Carbohydrate Research, 114 (1983) 306–310
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

Note

Isolation and alkaline degradation of some mono-*O*-methylsucroses

WAYNE MOODY, GEOFFREY N. RICHARDS,
Department of Chemistry and Biochemistry, James Cook University of North Queensland, Townsville 4811, Queensland (Australia)
NORMAN W. H. CHELTHAM, AND PADMINI SIRIMANNE
School of Chemistry, The University of New South Wales, P.O. Box 1, Kensington 2033 (Australia)

(Received October 25th, 1982; accepted for publication, November 16th, 1982)

This study continues the investigation of the mechanisms of the alkaline degradation of sucrose[1–3]. The ability of liquid chromatography (l.c.) to resolve mixtures of partially methylated sucrose derivatives[4] (see Fig. 1) made it possible to isolate and study the alkaline degradation of two pure mono-*O*-methylsucroses, thus supplementing alkaline-degradation studies performed on a mixture of the mono-*O*-methylsucroses[1]. In addition, it was found possible to clarify some assignments of ^{1}H-n.m.r. chemical-shifts for the methoxyl groups in *O*-methylsucroses[1,2]. The ^{13}C-n.m.r. spectra of the two mono-*O*-methylsucroses were consistent with their structures as found by chemical means.

EXPERIMENTAL

General methods. — Methods for permethylation, gas–liquid chromatography (g.l.c.), and ^{1}H-n.m.r. spectroscopy have been described[2], as has the method for the preparation of partially methylated sucrose derivatives[1]. ^{13}C-N.m.r. spectra were recorded, for solutions in D_2O, at 75.46 MHz with a Bruker CXP-300 instrument operated in the Fourier-transform mode at 33°. Chemical shifts are expressed in p.p.m. relative to Me_4Si, 1,4-dioxane, taken to be 67.4 p.p.m. on the Me_4Si scale, being the internal standard.

L.c. was conducted, with differential refractive index detection, as follows.

Analytical l.c. — For resolution of mono-*O*-methylsucroses: Waters Dextropak cartridge (10 × 0.8 cm) under radial compression (RCM 100) with water elution at 1.5 mL/min. For resolution of mono-*O*-methylfructoses: Waters μBondapak-NH_2 column eluted with 1:19 water–acetonitrile at 1 mL/min.

Preparative l.c. — Waters Prep LC/system 500 A, column Prep Pak C_{18} cartridge (30 × 5.7 cm), eluted with water at 250 mL/min.

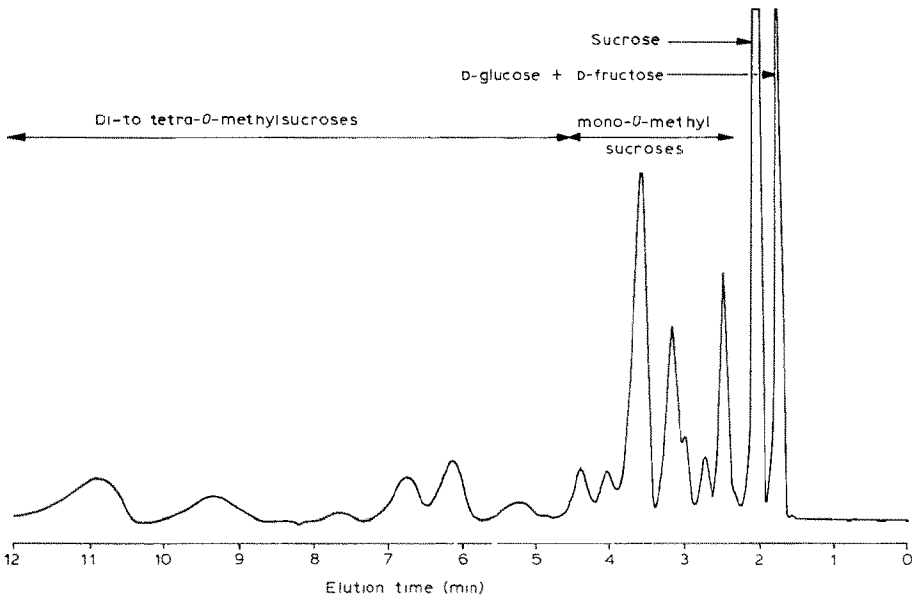

Fig. 1. L.c. profile of a mixture of methylated sucrose derivatives. (Column: Dextropak; solvent: water; flow-rate: 1.5 mL/min.)

Partially methylated sucrose (8 g) in water (50 mL) was passed through the preparative instrument, four fractions being collected. The second fraction (1.8 g) (mainly peaks 3 and 4 material; see Fig. 1) and the fourth fraction (2.0 g) (largely peak 7) were again passed through the preparative instrument, and nine fractions were collected from each. Final purification of peaks 3 and 7 material was achieved by using the (smaller) Dextropak column. Samples (~40 mg) of each were finally obtained as syrups containing only traces of other mono-*O*-methylsucroses. Peak 3, subsequently shown to be 3′-*O*-methylsucrose, showed $[\alpha]_D^{23}$ +53.0° (*c* 0.23, H_2O), and the ^{1}H-n.m.r. spectrum (D_2O) gave a chemical shift of 3.363 p.p.m. for the methoxyl protons. Peak 7, subsequently shown to be 4′-*O*-methylsucrose, showed $[\alpha]_D^{23}$ +52.9° (*c* 0.29, H_2O); the ^{1}H-n.m.r. spectrum (D_2O) gave a chemical shift of 3.368 p.p.m. for the methoxyl protons.

*Alkaline degradation of mono-*O*-methylsucroses.* — The alkaline degradations were performed on solutions of the mono-*O*-methylsucrose derivatives in 5.68M NaOH. The solutions were contained in stainless-steel reaction-vessels sealed with tight-fitting Teflon caps, and were suspended in an oil bath maintained at 100 ±0.1°. In experiments where air was displaced from the solution and the vessel by nitrogen, no significant effect on the rate of degradation was detected. Samples were taken at intervals, accurately weighed, and the pH adjusted to 6–7 with 60% aqueous acetic acid; they were then diluted to a known volume with water, and their optical rotations measured at 589 nm with a Perkin–Elmer 141 polarimeter. The data were fitted by the linear, least-squares method to a plot of ln α_{obs} against time, and k_{obs} was cal-

culated from the slope. Optical rotations for sucrose solutions containing sodium acetate in various proportions showed that the effect of sodium acetate on the optical rotation was negligible in the experimental range used.

RESULTS AND DISCUSSION

It was decided to isolate peaks 3 and 7 (see Fig. 1) for study, as they predominated in the mono-*O*-methyl region of a sample of deliberately undermethylated sucrose. Peak 3 on hydrolysis was shown by g.l.c.[1] and l.c., with reference to authentic compounds (including all of the possible mono-*O*-methyl-D-fructoses), to liberate glucose plus 3-*O*-methylfructose. Peak 7 yielded glucose plus 4-*O*-methylfructose. Peak 3 therefore consisted of 3′-*O*-methylsucrose, and peak 7, of 4′-*O*-methylsucrose.

It had been anticipated that methylation would occur most rapidly at O-1′, -2, and -3′ (the most acidic hydroxyl groups), followed by O-6 and -6′ (the least-hindered hydroxyl groups). Thus, the occurrence of 4′-*O*-methylsucrose as the

TABLE I

RATES OF ALKALINE DEGRADATION[a] AT 100°

Compound	10^5 k_{obs} s^{-1}	
Sucrose[b]	642	15
6,6′-Di-*O*-methylsucrose[b]	162	4
4′-*O*-Methylsucrose	63	3
3′-*O*-Methylsucrose	11	1
2,3,4,3′,4′-Penta-*O*-methylsucrose[b]	12.3	0.5
1′,4,6-Tri-*O*-methylsucrose[b]	4.8	0.2

[a]Solutions (2%) in 5.69M NaOH. [b]See ref. 3.

TABLE II

ASSIGNMENTS OF ^{1}H-N.M.R. CHEMICAL SHIFTS FOR METHOXYL GROUPS IN OCTA-*O*-METHYLSUCROSE

Peak	*Chemical shift*[a]	*Previous assignment*[b]	*Reassignment*[c]
I	3.173	1	
II	3.202	6	
III	3.235	6	
IV	3.250	4	2
V, VI	3.367	2, 3′	3, 4′
VII	3.531	4	
VIII	3.601	possibly 3	

[a]Shifts in p.p.m. downfield from internal Me_4Si, in benzene-d_6. [b]From refs. 1 and 2. [c]From present work.

TABLE III

CARBON-13 CHEMICAL-SHIFT ASSIGNMENT FOR 3′- AND 4′-*O*-METHYLSUCROSE

Compound	*Carbon-13 chemical-shifts for* D-*fructosyl unit*						
	Methoxyl	*C-1*	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*
Me β-D-fructofuranoside	—	60.0	104.7	77.7	75.9	82.1	63.6
Sucrose	—	62.3	104.4	77.4	74.9	82.2	63.2
Sucrose, 3′-*O*-methyl-	60.0	63.6	104.7	86.0	74.6	82.4	63.15
4′-*O*-methyl-	59.8	62.2	104.7	77.3	84.7	81.6	63.8

preponderant mono-*O*-methyl derivative was rather surprising. A possible explanation is that 4′-*O*-methylsucrose is less liable to further methylation than some of the other mono-*O*-methylsucroses.

3′-*O*-Methylsucrose proved to be relatively stable towards alkaline degradation, being degraded at a rate similar to those for 1′,4′,6′-tri-*O*-methylsucrose and 2,3,4,3′,4′-penta-*O*-methylsucrose[3]. This result is compatible with the proposed mechanism[1], which requires ionization of HO-1′ and HO-3′ for facile alkaline degradation of sucrose. 4′-*O*-Methylsucrose is more labile, being degraded at about half the rate of 6,6′-di-*O*-methylsucrose (see Table I).

Per(deuteriomethyl)ation of the 3′- and 4′-*O*-methylsucroses, followed by ^{1}H-n.m.r. spectroscopy, revealed almost identical chemical shifts for the methoxyl protons (3.363 and 3.368 p.p.m., respectively). Table II shows the (previously determined) chemical shifts[1] and assignments[1,2] for the methoxyl singlets observable in the ^{1}H-n.m.r. spectrum of octa-*O*-methylsucrose; the assignments of peaks IV, V, and VI were then tentative. The present work has now shown that the ^{1}H-n.m.r. peaks V and VI should be assigned to 3′- and 4′-*O*-methylsucrose, and that peak IV is therefore due to 2-*O*-methylsucrose. As 2-*O*-methylsucrose would disappear in alkaline degradation, this reassignment is still consistent with the observed alkali-lability[1] of peak IV material.

The ^{13}C-n.m.r. chemical-shifts of the fructofuranoside carbon atoms and of the methoxyl carbon atoms are shown in Table III. The assignments of the methyl β-D-fructofuranoside carbon atoms are those of Angyal and Bethell[5]. The chemical shifts shown in Table III are consistent with the peak 3 material's being 3′-*O*-methylsucrose, and peak 7 material's being 4′-*O*-methylsucrose. Thus, the resonance for C-3′ in peak 3 has undergone an α-shift downfield by some 8.6 p.p.m. The C-4′ resonance of the same compound has undergone a small β-shift upfield of ~0.3 p.p.m. Similar shifts (9.8 and 0.1 p.p.m.) apply to the spectrum of peak 7 material. In addition, the 4′-methoxyl group is apparently close enough to C-6 of the D-fructosyl moiety to bring about a downfield shift of ~0.6 p.p.m.

ACKNOWLEDGMENTS

The authors thank the Australian Development Assistance Bureau for the award of a Colombo Plan scholarship (to P.S.): Waters Associates (Australia) Pty., Ltd., and Mr. G. White for performing the preparative l.c.: Mr. V. Pickles and Dr. J. Saunders (School of Chemistry, University of New South Wales) for the ^{1}H- and ^{13}C-n.m.r. spectra, respectively; and Professor S. J. Angyal for his advice concerning the ^{13}C-n.m.r. spectra. Financial support was provided by the Australian Research Grants Committee and by the Sugar Research Institute, Mackay, Australia.

REFERENCES

1 M. Manley-Harris and G. N. Richards, *Carbohydr. Res.*, 90 (1981) 27–40.
2 M. Manley-Harris and G. N. Richards, *Carbohydr. Res.*, 82 (1980) 356–361.
3 M. Manley-Harris, W. Moody, and G. N. Richards, *Aust. J. Chem.*, 33 (1980) 1041–1047.
4 N. W. H. Cheetham and P. Sirimanne, *J. Chromatogr.*, 196 (1980) 171–175.
5 S. J. Angyal and G. S. Bethell, *Aust. J. Chem.*, 29 (1976) 1249–1265.