Carbohydrate Research, 79 (1980) 287–292

STRUCTURE OF TIGMOBIOSE

DEVESHWARI P. KHARE*, ANAKSHI KHARE, AND MAHESHWARI P. KHARE
Department of Chemistry, University of Lucknow, Lucknow (India)
(Received September 4th, 1979; accepted for publication, October 1st, 1979)

ABSTRACT

A new, nonreducing disaccharide, tigmobiose, has been isolated from the dried twigs of *Sarcostemma brevistigma*. On the basis of chemical and spectroscopic evidence, and identification of its sole hydrolysis product, its structure has been established as 2,6-dideoxy-β-D-*ribo*-hexopyranosyl 2,6-dideoxy-β-D-*ribo*-hexopyranoside (β-D-digitoxopyranosyl β-D-digitoxopyranoside).

INTRODUCTION

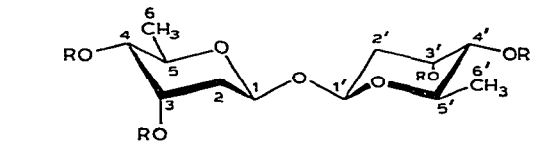
In a chemical investigation of the dried twigs of *Sarcostemma brevistigma*, pregnane glycosides were extracted. Mild hydrolysis[1] of these glycosides with acid gave a mixture of sugars from which the polar sugar brevobiose was isolated, and its structure was elucidated[2]. During the isolation of brevobiose by the earlier method, from fresh extracts of the plant, a new disaccharide of higher polarity was isolated in pure, crystalline state, and was designated tigmobiose (**1**).

RESULTS AND DISCUSSION

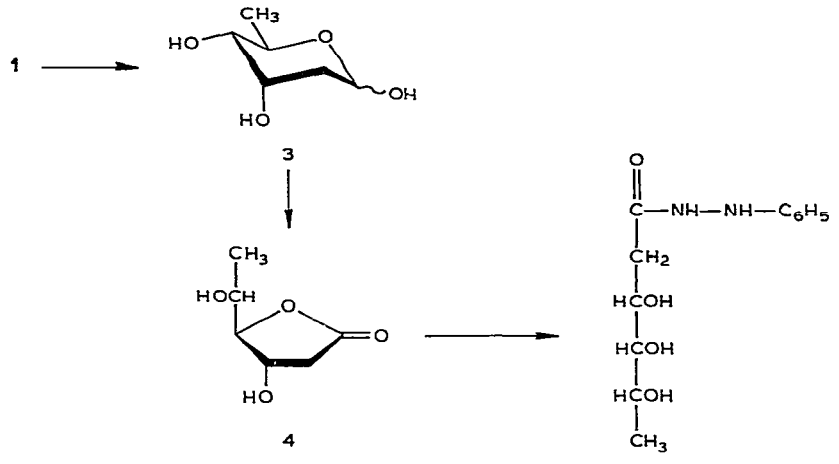
Tigmobiose (**1**) has m.p. 90–93°, $[\alpha]_D$ +19°, and an elemental analysis corresponding to that calculated for $C_{12}H_{22}O_7$. It does not reduce Fehling solution, and remains unaffected on treatment with bromine water. It exhibits positive tests characteristic of 2-deoxy sugars in the xanthydrol[3] and Keller–Kiliani[4] reactions. From these properties, it appeared to be a disaccharide having its monosaccharide units joined glycosidically through their anomeric carbon atoms.

To identify the two sugar units of this disaccharide, it was completely hydrolyzed with 5mM H_2SO_4 in 1,4-dioxane within 30 min at 50°. In t.l.c. and p.c., the hydrolyzate showed the presence of only one product, which was isolated in crystalline form, m.p. 99–104°, $[\alpha]_D$ +38°. A comparison of its rotation, m.p., and mobility in p.c. with those of an authentic specimen indicated it to be D-digitoxose[5]. For further characterization, the sugar from the hydrolyzate was oxidized with bromine–water to an amorphous lactone, $[\alpha]_D$ −32°, identical with D-digitoxono-1,4-lactone

*Doctoral candidate in the Department of Chemistry, University of Lucknow, Lucknow, India.



1 R = H
2 R = Ac



(**4**; lit.[5] $[\alpha]_D$ −32.1°), and different from canarono-[5] and boivinono-1,4-lactone[2]. For final confirmation, the hydrazide (**5**) was prepared from it; this possessed the same properties as an authentic specimen of D-digitoxonic acid hydrazide[5]. Tigmobiose (**1**) is thus a trehalose type of disaccharide involving two residues of D-digitoxose per molecule.

The ring size of the D-digitoxose (2,6-dideoxy-D-*ribo*-hexose) residues remained to be established; this was determined from a positive $NaIO_4$–benzidine test[6] that indicated the presence of vicinal-diol groupings in the molecule. The presence of a vicinal diol in a 2,6-dideoxy-*ribo*-hexose residue requires that HO-3 and HO-4 should be free, and, consequently, it is in the pyranoid form.

On acetylation, tigmobiose furnished tetraacetate **2**, m.p. 82–83°, $[\alpha]_D$ +42°. Its elemental analysis agreed with that calculated for $C_{20}H_{30}O_{11}$, and its mass spectrum displayed the highest-mass peak at *m/e* 445 (9.7%, M − 1), in agreement with the formula for a tetra-*O*-acetyltigmobiose.

The p.m.r. spectrum of **2** was typical of a single molecule of a di-*O*-acetyl-digitoxopyranoside. It was therefore inferred that this disaccharide was fully symmetrical, and that both of its D-digitoxopyranoside units were presumably linked glycosidically through their anomeric carbon atoms. The p.m.r. spectrum of **2** in $CDCl_3$ exhibited a two-proton signal at δ 5.88 as a double doublet (J 3.5 and 8 Hz), corresponding to two α-anomeric protons (H-1 and H-1′) coupling with the

adjacent methylene protons. Another two-proton signal appearing as a quartet (J 3 Hz) at δ 5.35 was assigned to H-3 and H-3′, and a double doublet (J 3 and 8 Hz) centered at δ 4.52 was attributed to H-4 and H-4′. An octet for two protons, centered at δ 3.98, was evidently due to H-5 and H-5′. Multiplets, ~2 protons each, at δ 2.32 and 1.60, were considered to be the signals for the equatorial and axial protons, respectively, of the two methylene groups. Two singlets, at δ 2.0 and 1.92 (six protons each), corresponded to the four acetyl groups, and a six-proton doublet (J 6 Hz) centered at δ 1.15 was assigned to the two, secondary methyl groups.

Double-resonance experiments were helpful in the confirmation of these assignments. Irradiation of the lowest-field signals at 530 Hz resulted in the collapse of the methylene multiplets, confirming the assignment of the anomeric protons (H-1 and H-1′). Similarly, irradiation of the secondary methyl doublet signal at 104 Hz caused collapse of the H-5, H-5′ unsymmetrical octet into a doublet (J 8 Hz) due to coupling of H-4 with H-5, and H-4′ with H-5′, indicating them to be present in *trans*-diaxial orientation in a chair conformation of the pyranoid moiety. Irradiation of the H-3, H-3′ signal at 482 Hz resulted in the collapse of the two multiplets corresponding to the two methylene groups, and of the H-4, H-4′ double doublet (J 8 and 3 Hz) into a simple doublet (J 8 Hz), reconfirming the *trans*-diaxial orientation of H-4 and H-5, as well as of H-4′ and H-5′, and also indicating H-3 and H-3′ to have equatorial dispositions. Conversely, irradiation of the H-4, H-4′ signal at 407 Hz caused collapse of the H-3, H-3′ signal to a triplet (J 3 Hz), further suggesting that the couplings between H-2*e*(2′*e*) and H-3(3′), H-2*a*(2′*a*) and H-3(3′), and H-4(4′) and H-3(3′) were all 3 Hz. The unsymmetrical octet for H-5, H-5′ resulted from two quartets originating from the coupling of H-5(5′) with CH_3-6 (CH_3-6′) (J 6 Hz) and with H-4(4′) (J 8 Hz). The p.m.r. spectrum thus confirms the structure of tigmobiose as β-D-digitoxopyranosyl β-D-digitoxopyranoside.

The molecular rotation of tigmobiose (Found: +52.8°; Calc.: +24°) [calculated from that (+12°) of methyl β-D-digitoxopyranoside[5]] was found to agree with the Klyne rule[7], further supporting the configurational assignment. This is the first time that a trehalose type of disaccharide has been found in Asclepiadaceae species; presumably, it exists in the plant in the free state, and would not be isolable in quantity (due to its difficult survival of the acid hydrolysis employed during isolation).

EXPERIMENTAL

General. — Melting points were determined in 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 I.R.-177 spectrophotometer, and p.m.r. spectra with a 90-MHz, Perkin–Elmer R-32 spectrometer for solutions in $CDCl_3$ (unless otherwise stated) with Me_4Si as the internal standard. Mass spectra were recorded with a JEOL High-resolution JMS-300 mass spectrometer. Sugars were detected in t.l.c. with 50% aq. H_2SO_4 reagent, and in p.c., with the vanillin–perchloric acid reagent[8]. Lactones

were detected with the NH_2OH–$FeCl_3$ reagent[9]. The adsorbent for t.l.c. was silica gel G (BDH), and, for column chromatography, silica gel for column (BDH), developed by the Duncan method[10]. Paper chromatography was performed on Whatman No. 1 filter paper, using 4:1 toluene–butanol saturated with water.

2,6-Dideoxy-β-D-ribo-*hexopyranosyl 2,6-dideoxy-β*-D-ribo-*hexopyranoside* (**1**). — Re-extraction of the dried twigs (4 kg) of *Sarcostemma brevistigma* according to an earlier method[11] afforded a mixture (5.11 g) of sugars which was chromatographed on silica gel (700 g). Fractions 128–132 (500 mL each) were eluted with 9:1 chloroform–methanol, and, on evaporation, afforded amorphous material (75 mg) containing **1**, which crystallized from acetone–ether as colorless rhombs (55 mg), m.p. 90–93°, $[\alpha]_D^{26}$ +19° (*c* 0.14, methanol). It did not reduce Fehling solution, and gave positive tests in the xanthydrol[3] and Keller–Kiliani[4] reactions, a blue coloration with vanillin–perchloric acid, and a positive $NaIO_4$–benzidine test[6].

Anal. Calc. for $C_{12}H_{22}O_7$: C, 51.80; H, 7.91. Found: C, 51.61; H, 8.01.

Periodate oxidation of **1**. — To a solution of crystalline **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 4 h at room temperature, diluted with water (0.4 mL), and evaporated under diminished pressure. The residue showed complete consumption of sugar by cochromatography in t.l.c. (9:1 chloroform–methanol).

3,4-Di-O-*acetyl-2,6-dideoxy-β*-D-ribo-*hexopyranosyl 3,4-di*-O-*acetyl-2,6-dideoxy-β*-D-ribo-*hexopyranoside* (**2**). — Crystalline **1** (20 mg) dissolved in anhydrous pyridine (0.4 mL) was mixed with acetic anhydride (0.3 mL), and the mixture was kept for 48 h at room temperature. The 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 hydrochloric acid, 2M sodium carbonate solution, and water, dried (anhydrous sodium sulfate), and evaporated to dryness, yielding an amorphous residue (16 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), giving an amorphous residue (12 mg) which crystallized as colorless rhombs (10 mg) from methanol; m.p. 82–83°, $[\alpha]_D^{26}$ +42° (*c* 0.33, methanol); p.m.r. data: δ 5.88 (dd, 2 H, *J* 3.5 and 8 Hz, H-1,1′), 5.35 (q, 2 H, *J* 3 Hz, H-3,3′), 4.52 (dd, 2 H, *J* 3 and 8 Hz, H-4,4′), 3.98 (unsym. octet ≡ superimposed dq, 2 H, *J* 8 and 6 Hz, H-5,5′), 2.32 (m, 2 H, H-2*e*,2′*e*), 2.0 (s, 6 H, 2 Ac), 1.92 (s, 6 H, 2 Ac), 1.60 (m, 2 H, H-2*a*,2′*a*), and 1.15 (d, 6 H, *J* 6 Hz, 2 sec. CH_3); *m/e* 445 (9.7%, M −1), 394 (2.5), 383 (3.1), 376 (3.1), 368 (3.2), 264 (2.8), 257 (7.5), 256 (18.1), 255 (100), 241 (2.9), 239 (3.2), 236 (11.8), 211 (3.7), 182 (1.4), 170 (7.0), 155 (4.6), 154 (5.7), 149 (3.4), 145 (6.2), 140 (4.5), 138 (6), 135 (1.5), 132 (4.3), 129 (3.2), 128 (6), 124 (4.2), 117 (5.3), 112 (8.4), 111 (6.8), 110 (4), 105 (7.6), 103 (4), 98 (9.3), 97 (7.8), 96 (1.8), 85 (4.8), 84 (5.4), 83 (6.1), 82 (8.1), 73 (2.3), 71 (6.2), 68 (19.5), 60 (4.5), 59 (1.2), 57 (7.5), and 56 (1.8).

Anal. Calc. for $C_{20}H_{30}O_{11}$: C, 53.81; H, 6.73. Found: C, 53.87, H, 6.88.

Mild hydrolysis of **1** *with acid.* — To a solution of crystalline **1** (10 mg) in 1:1 water–1,4-dioxane (0.5 mL) was added 5mM H_2SO_4 (0.5 mL), and the solution was

warmed for 30 min at 50°, cooled, and made neutral with freshly precipitated barium carbonate; the suspension was filtered, and the filtrate was evaporated to dryness under diminished pressure. The residue was extracted with hot acetone; evaporation of the extract yielded a syrup (9 mg) that exhibited one spot in t.l.c. (9:1 chloroform–methanol) and, in p.c., showed the same mobility as an authentic sample of D-digitoxose. For purification, it was sublimed under high vacuum. The sublimate crystallized from acetone–ether, giving colorless crystals (7 mg), m.p. 99–104°, $[\alpha]_D^{26}$ +38° (*c* 0.41, methanol). It reduced Fehling solution, and gave positive tests for a 2-deoxy sugar in the xanthydrol[3] and vanillin–perchloric acid reactions. Sugar **3**, obtained from the hydrolyzate of **1**, was thus identified as D-digitoxose[5].

Bromine–water oxidation of the sugar from the hydrolyzate of **1**. — A solution of the reducing sugar (7 mg), from the hydrolysis of **1**, in water (0.1 mL) was mixed with bromine (1.5 μL), and the mixture was shaken in a stoppered flask in the dark for 24 h at room temperature. The excess of bromine was then removed under diminished pressure, and the acidic mixture was made neutral with freshly precipitated siver carbonate. The suspension was filtered, and H_2S was passed through the filtrate to remove Ag^+ ions. The suspension was filtered, and the filtrate was evaporated to dryness under diminished pressure, yielding a dark-brown syrup (4 mg) which gave a spot (with the NH_2OH–$FeCl_3$ reagent) of lactone **4** that exhibited in cochromatography (t.l.c., 19:1 ethyl acetate–methanol) a mobility identical to that of the lactone prepared from authentic D-digitoxose. For purification, the crude lactone was sublimed under high vacuum, yielding a colorless syrup (3 mg), $[\alpha]_D^{26}$ −32° (*c* 0.18, acetone); lit.[5] $[\alpha]_D$ −32.1°.

Phenylhydrazide (**5**) *from the lactone obtained from the hydrolyzate of* **1**. — A solution of the lactone (2.5 mg) in absolute ethanol (0.02 mL) was mixed with freshly distilled phenylhydrazine (0.02 mL), and the solution was heated for 30 min at 100°. The viscous mass resulting was repeatedly triturated with absolute ether to remove the excess of phenylhydrazine. The ether-insoluble, brown powder crystallized from methanol–ether as colorless needles (1 mg), m.p. 123°, identified as D-digitoxonic acid phenylhydrazide; lit.[5] m.p. 123°.

ACKNOWLEDGMENT

The authors thank Dr. D. K. Kulshreshtha (C.D.R.I., Lucknow) for helpful discussions.

REFERENCES

1 S. Rangaswami and T. Reichstein, *Helv. Chim. Acta*, 32 (1949) 939–949.
2 D. P. Khare, S. S. Tiwari, A. Khare, and M. P. Khare, *Carbohydr. Res.*, 79 (1980) 279–286.
3 G. M. Barton, R. S. Evans, and J. A. F. Gardner, *Nature (London)*, 170 (1952) 249–250; R. Tschesche, G. Grimmer, and F. Seehofer, *Chem. Ber.*, 86 (1953) 1235–1241.
4 W. Nagata, C. Tamm, and T. Reichstein, *Helv. Chim. Acta*, 40 (1957) 41–61.
5 U. Eppenberger, H. Kaufmann, W. Stöcklin, and T. Reichstein, *Helv. Chim. Acta*, 49 (1966) 1492–1504; H. Allgeier, *ibid.*, 51 (1968) 668–682; A. Saner and H. Allgeier, *ibid.*, 52 (1969) 1655–1661; B. M. Kapur, H. Allgeier, and T. Reichstein, *ibid.*, 50 (1967) 2147–2171.

6 J. A. CIFONELLI AND F. SMITH, *Anal. Chem.*, 26 (1954) 1132–1134; H. T. GORDON, W. THORNBURG, AND L. N. WERUM, *ibid.*, 28 (1956) 849–855; D. F. MOWERY, *ibid.*, 29 (1957) 1560–1561.
7 W. KLYNE, *Biochem. J.*, 47 (1950) xli–xlii.
8 A. P. MACLENNAN, H. M. RANDALL, AND D. W. SMITH, *Anal. Chem.*, 31 (1959) 2020–2022.
9 M. ABDEL-AKHER AND F. SMITH, *J. Am. Chem. Soc.*, 73 (1951) 5859–5860.
10 G. R. DUNCAN, *J. Chromatogr.*, 8 (1962) 37–43.
11 F. SCHAUB, H. KAUFMANN, W. STÖCKLIN, AND T. REICHSTEIN, *Helv. Chim. Acta*, 51 (1968) 738–767.