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Oxypregnane-oligoglycosides from the Stems of *Stephanotis mucronata* (Asclepiadaceae)

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Abstract: Four new oxypregnane-oligoglycosides, mucronatosides A (**1**), B (**2**), C (**3**), and D (**4**), together with one known stephanoside E (**5**) were isolated from the stems of *Stephanotis mucronata* (Blanco) Merr.. Their chemical structures were determined on the basis of chemical and extensive spectroscopic methods including one-dimensional and two-dimensional NMR.

Keywords: *Stephanotis mucronata*, oxypregnane-oligoglycosides, mucronatoside.

Stephanotis mucronata (Blanco) Merr. (Asclepiadaceae) is distributed in the south region of China and used in Chinese folk medicine for the treatment of rheumatoid arthritis[1]. In order to isolate the biological active constituents, chemical studies on the constituents of this plant were undertaken and five oxypregnane-oligoglycosides, including four new compounds, mucronatosides A (**1**), B (**2**), C (**3**), and D (**4**), and one known compound, stephanoside E (**5**)[2], were obtained. This paper deals with the structural elucidation of the new compounds.

Mucronatosides A (**1**), $C_{49}H_{78}O_{18}$ [HRFABMS (negative) *m/z*: 953.5147 $[M-H]^-$, Calcd. 953.5110**],** amorphous powder**.** Its IR (KBr, ν) spectrum showed absorption of OH (3453 cm^{-1}) , C=O (1706 cm^{-1}), olefinic (1649 cm^{-1}) and C-O (1195 cm^{-1}) groups. In the ^{13}C NMR spectrum of **1**, the signals due to the aglycone moiety were in good agreement with those of kidjoladinin within the range of glycosylation shifts at C-3 (+6.4 ppm), C-2 (-3.1 ppm), and C-4 (-4.6 ppm)[3]. On acid hydrolysis, **1** afforded kidjoladinin and a sugar mixture. The ^1H and ^{13}C NMR data of **1** suggested the presence of acetyl and tigloyl substituents**,** and their linkages to steroidal nucleus were confirmed by HMBC. In the HMBC spectrum, the carbonyl signal of the acetyl group at δ 171.2 was correlated with the methine proton (H-20) at δ 4.60 (q, *J*=8.0 Hz) on an oxygen-bearing carbon (C-20) at δ 74.0, and that of the tigloyl group at δ 166.2 was correlated with the methine proton (H-12) at δ 4.56 (*dd*, *J*=14.0, 5.5 Hz) on an oxygen-bearing carbon (C-12) at δ 73.4, establishing that the acetyl group is located at C-20 and the tigloyl group at C-12 in **1**. The NMR spectral data also showed that **1** contained three anomeric carbon signals at δ 96.0, 99.5, and 104.2, corresponding to three anomeric protons at δ 4.81 (*dd*,

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1H, *J*=9.5, 2.0 Hz), 4.70 (*d*, 1H, *J*=9.5, 2.0 Hz), and 4.27 (*d*, 1H, *J*=10.0 Hz), respectively, indicating the presence of three sugar units, all with *β*-linkages. Thus, compound **1** was believed to be kidjoladinin 3-*O*-trioside. The HMQC and HMBC experiments allowed the assignments of ^{13}C resonances for the sugar moieties as shown in **Table 1**, starting from the anomeric proton signals at δ 4.81, 4.70, and 4.27, and from two groups of well recognized oxygenated methine protons (δ 3.21, 3.24). These data indicated that the sugar moieties were two *β*-cymarose and one *β*-thevetose[2]. For the sugar linkages, the following long-range correlations were observed in the HMBC spectrum: *δ* 96.0 [C-1′ of the *β*-cymaropyranose] and *δ* 3.80 (*m*, 1H) [H-3 of the aglycone], *δ* 99.5 [C-1″ of the *β*-cymaropyranose] and *δ* 3.21 (*dd*, 1H, *J*=12.0, 3.5 Hz) [H-4′of the *β*-cymaropyranose], *δ* 104.2 [C-1‴ of the *β*-thevetopyranose] and *δ* 3.24 (*dd*, 1H, *J*=12.0, 3.5 Hz) [H-4″ of the *β*-cymaropyranose]. Therefore, the sugar sequence was determined to be D-thevetose-(1→4)-D-cymarose-(1→4)-D-cymarose-C-3 of the aglycone. This is in good agreement with the FABMS spectral fragmentation pattern of **1**, which displayed at *m/z*: 953[M-H]$^-$, 708[M-H-83-162]$^-$, 647[M-H-162-144]$^-$, 503[M-H-162-144-144]$^-$. Consequently, the structure of **1** was established as kidjoladinin 3-*O*-*β*-D-thevetopyranosyl (1→4)-*β*-D-cymaropyranosyl (1 → 4)-*β*-D-cymaropyranoside.

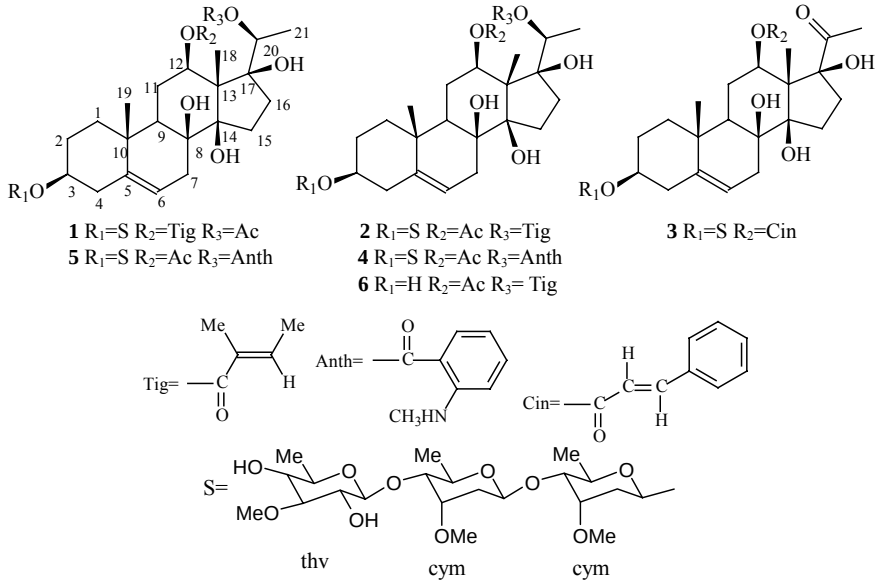
Mucronatosides B (**2**), $C_{49}H_{80}O_{18}$ [HRFABMS (negative) *m/z*: 955.5222 [M-H]$^-$, calcd. 955.5266**],** amorphous powder**.** Its molecular formula was 2H more than that of **1**. Its IR (KBr, ν) spectrum showed signals of OH (3462 cm^{-1}) , C=O (1706 cm^{-1}), olefinic (1649 cm^{-1}), and C-O (1151 cm^{-1}) groups. By comparing ^{13}C and ^{1}H NMR data with those of **1**, **2** was determined to have the same sugar sequence in its oligosaccharide moiety as that of **1**. This suggested that the aglycone part of **2** had 2H more than that of **1**, therefore the molecular formula of the aglycone of **2** was established as $C_{28}H_{44}O_8$. The ^{13}C NMR spectrum showed five quaternary carbons, but there was no olefinic carbon in the steroid nucleus, and the spectral analysis of the aglycone part in **2** revealed a distinct similarity with **1**, except that the double bond between C-5 and C-6 in **2** was reduced. On acid hydrolysis, **2** afforded a new aglycone, named mucronatin (**6**). The ^{13}C NMR assignments of **6** were identical to those of isokidjoladinin[4], except those of C-5 and C-6**,** and these data indicated there was no olefinic bond between C-5 and C-6 in **6**. The ^{1}H and ^{13}C NMR spectra of **2** also suggested the presence of acetyl and tigloyl substituents same as those in **1,** and their linkages to steroidal nucleus were confirmed by HMBC. The HMBC spectrum of compound **2** showed long-range correlations: H-12 of the aglycone at *δ* 4.58 (*dd*, 1H, *J*=13.5, 5.0 Hz) with C-1 of the acetyl group at *δ* 171.2, H-20 of the aglycone at *δ* 4.65 (*q*, 1H, *J*=7.5 Hz) with C-1 of the tigolyl group at *δ* 166.0, suggesting that the acetyl group was linked to C-12 and the tigloyl unit was linked to C-20. Therefore, **6** was determined to be 12-*O*-acetyl-20-*O*-tigoyl dihydrosarcostin (named mucronatin). In comparison with those of **6**, the glycosylation shifts were observed in the ^{13}C NMR spectrum of **2** at C-2 (-3.2ppm), C-3 (+5.6 ppm), and C-4 (-4.1 ppm), which indicated that the sugar moiety was linked to the C-3 hydroxyl group of the aglycone. Based on the above information, the structure of **2** was established as 12-*O*-acetyl-20-*O*-tigoyl dihydrosarcostin (mucronatin) 3-O-*β*-D-thevetopyranosyl (1→4) –*β*-D-cymaropyranosyl (1→4)-*β*-D-cymaropyranoside.

Mucronatosides C (**3**), $C_{51}H_{76}O_{17}$ [HRFABMS (negative) *m/z*: 959.4955 [M-H]$^-$,

calcd. 959.5004**],** amorphous powder**.** Its IR (KBr, ν) spectrum showed absorption of OH (3449 cm^{-1}) , C=O (1711 cm^{-1}), olefinic (1637 cm^{-1}), and C-O (1168 cm^{-1}) groups. On acid hydrolysis, **3** afforded a sugar mixture and kidjolanin, and ^{13}C NMR data of the latter were the same as those in the literature[5]. The ^{1}H NMR and ^{13}C NMR signals due to the sugar moieties of **3** were also superimposable on those of **1**. Therefore, the structure of **3** was established as kidjolanin 3-*O*-*β*-D-thevetopyranosyl (1→4)-*β*-D-cymaropyranosyl (1→4)-*β*-D-cymaropyranoside.

Mucronatosides D (**4**), $C_{52}H_{81}NO_{18}$ [HRFABMS (negative) *m/z*: 1006.5384 $[M-H]^-$, calcd. 1006.5375**],** amorphous powder**.** It showed an intense blue fluorescence in methanol solution. Its IR (KBr, ν) spectrum showed absorption of OH (3442 cm^{-1}), C=O(1732 cm^{-1}), olefinic (1675 cm^{-1}), and C-O (1151 cm^{-1}) groups. By comparing ^{1}H and ^{13}C NMR data with those of **2,** it was shown that **4** possessed the same sugar sequence in its oligosaccharide moiety and the same steroidal nucleus as those in **2**. The NMR spectra of **4** suggested the presence of acetyl and N-methylanthraniloyl substitutions, and their linkages to steroidal nucleus were confirmed by HMBC. The HMBC spectra of **4** showed long-rang correlations: H-12 of the aglycone at *δ* 4.63 (*dd*, 1H, *J*=13.5, 5.0 Hz,) with C-1 of the acetyl group at *δ* 171.2, H-20 of the aglycone at *δ* 4.81 (*q*, 1H, *J*=7.5 Hz) with C-1 of the N-methylanthraniloyl at δ 109.4, suggesting that the acetyl group was linked to C-12 and the N-methylanthraniloyl moiety was linked to C-20. Therefore, the aglycone of **4** was determined to be 12-*O*-acetyl-20-*O*-(N-methyl) anthraniloyl dihydrosarcostin. The sugar linkage at C-3 was determined by the HMBC experiment, which showed a correlation between H-3 of the aglycone at *δ* 3.61 with C-1′ of the inner cymarose at *δ* 95.4. Based on the above information, the structure of **4** was established as 12-*O*-acetyl-20-*O*-(*N*-methyl)anthraniloyl dihydrosarcostin 3-*O*-*β*-D-thevetopyranosyl (1→4)-*β*-D-cymaropyranosyl-(1→4)-*β*-D-cymaropyranoside.

Figure 1 The structures of compounds **1-6**



1 R_1=S R_2=Tig R_3=Ac
5 R_1=S R_2=Ac R_3=Anth

2 R_1=S R_2=Ac R_3=Tig
4 R_1=S R_2=Ac R_3=Anth
6 R_1=H R_2=Ac R_3= Tig

3 R_1=S R_2=Cin

Table 1 ^{13}C NMR Data of compounds **1-6** (in $CDCl_3$, δ ppm)

C	1	2	3	4	5	6	C	1	2	3	4	5
1	38.7	37.7	38.7	37.7	38.7	37.7	Cym					
2	28.9	28.7	28.8	28.7	28.9	31.9	1'	96.0	95.4	96.0	95.4	96.0
3	77.9	76.9	77.8	76.9	77.9	71.3	2'	35.3	35.4	35.4	35.4	35.4
4	38.7	33.6	38.7	33.9	38.7	37.7	3'	76.9	76.8	77.0	76.8	77.0
5	139.6	45.1	140.6	45.1	139.8	45.3	4'	82.5	82.5	82.5	82.6	82.5
6	118.2	24.5	117.6	24.5	118.1	24.4	5'	68.4	68.4	68.5	68.4	68.4
7	34.3	33.8	34.2	33.8	34.9	34.0	6'	17.7	18.1	18.5	18.3	18.4
8	74.0	75.6	74.2	74.6	74.1	75.7	OMe	57.9	57.8	58.0	57.8	57.9
9	43.1	46.2	43.6	46.1	43.2	46.2	Cym					
10	36.8	36.1	37.1	36.1	36.9	36.1	1"	99.5	99.5	99.6	99.5	99.5
11	24.6	23.6	24.1	23.7	24.8	23.7	2"	35.0	35.0	35.0	35.0	35.0
12	73.4	74.1	72.6	74.1	73.5	74.1	3"	76.7	76.7	76.9	76.8	76.9
13	55.9	56.3	57.9	56.4	56.1	56.4	4"	82.4	82.5	82.5	82.5	82.5
14	87.7	87.7	88.0	87.8	87.8	87.8	5"	68.2	68.3	68.3	68.2	68.3
15	31.8	31.6	31.9	31.8	32.2	31.6	6"	18.3	18.4	18.4	18.1	18.2
16	32.9	32.8	33.1	32.8	32.9	32.9	OMe	57.8	57.8	58.0	57.8	57.9
17	87.7	88.1	91.4	88.2	87.8	88.2	Thv					
18	10.2	10.7	9.4	10.9	10.3	10.8	1"'	104.2	104.3	104.3	104.2	104.3
19	18.1	12.4	18.2	12.4	18.2	12.5	2"'	74.5	74.5	74.6	74.5	74.6
20	74.0	73.9	209.2	73.7	73.9	73.9	3"'	85.2	85.2	85.2	85.1	85.2
21	14.9	15.0	27.4	15.2	15.1	15.1	4"'	74.5	74.6	74.2	74.6	74.6
12-*O*-acyl	Tig	Ac	Cin	Ac	Ac	Ac	5"'	71.5	71.6	71.6	71.5	71.6
1	166.2	171.2	165.7	171.2	171.2	171.2	6"'	18.1	17.8	17.8	17.7	17.8
2	128.5	21.7	117.5	21.7	21.7	21.7	OMe	60.7	60.7	60.7	60.7	60.7
3	137.9		145.4									
4	14.5		134.2									
5	12.1		128.1									
6			128.8									
7			130.4									
8			128.8									
9			128.1									
20-*O*-acyl	Ac	Tig		Anth	Anth	Tig						
1	171.2	166.0		109.4	109.6	166.0						
2	21.7	128.5		152.2	152.2	128.6						
3		138.0		111.1	110.9	137.9						
4		14.5		134.9	134.8	14.5						
5		12.2		114.5	114.4	12.2						
6				131.2	131.4							
7				166.8	167.2							
CH3				29.6	29.5							

Abbreviation: Cym=β-cymaropyranosyl, Thv=β-thevetopyranosyl

References

1. Zhenan Bencao New Edit Committee, *Zhenan Bencao New Edit.*, **1975**, 270.
2. K.Yoshikawa, N. Okada, Y. Kann, S. Arihara, *Chem. Pharm. Bull.*, **1996**, *44*, 1790.
3. H. Seto, T. Sasaki, K. Hayashi, H. Mitsuhashi, *Chem. Pharm. Bull.*, **1976**, *24*, 2185.
4. F. Abe, H. Okabe, T. Yamauchi, K. Honda, N. Hayashi, *Chem. Pharm. Bull.*, **1999**, *47*, 869.
5. K. Tsukamoto, K. Hayashi, H. Mitsuhashi, *Chem. Pharm. Bull.*, **1985**, *33*, 2294.

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