Carbohydrate Research, 38 (1974) 293–299
© Elsevier Scientific Publishing Company, Amsterdam – Printed in Belgium

Note

Quantitative determination of aldonic and uronic acids in mixtures as deuterium-labelled alditol acetates by gas chromatography–mass spectrometry

EERO SJÖSTRÖM, KLAUS PFISTER, AND EINO SEPPÄLÄ
Laboratory of Wood Chemistry, Helsinki University of Technology, SF-02150 Otaniemi (Finland)
(Received April 3rd, 1974; accepted for publication, May 10th, 1974)

During our studies on the composition of sulphite spent-liquors, it became necessary to determine aldonic and uronic acids in mixtures[1]. Reduction with sodium borohydride and subsequent g.l.c. of the alditol acetates has been used for the determination of aldonic acids[2], but this technique is not applicable to mixtures also containing uronic acids. This paper describes a method in which the reduction is effected with sodium borodeuteride. The deuterium-labelled alditol acetates formed from D-gluconic and D-glucuronic acid lactones were analysed by g.l.c.–m.s.

A number of different fragments are formed from alditol acetates[3] in electron-impact mass spectrometry but, for the purposes of illustration, only the symmetrical fragmentation will be discussed here. When the bond scission of deuterated D-glucitol

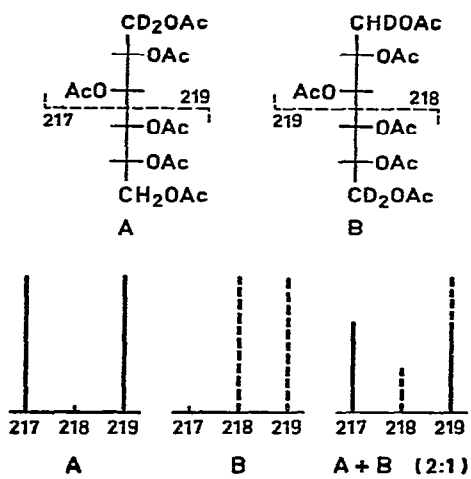

Fig. 1. Scheme illustrating the principle of the method. Compound A (originating from D-gluconic acid) gives the peaks at *m/e* 217 and 219. Compound B (originating from D-glucuronic acid) gives the peaks at *m/e* 218 and 219.

hexa-acetates occurs between C-3 and C-4, fragments are obtained corresponding to *m/e* 217 (undeuterated) and 218 (1D), which are characteristic for D-gluconic and D-glucuronic acids, respectively (Fig. 1). In addition, a common fragment at *m/e* 219 (2D) is formed. Assuming that deuterium has no significant influence on the fragmentation, the total number of fragments obtained is directly proportional to the amount of sample injected and is independent of the ratio between gluconic and glucuronic acid. The composition of the mixture can thus be determined simply from the ratios between the fragment intensities. In practice, however, this ideal case is not valid; owing to the lack of steric symmetry, the numbers of fragments formed from the ends of a molecule are not exactly the same. Further, the deuteration does not proceed with 100% yield, and traces of foreign fragments with equal weight can also be formed. However, these factors can be taken into account after determining the spectra of the reference substances, *i.e.*, the deuterated alditol acetates obtained from D-gluconic and D-glucuronic acids.

The calculations were based on mass-spectral data contained in the following peak groups: (*1*) *m/e* 187–188–189, (*2*) *m/e* 217–218–219, and (*3*) *m/e* 259–260–261, in which the intensities were comparatively strong; fragments *m/e* 187 and 259 are formed by loss[3] of AcOH plus ketene from the primary fragments of *m/e* 289 (cleavage of the C-2–C-3 or C-4–C-5 bonds) and 361 (cleavage of the C-1–C-2 or C-5–C-6 bonds). The intensity data in each peak group were corrected for the background as well as for the surplus intensity caused by the ^{13}C isotope, so that the additional (fourth) peak in each group (*e.g.*, *m/e* 220) was neglected. To compensate for variations in concentration, the peak intensity values were multiplied by a factor so that the sum of the peak intensities in each group reached the same, constant value (100 mm). These corrected intensity values are designated as A_i (gluconic acid), B_i (glucuronic acid), and M_i (mixture), where the index *i* refers to the mass number.

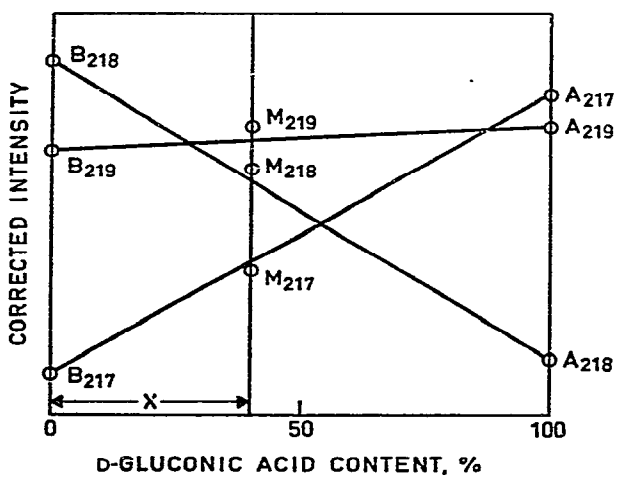

Fig. 2. Principle for the calculation of the D-gluconic acid content.

Fig. 2 illustrates the principle. The intensity values for the "pure" substances (A_i B_i) are set on the two vertical axes. The three lines obtained in each peak group correspond to the peak intensity variations as a function of the composition of the mixture.

The mass spectra of the deuterated alditols could not be reproduced entirely satisfactorily. Comparatively large and irregular variations were obtained after repeated scans, even within the same peak group. The determination of the gluconic acid content of a mixture was based on a number of intensity measurements. The deviations of the values from each respective calibration line [$\Delta M_i = (A_i - B_i)X - B_i - M_i$] were calculated and the sum of their squares was minimized (*cf.* Fig. 2). This gave the following expression for the gluconic acid content (X):

$$X = \frac{\Sigma (A_i - B_i)(M_i - B_i)}{\Sigma (A_i - B_i)^2} \cdot 100\%$$

Because the measurement of the A_i and B_i values also inlcudes an error, separate calculations were made according to which the best line was first determined by the method of least squares, *i.e.*, by minimizing the value of $\Delta A_i^2 + \Delta M_i^2 + \Delta B_i^2$. The final values (X) were then obtained as a result of minimizing the value of $\Sigma \Delta A_i^2 + \Sigma \Delta M_i^2 + \Sigma \Delta B_i^2$. This is much more complicated than calculating with the expression above, and no significant differences were obtained with our data.

Two series of experiments were carried out, separated by several months. In Series I, the D-gluconic (A) and D-glucuronic (B) acids were first reduced and acetylated and then mixed, whereas in Series II, mixtures containing known proportions of A and B were converted directly into alditol acetates. In Series I, repeated scans were taken from one injection, and in both series the injection of the sample was repeated several times. The results are presented in Table I.

For any single determination, the calculated value can deviate substantially from the known value, but the accuracy is improved when the scans are repeated several times (Series I) and the data in each peak group are taken into account. The values are further improved and become accurate to within 1–2% when, in addition to repeated scans, the injections are also repeated. No significant differences were found between the values from Series I and Series II. Consequently, any difference in yield between the reduction of D-glucono- and D-glucurono-lactones is negligible, in accordance with observations made separately[1].

Although the method was tested on D-gluconic and D-glucuronic acids only, it would seem obvious that the principle is generally applicable to the aldonic and uronic acids that can be reduced to alditols and separated as acetates by gas chromatography.

EXPERIMENTAL

All the chemicals used were analytical grade reagents or of purest available quality. The sugar acids were obtained as lactones (D-glucono-1,5-lactone and D-glucurono-6,3-lactone from Fluka AG, Switzerland), and the sodium borodeuteride

TABLE I

ANALYSIS OF D-GLUCONIC AND D-GLUCURONIC ACID MIXTURES AS DEUTERIUM-LABELLED ALDITOL ACETATES. THE D-GLUCONIC ACID CONTENTS WERE CALCULATED FROM THE PEAK INTENSITY VALUES IN THREE PEAK GROUPS (*m/e* 187-, 217-, AND 259-). DIRECTLY MEASURED, INTENSITY VALUES (mm) ARE REPORTED ONLY FOR ONE PEAK GROUP (*m/e* 217-).

Sample[a]	D-*Gluconic acid content* (%)	*Number of scans*	*Measured intensity values*[b] *for the peak group* m/e *217-*			D-*Gluconic acid content calculated from intensity values in the following peak groups* (m/e):			
			217	*218*	*219*	*187-*	*217-*	*259-*	*Av. value*[c]
I-A-1[1]	100	4	60.1	15.2	59.6	99.8	99.9	101.9	100.5
I-A-1[2]	100	4	63.9	15.8	66.3	100.1	99.5	98.7	99.4
I-A-1[3]	100	6	43.0	10.2	43.0	100.1	100.9	99.3	100.1
			Σ 167.0*	41.2*	168.9*	100*	100*	100*	100*
I-A-2[1]	100	3	34.8	7.0	32.2	100.8	105.3	99.4	101.8
I-A-2[2]	100	8	46.6	9.9	46.6	100.7	102.9	101.1	101.6
			Σ 81.4	16.9	78.8	100.7	103.9	100.0	101.5
I-B-3[1]	0	5	5.8	62.8	55.5	1.6	−0.2	0.6	0.7
I-B-3[2]	0	7	4.1	43.6	38.8	−0.9	0.2	−0.3	−0.3
			Σ 9.9*	106.4*	94.3*	0*	0*	0*	0*
I-B-4[1]	0	4	5.1	63.1	59.2	−0.2	0.6	1.5	0.6
I-B-4[2]	0	5	3.6	30.3	28.5	−1.3	4.0	1.9	1.5
			Σ 8.7	93.4	87.7	−0.9	1.7	1.7	0.9
I-AB-5[1]	46.0	6	22.8	32.1	44.3	44.8	46.0	47.5	46.1
I-AB-5[2]	46.0	7	16.3	22.2	31.4	44.8	47.2	49.0	47.0
I-AB-5[3]	46.0	6	25.2	33.6	46.2	45.7	47.6	48.9	47.4
I-AB-5[4]	46.0	4	37.6	53.0	71.8	45.6	45.8	46.0	45.8
			Σ 101.9	140.9	193.7	45.2	46.5	47.9	46.5

TABLE I (*Continued*)

Sample[a]	D-*Gluconic acid content* (%)	*Number of scans*	*Measured intensity values*[b] *for the peak group* m/e *217-*			D-*Gluconic acid content calculated from intensity values in the following peak groups* (m/e):			
			217	*218*	*219*	*187-*	*217-*	*259-*	*Av. value*[c]
I-AB-6[1]	90.0	7	43.7	15.2	46.3	88.6	92.0	89.0	89.9
I-AB-6[2]	90.0	6	79.9	28.1	84.2	88.8	91.8	91.2	90.6
I-AB-6[3]	90.0	7	33.7	11.5	34.7	88.4	92.9	90.2	90.5
I-AB-6[4]	90.0	6	80.9	27.7	84.8	89.8	92.6	91.2	91.2
			Σ 238.2	82.5	250.0	88.9	92.3	90.5	90.6
II-A-1[1]	100	2	47.3	13.0	43.0	98.8	99.8	101.5	100.1
II-A-1[2]	100	1	62.5	19.5	54.0	96.5	98.0	102.0	98.9
II-A-1[3]	100	1	52.0	16.0	58.0	98.6	94.1	99.5	97.4
II-A-1[4]	100	1	63.0	16.0	54.0	99.8	102.7	96.1	99.5
			Σ 224.8	64.5	209.0	98.4	98.5	99.7	98.9
II-A-2[1]	100	1	44.0	11.5	42.5	93.8	99.7	90.0	94.5
II-A-2[2]	100	2	55.5	16.0	53.8	96.5	97.6	101.4	98.5
II-A-2[3]	100	1	43.0	13.0	35.5	92.9	99.7	94.3	95.6
II-A-2[4]	100	1	29.5	6.5	24.0	96.2	106.6	105.4	102.7
			Σ 172.0	47.0	155.8	95.2	100.0	97.3	97.5
II-B-3[1]	0	1	7.5	70.5	62.0	−1.3	0.9	−1.0	−0.5
II-B-3[2]	0	1	5.5	43.5	36.0	−1.3	0.9	−0.2	−0.2
II-B-3[3]	0	1	5.0	40.0	34.0	−0.2	1.6	−3.4	−0.7
			Σ 18.0	154.0	132.0	−1.0	1.1	−1.3	−0.4
II-B-4[1]	0	1	4.5	29.5	25.0	1.1	3.8	9.0	4.6
II-B-4[2]	0	1	5.5	52.0	40.0	−0.8	−3.3	−2.0	−2.1
II-B-4[3]	0	1	2.5	16.5	17.0	0.7	9.0	6.0	5.2
			Σ 12.5	98.0	82.0	0.7	1.3	3.2	1.7

TABLE I (*Continued*)

Sample[a]	D-*Gluconic acid content* (%)	*Number of scans*	*Measured intensity values*[b] *for the peak group* m/e *217-*			D-*Gluconic acid content calculated from intensity values in the following peak groups* (m/e):			
			217	*218*	*219*	*187-*	*217-*	*259-*	*Av. value*[c]
II-AB-5[1]	59.3	1	9.0	14.5	20.5	62.8	42.2	62.8	55.9
II-AB-5[2]	59.3	1	22.0	24.5	31.0	54.9	53.2	58.2	55.5
II-AB-5[3]	59.3	1	42.0	44.0	61.0	53.0	55.8	65.6	58.1
			Σ 73.0	83.0	112.5	56.4	52.8	62.9	57.4
II-AB-6[1]	59.3	1	39.5	41.0	68.5	60.2	56.7	62.3	59.7
II-AB-6[2]	59.3	1	36.0	40.0	58.0	58.6	53.9	61.1	57.9
II-AB-6[3]	59.3	1	44.0	42.5	68.0	60.1	59.0	62.9	60.7
			Σ 119.5	123.5	194.5	59.7	56.6	62.1	59.5
II-AB-7[1]	39.4	1	8.0	17.0	19.0	37.4	31.7	39.4	36.2
II-AB-7[2]	39.4	1	25.0	43.5	50.0	36.6	37.7	32.5	35.6
II-AB-7[3]	39.4	1	22.0	32.0	37.5	35.3	43.5	37.9	38.9
			Σ 55.0	92.5	106.5	36.2	38.7	35.9	36.9
II-AB-8[1]	39.4	1	10.5	18.0	22.5	44.7	39.2	38.9	40.9
II-AB-8[2]	39.4	1	22.0	39.0	45.5	39.8	37.4	41.7	39.6
II-AB-8[3]	39.4	1	16.0	23.5	34.0	36.3	45.1	40.5	40.6
			Σ 48.5	80.5	102.0	39.6	40.2	40.7	40.2

[a]Samples marked with I and II were prepared from the same starting materials, but at different occasions. The samples in Series I were prepared (reduced and acetylated) separately from D-gluconic and D-glucuronic acids, and the alditol acetates thus obtained were then mixed. The samples in Series II were prepared after a direct reduction and acetylation of the acid (lactone) mixture. The injection was repeated for the same sample several times as denoted by an index after the sample number. [b]The data given are results from the same run (one injection) after one or several scans (average values). The sum of the spectra represent several injections of the sample. The reference values are marked with an asterisk. [c]These are average values for the three peak groups.

(Fluka AG) had a guaranteed degree of deuteration of at least 98%. The deuterium-labelled D-glucitol hexa-acetates used in Series I were prepared by repeated treatment[2] with sodium borodeuteride. The acetylation was performed in acetic anhydride–pyridine solution at room temperature overnight. The alditol acetates obtained were purified by recrystallisation from ethanol–light petroleum. In Series II, the D-gluconо- and D-glucurono-lactones were reduced and acetylated exactly as described earlier[2].

Mass spectra were recorded on a Perkin–Elmer 270 B mass spectrometer–gas chromatograph at 70 eV. The temperature of the ionization chamber was 180°. The samples were eluted into the mass spectrometer from the gas chromatograph fitted with stainless-steel columns (2 m × 1/8 in., 3% ECNSS-M on Chromosorb W-H.P.) and using helium with a flow rate of ~2 ml/min as carrier gas. The column temperature was maintained at 180°.

Peak heights were measured, with an accuracy of ~0.5 mm, manually from the chart.

REFERENCES

1 K. FORSS, K. PASSINEN, AND E. SJÖSTRÖM, *Abstr. Pap. Amer. Chem. Soc. Meeting*, 167 (1974); K. PFISTER AND E. SJÖSTRÖM, unpublished data.
2 E. SJÖSTRÖM, P. HAGLUND, AND J. JANSON, *Acta Chem. Scand.*, 20 (1966) 1718.
3 N. K. KOCHETKOV AND O. S. CHIZHOV, *Advan. Carbohyd. Chem.*, 21 (1966) 39; O. S. CHIZHOV, L. S. GOLOVKINA, AND N. S. WULFSON, *Izv. Akad. Nauk SSSR, Ser. Khim.*, (1966) 1915.