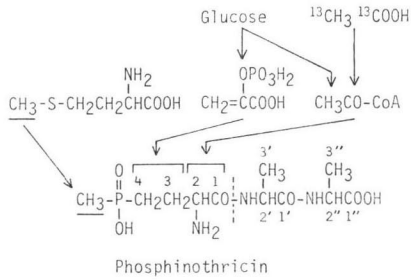
STUDIES ON THE BIOSYNTHESIS OF BIALAPHOS (SF-1293)

1. INCORPORATION OF ^{13}C- AND ^{2}H-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 moiety[3]. 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**.

Fig. 1. Biosynthetic pathway of bialaphos.



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-^{13}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-$^{13}C_2$]acetate. As seen in Fig. 2, only C-2 and C-1 exhibit ^{13}C-^{13}C coupling (J=53 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 $-CH_2-CH_2-P$ unit in **II** was glucose as in the case of 2-aminoethylphosphonic acid[4]. As expected, the ^{13}C NMR spectrum of **I** labeled with [U-$^{13}C_6$]glucose showed an AB-type ^{13}C-^{13}C 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

Fig. 2. The ^{13}C NMR spectrum of bialaphos labeled with sodium [1,2-$^{13}C_2$]acetate in D_2O.
The smaller splitting of C-2 is caused by coupling to phosphorous.

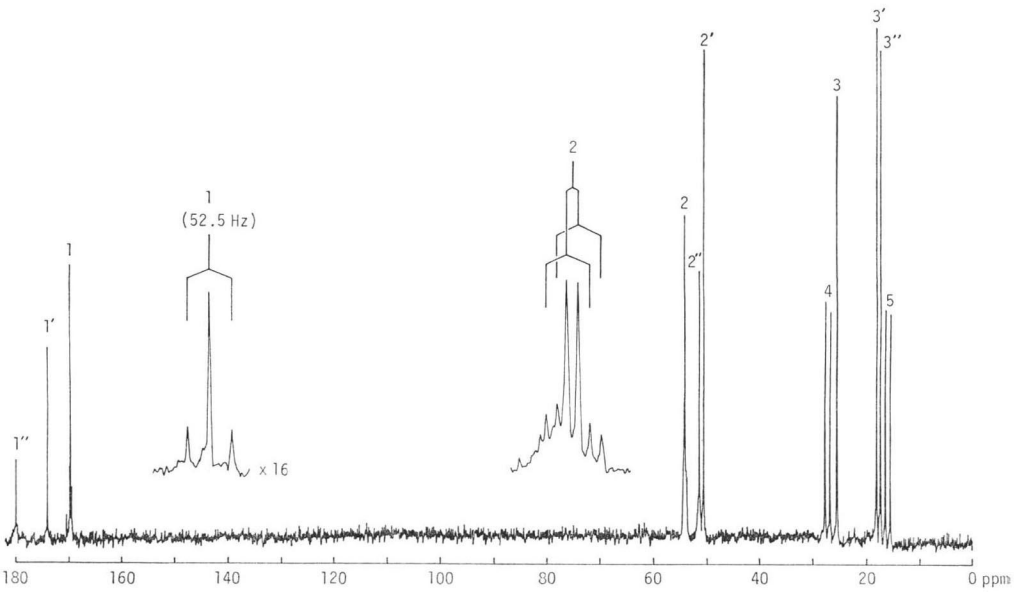


Fig. 3. The ^{13}C NMR spectrum of bialaphos labeled with [U-$^{13}C_6$]glucose in D_2O.
The chemical shifts are expressed in ppm downfield to internal dioxane.

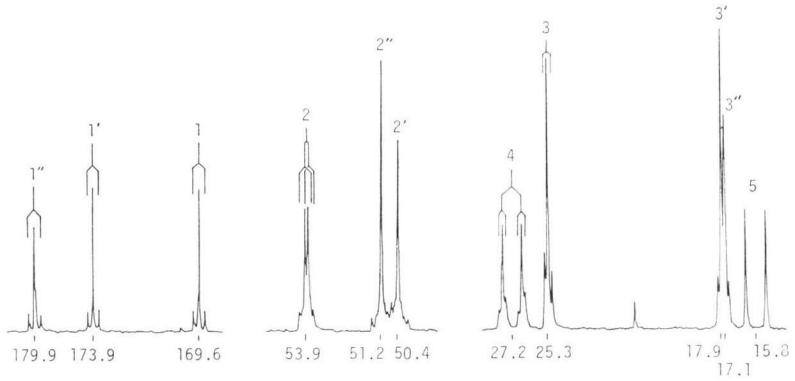
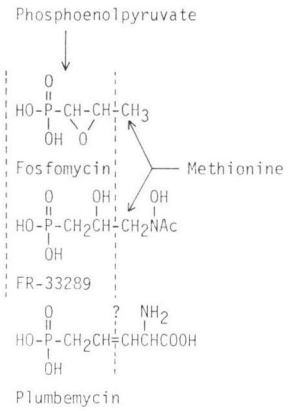


Fig. 4. The structures of representative antibiotics containing a phosphonic acid.



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 fosfomycin[5] and FR-33289[6] (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 plumbemycin[7] 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_{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 ($^3J_{C-P}$=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_3-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.

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