

formed with 2 ml of methanol in the Soxhlet manner for 2 h. Then the methanol was evaporated off, 2 ml of a mixture of sulfuric acid and ethanol was added to the test tube, and it was heated at 60°C for 30 min and was cooled, and the optical density was measured at 530 nm against the reaction mixture.

The blank experiment was carried out similarly using pure silica gel removed from an area of the plate corresponding to the area of the hederagenin spot.

To determine caulosides A and C in a combined preparation, 40  $\mu$ l of a methanolic solution of the preparation with a concentration of 2 mg/ml was deposited on a plate together with a synthetic mixture of caulosides A and C for the clearer identification of the spots, and a pure band of silica gel was left for the blank experiment. The determination was carried out in the way described above.

The samples of caulosides C and D were presented by N. I. Uvarova and N. S. Chetyrina.

#### SUMMARY

Using hederagenin and its glycosides — caulosides A, C, and D isolated from the Far Eastern plant *Caulophyllum robustum* Maxim — as examples, the spectrometric reactions with concentrated sulfuric acid and mixtures of sulfuric acid with ethanol and with glacial acetic acid have been studied. Conditions have been proposed for performing the reaction which exclude the influence of the carbohydrate components of the glycosides on the determination of hederagenin. The reaction with a mixture of sulfuric acid and ethanol has been used to determine the amounts of caulosides A and C in a combined preparation after their separation by the TLC method. The relative error of the determination did not exceed 5 rel. %.

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#### MECHANISM OF THE REACTION OF TRITERPENOIDS WITH SULFURIC ACID

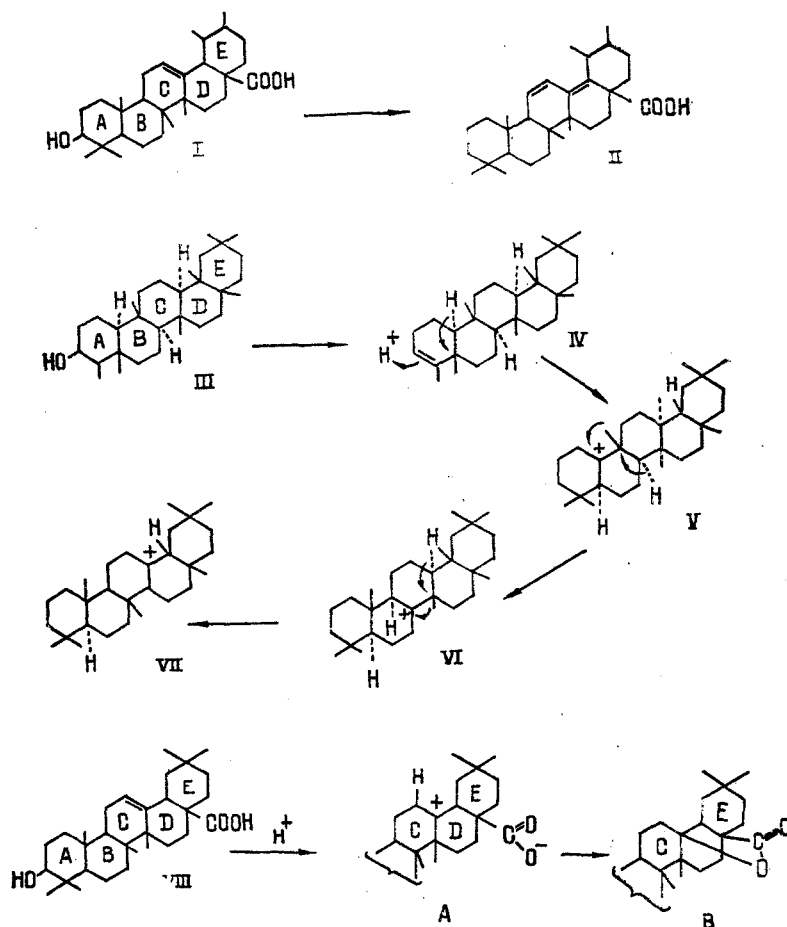
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The single maximum of triterpenoids in sulfuric acid solutions at 310 nm is due to the formation of a carbocation. Under the action of sulfuric acid, oleanolic acid undergoes lactonization at the COOH group and the  $\Delta^{12}$   $>C=C<$  bond.

Brieskorn et al. [1, 2] have reported studies of the Lieberman-Burchard reaction. On the basis of the results of these investigations, the authors came to the conclusion that under the reaction of sulfuric acid triterpenoids undergo dehydration in ring A, after which the double bond formed migrates until conjugation arises in rings C and D. Considering ursolic acid (I), in particular, the authors suggested that because of dehydration followed by migration of the double bond ursadienecarboxylic acid (II) is formed. The formation of the suggested diene was judged on the basis of the fact that the reaction product after isolation had an absorption maximum in ethanolic solution at 246 nm (Scheme 1).

Thus, according to the proposed mechanism, dehydration in ring A leads to the appearance of a double bond in it and then, as a consequence of intramolecular rearrangement, migrations of two types takes place: the  $\Delta^{12}$  double bond migrates into ring D and the double bond formed as a result of dehydration migrates from ring A to ring C and, in the final result, a conjugated system of double bonds —  $\Delta^{11}$  and  $\Delta^{13}$  — is formed. The absorption maximum of an



Scheme 1

ethanolic solution of the reaction product at 246 nm was identified by the authors with that for conjugated dienes (taking Woodward's rule into account).

It is known [3] that under the action of various acidic reagents such as phenol, hydrogen chloride, red phosphorus, and hydrochloric acid, a pentacyclic triterpene alcohol — epifriedelanol (III) — can undergo a number of transformations (see Scheme 1).

From the Scheme of transformations of epifriedelanol it can be seen that a dehydration reaction at the C<sub>3</sub> and C<sub>4</sub> positions is possible only if there is a hydrogen atom at C<sub>4</sub>. The greater mobility of this atom (as compared with the two H's at C<sub>2</sub>) can apparently be explained by the positive I-effect of the CH<sub>3</sub> group. The double bond formed through the splitting out of water from positions C<sub>3</sub> and C<sub>4</sub> then migrates into the C<sub>1</sub>(C<sub>10</sub>) position and the methyl group migrates from C<sub>3</sub> to C<sub>4</sub> with the formation of compound (V). Then the migration of the double bond continues from ring B into ring C with the formation of compound (VII), which is olean-13(18)-ene, i.e., the parent hydrocarbon of the amyrane series. Compound (VII) is a carbocation with a positive charge on carbon-13.

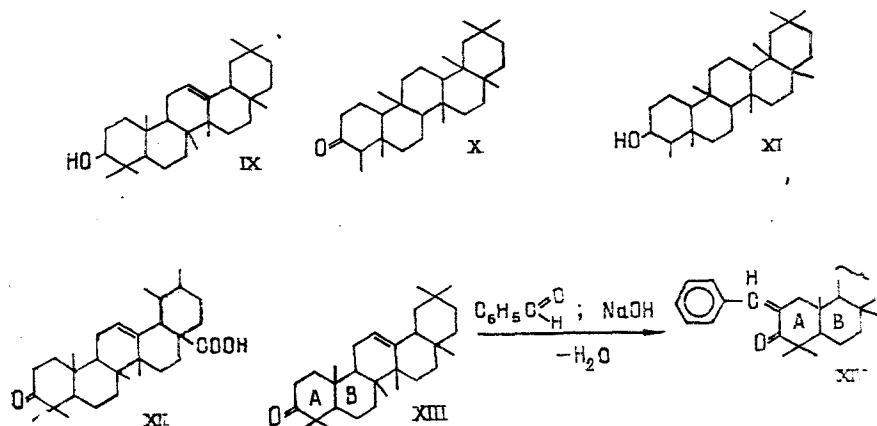
If we compare these transformations of epifriedelanol with the mechanism suggested by Brieskorn et al., some divergences become obvious. The first of them is that in the case of ursolic acid, and also for derivatives of  $\alpha$ - and  $\beta$ -amyrins, dehydration at the C<sub>3</sub>-C<sub>4</sub> positions is excluded because of the absence of hydrogen at C<sub>4</sub>. The second is that if the dehydration reaction takes place in the C<sub>2</sub>-C<sub>3</sub> positions, the migration of the double bond should occur in such a way as not to affect the methyl groups in the C<sub>8</sub>, C<sub>10</sub>, and C<sub>14</sub> positions, otherwise it would be necessary to consider for the proposed diene a structure alternative to that put forward by Brieskorn. All these facts give grounds for assuming the existence of a different mechanism of the reaction of triterpenoids with sulfuric acid.

It has been shown previously [4-6] that solutions of triterpenoids in sulfuric acid have a characteristic absorption band with a maximum at 310 nm. The results have been confirmed

by Kartnig and Micula [7] who have used this absorption band for the quantitative analysis of triterpenoids.

It was shown in these early papers [4-6] that the absorption band with its maximum at 310 nm is characterized for triterpenoids — derivatives of  $\alpha$ - and  $\beta$ -amyrins and of lupane and other representatives (more than 20 compounds). On the basis of these facts, a hypothesis has been put forward concerning the possible mechanism of the reaction of triterpenoids with sulfuric acid. Continuing these investigations, we have made an attempt to elucidate the mechanism of the reaction of triterpenoids with sulfuric acid.

We used oleanolic acid (VIII),  $\beta$ -amyrin (IX), friedelin (X) and friedelanol (XI), i.e., compounds belonging to different groups of triterpenoids.



Scheme 2

In sulfuric acid  $\beta$ -amyrin and oleanolic acid give a single maximum at 310 nm ( $\log \epsilon$  4.08 and 4.10, respectively;  $C = 0.004$  g in 100 ml of  $\text{H}_2\text{SO}_4$ ). The products of the reaction with sulfuric acid were isolated by the method of Brieskorn et al. [1, 2]. After the substances had been dissolved in ethanol, their UV spectra were measured at 220-420 nm. An ethanolic solution of oleanolic acid has a maximum at 246 nm, and an ethanolic solution of the product of the reaction of  $\beta$ -amyrin in  $\text{H}_2\text{SO}_4$  proved to be "transparent" possessing no characteristic absorption band.  $\beta$ -Amyrin acetate behaved similarly.

The question immediately arises: if the mechanism proposed by Brieskorn is correct, i.e., if a diene is formed, why is an anomaly observed in the case of  $\beta$ -amyrin? The structures given show that if dehydration took place with the subsequent migration of a double bond, in both cases one should expect absorption in the ultraviolet region (230-250 nm), which, in Brieskorn's opinion, would be due to the formation of a diene (in the present case, conjugated). This fact alone indicates the realization of a different reaction mechanism.

The products of the oxidation of oleanolic acid (3-ketooleanolic acid (XIII)) and 3-keto-ursolic acid (XII) also have maxima at 310 nm in sulfuric acid solutions. How does the dehydration reaction suggested by Brieskorn take place in this case? We have studied the product of the reaction of oleanolic acid with sulfuric acid with the aid of PMR spectroscopy in comparison with the spectra of the initial oleanolic acid and its solution in  $\text{H}_2\text{SO}_4$ . In the PMR spectrum of the initial oleanolic acid, the olefinic protons have chemical shifts of 5.3 ppm. In the PMR spectrum of a solution of oleanolic acid in  $\text{H}_2\text{SO}_4$ , no signals corresponding to olefinic protons are observed, but the signals of the aliphatic protons are shifted downfield as compared with the signals of the corresponding protons of oleanolic acid, which indicates the appearance of a positive charge in the molecule.

In the PMR spectrum of the product of the reaction of oleanolic acid with  $\text{H}_2\text{SO}_4$  in  $\text{CDCl}_3$ , likewise, there are no signals of olefinic protons. The reaction product was redissolved in  $\text{H}_2\text{SO}_4$  and the UV and PMR spectra of the resulting solution were investigated. In the UV spectrum, again, a maximum appeared at 310 nm, and in the PMR spectrum the signals of olefinic protons were absent.

The IR spectrum of the product of the reaction of oleanolic acid with sulfuric acid contained the following bands ( $\text{cm}^{-1}$ ): 1780, 1758 ( $\text{C}=\text{O}$  in a  $\gamma$ -lactone), 1160, 1120 (doublet,  $-\text{C}-\text{O}-\text{C}-$  bonds), 3400 ( $-\text{OH}$ ). These facts permit the assumption that, under the action of sulfuric acid, protonation of the olefinic bond of ring C takes place at  $\text{C}_{12}$  in accordance with Markovnikov's rule with the formation of a positive charge on  $\text{C}_{13}$  followed by lactonization with the carboxy group present in the  $\beta$  position with respect to the more substituted end of the double bond. Compound A is apparently formed in  $\text{H}_2\text{SO}_4$  solution, and compound B is a lactone formed after the dilution of the solution and the isolation of the product.

The presence of a carbocation is shown by the appearance of a coloration when triterpenoids are dissolved in sulfuric acid and by the maximum at 310 nm, which agrees well with literature information on carbocations [8]. Since the absorption at 310 nm proved to be characteristic for alcohols with an OH group in position  $\text{C}_3$  and their 3-keto derivatives (ursolic and 3-ketoursolic acids and oleanolic and 3-ketooleanolic acids, Table 1), we synthesized products from 3-ketooleanolic acid in which both hydrogens in the  $\text{C}_2$  position were substituted. For this purpose, 3-ketooleanolic acid was condensed with benzaldehyde.

The reaction product, consisting of 2-benzylidene-3-ketooleanolic acid (XIV) had characteristic absorption maxima in ethanol at 285 and 310 nm, which is explained by the presence of an aromatic nucleus in the conjugated system. The same compound had a characteristic absorption maximum in  $\text{H}_2\text{SO}_4$  at 310 nm.

Thus, in the case of substitution of positions 2 and 4, a 3-keto derivative has in sulfuric acid the characteristic maximum due to a carbocation, which is evidence in favor of the mechanism which we have suggested.

Finally, a substantial difference is observed in the UV spectra of sulfuric acid solutions of friedelin and friedelanol. In  $\text{H}_2\text{SO}_4$ , friedelanol has a maximum at 310 nm ( $\log \epsilon$  4.26) while friedelin does not absorb in sulfuric acid at all. The absorption of friedelanol is easily explained by Scheme 1, where it is shown that as the result of dehydration at  $\text{C}_3-\text{C}_4$  an olefinic double bond is obtained with the subsequent formation of a carbocation. In the case of friedelin, such a progress is impossible.

Thus, the experimental results that we have obtained permit the conclusion that on reaction with sulfuric acid triterpenoids containing olefinic double bonds form a carbocation with a characteristic absorption maximum at 310 nm. When a carboxy group is present in the  $\beta$  position with respect to the more substituted end of the double bond (ursolic and oleanolic acids), lactonization takes place with the breakage of the double bond.

#### EXPERIMENTAL

The UV spectra of the substances were measured on a SF-16 spectrophotometer, the IR spectra on a UR-20 instrument and the PMR spectra on a JNM-C60H1 spectrometer with a working frequency of 60 MHz.

TABLE 1. Absorption Maxima of Solutions of Triterpenoids in Sulfuric Acid

Substance	Position of the double bond	$\lambda_{\text{max}}$ , nm	$\log \epsilon$
Uvaol	$\Delta^{12}$ (ring C)	310	4.47
Ursolic acid	"	310	4.11
3-Ketoursolic acid	"	310	3.97
Ursolic acid acetate	"	310	4.17
$\beta$ -Amyrin	"	310	4.10
$\beta$ -Amyrin acetate	"	310	4.29
Oleanolic acid	"	310	4.08
Erythrodiol	"	310	4.19
3-Ketooleanolic acid	"	310	4
Taraxerol	$\Delta^{14}$ (ring D)	310	4.39
Taraxerone	"	310	4.33
Friedelanol	"	310	4.26
Friedelin	"	—	—
Lupeol	$\Delta^{20}$ (side-chain)	310	4.07
Lupenol	"	310	4.22
Betulifol	"	310	4.31
Betulinic acid acetate	"	310	3.94

UV Spectroscopy. Samples of the substances weighing 0.3-0.4 mg were dissolved in 10 ml of sulfuric acid (density 1.835), and the solutions obtained were thermostated at 70°C for 60 min. After the cooling of the reaction mixture, the spectra measurements were made relative to sulfuric acid of the same density.

Isolation of the Product of the Reaction of Oleanolic Acid with Sulfuric Acid. About 2 g of oleanolic acid was dissolved in 20 ml of H<sub>2</sub>SO<sub>4</sub> (density 1.835), the solution was thermostated at 70°C for 60 min and it was then cooled and poured onto 200 mg of crushed ice. The precipitate was separated off, washed with water, dried, and crystallized from ethanol.

The product of the reaction of  $\beta$ -amyrin with sulfuric acid was obtained similarly.

Condensation of 3-ketooleanolic Acid with Benzaldehyde. A mixture of 2.8 g (0.005 mole) of 3-ketooleanolic acid and 0.5 g (0.005 mole) of benzaldehyde was condensed in ethanolic NaOH solution (25 ml of ethanol and 1 g of NaOH in 1.5 ml of water) for 5-6 h, after which the reaction mixture was acidified with 10% HCl. The precipitate was separated off, washed with water, dried, and crystallized from ethanol) mp 185-186°C, C<sub>37</sub>H<sub>51</sub>O<sub>3</sub>.

#### SUMMARY

The single maximum of triterpenoids in sulfuric acid at 310 nm is due to the formation of a carbocation. Under the action of sulfuric acid oleanolic acid undergoes lactonization at the COOH group and the  $\Delta^{12} >C=C<$  bond.

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#### USE OF STEROID GLYCOSIDES FOR THE AFFINITY CHROMATOGRAPHY OF CHOLESTEROL

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A number of sorbents for the sorption of cholesterol has been synthesized from eight saponins of the spiro- and furostanol series immobilized on amino-Silochrome. The most effective have proved to be a sorbent containing capsicosin. Amino-Silochrome, and also sorbents containing digitonin, F-gitonin, and purpleagitoside cause hemolysis. The sorption capacity of the sorbents obtained falls on passing from saponins of the spirostanol series to saponins of the furostanol series, and also with a decrease in the length of the oligosaccharide moiety of the saponins.

The synthesis of an affinity sorbent of cholesterol using as the affinate digitonin oxidized with sodium periodate and immobilized on amino-Silochrome has been reported previously. In order to study the possibility of the use of other saponins for this purpose, we have investigated a series of steroid glycosides containing various aglycones and monosaccharide residues. Thus, we have considered saponins of the spiro- and furostanol series containing

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