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CHARACTERIZATION OF ALKALINE-DEGRADATION PRODUCTS OF SOME 3,4-DI- AND 3,4,6-TRI-METHYL ETHERS OF HEXOSES BY G.L.C.–MASS SPECTROMETRY OF *O*-TRIMETHYLSILYL DERIVATIVES*

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ABSTRACT

G.l.c.–mass spectrometry has been used to provide information on the *O*-trimethylsilyl derivatives of the products of alkaline degradation of 3,4-di- and 3,4,6-tri-*O*-methyl-D-glucose, and 3,4,6-tri-*O*-methyl-D-galactose. During reaction with sodium hydroxide–sodium borohydride mixtures, reduction occurs more rapidly than β-elimination and the only detectable products were the corresponding alditols and the epimeric 3-deoxyalditols. Extended reaction with sodium hydroxide alone, followed by treatment with sodium borohydride, gives mixtures of aldonic acids including the epimeric 3-deoxy-4-*O*-methylaldonic acids (metasaccharinic acids), 3-deoxyaldonic acids (with loss of the 4-*O*-methyl substituent), and 3,4-dideoxyaldonic acids. Possible reaction-pathways are discussed.

INTRODUCTION

3,4-Dideoxyhex-3-enitols or oligosaccharides terminated by residues of such unsaturated alditols have been isolated from the degradation of blood-group substances with sodium hydroxide–sodium borohydride mixtures[1]. There is reasonable evidence to suggest that the 3,4-dideoxyhex-3-enitols are formed from 3,4-di-*O*-substituted hexoses by base-catalyzed elimination of both 3- and 4-*O*-substituents to give a 3,4-dideoxyhex-3-enosulose, which is then reduced to an epimeric pair of 3,4-dideoxy-*erythro*- and 3,4-dideoxy-*threo*-hex-3-enitols. As part of a series of investigations on the base-catalyzed degradations of carbohydrates[2], and in an attempt to substantiate the proposed reaction-pathway for 3,4-dideoxyhex-3-enitol formation, we have studied the alkaline degradation of 3,4-di- (**1**) and 3,4,6-tri-*O*-methyl-D-glucose (**2**), and 3,4,6-tri-*O*-methyl-D-galactose (**3**) by (*a*) the action of sodium hydroxide–sodium borohydride mixtures and (*b*) the action of sodium hydroxide alone followed by treatment with sodium borohydride.

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‡Taken from the M.Sc. thesis of S.C. Tam, Trent University, 1973.

RESULTS AND DISCUSSION

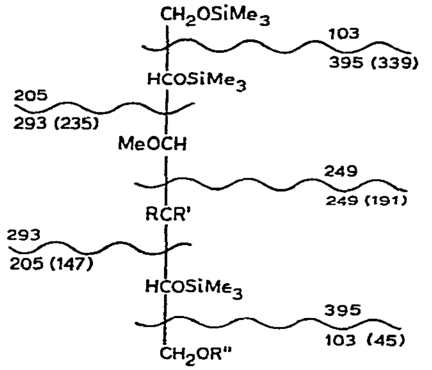
O-Methylated reducing sugars were treated with a sodium hydroxide–sodium borohydride mixture under the conditions employed by Kabat and Lloyd and their collaborators[1]. Examination of the reaction mixtures by g.l.c. of the *O*-trimethylsilyl (Me_3Si) derivatives indicated that the corresponding alditols were the major products and that in each case two minor products, but no 3,4-dideoxyhex-3-enitols, were also formed. Higher proportions of these other products were formed when the proportion of sodium hydroxide in the mixture with sodium borohydride was increased (Table I). G.l.c.–m.s. of the Me_3Si derivatives confirmed the identities of the alditols (**4**–**6**) formed by direct reduction with sodium borohydride and showed that the other products were Me_3Si derivatives of epimeric pairs of 3-deoxyhexitols (**7** and **8**, **9** and **10**, and **11**, and **12**, respectively). As indicated later, the mass spectra of the various Me_3Si alditols showed the fragment ions predicted from primary cleavage[3].

TABLE I

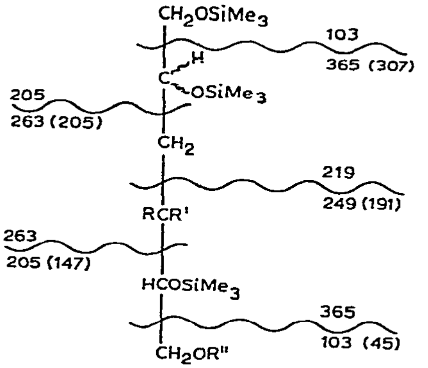
G.L.C. OF Me_3Si DERIVATIVES OF REACTION PRODUCTS OF *O*-METHYLATED SUGARS WITH SODIUM HYDROXIDE–SODIUM BOROHYDRIDE

Sugar	*Product*	*T[a] of Me_3Si derivative*		*Relative proportions*	
		at 130°	*145°*	(a)[b,c]	(b)[b,c]
3,4-Di-*O*-methyl-D-glucose (**1**)	**4**		0.81	m	1
	7		0.57	t	4
	8		0.61	t	9
3,4,6-Tri-*O*-methyl-D-glucose (**2**)	**5**	0.47		m	1.0
	9	0.34		t	3.0
	10	0.38		t	1.3
Tri-*O*-methyl-D-galactose (**3**)	**6**	0.47		m	14
	11	0.34		t	1.0
	12	0.38		t	1.1
Reference compound					
3,4-Di-*O*-methyl-D-glucitol			0.81		
3,4,6-Tri-*O*-methyl-D-glucitol		0.47			
3,4,6-Tri-*O*-methyl-D-galactitol		0.47			
(*E*)-3,4-Dideoxy-D-*threo*-hex-3-enitol			0.43		
(*Z*)-3,4-Dideoxy-D-*threo*-hex-3-enitol			0.43		
(*E*)-3,4-Dideoxy-*erythro*-hex-3-enitol			0.45		

[a] Retention times (*T*) are quoted relative to the Me_3Si derivative of methyl α-D-glucopyranoside. [b] Reaction with (*a*) 0.2M sodium hydroxide containing 1% of sodium borohydride and (*b*) M sodium hydroxide containing 1% of sodium borohydride. [c] t = traces, m = major product.



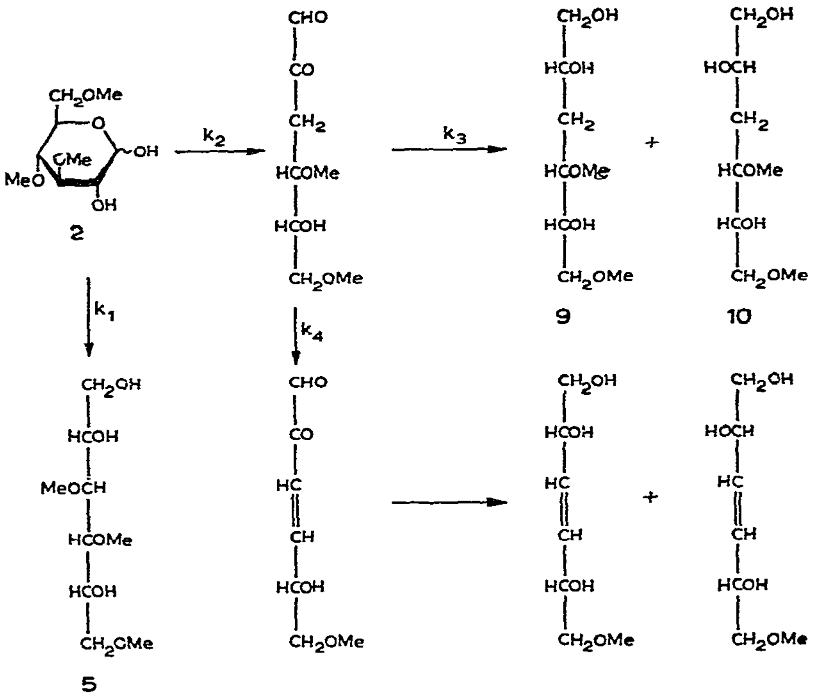
Fragmentation of Me_3Si derivatives of **4** (R = H, R′ = OMe, and R″ = $SiMe_3$), **5** (R = H, R′ = OMe, and R″ = Me), and **6** (R = OMe, R′ = H, and R″ = Me). Figures in parentheses indicate fragment ions from **5** and **6** where these are different from those from **4**.



Fragmentation of Me_3Si derivatives of **7** and **8** (R = H, R′ = OMe, and R″ = $SiMe_3$), **9** and **10** (R = H, R′ = OMe, and R″ = $SiMe_3$), and **11** and **12** (R = OMe, R′ = H, and R″ = Me). Figures in parentheses indicate fragment ions from **9**–**12** where these differ from those from **7** and **8**.

The reaction that may be anticipated when 3,4-di-*O*-substituted sugars are treated with sodium hydroxide–sodium borohydride mixtures are exemplified in Scheme 1 for 3,4,6-tri-*O*-methyl-D-glucose (**2**). The observed marked preference for reduction rather than base catalyzed-elimination requires that $k_1 > k_2$, and $k_3 \gg k_4$, and is in accord with the generally poor character of methoxyl as a leaving group in elimination reactions.

In order to obtain further evidence for the possible consecutive elimination of both 3-*O*- and 4-*O*-substituents during the base-catalyzed degradation of 3,4-di-*O*-substituted sugars, the reducing sugars were treated with oxygen-free sodium hydroxide until no further reducing sugar could be detected, and the reaction mixtures were then treated with sodium borohydride. The products, whose i.r. spectra showed strong bands at 1720 cm^{-1} (carboxyl), were converted into Me_3Si derivatives for



examination by g.l.c.–m.s. Four major peaks were observed in each case (Table II), although for the products from 3,4-di-*O*-methyl-D-glucose the incomplete resolution of two of the peaks (presumably Me_3Si derivatives of **19** and **20**) did not permit satisfactory spectra to be recorded. The mass spectra observed were consistent with the formation of a 3,4-dideoxy-6-*O*-methyl-hexonic acid (**13**–**15** respectively) and a

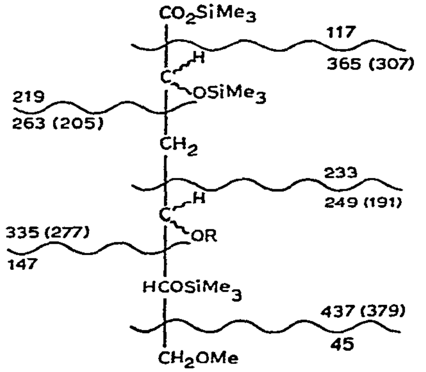
TABLE II

PROMINENT IONS IN THE MASS SPECTRA OF Me_3Si DERIVATIVES OF *O*-METHYLALDITOLS AND 3-DEOXY-*O*-METHYLALDITOLS

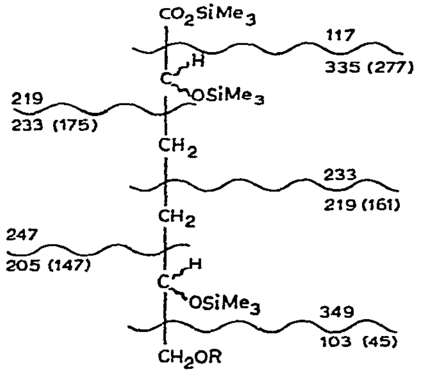
m/e	*Abundances (% of base peak at* m/e 73) *of ions from Me_3Si derivatives of compounds*			
	4	5–6[a]	7–8[a]	9–12[a]
293	6	4		
263			3	3
249	11	6		
235		8		
205	80	35	50	40
147[b]		50		30
103	50	40	30	15
45		30		30

[a] Average abundances are given for stereoisomeric compounds. [b] Peaks at *m/e* 147 arise in a variety of ways[13] and have low structural significance.

3-deoxy-6-*O*-methyl-hexonic acid (**16**–**18** respectively) from each methylated sugar. No chromatographic separation of stereoisomers of these types of compound was observed. The mass spectra of the Me_3Si derivatives of the additional products from the 3,4,6-trimethyl ethers (**2** and **3**) indicated that these were epimeric pairs (**21** and **22**, and **23** and **24**) of 3-deoxy-4,6-di-*O*-methylhexonic acids. In the case of the Me_3Si derivative of the 3-deoxyhexonic acid (**16**) formed from 3,4-di-*O*-methyl-D-glucose (**1**), the mass spectrum was essentially similar to that reported by Petersson[4] for the Me_3Si derivative of the metasaccharinic acid, 3-deoxy-D-*arabino*-hexonic acid. The mass spectra of the Me_3Si derivatives of the various 3-deoxy-*O*-methylhexonic acids showed analogous fragment-ions from primary cleavages, with some subsequent rearrangements with loss of trimethylsilanol which were supported by metastable peaks (see Experimental). The mass spectra of the Me_3Si derivatives of the various 3,4-dideoxyhexonic acids (**13**–**15**) were likewise analogous to that reported by Petersson[4] for the Me_3Si derivative of 3,4-dideoxypentonic acid.

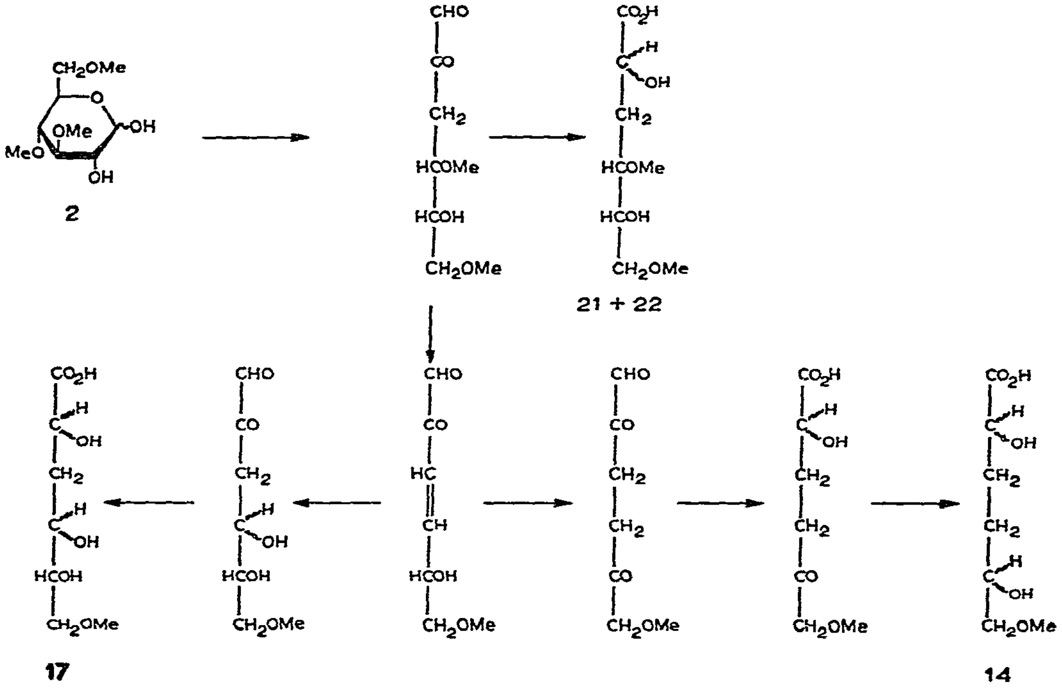


Fragmentation of Me_3Si derivatives of 3-deoxy-6-*O*-methylhexonic acids (**17** and **18**, R = $SiMe_3$ and 3-deoxy-4,6-di-*O*-methylhexonic acids (**21** and **22**, and **23** and **24**, R = Me) (ions in parentheses).



Fragmentation of Me_3Si derivatives of 3,4-dideoxyhexonic acids (**13**, R = $SiMe_3$; **14** and **15**, R = Me) (ions in parentheses).

The formation of the three types of aldonic acid may be accounted for in the sequence of reactions exemplified in Scheme 2. The epimeric pairs of 3-deoxy-4-*O*-methyl- (**19** and **20**) and 3-deoxy-4,6-di-*O*-methylhexonic acids (**21** and **22**, and **23** and **24**) (metasaccharinic acids) are clearly formed by the well known reaction[5] involving elimination of the 3-substituent followed by rearrangement of the resulting 3-deoxyglycosulose. The formation of 3-deoxy-6-*O*-methyl-hexonic acids (**16–18**) with loss of both the 3-*C*-substituent and the 4-*O*-substituent may result from a sequence of reactions involving elimination of methanol from the 3-deoxy-5-*O*-methyl- or 3-deoxy-4,6-di-*O*-methylglycosulose, base-catalyzed addition of water to the α,β-unsaturated system of the 3,4-dideoxyhex-3-enosulose, and rearrangement of the resulting 3-deoxyglycosulose. As far as we are aware, evidence for base-catalyzed hydration of 3,4-dideoxyhex-3-enosuloses has not been reported previously, but a precedent for such nucleophilic addition to a 3,4-dideoxyhex-3-enosulose has been reported by Anet and Ingles[6] in the addition of sulfite with subsequent rearrangement of the adduct to give a 3,4-dideoxy-4-sulfohexonic acid. The formation of 3,4-dideoxyhexonic acids may be accounted for by a similar elimination of 3-*C*- and 4-*O*-substituents to give the 3,4-dideoxyhex-3-enosuloses, which in this case rearrange by a vinylogous enolization, as in the alkaline conversion of santonin into santonic acid[7], to furnish hexos-2,5-diuloses. Benzilic acid-type rearrangement to the 3,4-dideoxy-5-hexosulonic acids and subsequent reduction by sodium borohydride would then afford the 3,4-dideoxyhexonic acids (**13–15**).



No evidence has been observed for the formation of 3,4-dideoxyhex-3-enitols when 3,4-di- and 3,4,6-tri-*O*-methylhexoses are treated with sodium hydroxide–sodium borohydride mixtures. However, the formation of 3,4-dideoxyhex-3-enosuloses, which are probable intermediates in the formation of 3,4-dideoxyhex-3-enitols[1], is clearly implied in the production of 3-deoxyhexonic acids (**16–18**) with loss of the 4-*O*-methyl substituent, and of 3,4-dideoxyhexonic acids (**13–15**) on extended treatment of the same methyl ethers with sodium hydroxide alone followed by reduction with sodium borohydride. The products (**4–12**) formed on treatment of 3,4-di- and 3,4,6-tri-*O*-methylhexoses (**1–3**) with sodium hydroxide–sodium borohydride mixtures suggest that reductions of both parent sugars and of 3-deoxyhexosuloses take place much more rapidly than elimination of *O*-methyl groups. Alkoxyl groups are generally poor leaving in β-elimination reactions as shown, for example, in studies on hexuronic esters[8], and it is probable that 3,4-di-*O*-glycosylhexoses would provide more appropriate model compounds for the study of 3,4-dideoxyhex-3-enitol formation when branched oligosaccharides are treated with sodium hydroxide–sodium borohydride mixtures.

EXPERIMENTAL

General methods. — Melting points are uncorrected. Optical rotations were measured with a Perkin–Elmer model 141 polarimeter at 20 ±2°. I.r. spectra were measured on a Unicam SP-200 spectrophotometer. N.m.r. spectra were recorded on a JEOL C-60HL spectrophotometer with tetramethylsilane or sodium 4,4-dimethyl-4-silapentane 1-sulfonate as internal standards. Mass spectra were recorded on an A.E.I. MS-12 mass spectrometer, with an inlet temperature of 200°, an ionization potential of 70 eV, and an ion-source temperature of ~150°. G.l.c. was carried out with a Pye model 104 chromatograph using a column of dichloromethylsilane-treated Celite coated with 10% of silicone gum SE-30 (operating temperature 145°). For g.l.c.–m.s., the gas chromatograph was connected to the mass spectrometer via a Watson–Biemann separator.

Methyl ethers. — 3,4-Di-*O*-methyl-D-glucose was prepared as described by Mitra *et al.*[9] and had m.p. 109–118° and $[\alpha]_D$ +73°→+76° (24 h, constant) (*c* 5.0, water) (lit.[9], m.p. 114–118°, $[\alpha]_D$ +80°→+76°); n.m.r. data (D_2O): τ 6.39 and 6.45 (two 3-proton singlets, 2 OMe). 3,4,6-Tri-*O*-methyl-D-glucose was prepared from 3,4,6-tri-*O*-methyl-D-glucal, essentially by the procedure of Kuhn and Baer[10], but using *m*-chloroperoxybenzoic acid, and had m.p. 76–78°, $[\alpha]_D$ +92°→+77.5° (20 h, constant) (*c* 1.0, water) (lit.[11], m.p. 76–77°, $[\alpha]_D$ +91.9°→+77.4°); n.m.r. data ($[^2H_6]$acetone): τ 6.43, 6.53, and 6.70 (three 3-proton singlets, 3 OMe). 3,4,6-Tri-*O*-methyl-D-galactose was similarly prepared from 3,4,6-tri-*O*-methyl-D-galactal, and had m.p. 86–89° and $[\alpha]_D$ +145°→+113.2° (24 h, constant) (*c* 1.0, water) (lit.[10], m.p. 88–89°, $[\alpha]_D$ +154°→+110°); n.m.r. data ($[^2H_6]$acetone): τ 6.50 (6-proton singlet, 2 OMe), 6.62 (3-proton singlet, OMe).

Treatment of sugars with sodium hydroxide–sodium borohydride mixtures. —

The sugar (40 mg) was dissolved in (*a*) 0.2M sodium hydroxide containing 1% of sodium borohydride (0.4 ml) or (*b*) M sodium hydroxide containing 1% of sodium borohydride (0.4 ml), and the solution was kept for 7 days at room temperature. Excess of borohydride was decomposed and sodium ions were removed by treatment with Amberlite resin IR-120 (H^+), the mixture was filtered, and methanol was evaporated several times from the filtrate to remove boric acid. The resulting syrup was trimethylsilylated by the method of Sweeley *et al.*[12], and the Me_3Si derivatives were analyzed by g.l.c. (see Table I) and by g.l.c.–m.s. In the mass spectra, all of the fragment-ions previously mentioned were observed and the more important ions are listed in Table II.

Treatment of sugars with sodium hydroxide followed by reduction with sodium borohydride. — The sugar (50 mg) was kept in oxygen-free 0.2M sodium hydroxide (5 ml) for 9 days at room temperature, sodium borohydride (0.1 g) was added, and after 1 day the reaction mixture was processed as already described to give a syrup [ν_{max}^{film} 1720 cm^{-1} (CO_2H)]. The syrup was trimethylsilylated and the Me_3Si derivatives were analyzed by g.l.c. (see Table III) and by g.l.c.–m.s. Molecular-ion (M^+) peaks were not seen in the mass spectra, but in most cases there were observed peaks at $M-15$ having intensities of ~0.3% of base peak, together with all of the fragment ions previously mentioned. Table IV lists major ions from primary fragmentations together with some less-intense ions of higher mass and those ions derived therefrom on subsequent rearrangement with loss of Me_3SiOH, for which decompositions metastable peaks were observed.

TABLE III

G.L.C. OF Me_3Si DERIVATIVES OF REACTION PRODUCTS OF *O*-METHYLATED SUGARS WITH SODIUM HYDROXIDE FOLLOWED BY SODIUM BOROHYDRIDE

Sugar	*Product*	T^a *of* Me_3Si *derivative*		*Relative proportions*
		at 130°	*145°*	
3,4-Di-*O*-methyl-D-glucose (1)	13		0.53	3
	16		1.01	3.5
	19		0.63	2
	20		0.74	1
3,4,6-Tri-*O*-methyl-D-glucose (2)	14	0.31		2.7
	17	0.60		2.7
	21	0.38		1
	22	0.43		1.7
3,4,6-Tri-*O*-methyl-D-galactose (3)	15	0.31		4.5
	18	0.60		3.2
	23	0.38		1.4
	24	0.43		1

[a] See Table I.

TABLE IV

PROMINENT IONS IN THE MASS SPECTRA OF Me_3Si DERIVATIVES OF 3-DEOXYHEXONIC AND 3,4-DIDEOXYHEXONIC ACIDS

m/e	*Abundances (% of base peak at* m/e 73) *of ions from* Me_3Si *derivatives of compounds*				
	13	**14–15**[a]	**16**	**17–18**[a]	**21–24**[a]
349	8	12			
[259][b]	9	13			
335	2		7	5	
[245][c]	5		45	25	
277		8			4
[187][d]		21			14
205	4		4		
147[e]		35		20	27
117	4	4	5	3	4
103	8		15		
45		30		16	24

[a]Average abundances are given for stereoisomeric compounds. [b,c,d]Figures in brackets refer to ions formed from the preceding primary fragments on rearrangement with loss of Me_3SiOH. Metastable peaks were observed at (*b*) m* 192–192.5 (349→259) [calc. 192.3], (*c*) m* 178.5–180 (335→245) [calc. 179.3], and (*d*) m* 126–126.5 (277→187) [calc. 126.3]. [e]Peaks at *m/e* 147 arise in a variety of ways[13] and have low structural significance.

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REFERENCES

1 K. O. LLOYD, E. A. KABAT, AND E. LICERIO, *Biochemistry*, 7 (1968) 2976, and earlier papers cited therein.
2 G. O. ASPINALL, R. KHAN, R. R. KING, AND Z. PAWLAK, *Can. J. Chem.*, 51 (1973) 1359, and earlier papers cited therein.
3 G. PETERSSON, *Tetrahedron*, 25 (1969) 4437.
4 G. PETERSSON, *Tetrahedron*, 26 (1970) 3413.
5 W. PIGMAN AND E. F. J. L. ANET, in W. PIGMAN AND D. HORTON (Eds.), *The Carbohydrates*, 2nd ed., Vol. 1A, Academic Press, New York, 1972, chapter 4.
6 E. F. J. L. ANET AND D. L. INGLES, *Chem. Ind.* (London), (1964) 1319; see also, E. F. J. L. ANET, *Advan. Carbohyd. Chem.*, 19 (1964) 181.
7 R. B. WOODWARD, F. J. BRUTSCHY, AND H. BAER, *J. Amer. Chem. Soc.*, 70 (1948) 4216.
8 G. O. ASPINALL AND P. E. BARRON, *Can. J. Chem.*, 50 (1972) 2203.
9 A. K. MITRA, D. H. BALL, AND L. LONG, JR., *J. Org. Chem.*, 27 (1962) 1601.
10 R. KUHN AND H. H. BAER, *Chem. Ber.*, 90 (1957) 1537.
11 R. L. SUNDBERG, C. M. MCCLOSKEY, D. E. REES, AND G. H. COLEMAN, *J. Amer. Chem. Soc.*, 67 (1945) 1080.
12 C. C. SWEELEY, R. BENTLEY, M. MAKITA, AND W. W. WELLS, *J. Amer. Chem. Soc.*, 85 (1963) 2497.
13 J. DIEKMAN, J. B. THOMSON, AND C. DJERASSI, *J. Org. Chem.*, 33 (1968) 2271.