andrewsii*.—Roots of this species of blue gentian were collected from flowering plants found in the mountains of New Hampshire, U. S. A., in September, 1963. The partially dried roots (77 g.) which had been kept at room temperature for about 7 days were heated with water (500 ml.) on a steam bath for 5 hr. The swollen roots were disintegrated in a Waring Blendor in the presence of added water (total volume 1000 ml.). The mixture was filtered through a linen cloth and the filtrate was concentrated to a volume of 500 ml. and treated with ethanol (1000 ml.). The polysaccharide which was precipitated at this stage was recovered (centrifuge) and purified by reprecipitation (twice) from aqueous solution with ethanol and then dried *in vacuo* after washing successively with ethanol, ether, and petroleum ether. This polysaccharide, which readily dissolved in water, showed $[\alpha]^{24}D$ +157° (*c* 1, water), and, upon hydrolysis by heating (sealed tube) with 0.5 *N* H_2SO_4 for 5 hr. in a boiling water bath, it gave rise to arabinose, galactose, glucose, and traces of rhamnose as revealed by paper chromatography.

Evaporation of the aqueous ethanolic solution from the first precipitation of the polysaccharide gave a sirupy product which was dissolved in methanol (250 ml.). After removing a small proportion of insoluble precipitate, the methanolic solution was concentrated to dryness and the yellowish brown residue was subjected to acetylation by heating for 2 hr. with a mixture of acetic anhydride (135 ml.) and anhydrous sodium acetate (11 g.). The reaction mixture was poured with stirring into water (1000 ml.) and, after the excess of acetic anhydride had decomposed, the acetylated product was extracted with chloroform (500 ml.). The chloroform solution was washed with an aqueous solution of sodium bicarbonate and with water. The dried ($MgSO_4$) chloroform extract was treated with charcoal, filtered, and concentrated to remove the solvent. The residue was dissolved in a small volume of methanol and the solution, after nucleation with β-gentiobiose octaacetate, was kept at room temperature for 2 days until crystallization was complete. The crystalline mass was diluted with methanol and the crystals were recovered by filtration. Recrystallization of the product from methanol gave β-gentiobiose octaacetate (2.0 g.), m.p. 195° and $[\alpha]^{25}D$ −6.4° (*c* 2, chloroform).

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Synthesis of α-Keto Acids from Diethyl Alkylidenemalonates

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An earlier paper[1] described the preparation of α-keto amides by epoxidation of ethyl alkylidenecyanoacetates and subsequent decarboxylation of the epoxy acids thus obtained. This procedure has now been extended to the synthesis of α-keto acids by the use of diethyl alkylidenemalonates.

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