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Nuclear Magnetic Resonance Spectroscopy. ^{13}C Fourier Transform Spectra of Δ^8- and Δ^9-Tetrahydrocannabinol

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ABSTRACT Carbon-13 nuclear magnetic resonance spectra have been taken of Δ^8- and Δ^9-tetrahydrocannabinol. The absorptions have been assigned to specific carbons with the aid of off-resonance and selective proton-decoupling experiments, as well as by chemical-shift comparison with model compounds.

The nuclear magnetic resonances (NMRs) of ^{13}C at the natural-abundance level have gradually achieved tremendous potential for spectroscopic analysis of the structures of quite complex natural products including carbohydrates, terpenes, steroids, amino acids, peptides, proteins, nucleosides, nucleotides, alkaloids, and others (1). The advantages of ^{13}C NMR (CMR) arise mainly because of the simplicity of proton-decoupled carbon spectra, the wide range of common chemical shifts, the sensitivity of these shifts to changes in molecular structure, and the general applicability of pulse methods utilizing the Fourier-transform technique (2) to large organic molecules. Carbon-13 is expected to be a wholly safe tracer to use for metabolic and biosynthetic studies in humans, which provides the advantage, through CMR spectra, of avoiding difficult and time-consuming degradations to locate the positions of tagged atoms as are necessary for ^{14}C.

The physiological effects of marijuana have stimulated considerable interest in the chemistry, molecular structure, and the mechanism of action of the components of *cannabis* (3). Because of the possible utility of CMR as a structural tool and potential tracer technique for these substances, we have investigated the CMR spectra of Δ^8- (**I**) and Δ^9-tetrahydrocannabinol (**II**), the latter compound being the major active constituent of *cannabis* (4).

EXPERIMENTAL

The CMR spectra of about 1 M solutions of **I** and **II** in deuteriochloroform were obtained in 10-mm spinning tubes with tetramethylsilane as internal standard. Field-frequency stability was achieved by locking on the deuterium resonance of the solvent. The ambient probe temperature was about 35°.

The spectrometer used was a modification of the one described by Weigert and Roberts (5) to permit pulse-Fourier transform operation, and it differs in many respects from those described (6). The 15.1-MHz output of a Hewlett–Packard synthesizer is gated by use of a Bruker BSV-2 pulse power amplifier unit. Preamplification of the pulsed 15.1-MHz signal is accomplished with the transmitter section of a Varian V-4311 RF unit before power amplification in the BSV-2 unit, the latter providing about 70 W of pulse power. A Bruker preamp and universal probe, modified to include field-sweep coils (to allow homogeneity tuning), is set up in a 12-in., 14-kG Varian magnet. Single-coil operation with passive diode gating (6*c*) provides 90° pulse widths of about 15 μsec for ^{13}C in a 10-mm sample tube. The 17.15-MHz local oscillator frequency is synthesized by mixing the 2.05-MHz lock unit reference frequency with the 15.1-MHz synthesizer output followed by buffer amplification. The insert is doubly tuned to 15.1 MHz and 9.2 MHz to enable internal deuterium stabilization. A 9.2-MHz Bruker time-shared deuterium lock unit is used, wherein the frequency is synchronized to the 1-MHz synthesizer clock for frequency stability. A Bruker receiver unit is used for both the lock and analytical channels. The output of the lock channel is coupled to the Varian flux stabilizer, while the analytical channel output is fed to a Varian pulse unit working into a 16 K Varian 620 *i* computer. The digital recorder was interfaced to the computer for spectral readout.

RESULTS AND DISCUSSION

CMR spectra of **I** and **II** are shown in Fig. 1, and the resulting spectral data are collected in Table 1. Off-resonance proton decoupling was used to obtain the spectral assignments for both **I** and **II**. Various decoupler frequencies were used to provide different residual splittings (7) for unambiguous determinations of the number of directly attached protons to each carbon. Six quaternary carbon resonances were observed for both **I** and **II**. The peaks arising from C1 and C4a were assigned on the basis of characteristic low-field shifts (8), although the proximity of these lines precluded their being individually differentiated. The resonance of C6 was also assigned on the basis of the low-field shift expected from direct attachment of oxygen to this carbon (9). Of the three remaining quaternary carbon resonances, that of C3 was assigned because of its insensitivity to the change in double-bond position between **I** and **II**. The two remaining and widely separated quaternary carbon resonances corresponding to C9 and C10b were distinguished on the bases of expected chemical shifts and additivity effects of the substituted phenyl ring (8).

The three remaining lines in the low-field unsaturated region result from carbons bearing a single hydrogen and correspond to C2, C4, and C8 (for **I**) or C10 (for **II**). These lines could be assigned by correlation of the reported proton spectra (10) with the carbon lines utilizing specific proton decoupling. The previous proton assignments (10) and double-resonance experiments also allowed a distinction to be made between the two aliphatic methine carbons, C6a and C10a. Thus, irradiation of H10a in both **I** and **II** resulted in marked sharpening of the high-field methine resonance and dictated its assignment to C10a. The five-carbon resonances in the aliphatic chain

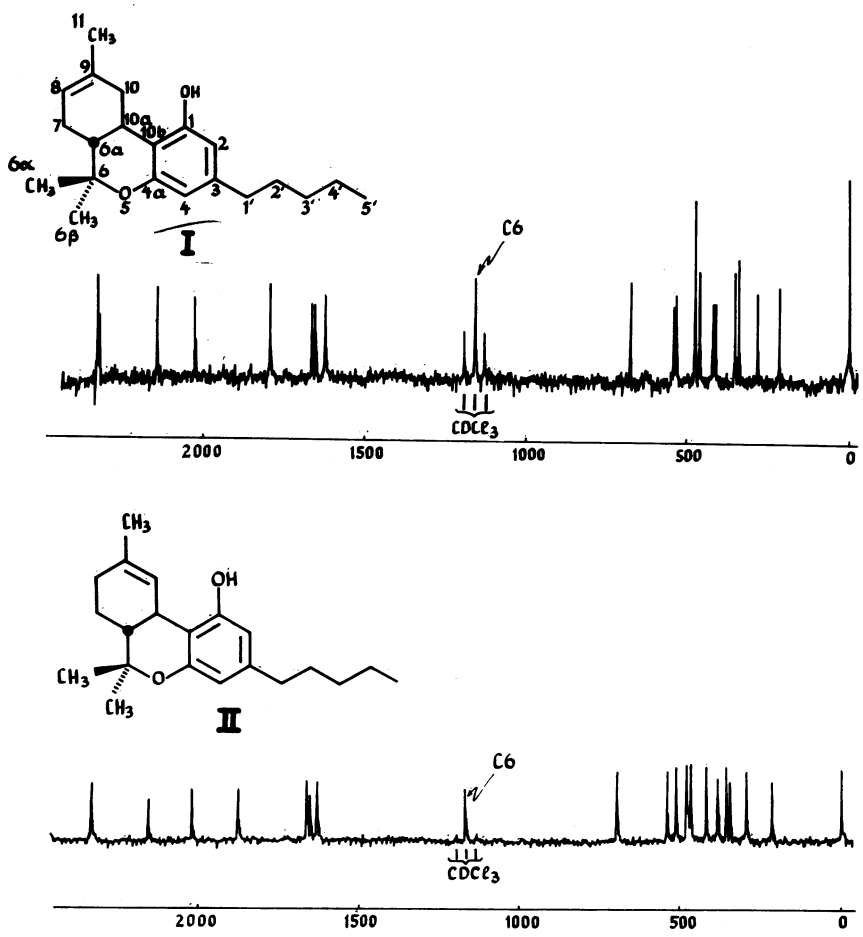

FIG. 1. CMR Fourier-transform spectra of I and II obtained with 0.8-sec acquisition times and about 5 min of spectral accumulation. The line at zero is the methyl of tetramethylsilane and the horizontal scale is in Hz.

could be identified by comparison of their positions with those of *n*-pentylbenzene (11). The close correspondance of shifts of these resonances in I and II further supports these identifications. A clear distinction between C2′ and C3′ could not be made because of the near-degeneracy of these lines in the model compound (11).

TABLE 1. *CMR chemical shifts of* I *and* II[a]

Carbon	I	II	Carbon	I	II
1	154.9[b]	154.6[e]	9	134.8	133.8
2	107.8	108.0	10	27.9[c]	124.2
3	142.6	142.7	10a	31.6	33.7
4	110.0	110.0	10b	110.4	109.3
4a	154.6[b]	154.4[e]	11	23.4	23.4
6	76.8	77.4	1′	35.5	35.5
6α	18.4	19.3	2′	30.6[d]	30.7[g]
6β	27.5	27.5	3′	31.6[d]	31.6[g]
6a	45.0	46.0	4′	22.6	22.6
7	36.0[c]	31.2[f]	5′	14.0	14.0
8	119.2	25.1[f]			

[a] All shifts are in ppm downfield from internal tetramethylsilane.

[b–g] These two assignments could be reversed (see *text*).

Difficulty was also encountered in deciding which is which of the two methylene resonances of the cyclohexene ring. The complexity of the spectra for the attached protons, the proximity of the proton chemical shifts, and carbon spectral complexity in the methylene region with partial proton decoupling obscured the results of selective proton decoupling experiments.

The four methyl-carbon resonances, however, could be identified by use of the selective decoupling technique based on the proton assignments (10). Except for C5′, the axial C6α methyl carbon in both I and II comes at the highest field. This is likely a result of steric interactions with H7α and H10a (12). Unsuccessful attempts were made to use lanthanide shift reagents to further elucidate and confirm assignments in I and II. No significant shifts were observed on addition of up to 0.4 mol of praseodymium tris(dipivaloylmethide) per mol of I. Similar results were obtained on addition of europium tris-(1,1,1,2,2,3,3-heptafluoro-7,7-dimethyl-4,6-octanedionate) to a solution of II. The absence of induced shifts may result from steric inhibition of complex formation for the oxygen atoms or some chemical reaction between the chelate and I and II.

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