Lignans and a Sesquiterpene Glucoside from *Carissa carandas* Stem

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Two new compounds, the sesquiterpene glucoside carandoside (**1**) and (6*S*,7*R*,8*R*)-7a-[(*β*-glucopyranosyl)oxy]lyoniresinol (**2**), were isolated from the stem of *Carissa carandas*, together with three known lignans. The structures of the isolated compounds were determined on the basis of spectroscopic evidence. Their DPPH free radical-scavenging activities were also evaluated.

Introduction. – The genus *Carissa* (Apocynaceae) is composed of 35 species widely distributed in Africa, Asia, and Australia [1][2]. *Carissa carandas* L. is a shrub commonly found in several Asian countries, such as India and Thailand [2–4]. In India, the roots of *C. carandas* have been traditionally used for diarrhea, stomachic, and anthelmintic properties [3][5]. Earlier studies showed that the roots of this plant possessed antiviral activity and contained several classes of secondary metabolites, including triterpenoids, steroids, cardenolides, and lignans [3][5–7]. In Thailand, *C. carandas* is known as 'Naam-Dang', and its stem has been used in folkloric medicine as a bitter tonic [4]. As part of our continuing studies on Thai medicinal plants [8], a chemical investigation of the stem of *C. carandas* has been conducted. In this article, we report the isolation and characterization of a new sesquiterpene glycoside named carandoside (**1**), and a hitherto unknown lignan glycoside, namely (6*S*,7*R*,8*R*)-7a-[(*β*-glucopyranosyl)oxy]lyoniresinol (**2**), along with three known lignans including (6*R*,7*S*,8*S*)-7a-[(*β*-glucopyranosyl)oxy]lyoniresinol (**3**) [9], carissanol (**4**) [10], and (−)-nortrachelogenin (**5**) [10] (*Fig. 1*). These compounds were also studied for their DPPH free radical scavenging activity.

Results and Discussion. – Carandoside (**1**) was obtained as a yellow amorphous solid. The *quasi*-molecular ion $[M+H]^+$ at *m*/*z* 413.2177 in the HR-ESI-MS of **1** indicated a molecular formula of $C_{21}H_{32}O_8$, and the IR absorptions at 3368 and 1650 cm^{-1} suggested the presence of OH groups and a conjugated C=O functionality, respectively. The ^{13}C-NMR and DEPT spectra of **1** revealed the presence of a glucose moiety, as indicated by the resonances at *δ*(C) 100.1 (C(1′))[1], 73.6 (C(2′)), 77.4 and 77.0 (C(3′) and C(5′)), 70.0 (C(4′)), and 61.2 (C(6′)) (*Table 1*). This, together with the molecular formula, suggested that **1** was a glucosidic sesquiterpene. For the aglycon part, the C-atom signals observed for four Me groups at *δ*(C) 9.7 (C(15)), 22.2 (C(14)), and 25.4 and 26.7 (C(12) and C(13)), two olefinic C-atoms at *δ*(C) 127.4 (C(4)) and

[1]) Arbitrary numbering. For systematic names, see *Exper. Part.*

Table 1. *^{1}H- and ^{13}C-NMR Data of Compound* **1**. At 300/75 MHz; δ in ppm, J in Hz.

Position[1])	δ(H)		δ(C)
	in (D_4)MeOH	in (D_6)DMSO	in (D_4)MeOH
1	–	–	180.3
2	5.73 (*s*)	5.64 (*s*)	103.3
3	–		189.1
4	–		127.4
5	–	–	159.3
6	2.08 (br. *d*, J = 12.9, H_α), 2.94 (br. *d*, J = 12.9, H_β)	1.97 (br. *d*, J = 12.9, H_α), 2.84 (br. *d*, J = 12.9, H_β)	28.1
7	1.25–1.29 (*m*)	1.22–1.27 (*m*)	51.7
8	1.71–1.75 (*m*, H_α), 1.52–1.56 (*m*, H_β)	1.45–1.49 (*m*, H_α), 1.66–1.70 (*m*, H_β)	22.3
9	1.22–1.25 (*m*, H_α), 2.26 (br. *d*, J = 13.2, H_β)	1.19–1.22 (*m*, H_α), 2.16 (br. *d*, J = 12.6, H_β)	36.8
10	–	–	43.3
11	–	–	71.9
12, 13	1.17 (*s*), 1.18 (*s*)	1.10 (*s*), 1.11 (*s*)	25.4, 26.7
14	1.37 (*s*)	1.32 (*s*)	22.2
15	1.83 (*s*)	1.77 (*s*)	9.7
1′	[a])	4.72 (*d*, J = 6.9)	100.1
2′	3.25–3.38 (*m*)	3.06–3.75 (*m*)	73.6
3′	3.25–3.38 (*m*)	3.06–3.75 (*m*)	77.0 or 77.4
4′	3.25–3.38 (*m*)	3.06–3.75 (*m*)	70.0
5′	3.25–3.38 (*m*)	3.06–3.75 (*m*)	77.0 or 77.4
6′	3.63–3.83 (*m*)	3.65–3.75 (*m*)	61.2

[a]) Hidden under solvent signal.

159.3 (C(5)) and a CO group at δ(C) 189.1 (C(3)) in **1** were reminiscent of carissone (11-hydroxyeudesma-4-en-3-one), an eudesmane-type sesquiterpene previously isolated from this plant [7] and *C. edulis* [11]. This was also supported by ^{1}H-NMR signals for Me groups at δ(H) 1.83 (Me(15)), 1.37 (Me(14)), and 1.17 and 1.18 (Me(12) and Me(13)) (*Table 1*), which correlated to their corresponding C-atoms in the HMQC spectrum. However, **1** differed significantly from carissone in that its C(1) and C(2) resonated at much higher frequencies, appearing as an olefinic C–O and C–H C-atom each at δ(C) 180.3 (*s*) and 103.3 (*d*). The former signal was assigned to C(1), and the latter to C(2), based on the HMBC correlations from Me(14) to C(1), and from H–C(2) to C(4) (*Fig. 2*). This was also in agreement with the γ-effect observed for C(9) (−4.5 ppm) in **1** as compared with its counterpart in carissone [12]. The glucose unit should be attached to C(1) of the aglycon, as evidenced by the three-bond coupling between H–C(1′) and C(1). The appearance of the anomeric H-atom (H–C(1′)) (in (D_6)DMSO) as a *doublet* (J = 6.9 Hz) at δ(H) 4.72 indicated a β-configuration. The relative configuration at C(7) and C(10) was then determined from the ROESY spectrum which showed cross peaks for the following pairs of H-atoms: H–C(7)/H_β–C(8), H–C(7)/H_β–C(9), H_β–C(8)/H_β–C(9), H_α–C(6)/Me(14), and H_β–C(6)/Me(15) (*Fig. 2*). Based on the above spectroscopic data, **1** was established as

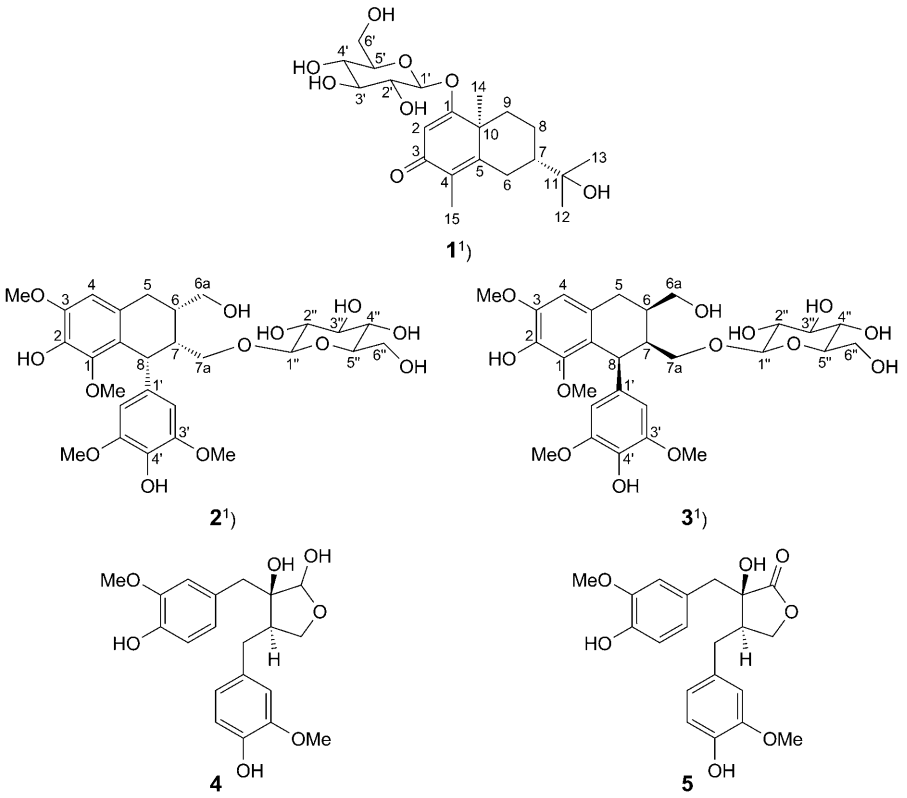

Fig. 1. *Structures of compounds* **1**–**5**

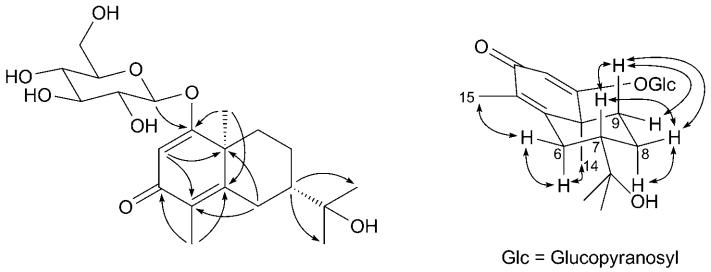

Fig. 2. *Key HMBC* (H → C) *and ROESY* (H ↔ H) *correlations for* **1**[1])

11-hydroxyeudesma-1,4-dien-3-on-1-yl β-glucoside and given the trivial name carandoside. In *Fig. 1*, the structure is shown with relative configuration. To the best of our knowledge, the structure of the aglycon itself was also unknown prior to this report.

Compound **2** was isolated as a yellow amorphous solid. The molecular formula was determined as $C_{28}H_{38}O_{13}$ by HR-ESI-MS ($[M + Na]^+$ at m/z 605.2216). The UV and MS data of **2** suggested that it had a structure similar to that of **3**, a lignan glycoside

obtained in this study and identified as (6*R*,7*S*,8*S*)-7a-[(*β*-glucopyranosyl)oxy]lyoniresinol by comparison of its physicochemical properties including UV, NMR, MS, and CD data with literature values [9]. The ^{1}H- and ^{13}C-NMR data of compound **2** closely resembled those of compound **3** (*Table 2*), indicating that **2** also contained the aglycon lyoniresinol connected to a glucose moiety through the C(7a)[1]) to C(1″) ether linkage. Nevertheless, several NMR spectral differences between these two compounds were noticed. The resonances for H–C(8) (*δ*(H) 4.19) and H–C(1″) (*δ*(H) 4.09) of **2** appeared at more upfield positions than their counterparts in **3** (*δ*(H) 4.36 and 4.23, resp.). Additionally, in the ^{13}C-NMR spectrum of **2**, C(7a) (*δ*(C) 72.0, *t*) was found to absorb at a higher frequency than C(4″) (*δ*(C) 71.5, *d*), whereas the reverse was true for compound **3**. Despite the above-mentioned spectral differences, **2** and **3** were not

Table 2. *^{1}H- and ^{13}C-NMR Data of Compounds* **2** *and* **3**. In (D_4)MeOH at 300 and 75 MHz, resp.; *δ* in ppm, *J* in Hz.

Position[1])	**2**		**3**		
	δ(H)	*δ*(C)	*δ*(H)	*δ*(C)	*δ*(C)[a])
1	–	147.5	–	147.5	147.6
2	–	138.8	–	138.9	138.9
3	–	148.7	–	148.6	148.6
4	6.53 (*s*)	107.8	6.51 (*s*)	107.8	107.9
5	2.62–2.64 (*m*)	33.8	2.55–2.68 (*m*)	33.8	33.8
6	1.60–1.70 (*m*)	41.2	1.60–1.70 (*m*)	40.6	40.6
6a	3.58 (*d*, *J* = 4.8)	66.2	3.50 (*d*, *J* = 6.0)	66.2	66.2
7	2.05–2.15 (*m*)	46.5	1.97–2.10 (*m*)	46.6	46.7
7a	3.52–3.54 (*m*), 3.86–3.88 (*m*)	72.0	3.37–3.42 (*m*), 3.82–3.86 (*m*)	71.5	71.5
8	4.19 (*d*, *J* = 6.3)	43.2	4.36 (*d*, *J* = 6.3)	42.7	42.7
9	–	126.2	–	126.4	126.4
10	–	130.2	–	130.2	130.2
1′	6.37 (*s*)	139.4	6.37 (*s*)	139.3	139.3
2′	–	107.1	–	106.9	106.9
3′	–	149.0	–	148.9	149.0
4′	–	134.6	–	134.5	134.5
5′	–	149.0	–	148.9	149.0
6′	6.37 (*s*)	107.1	6.37 (*s*)	106.9	106.9
1″	4.09 (*d*, *J* = 7.5)	104.2	4.23 (*d*, *J* = 7.8)	104.7	104.8
2″	3.15–3.18 (*m*)	75.0	3.18–3.19 (*m*)	75.1	75.2
3″	3.25–3.27 (*m*)	77.8	3.31–3.33 (*m*)	78.2	78.2
4″	3.27–3.28 (*m*)	71.5	3.24–3.26 (*m*)	71.6	71.7
5″	3.10–3.20 (*m*)	78.1	3.20–3.22 (*m*)	77.9	77.9
6″	3.64–3.68 (*m*), 3.80–3.83 (*m*)	62.7	3.57–3.58 (*m*), 3.60–3.75 (*m*)	62.8	62.8
1-OMe	3.29 (*s*)	60.1	3.28 (*s*)	60.2	60.2
3-OMe	3.81 (*s*)	56.6	3.78 (*s*)	56.6	56.6
3′-OMe	3.71 (*s*)	56.9	3.68 (*s*)	56.9	56.8
5′-OMe	3.71 (*s*)	56.9	3.68 (*s*)	56.9	56.8

[a]) From ref. [9].

distinguishable by 2D-NMR analysis as both showed similar patterns of HMBC correlations (*Fig. 3*). Moreover, the ROESY cross peaks obtained for H–C(6), H–C(7), and H–C(8) indicated that the relative configurations at C(6), C(7), and C(8) of compound **2** were identical with those of compound **3** (*Fig. 3*). However, compounds **2** and **3** were found to have opposite signs of optical rotation ($[\alpha]_D^{20} = -46.9$ *vs.* $+22.7$), suggesting the enantiomeric nature for their aglycons. Conclusive evidence came from the circular dichroism (CD) studies. It is known that for this class of lignans and their glucosides, the sign of the couplets at 287 and 273 nm reflects the orientation of the aryl substituent at C(8)[1]) [13–15]. In our study, compound **3** showed negative and positive peaks at 287 and 273 nm, respectively (*Fig. 4*), and was therefore assigned the (6*R*,7*S*,8*S*)-absolute configuration, consistent with the earlier report [9]. The opposite results were obtained for **2** (*Fig. 4*), indicating the (6*S*,7*R*,8*R*)-absolute configuration [13–15]. Hence, **2** was identified as (6*S*,7*R*,8*R*)-7a-[(*β*-glucopyranosyl)-oxy]lyoniresinol. It is interesting to note that **2** and **3** are diastereomeric glucosides with enantiomeric aglycons.

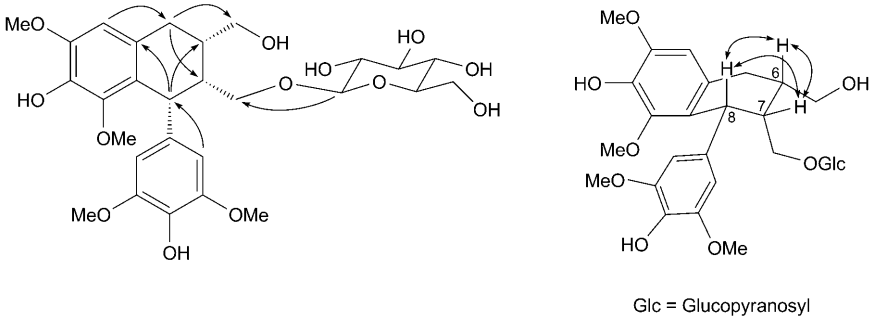

Fig. 3. *Key HMBC* (H → C) *and ROESY* (H ↔ H) *correlations for* **2**[1])

The other isolated compounds were identified as carissanol (**4**) [10], and (–)-nortrachelogenin (**5**) [10], by interpretation of their spectra and comparison with the literature data. It should be mentioned that compounds **3**–**5** were not identified from *C. carandas* in previous reports [3][6][7].

Compounds **1**–**5** were evaluated for their DPPH free radical scavenging activity [16]. All of them showed weak activity with IC_{50} values of 116.5, 21.5, 43.0, 12.7, and 30.2 μM, respectively, as compared with the positive control quercetin (IC_{50} 4.6 μM).

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Experimental Part

General. Column chromatography (CC): silica gel *60* (SiO_2; 70–230 mesh; *Merck*); *Sephadex LH 20* (*Pharmacia*). *C18* flash column: *VerSaPak*, *C18* cartridge (40 × 75 mm, 45–75 μm). Prep. HPLC: *Shimadzu LC-8A*, *C18*, column: *Shim-pack Prep-ODS* (20 × 250 mm, 5 μm), flow rate: 2 ml/min, UV

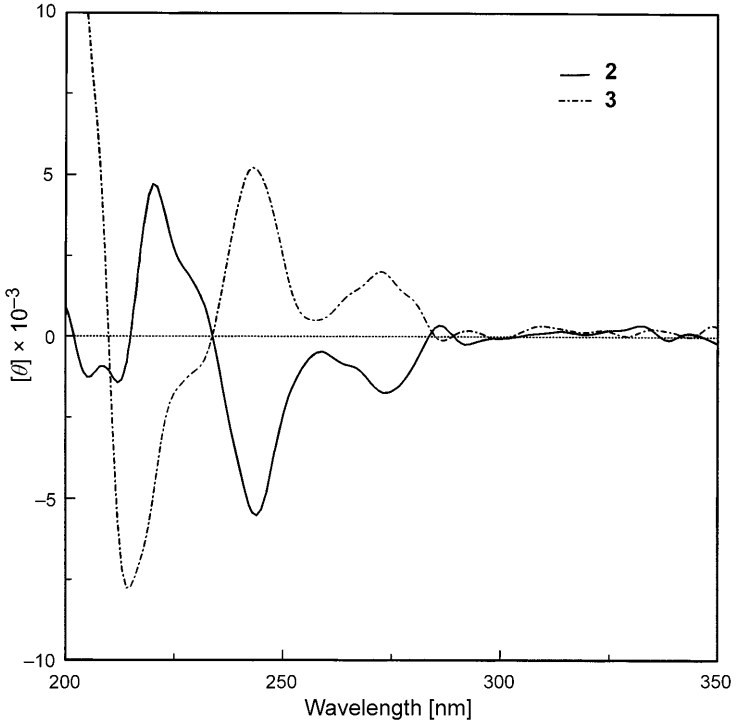

Fig. 4. *CD Curves of Compounds* **2** (—) *and* **3** (– · – · –)

detector: *SPD-10A* (at 254 nm). Optical rotations: *Perkin-Elmer 341* Polarimeter. UV Spectra: *Shimadzu UV-160A* spectrophotometer. CD Spectra: *Jasco J-715* spectropolarimeter. IR Spectra: *FT-IR Perkin-Elmer* spectrometer. NMR Spectra: *Bruker Avance DPX-300* spectrometer. ESI- and HR-ESI-MS: *Bruker microTOF* mass spectrometer.

Plant Material. Stems of *C. carandas* were collected from the Botanical Garden of the Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand, in October, 2006. A voucher specimen (RW 102549) has been on deposit with the Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Chulalongkorn University.

Extraction and Isolation. Dried and ground stems of *C. carandas* (2 kg) were extracted with MeOH ($3 \times 10\,l \times 4$ d) at r.t. The crude extracts were combined and dried under reduced pressure to yield a MeOH extract (149.5 g). The MeOH extract was then separated on a *C18* flash column (MeOH/H_2O 3:7) to give six fractions (*Fr. I–VI*). *Fr. IV* (790 mg) was further separated on *Sephadex LH-20* (eluted with MeOH) to afford seven fractions (*Fr. IV_A–IV_G*). *Fr. IV_B* (110 mg) was purified by HPLC (MeCN/MeOH/H_2O 1.4:0.9:7.7) to give compounds **1** (9 mg, t_R 109.7 min), **2** (12 mg, t_R 91.5 min), and **3** (23 mg, t_R 85.4 min). *Fr. IV_E* (130 mg) was subjected to HPLC (MeOH/H_2O 4:6) to afford six fractions (*Fr. IV_{E1}–IV_{E6}*). *Fr. IV_{E4}* (19 mg) was purified by repeated chromatography over a SiO_2 column with CH_2Cl_2/MeOH (100:0 → 98:2) as eluent to give compound **4** (5.2 mg). *Fr. V* (530 mg) was separated by *Sephadex LH-20* CC (eluted with MeOH) to give six fractions (*Fr. V_A–V_G*). *Fr. V_C* (120 mg) was further separated by HPLC (MeOH/H_2O 4:6) to afford six fractions (*Fr. V_{C1}–V_{C6}*). Purification of *Fr. V_{C4}* (40 mg) by SiO_2 CC with CH_2Cl_2/AcOEt (100:0 → 95:5) as eluent afforded compound **5** (25 mg).

Carandoside (=*(6*S**,8a*S**)-3,5,6,7,8,8a-Hexahydro-6-(2-hydroxypropan-2-yl)-4,8a-dimethyl-3-oxo-naphthalen-1-yl β-*D*-Glucopyranoside*; **1**). Yellow amorphous solid. $[\alpha]_D^{20} = -93.8$ ($c = 0.04$, MeOH). UV (MeOH): 218 (3.44), 243 (3.95). CD ($c = 4.85 \times 10^{-4}$, MeOH): +2737 (212), −3798 (232), −5318

(267), −2250 (323). IR (film): 3368, 1650, 1456. ^{1}H- and ^{13}C-NMR: *Table 1*. HR-ESI-MS: 413.2177 ($[M+H]^+$; $C_{21}H_{33}O_8^+$; calc. 413.2175).

(6S,7R,8R)-7a-[(β-Glucopyranosyl)oxy]lyoniresinol (= *[(1R,2R,3S)-1,2,3,4-Tetrahydro-7-hydroxy-1-(4-hydroxy-3,5-dimethoxyphenyl)-3-(hydroxymethyl)-6,8-dimethoxynaphthalen-2-yl]methyl β-Glucopyranoside*; **2**). Yellow amorphous solid. UV (MeOH): 224 (4.32), 278 (3.64). $[\alpha]_D^{20} = -46.9$ ($c = 0.04$, MeOH). CD ($c = 3.44 \times 10^{-4}$, MeOH): +13687 (220), −16095 (244), −5076 (274), +993 (286). IR (film): 3368, 1613, 1515. ^{1}H- and ^{13}C-NMR: *Table 2*. HR-ESI-MS: 605.2216 ($[M+Na]^+$; $C_{28}H_{38}NaO_{13}^+$; calc. 605.2210).

(6R,7S,8S)-7a-[(β-Glucopyranosyl)oxy]lyoniresinol (= *[(1S,2S,3R)-1,2,3,4-Tetrahydro-7-hydroxy-1-(4-hydroxy-3,5-dimethoxyphenyl)-3-(hydroxymethyl)-6,8-dimethoxynaphthalen-2-yl]methyl β-Glucopyranoside*; **3**). Yellow amorphous solid. $[\alpha]_D^{20} = +22.7$ ($c = 0.04$, MeOH). UV (MeOH): 225 (4.52), 279 (3.83). CD ($c = 3.44 \times 10^{-4}$, MeOH): −22594 (214), +15168 (243), +5828 (273), −366 (287). IR (film): 3368, 1613, 1515. ^{1}H- and ^{13}C-NMR: *Table 2*. ESI-MS: 605.90 ($[M+Na]^+$; $C_{28}H_{38}NaO_{13}^+$).

REFERENCES

[1] W. C. Evans, 'Trease and Evans Pharmacognosy', W. B. Saunders, Edinburgh, 2002, p. 32.
[2] J. D. Hooker, 'Flora of British India Vol. III', L. Reeve & Co., London, 1882, p. 630.
[3] S. C. Pakrashi, S. Datta, P. P. Ghosh-Dastidar, *Phytochemistry* **1968**, *7*, 495.
[4] Faculty of Pharmaceutical Sciences, Mahidol University, 'Siam Medicinal Plants: National Wisdom', Amarin Printing and Publishing, Bangkok, 1995, p. 56.
[5] R. S. L. Taylor, J. B. Hudson, N. P. Manandhar, G. H. N. Towers, *J. Ethnopharmacol*. **1996**, *53*, 105.
[6] R. Pal, D. K. Kulshreshtha, R. P. Rastogi, *Phytochemistry* **1975**, *14*, 2302.
[7] B. Singh, R. P. Rastogi, *Phytochemistry* **1972**, *11*, 1797.
[8] R. Wangteeraprasert, K. Likhitwitayawuid, *Heterocycles* **2008**, *75*, 403.
[9] Y.-L. Yang, F.-R. Chang, Y.-C. Wu, *Helv. Chim. Acta* **2005**, *88*, 2731.
[10] H. Achenbach, R. Waibel, I. Addae-Mensah, *Phytochemistry* **1983**, *22*, 749.
[11] H. Achenbach, R. Waibel, I. Addae-Mensah, *Phytochemistry* **1985**, *24*, 2325.
[12] G. T. Maatooq, D. K. Stumpf, J. J. Hoffmann, L. K. Hutter, B. N. Timmermann, *Phytochemistry* **1996**, *41*, 519.
[13] K. Ohashi, H. Watanabe, Y. Okumura, T. Uji, I. Kitagawa, *Chem. Pharm. Bull*. **1994**, *42*, 1924.
[14] J. Sakakibara, H. Ina, M. Yasue, *Yakugaku Zasshi* **1974**, *94*, 1377.
[15] P. B. Hulbert, W. K. Klyne, P. M. Scopes, *J. Chem. Res.*, *Miniprint* **1981**, *2*, 401.
[16] K. Likhitwitayawuid, C. Klongsiriwet, V. Jongbunprasert, B. Sritularak, S. Wongseripipatana, *Arch. Pharm. Res.* **2006**, *29*, 199.

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