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FIVE GLYCOSIDES FROM THE CHINESE DRUG 'TONG-GUANG-SAN': THE STEMS OF *MARSDENIA TENACISSIMA**

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Key Word Index—*Marsdenia tenacissima*; Asclepiadaceae; pregnane glycosides; tenacissosides A–E.

Abstract—Five new glycosides were isolated from the Chinese crude drug 'Tong-guang-san': the stems of *Marsdenia tenacissima* (Roth.) Wight et Arn. (Asclepiadaceae). The structures of tenacissosides A–E were deduced on the basis of chemical and spectral evidence as tenacigenin B-I 3-*O*-β-D-glucopyranosyl-(1→4)-3-*O*-methyl-6-deoxy-β-D-allopyranosyl-(1→4)-β-D-oleandropyranoside, tenacigenin B-II 3-*O*-β-D-glucopyranosyl-(1→4)-3-*O*-methyl-6-deoxy-β-D-allopyranosyl-(1→4)-β-D-oleandropyranoside, tenacigenin B-III 3-*O*-β-D-glucopyranosyl-(1→4)-3-*O*-methyl-6-deoxy-β-D-allopyranosyl-(1→4)-β-D-oleandropyranoside, tenacigenin B-IV 3-*O*-β-D-glucopyranosyl-(1→4)-3-*O*-methyl-6-deoxy-β-D-allopyranosyl-(1→4)-β-D-oleandropyranoside and tenacigenin B-V 3-*O*-β-D-glucopyranosyl-(1→4)-3-*O*-methyl-6-deoxy-allopyranosyl-(1→4)-β-D-oleandropyranoside, respectively.

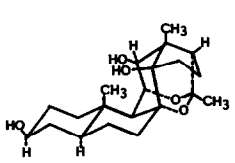
INTRODUCTION

The Chinese crude drug 'Tong-guang-san': the stems of *Marsdenia tenacissima* (Roth.) Wight et Arn. (Asclepiadaceae), native to Southwest China, has been used as an anti-asthmatic and anti-cancer drug. In 1981, tenacigenins A (**9**) and B (**8**) were isolated by Zhou and his co-workers as aglycones of crude glycosides from this drug [1], while in 1979 cissogenin (**10**) and its analogues were isolated from the same plant by Shinghal and his co-workers [2]. There remains a possibility that tenacigenin A (**9**) may be an artifact: since **9** may be formed from **8** by intramolecular ketalization under the acidic or basic conditions employed by Zhou *et al.* In this paper, we describe the isolation and structures of five new glycosides from *M. tenacissima* which consisted of C/D-*cis*-8β,14β-epoxypolyoxypregnane derivatives and a triose having a 3-*O*-methyl-2,6-dideoxy sugars.

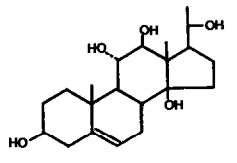
RESULTS AND DISCUSSION

The glycosides were isolated from the methanolic extract of the stems of *M. tenacissima*. The crude glycoside mixture was subjected to repeated silica gel, MCI gel and reversed phase gel column chromatography with various solvent systems and afforded tenacissosides A (**1**), B (**2**), C (**3**), D (**4**) and E (**5**). They showed positive Liebermann–Burchard and Keller–Kiliani reactions [3], which suggested that they were steroidal glycosides with a 2-deoxy sugar moiety in each molecule.

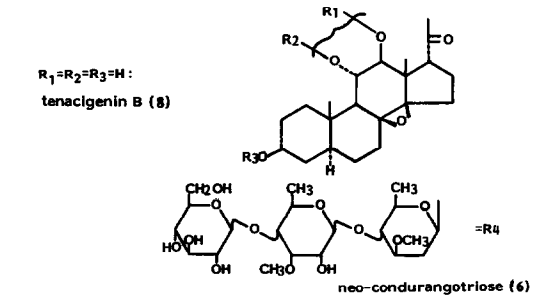
Tenacissoside A (**1**) has a molecular formula $C_{48}H_{74}O_{19}$ on the basis of elemental analysis. The 500 MHz ^1H NMR spectrum of **1** showed the signals due to each two secondary methyl and methoxyl groups of

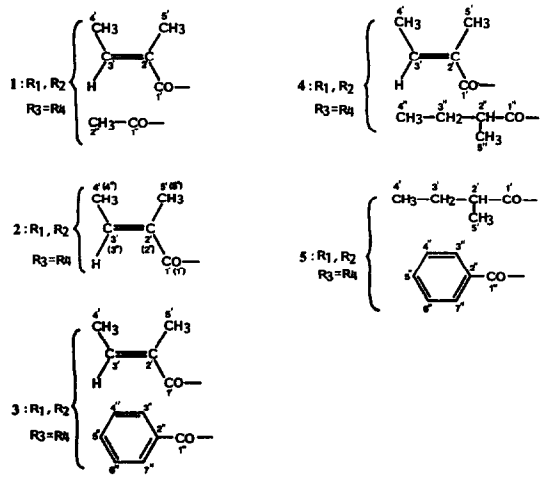


tenacigenin A (9)



cissogenin (10)





*Part 63 in the series "Studies on the Constituents of Asclepiadaceae Plants". For part 62 see Tsukamoto, S., Hayashi, K., Mitsuhashi, H., Snyckers, F. O. and Fourie, T. G. (1985) *Chem. Pharm. Bull.* **33**, 4807.

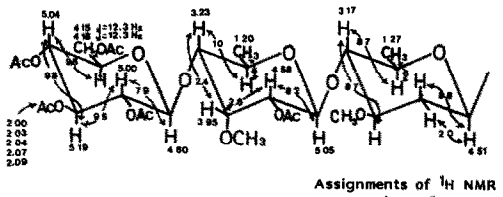
sugar moiety at δ1.26, 1.37, 3.38 and 3.60. In addition, all the glycosidic linkages were revealed to be β from the coupling constants of three anomeric proton signals at δ4.38 (1H, *d*, J=7.7 Hz), 4.57 (1H, *dd*, J=10.1, 2.0 Hz), 4.77 (1H, *d*, J=8.1 Hz). The ^{13}CNMR spectrum of **1** (Table 2) suggested that **1** contained neo-condurangotriose (**6**): β-D-glucopyranosyl-(1→4)-3-*O*-methyl-6-deoxy-β-D-allopyranosyl-(1→4)-D-oleandropyranose [4] as its sugar moiety. This was confirmed by the mild acidic hydrolysis (0.05 N H_2SO_4–50% MeOH, 60°, 60 min) of **1**. It afforded a single sugar component which was identified with authentic neo-condurangotriose (**6**) by TLC analysis. The ^{1}H NMR spectrum of tenacissoside A penta-*O*-acetate (**7**) showed the signal of the methine proton at C-4 of D-oleandrose at δ3.17 as a triplet (7 Hz) which excludes the possibility that this sugar moiety was dregeatriose (β-D-glucopyranosyl-(1→4)-3-*O*-methyl-6-deoxy-β-D-allopyranosyl-(1→4)-D-cymaropyranose) [4]. By acetylation, the C-2 methine signal of 3-*O*-methyl-6-deoxyallose moiety in **1** shifted downfield to δ4.58 (1H, *dd*, J=8.2, 2.8 Hz) and again suggested that the sugar moiety of **1** was **6**. The ^{13}C NMR chemical shifts (Table 2) due to the sugar moieties of the other four glycosides coincided exactly with those of **1** so that they were supposed to contain the same sugar moieties as that of **1**. The ^{13}C NMR spectrum (Table 1), showed that the signals of the aglycone moiety of **1** corresponded to those of tenacigenin B (**8**)-11,12-diester (named tenacigenin B-I), except the aglycone carbons of **1** at C-2 (−2.2 ppm), C-3 (+6.3) and C-4 (−4.2) (Table 1). This indicates that the sugar moiety is linked to the C-3 hydroxyl group of the aglycone. The same glycosidation shifts [5, 6] were observed in the other four glycosides (**2**–**5**) (Table 1) so that they were also shown to bear the sugar moiety at the C-3 hydroxyl group. The ^{1}H NMR spectrum of **1** showed the proton signal of the methine carbon bearing ester group at δ5.00 (1H, *d*, J = 10.3 Hz, 12-CHα) and 5.40 (1H, *t*, J = 10.3 Hz, 11-CHβ) and the signals due to acetyl and tigloyl groups at δ2.18 (3H, *s*, 2″-Me), 1.86 (3H, *br s*, 5′-Me), and 6.76 (1H, *qq*, J = 6.4, 1.7 Hz, 3′-CH). The ^{13}C NMR spectrum of **1** also showed the presence of these two groups (Table 1), so that the aglycone moiety of **1** was deduced to be the acetic and tiglic acid diesters of **8** at O-11 and O-12. Thus the structure of **1** was deduced to be tenacigenin B-I 3-*O*-β-D-glucopyranosyl-(1→4)-3-*O*-methyl-6-deoxy-β-D-allopyranosyl-(1→4)-β-D-oleandropyranoside. However, the order of the acetic and tiglic diester linkage at C-11 and C-12 of **1**, and also those of three diester glycosides (**3**, **4** and **5**) other than **2**, remained unsettled.

Tenacissoside B (**2**) has a molecular formula $C_{51}H_{78}O_{19}$ on the basis of elemental analysis. FABMS gave ion peaks at m/z 1033 [(M+K)$^+$]. The ^{1}H NMR spectrum of **2** showed a pattern similar to that of **1** except for the signals due to the diester groups which were deduced from the signals at δ1.67 (6H, *br s*, 5′- and 5″-Me), 1.69, 1.71 (3H, each *d*, J = 7 Hz, 4′- and 4″-Me), and 6.67, 6.71 (1H, each *qq*, J=7.0, 1.5 Hz, 3′- or 3″-CH) due to two tigloyl groups. The ^{13}C NMR spectrum (Tables 1 and 2) showed the signals due to two molecules of tigloyl group. Other signals except these were similar to those in **1**. In addition, the sugar component of **2**, obtained by mild acidic hydrolysis, was identified as **6**. Therefore, tenacissoside B (**2**) was deduced as 11,12-di-*O*-tigloyl tenacigenin B (named tenacigenin B-II) as its aglycone moiety and the structure of **2** was confirmed as tenacigenin B-II 3-*O*-β-D-glucopyranosyl-(1→4)-3-*O*-methyl-6-deoxy-β-D-allopyranosyl-(1→4)-β-D-oleandropyranoside.

Tenacissoside C (**3**) has a molecular formula $C_{53}H_{76}O_{19}$ on the basis of elemental analysis. FABMS gave ion peaks at m/z 1055 [(M+K)$^+$]. Its ^{1}H NMR spectrum was similar to that of **1**, other than the signals ascribable to the tigloyl and benzoyl groups at δ1.45 (3H, *d*, J=6.1 Hz, 4′-Me), 1.47 (3H, *br s*, 5′-Me), 6.56 (1H, *qq*, J=6.1, 1.2 Hz, 3′-CH), 7.52 (1H, *t*, J=7.3 Hz, 5″-CH), and 7.89 (2H, *d*, J=7.7 Hz, 3″- and 7″-CH). The ^{13}C NMR spectrum (Tables 1 and 2) was also analogous to that of **1** except for the signals due to the diester groups. On mild acidic hydrolysis, **3** afforded one sugar fragment which was identified as **6**. Therefore, the aglycone of **3** was deduced to be tiglic acid, and the benzoic acid diester of **8** at O-11 and O-12 positions, and this aglycone was named tenacigenin B-III. Thus, the structure of tenacissoside C (**3**) was deduced to be tenacigenin B-III 3-*O*-β-D-glucopyranosyl-(1→4)-3-*O*-methyl-6-deoxy-β-D-allopyranosyl-(1→4)-β-D-oleandropyranoside.

Tenacissoside D (**4**) has a molecular formula $C_{51}H_{80}O_{19}$ on the basis of elemental analysis. Its ^{1}H NMR spectrum showed a similar pattern as in **1** except that two diester groups were replaced by tigloyl and 2-methyl butanoyl groups whose signals appeared at δ1.75 (3H, *br s*, 5′-Me), 1.76 (3H, *d*, J=6.7 Hz, 4′-Me), 6.80 (1H, *qq*, J=6.7, 1.8 Hz, 3′-CH), 0.79 (3H, *t*, J=7.3 Hz, 4″-Me) and 0.97 (3H, *d*, J=7.0 Hz, 5″-Me). The ^{13}C NMR spectrum (Tables 1 and 2) was also analogous to that of **1** except for the signals assignable to tigloyl and 2-methyl butanoyl groups. On mild acidic hydrolysis, **4** afforded one sugar fragment which was identified as **6**. So the aglycone moiety of **4** was deduced as tiglic acid and the 2-methyl butanoic acid diester of **8** at the O-11 and O-12 positions, and this aglycone was named tenacigenin B-IV. Therefore, the structure of tenacissoside D (**4**) was deduced to be tenacigenin B-IV 3-*O*-β-D-glucopyranosyl-(1→4)-3-*O*-methyl-6-deoxy-β-D-allopyranosyl-(1→4)-β-D-oleandropyranoside.

Tenacissoside E (**5**) has a molecular formula $C_{53}H_{78}O_{19}$ on the basis of elemental analysis. Its ^{1}H NMR spectrum showed a resemblance to the signal pattern of **1** except that two diester groups were replaced by benzoyl and 2-methyl butanoyl groups whose signals appeared at δ7.42 (2H, *t*, J=7.7 Hz, 4″- and 6″-CH), 7.55 (1H, *t*, J=7.3 Hz, 5″-CH), 7.96 (2H, *d*, J=8.1 Hz, 3″- and 7″-CH), 0.55 (3H, *t*, J=7.3 Hz, 4′-Me) and 0.85 (3H, *d*, J=6.8 Hz, 5′-Me). The ^{13}C NMR spectrum (Tables 1 and 2) also showed an analogy to that of **1** except for the signals due to the diester groups. On mild acidic hydrolysis, **5** afforded one sugar fragment which was identified as **6**. Therefore, the aglycone of **5** was deduced as benzoic acid and the 2-methyl butanoic acid diester of **8** at the O-11 and O-12 position, and this aglycone was named tenacigenin B-V. Consequently,



Assignments of ^{1}H NMR for sugar moiety of **7** (δ in CDCl3)

Table 1. ^{13}C NMR chemical shifts of aglycone moieties

C	1	2	3	4	5	8
1	37.8	37.6	37.8	37.8	38.1	38.3
2	29.8 (−2.2)	29.9 (−2.1)	29.9 (−2.1)	29.5 (−2.5)	29.6 (−2.4)	32.0
3	76.1 (+6.3)	76.0 (+6.2)	76.0 (+6.2)	75.8 (+6.0)	75.9 (+6.1)	69.8
4	35.2 (−4.2)	35.1 (−4.3)	35.2 (−4.2)	35.1 (−4.3)	35.2 (−4.2)	39.4
5	43.9	43.8	43.9	43.8	43.9	44.3
6	27.2*	27.1*	27.2*	27.1*	27.2*	27.0*
7	25.2*	25.0*	25.2*	25.1*	25.2*	25.1*
8	66.7	66.6	66.8	66.7	66.8	66.8
9	51.8	51.7	51.8	51.6	51.7	51.8
10	39.3	39.2	39.4	39.3	39.4	39.4
11	69.1	69.0	69.1	68.9	68.9	68.9
12	75.1	74.9	75.9	74.8	75.9	75.2
13	46.1	46.2	46.4	46.3	46.4	46.2
14	71.7	71.6	71.8	71.6	71.7	71.6
15	32.1	32.0	32.1	32.1	32.1	32.1
16	27.2*	27.1*	27.2*	27.1*	27.1*	27.2*
17	59.7	59.6	59.8	60.0	60.0	60.0
18	12.9†	12.9†	13.1†	13.1†	13.1†	13.2†
19	16.8†	16.8†	16.8†	16.9†	17.0†	17.0†
20	210.2	210.2	210.3	210.0	210.1	209.8
21	30.2	30.2	30.2	29.9	29.9	29.8
	Tig.	Tig.	Tig.	Tig.	Bu.	
1′	167.1	167.2	167.4	167.3	175.7	
2′	129.0	128.9	128.8	128.5	41.4	
3′	138.1	138.0	138.2	138.5	15.4	
4′	12.1‡	11.8‡	11.8‡	12.0‡	11.5‡	
5′	14.2‡	14.1‡	14.0‡	14.2‡	26.2‡	
	Ac.	Tig.	Bnz.	Bu.	Bnz.	
1″	170.6	167.2	166.3	175.4	166.3	
2″	20.4	128.2	129.9	41.4	130.2	
3″		137.8	130.0	15.4	130.2	
4″		11.8§	128.8	11.6	129.0	
5″		14.1§	133.6	26.3	133.8	
6″			128.8		129.0	
7″			130.0		130.2	

δ values (ppm) from internal TMS in C_5D_5N. Glycosidation shifts are given in parentheses. Tig., tigloyl; Ac., acetyl; Bnz., benzoyl; Bu., 2-methyl butanoyl.

*†‡§values in each column may be interchangeable.

the structure of tenacissoside E (**5**) was deduced to be tenacigenin B-V 3-*O*-β-D-glucopyranosyl-(1→4)-3-*O*-methyl-6-deoxy-β-D-allopyranosyl-(1→4)-β-D-oleandropyranoside.

The structures of compounds **1**–**5** differed only in the ester parts of the aglycone moiety. Since we were not able to isolate the glycoside containing tenacigenin A, the possibility that **1** is an artifact still remained. The facile conversion of tenacigenin B type aglycone to tenacigenin A (**9**) by removal of the ester group makes this a difficult problem.

EXPERIMENTAL

Melting points were determined on a Kofler hot stage apparatus and are uncorr. Optical rotations were measured with a JASCO DIP-4 digital polarimeter at room temp. UV spectra were obtained in MeOH with a Shimadzu UV-220 spectrometer, and absorption maxima are given in nm. IR spectra were recorded on a JASCO A-102 spectrometer. FABMS were run on a JEOL JMS-DX 300 mass spectrometer. ^1H NMR spectra were run on JEOL GX-500 (500 MHz) in $CDCl_3$ or in a mixture of $CDCl_3$–CD_3OD (10:1), and ^{13}C NMR spectra on a FX-200 (50 MHz) or FX-90Q (22.5 MHz) spectrometer in C_5D_5N soln with TMS as an internal standard. CC was carried out on Wakogel C-100 (100 mesh), Wakogel C-200 (200 mesh), MCI GEL and Fujigel ODS-Q3 (reversed phase). Prep. HPLC was conducted with a Waters 6000A pump, U6K injector, detected by both $A_{254\,nm}$ and by refractive index, using two 30 cm columns of μ-Bondapak C-18 connected directly (solvent: H_2O–MeOH, 1:3, v/v). TLC was carried out on silica gel (Kiesel gel 60 F_{254}, Merck) and RP-18 F_{254} gel (reversed phase). Abbreviations are used for sugars in this section as follows: cym, cymarose; ole, oleandrose; allo, 6-deoxy-3-*O*-methyl-allose; glu, glucose.

Plant material. Tong-guang-san used in this research was obtained in Hong Kong market and was identified as *Marsdenia tenacissima* by Mr. Zhuang-xin Zhang of Kunming Institute of Botany, Kunming, Yunnan, China.

Extraction and isolation of glycosides. Ground stems of *Marsdenia tenacissima* were dried, pulverized (5.1 kg) and percolated with MeOH at room temp. A dark brown extract (509 g),

Table 2. ^{13}C NMR chemical shifts of sugar moieties

	1	2	3	4	5
Ole C-1	97.5	97.4	97.5	97.3	97.4
2	37.7	37.6	37.8	37.8	37.8
3	79.6	79.4	79.6	79.6	79.7
4	83.1	82.9	83.1	83.1	83.1
5	71.9*	71.6	71.8	71.8	71.9
6	18.2	18.1	18.3	18.2	18.3
3-OMe	57.1	57.0	57.1	57.1	57.2
Allo C-1	101.7	101.6	101.8	101.7	101.8
2	72.5	72.4	72.6	72.6	72.7
3	83.1	82.9	83.1	83.1	83.3
4	83.1	82.9	83.1	83.1	83.3
5	69.4	69.3	69.5	69.4	69.5
6	19.0	18.9	19.0	19.0	19.1
3-OMe	61.6	61.5	61.6	61.6	61.7
Glc C-1	106.4	106.2	106.5	106.3	106.5
2	75.3	75.2	75.4	75.3	75.5
3	78.2	78.0	78.3	78.2	78.3
4	71.7*	71.6	71.8	71.8	71.9
5	78.2	78.0	78.3	78.2	78.3
6	63.0	62.8	63.0	62.9	63.0

δ values (ppm) from internal TMS in C_5D_5N. Ole, β-D-oleandropyranosyl. Allo, 3-*O*-methyl-6-deoxy-β-D-allopyranosyl. Glc, β-D-glucopyranosyl.

*In each column may be interchangeable.

obtained by evaporation of MeOH *in vacuo*, was dissolved in $CHCl_3$ (1.0 l.), and this soln was dropped into hexane (3.2 l.). After the supernatant soln was removed by decantation, the remainder was dried. Finally a yellow crude glycoside mixture (391 g), which showed a positive Liebermann–Burchard and Keller–Kiliani reactions, was obtained. The crude glycosides mixture (40 g) was subjected to CC on silica gel with solvent of $CHCl_3$–MeOH (19:1, v/v) to separate the fraction 3, and rechromatographed on MCI GEL with MeOH to separate the fraction B (7.72 g: a mixture of **1**, **2**, **3**, **4** and **5**). The fraction B was rechromatographed on reverse phase gel (solvent H_2O–MeOH, 1:3) to separate the fraction X (360 mg: mainly **1**) and the fraction Y (1.36 g: a mixture of **2**, **3**, **4** and **5**). The fraction X was rechromatographed on reverse phase gel (solvent H_2O–MeOH, 4:9) to afford chromatographically pure **1** (158.5 mg). The fraction Y was rechromatographed on reverse phase gel (solvent H_2O–MeOH, 1:3) to give **2** (93.3 mg), **5** (86.6 mg) and the fraction Z (362.5 mg: a mixture of **3** and **4**) which was further separated by HPLC (flow rate: 1 ml/min) to give **3** (24.4 mg, R_t 13.8 min) and **4** (70.5 mg, R_t 14.4 min). R_f values of **1**, **2**, **3**, **4** and **5** on reverse phase gel (H_2O–MeOH, 1:3) were 0.44, 0.20, 0.17, 0.17, and 0.15, respectively.

Tenacissoside A (**1**). An amorphous white powder (mp 139.5–140.5°), $[\alpha]_D$ −16.3° (*c* 0.98; MeOH). Found: C, 59.40; H, 7.78. $C_{48}H_{74}O_{19}\cdot H_2O$ requires: 59.24; H, 8.00%. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3300 (OH), 1720 (C=O), 1700 (C=O), 1640 (C=C), 1100 (C–O–C). UV λ_{max}^{MeOH} nm (log ε): 216 (4.06). ^{1}H NMR: δ1.06 (3H, *s*, 19-Me), 1.11 (3H, *s*, 18-Me), 1.26, 1.37 (each 3H, *d*, *J* = 6 Hz, 6-Me of sugar moiety), 1.76 (3H, *d*, *J* = 6.4 Hz, 4′-Me), 1.86 (3H, *br s*, 5′-Me), 2.18 (3H, *s*, 2″-Me), 2.20 (3H, *s*, 21-Me), 2.94 (1H, *br d*, *J* = 7.3 Hz, 17-CHβ), 3.38, 3.60 (each 3H, *s*, 3-*O*-Me of sugar moiety), 3.92 (1H, *t*, *J* = 3.0 Hz, allo-3-CH), 4.38 (1H, *d*, *J* = 7.7 Hz, Glc-1-CH), 4.57 (1H, *dd*, *J* = 10.1, 2.0 Hz, ole-1-CH), 4.77 (1H, *d*, *j* = 8.1 Hz, allo-1-CH), 5.00 (1H, *d*, *J* = 10.3 Hz, 12-CHα), 5.40 (1H, *t*, *J* = 10.3 Hz, 11-CHβ), 6.76 (1H, *qq*, *J* = 6.4, 1.7 Hz, 3′-CH). ^{13}C NMR: see Tables 1 and 2.

Acetylation of **1**. A soln of **1** (10 mg) in pyridine (1 ml) was treated with Ac_2O (0.7 ml) and stirred at room temp. overnight. Usual work-up of the reaction mixture gave tenacissoside A penta-*O*-acetate **7** (12.1 mg) as a syrup.

Penta-O-acetate **7**. ^{1}H NMR: δ1.05 (3H, *s*, 19-Me), 1.10 (3H, *s*, 18-Me), 1.20, 1.27 (each 3H, *d*, *J* = Hz, 6-Me of sugar moiety), 1.75 (3H, *d*, *J* = 7.0 Hz, 4′-Me), 1.76 (3H, *br s*, 5′-Me), 1.85, 2.00, 2.03, 2.04, 2.07, 2.09 (each 3H, *s*, OAc), 2.19 (3H, *s*, 21-Me), 2.93 (1H, *br d*, *J* = 7.6 Hz, 17-CHβ), 3.17 (1H, *t*, *J* = 8.7 Hz, ole-4-CH), 3.23 (1H, *dd*, *J* = 9.5, 2.4 Hz, allo-4-CH), 3.27 (1H, *dq*, *J* = 9, 6 Hz, ole-5-CH), 3.35, 3.48 (each 3H, *s*, 3-*O*-Me of sugar moiety), 3.58 (1H, *m*, 3-CHα), 3.74 (1H, *ddd*, *J* = 10, 6, 3 Hz, Glc-5-CH), 3.90 (1H, *dq*, *J* = 10, 6 Hz, allo-5-CH), 3.95 (1H, *dd*, *J* = 2.8, 2.4 Hz, allo-3-CH), 4.15 (1H, *dd*, *J* = 12, 3 Hz, Glc-6-CH), 4.18 (1H, *dd*, *J* = 12, 4 Hz, Glc-6-CH), 4.51 (1H, *dd*, *J* = 9.8, 2.0 Hz, ole-1-CH), 4.58 (1H, *dd*, *J* = 8.2, 2.8 Hz, allo-2-CH), 4.60 (1H, *d*, *J* = 7.9 Hz, Glc-1-CH), 5.00 (1H, *d*, *J* = 10.1 Hz, 12-CHα), 5.00 (1H, *dd*, *J* = 9.5, 7.9 Hz, Glc-2-CH), 5.04 (1H, *t*, *J* = 9.8 Hz, Glc-4-CH), 5.05 (1H, *d*, *J* = 8.2 Hz, allo-1-CH), 5.19 (1H, *dd*, *J* = 9.8, 9.5 Hz, Glc-3-CH), 5.40 (1H, *t*, *J* = 10.1 Hz, 11-CHβ), 6.75 (1H, *qq*, *J* = 7.0, 2.0 Hz, 3′-CH).

Tenacissoside B (**2**). An amorphous white powder (mp 132.5–134.5°), $[\alpha]_D$ + 11.0° (*c* 1.02; MeOH). Found: C, 59.94; H, 7.73. $C_{51}H_{78}O_{19}\cdot 3/2H_2O$ requires: C, 59.92; H, 7.99%. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3300 (OH), 1700 (C=O), 1640 (C=C), 1100 (C–O–C). UV λ_{max}^{MeOH} nm (log ε): 218 (4.32). FABMS *m/z*: 1033 $[(M+K)^+]$. ^{1}H NMR: δ1.06 (3H, *s*, 19-Me), 1.12 (3H, *s*, 18-Me), 1.29, 1.36 (each 3H, *d*, *J* = 5.8 Hz, 6-Me of sugar moiety), 1.67 (6H, *br s*, 5′- and 5″-Me), 1.69, 1.71 (each 3H, *d*, *J* = 7 Hz, 4′- and 4″-Me), 2.22 (3H, *s*, 21-Me), 2.94 (1H, *br d*, *J* = 7.3 Hz, 17-CHβ), 3.37, 3.60 (each 3H, *s*, 3-*O*-Me of sugar moiety), 3.92 (1H, *br s*, allo-3-CH), 4.37 (1H, *d*, *J* = 7.9 Hz, Glc-1-CH), 4.57 (1H, *br d*, *J* = 9.2 Hz, ole-1-CH), 4.77 (1H, *d*, *J* = 8.2 Hz, allo-1-CH), 5.05 (1H, *d*, *J* = 10.4 Hz, 12-CHα), 5.46 (1H, *t*, *J* = 10.4 Hz, 11-CHβ), 6.67, 6.71 (each 1H, *qq*, *J* = 7.0, 1.5 Hz, 3′- or 3″-CH). ^{13}C NMR: see Tables 1 and 2.

Tenacissoside C (**3**). An amorphous white powder (mp 128.0–132.5°), $[\alpha]_D$ + 16.3° (*c* 0.97; MeOH). Found: C, 61.78; H, 7.65. $C_{53}H_{76}O_{19}\cdot H_2O$ requires: C, 61.49; H, 7.60%. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3300 (OH), 1700 (C=O), 1640 (C=C), 1600 (aromatic C=C), 1100 (C–O–C). UV λ_{max}^{MeOH} nm (log ε): 282 (3.00), 274 (3.00), 225 (4.36). FABMS *m/z*: 1055 $[(M+K)^+]$. ^{1}H NMR: δ1.16 (3H, *s*, 19-Me), 1.19 (3H, *s*, 18-Me), 1.28, 1.36 (each 3H, *d*, *J* = 6.1 Hz, 6-Me of sugar moiety), 1.45 (3H, *d*, *J* = 6.1 Hz, 4′-Me), 1.47 (3H, *br s*, 5′-Me), 2.27 (3H, *s*, 21-Me), 3.00 (1H, *br d*, *J* = 7.3 Hz, 17-CHβ), 3.38, 3.60 (each 3H, *s*, 3-*O*-Me of sugar moiety), 3.91 (1H, *br s*, allo-3-CH), 4.39 (1H, *d*, *J* = 7.8 Hz, Glc-1-CH), 4.57 (1H, *dd*, *J* = 10.4, 2.0 Hz, ole-1-CH), 4.78 (1H, *d*, *J* = 7.9 Hz, allo-1-CH), 5.25 (1H, *d*, *J* = 10.4 Hz, 12-CHα), 5.59 (1H, *t*, *J* = 10.4 Hz, 11-CHβ), 6.56 (1H, *qq*, *J* = 6.1, 1.2 Hz, 3′-CH), 7.38 (2H, *t*, *J* = 7.9 Hz, 4″- and 6″-CH), 7.52 (1H, *t*, *J* = 7.3 Hz, 5″-CH), 7.89 (2H, *d*, *J* = 7.7 Hz, 3″- and 7″-CH). ^{13}C NMR: see Tables 1 and 2.

Tenacissoside D (**4**). An amorphous white powder (mp 137.0–140.5°), $[\alpha]_D$ + 16.4° (*c* 0.94; MeOH). Found: C, 60.98; H, 7.83. $C_{51}H_{80}O_{19}\cdot 1/2H_2O$ requires: C, 60.88; H, 8.11%. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3300 (OH), 1720 (C=O), 1700 (C=O) 1640 (C=C), 1100 (C–O–C). UV λ_{max}^{MeOH} nm (log ε): 221 (4.07). ^{1}H NMR (500 MHz): δ0.79 (3H, *t*, *J* = 7.3 Hz, 4″-Me), 0.97 (3H, *d*, *J* = 7.0 Hz, 5″-Me), 1.05 (3H, *s*, 19-Me), 1.09 (3H, *s*, 18-Me), 1.29, 1.37 (each 3H, *d*, *J* = 6 Hz, 6-Me of sugar moiety), 1.75 (3H, *br s*, 5′-Me), 1.76 (3H, *d*, *J* = 6.7 Hz, 4′-Me), 2.23 (3H, *s*, 21-Me), 2.92 (1H, *br d*, *J* = 7.3 Hz, 17-CHβ), 3.37, 3.60 (each 3H, *s*, 3-*O*-Me of sugar moiety), 3.92 (1H, *t*, *J* = 2.8 Hz, allo-3-CH), 4.38 (1H, *d*, *J* = 7.9 Hz, Glc-1-CH), 4.58 (1H, *dd*, *J* = 9.8, 2.0 Hz, ole-1-

CH), 4.78 (1H, *d*, J = 7.9 Hz, allo-1-CH), 5.04 (1H, *d*, J = 10.1 Hz, 12-CHα), 5.40 (1H, *t*, J = 10.1 Hz, 11-CHβ), 6.80 (1H, *qq*, J = 6.7, 1.8 Hz, 3'-CH). ^{13}C NMR: Tables 1 and 2 .

Tenacissoside E (**5**). An amorphous white powder (mp 140.5–142.5°), $[\alpha]_D$ +26.2° (*c* 1.03; MeOH). Found: C, 61.14; H, 7.61). $C_{53}H_{78}O_{19}\cdot 3/2H_2O$ requires: C, 60.84; H, 7.80%. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400 (OH), 1720 (C=O), 1700 (C=O), 1600 (aromatic C=C), 1100 (C–O–C). UV λ_{max}^{MeOH} nm (log ε): 282 (3.00), 275 (3.07), 231 (4.13). ^{1}H NMR: δ0.55 (3H, *t*, J = 7.3 Hz, 4'-Me), 0.85 (3H, *d*, J = 6.8 Hz, 5'-Me), 1.08 (3H, *s*, 19-Me), 1.16 (3H, *s*, 18-Me), 1.28, 1.37 (each 3H, *d*, J = 6.0 Hz, 6-Me of sugar moiety), 2.28 (3H, *s*, 21-Me), 2.98 (1H, *br d*, J = 7.7 Hz, 17-CHβ), 3.38, 3.60 (each 3H, *s*, 3-*O*-Me of sugar moiety), 3.91 (1H, *br s*, allo-3-CH), 4.39 (1H, *d*, J = 7.7 Hz, Glc-1-CH), 4.58 (1H, *br d*, J = 9.8 Hz, ole-1-CH), 4.78 (1H, *d*, J = 7.7 Hz, allo-1-CH), 5.24 (1H, *d*, J = 10.3 Hz, 12-CHα), 5.53 (1H, *t*, J = 10.3 Hz, 11-CHβ), 7.42 (2H, *t*, J = 7.7 Hz, 4''- and 6''-CH), 7.55 (1H, *t*, J = 7.3 Hz, 5''-CH), 7.96 (2H, d, J = 8.1 Hz, 3''- and 7''-CH). ^{13}CNMR (50 MHz, C_5D_5N): Tables 1 and 2.

Acidic hydrolysis of **1**, **2**, **3**, **4** *and* **5** *with* 0.05 N H_2SO_4–50% MeOH. A soln of **1** (2 mg) in MeOH (3 ml) and 0.2 N H_2SO_4 (1 ml) was kept at 60° for 60 min, then H_2O (3 ml) was added and the whole was concd to 4 ml. The soln was warmed at around 60° for a further 60 min, then the soln was neutralized with $Ba(OH)_2$ aq. soln. The ppts were filtered off and the soln was evaporated to dryness to give a mixture of hydrolysis products of **1**. The other four glycosides, **2**, **3**, **4** and **5**, were acid-hydrolysed by the procedure described above. Every hydrolysate contained one sugar fragment, and it was identified as neo-condurangotriose (**6**) by TLC comparison with an authentic sample. R_f value of **6** was 0.11 ($CHCl_3$–MeOH, 4:1, v/v).

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