STUDIES ON THE BIOSYNTHESIS OF BIALAPHOS (SF-1293)
1. INCORPORATION OF 13C- AND 2H-LABELED PRECURSORS INTO BIALAPHOS

Sir:

Bialaphos (formerly called SF-1293) which is now being developed as an effective herbicide is a metabolite of Streptomyces hygroscopicus SF-1293. It has been proved to be identical with phosphinothricylalaninylalanine reported by Bayer et al. Bialaphos (I) is the only natural product to have the unique C-P-C bond in the phosphinothricyl moiety. In view of this structural characteristic, our efforts were directed toward understanding the mechanism of formation of the C-P bond. We wish to report herein our biosynthetic studies on I.

The metabolite is a tripeptide comprising two alanine residues and one phosphinothricin (II) (Fig. 1) which was the subject of our interest. Since the main framework of II is an amino acid consisting of four carbon atoms, biosynthetic studies were initiated by feeding structurally related 14C-labeled amino acids such as methionine, aspartic acid or glutamic acid to the fermentation broth of the producing organism; however, no significant incorporation was observed with these substances.

On the other hand, sodium [2-13C]acetate was incorporated selectively into C-2 of II (ca. 1.5 fold). This result was further corroborated by the experiment using sodium [1,2-13C2]acetate. As seen in Fig. 2, only C-2 and C-1 exhibit 13C-13C coupling (J=53 Hz) providing unequivocal evidence that these two carbon atoms in II originate from the intact acetate molecule. This finding strongly suggested that the origin of the remaining -CH2-CH2-P unit in II was glucose as in the case of 2-aminoethylphosphonic acid. As expected, the 13C NMR spectrum of I labeled with [U-13C6]glucose showed an AB-type 13C-13C coupling between C-4 and C-3 (J=33 Hz, see Fig. 3). This precursor was also incorporated into C-2 and C-1 probably after its degradation.
to acetic acid. Although the four carbons in II were labeled by glucose to the same degree, the absence of $^{13}$C-$^{13}$C coupling between C-3 and C-2 indicated that the two carbon pairs C-4 and C-3, and C-2 and C-1 were derived from different precursors. The $^{13}$C-coupling patterns of C-3', C-2', and C-1', and C-3'', C-2'' and C-1'' are reasonably explained by the incorporation of glucose into these carbons via pyruvic acid. The origin of the C-5 methyl was proved by the incorporation of CD$_3$-methionine into C-5 ($J_{C-P}$=93 and 90 Hz, respectively) and splitting patterns in the off-resonance decoupled spectrum. C-2 was also assigned based on long range coupling ($J_{C-P}$=14 Hz) with phosphorous. C-1 was identified by $^{13}$C-$^{13}$C coupling observed in the $^{13}$C NMR spectrum of I labeled with [U-$^{13}$C$_6$]glucose. All the carbons in the alanylalanine moiety were assigned by their chemical shift trends and splitting patterns in the off-resonance spectrum.

CH$_2$-P bond formation during the biosynthesis of I is believed to proceed via the demethyl derivative of phosphinothricin (MP-101). This derivative was recently isolated from the fermentation medium of the bialaphos producing organism. Details will be published elsewhere.

Acknowledgements

This work was supported in part by a Grant-in-Aid for Special Project Research, the Ministry of Education, Science and Culture, Japan.

The authors wish to express their thanks to Miss. T. TAKEI and Mrs. K. ITO for their technical assistance.

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(Received July 15, 1982)

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