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GERMACRANOLIDE FROM *Tanacetopsis mucronata*

M. B. Izbosarov,[a] B. Kh. Abduazimov,[a] I. M. Yusupova,[b]
B. Tashkhodzhaev,[b] A. D. Vdovin,[b] and N. D. Abdullaev[b]

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A sesquiterpene lactone of the germacrane series, mucrochin, has been isolated from the epigeal part of Tanacetopsis mucronata, *and its structure has been studied by IR and mass spectral methods and PMR spectroscopy. It structure has been determined unambiguously by x-ray structural analysis as 5,6-dihydroxy-5α,6β,7α,8β(H)-germacra-1(10),4(15), 1(13)-trien-8,12-olide. A boat-chair conformation with a configuration of the* $_1D^{14}$ $_{15}D_5$ *type has been established.*

Continuing an investigation [1-5] of the plant *Tanacetopsis mucronata*, we have isolated a sesquiterpene lactone of the germacrane type with mp 181-183°C, composition $C_{15}H_{20}O_4$, which has been called mucrochin. Its IR spectrum contained absorption bands at (cm^{-1}) 3455 (–OH group), 1735 (C=O of a γ-lactone), 1670 (double bond conjugated with a γ-lactone carbonyl), and 1658 (isolated C=C bond). In the mass spectrum we observed the peak of the molecular ion with *m/z* 264 (M^+) and peaks of ions with *m/z* 246 ($M^+ - H_2O$) and 228 ($M^+ - 2H_2O$) due to ejection of one and two molecules of water, and also fragments characteristic for breakdown of germacranolides under electron impact (see Experimental section).

In the PMR spectrum, two doublets, each with a ^3J value of 3 Hz, appeared at 6.63 and 5.56 ppm. On the addition of very small amounts of trifluoroacetic acid they shifted in the downfield direction, and this showed that they belonged to the protons of two secondary hydroxy groups. The PMR spectrum also contained the signals of the protons of a methyl group at a double bond, of exocyclic methylenes, of protons geminal to a hydroxy group, of a proton on a double bond of the main ring, and of a lactone proton (Table 1). These facts make it possible to assume that mucrochin is a germacranolide. The presence of the resonance lines of two protons at 3.55 and 4.4-4.6 ppm showed the secondary nature of the hydroxy groups. Double-resonance experiments confirmed that they were located vicinally.

Signals of the protons of an exomethylene group of a γ-lactone ring appeared in the form of doublets at 6.46 and 5.77 ppm with $^4J_{7,13} = 3.5$, $^4J_{7,13'} = 3.0$ Hz, which showed a *trans*-linkage of the γ-lactone ring of type S [6-9]. One of the protons geminal to a hydroxy group, H-6, gave a signal at 3.55 ppm in the form of a double doublet with broadened spin–spin coupling constants (SSCCs) of 10.5 and 3.0 Hz. The second hydroxy group had to be located at C-5. In the PMR spectrum of mucrochin taken at room temperature the resonance lines of H-5 and H-8 overlapped one another in the 4.4-4.6 ppm interval and the measured SSCC of H-5 with its neighbor, H-6, amounted to 10.5 Hz. In the light of the $^3J_{5,6}$ value of 10.5 Hz, we concluded that the hydroxy groups at C-5 and C-6 had a mutual *trans*-diequatorial orientation.

The *trans*-linkage of the γ-lactone ring and the fact that mucrochin is an 8,12-olide were shown by the nature of the splitting and the value of the SSCC between H-7 and H-8 and the H-9 and H-9' protons (see Table 1).

A chain of protons bound by a mutual spin–spin interaction, H-5, H-6, H-7, H-8, H_9, and H_{13} was established with the aid of double proton resonance. The results of these experiments also enabled us to determine the position of the exomethylene group at C-4, the isolated double bond at C-1–C-10, and the methyl function at C-10.

The H-7 proton has the α-orientation, and the SSCCs between the H-5, H-6, H-7, and H-8 protons showed their mutual *trans*-axial orientations (see Table 1). In the PMR spectrum the exomethylene protons at C-13 appeared with a difference between their chemical shifts, Δδ, of 0.69 (in C_5D_5N) and 0.78 ppm (in $CDCl_3$). This is characteristic for *trans*-linked lactones if in the γ-position to an exomethylene function there is a β-oriented hydroxy group [10-12] or an acyl group at C-6 or C-8 [13-15]. A closeness of the signals of the exomethylene protons at C-13 is observed when α-oriented hydroxy groups are present at C-6 or C-8 [16-19]. In view of the fact that in mucrochin the signals of the exomethylene protons H-13 and H-13' are not close, it is possible to propose for it a structure in which the hydroxy groups at C-5 and C-6 are α- and β-oriented, respectively.

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a) Pharmaceutical Institute, Tashkent-15, pr. Aibeka 45, tel.: 56-37-38; b) Institute of the Chemistry of Plant Substances, Academy of Sciences of the Republic of Uzbekistan, Tashkent, fax (371) 240 64 75. Translated from Khimiya Prirodnykh Soedinenii, No. 4, pp. 492-498, July-August, 1998. Original article submitted July 7, 1997.

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TABLE 1. Characteristics of the PMR Spectra of Mucrochin (100 MHz, δ, ppm, J, Hz)

Protons	Py-d_5	$CDCl_3$
1	5.39 br.t $J_{1,2}=7.4$, $J_{1,2'}=7.4$	5.3-5.5 m
2	1.8-2.4 m	
2′	1.8-2.4 m	
3	1.8-2.4 m	
3′	1.8-2.4 m	
5	4.4-4.6 m	4.21 d, $J_{5,6}=10$
6	3.55 dd $J_{5,6}=10.5$, $J_{6,OH}=3$	3.26 dd, $J_{5,6}=10$, $J_{6,OH}=6$
7	3.21 dt $J_{7,8}=7.5$,	3.09 dt, $J_{7,8}=8$, $J_{7,13}=3.5$, $J_{7,13'}=3.5$
8	4.4-4.6 m	4.34 ddd, $J_{7,8}=8$, $J_{8,9}=5$, $J_{8,9'}=11$
9	2.65 dd $J_{8,9}=5$, $J_{9,9}=11.5$	2.78 dd, $J_{8,9}=5$, $J_{9,9'}=11$
9′	1.8-2.4 m	1.96 t, $J_{8,9'}=11$, $J_{9,9'}=11$
13	6.46 d $J_{7,13}=3.5$	6.38 d, $J_{7,13}=3.5$
13′	5.77 d $J_{7,13'}=3$	5.6 d, $J_{7,13'}=3$
14	1.34 br.s	1.65 br.d
15	5.30 br.s	5.27 br.s
15′	5.11 br.s	5.17 br.s
5-OH	d, $J_{5,OH}=3$	
6-OH	5.56 d, $J_{6,OH}=3$	

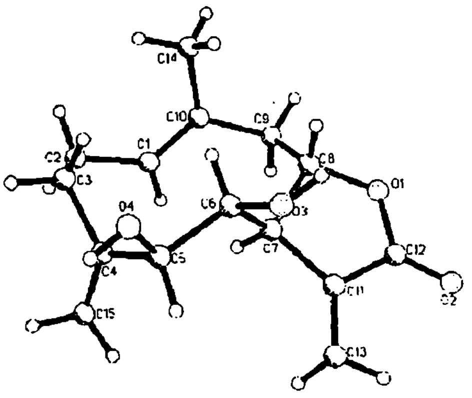
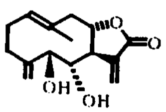


Fig. 1. Spatial structure of mucrochin.

It must be mentioned that deacetyllaurenobiolide, balchanolide, and mucronin, having the chair-chair conformation, and also mucrin, tanachin, and tamirin, having the boat-boat conformation with an α-oriented hydroxy group at C-6, have been isolated from *T. mucronata* previously [20]. For these substances the signals of the H-13 and H-13′ exomethylene protons are close, which is not the case for mucrochin. On the basis of biogenetic considerations and the diequatorial arrangement of the hydroxy groups, mucrochin possibly has the structure



A substance isolated from *Australiabum candidum* [22] has been assigned a similar structure but, as reported, has the state of aggregation of a colorless oil. According to our observations, mucrochin crystallizes very readily: on the ordinary evaporation of a solvent a white microcrystalline powder is formed. Moreover, there are differences in the IR spectra and slight deviations in the parameters of the PMR spectra. Configuration and conformation are not discussed in [22].

To establish the chemical composition and spatial structure of mucrochin unambiguously we carried out an x-ray investigation of a single crystal of it. The results showed that mucrochin has the structure given above, while its stereochemistry is shown in Fig. 1. From an analysis of torsion angles and Fig. 1 it can be seen that the ten-membered ring has a boat-chair conformation with the ${}_1D^{14}$ ${}_{15}D_5$ configuration. It must be mentioned that this is the first time that this type of confor-

TABLE 2. Intracyclic Torsion Angles of Germacranolides

Angles	Mucrochin	6-Epideacetyl-laurenobiolide	Parthenolide	Tanachin	Spiciformin	Eupaformin	Scorpiodine	Epoxyiso-acogermacrane
	[23]	[24]	[24]	[20]	[25]	[20]	[29]	[30]
	${}_1D^{14}$ ${}_{15}D^5$	${}_1D^{14}$ ${}_{15}D^5$	${}_1D^{14}$ ${}^{15}D_5$	${}^1D_{14}$ ${}^{15}D_5$	${}^1D_{14}$ ${}_{15}D^5$	${}_1D^{14}$ ${}_{15}D_5$	${}^1D_{14}$ ${}_{15}D_5$	${}_1D^{14}$ ${}^{15}D^5$
	b/c	b/b	c/c	b/b	c/b	b/c	c/c	c/c
C10C1C2C3	-75.5	-72	-109.7	-57.6	115	-80	98	98
C1C2C3C4	-49.7	-27	52.5	-33.8	-51	-58	-66	-69
C2C3C4C5	123.6	97	-89.5	103.1	92	92	91	87
C3C4C5C6	-62.6	-163	145.8	-160.9	-151	4	3	2
C4C5C6C7	-63.9	75	-121.3	119.2	70	-124	-121	-148
C5C6C7C8	153.1	65	95.8	-67.9	59	135	108	59
C6C7C8C9	-102.2	-112	-91.6	110.8	-134	-80	-96	56
C7C8C9C10	54.2	75	76.4	-73.0	60	61	60	-84
C8C9C10C1	81.0	102	106.8	66.3	55	96	50	101
C9C10C1C2	165.3	165	167	157.6	-163	168	-167	-154

Note. b/b — boat-boat; b/c — boat-chair; c/c — chair-chair; c/b — chair-boat.

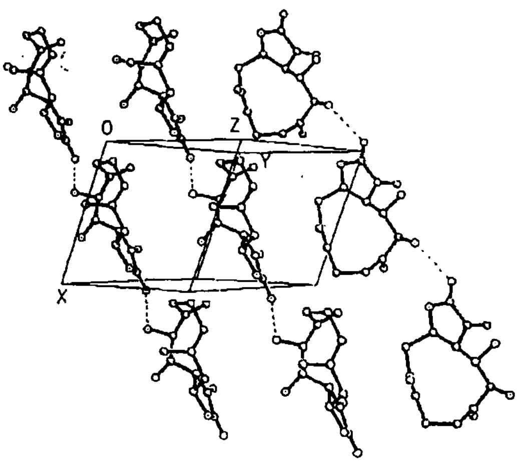


Fig. 2. Molecular packing of mucrochin.

mation has been found among germacranolides without an endocyclic double bond at C-4–C-5 and without a hydroxy or carbonyl group at C-3. The methyl group at C-10 and the hydroxyl at C-5 are β-oriented and the hydroxyl at C-6 is α-oriented. The exomethylenes at C-4 and C-11 are oriented downwards from the mean plane of the rings. The lactone ring has an envelope conformation.

No anomalous deviations are observed in the bond lengths and valence angles. The lengths of the $C_{sp^3}-C_{sp^3}$ bonds vary between 1.51 and 1.56 Å, but within the 3σ limits of error they agree with the generally accepted value of 1.54 Å [21]. The valence angles were determined with an error of 0.5°.

The presence of hydroxy groups favors the formation of $C{=}O\cdots H{-}O$ intermolecular hydrogen bonds between the carbonyl of the lactone ring and the hydrogen atom of the hydroxy group at C-5, as is shown by the O4···O2 distance of 2.81 Å (Fig. 2).

For comparison of the conformers we give the values of the torsion angles of germacranolide rings (Table 2). As can be seen from Table 2, mucrochin has a conformation differing from all four possible conformers of germacranolides. An analysis of the literature on the crystal structures of E,Z-germacranolides shows that the cyclodecadiene ring in the heliangolides can have two conformations — boat-chair and chair-chair, with four configurations: $^1D_{14}$ $^{15}D_5$, $^1D_{14}$ $_{15}D_5$, $_1D^{14}$ $^{15}D^5$, and $_1D^{14}$ $_{15}D_5$. The analysis that we have performed showed that in crystals the heliangolides do actually have two conformations: boat-chair and chair-chair, and these only with the configurations $_1D^{14}$ $_{15}D_5$ and $^1D_{14}$ $^{15}D^5$, respectively. However, no boat-chair conformation has ever been found among the 7,8-lactonized helioangolides and heliangolides with no lactone ring. All 6,7-lactonized heliangolides have the boat-chair conformation. A second chair-chair conformation has been found in 7,8-lactonized heliangolides and in heliangolides without a lactone ring.

We propose for the germacranolide isolated from *T. mucronata* the name mucrochin.

EXPERIMENTAL

IR spectra were taken on a UR-20 spectrophotometer (KBr), mass spectra on a MKh-1303 instrument, and PMR on a Tesla BS-567 A instrument. Chemical shifts are given on the δ scale relative to the signals of HMDS taken as 0. Thin-layer chromatography (TLC) was conducted on Silufol UV-254 plates with a 1% solution of vanillin in concentrated sulfuric acid as the revealing agent. Systems: benzene–alcohol (4:1) and ethyl acetate–hexane (3:2).

Isolation of Mucrochin (1). The resin from the mother solutions of tavulin and tanachin (4.69 g) was rechromatographed on silica gel in a ratio of 1:50 [3]. Elution was performed with hexane (fraction 1), hexane–chloroform (1:1) (fraction 2), and chloroform (fractions 3-70), the fractions having a volume of 100 ml. Fraction 2, on recrystallization, yielded colorless fine acicular crystals with mp 181-183°C (acetone–hexane). R_f 0.41 (ethyl acetate–hexane (3:2)), R_f 0.67 [benzene–alcohol (4:1)]. IR spectrum (KBr, cm^{-1}): 3455 (–OH), 1735 (C=O of a γ-lactone), 1658 (isolated C=C), 1364, 1319, 1281, 1253, 1150, 1113, 1085, 1034, 1014, 958, 917, 887, 816, 713, 664, 618. Mass spectrum, m/z (%): 264(M^+, $C_{15}H_{20}O_4$) (10.20), 246(M^+-H_2O) (12.26), 231(M^+-H_2O, $-CH_3$) (12.26), 228(M^+-2H_2O) (8.8), 202(M^+-

TABLE 3. Coordinates ($\times 10^4$) of the Nonhydrogen Atoms and Temperature Factors (U_{eq}) ($Å^2 \times 10^3$) of the Mucrochin Molecule

Atom	x	y	z	U_{eq}'
O1	1014(9)	-996(11)	9510(9)	54(3)
O2	-722(9)	589(10)	9264(10)	68(3)
O3	3205(8)	-875(11)	14609(8)	57(3)
O4	6593(8)	-710(10)	16679(7)	49(3)
C1	7065(13)	-1735(13)	11287(13)	48(4)
C2	8864(14)	-1870(14)	12765(15)	62(5)
C3	8737(13)	-1503(13)	14529(14)	53(5)
C4	7838(11)	298(13)	14498(11)	42(4)
C5	6233(11)	-219(13)	15005(11)	43(4)
C6	4544(11)	-908(12)	13833(11)	40(4)
C7	3730(11)	-484(13)	11964(10)	38(3)
C8	2721(12)	-1504(12)	10721(12)	42(4)
C9	3760(13)	-2054(13)	9678(13)	50(4)
C10	5629(14)	-2442(12)	10930(13)	49(5)
C11	2272(12)	466(12)	11502(11)	39(4)
C12	689(14)	66(14)	10012(14)	49(5)
C13	2334(14)	1518(13)	12175(13)	54(5)
C14	5663(16)	-3579(13)	11803(15)	63(6)
C15	8555(14)	660(14)	14104(15)	63(5)

H_2O, $-CO_2$) (10.20), 176($M^+ - H_2O$, $-CO_2$, $-C{=}CH_2$) (23.60), 149 (28.40), 148(19.60), 135(24.50), 121(40.41), 119(20.24), 117(16.16), 109(39.10), 108(16.3), 107(40.80), 105(28.30), 97 (61.20), 96(22.40), 95(44.80), 94(18.30), 93(44.40), 91(36.70), 84(14), 82(26.40), 81(67.35), 80(22.42), 79(38.80), 77(23.40), 71(30.61), 69(100), 68(40.80), 67(34.70), 55(40), 54(18.36), 53(34.70).

X-Ray Structural Analysis. The space group and the unit cell parameters were determined on a Syntex $P2_1$ four-circle automatic diffractometer (CuK_α, monochromator, $\theta/2\theta$ scanning, $I > 2\sigma(1)$: $a = 7.451(1)$, $b = 10.520(2)$, $c = 22.545(10)$ Å). Space group $P2_1$, $z = 2$, $d = 1.288$ g/cm^3. In the calculations we used 950 reflections. The structure was interpreted by the direct method employing the SHELXS-86 program [26] and was refined by the SHELXS-76 program [27] in the anisotropic approximation. Hydrogen atoms found theoretically were included in the calculation. The final values were $R = 0.064$ and $R = 0.066$ [sic]. All the calculations were performed on a personal computer of the IBM PC/AT type. The coordinates of the nonhydrogen atoms in the molecule are given in Table 3.

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