New phenol derivatives from *Ligularia stenocephala*

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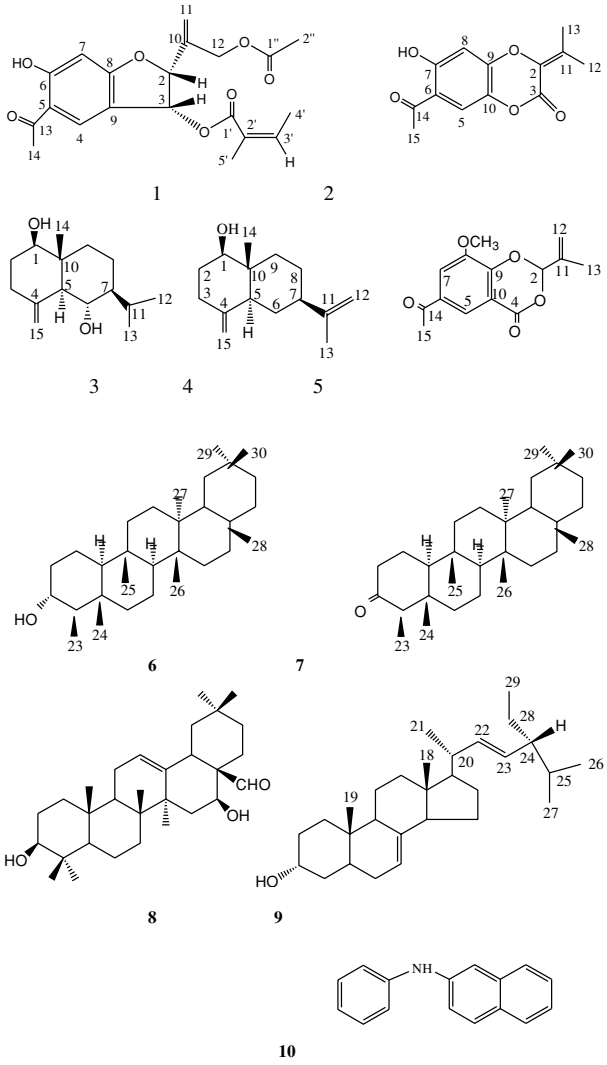
The C–3 2-methyl-but-2-enoic acid ester of 2-(1-1-acetoxyisopropenyl)-5-acetyl-3,6-dihydroxy-2,3-dihydro-benzofuran (**1**) and 6-acetyl-7-hydroxy-2-isopropylidene-benzo[1,4]dioxin-3-one (**2**), together with nine known compounds (**3**–**11**) were isolated from the roots of *Ligularia stenocephala*. Their structures were elucidated by spectroscopic methods (IR, MS, ^{1}H, ^{13}C and 2D NMR).

Keywords: *Ligularia stenocephala*, phenol derivatives

Ligularia stenocephala (Compositae) has long been used as a Chinese folk medicine in the treatment of edema and scrofula.[1] In a previous paper,[2] we have reported the isolation of three new benzofuran derivatives from *L. stenocephala*. In this paper, we report the isolation and structural elucidation of two new compounds C-3-2-methyl-but-2-enoic acid ester of 2-(1-1-acetoxyisopropenyl)-5-acetyl-3,6-dihydroxy-2,3-dihydro-benzofuran (**1**) and 6-acetyl-7-hydroxy-2-isopropylidene-benzo [1,4]dioxin-3-one (**2**), together with nine known compounds, 1β,6α-dihydroxy-4(15)-eudesmene (**3**),[3,4] β-dictyopterol (**4**),[5] 2-isopropenyl-6- acetyl-8-methoxy-1,3-benzodioxin-4-one (**5**),[6] friedelinol (**6**),[7] friedelin (**7**),[7] gummosogenin (**8**),[8] (24R)-stigmast-7,22(E)-dien-3α-ol (**9**),[9] plasticiser,[10] N-phenyl-2-naphthylamine (**10**)[11,12] from this plant.

The roots of *Ligularia stenocephala* (Maxim.) Matsum. et Koidz. were collected in Henan Province of China and identified by Professor Changshan Zhu, Henan Agriculture University, P. R. China. They were extracted with petroleum ether (60–90°C)-Et_2O-MeOH (1:1:1), then separated by silica gel column chromatography to give compounds **1 – 10**.

Compound **1** was obtained as a yellowish gum, with the molecular formula $C_{20}H_{22}O_7$ as determined by HR-ESI-MS $[M+NH_4]^+$. The IR spectrum suggested the presence of the hydroxyl group (3412 cm^{-1}), carbonyl groups (1743, 1716 and 1648 cm^{-1}), double bonds (3080 and 1638 cm^{-1}) and 1,2,4,5-tetrasubstituted benzene ring (1594, 1485, 1429 and 852 cm^{-1}). The UV spectrum (371.2 nm) also suggested the presence of a conjugated aromatic ring. Its ^{1}H NMR spectrum gave an acetoxy group signal at δ 2.03 (3H, s), typical signals of angeloyl group at δ 6.19 (1H, q, J = 7.5 H_Z), 2.02 (3H, d, J = 7.5 H_Z) and 1.91 (3H, s) which was further supported by ^{13}C NMR signals at δ 170.3, 20.6 and δ 166.9, 126.8, 140.2, 15.9, 20.4. Moreover, a significant fragment of $[M-100]^+$ at *m/z* 274 in EI-MS spectrum also indicated an angeloyl moiety in compound **1**. In addition, there were signals for an acetyl group at δ_H 2.65 (3H, s) in ^{1}H NMR and δ_C 203.9 (C=O), 26.9 (CH_3) in ^{13}C NMR, and a substituted 1-methyl-vinyl moiety at δ_H 5.41(1H, s), 5.36 (1H, s), 4.75 (1H, d, J = 13.5 Hz), 4.68 (1H, d, J = 13.5 Hz) in ^{1}H NMR and δ_C 139.8 (=C), 116.8 (=CH_2), 63.8 (O-CH_2). Combined with the IR spectrum, its ^{1}H NMR showed a downfield shift for a hydroxy proton (δ_H 12.05, s) which formed intramolecular hydrogen bond. Apart from the proton signals corresponding to the above groups, the ^{1}H NMR displayed two oxygenated methine signals at δ_H 6.31, 6.51 (each 1H, d, J = 2.7 Hz) and two singlet aromatic proton signals at δ_H 7.26 and 7.12 (each 1 H), which were located in the *para*-position of a benzene ring. In conjunction with the degree of unsaturation and the ^{1}H NMR, IR, UV spectra, the other signals in ^{13}C NMR at δ 77.4, 87.8, 109.5, 116.4, 126.8, 133.7, 152.5 and 157.6 established a partial structure of 2,3-dihydro-benzofuran ring. Its HMBC spectrum gave the long-range correlations between H-4 and C-13; H-14 and C-13 and C-5; H-2 and C-10; H-11 and H-12 with C-2; and the OH with C-6. These showed that the acetyl, substituted 1-methyl-vinyl and hydroxy were attached to C-5, C-2 and C-6, respectively. From the long-range correlations between H-12 and C-1", H-3 with C-1', the acetoxy and angeloyl groups were connected to C-12 and C-3. The structure of compound **1** including the relative stereo chemistry was elucidated as shown. In order to confirm this assignment, the NOE difference spectroscopy was obtained. The H-2 had a NOE with H-3 (7 %), which indicates a *cis*-geometry for H-2 and H-3. Therefore, the structure of **1** was determined as the C-3 2-methyl-but-2-enoic acid ester of 2-(1-1-acetoxyisopropenyl)-5-acetyl-3,6-dihydroxy-2,3-dihydro-benzofuran.



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Compound **2** was obtained as a white powder. Its molecular formula was assigned as $C_{13}H_{12}O_5$ from FAB-MS $[M+H]^+$ at *m/z* 249, which was confirmed by ^{13}C NMR and DEPT data. Its IR spectrum showed absorption bands for hydroxyl group (3352 cm^{-1}), carbonyl group (1737 cm^{-1}) and 1,2,4,5-tetrasubstituted benzene ring (1618, 1505, 1450 and 870 cm^{-1}). The UV (λ_{max}) spectrum (333.8 nm) also suggested the presence of a conjugated aromatic ring. The ^1H NMR spectrum contained the typical signals of acetyl group at δ_H 2.57 (3H, s) and hydroxy forming a intramolecular hydrogen bond at δ_H 12.4 (1H, s), combined with the singlet aromatic proton signals at δ_H 7.39 and 6.60 (each 1H). This showed that compound **2** had a similar 1-hydroxy-2-acetyl-4,5-disubstituted benzene ring as compound **1**. Furthermore, the signals in ^{13}C NMR at δ 202.6, 26.7 (acetyl) and δ 104.6, 114.7, 117.9, 132.8, 147.8 and 161.1 (benzene ring) confirmed this conclusion. Besides the signals mentioned above, ^1H NMR gave two downfield shift of methyl groups at δ 2.05 and 2.30 (each 3H, s), ^{13}C NMR displayed five signals at δ 155.4 (C), 138.2 (C), 131.9 (C), 21.3 (CH_3), 20.5 (CH_3). From the HMBC correlations between the methyl protons with three quarternarycarbon, the partial structure of an isopropylidene group was deduced. It was further supported by a important fragment *m/z* at 82 [33, $(CH_3)_2C{=}C{=}C{=}O^+$] in the EI-MS. Consideration of the molecular formula and the degres of unsaturation, C-2 and C-3 were connected to benzene ring by C–O–C bond. In addition, comparing with the known compounds caleteucrin[13] and 9-angeloyloxycalefolione[14], they had the similar structure except in position of the substituents at benzene ring. On the basis of the above evidence, the structure of compound **2** was confirmed as 6-acetyl-7-hydroxy-2-isopropylidene-benzo [1,4]dioxin-3-one.

Compounds **3**–**11** were identified by comparison of their ^1H and ^{13}C NMR and MS spectroscopic data with those reported in literatures previously.[3-12]

Experimental

Melting points were determined on a Kofler melting point instrument and are uncorrected. Optical rotations were taken on a Perkin-Elmer 341 polarimeter. IR spectra were determined on a Nicolet NEXUS 670 FT-IR spectrometer. ^1H NMR, ^{13}C NMR and 2D NMR spectra were measured on a Mercury Plus-300 BB spectrometer using TMS as the internal standard. HR-ESI MS were recorded on a Bruker APEXII mass spectrometer. EI-MS data were obtained on an HP-5988A GC/MS spectrometer. FAB MS data were obtained on a VG-ZAB-HS mass spectrometer (at 70 eV); Silica gel (200–300 mesh) was used for column chromatography and silica gel GF_{254} for TLC were made by the Qing-dao Marine Chemical Factory of China.

Extraction and isolation procedures

The air dried and powdered roots of *L. stenocephala* (3.1 kg) were extracted at room temperature with petroleum ether (60–90°C)-Et_2O-MeOH (1:1:1). The extract (165 g) was obtained after concentration. The extract was subjected to column chromatography over silica gel and eluted with a gradient of petroleum ether-EtOAc (30:1→0:1) to give **1** (34 mg), **2** (7 mg), **3** (5 mg), **4** (39 mg), **5** (12 mg), **6** (22 mg), **7** (13 mg), **8** (7 mg), **9** (9 mg), **10** (10mg) and **11** (5 mg).

2-Methyl-but-2-enoic acid ester of 2-(1-1-acetoxyisopropenyl)-5-acetyl-3,6-dihydroxy-2,3- dihydro-benzofuran (**1**): Yellowish gum, $[\alpha]_D^{23}$ –121.0°(*c* 3.8, $CHCl_3$). HR–ESI–MS: $[M+NH_4]^+$ Found: 392.1709, Calcd for $C_{20}H_{22}O_7 + NH_4$ 392.1704; UV λ_{max} ($CHCl_3$) 371.2 (logε, 1.69) nm; IR ν_{max}/cm^{-1}: 3400, 3080, 2956, 1743, 1716, 1648, 1594, 1485, 1429, 1372, 1227, 1145, 853; δ_H: (300 MHz, $CDCl_3$, TMS): 12.05 (1H, s, OH), 7.26 (1H, s, 4-H), 7.12 (1H, s, 7-H), 6.31 (1H, d, *J* = 2.7 Hz, 3-H), 6.19 (1H, q, *J* = 7.5 Hz, 3'-H), 5.41 (1H, s, 11-Ha), 5.36 (1H, s, 11-Hb), 5.21 (1H, d, *J* = 2.7 Hz, 2-H), 4.75 (1H, d, *J* = 13.5 Hz, 12-Ha), 4.68 (1H, d, *J* = 13.5 Hz 12-Hb), 2.65 (3H, s, 14-H), 2.03 (3H, s, 2''-H), 2.02 (3H, d, *J* = 7.5 Hz, 4'-H), 1.91 (3H, s, 5'-H). δ_C: (75 MHz, $CDCl_3$, TMS): 87.8 (C-2), 77.4 (C-3), 109.5 (C-4), 133.7 (C-5), 157.6 (C-6), 116.4 (C-7), 152.5 (C-8), 126.8 (C-9), 139.8 (C-10), 116.8 (C-11), 63.8 (C-12), 203.9 (C-13), 26.9 (C-14), 166.9 (C-1'), 126.8 (C-2'), 140.2 (C-3'), 15.9 (C-4'), 20.4 (C-5'), 170.3 (C-1''), 20.6 (C-2''). EI-MS *m/z* (rel int): 374 ($[M]^+$ 7), 274 ($[M\text{-angeloyl}]^+$ 22), 259 ($[274\text{-Me}]^+$ 20), 232 (8), 231 (6), 215 (5), 173 (3), 171 (3), 115 (5), 83 (100), 43 (60).

6-Acetyl-7-hydroxy-2-isopropylidene-benzo[1,4]dioxin-3-one (**2**): White powder, m.p. 175–176°C ($CHCl_3$); UV λ_{max} (Me_2CO): 333.8 (logε, 0.80) nm; IR ν_{max}/cm^{-1}: 3353, 3066, 2917, 2849, 1737, 1618, 1602, 1504, 1450, 1425, 1367, 1317, 1188, 870; δ_H: (300 MHz, $CDCl_3$, TMS): 12.4 (1H, s, OH), 7.39 (1H, s, 5-H), 6.60 (1H, s, 8-H), 2.57 (3H, s, 15-H), 2.30 (3H, s, 12-H), 2.05 (3H, s, 13-H). δ_C: (75 MHz, $CDCl_3$, TMS): 131.9 (C-2), 155.4 (C-3), 117.9 (C-5), 114.7 (C-6), 161.1 (C-7), 104.6 (C-8), 132.8 (C-9), 147.8 (C-10), 138.2 (C-11), 21.3 (C-12), 20.5 (C-13), 202.6 (C-14), 26.7 (C-15). FAB-MS *m/z* 249 $[M+H]^+$; EI-MS *m/z* (rel int): 248 ($[M]^+$ 91), 233 (74), 219 (18), 205 ($[M\text{-}CH_3CO]^+$ 61), 191 (3), 187 (4), 178 (4), 177 (12), 149 (27), 95 (23), 83 (16), 82 ($[(CH_3)_2C{=}C{=}C{=}O]^+$ 33), 43 (100).

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