Carbon-13 Nuclear Magnetic Resonance in Biosynthetic Studies of Lipids

By A. L. BURLINGAME, B. BALOGH,* J. WELCH, S. LEWIS, and D. WILSON
(*Space Sciences Laboratory, University of California, Berkeley, California* 94720)

Summary ^{13}C-labelled lipids (enrichment 32%) have been isolated from wild-type yeast, *Saccharomyces cerevisiae* (x2180), grown on media containing sodium [2-^{13}C]-acetate (50—60%); the ^{13}C n.m.r. spectrum of ^{13}C-enriched methyl palmitoleate is correlated with the natural abundance ^{13}C resonance signals assigned using additive bond parameters and structural analogies with reference compounds.

CARBON-13 labelled lipids were obtained from the yeast, *Saccharomyces cerevisiae* (x2180). The cells were grown aerobically for 18 h at 30 °C on a highly enriched medium containing 5% glucose, 0·5% bactopeptone, 1% yeast extract, 0·25% beef extract supplemented with amino-acids, pyrimidines and purines,[1] and 0·11% sodium [2-^{13}C]-acetate (50—60%). The cells were harvested, lyophilized, and extracted with chloroform–methanol (2:1) After saponification of the extract with methanolic sodium hydroxide, the fatty acids were separated from the non-saponifiable material and esterified, and the ester mixture was chromatographed. Fatty acid esters were identified by co-injection with standards and also by mass spectrometry, which indicated that ^{13}C had been incorporated in the yeast fatty acids.

The low resolution mass spectrum indicated a 30% overall ^{13}C enrichment in the collected methyl palmitoleate (16:1 Δ^9), but failed to reveal the exact positions of ^{13}C incorporation. A high resolution mass spectrum[2] showed that the relative intensities of the M and $M + 1$, m/e 74 and 74 + 1, m/e 57 and 57 + 1 ion peaks were consistent with 32% ^{13}C enrichment at 8 alternate sites in the carbon chain.

^{13}C enriched methyl palmitoleate (16:1 Δ^9) (1·2 mg) was subjected to ^{13}C n.m.r. analysis using commercial methyl palmitoleate (0·25 g; 99%) as reference. Instrumental details have been described elsewhere.[3]

As expected, the spectra show a three-fold accidental degeneracy (overlaps) due to the three groups of nearly equivalent carbons. The ^{13}C resonances of the CO_2 and OMe groups were easily recognized on the basis of well accepted chemical shift correlations.[4] The low signal intensity of the CO carbon is due to the diminished nuclear Overhauser enhancement and long T_1 for the pulse conditions. The methine carbons of the *cis*-double bond were identified by comparison with literature data for sp^2 unsaturated systems.[5,6] However, ^{13}C n.m.r. seems to be relatively insensitive to internal, double bond configuration, *i.e.*, *cis–trans* isomerism, unless unsymmetrical substitution

is present.[5] The α,β, and γ carbon resonances were identified near the predicted positions.[7] Constitutive bond parameters developed by Grant *et al.*[8] served for the calculation of C-16, C-15, C-14, C-13, C-5, and C-6 chemical shifts, whereas the rest of the ^{13}C signals were designated by structural analogy with methyl oleate.[9]

The ^{13}C n.m.r. chemical shifts (in p.p.m. from CS_2) are: C-1 (CO), 18·6; C-2 (α), 158·8; C-3 (β), 167·8; C-4 (γ), 165·6; C-9 and -10 (=CH), 62·9; C-5, -6, -7, and -12, 163·7; C-8, -11, and -13, 163·1; C-14, 161·0; C-15, 170·1; C-16, 178·8; OMe, 141·4.

The ^{13}C resonance assignments of the selectively enriched methyl palmitoleate compared with natural abundance spectra shows that alternate carbon atoms have been labelled, in agreement with well accepted biosynthetic rules.

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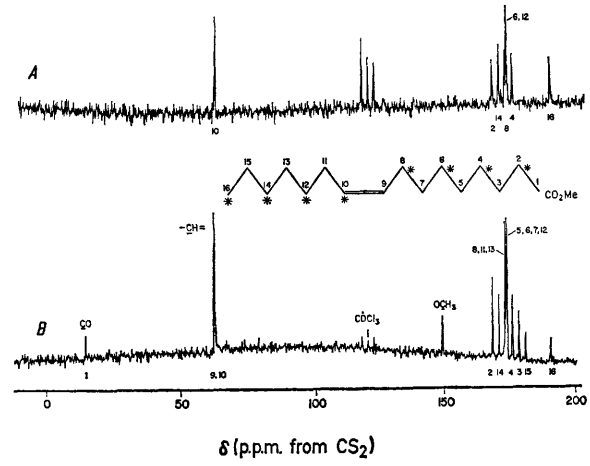

FIGURE. ^1H *noise decoupled* ^{13}C *n.m.r. spectra of methyl palmitoleate* (16:1 Δ^9). (A) *Selectively enriched compound.* (B) *Natural abundance spectrum. The central peak at* 115·7 *p.p.m. due to* $CDCl_3$ *is used as internal reference.*

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