Ozonolysis of the Hydroxy Ketone (IV) by the Method of [1]. The hydroxy ketone (IV) (60 mg) was ozonized in 10 ml of methylene chloride and the product was worked up as described in [1]. This led to the isolation of 10 mg of a neutral fraction and 31 mg of an acid fraction. The latter, consisting, according to TLC, of two components, was chromatographed on a column containing 9 g of silica gel 100/250 μ deactivated by the addition of 10% of water. Petroleum ether-diethyl ether (4:1) eluted 5 mg of a product apparently consisting of the hydroxy acid (IX). PMR spectrum (ppm): 0.74 (3H, s), 0.91 (3H, s), 0.94 (3H, s) [C₄- and C₁₀-CH₃], 1.12 (3H, s) (C₈-CH₃), 2.03 (1H, s, C₉-H), 12.27 (1H, s, COOH).

Then the same solvent eluted 23 mg of 8 α hydroxydriman-11-oil acid (VI) with mp 176-178°C.

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PRODUCTS OF THE OZONOLYSIS OF ISOABIENOL

A. N. Aryku, G. N. Mironov, M. N. Koltsa, and P. F. Vlad UDC 547.596/599

The ozonization of abienol in ethyl acetate in the presence of pyridine or in methanol followed by the reduction of the peroxides by methyl sulfide has given ambreinolide. On reduction of the peroxide products of the ozonolysis of iso-abienol in methanol with potassium tetrahydroborate, the main reaction product was 15,16-bisnorlabdane-8,13,14-triol. Dimethyl sulfide first caused the re-arrangement of the peroxide products of ozonization with the cleavage of the ordinary C-13-C-14 carbon bond, and then their reduction.

We [1] were the first to show that isoabienol (I) can be selectively oxidized with potassium permanganate to ambreinolide (II) - a valuable product for the perfumery industry [2]. With the discovery by Ekman et al. [3] of a readily available source of isoabienol, the question of its oxidation to ambreinolide has also acquired practical interest. A method for obtaining ambreinolide by the ozonization of isoabienol has been patented [4]. When ozonolysis was performed in absolute methanol at 0°C and the peroxides were

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hydrogenated over Lindlar catalyst, the yield of the δ -lactone (II) amounted to 96%, but it was somewhat lower (85%) if dimethyl sulfide was used as the reducing agent.

In order to simplify the process of ozonolyzing isoabienol (elimination of a hydrogenation stage), we ozonized isoabienol (I) in ethyl acetate in the presence of pyridine. In this case, as well, the reaction took place fairly unambiguously, but the yield of ambrienolide after chromatographic purification was only 72%. However, it must be mentioned that on the analogous purification of the product of the reaction that we performed under the conditions of [4] in methanol and reduction of the peroxides with dimethyl sulfide, the yield of ambreinolide (67%) also proved to be lower than that given in [4] (85%).



In view of the fact that to obtain perfume substances from ambreinolide it must be reduced to the diol (III) or to the hydroxy aldehyde (V) [2], we attempted to obtain the diol (III) by the direct reduction of the peroxide products of the ozonolysis of isoabienol in methanol with potassium tetrahydroborate. However, a crystalline alcohol differing in its properties from the diol (III) was unexpectedly isolated in 72% yield. Judging from its IR and PMR spectra, its molecule contained only hydroxy groups (primary, secondary, and tertiary), three methyl groups at quaternary carbon atoms, and one at a tertiary carbinol carbon atom. These facts and the elementary composition, $C_{18}H_{34}O_3$, led to the structure of 15,16-bis-norlabdane-8 α ,13,14-triol (IV) for the compound under investigation. This was confirmed by its mass spectrum in which, together with the peak of the molecular ion, having m/z 298, and the peaks of the usual ions for labdane diterpenoids [5], there were informative peaks of ions with m/z 280 (M - H₂O), 249 [M - (H₂O + CH₂OH)], 231 (249 - H₂O), 210 (M - C₄H₈O₂) and 192, a possible route to the formation of which is shown in the scheme given below.



Finally, the structure of the triol (IV) was confirmed by its oxidation with sodium periodate to the known hydroxy aldehyde (V), existing in the form of the cyclic semiacetal (VI), which, on vacuum distillation, split out water, and gave the unsaturated oxide (VII) with a strong ambergris odor [6].

The facts presented show that on the use of dimethyl sulfide for the reduction of the peroxide products of the ozonolysis of isoabienol (I) they first undergo a rearrangement with the cleavage of the ordinary C-13-C-14 carbon-carbon bond and then reduction, while potassium tetrahydroborate only reduces them.

For the general part, see [7]. Mass spectra were taken on a MKh 1320 spectrometer with a glass system for the introduction of the sample directly into the ion source at an ionizing energy of 70 eV.

<u>Production of Ambreinolide (I).</u> a) At -65 to -70°C, a mixture of ozone and oxygen was passed through a solution of 103 mg of isoabienol (I) in 10 ml of ethyl acetate and 0.1 ml of dry pyridine until ozone appeared at the outlet from the reaction flask. The solution was purged with nitrogen and was allowed to warm up to room temperature, after which 20 ml of a 10% solution of hydrochloric acid was added and the products were extracted with ether (3×25 ml). The extract was washed with water (3×20 ml), dried with anhydrous sodium sulfate, and filtered, and the solvent was distilled off. The residue (108 mg) was chromatographed on a column containing 3 g of silica gel 40/100 μ . Petroleum ether-diethyl ether (9:1) eluted 71 mg (72%) of ambreinolide (II), mp 139-140°C (from petroleum ether); it was identified by comparison with an authentic sample.

b. At 0°C, a mixture of ozone and oxygen was bubbled through a solution of 100 g of isoabienol (I) in absolute methanol (5 ml) until the appearance of a blue coloration of the solution. It was purged with nitrogen, and after the addition of 0.4 ml of methyl sulfide the mixture was stirred for 8 h. The methanol was distilled off, and the residue was diluted with chloroform, washed with water, dried with anhydrous sodium sulfate, and filtered, and the solvent was distilled off. The residue (101 mg) was chromatographed on a column containing 3 g of silica gel, and 62 mg (67%) of ambreinolide (II) with mp 139-140°C, was isolated.

<u>Production of 15,16-Bisnorlabdane-8 α ,13,14-triol (IV).</u> At -25 to -30°C, a mixture of ozone and oxygen was bubbled through a solution of 103 mg (0.36 mmole) of isoabienol (I) in 10 ml of dry methanol until a permanent blue coloration of the solution had appeared. The ozone was displaced by nitrogen, the solution was allowed to assume room temperature, 97 mg (1.8 mmole) of potassium tetrahydroborate was added, and the mixture was stirred for 1 h. Then 50 ml of a 1% solution of caustic potash was added and the products were extracted with ether (3 × 25 ml). The extract was washed with 20 ml of 10% solution of sulfuric acid, and with water to neutrality, and it was dried with anhydrous sodium sulfate and filtered, and the solvent was distilled off. The residue (80 mg) was chromatographed on a column containing 3 g of silica gel. Diethyl ether-petroleum ether (1:1) eluted 76 mg (72%) of the triol (IV), mp 134-135.5°C (from ethanol). IR spectrum (CHCl₃, cm⁻¹): 1040, 1063, 1123, very intense bands at 3100-3560 with a maximum at 3380, 3589 (OH groups): 1356, 1384 (gem-dimethyl group).

PMR spectrum (CDCl₃, δ , ppm): 0.68 (3H, s, CH₃ at C-10); 0.76 (3H, s), 1.05 (3H, s) [C(CH₃)₂]; 1.10 (3H, s, CH₃ at C-8), 3.50 (3H, m, H atoms at C-13 and C-14). Mass spectrum, m/z (%): 298 (M⁺, 20), 284(3), 280(6), 268(5), 267(13), 265(10), 249(13), 232(6), 231(25), 210(11), 209(9), 198(7), 196(6), 195(21), 193(9), 192(28), 191(29), 179(5), 178(7), 177(38), 151(9), 150(6), 149(22), 147(7), 145(20), 139(9), 138(15), 137(32), 135(15), 126(8), 125(33), 124(32), 123(35), 122(11), 121(26), 110(13), 109(69), 108(11), 107(27), 98(11), 97(28), 96(19), 95(73), 94(10), 93(31), 85(19), 84(12), 83(38), 82(30), 81(69), 80(7), 79(23), 73 (14), 72(15), 71(69), 70(20), 69(81), 68(17), 67(38), 59(6), 58(13), 57(47), 56(19), 55(73), 45(16), 44(23), 43(100), 42(13), 41(58). Found, %: C 72.76, H 11.74. C₁₈H₃₄O₃. Calculated, %: C 72.48, H 11.41.

Oxidation of the Triol (IV). A solution of 150 mg (0.1 mmole) of sodium periodate in 1.2 ml of water was added to a solution of 30 mg (0.1 mmole) of the triol (IV) in 1.2 ml of ethanol and 0.6 ml of saturated aqueous potassium carbonate solution. The reaction mixture was stirred for 6 h and was then diluted with water (10 ml) and extracted with ether $(3 \times 20 \text{ ml})$. The extract was washed with water, with 10% sulfuric acid solution (2 × 10 ml), and with water to neutrality and was dried and filtered, and the solvent was distilled off. This gave 25 mg (94%) of the semiacetal (VI) in the individual form according to TLC, with mp 195-196.5°C (from ethanol); it was identified by comparison with an authentic sample. According to the literature [5]: mp 196-197°C.

<u>Production of the Oxide (VII).</u> The semiacetal (VI) (25 mg) was distilled in vacuum at 100-120°C/2 mm in a rotating flask. This gave as the only product, according to TLC, 18 mg (77%) of unsaturated oxide (VII) with a pleasant ambergris smell, mp 82.5-84°C (from ethanol). According to the literature [6]: mp 83-84°C.

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SECONDARY-ION MASS SPECTROMETRY OF LYCOTETRAOSIDES OF THE SPIROSTAN

AND FUROSTAN SERIES

UDC 543.51+547.926

Yu. M. Mil'grom, V. L. Sadovskaya, Ya. V. Rashkes, and Yu. S. Vollerner

The secondary-ion mass spectra of seven steroid lycotetraosides have been obtained by the SIMS method. On the use of a glycerol matrix, all the compounds of the spirostan series, including those with an additional carbohydrate substituent a C-24, formed the $(M + H)^+$ ions, while a lycotetraoside of the furostan series formed the $(M - H_2O + H)^+$ ion. They subsequently decomposed by the alternative successive elimination of the terminal carbohydrate units of the lycotetraose. Substituents at C-25 and C-26 were ejected in the form of glucose molecules. Fragments of the aglycon and of the lycotetraoside moieties of the molecules were recorded. When NaCl was added to the same matrix, all the compounds gave preferentially the $(M + Na)^+$ and $(M + 2Na - H)^+$ ions.

The functions of the mass-spectrometric method in the study of the structures of the glycosides of the spirostan and furostan series have changed considerably during the last 15 years. Earlier, when exhaustive methylation of natural glycosides was the almost obligatory first stage of chemical investigations, the electron-impact (EI) mass spectra of these compounds gave rich information on the structures of the initial substances, thanks to the stability of the M⁺ ions and the presence of a large number of characteristic fragments [1]. However, with the appearance of other methods of demonstrating structures and, above all, with the use of ¹³C NMR spectroscopy, the modification of glycosides lost its previous urgency, but the possibilities of EI mass spectrometry of the native glycosides were limited to the production of spectra of triosides of the spirostan series containing, in addition to the weak peak of the M⁺ ion, the peaks of ions corresponding to the successive elimination of the three carbohydrate units and the peaks of the key fragments of the aglycon parts of the molecule [2]. In addition to this, the wide use of new methods of "mild" ionization (fast-atom bombardment (FAB) [3], and secondary-ion mass spectrometry (SIMS) [4]) substantially broadened the possibilities of mass spectrometry in the analysis of unmodified polar and thermally labile natural compounds of various classes such as proteins and peptides [5],

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