

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

Sir:

Bialaphos (formerly called SF-1293^{1,2}) 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 phosphinothricylalanylalanine reported by BAYER *et al.*³ Bialaphos (I) is the only natural product to have the unique C-P-C bond in the phosphinothricyl moiety³. 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 ^{14}C -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 [$2\text{-}^{13}\text{C}$]acetate was incorporated selectively into C-2 of II (*ca.* 1.5 fold). This result was further corroborated by the experiment using sodium [$1,2\text{-}^{13}\text{C}_2$]acetate. As seen in Fig. 2, only C-2 and C-1 exhibit ^{13}C - ^{13}C coupling ($J=53\text{ Hz}$) providing unequivocal evidence that these two carbon atoms in II originate from the intact acetic acid molecule. This finding strongly suggested that the origin of the remaining $-\text{CH}_2-\text{CH}_2-\text{P}$ unit in II was glucose as in the case of 2-aminoethylphosphonic acid⁴. As expected, the ^{13}C NMR spectrum of I labeled with [$\text{U-}^{13}\text{C}_6$]glucose showed an AB-type ^{13}C - ^{13}C coupling between C-4 and C-3 ($J=33\text{ Hz}$, see Fig. 3). This precursor was also incorporated into C-2 and C-1 probably after its degradation

Fig. 1. Biosynthetic pathway of bialaphos.

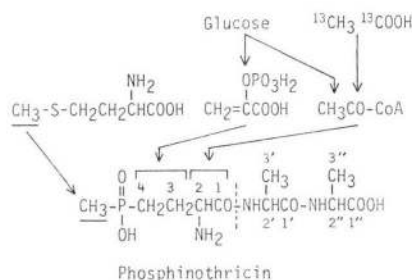


Fig. 2. The ^{13}C NMR spectrum of bialaphos labeled with sodium [$1,2\text{-}^{13}\text{C}_2$]acetate in D_2O . The smaller splitting of C-2 is caused by coupling to phosphorous.

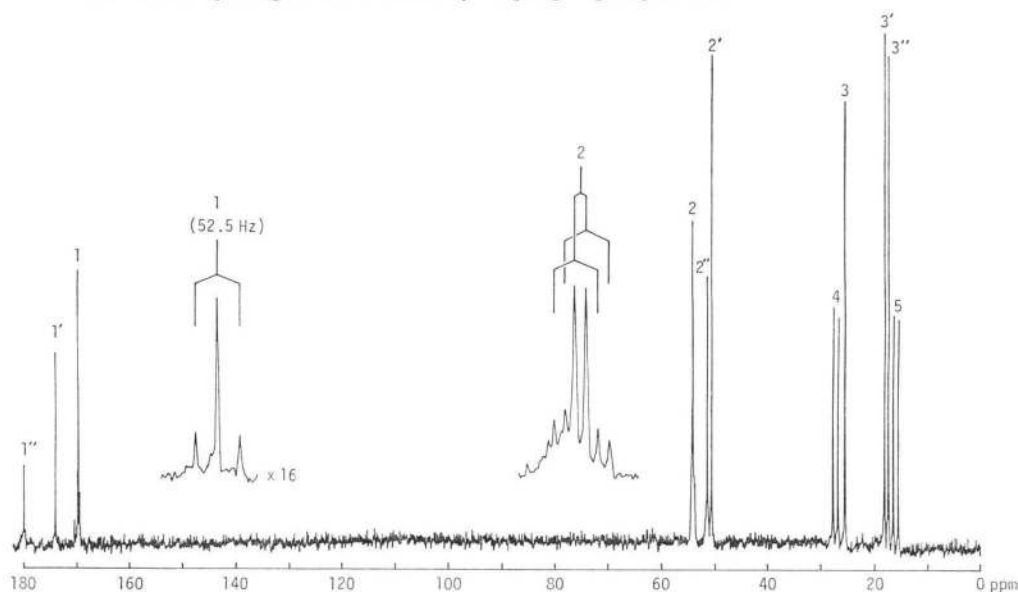


Fig. 3. The ^{13}C NMR spectrum of bialaphos labeled with $[\text{U-}^{13}\text{C}_6]\text{glucose}$ in D_2O . The chemical shifts are expressed in ppm downfield to internal dioxane.

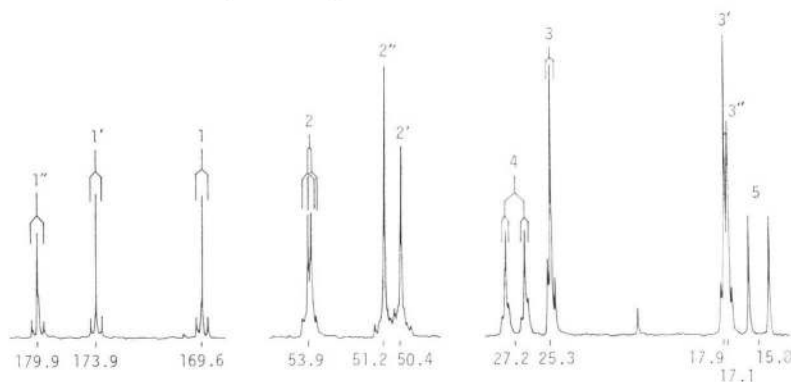
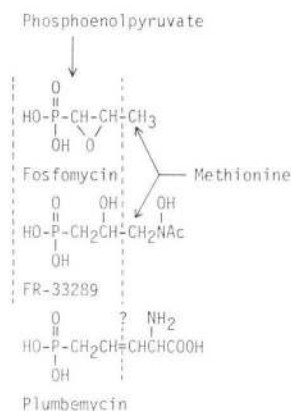


Fig. 4. The structures of representative antibiotics containing a phosphonic acid.



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 (δ_D 1.3) of **II**. Thus, the biosynthetic pathway of **I** is summarized as seen in Fig. 1.

The incorporation of glucose into a similar two carbon unit adjacent to a phosphonic acid moiety was also reported for fosfomycin⁵⁾ and FR-33289⁹⁾ (Fig. 4). Consequently it may be concluded that a common biosynthetic mechanism catalyzes the formation of a C-P bond in

the producing organism. In this regard, the biosynthesis of plumbemycin⁷⁾ which possesses a somewhat different carbon skeleton is worth investigating.

The assignment of the ^{13}C NMR spectrum of **I** was made as follows. C-5 and C-4 were distinguished from the other carbons by their direct coupling to phosphorous ($J_{\text{C-P}} = 93$ and 90 Hz, respectively) and splitting patterns in the off-resonance decoupled spectrum. C-2 was also assigned based on long range coupling ($^3J_{\text{C-P}} = 14$ Hz) with phosphorous. C-1 was identified by ^{13}C - ^{15}N coupling observed in the ^{13}C NMR spectrum of **I** labeled with $[\text{U-}^{13}\text{C}_6]\text{glucose}$. All the carbons in the alanylalanine moiety were assigned by their chemical shift trends and splitting patterns in the off-resonance spectrum.

$\text{CH}_3\text{-P}$ bond formation during the biosynthesis of **I** is believed to proceed *via* the demethyl derivative of phosphinothricin (MP-101)⁸⁾. 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. ITÔ for their technical assistance.

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(Received July 15, 1982)

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