Chemical Constituents from *Otostegia persica*

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(Received 17th December, 2005, revised 28th September. 2006)

Summary: Eleven compounds namely geraniol, eugenol, ceryl alcohol, hentriacontane, caffeic acid, p-hydroxybenzoic acid, β-sitosterol, β-sitosteryl acetate, β-amyrin, campesterol and stigmasterol were isolated from *Otostegia persica*, a 1-2 meters shrub distributed in south Iran and south-western Pakistan. The structures of all the compounds were established with the help of spectral data.

Introduction

The genus *Otostegia* belongs to the family *Labiatae,* which is an almost cosmopolitan family of about 180 genera and over 3500 species. The genus *Otostegia* comprises of about 20 species, which are generally shrubs or subshrubs glabrous or pubescent, with simple hair and having two distinct areas of concentration, one in the regions of Asia particularly areas of Pakistan, Afghanistan and the other in the mountains of East Africa. [1]. Until now, mono-, di-, sesquiterpenes [2-3], flavonoids, steroids and terpenoids [4-6] have been reported from this genus. The presence of these biologically and physiologically interesting classes of compounds prompted us to carry out phytochemical screening of *Otostegia persica,* which is a 1-2 meter high shrub, widespread in the south of Iran and south-western Pakistan.

In the present investigations, the methanolic extract of the aerial parts of *Otostegia persica*, showed positive activity in the brine shrimp lethality test [7]. Further biological screening of the methanolic extract revealed strong antioxidant as well as antibacterial activities against various strains of Gram-positive and Gram-negative bacteria [8]. This prompted us to carry out phytochemical studies on this plant, which resulted in the isolation of geraniol (**1**), eugenol (**2**), ceryl alcohol (**3**), hentriacontane (**4**), caffeic acid (**5**), p-hydroxybenzoic acid (**6**), β-sitosterol (**7**), β-sitosteryl acetate (**8**), β-amyrin (**9**), campesterol (**10**) and stigmasterol (**11**).

Results and Discussion

The column chromatography of the hexane soluble fraction of *Otostegia persica,* resulted in the isolation and characterization of compounds **1-4**, while ethyl acetate soluble fraction yielded compounds **5-11**. These compounds have not yet been reported from *Otostegia persica.*

Experimental

The IR spectra were recorded on JASCO A-302 spectrometer. The mass spectra were recorded on a Finnigan MAT-112 spectrometer and the NMR spectra ($CDCl_3$) 400 MHz for ^1H and 100 MHz for ^{13}C using TMS as internal standard were recorded on Bruker spectrometer. The aluminum sheets precoated with silica gel 60 F_{254} (20 x 20 cm, 0.2 mm thick; E-Merck) were used for TLC and flash silica (230-400 mesh) was used for column chromatography.

Extraction and Isolation

The *Otostegia persica* was collected from the southern part of Iran. The freshly collected aerial parts of the plant (25 kg) were extracted thrice with MeOH at room temperature. The methanolic extract was then evaporated under vacuo to yield a brownish residue, which was submitted for preliminary bioassay techniques. Later on, it was suspended in H_2O and then extracted successively with *n*-hexane, chloroform and ethyl acetate. The hexane fraction was chromatographed over silica gel using a mixture of *n*-hexane and ethyl acetate as eluent in increasing order of polarity. The fractions obtained from *n*-

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hexane and ethyl acetate (9: 1) yielded compounds **1-4**. On the other hand, the ethyl acetate fraction yielded compounds **5-11**, when treated in a similar fashion using *n*-hexane and ethyl acetate (7: 3) as the eluent. These compounds were further purified by thick layer chromatography using the same solvent system.

Geraniol (1)

The compound (**1**) was obtained as a colorless oil having b.p. 230-2 °C; EIMS *m/z* 154.2 ($C_{10}H_{18}O$, M^+), 136, 121, 93, and 69. The main signals in ^{1}H-NMR spectrum appeared at δ_H 5.31 (1H, br s), 5.17 (1H, dd, J = 8.12 and 5.2 Hz), 4.11 (2H, br s), 2.03 (4H, m), 1.80 (6H, s), 1.67 (3H, s). The other data coincided with the literature [9].

Eugenol (2)

The compound (**2**) was obtained as a colorless oil having b.p. 74-6 °C; EIMS *m/z* 164.2 ($C_{10}H_{12}O_2$, M^+), 149, 147, 131 and 115. The main signals in ^{1}H-NMR spectrum appeared at δ_H 6.81 (1H, s), 6.73 (1H, s), 6.59 (1H, s), 5.83 (1H, m), 5.12 (2H, m), 3.71 (3H, s) and 2.97 (2H, m). The other data coincided with the literature [10].

Ceryl alcohol (3)

The compound (**3**) was obtained as colorless crystals having m.p. 80-82 °C; EIMS *m/z* 382.7 ($C_{26}H_{54}O$, M^+), 367, 354, 351, and 326. The main signals in ^{1}H-NMR spectrum appeared at δ_H 3.39 (2H, br s), 1.25-1.33 (44H, br s), 1.41-1.49 (4H, br s), 0.85 (3H, t). The other data coincided with the literature [11].

Hentriacontane (4)

The compound (**4**) was obtained as colorless crystals having m.p. 66-68 °C; EIMS *m/z* 436.8 ($C_{31}H_{64}$, M^+), 421, 408, 406 and 380. The main signals in ^{1}H-NMR spectrum appeared at δ_H 0.83 (6H, t) and 1.23-1.33 (58 H, br s). The other data coincided with the literature [11].

Caffeic acid (5)

The compound (**5**) was obtained as colorless crystals having m.p. 194-196 °C; EIMS *m/z* 180.1 ($C_9H_8O_4$, M^+), 135 and 109. The main signals in ^{1}H-NMR spectrum appeared at δ_H 7.37 (1H, d, J = 11.2 Hz), 7.03-6.89 (3H, m) and 6.27 (1H, d, J = 11.2 Hz). The other data coincided with the literature [11].

p-Hydroxybenzoic acid (6)

The compound (**6**) was obtained as colorless crystals having m.p. 214-216 °C; EIMS *m/z* 138.1 ($C_7H_6O_3$, M^+), 93 and 76. The main signals in ^{1}H-NMR spectrum appeared at δ_H 7.97 (2H, br s) and 6.83 (2H, br s). The other data coincided with the literature [11].

β-Sitosterol (7)

The compound (**7**) was obtained as colorless crystals having m.p. 140-142 °C; $[\alpha]_D$ -37.0 ($CHCl_3$); EIMS *m/z* 414.7 ($C_{29}H_{50}O$, M^+), 399, 396 and 371. The main signals in ^{1}H-NMR spectrum appeared at δ_H 5.30 (1H, dd, J = 8.13 and 5.12 Hz), 3.29 (1H, m), 1.53-1.23 (29H, m), 1.23 (3H, s), 1.02 (3H, s), 0.93 (3H, d, J = 4.95 Hz), 0.91 (6H, d, 5.03 Hz), and 0.83 (3H, t). The other data coincided with the literature [12-14].

β-Sitosteryl acetate (8)

The compound (**8**) was obtained as colorless crystals having m.p. 126-128 °C; $[\alpha]_D$ -41.3 ($CHCl_3$); EIMS *m/z* 456.8 ($C_{31}H_{52}O_2$, M^+), 396, 381 and 353 . The main signals in ^{1}H-NMR spectrum appeared at δ_H 5.35 (1H, dd, J = 8.21 and 5.23 Hz), 4.12 (1H, m), 2.11 (3H, s), 1.57-1.25 (29H, m), 1.23 (3H, s), 1.01 (3H, s), 0.96 (3H, d, J = 5.11 Hz), 0.93 (6H, d, 5.23 Hz) and 0.87 (3H, t). The other data coincided with the literature [13].

β-Amyrin (9)

The compound (**9**) was obtained as colorless crystals having m.p.196-198 °C; $[\alpha]_D$ -88.3 ($CHCl_3$); EIMS *m/z* 426.7 ($C_{30}H_{50}O$, M^+), 411 and 408. The main signals in ^{1}H-NMR spectrum appeared at δ_H 5.31 (1H, m), 3.17 (1H, m), 1.21 (3H, s), 1.12 (6H, s), 1.09 (3H, s), 1.01 (3H, s), 0.98 (6H, s), 0.95 (3H, s) and 1.56-1.29 (23H, m). The other data coincided with the literature [15].

Campesterol (10)

The compound (**10**) was obtained as a gummy solid; $[\alpha]_D$ -32.9 ($CHCl_3$); EIMS *m/z* 400.7 ($C_{28}H_{48}O$, M^+), 385, 382 and 367. The main signals in ^{1}H-NMR spectrum appeared at δ_H 5.31 (1H, m), 3.22 (1H, m), 1.59-1.29 (27H, m), 1.25 (3H, s), 1.02 (3H, s), 0.95 (6H, br s) and 0.91 (6H, d, 5.23 Hz). The other data coincided with the literature [12-14].

Stigmasterol (11)

The compound (**11**) was obtained in crystalline form having m.p. 168-170 °C; $[\alpha]_D$ -50.3 ($CHCl_3$); EIMS *m/z* 412.7($C_{29}H_{48}O$, M^+), 397, 394 and 379. The main signals in ^{1}H-NMR spectrum appeared at δ_H 5.45 (1H, m), 5.41 (1H, dd, J = 11.12 and 4.32 Hz), 5.29 (1H, m), 3.27 (1H, m), 1.56-1.31 (28H, m), 1.23 (3H, s), 1.03 (3H, s), 0.93 (3H, d, J = 4.75 Hz) and 0.89 (6H, d, 5.11 Hz). The other data coincided with the literature [12-14].

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