Two New Bis-styryl Compounds from *Miliusa balansae*

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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
gastro-pathy 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\(_2\)O 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 iso-
lated from this plant and reported in a previous pa-
per [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

(+)-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 \(^{13}\text{C}\) 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. \(^1\text{H}\) and \(^{13}\text{C}\) NMR data of compound 2
were similar to those of 1 [2] with two exceptions. In-
stead of the signals of the double bond C-5/C-6 in the
spectra of 1, compound 2 exhibited two aliphatic me-
thane groups (\(\delta_H = 3.94, 4.65\); \(\delta_C = 54.1, 41.4\)). This

![Fig. 1. Structures of compounds 1–3.](image-url)
indicated that compound 2 formally was a [2+2] cy cloaddition 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 the missing $\Delta^2$ double bond. The existence of a symmetric dimer was further established by the fact that C-5/5′ ($\delta_C = 54.1$) showed not only a direct correlation ($^1J_{CH} = 41.4$) in the HMQC experiment but at the same time a long-range correlation ($^3J_{CH}$) to the protons at $\delta^H_1 = 3.94$ (H-5/5′) in the HMBC spectrum. Similarly, C-6/6′ ($\delta_C = 41.4$) showed $^1J_{CH}$ and $^3J_{CH}$ correlations to the protons H-6/6′ at $\delta_1 = 4.65$. 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 [$\delta_H = 3.94$ (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, 5,6′-cis, 5′,6′-cis, 6,5-trans), only the latter one represents a AAXX′ 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 styril 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 $C_{29}H_{36}O_7$ 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$_6$H$_7$O$_3$]$^+$ and prominent fragments at $m/z = 115$ [C$_5$H$_2$O$_3$]$^+$ (89.8 %), 103 [C$_4$H$_7$O$_3$]$^+$ (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 $\delta_C = 173$.
Table 2. $^1$H and $^{13}$C NMR data (500/125 MHz) of compound 3 in CDCl$_3$ ($\delta$ in Hz in parentheses)*.

<table>
<thead>
<tr>
<th>Position</th>
<th>$\delta_C$</th>
<th>$\delta_H$</th>
<th>CH long-range correlations (HMBC)$^b$</th>
<th>NOESY correlations</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>163.9</td>
<td>–</td>
<td>H-3, 1-OMe</td>
<td>–</td>
</tr>
<tr>
<td>2</td>
<td>160.3</td>
<td>–</td>
<td>H-3, 2-OMe</td>
<td>–</td>
</tr>
<tr>
<td>3</td>
<td>101.0</td>
<td>4.98 s</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>4</td>
<td>195.6</td>
<td>–</td>
<td>H-3, H-5, H-6/H-6'</td>
<td>H-5, 2-CH$<em>2$, H$</em>{phenyl}$ (w)</td>
</tr>
<tr>
<td>5</td>
<td>54.1</td>
<td>3.89 dd (11.1, 6.4)</td>
<td>H-6/H-6', H-5'</td>
<td>H-3, H-6/H-6', H$_{phenyl}$</td>
</tr>
<tr>
<td>6</td>
<td>41.1</td>
<td>4.66 dd (11.2, 7.5)</td>
<td>H-5, H-5', H-6', H$_{phenyl}$</td>
<td>–</td>
</tr>
<tr>
<td>7</td>
<td>138.6$^a$</td>
<td>–</td>
<td>H-5/5', H-6, H$_{phenyl}$</td>
<td>–</td>
</tr>
<tr>
<td>8, 12</td>
<td>127.9$^{**}$</td>
<td>7.22–7.35</td>
<td>H-6, H$_{phenyl}$</td>
<td>–</td>
</tr>
<tr>
<td>9, 11</td>
<td>128.6$^{***}$</td>
<td>7.22–7.35</td>
<td>H$_{phenyl}$</td>
<td>–</td>
</tr>
<tr>
<td>10</td>
<td>127.6</td>
<td>7.22–7.35</td>
<td>H$_{phenyl}$</td>
<td>–</td>
</tr>
<tr>
<td>2'</td>
<td>167.0</td>
<td>–</td>
<td>2'-OCH$_3$, H-3'a, H-3'b</td>
<td>H-3'b, H-5' (w), H$_{phenyl}$ (w)</td>
</tr>
<tr>
<td>3'</td>
<td>48.3</td>
<td>–</td>
<td>a: 2.75 d (15.7)</td>
<td>H-3'a, H-5' (w)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>b: 3.01 d (15.7)</td>
<td>H-3'a, H-5' (w)</td>
</tr>
<tr>
<td>4'</td>
<td>201.0</td>
<td>–</td>
<td>H-6'/6', H-3'a, H-3'b, H-5'</td>
<td>–</td>
</tr>
<tr>
<td>5'</td>
<td>53.9</td>
<td>4.14 dd (11.2, 7.5)</td>
<td>H-5, H-6/6'</td>
<td>H-6'/6', H-3'a (w), H-3'b, H$_{phenyl}$</td>
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<tr>
<td>6'</td>
<td>41.3</td>
<td>4.65 dd (11.2, 6.4)</td>
<td>H-5, H-5, H-5', H$_{phenyl}$</td>
<td>–</td>
</tr>
<tr>
<td>7'</td>
<td>138.5$^a$</td>
<td>–</td>
<td>H-5/5', H-6', H$_{phenyl}$</td>
<td>–</td>
</tr>
<tr>
<td>8', 12'</td>
<td>128.4$^{**}$</td>
<td>7.22–7.35</td>
<td>H-5', H$_{phenyl}$</td>
<td>–</td>
</tr>
<tr>
<td>9', 11'</td>
<td>128.9$^{***}$</td>
<td>7.22–7.35</td>
<td>H$_{phenyl}$</td>
<td>–</td>
</tr>
<tr>
<td>10'</td>
<td>127.3</td>
<td>7.22–7.35</td>
<td>H$_{phenyl}$</td>
<td>–</td>
</tr>
<tr>
<td>1-OMe</td>
<td>52.8</td>
<td>3.77 s</td>
<td>–</td>
<td>2-OMe (w)</td>
</tr>
<tr>
<td>2-OMe</td>
<td>56.7</td>
<td>3.41 s</td>
<td>–</td>
<td>H-3, 1-OMe (w)</td>
</tr>
<tr>
<td>2'-OMe</td>
<td>52.2</td>
<td>3.57 s</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

* $H_{phenyl}$ = 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 DPFQSE NOE as described in the text; $e$ multiplicity at 750 MHz: ddd (11.2, 7.6, 0.8 Hz); $a$, $b$; $c$, $d$; $e$, $f$: signals exchangeable within one column.

201.0, a methoxyl ester group with the signals at $\delta_C = 167.0$, $\delta_C = 52.2$/ $\delta_H = 3.57$ and a methylene group at $\delta_C = 48.3$/$\delta_H = 2.75$ and 3.01 was elucidated as 3-oxopropionic acid methyl ester by the HMBC correlations $\delta_C = 167.0$ (C-2')/$\delta_H = 3.57$ (2'-OCH$_3$), 2.75 (H-3'a), 3.01 (H-3'b) and $\delta_C = 201.0$ (C-4')/$\delta_H = 2.75$, 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 $\delta_H = 3.89$/$\delta_H = 4.65$, 4.66 and $\delta_H = 4.14$/$\delta_H = 4.65$, 4.66 and by the weak $^3$J$_{HH}$ correlation of the protons at $\delta_H = 3.89$ and 4.14. Thus these two protons as well as the overlapping protons at $\delta_H = 4.65$ 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 $\delta_C = 41.1$ (C-6) and 41.3 (C-6') which were directly bound to the protons at $\delta_H = 4.66$ (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 ($\delta_H = 4.65/4.66$), which were like those in compound 2 deshielded by the neighboring aromatic rings. The methin carbon at $\delta_C = 54.1$ (C-5) is connected to the (2E)-2-methoxy-4-oxo-2-but-2-en-oic acid methyl ester side chain, as deduced from the long-range coupling of C-4 ($\delta_C = 195.6$) to H-5 ($\delta_H = 3.89$) and the NOE between H-5 and H-3 ($\delta_H = 4.98$). Finally, the NOEs between H-5' ($\delta_H = 4.14$) and the two methylene protons of the 3-oxopropionic acid methyl ester side chain at $\delta_H = 2.75$ and 3.01 (H$_2$-3') indicated that this side chain is located at C-5' ($\delta_C = 53.9$). 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 DPFQSE NOE experiments (Double Pulsed Field Gradient Spin Echo NOE) were carried out at 750 MHz: Upon irradiation of H-5 ($\delta = 3.89$) a double doublet appeared in the NOE spectrum at $\delta = 4.66$ (H-6), which was clearly distinguishable from that at $\delta = 4.65 = (H-6')$ which appeared upon irradiation of H-5' ($\delta = 4.14$). 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' ($\delta = 7.29$, d, 7.5 Hz) and of H-5' to H-8/12 ($\delta = 7.26$, d, 7.7 Hz) confirmed this relative configuration.
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 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-H2O (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-CHCl3 (1–2 % EtOAc) to give compounds 2 (13 mg) and 3 (5 mg).

**Miliubisstyryl A (2)**

Colorless needles from EtOAc/CHCl3, m. p. 127–129 °C. – [α]D25 = +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−CH3]+, 460 (8.5) [M−CH2OH]+, 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 C28H28O8Na, [M + Na]+). – NMR: see Table 1.

**Miliubisstyryl B (3)**

Colorless needles from EtOAc/CHCl3, m. p. 208–210 °C. – [α]D25 = +35° (c = 0.1, MeOH). – IR (KBr): ν = 2957, 1743 (νC=O ester), 1680 (νC=O), 1620 (νC=C conjugated with C=O), 1437, 1221, 1170, 1080, 708 cm−1. – EIMS (70 eV): m/z (%) = 540 (0.5) [M]+, 391 (6.9) [M−COOCH3]+, 349 (31.7) [M−CH2-COOCH3]+, 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) [COOCH3]+. – HR-TOF-ESI-MS: m/z = 473.1577 (calcd. 473.1571 for C26H26O7Na, [M + Na]+), 923, 3213 [2M + Na]+. – NMR: see Table 2.

**Octacosanoic acid (CH3(CH2)26COOH)**


**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.


