Chemistry of Natural Compounds and Bioorganic Chemistry

Rhaposerine and rhaserolide, new sesquiterpene lactones from *Rhaponticum serratuloides*

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Two new crystalline guaianolides, *viz.*, rhaposerine and rhaserolide, were isolated from an ethanolic extract of the above-ground part of the plant *Rhaponticum serratuloides* by column chromatography along with sesquiterpene lactones, *viz.*, centaurepensin and acroptilin, which have been found in other plants. The chemical structures of the title lactones were established by ^{1}H and ^{13}C NMR spectroscopy and 2D ^{1}H—^{1}H (COSY) and ^{13}C—^{1}H (COSY and COLOC) NMR spectroscopy.

Key words: sesquiterpenoids, guaianolides, cynaropicrin, rhaposerine, rhaserolide, acroptilin, centaurepensin, 2D NMR spectroscopy, HPLC.

Plants of the *Rhaponticum* Adams genus (the *Compositae* P. F. Gmelin family) are rather poorly studied from the chemical standpoint. Noteworthy are the detailed studies of ecdysteroids present in *Rh. carthamoides*[1,2] and *Rh. integrifolium*[1] and of polyacetylene compounds from *Rh. carthamoides* and *Rh. centauroides.*[3]

Rhaponticum serratuloides (Georgi.) Bobr. is a herbaceous plant typical of the steppe zones of Kazakhstan and Russia. The well-known[4] guaianolide cynaropicrin (**1**)[5] was detected by TLC in extracts from the above-ground part of this plant and of *Rh. carthamoides* cultivated in Poland. Data on the presence of other sesquiterpene lactones in the plants of the *Rhaponticum* Adams genus are lacking in the literature.

We studied sesquiterpene lactones of the title plant which had come into bud. The plant was collected in Central Kazakhstan. The ethanolic extract of air-dried and dispersed raw material was purified from ballast compounds. The resulting concentrate of lac-

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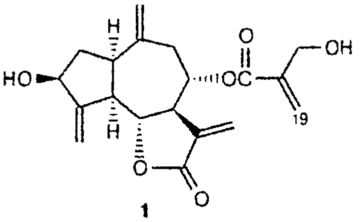
tones was chromatographed on silica gel. Fractions containing lactone **1**, the known guaianolide centaurepensin (**2**), acroptilin (**3**), and two new lactones, which we named rhaposerine (**4**) and rhaserolide (**5**), were successively isolated. New lactones **4** and **5** were obtained in 0.03% and 0.01% yields (with respect to the weight of the starting raw material), respectively.



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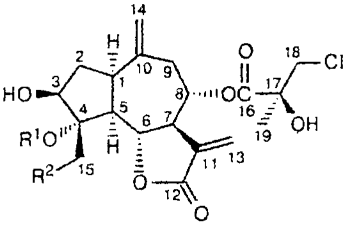
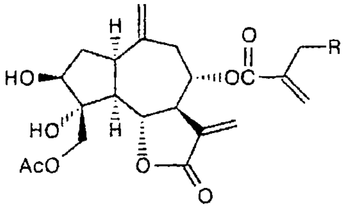


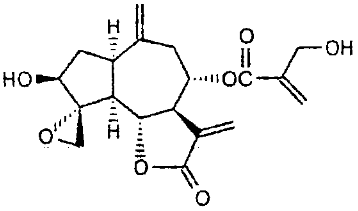
2—4

2: R¹ = H; R² = Cl
3: R¹ + R² = σ bond
4: R¹ = H; R² = OC²⁰(O)C²¹H₃



5, 6

5: R = H
6: R = OH



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Since lactones **2—5** are poorly soluble in $CDCl_3$, their ^1H and ^{13}C NMR spectra were recorded in Py-d_5. All the signals in the spectra (Tables 1 and 2) were assigned using 2D $^1H—^1H$ (COSY) and $^{13}C—^1H$ (COSY and COLOC) NMR spectroscopy, which allowed us to establish the molecular structures of new lactones **4** and **5**. These lactones differ only in the structures of the side chains. The following important cross-peaks in the COLOC spectrum of compound **4** made it possible to determine the position of the acetoxy group in the molecule: C(4)/H(5),2H(15) and C(20)/3H(21),2H(15).

Tables 1. ^1H NMR spectral data for compounds **2—5** (Py-d_5, Me_4Si)

H	δ			
Atom	**2**	**3**	**4**	**5**
1	3.97 (ddd)	3.28 (ddd)	3.86 (ddd)	3.81 (ddd)
2a	1.80 (dd)	2.10 (dddd)	1.81 (dd)	1.78 (dd)
2b	2.79 (dddd)	2.36 (dddd)	2.76 (dddd)	2.71 (dddd)
3	4.65 (br.d)	4.23 (br.d)	4.57 (br.d)	4.53 (br.d)
5	2.63 (dd)	2.19 (dd)	2.66 (dd)	2.64 (dd)
6	5.32 (dd)	4.93 (dd)	5.24 (dd)	5.20 (dd)
7	3.19 (dddd)	3.10 (dddd)	3.17 (dddd)	3.16 (dddd)
8	5.39 (ddd)	5.32 (ddd)	5.35 (ddd)	5.24 (ddd)
9a	2.59 (d)	2.47 (dd)	2.54 (d)	2.46 (d)
9b	2.94 (dd)	2.85 (dd)	2.92 (dd)	2.87 (dd)
13a	5.75 (d)	5.76 (d)	5.74 (d)	5.46 (d)
13b	6.18 (d)	6.15 (d)	6.14 (d)	6.09 (d)
14a	5.07 (nar.m)	5.07 (nar.m)	5.04* (nar.m)	5.01* (nar.m)
14b	5.12 (nar.m)	5.10 (nar.m)	5.10 (nar.m)	5.05 (nar.m)
15a	4.34 (d)	3.20 (d)	5.04* (s)	5.01* (s)
15b	4.78 (d)	3.39 (d)	5.04* (s)	5.01* (s)
18a	4.00 (d)	3.95 (d)	3.97 (d)	5.52 (nar.m)
18b	4.10 (d)	4.06 (d)	4.07 (d)	6.16 (nar.m)
19	1.70 (s)	1.65 (s)	1.67 (s)	1.88 (br.s)
21	—	—	1.81 (s)	1.78 (s)

Note. J/Hz: 1,2α = 8; 1,2β = 11; 1,5 = 8; 2α,2β = 14.5; 2α,3 = 1.5; 2β,3 = 6; 5,6 = 11; 6,7 = 9; 7,8 = 6.5; 7,13a = 3; 7,13b = 3.5; 8,9α = 1.5; 8,9β = 5; 9α,9β = 14.5; 15a,15b (for **2**) = 11.5; 15a,15b (for **3**) = 5; 18a,18b (for **2**, **3**, and **4**) = 11; 18a,18b (for **5**) = 1.5.

* The signals were observed separately when the solution was diluted in an NMR tube with an equal volume of $CDCl_3$; for 2 H(14), the signals were shifted downfield (**4**, δ: 5.06 (d, *J* = 1.8 Hz) and 5.15 (d, *J* = 1.8 Hz)); for 2 H(15), the signals were shifted upfield (**4**: 4.90 (d, *J* = 12.0 Hz) and 4.93 (d, *J* = 12.0 Hz)).

Table 2. ^{13}C NMR spectral data for compounds **2**—**5** and **7** (Py-d_5, Me_4Si)

C	δ				
atom	**2**	**3**	**4**	**5**	**7**
1	48.55 (d)	46.05 (d)	47.60 (d)	47.47 (d)	45.81 (d)
2	40.29 (t)	38.90 (t)	40.41 (t)	39.96 (t)	38.76 (t)
3	76.32 (d)	75.26 (d)	77.01 (d)	77.06 (d)	75.15 (d)
4	85.32 (s)	69.05 (s)	84.26 (s)	84.16 (s)	69.13 (s)
5	59.39 (d)	53.22 (d)	58.32 (d)	58.32 (d)	52.81 (d)
6	77.46 (d)	77.25 (d)	77.46 (d)	77.54 (d)	77.51 (d)
7	46.50 (d)	47.58 (d)	46.76 (d)	46.74 (d)	47.81 (d)
8	75.42 (d)	75.16(t)	75.47 (d)	74.57 (d)	74.32 (d)
9	35.06 (t)	36.08 (t)	35.50 (t)	35.75 (t)	36.82 (t)
10	144.57 (s)	142.80 (s)	144.48 (s)	144.62 (s)	142.15 (s)
11	138.87 (s)	138.53 (s)	138.90 (s)	139.06 (s)	138.57 (s)
12	169.03 (s)	168.98 (s)	169.08 (s)	169.20 (s)	169.03 (s)
13	121.05 (t)	121.26 (t)	121.00 (t)	120.68 (t)	121.35 (t)
14	117.06 (t)	117.98 (t)	117.02 (t)	116.55 (t)	117.71 (t)
15	51.23 (t)	48.89 (t)	67.50 (t)	67.46 (t)	48.48 (t)
16	173.45 (s)	173.33 (s)	173.44 (s)	166.52 (s)	165.72 (s)
17	75.23 (s)	75.27 (s)	75.37 (s)	136.80 (s)	143.07 (s)
18	52.19 (t)	52.05 (t)	52.17 (t)	126.30 (t)	60.93 (t)
19	24.29 (q)	24.19 (q)	24.28 (q)	18.26 (q)	127.74 (t)
20	—	—	171.13 (s)	171.14 (s)	—
21	—	—	20.85 (q)	20.86 (q)	—

The molecule of lactone **5** contains a residue of methacrylic acid, which is rather often present in molecules of acylated guaianolides from plants of the *Compositae* family.[6,7] An analog of lactone **5**, *viz.*, 18-hydroxy derivative **6** (15-dechloro-15-acetoxychloroyanerin), was isolated from the plant *Saussurea candicans* C. B. Clarke by Indian researchers.[8]

Centaurepensin **2** was discovered for the first time in the above-ground part of Russian centaury (*Centaurea repens* L.)[9] and was described under the name chlorohyssopifolin A by Spanish researchers.[10] Subsequently, the authors[10] demonstrated that it is identical with centaurepensin.[11] Acroptilin **3** is known as a constituent of the plant *Acroptilon repens* (L.) DC.[12] More recently, this compound was found in *Centaurea incana* Desf.[13] The structures and stereochemistry of lactones **2** and **3** have been established by X-ray diffraction analysis.[9,14] The structure of lactone **2** was reinvestigated,[15] and it was established that the absolute configuration of lactone **2** is opposite to that proposed initially.[9]

The unit cell parameters of the crystal of centaurepensin **2** from *Rh. serratuloides* correspond to those reported previously,[9,15] which indicates that it was centaurepensin **2** that was isolated from the plant under study. Particular attention which is given to this question is associated with the fact that a chromatographically inseparable oily mixture of lactones **2** and 17-epi-**2** was isolated from the plant *Centaurea conifera* L.[16] Based on the ^{1}H NMR data, the authors[16] believed that this mixture contained centaurepensin **2** and its 17-epimer as the major and minor components, respectively. The ^{1}H NMR spectra of these lactones (in the mixture) differ only slightly from one another. The ^{13}C NMR spectra of these compounds are also almost identical except for the signals for the C(10), C(13a), and C(19) atoms. However, comparison of our data on lactone **2** with the NMR data obtained previously[16] (Tables 3 and 4) indicates that the reverse is true, *viz.*, the inseparable mixture of lactones from *Centaurea conifera* L. contained 17-epi-**2** as the major component, while centaurepensin **2** was the minor component.

Table 3. Comparative ^{1}H NMR spectral data for lactone **2** and the components of the natural mixture of lactones **2** and 17-epi-**2**[9] ($CDCl_3$, δ, Me_4Si)

Group	**2**+17-epi-**2**		**2**
	major	minor	
HC(8)	5.16	5.21	5.20
$H_aC(13)$	5.83	5.57	5.55
$H_bC(13)$	6.24	6.22	6.21
$H_2C(14)$	4.82	5.01	5.00

Table 4. Comparative ^{13}C NMR spectral data for the components of the natural mixture of lactones **2** and 17-epi-**2**[9] and lactones **2**—**4** ($CDCl_3$, δ, Me_4Si, $C \approx 1$ mg mL^{-1})

C	**2**+17-epi-**2**		**2**	**3**	**4**
Atom	major	minor			
10	136.5	136.9	136.8	137.1	136.9
13	123.2	122.2	122.1	122.1	122.2
19	23.9	23.4	23.3	23.4	23.3

In connection with the aforesaid,[16] it should be noted that the interpretation of the NMR spectra of three guaianolides (**2**, 17-epi-**2**, and chloroyanerin) was performed without recourse to 2D COLOC NMR spectroscopy and required refinement for those atoms not bound to hydrogen atoms. Taking into account our results (see Table 2), the assignment[16] of the C(11) and C(12) atoms in all the above-mentioned three lactones should be reversed.

According to the ^{13}C NMR data, the configuration of the asymmetrical center at the C(17) atom in the molecule of rhaposerine **4** is identical with those in centaurepensin **2** and acroptilin **3** (see Table 4). The ^{13}C NMR spectral data for lactone **3** have been reported previously.[13] However, the authors mentioned that these data were ambiguous. We performed the complete interpretation of the ^{13}C NMR spectrum of lactone **3** using the 2D ^{1}H—^{1}H and ^{13}C—^{1}H (COSY and COLOC) NMR spectra. In addition, we carried out the interpretation of yet another known guaianolide, *viz.*, yanerin **7**,[13,17] whose cyclic portion coincides with that of acroptilin **3** (see Table 2).

The reversed-phase chomatographic behavior of lactones **2—5** in relation to the concentration of MeOH in the eluent was studied by HPLC:

MeOH	Capacity coefficient, *k'*			
(vol. %)	**2**	**3**	**4**	**5**
40	4.7	5.7	3.8	14.0
50	1.5	1.7	1.2	4.0

(the void volume of the column was 170 μL).

A decrease in the polarity of lactone **5** compared to that of the group of lactones **2—4**, which are similar in polarity, is readily seen. When eluted with 50% aqueous methanol, lactones **2—4** had close and small capacity coefficients (*k'*), while lactone **5** was eluted later and was readily separated from the above-mentioned three compounds. Elution with 40% aqueous methanol resulted in complete separation of lactones **2—4**, but the value of *k'* for lactone **5** substantially increased.

The UV spectra of lactones **2—5** were recorded in the region of 190—300 nm in the stop-flow mode. The spectra of compounds **2—4** are similar (a maximum at 196 nm and a shoulder at 216 nm). The spectrum of lactone **5** has an absorption maximum as a plateau at 198—208 nm, which is attributable to the fact that **5** contains a new chromophore, *viz.*, the methacrylic acid residue.

It is known that chloroguaianolides exhibit high biological activity.[18] Antiviral activity of compounds **2** and **4** was examined at the Institute of Microbiology and Virology of the Kazakh Academy of Medical Sciences (Almaty) by studying their effect on reproduction and infecting ability of influenza virus (strain A/FPV/Rostock/34) and virus of Newcastle disease (strain La Sota). Both lactones at a concentration of 5 μmol L^{-1} suppressed reproduction of influenza virus by more that 50%. At a concentration of 100 μmol L^{-1}, the reproduction was completely suppressed. Lactone **2** at a concentration of 100 μmol L^{-1} decreased the infecting ability of virus of Newcastle disease.

Experimental

The melting points were determined on a Boetius instrument. The IR spectra were obtained on a UR-20 instrument. The NMR spectra were recorded on a Bruker DRX-500 spectrometer (500.13 and 125.76 MHz for ^{1}H and ^{13}C NMR, respectively) using the standard Bruker software for recording 2D COSY and COLOC (9 Hz) NMR spectra. The high-resolution mass spectra (EI, 70 eV) were obtained on a Finnigan MAT 8200 instrument. The optical rotation was measured (at 580 nm) on a Polamat A polarimeter. The unit cell parameters of the crystal of lactone **2** were determined on a Syntex-P2$_1$ diffractometer.

Column chromatography was carried out on SiO$_2$ (KSK); the compound to sorbent ratio was ~1 : 20. The TLC analysis was performed on Silufol plates; spots were visualized by spraying with a 1% vanillin solution in H$_2$SO$_4$.

The above-ground part of the plant *Rhaponticum serratuloides* (Georgi.) Bobr. was collected when it came in flower in the neighborhood of Karaganda, dried in air, and dispersed.

Isolation of lactones. The raw material (1.3 kg) was extracted with boiling ethanol (4×4 L). The extracts were combined, filtered, and concentrated *in vacuo*. The resulting residue was dissolved in 50% aqueous ethanol (1 L) and nonpolar components were removed by treatment with light petroleum (4×0.8 L). Then the aqueous-ethanolic solution was extracted with benzene (5×0.8 L). The combined benzene extracts were concentrated to dryness and the residue (10.0 g) was chromatographed on a column. The fraction containing lactone **1** (600 mg) was eluted with a 20 : 1 PhH—EtOAc mixture. Elution with PhH—EtOAc mixtures (from 10 : 1 to 5 : 1) afforded four fractions containing lactones **2—5**, which were recrystallized from a mixture of light petroleum and acetone. Lactones **2—5** were obtained in yields of 104 mg (0.008% with respect to the air-dried starting raw material), 52 mg (0.004%), 390 mg (0.03%), and 130 mg (0.01%), respectively.

Centaurepensin (2): m.p. 221—223 °C, $[\alpha]^{23}{}_{580}$ +135.1° (*c* 0.73, THF) (*cf.* the published data: m.p. 214—215 °C,[9] 217—219 °C,[10] $[\alpha]^{24}{}_{\rm D}$ +107.8° (THF)[9], $[\alpha]_{\rm D}$ +97° (MeOH)[10]). The ^{1}H NMR spectral data are given in Tables 1 and 3. The ^{13}C NMR spectra are listed in Tables 2 and 4. The 2D COLOC spectrum has the following cross-peaks, which were used in the assignment of the signals of the atoms: C(10)/H(8), H(9a), H(9b); C(11)/H(6), H(7), H(8), H(13a); and C(12)/H(13a), H(13b).

The unit cell parameters are as follows: a = 10.47(1) Å, b = 9.25(1) Å, c = 11.49(2) Å; β = 113.1(1)°.

Acroptilin (3): m.p. 195—198 °C, $[\alpha]^{23}{}_{580}$ +110.7° (*c* 0.62, MeOH) (*cf.* the published data: m.p. 196 °C, $[\alpha]^{20}{}_{\rm D}$ +92.3° (EtOH)[12], 197—199 °C, $[\alpha]_{\rm D}$ +100° (MeOH)[11]). The ^{1}H NMR spectral data are given in Table 1. The ^{13}C NMR spectral data are listed in Tables 2 and 4.

Rhaposerine 4: m.p. 191—193 °C, $[\alpha]^{23}{}_{580}$ +214° (*c* 0.35, MeOH). Found (%): C, 55.03; H, 6.21; Cl, 7.65. $C_{21}H_{27}ClO_9$. Calculated (%): C, 54.96; H, 5.91; Cl, 7.72. IR (CHCl$_3$), ν/cm^{-1}: 3540 (OH), 3095, 1640 (C=CH$_2$), 1765 (γ-lactone), 1715 (C=O), 1215, 1140, 1060 (C—O). MS, m/z ($I_{\rm rel}$ (%)): 429 (6), 427 (19), 260 (30), 247 (19), 243 (24), 242 (23), 229 (30), 203 (23), 175 (26), 43 (100). Found, m/z: 427.11711 $[M - CH_3O]^+$. $C_{20}H_{24}ClO_8$. Calculated: 427.11596. The ^{1}H NMR spectral data are given in Table 1. The ^{13}C NMR spectral data are listed in Tables 2 and 4.

Rhaserolide 5: m.p. 153—155 °C, $[\alpha]^{23}{}_{580}$ +126° (*c* 0.44, CHCl$_3$). IR (CHCl$_3$), ν/cm^{-1}: 3500 (br) (OH), 3095, 1640, 1060 (C=CH$_2$), 1765 (γ-lactone), 1715 (C=O), 1215, 1140 (C—O). MS, m/z ($I_{\rm rel}$ (%)): 375 (19), 333 (2), 259 (15), 69 (100). Found, m/z: 375.14589 $[M - CH_3O]^+$. $C_{20}H_{23}O_7$. Calculated: 375.1443. Found, m/z: 333.13655 $[M - CH_2OAc]^+$. $C_{18}H_{21}O_6$. Calculated: 333.13380. The ^{1}H and ^{13}C NMR spectral data are given in Tables 1 and 2, respectively.

HPLC and UV spectra. Chromatography and UV spectroscopic studies of lactones **2—5** were performed on a Milikhrom (Ob'-4) microcolumn liquid chromatograph[19] (64×2-mm column; LiChrosorb RP-18 (Merck) as the sorbent, 5 μm; the column temperature was 30 °C). The eluent was prepared by mixing MeOH with 0.05 *M* aqueous H$_3$PO$_4$. The compounds were detected at λ = 200 nm. The rate of elution was 100 μL min^{-1}. To record the UV spectra, the eluent flow was stopped at the maximum of the chromatographic peak. The concentration of the samples was 1.2 mg mL^{-1} in MeOH; 1.4 μL of the solution was applied to the column.

A sample of yancrin **7**, whose ^1H NMR spectrum is identical with that reported previously,[17] was provided by E. K. Nurmukhmetova (Tomsk Medical University).

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