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MODIFICATION OF THE SESQUITERPENE LACTONES LEUKOMISIN AND AUSTRICIN BIOLOGICAL ACTIVITIES OF SOME OF THEIR DERIVATIVES

A. B. Plutno,[a] I. D. Sham'yanov,[a] M. I. Aizikov,[b] M. R. Prokhorova,[b] G. G. Galyust'yan,[a] and A. G. Kurmukov[b]

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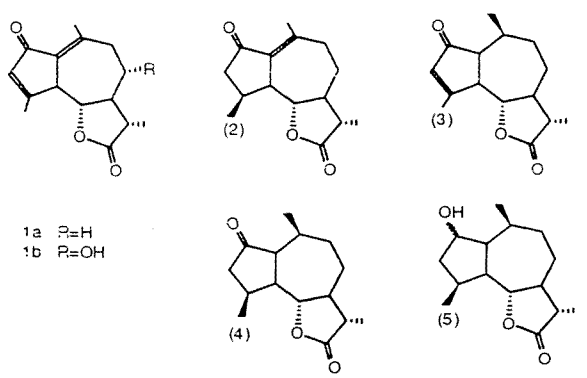
The acylation and relactonization reactions of austricin and the hydrolysis of leukomisin and austricin have been investigated. The results are given of a study of the antihypoxic and antiinflammatory effects of some of their derivatives.

Pharmacological investigations have shown the existence of a high angioprotector and antilipidemic activity in the sesquiterpene lactones leukomisin (**1a**) and austricin (**1b**). The antiatherosclerotic and angioprotector activities of leukomisin exceed those of prodektin, and its hypolipidemic action is comparable with that of miscleron — these drugs being widely used in medical practice [1]. It has also been established that a combination of antihypoxic and antiinflammatory activities gives a greater guarantee of the presence of angioprotector action in a substance [2, 3].

With the aim of studying the relationship between chemical structure and biological activity and also of finding new drugs, we have investigated the chemical modification of the initial molecules (**1a**) and (**1b**). To reveal the influence of various structural fragments of the molecule on the biological activity of a compound of type (**1**), we obtained a series of reduction products: 3,4-dihydro-, 1,10-dihydro-, tetrahydro-, and hexahydroleukomisins (**2**, **3**, **4**, and **5** respectively) [4].



In the present paper we give the results of an investigation of the acylation, hydrolysis, and relactonization of austricin (**1b**), the hydrolysis of leukomisin, and also an account of a comparative study of the antihypoxic and antiinflammatory activities of the hydrogenation products of leukomisin and some other derivatives of compounds (**1a**) and (**1b**).

It is known that in many cases the acylation of hydroxy groups of physiologically active compounds enhances their action [5-7], and modification of the structure of austricin therefore began with acylation. Esters of austricin (**1b**) were obtained by its interaction with acylating agents — benzenesulfonyl chloride and benzoic, succinic, and acetic anhydrides — by the following scheme:

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a) Institute of the Chemistry of Plant Substances, Academy of Sciences of the Republic of Uzbekistan, Tashkent, fax (3712) 89 14 75. b) Scientific-Research Institute of Cardiology, Ministry of Health of the Republic of Uzbekistan, Tashkent.
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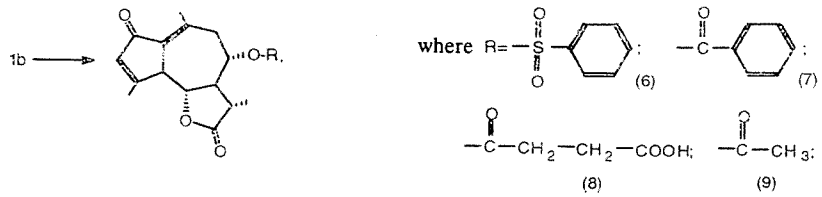


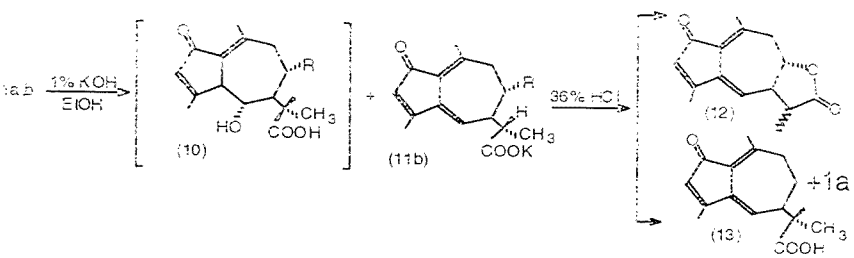
In this series the most active acylating agent proved to be benzenesulfonyl chloride. Reaction by the usual method (in pyridine solution at room temperature) took 15 min with the evolution of heat, by when the austricin (**1b**) had disappeared completely from the reaction mixture. However, under these conditions, a large amount of decomposition products was obtained, which made purification difficult and lowered the yield of desired product. To increase the yield of benzenesulfonylaustricin (**6**), the reaction conditions were varied. It was possible to achieve a considerable decrease in the amount of decomposition products and a corresponding improvement in the yield (to 60%) and in the purity of the product (**6**) by using as solvent a 1:1 mixture of pyridine and benzene.

Acylation by the anhydrides was performed in pyridine under various conditions. In the case of benzoic anhydride, the reaction took place only on heating (to 100°C), with the formation of benzoylaustricin (**7**) in a yield of 60%. Acylation by succinic anhydride was successfully accomplished at room temperature in 14 days. Austricin succinate (**8**) was obtained with a yield of 61%. The reaction of lactone (**1b**) with acetic anhydride was complete in 12 h (at 25°C), with a high yield (96%) and a high purity of the acetylaustricin (**9**).

It must be mentioned that this is the first time that compounds (**6-8**) have been obtained. Acetylaustricin (**9**) has been described previously [8, 9].

It is known that, under the action of bases, lactone rings open with the formation of salts of the corresponding hydroxy acids, the acidification of which leads to the reclosure of a lactone ring. However, depending on the structure, it is not always the initial lactones that are formed: so-called allo- compounds may be produced [10].

We have established that leukomisin (**1a**) and austricin (**1b**) are readily hydrolyzed by 1% solution of KOH in ethanol at room temperature, and subsequent acidification leads to the formation of the products shown in the scheme given below.



As experiments have shown, the behaviors of lactones (**1a**) and (**1b**) in the reactions studied differ substantially. In the first place, the rate of hydrolysis of the lactone ring of austricin proved to be far higher than that of leukomisin. Thus, austricin was hydrolyzed completely after 6 h under the given conditions, while the conversion of leukomisin was approximately 50%. On the hydrolysis of leukomisin followed by acidification of the reaction mixture, together with dehydroleukomisinic acid the initial compound (**1a**) was formed, which permits the unambiguous conclusion of the presence of the intermediate product (**10a**) [sic] in the reaction mixture.

Under analogous conditions, in the case of austricin only dehydroisoleukomisin (**12**) was formed, which showed the complete elimination of the hydroxy group in position 6 of austricin, in contrast to leukomisin.

The product obtained (**12**) was more stable in an alkaline medium than austricin. In the hydrolysis of austricin at pH 10, after six days the potassium salt of dehydroaustricinic acid (**11b**) and compound (**12**) were recorded, the initial (**1b**) being absent.

Thus, the hydrolysis reaction has enabled three new products to be obtained: potassium dehydroaustricinate (**11b**), dehydroleukomisinic acid (**13**), and dehydroisoleukomisin (**12**). The structures of all the derivatives synthesized were confirmed by spectral methods (IR, UV, mass, PMR).

TABLE 1. Antihypoxic and Antiinflammatory Effects of Leukomisin, Austricin, and Their Derivatives

Compound	Antihypoxic action			Antiinflammatory action		
	Prolongation of life, min	AHA, %	p	Degree of inflammation	AIA, %	p
Control	24.2±1.0	–	–	100±6.4	–	–
1b	29.9±2.0	23.5	0.01	51.4±9.4	48.6	0.01
8	30.4±1.9	25.6	0.01	65.4±10.6	47.2	0.001
9	26.4±1.4	9.1	0.1	–	–	–
11b	30.4±2.4	25.6	0.05	71.3±11.1	28.7	0.05
1a	30.7±2.3	26.8	0.05	55.3±9.4	44.7	0.01
2	35.5±2.6	46.7	0.001	52.8±8.6	47.2	0.001
3	31.6±1.8	30.6	0.001	–	–	–
4	30.5±2.3	26.0	0.01	36.2±7.6	63.8	0.001
5	31.4±2.4	29.8	0.01	106.7±10.0	6.7	0.5

To reveal the link between chemical structure and biological activity, we made a comparative study of the antihypoxic and antiinflammatory activities of the initial compounds (**1a**, **1b**) and of a series of the compounds synthesized (**2-5**, **8**, **9**, **11b**) (Table 1). Acylation led to no enhancement of the antihypoxic and antiinflammatory effect (compounds **8** and **9**). Opening of the lactone ring (compound (**11b**) and, especially, exhaustive reduction of the double bonds and of the keto group (compound **5**) considerably weakened the antiinflammatory activity. A rise in activity in comparison with the initial value was observed in the substances obtained by the stepwise hydrogenation of the double bonds with retention of the keto group (compounds **2**, **3**, and **4**). It must be mentioned that to show an antiinflammatory effect in the series of compounds of type (**1**), the presence of a lactone ring and of a carbonyl group in position 2 is necessary, while for the manifestation of an antihypoxic effect the presence of an α,β-unsaturated keto grouping of the guaiane skeleton of the molecule is sufficient.

EXPERIMENTAL

IR spectra were taken on a UR-20 instrument in KBr tablets. PMR spectra were taken on a Tesla BS 567 A instrument with a working frequency of 100 MHz, UV spectra on a Hitachi spectrometer in ethanol solution, and mass spectra on Kratos MS-25 RF chromato-mass spectrometer (United Kingdom). The course of the reactions and the purity of the products synthesized were monitored by TLC on Silufol UV-254 plates in various systems, the revealing agents being a 1% solution of potassium permanganate in 1% sulfuric acid and a 0.5% solution of vanillin in concentrated sulfuric acid.

Benzenesulfonylaustricin (6). With stirring, 3 ml of freshly redistilled benzenesulfonyl chloride was added to a solution of 1.0 g of austricin (**1b**) in 10 ml of pyridine–benzene (1:1). The reaction mixture was kept at room temperature for 30 min. Then it was poured onto ice and extracted with benzene. The benzene layer was washed twice with 5% HCl solution and then with 5% aqueous Na_2CO_3. The benzene extracts were dried and distilled. The residue (yellowish crystals) was twice recrystallized from ethanol, which gave pure benzenesulfonylaustricin with the composition $C_{21}H_{22}O_6$, mp 171°C (decomp.). TLC showed a single spot with R_f 0.46 in the hexane–acetone (3:1) system. Yield 60%. IR spectrum (cm^{-1}): 1790 (γ-lactone carbonyl), 1685 (ketonic carbonyl); 1642, 1625, 1460 (skeletal vibrations of an aromatic ring).

Mass spectrum (*m/z*, intensity, %): M^+ 402(30.8), 261 ($M^+-C_6H_5O_2H$; 15.4), 244 ($M^+-C_6H_5O_3H$; 54.0), 202(77.0), 187(54.0), 173(100), 171(77.0), 160(69.2), 145(84.6), 128(84.6), 115(69.2), 105(46.2), 91(69.2), 77(92.3).

PMR spectrum ($CDCl_3$, ppm): 1.13 (3H, d, J = 8 Hz., $C_{11}-CH_3$), 2.1 (3H, s, C_4-CH_3), 2.2 (3H, s, $C_{10}-CH_3$), 3.24(1H, d, J = 10 Hz, C_5-H), 3.57(1H, t, $^3J_{6.5} = {}^3J_{6.7}$ = 10 Hz, C_6-H), 4.37(1H, t.d., $^3J_{8.7} = {}^3J_{8.9\alpha}$ = 10 Hz, $^3J_{8.9\beta}$ = 2.5 Hz, C_8-H), aromatic protons: 7.56 (3H, m, ΣJ = 26 Hz, $2H_b + H_c$) and 7.9 (2H, d.d., $^3J_{a.b}$ = 8 Hz, $^4J_{a.c}$ = 25 Hz).

Benzoylaustricin (7). A solution of 0.51 g of austricin (**1b**) and 1.7 g of benzoic anhydride in 10 ml of pyridine was boiled under reflux for 5 h. The reaction mixture was poured onto ice. The oil so formed was washed with diethyl ether. The residue was recrystallized from ethanol (three times) to give benzoylaustricin with the composition $C_{22}H_{22}O_5$, mp 199-200°C, R_f 0.45 in the benzene–acetone (5:1) system. Yield 60%. IR spectrum (cm^{-1}): 1790 (γ-lactone C=O); 1720, 1285 (ester C=O); 1690 (ketonic C=O); 1645, 1625 (stretching vibrations of an aromatic ring).

Mass spectrum (*m/z*, intensity, %): M^+ 366 (9.0), 244 ($M^+-C_6H_5COOH$, 9.0), 229(16.7), 216(7.6), 201(12.0), 188(15.0), 173(13.6), 171(15.0), 160(25.7), 106(100), 92(36.4), 78(45.5), 54(37.9).

PMR spectrum (δ, $CDCl_3$, ppm): 1.26 (3H, d, J = 7 Hz, $C_{11}-CH_3$), 2.25 (3H, s, C_4-CH_3), 2.4 (3H, s, $C_{10}-CH_3$), 3.36 (1H, d, broad components, J = 10 Hz, C_5-H), 3.68 (1H, t, $^3J_{6.5} = {}^3J_{6.7}$ = 10 Hz, C−H), 5.05 (1H, t.d, $^3J_{8.7} = {}^3J_{8.9\alpha}$ = 9 Hz, $^3J_{8.9\beta}$ = 2.0 Hz, C_8-H), aromatic protons: 7.42 (3H, m, ΣJ = 25 Hz, $2H_b + H_c$) and 7.93 (2H, d.d, $^3J_{a.b}$ = 8 Hz, $^4J_{a.c}$ = 2.5 Hz).

Austricin Succinate (8). A mixture of 1 g of austricin and 0.87 g of freshly prepared succinic anhydride in 5.5 ml of pyridine was kept at room temperature for 14 days. Then the reaction mixture was poured onto ice. The yellowish crystals that deposited were recrystallized twice from ethanol, giving austricin succinate with the composition $C_{19}H_{22}O_7$, mp 156-157°C. Yield 61%.

IR spectrum (cm^{-1}): 3260 (COOH), 1768 (γ-lactone C=O), 1742 (ester C=O), 1690 (conjugated ketone C=O), 1645, 1620 ($C_3=C_4$, $C_1=C_{10}$).

Mass spectrum (*m/z*, intensity, %): M^+362 (14.1), 244 ($M^+-COOH-CH_2-CH_2-COOH$:100), 229(12.9), 216(11.6), 201(24.5), 187(28.3), 171(97.8), 159(89.6), 145(34.5), 128(32.2), 115(30.3), 91(56.1), 84(30.3), 77(32.2), 69(86.4), 55(69.0).

Acetylaustricin (9). Austricin acetate was obtained in the same way as as (**8**), but with the reaction mixture held for 12 h,; it had the composition $C_{17}H_{20}O_5$, mp 190-191°C. Yield 96%.

IR spectrum (cm^{-1}): 1790 (γ-lactone C=O), 1740 (ester C=O), 1690 (conjugated ketone C=O), 1640, 1620 ($C_3=C_4$; $C_1=C_{10}$).

Mass spectrum: M^+304 (83.3), 244(M^+-CH_3COOH; 83.3), 228 (30.0), 216 (30.0), 201 (40.0), 118 (36.7), 173 (40.0), 171 (53.3), 159 (60.0), 145 (33.3), 136 (40.0), 91 (100), 77 (60.0), 69 (60.0), 65 (46.7).

Formation of the Products of Hydrolysis and Relactonization (11b)-(13). General Procedure. The appropriate lactone was dissolved in ethanol with heating. The solution was cooled, and, with vigorous stirring, a 10% excess (by volume) of a 1% alcoholic solution of KOH was added (in the case of leukomisin, a 50% excess was taken). The mixture was stirred for 15 min, and then concentrated hydrochloric acid was added dropwise to give a pH of 8, whereupon potassium chloride precipitated in the form of small crystals, which were filtered off. The filtrate was evaporated, and on recrystallization from methanol−acetone−hexane the corresponding potassium salt (**11**) was obtained.

Acidification to pH 1-2 led in the case of austricin (**1b**) to dehydroisoleukomisin (**12**), and in the case of leukomisin (**1a**) to dehydroleukomisinic acid (**13**), which was isolated from the acidified reaction mixture by extraction with benzene, followed by evaporation of the solvent and recrystallization of the resulting mass from ethanol.

Potassium Salt of Austricin (11b). Obtained with a yield of 44%, composition $C_{15}H_{17}O_4K$, decomposing with carbonization at 245°C.

IR spectrum (paraffin oil, cm^{-1}): 1680 (conjugated ketone C=O); 1635 (−C=C−); 1590, 1465 (COO^-).

UV spectrum (max, nm): 236, 244 (log ε 4.28, 4.27; $C_{10}=C_1-C_5=C_6$ and $C_3=C_4-C_5=C_6$); 263 (log ε 4.31; C=C−CO−C=C), 322 (log ε 3.95 (COO^-).

PMR spectrum (CD_3OD, ppm): 1.17 (3H, d, J = 7 Hz, $C_{11}-CH_3$), 2.08 (3H, s, C_4-CH_3), 2.3 (3H, s, $C_{10}-CH_3$), 3.97 (1H, q, ^3J = 5; 7; C_8-H), 5.87 (1H, br.s, C_3-H), 5.98 (1H, d, J = 6 Hz, C_6-H).

Dehydroisoleukomisin (12). Obtained with a yield of 53%, composition $C_{15}H_{16}O_3$, mp 203-204°C.

IR spectrum (cm^{-1}): 1790 (γ-lactone C=O); 1690 (ketone C=O); 1633, 1605 (C=C).

Mass spectrum: 244 (M^+, 100), 216(6.9), 201(12.3), 187(23.4), 173(38.2), 159(40.2), 145(36.5), 128(32.4), 115(37.5), 91(32.4), 77(28.1), 69(28.5), 51(25.7), 44(61.0).

PMR spectrum ($CDCl_3$, ppm): 1.28 (3H, d, J = 8 Hz, $C_{11}-CH_3$), 2.10 (3H, s, C_4-CH_3), 2.40 (3H, s, $C_{10}-CH_3$), 4.50 (1H, t.d., $^3J_{8.7} = {}^3J_{8.9\alpha}$ = 10 Hz, $^3J_{8.9\beta}$ = 4 Hz, C_8-H), 5.83 (1H, d, J = 3 Hz, C_6-H), 6.07 (1H, s, C_3-H).

Dehydroleukomisinic Acid (13). Obtained with a yield of 65%, composition $C_{15}H_{18}O_3$, mp 219-220°C.

IR spectrum (cm^{-1}): 3300-2600 (carboxyl group OH); 1740 (carboxyl group C=O); 1675 (conjugated ketone C=O); 1630 (C=C).

Mass spectrum (*m/z*, intensity%): 246 (M^+ 45.6), 202 (23.2), 185 (12.0), 173 (90.6), 159 (29.5), 145 (67.1), 128 (40.2), 115 (42.9), 105 (62.4), 91 (39.3), 77 (33.5), 65 (20.8), 51 (26.1).

PMR spectrum (d−Py, ppm): 1.2 (3H, d, J = 6 Hz, $C_{11}-CH_3$), 1.85 (3H, s, C_4-CH_3), 2.26 (3H, s, $C_{10}-CH_3$), 6.05 (2H, superposition of the singlet signal of the olefinic proton at C_3 on the doublet of the analogous proton at C_6).

The pharmacological investigations were conducted on 287 white mice weighing 18-22 g. Hypoxia was caused by placing the animals in individual thermochambers with a volume of 250 cm^3. Death was judged visually from the cessation of respiratory movements. Formalin inflammation was obtained by the injection of 0.2 ml of a 1% solution of formalin into the muscle of the right rear paw. A day after nembutal euthanasia the paw was cut off, freed from skin, and weighed. The average increase in weight of the paws in a control group in relation to the average weight of the paws of intact mice was taken as 100%. From this we calculated the percentage increase in weight of a paw in the experimental groups. There were 20-25 mice in the intact and the control groups, and 6-10 animals in the experimental groups.

The substances were injected intraperitoneally: in the study of the antihypoxic effect, once 40 min before the beginning of hypoxia, and in the study of the antiinflammatory effect, over four days, once per day, the last time 40 min before the injection of formalin. In the first case the dose was 1 mg/kg, and in the second case it was 10 mg/kg — the optimum doses for leukomisin and austricin. The animals of the control groups received the solvent (an emulsion of gum arabic) in appropriate amounts in a similar way.

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