STUDIES ON THE BIOSYNTHESIS OF BIALAPHOS (SF-1293)
4. PRODUCTION OF PHOSPHONIC ACID DERIVATIVES, 2-HYDROXYETHYLPHOSPHONIC ACID, HYDROXYMETHYLPHOSPHONIC ACID AND PHOSPHONOFORMIC ACID BY BLOCKED MUTANTS OF STREPTOMYCES HYGROSCOPICUS SF-1293 AND THEIR ROLES IN THE BIOSYNTHESIS OF BIALAPHOS

Sir:

Bialaphos (formerly called SF-1293) is a metabolite produced by Streptomyces hygroscopicus SF-1293 and is presently being developed as a herbicide. As an approach to improve the production yield of bialaphos and thereby cutting down its production cost, we have been engaged in the biosynthetic studies of this compound. As a result we isolated phosphinic acid derivatives such as MP-101, MP-102, MP-103, MP-104 and MP-105 from the fermentation broth of the parent strain cultivated in the absence of cobalt ion or of a blocked mutant. Some of these metabolites, MP-101, MP-102 and MP-103, in particular, proved to be true biosynthetic intermediates by their very efficient transformation to the final product. MP-103 was converted to MP-102 via MP-101 and methylation of the phosphinic acid moiety in MP-102 took place at the final stage of bialaphos biosynthesis. Thus, it turned out that the reduction of phosphonic acid (H₂O₃P-C⁻) to phosphinic acid (H₂O₂P-C⁻) is the key step for the formation of the C-P-C bond in bialaphos. The detailed mechanism, however, remains as yet to be clarified. Further screening for biosynthetic intermediates resulted in the isolation of phosphonic acid derivatives situated in the earlier biosynthetic steps. We wish to report herein the isolation, structural elucidation and biological conversion of these metabolites, 2-hydroxyethylphosphonic acid, hydroxymethylphosphonic acid and phosphonoformic acid which were produced by cultivation in a similar way as reported previously of blocked mutants (NP-46, NP-213 and NP-221) obtained by treatment with nitrosoguanidine of the parent strain. Although these compounds had been prepared by chemical synthesis, they have never been found in nature.

Throughout the isolation process, fractions containing C-P compounds were detected by ³¹P NMR spectroscopy. Since the chemical shifts of the phosphorus atom in C-P compounds are very characteristic, ³¹P NMR enabled us to detect C-P compounds selectively and efficiently.

2-Hydroxyethylphosphonic Acid (HEP)

Filtered broth (470 ml, pH 3.5) of the mutant NP-46 was passed through a Dowex-50 (H⁺ form) column and then adsorbed on a Dowex-1 (Cl⁻ form) column which was, after washing with water, eluted with 2% NaCl solution. Fractions showing ³¹P signals (δp ca. 20) were combined and purified by Sephadex G-10 column chromatography. The combined fraction rich in a C-P compound was further purified by cellulose column chromatography (BuOH - AcOH - H₂O, 2:1:1) to give the monosodium salt of HEP (210 mg), C₃H₆O₄PNa, FD-MS (m/z) 149 (M⁺H)+, 171 (M⁺Na)+.

The ¹H NMR spectrum of HEP showed the presence of two contiguous methylene units adjacent to a phosphorus atom [δH 3.74 (2H) and 1.89 (2H), JHH = 8.3 Hz, JHP = 18.1 Hz and JHH = 1.89 Hz]. The downfield shift of one of the methylene signals suggested its linkage to a hydroxyl group. This partial structure is supported by the ¹³C and ³¹P NMR spectral data (δc-CH₂-OH 58.4 and P-CH₂ 32.5, Jc-P = 126.2 Hz, δp 19.0). Since this compound showed no doublet proton signal at δH c.7.0 characteristic to phosphinic acid derivatives, it is apparent that the phosphorus is present as a phosphonic acid function in this molecule. Thus the structure of HEP has been established as shown in Fig. 1 and is supported by identity with a synthetic sample.

Hydroxymethylphosphonic Acid (HMP)

This compound was produced by the blocked mutant NP-221. Filtered broth (2 liters) was passed through a Dowex-50 (H⁺ form) column and the effluent was adsorbed on an Amberlite IRA-45 (OH⁻ form) column. After washing with 0.5 N AcOH, HMP was eluted with 0.5 N HCl and a rich fraction was further purified by Sephadex G-10 (developed with H₂O) and Toypearl HW-40 (developed with H₂O) to give 50 mg of HMP, CH₅O₄P, FD-MS (m/z) 135 (M⁺H)+, 171 (M⁺Na)+. The structure of HMP has been determined by ¹H NMR (δH 3.70, JH-P = 7.3 Hz), ¹³C NMR (δc 56.7, Jc-P = 158.4 Hz) and ³¹P NMR (δp 18.0) spectral data.
was confirmed by chemical synthesis according to the method reported by PAGE\(^7\).

Phosphonofbrmic Acid (PF)

Since PF is unstable, all the isolation procedures were made in a cold room at 0\(^\circ\)C. Filtered broth (2 liters) of a mutant NP-213 (formerly called NTG-213) was passed through a column of Dowex-50 (H\(^+\) form) and the emuent was, after neutralization to pH 7.0 with NaOH, adsorbed on a Dowex-1 (Cl\(^-\) form) column which was developed with 0.5% NaCl and then with 3% NaCl solution. The latter fraction was further purified by repeated column chromatography on Sephadex G-10 to afford a pure sample of the trisodium salt of PF (150 mg), CO\(_5\)PNa\(_3\), FD-MS (m/z) 193 (M\(^+\)H\(^+\)), 215 (M\(^+\)Na\(^+\)). Its structure was established by \(^13\)C NMR (\(\delta_c\) 183.6, \(J_{c-p}=223\) Hz) and \(^31\)P NMR (\(\delta_p\) 0) spectroscopy and by direct comparison with a synthetic sample (Sigma Chemical Co.) known as an antiviral agent\(^8\).

It is important to note that PF can be regarded as a potential phosphinic acid derivative since it is very easily converted to phosphoric acid with evolution of CO\(_2\) under acidic condition\(^8\).

Transformation of the Isolated Phosphonic Acid Derivatives to Bialaphos

In order to know whether these metabolites are true biosynthetic intermediates, they were subjected to transformation experiments using appropriate blocked mutants (NP-46, NP-221 and NP-213) and another blocked mutant NP-47 which can not produce any C-P compound (S. IMAI et al. unpublished results). Although HEP, HMP and PF were not as good precursors as MP-103, they were clearly transformed to bialaphos as shown in Table 1. Thus NP-47 converted HEP, HMP and PF, NP-46 changed HMP...
and PF, and NP-221 transformed PF to bialaphos. On the other hand, NP-213 could not utilize these phosphonic acid derivatives as substrates. Based on these experimental results, the reaction sequence for the biosynthesis of bialaphos together with the blocked steps of the mutants are summarized as shown in Fig. 1.

It has been generally accepted that the C-P bond in naturally occurring C-P compounds is formed by intramolecular rearrangement of phosphoenolpyruvate to phosphonopyruvate which is then metabolized to phosphonoalanine and phosphonoacetaldehyde. However, since these phosphonic acids were not utilized at all by another blocked mutant (NP-213), we postulated in a previous paper that phosphonic acid derivatives would not be involved in the biosynthesis of bialaphos and that direct reduction of phosphoenolpyruvate to (hypothetical) phosphonoenolpyruvate precedes the C-P bond formation. Therefore, it is significant that NP-47 could transform phosphonoalanine, phosphonoacetic acid, and aminoethylphosphonic acid, a plausible biological equivalent of phosphonoacetaldehyde to bialaphos (Table 2). With these results in hand, phosphonic acid derivatives are concluded to play important roles for the formation of the phosphinic acids.

It is also interesting to note that HEP was converted to fosfomycin by its producing organism. Therefore, it may be concluded that there exists a common biosynthetic pathway which forms phosphonic acid derivatives possessing a two carbon unit such as HEP from phosphoenolpyruvate.

The mechanism converting phosphonoformic acid to a-keto MP-103 still remains to be clarified.

### Acknowledgment

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

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(Received July 23, 1984)

### References


