water or oxygen is eliminated under these conditions. In a typical preparation a quartz epr flat cell is attached to a Y cell[10] and the assembly is flushed with dry nitrogen. The cell is charged with 0.75 ml of HMPA, 50 μl of 1.6 *F* *n*-butyllithium in HMPA,[11] and 25 μl of 6.5×10^{-3} *M* biphenyl in HMPA by syringe. Both the epr [$a_{p\text{-H}} = 5.26$ G, $a_{o\text{-H}} = 2.58$ G, $a_{m\text{-H}} = 0.40$ G, line width = 40 mG] and electronic spectra of the solution are recorded in the flat cell.

The spectra obtained for the lithium salts of the hydrocarbon radical anions indicate that they are the expected "free ions."[12] However, epr and electronic spectra indicate that the magnesium salts exist as associated ion pairs in HMPA solution. For example, lithium anthracenide has epr parameters ($a_{H\text{-}9} = 5.24$, $a_{H\text{-}1} = 2.72$, and $a_{H\text{-}2} = 1.48$ G) and absorbs at 366 nm, whereas magnesium anthracenide has epr parameters ($a_{H\text{-}9} = 5.20$, $a_{H\text{-}1} = 2.71$, and $a_{H\text{-}2} = 1.48$ G) and absorbs at 340 nm.

The observations reported here suggest that many radical anions previously prepared with great difficulty can be easily prepared by one-electron reduction with readily available organometallic compounds in HMPA solution.

Acknowledgment. The Tulane University Chemistry Department and Computer Laboratory are thanked for their support. The epr spectrometer was purchased with funds supplied in part by the National Science Foundation.

(10) The Y cell is one side of Russell's H cell.[3]

(11) For most applications the organometallic reagent can be used as the more readily available hydrocarbon or ether solutions.

(12) M. Szwarc, "Carbanions, Living Polymers, and Electron Transfer Processes," Interscience, New York, N. Y., 1968, Chapters 5 and 6.

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Utilization of Carbon-13–Carbon-13 Coupling in Structural and Biosynthetic Studies. An Alternate Double Labeling Method

Sir:

We wish to report a new method utilizing ^{13}C–^{13}C coupling in structural and biosynthetic studies.[1,2] When a microbial metabolite of polyacetate origin is labeled with doubly labeled acetate ($^{13}CH_3{}^{13}CO_2H$), the ^{13}C–^{13}C coupling should be observed with the metabolite between the C–C bonds which had formed the acetic acid molecule. However, the coupling should not be observed between the C–C bonds formed by the condensation of acetic acid. In the proton-decoupled cmr spectrum of the labeled compound, each signal appears as a triplet, whose center peak is caused by the natural abundance peak.

(1) For earlier studies of ^{13}C–^{13}C couplings in organic molecules, see: K. D. Summerhays and G. E. Maciel, *J. Amer. Chem. Soc.*, **94**, 8348 (1972); F. J. Weigert and J. D. Roberts, *ibid.*, **94**, 6021 (1972); A. M. Ihrig and J. L. Marshall, *ibid.*, **94**, 1756 (1972).

(2) For biosynthetic studies in which ^{13}C–^{13}C couplings were observed from singly labeled precursors, see: M. Tanabe, T. Hamasaki, H. Seto, and L. F. Johnson, *Chem. Commun.*, 1539 (1970); M. Tanabe, T. Hamasaki, Y. Suzuki, and L. F. Johnson, *J. Chem. Soc., Chem. Commun.*, 212 (1973); A. I. Scott, C. A. Townsend, K. Okada, M. Kajiwara, P. J. Whitman, and R. J. Cushley, *J. Amer. Chem. Soc.*, **94**, 8267 (1972).

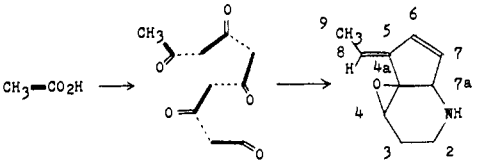

Figure 1. Dihydrolatumcidin and its biosynthetic pathway.

Information on the other C–C bonds formed by the condensation of acetic acid molecule may be obtained by labeling the metabolite using mixed labeled acetate (1:1 mixture of $^{13}CH_3CO_2H$ and $CH_3{}^{13}CO_2H$). Although four combinations between these two differently labeled acetates are possible, only one ($CH_3{}^{13}CO\cdots{}^{13}CH_2COR$) gives the desired ^{13}C–^{13}C coupling.[3] The results obtained by using doubly and mixed labeled acetate are complementary to each other and will give the complete sequence of the carbon skeleton of the metabolite. In addition to polyketides, the above mentioned "alternate double labeling method" can be applied to steroids and terpenes which are biosynthesized fron acetate *via* mevalonate.

From the biosynthetic point of view, one of the advantages of using doubly labeled acetate is that this method makes it possible to know the occurrence of any C–C bond fission during biosynthesis. The direction of the elongation of a polyketide chain can also be detected (R_1CH_2–$CO\cdots CH_2R_2$ or $R_1CH_2\cdots CO$–CH_2R_2).[5]

In order to test the "alternate double labeling method," we chose as a model compound dihydrolatumcidin (I) ($C_{10}H_{13}ON$), a metabolite of *Streptomyces reticuli* var. *latumcidicus*,[6] whose structure[7] and biosynthesis[8] had been previously established (Figure 1).

The proton-decoupled cmr spectrum of I labeled with doubly labeled acetate (90% enriched) exhibited very strong peaks due to ^{13}C–^{13}C coupling (Figure 2a). Likewise, the ^{13}C–^{13}C coupling with weaker intensity appears in the spectrum of I (Figure 2b) labeled with mixed labeled acetate (both 90% enriched). It should be noticed in this spectrum that C_{4a} and C_5 couple respectively to two carbons and that C_2 and C_9 do not couple to any carbon.

The coupling constants, chemical shift, and multiplicity on the off-resonance decoupling spectrum obtained with an unlabeled sample are summarized in Table I. From these data and the known chemical shift of carbons,[9] the following carbon sequences are very easily obtained from the sample labeled with doubly labeled acetate: $NC_{(2)}H_2C_{(3)}H_2$, $C_{(4)}HX$-

(3) In addition to the 1,2 couplings, 1,3 couplings ($^{13}CH_3CO\cdots{}^{13}CH_2COR$) may also occur. In this case, discrimination between the 1,2 and the 1,3 couplings becomes very important in the correlation of carbon signals. Fortunately, since the 1,2 coupling is much larger than the 1,3 or 1,4 coupling, with the exception of cyclopropane derivatives,[4] the magnitude of the coupling constant is very useful in making the assignment.

(4) J. B. Stothers, "Carbon-13 NMR Spectroscopy," Academic Press, New York, N. Y., 1972, p 370.

(5) H. Seto, L. W. Cary, and M. Tanabe, *J. Chem. Soc., Chem. Commun.*, 1289 (1973).

(6) Y. Sakagami, I. Yamaguchi, H. Yonehara, Y. Okimoto, S. Yamanouchi, K. Takiguchi, and H. Sakai, *J. Antibiot., Ser. A*, **11**, 6 (1958).

(7) Y. Kōno, S. Takeuchi, H. Yonehara, F. Marumo, and Y. Saito, *Acta Crystallogr., Sect. B*, 27, 2341 (1971).

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(9) L. F. Johnson and W. C. Jankowski, "Carbon-13 NMR Spectra," Wiley-Interscience, New York, N. Y., 1972; G. C. Levy and G. L. Nelson, "Carbon-13 Nuclear Magnetic Resonance for Organic Chemists," Wiley-Interscience, New York, N. Y., 1972.

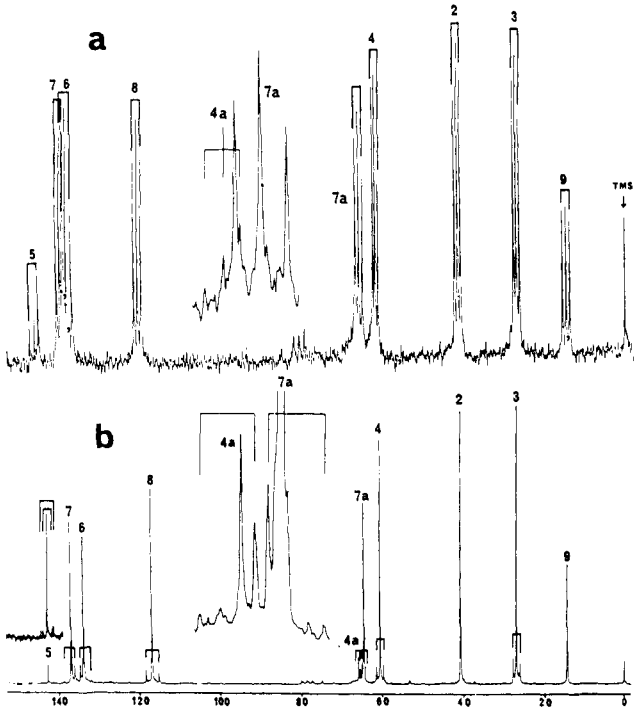

Figure 2. Proton noise-decoupled Fourier transform cmr spectrum of dihydrolatumcidin in $CDCl_3$. (a) From $^{13}CH_3{}^{13}CO_2Na$ (90% enriched), 55 mg. The precursor, diluted with unlabeled CH_3CO_2Na by 2.5 times in order to avoid excess labeling which would result in a complicated spectrum, was added to the fermentation broth of *S. reticuli* var. *latumcidicus* 14, 19, and 24 hr after inoculation. A 100-mg portion of the acetate was added to each 500-ml flask containing 100 ml of medium. The labeled dihydrolatumcidin was isolated by solvent extraction 63 hr after inoculation; pulse width, 25 μsec; acquisition time, 0.8 sec; 11,735 transients. (b) From a 1:1 mixture of $^{13}CH_3CO_2Na$ and $CH_3{}^{13}CO_2Na$ (both 90% enriched). A 100-mg portion of the precursor was added to each flask 14 hr after inoculation. A yield of 63 mg/five flasks was obtained; 62,325 transients.

Table I. Chemical Shift and Coupling Constant of Dihydrolatumcidin

Carbon	Chemical shift	Multiplicity on off-resonance decoupling	$J_{^{13}C-^{13}C}$, Hz: Doubly labeled	$J_{^{13}C-^{13}C}$, Hz: Mixed labeled
2	39.6[a]	t[b]	35.4	
3	25.9	t	35.4	43.4
4	59.2	d	30.7	43.4
4a	64.1	s	30.7	46.0, 57[c,d]
5	140.0	s	53.4	78.7, 57.9
6	131.7	d	53.0	68.1[c]
7	133.8	d	43.4	68.3
7a	63.1	d	43.8	46.1
8	114.9	d	44.8	78.7
9	14.0	q	44.8	

[a] Downfield from internal TMS. [b] s, singlet; d, doublet; t, triplet; q, quartet. [c] Small splittings or line broadening caused by long-range coupling were observed. [d] Due to the overlapping of the 7a signal, this value was obtained with the single labeled sample.[8]

$C_{(4a)}Y<$, $=C_{(5)}C_{(6)}H=$, $=C_{(7)}HC_{(7a)}HZ$, and $=C_{(8)}H$-$C_{(9)}H_3$, where X, Y, and Z represent oxygen or nitrogen. Although another combination between C_5 and C_6 is possible, namely $>C_{(5)}=C_{(6)}H$, the magnitude of the coupling constant ($J_{5,6}$ = 53.4 Hz) indicates clearly the latter possibility to be unlikely (*cf.* ethylene,[10] $J_{1,2}$ = 67.2 Hz; 1,3-butadiene,[11] $J_{1,2}$ = 68.8 Hz and $J_{2,3}$ = 53.7 Hz).

These carbon sequences can be further extended by utilizing the following sequences obtained with I labeled by mixed labeled acetate: $C_{(3)}H_2C_{(4)}HX$, $C_{(7a)}HZ$-$C_{(4a)}YC_{(5)}=C_{(8)}H$ and $C_{(6)}H=C_{(7)}H$. The very small coupling constant between C_4 and C_{4a} ($J_{4,4a}$ = 30.7 Hz), which is not in agreement with the expectation of rather large coupling constant due to the binding of these carbons to electronegative substituent,[4] can only be explained in terms of the epoxide structure. The presence of the epoxide is also supported by the large $^{13}C-H_4$ coupling constant ($J_{^{13}C-H}$ = 178 Hz). Therefore, the remaining unexplained bonds of the heteroatom on C_{7a} (which must be nitrogen) and the nitrogen bonding to C_2 must be connected to each other.

Thus, the complete structure of I has been established as shown in Figure 1. It should be emphasized that by the aid of the "alternate double labeling method" we have arrived at the correct structure without using any other information usually available by conventional methods such as ir, uv, and proton nmr spectroscopy.

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Gas-Phase Acidity of Monosubstituted Phenols

Sir:

Recent studies of the acidity of molecules in the gas phase have elucidated a number of glaring discrepancies between gas-phase and solution behavior. For example, in the gas phase toluene is a stronger acid than water, and the acidity order of the aliphatic alcohols is *reversed* from that observed in solution.[1,2] In this communication we report the relative gas-phase acidity of a number of monosubstituted phenols and examine the correlation with the corresponding ionization constants in water.

Using pulsed ion cyclotron resonance spectroscopy,[3] equilibrium constants can be measured for reactions such as

$$AH + B^- = BH + A^- \quad (1)$$

where AH and BH are Brønsted acids, and A^- and B^- are the corresponding conjugate bases.[4] Since acidity

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