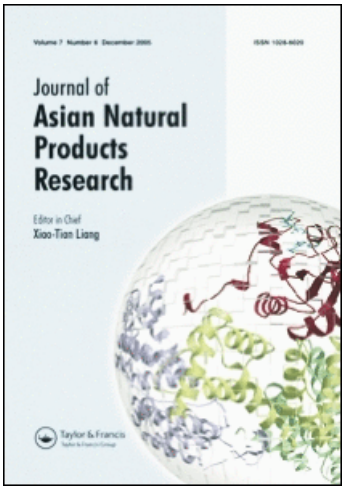


Journal of Asian Natural Products Research

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713454007

Two new compounds from *Trifolium resupinatum* var. *microcephalum*

Temine Sabudak[a]; Emel Isik[b]; Sevil Oksuz[c]
[a] Department of Environmental Engineering, Corlu Engineering Faculty, Namik Kemal University, Corlu, Tekirdag, Turkey [b] Edirne Science High School, Edirne, Turkey [c] Department of Chemistry, Pharmacy Faculty, Istanbul University, Istanbul, Turkey

To cite this Article Sabudak, Temine , Isik, Emel and Oksuz, Sevil(2008) 'Two new compounds from *Trifolium resupinatum* var. *microcephalum*', Journal of Asian Natural Products Research, 10: 11, 1017 — 1021
To link to this Article: DOI: 10.1080/10286020802278038
URL: http://dx.doi.org/10.1080/10286020802278038

Journal of Asian Natural Products Research
Vol. 10, No. 11, November 2008, 1017–1021

Two new compounds from *Trifolium resupinatum* var. *microcephalum*

Temine Sabudak[a]*, Emel Isik[b] and Sevil Oksuz[c]

[a]*Department of Environmental Engineering, Corlu Engineering Faculty, Namik Kemal University, Corlu, Tekirdag, Turkey;* [b]*Edirne Science High School, Edirne, Turkey;* [c]*Department of Chemistry, Pharmacy Faculty, Istanbul University, Istanbul, Turkey*

(*Received 4 December 2007; final version received 28 May 2008*)

An investigation of CH_2Cl_2 and EtOH extracts of *Trifolium resupinatum* L. var. *microcephalum* Zoh. has led to the isolation of two new compounds characterized as 4,15-dimethyl-2-(1,2-dihydroxyethyl)-hexadecene (**1**) and 1-undecene-1-*O*-β-2′,3′,4′,6′-tetraacetyl glucopyranoside (**2a**). Their structures were established by 1D and 2D NMR techniques, and mass spectroscopy.

Keywords: *Trifolium resupinatum* L. var. *microcephalum* Zoh; leguminosae; 4,15-dimethyl-2-(1,2-dihydroxyethyl)-hexadecene; 1-undecene-1-*O*-β-2′,3′,4′,6′-tetraacetyl glucopyranoside

1. Introduction

Trifolium is one of the most important genera of the Leguminosae family both in its agricultural value and the number of species, about 300 [1]. In Turkey, it is represented by 103 species [2]. Some *Trifolium* species such as *T. repens*, *T. arvense*, and *T. pratense* are used for the treatment of analgesic, expectoran, antiseptic, and rheumatism aches in Turkish folk medicine [3]. However, some of them are an important material for sheep and cattle in the Mediterranean region [4,5]. The aerial part of *Trifolium alexandrinum* is used as cattle feed and the seeds are used as an antidiabetic in Egypt [6]. In previous studies, saponins [7,8], flavonoids [4,5,8–10], chalcanol glucosides [11], and megastigmane glycosides [6] have been reported from the *Trifolium* species. Phytylester, steroids, lipids [12,13], and flavonoids [14] have also been isolated from *T. resupinatum* L. var. *microcephalum* Zoh. However, a study on the alcoholic extract of *T. resupinatum* var. *microcephalum* revealed anti-inflammatory and antioxidant activities in arthritic rats [15].

In this paper, we have reported the isolation and structure determination of two new compounds (**1** and **2**), an alkenol and an alkyl glucoside, from *T. resupinatum* var. *microcephalum* (Figure 1). They are reported for the first time from *Trifolium* var. *microcephalum*.

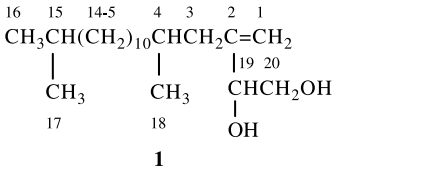
2. Results and discussion

In the ^{1}H NMR spectrum of **1**, the double doublets at δ 3.52 (H-20$_a$) and 3.69 (H-20$_b$) were attributed to the methylene protons adjacent to an OH group, while the methine proton adjacent to the OH group was observed as a double doublet at δ 4.18 (H-19). The methylene protons of the double bond were observed as two singlets at δ 4.96 (H-1$_a$) and 5.11 (H-1$_b$). In addition, the ^{1}H NMR

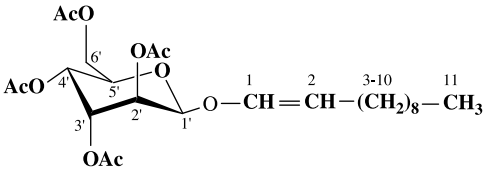
*Corresponding author. Email: tsabudak@yahoo.com

ISSN 1028-6020 print/ISSN 1477-2213 online

DOI: 10.1080/10286020802278038
http://www.informaworld.com



1



2a

Figure 1. Structures of compounds **1** and **2a**.

spectrum of **1** indicated two doublets at δ 0.85 (H-16/17) and 0.83 (H-18), and a multiplet centered at δ 1.19 (20H, 10 × CH_2 groups of the aliphatic chain), δ 1.54 (H-4), and δ 1.42 (H-15). The signal at δ 1.89 (H-3) indicated the presence of methylene protons adjacent to an olefinic carbon. The $^1H-^1H$ COSY spectrum of **1** confirmed the connectivity between H-19/H-20, H-20_a/H-20_b, H-1_a/H-1_b, H-4/H-18, and H-15/H-16/H-17. The molecular formula $C_{20}H_{40}O_2$ of compound **1** was determined on the basis of the HRMS and EIMS spectra. The HRMS showed a molecular ion peak at *m/z* 312.3038 $[M]^+$. In the EIMS spectrum of **1**, the fragmentation of the hydrocarbon chain was observed, from which the methyl positions could be deduced (Figure 2). The spectrum contained two major ion peaks at *m/z* 43 $[CH_3CHCH_3]^+$ and *m/z* 297 $[M - 15]^+[(C_{20}H_{40}O_2)—CH_3]^+$,

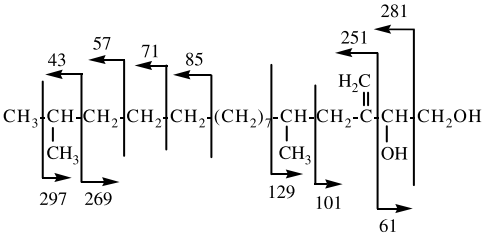


Figure 2. EIMS fragmentation patterns of compound **1**.

characteristic of methyl substitutions [16]. The EIMS spectrum also displayed ion peaks at *m/z* 269 and 297 associated with the peak at *m/z* 43, suggesting one isopropyl group at C-14; the ion peaks at *m/z* 101 and 129 were consistent with another methyl substitution at C-4. However, the prominent fragmentation ions at *m/z* 281, 251, and 61 suggested hydroxylation at C-19. The $[(M + 1) - 18]^+$ and $[(M + 1) - 36]^+$ peaks arising from the loss of H_2O were observed in the EIMS spectrum.

In the ^{13}C NMR spectrum, 20 carbon resonances were observed and assigned with the aid of the DEPT spectrum; the methyl branching was confirmed by the presence of signals for methine carbons (δ 32.9 and 33.0), methyl groups (δ 19.9, 19.9, and 22.8), and methylene groups α to the methine carbons (δ 37.4, 37.6, and 39.5 (C-3/5/14)) [12,16,17]. The signals at δ 110.7 (C-1) and 148.8 (C-2) for olefinic carbons and the signals at δ 75.2 (C-19) and 65.8 (C-20) for oxygen-bearing carbons were observed. All these data supported that compound **1** was 4,15-dimethyl-2-(1,2-dihydroxyethyl)-hexadecene.

Compound **2** could not be dissolved in $CDCl_3$ and other NMR solvents. For this reason, first, it was converted to its acetyl derivative (**2a**) and then its spectra were taken. Compound **2** was identified as an alkyl glucoside, 1-undecene-1-*O*-β-glucoside, by comparison of the ^{13}C NMR, HMQC, HMBC, ^1H NMR, $^1H-^1H$ COSY, HRMS, and EIMS spectra. In the ^1H NMR spectrum of **2a**, two *cis*-double bond protons at δ 6.58 (H-1, d, $J = 9.0$ Hz) and 5.35 (H-2, ddd, $J = 9.5$, 6.0, 9.0 Hz), an anomeric proton at δ 5.34 (H-1′), and four methyl protons at δ 2.00, 2.03, 2.06, 2.16 (12H, 4 × CH_3COO^-) were observed. The ^1H NMR spectral data of **2a** showed the resonances of protons of an acetylated sugar moiety at C-2′, C-3′, C-4′, and C-6′, as evidenced by downfield shifts of H-2′ (δ 5.07), H-3′ (δ 4.93), H-4′ (δ 5.07), and H-$6'_{a,b}$ (δ 3.99, 4.31) signals in comparison with β-glucopyranoside. The acetylated groups were conclusively confirmed by the HMBC spectrum (see Experimental section).

The ^{13}C NMR and HMQC spectra revealed the presence of five methyl groups at δ 19.6, 19.7, 19.8, 19.9 (CH_3COO^-) and δ 13.1 (C-11), two olefinic carbons at δ 130.2 (C-1) and δ 128.3 (C-2), four ester carbonyls at δ 168.9, 169.0, 169.9, 170.2 (CH_3COO^-), eight methylenes at δ 21.7, 23.9, 24.5, 27.3, 28.7, 30.8, 30.9, 31.2 (C-3–C-10) and δ 61.4 (C-6′), five methines in the region δ 104.0–71.4, including an anomeric carbon at δ 104.0. The HRMS of compound **2a** showed the $[M]^+$ at *m/z* 500.2662 in agreement with the molecular formula $C_{25}H_{40}O_{10}$. The EIMS spectrum of **2a** gave a major molecular ion at *m/z* 501 $[M + 1]^+$, which was consistent with the molecular formula $C_{25}H_{40}O_{10}$, as well as a strong ion fragment at *m/z* 167 $[(M - 2)\text{-Glu(OAc)}_4]^+$(100). The observation of a fragment ion peak at *m/z* 167 provided additional evidence of the sugar moiety. The prominent mass spectral fragmentations at *m/z* 125, 83, 41 were also consistent with the proposed structure. Thus, compound **2a** was characterized as 1-undecene-1-*O*-*β*-2′,3′,4′,6′-tetraacetyl glucopyranoside [18–20].

3. Experimental

3.1 General experimental procedures

Optical rotations were measured on a JASCO DIP-360 digital polarimeter. The NMR spectra were recorded in $CDCl_3$ on a Varian instrument at 300 and 500 MHz (Mercury plus) for ^{1}H NMR, ^{13}C NMR, DEPT, ^{1}H–^{1}H COSY, HMQC, and HMBC. HRMS was recorded with a Bruker (Micro TOF-Q) instrument. EIMS was measured on a VG Zabspec MS and a Jeol MS Route apparatus.

3.2 Plant material

T. resupinatum var. *microcephalum* was collected from Edirne, Turkey in May 2002 and identified by Dr N. Başak (Trakya University). A voucher specimen is deposited in the Herbarium of the Biology Department, Trakya University (EDTU 8328).

3.3 Extraction and isolation

Dried aerial parts of the plant material were macerated with CH_2Cl_2 and then with EtOH. The CH_2Cl_2 extracts were concentrated under reduced pressure to yield 35 g of a crude extract, which was chromatographed on silica gel column (CC), using petroleum ether and a gradient of CH_2Cl_2, EtOAc, and, finally, MeOH (100%). The collected similar fractions in CH_2Cl_2:EtOAc (90:10) were combined to produce one fraction, and this fraction was rechromatographed by CC. The single compounds were purified by preparative TLC [CH_2Cl_2:EtOAc (6:1.2)] to yield compound **1** (7.5 mg).

The EtOH extract was concentrated on a rotary evaporator under reduced pressure. The residual extract was diluted with water and then extracted with EtOAc to yield 27.9 g of a crude EtOAc extract. The EtOAc extract was chromatographed on silica gel column, using *n*-hexane and gradients of EtOAc, and, finally, MeOH (100%) as a solvent. The collected similar fractions in the 100% EtOAc were combined to produce one fraction, and this fraction was rechromatographed. The single compounds were purified by preparative TLC [hexane:EtOAc (15:85)] to afford **2** (12 mg). Compound **2** was converted to its acetyl derivative because it could not be dissolved in $CDCl_3$ and other NMR solvents. Then, the spectra of its acetyl derivative were recorded. In the acetylation of **2**, it was dissolved in pyridine (1 ml) and treated overnight with Ac_2O (1 ml).

3.3.1 4,15-Dimethyl-2-(1,2-dihydroxyethyl)-hexadecene (1)

$[\alpha]_D^{19}$ + 2.5 (*c* 0.7, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ: 4.96 (1H, s, H-1$_a$), 5.11 (1H, s, H-1$_b$), 1.89 (2H, m, H-3), 1.54 (1H, m, H-4), 0.95–1.44 (20H, m, H-5–H-14), 1.42 (1H, m, H-15), 0.85 (6H, d, J = 6.3 Hz, H-16/17), 0.83 (3H, d, J = 6.6 Hz, H-18), 4.18 (1H, dd, J = 3.3, 7.2 Hz, H-19), 3.52 (1H, dd, J = 7.2, 11.1 Hz, H-20$_a$), 3.69 (1H, dd, J = 3.6, 11.1 Hz, H-20$_b$); ^{13}C NMR and DEPT ($CDCl_3$)

δ: 110.7 (C-1), 148.9 (C-2), 32.9 (C-4), 33.0 (C-15), 19.9 (C-16), 19.9 (C-17), 22.8 (C-18), 75.2 (C-19), 65.8 (C-20), 37.4, 37.6, 39.5 (C-3/5/14), 22.9, 24.6, 24.9, 25.7, 28.1, 29.8, 33.1, 37.0 (C-6–C-13); ^{1}H–^{1}H COSY ($CDCl_3$) δ: 1.89/1.54 (H-3/H-4), 4.18/3.69 and 3.52 (H-19/H-20_a and H-20_b), 3.52/3.69 (H-20_a/H-20_b), 4.96/5.11 (H-1_a/H-1_b), 1.54/1.89 (H-4/H-3), 1.54/0.83 (H-4/H-18), 1.42/0.85 (H-15/H-16 and H-17); HRMS *m/z* 312.3038 $[M]^+$ (calcd for $C_{20}H_{40}O_2$, 312.3028); EIMS *m/z* (rel. int.%): 312 $[M]^+$(5) ($C_{20}H_{40}O_2$), 295 $[(M + 1)\text{-}H_2O]^+$(45), 277 $[(M + 1)\text{-}2H_2O]^+$(70), 297 $[M - CH_3]^+$(25), 281 $[M - CH_2OH]^+$(15), 263 $[281\text{-}H_2O]^+$(18), 45 $[(M + 2)\text{-}C_{17}H_{33}O_2]^+$(100), 57 $[M - C_{16}H_{31}O_2]^+$(85), 251 $[M - (—CH(OH)CH_2OH)]^+$(3), 61 $[M - C_{18}H_{35}]^+$ (3), 269 $[M - C_3H_7]^+$(4), 129 $[M - C_{13}H_{27}]^+$ (3), 101 $[M - C_{15}H_{31}]^+$(17), 85 $[M - C_{14}H_{27}O_2]^+$(27), 71 $[M - C_{15}H_{29}O_2]^+$(77).

*3.3.2 1-Undecene-1-*O*-β-2′,3′,4′,6′-tetraacetyl glucopyranoside (2a)*

$[\alpha]_D^{19}$ − 27.4 (c 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ: 6.58 (1H, d, $J = 9$ Hz, H-1), 5.35 (1H, ddd, $J = 9.5$, 6, 9 Hz, H-2), 1.94 (2H, m, H-3), 1.55 (2H, br s, H-4), 1.23–1.29 (12H, m, H-5–H-10), 0.85 (3H, t, $J = 6.5$ Hz, H-11), 5.34 (1H, d, $J = 6$ Hz, H-1′), 5.07 (1H, dd, $J = 8$, 11.5 Hz, H-2′), 4.93 (1H, dd, $J = 3.5$, 9.5 Hz, H-3′), 5.07 (1H, dd, $J = 3.5$, 8 Hz, H-4′), 4.42 (1H, m, H-5′), 3.99 (1H, dd, $J = 3$, 11.5 Hz, H-$6'_a$), 4.31 (1H, dd, $J = 6.5$, 11.5 Hz, H-$6'_b$), 2.0, 2.03, 2.06, 2.16 (12H, s, 4 × *CH*$_3$COO$^-$); ^{13}C NMR and HMQC ($CDCl_3$) δ: 130.2 (C-1), 128.3 (C-2), 21.7, 23.9, 24.5, 27.3, 28.7 (C-3/4/5/6/7/8/9/10), 13.1 (C-11), 104.0 (C-1′), 73.0 (C-2′), 75.6 (C-3′), 71.4 (C-4′), 71.6 (C-5′), 61.4 (C-6′), 19.4, 19.7, 19.8, 19.9 (4 × *CH*$_3$COO$^-$), 169.0, 169.0, 169.9, 170.2 (4 × CH$_3$*COO*$^-$); ^{1}H–^{1}H COSY ($CDCl_3$) δ: 0.85/1.23–1.29 (H-11/H-10), 1.94/1.23–1.29 (H-3/H-4), 3.99/4.31 (H-$6'_a$/H-$6'_b$), 4.42/5.07 (H-5′/H-4′), 5.07/4.93 (H-4′/H-3′), 4.93/5.07 (H-3′/H-4′ and H-2′), 6.58/5.35 (H-1/H-2), 5.35/1.94 (H-2/H-3), 4.42/3.99 and 4.31 (H-5′/H-$6'_a$ and H-$6'_b$); HRMS *m/z* 500.2662 $[M]^+$ (calcd for $C_{25}H_{40}O_{10}$, 500.2621); EIMS *m/z* (rel. int.%): 501 $[M + 1]^+$(1) ($C_{25}H_{40}O_{10}$), 502 $[M + 2]^+$(7), 327 $[(M\text{-}1)\text{-}(4 \times CH_3CO)]^+$ (10), 167 $[(M\text{-}2)\text{-}Glu(OAc)_4]^+$(100), 149 $[167\text{-}H_2O]^+$(29), 139 $[(167 + 1)\text{-}(—CH_2CH_3)]^+$(15), 125 $[(167 + 1)\text{-}(—CH_2CH_2\text{-}CH_3)]^+$(79), 83 $[(167 + 1)\text{-}(—(CH_2)_5CH_3)]^+$ (42), 41 $[(167 + 1)\text{-}(—(CH_2)_8CH_3)]^+$(27), 125 $[167\text{-}C_2H_2O]^+$(79). HMBC correlations: H-3/C-1, C-2, C-4; H-1/C-3; H-1′/C-1; H-2′/C-3′, C-1′; H-3′/C-2′, C-4′; H-4′/C-3′, C-2′; H-5′/C-6′; H-6′/C-5′.

References

[1] M. Zohary and D. Heler, *The genus* Trifolium, (Ahva Printing Press, Jerusalem, 1984), p. 606.

[2] Zohary, M., and Davis, P.H. (eds) (1970) Flora of Turkey. Vol. 3. Edinburg: Edinburg University Press, pp. 384–448.

[3] T. Baytop, *Teraphy with Medicinal Plants in Turkey*, (Istanbul University Press, Istanbul, 1984), p. 409.

[4] E. DeRijke, A. Zarfa Gomez, F. Ariese Udo, A.Th. Brinkman, and C. Goolijer, *J. Chromtogr. A* **932**, 55 (2001).

[5] W. Oleszek and A. Stochmal, *Phytochemistry* **61**, 165 (2002).

[6] K.M. Mohamed, H.M. Mohamed, K. Ohtani, R. Kasai, and K. Yamasaki, *Phytochemistry* **50**, 859 (1999).

[7] K.M. Mohamed, K. Ohtani, R. Kasai, and K. Yamasaki, *Phytochemistry* **40**, 1237 (1995).

[8] A.M. Simonet, A. Stochmal, W. Oleszek, and F.A. Macias, *Phytochemistry* **51**, 1065 (1999).

[9] L.Y. Foo, Y. Lu, A.L. Molan, D.R. Woodfield, and W.C. McNabb, *Phytochemistry* **54**, 539 (2000).

[10] B. Klejdus, D. Vitamvasova-Sterbova, and V. Kuban, *Anal. Chim. Acta* **450**, 81 (2001).

[11] K.M. Mohamed, H.A. Hassanean, K. Ohtani, R. Kasai, and K. Yamasaki, *Phytochemistry* **53**, 401 (2000).

[12] T. Sabudak, M.T.H. Khan, M.I. Choudhary, and S. Oksuz, *Nat. Prod. Res.* **24**, 665 (2006).

[13] T. Sabudak, E. Isik, and S. Oksuz, *Nat. Prod. Res.* **21**, 828 (2007).

[14] E. Isik, T. Sabudak, and S. Oksuz, *Chem. Nat. Comp.* **43**, 614 (2007).

[15] T. Sabudak, D. Dokmeci, F. Ozyigit, E. Isik, and N. Aydogdu, *Asian J. Chem.* **20**, 1491 (2008).
[16] P. Metzger, E. Villarreal-Rosalas, and E. Casadevall, *Phytochemistry* **30**, 185 (1991).
[17] M.S. Buchanan, T. Hasimoto, and Y. Asakawa, *Phytochemistry* **41**, 1371 (1996).
[18] C. Zeng Wang and D.Q. Yu, *Phytochemistry* **48**, 711 (1998).
[19] L.O.A. Manguro, I. Ugi, R. Hermann, and P. Lemmon, *Phytochemistry* **63**, 497 (2003).
[20] K. Kurashima, M. Fuji, Y. Ida, and H. Akita, *J. Mol. Catal. B: Enzymatic* **26**, 87 (2003).