ISOLATION AND STRUCTURES OF NITROGEN-FREE PLATENOLIDE GLYCOSIDES

I. THE 5-O-(DEOXY-3′-C-ACETYL-β-D-HEXOPYRANOSYL)-PLATENOLIDES I AND II

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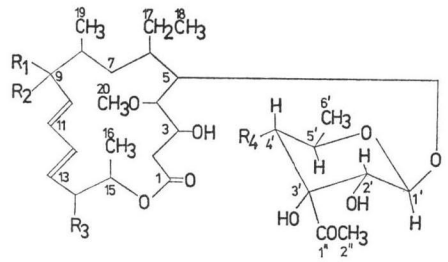
Four novel nitrogen-free glycosides of platenolides I and II were isolated as secondary shunt metabolites of the turimycin biosynthesis from the culture broth of an industrial strain of *Streptomyces hygroscopicus* IMET JA 6599. By spectral (MS, ^{1}H and ^{13}C NMR) studies the structures of the glycosides have been settled as 5-O-(4′,6′-dideoxy-3′-C-acetyl-β-D-hexopyranosyl)-platenolide I (DDAH-Pl-I), 5-O-(4′,6′-dideoxy-3′-C-acetyl-β-D-hexopyranosyl)-platenolide II (DDAH-Pl-II), 5-O-(4′,6′-dideoxy-3′-C-acetyl-β-D-hexopyranosyl)-14-hydroxyl-platenolide II (DDAH-OH-Pl-II) and 5-O-(6′-deoxy-3′-C-acetyl-β-D-hexopyranosyl)-platenolide II (DAH-Pl-II). A fifth glycoside, 5-O-(6′-deoxy-3′-C-acetyl-β-D-hexopyranosyl)-platenolide I (DAH-Pl-I) was identified through its MS data.

The microbial metabolism of differentiation is known to produce a more or less broad spectrum of secondary metabolites (idiolites). Even some industrial strains of antibiotic-yielding streptomycetes were shown to produce smaller amounts of concomitant satellite antibiotics and other secondary shunt products. Interest in the chemical structure of these secondary metabolites is primarily due to the fact that these products furnish information on the alternative pathways of secondary metabolism. Occasionally, the idiolites are novel compounds and as such they may possess unique biological activity or serve as starting materials for biological or chemical transformation into potentially active drugs.

In the present series of two papers we report on our investigations on the by-products of the turimycin biosynthesis, a leucomycin-analogue antibiotic[1], produced by the industrial strain R 27-158v-rek. 2 of *Streptomyces hygroscopicus* IMET JA 6599. The structure elucidation of isolated secondary metabolites has discovered a number of novel, nitrogen-free glycosides of platenolides I and II (Pl-I and Pl-II)[2]. Hitherto, these aglycones were known to occur solely either in the free state or in glycosides of aminosugars[3,4]. This first part deals with the isolation and structure elucidation of four of the novel glycosides containing 3-C-acetylated hexoses, the 5-O-(4′,6′-dideoxy-3′-C-acetyl-β-D-hexopyranosyl)-platenolides I and II (DDAH-Pl-I and DDAH-Pl-II), 5-O-(4′,6′-dideoxy-3′-C-acetyl-β-D-hexopyranosyl)-14-hydroxyl-platenolide II (DDAH-OH-Pl-II) and 5-O-(6′-deoxy-3′-C-acetyl-β-D-hexopyranosyl)-platenolide II (DAH-Pl-II). Additionally, 5-O-(6′-deoxy-3′-C-acetyl-β-D-hexopyranosyl)-platenolide I (DAH-Pl-I) was isolated in trace amounts. Their structures are displayed in Fig. 1.

Fig. 1. Chemical structures of platenolide glycosides.
5-O-(4′,6′-Dideoxy-3′-C-acetyl-β-D-xylohexopyranosyl)-platenolide I (DDAH-Pl-I; R_1/R_2=O, R_3=H, R_4=H),
5-O-(4′,6′-dideoxy-3′-C-acetyl-β-D-xylohexopyranosyl)-platenolide II (DDAH-Pl-II; R_1/R_2=H, OH, R_3=H, R_4=H),
5-O-(4′,6′-dideoxy-3′-C-acetyl-β-D-xylohexopyranosyl)-14-hydroxyl-platenolide II (DDAH-OH-Pl-II; R_1/R_2=H,OH, R_3=OH, R_4=H),
5-O-(6′-deoxy-3′-C-acetyl-β-D-glucohexopyranosyl)-platenolide I (DAH-Pl-I; R_1/R_2=O, R_3=H, R_4=OH),
5-O-(6′-deoxy-3′-C-acetyl-β-D-glucohexopyranosyl)-platenolide II (DAH-Pl-II; R_1/R_2=H, OH, R_3=H, R_4=OH)



Materials and Methods

Organism and conditions of fermentation

The industrial strain R 27-158 v-rek.2 of *Streptomyces hygroscopicus* IMET JA 6599 yielding high amounts of turimycin was cultivated according to KNÖLL *et al.*[5] on a complex medium in 200~2,000 liters fermentors under aerobic conditions for 96 hours.

Chromatography

TLC was carried out using Silufol sheets precoated with silica gel (Kavalier, CSSR); solvent 1: benzene - acetone (5: 3, v/v); solvent 2: benzene - $CHCl_3$ - methanol (6: 4: 1, v/v). For preparative chromatography, 3 cm×100 cm columns were filled with either Sephadex LH-20 in methanol or a mixture of silica gel H (type 60) - silica gel 60, 0.063~0.2 mm (1: 1, w/w, Merck, Darmstadt, F.R.G.) using the solvent system 1.

Instruments

Mass spectrometric analysis was carried out by means of a JEOL JMS-D 100 mass spectrometer at 75 eV using direct inlet system heated to 150~190°C. The molar composition of diganostic peaks in Table 2 was calculated from peak matching experiments. ^1H and ^{13}C NMR spectra were recorded with a Varian Associates model XL-100/15 FOURIER transform spectrometer equipped with Varian Disc Accessory at 100.1 and 25.16 MHz, respectively, $CDCl_3$ as the solvent. TMS was the internal standard. CD spectra were measured in ethanol at room temperature with a Dichrographe II (Roussel-Jouan, France).

Results and Discussion

Isolation of the Glycosides DDAH-Pl-I, DDAH-Pl-II, DAH-Pl-II, DDAH-OH-Pl-II and DAH-Pl-I

After the removal of the basic antibiotic turimycin by reextracting with 0.05 N H_2SO_4 the butylacetate extract of the 96-hours culture medium of strain R 27-158v-rek.2, the usual procedure for neutral lipophilic compounds was employed. The crude glycosides were separated from the residue of evaporated butylacetate layer by Sephadex LH-20 chromatography using methanol as the solvent followed by silica gel chromatography using solvent system 1. The individual fractions were further purified by repeated chromatography on silica gel and recrystallization from benzene - *n*-hexane. Each 3 g of the initial glycoside mixture obtained by chromatography on Sephadex LH-20 yielded about 50 mg of DDAH-Pl-I, about 1.1 g of DDAH-Pl-II, about 250 mg of DAH-Pl-II, about 50 mg of DDAH-OH-Pl-II and trace amounts of DAH-Pl-I as homogeneous substances. Up to 1 mg/ml (dissolved in methanol), they failed to exhibit any antimicrobial activity against *Bacillus subtilis* ATCC 6633 in standard agar plate diffusion tests.

Physicochemical Properties and Structures

The glycosides were differentiated from each other and concomitant components[6] through visuali-

Table 1. Physicochemical properties of DDAH-Pl-I, DDAH-Pl-II, DAH-Pl-II, DDAH-OH-Pl-II and DAH-Pl-I.

Compound	DDAH-Pl-I	DDAH-Pl-II	DAH-Pl-II	DDAH-OH-Pl-II	DAH-Pl-I
Appearance	Colourless crystals	Colourless crystals	Colourless crystals	Colourless crystals	—
m.p.	99~101°C	105~107°C	119~121°C	117~119°C	—
Formula	$C_{28}H_{44}O_{10}$	$C_{28}H_{46}O_{10}$	$C_{28}H_{46}O_{11}$	$C_{28}H_{46}O_{11}$	$C_{28}H_{44}O_{11}$
Mol. Wt. (MS)	540.2908 (M^+)	524.2951 (M^+-H_2O)	540.2920 (M^+-H_2O)	522.2825 (M^+-2H_2O)	556.2915 (M^+)
Elem.Anal.(%)	Found Calcd.	Found Calcd.	Found Calcd.	Found Calcd.	
C	62.12 62.20	61.84 61.97	59.99 60.20	60.01 60.20	—
H	8.50 8.21	8.56 8.54	8.33 8.30	8.10 8.30	—
UV λ_{max}^{EtOH}	280 nm	232 nm	230 nm	230 nm	
IR (KBr, cm^{-1})	970, 1000, 1010, 1025, 1050, 1065, 1080, 1110, 1120, 1160, 1245, 1270, 1310, 1340, 1355, 1380, 1415, 1590, 1630, 1675, 1710, 2925, 2970, 3400~3600	980, 1075, 1080, 1105, 1120, 1140, 1160, 1210, 1270, 1320, 1355, 1380, 1420, 1450, 1590, 1710, 2930, 2960, 3400~3600	980, 1010, 1025, 1075, 1120, 1165, 1210, 1270, 1305, 1350, 1375, 1410, 1450, 1590, 1710, 2870, 2925, 2950, 3400~3600	990, 1035, 1080, 1120, 1160, 1210, 1270, 1310, 1315, 1375, 1420, 1450, 1630, 1710, 2870, 2925, 2960, 3400 ~3600	
TLC (Rf)					
solvent 1	0.45 (0.65)	0.42 (0.60)	0.22 (0.37)	0.17 (0.26)	0.31
solvent 2	0.39	0.32	0.23	0.17	—

(in parentheses: two runs)

zation of TLC plates as red (DDAH-Pl-I), greyish-violet (DDAH-Pl-II and DAH-Pl-II), brown (DAH-Pl-I) and green-blue (DDAH-OH-Pl-II) spots after spraying with 1% vanillin in conc. H_2SO_4 at room temperature. Rf values and physicochemical properties are listed in Table 1.

The structure and stereochemistry of the glycosides as shown in Fig. 1 were elucidated by combined use of MS, ^1H, ^{13}C NMR spectral analysis and chemical modifications. Presented in Table 2 are some diagnostic *m/e* values for DDAH-Pl-I, DDAH-Pl-II, DDAH-OH-Pl-II, DAH-Pl-II and DAH-Pl-I and their derivatives. The ^{13}C chemical shift data and ^1H NMR parameters are listed in Tables 3 and 4, respectively.

Accurate mass measurements (Table 2) and chemical analysis provided the molecular formulas for the glycosides (Table 1), the aglycones (DDAH-Pl-I, DAH-Pl-I and DDAH-OH-Pl-II($-H_2O$): *m/e*367; $C_{20}H_{31}O_6$; (DDAH-Pl-II and DAH-Pl-II: *m/e* 369; $C_{20}H_{33}O_6$) and the sugar fragments (DDAH: *m/e*173; $C_8H_{13}O_4$, DAH: *m/e* 189; $C_8H_{13}O_5$).

Comparison of fragmentation patterns with other macrolide glycosides[2,3,7] as well as consideration of the presumed biosynthetic pathway of turimycin[2,8] have led us to the conclusion that all glycosides contain platenolides as the aglycones.

As expected cleavage of the sugar moieties occurs *via* formation of oxonium ions of the hexose fragments (*m/e* 173 and 189).

Another characteristic feature of the fragmentation is the loss of one acetyl group from the sugar moiety of DDAH-Pl-I and DDAH-Pl-II. Acetylation of DDAH-Pl-I, DDAH-Pl-II, DDAH-OH-Pl-II, DAH-Pl-II and DAH-Pl-I afforded, respectively, the corresponding acetates (Ac_2-DDAH-Pl-I, Ac_3-DDAH-Pl-II, Ac_4-DDAH-OH-Pl-II, Ac_4-DAH-Pl-II and Ac_3-DAH-Pl-I). The mass spectra of Ac_2-DDAH-Pl-I, Ac_3-DDAH-Pl-II and Ac_4-DDAH-OH-Pl-II show (see Table 2) that there is only one

Table 2. Diagnostic MS data of platenolide glycosides and their derivatives.

Fragment	*m/e* obtained from compound					
	DDAH-Pl-I	Ac_2-DDAH-Pl-I	DDAH-Pl-II	Ac_3-DDAH-Pl-II	H_2-DDAH-Pl-II	Ac_4-H_2-DDAH-Pl-II
M^+	540	624	542	668	—	712
M^+-H_2O (−HAc)	522	564	524	608	526	652
M^+-2H_2O ($-H_2O$, $-CH_2CO$)	—	—	—	—	—	—
M^+-COCH_3	497	—	499	—	—	—
M^+-COCH_3, $-H_2O$	—	—	—	—	—	—
Aglycone+O	367	409	369	454	369	454
Aglycone−O	351	393	353	437	353	437
Aglycone+O, $-H_2O$ (−HAc)	—	—	351	394	351	394
Sugar−O	173	215	173	215	175	259
Sugar−O, $-H_2O$	155	197	155	197	157	241

Fragment	*m/e* obtained from compound					
	DAH-Pl-II	Ac_4-DAH-Pl-II	DDAH-OH-Pl-II	Ac_4-DDAH-OH-Pl-II	DAH-Pl-I	Ac_3-DAH-Pl-I
M^+	558	726	558	726	556	682
M^+-H_2O (−HAc)	540	708	540	708	538	—
M^+-2H_2O ($-H_2O$, $-CH_2CO$)	522	666	522	666	—	—
M^+-COCH_3	—	—	—	—	—	—
M^+-COCH_3, $-H_2O$	497	—	497	—	495	—
Aglycone+O	369	454	—	—	367	409
Aglycone−O	353	437	369	495	351	393
Aglycone+O, $-H_2O$ (−HAc)	351	394	367	451	—	—
Sugar−O	189	273	173	215	189	273
Sugar−O, $-H_2O$	171	—	155	197	171	—

acylable hydroxyl function in the hexose unit. As compared with DDAH-OH-Pl-II the MS data of DAH-Pl-II revealed the acylable OH groups to be equally distributed between the sugar and the aglycone (*m/e* 273 and 454) while the former glycoside was found to possess three acylable OH functions in the aglycone and only one in the hexose moiety (*m/e* 495 and 215).

The fragmentation patterns of the sugar moieties of Ac_2-DDAH-Pl-I, Ac_3-DDAH-Pl-II and Ac_4-DDAH-OH-Pl-II disclosed the loss of one molecule of water suggesting the occurrence of an additional nonacylable tertiary hydroxyl group in this part of the molecules. Supporting evidence for this contention was provided by ^1H-^2H-exchange experiments giving rise to the expected shifts of the relevant *m/e* values (from 215 to 216 and, respectively, from 273 to 274, acetylated fragments of hexoses).

Upon reduction with $NaBH_4$, the *m/e* values of the glycosides and the sugar fragments increased for two mass units, except for DDAH-Pl-I whose *m/e* value rose by four units. This suggested the presence of oxo groups within the sugar moieties.

Treatment of either DDAH-Pl-I or DDAH-Pl-II with $NaBH_4$ yielded the same reduction product, 5-O-(4′,6′-dideoxy-3′-C-(1″-hydroxyethyl)-β-D-hexopyranosyl)-platenolide-II (H_2-DDAH-Pl-II). The products of reduction of the glycosides were readily acetylated to the corresponding acetates (Table 2).

Attempts of split off the 4,6-dideoxy-3-C-acetyl-D-hexopyranose unit (DDAH) by acidic hydrolysis of DDAH-Pl-II in aqueous media failed. Treatment of DDAH-Pl-II with 1% HCl in methanol for several days gave a mixture of at least 8 components. From this, trace amounts of the unstable 1-O-methyl glycoside of DDAH could be isolated by column chromatography on silica gel (M^+: *m/e* 204.0989; Calcd.: 204.0998 for $C_9H_{16}O_5$; M^+-COCH_3: *m/e* 161, $C_7H_{13}O_4$), Rf 0.37 (TLC, Silufol, solvent 1).

Conclusive evidence for the structure and stereochemistry was obtained by detailed analysis of the ^1H and ^{13}C NMR spectra. Except for the C–7 methylene group, the 100 MHz ^1H NMR spectra provided direct informations (chemical shift and/or ^1H-^1H coupling) about the sequences of protons within the aglycone moieties of the glycosides. To the partial structures thus derived, the respective ^{13}C resonances in the carbon-13 NMR spectrum could be consistently assigned. In the latter procedure, cross-correlations between the ^1H and ^{13}C spectra established *via* selective ^{13}C-^1H double resonance

Table 3. ^{13}C Chemical shifts* of platenolide glycosides and their aglycones.

Carbon	DDAH-Pl-I	Pl-I	DDAH-Pl-II	Pl-II	DAH-Pl-II	DDAH-OH-Pl-II
C- 1	174.78	173.81	174.35	173.91	174.33	174.11
C- 2	38.57	38.88	38.09	38.27	38.38	38.43
C- 3	67.71	67.31	68.26	67.90	68.44	68.20
C- 4	85.58	85.47	85.12	84.89	85.19	85.23
C- 5	79.44	71.13	79.50	70.88	79.41	79.43
C- 6	39.07	37.96	37.61	35.95	37.71	37.79
C- 7	33.82	32.99	31.78	31.19	31.83	31.59
C- 8	44.93	45.03	33.66	33.30	33.81	33.85
C- 9	203.15	203.52	73.39	72.98	73.56	73.33
C-10	122.76	122.74	130.38	130.13	130.49	132.47
C-11	142.78	142.77	134.15	133.66	134.17	134.07
C-12	131.87	131.93	131.32	130.85	131.38	133.03
C-13	140.64	140.66	132.61	132.53	132.64	133.03
C-14	41.80	41.55	41.82	41.52	41.80	77.71
C-15	68.92	68.86	69.11	68.84	69.19	72.23
C-16	20.27	20.28	20.07	19.91	20.11	17.36
C-17	21.25	22.65	20.36	21.53	20.43	20.40
C-18	11.67	11.46	11.97	11.37	11.94	12.04
C-19	17.64	17.66	14.87	14.69	14.96	15.11
C-20	61.60	61.75	61.41	61.35	61.35	61.47
C- 1′	103.44		103.29		102.52	103.22
C- 2′	72.76		72.64		71.44	72.68
C- 3′	80.65		80.59		83.16	80.71
C- 4′	40.80		40.67		73.03	40.76
C- 5′	67.50		67.42		74.21	67.47
C- 6′	20.60		20.61		17.62	20.63
C- 1″	210.49		210.62		208.15	210.82
C- 2″	24.71		24.78		24.36	24.81

* In ppm, relative to internal TMS, in dilute $CDCl_3$ solutions.

experiments, were of particular assistance. Thus, the sugar carbon resonances were easily separable from those of the aglycones through their significantly longer relaxation times (narrower lines). Comparison of the ^{13}C chemical shift values for the aglycones with published data on 16-membered macrolide antibiotics[8,9] suggested that the methylene carbon resonances appearing at 33.82 ppm in the spectrum of DDAH-Pl-I and at 31.78 ppm in that of DDAH-Pl-II are both due to a CH_2 group in position 7 of a macrolide skeleton and thus the elemental composition of the aglycones of DDAH-Pl-I, DAH-Pl-II and DDAH-Pl-II corresponds to the known structures of platenolides I and II (Pl-I and Pl-II), respectively.

DDAH-OH-Pl-II differed with respect to DDAH-Pl-II by the presence of an additional OH function within the aglycone, the glycoside DAH-Pl-II by an additional OH group within the sugar moiety. In accord with the observed changes in the ^1H-^1H coupling patterns and substitution effects noted at the respective ^{13}C resonances (see Tables 3 and 4) the site of hydroxylation was unambiguously identified as C–14 for DDAH-OH-Pl-II and C–4′ for DAH-Pl-II. Furthermore, the observed few changes in the carbon-13 chemical shift values in the aglycone moieties with respect to those of the free aglycones are readily understood in terms of glycosidation at C–5 (*cf.* the α and β substitution shift at C–4, C–3 and C–5 resonances) and minor alterations in the preferred conformation of the macrolide rings.

Table 4. ^1H NMR parameters of platenolide glycosides.*

Proton	DDAH-Pl-I	DDAH-Pl-II	DAH-Pl-II	DDAH-OH-Pl-II
C–2H$_A$ ($^2J_{AB}$; $^3J_{2A,3}$)	2.73 (16.0; 10.5)	2.64 (15.0; 10.3)	2.63 (15.0; 10.5)	2.68 (15.0; 10.4)
C–2H$_B$ ($^3J_{2B,3}$)	2.23 (1.6)	2.25 (2.0)	2.22 (2.0)	2.27 (2.0)
C–3H ($^3J_{3,4}$)	3.74 (1.4)	3.73 (2.0)	3.73 (2.0)	3.72 (2.0)
C–4H ($^3J_{4,5}$)	3.13 (9.3)	3.09 (8.5)	3.07 (8.5)	3.08 (8.5)
C–5H ($^3J_{5,6}$)	4.14 (0.9)	4.15 (0.5)	4.12 (0.5)	4.13 (0.5)
C–9H ($^3J_{8,9}$; $^3J_{9,10}$)	—	4.12 (4.3; 8.5)	4.13 (4.1; 8.5)	4.13 (4.3; 8.2)
C–10H ($^3J_{10,11}$)	6.27 (15.0)	5.68 (15.0)	5.68 (15.0)	5.70 (15.0)
C–11H ($^3J_{11,12}$)	7.23 (10.3)	6.22 (10.2)	6.20 (10.2)	6.25 (10.0)
C–12H ($^3J_{12,13}$)	6.17 (15.0)	6.01 (15.0)	6.0 (15.0)	6.15 (15.0)
C–13H ($^3J_{13,14A}$; $^3J_{13,14B}$)	6.08 (4.5; 4.5)	5.55 (9.5; 4.5)	5.55 (9.5; 4.5)	5.54 (8.3; —)
C–14H$_A$ ($^2J_{AB}$; $^3J_{14A,15}$)	2.60 (13.0; 10.8)	2.0 (13.0; 10.5)	2.0 (13.0; 11.0)	3.88 (—; 8.4)
C–14H$_B$ ($^3J_{14B,15}$)	2.35 (2.6)	2.4 (3.5)	2.4 (3.5)	—
C–15H ($^3J_{15,16}$)	5.19 (6.5)	5.26 (6.4)	5.26 (6.2)	4.98 (6.4)
C–16H (Me)	1.31	1.29	1.29	1.40
C–18H (Me) ($^3J_{17,18}$)	0.89 (6.8)	0.97 (6.8)	0.97 (6.8)	0.99 (6.8)
C–19H (Me) ($^3J_{8,19}$)	1.17 (7.0)	1.00 (6.8)	1.00 (6.8)	1.03 (6.8)
C–20H (OMe)	3.54	3.49	3.48	3.48
C–1′H ($^3J_{1',2'}$)	4.64 (7.8)	4.69 (7.8)	4.71 (7.8)	4.70 (7.8)
C–2′H	3.69	3.71	3.68	3.70
C–4′H$_A$ ($^2J_{AB}$; $^3J_{4'A,5'}$)	1.65 (14.0; 8.6)	1.65 (14.0; 8.5)	3.50 (—; 9.8)	1.65 (14.0; 8.5)
C–4′H$_B$ ($^3J_{4'B,5'}$)	1.55 (4.5)	1.55 (4.5)	—	1.55 (4.5)
C–5′H ($^3J_{5',6'}$)	3.96 (6.2)	3.96 (6.2)	3.62 (6.2)	4.00 (6.2)
C–6′H (Me)	1.20	1.20	1.29	1.22
C–2″H (Me)	2.26	2.26	2.32	2.28

* 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.

In accord with MS observations (*vide supra*), ^{1}H and ^{13}C NMR spectra disclosed that DDAH-Pl-I, DDAH-Pl-II and DDAH-OH-Pl-II feature a common sugar moiety. Its structure and relative stereochemistry at C–1′, C–2′ and C–5′ could be inferred unambiguously from the combined analysis of the proton and carbon-13 results. The sequence and steric disposition of hydrogen atoms (*i.e.* C–1′ H_{ax}, C–2′ H_{ax}, C–5′ H_{ax}) were readily available from ^{1}H–^{1}H double resonance experiments. The lack of $^{3}J_{2',3'}$ and $^{3}J_{3',4'}$ couplings in the ^{1}H spectra and the occurrence of a quarternary carbon resonance at 80.6 ppm indicated geminal disubstitution at C–3′ of DDAH-Pl-I, DDAH-Pl-II and DDAH-OH-Pl-II with one substituent being an OH group. The nature of the second geminal substituent ($COCH_3$) followed from the occurrence of an oxo carbon resonance at 210.5 ppm (DDAH-Pl-I and DDAH-Pl-II) and an acetyl methyl signal at 24.7 ppm with a corresponding singlet ^{1}H methyl resonance at 2.24 ppm.

This finding is in complete agreement with the easy loss of one acetyl unit in the mass fragmentation process (*vide supra*) and also received further support by reduction experiments (*vide supra*): ^{1}H and ^{13}C NMR of the reduction product H_2-DDAH-Pl-II attested the presence of unchanged macrolide skeleton and the formation of $CH(OH)CH_3$ side chain at C–3′. ^{1}H and ^{13}C NMR data furnished no direct evidence as to the relative stereochemistry at C–3′ and the actual ring conformation ($^{4}C_1$ or $^{1}C_4$) of sugar units. The CD spectra of DDAH-Pl-II, DAH-Pl-II and DDAH-OH-Pl-II showed (+) COTTON effect at 285 nm with amplitudes of $\theta = +794$, $\theta = +1255$ and, respectively, $\theta = +826$ attributable to the oxo group of the side chain at C–3′. According to available CD data on related branched sugars[10] the positive sign suggests S configuration at C–3′. If it is assumed that the sugar ring is in its 'normal' $^{4}C_1$ conformation, this settles the structure and stereochemistry of the carbohydrate moieties as 4,6-dideoxy-3-C-acetyl-D-xylohexopyranose (DDAH) and 6-deoxy-3-C-acetyl-D-glucohexopyranose (DAH).

The structure and the stereochemistry of the novel glycosides are displayed in Fig. 1. Support to this contention seems to be available from the lack of stereospecific four-bond ^{1}H-^{1}H coupling between C–3′ (OH) and the axial C–4′ methylene proton in the ^{1}H NMR spectrum of the glycosides recorded in DMSO-deuteroacetone solution, an observation that suggests equatorial orientation for C–3′ (OH) (*i.e.* $^{4}C_1$ ring conformation)[10]. It must be noted, however, because of the uncertainties in the configurational assignment at the quarternary carbon atoms of branched sugars, the determination of the exact stereochemistry of DDAH and DAH requires further studies.

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