Carbohydrate Research, 81 (1980) 275–283

STRUCTURE OF SARCOBIOSE

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(Received October 31st, 1979; accepted for publication, November 20th, 1979)

ABSTRACT

A new, nonreducing disaccharide, sarcobiose, has been isolated from the dried twigs of *Sarcostemma brevistigma*. On the basis of chemical and spectroscopic evidence and identification of its hydrolysis product, its structure has been established as 3,4-anhydro-2,6-dideoxy-β-D-*lyxo*-hexopyranosyl 6-deoxy-3-*O*-methyl-β-D-allopyranoside.

INTRODUCTION

In a chemical investigation of the dried twigs of *Sarcostemma brevistigma* (Family: Asclepiadaceae), pregnane glycosides were extracted[1]. Mild, acid hydrolysis[2] of these glycosides gave a mixture of sugars which was separated on a column of silica gel, affording very small quantities of two new sugars, brevobiose and sarcobiose, insufficient for detailed chemical studies. During the isolation of larger quantities of these sugars by us (using an earlier method), from fresh extract of the plant, the new, polar sugar tigmobiose, and additional quantities of brevobiose and sarcobiose were obtained. The structures of brevobiose[3] and tigmobiose[4] have been elucidated. In the present paper, the structure of sarcobiose (**1**) is reported.

RESULTS AND DISCUSSION

Sarcobiose (**1**), m.p. 103–104° and 169–172°, $[\alpha]_D$ +68°, $C_{13}H_{22}O_7$ (e.i.-m.s., M^+ *m/e* 290; isobutane c.i.-m.s., M + H^+ *m/e* 291), contains a methoxyl group (p.m.r. spectrum), and does not reduce Fehling solution. The molecular formula, $C_{13}H_{22}O_7$, for this new sugar suggested that it could be an anhydride of a biose, $C_{13}H_{24}O_8$, containing two hexosyl units. If the assumption that it is a disaccharide is correct, then a double bond, a carbonyl group, or an epoxide ring must be present in the molecule. The absence of any evidence for a double bond (negative tetranitromethane reaction[5]) and a carbonyl group (i.r. spectrum) led us to postulate the presence of an epoxide ring in the molecule. Confirmation was forthcoming from positive Buchanan–Schwarz[6] and Ross[7] tests, diagnostic for an epoxide ring. The

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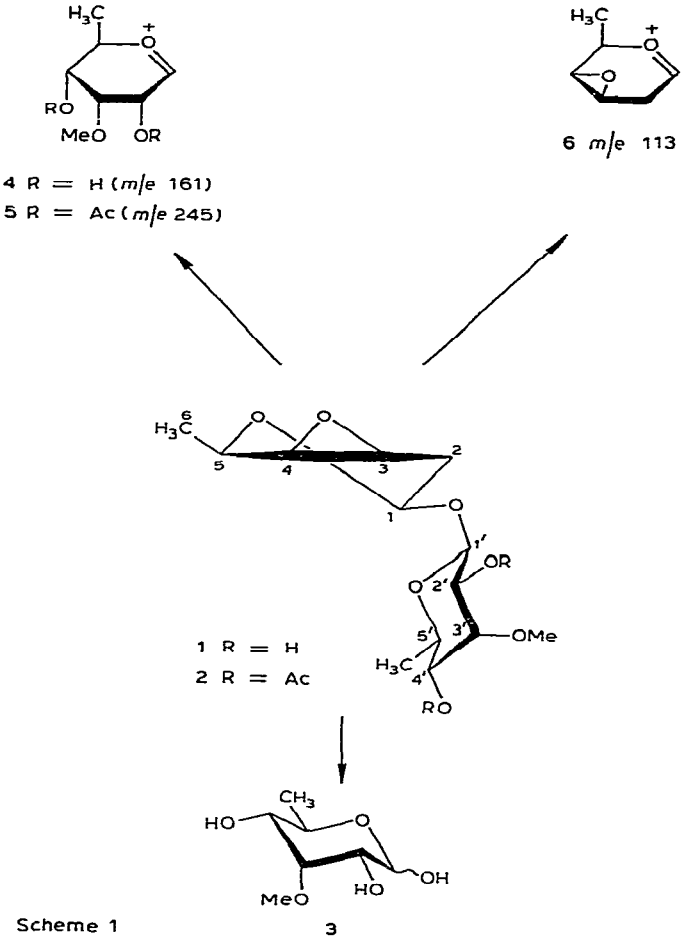
same conclusion was arrived at by an accounting of the seven oxygen atoms in the sarcobiose molecule, where two oxygen atoms are present as hydroxyl groups (per-acetylation results), one as a methoxyl group, two as the ring-oxygen atoms of the two hexose units, and one involved in the glycosidic linkage, indicating that the seventh (unacetylatable) oxygen function in sarcobiose is present in an epoxide ring.



Scheme 1

The peracetylation of **1** furnished a di-*O*-acetyl derivative (**2**), $C_{17}H_{26}O_9$ (e.i., M^+ *m/e* 374; isobutane c.i., $M + H^+$ *m/e* 375), m.p. 79–80°, $[\alpha]_D$ +68°, indicating the presence of only two acetylatable hydroxyl groups in the molecule. These hydroxyl groups are not, however, vicinal, as indicated by a negative $NaIO_4$–benzidine test[8]. Sarcobiose displayed positive tests for 2-deoxy sugars, *viz.*, the xanthydrol reaction[9] and Keller–Kiliani reaction[10], suggesting that it contains at least one 2-deoxyhexose moiety. It was, therefore, subjected to mild treatment with acid according to Mannich and Siewert[11], but this failed to liberate any free sugar. However, drastic hydrolysis with acid by the Kiliani method[12] (2-deoxy sugars are decomposed under these

conditions) yielded a crystalline, reducing sugar (**3**, see Scheme 1), m.p. 112–113°, which was identified as 6-deoxy-3-*O*-methyl-D-allose[13] by mixed m.p., and cochromatography in p.c., with an authentic sample. Further identification was achieved by comparison, by t.l.c., of the lactone prepared from it with authentic 6-deoxy-3-*O*-methyl-D-allono-1,4-lactone; their identity suggested that the epoxide ring was present in the 2-deoxy sugar unit.

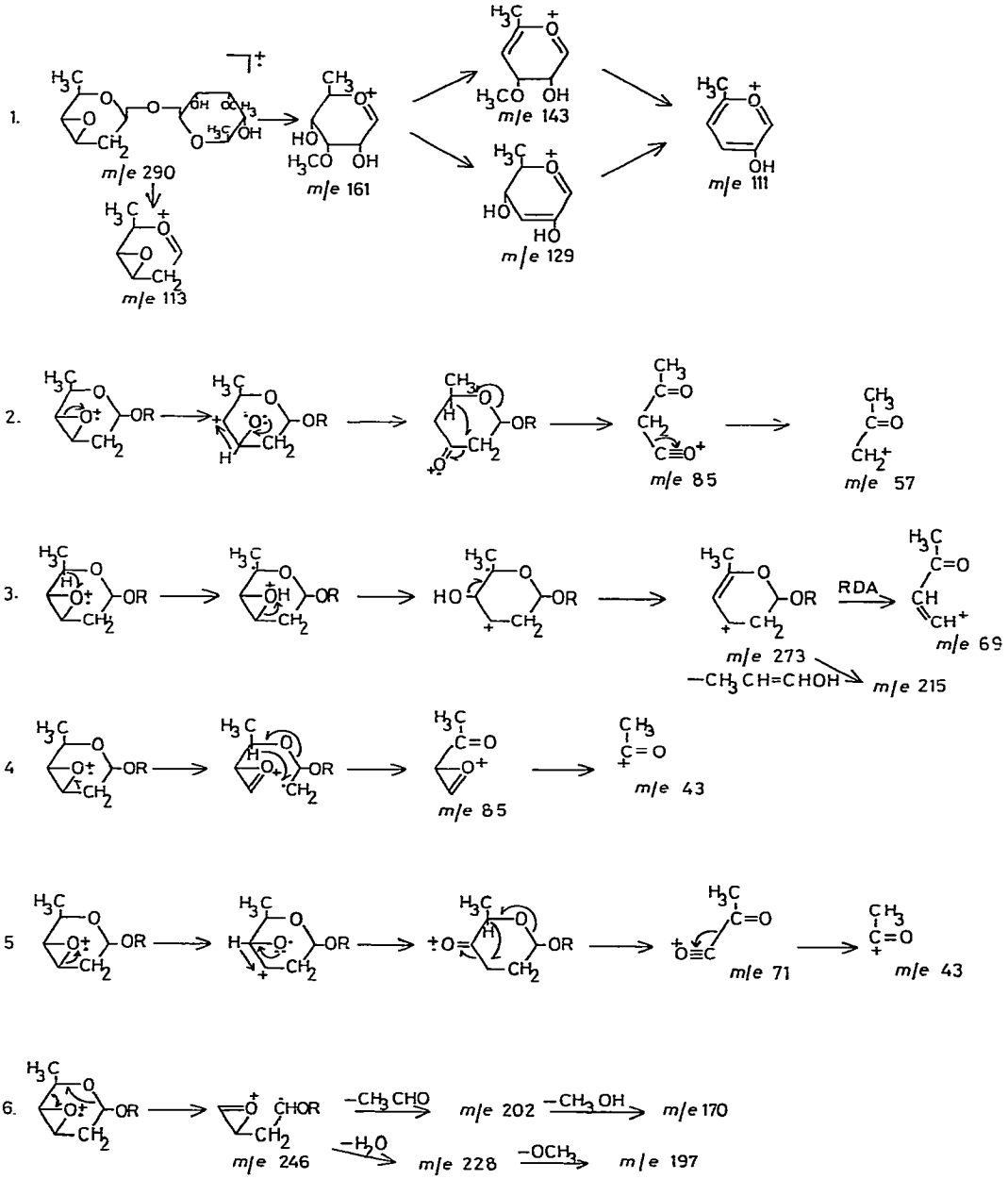
Two prominent, fragment-ion peaks, at *m/e* 161 and 113, in the mass spectrum of sarcobiose are respectively diagnostic[14] of the two hexose fragments **4** ($C_7H_{13}O_4$) and **6** ($C_6H_9O_2$) (see Scheme 1). As fragment **4** was derived from 6-deoxy-3-*O*-methyl-D-allose, the other fragment must have originated from the rest of the molecule, which should be a dideoxyhexose residue carrying the epoxide ring. This conclusion was supported by corresponding peaks at *m/e* 245 for **5**, and *m/e* 113 for **6**, in the mass spectrum of di-*O*-acetylsarcobiose.

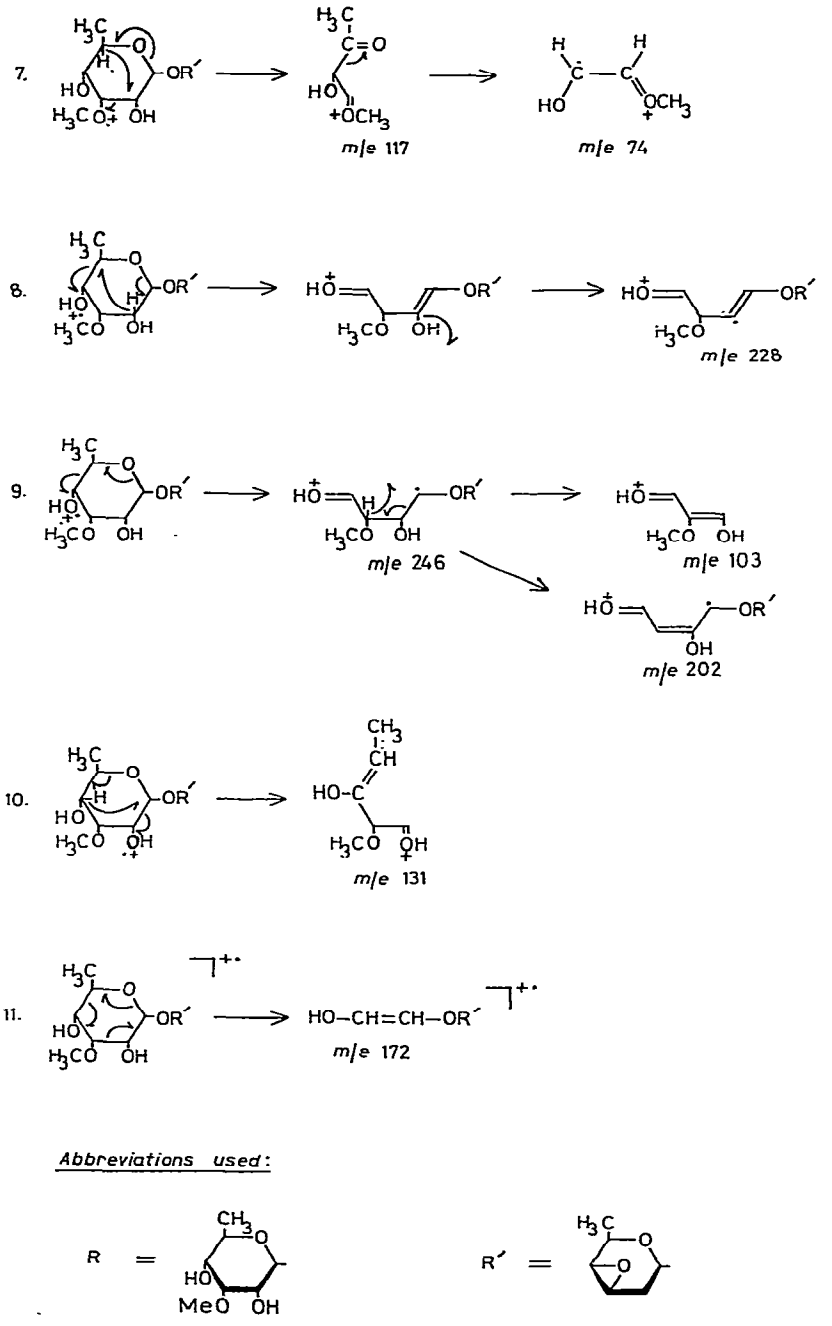
As **1** is nonreducing, it should be a biose of the trehalose type, with its monosaccharide constituents joined together by a glycosidic (1↔1)-linkage. Furthermore, it was anticipated that, if both hexose units in sarcobiose are present in the pyranose form, the 6-deoxy-3-*O*-methyl-D-allose would assume a chair, and the anhydrodideoxyhexose a half-chair, conformation.

A close analysis of the p.m.r. spectra of **1** and its diacetate **2** not only confirmed these assumptions and ascertained the configuration of the glycosidic linkage but also led to characterization of the anhydrodideoxyhexose moiety. In the p.m.r. spectrum of **2**, two three-proton doublets centered at δ 1.15 (J 7 Hz) and 1.3 (J 7 Hz) were attributed to two secondary methyl groups, and two one-proton multiplets, at δ 2.4–2.6 and δ 1.55–1.9, to a methylene group. Its three singlets, of three protons each, at δ 3.48, 2.05, and 2.03, were assigned to a methoxyl group and two acetyl groups, respectively. Of these, the signals for a methoxyl group, two acetyl groups, and a secondary methyl group must have arisen from the 6-deoxy-3-*O*-methyl-D-allose. The remaining, secondary methyl group and a methylene group must, therefore, be parts of the dideoxyhexose moiety. In the p.m.r. spectrum of **1**, a one-proton doublet centered at δ 4.78 (J 8 Hz) was assigned to the anomeric proton (H-1′) of **3**. The large coupling constant (J 8 Hz) of this anomeric proton corresponded to axial–axial coupling between H-1′ and H-2′, suggesting that the conformation of the 6-deoxy-3-*O*-methyl-D-allose[15] is $^4C_1$, and that it is linked to the other hexose through a β-glycosidic linkage[13,15]. A double doublet centered at δ 5.28 (J 4 and 9 Hz) was attributed to the anomeric proton of the anhydrodideoxyhexose moiety on the basis of double-resonance experiments, where irradiation at 475 Hz led to the collapse of the methylene multiplets centered at δ 1.7 and 2.18. Thus, the methylene group in this moiety was present next to the anomeric proton, *i.e.*, this moiety is a 2,6-dideoxyhexose residue. Assuming it to be the D enantiomer, the smaller J value (4 Hz) of the H-1 signal referred to H-1*a*, H-2*qe* coupling (*q* = *quasi*) and the larger J value (9 Hz) to H-1*a*, H-2*qa* coupling, from which it was inferred that this hexose exists in the $^OH_1$ conformation[16], and is joined to the other hexose through a β-glycosidic linkage.

The same conclusions could be drawn from the two anomeric-proton splittings

in the p.m.r. spectrum of di-*O*-acetylsarcobiose (**2**), where the H-1 signal appeared at δ 5.79 as a double doublet (J 4 and 9 Hz), and the H-1′ signal, at δ 4.82 as a doublet (J 8 Hz). An important signal in the spectrum of diacetate **2** was the double doublet centered at δ 3.16 (J 3 and 8 Hz). In view of its chemical shift and splitting pattern, this signal was assigned to the proton located at C-3 of the C-3–C-4 epoxide ring. This assignment was supported by the results of inspection of a Dreiding model of the





Scheme 2. Mass-spectral fragmentation of sarcobiose.

anhydro sugar, which showed that the dihedral angles between H-2*qe* and H-3, H-2*qa* and H-3, and H-3 and H-4 are such as to show coupling constants of the order of ~3, ~0, and ~8 Hz, respectively, which are quite close to the respective *J* values observed. Identification of the signals of H-5 and H-5′ present in the multiplet between δ 3.73 and 4.03 in the spectrum of diacetate **2** was achieved by a double-resonance experiment. Characterization of a prominent doublet centered at δ 3.95 (*J* 8 Hz) in the partly collapsed multiplet obtained after irradiation of the doublet of the secondary methyl group at δ 1.31 identified the signals of CH_3-5′ and H-5′, and confirmed an axial–axial orientation between H-4′ and H-5′ on a pyranose ring. Similarly, appearance of a narrow doublet centered at δ 3.91 (*J* 2.5 Hz) in the partly collapsed multiplet between δ 3.73 and 4.03 after irradiation of the other secondary methyl group signal, at δ 1.15, not only identified the CH_3-5 signal but also that of H-5. The coupling-constant value of the order of 2.5 Hz for H-5, observed in the irradiated signal, also confirmed a cis orientation between H-4 and H-5 in the $^{O}H_{1}$ conformation. As the chemical shift of the H-5 signal has virtually the same position as H-5′, it was concluded that H-5 is not deshielded by the ring-oxygen atom of the epoxide (already decided from the coupling constant of 2.5 Hz between H-4 and H-5 in cis orientation). On the basis of these results, it was concluded that the 2-deoxy-hexosyl unit of sarcobiose is 3,4-anhydro-2,6-dideoxy-β-D-*lyxo*-hexopyranosyl.

Sarcobiose (**1**) is, therefore, 3,4-anhydro-2,6-dideoxy-β-D-*lyxo*-hexopyranosyl 6-deoxy-3-*O*-methyl-β-D-allopyranoside.

In the mass spectrum of sarcobiose, many prominent peaks can be explained by standard, decomposition pathways[17], which fully support the proposed structure of this disaccharide. A partial-fragmentation pattern for the structure proposed for sarcobiose is presented in Scheme 2, which accounts for most of the major peaks in the spectrum.

EXPERIMENTAL

General. — All melting points were determined on a Boetius micro melting-point apparatus and are uncorrected. Optical rotations were measured, in a 1-dm tube, with a Jasco-Dip 180 automatic polarimeter. I.r. spectra were recorded with a Perkin–Elmer IR-177 spectrophotometer, and p.m.r. spectra with a 90-MHz, Perkin–Elmer R-32 spectrometer for solutions in $CDCl_3$ (unless otherwise mentioned), with Me_4Si as the internal standard. The sugars were made visible in t.l.c. with 50% aq. H_2SO_4 reagent, and, in p.c., with vanillin–perchloric acid reagent[18]. The lactone was made visible with NH_2OH–$FeCl_3$ reagent[19]. The adsorbent for t.l.c. was Silica Gel G (BDH) and, for column chromatography, silica gel for column (BDH). Paper chromatography was performed on Whatman No. 1 filter paper, using 4:1 toluene–1-butanol saturated with water.

3,4-Anhydro-2,6-dideoxy-β-D-lyxo-*hexopyranosyl 6-deoxy-3-*O*-methyl-*β-D-*allopyranoside* (**1**). — Reextraction of the dried twigs (4 kg) of *Sarcostemma brevistigma* according to an earlier method[20] afforded a sugar mixture (5.11 g); this was chro-

matographed on silica gel (700 g). Fractions 17–51 (500 mL each) eluted with 97:3 chloroform–methanol afforded an amorphous substance (400 mg) containing sarcobiose admixed with two other sugars. This mixture was rechromatographed on fine silica gel[21] (15 g; 8 min). Elution was conducted with 99:1 chloroform–methanol (collection of 20-mL fractions). Fractions 16–24 afforded amorphous substance (120 mg) containing sarcobiose, which crystallized from acetone–pentane as colorless rhombs (60 mg), m.p. 103–104° and 169–172°, $[\alpha]_D^{26}$ +68° (*c* 0.7, methanol). It did not reduce Fehling solution, and gave positive tests in the xanthydrol[9] and Keller–Kiliani[10] reactions, and a blue coloration with vanillin–perchloric acid. It also gave positive Buchanan–Schwarz[6] and Ross[7] tests for epoxide. It showed ν_{max}^{KBr} 3520–3392, 2976, 2924, 2872, 1445, 1370, 1320, 1169, 1130, 1105, 1073, 1056, 1028, 915, 868, and 848 cm^{-1}; p.m.r. data ($CDCl_3$–pyridine-d_5): δ 5.29 (dd, 1 H, *J* 4 and 9 Hz, H-1), 4.79 (d, 1 H, *J* 8 Hz, H-1′), 4.25 (m, 1 H, H-5′), 3.65–4.0 (m, 3 H, H-4,5,3′), 3.57 (s, 3 H, OCH_3), 3.05–3.4 (m, 3 H, H-3,2′,4′), 2.03–2.3 (m, 1 H, H-2*e*), 1.6–1.8 (m, 1 H, H-2*a*), 1.33 (d, 3 H, *J* 5 Hz, sec. CH_3), and 1.26 (d, 3 H, *J* 5 Hz, sec. CH_3); e.i.-m.s.: *m/e* 290 (M^+, 15%), 273 (7), 272 (5), 262 (3), 259 (3), 246 (2.6), 228 (6.6), 217 (1.5), 215 (9.6), 202 (7.4), 197 (3), 173 (12.6), 172 (100), 170 (16.2), 161 (10.8), 155 (7.5), 154 (8), 143 (4), 131 (7.6), 129 (9), 128 (12.5), 126 (11.4), 118 (10), 117 (3), 113 (31.4), 112 (5.4), 111 (7), 103 (21.6), 99 (11.3), 96 (6.5), 95 (13), 87 (38.8), 85 (36), 81 (5.8), 78 (5), 74 (100), 71 (22), 69 (45.6), 57 (25), 45 (18), 43 (40), and 41 (11); isobutane c.i.-m.s.: *m/e* 291 ($M + H^+$, 33%), 289 (26.6), 274 (71), 273 (100), 259 (52), 255 (33), 241 (52.3), 223 (33), 215 (71), 197 (51), 179 (30), 177 (100), 161 (50), 155 (19), 153 (19), 146 (25), 145 (28), 143 (25.7), 131 (33), 129 (38), 115 (29), 113 (98), 111 (28), 103 (14.2), 101 (50), 99 (50), 98 (50), 97 (100), 95 (50), 87 (23.8), 85 (42.7), 84 (15), 83 (19), 74 (10), 73 (100), 71 (19), 59 (100), 58 (100), 57 (100), 56 (76), and 43 (100).

Anal. Calc. for $C_{13}H_{22}O_7$: C, 53.79; H, 7.59. Found: C, 53.55; H, 7.48.

Kiliani hydrolysis of sarcobiose (**1**). — A solution of crystalline sarcobiose (10 mg) in Kiliani mixture (0.7 mL; 3.5 parts of glacial acetic acid + 5.5 parts of water + 1 part of conc. hydrochloric acid) was heated for 1 h at 100°, cooled, and evaporated to dryness over KOH in a vacuum desiccator. The residue was dissolved in water (1 mL), the acid neutralized with freshly precipitated silver carbonate, the suspension filtered, H_2S passed through the filtrate (to remove Ag^+ ions), and the suspension filtered through a thin layer of decolorizing charcoal. The filtrate was evaporated to dryness, and the residue was sublimed under high vacuum. The sublimate crystallized from acetone–ether; 2 mg, m.p. 112–113°. This reducing sugar gave a brown spot with the Partridge reagent[22], and reduced ammoniacal silver nitrate. It was identified as 6-deoxy-3-*O*-methyl-allose (**3**) by mixed m.p., and cochromatography in p.c., with an authentic sample.

Bromine–water oxidation of the sugar from the hydrolyzate of sarcobiose (**1**). — A solution of the reducing sugar (1.5 mg) from the hydrolyzate of sarcobiose in water (0.05 mL) was mixed with bromine (0.5 μL) and shaken in a stoppered tube in the dark for 24 h at room temperature. The excess of bromine was then removed under

diminished pressure, and the acid in the mixture was neutralized with freshly precipitated silver carbonate, and the suspension filtered; H_2S was passed through the filtrate (to remove Ag^+ ions), and the suspension was filtered. The filtrate was evaporated to dryness under diminished pressure, yielding a dark-brown, syrupy residue (0.5 mg) which gave a spot (NH_2OH–$FeCl_3$ reagent) of a lactone exhibiting identical mobility in p.c. and t.l.c. (19:1 ethyl acetate–methanol) to that of the lactone prepared from an authentic sample of **3**.

*3,4-Anhydro-2,6-dideoxy-β-*D-lyxo-*hexopyranosyl 2,4-di-*O*-acetyl-6-deoxy-3-*O*-methyl-β-*D*-allopyranoside* (**2**). — Crystalline sarcobiose (35 mg) dissolved in anhydrous pyridine (0.8 mL) was mixed with acetic anhydride (0.6 mL), and the mixture was kept for 48 h at room temperature. Pyridine and the excess of acetic anhydride were then removed under diminished pressure. The viscous residue was dissolved in chloroform, and the solution successively washed with 2M HCl, 2M Na_2CO_3 solution, and water, dried (anhydrous sodium sulfate), and evaporated to dryness, yielding an amorphous residue (30 mg) which gave two spots in t.l.c. (1:4 ethyl acetate–benzene). The major spot was separated by preparative t.l.c. (1:4 ethyl acetate–benzene), yielding an amorphous residue (22 mg) which crystallized as colorless rhombs (20 mg) from methanol, m.p. 79–80°, $[\alpha]_D^{26}$ +68° (*c* 0.76, methanol); p.m.r. data: δ 5.78 (dd, 1 H, *J* 4 and 9 Hz, H-1), 4.83 (d, 1 H, *J* 8 Hz, H-1′), 4.2–4.5 (m, 2 H, H-2′,4′), 3.73–4.03 (m, 3 H, H-4,5,5′), 3.48 (s, 3 H, OCH_3), 3.33–3.5 (m, 1 H, H-3′), 3.16 (dd, 1 H, *J* 3 and 8 Hz, H-3), 2.05 (s, 3 H, OAc), 2.03 (s, 3 H, OAc), 2.4–2.6 (m, 1 H, H-2*qe*), 1.55–1.9 (m, 1 H, H-2*qa*), 1.31 (d, 3 H, *J* 6 Hz, CH_3-5), and 1.15 (d, 3 H, *J* 6 Hz, CH_3-5′); e.i.-m.s.: *m/e* 374 (M^+, 10.6%), 357 (0.9), 343 (4.6), 332 (1), 331 (1.2), 316 (3), 315 (11.4), 314 (11.4), 287 (5.3), 282 (3.1), 270 (11), 257 (21.2), 245 (21.2), 238 (6.3), 215 (74), 214 (100), 212 (20), 203 (93), 202 (31), 197 (19), 183 (55), 173 (35.9), 171 (31.9), 155 (25.5), 154 (71), 152 (72.3), 143 (40.3), 129 (100), 127 (29.7), 125 (35.9), 124 (29.7), 116 (93.6), 115 (38.2), 114 (55.5), 113 (100), 112 (100), 111 (100), 101 (70), 100 (70.2), 99 (40.3), 97 (57.4), 96 (100), 95 (100), 93 (17), 87 (100), 85 (100), 83 (72), 81 (72.3), 79 (100), 78 (31.9), 74 (100), 71 (65.9), 69 (100), 68 (85), and 59 (100); isobutane c.i.-m.s.: *m/e* 375 ($M + H^+$, 26%), 371 (15), 357 (33), 353 (16), 343 (16), 317 (50), 316 (100), 315 (100), 313 (33), 287 (50), 283 (66), 271 (8), 267 (14.6), 257 (16), 246 (23), 245 (100), 239 (16), 225 (50), 223 (60), 219 (66.6), 203 (100), 200 (20), 187 (13), 179 (10), 173 (16), 161 (16), 155 (36.6), 145 (8), 127 (13), 115 (20), 113 (60), 111 (26), 98 (26), 97 (100), 95 (60), 85 (60), 80 (23.3), 73 (66.6), 61 (20), 59 (83), 58 (83), and 57 (100).

Anal. Calc. for $C_{17}H_{26}O_9$: C, 54.55; H, 6.95. Found: C, 54.36; H, 6.88.

ACKNOWLEDGMENTS

The authors thank Prof. L. Mester (Institut de Chimie des Substances Naturelles, Gif-sur-Yvette, France) for providing the elemental analysis of sarcobiose, and for kindly arranging the recording of the e.i. and c.i. mass spectra of sarcobiose and its diacetate. The authors are indebted to Prof. T. Reichstein (Organisch Chemi-

sche Anstalt, Basel University, Switzerland) for kindly supplying the authentic sample of 6-deoxy-3-*O*-methyl-D-allose. Thanks are also due Dr. D. K. Kulshreshtha (C.D.R.I., Lucknow) for helpful discussions.

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