Note

Mass spectrometry of methylated pseudoaldobiouronic acids

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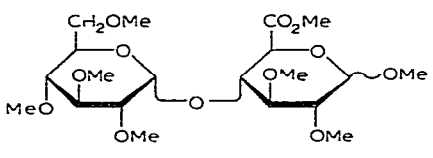
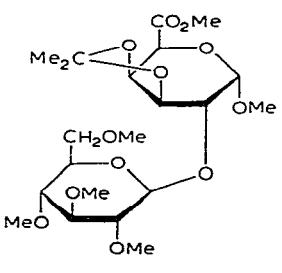
Recently, the isolation and partial elucidation of the structure of a novel pseudoaldobiouronic acid from human bile were reported[1] This compound has been tentatively identified as 4-*O*-α-D-glucofuranosyl-D-glucuronic acid Linked glycosidically to the carboxyl groups of bilirubin, it constitutes one of the major, naturally occurring conjugates of this bile pigment The finding of this biologically important pseudoaldobiouronide prompted an investigation into the possibility of identifying pseudoaldobiouronic acids by mass spectrometry In particular, the question arose as to whether these compounds fragmented by pathways similar to those observed with neutral disaccharides[2,3], aldobiouronic acids[1,4], and aldotriouronic acids[5], thus permitting a detailed analysis of their structures with respect to type of glycosidic linkage and size of sugar ring

Because of the scarcity[1] of suitable reference compounds, only two substances were analyzed by mass spectrometry These were methyl [methyl 2,3-di-*O*-methyl-4-*O*-(2,3,4,6-tetra-*O*-methyl-α-D-glucopyranosyl)-D-glucopyranosid]uronate (**1**) and methyl [methyl 3,4-*O*-isopropylidene-2-*O*-(2,3,4,6-tetra-*O*-methyl-β-D-glucopyranosyl)-α-D-galactopyranosid]uronate (**2**)



1



2

The fragments observed for **1** and **2** are listed in Table I They are identified by the symbols introduced by Kováčik *et al* [4]. The mass spectra reveal the occurrence of essentially all the fragments known to be produced under similar conditions from methylated derivatives of neutral disaccharides[2,3] and aldobiouronic acids[1,4] Thus,

they support the hypothesis that all types of disaccharides fragment along similar pathways

Pseudoaldobiouronic acids are easy to distinguish from neutral disaccharides

TABLE I

MASS SPECTRA OF METHYLATED PSEUDOALDOBIOURONIC ACIDS

Compound **1** (*mol wt*, *468*)		*Compound* **2** (*mol. wt*, *480*)		*Assignment*
m/e	*Relative intensity* (%)	m/e	*Relative intensity* (%)	
		465	1 5	*baO*
437	0 1	449	0 6	baA_1
436	0 01			$M^+ - CH_3OH$
		406	0 4	abB_1
367	0 05	379	1 4	abD_1
319	0 1			abF_1
306	1 2			
305	0 9			baF_1
293	27 6	305	31 2	abJ_1
		275	2 7	
247	0 4			
233	14 0	245	4 3	bA_1
		231	2 4	
219	1 7	219	2 4	aA_1
201	15 0			bA_2
187	14 9	187	46 1	aA_2
173	2 8			bC_2
		173	4 4	
169	5 1			bA_3
161	1 0			aB_3, bB_3
155	3 2	155	4 0	aA_3
145	4 8	145	6 6	
141	3 0			
131	2 1			
129	4 4			
127	4 0	127	12 5	aC_3
111	8 1	111	12 5	aA_4
101	34 9	101	78 5	F_1, G_1
89	9 3			
88	*100 0*	88	*100 0*	H_1
85	5 6			
75	25 6			bJ_1
		75	29 0	aJ_1
73	7 4			H_2
71	11 3	71	31 4	D_3 following the nomenclature of Heyns *et al* [2]
59	4 4			CH_3 CH_3 $C=\overset{+}{O}H$
		59	23 2	
45	19 1	45	86 5	$H_2C=\overset{+}{O}CH_3$
		43	96 5	$CH_3C\equiv\overset{+}{O}$

by virtue of a number of fragments that contain the group $-COOCH_3$ as opposed to $-CH_2OCH_3$ Thus, comparison of corresponding, fully methylated derivatives, *e g*, compound **1** with octa-*O*-methylmaltose, would reveal differences of 14 mass units between corresponding fragments of type bA_1, $M^+ - CH_3OH$, abD_1, baF_1, abJ_1, bA_1, bA_2, bA_3, bC_2, and bC_3

Differentiation between pseudoaldobiouronic acids and aldobiouronic acids is less-readily accomplished, and the identification of a fully methylated pseudoaldobiouronic acid rests almost exclusively on the occurrence of a prominent ion abJ_1 of mass 293 In contrast, the corresponding fragment derived from an aldobiouronic acid would appear at *m/e* 279, and the mass position 293 would be either free or occupied by a minor ion species only[1]

The presence of a signal at *m/e* 305 (baF_1) might appear to provide another means of identifying a pseudoaldobiouronic acid, as opposed to an aldobiouronic acid However, this criterion seems less reliable for the following reasons (*1*) Although the occurrence of an ion abF_1 of mass 305 has never been reported for aldobiouronic acids[1 4], the presence of just such a fragment (occurring at *m/e* 319) in the spectrum of compound **1** nevertheless opens the possibility that it might also be produced from certain aldobiouronic acids (*2*) The relatively low abundance of the fragment abF_1 in the spectrum of fully methylated pseudoaldobiouronic acids (*e g* **1**) provides for the eventuality that this ion might remain undetected in cases where only very small amounts of the sample are available for analysis The fact that the fragment baF_1 is not observed in the spectrum of compound **2** is not at variance with the idea that it is an obligatory signal with fully methylated pseudoaldobiouronic acids, the absence of this particular ion is easily explained by the presence of the isopropylidene group, which prevents compound **2** from being fragmented along pathway *F*

The fragment abD_1 (*m/e* 367 with compound **1** and at *m/e* 379 with **2**) needs special mention because of the ease with which it could be mistaken for the key fragment baD_1 characteristic of (1→6)-linked aldobiouronic acids (*m/e* 367 with fully methylated derivatives)[1 4] However, it should be relatively easy to distinguish between the two alternatives since, with (1→6)-linked aldobiouronic acids, baD_1 is quite prominent, whereas its counterpart derived from pseudoaldobiouronic acids tends to be a minor species

It has emerged from several studies that the only fragment which allows unambiguous differentiation between a (1→2)- and a (1→4)-linkage in neutral disaccharides[3] and aldobiouronic acids[4] is the fragment aB_3 or bB_3 occurring at *m/e* 161 The presence of this ion is indicative of a (1→4)-linkage, whereas its absence is taken as evidence for a (1→2)-linked compound It is a particularly rewarding result of the present study to find that this rule is equally applicable to the analysis of pseudoaldobiouronic acids This is deduced from the observation of a fragment of mass 161 in the spectrum of compound **1**, which is absent from that of **2** (Table I)

All of the above results are consistent with the contention that mass-spectrometric identification and structure elucidation of methylated pseudoaldobiouronic acids is feasible

EXPERIMENTAL

Preparation of compound **1** — 4-*O*-α-D-Glucopyranosyl-D-glucuronic acid hepta-acetate[6] was a gift from Dr Y Hirasaka, Chugai Pharmaceutical Co Ltd, Tokyo, Japan, and was converted into its fully methylated derivative essentially as described[1] for the preparation of hepta-*O*-methylaldobiouronic acid methyl esters The crude product was purified by preparative thin-layer chromatography on silica gel PF_{254} (Merck) with benzene–ethanol (5 1) The major fraction (R_F 0 65), eluted with chloroform, gave 2 3 mg of compound **1**

Preparation of compound **2.** — The acetyl groups in methyl [methyl 3,4-*O*-isopropylidene-2-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-α-D-galactopyranosid]-uronate[7] (a gift from Dr Š Bauer, Institute of Chemistry, Slovak Academy of Sciences, Bratislava, Czechoslovakia) were exchanged for methyl by direct methylation with methyl sulphate and barium oxide[1] P l c, as described above, yielded 3 0 mg of compound **2**

Mass spectrometry — Spectra were recorded with an LKB gas chromatograph–mass spectrometer type 9000 at an ionising potential of 70 eV Samples were dissolved in dichloromethane and injected into a column (2 5 m × 4 mm) of 1% SE-30 on Chromosorb W packed in a glass tube Further conditions were carrier gas, He, 30 ml/min, injector-block temperature, 220°, column temperature, 155°, separator temperature, 250°, ion-source temperature, 270° Compounds **1** and **2** each gave a single peak on g l c, and each mass spectrum was taken at the maximum peak height

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