CHEMICAL CONSTITUENTS FROM LEAVES OF ALLELOPATHIC CULTIVAR SUNFLOWER IN CHINA

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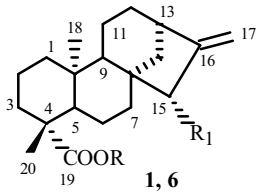
Sunflower, a typical allelopathic plant, is wellknown for producing a variety of secondary metabolites, some of which possess a wide variety of biological properties, including allelopathic activity [1–3]. To study the chemical constituents in sunflower leaves in China and find useful active chemicals from its body, from the methanolic extracts of the leaves of cultivar sunflower (*Helianthus annuus* L.) eight compounds, (–)-kaur-16-en-19-oic acid (**1**), (6*R*,10*R*)-6,10,14-trimethyl-2-pentadecanone (**2**), α-tocopherol (**3**), dehydrocostus lactone (**4**), (–)-α-tocospirone (**5**), angeloygrandifloric acid (**6**), *trans*-phytol (**7**), and 3(20)phytene-1,2-diol (**8**), were isolated. Here we describe the isolation and characterization of these compounds **1**–**8**. Among them, compound **2**, **5**, and **8** were isolated from this plant for the first time.

Leaves of *Helianthus annuus* L. were collected in September 2003 in Wuzhong city, Ningxia Province, China and were identified by Mr. Z. H. Wu at Northwest Institute of Botany, Yangling, Shaanxi, and were deposited at Natural Medicine Chemistry Research Centre, College of Sciences, Northwest A &F University.



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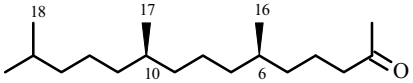

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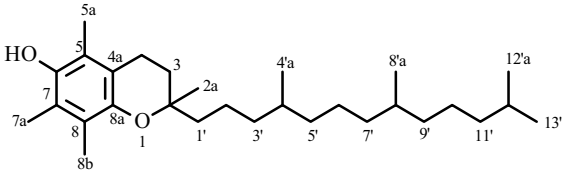

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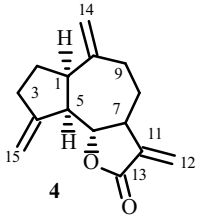
1: R = R_1 = H; **6:** R = H, R_1 = Ang



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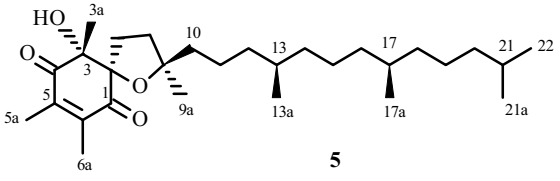

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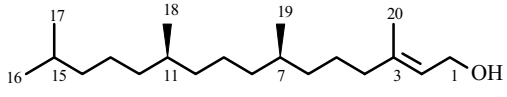

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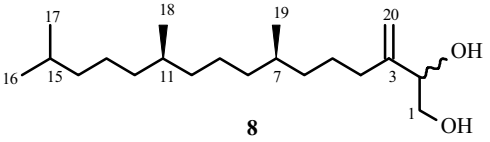

8

Extraction and Isolation. The dried and powdered leaves (20 kg) were extracted three times with MeOH at room temperature, and the combined extracts were concentrated *in vacuo* to give a deep green gum (2230 g), which was partitioned between $CHCl_3$ and H_2O. After removal of the organic solvent under reduced pressure, the resulting residue (498 g) was

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subjected to CC (200–300 mesh, Qingdao Marine Chemical Ltd., Qingdao, P. R. China) on silica gel eluting with $CHCl_3$/MeOH mixtures of increasing polarity to yield 8 fractions (frs 1–8) according to TLC analysis.

Fraction 2 was subjected to CC on silica gel eluting with petroleum ether/EtOAc (50:1 to 10:1) to provide four subfractions (A–D). Subfraction A was separated by silica gel CC using petroleum ether/EtOAc (40:1) to afford compound **1** (3.15 g). Subfraction B was rechromatographed over silica gel CC with petroleum ether/$CHCl_3$ (6:1) and on a Sephadex LH-20 column ($CHCl_3$/MeOH 1:1), followed by preparative TLC (petroleum ether/ether, 7:1) to give compound **2** (13 mg). Subfraction C was rechromatographed over silica gel column using petroleum ether/EtOAc (50:1), then on a Sephadex LH-20 column ($CHCl_3$/MeOH, 1:1) to furnish compound **3** (14 mg). Subfraction D was rechromatographed over silica gel column eluting with petroleum ether/$CHCl_3$ (1:2.5), and on a followed by Sephadex LH-20 column ($CHCl_3$/MeOH, 1:1), preparative TLC (petroleum ether/ether, 2.5:1), yielding compound **4** (12 mg). Four fractions (E–G) were obtained from fraction 3 by CC on silica gel eluted with petroleum ether/EtOAc mixtures of increasing polarity (20:1, 10:1, 3:1).

Fraction E was rechromatographed over a silica gel column using petroleum ether/acetone (60:1) and on a Sephadex LH-20 column ($CHCl_3$/MeOH, 1:1), and further purified by preparative TLC (petroleum ether/EtOAc, 5:1), yielding **5** (17 mg). Fraction F was chromatographed over a silica gel eluting with petroleum ether/EtOAc (15:1) to afford a mixture containing compound **6** (258 mg), which was further purified by recrystallization in petroleum ether/$CHCl_3$. Fraction G was chromatographed over a silica gel column using petroleum ether/$CHCl_3$ (3:2), then on a Sephadex LH-20 column ($CHCl_3$/MeOH, 1:1) to give compounds **7** (91 mg) and **8** (18 mg).

Melting points were obtained on a XRC-1 apparatus and uncorrected. Optical rotations were measured on a Horiba SEPA-300 polarimeter. NMR spectra were recorded on Bruker AV-400 and DRX-500 spectrometers with TMS as an internal standard. IR spectra were obtained with a Bruker Tensor 27 FT-IR with KBr pellets. UV spectrum was measured on a Shimadzu double-beam 210A spectrometer. MS (EI, FAB) were recorded with a VG Autospec-3000 spectrometer, *m/z* (rel. int.). ESI and HR-ESI-MS were recorded with an API QSTAR Pulsar 1 spectrometer and Sephadex LH-20 (Amersham Biosciences, Uppsala, Sweden).

Compound **1**, yield 0.0158%, $C_{20}H_{30}O_2$, colorless crystals, mp 179–180°C; $[\alpha]_D^{24.5}$ –111° (*c* 1.6, $CHCl_3$); +TOF-MS *m/z*: 303 $[M + H]^+$, were identical to those recorded for an authentic specimen of (–)-kaur-16-en-19-oic acid [4, 5].

Compound **2**, yield 0.000065%, $C_{18}H_{36}O$, FAB$^+$MS *m/z*: 269 $[M]^+$ (100), ^1H and ^{13}C NMR. These data were identical to those recorded for an authentic specimen of (6*R*,10*R*)-6,10,14-trimethyl-2-pentadecanone [6].

Compound **3**, yield 0.00007%, $C_{20}H_{50}O_2$, colorless oil, UV (λ_{max}, MeOH, nm): 291, 298; EI-MS *m/z* (%): 430 $[M]^+$ (100), 205 (26), 165 (78), 164 (58), 136 (10), 121 (11), was identified as α-tocopherol by comparison of spectral data with those in the literature [7, 8].

Compound **4**, yield 0.00006%, $C_{15}H_{18}O_2$, colorless needles (EtOAc), mp 49°C; IR (ν_{max}, cm^{-1}): 1762, 1644; +TOF-MS *m/z*: 231 $[M + 1]^+$, which was determined as dehydrocostus lactone [9].

Compound **5**, yield 0.00006%, $C_{29}H_{50}O_4$, colorless oil, UV (λ_{max}, MeOH): 251.4; IR (ν_{max}, cm^{-1}): 3487 (OH), 1697, 1679 (C = O), 1622; FAB $^+$MS *m/z*: 463 $[M]^+$ (100), 445 $[M+H - H_2O]^+$ (35), 419 (85), 402 (15), 352 (8), 237 [M–side chain]$^+$ (8), 167 (23). These data were identical to those recorded for an authentic specimen of (–)-α-tocospirone [10].

Compound **6**, yield 0.00129%, $C_{25}H_{36}O_4$, colorless crystals, mp 196–198°C; IR (ν_{max}, cm^{-1}): 3200–2500, 1702, 1250, 1040, 1005 and 896; EI-MS *m/z* (%): 400 $[M]^+$ (8), 300 (65), 285 (70), 272 (34), 255 (22), 83 (100). These data were identical to those recorded for an authentic specimen of angeloygrandifloric acid [4].

Compound **7**, yield 0.000455%, $C_{20}H_{40}O$, EI-MS *m/z* (%): 296 $[M]^+$ (2), 278 $[M - H_2O]^+$ (5), 196 (7), 179 (5), 137 (11), 123 (63), 111 (33), 95 (42), 81 (57), 71 (100), and ^1H and ^{13}C NMR. These data were identical to those recorded for an authentic specimen of *trans*-phytol [11].

Compound **8**, yield 0.00009%, $C_{20}H_{40}O_2$, colorless oil, IR (ν_{max}, cm^{-1}): 3400, 1640 and 890; FAB+MS *m/z*: 313 $[M + 1]^+$ (18), 295 $[M+1 - H_2O]^+$ (18), 277 (6), 83 (32); ^1H NMR These data were identical to those of 3(20)phytene-1,2-diol [11, 12].

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