Chemistry of Natural Compounds, Vol. 41, No. 5, 2005

SESQUITERPENE LACTONES FROM *Stizolophus balsamita* AND THEIR BIOLOGICAL ACTIVITY

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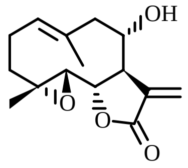
UDC 547.314

Four sesquiterpene lactones with the germacrane structure were isolated from the aerial part of Stizolophus balsamita. *X-ray structure analyses showed that two of them were previously known balsamin derivatives. A third was a crystalline isomorphous mixture of the known lactone stizolin and 11,13-dihydrostizolin in a 53:47 ratio. The biological activities of the balsamin derivatives were investigated.*

Key words: *Stizolophus balsamita*, germacranolides, PMR, x-ray structure analysis, antimicrobial activity.

Stizolophus balsamita (Lam.) Cass. ex Takht. is an annual herbaceous plant that grows in southern Kazakhstan [1]. The germacrane sesquiterpene lactones stizolicin [2-5], stizolin (**1**) [6-8], and balsamin (**2**) [7, 8] have been previously isolated from it. The composition of the essential oil isolated from the aerial part by steam distillation has been described [9].

S. balsamita collected in Kurdai Region of South-Kazakhstan District was extracted with water by the known method [10] in order to study further its chemical composition. Chromatographic separation of the resulting resin with benzene:ethylacetate isolated three crystalline compounds. They were γ-lactones according to their IR spectra. X-ray structure analysis showed that the first two had the structural formulas of **3** (9-epibalsamin) and **4** (9-epi-4′-hydroxybalsamin). These same structures were proposed for lactones isolated from the aerial part of *Centaurea coronopifolia* Lam. by Oksuz and Ayyildiz [11].



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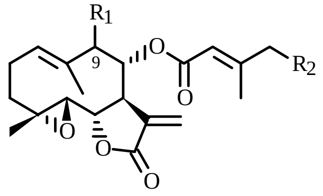
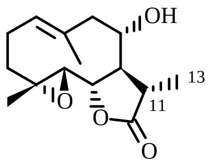


2 - 4

2: $R_1 = \beta$–OH, R_2 = H
3: R = α–OH, R_2 = H
4: $R_1 = \alpha$–OH, R_2 = OH



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The XSA confirmed that the structures proposed for **3** and **4** were correct (Figs. 1 and 2).

Figure 3 shows the third isolated compound, which is a crystalline mixture of two compounds, stizolin (**1**) and 11,13-dihydrostizolin (**5**). This was also confirmed by the PMR spectrum. 11,13-Dihydrostizolin was isolated from a natural source for the first time. It was described earlier as the product of reacting **1** and $NaBH_4$ [6].

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TABLE 1. Crystal Data and Refinement Parameters for **1-5**

Parameter	**3**	**4**	**1**+**5**
Empirical formula	$C_{20}H_{26}O_6$	$C_{20}H_{27}O_7$	-
System	Monoclinic	Monoclinic	Orthorhombic
Space group	$P2_1$	$P2_1$	$P2_12_12_1$
Instrument	Syntex $P2_1$	Bruker P4	Bruker P4
Radiation, scanning mode	Mo Kα, θ/2θ	Mo Kα, θ/2θ	Mo Kα, θ/2θ
Scanning region, θ (°)	2.35-25.02	2.32-25.00	2.14-25.00
Cell constants			
a, Å	8.320 (3)	8.358 (1)	7.2737 (7)
b, Å	6.816 (2)	6.813 (1)	10.138 (1)
c, Å	17.606 (7)	17.736 (3)	19.036 (2)
β, (°)	99.53 (3)	98.43 (1)	90
Cell volume, Å^3	984.6 (6)	999.0 (3)	1403.6 (2)
Z, ρ (calc.), g/cm^3	2, 1.222	2, 1.261	4, 1.241
μ, mm^{-1}	0.090	0.095	0.089
Crystal dimensions, mm	1.00×0.54×0.17	0.43×0.32×0.04	0.75×0.26×0.26
Number of reflections, meas./ind.	1940/1879	2047/1908	1448/1448
Absorption calc.	From facetting	From facetting	-
Transmission	0.9837-0.9503	0.9962-0.9603	-
Number of reflections with $I > 2\sigma(I)$	1458	1361	1197
Number of refined parameters	236	246	199
R_1 for [F>4σ (F)]	0.0420	0.0640	0.0391
wR_2 for all reflections	0.1002	0.1923	0.1126
GOOF	1.037	1.032	1.047
Extinction coefficient	0.018 (6)	0.03 (1)	0.003 (2)

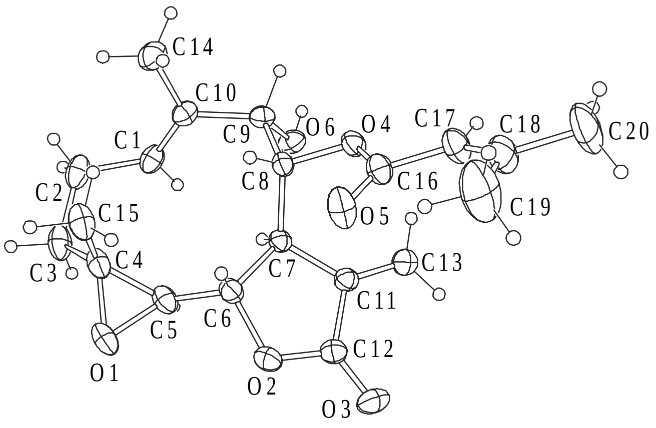

Fig. 1. Molecular structure of **3**.

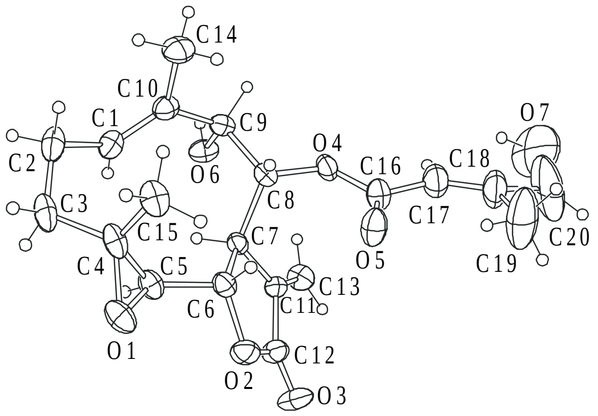

Fig. 2. Molecular structure of **4**.

The conformations of the 10-membered rings in **3** and **4** are practically the same and similar to that in the literature for the *p*-bromobenzoate of tomenphantin A [12]. The conformation of the 10-membered ring in the **1**+**5** cocrystal differed slightly from the two preceding ones, especially the C4-C5-C6-C7 and C6-C7-C8-C9 angles (Tables 1 and 2). However, it is similar to that in eupahyssopin diacetate [13]. The orientations of the esters in **3** and **4** are practically the same (Tables 1 and 2).

TABLE 2. Selected Torsion Angles (deg) in **3**, **4**, **1**+**5**

Angle	**3**	**4**	**1**+**5**	[12]	[13]
C(10)-C(1)-C(2)-C(3)	-115.8 (4)	-115.5 (7)	-104.1 (4)	-115.5	-97.8
C(1)-C(2)-C(3)-C(4)	44.5 (5)	43.7 (8)	51.3 (4)	45.6	51.9
C(2)-C(3)-C(4)-C(5)	-93.6 (4)	-92.0 (7)	-84.6 (4)	-97.7	-82.6
C(3)-C(4)-C(5)-C(6)	143.1 (3)	143.0 (6)	146.5 (3)	147.6	146.3
C(4)-C(5)-C(6)-C(7)	-99.5 (4)	-101.1 (7)	-134.1 (3)	-100.5	-133.2
C(5)-C(6)-C(7)-C(8)	100.8 (3)	102.1 (6)	98.4 (3)	95.2	97.8
C(6)-C(7)-C(8)-C(9)	-122.1 (3)	-122.5 (5)	-82.6 (3)	-121.0	-77.7
C(7)-C(8)-C(9)-C(10)	82.7 (3)	81.8 (6)	70.6 (3)	88.2	57.6
C(2)-C(1)-C(10)-C(9)	165.2 (3)	165.1 (6)	170.2 (3)	162.9	173.4
C(8)-C(9)-C(10)-C(1)	-86.1 (4)	-84.6 (7)	-111.6 (4)	-87.1	-111.1
C(7)-C(8)-O(4)-C(16)	-73.6	-75.3	-	-76.8	140.1

TABLE 3. Antimicrobial Activities

Sample	Diameter of growth inhibition zone, mm			
	Staphylococcus aureus	*Bacillis subtilis*	*Esherichia coli*	*Candida albicans*
3	14±0.2	16±0.1	14±0.2	15±0.1
4	13±0.1	15±0.3	15±0.1	16±0.2
Gentamicin	26±0.1	24±0.1	23±0.2	
Nystatin				22±0.1

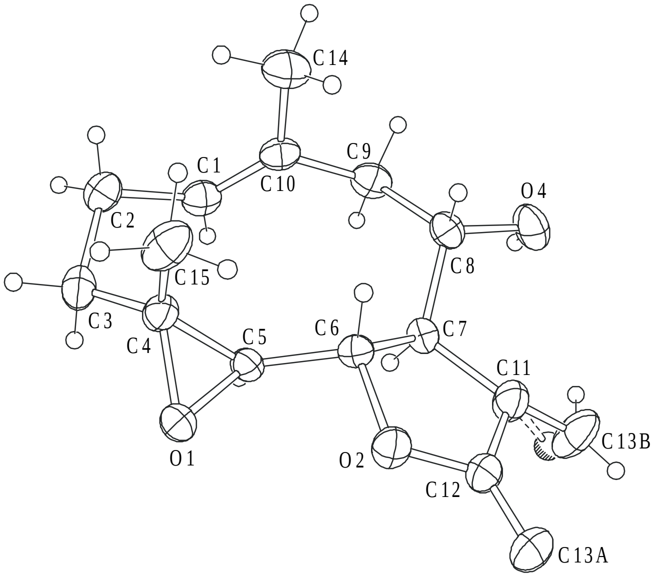

Fig. 3. XSA of the crystalline mixture of **1** and **5** (atoms that do not coincide: **C13B**, C-13 in **1**; **C13A**, C-13 in **5**).

The lactone rings in the studied compounds are rather planar. The mean-square deviation of the atoms from the plane of the ring is about 0.046 Å in **4**, 0.075 Å in **1**+**5**. The bond lengths in the studied compounds are normal. The C7–C8 bond [1.556(4) and 1.561(7) Å] in germacranes **4** and **3** is slightly longer than that in the **1**+**5** cocrystal [1.541(4) Å]. Apparently this is due to replacing the hydroxyl by the ester.

The supramolecular one-dimensional motif in **3** and **4** is the same. Infinite head-to-tail chains are oriented along the *a* axis through OH...O-bonds with parameters O6–H...O3: H...O 1.96, 1.96; O...O 2.764(4), 2.766(6) Å; O–H...O 168, 168°.

The H4...O3 and O4...O3 distances in cocrystal **1**+**5** are increased to 2.52 and 3.241(4) Å. These are not typical of an ordinary H-bond.

The antimicrobial and antitrichomonal activities of **3** and **4** were investigated.

The diffusion method in agar-agar was used to study the antimicrobial activities [14]. The reference compounds were the antibiotic gentamycin and the fungicide nystatin. Table 3 presents the results.

Table 3 shows that all samples exhibited generally moderate antimicrobial activity toward one or another bacterial strain and antifungal activity toward *Candida albicans*.

The antitrichomonal activities of the samples toward *Trichomonas vaginalis* were studied in liquid nutrient medium. None of the compounds exhibited antitrichomonal activity.

EXPERIMENTAL

IR spectra were recorded on an Avatar 360 instrument (Thermo Nicolet). NMR spectra were recorded in $CDCl_3$ solutions on a Bruker DRX-500 spectrometer (working frequency 500.13 MHz for ^1H, 125.76 MHz for ^{13}C).

High-resolution mass spectra (EI, 70 eV) were obtained in a Finnigan MAT 8200 instrument. Optical rotation was measured (at 580 nm) on a Polamat A polarimeter. Column chromatography was performed over SiO_2 (Armsorbsil 100/160) using benzene:ethylacetate mixtures with the volume of the latter increasing from 0 to 100%. TLC used Silufol plates that were developed with saturated aqueous $KMnO_4$ solution. Melting points were determined on a Boetius instrument.

The aerial part of *S. balsamita* was collected in Kurdai Region of South-Kazakhstan District on June 27-30, 2003, was dried in air without exposure to direct sunlight, and was ground. The herbarium index of the sample is 2003.06.28.01.01 (Herbarium Foundation of the Phytochemistry Institute, MES, RK). A weighed portion of raw material (3 kg) was soaked three times with hot water. The aqueous extract was exhaustively extracted with $CHCl_3$. The solvent was removed. The resulting resin (104.6 g) was separated by column chromatography. Oily substances that were not lactones (IR spectrum) were found in the first fractions.

9*α*-Epibalsamin (3). Elution of the column by benzene:ethylacetate (75:25) produced colorless crystals of **3**, 1.28 g (0.043% yield based on air-dried raw material), mp 227-223°C, $[\alpha]_{580}^{20}$ -168°.

IR spectrum (KBr, ν, cm^{-1}): 3404 (OH), 1746 (γ-lactone C=O), 1698 (C=C).

PMR spectrum (500 MHz, δ, $CDCl_3$) agreed with that published [11].

Crystalline Mixture of Lactones 1 and 5. Elution of the column by benzene:ethylacetate (65:35) gave colorless crystals (mixture of **1** and **5**), 0.06 g (0.02% yield based on air-dried raw material), mp 185-190°C.

IR spectrum (KBr, ν, cm^{-1}): 3541 (OH), 2978, 2939, 2906, 2869, 1749 (γ-lactone C=O), 1645 (C=C), 1443, 1391, 1336, 1285, 1270, 1214, 1200, 1188, 1151, 1121, 1064, 1030, 1001, 963, 951, 894, 881, 838.

PMR spectrum contained all signals for **1** and **5** given in the literature [6].

Lactone 4. Elution of the column by benzene:ethylacetate (25:75) gave fractions in which colorless crystals of **4** formed, 0.40 g (0.013% yield based on air-dried raw material), mp 210-213°C, $[\alpha]_{580}^{20}$ -77°.

Mass spectrum (*m*/*z*, *I*, %): 378 (1) $[M]^+$, 363 (5), 295 (19), 279 (6), 262 (22), 244 (10), 234 (7), 226 (4), 219 (5), 215 (10), 204 (21), 201 (7), 199 (3), 191 (8), 187 (9), 179 (37), 175 (12), 173 (8), 169 (6), 165 (20), 162 (16), 159 (10), 157 (5), 151 (13), 149 (15), 139 (13), 135 (13), 125 (13), 123 (18), 117 (11), 109 (27), 107 (17), 105 (14), 100 (35), 95 (3), 93 (24), 91 (21), 84 (48), 82 (57), 79 (18), 77 (15), 71 (67), 69 (50), 65 (9), 55 (53), 53 (37), 43 (100), 41 (55), 39 (35).

IR spectrum (KBr, ν, cm^{-1}): 3420 (OH), 2994, 2940, 2870, 1745 (γ-lactone C=O), 1657 (C=C), 1440, 1385, 1361, 1338, 1293, 1250, 1223, 1151, 1105, 1059, 1018, 1003, 968, 947, 889, 868, 848, 835, 820, 798, 749, 668, 618, 582.

^{13}C NMR ($CDCl_3$, 125.76 MHz): 111.82 (d, C-1), 35.89 (t, C-2), 23.52 (t, C-3), 60.68 (s, C-4), 75.47 (d, C-5), 75.47 (d, C-6), 80.64 (d, C-7), 66.76 (d, C-8), 39.99 (d, C-9), 133.66 (s, C-10), 160.49 (s, C-11), 169.61 (s, C-12), 125.54 (t, C-13), 17.09 (q, C-14), 15.63 (q, C-15), 166.06 (s, C-1′), 124.64 (d, C-2′), 133.49 (s, C-3′), 66.61 (t, C-4′), 13.9 (q, C-5′).

PMR spectrum agreed with that described for lactone **4** [11].

X-ray structure analysis was performed on a Bruker P4 diffractometer (Mo Kα-radiation, graphite monochromator, 2θ/θ-scanning, 2θ < 50°).

Table 1 lists the crystallographic data for **3**, **4**, and **1**+**5** and the parameters of the x-ray diffraction experiments. Note that **3** and **4** are isostructural. The structures were solved by direct methods using the SHELXS-97 programs and were refined by anisotropic-isotropic least-squares methods using the SHELXL-97 program. Positions of H atoms, with the exception of

O4–H in **1**+**5**, the position of which was found in a difference synthesis, were calculated geometrically and refined in agreement with the positions of the heavy atoms.

The large thermal factors of the atoms in the ester substituent in the 4-position of **4** imposed geometric limitations on the refined interatomic distances for this substituent. For this same reason it was practically impossible to determine the type of terminal atom. According to the NMR, this was hydroxyl O-7, which did not form H-bonds in the crystal.

It should be noted that the refined structure of **1**+**5** contains a pyramidal C-11 with a C11–C13 bond length of about 1.4 Å, which is intermediate between double and single bonds. This fact and the peaks in the difference synthesis led to the conclusion that **1** and **5** had cocrystallized in a 53(3):47(3) ratio according to the final refinement. For this reason limitations were imposed on the refined C11–C13A and C11–C13B distances.

The XSA data were deposited as CIF files in the Cambridge Crystallographic Data Center (CCDC 273875, 273876, and 273874 for **3**, **4**, and **1**+**5**, respectively).

Antimicrobial Activity. The antimicrobial activities of **3** and **4** were studied toward gram-positive bacteria strains *Staphylococcus aureus*, *Bacillis subtilis*, the gram-negative strain *Escherichia coli*, and the yeast fungus *Candida albicans*. The studied samples were dissolved in ethanol (96%, 1 mg/mL). The references were the broad-spectrum antibiotic lincomycin (30% aqueous solution) and the fungicide nystatin (0.5 mg/mL).

Cultures were grown in liquid medium at pH 7.3 ± 0.2 at 30-35°C for 18-20 h and were started 1:1000 in sterile isotonic NaOAc solution (0.9%). They were placed (1 mL) in dishes with the corresponding selected nutrient media for the studied test-strain and innoculated by the "uniform field" method. Depressions 6.0 mm in size were formed on the agar surface after drying. Solutions of the studied samples and references (10 μL) were placed in these. The control was ethanol (96%) in equal volumes. Cultures were incubated at 37°C. Growing cultures were counted after 24 h.

The antimicrobial activities of the samples were estimated from the diameter of the growth-inhibited zones of the test-strains (mm). Zones less than 10 mm in diameter and uniform growth in the dish were considered indicative of a lack of antimicrobial activity; 10-15 mm, weak activity; 15-20, moderate activity, >20 mm, distinct activity. Each sample was tested in three parallel experiments. Statistical treatment used parametric statistics methods with calculation of the arithmetic mean and its standard deviation.

ACKNOWLEDGMENT

We thank the RFBR (project 02-07-90322) for financial assistance in obtaining a license for the Cambridge Crystallographic Database.

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