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FULL ASSIGNMENT OF THE PROTON AND CARBON-13 NMR SPECTRA OF 2,3,6 -TRI-O-METHYL-β-CYCLODEXTRIN

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ABSTRACT - The proton and carbon-13 NMR spectra of 2,3,6 -tri-O-methyl-β-cyclodextrin in deuteriochloroform have been fully and unambiguously assigned using homonuclear and selective heteronuclear spin decoupling and two-dimensional homo- and heteronuclear correlation NMR spectroscopy. Corrections are made to some earlier literature assignments.

INTRODUCTION

The properties of cyclodextrins and their ability to complex with suitable guest molecules in solution and in the solid state have been well documented.[1] Pharmaceutical interest in β-cyclodextrin has recently been extended to methods of improving the often low aqueous solubility of its complexes with apolar drug molecules. In particular, methylation of β-cyclodextrin to form the 2,3,6-tri-O-methyl derivative (I) significantly improves aqueous solubility in addition to conferring solubility in organic solvents such as chloroform. NMR investigations into the effect of guest molecules on the ^{1}H and ^{13}C chemical shifts of the parent β-cyclodextrin molecule have unequivocally verified inclusion of the guest molecule within the host cavity,[2,3] and NMR data have been used in conformational analysis of the complexes.[4] While it has been demonstrated that 2,3,6-tri-O-methyl-β-cyclodextrin retains complexing ability, detailed NMR investigations into the nature of host-guest interactions have not so far been published.[5,6] This has been due to difficulty in resolving proton signals, particularly those from H-3 to H-6 of the macrocycle, which are close to or overlapping with the methoxy proton signals. ^{13}C assignments for C-1 to C-6 of 2,3,6-tri-O-methyl-β-cyclodextrin have been proposed by Gagnaire *et. al*,[7] but have subsequently been re-examined and questioned by Szejtli *et. al*.[8] This paper reports the unambiguous assignment of all the carbon-13 and proton resonances in this molecule by methods which do not rely on spectral comparisons with model compounds.

RESULTS and DISCUSSION

The proton NMR spectrum (Figure 1a) of (I) at 360 MHz clearly shows the resonances due to the protons of three methoxy groups. The highest frequency doublet at 5.12 δ can be assigned with certainty to the acetal proton H-1. Other clear proton resonances are centred at 3.18, 3.79 and 3.84 δ, and the remaining three proton resonances are partly obscured by two of the methoxy resonances. The H-2 resonance and the position of the H-3 resonance were identified by homonuclear double resonance experiments. Irradiation of the H-1 resonance simplifies only the doublet of doublets at 3.18 δ to a doublet therefore this resonance is assigned to proton H-2. Irradiation of the H-2 resonance collapses the H-1 resonance to a singlet and causes a change in the appearance of the spectrum around 3.5 δ. Irradiation at this position simplifies the H-2 resonance to a doublet indicating that the central part of the H-3 proton resonance is obscured by a methoxy resonance. The position of the resonances due to H-3 and H-4 are clearly revealed by the contour plot (Figure 1b) from a two-dimensional proton COSY experiment.[9] Off-diagonal peaks correlate the H-2 resonance with the H-3 resonance which appears as a triplet centred at 3.50 δ, the outer lines of which are visible in Figure 1a. Also clear is the correlation of this resonance with the H-4 resonance, which appears as a triplet at 3.60 δ, also discernable in Figure 1a. The experimental conditions do not yield the correlation of the H-4 and H-5 resonances but show, but not assign, the positions of the protons H-5, H-6a and H-6b. The assignment of these and of the methoxy resonances was made in conjunction with carbon-13 NMR spectra.

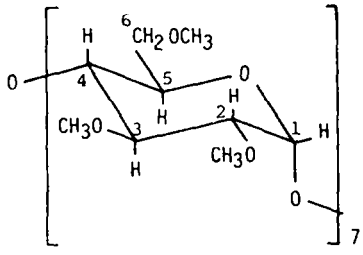
The carbon-13 NMR spectrum (Figure 2a) at 90 MHz of a 0.02 M solution in deuteriochloroform is fully resolved. Resonances were identified as due to CH, CH_2 or CH_3 groups from spectra obtained using the DEPT pulse sequence.[10] From these spectra the resonance at 71.0 δ can be unambiguously assigned to the methylene carbon C-6.

The assignment of resonances corresponding to carbon atoms C-1 to C-5 and the correlation of the methoxy carbon and proton resonances was made using a two-dimensional heteronuclear correlation experiment[11] based on the DEPT pulse sequence and utilising one bond proton-carbon couplings. A contour plot of the carbon chemical shift region from 55 δ to 100 δ is shown in Figure 2b. The assignments of C-1 to C-4 are made by direct correlation with the resonances of the protons H-1 to H-4 previously assigned. The remaining CH resonance therefore corresponds to C-5 and thus the multiplet at 3.79 δ in the proton spectrum is assigned to H-5. The methylene carbon atom C-6 correlates with the remaining two proton resonances at 3.56 δ and 3.84 δ (Table 1). The digital resolution in the proton dimension allows only proton homonuclear couplings in excess of *ca.* 7 Hz to be visible. However a first order analysis of a resolution enhanced one-dimensional proton spectrum gave the inter-proton coupling constants as follows:

$^3J_{1,2}$ = 3.6 Hz $^3J_{2,3}$ = 9.7 Hz $^3J_{3,4}$ = 8.6 Hz $^3J_{4,5}$ = 9.5 Hz

$^3J_{5,6a}$ = 1.3 Hz $^3J_{5,6b}$ = 4.0 Hz $^2J_{6a,6b}$ = 10.3 Hz

These values are in good agreement with those reported[12] for β-cyclodextrin and its peracetyl derivative and confirm the conformation and configuration of the sugar ring. Internal rotation gives time averaged values for the H-5/H-6a and H-5/H-6b coupling constants. It has been



(I)

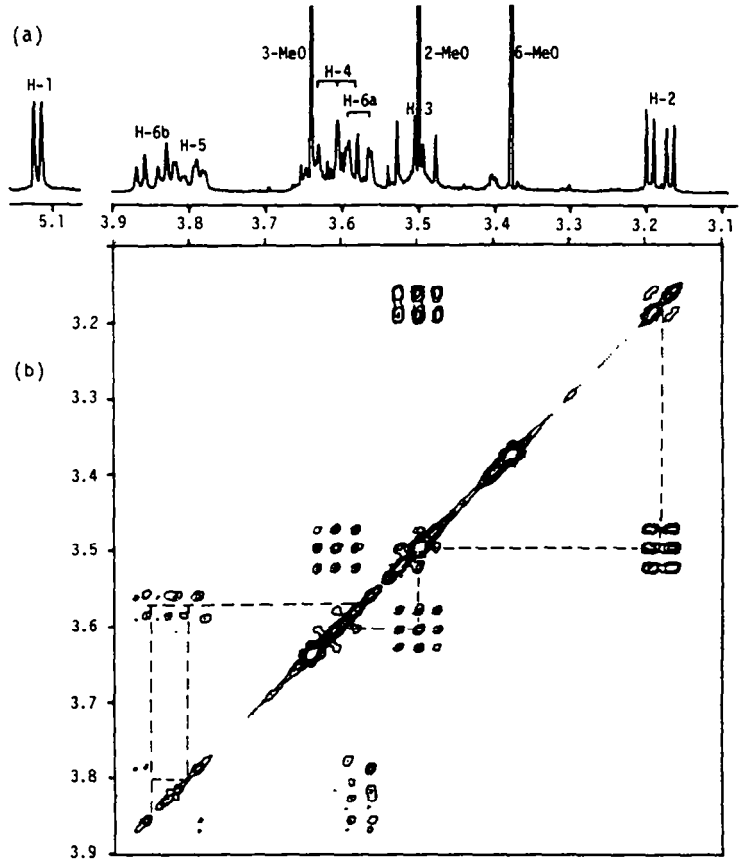


Figure 1. (a) H 360 MHz NMR spectrum of compound (I): (15 mg in 0.4 ml $CDCl_3$)
(b) Two-dimensional H COSY NMR spectrum of (I).

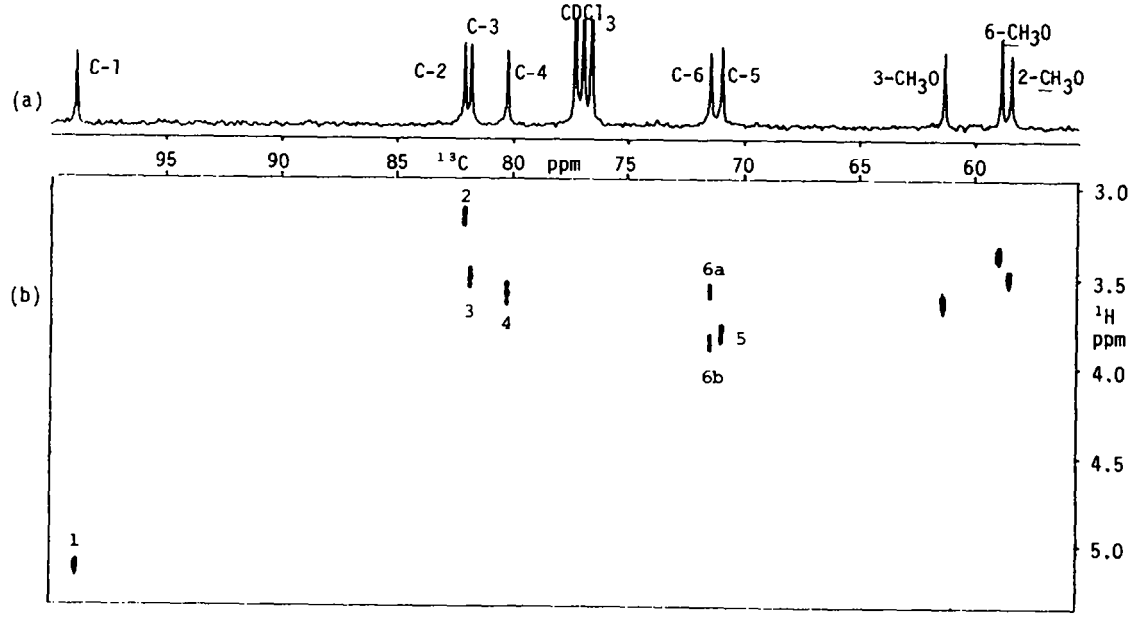
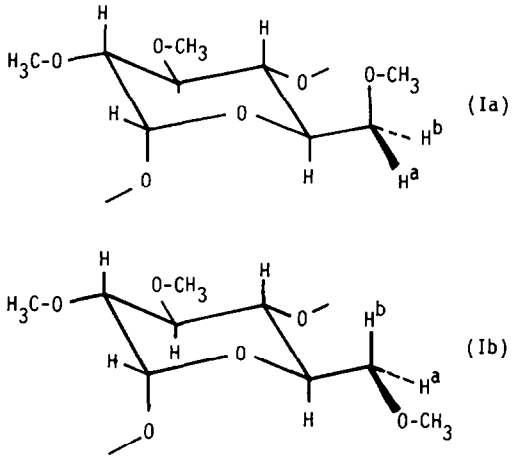


Figure 2. (a) ^{13}C 90 MHz NMR spectrum (proton decoupled) of compound (I)
(b) Two-dimensional ^{1}H-^{13}C correlation NMR spectrum (contour plot) of (I)

TABLE 1. Carbon and Proton Chemical Shifts† of Compound (1)

Proton	δ	Carbon	δ
H-1	5.12	C-1	98.4
H-2	3.18	C-2	81.6
H-3	3.50	C-3	81.4
H-4	3.60	C-4	79.7
H-5	3.79	C-5	70.5
H-6a	3.56	C-6	71.0
H-6b	3.84		
2-CH_3O	3.50	2-CH_3O	58.0
3-CH_3O	3.64	3-CH_3O	60.9
6-CH_3O	3.37	6-CH_3O	58.4

†(p.p.m. from TMS)



(Ia)

(Ib)

suggested[13] that significant contributions arise from the *gauche-gauche* (1a) and *gauche-trans* (1b) conformations. This would lead to a smaller value for the H-5/H-6a coupling constant as in neither conformation does H-6a occupy a position *trans* to H-5.

The assignments of the methoxy carbon resonances and thence the methoxy proton resonances were made from fully proton coupled and low power selectively proton decoupled carbon-13 NMR spectra. The methoxy region, 56-62 δ, (Figure 3) shows the long range proton coupling superimposed on the central pair of lines of each methoxy carbon quartet. This arises from 3 bond proton-coupling through oxygen. Thus the triplet fine structure on the resonance centred at 58.4 δ allows the assignment of this resonance to the methoxy group on C-6. Low power irradiation of the H-2 proton resonances removes the long range coupling to the resonance centred at 58.0 δ and similar irradiation of the H-3 proton resonance removes the long range coupling to the resonance centred at 60.9 δ, thus identifying these carbon resonances as due to the C-2 and C-3 methoxy groups respectively. The other intensity and multiplicity changes in these spectra arise from population transfer and spin tickling effects caused by the proximity to the irradiation position of carbon-13 satellites of other proton resonances. The identification of the corresponding proton resonances (Table 1) follows from the two-dimensional proton-carbon correlation spectrum.

CONCLUSION

This full assignment of the proton and carbon NMR spectra (Table 1) demonstrates that the recently proposed assignments[8] for C-3 and C-4 should be reversed in accordance with the earlier assignments of Gagnaire.[7] The assignments for the remaining ring carbon atoms is confirmed and the methoxy carbon resonances have also been assigned. The proton spectrum has been fully resolved and requires that the previous assignments[14] of the 2- and 3-methoxy proton resonances be reversed. These results demonstrate that it is not always advisable to rely on chemical shift assignments based solely on incremental substituent effects.

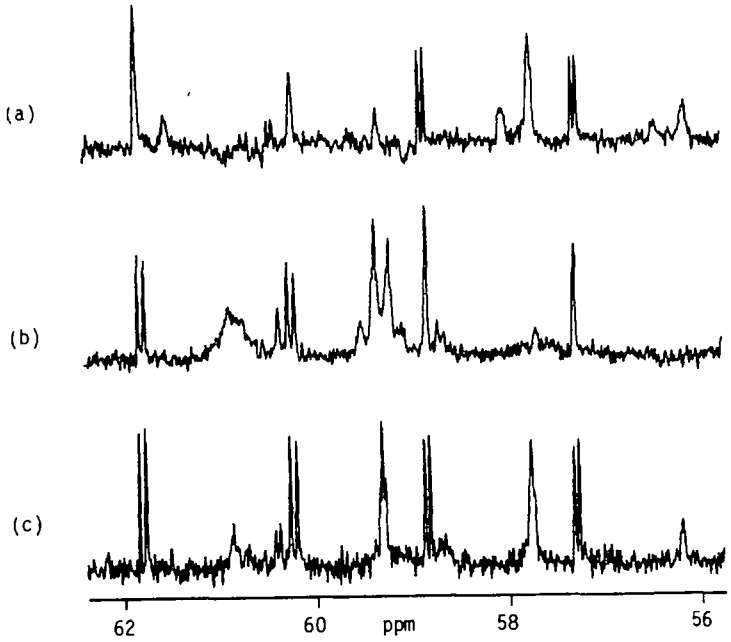


Figure 3. ^{13}C NMR spectra of compound (I): (a) with selective low power irradiation of H-3, (b) with selective low power irradiation of H-2, (c) fully proton coupled.

EXPERIMENTAL

Materials

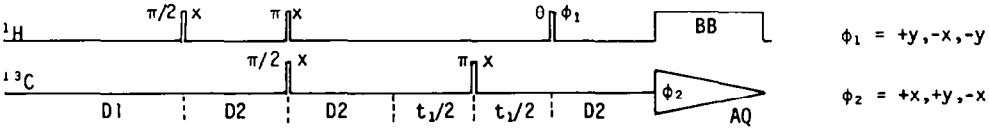
2,3,6-tri-O-methyl-β-cyclodextrin was synthesised by a reported procedure,[8] and the crude product recrystallised twice from n-hexane (m.p. 86.0 - 87.5, lit.[6] m.p. 88°).

NMR Spectroscopy

NMR spectra were recorded at ambient temperature in deuteriochloroform on a Bruker WH360 spectrometer equipped with an Aspect 2000 computer, using the DISNMRP Program version 820601.6 and operating at 360.13 MHz for protons and 90.56 MHz for carbon-13 nuclei. Selectively proton decoupled carbon-13 NMR spectra were obtained using low decoupling power of 41 dB below 0.5 watt to remove long range couplings only.

Two-dimensional proton homonuclear correlation spectra (COSY) were obtained using the pulse sequence D1–90°–t_1–D2–θ°–D2–AQ, with phase cycling for N-type selection, where D1 is a relaxation delay, D2 is a fixed delay to enhance small couplings, t_1 is the incremented delay and θ is the observation pulse. Typical parameters: SW1 = ±250 Hz; SI = IK; 256 FIDs; D1 = 2s; D2 = 0.01 s; Δt_1 = 0.002; θ = 60°: F1 data zero filled from 256 W to 512 W, processed using sine-bell squared window in both dimensions and symmetrized. Resolution: 1.0 Hz/point.

Two-dimensional proton-carbon correlation spectra were obtained using the pulse sequence[11] (DEPT 2D) with phase cycling as indicated and with all phases incremented by 90° to give a 16 phase



cycle, and with proton broad-band decoupling throughout the acquisition period AQ. Typical parameters: SW1(H) = ±450 Hz; SW2(C) = 5000 Hz; SI = 2K; 128 FIDs; D1 = 3s; D2 = $1/2^1J_{CH}$ = 0.0037 s; Δt_1 = 0.0005 s; θ = 55°; F1 data zero-filled from 128 W to 256 W and processed as above (COSY) without symmetrization. ^{1}H resolution: 3.5 Hz/point, ^{13}C resolution: 4.9 Hz/point.

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