HETEROCYCLES, Vol. 87, No. 3, 2013, pp. 583 - 589. © 2013 The Japan Institute of Heterocyclic Chemistry
Received, 27th November, 2012, Accepted, 11th January, 2013, Published online, 30th January, 2013
DOI: 10.3987/COM-12-12642

AURONES AND ISOAURONES FROM THE FLOWERS OF *ROSA DAMASCENA* AND THEIR BIOLOGICAL ACTIVITIES

Xue-Mei Gao,[1] Li-Ying Yang,[1,2] Xiang-Zhong Huang,[1] Li-Dan Shu,[1] Yan-Qiong Shen,[1] Qiu-Fen Hu,[1*] and Zhang-Yu Chen[1,2*]

[1] Key Laboratory of Chemistry in Ethnic Medicinal Resources, State Ethnic Affairs Commission & Ministry of Education, Yunnan University of Nationalities; [2] Key Laboratory of Tobacco Chemistry of Yunnan Province, Yunnan Academy of Tobacco Science, Kunming 650106, P. R. China. E-mail: huqiufena@yahoo.com.cn; chenzy@ynzy-tobacco.com

Abstract – One new aurone, one new isoaurone, damaurones A and B (**1** and **2**), and five known compounds (**3**-**7**) were isolated from the flowers of *Rosa damascena*. Their structures were elucidated by spectroscopic methods, including extensive 1D- and 2D- NMR techniques. Compound **1** is the first naturally occurring aurone derivatives bearing an acetyl group. Compounds **1-7** were tested for their anti-HIV-1 activities and cytotoxicities. The results showed that compound **1** has significant potential anti-HIV-1 activity with therapeutic index (TI) values above 80, and showed high cytotoxicities against NB4 and MCF7 cell lines with IC_{50} values of 3.4 and 2.6 μM, respectively.

INTRODUCTION

The genus of *Rosa* is one of the most important ornamental plants in the world. Some species of *Rosa*, such as *R. rugosa*, *R. damascena*, and *R. centifolia,* are renowned for their beautiful flowers and fine fragrance, and had widely been cultivated in several areas of Yunnan Province for rose oil and ornamental flowers commercially.[1,2] Meanwhile, their petals and buds also have been used as food or medicine for treating stomachache, diarrhoea and women's diseases.[3,4] The previous phytochemical researches have revealed that tannins,[5,6] flavonoids,[7-9] as well as terpenoids[10,11] are major components isolated from plants of this genus.

In our previous studies, some aurones were isolated from the flower of *R. rugosa*.[9] With the aim of continuing efforts to multipurpose utilization of the genus of *Rosa* and identify bioactive natural products from these plants, we have carried out phytochemical investigation on the flowers of *R. damascena*. As a

result, one new aurone (**1**), one new isoaurone, (**2**), and five known compounds (**3**-**7**) were isolated from this plant. Compound **1** is the first naturally occurring aurone derivatives bearing an acetyl group. In addition, the anti-HIV-1 activities and cytotoxicities of compounds **1**-**7** were evaluated. Compound **1** showed significant potential anti-HIV-1 activity with therapeutic index (TI) value above 80, and showed high cytotoxicities against NB4 and MCF7 cell with IC_{50} values of 3.4 and 2.6 μM, respectively.

RESULTS AND DISCUSSION

A 70% aqueous methanol extract prepared from the flowers of *R. damascena* was subjected repeatedly to column chromatography on silica gel, Sephadex LH-20, RP-18 and preparative HPLC to afford one new aurone, damaurone A (**1**), one new isoaurone, damaurone B (**2**), together with five known compounds. The structures of the compounds **1**-**7** were as shown in Figure 1, and the ^{1}H and ^{13}C NMR data of compounds **1** and **2** were listed in Table 1. Compared with literature data, the known compounds were identified as: 4′,6-dihydroxy-4-methoxyisoaurone (**3**),[12] cernuoside (**4**),[13] 6,7,3′,4′-tetrahydroxyaurone (**5**),[14] maritimein (**6**),[15] 4,6,10,4′-tetrahydroxyaurone (**7**).[16]

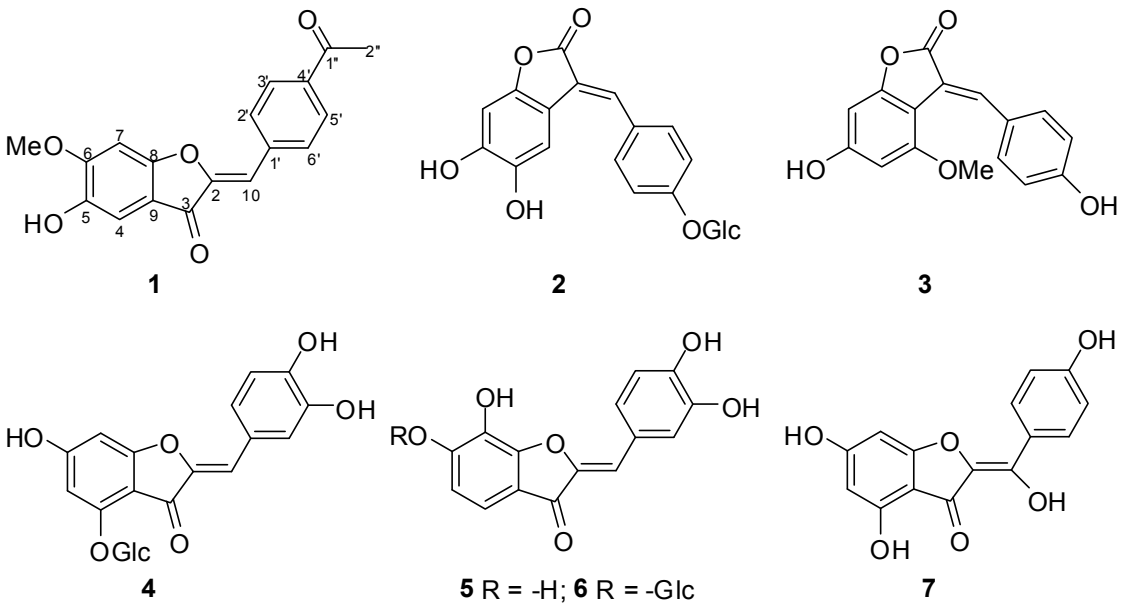

Figure 1. The structures of compounds **1** - **7**

Compound **1** was obtained as yellow gum. Its molecular formula was determined as $C_{18}H_{14}O_5$ by HR-ESI-MS *m/z* 309.0756 $[M-H]^-$ (calcd 309.0763). The ^{1}H and ^{13}C NMR spectrum of **1** (Table 1) displayed 18 carbon signals and 14 proton signals, respectively, corresponding to an aurone nucleus[9,17] (δ_C 147.8 s,

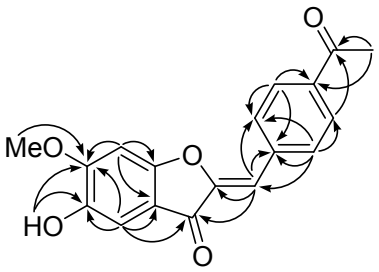

Figure 2 Selected HMBC (↷) correlations of **1**

180.5 s, 114.6 d, 141.1 s, 150.0 s, 103.3 d, 159.9 s, 117.2 s, 110.9 d, 133.0 s, 132.4 d (2C), 122.6 d (2C), 137.2 s), one methoxy group (δ_C 55.8, δ_H 3.80), one phenolic hydroxy proton (δ_H 10.82), and one acetyl group (δ_C 199.3 s, 28.4 q; δ_H 2.54). Strong absorption bands accounting for hydroxy group (3420 cm^{-1}), carbonyl group (1688, 1665 cm^{-1}) and aromatic groups (1638, 1592, 1497, 1442 cm^{-1}) could also be observed in its IR spectrum. The UV spectrum of **1** showed absorption maxima at 352, 264 and 210 nm, which confirmed the existence of the aromatic functions.

The HMBC correlations (Figure 2) of H-10 (δ_H 6.81) with C-2 (δ_C 147.8), C-3 (δ_C 180.5), C-1′ (δ_C 133.0), C-2′ (δ_C 132.4), of H-2′ (δ_H 7.79) with C-10 (δ_C 110.9), and of H-4 (δ_H 7.34) with C-3 (δ_C 180.5), also supported the aurone nucleus. The signals for four coupled aromatic protons at δ_H 7.79 (d, J = 8.8 Hz, 2H), and 8.12 (d, J = 8.8 Hz, 2H), suggested a 4′-monosubstituted for C ring. The proton signals for two singlets at δ_H 7.34 (s, 1H), and δ_H 6.60 (s, 1H) also revealed that the substituents for B-ring should be located at C-5, and C-6. The HMBC correlations of the hydroxy proton signal, δ_H 10.82 with C-4 (δ_C 114.6), C-5 (δ_C 141.1), C-6 (δ_C 150.0), suggested the attachment position of the phenolic hydroxy group at C-5. The HMBC correlation of the methoxy proton signal (δ_H 3.80) with C-6 (δ_C 150.0) suggested the methoxy group located at C-6. The assignment of the acetyl group at C-4′ was also supported by the HMBC correlations of H-2″ (δ_H 2.54) with C-4′ (δ_C 137.2), and of H-3′, 5′ (δ_H 8.12) with C-1″ (δ_C 199.3). Thus, the structure of **1** was established as 4′-acetyl 5-hydroxy-6-methoxyaurone, and given the trivial name of damaurone A. Damaurone A is the first naturally occurring aurone derivative bearing an acetyl group.

Table 1. ^1H and ^{13}C NMR data of compounds **1**and **2**

No.	Compound **1** [a]		Compound **2** [b]	
	δ_C (m)	δ_H (m, J, Hz)	δ_C (m)	δ_H (m, J, Hz)
2	147.8 s		169.9 s	
3	180.5 s		120.0 s	
4	114.6 d	7.34, s	109.4 d	6.59, s
5	141.1 s		145.0 s	
6	150.0 s		147.4 s	
7	103.3 d	6.60, s	105.1 d	6.33, s
8	159.9 s		154.5 s	
9	117.2 s		118.8 s	
10	110.9 d	6.81, s	142.6 d	7.79, s
1′	133.0 s		126.9 s	
2′,6′	132.4 d	7.79, d, J=8.8	132.3 d	8.11, d, J=8.8
3′,5′	122.6 d	8.12, d, J=8.8	115.1 d	6.91, d, J=8.8
4′	137.2 s		161.1 s	
1″	199.3 s		104.3 d	5.25, d, J=7.3
2″	28.4 q	2.54, s	75.8 d	3.44 m
3″			78.4 d	3.42 m
4″			71.4 d	3.32 m
5″			78.1 d	3.21 m
6″			62.7 t	3.53, 3.68 m
OMe-6	55.8 q	3.80, s		
OH-5		10.82, s		

[a] obtained in C_5ND_5 (500 and 125 M Hz), [b] obtained in CD_3OD (400 and 100 M Hz).

Since the configuration of the olefinic bond in aurones can be established on the basis of the chemical shift of olefic methine resonance as it absorbs at 119.9-121.5 ppm in *E*-aurones and at 105.9-112.8 ppm

in *Z*-aurones.[18] The configuration of the olefinic bond in **1** was defined as *Z* by the chemical shift value of carbon atom C-10.

Compound **2** was obtained as a yellow solid. It gives a parent ion by HR-ESIMS at *m/z* 431.0971 $[M-H]^-$ (calcd for 431.0978) corresponding to a molecular formula of $C_{21}H_{20}O_{10}$, requiring twelve degrees of unsaturation. The ^{1}H NMR spectrum of **2** showed the presence of an AA′BB′ aromatic system at δ_H 8.11 (2H, d, J = 8.8 Hz, H-2′,6′) and 6.91 (2H, d, J = 8.8 Hz, H-3′,5′), two *non*-coupled aromatic protons at δ_H 6.59 (1H, s, H-4) and 6.33 (1H, s, H-7), an isolated olefinic proton at δ_H 7.79 (1H, s, H-10), and a glucosyl moiety [δ_H 5.25 (1H, d, J = 7.3, H-1″); δ_H 3.21 ~ 3.68 (6H, m, H-2″, H-3″, H-4″, H-5″, H-6″)]. The ^{13}C NMR spectrum of **2** also revealed the presence of an isoaurone nucleus,[19,20] and a glucosyl moiety (Table 1). The HMBC correlations of the deshielded H-10 (δ_H 7.79) coupled to C-2 (δ_C 169.9) suggested that the structure of **2** could be an isoaurone too. The long-range correlations in the HMBC spectrum between H-1″ (δ_H 5.25 d) and C-4′ (δ_C 161.1 s) indicated the glucosyl was linked to C-4′, and the coupling constant value of H-1″ (J = 7.3 Hz) indicated that the glucosyl moiety was connected to the aglycone by a β-linkage.[20,21] The configuration of *E*- and *Z*-isoaurones is determined on the basis of the chemical shift of H-10, which is anisotropically and diamagnetically affected by the C-2 carbonyl group. It is known that the chemical shift of H-10 in *Z*-isoaurone resonates at higher field (7.4~7.5 ppm) than in the *E*-isomer (7.8~8.0 ppm).[22] Therefore, the H-10 chemical shift at δ_H 7.79 suggested that **2** is *E*-isoaurone. Since no other substituent signals were observed, two hydroxy groups should be located at C-5 and C-6 to support the ^{1}H NMR signals of two *non*-coupled aromatic protons for aromatic ring B. The NMR signals of **2** were also agreed with the previously reported 6,7-dihydroxy substituted isoaurones.[20] On the basis of the above evidence, the structure of **2** was established as shown, and given the name as damaurone B.

Since certain of the aurone derivatives exhibit potential cytotoxicities and anti-HIV-1 activities. Compounds **1**-**7** were tested for their cytotoxicities and anti-HIV-1 activities.

Table 2. Anti-HIV activity of compounds **1** - **7**

compounds	CC_{50} (μM)	EC_{50} (μM)	TI [a]
1	>200	2.43± 0.06	>82.3
2	>200	6.27± 0.03	>31.9
3	>200	4.15± 0.05	>48.2
4	96.5± 0.13	3.68± 0.08	26.2
5	>200	11.9± 0.09	>16.8
6	>200	12.84± 0.04	>15.6
7	>200	9.53± 0.05	>21.0
AZT	>200	0.045± 0.06	>4444.4

[a] TI (therapeutic index) = CC_{50}/EC_{50}.

For anti-HIV-1 activity assay, the cytotoxicity against C8166 cells (CC_{50}) was assessed using the MTT method and anti-HIV-1 activity was evaluated by the inhibition assay for the cytopathic effects of HIV-1 (EC_{50}), using AZT as a positive control (EC_{50} = 0.045 μM and CC_{50} > 200 μM).[23] The results are shown in Table 2. Compound **1** showed

significant potential anti-HIV-1 activity with therapeutic index (TI) value above 80. Compounds **2** and **3** also showed moderate anti-HIV-1 activitiy with TI values above 30.

The cytotoxicity tests for the isolates were performed using a previously reported procedure.[24] All treatments were performed in triplicate. In the MTT assay, the IC_{50} was defined as the concentration of the test compound resulting in a 50% reduction of absorbance compared with untreated cells. The cytotoxic abilities against NB4, A549, SHSY5Y, PC3, and MCF7 tumor cell lines by MTT-assay (with taxol as the positive control) were shown in Table **3**. The results showed that compound **1** showed high cytotoxicity against NB4 and MCF7 cell lines with IC_{50} values of 3.4 and 2.6 μM, respectively.

Table 3. The cytotoxicities data for the compounds **1** - **7**

Compounds	NB4	A549	SHSY5Y	PC3	MCF7
1	3.4±0.06	6.9±0.05	>10	8.2±0.07	2.6
2	>10	>10	>10	>10	>10
3	8.9±0.09	>10	6.5±0.04	8.6±0.06	2.6
4	>10	>10	>10	>10	>10
5	8.1±0.10	>10	>10	8.8±0.09	7.9
6	>10	>10	>10	>10	>10
7	8.5±0.12	>10	8.7±0.08	>10	>10
Taxol	0.03±0.01	0.02±0.02	0.2±0.01	0.2±0.01	0.1

EXPERIMENTAL

General. UV spectra were obtained using a Shimadzu UV-2401A spectrophotometer. IR spectra were obtained in KBr disc on a Bio-Rad Wininfmred spectrophotometer. ESI-MS were measured on a VG Auto Spec-3000 MS spectrometer. ^{1}H, ^{13}C and 2D NMR spectra were recorded on Bruker DRX-500 or 400 instrument with TMS as internal standard. Column chromatography was performed on silica gel (200-300 mesh), or on silica gel H (10～40 μm, Qingdao Marine Chemical Inc., China). Further separation used an Agilent 1100 HPLC equipped with ZORBAX-C_{18} (21.2 mm × 250 mm, 7.0 μm) column and DAD detector.

Plant material. The flowers of *Rosa damasecena*. Mill. were collected in Kunming Herb Medicine Market, in September 2010. The identification of the plant material was verified by Prof. Chen Y. J (Yunnan Nationalities University). A voucher specimen (YNNI 10-9-87) has been deposited in our laboratory.

Extraction and Isolation. The air-dried and powdered flowers of *R. damascena* (4.2 kg) were extracted four times with 70% MeOH (4 × 20 L) at room temperature and filtered. The crude extract (155 g) was applied to silica gel (200–300 mesh) column chromatography, eluting with $CHCl_3$-$(CH_3)_2CO$ gradient system (20:1, 9:1, 8:2, 7:3, 6:4, 5:5), to give six fractions A–F. The further separation of fraction C (7:3, 15.6 g) by silica gel column chromatography, eluted with $CHCl_3$-MeOH (9:1, 8:2, 7:3, 6:4, 1:1), yielded mixtures D1–D5. Fraction D1 (8:2, 1.57 g) was subjected to preparative HPLC (30% MeOH, flow rate 12 mL/min) to give **1** (22.4 mg) and **3** (18.2 mg). The further separation of fraction F (1:1, 35.6 g) by silica

gel column chromatography, and preparative HPLC (20% MeOH, flow rate 12 mL/min) give **2** (16.4 mg), **4** (22.8 mg), **5** (15.2 mg), **6** (11.4 mg), and **7** (14. 5 mg).

Anti-HIV1 Assays. The cytotoxicity assay against C8166 cells (CC_{50}) was assessed using the MTT method and anti-HIV-1 activity was evaluated by the inhibition assay for the cytopathic effects of HIV-1 (EC_{50}).[23]

Cytotoxicity Assay. The cytotoxicity tests for the isolates were performed against NB4, A549, SHSY5Y, PC3 and MCF7 tumor cell lines by MTT-assay (with taxol as the positive control).[24]

Damaurone A (1). Obtained as a yellow gum; UV (MeOH), λ_{max} (log ε) 352 (3.28), 264 (3.80), 210 (4.07) nm; IR (KBr) ν_{max} 3420, 2908, 2876, 1688, 1665, 1638, 1592, 1497, 1442, 1267, 1145, 1073, 862, 784 cm^{-1}; ^1H NMR and ^{13}C NMR data (C_5ND_5, 500 MHz and 125 MHz, respectively), **Table 1**; ESIMS (negative ion mode) *m/z* 309 $[M-H]^-$; HRESIMS (negative ion mode) *m/z* 309.0756 $[M-H]^-$ (calcd 309.0763 for $C_{18}H_{13}O_5$).

Damaurone B (2). Obtained as yellow gum; UV (MeOH) max (log ε) 390 (3.32), 260 (3.58), 210 (4.02) nm; IR (KBr) ν_{max} 3423, 2911, 2864, 1675, 1657, 1625, 1543, 1436, 1352, 1142, 908, 858, 779 cm^{-1}; ^1H NMR and ^{13}C NMR data (CD_3OD, 400 and 100 MHz), see Table 1; negative ESIMS *m/z* 431 $[M-H]^-$; negative HRESIMS *m/z* 431.0971 $[M-H]^-$ (calcd for $C_{21}H_{19}O_{10}$, 431.0978).

ACKNOWLEDGMENT

This project was supported financially by the Excellent Scientific and Technological Team of Yunnan High School (2010CI08), the Yunnan University of Nationalities Green Chemistry and Functional Materials Research for Provincial Innovation Team (2011HC008), and Open Research Fund Program of Key Laboratory of Chemistry in Ethnic Medicine Resource (Yunnan University of Nationalities) (2010XY08).

REFERENCES

1. X. H. Zhang and Z. Z. Xie, *Chin. J. Shandong. Forest. Sci. Tech.*, 2012, **42**, 5.
2. J. L. Lu, *China Flowers Horticul.*, 2012, **11**, 26.
3. K. Shibata, *A Cyclopedia of Useful Plant and Plant Products*: Enlarged and Revised Edition, The Hokuryukan, Tokyo, 1957, p. 612.
4. Y. Jiang, *Flora of China*, Chinese Science Press: Beijing, 1977.
5. S. Ochir, B. Park, M. Nishizawa, T. Kanazawa, M. Funaki, and T. Yamagishi, *J. Nat. Med.*, 2010, **64**, 383.
6. K. H. Park, K. Sung, S. E. Choi, J. H. Kwon, H. Myung, and M. W. Lee, *Chem. Pharm. Bull.*, 2010, **58**, 1227.

7. E. A. Porter, A. A. van den Bos, G. C. Kite, N. C. Veitch, and M. S. Simmonds, *Phytochemistry*, 2012, **81**, 90.
8. E. K. Kwon, D. Y. Lee, H. Lee, D. O. Kim, N. Baek, Y. E. Kim, and H. Y. Kim, *J. Agric. Food Chem*., 2010, **58**, 882.
9. X. M. Gao, L. Y. Yang, L. D. Shu, Y. Q. Shen, Y. J. Zhang, and Q. F. Hu, *Heterocycles*, 2012, **85**, 1925.
10. J. Q. Yuan, X. Z. Yang, J. H. Miao, C. P. Tang, C. Q. Ke, J. B. Zhang, X. J. Ma, and Y. Ye, *Molecules*, 2008, **13**, 2229.
11. N. Zeng, Y. Shen, L. Z. Li, W. H. Jiao, P. Y. Gao, S. J. Song, W. S. Chen, and H. W. Lin, *J. Nat. Prod*., 2011, **74**, 732.
12. N. T. Dat, X. J. Jin, Y. S. Hong, and J. J. Lee, *J. Nat. Prod*., 2010, **73**, 1167.
13. F. Imperato, *Cell. Mol. Life Sci*., 1982, **38**, 67.
14. M. Sashida, K. Ogawa, M. Kitada, H. Karikome, Y. Mimaki, and H. Shimomura, *Chem. Pharm. Bull*., 1991, **39**, 709.
15. J. B. Harborne and T. A. Geissman, *J. Am. Chem. Soc*., 1956, **78**, 829.
16. U. V. Mallavadhani and A. Mahapatra, *Nat. Prod. Res*., 2005, **19**, 91.
17. L. Huang, M. E. Wall, M. C. Wani, H. Navarro, T. Santisuk, V. Reutrakul, E. K. Seo, N. R. Farnsworth, and A. D. Kinghorn. *J. Nat. Prod*., 1998, **61**, 446.
18. P. K. Agrawal, *Carbon-13 NMR of Flavonoids*, Elsevier, Amsterdam, 1989, p. 564.
19. N. T. Dat, X. J. Jin, Y. S. Hong, and J. J. Lee, *J. Nat. Prod*., 2010, **73**, 1167.
20. X. M. Gao, L. D. Shu, L. Y. Yang, Y. Q. Shen, M. Z. Cui, X. M. Li, and Q. F. Hu, *Heterocycles*, 2013, **87**, 125.
21. S. Sianne, B. N. Zhou, E. G. Thomas, L. S. Jessica, and G. I. K. David, *J. Nat. Prod*., 2000, **63**, 457.
22. S. Venkateswarlu, G. K. Panchagnula, M. B. Guraiah, and G. V. Subbaraju, *Tetrahedron*, 2006, **62**, 9855.
23. J. H. Wang, S. C. Tam, H. Huang, D. Y. Yang, Y. Y. Wang, and Y. T. Zheng, *Biochem. Biophys. Res. Commun.*, 2004, **317**, 965.
24. T. Mosmann, *J. Immunol. Methods*, 1983, **65**, 55.