$CDCl_3$) δ (ppm) 8.92 (dd, J=4.2 & 1.8 Hz, 1H, H of C-2 in quinoline), 8.17-7.3 (m, 5H, Hs of quinoline), 5.41 (m, 2H, -CH=CH-), 3.32 (t, J=7.2 Hz, 2H, CH_2CO), 2.02 (m, 2H, CH_2-C=), 1.77 (m, 2H, CH_2 of C-2 in hept-5-enyl group), 1.61 (d, J=4.3 Hz, 3H, CH_3), 1.47 (m, 2H, CH_2 of C-3 in hept-5-enyl group); IR (neat) 1680 cm^{-1} for CO; mass spectrum: m/e (relative intensity), 253 (M^+, 15), 252 (M^+-1, 13), 225 (M^+-CO, 7), 198 (M^+-C_4H_7, 8), 184 (76), 171 (12), 156 (quinolinylCO$^+$, 100), 128 (quinolinyl$^+$, 54).

8-Quinolinyl oct-6-enyl ketone. ^{1}H-NMR (200 MHz, $CDCl_3$) δ (ppm) 8.93 (dd, J=4.1 & 1.7 Hz, 1H, H of C-2 in quinoline), 8.2-7.3 (m, 5H, Hs of quinoline), 5.38 (m, 2H, -CH=CH-), 3.32 (t, J=7.4 Hz, CH_2-CO), 1.99 (m, 2H, CH_2-C=), 1.76 (m, 2H, CH_2 of C-2 in oct-6-enyl group), 1.62 (d, J=4.8 Hz, 3H, CH_3), 1.38 (m, 4H, CH_2 of C-3 and C-4 in oct-6-enyl group); IR (neat) 1680 cm^{-1} for CO; mass spectrum: m/e (relative intensity), 267 (M^+, 5), 266 (M^+-1, 9), 239 (M^+-CO, 5), 212 (M^+-C_4H_7, 2), 198 (10), 184 (63), 171 (26), 156 (quinolinylCO$^+$, 100), 128 (quinolinyl$^+$, 55).

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Carbon-13 Two Dimensional INADEQUATE Experiment of Cholestane

Sueg-Geun Lee

Korea Research Institute of Chemical Technology, P.O. Box 9, Daedeogdanji, Taejon 305-606

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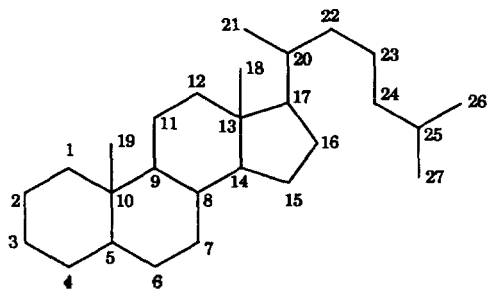
Cholestane (**1**) is one of the most important parent compound in the family of steroids which are of great biological interests, and still new steroids are continuously being isolated from plants. Because many of their proton resonances fall in a fairly narrow shift range, the carbon chemical shifts are in general far more informative than proton resonances for structural analysis of steroids. Therefore, the early study of steroids was concentrated on the carbon-13 NMR and also was greatly facilitated by using many substitution products which permitted a reasonable assignment of the carbon resonances in terms of the known substitution effects on conformationally fixed cyclohexane rings.[1-4] The analysis of the carbon-13 spectra of a series of steroids by Roberts and his co-workers is a particularly elegant example of this application and many of their data provide a good basis for the carbon-13 spectra of related materials.[1,2] However, the contradictory assignments in the parent hydrocarbon, cholestane (**1**), exist in the literature; such as carbons 12 and 16.[1,3,4] In addition, the chemical shifts of carbons 4 and 6, of carbons 18 and 19, and of carbons 10 and 22 are reported to be overlapped in the 100 MHz NMR (25 MHz at carbon).[3,4] Although unambiguous assignment of the parent compound, **1**, is absolutely prerequisite before attempting the exact evaluation of substitution effects, no high field NMR of cholestane (**1**) has ever been studied.

In order to elucidate the carbon skeleton of organic molecules, there are numerous indirect ways nowadays.[5] For example, in the case of protonated carbons the combination of COSY and HETCOR or long range HETCOR experiment provides the necessary information required to establish the carbon connectivity.[5] Although the application of long range HETCOR for the assignment of quaternary carbons is very useful, the result may be ambiguous because of the uncertainties regarding the bond length of the polarization pathway. In addition, this indirect approach fails and some ambiguities remain further in the assignment of carbon resonances when the proton spectra do not exhibit well resolved



Cholestane (1)

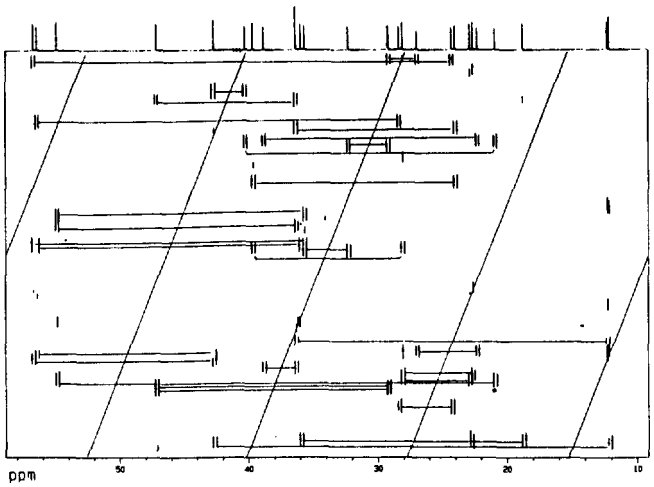


Figure 1. The 2D-INADEQUATE spectrum of cholestane (**1**). Brucker AMX-500 spectrometer, sample: 93 mg in 0.25 m*l* $CDCl_3$ (1 M), relaxation delay 2 s, 480 scans, total 20 hrs acquisition. The data were acquired with 4 K×64 data points multiplied by sine window in F2 shifted by π/3 and sine square in F1 dimension without shift and followed by zero-filling to give 2 K×256 data matrix.

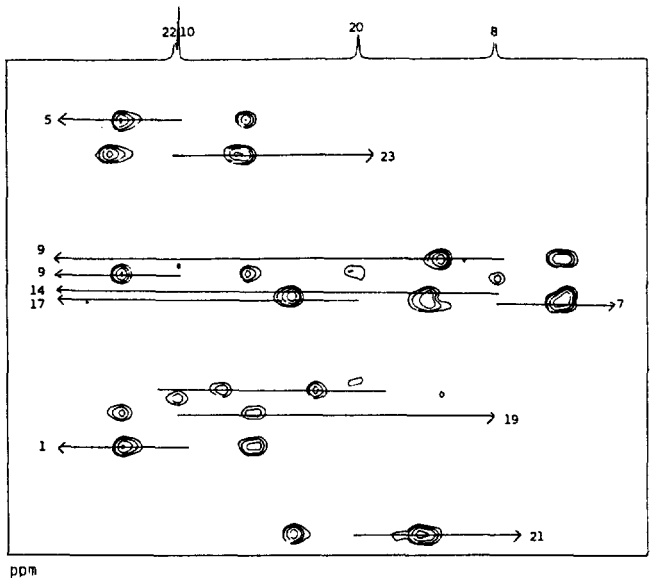


Figure 2. The expanded contour mpa of the regin 36.64-35.30 ppm in the Figure 1.

signals. All of these limitation mentioned above could be solved if we could measure the direct carbon-carbon correlations. In this note we now report complete and experimentally proved assignments of carbon-13 NMR spectrum of the cholestane (**1**) by using two-dimensional INADEQUATE experiment.[6]

The pulse sequence employed was the 90°-τ-180°-τ-90°-t_1-90°-t_2 sequence[6] with quadrature detection in F1 dimension.[7] The representative carbon-13 2D INADEQUATE spectrum of cholestane (**1**) obtained at 125.76 MHz (Bruker AMX-500 spectrometer) is shown in the Figure 1. The expanded region (36.64-35.30 ppm) of the carbons 22, 10, 20 and 8 is shown

Table 1. Chemical Shifts[a] of Cholestane, **1**

Position	Chemical shift[a] (ppm)	Position	Chemical shift[a] (ppm)
1	38.79	15	24.25
2	22.27	16	28.33
3	26.92	17	56.44
4	29.14	18	12.13
5	47.12	19	12.27
6	29.19	20	35.90
7	32.25	21	18.74
8	35.62	22	36.29
9	54.88	23	23.98
10	36.28	24	39.61
11	20.91	25	28.06
12	40.23	26	22.85
13	42.66	27	22.61
14	56.72		

[a]The solvent was $CDCl_3$, which served also as an internal reference for the ^{13}C spectrum (δ=77.0). The digital resolution of spectrum was 0.003 ppm.

in the Figure 2. While the choice of the fixed duration, 1/4J (6.25 ms), could be easily optimized because of only one kind of hybridization in the molecule, the selection of relaxation delay needed to know the longest T_1 value. Although it is generally known to give 1.5 T_1 of the most slowly relaxing carbon for all the connectivities of possible responses, the less than one fourth of the relaxation delay (2 s) the longest T_1 of carbon 10 (8.7 s)[8] showed all the correlations in this experiment (*vide infra*). Under the normal condition of double quantum spectra (2F2=F1), all the possible responses will be within the F1 frequency range and the skew diagonal passes the middle of the responses correlating two carbons with the slope of 2. The Figure 1 was recorded with the condition of F2=2F1 to increase the digital resolution of F1 dimension with the small number of experiments. That is, the spectrum folded twice in F1 dimension and the skew diagonals (slope is two) could be drawn as in Figure 1. Although the more skew diagonals appeared in the Figure 1 than the usual unfolded spectrum, the principles governing where a response will appear are same regardless of whether a spectrum will be folded or not. Beginning with the carbon resonating furthest upfield at 12.13 ppm (Figure 1), we can easily identify that this peak is responsible for the carbon 18 referring to the multiplicities of carbon spectrum, determined by DEPT experiment.[9] That is, the carbon 18 and 19 both have correlations with quaternary carbon 13 and 10, respectively. Then each quaternary carbon has correlation peaks with one methylene carbon and two methine carbons to give no difference between the 18 and 19 carbons. However, the further comparison between each methylene carbon clearly shows the difference; while the methylene derived from the carbon 18 has one more methylene corrlation followed by methine correlation (54.88 ppm), the other has three more methylene correlations. We can continue to assemble carbon connectivities to assign the complete and self-consistent structure of cholestane. By using this way, all the connectivities are drawn parallel to F2 axis

as shown in the Figure 1 except those between the carbon 20 and 22 (*vide infra*) and the assignments of all the carbon chemical shifts are shown in the Table 1. These data clearly show that the previously reported assignments were found to be in error and unresolved.[10] In the case of the carbon 20 and 22 (see Figure 2), the response is identified only by the inner resonances of the doublet,[11] the outer resonances being the noise threshold in the spectrum because of a strongly coupled AB pattern (see Figure 2). This two time folding resolves all the double quantum correlation peaks of cholestane (**1**) clearly enough to discriminate in the F1 dimension with 64 increments.[12]

On the basis of this study, even without any prior knowledge of structure, we can deduce the cholestane (**1**) carbon skeleton correctly using the spectrum shown in the Figure 1 which has no connectivity breacks. This is not always the case in organic molecules because of the presence of heteroatoms and AB pattern of strong coupling. However, most of steroids does not have many heteroatoms which is a main problem of disconnection of correlations. Considering the sensitivity,[13] the choice of carbon-13 2D-INADEQUATE experiment was just inadequate until recently. However, in view of our result which required only 20 hours experiment time with 93 mg of sample, there is no reason not to use this experiment for the structure determination of steroids, especially. Further studies are currently in progress to evaluate quantitative aspects of this experiment.

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11. The presence of this connection is also proven by the long range CH-correlation spectrum which showed the correlation peak between the methyl protons of 21 and the carbon of 22.
12. In order of increase F1 dimension resolution, three times folding experiment was also carried out under the same condition. The result showed that some of the overlapped peaks in the Figure 1 were resolved better, but some of previously well resolved peaks were overlapped instead.
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