# PREPARATION OF CERTAIN DERIVATIVES OF URSOLIC ACID AND THEIR ANTIMICROBIAL ACTIVITY

N. I. Zaletova, A. N. Shchavlinskii,
O. N. Tolkachev, S. A. Vichkanova,
T. V. Fateeva, N. M. Krutikova,
I. V. Yartseva, N. A. Klyuev

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For complex utilization of raw material and search for a new biologically active compounds, we studied the industrial wastes from the production of an antitumorigenic preparation rosevin, in which about 18% of ursolic acid (I$\alpha$) was found. Compound I was isolated and purified from the wastes by the method described in [7]. As the raw material for the preparation of Ia, we used the wastes from the production of thyme extracts, in which the content of triterpenoid compounds (ursolic and olenaoic acids) is 4.5% [1]. If the broad spectrum of the biological action of Ia (anti-inflammatory [3], hypocholesteremic, antiatherosclerotic [4], antimicrobial [10]), data on the use of salts of Ia as emulsifiers [14], and also data on the biological activity of certain derivatives of Ia [4, 6] are considered, it is seen that the isolation and use of Ia as the starting material for the synthesis of biologically active compounds is interesting.

The oxidation products of triterpenoids of ursan and oleanan series have antimicrobial activity; derivatives of oleanan, olean-18-en-3-on-28-oic acid (moronic acid) are active towards Staphylococcus aureus and Bacillus subtilis [9]. The oxidation of ursolic acid acetate (Id) by chromic anhydride in a mixture of glacial acetic acid and benzene is also known. It proceeds with the formation of a mixture of 3-O-acetyl-11-oxoursolic acid (Ie, yield 50%) and 3-O-acetyl-12-oxours-13,28-lactone (If, yield 20%) [12]. However, data on the antimicrobial activity of the Ia derivatives are not available. We obtained several oxygen-substituted derivatives of Ia by oxidation with chromic acid and $KMnO_4$ in different media to study their biological activity.

In the oxidation of Ia by chromic acid in acetone, ursonic acid (Ib) was obtained in a yield of 90%, and was converted into 3-oximino-12-enurs-28-oic acid (Ig). In glacial AcOH, the oxidation of Ia proceeds more extensively, and the main reaction product is 11-oxoursonic acid (Ic). Oxidation of Ia by $KMnO_4$ in an alkaline solution of tert-butanol gave 11,12-dehydro-13,28-lactone of ursolic acid (IIa) as the only reaction product; the compound had already been isolated together with its 3-O-acetyl derivative from certain types of eucalyptus [8]. In the oxidation of Ib by the action of $KMnO_4$ in glacial AcOH, 11,12-dehydro-13,28-lactone of ursonic acid (IIb) is obtained. The closing of the lactone ring with shifting of the double bond to the C(11)—C(12) position had already been observed in the reduction of 11-oxoursolic acid (Ih) by metallic sodium in ethanol [8], or by $LiAlH_4$ in anhydrous ether [12]. Compounds Ic and IIb were synthesized for the first time.

The structure of the compounds obtained was confirmed by the data of IR and PMR spectra and also by the data of molecular mass spectroscopy with ionization of the molecules under an electron impact. It is known from the literature sources (on the example of ursan [13]) that compounds with such a structure have in their mass spectra fairly intense peaks of molecular ions and two types of fragmentary ions formed as the result of degradation of C—C $\sigma$-bonds in ring C at position C(9)—C(11) and C(8)—C(14) (see Table 1). The fragment including rings A and B is designated as a, the fragmentary ion including rings C, D, and E is designated as a'. Table 1 shows that fragmentary ion a' has a higher intensity than

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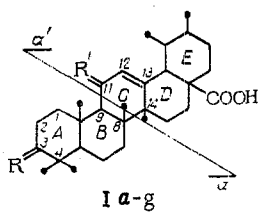
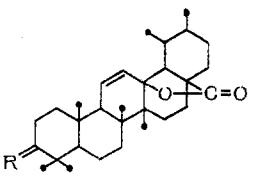
TABLE 1. Characteristic Ions in Electron Impact Mass Spectra of Compounds I$a$-e, II$a$, b

| Arbitrary designation of ions | Ia | | Ib | | Ic | | Id | | Ie | | IIa | | IIb | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | A | B | A | B | A | B | A | B | A | B | A | B | A | B |
| $[M+1]^+$ | 457 | — | 455 | — | 469 | 14 | | | | | 455 | 33 | 453 | 30 |
| $M^+$ | 456 | 9 | 454 | 9 | 468 | 43 | 498 | 9 | 512 | 37 | 454 | 100 | 452 | 100 |
| $[M-CO]^+$ | | | | | 440 | 16 | | | 484 | 5 | 426 | 25 | 424 | 12 |
| $[M-HOCOCH_3]^+$ | | | | | | | 438 | 8 | 452 | 10 | | | | |
| $[M-CO_2]^+$ | | | | | | | | | | | 410 | 96 | 408 | 46 |
| $[a'+1]^+$ | 249 | 23 | 249 | 20 | 263 | 16 | 249 | 53 | 263 | 19 | 247 | — | 247 | — |
| $[a']^+$ | 248 | 100 | 248 | 100 | 262 | 100 | 248 | 100 | 262 | 100 | 246 | 5 | 246 | 5 |
| $a^+$ | 208 | 7 | 206 | 5 | 206 | 5 | 250 | 8 | 250 | — | 208 | 11 | 206 | — |
| $[a-H]^+$ | 207 | 29 | 205 | 16 | 205 | 8 | 249 | 58 | 249 | 5 | 207 | 15 | 205 | 7 |
| $[a-COOH]^+$ | 203 | 31 | 203 | 34 | 217 | 23 | 203 | 67 | 217 | 24 | | | | |
| $[a-CO_2]^+$ | | | | | | | | | | | 202 | 13 | 202 | 6 |

Note. A) m/z; B) $I/I_{max}$, intensity in percent with respect to intensity of maximal peak of an ion; peaks of ions with intensity ≥5% are listed; peaks of ions up to 200 amu are taken into account.




I $a$-g




II $a$, b

Ia: R = OH, H, R' = 2H; Ib: R = O, R' = 2H; Ic: R = R' = O; Id: R = OAc, H; R' = 2H; Ie: R = OAc, H, R' = O; Ig: R = NOH, R' = 2H; Ih: R = OH, H, R' = O; IIa: R = OH, H; IIb: R = O.

fragment $a$ (exceptions are compounds II$a$, b in the presence of a π-bond in position C(10)—C(12)). In addition to the above ions, in the mass spectrum specific fragmetary ions are observed, characterizing the presence of a lactone ring and a carboxylic group. For compounds II$a$, b splitting of the $CO_2$ group is observed from both the molecular ion and the fragmentary ion a'. In the presence of a carboxylic group, the $CO_2$ group is eliminated from ion a'. It should be noted that in compounds Ic-e, IIa, b, elimination of a neutral CO particle from $M^+$ is observed (for Ic, e this process is related to the contraction of ring C to a 5-membered ring), while in compounds IIa, b, this involves elimination of CO from the lactonic group.

## EXPERIMENTAL (CHEMICAL)

The course of the processes and the purity of compounds obtained were controlled by TLC on Silufol UV-254 plates in a 9:1 $CHCl_3$—MeOH system. The plates were sprayed by a 3% alcoholic solution of silicotungstic acid. The preparative synthesis of the compounds was carried out on columns with silica gel L 100/250 (CSSR). The mass spectra were obtained on a MAT-311A apparatus from the firm Varian under standard conditions of exposure [5]. The IR spectra were run on a UR-20 spectrophotometer (GDR) in the form of a suspension in mineral oil, and PMR spectra on a Brucker WH-360 spectrometer, MHz in $CDCl_3$, using TMS as internal standard. The melting points were determined on a microheating Boetius plate.

3-Oxo-12-enurs-28-oic acid (Ursonic Acid) (Ib). A 2.0 g portion (6 mmoles) of $K_2Cr_2O_7$ in 30 ml of 30% $H_2SO_4$ is added in the course of 10 min, with continuous stirring at room temperature, to a solution of 2.0 g (4 mmoles) of Ia in 200 ml of acetone. The reaction mixture is stirred for another 10 min, and then poured into ice water. The precipitate that

separates is filtered, washed with water, and dried. Yield 1.8 g (90%) of Ib, mp 271-275°C (ethanol). IR spectrum, $\lambda_{max}$, $cm^{-1}$: 1695, 1685. PMR spectrum, δ, ppm: 5.26 m ($H_{(12)}$). $M^+$ 454. Found, %: C 79.19; H 10.18. $C_{30}H_{46}O_3$. Calculated, %: C 79.29; H 10.13.

3,11-Dioxo-12-enurs-28-oic Acid (Ic). A 1 g portion (3 mmoles) of $K_2Cr_2O_7$ in 20 ml of 30% $H_2SO_4$ is added to a solution of 1 g (2 mmoles) of Ia in 90 ml of glacial AcOH. The mixture is stirred for 1.5 h, keeping the temperature of the reaction mixture at 45-50°C. On the chromatogram two spots were noted: the starting compound with $R_f$ 0.45 and the oxidation product with $R_f$ 0.69. At the end of the reaction, the reaction mixture is poured into double the volume of ice water, the precipitate that separates is filtered, washed with water, dried and chromatographed on a column with silica gel. After the elution with a petroleum ether–$CHCl_3$ (3:2) mixture, 0.72 g (72%) of Ic is obtained. White, fine-crystalline precipitate, mp 279-283°C (aqueous acetone). The compound is soluble in $CHCl_3$, acetone, benzene, sparingly soluble in alcohols, and insoluble in water. IR spectrum, $\lambda_{max}$, $cm^{-1}$: 1715, 1700, 1660, 1625. PMR spectrum, δ, ppm: 5.63 s ($H_{(12)}$). $M^+$ 468. Found, %: C 76.56; H 9.40. $C_{30}H_{44}O_4$. Calculated, %: C 76.79; H 9.40.

11,12-Dehydro-13,28-lactone of Ursolic Acid (IIa). A 0.1 g portion of KOH and 0.5 g of Ia are dissolved in 40 ml of tert-butanol. The solution is heated to 70°C and 10 ml of a 5% aqueous solution of KOH are added dropwise with continuous stirring. The temperature of the reaction mixture is kept at 70-75°C for 12 h up to the appearance of the main spot with $R_f$ 0.86 on the chromatogram. After the end of the reaction, the brown $MnO_2$ precipitate is filtered, and washed with tertbutyl alcohol. The filtrate is acidified by 5% HCl to pH 5.0 and evaporated *in vacuo* to half its volume. The gelatinous precipitate that separates is extracted by $CHCl_3$ (3 × 30 ml). The chloroform extract is washed with water, dried over anhydrous $Na_2SO_4$, the solvent is distilled *in vacuo*, and the residue is chromatographed on a column. After elution with a pe-troleum ether–$CHCl_3$ (85:15) mixture, 0.33 g (66%) of IIa is obtained, mp 244-246°C (aqueous MeOH). IR spectrum, $\lambda_{max}$, $cm^{-1}$ ($CHCl_3$): 3620, 1750. PMR spectrum, δ, ppm: 3.21 m($H_{(3)}$), 5.53 dd ($H_{11}$, $J_{11,12}$ 10.14 Hz $J_{11,9}$ 3.29 Hz) 5.95 dd ($H_{(12)}$, $J_{12,9}$ 1.29 Hz), $M^+$ 454. Found. %: C 79.26; H 10.32. $C_{30}H_{46}O_3$. Calculated, %: C 79.23; H 10.13.

3-O-Acetyl-11,12-dehydro-13,28-lactone of ursolic acid is obtained by the method described in [11]. mp 261-263°C (acetone). $M^+$ 496. IR spectrum. $\lambda_{max}$, $cm^{-1}$: 1750, 1725, 1240.

11,12-Dehydro-13,28-lactone of Ursonic Acid (IIb). A 20 ml portion of a 5% aqueous solution of $KMnO_4$ is added with continuous stirring to a solution of 1 g of Ib in 80 ml of glacial acetic acid. The temperature of the reaction mixture is kept for 4 h at 45-50°C up to the appearance of a spot with $R_f$ 0.95 on the chromatogram. The brown $MnO_2$ precipitate is separated, washed with AcOH, and to the filtrate 100 ml ofwater are added. The filtrate is extracted by $CHCl_3$ (3 × 50 ml), the extract is evaporated *in vacuo*, and the residue is chromatographed on a column. After elution with a petroleum–$CHCl_3$ (4:1) mixture, 0.6 g (60%) of IIb is obtained; a white crystalline substance, soluble in $CHC_3$, acetone, sparingly soluble in alcohols, and insoluble in water. Mp 224-226°C (aqueous acetone). IR spectrum, $\lambda_{max}$, $cm^{-1}$: 1750, 1695. PMR spectrum, δ, ppm: 5.58 dd ($H_{11}$, $J_{11,12}$, 10.15 Hz, $J_{11,9}$, 3.10 Hz) 5.95 dd ($H_{12}$, $J_{12,9}$ 1.25 Hz). $M^+$ 452. Found, %: C 79.86; H 9.80. $C_{30}H_{44}O_3$. Calculated, %: C 79.65 H 9.73.

3-O-Acetyl-12-enurs-28-oic acid (Id) is obtained by the method described in [11]. Mp 260-263°C (benzene). IR spectrum, $\lambda_{max}$, $cm^{-1}$: 1725, 1690, 1240. $M^+$ 498.

3-O-Acetyl-11-oxoursolic acid (Ie) and 3-O-acetyl-12-oxours-13,28-lactone-28-oic acid (If) are obtained by a method described in [12]. For compound Ie, mp 323-326°C (MeOH). IR spectrum, $\lambda_{max}$, $cm^{-1}$: 1725, 1817, 1625, 1240. $M^+$ 512. For compound If, mp 277-279°C (aqueous acetone). IR spectrum, $\lambda_{max}$, $cm^{-1}$: 1775, 1724, 1710, 1240. $M^+$ 512.

11-Oxoursolic Acid (Ih). A 50 ml portion of 10% $H_2SO_4$ is added to a solution of 1 g of Ie in 50 ml of MeOH, and the mixture is boiled for 1 h. The reaction mixture is diluted with double the volume of water, the precipitate that separates is filtered, washed with water, and dried. Yield quantitative. Mp 242-245°C (ethanol). IR spectrum, $\lambda_{max}$, $cm^{-1}$: 3620-3628, 1690, 1660. $M^+$ 470.

3-Oximino-12-enurs-28-oic acid (Ig) is obtained by the method described in [15]. Mp 273-276°C (ethanol). IR spectrum, $\lambda_{max}$, $cm^{-1}$: 1695, 1665. $M^+$ 469.

## EXPERIMENTAL (BIOLOGICAL)

The antimicrobial activity of compounds Ia-h, IIa, b was studied by the method of double serial dilutions [2] in a Hottinger bullion for *Staphylococcus aureus* 209P, *Escherichia coli* M17, *Proteus vulgaris*, *Pseudomonas aeruginosa* 165. In the determination of the antifungal activity towards *Microsporum lanosum*, *Candida albicans* 1755, Saburo medium was used. After they had been inoculated by the corresponding cultures of microorganisms, the test tubes were incubated at 37-38°C (for growing the bacteria) and at 28-30°C (for growing mycelial and yeast-like fungi). The antimicrobial activity (in μg/ml) was determined from maximal dilution at which the growth of the microorganisms was no longer observed visually.

The results of the investigations showed that several of the compounds have antimicrobial activity towards Gram-positive bacteria. Thus, five of the compounds out of ten (Ia, c,g,h, and IIb) inhibited the growth of *St. aureus* in concentrations of 250 μg/ml, and Id, 1000 μg/ml. In the case of gram-negative microorganisms, the effect was less pronounced and appeared at a concentration of 500 and 1000 μg/ml.

In our experiments, the starting compound (Ia) inhibited the growth of *St. aureus* (250 μg/ml) and *Ps. aeruginosa* (1000 μg/ml) only. When a carbonyl group was introduced into the C(11) position of the molecule of Ia (Ih), the activity towards *St. aureus* was retained, the activity towards *Ps. aeruginosa* doubled, and an activity appeared towards Gram-negative bacteria, such as *E. coli* (500 μg/ml), *Pr. vulgaris* (1000 μg/ml) and mycelial fungi *M. lanosum* (1000 μg/mg) When the hydroxylic group in the molecule of ursolic acid was replaced by a carbonyl group (Ib), the bacteriostatic action disappeared completely. It was interesting that Ia has a bacteriostatic action, but does not inhibit the growth of the mycelial and yeast-like fungi. Acetylation of Ia does not change the fungistatic action. The formation of a lactone ring in a molecule of Ia with shift of the double bond to positions C(11)–C(12) imparted antifungal properties to Ia: at a concentration of 250 μg/ml, IIa inhibits the growth *M. lanosum* and *C. albicans*. When the alcoholic group of IIa is oxidized to a carbonyl group (IIb), the fungistatic effect decreases by a factor of 2-4.

With ursolic acid as an example it was thus shown that an appreciable change in the biological activity of the compound is possible. The regularities found, taking into account the literature data on the high biological activity of certain triterpenoids, can be used to produce new medicinal preparations with given properties.

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