The decomposition of the diazotic acid **1** is entirely in accord with the proposed decomposition of diazotate salts with hydroxylic species.[4] Decomposition of **2** affords less olefinic products than decomposition of diazonium carboxylates[3] or diazonium hydroxides[4] because the nitrate counterion is less basic than the carboxylate or hydroxide counterions.

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Biosynthetic Studies with Carbon-13. Asperlin

Sir:

We report on the biosynthesis of the antibiotic lactone asperlin[1] isolated from growing cultures of *Aspergillus nidulans* (NRRL 3134) supplemented with sodium 2-^{13}C-acetate (61%).

In the cmr spectrum (Figure 1) of the labeled antibiotic obtained at 25.15 MHz in dioxane as the lock signal with simultaneous proton noise decoupling (pnd), the carbons appear as singlets. Signals from C_2, C_4, C_6, C_8, and C_{10} of increased intensity from these isotope-enriched sites are evident as are the resonances from the carbons at natural abundance at C_1, C_3, C_5, C_7, and C_9. The labeling pattern indicated verifies the tetracetyl origin of the eight-carbon epoxy γ-lactone in the antibiotic.

Application of off-resonance continuous-wave (cw) decoupling aided in unequivocal assignment of the carbon signals in asperlin. The magnitude of the residual splitting (J_r) is given by expression 1 derived by Ernst,[2]

$$J_r \approx \frac{\Delta f J}{\gamma^{H_2}/2\pi} \qquad (1)$$

where Δf is the separation of the proton signal from the applied decoupling frequency (solvent signal) in hertz, J is the ^{13}C–H coupling constant, and $\gamma^{H_2}/2\pi$ is the decoupling field strength.

The proton spectrum of asperlin shows H_8 (δ 1.31) further separated from the dioxane proton (δ 3.70) than the acetyl-methyl H_{10} protons (δ 2.10). In the cw spectrum of asperlin in dioxane (Figure 2), these methyl carbons C_8 and C_{10} appear as quartets, and the assignments are based on J_r = 13.5 Hz as observed for C_8 relative to J_r = 10.0 Hz for C_{10}.

C_4, C_5, C_6, and C_7 are in similar chemical environments and the shift assignments as indicated in Figure 1 were made from the cw decoupled spectrum in Figure 3. The H_6, H_7, H_5, and H_4 shifts are very near the dioxane proton shift, and J_r for the corresponding carbons will be very small. However, in benzene (δ 7.37) the proton shift separations are much larger (H_4, δ 5.22; H_5, δ 3.90; H_6, δ 3.05; H_7, δ 2.93) and the relative magnitudes of J_r increase in that order. These large shift differences facilitate the assignment as shown in Figure 3 with $J_r(C_6)$ = 36 Hz > $J_r(C_7)$ = 32 Hz and $J_r(C_5)$ = 24 Hz > $J_r(C_4)$ = 15 Hz. All of the observed J_r values are in good agreement with values calculated from (1).[3] This multiple-solvent technique in cw decoupling allows unequivocal specification of the 2-^{13}C-acetate origin of C_4 and C_6 as well as C_8 and C_{10}, despite their similar cmr chemical shifts and identical splitting patterns when cw decoupled.

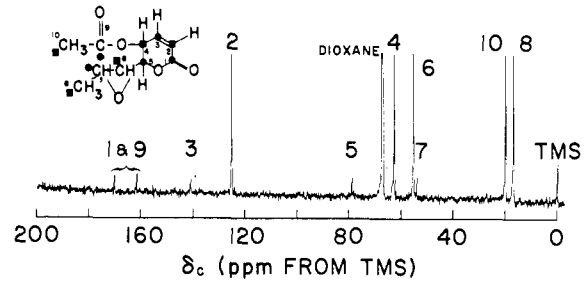

Figure 1. The proton noise-decoupled cmr spectrum (25.15 MHz) of asperlin from sodium 2-^{13}C-acetate. The spectrum was obtained on 153 mg in 1.0 ml of dioxane in an 8-mm spinning tube. A V-3530 RF/AF sweep unit with a Spectro System 100 was used to accumulate 107 scans of 5030 Hz (200 ppm) at 200 sec/scan. Dioxane was used as the homonuclear lock signal. Chemical shifts were measured relative to the ^{13}C signal of dioxane and converted to parts per million from dissolved TMS (δ_c) using δ_c(dioxane) = 67.4 ppm.

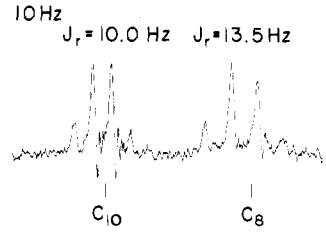

Figure 2. Continuous-wave decoupled cmr spectrum of asperlin from sodium 2-^{13}C-acetate, 153 mg/1.0 ml of dioxane; 57 scans of 252 Hz at 26 sec/scan were accumulated.

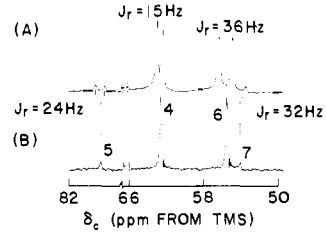

Figure 3. Portions of the cmr spectra of asperlin from sodium 2-^{13}C-acetate in benzene, 153 mg/1.0 ml; lock signal, benzene: (A) cw decoupled, 2052 scans of 1006 Hz, 26 sec/scan; (B) pnd, 56 scans of 1006 Hz, 26 sec/scan.

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(3) Using the observed $J_{^{13}C-H}$ coupling constants from Table I and $\gamma^{H_2}/2\pi$ set at 2100 Hz in these experiments, (1) yielded calculated J_r values for C_8 of 14.3, C_{10} of 9.9, C_4 of 15.5, C_5 of 24, and C_6 of 31.9 Hz, respectively. $J_{^{13}C-H}$ at C-5 of 145 Hz was estimated by the method of E. R. Malinowski, *J. Amer. Chem. Soc.*, **83**, 4479 (1961).

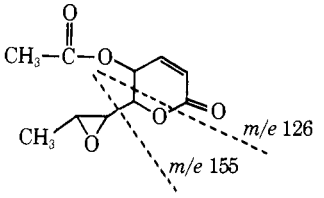
Isotope enrichment in asperlin was estimated to be 9% ± 1 per labeled carbon by comparison of signal intensities in the cmr spectrum. Enrichments determined by the proton–^{13}C satellite band areas are in Table I, which shows about 10 ± 0.5% per labeled carbon.

Table I. ^{13}C Enrichment Levels of Labeled Asperlin by ^{13}C–H Satellite Area

Carbon	$J_{^{13}C-H}$	Enrichment
C_8–H_3	126	11.5
C_{10}–H_3	130	10.9
C_4–H	152	10.0
C_2–H	174	10.8
C_6–H	165	*a*

[a] Complexity of satellite band prevented estimation of this value.

The mass spectrometric analysis demonstrated a difference in the ratios M + 1/M for the labeled and unlabeled asperlins of 0.366. This corresponds to an average of 7.3% ^{13}C enrichment at each of five positions, or a total of 8.4% ^{13}C (with natural abundance of 1.1% ^{13}C included) at each of the labeled carbons. Thus the approximate value of 8–10% ^{13}C deduced by nmr is confirmed and more accurately established by mass spectrometry.



asperlin, M = *m/e* 212

Qualitative distribution of the label could be observed by inspection of the isotope content of major fragments in the mass spectrum of labeled asperlin. For example, part of the label was located on the C_6, C_7, C_8 substituent, as indicated by the lower ^{13}C enrichment in fragments of mass 155 and 126. The label at the acetate could be observed directly in the CH_3CO peak (*m/e* 43), as well as indirectly by the loss of ketene from M, *m/e* 155, and *m/e* 126 to give *m/e* 170, 113, and 84, respectively, each with a reduced isotope enrichment. The latter two peaks nevertheless still contained some additional ^{13}C above natural abundance, demonstrating the presence of label also at carbons 1–4.

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Secondary Hydrogen–Deuterium Isotope Effects in the Fluorine Nuclear Magnetic Resonance Shifts of Methyl-*p*-fluorophenylcarbonium Ions[1,2]

Sir:

We have observed substantial secondary β-deuterium isotope effects on the fluorine nmr shifts of *p*-fluorophenylmethyl(H_3 and D_3)carbonium ions. These isotope effects are analogs of the well-known β-deuterium isotope effects on solvolysis rates,[3] although our measurements involve no state change, *i.e.*, are a physical property (presumably a ground-state charge distribution measure[4]) of the carbonium ion state. The effects of normal substituents on F nmr shifts of *p*-fluorophenyl derivatives have been found to show a remarkable correspondence to their effects on chemical reaction rates and equilibria.[5]

Measurable F nmr secondary β-deuterium isotope effects have been found (*cf.* Table I) only for *p*-fluorophenyl-labeled systems (ion or neutral) for which substantial stabilization by (methyl group) hyperconjugative interaction may be expected. Our results are consistent with those of Traficante and Maciel[6] for *m*- and *p*-fluorotoluene and their CD_3 derivatives, and, in the carbonium ions, the isotope effects are more than an order of magnitude larger than that found for the *p*-fluorotoluene system.

Although the secondary β-deuterium isotope effects on solvolysis rates have been generally interpreted in terms of hyperconjugative stabilization of the solvolysis transition state,[3,7] an interpretation in terms of nonbonded steric interactions has been advanced.[8,9] Steric isotope effects are undoubtedly involved in the rates of racemization of 9,10-dihydro-4,5-dimethylphenanthrene.[10]

In the present study of carbonium ions two lines of evidence (in addition to the single-state physical property nature of our measurement) have been obtained which indicate little or no steric interaction effects are involved in the observed β-deuterium F nmr shifts. (1) For the carbonium series I the β-deuterium isotope effect does not follow the steric size sequence X = H < OH, NH_2, < Me < CF_3, but instead follows the electronic sequence X = NH_2 < OH < Me < H < CF_3.[11] This latter order is expected for increased hyperconjugative demand from the carbinyl

(1) This work was supported in part by the National Science Foundation. We are also grateful for support from NSF which made available to the UCI chemistry department the nmr spectrometer.

(2) Preliminary reports of this work were made at the Gordon Conference on Chemistry and Physics of Isotopes, Holderness, N. H., June 1968, and the Conference on Carbonium Ions, Cleveland, Ohio, Oct 1968.

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