# INVESTIGATION OF THE CHEMICAL COMPOSITION OF SAUSSUREA FROLOVII, LDB.

## I. The Triterpene Alcohol Saussurol

A. T. Troshchenko and V. S. Kobrin

Khimiya Prirodnykh Soedinenii, Vol. 1, No. 4, pp. 256-262, 1965

Saussurea frolovii Lbd., the costus plant, is a plant of the Compositae family inhabiting the low zone of the Alpine region in pine and larch woods of the Altai, the Tuva autonomous region (Sayany), the Mongolian People's Republic, and also the Kazakh SSR (Tarbagatai) [1, 2].

Various representatives of the genus Saussurea DC contain alkaloids, essential oils, tanning agents, bitter principals, and the like [2-7]. Information on the physiological activity of plants of this genus is given in the literature [2, 8-10].

We have investigated the plants in the flowering phase collected at a height of 2600-2800 m above sea level on the southwestern slopes of the Kurai range (Gorno-Altai autonomous region).

Qualitative reactions confirmed that the roots, stems, leaves, and also the flowers of the costus plant contained a small amount of alkaloids.

Extraction with petroleum ether of the epigeal part of the plant (stems and leaves without inflorescences) gave a substance of composition $C_{30}H_{50}O$ giving positive Keller-Kiliani, Legal, and Ballier tests for triterpene compounds [11].

The IR spectrum of the compound obtained absorbed at 3627 cm$^{-1}$, which is characteristic for a secondary hydroxyl group in the β-(equatorial) position at the $C_3$ or $C_{11}$ carbon atom of triterpene compounds [13] (Fig. 1).*

The presence of a hydroxyl group in $C_{30}H_{50}O$ was confirmed by the preparation of acetyl, benzoyl, 3, 5-dinitrobenzoyl, and α-naphthylurethane derivatives.

Oxidation of the triterpene alcohol isolated with chromic anhydride led to a ketone and showed that the original substance was a secondary alcohol. The IR spectrum of the ketone had an absorption band at 1716 cm$^{-1}$ which is characteristic for a ketone group in a six-membered ring [14].

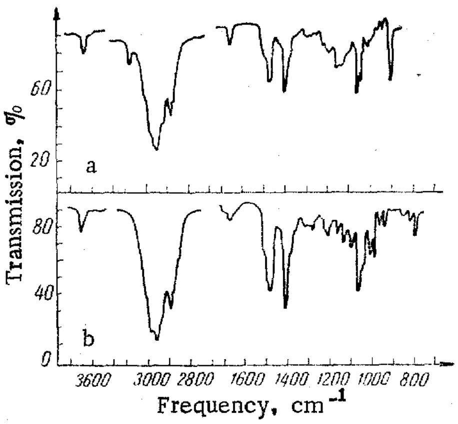
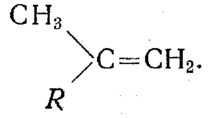


Fig. 1. IR spectrum of saussurol (a) and isosaussurol (b).

An oxime was prepared from the ketone obtained, and saponification of this gave the initial ketone.

The isolated triterpene alcohol has been called saussurol. The spectrum of saussurol has an absorption band in the 3050-3060 cm$^{-1}$ region which is retained in all the derivatives obtained from it. This band is characteristic for compounds containing a cyclopropane ring with a free methylene group in the molecule [15].

However, the existence of vibrations in the 3015-3075 cm$^{-1}$ region may be due in individual cases to the presence of a double bond [16]. These vibrations generally relate to the group



$$\overset{CH_3}{\underset{R}{>}}C{=}CH_2.$$

In the case of compound $C_{30}H_{50}O$, this is unlikely, since on oxidation with chromic acid such an unsaturated grouping should be oxidized as well as the secondary alcohol group. We have oxidized saussurol repeatedly and in no case has a second carbonyl group been observed, although its appearance could be expected if an isopropenyl group were present.

The IR spectrum also has vibrations in the 1650 cm$^{-1}$ region. This frequency is most often connected with unsaturation in ring C. Nevertheless, this type of double bond is more reliably characterized by the absorption in the UV spectrum at 210-225 mμ [17]. The compound that we isolated exhibited no absorption in this region.

---

* In Figs. 1 and 2, the 700-800 cm$^{-1}$ region was recorded in KBr at a concentration of 0.5% with an NaCl prism and the 2600-3800 cm$^{-1}$ region in $CCl_4$ at a concentration of 1%, d = 0.1, with a LiF prism.

The oxidation with hydrogen peroxide in acetic acid which is characteristic for unsaturated compounds of the triterpene series has not been observed in the case of saussurol.

The weak reaction with tetranitromethane (as compared with the analogous reactions of β-amyrin and taraxerol) cannot unambiguously confirm either the presence or the absence of a double bond.

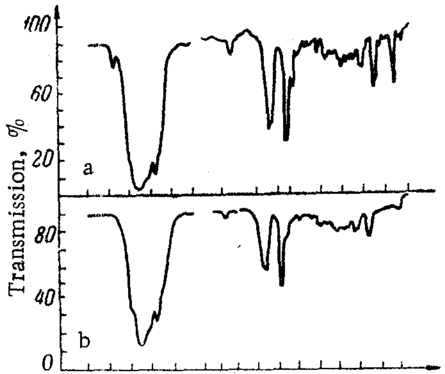
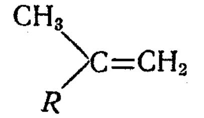


Fig. 2. IR spectrum of saussurone (a) and isosaussurane (b).

In experiments on the isomerization of saussurol acetate by the passage of gaseous hydrogen chloride [18], the absorption band in the 3050-3060 cm$^{-1}$ region disappears, while the absorption band at 1650 cm$^{-1}$ is retained (cf. Fig. 1).

All this indicates the absence of a  $(CH_3)(R)C{=}CH_2$ group from the compound and the presence of a cyclopropene ring with a free double bond. Moreover, the acetate obtained in the isomerization reaction and the alcohol give a more intense coloration with tetranitromethane.

The reduction of the ketone (saussurone) by the Wolff-Kizhner method leads to a hydrocarbon, which has been called saussurane, the spectrum of which retains the vibrations in the 3050-3060 cm$^{-1}$ region. The vibrations in the 1025 cm$^{-1}$ region may be assigned to a cyclopropane ring [15]. The isomerization of the hydrocarbon by treatment with anhydrous hydrogen chloride in chloroform gave isosaussurane, the IR spectrum of which lacks the absorption band at 3050-3060 cm$^{-1}$ and is somewhat different in the 850-1050 cm$^{-1}$ region. This may confirm the presence in saussurol of a cyclopropane grouping (Fig. 2).

The results of a comparison of the properties of the alcohol isolated and its derivatives with data in the literature permit the assumption that we have obtained a previously unknown compound.

The petroleum ether extract after the separation of saussurol was separated into acidic and neutral fractions by the usual method. Saussurol acetate was isolated by chromatography of the neutral fraction on alumina.

## Experimental

Isolation and properties of saussurol. The air-dry epigeal part (6 kg) of Saussurea frolovii Ldb. was extracted with petroleum ether (40-70°). After the extract had been concentrated and allowed to stand for a week, a white precipitate (13 g) deposited.

Chromatography on plates with a fixed layer of silica gel in the di-isopropyl ether-acetone (10 : 1) system [12], and subsequent showing up of the spots with concentrated sulfuric acid showed the individual nature of the compound isolated, which consisted of colorless needle-like crystals (12 g) with mp 212.5-213° (from alcohol), $[\alpha]_D^{22}$ +69.9° (c 0.4).

IR spectrum: 3627 (OH group), 3050 ($CH_2$ of a cyclopropane ring or a double bond), 1650 cm$^{-1}$ (double bond).

Saussurol does not absorb in the UV region.

Found, %: C 84.42, 84.75; H 11.81, 11.84. Calculated for $C_{30}H_{50}O$, %: C 84.44; H 11.81.

The alcohol is sparingly soluble in the majority of organic solvents and is completely insoluble in water.

Preparation of saussuryl acetate. The alcohol (1 g) was acetylated by heating on a boiling water bath for 3 hr with acetic anhydride (12.5 ml) in pyridine (38 ml). The reaction mixture was treated by the usual method. The acetate (0.9 g) had mp 246-248° (from acetone), $[\alpha]_D^{22}$ +140.3° (c 0.9).

IR spectrum: 3060 cm$^{-1}$ ($CH_2$ of a cyclopropane ring or a double bond), 1740, 1255 cm$^{-1}$ (acetyl group), 1650 cm$^{-1}$ (double bond). With tetranitromethane it gave a faint yellow coloration, and the UV spectrum had no absorption characteristic for double bonds (from 215 mμ).

Found, %: C 81.76, 81.86; H 11.12, 11.16; $CH_3CO$ 936, 942. Calculated for $C_{32}H_{52}O_5$, %: C 81.99; H 11.18; $CH_3CO$ 9.18.

Oxidation of saussuryl acetate with hydrogen peroxide [18]. The acetate (0.38 g) was heated on a boiling water bath for 1 hr with a mixture of perhydrol (25 ml) and glacial acetic acid (20 ml). After the completion of the reaction, the precipitate which was deposited was separated off and recrystallized from acetone. From its melting point and IR spectrum, the compound obtained was identical with saussuryl acetate. A mixed sample gave no depression of the melting point.

Alkaline hydrolysis of saussuryl acetate. The acetate (0.05 g) was hydrolyzed with a 10% alcoholic solution of caustic potash (3 ml' by boiling for 3 hr. The reaction mixture was poured on to ice, and the precipitate which was deposited was filtered off, washed with water, and dried. After recrystallization from ethyl alcohol, the product (0.04 g) had mp 212-214°. From its IR spectrum and a mixed melting point, the alcohol obtained was identical with saussurol.

Preparation of saussuryl benzoate. The alcohol (0.1 g) was benzoylated by being heated on a boiling water bath for 3 hr with benzoyl chloride (1 ml) in a mixture of benzene (3 ml) and pyridine (1 ml). After cooling, the reaction mixture was treated in the usual way and was chromatographed on alumina (neutral, activity grade II, 20 g). The fraction eluted with petroleum ether gave a compound (0.07 g) with mp 281-282° (from acetone), $[\alpha]_D^{22}$ +61.4° (c 0.1). IR spectrum: 3055 cm$^{-1}$ ($CH_2$ of a cyclopropane ring or a double bond), 1720, 1280 (benzoyl group), 1650 cm$^{-1}$ (double bond).

Found, %: C 84.29, 83.98; H 10.61, 10.36. Calculated for $C_{37}H_{54}O_2$, %: C 83.72; H 10.25.

Saponification of saussuryl benzoate. The benzoate (0.08 g) was hydrolyzed with a 10% solution of caustic potash in alcohol (5 ml) at the boil for 3.5 hr. The reaction mixture was poured on to ice. The precipitate which was deposited (0.06 g) proved to be absolutely identical with saussurol in respect of its melting point, IR spectrum, and mixed melting point.

Preparation of the 3, 5-dinitrobenzoyl derivative. Saussurol (0.3 g) was mixed with absolute benzene (20 ml) and absolute pyridine (1 ml). Freshly prepared [19] 3, 5-dinitrobenzoyl chloride (0.4 g) was added to the mixture. The reaction mixture was worked up in the usual way. The dinitrobenzoate had mp 241-241.5° (from a mixture of dichloroethane and methanol). IR spectrum: 3060 cm$^{-1}$ ($CH_2$ of a cyclopropane ring or a double bond), 1730, 1270 cm$^{-1}$ (benzoyl group), 1650 (double bond), 1570, 1355 cm$^{-1}$ (nitro group).

Found, %: C 71.66, 71.42; H 8.44, 8.67; N 4.92, 4.90. Calculated for $C_{37}H_{52}O_6$, %: C 71.58; H 8.44; N 4.51.

Preparation of the α-naphthylurethane. Saussurol (0.5 g) was charged into a flask with absolute benzene (20 ml) and α-naphthyl isocyanate (0.4 ml). The reaction mixture was boiled for 12 hr. After the completion of the reaction, the solvent was distilled off to dryness, and the residue was recrystallized from ethyl alcohol (twice) and then from diethyl ether. The melting point of the urethane obtained (0.3 g) was 235-236.5°. IR spectrum: 3470 cm$^{-1}$ (amino group), 3055 ($CH_2$ of a cyclopropane ring or a double bond), 1650 cm$^{-1}$ (double bond).

Found, %: C 82.46, 82.31; H 9.49, 9.42; N 2.78, 2.85. Calculated for $C_{41}H_{57}O_2$, %: C 82.64; H 9.64; N 2.35.

Oxidation of saussurol with chromic anhydride [18]. Saussurol (0.17 g) was mixed with glacial acetic acid (15 ml) and absolute benzene (3 ml). With vigorous stirring, chromic anhydride (0.04 g) in the minimum amount of water (0.2 ml) was added to the mixture. This was stirred at room temperature for 20 hr and was then poured on to ice. The precipitate was filtered off, washed with water, and dissolved in diethyl ether (70 ml). The ethereal solution was treated with 5% NaOH solution to eliminate acidic compounds and was then washed with water and dried over magnesium sulfate. The saussurone (0.16 g) obtained after the distillation of the solvent was chromatographed on alumina (neutral, activity grade III, 75 g); the fractions eluted by a mixture of petroleum ether and ether (19 : 1) had mp 175-176° (from a mixture of methanol and chloroform), $[\alpha]_D^{22}$ +135.4° (c 0.9). IR spectrum: 3060 cm$^{-1}$ ($CH_2$ of a cyclopropane ring or a double bond), 1716 (carbonyl group in a six-membered ring), 1650 cm$^{-1}$ (double bond).

Found, %: C 84.70, 84.55; H 11.45, 11.63. Calculated for $C_{30}H_{48}O$, %: C 84.84; H 11.39.

Preparation of the oxime. An alcoholic solution of hydroxylamine (3.3 ml, containing 0.002 mole of hydroxylamine) was added to saussurone (0.3 g) in alcohol (2 ml). The mixture was heated on a boiling water bath for 2 hr. On cooling, the reaction mixture gave an oxime (0.18 g) with mp 230-231° (from alcohol). IR spectrum: 3350 cm$^{-1}$ (OH of an oxime), 3060 ($CH_2$ of a cyclopropane ring or a double bond), 1645 cm$^{-1}$ (C=N of oximes).

Found, %: C 81.65, 81.70; H 10.86, 10.89; N 3.78, 3.78. Calculated for $C_{30}H_{50}NO$, %: C 81.56; H 11.64; N 3.17

Saponification of the oxime. A mixture of the oxime (0.1 g) and 0.01 N alcoholic hydrochloric acid (5 ml) was boiled for 5 hr. After cooling, the reaction mixture was treated with water (10 ml) and extracted with ether (5 × 20 ml). The ethereal solution was washed with water, with 1% sodium carbonate solution and with water again, and was dried over magnesium sulfate. After the distillation of the ether, saussurone (0.07 g) was obtained.

Wolff-Kizhner reduction of saussurone [20]. A mixture of saussurone (0.55 g) in diethyleneglycol (45.5 ml) and 85% hydrazine hydrate was boiled in a flask fitted with a reflux condenser for 30 min. After the addition of caustic potash (0.58 g), the mixture was boiled for a further 1 hr. The condenser was replaced by one for downward distillation, and the water and the excess of hydrazine hydrate were distilled off from the reaction mixture until the temperature in the flask had reached 190°. The reaction mixture was boiled at this temperature for a further 2.5 hr under reflux. It was then poured on to ice, and the precipitate was chromatographed on alumina (neutral, activity grade I-II, 120 g). The solid residue obtained from the fractions eluted by petroleum ether gave a hydrocarbon (0.48 g) with mp 170-171° (from a mixture of methanol and chloroform), $[\alpha]_D^{22}$ +133.8° (c 0.8). IR spectrum: 3060 ($CH_2$ of a cyclopropane ring or a double bond), 1650 $cm^{-1}$ (double bond). With tetranitromethane, the substance gave a feeble yellow coloration.

Found, %: C 88.20, 88.05; H 12.13, 12.06. Calculated for $C_{30}H_{50}$, %: C 87.73; H 12.27.

Isomerization of saussurane. Saussurane (0.07 g) was dissolved in absolute chloroform (20 ml). At 0-2°, a slow current of dry gaseous hydrogen chloride was bubbled into the solution for 4 hr. The chloroform solution was washed with water, 5% sodium carbonate solution, and water again, and was dried over magnesium sulfate. The residue left after distillation of the solvent was chromatographed on alumina (neutral, activity grade I, 60 g). The fractions eluted by petroleum ether gave a hydrocarbon (0.06 g) having mp 163-164° (from a mixture of chloroform and methanol), $[\alpha]_D^{22}$ +121.2° (c 0.8). IR spectrum: no bands in the 3050-3060 $cm^{-1}$ region, and changes observed in the 1050-850 $cm^{-1}$ region. With tetranitromethane the substance gave a weak yellow coloration.

Isomerization of saussuryl acetate. The substance (0.3 g) was dissolved in absolute chloroform (50 ml). A weak current of gaseous hydrogen chloride was passed into the solution at 0-2° for 4 hr. After the end of the reaction, the chloroform solution was treated as in the case of the isomerization of saussurane. The isosaussuryl acetate obtained after the distillation of the solvent (0.27 g) had mp 201.5-203° (from acetone), $[\alpha]_D^{22}$ +98.5° (c 0.9). IR spectrum: vibrations in the 3050-3060 $cm^{-1}$ region absent; 1740, 1255 $cm^{-1}$ (acetyl group). The substance did not adsorb in the UV region; with tetranitromethane it gave a weak yellow coloration.

Found, %: C 81.80, 81.78; H 11.14, 11.22; $CH_3CO$ 9.15, 9.47. Calculated for $C_{32}H_{52}O_2$, %: C 81.99; H 11.18; $CH_3CO$ 9.18.

Hydrolysis of isosaussuryl acetate. The substance (0.16 g) was subjected to hydrolysis by the method described for saussuryl acetate. The isosaussurol obtained as a result of the reaction (0.12 g) had mp 206-207° (from alcohol), $[\alpha]_D^{23}$ +66.2° (c 0.8). IR spectrum: 3628 (OH group), 1650 $cm^{-1}$ (double bond).

The alcohol did not absorb in the UV region and gave a weak yellow coloration with tetranitromethane.

Found, %: C 84.19, 84.29; H 11.62, 11.40. Calculated for $C_{30}H_{50}O$, %: C 84.44; H 11.81.

Isolation of saussuryl acetate from the petroleum ether extract. After removal of the saussurol, the petroleum ether extract was concentrated to 2 liters and was separated into acidic and neutral fractions by the usual method.

The acidic part of the extract gave a viscous water-insoluble dark green mass (3 g).

The neutral part, a viscous yellow-green mass (230 g), was roughly separated by chromatography on alumina (neutral, activity grade III, 1 kg) into four fractions: A-I (61.32 g, pale yellow mass); A-II (99.37 g, dark orange mass); A-III (12.79 g, yellow mass); A-IV (55.30 g, dark green mass).

Fractions A-II and A-III were combined and rechromatographed on alumina (neutral, activity grade II, 100 g of substance, 750 g of alumina).

Five combined fractions were obtained: B-1 (66.29 g, pale lemon yellow); B-2 (6.27 g, white mass with a greenish tinge); B-3 (2.59 g, bright yellow oil from which crystals were deposited on standing); B-4 (10.76 g, orange oil); and B-5 (10.10 g, bright yellow viscous mass).

The crystals that were deposited from fraction B-3 (1.59 g) were separated off, and the mother liquors were evaporated to dryness in vacuum. The residue obtained, after recrystallization from benzene, gave an additional amount of crystalline product (0.94 g) identical with that which had been deposited directly. The two fractions were combined and, after the three recrystallizations from acetone, proved to be completely identical with saussuryl acetate (in melting point, IR spectrum, mixed melting point, chromatographic behavior, and the results of a comparison of the saponification products).

The IR spectra were taken on a UR-10 instrument, the UV spectra on a SFD-2 instrument in the region from 215 mμ [17]. Solvent $CCl_4$, concentration 5%, layer thickness 0.1 mm. The angles of rotation were determined in chloroform and the melting points in sealed capillaries.

## Summary

A new triterpene alcohol saussurol and its O-acetate have been isolated from *Saussurea frolovii* Lbd.

## REFERENCES

1. P. N. Krylov, Flora of Western Siberia [in Russian], Vol. II, Tomsk, 2928, 1949.
2. Flora of the USSR [in Russian], Vol. 27, Moscow and Leningrad, 384, 1962.
3. C. Wehmer, Die Pflanzenstoffen, Jena, 1253, 1931.
4. L. Crabalona, Bull., 2, 257, 1948; P. S. Rao and P. S. Varma, J. Sci. Ind. Res. (India), 10B, 166, 1955; M. Romanuk, V. Herout, and F. Storm., collection: 23, 2188, 1958; R. S. Joshi, A. S. Bawdekar, G. H. Kalkarni, A. S. Rao, G. R. Kelkar, and S. C. Bhattacharyya, Perfum. and Essent. Oil Res., 52, 773, 1961.
5. K. S. Salooja, V. N. Sharma, and S. Siddique, J. Sci. Ind. Res. (India), 9B, 1950.
6. A. P. Roekhov, Byull. n.-i. khim.-farm. in-ta. no. 3-4, 1930.
7. N. D. Strel'nikova, G. E. Alekseeva, and N. V. Syrneva, collection: New Medicinal Plants of Siberia, Medical Preparations from Them, and Their Use [in Russian], no. 5, Tomsk, 61, 1959.
8. A. L. Korolenko, collection: New Medicinal Plants of Siberia, Medical Preparations from Them, and Their Use [in Russian], no. 5, Tomsk, 194, 1959.
9. R. N. Chopra and De Premankur., Indian Med. Gaz., 59, 540, 1924.
10. F. I. Ibragimov and V. Ibragimova, Basic Medicinal Agents of Chinese Medicine [in Russian], Moscow, 234, 1960.
11. K. Bauer, Die organische Analyse [Russian translation], Moscow, 432, 1953.
12. R. Tschesche, F. Lampert, and G. Snatzke, J. Chromatography, 5, 217, 1961.
13. A. R. H. Cole, et al., J. Chem. Soc., 1218, 1959.
14. A. R. H. Cole and R. L. S. Willix, J. Chem. Soc., 1213, 1959.
15. A. R. H. Cole, J. Chem. Soc., 3807, 1954.
16. R. N. Jones, et al., J. Am. Chem. Soc., 70, 2024, 1948; R. N. Jones, et al., J. Am. Chem. Soc., 72, 86, 1950.
17. P. Bladon, H. B. Henbst, and G. W. Wood, J. Chem. Soc., 2737, 1952; T. G. Halsall, Chem. Ind. 867, 1951.
18. D. H. R. Barton and P. de Majo, J. Chem. Soc., 2178, 1953.
19. N. Cheronis, Micro- and Semi-Micro Methods of Organic Chemistry [in Russian], Moscow, 235, 1960.
20. P. Sengupta and H. N. Khastgir, Tetrah., 19, 123, 1963.

1 March 1965

Novosibirsk Institute of Organic Chemistry
Siberian Division AS USSR