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Sulfur-containing and dimeric flavanols from *Glycosmis montana*

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Abstract—Two novel sulfur-containing flavanols, glymontanines A and B, and two new flavanol dimers, montahomobisflavans A and B, were isolated from the twigs and leaves of *Glycosmis montana*. Their structures were elucidated by spectroscopic methods including 2D NMR analysis.

The genus *Glycosmis* (Rutaceae-Aurantioideae) was shown to be a rich source of diverse amides characterized by sulfur-containing acid moieties, which to date have exclusively been isolated from this genus.[1] Some sulfur-containing amides showed antimalarial, antifungal, and insecticidal activity.[2–6] In our search for bioactive substances from *Glycosmis montana*, we isolated two novel sulfur-containing flavanols (glymontanines A and B) and two new dimeric flavanols in which two units are linked through a methylene bridge at the 8,8′- and 8,6′-positions (montahomobisflavans A and B). Glymontanines A and B are the first examples of flavonoids with sulfur-containing amide moiety in nature. Here we report on the isolation and structural elucidation of these compounds from *G. montana*.

The aerial parts of *G. montana* were extracted with MeOH at room temperature and filtered. The filtrate was concentrated and extracted with $CHCl_3$ and EtOAc in turn. The EtOAc extract (25 g) was chromatographed over D101 porous resin and eluted with gradient aqueous ethanol. The fraction eluted with EtOH/H_2O (1:1) was further subjected to Sephadex LH-20 (MeOH) and reversed-phase HPLC chromatographic separation (RP-18, with a gradient starting from 20% to 40% methanol). Four compounds, glymontanines A (**1**, 9 mg) and B (**2**, 8 mg), and montahomobisflavans A (**3**, 32 mg) and B (**4**, 15 mg) were obtained.

Glymontanine A (**1**), was obtained as colorless powder. The molecular formula was determined to be $C_{19}H_{21}NO_8S$ by negative HRESIMS (found 423.1009, calcd 423.0988).[8] The ^{1}H and ^{13}C NMR (Tables 1 and 2) spectra of **1** was similar to those of 4′-*O*-methylgallocatechin (**5**),[7] which were obtained in a large amount (900 mg) and identified in our work. The absence of one methine carbon signal in the ring-A, and instead, the presence of a quaternary carbon signal at δ 104.4, indicating a probable ring-A substitution. The methyl signal at δ 12.2 in ^{13}C NMR and a singlet signal at δ 2.25 (3H) in ^{1}H NMR suggested the occurrence of a methylthio group (SMe). An additional proton signal at δ 9.77 in C_5D_5N, exchangeable with D_2O, was determined to be NH group, which has no HMQC correlations with carbon. The HMBC cross-peaks from the SMe and the methylene protons of NH–CH_2–Ar to the quaternary carbon signal at δ 172.5 indicated the connectivity of the side chain, which was confirmed by the two HMBC cross-peaks in C_5D_5 N, from the NH group to the quaternary carbon signal at δ 169.3 and the methylene carbon signal of NH–CH_2–Ar at δ 35.5. The linkage of the side chain to the flavan backbone was also determined by HMBC experiment. The H-2 proton showed long-range correlation with the carbon at δ 154.6, which can only be assigned to C-8a. The methylene protons of NH–CH_2–Ar showed correlations with C-7, C-8, and C-8a, suggesting a C-8 substitution. This was also confirmed by the cross-peaks from the ring-A residual aromatic proton to C-5 and C-7. The coupling constant ($J = 6.0$ Hz) for H-2 defined the 2,3-*trans* stereochemistry. The 2*R*, 3*S* configuration was assigned to **1** based on the negative Cotton effect in the diagnostic wavelength region of the CD spectra ($[\theta] = -5632$, 285 nm).[12]

Keywords: Flavanol; *Glycosmis montana*; Glymontanine; Montahomobisflavan.

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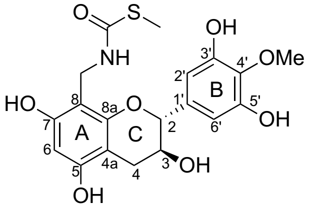

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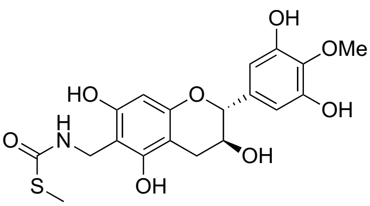

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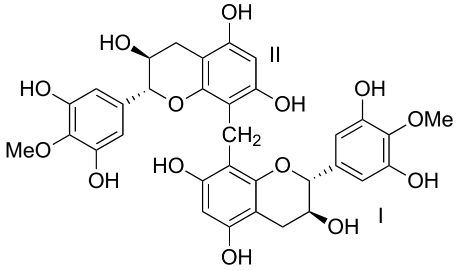

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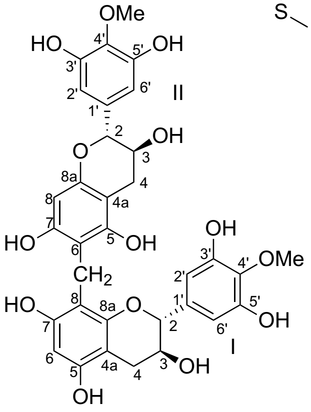

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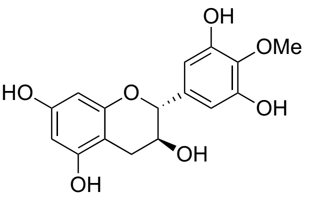

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Table 1. ^{1}H (400 MHz) data of compounds **1**–**4** in CD_3OD (δ, J in ppm)

No.	**1**	**2**	**3** (I II)	**4** (I)	**4** (II)
2	4.76 d (6.0)	4.59 d (6.7)	4.50 d (7.2)	4.75 d (7.2)	4.54 d (6.8)
3	4.02–4.06 m	3.96–4.01 m	3.92–3.97 m	4.07–4.10 m	3.90–3.97 m
4ax	2.55 dd (16.3, 6.5)	2.55 dd (16.3, 7.2)	2.49 dd (16.4, 8.0)	2.57 dd (16.5, 7.9)	2.47 dd (16.3, 7.4)
4eq	2.67 dd (16.3, 4.9)	2.78 dd (16.3, 5.1)	2.79 dd (16.4, 5.4)	2.88 dd (16.3, 5.2)	2.76 dd (16.5, 5.2)
6	5.99 s		5.97 s	6.07 s	
8		5.96 s			5.95 s
2′/6′	6.38 s	6.38 s	6.38 s	6.48 s	6.37 s
–OMe	3.76 s	3.77 s	3.79 s	3.81 s	3.77 s
–CH$_2$–	4.43 d (15.7) 4.29 d (15.7)	4.29 s	3.75 s	3.68 s	
–SMe	2.25 s	2.29 s			
–NH–	9.77 br s[a]	10.5 br s[a]			

[a] Values in C_5D_5N.

Table 2. ^{13}C (100 MHz) data of compounds **1**–**4** in CD_3OD (δ, in ppm)

No.	**1**	**2**	**3** (I II)	**4** (I)	**4** (II)
2	82.3 d	82.4 d	83.1 d	84.0 d	82.4 d
3	68.2 d	68.6 d	68.4 d	68.0 d	68.7 d
4	27.2 t	28.0 t	28.2 t	28.2 t	28.2 d
5	157.1 s	155.9 s	155.5 s	155.8 s	154.5 s
6	96.6 d	106.9 s	96.9 d	97.4 d	108.1 s
7	156.2 s	156.5 s	155.2 s	154.9 s	154.8 s
8	104.4 s	95.8 d	106.5 s	106.6 s	96.2 d
4a	100.7 s	100.7 s	101.2 s	101.5 s	102.0 s
8a	154.6 s	156.0 s	153.2 s	152.6 s	154.7 s
1′	136.7 s	136.7 s	136.6 s	135.1 s	136.7 s
2′/6′	107.0 d	107.3 d	107.6 d	107.8 d	107.4 d
3′/5′	151.7 s	151.6 s	151.5 s	151.8 s	151.5 s
4′	136.4 s	136.4 s	136.1 s	136.9 s	136.4 s
–OMe	60.8 q	60.8 q	60.8 q	60.8 q	60.8 q
–CH$_2$–	35.3 t	35.4 t	16.8 t	17.6 t	
–C(O)–	172.5 s	172.8 s			
–SMe	12.2 q	12.2 q			

The negative HRESIMS of **2** gave the molecular formula $C_{19}H_{21}NO_8S$ (found 423.0997, calcd 423.0988). The ^{1}H and ^{13}C NMR data (Tables 1 and 2) as well as 2D correlations[9] revealed that both the functional groups and the skeleton present in **2** were similar to those observed in **1**, suggesting that **2** was a conformation isomer of **1**. In its HMBC spectra,[9] the H-2 proton doublet at δ 4.59 correlated with the carbon signal at δ 156.0, which corresponds thus to C-8a. This carbon showed correlation with the residual aromatic proton located at δ 5.96, which was then attributed to H-8, indicating a C-6 substitution. This was confirmed by the presence of correlations between C-5, C-6, and C-7 carbons with the methylene protons of NH–CH_2–Ar. Glymontanine B was then concluded to be the isomer of **1** in which the side chain is linked to the C-6 carbon.

Careful inspection of the NMR data (Tables 1 and 2) for montahomobisflavan A (**3**) indicated that the flavanol unit was the same as those of **1** and **2**. The singlet signal at δ 3.75 of the methylene group was characteristic of a proton located between two deshielding groups. On the contrary, the carbon signal of the CH_2 group (δ 16.8) was not influenced by the presence of such deshielding groups. From the aromatic ring-A, only one proton signal was observed as a singlet at δ 5.97. The negative HRFABMS[10] of **3** gave the molecular formula $C_{33}H_{32}O_{14}$ corresponding to two flavanol units linked by a methylene group. There are three possible isomers: the 6–6, 6–8, and the 8–8 regio-isomer. Since we observed only one flavanol unit signal, we can assign either 6–6 or 8–8 isomer to **3**. The HMBC[10] cross-peaks from the methylene protons of Ar–CH_2–Ar to C-7, C-8, and C-8a suggesting a C-8 substitution. The ring-A residual aromatic proton showed correlations to C-5 and C-7, which confirmed **3** to be the 8–8 isomer.

Montahomobisflavan B (**4**) was obtained as colorless powder. Its molecular formula was determined to be $C_{33}H_{32}O_{14}$ by negative HRFABMS.[11] The NMR signals of **4** appeared (Tables 1 and 2) in pairs at the same region as those of **3**, which suggested **4** to be the nonsymmetrical 6–8 isomer. The presence of a correlation peak in the HMBC[11] experiment from the methylene protons of Ar–CH_2–Ar to C-8 (I) and C-8a (I) demonstrated that the methylene bridge is attached to C-8 of unit I. Detailed analysis of the 1D and 2D (HMQC and HMBC)[11] data allowed us to assign all protons and carbons.

The same 2*R*, 3*S*-configuration depicted in structure **2**–**4** was based on the coupling constant for H-2 and the negative Cotton effect at around 280 nm of their CD spectra.

The mechanism of the formation of these flavanol derivatives is suggested to be similar to that described elsewhere.[13–15] The structure of the intermediates may be 4′-*O*-methylgallocatechine substituted by aldehydic unit at C-6 and/or C-8, and then react with another 4′-*O*-methylgallocatechine to form montahomobisflavans A and B. The –CO–S–Me group of the thiocarbamate unit has been proposed to come from cysteine by β-oxidation.[5] The intermediates bind to the thiocarbamate moiety, giving rise to glymontanines A and B.

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8. Glymontanine A (1): Colorless powder; $[\alpha]_D^{23}$: −18.4 (*c* 0.45, MeOH); CD (MeOH): $[\theta]_{285}$ −5632; ^{1}H NMR and ^{13}C NMR see Tables 1 and 2; negative HRESIMS *m*/*z* found 423.1009 (calcd for $C_{19}H_{21}NO_8$ S: 423.0988); HMBC: methylene protons of NH–CH_2–Ar to C-7, 8, 8a, and –C(O)–, methyl protons to –C(O)–, –NH– to –C(O)–, and methylene group of NH–CH_2–Ar (in C_5D_5N), H-6 to C-5 and 7, H-2 to C-8a, 3, 4, 1′, and 2′/6′, H-4 to C-2, 3, 5, 4a, and 8a, H-2′/6′ to C-2, 1′, and 3′/5′, and methoxy protons to C-4′.
9. Glymontanine B (2): Colorless powder; $[\alpha]_D^{23}$: −13.3 (*c* 0.25, MeOH); CD (MeOH): $[\theta]_{286}$ −4701; ^{1}H NMR and ^{13}C NMR see Tables 1 and 2; negative HRESIMS *m*/*z* found 423.0997 (calcd for $C_{19}H_{21}NO_8S$: 423.0988); HMBC: methylene protons of NH–CH_2–Ar to C-5, 6, 7, and –C(O)–, methyl protons to –C(O)–, –NH– to –C(O)–, and methylene group of NH–CH_2–Ar (in C_5D_5N), H-8 to C-7 and 8a, H-2 to C-8a, 3, 4, 1′, and 2′/6′, H-4 to C-2, 3, 5, 4a, and 8a, H-2′/6′ to C-2, 1′, and 3′/5′, and methoxy protons to C-4′.
10. Montahomobisflavan A (3): Colorless powder; $[\alpha]_D^{23}$: −44.1 (*c* 1.0, MeOH); CD (MeOH): $[\theta]_{282}$ −8211; ^{1}H NMR and ^{13}C NMR see Tables 1 and 2; negative HRFABMS *m*/*z*: found 651.1720 ($[M-H]^-$) (calculated for $C_{33}H_{31}O_{14}$: 651.1714); HMBC: methylene protons of Ar–CH_2–Ar to C-7, 8, and 8a, H-6 to C-5, 7, 8, and 4a, H-2 to C-8a, 3, 4, 1′, and 2′/6′, H-4 to C-2, 3, 5, 4a, and 8a, H-2′/6′ to C-2, 1′, and 3′/5′, and methoxy protons to C-4′.
11. Montahomobisflavan B (4): Colorless powder; $[\alpha]_D^{23}$: −26.6 (*c* 0.61, MeOH); CD (MeOH): $[\theta]_{283}$ −7305; ^{1}H NMR and ^{13}C NMR see Tables 1 and 2; negative HRFABMS *m*/*z*: found 651.1722 ($[M-H]^-$) (calculated for $C_{33}H_{31}O_{14}$: 651.1714); HMBC: methylene protons of Ar–CH_2–Ar to C-8 (I), 8a (I), 5 (II), 6 (II), and 7 (II), H-6 (I) to C-5 (I), 7 (I), 8 (I), and 4a (I), H-8 (II) to C-6 (II), 7 (II), 4a (II), H-2 (I II) to C-8a (I II), 3 (I II), 1′(I II), and 2′/6′ (I II), H-4 (I

II) to C-2 (I II), 5 (I II), 4a (I II), and 8a (I II), H-2′/6′ (I II) to C-2 (I II), 1′ (I II), and 3′/5′(I II), and methoxy protons (I II) to C-4′ (I II).

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