New Alkaloids from *Annona purpurea*

Fang-Rong Chang, Chung-Yi Chen, Po-Hsun Wu, Reen-Yen Kuo, Yuh-Chwen Chang, and Yang-Chang Wu*

Graduate Institute of Natural Products, Kaohsiung Medical University, Kaohsiung 807, Taiwan, Republic of China

Received November 5, 1999

Three new alkaloids, promucosine (**1**), romucosine F (**2**), and romucosine G (**3**), along with 28 known compounds, were isolated from the MeOH extract of stems of *Annona purpurea*. The structures of **1**−**3** were determined on the basis of spectral data and chemical evidence.

Annona purpurea Moc. and Sessé ex Dunal (Annonaceae) is a bushy tree native to Middle and South America. Its fruit is edible, and its wood is used for the manufacture of paper. Various parts of the tree are employed in traditional medicine. The fruit juice is a remedy for fever, chills, and jaundice, and a decoction of the inner bark is prescribed in cases of dysentery and edema. The powdered seeds were reported to be insecticidal, with the methylene chloride extract of the leaves exhibiting strong toxicity toward larvae of the crustacean *Artemia salina* (brine shrimp), and pronounced activity against larvae of the mosquito, *Aedes aegypti*, a vector of yellow fever.[1] In previous phytochemical studies, several aporphine alkaloids [2−4] and annonaceous acetogenins [1,5] had been identified from this species. Among these, 7-dehydroaporphine alkaloids exhibited significant antiplatelet aggregation activity.[3,4,6] In this paper, we report herein three new alkaloids, promucosine (**1**), romucosine F (**2**), and romucosine G (**3**), along with 28 known compounds. From the methanolic extract of the stems of *A. purpurea*, the following known compounds were isolated: seven aporphines, (+)-norpurpureine,[4] (−)-norglaucine,[7] (+)-northalbaicalidine,[8] (+)-thalicsimidine [(+)-purpureine],[8] (−)-lirinidine,[9] (+)-apoglaziovine,[10] and (+)-isocorydine;[11] four oxoaporphines, lysicamine,[11] liriodenine,[11] oxopurpureine,[12] and oxoglaucine;[11] one proaporphine, (+)-stepharine;[11] three morphinandienones, (−)-pallidine,[13] (−)-norpallidine,[14] and (+)-*O*-methylflavinantine;[15,16] one benzylisoquinoline, (+)-reticuline;[17] one isoquinolone, thalifoline;[18] one lactam, squamolone;[11] one acetogenin, purpurenin;[19] five benzenoids, methylparaben,[18] isovanillic acid,[18] vanillin,[18] isovanillin,[18] and *p*-methoxybenzoic acid;[18] and four steroids, β-sitosterol,[11] stigmasterol,[11] β-sitosteryl-β-D-glucoside,[18] and stigmasteryl-β-D-glucoside.[18] The identities of the known compounds were verified by comparing UV, IR, ^{1}H NMR, ^{13}C NMR, and MS data with published values.[4,7−19]

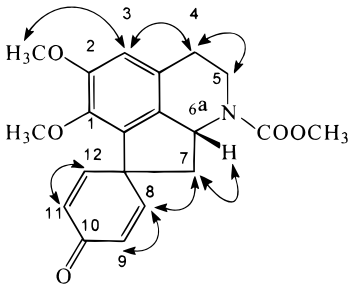
Results and Discussion

Compound **1** was obtained as a brown powder. The molecular formula, $C_{20}H_{21}O_5N$, was determined by HREIMS measurement. Its UV absorption maxima at λ 217, 267, and 303 nm and a signal at δ 185.1 in the ^{13}C NMR spectrum represented a 1,2-oxygenated proaporphine skeleton in the molecule.[11] An IR band at 1710 cm^{-1} and a signal at δ 155.4 in the ^{13}C NMR spectrum indicated that a carbamate moiety was present.[20] The mass fragment at *m*/*z* 267 [M − (CH_2−N−$COOCH_3$) + H]$^+$ was further characteristic of a *N*-carbamate group. The ^{1}H NMR spectrum indicated the presence of singlets at δ 3.84, 3.63, and 3.77, corresponding to two methoxy groups and one *N*-(methoxycarbonyl) group. Five aromatic protons, one singlet at δ 6.71 (1H) and four double doublets at δ 7.02 (1H, dd, J = 10.0, 2.8 Hz), 6.85 (1H, dd, J = 10.0, 2.8 Hz), 6.40 (1H, dd, J = 10.0, 2.0 Hz), and 6.31 (1H, dd, J = 10.0, 2.0 Hz), were indicative of aromatic protons of a 1,2-oxygenated proaporphine.[11] The seven proton signals at δ 4.86 (1H, m), 4.38 (1H, m), and 3.05−2.35 (5H, m) for the aliphatic protons were consistent with a *N*-(methoxycarbonyl)proaporphine. Two significant downfield signals at δ 4.86 (1H, m) for H-6a and δ 4.38 (1H, m) for H-5a indicated an electron-withdrawing group bonded to the nitrogen atom. The COSY and NOESY (Figure 1) experiments were useful in establishing the structure of **1**. Significant correlated sequences of OMe-1/OMe-2/H-3/H-4/H-5, H-6a/H-7/H-8/H-9, and H-11/H-12 were observed in the NOESY spectrum (Figure 1). The ^{13}C NMR spectrum, showed 10 aromatic carbons between δ 153.5 and 112.3; two methoxys at δ 60.4 and 55.9; three methylenes at δ 53.8, 30.3, and 28.5; two carbonyl carbons at δ 185.1 and 155.4; one carboxylic methyl carbon at δ 53.2; one quaternary carbon at δ 38.8; and one methine at δ 54.5, in agreement with structure **1**. Compound **1** is a novel alkaloid, which has been named promucosine and is the first example of a natural *N*-(methoxycarbonyl)proaporphine.

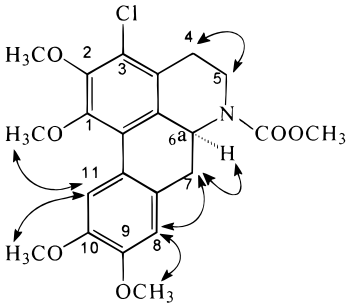
Compound **2** was obtained as white needles. Its molecular formula was established as $C_{22}H_{24}O_6NCl$ by HREIMS measurement. The presence of a 1,2,9,10-oxygenated aporphine skeleton in the molecule was deduced by its UV absorption maxima at 222, 280, and 305 nm.[21] The IR absorption at 1680 cm^{-1} and a signal at δ 153.4 in the ^{13}C NMR spectrum suggested the presence of a carbamate moiety.[20] The EIMS spectrum showed that one chlorine is present in alkaloid **2** with a 3-to-1 ratio at *m*/*z* (rel int %): 433 (65, M$^+$)/435; 397 (85, [M − HCl]$^+$); 345 (100)/347. The molecular ion was seen at *m*/*z* 433 (65, M$^+$)/435, and the base peak was at *m*/*z* 345 (100)/347 due to loss of (CH_2−N−$COOCH_3$ + H) from the molecular ion. The ^{1}H NMR spectrum shows the presence of singlets at δ 3.96, 3.94, 3.93, 3.73, and 3.77, corresponding to four methoxy groups and one *N*-(methoxycarbonyl) group, respectively. Two aromatic protons at δ 8.07 (1H, s) and 6.79 (1H, s) were observed. The typical seven-proton signals at δ 4.75 (1H, dd, J = 14.0, 4.3 Hz), 4.46 (1H, m), and 3.07−2.64 (5H, m) for the aliphatic protons were consistent with the features of *N*-(methoxycarbonyl)aporphine.[20] Two significant downfield signals at δ 4.75 (1H, dd, J = 14.0, 4.3 Hz) for H-6a and δ 4.46 (1H, m) for H-5a indicated an electron-withdrawing group bonded to the nitrogen atom.[20] To confirm the structure of **2**, the NOESY spectrum was examined. The typical deshielding aromatic proton reso-

* To whom correspondence should be addressed. Tel.: 886-7-3121101, ext. 2197. Fax: 886-7-3114773. E-mail: yachwu@cc.kmu.edu.tw.

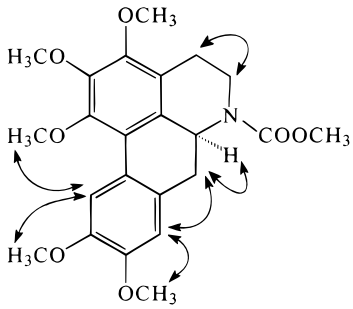
10.1021/np990548n CCC: $19.00 © 2000 American Chemical Society and American Society of Pharmacognosy
Published on Web 04/29/2000



1



2



3

Figure 1. The 2D NOESY response of **1**, **2**, and **3**.

nance at δ 8.07 (s, H-11) showed correlations to two methoxy groups at δ 3.73 (OMe-1) and 3.94 (OMe-10) (Figure 1). The sequence of a methoxy at δ 3.73 (OMe-9), the aromatic proton at δ 6.79 (H-8), a methylene at δ 3.07−2.64 (H-7), and a methine at δ 4.75 (H-6a) were also confirmed by NOESY (Figure 1). The chlorine atom should, therefore, be located at the 3-position. The ^{13}C NMR spectrum showed signals for 12 aromatic carbons between δ 151.3 and 111.0; four methoxys at δ 61.0, 56.1, 55.4, and 53.8; three methylenes at δ 46.6, 38.2, and 31.1; one carbonyl carbon at δ 153.4; one carboxylic methyl carbon at δ 53.3; and one methine at δ 57.0, in agreement with the proposed structure **2**, which is named romucosine F. Although the majority of the natural organohalogens are metabolites from organisms living in marine environments, several strains of different genera of wood- and forest litter-degrading basidomycetes are also known to produce halogenated compounds.[22]

Compound **3** was obtained as white needles. The molecular formula, $C_{23}H_{27}O_7N$, was deduced by HREIMS measurement. The presence of a 1,2,3,9,10-oxygenated aporphine skeleton in the molecule was deduced by its UV absorption maxima at 233, 282, and 312 nm.[23] The presence of a carbamate moiety was suggested by an IR band at 1650 cm^{-1}, a signal at δ 154.7 in the ^{13}C NMR spectrum,[20] and a mass fragment at *m*/*z* 341 [M − (CH_2−N−$COOCH_3$) + H]$^+$. The ^{1}H and ^{13}C NMR spectra of **3** were similar to those of **2** except for the presence of a methoxy signal, indicated by signals at δ 3.90 in ^{1}H NMR and δ 59.8 in ^{13}C NMR. Significant correlated sequences of OMe-3/OMe-2/OMe-1/H-11/OMe-10/OMe-9/H-8/H-7/H-6a and H-4/H-5 were observed in the NOESY spectrum (Figure 1), confirming the positions of the methoxy groups. Thus, the structure of **3** was determined as illustrated and named romucosine G (**3**). Treatment of (+)-norpurpureine with triethylamine and methyl chlorocarbonate gave a compound whose mp, UV, TLC, and ^{1}H NMR data were the same as those of **3**.

We have previously reported *N*-(methoxycarbonyl)aporphinoid alkaloids such as romucosine to have significant antiplatelet aggregation activity.[18,24] It appears that these aporphine alkaloids act by a different mechanism from that of aspirin, which is known to be a cyclooxygenase inhibitor.[18] The antiplatelet aggregation activity of these compounds needs to be further investigated.

Experimental Section

General Experimental Procedures. Optical rotations were measured with a JASCO DIP-370 digital polarimeter. Melting points were determined using a Yanagimoto micromelting point apparatus and are uncorrected. The IR spectra were measured on a Hitachi 260-30 spectrophotometer. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra (all in $CDCl_3$) were recorded with Varian NMR spectrometers, using TMS as internal standard. LRFABMS and LREIMS were obtained with a JEOL JMS-SX/SX 102A mass spectrometer or a Quattro GC/MS spectrometer having a direct inlet system. HRFABMS were measured on a JEOL JMS-HX 110 mass spectrometer. Si gel 60 (Macherey-Nagel, 230−400 mesh) was used for column chromatography, precoated Si gel plates (Macherey-Nagel, SIL G_{25} UV_{254}, 0.25 mm) were used for analytical TLC, and precoated Si gel plates (Macherey-Nagel, SIL G/UV_{254}, 0.25 mm) were used for preparative TLC. Plates were visualized by spraying with Dragendorff's reagent or with 50% H_2SO_4 and then heating on a hot plate. HPLC was performed on a JASCO PU-980 apparatus equipped with a UV-970 detector. Develosil ODS-5 (250 × 4 mm i.d.) and preparative ODS-5 (250 × 20 mm i.d.) columns were used for analytical and preparative purposes, respectively.

Plant Material. *A. purpurea* was collected from Chia-Yi City, Taiwan, in September 1997. A voucher specimen is deposited in the Graduate Institute of Natural Products, Kaohsiung Medical University, Kaohsiung, Taiwan.

Extraction and Isolation. The air-dried stems (15.0 kg) of *A. purpurea* were extracted repeatedly with MeOH at room temperature. The combined MeOH extracts were evaporated and partitioned to yield $CHCl_3$ and aqueous extracts. The bases in the $CHCl_3$ solution were extracted with 3% HCl to yield the $CHCl_3$ layer (Part A) and the HCl-solution layer. The HCl-solution layer was basified with NH_4OH and then extracted with $CHCl_3$ (Part B). Part B was dried and evaporated to leave a brown viscous residue. The residue was further separated by column chromatography on Si gel with gradient systems of *n*-hexane−$CHCl_3$ (*n*-hexane−$CHCl_3$ 4:1 to $CHCl_3$) and $CHCl_3$−MeOH ($CHCl_3$ to $CHCl_3$−MeOH 4:1) to yield 100 fractions of 120 mL each, which were further combined into 10 fractions according to their TLC patterns. Each fraction was chromatographed a second time over Si gel and purified by further Si gel column chromatography, recrystallization, preparative TLC, or reversed-phase HPLC (Develosil ODS-5 column; 250 × 4 mm i.d.; 75:25 MeOH−water; flow rate of 1 mL/min) to yield 31 compounds. The yield amount, TLC, or HPLC data for the new compounds are as follows: **1** (3.5 mg; $CHCl_3$−MeOH 20:1, R_f 0.33; t_R 3.860 min), **2** (2.4 mg; $CHCl_3$−MeOH 20:0.5, R_f 0.43; t_R 21.556 min), **3** (3.8 mg; $CHCl_3$−MeOH 20:1, R_f 0.41; t_R 21.776 min).

Promucosine (1): brown powder; mp 125−127 °C; $[\alpha]^{25}_D$ +147.0° (*c* 0.07, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 217 (3.78), 267 (4.45), 303 (3.87) nm; IR (KBr) ν_{max} 1710 (C═O), 1660 (C═O) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) 7.02 (1H, dd, *J* = 10.0, 2.8 Hz, H-12), 6.85 (1H, dd, *J* = 10.0, 2.8 Hz, H-8), 6.71 (1H, s, H-3), 6.40 (1H, dd, *J* = 10.0, 2.0 Hz, H-9), 6.31 (1H, dd, *J* = 10.0, 2.0 Hz, H-11), 4.86 (1H, m, H-6a), 4.38 (1H, m, H-5a), 3.84 (3H, s, OMe-2), 3.77 (3H, s, *N*-COOMe), 3.63 (3H, s, OMe-1); ^{13}C NMR (100 MHz, $CDCl_3$) 185.1 (s, C-10), 155.4 (s, *N*-*CO*OMe), 153.5 (d, C-8 and C-12), 150.2 (s, C-2), 148.7 (s, C-1), 132.6 (s, C-3b), 129.9 (s, C-3a), 128.5 (d, C-9 and C-11), 127.5 (s, C-3c), 112.3 (d, C-3), 60.4 (q, OMe-1), 55.9 (q, OMe-2), 54.5 (d, C-6a), 53.8 (t, C-5), 53.2 (q, *N*-COO*Me*), 38.8 (s, C-7a), 30.3 (t, C-7), 28.5 (t, C-4); EIMS (70 eV) *m*/*z* (rel int %) 355 ($[M]^+$, 65), 340 (100), 296 (17), 267 (27), 253 (20); HREIMS *m*/*z* $[M]^+$ 355.1415 (calcd for $C_{20}H_{21}O_5N$, 355.1419).

Romucosine F (2): white needles; mp 140−142 °C; $[\alpha]^{25}_D$ +134.0° (*c* 0.05, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 222 (4.35), 280 (4.21), 305 (3.76) nm; IR (KBr) ν_{max} 3018, 1680 (C═O) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) 8.07 (1H, s, H-11), 6.79 (1H, s, H-8), 4.75 (1H, dd, *J* = 14.0, 4.3 Hz, H-6a), 4.46 (1H, m, H-5a), 3.96 (3H, s, OMe-2), 3.94 (3H, s, OMe-10), 3.93 (3H, s, OMe-9), 3.77 (3H, s, *N*-COOMe), 3.73 (3H, s, OMe-1); ^{13}C NMR (100 MHz, $CDCl_3$) 153.4 (s, *N*-*CO*OMe), 151.3 (s, C-1), 149.4 (s, C-2), 144.1 (s, C-9), 142.0 (s, C-10), 135.5 (s, C-3), 130.2 (s, C-7a), 130.1 (s, C-3a), 129.5 (s, C-11a), 124.7 (s, C-3b), 120.1 (s, C-11b), 113.7 (d, C-11), 111.0 (d, C-8), 61.0 (q, OMe-1), 56.1 (q, OMe-10), 55.4 (q, OMe-9), 53.8 (q, OMe-2), 57.0 (d, C-6a), 53.3 (q, *N*-COO*Me*), 46.6 (t, C-5), 38.2 (t, C-7), 31.1 (t, C-4); EIMS (70 eV) *m*/*z* (rel int %) 435 (M + 2, 21), 433 ($[M]^+$, 65), 399 (24), 397 (85), 347 (34), 345 (100); HREIMS *m*/*z* $[M]^+$ 433.1299 (calcd for $C_{22}H_{24}O_6NCl$, 433.1292).

Romucosine G (3): white needles; mp 135−136 °C; $[\alpha]^{25}_D$ +44.0° (*c* 0.08, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 233 (4.33), 282 (4.04), 303 (3.54) nm; IR (KBr) ν_{max} 3030, 1650 (C═O) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) 8.03 (1H, s, H-11), 6.78 (1H, s, H-8), 4.72 (1H, dd, *J* = 13.6, 4.2 Hz, H-6a), 4.45 (1H, m, H-5a), 3.97 (3H, s, OMe-2), 3.94 (3H, s, OMe-10), 3.93 (3H, s, OMe-9), 3.90 (3H, s, OMe-3), 3.77 (3H, s, *N*-COOMe), 3.72 (3H, s, OMe-1); ^{13}C NMR (100 MHz, $CDCl_3$) 154.7 (s, *N*-*CO*OMe), 152.3 (s, C-1), 150.8 (s, C-3), 148.2 (s, C-2), 145.4 (s, C-9), 144.2 (s, C-10), 133.2 (s, C-3a), 131.7 (s, C-3b), 131.1 (s, C-7a), 130.9 (s, C-11a), 125.1 (s, C-11b), 113.5 (d, C-11), 112.3 (d, C-8), 61.4 (q, OMe-2), 60.1 (q, OMe-1), 59.8 (q, OMe-3), 57.5 (d, C-6a), 56.0 (q, OMe-10), 55.7 (q, OMe-9), 53.2 (q, *N*-COO*Me*), 50.8 (t, C-5), 39.2 (t, C-7), 34.4 (t, C-4); EIMS (70 eV) *m*/*z* (rel int %) 429 ($[M]^+$, 73), 397 (100), 341 (84), 327 (12), 278 (18); HREIMS *m*/*z* $[M]^+$ 429.1763 (calcd for $C_{22}H_{24}O_6NCl$, 429.1788).

Preparation of *N*-(Methoxycarbonyl)norpurpureine [Romucosine G (3)]. (+)-Norpurpureine (20 mg) in dry CH_2Cl_2 (10 mL) was treated with triethylamine (5 μL), with stirring at 0 °C, for 10 min, and then methyl chlorocarbonate (2 mL) was slowly added. The reaction mixture was stirred for 10 min, and H_2O was added to quench excess reagent. The mixture was partitioned with $CHCl_3$, then passed through a disposable pipet (0.6 × 6 cm) containing Si gel (230−400 mesh), and eluted with 10 mL of $CHCl_3$. Elution with $CHCl_3$ afforded a brown amorphous powder (5 mg) that was identical with **3** (mixed mp, co-TLC, UV, IR, ^1H and ^{13}C NMR).

Acknowledgment. This investigation was supported by a grant from the National Science Council of the Republic of China.

References and Notes

(1) Cepleanu, F.; Ohtani, K.; Hamburger, M.; Gupta, M. P.; Solis, P.; Hostettmann, K. *Helv. Chim. Acta* **1993**, *76*, 1379−1388.
(2) Sonnet, P. E.; Jacobson, M. *J. Pharm. Sci.* **1971**, *60*, 1254−1256.
(3) Chang, F. R.; Wei, J. L.; Teng, C. M.; Wu, Y. C. *J. Nat. Prod.* **1998**, *61*, 1457−1461.
(4) Chang, F. R.; Wei, J. L.; Teng, C. M.; Wu, Y. C. *Phytochemistry* **1998**, *49*, 2015−2018.
(5) Chavez, D.; Mata, R. *J. Nat. Prod.* **1998**, *61*, 580−584.
(6) Chia, Y. C.; Chen, K. S.; Chang, Y. L.; Teng, C. M.; Wu, Y. C. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 3295−3300.
(7) Baarscher, W. H.; Arndt, R. R.; Pachler, K. J.; Weisbach, A.; Douglas, B. *J. Chem. Soc.* **1964**, 4478−4481.
(8) Guinaudeau, H.; Leboeuf, M.; Cave, A. *J. Nat. Prod.* **1976**, *38*, 275−338.
(9) Hui, Y. H.; Rupprecht, J. K.; Liu, Y. M.; Anderson, J. E.; Smith, D. L.; Chan, C. J.; McLaughlin, J. L. *J. Nat. Prod.* **1989**, *52*, 463−477.
(10) Cave, M. P.; Wakisaka, K.; Noguchi, I.; Edie, D. L.; Darocha, A. I. *J. Org. Chem.* **1974**, *38*, 3588−3591.
(11) Chen, C. Y.; Chang, F. R.; Wu, Y. C. *J. Chin. Chem. Soc.* **1997**, *44*, 313−319.
(12) Sonnet, P. E.; Jacobson, M. *J. Pharm. Sci.* **1975**, *38*, 275−281.
(13) Kametani, T.; Ihara, M.; Honda, T. *J. Chem. Soc., Chem. Commun.* **1969**, 1031−1035.
(14) Shamma, M.; Chinnasamy, P.; Hussain, S. F.; Khan, F. *Phytochemistry* **1976**, *15*, 1802−1803.
(15) Lajis, N. H.; Mahmud, Z.; Toia, F. R. *J. Nat. Prod.* **1991**, *54*, 612−614.
(16) Etse, J. T.; Waterman, P. G. *Phytochemistry* **1986**, *25*, 1903−1905.
(17) Yang, T. H.; Chen, C. M. *J. Chin. Chem. Soc.* **1970**, *17*, 243−245.
(18) Chen, C. Y.; Chang, F. R.; Wu, Y. C. *J. Chin. Chem. Soc.* **1999**, *46*, 77−86.
(19) Fujimoto, Y.; Murasaki, C.; Shimada, H.; Nishioka, S.; Kakinuma, K.; Singh, M.; Gupta, Y. K.; Sahai, M. *Chem. Pharm. Bull.* **1994**, *42*(2), 1175−1184.
(20) Chen, Y. Y.; Chang, F. R.; Wu, Y. C. *J. Nat. Prod.* **1996**, *59*, 904−906.
(21) Chalandre, M. C.; Jacquemin, H.; Bruneton, J. *J. Nat. Prod.* **1985**, *48*, 333−334.
(22) Field, J. A.; Verhagen, F. J. M.; De Jong, E. *Trends. Biotechnol.* **1995**, *13*, 451−455.
(23) Guinaudeau, H.; Leboeuf, M.; Cavé, A. *J. Nat. Prod.* **1976**, *38*, 275−338.
(24) Wu, Y. C.; Chang, F. R.; Chao, Y. C.; Teng, C. M. *Phytotherapy Res.* **1998**, *12*, S39−S41.

NP990548N