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Guaiasistanol: A new guaiane sesquiterpenoid from *Teucrium persicum* Boiss

A. Miri[1,2] and J. Sharifi-Rad[1,2]✉

[1] Zabol Medicinal Plants Research Center, Zabol University of Medical Sciences, Zabol 61615-585, Iran
[2] Department of Pharmacognosy, Faculty of Pharmacy, Zabol University of Medical Sciences, Zabol 61615-585, Iran

Corresponding author: Javad Sharifi-Rad, Zabol Medicinal Plants Research Center, Zabol University of Medical Sciences, Zabol 61615-585, Iran. E-mail: javad.sharifirad@gmail.com

Abstract

In this study, a new guaiane type skeleton sesquiterpenoid named guaiasistanol (6α, 10α-epoxy-4α-hydroxyguaiane) was isolated from chloroform part of the *Teucrium persicum* extract, also chrysothol. Spectroscopic means explained the structures. This compound was evaluated for inhibitory activity against acetylcholinesterase and it showed a moderate activity with 28% inhibition.

Key words: *Teucrium persicum* Boiss., guaiane sesquiterpenes, chrysothol, guaiasistanol, acetylcholinesterase inhibition.

Introduction

The genus of *Teucrium* belongs with Labiatae family. *T. persicum* is an endemic plant of Iran; its local name is "Marv-e-talkh" and traditionally used for treatment of abdominal pains and headaches (1). *Teucrium* species are used in folk medicines for centuries as anti-diabetes, obesity, hyperlipidemia, inflammation, and rheumatoid (2,3). The search of biologically active component of plants has always been great interest to researcher (4–9). Several studies about the components of *Labiatae* species were carried out, and many of them showed the presence of guaiane sesquiterpenes but to date, nothing has been reported on the isolation of three-cycle ring guaian skeleton type from *Teucrium* (10). This study was carried out to determine the constituents of chloroform fracting methanolic extract of the plant.

Materials and methods

General

1H-NMR and 13C-NMR spectra were measured on Bruker 500 MHz spectrometer and chemical shifts expressed as δ relative to TMS as internal standard. Mass spectra were measured with Agilent 5973, EI 70 eV. The infra-red spectra were got on Shimadzu 470 spectrophotometer (Shimadzu Corporation, Tokyo, Japan). Optical rotation was carried out on Perkin Elmer 241 polarimeter at 25 °C.

Plant Material

Aerial parts of plant were collected in September 2009, at full flowering stage, on Lar Mountain, 1200 m altitude, Iran. Voucher specimen (No. 397) has been deposited at central Herbarium of Medicinal Plants in Iran.

Extraction and Isolation

Air-dried chopped plant material (1000 g) was extracted with 85% methanol. The extract was concentrated by rotaryavaporator. The residue (125 g) was partitioned with water and chloroform (85 g residue). The $CHCl_3$ extract was chromatographed on a column (7 × 30 cm) of silica gel 35–70, eluted with hexane and EtOAc, in gradient method, 200 mL each fraction. Fraction 23 (250 mg) was purified by CC (1.6 × 35 cm) on silica gel G60 230–400 Eluted with hexane-EtOAc gradient method, 5 mL each fraction, to give 25 fractions. Fractions 12 to 19 (230 mg) were combined according to their TLC profiles and further purified by PTLC (mobile phase: Hex: EtOAc (2.5:4.5), Merck Kieselgel 60 F254, 500 μ film thickness, reagent: anisaldehyde) to afford compounds 1 and 2. Band compounds 1 and 2 were further purified four times on sephadex LH-20 column, employing 100% MeOH, to give compounds 1 and 2 (50, 70 mg and oily residue, respectively).

Bioassay of AChE Inhibitory Activity

The AChE inhibitory activity of this compound was examined by the spectrophotometric method (11,12). Acetylthiocholine iodide (Sigma, St. Louis, MO, USA) was used as substrate in the test. The compound was dissolved in 5% dimethyl sulfoxide (DMSO). The reaction mixture, consisting of 110 μL phosphate buffer (pH = 8), 10 μL of tested compound solution (2000 $\mu mol \cdot L^{-1}$), and 40 μL AChE solution (0.04 U/100 μL), was mixed and incubated for 15 min at 30 °C. The reaction was initiated by the addition of 20 μL 5,5-dithiobis-2-nitrobenzoic acid (6.25 $mmol \cdot L^{-1}$) and 20 μL acetylthiocholine. The hydrolysis of acetylthiocholine was monitored at 405 nm after 35 min. Tacrine (Sigma-Aldrich Corp., St. Louis, Mo., USA) was used as positive control. All the reactions were done in triplicate. The percentage inhibition was calculated as follows: % inhibition = (E−S)/E × 100 (E is the activity of the enzyme without test compound and S is the activity of enzyme with test compound).

Results and Discussion

Chrysothol, Oily Residue, $C_{15}H_{26}O_2$, ^{1}H-NMR and ^{13}C-NMR Spectral Data of Chrysothol

Chrysothol, oily residue, $C_{15}H_{26}O_2$, ^{1}H-NMR and

^{13}C-NMR spectral data of chrysothol in $CDCl_3$ are shown in Table 1. HSQC(F1, F2): (δ, δ)(20.16–1.77), (21.1–0.9), (20.21–0.92), (21.9–1.15), (23.8–1.48), (25.7–1.38), (32.6–1.67), (37.5–1.7, 1.4), (38.4–1.36), (48.1–2.14, 2.07), (53.17–2.3), (68.1–2.3), (74.4– -), (74.5– -), (75.9–3.99). EIMS: *m/z* 238 $[M]^+$, 223 $[M-CH_3]^+$, 220 $[M-H_2O]^+$, 195 $[M-C_3H_7]^+$. Peak match: *m/z* 238.19 (calcd. *m/z* 238.1932 for $C_{15}H_{26}O_2$). IR ν_{max} ($CHCl_3$) cm^{-1}: 3410 (OH).

Guaiasistanol, Oily Residue, $C_{15}H_{26}O_2$, 1D and 2D ^{1}H-NMR, ^{13}C-NMR Spectral Data in $CDCl_3$ and ^{13}C-NMR in DMSO-d_6

Guaiasistanol, oily residue, $C_{15}H_{26}O_2$, 1D and 2D ^{1}H-NMR, ^{13}C-NMR spectral data in $CDCl_3$ and ^{13}C-NMR in DMSO-d6 is shown in Table 2. HSQC (F1, F2): (δ, δ) (16. 61–1.37), (16.67–1.31), (20.7–0.93), (20.87–0.93), (22.87–1.43, 1.83), (24.65–1.49, 2.23), (31.97–1.64), (34.44–1.14, 2.08), (41–2.23, 0.95), (49.57–0.85), (60.03–2.99), (68.24–2.86) and (70.31–3.64). EIMS: *m/z* 237.2 $[M-H]^+$, 223 $[M-CH_3]^+$, 220 $[M-H_2O]^+$, 195 $[M-C_3H_7]^+$, (calcd. *m/z* 238.1932 for $C_{15}H_{26}O_2$). IR ν_{max} ($CHCl_3$) cm^{-1}: 3442 (OH).

Purified Compounds by Analysis of Their Spectral Data

The purified compounds were identified as guaian type skeleton by analysis of their spectral data and supported by comparison with paper. Compound 1 (Figure 1), oily and colorless, $C_{15}H_{26}O_2$, was recognized as chrysothol, previously isolate from *Salvia bucharica*, *Cleome droserfolia* (Forssk.) Del, *Chrysothamnus viscidiflorus* (13–15). Compound 2 (Figure 1) was isolated as colorless oily compound with $[\alpha]_D^{25}$ +13.0 ($CHCl_3$, C = 0.24). The molecular formula, $C_{15}H_{26}O_2$, was established by EI-MS (*m/z* 238 $[M]^+$) and 13C-NMR data, DEPT90 and 135 which corresponds to three unsaturation, and the absence of any types of double bond was showed that compound 2 is corporate a three cyclic ring system. The presence of three carbon atoms connected to oxygen (δ 58.92, 68.72 and 59.39), 13C-NMR data, and one hydroxyl group, peak 3442 cm^{-1} in IR spectrum, and only two oxygen atoms suggested an epoxide ring. The EI-MS spectrum showed, fragments at 237.2$[M-H]^+$, 219.2$[M-H_2O]^+$, 196.2[M−isopropyl]$^+$.

The ^{13}C-NMR and DEPT experiments spectra data in $CDCl_3$ and DMSO-d6 (Table 2) showed 15 carbon atoms of molecule, including four methyl groups, four methy-

Table 1. ^{13}C- and ^{1}H-NMR data (125 MHz, 500 MHz, respectively, δ-values, in $CDCl_3$) of compound 1.

Position No.	CH_n	C-NMR			H-NMR
		All signals	DEPT		
			DEPT-90	DEPT-135	
1	CH	53.17	53.17	PS*	2.32
2	CH_2	23.88	-	NS§	1.51
3	CH_2	48.12	-	NS	2.14, 2.07
4	C	74.58	-	PS	-
5	CH	68.1	68.06	PS	2.32
6	CH	75.94	75.94	PS	3.99 (1H, br.dd, *J* = 1.5Hz)
7	CH	38.4	38.5	PS	1.39 (br.dd, *J* = 9.1, 3.1 Hz)
8	CH_2	20.16	-	NS	1.77
9	CH_2	37.51	-	NS	1.72, 1.4
10	C	74.4	-	PS	-
11	CH	32.62	32.62	PS	1.67
12	CH_3	21.1	-	PS	0.93 (3H, d, *J* = 6.7 Hz)
13	CH_3	20.21	-	PS	0.92 (3H, d, *J* = 6.7 Hz)
14	CH_3	21.91	-	PS	1.15 (3H, s)
15	CH_3	25.7	-	PS	1.38 (3H, s)

*PS: Positive signal; §NP: Negative signal.

Table 2. ^{13}C- and ^{1}H-NMR data (125 MHz, 500 MHz, respectively, δ-values) of compound 2.

Position No.	CH_n	in $CDCl_3$				in DMSO-d_6		
		H-NMR	C-NMR			C-NMR		
			All signals	DEPT		All signals	DEPT	
				DEPT-90	DEPT-135		DEPT-90	DEPT-135
1	CH	2.99	60.03	PS*	PS	59.09	PS	PS
2	CH_2	1.49(2.23)	24.65	-	NS§	24.19	-	NS
3	CH_2	1.14(2.08)	34.44	-	NS	34.16	-	NS
4	C	-	-	-	-	58.92	-	PS
5	CH	2.86(1H, d, *J* = 8.25 Hz)	68.24	PS	PS	67.69	PS	PS
6	CH	3.64(1H, d, *J* = 8.25 Hz)	70.31	PS	PS	68.72	PS	PS
7	CH	0.85(1H)	49.57	PS	PS	48.95	PS	PS
8	CH_2	1.43(1.83)	22.87	-	NS	22.65	-	NS
9	CH_2	0.95(2.23)	41	-	NS	40.60	-	NS
10	C	-	-	-	-	59.39	-	PS
11	CH	1.64	31.97	PS	PS	31.52	PS	PS
12	CH_3	0.9 (3H)	20.87	PS	PS	20.53	PS	PS
13	CH_3	0.93(3H)	20.72	PS	PS	20.53	PS	PS
14	CH_3	1.37(3H, s)	16.61	PS	PS	16.43	PS	PS
15	CH_3	1.31(3H, s)	16.67	PS	PS	16.52	PS	PS

*PS: Positive signal; §NP: Negative signal.

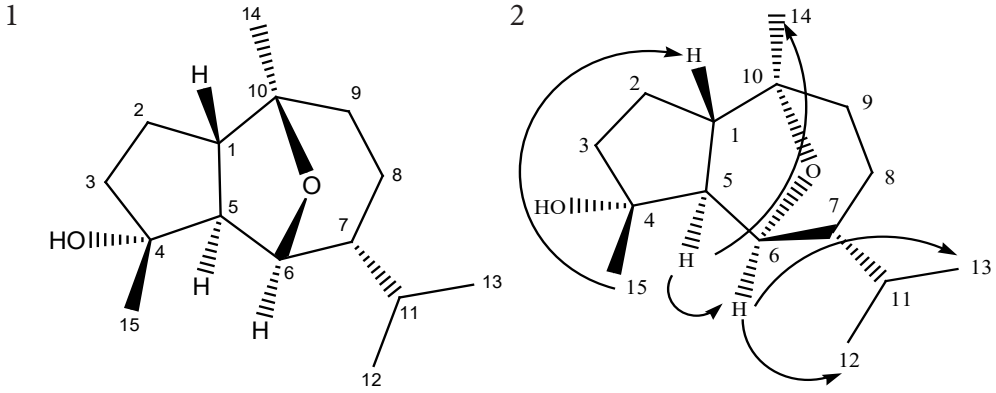

Figure 1. Structures of the isolated compounds 1 and 2.

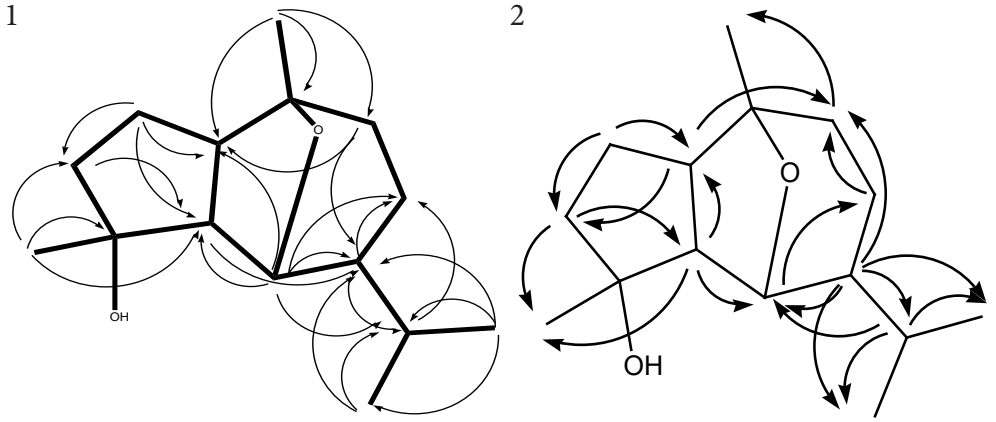

Figure 2. Long range correlations observed in the HMBC spectra of compounds 1 and 2.

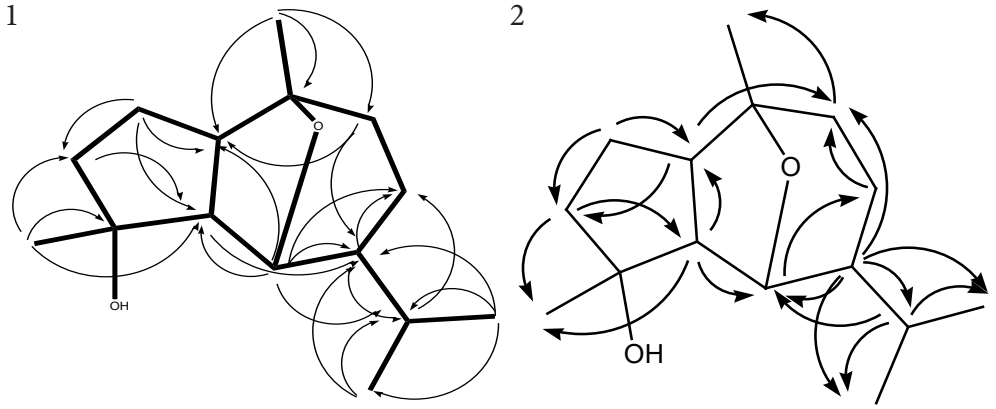

Figure 3. Long range correlations observed in the HMBC spectra of compounds 1 and 2.

lenes, five methines and two quaternary carbons.[1]H-NMR confirmed above EI-MS and [13]C-NMR data with two methyl signals (δ 0.93, 0.90), two tertiary methyl signals at 1.37 (Me-14) and 1.31 (Me-15), and a doublet at δ 3.64 (J = 8.25 Hz).

The other protons and carbons were determined by 2D experiments (HMQC and HMBC). The assignments of all protons signals and their connectivity to adjacent protons and carbons signals were established from the results of the 2D HH-COSY (Figure 2), HMBC (Figure 3) and NOE.

Interpretation of all compound 2 spectra were showed a guaian skeleton similar to compound 1 structure, therefore difference of these two compounds must be stereochemistry. Stereochemistry of compound 2 was determined on the NOE experiment and biosynthesis route. The H1 proton showed a cross peak with H14. The H6 proton showed, cross peaks with H13, H12 and H5, also clear NOE correlations were observed among H5 with H6 and H15. Therefore, H5, H6, H12 and H15 were on the same face of the molecule and H1 and H14 were the same orientation. The biogenetic route of guaian skeleton suggested the presence of intermediate carbocation ion that it can attack from any face of molecule, thus Oxygen Bridge can be any configuration α or β, and in bibliographical search, we control any possible structure and related configurations. Optical rotation of this compound is the same as compound 1, therefore, the oxygenated bridge will be α, finally compound 2 was identified to be 6α, 10α-epoxy-4α-hydroxy guaiane, named guaiasistanol (Figure 1).

Conclusions

The chemical investigation of *Teucrium persicum* led to the isolation of a new guaiane type skeleton sesquiterpenoid named guaiasistanol (6α, 10α-epoxy-4α-hydroxyguaiane; *1S,2S,3R,6S,7R,10R)-3,7-dimethyl-*

10-(propan-2-yl)-11-oxatricyclo[5.3.1.0^{2,6}]undecan-3-o). This compound was isolated for the first time from Teucrium genus. Evaluation of the inhibitory activity against acetylcholinesterase of the new compound showed that it exhibited a moderate activity with 28% inhibition.

References

1. Miri, A., Monsef-Esfahani, H.R., Amini, M., Amanzadeh, Y., Hadjiakhoondi, A. and Hajiaghaee, R., Determination of Phenolics and Flavonoid Contents, Antioxidant Capacity and Major Flavonoids Structure in *Teucrium perscicum* Boiss. *J. Anim. Vet. Adv.* 2011, **10**:1258-1261. doi: 10.3923/javaa.2011.1258.1261.

2. Masoudi, S., Aghajani, Z., Rustaiyan, A., Feizbakhsh, A. and Kakhky, A.M., Volatile constituents of *Teucrium persicum* Boiss., *Thymus caucasicus* Willd. ex Ronniger subsp. Grossheimii (Ronniger) Jalas. and *Marrubium crassidens* Boiss. Three Labiatae herbs growing wild in Iran. *J. Essent. Oil Res*. 2009, **21**:5–7. doi: 10.1080/10412905.2009.9700093.

3. Miri, A., Monsef-Esfahani, H.R., Amini, M., Amanzadeh, Y., Hadjiakhoondi, A., Hajiaghaee, R. and Ebrahimi, A., Comparative Chemical Composition and Antioxidant Properties of the Essential Oils and Aromatic Water from *Teucrium persicum* Boiss. *Iran. J. Pharma. Res.* 2012, **11**:573–581.

4. Rad, J.S., Alfatemi, S.M.H. and Rad, M.S., *In vitro* assessment of antibacterial activity of *Salicornia herbacea* L. seed extracts against multidrug resistant gram-positive and gram-negative bacteria. *Int. J. Biosci.* 2014, **4**:217–222.

5. Sharifi-Rad, J., Miri, A., Hoseini-Alfatemi, S.M., Sharifi-Rad, M., Setzer, W.N. and Hadjiakhoondi, A., Chemical Composition and Biological Activity of *Pulicaria vulgaris* Essential Oil from Iran. *Nat. Prod. Commun*. 2014, **9**:1633–1636.

6. Rad, J.S., Alfatemi, S.M.H., Rad, M.S. and Iriti, M., *In-vitro* antioxidant and antibacterial activities of *Xanthium strumarium* L. extracts on methicillin-susceptible and methicillin-resistant *Staphylococcus aureus*. *Ancient Sci. Life* 2013, **33**:107–111. doi: 10.4103/0257-7941.139050.

7. Sharifi-Rad, J., Hoseini-Alfatemi, S.M., Sharifi-Rad, M. and Setzer, W.N., Chemical composition, antifungal and antibacterial activities of essential oil from *Lallemantia royleana* (Benth. In Wall.) Benth. *J. Food Safety* 2015, 35(1):19-25. doi:10.1111/jfs.12139.

8. Sharifi-Rad, J., Sharifi-Rad, M., Hoseini-Alfatemi, S.M. Iriti, M., Sharifi-Rad, M. and Sharifi-Rad, M., Composition, Cytotoxic and Antimicrobial Activities of *Satureja intermedia* C.A.Mey Essential Oil. *Int. J. Mol. Sci.* 2015, **16(8)**:17812-17825. doi: 10.3390/ijms160817812.

9. Sharifi-Rad, J., Hoseini-Alfatemi, S.M., Sharifi-Rad, M., Sharifi-Rad, M., Iriti, M., Sharifi-Rad, M., Sharifi-Rad, R. and Raeisi, S., Phytochemical Compositions and Biological Activities of Essential Oil from *Xanthium strumarium* L.. *Molecules* 2015, **20(4)**:7034-7047. doi: 10.3390/molecules20047034.

10. Bruno, M., Torre, M.C.D.L., Rodríguez, B. and Omar, A.A., Guaiane sesquiterpenes from *Teucrium leucocladum*. *Phytochemistry* 1993, **34**:245–247. doi: 10.1016/S0031-9422(00)90812-4.

11. Zhang, S.S., Ma, Q.Y., Zou, X.S., Dai, H.F., Huang, S.Z., Luo, Y., Yu, Z.F., Luo, H.R. and Zhao, Y.X., Chemical constituents and their *in vitro* acetylcholinesterase inhibitory activities from the fungus *Amauroderma amoiensis*. *Planta Med*. 2013, **79**:87-91.

12. Adewusi, E.A., Fouche, G. and Steenkamp, V., Cytotoxicity and acetylcholinesterase inhibitory activity of an isolated crinine alkaloid from *Boophane disticha* (Amaryllidaceae). *J. Ethnopharmacol.* 2012, **143**:572-578. doi: 10.1016/j.jep.2012.07.011.

13. Ahmad, V., Zahid, M., Ali, M., Jassbi, A., Abbas, M., Ali, Z. and Iqbal, M., Bucharioside and buchariol from *Salvia bucharica*. *Phytochemistry* 1999, **52**:1319-1322. doi: 10.1016/S0031-9422(99)00389-1.

14. Ahmed, A.A., Hegazy, M.E.F., Hassan, N.M., Wojcinska, M., Karchesy, J., Pare, P.W., Mabry, TJ., Constituents of *Chrysothamnus viscidiflorus*. *Phytochemistry* 2006, **67**:1547-1553. doi: 10.1016/j.phytochem.2006.03.021.

15. El-Askary, H.I., Terpenoids from *Cleome droserifolia* (Forssk.) Del. *Molecules* 2005, **10**: 971-977. doi: 10.3390/10080971.