PERGAMON

Phytochemistry 51 (1999) 909–912

PHYTOCHEMISTRY

# Iridoids from *Centranthus longiflorus* subsp. *longiflorus*

L.Ö. Demirezer[a,*], Z. Güvenalp[b], H.-J. Schiewe[c], I. Strietzel[c], M. Harmandar[b], A. Zeeck[c]

[a]*Hacettepe University, Department of Pharmacognosy, Faculty of Pharmacy, 06100 Ankara, Turkey*
[b]*Atatürk University, Department of Organic Chemistry, Faculty of Education, Erzurum, Turkey*
[c]*Georg-August University, Institute of Organic Chemistry, Tammannstraße 2, D-37077 Göttingen, Germany*

Received 4 January 1999; accepted 19 February 1999

## Abstract

An investigation of the extracts from *Centranthus longiflorus* subsp. *longiflorus* has led to the isolation of seven substances. A new iridolactone has been isolated from the aerial parts of this plant and was elucidated by analysis of the spectroscopic data and named longiflorone. The constitution of the previously described valtrate hydrine B8 was elucidated via 2D NMR analysis for the first time. Additional compounds isolated were patrinoside, kanokoside A, oleanolic acid, sitosterol and quercetin 3-*O*-rutinoside. © 1999 Elsevier Science Ltd. All rights reserved.

*Keywords:* *Centranthus longiflorus* subsp. *longiflorus*; Valerianaceae; iridoids; valepotriate; flavonoid; triterpenes; longiflorone

## 1. Introduction

The genus *Centranthus* belongs to the Valerianaceae and is represented by three species in the Flora of Turkey (Davis, 1972). Plants of the this family are well known for their sedative properties (Hölzl, 1997) and *Centranthus longiflorus* subsp. *longiflorus* is commonly used in Turkey as remedy which shows similar effects. Cyclopentane monoterpenes, valepotriates, anthocyanin pigments and glyceridic acids were previously isolated constituents from *Centranthus* species (Stoll & Seebeck, 1957; Handjieva, Popov & Marekov, 1978; Adzet & Raynaud, 1971; Khandzieva & Zaikin, 1978; Marekov, Popov & Khandzieva, 1984; Hölzl, 1997). In this study a new iridolactone was isolated together with a valepotriate and two known iridoid glycosides. In addition two steroids and a flavonol glycoside were identified. All the compounds isolated were assigned by means of spectral evidence (UV, IR, NMR, MS).

## 2. Results and discussion

The aerial parts of *Centranthus longiflorus* subsp. *longiflorus* were extracted with methanol. The concentrated methanol extract was suspended in water. This suspension was partitioned with petroleum, $Et_2O$, EtOAc and *n*-BuOH, respectively. The concentrated petroleum extract, after repeated column chromatography, yielded oleanolic acid (Bischoff, Jeger & Ruzieka, 1949) and sitosterol (Rubinstein, Goad, Clague & Mullheirn , 1976). The *n*-BuOH extract, after repeated Si Gel and Sephadex LH-20 column chromatography and RP-18 MPLC, led to the quercetin 3-*O*-rutinoside (Wenckert & Gottlieb, 1977), 7-hydroxy-11-glucosyl-1-isovaleryl-iridoid (patrinoside), 8,10-dihydroxy-6,7-epoxy-11-glucosyl-1-isovaleryl-iridoid (kanokoside A) (Nishiya, Kimura, Takeya & Itokawa, 1992) and valtrate hydrine B8 (**2**) (Koch & Hölzl, 1985). Patrinoside and kanokoside A have been isolated before only from *Valeriana officinalis* and valtrate hydrine B8 has been isolated before only from *Valeriana alliarifolia*. More remarkable is a new iridolactone, named longiflorone (**1**).

---

* Corresponding author.

0031-9422/99/$ - see front matter
PII: S0031-9422(99)00152-1

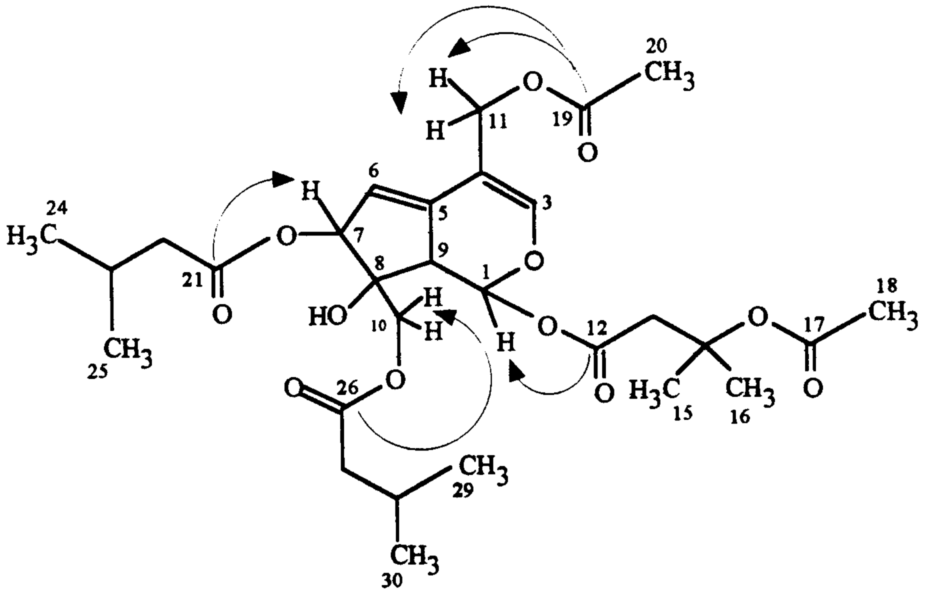
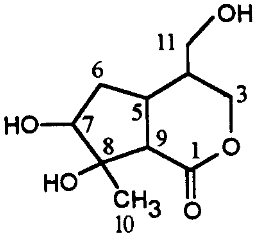


Fig. 1



**1**

Compound **1** was obtained in crystalline form and exhibited the molecular formula $C_{10}H_{16}O_5$ (DCI-MS: *m*/*z* 234 $[M+NH_3+H]^+$). The IR ($\nu=3395$, 2490, 1705 $cm^{-1}$) absorptions and $^1H$ NMR data indicated an iridolactone (Marini-Bettolo, et al., 1983). Because of the value of coupling constants ($>10$ Hz) and HETCOR correlations the signals at $\delta_H=4.54/4.19$, 3.58/3.45 and 2.04/1.92 were interpreted as diastereotopic protons of three methylene groups. From a COSY experiment a chain resulted, which starts with the methylene protons at $\delta=4.54/4.19$, followed by a methine proton ($\delta=1.78$), a second methine proton ($\delta=2.50$), further methylene protons ($\delta=2.04/1.92$) and finally the methine proton at $\delta=3.71$. Two branching points in this chain are proved by correlations between the protons at $\delta=1.78$ and the methylene protons at $\delta=3.58/3.45$ as well as between $\delta=2.50$ and $\delta=2.86$. The $^{13}C$ NMR spectrum shows the expected signals of three methylene ($\delta=69.1$, 62.7, 38.9), four methine ($\delta=80.7$, 50.6, 42.4, 36.2) and one methyl ($\delta=22.3$). Two quaternary carbon atoms appear at $\delta=85.4$ (adjacent to an oxygen atom) and $\delta=175.4$ (lactone carbonyl), respectively. The COLOC experiment confirms both the COSY derived chains as well as the position of the quaternary carbon atoms, resulting in a bicyclic structure. Thus this compound was identified as 7,8-dihydroxy-4-hydroxymethyl-8-methyl-perhydrocyclopenta[c]pyran-1-one (**1**). To the best of our knowledge, this structure is here described for the first time.

The amorphous powdery compound **3** displays absorption bands in the IR spectrum at $\nu=3347$, 2962 and 1738 $cm^{-1}$, which hints to the presence of hydroxyl groups, aliphatic CH and ester. On the basis of the DCI-MS (*m*/*z* 600 $[M+NH_3+H]^+$), $^1H$ and $^{13}C$ NMR data the molecular formula was determined to be $C_{29}H_{42}O_{12}$. Due to the comparison of the $^{13}C$ NMR spectrum of **2** with literature data (El-Naggar & Beal, 1980), we were able to assign the signals to a valepotriate core, one acetyl, two isovaleryl and a 3-acetyloxy-isovaleryl residue. This assignment was confirmed by the analysis of the $^1H$- $^1H$-*J* coupling constants and 2D NMR experiments (HSQC, COLOC). The connectivities between the various side chains and the valepotriate core were determined via an HMBC experiment (Fig. 1), proving the structure of **3**, as previously described for valtrate hydrine B8 (Koch & Hölzl, 1985).

## 3. Experimental

### 3.1. Plant material

Plant material was collected from Erzurum, eastern Anatolia, in the vicinity of Ispir on July 6, 1995. A voucher specimen is deposited in the Herbarium of Hacettepe University, Faculty of Pharmacy (HÜEF 96022).

### *3.2. Extraction and isolation of longiflorone (**1**), kanokoside A (2), patrinoside, quercetin 3-O-rutinoside and sitosterol*

Air dried aerial parts of the plant (420 g) were extracted twice with methanol at 50°C. After evaporation of the solvent under vacuum, the residue (60 g) was suspended in water, degreased with petroleum and extracted with diethyl ether, ethyl acetate and *n*-butanol. The petroleum extract was chromatographed on a Si Gel column using a hexane–ethyl acetate gradient (19:1 → 7:3). Sitosterol containing fractions were chromatographed with $CHCl_3$–MeOH (19:1, 9:1) and petroleum-ethyl acetate (7:3), respectively, to yield sitosterol (11 mg) as an amorphous powder identified by spectral ($^1$H-NMR and $^{13}$C-NMR) data. The *n*-butanol phase was lyophilized to yield 15 g of crude extract, which was chromatographed on Sephadex LH-20, eluting with methanol to yield two main fractions A (10 g) and B (1 g). Longiflorone (**1**) precipitated as crystals (40 mg) from fraction A. Fraction A, which was rich in iridoids, was subjected to Si Gel chromatography using $CHCl_3$–MeOH–$H_2O$ (40:10:1) to yield six main fractions, A1–A6. Fraction A4 (1.250 g) was applied to MPLC with RP-18 using an $H_2O$–MeOH gradient solvent system (40–80% MeOH) at a flow rate 5 ml/min. Five fractions, A4a–A4e were obtained. Patrinoside (15 mg) was obtained as an amorphous powder by rechromatographing the MPLC fraction A4b on a Si Gel column using $CHCl_3$–MeOH–$H_2O$ (40:10:1) as eluent. Fraction A4c was pure kanokoside A (80 mg) (**2**). The flavonoid glycoside, quercetin 3-*O*-rutinoside (35 mg) was isolated from fraction B after a series of chromatographic procedures. Patrinoside, kanokoside A and quercetin 3-*O*-rutinoside were identified by spectral ($^1$H-NMR and $^{13}$C-NMR) data.

### *3.3. Longiflorone (**1**), 7,8-dihydroxy-4-hydroxymethyl-8-methyl-perhydrocyclopenta[c]pyran-1-one*

Crystalline, Mp 163–165°; $[\alpha]_D^{20}$ +61.9° (MeOH; *c* 1.1); UV $\lambda_{max}^{MeOH}$: end absorption; IR $\nu_{max}^{KBr}$ cm$^{-1}$: 3395 (OH), 2940 (C–H), 1705 (C=O), DCI-MS *m/z* 234 $[M+NH_3+H]^+$ (calcd. for $C_{10}H_{16}O_5$ 216); $^1$H NMR (300 MHz, $CD_3OD$): $\delta$ 1.48 (3H, s, H-10), 1.78 (1H, m, H-4), 1.92 (1H, ddd, $J=13.5$ and 8.0 and $<1$, H-6a), 2.04 (1H, ddd, $J=13.5$ and 9.5 and 4.0 H-6b), 2.50 (1H, m, H-5), 2.86 (1H, d, $J=11.0$, H-9), 3.45 (1H, dd, $J=11.0$ and 8.0, H-11a), 3.58 (1H, dd, $J=11.0$ and 5.5, H-11b), 3.71 (1H, dd, $J=4.0$ and $<1$, H-7), 4.19 (1H, dd, $J=10.5$ and 6.0, H-3a), 4.54 (1H, dd, $J=10.5$ and 3.0, H-3b); $^{13}$C NMR (75.5 MHz, $CD_3OD$): $\delta$ 175.4 (C-1), 69.1 (C-3), 42.4 (C-4), 36.2 (C-5), 38.9 (C-6), 80.7 (C-7), 85.4 (C-8), 50.6 (C-9), 22.3 (C-10), 62.7 (C-11).

### *3.4. Kanokoside A (**2**)*

$^{13}$C NMR (75.5 MHz, $CD_3OD$): $\delta$ 90.5 (C-1), 142.6 (C-3), 109.2 (C-4), 35.4 (C-5), 60.1 (C-6), 59.7 (C-7), 80.1 (C-8), 43.3 (C-9), 67.0 (C-10), 69.5 (C-11), 172.9 (C-12), 44.1 (C-13), 26.8 (C-14),, 22.6 (C-15), 22.6 (C-16), 102.0 (C-1′), 75.1 (C-2′), 78.2 (C-3′), 71.8 (C-4′), 77.9 (C-5′), 62.8 (C-6′).

### *3.5. Extraction and isolation of valtrate hydrine B8 (**3**) and oleanic acid*

The air dried herb (300 g) was extracted with chloroform. This extract was chromatographed on a Si Gel column with petroleum ether–ethylacetate–acetone (50:1:1) as solvent system. Oleanolic acid containing fractions were chromatographed on Si Gel with hexane–ethyl acetate (7:3) and dichloromethane–acetone–ethyl acetate (50:1:1, 25:1:1), respectively, to yield pure crystalline oleanolic acid. The fractions containing **3** were chromatographed on Si Gel with dichloromethane–ethylacetate–acetone (50:1:1), chloroform–methanol (19:1), chloroform, hexane–ethylacetate (7:3), petroleum–ethylacetate (4:1) solvent systems, respectively. The last fraction containing **3** was dissolved in hexane and precipitated with diethyl ether to give valtrate hydrine B8 (200 mg) as an amorphous powder.

### *3.6. Valtrate hydrine B8 (**3**), 11-acetyloxy-1-[3-acetyloxy-isovaleryl]-8-hydroxy-7,10-isovaleryl-7,9-dihydrocyclopenta[c]pyran*

Powder, $[\alpha]_D^{20}$ +48.0° (MeOH; *c* 1.8); UV $\lambda_{max}^{MeOH}$ nm (ε): 202 (30567), 250 (37830), IR $\nu_{max}^{KBr}$ cm$^{-1}$: 3447 (OH), 2962 (C–H), 1738 (C=O); DCI-MS *m/z* 600 $[M+NH_3+H]^+$ (calcd for $C_{29}H_{42}O_{12}$ 582.6); $^1$H NMR (300 MHz, $CD_3OD$): $\delta$ 0.91 (6H, m, $Me_2CHCH_2$–), 0.96 (6H, s, $Me_2CHCH_2$–), 1.54 (3H, s, Me), 1.56 (3H, s, Me), 1.96 (3H, s, Me), 2.00 (3H, s, Me), 2.05 (2H, m, H-23, H-28), 2.16 (2H, m, H-22a, H-22b), 2.21 (2H, m, H-27a, H-27b), 2.93 (1H, dd, $J=10.0$ and 2.5, H-9), 2.97 (1H, d, $J=14.5$, H-13a), 3.11 (1H, d, $J=14.5$, H-13b), 4.32 (2H, s, H-10a and H-10b), 4.65 (1H, d, $J=12$, H-11a), 4.77 (1H, d, $J=12.0$, H-11b), 5.47 (1H, d, $J=2.5$, H-7), 5.67 (1H, dd, $J=2.5$ and 2.5, H-6), 6.13 (1H, d, $J=10.0$, H-1), 6.77 (1H, s, H-3). $^{13}$C NMR (75.5 MHz, $CD_3OD$): $\delta$ 94.1 (C-1), 149.3 (C-3), 110.5 (C-4), 140.6 (C-5), 118.7 (C-6), 84.5 (C-7), 80.7 (C-8), 49.6 (C-9), 66.7 (C-10), 61.9 (C-11), 169.2 (C-12), 44.6 (C-13), 80.5 (C-14), 26.9 (C-15), 27.0 (C-16), 172.3 (C-17), 22.3 (C-18), 172.6

(C-19), 20.8 (C-20), 173.5 (C-21), 44.3 (C-22), 26.9 (C-23), 22.7 (C-24), 22.7 (C-25), 174.3 (C-26), 44.0 (C-27), 26.7 (C-28), 22.8 (C-29), 22.8 (C-30).

## References

Adzet, T., & Raynaud, J. (1971). *Bull. Trav. Soc. Pharm. Lyon*, *15*, 134.

Bischoff, B., Jeger, O., & Ruzieka, L. (1949). *Helv. Chim. Acta*, *32*, 1911.

Davis, P. H. (1972). In *Flora of Turkey and East Aegean Islands*, *vol. 4*.

El-Naggar, J., & Beal, J. L. (1980). *J. Nat. Prod.*, *43*, 649.

Handjieva, N., Popov, S., & Marekov, N. (1978). *Phytochemistry*, *17*, 561.

Hölzl, J. (1997). In P. J. Houghton, *Valerian. The Genus Valeriana*. Amsterdam: Harwood Academic Publishers.

Khandzieva, N., & Zaikin, V. G. (1978). *Planta Medica*, *34*, 203.

Koch, U., & Hölzl, J. (1985). *Planta Medica*, *2*, 172.

Marekov, N., Popov, S., & Khandzieva, N. (1984). *Stud. Org. Chem.*, *17*, 313.

Marini-Bettolo, G. B., Nicoletti, M., Messana, I., Patamia, M., Galeffi, C., Ogukava, J. U., Pottalone, G., & Vaciago, A. (1983). *Tetrahedron*, *39*, 323.

Nishiya, K., Kimura, T., Takeya, K., & Itokawa, H. (1992). *Phytochemistry*, *31*, 3511.

Rubinstein, I., Goad, L. J., Clague, A. D. H., & Mullheirn, J. (1976). *Phytochemistry*, *15*, 195.

Stoll, A., & Seebeck, E. (1957). *Liebigs Ann. Chem.*, *603*, 158.

Wenckert, E., & Gottlieb, H. G. (1977). *Phytochemistry*, *16*, 1811.