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Guaianolides from *Centaurea nicolai*: antifungal activity

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Abstract

A new guaianolide, 3-deacetyl-9-O-acetylsalograviolide A, along with four known closely related lactones, salograviolide A, 9-O-acetylsalograviolide A, kandavanolide and salograviolide B were detected in the aerial parts of the flowering plant *Centaurea nicolai*. Antifungal tests performed on salograviolide A and its 9-O-acetyl and 3-O-deacetyl-9-O-acetyl derivatives revealed inhibitory activity against *Aspergillus niger*, *A. ochraceus*, *Penicillium ochrochloron*, *Cladosporium cladosporoides*, *Fusarium tricinctum* and *Phomopsis helianthi*. Neither of them was active against *Trichoderma viride*.

Keywords: *Centaurea nicolai*; Asteraceae; Aerial parts; Sesquiterpene lactones; Guaianolides; Antifungal activity

1. Introduction

In course of our phytochemical study of the Yugoslavian wild-growing species of genus *Centaurea* (Tešević, Djoković, Vajs, Marin & Milosavljević, 1994; Tešević, Vajs, Janaćković et al., 1998; Tešević et al., 1998), we investigated aerial parts of *C. nicolai* Bald, an endemic species, occurring in Yugoslavia (Montenegro) and Albania (Šilić, 1988), not studied before. These examinations, comprising isolation and characterisation of the lipophilic compounds (mainly sesquiterpene lactones) and evaluation of antifungal activities of the major compounds, are reported in this paper.

2. Results and discussion

Since the species of this genus have been shown to contain sesquiterpene lactones, some of them being biologically active (Kaij-a-Kamb, Amoros & Girre, 1992, and references therein), the extract of aerial parts was prepared by the usual procedure used for the isolation of these compounds (Bohlmann, Zdero, King & Robinson, 1979). Silica gel column chromatography of the extract afforded five guaianolides (**1**–**5**). Kandavanolide (**1**) (Rustaiyan & Ardebili, 1984), salograviolide A (**2**) (Daniewski et al., 1993; Daniewski, Nowak, Routsi, Rychlewska, Szczepanska & Skibicki, 1992) (also named 9β-hydroxykandavananolide (Rustaiyan and Ardebili, 1984)) and salograviolide B (**5**) (Daniewski et al., 1993) were identified by similarity of their spectral data to those published.

In addition, we isolated syringaldehyde (Borges-del-Castillo, Bradley-Delso, Manresa-Ferrero, Vazquez-Bueno & Rodriguez-Luis, 1983), matairesinol (lignan) (Youssef & Frahm, 1995), aplotaxene (Cossy & Aclinou, 1990) and two binary mixtures, α- + β-amyrin

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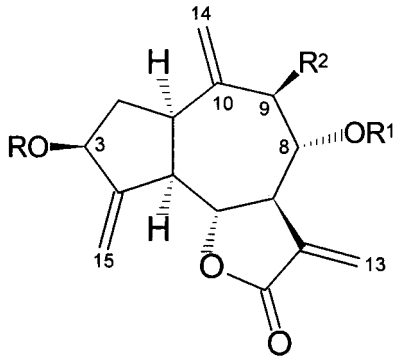

	R	R^1	R^2
1	Ac	H	H
2	Ac	H	OH
3	H	H	OAc
4	Ac	H	OAc
5*	Ac	H	H

* 10β, 14β-epoxy

(~1:4, respectively) and stigmasterol + β-sitosterol (~1:2, respectively).

The ^{1}H NMR spectral data of **3** and **4** (Table 1), assigned by comparison to the spectrum of **2**, were in accordance with the same type of (guaia-11(13),10(14),4(15)-trien-12,6α-olide) skeleton, oxygenated (as in **2**) at 3β-, 8α- and 9β-positions. Lactone **3**, with the same molecular formula as **2** ($C_{17}H_{20}O_6$), was identified as 3-deacetyl-9-O-acetylsalograviolide A on the basis of upfield and downfield shifts of H-3 and H-9, respectively, in comparison to those in **2** (Table 1). The ^{13}C NMR data of **3**, when compared to those of **2** and **4** (Table 2) also fit to the proposed structure. It should also be noted that upfield acetylation shifts of olefinic carbon in **3** and **4** ($\Delta\delta$ ~−4), resonating in **2** at δ 147.7 indicated that this signal (originally assigned to C-11 (Daniewski et al., 1992)) should be reassigned to C-10. At the same time, the signal assigned to C-10 (Daniewski et al., 1992), occurring in **2**, **3** and **4**, at approximately the same chemical shift (δ ~136) should be assigned to C-11.

Lactone **4** ($C_{19}H_{22}O_7$) contained two acetoxy functions. Chemical shift of H-3 (δ 5.58), almost analogous to that in **1**, accorded with 3β-acetoxy position in **3**. The chemical shifts of H-8, H-9 and H-13/13′ (δ 3.76, 4.96 and ~6.36, respectively) indicated 8α-OH,9β-OAc substitution pattern. According to our knowledge, this compound, prepared previously by partial acetylation of **2** (Rustaiyan and Ardebili, 1984), was not isolated before from the natural sources.

Table 1
^{1}H 200 MHz NMR data of compounds **2**–**4** in $CDCl_3$ (δ, mult., *J*, Hz)

H	**2**[a]	**3**	**4**
1	2.92[b]	⩾3 m[b]	⩾3 m[b]
2α	2.58	2.40 dt, 14.0, 8.0	2.56 dt, 14.5, 8.4
2β	1.84	1.83 dt, 14.0, 6.0	1.86 dt, 14.5, 5.0
3	5.55	4.59 *m*	5.58 m
5	2.92[b]	⩾3 m[b]	⩾3 m[b]
6	3.93[b]	3.99 t, 9.6	3.96 t, 9.5
7	2.97[b]	2.96 m[b]	2.97 m[b]
8	3.48	3.77 dd, 8.8, 10.0	3.76 ddd, 3.2, 8.6, 10.2
9	3.99[b]	4.93 d, 8.8	4.96 d, 8.6
13′	6.34	6.33[b]	6.36[b]
13	6.40	6.33[b]	6.36[b]
14′	5.20	5.28[b]	5.16 *br s*
14	5.50	5.28[b]	5.26 d, ~1
15′	5.31	5.35 t, 1.9	5.32 t, ~2
15	5.47	5.43 t, 1.9	5.48 t, ~2
OAc	2.13	2.16 s	2.11 s, 2.17 s
OH		~3[b]	2.76 d, 3.2

[a] Multiplicities same as those reported (Daniewski et al., 1992).
[b] Overlapping signals.

The antifungal in vitro activity test against seven fungi (*Aspergillus niger*, *A. ochraceus*, *Penicillium ochrochloron*, *Cladosporium cladosporoides*, *Fusarium tricinctum*, *Phomopsis helianthi* and *Trichoderma viride*) on lactones **2**, **3** and **4** was carried out using a modified agar dilution method (Ishi, 1995). The tested lactones exhibited inhibitory activity against all strains, with exception of *Trichoderma viride*, where no inhibition was observed under the applied experimental

Table 2
^{13}C 50 MHz NMR chemical shifts (δ) of **2**–**4** in $CDCl_3$[a]

C	**2**	**3**	**4**
1	48.7[b]	48.8[b]	48.8[b]
2	36.1	38.1	36.0
3	74.6	73.6	74.5
4	146.9	152.0	147.4
5	47.0[b]	48.3[b]	47.8[b]
6	79.4	78.8	78.8
7	40.9	40.9	41.1
8	77.6	75.2	75.2
9	79.7	81.0	81.0
10	147.7	143.8	143.7
11	136.1	136.3	135.9
12	171.1	170.0	170.6
13	125.3	125.1	125.5
14	112.5[b]	113.6	113.8
15	112.4[b]	111.9	113.1
OAc	170.4, 20.9	169.6, 21.1	170.6, 169.9, 21.1, 21.0

[a] Assigned by analogy with the published data (Daniewski et al., 1992); the original assignments of C-10 and C-11 are interchanged.
[b] The assignments can be interchanged within the column.

Table 3
Fungicidal activity (MIC, μg/ml) of **2**, **3** and **4**

Fungal species	**2**	**3**	**4**
Aspergillus niger	6.25	6.25	3.13
Aspergillus ochraceus	3.13	3.13	0.78
Penicillium ochrocloron	25	25	6.25
Cladosporium cladosporoides	3.13	3.13	0.78
Fusarium tricinctum	12.5	12.5	6.25
Phomopsis helianthi	1.56	1.56	0.78
Trichoderma viride	–[a]	–	–

[a] (–) no inhibition observed.

conditions (Table 3). In all cases where the activity was observed, lactone **4** exhibited the lowest MICs.

3. Experimental

3.1. General

MPs: uncorr. CC: silica gel 60 (Merck), 0.063–0.200 mm. TLC: Kieselgel 60 GF_{254}, layer thickness 0.25 and 0.5 mm. IR: transparent dry films (Perkin-Elmer FT IR spectrometer 1725X). ^{13}C and ^{1}H NMR: at 50 and 200 MHz, respectively (Varian Gemini 2000). DCIMS: double focusing mass spectrometer (Finnigan MAT 8230, BE geometry), 150 eV (isobutane).

3.2. Plant material

The plant material was collected during the flowering season (July 1997) at the south slopes of mountain Rumija (altitude of 560 m), situated at the Adriatic coast, Montenegro. Voucher specimen (No 0797CN) was deposited in the herbarium of The Botanical Garden, ''Jevremovac'', Faculty of Biology, University of Belgrade.

3.3. Bioassays

The bioassays were performed using the following fungi: *Aspergillus niger* (ATCC 6275), *A. ochraceus* (ATCC 12066), *Cladosporium cladosporoides* (ATCC 13276), *Phomopsis helianthi* (ATCC 201540), *Penicillium ochrochloron* (ATCC 9112), *Fusarium tricintum* (CBS 14478) and *Trichoderma viride* (IAM 5061), originating from mycoteca of the Mycological Laboratory, Department of Plant Physiology, Institute for Biological Research ''Siniša Stankovic'', Belgrade. The modified mycelial growth test with malt agar (MA) was used (Ishi, 1995). Each fungal species was previously cultured on potato dextrose agar (PDA) and MA at 20°C and the cultures were stored at +4°C and subcultered once a month (Booth, 1971). Initial concentration (1 mg/ml) of samples was made in MeOH. Serial dilutions (50–0.4 μg/ml) of samples were prepared and added into molten MA and poured in Petri dishes (90 mm diameter). All fungal strains were tested in duplicate. After 24 h the fungi were inoculated at the center of plates containing tested lactones. Petri dishes with MeOH were used as a control. After incubation for three weeks at 20°C, mycelial growth of the fungal species was evaluated by measuring the colony diameter. The percentage of inhibition was obtained by comparison of the colony diameter of fungicide-amended medium to that of the control. MICs, corresponding to 100% of inhibition, are listed in Table 3.

3.4. Extraction and isolation

A crude extract (46 g) of air-dried aerial parts (2.1 kg) was obtained by extraction with freshly distilled solvents (7 l): Et_2O(peroxides free)–petrol–MeOH (1:1:1) at room temperature (24 h), followed by treatment with MeOH to remove long chain saturated hydrocarbons by the usual procedure (Bohlmann et al., 1984).

The whole quantity of the crude extract was applied to a CC and the elution was started with petrol. The polarity of the solvent was gradually increased by addition of Et_2O.

Lactone **1** (5 mg) was isolated from the fraction eluted with petrol–Et_2O, 1.5:8.5 after two CCs (CH_2Cl_2–MeOH, 9.5:0.5) followed by prep TLC (CH_2Cl_2–MeOH, 9.5:0.5). Lactone **2** (42 mg) crystallised directly from the fraction eluted with neat Et_2O. Two CCs (toluene–Et_2O–MeOH, 7:2:1) of the mother liquor afforded **3** (16 mg) and a less polar fraction, which upon repeated CC (same conditions as above) and prep TLC yielded **5** (4 mg). Compound **4** (10 mg) was isolated in form of colorless crystals, $[\alpha]_D^{24}+8.80$ (MeOH, c0.102), m.p. 124°C, from the fraction eluted with petrol–Et_2O, 1:9.

3-O-deacetyl-9-O-acetylsalograviolide A (**3**): colorless solid; $[\alpha]_D^{22}+29.80$ (MeOH; c0.0436); IR ν_{max} cm^{-1}: 3407 (OH), 1747 (C═O, α,β-unsat. γ-lactone, OAc), 1658 (C═C), 1267, 1237, 1155; 1081, 1044; ^{1}H and ^{13}C NMR (see Tables 1 and 2); DCIMS, m/*z* (rel. int.): 321 $[M+H]^+$ (100), $C_{17}H_{20}O_6$, 303 $[M+H\text{-}18]^+$ (15), 261 $[M+H\text{-}60]^+$ (94), 243 $[M+H\text{-}18\text{-}60]^+$ (13).

Aplotaxene (20 mg), eluted with neat petrol was purified using prep TLC (petrol–Et_2O, 9.5:0.5). Matairesinol (6 mg) was isolated by means of prep TLC (CH_2Cl_2–MeOH, 9.5:0.5) from the fraction eluted with petrol–Et_2O, 8.5:1.5. Syringaldehyde (3 mg), eluted with the neat Et_2O (after the fraction containing lactones **2**, **3** and **6**) was purified by silica gel CC (toluene–Et_2O–MeOH, 7:2:1), followed by prep TLC (CH_2Cl_2–MeOH, 9.5:0.5). The mixtures of α+β-amyrin (1:4, 120 mg) and β-sitosterol+stigmasterol

(1:2, 48 mg) were isolated from the fractions eluted with petrol–Et_2O, 3.5:6.5 and 2:3, respectively.

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