[CONTRIBUTION FROM THE DEPARTMENT OF MARINE BIOLOGY, SCRIPPS INSTITUTION OF OCEANOGRAPHY, UNIVERSITY OF CALIFORNIA, LA JOLLA, CALIFORNIA]

The Plant Sulfolipid. IX. Sulfosugar Syntheses from Methyl Hexoseenides[1,2]

BY J. LEHMANN[3] AND A. A. BENSON

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Bisulfite ion in neutral solution added rapidly to the olefinic bonds of methyl glucoseenide, methyl mannoseenide, and phosphoenolpyruvic acid. The sulfonic acids, methyl 6-sulfo-α-D-quinovoside, methyl 6-sulfo-α-D-rhamnoside, and sulfoacetic acid were produced in good yields. Sulfonation of the terminal methylene carbon atom and its inhibition by hydroquinone revealed a cyclic free-radical mechanism for the addition reaction. The reaction in HTO solution at 0° gave methyl sulfoquinovoside with 2% of the theoretical tritium content. The isotope effect involved in sulfite ion addition to acrylamide, 22%, indicated a different mechanism. Synthesis of methyl α-D-mannoseenide, its triacetate and isopropylidene derivative, are reported. Biosynthetic implications of free-radical-mediated additions to glycoseenides and enol derivatives for the formation of sulfonic and phosphonic acids are discussed.

Introduction

6-Sulfo-D-quinovose[4] of the sulfolipid occurring in green plants has been synthesized by displacement reactions of tosyl or iodo derivatives with sodium sulfite.[5-7] These reactions, however, offered little implication for its mechanism of biosynthesis. Zill and Cheniae[8] suggested that the sulfosugar may be the product of sulfonyl group transfer to a nucleotide-bound 5,6-glucoseenide. As a model for this reaction, we have studied the reaction of bisulfite ion with methyl α-D-glucoseenide.[9,10]

The glycoseenides are acid-labile enol acetals. These olefinic glycosides were prepared by dehydrohalogenation of the 6-iodoglycosides using silver fluoride[9] or sodium methylate.[11]

Results and Discussion

The Displacement Reaction.—The displacement of iodine in methyl 6-iodo-6-deoxy-α-D-glucoside by sodium sulfite in ^3H-water proceeded with a low (0.4%) but reproducible incorporation of tritium. The fact that the reaction is accompanied by an initial pH drop suggested that the displacement reaction actually proceeds, at least partially, in two stages: (1) the elimination of HI, and (2) addition of bisulfite ion to the double bond The possible intermediate formation of an anhydro ring[12] between C-6 and C-3 or C-2 was excluded by the reaction of methyl 2,3-isopropylidene-6-iodo-6-deoxy-α-D-mannoside with sodium sulfite in water to yield a crystalline complex of sodium iodide and methyl 2,3-isopropylidene-6-sulfo-α-D-rhamnoside sodium salt. The same sulfosugar derivative was obtained as a sirup upon reaction of methyl 2,3-isopropylidene-α-D-mannoseenide[13] with sodium bisulfite. Both yielded methyl 6-sulfo-α-D-rhamnoside sodium salt after autohydrolysis of the free acid in water and neutralization with sodium bicarbonate. The same product was obtained from methyl 6-iodo-6-deoxy-α-D-mannoside and sodium sulfite.

Sulfite Addition to Glycoseenides.—Methyl 6-sulfo-α-D-quinovoside and methyl 6-sulfo-α-D-rhamnoside are products of addition of bisulfite to the double bonds of methyl α-D-glucoseenide and methyl α-D-mannoseenide. Chromatographic analysis of the reaction mixtures showed that the reactions were essentially complete in 5 min. at room temperature in aqueous solutions at pH 6.4–7.0. After 2 hr. the sulfoglycosides were isolated in good yields. ^{35}S-Labeled sulfosugars are therefore directly available from bisulfite-^{35}S for use in metabolic degradation experiments.

TABLE I
TRITIUM INCORPORATION IN BISULFITE ADDITION REACTIONS

Reactant	Methyl glucoseenide		Acrylamide	Methyl 6-deoxy-6-iodoglucoside
Product	Methyl sulfoquinovoside		Sulfopropionamide	Methyl sulfoquinovoside
Temperature	0°	40°	40°	100°
Tritium yield[a]	2.09	3.08	23.7	0.4
	1.91	3.09	20.6	
	2.01	3.20		

[a] Yields are given in per cent of theoretical tritium content. All figures represent independent preparations.

Mechanism.—The reaction conditions required and the nature of the products of bisulfite addition to olefins are determined by the mechanism involved. Acrylamide, like acrylonitrile,[14] added bisulfite rapidly at pH 6.4 to form 3-sulfopropionamide in good yield. The established nucleophilic addition of sulfite to the carbonium structure proceeded in tritiated water with a subsequent tritium incorporation of 22% (Table I).

$$HTO + SO_3^{-2} + [{}^+CH_2CH{=}C(NH_2)O^-] \longrightarrow {}^-O_3SCH_2CHTCONH_2 + OH^-$$

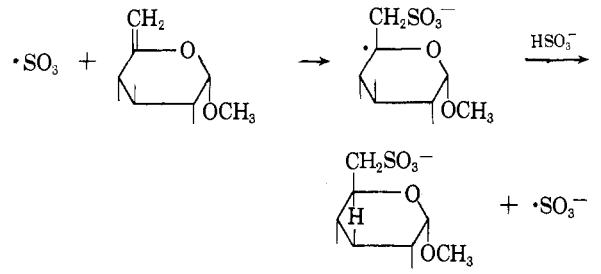
The addition of bisulfite to methyl glucoseenide in tritiated water at pH 6.4 and room temperature to form methyl 6-sulfoquinovoside-5-*t* resulted in incorpo-

(1) This work was supported by a grant from the National Science Foundation.
(2) Methyl 6-deoxyhexopyranosid-5-ene.
(3) Organisch chemisches Institut der Universität Freiburg, i. Br.
(4) D-Quinovose is 6-deoxy-D-glucose.
(5) M. Miyano and A. A. Benson, *J. Am. Chem. Soc.*, **84**, 59 (1962).
(6) B. Helferich and O. Ost, *Z. physiol. Chem.*, **83**, 114 (1963).
(7) R. L. Whistler and D. G. Medcalf, *Arch. Biochem. Biophys.*, **105**, 1 (1964).
(8) L. P. Zill and G. Cheniae, *Ann. Rev. Plant Physiol.*, **13**, 225 (1962).
(9) B. Helferich and E. Himmen, *Ber.*, **61**, 1825 (1928).
(10) Methyl 6-deoxy-α-D-glucopyranosid-5-ene. It should be pointed out that although C-5 is not asymmetric the structure is defined as a D-sugar.
(11) K. Freudenberg and K. Raschig, *Ber.*, **62**, 373 (1929).
(12) Formation of a four-membered 4–6 anhydro ring could not be detected when this iodoglucoside was treated with sodium methoxide in absolute methanol to yield methyl 2,3-isopropylidene-α-D-mannoseenide.
(13) Methyl 2,3-isopropylidene-α-D-mannoseenide was found particularly sensitive to acid. Carbonic acid catalyzed hydrolysis and rearrangement to 2,3-isopropylidene-5-keto-6-deoxy-D-mannofuranose.
(14) M. Morton and H. Landfield, *J. Am. Chem. Soc.*, **74**, 3523 (1952).

ration of 3% of the theoretical amount of tritium.[15] At pH 8 acrylamide still reacted rapidly with sulfite but the glucoseenide did not react at all. It appears that the two sulfonation reactions are different.

No 5-sulfoglycoside was observed as product of bisulfite addition to glycoseenides. It would be anticipated as a result of electrophilic proton attack at the negatively charged C-6 induced by the ring oxygen. Nucleophilic addition of sulfite ion should also lead to sulfonation at C-5.

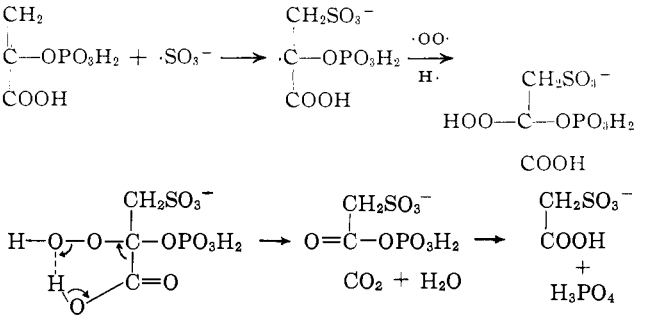
The radical-mediated addition of bisulfite ion to olefins was recognized by Kharasch, *et al.*[16,17] It leads to ω-addition of the sulfite radical followed by hydrogen extraction by the product. The radical



mechanism for the addition reaction of bisulfite to glucoseenide was substantiated by its observed inhibition by hydroquinone. It was further supported by the observed ultraviolet light requirement for analogous reactions, the addition of sodium hypophosphite and dibutyl phosphite to methyl glucoseenide. Quantitative addition of these compounds was achieved during 4 hr. ultraviolet irradiation.[18] It thus appears from the required reaction conditions and from the nature of the products that bisulfite addition to methyl glucoseenide is an obligatory free-radical reaction.

The stereospecificity of the free-radical addition to the planar methylidene group of the glucoseenide was demonstrated by the purity of the sulfosugar product. The alternate product which might be anticipated, methyl 6-sulfo-6-deoxy-β-L-idopyranoside, was not evident. The initial attack by the sulfite radical, therefore, yields an equatorial sulfomethylene group. Subsequent hydrogen extraction by the radical intermediate occurs with assumption of the D-configuration.

The production of sulfoacetic acid upon addition of bisulfite-^{35}S to the biological metabolite, phosphoenolpyruvate, lends further support to the free-radical mechanism. It is apparent that reaction of oxygen with the primary radical product leads to hydroperoxide formation and subsequent decarboxylation to form sulfoacetic acid in good yield. The anticipated reaction product, 3-sulfo-2-O-phospholactic acid, was obtained in much lower yield. A small amount (less than 1% of sulfoglycoside formed) of sulfoacetic acid was also detected in reaction mixtures of glycoseenides with bisulfite-^{35}S. It is probable that in this case also hydroperoxide formation leads to a C-C bond



cleavage and subsequent formation of sulfoacetic acid following a similar reaction mechanism.

Biological Significance.—The radical-mediated sulfonations of glycoseenides and phosphopyruvate proceed so readily that it is tempting to suspect occurrence of such reactions in the chloroplast where free-radical formation occurs and sulfosugars are found. Certainly the photochemical apparatus and the very high oxygen tension surrounding it would provide conditions conducive to such utilization of sulfite radicals. Sulfolipid appears to be absent in heterotropically grown organisms and those which do not produce oxygen. Sulfoacetic acid occurring in alkaloids of *Erythrina crista-galli* L.[19] also could have been formed by such reactions.

Whether these sulfonic acids might be formed by sulfuryl group transfer from phosphoadenylyl sulfate as suggested by Zill and Cheniae[8] is open to investigation. Phosphoryl group transfer from adenosine triphosphate to enol acceptors could also be involved in synthesis of the phosphonic acids occurring in nature.[20] A radical-addition mechanism could involve participation of a pyridine nucleotide or flavin semiquinone intermediate.

The probable product of the sulfonation, a nucleotide diphosphosulfoquinovose, has been recognized among the ^{35}S-labeled components of plant extracts.[21] The reaction of bisulfite-^{35}S with glucoseenides will provide a means for recognizing an intermediate such as a nucleotide diphosphoglucoseenide in plant extracts. Sulfoglycosylation of a diglyceride, as demonstrated by Neufeld[22] in the case of galactosylation of diglycerides, is the most probable pathway for phytosynthesis of the sulfolipid. The nucleotide diphosphosulfoquinovose would be the sulfoglycosyl donor in such a reaction.

Experimental

Methyl 6-Sulfo-α-D-quinovoside.—To 0.35 g. of methyl α-D-glucoseenide[9] was added a solution of 0.8 g. of sodium sulfite and 0.4 g. of sodium metabisulfite in 10 ml. of water. After 2 hr. at room temperature the reaction mixture was evaporated to dryness under reduced pressure. The product was extracted by shaking with 20 ml. of absolute methanol; the inorganic salts were separated by centrifugation. The supernatant solution was evaporated to dryness, taken up in minimal water, and passed through a Dowex 50 H^+ column. Sulfur dioxide was removed quantitatively from the acidic solution by distilling *in vacuo* one-eighth of the solution into a receiver containing sodium hydroxide. The acidic solution was either neutralized with

(15) The high tritium isotope effect of the addition reaction could be explained as a combination of an equilibrium isotope effect for bisulfite tritium exchange, $HSO_3^- - T^- \rightleftharpoons TSO_3^- + H^+$, and a kinetic isotope effect in a radical chain reaction.

(16) M. S. Kharasch, E. M. May, and F. R. Mayo, *J. Org. Chem.*, **3**, 175 (1938).

(17) M. S. Kharasch, R. T. E. Schenck, and F. R. Mayo, *J. Am. Chem. Soc.*, **61**, 3092 (1939).

(18) Unpublished observations.

(19) K. Folkers, F. Koniuszy, and J. Shavel, Jr., *J. Am. Chem. Soc.*, **66**, 1083 (1944). Phytosynthesis of bound sulfoacetic acid from $^{35}SO_4^{-2}$ by leaves of *E. crista-galli* L. has been confirmed (R. Prasad, private communication).

(20) M. Horiguchi and M. Kandatsu, *Bull. Agr. Chem. Soc. Japan*, **24**, 565 (1960).

(21) I. Shibuya, T. Yagi, and A. A. Benson, "Studies on Microalgae and Photosynthetic Bacteria," edited by Japan Society of Plant Physiology, University of Tokyo Press, 1963, p. 627.

(22) E. Neufeld, *Federation Proc.*, **22**, 464 (1963).

sodium bicarbonate or with freshly distilled cyclohexylamine depending on the salt desired. After evaporation to dryness under reduced pressure the sodium salt was crystallized from 60% aqueous methanol by careful addition of acetone; the cyclohexylammonium salts were crystallized from absolute ethanol–toluene. Yields were 65–85%.

The methyl 6-sulfoquinovoside salts exhibited physical properties (infrared, melting point, $[\alpha]_D$) identical with those of the compounds prepared previously by displacement reaction.[5-7]

Methyl 6-Sulfo-α-D-rhamnoside Sodium Salt Monohydrate.—The same procedure was used for reaction of bisulfite with methyl α-D-mannoseenide. The methyl 6-sulfo-α-D-rhamnoside sodium salt, colorless prisms, was crystallized from 60% aqueous methanol by addition of acetone; $[\alpha]^{22}D$ +52 (*c* 0.65, water).

Anal. Calcd. for $C_7H_{13}O_8SNa \cdot H_2O$ (298.3): C, 28.19; H, 5.07; S, 10.75; Na, 7.70; H_2O, 6.05. Found: C, 28.28; H, 4.90; S, 10.62; Na, 7.76; H_2O, 6.30.

Methyl 6-Sulfo-α-D-quinovoside-^{35}S.—Sulfur dioxide-^{35}S was prepared by igniting 1 mc. of elemental ^{35}S (specific activity, 1 mc./mg. of S) at 400–500° in oxygen in a sealed 7-ml. quartz tube. The sulfur dioxide was distilled into a receiver containing 17 mg. of sodium sulfite in 1 ml. of water. To this was added excess (40 mg.) methyl glucoseenide and the mixture allowed to react for 2 hr. Two-dimensional paper chromatograms and their radioautograms were prepared in the usual manner. The small amount of bisulfite ion involved in these reactions resulted in appreciable oxidation to sulfate by autoxidation. When the sulfite ^{35}S was kept under nitrogen the yield of methyl sulfoquinovoside, R_f 30 and 13 (in phenol–water and in butanol–propionic acid–water solvents), based upon the amount of sulfate remaining in the reaction mixture, was 80%.

Sulfoacetic Acid-^{35}S from Phosphoenolpyruvate.—To a solution of $^{35}SO_2$ (1 mc.) and 17 mg. of sodium sulfite in 1 ml. of water was added 80 mg. of phosphoenolpyruvate tricyclohexylammonium salt. After 4 hr. at pH 6.4 and 25° an aliquot portion of the solution was chromatographed in phenol–water (100:38, w./w.) and in butanol–propionic acid–water (142:71:100, v./v.). The primary product (>90% of the organic products) cochromatographed with authentic sulfoacetic acid, R_f 10, 21. It was cocrystallized from aqueous acetone with sulfoacetic acid cyclohexylammonium salt. Successive recrystallizations did not alter the specific activity obtained with the initial product.

A second compound, 10% of the sulfoacetic acid formed, could be detected in the reaction mixture. It exhibited the same R_f values and electrophoretic mobilities at pH 2 and 6 as sulfoacetic acid and was shown to undergo spontaneous decomposition to sulfoacetic acid. Reduction with sodium borohydride in aqueous solution yielded sulfolactic acid which was identified by cocrystallization with sulfolactic acid dicyclohexylammonium salt prepared by treatment of cysteic acid with nitrous acid. The specific activity remained unchanged upon three successive crystallizations.

Anal. Calcd. for $C_{15}H_{32}O_6N_2S$ (368.4): C, 48.9; H, 8.76; N, 7.60. Found: C, 49.19; H, 8.88; N, 7.55.

A smaller amount, <5%, of a different product, R_f 4, 14, was observed in radiograms of the products. Its identity as sulfolactic acid O-phosphate ester was deduced from the following observations. Acid hydrolysis produced a product with electrophoretic mobility and R_f values corresponding to those of sulfolactic acid.[21] Treatment with Polidase-S (Schwarz BioResearch, Inc., Orangeburg, N. Y.), an acid phosphatase, yielded the same product. No sulfate ion or sulfoacetic acid was observed as a result of these treatments.

Methyl 6-Sulfo-α-D-quinovoside-5-*t*.—The sulfonation of glucoseenide was carried out as above with the exception that ^{3}H-water (1–10 mc./ml.) was used as solvent. After the reaction it was evaporated *in vacuo* from the frozen mixture.

Methyl 6-sulfo-α-D-quinovoside-5-*t* was also prepared from methyl 6-iodo-6-deoxy-α-D-glucoside and sodium sulfite by the method of Helferich and Ost[6] using ^{3}H-water (10 mc./ml.) as solvent.

The cyclohexylammonium salts of the products were recrystallized until constant specific activity was obtained. This was usually the case after two recrystallizations. Tritium activity of the crystalline product and of aliquot portions of the water used was determined using 3 ml. of absolute methanol as solvent in 12 ml. of PPO–POPOP–toluene liquid scintillator. Results are shown in Table I.

3-Sulfopropionamide-2-*t*.—Acrylamide (0.07 g.) was treated with a solution of sodium metabisulfite (0.2 g.) and sodium sulfite (0.4 g.) in 5 ml. of ^{3}H-water (1–10 mc./ml.). The sulfonic acid was isolated as the cyclohexylammonium salt as described for methyl 6-sulfo-6-deoxy-α-D-quinovoside-5-*t*. Its chromatographic coordinates, R_f 40, 20, were determined using an ^{35}S-labeled product. The salt was recrystallized from ethanol–toluene, yield 90%, m.p. 131–132°. Its ammonium salt, m.p. 179°, corresponded to that prepared by Kharasch, *et al.*,[23] from the acid anhydride. Tritium content of the product was measured as above and reported in Table I.

Methyl 2,3-Isopropylidene-6-sulfo-α-D-rhamnoside Sodium Salt Monohydrate Sodium Iodide.—Methyl 6-deoxy-6-iodo-2,3-isopropylidene-α-D-mannoside (3.5 g.) was stirred vigorously in a refluxing solution of sodium sulfite (2.5 g.) in water (15 ml.). The heavy, oily drops dissolved slowly after a few hours. Boiling under reflux was continued for 16 hr. The reaction mixture was evaporated to dryness and the residue extracted with absolute methanol. On evaporation of the methanol solution a crystalline residue was obtained which was crystallized by dissolving it in 95% ethanol and adding toluene until incipient precipitation. The precipitate was redissolved by heating and crystallized at 0°. The crystalline product contained sodium iodide which would neither be removed by washing with acetone nor by recrystallization. The yield was 3.7 g. or 75%, $[\alpha]^{25}D$ +16 (*c* 2.5, water).

Anal. Calcd. for $C_{10}H_{17}O_8SNa \cdot NaI \cdot H_2O$ (488.25): C, 24.59; H, 3.92; S, 6.65; I, 25.92; Na, 9.42; H_2O, 3.7. Found: C, 24.74; H, 3.79; S, 6.72; I, 25.93; Na, 9.35; H_2O, 4.4.

Separation of sodium iodide and the sulfosugar derivative was achieved by paper chromatography in the butanol–propionic acid–water (142:71:100, v./v.) solvent. The compound was autohydrolyzed by passing through Dowex 50 H^+. From the hydrolysate methyl 6-sulfo-α-D-rhamnoside sodium salt was obtained by neutralization with sodium bicarbonate, evaporation, and crystallization from water–acetone.

Methyl 6-Deoxy-6-iodo-α-D-mannoside and Methyl 6-Deoxy-6-iodo-2,3,4-triacetyl-α-D-mannoside.—Both compounds were prepared according to the method reported by Raymond and Schroeder.[24] The yield of triacetate, crystallized from 95% ethanol, m.p. 91–92°, $[\alpha]^{25}D$ +37.0° (*c* 0.6, chloroform) was 45% based upon methyl 6-deoxy-6-iodo-α-D-mannoside taken.

Anal. Calcd. for $C_{13}H_{19}O_8I$ (430.15): C, 36.30; H, 4.45; I, 29.50. Found: C, 36.12; H, 4.48; I, 29.51.

Deacetylation carried out with a catalytic amount of sodium methoxide in absolute methanol yielded 76% of methyl 6-deoxy-6-iodo-α-D-mannoseenide, m.p. 120–122°, $[\alpha]^{25}D$ +55.5° (*c* 0.4, water) after crystallization from methanol–ether.

Anal. Calcd. for $C_7H_{13}O_5I$ (304.08): C, 27.65; H, 4.31; I, 41.73. Found: C, 27.59; H, 4.28; I, 41.90.

Methyl 6-Deoxy-6-iodo-2,3-isopropylidene-α-D-mannoside.—Methyl 6-deoxy-6-iodo-α-D-mannoside (4 g.) was dissolved in acetone (100 ml.) and shaken with anhydrous cupric sulfate (4 g.). Concentrated sulfuric acid (0.2 ml.) was added to the stirred solution and agitation continued 16 hr. at room temperature. After addition of a small excess of concentrated ammonia the inorganic material was removed by filtration. The filtrate was evaporated under reduced pressure whereupon the remaining sirup crystallized spontaneously. The compound was recrystallized from petroleum ether (80–100°) or ethanol–water, yield 3.4 g., 75% of theory, m.p. 109–110°, $[\alpha]^{25}D$ +44° (*c* 5.5, 95% ethanol).

Anal. Calcd. for $C_{10}H_{17}O_5I$ (344.16): C, 34.89; H, 4.98; I, 36.86. Found: C, 35.00; H, 5.02; I, 37.05.

Methyl 2,3,4-Triacetyl-α-D-mannoseenide.—The method of Helferich and Hinmen[9] for conversion of the corresponding glucose analog to glucoseenide was followed for preparation of methyl 2,3,4-triacetyl-α-D-mannoseenide from methyl 6-iodo-6-deoxy-2,3,4-triacetyl-α-D-mannoside in 54% yield, m.p. 73°, $[\alpha]^{25}D$ +14.5° (*c* 0.4, water).

Anal. Calcd. for $C_{13}H_{18}O_8$ (302.30): C, 51.67; H, 6.00. Found: C, 51.48; H, 5.97.

The methyl α-D-mannoseenide was obtained as a sirup by catalytic deacetylation with sodium methoxide in absolute methanol. It could not be crystallized; however, it gave methyl 6-sulfo-α-D-rhamnoside upon bisulfite addition (*vide supra*). It was identical with the sulforhamnoside prepared from the 6-iodomannoside by the method of Helferich and Ost.[6]

(23) M. S. Kharasch, T. H. Chao, and H. C. Brown, *J. Am. Chem. Soc.*, **62**, 2393 (1940).

(24) A. L. Raymond and E. F. Schroeder, *ibid.*, **70**, 2785 (1948).

Methyl 6-Deoxy-2,3-isopropylidene-α-D-mannoseenide.—Methyl 6-deoxy-6-iodo-α-D-mannoside (1 g.) was heated 4 hr. under reflux in absolute methanol (50 ml.) in which sodium (1.5 g.) has been dissolved. Most (40 ml.) of the methanol was removed by distillation under reduced pressure. The residue was taken up in chloroform (50 ml.), washed free of alkali with cold water, and dried over sodium sulfate. The chloroform was evaporated under reduced pressure and the remaining oil was distilled at 0.001 mm. with an oil bath temperature of 100–110°. The compound is extremely sensitive to acid-catalyzed hydrolysis; even carbonic acid or exposure to air resulted in hydrolysis, rearrangement, and crystallization of 6-deoxy-2,3-isopropylidene-5-keto-D-mannofuranose. This instability is responsible for an incorrect analysis.

The compound, when freshly prepared, showed the typical infrared absorption for C=C and decolorized bromine water instantaneously. The yield of mannoseenide was 0.50 g., 80% of theory, $[\alpha]^{24}D$ +51° (*c* 0.4, methanol–water, 2:1, v./v.). In 0.01 *N* hydrochloric acid in methanol–water (2:1, v./v.) containing 0.4% of the compound, the specific rotation decreased during 16 hr. from +45 to −23°.

Anal. Calcd. for $C_{10}H_{16}O_5$: C, 55.54; H, 7.46. Found: C, 54.23; H, 7.41.

The methyl 2,3-isopropylidene-α-D-mannoseenide was also prepared in lower yield by using silver fluoride in pyridine[9] for dehydroiodination of methyl 6-iodo-6-deoxy-2,3-isopropylidene-α-D-mannoside.

6-Deoxy-2,3-isopropylidene-5-keto-D-mannofuranose.—Methyl 6-deoxyisopropylidene-α-D-mannopyranoseenide (1 g.) was dissolved in 0.01 *N* hydrochloric acid (methanol–water, 2:1, v./v.) and left standing at room temperature for 16 hr. The acid was neutralized with sodium bicarbonate and the solution was evaporated to dryness under reduced pressure. The residue was extracted with boiling ether and the compound was induced to crystallize by addition of petroleum ether. It was recrystallized from ether–petroleum ether to give firm needles, m.p. 147–149°, $[\alpha]^{23}D$ −28° (*c* 0.5, methanol–water, 2:1, v./v.) in 0.6 g. yield.

Anal. Calcd. for $C_9H_{14}O_5$ (202.20): C, 53.44; H, 6.98. Found: C, 53.18; H, 7.07.

The compound did not decolorize bromine water but reduced ammoniacal silver nitrate solution. It showed typical infrared carbonyl absorption. It formed an amorphous bis (*p*-nitrophenylhydrazone).

Anal. Calcd. for $C_{21}H_{24}O_7N_6$ (470.30): C, 53.39; H, 5.12; N, 17.78. Found: C, 53.11; H, 5.62; N, 16.15.

6-Deoxy-L-gulitol.—6-Deoxy-2,3-isopropylidene-5-keto-D-mannofuranose (0.10 g.) was reduced with an excess of sodium borohydride in water. After standing 7 hr. at room temperature, the remaining borohydride was decomposed by addition of acetic acid. Sodium ions were removed by passing through a column of Dowex 50 H^+, and boric acid and acetic acid were driven off by repeatedly distilling absolute methanol from the residue. The remaining sirup crystallized from methanol; yield, 0.059 g. (73% of theory), m.p. 127°, m. m.p. with authentic compound, 127–128°. Müller and Reichstein[25] reported m.p. 127°. The infrared spectrum of the compound was identical with that of a reference sample of 1-deoxy-D-glucitol (or 6-deoxy-L-gulitol).[26] A trace of a second 6-deoxyhexitol was detected by paper chromatography. It showed the same chromatographic properties as L-rhamnitol. This demonstrated the position of the keto group in 6-deoxy-5-keto-2,3-isopropylidene-D-mannofuranose.

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(25) H. Müller and T. Reichstein, *Helv. Chim. Acta*, **21**, 251 (1938).
(26) Kindly provided by Dr. A. B. Foster, University of Birmingham.

[CONTRIBUTION FROM THE DANIEL SIEFF RESEARCH INSTITUTE, THE WEIZMANN INSTITUTE OF SCIENCE, REHOVOTH, ISRAEL]

Synthetic Studies on Sphingolipids. X.[1] Synthesis of Psychosine[2]

BY DAVID SHAPIRO, ELIEZER S. RACHAMAN, AND TUVIA SHERADSKY

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The synthesis of psychosine (VIa) is described. N-Dichloroacetyl-3-O-benzoylsphingosine (IVa) was condensed with acetobromogalactose, and the resulting product deacylated by sodium methoxide to the N-dichloroacetylcerebroside (Va) which was hydrolyzed to VIa by warming with 0.3 *N* barium hydroxide at 70–80° for 90 min. By the same procedure glucopsychosine and dihydropsychosine were synthesized. The latter glycoside could also be obtained by hydrogenolysis of the N-benzyloxycarbonyl derivative Vc.

Psychosine (1-D-sphingosyl β-D-galactoside, VIa) is an alkaline hydrolysis product of the cerebrosides (I) in which the amino group forms an amide with a long-chain fatty acid.[3,4] On the basis of results obtained from enzymatic studies Cleland and Kennedy[5] have recently postulated that this galactoside is an intermediate in the biosynthesis of cerebrosides and other more complex glycosphingolipids. We wish to report the synthesis of psychosine and its dihydro derivative.

In a recent communication[6] we have demonstrated that in order to assure an unequivocal synthesis of a sphingolipid involving substitution at carbon 1 of the sphingosine molecule it is necessary to block both the amino and the secondary hydroxyl group. Thus, the synthesis of cerasine (I) and other natural cerebrosides[7] was achieved by glycosidation of the disubstituted sphingosine (II).

The hydrolysis of cerebrosides of type I is known to proceed sluggishly giving psychosine in low yield and of poor quality.[8] In contradistinction to the synthesis of cerebrosides in which the amide grouping is retained as part of the molecule, the protecting N-acyl in the synthesis of psychosine must be such as to undergo mild hydrolysis. On the other hand, hydrolysis should not take place prior to the removal of the benzoyl group, since in such a case it would be difficult to avoid, during the course of the synthesis, O→N benzoyl migration which proceeds rapidly at a pH slightly above 7. We therefore undertook a systematic investigation of such protective groups which were likely to meet these requirements. To this end we prepared the 3-O-, N-protected bases IVa–h as possible aglucons in the proposed synthesis. Some of these protective groups are well known in the chemistry of amino acids.[9] Sphingosine is, however, a much stronger

(1) For part IX see D. Shapiro and E. S. Rachaman, *Nature*, **201**, 878 (1964).
(2) Taken from part of a thesis submitted by E. S. Rachaman to the Senate of the Hebrew University, Jerusalem, in partial fulfillment of the requirements for the Ph.D. degree, April, 1964.
(3) I. Pryde and R. W. Humphrey, *Biochem. J.* **18**, 661 (1924).
(4) I. Pryde and R. W. Humphrey, *ibid.*, **20**, 825 (1926).
(5) W. W. Cleland and E. P. Kennedy, *J. Biol. Chem.*, **235**, 45 (1960).
(6) See paper in ref. 1.
(7) D. Shapiro and H. M. Flowers, *J. Am. Chem. Soc.*, **83**, 3327 (1961).
(8) E. Klenk and R. Harle, *Z. Physiol. Chem.*, **178**, 221 (1928).