Two New Dimeric Coumarins Isolated from *Murraya exotica*

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Bismurrangatin and murramarin A, two new coumarins, were isolated from the vegetative branches of *Murraya exotica*. Murramarin A is a rare type of bicoumarin that connects two coumarin moieties by orthoester structure. The structures were elucidated based on spectroscopic methods, especially by 2D-NMR experiments.

Key words *Murraya exotica*; bicoumarin; bismurrangatin; murramarin A

Murraya species have been used in different folk medicines in India, Australia and South Africa.[1,2] A decoction of the leaves is a remedy for bruises and has been used to treat certain fungoid skin troubles; the leaves and fruits are astringent, antidysenteric and act as a febrifuge; the leaves have been used as a tonic, a toothache remedy, an emmenagouge, stimulant and astringent; an infusion of the leaves or bark is regarded as antidiarrheic and antidysenteric.[3] Moreover, *M. exotica* has been tested successfully as an anticancer drug.[4] While *Murraya* species have been used in folk medicine, *M. exotica* has been considered similar to or identical to *M. paniculata*. However, recent studies have shown that this species may be a distinct taxon. Currently, studies of the constitution of *Murraya* plants have been tackled from the point of view of chemotaxonomy. In continuing further investigation of the constituents of Rutaceous plants, we examined the constituents of *M. exotica* cultivated and collected in Egypt and isolated two new bicoumarins named bismurrangatin (**1**) and murramarin A (**4**). This paper analyzes the structural elucidation of these compounds.

Bismurrangatin (**1**) was isolated as a colorless oil, $[\alpha]_D$ +2.5° (MeOH) and analyzed for $C_{30}H_{30}O_9$ from the quasi-molecular ion at *m/z* 557.1780 (Calcd for $C_{30}H_{30}O_9Na$) in its high-resolution (HR)-FAB-MS. The IR (1730, 1608 cm^{-1}) and UV (264, 316 nm) spectral data suggested the presence of a 7-oxygenated coumarin nucleus.[5] The ^{1}H-NMR showed signals of four pairs of AB-type doublets [δ 7.23, 5.95 (each 1H, J=9.5 Hz, H-4′, H-3′); 6.95, 6.57 (each 1H, J=8.4 Hz, H-5′, H-6′); 7.33, 6.01 (each 1H, J=9.5 Hz, H-4, H-3); 7.00, 6.54 (each 1H, J=8.8 Hz, H-5, H-6)] as well as two methoxyl singlets at δ 3.91 and 3.88, suggesting the presence of two 7-methoxy-8-substituted coumarin nuclei. The presence of two isopropenyl groups was determined by the ^{1}H-NMR signals at δ 1.72, 1.69 (each 3H, s), 4.63 (1H, s), 4.60 (1H, s) and 4.72 (2H, s), in addition to the ^{13}C-NMR signals at δ 17.4 (q), 17.5 (q), 113.9 (t), 114.0 (t), 143.4 (s) and 143.8 (s). The two pairs of doublets at δ 5.44 and 5.00 (each 1H, J=8.1 Hz, H-9′, H-10′) and δ 5.10 and 5.02 (each 1H, J=8.8 Hz, H-9, H-10) revealed the presence of two pairs of benzylic and allylic protons. These data suggested the structure of bismurrangatin (**1**) to be composed of two 7-methoxy-8-(1,2-dihydroxy-3-methyl-3-butenyl) coumarins nuclei such as murrangatin (**2**)[6] and minumicrolin (**3**)[7] moieties, and the linkage of the two nuclei through an ether bond was presumed. Full assignment of the ^{1}H- and ^{13}C-NMR signals of bismurrangatin (**1**) was made using heteronuclear multiple quantum coherence (HMQC) and heteronuclear multiple bond correlation (HMBC) experiments. In the HMBC experiment (Fig. 1), key correlations were observed: the benzylic proton signal at δ 5.10 (H-9) showed cross peaks with the carbon sig-

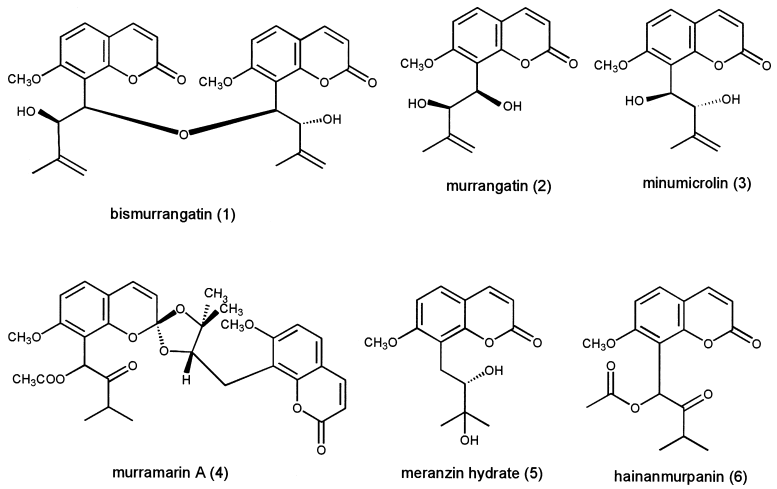

Chart 1

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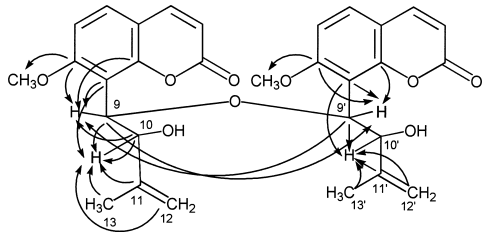

Fig. 1. Key C–H Long-Range Correlations in the HMBC Spectrum of **1**

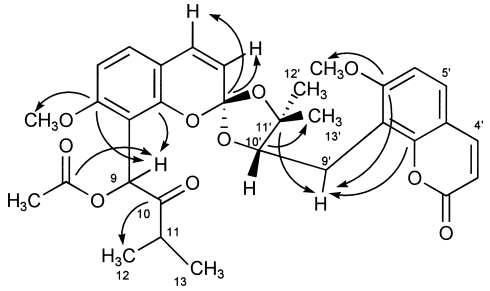

Fig. 2. Key C–H Long-Range Correlations in the HMBC Spectrum of **4**

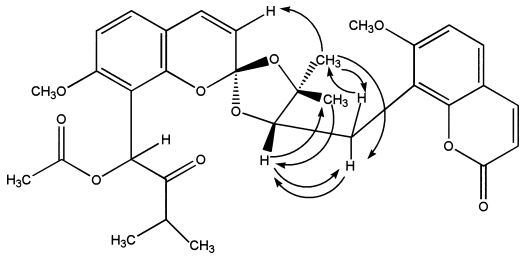

Fig. 3. Key NOEs of **4**

nals at δ 76.8 (C-10), 78.7 (C-9′), 114.9 (C-8), 161.5 (C-7) and 152.2 (C-8a); another benzylic proton signal at δ 5.44 (C-9′) showed cross peaks at δ 76.9 (C-9), 114.4 (C-8′), 160.9 (C-7′) and 153.5 (C-8′a); the proton signal at δ 5.02 (H-10) showed cross peaks at δ 76.9 (C-9), 114.9 (C-8), 143.4 (C-11), 114.0 (C-12) and 17.4 (C-13); the signal at δ 5.00 (H-10′) showed peaks at δ 78.7 (C-9′), 114.4 (C-8′), 143.8 (C-11′), 113.9 (C-12′) and 17.5 (C-13′). In conclusion, the structure of bismurrangatin was determined to be **1**, connected by an ether bond between the C-9 and C-9′ of murrangatin (**2**) and minumicrolin (**3**) moieties. In our previous paper,[8] we reported the revision of the relative configurations for the α-glycol moieties of murrangatin (**2**) and minumicrolin (**3**) to *threo* and *erythro*, respectively. In comparison to the ^{1}H-NMR spectra of **2** and **3**, the signals due to the allylic methyl and *exo*-methylene of **3** resonated at lower fields than those of **2**. Furthermore, the signals indicated significant differences due to *exo*-methylene protons that were observed as two singlets in the spectrum in contrast to an almost singlet signal, as in that of **3**. In the ^{1}H-NMR spectrum of bismurrangatin (**1**), allylic methyl and *exo*-methylene signals were observed at δ 1.72 (13′-Me) and 4.72 (2H, s, H-12′), and 1.69 (13-Me), 4.63 (1H, s, H-12) and 4.60 (1H, s, H-12). Based on these spectral data, the structure of **1** was presented as shown in Chart 1, consisting of murrangatin (**2**) and minumicrolin (**3**) with the exception of the absolute stereochemistry.

Murramarin A (**4**), a colorless oil, $[\alpha]_D$ +96° (MeOH), was assigned the molecular formula $C_{32}H_{34}O_{10}$ ($[M]^+$ *m/z* 578.2147) by HR-EI-MS. The IR (1724, 1610 cm^{-1}) and UV [220, 240 (sh), 258, 300 (sh), 312, 326 (sh) nm] spectral data indicated the presence of a 7-oxygenated coumarin nucleus.[5] The ^{1}H-NMR spectrum showed the presence of four pairs of doublets at δ 7.96 and 6.27 (each 1H, d, J=9.5 Hz), 7.58 and 7.08 (each 1H, d, J=8.8 Hz), 6.82 and 5.59 (each 1H, d, J=9.9 Hz), and 7.31 and 6.70 (each 1H, d, J=8.8 Hz), suggesting the existence of two 7-oxygenated-8-substituted coumarin units. However, in the ^{13}C-NMR spectrum, only a single lactone carbonyl carbon signal (δ 160.2, C-2′) and orthoester carbon signal (δ 116.2, C-2) were observed. These data indicated that one of the two lactone carbonyls in **4** was replaced by an orthoester structure similar to the known spirobicoumarins, rivulobirins B, C[9] and paradisin C.[10] The presence of benzylic methylene and a vicinal methine proton was indicated by signals at δ 3.05 (1H, dd, J=9.5, 14.3 Hz), 2.87 (1H, dd, J=2.6, 14.3 Hz) and 4.32 (1H, dd, J=2.6, 9.5 Hz). On irradiation of the methoxy signals at δ 3.75 and 3.95, 6% and 5% increments were observed on the aromatic proton signals at δ 6.70 and 7.08, respectively, indicating the location of these aromatic protons at C-6 and/or C-6′. The presence of an isobutyl group was also suggested by the ^{1}H-NMR [δ 0.41 (3H, br s), 0.80 (3H, d, J=7.0 Hz), 2.23 (1H, t, J=7.0 Hz)] and ^{13}C-NMR [δ 205.4 (s), 35.0 (d), 19.0 (q), 16.6 (q)] signals. The remaining three proton signals were assigned as follows: at δ 1.94 an acetyl, and at δ 1.47 and 1.26 geminal methyls, along with one proton singlet at δ 6.59 as benzylic methine attached to the carbon substituted with both oxyen and carbonyl. The relations of these moieties and two 7-methoxycoumarin were determined by extensive 2D-NMR experiments. The C–H correlation between δ 6.82 (H-4)/116.2 (C-2) in the HMBC spectrum (Fig. 2) and the NOE correlation between H-3 (δ 5.59)/12′-Me (δ 1.26) suggested the presence of an orthoester structure in **4**. The linkage was also presumed by the correlation of 12′-Me (δ 1.26) and C-10′ (δ 82.5), H-9′ (δ 3.05) and C-7′ (δ 160.1), C-8′a (δ 152.7) and C-11′ (δ 82.7). The relative configurations of the C-2 and C-10′ in **4** were confirmed by the analysis of its NOESY spectrum (Fig. 3). The entire structure was established by the ^2J and ^3J correlations observed in the HMBC spectrum (Fig. 2), and it was clarified to be composed of meranzin hydrate (**5**)[11] and hainanmurpanin (**6**).[12] Spirocoumarins containing an orthoester structure have been isolated from *Pleurospermum rivulorum* (Umbelliferae),[9] *Citrus paradisi* (Rutaceae),[10] and *Murraya exotica*.[13] Murramarin A is the second bicoumarin linked by orthoester, and it does not contain a furanocoumarin moiety. Further investigation of other constituents is in progress.

Experimental

General Remarks UV spectra were recorded on a UVIDEC-610C double-beam spectrophotometer (JASCO) in MeOH. IR spectra were measured on an IR-230 (JASCO) in $CHCl_3$, and optical rotations on a DIP-370 (JASCO) in MeOH at 25 °C. MS were taken with a HX-110 (JEOL) or JMS-700 (JEOL) spectrometer with a direct inlet system. ^{1}H- and ^{13}C-NMR, NOE, HMQC and HMBC (J=8 Hz) spectra were recorded on JNM A-400, A-600, and/or ECP-500 (JEOL) spectrometers in $CDCl_3$. Preparative TLC was carried out on Kieselgel 60 F_{254} (Merck).

Plant Material Vegetative branches of *M. exotica*, collected from the Botanical Garden of Aswan in southern Egypt in January 1998, were used as the plant material in this study. A voucher specimen has been deposited at

the Herbarium of the Botany Department, Aswan, Faculty of Science, South Valley University, Aswan, Egypt, 10809.

Extraction and Isolation The dried and powdered vegetative branches (1 kg) of *M. exotica* were extracted with acetone (2.6 l×3) at room temperature for 7 d. The extract was evaporated under reduced pressure to give an acetone extract (75.4 g). The extract was chromatographed on silica gel (9×19 cm), with successive elution with hexane, toluene, CH_2Cl_2, CH_2Cl_2–acetone (9 : 1), CH_2Cl_2–acetone (8 : 2), acetone and MeOH. The CH_2Cl_2 eluate (4.02 g) was submitted to centrifugal chromatography and eluted with $CHCl_3$ containing increasing amounts of acetone. The $CHCl_3$ eluate was submitted to repeated PTLC [solvent: acetone–benzene (2 : 8), acetone–$CHCl_3$ (1 : 9), AcOEt–benzene (3 : 7)] to give bismurrangatin (**1**) (5 mg) and murramarin A (**4**) (3.7 mg).

Bismurrangatin (**1**): Colorless oil. $[\alpha]_D$ +2.5° (c=0.14, MeOH). EI-MS m/z: 463 (15), 259 (71), 242 (58), 231 (82), 205 (100, base peak), 203 (22), 189 (71), 131 (25). FAB-MS m/z: 557 $[M+Na]^+$. HR-FAB-MS Calcd for $C_{30}H_{30}O_9Na$ 557.1788. Found 557.1780. UV (λ_{max}, MeOH): 204, 264, 316 nm. IR ($CHCl_3$, cm^{-1}): 3510 (br), 1730, 1608. ^{1}H-NMR ($CDCl_3$, δ): 7.33 (1H, d, J=9.5 Hz, H-4), 7.23 (1H, d, J=9.5 Hz, H-4′), 7.00 (1H, d, J=8.8 Hz, H-5), 6.95 (1H, d, J=8.4 Hz, H-5′), 6.57 (1H, d, J=8.4 Hz, H-6′), 6.54 (1H, d, J=8.8 Hz, H-6), 6.01 (1H, d, J=9.5 Hz, H-3), 5.95 (1H, d, J=9.5 Hz, H-3′), 5.44 (1H, d, J=8.1 Hz, H-9′), 5.10 (1H, d, J=8.8 Hz, H-9), 5.02 (1H, d, J=8.8 Hz, H-10), 5.00 (1H, d, J=8.1 Hz, H-10′), 4.72 (2H, s, H-12′), 4.63 (1H, s, H-12), 4.60 (1H, s, H-12), 3.91 (3H, s, 7′-OMe), 3.88 (3H, s, 7-OMe), 1.72 (3H, s, 13′-Me), 1.69 (3H, s, 13-Me). NOE: irradiation at δ 3.88—15% enhancement at δ 6.54; irradiation at δ 3.91—15% enhancement at δ 6.57; irradiation at δ 5.44, 5.10, 5.02 and 5.00—no enhancement observed on any proton signals. ^{13}C-NMR ($CDCl_3$, δ): 161.5 (s, C-7), 160.9 (s, C-7′), 160.4 (s, C-2′), 160.1 (s, C-2), 153.5 (s, C-8′a), 152.2 (s, C-8a), 143.8 (s, C-11′), 143.4 (s, C-11), 143.1 (d, C-4), 143.0 (d, C-4′), 128.2 (d, C-5), 128.1 (d, C-5′), 114.9 (s, C-8), 114.4 (s, C-8′), 114.0 (t, C-12), 113.9 (t, C-12′), 112.3 (d, C-3), 112.4 (d, C-3′), 111.9 (s, C-4′a), 111.5 (s, C-4a), 107.6 (d, C-6), 106.9 (d, C-6′), 78.7 (d, C-9′), 77.3 (d, C-10′), 76.9 (d, C-9), 76.8 (d, C-10), 56.2 (q, 7′-OMe), 56.0 (q, 7-OMe), 17.5 (q, 13′-Me), 17.4 (q, 13-Me).

Murramarin A (**4**): Colorless oil. $[\alpha]_D$ +96° (c=0.135, MeOH). EI-MS m/z: 578 (M^+, 16), 507 (26), 368 (10), 256 (15), 243 (19), 210 (12), 206 (14), 205 (base peak, 100), 203 (17). HR-EI-MS Calcd for $C_{32}H_{34}O_{10}$ 578.2150. Found 578.2147. UV (λ_{max}, MeOH): 220, 240 (sh), 258, 300 (sh), 312, 326 (sh) nm. IR ($CHCl_3$, cm^{-1}): 1724, 1610. ^{1}H-NMR (DMSO-d_6, δ): 7.96 (1H, d, J=9.5 Hz, H-4′), 7.58 (1H, d, J=8.8 Hz, H-5′), 7.31 (1H, d, J=8.8 Hz, H-5), 7.08 (1H, d, J=8.8 Hz, H-6′), 6.82 (1H, d, J=9.9 Hz, H-4), 6.70 (1H, d, J=8.8 Hz, H-6), 6.59 (1H, s, H-9), 6.27 (1H, d, J=9.5 Hz, H-3′), 5.59 (1H, d, J=9.9 Hz, H-3), 4.32 (1H, dd, J=2.6, 9.5 Hz, H-10′), 3.95 (3H, s, 7′-OMe), 3.75 (3H, s, 7-OMe), 3.05 (1H, dd, J=14.3, 9.5 Hz, H-9′), 2.87 (1H, dd, J=14.3, 2.6 Hz, H-9′), 2.23 (1H, t, J=7.0 Hz, H-11), 1.94 (3H, s, CH_3CO–), 1.47 (3H, s, 13′-Me), 1.26 (3H, s, 12′-Me), 0.80 (3H, d, J=7.0 Hz, 12-Me), 0.41 (3H, br s, 13-Me). ^{13}C-NMR (DMSO-d_6, δ): 205.4 (s, C-10), 169.2 (s, CH_3CO–), 160.2 (s, C-2′), 160.1 (s, C-7′), 158.3 (s, C-7), 152.7 (s, C-8′a), 150.7 (s, C-8a), 144.5 (d, C-4′), 129.2 (d, C-5), 127.9 (d, C-4), 127.7 (d, C-5′), 117.4 (d, C-3), 116.2 (s, C-2), 113.3 (s, C-4′a), 112.6 (d, C-3′), 112.4 (s, C-8′), 112.0 (s, C-4a), 109.1 (s, C-8), 108.0 (d, C-6′), 104.4 (d, C-6), 82.7 (s, C-11′), 82.5 (d, C-10′), 68.6 (d, C-9), 56.3 (q, 7′-OMe), 56.2 (q, 7-OMe), 35.0 (d, C-11), 25.7 (q, 13′-Me), 22.5 (t, C-9′), 22.3 (q, 12′-Me), 20.3 (q, CH_3CO–), 19.0 (q, C-12), 16.6 (q, C-13).

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