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STRUCTURE OF "SUGAR T"

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ABSTRACT

The structure of a disaccharide, "sugar T", isolated from dried stems and twigs of *Gymnema tingens*, has been elucidated as 3,4-anhydro-2,6-dideoxy-β-D-*ribo*-hexopyranosyl 6-deoxy-3-*O*-methyl-β-D-allopyranoside on the basis of chemical and spectroscopic evidence, and identification of its hydrolysis products.

INTRODUCTION

In the chemical investigation of the shade-dried stems and twigs of *Gymnema tingens* (Family: Asclepiadaceae), pregnane glycosides were extracted[1]. Mild, acid hydrolysis[2] of these glycosides gave a mixture of sugars from which a less polar, crystalline sugar (**1**) was isolated by column chromatography on silica gel, and preparative t.l.c. The structure of **1** is now reported.

RESULTS AND DISCUSSION

The sugar (**1**), m.p. 203–206°, $[\alpha]_D$ +129°, $C_{13}H_{22}O_7$ (M^+, *m/e* 290), which contained a methoxyl group (p.m.r. spectrum), did not reduce Fehling solution, and exhibited the characteristic tests of 2-deoxy sugars in the xanthydrol[3] and Keller–Kiliani[4] reactions. From its molecular formula, it appeared to be an anhydride of a biose, $C_{13}H_{24}O_8$, containing two hexose units. Drastic, acid hydrolysis by the Kiliani method[5] (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 by mixed m.p., and cochromatography in p.c., with an authentic sample. Further identification was achieved by t.l.c. comparison of the lactone prepared from it with authentic 6-deoxy-3-*O*-methyl-D-allono-1,4-lactone. On comparison of the m.p., specific rotation, and i.r. and mass spectra of **1**, it was found to be identical with the "sugar T" isolated by Reichstein *et al.* from *Dregea volubilis*[6] and from *Dregea abyssinica*[7]. Except for the u.v. data (end absorption at 200 nm, log ε 2.86), the mass and i.r. spectra, and identification of **3** as one of the units of this disaccharide[7,8], no other data and no interpretation of its structure are mentioned in

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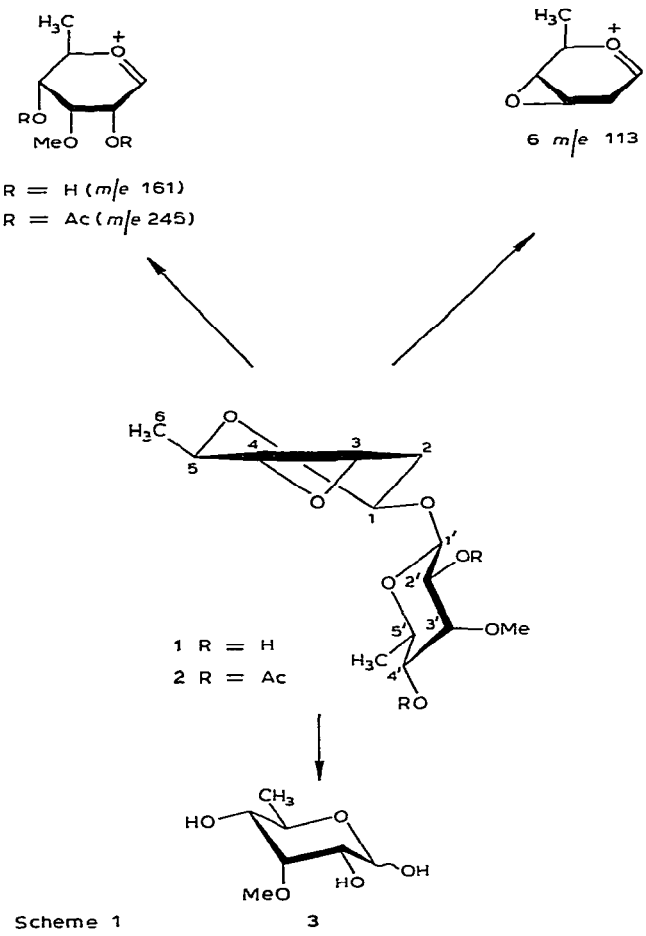


Scheme 1

the literature. It was, therefore, of interest to undertake a detailed study of this novel sugar with a view to elucidating its structure. Mild, acid hydrolysis of **1** by the method of Mannich and Siewert[9] did not liberate a free sugar, but isolation of a normal sugar (OH-2), *viz.*, **3**, only after drastic, acid hydrolysis indicated this nonreducing disaccharide to be of the trehalose type, with its two monosaccharide units joined by a glycosidic linkage through their reducing groups.

If the assumption that "sugar T" is a disaccharide is correct, then a double bond, a carbonyl group, or an epoxide ring must be present in the molecule. As "sugar T" did not exhibit any selective absorption in the u.v. spectrum, except for the end absorption at 200 nm, the possibility of the presence of a double bond or carbonyl group in the molecule was excluded. These conclusions were supported by the negative tetranitromethane test[10] given by the diacetate of "sugar T", and the absence of a carbonyl stretching band from its i.r. spectrum, and so the presence of an epoxide ring in the molecule was postulated. The same conclusions were arrived at by an accounting of the seven oxygen atoms in the molecule. In **1**, two oxygen

atoms are present as acetylatable hydroxyl groups (peracetylation results), one as a methoxyl group, two as the ring-oxygen atoms of the two hexose units, and one involved in the glycoside linkage. The seventh (unacetylatable) oxygen function in the molecule can, therefore, be present only as an epoxide ring. Further confirmation in this regard was afforded by positive Buchanan–Schwarz[11] and Ross[12] tests, diagnostic for an epoxide ring, of "sugar T". Identification of 6-deoxy-3-*O*-methyl-D-allose as one of the hexose units of "sugar T" suggested that the epoxide ring was present in the other part of this disaccharide.

Peracetylation of **1** afforded a crystalline di-*O*-acetyl derivative (**2**), $C_{17}H_{26}O_9$ (M^+, *m/e* 374), m.p. 127–128°, $[\alpha]_D$ +71°, indicating the presence of only two acetylatable hydroxyl groups in the molecule. These hydroxyl groups were not, however, vicinal, as indicated by the negative $NaIO_4$–benzidine test[13] given by "sugar T". Obviously, these two hydroxyl groups belonged to the 6-deoxy-3-*O*-methyl-D-allosyl group, and the other hexose unit of **1** could not, therefore, contain a free hydroxyl group. The nonreducing "sugar T" was thus inferred to be a (1↔1) disaccharide of **3** and an anhydroaldohexose. Furthermore, if both of these hexose units in **1** are present in the pyranose form, it would be anticipated that the 6-deoxy-3-*O*-methyl-D-allosyl group would assume a chair conformation, and the anhydrohexose, a half-chair conformation (see Scheme 1).

The two fragment-ion peaks, at *m/e* 161 and 113, in the mass spectrum of **1** were respectively diagnostic[14] of its two hexose fragments, **4** ($C_7H_{13}O_4$) and **6** ($C_6H_9O_2$) (see Scheme 1). As fragment **4** was derived from **3**, the other fragment must have originated from the rest of the molecule, which should be a dideoxyhexose carrying the epoxide ring. This conclusion was supported by corresponding peaks at *m/e* 245 for **5** and at 113 for **6** in the mass spectrum of **2**. Moreover, the mass-fragmentation patterns of **1** (base peak, *m/e* 172) and its diacetate **2** (base peak, *m/e* 203) were virtually identical with those of sarcobiose[15] (base peak, *m/e* 172) and its diacetate[15] (base peak, *m/e* 203).

On the basis of these results, it was anticipated that "sugar T" has the same gross structure as sarcobiose[15], isolated earlier in this laboratory from *Sarcostemma brevistigma*, *i.e.*, both contain a 6-deoxy-3-*O*-methyl-D-allosyl and a 3,4-anhydro-2,6-dideoxyhexosyl group. These two bioses had low polarity, and showed close mobilities in p.c. These results led to the conclusion that "sugar T" and sarcobiose are stereoisomers, differing either in the configuration of the glycosidic linkage or of the 3,4-epoxide ring in the 2,6-dideoxyhexose moiety.

A close analysis of the p.m.r. spectrum of diacetate **2** not only confirmed these assumptions but also ascertained the configuration of the glycosidic linkage and of the epoxide ring, and characterized the dideoxyhexose moiety. In this p.m.r. spectrum, two 3-proton doublets centered at δ 1.15 (J 6 Hz) and at 1.31 (J 6 Hz) were attributed to the two secondary methyl groups, and two 1-proton multiplets in the regions of δ 2.25–2.60 and 1.55–1.90, to a methylene group. The 3-proton singlet at δ 4.70 was assigned to a methoxyl group, and two other singlets (3 H each), at δ 2.05 and 2.03, to two acetyl groups in the molecule. Of these, the signals for the methoxyl group,

two acetyl groups, and one secondary methyl group must have arisen from 6-deoxy-3-*O*-methyl-D-allose (**3**). The remaining secondary methyl group and the methylene group must, therefore, be part of the dideoxyhexose moiety. A 1-proton doublet at δ 4.82 (J 8 Hz) could be assigned to the anomeric proton (H-1′) of **3**. The large coupling-constant (8 Hz) of this anomeric proton corresponded to the axial–axial coupling between H-1′ and H-2′, suggesting that the conformation of the 6-deoxy-3-*O*-methyl-D-allose[16] was $^4C_1$, and that it was linked to the other hexose through a β-glycosidic linkage[16], as in sarcobiose[15]. A double doublet centered at δ 5.79 (J 3.5 and 9 Hz) was attributed to the anomeric proton (H-1) of the 3,4-anhydro-2,6-dideoxyhexose moiety. Assuming it to be the D enantiomer, the smaller J value (3.5 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 the hexopyranose bearing the epoxide ring exists in the $^OH_1$ conformation[17], and is joined to the other hexose through a β-glycosidic linkage. Another, prominent, 1-proton signal in the spectrum was a double doublet centered at δ 3.15 (J 3 and 8 Hz). In view of the chemical shift and splitting pattern, and its analogy with a p.m.r. signal of sarcobiose acetate[15], 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 an inspection of a Dreiding model of the 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 ~3, ~0, and ~8 Hz, respectively, which are quite close to the values observed. Identification of the signals of H-5 and H-5′ present in the multiplets between δ 4.2 and 4.5, and 3.8 and 4.1, in the spectrum was achieved by a double-resonance experiment. Thus, irradiation of the (secondary methyl) doublet at δ 1.31 led to partial collapse of the multiplet in the region δ 3.8–4.1 to a doublet centered at δ 3.95 (J 8 Hz), which could be assigned to H-5′, and the related, secondary methyl doublet to CH_3-5′. This also confirmed an axial–axial orientation between H-4′ and H-5′ on the $^4C_1$ part. Similarly, appearance of a broad singlet at δ 4.4 in the partly collapsed multiplet between δ 3.8 and 4.1 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 low value of the coupling constant between H-5 and H-4, as manifested by the broad singlet in the irradiated signal of H-5, indicated a trans orientation of H-4 and H-5 in the $^OH_1$ conformation of the anhydrohexose unit. As the H-5 signal appeared at a field lower than that of H-5′, it was inferred that H-5 was deshielded by the epoxide ring-oxygen atom, a conclusion in full agreement with the orientation of H-4 and H-5 just derived. On the basis of these results, it was concluded that the 2-deoxyhexopyranosyl unit in "sugar T" is 3,4-anhydro-2,6-dideoxy-β-D-*ribo*-hexopyranosyl.

The p.m.r. spectrum of **1** in pyridine-d_5 displayed some interesting features. The anomeric proton (H-1′), the methoxyl group, and one of the secondary methyl groups gave rise to sets of signals: H-1′ centered at δ 4.97 and 4.95 (d, J 9 Hz), OCH_3 at δ 3.58 and 3.56 (s), and secondary methyl at δ 1.315 and 1.305 (d, J 5 Hz). On shaking with D_2O, however, one set of doublets due to a secondary methyl group

TABLE I

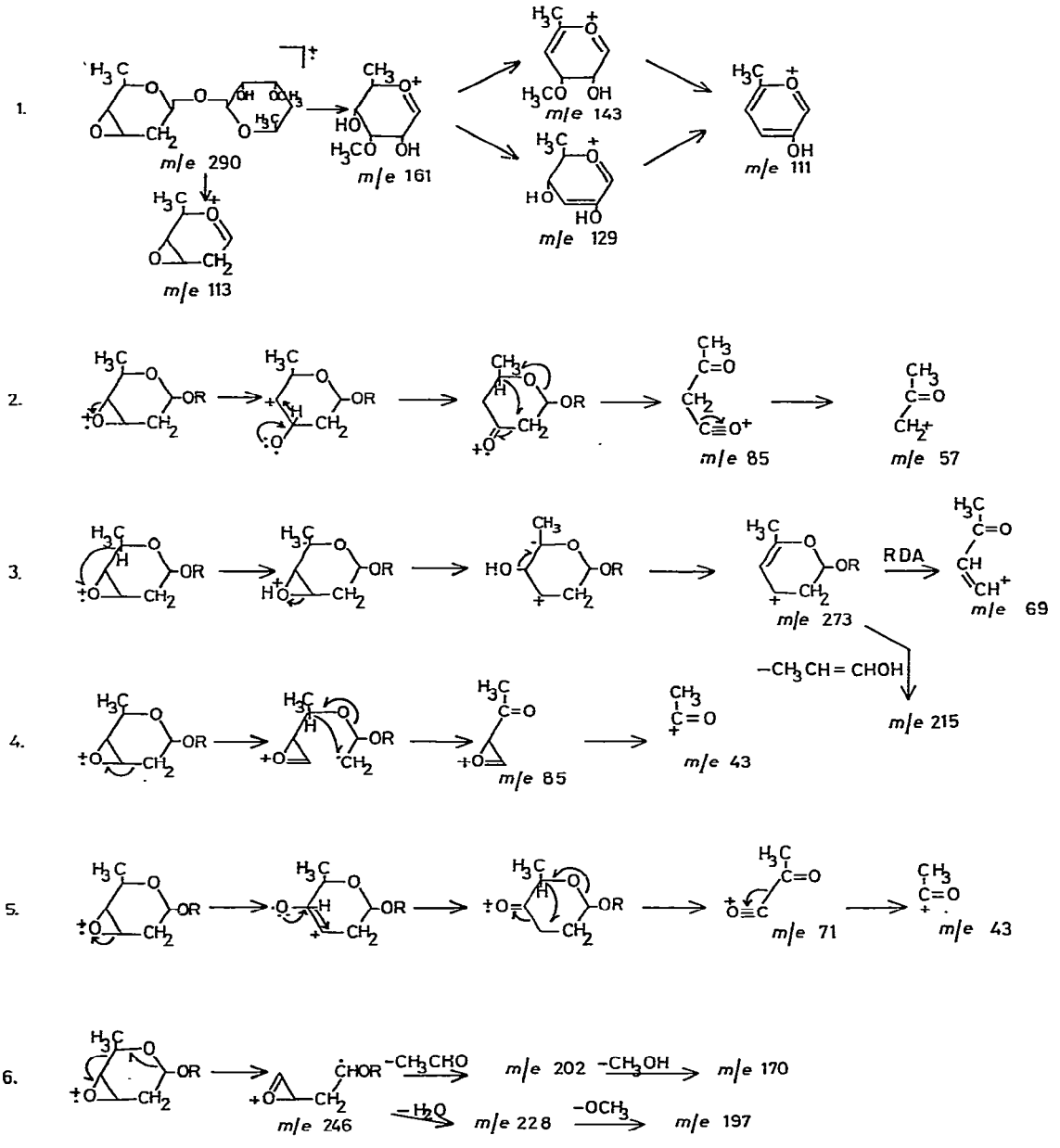
FOUND AND CALCULATED VALUES OF THE SPECIFIC ROTATIONS OF THE ANHYDRODIDEOXYHEXOSYL GROUPS IN SARCOBIOSE AND "SUGAR T" (1)

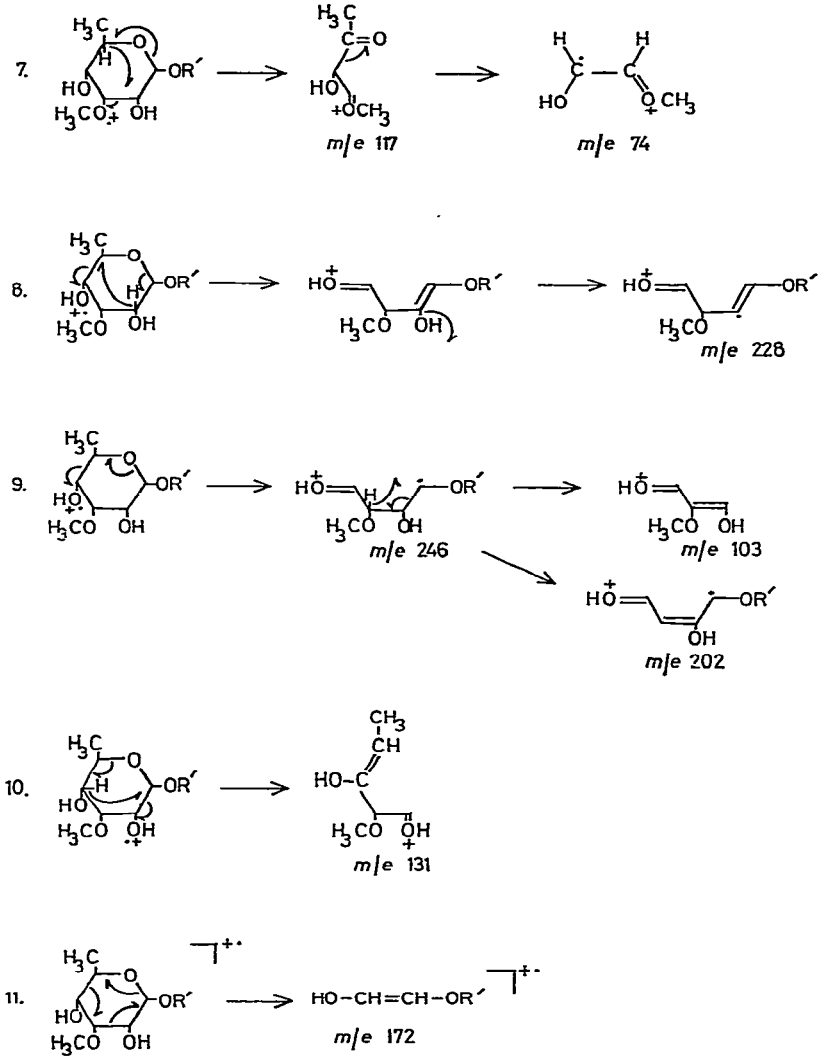
Name	*Natural sugar*		*Methyl 6-deoxy-3-O-methyl-β-D-allo-pyranoside*[19]		*3,4-Anhydro-2,6-dideoxy-β-D-hexo-pyranosyl component*	
	Found (degrees)		*Reported*[19] *(degrees)*		*Calculated (degrees)*	
	$[\alpha]_D$	$[M]_D$ I	$[\alpha]_D$	$[M]_D$ II	$[M]_D$ I − II	$[\alpha]_D$
Sarcobiose	+68	+197	−37	−71	+268	+206
"Sugar T" (1)	+129	+374	−37	−71	+445	+342

disappeared, whereas two methoxyl singlets remained intact. The H-1′ signal could not be deciphered properly; as this feature was restricted only to the protons of the 6-deoxy-3-*O*-methyl-D-allopyranosyl group, it was evident that pyridine was being bound to the free hydroxyl groups of the sugar, giving rise to two species in equilibrium, one free and the other bound to pyridine. The chemical shifts of the protons in the anhydrohexose unit, having no free hydroxyl group, remained unaffected by this solvent, and afforded valuable information regarding the structure of this unit. Thus, a double doublet centered at δ 5.48 (J 4 and 9 Hz) was attributed to the anomeric proton (H-1) of the 3,4-anhydro-2,6-dideoxyhexopyranosyl moiety on the basis of double-resonance experiments, whereby irradiation at 489 Hz led to the collapse of the methylene multiplets at δ 2.2–2.5 and 1.61–1.91. The methylene group in this moiety is, therefore, present next to this anomeric proton. In addition, the corresponding, secondary methyl group signals suggested that this moiety was a 2,6-dideoxyhexopyranosyl residue. The two coupling-constants (J 4 and 9 Hz), respectively attributed to the coupling between H-1*a* and H-2*qe*, and H-1*a* and H-2*qa*, corroborated the finding that the anhydrohexose exists in the $^{O}H_1$ conformation, and led to the conclusion that it is joined to the other hexosyl group through a β-glycosidic linkage.

These results indicated that, in **1**, the 6-deoxy-3-*O*-methyl-D-allopyranosyl and 3,4-anhydro-2,6-dideoxyhexopyranosyl groups are both joined by a β-glycosidic linkage. By applying the Klyne rule[18], the specific rotations of the 3,4-anhydro-2,6-dideoxy-β-D-hexopyranosyl groups in sarcobiose[15] and "sugar T" were calculated by using the known value of the specific rotation of methyl 6-deoxy-3-*O*-methyl-β-D-allopyranoside[19] (see Table I). The large difference in the specific rotations of the β-glycosidically linked, anhydro sugars in "sugar T" and sarcobiose suggested that the epoxide rings in these anhydrohexose units have different configurations, and lent support to the conclusions derived from the p.m.r.-spectral analysis. Thus, "sugar T" (**1**) is 3,4-anhydro-2,6-dideoxy-β-D-*ribo*-hexopyranosyl 6-deoxy-3-*O*-methyl-β-D-allopyranoside.

In the mass spectrum of "sugar T", many prominent peaks can be explained by standard, decomposition pathways[20] that fully support the structure proposed for this disaccharide. A partial, fragmentation pattern for the proposed structure fo "sugar T" is presented in Scheme 2, which accounts for most of the major peaks in the spectrum.





Scheme 2. Mass-spectral fragmentation of "sugar T".

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 reagent, and the 2-deoxy sugar, in p.c., with vanillin–perchloric acid reagent[21]. The lactone was made visible with NH_2OH–$FeCl_3$ reagent[22]. 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-ribo-*hexopyranosyl 6-deoxy-3*-O-*methyl-β*-D-*allo-pyranoside* (**1**). — Shade-dried stems and twigs of *Gymnema tingens* were extracted according to an earlier method[23]. Mild, acid hydrolysis[2] of the isolated glycosides afforded a sugar mixture (5 g) which was chromatographed on silica gel (500 g). Fractions 12–21, eluted with 49:1 chloroform–methanol (collection of 500-mL fractions) afforded a viscous material (305 mg) containing **1** admixed with two other sugars of close mobilities in t.l.c. Pure **1** was isolated from this mixture by preparative t.l.c. on plates of Silica Gel G, using 19:1 chloroform–methanol. The fastest band in t.l.c. was scraped off, and eluted with methanol. For further purification, the viscous material (110 mg) obtained from preparative t.l.c. was rechromatographed on silica gel (25 g). Fractions 10–20, eluted with 24:1 chloroform–methanol (collection of 30-mL fractions), afforded pure, amorphous "sugar T" (80 mg), which crystallized from acetone–ether as colorless rhombs (65 mg), m.p. 203–206°, $[\alpha]_D^{25}$ +129° (*c* 0.63, methanol). It gave a blue coloration (for 2-deoxy sugar) with vanillin–perchloric acid spray, did not reduce Fehling solution, but gave positive Buchanan–Schwarz[11] and Ross[12] tests for epoxide. It showed ν_{max}^{KBr} 3280, 2850, 2800, 1430, 1410, 1365, 1342, 1245, 1180, 1141, 1118, 1070, 1038, 1022, 978, 910, 840, and 818 cm^{-1}; p.m.r. data (pyridine-d_5): δ 5.48 (dd, 1 H, *J* 4 and 9 Hz, H-1), 4.97 and 4.95 (2 d, 1 H, *J* 9 Hz, H-1′), 4.1–4.25 (m, 1 H, H-5), 3.78–4.05 (m, 4 H, H-4,2′3,5′), 3.58 and 3.56 (2 s, 3 H, OCH_3), 3.3–3.5 (m, 2 H, H-3,4′), 2.2–2.5 (m, 1 H, H-2*e*), 1.61–1.91 (m, 1 H, H-2*a*), 1.37 (d, 3 H, *J* 5 Hz, sec. CH_3), 1.315 and 1.305 (2 d, 3 H, *J* 5 Hz, sec. CH_3) [after shaking with D_2O the major change was δ 1.31 (d, 3 H, *J* 5 Hz, sec. CH_3); before shaking with D_2O, this signal was two sets of doublets; no other change in the spectrum]; *m/e* 290 (M^+, 12%), 273 (3), 272 (4), 262 (3), 259 (3), 246 (2.4), 228 (7.2), 217 (1.4), 215 (5.8), 202 (2), 197 (3.2), 173 (9.1), 172 (100), 170 (2.8), 161 (5.8), 155 (6.5), 154 (7.3), 143 (1.7), 131 (2.8), 129 (3.8), 128 (12.5), 126 (3.9), 118 (8.4), 117 (5), 113 (29.1), 112 (2.2), 111 (3.7), 103 (12.7), 99 (7.5),

96 (5.5), 95 (13.2), 87 (24.2), 85 (17.8), 81 (4.6), 78 (6.4), 74 (55.7), 71 (12.1), 69 (40.8), 57 (14.1), 45 (16.2), 43 (38.4), and 41 (12).

Anal. Calc. for $C_{13}H_{22}O_7$: C, 53.79; H, 7.59. Found: C, 53.58; H, 7.51.

Kiliani hydrolysis of **1**. — Crystalline "sugar T" (10 mg) was dissolved in the Kiliani mixture[5] (0.7 mL; 3.5 parts of glacial acetic acid + 5.5 parts of water + 1 part of conc. hydrochloric acid), and the solution 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; 3 mg, m.p. 112–113°. This reducing sugar gave a brown spot with the Partridge reagent[24], and reduced ammoniacal silver nitrate. It was identified as 6-deoxy-3-*O*-methyl-D-allose by mixed m.p., and cochromatography in p.c., with an authentic sample.

Bromine–water oxidation of the sugar from the hydrolyzate of "sugar T". — A solution of the reducing sugar (2 mg; from the hydrolyzate of "sugar T") in water (0.06 mL) was mixed with bromine (0.6 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, the acidic mixture was 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. The filtrate was evaporated to dryness under diminished pressure, yielding a dark-brown, syrupy residue (1 mg) which gave a spot (NH_2OH–$FeCl_3$ reagent) of lactone exhibiting mobility in p.c. and t.l.c. (19:1 ethyl acetate–methanol) identical to that of the lactone prepared from an authentic sample of **3**.

*3,4-Anhydro-2,6-dideoxy-β-*D-ribo-*hexopyranosyl 2,4-di-*O*-acetyl-6-deoxy-3-*O*-methyl-β-*D-*allopyranoside* (**2**). — Crystalline "sugar T" (30 mg) dissolved in anhydrous pyridine (0.7 mL) was mixed with acetic anhydride (0.5 mL), and the solution 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 was successively washed with 2M HCl, 2M sodium carbonate solution, and water, dried (anhydrous sodium sulfate), and evaporated to dryness, yielding an amorphous residue (26 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 (20 mg) which crystallized from methanol as colorless rhombs (18 mg), m.p. 127–128°, $[\alpha]_D^{26}$ +71° (*c* 0.55, methanol); p.m.r. data: δ 5.79 (dd, 1 H, *J* 3.5 and 9 Hz, H-1), 4.82 (d, 1 H, *J* 8 Hz, H-1′), 4.7 (s, 3 H, OCH_3), 4.2–4.5 (m, 2 H, H-5,2′), 3.8–4.1 (m, 3 H, H-4,5,4′), 3.34–3.5 (m, 1 H, H-3′), 3.15 (dd, 1 H, *J* 3 and 8 Hz, H-3), 2.28–2.6 (m, 1 H, H-2*qe*), 2.05 (s, 3 H, OAc), 2.03 (s, 3 H, OAc), 1.55–1.9 (m, 1 H, H-2*qa*), 1.31 (d, 3 H, *J* 6 Hz, CH_3-5), 1.15 (d, 3 H, *J* 6 Hz, CH_3-5′); *m/e* 374 (M^+, 21.4%), 357 (1), 343 (3.5), 332 (5.7), 331 (1), 316 (2.6), 315 (7.8), 314 (10.7), 287 (5.7), 282 (3.5), 270 (7.1),

257 (17.8), 245 (20), 244 (28.5), 238 (8), 215 (56), 214 (57), 212 (21.4), 203 (100), 202 (28.5), 197 (28.5), 183 (35.7), 173 (25), 172 (29), 171 (21.4), 155 (21), 154 (32), 152 (57), 143 (25), 141 (21.4), 129 (57), 127 (14.2), 125 (14.5), 124 (21.4), 116 (100), 115 (42.8), 114 (25), 113 (100), 112 (100), 111 (57), 101 (25), 100 (32), 99 (57), 97 (32), 96 (71.4), 95 (100), 93 (25), 87 (71), 85 (100), 84 (17.5), 83 (32), 81 (46.6), 78 (17), 74 (72), 73 (14.4), 71 (57), 69 (57), 68 (28.5), and 59 (36).

Anal. Calc. for $C_{17}H_{26}O_9$: C, 54.55; H, 6.95. Found: C, 54.34; H, 6.83.

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