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SUBSTITUTED GLUCOSYLAMINES CONTAINING THE INDOLE NUCLEUS

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Abstract—1-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)indoline (III) has been prepared by a stereospecific synthesis from 3,4,6-tri-O-acetyl-α-D-glucopyranose 1,2-(ethylorthoacetate) and indoline. The corresponding acetylated glucosylamines from 2-methylindoline and 5-nitroindoline have been prepared using the Suvorov procedures; their NMR spectra are compared with those of 2,3,4,6-tetra-O-acetyl-N-*p*-nitrophenyl-β-D-glucopyranosylamine (VI) and III, and are used as evidence for the respective structures.

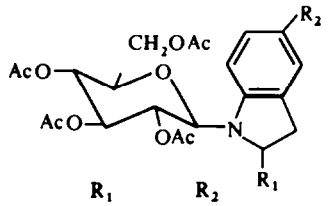
INTRODUCTION

THE isolation[1] of the pyrrolopyrimidine nucleoside tubercidin has focussed attention on related types of compound.[2,3] We have commenced a programme of synthesis of nucleoside-type derivatives containing the indole nucleus. Two groups of workers[4–8] have recently reported the synthesis of related compounds.

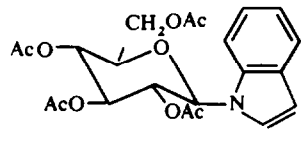
DISCUSSION

Preparative methods. Following the procedures outlined,[4] 1-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-2-methylindoline (I) and 1-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-5-nitroindoline (II) have been prepared together with the known 1-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)indoline (III) and 1-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)indole (IV). These procedures involve reacting (a) penta-O-acetyl-β-D-glucopyranose, (b) its α-anomer, or (c) 2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl bromide with the appropriate indoline. The yields using (c) were very low (<1%). An additional method used by these authors involves (d) reacting D-glucose with the appropriate indoline, and acetylating the product. We have found this the most convenient method of preparing acetylated glucosylindolines.

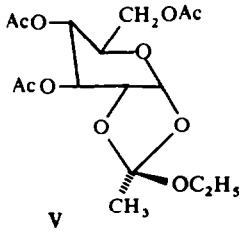
Kochetkov *et al.*[9] have used 3,4,6-tri-O-acetyl-α-D-glucopyranose 1,2-(ethyl orthoacetate)[10] (V) in the synthesis of acetylated glycosides. When indoline was reacted with V under Kochetkov's conditions, III was obtained as a minor product, while acetylation of the major product afforded III. Modification of the reaction conditions by reacting in ethanol at 50° in the absence of a catalyst led to III as the major product, a small quantity of the partially acetylated compound being obtained. Kochetkov *et al.*[9] state that the use of V leads stereospecifically to 1,2-*trans*-glycosides; the above methods accordingly give only the β-D-anomer.



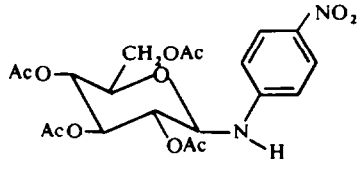
	R_1	R_2
I	CH_3	H
II	H	NO_2
III	H	H



IV



V



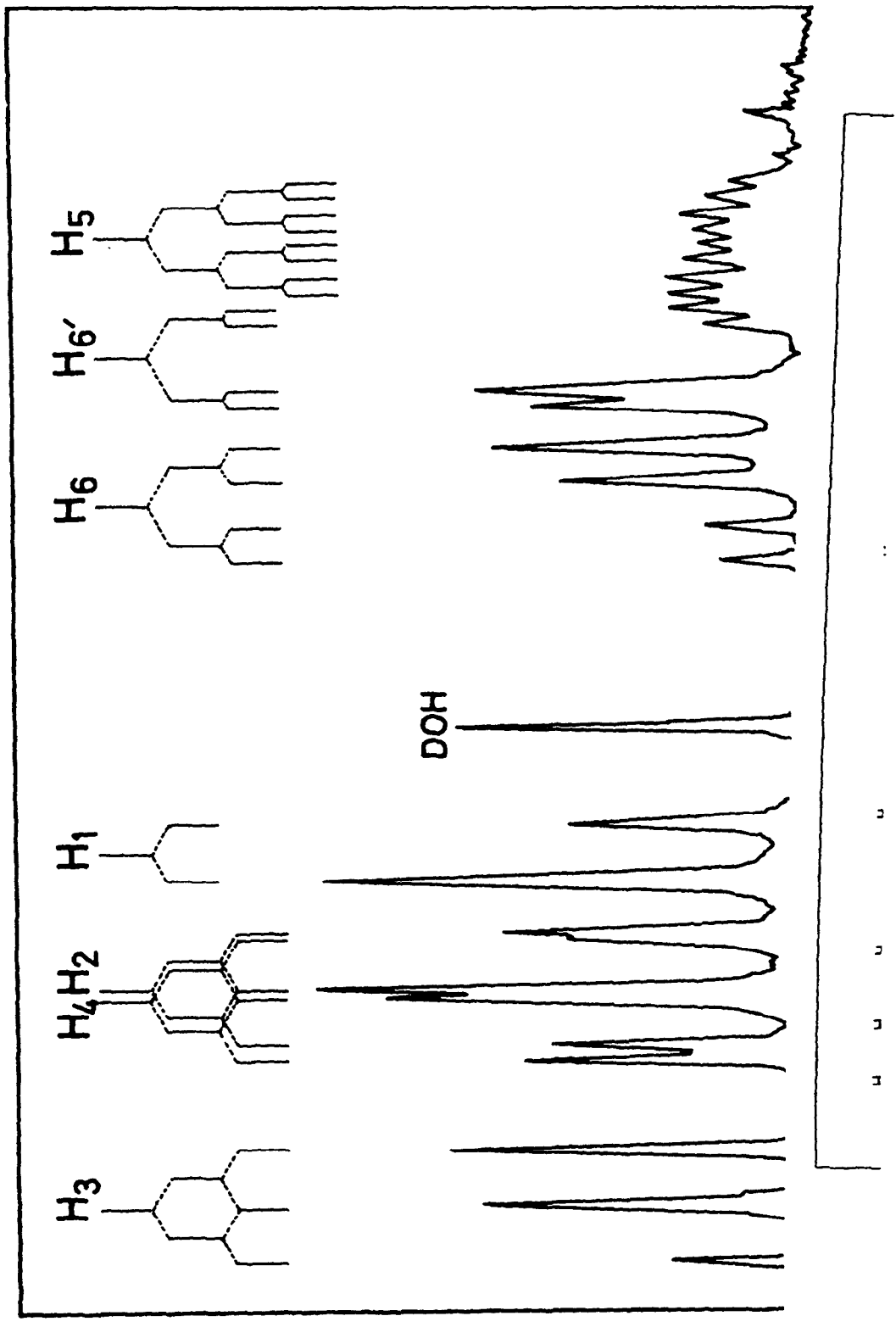
VI

Mass spectra. The mass spectra of compounds I, II, III, IV and 2,3,4,6-tetra-O-acetyl-N-*p*-nitrophenyl-β-D-glucopyranosylamine (VI) have been studied, and these will be discussed in a future communication. All of the above compounds show large molecular ion peaks. In addition major fragments are found corresponding to the acetylated glucopyranosyl[11] (*m/e* 331) and to the nitrogen-containing moieties. These fragments support the structures proposed for these compounds.

NMR spectra. Configurational and conformational proofs of the structure of glucosylamines have been conclusive in few cases. Suvorov and Preobrazhenskaya[4] have based their structure of III on the grounds of the stereospecicifity of the Koenigs–Knorr reaction, the thermodynamic preference for the equatorial orientation of the indoline moiety, and on the basis of Hudson's rules for the superimposition of molecular rotations. Walton *et al.*[6] have put forward NMR proof of the anomeric configuration and sugar conformation in 1-(β-D-glucopyranosyl)indole. We have analysed the NMR spectra of I, II, III, IV and VI, in order to obtain information on their stereochemistry. NMR results are summarized in Table 1. Compound VI[12,13] was included in this investigation because its anomeric configuration is known from optical rotation and other data to be β-D, and because the NMR signals in $CDCl_3$ are well separated.

In the NMR spectrum of VI dissolved in $CDCl_3$ the multiplets at τ 1·90 and 3·34 are due to the aromatic protons adjacent to the nitro and amino groups respectively.[14,15] The doublet at τ 4·36 with a splitting of 7·6 Hz is due to the NH proton; after D_2O exchange this doublet disappears, and the triplet at τ 5·14 reduces to a doublet of splitting 8·9 Hz (Fig. 1). This triplet is thus due to H_1, and H_1 and H_2 must have a *trans*-diaxial orientation.

The three triplets in the region τ 4·5–5·1 are due to H_2, H_3 and H_4. Because the spectrum retains its first-order structure, and from line intensity considerations, H_3 must be that at lowest field giving $J/\Delta\nu$ the smallest possible value. An assignment of the resonances of H_2 and H_4 can be made from the magnitude of their splittings.


H5
H6'
H6
DOH
H1
H4H2
H3

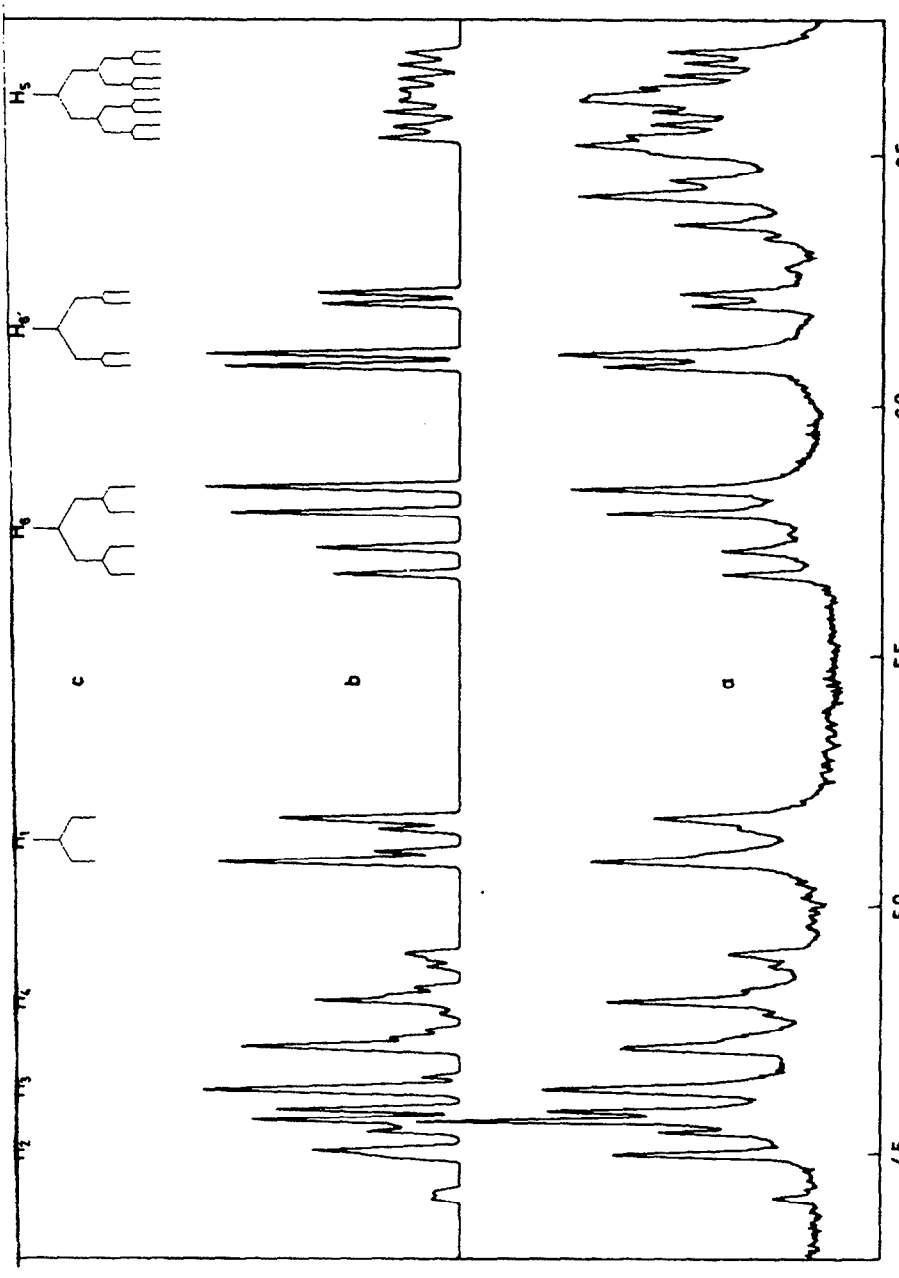


FIG. 2 (a) 100 MHz NMR spectrum of III in C_6H_6, range τ 4·3–6·8.
(b) Theoretical spectrum of III, calculated using parameters from Table 1.
(c) First-order analysis* of H_1, H_5, H_6 and $H_{6'}$.

* The first-order analysis of the H_2, H_3 and H_4 multiplet is complicated and has been omitted from the figure.

TABLE 1*

Compound	Solvent	τ_1	τ_2	τ_3	τ_4	τ_5	τ_6	$\tau^{6'}$	τ_{acetyl}	$\tau_{aromatic}$	τ_{other}	J_{12}	J_{23}	J_{34}	J_{45}	J_{56}	$J_{56'}$	$J_{66'}$	J_{other}
I	$CDCl_3$	5·06	4·52	4·61	4·88	6·24	5·77	5·88	7·98 8·00 8·00 8·16	H_4 3·44 (d) H_5 ~2·9 (t) H_6 3·31 (t) H_7 ~2·9 (d)	H_2 5·96 (m) H_3 6·84 (q) $H_{3'}$ 7·47 (q) Me 8·79 (d)	8·9	8·0	8·5	9·7	4·6	2·4	−11·8	J_{23} 6·3 $J_{23'}$ 2·2 $J_{33'}$ −15·2
III	C_6H_6	5·13	4·52	4·63	4·80	6·62	5·76	6·14	8·22† 8·24 8·33 8·33	Obscured by solvent	H_2, $H_{2'}$ ~6·5 (m) H_3, $H_{3'}$ 7·37 (m)	9·0	9·6	9·0	9·8	5·2	2·2	−12·3	
VI	$CDCl_3$	5·14	4·94	4·60	4·93	6·10	5·69	5·91	7·94 7·94 7·94 7·94	H_3, H_5 1·90 (m) H_2, H_6 3·34 (m)	N—H 4·36 (d)	8·9	9·0	9·1	9·7	5·6	2·3	−12·3	J_{N1} 7·6

* τ_1, τ_2, etc. refer to the chemical shifts of the protons at C_1, C_2, etc. of the sugar ring. J(Hz) values refer to splittings, except for III in C_6H_6 where coupling constants are shown. Multiplicity of signals: d = doublet, t = triplet, q = quartet, m = multiplet.

† Cf. the τ_{acetyl} values found[17] for III in DMSO.

The triplet at τ 4·93 has splittings of 9·1 and 9·7 Hz, and that at τ 4·94 splittings of 8·9 and 9·0 Hz. Analysis of the H_5 multiplet yields a H_4–H_5 interaction of 9·7 Hz. This shows the low-field triplet to be due to H_4.

H_5, H_6 and $H_{6'}$ are found as an ABX pattern, with an additional splitting (J_{45}) due to the H_4–H_5 interaction, between τ 5·6 and 6·2. Analysis of these signals gave the following coupling constants $J_{56} = 5{\cdot}6$, $J_{56'} = 2{\cdot}3$ and $J_{66'} = -12{\cdot}3$ Hz, and chemical shifts τ_5 6·10, τ_6 5·69 and $\tau_{6'}$ 5·91. All four acetyl signals are found at τ 7·94.

All of the coupling constants of the sugar moiety ring protons are of the order of 9 Hz. This requires the Reeves C 1 conformation as there are no axial-equatorial or equatorial–equatorial splittings. Furthermore, J_{12} being 8·9 Hz indicates the β-D-configuration. From a study of other peaks there is no evidence of the presence of α-D-anomer.

The spectra of II, III and IV in $CDCl_3$ had the H_1, H_2, H_3 and H_4 signals appearing as a complex multiplet which allowed no detailed assignment of signals to be made. Spectra of these compounds were also obtained using benzene, pyridine, dimethylsulphoxide and acetone solutions; only in the case of III in benzene was any improvement obtained.

The spectrum of III in benzene (Fig. 2a) allowed first-order parameters to be obtained for the sugar moiety protons. These parameters were varied until agreement between a computed spectrum (Fig. 2b) and the observed spectrum was reached.

The spectrum of I in $CDCl_3$ gave parameters which agree very favourably with those obtained for III and VI. In each case where parameters have been found, the anomeric splitting is of the order of 9 Hz (Table 1). Thus the β-D-configuration and Reeves C 1 conformation have been established for I, III and VI.

As compounds I, II, III, IV and VI have been prepared by similar methods, possess similar optical rotations (Experimental), fragment in identical fashions under the conditions used in mass spectrometry, and show comparable parameters for the sugar-moiety protons in their NMR spectra (Table 1 and Experimental), we have advanced analogous structures for each with β-D-configuration and C 1 conformation.

Tests for physiological activity. Compounds I, II and III proved to be non-toxic inactive when tested for anti-tumour activity; this work was undertaken as part of the programme of the United States Institutes of Health.

EXPERIMENTAL

NMR spectra were obtained on a Varian HA-100 Spectrometer from dilute solns in benzene and $CDCl_3$. TMS was used as internal standard and the probe temp was 32°. The spectra, except that of I, were recorded several times on an expanded scale and the line positions averaged. ABX patterns were analysed following the usual procedures.[16] All other NMR parameters were obtained on a first-order basis. The results were estimated to have the following accuracy: splittings $J \pm 0{\cdot}2$ Hz, $\tau \pm 0{\cdot}01$ ppm. IR spectra were recorded on a Perkin–Elmer 237 spectrometer as hexachlorobutadiene mulls on NaCl plates. M.ps were determined on a Fisher–Johns m.p. apparatus and are uncorrected. Mass spectra were determined with an A.E.I. MS9 spectrometer, using the direct insertion technique.

TLC was carried out on silica-gel plates with the following solvent systems (both v/v): *a* $CHCl_3$–C_6H_6 (3:1), *b* $CHCl_3$–EtOAc (5:2). Plates were sprayed with *p*-dimethylaminobenzaldehyde in HCl (Ehrlich reagent), or 2N H_2SO_4. Column chromatography was carried out on silica using $CHCl_3$–C_6H_6 (3:1) as

solvent. Paper chromatography was performed on Whatman No. 1 paper, eluted with 1-BuOH–EtOH-water (4:1:5, upper layer) and sprayed with Ehrlich reagent or *p*-anisidine hydrochloride in 1-BuOH.

Synthesis of III *from* 3,4,6-*tri*-O-*acetyl*-α-D-*glucopyranose* 1,2-(*ethyl orthoacetate*)[10] (V)

A mixture of V (150 mg, 0·40 mmole), indoline (47 mg, 0·40 mmole) and anhyd EtOH (2 ml) was stirred with gentle warming (50°) until soln was complete. After 2 hr at room temp the solvent was removed *in vacuo*, and the syrup crystallized from ether–light petroleum (b.p. 40–60°). TLC (solvent *b*) showed III to be the major product. The minor product which afforded III on acetylation is possibly 1-(3,4,6-tri-O-acetyl-β-D-glucopyranosyl)indoline. Recrystallization of III from EtOH gave 13 mg white needles (7%), which proved on TLC and mixed melt to be identical with those produced by one of the Suvorov procedures, m.p. 119–120°, $[\alpha]_D^{20}$ +8° (*c* 2·1 in $CHCl_3$) [lit.[4] m.p. 117·5–118·5°, $[\alpha]_D^{20}$ +11° (*c* 6 in $CHCl_3$)]. (Found: C, 58·8; H, 6·2; N, 3·2. Calc. for $C_{22}H_{27}NO_9$: C, 58·8; H, 6·1; N, 3·1%). The IR spectrum showed the following bands: ν_{max}^{HCBD} cm^{-1} 1750–1760, 1180–1260 (acetyl); 2840–3090 (C—H stretching; for penta-O-acetyl-β-D-glucopyranose 2895–2960, for indoline 2840–3010); absence of N—H stretching at 3360. NMR of III in $CDCl_3$: H_1, H_2, H_3 and H_4 of the sugar ring give rise to complex multiplets (τ 4·6–5·1); parameters for the remaining protons [sugar ring protons, H_5 at τ 6·25 (J_{45} = 9·7, J_{56} = 4·3, $J_{56'}$ = 2·8), H_6 at τ 5·75 ($J_{66'}$ = −12·3), $H_{6'}$ at τ 5·96; aromatic ring protons, H_4 at τ 3·46 (doublet), H_5 at τ 2·95 (triplet), H_6 at τ 3·29 (triplet), H_7 at τ 2·92 (doublet); indolinic protons, H_2 and $H_{2'}$ at τ 6·39 (multiplet), H_3 and $H_{3'}$ at 7·05 (multiplet)] resemble those reported for benzene soln (Table 1). Acetyl protons at τ 7·99, 8·00, 8·03 and 8·05; compare values for DMSO soln.[17]

1-(2,3,4,6-*tetra*-O-*acetyl*-β-D-*glucopyranosyl*)*indole* (IV)

Compound III was dehydrogenated by the action of chloranil in xylene[4] to give IV (25% yield). Dehydrogenation using 2,3-dichloro-5,6-dicyano-1,4-benzoquinone in xylene[6] gave IV (78% yield) as white needles, m.p. 134–135°, $[\alpha]_D^{20}$ +2° (*c* 2·2 in $CHCl_3$) [lit.[4] m.p. 149°, $[\alpha]_D^{20}$ +1·5° (*c* 5·5 in $CHCl_3$)]. (Found: C, 58·8; H, 5·7; N, 3·2. Calc. for $C_{22}H_{25}NO_9$: C, 59·1; H, 5·6; N, 3·1%). NMR of IV in $CDCl_3$ and C_6H_6: H_1, H_2, H_3 and H_4 of the sugar ring give rise to complex multiplets (τ 4·3–4·8 and τ 4·4–4·8 respectively). The remaining protons are assigned as follows. In $CDCl_3$: sugar ring protons, H_5 at τ 6·02 (J_{45} = 9·6, J_{56} = 5·2, $J_{56'}$ = 2·2), H_6 at τ 5·71 ($J_{66'}$ = −12·2), $H_{6'}$ at τ 5·86; aromatic ring protons at τ 2·37–2·97 (multiplet); indolic protons, H_2 at τ 2·82 (J_{23} = 3·5), H_3 at τ 3·46. In C_6H_6: sugar ring protons, H_5 at τ 6·52 (J_{45} = 9·7, J_{56} = 5·0, $J_{56'}$ = 2·5), H_6 at τ 5·77 ($J_{66'}$ = −12·4), $H_{6'}$ at τ 6·06; the aromatic and indolic proton signals are obscured by the solvent signals. Acetyl protons at τ 7·95, 7·96, 8·00 and 8·36 in $CDCl_3$, and at τ 8·27, 8·28, 8·33 and 8·62 in C_6H_6; compare values for DMSO soln.[17]

A sample of IV recovered from the benzene soln used in the NMR study had m.p. 159·5°. Seeding a small quantity of the low melting compound in benzene with a crystal of the higher melting material resulted in crystals, m.p. 157·5°. Suvorov and Preobrazhenskaya[4] report two crystalline forms melting at 149° and 158°. IR spectra of the compounds melting at 135° and 159° were identical: ν_{max}^{HCBD} cm^{-1} 1742–1750, 1200–1255 (acetyl); 2890–3140 (C—H stretching; for penta-O-acetyl-β-D-glucopyranose 2895–2960, for indole 2880–3110); N—H stretching band at 3360 absent.

1-(2,3,4,6-*tetra*-O-*acetyl*-β-D-*glucopyranosyl*)-2-*methylindoline* (I)

(a) 2-Methylindoline (2g, 15·0 mmole), penta-O-acetyl-β-D-glucopyranose (3g, 7·69 mmole) and AcOH (2 ml) were dissolved in EtOH (40 ml) with gentle warming and allowed to stand for 1 week at room temp. TLC (solvent system *a*) showed the presence of both N-acetyl-2-methyl-indoline and I. After removal of one-half of the solvent *in vacuo* and cooling overnight, the crystals deposited were filtered, washed with cold EtOH and recrystallized from EtOH to give white needles, m.p. 135–136°, $[\alpha]_D^{20}$ −5° (*c* 0·84 in $CHCl_3$). (Found: C, 59·2; H, 6·3; N, 3·1. $C_{23}H_{29}NO_9$ requires: C, 59·6; H, 6·3; N, 3·0%).

Concentration of the mother liquors gave a further crop of crystals; total yield 0·3 g (8·5%). A soln of I in EtOH gave positive Molisch and Ehrlich tests. Paper chromatography showed de-O-acetylated material I to have R_f 0·85. The IR spectrum showed the following bands: ν_{max}^{HCBD} cm^{-1} 1740–1760, 1215–1270 (acetyl); 2840–3020 (C—H stretching; for penta-O-acetyl-β-D-glucopyranose 2895–2960, for 2-methyl-indoline 2840–3070); N—H stretching at 3360 absent.

(b) 2-Methylindoline (2·9 g, 21·8 mmole) and D-glucose (3·05 g, 17·0 mmole) were dissolved with warming in methanol (65 ml) containing a trace of HCl,[13] and the mixture was heated on a water bath for 15 min. After neutralization of the HCl with NH_4OH, the solvent was removed *in vacuo*. The resultant syrup was dried over P_2O_5 *in vacuo* and acetylated by the action of a 1:1 mixture of pyridine and acetic anhydride

(20 ml) at0°. Pouring into ice-water and recrystallizing from EtOH gave white needles identical with those in (a) above; yield 1·2 g (15%).

1-(2,3,4,6-*tetra*-O-*acetyl*-β-D-*glucopyranosyl*)-5-*nitroindoline* (II)

5-Nitroindoline (2·75 g, 16·7 mmole), D-glucose (3g, 16·7 mmole) and water (1 ml) were stirred on a boiling water bath for 1 hr after a clear soln had been obtained. On cooling, the reaction mixture solidified. The solid was dissolved in MeOH (20 ml), filtered and taken to dryness *in vacuo* to give a yellow frothy mass. After drying over P_2O_5 this was acetylated in pyridine and Ac_2O. Yellow crystals were collected at the pump, dried and recrystallized from EtOH to give pale yellow needles (3·1 g, 38% yield), m.p. 162–163° (dec.), $[\alpha]_D^{20}$ +30° (*c* 0·4 in $CHCl_3$). (Found: C, 52·6; H, 5·2; N, 4·9. $C_{22}H_{26}N_2O_{11}$ requires: C, 53·4; H, 5·3; N, 5·7%). The IR spectrum gave the following bands: ν_{max}^{HCBD} cm^{-1} 1750–1760, 1215–1295 (acetyl); 2870–3065 (C—H stretching); 1340, 1515 (NO_2); N—H stretching at 3360 absent. NMR of II in $CDCl_3$: H_1, H_2, H_3 and H_4 of the sugar ring give rise to complex multiplets (τ 4·6–5·0). The remaining protons are assigned as follows: sugar ring protons, H_5 at τ 6·12 (J_{45} = 9·8, J_{56} = 5·0, $J_{56'}$ = 2·2), H_6 at τ 5·74 ($J_{66'}$ = −12·4), $H_{6'}$ at τ 5·89; aromatic ring protons, H_4 at τ2·06 (J_{46} = 2·0), H_6 at τ 1·92 (J_{67} = 9·0), H_7 at τ 3·46; indolinic protons, H_2 and $H_{2'}$ at τ 6·20 (multiplet), H_3 and $H_{3'}$ at τ 6·94 (multiplet). Acetyl protons at τ 7·96, 7·98, 7·98 and 8·06.

Reaction of 2,5-dimethylindoline or 5-methylindoline with either D-glucose or penta-O-acetyl-β-D-glucopyranose failed to yield the required glucosylindoline.

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