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CYCLOCLARKEANOL, A NEW TRITERPENE FROM *EUPHORBIA CLARKEANA*

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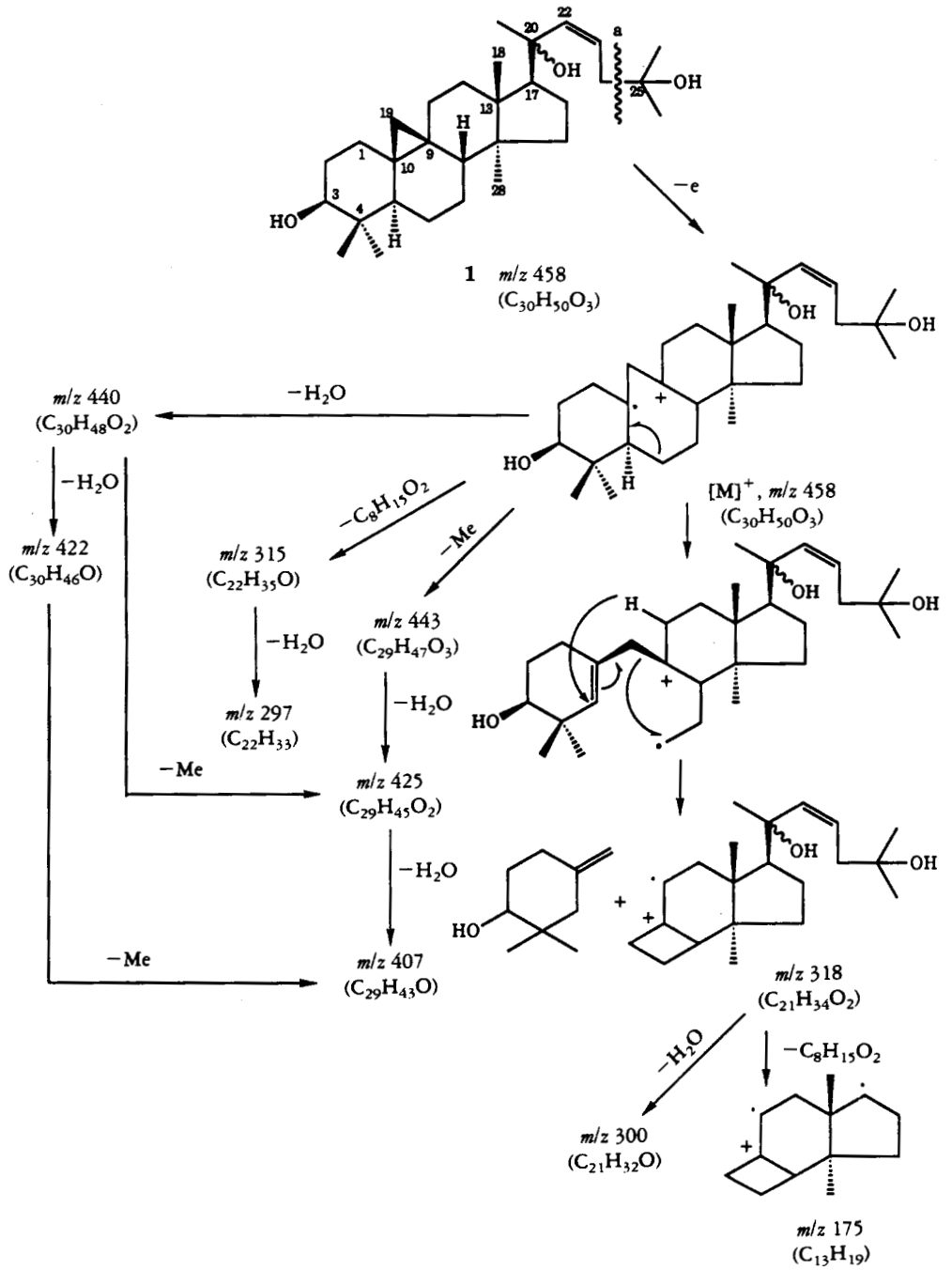
ABSTRACT.—Phytochemical studies on *Euphorbia clarkeana* have resulted in the isolation of cycloartanol, cycloart-23-ene-3β,25-diol, 20-acetylingenol-3-decadienoate, and a new cycloartane-type triterpene, cycloclarkeanol. Its structure is elucidated as cycloart-22-ene-3β,20ϵ,25-triol [**1**] on the basis of chemical and spectral studies.

The genus *Euphorbia* (Euphorbiaceae) consists of about 2000 species occurring in the form of lacticiferous herbs, shrubs, and small trees, inhabiting the tropical and temperate zones of Asia and other parts of the world. A large number of these are used in indigenous medicine for the treatment of a variety of ailments including cancer, rheumatism, neuralgia, asthma, and bacterial infections (1). The many uses of *Euphorbia* prompted us to carry out phytochemical studies on various *Euphorbia* species. One of these is *Euphorbia clarkeana* Hook. f., an annual, glabrous, prostrate herb with many branches, which is widely distributed in fields, sandy soil, and as a garden weed in India, Afghanistan, and Pakistan (2). So far no report of chemical work on this plant has appeared. Systematic study of the hexane extract of *E. clarkeana* has resulted in the isolation of cycloartanol, cycloart-23-ene-3β,25-diol, 20-acetylingenol-3-decadienoate, and a new cycloartane triterpene named cycloclarkeanol [**1**]. We now report the structure elucidation of **1**.

Cycloclarkeanol [**1**] crystallized as colorless needles from a mixture of $CHCl_3$ and MeOH, mp 190–192°; $[\alpha]_D$ 37.84° ($c = 0.21$, $CHCl_3$). Its ir spectrum showed absorptions at 3460–3440 (OH group), 3065 and 1630 (C=C), 3040 (CH_2 asymmetric stretching of a cyclopropane ring), and 1380 (*gem*-dimethyl). The hrms gave a molecular ion peak at *m/z* 458.3753 consistent with the molecular formula $C_{30}H_{50}O_3$ (calcd 458.3747), indicating the presence of six double bond equivalents in the molecule. The ^{1}H-nmr spectrum ($CDCl_3$, 400 MHz) showed signals for olefinic protons (d at δ 5.48, $J = 7.05$ Hz and m at δ 5.40 Hz), a methine proton attached to the carbon bearing the hydroxyl group (δ 3.26, 1H, dd, $J_{a,a} = 10.78$ Hz and $J_{a,e} = 4.58$ Hz), seven tertiary methyls at δ 1.39, 0.87, 0.80, 1.33, and 0.95), and a pair of doublets, indicative of a cyclopropane ring bearing two non-equivalent hydrogen atoms (δ 0.33, $J = 4.26$ Hz and δ 0.54, $J = 4.26$ Hz). The ^{13}C-nmr spectrum showed 30 carbon atoms; multiplicities were determined by DEPT experiments (3,4), which revealed the presence of 7 methyl, 10 methylene, 6 methine carbon atoms, and 7 quaternary carbons. Both the ^{1}H- and ^{13}C-nmr spectra indicated the presence of one secondary and two tertiary hydroxyl groups, which was further confirmed by acetylation of **1** to a monoacetate showing a molecular ion peak at *m/z* 500.3839 and a broad band at 3460 cm^{-1} (OH) in the ir spectrum.

Structural information was obtained from the mass spectrum of **1** (Scheme 1). It showed a predominant ion peak, typical for the cleavage of cycloartane triterpenes at *m/z* 318.2520 ($C_{21}H_{34}O_2$) (5–7). Fragment peaks appeared at *m/z* 315.2638 and 175.1461 and are attributable to the loss of the side chain



SCHEME 1

moiety ($C_8H_{15}O_2$) from the parent ion. These data indicate that two of the three hydroxyl groups and the olefinic bond are present in the side chain, and the remaining hydroxyl group is in ring A/B. The peak at *m/z* 399.3251 $[M-C_3H_2O]^+$ results from allylic cleavage of the C-24–C-25 bond in **1** and allows us to place the double bond at C-22 (8). Comparison of the chemical shift values of the side chain carbon signals with those of stigmasterol reveals downfield shifts of methyl groups

at C-20 and C-25 in **1** (9). Therefore the tertiary hydroxy groups can be assigned to these positions.

The secondary hydroxyl group was assigned to C-3 on the basis of biogenetic analogy and also by ^{1}H-^{1}H-correlated spectroscopy. The carbinylic proton at δ 3.26 showed cross peaks with two other protons, limiting it to positions 1 or 3. However, the chemical shifts of C-2, C-4, and the methyl carbons attached to C-4 showed close similarity to those in cycloartanol, providing evidence for the presence of a hydroxyl group at C-3 rather than at C-1. The coupling constants of the carbinylic proton signal were in accord with its axial and α orientation; therefore structure **1** could be assigned to cycloclarkeanol. The ^{13}C and ^{1}H-^{13}C correlated spectra were in agreement with the assigned structure. The configuration at C-20 could not be determined in the present work.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—The ir spectra were recorded on a JASCO A-302 spectrometer, and hrms and eims were determined on a Finnigan MAT-312 mass spectrometer connected to a PDP 11/34 (DEC) computer system. The ^{1}H- and ^{13}C-nmr spectra were recorded on a Bruker AM-400 spectrometer with TMS as internal reference. DEPT experiments were carried out with last pulse angle θ = 45°, 90°, and 135°. The quaternary carbons were determined by subtracting these spectra from the broad-band ^{13}C-nmr spectrum.

PLANT MATERIAL.—*E. clarkeana* was collected in Karachi, Pakistan and was identified by Prof. Dr. S.I. Ali, Plant Taxonomist, Department of Botany, University of Karachi, where a voucher specimen is deposited.

ISOLATION OF COMPOUNDS.—Freshly collected whole plant material (20 kg) was chopped and extracted thrice with MeOH (total 60 liters). The whole residue of the combined MeOH extract was partitioned with H_2O and hexane. The hexane fraction (12 g) was chromatographed over Si gel (480 g) and successively eluted with increasing polarities of a mixture of hexane and $CHCl_3$. The hexane-$CHCl_3$ (7:3) eluate yielded an oily residue which on further purification by plc in the same solvent system provided 20-acetylingenol-3-decadienoate (18 mg). The eluates from hexane-$CHCl_3$ (6:4 and 5.5:4.5) yielded crystalline residues, which on repeated crystallization from hexane/$CHCl_3$ gave cycloartanol (24 mg) and a mixture of triterpenes. The latter were resolved through flash cc over Si gel using hexane-$CHCl_3$ (7:3) as solvent system to yield cycloart-23-ene-3β,25-diol (20 mg) and cycloclarkeanol (22 mg).

Cycloclarkeanol [**1**].—Mp 190–192°; [α]D 37.84 (c = 0.21, $CHCl_3$); ir ($CHCl_3$) ν max cm^{-1} 3460–3440, 3065, 3040, 1630, 1380, 1015; hrms *m/z* 458.3753 ($C_{30}H_{50}O_3$ requires 458.3747); ms *m/z* (rel. int. %) $[M]^+$ 458 (18), 443 (16), 440 (38), 425 (45), 399 (16), 318 (26), 315 (32), 175 (54); ^{1}H nmr ($CDCl_3$, 400 MHz) δ 5.68 (1H, d, J = 7.05 Hz, H-22), 5.40 (1H, m, H-23), 3.26 (1H, dd, $J_{a,a}$ = 10.78 Hz and $J_{a,e}$ = 4.58 Hz, H-3), 1.39 (3H, s, Me-21), 1.33 (6H, s, Me-26 and Me-27), 0.95 (6H, s, Me-18 and Me-30), 0.87 (3H, s, Me-28), 0.80 (3H, s, Me-29), 0.54–0.33 (2H, dd, J = 4.20 and 4.26 Hz, H_2-19); ^{13}C-nmr ($CDCl_3$, 100.61 MHz) δ 31.87 (C-1), 30.26 (C-2), 78.84 (C-3), 40.39 (C-4), 47.03 (C-5), 21.02 (C-6), 28.10 (C-7), 47.89 (C-8), 19.93 (C-9), 26.17 (C-10), 26.01 (C-11), 35.59 (C-12), 45.27 (C-13), 48.82 (C-14), 32.68 (C-15), 26.42 (C-16), 52.10 (C-17), 17.98 (C-18), 29.81 (C-19), 73.01 (C-20), 24.26 (C-21), 134.47 (C-22), 130.68 (C-23), 39.38 (C-24), 72.28 (C-25), 29.39 (C-26), 29.44 (C-27), 19.22 (C-28), 25.36 (C-29), 14.01 (C-30). The assignments are made through comparison with published ^{13}C-nmr spectra of related compounds (10,11) and confirmed by 2D ^{1}H-^{13}C COSY experiment.

ACETYLATION OF **1**.—Compound **1** (5.0 mg) was dissolved in pyridine (1.0 ml) and refluxed with Ac_2O (2.5 ml) for 30 min. The reaction mixture was worked up in the usual manner to afford **1** acetate: hrms *m/z* 500.3839 ($C_{32}H_{52}O_4$ requires 500.3852); eims *m/z* (rel. int.%) $[M]^+$ 500 (18), 318 (28), 315 (24), 175 (45); ^{1}H nmr ($CDCl_3$, 400 MHz) δ 5.69 (1H, d, J = 7.10 Hz, H-22), 5.40 (1H, m, H-23), 4.46 (1H, dd, $J_{a,a}$ = 10.75 Hz and $J_{a,e}$ = 4.81 Hz, H-3), 2.11 (3H, s, OAc).

Cycloartanol.—Mp 100–102°; [α]D 49.9° (c = 0.23, $CHCl_3$); eims *m/z* (rel. int. %) $[M]^+$ 428 (12), 413 (18), 410 (25), 395 (15), 355 (10), 315 (25), 288 (30), 273 (40), 297 (35), 175 (45). The physical and spectral data coincided with that reported in literature (6,7,10,12).

Cycloart-23-ene-3β,25-diol.—Mp 196–198°; [α]D 33.95° (c = 1.78, $CHCl_3$); ms *m/z* (rel. int. %) $[M]^+$ 442 (12), 427 (10), 424 (15), 409 (20), 391 (18), 315 (35), 302 (40), 294 (42), 287 (62), 175 (80). The physical and spectral data agree with those in the literature (6,11,13).

20-Acetylingenol-3-decadienoate.—[α]D 3.92° (c = 0.21, $CHCl_3$); eims *m/z* (rel. int. %) 540 (5),

522 (3), 480 (4), 438 (3), 408 (35), 390 (25), 374 (5), 330 (20), 312 (15), 202 (80), 184 (10). The physical and spectral data correspond to those of 20-acetylingenol-3-decadienoate reported in the literature (14).

LITERATURE CITED

1. E. Hecker, *Pure Appl. Chem.*, **49**, 1423 (1977).
2. E. Nasir and S.I. Ali, "Flora of Pakistan," Department of Botany, University of Karachi, Pakistan, 1986, pp. 102–103.
3. R. Benn and H. Gunther, *Angew. Chem., Int. Ed. Engl.*, **22**, 350 (1983).
4. J.N. Shoolery, *J. Nat. Prod.*, **47**, 226 (1984).
5. R.T. Aplin and G.M. Hornby, *J. Chem. Soc. B*, 1078 (1966).
6. J.S. Shannon, *Proc. R. Aust. Chem. Inst.*, 324 (1964).
7. F.W. McLafferty, *Chem. Commun.*, 78 (1966).
8. Y.M. Sheikh and C. Djerassi, *Tetrahedron*, **30**, 4095 (1974).
9. H.L. Holland, P.R.P. Diakow, and G.J. Taylor, *Can. J. Chem.*, **56**, 3121 (1978).
10. F.K. Huu, M. Sangare, V.M. Chari, A. Bekaert, M. Devys, M. Barbier, and G. Lukacs, *Tetrahedron Lett.*, 1787 (1975).
11. A.Q. Khan, Z. Ahmed, S.N.H. Kazmi, and A. Malik, *Planta Med.*, **53**, 577 (1987).
12. G. Berti, F. Bottari, R. Narsili, I. Morelli, and M. Polvani, *Tetrahedron Lett.*, **2**, 125 (1967).
13. A.S. Chowla, V.K. Kapoor, and P.K. Sangala, *Planta Med.*, **34**, 109 (1978).
14. J.G. Urones, P.B. Barcala, M.J.S. Cuadrado, and I.S. Marcos, *Phytochemistry*, **27**, 207 (1988).

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