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REDUCTION OF THE NATURAL SESQUITERPENE LACTONE LEUCOMISIN

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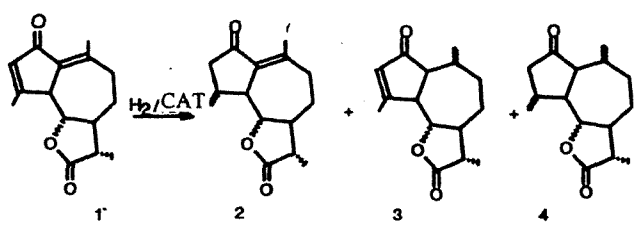
The hydrogenation of the natural sesquiterpene lactone leucomisin has been investigated; a nontrivial procedure for the qualitative and quantitative analysis of the reaction products by the HPLC method has been developed.

The catalytic hydrogenation of sesquiterpene lactones with the guaiane type of skeleton has not been studied systematically. Some scattered facts are given in reports on the determination of the structures of new compounds isolated from plant raw materials. Thus, the exhaustive hydrogenation of the system of double bonds present in cross-conjugation with the keto group of the five-membered ring for guainolides of type (1) in ethanol and ethyl acetate leads to the corresponding tetrahydro product [1-5] while when 1 mole-equivalent of hydrogen is adsorbed a mixture of two dihydro derivatives is formed in various ratios [6-8]. No quantitative investigations of the reaction described were performed. Stereochemistry was not taken into account.

Thus, the catalytic hydrogenation of guaianolides with a system of double bonds present in cross-conjugation with the carbonyl group of the five-membered ring is of interest from the point of view of comparing the reactivities of the two multiple bonds of such a complex system and of considering the stereochemistry of the process.



We have investigated the hydrogenation of the natural sesquiterpene lactone leucomisin [1]. In view of the presence in the (1) molecule of four reaction centers possessing different capacities for undergoing reduction, the reaction was carried by two methods: 1) as far as the complete disappearance of the initial (1); and (2) until the absorption of hydrogen ceased.

The use of the first method with PtO_2 or Raney Ni as catalyst led to three products: 3,4-dihydroleucomisin (2), 1,10-dihydroleucomisin (3), and tetrahydroleucomisin (4). Compounds (2) and (3) were new, while compound (4) had been obtained previously on the exhaustive hydrogen of leucomisin over PtO_2 [9].

We selected the HPLC method for the qualitative and quantitative investigation of the products of this reaction. The use of the reversed variant of HPLC with, as eluents, MeOH—H_2O (20:80), THF—H_2O (5:95), THF—H_2O (1:2), *iso*-PrOH—H_2O (5:95), and H_2O did not lead to any acceptable separation of the compounds mentioned, apparently because of their very low solubility in the eluting mixture. We therefore used the direct variant of HPLC with nine mixed eluents — hexane with ethanol, butanol, methanol, and tetrahydrofuran in various ratios, and also three-component mixtures of these solvents. In all cases good separation of components (1), (2) and (3) was observed. However, it was impossible to separate compounds (2) and (4). A chromatogram for the hexane—EtOH (95:5) system, which proved to be the best, is given in Fig. 1.

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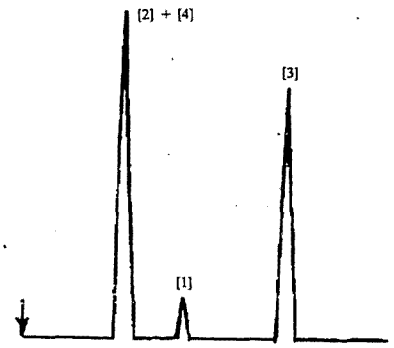


Fig. 1. Chromatogram for a mixture of compounds (**1**)-(**4**).

In column liquid chromatography, the impregnation of the sorbent with $AgNO_3$ promotes the separation of isomeric compounds containing double bonds [10].

The use of a column impregnated with 5% of $AgNO_3$ and the eluting mixture hexane—BuOH-1 (95:5) led to an increase in the retention times of all the components in comparison with the nonimpregnated column, but no separation of compounds (**2**) and (**4**) was achieved.

It was possible to perform a qualitative and quantitative analysis of the reaction mixture from the hydrogenation of leucomisin thanks to the different sensitivities of the UV detector of the chromatograph to compounds (**2**) and (**4**) at λn = 230 nm. The sensitivity coefficient of the detector, K_i is defined as the ratio of the area of the chromatographic peak of the *i*-th compound S_i to the mass of this compound introduced into the chromatographic system, M_i:

$$K_i = S_i/M_i$$

It was established that $K_4 = 0.015 \cdot 10\ 5^{-3}$ cm^2/mg [sic] and $K_2 = 4.59 \cdot 10\ 5^{-3}$ cm^2/mg [sic] at λ = 230nm. Consequently, $K_2/K_4 = 306$, i.e., the sensitivity of the detector of the chromatograph for compound (**2**) was 306 times greater than for compound (**4**).

We determined the contribution of each component to the area of the peak S_{2+4} under the condition that the percentage ratio (**4**):(**2**) = 84:6.

$$S_2 = K_2 \cdot 6 = 27.54$$
$$S_4 = K_4 \cdot 84 = 1.26\ ;\ S_{2+4} = S_2 + S_4 = 28.8$$

The contribution of compound (**4**) to the area of the peak S_{2+4} was determined from the formula: $S_4 \cdot 100/S_{2+4}$; it was 4.3%, which does not exceed the experimental error of 5%.

Consequently, it may be considered that at the given wavelength of a UV detector, λ = 230 nm, compound (**4**) makes no appreciable contribution to the area (height) of the S_{2+4} peak and the value of S_{2+4} is practically equal to S_2. Thus, the masses of (**2**) and (**3**) in a sample were determined directly from a chromatogram using the straight-line calibration curves $S_i = f(M_i)$ (Fig. 2), and the mass of compound (**4**) was determined as the difference:

$$M_4 = M_{2+3+4} - (M_2 + M_3),$$

provided that the initial (**1**) was absent from the sample being analyzed.

As can be seen from the results presented, by varying the catalysts and solvents it is possible to achieve changes in the ratio of (**2**), (**3**), and (**4**) in the reaction mixture.

The results of quantitative investigations are given in Table 1.

In addition to the quantitative investigations, we performed qualitative monitoring of the dynamics of the accumulation of products (**2-4**) by the HPLC method (using the reaction in ethanol over PtO_2 as an example). Its results permitted the conclusion that under these conditions the C_1—C_{10} double bond of leucomisin is hydrogenated more readily than the double bond of the five-membered ring, since compound (**3**) was the first to appear in the reaction mixture; after some time, compound

TABLE 1

Conditions	Products, %		
	2	3	4
PtO_2/EtOH	13	30	57
PtO_2/acetone	18	25	57
PtO_2/AcOEt	34	18	48
Raney Ni/ACOET	6	10	84

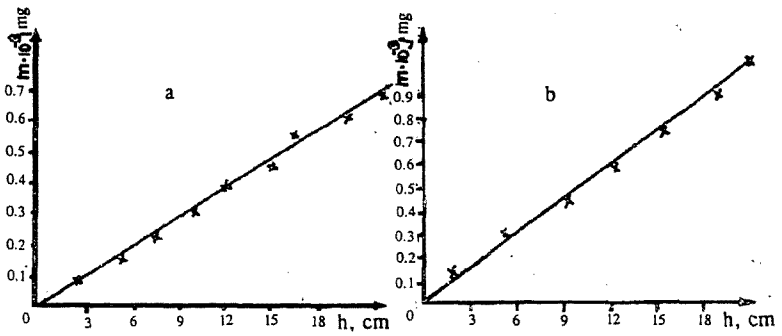


Fig. 2. Graph of the dependence of the height of the chromatographic peaks (**h**) of compounds (**2**) (*a*) and (**3**) (*b*) on the masses of them introduced into the chromatographic system.

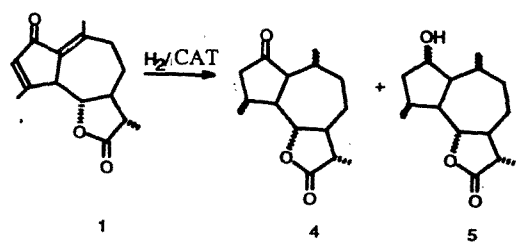
(**2**) was also recorded. This is apparently connected with the high resonance energy and the correspondingly high stability of the cyclopentenone fragment of the leucomisin molecule.

Subsequently, before the disappearance of the initial (**1**), the parallel reactions of the reduction of (**1**) to (**2**) and (**3**), and of (**2**) and (**3**) to (**4**), took place.

It is known that catalytic heterogeneous hydrogenation is a diastereoselective process, since the addition of hydrogen takes place in the *syn* manner from the less sterically hindered side [11]. In our case, each compound isolated was one of the possible geometric isomers, and the individuality of each of them was confirmed by the results of physical methods of investigation.

A change of catalyst in the hydrogenation of leucomisin did not lead to a change in the stereochemistry of the products obtained. The complete identity of the corresponding compounds obtained with the use of PtO_2 and Raney Ni catalyst was shown by direct comparison of their IR spectra.

Thus, we have established that the catalytic heterogeneous reduction of the multiple bonds of leucomisin (**1**) is a diastereoselective process.



The more far-reaching hydrogenation of leucomisin (**1**) by the second method (until the absorption of hydrogen had ceased completely) led to two products: tetrahydroleucomisin (**4**) and hexahydroleucomisin (**5**) in a ratio of approximately 1:1 (qualitative evaluation from the size of the spots in TLC).

Examples are given in the literature of the catalytic reduction of the carbonyl group to a hydroxy group in compounds of this class [12].

The weak absorption of compound (**4**) and the absence of absorption by compound (**5**) in the near region of the UV spectrum did not permit the mixtures to be analyzed by the HPLC method.

Hexahydroleucomisin (**5**) is a mixture of two epimers with respect to the hydroxy group (in a ratio of 1:1), and these were separated in the form of their acetyl derivatives by the TLC method. With the aim of the complete conversion of (**4**) into (**5**) we varied the hydrogenation conditions. An increase in the reaction time to more than one hour did not lead to an

appreciable change in the (4):(5) ratio, which is apparently connected with a blockage of the active centers of the catalyst during the reaction of the hydroxy group of hexahydroleucomisin (5).

A variation of the catalysts (PtO_2, Pt/SiO_2, $Pd/CaCO_3$, $Pd/BaSO_4$, Ni/Al_2O_3) of the solvents (EtOH, AcOEt, AcOAc, AcOH, acetone), and of the temperature conditions (25°C, 55°C) likewise did not lead to an increase in the yield of (5).

It had been shown previously that in hydrogenation over PtO_2 the addition of small amounts of Fe^{3+}, Cu^{2+}, Co^{2+}, Sn^{2+}, and Sn^{4+} salts promotes the reduction of a carbonyl group, and in some cases an inhibition of the hydrogenation of multiple carbon–carbon bonds is observed, the most powerful action being exerted by Sn^{2+} salts [13].

As established in our experiments, the addition of $SnCl_2$ in amounts of $0.1 \cdot 10^{-4}$, $0.2 \cdot 10^{-4}$, and $0.1 \cdot 10^{-5}$ mole per mole of (1) and 0.1 mole of PtO_2 did not cause an appreciable change in the ratio of the products (4):(5). Moreover, the addition of $0.1 \cdot 10^{-2}$ mole of $SnCl_4$ per mole of compound (1) and 0.1 mole of PtO_2 exerted an inhibiting action. Thus, on hydrogenation under the above conditions in AcOH for 8 h, the initial (1) did not disappear completely and the only product was 3,4-dihydroleucomisin (2).

It was possible to achieve the complete conversion of (4) to (5) by using as reagent sodium tetrahydroborate in ethanol [14]. The reaction took place quantitatively and, as on hydrogenation over PtO_2, both epimers of (5) with respect to the hydroxy group were obtained, in a ratio of 1:1.

Thus, as a result of the catalytic hydrogenation of the natural sesquiterpene lactone leucomisin (1) four products (three of them being new) have been isolated and characterized, and a nontrivial procedure for the quantitative analysis of the mixture of unseparated components by the HPLC method has been developed, by means of which it has been shown that the composition and ratio of the products depend on the reaction conditions. It has been established that the double bond of the seven-membered ring of (1) is hydrogenated more readily than the double bond of the five-membered ring and also that the catalytic hydrogenation of leucomisin (1) is a diastereoselective process and the stereochemistry of the product does not depend on the choice of catalysts; conditions have been found for the selective reduction of the carbonyl group of tetrahydroleucomisin (4) to a hydroxy group.

EXPERIMENTAL

NMR spectra were taken in deuterochloroform on a Tesla BS 567A/100 MHz spectrometer, 0 — HMDS; IR spectra on a UR-20 spectrophotometer; UV spectra on a Hitachi spectrophotometer; and mass spectra on a Kratos MS-25 RF chromato-mass spectrometer. HPLC analysis was conducted on a Milikhrom instrument. For reversed-phase chromatography we used a KAX-2 column with Separon C_{18}, 5.0 μm, 64 × 2 mm, NTP 5100. For direct-phase chromatography we used a KAX-1 column with Silosorb 600, 4.0 μm, 64 × 2 mm, NTP 3600.

The optimum eluting mixture was hexane—ethanol (95:5), and the working wavelength of the UV detector was λ = 230 nm.

General Hydrogen Procedure. Method 1. Hydrogenation was conducted in a closed "duck" in the solvents ethanol, ethyl acetate, and acetone with vigorous shaking under a pressure of hydrogen somewhat exceeding atmospheric pressure. The ratio of the weight of the catalyst to the weight of (1) for PtO_2 was approximately 1:10, and for Raney Ni 1:2. After the absorption of one mole-equivalent of hydrogen, the reaction mixture was tested by TLC, and if the initial compound was absent it was subjected to further treatment: filtration of the catalyst and elimination of the solvent, followed by chromatography of the mass so obtained on a column of silica gel in the hexane—benzene system with a gradient of benzene. Compound (4) issued first, and then a mixture of (4) and (2) from which (2) was isolated by recrystallization from ethanol, and then (3), which was additionally purified by recrystallization from ethanol.

Method 2. Hydrogenation was conducted in a closed "duck" in the solvents ethanol, ethyl acetate, acetone, acetic acid, acetic anhydride, and pyridine and mixtures of them with vigorous shaking under a pressure of hydrogen somewhat exceeding atmospheric. The ratio of the mass of (1) to the mass of (2) was approximately 10:1. After 2 h, the mixture was subjected to working up as in method (1). On chromatography, compound (4) issued first, and in a mixture of (4) and (5), followed, on elution with pure benzene, by the pure compound (5).

Reduction of (4) to (5) with Sodium Tetrahydroborate. At room temperature, $NaBH_4$ was added in portions to a solution of (4) in ethanol until the initial substances had disappeared completely from the reaction mixture (checked by TLC). Then the reaction mixture was diluted with water and extracted with benzene. The extract was washed with water and was dried

over Na_2SO_4. After the solvent had been distilled off, compound (**5**) was isolated in the form of a colorless oil with a purity of 98%.

3,4-Dihydroleucomisin (2) — white acicular crystals with mp 209°C. PMR (100 MHz, $CDCl_3$, ppm, J, Hz): 0.92 (3H, d), 1.20 (3H, d), 2.28 (3H, d) 3.72 (1H, t). IR spectrum (KBr, ν, cm^{-1}): 1770, 1708, 1610. Mass spectrum (EI, 70 eV), m/z (I_{rel}, %): 248 (M^+, 90), 175 (100). UV spectrum, (EtOH, λ_{max}, nm): 252 (log ε 4.17).

1,10-Dihydroleucomisin (3) — white acicular crystals with mp 156°C. PMR (100 MHz, $CDCl_3$, ppm, J, Hz): 0.68 (3H, d), 1.2 (3H, d), 2.19 (3H, d), 3.02 (1H, t), 4.27 (1H, t), 5.98 (1H, s). IR spectrum (KBr, ν, cm^{-1}): 1775, 1703, 1628. Mass spectrum (EI, 70 eV), m/z (I_{rel}, %): 248 (M^+, 83), 96 (100). UV spectrum (EtOH, λ_{max}, nm): 228 (log ε 4.21).

Tetrahydroleucomisin (4) — white acicular crystals with mp 150°C. PMR (100 MHz, $CDCl_3$, ppm, J, Hz): 0.88 (6H, t (superposition of two doublets)), 1.92 (3H, d), 4.2 (1H, t). IR spectrum (KBr, ν, cm^{-1}): 1760, 1730. Mass spectrum (EI, 70 eV), *m/z* (I_{rel}, %): 250 (M^+, 44), 97 (100). UV spectrum (EtOH, λ_{max}, nm) 215 (log ε 2.19).

Hexahydroleucomisin (5) — colorless oil. PMR (100 MHz, $CDCl_3$, ppm, J, Hz): 1.0 (9H, multiplet), 4.25 (2H, distorted quartet). IR spectrum (KBr, ν, cm^{-1}): 3485 — broad and intense; 1770. Mass spectrum (EI, 70 eV), *m/z* (I_{rel}, %: 253 (M^+ + H 5), 234 (M^+ - H_2O 34), 209 (100).

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