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DITERPENOIDS OF *Pulicaria salviifolia*
IV. STRUCTURES OF SALVICINOLIDE AND SALVICINOLIN

K. A. Éshbakova, G. V. Sagitdinova, M. G. Levkovich, B. F. Rasulev, N. D. Abdullaev, and V. M. Malikov

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Two new clerodanolides — salvicinolide and salvicinolin — have been isolated from the epigeal part of Pulicaria salviifolia Bge *in* Mem *(fam. Compositae). Their structures have been established by an investigation of their spectral and physicochemical characteristics. It has been found that salvicinolide is capable of cyclizing at the C-6 and C-19 carbon atoms with the formation of a second lactone ring. The structure of 6α-hydroxy-19-carboxy-*trans*-clero-3,13-dien-15-olide is proposed for salvicinolide, and 6α,13-dihydroxy-19-carboxy-*trans*-cleroda-3,13-diene for salvicinolin.*

We have previously reported the determination of the structure of a new diterpene acid of the clerodane type — salvicinin — isolated from *P. salviifolia* [1]. Continuing an investigation of the terpenoids of this plant, we have isolated two new clerodanolides, which we have called salvicinolide (**1**) and salvicinolin (**2**).

Salvicinolide has the composition $C_{20}H_{28}O_5$, mp 148-150°C. Its IR spectrum contains the absorption bands of a hydroxy group (3400 cm^{-1}) and of the carbonyl of an α,β-unsaturated γ-lactone (1752, 1669) cm^{-1} and regions at 755 and 1669 cm^{-1} that are characteristic for an α,β-unsaturated carboxylic acid. The molecular mass of (**1**), 348, was established by mass spectrometry.

The PMR spectrum of (**1**), taken in $CDCl_3$, was similar in nature to those of the clerodane derivatives isolated previously from this plant [1-3]. In the strong-field region at 0.81, 1.20, and 0.71 ppm we observed the signals of protons corresponding to three methyl groups: CH_3-17, -18, and -20 (Table 1). Within the region of resonance of protons located geminally to hydroxy groups we observed a one-proton triplet with broadened components at 3.75 ppm having Σ^3J = 11 Hz. A 2H broadened singlet was observed at 4.73 ppm. Besides these, at 7.06 and 7.10 ppm there were two poorly resolved resonance lines forming a generalized envelope with an intensity of 2H and having its center at 7.08 ppm, and a singlet also with an integral intensity of 2H at 8.47 ppm. The form and position in the spectrum of the latter and its half-width of 20 Hz showed the presence in the (**1**) molecule of two functional groups with mobile protons.

On the whole, the PMR spectrum of (**1**) taken in $CDCl_3$ was characterized by poor resolution and the mutual superposition of some signals. This problem was solved by recording the spectrum of a solution of (**1**) in CD_3OD both at room temperature and at 60°C. Two signals appeared in the region of resonance of olefinic protons: a doublet of doublets at 6.83 ppm with Σ^3J = 8.0 Hz, and a poorly resolved multiplet at 7.30 ppm. Under double-resonance conditions, with saturation of the resonance transitions of the methylene protons at ~2.0 ppm, the first was converted into a singlet, which permitted us to assign it to H-3. The double-resonance experiments also showed that the multiplicity of the signal at 7.30 ppm (half-width 3.5 Hz) was due to long-range spin-spin coupling with methylene protons resonating at 4.77 and ~2.2 ppm. On this basis, the multiplet at 7.30 ppm and the doublet of triplets at 4.77 ppm were assigned to H-14 and 2H-16, respectively. Thus, it was established that the H-14 olefinic proton formed in the spectrum a poorly resolved quintet structure which can be explained by triplet/triplet splitting with the H-12 and H-16 methylene protons having spin-spin coupling constants of 1.5 and 2.0 Hz. Correspondingly, the H-12 methylene protons were observed at ~2.0 ppm after the decoupling of spin-spin interaction with H-14 and H-16. When the H-12 proton was suppressed, the H-14 signal showed residual triplet splitting, and the H-16 signal doublet splitting with the SSCC ^4J = 2.0 Hz.

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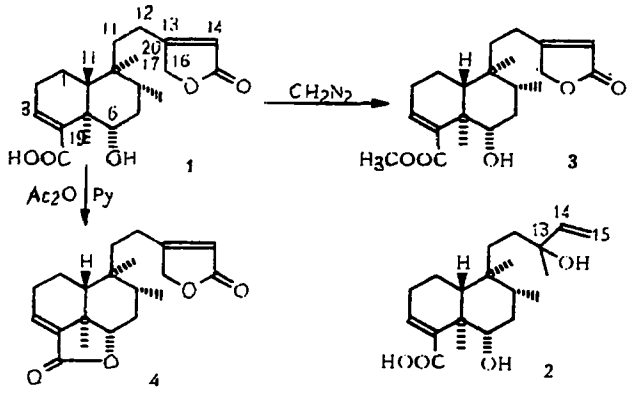

On passage to the more polar solvent CD_3OD, the signal appearing in the PMR spectrum of (1) in $CDCl_3$ at 3.75 ppm in the form of a broadened triplet acquired the structure of a distinct doublet of doublets at 3.58 ppm with ^3J = 8.5 and 6.5 Hz. This change was obviously due to a replacement of the mobile proton of a secondary hydroxy group by deuterium, which liquidated a possible spin-spin interaction between the protons of the secondary hydroxy group and the hydrogen atom located geminally to the hydroxylic function. Moreover, an intramolecular hydrogen bond between the secondary hydroxy and the carboxy groups, the formation of which is more favored in $CDCl_3$, may be appreciably destabilized on passing to the more polar medium. The structure of the splitting of the signal under consideration in the spectrum at 3.58 ppm unambiguously showed its assignment to H-6, at which the secondary hydroxy group is located. It has the α-equatorial orientation, as was shown by the magnitudes of the vicinal SSCCs between the H-6 and 2H-7 protons — 8.5 and 6.5 Hz.

In addition to establishing that (**1**) was a compound of the clerodane series, the experimental facts given above revealed the presence and position in the salvicinolide molecule of three main oxygen-containing functions: a carboxy group conjugated with a double bond at C-4, a secondary hydroxy group at C-6, and an α,β-conjugated carbonyl in the five-membered ring of a γ-lactone in the side-chain of the compound under investigation.

On methylation with diazomethane under the usual conditions, salvicinolide formed the methyl ester (**3**), and on its treatment with acetic anhydride in pyridine at room temperature, in place of the expected acetate at the secondary hydroxyl, the dilactone (**4**) was formed. In the IR spectrum of the latter, together with the absorption band of the carbonyl of the initial substance at 1752 cm^{-1}, an intense absorption band of a γ-lactone carbonyl appeared at 1760 cm^{-1} and the absorption band of the secondary hydroxyl had disappeared completely.

The molecular mass of substance (**4**) had decreased by 18 units (M^+ 330), which is possible on dehydration followed by cyclization at C-6 and C-19 with the formation of a lactone ring.

As can be seen from Table 1, on comparing the characteristics of the PMR spectra of (**1**) and (**3**) in $CDCl_3$ the greatest changes in the chemical shifts were observed for the H-3 signal. The diamagnetic effect of the methylation of the carboxy group on this proton amounted to 0.36 ppm, which again confirmed the position of this function at C-4 of the (**1**) molecule. The same tendency was observed in a comparison of the characteristics of the spectra of (**1**) and (**4**), but here the diamagnetic change in the chemical shift was almost twice as great, amounting to 0.62 ppm. This fact mainly shows a substantial change in the electron density distribution at the double bond formed between the C-3 and C-4 carbon atoms on the cyclization of the carboxy group to form a lactone ring during attempted acetylation. Such a tendency to lactonization has been reported previously for salvicin [1].

Thus, for salvicinolide we propose the structure 6α-hydroxy-19-carboxy-*trans*-cleroda-3,13-dien-15-olide.

Salvicinolin (**2**) had mp 160-161°C. Its IR spectrum contained absorption bands at 3200-3600 cm^{-1} (hydroxy group) and 1750 and 1680 cm^{-1} (carbonyl of an α,β-unsaturated γ-lactone).

The characteristics of its PMR spectrum (see Table 1) permitted the assignment of salvicinolin, as well, to the clerodane series, but, in contrast to salvicinolide, it has an aliphatic side-chain.

The structure of the aliphatic side-chain of (**2**) became clear on the basis of an investigation of its PMR spectrum. A doublet of doublets at 5.01 ppm (J = 10 Hz) and 5.14 ppm (J = 16 Hz) related to the C-15 protons of a C-15 exomethylene grouping for which the constants of vicinal interaction were large and those of geminal interaction were close to zero. A one-proton doublet of doublets at 5.82 ppm (J = 16.0 and 10.0 Hz) related to the proton at C-14 vicinal to them while the absence of additional splitting in the signal of this proton showed that the neighboring C-13 carbon atom was quaternary. This agreed well with the fact that at 1.22 pm there was the signal of methyl C-16 at a carbon atom connected with an oxygen function.

TABLE 1. Chemical Shifts (δ, ppm) and Spin-spin Coupling Constants of the Protons (Hz) in the PMR Spectra of Salvicinolide (**1**) and Its Derivatives (**3** and **4**) and Salvicinolin (**2**)

Proton	Substance and solvent				
	1 $CDCl_3$	**1** CD_3OD	**3** $CDCl_3$	**4** $CDCl_3$	**2** $CDCl_3$
H-3	7.06 br.t Σ^3J=7.5	6.83 t, Σ^3J=8.0	6.70 t, Σ^3J=8.0	6.44 t, Σ^3J=7.0	7.0 t, Σ^3J=7.5
H-6	3.75 br.t Σ^3J=11.0	3.58 dd ^3J=8.5; 6.5	3.62 dd, ^3J=11; 5.0	3.66 dd, ^3J=11.4; 4.1	3.60 br.t Σ^3J=12.0
H-14	7.10 br.s	7.30 tt, ^4J=2.0; 1.5	7.05 tt, ^4J=1.5; 2.0	7.05 tt, ^4J=2.5; 2.0	5.82 dd, ^3J=16.0; 10.0
2H-15	–	–	–	–	5.14 d, ^3J=16.0 5.01 d, ^3J=10.0
2H-16	4.73 br.s	4.77 dt ^4J=2.0; 1.5	4.73 dt ^4J=2.0; 1.5	4.72 dt ^4J=2.0; 1.8	1.22 s (3H)
CH_3-17	0.81 br.d ^3J=5.5	0.83 d. ^3J=5.5	0.80 d. ^3J=5.5	0.94 d., ^3J=5.5	0.68 d. ^3J=5.5
CH_3-18	1.20 s	1.18 s	1.17 s	0.97 s	1.12 s
CH_3-20	0.71 s	0.72 s	0.70 s	0.82 s	0.64 s

The presence of an *ABC*-exomethylene system for the H-14 and 2H-15 protons was also confirmed by the double-resonance method.

Thus, the combination of spectral characteristics permits us to propose for salvicinolide the structure of 6α,13-dihydroxy-19-carboxy-*trans*-cleroda-3(4), 14(15)-diene.

For both lactones the ring linkage is given from biogenetic considerations by analogy with salvicin, isolated from this plant [4].

EXPERIMENTAL

The conditions for taking the spectra and for separation and isolation are given in [1]. System for TLC: chloroform–ethyl acetate (1:1); Silufol plates (Chemapol). All the substances described gave a claret coloration when a chromatogram was sprayed with a 1% solution of vanillin in concentrated sulfuric acid, and they decolorized an aqueous solution of potassium permanganate.

Isolation of Salvicinolide. When the fraction was rechromatographed on a column of silica gel (Chemapol) the chloroform–ethyl acetate (3:1) system eluted salvicinolide: $C_{20}H_{28}O_5$, M^+ 348, mp 148-150°C, $[\alpha]_D^{22}$ −100° (*c* 0.5; ethanol), R_f 0.18 (yield 0.35 g). IR spectrum (ν_{max}, cm^{-1}): 1557, 1669, 1752, 3400. UV spectrum: λ_{max} 210 nm (log ε 2.98) in alcohol). Mass spectrum, *m/z*: M^+ 348, 330 (M^+-H_2O), 312 (M^+-$2H_2O$), 297 (M^+-$2H_2O$-CH_3), 260 (M^+-CH_3-COOH).

Isolation of Salvicinolin. Elution at a solvent ratio of 2:1 yielded salvicinolin (**2**) with mp 161-162°C, $[\alpha]_D^{22}$ −48° (*c* 0.5; ethanol), R_f 0.15 (yield 0.4 g). IR spectrum (ν_{max}, cm^{-1}): 1680, 1750, 3200-3600. Mass spectrum, *m/z* (%): 318 (5), 300 (M^+-H_2O) (20), 285 (M^+-H_2O-CH_3) (15), 219 (100).

Methyl Ester of Salvicinolide (3). A solution of 50 mg of salvicinolide in absolute ethanol was treated with 6 ml of an ethereal solution of diazomethane, and after 6 h the solvent was distilled off and the residue was separated by column chromatography on silica gel with chloroform as eluent. This gave 80 mg of a product with the composition $C_{21}H_{30}O_5$ — an oily substance, R_f 0.7. IR spectrum (ν_{max}, cm^{-1}): 1745, 1760, 3200-3400.

Cyclization of Salvicinolide to (4). A solution of 50 mg of salvicinolide in 3 ml of pyridine was treated with 3 ml of acetic anhydride and was left at room temperature for a day. After the usual work-up, the reaction product was purified by column chromatography on silica gel with gasoline and gasoline–ethyl acetate as eluents. This gave 30 mg of a product with the composition $C_{20}H_{26}O_5$ — an oily substance, R_f0.8. IR spectrum (ν_{max}, cm^{-1}) 1680, 1752, 1760.

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