THE STRUCTURE OF ARNIFOLIN, SESQUITERPENE LACTONE FROM Arnica folio NUTT. AND Arnica montana L.

R. I. Evstratova, V. I. Sheichenko,
K. S. Rybalko, and A. I. Ban'kovskii

UDC 615.256.54.011.5

Leafy arnica and mountain arnica are used in gynecological practice in the form of tinctures as styptic media. From the leaves and flowering calathides of these plants we have isolated a colorless crystalline material of composition $C_{20}H_{26}O_6 \cdot H_2O$, mp 128-132°C (dec.), $[\alpha]_D^{20} + 52.6°C$ (c 3.0; alcohol), which stimulates the smooth muscle of the uterus, i.e., a primary nutrient of arnica [1]. We have called the material arnifolin. The individuality of arnifolin was demonstrated by constancy of constants upon recrystallization from various solvents and by chromatography.

Arnifolin (I) does not dissolve in bases in the cold but dissolves easily upon heating. Upon short heating in weak solutions 1 g-eq. of base is consumed, and upon acidification arnifolin is again formed. In more concentrated bases and upon more prolonged heating 2 g-eq of base are consumed. Tiglic acid (II, $C_5H_8O_2$, mp 61-63°, identified by IR and NMR spectra, was isolated from the hydrolysis products.

Arnifolin forms an oxime, mp 160° (dec.), and gives a positive Zimmermann test for the $CO-CH_2$ grouping.

Upon hydrogenation of arnifolin in the presence of both Pt and Ni catalysts in alcohol, 2 moles of hydrogen are consumed and the tetrahydro derivative (III) is formed, mp 64-66° (hydrate form), which after drying is a colorless, glassy product of composition $C_{20}H_{30}O_6$. (See scheme on next page.)

In the IR spectrum of arnifolin are observed absorption bands at 3578, 3384, 3230 cm^{-1} (OH group), 1712 cm^{-1} (α,β-unsaturated ester), and 1655 (C=C), and also a broad band at 1754 cm^{-1}. In the tetrahydro derivative, in addition to absorption bands of the OH group, absorption maxima are present at 1770 cm^{-1} (α-lactone), 1750 cm^{-1} (cyclopentanone), and 1725 cm^{-1} (ester).

It follows from these data that arnifolin is an ester of a sesquiterpene hydroxyketolactone and tiglic acid. The broad band at 1754 cm^{-1} in arnifolin is due to merging of absorption bands of the α-lactone and cyclopentanone.

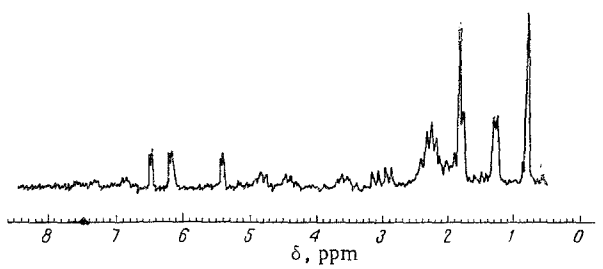


Fig. 1. NMR spectrum of arnifolin in $CDCl_3$. All NMR spectra were taken on a 100 MHz INM-4H-100 instrument.

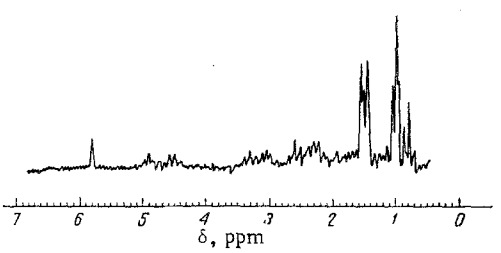
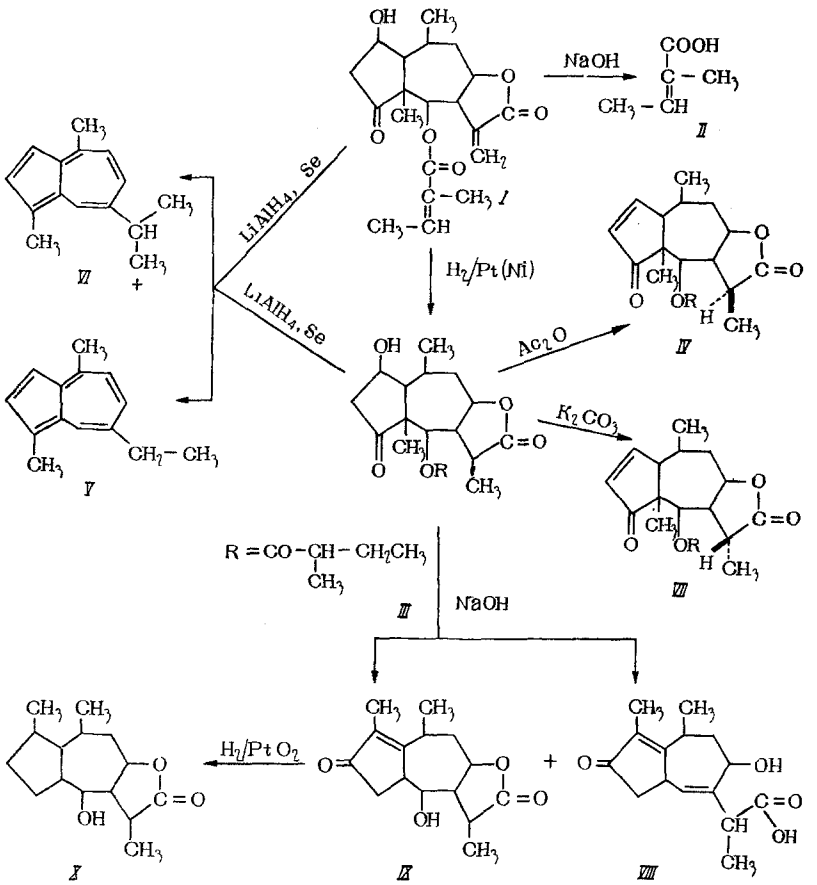


Fig. 2. NMR spectrum of tetrahydroarnifolin in $CDCl_3$.

All-Union Scientific-Research Institute of Medicinal Plants, Moscow. Translated from Khimiko-Farmatsevticheskii Zhurnal, No. 9, pp. 39-45, September, 1969. Original article submitted March 17, 1969.



The UV spectrum of arnifolin contains an absorption maximum at 219 mμ, ε 14,410, characterizing the presence of conjugated double bonds; this absorption maximum is not present in (III).

Treatment of tetrahydroarnifolin with acetic anhydride yields anhydrotetrahydroarnifolin (IV), $C_{20}H_{28}O_5$, mp 122-126°; ν_{max}: 1765 (γ-lactone), 1730 (ester), 1710 and 1585 cm^{-1} (cyclopentenone). The presence of the latter is confirmed by the UV spectrum (λ_{max} 226 mμ, ε 8,673), characteristic for an α,β-cyclopentenone [2-5]. Consequently, the hydroxyl is situated in a five-membered ring.

Dehydrogenation of arnifolin and the tetrahydro derivative over selenium at 310-360° for 1, 2, and 6 h gave only traces of a blue material; no other aromatic derivatives were obtained. Dehydrogenation over selenium of the lithium aluminum hydride reduction product of arnifolin and tetrahydroarnifolin led to chamazulene (V) and guaiazulene (VI), but also in very low yield. It is known that sesquiterpene lactones of the ambrozan type [6] often give such dehydrogenation results. The similarity of arnifolin to sesquiterpenes of this type is confirmed by NMR spectral data of arnifolin which show the characteristic methyl singlet at 0.83 ppm (angular methyl).

The NMR spectrum of arnifolin (Fig. 1) also contains the signal of the vinyl proton as a quartet at 6.86 ppm and signals of two vinyl methyls at about 1.85 ppm, $-2CH_3-C=C$. These signals virtually co-

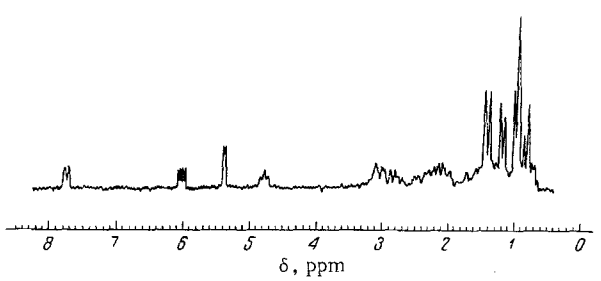


Fig. 3. NMR spectrum of anhydrotetrahydroarnifolin in $CDCl_3$.

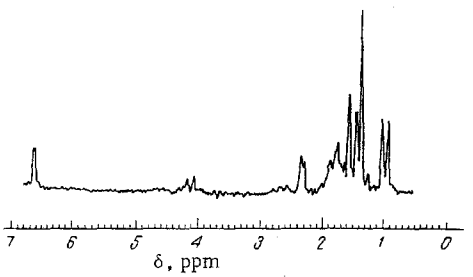


Fig. 4. NMR spectrum of the hydroxyketoacid in pyridine.

incide with the signals of tiglic acid. In addition, the spectrum of arnifolin contains a doublet at 1.32 ppm, $-CH_3-CH$, and a pair of doublets at 6.15 and 6.43 ppm (J = 3 Hz), each corresponding to one proton, which characterizes the exocyclic methylene in conjugation with a lactone carbonyl.

Signals of vinyl protons are absent in the NMR spectrum of (III) (Fig. 2), and a signal of the methyl of the ethyl group at 0.88 ppm and signals corresponding to 6 H at 1.5 ppm, $-2CH_3-CH$, have appeared; the signal of its angular methyl is shifted slightly to weaker field and appears as a singlet at 1 ppm partially superimposed on the signal of the CH_3-CH doublet at 1.02 ppm.

In the NMR spectrum of (IV) the position of the ethyl group methyl signal has not changed at 0.88 ppm. The C_{10} methyl appears as a singlet at 1.03 ppm and the C_{11} methyl is a doublet at 1.04 ppm; the doublets at 1.5 and 1.27 ppm (J = 7 Hz) correspond to the remaining two methyls.

The doublet at 5.38 ppm (J = 2 Hz) in the NMR spectrum of arnifolin corresponds to the ester proton; in (III) it is shifted to weak field and is expressed as a singlet at 5.7 ppm; in (IV) (Fig. 3) it is a singlet at 5.42 ppm. The lactone proton in (I) appears as a triplet with broadened components at 4.84 ppm and its position does not change in (III) and (IV). The quartet at 4.42 ppm ($\Sigma JH_1 = 23$ Hz) is assigned to the hemihydroxyl proton (at C_1); it is also present in (III) as a quarter at 4.5 ppm ($\Sigma JH_1 = 24$ Hz); this signal is absent in (IV), and two new signals appear instead: a quartet at 6.07 ppm (JH_2, $H_1 = 6$ Hz, $JH_{2,9} = 3$ Hz) and 7.76 ppm (JH_1, $H_2 = 6$ Hz, JH_1, $H_9 = 1.5$ Hz). These signals indicate that the double bond is located in the five-membered ring and is situated in the C_1-C_2 position [2-5].

Treatment of tetrahydroarnifolin with a K_2CO_3 solution with heating also leads to anhydrotetraarnifolin (VII), $C_{20}H_{28}O_5$, ν_{max} 1780 (γ-lactone), 1745 (ester), 1710 and 1585 cm^{-1} (cyclopentenone), λ_{max} 226 mμ, ε 6,322, but having a different NMR spectrum. The difference in the positions of the methyl group signals is evidently associated with rearrangement around the asymmetric center at C_{11} upon heating with K_2CO_3, which is already known [7].

Cleavage of the hydroxyl upon treatment with both acid and base is possible if it is located in a β-position to the carbonyl, i.e., the hydroxyl is located at C_1 [8, 9].

Hydrolysis of tetrahydroarnifolin in the presence of NaOH gives two materials: 1) a hydroxylactone of composition $C_{15}H_{20}O_4$, mp 294-296°, ν_{max} 3500 (OH), 1758 (γ-lactone), 1699 (cyclopentenone), 1624 cm^{-1} (C=C); UV spectrum λ_{max} 237 mμ, ε 15,248; 2) an acid of composition $C_{15}H_{20}O_4$, mp 176-178°, ν_{max} 3450 (OH), 2650, 2550 (carboxyl OH), 1705 (carboxyl C=O), 1690 (cyclopentenone), 1670 cm^{-1} (C=C); λ_{max} 250 mμ, ε 6205.

The NMR spectrum of the acid (Fig. 4) contains a signal of a secondary methyl as a doublet at 0.95 ppm, a signal of a methyl at a double bond as a singlet at 1.38 ppm, signals of protons of the $CH_3-\underset{|}{CH}-\underset{|}{C}=O$ group as a doublet at 1.55,ppm and a quartet at 4.12 ppm, and a one-proton vinyl proton as a doublet at 6.56 ppm.

The NMR spectrum of the lactone contains signals of two secondary methyl groups at C_8 and C_{11} as doublets at 1.12 and 1.43 ppm, respectively; the singlet at 1.73 ppm corresponds to the vinyl methyl.

As a result of the IR, NMR, and UV spectral data, the hydroxyacid and hydroxyketolactone must consequently have the structural formulas (VIII) and (IX).

An analogous rearrangement, occurring upon heating with bases, is described for certain amrbosanolides, for example, tenulin and helenalin [10, 11]; the products formed here were called neotenulin and neohelenalin.

Upon hydrogenation of (IX) over Pt catalyst total reduction of the keto group occurs and a hydroxylactone (X) of composition $C_{14}H_{24}O_3$, mp 203-205°, is formed. Ease of reduction of the keto group to the hydrocarbon is also characteristic for similar neocompounds.

We propose structure (I) for arnifolin on the basis of the indicated reactions and also from IR, UV, and NMR spectral data.

EXPERIMENTAL

Isolation of Arnifolin. The leaves and flowery calathides of Arnica foliosa Nutt., gathered at the introductory nursery of the All-Union Scientific-Research Institute of Medicinal Plants near Moscow during

the blooming phase, were digested three times with hot water (75%) for 30 min. The aqueous extracts were shaken with chloroform, the latter were distilled to dryness, and ether was added to the obtained resin. The precipitated lightly cream-colored crystals were recrystallized three times from alcohol, mp 128-137° (dec.); $[\alpha]_D^{20}$ + 52.6° (c 3.0; alcohol) [1]. Yield was 0.1% from the leaves and 0.2% from the flowers. Found, %: C 63.15, 63.47; H 7.51, 7.44; M (according to Rast) 374.9, 362. H_2O 4.4 and 4.5% (according to Fischer). $C_{20}H_{26}O_6 \cdot H_2O$. Calculated, %: C 63.15; H 7.36; M 380; H_2O 4.7%.

Chromatography on a thin layer of aluminum oxide of Grade IV activity in a system of petroleum ether – benzene – chloroform – methanol (5 : 4 : 1 : 2) gave one clear spot with R_f 0.5; chromatography on paper impregnated with a 10% solution of formamide in methanol by the descending method in a system of petroleum ether – benzene – methanol (5 : 4 : 2) also gave one clear spot with R_f 0.58: the indicator in this case and the other case was a 1% solution of $KMnO_4$ in a 1% solution of H_2SO_4.

Column chromatography of arnifolin on neutral aluminum oxide of Grade IV activity (elution with chloroform) gave one material, arnifolin.

The Zimmermann reaction with m-dinitrobenzene in an alcoholic solution of KOH was positive.

Arnifolin was also isolated from leaves and flowers of A. montana L.

The oxime of arnifolin is a finely crystalline precipitate, mp 160° (dec.).

Hydrolysis of Arnifolin. A. We boiled 0.137 g of arnifolin, 10 ml of alcohol, and 10 ml of a 0.1 N solution of NaOH for 10 min. During saponification 1 mole of NaOH was consumed.

B. We boiled 0.18 g of arnifolin, 10 ml of alcohol, and 15 ml of a 0.5 N solution of NaOH for 20 min. Upon saponification 2 moles of NaOH was consumed. Loss of base was not observed upon more prolonged boiling and addition of additional 0.5 N solution of NaOH.

C. We boiled 1 g of arnifolin, 0.8 g of K_2CO_3, 35 ml of alcohol, and 25 ml of water on a water bath at 50-60° for 1 h, left the mixture at room temperature for 24 h, then diluted it three-fold with water and extracted with chloroform; the chloroform solution was washed with 0.5% HCl and water and the chloroform was distilled to dryness. We obtained 0.08 g of arnifolin (identified by IR spectrum and thin layer chromatography). The mother solution after extraction with chloroform was acidified with a 15% solution of HCl to pH 1.0 and extracted with chloroform; the latter was washed with a 5% solution of K_2CO_3, then with water to a neutral reaction, and evaporated to dryness. We obtained 0.9186 g of arnifolin.

E. We heated 1 g of arnifolin and 100 ml of a 4% solution of NaOH at 50-60° for 3 h (cherry color), and after cooling acidified the mixture with a 10% solution of HCl to pH 1.0, extracted with chloroform, and washed the chloroform with a 4% solution of $NaHCO_3$ (color passes into the soda solution), then with water to a neutral reaction; the chloroform was distilled to dryness and traces of a resin were obtained. The soda solution was acidified, treated with chloroform, then with water to a neutral reaction, and the chloroform was distilled. We obtained 0.38 g of a dark crystalline mass. The crystals were purified by sublimation at 70° (10 mm) to give 0.1 g of (II), mp 61-63°. Found, %: C 59.83, 59.92; H 8.04, 7.85. $C_5H_8O_2$. Calculated, %: C 60.0; H 8.00. ν_{max} 2550; 2700; 1690; 1650 cm^{-1}.

Hydrogenation of Arnifolin. A. We hydrogenated 2 g of arnifolin in 100 ml of alcohol over 0.11 g of PtO_2 (Adams) until absorption of hydrogen ceased (the reaction is over in 1 h); 270 ml of hydrogen was absorbed (276 ml calculated for two double bonds). The catalyst was filtered and the alcohol was evaporated to dryness. A thick colorless liquid was obtained which partially crystallized after careful prolonged drying, mp 64-66°; after drying in a vacuum pistol it became a glassy product. Found, %: C 65.74, 65.34; H 8.35, 8.32. $C_{20}H_{30}O_6$. Calculated, %: C 65.55; H 8.25.

B. We hydrogenated 2 g of arnifolin in 100 ml of alcohol in the presence of Raney Ni catalyst. The amount of hydrogen absorbed was 270 ml (276 ml calculated for two double bonds) and hydrogenation did not proceed further. The catalyst was filtered and the alcohol was removed to give a glassy product. Treatment with ether yielded crystals which melted upon drying in a vacuum pistol at 70° (0.5 mm). Found, %: C 65.57, 65.63; H 8.31, 8.27.

Acetylation of Tetrahydroarnifolin. We heated 0.5 g of tetrahydroarnifolin, 5 ml of acetic anhydride, and 10 ml of pyridine for 1.5 h at 60-70°; after cooling the reaction mixture was diluted with water and extracted with ether and the extract was washed with a 5% solution of HCl, then with water to a neutral reac-

tion; the ether was evaporated to give a yellow resin which crystallized upon standing under vacuum; the crystals were washed with ether, mp 122-126° (IV). Found, %: C 68.60, 68.71; H 8.15, 8.19. $C_{20}H_{28}O_5$. Calculated, %: C 68.94; H 8.10.

The mother solution was chromatographed on aluminum oxide of Grade IV activity. The first benzene fraction yielded a glassy material having R_f 0.8 upon thin layer chromatography in a system of benzene – methanol (9:1). Found, %: C 68.79, 68.62; H 8.17, 8.24.

Hydrolysis of Tetrahydroarnifolin. A. We heated 0.7 g of tetrahydroarnifolin with 20 ml of alcohol, 1.4 g of K_2CO_3, and 15 ml of water at 50° for 2 h, evaporated the alcohol under vacuum at 50° over 40 min, and after cooling acidified the mixture with a 20% solution of HCl to pH 1.0; the product was extracted with chloroform, the chloroform solution was washed with a 5% solution of $NaHCO_3$ and water, and the chloroform was distilled in vacuum. We obtained 0.5 g of a lightly cream-colored thick liquid. Upon chromatography on neutral aluminum oxide of Grade IV activity the benzene fraction yielded 0.35 g of a colorless glassy material having R_f 0.8 (benzene – methanol, 9:1) (VII). Found, %: C 68.78, 68.91; H 8.25, 8.16. $C_{20}H_{28}O_5$. Calculated, %: C 68.94; H 8.10.

B. We heated 2.5 g of tetrahydroarnifolin and 100 ml of a 4% solution of NaOH at 50-55° for 40 min and upon cooling acidified the reaction mixture with a 10% solution of HCl to pH 1.5. The mixture was extracted with ether, the ether was washed with water, and removal of the ether gave traces of a resin.

The mother solution after treatment with ether was extracted several times with ethyl acetate; the latter was washed with water and evaporated to dryness to give 0.2 g of almost colorless crystals having mp 205-214°, R_f 0.63 and 0 (system benzene – alcohol, 8:2) in a thin layer of neutral aluminum oxide of Grade IV activity; the developer was a 1% solution of $KMnO_4$ in a 1% solution of H_2SO_4. The crystals were washed with benzene. From the benzene was obtained 0.03 g of colorless crystals of the hydroxyketoacid having mp 176-178° (VIII). Found, %: C 68.39, 67.89; H 7.79, 7.76. $C_{15}H_{20}O_4$. Calculated, %: C 68.16; H 7.63.

Crystals insoluble in benzene were recrystallized from alcohol to give colorless crystals of the hydroxyketolactone having mp 294-295° (IX). Found, %: C 68.15, 68.37; H 7.80, 7.83. $C_{15}H_{20}O_4$. Calculated, %: C 68.16; H 7.63.

Hydrogenation of (IX). A solution of 0.2 g of (IX) in 20 ml of alcohol and 0.1 g of PtO_2 was hydrogenated for 1 h. The amount of hydrogen absorbed amounted to 30 ml; removal of the alcohol and catalyst yielded 0.17 g of a yellowish resin which was next chromatographed on neutral aluminum oxide of Grade IV activity. From the benzene fraction was obtained 0.07 g of colorless crystals having mp 203-205° (X). Found, %: C 71.32; H 10.00. $C_{15}H_{24}O_3$. Calculated, %: C 71.42, H 9.52.

Dehydrogenation of Arnifolin. We boiled 1.2 g of arnifolin, 1 g of lithium aluminum hydride, and 350 ml of dioxane for 6 h and upon cooling carefully decomposed unreacted lithium aluminum hydride with water. Distillation of the solvent yielded about 1 g of a glassy yellowish product which was dehydrogenated in the presence of 1 g of selenium at 320-360° for 3 h; the reaction product was extracted with petroleum ether and chromatographed on neutral aluminum oxide of Grade I activity. Two fractions were obtained in low yield: blue and blue-violet, identified by thin layer chromatography as chamazulene (V) and guaiazulene (VI). The same results were obtained upon dehydrogenation of tetrahydroarnifolin, also preliminarily reduced with lithium aluminum hydride.

LITERATURE CITED

1. K. S. Rybalko, E. A. Trutneva, and P. N. Kibal'chich, Aptechn. Delo, No. 4, 32 (1965).
2. W. Herz and M. V. Lalshmikantham, Tetrahedron, 21, 1711 (1965).
3. W. Herz, S. Rajappa, et al., ibid., 22, 1907 (1966).
4. E. Dominguez and J. Romo, ibid., 19, 1415 (1963).
5. W. Herz, A. Romo de Vivar, et al., ibid., 1159.
6. W. Herz, K. Veda, and S. Inajama, ibid., 483.
7. W. Dauben, J. S. Schwarz, et al., J. Amer. Chem. Soc., 82, 2239 (1960).
8. K. S. Rybalko, Zh. Obshch. Khim., 33, 2734 (1963).
9. W. Herz, V. Kishida, et al., Tetrahedron, 20, 979 (1964).
10. W. Herz, V. Ronde, et al., J. Amer. Chem. Soc., 84, 3857 (1962).
11. W. Herz, A. Roma de Vivar, et al., J. Amer. Chem. Soc., 85, 19 (1963).