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STRUCTURE OF BREVOBIOSE

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ABSTRACT

A new disaccharide, brevobiose (**1**), has been isolated from the dried twigs of *Sarcostemma brevistigma*. The structure of **1** has been established as 4-*O*-(6-deoxy-2-*O*-methyl-β-D-allopyranosyl)-D-boivinose on the basis of chemical and spectroscopic evidence, and identification of its hydrolysis products.

INTRODUCTION

In the chemical investigation of the dried twigs of *Sarcostemma brevistigma*, pregnane glycosides were extracted[1]. Mild hydrolysis[2] of these glycosides with acid gave a mixture of sugars which was separated by chromatography on a column of silica gel. The structure of a new, crystalline disaccharide named brevobiose (**1**), $C_{13}H_{24}O_8 \cdot H_2O$, m.p. 80–84°, $[\alpha]_D$ +45°, is now reported. It reduces Fehling solution, and exhibits positive tests for a 2-deoxy sugar in the xanthydrol reaction[3] and the Keller–Kiliani reaction[4], indicating it to contain at least one 2-deoxy sugar residue in the molecule. Several disaccharides consisting of residues of a 2,6-dideoxyhexose and a 6-deoxyhexose had earlier been reported from various Asclepiadaceae species[5]. For the identification of the units of such disaccharides, acid hydrolysis is only partly suitable, as it requires relatively drastic conditions under which the 2-deoxy sugar is mostly decomposed, although it permits the acquiring of undamaged, normal (2-hydroxy) sugars. In order to identify the 2-deoxy sugar, such disaccharides are first oxidized with bromine water to the bionic acid lactone, and then this is hydrolyzed with acid, to afford two units, the reducing end as a lactone, and the nonreducing end as the free sugar which can then be identified.

RESULTS AND DISCUSSION

In the p.m.r. spectrum of the monohydrate of **1** in pyridine-d_5, a double doublet centered at δ 5.72 (J 9 and 3 Hz) was assigned to the anomeric proton of the 2-deoxyhexose moiety. Its large coupling-constant (9 Hz) was typical of an axial proton of a pyranoid, deoxy sugar. Its assignment as the signal of H-1 was confirmed by double-irradiation experiments, which showed collapse of the methylene proton signals in

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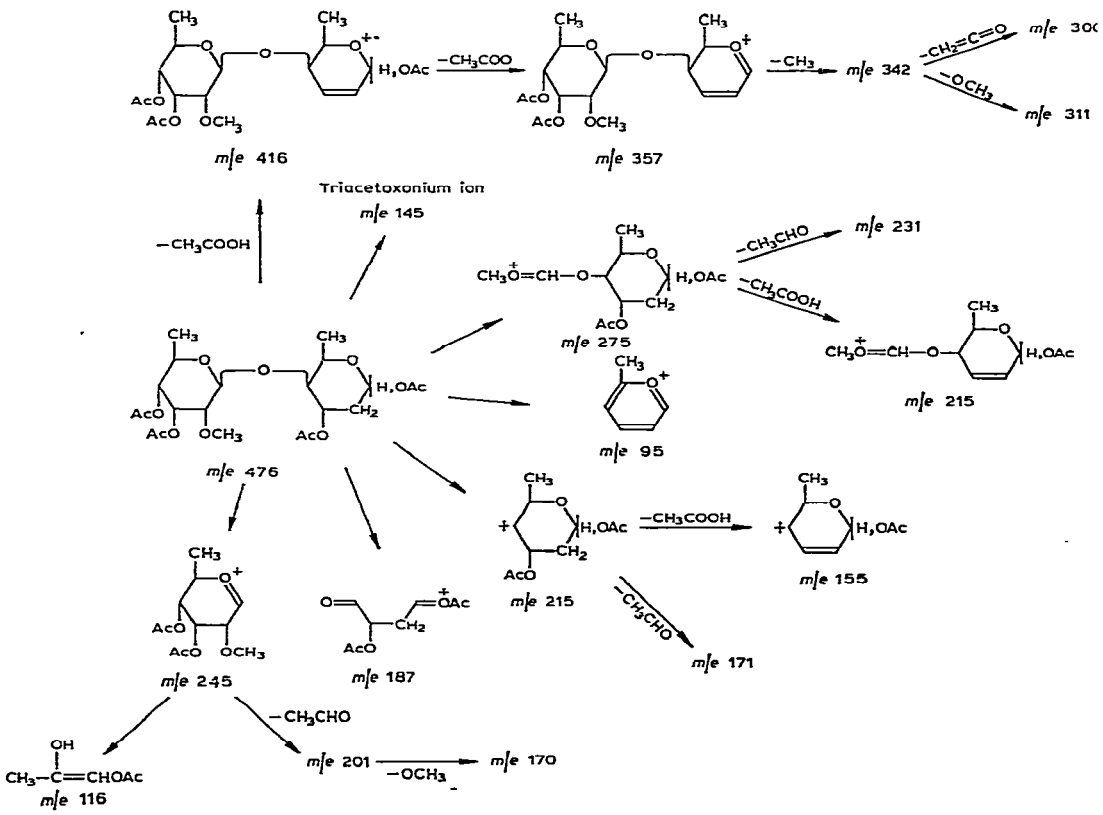
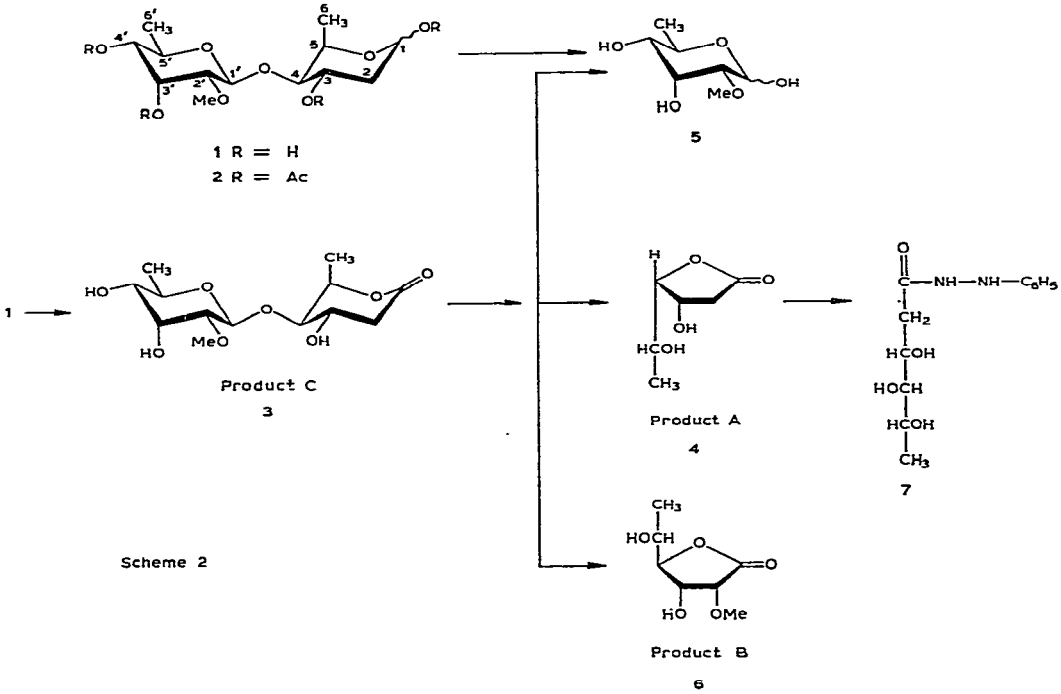
the range of δ 2.32–1.87 when irradiation at 515 Hz was conducted. The signal for the other anomeric proton (H-1'), expected at $\delta \sim 5.0$, could not be observed, as it was obscured by a broad hump in the region of δ 4.6–5.3, presumably due to moisture (originating from the water of crystallization in the hydrate of **1**). A three-proton singlet at δ 3.73 was assigned to a methoxyl group, and two sets of doublets (J 6 Hz), of three protons each, at δ 1.43 and 1.47, to two secondary methyl groups, suggesting the 6-deoxy nature of both monosaccharide units in **1**. Two ill-resolved multiplets (probably 2-proton), at δ 2.32–2.42 and 1.87–2.18, suggested the presence of one methylene group in the molecule. These data led to the conclusion that the monomethoxy disaccharide **1** contains one 2,6-dideoxyhexose residue present in the pyranoid form and a 6-deoxyhexose unit.

The mass spectrum of the peracetate (**2**) of brevobiose displayed its highest-mass peak at *m/e* 416 (0.24%, M — AcOH), in agreement with the formula $C_{21}H_{32}O_{12}$. Two prominent peaks, at *m/e* 245(36) and 215(64), diagnostic of the two hexose fragments[6] of **2**, suggested the presence of its lone methoxyl group in the 6-deoxyhexose unit (and not in the 2,6-dideoxyhexose moiety). On the same basis[6], assignments of other abundant peaks of the mass spectrum of **2** are given (see Scheme 1).



Scheme 1

Drastic hydrolysis of brevobiose (**1**) with acid by the Kiliani method[7] furnished an amorphous, reducing sugar (**5**, see Scheme 2). As 2-deoxy sugars are decomposed under these conditions, the sugar remaining should be that from the 6-deoxyhexose unit. It gave a negative Triphenyltetrazolium Blue test[8], which suggested that its 2-hydroxyl group was blocked[9]. Hence, the methoxyl group, manifested in the p.m.r. spectrum of **1**, was located at C-2 of this hexose. Sublimation of the sugar in high vacuum, followed by recrystallization from acetone, gave a few mg of crystals of **5**, m.p. 107–108°. On comparison of its m.p. with those of the 6-deoxy-2-*O*-methyl-hexoses reported in the literature, it was found to be identical with 6-deoxy-2-*O*-methyl-D-allose (D-javose, **5**; lit.[10] m.p. 107–110°). In the absence of an authentic sample of 6-deoxy-2-*O*-methyl-D-allose, and for further support of its identity, a comparison of the mobility of this sugar in p.c. with that of 6-deoxy-3-*O*-methyl-D-allose was made, and, as reported in the literature[10], they showed identical mobility (which, however, differed in t.l.c.).



Scheme 2

The oxidation of **1** with bromine water led to the formation of three products, designated A, B, and C in decreasing order of R_F value in t.l.c. These were separated by preparative t.l.c.

Product A (**4**) showed a strong i.r. band at 1780 cm^{-1}, indicative of a γ-lactone grouping[11]. Its p.m.r.-spectral analysis suggested that it was a 2,6-dideoxyhexono-1,4-lactone. It showed signals for eight protons in all. A doublet (J 7 Hz) centered at δ 1.23 gave rise to a singlet on irradiation of a 1-proton multiplet at δ 3.90. These

signals were, therefore, respectively assigned to the methyl group on C-5 and to H-5. Two double doublets, 1 H each, centered at δ 2.4 (J 18 and 4 Hz) and δ 2.88 (J 18 and 7 Hz), were assigned to the axial and equatorial protons on C-2 (methylene group of the lactone). On irradiation of a 1-proton multiplet at δ 4.55, both of these double doublets collapsed to doublets; this multiplet was therefore attributed to H-3. The remaining, narrow multiplet, at δ 4.17, could now be assigned to H-4.

Thus, it was clear that product A was a 1,4-lactone derived from the 2,6-dideoxyhexose moiety of **1**. This 2,6-dideoxyhexose could be any one of the four isomers, *viz.*, canarose[5,12,13], digitoxose[5,12,13], 2-deoxyfucose[5], and 2,6-dideoxy-D-*xylo*-hexose (boivinose[14]). However, product A was found to differ from the lactones from D-canarose and D-digitoxose in t.l.c., and from 2-deoxy-L-fucono-1,4-lactone on the basis of a different magnitude of specific rotation. Moreover, the m.p. (136–137°) of the phenylhydrazide (**7**) of product A was different from those reported in the literature[5] for the phenylhydrazides derived from the lactones of D-canaronic acid, D-digitoxonic acid, and 2-deoxy-L-fuconic acid. Product A was, therefore, confirmed to be the hitherto-unreported boivinono-1,4-lactone, and, hence, the 2,6-dideoxyhexose moiety of brevobiose is boivinose. As only D-boivinose[14] has so far been found to occur in plants, it is presumed by analogy that the D enantiomer is present in brevobiose.

Product B (**6**) was also a γ-lactone (ν_{max} 1780 cm^{-1}), and was found to be identical with 6-deoxy-2-*O*-methylallono-1,4-lactone by direct comparison (t.l.c.) with the lactone prepared by bromine–water oxidation of **5**.

Product C (**3**) was obtained in the smallest proportion; it showed a lactone band at 1736 cm^{-1}, characteristic of a δ-lactone[11]. Kiliani hydrolysis[7] of product C yielded a reducing sugar identical with 6-deoxy-2-*O*-methyl-D-allose (**5**), plus a lactone identical with boivinono-1,4-lactone (**4**) in p.c. and t.l.c.

These results suggested that a 6-deoxy-2-*O*-methyl-D-allosyl group constitutes the nonreducing end of brevobiose, and a boivinose residue the reducing end. Furthermore, the presence of a δ-lactone residue in product C, and formation therefrom of γ-lactone **4** on acid hydrolysis, indicated that, in product A, 6-deoxy-2-*O*-methyl-D-allose is glycosidically linked (presumably β) to C-4 of boivinono-δ-lactone. On liberation, the δ-lactone changed to the γ-lactone, as the 4-hydroxyl group was now available for lactonization.

The formation of products C (**3**), B (**6**), and A (**4**) during bromine–water oxidation could now be explained. The initial reaction was oxidation at the reducing end of the disaccharide to give brevobiono-1,5-lactone (**3**), which underwent hydrolysis by the action of the HBr present in the reaction mixture, with the formation of boivinono-1,4-lactone (**4**) and 6-deoxy-2-*O*-methyl-D-allose (**5**). Oxidation of the latter gave 6-deoxy-2-*O*-methyl-D-allono-1,4-lactone (**6**).

The ring size of the 6-deoxy-2-*O*-methyl-D-allosyl group in **1** still remained to be established. A strongly positive $NaIO_4$–benzidine test[15] for brevobiose, and also its complete reaction with $NaIO_4$ in solution within 10 min (t.l.c.; no **1** left) confirmed

the presence of a vicinal-diol grouping in the molecule. Its presence in the 2,6-dideoxy sugar residue (of boivinose), with its 4-hydroxyl group engaged in the glycosidic linkage, is precluded. The diol grouping should, therefore, be present in the 6-deoxy-2-*O*-methyl-D-allosyl group, which means that HO-3 and HO-4 thereof are free and, consequently, it is in the pyranose form. The difficult hydrolyzability of brevobiose and its lactone with acid led to the same conclusion.

In the light of the foregoing evidence, the structure of brevobiose was established as 4-*O*-(6-deoxy-2-*O*-methyl-β-D-allopyranosyl)-D-boivinose (**1**), a structure also in conformity with the observation that, in the disaccharides isolated from other species of Asclepiadaceae, the reducing end is invariably the 2,6-dideoxyhexose residue, and the nonreducing end, the 6-deoxyhexosyl group, joined by a (1→4)-glycosidic linkage.

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. Mass spectra were recorded with a JEOL High Resolution JMS-300 mass spectrometer. The sugars were made visible, in t.l.c., with 50% aq. H_2SO_4. In p.c., the 2-deoxy sugars were detected with the vanillin–perchloric acid reagent[16], and 2-hydroxy sugars with the Partridge reagent[17]. The lactones were detected in t.l.c. and p.c. with the NH_2OH–$FeCl_3$ reagent[18]. The adsorbent for t.l.c. was silica gel G (BDH) and, for column chromatography, silica gel for columns (BDH) developed by Duncan's method[19]. Paper chromatography was performed on Whatman No. 1 filter-paper, using 4:1 toluene–butanol saturated with water. Light petroleum refers to the fraction having b.p. 40–60°.

4-O-(6-Deoxy-2-O-methyl-β-D-allopyranosyl)-D-boivinose (**1**). — Shade-dried, powdered twigs (4 kg) of *Sarcostemma brevistigma* were extracted by the earlier method[20] for pregnane glycosides, using 50–95% ethanol. The ethanolic extracts were combined, and concentrated under diminished pressure, and the concentrate was extracted successively with light petroleum (500 mL), ether (500 mL), 4:1 chloroform–ethanol (700 mL), and 3:2 chloroform–ethanol (700 mL). The last three extracts were separately evaporated to dryness, and the resulting residues from the extracts with ether (3 g), 4:1 chloroform–ethanol (10 g), and 3:2 chloroform–ethanol (7 g) were rich in glycosides; they were hydrolyzed with 25mM H_2SO_4 in 50% methanol[2] to obtain the genins and sugars. The combined sugars (5.11 g) obtained from these extracts were chromatographed on silica gel (700 g), using 23:2 chloroform–methanol as the eluant, and collecting 500-mL fractions. Evaporation of fractions 122–126 gave a residue (85 mg) that crystallized from acetone–ether, giving brevobiose (**1**, 75 mg) as colorless rhombs, m.p. 80–84°, $[\alpha]_D^{26}$ +45° (*c* 0.8, methanol). It reduced Fehling solution, gave positive tests in the xanthydrol[3] and

Keller–Kiliani[4] reactions, and a blue coloration with vanillin–perchloric acid. It also gave a positive $NaIO_4$–benzidine test[15]. P.m.r. data (60 MHz, pyridine-d_5): δ 5.72 (dd, 1 H, J 9 and 3 Hz, H-1), 4.8–5.4 (broad hump, moisture), 3.9–4.4 (m, 5 H, not assigned), 3.50–3.68 (m, 2 H, not assigned), 3.73 (s, 3 H, OCH_3), 2.32–2.42 and 1.87–2.18 (2 m, 4 H, 2 OH and CH_2), 1.47 (d, 3 H, J 6 Hz, CH_3), and 1.43 (d, 3 H, J 6 Hz, CH_3).

Anal. Calc. for $C_{13}H_{24}O_8 \cdot H_2O$: C, 47.84; H, 8.03. Found: C, 47.03, 47.40; H, 8.01, 7.95.

Compound **1** was peracetylated on a microscale in the usual way, giving tetra-acetate **2**: *m/e* 476 (M^+ not observed), 416 (0.24%), 387 (1.6), 359 (6.4), 357 (4.4), 342 (2.4), 331 (2.8), 326 (2.8), 317 (0.8), 315 (0.84), 311 (0.61), 300 (1), 287 (0.68), 275 (6.4), 261 (2.4), 257 (2), 245 (36), 231 (6.4), 215 (64), 201 (12), 187 (10), 171 (17.2), 170 (20), 155 (68), 145 (32), 128 (28), 116 (84), 104 (3.2), 95 (100), 87 (60), 85 (56), 74 (80), 68 (100), 59 (28), and 43 (100).

Periodate oxidation of **1**. — To a solution of **1** (2 mg) in methanol (0.2 mL) was added a solution of sodium metaperiodate (6 mg) in water (0.1 mL), and the mixture was kept for 10 min at room temperature, diluted with water (0.4 mL), and evaporated under diminished pressure. By cochromatography, the residue showed complete consumption of brevobiose (t.l.c., 9:1 chloroform–methanol).

Kiliani hydrolysis of **1**. — Crystalline **1** (6 mg) was dissolved in the Kiliani mixture (0.4 mL; 7 parts of glacial acetic acid + 11 parts of water + 2 parts of conc. hydrochloric acid), and the solution 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 solution made neutral 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 through a thin layer of decolorizing charcoal. The filtrate was evaporated to dryness, and the residue sublimed under high vacuum. The sublimate crystallized from acetone; 1 mg, m.p. 107–110°. This reducing sugar gave a brown spot with the Partridge reagent, and reduced ammoniacal silver nitrate, but did not give any coloration with Triphenyltetrazolium Blue reagent[8]. It exhibited the same mobility as an authentic sample of 6-deoxy-3-*O*-methyl-D-allose in p.c., but a lower mobility in cochromatography (t.l.c.; 18:1:1 ethyl acetate–isopropyl alcohol–methanol). The reducing sugar was thus identified as 6-deoxy-2-*O*-methyl-D-allose.

Bromine–water oxidation of **1**. — A solution of **1** (50 mg) in water (0.8 mL) was mixed with bromine (13 μL), and shaken in a stoppered flask in the dark for 24 h at room temperature. The excess of bromine was then removed under diminished pressure, the acidic mixture was made neutral with freshly precipitated silver carbonate, and the suspension was 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 which gave three spots, of lactones A, B, and C in decreasing order of their mobilities in t.l.c. (19:1 ethyl acetate–methanol; with NH_2OH–$FeCl_3$ reagent).

Separation by preparative t.l.c., using 19:1 ethyl acetate–methanol, of the mixture of lactones (40 mg) afforded lactone A (10 mg), lactone B (9 mg), and lactone C (5 mg), all as syrups.

Lactone A (**4**). — This syrupy product had $[\alpha]_D^{26}$ −15.8° (*c* 0.38, acetone); $\nu_{max}^{CH_2Cl_2}$ 3580 (ass. OH), 2980, 1780 (C=O, γ-lactone), 1582–1568, 1190, 1165, 1100, 1015, 955, and 850 cm^{-1}; p.m.r. data: δ 4.55 (m, 1 H, H-3), 4.17 (m, 1 H, H-4), 3.9 (m, 1 H, H-5), 3.35–3.70 (hump, 2 H, 2 OH), 2.88 (dd, 1 H, *J* 18 and 7 Hz, H-2*e*), 2.4 (dd, 1 H, *J* 18 and 4 Hz, H-2*a*), and 1.23 (d, 3 H, *J* 7 Hz, CH_3).

Phenylhydrazide (**7**) *of lactone A*. — A solution of lactone A (**4**, 6 mg) in absolute ethanol (0.05 mL) was mixed with freshly distilled phenylhydrazine (0.04 mL), and the mixture was heated for 30 min at 100°. The viscous mass was cooled, and repeatedly triturated with absolute ether (to remove the excess of phenylhydrazine), yielding a brown powder. This residue (5 mg) crystallized from methanol–ether as colorless needles (3 mg), m.p. 136–137°; ν_{max}^{KBr} 3160, 2920, 2800, 2660, 1602, 1582, 1488, 1438, 1310, 1228, 1159, 1100, 1075, 1040, 1022, 1000, 886, 858, 767, 725, and 680 cm^{-1}.

Lactone B (**6**). — This compound had $[\alpha]_D^{26}$ 0° (*c* 0.33, acetone); $\nu_{max}^{CH_2Cl_2}$ 3500 (ass. OH), 2928, 1785 (C=O, γ-lactone), 1586, 1570, 1460, 1380, 1175, 1135, 1100, 1030, 1000, 960, 930, 860, and 835 cm^{-1}; p.m.r. data: δ 4.5 (d, 1 H, *J* 7.5 Hz, H-2), 3.85–4.20 (m, 2 H, H-4,5), 3.4 (s, 3 H, OCH_3), 2.72 (dd, 1 H, *J* 4 and 8 Hz, H-3), 1.25 (d, 3 H, *J* 7 Hz, CH_3), and 1.85 (broad hump, 2 H, 2 OH). Lactone B had the same mobility in t.l.c. (19:1 ethyl acetate–methanol) as the lactone prepared from reducing sugar **5**, obtained by Kiliani hydrolysis[7] of **1**. Lactone B was thus identified as 6-deoxy-2-*O*-methyl-D-allono-1,4-lactone (**6**).

Lactone C (**3**). — This compound showed $\nu_{max}^{CH_2Cl_2}$ 3558 (ass. OH), 2928, 1736 (C=O, δ-lactone), 1575, 1450, 1372, 1170, 1100, 1020, 860, and 830 cm^{-1}.

Kiliani hydrolysis[7] *of lactone C*. — Lactone C (**3**, 3 mg), recovered from the solution employed for recording the i.r. spectrum, was mixed with the Kiliani mixture (0.2 mL), and the solution was heated for 1 h at 100°, cooled, and evaporated over KOH in a vacuum desiccator. The residue, giving a positive test for reducing sugar with the Partridge reagent and for a lactone with the NH_2OH–$FeCl_3$ reagent, was applied directly in p.c. and t.l.c. for identification of its constituents.

The lactone from the hydrolyzate of lactone C (**3**) exhibited a mobility in t.l.c. (19:1 ethyl acetate–methanol) identical to that of lactone A (**4**).

The reducing sugar from the hydrolyzate of lactone C exhibited in p.c. and t.l.c. a mobility identical to that of the sugar from the Kiliani hydrolysis of **1**, identified as 6-deoxy-2-*O*-methyl-D-allose (**5**).

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