Two New Bis-styryl Compounds from *Miliusa balansae*

Do Thu Huong[a], Nguyen Thi Hong Van[a], Christine Kamperdick[b], Nguyen Thi Hoang Anh[a], and Tran Van Sung[a]

[a] Institute of Chemistry, Vietnamese Academy of Science and Technology, 18 Hoang Quoc Viet, Cau Giay, Hanoi, Vietnam
[b] Leibniz Institute for Age Research – Fritz Lippman Institute, Jena, Germany

Reprint requests to Prof. Dr. Tran Van Sung. Fax: 00 84 4 8361283. E-mail: tvs@ich.vast.ac.vn

Z. Naturforsch. **2008**, *63b,* 335 – 338; received July 17, 2007

Two new compounds named miliubisstyryl A and miliubisstyryl B were isolated from leaves and branches of *Miliusa balansae* Fin. & Gagn. (Annonaceae) in addition to octacosanoic acid. Their structures were elucidated by spectroscopic methods, especially 2D NMR spectroscopy. The rare cyclobutane skeletons of these compounds are derived from a styryl compound which is also present in this plant.

Key words: Miliusa balansae, Miliubisstyryl A, Miliubisstyryl B

Introduction

The plant *Miliusa balansae* Fin. & Gagn. is a shrub of the family Annonaceae. This plant is used for gastropathy and glomerulonephropathy in Chinese traditional medicine [1]. From this plant three homogentisic acid derivatives, miliusate [1, 2], miliusol and miliusolid [3], four flavanones, two dihydrochalcones and two styryl derivatives [2] were isolated. In this paper the isolation and structure determination of two new bis-styryl compounds (**2**, **3**) are described.

Results and Discussion

The extract of leaves and branches (MeOH-H_2O 95 : 5) of *M. balansae* was partitioned between water and organic solvents of increasing polarity (*n*-hexane, EtOAc and BuOH). Three compounds were isolated from the EtOAc extract using column chromatography on silica gel. Besides octacosanoic acid, two new bis-styryl compounds named miliubisstyryl A (**2**) and miliubisstyryl B (**3**) were obtained. Their structures are closely related to the structure of the styryl derivative (2*E*, 5*E*)-2-methoxy-4-oxo-6-phenyl-hexa-2,5-dienoic acid methyl ester (**1**, $C_{14}H_{14}O_4$), which has been isolated from this plant and reported in a previous paper [2].

Miliubisstyryl A (**2**) was isolated as needles. The molecular formula of $C_{28}H_{28}O_8$ and the molecular weight of *m/z* = 492 were obtained by high resolution of the $[M+Na]^+$ peak at *m/z* = 515.1676 in the

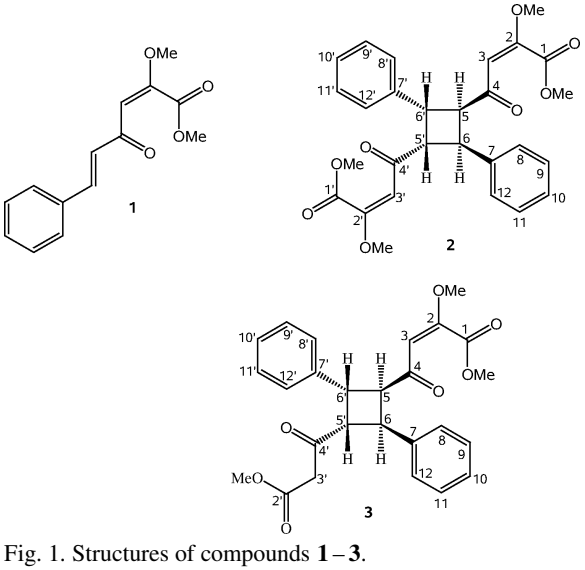

Fig. 1. Structures of compounds **1** – **3**.

(+)-ESI-MS. The EIMS showed a weak molecular ion at *m/z* = 492 (0.9 %) and a base peak $[M-C_6H_7O_4]^+$ at *m/z* = 349. The ^{13}C NMR spectrum revealed only 14 carbon signals. Thus, compound **2** consisted of two equivalent $C_{14}H_{14}O_4$ moieties which had the same molecular formula like the previously isolated styryl derivative **1**. ^{1}H and ^{13}C NMR data of compound **2** were similar to those of **1** [2] with two exceptions. Instead of the signals of the double bond C-5/C-6 in the spectra of **1**, compound **2** exhibited two aliphatic methine groups (δ_H = 3.94, 4.65; δ_C = 54.1, 41.4). This

Table 1. ^{1}H and ^{13}C NMR data (500/125 MHz) of compound **2** in $CDCl_3$ (*J* in Hz in parentheses)[a].

Position	δ_C	δ_H	CH long-range correlations (HMBC)	NOESY correlations
1/1′	164.0	–	H-3/3′, 1/1′-OCH_3	–
2/2′	160.1	–	H-3/3′, 2/2′-OCH_3	–
3/3′	101.1	5.01 s	–	2/2′-OCH_3, H_{phenyl}(w)
4/4′	195.8	–	H-3/3′, H-5/5′, H-6/6′	–
5/5′	54.1	3.94 m[b] (10.7;7.4)	H-5′/5, H-6/6′	H-6/6′, H-3/3′, H_{phenyl}
6/6′	41.4	4.65 m[b] (10.7;7.4)	H-5/5′, H-6′/6, H_{phenyl}	H-5/5′, H_{phenyl}
7/7′	139.0	–	H-5/5′, H-6/6′, H_{phenyl}	–
8/8′/12/12′	128.1	7.27 – 7.32	H-6/6′, H_{phenyl}	H-5/5′, H-6/6′, H-3 (w)
9/9′/11/11′	128.6	7.27 – 7.32	H_{phenyl}	
10/10′	127.2	7.22 – 7.23	H_{phenyl}	
1/1′-OCH_3	52.8	3.75 s	–	
2/2′-OCH_3	56.7	3.42 s	–	H-3/3′

[a] H_{phenyl} = overlapping aromatic protons H-8–H-12, H-8′–H-12′; w = weak; [b] multiplet of higher order (AA′XX′ spin system) similar to dd.

indicated that compound **2** formally was a [2+2] cycloaddition dimer of compound **1** resulting in a cyclobutane ring with the linkage between the monomers at C-5 and C-6. Analysis of the CH long-range correlations from the HMBC spectrum and the NOE data from the NOESY experiment (Table 1) confirmed that the monomeric moiety of **2** was identical with compound **1** regarding of the missing Δ^5 double bond. The existence of a symmetric dimer was further established by the fact that C-5/5′ (δ_C = 54.1) showed not only a direct correlation ($^1J_{CH}$) in the HMQC experiment but at the same time a long-range correlation ($^{2,3}J_{CH}$) to the protons at δ_H = 3.94 (H-5/5′) in the HMBC spectrum. Similarly, C-6/6′ (δ_C = 41.4) showed $^1J_{CH}$ and $^{2,3}J_{CH}$ correlations to the protons H-6/6′ at δ_H = 4.65. This dimer may have been formed by head-to-head (C-5–C-5′, C-6–C-6′ connection) or head-to-tail (C-5–C-6′, C-6–C-5′ connection) linkage. The kind of connection of the monomeric moieties could not be deduced from the CH long-range correlations because of the equivalence of both halves. The proton multiplicities were expected to be different for both isomers. In the head-to-head isomer, the $^3J_{HH}$ coupling partners of each cyclobutane proton are one chemically equivalent proton and one non-equivalent proton. Thus the signal multiplicity should be influenced by only one large coupling constant, as couplings between equivalent protons usually do not appear in the ^{1}H NMR spectra. But H-5 and H-6 both appeared as multiplets of higher order similar to a double doublet with two large coupling constants [δ_H = 3.94 (10.7, 7.4); 4.65 (10.7, 7.4)]. This is in correspondence with the structure of the head-to-tail isomer, where each proton is neighbored by two protons, which are non-equivalent. The relative configuration could be deduced from symmetry considerations. Among the 3 stereo isomers (3 pairs of enantiomers) with chemically equivalent protons (all-*cis;* all-*trans;* 5,6-*cis*, 6,5′-*trans*, 5′,6′-*cis*, 6′,5-*trans*), only the latter one represents a AA′XX′ spin system as observed in the ^{1}H spectrum, whereas the other two isomers are A_2X_2 spin systems which would result in triplet signals for both H-5/5′ and H-6/6′. Consequently, the structure of compound **2** was established as depicted in Fig. 1: a symmetric head-to-tail dimer of the styryl derivative **1**. In our opinion, compound **2** can not be an artifact, because the extraction and purification of this compound has been carried out under mild, neutral conditions. In addition, we could not isolate compound **2** from **1** after four weeks standing in the same solution.

Compound **3** was isolated as colorless crystals. The ESI-MS of this compound gave a molecular formula as $C_{26}H_{26}O_7$ by high resolution of the $[M+Na]^+$ peak at *m/z* = 473.1577. The EIMS indicated a weak molecular ion peak at *m/z* = 450 (0.49 %), a base peak at *m/z* = 143 $[C_6H_7O_4]^+$ and prominent fragments at *m/z* = 115 $[C_5H_7O_3]^+$ (89.8 %), 103 $[C_4H_7O_3]^+$ (64.0 %). In accordance with the molecular formula the ^{13}C NMR spectra showed 26 signals. Many of them had similar chemical shifts as those in compound **2** (C-1–C-12, C-7′–C-12′). Together with a difference of 2 carbons compared to compound **2**, these data suggested a similar structure of **3** with a cyclobutane with two phenyl groups and two aliphatic side chains where one side chain has 2 carbons less, so that the compound is no longer symmetrical. The structural identity of the longer side chain with that of compound **2** was proved not only by the very similar chemical shifts but also by the CH long-range correlations in the HMBC experiment and the NOE in the NOESY spectrum (Table 2). The structure of the shorter side chain with the additional signals of a non-conjugated keto group at δ_C =

Table 2. ^{1}H and ^{13}C NMR data (500/125 MHz) of compound **3** in $CDCl_3$ (J in Hz in parentheses)[a].

Position	δ_C	δ_H	CH long-range correlations (HMBC)[b]	NOESY correlations
1	163.9	–	H-3, 1-OMe	–
2	160.3	–	H-3, 2-OMe	–
3	101.0	4.98 s	–	H-5, 2-OCH_3, H_{phenyl} (w)
4	195.6	–	H-3, H-5, H-6/H-6′	–
5	54.1	3.89 dd[c] (11.1, 6.4)	H-6/H-6′, H-5′	H-3, H-6/6′, H_{phenyl} [d]
6	41.1	4.66 dd (11.2, 7.5)	H-5, H-5′, H-6′, H_{phenyl}	
7	138.6*	–	H-5/5′, H-6, H_{phenyl}	–
8, 12	127.9**	7.22 – 7.35	H-6, H_{phenyl}	H_{phenyl}
9, 11	128.6***	7.22 – 7.35	H_{phenyl}	H_{phenyl}
10	127.6	7.22 – 7.35	H_{phenyl}	H_{phenyl}
2′	167.0	–	2′-OCH_3, H-3′a, H-3′b	–
3′	48.3	a: 2.75 d (15.7) b: 3.01 d (15.7)	–	H-3′b, H-5′ (w), H_{phenyl} (w) H-3′a, H-5′ (w)
4′	201.0	–	H-6/6′, H-3′a, H-3′b, H-5′	–
5′	53.9	4.14 dd[e] (11.2, 7.5)	H-5, H-6/6′	H-6/6′, H-3′a (w), H-3′b, H_{phenyl} [d]
6′	41.3	4.65 dd (11.2, 6.4)	H-5, H-6, H-5′, H_{phenyl}	
7′	138.5*	–	H-5/5′, H-6′, H_{phenyl}	–
8′, 12′	128.1**	7.22 – 7.35	H-6′, H_{phenyl}	
9′, 11′	128.9***	7.22 – 7.35	H_{phenyl}	
10′	127.3	7.22 – 7.35	H_{phenyl}	
1-OMe	52.8	3.77 s	–	2-OMe (w)
2-OMe	56.7	3.41 s	–	H-3, 1-OMe (w)
2′-OMe	52.2	3.57 s	–	–

[a] H_{phenyl} = overlapping aromatic protons H-8–H-12, H-8′–H-12′; w = weak; [b] correlations of the signals of H-6 (4.66) and H-6′ (4.65) not resolved; [c] multiplicity at 750 MHz: ddd (11.2, 6.3, 1.0 Hz); [d] correlations of the signals of H-6 (4.66) and H-6′ (4.65) were not resolved in the NOESY spectrum. The protons were resolved in the DPFGSE NOE as described in the text; [e] multiplicity at 750 MHz: ddd (11.2, 7.6, 0.8 Hz); *, **, ***: signals exchangeable within one column.

201.0, a methoxyl ester group with the signals at δ_C = 167.0, δ_C = 52.2 / δ_H = 3.57 and a methylene group at δ_C = 48.3 / δ_H = 2.75 and 3.01 was elucidated as 3-oxopropionic acid methyl ester by the HMBC correlations δ_C = 167.0 (C-2′) / δ_H = 3.57 (2′-OCH_3), 2.75 (H-3′a), 3.01 (H-3′b) and δ_C = 201.0 (C-4′) / δ_H = 2.75, 3.01 (H-3′a/b). The presence of a cyclobutane ring was confirmed by the ^{1}H-^{1}H-COSY correlations ($^{3}J_{HH}$) of δ_H = 3.89 / δ_H = 4.65, 4.66 and δ_H = 4.14 / δ_H = 4.65, 4.66 and by the weak $^{4}J_{HH}$ correlation of the protons at δ_H = 3.89 and 4.14. Thus these two protons as well as the overlapping protons at δ_H = 4.65 and 4.66 are diagonal across from each other. The location of the different side chains was deduced from the CH long-range correlations.

The carbons at δ_C = 41.1 (C-6) and 41.3 (C-6′) which were directly bound to the protons at δ_H = 4.66 (H-6) and 4.65 (H-6′), both showed long-range correlations to the aromatic protons and thus each carried a phenyl group. This was further confirmed by the high chemical shifts of H-6′ and H-6 (δ_H = 4.65/4.66), which were like those in compound **2** deshielded by the neighboring aromatic rings. The methin carbon at δ_C = 54.1 (C-5) is connected to the (2*E*)-2-methoxy-4-oxo-but-2-en-oic acid methyl ester side chain, as deduced from the long-range coupling of C-4 (δ_C = 195.6) to H-5 (δ_H = 3.89) and the NOE between H-5 and H-3 (δ_H = 4.98). Finally, the NOEs between H-5′ (δ_H = 4.14) and the two methylene protons of the 3-oxopropionic acid methyl ester side chain at δ_H = 2.75 and 3.01 (H_2-3′) indicated that this side chain is located at C-5′ (δ_C = 53.9). Full analysis of the CH long-range correlations (Table 2) confirmed this constitution. The relative configuration of the cyclobutane ring could not be deduced from the 2D NOESY experiment due to overlapping of H-6 and H-6′. Thus, one-dimensional DPFGSE-NOE experiments (Double Pulsed Field Gradient Spin Echo NOE) were carried out at 750 MHz: Upon irradiation of H-5 (δ = 3.89) a double doublet appeared in the NOE spectrum at δ = 4.66 (H-6), which was clearly distinguishable from that at δ = 4.65 = (H-6′) which appeared upon irradiation of H-5′ (δ = 4.14). Thus, H-5 is *cis* to H-6 and *trans* to H-6′, and H-5′ is *cis* to H-6′ and *trans* to H-6, resulting in the same relative configuration as suggested for compound **2**. Further NOEs of H-5 to the aromatic protons H-8′/12′ (δ = 7.29, d, 7.5 Hz) and of H-5′ to H-8/12 (δ = 7.26, d, 7.7 Hz) confirmed this relative configura-

tion and enabled the assignment of these overlapping aromatic signals. The very close correspondence of the coupling constants of the cyclobutane protons of compounds **2** and **3** additionally confirmed their identical relative configurations. The similarity of the structures indicated that both compounds were closely related in their biosynthesis with compound **1** as their precursor.

Experimental Section

General

EIMS: 70 eV, HP 5989 B-Engine, HR-TOF-ESI-MS: QStar Pulsar (Applied Biosystems). FTIR: Nicolet IMPACT 410. NMR: Bruker Avance 500 (500/125 MHz); Varian ^{Unity}*INOVA* 750 MHz. The DGFGSE NOE (Double Pulsed Field Gradient Spin Echo NOE) was acquired with a mixing time of 1.0 s. TLC: silica gel $60F_{254}$ (Merck).

Plant material

Leaves and branches of *Miliusa balansae* Fin. & Gagn. were collected in Hoa Binh province, North Vietnam, in June 1999 and identified by Prof. Dr. Nguyen Tien Ban, Institute of Biological Resources and Ecology, Vietnamese Academy of Science and Technology. A voucher specimen (Nr. 2142) is deposited at this Institute.

Extraction and isolation

The dried and ground leaves and branches (750 g) were extracted four times with MeOH-H_2O (95 : 5) at r. t. The organic solvent was evaporated under reduced pressure and the residue extracted with *n*-hexane, EtOAc and *n*-BuOH successively, yielding *n*-hexane (15 g), EtOAc (19 g) and *n*-BuOH (11 g) extracts. The EtOAc extract was separated on a silica gel column (6 × 100 cm, 350 g, 230 – 400 mesh) with solvents of increasing polarity (0 – 100 % EtOAc in *n*-hexane) to yield 113 fractions. By eluting with 60 % EtOAc, fraction 81 (350 mg) was received. This fraction was crystallized from EtOAc to give octacosanoic acid (20 mg). Fraction 113 (150 mg, eluting with 100 % EtOAc) was fractionated on a silica gel column (1 × 35 cm, 20 g, 230 – 400 mesh) with EtOAc-$CHCl_3$ (1 – 2 % EtOAc) to give compounds **2** (13 mg) and **3** (5 mg).

*Miliubisstyryl A (**2**)*

Colorless needles from EtOAc/$CHCl_3$, m. p. 127 – 129 °C. – $[\alpha]_D^{25}$ = +8.5° (*c* = 0.1, MeOH). – IR (KBr): ν = 2920, 1744, 1678, 1607, 1431, 1216, 1168, 1085, 848 cm^{-1}. – EIMS (70 eV): *m/z* (%) = 492 (0.9) $[M]^+$, 477 (3.0) $[M-CH_3]^+$, 460 (8.5) $[M-CH_3OH]^+$, 433 (9.5), 401 (5.3), 369 (5.6), 349 (100), 317 (27.1), 299 (7.4), 257 (6.9), 245 (19.7), 215 (22.07), 171 (38.3), 143 (94.1), 115 (86.1), 103 (31.2), 69 (35.7), 59 (9.3). – HR-TOF-ESI-MS: *m/z* = 515.1690 (calcd. 515.1676 for $C_{28}H_{28}O_8Na$, $[M + Na]^+$). – NMR: see Table 1.

*Miliubisstyryl B (**3**)*

Colorless needles from EtOAc/$CHCl_3$, m. p. 208 – 210 °C. – $[\alpha]_D^{25}$ = +35° (*c* = 0.1, MeOH). – IR (KBr): ν = 2957, 1743 ($\nu_{C=O}$ ester), 1680 ($\nu_{C=O}$), 1620 ($\nu_{C=C}$ conjugated with C=O), 1437, 1221, 1170, 1080, 708 cm^{-1}. – EIMS (70 eV): *m/z* (%) = 450 (0.5) $[M]^+$, 391 (6.9) $[M-COOCH_3]^+$, 349 (31.7) $[M-CH_2\text{-}COOCH_3]^+$, 326 (33.8), 317 (20.9), 307 (6.2), 245 (13.4), 215 (29.1), 187 (37.1) 143 (100), 131 (78.9) $[cinnamoyl]^+$, 115 (89.81), 103 (64.0) $[styryl]^+$, 69 (42.6), 59 (27.2) $[COOCH_3]^+$. – HR-TOF-ESI-MS: *m/z* = 473.1577 (calcd. 473.1571 for $C_{26}H_{26}O_7Na$, $[M + Na]^+$), 923. 3213 $[2M + Na]^+$. – NMR: see Table 2.

Octacosanoic acid ($CH_3(CH_2)_{26}COOH$)

Solid, m. p. 90 – 92 °C. – ^1H NMR ($CDCl_3$, 500 MHz): δ = 2.35 (t, CH_2), 1.63 (quin, CH_2), 1.20 – 1.38 (CH_2), 0.88 (t, CH_3). – ^{13}C NMR ($CDCl_3$, 125 MHz): δ = 178.6, 33.8, 31.9, 29.7, 29.6, 29.5, 29.3, 29.2, 29.1, 24.7, 22.7, 14.1. ^1H and ^{13}C NMR data are in agreement with reference data [4]. – EIMS (70eV): *m/z* = 424 $[M]^+$, 396 $[M-(CH_2)_2]^+$, 382 $[M-(CH_2)_3]^+$, 368 $[M-(CH_2)_4]^+$, 356 $[M-(CH_2)_5]^+$, 340 $[M-(CH_2)_6]^+$. – IR (KBr): ν = 2920, 2852, 1705 (COOH), 1405 cm^{-1}.

Acknowledgements

We wish to thank Mr. Dang Vu Luong, Institute of Chemistry, Hanoi, and Dr. J. Schmidt, Institute for Plant Biochemistry, Halle/S., Germany, for NMR and MS measurements, respectively.

[1] R. Wu, Q. Ye, N. Y. Chen, G. L. Zhang, *Chinese Chemistry Letters* **2001**, *12*, 247 – 248.

[2] C. Kamperdick, N. H. Van, T. V. Sung, *Phytochemistry* **2002**, *61*, 991 – 994.

[3] D. T. Huong, C. Kamperdick, T. V. Sung, *Journal of Natural Products* **2004**, *67*, 445 – 447.

[4] S. Satish, D. S. Bhakuni, *Phytochemistry* **1972**, *11*, 2888 – 2889.