



A New Isoprenylated Aurone from the Flowers of *Rosa damascene* and Its Cytotoxicities

HUAN WANG, JUAN-XIA YANG, JIE LOU, LIMEI LI, GUI-YOU LIU, QIU-FEN HU, YAN-QING YE and XUE-MEI GAO*

Key Laboratory of Chemistry in Ethnic Medicinal Resources, State Ethnic Affairs Commission and Ministry of Education, Yunnan Minzu University, Kunming 650031, P.R. China

*Corresponding author: E-mail: gao_xuemei@hotmail.com

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A phytochemical investigation of the flowers of *Rosa damascena* resulted in the isolation of a new isoprenylated aurone (*Z*)-2-(4-methoxybenzylidene)-7,7-dimethyl-7,8-dihydro-2*H*-furo [2,3-*f*]chromene-3,9-dione (**1**). Its structure was elucidated by spectroscopic methods, including extensive 1D- and 2D- NMR experiments. Compound **1** was tested for their cytotoxicities against five human tumor cell lines, NB4, A549, SHSY5Y, PC3 and MCF7 and it showed potential cytotoxicity against NB4 and SHSY5Y cell lines with IC₅₀ values of 4.8 and 3.4 μM, respectively.

Keywords: *Rosa damascene*, Isoprenylated aurone, Cytotoxicities.

INTRODUCTION

The genus *Rosa* has long been appreciated for the utilization of gardening, food and medication¹. The fruits, petals and buds of some species are manufactured as herbal tea, drinks, juice, jam and medicine to treat stomachache, diarrhea and gynecological disease^{2,3}. *Rosa damascene* Mill., as one of the most important *Rosa* species, is widely cultivated in the world. Their flowers are renowned for fine fragrance and commercially processed for rose oil to manufacture high quality perfume. Many reports are available on the studies of the volatile constituents from petals oil of this species and phenolics were found to be the major components from this plant³⁻⁷. In order to identify bioactive natural compounds and multipurpose utilize the species *R. damascene*, we carried out phytochemical investigation on the flowers of this species. Separation of the ethyl acetate-soluble from an 95 % EtOH extract of the flowers afforded a new isoprenylated aurone (*Z*)-2-(4-methoxybenzylidene)-7,7-dimethyl-7,8-dihydro-2*H*-furo [2,3-*f*]chromene-3,9-dione (**1**). Compound **1** were tested for its cytotoxicities against five human tumor cell lines, NB4, A549, SHSY5Y, PC3 and MCF7. In this paper, we present the structure elucidation of compound **1** and its biological evaluation.

EXPERIMENTAL

Column chromatography (CC): silica gel H (200-300 mesh; Yantai Institute of Chemical Technology, P.R. China), Chromatorex RP-18 gel (20-45 mm; Fuji Silysia Chemical, Ltd., Japan) and Sephadex LH-20 (GE Healthcare Amersham

Biosciences, Sweden). TLC: precoated silica gel GF₂₅₄ plates (10-40 μm; Yantai Institute of Chemical Technology, China). HPLC: Agilent 1200 (Agilent Technologies, USA), Sepax Amethyst C₁₈ column (10 × 150 mm, 5 μm; Sepax Technologies, Inc., USA). UV Spectra: Shimadzu UV-2401PC spectrophotometer; λ_{max} (log ε) in nm. IR spectra: Nicolet Avatar-360 spectrometer; ν_{max} in cm⁻¹. NMR spectra: Bruker DRX-500 instruments; δ in ppm rel. to residual solvent peaks of C₅D₅N (δ(H) 8.74, 7.58, 7.22 δ(C) 150.3, 135.9, 123.9); J in Hz. ESI-MS and HR-ESI-MS: VG Auto Spec-3000 mass spectrometer; in *m/z* (rel. %).

The flowers of *Rosa damascena* Mill. were collected in Hanzhoung county, Shaanxi Province, China in September 2012. The plant was identified by Prof. Y.-J. Chen (Yunnan Minzu University). A voucher specimen (YNNI 12-09-47) has been deposited in our laboratory.

Extraction and isolation: The air-dried and powdered flowers of *R. damascena* (2.2 kg) were extracted three times with 95 % EtOH (3 × 4 L) at room temperature. The combined residue, after removal of solvent, was partitioned between ethyl acetate and water. The ethyl acetate crude extract (63.1 g) was applied to silica gel (200-300 mesh) column chromatography, eluting with CH₂Cl₂-(CH₃)₂CO gradient system (50:1, 20:1, 9:1, 8:2, 7:3, 6:4, 5:5), to give seven frs. A-G. The further separation of fr. C (8:2, 8.6 g) by silica gel column chromatography, eluted with CHCl₃-MeOH (9:1, 8:2, 7:3, 6:4, 1:1), yielded mixtures C1-C6. Fr. C1 (9:1, 1.6 g) was subjected to Sephadex LH-20, Chromatorex RP-18 gel and then preparative HPLC (50-60 % MeOH, 3 mL/min) to give **1** (13.2 mg).

(Z)-2-(4-Methoxybenzylidene)-7,7-dimethyl-7,8-dihydro-2H-furo [2,3-f]chromene-3,9-dione (1): Obtained as a yellow gum; UV (MeOH), λ_{\max} (log ϵ) 352 (3.35), 265 (4.01), 210 (4.22) nm; IR (KBr, ν_{\max} , cm^{-1}) 3428, 2913, 2872, 1695, 1664, 1610, 1545, 1489, 1423, 1266, 1147, 1072, 873, 781; ^1H NMR and ^{13}C NMR data: see Table-1; ESI-MS (positive ion mode) m/z 373 $[\text{M} + \text{Na}]^+$; HR-ESI-MS (positive ion mode) m/z 373.1059 $[\text{M} + \text{Na}]^+$ (calcd 373.1052 for $\text{C}_{21}\text{H}_{18}\text{NaO}_5$).

RESULTS AND DISCUSSION

The ethyl acetate-soluble fraction from a 95 % EtOH extract of *R. damascene* were purified by repeated column chromatography on silica gel, *Sephadex* LH-20, RP-18, as well as preparative HPLC, to yield a new isoprenylated aurone (**1**). Its structure was shown in Fig. 1 and its ^1H and ^{13}C NMR spectroscopic data were listed in Table-1.

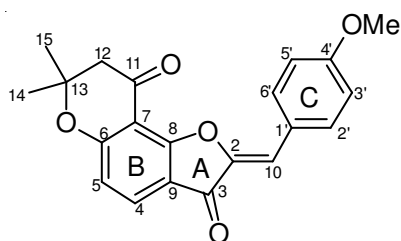


Fig. 1. Structure of compound **1**

Compound (**1**) was obtained as a yellow gum. The ESI-MS spectrum showed a molecular ion at m/z 373 and the molecular formula was determined as $\text{C}_{21}\text{H}_{18}\text{O}_5$ from the HR-ESI-MS spectrum by m/z 373.1059 $[\text{M} + \text{Na}]^+$; calcd 373.1052 for $\text{C}_{21}\text{H}_{18}\text{NaO}_5$, requiring 13 degrees of unsaturation. The UV spectrum of **1** exhibited absorption maxima at 210, 265 and 352 nm, which confirmed the existence of the aromatic functions. The IR absorptions of **1** implied the presence of $\nu(\text{OH})$ (3428 cm^{-1}), $\nu(\text{C}=\text{O})$ ($1695, 1664\text{ cm}^{-1}$) and aromatic ring ($1610, 1545, 1489\text{ cm}^{-1}$) moieties. The ^1H NMR spectrum (Table-1) showed signals of a methoxy group at δ_{H} 3.82 (s, 3 H), a group of aromatic AA'BB' spin system at δ_{H} 7.88 (d, $J = 8.8$ Hz, 2 H) and 6.89 (d, $J = 8.8$ Hz, 2 H), an aromatic AB spin system at δ_{H} 7.75 (d, $J = 8.6$ Hz, 1 H) and 6.74 (d, $J = 8.6$ Hz, 1 H), an olefinic proton at δ_{H} 6.65 (s, 1 H), a broad singlet at δ_{H} 2.64 (2 H) and two methyl singlets at δ_{H} 1.55 (s, 6 H). The ^{13}C NMR spectrum exhibited 21 carbon signals attributable to two carbonyl groups, 14 sp^2 carbons, one oxygenated quater-

nary sp^3 carbon, one methene sp^3 carbon and two methyl carbons (Table-1). These data suggest that **1** is an isoprenylated flavone or aurone bearing one methoxy group. Two groups of HMBC correlations from the olefinic proton H-C(10) (δ_{H} 6.65) to the carbonyl carbon C(3) (δ_{C} 181.3), C(2) (δ_{C} 146.5), C(1') (δ_{C} 126.2) and C(2' and 6') (δ_{C} 131.0) and from H-C(4) (δ_{H} 7.75) to C(3) clearly confirmed that **1** is an aurone (Fig. 2). The AA'BB' spin system in the ^1H NMR and the HMBC correlation of the methoxy proton (δ_{H} 3.82) with C(4') (δ_{C} 160.8) indicated that the methoxy group attached to C(4') of the C-ring. According to the molecular formula and the 13 degrees of unsaturation, in addition to the 11 degrees of unsaturation for the aurone skeleton of A-C rings, there should be another oxygen-containing ring with a carbonyl group (δ_{C} 191.2) existed in compound **1**. The oxygen-containing ring should be a six-membered 2,2-dimethylpyran ring, since two singlet methyl groups obviously observed in the ^1H - and ^{13}C NMR spectra. The carbonyl was determined to be at C(11) in the pyran ring, which can be deduced from the key HMBC correlations from CH_3 (14, 15) (δ_{H} 1.55) to C(12) (δ_{C} 48.4) (Fig. 2). And the ring was established to be fused at C(6) (δ_{C} 165.1) and C(7) (δ_{C} 116.5) of B-ring by the existence of aromatic AB spin system of H-C(4) with H-C(5) (δ_{H} 6.74) in the B-ring and the key HMBC correlation from CH_2 (12) (δ_{H} 2.64) to C(7) (Fig. 2). The configuration of the olefinic bond in **1** was defined as Z by the chemical shift value of C(10) (δ_{C} 112.4), since the configuration of the olefinic bond in aurones can be established on the chemical shift of olefinic methine resonance at δ_{C} 119.9-121.5 in E-aurones and 105.9-112.8 in Z-aurone⁸. Thus, the structure of **1** was elucidated as (Z)-2-(4-methoxybenzylidene)-7,7-dimethyl-7,8-dihydro-2H-furo [2,3-f]chromene-3,9-dione.

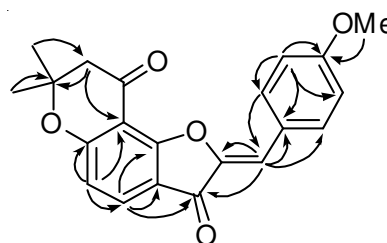


Fig. 2. Key HMBC (\curvearrowright) correlations of **1**

Compound **1** was tested for its cytotoxicities against five human tumor cell lines, NB4, A549, SHSY5Y, PC3 and MCF7, using a previously reported procedure with taxol as the positive

TABLE-1
 ^1H - AND ^{13}C NMR DATA OF COMPOUND **1** (500 AND 125 MHz, IN $\text{C}_5\text{D}_5\text{N}$)

| No. | δ_{C} (mult) | δ_{H} (mult, J) | No. | δ_{C} (mult) | δ_{H} (mult, J) |
|-----|----------------------------|----------------------------------|--------|----------------------------|----------------------------------|
| 2 | 146.5 (s) | - | 11 | 191.2 (s) | - |
| 3 | 181.3 (s) | - | 12 | 48.4 (t) | 2.64 (br. s) |
| 4 | 128.2 (d) | 7.75 (d, $J = 8.6$) | 13 | 80.9 (s) | - |
| 5 | 113.7 (d) | 6.74 (d, $J = 8.6$) | 14, 15 | 26.5 (q) | 1.55 (s) |
| 6 | 165.1 (s) | - | 1' | 126.2 (s) | - |
| 7 | 116.5 (s) | - | 2', 6' | 131.0 (d) | 7.88 (d, $J = 8.8$) |
| 8 | 162.9 (s) | - | 3', 5' | 115.4 (d) | 6.89 (d, $J = 8.8$) |
| 9 | 115.3 (s) | - | 4' | 160.8 (s) | - |
| 10 | 112.7 (d) | 6.65 (s) | OMe-4' | 56.2 (q) | 3.82 (s) |

control⁹. Compound **1** showed potential cytotoxicity against NB4 and SHSY5Y cell lines with IC₅₀ values of 4.8 and 3.4 μM, respectively.

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