ISOLATION AND STRUCTURES OF NITROGEN-FREE PLATENOLIDE GLYCOSIDES

II. THE 5-O-(α-MYCAROSYL)- AND 5-O-(3′-DEMETHYL-β-MYCAROSYL)-PLATENOLIDES I AND II

U. GRÄFE, W. SCHADE, W. IHN, G. REINHARDT,
K. DORNBERGER and H. THRUM

Zentralinstitut für Mikrobiologie und experimentelle Therapie der Akademie der Wissenschaften der DDR, DDR-69 Jena, Beutenbergstrasse 11, G.D.R.

and L. RADICS

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

(Received for publication October 16, 1979)

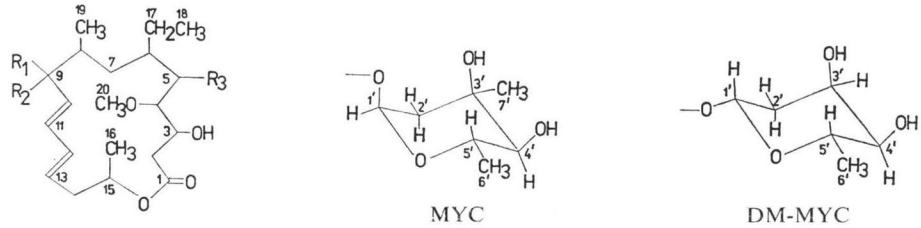
Three novel glycosides of platenolides I and II containing either mycarose (2,6-dideoxy-3-C-methyl-L-ribohexopyranose) or 3-demethyl-mycarose (2,6-dideoxy-L-ribohexopyranose) were isolated as the shunt products of turimycin biosynthesis by an industrial strain of *Streptomyces hygroscopicus* IMET JA 6599. By means of MS, ^{13}C and ^{1}H NMR spectroscopic studies, their structures were assigned as 5-O-(α-mycarosyl)-platenolide I (MYC-Pl-I), 5-O-(α-mycarosyl)-platenolide II (MYC-Pl-II) and 5-O-(3′-demethyl-β-mycarosyl)-platenolide II (DM-MYC-Pl-II). The occurrence of 3-demethyl-mycaroside amongst the shunt metabolites is discussed in terms of its biosynthesis.

Besides the leucomycin-type antibiotic turimycin the industrial strain of *Streptomyces hygroscopicus* IMET JA 6599-R 27-158v-rek.2 was shown to produce a wide spectrum of other secondary metabolites, including diverse nitrogen-free glycosides of platenolides I and II containing 3-C-acetylated hexoses[1]. These results made it tempting to search for platenolide glycosides of the mycarose (2,6-dideoxy-3-C-methyl-L-ribohexopyranose)[2], a common sugar constituent of turimycin. The present communication deals with the isolation and structure elucidation of three novel nitrogen-free glycosides of the platenolides: 5-O-(α-mycarosyl)-platenolide I (MYC-Pl-I), 5-O-(α-mycarosyl)-platenolide II (MYC-Pl-II) and 5-O-(3′-demethyl-β-mycarosyl)-platenolide II (DM-MYC-Pl-II). The structure and stereochemistry of the novel glycosides are displayed in Fig. 1. The altered stereochemistry of glycosidic bond in DM-MYC-Pl-II provides useful information on the tentative biosynthetic pathway of the 2,6-dideoxy-L-ribohexopyranose (digitoxose)[3].

Materials and Methods

The organism, conditions of fermentation, chromatographic procedures, chemical derivatizations and instruments employed in the present study were identical with those described in part I of this series[1].

Fig. 1. Chemical structure of platenolide glycosides.
MYC-Pl-I (R_1/R_2=O; R_3=mycarose (MYC)), MYC-Pl-II (R_1/R_2=H,OH; R_3=mycarose (MYC)) and DM-MYC-Pl-II (R_1/R_2=H,OH; R_3=3-demethyl-mycarose (DM-MYC)).



Results and Discussion

Isolation and Physicochemical Properties of Glycosides

MYC-Pl-I, MYC-Pl-II and DM-MYC-Pl-II were isolated from the butylacetate extract of fermentation broth of the *Streptomyces hygroscopicus* IMET JA 6599-R 27-158v-rek.2 as described previously[1]. Isolation and purification were carried out by column chromatography on Sephadex LH-20 and silica gel. From 3 g of a glycosidic mixture obtained by chromatography on Sephadex LH-20[1] approximately 50 mg of MYC-Pl-I, 150 mg of MYC-Pl-II and 50 mg of DM-MYC-Pl-II were isolated. The physicochemical characteristics of novel glycosides are collected in Table 1. MYC-Pl-I was differentiated from MYC-Pl-II and DM-MYC-Pl-II by its IR absorption (1500~1720 cm^{-1}) and UV band at 280 nm characteristic of the α, β, γ, σ-dienone chromophore.

Structure Elucidation

The structure and stereochemistry of the new products were determined by means of MS, ^1H and ^{13}C NMR spectral analysis. Shown in Table 2 are some diagnostic *m/e* values for MYC-Pl-I, MYC-

Table 1. Physicochemical properties of MYC-Pl-I, MYC-Pl-II and DM-MYC-Pl-II.

	MYC-Pl-I		MYC-Pl-II		DM-MYC-Pl-II	
Appearance	colourless crystals		colourless crystals		colourless crystals	
Formula	$C_{27}H_{44}O_9$		$C_{27}H_{46}O_9$		$C_{26}H_{44}O_9$	
Mol. Wt. (MS)	512.2993 (M^+)		496.3055 (M^+-H_2O)		482.2913 (M^+-H_2O)	
m.p. (°C)	114~116		191~192		109~110	
Elem. Anal. (%)	Found	Calcd.	Found	Calcd.	Found	Calcd.
C	63.11	63.25	62.84	63.01	62.26	62.37
H	8.74	8.65	8.91	9.01	8.58	8.86
IR (KBr, cm^{-1})	960, 1005, 1060, 1120, 1160, 1175, 1250, 1270, 1320, 1360, 1375, 1405, 1455, 1590, 1630, 1675, 1710, 2875, 2925, 2960, 3500, 3600, 3680		985, 1000, 1050, 1070, 1120, 1140, 1155, 1170, 1205, 1250, 1260, 1315, 1370, 1405, 1450, 1590, 1710, 2850, 2930, 2960, 3480, 3600, 3680		990, 1005, 1075, 1125, 1140, 1160, 1205, 1250, 1270, 1315, 1360, 1370, 1400, 1450, 1590, 1710, 2870, 2930, 2960, 3500, 3600, 3680	
UV λ_{max}^{EtOH} (nm)	280		232		232	
Rf, in parentheses: two runs	0.36 (0.55)		0.28 (0.46)		0.14 (0.21)	

Table 2. Diagnostic *m/e* values of the glycosides and their acetylated derivatives.

Fragment	*m/e* values of compound					
	MYC-Pl-I	Ac_2-MYC-Pl-I	MYC-Pl-II	Ac_3-MYC-Pl-II	DM-MYC-Pl-II	Ac_4-DM-MYC-Pl-II
M^+	512	596	514	640	500	668
M^+-H_2O	494	578	496	622	482	—
Aglycone +O	367	409	369	454	369	454
Aglycone −O	351	393	353	437	353	437
Aglycone +O−H_2O (−HAc)	—	—	351	393	351	393
Sugar−O	145	187	145	187	131	215
Sugar−O −H_2O	127	169	127	169	113	—
Sugar−O −$2H_2O$	109	—	109	—	95	—

Table 3. ^{13}C Chemical shift data of glycosides (in ppm, relative to internal TMS, in dilute $CDCl_3$ solutions).

Carbon	MYC-Pl-I	MYC-Pl-II	DM-MYC-Pl-II
C- 1	173.76	174.34	174.44
C- 2	38.53	38.12	38.19
C- 3	67.75	68.28	68.26
C- 4	85.90	85.12	85.78
C- 5	80.63	80.12	79.09
C- 6	38.65	37.32	36.97
C- 7	34.25	32.25	32.07
C- 8	44.89	33.83	33.46
C- 9	202.62	73.42	73.42
C-10	122.28	130.43	130.01
C-11	143.13	134.05	134.45
C-12	131.71	131.47	131.36
C-13	141.09	132.47	132.72
C-14	41.48	41.76	41.84
C-15	68.91	69.15	69.37
C-16	20.27	20.07	20.13
C-17	22.31	21.73	20.13
C-18	11.75	11.93	11.93
C-19	17.66	15.22	14.74
C-20	62.13	61.84	61.81
C- 1′	100.19	99.84	98.96
C- 2′	41.48	41.42	38.50
C- 3′	70.10	70.08	69.03*
C- 4′	76.54	76.64	73.35
C- 5′	66.37	66.34	68.59*
C- 6′	17.76	17.86	17.97
C- 7′	25.51	25.51	—

* Assigments may be interchanged.

Pl-II and DM-MYC-Pl-II and their acetylated derivatives. ^{13}C Chemical shifts data and ^1H parameters are collected in Tables 3 and 4, respectively.

Chemical analysis and accurate mass measurements (Table 2) afforded the molecular formula for the glycosides (see Table 1), the aglycones (*m/e* 367, $C_{20}H_{31}O_6$; *m/e* 369, $C_{20}H_{33}O_6$) and the sugar fragments (*m/e* 145, $C_7H_{13}O_3$; *m/e* 131, $C_6H_{11}O_3$). The similarity of fragmentation patterns with those of other platenolide glycosides[1] showed that platenolide I (Pl-I) and platenolide II (Pl-II) constitute the aglycone part of these molecules, too. Acetylation of MYC-Pl-I, MYC-Pl-II and DM-MYC-Pl-II afforded, respectively, the diacetate (Ac_2-MYC-Pl-I), triacetate (Ac_3-MYC-Pl-II) and tetraacetate (Ac_4-DM-MYC-Pl-II). MS analysis revealed that MYC-Pl-I and MYC-Pl-II contain one, whereas DM-MYC-Pl-II possesses two acylable OH functions within the hexose unit.

Evidence for the presence of a second, non-acylable hydroxyl group in MYC-Pl-I and MYC-Pl-II was provided by the loss of two molecules of water from the hexose fragments (*m/e* 127 and 109) and also by ^1H-^2H exchange experiments which resulted in the expected shift of the *m/e* value of monoacetylated hexose fragment (from 187 to 188).

Reduction with $NaBH_4$ of MYC-Pl-I yielded a 1: 1 mixture of C-9 stereoisomers of MYC-Pl-II with Rf 0.35 and Rf 0.28 on TLC suggesting the identity of the latter compound with the naturally occurring glycoside. After separation by preparative TLC on silica gel G (solvent: benzene - acetone, 5: 3, v/v, two runs) the two components displayed mass spectra which proved identical with that of naturally occurring MYC-Pl-II.

Table 4. ^1H NMR parameters of glycosides*

Proton	MYC-Pl-I	MYC-Pl-II	DM-MYC-Pl-II
C- $2H_A$ ($^2J_{AB}$; $^3J_{2A,3}$)	2.74 (16.1; 10.5)	2.66 (15.1; 10.4)	2.66 (15.0; 10.6)
C- $2H_B$ ($^3J_{2B,3}$)	2.22 (1.5)	2.22 (1.8)	2.19 (1.7)
C- 3H ($^3J_{3,4}$)	3.74 (1.8)	3.69 (1.8)	3.72 (1.7)
C- 4H ($^3J_{4,5}$)	3.00 (9.3)	2.95 (8.7)	2.97 (9.0)
C- 5H ($^3J_{5,6}$)	4.05 (0.6)	4.04 (0.5)	4.05 (0.8)
C- 9H ($^3J_{8,9}$; $^3J_{9,10}$)	—	4.14 (3.9; 8.4)	4.12 (4.3; 9.5)
C-10H ($^3J_{10,11}$)	6.25 (15.0)	5.70 (14.7)	5.68 (14.9)
C-11H ($^3J_{11,12}$)	7.27 (10.2)	6.20 (9.9)	6.21 (9.6)
C-12H ($^3J_{12,13}$)	6.18 (15.0)	6.04 (14.0)	6.05 (14.0)
C-13H ($^3J_{13,14A}$; $^3J_{13,14B}$)	6.07 (4.5; 4.5)	5.58	5.60
C-$14H_A$ ($^2J_{AB}$; $^3J_{13,14B}$)	2.6 (13.0; 10.8)	2.1 (13.5; 10.1)	2.1 (13.5; 11.0)
C-$14H_B$ ($^3J_{14B,15}$)	2.35 (2.6)	2.5 (3.3)	2.5 (3.4)
C-15H ($^3J_{15,16}$)	5.19 (6.5)	5.24 (6.3)	5.25 (6.3)
C-16H (Me)	1.31	1.29	1.25
C-18H (Me) ($^3J_{17,18}$)	0.92 (6.8)	1.00	0.97
C-19H (Me) ($^3J_{8,19}$)	1.17 (7.0)	1.02 (6.7)	0.98 (6.8)
C-20H (OMe)	3.53	3.47	3.47
C-1′H ($^3J_{1',2'A}$; $^3J_{1',2'B}$)	5.16 (3.7; 1.5)	5.17 (3.8; 1.5)	5.04 (9.7; 2.3)
C-$2'H_A$ ($^2J_{AB}$; $^3J_{2'A,3'}$)	1.82 (14.5; —)	1.81 (14.4; —)	1.71 (13.8; 3.1)
C-$2'H_B$ ($^3J_{2',B,3'}$)	2.10 (—)	2.08 (—)	2.10 (3.4)
C-3′H ($^3J_{3',4'}$)	—	—	3.5 (3.4)
C-4′H ($^3J_{4',5'}$)	2.96 (9.6)	2.94 (9.6)	3.24 (9.5)
C-5′H ($^3J_{5',6'}$)	3.78 (6.5)	3.77 (6.1)	3.68 (7.3)
C-6′H (Me)	1.27	1.28	1.29
C-7′H (Me)	1.24	1.23	—

* in $CDCl_3$ solutions. Chemical shift values in ppm, relative to internal TMS. ^1H-^1H coupling constants in Hz as obtained from first order approximations. Mutual interproton couplings are given only once.

The hydrolysis of MYC-Pl-I and MYC-Pl-II with 0.1 N HCl for 3 days at room temperature afforded the free sugar. By MS analysis, the TLC spot with Rf 0.15 was found to be identical with mycarose.

The structure and the stereochemistry of the novel glycosides MYC-Pl-I, MYC-Pl-II and DM-MYC-Pl-II were unambiguously established by detailed analysis of the ^1H and ^{13}C NMR spectra aided by extensive ^1H-^1H double resonance experiments and cross-correlations between ^1H and ^{13}C NMR spectra. The pertinent spectral parameters are displayed in Tables 3 and 4.

Comparison of the ^1H and ^{13}C NMR results with the corresponding values obtained by us for the 5-O-(4′,6′-dideoxy-3′-C-acetyl-β-D-hexopyranosyl)-platenolides I and II[1] clearly showed that MYC-Pl-II and DM-MYC-Pl-II constitute two different glycosides of platenolide II[4], whereas MYC-Pl-I contains platenolide-I[4] as the aglycone. Analysis of the ^1H and ^{13}C NMR parameters, furthermore, revealed that MYC-Pl-I and MYC-Pl-II feature a common sugar moiety, identified, through the ^1H-^1H coupling patterns and ^{13}C chemical shift values, as mycarose[2]. The structure of sugar and the orientation of sugar protons in DM-MYC-Pl-II (*i.e.* C-1′H_{ax}, C-3′H_{eq}, C-4′H_{ax} and C-5′H_{ax}) readily followed from the ^1H NMR results (see Table 4).

The finding of mycarosyl- and 3-C-acetyl hexopyranosyl-platenolides[1] in the fermentation broth

of an industrial strain of the macrolide antibiotic-producing *Streptomyces hygroscopicus* suggests excessive formation of the aglycone precursors giving rise to the induction of alternative pathways of the secondary metabolism. As shown in our preceding work[5], this high-producing *Streptomyces* strain contains an increased level of enzymes that produce the building units of macrocyclic aglycone, a feature which may explain the production of platenolides I and II in large quantities.

Furthermore the 3-demethyl-L-mycarose, previously established as the constituent of a polyene antibiotic (digitoxose)[3], seems to represent a shunt metabolite of mycarose biosynthesis[6,7,8]. As noted above, the relative stereochemistry derived from ^{1}H-^{1}H coupling constants suggests β-configuration for the glycosidic linkage of DM-MYC-Pl-II in contrast to the appropriate derivatives of the mycarose in which the sugar is bound α-glycosidically. Since the mycarose biosynthesis is known to start with the α-anomer of dTDP-glucose[6,7], the phenomenon could be explained perhaps by assuming the β-anomer of dTDP-glucose to be the biogenetic ancestor of 3-demethyl-mycarose, thus giving rise to the formation of β-configurated derivatives.

Acknowledgements

The authors are grateful to Dr. KAJTÁR-PEREDY and Dr. E. BAITZ-GÁCS (NMR Laboratory, Central Research Institute of Chemistry) for their assistance in the compilation of NMR data. The technical assistance of Miss E. STEIN and Mrs. M. RÓZSA is greatly appreciated.

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