Homogentisic Acid Derivatives from *Miliusa balansae*

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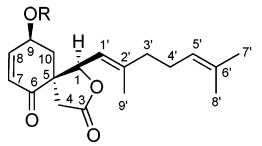
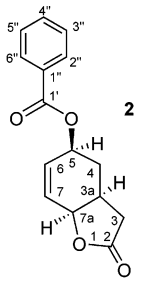
The new homogentisic acid derivatives miliusol (**1b**) and miliusolide (**2**) from *Miliusa balansae* were isolated and structurally determined by spectroscopic means. The relative configurations of the new **1b** and its known acetate **1a** were established. Furthermore, the symmetric ether bis(2-hydroxyphenyl)-methyl ether **3**, which was isolated for the first time from a natural source, the known flavonoids pachypodol and chrysosplenol C, and sodium benzoate were identified.

The plant *Miliusa balansae* Fin. & Gagn. is a shrub of the family Annonaceae.[1] In Chinese traditional medicine, the plant is used to treat various diseases, for example, gastropathy and glomerulonephropathy.[2] Recently, a new geranyl homogentisic acid derivative named miliusate (**1a**) with a very unusual spiro structure was isolated from this plant, but the relative configuration was not determined.[2] In a previous study, in addition to miliusate (**1a**) and some known flavanones and dihydrochalcones, we elucidated the structures of two new compounds, 3,4-dimethoxy-6-styrylpyran-2-one and (2*E*,5*E*)-2-methoxy-4-oxo-6-phenylhexa-2,5-dienic acid methyl ester, from the title plant collected in Hoa Binh Province.[3] In this paper, we report on the constituents from *M. balansae* collected in Hai Duong Province. Instead of miliusate (**1a**), we found the free alcohol miliusol (**1b**) and established its relative configuration. A further new homogentisic acid derivative (**2**), miliusolide, and a symmetric ether (**3**), which was not yet isolated from a natural source, are also described.



1a R = Ac
1b R = H



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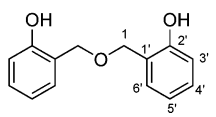

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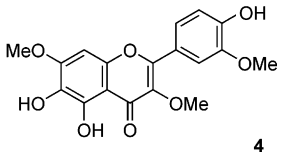

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Miliusol (**1b**) was obtained as an oil. The molecular formula was determined as $C_{18}H_{24}O_4$ from the $[M + Na]^+$ peak at *m*/*z* 327 in the HRESITOFMS. The ^1H and ^{13}C NMR spectra were similar to those of miliusate (**1a**) except for the missing acetyl group and the associated upfield shift of H-9 (δ 4.61). Full analysis of HH-COSY and HMBC experiments (Table 1) confirmed **1b** to be the hitherto unknown free alcohol of miliusate (**1a**). The compound was named miliusol. The relative configuration was deduced

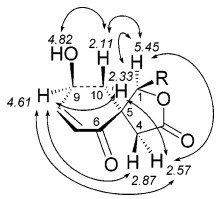

Figure 1. NOE enhancements observed in the NOESY experiment of miliusol (**1b**). R = −CH=C(CH$_3$)−CH$_2$−CH$_2$−CH=C(CH$_3$)$_2$.

from the NOESY spectrum, in which a NOE enhancement between H-1 (δ 5.45) and H-4A (δ 2.57) established the *cis*-relationship of these protons. In Figure 1 they are depicted at the back of the five-membered ring. Further NOE interactions of the methylene protons H-10A and H-10B (δ 2.11 and 2.33) with these two background protons showed that the bond C-9/C-10 of the cyclohexene is also located in the background, resulting in the depicted configuration for C-5. Proton H-9 gave NOE interactions to H-4A and H-4B (δ 2.57 and 2.87) and thus must be oriented downward. This was further supported by the NOESY correlations H-9/H-10B and OH-9/H-10A, indicating the *cis*-relationships of both pairs. As expected, the C-9-hydroxy group assumed an equatorial configuration, because the observed *W*-coupling of about 1 Hz between H-8 and H-10B is possible only in the case of the equatorial conformation of H-10B. Finally, the NOESY correlation H-1′/H$_2$-3′ confirmed the *E*-configuration of the $\Delta^{1'}$-double bond. Miliusate (**1a**) is proposed to have the same relative configuration as miliusol (**1b**). All the above-mentioned NOE effects for **1b** were also observed in the NOESY spectrum of miliusate (**1a**), but the correlations of H-4A (δ 2.27) and H-10A (δ 2.26) could not be distinguished due to overlapping. Also, the HH coupling constants of **1a** in $CDCl_3$[3] are in very close correspondence to those of **1b** in the same solvent. All these data support the same relative configuration of both **1b** and **1a**.

Miliusolide (**2**) was isolated as colorless crystals. The molecular formula was determined as $C_{15}H_{14}O_4$ from the $[M + Na]^+$ peak at *m*/*z* 281 in the HRESITOFMS. A benzoyl ester moiety was deduced from the base peak in the EIMS at *m*/*z* 105 and the characteristic chemical shifts in the ^1H and ^{13}C NMR spectra (Table 2). For the alcohol moiety, the spectra exhibited a *cis* double bond (δ_C 134.0, 125.3, δ_H 6.20, 6.08, $^3J_{HH}$ = 10.2 Hz), two oxygen-substituted methine groups (δ_C 74.2, 67.7, δ_H 4.84, 5.57), and one ester or lactone group (δ_C 175.5). The HH-COSY experiment revealed the spin system H-3α/β−H-3a−H-4α/β−H-5−H-6−H-7−H-7a. An additional correlation ($^3J_{HH}$)

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Table 1. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) Data of Miliusol (**1b**) in Acetone-d_6 (δ in ppm, J in Hz)[a,b]

position	δ_C	δ_H	HH correlations[c]	C−H long-range correlations[d]	NOE enhancements[e]
1	82.5	5.45 d (9.8)	H-1′	H-4A (w), H-4B, H-10A, H-10B, H_3-9′ (w)	H-4A (w), H-10A, H-10B, H_3-9′
3	175.1			H-1, H-4A, H-4B	
4	38.8	A: 2.57 d (17.2)	H-4A	H-1 (w), H-10A, H-10B	H-1 (w), H-4B, H-9 (w), H-10B
4		B: 2.87 d (17.2)	H-4B		H-4A
5	53.5			H-1, H-4A, H-4B, H-7, H-9 (w), H-10A, H-10B, H-1′ (vw), H_3-9′ (w)	
6	197.7			H-1, H-4A, H-4B, H-8, H-10A, H-10B	
7	128.3	5.84 dd (10.2, 1.6)	H-8, H-9 (w)	H-9 (w)	H-8
8	153.2	6.98 ddd (10.1, 2.9, 0.8)	H-7, H-9, H-10B (w)	H-9 (w), H-10A, H-10B	H-7, H-9
9	64.3	4.61 m ($\Delta\nu_{1/2}$ = 14 Hz)	H-8, H-10A, H-10B	H-7, H-10A, H-10B	H-4A (w), H-8, H-10A (w), H-10B
10	40.36[f]	A: 2.11 dd (13.4, 7.3)	H-9, H-10A, H-10B	H-1, H-4A, H-4B, H-8	H-1 (w), H-9 (w), H-10B
10		B: 2.33 ddd (13.4, 5.1, 1.1)	H-8 (w), H-9		H-1, H-4A, H-9, H-10A
1′	120.1	5.27 dq (9.8, 1.2)	H-1, H_2-3′ (w), H_3-9′	H-3′, H_3-9′	H_2-3′
2′	144.1			H-1, H_2-3′/4′, H_3-9′	
3′	40.34[f]	2.00−2.08 m	H-1′, H_3-9′	H-1′, H_2-4′, H-5′, H_3-7′ (vw), H_3-8′ (vw), H_3-9′	H-1′, H-5′,
4′	26.8	2.00−2.08 m	H-5′, H_3-7′, H_3-8′ (w)	H_2-3′, H-5′	H-5′, H-8′
5′	124.4	5.02 tsept (6.9, 1.4)	H_2-4′, H_3-7′, H_3-8′	H_2-3′/4′, H_3-7′, H_3-8′	H_2-3′/H_2-4′, H_3-7′
6′	132.2			H_2-4′, H_3-7′, H_3-8′	
7′	25.8	1.63 d (1.1)	H_2-4′, H-5′	H-5′, H_3-8′	H-5′
8′	17.7	1.57 d (0.6)	H_2-4′, H-5′	H-5′, H_3-7′	H_2-3′/4′,
9′	16.9	1.73 d (1.4)	H-1′, H_2-3′/4′	H-1′, H_2-3′	H-1
OH-9		4.82 d (5.2)			H-9, H-10A (w)

[a] ^{1}H and ^{13}C NMR data in $CDCl_3$ are in the Experimental Section. [b] The correlations of C-10 and C-3′ at δ 40.36 and 40.34 as well as the correlations of H_2-3′ and H_2-4′ (δ 2.00−2.08) were not resolved. [c] From HH-COSY experiment. [d] From ^{1}H−^{13}C HMBC experiment. [e] From NOESY experiment. [f] Exchangeable. (w) = weak. (vw) = very weak. H-4A = H-4(*pro-R**), H-4B = H-4 (*pro-S**); H-10A = H-10 (*pro-S**), H-10B = H-10 (*pro-R**).

Table 2. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) Data of Miliusolide **2** in $CDCl_3$ (δ in ppm, J in Hz)

position	δ_C	δ_H	HH correlations[a]	C−H long-range correlations[b]	NOE enhancements[c]
2	175.5			H-3α, H-3β	
3	35.9	α: 2.87 dd (17.4, 8.3)	H-3β, H-3a	H-4α (w), H-4β (w)	H-3β, H-3a, H-7a (w)
		β: 2.50 dd (17.4, 2.8)	H-3α, H-3a		H-3α, H-3a, H-4α, H-4β
3a	31.7	2.77 m	H-3α, H-3β, H-4α, H-4β, H-7a	H-3α, H-3β, H-4α, H-4β, H-7 (w)	H-3α, H-3β, H-4α, H-4β, H-5, H-7a
4	29.4	α: 2.23 dt (12.8, 4.8)	H-3a, H-4β, H-5	H-3α, H-3β, H-6	H-3β, H-3a, H-4β, H-5
		β: 1.72 ddd (12.6, 11.9, 9.0)	H-3a, H-4α, H-5		H-3β, H-3a, H-4α, H-5 (w)
5	67.7	5.57 dddd (9.0, 5.2, 3.7, 2.0)	H-4α, H-4β, H-6 (w), H-7 (w), H-7a (w)	H-4α, H-4β, H-7	H-3a, H-4α, H-4β, H-6, H-7a (w), H-2″/6″ (w)
6	134.0	6.20 dm (10.2)	H-4α (w), H-5, H-7, H-7a (w)	H-4α, H-4β, H-5 (w), H-7a (w)	H-5, H-7, H-7a (w), H-2″/6″ (w)
7	125.3	6.08 ddd (10.2, 3.6, 1.9)	H-3β (w), H-5 (w), H-6, H-7a	H-5, H-7a (w)	H-5 (w), H-7a
7a	74.2	4.84 m	H-3a, H-5 (w), H-6 (w), H-7	H-3α, H-3β, H-4α, H-4β, H-6	H-3α, H-3a, H-4α (w), H-5 (w), H-7
1′	165.9			H-5 (w), H-2″/6″, H-3″/5″	
1″	129.8			H-3″/5″	
2″/6″	129.7	8.03 dd (8.3, 1.1)	H-3″/5″, H-4″	H-6″/2″, H-4″	H-3β, H-5 (w), H-6 (w)
3″/5″	128.5	7.46 ddd (8.0, 7.5, 1.5)	H-2″/6″, H-4″	H-5″/3″	
4″	133.4	7.59 tt (7.4, 1.3)	H-2″/6″, H-3″/5″	H-2″/6″	

[a] From HH-COSY experiment. [b] From ^{1}H−^{13}C HMBC experiment. [c] From NOESY experiment. (w) = weak.

between H-3a and H-7a indicated a ring closure. The benzoyl moiety was located at C-5 (δ 67.7) because of the CH long-range correlation in the HMBC experiment between the benzoyl carbonyl carbon C-1′ (δ 165.9) and H-5 at δ 5.57. The number of double-bond equivalents in the molecular formula indicated an additional ring closure via an oxygen bridge between C-2 (δ 175.5) and C-7a (δ 74.2), which was confirmed by the characteristic IR band for a γ-lactone at 1781 cm^{-1}. These data led to the structure 5-benzoyloxy-3a,4,5,7a-tetrahydro-3*H*-benzofuran-2-one for **2**. The relative configuration was obtained from the NOESY experiment, where the strong interactions H-3a/H-7a and H-3a/H-5 revealed that all these protons have *cis*-relationships to give the configuration 3a*S**,5*S**,7a*R**. This benzoyl ester (**2**) and the free alcohol are new compounds. An isomeric alcohol with the absolute configuration 3a*S*,5*R*,7a*R* and its enantiomer are known as synthetic products.[4]

The ^{1}H and ^{13}C NMR spectra of compound **3** showed a 1,2-disubstituted benzene ring (ABCD spin system at δ_H 7.24−6.88), a phenolic hydroxyl group (δ_H 6.80), and an oxygen-substituted CH_2 group (δ_H 4.74, s, δ_C 70.5), suggesting the structure 2-(hydroxymethyl)phenol, which is a common natural product. However, the molecular ion in the EIMS was found at *m*/*z* 230, and the carbon shift of the CH_2 group in **3** is about 8 ppm higher than that of the of hydroxymethyl alcohol,[5] suggesting a symmetric ether.

The carbon shifts in $CDCl_3$ were in correspondence with the synthetic compound bis(2-hydroxyphenyl)methyl ether in acetone-d_6,[5] which has not yet been found as a natural product.

In addition, the flavone pachypodol was identified by comparison of the ^{13}C NMR data in DMSO-d_6 with reference data.[6] The structure of chrysosplenol C[7] (**4**) was identified by analysis of the HMBC experiment. Sodium benzoate was identified from the ^1H and ^{13}C NMR spectra[8] and its $[M + Na]^+$ peak at *m/z* 167 in the ESIMS.

Experimental Section

General Experimental Procedures. Melting points are uncorrected and were measured on a Botius HMK melting point apparatus. The optical rotations were recorded on a JASCO DIP 1000 polarimeter. FTIR spectra were measured on a Nicolet Impact 410 spectrometer. NMR spectra were acquired on a Bruker Avance 500 spectrometer. EIMS was measured on an HP 5989B mass spectrometer at 70 eV. HRESITOFMS were measured on a QStar Pulsar spectrometer (Applied Biosystems).

Plant Material. Leaves and branches of *M. balansae* were collected near Hoang Hoa Tham, Chi Linh District, Hai Duong Province, Vietnam, in October 2000, and identified by Mr. Ngo Van Trai, Institute of Materia Medica, Hanoi. A voucher specimen (No. TC023) is deposited at the Institute of Pharmacy, Hanoi.

Extraction and Isolation. Air-dried and ground leaves and branches (2.3 kg) of *M. balansae* were extracted several times with MeOH−H_2O (95:5) at room temperature to give 200 g of a MeOH extract after evaporation of the solvent. This extract (160 g) was redissolved in water and subjected to liquid−liquid partitioning with EtOAc and *n*-BuOH, successively, giving 110 g of EtOAc extract and 40 g of *n*-BuOH extract. The EtOAc extract was separated by column chromatography on silica gel (230−400 mesh) using solvent mixtures of increasing polarity (*n*-hexane, EtOAc, and MeOH), giving 10 fractions. Fraction 2 (2 g) was further purified by column chromatography on silica gel with CH_2Cl_2 to give 3 mg of compound **3**. Fraction 4 (5.7 g) was crystallized from CH_2Cl_2 to give 1.2 g of pachypodol. The mother liquor contained miliusolide (**2**), which was purified by column chromatography on silica gel using mixtures of *n*-hexane−EtOAc with increasing polarity to give 15 mg of **2**. Fraction 5 (11 g) was chromatographed on silica gel with solvent mixtures with increasing polarity (*n*-hexane, EtOAc, and MeOH). An aliquot (60 mg) of the fraction containing miliusol (**1b**, 7.04 g) was purified by preparative TLC (silica gel, thickness 5 mm) using *n*-hexane−EtOAc−MeOH (9:1:0.5) and yielded 30 mg of **1b**. Another fraction (4 g) of fraction 5 gave 20 mg of sodium benzoate. Fraction 6 (3 g) was crystallized from CH_2Cl_2−MeOH and yielded 2.5 g of chrysosplenol C (**4**).

Miliusol (1′*E*)-(1*R,5*R**,9*S**)-9-hydroxy-1-(2,6-dimethylhepta-1,5-dienyl)-3,6-dioxo-2-oxa-spiro[4.5]dec-7-ene (1b):** colorless oil; $[\alpha]^{23}_D$ +38° (*c* 0.5, $CHCl_3$); IR (neat) ν_{max} 3444, 2923, 1777, 1671, 1445, 1379, 1212, 1044, 983 cm^{-1}; ^1H and ^{13}C NMR data in acetone-d_6, see Table 1; ^1H NMR ($CDCl_3$, 500 MHz) δ 6.89 (1H, ddd, J = 10.1, 4.0, 0.9 Hz, H-8), 6.01 (1H, dd, J = 10.1, 1.1 Hz, H-7), 5.58 (1H, d, J = 10.3 Hz, H-1), 5.15 (1H, dq, J = 10.3, 1.2 Hz, H-1′), 5.00 (1H, m, H-5′), 4.57 (1H, m, H-9), 3.21 (1H, d, J = 17.2 Hz, H-4B), 2.39 (1H, br s, OH-9), 2.34 (1H, ddd, J = 14.3, 4.4, 1.1 Hz, H-10B), 2.30 (1H, d, J = 17.3 Hz, H-4A), 2.22 (1H, dd, J = 13.8, 5.0 Hz, H-10A), 2.02−2.07 (4H, m, H_2-3′, H_2-4′), 1.72 (3H, d, J = 1.5 Hz, H_3-9′), 1.68 (3H, s, H_3-7′), 1.59 (3H, s, H_3-8′); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 196.3 (C-6), 174.9 (C-3), 148.2 (C-8), 145.5 (C-2′), 132.2 (C-6′), 129.1 (C-7), 123.2 (C-5′), 118.4 (C-1′), 82.0 (C-1), 63.2 (C-9), 52.3 (C-5), 39.7, 39.5 (C-10, C-3′), 38.0 (C-4), 26.0 (C-4′), 25.7 (C-7′), 17.7 (C-8′), 16.9 (C-9′); EIMS *m/z* 304 $[M]^+$ (0.5), 286 $[M - H_2O]^+$ (0.6), 245 (4), 218 (2), 190 (4), 153 (37), 135 (32), 107 (60), 84 (53), 69 (100), 55 (75); HRESITOFMS *m/z* 327.15625 $[M + Na]^+$, calcd for $C_{18}H_{24}O_4Na$ 327.15668.

Miliusolide (3a*S,5*S**,7a*R**)-5-Benzoyloxy-3a,4,5,7a-tetrahydro-3*H*-benzofuran-2-one) (2):** colorless crystals, mp 120−121 °C (EtOAc); IR (KBr) ν_{max} 2931, 1781, 1726, 1270, 1172 cm^{-1}; ^1H and ^{13}C NMR data, see Table 2; EIMS *m/z* 259 $[M + H]^+$ (3), 258 $[M]^+$ (0.5), 214 $[M - CO_2]^+$ (4), 199 (4), 123 (6), 119 (4), 105 (100), 92 (41), 91 (26), 77 (63), 51 (26); HRESITOFMS *m/z* 281.07823 $[M + Na]^+$, calcd for $C_{15}H_{14}O_4$-Na 281.07843.

Chrysosplenol C (4′,5,6,-trihydroxy-3,3′,7-trimethoxyflavone) (4): EIMS *m/z* 360 $[M]^+$. The compound was identified by analysis of the HMBC experiment. ^1H NMR (DMSO-d_6, 500 MHz) δ 12.35 (1H, s, OH-5), 9.89 (1H, s, OH-4′), 8.70 (1H, s, OH-6), 7.67 (1H, d, J = 2.0 Hz, H-2′), 7.62 (1H, dd, J = 8.4 and 2.1 Hz, H-6′), 6.96 (1H, d, J = 8.4 Hz, H-5′), 6.89 (1H, s, H-8), 3.91 (3H, s, OMe-7), 3.87 (3H, s, OMe-3′), 3.81 (3H, s, OMe-3); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 178.1 (C-4), 155.5 (C-2), 154.5 (C-7), 149.7 (C-4′), 148.8 (C-9), 147.5 (C-3′), 145.6 (C-5), 137.6 (C-3), 129.6 (C-6), 122.6 (C-6′), 121.0 (C-1′), 115.6 (C-5′), 112.0 (C-2′), 105.5 (C-10), 91.0 (C-8), 59.7 (OMe-3), 56.3 (OMe-7), 55.8 (OMe-3′).

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References and Notes

(1) Ho, P. H. *Cay co Vietnam (An Illustrated Flora of Vietnam)*; Nha xuat ban tre (Youth Publishing House): Ho Chi Minh City, 1999; Vol. I, p 272.
(2) Wu, R.; Ye, Q.; Chen, N. Y.; Zhang, G. L. *Chin. Chem. Lett.* **2001**, *12*, 247−248.
(3) Kamperdick, C.; Van, N. H.; Sung, T. V. *Phytochemistry* **2002**, *61*, 991−994.
(4) Bäckvall, J.-E.; Gatti, R.; Schink, H. E. *Synthesis* **1993**, *3*, 343−348.
(5) Fisher, T. H.; Chao, P.; Upton, G. C.; Day, A. J. *Magn. Reson. Chem.* **1995**, *33*, 717−723.
(6) Itokawa, H.; Suto, K.; Takeya, K. *Chem. Pharm. Bull.* **1981**, *29*, 254−256.
(7) Timmermann, B. N.; Mabry, T. J. *Biochem. Syst. Ecol.* **1983**, *11*, 37−39.
(8) Kalinowski, H.-O.; Berger, S.; Braun, S. *Carbon-13 NMR Spectroscopy*; John Wiley & Sons: New York, 1988; p 314.

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