STRUCTURE AND BIOLOGICAL ACTIVITY OF α-SANTONIN CHLORO-DERIVATIVES

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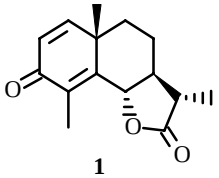
2-Chloro-4,5α-epoxy-α-santonin and 2-chloro-α-santonin, the molecular structure of which was confirmed by an XSA, were produced by reacting α-santonin and chlorine in aqueous acetonitrile. It was found that 2-chloro-α-santonin is highly effective against trichomonas.

Key words: sesquiterpene lactones, α-santonin, chlorination, XSA, NMR.

One of the most efficient synthetic pathways to chloro-derivatives of terpenoids is the reaction of terpene enones with gaseous chlorine [1-3]. Japanese researchers observed that the chemo- and stereoselectivity of the reaction of chlorine with α-santonin (**1**) depended on the nature of the solvent [1].



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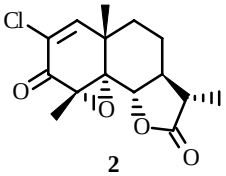

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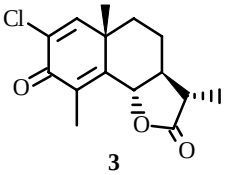

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In order to expand the list of available potentially biologically active chloro-derivatives of lactone **1**, we carried out for the first time the chlorination of **1** in aqueous acetonitrile. Two reaction products, chloroepoxide **2** and chloride **3**, were isolated in yields of 20 and 60%, respectively.

The structures of chlorination products **2** and **3** were confirmed by UV, IR, and PMR spectra (see Experimental). The molecular structure of **3** was established by an x-ray structure analysis (XSA). It has the structure 2-chloro-3-oxo-7α,6,11β(H)-eudesm-1,4-dien-12,6-olide (Fig. 1).

Six-membered rings A and B are pseudo-*trans*-fused. Ring A is planar within 0.02 Å. Ring B has the 7α,10β-chair conformation. Ring B is *trans*-fused to the lactone (C). Ring C has the 7α-envelope conformation. The conformation of **3** is similar to that of the previously studied 2-bromo-α-santonin [4]. Differences in the corresponding torsion angles are less than 2.8°. Table 1 gives the atomic coordinates of **3**.

The preparation in two steps of **3** from **1** by chlorination in $CHCl_3$ followed by dehydrochlorination in the presence of *N*,*N*-dimethylaniline has been described [1]. Four steps were required to prepare **2** [1].

The reason that **3** forms upon chlorination in aqueous CH_3CN is that the solvent itself is weakly basic. Scheme 1 shows the probable pathway for formation of **2**. The appearance of the epoxy group in the 4,5-position can be explained by the presence of water in the reaction mixture. In order to check the correctness of this hypothesis, we carried out 10 model experiments using weighed portions (25 mg) of **1** for each experiment and CH_3CN:H_2O solvent mixtures with water content from 0 to 50%. Then, chlorination was carried out under identical conditions until starting **1** was fully converted. The solvent was distilled off, The solid was extracted with ethylacetate. The resulting extracts were left in vials until the solvent was completely evaporated. The solids were analyzed by HPLC. Table 2 gives the analytical results.

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TABLE 1. Atomic Coordinates (Fractional × 10^4) of **3**

Atom	x	y	z
Cl-1	310 (1)	17 (1)	1063 (1)
O-1	-220 (3)	-3179 (3)	-1705 (1)
O-2	-1697 (4)	-4441 (3)	-2410 (1)
O-3	231 (4)	-3180 (3)	546 (1)
C-1	286 (5)	730 (4)	-59 (1)
C-2	276 (5)	-418 (4)	328 (1)
C-3	244 (4)	-2143 (4)	176 (1)
C-4	241 (4)	-2536 (3)	-446 (1)
C-5	155 (4)	-1361 (3)	-840 (1)
C-6	-135 (4)	-1546 (3)	-1479 (1)
C-7	-1989 (5)	-828 (4)	-1646 (1)
C-8	-1947 (6)	989 (4)	-1559 (1)
C-9	-1525 (5)	1305 (4)	-919 (1)
C-10	209 (4)	434 (3)	-691 (1)
C-11	-2282 (5)	-1560 (4)	-2238 (1)
C-12	-1429 (5)	-3202 (5)	-2150 (1)
C-13	-4237 (6)	-1660 (6)	-2450 (2)
C-14	1972 (5)	1195 (4)	-951 (2)
C-15	278 (6)	-4328 (4)	-571 (2)

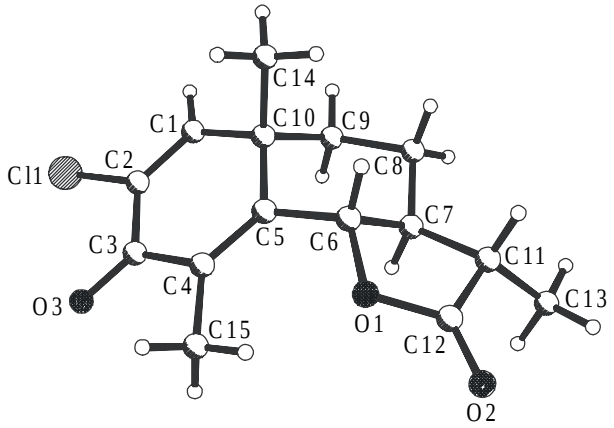
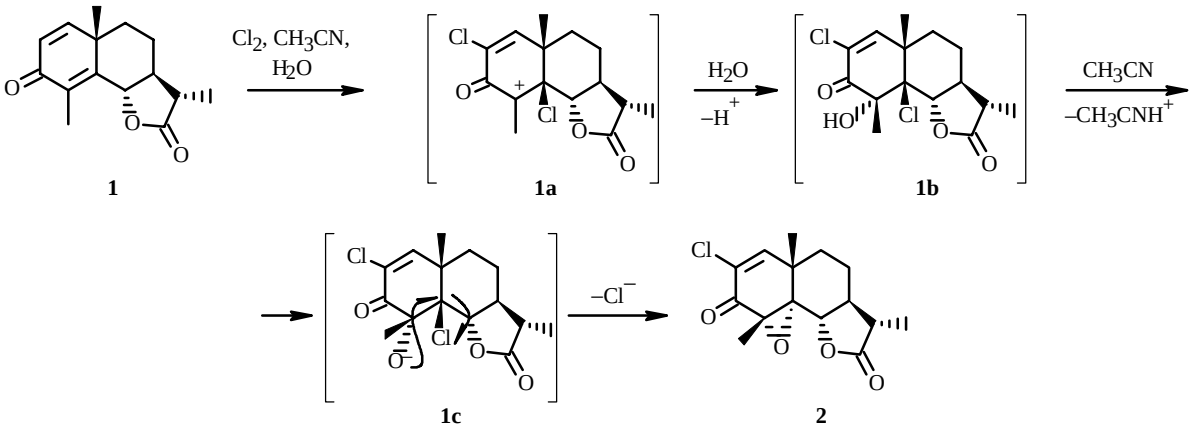


Fig. 1. Molecular structure of **3** from XSA.



Scheme 1.

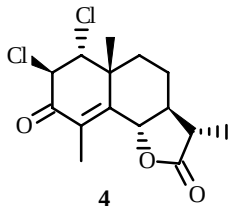
TABLE 2. Content of **2** and **3** (HPLC) in Chlorination Product Mixtures of α-Santonin (**1**) as a Function of Solvent Water Content

Solvent: $CH_3CN:H_2O$, ratio	Content in total products	
	2	**3**
100:0	0	85.04
95:5	1.93	78.93
90:10	7.09	73.21
85:15	14.89	67.71
80:20	22.15	61.95
75:25	28.91	57.03
70:30	32.08	51.84
65:35	39.45	46.38
60:40	46.28	42.0
50:50	52.20	36.51

Reaction time 45 min.

The results of model experiments on α-santonin chlorination in $CH_3CN:H_2O$ mixtures and a study of the qualitative and quantitative composition of the reaction mixtures by HPLC agreed with the proposed mechanism and the effect of water on the yield of **2**.

The biological activities were studied by the literature method [5]. We also prepared by published methods [1] the two known α-santonin chloro-derivatives 1α,2β-dichlorosantonin (**4**) and 5β-chloro-4α-methoxysantonin (**5**).



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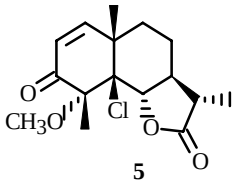

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Compound **3** was also prepared by a previously unpublished method using dehydrochlorination of **4** by boiling in pyridine.

α-Santonin chloro-derivatives **2-5** were screened in vitro for antitrichomonal activity toward *Trichomonas vaginalis*. The screening results show that derivative **3** is notable because the reference preparation did not exhibit activity toward the test-strain at the tested doses.

Compound	200 $\mu g/mL$
1	Resistant
2	Slight decrease in trichomonad amount
3	Trichomonas absent
4	Resistant
5	Slight decrease in trichomonad amount
Metronidazole	Resistant

All compounds lacked antitrichomonal activity at 50 μg/mL. At 100 μg/mL, only **3** caused a quantitative decrease in trichomonas with destruction of the membrane integrity.

α-Santonin does not possess antitrichomonal activity. Adding a methoxy and an epoxide ring with a Cl atom on C-2 slightly decreased the activity. The activities of the tested compounds were completely lost or reduced without double bonds at C-1 and C-4.

The results showed that a Cl atom on C-2 in the dienone fragment of α-santonin is required for antitrichomonal activity to be exhibited.

The XSA data were deposited as CIF files in the Cambridge Crystallographic Data Centre (CCDC281686).

EXPERIMENTAL

α-Santonin (**1**), mp 169-171°C (ethylacetate), $[\alpha]_D^{20}$ -172° (*c* 0.2, MeOH) was isolated from the aerial part of *Artemisia cina* Berg. [6] and used for the chemical modifications.

The course of reactions and purity of products were monitored using TLC on Silufol plates with development by spraying with vanillin (1%) in H_2SO_4 and aqueous $KMnO_4$ (1%).

The resulting derivatives were purified by column flash-chromatography over silica gel (Chemapol, 40/100) with elution by petroleum ether:ethylacetate mixtures with increasing (from 0 to 50%) content of the latter.

IR spectra were recorded on a Vector 22 instrument; NMR spectra, on a Bruker DRX-500 spectrometer (working frequency 500.13 MHz for ^1H; 125.76 MHz for ^{13}C). Signals were assigned using ^{13}C NMR spectra recorded using J-modulation (proton decoupling and opposite phase for signals with even and uneven numbers of bonded protons with tuning at J = 135 Hz).

The XSA was performed on a Bruker P4 diffractometer (Mo Kα-radiation, graphite monochromator, 2θ/θ-scanning, 2θ < 55° and 50°). The structure was solved by direct methods using the SHELXS-97 program. The positions of H atoms were calculated geometrically. The final structure factors were refined over all F^2 by anisotropic-isotropic (for H atoms) full-matrix least-squares methods using the SHELXS-97 program.

The α-santonin chloro-derivatives were analyzed by HPLC using a High Pressure Laboratory Chromatograph (Laboratorni Pristroje Prana, Czech Rep.), a steel column (150 × 4 mm, Elsiko, Moscow), Separon SGX C18 stationary phase (5 μm), UV detection at 254 nm, and sample volume 20 μL. The mobile phase was CH_3OH:H_2O (1:1) at 20 MPa and flow rate 1 mL/min.

Chlorination of α-Santonin (1) (General Method). A stream of dry gaseous Cl_2 was passed through a solution of **1** (1.0 g, 4 mmol) in aqueous CH_3CN (15 mL) at 20°C until the starting material was completely converted (about 1 h according to TLC). The solvent was evaporated using a water aspirator. The solid was chromatographed over a column of silica gel (KSK, <0.3 mm) at a 1:10 compound:sorbent ratio using petroleum ehter:ethylacetate mixtures with increasing content (from 0 to 50%) of the latter.

Lactone 2. Colorless crystalline compound, mp 236-238°C (ethylacetate), yield 0.24 g (20%).

UV spectrum (EtOH, λ_{max}, log ε, nm): 221 (3.72), 252 (3.88).

PMR spectrum (acetone-d_6, δ, ppm, J/Hz): 6.90 (s, H-1), 4.84 (d, J = 12.0, H-6), 3.06 (dddd, J = 12.0, 12.0, 12.0, 4.0, H-7), 2.18 (dddd, J = 13.0, 4.5, 4.0, 2.5, H-8a), 1.89 (dddd, J = 13.0, 13.5, 12.0, 4.5, H-8b), 2.83 (dddq, J = 14.0, 13.5, 4.5, 1.3, H-9a), 2.03 (ddd, J = 14.0, 2.5, 4.5, H-9b), 2.58 (dq, J = 12.0, 7.0, H-11), 1.22 (d, 3H, J = 7.0, H-13), 1.67 (d, 3H, J = 1.3, H-14), 2.11 (s, 3H, H-15).

HPLC: retention time t_R = 41.84 min, 91.74% pure.

Lactone 3. Colorless crystals, mp 235-237°C (ethylacetate), yield 0.68 g (60%).

IR spectrum (KBr, ν, cm^{-1}): 2972, 2943, 2877 (CH), 1779 (C=O), 1767 (γ-lactone C=O), 1655 (C=C), 1617, 1455, 1379, 1355, 1331, 1296, 1272, 1243, 1211, 1175, 1145, 1104, 1066, 1038, 994, 966, 928, 893, 870, 843, 787, 766, 731, 658, 607 (C–Cl), 579, 554, 507.

UV spectrum (EtOH, λ_{max}, nm, log ε): 250 (4.16).

PMR spectrum (acetone-d_6, δ, ppm, J/Hz): 7.17 (s, 1H, H-1), 5.05 (dq, 1H, J = 11.0, 1.3, H-6), 1.93 (dddd, 1H, J = 12.0, 11.5, 11.5, 3.5, H-8a), 1.86 (dddd, 1H, J = 12.0, 12.0, 12.0, 4.0, H-8b), 2.05-2.10 [overlapping s, CH_3 (15), H-9a], 1.61 (dddq, 1H, J = 13.0, 13.0, 4.5, 0.7, H-9b), 2.62 (dq, 1H, J = 12.0, 7.0, H-11), 1.19 (d, 3H, J = 7.0, H-13), 1.44 (d, 3H, J = 0.7, H-14), 2.09 (d, 3H, J = 1.3, H-15).

^{13}C NMR spectrum (acetone-d_6, δ, ppm, J/Hz): singlets at 130.69 (C-2), 179.89 (C-3), 127.76 (C-4), 153.79 (C-5), 44.16 (C-10), 177.87 (C-12); doublets at 152.41 (C-1), 81.35 (C-6), 41.08 (C-7), 54.35 (C-11); triplets at 23.17 (C-8), 38.71 (C-9); quartets at 11.69 (C-13), 12.65 (C-14), 25.07 (C-15).

HPLC: retention time t_R = 19.38 min, purity 98.4%.

Lactone 4. Colorless crystals, mp 236-238°C (methanol), yield 1.10 g (85%).

IR spectrum (KBr, ν, cm^{-1}): 2973, 2932, 2869, 1772 (γ-lactone C=O), 1697, 1625 (C=O, C=C), 1461, 1381, 1355, 1323, 1310, 1295, 1277, 1229, 1201, 1167, 1154, 1134, 1097, 1068, 1029, 982, 905, 870, 840, 822 (C–Cl), 767, 754, 727, 692, 651, 610 (C–Cl), 595, 581, 543, 510.

UV spectrum (EtOH, λ_{max}, nm, log ε): 251 (4.10).

PMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 4.68 (d, 1H, J = 4.0, H-1), 5.43 (d, 1H, J = 4.0, H-2), 4.38 (d, 1H, J = 4.0, H-6), 2.05 (s, 3H, CH_3-4), 1.60 (s, 3H, CH_3-10), 1.37 (d, 3H, J = 11.0, CH_3-11).

HPLC: retention time t_R = 37.20 min, purity 98.05%.

Lactone 5. Amorphous compound, mp 232-234°C (ethylacetate), yield 0.83 g (65%).

IR spectrum (KBr, ν, cm^{-1}): 1782 (γ-lactone C=O), 1691.

UV spectrum (EtOH, λ_{max}, nm, log ε): 231 (3.80).

PMR spectrum (acetone-d_6, δ, ppm, J/Hz): 6.30 (d, 1H, J = 11.0, H-1), 5.89 (d, 1H, J = 11.0, H-2), 4.41 (d, 1H, J = 12.0, H-6), 3.10 (s, 3H, CH_3O-5), 1.69 (s, 3H, CH_3-4), 1.48 (s, 3H, CH_3-10), 1.23 (d, 3H, J = 7.0, CH_3-11).

HPLC: retention time t_R = 34.89 min, purity 90.7%.

Antitrichomonal activity *in vitro* was investigated in liquid nutrient medium CPLM, to which at 37°C were added the compounds at doses of 50, 100, and 200 μg/mL (3% ethanol solvent did not cause visible changes in the vitality of the specimens). Then isolates of *Trichomonas vaginalis* obtained two days (incubation at 37°C) after isolation from mucus membranes of urogenital organs of patients suffering from trichomonas were added. The tubes were incubated at 37°C and investigated after 20 and 240 h in a Goryaev chamber. The mobility, amount, and presence or absence of flagella were evaluated. The reference preparation was metronidazole at analogous concentrations [5].

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