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New sesquiterpenes from the roots of *Ligularia virgaurea*

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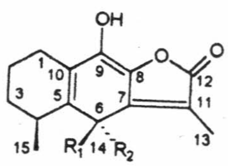
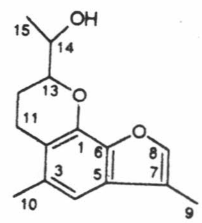
Phytochemical reinvestigation of the petroleum ether-Et_2O extract of the roots of *Ligularia virgaurea* has resulted in the isolation of three new sesquiterpenes, named virgauride 1, epivirgauride 2, and virgauronin 3, together with two known compounds, cacalone and epicacalone. Their structures have been elucidated on the basis of their spectroscopic studies (UV, IR, MS, ^{1}H, ^{13}C NMR and DEPT) including 2D NMR experiments (HMQC and HMBC).

Ligularia virgaurea has been used as a traditional medicine for a long time in China[1]. In previous papers[2,3], we have reported the structures of five new sesquiterpenes and a new benzofuranosesquiterpene dimer from the rhizomes of this plant. In the present phytochemical reinvestigation of the same plant, three new sesquiterpenes, named virgauride **1**, epivirgauride **2** and virgauronin **3**, together with two known compounds, cacalone **4** and epicacalone **5**, have been obtained. Details of isolation and structural elucidation of these compounds form the subject of this paper.



	R_1	R_2
1	CH_3	H
2	H	CH_3



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Results and Discussion

The petroleum ether (60-90°C)-Et_2O (2:1) extract of the roots of *L. virguarea* was subjected to separation by column chromatography over silica gel and preparative TLC to give three new sesquiterpenes **1, 2** and **3**, in addition to the two previously reported compounds, cacalone **4** and epicacalone **5**.

Compounds **1** and **2**, colourless platelets, behaving as a pure compound in TLC and HPLC, were obtained as an epimeric mixture (*ca* 1:1) by ^{13}C NMR spectrum, which exhibited two sets of signals with close chemical shifts. The IR spectrum showed absorption bands for hydroxyl group (3384 cm^{-1}), α,β-unsaturated γ-lactone (1792 cm^{-1}) and double bond (1634 cm^{-1}). The molecular formula $C_{15}H_{18}O_3$ was deduced from its EIMS (m/z 246 $[M]^+$) together with ^{13}C NMR and DEPT spectra which indicated the presence of three methyls, three methylenes, two methines and seven quaternary carbon atoms for each compound (see Table I). The ^{1}H NMR spectra of **1** and **2** showed the presence of two secondary methyl groups at δ 1.14 (d, 3H, J=7.1 Hz, H-15) and 1.59 (d, 3H, J=7.6 Hz, H-14), a vinyl methyl group at 2.19 (s, 3H, H-13), and a methine group at 3.70 (q, 1H, J=7.6 Hz, H-6). The long wave maxima in the UV spectrum (λ_{max} 212 and 287 nm), the significant absorption band at 1792 cm^{-1} in its IR spectrum, and the double bond and carbonyl resonance signals at δ 125.1 (C, C-7), 123.2 (C, C-11) and 178.4 (C, C-12) in the ^{13}C NMR spectrum suggested the existence of an α,β-unsaturated γ-lactone moiety in the molecules of **1** and **2**. Detailed examination of the ^{1}H and ^{13}C NMR data of **1** and **2** showed close similarities with those of Adenostylide[4]. However, the oxygenated quaternary carbon signal (at δ 74.3, C,C-6) of Adenostylide was absent in **1** and **2**, while a methine resonance signal at δ 39.2 (CH, C-6) was visible in the ^{13}C NMR spectrum. This ob-

Table I—^{13}C NMR chemical shifts of compounds **1, 2** and **3** (100.16 MHz, ppm)

Carbon	1[a]	2[a]	DEPT	3	DEPT
1	22.8	23.2	CH_2	142.6	C
2	16.3	16.4	CH_2	131.3	C
3	29.5	29.8	CH_2	115.8	C
4	28.8	28.9	CH	111.3	CH
5	135.3	135.4	C	128.2	C
6	39.2	39.2	CH	140.4	C
7	125.1	125.1	C	116.7	C
8	137.6	137.7	C	140.8	CH
9	137.3	137.3	C	7.9	CH_3
10	124.6	124.9	C	19.6	CH_3
11	123.2	123.3	C	22.5	CH_2
12	178.4	178.4	C	21.3	CH_2
13	14.0	14.3	CH_3	68.6	CH
14	16.1	16.3	CH_3	79.3	CH
15	20.9	20.9	CH_3	17.9	CH_3

$CDCl_3$ as solvent; TMS as internal standard.
[a]Assignments for the corresponding carbons of **1** and **2** are interchangeable.

servation was strongly supported by the fact that an additional methine signal at δ 3.70 (q, 1H, J=7.6 Hz, H-6) in the ^{1}H NMR spectrum was observed. The structures of compounds **1** and **2** were unambiguously determined, and the UV, IR and EIMS data also supporting the proposed structures. Compounds **1** and **2** (epimeric at C-6) were named as virgauride and epivirgauride, respectively.

Compounds **3**, named as virgauronin, was obtained as a colourless gum. Its IR spectrum showed absorption bands at 3411 (hydroxyl group), 1659 (double bonds), 1634, 1581 and 1448 (aromatic rings), and 1098 and 1040 cm^{-1} (C–O and C–O–C). The molecular ion peak at m/z 246 $[M]^+$ in its EIMS suggested the molecular formular $C_{15}H_{18}O_3$ (7 unsaturations) for **3**, and this suggestion was confirmed by ^{13}C NMR and DEPT spectra which indicated the presence of 15 carbon atoms including three methyls, two **sp**^{3} methylenes, two **sp**^{3} oxymethines, two **sp**^{2} methines, and six **sp**^{2} quaternary carbons in the molecule (see Table I). Its ^{1}H NMR spectrum showed the presence of a secondary methyl group at δ 1.33 (d, J=6.4 Hz), a methyl group on the β-position of the furan ring at δ 2.19 (s), an aromatic methyl group at 2.30 (s), two oxymethine groups at 3.99 (dq, J= 11.1 and 2.2 Hz) and 4.23 (ddd, J=10.2, 6.5, and 3.7 Hz), and a benzene proton and an α-proton of the furan ring at δ 6.89 (s) and 7.33 (s). Considering all the above spectral signals, in conjunction with the number of unsaturations, compound **3** was suggested to be a benzofuranosesquiterpene containing an additional ring in the molecule. As indicated from DEPT spectrum, 17 out of the total 18 hydrogen atoms in the molecule are connected to the carbon atoms, suggesting thereby that only one hydroxyl group exists in **3**. So, the third oxygen atom must be present in the third ring (two oxygen atoms have been accounted for the hydroxyl group and furan ring). The signals at δ 3.99 (dq) and 4.23 (ddd) and their multiple patterns in the ^{1}H NMR indicated the existence of CH_3-CH(OH)-CH(-O-)-CH_2-moiety in **3**, and this proposal was further confirmed by the direct ^{1}H/^{13}C chemical shift correlation (HMQC) experiment. In the HMQC spectra, the signals at δ 111.3 (C-4), 140.8 (C-8), 22.5 (C-11), 21.3 (C-12), 68.6 (C-13), and 79.3 (C-14) correlated with the corresponding protons at δ 6.89 (H-4), 7.33 (H-8), 2.80 (H-11), 2.71 (H-12), 4.23 (H-13) and 3.99 (H-14). Meanwhile, the methyl carbons which resonated at δ 7.9 (C-9), 19.6 (C-10), 17.9 (C-15) were coupled with protons at δ 2.19 (H-9), 2.30 (H-10), and 1.33 (H-15), respectively.

Long-range correlations between ^{1}H- and ^{13}C-nuclei were established by HMBC experiment which provided conclusive evidence to support the proposed structure for compound **3**. The significant ^2J and ^3J cross peaks exhibited at C-2, C-3 and C-4/H-10, C-5/H-8, C-7/H-4 and H-8, C-5, C-7 and C-8/H-9, and C-13/H-15. The above cross peaks supported the proposed structure of compound **3**.

In conclusion, all the above spectral assignments are consistent with the proposed structure for compound **3**.

The known compounds **4** and **5** had identical ^{1}H, ^{13}C NMR and DEPT spectral data[5] with those of cacalone and epicacalone previously isolated from *Cacalia adenostyloides*[4] and *Cacalia decomposita*[6,7].

Experimental Section

General. Melting points were determined on a Kofler micro melting point apparatus and are uncorrected. ^{1}H (400 MHz), ^{13}C NMR (100.16 MHz), DEPT and 2D NMR (HMQC, HMBC) spectra were measured on a Bruker AM-400 NMR spectrometer in $CDCl_3$ with TMS as internal standard. EIMS data were obtained on an HP 5988 A GC/MS spectrometer. IR spectra and optical rotations were recorded on a Nicolet FT-170 SX and a J-20C instrument, respectively, UV spectra in MeOH were scanned on a UV-240 spectrometer.

The roots of *L. virgaurea* were collected in Zhang country, Gansu province, P.R. China in August 1988 and identified by Prof. Ru-Neng Zhao. A voucher specimen has been deposited at the Herbarium of the Pharmacognosy Department, Lanzhou Medical College.

Extraction and isolation. The air-dried roots of *L. virgaurea* (5.0 kg) were pulverized and extracted with petroleum ether (60-90°C)-Et_2O (2:1) at room temperature two times (one week each time) yielding 250 g of the crude extract. A part (70 g) of this was chromatographed over silica gel (200-300 mesh, 950 g) column, and eluted successively with a petroleum ether (60-90°C). EtOAc (50:1-1:1) gradient. Evaporation of solvent from the CC fractions (250 mL each), combined according to the TLC monitoring, gave eight major crude fractions. From fraction VI (2.6 g; eluted with petroleum ether-EtOAc, 7:1), 30 mg of compounds **1** and **2** were obtained by repeated CC over silica gel (200-300 mesh, 80 g) eluted with petroleum ether-EtOAc (6:1) and then further purified by preparative TLC using petroleum ether-EtOAc (6:1) (developed two times). Fraction VIII (7.2 g) eluted with petroleum ether-EtOAc, 3:1) was rechromatographed using petroleum ether-EtOAc (4:1) as eluent to give two fractions, which were further separated by preparative TLC using petroleum ether-EtOAc (4:1 and 3:1) as development solvents to afford compound **3** (25 mg), and a mixture of **4** and **5** (100 mg), respectively.

Virgauride and epivirgauride (1 and 2). Colourless platelets, mp 139-141°C; UV (MeOH): 212 (log ε 4.62), 287 nm (3.56); IR (KBr): 3429, 3384, 2931, 2888, 1792, 1634, 1453, 1420, 1336, 1309, 1255, 1228, 1167, 1094, 1020, 990, 950 cm^{-1}; EIMS: *m/z* 246 $[M]^+$ (22%), 231 (30), 218 (47), 203 (100), 185 (7), 157 (6), 115 (5), 91 (7), 77 (6); ^{1}H NMR ($CDCl_3$): 1.14 (d, *J*=7.1 Hz, 3H, H-15), 1.59 (d, *J*=7.6 Hz, 3H, H-14), 1.80 (m, 4H, H-2 and 3), 2.19 (s, 3H, H-13), 2.46-2.55 (m, 2H, H-1), 3.03 (m, 1H, H-4), 3.70 (q, *J*=7.6 Hz, 1H, H-6), 5.82 (s, 1H, OH); ^{13}C NMR and DEPT data, see Table I.

Virgauronin 3. Colourless gum; $[\alpha]_D^{20}$ −25.0° (*c* 0.60, $CHCl_3$); IR (film): 3411, 2969, 2926, 1659, 1634, 1581, 1480, 1448, 1367, 1237, 1098, 1040 cm^{-1}; EIMS: *m/z* 246 $[M]^+$ (86%), 231 (1), 228 (2), 201 (82), 175 (100), 147 (4), 115 (16), 91 (10), 77 (6), 55 (3); ^{1}H NMR ($CDCl_3$): δ 1.33 (d, *J*=6.4 Hz, 3H, H-15), 2.19 (s, 3H, H-9), 2.30 (s, 3H, H-10), 2.71-2.76 (m, 2H, H-12), 2.80-2.85 (m, 2H, H-11), 3.99 (dq, *J*=11.1, 2.2 Hz, 1H, H-14), 4.23 (ddd, *J*=10.2, 6.5, 3.7 Hz, 1H, H-13), 6.89 (s, 1H, H-4), 7.33 (s, 1H, H-8); ^{13}C NMR and DEPT data, see Table I.

Acknowledgement

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