B. Kh. Abduazimov, B. Tashkhodzhaev, S. Nasírov, I. D. Sham'yanov, M. R. Yagudaev, V. M. Malikov, and S. N. Aminov

A new sesquiterpene lactone of the eudesmane series, mucrolide, has been isolated from the epigeal part of <u>Tanaceptopsis mucronata</u> and its constitution has been established on the basis of chemical transformations and IR, mass, and PMR spectra. Its spatial structure has been reliably determined by x-ray structural analysis as 1β , 6α -dihydroxy- 5α , 7α (H)-eudesmane-3,11(13)-dien-8, 12-olide.

Continuing an investigation [1-4] of the plant <u>Tanacetopsis</u> <u>mucronata</u>, we have isolated a new sesquiterpene lactone, which has been called mucrolide (I).

The IR spectrum of (I) contained absorption bands at (cm^{-1}) 3450 (OH group), 1750 (C=O of a γ -lactone), 1672 (C=C conjugated with the carbonyl of a γ -lactone), and 1655 (isolated C=C). The mass spectrum of (I) contained the peak of the molecular ion with m/z 264 (M⁺), the peaks of ions with m/z 246 (M-H₂O) and 228 (M-2H₂O) due to the splitting out of one and two water molecules, and also those of fragments characteristic for the breakdown of eudesmanolides under electron impact (see the Experimental section).

In the PMR spectrum of (I) in C_5D_5N the signals of the protons of the hydroxy groups appeared at 6.24 and 6.12 ppm in the form of doublets with J = 5 Hz, the higher-field one being superposed on the signal of the exocyclic methylene group of the lactone ring. These signals of the protons of the hydroxy groups disappeared when trifluoroacetic acid was added (Table 1). At the same time, the signal at 3.78 ppm became a triplet and the pattern (nature of the splitting) of the three-proton signal at 4.0-4.3 ppm changed perceptibly, which showed the superposition of the signals of gem-hydroxylic and lactone protons.

In the PMR spectrum there were also the signals of the protons of a methyl group at a double bond, of an angular methyl group, and of a proton at a double bond at 2.06, 0.98, and 5.26 ppm, respectively.

The secondary nature of the hydroxy groups was confirmed by the acetylation of (I), as the result of which a diacetyl derivative with the composition $C_{19}H_{24}O_6$ (II) was formed. The PMR spectrum of (II) taken in CDCl₃ contained the signals of the methyl protons of acetyl groups at 2.06 and 2.00 ppm. In this spectrum the lactone proton appeared in the form of a one-proton triplet of doublets at 4.05 ppm with two large $(J_1 = J_2 = 10 \text{ Hz})$ and one small (J_3 = 4 Hz) SSCCs, which showed a trans-diaxial interaction of the lactone proton, H8, with the vicinal protons, H7 and H9. Thus, the lactone ring is trans-linked at the C7-C8 bond. In the PMR spectrum of (II) there were also the signals of the protons of methyl groups (singlets of 3 H each at 0.97 and 1.61 ppm) and of the exocyclic methylene group of the lactone ring (doublets of 1 H each at 5.34 and 6.03 ppm with $J_{7,13a} = J_{7,13b} =$ 3 Hz), and of a gem-acyl proton (doublet of doublets, 1 H, at 4.84 ppm with $J_1 = J_2 = 10$ Hz). In this spectrum the signals of the olefinic protons and of one of the gem-acyl protons were superposed at 5.30 ppm. A similar superposition of the signals of these protons was observed in the PMR spectrum of (II) in C₅D₅N. The signals of the protons of the exomethylene group of the γ -lactone ring, appeared in the form of doublets at 5.40 and 6.04 ppm with $J_{7,13a} = J_{7,13a} = 3$ Hz. The shift of the signal of the exomethylene protons in (II) relative to the initial (I) showed the presence of an α -oriented hydroxy group at C6 [5], and the value of the SSCC between H7 and H13 confirmed the trans linkage of the lactone ring at the C7-C8 bond [6-8]. The H6 gem-acyl proton gave a signal at 5.49

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Fig. 1. Spatial structure of mucrolide.

TABLE 1. Chemical Shifts of the Protons of Mucrolide, of the Diacetyl Derivative of Mucrolide, and of its Isomers (I, II, III, and IV-VI)

Proton	1		11		IV [13]	V [14]	VI [15]
	C,D,N	C ₅ D ₂ N*	C₁D₅N	CDCI.	CDCI,	CDCI3	CDCI3
H-1 H-3 H-6 H-7 H-8 H-13 H-13 H-13 H-14 H-15 OH OAc	3,78 t.d 5,28 m 4,0-4,3 m 2,64 m 4,0-4,3 m 6,14 m 6,14 m 0,98 s 2,10 s 6,24 d 6,12 d	3.78 d.d 5.28 m 4.16 m 2.64 m 4.16 m 6.14 m 0.98 s 2.10 s	4.90 4.90 m 5.49 m 2.72 m 4.15 t.d 6.04 d 5.40 d 0.82 s 1.57 s 	4,84 d.d 5,33 m 2,69 m 4,65 t.d 6,03 d 5,34 d 0,96 s 1,61 s	3,63 d. d 4.71 d 2,55 q.d 4,09t.d 6,03 d.d 6,03 d.d 1,05 s 1,97 d.d	2,58t.d 4.13t.d 2,59q.d 3.98t.d 6,18d.d 5,98d.d 0,80s 5,10br.s 4.77 br.s 2,16d	3,7 d.d 5,34 b rrs 2,42 q.d 4,05 t.d 6,09 d 5,45 d 0,89 1,69

s) singlet; d) doublet; t) triplet; d.d) doublet of doublets; t.d) triplet of doublets; q.d) quartet of doublets; br.s) broadened singlet; m) multiplet. *With trifluoroacetic acid: II) $1\alpha 2\alpha = 8$; $1\alpha 2\beta = 10$; 1.0H = 4; $5\alpha 6\beta = 6\beta 7\alpha = 10$; $7\alpha 8\beta =$ $8\beta 9\alpha = 12$; $7\alpha 13 = 3$; $8\beta 9\beta = 3.8$; IV) $1\alpha 2\alpha = 5$; $1\alpha 2\beta = 6\beta 7\alpha =$ $7\alpha 8\beta = 11$; 7α , 13 = 3; $8\beta 9\beta = 12$; $8\beta 9\beta = 4$; V) $1\alpha 2\alpha = 4.5$; $1\alpha 2\beta = 11$; 1, 0H = 4; $5\alpha 6\beta = 6\beta 7\alpha = 10$; 6β , 0H = 2; $7\alpha 13 = 3$; $7\alpha 8\beta = 8,9\alpha = 12$; $8\beta 9\beta = 3.5$; VI) $1\alpha 2\alpha = 7$; $1\alpha 2\beta = 10$; $5\alpha 6\beta =$ $6\alpha 6\beta = 6\beta 7\alpha = 7\alpha 8\beta = 8\beta 9\alpha = 12.5$; $5\alpha 6\alpha = 6\alpha 7\alpha = 7\alpha 13 = 3$; $8\beta 9\beta = 4$.

ppm and was characterized by a triplet with $\Sigma J = 20$ Hz, which was due to its trans-diaxial interaction with the H5 and H7 protons. This showed the α -orientation of the H5 proton.

Thus, the presence of a methyl group at a double bond and of an angular methyl group and also the existence of an olefinic proton unambiguously showed the position of the double bond in the eudesmane skeleton of mucrolide at C3 and C4 and the presence of a secondary hydroxy group at C1.

Without knowing a priori the conformation of ring A it is impossible to determine the orientation of the methyl group at C10 from the results of PMR spectroscopy. In view of this, the question of the trans or cis linkage of rings A and B remained unanswered. The elucidation of the orientation of the hydroxy group at C1 was difficult, since the existence



Fig. 2. Packing of the mucrolide molecules.

of one diaxial SSCC of the gem-hydroxylic HI proton with the neighboring protons can still not serve as a basis for determining its α - or β -orientation, because the SSCC depends on the conformation of ring A. Consequently, on the basis of the spectral characteristics it is possible to suggest, as a minimum, two alternative structures for mucrolide, in the form of (I) and (III)



In order to determine the structure of mucrolide unambiguously we carried out an x-ray structural analysis, and this confirmed structure (I) for this compound. The spatial structure of the (I) molecule is shown in Fig. 1 in the projection of the minimum overlapping of the atoms [for illustrating the molecule of (I) we used the PLUTO-78 and MOLDRAW programs*] and the values of the individual torsion angles are also given, in a separate formula.

As can be seen from Fig. 1, in (I) rings A and B are trans-linked and the orientation of the hydroxy group at Cl is β -equatorial and of that at ClO α -axial. Ring A adopts the rarely found sofa conformation with the β departure of the ClO atom from the plane of the other five atoms, Cl, C2, C3, C4, and C5. Ring B has the chair conformation. The lactone ring is close to the (C7)-envelope conformation, but this confirmation is considerably distorted (Fig. 1). Thus, mucrolide is 1β , 6α -dihydroxy- 5α ,7a(H)-eudesmane-3,11(13)-dien-8, 12-olide.

It is interesting to note that the eudesmanolides mucrolide (7,8-lactone) and isosilerolide (6,7-lactone) [10] with the trans linkage of rings A/B and the C3=C4 position of the double bond have the sofa configuration of ring A, regardless of the α - or β orientation of the methyl group at C10. At the same time, the departure of the C10 atom

^{*}The MOLDRAW program [9] was kindly supplied by Prof. D. Viterbo (University of Turin, Italy).

Atom	<u>x</u>	v	z
Atom C1 C2 C3 C4 C5 C6 C7 C8 C9 C10 C11 C12 C13 C14 C15 O1 C2 C3 C7 C8 C9 C10 C11 C12 C1 C1 C1 C1 C2 C3 C4 C5 C5 C7 C7 C7 C7 C7 C7 C7 C7 C7 C7	x 3167 (3) 3043 (3) 1544 (3) 0029 (5) 0229 (2) -1299 (3) -0540 (3) 0525 (5) 2026 (3) 1752 (2) -2012 (3) -0931 (3) -2575 (5) 1654 (4) -1635 (4) 4700 (3) -2442 (3) 0526 (2) -2442 (3)	y 7761 (2) 7347 (2) 7141 (2) 7361 (1) 7764 (2) 8240 (3) 8646 (2) 9450 (2) 9234 (2) 9234 (2) 9234 (2) 9234 (3) 9341 (3) 9464 (3) 7205 (4) 8275 (3) 7387 (5) 9095 (2)	z 2341 (2) 3406 (2) 3735 (2) 3191 (2) 2124 (1) 1636 (2) 0697 (2) 0697 (2) 0697 (2) 0697 (2) 2077 (1) -0065 (2) -0065 (2) -0065 (2) 2896 (3) 3649 (3) 2187 (2) 1513 (2) -0690 (1)
04	-1261(2)	10270 (2)	1534 (2)

TABLE 2. Coordinates ($\times 10^4$) of the Nonhydrogen Atoms in the Structure of (I)

from the plane of the other five atoms in the α - or the β -direction corresponds to the analogous orientation of the methyl group (C14H₃).

Analysis of the geometry of the molecule of (I) in comparison with related eudesmanolides [10, 11] showed that the bond lengths and valence angles agreed well with those found in the related compounds and with the standard values [12]. The errors in the determination of the bond lengths and valence angles did not exceed 0.007 Å and 0.4°, respectively.

The packing of the mucrolide molecules is shown in Fig. 2 in projection on the (100) plane. An analysis of intermolecular contacts has shown that both the hydroxy groups and the carbonyl group are involved in intermolecular H-bonds. Helical chains around 2, screw axes along the z axis are formed through the $01-H\cdots04$ H-bonds ($0\cdots$ distance 2.76 Å, $H\cdots0$ distance 1.90 Å, and $0-H\cdots0$ angle 155°). These helices, in their turn, are linked to one another through $02-H\cdots01$ H-bonds (2.76 and 1.97 Å and 155°) by translation along the x axis. Thus in the crystal of (I) the molecules are joined by H-bonds into layers parallel to (010).

EXPERIMENTAL

IR spectra were taken on a UR-20 spectrometer (KBr), mass spectra on a MKh 1303 instrument, and PMR spectra on Varian XL-200 and Tesla BS-567A instruments. Chemical shifts are given in the δ scale relative to the signal of HMDS, taken as 0. Thin-layer chromatography (TLC) was conducted on Silufol UV-254 plates with visualization by means of sulfuric acid containing 1% of vanillin. System 1 was ethyl acetate-hexane (3:2) and system 2 ethyl acetate-hexane (2:3).

Isolation of Mucrolide. The resin from the mother liquors of tavulin was rechromatographed on KSK silica gel in a ratio of 1:20. Elution was performed with hexane-ethyl acetate (96:4) with a subsequent increase in the concentration of the latter, the volume of the eluates being 400 ml. Fractions 6-8 yielded mucrolide with mp 230-232°C (from ethyl acetate); R_f 0.21 (system 2), R_f 0.53 (system 1); mass spectrum, m/z: 264 (M⁺) (63), 246 (M - H₂O) (15), 231 (M - H₂O - CH₃) (7.5), 228 (M - 2H₂O) (15), 218(8), 213(9.6), 191(10.4), 176(26.9), 175(15.4), 166(34.6), 164(34.6), 150(38.5), 108(92), 107(69), 106(42), 97(100).

<u>Acetylation of Mucrolide</u>. With stirring, 3 ml of acetic anhydride was added to a solution of 65 mg of mucrolide in 2 ml of pyridine. The reaction was continued for 2 h at room temperature. After the usual working up, a product $C_{19}H_{24}O_6$ (from mass spectra), R_f 0.68 (system 2) was isolated).

<u>X-Ray Structural Analysis.</u> Colorless crystals of mucrolide in the form of elongated prisms were grown from solution in ethyl acetate-hexane. The parameters of the elementary cell and the space group were determined on a Syntex-P2₁, four-circle diffractometer using CuK_{α} radiation: α = 8.296(2), b = 12.400(4), c = 13.366(4) Å; Z = 4; d_{calc} = 1.279 g/cm³; space group P2₁2₁2₁.

After preliminary treatment, the set of experimental reflections obtained on the above diffractometer consisted of 1240 structural amplitudes. The structure was interpreted by the direct method according to the SHELXS-86 program* and was refined according to the SHELX-76 program [16] in the full-matrix anisotropic approximation for the nonhydrogen atoms. The positions of the hydrogen atoms were calculated or, in the case of the OH groups, found in an electron-density difference synthesis and were refined in the isotropic approximation. Table 2 gives the coordinates of the nonhydrogen atoms from the last stage of the MLS refinement (R = 0.066 and $R_w = 0.071$).

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