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DEUTERIUM INCORPORATION IN SACCHARINIC ACID FORMATION

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ABSTRACT

Deuterated 3-deoxy-2-*C*-hydroxymethyl-D-*erythro*-pentonic and 3-deoxy-2-*C*-hydroxymethyl-D-*threo*-pentonic acids, 3-deoxy-D-*ribo*-hexonic and 3-deoxy-D-*arabino*-hexonic acids, and 2-*C*-methyl-D-ribonic acid have been isolated from the degradations in aqueous barium deuteroxide of maltose, turanose, and inulin, respectively. The position and extent of deuterium incorporation in these products have been examined by using mass-spectral and n.m.r. methods.

INTRODUCTION

The anaerobic degradation of 4-*O*-substituted hexoses yields[1–3] mainly 3-deoxy-2-*C*-hydroxymethyl-D-*erythro*-pentonic acid (**1**) and 3-deoxy-2-*C*-hydroxymethyl-D-*threo*-pentonic acid (**2**). 3-Deoxy-D-*ribo*-hexonic (**3**) and 3-deoxy-D-*arabino*-hexonic acid (**4**) are known to be the major products of the degradation of 3-*O*-substituted hexoses[3–5], and 1-*O*-substituted hexuloses are characteristically degraded[3,6] to give 2-*C*-methyl-D-ribonic acid (**5**).

A consideration of the accepted mechanism of alkaline degradation[7] suggests that degradations performed in barium deuteroxide will yield saccharinic acids incorporating carbon-bound deuterium. There is already some evidence to support this suggestion[8,9]. The present work describes an examination of the position and extent of this deuterium incorporation and was undertaken as a preliminary to the production of saccharinic acids similarly labelled with tritium.

EXPERIMENTAL

Degradations in barium deuteroxide. — Standards of **6**, **7**, **12**, **13**, and **14** were prepared as previously reported[18]. Maltose (2.2 g; 60 mg/ml), turanose (8 mg; 2 mg/ml), and inulin (8 mg; 2 mg/ml) were each degraded at 50° in 0.25M barium deuteroxide (prepared from barium oxide and deuterium oxide) under an atmosphere of oxygen-free nitrogen. After freeze-drying, the turanose and inulin reaction mixtures were fractionated by elution from a column (140.0 × 0.6 cm; bed volume, 37 ml) of Dowex-AG1 X8 (AcO^-) resin (200–400 mesh), using 0.5M acetic acid (0.8 ml/min)[19],

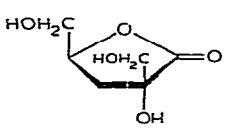
to give **3**, **4**, and **5**, respectively. The maltose reaction product was fractionated on a larger column (47.0 × 4.0 cm, bed volume, 590 ml) of resin, using 0.5M acetic acid (1.60 ml/min), to give fractions containing **1** and **2**. Each of these fractions was re-chromatographed on this latter column, using 0.08M sodium acetate[19] (1.60 ml/min), to give **1** and **2**, respectively. Compounds **1**–**5** were converted into their respective lactones by rotary evaporation of their aqueous solutions to dryness[18].

Gas-liquid chromatography. — A Pye 104 (model 64) gas chromatograph fitted with dual flame-ionisation detectors was used. The carrier gas was "white spot" nitrogen (British Oxygen Co. Ltd.) and glass coils (250 × 0.25 cm; coil diameter, 18 cm) packed with 10% SE-30 on Celite (100–120 mesh) were used. For preparative fractionation, samples were trapped in a glass U-tube and then taken up in hexane (redistilled). All purified *O*-trimethylsilyl (TMS) derivatives of the deuterated materials were chromatographically homogeneous under a variety of elution conditions and were chromatographically indistinguishable from the TMS derivatives of their authentic, undeuterated counterparts.

Mass spectrometry. — An A.E.I. MS-9 double-focusing instrument fitted with a direct-insertion probe was used. All spectra were recorded at 70 eV at a source temperature of 220°. All precise mass-measurements were subject to an experimental error of ±0.005 a.m.u.

RESULTS AND DISCUSSION

Deuterated 3-deoxy-2-*C*-hydroxymethyl-D-*erythro*-pentonic acid (**1**-*d*) and deuterated 3-deoxy-2-*C*-hydroxymethyl-D-*threo*-pentonic acid (**2**-*d*) were isolated from the degradation of maltose with aqueous barium deuteroxide under an atmosphere of nitrogen. Acids **1**-*d* and **2**-*d* were converted into 3-deoxy-2-*C*-hydroxymethyl-D-*erythro*-pentono-1,4-lactone (**6**-*d*) and 3-deoxy-2-*C*-hydroxymethyl-D-*threo*-pentono-1,4-lactone (**7**-*d*), respectively. The 100-MHz n.m.r. spectra of **6**-*d* and **7**-*d* in deuterium oxide were compared with the spectra for the corresponding undeuterated compounds **6** and **7**, respectively. In the spectra of **6** and **7**, H-4 gave a complex signal (centred at δ 4.88 for **6**), which was part of an AMX system involving H-5,4,5′



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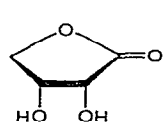

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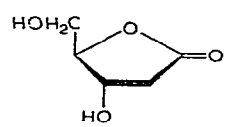

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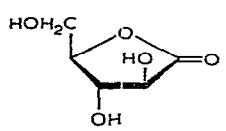

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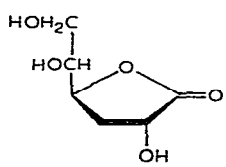

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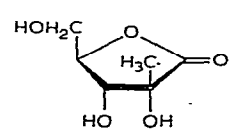

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(**6**: $J_{4,5}$ 3.2, $J_{4,5'}$ 5.7, $J_{5,5'}$ 13.0 Hz, H-5 δ 4.15, H-5′ 3.43; **7**: $J_{4,5}$ 2.8, $J_{4,5'}$ 5.6, $J_{5,5'}$ 13.0 Hz, $\Delta_{5,5'}$ 72 Hz). H-4 was also coupled to the two protons at C-3, and in the spectrum of **6** the signal for H-3,3′ appeared as a doublet (**6**: H-3, H-3′ δ 2.32,

TABLE I

MASS-SPECTRAL DATA OF COMPOUNDS **6**(TMS), **6**-*d*(TMS), **7**(TMS), AND **7**-*d*(TMS)[a]

m/e	*Relative intensity* (%)				m/e	*Relative intensity* (%)			
	6	**6**-*d*	**7**	**7**-*d*		**6**	**6**-*d*	**7**	**7**-*d*
384				0.2	306	0.6	0.1	0.6	0.3
383		0.2		0.6	305	1.7		1.6	
382		0.6		1.4	295		0.1		0.1
381		1.2	0.2	2.8	294		0.3	0.2	0.6
380	0.5	0.4	0.4	0.9	293	0.4	0.3	0.5	0.8
379	1.0	0.1	0.8	0.1	292	1.8	0.8	1.5	1.4
378	3.5	0.2	2.7		291	0.6		1.1	
369				0.1	279		0.1		0.3
368		0.3		0.5	278		0.4	0.5	0.8
367		0.6		1.2	277	0.2	0.5	0.8	1.8
366		1.5	0.2	2.4	276	1.0	1.1	3.3	2.3
365	0.7	0.5	0.4	0.8	275	1.6	0.7	1.9	1.7
364	1.3	0.1	0.8	0.1	274	0.9	0.1	0.8	0.4
363	4.0	0.2	2.6		273	3.9	0.3	3.2	0.1
354		0.2		0.5	248				7.2
353		0.9		1.7	247		5.3	5.1	12.1
352	0.9	4.0	0.6	7.6	245	17.5		16.8	
351	2.5	8.4	2.2	15.3	243			5.3	
350	11.5	25.2	10.0	47.2	231	7.7			
349	19.7	4.6	17.1	7.5	219				7.6
348	62.2	4.1	54.0	2.8	217	9.6		8.9	
347				0.1	155	6.1			
340		0.1		0.4	149	9.6		7.0	5.7
339		0.3		0.7	148	5.7		5.6	6.9
338		0.6		1.5	147	37.2	28.5	33.2	29.0
337	0.4	0.2	0.3	0.6	133	11.4	7.2	10.5	9.5
336	1.0	0.1	0.5	0.2	132				7.9
335	2.6	0.2	1.8	0.3	131	5.7	13.4	5.5	26.7
334	0.8		0.2		130				5.7
333	1.1		0.3		129	30.0		26.2	
332					117	30.8	21.4	26.3	36.2
323				0.2	116	11.0	6.0	11.4	16.3
322		0.2		0.6	105				6.3
321		0.3	0.2	0.9	104		8.7		46.7
320	0.3	1.1	0.6	2.3	103	28.0	10.0	42.2	20.7
319	1.0	0.3	1.1	0.7	101	7.9		7.1	8.0
318	2.6	0.2	2.5	0.1	89	6.6		5.5	6.8
317	0.8		0.5		85				15.5
309		0.1		0.4	82				18.4
308		0.2	0.2	0.7	77				42.0
307	0.4	0.9	0.6	1.8	75	19.3	13.1	19.9	18.0
					74	8.7	10.1	10.9	16.2
					73	*100*	*100*	*100*	*100*

[a]Below *m/e* 273, only those peaks of relative intensity >5% are included.

$J_{3,4}$ 7.8 Hz). However, the spectrum of **7** showed H-3,3′,4 as a typical AMX system (**7**: $J_{3',4}$ 9.2, $J_{3,4}$ 7.0, $J_{3,3'}$ 13.9 Hz, $\Delta_{3,3'}$ 46 Hz). Both **6** and **7** gave H-2^1,$2^{1'}$ as a doublet (**6**: centred at δ 3.75) but $J_{2^1,2^{1'}}$ could not be measured due to overlap with H-5,5′.

The assignments were confirmed by the spectra of **6** in methyl sulphoxide-d_6 and of its 2,2^1-*O*-isopropylidene derivative (**8**) in benzene-d_6. For each compound, H-3,3′,4 appeared as a typical AMX system.

The spectra in deuterium oxide of **6**-*d* and **7**-*d* showed almost total collapse of the H-3,3′ signal. Integration showed that, in each spectrum, this signal had decreased to 5% of the value for **6** and **7**. Similarly, the signal for H-2^1,$2^{1'}$ showed a 50% decrease in intensity. Carbon-bound deuterium was thus shown to be present at C-3 and C-2^1 in **6**-*d* and **7**-*d*.

These observations were confirmed by the mass spectra of the *O*-trimethylsilylated (TMS) derivatives of **6**, **6**-*d*, **7**, and **7**-*d* (Table 1). As anticipated, the low-mass regions of the spectra exhibited many of the ion peaks previously recorded with TMS derivatives[10,11]. Each sample gave a molecular ion (M), with which was associated the well-known[10,11] M−15 ion peak. The next peak, at *m/e* 348 (M−30), was the most intense with the exception of the base peak (*m/e* 73 in all the spectra). No M−30 peak was seen in the spectra of the TMS derivatives of D-erythrono-1,4-lactone[12,13] (**9**) and 2-deoxy-D-*erythro*-pentono-1,4-lactone[13] (**10**), or, to a significant degree, in the spectrum of any trimethylsilylated aldono-1,4-lactone previously reported[12]. Precise measurements of mass (Table II) showed that the M−30 ion was formed from M by loss of formaldehyde. A strong metastable peak (calc.: 320.5; found: 320–321) confirmed this assignment. Formaldehyde is probably lost by a McLafferty type rearrangement[14] (Fig. 1). A metastable peak (calc.: 290.5; found 290–291) indicated that this M−30 species is degraded, at least partially, by further loss of formaldehyde to give the ion at *m/e* 318. The formaldehyde presumably arises as a result of a rearrangement involving cleavage of the C-4–C-5 bond. A peak at *m/e* 355 recorded the presence of the M−43 ion. A similar M−43 peak was seen previously in the spectra of aldono-1,4-lactones(TMS)[12], and a metastable peak observed in the spectrum of **9**(TMS) confirmed that this M−43 ion arose from the M−15 ion by loss of carbon monoxide. The mode of fragmentation shown in Fig. 2 would account for the fact that no ion of *m/e* 233 (M−43) was seen[13] in the spectrum of **10**(TMS). A small peak at *m/e* 333 corresponded to the M−45 ion, which could have arisen from either the M−15 or M−30 species. No M−45 ion peak was observed in the spectrum of **9**(TMS)[12], but **10**(TMS)[13] gave a small peak at *m/e* 231, suggesting that the M−45 ion arises by loss of formaldehyde from C-5. A metastable peak was seen, which indicated that either or both of the transitions *m/e* 348→305 and *m/e* 319→292 occurred. The ion *m/e* 273 arises by loss of trimethylsilanol (90 a.m.u.) from the ion *m/e* 363, and a metastable peak confirmed that this transition occurred (calc.: 205.5; found: 205–206). A similar reaction accounted for the transition *m/e* 335→245 (metastable calc.: 179.1; found: 179–180). Such loss of trimethylsilanol has been shown to include non-specific abstraction of a hydrogen atom[15].

TABLE II

ELEMENTAL COMPOSITION BY PRECISE MASS MEASUREMENT

Calculated	*Found*	*Elemental composition*
378.171	**6**: 378.170; **7**: 378.167	$C_{15}H_{34}O_5Si_3$
348.159	**6**: 348.156; **7**: 348.155	$C_{14}H_{32}O_4Si_3$
318.149	**6**: 318.149; **7**: 318.146	$C_{13}H_{30}O_3Si_3$
117.037	**6**: 117.035	$C_4H_9O_2Si$
381.190	**6**-*d*: 381.186; **7**-*d*: 381.187	$C_{15}H_{31}D_3O_5Si_3$
350.173	**6**-*d*: 350.173; **7**-*d*: 350.176	$C_{14}H_{30}D_2O_4Si_3$
320.162	**6**-*d*: 320.159	$C_{13}H_{28}D_2O_3Si_3$
322.146	**12**: 322.145	$C_{12}H_{30}O_4Si_3$
292.135	**12**: 292.135; **13**: 292.133	$C_{11}H_{28}O_3Si_3$
219.089	**11**: 219.086	$C_8H_{19}O_3Si_2$

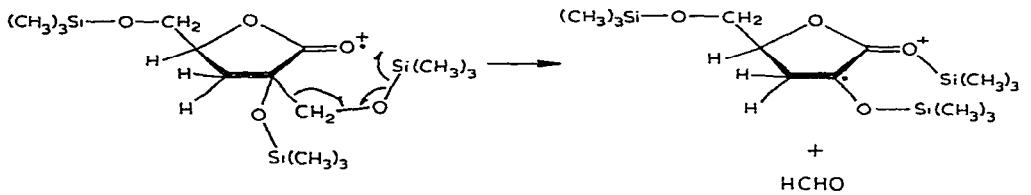

Fig. 1. Degradation of the *m/e* 378 ion by a McLafferty rearrangement.

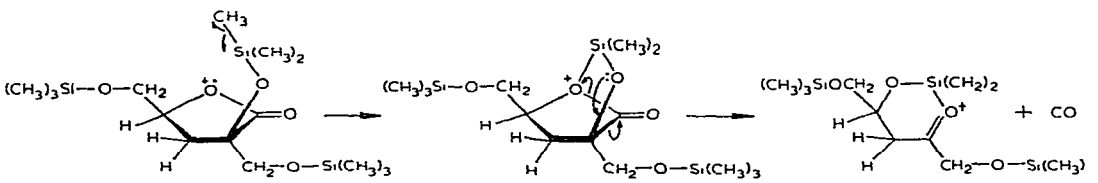

Fig. 2. Formation of the M−43 ion from the M−15 ion.

In the mass-spectral degradation of α-D-glucopyranose(TMS), DeJongh *et al.*[16] showed that the ion *m/e* 117 possessed the elemental composition $C_4H_9O_2Si$ and arose from C-5 and C-6. This ion was shown[16], by specific deuterium labelling at C-6, to contain both H-6 atoms. An ion of identical composition (Table II) was observed in the spectra of **6** and **7**, and is assumed to also arise from C-4 and C-5. This assumption is consistent with the observation that, whereas no *m/e* 117 peak was found in the spectrum of **9**(TMS)[13,14], a 117 peak was seen in the spectrum of D-arabinono-1,4-lactone (**11**)(TMS)[13,14]. De Jongh and his co-workers[16] showed that the *m/e* 117 ion was subsequently degraded to *m/e* 89 by loss of carbon monoxide, and a metastable peak (calc.: 67.7; found: 67.2–68.0) indicated the occurrence of the same degradation.

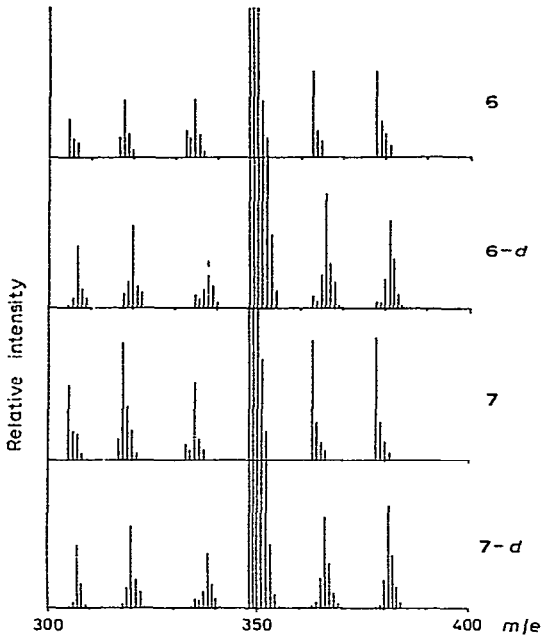

Fig. 3. The high-mass region of the spectra of 6(TMS), 6-*d*(TMS), 7(TMS), and 7-*d*(TMS).

The high-mass region (Fig. 3) of the spectra of 6-*d*(TMS) and 7-*d*(TMS) revealed that each of these samples was actually a mixture of molecules that were either unsubstituted (*m/e* 378) or contained 1–4 atoms of deuterium (*m/e* 379, 380, 381, and 382, respectively). The most-intense peak in this range was at *m/e* 381, corresponding to an ion containing three atoms of deuterium. Associated with the molecular-ion range was a similar group of peaks resulting from the loss of 15 a.m.u. Here, the ion at *m/e* 366 gave the most-intense peak but, as the formation of this ion is dependent on the fragmentation of the trimethylsilyl group only, it yields no information on the position of deuterium labelling in the molecules. The strongest peak in the M−30 range was at *m/e* 350, due to an ion containing two atoms of deuterium. This could arise from ions at *m/e* 382, 381, or 380 by loss of 32, 31, or 30 a.m.u., respectively, but data (Table III) showing the relative abundance of the individual species in the M and M−30 ranges, calculated from the relative intensities of Table I, suggest that the majority of the *m/e* 350 ions are derived from those at *m/e* 381 by loss of 31 a.m.u. Since this labelled formaldehyde is known to arise by cleavage of the C-1–C-2[1] bond, the presence of deuterium at C-2[1] is confirmed.

The M−30 range of ions gives rise to the M−60 ions by loss of formaldehyde and, as the most abundant of these M−60 ions (*m/e* 320) contains two atoms of deuterium, the formaldehyde lost must be unlabelled. It is probable that this formaldehyde originates from C-5, and so the presence of deuterium at this position seems unlikely. This conclusion was confirmed by the appearance of the ion peak at

TABLE III

RELATIVE ABUNDANCE OF IONIC SPECIES[a]

Ion m/e	*Relative abundance* (%)					*Ion* m/e	*Relative abundance* (%)	
	6-*d*	**7**-*d*	**12**-*d*	**13**-*d*	**14**-*d*		**6**-*d*	**7**-*d*
382	16	4	0	0	6	352	0	0
381	44	65	1	5	72	351	2	1
380	23	25	81	81	20	350	59	83
379	2	3	15	8	3	349	17	14
378	13	2	3	6	0	348	22	2

[a]Values obtained from mass spectra, after correcting peak intensities for the occurrence of natural isotopes.

m/e 117. This ion contains both the C-4 and C-5 groups but can contain no deuterium, since no shift of the *m/e* 117 peak to a higher value was observed. The ion peak found at *m/e* 103 in the spectra of **6** and **7** was seen here accompanied by a similarly intense peak at *m/e* 104. As the *m/e* 104 ion can be obtained by simple α-cleavage of the C-1–C-2[1] or C-4–C-5 bonds, the presence of deuterium at either C-5 or C-2[1] is confirmed. The ion peaks at *m/e* 247 and 273, arising from non-specific loss of trimethylsilanol[15], did not yield any information on the position of deuterium incorporation.

The combined n.m.r. and mass-spectral evidence showed conclusively that **6**-*d* and **7**-*d* contained carbon-bound deuterium at C-3 and C-2[1] only.

Compounds **3**-*d* and **4**-*d* were converted into 3-deoxy-D-*ribo*-hexono-1,4-lactone (**12**-*d*) and 3-deoxy-D-*arabino*-hexono-1,4-lactone (**13**-*d*), respectively. The 70eV mass-spectral data for the TMS ethers of **12**-*d* and **13**-*d* are shown in Table IV, together with those of the corresponding undeuterated materials **12**(TMS) and **13**(TMS). Rather weak peaks for the molecular ions were seen, and no M−30 ions were recorded [*cf.* **6**(TMS) and **7**(TMS)]. Ions were recorded at *m/e* 363 (M−15) and 335 (M−43) and are generated by the pathways described above. The ion peaks at *m/e* 322 and 292 could not be interpreted, even though precise measurements of mass suggested that these peaks were due to ions of elemental composition $C_{12}H_{30}O_4Si_3$ and $C_{11}H_{28}O_3Si_3$, respectively. The ion at *m/e* 273 was formed, as previously, by loss of trimethylsilanol from the (M−15) ion at *m/e* 363. Of more interest was the ion at *m/e* 219, which was seen to originate in a manner similar to the *m/e* 117 ion seen in the spectra of **6**(TMS) and **7**(TMS). A metastable peak was observed, confirming this assignment (calc.: 132.2; found: 132–133). The *m/e* 219 ion was degraded to the *m/e* 191 species (metastable calc.: 166.8; found: 166–167) by loss of carbon monoxide, as previously.

The spectra of the deuterated samples **12**-*d*(TMS) and **13**-*d*(TMS) showed that the most-abundant molecular ion was that at *m/e* 380, in contrast to the *m/e* 381 ion observed in the spectra of **6**-*d*(TMS) and **7**-*d*(TMS) (Table III). The ion at *m/e* 219 showed that no deuterium was bound at C-4, C-5, or C-6. Similarly, the appearance

of the ion at *m/e* 103 confirmed that no deuterium was to be found at C-6. No ion could be identified that would characterise either C-2 or C-3 uniquely. Nevertheless, it can be concluded that all the deuterium incorporated in **3-*d*** and **4-*d*** is bound to

TABLE IV

MASS-SPECTRAL DATA OF COMPOUNDS **12**(TMS), **12-*d***(TMS), **13**(TMS), AND **13-*d***(TMS)[a]

m/e	*Relative intensity* (%)				m/e	*Relative intensity* (%)			
	12	**12-*d***	**13**	**13-*d***		**12**	**12-*d***	**13**	**13-*d***
383		0.04		0.05	278		0.14		0.27
382		0.18		0.14	277		0.26	0.10	0.97
381	0.3	0.40		0.32	276	0.3	0.60	0.20	1.35
380	1.2	1.1	0.01	0.80	275	2.1	1.7	0.90	4.3
379	2.5	0.20	0.03	0.12	274	4.5	0.42	1.0	0.87
378	7.6		0.08	0.05	273	19.2	0.12	4.4	0.12
368				0.10	248				
367		0.20		0.57	247	11.5			
366	0.1	0.42		0.85	246	18.3		8.95	
365	0.7	1.1	0.08	2.0	245	9.5			
364	1.4	0.20	0.12	1.4	231				
363	4.5		0.44	0.05	219	9.0			
341					206	5.7			
339				0.15	205	15.3	6.2	11.1	9.0
338		0.10		0.30	191	5.2			
337	0.1	0.18	0.04	0.72	189	9.5	5.5		
336	0.3	0.04	0.06	0.15	155	15.0		6.4	
335	1.0	0.10	0.22	0.12	149	10.6	7.4		
334	0.1	0.04		0.05	148	7.5			
333	0.6	0.08	0.08		147	18.2	16.3	22.5	19.0
325	1.3			0.15	133	11.6	6.7	6.1	7.5
324	0.5	0.12	0.14	0.35	131	10.9	12.0	5.4	17.5
323	1.0	0.18	0.28	0.81	130	9.1	8.4		10.0
322	3.5	0.60	0.92	2.0	129	31.4		38.8	
321		0.08		0.10	117	13.0	11.0	6.4	7.5
320		0.08		0.05	103	18.8	13.4	17.9	16.0
319	0.1	0.08		0.10	101	6.9			
318	0.2	0.12	0.04	0.22	97		5.3		
317	0.6	0.04	0.10	0.05	83		6.6		
309		0.04		0.07	81		6.0		
308	0.1	0.08	0.04	0.15	75	22.2	14.1	14.2	14.0
307	0.6	0.22	0.12	0.37	74	13.9	9.6	8.9	9.0
306	0.1	0.08		0.05	73	*100*	*100*	*100*	*100*
305	0.6	0.10	0.10	0.07					
295	0.3	0.10	0.08	0.17					
294	1.7	0.22	0.40	0.50					
293	3.2	0.42	0.70	0.97					
292	11.7	1.1	2.65	3.4					
291	0.1	0.12		0.07					
290		0.04		0.10					
280		0.12		0.12					
279		0.10		0.15					

[a]Below *m/e* 273, only those peaks of relative intensity >5% are included.

C-2 and/or C-3. For the molecule of mass 381 a.m.u., obviously all three hydrogen atoms at C-2 and C-3 had been replaced with deuterium.

Deuterated 2-*C*-methyl-D-ribono-1,4-lactone (**14-*d***) was prepared from deuterated 2-*C*-methyl-D-ribonic acid (**5-*d***), and the mass-spectral data (70eV) for the TMS

TABLE V

MASS-SPECTRAL DATA OF COMPOUNDS **14**(TMS) AND **14-*d***(TMS)[a]

m/e	*Relative intensity* (%)		m/e	*Relative intensity* (%)	
	14	**14-*d***		**14**	**14-*d***
383		1.7	263		6.0
382		4.1	260	6.5	
381	1.0	11.5	235	7.0	
380	4.0	4.6	234		8.5
379	8.0	0.90	231	9.0	
378	26.0		219	9.0	13.1
367		0.40	218	23.0	24.2
366		1.3	217	54.2	43.0
365		0.60	203	6.1	
364	1.0		191	7.0	
363	2.5		150		14.0
339		0.40	149	7.0	
338		1.2	148	6.0	9.0
337		0.80	147	40.6	47.2
336	1.0	0.60	145		10.5
335	2.0	0.30	143	7.0	
333	1.0		141		17.0
310		0.26	133	7.2	10.0
309		0.95	131		11.0
308		4.8	129	5.0	7.0
307	1.0	4.2	119		7.5
306	2.0		117	35.5	27.5
305	5.0		116	6.0	
294		0.31	111		10.0
293		0.23	105		7.0
292	1.0		104		9.0
291	2.0	0.12	103	9.0	23.0
290		0.31	98		10.0
289		0.27	97		19.0
279		0.12	95		9.0
277		0.10	91		11.1
276		0.28	87	18.5	
275		0.17	85		5.5
274	1.0		83		27.5
273	4.1		82		7.0
			77	7.2	15.0
			76		14.0
			75	17.0	31.0
			74	9.4	10.0
			73	*100*	*100*

[a]Below *m/e* 273, only those peaks of relative intensity >5% are included.

derivatives of **14**-*d* and the undeuterated material (**14**) are given in Table V. A strong peak for the molecular ion was seen for each compound and the most-abundant molecular ion in the deuterated material was that at *m/e* 381. Once again, ion peaks were seen for **14**(TMS) at *m/e* 363 (M−15), 335 (M−43), and 333 (M−45), but few of the other peaks could be readily interpreted. Nevertheless, the appearance of the *m/e* 117 ion in each spectrum demonstrated that no deuterium was bound to either C-4 or C-5 of **14**-*d* and was confirmed by the appearance of the *m/e* 103 peak in the spectrum of **14**-*d*. This ion arises from C-5 and would have appeared at higher mass if deuterium was bound at this site. The strong peak at *m/e* 129 seen in the spectra of **6**(TMS), **7**(TMS), **12**(TMS), and **13**(TMS) was not seen in the spectra of **14** or **14**-*d*. This suggests that this ion is characteristic of the C-3 deoxy function in compounds **6**, **7**, **12**, and **13**—an observation that is consistent with seeing the ion *m/e* 129 at 131 in the spectra of **6**-*d*(TMS), **7**-*d*(TMS), **12**-*d*(TMS), and **13**-*d*(TMS). An ion *m/e* 129 was shown previously to arise from C-4, C-5, and C-6 of α-D-glucopyranose (TMS)[16]. The strong peak at *m/e* 217 for **14**(TMS) and **14**-*d*(TMS) could not be assigned, although characteristic *m/e* 217 peaks have been reported previously[16,17]. Nevertheless, it was possible to show that any deuterium incorporated in **5**-*d* must be bound at either C-3 or C-2[1].

The distribution of deuterium in **6**-*d* and **7**-*d* is given in Table III, and a consideration of the reaction mechanism that operates in the degradation of maltose demonstrates the significance of this distribution. The stages at which deuterium can be incorporated into the molecules are shown in Fig. 4. It will be seen that any deuterium incorporated in reaction 1_r must necessarily be lost in the forward reaction 1_f. Deuterium can also be incorporated at C-1 [ultimately C-2[1] of the 3-deoxy-2-*C*-hydroxymethyl-D-pentonic acid] in the reaction 2_f. Reversal of this process with C–H bond cleavage at C-1 would lead to complete deuteration at this site. As in reaction 1, any deuterium gained in reaction 3_r is lost again in the forward reaction 3_f. The β-elimination of the *O*-glucosyl anion (reaction 4) is effectively irreversible, as in the subsequent dicarbonyl rearrangement (reaction 6). This means that, although the two species formed in reactions 4 and 5_f are in equilibrium, neither of them is in equilibrium with the rest of the reaction mixture. Since the combined mass-spectral and n.m.r. evidence presented here shows that 60–80% of all the molecules contain two atoms of deuterium at C-3, reaction 6 must proceed more slowly than equilibrium reaction 5. Furthermore, if reaction 4 was rate-determining, then a higher degree of deuterium incorporation would be anticipated to occur at C-2[1] than that observed. Thus, the findings of the deuterium-labelling experiments performed here suggest that, in the formation of 3-deoxy-2-*C*-hydroxymethyl-D-pentonic acid from maltose degraded at 50° in 0.25M $Ba(OD)_2$ under nitrogen, the rate-determining step is the dicarbonyl rearrangement shown here as reaction 6.

In each of the degradations examined, it was possible to demonstrate that deuterium had been transferred from the reaction solvent to the saccharinic acid products. In each of these reaction products, the position of the carbon-bound deuterium within the molecules was as predicted from a consideration of the Nef–Isbell

theory[7]. The work thus provided a basis for the corresponding production of tritium-labelled reaction products on alkaline degradation to be described in a subsequent publication.

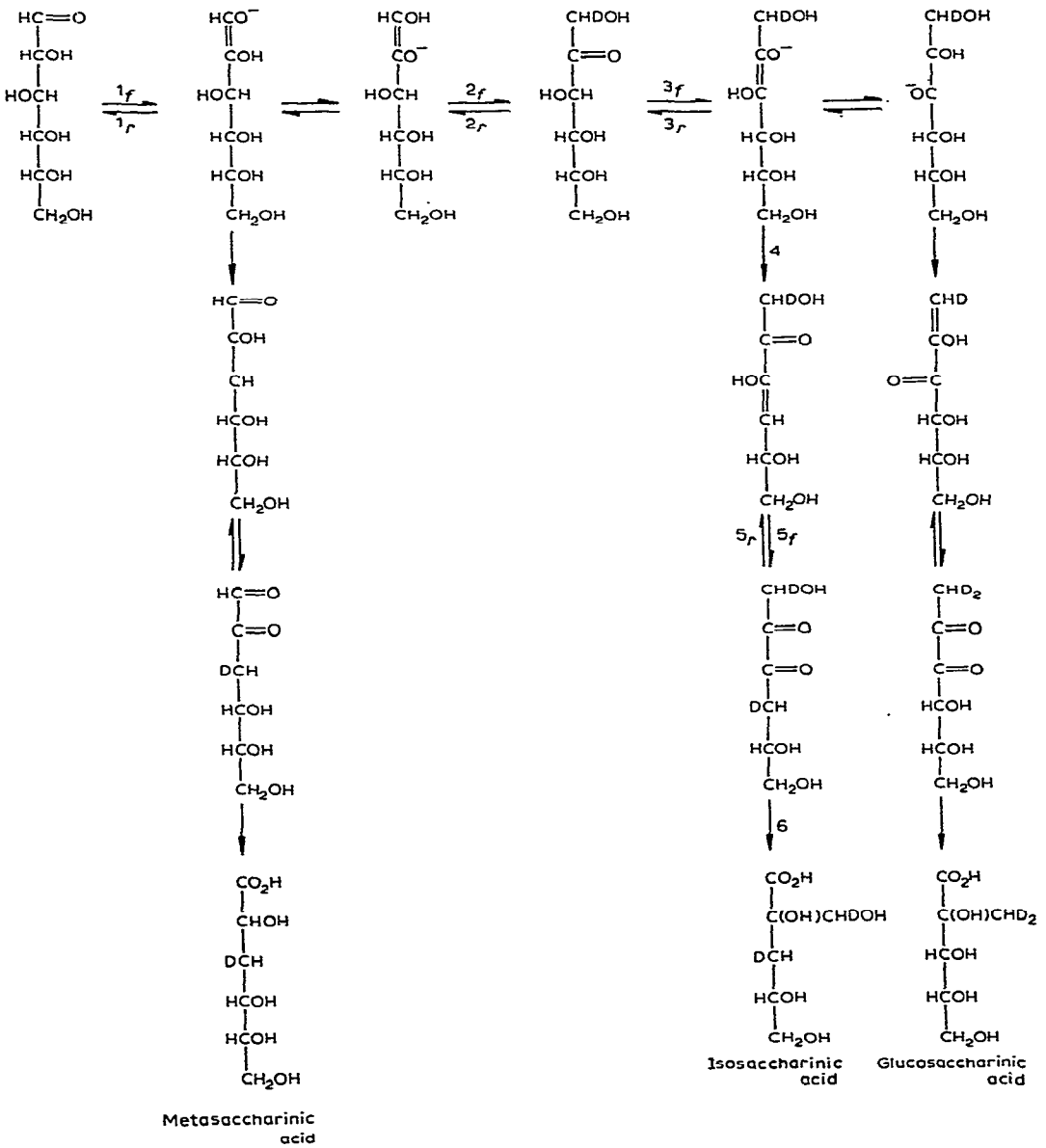

Fig. 4. Deuterium incorporation in saccharinic acid production. For convenience, the reaction pathways are shown originating from D-glucose, although metasaccharinic acid was obtained from turanose, isosaccharinic acid from maltose, and glucosaccharinic acid from inulin.

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REFERENCES

1 R. L. WHISTLER AND J. N. BEMILLER, *J. Amer. Chem. Soc.*, 82 (1960) 3705.
2 G. MACHELL AND G. N. RICHARDS, *J. Chem. Soc.*, (1960) 1932.
3 R. L. WHISTLER AND J. N. BEMILLER, *Advan. Carbohyd. Chem.*, 13 (1958) 28.
4 G. MACHELL AND G. N. RICHARDS, *J. Chem. Soc.*, (1960) 1938.
5 E. F. L. J. ANET, *J. Amer. Chem. Soc.*, 82 (1960) 1502.
6 A. ISHIZU, B. LINDBERG, AND O. THEANDER, *Carbohyd. Res.*, 5 (1967) 329.
7 H. S. ISBELL, *J. Res. Nat. Bur. Stand., Sect. A*, 32 (1944) 45.
8 H. S. ISBELL, H. L. FRUSH, C. W. R. WADE, AND C. E. HUNTER, *Carbohyd. Res.*, 9 (1969) 163.
9 M. S. FEATHER AND J. F. HARRIS, *Carbohyd. Res.*, 15 (1970) 304.
10 J. DIEKMAN, J. B. THOMPSON, AND C. DJERASSI, *J. Org. Chem.*, 33 (1968) 2271.
11 A. G. SHARKEY, JR., R. A. FRIEDEL, AND S. H. LANGER, *Anal. Chem.*, 29 (1957) 770.
12 G. PETERSSON, O. SAMUELSON, K. ANJOU, AND E. SYDOIS, *Acta Chem. Scand.*, 21 (1967) 1251.
13 A. KO, Ph.D. Thesis, University of Birmingham, 1969.
14 G. PETERSSON, *Tetrahedron*, 26 (1970) 3413.
15 J. DIEKMANN, J. B. THOMPSON, AND C. DJERASSI, *J. Org. Chem.*, 32 (1967) 3904.
16 D. C. DEJONGH, T. RADFORD, J. D. HRIBAR, S. HANESSIAN, M. BIEBER, G. DAWSON, AND C. C. SWEELEY, *J. Amer. Chem. Soc.*, 91 (1969) 1728.
17 S. KARADY AND S. H. PINES, *Tetrahedron*, 26 (1970) 4527.
18 R. L. WHISTLER AND J. N. BEMILLER, *Methods Carbohyd. Chem.*, 2 (1963) 477.
19 O. SAMUELSON AND L. THEDE, *J. Chromatogr.*, 30 (1967) 556.