BIOCONVERSION OF 5-O-(4′, 6′-DIDEOXY-3′-C-ACETYL-β-D-XYLOHEXOPYRANOSYL)-PLATENOLIDE-II BY *STREPTOMYCES HYGROSCOPICUS* IMET JA 6599-NG-33-354r

Sir:

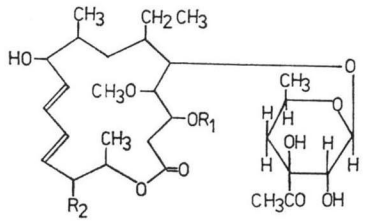
In recent publications, considerable attention has been paid to the acylation steps involved in the biosynthesis of 16-membered macrolide antibiotics[1,2]. To elucidate either the final steps of antibiotic biosynthesis or to manipulate the composition of complex antibiotic mixtures, microbial acylations of both the 3-OH group of the aglycone and the 4-OH group of the mycarose moiety were studied. These efforts prompted us to investigate the bioconversion of the 5-O-(4′, 6′-dideoxy-3′-C-acetyl-β-D-xylohexopyranosyl)-platenolide-II (DDAH-Pl-II, Fig. 1a), isolated previously from the fermentation broth of *Streptomyces hygroscopicus* IMET JA 6599-R 27-158v[3], by *S. hygroscopicus* IMET JA 6599-NG-33-354r, the genetic ancestor of the former strain. In contrast to the former, strain NG-33-354r is known to produce 3-O-acylated derivatives of turimycin (Fig. 1b), a leucomycin-type antibiotic[4].

The present communication deals with the isolation and structure determination of four novel bioconversion products of DDAH-Pl-II formed through either the acetylation or propionylation of the 3-OH group with the concomitant hydroxylation at C-14 (Fig. 1a).

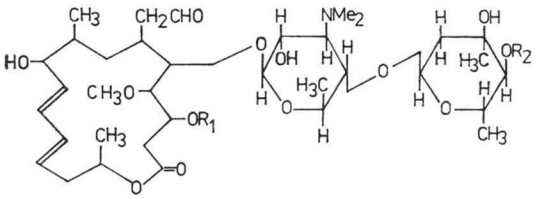
The strain NG-33-354r was grown in 500-ml shake flasks each containing 50 ml of a medium composed as follows: potato starch 3%, D-glucose 0.7%, beet molasses 0.5%, extracts of soya bean meal 2% and of dry yeast 0.3%, $MgSO_4 \cdot 7H_2O$ 0.05%, $MnSO_4 \cdot 2H_2O$ 0.05%, $CaCO_3$ 0.3% and DDAH-Pl-II (1 mg/ml, sterilized separately by filtration in methanolic solution over bacterial filters; the final concentration of methanol in the culture was 1%), pH 6.2. After 96 hours of cultivation on rotary shakers (240 rpm, 5-cm stroke, 25°C) the culture medium was extracted twice with *n*-butylacetate. Fig. 2a displays that control cultures of strain NG-33-354r grown without DDAH-Pl-II lack chromatographically detectable amounts of platenolide glycosides. Detailed investigations on the spectrum of secondary metabolites produced showed that in addition to

Fig. 1.

(a) Chemical structures of bioconversion products of 5-O-(4′,6′-dideoxy-3′-C-acetyl-β-D-xylohexopyranosyl)-platenolide-II (DDAH-Pl-II, R_1: H, R_2: H); 3-O-Pr-DDAH-Pl-II (R_1: COC_2H_5, R_2: H); 3-O-Ac-DDAH-Pl-II (R_1: $COCH_3$, R_2: H); 3-O-Pr-14-OH-DDAH-Pl-II (R_1: COC_2H_5, R_2: OH); 3-O-Ac-14-OH-DDAH-Pl-II (R_1: $COCH_3$, R_2: OH); 14-OH-DDAH-Pl-II (R_1: H, R_2: OH), for the abbreviations see in the text.



(b) Chemical structures of turimycin produced by *S. hygroscopicus* IMET JA 6599-NG-33-354r (R_1: acetyl, propionyl, respectively; R_2: acetyl, propionyl and isovaleryl, respectively).



0.5 mg/ml of turimycin approx. 0.01 mg/ml of shunt metabolites are formed, an amount which is much lower than the level of bioconversion products of DDAH-Pl-II. The turimycin was separated from the products of bioconversion of DDAH-Pl-II by column chromatography on Sephadex LH-20 using methanol as solvent[5]. The glycoside fraction was subsequently chromatographed on Merck TLC plates precoated with silica gel (benzene - acetone, 5: 3, v/v, as solvent). Staining was performed with a solution of 1% vanillin in conc. H_2SO_4 at room temperature. Calculations of the quantitative ratios of spots were based on measurements of light absorption 30 minutes after staining with vanillin reagent using the image analyzer Quantimet 7200 (Image Analyzing Company Ltd., England). A typical chromatogram (Fig. 2b) displayed six different spots: Rf 0.50; 3-O-propionyl-DDAH-Pl-II (3-O-Pr-DDAH-Pl-II, 16%); Rf 0.46; 3-O-acetyl-DDAH-Pl-II (3-O-Ac-DDAH-Pl-II, 26%); Rf 0.42; DDAH-Pl-II (original substance, 35%); Rf 0.32; 3-O-propionyl-14-hydroxyl-DDAH-Pl-

Fig. 2. Thin-layer chromatograms of bioconversion products of DDAH-Pl-II.

(a) Extracts from 96-hour cultures.
A: control culture without DDAH-Pl-II (only turimycin detectable).
B: culture fed with DDAH-Pl-II (mixture of turimycin, DDAH-Pl-II and its bioconversion products).

(b) 1. 3-O-Pr-DDAH-Pl-II; 2. 3-O-Ac-DDAH-Pl-II; 3. DDAH-Pl-II (original substance fed to the medium at zero hour); 4. glycoside fraction obtained by LH-20 chromatography of crude bioconversion mixture (see B); 5. 3-O-Pr-14-OH-DDAH-Pl-II; 6. 3-O-Ac-14-OH-DDAH-Pl-II; 7. 14-OH-DDAH-Pl-II.

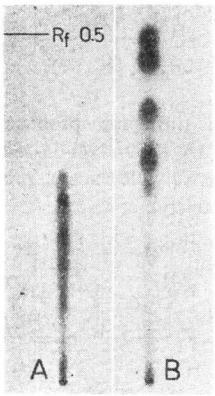


Fig. 2a

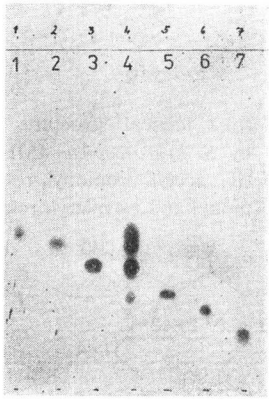


Fig. 2b

II (3-O-Pr-14-OH-DDAH-Pl-II, 15%); Rf 0.28; 3-O-acetyl-14-hydroxyl-DDAH-Pl-II (3-O-Ac-14-OH-DDAH-Pl-II, 5%) and Rf 0.22; 5-O-(4′, 6′-dideoxy-3′-C-acetyl-β-D-xylohexopyranosyl)-14-hydroxyl-platenolide-II (14-OH-DDAH-Pl-II, 3%).

Under the conditions of fermentation used for the investigations, addition of up to 0.4% of sodium butyrate as an effector of 3-O-acylations in macrolide biosynthesis[5] had no effect on the yield and the ratios of bioconversion products. But this result does not necessarily exclude the possibility that there is an inhibitory effect of butyrate on acylations of platenolide glycosides under other conditions. Further investigations are carried out to elucidate this problem in detail.

The components of the bioconversion mixture of DDAH-Pl-II were separated by column chromatography on silica gel H (type 60)/silica gel 60, 0.063 ~ 0.2 mm (1 : 1, w/w) using benzene - acetone (5 : 3, v/v) as the solvent. According to MS data (Table 1), the microbial transformation affected DDAH-Pl-II exclusively within the aglycone part. Accurate mass measurements provided the molecular formulas of the bioconversion products 3-O-Ac-DDAH-Pl-II: M^+ $C_{30}H_{48}O_{11}$; 3-O-Pr-DDAH-Pl-II: M^+ $C_{31}H_{50}O_{11}$; 3-O-Ac-14-OH-DDAH-Pl-II: M^+ $C_{30}H_{48}O_{12}$ and 3-O-Pr-14-OH-DDAH-Pl-II: M^+ $C_{31}H_{50}O_{12}$. Acetylations of 3-O-Ac-DDAH-Pl-II and 3-O-Ac-14-OH-DDAH-Pl-II led to the known tri- and tetra-acetates3,9,2′-O-Ac_3-DDAH-Pl-II and, 3,9,14, 2′-O-Ac_4-14-OH-DDAH-Pl-II[3] respectively. The aglycone fragments of 3-O-Pr-DDAH-Pl-II and

Table 1. Diagnostic *m/e* values of the bioconversion products of DDAH-Pl-II and their acetylated derivatives (measurements with a JEOL JMS-D 100[8]).

1. DDAH-Pl-II; 2. 3-O-Ac-DDAH-Pl-II; 3. 3,9,2′-O-Ac_3-DDAH-Pl-II; 4. 3-O-Pr-DDAH-Pl-II; 5. 9,2′-O-Ac_2-3-O-Pr-DDAH-Pl-II; 6. 14-OH-DDAH-Pl-II; 7. 3-O-Ac-14-OH-DDAH-Pl-II; 8. 3,9,14,2′-O-Ac_4-14-OH-DDAH-Pl-II; 9. 3-O-Pr-14-OH-DDAH-Pl-II; 10. 9,14,2′-O-Ac_3-3-O-Pr-14-OH-DDAH-Pl-II

	m/e values									
	1	2	3	4	5	6	7	8	9	10
M^+	542	584	668	598	682	558	600	726	614	740
M^+-H_2O (−HAc)	524	566	608	580	622	540	582	666	596	680
M^+-H_2O $-COCH_2$	481	523	—	537	—	497	539	—	553	—
Aglycone+O	369	411	453	425	467	—	—	—	—	—
Aglycone+O $-H_2O$(−HAc)	351	393	393	407	—	367	—	451	—	—
Aglycone−O	353	395	437	409	—	369	411	495	425	509
Sugar−O	173	175	215	173	215	173	173	215	173	215
Sugar−O−H_2O	155	155	197	155	197	155	155	197	155	197

3-O-Pr-14-OH-DDAH-Pl-II differed from the corresponding 3-O-acetyl derivatives by 14 mass units suggesting the presence of a propionyl group. Conclusive evidence for the correctness of the structures proposed in Fig. 1 was obtained from C-13 NMR data obtained with a Varian XL-100/15 FT NMR spectrometer[3]. Compared with those of DDAH-Pl-II and 14-OH-DDAH-Pl-II, the C-13 spectra of bioconversion products displayed additional signals due to the acyl group and characteristic acylation shifts of resonances due to C-1, C-2, C-3 and C-4 in full accord with an acylation at C-3-OH.

Discussion

The observed bioconversion of DDAH-Pl-II by the strain NG-33-354r suggests low substrate specificity of the 3-O-acylating enzyme involved in the biosynthesis of turimycin by this mutant[4]. Obviously, this enzyme converts not only the amino-sugar substituted aglycones, *i.e.* the intermediates of turimycin biosynthesis[6], but also the nitrogen-free products of secondary shunt pathways. Therefore, it seems likely that acylation of the 3-OH group may occur during several intermediary steps in the biosynthetic pathway of the antibiotic[7]. The occurrence of 14-hydroxylated derivatives of DDAH-Pl-II among the bioconversion products supports earlier contentions concerning the biogenetic origin of 14-OH-DDAH-Pl-II *via* hydroxylation of DDAH-Pl-II[3]. The lack of conversion within the hexose moiety, on the other hand, demonstrates the 4′, 6′-dideoxy-3′-C-acetyl-D-xylohexopyranose to be the final product rather than an intermediate in the biosynthesis of 3-C-acetylated sugars by *S. hygroscopicus*.

U. GRÄFE
G. REINHARDT
W. SCHADE
P. MÜHLIG
H. THRUM

Zentralinstitut für Mikrobiologie und experimentelle Therapie
der Akademie der Wissenschaften der DDR,
Beutenbergstrasse 11,
DDR-6900 Jena,
German Democratic Republic

L. RADICS

NMR Laboratory,
Central Research Institute of Chemistry
of the Hungarian Academy of Sciences,
H-1525 Budapest, P. O. Box 17, Hungary

(Received April 7, 1980)

References

1) ŌMURA, S.; C. KITAO, H. HAMADA & H. IKEDA: Bioconversion and biosynthesis of 16-membered macrolide antibiotics. X. Final steps in the biosynthesis of spiramycin, using enzyme inhibitor: cerulenin. Chem. Pharm. Bull. 27: 176～183, 1979
2) MIYAGAWA, K.; M. SUZUKI, E. HIGASHIDE & M. UCHIDA: Effect of aspartic acid family amino acids on production of maridomycin. III. Agric. Biol. Chem. 43: 1103～1109, 1979
3) GRÄFE, U.; W. SCHADE, W. IHN, G. REINHARDT, K. DORNBERGER, H. THRUM & L. RADICS: Isolation and structures of nitrogen-free platenolide glycosides. I. The 5-O-(deoxy-3′-C-acetyl-β-D-hexopyranosyl)-platenolides I and II. J. Antibiotics 33: 566～573, 1980
4) HAUPT, I.; H. FRICKE, J. ČERNA & J. RYCHLIK: Effect of leucomycin-like macrolide antibiotic turimycin on ribosomal peptidyltransferase from *Escherichia coli*. J. Antibiotics 29: 1314～1319, 1976
5) KITAO, C.; J. MIYAZAWA & S. ŌMURA: Induction of the bioconversion of leucomycins by glucose and its regulation by butyrate. Agr. Biol. Chem. 43: 833～839, 1979
6) FURUMAI, T.; K. TAKEDA & M. SUZUKI: Studies on the biosynthesis of basic 16-membered macrolide antibiotics, platenomycins. IV. Biosynthesis of platenomycin. J. Antibiotics 28: 789～797, 1975
7) KITAO, C.; H. HAMADA, H. IKEDA & S. ŌMURA: Bioconversion and biosynthesis of 16-membered macrolide antibiotics. XV. Final steps in the biosynthesis of leucomycins. J. Antibiotics 32: 1055～1057, 1979