Sir:

Compounds possessing a C-P or C-P-C bond are very rare among the natural products and their formation mechanisms remain as yet to be investigated in detail. We report here the first isolation of compounds with a H-P-C bond and their involvement in the C-P-C bond formation.

Bialaphos (formerly called SF-1293) is a tripeptide, phosphinothricylalanylalanylalane (Fig. 1) produced by Streptomyces hygroscopicus SF-1293 and S. viridochromogenes and its use as a herbicide is now being actively investigated. The metabolite is characterized by the presence of a C-P-C bond in the phosphinothricin moiety. During biosynthetic studies on this compound in order to shed light on the formation mechanism of carbon-phosphorous bonds, we found the accumulation in the fermentation broth of two new metabolites named MP-101 and MP-102 containing a H-P-C bond which, to the best of our knowledge, has never been found in nature.

When S. hygroscopicus was cultivated in the absence of Co++, accumulation of a new type of compounds was detected by direct analysis of the broth filtrate by 31P NMR spectroscopy (Fig. 2). Thus, new signals were observed at $\delta_p$ 27.4 and 29.7 in the proton noise decoupled spectra; these chemical shifts are characteristic to $-\text{C-P(O)OH}$ structures. Without proton decoupling these signals collapsed to doublets with very large coupling constants ($J_{H-P} = 525$ Hz) suggesting the direct linkage of the phosphorous to a hydrogen atom. The 31P NMR analysis of the fermentation broth also showed that the amount of MP-102 ($\delta_p$ 29.7) reached its maximum level 4~5 days after the initiation of the fermentation and that it gradually changed to MP-101 ($\delta_p$ 27.4). The maximum yield of MP-101 was obtained after a further 6~8 days.

These two compounds were isolated as follows. The broth filtrate of S. hygroscopicus was passed through a column of Dowex-50 (H+) and the column was washed successively with water. Appropriate washing fractions containing C-P compounds were detected by 31P NMR were passed through a column of Dowex-1 (CH3COO−). After washing the column with water, C-P compounds were eluted with 0.3 N acetic acid. Concentration of the eluate and crystallization of the residue from water gave MP-102 (from the fermentation broth of 6th day) or MP-101 (from the fermentation broth of 8th day). Their physicochemical properties are as follows.

MP-101, C12H20O6N3P, (M+H)+ 310 (FD-MS), $[\alpha]_D^{20} = +28.9^\circ$ (c 1, 1 N HCl), mp 226-228°C (dec.). Found: C 39.02, H 6.62, N 13.70, P 9.95, positive to ninhydrin.

MP-102, C10H16O4N3P, (M+H)+ 168 (FD-MS), $[\alpha]_D^{20} = +76.1^\circ$ (c 1, 1 N HCl), mp 211-213°C (dec.). Found: C 29.20, H 5.99, N 8.38, P 18.60, positive to ninhydrin.
In the $^1$H NMR spectrum taken in D$_2$O, MP-101 showed a very characteristic doublet signal at $\delta$H 7.00 ($J_{H-P}=525$ Hz) due to a H–P(O)OH–C partial structure. The remaining part of MP-101, –CH$_2$CH$_2$CH(NH$_2$)COOH, including its L-configuration was proved by spin decoupling experiments ($\delta$H H-2:4.20, H-3:2.15 and H-4:1.70) and by comparison to 2-amino-4-phosphonobutyric acid$^1$, $^2$-amino-4-phosphonothricin$^3$, and bialaphos (Fig. 3). Consequently, MP-102 is a tripeptide comprising MP-101 and two moles of L-alanine as shown in Fig. 1.

MP-101 and MP-102 did not show any biological activity tested so far except that the former inhibited only the growth of the producing organism at the concentration of 10 $\mu$g/ml.

Both the compounds were quantitatively converted to bialaphos as shown in Table 1 by a mutant (NTG-213) of the producing organism of bialaphos which is blocked at an early step of the biosynthetic pathway. This experimental result clearly shows that the formation of a H–P bond is a prerequisite for the methylation of phos-
Table 1. Transformation of MP-101 and MP-102 to bialaphos by washed mycelia of a mutant (NTG 213) of *Streptomyces hygroscopicus* SF-1293.

<table>
<thead>
<tr>
<th>Precursor added</th>
<th>Concentration (mm)</th>
<th>Amount of bialaphos produced (mm)</th>
<th>Conversion rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MP-101</td>
<td>0.143</td>
<td>0.173</td>
<td>121</td>
</tr>
<tr>
<td></td>
<td>0.057</td>
<td>0.053</td>
<td>93</td>
</tr>
<tr>
<td></td>
<td>0.029</td>
<td>0.034</td>
<td>117</td>
</tr>
<tr>
<td></td>
<td>0.014</td>
<td>0.016</td>
<td>114</td>
</tr>
<tr>
<td>MP-102</td>
<td>0.077</td>
<td>0.071</td>
<td>92</td>
</tr>
<tr>
<td></td>
<td>0.031</td>
<td>0.025</td>
<td>81</td>
</tr>
<tr>
<td></td>
<td>0.016</td>
<td>0.012</td>
<td>75</td>
</tr>
<tr>
<td></td>
<td>0.008</td>
<td>0.008</td>
<td>100</td>
</tr>
</tbody>
</table>

The reaction was carried out in phosphate buffer (pH 6.5, 50 mm) at 28°C for overnight. The amount of bialaphos was determined by biological activity against *Bacillus subtilis*. The transformation product was confirmed to be bialaphos by TLC analysis.

It has been generally accepted that phosphonic acid derivatives such as fosfomycin and FR-332893 are formed by the intramolecular rearrangement of phosphoenolpyruvate. In the case of bialaphos, however, several experimental evidences in our hand (to be published elsewhere) are in favor of the hypothesis that prior to the formation of the C-P bond, the reduction of a phosphate ester (most probably phosphoenolpyruvate) takes place to give a phosphite ester which would then rearrange to form a phosphinic acid intermediate for MP-101 and MP-102.

The purification of the enzyme catalyzing this reduction which may be named "phosphate reductase" is now under way.

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References


