STRUCTURE OF LAPPACONITINE

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UDC 547.944/945

The alkaloid lappaconitine has been isolated from plants of the genus Aconitum [1-3]. The composition $C_{32}H_{44}O_8N_2$ and the expanded formula $C_{18}H_{20}(OH)_2(OCH_3)_3(N-C_2H_5)(OCOC_6H_4NHCOCH_3)$ have been established for it. Alkaline hydrolysis of the base gives an amino alcohol, lappaconine, $C_{23}H_{37}O_6N$ [2, 3]. A recent paper in which, on the basis of chemical and spectral results and an x-ray structural analysis, structure I was proposed for lappaconine [4, 5] has induced us to publish the information obtained in a study of the structure of lappaconitine from Aconitum leucostomum (excelsum).

Acetylation of lappaconine confirmed the presence of three hydroxyl groups, and the NMR spectrum of the base showed the presence of three methoxy groups and an N-ethyl group. The oxidation of I with potassium permanganate [6] gave oxolappaconine (II), containing, according to its IR spectrum, a lactam carbonyl in a six-membered ring. In the mass spectrum of lappaconine, the maximum peak is that of the ion M-31, the second is M-15 (8%), and the third (6%) is the peak of the molecular ion, the other peaks being very small. In the mass spectrum of oxolappaconine, the peak of the molecular ion is the strongest, and the peak corresponding to the fragment M-15 amounts to approximately 30% of the maximum peak. The results of a comparison of these spectra with those of other Aconitum alkaloids and their oxo derivatives shows that lappaconitine is a diterpene alkaloid based on the lycoctonine skeleton [7, 8].

According to the expanded formula of lappaconine, the basic skeleton of the alkaloid, which consists of a system of six rings (similar to that of lycoctonine) contains 18 carbon atoms. The lycoctonine skeleton, however, consists of 19 carbon atoms.

In view of the rigidity of the lycoctonine system, the presence of only 18 carbon atoms in the lappaconine skeleton is probably due to the absence of the C-19 methylene group, since the contraction of one of the rings (apart from ring A) would lead to a considerable strain in the system. Furthermore, as mentioned above, the mass spectra of lappaconine and of oxolappaconine are characteristic for the lycoctonine skeleton.

We have been unable to find a C-19 methylene group in any product of the transformation of lappaconine. A methyl group at C_4 is excluded on the basis of the NMR spectra.

The oxidation of oxolappaconine with chromic anhydride in acetone gave a product (III) with the composition $C_{23}H_{33}O_7N$. Its spectrum has, in addition to a band at 1640 cm^{-1} (CO−N <), a band at 1737 cm^{-1} showing the presence of a carbonyl group in a five-membered ring. According to this, the presence of a secondary hydroxy group in the base could be assumed. However, the oxidation of oxolappaconine with periodic acid forms a product identical with that obtained above in the oxidation of oxolappaconine with chromic anhydride. The extreme difficulty of the oxidation of lappaconine itself with various oxidizing agents specific for hydroxy groups also raises doubts as to whether a secondary hydroxy group is present in the alkaloid.

Consequently, lappaconine has a glycol system which is readily cleaved under the action of chromic anhydride. The presence of a diol system with two tertiary hydroxy groups is confirmed by other facts that are mentioned below. The two adjacent hydroxy groups are probably located at C-7 and C-8 or at C-8 and C-9. The C-7 and C-8 positions are excluded, since bases with such a diol system are readily oxidized by periodic acid, give a complex mixture of products on acetylation and, finally, their seco products have a

Institute of the Chemistry of Plant Substances, Academy of Sciences of the Uzbek SSR. Translated from Khimiya Prirodnykh Soedinenii, No. 5, pp. 583-588, September-October, 1970. Original article submitted June 8, 1970.

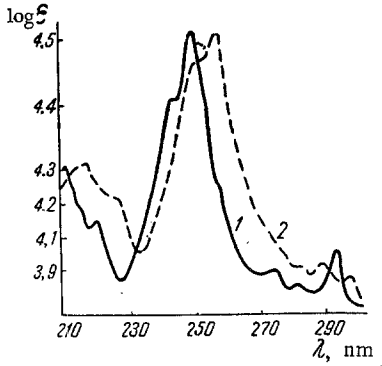


Fig. 1. UV spectrum of 1) phenanthrene and 2) trimethylphenanthrene.

characteristic mass spectrum (compare with the spectra of the corresponding products from delphatine, lycoctonine, and browniine). In contrast to this, lappaconine and oxolappaconine are oxidized by periodic acid with difficulty and form a triacetyl derivative. The presence of only one carboxy group in the oxidation product is unexpected. In an ethanolic solution of hydrochloric acid, the compound gave a substance differing from the starting material by one methylene group. Its NMR spectrum had the signals of only two methoxy groups although there are three in the starting material. Hydrolysis of a methoxy group may possibly be connected with the influence of the carbonyl group. Oxidation of oxolappaconine with lead tetraacetate gave a product IV with the composition $C_{23}H_{31}O_7N$. Its IR spectrum has absorption bands at (cm^{-1}): 3490 (OH), 3065 (double bond), 1705 (C=O), and 1650 (CO−N<). In an alcoholic solution of hydrochloric acid, saponification of one of the methoxy groups also takes place in IV, but much more rapidly than in the product of oxidation with chromic anhydride. Hydrogenation of IV over a platinum catalyst led to a tetrahydro derivative $C_{23}H_{35}O_7N$ whose IR spectrum lacked the absorption bands of the double bond and of the carbonyl group at 1705 cm^{-1}.

Dehydrogenation of lappaconine with selenium gave trimethylphenanthrene $C_{17}H_{16}$ (Fig. 1). Formation of a phenanthrene derivative when lappaconine has a lycoctonine skeleton confirms the presence of a hydroxy group at C-8.

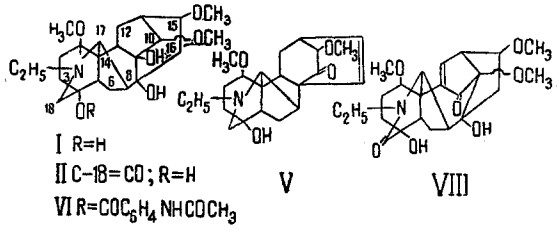
In the mass spectrum of lappaconine, the peak of the ion M-31 is the maximum peak. This shows that the methoxy group is present at C-1 [7].

Treating lappaconine with sulfuric acid gave an amorphous product differing from the starting material by the elements of water and methanol. The IR spectrum of this product had the absorption band of a carbonyl group at 1720 cm^{-1}. The NMR spectrum has signals due to an N-ethyl group (three-proton triplet at 0.99 ppm) and two methoxy groups (three-proton singlets at 3.20 and 3.46 ppm), and also those of two olefinic protons (one-proton doublet at 5.48 ppm and one-proton triplet at 6.49 ppm). The appearance in the reaction product of a carbonyl group in a six-membered ring, according to the IR spectrum, and elimination of a molecule of water could result from pinacolone type rearrangement. There is no absorption in the 220-250 nm region, which shows the absence of conjugation between the carbonyl group and the double bond. The elimination of a molecule of methanol apparently takes place at the expense of the methoxy group at C-15, whose mobility was known [9, 10]. A combination of these results and the nature of the spin−spin coupling between the olefinic protons (a similar pattern is observed in the NMR spectrum of isopyrotalatisamine [11]) suggests structure V for this product.

Oxidation of lappaconitine (VI) with chromic anhydride in acetone gave a product VII with the composition $C_{32}H_{42}O_8N_2$ which, after saponification of an ester group, proved to be identical with the oxidation product of lappaconine under the same conditions. Pyrolysis of lappaconitine in vacuo led to products differing from the starting material by the elements of water and containing in the IR spectrum the absorption band of a carbonyl group at 1735 cm^{-1}.

Loss of the elements of water and the formation of a carbonyl group is undoubtedly connected with a rearrangement process in which the diol system participates. These results show that in lappaconitine acetylanthranilic acid esterifies the third tertiary hydroxy group, which is present, according to x-ray structural analysis, at C-4 [5].

As mentioned above, when the diol system in oxolappaconine is cleaved, only one carbonyl group appears. The authors of a paper on the structure of lappaconine [4] explained this by the aldol condensation of the carbonyl at C-8 with the hydrogen at C-10, which is activated by an α-carbonyl, and they give the structural formula VIII. They were unable to detect absorption characteristics for an α,β-unsaturated ketone in the UV spectrum of this compound. Consequently, a further chemical study of the oxidation products of lappaconine and of oxolappaconine is necessary to elucidate their structures.



EXPERIMENTAL

The NMR spectra were taken on a JNM-4H-100/100 MHz instrument in deuterochloroform with HMDS as internal standard (δ scale), and the mass spectra on an MKh-1303 instrument fitted with a system for the direct introduction of the sample into the ion source. The purity of all the products was checked by chromatography in a thin layer of ShSK silica gel in a benzene–methanol (4 : 1) system.

Triacetyllappaconine. The substance was obtained by heating a mixture of lappaconine, acetic anhydride, and p-toluenesulfonic acid for 1 h; mp 190-193°C (from ether). NMR spectrum: 1.01 ppm (triplet, $N-CH_2CH_3$), 1.89, 1.99, and 2.04 ppm (singlets, $3OCOCH_3$). Mol. wt. 549.

Oxolappaconine (II). A solution of 0.8 g of lappaconine in 130 ml of a mixture of acetone and water (4 : 1) was mixed with a solution of 0.8 g of potassium permanganate in 450 ml of a mixture of acetone and water (1 : 1). The mixture was stirred for 5 min, and the excess potassium permanganate was decomposed with sodium sulfite. The solution was filtered and evaporated until all the acetone had been eliminated. Then the reaction product was extracted with ether. Distillation of the solvent gave a crystalline substance with mp 178-180°C (from ether). IR spectrum: 1633 cm^{-1}; NMR spectrum: 1.12 ppm (triplet, $N-CH_2CH_3$). Mol. wt. 437.

Oxidation of II with Chromic Anhydride. A solution of 0.65 g of oxolappaconine in 50 ml of acetone was mixed with 0.6 g of chromic anhydride in 50 ml of acetone with ice-water cooling. The mixture was left at room temperature for 48 h. Then the acetone was driven off and the residue was dissolved in 10% H_2SO_4. The excess chromic anhydride was decomposed with sodium sulfite and the reaction product was extracted with chloroform. This gave the crystalline substance III with mp 246-248°C (ether–methanol). Mol. wt. 435.

seco-Oxolappaconine. A solution of 0.1 g of oxolappaconine in 15 ml of water was mixed with a solution of 0.1 g of periodic acid in 3 ml of water. The mixture was left for 24 h and then made alkaline with sodium carbonate and extracted with chloroform. The reaction product was passed through a column of alumina and eluted with chloroform. This gave a crystalline substance with mp 248-250°C (ether–methanol), identical with product III.

Hydrolysis of III. A solution of 0.1 g of III in 10 ml of ethanolic HCl was left at room temperature for 24 h. The solvent was evaporated, the residue was dissolved in water, and the solution was made alkaline with sodium carbonate and extracted with ether. A crystalline substance deposited with mp 269-271°C (acetone). NMR spectrum: 1.11 ppm (triplet, $N-C_2H_5$); 3.20 and 3.46 ppm (singlets, $2OCH_3$). Mol. wt. 421.

Oxidation of II with Lead Tetraacetate. A solution of 0.15 g of II in 9 ml of acetic acid was mixed with a solution of 0.2 g of lead tetraacetate in 6 ml of acetic acid. After 12 h, 6 ml of saturated sodium acetate solution, 0.1 g of potassium iodate, and an excess of sodium thiosulfate were added to the solution and it was extracted with chloroform. The extract was washed with 4% caustic soda solution and then with water. The solvent was distilled off and the residue was separated on a column of silica gel. Elution with a mixture of benzene and ether (1 : 2) gave product IV with mp 214-216°C. NMR spectrum: 1.09 ppm (triplet, $N-CH_2CH_3$), 3.19, 3.41, and 3.58 ppm (singlets, $3OCH_3$), 6.88 ppm (doublet, 1H). Mol. wt. 433.

Hydrogenation of IV. A 30-mg quantity of IV was hydrogenated in 10 ml of ethanol by Adams' method, and a crystalline substance with mp 251-253°C was isolated. IR spectrum, cm^{-1}: 3510, 3460, and 3430 (OH), and 1640 (CO–N <). Mol. wt. 437.

Dehydrogenation of Lappaconine with Selenium. A mixture of 9 g of lappaconine and 9 g of selenium was heated in an atmosphere of nitrogen at 300-310°C for 8 h. After cooling, the reaction products were extracted with benzene (200 ml). The extract was washed, first with 10% H_2SO_4, then with 5% alkali solution,

and finally with water. The benzene was distilled off and the residue was treated with petroleum ether. The petroleum ether fraction was chromatographed on a column of alumina and eluted with petroleum ether. The movement of the substances in the column was observed in UV light. The fractions containing a substance fluorescing violet gave a picrate which was decomposed on a column of alumina and eluted with ether. This gave a colorless oil which could not be crystallized. NMR spectrum: 2.50 ppm (singlet, CH_3), 2.61 ppm (singlet, $2\,CH_3$). Mol. wt. 220.

Oxidation of Lappaconitine with Chromic Anhydride. With cooling, a solution of 0.3 g of the base in 50 ml of acetone was mixed with a solution of 0.3 g of chromic anhydride in 15 ml of acetone. The mixture was left at room temperature for 48 h. The acetone was driven off and the residue was dissolved in 10% H_2SO_4. The excess chromic anhydride was decomposed with sodium sulfite. The acid solution was made alkaline with sodium carbonate and extracted with chloroform. The solvent was distilled off and the residue was treated with methanol. This gave 0.21 g of the initial base. The residue from the evaporation of the mother solution (0.09 g) was chromatographed on a column of alumina, the reaction product being eluted with ether. A crystalline substance (VII) with mp 189-192°C (ether–methanol) deposited. IR spectrum: 1745 cm^{-1} (cyclopentanone). Mol. wt. 582.

Saponification of VII. A mixture of 0.05 g of VII and 8 ml of 5% methanolic alkali was heated in a water bath for 30 min. The solvent was evaporated off and the residue was dissolved in water and extracted with ether. This gave a crystalline substance with mp 178-180°C (n-hexane–acetone). IR spectrum: 1743 cm^{-1} (cyclopentanone). Mol. wt. 421.

Oxidation of Lappaconine with Chromic Anhydride. With cooling, a solution of 0.3 g of I in 10 ml of acetone was mixed with 0.3 g of chromic anhydride in 15 ml of acetone. The mixture was left for 48 h at room temperature. The acetone was driven off, the residue was dissolved in 10% H_2SO_4, and the excess chromic anhydride was decomposed with sodium sulfite. The solution was made alkaline with sodium carbonate and extracted with chloroform. The residue from the distillation of the chloroform was separated on a column of alumina, elution being carried out with ether–methanol (20:1). A crystalline substance was isolated which was identical with the product obtained in the preceding experiment.

Pyrolysis of Lappaconitine. The base (0.3 g) was heated in vacuo at 225-230°C for 15 min. After cooling, the reaction mixture was dissolved in 5% H_2SO_4, washed with chloroform, made alkaline with sodium carbonate, and extracted with chloroform. After distillation of the solvent and treatment with ether, a crystalline substance deposited with mp 220-222°C (ether). IR spectrum: 1735 cm^{-1} (CO). Mol. wt. 566. NMR spectrum, ppm: 1.02 (triplet, $N-CH_2CH_3$), 2.14 (singlet, $OCOCH_3$), 3.22, 3.30, and 3.52 (singlet, $3\,OCH_3$).

Anhydrodemethanollappaconine. A solution of 0.15 g of lappaconine in 10 ml of 50% H_2SO_4 was heated in a water bath for 2 h. After cooling, the acid solution was made alkaline with sodium carbonate and extracted with chloroform. The solvent was driven off and the residue was treated with ether. This gave 0.09 g of the initial lappaconine. The mother solution (0.055 g), after evaporation, was chromatographed on a column of alumina and eluted with ether. This gave 0.043 g of an amorphous product (V). IR spectrum: 1720 cm^{-1} (CO in a six-membered ring). NMR spectrum, ppm: 0.99 (triplet, $N-CH_2CH_3$); 3.20 and 3.46 (singlets, $2\,OCH_3$), 5.48 (doublet, 1H), 6.49 (triplet, 1H). Mol. wt. 373.

CONCLUSIONS

A structure has been proposed for lappaconitine on the basis of its chemical and spectral characteristics.

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