Chemistry of Natural Compounds, Vol. 47, No. 3, July, 2011 [Russian original No. 3, May–June, 2011]

CHEMICAL CONSTITUENTS FROM THE ROOTS OF *Uvaria rufa*

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UDC547.944/945+547.972

There are approximately 150 species of plants in the genus *Uvaria* (Annonaceae) distributed in tropical areas. Fourteen species have been found in Thailand [1]. *Uvaria rufa* Blume, locally knows as "Pe-Puan-Noi", is a climber shrub plant distributed mainly in tropical rainforest areas of Thailand. An alcoholic tincture of its roots is used ethnomedically as ecbolic [2]. Previous phytochemical studies of this plant have revealed the presence of flavonoids, tectochrysin, 7-*O*-methylwogonine, and 6,7-*O*,*O*-dimethylbaicalein from the bark [3] and 2,5-dihydroxy-7-methoxyflavanone from the roots [4]. (*E*)-3,7-Bisbenzoyloxyhept-4-en-1,2,6-triol [5] and highly oxygenated cyclohexenes (zeylenol, ellipeiosol B, and ferrudiol) [2] were also isolated from the leaves of this plant. In continuation of our phytochemical investigation of this plant, we isolated 13 compounds (**1–13**), one flavonoid (**8**), and four alkaloids (**9**, **11–13**) were isolated from this plant for the first time. To our knowledge, this is the first report on the alkaloid constituents of *U. rufa*.

Plant Material. The roots of *U. rufa* were collected from Nakornpanom Province in Northeastern part of Thailand in July 2004, and identified by Prof. Dr. Obchan Thaithong through comparison with the herbarium specimen (BCU 005767) available at the Department of Botany, Chulalongkorn University.

Extraction and Isolation. The air-dried powdered roots (1.2 kg) of *U. rufa* were defatted with *n*-hexane and extracted with CH_2Cl_2 and MeOH at room temperature. Evaporation of CH_2Cl_2 under reduced pressure gave a brown colored residue (17.3 g), and evaporation of MeOH gave a dark brown solid (67.9 g). The methanolic extract was partitioned with *n*-butanol and water. The *n*-butanolic extract was evaporated under reduced pressure to yield a brown solid (4.0 g). The CH_2Cl_2 extract (15.0 g) was subjected to vacuum liquid chromatography (VLC) over silica gel (Merck Art 7730) using hexane, CH_2Cl_2, EtOAc, and MeOH with increasing polarity. A total of eight fractions was collected (A–H). From VLC, fraction B was recrystallized from EtOAc–CH_2Cl_2 (1:24) to yield colorless needles of 2,5-dihydroxy-7-methoxyflavanone (**1**, 1.1 mg) [4] and yellowish rhombic crystals of tectochrysin (**2**, 310.0 mg) [3]. The mother liquor of this fraction was chromatographed on silica gel column using a stepwise gradient elution of hexane, CH_2Cl_2, and EtOAc, and further purified by HPLC (Cosmosil 5C18-ARII, 10 × 250 mm, MeOH–H_2O (1:1)) to furnish pinostrobin (**3**, 1.7 mg) [6]. Similary, fraction A was also subjected to column chromatography over silica gel (hexane–CH_2Cl_2, 1:1) and further purified with chromatotron using a stepwise gradient of EtOAc in hexane to give benzyl benzoate (**4**, 2.1 mg) and 2-methoxybenzyl benzoate (**5**, 14.6 mg) [7]. Repeated VLC of fraction C using a stepwise gradient elution of EtOAc in hexane yielded seven fractions (C 1–7). Fraction C3 was separated by chromatotron eluting with a gradient between hexane and EtOAc to afford 6,7-*O,O*-dimethylbaicalein (**6**, 1.0 mg) [3]. Similarly, repeated CC of fraction C5 eluted with hexane–CH_2Cl_2 (7:3) yielded 7-*O*-methylwogonine (**7**, 21.2 mg) [3]. HPLC (Cosmosil 5C18-ARII, 10 × 250 mm, MeOH–H_2O (7:3)) was performed for purification of fraction C4 to yield an inseparable mixture of 85.6 mg of 2,5-dihydroxy-6,7-dimethoxyflavanone (**8** [8] and **6**).

The butanolic extract (14.0 g) was similarly chromatographed on silica gel VLC using a stepwise gradient elution of MeOH in CH_2Cl_2 to yield eight fractions (B1-8). Fraction B8, eluted with pure MeOH, gave an orange powder of oxoanolobine (**9**, 42.0 mg) [9]. Repeated column chromatography of B5, eluted with EtOAc–CH_2Cl_2 (1:4) and further purified by HPLC (Cosmosil 5C18-ARII, 10 × 250 mm, MeOH–H_2O (3:2)), afforded liriodenine (**10**, 1.2 mg) and lanuginosine (**11**, 2.9 mg) [10, 11]. Similarly, fraction B6 eluted with 100 % MeOH was purified by flash silica gel column chromatography to furnish roemerine (**12**, 5.7 mg) [12] and roemeroline (**13**, 0.5 mg) [13].

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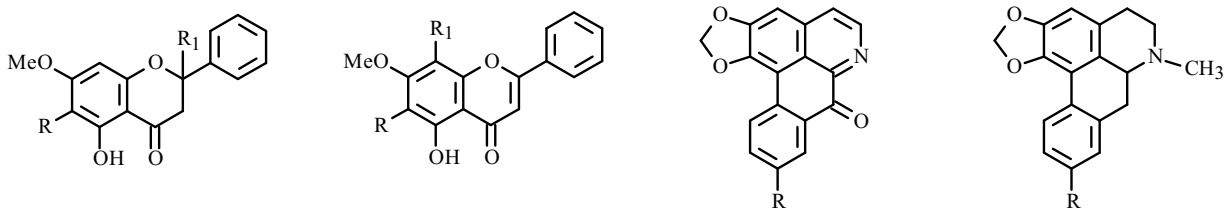
 0009-3130/11/4703-0474



1, 3, 8 **2, 6, 7** **9 - 11** **12, 13**

1: R = H, R_1 = OH; **2:** R = R_1 = H; **3:** R = R_1 = H; **6:** R = OMe, R_1 = H; **7:** R = H, R_1 = OMe
8: R = OMe, R_1 = OH; **9, 13:** R = OH; **10, 12:** R = H; **11:** R = OMe

Identification of all isolated compounds was confirmed by means of spectroscopic methods (MS, ^{1}H, ^{13}C NMR, and 2D NMR) as well as comparison with literature data.

2,5-Dihydroxy-6,7-dimethoxyflavanone (8): yellowish solid. ^{1}H NMR (400 MHz, $CDCl_3$, δ): 12.26 (1H, s, 5-OH), 8.42–8.44 (2H, m, H-2′ and H-6′), 7.95–8.01 (3H, m, H-3′, H-4′ and H-5′), 6.66 (1H, s, H-8), 3.56–3.58 (2H, m, H-3), 4.41 (3H, s, 7-OCH_3), 4.37 (3H, s, 6-OCH_3). ^{13}C NMR (100 MHz, $CDCl_3$, δ): 194.4 (C-4), 160.8 (C-7), 155.0 (C-9), 154.7 (C-5), 130.8 (C-6), 129.4 (C-3′, C-4′ and C-5′), 128.7 (C-2′ and C-6′), 102.4 (C-10), 101.7 (C-2), 92.6 (C-8), 61.1 (6-OCH_3), 56.1 (7-OCH_3), 48.4 (C-3).

Oxoanolobine (9): orange amorphous solid; ESI-MS *m/z* 292 $[M + H]^+$. UV (MeOH, λ_{max}, nm): 378, 314, 269, 247, 218. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm, J/Hz): 10.34 (1H, s, 9-OH), 8.79 (1H, d, J = 4.8, H-5), 8.51 (1H, d, J = 8.8, H-11), 8.03 (1H, d, J = 5.2, H-4), 7.71 (1H, d, J = 2.8, H-8), 7.30 (1H, dd, J = 2.6, 8.6, H-10), 7.50 (1H, s, H-3), 6.46 (2H, s, 1,2-OCH_2O). ^{13}C NMR (100 MHz, DMSO-d_6, δ): 158.2 (9-OH), 152.0 (C-2), 147.2 (C-1), 144.9 (C-3a), 136.0 (C-6a), 133.0 (C-7a), 124.5 (C-11a), 113.0 (C-8), 129.4 (C-11), 124.7 (C-4), 122.8 (C-10), 122.0 (C-1b), 106.9 (C-1a), 102.6 (C-3).

Lanuginosine (11): orange-yellow solid. UV (MeOH, λ_{max}, nm): 269, 246, 219. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm, J/Hz): 8.81 (1H, d, J = 5.2, H-5), 8.57 (1H, d, J = 8.8, H-11), 8.04 (1H, d, J = 5.2, H-4), 7.80 (1H, d, J = 2.8, H-8), 7.48 (1H, dd, J = 3.2, 8.8, H-10), 7.53 (1H, s, H-3), 6.48 (2H, s, 1,2-OCH_2O), 3.93 (9-OCH_3). ^{13}C NMR (100 MHz, DMSO-d_6, δ): 159.4 (9-OCH_3), 151.9 (C-2), 147.7 (C-1), 144.4 (C-3a), 136.0 (C-6a), 132.7 (C-7a), 126.1 (C-11a), 125.2 (C-4), 122.4 (C-1b and C-10), 106.4 (C-1a), 102.7 (C-3).

Roemeroline (13): brown solid; ESI-MS *m/z* 296 $[M + H]^+$. ^{1}H NMR (400 MHz, CD_3OD, δ, ppm, J/Hz): 7.80 (1H, d, J = 8.0, H-11), 6.63 (1H, s, H-8), 6.61 (1H, m, H-10), 6.42 (1H, s, H-3), 5.94 and 5.81 (2H, s, 1,2-OCH_2O), 2.49 (3H, s, N-CH_3). ^{13}C NMR (100 MHz, CD_3OD, δ): 156.6 (9-OH), 147.2 (C-2), 141.6 (C-1), 135.6 (C-7a), 125.6 (C-3a or C-1b), 124.7 (C-3a or C-1b), 121.9 (C-11a), 114.2 (C-8), 112.9 (C-10), 61.8 (C-6a), 52.9 (C-5), 42.1 (N-CH_3), 33.7 (C-7), 27.6 (C-4).

ACKNOWLEDGMENT

The authors are grateful to the Graduate School of Chulalongkorn University for fellowships and financial support.

REFERENCES

1. T. Smitinand, *Thai Plant Names* (*Botanical Names-Vernacalar Names*). Khongrit Ekavibhata, Bangkok, 1980, p. 340.
2. F. A. Tudla, A. M. Aguinaldo, K. Krohn, H. Hussain, and A. P. G. Macabeo, *Biochem. Syst. Ecol.*, **35**, 45 (2007).
3. V. Lojanapiwatna, K. Promsuwansiri, B. Suwannatip, and P. Wiriyachitra, *J. Sci. Soc. Thailand*, **7**, 83 (1981).
4. K. Chantrapromma, C. Pakawatchai, B. W. Skelton, A. H. White, and S. Worapatamasri, *Aust. J. Chem*., **42**, 2289 (1989).
5. A. Chayunkiat, P. Wiriyachitra, and W. Taylor, *J. Sci. Soc. Thailand*, **10**, 239 (1984).
6. P. Tuchinda, V. Reutrakul, P. Claeson, U. Pongprayoon, T. Sematong, T. Santisuk, and W. C. Taylor, *Phytochemistry*, **59**, 169 (2002).

7. M. H. H. Nkunya, *J. Nat. Prod.*, **48**, 999 (1985).
8. J. F. Stevens, M. Ivancic, M. L. Deinzer, and E. Wollenweber, *J. Nat. Prod*., **62**, 392 (1999).
9. C. H. Phoebe, P. L. Schiff, J. E. Knapp, and D. J. Slatkin, *Heteocycles*, **14**, 1977 (1980).
10. Z. Zhang, H. N. Elsohly, M. R. Jacob, D. S. Pasco, L. A. Walker, and A. M. Clark, *J. Nat. Prod*., **65**, 856 (2002).
11. M. R. Khan, M. Kihara, and A. D. Omoloso, *Eupomatia Laurina. Pharm. Biol*., **41**, 277 (2003).
12. M. You, D. B. M. Wickramaratne, G. L. Silva, H. Chai, T. E. Chagwedera, N. R. Farnsworth, G. A. Cordell, A. D. Kinghorn, and J. M. Pezzuto, *J. Nat. Prod*., **58**, 598 (1995).
13. J. A. Lopez, J. G. Laurito, A. M. Brenes, F. T. Lin, M. Sharaf, L. K. Wong, and P. L. Jr. Schiff, *Phytochemistry*, **29**, 1899 (1990).