STUDIES OF ^{15}N-LABELED AMINO SUGARS[1]. THE SYNTHESIS AND MASS SPECTROMETRY OF DERIVATIVES OF 6-AMINO-6-DEOXY-D-GLUCOSE-*6*-^{15}*N**

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ABSTRACT

Derivatives of 6-amino-6-deoxy-D-glucose-*6*-^{15}*N* have been synthesized in high yield and high chemical and isotopic purity by reaction of the 6-*O*-*p*-tolylsulfonyl or 6-deoxy-6-iodo derivative of 1,2:3,5-di-*O*-isopropylidene-α-D-glucofuranose with potassium phthalimide-^{15}*N*. The infrared and mass spectra of some of these derivatives are discussed. Comparisons of the mass spectra of the ^{14}N compounds and ^{15}N-labeled derivatives afforded confirmation of the pathways of fragmentation.

INTRODUCTION

Derivatives labeled with heavy isotopes such as ^{2}H, ^{3}H, ^{13}C, and ^{14}C have played an important role in the development of carbohydrate chemistry and in its application to biochemical problems. However, despite the very considerable importance[2] of amino sugars in biochemical processes and in relation to carbohydrate-containing antibiotics, very few examples are known of amino sugars that are highly enriched with ^{15}N, apparently because many of the popular methods for their synthesis employ a large excess of the nitrogen-introducing reagent, for example, ammonia or hydrazine. Such symmetrical, multi-nitrogen species as hydrazine and the azide ion are unsuitable for the introduction of ^{15}N in high enrichment, as double labeling would be required.

The availability of ^{15}N-labeled amino sugars as reference materials is important from several points of view, including the biosynthesis of carbohydrate-containing antibiotics and of physiologically active polymers of amino sugars, and the application of mass spectrometry to these problems. With respect to the exploitation of ^{15}N magnetic resonance parameters in structural and conformational characterization, the natural abundance and n.m.r. sensitivity of ^{15}N are so low that highly enriched derivatives are a virtual necessity for successful work by existing techniques[3].

A synthesis of 1,1-bis(acetamido)-1-deoxy-L-erythritol containing 6% of ^{15}N has been reported[4], and highly enriched, singly- and doubly-labeled sugar osazones[5] and formazans[6,7] have been described.

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In the present work, an efficient, high-yielding synthesis of amino sugars was desired, and so nucleophilic substitution of suitable leaving-groups by phthalimide-^{15}N ion was employed[8]; potassium phthalimide-^{15}N is readily available with enrichment greater than 99%. The method has been applied initially to the synthesis of derivatives of 6-amino-6-deoxy-D-glucose-6-^{15}N, which, in its unlabeled form, occurs naturally[9] in kanamycin.

RESULTS AND DISCUSSION

The most convenient starting-material for the synthesis of derivatives of 6-amino-6-deoxy-D-glucose-6-^{15}N was found to be 1,2:3,5-di-*O*-isopropylidene-6-*O*-*p*-tolylsulfonyl-α-D-glucofuranose (**1**), which was prepared in 94–98% yield by acid-catalyzed reactions of 1,2-*O*-isopropylidene-6-*O*-*p*-tolylsulfonyl-α-D-glucofuranose with 2,2-dimethoxypropane or mixtures of it with acetone. However, 6-deoxy-6-iodo-1,2:3,5-di-*O*-isopropylidene-α-D-glucofuranose (**2**) may also be used as an intermediate. In the present work, **2** was prepared in 73% yield by treatment of **1** with sodium iodide in butanone, although this derivative (**2**) may also be synthesized directly in at least 25% yield by rearrangement of 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose by methyltriphenoxyphosphonium iodide[10,11], or, less conveniently, by treatment of the corresponding 6-bromo-6-deoxy analog with sodium iodide[12].

Treatment of **1** or **2** with 1.05–1.2 molecular equivalents of potassium phthalimide or its ^{15}N-labeled form in dry hexamethylphosphoramide at 100° gave crystalline 6-deoxy-1,2:3,5-di-*O*-isopropylidene-6-phthalimido-α-D-glucofuranose (**3** or **3**-^{15}N) in yields of 81–84%. The use of a slight excess (0.2 molecular equivalent) of the potassium phthalimide generally gave a purer product. However, use of boiling *N*,*N*-dimethylformamide as the reaction solvent did not result in significantly lower yields of **3**. After two recrystallizations, the purity of **3** was determined by differential scanning calorimetry[13] (d.s.c.). The d.s.c. thermogram (see Fig. 1a) shows a single, fairly sharp maximum, and analysis of the curve (see Fig. 1b and 1c) gave a heat of fusion of 29.2 kJ.mole^{-1} (6.99 kcal.mole^{-1}) and a purity value of 99.58 mole %.

The infrared spectra of **3** and **3**-^{15}N are very similar, except that **3** shows a strong absorption band at 1400 cm^{-1} whereas **3**-^{15}N shows it at 1388 cm^{-1}. These bands have therefore been assigned to C–^{14}N and C–^{15}N stretching vibrations[14], respectively. The isotopic shift (12 cm^{-1}) is considerably smaller than the value (47 cm^{-1}) calculated from the relationship $\nu(C-^{15}N) = \nu(C-^{14}N)\sqrt{(14/15)}$, which depends on the assumption that the force constant for each type of carbon–nitrogen bond is the same[15].

When **3** and **3**-^{15}N were heated with hydrazine hydrate in boiling ethanol, the *N*,*N*-phthaloyl group was smoothly removed, giving syrupy 6-amino-6-deoxy-1,2:3,5-di-*O*-isopropylidene-α-D-glucofuranose (**4** and **4**-^{15}N) in yields of 83–97%. Compound **4** was characterized as its known, crystalline, *p*-toluenesulfonate (salt), which has been reported[16] to be one of the several products isolated from the reaction of **1** with ammonia. The amines **4** and **4**-^{15}N were also converted into their crystalline *N*-acetyl (**5** and **5**-^{15}N) and *N*-(trifluoroacetyl) (**6** and **6**-^{15}N) derivatives.

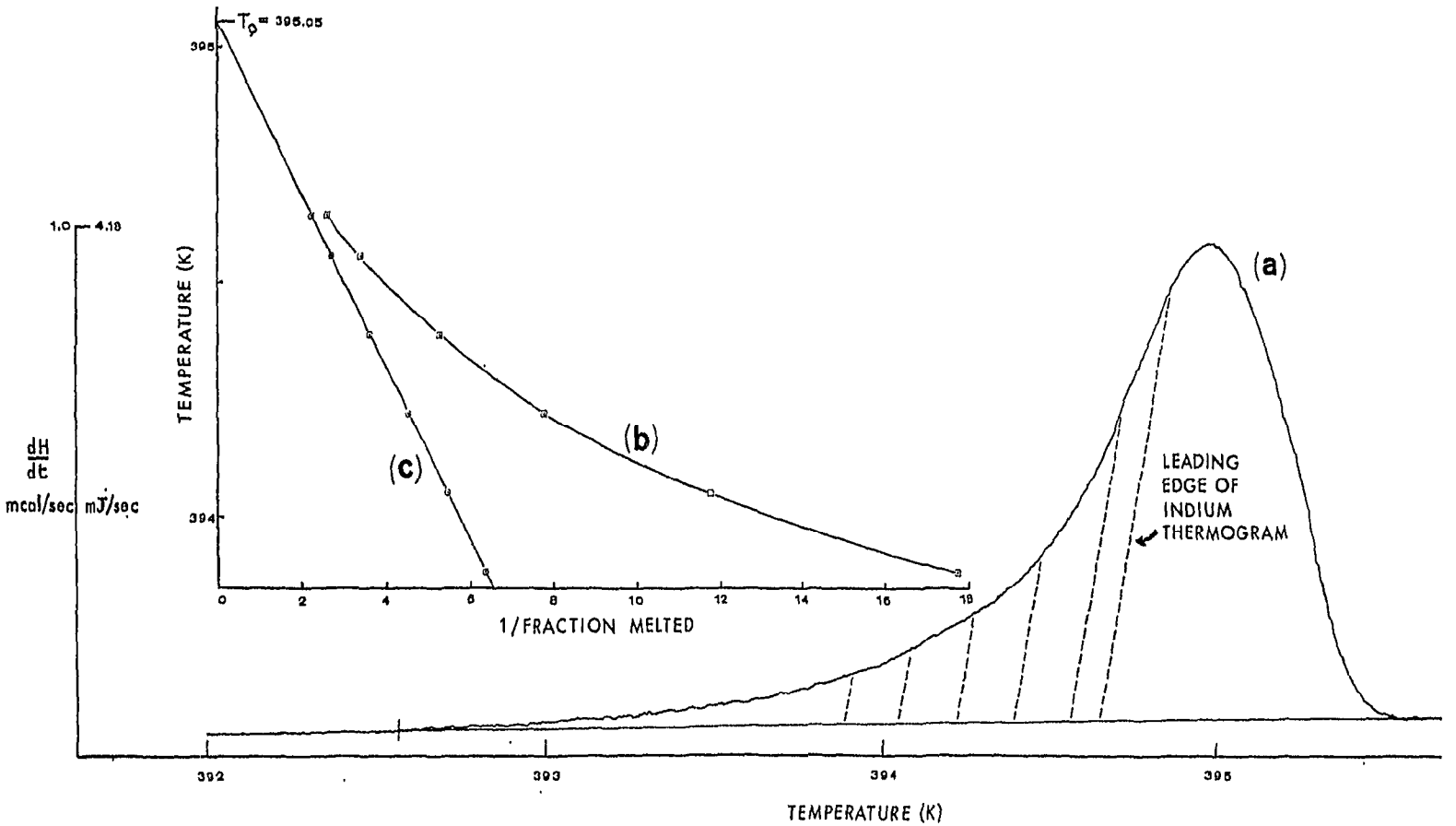
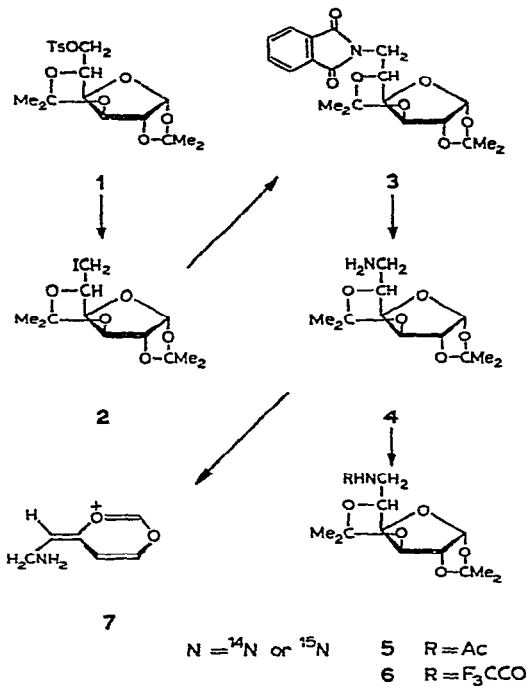


Fig. 1. (*a*) Thermogram from differential scanning calorimetry of 6-deoxy-1,2:3,5-di-*O*-isopropylidene-6-phthalimido-α-D-glucofuranose (**3**). Plot of the reciprocal of the fraction melted against temperature (*b*) before linearization, and (*c*) after linearization.



MASS-SPECTRAL STUDIES

Previous studies of the mass spectra of amino sugars (predominantly ^{14}N) have been concentrated on their *N*-acetyl and *N*-acetyl-d_3 derivatives[17–20]. In the present work, the mass spectra of the free amines (**4** and **4**-^{15}N) and of their *N*,*N*-phthaloyl derivatives (**3** and **3**-^{15}N) have been studied.

The mass spectra of **4** and **4**-^{15}N (see Table I) indicate at least four different pathways of fragmentation under electron impact. The most characteristic pathway involves scission of the C-5–C-6 bond to give an extremely abundant, resonance-stabilized ion at *m/e* 30 [*m/e* 31]* ($CH_2=NH_2^+$) together with *m/e* 229 (M−30)[M−31]. Fragmentation of the latter by loss of acetone[21] then gives an ion at *m/e* 171 (M−30−58)[M−31−58] that may suffer cleavage of a methyl radical from the remaining isopropylidene group to give a peak at *m/e* 156 (M−30−58−15) [M−31−58−15]. A different series of ions results if this cleavage occurs first: *m/e* 244[245] (M−15)→*m/e* 202[203](M−15−42), 184[185](M−15−60), 159[160] (M−15−85), and 186[187](M−15−58). The last four ions arise, respectively, by loss either of ketene[22a], acetic acid[21], or a 2-methyl-*m*-dioxolenium cation (*m/e* 85) from the isopropylidene glycol moiety that has previously lost a methyl radical, and by elimination of acetone from the other isopropylidene group[21].

Elision of the terminal aminomethylene group from the ions of *m/e* 202, 186,

*The corresponding fragment ion derived from the ^{15}N-labeled derivative is shown here and hereafter in brackets, either in terms of *m/e* or as an assignment, according to whether the mass of the fragment is or is not changed by the labeling.

and 184 can then give the ions at *m/e* 172(M−15−42−30)[M−15−42−31], 156 (M−15−58−30) [M−15−58−31], and 154(M−15−60−30)[M−15−60−31], respectively. Elimination of acetic acid from the ion of *m/e* 186 or of acetone from that of *m/e* 184 gives an ion having *m/e* 126[127](M−15−58−60), to which structure **7** was assigned. The formation of some of the foregoing ions can also be initiated by loss of acetone from **4**, to give *m/e* 201[202](M−58). Decomposition of this ion can proceed by loss of a methyl radical and ketene, to give *m/e* 144[145](M−58−15−42), or by loss of a further molecule of acetone, to give *m/e* 143[144](M−58−58).

The observation of weak ion-currents at *m/e* 215(M−44)[M−45] and 157 (M−44−58) [M−45−58] suggests that elision of protonated ethylenimine is a minor pathway. Weak, pressure-sensitive M+1 ions were observed both for the amines **4** and **4**-^{15}N and their *N,N*-phthaloyl derivatives **3** and **3**-^{15}N. Additionally, in the low-mass range, all of these compounds showed strong ion-currents at *m/e* 43 (acetylium), 59 (protonated acetone), 85 (2-methyl-*m*-dioxolenium) and its partial precursor at 100 (2,2-dimethyl-*m*-dioxolene cation), 113 (2,2-dimethyl-*m*-dioxenium), and 129 (**19**, protonated 4-formyl-2,2-dimethyl-*m*-dioxolene)[21].

The mass spectra of the *N,N*-phthaloyl derivatives **3** and **3**-^{15}N (see Table I) showed similar series of ions due to the loss of methyl radicals, ketene, acetone, acetic acid, or 2-methyl-*m*-dioxolenium, or various combinations of these moieties. In each case, the proposed nitrogen-containing ions from **3**-^{15}N were observed at one mass unit greater than those from **3**. However, it appears that cleavage of the phthalimido group, or parts of it, occurs via several different mechanisms. Scission of the C-5–C-6 bond gives a phthalimidomethylene cation[23] having *m/e* 160[161] and the carbohydrate ion at *m/e* 229 (M−160)[M−161] that was also obtained from the amines **4** and **4**-^{15}N. Loss of acetone from this ion gives an ion at *m/e* 171.

Cleavage within the phthalimido group gives a dehydrobenzoyl radical-cation (**8**) at *m/e* 104, and an isocyanate ion (**9**), at *m/e* 285[286](M−104) that loses acetone to give *m/e* 227[228] (M−104−58). However, a peak having *m/e* 105, corresponding to benzoyl ion, was also observed.

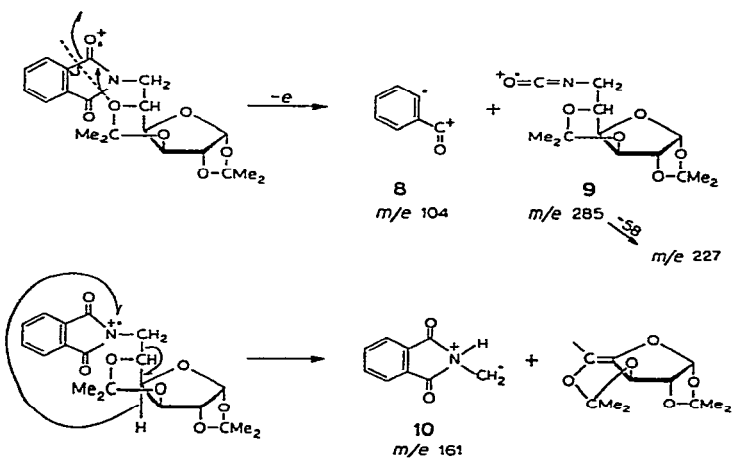


TABLE I

MASS-SPECTRAL DATA FOR 6-AMINO-6-DEOXY-1,2:3,5-DI-*O*-ISOPROPYLIDENE-α-D-GLUCOFURANOSE DERIVATIVES

m/e	*Relative intensities*[a] *for derivative*				*Assignments for* 3-^{15}N
	4	4-^{15}N,	3	3-^{15}N	
30	20.2				
31		43.1			
43	71.8	100.0	35.5	11.1	Ac^+
55	14.8	17.4	3.7	2.0	
56	7.7	6.0	1.5	0.45	
57	11.3	22.8	2.0	1.3	
58	15.1	1.8	4.8	1.2	
59	53.4	56.3	21.6	8.7	$MeAc+H^+$
60	7.0	5.2	0.98	0.41	
61	1.6	4.9	0.59	0.28	
68	7.8	9.3	3.7	2.1	
69	6.0	9.2	1.5	0.61	
70	7.3	3.7	0.70	0.18	
71	9.9	12.6	2.7	2.2	
72	7.4	2.3			
73	7.3	12.3	1.7	1.3	
74			0.52	0.51	
75			0.85	0.41	
76			3.2	1.3	$C_6H_4^{+\cdot}$
77			3.3	2.1	$C_6H_5^+$
78			0.62	0.35	
80	1.9				
81	5.9	6.6	2.4	1.4	
83	6.0	7.9	0.73	0.98	
84	14.9	10.4	5.3	4.4	M−87−161−58
85	26.5	31.1	7.8	4.6	Me-dioxolenium
89		6.7			
97	7.8	7.3	2.2	2.2	
98	5.4	3.8			
99	5.0	5.9			
100	18.4	11.3	7.7	6.2	Me_2−dioxolene$^+$
101	8.9	5.5			
102		7.4			
104			3.5	1.6	$C_6H_4CO^+$
105			2.8	2.5	$C_6H_5CO^+$
113	100.0	99.4	100.0	100.0	Me_2−dioxenium
114	17.3	16.8	12.6	14.2	
126	7.6				
127	8.6	6.7			
129	14.8	9.8	13.9	11.2	Me_2−dioxolene-$CHOH^+$
130			6.7	1.3	
131				3.8	$NCC_6H_4CO^+$
142	8.0	4.0	6.2	5.3	M−87−161
143	5.5	5.0			
144	2.7	3.6			
145		3.0			
148			2.0		
149				4.0	phthalimide$+H^+$

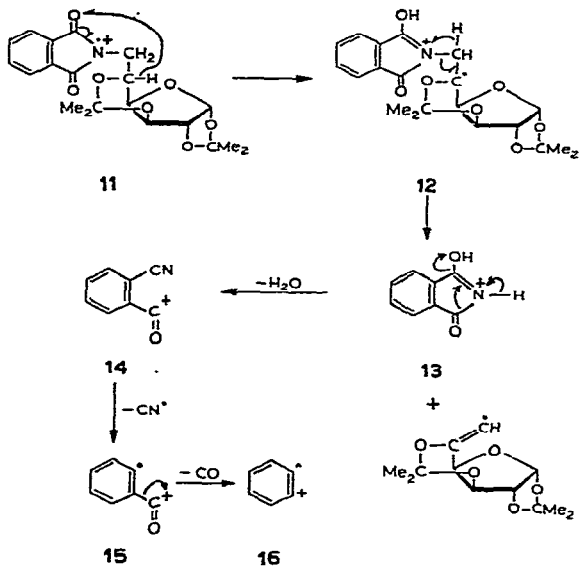
TABLE I (continued)

m/e	Relative intensities[a] for derivative				Assignments for 3-^{15}N
	4	4-^{15}N	3	3-^{15}N	
154	4.1	2.4			
155	3.9	5.0			
156	3.4	3.2			
157	1.7	3.2			
158	1.3				
159	3.4				
160	4.9	4.4	28.2		
161		3.7	9.0	19.5	phthalimide + CH_2^+
162				7.6	phthalimide + $CH_2^{+\cdot}$
171	14.4	13.3	4.3	6.3	M − 161 − 58
172	5.3	5.9			
184	2.4				
185	3.2	1.8			
186	2.6				
187		2.5			
190			9.0	10.3	
201	1.7				
202	1.6	2.8	4.2		
203		1.2		5.5	Phthalimidopropenyl epoxide$^+$
215	1.3	1.4			
227			3.2		
228				3.1	M − 58 − 104
229	15.3	15.0	6.4	8.5	M − 161
230	2.5	2.2			
231			5.6		
232				8.1	M − 58 − 15 − 85
244	3.4				
245	0.7	4.8			
256			4.8		
257				6.4	M − 58 − 15 − 60
260	1.5				
272			21.7		
273			7.2	25.6	M − 58 − 59
274			5.0	9.2	M − 58 − 58
275				4.2	M − 15 − 42 − 58
285			2.3		
286				3.5	M − 104
302			12.2		
303			2.0	14.8	M − 87
304				2.9	
316			1.5		
317				1.4	M − 15 − 58
331			0.79		
332			0.79	0.30	M − 58
333				0.67	M − 15 − 42
374			11.5		
375			2.1	10.3	M − 15
376				2.2	
390			0.83		
391				0.22	M + 1

[a]Percent of base peak.

The formation of a nitrogen-containing ion of *m/e* 161[162] (protonated phthalimidomethylene radical-ion, **10**) is attributed to a McLafferty rearrangement[24] of the molecular ion, in which H·-4 migrates to the nitrogen atom with concurrent homolysis of the C-5–C-6 bond, the charge remaining exclusively at this atom. Similar rearrangements have been postulated for the mass-spectral fragmentation of *N*-butylsuccinimide and related derivatives[22b].

An alternative rearrangement and degradation of the molecular ion (**11** → **12** → **13**) involves successive migration of hydrogen atoms from C-5 to a carbonyl oxygen atom and from C-6 to the nitrogen atom with concomitant fission of the N–C-6 bond.

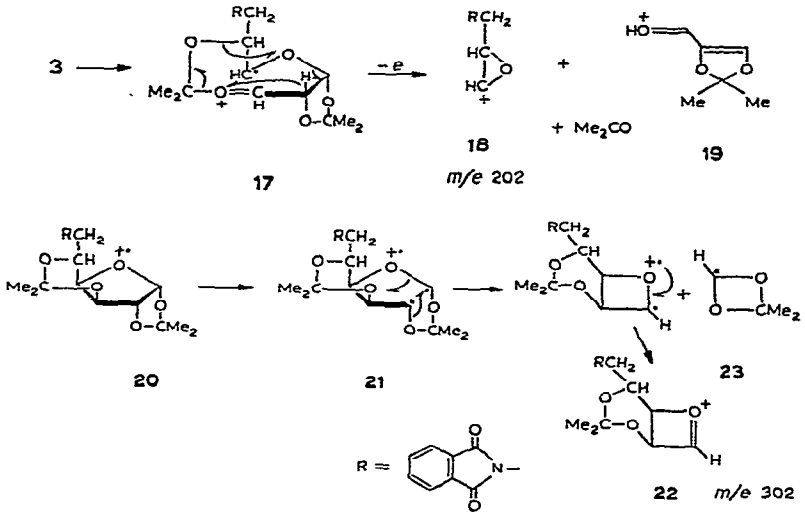


The resulting protonated phthalimide **13**, *m/e* 148[149], has been shown to be a common intermediate in the fragmentation of *N*,*N*-phthaloylamino acids[25]. Dehydration of **13** gives the cyanobenzoyl ion (**14**), *m/e* 130[131], that can lose[25] a cyanide radical to give the dehydrobenzoyl radical ion (**15**), *m/e* 104. Expulsion[26] of carbon monoxide from the latter ion gives a radical ion $C_6H_4^{+\cdot}$ that has been interpreted[22c] as being a benzyne ion (**16**).

The formation of a nitrogenous ion (**18**) at *m/e* 202[203] appears to be due to the complex fragmentation-mechanism (**11** → **17** → **18** + **19**) proposed[21] for fragmentation of 1,2:3,5-di-*O*-isopropylidene-α-D-xylofuranose.

The presence of a nitrogen-containing ion at *m/e* 302[303](M − 87) seems unusual. It can be rationalized in terms of the mechanism **20** → **21** → **22** + **23**, in which an initial homolysis of the C-1–C-2 bond of the molecular ion **20** is followed by ring contraction and scission, to give the oxetane cation (**22**), *m/e* 302[303], and the 1,3-dioxacyclobutyl radical (**23**). The cation **22** may then suffer loss of the phthalimido-

methylene group to give the ion at *m/e* 142 (M−87−160)[M−87−161], which may lose acetone to give that at *m/e* 84.



The mass spectra of the ^{15}N-labeled derivatives provide further support for the assignments proposed[21], for the spectra of simple *O*-isopropylidenehexoses, that were based on deuterium labeling[21,27] and high-resolution spectra[21].

All of the compounds in the present study had n.m.r. spectra that were consistent with the structures proposed. The parameters obtained by analysis of these spectra will be reported in the near future[28].

EXPERIMENTAL*

General. — Evaporations were performed under diminished pressure, and optical rotations were measured for solutions in chloroform, unless stated otherwise. Infrared spectra were recorded with a Perkin–Elmer grating spectrometer Model 257, and their absorption maxima are given in cm^{-1} with intensity indicated as m (moderate), s (strong), or w (weak), together with probable assignments.

Mass spectra were recorded, at an ionizing potential of 70eV, with a sample inlet temperature of 50–110° and an ionization chamber temperature of 200–290°, either with a CEC Model 21-491 or an LKB Model 9000 spectrometer. Thin-layer chromatography (t.l.c.) was performed on layers of Silica Gel G (E. Merck, Darmstadt).

Differential scanning calorimetry (d.s.c.) was conducted with a Perkin–Elmer calorimeter Model DSC-1B that had been calibrated against indium metal (purity, 99.9999%) by assuming its m.p. to be 429.5°K, and its heat of fusion, 3.26 $kJ.mol^{-1}$.

*Mention of commercial instrumentation in this publication does not imply recommendation or endorsement by the National Bureau of Standards, nor does it imply that the equipment identified is necessarily the best available for the purpose.

D.s.c. thermograms were obtained by using the maximum sensitivity of the instrument and a scanning rate of 0.625°K/min, starting at 391°K. The thermograms (see Fig. 1a) were divided into seven segments, and the area of each was measured in duplicate by planimetry. A curved plot (see Fig. 1b) of the reciprocal of the fraction melted against the absolute temperature of the sample was obtained. This plot was then linearized by applying to each fractional area a positive correction equal to 12% of the total area under the thermogram curve. The mole percentage of (total) impurity was calculated from the function $100\Delta H_f \Delta T/RT_0^2$, where ΔH_f, the heat of fusion of the sample, was obtained from the corrected, total area of the curve; ΔT, the melting-point depression, from the slope of the linearized plot (see Fig. 1c); and T_0, the true melting-point, from the intercept of the plot.

*1,2:3,5-Di-*O*-isopropylidene-6-*O*-p-tolylsulfonyl-α-*D*-glucofuranose* (**1**). — (*A*) *By use of acetone and 2,2-dimethoxypropane.* Concentrated sulfuric acid (1.25 ml) was added dropwise to a stirred mixture of 1,2-*O*-isopropylidene-6-*O*-*p*-tolylsulfonyl-α-D-glucofuranose (25 g), dry acetone (550 ml), anhydrous copper(II) sulfate (50 g), and Drierite (25 g). After 8 h, 2,2-dimethoxypropane (100 ml) and molecular sieve 4A (50 g) were added to the mixture, which was stirred for a further 20 h. An excess of powdered calcium oxide was added, and the suspension was stirred for 5 min and filtered through Filter-Cel; the solids were thoroughly washed with acetone, and the filtrate and washings were combined and evaporated to a mobile, yellow syrup that crystallized spontaneously on dissolution in warm ethanol followed by dilution with water. Crystallization at −12° overnight gave prisms of **1** that were washed with cold aqueous ethanol and petroleum ether, and dried (27.1 g, 98%), m.p. 85–86°. After crystallization from aqueous ethanol, it had m.p. 86–87°; lit.[29] m.p. 87°. The structure and purity of the product were confirmed by p.m.r. spectroscopy at 60 and 100 MHz.

(*B*) *By use of 2,2-dimethoxypropane.* To a stirred mixture of 1,2-*O*-isopropylidene-6-*O*-*p*-tolylsulfonyl-α-D-glucofuranose (1.06 g), 2,2-dimethoxypropane (20 ml), and molecular sieve 4A (3 g) was added concentrated sulfuric acid (50 μl) dropwise. After 4 h, the mixture was treated with calcium oxide, and processed as in (*A*) to give crystalline **1** (1.104 g, 94%), m.p. 86–87°.

*6-Deoxy-6-iodo-1,2:3,5-di-*O*-isopropylidene-α-*D*-glucofuranose* (**2**). — A mixture of **1** (1 g), purified butanone, and dry sodium iodide (10 g) was boiled under reflux for 3 h with stirring, and then concentrated almost to dryness. The resulting pale-yellow suspension was diluted with dichloromethane (100 ml), washed successively with sodium thiosulfate solution and water (twice), filtered through a filter paper wetted with dichloromethane, and the filtrate evaporated to dryness. T.l.c. of the pale-yellow oil indicated the presence of one major component and two minor impurities. The oil was therefore purified by chromatography on a column (18 × 2 cm) of Silica Gel (100–200 mesh) prepared with petroleum ether. Elution with 1:1 (v/v) dichloromethane–petroleum ether, and evaporation of the second fraction, yielded **2** as a pale-yellow, mobile syrup (654 mg, 73%) that has thus far resisted attempts at crystallization. It was homogeneous by t.l.c. and by p.m.r. spectroscopy, and had $[\alpha]_D^{32}$ +34.4° (*c* 0.52, ethanol); lit.[12] $[\alpha]_D^{18}$ +30.9° (in ethanol).

Anal. Calc. for $C_{12}H_{19}IO_5$: C, 38.9; H, 5.2; I, 34.3. Found: C, 39.1; H, 5.2; I, 35.1.

6-Deoxy-1,2:3,5-di-O-isopropylidene-6-phthalimido-α-D-glucofuranose (**3**). — (*A*) *From* **1**. A mixture of **1** (1.0 g), potassium phthalimide (473 mg, 1.05 molecular equivalents), and dry hexamethylphosphoramide (20 ml) was stirred and heated for 2 h at 100°. The pale-yellow solution was then cooled to room temperature and diluted with dichloromethane (100 ml) to give a gel that was successively washed with cold, 5% sodium hydroxide solution (200 ml) and water (4×300 ml). The colorless, organic layer was evaporated to a syrup which crystallized from aqueous ethanol, to give **3** as brilliant, fine needles, 772 mg (83%), m.p. 121–123°. Recrystallized from aqueous ethanol and then from aqueous acetone, it had m.p. 124–125° $[\alpha]_D^{29}$ +22.1° (*c* 0.58); ν_{max}^{Nujol} 1773 m and 1715 s (fused lactam ring C=O), 1611 w and 1602 w (Ar) cm^{-1}; $\nu_{max}^{CHCl_3}$ (*c* 3.25) 1775 m and 1718 s (C=O), 1615 w (Ar), 1400 s (mean of five spectra, C–^{14}N), 1158 w cm^{-1}.

Anal. Calc. for $C_{20}H_{23}NO_7$: C, 61.7; H, 6.0; N, 3.6. Found: C, 61.7; H, 5.8; N, 3.7.

A similar preparation in which boiling, dry *N,N*-dimethylformamide (15 ml) was used for 1.5 h as the reaction medium (instead of hexamethylphosphoramide) afforded an 80% yield of crystalline **3**, m.p. 123.5–124.5°.

The ^{15}N-labeled derivative (**3**-^{15}N) was prepared by heating a mixture of **1** (878 mg), potassium phthalimide-^{15}N (473 mg, 1.2 molecular equivalents; enrichment, 99.5 atom-%), and dry hexamethylphosphoramide (15 ml), with stirring, for 2 h at 100°. Isolation of the product as before gave **3**-^{15}N as colorless needles (619 mg, 84%), m.p. 123.5–124.5°. After recrystallization from hot aqueous ethanol, it had m.p. 124–125° and $[\alpha]_\lambda^{34}$ +22.3, +22.8, +26.3, +46.0, and +83.7°, at values of λ of 589, 578, 546, 436, and 365 nm, respectively (*c* 0.57); $\nu_{max}^{CHCl_3}$ (*c* 3.25) 1775 m and 1718 s (fused lactam ring C=O), 1615 w (Ar), 1388 s (mean of five spectra, C-^{15}N), 1151 w cm^{-1}.

Anal. Calc. for $C_{20}H_{23}{}^{15}NO_7$: C, 61.5; H, 6.0; ^{15}N, 3.8. Found: C, 61.6; H, 6.0; ^{15}N, 3.9.

(*B*) *From* **2**. A mixture of the syrupy iodo derivative **2** (191 mg) with potassium phthalimide (120 mg, 1.2 molecular equivalents) and dry hexamethylphosphoramide (5 ml) was heated and stirred for 2 h at 100°. Isolation of **3** as in (*A*) furnished colorless needles (163 mg, 81%), m.p. 124–125°, undepressed by admixture with **3** prepared as described in (*A*), and $[\alpha]_D^{26}$ +22.3° (*c* 0.66).

6-Amino-6-deoxy-1,2:3,5-di-O-isopropylidene-α-D-glucofuranose (**4**). — A solution of **3** (3.253 g) in ethanol (35 ml) and 85% hydrazine hydrate (1.0 ml, 2.1 molecular equivalents) was boiled under reflux, whereupon the solution turned yellow almost immediately, and then, after 5 min, suddenly became semi-solid. Ethanol (30 ml) was added, the cake of solid material was broken up, and the suspension was boiled under reflux for 2 h with stirring. T.l.c. indicated that the reaction was complete within 1.5 h. The suspension was cooled, and evaporated to a colorless solid to which were successively added 5% potassium hydroxide solution (100 ml) followed

by potassium carbonate (~ 10 g). The resultant solution was extracted wit hether (100 ml, 2 × 50 ml), and the extracts were combined, dried (potassium carbonate), and evaporated to give the amine **4** as a colorless syrup (2.105 g, 97%) that has not crystallized thus far. It reacted with ninhydrin at room temperature, giving a purple coloration that changed to dark red on heating; it was homogeneous by t.l.c., and showed $[\alpha]_D^{21}$ +39.2° (*c* 1.12).

Anal. Calc. for $C_{12}H_{21}NO_5$: C, 55.6; H, 8.2; N, 5.4. Found: C, 55.4; H, 8.4; N, 5.5.

The ^{15}N-labeled amine (**4**-^{15}N) was prepared in a similar way (yield, 83%).

Anal. Calc. for $C_{12}H_{21}{}^{15}NO_5$: C, 55.4; H, 8.1; ^{15}N, 5.8. Found: C, 55.4; H, 8.3; ^{15}N, 5.3.

*6-Amino-6-deoxy-1,2:3,5-di-*O*-isopropylidene-*α*-*D*-glucofuranose* p-*toluenesulfonate.* — To a solution of the amine **4** (166 mg) in acetone (5 ml) was added *p*-toluenesulfonic acid (111 mg, 1 molecular equivalent) and anhydrous ether (15 ml). On scratching part of the solution on a ground-glass surface, the salt of *p*-toluenesulfonic acid crystallized as fluffy, colorless needles (162 mg, 59%), m.p. 162–164° (dec.), $[\alpha]_D^{22}$ +28.0° (*c* 0.45, water); lit.[16] m.p. 172.5°, $[\alpha]_D^{20}$ +30.96° (*c* 4.0, water). The m.p. of the product varied according to the method of recrystallization or drying.

Anal. Calc. for $C_{19}H_{29}NO_8S$: C, 52.9; H, 6.8; N, 3.2; S, 7.4. Found: C, 52.9; H, 6.9; N, 3.2; S, 7.8.

*6-Acetamido-6-deoxy-1,2:3,5-di-*O*-isopropylidene-*α*-*D*-glucofuranose* (**5**). — The amine **4** (518 mg) was treated with dry pyridine (3 ml), followed by acetic anhydride (3 ml). By t.l.c. of the solution, the reaction was found to be essentially complete within 5 min; after 1 h at room temperature, the solution was poured into sodium hydrogen carbonate solution. The mixture was then extracted with dichloromethane (3 × 50 ml), and the extracts were combined, successively washed with 5% copper(II) sulfate solution (50 ml) and water (50 ml), dried (sodium sulfate), and evaporated to a pale-yellow syrup (608 mg) that crystallized on being kept for several weeks at 0°. Recrystallization from ether–hexane yielded long needles of **5** (536 mg, 89%), m.p. 154–155°. Decolorization of the product with charcoal in ethanol, followed by further recrystallizations from ethanol–hexane and from ethyl acetate, afforded bipyramidal prisms, m.p. 156–157°, $[\alpha]_D^{20}$ +27.0° (*c* 0.48); ν_{max}^{film} 3285 m and 1547 m (NH), and 1652 cm^{-1} (amide C=O).

Anal. Calc. for $C_{14}H_{23}NO_6$: C, 55.8; H, 7.7; N, 4.6. Found: C, 56.1; H, 7.9; N, 4.7.

The ^{15}N-labeled derivative (**5**-^{15}N) was prepared similarly; m.p. 155–156°.

Anal. Calc. for $C_{14}H_{23}{}^{15}NO_6$: C, 55.6; H, 7.7; ^{15}N, 5.0. Found: C, 56.9; H, 7.8; ^{15}N, 4.7.

Compounds **5** and **5**-^{15}N were unstable and tended to decompose during storage or drying.

*6-Deoxy-1,2:3,5-di-*O*-isopropylidene-6-(trifluoroacetamido)-*α*-*D*-glucofuranose* (**6**). — A solution of the amino derivative **4** (298 mg) in dry pyridine (4 ml) was cooled to 0°, and trifluoroacetic anhydride (2 ml) was added with swirling and cooling of the

solution. The viscous mixture was kept for 10 min at 0° and for 50 min at room temperature, diluted with dichloromethane (125 ml), washed twice with ice-cold, saturated sodium hydrogen carbonate solution and thrice with water, and dried (sodium sulfate). Evaporation of the pale-brown solution yielded a partially crystalline syrup (299 mg, 73%) that was recrystallized from ether–hexane, giving 187 mg (46%) of **6**, m.p. 139–141°. Decolorization of the product in ethanol followed by recrystallization from ether–hexane gave irregular, hexagonal prisms of **6**, m.p. 141–142°, $[\alpha]_D^{29}$ +36.3° (*c* 1.28); ν_{max}^{film} 3400 m and 1570 m (NH), and 1720 s cm^{-1} (amide C=O).

Anal. Calc. for $C_{14}H_{20}F_3NO_6$: C, 47.3; H, 5.7; N, 3.9. Found: C, 47.4; H, 5.6; N, 4.0.

Compound **6**-^{15}N was prepared in a similar manner, and had m.p. 140–141°.

Anal. Calc. for $C_{14}H_{20}F_3{}^{15}NO_6$: C, 47.2; H, 5.7; ^{15}N, 4.2. Found: C, 47.5; H, 5.7; ^{15}N, 3.9.

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