Chemistry of Natural Compounds, Vol. 46, No. 4, 2010

SECONDARY METABOLITES FROM THE STEMS OF *Artocarpus heterophyllus*

C.-Y. Chen,[1] M.-J. Cheng,[2] S.-H. Kuo,[1] S.-Y. Kuo,[1] and W.-L. Lo[1]*

UDC 547.972:918

Artocarpus species (Moraceae) are evergreen trees with a milky juice and large edible fruits. There are about 50 species in tropical Asia, Polynesia, and the Pacific islands [1, 2]. *Artocarpus* species have been used in folk medicine for their anti-inflammatory, antioxidant, antibacterial, and antiplatelet activities [3–6]. Plants of the genus *Artocarpus* are rich in prenylflavonoids [3, 7–16].

To further understand the chemotaxonomy of the genus *Artocarpus* and to continue searching for novel bioactive metabolites from Moraceae plants, *A. heterophyllus* was chosen for a phytochemical investigation.

Carefull examination of this plant has resulted in the isolation of nine constituents, **1–9**. Compounds **3–9** were isolated from this species for the first time. The structures of these compounds were established by means of spectral experiments.

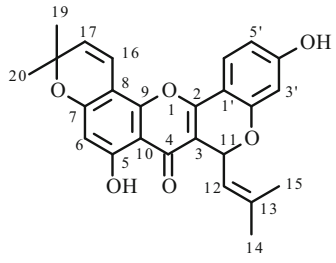
Cyclomorusin (**1**) [3] and cycloartocarpin A (**2**) [17] were obtained as colorless needles, whereas brosimone I (**3**) [18] was isolated as a yellow powder with mp 150–152°C. The IR and NMR data of these compounds were consistent with corresponding authentic samples. It is noteworthy that brosimone I (**3**) was isolated for the first time from this plant.

Stearic acid (**4**) [19], palmitic acid (**5**) [19], and linoleic acid (**6**) [19] were isolated as colorless needles. They were identified by comparison of their physical and spectral data with reported values [19].

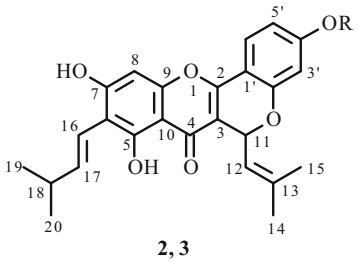
Squalene (**7**) was obtained as a colorless oil. The IR and ^{1}H NMR data corresponded with literature values [20].

Two steroids, β-sitosterol (**8**) [21] and stigmasterol (**9**) [21]), gave a positive Liebermann-Burchard test. The molecular formula of compounds **8** and **9** was determined to be $C_{29}H_{50}O$ (414) and $C_{29}H_{48}O$ (412), respectively, from EI-MS and ^{13}C NMR spectra. These two compounds were further identified by comparison of the IR and ^{1}H NMR spectra with the corresponding literature data.

Brosimone I (3). Yellowish powder; mp 150–152°C; $[\alpha]_D^{25}$ +100° (*c* 0.12, acetone). UV (MeOH, λ_{max}, nm) (log ε): 260 (4.91), 290 (4.21), 370 (4.25). IR (KBr, ν_{max}, cm^{-1}): 3400 (OH), 1652 (C=O). ^{1}H NMR (500 MHz, acetone-d_6, δ, ppm, J/Hz): 1.08 (6H, t, J = 6.8, CH_3-19, 20), 1.68, 1.93 (each 3H, d, J = 1.5, CH_3-14, 15), 2.43 (1H, m, H-18), 5.45 (1H, m, H-12), 6.20 (1H, d, J = 9.0, H-11), 6.40 (1H, d, J = 2.0, H-3′), 6.58 (1H, s, H-8), 6.51–6.60 (2H, m, H-5′, 16), 6.74 (1H, dd, J = 16.0, 7.0, H-17), 7.65 (1H, d, J = 8.0, H-6′), 13.70 (1H, s, OH-5, D_2O exchangeable). EI-MS *m/z* (rel. int): 420 $[M]^+$ (52), 403 (11), 377 (13), 321 (100).



1



2, 3

2: R = CH_3; **3:** R = H

1) School of Medicine and Health Sciences, Fooyin University, Kaohsiung, Taiwan 831, R.O.C., e-mail: low463@gmail.com; 2) Bioresource Collection and Research Center (BCRC), Food Industry Research and Development Institute (FIRDI), Hsinchu, Taiwan 300, R.O.C., e-mail: cmj0404@gmail.com. Published in Khimiya Prirodnykh Soedinenii, No. 4, pp. 538–539, July–August, 2010. Original article submitted March 23, 2009.

From the above evidence, the structure of **3** was elucidated as 4′,5,7-trihydroxy-6-((*E*)-3-methyl-but-1-enyl)-11-(2-methylpropenyl)-6*H*-chromeno[4,3-b]chromen-4-one, a known flavone named brosimone I, which was further confirmed by COSY, NOESY, HSQC, and HMBC plots.

All the above-mentioned compounds were identified by comparison of their spectral data (UV, IR, ^{1}H NMR, MS) with the corresponding values in the literature or with authentic samples. Among them, all known compounds except **1** and **2** were isolated from this plant for the first time.

Many bioactive prenylflavonoids isolated from *Artocarpus* species have been reported in recent studies [7–16]. Most of the compounds possess anti-inflammatory, antioxidant, antibacterial, and antiplatelet activities [3–6]. It is worth examining some minor bioactive metabolites from *Artocarpus* species further.

General Experimental Procedures. Melting points were determined with a YANACO micro-melting point apparatus and were uncorrected. IR spectra were taken on a Hitachi 260-30 spectrophotometer. UV spectra were obtained on a JASCO UV-240 spectrophotometer. EI-MS spectra were recorded on a VG Biotech Quattro 5022 spectrometer. HR-EI-MS were recorded on a JEOL JMX-HX 110 mass spectrometer. ^{1}H NMR and ^{13}C NMR spectra were measured on Varian Gemini 200 and Varian Unity Plus 400 spectrometers and are given in parts per million (δ) downfield from internal TMS. Si gel 60 (Merck 70–230 mesh, 230–400 mesh) was used for column chromatography, and Si gel 60 F_{254} (Merck) for TLC.

Plant Material. Dried stems of *A. heterophyllus* were collected from Fooyin University′s campus in May 2001. A voucher specimen (Lo 001) was deposited in the Herbarium of the School of Medicine and Health Sciences, Fooyin University, Kaohsiung County, Taiwan, Republic of China.

Extraction and Separation of Compounds. The air-dried stems of *A. heterophyllus* (9.5 kg) were extracted repeatedly with MeOH (8 × 4 L) at room temperature. The combined MeOH extracts were evaporated under reduced pressure to yield a brownish viscous residue (187.2 g). The MeOH extract suspended in H_2O (1 L) was partitioned with $CHCl_3$ (2 L × 5) to give fractions soluble in $CHCl_3$ (Part A) (102.3 g) and H_2O (Part B) (46.5 g). The $CHCl_3$-soluble fraction (Part A) (102.3 g) was chromatographed over silica gel CC (3.4 kg) and eluted with increasing polarity of $CHCl_3$–MeOH (50:1, 30:1, 20:1, 10:1, 4:1) mixtures to obtain 100 fractions of 125 mL each. The fractions were combined into 14 fractions (A1–A14) based on TLC. Fraction A3 (*n*-hexane–acetone, 60:1, 10.4 g) was resubjected to silica gel CC (360.7 g), eluting with *n*-hexane–acetone (20:1) and gradually increasing polarity with acetone (60 frs., 150 mL each) to give 12 fractions (A3-a-A3-l) based on TLC. Fraction A3-d (*n*-hexane–EtOAc, 10:1, 2.4 g) was subjected to silica gel CC (5.5 g), eluting with *n*-hexane–EtOAc (5:1) to give **4** and **5** (900.2 mg, R_f 0.52). Fraction A5 (*n*-hexane–acetone, 45:1, 10.1 g) was resubjected to silica gel CC (350.4 g), eluting with *n*-hexane–acetone (25:1) and gradually increasing polarity with acetone (55 fr., 200 mL each) to give 10 fractions (A5-a-A5-j) based on TLC. Fraction A5-c (*n*-hexane–EtOAc, 9:1, 2.1 g) was subjected to silica gel CC (5.5 g), eluting with *n*-hexane–EtOAc (3:1) to give **6** (800.2 mg, R_f 0.45) and **7** (50.3 mg, R_f 0.72). Fraction A6 (*n*-hexane–EtOAc, 40:1, 15.5 g) was resubjected to silica gel CC (550.6 g), eluting with *n*-hexane–acetone (20:1) and gradually increasing polarity with acetone (65 frs., 250 mL each) to give 12 fractions (A6-a-A6-l) based on TLC. Fraction A6-e (*n*-hexane–Me_2CO, 25:1, 2.6 g) was subjected to silica gel CC (6.3 g), eluting with *n*-hexane–EtOAc (6:1) to afford a mixture of **8** and **9** (500.5 mg, R_f 0.45). Fraction A7 (*n*-hexane–EtOAc, 40:1, 7.5 g) was resubjected to silica gel CC (260.6 g), eluting with *n*-hexane–acetone (20:1) and gradually increasing polarity with acetone (50 frs., 150 mL each) to give 9 fractions (A7-a-A7-i) based on TLC. Fraction A7-e (*n*-hexane–EtOAc, 4:1, 1.7 g) was subjected to silica gel CC (5.5 g), eluting with *n*-hexane–Me_2CO (4:1) to give **1** (100.4 mg, R_f 0.55). Fraction A8 (*n*-hexane–acetone, 35:1, 13.7 g) was resubjected to silica gel CC (480.6 g), eluting with *n*-hexane–acetone (15:1) and gradually increasing polarity with acetone (55 fr., 200 mL each) to give 8 fractions (A10-a-A10-h) based on TLC. Fraction A10-d (*n*-hexane–Me_2CO, 9:1, 60.8 mg) was resubjected to preparative TLC (*n*-hexane–Me_2CO, 4:1) to give **3** (10.6 mg, R_f 0.50). Fraction A9 ($CHCl_3$–EtOAc, 35:1, 3.7 g) was resubjected to silica gel CC (130.6 g), eluting with *n*-hexane–$CHCl_3$ (1:7) and gradually increasing polarity with $CHCl_3$–MeOH mixture (40:1, 30:1, 15:1, 4:1; 35 fr., 100 mL each) to give **9** fractions (A12-a-A12-i) based on TLC. Fraction A12-e ($CHCl_3$–EtOAc, 15:1, 50.7 mg) was subjected to silica gel CC (2.1 g), eluting with $CHCl_3$–MeOH (9:1) to give **2** (3.1 mg, R_f 0.40).

ACKNOWLEDGMENT

The first two authors (C.-Y. Chen and M.-J. Cheng) contributed equally to this work and should both be regarded as first author of this publication. This investigation was supported by a grant from the National Science Council of the Republic of China NSC-94-2320-B-242-010 (W.-L. Lo).

REFERENCES

1. M. T. Kao, *Artocarpus in Flora of Taiwan*, Editorial committee of the Flora of Taiwan, Taipei, Taiwan, 2nd Ed., Vol. **II**, 1993, p. 136.
2. Y. H. Wang, A. J. Hou, L. Chen, D. F. Chen, H. D. Sun, Q. S. Zhao, K. F. Bastow, Y. Nakanish, X. H. Wang, and K. H. Lee, *J. Nat. Prod.*, **67**, 757 (2004).
3. B. L. Wei, J. R. Weng, P. H. Chiu, C. F. Hung, J. P. Wang, and C. N. Lin, *J. Agric. Food Chem*., **53**, 3867 (2005).
4. F. Shahidi, *Biofactors,* **13**, 179 (2000).
5. M. R. Khan, A. D. Omoloso, and M. Kihara, *Fitoterapia*, **74**, 501 (2003).
6. J. R. Weng, S. C. Chan, Y. H. Lu, H. C. Lin, H. H. Ko, and C. N. Lin, *Phytochemistry,* **67**, 824 (2006).
7. M. I. Chung, C. M. Lu, P. L. Huang, and C. N. Lin, *Phytochemistry*, **40**, 1279 (1995).
8. B. R. Barik, T. Bhaumik, A. K. Dey, and A. B. Kundu, *Phytochemistry*, **35**, 1001 (1994).
9. C. N. Lin and C. M. Lu, *Tetrahedron Lett*., **34**, 8249 (1993).
10. C. M. Lu and C. N. Lin, *Phytochemistry*, **33**, 909 (1993).
11. Y. Hano, M. Aida, and T. Nomura, *J. Nat. Prod*., **53**, 391 (1990).
12. R. H. V. Mourao, J. Xavier Filho, E. W. Alves, E. G. Orellano, D. F. de Melo, M. E. F. de Aragao, M. da G. S. Lima, and A. Prouvost-Danon, *Acta Farm. Bonaerense*, **18**, 41 (1999).
13. B. Barik, T. Bhaumik, A. K. Dey, and A. B. Kundu, *J. Indian Chem. Soc.,* **74**, 163 (1997).
14. M. Aida, K. Shinomiya, K. Matsuzawa, Y. Hano, and T. Nomura, *Heterocycles*, **39**, 847 (1994).
15. M. K. Hossain, M. A. Rahman, A. J. Mian, and A. K. M. M. Rahman, *J. Bangladesh Acad. Sci.,* **14**, 49 (1990).
16. Y. Hano, M. Aida, M. Shiina, T. Nomura, T. Kawai, H. Ohe, and K. Kagei, *Heterocycles*, **29**, 1447 (1989).
17. C. M. Lu and C. N. Lin, *Phytochemistry*, **35**, 781 (1994).
18. F. Ferrari, I. Messana, and M. C. Mesquita de Araujo, *Planta Med*., **55**, 70 (1989).
19. N. H. El-Sayed, N. H. Mahmoud, E. A. Soher, K. H. Mohamed, and T. J. Mabry, *Rev. Latinoam. Quim.,* **27**, 1 (1999).
20. M. Y. Rios and C. Terpenes, *Chem. Nat. Comp.,* **41**, 297 (2005).
21. C. Y. Chen, F. R. Chang, and Y. C. Wu, *J. Chin. Chem. Soc*., **44**, 313 (1997).