Natural Abundance $^{13}$C–$^{13}$C Coupling Constants Observed via 1D and 2D INADEQUATE Experiments in Stigmasterol and 1,4-Androstadiene-3,11,17-trione

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For two steroids, stigmasterol and 1,4-androstadiene-3,11,17-trione, natural abundance $^{13}$C–$^{13}$C coupling constants are determined by 1D and 2D INADEQUATE experiments. Besides full assignment of the $^{13}$C resonances of these steroids is achieved.

Recent advances in pulsed NMR methods [1–2] and the availability of high-field spectrometers with enhanced sensitivity have extended the scope and utility of $^{13}$C–$^{13}$C coupling constant measurements at natural abundance to structural elucidation of medium molecular weight molecules [3–7]. Molecular carbon framework connectivity is the direct experimental result from the two-dimensional INADEQUATE pulse sequence [8], while precise one-bond $^{13}$C–$^{13}$C coupling constants are usually derived from a separate high-resolution one-dimensional experiment [9] using different experimental parameters.

Particular $^1J(C-C)$ values have already been known as a result of biosynthetic studies [10]; however, the first complete $^{13}$C–$^{13}$C coupling patterns for steroidal molecules [11–12] and the first structural application of the INADEQUATE experiment in this field have been reported only recently [13]. In the present work we report $^1J(C-C)$ patterns for stigmasterol 1 and 1,4-androstadiene-3,11,17-trione 2 (Figs. 1 and 2 and Table I).

Chemical shift assignments in the case of 1, based on the INADEQUATE experiment alone, are in excellent agreement with previous results on hormonal steroids [14] and sterols [15–16].

The determination of carbon-carbon coupling constants on complex natural products and at natural abundance requires carefully designed experiments. Problems are associated with a number of factors discussed earlier [3]. The most important among them appears to be the necessity to ensure long T$_2$ values for proton bearing carbon atoms. Only in this way can the closely spaced satellite lines be sharp enough for unambiguous analysis. Sometimes the consecutive use of 1D and 2D experiments is the only remedy for interpretation difficulties caused by accidental overlapping.

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**Fig. 1.** Stigmasterol 1. One-bond $^{13}$C–$^{13}$C coupling constants (in Hz) measured at 100.6 MHz and 70 °C in pyridine-$d_5$. $^{13}$C NMR Spectra, Stigmasterol, 1,4-Androstadiene-3,11,17-trione

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Table I. $^{13}$C chemical shifts measured at 100.6 MHz and 70 °C, relative to $^{13}$C=0 (pyridine-d$_5$, solvent, 149.9 ppm). Longitudinal relaxation times were determined by the inversion recovery method at 25.16 MHz and 90 °C at the concentration and in the solvent as given for Fig. 1. A three parameter non-linear least squares fit was used. All $T_1$ values are in sec. $T_1$ values are not reported for lines that are not clearly resolved.

![Table I](image)

The most interesting coupling information displayed by the two steroidal systems is attributed to the dramatic changes of the $J(C-C)$ values of angular methyl groups upon introduction of adjacent sp$_2$-hybridized carbon centers. Thus, $J(C_9-C_{19})$ and $J(C_{13}-C_{18})$ in 1 are reduced from 35.2 and 35.4 Hz to 32.9 and 32.7 Hz, respectively, in 2. It is also worth noting that $J(C_8-C_9)$ is smaller by 2.2 Hz in 2 than in 1.

The presence of the oxygen atom at C-3 in 1 and the carbonyl group in ring D in 2 enhance the $J(C-C)$ values by about 3 Hz (for similar findings see [17]).

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