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PROTON MAGNETIC RESONANCE SPECTRA OF METHYL β-D-GLUCOPYRANOSIDE TETRANITRATE AND β-CELLOBIOSE OCTANITRATE

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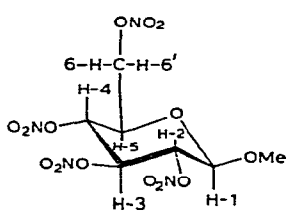
ABSTRACT

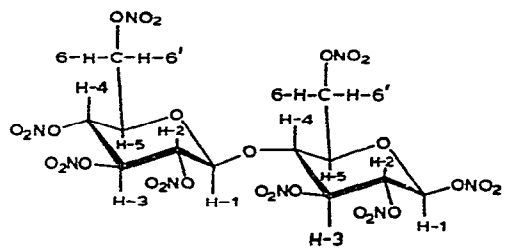
The ^{1}H-n m r spectra of methyl β-D-glucopyranoside tetranitrate and β-cellobiose octanitrate have been obtained with a 220-MHz spectrometer operated in the Fourier-transform mode Interpretation of the spectra from first-order analysis is presented The chemical shifts and coupling constants of all protons in these two molecules have been calculated with the LAOCN 3 computer program The simulated spectra obtained with the KOMBIP program are given

INTRODUCTION

Nuclear magnetic resonance has proved to be an extremely fruitful technique for studying the structure and conformation of many carbohydrates and their derivatives[1] One class of carbohydrate derivatives that seems to have escaped general interest is the nitrates, typified by methyl β-D-glucopyranoside tetranitrate (**1**) and β-cellobiose octanitrate (**2**) These two D-glucosides may be considered to be structurally very closely related to the fundamental unit of cellulose nitrate Considering the commercial importance of this polysaccharide ester, it seemed desirable to



Methyl β-D-glucopyranoside tetranitrate (1)



β-Cellobiose octanitrate (2)

establish the p m r -spectral assignments of these simple D-glucosides with a view to utilizing this information in the spectral analysis of cellulose nitrate

EXPERIMENTAL

Preparation of methyl β-D-glucopyranoside tetranitrate (**1**) — The procedure for the nitration of methyl β-D-glucopyranoside (purchased from K & K Laboratories, Inc , Plainview, N Y , U S A) was essentially that described by Honeyman and Morgan[2]. Two recrystallizations from methanol gave the tetranitrate as white prisms in 77% yield Thin-layer chromatography on sheets (20 × 20 cm) of Eastman Chromatogram Silica Gel with 15 4 1 (v/v) 1,2-dichloroethane–dichloromethane–ethyl ether revealed the presence of a single component, R_F 0 88, m p 117° (uncorr), $[\alpha]_D^{25}$ +13 2° (*c* 3, acetone) {lit [2] m p 116 5° (uncorr), $[\alpha]_D^{20}$ +11 1° (*c* 4, chloroform)}

Anal Calc for $C_7H_{10}N_4O_{14}$ C, 22 46, H, 2 67, N, 14 97 Found C, 22 80, H, 2 75, N, 14 58

Preparation of β-cellobiose octanitrate (**2**) — Cellobiose (purchased from Eastman Kodak, Rochester, N Y , U S A) was used as received Cellobiose (5 g) was added in portions to a chilled nitrating mixture consisting of 98 7% nitric acid (35 ml) and acetic anhydride (35 ml) After reaction for 45 min at 0°, the mixture was quickly added to stirred ice–water The resulting solid was filtered off, and successively washed with water (several times), 1% sodium hydrogencarbonate solution, and water until neutral The product was dried to constant weight *in vacuo* over phosphorus pentaoxide yield 10 1 g (98% of the theoretical) Part of this product (2 g) was dissolved in 2 1 1,2-dichloroethane–petroleum ether (25 ml), the solution was added to a column (2 7 × 40 cm) of silica gel, and the column was eluted with the same solvent From the first seven 50-ml fractions of eluate was obtained compound **2** (1 1 g), m p 117°, $[\alpha]_D^{25}$ + 36° (*c* 3, acetone) {lit [3] m p 140°, $[\alpha]_D^{20}$ +22 1° (*c* 6, acetone)}

Anal Calc for $C_{12}H_{14}N_8O_{27}$ C, 20 51 H, 1 99, N, 15 95 Found C, 20 40, H, 2 10, N, 15 65

The disagreement between our values for m p and $[\alpha]_D$ and those reported in the litrature[3] can be traced to the different methods of purification We purified our crude products by chromatography on Silica gel columns, whereas previous workers applied the method of recrystallization from hot methanol By using the latter method, we also obtained a material melting at 140°, but it gave two spots on thin-layer chromatograms In addition, the material melting at 140° shows the presence of a methyl group (by n m r spectroscopy) and a hydroxyl group by i r spectroscopy It appears that the treatment with hot methanol in the recrystallization step results in denitration at the anomeric carbon atom, followed by partial methyl glycosidation In our judgment, the compound reported to melt at 140° is probably a mixture of cellobiose octanitrate, cellobiose 2,3,6,2′,3′,4′,6′-heptanitrate, and methyl celiobioside heptanitrate

Measurements of n m r spectra — Solutions [2% (w/v)] of the nitrates in acetone-d_6 were prepared A trace of tetramethylsilane was used as the internal

standard Shifts are reported both in Hz and in p p m relative to Me_4Si downfield shifts are positive Spectra were recorded at the Rockefeller University, New York City, with a 220-MHz Varian spectrometer (model HR 220) operating in the Fourier-transform mode Each spectrum represents the composite of 32 scans The resulting spectra of methyl β-D-glucopyranoside tetranitrate and β-cellobiose octanitrate are reproduced in Figs 1 and 3, respectively

The LAOCN 3 program for the analysis of high-resolution, n m r spectra was obtained from the Quantum Chemistry Program Exchange, QCPE No 111 The KOMBIP program for the generation of Lorentzian/Gaussian line-shape and stick-plot n m r spectra was obtained from the Quantum Chemistry Program Exchange, QCPE No 205

INTERPRETATION OF SPECTRA

Methyl β-D-glucopyranoside tetranitrate (**1**) — The ^{1}H-n m r spectrum of a solution of methyl β-D-glucopyranoside tetranitrate in acetone-d_6 is shown in Fig 1 The signals of each proton are completely resolved, and can be interpreted by a pseudo-first-order analysis, as indicated on the spectrum

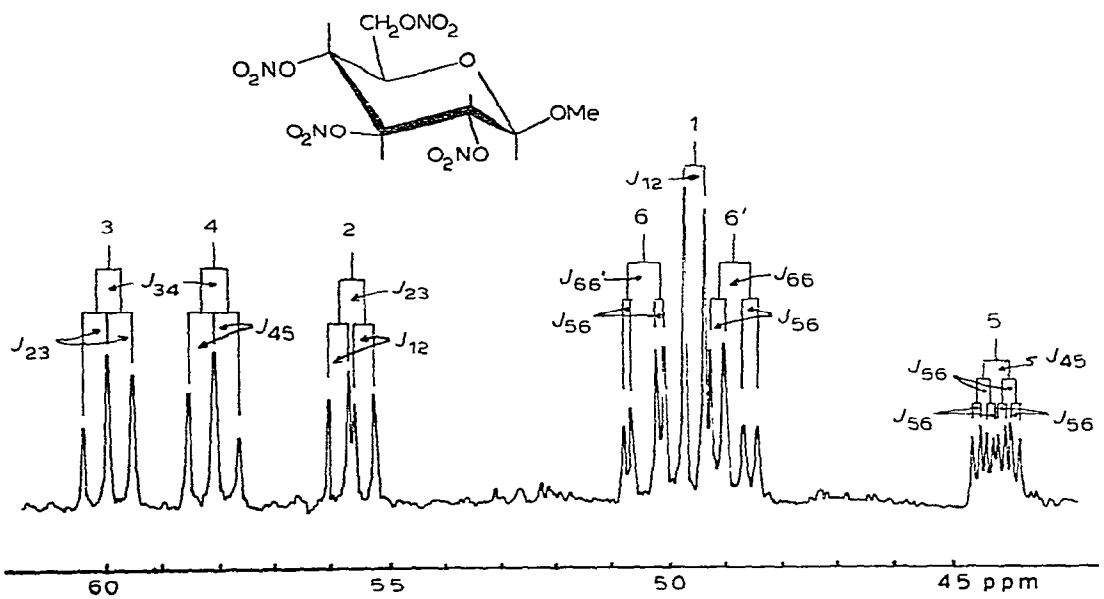


Fig 1 Experimental spectrum of methyl β-D-glucopyranoside tetranitrate (**1**) and first-order spectral assignments

The methoxyl protons exhibit a readily identifiable singlet at 3 55 p p m (781 Hz) Proton 1 gives a doublet at 4 96 p p m (1091 Hz) It is coupled to proton 2, with $J_{1\,2}$ 8 0 Hz The only other pattern in the spectrum that exhibits such a splitting

is the doublet of doublets centered at 5 37 p p m (1225 Hz) Hence, we conclude that this must be due to proton 2 This pattern also shows that $J_{2\,3} = 9\,6$ Hz

The two triplets located downfield at 6 00 p p m (1320 Hz) and 5 81 p p m (1278 Hz) show slightly different splittings Each triplet is the result of coupling by two different protons The two couplings at 6 00 p p m are both 9 6 Hz, whereas the two couplings at 5 81 p p m are 9 6 and 10 0 Hz The asymmetry of the line intensities of each triplet and the overall symmetry of the pair of triplets are due to second-order effects, and give evidence that these two protons are mutually coupled, with J 9 6 Hz As $J_{2,3}$ also equals 9 6 Hz, we conclude that the shift of proton 3 is 6 00 p p m Proton 4 resonates at 5 81 p p m , with $J_{3\,4}$ 9 6 Hz and $J_{4\,5}$ 10 0 Hz

Proton 4 is coupled to proton 5, with $J_{4\,5}$ 10 0 Hz This coupling is also found in the fine structure of the octet at 4 44 p p m (977 Hz) We would expect proton 5 to resonate at high field, as it is bonded to a carbon atom that does not bear a nitrate group The nitrate group causes a downfield shift The fine structure of proton 5 at 4 44 p p m also shows splittings of 6 2 and 3 0 Hz, caused by coupling with the two methylene protons, H-6 and H-6′, of the $-CH_2-ONO_2$ group

Protons 6 and 6 resonate at 5 06 p p m (1113 Hz) and 4 90 p p m (1078 Hz) Two different shifts arise because of molecular asymmetry The magnitude of the mutual coupling between these two protons is 13 0 Hz, typical of geminal proton–proton couplings The fine splittings ($J_{5\,6}$ 3 0 Hz and $J_{5\,6}$ 6 2 Hz) are identical to those found in the high-field multiplet of proton 5, confirming this conclusion

A summary of these assignments and their refinement through the LAOCN 3 program are compiled in Table I For **1**, the spectrum generated from computer calculations is shown in Fig 2

TABLE I

^{1}H-N M R CHEMICAL SHIFTS AND COUPLING CONSTANTS[a] OF METHYL β-D-GLUCOPYRANOSIDE TETRANITRATE[b] (1)

	First-order analysis		*Computer-calculated values (LAOCN 3)*[c]	
	p p m	*Hz*	*Best values (Hz)*	*Probable error (Hz)*
H-1	4 96	1091 2	1092 468	0 010
H-2	5 57	1225 4	1225 917	0 011
H-3	6 00	1320 0	1319 684	0 011
H-4	5 81	1278 2	1279 899	0 011
H-5	4 44	976 8	974 907	0 010
H-6	5 06	1113 2	1110 762	0 011
H-6′	4 90	1078 0	1077 949	0 011
$J_{1\,2}$		8 0	7 947	0 015
$J_{2\,3}$		9 6	9 847	0 015
$J_{3\,4}$		9 6	9 593	0 015
$J_{4\,5}$		10 0	9 938	0 015
$J_{5\,6}$		3 0	2 806	0 015
$J_{5\,6}$		6 2	5 527	0 015
$J_{6,6}$		−13 0	−12 606	0 015

[a]At 220 MHz [b]In acetone-d_6 , reference standard. Me_4Si [c]Root mean-square error = 0 122 Hz.

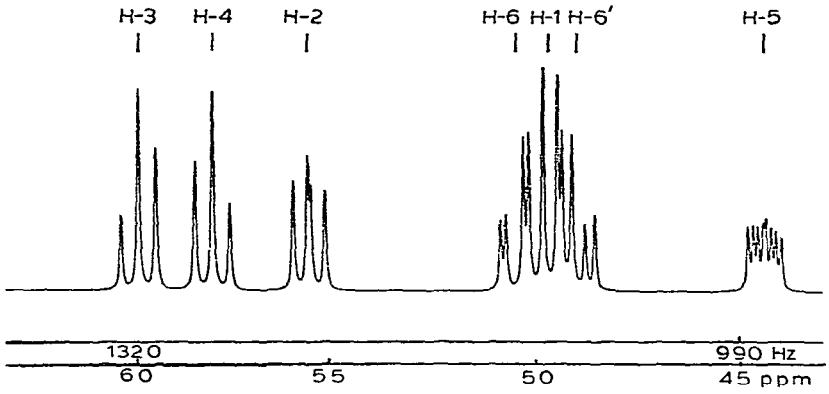


Fig 2 Simulated spectrum of methyl β-D-glucopyranoside tetranitrate (1) obtained from the LAOCN 3 and KOMBIP programs

β-Cellobiose octanitrate (2) — The ^{1}H-n m r spectrum of a solution of cellobiose octanitrate in acetone-d_6 is shown in Fig 3. The spectrum is clearly resolved, except for the signals at 4 5 p p m (990 Hz) and, possibly, at 6 1 p p m (1342 Hz), where some complex features are noticeable Consequently, care must be exercised in the first-order analysis Some internal spacings of the multiplets will not necessarily give the true coupling-constants

Protons 1A and 1B are the only protons that can give rise to a doublet in the spectrum Doublets appear at 6 48 p p m (1426 Hz) and 5 36 p p m (1179 Hz) We have assigned the signal at 5 36 p p m to 1B and the signal at 6 48 p p m to 1A, because proton 1A is bonded to a carbon atom that carries a (highly deshielding) nitrate group Assuming that the spacings correspond sufficiently well to the actual coupling-constants, we observed that proton 1A is coupled to proton 2A with $J^{A}_{1,2}$ 8 1 Hz, whereas proton 1B is coupled to proton 2B with $J^{B}_{1\,2}$ 7 9 Hz The only other place where such couplings can be found is in the multiplet region between 5 74 and 5 57 p p m The splitting pattern in this region indicates that the chemical shift of proton 2A is at 5 60 p p m (1232 Hz) and that of protons 2B at 5 71 p p m (1256 Hz) The fine structure also shows that $J^{A}_{2,3}$ and $J^{B}_{2,3}$ are very close, and equal to 9 7 and 9 8 Hz, respectively

The only region of the spectrum that shows coupling of this magnitude, 9 7–9 8 Hz, comprises the two sets of triplets centered at 6 07 p p m (1335 Hz) and 5 91 p p m (1300 Hz) The double intensity of the low-field triplet (6 07 p p m) clearly indicates the presence of two protons having comparable coupling There is a hint, however, of the possibility of higher-order effects from the features observed for the 6 07-p p m triplet From the magnitude of the spacings at 6 07 and 5 91 p p m , we deduced that these signals must be associated with protons 3A, 3B, and 4B We excluded 4A, because it is attached to a carbon atom that does not bear a nitrate group and, hence, is expected to resonate at a much higher field. The triplet centered

at 5 91 p p m shows the low-field line to be much greater in intensity than the high-field line, giving evidence that this proton is coupled to a proton resonating at lower field

As 4A, which is coupled to 3A, resonates at higher field, we conclude that the signal at 5 91 p p m does not arise from proton 3A, and must, therefore, be assigned to either proton 3B or 4B Two arguments may be advanced to assign the signal at 5 91 p p m to proton 4B Firstly the relative chemical-shifts of the ring protons on ring B of cellobiose octanitrate would be expected to parallel those of methyl β-D-glucopyranoside tetranitrate, *i e*, the sequence of the signals should proceed in the order 3B 4B 2B towards higher field Secondly, placing the signal of proton 4B in coincidence with that of proton 3B at 6 07 p p m would be expected to yield a non-first-order splitting-pattern at 6 07 p p m considerably more complex than is actually observed To summarize, we conclude that the double-intensity signal at 6 07 p p m originates from protons 3A and 3B, whereas the signal observed at 5 91 p p m arises from proton 4B

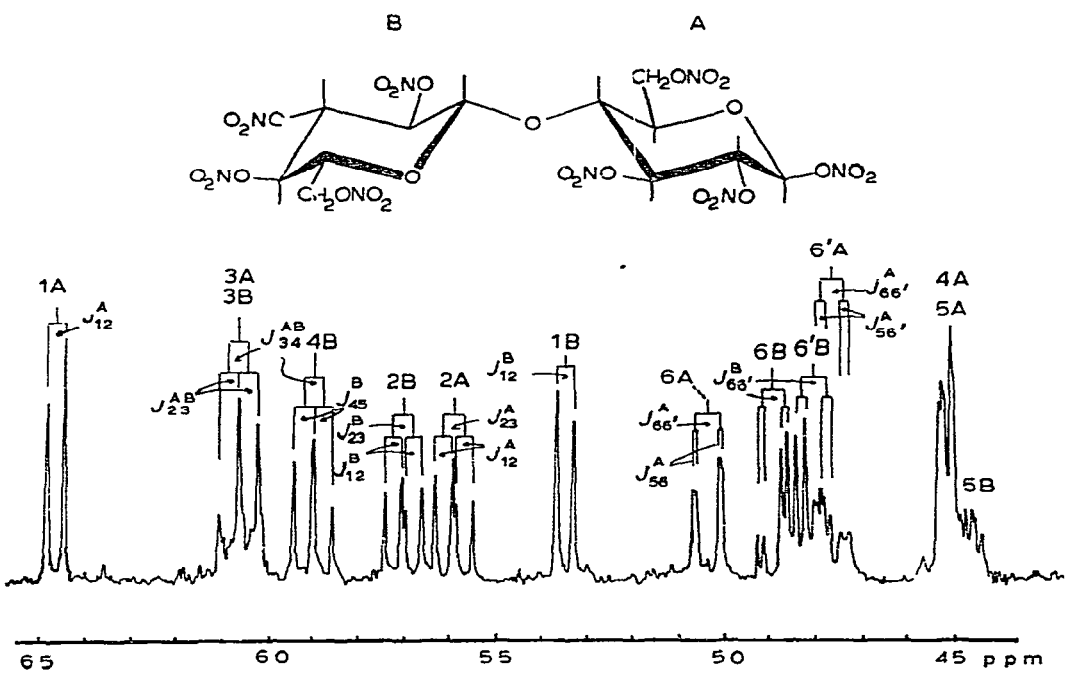


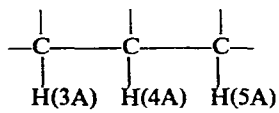
Fig 3 Experimental spectrum of β-cellobiose octanitrate (**2**), and first-order spectral assignments

Interpretation of the splittings in the region between 5 1 p p m (1120 Hz) and 4 7 p p m (1030 Hz) is clearly illustrated in Fig 3 The fine details of the splittings give evidence that all of these lines belong to the 6A, 6'A, 6B, and 6'B protons Here, we find spacings corresponding to $J_{6,6'}$ 12 3 Hz, and another, to $J_{6,6'}$ 11 7 Hz These

absolute values are typical of geminal, proton–proton couplings It is apparent from the spectrum that the proton at 5 05 p p m (1111 Hz) is coupled to the proton at 4 77 p p m (1049 Hz), as both exhibit a spacing of 12 4 Hz Similarly, the proton at 4.90 p p m (1078 Hz) is coupled to that at 4 81 p p m (1058 Hz) with a spacing of 11 7 Hz From the spectrum, it is not immediately apparent which pair of protons belongs to which ring We note, however, that the B ring much more resembles that of methyl β-D-glucopyranoside tetranitrate than does the A ring Comparison of the spectrum of this compound with that of the octanitrate led us to assign 5 05, 4 90, 4 81, and 4 77 p p m to protons 6A, 6B, 6'B, and 6'A, respectively The fine structure in this region indicates the following couplings $J^{A}_{5\,6'}$ 1 1 Hz, $J^{A}_{5\,6}$ 4 0 Hz, $J^{B}_{5\,6}$ 3 0 Hz, and $J^{B}_{5,6}$ 4 9 Hz In the 4 5-p p m region, despite overlapping with the signals of the 4A and 5A protons it seems reasonable to identify the five highest-field peaks as belonging to the octet of the 5B proton The measured spacings appear to correspond with those found for the 6B and 6'B protons

A summary of the assignments from the first-order analysis of the observed spectrum is given in Table II Except for protons 3A and 3B at 6 07 p p m and protons 4A, 5A, and 5B in the neighborhood of 4 5 p p m , all other chemical shifts and coupling constants appear to be fairly well established by the results of this first-order analysis

Interestingly, the assignments deduced from the first-order analysis suggest a classical case for the possibility of virtual long-range, spin–spin coupling[4] for protons 3A, 4A, 5A, 6A, or 6'A For example, in the 3-proton arrangement shown, with $|\nu_{3A}-\nu_{4A}| > J_{3\,4A}$ and $J_{3\,5A}=0$, the 3A proton resonance is expected to be com-



plicated by virtual long-range coupling due to the fact that the chemical-shift difference between protons 4A and 5A approaches zero, $|\nu_{4A}-\nu_{5A}| \sim 0$ Indeed, as already mentioned, we see evidence of additional signals at 6 07 p p m , the region assigned to the 3A proton Similarly, the broadening of the 6'A proton signals at 4 76 p p m could also be an indication of the occurrence of virtual long-range coupling In fact, expansion of the spectrum from 1 mm per Hz to 5 mm per Hz showed that the apparent doublet at 4 76 p p m is actually a triplet caused by higher-order effects

By repeated application of the combined LAOCN 3 and KOMBIP programs, we succeeded in faithfully reproducing the experimental spectrum, as illustrated in Fig 4 Each ring of β-cellobiose octanitrate has seven protons (seven-spin system), which corresponds to the limit allowable with the LAOCN 3 program Consequently, the computer analysis had to be performed for the protons of each ring separately The computer tracings for protons of the individual rings are shown in Figs 5 and C The simulated spectrum for the protons of ring A confirms the occurrence of higher-order effects in the regions of 6 07 and 4 76 p p m To establish conclusively the

TABLE II

^{1}H-N M R. CHEMICAL SHIFTS AND COUPLING CONSTANTS[a] OF β CELLOBIOSE OCTANITRATE[b] (2)

	First-order analysis				*Computer-calculated values (LAOCN 3)*[c]			
	Ring A		*Ring B*		*Ring A*		*Ring B*	
	p p m	*Hz*	*p p.m*	*Hz*	*Best values (Hz)*	*Error (Hz)*	*Best values (Hz)*	*Error (Hz)*
H 1	6 48	1425 6	5 36	1179 2	1422 916	0 013	1176 321	0 012
H-2	5 60	1232 0	5 71	1256.2	1230 455	0 013	1254 341	0 012
H 3	6 07	1335 4	6 07	1335 4	1331 279	0 011	1332 304	0 012
H 4	4 50	990 0	5 91	1300.2	991 797	0 018	1298 530	0 012
H-5	4 50	990 0	4 50	990.0	994 781	0 018	984 630	0 011
H-6	5 05	1111 0	4 90	1078.0	1107 670	0 014	1074 860	0 014
H-6′	4 77	1049 4	4 81	1058 2	1048 441	0 012	1058 501	0 014
$J_{1,2}$		7 5		7 0	8 017	0 019	7 818	0 016
$J_{2,3}$		8 5		8.5	9 458	0 017	9 617	0 017
$J_{3,4}$		8.5		8.5	9 043	0 027	9 519	0 016
$J_{4,5}$		?		8 5	9 348	0 017	9 816	0 017
$J_{5,6}$		1 0		2 8	1 968	0 024	2 914	0 018
$J_{5,6'}$		4 0		4 0	4 933	0 025	4 806	0 018
$J_{6,6'}$		−11 2		−10 5	−11 931	0 018	−11 845	0.016

[a]At 220 MHz [b]In acetone-d_6, reference standard Me_4Si [c]Root mean-square error ring A = 0 155 Hz, ring B = 0 134 Hz

relative positions of the 4A and 5A proton signals, we have varied their chemical shifts and permuted these values over a small range (2–4 Hz) As shown in Fig 7, a reversal of the two main peaks in this region is observed, and comparison of the signal pattern with that shown in Fig 6 leaves no doubt that the assignment for these two protons is correct The final assignments from the computer analysis are given in Table II

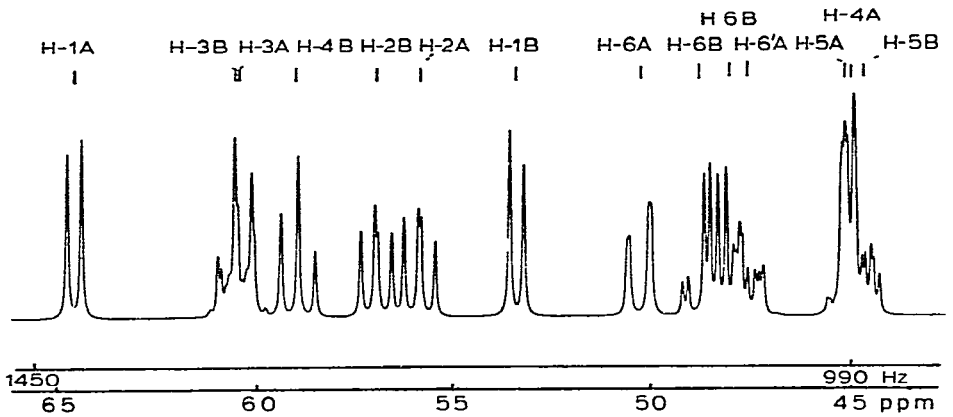


Fig 4 Simulated spectrum of β-cellobiose octanitrate (2) obtained from the LAOCN 3 and KOMBIP programs

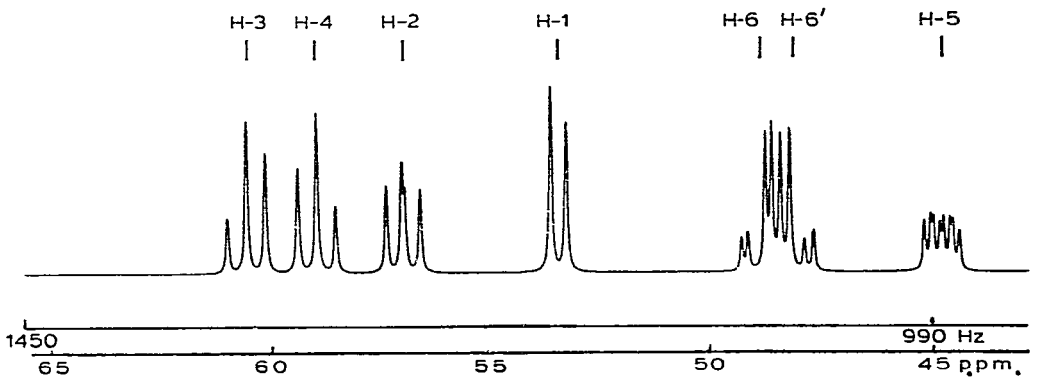


Fig 5 Simulated spectrum for ring B of β-cellobiose octanitrate (2) obtained from the LAOCN 3 and KOMBIP programs

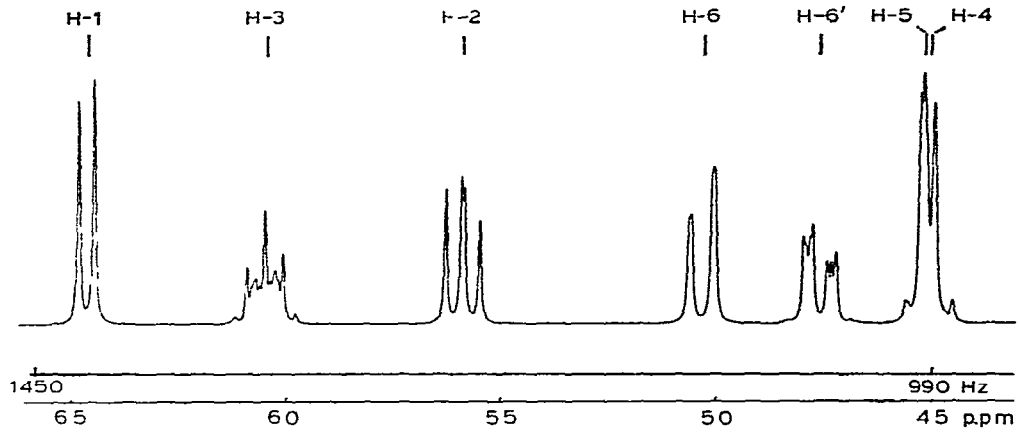


Fig 6 Simulated spectrum for ring A of β-cellobiose octanitrate (2) obtained from the LAOCN 3 and KOMBIP programs

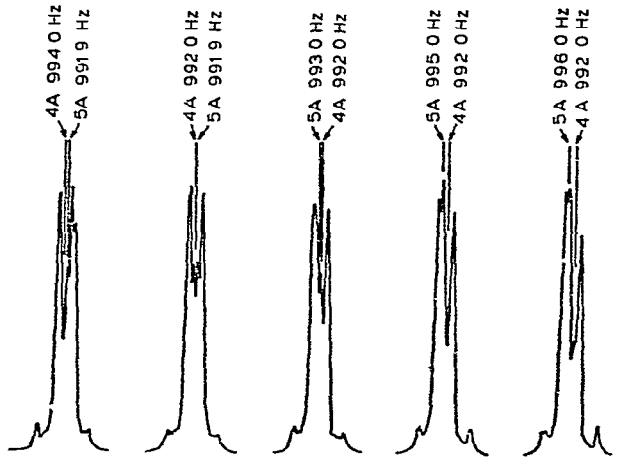


Fig 7 Effect of small variations in chemical shifts on the peak shape of the 4A and 5A protons

DISCUSSION

With the help of the LAOCN 3 program, we have accomplished a complete assignment of the chemical shifts and coupling constants for all the protons of methyl β-D-glucopyranoside tetranitrate and β-cellobiose octanitrate We observe that the magnitude of the coupling constants for all the ring protons in both compounds ranges from 8 to 10 Hz If these values are compared with those tabulated for chair forms of many carbohydrate derivatives[5], it may be concluded that the steric relationship between the protons on vicinal carbon atoms is anti-periplanar, and that

the pyranose residues in methyl β-D-glucopyranoside tetranitrate and β-cellobiose octanitrate are all in the $^4C_1$(D) conformation

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