STRUCTURE OF A PENTACYCLIC TRITERPENE ALCOHOL FROM *Centaurea aquarrosa*

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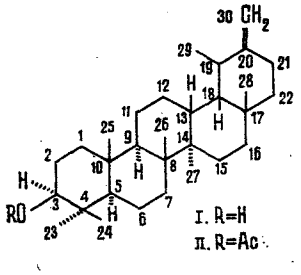
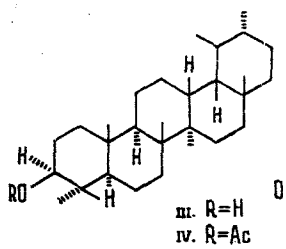
UDC 547.914.4

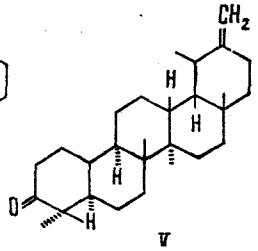
The present paper describes the determination of the structure of a previously unknown pentacyclic triterpene (I) isolated from *Centaurea squarrosa* Willd. [1].



I. R=H
II. R=Ac



III. R=H
IV. R=Ac



V

IR spectrum of the triterpene (I) (Fig. 2) shows the absorption bands of an alcoholic hydroxyl (3400 cm^{-1}) and of a $-C=CH_2$ group (1642, 3090, and 882 cm^{-1}).

The presence of an alcoholic hydroxyl was confirmed by the acetylation of (I) to the corresponding acetate (II) and by its oxidation with chromium trioxide to the ketone (V). The presence in the IR spectrum of the oxidation product (V) of an absorption band of a ketonic carbonyl at 1704 cm^{-1} and the absence of absorption bands of an aldehyde group shows the secondary alcoholic nature of the hydroxyl. The PMR spectrum of the acetate (II) showed a three-proton singlet of acetyl protons at 2.00 ppm and the paramagnetic shift of the signal of a hydrogen geminal to a hydroxy group — a one-proton quartet at 3.20 ppm with J_{AX} = 10 Hz and J_{BX} = 5 Hz (Fig. 2) — had changed into a quartet at 4.52 ppm.

In the UV spectrum of (I) there was no selective absorption in the 220-400 nm region. The appearance of a characteristic maximum at 308 nm after the brief heating of its sulfuric acid solution showed that the alcohol belonged to the series of pentacyclic triterpenes [2].

Analysis of the PMR spectra of (I) (see Fig. 2) and (II) (see [3, 4]) enabled the number of methyl groups attached to saturated carbon atoms to be determined (seven), and also the type of unsaturation and the number of hydrogen atoms on the double bond to be determined (two). The signal of the protons at 4.65 ppm with J = 1-2 Hz is characteristic for methylene protons at an exocyclic double bond [5]. It was absent from the spectrum of the product of the catalytic hydrogenation of (III) which, in contrast to the hydrogenation of olean-12-enes and urs-12-enes takes place with great readiness [6]. This brought the number of methyl groups up to eight.

Table 1 gives the results of a mass-spectrometric analysis of the compounds mentioned above (I-IV).

The ions most characteristic of pentacyclic triterpenes on mass-spectrometric fragmentation are ions a and $[a - ROH]^+$, the peaks of which have the highest intensities in the mass spectra [4, 7]. A comparison of the m/e values of the ions a, b, c, d, e, etc., en-

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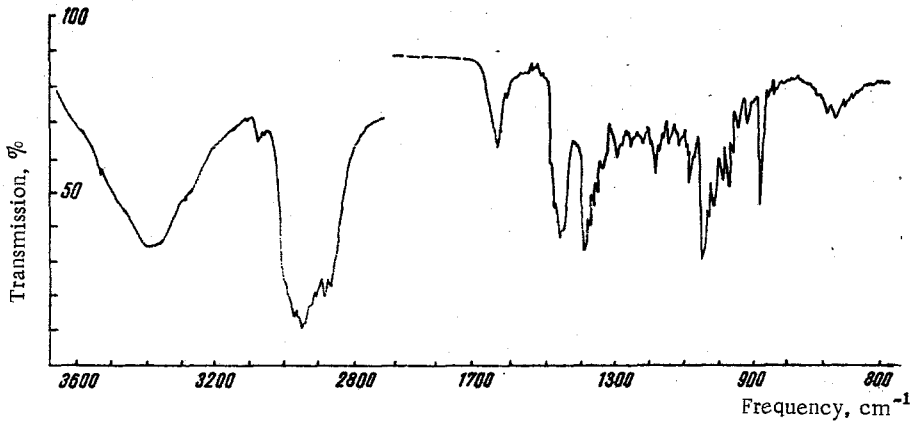


Fig. 1. IR spectrum of the triterpene (I) (in KBr).

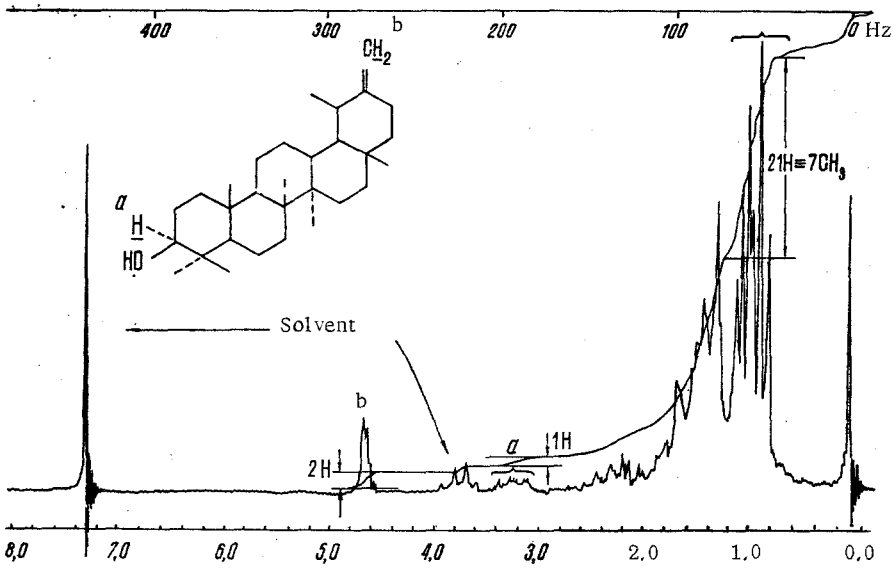
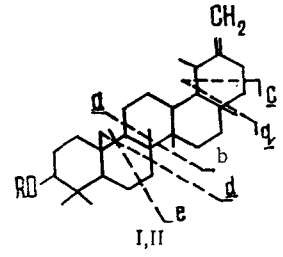
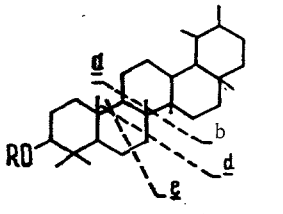


Fig. 2. PMR spectrum of the triterpene (I) (in $CDCl_3$).

TABLE 1. Main Ions Formed in the Fragmentation under Electron Impact of the Molecular Ions of (I-IV)*



I,II



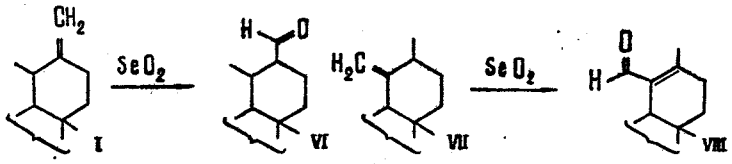
III,IV

Type of ion	I	II	III	IV
M^+	426(100)	468(88)	428(63)	470(39)
$M-Me\cdot]^+$	411(9)	453(5)	413(12)	455(5)
$[M-ROH]^+$	408(9)	408(24)	410(12)	410(21)
$[M-Me\cdot-ROH]^+$	393(6)	393(11)	395(7)	395(16)
a	207(100)	249(24)	207(100)	249(34)
$[a-\overline{R}OH]^+$	189(88)	189(100)	189(50)	189(100)
b	218(17)	218(28)	—	—
c	357(20)	399(16)	—	—
d	257(8)	257(5)	259(4)	259(6)
e	272(8)	272(5)	274(6)	274(3)
q	344(7)	386(6)	—	—
$[M-29]^+$	397(5)	439(3)	—	—
$[M-C_8H_{15}]^+$ x	315(12)	357(11)	—	—
	—	365(5)	—	367(2)

*Relative intensities of the peaks of the corresponding ions are shown in parentheses.

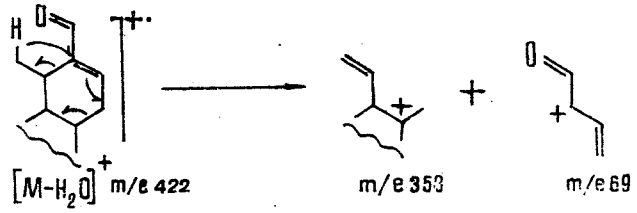
ables us to suggest that the double bond is present in ring E between carbon atoms 20 and 30 (structure I) or between carbon atoms 19 and 29 (VII).

The formation of ion c is evidence in favor of structure (I). To confirm the correctness of this conclusion, (I) was readily oxidized with selenium dioxide to the corresponding aldehyde (VI). In the case of structure (VII), the formation with somewhat less ease [8] of the aldehyde (VIII) would have been expected (Scheme 1).



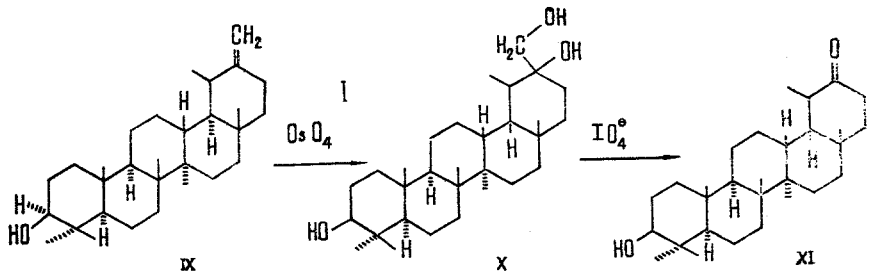
Scheme 1

The structure of the aldehyde (VI), giving a positive reaction with dinitrophenylhydrazine, was confirmed by its IR, UV, PMR, and mass spectra. The UV spectrum of (VI) shows an absorption maximum at 233 nm (EtOH) with ε = 6600, which is characteristic for structure (VI) (see [9, 10]. This value is also in agreement with the value expected for α,β-disubstituted α,β-unsaturated aldehydes calculated according to the Woodward–Fieser rule [11] and excludes the possibility of a tetrasubstituted double bond. The PMR spectrum of the aldehyde contained, in addition to the signal of the aldehydic proton at 9.37 ppm, a one-proton multiplet at 6.5-6.9 ppm. The latter corresponds to a proton at a C=C double bond conjugated with a carbonyl group. The presence in the mass spectrum of the aldehyde (VI) of strong peaks of ions with m/e 353 and 69, which are probably formed as the result of a McLafferty rearrangement [12], is one more piece of evidence in favor of structure (VI). The rearrangement probably takes place as shown in Scheme 2.



Scheme 2

Thus, the structural difference between the triterpene (I) and taraxasterol (IX), a long-known pentacyclic triterpene [10], must be sought in the stereochemistry of the rings and substituents.



Scheme 3

The ketone (V) shows dichroic absorption at 293 nm (291 nm, EtOH) with $\Delta\varepsilon$ = +0.7632 (+0.505; EtOH) (Fig. 3a), from which it may be concluded the rings A, B, and C are trans-linked [13, 14]. The nature of the ORD curves of (I) and (III) (Fig. 3b) and a comparison of them with the ORD curves of a number of pentacyclic triterpenes [13] permit us to suggest the trans configuration for rings C and D, as well.

A proof of the cis-linkage of rings D and E was obtained from a structural analysis of the ketone (XI). In order to obtain the latter, the triterpene (I) was first oxidized with osmic acid to the triol (X) (Scheme 3). In the mass spectrum of the latter, the maximum intensity was possessed by the peak of the ion $[M - CH_2OH]^+$ with m/e 429, formed as a result of the cleavage of the glycol bond [17], which is one more proof of the presence of an exocyclic methylene group in the triterpene (I). The triol (X) was characterized mass-spec-

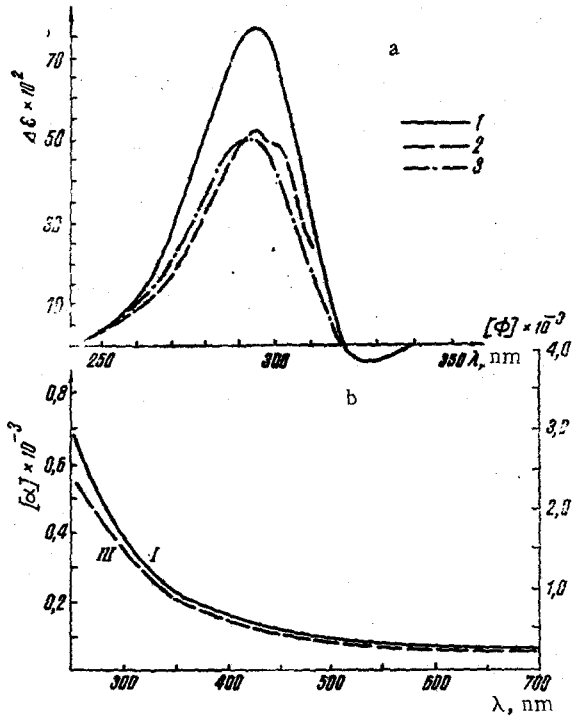
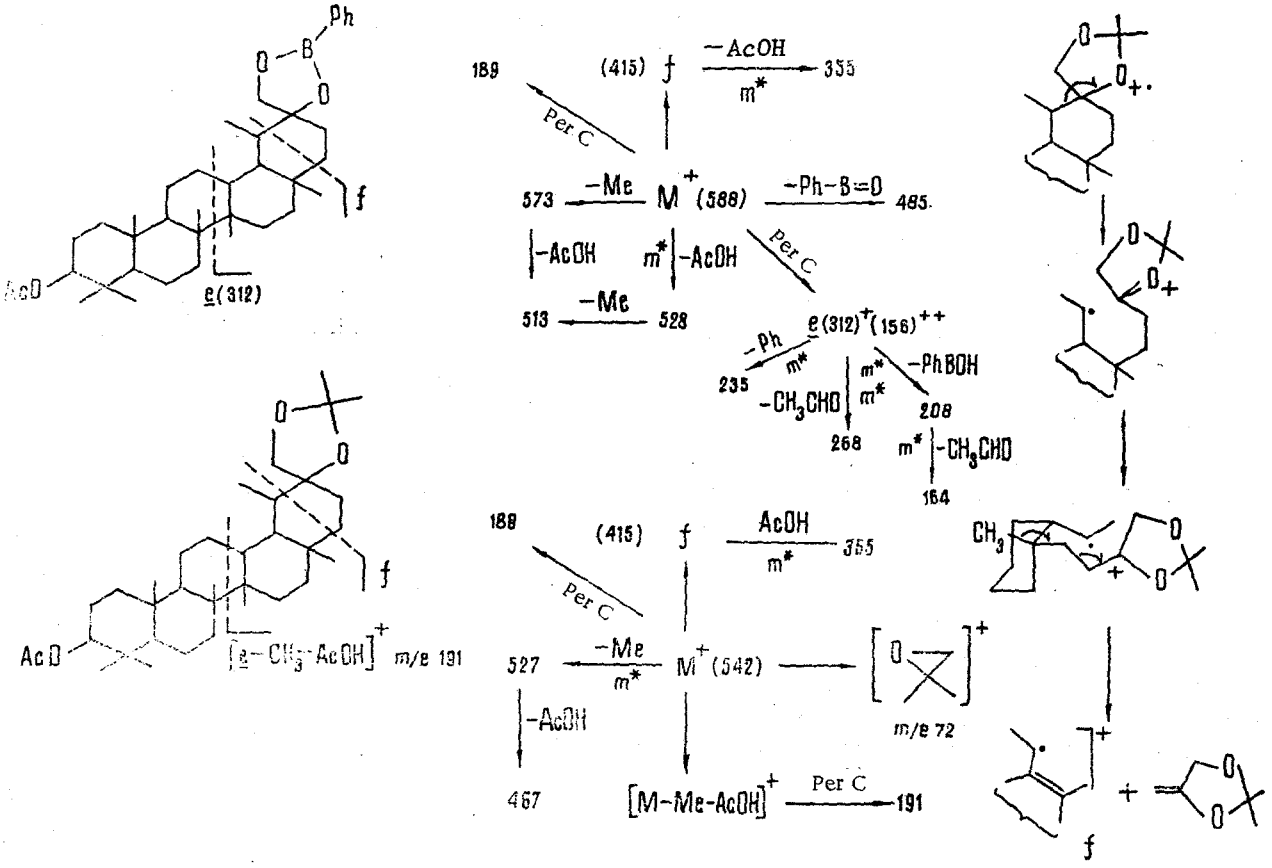


Fig. 3. CD curves of the ketone (V) (a) and ORD curves of the triterpene (I) and the product of its hydrogenation (b): 1) in $CHCl_3$; 2) in dioxane; 3) in EtOH.



Scheme 4. (*-doubly charged ion).

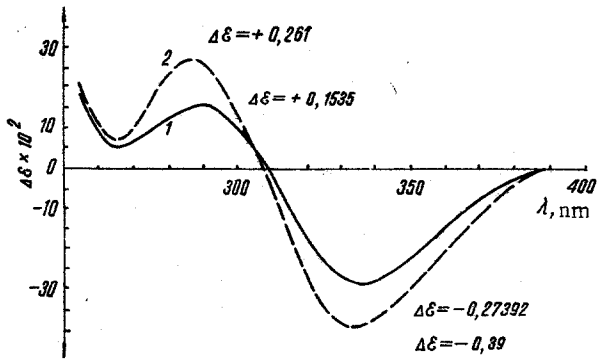
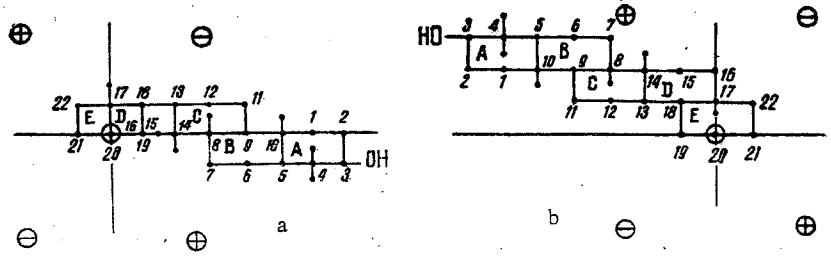


Fig. 4. CD curve of the ketone (XI) in $CHCl_3$ (1) and in EtOH (2).

trometrically in the form of the acetates of the benzeneboronate (XII) and of the isopropylidene ketal (XIII). Scheme 4 shows the pathways of their mass-spectrometric fragmentation.

The triol (X) was oxidized with sodium periodate to the ketone (XI) and formaldehyde, which was identified spectrophotometrically [17].

Scheme 5* shows the application of the octant rule [22] to the ketone (XI) with the cis configuration of rings D and E (a) and to the corresponding ketone obtained from taraxasterol (IX) with the trans linkage of rings D and E (b).



Scheme 5

For the ketone (XI) a fairly weak Cotton effect in the CD spectrum at 290-300 must be expected, while in case b (see Scheme 5) a very strong effect is assumed. Figure 4 shows the CD curves of the ketone (XI) obtained. It can be seen that the additional maximum at 290 nm (288 nm in EtOH) has the relatively small value of $\Delta\varepsilon$ = +0.15348 (+0.261; EtOH).

The negative maximum at 335 nm (333 nm, EtOH) can be explained both by the formation of an intermolecular hydrogen bond of the hydroxyl at C_3 with the carbonyl group and by the existence of ring E in the "twist" form. A whole series of similar examples of the appearance of complex Cotton effects is known which are explained either by a conformational equilibrium or by asymmetric solvation [15]. In the present case, the bands of opposite signs should be considered as the result of a conformational equilibrium.

The conclusion that the hydroxyl has the 3β configuration was made by comparing the PMR spectra of (I) (see Fig. 2) and (III) with the PMR spectrum of methyl bryonolate, the 3β configuration of the alcoholic hydroxyl of which has been demonstrated [19]. The spectra of these compounds show a one-proton quartet at 3.20 ppm (J_{AX} + 10 Hz, J_{BX} = 5 Hz) which corresponds to axial hydrogen present in the geminal position to an alcoholic hydroxyl.

The methyl group at C_{19} is obviously equatorial, since the ketone (XI) does not epimerize at C_{19} under conditions in which epimerization takes place for 30-nortaraxastan-20-one [10]. This becomes completely understandable if one bears in mind the fact that in this case rings D and E are in the cis configuration. The passage of the C_{29} methyl group into the axial position is hindered because of steric interaction with the C_{27} methyl group at C_{14}.

*In order to simplify Scheme 5, no account has been taken of the distortion of rings A and B as a consequence of the 4,4-dimethyl effect [14].

Thus, the totality of the facts given permit triterpene (I) to be assigned the structure of urs-20(30)-en-3β-ol (I).

EXPERIMENTAL

For column chromatography and TLC we used type KSK silica gel. The plates for TLC were prepared by the method of Bergel'son et al. [20] and were run in the solvent system $CHCl_3$—benzene—EtOAc (3:2:1). The substances on the chromatograms were revealed with 1) 40% H_2SO_4 followed by heating at 180-200°C; 2) a mixture of H_2SO_4 and CH_3COOH (1:1) [21]; 3) a 0.4% solution of 2,4-dinitrophenylhydrazine in 2 N HCl [22], and 4) a mixture of periodate and the Schiff reagent [23].

The IR spectra were taken on a UR-20 spectrophotometer (Zeiss), the PMR spectra on a Varian-60A spectrometer at 60 MHz in $CDCl_3$ with Me_4Si as internal standard, the UV spectra on a Specord UV-Vis spectrophotometer in EtOH (c 0.01 mg/ml, ℓ 1 cm), the ORD curves on a Jasco ORD/UV-5 spectropolarimeter in $CHCl_3$, EtOH, and dioxane (c 1 mg/ml, ℓ 1 cm), and the mass spectra on an LKB-9000 mass spectrometer with an energy of the ionizing electrons of 70 eV and on an MKh-1303 mass spectrometer (40 eV).

The formaldehyde was determined by Renkonen's method [17] on a SF-4 spectrophotometer. The EtOH for optical measurement was redistilled after being boiled under reflux over dinitrophenylhydrazine, the $CHCl_3$ over P_2O_5, and the dioxane over KOH.

The Triterpene Alcohol (I). The isolation of (I) has been described previously [1]. mp 197-198°C (methanol—acetone; 4:1); R_f 0.67 (systems 1 and 2); $[\alpha]_D^{20}$ 71 ± 2.5° (c 1.09; chloroform).

The Acetate (II). A mixture of 54.2 mg of the triterpene (I), 1.5 ml of pyridine, and 0.1 ml of Ac_2O was kept at 25°C for 24 h and at 70°C for 2 h, and then the solvent was evaporated off (40°C/0.05 mm, and the residue was chromatographed on a column (30 × 1 cm) containing 10 g of silica gel (60-80 mesh). The acetate (II) was eluted with 50 ml of $CHCl_3$, and the solvent was evaporated, giving 51.4 mg of the acetate (II), R_f 0.83 (chromogenic systems 1 and 2). mp 214-215°C (acetone), $[\alpha]_D^{24}$ +100 ± 3° (c 0.87; chloroform); IR spectrum,* ν_{max}^{KBr}, cm^{-1}: 1729 (str. v. of an acetyl C=O), 1251 (str. v. of C—O), 1642 (str. v. $-\underset{|}{C}=CH_2$, 3088 and 889 (str. and nonpl. def. v. $=C\begin{matrix}H\\H\end{matrix}$) cm^{-1}.

PMR spectra, δ, ppm: 0.89, 0.91, 0.97, 1.00, 1.06, 1.12 (seven C—Me's); 2.00 (3H, singlet, $\underline{CH_3}-\overset{O}{\overset{|}{C}}-C-$), 3.50 (1H, quartet, J_{AX} = 10 Hz, J_{BX} = 5 Hz, $H-\underset{OAc}{\overset{|}{C}}-\underset{H}{\overset{|}{C}}-\underline{H}$); 4.62 (2H, multiplet $=CH_2$).

Hydrogenation of (I). A room temperature and atmospheric pressure, 75.0 mg of (I) in 10 ml of mixture of EtOH and petroleum ether (3:2) and 0.3 ml of glacial acetic acid was hydrogenated over 20 mg of PtO_2 at room temperature and atmospheric pressure for 5 h. The catalyst was filtered off, and, after evaporation of the filtrate and drying of the residue, (30°C/0.05 mm), 66.6 mg of (III) was obtained. R_f 0.68 (systems 1 and 2). mp 203-205°C (acetone). PMR spectrum: δ 0.78-1.13 (eight C—Me's), 3.20 (1 H, quartet, J_{AX} = 10 Hz, J_{BX} = 5 Hz, $H-\underset{OH}{\overset{|}{C}}-\underset{H}{\overset{|}{C}}-\underline{H}$).

Acetylation of (III). Compound (III) (17.2 mg) was acetylated similarly to (I). This gave 16.6 mg of (IV) with R_f 0.83 (systems 1 and 2). mp 212-214°C. IR spectrum, ν_{max}^{KBr} 1730 (str. v. of an acetyl C=O), 1250 (str. v. of C=O).

Hydrogenation of (II). Compound (II) (6.2 mg) was hydrogenated in a similar manner to (I). This gave 6.0 mg of (IV). R_f 0.83 (systems 1 and 2). mp 212-214°C.

*Here and below, in the description of the IR spectra the following abbreviations have been adopted: str.) stretching; def.) deformation; nonpl.) nonplanar; v) vibrations.

Oxidation of (I) with Chromium Trioxide [24]. To a solution of 52 mg of (I) in 8 ml of acetone (redistilled over $KMnO_4$) was added 0.08 ml of a solution of the oxidizing agent (a mixture of 2.67 mg of chromium trioxide and 2.3 ml of H_2SO_4 diluted with water to 10 ml), and the mixture was kept in an atmosphere of nitrogen at room temperature for 5 min. Then it was diluted with 40 ml of water, and the white precipitate that deposited was filtered off and dried in vacuum over KOH. This gave 46.2 mg of (V). R_f 0.82 (systems 1 and 2); mp 178-179°C (EtOAc); $[\alpha]_D^{15}$ +90 ± 5° (c 0.23; CCl_4); IR spectrum, ν_{max}^{Nujol}, cm^{-1}: 1704 (str. v. —C=O), 1640 (str. v. —C=CH_2), 3080, 890 (str. and nonpl. def. v. =C<H/H) cm^{-1}; mass spectrum,* m/e: 424 (100), 409 (10), 395 (4), 355 (29), 313 (15), 205 (12), 218 (60).

PMR spectrum, δ, ppm: 0.87-1.10 (seven C—Me's), 4.58 (2H, multiplet =C<u>H</u>$_2$), quartet at 3.20 absent. The ORD and CD curves are given in Fig. 3.

Oxidation of (I) with Selenium Dioxide. A mixture of 120 mg of (I) and 150 mg of SeO_2 was boiled in 12 ml of a mixture of dioxane and acetic acid (1:1) for 1 h. After cooling, 15 ml of H_2O was added and the reaction mixture was extracted with benzene (3 × 30 ml). The combined extract was evaporated. The residue was chromatographed on plates (30 × 20 cm) coated with silica gel in the $CHCl_3$—benzene—EtOAc (3:2:1) system. Not more than 50 mg of substance was deposited on these plates. The substance, which showed up without the use of a revealing agent in the form of a light yellow band, was eluted from the adsorbent with 60 ml of $CHCl_3$—MeOH—ether (1:1:1). This gave 50.2 mg of the aldehyde (VI). R_f 0.62 (systems 1-3), mp 228-229°C (MeOH); IR spectrum, ν_{max}^{KBr}, cm^{-1}: 1680 (str. v. of C=O conjugated with C=C bond), 1666 (str. v. of C=O conjugated with C=C bond of an associated aldehydic carbonyl) 2735, 2840 (str. v. of aldehydic C—H bond), 1640 (str. v. of C=C), 3480 (str. v. of O—H of an associated alcoholic hydroxyl); ν_{max}^{Nujol} 1659 and 1679 cm^{-1} (aldehydic carbonyl bound and not bound by an intermolecular hydrogen bond with an alcoholic hydroxyl), 3480 (str. v. O—H of an associated alcoholic hydroxyl); $\nu_{max}^{CHCl_3}$ 1670 (str. v. of C=O of a conjugated aldehydic carbonyl), 3590 (str. v. of O—H of an unassociated alcoholic hydroxyl). PMR spectrum, δ, ppm: 0.64-1.13 (seven C—Me's); 9.37 (1H, singlet, H—C(=O)—); 6.5-6.9 (1H, multiplet, H—C=C—CHO); 3.19 (1H, quartet, J_{AX} = 10 Hz, J_{BX} = 5 Hz, H—C(OH)—C(H)—H). Mass spectrum, m/e: 440 (23), 422 (100), 407 (18), 379 (26), 353 (7), 340 (12), 207 (7), 189 (7), 69 (95).

Oxidation of (I) with Osmic Acid [25]. To a solution of 160 mg of (I) in 18 ml of pyridine—dioxane (1:8) was added 270 mg of osmic acid in 16 ml of dioxane. The reaction mixture was kept at room temperature for 18 h, and then 65 ml of $Na_2S_2O_5$ solution (8 g of $Na_2S_2O_5$ was dissolved in 50 ml of H_2O and 15 ml of NaOH) was added. After 1.5 h, the precipitate was filtered off and was washed with $CHCl_3$, MeOH, and dioxane (20-ml portions). The filtrate was evaporated to dryness and the residue was extracted with chloroform. The chloroform-insoluble matter was dissolved in 10 ml of H_2O and the solution was extracted with chloroform. The aqueous layer was evaporated and the residue was dissolved in 30 ml of a mixture of $CHCl_3$ and MeOH (2:1), the solid being separated by centrifuging, and the supernatant liquid being combined with the chloroform extracts, which were then evaporated to dryness. This gave 170 mg of the triol (X). R_f 0.1 (systems 1, 2, and 4); mp 266-268°C ($CHCl_3$). Mass spectrum, m/e: 460 (0.5), 441 (10), 429 (53), 411 (15), 410 (12), 373 (38), 355 (17), 341 (3), 207 (60), 189 (56).

Oxidation of (II) with Osmic Acid. The acetate (II) (10 mg) was oxidized in the same way as (I). The resulting acetate of the diol (XIV) [9.2 mg, R_f 0.21 (systems 1, 2, and 4)] was analyzed mass-spectrometrically in the form of the benzeneboronate (XII) and the isopropylidene ketal (XIII).

Preparation of the Benzeneboronate [26] (XII). A solution of 2.4 mg of (XIV) in 2 ml of absolute acetone was treated with 0.3 mg of benzeneboronic anhydride. The mixture was kept for 24 h, after which the solvent was evaporated off to give 2.6 mg of (XII) with mp 205-208°C.

*Here and below, in the description of the mass spectra the relative intensities of the peaks are shown in parentheses.

Preparation of the Acetonide [25] (XIII). A solution of 5.2 mg of (XIV) in 5 ml of absolute acetone was treated with 250 mg of anhydrous copper sulfate. After the mixture had been heated for an hour at 50°C, the suspension was centrifuged. The supernatant was evaporated to give 4.6 mg of (XIII) with mp 266-269°C.

Periodate Oxidation [10] of (X). To a solution of 32 mg of the triol (X) in 20 ml of EtOH was added 80 ml of a solution of $NaIO_4$ (to 400 mg of HIO_4 in 4 ml of H_2O was added 80 ml of NaOH in 60 ml of H_2O, and the solution was diluted to 20 ml, pH 6.9). The reaction mixture was poured into 5 ml of H_2O and extracted with ether (10 ml × 3). The combined extract was evaporated and the residue was chromatographed on a column (20 × 1 cm) containing silica gel (60-80 mesh). This gave 26.8 mg of (XI). R_f 0.59 (systems 1-3), mp 235-236°C. IR spectrum, $\nu_{max}^{CHCl_3}$, cm^{-1}: 1695 and 1730 (str. v. of C=O of a carbonyl ketone bound and nonbound by an intermolecular hydrogen bond with the hydroxyl at C_3); ν_{max}^{KBr}, cm^{-1}: 1675 and 1707 (str. v. of C=O). Mass spectrum, m/e: 428 (62), 410 (100), 395 (53), 367 (33), 355 (2), 341 (13), 328 (40), 207 (51), 189 (98).

SUMMARY

The structure of a previously unknown pentacyclic triterpene isolated from *Centaurea squarrosa* Willd. has been established. It is urs-20(30)-en-3β-ol (I).

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