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A NEW MACROCYCLIC DITERPENE ESTER FROM THE LATEX OF *EUPHORBIA TIRUCALLI*

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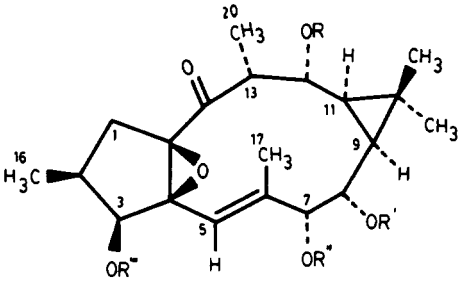
ABSTRACT.—A new ingol ester was isolated from the fresh and undried latex of *Euphorbia tirucalli*. Its structure has been assigned as 3,7,12-tri-*O*-acetyl-8-isovaleryl-ingol [**1**] on the basis of its spectroscopic data as well as those of its hydrolysis products.

Ingol esters, which are macrocyclic diterpenes based on the parent hydrocarbon lathyrane, are thought to be biogenetically related to the compounds of the tigliane, daphnane, and ingenane types (1,2). All of these have been frequently reported from the plants of the families Euphorbiaceae and Thymelaeaceae and claim intrinsic interest because of their diverse biological activities (3). *Euphorbia tirucalli* L. (Euphorbiaceae) is a small tree common in tropical and subtropical regions of Asia. Its various parts are reputed for their medicinal properties in the indigenous system of medicine (4). It has been well worked out for diterpenes, and a number of ingenol and phorbol esters have so far been reported (5–10). This manuscript describes the isolation and structure elucidation of a new ingol ester.

The diterpene **1** showed $[\alpha]_D$ +92°. Its ir spectrum showed intense absorption at 1730 cm^{-1} (ester carbonyl), 1705 cm^{-1} (ketone), 3055, 1650, and 810 cm^{-1} (trisubstituted double bond). The hrms indicated the molecular ion peak at *m/z* 576.6827, consistent with the molecular formula $C_{31}H_{44}O_{10}$ (calcd 576.6830) indicating ten double bond equivalents in the molecule. The molecular ion peak was also confirmed by fdms. Its ^{1}H-nmr spectrum ($CDCl_3$, 300 MHz) showed the presence of three acetate groups (δ 2.01, 2.08, 2.11; 3 × 3H, singlets) and one isovalerate group (δ 2.21, 2H, doublet, H-2′; 1.43, 1H, m, H-3; 0.99, 6H, doublet, H-4′ and H-5).

Further signals were observed at δ 5.57 (olefinic proton), 5.37, 5.08, 4.88, and 4.56 (protons geminal to ester functions), 0.86, 1.11, and 1.91 (tertiary methyl groups), 0.95, 1.08 (secondary methyl groups). The ^{13}C-nmr spectrum ($CDCl_3$, 75.3 MHz) showed the presence of 31 carbon atoms; their multiplicity assignments were determined by carrying out multipulse 1D DEPT experiments using last polarization pulse angle θ = 45°, 90°, and 135°. It showed the presence of ten methine, two methylene, and ten methyl carbons.

On hydrolysis the four ester groups behaved differently. Two of the acetates were readily cleaved initially to give mono-acetyl isovaleryl derivative **2**, while further heating for 30 min resulted in the formation of two additional products. All these were isolated by preparative tlc. Compound **2** showed molecular ion peak at *m/z* 492.6114 ($C_{27}H_{40}O_8$). The baseline compound **3** after acetylation was found to be 3,7,8,12-tetra-*O*-acetyl ingol [**4**]: $[M]^+$ 534.6051 ($C_{28}H_{38}O_{10}$). The reaction product, R_f 0.15, originated from the removal of isovaleryl group from **2** and was identified as 12-*O*-acetyl ingol [**5**]: $[M]^+$ 408.4910 ($C_{22}H_{32}O_7$). The identification of both **4** and **5** was fully authenticated by comparison of their spectral data with those reported in literature (11,12). The facile cleavage of two acetyl groups on hydrolysis led us to assign them to C-3 and C-7 (13,14). Further evidence was provided by the chemical shifts of H-3 and H-7 in the ^{1}H-nmr spectrum of **1**, which showed complete agreement with those reported



1 R=R″=R‴=Ac, R′=$COCH_2CH(Me)_2$
2 R=Ac, R″=R‴=H, R′=$COCH_2CH(Me)_2$
3 R=R′=R″=R‴=H
4 R=R′=R″=R‴=Ac
5 R=Ac, R′=R″=R″=H

in the literature for 3,7-diacetyl ingol esters (12,13). The isovaleryl residue in **1** and **2** must, therefore, be at C-8. The possibility of trans esterification during partial hydrolysis was ruled out by reesterification of **2** with Ac_2O and pyridine to the parent compound **1**.

The assignments of chemical shifts of protons at C-1, C-2, C-3, C-7, C-8, C-12, C-13, C-16, and C-20 were made through a series of homodecoupling experiments as described earlier (12) for related compounds. Furthermore, irradiation of the signals at δ 4.56 (H-8) and δ 4.88 (H-12) induced the double doublets at δ 1.21 and δ 1.29 to collapse into doublets (J = 8.5 Hz), indicating that these signals were due to H-9 and H-11, respectively. Although the vinylic proton appeared as a broad singlet, the allylic coupling with the methyl group at δ 1.91 was evident in the 2D-J resolved ^{1}H-nmr spectrum and was confirmed by irradiation at δ 5.57, which converted the broad singlet at δ 1.91 into a sharp singlet and vice versa. The coupling interactions established through selective homodecoupling experiments were also illustrated by homonuclear ^{1}H-^{1}H correlated spectroscopy (COSY-45°) (15).

Conclusive evidence for the location of ester functions and specific assignments of the carbon resonances of the ester groups was provided by 2D long-range ^{1}H-^{13}C shift correlated spectrum (^{1}H-^{13}C-COLOC) (16). The attachment of the acetyl group at C-3, C-7, and C-12 was clearly illustrated by cross peaks of acetyl carbonyls at δ 170.89, 170.70, and 169.79 with protons at C-12, C-3, and C-7 as well as additional cross peaks with methyl groups at δ 20.54, 20.94, and 20.99, respectively. The isovaleryl carbonyl at δ 176.00 showed a cross peak with H-8, in addition to two further cross peaks with H-2′ and H-3′. The assignments of chemical shifts of carbons were made by comparison with the published ^{13}C-nmr spectra of ingol esters (17,18) and confirmed by ^{1}H-^{13}C-heteronuclear chemical shift correlated spectroscopy (Hetero-COSY).

Although a variety of ingol esters have been isolated from the genus *Euphorbia*, (19,20) the present report constitutes the first example of the natural occurrence of an ingol ester with an isovalerate moiety, and its isolation may be of chemotaxonomic significance.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—All ir spectra were recorded on JASCO A-302 spectrometer. Hrms were recorded on Finnigan MAT-312 mass spectrometer connected to a PDP 11/34 (DEC) computer system. The ^{1}H-nmr spectra were recorded at 300 MHz on a Bruker AM-300 spectrometer with TMS as internal reference. The DEPT experiments were carried out with θ = 45°, 90°, and 135°. The quaternary carbons were determined by subtraction of these spectra from the broad band ^{13}C-nmr spectrum. The two-dimensional COSY-45° experiment was

acquired at 300 MHz with sweep width of 4000 Hz (2K data points in $\omega 2$) and 2000 Hz (256 t_1 values zero-filled to 1K) in $\omega 1$. The heteronuclear two-dimensional ^1H-^{13}C chemical shift correlation experiments were carried out at 300 MHz with sweep width of 12820 Hz (2K data points in $\omega 2$) and 1024 Hz (256 t_1 values zero-filled to 1K) in $\omega 1$. In both of the 2D experiments a 2-sec relaxation delay was used, and 16 transients were performed for each t_1 value.

PLANT MATERIAL.—The plant material (latex) was collected in Karachi, Pakistan, and was identified by Prof. S.I. Ali, the plant taxonomist, Department of Botany, University of Karachi, where a voucher specimen is deposited.

ISOLATION PROCEDURES.—The fresh latex (2 kg) was directly tapped from incisions into a flask containing Me_2CO. After standing overnight at 4°, the coagulated residue was removed and used. The Et_2O-soluble fraction obtained from the Me_2CO-insoluble residue was subjected to cc over activated Si gel. Elution was carried out with a mixture of hexane/Et_2O, using increasing order of polarity. The eluate obtained from hexane-Et_2O (6.5:3.5) was subjected to preparative tlc on Si gel (GF-254) precoated plates with hexane-$CHCl_3$-Et_2O (6:2.5:1.5) as the solvent system. This afforded a pure diterpene **1** (R_f = 0.5) (120 mg): $[\alpha]D$ +92° ($CHCl_3$); ir ($CHCl_3$) ν max cm^{-1} 1730 (ester carbonyl), 1705 (ketone), 3055, 1650, and 810 (trisubstituted double bond); ms *m/z* (rel. int. %) 576 (23), 516 (8), 475 (20), 432 (10), 372 (12), 330 (18), 312 (15), 295 (11), 165 (22), 109 (42), 85 (100), 69 (45); ^1H nmr ($CDCl_3$) δ 5.57 (1H, br s, H-5), 5.37 (1H, d, J = 8.5 Hz, H-3), 5.08 (1H, d, J = 1.8 Hz, H-7), 4.88 (1H, dd, J = 11.0, 3.9 Hz, H-12), 4.56 (1H, dd, J = 10.8, 1.8 Hz, H-8), 2.98 (1H, m, H-13), 2.76 (1H, dd, J = 14.8, 8.6 Hz, H-1α), 2.44 (1H, m, H-2), 2.11 (3H, s, 3-OCO*Me*), 2.08 (3H, s, 12-OCO*Me*), 2.01 (3H, s, 7-OCO*Me*), 1.91 (3H, br s, H-17), 1.67 (1H, d, J = 14.7 Hz, H-1β), 1.11 (3H, s, H-19), 1.08 (3H, d, J = 6.8 Hz, H-20), 0.95 (3H, d, J = 7.5 Hz, H-16), 0.86 (3H, s, H-18), 2.21 (2H, d, H-2′), 1.43 (1H, m, H-3′), 0.99 (6H, d, J = 6.6 Hz, H-4′ and H-5′); ^{13}C-nmr ($CDCl_3$, 75.3 MHz) δ 31.34 (C-1), 29.10 (C-2), 76.30 (C-3), 73.46 (C-4), 116.97 (C-5), 136.78 (C-6), 76.80 (C-7), 71.14 (C-8), 24.87 (C-9), 19.29 (C-10), 30.73 (C-11), 70.77 (C-12), 42.90 (C-13), 207.0 (C-14), 71.72 (C-15), 169.96 (C-16), 17.30 (C-17), 29.67 (C-18), 16.20 (C-19), 11.98 (C-20), 169.79 (7-OCOMe), 170.70 (3-OCOMe), 170.89 (12-OCOMe), 20.99 (7-OCO*Me*), 20.94 (3-OCO*Me*), 20.54 (12-$OCOCH_3$), 176.00 (C-1′), 26.69 (C-2′), 40.85 (C-3′), 22.6 (C-4′ and C-5′).

BASE-CATALYZED HYDROLYSIS OF **1**.—Compound **1** (100 mg) was hydrolyzed with 0.1 M KOH in MeOH at 50° for 30 min. Three hydrolysis products were isolated from the reaction mixture by means of tlc on Si gel (GF-254) using hexane-Et_2O-$CHCl_3$ (5:2:3) as solvent system. The baseline product **3** was eluted and acetylated with pyridine-Ac_2O (1:2). The resulting acetate was identified as 3,7,8,12-tetra-*O*-acetyl ingol [**4**]. The second product **5** (R_f 0.15) was identified as 12-*O*-acetyl ingol. Finally, the reaction product (R_f 0.2) exhibited the following spectral data and was identified as 12-*O*-acetyl-8-isovaleryl ingol [**2**]: $[\alpha]D$ +45° ($CHCl_3$); ir ($CHCl_3$) ν max cm^{-1} 1725 (ester carbonyl), 1705 (ketone), 3050, 1635, and 805 (trisubstituted double bond); ms *m/z* (rel. int. %) 492 (15), 474 (10), 432 (20), 393 (17), 330 (7), 312 (18), 295 (10), 181 (15), 85 (100), 69 (35); ^1H-nmr ($CDCl_3$) δ 5.67 (1H, br s, H-5), 4.88 (1H, dd, J = 11.1, 4.0 Hz, H-12), 4.45 (1H, dd, J = 10.9, 1.7 Hz, H-8), 4.35 (1H, d, J = 8.5 Hz, H-3), 4.24 (1H, d, J = 1.7 Hz, H-7), 2.94 (1H, m, H-13), 2.74 (1H, dd, J = 14.8, 8.6 Hz, H-1α), 2.45 (1H, m, H-2), 2.08 (3H, s, OCO*Me*), 1.93 (3H, br s, H-17), 1.65 (1H, d, J = 14.7 Hz, H-1β), 1.11 (3H, s, H-19), 1.07 (3H, d, J = 6.8 Hz, H-20), 0.95 (3H, d, J = 7.1 Hz, H-16), 0.88 (3H, s, H-18), 0.99 (6H, d, J = 6.9 Hz, H-4′ and H-5′), 2.20 (2H, d, H-2′), 1.41 (1H, m, H-3′).

ACETYLATION OF **2**.—Compound **2** (10 mg) was refluxed with Ac_2O (3 ml) in pyridine (1.5 ml) for 45 min. The reaction mixture was worked up as usual and the product purified through preparative tlc. It exhibited identical R_f, $[\alpha]D$, and ^1H-nmr spectra values identical to those of **1**.

LITERATURE CITED

1. H. Lotter, H.J. Opferkuch, and E. Hecker, *Tetrahedron Lett.*, **20**, 77 (1979).
2. W. Adolf and E. Hecker, *Isr. J. Chem.*, **16**, 75 (1977).
3. E. Hecker, *Pure Appl. Chem.*, **49**, 1423 (1977).
4. W. Dymock, "Pharmacographia Indica," Zain Packaging Industries, Karachi, 1883, Vol. 3, p. 252.
5. G. Fuerstenberger and E. Hecker, *Tetrahedron Lett.*, 925 (1977).
6. G. Fuerstenberger and E. Hecker, *Experientia*, **33**(8), 986 (1977).
7. A.D. Kinghorn, *J. Nat. Prod.*, **41**, 648 (1978).
8. A.D. Kinghorn, *J. Nat. Prod.*, **42**, 112 (1979).
9. G. Furstenberger and E. Hecker, *Z. Naturforsch.*, **40c**, 631 (1985).
10. G. Furstenberger and E. Hecker, *J. Nat. Prod.*, **46**, 386 (1986).

11. R.R. Uphadyay and E. Hecker, *Phytochemistry*, **14**, 2514 (1976).
12. K. Abo and F.J. Evans, *Planta Med.*, **43**, 392 (1981).
13. K. Abo and F.J. Evans, *Phytochemistry*, **20**, 2535 (1981).
14. K.A. Abo and F.J. Evans, *J. Nat. Prod.*, **45**, 365 (1982).
15. Atta-ur-Rahman, "Nuclear Magnetic Resonance," Springer-Verlag, New York, 1986, p. 262.
16. Atta-ur-Rahman, "One and Two Dimensional NMR Spectroscopy," Elsevier, New York, 1989, p. 404.
17. J.D. Connolly, C.O. Fakunle, and D.S. Rycroft, *J. Chem. Res., Synop.*, 368 (1984).
18. J.D. Connoly, C.O. Fakunle, and D.S. Rycroft, *Tetrahedron Lett.*, **25**, 3773 (1984).
19. H.J. Opferkuch and E. Hecker, *Tetrahedron Lett.*, 3611 (1973).
20. M. Hergerhahn, S. Kusumoto, and E. Hecker, *Experientia*, **30**, 1438 (1974).

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