Asian Journal of Chemistry Vol. 21, No. 3 (2009), 2253-2258

Two Diterpenoids From *Scutellaria orientalis* L. subsp. *porphyrostegia* Edmondson

ÇIGDEM KARABACAK*, TAHIR TILKI and MUSTAFA CENGIZ
Department of Chemistry, Faculty of Arts and Sciences
Süleyman Demirel University, 32260 Isparta, Turkey
E-mail: cigdem@fef.sdu.edu.tr

In this study, two diterpenoids *viz.*, Ajugarin V and Scutenisin were isolated from the acetone extract of *S. orientalis* L. subsp. *porphyrostegia* Edmondson. The structure of these compounds were elucidated. The compounds were 4α,18-epoxy-6α-acetoxy-neoclerod-13-en-15,16-olide and (13R*)-6α,7β-diisobutryloxy-4α,18; 8β,13-diepoxy-19-hydroxy-neoclerod-15,16-olide.

Key Words: *Scutellaria orientalis* L. subsp. *porphyrostegia* Edmondson, Ajugarin V, Scutenisin.

INTRODUCTION

Terpenoids (or isoprenoids), a subclass of the prenyllipids (terpenes, phenylquinones and sterols), represent the oldest group of small molecular products synthesized by plants and are probably the most widespread group of natural products[1]. Labiatae family plants have been used widely as folk medicine in Turkey. This plant is used in folk medicine as a tonic, haemostatic, antidiarrheaic for the treatment of bronchitis, tumors, liver cirrhosis, heptitis and other diseases[2-5].

The genus *Scutellaria*, Labiatae, occurs with 360 species. The genus is widespread in temperate regions and on tropical mountains. Most are annual or perennial herbaceous plants from 5 cm to 1 m tall, but a few are subshrubs; some are aquatic.

Many species of *Scutellaria* genus have been shown that chemical constituents of their roots are rich in flavonoids[6]. Several neo-clerodane diterpenoids with antifungal and antifeedant activities have been isolated from Labiatae genera including *Scutellaria*. So, this genus have attracted much attention recently[7,8]. A number of biologically active neo-clerodane diterpenoids have been isolated during the last decade from *Scutellaria* species. Their structures were elucidated by means of NMR spectroscopy and other spectroscopic methods[9].

Recently, 17 flavonoids were isolated from the roots of *Scutellaria luzonica* Rolfe. These compounds include 13 flavones, 2 flavanones, a chalcone and a flavone glucoside[10]. Two diterpenoids have been isolated from *Scutellaria rubicunda* subsp. *linneana*: scutecyprol B and scutalbin C. Both compounds were tested for antifeedant activity[11]. Four new neoclerodane-type diterpenoids, scutellones, B, G, H and I, have been isolated from aerial parts of *Scutellaria rivularis* WALL. and their structures

also elucidated on the basis of spectral and chemical evidence[12]. Seven previously known neo-clerodanes have been isolated from *Scutellaria alpina* subsp. *javalambrensis* together with a new diterpenoid, 11-deacetylscutalpin D. The antifeedant activity of some of the isolated diterpenoids was assessed against larvae[13]. A new neo-clerodane diterpenoid, 11-episcutecolumnin C, has been isolated from the acetone extract of the aerial parts of *Scutellaria columnae* var. *columnae*, in addition to the previously known diterpenes, 11-episcutecyprin, scutegalin D, scutecolumnin C, scutecyprol B, scutaltisin and the iridoid glycoside globularin[14]. The diterpenoid fraction occurring in the acetone extract of the aerial parts of *Scutellaria parvula* has been investigated and only one neoclerodane diterpenoid, scuteparvin was isolated[6]. Two new iridoid glycosides, scalbidoside and albidoside were isolated from the methanolic extract of the aerial parts of *Scutellaria albida* subsp. colchica along with the known iridoid glucosides catalpol, globularin, scutellarioside II and mussaenosidic acid[15]. A new neo-clerodane diterpenoid, scuteselerin has been isolated from the aerial parts of *Scutellaria seleriana*, besides the known flavone oroxylin A. The structure of the new diterpenoid was established by spectroscopic methods[16]. A new neo-clerodane diterpenoid, scutorientalin D, was isolated from the acetone extract of the aerial parts of *Scutellaria orientalis* subsp. *pinnatifida*[17]. Two new neo-clerodane diterpenoids, scutalpins N and O, have been isolated from *Scutellaria alpina*, together with six previously known neo-clerodanes[18]. An acetone extract of the aerial parts of *Scutellaria orientalis* subsp. *pinnatifida* provide a new neoclerodane, scutorientalin E, whose structure was established by spectroscopic means and by comparison with related compounds[19]. Three neo-clerodane diterpenoids, named barbatins A-C and the neo-clerodane diterpenoid nicotinyl ester, named scutebarbatine B, were isolated from the whole plant of *Scutellaria barbata* D.Don[20]. Three new diterpenoids, scutelaterins A-C, have been isolated from *Scutellaria lateriflora*. In addition, the already known neo-clerodanes ajugapitin and scutecyprol A were also found[21]. Two new phenethyl alcohol glycosides, darendoside A and B were isolated from the methanolic extract of the aerial parts of *Scutellaria orientalis* subsp. pinnatifida, along with 4 known glycosides, syringin, martynoside, leucosceptoside A and verbascoside[22]. A new flavonoid glycoside, viscidulin III-2i-O-β-D-glucoside and three known flavones, 5,7,2i,5i-tetrahydroxyflavone, (-)-eriodictyol and rivularin were isolated from the roots of *Scutellaria baicalensis* fort he first time[23]. A new neo-clerodane, 11-episcutecyprin has been isolated from the aerial parts of *Scutellaria columnae* var. *columnae*, in addition to the known diterpene scutegalin D[24]. Three neo-clerodane diterpenoids, jodrellin A, jodrellin B and clerodin have been isolated from *Scutellaria* spp. and assayed for antifungal activity[25]. Four new flavonoids were isolated from the root of *Scutellaria rivularis* WALL, together with 7-O-β-D-glucuronopyranosides of baicalein, wogonin, carthamidin and isocarthamidin[26]. Four new flavanones and a new chalcone were isolated from the root of *Scutellaria discolour* COLEBR., together with wogonin, norwogonin, 5,7,2i-trihydroxy-8-methoxyflavone, 5,7-dihydroxy-8,2i-dimethoxyflavone and wogonin 7-O-glucuronide[27]. From the root of *Scutellaria*

scandens BUCH.-HAM. ex D.Don, five new flavanones were isolated, together with oroxylin A, dihydrooroxylin A, wogonin, chrysin, baicalein, dihydrobaicalein, norwogonin, wogonin 7-O-glucuronide, chrysin 7-O-glucuronide, baicalin and dihydrobaicalin[28].

In search for new neo-clerodane diterpenoids in *Scutellaria* plants, *Scutellaria orientalis* L. subsp. *porphyrostegia* Edmondson grown near the city of Siirt, Turkey, has been investigated.

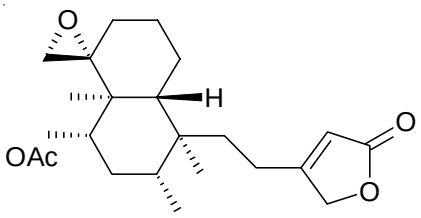
EXPERIMENTAL

Plant materials of *Scutellaria orientalis* L. subsp. *porphyrostegia* Edmondson were collected in July, 2005 near Siirt, Turkey.

Extraction and isolation of the diterpenoids: Dried and finely powdered aerial parts of *Scutellaria orientalis* L. subsp. *porphyrostegia* Edmondson (450 g) were extracted with Me_2CO (3 × 5 L) at room temperature for 1 week. After filtration, the solvent was evaporated *in vacuo* to dryness, yielding a residue (3 g), which was subjected to column (silica gel 60, 70-230 mesh, E. Merck) and eluted with a petrol ether-EtOAc. The fractions eluted with petrol ether-EtOAc (7:3) gave Ajugarin V(**1**) (32 mg) which gave only one spot on thin layer chromatography (TLC, 60 F_{254}, Merck) with several eluents. Elution with petrol ether-EtOAc (9:1) gave crude (**2**) (28 mg) which was recrystallized from ethanol to yield pure scutenisin (21 mg).

RESULTS AND DISCUSSION

Repeated chromatography of an acetone extract of the aerial parts of *Scutellaria orientalis* L. subsp. *porphyrostegia* Edmondson provided a neo-clerodane diterpenoid, Ajugarin V(**1**), which previously isolated from *Ajuga remota*[29] but it has been isolated from *Scutellaria orientalis* L. subsp. *porphyrostegia* Edmondson for the first time.



Structure of ajugarin V(**1**)

Compound **1** was isolated as white needles, m.p. 217-218 ºC and its high-resolution MS showed the m.f. of compound **1** to be $C_{22}H_{32}O_5$. The MS exhibited a molecular ion at M^+ 382.2445; Calcd. 382.2438; IR (KBr, ν_{max}, cm^{-1}): 2960, 1730 (C=O), 1450, 1375, 1155, 1025 (C-O-C).

Ajugarin V ($C_{22}H_{32}O_5$) showed IR absorptions consistent with the presence of ester and ether groups. The ^1H NMR spectrum showed signals of two tertiary methyl

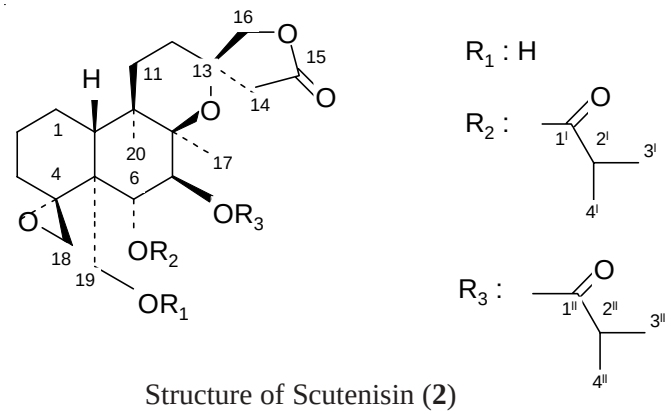
groups (δ 0.84, 3H, s and δ 0.96, 3H, d), a secondary methyl group (δ 1.10, 3H, d), two protons of a methylene epoxide (δ 3.14, 1H, dd and δ 2.32, 1H, d) and a proton (δ 5.12, 1H, dd) geminal to an esterified secondary hydroxy group. These data were similar to those of Ajugarin V, isolated from *Ajuga remota*. The ^{13}C NMR shows the presence of 3 methyl groups at δ 14.13, 15.54 and 18.80 ppm, one of which is assigned to the secondary methyl group (Table-1), this conclusion supported by the ^{1}H NMR signals appearing at δ 0.96 ppm (3H, d) and the others to the tertiary methyl groups at δ 0.84 (3H, s) and δ 1.10 ppm (3H, d).

TABLE-1
^{1}H AND ^{13}C NMR SPECTRAL DATA OF COMPOUND **1**

H	δ ppm	H	δ ppm	C	δ ppm	C	δ ppm
1	1.65 s	12	2.22 m	1	20.81 t	11	35.08 t
2	1.27 s	14	5.90 m	2	24.79 t	12	20.81 t
3	1.57 s	16	4.72 d	3	31.93 t	13	170.22 s
6	5.12 m	17	1.10 d	4	66.50 s	14	-
7	-	18	3.14 m	5	41.73 s	15	175.51 s
8	1.91 s		2.32 d	6	73.20 d	16	73.20 t
10	1.98 m	19	0.84 s	7	31.93 t	17	15.54 q
11	1.27 s	20	0.96 s	8	35.08 d	18	51.83 t
	1.44 s			9	41.73 s	19	14.13 q
				10	-	20	18.80 q

The neoclerodane diterpenoids synthesized by species of *Ajuga* and *Scutellaria* always show the oxigenated heterocyclic system. All of the findings confirm that the genera *Scutellaria* and *Ajuga* are closely related taxonomically[6,16].

Repeated chromatography of an acetone extract of the aerial parts of *Scutellaria orientalis* L. subsp. *porphyrostegia* Edmondson provided a neo-clerodane diterpenoid, Scutenisin (**2**), which previously isolated from *Scutellaria orientalis* subsp. *sintenisii*[7] but it has been isolated from *Scutellaria orientalis* L. subsp. *porphyrostegia* Edmondson for the first time.



Structure of Scutenisin (**2**)

Compound **2** was isolated as yellow needles, m.p. 249-251 ºC and its high-resolution MS showed the molecular formula of **2** to be $C_{28}H_{42}O_9$. IR (KBr, ν_{max}, cm^{-1}): 3430 (OH), 2920 and 2850 (C-H *str.*), 1733 (C=O *str.*), 1654 (α-β-unsaturated-δ-lactone), 1272 (epoxide), 1026 (C-O-C symetric *str.*).

Scutenisin ($C_{28}H_{42}O_9$) showed IR absorptions consistent with the presence of hydroxyl and ester groups (Table-2). The ^1H NMR spectrum showed signals of a free hydroxyl group at the C-19 position. The NMR data indicated the presence of methyl groups and a free hydroxyl group together with characteristic signals of a neo-clerodane diterpene like other neo-clerodane derivates previously isolated from Scutellaria plants (Table-2).

TABLE-2
^1H AND ^{13}C NMR SPECTRAL DATA OF COMPOUND **2**

H	δ ppm	H	δ ppm	H	δ ppm
1α	1.71	Me-17	1.18 s	1	22.70 t
1β	1.61	18	2.25 d	2	29.67 t
2α	1.99 m	19-OH	2.29 m	3	31.93 t
2β	1.47	Me-20	0.86 s	12	29.37 t
3β	1.11	Me-3', Me-3"	1.15 d, 1.11 d	13	76.70 s
10β	2.12 dd	Me-4', Me-4"	1.13 d, 1.04 d	16	77.33 t
14	2.53 d			20	14.13 q

Conclusion

In conclusion, during the systematic phytochemical investigations on plants of the family Labiatae, *Scutellaria orientalis* L. subsp. *porphyrostegia* Edmondson, an endemic species in Turkey has been examined. The acetone extracts of the aerial parts of this plant yielded a neo-clerodane diterpenoids, Ajugarin V and scutenisin, whose structures were elucidated by means of spectroscopic methods.

ACKNOWLEDGEMENTS

The authors are grateful for financial support by The Scientific and Technical Research Council of Turkey (Project no: 106T228). Thanks are also due to Dr. Mehmet Cicek for plant material.

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(*Received*: 17 April 2008; *Accepted*: 20 November 2008) AJC-7052