Communications to the editor

THE PLATENOLIDES I AND II AS PRECURSORS OF TURIMYCIN

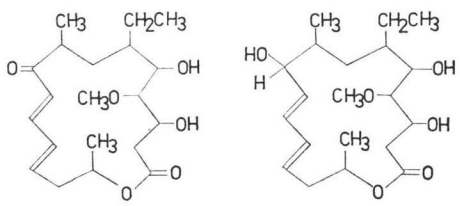
Sir:

Recently we isolated a number of glycosides of the platenolides I (**I**) and II (**II**) from cultures of *Streptomyces hygroscopicus* IMET JA 6599-R 27-158 v which also yields significant amounts of the leucomycin-type antibiotic turimycin[1,2,3]. As was shown by FURUMAI and SUZUKI[4] the platenolides play a role as biosynthetic precursors of the platenomycins whose chemical structures are similar to the turimycin complex[2,4]. Both **I** and **II** are formed by mutant strains of *Streptomyces platensis* subsp. *malvinus* which are incapable of producing the complete 16-membered macrolide antibiotics[5]. Until now there has been no experimental evidence of accumulated platenolides in fermentations of antibiotic-producing strains and, respectively, in cultures of other macrolide-producing organisms.

These results prompted us to first search for the platenolides I (**I**) and II (**II**) (Fig. 1) in the fermentation broth of *S. hygroscopicus* IMET JA 6599-R 27-158 v and, secondly, to demonstrate their possible involvement in the biosynthesis of turimycin by means of blocked mutants of this organism.

We succeeded in isolating **I** and **II** from 96-hour fermentations of this strain using a modified procedure described previously for the isolation of platenolide glycosides[3]. Accordingly, the culture liquid (pH 8.2) was extracted with 0.2 volumes of butylacetate and the turimycin was removed by reextracting the organic layer with 0.05 N H_2SO_4 followed by washing with $NaHCO_3$ (0.05 M). The residue of evaporated butylacetate extract was subsequently separated into a glycoside fraction, a fraction containing the platenolides I and II and several unidentified products by column chromatography on Sephadex LH-20 using methanol as eluent. The crude platenolide fraction was further purified by column chromatography on silica gel H (type 60)/silica gel 60, 0.05 ~ 0.2mm (1: 1, w/w, purchased from Merck, Darmstadt, F.R.G.) using benzene - acetone (5: 3, v/v) as solvent.[3]

Fig. 1. Chemical structures of platenolides I and II.



Pl-I Pl-II

From each 30 liters of butylacetate extract of the culture broth about 50 mg of pure **I** and trace amounts (5 mg) of **II** were obtained. The identification was accomplished by combined use of IR, UV, MS and ^1H NMR spectrometric investigations comparing the data with those reported in the literature[4] or recorded with samples of authentic material. Furthermore, during TLC (Silufol sheets purchased from Kavalier, Č.S.S.R., solvent: benzene - acetone, 5: 3, v/v) our preparations gave Rf values identical with authentic samples (**I**: Rf 0.33, scarlet staining with 1% vanillin in conc. H_2SO_4; **II**: Rf 0.26, violet staining).

In another series of experiments we checked some mutants of *S. hygroscopicus* IMET JA 6599, incapable of producing turimycin, either for the formation of platenolides or for their capacity to convert **I** and **II** into turimycin.

Thus, substantial amounts of platenolide II (**II**) were isolated from cultures of the mutant Sm 5 by means of the procedure described above[3]. On the other hand, feeding of **I** and **II** to cultures of the blocked strains Re 9, UV 53 and NG 11 afforded the production of turimycin as shown in Fig. 2. The formation of antibiotic (turimycin) after feeding **I** and **II** was assayed by the usual cup-plate method on complex medium using *Bacillus subtilis* ATCC 6633 as the test organism. The appearance of an inhibition zone along the agar strips was considered as a sign of turimycin production. The turimycin complex, produced by feeding the platenolides I and II to submerged cultures of the mutant Re 9 on a complex medium, was extracted from the culture broth and identified by comparing its behaviour on TLC with authentic material.

The results provide supporting evidence to the conclusion[4,5] that both **I** and **II** are common

intermediates of biosynthesis not only of the platenomycins but also of other macrolide antibiotics of the leucomycin-carbomycin group. Moreover, in accord with preceding results[3] the finding of the platenolides I and II in fermentations of *S. hygroscopicus* IMET JA 6599-R 27-158 v suggests that in this industrial strain there is no limitation of turimycin biosynthesis by the production of the macrolide ring precursors. This may be due to the greatly improved supply of precursors of the macrocyclic aglycone in this high-producing mutant[6].

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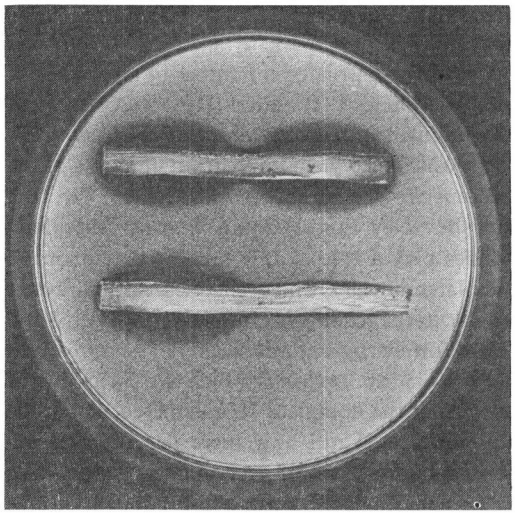
Fig. 2. Production of turimycin by the mutant strain Re 9 after feeding the platenolide I (upper left), sterile culture liquid of platenolides-producing mutant Sm 5 (upper right), platenolide II (lower left) and sterile culture liquid of the non-platenolide-producing mutant UV 53 (lower right).



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