Assignment of the ^{1}H N.M.R. Spectrum of Heptamethyl Dicyanocobyrinate in Chloroform Solution by Two-dimensional ^{13}C/^{1}H Chemical Shift Correlation Spectroscopy

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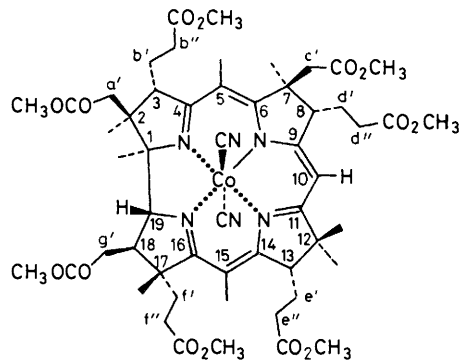
The ^{1}H n.m.r. chemical shifts of the vitamin B_{12} derivative heptamethyl dicyanocobyrinate ('cobester') in $CDCl_3$ have been determined by two-dimensional ^{13}C/^{1}H chemical shift correlation spectroscopy at 75/300 MHz. For reasons of sensitivity a pulse scheme was employed which eliminates homonuclear spin-spin coupling in the F_1 dimension between protons bonded to different carbon atoms. Large differences in ^{1}H chemical shifts and changes of signal sequences are observed relative to Battersby's study of cobester solutions in C_6D_6.

Heptamethyl dicyanocobyrinate ('cobester') is an important compound in chemical and biosynthetic studies of vitamin B_{12}.[1,2] Its ^{13}C n.m.r. spectrum has been completely assigned for solutions in both deuteriochloroform[3] and hexadeuteriobenzene.[4] A ^{1}H n.m.r. analysis at 400 MHz has also been carried out, but only for the C_6D_6 solution.[4] This analysis required an extensive set of experiments to be carried out, *viz.* n.O.e. difference spectroscopy, decoupling difference measurements, selective ^{13}C{^{1}H}-decoupling, T_1 determinations, and investigation of a number of specifically deuteriated derivatives.



Not only is $CDCl_3$ more common as a solvent than C_6D_6 but, as we found in a study of a series of dicyanocobyrinic acid methyl ester amides,[5] cobester derivatives of higher polarity dissolve better in it. Moreover, substantial differences in ^{1}H chemical shifts are to be expected between those recorded for $CDCl_3$ solutions and those for C_6D_6. For these reasons we undertook an analysis of the ^{1}H n.m.r. spectrum of cobester in $CDCl_3$. Since we did not have a number of suitably deuteriated derivatives at our disposal and since the spectrum of the $CDCl_3$ solution shows more severe overlap of resonances than the one of the C_6D_6 solution, we decided to perform an indirect analysis *via* two-dimensional (2D) heteronuclear shift correlation spectroscopy. By this means, the secure assignment of the ^{13}C n.m.r. spectrum[3] leads to an unambiguous assignment of the ^{1}H chemical shifts.

The cross-sections in the F_1 dimension of a 2D ^{13}C/^{1}H shift correlation data matrix contain the resonances of the protons bonded to the individual ^{13}C nuclei. In the original version[6-9] of the experiment, both the ^{1}H chemical shifts and, given sufficient digital resolution, the H,H-coupling constants can be obtained from these cross-sections. The procedure, however,

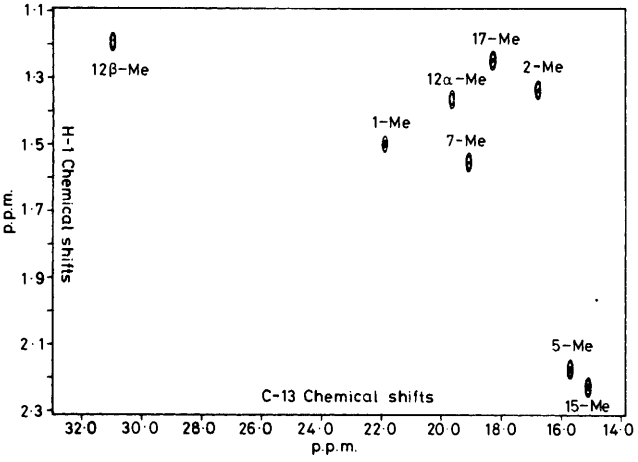


Figure 1. Contour plot (methyl region) of the ^{13}C/^{1}H chemical shift correlation matrix for the solution of cobester in $CDCl_3$. The minimum level was chosen high enough to suppress methylene peaks in the lower left quadrant

has the disadvantage of low sensitivity when the proton resonances are split into many-line multiplets by a large number of coupling partners. In the cobester molecule some protons are coupled to four non-equivalent neighbouring nuclei and thus give rise to complicated multiplets which are the cause of the low signal/noise ratio in the 2D ^{13}C/^{1}H-correlation spectrum. This situation can be improved by employing one of the recent pulse schemes, by Bax[10] or by Rutar,[11] which suppress ^{1}H,^{1}H-coupling between protons bonded to different ^{13}C nuclei, thus yielding better signal/noise ratios at the expense of ^{1}H,^{1}H coupling information. For reasons of sensitivity, we decided to apply this technique.

Results and Discussion

Various sections of the ^{13}C/^{1}H chemical shift correlation matrix are represented as contour plots in Figures 1—3. Figure 1 shows very clearly the assignment of the ^{1}H C-methyl resonances which previously was difficult to obtain[3] by selective ^{13}C{^{1}H}-decoupling because of the similar ^{1}H chemical shifts, especially of the 2- and 12α-CH_3 groups. Since all the C-methyl groups in the one-dimensional ^{1}H n.m.r. spectrum appear as singlet resonances, their appearance in the 2D ^{13}C/^{1}H correlation experiment with 'homo-nuclear broadband

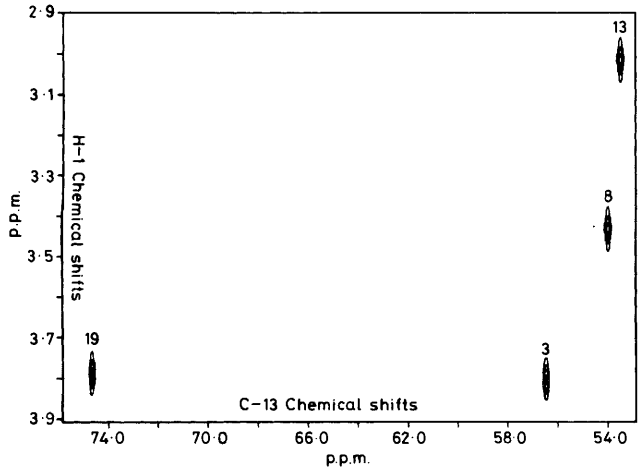


Figure 2. Contour plot (methine region) of the $^{13}C/^{1}H$ shift correlation matrix of cobester in $CDCl_3$

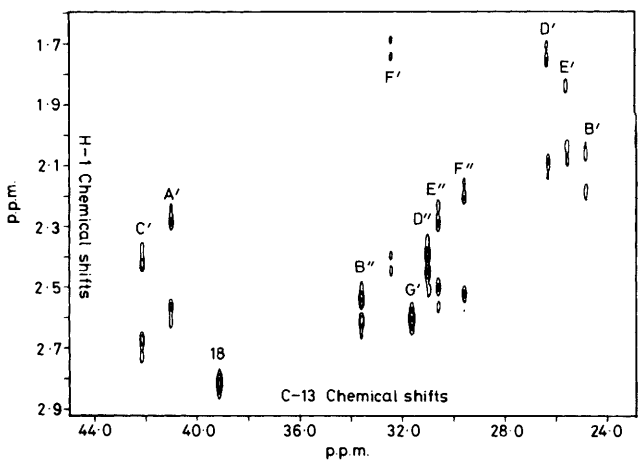


Figure 3. Contour plot (methylene region) of the $^{13}C/^{1}H$ shift correlation matrix of cobester in $CDCl_3$

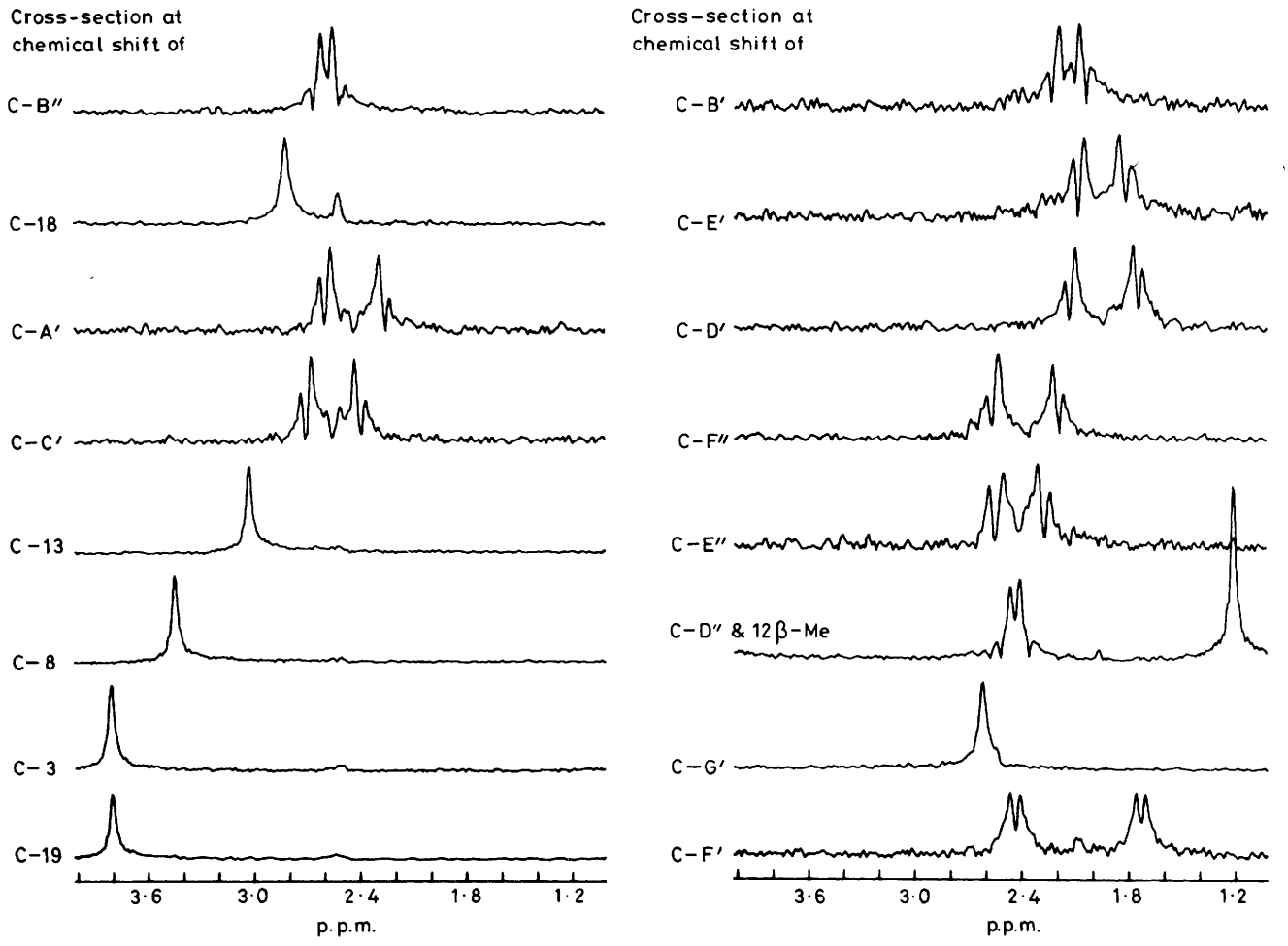


Figure 4. Cross-sections through the correlation matrix at the ^{13}C chemical shifts of methine and methylene carbons, showing chemical shifts of connected protons

decoupling' is the same as in a conventional experiment. The situation is different for the methine moieties H-C(19), H-C(3), H-C(8) and H-C(13) (Figure 2). While in the proton spectrum, and hence also with conventional $^{13}C/^{1}H$ correlation, they would appear as the X parts of AX and ABX or AMX spin systems, respectively, the pulse sequence applied in this study effectively decouples the neighbouring protons with a concomitant increase of the signal/noise ratio.

The advantage of 2D $^{13}C/^{1}H$ correlation is demonstrated well by the H-C(19) and H-C(3) resonances. The signals of the

Table. Results of the 2D $^{13}C/^{1}H$ correlation experiment for heptamethyl dicyanocobyrinate in $CDCl_3$ solution

Carbon	^{13}C Chemical shift in $CDCl_3$*†	^{1}H Chemical shift(s) in $CDCl_3$	^{1}H Chemical shift(s) in C_6D_6‡
10	91.1	5.59	5.74
19	74.7	3.80	4.02
3	56.6	3.82	3.93
8	54.1	3.45	3.71
13	53.6	3.03	2.81
g-OMe	52.25	3.760	
Other OMe	51.71	3.697	3.46—3.28
	51.70	3.717	
	51.67	3.688	
	51.63	3.684	
	51.50	3.675	
	51.46	3.629	
c′	42.2	2.71, 2.41	2.55, 2.55
a′	41.1	2.60, 2.28	2.94, 2.28
18	39.2	2.83	2.89
b″	33.7	2.64, 2.53	2.52, 2.52
f′	32.5	2.44, 1.74	2.46, 1.82
g′	31.7	2.62, 2.62	2.41, 2.27
d″	31.1	2.49, 2.38	2.65, 2.37
12β-Me	31.0	1.21	0.98
e″	30.7	2.54, 2.28	2.74, 2.38
f″	29.6	2.56, 2.20	2.77, 2.46
d′	26.4	2.13, 1.75	2.12, 1.81
e′	25.6	2.08, 1.83	2.19, 1.86
b′	24.9	2.22, 2.06	1.98, 1.98
1-Me	21.9	1.52	1.51
12α-Me	19.7	1.38	1.11
7-Me	19.2	1.57	1.39
17-Me	18.3	1.27	1.00
2-Me	16.8	1.36	1.17
5-Me	15.7	2.19	2.10
15-Me	15.2	2.24	2.24

* The ^{13}C signal sequence is identical for $CDCl_3$ and C_6D_6 solutions. † From ref. 3. ‡ From ref. 4.

protons at C-3 and C-19 are overlapping in the 1D spectrum even at 500 MHz observation frequency (in $CDCl_3$). Owing to the large shift difference of the respective carbon atoms, the assignment ($\delta_{3\text{-}H} > \delta_{19\text{-}H}$) is made very easy by the 2D experiment (Figure 2).

Finally, the advantages of the method are most clearly seen in Figure 3 from which the ^{1}H chemical shifts of the 22 side-chain methylene protons can be obtained without any difficulty despite the 'moderate' ^{1}H observation frequency of 300 MHz. There are 21 different chemical shifts for these methylene protons, only the two protons connected to C-g′ having shifts so similar that they cannot be resolved. This contrasts with the ^{1}H spectrum in C_6D_6 solution[4] where several accidental chemical shift equivalences are observed.

In Figure 3, an exception to the 'broadband decoupling' effect of Bax'[10] and Rutar's[11] pulse sequences has to be noted. The three proton pulses at the centre of the evolution period, which have been added to the conventional $^{13}C/^{1}H$ correlation pulse sequence, have the effect of eliminating H,H-coupling to protons which are not bonded to a ^{13}C nucleus. This affects vicinal or more remote protons since in ^{13}C n.m.r. spectroscopy only mono-^{13}C isotopomers are observed because of the low natural abundance of this nucleus. In the case of anisochronous geminal protons, however, the situation is different: the protons are both bonded to the ^{13}C nucleus of the same isotopomer, hence their mutual spin coupling is not suppressed by the pulse sequence. The cross-sections in the F_1 dimension of the $^{13}C/^{1}H$ shift correlation matrix (Figure 4) therefore show homonuclear coupling between protons that belong to the same methylene group. This leads to an AX or AB pattern appearance of the F_1 cross-sections, with second-order artefacts in the AB case. In spite of this complication, the signal/noise ratio of the methylene region was still better in the 2D spectrum which had been acquired in 13 h using the 'broadband decoupling' pulse sequence than in a spectrum obtained in 64 h by the conventional technique.

The digital resolution in the experiment just described was not sufficient to show all the individual cross-peaks in the methoxy region. A second experiment was therefore carried out in which a $^{13}C/^{1}H$ shift correlation was obtained only for the seven methoxy resonances. The result is shown in Figure 5. This demonstrates that with appropriate digital resolution very small chemical-shift differences (0.01 p.p.m. or less) are resolvable and that correlations are possible which would be unobtainable by selective decoupling. However, apart from the methoxy group of side-chain g, specific assignments cannot be made for the ^{1}H or ^{13}C methoxy signals. Figure 5 shows that, with one exception, increasing ^{1}H shielding of methoxy groups correlates with increasing ^{13}C shielding. It is questionable though that minute shielding differences such as the present ones will be of use in structure elucidations of unknown derivatives.

The Table summarizes the ^{1}H chemical shifts for the solution of heptamethyl dicyanocobyrinate in $CDCl_3$ and a comparison with Battersby's data[4] for the C_6D_6 solution is made. When the solvent is changed, most of the protons suffer chemical-shift changes of several tenths of a p.p.m. in either direction and, as a consequence, the signal sequence is substantially different in the two cases. It is not easy, however, to offer an explanation in terms of specific solute–solvent interactions because of the large number of functional groups in heptamethyl dicyanocobyri-

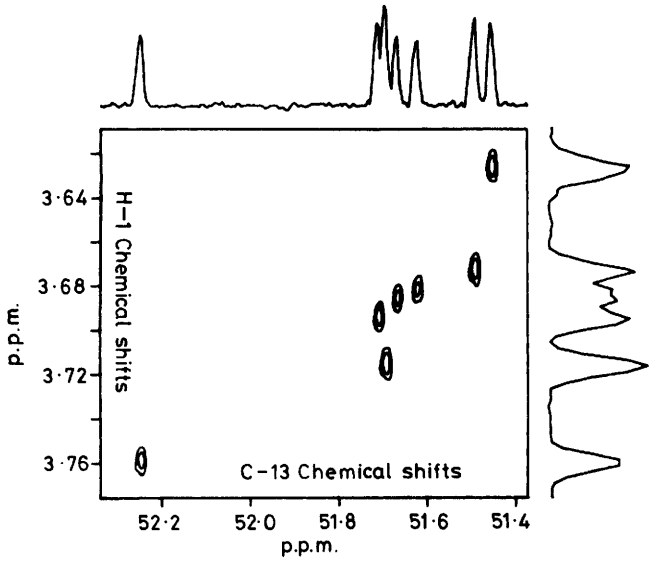


Figure 5. Very high resolution $^{13}C/^{1}H$ shift correlation (methoxy region) of cobester in $CDCl_3$. The projections of the F_2 (^{13}C) and of the F_1 (^{1}H) traces are displayed at the top and at the right, respectively.

nate. The correctness of Battersby's assignments of the cobester proton resonances in C_6D_6 solution was proved by applying $^{13}C/^{1}H$ chemical shift correlation to a sample of *ca.* 90 mg cobester in 0.5 ml C_6D_6. In general, very good agreement was found between Battersby's[4] results and ours. Only the unavoidable difference in concentration between the two solutions led to crossover of closely lying signals ($\Delta\delta \leqslant 0.05$ p.p.m.) in three instances. The present study shows that it is not possible simply to transfer assignments of proton signals obtained from a spectrum of cobester in one solvent to a spectrum taken in a different one. But since it is relatively easy to follow the dependence of the ^{13}C signal sequence as a function of the solvent, it should then also be possible to measure the proton chemical shifts in any solvent *via* $^{13}C/^{1}H$-correlation in a single overnight experiment.

Experimental

Heptamethyl dicyanocobyrinate was available from an earlier study.[3] A solution of 99 mg of compound in 0.5 ml $CDCl_3$ (concentration: 0.18 M) was studied in a 5 mm sample tube on a Bruker AM 300 n.m.r. spectrometer (^{1}H: 300.13 MHz, ^{13}C: 75.47 MHz) interfaced to an ASPECT 3000 computer. The lowest frequency ^{1}H resonance (12β-Me, $\delta_H = 1.21$) and the highest frequency ^{13}C methoxy resonance (g-OMe, $\delta_C = 52.25$) were used as chemical shift references. The chemical shift ranges covered in the $^{13}C/^{1}H$ correlation experiment were $\delta_C = 95.0 - 10.1$ (spectral width = 6 410 Hz in the F_2 dimension) and $\delta_H = 4.04—1.04$ (spectral width ± 450 Hz in the F_1 dimension). The pulse sequence used was:[11]

$D_1 - 90°_x(^{1}H) - t_1/2 - 90°_y(^{1}H) - D_3 - 180°_x(^{1}H),$
$180°_x(^{13}C) - D_3 - 90°_y(^{1}H) - t_1/2 - D_3 - 90°_x(^{1}H),$
$90°_x(^{13}C) - D_4$

followed by acquisition with broadband proton decoupling. The delays were $D_1 = 0.5$ s (for relaxation), $D_3 = 1/(2\,^{1}J_{CH}) = 3.70$ ms, $D_4 = 1/(3.5\,^{1}J_{CH}) = 2.12$ ms and $t_1/2$ (variable) from 3 μs to 71 ms in 128 increments of 555 μs. 488 Scans were accumulated for each value of $t_1/2$, preceded by one dummy scan. The resulting data matrix (2K times 128 points) was multiplied by a sine bell function and zero-filled once in both dimensions yielding, after double Fourier transformation, a 512-K matrix of real points (magnitude representation) with digital resolutions of 0.041 p.p.m./point on the ^{13}C and 0.012 p.p.m./point on the ^{1}H chemical shift axis. The 90° pulse widths were 7.5 μs (^{13}C, observe pulse) and 14.3 μs (^{1}H, decoupler pulse). The decoupler power was set at *ca.* 10 W for the decoupler pulse and at *ca.* 2 W for broadband decoupling. Bruker standard DISNMRP software (version 831101.1) was used. The total measuring time was *ca.* 13 h.

A second experiment was carried out for obtaining the detailed correlation between the ^{13}C and ^{1}H chemical shifts of the methoxy groups. The conventional pulse sequence:[6-9]

$D_1 - 90°_x(^{1}H) - t_1/2 - 180°_x(^{13}C) - t_1/2 - D_3 - 90°_x(^{1}H),$
$90°_x(^{13}C) - D_4 -$ (acquisition with broadband decoupling)

was used with $D_1 = 1.3$ s, $D_3 = 3.36$ ms, $D_4 = 1.92$ ms and $t_1/2 = 3$ μs to 320 ms in 64 increments of 5 ms. 48 Scans plus 2 dummy scans were accumulated into 512 words of computer memory. Spectral widths were 160 Hz in the F_2 dimension ($\delta_C = 52.98 - 50.86$) and ± 50 Hz in the F_1 dimension ($\delta_H = 3.86 - 3.53$). Zero filling in both dimensions gave a 64K real point matrix, digital resolution: 0.0041 p.p.m./point (^{13}C) and 0.0026 p.p.m./point (^{1}H). The g-methoxy signal was taken as internal reference ($\delta_C = 52.25$, $\delta_H = 3.760$) for the methoxy region correlation matrix. The total acquisition took *ca.* 3 h. Parameters not mentioned had the same values as in the first experiment.

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Received 16*th January* 1984; *Paper* 4/082