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A NEW PENTACYCLIC TRITERPENE FROM *CALOTROPIS PROCERA*

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ABSTRACT.—Calotropenyl acetate, a new pentacyclic triterpene of the ursane series isolated from the flowers of *Calotropis procera*, is assigned structure **1** on the basis of chemical and spectral studies.

Calotropis procera R.Br. (Asclepiadaceae) grows widely in tropical regions of Asia and Africa. The milky juice of this plant is used by the natives of India as a purgative, while the flowers are considered digestive, stomachic, tonic, and useful in cough, asthma, catarrh, and loss of appetite. The root bark is said to promote secretions and to be useful in treating skin diseases, enlargement of the abdominal viscera, intestinal worms, ascites, and anasarca (1). A literature survey shows that only one terpenoidal constituent has so far been reported from this plant (2). A systematic study on the fresh and undried flowers has resulted in the isolation of a new pentacyclic triterpene that is provisionally named calotropenyl acetate. Its structure has been elucidated as urs-19(29)-en-3β-yl acetate through chemical and spectroscopic studies.

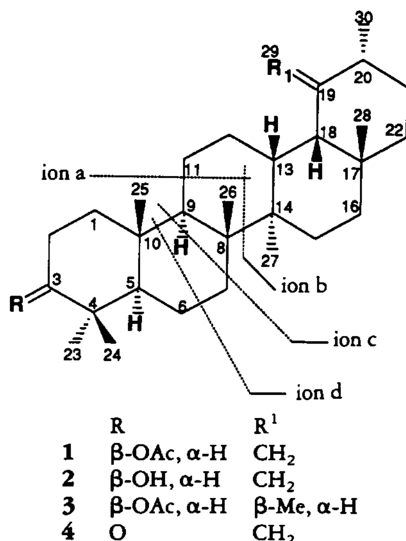
RESULTS AND DISCUSSION

Calotropenyl acetate [**1**]: mp 198°; [α]D +8.9°. The hrms gave a molecular ion peak at 468.7677 corresponding to the molecular formula $C_{32}H_{52}O_2$ (calcd 468.7660). The ir spectrum ($CHCl_3$) showed an acetate group (1725 and 1320 cm^{-1}) and a methylene group (1640 and 880 cm^{-1}). The ^{13}C-nmr spectrum showed 32 carbon atoms; the multiplicities of these were determined by using DEPT experiments (3) which revealed the presence of 7-methyl, 11-methylene, and 6-methine carbons. The ^{1}H-nmr (300 MHz) spectrum showed signals for vinylic protons (broad singlets at δ 4.6 and 4.68, 1H each), an equatorial acetoxy group (double doublet at δ 4.52, 1H, 3α-H and singlet at δ 2.08, 3H, $OCOCH_3$), one secondary methyl group (doublet at δ 0.91, $J=6.5$ Hz), and six tertiary methyls (singlets at δ 0.82, 0.83, 1.0, 1.01, 1.03). The mass spectrum of **1** is also characteristic of pentacyclic triterpenes of the ursane series in which rings A, B, C, and D are saturated. The major ions a and b were generated by transfer of hydrogen from C-26 to C-11 accompanied by cleavage of 9-11 and 8-14 bonds with charge retention on either of the resulting fragments (4,5). The important fragmentations are shown in Figure 1.

Alkaline hydrolysis of calotropenyl acetate provided the free alcohol, calotropenol [**2**], while catalytic reduction afforded the corresponding dihydro derivative, calotropanyl acetate [**3**]. Oxidation of **2** with Jones reagent gave calotropenone [**4**]. The latter gave a positive Zimmermann test indicating the presence of the 3-oxo group (6). The $NaBH_4$ reduction of **4** regenerated the parent alcohol **2**, confirming the equatorial orientation of the hydroxyl group in **2** and, hence, the acetoxy group in **1**. The configuration of the hydroxyl group in **2** was also indicated by the characteristic downfield shift of the 3α-H from δ 3.23 to δ 4.52 upon acetylation. In both **1** and **2** the 3α-H appeared as a double doublet with $J_{ax,ax}=9.8$ Hz, $J_{ax,eq}=4.7$ Hz, owing to coupling with protons at C-2.

Further evidence for the structure of **1** is provided by study of its dihydro derivative **3**. Its ^{1}H-nmr spectrum showed the absence of olefinic protons but gave the signal of an additional methyl group (δ 0.86, d, $J=6.9$ Hz). The reduction of the methylene group was expected to form a methyl group with α or β configuration. However, the former was formed in trace amount due to steric crowding of the α-oriented methyl group at C-

8. The β configuration of the methyl group at C-19 was confirmed by a very strong nOe interaction (16.6%) between 19-αH and 27-H_3. Alkaline hydrolysis of **3** gave calotropanol, which was identified as 3β-hydroxyursane through comparison of physical and spectral data with those reported in literature (7,8). The formation of **3** from **1** left only position 19(29) for the vinylic double bond. The stereostructures of **1** and its derivatives were, therefore, as shown in Figure 1.



	R	R^1
1	β-OAc, α-H	CH_2
2	β-OH, α-H	CH_2
3	β-OAc, α-H	β-Me, α-H
4	O	CH_2

FIGURE 1. Mass spectrometric fragmentation of **1**.

Conclusive evidence for the structure of **1** is derived from the results of extensive 2D-nmr experiments. Hetero-COSY experiments (9) were carried out to identify the relationship between carbons and their respective protons. The signals of C-3, C-29, C-30, C-21, C-12, and C-13 in the ^{13}C-nmr spectrum could be easily correlated with the corresponding protons in the ^{1}H-nmr spectrum. The coupling interaction was illustrated by ^{1}H-^{1}H correlated spectroscopy (COSY 45°) (9), which showed the connectivity of 3αH to both protons at C-2 and the connectivity of 20-H with 30-H_3.

The position of the double bond and the stereochemistry at C-18 (*cis*-linkage of ring D and E) were authenticated by nOe difference measurements at certain points. Irradiation at δ 4.68 (29-H_2) resulted in 9.7% nOe at δ 1.47 (12-H_2), 8.1% nOe at δ 0.91 (30-H_3), and 11.2% nOe at δ 1.03 (27-H_3). Irradiation at δ 0.91 (30-H_3) resulted in 7.79% nOe at δ 4.68 (29-H_2) and 6.7% nOe at δ 1.6 (21-H_2). Irradiation at δ 1.47 (12-H_2) resulted in 10.3% nOe at δ 4.68 (29-H_2) and 9.05% nOe at δ 1.41 (11-H_2). These nOe interactions are summarized in Figure 2. The nOe interaction between the

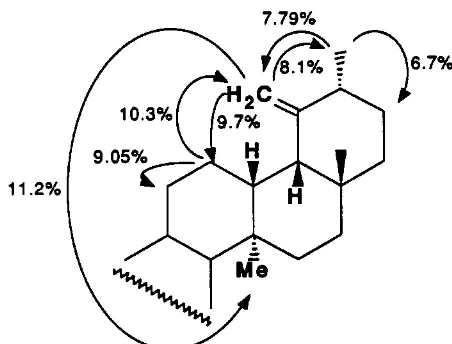


FIGURE 2. NOe interactions for **1**.

vinylic protons, 30-H_3 and 27-H_3 not only proved the position of the double bond but also confirmed the β configuration of 18-H. In taraxasterol where the double bond is at 20(30) and the D/E ring junction is *trans*, no such interactions are observed.

Biogenetic evidence suggesting the structure of **1** is provided by the recent isolation of a similar triterpene in which the vinylic double bond and the methyl group in the ring E have interchanged positions (7). To the best of our knowledge this is the first report of a naturally occurring pentacyclic triterpene with an exocyclic double bond at 19(29); its isolation may be significant in chemotaxonomic study.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mps are uncorrected; ir spectra were recorded in $CHCl_3$ with a Jasco IRA-1 spectrophotometer and hrms on a Finnigan MAT 312 double focusing mass spectrometer coupled to a PDP 11/34 computer system. ^{1}H-nmr spectra were recorded on a Bruker Aspect AM-300 spectrometer with TMS as internal reference. The DEPT experiments were carried out with $\theta = 45°$, 90°, and 135°; the quaternary carbons were determined by subtraction of these spectra from the broad band ^{13}C-nmr spectrum.

For nOe measurements, the sample was frozen under liquid nitrogen and degassed. A lower decoupler power of maximum 0.2 watts with 35 attenuations in dbs was used. The pre-irradiation time was 11 sec which is the sum of three delays as used in the nOe difference program of Bruker. The impulse length of 10 microseconds was maintained to avoid saturation. The two-dimensional COSY-45° experiments were acquired at 300 MHz with a sweep width of 4000 Hz (2K data points) in ω_2 and 2000 Hz (256 t_1 values zero-filled to 1K) in ω_1. The heteronuclear 2D ^{1}H-^{13}C chemical shift correlation experiments were carried out at 300 MHz with a sweep width of 12820 Hz (2K data points) in ω_2 and 1024 Hz (256 t_1 values zero-filled to 2K) in ω_1. In both the 2D experiments a 2-sec relaxation delay was used, and 16 transients were performed for each t_1 value.

PLANT MATERIAL.—The flowers of *C. procera* were collected from the Karachi region and identified by Dr. S.I. Ali, Department of Botany, University of Karachi. A voucher specimen has been deposited in the Herbarium of the Department of Botany, University of Karachi.

ISOLATION PROCEDURES.—The freshly collected plant material (20 kg) was extracted with EtOH at room temperature. The gummy residue obtained from the EtOH extract was partitioned between $CHCl_3$ and H_2O. The hexane-soluble portion of the $CHCl_3$ fraction was column chromatographed over Si gel. The column was eluted with various solvent gradients of increasing polarities. The fraction eluted with hexane-$CHCl_3$ (75:25) was evaporated and further purified through preparative layer chromatography using a solvent of hexane-Et_2O (55:45). Fractional crystallization yielded calotropenyl acetate [**1**] (190 mg), mp 198°; $[\alpha]_D$ +8.9° (c = 0.415, $CHCl_3$); ir 1725, 1350, 1640, 880 cm^{-1}; ms *m/z* (rel. int.) $[M]^+$ 468 (45), $[M-Me]^+$ 453 (7), $[M-HOAc]^+$ 408 (10), $[M-Me-HOAc]^+$ 393 (8), $[\text{ion a}]^+$ 249 (17), $[\text{ion a}-HOAc]^+$ 189 (100), $[\text{ion b}]^+$ 218 (51), $[\text{ion c}]^+$ 257 (4), $[\text{ion d}]^+$ 272 (5); ^{1}H nmr ($CDCl_3$) 4.6–4.68 (2H, br s, H-29), 4.52 (3H, s, dd, $J_{ax,ax}$ = 9.8 Hz, $J_{ax,eq}$ = 4.7 Hz, H-3), 2.08 (3H, s, OCOMe), 1.03 (3H, s, H-27), 1.01 (3H, s, H-23), 1.0 (3H, s, H-26), 0.91 (3H, d, J = 6.5 Hz, H-30), 0.83 (3H, s, H-25), 0.82 (6H, s, H-24, 28); ^{13}C nmr ($CDCl_3$) C-1 (38.54), C-2 (23.76), C-3 (80.95), C-4 (37.85), C-5 (55.54), C-6 (18.26), C-7 (34.10), C-8 (41.01), C-9 (48.78), C-10 (37.57), C-18 (59.40), C-19 (154.57), C-20 (39.44), C-21 (31.22), C-22 (38.97), C-23 (28.0), C-24 (16.54), C-25 (15.96), C-26 (16.37), C-27 (25.54), C-28 (28.10), C-29 (107.24), C-30 (19.53), OCOMe (170.8), OCO*Me* (21.20).

HYDROLYSIS OF CALOTROPENYL ACETATE.—The acetate **1** (100 mg) was refluxed with 5% ethanolic KOH (20 ml) for 3 h. The reaction mixture was extracted with $CHCl_3$. The $CHCl_3$ solution, after washing with H_2O and drying over anhydrous Na_2SO_4, was evaporated to afford calotropenol (97.28 mg) which was crystallized from $CHCl_3$-C_6H_6 (1:1) as colorless needles, mp 168°; $[\alpha]_D$ +37.26° (c = 0.161, $CHCl_3$); ir 3420, 1645, 890 cm^{-1}; hrms 426.3854 ($C_{30}H_{50}O$); ms *m/z* (rel. int.) $[M]^+$ 426 (32), $[M-Me]^+$ 411 (8), $[M-H_2O]^+$ 408 (14), $[M-Me-H_2O]^+$ 393 (5), $[\text{ion a}]^+$ 207 (100), $[\text{ion a}-H_2O]^+$ 189 (72), $[\text{ion b}]^+$ 218 (6), $[\text{ion d}]^+$ 272 (8); ^{1}H nmr ($CDCl_3$) 4.6–4.7 (2H, br s, H-29), 3.23 (1H, dd, $J_{ax,ax}$ = 9.2 Hz, $J_{ax,eq}$ = 4.3 Hz, H-3), 1.05 (3H, s, H-27), 1.02 (3H, s, H-23), 0.99 (3H, s, H-26), 0.93 (3H, d, J = 6.2 Hz, H-30), 0.84 (3H, s, H-25), 0.82 (6H, s, H-24, 28).

OXIDATION OF CALOTROPENOL.—Calotropenol (20 mg) was dissolved in Me_2CO (40 ml) and treated with freshly prepared Jones reagent (5 ml). The reaction mixture was stirred at room temperature till the reaction was completed (tlc monitoring). It was diluted with H_2O and extracted with Et_2O. Removal of solvent from the Et_2O extract and crystallization from Et_2O/MeOH yielded calotropenone (15.35 mg), mp 130°; $[\alpha]_D$ +27.5° (c = 0.124, $CHCl_3$); ir 1705, 1640, 885 cm^{-1}; hrms 424.3714 ($C_{30}H_{48}O$);

ms *m/z* (rel. int.) $[M]^+$ 424 (20), $[M-Me]^+$ 409 (12), $[\text{ion a}]^+$ 205 (100), $[\text{ion b}]^+$ 218 (35), $[\text{ion c}]^+$ 257 (15), $[\text{ion d}]^+$ 272 (8); ^1H nmr ($CDCl_3$) 4.6–4.7 (2H, br s, H-29), 1.05 (3H, s, H-27), 1.01 (3H, s, H-23), 0.98 (3H, s, H-26), 0.93 (3H, d, $J=6.7$ Hz, H-30), 0.83 (3H, s, H-23), 0.81 (6H, s, H-24, 28).

REDUCTION OF CALOTROPENONE.—Calotropenone (10 mg) was dissolved in 2 ml of EtOH, and 0.5 mg of $NaBH_4$ was then added with stirring (1 h). After dilution with H_2O, the mixture was extracted with $CHCl_3$, and the $CHCl_3$ layer was washed with H_2O, dried, and evaporated. Crystallization from $CHCl_3$ yielded **2**, mp 167°; $[\alpha]D$ +38.06° ($c=0.151$, $CHCl_3$).

CATALYTIC REDUCTION OF CALOTROPENYL ACETATE.—Calotropenyl acetate (20 mg) in glacial HOAc (6.0 ml) and dry Et_2O (10 ml) was shaken with H_2 and PtO_2 (100 mg) for 1 h. The catalyst was filtered off, and the filtrate was extracted with Et_2O. The Et_2O layer was successively washed with H_2O, dilute $NaHCO_3$ solution, and again with H_2O. Drying (Na_2SO_4) and removal of solvent provided a crystalline residue that was recrystallized from MeOH to obtain the corresponding saturated acetate. Upon hydrolysis with 3% ethanolic KOH, it provided calotropanol, which on recrystallization from Et_2O melted at 205° and showed $[\alpha]D$ −12° ($c=0.14$, $CHCl_3$); ir 3450 cm^{-1}; hrms 428.4012 ($C_{30}H_{52}O$); ms *m/z* (rel. int.) $[M]^+$ 428 (20), $[M-Me]^+$ 413 (14), $[M-H_2O]^+$ 410 (10), $[M-Me-H_2O]^+$ 395 (15), $[\text{ion a}]^+$ 207 (100), $[\text{ion a}-H_2O]^+$ 189 (40), $[\text{ion c}]^+$ 259 (12), $[\text{ion d}]^+$ 274 (6).

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