SPATIAL STRUCTURE OF THE DIMERIC SESQUITERPENE GERMACRANE LACTONE MIKAGOYANOLIDE

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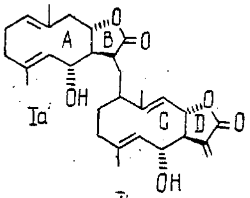
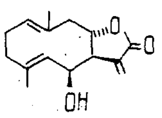
UDC 547.992.547.37+548.737

The dimeric sesquiterpene lactone mikagoyanolide (I) has been isolated from the plant Tanacetopsis mucronata. The spatial structure of (I) has been determined by the x-ray structural method (R = 0.086, 1161 reflections). The dimeric lactone is formed from cyclodecadienes of the germacrolide and melampolide types having chair–chair and boat–chair conformations. The conformational state of the free molecule of (I) has been calculated by the method of molecular mechanics.

Continuing an investigation of the plant Tanacetopsis mucronata, from the total nonpolar material we have isolated a compound (I) with mp 200°C and the composition $C_{30}H_{40}O_6$. The physicochemical constants, and also the mass, PMR, and ^{13}C NMR spectra of (I) and its acetate showed that the compound isolated was the previously known dimeric germacranolide mikagoyanolide [1].



I



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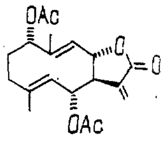

III

In order to determine the conformations of the cyclodecadiene lactone rings and to establish the mutual positions in space of the two linked sesquiterpene monolactones in the molecule of (I), we made an x-ray structural investigation (XSI). The spatial structure of mikagoyanolide in a projection on the plane of the ab axes of the unit cell is shown in Fig. 1. As can be seen from Fig. 1, the results of the XSI confirmed those of NMR spectroscopy [1]: the cyclodecadiene rings are trans-linked with the lactone rings (A/B and C/D) in the (I) molecule, and the hydroxy groups at the C6 and C6' atoms are α-oriented. Mikagoyanolide may be arbitrarily considered as a dimer consisting of the monomers (Ia) (rings A and B) and (Ib) (rings C and D) formed through a covalent C13–C1' bond.

The torsional angles characterizing the conformation of the (I) molecule are given in Table I. The values of the torsional angles of the C2–C1=C10–C9 and C3–C4=C5–C6 double bonds (159 and 163°) show that the cyclodecadiene A belongs to the germacrolide type – one of the four types of isomers with respect to the dienic bond in the germacranolides [2].* The methyl groups at C10 and C4 are β-oriented relative to the mean-square plane of the germacrane ring and have the syn arrangement with respect to one another; consequently, the cyclodecadiene ring A assumes the chair-chair conformation with the $^{14}D_1$, $_5D^{15}$ configuration. A comparison of the values of the torsional angles of ring A with those observed in the germacrolide ivaxillin [3] confirmed what has been said above. In deacetyllaurenobiolide (II),

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*With respect to the geometric isomerism of the C1=C10 and C4=C5 double bonds, the germacranolides are divided into four groups: germacrolides (trans-trans), melampolides (cis-trans), heliangolides (trans-cis), and cis,cis-germacranolides (cis-cis).

Institute of Chemistry of Plant Substances, Uzbekistan Republic of Sciences, Tashkent.
Translated from Khimiya Prirodnykh Soedinenii, No. 2, pp. 213-217, March-April, 1993. Original article submitted June 29, 1992.

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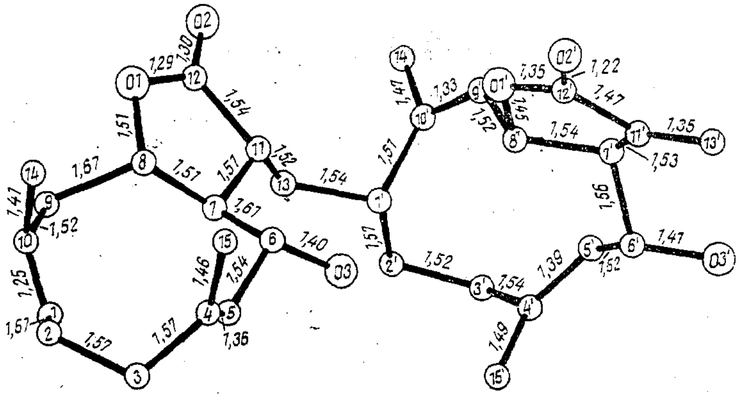

Fig. 1. Spactial structure of mikagoyanolide.

TABLE 1. Torsional Angles ϕ (degrees) in the Structure of (I)*

Angle	φ_1	φ_2	φ_3	Angle	φ_1	φ_2	φ_3
C10C1C2C3	—133	—99	—99	C10'C1'C2'C3'	57	52	54
C1C2C3C4	62	44	47	C1'C2'C3'C4'	42	41	41
C2C3C4C5	—84	-86	—86	C2'C3'C4'C5'	—111	—109	—107
C3C4C5C6	159	171	171	C3'C4'C5'C6'	165	169	169
C4C5C6C7	—124	-128	-133	C4'C5'C6'C7'	—120	—115	—119
C5C6C7C8	73	76	76	C5'C6'C7'C8'	60	46	48
C6C7C8C9	—115	—92	—87	C6'C7'C8'C9'	—102	—89	—88
C7C8C9C10	105	81	80	C7'C8'C9'C10'	120	123	121
C8C9C10C1	—91	—109	—114	C8'C9'C10'C1'	5	2	1
C9C10C1C2	163	168	168	C9'C10'C1'C2'	—119	—119	—120
C7C8O1C12	—10	—20	-24	C7'C8'O1'C12'	—19	—19	—20
C8O1C12C11	—1	6	7	C8'O1'C12'C11'	10	4	4
O1C12C11C7	12	9	13	O1'C12'C11'C7'	3	14	15
C12C11C7C8	—17	—20	—25	C12'C11'C7'C8'	—14	—24	-25
C11C7C8O1	17	24	28	C11'C7'C8'O1'	19	25	26
C13C11C12O2	—40	—45	—36	C13'C11'C12'O2'	1	15	17

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*ϕ_1 are the initial torsional angles obtained by XSI; ϕ_2 are the torsional angles at $\phi = -118°$; and ϕ_3 are the torsional angles at $\phi = 83°$.

structurally identical with moiety (Ia), the chair—chair conformation of the ten-membered ring likewise predominates [4].

In the second moiety of the mikagoyanolide molecule, (Ib), the methyl groups at C15' and C14' have the β- and α-orientations relative to the plane of the germacrane ring and have the anti arrangement with respect to one another. The torsional angles of the C8'—C9'=C10'—C1' and C3'—C4=C5'=C6' double bonds are 5 and 165°, respectively, which permits the cyclohexadiene C to be assigned to the melampolide type (cis-trans), although in the melampolides the double bond usually has the C1=C10 position. Consequently, the germacrane ring C of the (I) molecule has a boat—chair conformation with the $^{14}D_9$, $^5D_{15}$ configuration, as in tulirinol (III) [5].

The trans-linked lactone rings B and D have as their respective conformations an envelope with C_S symmetry and a conformation intermediate between envelope and half-chair (but closer to envelope). The degrees of asymmetry $\Delta C_S(7)$ and $\Delta C_S(8')$ according to [6] are 0.9 and 2.9°. The deviations of the C7 and C8' atoms in rings B and D from the planes of the other atoms amounted to 0.28 and 0.30 Å, respectively.

The mutual positions of the two labile monolactone moieties (Ia) and (Ib) in the (I) molecule determine the torsional angles of the C11—C13 and C1'—C13 bonds. To evaluate the degree of lability of moieties (Ia) and (Ib) we performed calculations by the method of molecular mechanics (MM) [7]. Rotation was brought about by a change in the torsional angle ϕ = C7—C11—C13—C1' by 360° in 10° steps, the starting point being the XSI results. No special conditions were imposed for the change in the conformations of the germacrane and lactone rings. The results of the calculation showed that at two values of ϕ, −118° and 83° (the initial angle according to the XSI result was −107°), the (I) molecule has minimum

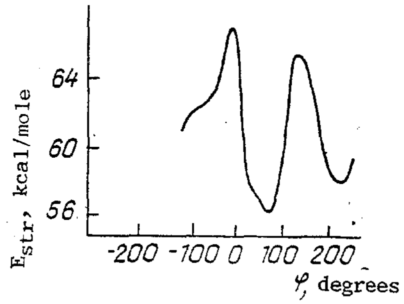

Fig. 2. Dependence of the strain energy E_{str} (kcal/mole) on the angle of rotation φ around the C11—C13 bond of the molecule of (I) (along the axis of abscissas are given the values of the torsional angle φ = C7—C11—C13—C1').

TABLE 2. Valence Angles ω (°) in the Structure of (I)

Angle	ω	Angle	ω	Angle	ω
C10C1C2	95(3)	C14C10C0	102(5)	O3′C6′C5′	112(1))
C3C2C1	101(2)	C12C11C7	101(1)	O3′C6′C7′	112(1)
C4C3C2	110(2)	C13C11C7	117(1)	C8'C7'C6'	110(1)
C5C4C3	115(1)	C13C11C12	110(1)	C11′C7′C6′	113(1)
C15C4C3	117(1)	O1C12C11	112(2)	C11′C7′C8′	102(1)
C15C4C5	122(1)	O2C12C11	118(2)	C9′C8′C7′	113(1)
C6C5C4	124(1)	O2C12O1	130(2)	O1′C8′C7′	106(1)
C7C6C5	107(1)	C1′C13C11	115(1)	O1′C8′C9′	107(1)
O3C6C5	109(1)	C12O1C8	113(2)	C10′C9′C8′	128(1)
O3C6C7	101(1)	C2′C1′C13	109(1)	C9′C10′C1′	124(1)
C8C7C6	106(1)	C10′C1′C13′	110(1)	C14′C10C1′	118(1)
C11C7C6	112(1)	C10′C1′C2′	114(1)	C14′C10′C9′	118(1)
C11C7C8	109(1)	C3′C2′C1′	117(1)	C12′C11′C7′	106(1)
C9C8C7	118(1)	C4′C3′C2′	110(1)	C13′C11′C7′	129(1)
O1C8C7	102(1)	C5′C4′C3′	116(1)	C13′C11′C12′	125(1)
O1C8C9	99(2)	C15′C4′C3′	115(1)	O1′C12′C11′	112(1)
C10C9C8	106(2)	C15′C4′C5′	128(1)	O2′C12′C11′	125(1)
C9C10C1	105(3)	C6′C5′C4′	124(1)	O2′C12′O1′	123(1)
C14C10C1	139(5)	C7′C6′C5′	101(1)	C12′O1′C8′	109(1)

strain energies of 57.8 and 55.7 kcal/mole, respectively. The transition barrier amounts to 8-10 kcal/mole (Fig. 2). With such a height of the barrier both conformers of mikagoyanolide should be observed in solution [8], since the real barrier must be considerably lower than the calculated one because of the complexity and diversity of the conformational transitions.

In the optimization of the geometry of the (I) molecule by the MM method, a change was noted in the endocyclic torsional angles of the germacrane ring A in moiety (Ia) as compared with the initial XSI figures (up to 33°), although no such changes are observed in moiety (Ib) (Table 1). Thus, on the whole, the germacrane rings retained their chair—chair (ring A), and boat—chair (ring C) conformations, regardless of their positions in either of the two calculated conformational minima. However, after optimization, the lactone ring B in the (I) molecule slightly changed its conformation from envelope in the direction of half-chair, as was clearly indicated by the value of the degree of asymmetry: $\Delta C_s(7)$ = 0.9°, $\Delta C_2(O1)$ = 7.7° before, and $\Delta C_s(6)$ = 8.3°, $\Delta C_2(12)$ = 2.1° after optimization. No conformational changes were observed in D after optimization ($\Delta C_s(7)$ = 3.6°, $\Delta C_2(12)$ = 7.9°).

The bond lengths are shown in Fig. 1, and the valence angles are given in Table 2. The observed anomalies in moiety (Ia), particularly in the C1—C2 and C8—C9 bonds (1.67 Å) and in the angles C9C10C14 (102°) and C1C10C14 (139°), are due to the large thermal vibrations of the C1, C10, and C14 atoms and the limited nature of the experimental results because of the low quality of the single crystal studied. No anomalies in the valence angles and bond lengths were observed in the other parts of the molecule, and they agree with the standard values [9]. The mean-square errors of determination for the bond lengths do not exceed 0.03 Å.

In the (I) crystal, the O3 hydroxy group of the initial molecule forms an intermolecular H-bond with the O2' carbonyl group of the molecule transformed by a 2nd-order axis of

TABLE 3. Coordinates ($\times 10^4$) of the Basis Atoms of the Structure of (I)

Atom	x	y	z	Atom	x	y	z
C1	5488(23)	6556(27)	−1501(11)	C1′	6095(7)	1680(10)	−750(3)
C2	4522(24)	7340(13)	−1457(7)	C2′	6373(10)	901(11)	−1105(4)
C3	3625(19)	6600(13)	−1500(7)	C3′	5827(12)	−94(11)	−1078(4)
C4	3643(12)	5819(11)	−1128(6)	C4′	4712(12)	63(11)	−1005(4)
C5	4241(13)	5010(11)	−1191(5)	C5′	4375(11)	−272(8)	−661(4)
C6	4645(10)	4341(8)	−831(4)	C6′	3379(9)	21(9)	−420(4)
C7	5838(10)	4334(9)	−873(3)	C7′	3711(8)	531(8)	3(3)
C8	6179(15)	5350(12)	−724(6)	C8′	4377(8)	1437(8)	−92(3)
C9	6720(17)	6104(13)	−1079(11)	C9′	5481(9)	1237(8)	−21(3)
C10	5952(35)	6895(20)	−1187(20)	C10′	6219(9)	1302(8)	−301(3)
C11	6309(9)	3551(10)	−595(4)	C11′	2844(9)	999(9)	245(3)
C12	7162(13)	4147(22)	−389(6)	C12′	3169(13)	2 05(10)	362(4)
C13	6721(8)	2630(10)	−813(4)	C13′	1975(10)	576(12)	365(4)
C14	5742(43)	7321(48)	−787(23)	C14′	7224(10)	1019(11)	−159(4)
C15	3392(12)	6175(13)	−702(7)	C15′	4159(11)	670(12)	−1323(4)
O1	7078(16)	5091(14)	−457(6)	O1′	4086(6)	2211(5)	207(2)
O2	7869(9)	36 5(12)	−200(3)	O2′	2700(6)	2585(6)	585(2)
O3	4423( )	3347(7)	−921(3)	O3′	2758(7)	−807(7)	−346(3)

symmetry passing through the origin of coordinates (the O3···O2' distance is 2.82 Å), creating a chain of molecules. The translation of the chain along the a and b axes forms a two-dimensional lattice in the ab plane thanks to the H-bonds between the O3' hydroxyl and the O2 carbonyl (the O3'···O2 distance is 2.75 Å).

EXPERIMENTAL

Isolation of Mikagoyanolide. The total nonpolar material from Tanacetopsis mucronata (after rough chromatography) (27 g) was chromatographed on silica gel in a ratio of 1:17. The hexane—ethyl acetate (9:1) system was used for elution, with a subsequent increase in the concentration of ethyl acetate to (3:2), and fractions 147-151 were collected, which contained dimer (I) with a molecular mass of 496 (determined mass spectrometrically) and mp 200°C, R_f 0.56 (hexane—ethyl acetate (1:1) system).

X-Ray Structural Investigation. Colorless crystals of (I) in rhombic form were grown from hexane—ethyl acetate (1:1) solution. The space group, the parameters of the unit cell, and the intensities of the reflections were measured on a Syntex-$P2_1$, automatic four-circle diffractometer at room temperature using CuK_α radiation: crystals tetragonal, a = b = 13.411(3), c = 31.567(9) Å, d_{calc} = 1.17 g/cm³; space group $P4_12_12$, Z = 8.

The complete set of experimental reflections (2332) with $\theta < 58°$ was obtained on the above-mentioned diffractometer ($\theta < 2\theta$ scanning). In the primary treatment of the results, weak reflections with $I \le 2\sigma(I)$ were excluded. In the calculations we used 116 reflections with $|F| > 4\sigma(|F|)$. The structure was determined by the direct method using the SHELXS-86 program [10] and was refined in the full-matrix isotropic-anisotropic approximation by the SHELX-76 program [11] (both programs in the PC MSDOS version). The H atoms were located geometrically and were refined isotropically. The final value of the divergence factor was R = 0.086 (R_w = 0.082). The coordinates of the non-hydrogen atoms are given in Table 3.

The conformational calculations were performed on an IBM PC AT personal computer using the PC MODEL and MMX-86 programs [12] with complete optimization of the geometry of the molecule using the programs of potential parameters given in this version.

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DEVELOPMENT OF A METHOD FOR THE QUANTITATIVE DETERMINATION OF NEFROTSIZIN (LUTEOLIN 7-O-β-D-GLUCOPYRANOSIDE) IN THE LEAVES OF *Ferula varia*

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UDC 615.322(045):547.972.3:543.42

A method is proposed for determining nefrotsizin (luteolin 7-O-β-D-glucopyranoside) in the radical leaves of Ferula varia. which is based on the extraction of nefrotsizin from the raw material, its identification by TLC, elution from the plate, and spectrophotometric determination.

Luteolin 7-O-β-D-glucopyranoside has been isolated in the Institute of the Chemistry of Plant Substances of the Uzbekistan Republic Academy of Sciences from radical leaves of Ferula varia (Schrenk.) Trautv, family Apiceae, and at the present time, under the name nefrotsizin (I), is undergoing clinical trials as a hyperazotemic agent [2].

Nefrotsizin is an odorless microcrystalline powder, yellow or grayish yellow with a greenish tinge. It is insoluble in water, very slightly and slowly soluble in alcohols, and most soluble in aqueous alcohols (60 and 80% ethanol, 80% methanol). The UV spectrum of (I) in 80% ethanol in the 250-400 nm region has absorption maxima at 256 and 253 nm and a shoulder at 268 nm.

The procedure that has been developed for the quantitative determination of (I) in the radical leaves of F. varia is based on spectrometry in combination with TLC. During the development of the method the following stages were studied: the extraction of (I) from the plant raw material; 2) its chromatographic separation from accompanying substances; and 3) the elution of (I) from the sorbent and its spectrophotometric determination.

For this study, as the standard substance we used (I) corresponding to the requirements of the draft Provisional Pharmaceutical Standard for this preparation.

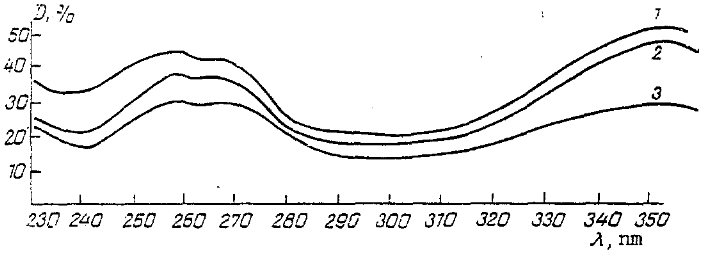

Fig. 1. UV spectrum of (I) in 80% ethanol: 1) solution of standard (I); 2) eluate of the zone of the standard sample of (I) from a plate; 3) eluate of the zone from a plant extract.

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Translated from Khimiya Prirodnykh Soedinenii, No. 2, pp. 217-220, March-April, 1993. Original article submitted June 29, 1992.