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Note

Separation of aldononitrile acetates of neutral sugars by gas–liquid chromatography and its application to polysaccharides

Gas–liquid chromatography (GLC) methods have been used extensively for the identification and quantitation of neutral sugars in plant polysaccharides and biological materials. The most commonly employed derivatives of the reducing neutral sugars used for GLC are the trimethylsilyl (TMS) derivatives[1–7] and their methyl glycosides[8], and their acetyl- and trifluoroacetates[9,10] and the alditol acetates[11,12]. All but the last of these derivatives give rise to more than one product because of the possible formation of α- and β-anomers and of pyranose and furanose rings. CLAMP *et al.*[6], and SWEELEY *et al.*[2], reported that the TMS derivatives of the free sugars or their methyl glycosides can yield as many as four peaks for each neutral sugar, thereby producing a complex chromatogram which makes the identification and quantitation difficult. Also the TMS derivatives are not very stable[13] and therefore cannot be preserved. On the other hand, the acetates and alditol acetates of the neutral sugars are quite stable. However, in the xylose series the acetylated alditols have a limited use as a result of the symmetry of xylitol as reported by GUNNER *et al.*[14]. Hence 2-O-methyl-D-xylose gives the same derivative as the 4-O-isomer and 2,3-di-O-methyl-D-xylose gives the same derivative as the 3,4-di-O-isomer. LANCE AND JONES[15] in their paper on gas chromatography of derivatives of the methyl ethers of D-xylose mentioned that the acetylated nitriles gave single peaks.

This paper reports the GLC separation of the acetylated aldononitriles, which are stable derivatives of reducing sugars and give single, fairly well separated peaks. The trimethylsilylated aldononitriles[16], which also give single peaks, were not used since they were unstable and thus could not be preserved for future use. The use of the acetylated aldononitriles, when applied to the characterization and quantitation of the neutral sugars from gum arabic, which is an acid polysaccharide, and guar gum (Jaguar), a neutral polysaccharide, gave results comparable with those obtained by the use of the TMS derivatives and also with those of the reported values[17,18].

Experimental

Materials. Pyridine, acetic anhydride, hydroxylamine hydrochloride and gum arabic were obtained from Applied Science Laboratories, Inc., State College, Pa. Tri-sil was obtained from Pierce Chemical Co., Rockford, Ill. All sugars were obtained from Sigma Chemical Co., St. Louis, Mo., and guar gum (Jaguar) was obtained from Stein, Hall and Co., Inc., New York, N. Y.

Drying procedures. 100 ml of ACS reagent grade chloroform and hexane were stored separately over 10 g of anhydrous sodium sulfate for 24 h and distilled. All

sugars and gums were dried in a vacuum desiccator at 30° to constant weight.

Gas chromatography. GLC analyses of aldononitrile acetates were carried out with a Series 200 Varian Aerograph, a Model SRG Sargent-Welch recorder, and a disc integrator (Disc Instruments, Model 204). A stainless-steel column (5 ft. × 1/8 in.) packed with 10% (w/w) LAC-4R-886 polyester wax (purged for two days at 200°) on 100–200 mesh acid-washed Chromosorb W was used and the instrument was operated isothermally at 190°. Nitrogen was used as the carrier gas at a flow-rate of 75 ml/min. The injector and detector temperatures were maintained at 230°.

Preparation of aldononitrile acetates of standard sugars. A dried mixture of twelve monosaccharides containing 1 mg of each sugar was dissolved in pyridine (0.6 ml) and treated with 12 mg of dried hydroxylamine hydrochloride and heated in a sealed glass ampoule at 90° for 30 min. After cooling the top part of the ampoule was opened and acetic anhydride (1.8 ml) was added and heating continued for another 30 min in the resealed ampoule. The cooled solution was evaporated to dryness under diminished pressure at 40°. The residue was dissolved in 0.1 ml of dry chloroform and a 1-μl sample was injected into the chromatograph with a 10-μl Hamilton syringe.

Hydrolysis of gums. 2 mg of dried gum were hydrolysed with 1 ml of 0.15 N H_2SO_4 in a sealed ampoule at 95° for 36 h. After cooling the ampoule was opened and the solution transferred to a 50-ml beaker and the pH adjusted to between 4.5 and 5 with $BaCO_3$. The precipitate was removed by filtration through a sintered glass funnel. The filtrate and the washings were evaporated to dryness under diminished pressure at 40°. The residue was dissolved in 1 ml of distilled water and divided into two equal portions (0.5 ml) which were freeze-dried in two 5-ml glass ampoules. Each portion contained 1 mg of the hydrolysed gum. The sugars in one portion were derivatised to aldononitrile acetates and the other to the TMS ethers as described below.

Preparation of aldononitrile acetates of sugars in the gum hydrolysates. The neutral sugars in 1 mg of hydrolysed gum were derivatised to the aldononitrile acetates by the procedure used for standard sugars. The quantities of various reagents used were: pyridine (5 drops), hydroxylamine hydrochloride (1 mg), and acetic anhydride (15 drops). The residue at the end of the reaction was dissolved in 0.05 ml of dry chloroform and a 4-μl sample was used for GLC analysis.

Preparation of TMS ethers of sugars in the gum hydrolysates. The sugars in 1 mg of hydrolysed gum and the standard sugars were separately derivatised to the TMS ethers and chromatographed by the procedure of VARMA *et al.*[13], except that the temperature programming used was 2°/min instead of 10°/min. The residue at the end of the reaction was extracted with 0.05 ml of dry hexane and a 4-μl sample was used for injection.

Results and discussion

Fig. 1 illustrates the GLC separation of the aldononitrile acetates of a mixture containing twelve standard sugars. The retention times of the aldononitrile acetates are shown in Table I. Figs. 2 and 3 represent typical chromatograms for the GLC separation of the aldononitrile acetates of neutral sugars from gum arabic and guar gum, respectively. Figs. 4 and 5 show typical chromatograms for the GLC separation of the TMS ethers of the sugars from gum arabic and guar gum, respectively. The

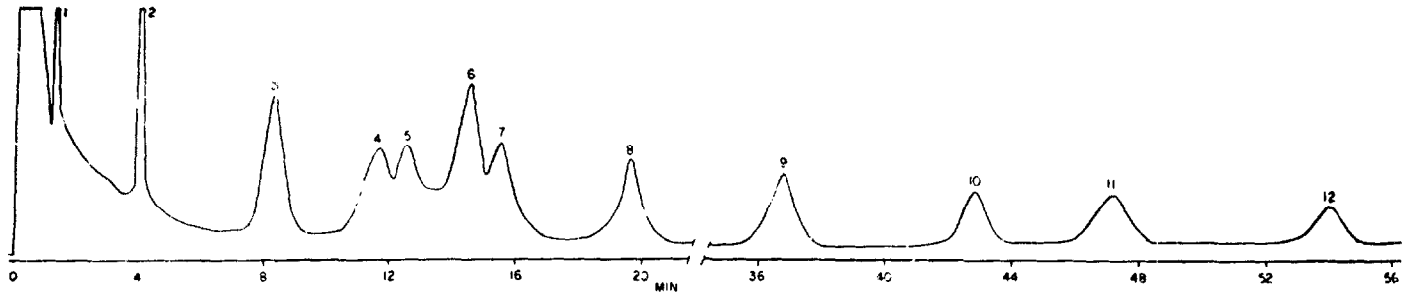

Fig. 1. GLC separation of the aldononitrile acetates of the mixture of standards. 1 = Glyceraldehyde; 2 = erythrose; 3 = rhamnose; 4 = fucose; 5 = ribose; 6 = lyxose; 7 = arabinose; 8 = xylose; 9 = mannose; 10 = 2-deoxygalactose; 11 = glucose; 12 = galactose.

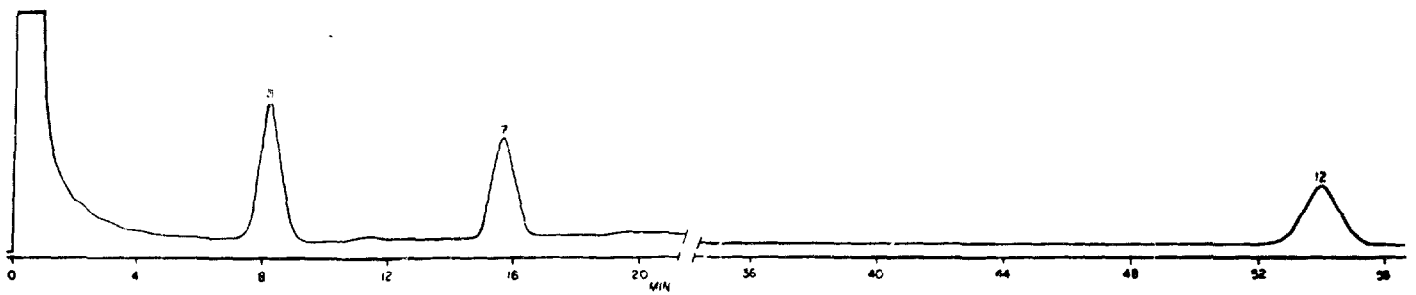

Fig. 2. GLC analysis of the aldononitrile acetates of the neutral sugars from gum arabic. 3 = Rhamnose; 7 = arabinose; 12 = galactose.

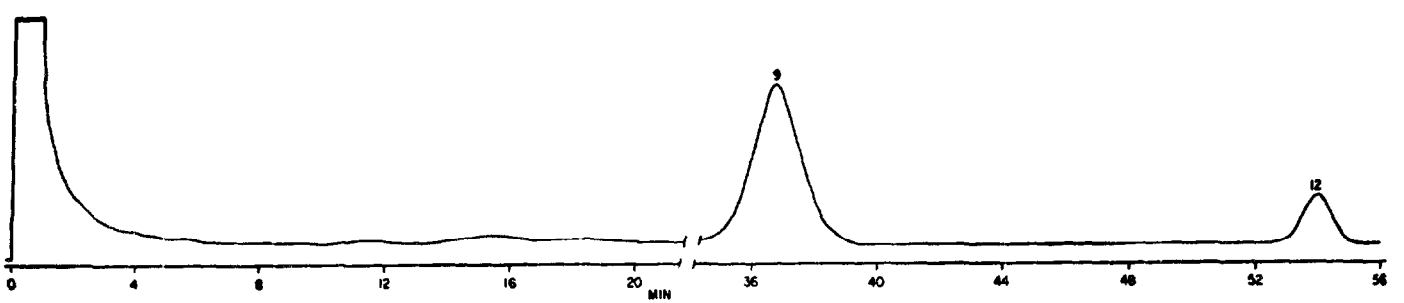

Fig. 3. GLC analysis of the aldononitrile acetates of the sugars from guar gum. 9 = Mannose; 12 = galactose.

TABLE I

RETENTION TIMES OF ALDONONITRILE ACETATES OF NEUTRAL SUGARS

Sugar	*Retention time (min)*
Glyceraldehyde	1.2
Erythrose	3.9
Rhamnose	8.3
Fucose	11.7
Ribose	12.6
Lyxose	14.6
Arabinose	15.6
Xylose	19.6
Mannose	30.8
2-Deoxygalactose	42.8
Glucose	47.2
Galactose	54.0

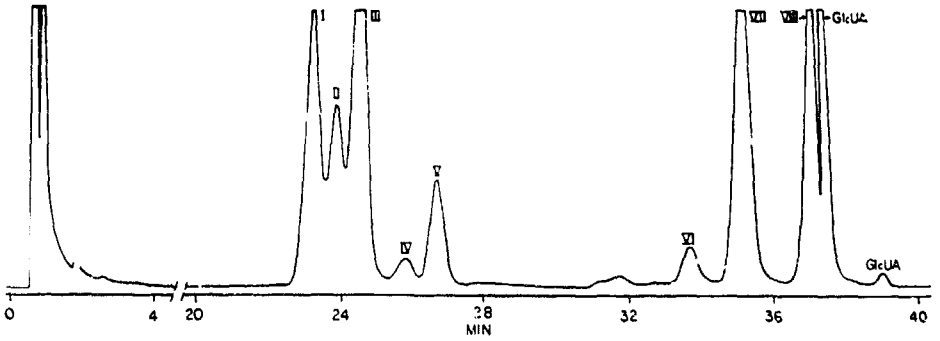

Fig. 4. GLC analysis of TMS ethers of sugars from gum arabic. I, III, IV = Arabinose; II, V = rhamnose; VI, VII, VIII = galactose.

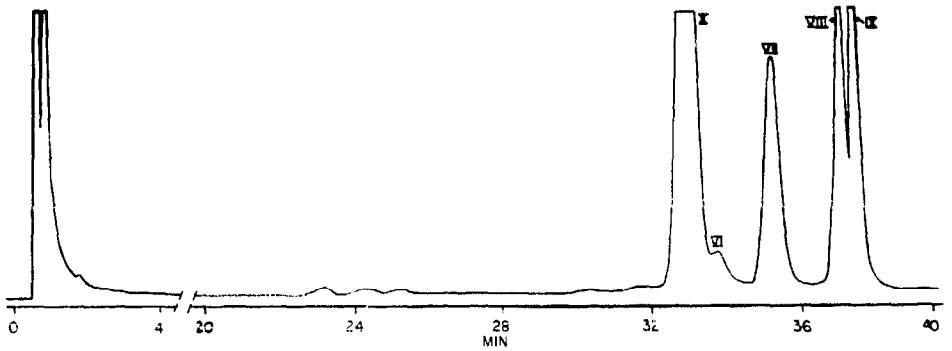

Fig. 5. GLC analysis of TMS ethers of sugars from guar gum. IX, X = Mannose; VI, VII, VIII = galactose.

TMS ether peaks for various sugars were identified and quantitation was done by comparison with authentic sugars. The retention times of the TMS ethers of arabinose, rhamnose, mannose and galactose are shown in Table II. The reported

percentage of rhamnose, arabinose and galactose in gum arabic[17] and of mannose and galactose in guar gum[18] and those determined (peak area method) by the use of aldononitrile acetates and the TMS ethers are shown in Table III. The results obtained by the two methods are in good agreement with each other.

TABLE II

RETENTION TIMES OF TMS DERIVATIVES OF NEUTRAL SUGARS

Sugar	*Retention time (min)*		
	Peak 1	*Peak 2*	*Peak 3*
Arabinose	23.2	24.5	25.8
Rhamnose	23.8	26.7	—
Mannose	33.0	37.5	—
Galactose	33.7	35.2	37.1

TABLE III

PERCENTAGE OF NEUTRAL SUGARS IN GUM ARABIC AND GUAR GUM OBTAINED BY THE ALDONONITRILE ACETATE AND TMS ETHER METHODS AND THE REPORTED VALUES

Sugar (%)	*Gum arabic*			*Guar gum*		
	Reported values[a,b]	*Aldononitrile acetate method*	*TMS ether method*	*Reported values*[18]	*Aldononitrile acetate method*	*TMS ether method*
Rhamnose	13.52	12.20	12.29	—	—	—
Arabinose	18.55	16.71	16.83	—	—	—
Mannose	—	—	—	63.00	61.82	62.10
Galactose	51.94	47.05	46.99	35.00	34.31	34.58

[a] Percentage was calculated from the molecular structure of gum arabic as given in ref. 17.
[b] It should be kept in mind that different samples of the same gum may vary slightly in their sugar content.

The aldononitrile acetates of neutral sugars were prepared from the sugar oximes[2], which, without isolation, were simultaneously acetylated and dehydrated to the nitrile by heating them in pyridine and acetic anhydride. These derivatives were found to be very stable for several months when stored in a vacuum desiccator at room temperature (25°). Our experience with four-months-old aldononitrile acetates showed that they had not undergone any decomposition. Also, determination could be done on as little as 2–5 μg of the neutral reducing sugar.

For the hydrolysis of gums our studies (varying time, temperature and acidity) showed that the best compromise conditions for all constituent sugars for maximal release and minimal destruction were heating at 95° with 0.15 N H_2SO_4 for 36 h.

The retention times of 2-deoxyribose and ribose were very close to each other and thus these were not separated. Also, 2-deoxyglucose and xylose had the same retention times. However, the two sugars of each pair do not occur together as components of a single biological material. The aldononitrile acetate of glucuronic

acid, one of the components of gum arabic, could not be detected in the gas chromatogram. For some unknown reason the aldononitrile acetate of standard glucuronic acid could not be prepared under the conditions used. Glucosamine also did not form its aldononitrile acetate. However, its preparation is described by RESTELLI *et al.*[19], under different experimental conditions. Currently, the conditions for the preparation of aldononitrile acetates of glucuronic acid and glucosamine are being worked out and the application of this method to the determination of sugars from glycoproteins and acid mucopolysaccharides is in progress.

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