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Mass Spectrometric Identification of Aldonolactones as Trimethylsilyl Ethers

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Trimethylsilyl ethers of aldonolactones are useful for the identification of aldonic acids by mass spectrometry. Peaks recorded for the molecule ion (M) and for mass M−15 give a reliable determination of the molecular weight. Significant differences exist between 1,4- and 1,5-lactones. All diastereomers studied can be well distinguished from each other.

Ion exchange chromatography has been used for the isolation of aldonic acids from sulfite waste liquors,[1,2] from hydrolyzates from oxidized cotton,[3] and from wood pulps.[4] With these complex mixtures the separations have to be carried out in several steps to achieve a clean-cut separation. An identification of the isolated acid fractions by classical methods is difficult unless rather large amounts of substances are isolated. The separation process must, therefore, be carried out on a fairly large scale, which is a serious complication in all chromatographic separations of compounds with very similar properties. A combination of various chromatographic methods including gas chromatography has been used for identification [5] but the reliability of such identifications can of course be questioned.

The purpose of the present work was to study the application of mass spectrometric identification of aldonic acids. The preparation of trimethylsilyl (TMS) derivatives according to the procedure devised by Sweeley *et al.*[6] is a most convenient method for transforming the aldonic acids into volatile compounds suitable for gas-liquid chromatography. Separations of TMS-derivatives from various aldonic acids by gas chromatography have also been reported.[6,7] As shown in this paper, these derivatives are also suitable for identification by means of mass spectrometry.

EXPERIMENTAL

Preparations. The trimethylsilyl derivatives were prepared according to Sweeley *et al.*[6] with slight modifications. The sample (5—20 mg) was dissolved or suspended in 0.5—2 ml pyridine and then about 0.5 ml hexamethyldisilazane and 0.3 ml trimethylchlorosilane were added. The reaction mixture was shaken for 2 min. If dissolution was difficult the mixture was warmed carefully. The solvent was removed in a rotating vacuum evaporator at a temperature not exceeding 50°C. After dissolution in diethyl ether or hexane to a concentration of about 1 mg/ml the sample was applied to the chromatographic column.

The 1,4-lactones of threonic and lyxonic acids were prepared according to Hardegger *et al.*[8] and glucono-1,4-lactone according to Isbell and Frush.[9] The other lactones used were from various commercial sources or prepared in connection with previously published work.[1,5] The TMS-ethers of arabinono-1,5-lactone and xylono-1,5-lactone were separated by gas chromatography from the product mixtures obtained on trimethylsilylation of the residues after evaporation of an aqueous solution of the corresponding calcium salts in the presence of hydrochloric acid.[6]

TMS-ethers of the following compounds were investigated:
Tetronolactones: Erythrono-1,4-lactone, threono-1,4-lactone.
Pentonolactones: Ribono-1,4-lactone, arabinono-1,4-lactone, xylono-1,4-lactone, lyxono-1,4-lactone, arabinono-1,5-lactone, xylono-1,5-lactone.
Hexonolactones: Glucono-1,4-lactone, mannono-1,4-lactone, gulono-1,4-lactone, galactono-1,4-lactone, talono-1,4-lactone, glucono-1,5-lactone.
Heptonolactones: D-*Glycero*-D-*gulo*-heptono-1,4-lactone, D-*glycero*-L-*manno*-heptono-1,4-lactone.

Gas chromatography — mass spectrometry. LKB 9000 gas chromatograph — mass spectrometer was used in all experiments.

The GLC column was a 300 × 0.3 cm i.d. glass column filled with silanized and acid-washed 100—120 mesh Chromosorb P with 1 % SE 30 as stationary phase. The carrier gas, helium, was used at a flow rate of 30 ml/min. All experiments were performed at a constant column temperature, chosen in the range 120°C (tetronolactones) to 180°C (heptonolactones). The gas chromatograms were recorded by measurement of the total ion current. The amounts of TMS-ethers injected were 1—5 μg.

An electron energy of 70 eV was employed. The temperature of the molecule separators was 200—220°C and the temperature of the ion source 250°C. The exit slit was set to 0.05 mm and the collector slit to 0.1 mm. The scanning time over the mass range 12—600 was about 5 sec.

A linear correction for column bleeding was applied.

RESULTS AND DISCUSSION

1,4-Lactones of varying molecular weight. Mass spectra of 1,4-lactones of aldonic acids containing 4, 5, 6, and 7 carbon atoms are reproduced in Fig. 1. As indicated in the figure the regions of higher mass number were enlarged by a factor of 10 for the higher aldonic acids. The mass number (m/e) of the base peak was in all spectra equal to 73 which corresponds to the $(CH_3)_3Si$-ion. TMS-derivatives of straight chain secondary alcohols have earlier been shown to give rise to a prominent peak at this position.[10]

The molecule ion (M) was recorded as a significant peak at a mass in agreement with that calculated for the fully trimethylsilylated derivatives: 262, 364, 466, and 568, respectively. The intensity was lower with D-*glycero*-D-*gulo*-heptono-1,4-lactone and D-*glycero*-L-*manno*-heptono-1,4-lactone than with the other lactones. Several fragment ions can be used to confirm the determination of molecular weight. The ion M—15 obtained when one methyl group is split off gave with several aldonolactones a more intense peak than

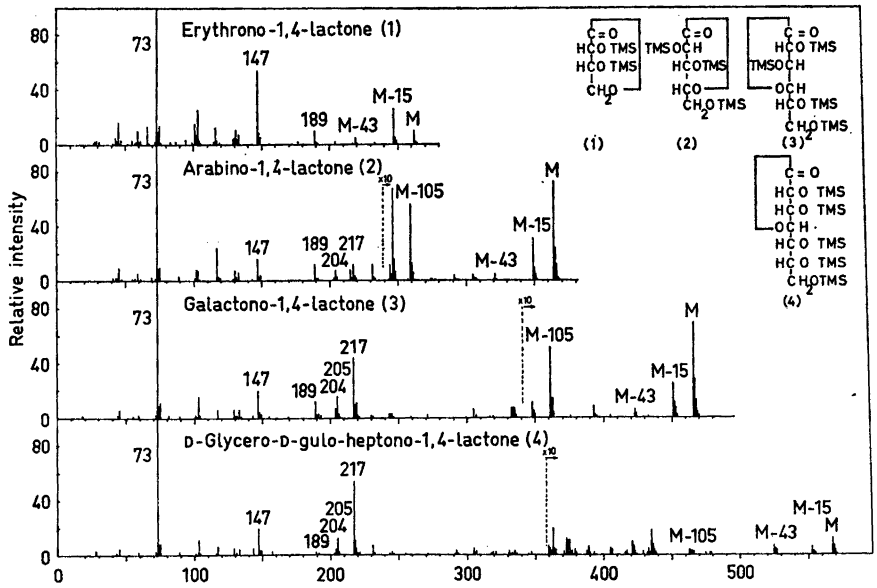

Fig. 1. Mass spectra of TMS-derivatives of four aldono-1,4-lactones with 4, 5, 6, and 7 carbon atoms.

that due to the molecule ion. Similar observations have been made in earlier studies of TMS-derivatives of other compounds.[10,11] The results indicate that the molecule ion and the fragment ion M—15 give a reliable determination of the molecular weight of aldono-lactones. In the upper range of the spectrum peaks corresponding to ions containing heavy isotopes of silicon and carbon were recorded.

In the lower part of the spectrum several ions (m/e = 45, 59, 73, 75, 89, and 103) previously shown to be characteristic of TMS-derivatives of other compounds [10] were recorded with all aldonolactones investigated. An intense peak at mass 147 was recorded with all species. This peak has earlier been recorded with other polyhydroxy compounds.[10,12] For all 1,4-lactones investigated significant peaks were recorded at masses 217, 204, and 189. The upper part of the mass spectrum exhibited several specific peaks. One metastable peak recorded for erythronolactone indicates that M—43 is formed from M—15. The splitting off from various heavy ions of trimethylsilanol explains the existence of several pairs of peaks with mass differences equal to 90. This type of fragmentation has earlier been reported with other TMS-derivatives.[11]

Size of the lactone ring. With lactones from aliphatic monohydroxy acids the most favored cleavage is a rupture of the bond between the side chain and the ring.[13,14] It is thus to be expected that great differences will exist for the TMS-derivatives of aldonolactones of different ring size.

A comparison between the mass spectrum of the TMS-ether of glucono-1,4-lactone and that of the 1,5-lactone is given in Fig. 2. The fragmentation

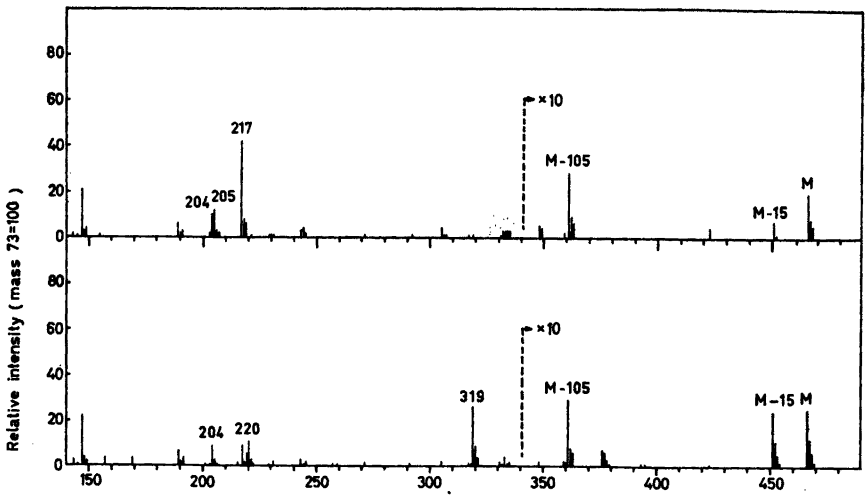

Fig. 2. The higher mass range of mass spectra from TMS-derivatives of glucono-1,4-lactone (upper diagram) and glucono-1,5-lactone (lower diagram).

pattern is more complicated than for the lactones of monohydroxy acids. There are indications that fragment ions from the side chain are formed. A significant peak at mass 205 was recorded with all 1,4-lactones derived from hexonic acids.

A prominent ion was obtained with 1,4-lactones of hexonic acids at $m/e = 217$ whereas glucono-1,5-lactone gave a characteristic peak at $m/e = 319$. The difference (102) corresponds to one carbon atom in the lactone ring. The peak at mass 319 was obtained only for the 1,5-lactone, which also gave rise to a significant peak at $m/e = 220$. A characteristic peak at this mass was recorded also for arabinono-1,5-lactone and xylono-1,5-lactone. This ion was not formed from the corresponding 1,4-lactones.

These observations demonstrate the usefulness of mass spectrometry of TMS-derivatives for differentiating between 1,4-lactones and 1,5-lactones of aldonic acids.

Identification of diastereomeric aldonic acids. The only differences in mass spectra of various diastereomers are differences in intensity recorded for various fragmentation products. With TMS-derivatives of 1,4-lactones from diastereomeric aldonic acids marked differences were recorded for many ions and for a given aldonic acid the ratio of the intensities for a particular pair of ions was characteristic of that acid. A reliable identification of an unknown compound could therefore be obtained by comparing its mass spectrum with those recorded for authentic samples of various diastereomers. The ratios of the intensities of characteristic pairs of ions were tabulated and in order to reduce errors arising from concentration variations in the ion source during the scanning of the spectrum the ratios were calculated for ions with comparatively small differences in mass number. Results obtained with the 1,4-lactones of the four pentonic acids are given in Table 1. To illus-

Table 1. Intensity ratios for selected pairs of *m/e*-values for TMS-derivatives of 1,4-lactones of pentonic acids.

$(m/e)_1$:$(m/e)_2$	Ribonic acid (1.23)	Arabinonic acid (1.00)		Xylonic acid (1.05)	(1.03)	Lyxonic acid (1.58)	Unknown acid (1.07)
349:364	1.08	0.42	0.44	0.74	0.86	0.42	0.86
117:147	0.82	1.53	1.49	1.18	1.13	0.78	1.16
189:204	1.07	1.46	1.40	1.70	1.84	0.79	1.93
215:217	0.70	0.65	0.60	0.44	0.48	0.34	0.47
102:103	0.77	1.06	0.99	0.64	0.62	1.09	0.59
244:246	0.60	0.18	0.17	2.10	2.43	0.07	2.29
291:231	0.26	0.03	0.03	0.04	0.05	0.04	0.05

Values within parentheses refer to the retention times at 140°C relative to that of 2,3,5-tri-*O*-trimethylsilyl-D-arabinono-1,4-lactone.

trate the application of this technique for practical identification purposes the data obtained with an unknown organic acid isolated by ion exchange chromatography from a hydrolyzate of unbleached sulfate pulp [4] were included in the table. A tentative identification by liquid chromatography indicated that the acid was xylonic acid. The chromatographic retention time and the mass number of the molecule ion as well as other characteristics of the mass spectrum confirmed that the acid was a pentonic acid. As seen from the table the relative intensities of various ions gave a final confirmation of the identity. The deviations between the ratios determined with an authentic sample and those determined with the unknown sample were about the same as those obtained in duplicate runs with authentic samples of arabinonic acid and xylonic acid (Table 1).

A comparison between the mass spectra of galactono-1,4-lactone (Fig. 1) and glucono-1,4-lactone (Fig. 2) shows that these compounds are easily distinguished from each other. The same holds true also for the 1,4-lactones of the other hexonic acids studied (mannonic acid, gulonic acid and talonic acid). Likewise, erythrono-1,4-lactone and threono-1,4-lactone as well as the 1,4-lactones of the two heptonic acid studied gave mass spectra with marked differences in intensity of several characteristic ions.

From these results it is evident that mass spectrometry of TMS-derivatives of unknown polyhydroxy acids permits a decision as to whether the acid is an aldonic acid or not. If the acid is an aldonic acid its identity can be conveniently established provided that reference spectra obtained with the same apparatus are available. Likewise, the size of the lactone ring can be established. Since several aldonic acids can be well separated by gas chromatography the combination of gas chromatography with mass spectrometry can be used to simplify the separation procedure as well.

Complete mass spectra of the compounds investigated are available on request from the Department of Engineering Chemistry, Chalmers Tekniska Högskola.

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