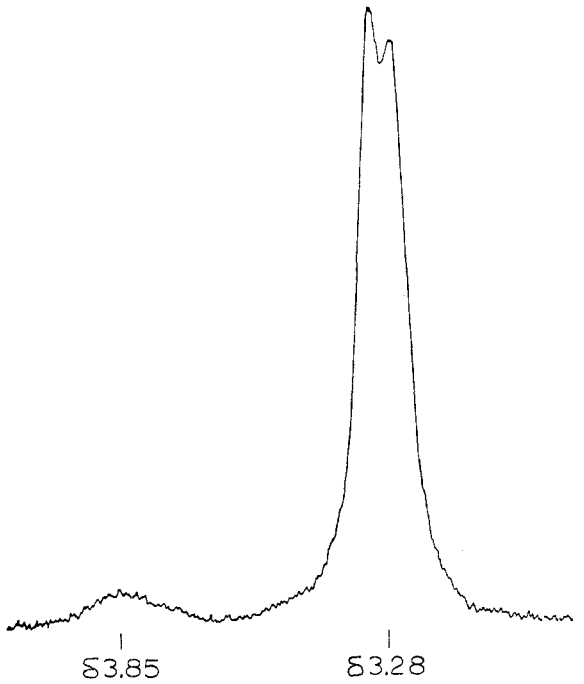

Figure 1.—Axial (δ 3.28) and equatorial (δ 3.85) HCO resonances of cyclohexanol-2,2,6,6-d_4 (3 M in CS_2) at −83°.

Perusal of Table I indicates no dramatic solvent effects (in the solvents used) although some variation is noted. The A value of hydroxyl is larger in the hydroxylic solvent CD_3OD, as expected. Some error is introduced into the A value determined in CD_3OD because of a slight overlap of the CHD_2OD impurity resonance with the axial H–C–O resonance of cyclohexanol-2,2,6,6-d_4.

These data provide an opportunity for a meaningful comparison albeit at a low temperature of the A value of hydroxyl with other oxygen-containing functionalities (Table II). Although the effective group

TABLE II

A VALUES OF VARIOUS OXYGEN-CONTAINING FUNCTIONALITIES

Group	A value, kcal/mol[a]	Group	A value, kcal/mol[a]
–OTs	0.52	–OC(=O)H	0.59
$-OCD_3$	0.55	–OAc	0.71
$-OSO_2CH_3$	0.56	–OH	0.97[b]

[a] All concentrations approximately 2½ M. Solvent is CS_2 except for OTs and OSO_2CH_3 in which case it is approximately 50:50 by volume CS_2–$CDCl_3$; see ref 3. [b] This work.

radius of hydroxyl is almost certainly smaller than the other functionalities, it has a significantly higher A value. The effect of intermolecular association is evident. It is also clear from Table II that the A values of functionalities with oxygen bonded to the cyclohexane ring are not all of the same magnitude.

Experimental Section

Nmr spectra were obtained using a Varian HR-60A spectrometer equipped with a custom-built variable-temperature probe. Spectral calibrations were performed using the audio-modulation technique. Temperature measurements were performed using a calibrated copper-constantan thermocouple.

Cyclohexanol-2,2,6,6-d_4 was prepared by the lithium aluminum hydride reduction of cyclohexanone-2,2,6,6-d_4.[6]

Registry No.—Cyclohexanol-2,2,6,6-d_4, 21273-03-0.

Acknowledgment.—We thank Research Corporation (Cottrell Grant) and the National Science Foundation (COSIP Grant) for support of this work.

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Biosynthetic Studies with Carbon 13. Piericidin A

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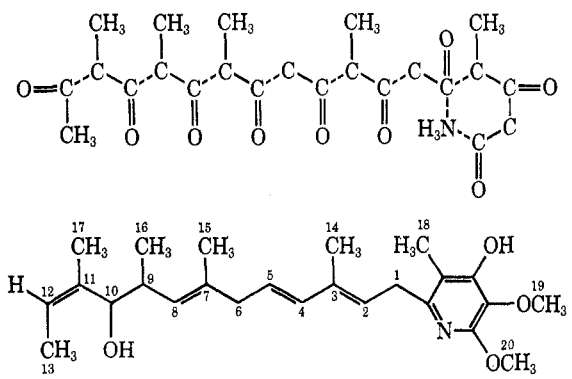
The antibiotic piericidin A is a naturally occurring insecticide which is produced by *Streptomyces mobaraensis*.[1] Its structural and stereochemical formulation (I) is due to the work of Takahashi and coworkers.[2] Biosynthetic studies conducted with carbon 14 labeled precursors indicated that the carbon chain of Piericidin A is formally derived by condensation of five propionate and four acetate units, presumably *via* an acetate starter and the methylmalonyl pathway.[3] A useful



I

procedure for biosynthetic studies of microbial metabolites is the nondegradative ^{13}C proton satellite method.[4] We wish to report that the production of piericidin A in the presence of ^{13}C-methyl labeled propionate ($^{13}CH_3CH_2CO_2Na$) affords direct information on the biological origin of the methyl groups in the antibiotic. This information can be obtained by the ^{14}C method; however, limitations on chemical degradative methods preclude identification of specific labeled carbon atoms.

Streptomyces mobaraensis fermentations in the pre-

(1) S. Tamura, N. Takahashi, S. Miyamoto, R. Mori, S. Suzuki, and J. Nagatsu, *Agr. Biol. Chem.* (Tokyo), **27**, 576 (1963).

(2) (a) N. Takahashi, A. Suzuki, and S. Tamura, *J. Amer. Chem. Soc.*, **87**, 2066 (1965); (b) N. Takahashi, A. Suzuki, and S. Tamura, *Agr. Biol. Chem.* (Tokyo), **30**, 1 (1966); (c) N. Takahashi, S. Yoshida, A. Suzuki, and S. Tamura, *ibid.*, **32**, 1108 (1968).

(3) (a) N. Takahashi, Y. Kimura, and S. Tamura, *Tetrahedron Lett.*, 4659 (1968); (b) Y. Kimura, N. Takahashi, and S. Tamura, *Agr. Biol. Chem.* (Tokyo), **33**, 1507 (1969).

(4) (a) M. Tanabe and G. Detre, *J. Amer. Chem. Soc.*, **88**, 4515 (1966); (b) D. Desaty, A. G. McInnes, D. G. Smith, and L. C. Vining, *Can. J. Biochem.*, **46**, 1293 (1968). (c) A. G. McInnes, D. G. Smith, L. C. Vining, and J. L. C. Wright, *Chem. Commun.* 1669 (1968).

viously reported C[4] medium[1] supplemented with 56% $^{13}CH_3CH_2CO_2Na$ (100 mg/40 ml) yielded after a 24-hr incubation isotopically enriched piericidin A. In the nmr spectrum of piericidin A, the C_{14}, C_{15}, C_{16}, C_{17}, and C_{18} methyl resonances are resolved and their positions can be assigned,[2c] thereby allowing most of their corresponding satellites in the labeled compound to be readily located and identified, and their intensities measured. The source of the methoxyl groups in the antibiotic was determined by additional experiments with 56% enriched [$^{13}CH_3$]-methionine (100 mg/40 ml). The nmr data for the labeled piericidins are summarized in Table I.

TABLE I

NMR DATA FOR PIERICIDIN A

			—100-MHz yield[a]—		—60-MHz yield—	
	τ	J^{13}_{CH}, Hz	Upfield satellite	Downfield satellite	Upfield satellite	Downfield satellite
			^{13}C-Propionate			
C_{14} CH_3	8.20	126	8.7[b]	10.1[c]	*d*	*e*
C_{15} CH_3	8.36	124	*d*	10.5[c]	9.8	*e*
C_{16} CH_3	9.18	128	9.6	*f*	12.4	*d*
C_{17} CH_3	8.24	126	7.3[b]	10.5[c]	*e*	*e*
C_{18} CH_3	7.90	130	9.1[g]	*e*	10.2	8.7
			^{13}C-Methionine			
C_{19} OCH_3	6.04	147	15.3	17.3		
C_{20} OCH_3	6.14	146	17.2	17.2		

[a] The yields are expressed a atom per cent excess ^{13}C. The incorporation yields were determined by comparing the area of the satellite peak with the area of the unlabeled carbon-1 methylene protons as an internal standard. Yields represent the area determined *via* a single scan on the Varian HA-100 and A-60A, respectively. Incorporation values are ±15% error. [b] This is an approximate value, since an impurity peak gives an overlapping signal at τ 8.75. [c] Spin decoupling proved that the C_9 proton which appears in this region of the spectrum, does not overlap with this downfield satellite peak. [d] This satellite signal was observed; however, owing to an impurity signal and/or overlapping signals, this yield was not calculated. [e] This satellite signal was completely obscured by overlapping signals. [f] This downfield satellite signal overlapped the upfield satellite signal of the C_{18} CH_3 group. [g] This signal appeared at τ 8.6 together with the C_{16} CH_3 downfield signal. This yield was approximated by subtracting the upfield C_{16} CH_3 area from the total peak area.

These data unequivocally show that five C-methyl groups are biosynthetically derived from the methyl group of propionate and the terminal C_{13} methyl group is not propionate derived. The nearly equal labelling pattern observed in the methyl groups along the chain implies that only a single polyketide chain is assembled subsequent to nitrogen introduction to form the pyridine ring. No other biogenetic unit appears to be involved. These results amplify and are in accord with the ^{14}C biosynthesis work.

We observed that incorporation of [$^{13}CH_3$]-methylmalonic acid into piericidin A was very low, since satellite bands could not be observed with a single scan. The poor incorporation is probably due to a cell membrane permeability effect. A similar result was observed in the biosynthesis of erythromycin.[5]

The general use of $^{13}CH_3CH_2CO_2Na$ and nmr for establishing the origin of methyl groups derived from propionate in microbial metabolites is a useful technique and high incorporation yields can be generally anticipated. The method is a useful complement to the radio carbon method.

(5) S. M. Friedman, T. Kaneda, and J. W. Corcoran, *J. Biol. Chem.*, **239**, 2396 (1964).

Registry No.—Piericidin A, 24467-35-4.

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Diels–Alder Reaction of Tetrachloroethylene with Anthracene

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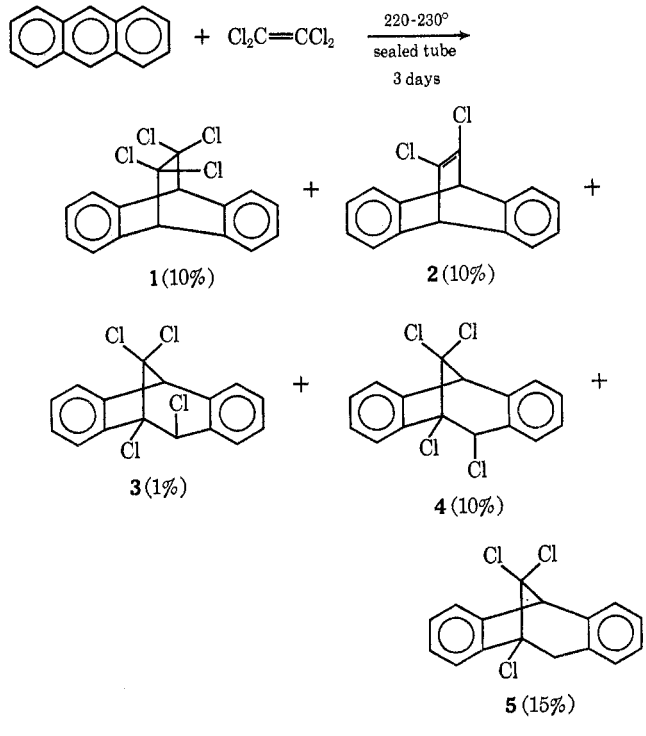
In connection with other experiments we wished to synthesize 11,11,12,12-tetrachloro-9,10-dihydro-9,10-ethanoanthracene (**1**). The only mention in the literature of this compound is the report of Russian workers[1,2] that **1** results from the Diels–Alder reaction of tetrachloroethylene with anthracene. We have repeated



this reaction and find that indeed **1** (mp 205–206°) is produced, albeit in a mixture with a number of other compounds. One of these other characterizable products is 11,12-dichloro-9,10-dihydro-9,10-ethenoanthracene (**2**), which from the melting point (179–180°) appears to be the compound the earlier workers assigned as **1**. The conclusion is supported by dipole-

(1) V. M. Zonoastrova and B. A. Arbuzov, *Dokl. Akad. Nauk SSSR*, **60**, 59 (1948).

(2) B. A. Arbuzov and A. N. Vereshchagin, *Bull. Acad. Sci. USSR*, 936 (1964).