Arabian Journal of Chemistry (2013) 6, 295–298

King Saud University

Arabian Journal of Chemistry

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ORIGINAL ARTICLE

Isolation and characterization of triterpenes from the leaves of *Orthosiphon stamineus*

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Received 6 September 2010; accepted 12 October 2010
Available online 18 October 2010

KEYWORDS

Orthosiphon stamineus;
Lamiaceae;
Local medicinal plant;
Triterpenes

Abstract *Orthosiphon stamineus,* Benth, belonging to the family Lamiaceae, is a medicinal plant growing wild in tropical countries. Seven triterpenes, ursolic acid, oleanolic acid, betulinic acid, hydroxybetulinic acid, maslinic acid, α-amyrin and β-amyrin have been isolated from the leaves of *Orthosiphon stamineus*. The structures of these compounds have been established by spectroscopic methods. α-Amyrin was isolated from this plant for the first time.

1. Introduction

Orthosiphon stamineus, Benth, is native to tropical Eastern Asia where it is locally known as 'Misai kucing' or 'Kumis kucing'. It is used for treatment of wide range of diseases. In Indonesia the leaves of this plant are used for treatment of rheumatism, diabetes, urinary lithiasis, edema, eruptive fever, influenza, hepatitis, jaundice, biliary lithiasis, and hypertension (Sumaryono et al., 1991; Tezuka et al., 2000; Shibuya et al., 1999) and in Malaysia, it is cultivated and the leaves are used as remedy for urinary system ailments (Liu et al., 2003). The recent surge of interest in chemistry of this plant has led to the isolation of more than 50 components with different biological activities.

The chemical constituents of *Orthosiphon stamineus* include highly-oxygenated isopimarane-type diterpenoids, methoxylated flavones, caffeic acid derivatives and triterpenoids (Tezuka et al., 2000). The present work deals with the isolation, structure elucidation and identification of the pentacyclic terpenoids from the methanol extract of leaves of locally grown *Orthosiphon stamineus*.

2. Experimental

2.1. General

IR spectra were recorded (KBr discs) on a FT-IR spectrometer, validation (ν_{max} in cm^{-1}). ^1H NMR spectra were recorded on a Bruker R-32 (300 MHz) instrument in $CDCl_3$ with TMS as an internal standard (chemical shifts in δ, ppm). UV spectra were recorded on HATACHI, U-2000 spectrophotometer Ultrospeck in methanol (λ_{max} in nm). TLC was performed with silica gel GF_{254}. All solvents were analytical reagent grade.

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Peer-review under responsibility of King Saud University.
doi:10.1016/j.arabjc.2010.10.009

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2.2. Plant material

The leaves of *Orthosiphon stamineus* were collected from the Island of Penang. The plant was identified and voucher specimen (041) was deposited in the herbarium of the School of Biology, University Sains Malaysia.

2.3. Extraction

Dried leaves of the plant (1.5 kg) were milled into powder and then extracted with methanol (10 L) in a Soxhlet extractor for 36 h. The extract was evaporated in a rotatory evaporator and dried by vacuum pump. The methanolic extract (50 g) was suspended on water and extracted successively with hexane, chloroform, ethyl acetate, and butanol to yield hexane (4 g), chloroform (10.5 g), ethyl acetate (7.4 g) and BuOH-soluble (4.23 g) fractions, respectively. Chloroform soluble fraction (8 g) was subjected to chromatography on silica gel (60–120 mesh, Merck) eluted with ethyl acetate-hexane (7:3) solvent system. Repeated chromatography to give five major fractions (Fraction-1, 0.110 g; Fraction-2, 0.143 g; Fraction-3, 1.229 g; Fraction-4, 0.059 g; Fraction-5, 0.125 g)

2.4. Ursolic acid (*1*)

Obtained from column chromatography it was further purified by preparative TLC over silica gel GF_{254} using ethyl acetate-hexane (3:2) as developing solvent. It was crystallized from methanol–water as white powder (**1**, 8 mg); m.p. 195 °C [lit. (Galgon et al., 1999) m.p. 197 °C]; (M^+, 456); UV: 210 nm; IR (KBr): 3435, 3243, 3019, 2827, 2679, 2542, 1643, 1621, 1600, 1528, 1380, 1347, 1304, 1293, 1216, 963, 898, 843 cm^{-1}; ^1H NMR ($CDCl_3$): 0.73, 0.77, 0.79, 0.92, 0.93, 0.94 and 1.00 (7s, 21H, all $-CH_3$), 1.37 (m, 2H, H-21), 1.38 (m, 2H, H-16), 1.45 (m, 2H, H-20), 1.51 (m, 4H, H-18, H-19 and H-15), 2.09 (m, 3H, H-1 and H-9), 2.13 (m, 2H, H-14), 3.17 (t, 2H, J = 7 Hz, H-2), 3.38 (s, 2H, H-7), 4.56 (s, 2H, H-11), 4.59 (s, 1H, H-12). Fraction-1 was identified as ursolic acid (**1**).

2.5. Oleanolic acid (*2*)

Isolated fraction was further purified by preparative TLC over silica gel GF_{254} using ethyl acetate-hexane (3:2) as developing solvent. It was crystallized from methanol as white powder (**2**, 6 mg); m.p. 173 °C [lit. (Galgon et al., 1999) m.p. 172 °C]; (M^+, 360); UV: 209 nm; IR (KBr): 3331, 3161, 3095, 2876, 2783, 2717, 2657, 1906, 1846, 1660, 1616, 1561, 1512, 1435, 1326, 1265, 1227, 1106, 1024, 964, 843, 810 cm^{-1}; ^1H NMR ($CDCl_3$): ^1H NMR ($CDCl_3$): 0.79, 0.89, 0.91, 0.92, 0.97, 1.08 and 1.34 (7s, 21H, all $-CH_3$), 1.37 (m, 2H, H-21), 1.38 (m, 2H, H-16), 1.51 (m, 5H, H-18, H-19 and H-15), 2.09 (m, 3H, H-1 and H-9), 3.17 (t, 1H, J = 7 Hz, H-2), 3.38 (s, 2H, H-7), 4.56 (s, 2H, H-11), 4.59 (s, 1H, H-12). Fraction -2 was identified as oleanolic acid (**2**).

2.6. Fraction-3

Isolated from column was further purified by preparative TLC over silica gel GF_{254} using ethyl acetate-hexane-acetone (7:3:5) as developing solvent to give compound **3** (5 mg) and compound **4** (6 mg).

2.6.1. Betulinic acid (*3*)

It was crystallized from water as white powder (6 mg); m.p 297 °C (dec) [lit. (Ye et al., 1998) m.p. 197 °C]; (M^+, 458); UV: 206 nm; IR (KBr): 3473, 3063, 2953, 2887, 2712, 1682, 1643, 1457, 1375, 1221, 1194, 1106, 1035, 980, 876, 871, 789 cm^{-1}; ^1H NMR ($CDCl_3$): 0.65, 0.77, 0.98, 1.14 and 1.34 (5s, 15H, all tertiary $-CH_3$), 1.37 (m, 2H, H-21), 1.38 (m, 2H, H-16), 1.45 (m, 2H, H-20), 1.51 (m, 4H, H-18, H-19 and H-15), 2.09 (m, 3H, H-1 and H-9), 2.13 (m, 2H, H-14), 3.17 (t, 2H, J = 7 Hz, H-2), 3.38 (s, 2H, H-7), 4.56 (s, 2H, H-11), 4.59 (s, 2H, H-12). Compound **1** was identified as betulinic acid (Knight, 1974) (**3**).

2.6.2. Hydroxybetulinic acid (*4*)

It was crystallized from xylene. It was a colourless amorphous solid; m.p. 270 °C (lit Knight, 1974 m.p. 271–274 °C); (M^+, 473), UV: 252 nm, IR (KBr):3435, 2926, 2838, 2712, 1742, 1665, 1463, 1380, 1254, 1161, 1139, 1112, 1041, 1013, 975., 823, 794, 728, 580 cm^{-1}. ^1H NMR ($CDCl_3$): 0.81, 0.84, 1.01, 1.18 and 1.40 (5s, 15H, all tertiary $-CH_3$), 1.37 (m, 2H, H-21), 1.35 (m, 2H, H-16), 1.43 (m, 2H, H-20), 1.55 (m, 4H, H-18, H-19 and H-15), 2.14 (m, 3H, H-1 and H-9), 2.16 (m, 2H, H-14), 3.19 (t, 2H, J = 7 Hz, H-2), 3.48 (s, 2H, H-7), 4.50 (s, 2H, H-11), 4.69 (s, 2H, H-12). Compound **2** was identified as hydroxybetulinic acid (**4**).

2.7. Fraction-4

It was purified by preparative TLC over silica gel GF_{254} using ethyl acetate-hexane-petroleum spirit (7:3:2) as developing solvent to give compound 5 (6 mg) and compound 6 (4 mg).

2.7.1. α-Amyrin (*5*)

It could not be crystallized from any solvent. It was a colourless amorphous solid. (M^+, 426), UV: 274, 257, 243 nm, IR (KBr):3446, 3035, 2969, 2931, 2849, 2717, 1758, 1645, 1452, 1369, 1309, 1254, 1139, 1084, 1024, 969, 887, 832, 794, 728, 580 cm^{-1}. ^1H NMR (δ values, $CDCl_3$): 0.86 (s, 3H, $-CH_3$), 0.88 (d, 3H, J = 7 Hz, $-CH_3$), 1.08 (d, 3H, J = 7.0 Hz, $-CH_3$), 1.26–1.29 (d, 6H, J = 7 Hz, two secondary $-CH_3$), 1.33 (s, 3H, $-CH_3$ at H-29) 1.47 (m, 2H, H-16), 1.51 (m, 2H, H-21), 1.60 (d, 1H, J = 11.5 Hz, H-18), 1.68 (s, 7 H, $-CH_3 \times 2$ and H-5), 1.80 (m, 1H, H-19), 1.83 (m, 2H, H-22), 1.85 (m, 2H, H-15), 2.01 (d, 2H, J = 11.5 Hz, H-1), 2.04 (s, 1H, H-9), 2.17-2.24 (d, 2H, J = 11.5 Hz, H-1 and H-16), 3.53 (s, 2H, H-7), 3.67 (m, 2H, H-2), 5.11 (s, 2H, H-11), 5.13 (s, 1H, H-12). Compound **1** was identified as α-amyrin (Ikuta et al., 1995) (**5**).

2.7.2. β-Amyrin (*6*)

It could not be crystallized from any solvent. It was a dark pink colour amorphous solid. (M^+, 426), UV: 245 nm, IR (KBr): 3452, 3035, 2964, 2920, 2843, 2717, 2669, 2032, 1753, 1660, 1452, 1369, 1309, 1260, 1128, 1084, 1030, 980., 882, 838, 728, 586 cm^{-1}, ^1H NMR (δ values, $CDCl_3$): 0.87 (s, 3H, $-CH_3$), 0.89 (d, 3H, J = 7 Hz, H-30), 1.08 (d, 3H, J = 7.0 Hz, H-27), 1.27–1.30 (d, 6H, J = 7 Hz, two secondary $-CH_3$), 1.48 (m, 2H, H-21), 1.52 (d, 1H, J = 11.5 Hz, H-18), 1.69 (s, 20H, $-CH_3 \times 5$, H-2, H-7 and H-5), 1.83 (m, 2H, H-19), 1.86 (m, 2H, H-22), 1.90 (m, 2H, H-15), 2.05 (d, 2H, J = 11.5 Hz, H-1), 2.19 (s, 1H, H-9), 2.23–2.26 (d, 2H,

J = 11.5 Hz, H-1 and H-16), 5.14 (s, 2H, H-11), 5.55 (s, 1H, H-12). It was identified as β-amyrin (Ikuta et al., 1995) (**6**).

2.8. Maslinic acid (7)

Obtained from column chromatography it was further purified by preparative TLC over silica gel GF_{254} using chloroform-methanol (95:5) as developing solvent. This compound was crystallized from petroleum spirit. It was a colourless needles (4 mg); m.p. 248^{0}C (lit[9] m.p. 248–250 °C); (M^+, 473); UV: 227 nm, IR (KBr):3435, 2926, 2838, 2712, 1742, 1665, 1463, 1380, 1254, 1161, 1139, 1112, 1041, 1013, 975., 823, 794, 728, 580 cm^{-1}. ^{1}H NMR (DMSO-d_6):0.89, 0.95, 0.97. 0.99, 1.03, 1.23, 1.25 (each, s, 3H, H-23, H-24, H-25, H-26, H-27, H-28, H-29 and H-30), 3.26 (dd, 1H, J = 4.5 and 14.5 Hz), 3.35 (d, 1H, J = 9 Hz, H-3α), 4.10 (ddd, 1H, J = 4.5, 9 and 11.0 Hz, H-2β), 5.44 (s, br, 1H). It was characterized as maslinic acid (**7**).

3. Results and discussion

The methanol extract of the leaves was extracted with *n*-hexane, chloroform, ethyl acetate and *n*-butanol. The chloroform fraction was purified and seven compounds were obtained (Fig. 1). By means of spectroscopic analysis, they were identified as ursolic acid (**1**), oleanolic acid (**2**), betulinic acid (**3**), hydroxybetulinic acid (**4**), α-amyrin (**5**), β-amyrin (**6**) and maslinic acid (7). α-Amyrin (**5**) was isolated from this plant for the first time.

α-Amyrin (**5**) was obtained as an orange amorphous solid. High resolution mass spectrum exhibited molecular ion at m/z 426, which is consistent with the molecular formula $C_{30}H_{50}O$. UV spectra displayed characteristic absorption bands for conjugated double bond 257 nm. IR spectra of compound (**5**) showed frequencies at 3446 cm^{-1} and 3035–2717 cm^{-1} indicating the presence of hydroxyl group and C–H in conjugation and the absorption peaks at 1665, 1452 and 1369 cm^{-1} indicated the presence of unsymmetric ethylenic double bond, aromatic rings and aromatic –CH_3 group, respectively.

The ^{1}H NMR spectrum showed five tertiary methyl signals at δ 0.86, 0.88, 1.08 and 1.68 indicating the triternoidal nature of the compound. It also exhibited one double bond proton multiplet at δ 5.13 along with two secondary methyl signal at δ 1.26–1.29 indicating the isopropenyl residue of the skeleton. The secondary hydroxyl group was assigned to C-3 biogenetic grounds in β- and equatorial configuration based on larger coupling constants at δ 3.5. The downfield shift of one of the methyl groups at δ 1.33, compared with 3 β-hydroxyurs-12-ene (Galgon et al., 1999) was allowed to assign one of the methyl group to C-19. Further evidence for the structure was obtained from ^{13}C NMR analysis in which the DEPT experiment showed that the isolated compounds had eight CH_3 groups, ten CH_2 groups, four –CH groups and one –CH–C group. It is known that the α-amyrin is widely distributed in the plant kingdom, however, to the best of our knowledge, the isolation triterpenes from *Orthosiphon stamineus* has not been reported elsewhere. All the isolated known triterpenes were identified on the basis of UV, IR and ^{1}H NMR data and compared with literature.

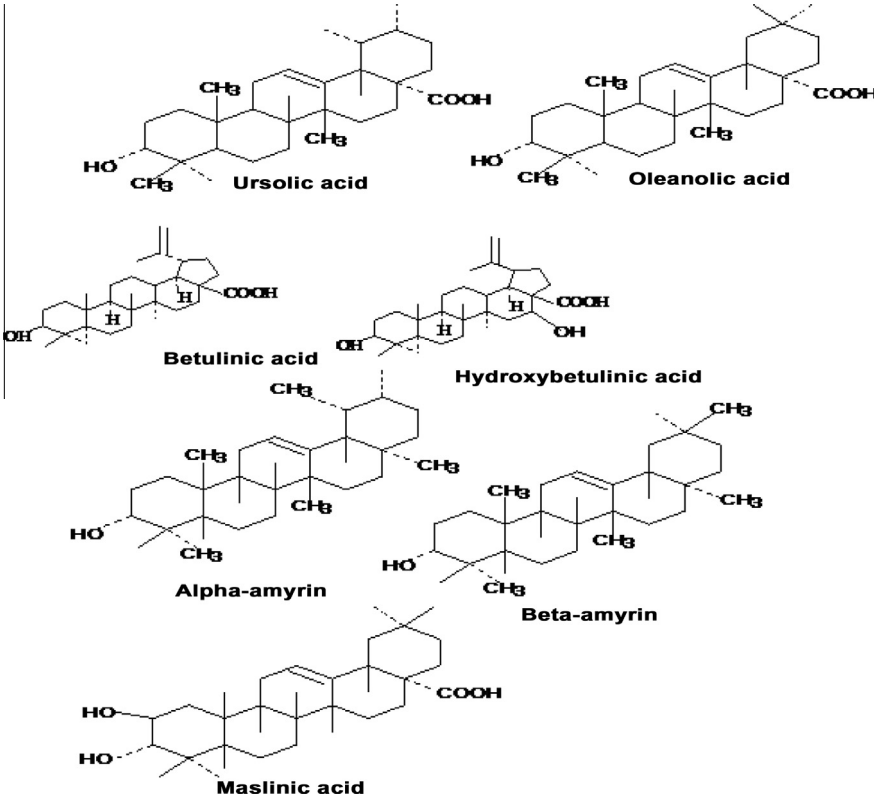

Figure 1 Chemical structures of triterpenoids.

Acknowledgements

The authors are grateful to Mr. Khoo Kay Hock and Mr. Yee Chin Leng of the School of Chemistry, Universiti Sains Malaysia, Malaysia for their help in connection with ^{1}H NMR and mass spectra. One of the authors (M. Amzad Hossain) is grateful to Universiti Sains Malaysia, Malaysia for providing a fellowship.

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