Carbohydrate Research, 73 (1979) 302–308

Note

Bromine oxidation of α,α- and β,β-trehalose

ELISABETH SCHOLANDER

Department of Chemistry, College of Agriculture, Swedish University of Agricultural Sciences, S-750 07 Uppsala (Sweden)

(Received October 11th, 1978; accepted for publication, November 14th, 1978)

Oxidation of secondary hydroxyl groups in carbohydrates provides useful intermediates for the synthesis of amino sugars and other functional derivatives. When methyl glycopyranosides are oxidized with aqueous bromine, ketoglycosides are formed[1]. The symmetry of α,α- and β,β-trehalose (**1α** and **1β**) makes them ideal models for the study of chemical modifications of disaccharides. The 3-keto derivative of α,α-trehalose has been obtained by microbial oxidation[2]. Other non-symmetrical and symmetrical analogues of α,α-trehalose have been prepared for studies of structural features affecting the sweetness of sugars[3,4] and enzyme specificity[3,5].

The trehaloses were oxidized with aqueous bromine at room temperature and pH 7.0; the bromine–sugar molar ratio was 2:1. The resulting reaction mixtures were treated with methoxyamine hydrochloride, to convert the keto derivatives obtained into their more stable *O*-methyloximes[1]. The methoximated products were fractionated by column chromatography on silica gel and identified by n.m.r. spectroscopy (Table I) and by g.l.c.–m.s. (Table II) of their trimethylsilyl derivatives, the spectral data being compared with those of the methoximated methyl hexopyranosiduloses[1]. The yields of the keto derivatives were determined by g.l.c. of the trimethylsilylated *O*-methyloximes[6] (Table III), some of which were obtained as mixtures of *syn* and *anti* forms, resulting in two g.l.c. peaks from each keto compound. The signals from the two geometric isomers generally coincided in the n.m.r. spectra. Only the *O*-methyloxime of **2β** was separated into the two forms by column chromatography. As the *O*-methyloxime was used merely for protection, a routine chromatographic separation of the *syn* and *anti* forms was not necessary.

From the reaction mixture obtained by bromine oxidation of α,α-trehalose, followed by methoximation, the *O*-methyloximes of the 2- and 4-keto derivatives (**2α** and **4α**) and the 2,2′-, 2,4′-, and 4,4′-diketo derivatives (**2,2′α**, **2,4′α**, and **4,4′α**) were obtained. No α,α-3-keto derivative could be detected.

The reaction mixture obtained by oxidation of β,β-trehalose, followed by methoximation, was more complex, but pure *O*-methyloxime of the 4-keto derivative (**4β**) and fractions containing mainly the *O*-methyloximes of the 2- and 3-keto

TABLE I

^{1}H-N.M.R. SPECTRAL DATA FOR METHOXIMATED KETO DERIVATIVES OF α,α- AND β,β-TREHALOSE IN D_2O

		2α	4α	2,2′α	2,4′α		4,4′α	2β[a]		3β	4β
					2-keto	*4-keto*					
Chemical shifts (p.p.m.)											
Oxidized residue	H-1[a]	6.28 s	5.49 s	6.33 s	6.31 s	5.40 d 5.34 d	5.49 d 5.39 d	6.28 s	5.60 s	5.33[b] d	5.01 d
	H-2	–	4.13 dd	–	–	4.03 dd	3.70–4.15	–	–	4.72[b] d	3.94 dd
	H-3	4.58 d	4.46 d	4.46 d	4.49 d	4.10 d	4.33 d	4.51 d	4.62 d	–	4.26 d
	H-4		–			–	–		4.24 dd	4.55 d	–
	H-5	3.40–4.10	5.17 dd	3.40–4.00	3.45–4.10	5.02 dd	5.06 dd	3.70–4.10			4.98 dd
	H-6						3.75 dd		3.20–4.10	3.25–4.15	
	H-6′		3.40–4.20			3.45–4.10	3.99 dd				3.20–4.05
	N-OCH_3	3.90 s	4.00 s	3.91 s	3.92 s	3.92 s	3 91 s	3.92 s	3.99 s	3.95 s	3.91 s
Glucose residue	H-1	5.26 d	5.38 d					4.64 d	4.88 d	4.85 d	4.83 d
	H-2–H-6′	3.40–4.10	3.40–4.20					3.70–4.10	3.20–4.10	3.25–4.15	3 20–4.05
Coupling constants (Hz)											
Oxidized residue	$J_{1,2}$[a]		2.3			3.2(3.2)	2.2 (2.3)			4.8	5.6
	$J_{2,3}$		5.2			5.2	3.8				5.3
	$J_{3,4}$	9.3		9.3	9 5			9.2	8.3		
	$J_{4,5}$								9.3	7.1	
	$J_{5,6}$		3.2			3 5	2.8				4.2
	$J_{5,6'}$		7.3			6.1	8.2				6.6
	$J_{6,6'}$						—12.2				
Glucose residue	$J_{1,2}$	3 1	3.2					8.0	7 7	7.4	7.6

[a]*Syn* or *anti* forms. [b]Mutual assignment uncertain.

TABLE II

PERTINENT FRAGMENTS[a] (RELATIVE INTENSITIES) IN THE MASS SPECTRA OF TRIMETHYLSILYLATED, METHOXIMATED KETO DERIVATIVES OF α,α- AND β,β-TREHALOSE

2α *m/e*	4α *m/e*	2,2'α *m/e*	2,4'α[b] *m/e*	4,4'α *m/e*	2β[b] *m/e*	3β *m/e*	4β *m/e*
217 (17)	204 (11)	214 (21)	217 (10)	272 (10)	204 (38)	204 (39)	204 (30)
243 (11)	271 (13)	217 (25)	316 (56)	287 (16)	217 (77)	217 (58)	217 (30)
263 (18)	271 (14)	256 (18)	406 (21)	316 (100)	243 (20)	243 (17)	243 (14)
271 (17)	316 (70)	285 (45)	422 (10)	346 (10)	256 (18)	271 (24)	271 (19)
316 (10)	331 (10)	290 (61)	495 (2)	406 (35)	263 (24)	287 (18)	316 (80)
331 (46)	361 (100)	304 (27)	797 (1)	418 (5)	271 (20)	316 (100)	331 (13)
361 (100)	406 (16)	307 (21)	813 (2)	422 (5)	316 (31)	331 (27)	346 (10)
406 (13)	422 (1)	316 (64)	828[c](3)	495 (5)	331 (57)	346 (17)	361 (100)
451 (1)	451 (2)	406 (100)		797 (3)	361 (71)	361 (96)	406 (20)
495 (1)	495 (3)	422 (42)		813 (3)	375 (25)	375 (71)	422 (2)
873[c](3)	873[c](1)	495 (1)		828[c](5)	406 (38)	406 (41)	495 (1)
		813 (3)			451 (1)	422 (2)	
		828[c](23)			495 (1)	495 (2)	

[a] $m/e > 200$. [b] 73 (100%). [c] Molecular ion.

TABLE III

CARBONYL COMPOUNDS FROM OXIDIZED α,α- AND β,β-TREHALOSE ANALYSED BY G.L C. OF THE TRIMETHYLSILYLATED *O*-METHYLOXIMES

Compound	*Yield (%)*	*Retention*[a]
1α	40	1.80
2α	23	1.71
4α	23	1.71 (63%) 1.75 (37%)
2,2′α	4	1.64
2,4′α	4	1.64 (65%) 1.68 (35%)
4,4′α	5	1.55 (60%) 1.58 (40%)
1β	25	3.96
2β	9	3.62 (29%) 3.71 (71%)
3β	13	3.40 (63%) 3.48 (37%)
4β	18	3.28
x,yβ[b]	9	2.51–3.20

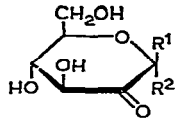
[a]Relative to trimethylsilylated *myo*-inositol. Two values refer to the *syn* and *anti* forms. [b]x,y = 2, 3, or 4.

derivatives (2β and 3β) were obtained by column chromatography. All three keto-glycosyl moieties could also be detected by g.l.c.–m.s. and n.m.r. spectroscopy in different fractions containing diketo compounds. It was not considered important in this investigation to prepare all of the different keto derivatives of β,β-trehalose in the pure state, as their identity was easily established by comparing spectral data of the fractions obtained with those of the methoximated methyl β-hexopyranosiduloses.

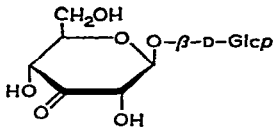
The total yield of monoketo derivatives of α,α-trehalose was ~50%, and the total yield of α,α-compounds containing one keto group in each ring was ~15%. No compound having more than one keto group in the same ring was found. No glucose or gluconic acid could be detected in the reaction mixtures, which indicates that, under the conditions used, the glycosidic linkage is not cleaved.

It has been proposed that the mechanism for the bromine oxidation of secondary alcohols involves a rate-determining hydride transfer from carbon[7,8]. The oxidation of methyl glycopyranosides is stereospecific; oxidation at ring carbons where the hydrogen is axial is hindered by a bulky *syn*-axial substituent. Consequently, in any α-D-glucopyranoside in the $^4C_1$ conformation, the aglycon should protect C-3 from oxidation. The foregoing results indicate that the trehaloses conform to this pattern. In α,α-trehalose, where the aglycon of either ring is axial, no oxidation occurs at C-3 or C-3′; in the β,β-isomer, where all ring substituents are equatorial, oxidation occurs at all secondary positions.

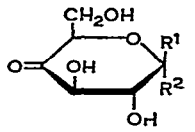
As the keto derivatives of α,α-trehalose are easily separated as *O*-methyloximes



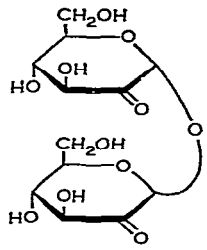
2α R^1 = H
R^2 = α-D-Glcp
2β R^1 = β-D-Glcp
R^2 = H



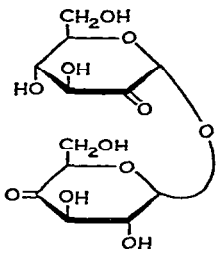
3β



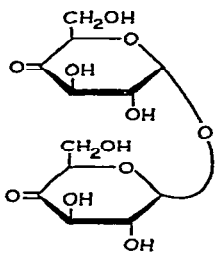
4α R^1 = H
R^2 = α-D-Glcp
4β R^1 = β-D-Glcp
R^2 = H



2,2'α



2,4'α



4,4'α

and regenerated by mild hydrolysis with acid[1], they have potential value in the synthesis of, for instance, biologically active amino[9] derivatives.

EXPERIMENTAL

General methods. — Melting points are corrected. Solutions were concentrated at reduced pressure below 40°. Optical rotations were measured with a Perkin–Elmer 141 polarimeter, and n.m.r. spectra were recorded with a Varian HA-100 D spectrometer. G.l.c. was performed with a Varian 2700 instrument, fitted with a flame-ionization detector. Separations were performed on glass columns (240 × 0.15 cm) containing 3% of OV-1 on Varaport 30 (100–200 mesh) at (*a*) 150 → 275° (6°/min) for the trimethylsilylated *O*-methyloximes from α,α-trehalose, and (*b*) 200 → 275° (2°/min) for those from β,β-trehalose. Detector responses were determined only for the trehaloses (0.56) and the *O*-methyloximes of **2α** (0.45) and **2,4'α** (0.42). The responses were determined by reference to trimethylsilylated *myo*-inositol. The responses of isomers were assumed to be equal. Peak areas were measured with an Autolab minigrator.

The mass-spectral data were obtained at 20 eV with a Varian MAT CH 7 mass spectrometer and a Varian 1740 gas chromatograph. The spectra of *syn* and *anti* forms of the trimethylsilylated methoxime derivatives showed only minor differences, and data are given only for the preponderating geometric isomers.

Electrophoresis was performed on Whatman No. 1 paper with 0.5M sodium acetate buffer (pH 4.5) at 25°. Detection was effected with silver nitrate–sodium hydroxide.

Bromine oxidations. — The bromine oxidations and methoximations were performed as previously described[1]. The salts were removed from the reaction mixtures by batchwise deionization; Dowex-50W X8 (H^+) and Dowex-1 X8 (HO^-) resins were added in portions, keeping the pH at 7, to avoid degradation of the *O*-methyloximes. The resulting solutions were evaporated to dryness and the residues charged on to columns of silica gel (Merck 60, 230–400 mesh). The products were eluted with acetonitrile–ethanol–water (7:1:1); the fractionation was monitored by t.l.c, with detection by anisaldehyde–sulphuric acid. Yields were not optimized, and only pure fractions of each oxime were collected.

(*a*) *α,α-Trehalose.* On oxidation of α,α-trehalose (4.00 g) with bromine (3.2 g) in water (250 ml), the following compounds were obtained.

α-D-Glucopyranosyl α-D-*arabino*-hexopyranosidulose (**2α**). The *O*-methyloxime of **2α** (243 mg) was an amorphous powder, $[\alpha]_{578}^{20}$ +195° (*c* 0.4, water).

Anal. Calc. for $C_{13}H_{23}NO_{11}$: C, 42.3; H, 6.3; N, 3.8. Found: C, 42.2; H, 6 5; N, 3.6.

α-D-Glucopyranosyl α-D-*xylo*-hexopyranosid-4-ulose (**4α**). The *O*-methyloxime of **4α** (164 mg) was an amorphous powder, $[\alpha]_{578}^{20}$ +184° (*c* 0.4, water).

Anal. Found: C, 42.2; H, 6.4; N, 3.9.

α-D-*arabino*-Hexopyranosylulose α-D-*arabino*-hexopyranosidulose (**2,2'α**). The bis(*O*-methyloxime) of **2,2'α** (59 mg) was a syrup, $[\alpha]_{578}^{20}$ +257° (*c* 0.4, water).

Anal. Calc. for $C_{14}H_{24}N_2O_{11}$: C, 42.4; H, 6.1; N, 7.1. Found: C, 41.2; H, 6.1; N, 6.8.

α-D-*arabino*-Hexopyranosylulose α-D-*xylo*-hexopyranosid-4-ulose (**2,4'α**). The bis(*O*-methyloxime) of **2,4'α** (95 mg) was a syrup, $[\alpha]_{578}^{20}$ +210° (*c* 0.4, water).

Anal. Found: C, 42.1; H, 6.3; N, 7.0.

α-D-*xylo*-Hexopyranosyl-4-ulose α-D-*xylo*-hexopyranosid-4-ulose (**4,4'α**). The bis (*O*-methyloxime) of **4,4'α** (52 mg) was a syrup, $[\alpha]_{578}^{20}$ +185° (c. 0.3, water).

Anal. Found: C, 42.1; H, 6.3; N, 7.0.

(*b*) *β,β-Trehalose.* On oxidation of β,β-trehalose (2.00 g) with bromine (1.6 g) in water (125 ml), diketo and the following monoketo derivatives were obtained

β-D-Glucopyranosyl β-D-*xylo*-hexopyranosid-4-ulose (**4β**). The *O*-methyloxime of **4β** was obtained as its monohydrate (156 mg), m.p. 138–141° (from aqueous ethanol), $[\alpha]_{578}^{20}$ —34° (*c* 0.9, water).

Anal. Calc. for $C_{13}H_{23}NO_{11}\cdot H_2O$: C, 40.3; H, 6.5; N, 3.6. Found: C, 40 0; H, 6.6; N, 3.6.

β-D-Glucopyranosyl β-D-*arabino*-hexopyranosidulose (**2β**) and β-D-glucopyranosyl β-D-*ribo*-hexopyranosid-3-ulose (**3β**) were obtained in mixtures together with small proportions of **4β**.

The n.m.r. and mass-spectral data for the methoximated keto derivatives from **1α** and **1β** are given in Tables I and II.

ACKNOWLEDGMENTS

The author thanks Professor Olof Theander and Docent Olle Larm for valuable discussions, and Mr. Rolf Andersson and Mr. Suresh Gohil for recording the n.m.r. and mass spectra, respectively. The work was supported by KABI, Stockholm, Sweden.

REFERENCES

1 O. Larm, E. Scholander, and O. Theander, *Carbohydr. Res.*, 49 (1976) 69–77.
2 S. Fukui and R. M. Hochster, *Can. J. Biochem. Physiol.*, 41 (1963) 2363–2371.
3 L. Hough and A. C. Richardson, *Pure Appl. Chem.*, 49 (1977) 1069–1084, and references therein.
4 C. K. Lee and M. G. Lindley, *Carbohydr. Res.*, 63 (1978) 277–282.
5 J. Defaye, H. Driguez, B. Henrissat, J. Gelas, and E. Bar-Guilloux, *Carbohydr. Res.*, 63 (1978) 41–49.
6 C. C. Sweeley, R. Bentley, M. Makita, and W. W. Wells, *J. Am. Chem. Soc.*, 85 (1963) 2497–2507.
7 N. Venkatasubramanian and V. Thiagarajan, *Tetrahedron Lett.*, (1968) 1711–1714.
8 I. R. L. Barker, W. G. Overend, and C. W. Rees, *J. Chem. Soc.* (1964) 3263–3267.
9 F. Arcamone and F. Bizioli, *Gazz. Chim. Ital*, 87 (1957) 896–902; S. Umezawa, *MTP Int Rev. Sci., Org. Chem. Ser. Two*, 7 (1976) 149–200.