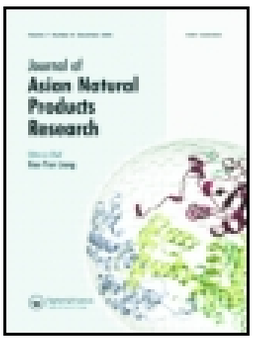

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Constituents of *Aegle marmelos* from Myanmar

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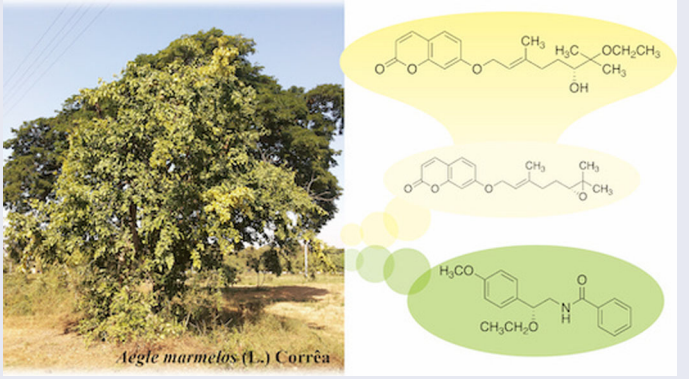
ABSTRACT

Five compounds (**1**–**5**), including three coumarins (**1**–**3**) and two alkaloids (**4**,**5**) were isolated during the first investigation of the stem bark of *Aegle marmelos* (L.) Corrêa. collected in Myanmar. Their structures were determined by NMR spectra analysis. Among them, 7′-*O*-ethylmarmin (**1**) and 2-*O*-ethyltembamide (**5**) were identified as new compounds. Skimmianine (**4**) showed moderate cytotoxicity against a HeLa cell line, and 7′-*O*-ethylmarmin (**1**), marmin (**2**), and (+)-epoxyaurapten (**3**) displayed weak radical scavenging activity according to a DPPH scavenging assay.



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KEYWORDS
Aegle marmelos; Rutaceae; coumarins; cytotoxicity; radical scavenging activity

1. Introduction

Aegle marmelos (L.) Corrêa is a small to medium-sized deciduous, aromatic tree belonging to the family Rutaceae. It is widely distributed in India, Bangladesh, Egypt, Malaysia, Myanmar, Pakistan, Sri Lanka, and Thailand [1]. Its roots, stem bark and fruits exhibit a wide range of medicinal properties including antidiabetic, anticancer,

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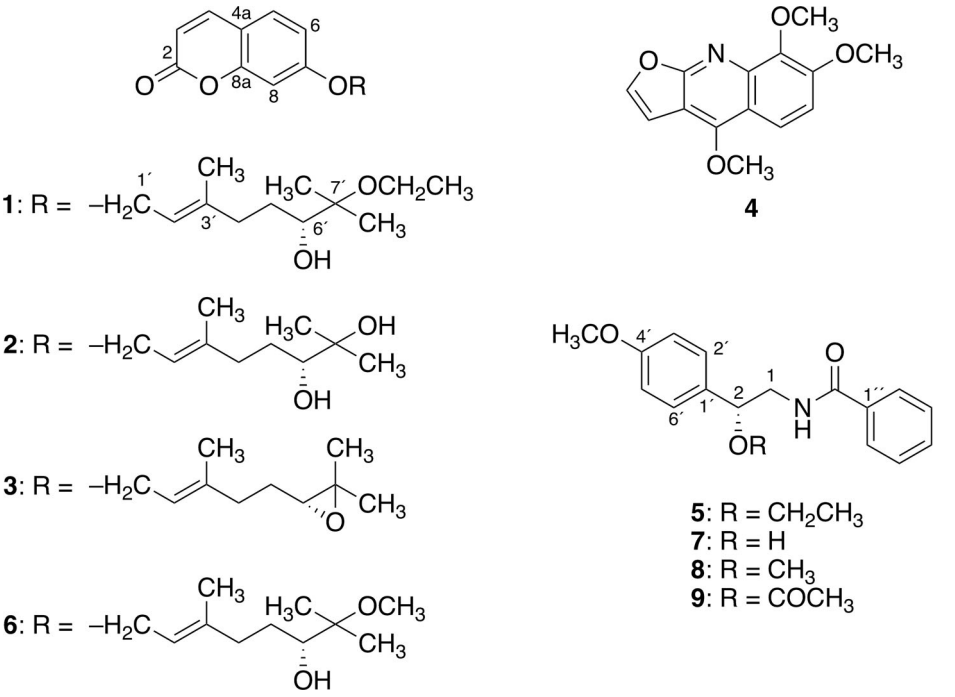


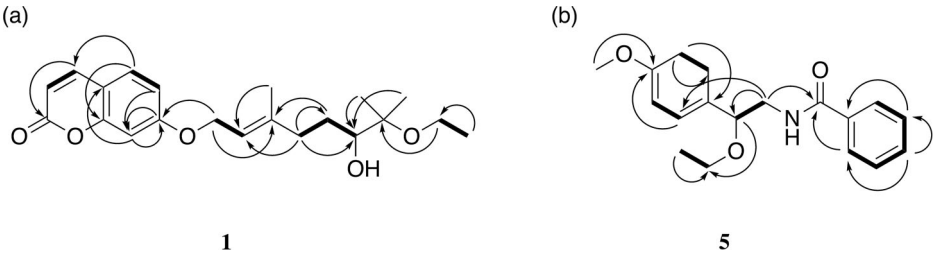
Figure 1. Structures of compounds **1**–**5** isolated from *A. marmelos* and related metabolites **6**–**9**.

antibacterial, antifungal, antipyretic, analgesic, antioxidant, cardio protective, radioprotective, antidiarrheal, antidysentery, antiulcer, and wound-healing activities [2]. In particular, an active principle occurring in the leaf aqueous extract of *A. marmelos* shows insulin-like-hypoglycemic activity. Leaves are also used to treat jaundice. In addition, an alcoholic extract of the seeds has shown antiallergic activity [2]. Several coumarins, including xanthotoxol and alpha-fagarine, hydroxyamides such as tembamide (**7**), sterols, and essential oils have been isolated from different parts of the plant [3–6]. In this report, we describe the isolation and structure determination of five compounds (**1**–**5**) from the ethanolic extract of *A. marmelos* stem bark, which constitutes, to the best of our knowledge, the first investigation on this plant collected in Myanmar (Figure 1).

2. Results and discussion

Repetitive column chromatographic and HPLC separations of the EtOH extract of *A. marmelos* over silica gel afforded two new compounds, i.e., 7′-*O*-ethylmarmin (**1**) and 2-*O*-ethyltembamide (**5**), together with the three known compounds, (+)-marmin (**2**) [6], (+)-epoxyaurapten (**3**) [6], and skimmianine (**4**) [7], which were identified by comparison with the literature data. The structures of the new compounds **1** and **5** were determined based on ^{1}H- and ^{13}C-NMR spectral data and ^{1}H-^{1}H COSY and HMBC correlations (Figure 2).

Compound **1**, with $[\alpha]_D^{27}$ +32.0 (*c* 0.03, $CHCl_3$), had the molecular formula $C_{21}H_{28}O_5$ as determined by the HR-DART-MS which exhibited a pseudo-molecular



1 **5**

Figure 2. ^{1}H-^{1}H COSY correlations (bold lines) and (^{1}H → ^{13}C) HMBC correlations (curved arrows) of (a) compound **1** and (b) compound **5**.

ion peak at *m/z* 361.2020 corresponding to $[M+H]^+$. Moreover, the hydrogen and carbon numbers were deduced from the ^{1}H- and ^{13}C-NMR spectra, respectively. The IR spectrum exhibited absorption bands at 3471, 3082, and 1733 cm^{-1}, indicating the presence of hydroxyl group, double bond, and carbonyl group of a ester. The ^{1}H-NMR spectrum (600 MHz, $CDCl_3$) of **1** showed the typical splitting pattern of a pair of coumarin H–3 and H–4 at δ 6.24 (1H, d, $J = 9.4$ Hz) and 7.63 (1H, d, $J = 9.4$ Hz), along with three aromatic protons at δ 7.36 (1H, d, $J = 8.5$ Hz; H–5), 6.85 (1H, dd, $J = 2.4$, 8.5 Hz; H–6) and 6.82 (1H, d, $J = 2.4$ Hz; H–8). The ^{1}H-NMR spectrum of compound **1** also showed a singlet methyl signal attached on an olefinic carbon at δ 1.78 (3′-Me), two singlet methyl signals at δ 1.12 and 1.09 (7′-Me x 2), and signals of ethoxy group at δ 3.43 (2H, q, $J = 7.0$ Hz) and 1.15 (3H, t, $J = 7.0$ Hz). The ^{13}C-NMR spectrum displayed 21 signals: eleven were assignable to sp^2 carbons, including a CO and two oxygenated carbons. The remaining signals were attributable to an oxygenated methylene, three methylenes, an oxygenated methine, an oxygenated quaternary carbon and four methyl carbons. These NMR data were combined by the HMBC correlations to suggest coumarin moiety and a prenyl side chain shown in Figure 2(a). Moreover, the HMBC correlations revealed that the ethoxy group was attached to a quaternary carbon of C-7′ of the side chain, and the prenyl chain was attached to C-7 position as an ether to confirm the structure of compound **1**. This compound corresponds to an 7′-*O*-ethyl analog of marmin (**2**) and the stereochemistry of the hydroxy group at C-6′ was speculated to be *R*, since (+)-epoxyaurapten (**3**) [6] was obtained at the same time, together with their similar $[\alpha]_D$ and NMR values.

Compound **5**, $[\alpha]_D^{20}$ +10.2 (*c* 0.03, $CHCl_3$), had the molecular formula $C_{18}H_{21}NO_3$ as determined by the HR-DART-MS which exhibited a pseudo-molecular ion peak at *m/z* 300.1591 corresponding to $[M+H]^+$. The ^{1}H-NMR spectrum (600 MHz, methanol-d_4) of **1** showed signals corresponding to protons on 1,4-disubstituted benzene ring and monosubstituted benzene ring (δ 7.28 (2H, d, $J = 8.6$ Hz; H–2′,6′), 6.92 (2H, d, $J = 8.6$ Hz; H–3′,5′), 7.75 (2H, br d, $J = 7.5$ Hz; H–2″,6″), 7.44 (2H, br d, $J = 7.5$ Hz; H–3″,5″), and 7.53 (1H, t, $J = 7.0$ Hz; H–4″). Moreover, signals of an unequivalent methylene protons (δ 3.56 (1H, dd, $J = 13.6$, 5.2 Hz) and 3.52 (1H, dd, $J = 13.6$, 7.9 Hz); H–1) and a proton on a carbon attached by an oxygen group (δ 4.51 (1H, dd, H–2) to form a partial structure –CH_2–CH(OR)–. This compound also had an ethoxy (δ 3.45 − 3.34 (2H, m), and 1.16 (3H, t, $J = 7.0$ Hz)) and a methoxy (δ 3.78 (3H, s)) groups. Compound **5** must have an amide moiety since IR absorptions at 1652, 1611, 1579, 1509, and 1246 cm^{-1}. These partial structures were combined on

the basis of the HMBC correlations as shown in Figure 2(b) to suggest that an amide of a benzoic acid and a tyramine analog. The stereochemistry of C-2 remains unresolved, but it can be *R* since the specific rotation of both compound **5** and 7′-*O*-methyltembamide (**8**) are very similar [8].

Moreover, comparison of the NMR data of compounds **1** and **5** with that of 7′-*O*-methylmarmin (**6**), which was isolated from a methanol extract of the roots of *A. marmelos* [6], and synthetic tembamide (**7**) [9] and natural acetyltembamide (**9**) isolated from an acetone extract of aerial parts of *Piper guayranum* (Piperaceae) [10], respectively, revealed that the spectral data of each set of compounds were almost same except for the signals of methoxy and ethoxy groups, which allowed to confirm the structures of the new compounds as 7′-*O*-ethylmarmin (**1**) and 2-*O*-ethyltembamide (**5**). Both compounds **1** and **6** can be envisaged as the products of the nucleophilic ring opening of the epoxide moiety of **3** by alcohols. This suggests that **1** and **6** may be artifacts, whereas compound **6** was previously claimed to be a naturally occurring metabolite [6]. The low amounts of compounds **1** and **3** made it difficult to prove whether compound **1** is an artifact. On the other hand, compound **5** also has an ethoxyl group at the benzyl position, whereas compounds **7** and **8** are reported to possess hydroxy and methoxy groups, respectively, though compound **8** was obtained from ethanol extract of *Zanthoxylum hyemale*. If compound **5** is an artifact derived from compound **7**, it would be a racemic modification, since this reaction is not stereoselective. These observations suggest that **1** and **5** were generated enzymatically, and thus, these compounds are most likely naturally occurring.

Skimmianine (**4**) showed moderate cytotoxicity (IC_{50} 55 µM) against HeLa cells in a standard MTT test [11], whereas (+)-epoxyaurapten (**3**) and 2-*O*-ethyltembamide (**5**) exhibited very low activity (IC_{50} >100 µM). The other isolated compounds could not be tested due to their insolubility in saline solution containing 10% DMSO. In the DPPH test [12], 7′-*O*-ethylmarmin (**1**), marmin (**2**), and (+)-epoxyaurapten (**3**) displayed weak radical scavenging activity (EC_{50} > 100 µM), whereas skimmianine (**4**) and 2-*O*-ethyltembamide (**5**) showed no activity.

3. Experimental

3.1. General experimental procedures

UV spectra were recorded in MeOH on a JASCO V-560 UV–vis spectrophotometer (Tokyo, Japan). IR spectra were recorded using KBr pellets on a JASCO FT/IR-410 spectrophotometer (Tokyo, Japan). Optical rotations were measured on a JASCO P-2200 polarimeter (Tokyo, Japan). ^{1}H- and ^{13}C-NMR spectra were recorded in $CDCl_3$ or in methanol-d_4 on a Bruker AvanceIII HD600 spectrometer (MA, USA) equipped with a Prodigy liquid nitrogen cryoprobe. Chemical shifts for ^{1}H- and ^{13}C-NMR spectra are given in ppm (δ) relative to the TMS signal (δ_H 0.00) and solvent signals (δ_C 77.0 for $CDCl_3$ and 49.0 for methanol-d_4, respectively) as internal standards. Coupling constants (*J*) are expressed in Hz. Positive mode DART-MS was scanned on an Exactive Plus (Thermo Scientific Inc., MA, USA) equipped with DART ion source (AMR Inc., Tokyo, Japan). EI- and FAB-MS data were determined with JEOL JMS MS-700 and HX-110 instruments (Tokyo, Japan), respectively. *m*-Nitrobenzyl

alcohol (TCI, Tokyo, Japan) was used as a matrix for FAB-MS. Column chromatographic separations were carried out on silica gel (BW-820MH, Fuji Silysia, Seto, Japan). Analytical TLC was performed on precoated silica gel 60 F_{254} plates (Merck, Darmstadt, Germany, Art. 5715). Spots were visualized under UV light at 254 nm and by spraying with anisaldehyde–sulfuric acid reagent, followed by heating at 200 °C for 1 min. Semipreparative HPLC separations were performed on a chromatograph equipped with a multiwavelength detector (JASCO, Tokyo, Japan) using a Develosil ODS UG-5 column (ϕ20 × 250 mm, Nomura Chemical, Seto, Japan). All other chemicals are analytical grade products of TCI (Tokyo, Japan) and Kanto Chemical Company (Tokyo, Japan).

3.2. Plant material

Stem barks of *Aegle marmelos* (L.) Corrêa (Rutaceae) were collected in October 2015 in the Sagaing Region, Myanmar. The plant was identified by Professor Dr Soe Myint Aye from the Department of Botany, University of Mandalay, Myanmar. A voucher specimen (N-7) was deposited at the Department of Chemistry, University of Mandalay, Myanmar.

3.3. Extraction and isolation

Air-dried ground plant material (1 kg) was extracted with EtOH (7 L) over one week. Filtration of the suspension and solvent evaporation under vacuum produced a sticky residue (96.6 g), which was partitioned between water and EtOAc to give an EtOAc-soluble oily residue (26.4 g) after solvent evaporation. A part of this fraction (2.0 g) was separated by a column chromatography on a silica gel column using a gradient of EtOAc in hexane as eluent mixtures to give 26 main fractions (A1–A26). Semipreparative HPLC separation of fraction A7 (178 mg) using a gradient mixture of MeOH–H_2O (2:98, v/v) and MeOH (from 50:50 to 0:100, v/v) yielded compounds **1** (4.1 mg), **2** (5.1 mg), **3** (5.2 mg), **4** (0.8 mg), and **5** (11.7 mg), respectively.

3.3.1. 7-O-Ethylmarmin (1)

Colorless oil. $[\alpha]_D^{27}$ +32.0 (*c* 0.03, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 215.0 (sh, 2.95), 324.0 (3.29) nm; IR (KBr) ν_{max} 3471, 3082, 2974, 2876, 1733, 1613, 1556, 1402, 1278, 1231, 1125, 1071, 835, 755 cm^{-1}. ^{1}H-NMR (600 MHz in $CDCl_3$) δ 7.63 (1H, d, J = 9.4 Hz, H–4), 7.36 (1H, d, J = 8.5 Hz, H–5), 6.85 (1H, dd, J = 2.4, 8.5 Hz, H–6), 6.82 (1H, d, J = 2.4 Hz, H–8), 6.24 (1H, d, J = 9.4 Hz, H–3), 5.52 (1H, t, J = 6.5 Hz, H–2′), 4.61 (2H, d, J = 6.5 Hz, H–1′), 3.43 (2H, q, J = 7.0 Hz, 7′-O$\underline{CH_2}CH_3$), 3.40 (1H, dd, J = 2.4, 10.0 Hz, H–6′), 2.41 (1H, ddd, J = 6.6, 9.7, 14.9 Hz, H–4′), 2.14 (1H, ddd, J = 4.8, 10.1, 14.9 Hz, H–4′),1.78 (3H, s, 3′-Me), 1.58 − 1.53, 1.50 − 1.44 (each 1H, m, H–5′), 1.15 (3H, t, J = 7.0 Hz, 7′-O$CH_2\underline{CH_3}$), 1.12 (3H, s, 7′-Me) 1.09 (3H, s, 7′-Me),; ^{13}C-NMR (150 MHz in $CDCl_3$) δ 143.4 (d, C–4), 142.5 (s, C–3′), 128.7 (d, C–5), 118.7 (d, C–2′), 113.2 (d, C–6), 112.9 (d, C–3), 112.4 (s, C–4a), 101.6 (d, C–8), 78.0 (s, C–7′), 76.4 (d, C–6′), 65.5 (t, C–1′), 56.4 (t, 7′-O$\underline{CH_2}CH_3$), 36.6 (t, C–4′), 29.2 (t,

C–5′), 19.3, 21.5 (each q, 7′-Me), 16.9 (q, 3′-Me), 16.1 (q, 7′-$OCH_2\underline{C}H_3$); HR-DART-MS: m/z 361.2020 $[M+H]^+$ (calcd for $C_{21}H_{29}O_5$, 361.2010).

3.3.2. 2′-O-Ethyltembamide (5)

Colorless oil; $[\alpha]_D^{27}$ +10.2 (c 0.02, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 227.0 (3.28), 273.5 (2.39), 321.5 (2.47) nm; IR (KBr) ν_{max} 3316, 3063, 2968, 2842, 1652, 1611, 1579, 1509, 1246, 1095, 831, 711 cm^{-1}. ^{1}H-NMR (600 MHz in methanol-d_4) δ 7.75 (2H, br d, $J=7.5$ Hz, H–2″,6″), 7.53 (1H, t, $J=7.0$ Hz, H–4″), 7.44 (2H, br d, $J=7.5$ Hz, H–3″, 5″), 7.28 (2H, d, $J=8.6$ Hz, H–2′,6′), 6.92 (2H, d, $J=8.6$ Hz, H–3′, 5′), 4.51 (1H, dd, $J=5.2$, 7.9 Hz, H–2), 3.78 (3H, s, 4′-OMe), 3.56 (1H, dd, $J=13.6$, 5.2 Hz, H–1), 3.52 (1H, dd, $J=13.6$, 7.9 Hz, H–1), 3.45 − 3.34 (2H, m, 2-$OC\underline{H}_2CH_3$), 1.16 (3H, t, $J=7.0$ Hz, 2-$OCH_2C\underline{H}_3$); ^{13}C-NMR (150 MHz in methanol-d_4) δ 170.3 (s, $\underline{C}ONH$), 161.0 (s, C–4′), 135.8 (s, C–1″), 133.5 (s, C–1′), 132.6 (d, C–4″), 129.5 (d, C–3″,5″), 129.1 (d, C–2′,6′), 128.2 (d, C–2″,6″), 114.9 (d, C–3′, 5′), 81.1 (d, C–2), 65.1 (t, 2-$O\underline{C}H_2CH_3$), 55.7 (q, OMe), 47.5 (t, C–1), 15.6 (q, 2-$OCH_2\underline{C}H_3$); HR-DART-MS: m/z 300.1591 $[M+H]^+$ (calcd for $C_{18}H_{22}NO_3$, 300.1594).

3.4. Cytotoxicity assay

The cytotoxic effects of the isolated compounds against HeLa (cervix adenocarcinoma) cell lines were assayed by the MTT colorimetric method using a cell counting kit-8 that was based on the tetrazolium salt/formazan system [11]. HeLa cells (JCRB9004, JCRB Cell Bank, Tokyo, Japan) were obtained from the Japanese Collection of Research Bioresources cell bank. Cells were cultured in minimum essential media (MEM, Merck KGaA, Darmstadt, Germany) supplemented with 10% fetal bovine serum. For the cytotoxicity assay, cells were seeded at a density of 5×10^3 cells/well in 0.2 ml of medium in 96-multiwell plates and adhered. Subsequently, samples were dissolved in saline solution containing 10% DMSO and sterilized by filtration. Then, series of diluted samples (0.2 ml) were added to the cells. The plates were incubated under a humid atmosphere of 5% CO_2 (v/v) and > 95% humidity (v/v) at 37 °C for 48 h. Cell counting kit-8 (20 µl) was added to each well, and the microplates were incubated for 1 h. Subsequently, cell densities were measured at 450 nm using a Bio-RAD Model 550 Microplate Reader (CA, USA), and cisplatin was used as the cytotoxic reference compound.

3.5. Radical-scavenging activity assay

The antiradical effects of the isolated compounds were assayed by the DPPH scavenging activity test [12]. A total of 0.5 ml of test solution at various concentrations (1–100 µM), 1 ml of test solution at various concentrations (1–100 µM) and 1 ml of 0.1 M acetate buffer (pH 5.5) were mixed in a test tube. Then, 0.5 ml of a 0.5 mM DPPH solution was added to the mixture, which was then homogenized using a vortex mixer in the dark place, shielded from UV light, and incubated for 30 min at room temperature. Subsequently, the absorbance (A) of the mixture at 517 nm was measured using a spectrophotometer. Vitamin C was used as the reference antiradical

compound in the same concentration range as the tested compounds. A control solution was prepared in the same manner as the test mixture. The free-radical scavenging activity [AA%] of each sample and the reference standard was determined according to the following formula: $[AA\%] = 100 - [(A_{sample} - A_{blank}) \times 100/A_{control}]$, where A_{sample} is the absorbance of the sample with DPPH, A_{blank} is the absorbance of the sample without DPPH, and $A_{control}$ is the absorbance of DPPH in EtOH. The concentration (μM) of the sample that reduced 50% DPPH (EC_{50}) was determined by plotting the percentage of inhibition against the sample concentration.

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Disclosure statement

No potential conflict of interest was reported by the authors.

References

[1] V.B. Lambole, K. Murti, U. Kumar, B.S.P. Bhatt, and V. Gajera, *Int. J. Pharm. Sci. Rev. Res.* **5**, 67 (2010).
[2] S.C. Verma, R. Rani, E. Vashishth, K. Basu, P. Pant, and M.M. Padhi, *Res. J. Pharmacogn. Phytochem.* **5**, 236 (2013).
[3] C.P. Khare, *Indian Medicinal Plants: An Illustrated Dictionary* (Springer-Verlag, Berlin/Heidelberg, 2007), p. 21.
[4] A. Shoeb, R.S. Kapil, and S.P. Popli, *Phytochemistry* **12**, 2071 (1973).
[5] S. Laphookhieo, C. Phungpanya, C. Tantapakul, S. Techa, S. Tha-In, and W. Narmdorkmai, *J. Braz. Chem. Soc.* **22**, 176 (2011).
[6] X.W. Yang, M. Hattori, and T. Nanba, *J. Chin. Pharm. Sci.* **5**, 68 (1996).
[7] E.M. Cardoso-Lopes, J.A. Maier, M.R.D. Silva, L.O. Regasini, S.Y. Simote, N.P. Lopes, J.R. Pirani, V.D.S. Bolzani, and M.C.M. Young, *Molecules* **15**, 9205 (2010).
[8] N.F. Moura, A.F. Morel, E.C. Dessoy, N. Zanatta, M.M. Bürger, N. Ahlert, G.P. Porto, and B. Baldisserotto, *Planta Med.* **68**, 534 (2002).
[9] J.S. Yadav, P.T. Reddy, S. Nanda, and A.B. Rao, *Tetrahedron Asym.* **12**, 3381 (2002).
[10] A. Maxwell, and D. Ramprasad, *J. Nat. Prod.* **52**, 411 (1989).
[11] M. Ishiyama, M. Shiga, K. Sasamoto, M. Mizoguchi, and P. He, *Chem. Pharm. Bull.* **41**, 1118 (1993).
[12] M.S. Blois, *Nature* **181**, 1199 (1958).