[CONTRIBUTION FROM THE NATIONAL INSTITUTE OF ARTHRITIS AND METABOLIC DISEASES, NATIONAL INSTITUTES OF HEALTH]

Migration of a Mesitoyl Group from C_1 to C_2 in α-D-Glucopyranose. Derivatives of 2-O-Mesitoyl-D-glucose

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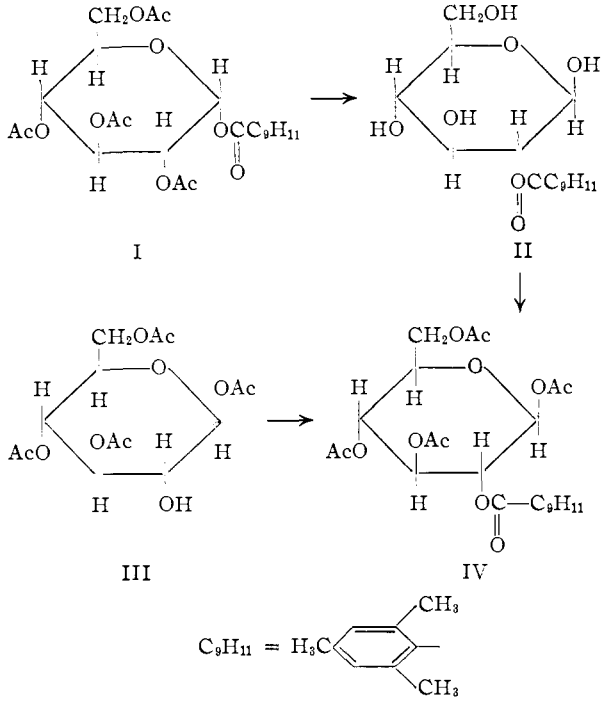
Treatment of 2,3,4,6-tetra-O-acetyl-1-O-mesitoyl-α-D-glucopyranose with methanolic ammonia at a low temperature results in loss of the acetyl groups and migration of the mesitoyl group. The product, a crystalline mono-O-mesitoyl-D-glucose, was converted to D-glucose phenylosotriazole and to a crystalline tetraacetate which was independently synthesized through mesitoylation of 1,3,4,6-tetra-O-acetyl-β-D-glucopyranose. The acyl migration product is, therefore, 2-O-mesitoyl-D-glucose. Some implications of this acyl migration are discussed.

In the course of some previous work, designed to throw light upon certain features of the structure of the remarkably sweet natural glucoside, stevioside,[2] the two anomeric 2,3,4,6-tetra-O-acetyl-1-O-mesitoyl-D-glucopyranoses[3] were synthesized in pure form. The β-anomer of this pair was deacetylated with methanolic ammonia to give 1-O-mesitoyl-β-D-glucopyranose in crystalline form, a stable, non-mutarotating substance, which on reacetylation gave the original 2,3,4,6-tetra-O-acetyl-1-O-mesitoyl-β-D-glucopyranose. We wish now to report the contrasting behavior of the α-anomer upon deacetylation.



When 2,3,4,6-tetra-O-acetyl-1-O-mesitoyl-α-D-glucopyranose (I) is treated with methanolic ammonia at a low temperature, a mono-O-mesitoyl-D-glucose is obtained which mutarotates in water, $[\alpha]^{20}D$ +24.0° (13.5 min.) → +45.5° (19 hr.),[4] and on reacetylation, affords a tetra-O-acetyl-O-mesitoyl-D-glucose which is not identical with the original starting material. While 1-O-mesitoyl-β-D-glucopyranose reduced lead tetraacetate at practically the same rate as the anomeric methyl D-glucopyranosides, the new O-mesitoyl-D-glucose was oxidized by this reagent more rapidly. These facts indicate that the mesitoyl group had probably migrated during the course of the deacetylation, leaving C_1 unsubstituted. With phenylhydrazine, the new mesitoyl-D-glucose gave D-glucose phenylosazone; the identity of the latter was confirmed by conversion to D-glucose phenylosotriazole.[5] The loss of the mesitoyl group on osazone formation may be taken as presumptive evidence that it occupied the C_2-position.[6] Confirmation was obtained through mesitoylation of the known 1,3,4,6-tetra-O-acetyl-β-D-glucose[7,8] (III). The product IV thus obtained was identical with that which had been prepared through the acetylation of the mono-O-mesitoyl-D-glucose.

This migration of a mesitoyl group from α-C_1 to C_2 under such mild conditions appears at first glance to be somewhat surprising. If the mesitoyl group with its sterically hindered carbonyl can undergo the shift with such ease, normal acyl groups should migrate even more rapidly. Indeed, Lemieux and Brice[9] have predicted on other grounds that 1,3,4,6-tetra-O-acetyl-α-D-glucose should rearrange to 2,3,4,6-tetra-O-acetyl-D-glucose. It is probably significant that we have been able to find only one example of a 1-O-acyl-α-D-glucopyranose in the literature,[10] namely, the α-glucogallin (1-O-galloyl-α-D-glucopyranose) which has recently been synthesized by Schmidt and Herok.[11] While the syntheses carried out by these authors appear to speak unequivocally for the structure which they have assigned, their product mutarotates in aqueous solution—a fact which, as they recognize, is difficult to explain.

Richtmyer and Hudson[12] found that the hydroly-

(1) Chemical Foundation Fellow, 1953-1955.

(2) H. B. Wood, Jr., and H. G. Fletcher, Jr., THIS JOURNAL, **78**, 207 (1956).

(3) Mesitoyl = 2,4,6-trimethylbenzoyl.

(4) Rotations are specific rotations for the D line of sodium at 20°; concentration is expressed in g. of substance per 100 ml. of solution.

(5) R. M. Hann and C. S. Hudson, THIS JOURNAL, **66**, 735 (1944).

(6) It will be recalled that even such stable substituents as the o-methyl group are removed from the C_2-position of aldoses during the course of osazone formation although the conditions are so mild as to leave radicals at other positions undisturbed.

(7) E. Hardegger and J. de Pascual, *Helv. Chim. Acta*, **31**, 281 (1948).

(8) R. U. Lemieux and G. Huber, *Can. J. Chem.*, **31**, 1040 (1953).

(9) R. U. Lemieux and C. Brice, *ibid.*, **33**, 109 (1955).

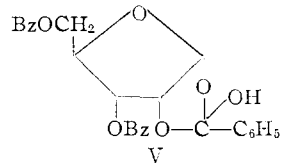
(10) A limited number of 1-O-acyl-β-D-glucoses having a hydroxyl group free at C_2 are known; *cf.* the 1-O-benzoyl-β-D-glucopyranose of L. Zervas, *Ber.*, **64**, 2289 (1931). Like our 1-O-mesitoyl-β-D-glucose (ref. 2) they are not sterically suitable for C_1 to C_2 acyl migration and show the normal stabilities of sugar esters.

(11) O. Th. Schmidt and J. Herok, *Ann.*, **587**, 63 (1954).

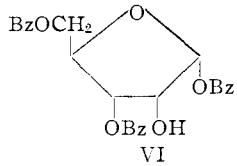
(12) N. K. Richtmyer and C. S. Hudson, THIS JOURNAL, **58**, 2534 (1936).

sis of acetochloroceltrobiose gave, in addition to the expected anomeric heptaacetates, a third heptaacetate which did not mutarotate in chloroform but gave β-celtrobiose octaacetate on acetylation. This substance might be 1,3,6-tri-*O*-acetyl-4-*O*-(tetra-*O*-acetyl-β-D-glucopyranosyl)-β-D-altrose.

Finally Ness and Fletcher[13] have found that the hydrolysis of 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl bromide affords a tribenzoyl-D-ribose that rearranges in aqueous pyridine to 2,3,5-tri-*O*-benzoyl-D-ribose.[14] On various grounds, this alkali-labile tribenzoate was assigned structure V. However, when the ease of migration of acyl groups from C_1 to C_2 is recognized, it is seen that formula VI, 1,3,5-tri-*O*-benzoyl-α-D-ribose, adequately rationalizes the properties of the substance. An investigation designed to clarify this latter case is under way in this Laboratory.



V



VI

The migration of phosphate from the α-C_1-position of D-glucopyranose to C_2 through the action of ammonia on uridine diphosphoglucose has been reported by Paladini and Leloir.[15] More recently, Wright and Khorana[16] have shown a similar migration caused by the action of dicyclohexylcarbodiimide on α-D-ribofuranose 1-phosphate, the anomeric (*trans*) phosphate being unaffected. In a formal sense, at least, these phosphate migrations can be construed as analogs of the acyl migration reported in the present paper.

Reaction of 2-*O*-mesitoyl-D-glucose with methanolic hydrogen chloride gave rise to a methyl 2-*O*-mesitoyl-D-glucopyranoside rotating $[\alpha]^{20}D$ +54.2° in water. Monomesitoylation of methyl 4,6-*O*-benzylidene-α-D-glucopyranoside followed by hydrogenolysis of the acetal linkages gave a crystalline product which consumed one mole of lead tetraacetate and was, therefore, methyl 2-*O*-mesitoyl-α-D-glucopyranoside. Since this latter showed $[\alpha]^{20}D$ +119° in chloroform, it is probable that the glucoside of $[\alpha]^{20}D$ +54.2° is the β-isomer. It may be noted in passing that 2-*O*-mesitoyl-D-glucose, its tetraacetate and the corresponding methyl glucoside were all obtained as the β-anomers; the bulky mesitoyl group at C_2 may tend to favor the β-forms.

Experimental[17]

2-*O*-Mesitoyl-β-D-glucose (II).—2,3,4,6-Tetra-*O*-acetyl-1-*O*-mesitoyl-α-D-glucose (I)[2] (5.5 g.) was dissolved in absolute methanol (450 ml.) and the solution cooled to 0°. A slow stream of dry ammonia was passed into the solution through a fritted glass disperser at such a rate that the temperature did not rise above 5°. After the reaction mixture was saturated it was left at −5° for 16 hr. and then concentrated *in vacuo* at room temperature to a thick sirup. Acetamide was removed by sublimation at 50–60° (bath) and 0.1 mm. pressure. From ethyl acetate–pentane the residue gave 1.1 g. of fine needles melting at 178–184°. Retreatment of the material in the mother liquor with methanolic ammonia led to the isolation of a second crop (0.6 g., m.p. 178–185°) which raised the total yield to 47%. Recrystallization from ethyl acetate–pentane gave material melting at 180–187°—a range which remained unchanged on further recrystallization. In water (*c* 0.94) the pure product rotated +24.0° (13.5 min.),[18] 27.8° (17 min.), 41.0° (112 min.), 44.6° (180 min.), 45.5° (515 min.) and 45.5° (1140 min.).

Anal. Calcd. for $C_{16}H_{22}O_7$: C, 58.88; H, 6.80. Found: C, 58.83; H, 7.09; acid (volatile with steam after alkali treatment and acidification), 1.09 equivalents per mole compound.

D-Glucose Phenylosazone and D-Glucose Phenylosotriazole from 2-*O*-Mesitoyl-D-glucose (II).—A solution of 100 mg. of 2-*O*-mesitoyl-D-glucose, 300 mg. of sodium acetate and 200 mg. of phenylhydrazine hydrochloride in 2 ml. of water was heated at 100° for 19 min. The yellow, crystalline precipitate, removed from the cooled mixture, was washed with 5 ml. of 5% acetic acid and then with water. Recrystallized immediately from aqueous ethanol and dried *in vacuo* over P_2O_5 it melted at 207–209° dec. either alone or in admixture with an authentic specimen of D-glucose phenylosazone. A sample treated with acidic copper sulfate solution, as described by Hann and Hudson,[5] gave a tan-colored, crystalline product which was purified according to the directions of these authors. After drying *in vacuo* over P_2O_5 at 78° the material melted at 196–198°; admixture with authentic D-glucose phenylosotriazole failed to depress this melting point.

1,3,4,6-Tetra-*O*-acetyl-2-*O*-mesitoyl-β-D-glucose (IV). (a) **From 2-*O*-Mesitoyl-D-glucose (II).**—2-*O*-Mesitoyl-D-glucose (100 mg.) was acetylated with acetic anhydride in pyridine solution in the usual fashion to give a product which crystallized from ethyl acetate–pentane as clear prisms melting at 189–190°. Recrystallization from ethanol–pentane afforded 83.6 mg. (55%) of pure product melting at 190–191° and rotating +38.2° ($CHCl_3$, *c* 1.3). Mixed with 1,3,4,6-tetra-*O*-acetyl-2-*O*-mesitoyl-β-D-glucose prepared as described in (b) below it melted at 189–190°.

(b) **From 1,3,4,6-Tetra-*O*-acetyl-β-D-glucose (III).**—Anhydrous mesitoic acid[19] (600 mg.) was mixed with 5 ml. of thionyl chloride and the solution boiled under reflux until the evolution of hydrogen chloride had practically ceased. Excess thionyl chloride was distilled off *in vacuo* at 100°, the final traces being removed by azeotroping benzene from the residue. The pale yellow mesitoyl chloride was cooled to 0°, diluted with 2 ml. of pyridine and then 1,3,4,6-tetra-*O*-acetyl-β-D-glucose[20] (1 g.) added. The reaction mixture was held at 35° for 15 hr. and then at 50° for 5 hr., earlier experiments having shown that milder conditions failed to effect the introduction of the mesitoyl group. The deep purple solution was cooled and diluted with 0.5 ml. of water; washing with water removed most of the color from the fine, needle-like precipitate (m.p. 187–189°, 600 mg., 42%). Three recrystallizations from ethanol–pentane gave pure material melting at 190–191° and rotating +38.1° ($CHCl_3$, *c* 0.96).

Anal. Calcd. for $C_{24}H_{30}O_{11}$: C, 58.29; H, 6.12. Found: C, 58.07; H, 6.34.

Methyl 2-*O*-Mesitoyl-β-D-glucopyranoside.—2-*O*-Mesitoyl-β-D-glucose (428 mg.) was dissolved in 17.3 ml. of methanol containing 5% by weight of hydrogen chloride and the

(13) R. K. Ness and H. G. Fletcher, Jr., THIS JOURNAL, **76**, 1663 (1954).

(14) The same alkali-labile substance had been discovered earlier and independently by F. Weygand and F. Wirth [*Chem. Ber.*, **85**, 1000 (1952)] in this fashion and also through the acid hydrolysis of partially benzoylated adenosine. These authors considered the substance to be 2,3,5-tri-*O*-benzoyl-D-ribose.

(15) A. C. Paladini and L. F. Leloir, *Biochem. J.*, **51**, 426 (1952).

(16) R. S. Wright and H. G. Khorana, THIS JOURNAL, **77**, 3423 (1955).

(17) Melting points are corrected.

(18) The low rate of solution of the substance in water prevented earlier readings.

(19) R. P. Barnes, "Organic Syntheses," Coll. Vol. III, John Wiley and Sons, Inc., New York, N. Y., 1955, p. 555.

(20) Hardegger and de Pascual (ref. 7), who made this substance from 1,2-anhydro-3,4,6-tri-*O*-acetyl-α-D-glucose, gave m.p. 131° and $[\alpha]D$ +28° (*c* 1.3, $CHCl_3$). Lemieux and Huber (ref. 8) made the same substance through treatment of 3,4,6-tri-*O*-acetyl-α-D-glucopyranosyl chloride with silver acetate; they reported m.p. 136–137° and $[\alpha]D$ +26° (*c* 0.8, $CHCl_3$). We find m.p. 138–139° and $[\alpha]^{20}D$ +23.1° (*c* 1.08, $CHCl_3$) on two samples prepared by the procedure of Lemieux and Huber and subjected to diverse purification procedures.

resulting solution held at 46–50°. After nine days the rotation was nearly constant (+2.71° in a 1.5-dm. tube at 20°)[21] and acid was then removed with Duolite A-4. Concentration *in vacuo* gave a crystalline residue; from ethanol–pentane the product (330 mg., 74%) crystallized as fine needles melting at 150–156°. Recrystallization from ethanol–pentane afforded pure material melting at 156–157° and rotating +54.2° in chloroform (*c* 0.88).

Anal. Calcd. for $C_{17}H_{24}O_7$: C, 59.99; H, 7.11. Found: C, 59.97; H, 7.27.

Methyl 4,6-*O*-Benzylidene-2-*O*-mesitoyl-α-D-glucopyranoside.—A solution of 2.1 g. of mesitoyl chloride in 10 ml. of dry pyridine was cooled to 0° and treated dropwise with a solution of 2.1 g. of methyl 4,6-*O*-benzylidene-α-D-glucopyranoside[22] in 10 ml. of dry pyridine. The solution was slowly warmed to room temperature and left at 22° overnight. Ten drops of water was added and 30 min. later the mixture was diluted with methylene chloride. The solution was extracted with cold 3 *N* sulfuric acid, aqueous sodium bicarbonate and, finally, dried with sodium sulfate. After concentration there was obtained a solid residue; crystallization from warm methanol gave 3.3 g. of crude crystalline material. Recrystallization from absolute ethanol afforded mesitoic anhydride; addition of pentane to the ethanolic mother liquor led to the formation of fine needles (1.5 g., 47%) melting at 193–196°. Recrystallization from ethanol–pentane gave pure methyl 4,6-*O*-benzylidene-2-*O*-mesitoyl-α-D-glucopyranoside melting at 195–196° and rotating +95.0° in chloroform (*c* 1.0).

Anal. Calcd. for $C_{24}H_{28}O_7$: C, 67.27; H, 6.59. Found: C, 67.48; H, 6.79.

Methyl 2-*O*-Mesitoyl-α-D-glucopyranoside.—The benzylidene derivative (1.07 g.) was reduced in a mixture of 30 ml. of methanol and 10 ml. of ethanol in the presence of palladium black (0.5 g.) at room temperature and pressure. The reaction being completed in 50 min., the catalyst was removed and the solution concentrated to a sirup which crystallized spontaneously. From ethanol–pentane 844 mg. (99%) of crude product was obtained in two crops. Recrystallization from the same solvent mixture gave pure material melting at 164–165° and rotating +119° in chloroform (*c* 1.1).

Anal. Calcd. for $C_{17}H_{24}O_7$: C, 59.99; H, 7.11. Found: C, 60.20; H, 7.21.

A sample of the product was dissolved in glacial acetic acid and treated with lead tetraacetate in the usual fashion. Analysis of aliquots at 2, 8 and 22 days showed the consumption of 0.134, 0.64 and 1.05 moles of oxidant.

Acknowledgment.—Microanalysis were carried out in the Institutes' Microanalytical Laboratory under the direction of Dr. W. C. Alford.

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(21) A rapid, initial levomutarotation was followed by a gradual dextromutarotation. In another run, made with 1% methanolic hydrogen chloride at 20°, the reaction was halted after the initial phase of the mutarotation was complete. A crystalline product, melting at 165–166° and rotating −13° in chloroform (*c* 0.47), was obtained in 92% yield. Combustion analysis (calcd. for $C_{17}H_{24}O_7$: C, 59.99; H, 7.11. Found: C, 59.99; H, 7.19) and the formation of formaldehyde (albeit in low yield) during oxidation with lead tetraacetate suggest that this substance is a methyl 2-*O*-mesitoyl-D-glucofuranoside.

(22) We are indebted to Dr. James W. Pratt of this Laboratory for our supply of this substance.

[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, PURDUE UNIVERSITY]

Cacao Polysaccharides[1]

BY ROY L. WHISTLER, EDWARD MASAK, JR., AND R. A. PLUNKETT

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Two distinct hot-water-soluble polysaccharides are extractable from mature caracas cacao fruit husk and seed. Preliminary work indicates the husk polysaccharide to be composed mainly of L-rhamnose, L-arabinose, D-galactose and D-mannose plus small amounts of glucose, xylose and an unidentified pentose. The seed polysaccharide contains the same major components but in different proportions.

Much of the previous characterization of the cacao bean, *Theobroma cacao*, the commercial source of cocoa and chocolate, has been confined to the ethanol-extractable sugars before[2,3] and after[4,5] fermentation and processing. The alcohol-extractable sugars are found to be D-glucose, D-fructose and D-galactose together with oligosaccharides composed of these monosaccharide units. However, little information has been reported on the ethanol-insoluble mucilages. The mucilage content of the bean is said to be 0.88% before commercial fermentation and 4.3% after fermentation.[6] The husk, which accounts for 76% of the fruit, is reported to contain as high as 8% mucilage and gums[3] before fermentation.

To obtain further information about these mucilages, the hot-water-soluble polysaccharides present in the mature Costa Rica Caracas cacao fruit husk and seed were extracted in yields of 2.0 and 0.3%, respectively, of the total dry fruit. Some differences between the polysaccharides are shown in Table I. The two polysaccharides are precipitated from solution at different potassium chloride concentrations. Quantitative paper chromatography indicates that the husk polysaccharide contains rhamnose, galactose, arabinose and mannose with a trace amount of glucose in the ratio of 11.5:11.5:4:3 while the polysaccharide isolated from the seed contains the same sugar units in the ratio of 3:2:2.5:1. The short gum-forming proper-

TABLE I

PROPERTIES OF POLYSACCHARIDES

Properties	Seed polysaccharide	Husk polysaccharide
$[\alpha]^{25}D$	+106°	+114°
Intrinsic viscosity	6.73	10.12
*p*H, 1% solution	6.85	6.35
Ash, natural	8.3	8.2
Ash, after dialysis	0.2	0.3
Phosphate test	+	+
Sulfate test	−	−
Uronic acid	−	−
Nitrogen, %	3.68	0.557

(1) Journal Paper No. 858 of the Purdue University Agricultural Experiment Station.

(2) E. M. Chatt, "Cocoa," Interscience Publishers, Inc., New York, N. Y., 1954, p. 89.

(3) E. C. Humphries, *Ann. Botany*, **7**, 45 (1943).

(4) J. Cerbulis, *Arch. Biochem.*, **49**, 442 (1954).

(5) H. Thaler, *Naturwiss.*, **41**, 432 (1954).

(6) A. W. Knapp, "Cacao Fermentation," Babe, Sons and Curnow, London, 1937.