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Sesquiterpene Lactones and Penta Methoxylated Flavone from *Artemisia kulbadica*

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The aerial parts of *Artemisia kulbadica* afforded a germacranolide and a guaianolide type sesquiterpene lactones together with a pentamethoxylated flavone. The structures were elucidated by spectropic methods, including ^{1}D and ^{2}D NMR analysis.

Key Words: *Artemisia kulbadica*, Compositae, Guaianolide, Sesquiterpene lactones, Flavones, Germacranolide.

INTRODUCTION

Artemisia is a genus of small herbs or shrubs found in Northern temperate regions. It belongs to the important family compositae (Asteraceae), one of the most bulky vegetal groupings, which comprises about 1000 genera and over 20000 species. Within this family, *Artemisia* is included into the tribe Anthemideae and comprises itself over 400 species. The 400 species of *Artemisia* are mainly found in Asia, Europe and North America. They are mostly perennial herbs and shrubs dominating the vast communities of Asia[1], 150 recorded for China about 50 reported to occur in Japan and 35 species of the genus are found in Iran, of which two are endemic: *A. melanolepis* Boiss and *A. kermanensis* Podl[2]. The genus *Artemisia* has always been of great botanical and pharmaceutical interest and is useful in traditional medicines for the treatment of the variety of diseases and complaints.

A. annua is a traditional medicinal herb of China. It is presently being cultivated on a commercial scale in China and Vietnam for its antimalarial sesquiterpene lactones artemisinin and its essential oil[3,4].

The genus *Artemisia* including some Iranian species has been studied chemically and presence of monoterpenes, sesquiterpene, especially sesqiterpene lactones and essential oils are reported. Acyclic monoterpenes and monoterpene hydroperoxides have been found in the aerial parts of *A. aucheri* Boiss[5]. The extract of the aerial parts of *A. diffusa krasch*. ex poljakov, afforded in addition to a monoterpene lactones, filifolide A, several eudesmanolides and a new type of sesquiterpene lactones with unusual carbon skeletone, an eight-member ring (tehranolide)[6]. The antimalarial properties of crude extract of the same species (*A. diffusa*) has been studied. The study especially examined the inhibitory effects of the extracts on developmental stages of *in vivo* of *Plasmodium berghei* on the mice body[7].

The hydrodistilled volatile oil from the aerial parts of *A. kullbadica* Boiss and Buhse was investigated by a combination of GC and GC/MS. Twenty- seven compounds were identified 92.9 % of the total oil. Sabinene (25.1 %), *trans*-thujone (18.7 %) and γ-cadinene (16.0 %) were the main components the antimicrobial was determined against six bacterial strains and one fungal[8].

EXPERIMENTAL

IR spectra were taken on a Thermo Nicolet Nexus 870 FT-IR spectrometer. ^{1}H and ^{13}C NMR spectra were recorded at 500 and 125 MHZ, respectively, with TMS as internal standard on a Bruker AM 500 instrument, under Aspect X 32 control. The ^{2}D NMR spectra were abtained using Bruker's microprograms. Low-resolution and high-resolution mass spectra were recorded on an MAT-5973 spectrometer. Silica gel 60 (70-230 and 230-400 mesh) and TLC was performed with kieselgel 60 F_{254} (Merck aluminium support plates) and spot were detected after spraying with a 15 % H_2SO_4 solution in MeOH.

Artemisia kulbadica was collected in September 2009 from their natural habitats in North East of Iran province of Khorassan. Voucher specimens are deposited at the Herbarium of the Research Instituted of Forests and Rangelands (TARI), Tehran, Iran.

Extraction and isolation: Ground aerial parts (500 g) were extracted with Et_2O/MeOH/petrol (1:1:1) (2 × 5 L) at room temperature. for 3 days to give 45 g (8.3 % yield) of crude extract which was suspended in EtOH (300 mL) at 55 °C, diluted with H_2O (250 mL) and extracted successively with

n-hexane (3 × 650 mL) and $CHCl_3$ (3 × 450 mL). The $CHCl_3$ extract on evaporation at reduced pressure furnished a residue (10 g) which was chromatographed over silica gel (200 g) using *n*-hexane with increasing amounts of EtOAc (0-100 %) and EtOAc/MeOH (9:1), 20 fractions being collected which were monitored by TLC. Fractions 5-6 of the rechromatographed which exhibited two spots on TLC afforded after repeated purification by CC to yield 169 mg of compound **1**.

Fraction 7-8 which showed a major spot on TLC was decolourized with charcoal in hexane-EtOAc. Filtration, evaporation at reduce pressure and recrystallization from *n*-heptane-EtOAc (3:1) gave 54 mg of pure compound (**2**). Fraction 9-11 were reunited and rechromatographed on silica gel (230-400 mesh) using C_6H_{14}-EtOAc (7:1) to yield 70 mg of compound **3**.

4α-Hydroxy-guaia-1(10),5(6)-dien-12,8-olide (1): Yellow crystals, m.p. 132-133 ºC; IR (KBr, ν_{max}, cm^{-1}): 3477 (OH), 1751 (γ-lactone); MS (m/z) (rel. int.): 248 $[M]^+$ (43), 230 $[M-H_2O]^+$ (56), 215 $(M-H_2O-Me]^+$ (100), 156 (37), 43 (76); ^1H and ^{13}C NMR spectra (Table-1).

TABLE-1
NMR DATA OF COMPOUND **1***

Position	δ_C	δ_H
1	137.2 s	–
2	45.5 t	2.77 br dd (15.0, 13.0), 2.83 m
3	25.9 t	1.89 m, 1.81 m
4	70.7 s	–
5	150.9 s	–
6	124.3 d	6.02 d (3.0)
7	52.4 d	1.72 ddd (3.0, 10.0, 12.0)
8	82.9 d	5.32 ddd (2.0, 4.0, 10.0)
9	39.9 t	2.01 dd (14.0, 4.0), 1.54 dd (14.0, 2.0)
10	134.9 s	–
11	41.5 d	2.27 dq (7.0, 12.0)
12	178.8 s	–
13	12.7 q	1.20 d (7.0)
14	14.9 q	2.14 brs
15	30.8 q	1.55 s

*Chemical shifts were determined at 500 (^1H) and 125 (^{13}C) Mhz in $CDCl_3$. ^1H and ^{13}C NMR Chemical shifts refer to $CHCl_3$ at 7.26 ppm and $CDCl_3$ at 77.0 ppm, respectively. *J* values hertz are in parentheses multiplicities astablished by DEPT experiments.

3-Oxogermacr-1(10)-(E)-en-12,6α-olide (2): $C_{15}H_{22}O_3$, white crystals, m.p. 169-171 ºC; IR (KBr, ν_{max}, cm^{-1}): 1759 (γ-lactone), 1704 (ketone), 1659 (C=C); MS (m/z) (rel. int.): 250 $[M]^+$ (15), 235 $[M-Me]^+$ (11) 193 (47), 108 (77), 67 (88), 55 (36), 41 (100); ^1H and ^{13}C NMR spectra Table-2.

5-Hydroxyl-6,7,8,2',4'-pentamethoxy-flavone (3): $C_{20}H_{20}O_8$, yellow crystals, m.p. 159-160 ºC; IR (KBr, ν_{max}, cm^{-1}): IR (KBr, ν_{max}, cm^{-1}): 3433, 1656, 1592, 1470. MS (m/z) (rel. int.): 388 $[M]^+$ (100), 373 $[M-Me]^+$ (85), 345 $[M-MeCo]^+$ (16), 165 (27), 149 (14), 69 (16); ^1H and ^{13}C NMR spectra (Table-3).

RESULTS AND DISCUSSION

Compound **1** displayed strong IR bands at 3477 and 1751 cm^{-1}, which suggest the presence of hydroxyl and lactones functions. The mass spectrum (EI) showed a molecular peak at m/z 248, which agrees with the molecular formula $C_{15}H_{20}O_3$. The ^1H NMR spectrum of 1 (Table-1) indicated that 11,13-dihydro guaianolide is present as followed from the signals and δ 2.27 dq and 1.20 d (3H). Spin decoupling allowed the assignment of all signals. Starting with that of H-11, that of H-7 could be determined. As the latter was coupled with a doublet at δ 6.02 (H-6) and a doublet of triplets at α 5.32 (H-8), subsequent decouplings led to the whole sequence. The ^{13}C NMR spectrum showed 15 signals corresponding to three-CH_3, three-CH_2, four-CH and five quaternary carbons, as deduced from a DEPT experiment, in agreement with the molecular formula obtained from the mass spectrum. ^{13}C NMR spectrum also indicated the presence of a lactones moiety,

TABLE-2
NMR DATA OF COMPOUND **2***

Position	δ_C	δ_H
1	116.7 d	5.47 br dd (8.4, 8.4)
2	42.5 t	3.01 br dd (13.0, 8.5), 3.03 br dd (13.0, 8.1)
3	208.1 s	–
4	40.0 d	2.83 m
5	39.8 t	1.51 ddd (13.0, 12.7, 3.6) 1.96 ddd (13.0, 12.9, 2.4)
6	82.0 d	3.53 dd (12.0, 2.8)
7	41.3 d	2.16 dd (11.0, 7.3)
8	24.0 t	1.71 ddd (13.8, 4.1, 3.1) 1.34 m
9	37.2 t	2.10 ddd (12.9, 13.0, 4.9) 2.28 ddd (3.7, 13.0, 3.6)
10	141.9 s	–
11	35.5 d	2.77 dq (7.3, 7.3)
12	178.7 s	–
13	10.2 q	1.14 d (13.0)
14	15.8 q	1.58 brs
15	18.4 q	1.04 d (6.7)

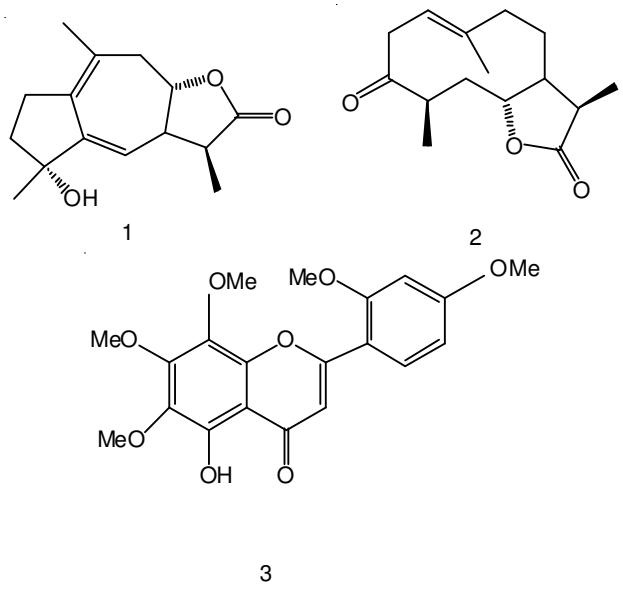
*Chemical shifts were determined at 500 (^1H) and 125 (^{13}C) Mh_z in $CDCl_3$. ^1H and ^{13}C NMR Chemical shifts refer to $CHCl_3$ at 7.26 ppm and $CDCl_3$ at 77.0 ppm, respectively. *J* values hertz are in parentheses multiplicities astablished by DEPT experiments.

TABLE-3
NMR DATA OF COMPOUND 3*

Position	δ_C	δ_H
2	158.7 s	–
3	90.3 d	6.46 s
4	178.8 s	–
5	155.8 s	–
6	148.8 s	–
7	151.4 s	–
8	152.7 s	–
9	152.2 s	–
10	106.5 s	–
1'	138.8 s	–
2'	132.3 s	–
3'	111.4 d	7.65 d (2.0)
4'	122.9 s	–
5'	122.1 d	7.70 dd (2.0, 8.5)
6'	110.9 d	6.95 d (8.5)
OMe-6	55.9 q	3.93
OMe-7	56.3 q	3.93
OMe-8	56.3 q	3.94
OMe-2'	60.8 q	3.83
OMe-4'	60.1 q	3.89

*Chemical shifts were determined at 500 (^1H) and 125 (^{13}C) Mhz in $CDCl_3$. ^1H and ^{13}C NMR chemical shifts refer to $CHCl_3$ at 7.26 ppm and $CDCl_3$ at 77.0 ppm, respectively. *J* values hertz are in parentheses multiplicities astablished by DEPT experiments.

which showed signals at δ 178.8 (C-12), 41.5 (C-11) and 12.7(C-13). The two methyl singlets in the ^{1}H NMR (δ 2.14 and 1.55 ppm) correlated with carbon signals at δ 14.9 (C-14) and 30.8 (C-15). The configuration at C-4, C-8 and C-11 could be deduced from the observed couplings and from the observed NOESY. Therefore, the structure of 1 was 4α-hydroxyl-guaia 1(10),5(6)-dien-12,6α-olide.



Structures of (**1**): 4α-Hydroxy-guaia-1(10),5(6)-dien-12,8α-olide; (**2**): (4R*,6R*,7S*,11R*)-3-Oxogermacr-1(10)-(E)-en-12,6α-olide; (**3**): 5-Hydroxy-6,7,8,2',4'-penta methoxy-flavone

The mass spectrum of compound (**3**) showed $[M]^+$ at m/z 388, corresponding to the molecular formula $C_{20}H_{20}O_8$ in agreement with the ^{13}C NMR spectrum and DEPT experiment, which showed 20 signals, five of them corresponding to CH_3, four to CH and eleven to quaternary carbons. In the ^{1}H NMR spectrum of 3 (Table-3) obtained in $CDCl_3$ a three-proton ABC system was present (δ 7.65, d, *J* = 2.0 Hz; 7.70, dd, *J* = 8.5, 2.0 Hz; 6.95, d, *J* = 8.5 Hz), typical of the 2',4'-disubstituted ring B of a flavonoid unit. Ring C showed only a resonance as one-proton singlet at δ 6.46. Moreover the presence of a hydroxyl proton signal at δ 12.58 indicated that the hydroxyl group must be at C-5.

The isolation and charactrization of compound **2** from *Artemisia absinthium* and *Ajania fruticulosa* has already been reported[9,10].

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