Carbon-13 Two-Dimensional INADEQUATE Experiment of Coprostane

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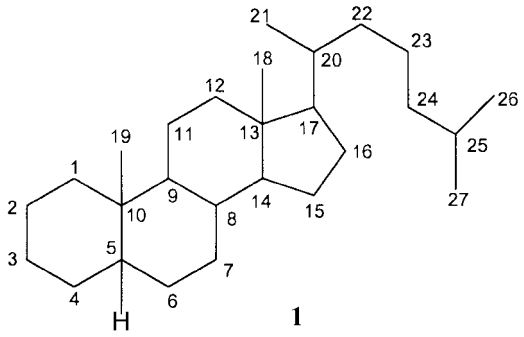
Received January 26, 2001

Keywords : Carbon-13 chemical shift, Coprostane, Cholestane, 2D-INADEQUATE.

A complete structural determination of complex natural products such as terpenes, alkaloids, and steroids still needs methods beyond already frequently used because of the amount of sample or the limitations of methods.[1] In almost all methods we are using, the chemical shift has been the first and most important key element. However, we have seen that this quantity, unfortunately, can not be predicted or determined with sufficient accuracy to allow distinction between carbons having very similar chemical environments. Therefore, there remains a significant number of known natural products for which full spectral assignments have not been reported and the chemical shifts were assigned on the basis of comparison with the assigned spectra of parent compounds using the direct additivity and substituent effects.[2] In view of the increasing use of spectral databases both for identification of known compounds and for prediction of chemical shifts for postulated new structures, it is obviously important to have complete and unambiguous assignments for the parent compounds and as many related compounds as possible.[3] Previously, we reported complete and unambiguous assignments for one of the most important parent compounds, cholestane, in the family of steroids by using two-dimensional INADEQUATE experiment.[4]

Coprostane (**1**), which has a same skeletal fragment as cholestane but having the *cis*-A/B ring fusion, is also a important parent compound and the chemical shift difference compared with cholestane could be applicable to all the 5α- and 5β-series of steroids as a good basis. Although pronounced chemical shift differences may be expected for many carbons in the A and B rings and for C-19 methyl carbon, the respective difference of the two carbons, C-19 and C-9, is reported only and used as an elegant stereochemical parameter of the A/B ring junction.[5] This 11-14 ppm ^{13}C shielding caused by γ-*gauche* effect was also applicable to distinguish the stereochemistry of several 3-oxo-steroids.[5] Even though the shielding parameter of some additional centers in these compounds provides further supporting evidence for many unknown steroids, no additional parameters were reported because their assignment requires a complete and unambiguous spectral analysis. These facts stimulated us to perform the complete assignment of the coprostane (**1**). Although there are numerous indirect ways to elucidate the carbon skeleton of organic molecules, only can one method, INADEQUATE, measure the direct carbon-carbon connectivities unambiguously. In this note we now report complete and unambiguous assignments of carbon-13 NMR spectrum of the coprostane (**1**) by using two-dimensional INADEQUATE experiment.



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The INADEQUATE pulse sequence, 90°-τ-180°-τ-90°-t_1-90°-t_2,[6] was employed with quadrature detection in F1 dimension.[7] The carbon-13 2D INADEQUATE spectrum of coprostane (**1**) in $CDCl_3$ (1 M) obtained at 125.78 MHz (Bruker AVANCE-500 spectrometer) is shown in the Figure 1. The choice of the fixed duration, 1/4J, for the maximum double quantum was 6.25 ms because of only sp^3 hybridization in the molecule. The 2 s of relaxation delay was enough to observe all the connectivities of possible responses in this experiment. 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 (64). Therefore, the spectrum is folded twice in the F1 dimension and the skew diagonal could be drawn as in the Figure 1. Under the normal condition of double quantum spectra (2F2=F1), all the possible responses will be within the F1 frequency range and one skew diagonal only passes the middle of all the responses correlating two carbons with the slope of 2. Although 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. Starting with two quaternary carbons (δ 35.36 and 42.70) determined by DEPT experiment,[8] we can easily identify that the peak at δ 35.36 is the carbon-10 because the carbon-13 has two methylene connections while the carbon-10 has four methylene connections consecutively. In the Figure 1, the carbon-13 showed consecutive two methylene correlation peaks but the carbon-10 showed only three methylene correlation peaks consecutively because of the strongly coupled AB pattern between carbon-3 and 4.[9] However, the distinction of carbon-3 and 4 was easily solved because the former had the correlation peak with the methyl-

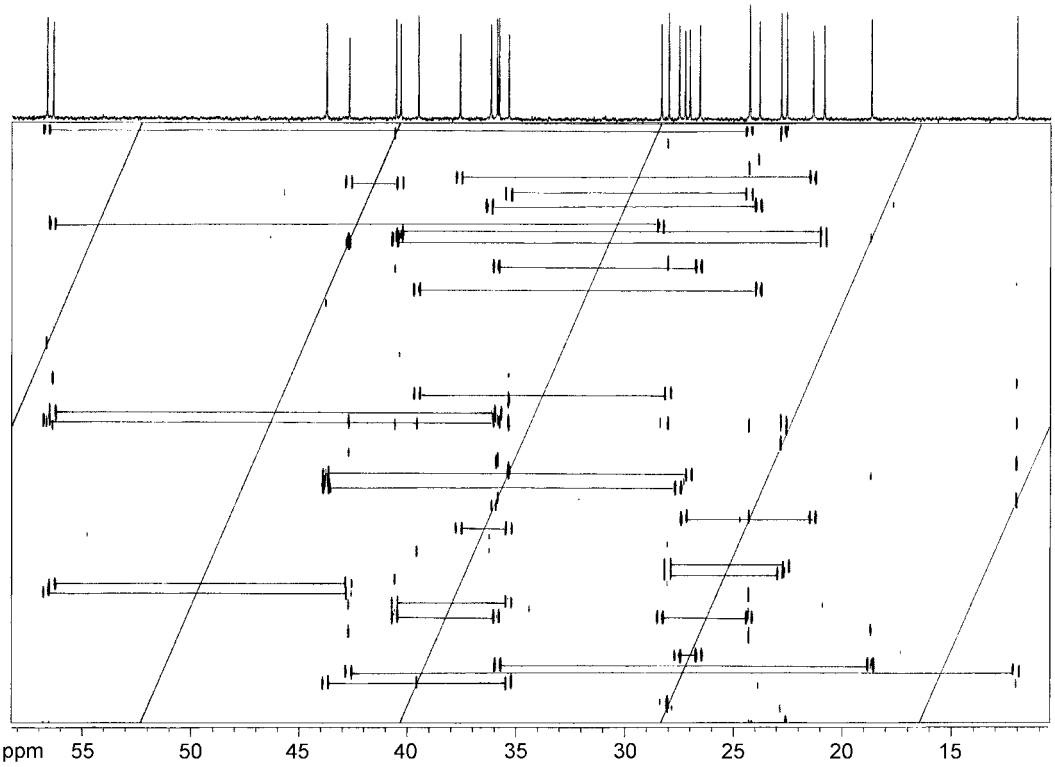

Figure 1. The 2D-INADEQUATE spectrum of coprostane (1) in $CDCl_3$ (1 M). Bruker AVANCE-500 spectrometer, 2 s relaxation delay, 512 scans, total 21.5 hours acquisition. The raw data were acquired with 4K × 64 data points and multiplied by $\pi/2$ shifted sine square window in F2 and unshifted sine square window in F1 and followed by zero-filling to give 2K × 256 data matrix before Fourier transformation.

ene carbon-2 (δ 21.34), but the latter showed correlation peak with methine carbon-5 (δ 43.75). One more missing correlation was observed between the carbon-20 and 22 and this connectivity was proven by the long range CH correlation peak between the methyl proton-21 and carbon-22. All the rest of the connectivities are drawn parallel to F2 axis as shown in the Figure 1.

The assignments of all the carbon chemical shifts are shown in the Table 1. The concentration of sample for the reported chemical shifts was 0.1 M. It was anticipated that there would be some dilution shift. In fact, the chemical shifts of 0.1 M solution of **1** were observed at higher field than those of 1 M solution, but the order of chemical shifts was not changed.[10] In order to compare directly the chemical shifts of coprostane (**1**) with those of cholestane, previously reported the chemical shifts of cholestane were also remeasured at concentration of 0.1 M and reported in the Table 1. This was considered important because we wished to have chemical shifts measured under comparable conditions to probe the effect of changes in the A/B ring fusion on all the carbon chemical shifts. Although the chemical shifts of cholestane at 0.1 M solution were different from those of 1 M solution,[4] the order of chemical shifts also was not changed as in the case of coprostane (**1**). First of all, these data clearly showed that the previously reported some assignments, which were used as a basis of all the parameters,[11] were found to be in error. The assignments of carbon-3 and 4 and carbon-15 and 19 were reversed. The well

Table 1. Chemical Shifts of Cholestane and Coprostane (1)

Position	Chemical Shift (ppm)[a]		Position	Chemical Shift (ppm)[a]	
	5α-[b]	5β-		5α-	5β-
1	38.68	37.59	15	24.19	24.27
2	22.19	21.34	16	28.26	28.34
3	26.85	27.26	17	56.27	56.36
4	29.05	27.03	18	12.07	12.04
5	47.02	43.75	19	12.22	24.29
6	29.10	27.54	20	35.81	35.80
7	32.18	26.58	21	18.66	18.67
8	35.51	35.89	22	36.22	36.18
9	54.75	40.53	23	23.83	23.82
10	36.17	35.36	24	39.51	39.51
11	20.80	20.84	25	28.01	28.01
12	40.11	40.32	26	22.82	22.82
13	42.57	42.70	27	22.56	22.56
14	56.62	56.65			

[a]The concentration of samples was 0.1 M and solvent was $CDCl_3$, which served also as an internal reference for ^{13}C spectrum (δ=77.0). The digital resolution of spectra was 0.003 ppm. [b]The assignments were based on the reference 4.

defined chemical shift differences of carbon-19 and 9 between two isomers were 12.07 and 14.22 ppm, respectively. The other possible shift difference at carbon-7 due to the *γ-gauche* effect with carbon-4 was observed by 5.6 ppm. However, the carbon-2 and 4, which were the counterparts

of carbon-9 and 7, showed small shieldings, 0.85 and 2.02 ppm in coprostane (**1**), respectively, because of the absence of *γ-gauche* effect with carbon-19. The change due to A/B ring fusion in this parent steroid caused the deshielding of 11 carbons and the shielding of 12 carbons. The remote carbons from the carbon-24 to 27 were not affected at all by the change of A/B ring fusion.

In conclusion, we reported here precise shielding and deshielding for each carbon of parent steroids, cholestane and coprostane (**1**), by the complete and unambiguous assignments of carbon-13 spectra. Further studies are in progress to evaluate how far this parameter could be applicable to the 5α- and 5β-series of steroids as a good basis and will be discussed later.

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