# THE STRUCTURE OF THE SESQUITERPENE LACTONE REPIN

R. I. Evstratova, K. S. Rybalko,
and V. I. Sheichenko

UDC 615.256.54.011.5

The isolation from Acroptilon repens L. (DC) of two sesquiterpene lactones, repin and acroptilin, has been reported previously [1-3]. From Centaurea hyrcanica Bornm. we have isolated repin [4], acroptilin, and a lactone related to them with mp 218-220°C (from ethanol), $[\alpha]_D^{20}$ + 104.7° (c 1.91; dioxane); in the NMR spectrum of the latter (Fig. 1) a singlet (3H) at 1.52 ppm relates to a methyl group on a quaternary carbon atom, two pairs of doublets at 3.68 and 3.85 ppm (J = 11 Hz) and at 3.97 and 4.40 ppm (J = 12 Hz) to two $CH_2OH$ groups; and two doublets at 5.57 ppm and 6.03 ppm (J = 4 Hz) to an exocyclic methylene group in conjugation with the carbonyl of a γ-lactone; the signal of the lactone proton is apparently located in the 4.8-5.3 ppm region.

The present paper gives the results of a chemical study of repin with the composition $C_{19}H_{22}O_7$, mp 154-156°C, $[\alpha]_D^{20}$ + 101.2° (c 2.57; chloroform). The IR spectrum of repin has absorption bands at (cm$^{-1}$) 3480 (OH), 3080, 3065, 1280, 1227, 1146 (epoxy group), 1770 (C=O of a γ-lactone), 1745 and 1240 (C=O), 1660 and 1640 (C=C). UV spectrum: $\lambda_{max}$ 200 nm, ε 15,827 (α-methylene-γ-lactone).

With acetic anhydride, repin formed a monoacetyl derivative (II), $C_{21}H_{24}O_8$, as a vitreous substance. Its IR spectrum lacked the absorption band of an OH group. Hydrogenation over a Pt catalyst in ethanol gave a vitreous substance (III), which was dehydrogenated over Se at 260-310°C, as a result of which chamazulene (IV) was isolated.

When repin was saponified, two moles of alkali were consumed. From the hydrolysis products were isolated a dihydroxy lactone (V) with the composition $C_{15}H_{18}O_5$, mp 162-164°C, and an acid (VI), $C_4H_6O_3$, and

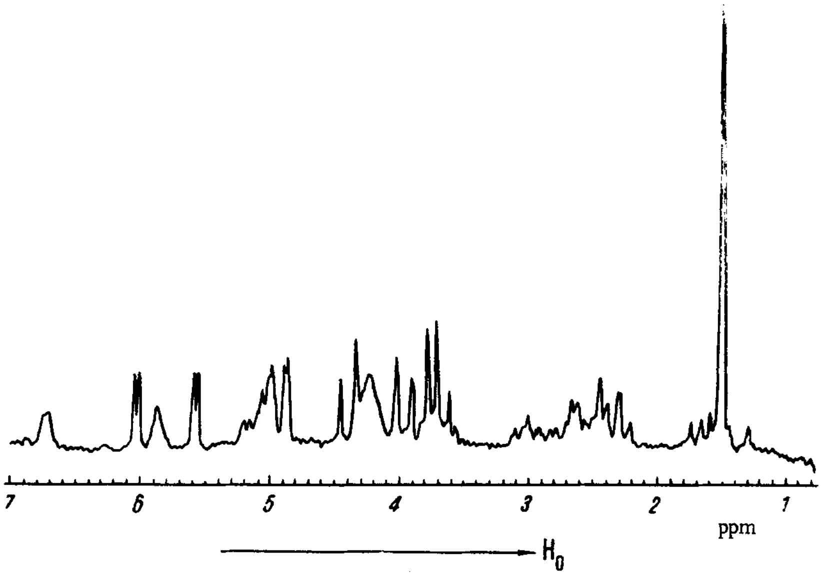


Fig. 1. NMR spectrum of the lactone with mp 218-220°C.

---

All-Union Scientific-Research Institute of Medicinal Plants. Translated from Khimiya Prirodnykh Soedinenii, No. 4, pp. 451-461, July-August, 1972. Original article submitted January 6, 1972.

TABLE 1. Chemical Shifts and Coupling Constants of the Protons of Repin and Its Derivatives, ppm (J in Hz)

| Substance | Protons | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | $H_2$ | $H_4$ | $H_6$ | $H_{10}$ | $H_{13,13'}$ | $H_{14,14'}$ | $H_{15,15'}$ | others |
| I | q 4,02 (J=4,5; 6,5) | q 4,68 (J=9,11) | m 5,16 | q 2,12 (J=9,11) | d 6,24 (J=4) d 5,62 (J=3,5) | br. s 5,24, 5,02 | 2 d 3,34 3,14 (J=4,5) | s 1,66 (3H);<br>2 d 3,22 2,87 (J=6) |
| II | m 4,86 | q 4,35 (J=8,5; 11) | m 5,03 | q 2,01 (J=8,11) | d 6,16 (J=4); d 5,52 (J=3,5) | 2 br. s 5,13, 4,92 | 2 d 3,24, 3,01 (J=4,5) | 2 d 2,74 3,08 (J=6)<br>s 1,98,<br>s 2,54 (3H) |
| V | s 3,77 | t 4,30 (J=10; 10) | m 3,86 | t 2,74 (J=10; 10) | q 6,59 (J=1,8; 3) q 6,37 (J=1,6; 3) | 2 br. s 5,12, 5,01 | 2 d 4,36, 4,18 (J=12) | |
| VI | — | — | — | — | — | — | — | s 1,58 (3H)<br>2 d 3,13, 2,82 (J=6; 6) |
| VII | — | — | — | — | — | — | — | s 1,28 (3H)<br>s 3,72 (3H)<br>2 d 3,48; 3,74 (J=12) |
| VIII | s 3,54 | t 4,30 (J=10; 10) | m 4,84 | t 2,63 (J=10; 10) | d 6,25 (J=4); d 5,74 (J=3) | br. s 5,13 (2H) | 2 d 4,55, 4,37 (J=12) | 2 s 2,13, 2,06 |
| XII | d 3,48 (J=2,6) | t 4,45 (J=10; 10) | m 5,11 | t 2,44 (J=10; 10) | d 1,26 (J=7) | d 0,96 (J=6) | d 4,46 d 4,35 (J=12) | s 2,05 (6H) |

Note. Solvent – $CDCl_3$ [spectrum of V taken in [D]pyridine relative to TMS and the spectrum of (II) relative to HMDS]; q – quartet; m – multiplet; t – triplet; d – doublet; s – singlet; br.s – broadened singlet.

consequently repin has the structure of an ester.

The NMR spectrum of the acid (Fig. 3) shows a 3H singlet at 1.66 ppm and two doublets, each of one proton unit, at 3.13 and 2.82 ppm (J = 6 Hz), which permit the assumption of structure (VI) for the acid. When the acid was methylated with diazomethane in moist methanol, the signals of the epoxide protons disappeared, and the signals of the protons of a hydroxymethylene group appeared – two doublets at 3.48 and 3.74 ppm (J = 12 Hz). An upfield chemical shift of the signal of the methyl group was observed (Table 1), which can be explained by the opening of the epoxide ring with the formation of two hydroxy groups. On this basis, we consider that the methyl ester of the acid isolated corresponds to structure (VII).

Thus, in the NMR spectrum of (I) (Fig. 2a), the singlet in the strong field at 1.66 ppm and the doublets at 3.22 and 2.87 ppm relate to the acyl residue. The NMR spectrum of repin also shows the signals of the protons of an exocyclic methylene group conjugated with the carbonyl of a $\gamma$-lactone ring – a pair of doublets at 6.24 and 5.62 ppm. Broadened singlets in the 5.24 and 5.02 ppm region are the signals of the protons of a second exocyclic methylene group; a quartet in the 4.02 ppm region is the signal of a hemihydroxy proton, and in the spectrum of acetylrepin it is shifted downfield (see Table 1). The multiplet at 5.16 ppm – the signal of a hemiacyl proton – is shifted upfield in the spectrum of (V), and the signals of the protons of the methylene group attached to the $\gamma$-lactone ring approach one another to a considerable extent, which indicates the $\beta$ position of the acyl residue with respect to the methylene of the $\gamma$-lactone ring [5]. A quartet at 4.68 ppm in (I) is the signal of the lactone proton; the signal changes little in the spectra of all the repin derivatives.

The dihydroxy lactone (V) formed a diacetate (VIII) with the composition $C_{19}H_{22}O_7$, mp 112-113°C the IR spectrum of which lacked the absorption band of a hydroxyl; i.e., compound (V) has only two hydroxyls. It follows from a comparison of the NMR spectra of (V) and (VIII) with that of (I) that considerable

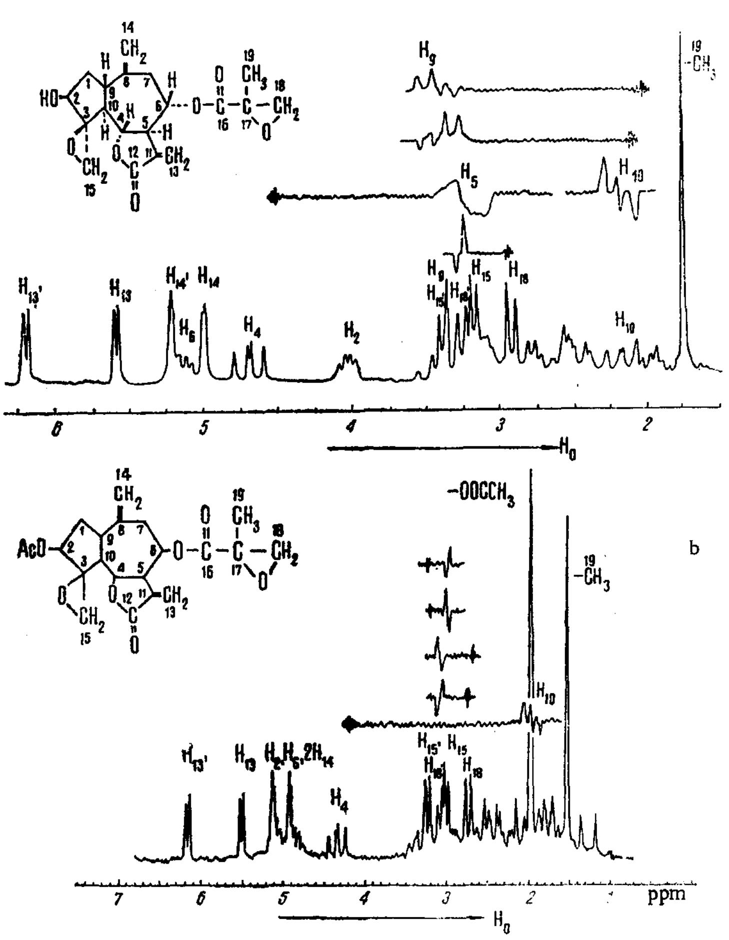


Fig. 2. NMR spectra of repin (I) (a) and of its monoacetyl derivative (II) (b).

changes took place on hydrolysis. In the spectrum of (V) the signal of the hemihydroxyl proton at $C_2$ had disappeared, while a new signal had appeared – a broadened singlet at 3.77 ppm in the acetyl derivative (VIII) not undergoing a downfield shift, since this signal corresponds to a proton attached to oxygen but not in a hydroxy group. In (V) a signal typical for a–$CH_2OH$ group (AB quartet in the 4.1-4.4 ppm region) had appeared, and in (VIII) it had shifted downfield (Fig. 4a).

The composition of (V), $C_{15}H_{18}O_5$, the presence of two hydroxy groups, and the absence of the absorption band of a carbonyl group in the IR spectrum, apart from the lactone carbonyl group, gives ground for assuming that (V) contains an oxide bridge. The oxidation of (V) over a Pt catalyst in acetic acid formed a mixture of products; two of them were successfully isolated with the compositions $C_{15}H_{24}O_5$ (IX) and $C_{15}H_{22}O_5$ (X). The latter formed a diacetate (XII), $C_{19}H_{26}O_7$, with mp 125-127°C. The IR spectrum of (XII) has no absorption band of an OH group. The acetylation of (IX) also gave a diacetate, (XI), $C_{19}H_{28}O_7$, in the IR spectrum of which there was the absorption band of a hydroxyl, showing that (IX) contains three hydroxy groups, one of which is tertiary. Consequently, in the hydrogenation of (V) the oxide ring opened with the formation of a tertiary alcohol; i.e., the oxide bridge is connected with a tertiary carbon atom. A hydroxymethylene group may arise in (V) by the hydrolysis of an ester group or by the opening of the epoxide ring. The presence of a second ester group in (I) is excluded by the results of the reactions performed and the features of the NMR spectrum. It remains to be assumed that repin has a second epoxide bridge, which agrees with the NMR spectra of (I) and (II) (Fig. 2) which each have two doublets in the 3.1-3.3 ppm region corresponding to the protons of an epoxide. On hydrolysis, the oxide brdige opens with the formation of an epoxide in a different position.

The production of chamazulene on dehydrogenation, and also the spin–spin coupling constant of $H_{13}$ and $H_{13}'$ (J = 3.5 Hz) in the NMR spectrum of (I) permits the conclusion that repin has either a guaiane or a germacrane carbon skeleton. Integration of the signals of the NMR spectra of (II) and (VIII) confirmed the presence in acetylrepin of 24 and in the acetyl dihydroxy lactone of 22 hydrogen atoms. With this num-

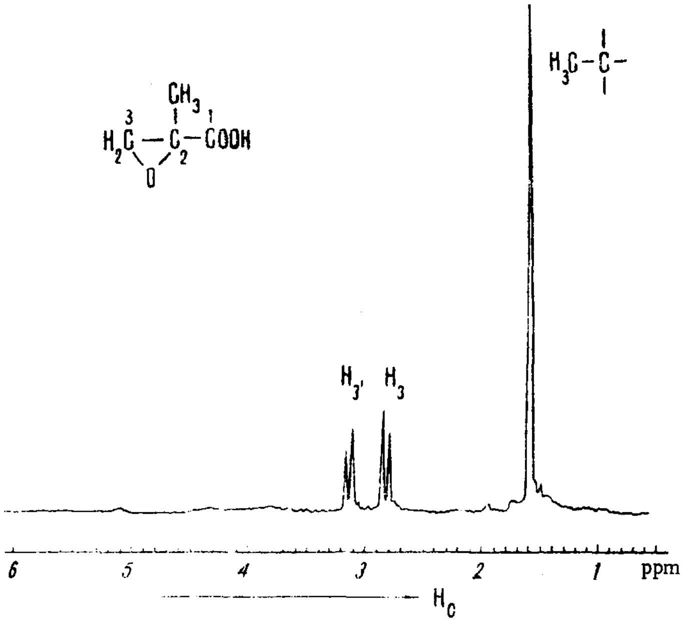


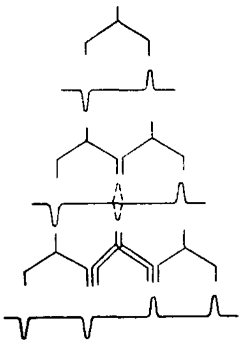
Fig. 3. NMR spectrum of the acid (IV).

ber of protons and the presence of the functional groups mentioned, a monocyclic (germacrane) structure for (I) is excluded. Thus, repin has a guaiane carbon skeleton.

The multiplicity of the signal of the lactone proton in (I) (Fig. 2) shows that the lactone ring is at $C_4-C_5$ and, consequently, the acyl group is located at $C_6$ ($\beta$ position with respect to the exocyclic methylene of the $\gamma$-lactone ring).

By means of the INDOR method it has been possible to determine the structure of the signal of the $H_{10}$ proton (see Figs. 2 and 4b). This signal forms quartets in the NMR spectra of (I) and (II) and triplets in the spectra of the other derivative, which means that it interacts with only one proton in addition to the lactone proton. The invariability of the multiplicity of the signal in the hydrogenated derivative (XII) (see Fig. 4b) excludes the location of the exocyclic methylene group in repin at $C_3$. Consequently, only one position remains for it – $C_8$; in repin the epoxide bridge is attached to $C_{15}$.

It was possible to determine the structure of the $H_9$ proton by the same method. The distance between the first heteropolar signals, if each line represents one transition, should correspond to the coupling constant between the protons in the lines of which INDOR is observed [6]; i.e., $J_{9,10} = 9$ Hz. However, in the INDOR spectrum of the $H_9$ proton the distance between the heteropolar signals is 18 Hz, which is equal to $2J_{9,10}$. The absence of a signal of polarity opposite to the first can be explained by the coincidence of INDOR signals of different polarities at a distance of 9 Hz from the first line. This shows that the inner lines of the quartet correspond to more than one transition; i.e., $H_9$ interacts with more than two protons, while only the position at $C_2$ remains for the OH group. The nature of the signal of the hemihydroxy proton agrees with those considered. The multiplicity of the signal of the $H_{10}$ proton shows that there is no proton at $C_3$ in (I) and all its derivatives.



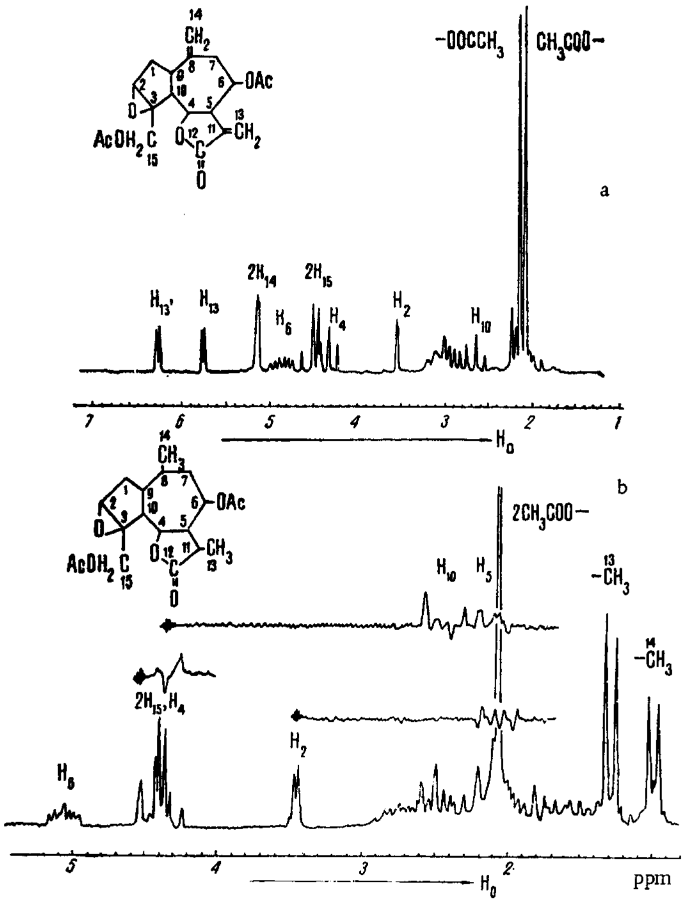
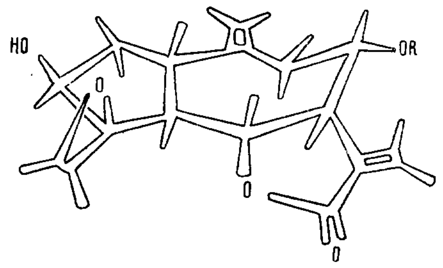


Fig. 4. NMR spectrum of the diacetates (VIII) (a) and (XII) (b).

The large coupling constant of the $H_4$ and $H_{10}$ protons ($J = 11$ Hz) shows that they are in the trans position with respect to one another: the coupling constant of the $H_4$ and $H_5$ ($J = 9$Hz) by itself does not show either the cis or the trans position [7], but in the hydrogenated product (XII) it has increased ($J = 10$ Hz) which now permits the conclusion that the $H_4$ and $H_5$ protons are in the transoid position.

The considerable shift in the signals of the vinyl protons of the γ-lactone ring when the substituent in position $C_5$ is substituted (see Table 1) shows the spatial propinquity of the methylene group of the γ-lactone ring and the acyl group, which is possible only if the $H_5$ and $H_6$ protons are in the trans position.

To determine the configuration of the OH group the spectra of repin with additions of a paramagnetic salt were recorded.* A large downfield shift was observed for the signals of the $H_4$ and $H_9$ protons ($\Delta\delta$ 0.7 and 0.4 ppm). This confirms the projection of the hydroxy group and of the $H_4$ and $H_9$ protons in the same direction from the plane of the five-membered ring.



---

*The salt used was europium tris(dipivalomethanate), which was synthesized at Patrice Lumumba University by V. P. Zvolinskii and V. F. Zakharov.

If the methylene group of the epoxide ring and the hydroxy group were in the cis position, a large difference would be expected in the shifts of the $H_{15}$ and $H_{15}''$ protons, since the distances from them to the hydroxy group differ by a factor of approximately two. The proton closest to the hydroxy group and the $H_2$ proton should be shifted to approximately the same extent because of the similarity of the distances of these protons to the hydroxy group [8]. However, the signals from the protons at $C_{15}$ are shifted similarly ($\Delta\delta$ 1 ppm), while the $H_2$ signal is shifted to a considerably greater extent ($\Delta\delta$ 2.5 ppm). This shows that the $C_{15}$ protons are present in the trans position with respect to the hydroxy group.

On the basis of the facts presented, we propose structure (I) as the most probable for repin.

## EXPERIMENTAL

The IR spectra were taken on a UR-10 spectrometer in paraffin oil, the UV spectra on a Hitachi EP-3T, and the NMR spectra on a HA-100D instrument. Thin-layer chromatography (TLC) was performed on neutral alumina (activity grade IV) in the benzene–methanol (9 : 1) and ether systems; the chromogenic agent was a 0.5% solution of $KMnO_4$ in 0.5% $H_2SO_4$. The results of the microanalyses corresponded to the calculated figures.

Isolation of Repin from Acroptilon repens L. (DC). The total lactones after the isolation of the acroptilin from them [3] were dissolved in benzene, and the crystals that subsequently deposited were twice recrystallized from ethanol and were dried over $P_2O_5$ at 75°C (10 mm Hg). Composition $C_{19}H_{22}O_7$, mp 154-156°C, $[\alpha]_D^{20}$ + 101.2° (c 2.57; chloroform); on TLC in the petroleum ether–benzene–methanol (5 : 4 : 2 : 1) system* it showed one spot with $R_f$ 0.29, and in the benzene–methanol system one spot with $R_f$ 0.54.

Isolation of Repin from Centaurea hyrcanica Bornm. The leaves and flower heads (1 kg) were steeped in hot water (80-90°C). The lactones were isolated from the aqueous extract with chloroform, and the extract was evaporated to dryness. On TLC, the combined lactones gave four spots with $R_f$ 0.1, 0.3, 0.5, and 0.9. When a mixture of ethanol and ether (1 : 5) was added to the combined lactones, slightly yellowish crystals deposited; after two recrystallizations from ethanol they had mp 155-156°C, $[\alpha]_D^{18}$ + 85.32°(c 1.72; chloreform) and showed one spot on TLC in the benzene–methanol system with $R_f$ 0.56. A mixture with the repin from Acroptilon repens gave no depression of the melting point and their IR spectra were identical.

Isolation of Acroptilin from Centaurea hyrcanica Bornm. After the precipitation of the repin (114 g), the combined lactones were chromatographed on 1800 g of neutral $Al_2O_3$ (activity grade IV) and were eluted first with ether, then with ether–ethanol (9 : 1, 8 : 2, 1 : 1) and with ethanol, 300-ml fractions being collected. Fractions 6-8 gave 5 g of colorless crystals with mp 151-155°C, $R_f$ 0.5. Fractions 9-11 deposited 1.2 g of colorless crystals with mp 187-191°C, $R_f$ 0.28; after recrystallization from ethanol, mp 194-196°C. A mixture with the acroptilin isolated from Acroptilon repens gave no depression of the melting point, and their NMR and IR spectra were identical.

Fractions 11-13 [ether–ethanol (9 : 1)] deposited colorless crystals with mp 210-220°C; after recrystallization from ethanol mp 218-220°C, $R_f$ 0.1. Yield 0.08 g. IR spectrum, $\gamma_{max}$, cm$^{-1}$: 3540, 3470 (OH), 1750 ($\gamma$-lactone), 1665 and 1640 (C = C).

Hydrolysis of Repin. Preparation of the Dihydroxy Lactone (V) and of the Acid (VI). A mixture of 0.7 g of repin and 35 ml of a 4% solution of KOH was left at room temperature for a day and was then acidified to pH 1 with 10% $H_2SO_4$ solution and was extracted first with ether and then with ethyl acetate. Each extract was washed five times with 5% $Na_2CO_3$ solution and with water to neutrality. Elimination of the ether yielded traces of a vitreous substance. The sodium carbonate extract from the ethereal solution was acidified with 5% HCl, the acid liberated was extracted with ether, and the extract was washed with water five times. The solution then yielded a faintly yellowish noncrystalline product which, after drying over $P_2O_5$ for three days, was found by microanalysis to have the composition $C_4H_6O_3$ (VI).

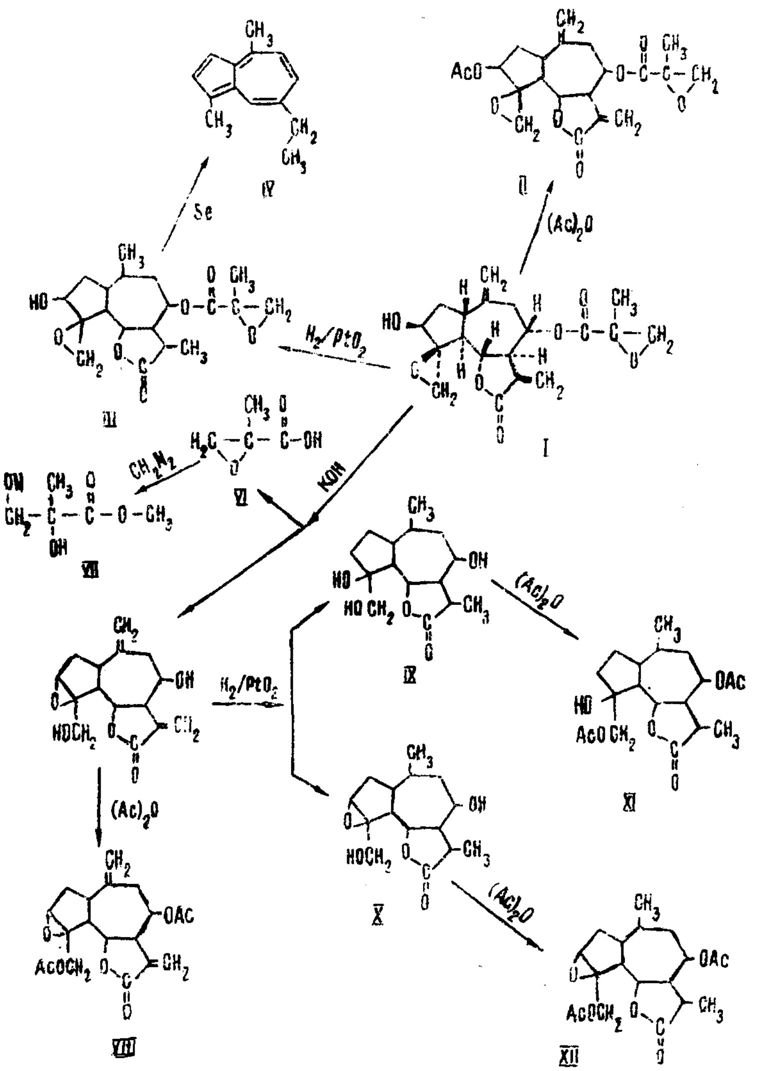
The ethyl acetate extract yielded 0.15 g of a colorless vitreous substance (V) with the composition $C_{15}H_{18}O_5$, mp 162-164°C (from ethanol). IR spectrum, $\gamma_{max}$, cm$^{-1}$: 3380, 3440 (OH), 1770 ($\gamma$-lactone), 1650 (C = C).

Methylation of (VI). Preparation of the Methyl Ester (VII). An excess of a solution of diazomethane in ether was added in small portions to a solution of 0.03 g of (VI) in 5 ml of methanol until decoloration ceased, and the mixture was left for a day. Then the solvent was evaporated off at room temperature and

---

*As in Russian original – Publisher.

Scheme of the Reactions of Repin



the residue was dried over $P_2O_5$ for two days.

Acetylation of (V). Preparation of (VIII). A mixture of 0.5 g of the dihydroxy lactone, 5 ml of acetic anhydride, and 10 ml of pyridine was left at room temperature for 18 h and was then diluted with water (1 : 5), and the reaction product was extracted with a mixture of ether and benzene (1 : 1), after which the extract was washed three times with 5% $NaHCO_3$ solution and then with water to neutrality. The residue after the elimination of the solvent was treated with ether, and the colorless crystals that deposited were recrystallized from ethanol; composition $C_{19}H_{22}O_7$ (VIII), mp 112-113.5°C. IR spectrum, $\gamma_{max}$, cm$^{-1}$: 1765 ($\gamma$-lactone), 1745 and 1235 (—OCO), 1647 (C = C).

Hydrogenation of (V). Preparation of (IX) and (X). Compound (V) (1.0 g) in 20 ml of acetic acid with 0.1 g of $PtO_2$ was hydrogenated until the absorption of hydrogen ceased. The reaction mixture was diluted with water and neutralized with $Na_2CO_3$; the reaction product was extracted first with chloroform and then with ethyl acetate. On TLC in the benzene—methanol system of the chloroform fraction, three spots with Rf 0.41, 0.51, and 0.25 were revealed; the ethyl acetate fraction gave two spots with Rf 0.1 and 0.25.

The two substances with $R_f$ 0.41 and 0.25 were isolated by the chromatographic separation of the chloroform fraction on neutral alumina (activity grade IV). The substance with $R_f$ 0.41 was a colorless vitreous product with the composition $C_{15}H_{22}O_5$ (X), and the substance with $R_f$ 0.25 was also a vitreous product and had the composition $C_{15}H_{24}O_5$ (IX).

Acetylation of (X). Preparation of (XII). A mixture of 0.2 g of (IX), 1 ml of acetic anhydride, and 2 ml of pyridine was left overnight at room temperature. Then the reaction mixture was diluted with water and extracted with ethyl acetate, and the extract was washed with 5% HCl solution and with water to neutrality, after which evaporation of the solvent gave a vitreous substance which then crystallized. The crystals were washed with benzene and then had the composition $C_{19}H_{26}O_7$ (XII), mp 125-127°C; IR spectrum, $\gamma_{max}$, $cm^{-1}$: 3470-3520 (OH, 1780-1720 ($\gamma$-lactone and $-OCO$).

Dehydrogenation. The hydrogenation of 1 g of repin in 100 ml of ethanol in the presence of 0.06 g of $PtO_2$ was performed until the absorption of hydrogen ceased (2 moles were absorbed). After the elimination of the catalyst and the solvent, a vitreous product (III) was formed, which was then dehydrogenated in the presence of 1 g of selenium at 260-310°C for 1 h. The reaction product was extracted with petroleum ether and chromatographed on neutral alumina (activity grade II). This gave a viscous blue liquid (IV) which was identified by its $R_f$ value on TLC in comparison with an authentic sample as chamazulene. A solution of 0.05 g of (V) in ethanol was mixed with a solution of 0.05 g of trinitrobenzene in ethanol, giving a precipitate of black needles which, after washing with ethanol, had mp 128-130°C.

## SUMMARY

1. Three sesquiterpene lactones have been isolated from Centaurea hyrcanica: repin, acroptilin, and a new lactone related to them with mp 218-220°C (ethanol), $[\alpha]_D^{20}$ + 104.7° (c 1.91; dioxane).

2. Structure (I) has been proposed for repin on the basis of the reactions performed, of an interpretation of the NMR spectra of compounds (I-XII) using the INDOR method, and also of a study of NMR spectra with additions of a paramagnetic salt.

## LITERATURE CITED

1. R. I. Evstratova, R. Ya. Rzazade, and K. S. Rybalko, Khim. Prirodn. Soedin., 290 (1966).
2. R. I. Evstratova, K. S. Rybalko, and R. Ya. Rzazade, Khim. Prirodn. Soedin., 284 (1967).
3. R. I. Evstratova, V. I. Sheichenko, E. Ya. Kiseleva, K. S. Rybalko, and A. I. Ban'kovskii, Khim. Prirodn. Soedin., 273 (1971).
4. R. I. Evstratova, M. N. Mukhametzhanov, V. I. Sheichenko, A. I. Shreter, and D. A. Pakaln, Khim. Prirodn. Soedin., 186 (1969).
5. K. S. Rybalko, A. I. Ban'kovskii, and V. I. Sheichenko, Tr. VILR [Proceedings of the All-Union Institute of Medicinal Plants], Khimiya, 15, 237 (1969).
6. V. I. Sheichenko and K. S. Rybalko, Khim. Prirodn. Soedin., 687 (1970).
7. Z. Samek, Abstracts of Lectures at the 7th International Symposium on the Chemistry of Natural Compounds [in Russian], Riga (1970), p. 708.
8. G. H. Wahl and M. R. Peterson, Chem. Commun., 1970, No. 18, 1167.