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THE ISOLATION OF 6-*O*-ACETYL-2,3,4-TRI-*O*-[(+)-3-METHYLVALERYL]-β-D-GLUCOPYRANOSE FROM TOBACCO*

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ABSTRACT

A crystalline mixed ester, 6-*O*-acetyl-2,3,4-tri-*O*-[(+)-3-methylvaleryl]-β-D-glucopyranose, was obtained from Turkish tobacco by column chromatography and liquid–liquid partitions of a hexane extract. Saponification of the ester gave glucose, identified by paper chromatography, and acetic and 3-methylpentanoic (3-methylvaleric) acids, identified by gas chromatography and by the formation of their *p*-phenylphenacyl esters. Exhaustive methylation of the mixed ester, followed by saponification of the methylated ester gave methyl β-D-glucopyranoside. Confirmation of the structure of the ester was provided by its synthesis from D-glucose, (+)-3-methylvaleric acid, and acetic acid in 43% yield.

DISCUSSION

Organic acids of low molecular weight are obtained from the volatile oils of tobacco[1–9], small amounts occurring seemingly in the free state[1–3,9]. However, these acids generally are found as esters of aliphatic alcohols, terpene alcohols, and polyhydric alcohols, such as glycerol and D-glucitol[4–8].

The isolation of 6-*O*-acetyl-2,3,4-tri-*O*-[(+)-3-methylvaleryl]-β-D-glucose marks the first time that D-glucose, esterified with low-molecular-weight fatty acids, has been isolated in a pure crystalline form from tobacco. The occurrence of this ester in Nature is unique, because it is a mixed ester which still exhibits reducing properties, and a naturally occurring sugar ester, esterified to the degree found in this compound, has never been reported.

The glucose ester was isolated from a hexane extract of Turkish tobacco, grown in the Souyalassian region of Greece, as follows: the extract was partitioned between 9:1 methanol–water and hexane on a Podbielniak centrifugal extractor[10], and the material from the 9:1 methanol–water layer was chromatographed on a Magnesol–Celite column. The effluent was thoroughly fractionated by partitions and chromatography to give the mixed tetraester of D-glucose.

*Dedicated to the memory of Professor M. L. Wolfrom.

The i.r.-absorption spectrum (Fig. 1) of this compound showed that it was a sugar ester containing at least one free hydroxyl group. On saponification, the sugar moiety was identified as a glucose by paper chromatography with the procedure of Partridge[11] and that of Hough, Jones, and Wadman[12]. Gas–liquid chromatography[13] showed the acids to be mainly (a) six-carbon acid(s) with a small amount of acetic or formic acid. The acids were converted into their *p*-phenylphenacyl esters by the method of Shriner and Fuson[14], and separated chromatographically according to Kirchner, Prater, and Haagen-Smit[15]. *p*-Phenylphenacyl acetate and *p*-phenylphenacyl (+)-3-methylvalerate were obtained.

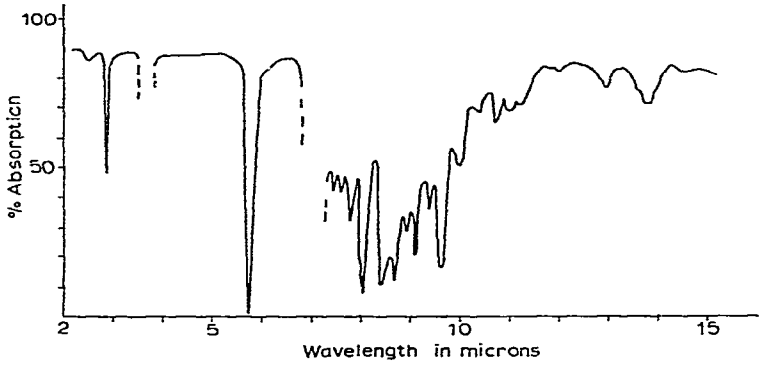

Fig. 1. I.r. spectrum of 6-*O*-acetyl-2,3,4-tri-*O*-[(+)-3-methylvaleryl]-β-D-glucose determined on a Nujol mull.

Methylation of the isolated D-glucose ester with methyl iodide and silver oxide[16] followed by saponification yielded methyl β-D-glucopyranoside. This showed that only one free hydroxyl group was present and that it was attached to the C-1 of the D-glucose molecule. This likewise indicated the presence of four ester groups. Furthermore, the elementary analysis and the saponification equivalent (118) indicated the presence of three 3-methylvaleryl groups and one acetyl group. The position of the acetyl group was determined by synthesis, and the β-D-configuration was established by mutarotation of the natural and synthetic products.

The glucose ester, 6-*O*-acetyl-2,3,4-tri-*O*-[(+)-3-methylvaleryl]-β-D-glucopyranose, was synthesized by a modification of the method used by Reynolds and Evans[17] for the synthesis of α- and β-D-glucose 1,2,3,4-tetraacetate and of the method of Wolfrom and Christman[18] involving further esterification after detritylation. D-Glucose was converted into the 6-*O*-trityl derivative, which was further esterified with (+)-3-methylvaleryl chloride to give 1,2,3,4-tetra-*O*-[(+)-3-methylvaleryl]-6-*O*-trityl-D-glucose. The primary hydroxyl group at C-6 was regenerated by detritylation with hydrogen bromide, and then esterified with acetyl chloride. The (+)-3-methylvaleryl group was removed from the 1-position with hydrogen bromide (more drastic conditions than in the detritylation). The resulting 1-bromo compound was treated with silver carbonate to give 6-*O*-acetyl-2,3,4-tri-*O*-[(+)-3-methylvaleryl]-α- and -β-D-glucose. The β anomer crystallized, whereas the α anomer remained as a syrup. With time, the

α anomer mutarotated to the α,β-equilibrium mixture from which an additional quantity of the β anomer crystallized. The yield of the β anomer was 43% based on D-glucose.

The synthetic product was shown to be identical with the ester isolated from tobacco. Thus, the structure of the isolated material has been unequivocally identified as 6-*O*-acetyl-2,3,4-tri-*O*-[(+)-3-methylvaleryl]-β-D-glucopyranose.

A small amount of a crystalline by-product was obtained from this synthesis. The i.r. spectrum and the elementary analysis suggested that it was 2,3,4,6-tetra-*O*-[(+)-3-methylvaleryl]-D-glucose, which was confirmed by synthesis.

EXPERIMENTAL

General. — All melting points were determined with a Fisher–Johns melting-point apparatus. Melting points and boiling points were uncorrected. The elementary analyses were performed by Huffman Microanalytical Laboratories, Wheatridge, Colorado.

Isolation and identification of 6-O-acetyl-2,3,4-tri-O-[(+)-3-methylvaleryl]-β-D-glucopyranose from tobacco. — The tobacco (55 kg) was shredded on a small cutting machine and extracted three times in a stainless-steel column with hexane at 30°. The combined hexane extracts were concentrated to 40 gallons on a Turba-Film evaporator. The hexane extract was passed through a Podbielniak extractor[10] where it contacted a stream of 9:1 methanol–water. The methanol–water extract was concentrated to a small volume on the Turba-Film evaporator, and water was removed at 45–50° under 28 mm pressure to give an oil (yield 438 g).

This material was divided into three parts (125, 153, and 160 g, respectively). Each of these was dissolved in 1:1 hexane–ether (500 ml) and the solution was passed through a column of Magnesol–Celite (5:1) prewashed with 1:1 hexane–ether (1000 ml). Development was effected with the same solvent mixture (6–8 liters). The column of adsorbent was extruded after collecting 6 l of effluent, and cut into 3 sections as indicated by the colored zones. The sections were eluted with methanol, and the solvent was removed under reduced pressure. The combined yields from the three columns were: 70 g from effluent (Fraction A), 120 g from bottom zone (Fraction B), 172 g from middle zone (Fraction C), and 29 g from top zone (Fraction D). Total yield was 391 g (89%).

Fraction A was partitioned between three 800-ml layers of hexane and four 800-ml layers of 9:1 methanol–water. Evaporation under diminished pressure of the combined lower layers yielded a syrup (47.4 g). It was dissolved in 1:1 hexane–ether (800 ml) and divided into 8 fractions (each 100 ml). Each fraction was passed through a silicic acid column (14 × 8 cm) which had been activated by washing with 0.2, 1.0, 0.8, and 1.0 vol. (solvent needed to wet the entire column) of ether, 1:1 ether–acetone, ether, and hexane, respectively. The column was developed with hexane–ether solutions; the amount of ether was increased from 10% to 100%, as needed to elute the material from the column. After evaporation of the solvent, similar syrupy fractions of the 8 separate chromatograms were combined according to their i.r.

spectra: 1.4 g (Fraction 1), 8.9 g (Fraction 2), 22.5 g (Fraction 3), and 10.5 g (Fraction 4).

The material from Fraction 3 was partitioned between 25 100-ml hexane–ether (3:1) layers and 25 100-ml methanol–water (3:1) layers, with an H. O. Post countercurrent-distribution apparatus[19]. After evaporation at 50° under diminished pressure, the material from upper layers 3–12, 13–16, and 17–22 was combined to give Fractions A, B, and C (3.13 g, 1.95 g, and 2.38 g, respectively). Fractions A and B partially crystallized, and were filtered off after the addition of pentane. More crystalline material was obtained from the filtrate and Fraction C. Recrystallization from hexane of the total solid obtained gave needles (1.2 g), m.p. 104–106°, $[\alpha]_D^{25}$ +30.21° (*c* 4.7, chloroform), $[\alpha]_D^{25}$ +15.45 → +54.50° (*c* 4.0, 4:1 methanol–water), constant after 25 h. This substance also gave a positive Somogyi[20] test for reducing sugars.

Anal. Calc. for $C_{26}H_{44}O_{10}$: C, 60.44; H, 8.58; Mol. wt. 516.6. Found: C, 60.16, 60.41; H, 8.56, 8.61; Mol. wt., 415 (with dec.; Rast method).

To a solution of the ester (100 mg) in methanol, cooled to −20°, was added a solution of 0.2M sodium methoxide in methanol (0.3 ml). After being kept at −20° for 62 h, acetone (6 ml) was added. A white solid precipitated. It was filtered off, dissolved in methanol, and the solution was concentrated under diminished pressure to give a syrup (29.5 mg). The filtrate was concentrated to give the sodium salts of the acids.

Identification of the sugar moiety. — The syrup was chromatographed according to the method of Partridge[11] along with D-glucose, D-ribose, D-xylose, D-arabinose, and D-rhamnose, on Whatman No. 1 paper with 4:1.1:1.9 butyl alcohol–ethanol–water (v/v) for 40 h. The spots were revealed[12] by spraying with a saturated solution of *p*-anisidine hydrochloride in butyl alcohol followed by drying at 100°. The syrup gave only one spot which coincided exactly with D-glucose. It was compared in a second chromatogram with maltose, sucrose, D-fructose, D-mannose, and D-glucose, and gave one spot coinciding with D-glucose.

Identification of acids. — The sodium salts of the acids were converted into the methyl esters by heating at reflux with methanol (10 ml) containing 10 drops of concentrated sulfuric acid. The esters were chromatographed on a Perkin–Elmer Vapor Fractometer, Model 154-B with a column (1 m) packed with purified Celite containing 20% of the adipate polyester of di-(2-hydroxyethyl) ether[13]. The chromatogram showed the presence of methyl acetate (or methyl formate) and (a) methyl ester(s) of a C_6 acid.

To a solution of the ester (100 g) in acetone (15 ml) cooled to 0° was added 0.1M sodium hydroxide (25 ml), and the mixture was kept for 2 h at 0°. It was neutralized with 0.1M hydrochloric acid (phenolphthalein). A control experiment was performed with glucose (33 mg) instead of the glucose ester to correct for any acid formation due to the action of alkali on the glucose portion. The saponification equivalent was found to be 118.2.

The sodium salts of the acids were recovered, dissolved in water (2 ml), and neutralized with two drops of dilute hydrochloric acid. Ethanol (5 ml) and *p*-phenylphenacyl bromide (200 mg) were added and the solution was heated at reflux for 1 h.

The reaction mixture was evaporated to dryness. The residue was dissolved in benzene and the solution was chromatographed on a silicic acid column (14 × 2 cm) according to the procedure of Kirchner, Prater, and Haagen-Smit[15]. The column was developed with 1:1 hexane–benzene, and fractions of 20 ml were collected. Fractions 4–6 yielded *p*-phenylphenacyl (+)-3-methylvalerate (51 mg), m.p. 46° (lit.[21]: m.p. 47°). This m.p. was not depressed when the product was mixed with an authentic sample prepared from (+)-3-methylvaleric acid. Fractions 8 and 9 gave 11 mg of *p*-phenylphenacyl acetate, m.p. 110–111°. A mixed melting point with an authentic sample gave no depression. The i.r.-absorption spectra of both esters were identical with those of the corresponding authentic samples.

Methylation of the ester[16]. — A mixture of the ester (300 mg), methyl iodide (2 ml), and silver oxide (200 mg) was warmed (oil bath) with vigorous stirring. Additional methyl iodide (2-ml portions) was added periodically over two days. Ether was added, the suspension was filtered, and evaporation of the filtrate gave a syrup (300 mg). The i.r. spectrum showed no hydroxyl peak. The methylated product was saponified in acetone (25 ml) at 0° with 0.1M sodium hydroxide (49 ml). After neutralization with 0.1M hydrochloric acid and evaporation to dryness under diminished pressure, the residue was dissolved in water (20 ml) and the solution was deionized by successive passage through (6 × 1.2 cm) columns of Amberlite IR-120 and Duolite A-4. The final effluent was concentrated to dryness under reduced pressure to give a syrup (100 mg). This was dissolved in abs. ethyl alcohol and the solution was chromatographed on a (6 × 1.2 cm diameter) column of Florex XXX–Celite (5:1 by wt.) prewashed with abs. ethyl alcohol (50 ml). The column was developed with abs. ethyl alcohol and 25-ml fractions were collected. On the basis of the i.r. spectra, fractions 3–6 were combined, dissolved in ethyl alcohol, and kept to crystallize. Recrystallization from ethyl alcohol gave methyl β-D-glucopyranoside (60 mg), m.p. 112–113°, $[\alpha]_D^{25}$ −32.5° (*c* 3.0, water). A mixed m.p. with an authentic sample[22] gave no depression. The i.r. absorption spectra of both products were identical.

(+)-*3-Methylvaleryl chloride.* — Attempts to resolve racemic 3-methylvaleric acid according to the methods of Levene and Marker[23] were unsatisfactory for our purpose. The dextrorotatory 3-methylvaleric acid (120 g), a known constituent of tobacco, was obtained from tobacco (70 kg) according to the procedure of Sabetay and Panouse[4], b.p. 196°, $[\alpha]_D^{25}$ +7.40° (lit.[25]: b.p. 196–198°, $[\alpha]_D^{25}$ +7.94).

(+)-3-Methylvaleric acid (20 g) was mixed with thionyl chloride (13 ml) and kept for 15 h. The product was distilled on a short glass helices-packed column to give (+)-3-methylvaleryl chloride, b.p. 140–142°.

1,2,3,4-Tetra-O-[(+)-*3-methylvaleryl*]-*6*-O-*trityl*-D-*glucose.* — D-Glucose (5 g), powdered and dried over phosphorus pentoxide, and chlorotriphenylmethane (8 g recrystallized from acetyl chloride by the procedure of Reynolds and Evans[17]), were dissolved in anhydrous pyridine (45 ml) by continuous shaking for 5 h. The solution was kept for 15 h and freshly prepared (+)-3-methylvaleryl chloride (16 g) was added carefully. After being kept for 7 h at room temperature, the reaction mixture was poured into ice–water (200 ml), and the aqueous mixture was extracted

twice with equal portions of ether. The ether extracts were combined, concentrated, decolorized with Darco G-60, and dried with sodium sulfate. Removal of the ether under reduced pressure gave a crude syrup product (25 g, incompletely dry).

1,2,3,4-Tetra-O-[(+)-*3-methylvaleryl*]-D-*glucose.* — The trityl ester was dissolved in acetic acid (60 ml) and the solution was cooled in an ice bath. Acetic acid (12 ml) saturated with hydrogen bromide gas at 0° was added. Bromotriphenylmethane precipitated and was immediately removed by filtration. The filtrate was quickly poured into ice–water (300 ml) and thoroughly stirred. The oily layer was dissolved in ether, decolorized with Darco G-60, and dried with sodium sulfate. Evaporation gave a noncrystalline product (17 g), contaminated with a small percentage of triphenylcarbinol.

6-O-Acetyl-1,2,3,4-tetra-O-[(+)-*3-methylvaleryl*]-D-*glucose.* — The crude 1,2,3,4-tetraester was immediately dissolved in anhydrous pyridine (25 ml) and treated with acetyl chloride (4 ml). After being kept for 4 h at room temperature, the reaction mixture was poured into ice–water (200 ml). The solid product was dissolved in ether, the solution was decolorized with Darco G-60, and dried with sodium sulfate. After filtration, evaporation of the filtrate under reduced pressure gave a crude syrup (16.4 g).

6-O-Acetyl-2,3,4-tri-O-(+)-*3-methylvaleryl*]-D-*glucopyranosyl bromide.* — The mixed ester just described was dissolved in 1,1,2-trichloroethane (25 ml). Acetic acid (15 ml) saturated at 0° with hydrogen bromide was added, and the mixture was kept for 24 h. The solvents were removed under diminished pressure, and benzene was added to form an azeotrope with acetic acid to yield 16.5 g of crude product. The free (+)-3-methylvaleric acid was not removed from the product at this time.

6-O-Acetyl-2,3,4-tri-O-[(+)-3-*methylvaleryl*]-β-D-*glucose.* — The glycosyl bromide was dissolved in acetone (45 ml), water (1 ml) and freshly prepared silver carbonate (9 g) were added, and the mixture was shaken continuously on a water bath for 7 h at 50°. The mixture was filtered through a short column (7 cm × 1.5 cm) containing a mixture of Darco G-60, Celite, and sodium sulfate (1:1:1). The filtrate was concentrated to give an almost colorless syrup (14.5 g) still containing one-mole equivalent of (+)-3-methylvaleric acid.

This crude product was dissolved in pentane and kept for 15 h at −15°, whereby 1.1 g of material crystallized. Recrystallization from hexane gave fluffy white needles, m.p. 105–106°; $[\alpha]_D^{25}$ +30.90° (*c* 2.32, chloroform), $[\alpha]_D^{25}$ +16.01 → +55.00° (*c* 1.82, 4:1 methanol–water, constant after 25 h). A mixed melting point with the glucose ester isolated from tobacco showed no depression. The crude mother liquor of the product described above had $[\alpha]_D^{25}$ +50.90 → +39.52° (*c* 5.0, 9:1 methanol–water). It was partitioned between 40 10-ml layers of pentane and 40 10-ml layers of 3:1 methanol–water. The solvent was removed from the upper and lower layers under reduced pressure, and the material in these layers was combined according to their i.r.-absorption spectra. The material in lower layers 15–39 and upper layers 25–39 were combined (6.1 g) which gave additional crystalline product (5.1 g), for a total yield of 6.2 g (43% based on D-glucose).

Lower layers 0–14 yielded a material (3.6 g) which contained mostly (+)-3-methylvaleric acid. Upper layers 0–4 gave 1.8 g of syrup which showed a weak i.r. absorption for a hydroxyl group. This indicated that the reactions involving the formation of a free hydroxyl group on C-1 were incomplete.

Upper layers 5–24 gave a material (80 mg) which crystallized. Recrystallization from hexane gave 52 mg of crystals, m.p. 52–53°. The i.r. spectrum of this material suggested that it was 2,3,4,6-tetra-*O*-[(+)-3-methylvaleryl]-D-glucose, formed by the incomplete tritylation, or by detritylation in the initial esterification step. This substance was then synthesized as described below. A mixed melting point of 2,3,4,6-tetra-*O*-[(+)-3-methylvaleryl]-D-glucose and of the crystalline material from upper layers 5–24 gave no melting-point depression.

2,3,4,6-*Tetra*-O-[(+)-3-*methylvaleryl*]-D-*glucose*. — D-Glucose (2 g) was mixed with anhydrous pyridine (24 ml) and heated on the steam bath for 30 min. On cooling, (+)-3-methylvaleryl chloride (8 ml) was added. The mixture was heated for 1 h at 60°, and then poured into cold water (200 ml). The ester was extracted with ether; the ether solution was washed with 5% sulfuric acid (10 ml) and water (20 ml), and dried with sodium sulfate. Subsequent removal of the ether gave 5.65 g of the glucose ester. The i.r. spectrum showed no hydroxyl group. The penta(methylvaleryl)-glucose was dissolved in 1,1,2-trichloroethane (6 ml) and treated for 2 h with acetic acid (6 ml) saturated with hydrogen bromide at 0°. Toluene (15 ml) was added, and the mixture was concentrated under diminished pressure to give the glycosyl bromide (4.5 g). This compound was dissolved in dry acetone (10 ml) and the solution was cooled to 0°. Freshly prepared silver carbonate (2 g) and water (0.25 ml) were added. The reaction mixture was continuously stirred for 45 min at 0°, heated for several min at 55° and then filtered. The filtrate was concentrated to a syrup, which partially crystallized at −15°. The crystalline product was filtered and recrystallized from hexane–petroleum ether, m.p. 52–53°, $[\alpha]_D^{25}$ +22.67 → +62.00° (*c* 3.0, 4:1 methanol–water, constant after 27 h).

Anal. Calc. for $C_{30}H_{52}O_{10}$: C, 62.91; H, 9.51. Found: C, 63.14; H, 9.09.

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