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SUBSTITUTED GLYCOSYLAMINES CONTAINING THE INDOLE NUCLEUS

MASS SPECTRAL STUDIES OF ACETYLATED GLUCOPYRANOSYLAMINE DERIVATIVES

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Abstract—The mass spectra of nine acetylated glucopyranosylamine derivatives (of which four are new) are compared with the mass spectra of nucleosides and acetylated sugars.

INTRODUCTION

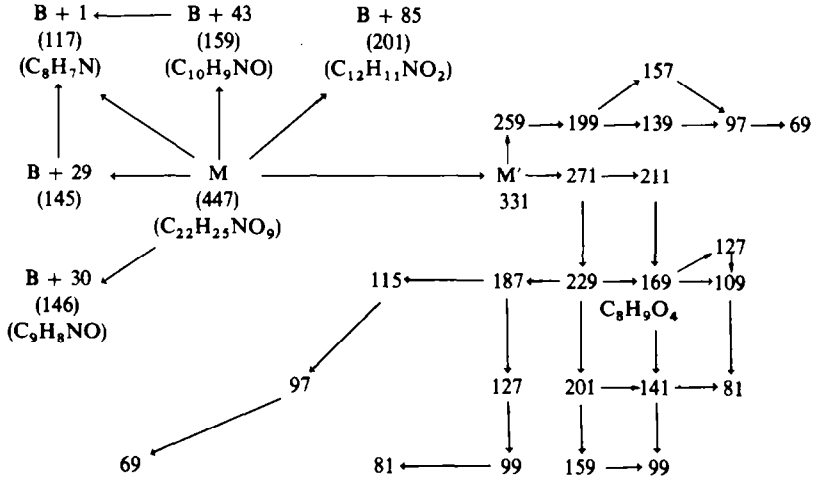
FOR a number of years nucleoside analogues such as 1-(β-D-arabinofuranosyl)-cytosine, 9-(β-D-ribofuranosyl)-6-mercaptopurine, psicofuranine and angustmycin A [6-amino-9-(6-deoxy-β-D-*erythro*-hex-5-enofuran-2-ulosyl)purine] have been known to exhibit antitumour activity. More recently certain 7-deazanucleoside analogues such as tubercidin and toyocamycin have been shown to exhibit the same property.[1] Certain 1-(β-D-ribofuranosyl)benzimidazoles exhibit antiviral activity,[2] and it has been suggested that 5,6-dichloro-1-(β-D-ribofuranosyl)benzimidazole prevents the synthesis of protein by interfering with a preliminary synthesis of RNA.[3] Compounds containing the indole nucleus, tryptophan and serotonin for example, are physiologically active, and we have evidence that certain tricyclic compounds containing the indole skeleton show antitumour activity. The synthesis of glycosyl derivatives of such compounds has been commenced with an evaluation of their antitumour potential in view.

The past decade has seen many advances in the mass spectrometric identification of sugars and of nucleosides. Since the early work of Biemann and McCloskey[4] several nucleoside derivatives have been studied by electron-impact (EI) mass spectrometry,[5–7] but no detailed fragmentation pathway has been published. Recently the technique of field ionization (FI) mass spectrometry has been applied to nucleosides.[8] The production of molecular ions of low energy gives rise to an uncomplicated spectrum showing relatively intense M, B + 1, B + 2 and M′ ions (B and M′ have *m/e* corresponding to the nitrogen-containing base less one proton and to the sugar portion of the nucleoside respectively). EI mass spectra of nucleosides are complex, usually showing low intensity M and M′ ion peaks and many other peaks of relatively high intensity.

It was felt that a study of the EI mass spectra of a series of acetylated glucosylamine derivatives would contribute to an understanding of the fragmentation of substituted nucleosides and of sugars, and would assist the mass spectrometric identification of nucleosides and glycosylamine derivatives.

RESULTS AND DISCUSSION

EI mass spectra of fully acetylated β-D-glucopyranosyl derivatives of (I) indoline, (II) 2-methylindoline, (III) 5-nitroindoline, (IV) indole, (V) 2-methylindole, (VI) 5-nitroindole, (VII) 1,2,3,4-tetrahydroquinoline, (VIII) 6-aminoquinoline and (IX) *p*-nitroaniline (Figs 1–4) show a large number of peaks; the relative intensities of some of these are presented in Table 1. The spectra show prominent M, M′, B + 85, B + 43, B + 30, B + 29 and B + 1 peaks, as well as those typical of most of the several fragmentation paths reported for acetylated and acetylated partially methylated sugars.[9–11] A possible fragmentation pathway is presented in Scheme 1; the *m/e* values given in parenthesis refer specifically to the spectrum of compound IV.

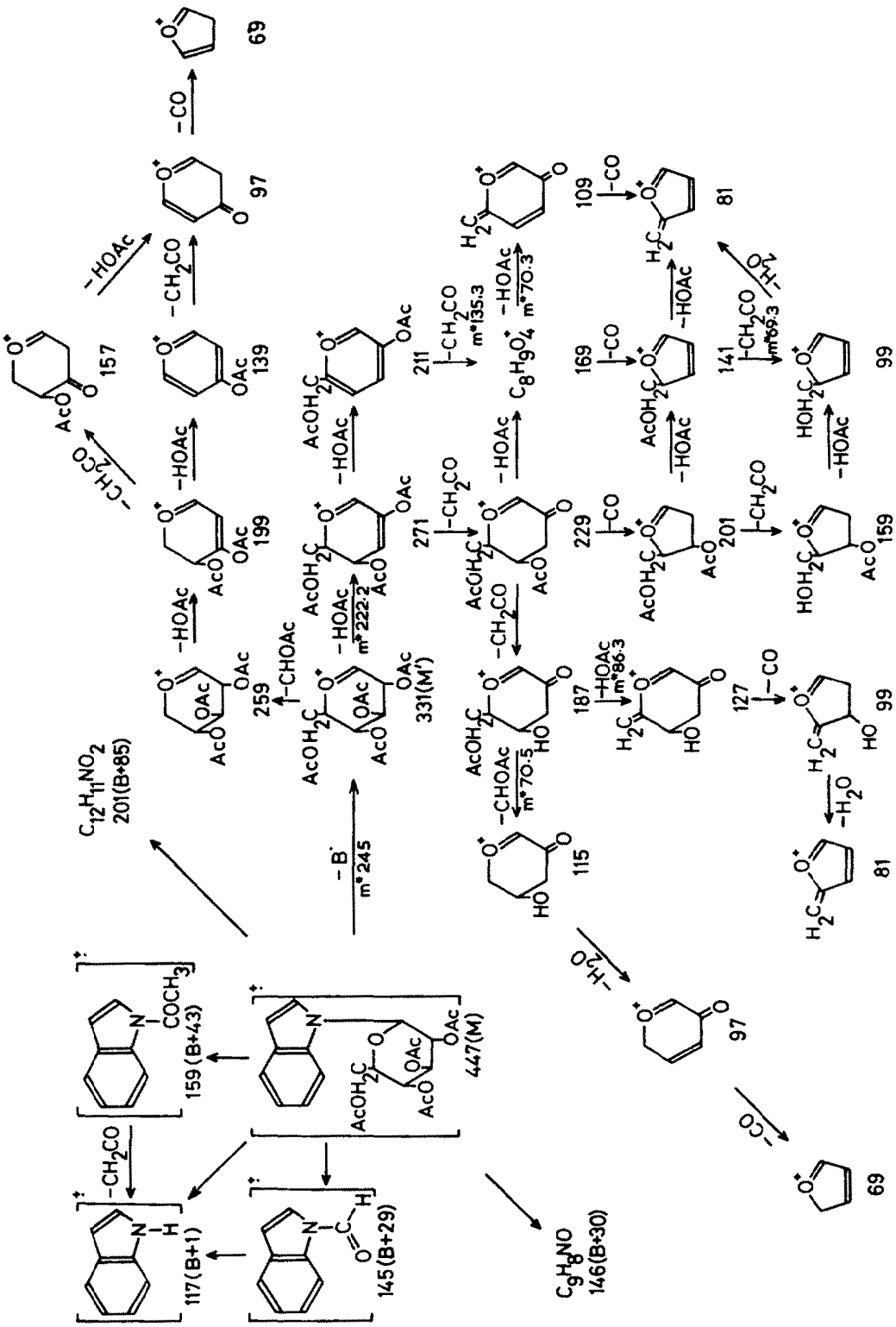


SCHEME 1. A possible fragmentation pathway for substituted glucopyranosylamines. *m/e* Values given in parentheses and formulae refer to fragments of IV.

The relative intensity of peaks corresponding to *m/e* values of M − 73, B + 85, B + 43, B + 30, M′ − 88 and M′ − 190 for the three indolic compounds (IV–VI) is very much less than for the remaining compounds. Also the ratio of relative intensities B + 85:B + 84 and B + 30:B + 29 is less than unity for IV–VI, but larger than unity for the remaining compounds.

Direct fragmentation of the glycosidic bond would lead to M′ and B peaks. Although both of these fragments are found in the spectra of I–IX, the peak at *m/e* corresponding to B + 1 is of much greater intensity than that at B. Biemann and McCloskey[4] suggest that for nucleosides the hydrogen transferred is of hydroxyl origin. Although loss of ketene from the molecular ion would give rise to an hydroxyl-containing fragment (M − 42), metastable scanning of the B + 1 ion in IV shows that the M − 42 ion is not a precursor ion, but that the molecular ion itself is one. Eggers *et al.*[6] have suggested an alternative mechanism resulting in cleavage of the N—C_1 bond[12] with transfer of the C_2 hydrogen of the sugar moiety, and this may operate here.

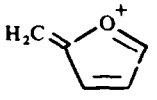
Penta-O-acetyl-β-D-glucopyranose (X) gives a base peak at *m/e* 115 and other major peaks at *m/e* 200, 169, 157, 140, 109 and 98. The major peaks for the sugar moiety of I–IX are found at *m/e* 331, 169 (the base peak in several cases), 141, 139, 127,



SCHEME 2. Possible fragmentation pathway for IV.

115, 109, 97 and 81. With the exception of the fragmentation pathway *m/e* 242 → 200 → 140 → 98, which is of low intensity (probably due to the greater ability of the base to hold a positive charge compared with the sugar ring), the several pathways suggested[9] for the fragmentation of X are present in the spectra of I–IX.

The peaks at *m/e* 81 and 69 are more prominent in the spectra of I–IX than in the spectrum of X. In a recent paper Rosenthal,[13] who carried out mass spectral studies on unsaturated sugars, postulated the pyronium ion structure for the *m/e* 81 ion. Although no proof of the structures of postulated fragments can be given without isotope labelling, XI is a likely structure arising from I–IX; fitting the sequence *m/e* 201 → 141 → 81 to the remainder of the fragmentation pathway (Schemes 1, 2) requires the loss of a molecule of carbon monoxide,[14] giving rise to a furanoid ring.



XI *m/e* 81

The fragmentation pathway suggested in Schemes 1 and 2 is postulated for compound IV from the evidence given above, the position of prominent peaks caused by metastable transitions, metastable scans for ions at *m/e* 109, 169 and 117, and accurate mass measurements. The limitations of such a scheme are realised; *for most of the structures suggested above, several isomeric structures are possible.* The intensity of several key fragments is low, as can be seen from Table 1, but many of the fragmentations suggested are supported by metastable peaks.

The results discussed above are substantiated by the mass spectra of other acetylated glycosylamine derivatives containing the same bases, but different sugar moieties.[15] From these results the structures of similar compounds may be elucidated by mass spectrometry.

EXPERIMENTAL

Mass spectra were determined at 70 eV with an A.E.I. MS 9 spectrometer at temps varying between 100° and 200°, using the direct insertion technique. IR spectra were recorded on a Perkin–Elmer 237 spectrometer as hexachlorobutadiene mulls on NaCl plates. NMR spectra were determined on a Varian HA-100 spectrometer from dilute solutions in $CDCl_3$ using TMS as internal standard. (*J* refers to splittings estimated to be accurate to ±0·3 Hz. Chemical shifts are estimated to be correct to ±0·01 ppm).

TLC was carried out on silica gel plates using the following solvent systems (both v/v): *a* $CHCl_3$–AcOEt (5:2), *b* C_6H_6–AcOEt (19:1). Components were made visible by spraying with *p*-dimethylaminobenzaldehyde in HCl (Ehrlich reagent) or by charring after being sprayed with 2N H_2SO_4. M.ps were obtained on a Fisher–Johns m.p. apparatus and are uncorrected. Optical rotations are given as equilibrium values, measured at 25°.

The syntheses of I, II, III, IV[16–19] and IX[20,21] have been described.

1-(2,3,4,6-*Tetra*-O-*acetyl*-β-D-*glucopyranosyl*)-2-*methylindole* (V). II (0·71 g, 1·53 mmole) and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ; 0·35 g, 1·54 mmole) were heated under reflux in dry *m*-xylene (25 ml) for 3 hr. The hot reaction mixture was filtered, the volume of filtrate reduced to 10 ml *in vacuo*, and the concentrated soln allowed to cool. After separation of the brown precipitated crystals (100 mg), the xylene volume was further reduced, the soln cooled to 0° and a second crop of the solid collected. TLC showed that the product (V; 400 mg, 57%), which was homogeneous, contained both indole and sugar moieties (R_f slightly less than that of II, but stained red with Ehrlich reagent as opposed to the yellow of II).

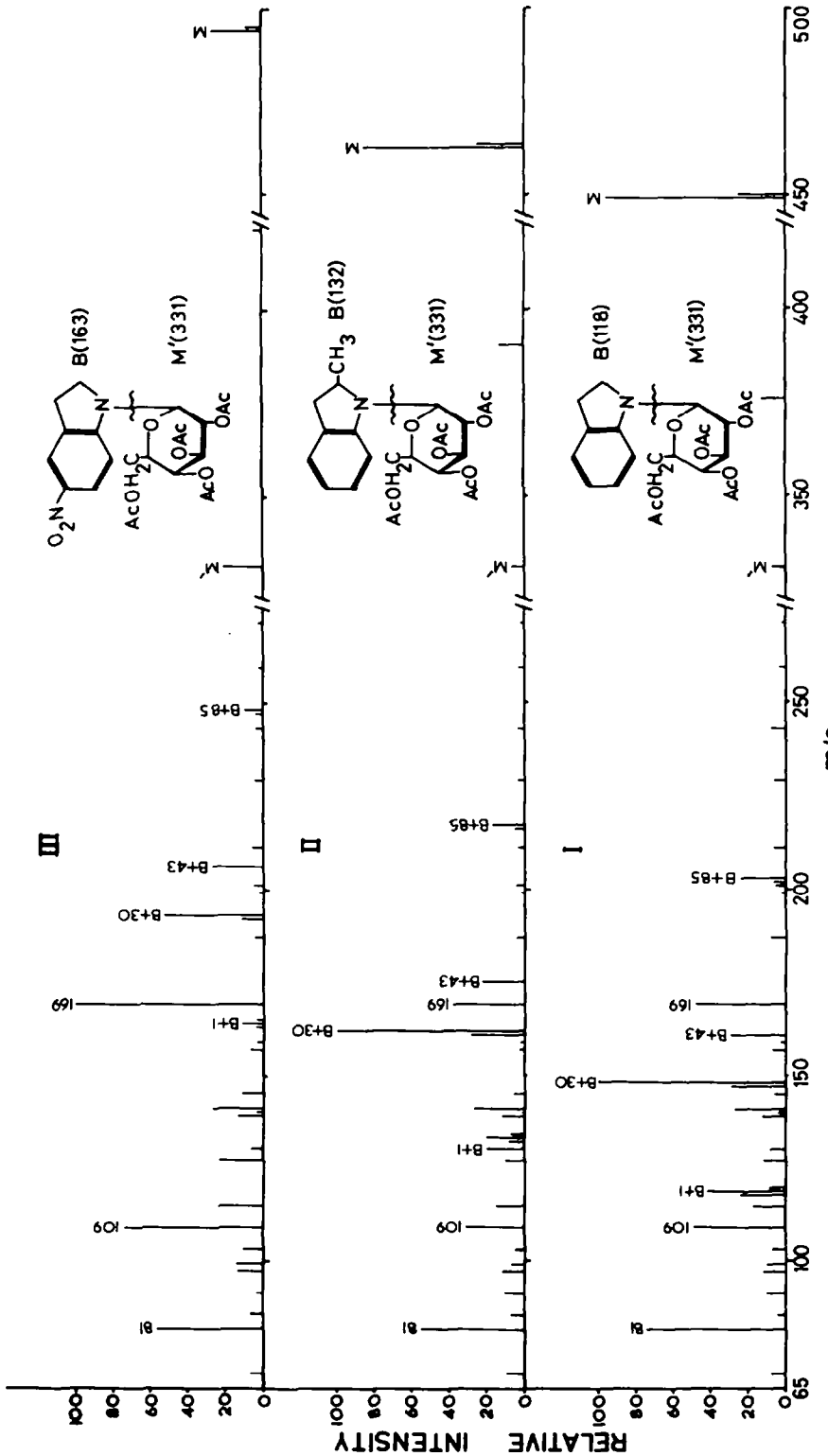


FIG 1. 70 eV EI Mass spectra of I, II, III.

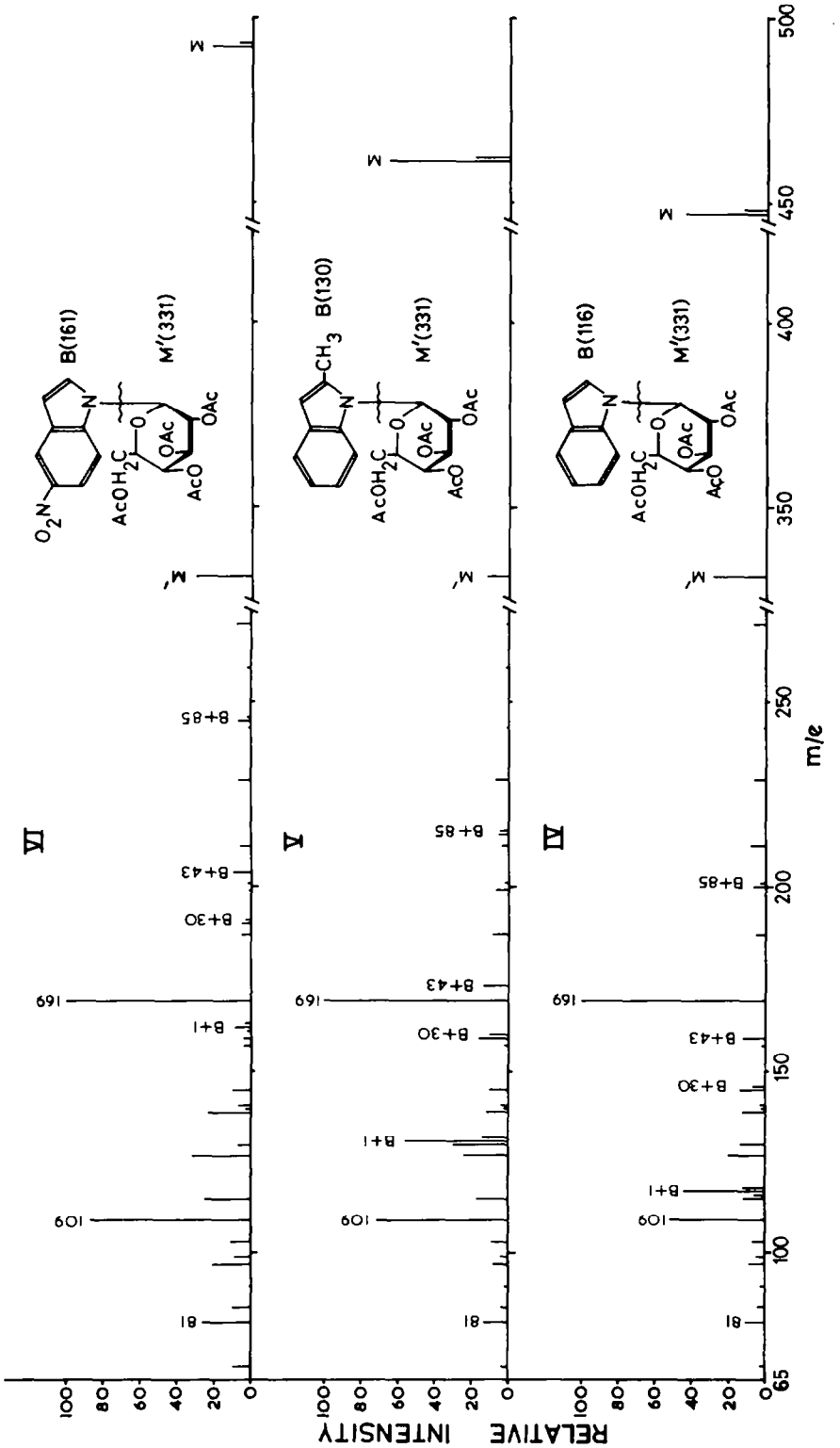


FIG 2. 70 eV EI Mass spectra of IV, V, VI.

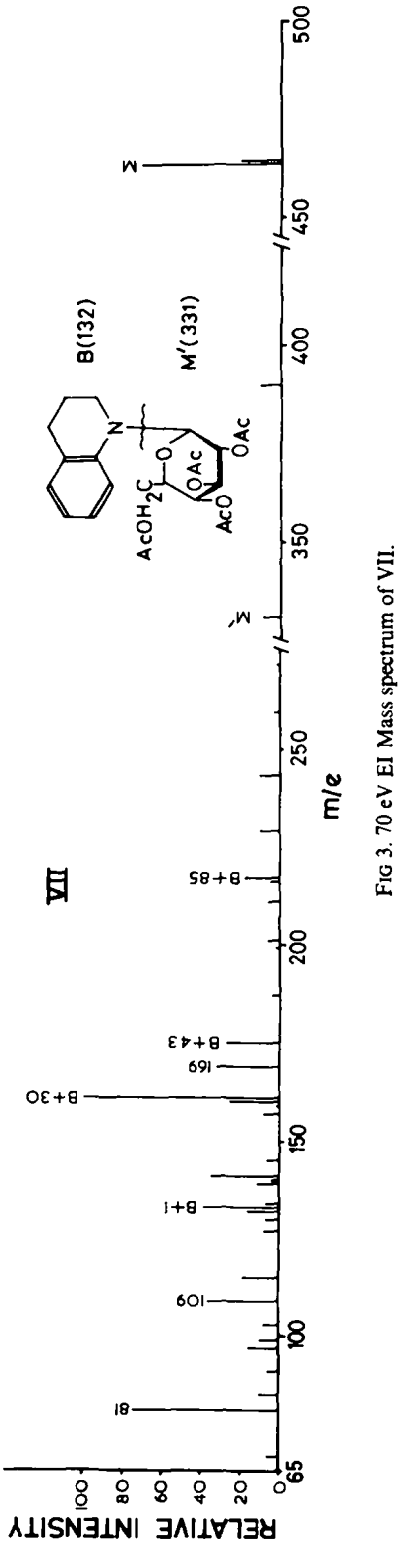


FIG 3. 70 eV EI Mass spectrum of VII.

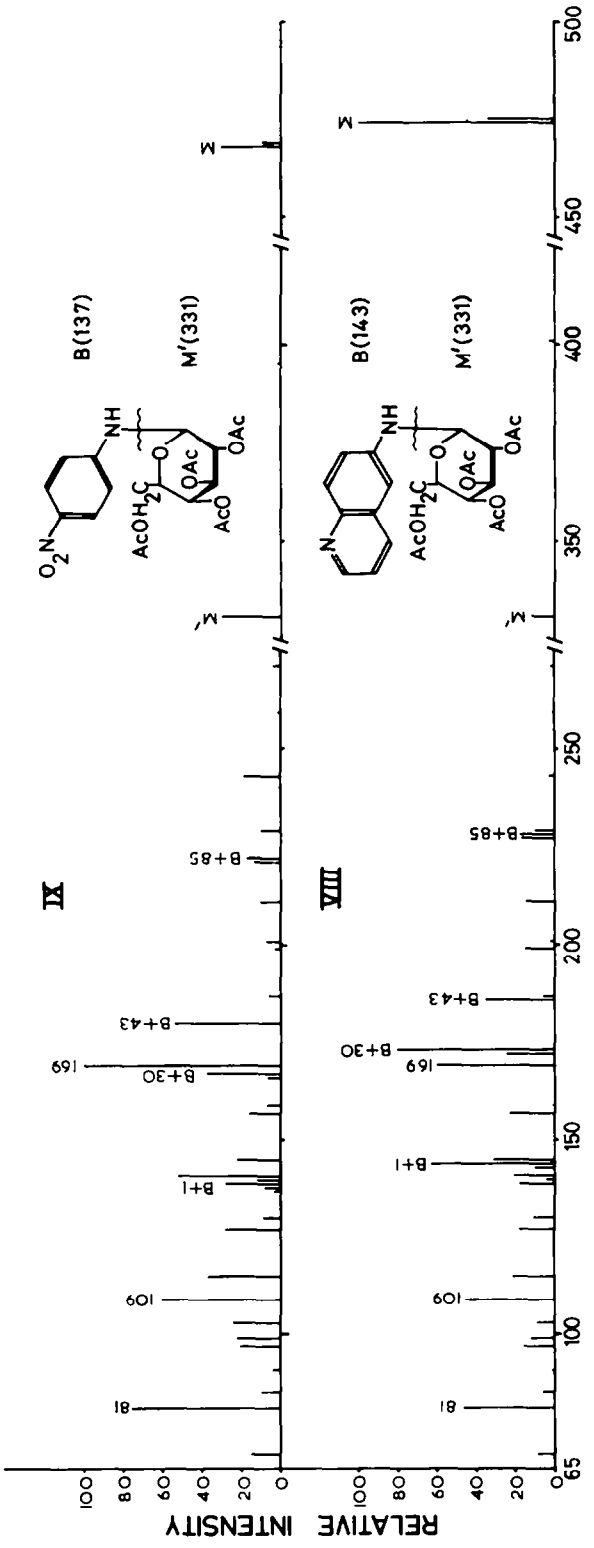


FIG. 4. 70 eV EI Mass spectra of VIII, IX.

TABLE 1. MASS SPECTRA OF SUBSTITUTED GLUCOSYLAMINES (I–IX): RELATIVE INTENSITIES OF PEAKS

	I	II	III	IV	V*	VI	VII	VIII	IX
M + 1	25	25	8	12	19	7	21	34	9
M	96	85	25	44	66	22	71	100	30
M-73	13	13	4	<1	<1	≪1	11	3	2
331(M')	7	7	23	29	12	30	9	12	30
271(M'-60)	1	1	2	7	1	8	1	2	4
259(M'-72)	3	3	2	<1	<1	2	3	1	<1
243(M'-88)	4	4	3	<1	≪1	≪1	6	4	10
229(M'-102)	3	3	4	6	7	7	3	10	10
211(M'-120)	3	3	5	8	4	6	6	15	10
B + 85	24	17	9	3	4	2	18	18	17
B + 84	6	5	3	7	5	7	5	17	13
201(M'-130)	5	4	4	3	<1	2	6	2	7
199(M'-132)	1	1	1	<1	1	1	2	15	3
187(M'-144)	6	4	4	5	7	5	4	13	6
169(M'-162)	48	38	100	100	100	100	48	60	100
B + 43	29	22	26	12	14	10	27	35	54
159(M'-172)	1	2	3	12	16	4	1	<1	7
157(M'-174)	3	3	9	2	2	4	8	23	12
B + 30	100	100	52	7	10	3	100	80	37
B + 29	29	28	11	14	16	5	26	24	6
145(M'')	6	6	11	14	10	10	6	31	22
141(M'-190)	27	22	27	3	4	7	35	21	52
140(M'-191)	4	1	3	2	2	3	4	4	11
139(M'-192)	12	12	13	13	12	23	11	18	28
130(M'-201)	8	10	6	14	30	7	7	11	9
127(M'-204)	12	10	23	20	24	32	8	18	28
B + 2	8	7	3	12	14	3	7	31	28
B + 1	42	20	11	44	56	8	39	63	8
B	24	8	3	6	30	1	17	10	3
115(M'-216)	17	15	24	12	17	25	22	21	37
109(M'-222)	49	31	74	52	71	88	36	46	61
103(M''-42)	7	5	11	7	9	11	8	9	24
99(M'-232)	10	7	14	5	4	9	9	12	22
97(M'-234)	12	12	13	9	8	21	11	16	20
91(M'-240)	10	10	3	3	2	1	6	1	4
85(M'-246)	4	7	6	4	4	10	5	6	10
81(M'-250)	74	55	56	11	13	26	75	46	76
69(M'-262)	7	9	6	3	4	10	6	9	15

* Relative intensities given for V differ slightly from those quoted in a preliminary abstract of this work [A. A. Magnin and A. M. Stephen, *S. Afr. Medical J.* **44**, 150 (1970)].

A soln of V in hot benzene was treated (twice) with charcoal, and the filtrate concentrated to give light brown crystals. Recrystallization yielded pale yellow needles, m.p. 160–161°, $[\alpha]_D$ −3° (*c* 6·7 in $CHCl_3$). (Found: C, 59·9; H, 6·0; N, 2·8. $C_{23}H_{27}NO_9$ requires: C, 59·9; H, 5·9; N, 3·0%). The IR spectrum showed the following bands: ν_{max}^{HCBD} cm^{-1} 1735–1755, 1210–1255 (acetyl); 2840–3080 (C—H stretching; for X 2895–2960, for 2-methylindole 2840–3080); N—H stretching at 3390 absent. NMR: H_1, H_2, H_3 and H_4 of the sugar ring gives rise to complex multiplets (τ 4·2–4·8). Sugar ring protons, H_5 at τ 6·09 (J_{45} = 9·6, J_{56} = 4·4, $J_{56'}$ = 2·6), H_6 at τ 5·70 ($J_{66'}$ = 12·2) and $H_{6'}$ at τ 5·83; aromatic ring protons as complex multiplets (τ 2·3–3·1); indolic proton H_3 at τ 3·79, Me at τ 7·53; acetyl protons at τ 7·97, 7·98, 8·03 and 8·39 (broad).

1-(2,3,4,6-*Tetra*-O-*acetyl*-β-D-*glucopyranosyl*)-5-*nitroindole* (VI). III (4·94 g, 10·0 mmole) and DDQ (2·27 g, 10·0 mmole) in *m*-xylene (120 ml) were treated as above. Removal of one-half of the xylene *in vacuo* resulted in the deposition of unchanged III (as shown by m.p. and NMR). Further concentration of the mother liquors afforded a second crop of crystals which was shown by NMR to be a mixture of III and VI (III and VI have the same R_f using either *a* or *b*). Yet further concentration gave VI as yellow crystals, shown by NMR to be free of III, which upon recrystallization from EtOH gave yellow needles (0·4 g, 8%), m.p. 195–197°, $[\alpha]_D$ +7° (*c* 2·0 in $CHCl_3$). (Found: C, 53·9; H, 4·9; N, 5·5. $C_{22}H_{24}N_2O_{11}$ requires: C, 53·7; H, 4·9; N, 5·7%). The IR spectrum showed the following bands: ν_{max}^{HCBD} cm^{-1} 1740–1760, 1205–1245 (acetyl); 2860–3120 (C—H stretching); 1335, 1525 (NO_2); N—H stretching at 3300 absent. NMR: H_1, H_2, H_3 and H_4 of the sugar ring give rise to complex multiplets (τ 4·2–4·8). Sugar ring protons, H_5 at τ *ca.* 5·9 (J_{56} = 4·8, $J_{56'}$ = 2·0), H_6 at τ 5·63 ($J_{66'}$ = 12·2) and $H_{6'}$ at τ 5·82; aromatic ring protons, H_4 at τ 1·46 (J_{46} = 2·4), H_6 at τ 1·86 (J_{67} = 9·4) and H_7 at τ 2·49; indolic protons, H_2 at τ 2·59 (J_{23} = 3·6) and H_3 at τ 3·27; acetyl protons at τ 7·92, 7·92, 7·97 and 8·32.

1-(2,3,4,6-*Tetra*-O-*acetyl*-β-D-*glucopyranosyl*)-1,2,3,4-*tetrahydroquinoline* (VII). 1,2,3,4-Tetrahydroquinoline (10 g, 75·3 mmole) and D-glucose (13·5 g, 75·0 mmole) were heated under reflux for 15 min in MeOH (300 ml) containing a trace of HCl.[20] Removal of one-half of the solvent *in vacuo* and cooling to 0° overnight afforded white crystals. These were collected, dried *in vacuo* over P_2O_5, and acetylated with a 1:1 mixture of pyridine and Ac_2O (20 ml). The product was recrystallized from EtOH to give VII (3·5 g) as white needles, m.p. 133–135°, $[\alpha]_D$ +70° (*c* 4·4 in $CHCl_3$). (Found: C, 59·7; H, 6·5; N, 3·0. $C_{23}H_{29}NO_9$ requires: C, 59·7; H, 6·3; N, 3·0%). The IR spectrum showed the following bands: ν_{max}^{HCBD} cm^{-1} 1720–1755, 1215–1250 (acetyl); 2900–3040 (C—H stretching; for X 2895–2960, for 1,2,3,4-tetrahydroquinoline 2780–3040); N—H stretching at 3410 absent. NMR: H_1, H_2, H_3 and H_4 of the sugar ring give rise to complex multiplets (τ 4·5–5·0). Sugar ring protons, H_5 at τ 6·26 (J_{45} = 9·8, J_{56} = 4·8, $J_{56'}$ = 2·8), H_6 at τ 5·75 ($J_{66'}$ = 12·0) and $H_{6'}$ at τ 5·92; aromatic ring protons, complex multiplets at τ 2·8–3·4; methylene protons, three multiplets at τ 6·73, 7·30 and *ca.* 8·1; acetyl protons at τ 7·96, 7·96, 7·99 and 8·07.

Removal of all the solvent used in the condensation of glucose and base, and treatment of the yellow syrupy residue as before, gave VII (12·0 g, total yield 43%), identical (m.p., mixed m.p. and TLC) with the product just described.

1-(2,3,4,6-*Tetra*-O-*acetyl*-β-D-*glucopyranosyl*)-6-*aminoquinoline* (VIII). 6-Aminoquinoline (0·52 g, 3·6 mmole) and D-glucose (0·5 g, 2·8 mmole) were treated as above to give white needles from EtOH (0·64 g, 48%), m.p. 217–218°, $[\alpha]_D$ −6° (*c* 21·9 in $CHCl_3$). (Found: C, 58·1; H, 5·7; N, 5·7. $C_{23}H_{26}N_2O_9$ requires: C, 58·2; H, 5·5; N, 5·9%). The IR spectrum showed the following bands: ν_{max}^{HCBD} cm^{-1} 1740–1765, 1210–1255 (acetyl); 2900–3080 (C—H stretching; for X 2895–2960, for 6-aminoquinoline 2980–3020); N—H stretching at 3250, (for 6-aminoquinoline 3300, 3390); NMR: sugar ring protons, H_1 at τ 5·12 (J_{12} = 9·0), H_2 at τ 4·87 (J_{23} = 8·9), H_3 at τ 4·75 (J_{43} = 9·0), H_4 at τ 4·89 (J_{45} = 9·6), H_5 at τ 6·07 (J_{56} = 5·7, $J_{56'}$ = 2·4), H_6 at τ 5·68 ($J_{66'}$ = 12·2) and $H_{6'}$ at τ 5·91; N—H at τ 4·79 (J_{N-1} = 8·8); aromatic ring protons, H_5 at τ 3·13 (J_{57} = 2·2), H_2, H_3, H_4, H_7, H_8 at τ 2·0–3·0 (complex multiplets); acetyl protons at τ 7·96, 7·96, 7·96 and 7·98.

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