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Note

Chemical-ionization mass spectra of per-*O*-acetylaldononitriles and methylated aldononitrile acetates

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Per-*O*-acetylaldononitriles have been used as derivatives for the separation and identification of mixture of aldoses by gas–liquid chromatography (g.l.c.)[1,2] and by gas–liquid chromatography–mass spectrometry[3–5]. Electron-impact mass spectrometry (e.i.–m.s.) does provide characteristic peaks from the aldononitrile peracetates for ready interpretation, although it seldom yields the molecular ion. The research reported here describes analysis of per-*O*-acetylaldononitriles by chemical-ionization mass spectrometry[6]. In contrast to the electron-impact spectra, where molecular ions are not normally seen, chemical-ionization spectra show $M+1$ peaks in each instance. The base peak in each spectrum is at $(M+1)-60$ and results from the loss of the elements of acetic acid from the per-*O*-acetylaldononitriles.

A mixture containing the aldononitrile peracetates from D-rhamnose (**1**), L-fucose (**2**), D-arabinose (**3**), D-xylose (**4**), D-mannose (**5**), D-glucose (**6**), and D-galactose (**7**) was separated and analyzed by g.l.c.–c.i.–m.s. The chromatographic nonseparation of **2** and D-ribose (**8**) is resolved by the m.s., which permits separation of the ions characteristic of each component (Table I). G.l.c. was performed on a mixed liquid-phase consisting of 3% of OV-225 and 2.5% of tetramethylcyclobutanediol succinate on 80–100 mesh Supelcoport, with helium (20 ml/min) as the carrier gas; this system provides two major benefits: short retention-times and minimal bleeding of the liquid phase into the detection system.

For all of the foregoing aldononitrile peracetates, relative intensities of principal ions and their mass-to-charge ratios (*m/e* values) are reported in Table I.

Methylation of laminaran according to Lindberg[7] gave a mixture of partially methylated sugars that was converted by hydrolysis and derivatization into 3,5,6-tri-*O*-acetyl-2,4-di-*O*-methyl-D-glucononitrile (**9**), 3,5-di-*O*-acetyl-2,4,6-tri-*O*-methyl-D-

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TABLE I

RELATIVE INTENSITIES OF [M + 1] AND FRAGMENT-IONS FROM MASS SPECTROSCOPY OF PER-*O*-ACETYLALDONONITRILES BY CHEMICAL IONIZATION

Parent sugar	m/e																				
	81	91	115[a]	127[a]	129[a]	145[a]	187[a]	212[a]	228	256	257	270	271	274	286	316	328	329	330	346	388
1	1	3	2		6	3		2	1			100	16			1			1		
2	1	11			9	11			11			100	18						55		
3			5	6			5			100	10			23		10					
4			2	1	1	3	2			100	10					5					
5			4	2		4	4	4									100	20		2	4
6	20		23	40		3				3					1		100	20		30	37
7				13				25							21		100	71		29	50
8	17	13				12				100	21			13		21					

[a]Fragment ions that also appear in the electron-impact mass spectra[3].

gluconpremitrile (**10**), and 5-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucononitrile (**11**). Ratios for **9**, **10**, and **11** were[8] 1:30:2, respectively, as determined with a Varian–Aerograph Model 2400 gas chromatograph, interfaced with an Autolab System 1 integrator. Although the electron-impact mass spectra of the acetylated aldononitrile methyl ethers did not give molecular ions, the chemical ionization-spectra afforded readily identifiable [M + 1] and [M + 1] − 60 peaks. As a confirmation of the foregoing, electron-impact mass spectra of the methylated glucononitrile acetates were compared to those published by Dmitriev *et al.*[3].

Chemical-ionization mass spectra of an aldononitrile peracetate were recently used by this laboratory to identify an unusual D-arabinose component in hyaluronic acid of bovine brain[9].

EXPERIMENTAL

Mass-spectral and gas-chromatographic conditions. — Chemical-ionization mass spectra of per-*O*-acetylaldononitriles were recorded with a Varian MAT 112, double-focusing mass spectrometer. Spectral recording was interfaced with a Varian 620/L-100 data system. The c.i.–m.s. source temperature was 230°, the ionizing potential was 145 eV, the filament emission-current was 1.5 mA, the accelerating voltage was −820 V; and a reagent-gas source-pressure of 7×10^{-5} torr was used in g.l.c. mode. Electron-impact mass spectra were performed with similar instrument settings, except that the ionization potential was 80 eV and the source pressure was 2×10^{-6} torr in the g.l.c. mode. Samples were introduced into the mass spectrometer by means of a Brunnee[10] separator coupled to a Varian–Aerograph 1400 gas chromatograph equipped with a 1.85-m glass column (2 mm i.d.). The column was packed with 3% of OV 225/2.5% tetramethylcyclobutanediol succinate on Supelcoport (80–100 mesh) (Supelco, Inc., Bellefonte, Pa.) and was temperature-programmed from 190–230° at 2°/min at a carrier-gas (helium) flow-rate of 20 ml/min. Isobutane reagent-gas was introduced directly into the combination e.i.–c.i. source through tubing and valves designed by Varian MAT.

Preparation and separation of aldononitrile acetates. — The peracetylated aldononitriles were prepared by a modification of the method of Lance and Jones[1], and Varma[2]. To 15 mg of a dried mixture of eight monosaccharides were added 1.5 ml of dry pyridine and 25 mg of dry hydroxylamine hydrochloride in a Reacti-Vial® (5 ml, Pierce Chemical, Rockford, Ill.,) which was placed in an oven for 5 min at 90°. The warm vial-cap was again tightened to assure containment of hydroxylamine, and the vial was kept in the oven for another 40 min. After cooling to room temperature, the vial was opened and 2.5 ml of redistilled acetic anhydride was added. After preliminary warming and tightening of the cap as before, the mixture was again heated for 60 min at 100°. The contents were evaporated to dryness under a stream of nitrogen. To the dried residue were added nanograde chloroform (1 ml) and 3M hydrochloric acid (1 ml). The mixture was centrifuged, and the lower (chloroform) layer was washed twice with distilled water (1 ml), once with 0.5M sodium hydrogencarbonate (1 ml), and again with distilled water (1 ml). The chloroform layer was dried with anhydrous soldium sulfate, and an aliquot injected into the gas chromatograph.

Retention times for the individual sugar derivatives, under the chromatographic conditions and column packing described, were (min): **1**, 8.1; **2**, 9.9; **3**, 11.4; **4**, 12.8; **5**, 18.4: **6**, 20.3; **7**, 21.8; and **8**, 9.9. Mass-spectral data are recorded in Table I for **1–8**.

Methylation analysis of laminaran. — Laminaran (Sigma Chemical Co., St. Louis, Mo.) was methylated according to the procedures described by Lindberg[7] and then hydrolyzed with 2M trifluoroacetic acid[11]. After removal of aqueous trifluoroacetic acid by evaporation, the hydrolyzate was converted into methylated aldononitrile acetates as already described. The g.l.c. column was programmed at 2°/min from 175–220°. Mass spectral data are recorded in Table II for **9–11**. Retention times for the methylated aldononitrile acetates were (min): **9**, 12; **10**, 9.8; **11**, 6.7.

TABLE II

RELATIVE INTENSITIES OF M+1 AND FRAGMENT-IONS FROM MASS SPECTROMETRY OF PARTIALLY METHYLATED GLUCONONITRILE ACETATES BY CHEMICAL IONIZATION

	m/e								
	184	203	205	216	244	272	276	304	332
9[a]			16.2	8.1		100			28.8
10[b]		5.9			100			48.1	
11[c]	44.2			100			36.5		

[a]**9** = 3,5,6-tri-*O*-acetyl-2,4-di-*O*-methylgluconoitrile. [b]**10** = 3,5-di-*O*-acetyl-2,4,6-tri-*O*-methylgluconoitrile. [c]**11** = 5-*O*-acetyl-2,3,4,6-tetra-*O*-methylgluconoitrile.

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