Biosynthetic Studies with Carbon-13. Carbon-13 Nuclear Magnetic Resonance Spectra of Radicinin

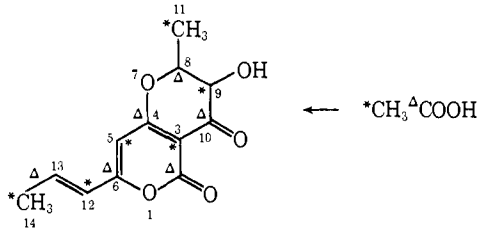
Sir:

Carbon-13 nmr spectroscopy is a useful technique for the identification of specific carbon atoms in complex organic molecules. The large differences in carbon chemical shift in ^{13}C nmr spectra make individual carbon atoms distinguishable.[1] The use of ^{13}C-labeled substrates simplifies biosynthetic studies since the ^{13}C isotopic excess incorporated at different sites can be located and identified by characteristic chemical shifts and an increased signal intensity over the natural abundance peaks.

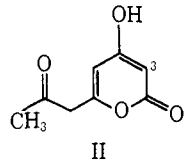
Recent advances in instrumental techniques have permitted the routine measurement of ^{13}C nmr spectra at 25.15 MHz on a HA-100 spectrometer with homonuclear lock in 8- or 12-mm sample tubes. The spectra have been greatly simplified and enhanced in signal-to-noise by simultaneously using proton noise decoupling[2,3] and selected solvent peaks such as dioxane, DMSO, or benzene for the homonuclear lock signal; the resulting fully proton decoupled spectra appear as singlets.

When using a solvent peak for lock, it is possible to switch from noise decoupling to single frequency or continuous wave (CW) decoupling when the decoupler is tuned to the exact frequency for irradiation of the solvent protons. Then most ^{13}C signals arising from CH_3, CH_2, or CH groups whose proton shifts are not very near the solvent proton shift give characteristic close-spaced multiplets that allow appropriate assignments to be made.

We report the application of the ^{13}C nmr method to the elucidation of the biosynthetic pathway of the microbial metabolite radicinin (I).[4] A recent total synthesis of *dl*-radicinin[5] verifies the structure as deduced from earlier chemical and spectroscopic evidence.[6] Previous speculations on the biosynthesis of radicinin have centered on the tetraacetic acid lactone II which when



I



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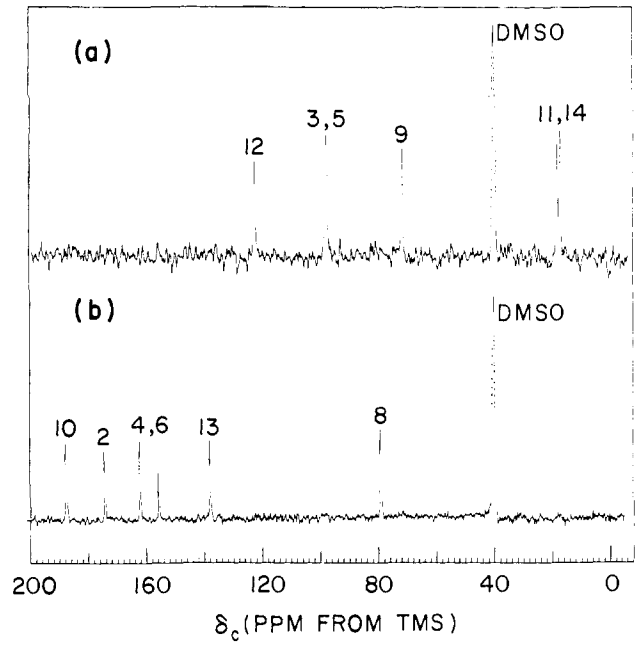

Figure 1. Carbon-13 nmr spectrum (25.15 MHz) of radicinin from $^{13}CH_3COONa$: (a) 57 mg/1.0 ml of DMSO, 8-mm tube, proton noise decoupled (PND) with 14 scans of 5030 Hz (200 ppm) at 50 sec/scan, lock signal DMSO. A V-3530 RF/AF sweep unit was used with a Spectro-System 100 for multiscan averaging. Chemical shifts were measured relative to the ^{13}C signal of DMSO and converted to ppm from dissolved TMS (δ_c) using δ_c(DMSO) 40.4 ppm. (b) 30 mg/1.0 ml of DMSO, 8-mm tube, PND with 100 scans of 5030 Hz (200 ppm) at 50 sec/scan, lock signal DMSO; chemical shifts in δ_c.

attached with an additional acetoacetic acid unit at C_3 *via* coenzyme A esters forms radicinin.[7]

Six-day cultures of *Stenphylium radicinum* were inoculated with 150 mg of sodium acetate-1-^{13}C (56.7%) or sodium acetate-2-^{13}C (61.6%) per 100 ml of culture broth. Fermentations were terminated 21 days after inoculation and the isotope enriched radicinins were isolated by chloroform extraction and purified by crystallization. Yields of 30 mg/100 ml of broth of labeled radicinins were obtained for nmr studies.

The ^{13}C nmr spectrum (a) in Figure 1 clearly indicates that six carbons, C_3, C_5, C_9, C_{11}, C_{12}, and C_{14}, of radicinin obtained from sodium acetate-2-^{13}C as precursor are derived from the methyl group of acetate, since only six peaks are evident and no peaks from carbon atoms at natural abundance are discernible. Spectrum b in Figure 1 also conclusively shows that the other six carbons of the labeled radicinin obtained from the sodium acetate-1-^{13}C are enriched at C_2, C_4, C_6, C_8, C_{10}, and C_{13} and must come from the carboxyl of acetate as indicated by the six peaks very evident from this ^{13}C enriched material. Our results confirm and fully support the polyacetate origin of radicinin with the expected alternating labeling of carbon atoms from either the methyl or carboxyl group of acetate.

The assignments of ^{13}C chemical shifts in spectra a and b generally follow from previously recognized trends where methyls exhibit high-field signals and carbonyls show low-field signals.[8] The CW decoupling

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(2) R. R. Ernst, *J. Chem. Phys.*, **45**, 3845 (1966).

(3) L. F. Johnson and M. E. Tate, *Can. J. Chem.*, **47**, 63 (1969).

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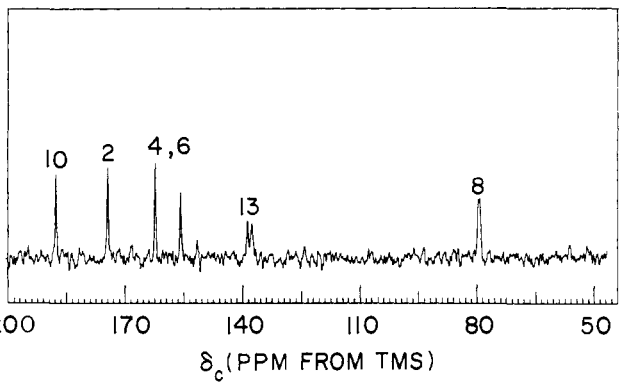

Figure 2. CW decoupled ^{13}C nmr spectrum of radicinin from $CH_3{}^{13}COONa$. Concentration same as in Figure 1b; 37 scans of 3775 Hz (150 ppm) at 50 sec/scan; lock signal DMSO; chemical shifts in δ_c.

technique was employed to obtain more information on spectrum b. Figure 2 shows the appearance of the methine carbons at C_8 and C_{13} as close-spaced doublets with the carbonyls and quaternary carbons remaining as singlets.

Nuclear Overhauser effects preclude precise calculation of incorporation yields by integration of peak areas since peak enhancements of up to 2.98 have been observed.[9] This effect may be responsible for the different spectral line intensities observed for C_4 and C_6 in Figure 1b. Incorporation yields can be approximated by relative comparison with natural abundance peaks, particularly with carbon atoms of similar substitution pattern which can undergo relaxation by similar mechanisms. Since natural abundance peaks in the labeled radicinins were not readily visible with the small number of scans employed, an incorporation yield of ~17% was determined by integration of the proton–^{13}C satellite bands of the C_{11} and C_{14} methyl groups ($J_{^{13}C-H}$ = 126 Hz) clearly evident in the proton spectrum at 60 MHz. Other $J_{^{13}C-H}$ values observed were (C_5) 172, (C_9) 148, and (C_{12}) 162 Hz. Detection and integration of ^{13}C satellite peaks serve as an alternate method of biosynthetic studies.[10]

The application of ^{13}C nmr spectroscopy to biological isotopic tracer studies offers many distinct advantages over conventional radioactive tracer methods.

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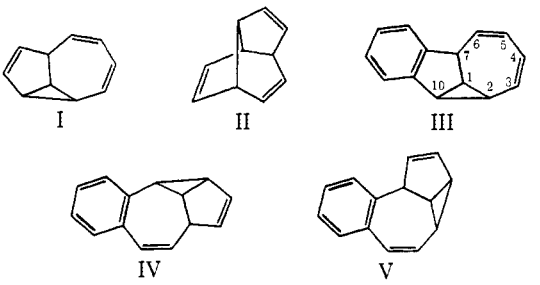
Thermal Reactions of Some Tricyclo[5.3.0.0^{2,10}]decatriene Derivatives

Sir:

Tricyclo[5.3.0.0^{2,10}]decatriene (I) is one of the $C_{10}H_{10}$ isomers which can be related to [10]annulene by a simple valence bond reorganization. However, the isolation of I poses a difficult challenge because the molecule contains a *cis*-divinylcyclopropane unit. Thus the activation energy for the Cope rearrangement of I is expected to be less than ~23 kcal/mol[1] and any thermal process involving [10]annulene is not likely to compete with facile rearrangement to II. In order to study transformations which might lead to [10]annulenes under more favorable circumstances, we have examined the thermal behavior of the benzo derivatives III,[2] IV, and V of tricyclo[5.3.0.0^{2,10}]decatriene. Compounds III and IV are expected to resist Cope rearrangement owing to the strategic presence of a benzene ring, while V should be unstable for the same reasons as I.



A synthetic route to the hitherto unknown structures IV and V is described in Scheme I. Benzotropylium ion[3] is condensed with diethyl malonate to afford a 2:3 mixture of VIa and VIIa. The isomers are separated by crystallization of VIb and VIIb, which are then converted into the cyclopropyl ketones VIIIa and IXa *via* copper-catalyzed decomposition of the diazoketones VIc and VIIc. The desired olefin IV[4] is obtained from the tosylhydrazone IXb upon treatment with methyllithium.[5] In the case of tosylhydrazone VIIIb, however, the divinylcyclopropane V cannot be isolated under the conditions necessary for olefin formation (several hours at 0°, 10–15% yield) and the sole hydrocarbon product is the rearranged isomer X.[4] As expected, a similar rearrangement occurs when the parent tosylhydrazone XI[6] is treated with methyllithium, and II[7] is the only detectable $C_{10}H_{10}$ product at 0°.

(1) The activation energy for the rearrangement of a model compound, *endo*-6-vinylbicyclo[3.1.0]hex-2-ene, is 23 kcal/mol: J. M. Brown, *Chem. Commun.*, 227 (1965). The additional constraints present in I compared to the model are expected to lower the magnitude of $\Delta S^{\ddagger}$ but the effect on $\Delta H^{\ddagger}$ is not easily predictable. A somewhat more facile rearrangement appears likely in the case of I.

(2) E. Vedejs, *Tetrahedron Lett.*, 2633 (1968).

(3) Benzotropylium tetrafluoroborate is easily prepared from trityl tetrafluoroborate and benzocycloheptatriene in methylene chloride solution.

(4) Compound IV: mp 51°; λ_{max}^{EtOH} 211 nm (ϵ 24,600), 234 nm (ϵ 19,400), 262 nm (ϵ 6250); nmr ($CDCl_3$) τ 2.90 (4 H, m), 3.82 (1 H, d of d, J = 11, 1 Hz), 4.00 (1 H, d of d, J = 11, 8.5 Hz), 4.51 (1 H, m), 4.84 (1 H, m), 6.30 (1 H, m), 7.7–8.1 (3 H, m). Compound X: mp 79°; λ_{max}^{EtOH} 265 nm (ϵ 800), 272 nm (ϵ 1100), 278 nm (ϵ 1100); nmr (CCl_4) τ 3.05 (4 H, m), 3.40 (1 H, d of d, J = 3, 6 Hz), 4.37 (3 H, m), 6.41 (1 H, m), 6.59 (1 H, m), 6.99 (1, H, m), 7.46 (1, H, m).

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(6) XI is available in the usual way as a mixture of stereoisomers from tricyclo[5.3.0.0^{2,10}]deca-3,5-dien-9-one: W. von E. Doering, B. M.