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Gas–liquid chromatographic separation of heptono-1,4-lactones as trimethylsilyl derivatives*

The separation of glyconolactones is of interest in studies of the pulping and bleaching of wood products[1], in gas–liquid chromatographic procedures for the analysis of uronic acids[2] and neutral glycoses[3], and for the identification of aldonic acids in natural products[4]. The present investigation was initiated in order to devise a convenient method for the detection and tentative identification of aldoheptoses in bacterial cell walls[5]. The procedure involves the identification of the heptono-1,4-lactone produced by mild oxidation of the aldoheptose released on hydrolysis of the starting material.

Paper chromatographic[6] and ion-exchange chromatographic[7,8] methods for the separation of aldonic acids and their lactone derivatives have been reported, however, the separation of heptonolactones is probably best achieved by gas–liquid chromatography of their trimethylsilyl derivatives[2,3,9]. The characterization of trimethylsilylated glyconolactones, isolated by gas–liquid chromatography, has been made by their mass spectrographic analysis[10].

All sixteen possible heptono-1,4-lactones were prepared by applying the Kiliani–Fischer cyanohydrin synthesis (see the review by HUDSON[11]) to all eight of the possible D-aldohexoses. Each aldohexose gave rise to the expected two epimeric heptonic acids which, after lactonization[3] and trimethylsilylation, were separated by gas–liquid chromatography and the two components were each collected. The IR spectrum of every collected derivative showed an absorption band in the carbonyl region at 1784 cm^{-1}, characteristic of a saturated 1,4-lactone structure. The identity of each component was established by gas–liquid chromatography of its corresponding hepta-O-acetylheptitol derivative, prepared by acetylation of the borohydride reduction product of the detrimethylsilylated heptonolactone, using authentic hepta-O-acetylheptitols as reference standards[12].

Table I records the gas chromatographic retention times of the sixteen 2,3,5,6,7-penta-O-trimethylsilylheptono-1,4-lactone derivatives relative to 2,3,5-tri-O-trimethylsilyl-D-ribono-1,4-lactone. The separation of the derivatives on several types of column packing materials was investigated but none provided a complete separation of all sixteen heptonolactone derivatives and the neopentylglycol sebacate polyester liquid phase column appeared to give the most satisfactory separations. Although some overlap of the peaks due to the heptonolactone derivatives occurs, the epimeric derivatives arising from each parent aldohexose following the cyanohydrin reaction were in each case well separated from each other.

The tentative identification of aldoheptoses in the hydrolyzates of bacterial cell walls and other biological materials can be made using the gas chromatographic procedure described above to analyse the heptonolactone components produced by mild oxidation[3] of the aldoheptose components in the hydrolyzates. All the trimethylsilyl derivatives of the heptono-1,4-lactones have greater retention times than the

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TABLE I

GAS–LIQUID CHROMATOGRAPHIC SEPARATION OF 2,3,5,6,7-PENTA-O-TRIMETHYLSILYLHEPTONO-1,4-LACTONES

Parent aldohexose	*2,3,5,6,7-Penta-O-trimethylsilylheptono-1,4-lactone derived by cyanohydrin synthesis on an aldohexose (TMS derivative)*	*Relative retention time*[a]
D-Allose	D-Glycero-D-*allo*-heptono-1,4-lactone	3.52
	D-Glycero-D-*altro*-heptono-1,4-lactone	2.98
D-Altrose	D-Glycero-D-*gluco*-heptono-1,4-lactone	2.00
	D-Glycero-D-*manno*-heptono-1,4-lactone	3.64
D-Glucose	D-Glycero-D-*gulo*-heptono-1,4-lactone	3.90
	D-Glycero-D-*ido*-heptono-1,4-lactone	3.29
D-Mannose	D-Glycero-D-*galacto*-heptono-1,4-lactone	2.30
	D-Glycero-D-*talo*-heptono-1,4-lactone	2.73
D-Gulose	D-Glycero-L-*galacto*-heptono-1,4-lactone	1.95
	D-Glycero-L-*talo*-heptono-1,4-lactone	2.14
D-Idose	D-Glycero-L-*ido*-heptono-1,4-lactone	3.63
	D-Glycero-L-*gulo*-heptono-1,4-lactone	2.68
D-Galactose	D-Glycero-L-*manno*-heptono-1,4-lactone	3.40
	D-Glycero-L-*gluco*-heptono-1,4-lactone	2.24
D-Talose	D-Glycero-L-*altro*-heptono-1,4-lactone	2.66
	D-Glycero-L-*allo*-heptono-1,4-lactone	2.24

[a] Relative retention time quoted with reference to 2,3,5-tri-O-trimethylsilyl-D-ribono-1,4-lactone (= 1.00). Retention time 3.5 min.

trimethylsilyl derivatives of hexonolactones and pentonolactones so that the aldoheptoses may be analyzed in the presence of aldohexoses and aldopentoses.

Experimental

Synthesis of heptono-1,4-lactones. To a solution of aldohexose (0.5 g) in water (4 ml) cooled to 0° was added a fresh solution of sodium cyanide (0.25 g) in water (3 ml) and the mixture was kept at 5° for 24 h. The reaction mixture was boiled gently for 5 h and water was added at intervals to maintain a total volume of about 10 ml. The cooled solution was passed down a column of Rexyn 101 (H^+) ion-exchange resin (25 ml) and the eluate and washing after concentration to near dryness were treated with a few drops of 2 *N* hydrochloric acid and were reconcentrated to dryness under reduced pressure, below 60°. The residue containing mixed heptono-1,4-lactones was used for gas chromatographic studies.

Gas–liquid chromatography. Separation of the 2,3,5,6,7-penta-O-trimethylsilylheptono-1,4-lactone derivatives was made with a Hewlett-Packard Model 402 gas chromatograph with a hydrogen flame detector, and fitted with glass U tubes (5 ft. × 6 mm × 3 mm I.D.) packed to each end with 10 % (w/w) neopentylglycol sebacate polyester on 80–100 mesh acid-washed Chromosorb W. The column was maintained at 190° and development was made with helium at a flow rate of 90 ml/min. The flash heating system was not used and in preparative runs the derivatives were collected from the exit of the column in drawn-out glass tubes (10 cm × 3 mm I.D.) in which the compounds were readily condensed.

The heptono-1,4-lactones (*ca.* 10 mg) were trimethylsilylated in acetonitrile (0.2 ml) by treatment at 80° for 2 h with bis(trimethylsilyl)trifluoroacetamide con-

taining 1 % trimethylchlorosilane (0.2 ml) (Regisil®, Regis Chemical Co., Chicago, Ill.) and the mixture was injected directly onto the top of the gas chromatographic column packing material.

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