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Note

Reactivities at the O-2, O-3, and O-6 positions of cycloamyloses in Hakomori methylation

C. Trinadha Rao and Josef Pitha*
National Institutes of Health, NIA/GRC, Baltimore, MD 21224 (U.S.A.)

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Methylation of cycloamyloses (cyclodextrins) has been used to obtain derivatives of improved characteristics for application in pharmacy[1] and chromatography[2,3]. Well established procedures for several methylated cycloamyloses are in use. For example, the Kuhn–Trischmann procedure uses $Ba(OH)_2$ as a base and Me_2SO and *N,N*-dimethylformamide (DMF) as solvents and yields products in which primarily the C-2 and C-6 hydroxyl groups are methylated[4–8]. The Hakomori and Brimacombe procedures produce fully methylated derivatives of cycloamyloses; in these procedures Me_2SO or DMF are used as solvents and cycloamyloses are fully converted into their alkoxide forms[9–11]. Recently, heptakis(2-*O*-methyl)cyclomaltoheptaose has also become accessible by a procedure analogous to Hakomori methylation but in which the carbohydrate was only partially converted into the alkoxide form[12].

Analysis of the course of methylation of cycloamyloses should be helpful in understanding the effects of reaction conditions on the relative reactivities of the three hydroxyl groups at C-2, C-3, and C-6. Here, the course of methylation of three cycloamyloses, namely cyclomaltohexaose, cyclomaltoheptaose, and cyclomaltooctaose, under Hakomori conditions, that is, with an excess of base in Me_2SO, was investigated and the results compared with the course of methylation using limited base concentration in the same solvent as described[12] while our work was in progress.

To analyze the course of methylation, a very simple process involving ^{13}C-n.m.r. spectroscopy was used. A pulse of highly enriched (99%) ^{13}C-labelled methyl iodide was introduced at four different stages of the Hakomori procedure performed with an excess of base (3.7 mol per mol of glucose residues of the cycloamylose) and unlabeled methyl iodide containing only 1.1% of ^{13}C-isotope (5.2 mol per mol of glucose residues of the cycloamylose), compare Table I and Experimental section. Fully methylated cycloamyloses were then isolated and the positions and extent to which the ^{13}C-methyl groups were incorporated was established by measurement of ^{13}C-n.m.r. spectra. The peaks of the three methyl carbons at O-2, O-3, and O-6 are readily distinguished in the ^{13}C-n.m.r. spectra and have been unambiguously attributed previously[13,14]. Introducing a pulse of ^{13}C-labeled methyl iodide at a particular stage during the course of the reaction, and simply measuring the relative intensities of the 2-*O*, 3-*O*, and 6-*O*-methyl carbons in the

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TABLE I

Stages of addition of ^{13}C-labeled and unlabeled methyl iodide during Hakomori methylation of cycloamyloses

Time (h)	*Quantity of methyl iodide (mL)*			
	Experiment 1	*Experiment 2*	*Experiment 3*	*Experiment 4*
0	0.1 (^{13}C)	0.1	0.1	0.1
1	0.4	0.4	0.4	0.4
2	0.1	0.1 (^{13}C)	0.1	0.1
3	0.4	0.4	0.4	0.4
4	0.1	0.1	0.1 (^{13}C)	0.1
5	0.4	0.4	0.4	0.4
6	0.1	0.1	0.4	0.1 (^{13}C)
7	0.4	0.4	0.4	0.4

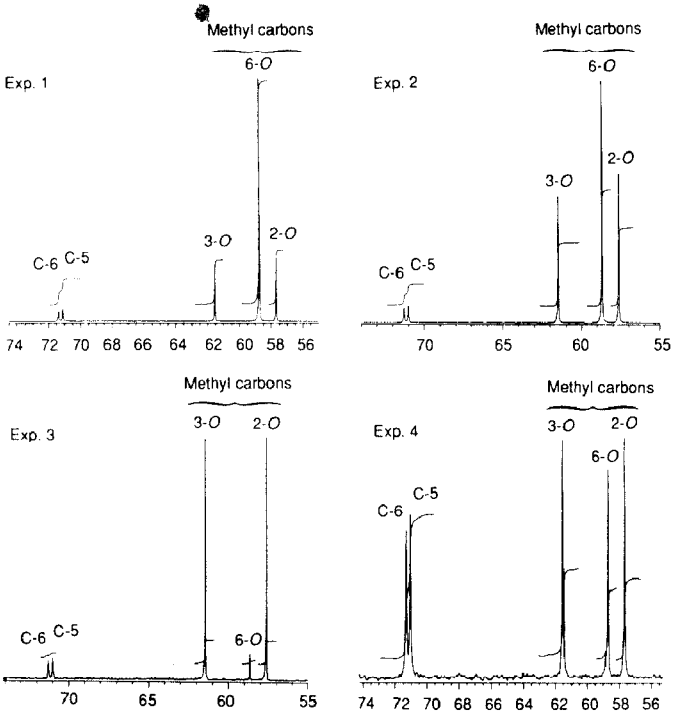

Fig. 1. ^{13}C-N.m.r. spectra of cyclomaltohexaose at different stages of methylation (see Table I for details) by ^{13}C-labeled and unlabeled methyl iodide.

TABLE II

Analysis of ^{13}C-n.m.r. spectra of ^{13}C-labeled permethyl cycloamyloses

Permethylated derivative of	*Position of O-methyl group*	*(p.p.m.)*		*Relative intensities of methyl group signals*[a]			
				Exp. 1	*Exp. 2*	*Exp. 3*	*Exp. 4*
Cyclomaltohexaose	2	57.62		18	30	49	35
	6	58.67		69	46	5	30
	3	61.46		13	24	46	35
			^{13}C Enhancement[b]	11	12	9	4
			k_2/k_3	1	1	1	1
			k_2/k_6	0.2	0.6	10	1
Cyclomaltoheptaose	2	58.51		29	31	40	33
	6	58.97		44	42	20	33
	3	61.48		27	28	39	35
			^{13}C Enhancement[b]	10	10	10	1
			k_2/k_3	1	1	1	0.9
			k_2/k_6	0.7	0.7	2	1
Cyclomaltooctaose	2	58.44		22	26	31	33
	6	58.75		58	50	39	33
	3	61.10		20	23	30	34
			^{13}C Enhancement[b]	7	12	13	1
			k_2/k_3	1	1	1	0.98
			k_2/k_6	0.39	0.53	0.78	1

[a] Normalized to a total of 100%. [b] Enhancement of methyl signals due to external ^{13}C incorporation. Measured from the ratios of the intensities of methyl group signals to the signals of C-5 and C-6 carbons in the ^{13}C-n.m.r. spectra of the permethylated cycloamylose.

^{13}C-n.m.r. spectrum, affords a snapshot picture of the relative reactivities of the alkoxides at that reaction stage. The ^{13}C-n.m.r. spectra obtained during methylation of cyclomaltohexaose are shown in Fig. 1. Similar spectra were obtained for cyclomaltoheptaose and cyclomaltooctaose and all results are summarized in Table II. The relative reactivities at O-2 and O-6 (k_2/k_6) and those between O-2 and O-3 (k_2/k_3) were measured from the ratios of the integrations of the corresponding methyl signals in the ^{13}C-n.m.r. spectra. As may be seen from Table II, the initital reactivities at the secondary O-2 and O-3 positions were lower than that at the primary O-6 position in all three cycloamyloses. This is in contrast to the reaction where only a limited quantity of the base was used[12] (one mol of NaH per mol of glucose residues of cyclomaltoheptaose). Under those conditions, only the most acidic C-2 hydroxyl groups formed anions that were subsequently methylated. In the present work, by using an excess of base, all of the anions at O-2, O-3, and O-6 are simultaneously generated and the O-6 positions, being sterically more accessible[15] by virtue of being primary, are the ones that are methylated more readily in the initital stages of methylation. Among the secondary hydroxyl groups O-2 seems to be slightly more reactive than O-3. As the methylation progressed, the order of reactivities was, as expected, eventually reversed.

EXPERIMENTAL

Procedures. — The cycloamyloses were dried at 110° *in vacuo* for 12 h prior to reaction. An 80% dispersion of NaH in mineral oil (0.69 g, 23 mmol) was washed with dry hexane, and under an atmosphere of dry argon anhydrous Me_2SO (10 mL) was added to it. The mixture was stirred for 75 min at 60–65°. A solution of the dry cycloamylose (1 g, corresponding to 6.17 mmol of glucose residues) in dry Me_2SO (10 mL) was then slowly introduced at room temperature and the resulting suspension vigorously stirred for 2 h. This procedure was carried out likewise in four separate flasks simultaneously with each cycloamylose. Methyl iodide, unlabeled and ^{13}C-labeled (99 atom%, Aldrich), was introduced at 1-h intervals in the quantities described in Table I. After completion of the addition the mixture was stirred for further 15 h at room temperature, decomposed with water (40 mL), and extracted into $CHCl_3$ (2 × 20 mL). The $CHCl_3$ layer was evaporated, water (40 mL) was added to the residue and the product extracted into ether (2 × 40 mL). The dried ($MgSO_4$) ether layer upon evaporation gave a crude product, which was flash chromatographed on silica gel (120 g, 230–400 mesh) with 20:1 CH_2Cl_2–MeOH. Fractions containing the permethylated compound and some undermethylated products (slightly more polar by t.l.c.) were pooled and evaporated.

The mixture thus obtained (dried for 5 h at 70° *in vacuo*) was subjected to further methylation by a procedure similar to that described (NaH, 0.35 g, 12 mmol, and MeI, 1 mL, were used for the reaction). The permethylated product was once again purified by flash chromatography; no undermethylated components were then detected by t.l.c. The average yields of permethylated cyclomaltohexaose, cyclomaltoheptaose, an cyclomaltooctaose were, 80, 81, and 85%, respectively. The ^{13}C-n.m.r. spectra of the samples

were recorded in $CDCl_3$ on a 90-MHz instrument (Spectral data services, Inc., Champaign, Illinois).

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