Triterpenoid Saponins from *Metadina trichotoma*

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Two new 27-nor-triterpene glycosides, pyrocincholic acid 3β-*O*-β-D-glucopyranosyl-(1 → 4)-β-D-quinovopyranosyl-28-*O*-β-D-glucopyranoside (Metatrichoside A, **1**), pyrocincholic acid 3β-*O*-β-D-glucopyranosyl-(1 → 4)-β-D-quinovopyranosyl-28-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside (Metatrichoside B, **2**), together with pyrocincholic acid 3β-*O*-β-D-quinovopyranosyl-28-*O*-β-D-glucopyranoside (**3**), pyrocincholic acid 3β-*O*-β-D-quinovopyranosyl-28-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside (**4**), quinovic acid 3β-*O*-β-D-quinovopyranoside (**5**), quinovic acid 3β-*O*-β-D-quinovopyranosyl-28-*O*-β-D-glucopyranoside (**6**), quinovic acid 3β-*O*-β-D-glucopyranoside (**7**) and quinovic acid 3β-*O*-β-D-glucopyranosyl-28-*O*-β-D-glucopyranoside (**8**) were isolated from the barks of *Metadina trichotoma*. Their structures were mainly determined by mass spectrometric and 1D and 2D NMR spectroscopic methods. Compound **5** and **6** showed cytotoxic activities towards the A549 non-small-cell lung cancer cell line (IC_{50} = 8.43 and 6.06 μm), and the methanol extract inhibited the activity of cathepsin B with an IC_{50} value of 0.77 μg mL^{-1}.

Key words: Metadina trichotoma, Triterpenoid Saponins, Metatrichoside A, Metatrichoside B

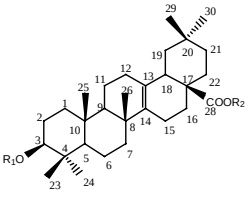
Introduction

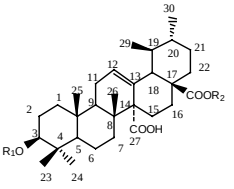
Metadina trichotoma (Zoll. et. Mor.) Bakh. belongs to the Rubiaceae and is the unique species in the genus *Metadina*, which spreads widely in Southwest China, Vietnam, and India *etc.* [1]. Up to now, there has been no report on its chemical constituents. In this paper, we report the structure elucidation of compounds **1** and **2**, and the bioactivity of the methanol extract and compounds **1** – **8**.

Results and Discussion

Compound **1** was found to possess the molecular formula $C_{47}H_{76}O_{17}$ by HR-TOF-MS (*m/z* = 911.5016 [M–1]$^-$, calcd. 911.5004), which was confirmed by ^{13}C and DEPT NMR spectra. The six tertiary methyl groups (δ_H = 1.29, 1.08, 0.80, 1.12, 0.90 and 0.88) observed in the ^{1}H NMR spectrum as well as the six Me signals (δ_C = 28.2, 16.7, 16.7, 20.9, 32.4, 25.0) in the ^{13}C NMR spectrum indicated that compound **1** is a triterpenoid saponin. The data of the aglycone of **1** in the ^{13}C NMR spectra (see Table 1) were consistent with those of pyrocincholic acid [2].

For the sugar moieties, three anomeric protons [δ = 5.37 (d, *J* = 7.5 Hz, 1′-H), 4.80 (d, *J* = 7.6 Hz, 1″-H), 6.31 (d, *J* = 8.1 Hz, 1‴-H)] in the ^{1}H NMR spectrum





1 - 4 **5 - 8**

1	R_1 = β-D-glc(1 → 4)β-D-qui	R_2 = β-D-glc
2	R_1 = β-D-glc(1 → 4)β-D-qui	R_2 = β-D-glc(1 → 6)β-D-glc
3	R_1 = β-D-qui	R_2 = β-D-glc
4	R_1 = β-D-qui	R_2 = β-D-glc(1 → 6)β-D-glc
5	R_1 = β-D-qui	R_2 = H
6	R_1 = β-D-qui	R_2 = β-D-glc
7	R_1 = β-D-glc	R_2 = H
8	R_1 = R_2 = β-D-glc	

Fig. 1. Structures of compounds **1** – **8**.

and the corresponding three anomeric carbon signals [δ = 106.0 (C-1′), 105.0 (C-1″), 95.8 (C-1‴)] in the ^{13}C NMR spectrum suggested that compound **1** contained three sugars. In the HSQC-TOCSY spectrum, the proton signal at δ = 5.37 (d, *J* =7.5 Hz, 1′-H) correlated with the carbon signals at δ = 106.0, 75.3, 78.6, 71.3, 78.3, and 62.8, and the proton signal at δ = 6.31 (d, *J* = 8.1 Hz, 1‴-H) correlated with the carbon signals at δ = 95.8, 74.3, 79.3, 71.7, 78.9, and 62.4 indicat-

	1	2		1	2
H-3	3.29 (dd, 4.0, 11.6)	3.28 (dd, 3.9, 10.9)	Me-30	0.88 (s)	0.86 (s)
Me-23	1.29 (s)	1.27 (s)	H-1′	5.37 (d, 7.5)	5.35 (d, 7.5)
Me-24	1.08 (s)	1.06 (s)	H-1″	4.80 (d, 7.6)	4.80 (d, 7.5)
Me-25	0.80 (s)	0.80 (s)	H-1‴	6.31 (d, 8.1)	5.00 (d, 7.6)
Me-26	1.12 (s)	1.12 (s)	H-1⁗	–	6.22 (d, 8.1)
Me-29	0.90 (s)	0.88 (s)	Me (Qui)	1.58 (d, 6.6)	1.59 (d, 5.6)

Table 1. ^{1}H NMR data of **1** and **2** (500 MHz; in C_5D_5N).

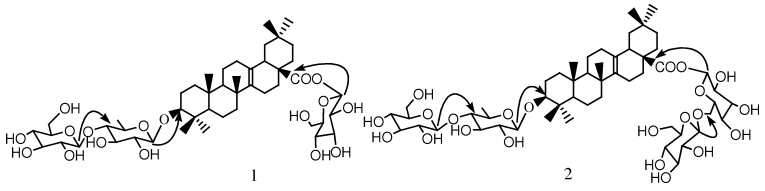


Fig. 2. Key HMBC correlations of compounds **1** and **2**.

ing that compound **1** contains two glucoses. The proton signal at δ = 4.80 (d, J = 7.6 Hz, 1″-H) correlated with the carbon signals at δ = 105.0, 76.5, 78.0, 83.7, 71.8, and 18.6, suggesting that this sugar might be a C-4″ substituted quinovose [3], which was confirmed by the correlation between δ = 83.7 (C-4″) and δ = 5.37 (d, J = 7.5 Hz, 1′-H) in the HMBC spectrum (Fig. 2). In the HMBC spectrum, the correlations between signals at δ = 83.7 (C-4″) and δ = 5.37 (d, J = 7.5 Hz, 1′-H), δ = 89.3 (C-3) and δ = 4.80 (d, J = 7.6 Hz, 1″-H), and δ = 176.7 (C-28) and δ = 6.31 (d, J = 8.1 Hz, 1‴-H) indicated that the link order between the aglycone and the sugars is C-1′ (Glc) → C-4″ (Qui), C-1″ (Qui) → C-3 and C-1‴ (Glc) → C-28 (Fig. 2). The ^{1}H and ^{13}C data of the anomeric protons and carbons indicated that each sugar was a pyranosyl unit in the β configuration.

Thus, the structure of **1** was determined to be pyrocincholic acid 3β-*O*-β-D-glucopyranosyl-(1 → 4)-β-D-quinovopyranosyl-28-*O*-β-D-glucopyranoside, named Metatrichoside A, a new 27-nor-triterpene glycoside.

Compound **2** was found to possess the molecular formula $C_{53}H_{86}O_{22}$ by HR-TOF-MS (m/z = 1073.5526 $[M–1]^-$, calcd. 1073.5532), which was confirmed by ^{13}C and DEPT NMR spectra. The ^{1}H and ^{13}C NMR spectra of **2** indicated that the aglycone was the same as that of **1**, *viz.* pyrocincholic acid [2].

For the sugar moieties of **2**, four anomeric protons [δ = 5.35 (d, J = 7.5 Hz, 1′-H), 4.80 (d, J = 7.5 Hz, 1″-H), 5.00 (d, J = 7.6 Hz, 1‴-H), and 6.22 (d, J = 8.1 Hz, 1⁗-H)] in the ^{1}H NMR spectrum and the corresponding four anomeric carbon signals [δ = 106.0 (C-1′), 105.0 (C-1″), 105.3 (C-1‴), 95.7 (C-1⁗)] in the ^{13}C NMR spectrum suggested that compound **2** contains four sugars. In the HSQC-TOCSY spectrum, the proton signal at δ = 5.35 (d, J = 7.5 Hz, 1′-H) correlated with the carbon signals at δ = 106.0, 75.2, 78.8, 71.1, 78.5, and 62.8, and the proton signal at δ = 5.00 (d, J = 7.6 Hz, 1‴-H) correlated with the carbon signals at δ = 95.7, 74.1, 78.5, 71.7, 78.5, and 69.6, indicating that compound **2** contains two glucoses. The proton signal at δ = 4.80 (d, J = 7.5 Hz, 1″-H) correlated with the carbon signals at δ = 105.0, 76.7, 78.0, 83.6, 72.6, and 18.7, showing that this sugar unit was also a C-4″ substituted quinovose [3], and the proton signal at δ = 6.22 (d, J = 8.1 Hz, 1⁗-H) correlated with the carbon signals at δ = 95.7, 74.1, 78.5, 71.7, 78.5, and 69.6, indicating that this might be a C-6⁗ substituted glucose, which was confirmed by the correlation between δ = 69.6 (C-6⁗) and δ = 5.00 (d, J = 7.6 Hz, 1‴-H) in the HMBC spectrum (Fig. 2). In the HMBC spectrum, the correlations between signals at δ = 83.6 (C-4″) and δ = 5.35 (d, J = 7.5 Hz, 1′-H), δ = 89.3 (C-3) and δ = 4.80 (d, J = 7.5 Hz, 1″-H), δ = 69.6 (C-6⁗) and δ = 5.00 (d, J = 7.6 Hz, 1‴-H), and δ = 176.9 (C-28) and δ = 6.22 (d, J = 8.1 Hz, 1⁗-H) indicated that the link order between the aglycone and the sugars is C-1′ (Glc) → C-4″ (Qui), C-1″ (Qui) → C-3, C-1‴ (Glc) → C-6⁗ (Glc), and C-1⁗ (Glc) → C-28 (Fig. 2). The ^{1}H and ^{13}C data of anomeric protons and carbons show that each sugar is a pyranosyl unit in the β configuration.

In this way the structure of **2** was determined to be pyrocincholic acid 3β-*O*-β-D-glucopyranosyl-(1 → 4)-β-D-quinovopyranosyl-28-*O*-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranoside, named Metatrichoside B, a new 27-nor-triterpene glycoside.

Six known compounds **3**–**8** were determined to be pyrocincholic acid 3β-*O*-β-D-quinovopyranosyl-28-*O*-β-D-glucopyranoside (**3**) [3], pyrocincholic acid 3β-*O*-β-D-quinovopyranosyl-28-*O*-β-D-

	1	**2**			**1**			**2**	
1	38.4	Glc($1' \rightarrow 4''$)	38.6	Glc($1' \rightarrow 4''$)	16	23.8	–	24.6	$1''''$ 105.3
2	26.8	$1'$ 106.0	27.0	$1'$ 106.0	17	45.8	–	45.8	$2''''$ 75.2
3	89.3	$2'$ 75.3	89.3	$2'$ 75.2	18	39.6	–	39.6	$3''''$ 78.3
4	39.8	$3'$ 78.6	39.8	$3'$ 78.8	19	41.6	–	41.6	$4''''$ 71.8
5	55.9	$4'$ 71.3	56.0	$4'$ 71.1	20	30.7	–	30.7	$5''''$ 78.0
6	18.7	$5'$ 78.3	19.1	$5'$ 78.5	21	34.4	–	34.6	$6''''$ 62.8
7	39.8	$6'$ 62.8	39.8	$6'$ 62.8	22	31.7	Glc($1''' \rightarrow 28$)	31.4	Glc($1'''' \rightarrow 28$)
8	38.1	Qui($1'' \rightarrow 3$)	38.2	Qui($1'' \rightarrow 3$)	23	28.2	$1'''$ 95.8	28.3	$1'''$ 95.7
9	56.5	$1''$ 105.0	56.7	$1''$ 105.0	24	16.7	$2'''$ 74.3	16.6	$2'''$ 74.1
10	37.3	$2''$ 76.5	37.3	$2''$ 76.7	25	16.7	$3'''$ 79.3	16.8	$3'''$ 78.5
11	18.1	$3''$ 78.0	18.2	$3''$ 78.0	26	20.9	$4'''$ 71.7	21.0	$4'''$ 71.7
12	32.2	$4''$ 83.7	32.2	$4''$ 83.6	–	–	–	–	–
13	130.3	$5''$ 71.8	130.4	$5''$ 72.6	28	176.7	$5'''$ 78.9	176.9	$5'''$ 78.5
14	137.0	$6''$ 18.6	137.1	$6''$ 18.7	29	32.4	$6'''$ 62.4	32.5	$6'''$ 69.6
15	21.1	–	21.1	Glc($1''' \rightarrow 6''''$)	30	25.0	–	25.1	–

Table 2. ^{13}C NMR data of **1** and **2** (125 MHz; in C_5D_5N).

glucopyranosyl-(1 → 6)-β-D-glucopyranoside (**4**) [4], quinovic acid 3β-*O*-β-D-quinovopyranoside (**5**) [5], quinovic acid 3β-*O*-β-D-quinovopyranosyl-28-*O*-β-D-glucopyranoside (**6**) [5], quinovic acid 3β-*O*-β-D-glucopyranoside (**7**) [5], quinovic acid 3β-*O*-β-D-glucopyranosyl-28-*O*-β-D-glucopyranoside (**8**) [6], by the same method as described above.

Compounds **1**–**8** and the methanol extract were tested for *in vitro* activity in CCLT and CAT-B assays. Compounds **5** and **6** showed cytotoxic activity towards the A549 non-small-cell lung cancer cell line (IC_{50} = 8.43 and 6.06 μm) and the methanol extract inhibited the activity of cathepsin B with an IC_{50} value of 0.77 μg mL^{-1}.

Experimental Section

General

Melting points were obtained on a SEISAKUSHO-1240 micromelting point apparatus and are uncorrected. Optical rotations were taken on a Horiba SEAP-300 polarimeter. ^{1}H, ^{13}C NMR and 2D NMR spectra were recorded on a Bruker AM-400 or a DRX-500 NMR spectrometer with TMS as internal standard (δ in ppm, J in Hz). MS data were obtained on a VG Autospec-3000 spectrometer.

Plant materials

The barks of *M. trichotoma* were collected from the Xishuangbanna district, Yunnan Province, People's Republic of China, in September 2002. The specimen was identified by Associate Prof. Wang Hong at Xishuangbanna Tropic Botanical Garden, the Chinese Academy of Sciences.

Extraction and isolation

The air-dried and powdered barks (9.0 kg) of *M. trichotoma* were extracted at r. t. three times with MeOH and the solution was then concentrated under reduced pressure. The concentrated MeOH extract (1668 g) was dissolved in hot water and extracted with petroleum ether, AcOEt and *n*-BuOH, respectively, to afford 19 g petroleum ether extract, 85 g AcOEt extract, 780 g *n*-BuOH extract and 884 g water extract. The AcOEt part was purified by CC (1.7 kg SiO_2; $CHCl_3$/MeOH/H_2O mixtures of increasing polarity), giving fractions (Fr.) 1–8. Fr. 6 was eluted with $CHCl_3$/CH_3OH/H_2O 9 : 1 : 0.1 to afford **5** 368 mg) and **7** (34 mg). Fr. 7 was subjected to repeated CC (SiO_2: $CHCl_3$/CH_3OH/H_2O 8 : 2 : 0.2; Rp-18: CH_3OH/H_2O 7.5 : 2.5) to afford **6** (40 mg), **8** (38 mg) and **3** (22 mg). The *n*-BuOH part was subjected to CC (3.5 kg SiO_2; $CHCl_3$/MeOH/H_2O mixtures of increasing polarity), giving parts 1–7. Part 3 was subjected to repeated CC (SiO_2: $CHCl_3$/CH_3OH/H_2O 8 : 2 : 0.2; Rp-18: CH_3OH/H_2O 7 : 3) to yield **1** (29 mg) and **4** (36 mg). Part 4 was subjected to repeated CC (SiO_2: $CHCl_3$/CH_3OH/H_2O 7 : 3 : 0.3; Rp-18: CH_3OH/H_2O 6.5 : 3.5) to yield **2** (35 mg).

*Metatrichoside A (**1**):* White powder. – M. p. 200–202 °C. – $[\alpha]_D^{27}$ = −9.1 (c = 0.46, MeOH). – ^{1}H and ^{13}C NMR spectral data see Table 1 and 2, respectively. – HR-TOF-MS: *m/z* = 911.5016 (calcd. 911.5004 for $C_{47}H_{76}O_{17}$, $[M-1]^-$). – FAB$^-$ MS: *m/z* = 911 $[M-1]^-$, 749 $[M-Glc]^-$, 587 $[M-2^*Glc1+1]^-$.

*Metatrichoside B (**2**):* White powder. – M. p. 215–217 °C. – $[\alpha]_D^{27}$ = −25.7 (c = 0.77, MeOH). – ^{1}H and ^{13}C NMR spectral data see Table 1 and 2, respectively. – HR-TOF-MS: *m/z* = 1073.5526 (calcd. 1073.5532 for $C_{53}H_{86}O_{22}$, $[M-1]^-$). – FAB$^-$ MS: *m/z* = 1074 $[M]^-$, 912 $[M-Glc+1]^-$, 749 $[M-2^*Glc+1]^-$, 587$[M-3^*Glc+1]^-$.

*Pyrocincholic acid 3β-O-β-D-quinovopyranosyl-28-O-β-D-glucopyranoside (**3**):* White powder. – M. p. 214–216 °C. – $[\alpha]_D^{27}$ = −23.3 (c = 0.52, MeOH).

*Pyrocincholic acid 3β-O-β-D-quinovopyranosyl-28-O-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside (**4**):* White powder. – M. p. 197–199 °C. – $[\alpha]_D^{27}$ = −12.6 (c = 0.23, MeOH).

*Quinovic acid 3β-O-β-*D*-quinovopyranoside (**5**):* White needles. – M. p. 193 – 195 °C. – $[\alpha]_D^{23}$ = +52.3 (*c* = 0.60, MeOH).

*Quinovic acid 3β-O-β-*D*-quinovopyranosyl-28-O-β-*D*-glucopyranoside (**6**):* White powder. – M. p. 200 – 202 °C. – $[\alpha]_D^{23}$ = +39.9 (*c* = 0.68, MeOH).

*Quinovic acid-3β-O-β-*D*-glucopyranoside (**7**):* White needles. – M. p. 247 – 249 °C. – $[\alpha]_D^{18}$ = +45.8 (*c* = 0.64, MeOH).

*Quinovic acid 3β-O-β-*D*-glucopyranosyl-28-O-β-*D*-glucopyranoside (**8**):* White powder. – M. p. 297 – 299 °C. – $[\alpha]_D^{18}$ = +33.4 (*c* = 0.65, MeOH).

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