PERGAMON

Phytochemistry 58 (2001) 1149–1152

PHYTOCHEMISTRY

www.elsevier.com/locate/phytochem

Monoterpene glucosides from *Origanum syriacum*

M.S. Kamel[a,*], M.H. Assaf[a], H.A. Hasanean[a], K. Ohtani[b], R. Kasai[b], K. Yamasaki[b]

[a]*Department of Pharmacognosy, Faculty of Pharmacy, Assiut University, Assiut 71526, Egypt*
[b]*Institute of Pharmaceutical Sciences, Hiroshima University School of Medicine, 1-2-3 Kasumi, Minami-Ku, Hiroshima 734, Japan*

Received 19 June 2001; received in revised form 14 August 2001

Abstract

From the aerial parts of *Origanum syriacum*, three new monoterpene glucosides thymoquinol 2,5-*O*-β-diglucopyranoside (**3**), carvacrol 2-*O*-β-glucopyranosyl-(1→2)-β-glucopyranoside (**4**) and *p*-menth-1-ene-3,4-diol 4-*O*-β-glucopyranoside (**5**) together with two known monoterpene glucosides thymoquinol 2-*O*-β-glucopyranoside (**1**), thymoquinol 5-*O*-β-glucopyranoside (**2**) have been isolated. The structures of the isolated compounds were verified by means of MS and NMR spectral analyses.

Keywords: *Origanum syriacum*; Lamiaceae; Thymoquinol glucosides; Carvacrol glucoside

1. Introduction

Origanum syriacum L. (Lamiaceae) is an aromatic, herbaceous and perennial plant growing wild in the Sinai Desert of Egypt (Tackholm, 1974). The essential oils and the constituents of many *Origanum* species have been intensively studied (Sendra et al., 1980; Ravid et al., 1983; Akgul et al., 1987; Harvala et al., 1987; Halim et al., 1991; Afsharypuor et al., 1997). In folk medicine, *Origanum* species are used as powerful disinfectants, flavouring agents, in perfumes and in scenting soaps (Gunther, 1949; Chiej, 1984; Kotb, 1985). It was reported that thymol and carvacrol represent the major constituents of the essential oils of *Origanum* species (Sarer et al., 1982). From Egyptian majorana (*Origanum vulgare*), arbutin and methyl arbutin have been already isolated and quantitatively estimated (Assaf et al., 1987). The present study deals with isolation and assignment of three new and two known monoterpene glucosides from the aerial parts of *Origanum syriacum* L.

2. Results and discussion

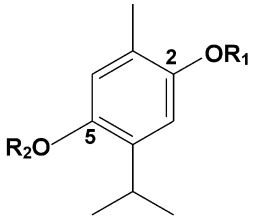
The ethanolic extract of the aerial parts of *O. syriacum* was deffated with CH_2Cl_2 and the aqueous layer was subjected to a column chromatography of Diaion HP-20. The methanol eluate was repeatedly chromatographed on columns of silica gel and then by MPLC and HPLC to afford 5 glycosides.

The structure of compound **1** was determined as thymoquinol 2-*O*-β-glucopyranoside while that of **2** was identified as thymoquinol 5-*O*-β-glucopyranoside previously isolated from *Schisandrae chinensis* (Yahara et al., 1993). The enzymatic hydrolysis of compounds **1** and **2** produced thymoquinol as aglycone (**1a**). In general, this is the first report for isolation of compounds **1** and **2** from family lamiaceae. The molecular formula of compound **3** was deduced as $C_{22}H_{34}O_{12}$ from HR FAB-MS spectrometry (see Experimental). The enzymatic hydrolysis of **3** gave also the aglycone **1a**. ^{13}C NMR spectrum (Table 1) and DEPT experiment of **3** displayed the presence of two unsubstituted β-glucopyranosyl units from the signals at δ 104.0 (C-1, 1′); 75.1, 75.0 (C-2, 2′); 78.6 (C-3, 3′); 71.3, 71.4 (C-4, 4′); 78.7 (C-5, 5′) and 62.4 (C-6, 6′) (Bradbury et al., 1984) together with 10 carbon signals for the aglycone as follows: 3 methyl groups (δ 16.4, 23.3 and 23.2), 3 methines (δ 119.1, 114.9 and 26.8) and 4 quaternary carbons (δ 152.3, 150.9, 137.0 and 126.2). Comparison of the ^{13}C NMR data of **3** with those of **1**, **2** and **1a** (Table 1) revealed that compound **3** is a bisdesmoside and its aglycone is 2,5-disubstituted thymoquinol. From ^{1}H NMR spectrum of **3**, the two singlets at δ 7.60 and 7.50 were assigned for H-3 and H-6, respectively (Metwally et al., 1986). The one proton

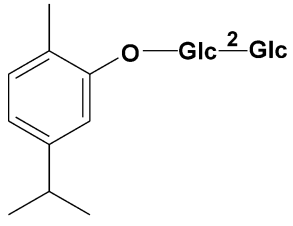
* Corresponding author. Tel.: +20-088-332-196; fax: +20-088-332-776.
E-mail address: mkamel@mailcity.com (M.S. Kamel).

0031-9422/01/$ - see front matter
PII: S0031-9422(01)00386-7

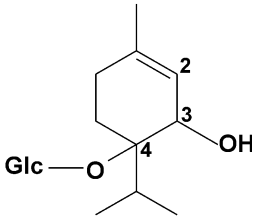
multiplet at δ 3.80 together with two methyl doublets at δ 1.26 and 1.21 were obvious for H-8, 9 and 10, respectively and the methyl singlet at δ 2.40 was assigned for H-7 (Laswell et al., 1977). The β-configuration of the glucopyranosyl units was deduced from the coupling constants (6.8, 7.3 Hz) of the anomeric proton doublets at δ 5.46 and 5.47, respectively in the ^{1}H NMR spectrum of **3**. The negative FAB-MS spectrum of 3 exhibited M^+ at m/z 489 $[M–H]^-$ as well as significant peaks at m/z 327 $[M–H\text{-glucose}]^-$ and 165 $[\text{glucose}]^-$. Consequently the structure of compound 3 was assigned as thymoquinol 2,5-*O*-β-diglucopyranoside.



	R1	R2
(1)	Glc	H
(1a)	H	H
(2)	H	Glc
(3)	Glc	Glc



(4)



(5)

The molecular formula of compound **4** was deduced as $C_{22}H_{34}O_{11}$ from HR FAB–MS spectrometry (see Experimental). ^{13}C NMR spectrum (Table 1) and DEPT experiment of **4** displayed the presence of one unsubstituted β-glucopyranosyl unit from the signals at δ 106.5 (C-1′), 76.8 (C-2′), 78.6 (C-3′), 71.3 (C-4′) 78.7 (C-5′) and 62.4 (C-6′), one substituted β-glucopyranosyl unit from the signals at δ 100.1 (C-1), 83.4 (C-2), 78.1 (C-3), 71.1 (C-4), 78.3 (C-5) and 62.2 (C-6) (Bradbury et al., 1984) together with 10 carbon signals for the aglycone as follows: 3 methyl groups (δ 16.3, 24.3 and 24.0), 4 methines (δ 130.7, 119.8, 112.8 and 34.2) and 3 quaternary carbons (δ 156.4, 147.9 and 124.9). ^{1}H NMR spectrum of **4** revealed the presence of one methyl singlet (δ 2.5) and two methyl doublets (δ 1.15 and 1.16) assignable for Me-7, 9 and 10, respectively. Moreover, the two doublet signals at δ 5.5 and 5.6 ($J = 7.6$ and 7.2 Hz, respectively) deduced the β-configuration of the glucopyranosyl units. ^{13}C NMR signals of the aglycone were similar to those reported for carvacrol (Zechmeister, 1979). The downfield shift of C-2 (δ 83.4) of the inner glucopyranosyl residue indicated its substitution with the terminal glucopyranosyl unit at this position (Bradbury et al., 1984). The negative FAB–MS spectrum of **4** exhibited M^+ at m/z 473 $[M–H]^-$ as well as significant peaks at m/z 311 $[M–H\text{-glucose}]^-$ and 149 $[M–H–2\ \text{glucose}]^-$. Consequently the structure of compound **4** was assigned as carvacrol 2-*O*-β-glucopyranosyl-(1→2)-β-glucopyranoside.

Table 1
^{13}C NMR spectral data of compounds **1–5** (100 MHz,C_5D_5N)

C	1	1a[e]	2	3	4	5
1	137.2	133.8	133.6	137.0	124.9	143.4
2	148.7	148.3	150.5	150.9	156.4	123.4
3	120.1	118.4	117.6	119.1	119.8	72.8
4	122.8	122.6	126.1	126.2	147.9	79.8
5	152.0	149.6	151.9	152.3	112.8	33.1
6	112.9	113.7	115.9	114.9	130.7	25.2
7	16.4	16.4	16.2	16.4	16.3	16.9
8	26.4	27.4	27.3	26.8	34.2	34.1
9	23.5[a]	23.2	22.9[a]	23.3[a]	24.3[a]	21.4[a]
10	23.3[a]	23.2	22.8[a]	23.2[a]	24.0[a]	21.2[a]
Glc						
1	104.5		104.3	104.0	100.1	97.9
2	75.1		75.0	75.1[b]	83.4	75.3
3	78.3[b]		78.2[b]	78.6[c]	78.1[a]	78.1[b]
4	71.4		71.3	71.3[d]	71.1[b]	71.9
5	78.6[b]		78.4[b]	78.7[c]	78.3[a]	78.5[b]
6	62.4		62.3	62.4	62.2[c]	62.7
Glc′						
1′				104.0	106.5	
2′				75.0[b]	76.8	
3′				78.6[c]	78.6[a]	
4′				71.4[d]	71.3[b]	
5′				78.7[c]	78.7[a]	
6′				62.4	62.4[c]	

[a, b, c, d] Assignments may be interchangeable in each column.
[e] Data published for the first time for thymoquinol.

The molecular formula of compound **5** was deduced as $C_{16}H_{28}O_7$ from HR FAB-MS spectrometry (see Experimental). ^{13}C NMR spectrum (Table 1) and DEPT experiment of **5** displayed the presence of one β-glucopyranosyl unit from the signals at δ 97.9 (C-1), 75.3 (C-2), 78.1 (C-3), 71.9 (C-4) 78.5 (5) and 62.7 (C-6) (Bradbury et al., 1984) together with 10 carbon signals for the aglycone as follows: 3 methyl groups (δ 16.9, 21.4 and 21.2), 2 methylene groups (δ 33.1 and 25.2), 3 methines (δ 123.4, 72.8 and 34.1) and 2 quaternary carbons (δ 143.4 and 79.8). The carbon signals at δ 72.8 and 79.8 indicated the attachment of two hydroxyl groups to these carbons (Breitmaier et al., 1987). On the other hand, ^{1}H NMR spectrum of **5** revealed the presence of one methyl singlet (δ 1.5) and two methyl doublets (δ 0.87) assignable for Me-7, 9 and 10 respectively. The broad singlets at δ 5.5 and 4.7 were assigned for H-2 and 3 respectively proving the attachment of the hydroxyl

groups to C-3 and C-4 of the aglycone together with the location of the double bond between C-1 and 2. Furthermore, comparison of ^{13}C NMR spectrum of **5** with *p*-menth-1-ene-4-ol confirmed the location of the hydroxyl group at C-3 of **5** from its downfield shift to δ 72.8 (Zechmeister, 1979). Moreover, the one proton multiplet signal at δ 3.9 in the ^1H NMR spectrum of **5** was interpreted for H-8 while the four protons multiplet at δ 1.8 – 2.2 was assigned for two methylene groups (H-5 and 6). The doublet signal at δ 5.16 ($J=8$ Hz) deduced the β-configuration of the glucopyranosyl unit. The attachment of the β-glucosyl moiety to C-4 of the aglycone was based on the downfield shift of this carbon (δ 79.8) in addition to the upfield shift of the anomeric carbon of the glucosyl unit (δ 97.9) that is characteristic for the attachment of the glucosyl moiety to a tertiary alcohol (Zhou et al., 1981; Mohamed et al., 1994). In general, the absolute configuration of the chiral center C-3 could not be determined because of the location of both a double bond and a quaternary chiral center (C-4) vicinal to it. The negative FAB-MS spectrum of **5** exhibited M^+ at *m/z* 331 $[M–H]^-$ as well as a significant peak at *m/z* 169 $[M–H\text{-glucose}]^-$. Consequently the structure of compound **5** was assigned as *p*-menth-1-ene-3, 4-diol 4-*O*-β-glucopyranoside.

3. Experimental

^1H and ^{13}C NMR (TMS as int. standard): 400 MHz and 100 MHz respectively were recorded on a Jeol JNM α-400 spectrometer. FAB MS spectra were taken on a Jeol JMS-SX 102 spectrometer by direct inlet method at an ionizing voltage of 70 eV MPLC: RP-18 column (20 mm i.d. ×40 cm); flow rate of mobile phase 3 ml/min. HPLC: D-ODS-5 and polyamine columns (each 20 mm i.d. ×25 cm, YMC) with a Toyo Soda high speed chromatograph HLC-803 D pump and a Tosoh refraction index (RI-8) detector; flow rate of mobile phase 6 ml/min, injection vol. 0.8–1.0 ml. On the ODS column, 60% MeOH (I) and 55% MeOH (II) were used while 90% MeCN (III) was used on the polyamine column. CC: Kieselgel 60 (70–230 mesh, Merck) and Diaion HP 20 (Mitsubishi). TLC: silica gel 60 precoated plates F-254 and HPTLC using RP-18 precoated plates, F-254 s (Merck).

3.1. Plant material

The aerial parts (stems and leaves) of *O. syriacum* L. (Lamiaceae) were collected from Saint Kathrine, Sinai desert, Egypt in May 1997. The plant was identified by Dr. Salah El-Naggar, Department of Botany, Faculty of Science, Assiut University, Assiut, Egypt. A voucher specimen is deposited at the Herbarium of Department of Pharmacognosy, Faculty of Pharmacy, Assiut University, Assiut, Egypt.

3.2. Extraction and isolation of compounds (*1–5*)

The air-dried powdered aerial parts of *O. syriacum* (2 kg) were extracted with 70% EtOH. The dried ethanolic extract (450 g) was suspended in H_2O and deffated with CH_2Cl_2. The aq. fr. was applied to a column of Diaion HP-20 and eluted with H_2O, MeOH and acetone successively. The MeOH eluate (30 g) was chromatographed by silica gel CC using CH_2Cl_2–MeOH–H_2O (85:15:1) and finally with (70:30:3) to give 5 fractions. Fraction 2 (500 mg) was separated by MPLC on RP-18 using 65% MeOH and HPLC using ODS column and solvent I to afford compounds **1** (white powder, 90 mg), **2** (white powder, 40 mg) and **5** (white powder, 15 mg). Fraction 3 (350 mg) was subjected to HPLC using first ODS column with solvent II and then Polyamine column with solvent III to give compounds **3 (**white powder, 60 mg) and **4** (white powder, 40 mg).

3.2.1. Compound (*1*)

Thymoquinol 2-*O*-β-glucopyranoside, HR FAB-MS (negative) *m/z*: 327.1469 $[M–H]^-$ $C_{16}H_{23}O_7$ (req. 327.1444). ^{13}C NMR (C_5D_5N, Table 1). ^1H NMR (C_5D_5N): δ 7.50 (1H, *s,* H-3), 7.10 (1H, *s*, H-6), 5.40 (1H, *d*, $J=6.8$ Hz, H-1 Glc), 3.82 (1H, *m*, H-8), 2.40 (3H, *s*, Me-7), 1.21 and 1.20 (each 3H, *d*, $J=6.1$ and 6.6 Hz, respectively, Me-9 and 10).

3.2.2. Compound (*1a*)

Thymoquinol, ^{13}C NMR (C_5D_5N, Table 1). ^1H NMR (C_5D_5N): δ 7.20 (1H, *s,* H-3), 7.10 (1H, *s*, H-6), 3.70 (1H, *m*, H-8), 2.40 (3H, *s*, Me-7), 1.30 (6H, *d*, $J=7.1$ Hz, Me-9 and 10).

3.2.3. Compound (*2*)

Thymoquinol 5-*O*-β-glucopyranoside, HR FAB-MS (negative) *m/z*: 327.1419 $[M–H]^-$ $C_{16}H_{23}O_7$ (req. 327.1444). ^{13}C NMR (C_5D_5N, Table 1). ^1H NMR (C_5D_5N): δ 7.60 (1H, *s,* H-3), 7.0 (1H, *s*, H-6), 5.40 (1H, *d*, $J=6.4$ Hz, H-1 Glc), 3.60 (1H, *m*, H-8), 2.40 (3H, *s*, Me-7), 1.32 (6H, *d*, $J=6.8$ Hz, Me-9 and 10).

3.2.4. Compound (*3*)

Thymoquinol 2,5-*O*-β-diglucopyranoside, $[\alpha]_D^{25}$ −12.5° (MeOH; c 1.20). HR FAB–MS (negative) *m/z*: 489.1955 $[M–H]^-$ $C_{22}H_{33}O_{12}$ (req. 489.1972). ^{13}C NMR (C_5D_5N, Table 1). ^1H NMR (C_5D_5N): δ 7.60 (1H, *s,* H-3), 7.50 (1H, *s*, H-6), 5.47 (1H, *d*, $J=7.3$ Hz, H-1 Glc), 5.46 (1H, *d*, $J=6.8$ Hz, H-1′ Glc), 3.80 (1H, *m*, H-8), 2.40 (3H, *s*, Me-7), 1.26 and 1.21 (each 3H, *d*, $J=7.1$ Hz, Me-9 and 10).

3.2.5. Compound (*4*)

Carvacrol 2-*O*-β-glucopyranosyl-(1→2)-β-glucopyranoside, $[\alpha]_D^{25}$ −25.1° (MeOH; c 1.60). HR FAB–MS (negative) *m/z*: 473.2058 $[M–H]^-$ $C_{22}H_{33}O_{11}$ (req. 473.2023). ^{13}C NMR (C_5D_5N, Table 1). ^1H NMR

(C_5D_5N): δ 7.40 (1H, *bs,* H-3), 7.04 (1H, *d*, $J=8$ Hz, H-6), 6.76 (1H, *bd*, $J=7.6$ Hz, H-5), 5.60 (1H, *d*, $J=7.2$ Hz, H-1 Glc), 5.50 (1H, *d*, $J=7.6$ Hz, H-1′ Glc), 2.80 (1H, *m*, H-8), 2.50 (3H, *s*, Me-7), 1.16 and 1.15 (each 3H, *d*, $J=6.8$ Hz, Me-9 and 10).

3.2.6. Compound (**5**)

p-Menth-1-ene-3,4-diol 4-*O*-β-glucopyranoside, $[\alpha]_D^{25}$ −18.9° (MeOH; *c* 1.50). HR FAB–MS (negative) *m*/*z*: 331.1758 $[M-H]^-$ $C_{16}H_{27}O_7$ (req. 331.1757). ^{13}C NMR (C_5D_5N, Table 1). ^1H NMR (C_5D_5N): δ 5.50 (1H, *bs,* H-2), 5.16 (1H, *d*, $J=8.0$ Hz, H-1 Glc), 4.70 (1H, *bs*, H-3), 3.90 (1H, *m*, H-8), 1.80–2.20 (4H, *m*, H-5 and 6), 1.50 (3H, s, H-7), 0.87 (6H, *d*, $J=6.8$ Hz, Me-9 and 10).

3.2.7. Enzymatic hydrolysis of compound **1**

Compound **1** (20 mg) was dissolved in 0.5 ml MeOH and a solution of β-glucosidase (100 mg) in 20 ml water was added. The mixture was extracted with diethyl ether (3 times ×20 ml) after stirring at 37 °C for 2 days. The combined ethereal extracts were evaporated and the residue was purified by HPLC using 95% MeCN on polyamine column to afford the aglycone **1a** (6 mg). Compounds **2** and **3** have been subjected to same procedure as **1** to afford the aglycone **1a**.

Acknowledgements

The authors are grateful to the Japan Society for Promotion of Science (JSPS) and a Grant-in-Aid, Ministry of Education and Culture, Japan for the financial support of this work. Also, we would like to thank the Research Center of Molecular Medicine of the Hiroshima University School of Medicine, Japan, for NMR measurements.

References

Afsharypuor, S., Ebrahim, S., Erfan, M., 1997. Volatile constituents of *Origanum vulgare* ssp. *Viride* (Syn. *O. heracleoticum*) from Iran. Planta Medica 63, 179–180.

Akgul, A., Bayrak, A., 1987. Constituents of essential oils from *Origanum* species growing wild in Turkey. Planta Medica 53 (4), 114.

Assaf, M.H., Ali, A.A., Makboul, A.M., Beck, J.P., Anton, R., 1987. Preliminary study of phenolic glycosides from *Origanum majorana*, quantitative estimation of arbutin, cytotoxic activity of hydroquinone. Planta Medica 53 (4), 343–345.

Bradbury, H., Jenkins, J., 1984. Determination of the structures of trisaccharides by ^{13}C-NMR spectroscopy. Carbohydr. Res. 126, 125–156.

Breitmaier, E., Voelter, W., 1987. Carbon-13 NMR spectroscopy, 3rd. Edition. Verlagsgesellschaft mbH: Weinheim, Germany, D-6940.

Chiej, R., 1984. The Macdonald Encyclopedia of Medicinal Plants. Macdonald Co. Ltd, London, pp. 212–297.

Gunther, E., 1949. The Essential Oils, Vol. 3, 535. Van Nostrand Co, New York.

Halim, A.F., Mashaly, M.M., Zaghloul, A.M., Abdel-Fattah, H., De Potter, H.L., 1991. Chemical constituents of the essential oils of *Origanum syriacum* and *Stachys aegyptiaca*. International Journal of Pharmacognosy 29 (1), 1–5.

Harvala, C., Menounos, P., Argyriadou, N., 1987. Essential oil from *Origanum dictamnus*. Planta Medica 53 (4), 107–109.

Kotb, F., 1985. Medicinal plants in Libya, 632, Arab Encyclopedia House.

Laswell, W, Hufford, C., 1977. Aromatic constituents from *Uvaria chamae*. Phytochemistry 16, 1439–1441.

Metwally, M., Dawidar, A., Metwally, S., 1986. A new thymol derivative from *Pulicaria undulata*. Chemical Pharmaceutical Bulletin 34 (1), 378–379.

Mohamed, K., Ohtani, K., Kasai, R., Yamasaki, K., 1994. Dolabellane diterpene glucosides from *Chrozophora obliqua*. Phytochemistry 37, 495–500.

Ravid, U., Putievsky, E., 1983. Essential oils of *Majorana syriaca*, *Coridothymus capitatus* and *Satureja thymbra* plants growing wild in Israel. Planta Medica 49, 248–249.

Sarer, E., Schefler, J., Baerheim, S., 1982. Monoterpenes of the essential oil of *Origanum majorana*. Planta Medica 46, 236–239.

Sendra, J.M., Cunat, P., 1980. Volatile constituents of Spanish *Origanum* (*Coriandothymus capitatis*) essential oil. Phytochemistry 19, 89–92 and 1513–1517.

Tackholm, V., 1974. Students Flora of Egypt, 2nd edn., 562. Cooperative Printing Co, Beirut.

Yahara, S., Sakamoto, C., Nohara, T., Niiho, Y., Nakajima, Y., Ito, H., 1993. Thymoquinol 5-*O*-beta-D-glucopyranoside, kaempferol 3-*O*-beta-rutinoside and protocatechuic acid from fruit of *Scisandrae chinensis*. Shoyakugaku zassi 47 (4), 420–422.

Zechmeister, L., 1979. Progress in the Chemistry of Organic Natural Products, Vol. 36, 24. Springer-Verlag, Wien, New York.

Zhou, J., Wu, M., Taniyasu, S., Besso, H., Tanaka, O., Saruwatari, Y., Fuwa, T., 1981. Dammarane saponins of Sanchi-ginseng, root of *Panax notoginseng* (Burk.) F. H. Chen (Araliaceae): structures of new saponins, notoginsenosides-R1 and -R2, and identification of ginsenosides-Rg2 and Rh-1. Chemical Pharmaceutical Bulletin 29, 2844–2850.