DITERPENOIDS OF *Pulicaria salviifolia*.

I. STRUCTURES OF SALVIN AND SALVININ*

M. R. Nurmukhamedova, Sh. Z. Kasymov,
N. D. Abdullaev, G. P. Sidyakin, and
M. R. Yagudaev

UDC 547.595.9

Two new diterpenoids of the clerodane series — salvin and salvinin — have been isolated from the epigeal part of *Pulicaria salviifolia* Bgl. in Mem. A structure and a most probable stereochemistry are proposed.

We have investigated *Pulicaria salviivolia* Bgl. in Mem., family Asteraceae, growing on the territory of the Uzbek SSR [1].

From a chloroform extract of the epigeal part of the plant gathered in 1981 we have isolated two substances of lactone nature which have been called, respectively, salvin (I) and salvinin (II).

Salvin was the main component of the combined lactones (making 0.14% of the weight of the dry raw material) and it had the elementary composition $C_{20}H_{26}O_3$ (M^+ 314) and contained a furan ring according to the Ehrlich color reaction [2]. UV spectrum: $\lambda_{max}^{C_2H_5OH}$ 220 nm (log ε 4.12); IR spectrum: ν_{max}^{KBr} 3155, 3140, 1590, 1510, 880 cm^{-1}. The mass spectrum — m/z 95 and 81 — indicated substitution in the β position, as was also shown by the PMR spectrum — 6.25 ppm (1 H, br.s), 7.21 ppm (1 H, br.s), and a broadened triplet at 7.36 ppm with Σ^3J = 3.5 Hz [3-7]. In the mass spectrum of (I), the peak of the ion with m/z 81, corresponding to a pyrylium ion, was the 100% peak, which is characteristic for an unsubstituted side chain [8]. The PMR spectrum of salvin (see below) contained three signals from the protons of methyl groups: Singlets at 0.88 and 1.04 ppm and a doublet at 1.01 ppm with ^3J = 7.3 Hz. The lactone nature of salvin was shown unambiguously by the IR spectrum (1785, 1690 cm^{-1}) and by the PMR spectrum — the lactone proton resonated in the form of a quartet with ^3J = 10.8 and 3.8 Hz. A one-proton triplet at 6.48 ppm with ^3J = 7.2 Hz corresponded with respect to the size of its chemical shift to an olefinic proton at a double bond conjugated with a carbonyl group. In the PMR spectrum of the alcohol (IV) — the product of the reduction of (I) with $LiAlH_4$ — where this conjugation was destroyed, the signal of the olefinic proton showed a considerable diamagnetic shift ($\Delta\delta$ = 0.96 ppm) and resonated at 5.52 ppm. Overlapping multiplets from the methylene and methine protons of salvin with a total intensity of 12 H occupied the part of the spectrum between 1.3 and 2.7 ppm.

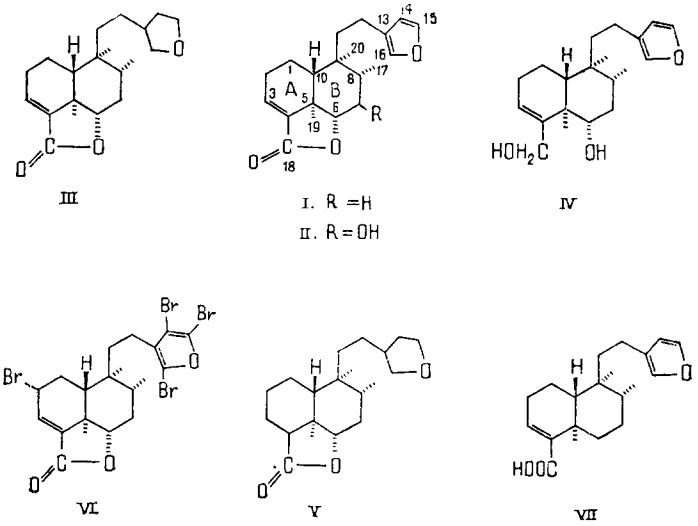
Salvin was unreactive in relation to osmium tetroxide and to p-chlorobenzoic acid. When salvin was hydrogenated over a platinum catalyst in acetic acid, the double bonds of the furan ring were saturated (III), but the double bond of the hydronaphthalene system was hydrogenated with difficulty to form a hexahydro derivative (V).

Attempts at the allyl bromination of salvin led to the bromination of the more reactive furan double bonds. Only when the reaction was carried out at −7°C was it possible to obtain a tetrabromo derivative of salvin (VI), and this one bromine atom was present in the β position to the double bond of ring A. In the PMR spectrum of compound (VI), the signals of the protons of the furan ring were absent. The signal of the olefinic proton appeared at 6.53 ppm in the form of a doublet with ^3J = 3.8 Hz. The proton in the geminal position to the bromine atom (H-2) gave a multiplet at 5.10 ppm. Under the conditions of double nuclear magnetic resonance with saturation of the resonance transitions of the nuclei of the H-3 olefinic proton (ν = 653 Hz) the multiplet at 5.10 ppm was converted into a quartet with ^3J = 6.5 and 2.3 Hz.

*Presented at the VIIth Indo-Soviet Symposium on the Chemistry of Natural Compounds [16].

Institute of the Chemistry of Plant Substances, Academy of Sciences of the Uzbek SSR, Tashkent. Translated from Khimiya Prirodnykh Soedinenii, No. 2, pp. 201-205, March-April, 1985. Original article submitted April 14, 1982.

 0009-3130/85/2102-0188$09.50



III

I. R = H
II. R = OH

IV

VI

V

VII

Consequently, H-2 interacted vicinally not only with the olefinic proton but also with the two neighboring methylenic protons.

Thus, all the facts given above in combination permit salvin (I) to be assigned to the diterpenoid lactones of the clerodane series [9-13]. This is also shown by the fact that when (I) was hydrogenated over a platinum catalyst in acetic acid, the double bonds of the furan ring were readily saturated (III), while the conjugated double bond was hydrogenated with difficulty to form the hexahydro derivative (IV) [14]. Below we give the characteristics of the PMR spectra of salvin (I) and salvinin (II) in $CDCl_3$ (δ, ppm, 0 — TMS):

	Salvin (*I*)	Salvinin (*II*)
H-3	6,48 t $\Sigma\,^3J = 7,2$ Hz	6,46 t, $\Sigma J^3 = 7,2$ Hz
H-6	3,74 q $^3J = 12,2$ and 3,8 Hz	3,67 d, $^3J = 2,9$ Hz
H-7		4,42 q, $^3J = 3\,3$ Hz 2,9 Hz
H-14	6,25 br.s	6,26 br.s. c
H-15	7,36 br.t $\Sigma J = 3,5$ Hz	7,37 br.t тр. $\Sigma J = 3,5$ Hz
H-16	7,21 br.s	7,22 br.s c
H-17	1,01 d $J = 7,3$ Hz	1,15 d, $^3J = 7,3$ Hz
H-19	1,04 s	1,35 s
H-20	0,88 s	1,06 s

Using the double resonance procedure it was established that the lactone proton interacted vicinally only with the two methylene protons at ~1.9 ppm with ^3J = 12.2 and 3.8 Hz. It follows from this, in view of the presence in the molecule of (I) of two tertiary and one secondary methyl groups that the lactone ring was formed at the C-4 and C-6 carbon atoms. The lactone proton, interacting axially-axially (^3J = 12.2 Hz) and axially-equatorially (^3J = 3.8 Hz) with the neighboring methylene protons has the β-axial orientation.

Salvinin (II), with the composition $C_{20}H_{26}O_4$, M^+ 330, had a maximum at 220 nm (log ε 4.08) in its UV spectrum. In its IR spectrum, absorption bands at 3150, 1570, 1510, and 880 cm^{-1} corresponded to a furan ring; a band at 3410-3430 cm^{-1} was characteristic for a hydroxy group; and bands at 1750 and 1680 cm^{-1} corresponded to the system of an α,β-unsaturated γ-lactone. The mass spectrum of (II), just as in the case of salvin (I), had the peaks of ions with m/z 95 (88%) and 81 (100%), due to the presence of a furan ring. From the characteristics given and its chromatographic behavior, salvinin (II) was close to salvin (I), and their difference was due to the presence of a hydroxy group in the molecule of (II)

The PMR spectrum of salvinin was characterized by the following signals (see above): Singlets of 3 H each at 1.06 and 1.35 ppm and a doublet with ^3J = 7.3 Hz at 1.15 ppm — tertiary and secondary methyl groups; a group of multiplets with an intensity of eleven proton units between 1.4 and 2.4 ppm — methylene and methine protons; broadened one-proton singlets at 6.26 and 7.22 ppm and a triplet with ΣJ = 3.5 Hz at 7.37 ppm — the protons of a β-substituted furan ring; and a one-proton triplet at 6.46 ppm with $\Sigma\,^3J$ = 7.2 Hz — an olefinic proton at a conjugated double bond. The signal of the lactone proton appeared at 3.67 ppm in the form of a doublet with ^3J = 2.9 Hz. The other one-proton signal at 4.42 ppm, appearing in the form of a

quartet with Σ^3J = 6.2 Hz, was assigned to the proton in the geminal position to the secondary hydroxy group.

It was established with the aid of the double-resonance procedure that the proton in the geminal position to the hydroxy group interacted vicinally with the lactone proton (3.67 ppm) with ^3J = 2.9 Hz, and with H-8 (~1.8 ppm) with ^3J = 3.3 Hz. The latter in its turn interacted vicinally with the protons of the secondary methyl group. This experiment enabled us to determine the position of the secondary hydroxy group at C-7 in the salvinin (II) molecule. The hydroxy group at C-7 and the methyl group at C-8 have the α orientation. The protons at C-6, C-7, and C-8 are β-orientated. This was shown by the set of spin-spin coupling constants found above for the CH(O)—CH(OH)— $CH(CH_3)$ fragment of the salvinin molecule.

Together with salvin and salvinin, from the polar fraction of the plant we isolated a known diterpenoid acid of the clerodane series — hardwickiic acid (VII) — identified from its physicochemical constants and its IR spectrum [15]. By analogy with hardwickiic acid, we assume the trans linkage of rings A and B as the most probable for compounds (I) and (II).

On the basis of what has been said, salvin is 15,16-epoxy-trans-cleroda-3,13(16),14-trien-19,6β-olide, and salvinin is 7-hydroxy-15,16-epoxy-trans-cleroda-3,13(16),14-trien-19,6β-olide.

EXPERIMENTAL

UV spectra were recorded on a Hitachi spectrophotometer (in ethanol), IR spectra on a UR-20 instrument (as tablets with KBr and as films), mass spectra on an MKh 1303 instrument, and CD spectra on a JASCO-20 spectropolarimeter. PMR spectra were recorded on a JNM-4H100 spectrometer in $CDCl_3$, δ, ppm, 0 — TMS. For TLC monitoring we used Silufol plates in the hexane—ethyl acetate (1:1) system with a 1% solution of vanillin in concentrated H_2SO_4 as the revealing agent.

Isolation of Salvin and Salvinin. The dried and comminuted part of the plant *Pulicaria salviifolia* collected in September, 1981 (village of Khonabad, Papskii region, Namanganskaya province) (9 kg) was exhaustively extracted with chloroform (40 liters) at room temperature. The concentrated chloroform extract was dissolved in 2.5 liters of ethanol, 1.6 liters of hot water was added, and the mixture was left for 12 h. The precipitate that deposited was filtered off. According to TLC, the resinous residue did not contain the desired compounds. The solution was filtered and concentrated. The weight of the total material of the concentrate was 130 g. This combination of extractive substances was deposited on a column of silica gel (particle size 0.16-0.2 μm) and was eluted with hexane—diethyl acetate (9:1), one-liter fractions being collected. Fractions 9-20 yielded 13 g of salvin. The amount of ethyl acetate was gradually increased, and at a ratio of hexane to ethyl acetate of 6:1 fractions 70-85 yielded 4 g of salvinin.

Salvin (I) formed a white crystalline substance, $C_{20}H_{26}O_3$, M^+ 314, mp 128°C (hexane—ethyl acetate (6:1)), $[\alpha]_D^{20}$ — 110.4° (c 0.42; methanol), R_f 0.57; ν_{max}^{KBr} (cm^{-1}); 1785, 1690, 1590, 1502, 880. Mass spectrum, m/z (%): 314 [M^+] (10), 299 [$M - CH_3$]$^+$ (11), 219 [M — CH_2 — CH_2 — furan]$^+$ (10), 95 [CH_2 — CH_2 — furan]$^+$ (95), 81 [pyrylium]$^+$ (100). CD: $[Q]_{208}$ = —45,000: Δε = —13.6. $[Q]_{253}$ = +10,000; Δε = +4.06.

Salvinin (II) formed a white crystalline substance. It was unstable, and on standing in the air in the course of a few days it became yellow and polymerized. $C_{20}H_{26}O_4$, M^+ 330, mp 127°C (hexane—ethyl acetate (4:1), $[\alpha]_D^{20}$ — 128° (c 0.22; methanol). R_f 0.41. ν_{max}^{KBr} (cm^{-1}); 3410-3430, 3150, 1750, 1680, 1570, 1510. Mass spectrum, m/z (%): 330 [M^+] (77); 315 [M^+ — 15] (72); 287 [$M^+ - CH_2CH_2CH_3$]$^+$ (38); 235 [M^+ — CH_2 — CH_2 — furan] (34); 95 [CH_2 — CH_2 — furan]$^+$ (88), 81 [pyrylium] (100). CD: $[Q]_{255}$ = +7280; Δε = +2.20; $[Q]_{210}$ = —52950; Δε = —16.0.

Hardwickiic Acid (VII). When the column was eluted further with hexane—ethyl acetate (4:1), fractions 91-100 yielded a complex mixture of substances from which, on several-times-repeated rechromatography a very small amount (10 mg) of hardwickiic acid was isolated — $C_{20}H_{28}O_3$, mp 140-142°C, $[\alpha]_D^{20}$ — 96° (c 0.19; methanol). IR spectrum: ν_{max}^{KBr} (cm^{-1}): 3170, 1680, 1510, 1502, 840.

Hydrogenation of Salvin (III). In 15 ml of acetic acid in the presence of 20 mg of platinum dioxide, 100 mg of the substance was hydrogenated for 1 h. This gave 80 mg of total product, which was purified by column chromatography on silica gel with elution by the hexane—ethyl acetate (5:1) system, leading to 40 mg of the oily product (III) (yield 40%). IR spectrum: ν_{max}^{KBr} (cm^{-1}): 3460-3500, 2950-2980, 1790. PMR ($CDCl_3$), ppm: 6.40, t, Σ^3J = 7.2 Hz (H-3);

0.84 and 1.02 (singlets, CH_3-20, CH_3-19). Mass spectrum, m/z: 318, 303, 300, 215. More prolonged hydrogenation led to the breakdown of (III) and the appearance in very small amounts of the hexahydro derivative (V), which was characterized mass-spectrometrically.

Reduction of Salvin with $LiAlH_4$ to (IV). At room temperature, 170 mg of $LiAlH_4$ was added in portions to a solution of 200 mg of salvin in absolute ether. The excess of $LiAlH_4$ was decomposed with water 15-20 min after the end of the reaction. The reaction products were extracted with ether and were purified by column chromatography in which elution was performed with the solvent system hexane—ethyl acetate (9:1) with a gradual increase in the concentration of the latter. The weight of the main product (IV) (an oil) was 50 mg (25%). IR spectrum, ν_{max}^{KBr} (cm^{-1}); 3290-3320, 2880, 1710, 1510, 1460, 880. PMR ($CDCl_3$), ppm: 6.20 (br.s, H-14); 7.14 (br.s, H-16); 7.30 (br.t, H-15); 5.54 (t, Σ^3J = 7.2 Hz, H-3); 3.98 and 4.24 (2 d, 1 H each, ^3J = 12.6 Hz, CH_2OH at C-4); 3.64 (t, 1 H, Σ^3J = 15.5 Hz, H-6); 0.74 and 1.04 (singlets 3 H each, CH_3-19 and CH_3-20); 0.87 (d, 3 H, CH_3-17). Mass spectrum, m/z: 318, 95, 81.

Bromination of Salvin to give (VI). With stirring, a solution of N-bromosuccinimide in absolute chloroform was added dropwise to a solution of 250 mg of salvin in absolute chloroform cooled to −7°C. The reaction took 15 min, as was monitored by TLC. The excess of N-bromosuccinimide was filtered off. The product was purified by column chromatography, and the yield of the oily product (VI) was 50 mg (20%). The bromine derivative of salvin was unstable and the substance decomposed completely in 3-4 h. PMR ($CDCl_3$): 6.53 (d, 1 H, ^3J = 3.5 Hz); 5.10 (m, 1 H, ^3J = 6.5, 3.8, and 2.3 Hz); 0.86 and 1.02 (singlets, 3 H each, CH_3-19 and CH_3-20); 0.99 (d, 3 H, ^3J = 7.3 Hz, CH_3-17).

SUMMARY

Two new diterpenoid lactones of the clerodane series — salvin and salvinin — have been isolated from the epigeal part of *Pulicaria salviifolia*. By the performance of chemical transformations and on the basis of the results of a study of IR, UV, mass, and PMR spectra structures and most probable stereochemistries have been proposed for them.

LITERATURE CITED

1. The Flora of Uzbekistan [in Russian], Tashkent, Vol. VI (1962), p. 95.
2. T. Reichstein, Helv. Chim. Acta, 15, 1110 (1932).
3. D. Billet, M. Durgeat, S. Heitz, and A. Ahond, Tetrahedron Lett., 44, 3825 (1975).
4. S. R. Wilson, L. A. Neubert, and J. C. Huffman, J. Am. Chem. Soc., 98, 3669 (1976).
5. F. Bohlmann, M. Grenz, P. Wegner, and J. Jakupovic, Ann. Chem., 11, 2008 (1983).
6. G. Y. Papanov and P. Malakov, Phytochemistry, 22, No. 12, 2787 (1983).
7. G. Y. Papanov, P. Malakov, and F. Bohlmann, Phytochemistry, 20, 170 (1981).
8. H. Wagner, R. Seitz, and H. Lotter, J. Org. Chem., 43, No. 17, 3339 (1978).
9. R. McGrindle, E. Nakamura, and A. Anderson, J. Chem. Soc. Perkin Trans. I, 1590 (1976).
10. P. R. Jefferies and T. G. Payne, Tetrahedron Lett., 48, 4777 (1967).
11. A. Rustaiyan, E. Simozar, A. Ahmadi, M. Grenz, and F. Bohlmann, Phytochemistry, 20, 2772 (1981).
12. W. Vichnewski, R. Murari, W. Herz, Phytochemistry, 18, 129 (1979).
13. T. Anthonsen and R. McGrindle, Acta Chem. Scand., 23, No. 3, 1068 (1969).
14. T. Anthonsen, M. S. Henderson, H. Martin, and R. D. H. Murray, Can. J. Chem., 51, No. 9, 1332 (1973).
15. R. Misra, R. C. Pandey, and Sukh Dev., Tetrahedron Lett., 49, 3751 (1964).
16. M. R. Nurmukhamedova, Sh. Z. Kasymov, G. P. Sidyakin, and N. D. Abdullaev, in: VIIth Indo-Soviet Symposium on the Chemistry of Natural Compounds. Abstracts of Lectures [in Russian], Tbilisi (1983).