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PREPARATION OF α-SANTONIN AND ESTAFIATIN DERIVATIVES AND THEIR EFFECT ON BIOCHEMILUMINESCENCE KINETICS

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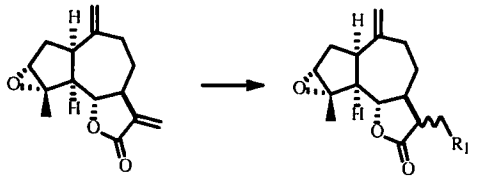
The anti-oxidant activity (AOA) of α-santonin, estafiatin, and their derivatives is studied by induced biochemiluminescence. A relationship is found between the AOA and molecular structure of the synthesized compounds.

One explanation for many pathological processes is the development of free-radical oxidation (FRO) in an organism [1]. Despite the widespread notion that phenolic compounds exhibit anti-oxidant activity (AOA), several studies have demonstrated that peroxide oxidation of lipids (POL) is inhibited *in vitro* by sesquiterpene lactones and their derivatives [2-4]. The AOA *in vitro* of the sesquiterpene lactones alantolactone and isoalantolactone in addition to the potential AOA of compounds of plant origin have been reported [5]. The ability to estimate the inhibition has been demonstrated [6].

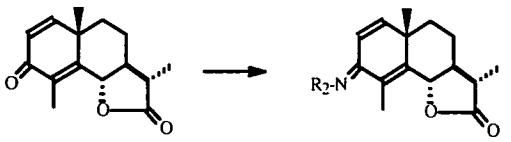
We chemically modified estafiatin (**1**) in order to study the ability to alter the AOA of sesquiterpene lactones. In absolute ethanol, **1** reacts with amines to produce Michael addition products, in particular, with morpholine, piperidine, and benzylamine, according to the reaction:



1 **2-4**

2: R_1= -N$(CH_2)_2$O
3: R_1= -N⟨⟩
4: R_1= -NH-CH_2-C_6H_5

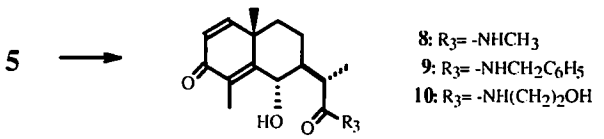
Another sesquiterpene lactone, α-santonin (**5**), was also used in condensation reations. According to the literature [7], it can be used to synthesize estafiatin. Condensation of α-santonin at the carbonyl group forms the imines **6** and **7**:



5 **6, 7**

6: R_2= -OH
7: R_2=NH-CO-NH_2

The lactone ring of α-santonin was opened to give compounds **8-10**:



8 -10

8: R_3= -$NHCH_3$
9: R_3= -$NHCH_2C_6H_5$
10: R_3= -$NH(CH_2)_2$OH

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TABLE 1. ICL Parameters of Lipids in the Presence of Compounds **1-10**

Compound	N, arb. units	τ, min	slope	N, arb. units
1	2.11±0.08	2.9±0.14	3.2±0.19	7.0±0.61
2	5.5±0.5	1.57±0.07	7.0 ±0.28	32.5±2.15
3	5.75±0.6	1.8±0.09	4.5 ±0.29	16.25±1.5
4	5.41±0.4	1.7±0.11	5.6 ±0.22	17.5±1.4
5	2.75±2.01	1.86±0.12	6.64 ±0.58	6.41±0.44
6	2.4±0.11	2.0±0.08	8.0 ±0.05	15.2±1.2
7	2.5±0.12	1.6±0.09	4.0 ±0.15	10.8±1.1
8	3.6±0.09	1.0±0.08	9.0±0.08	43.75±2.1
9	3.0±0.1	2.1±0.07	6.0±0.11	9.0±0.08
10	3.5±0.19	1.5±0.06	9.0±0.07	37.5±3.5
Ionol	2.17±0.13	7.64±0.15	2.69±0.13	6.34±0.51
Control	2.6±0.1	2.0±0.09	3.5±0.29	7.1±0.55

Estafiatin derivatives lose the AOA activity of the lactone itself (Table 1). In particular, the decrease in the latent period by 1.6 times and greater on going from **1** to its derivatives **2-4** can be explained by the lack of labile H atoms on the exo-methylene group, which apparently inhibits FRO. This was demonstrated using alantolactone and isoalantolactone as examples. The induced chemiluminescence (ICL) parameters of all estafiatin derivatives change substantially. The rapid and slow light pulses increase and the rate of FRO *in vitro* increases steadily. The anti-oxidant ionol (0.19 mM) was used as a standard. We found a significant increase of pro-oxidation activity for derivative **5** if a methyl group was introduced into it (**8**). In this instance, the intensity of slow luminescence increases by 6.16 times compared with the control. An analogous increase in ICL is observed for compounds **2** and **10**, which contain the morpholine ring. The slow luminescence increases by 4.0 and 5.23 times, respectively.

The slope corresponding to the rate of slow luminescence increases for compounds **2**, **8**, and **10** (Table 1). The induction period uncharacteristically remains the same for the whole series of studied exogeneous anti- and pro-oxidants. However, the latent period of the synthetic anti-oxidant ionol (c = 0.19 mM) increases by 3.8 times compared with the control.

Thus, the study of the effect of estafiatin, α-santonin, and their derivatives on ICL established a relationship between the molecular structure and their AOA. This makes it possible to modify them chemically in order to increase the biological activity.

EXPERIMENTAL

The AOA of all compounds was estimated by ICL of a 10% homogenate of laboratory animal brain tissue [8]. All compounds were dissolved in ethanol (10 mg/ml).

Estafiatin is a colorless crystalline compound, $C_{15}H_{18}O_3$, mp 102-104°C (ether), $[\alpha]_D^{20}$ = -10.3° (c = 0.01, $CHCl_3$), that is isolated from noble yarrow (*Achillea nobilis* L.) by the literature method [9].

α-Santonin is a colorless crystalline compound, $C_{15}H_{18}O_3$, mp 169-171°C (ether), $[\alpha]_D^{20}$ = -108.6° (c = 0.01, $CHCl_3$), that is isolated from *Artemisia gracilescens* by the literature method [10].

Melting points were determined on a Boetius stage.

IR spectra were recorded on a UR-20 spectrometer (KBr pellets, $CHCl_3$ solutions); UV spectra, on a SF-26 instrument in C_2H_5OH; PMR spectra, on a Bruker WP-200 SY instrument in $CDCl_3$.

Elemental analyses of synthesized compounds agreed with those calculated.

3,4-Epoxy-13-morpholin-guai-10(14)-en-6,12-olide (2). A solution of estafiatin (**1**, 300 mg, 1.2 mmole) in absolute ethanol (2.25 ml) was treated with morpholine (0.152 ml, 1.4 mmole). The reaction mixture was stirred in boiling alcohol for 1 h. Ethanol was removed under vacuum. The solid was recrystallized. Yield of compound **2**, 332 mg, 83%, $C_{19}H_{27}O_4N$, mp 74-76°C (ethanol), R_f 0.32 (ethylacetate—hexane, 3:2), $[\alpha]_D^{20}$ = -2.91° (c = 0.05, $CHCl_3$).

IR spectrum (ν, cm^{-1}): 2945, 1770, 1640, 1460, 1310, 1180, 1125, 1080, 920, 880, 835.

Calc., %: C 68.46, H 8.10, N 4.20.

Found, %: C 68.36, H 8.21, N 4.11.

PMR spectrum (δ, ppm, $CDCl_3$): 1.59 s (3H, CH_3-4), 2.01 m (2H, H-13a, H-13b), 2.89 br. s (1H, H-3), 3.12 t (1H, J = 10 Hz, H-6), 4.39 d (1H, J = 2.5 Hz, H-14), 4.39 d (1H, J = 2.5 Hz, H-14′), 3.37 m [8H, $N(CH_2)_2O(CH_2)_2$].

3,4-Epoxy-13-piperidin-guai-10(14)-en-6,12-olide (3). A solution of estafiatin (**1**, 300 mg, 1.2 mmole) in ethanol (2.25 ml) at room temperature was treated with stirring with piperidine (0.145 ml, 1.4 mmole). The reaction was carried out for 45 min. The solvent was removed in vacuum. Compound **3** was recrystallized from alcohol. Yield of substance **3**, 338 mg, 85%, mp 85-88°C (ethanol), $C_{20}H_{29}O_3N$, R_f 0.55 (ethylacetate—hexane, 3:2).

IR spectrum (ν, cm^{-1}): 2940, 1770, 1640, 1450, 1175, 1015, 915, 830.

Calc., %: C 72.50, H 8.76, N 4.22.

Found, %: C 72.70, H 8.43, N 4.42.

PMR spectrum (δ, ppm, $CDCl_3$): 1.56 s (3H, CH_3-4), 2.65 br d (2H, J = 2.5 Hz, H-13a, H-13b), 2.87 br s (1H, H-3), 3.12 t (1H, J = 10 Hz, H-6), 4.53 d (1H, J = 2.5 Hz, H-14), 4.53 d (1H, J = 2.5 Hz, H-14′), 2.78 m [10H, $N(CH_2)_5$].

3,4-Epoxy-13-benzylamine-guai-10(14)-en-6,12-olide (4). A solution of estafiatin (**1**, 300 mg, 1.2 mmole) in ethanol (2.25 ml) at room temperature was treated with benzylamine (0.157 ml, 1.44 mmole). The reaction was carried out for 1.5 h. Yield of **4**, 407 mg, 96%, $C_{22}H_{27}O_3N$, mp 97-99°C (alcohol), R_f 0.44 (ethylacetate—hexane, 3:2), $[\alpha]_D^{20} = -1.22°$ (c = 0.05, $CHCl_3$).

IR spectrum (ν, cm^{-1}): 2935, 2865, 1760, 1640, 1455, 1330, 1270, 1185, 1170, 1085, 1025, 1010, 915, 830, 750, 715.

Calc., %: C 74.57, H 7.90, N 3.95.

Found, %: C 74.22, H 7.80, N 3.96.

PMR spectrum (δ, ppm, $CDCl_3$): 1.53 s (3H, CH_3-4), 2.53 m (2H, H-13a, H-13b), 2.84 br s (1H, H-3), 3.03 br t (1H, J = 9 Hz, H-6), 4.50 br s (1H, H-14a), 4.56 br s (1H, H-14b), 3.43 s (1H, NCH_2Ph), 7.09 br s (5H, NCH_2Ph).

α-Santonin Oxime (6). α-Santonin (**5**, 2 g, 8 mmole) was dissolved in methanol (30 ml). Metallic sodium (0.36 g, 16 mmole) was dissolved in methanol (15 ml) in a separate flask and treated with hydroxylamine hydrochloride (1.1 g, 16 mmole) in methanol (20 ml). The NaCl was filtered off. The mother liquor (hydroxylamine) was added to the flask with solution of **5**. The mixture was boiled for one week. The solvent was removed. The solid was chromatographed on a silica-gel column. Yield of compound **6**, 600 mg, 28.3%, $C_{15}H_{19}O_3N$, mp 224-226°C (alcohol), $[\alpha]_D^{22} = +6.4°$ (c = 0.04, $CHCl_3$).

IR spectrum (ν, cm^{-1}): 3430, 2970, 2870, 1730, 1660. PMR spectrum (δ, ppm $CDCl_3$): 1.23 (d, J = 7 Hz, CH_3-11), 1.24 (s, CH_3-10), 2.12 (d, J = 2 Hz, CH_3-4), 2.30 (d, J = 11 and 6 Hz, H-11), 4.76 (d, J = 10 and 2 Hz, H-6), 6.02 (d, J = 9 Hz, H-1), 6.92 (d, J = 9 Hz, H-2).

Eudesm-1(2),4(5)-dien-6,12-olide-3-semicarbazone (7). Semicarbazide (180 mg, 1.6 mmole) and anhydrous NaOAc (130 mg, 1.6 mmole) were boiled in alcohol (3 ml). The boiling mixture was filtered. The filtrate was treated with α-santonin (200 mg, 0.8 mmole) in ethanol (3 ml). The solvent was removed under vacuum. The solid was extracted with ethylacetate. The extract was washed with water and dried over Na_2SO_4. The organic layer was filtered to remove desiccant and evaporated under vacuum. The solid (0.23 g) was mixed with silica gel (2 g) and separated by chromatography.

Elution of the column by a mixture of ethylacetate—benzene (3:7) isolated colorless crystalline $C_{16}H_{22}O_3N_2$ in 70% yield, mp 53-55°C (alcohol), $[\alpha]_D^{20} = +13.5°$ (c = 0.01, ethanol).

IR spectrum (ν, cm^{-1}): 3400, 3000, 2480, 2000, 1750, 1620, 1450, 1300, 1250, 1000, 800, 700.

Calc., %: C 63.1, H 6.78, N 4.8.

Found, %: C 63.6, H 6.2, N 4.28.

PMR spectrum (δ, ppm, $CDCl_3$): 1.14 (3H, s, CH_3-10), 1.14 (3H, d, J = 6 Hz, CH_3-11), 2.43 (3H, s, CH_3-4), 4.87 (1H, d, J = 10 Hz, H-6), 5.04 (3H, br s, $-HNCONH_2$), 6.05 (1H, d, J = 10 Hz, H-1), 7.27 (1H, J = 10 Hz, H-2).

6-Hydroxy-α-santoninic Acid 12-methylamide (8). Reaction of α-santonin with aqueous methylamine in ethanol for 20 h produces amide **8** in 64% yield, colorless crystals, $C_{16}H_{23}O_3N$, mp 159-161°C (ethanol), $[\alpha]_D^{22} = +36.8°$ (c = 0.26, $CHCl_3$).

IR spectrum (ν, cm^{-1}): 3540, 3450, 1680, 1650, 1630, 1620.

PMR spectrum (δ, ppm, $CDCl_3$): 0.96 (s, CH_3-10), 1.35 (d, J = 7.5 Hz, CH_3-11), 2.69 (s, CH_3-4), 2.92 (d, J = 5.0 Hz, $NH\underline{CH_3}$), 3.51 (q, J = 6.5 and 4 Hz, $\underline{NH}CH_3$), 4.76 (br. d, J = 10 Hz, H-6), 6.34 (d, J = 10 Hz, H-2), 6.56 (d, J = 10 Hz, H-1).

α-Santoninic Acid Benzylamide (9). A solution of α-santonin (100 mg, 0.4 mmole) in ethanol (1 ml) was treated with benzylamine (0.044 ml, 0.44 mmole). The reaction was carried out at 70-80°C for 2 h. Solvent was distilled off. The solid was recrystallized. Yield of compound **9**, 70 mg, colorless crystals, $C_{22}H_{27}O_3N$, 50%, mp 176-179°C (ethanol), R_f 0.56

(ethylacetate—hexane, 2:3), $[\alpha]_D^{20} = +99.3°$ ($c = 0.01$, ethanol).

IR spectrum (ν, cm^{-1}): 3550, 3400, 2940, 2300, 1660, 1480, 1450, 1300, 1100, 700.

Calc., %: C 69.9, H 7.9, N 3.9.

Found, %: C 69.39, H 7.16, N 4.0.

α-Santoninic Acid Monoethanolamide (10). A solution of α-santonin (300 mg, 1.2 mmole) in ethanol (2 ml) was treated with monoethanolamine (0.15 ml, 1.4 mmole). The mixture was refluxed for 15 h. The alcohol was removed under vacuum. The solid was dissolved in ethylacetate and treated with aqueous HCl (3%). The organic layer was dried over Na_2SO_4. The desiccant was filtered off. The solvent was distilled. The solid (0.4 g) was mixed with silica gel (8 g) and separated by chromatography. Elution by ethylacetate—benzene (1:1) gave compound **10**, 304 mg, colorless crystals, $C_{17}H_{24}O_4N$, 81%, mp 134-137°C (ethanol), R_f 0.24 (ethylacetate—hexane, 3:2), $[\alpha]_D^{22} = +5.09°$ ($c = 0.04$, $CHCl_3$).

IR spectrum (ν, cm^{-1}): 3540, 3450, 3000, 2800, 2390, 1680, 1660, 1480, 1340, 1270, 1100, 900, 740.

Calc., %: C 66.6, H 7.8, N 4.5.

Found, %: C 66.2, H 7.6, N 4.7.

PMR spectrum (δ, ppm, $CDCl_3$): 1.03 (3H, s, CH_3-10), 1.37 (3H, d, J = 7.5 Hz, CH_3-11), 2.61 (3H, s, CH_3-4), 3.55 [1H, q, J_1 = 7.5, J_2 = 4 Hz, $-CONH(CH_2)_2OH$], 3.78 [1H, q, J_1 = 12.5 Hz, J_2 = 5 Hz, $-CONH(CH_2)_2OH$], 4.01 [1H, t, J = 5 Hz, $-CONH(CH_2)_2OH$], 5.05 [1H, br s, $-CONH(CH_2)_2OH$], 4.77 (1H, br. d, J = 11 Hz, H-6), 6.32 (1H, d, J = 10 Hz, H-1), 6.53 (1H, d, J = 10 Hz, H-2).

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