Chemistry of Natural Compounds, Vol. 45, No. 6, 2009

CHEMICAL CONSTITUENTS FROM THE LEAVES OF *Michelia compressa* VAR. *formosana*

H. M. Wang,[1,2] W. L. Lo,[3] Y. C. Lu,[4] Y. T. Yeh,[4] L. Y. Huang,[4] J. C. Huang,[5] and C. Y. Chen[3]*

UDC 547.944/945

The genus *Michelia* (Magnoliaceae) consists of about 30 species. One of the species is native to Taiwan. *Michelia compressa* var. *formosana* is an evergreen tree, especially distributed in Taiwan, Japan, and Ryukyu Islands. *Michelia* species have been used by indigenous peoples for the treatment of cancer. For example, *Michelia champaca* has been used in India for the treatment of abdominal tumors and *M. hypoleuca* and *M. officinalis* for carcinomatous sores and leukemia, respectively, in China [1]. There is only one paper describing a constituent (ceryl alcohol) from the leaves of *M. compressa* [2]. Recently, we reported four aporphines, one oxoaporphine, two amides, one lignan, two neolignans, one benzenoid and two steroids from the stems of this plant [3]. In continuation of a program studying chemotaxonomy and biologically active components from Magnoliaceous plants [3–7], four aporphines, (–)-anonaine [8], (–)-*N*-acetylanonaine [9], (–)-*N*-formylanonaine [10], (–)-*N*-acetylnornuciferine [11]; two oxoaporphines, liriodenine [8], oxoxylopine [12]; one lignan, (+)-syringaresinol [13]; two amides, *N*-*trans*-feruloyltyramine [14], *N*-*cis*-feruloyltyramine [15]; seven benzenoids, 4-hydroxybenzaldehyde [16], 4-hydroxybenzoic acid [16], methylparaben [16], syringaldehyde [16], syringic acid [16], eugenol [17], ferulic acid [16]; two chlorophylls, pheophorbide a [18], aristophyll-C [19]; and two steroids, β-sitosterol, stigmasterol [20], are isolated from the leaves of *M. compressa*. In addition to (–)-anonaine, liriodenine, (+)-syringaresinol, *N*-*trans*-feruloyltyramine, *N*-*cis*-feruloyltyramine, β-sitosterol, and stigmasterol, all of these compounds were isolated for the first time from this source [21–27].

Anti-oxidant properties elicited by plant species have a full range of perspective applications in human health care. In recent years, the prevention of cancer and cardiovascular diseases has been associated with the ingestion of fresh fruits, vegetables, or teas rich in natural anti-oxidants [28]. Experimental evidence suggests that free radicals and reactive oxygen species (ROS) have been implicated in more than 100 diseases, including malaria, acquired immunodeciency syndrome, heart disease, stroke, arteriosclerosis, diabetes, cancer, and gastric ulcer [29–31]. Anti-oxidants can protect the human body from free radicals and ROS effects and retard the progress of many chronic diseases as well as lipid oxidative rancidity in foods [32, 33]. However, BHA and BHT, the most commonly used anti-oxidants at present, are suspected of being responsible for liver damage and carcinogenesis [34, 35]. Therefore, the exploration and utilization of more effective anti-oxidants and anti-bacterial compounds from natural sources are desired. It has been reported that anti-oxidants and radical scavengers inhibit these processes [36].

The leaves of *M. compressa* var. *formosana* were collected from Chiayi County, Taiwan, May 2006. Plant material was identified by Dr. Fu-Yuan Lu (Department of Forestry and Natural Resources College of Agriculture, National Chiayi University). A voucher specimen was deposited in the School of Medicine and Health Sciences, Fooyin University, Kaohsiung County, Taiwan. The air-dried leaves of *M. compressa* var. *formosana* (7.3 kg) were extracted with MeOH (50 L × 5) at room temperature, and a MeOH extract (249.5 g) was obtained upon concentration under reduced pressure. The MeOH extract, suspended in H_2O (1 L), was partitioned with $CHCl_3$ (2 L × 5) to give fractions soluble in $CHCl_3$ (112.2 g) and H_2O (87.3 g).

1) Faculty of Fragrance and Cosmetics, Kaohsiung Medical University, Kaohsiung 807, Taiwan; 2) Center for Excellence in Environmental Medicine, Kaohsiung Medical University, Kaohsiung 807, Taiwan; 3) School of Medical and Health Sciences, Fooyin University, Kaohsiung County 831, Taiwan, fax: +886 7 7863667, e-mail: xx377@mail.fy.edu.tw; 4) Department of Medical Technology, Fooyin University, Kaohsiung County 831, Taiwan; 5) Department of Forestry and Natural Resources, College of Agriculture, National Chiayi University, Chiayi 600, Taiwan. Published in Khimiya Prirodnykh Soedinenii, No. 6, pp. 776–777, November–December, 2009. Original article submitted May 6, 2008.

0009-3130/09/4506-0931

The $CHCl_3$-soluble fraction was chromatographed over silica gel (900 g, 70–230 mesh) using *n*-hexane–$CHCl_3$–MeOH mixtures as the eluent to produce five fractions. Part of fraction 1 (25.12 g) was subjected to silica gel chromatography, by eluting with *n*-hexane–EtOAc (50:1), enriched gradually with EtOAc, to furnish three fractions (1-1–1-5). Fraction 1-1 (8.32 g) was further purified on a silica gel column using *n*-hexane–EtOAc mixtures to obtain eugenol (38 mg), methylparaben (23 mg), and ferulic acid (24 mg). Fraction 1-2 (2.23 g) was further purified on a silica gel column using *n*-hexane–EtOAc mixtures to obtain pheophorbide a (25 mg) and aristophyll-C (51 mg). Fraction 1-3 (3.81 g) was further purified on a silica gel column using *n*-hexane–$CHCl_3$ mixtures to obtain 4-hydroxybenzaldehyde (24 mg), 4-hydroxybenzoic acid (25 mg), syringaldehyde (12 mg), and syringic acid (25 mg). Fraction 1-4 (4.57 g) was further purified on a silica gel column using *n*-hexane–EtOAc mixtures to obtain β-sitosterol and stigmasterol (93 mg). Part of fraction 2 (10.33 g) was subjected to silica gel chromatography by eluting with *n*-hexane–EtOAc (40:1), enriched with EtOAc, to furnish two further fractions (2-1–2-5). Fraction 2-1 (5.24 g) was further purified on a silica gel column using *n*-hexane–EtOAc mixtures to obtain liriodenine (49 mg). Part of fraction 2-3 (3.71 g) was further purified on a silica gel column using *n*-hexane–EtOAc mixtures to obtain (+)-syringaresinol (12 mg). Part of fraction 3 (14.11 g) was subjected to silica gel chromatography by eluting with *n*-hexane–EtOAc (20:1), enriched with EtOAc, to furnish three further fractions (3-1–3-5). Fraction 3-2 (6.11 g) was further purified on a silica gel column using *n*-hexane–EtOAc mixtures to obtain oxoxylopine (24 mg). Part of fraction 4 (37.04 g) was subjected to silica gel chromatography by eluting with $CHCl_3$–MeOH (100:1), enriched with MeOH, to furnish three fractions (4-1–4-5). Fraction 4-1 (19.12 g) was further purified on a silica gel column using $CHCl_3$–MeOH mixtures to obtain (–)-anonaine (51 mg) and (–)-*N*-acetylanonaine (78 mg). Fraction 4-2 (9.58 g), eluted with $CHCl_3$–MeOH (80:1), was further separated using silica gel column chromatography and preparative TLC ($CHCl_3$–MeOH (100:1) and gave (–)-*N*-formylanonaine (13 mg) and (–)-*N*-acetylnornuciferine (15 mg). Fraction 4-4 (7.11 g), eluted with $CHCl_3$–MeOH (100:1), was further separated using silica gel column chromatography and preparative TLC ($CHCl_3$–MeOH (80:1) and gave *N-trans*-feruloyltyramine (22 mg) and *N-cis*-feruloyltyramine (12 mg).

The ferrous ion-chelating potential of chlorophyll was investigated according to the method of [36]. Briefly, the test samples at 100 μM final concentrations dissolving in DMSO were added to a solution of 2 mM $FeCl_2 \cdot 4H_2O$ (0.05 mL). The reaction was initiated by the addition of 5 mM ferrozine (0.2 mL), and the mixture was vigorously shaken and left standing at room temperature for 10 min. After the mixture had reached equilibrium, the absorbance of the mixture was read at 650 nm against a blank. EDTA was used as a positive control. The data was expressed as a mean of three experiments. The metal chelating inhibition activity of (–)-*N*-acetylanonaine was 32.8% and that of parthenolide 28.4% at 100 (μM).

ACKNOWLEDGMENT

This investigation was supported by a grant from the National Science Council of the Republic of China NSC-96-2320-B-242-005 (C.-Y. Chen) and a grant from the Center of Excellence for Environmental Medicine KMU-EM-97-2.1.b (H.-M. Wang).

REFERENCES

1. J. L. Hartwell, *Lloydia*, **33**, 97 (1970).
2. T. Koyama and T. Morikita, *Kumamoto Pharm. Bull.*, **2**, 69 (1955).
3. W. L. Lo, Y. C. Wu, T. J. Hsieh, S. H. Kuo, H. C. Lin, and C. Y. Chen, *Chin. Pharm. J.*, **56**, 69 (2004).
4. C. Y. Chen, L. Y. Huang, L. J. Chen, W. L. Lo, S. Y. Kuo, Y. D. Wang, S. H. Kuo, and T. J. Hsieh, *Chem. Nat. Comp.*, **44**, 137 (2008).
5. C. Y. Chen, T. Z. Liu, W. C. Tseng, F. J. Lu, R. P. Hung, C. H. Chen, and C. H. Chen, *Food Chem. Toxicol.*, **46**, 2694 (2008).
6. R. J. Lin, W. L. Lo, Y. D. Wang, and C. Y. Chen, *Nat. Prod. Res*., **22**, 1055 (2008).
7. B. H. Chen, P. Y. Wu, T. F. Fu, H. M. Wang, and C. Y. Chen, *J. Nat. Prod.,* **72**, 950 (2009).
8. C. Y. Chen, F. R. Chang, and Y. C. Wu, *J. Chin. Chem. Soc.*, **44**, 313 (1997).
9. M. K. Pyo, H. S. Yun-Choi, and Y. J. Hong, *Planta Med.* **69**, 267 (2003).
10. F. R. Chang, C. Y. Chen, T. J. Hsieh, C. P. Cho, and Y. C. Wu, *J. Chin. Chem. Soc.*, **47**, 913 (2000).
11. C. D. Hufford, *Phytochemistry*, **15**, 1169 (1976).

12. S. K. Talapatra, A. Patra, and B. Talapatra, *Chem. Ind.*, 1056 (1969).
13. C. Y. Chen, T. Y. Wu, F. K. Chang, and Y. C. Wu, *J. Chin. Chem. Soc.*, **45**, 629 (1998).
14. C. Y. Chen, F. R. Chang, H. F. Yen, and Y. C. Wu, *Phytochemistry*, **49**, 1443 (1998).
15. N. Fukuda, M. Yonemitsu, and T. Kimura, *Chem. Pharm. Bull*., **31**, 156 (1983).
16. C. Y. Chen, F. R. Chang, C. M. Teng, and Y. C. Wu, *J. Chin. Chem. Soc.*, **46**, 77 (1999).
17. L. H. Chen and Y. H. Kuo, *J. Chin. Chem. Soc.*, **32**, 169 (1985).
18. Y. Nakatani, G. Ourisson and J. P. Beck, *Chem. Pharm. Bull*., **29**, 2261 (1981).
19. Y. Y. Chan, Y. L. Leu, and T. S. Wu, *Chem. Pharm. Bull*., **47**, 887 (1999).
20. Y. H. Kuo and M. H. Yeh, *J. Chin. Chem. Soc.*, **44**, 379 (1997).
21. K. Ito, *Yakugaku Zasshi*, **80**, 705 (1960).
22. S. S. Yang, W. Y. Huang, L. C. Lin, and P. Y. Yeh, *Huaxue*, 144 (1961).
23. M. Tomita and H. Fukukawa, *Yakugaku Zasshi*, **82**, 925 (1962).
24. T. H. Yang, *Yakugaku Zasshi*, **82**, 794 (1962).
25. T. H. Yang, *Yakugaku Zasshi*, **82**, 798 (1962).
26. T. H. Yang, *Yakugaku Zasshi*, **82**, 804 (1962).
27. M. Ogura, G. A. Cordell, and N. R. Farnsworth, *Phytochemistry*, **17**, 957 (1978).
28. D. F. Wang, J. Li, C. H. Wang, G. W. Zhao, Y. Jin, D. D. Chen, and S. Ye, *J. Tea Sci.*, **20**, 45 (2000).
29. B. Halliwell, *Am. J. Med*., **91**, 14 (1991).
30. M. G. L. Hertog, P. C. H. Hollman, M. B. Katan, and D. Kromhout, *Nutr. Cancer Int. J.*, **20**, 21 (1993).
31. J. E. Kinsella, E. Frankel, B. German, and J. I. Kanner, *Food Technol*., **47**, 85 (1993).
32. S. Sasaki, T. Ohta, and E. A. Decker, *J. Agric. Food Chem*., **44**, 1682 (1996).
33. A. L. Branen, *J. Am. Oil Chem. Soc.*, **52**, 59 (1975).
34. N. Ito, S. Fukushima, A. Hasegawa, M. Shibata, and T. Ogiso, *J. Natl. Cancer Inst.*, **70**, 343 (1983).
35. T. Nakagawa, T. Yokozawa, K. Terasawa, S. Shu, and L. R. Juneja, *J. Agric. Food Chem*., **50**, 2418 (2002).
36. E. A. Decker and B. Welch, *J. Agric. Food Chem*., **38**, 674 (1990).