

THREE NEW FURANONE-TYPE HELIANGOLIDES FROM *CALEA TERNIFOLIA* AND THE MOLECULAR STRUCTURE OF 8 β -ANGELOYLOXY-9 α -HYDROXYCALYCVLATOLIDE

NIKOLAUS H. FISCHER*, IHL-YOUNG LEE, FRANK R. FRONCZEK,

Department of Chemistry, Louisiana State University, Baton Rouge, LA 70803

GIACOMO CHIARI,

Istituto di Mineralogia, Cristallografia e Geochimica dell' Università,
Via San Massimo 22, 10123 Torino, Italy

and LOWELL E. URBATSCH

Department of Botany, Louisiana State University, Baton Rouge, LA 70803

ABSTRACT.—Three populations of *Calea ternifolia* var. *calyculata* from Mexico were chemically analyzed for their terpenoid constituents. Three new furanone-type heliangolides were isolated: 8 β -angeloyloxy-9 α -hydroxycalyculatolide (**2**), 8 β -methylacryloyloxy-9 α -hydroxycalyculatolide (**3**), and 15-hydroxy-11,13-dihydro-11 α ,13-epoxyatripliciolide-8 β -O-angelate (**6**). Their structures were determined by nmr and mass spectral methods, and the molecular structure of compound **2** was established by single-crystal X-ray diffraction.

In continuation of our biochemical systematic study of the genus *Calea* of the subtribe Galinsogineae (Asteraceae; Heliantheae) (1, 2), we have analyzed three different populations of *Calea ternifolia* Kunth var. *calyculata* (Robinson) Wussow, Urbatsch, & Sullivan (syn. *Calea albida*) (3). We wish to report the structures of three new furanone-germacranolides, which were characterized by nmr and mass spectral correlations with known compounds, and the molecular structure of **2** was determined by single crystal X-ray diffraction.

RESULTS AND DISCUSSION

CHEMICAL AND SPECTROSCOPIC CHARACTERIZATIONS.—8 β -Angeloyloxy-9 α -hydroxycalyculatolide¹ (**2**): A collection of *C. ternifolia* var. *calyculata* from San Luis

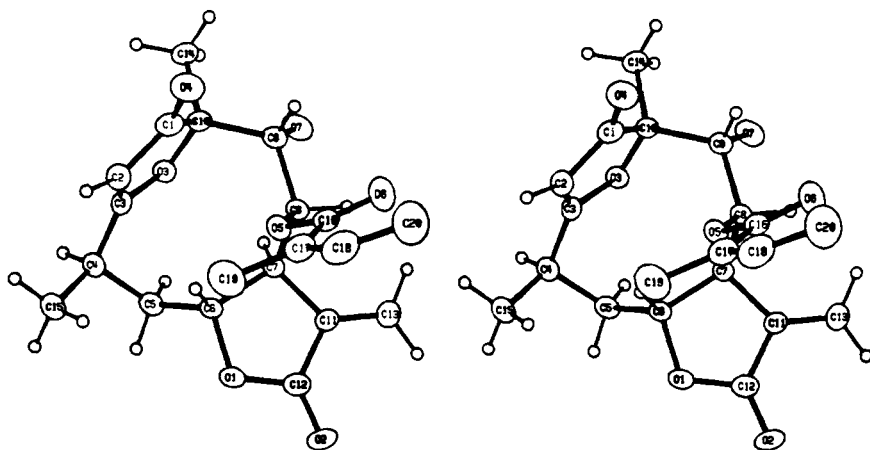


FIGURE 1. Stereoscopic representation of the 8 β -angeloyloxy-9 α -hydroxycalyculatolide molecule.

¹The name calyculatolide is reserved for the not yet known unsubstituted furanone-germacranolide skeleton.

Potosi, Mexico, provided two new compounds which we named 8 β -angeloyloxy-9 α -hydroxycalyculatolide and 8 β -methylacryloyloxy-9 α -hydroxycalyculatolide. The calyculatolide (**2**, C₂₀H₂₄O₇, mp 175° (dec.) exhibited pmr spectral absorptions that were very similar to 9 α -acetoxyzexbrevin (**1**), a compound of known X-ray structure (4) which had previously been isolated from *C. ternifolia* var. *calyculata* from the state of Nuevo Leon, Mexico (5). The spectral parameters of the two compounds differed in the chemical shifts of the H-9 absorptions, which in **1** appeared at 5.38 ppm, together with an acetate methyl singlet at 2.20 ppm. In the calyculatolide (**2**), the acetate signal was missing and H-9 represented a doublet of a doublet at 4.19 ppm ($J=7.5$; 6 Hz), suggesting the presence of a hydroxyl group at C-9; this was also supported by an ir OH-absorption at 3420 cm⁻¹ as well as a mass spectral peak at m/z 358 (M-H₂O). The presence of an angelate ester side-chain at C-8 was indicated by diagnostic pmr and ms signals. The new compound exhibited methyl absorptions, doublets of quartets at 1.92 (C-2'-methyl) and 1.82 ppm (C-3'-methyl), respectively, and a one-proton quartet of a quartet at 6.15 ppm, which are typical for the angelate moiety. Furthermore, ms peaks at m/z 276 (M-B), 83 (B'), and 55 (B'') were in accord with the above assignments.

The great similarity of the nmr and cd parameters of 9 α -acetoxyzexbrevin and the calyculatolide (**2**) suggests the same skeletal arrangement, including all stereochemical centers of the two compounds. Therefore, 8 β -angeloyloxy-9 α -hydroxycalyculatolide must have structure **2**. This was confirmed by single-crystal X-ray diffraction of **2**, which will be described later.

8 β -Methylacryloyloxy-9 α -hydroxycalyculatolide (3): A minor gummy constituent, C₁₉H₂₄O₇, which could not be freed from **2**, exhibited pmr signals very similar to those of the calyculatolide (**2**). The two compounds had nearly identical pmr parameters for the medium-ring protons, but they differed in the ester side-chain at C-8. Compound **3** showed pmr and ms signals typical for the methacrylate moiety (A'). A broadened three-proton singlet at 1.89 ppm and a pair of broad one-proton signals at 5.64 and 5.98 ppm, together with diagnostic ms peaks at m/z 69 (A') and 41 (A''), were in accord with a methacrylate group at C-8. Therefore, by spectral correlation with **2** the stereochemical representation **3** is suggested for the new lactone.

15-Hydroxy-11,13-dihydro-11 α ,13-epoxyatripliciolide-8 β -O-angelate (6): Plant material from a population of *C. ternifolia* var. *calyculata* from near Guadalajara, Jalisco, Mexico (Wussow-Landry No. 336), provided a new crystalline compound (**6**), C₂₀H₂₂O₈, mp 118-121°. The pmr spectrum exhibited absorptions that were similar to those of a compound (**9**) of known X-ray structure (5) previously isolated from another population of this taxon. Detailed spin decoupling experiments of the new substance indicated the lack of a hydroxyl function at C-9 (Table 1). Instead, an OH-group was present at C-15. The attachment of an angelate ester group at C-8 was supported by pmr chemical shifts (Table 1) and diagnostic signals at m/z 83 (B') and 55 (B''). Based on the pmr, ms, and cd-spectral correlations of the new compound with the parameters of **9** (5), we assign it the stereostructure **6**. Slightly more polar fractions contained a gummy mixture of compound **6** and a minor analog which, on the basis of pmr and ms data, had to be represented by structure **7**. Since this minor component could not be enriched due to lack of material, its physical data are not recorded.

Less polar fractions of the above plant collection provided a crystalline compound (**4**), C₂₀H₂₂O₇, mp 88-91°. Pmr and ms correlations of **4** with the parameters of compound **9**, a lactone from another population of *C. ternifolia* var. *calyculata* (5) with established X-ray structure (5), permitted complete structural assignment of **4**. The pmr data suggested that compound **4** is identical with a lactone previously described as a constituent of *C. pilosa* from Brazil (6). Although the compound reported in reference (6) was a gum and compound **4** is crystalline (mp 88-91°), their physical parameters

TABLE 1. Pmr Parameters^a of Compounds **2**, **3**, and **6**

Atom	Compound		
	2	3	6
H-2	5.56 s	5.56 s	5.69 s
H-4	3.03 brp (7)	3.03 brp (7)	—
H-5a	2.62 m	2.62 m	6.30 m
H-5b	2.10 br d (14)	2.10 brd (14)	—
H-6	4.52 dd (9.6; 4.8)	4.45 dd (9.6; 4.8)	5.33 m
H-7	3.79 m	3.81 m	3.35 m
H-8	5.12 brd (4.8)	5.07 brd (4.8)	5.14 m
H-9a	4.19 d (4.8)	4.18 d (4.8)	2.43 dd (18.5; 7.5)
H-9b	—	—	2.20 dd (18.5; 5.0)
C ₄ -CH ₃	1.39 d (7.0)	1.39 d (7.0)	4.43 brs (-CH ₂ O-)
C ₁₀ -CH ₃	1.5 s	1.64 s	1.47 s
H-13a	6.35 d (3)	6.35 d (3)	3.36 s (6.0)
H-13b	5.78 d (3)	5.78 d (3)	3.30 d (6.0)
C-2'-CH ₃	1.82 sq (1.5)	—	1.83 brq (2)
C-3'-CH ₃	1.92 dq (7.5; 1.5)	1.89 brs	1.95 dq (8; 2)
H-3'a	6.15 qq (7.5; 1.5)	5.98 brs	6.17 qq (8; 2)
H-3'b	—	5.64 brs	—

^aSpectra were run at ambient temperatures in CDCl₃ at 200 MHz. TMS was used as internal standard. Values are in PPM (δ) and signals are designated as follows: singlets, s; doublet, d; triplet, t; quartet, q; pentet, p; heptet, h; multiplet, m; broad singlet, brs. Figures in parentheses are coupling constants or line separations in Hertz.

strongly support structural identity. The cmr assignments of lactone **4** are summarized in Table 2.

A third collection of *C. ternifolia* var. *calyculata* from Jalisco, Mexico (Wussow-Landry No. 338), contained the chromene **10** that was identified by comparison of the pmr, ir, and uv spectra with data described in the literature (7). From more polar fractions, compounds **4**, **5**, **6**, and **8** were isolated. The pmr parameters for the crystalline compounds **5** and **8** were in good agreement with the data reported in the literature (6) as gummy constituents. More detailed deliberations with regard to the stereochemistry at C-11 of the 11,13-epoxylactones described in reference (6) were presented in an earlier paper (5).

STRUCTURE DESCRIPTION OF **2**.—Attempted repurification by preparative tlc of a crystalline sample of lactone **2** led to irreversible absorption of the compound. Therefore, a single crystal from a chromatographic fraction containing a mixture of **2** and **3** had to be used for the X-ray diffraction studies.

The conformation of **2** is given by the torsion angles in Table 3, in which it is compared to the conformation of the previously described 9 α -acetoxyzexbrevin (**1**). The

TABLE 3. Torsion angles of Compounds **1** and **2**

Atoms	Angle (2)	Angle (1)	Atoms	Angle (2)	Angle (1)
C1-C2-C3-O3 . . .	-5.8	-6.3	C6-C7-C8-C9 . . .	-96.7	-91.6
C2-C3-O3-C10 . . .	5.3	6.5	C7-C8-C9-C10 . . .	65.5	70.1
C3-O3-C10-C1 . . .	-2.5	-3.7	C8-C9-C10-C1 . . .	65.3	61.4
O3-C10-C1-C2 . . .	-0.7	0.3	C9-C10-C1-C2 . . .	-118.7	-115.4
C10-C1-C2-C3 . . .	3.8	3.4	O1-C6-C7-C11 . . .	-2.4	-20.3
C1-C2-C3-C4 . . .	165.6	165.6	C6-C7-C11-C12 . . .	1.4	21.6
C2-C3-C4-C5 . . .	-123.6	-125.3	C7-C11-C12-O1 . . .	0.2	-15.2
C3-C4-C5-C6 . . .	44.3	50.5	C11-C12-O1-C6 . . .	-1.9	1.1
C4-C5-C6-C7 . . .	-109.9	-109.0	C12-O1-C6-C7 . . .	2.8	12.8
C5-C6-C7-C8 . . .	116.6	101.7	O2-C12-C11-C13 . . .	1.5	-18.3

two are seen to be virtually identical with the exception of the lactone ring. The rms difference in the first 14 torsion angles in Table 3, describing the germacrane and furanone rings, is 4.7°. However, while the lactone ring of **2** is nearly planar, with the sum of five endocyclic torsion angle magnitudes ($\sum|\omega|$) 8.7°, the corresponding ring of **1** is distinctly nonplanar, having $\sum|\omega|=71.0^\circ$, and C-7 at the flap of an envelope conformation. As the substituents at C-8 are so similar, the difference in lactone conformation is most likely a result of the difference in substitution at C-9. In **2**, the hydroxyl substituent at C-9 is involved in an intermolecular hydrogen bond [O . . . O 2.996(5)Å] with the lactone carbonyl oxygen atom O2, while in **1**, acetylation of the hydroxyl group precludes such interaction. Apparently, the shift of lactone conformation from envelope to planar in **2** allows more favorable geometry for this intermolecular interaction.

EXPERIMENTAL

PLANT MATERIAL.—Dried aerial parts of *C. ternifolia* var. *calyculata* were extracted with CH₂Cl₂, and worked up according to a standard procedure (8) to give the crude terpenoid syrup which was then chromatographed over silica gel taking 50 ml fractions. Petroleum ether was used as eluant followed by petroleum ether-EtOAc mixtures (90:10, 80:20, 70:30, etc.). The crude extracts of three different populations of *C. ternifolia* var. *calyculata* were analyzed.

A. Collection J. Wusslow and G. Landry No. 320: collected on October 16, 1980, in Mexico: San Luis Potosi, along small paved road to Canoas; 2.2 miles North of junction with Hwy. 70. 200 g of dried plants gave 2.77 g of crude syrup. Chromatography provided 10 mg **2** and 8 mg of a mixture of **2** and **3**.

B. Collection J. Wussow and G. Landry No. 336: collected on October 21, 1980, in Mexico: Jalisco, in oak-grass savanna region, ca. 4 miles north of north edge of Guadalajara on Hwy. 50 to Zacatecas. Dried aerial parts (400 g) gave 4.1 g of the crude syrup. Chromatography afforded 145 mg **4** from fractions 8-9 and 20 mg **6** from fractions 19-20.

C. Collection J. Wussow and G. Landry No. 338: collected on October 21, 1980, in Mexico: Jalisco, along Hwy. 15 to Tepic, 11.9 miles west of west junction with bypass around Guadalajara and Hwy. 15 to Tepic. The crude syrup obtained from 136 g of dried aerial parts consisted of 10 mg **10** from fractions 1-3, 90 mg **4** from fractions 13-14, 15 mg **5** from fraction 16, 15 mg **8** from fractions 21-22, and 10 mg **6** from fractions 24 and 25. Voucher specimens are deposited at Louisiana State University, Baton Rouge, LA.

CHEMICAL DATA.—8β-Angeloyloxy-9α-hydroxycalyculatolide (**2**): C₂₀H₂₄O₇, mp 175° (dec.), cd (c, 5.1 × 10⁻⁴, MeOH), [θ]₂₂₁ -1.1 × 10⁴, [θ]₂₆₅ +3.8 × 10³, [θ]₃₁₀ +1.5 × 10³; ir ν max (CHCl₃) 3420 (OH), 1770 (γ-lactone), 1720 (ester), 1690 (ketone), 1596 (double bond); ms m/z (rel. int.) 376 (5.3, M⁺), 358 (0.4, M-H₂O), 276 (0.7, M-B), 83 (100, B'), 55 (11.5, B'').

Anal. calcd. for C₂₀H₂₄O₇: 376.1521. Found (ms): 376.1490.

11,13-Dihydro-11α,13-epoxyatripliciolide-8 β-O-angelate (**4**) (**6**): C₂₀H₂₂O₇, mp 88-91°; uv, λ max (EtOH) 262 nm (ε 4.35 × 10³); cd (c, 5.15 × 10⁻⁴, MeOH), [θ]₂₂₈ +1.0 × 10⁴, [θ]₂₉₂ -2.9 × 10³, [θ]₃₂₅ +1.4 × 10³; ir ν max (CHCl₃) 1792 (γ-lactone), 1716 (ester), 1708 (ketone), 1650, 1593 (double bonds); ms (70 eV) m/z (rel. int.) 374 (17.7, M⁺), 358 (0.5, M-16), 274 (0.9, M-B), 83 (100, B'), 55 (33.5, B'').

Anal. calcd for C₂₀H₂₂O₇: 374.1365. Found (ms): 374.1365.

11,13-Dihydro-11 α ,13-epoxyatripliciolide-8 β -O-methacrylate (**5**) (6): C₁₉H₂₀O₇, mp 155-158°; uv λ max (EtOH) 262 nm (ϵ 4.1 \times 10³); cd (c, 6.28 \times 10⁻⁴, MeOH), [θ]₂₃₅ +7.4 \times 10³, [θ]₂₉₅ -2.1 \times 10³, [θ]₃₂₅ +1.1 \times 10³; ir ν max (CHCl₃) 1793 (γ -lactone), 1718 (unsaturated ester), 1708 (ketone), 1591 (enolic double bond); ms (70 eV) m/z (rel. int.) 360 (24.5, M⁺), 274 (1.4, M-A), 69 (100, A'), 41 (21.7, A'').

Anal. calcd for C₁₉H₂₀O₇: 360.1208. Found (ms): 360.1232.

15-Hydroxy-11,13-dihydro-11 α ,13-epoxyatripliciolide-8 β -O-angelate (**6**): C₂₀H₂₂O₈, mp 118-121°; uv λ max (EtOH), 262 nm (ϵ , 7200); cd (c, 5.8 \times 10⁻⁴, MeOH), [θ]₂₂₇ +1.3 \times 10⁴, [θ]₂₆₄ +2.8 \times 10³, [θ]₂₉₃ -3.2 \times 10³, [θ]₃₂₉ +1.8 \times 10³; ir ν max (CHCl₃) 3440 (OH), 1762 (γ -lactone), 1723 (ester), 1704 (ketone), 1597 (double bond); ms m/z (rel. int.) 390 (11.3, M⁺), 372 (0.9, M-H₂O), 290 (0.7, M-B), 138 (29.4), 83 (100, B'), 55 (31.0, B'').

Anal. calcd for C₂₀H₂₂O₈: 390.1313. Found (ms): 390.1300.

9 α -Hydroxy-11 α ,13-epoxyatripliciolide-8 β -O-angelate (**8**) (6): C₂₀H₂₂O₈, mp 205-207°; cd (c, 4.1 \times 10⁻⁴, MeOH), [θ]₂₂₉ +2.6 \times 10⁴, [θ]₂₆₀ -951, [θ]₂₈₂ +3.56 \times 10³, [θ]₃₁₅ +6.7 \times 10³; ir ν max (CHCl₃) 3420 (OH), 1793 (γ -lactone), 1712 (ester), 1697 (unsaturated ketone), 1650, 1595 (double bonds); ms m/z (rel. int.) 390 (6.7, M⁺), 290 (1.6, M-B), 83 (100, B').

Anal. calcd for C₂₀H₂₂O₈: 390.1313. Found (ms): 390.1302.

X-RAY DATA.—A colorless crystal of dimensions 0.32 \times 0.40 \times 0.40 mm, grown by evaporation from EtOAc solution, was used for data collection on an Enraf-Nonius CAD4 diffractometer equipped with CuK α radiation (λ =1.54184 \AA) and a graphite monochromator. Crystal data are: C₂₀H₂₄O₇, MW=376.4 (for major product), orthorhombic space group P2₁2₁2₁, a =14.466(2), b =14.491(3), c =8.883(1) \AA , Z=4, d_c =1.323 g cm⁻³, μ (CuK α)=7.98 cm⁻¹. Intensity data were measured by ω -2 θ scans of variable speed design to yield I \approx 50 σ (I) for all significant reflections. One octant of data having 2° \leq θ \leq 70° was measured. Data reduction included corrections for background, Lorentz, and polarization

TABLE 4. Coordinates for Nonhydrogen Atoms of Compound **2**

Atom	x	y	z
O1	0.1295(2) ^a	0.4473(2)	0.7988(3)
O2	0.1302(3)	0.5862(2)	0.9018(3)
O3	0.1463(2)	0.3109(2)	0.3572(3)
O4	-0.0861(3)	0.2903(2)	0.2394(4)
O5	-0.0314(2)	0.4507(2)	0.4900(3)
O6	-0.1192(2)	0.5642(2)	0.4015(4)
O7	0.1436(2)	0.4886(2)	0.1983(4)
C1	-0.0121(4)	0.2854(3)	0.3013(5)
C2	0.0185(4)	0.2317(3)	0.4303(5)
C3	0.1068(4)	0.2532(3)	0.4603(5)
C4	0.1660(4)	0.2357(3)	0.5923(5)
C5	0.2022(3)	0.3305(3)	0.6477(5)
C6	0.1260(3)	0.4030(3)	0.6511(4)
C7	0.1305(3)	0.4822(2)	0.5328(4)
C8	0.0503(3)	0.4874(3)	0.4178(4)
C9	0.0638(3)	0.4432(3)	0.2609(4)
C10	0.0751(3)	0.3380(3)	0.2519(4)
C11	0.1322(3)	0.5674(3)	0.6275(4)
C12	0.1312(3)	0.5392(3)	0.7883(5)
C13	0.1339(4)	0.6536(3)	0.5878(6)
C14	0.1032(4)	0.3071(3)	0.0940(5)
C15	0.1145(5)	0.1820(3)	0.7149(6)
C16	-0.1119(3)	0.4969(3)	0.4781(4)
C17	-0.1854(3)	0.4536(3)	0.5687(5)
C18	-0.2719(4)	0.4902(5)	0.5758(6)
C19	-0.1615(4)	0.3705(4)	0.6531(7)
C20 ^b	-0.3093(6)	0.5645(6)	0.5125(12)

^aEstimated standard deviations in the least significant digits are shown in parentheses.

^bPopulation=0.60.

effects; Ψ scans of reflections near $\chi=90^\circ$ indicated that absorption effects were insignificant. A total of 2013 unique data were obtained, of which 1763 had $I>3\sigma(I)$ and were used in the refinement.

The structure was solved by use of the program RANT after the usual direct methods procedures failed. Structure completion and initial refinement were carried out using the SHELXTL system, and final refinement was done using the Enraf-Nonius SDP. All nonhydrogen atoms were treated anisotropically. Hydrogen atoms, except for those on C-18 and C-20 of the angelate substituent, were located from difference maps and included as fixed contributions to the structure factors. Refinement was carried out by full matrix least squares with unit weights.

Refinement of the ester substituent at C-8 as angelate led to problems with atom C-20. The C-18 to C-20 distance refined to quite short values ($\sim 1.3\text{\AA}$), and the thermal parameter of C-20 became unreasonably large. A disorder involving partial occupation of angelate and methacrylate had been previously suspected based on nmr data, and it became clear that the observed electron density could be accounted for reasonably well by a model involving superposition of *S-cis* angelate and *S-trans* methacrylate in an approximately 3:2 ratio. After data collection, the crystal was dissolved, and its pmr spectrum recorded. Integration of characteristic resonances of methacrylate and angelate indicated the appropriateness of a 3:2 ratio. The occupancy factor for C-20 was set at 0.60, and refinement was continued. Convergence was achieved with $R=0.049$, with maximum residual density 0.21 e\AA^{-3} .

ACKNOWLEDGMENTS

We wish to thank Dr. James Wussow and Mr. Gary Landry for plant collections and Helga D. Fischer for technical assistance. This work was supported in part by a grant from the National Science Foundation (Grant DEB 78-04265) awarded to L.E.U.

LITERATURE CITED

1. T.F. Stuessy, in: "The Biology and Chemistry of the Compositae," vol. 2. Ed. by V.H. Heywood, J.B. Harborne, and B.L. Turner, London: Academic Press, 1977, p. 621.
2. J.R. Wussow and L.E. Urbatsch, *Brittonia*, **30**, 477, 1978.
3. J.R. Wussow, "A Systematic Study of the Mexican, Central and South American Species of *Calea*." Dissertation, Louisiana State University, Baton Rouge Campus, 1981.
4. F.R. Fronczek, I.Y. Lee, and N.H. Fischer, *J. Nat. Prod.*, **46**, 104, 1983.
5. I.Y. Lee, F.R. Fronczek, A. Malcolm, N.H. Fischer, and L.E. Urbatsch, *J. Nat. Prod.*, **45**, 311, 1982.
6. F. Bohlmann, U. Fritz, R.M. King, and H. Robinson, *Phytochemistry*, **20**, 743, 1981.
7. L. Quijano, J.S. Calderon, and T. Rios, *Rev. Latinoamer. Quim.*, **8**, 90, 1977.
8. N.H. Fischer, R.A. Wiley, H.N. Lin, K. Karimian, and S.M. Politz, *Phytochemistry*, **14**, 2241, 1975.

Received 26 October 1982