Communications to the editor

UTILIZATION OF ^{13}C-^{13}C COUPLING IN STRUCTURAL AND BIOSYNTHETIC STUDIES

IV. PENICILLIC ACID[1]

Sir:

An approach to the detailed investigation on mechanisms involved in the biosynthesis of microbial metabolites has been facilitated by the use of ^{13}C-^{13}C coupling[2,3] in combination with cmr spectroscopy. This new technique has proved to be useful as well in structural elucidation[1,4] of polyketide metabolites.

We report on the biosynthetic study of penicillic acid[5] utilizing ^{13}C-^{13}C coupling. ^{13}C-Doubly labeled sodium acetate (90 % enriched $^{13}CH_3{}^{13}CO_2Na$, 100 mg per 100 ml culture broth) was added to surface cultures of *Penicillium cyclopium* in three 500 ml Erlenmeyer flasks 7 days after inoculation. After a further 14 days, the labeled penicillic acid was isolated as previously described.[6]

In the proton noise-decoupled cmr spectrum of the labeled penicillic acid (Fig. 1a), ^{13}C-^{13}C coupling was observed between C- 2 and 3 as well as between 5 and 7. From this result (Table 1), it is evident that C-2 and 3 (J_{c-c}= 78 Hz), and C-5 and 7 (J_{c-c}=45 Hz) are derived from the same molecule of doubly labeled acetic acid without cleavage of the C-C bond.[4] Incorporation of this precursor into C-1f, 4 and 6 with concomitant cleavage of the C-C bond of the acetic acid molecule is demonstrated by the increased signal intensity of these carbon signals relative to that of the unlabeled methoxyl signal. Since orsellinic acid is known to be an intermediate in penicillic acid biosynthesis, the biosynthetic pathway of the metabolite can be depicted as shown in Fig. 2. The cleavage of the C-C bond of orsellinic acid should occur at (*a*) but not at (*b*), since if the latter cleavage occurs, ^{13}C-^{13}C coupling should be observed between C-1 and 2, and C-3 and 4 in addition to the observed coupling between C-5 and 7. This result is in agreement with the conclusion of MOSBACH[7] obtained by using 2- and carboxy-^{14}C orsellinic acid as substrates.

Table 1. Carbon-13 chemical shift and coupling constant of carbon-13 labeled penicillic acid.

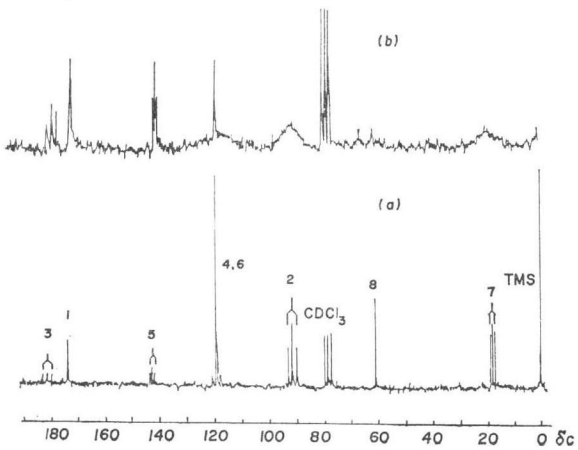
Carbon	Chemical shift (δc) ppm from $(CH_3)_4Si$	Coupling constant (J_{c-c}) Hz	Multiplicity on off-resonance decoupling[a]
1	171.52	—	s
2	89.99	78	d
3	178.17	77	s
4	117.58	—	s*
5	139.86	44	s
6	117.58	74	t
7	17.30	45	q
8	59.65	—	q

* Due to the overlapping of the C-6 signal, this information was obtained by the undecoupled spectrum. Detail see in the text.

([a]) s: singlet, d: doublet, t: tripled and q: quartet

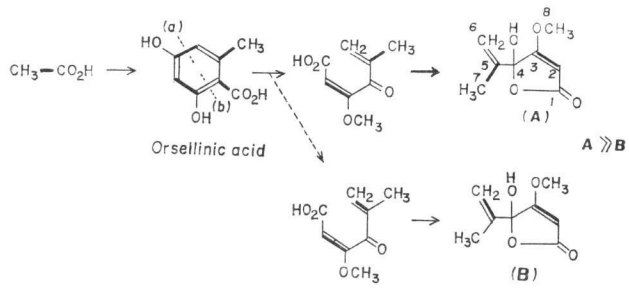
Fig. 1. ^{13}C-nmr spectrum of labeled penicillic acid in $CDCl_3$

(*a*) Proton noise-decoupled. Pulse width 20 μsec. Acquisition time 0.8 sec. 35,242 transients.
(*b*) Undecoupled. 66,915 transients.
Tne spectra were taken on a Varian XL-100 spectrometer.



The signal assignments were based on known chemical shifts of carbons[8] as well as on the results of single frequency off-resonance decoupling. The assignment of the C-3 signal (δ_c=178.17 ppm), which surprisingly absorbs at a very low field position, is rationalized by the fact that it is an oxygenated carbon that is deshielded in the β-position of an α, β-unsaturated lactone. Two β-oxygens further deshield the C-3 carbon. Similar chemical

Fig. 2. Biosynthetic pathway of penicillic acid



shifts were observed with althiomycin[9] and its degradation product, 4-methoxy-Δ^3-pyrrolin-2-one.[9]

Notwithstanding several attempts, the C-4 and 6 signals could not be resolved, but the coincidental overlapping of these two signals was confirmed as follows: (1) the signal collapsed to a triplet on off-resonance decoupling, which indicated the presence of the C-6 methylene group; (2) in the undecoupled spectrum (Fig. 1b), in which all protonated carbon signals collapsed to very broad signals, a singlet with decreased intensity remained at the same position. This indicated the presence of the C-4 quaternary carbon. The small satellite peaks on both sides of the signals of C-4 and 6 at δ_c=117.58 ppm in the proton noise-decoupled spectrum disappeared in the undecoupled spectrum. Thus, these small peak must be caused by ^{13}C-^{13}C coupling of the C-6 signal (J_{c-c}=74 Hz).* This result can be reasonably explained by assuming the formation of two differently labeled penicillic acids (*A* and *B* in Fig. 2). It should be noted that the satellite peak intensities of C-6 are weaker compared to the C-2, 3, 5 and 7 satellites. Therefore, it follows that partial conversion of *A* to *B* occurs at some step of the biosynthetic pathway. The penicillic acid *A* and *B* are not interconvertible.

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* This is probably due to the weak intensity of the satellite signals expected for C-5. The coupling between C-5 and 6 was not observed with C-5 at the signal to noise level attained.

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