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A NEW CYCLOARTANE-TYPE TRITERPENE FROM *PENTATROPIS SPIRALIS*

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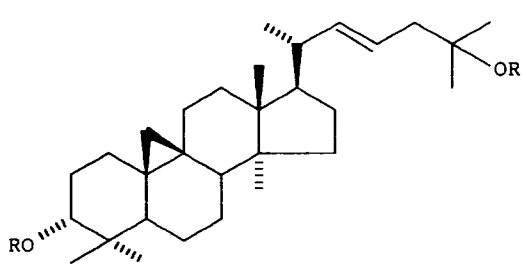
ABSTRACT.—A new triterpene has been isolated from *Pentatropis spiralis*. Its structure was established as cycloart-22-ene-3α,25-diol [**1**] through chemical and spectroscopic studies including 2D nmr. Two known triterpenes, cycloeucalenol and 24-methylenecycloartanol, were also isolated.

Pentatropis spiralis Decne. (syn. *Asclepias spiralis* Forssk., *Pentatropis cynanchoides* R.Br.) (Asclepiadaceae) is a slender climber with a thin root stock distributed in tropical regions of Asia, Africa, and Australia. Several species of this genus contain biologically active compounds (1,2). An EtOH extract of one of the species of this genus is widely used in folk medicine for the treatment of cancer and warts (3). *P. spiralis* is used in the indigenous system of medicine as a purgative. The decoction of dry root is an astringent, cooling, and alterative and is also used as a remedy in gonorrhea (4). The present paper describes the isolation and structure of a new cycloartane-type triterpene, cycloart-22-ene-3α,25-diol [**1**], along with two known triterpenes, cycloeucalenol and 24-methylenecycloartanol, which have been isolated for the first time from this genus.

RESULTS AND DISCUSSION

Cycloart-22-ene-3α,25-diol [**1**], mp 188°, $[\alpha]_D$ +38.5° ($CHCl_3$), was found to have the molecular formula $C_{30}H_{50}O_2$ by fdms and hrms ($[M]^+$ *m/z* 442.3870, calcd 442.3980) indicating six double bond equivalents in the molecule. The ir spectrum suggested two hydroxyl groups (3590 and 3440 cm^{-1}) and a cyclopropane ring (3045 cm^{-1}). The ^1H-nmr (300 MHz) spectrum showed signals due to six tertiary (δ 0.86, 0.88, 0.96, 0.97, 1.30, and 1.31, all singlets) and a secondary (δ 0.84, d, $J = 6.4$ Hz) methyl group. A triplet at δ 3.34 ($J = 2.6$ Hz) was indicative of a proton geminal to a hydroxyl group. Furthermore, the spectrum showed a multiplet at δ 5.59 for two olefinic protons and a pair of doublets at δ 0.30 and 0.50 ($J = 4.5$ Hz) for cyclopropane methylene protons. The ^{13}C-nmr spectrum showed 30 carbon atoms. The multiplicity assignments were made by DEPT experiments (5,6) which revealed the presence of seven methyl, ten methylene, and seven methine carbon atoms.

The presence of two hydroxyl groups in **1** was concluded from the preparation of the diacetate **2**. The mass spectra of **1**



1 R=H
2 R=Ac

and **2** showed the losses of $2 \times H_2O$ and $2 \times$ HOAc molecules, respectively, which indicated the presence of two acylable hydroxyl groups in **1**. The ^{1}H-nmr spectra of **1** and **2** showed a signal for only one carbinylic proton, suggesting the tertiary nature of the second hydroxyl group. This was also confirmed by ^{13}C nmr, showing one CH at δ 76.88 and a quaternary carbon at δ 70.77, characteristic for carbons bearing a hydroxyl group. The triplet at δ 3.34 ($J = 2.6$ Hz) in the ^{1}H-nmr spectrum of **1** was indicative of an equatorial carbinylic proton interacting with two adjacent axial and equatorial protons (7,8). In the ^{13}C-nmr spectrum of **1** an expected shielding and deshielding effect of the 3α-hydroxyl group on various carbon atoms of ring A also indicated α and axial orientation of the hydroxyl group (9).

Further insight into the structure of **1** was achieved from the mass spectrum of **1**. The spectrum showed a daughter ion peak at *m/z* 355.300 ($C_{25}H_{39}O$) corresponding to the elimination of C_5H_9 moiety from $[M-18]^+$, which is characteristic of 4,4-dimethyl-9:19-cyclosterol (10). Another characteristic process involved elimination of ring A (10,11), which was visible in the spectrum at *m/z* 302.2650 $[M-C_9H_{16}O]^+$. The presence of monounsaturated side chain was evident from the fragment ion at *m/z* 313.2564 ($C_{22}H_{33}O$) in the spectrum of **1**, presumably obtained by the loss of $C_8H_{15}O$ with two hydrogens transferred from the ring system (10). This fragment peak at *m/z* 313 revealed the presence of a quaternary hydroxyl function in the side chain. The possible position of the hydroxyl group is at C-20 or C-25. The former possibility could be eliminated by the presence of an α-hydroxy isopropyl moiety (δ 1.30, 1.31, each s, 3H).

The remaining problem was to locate the position of the 1,2-disubstituted double bond in the side chain of **1**. It was assigned to C-22 on the basis of ^{1}H-nmr data. The multiplet at δ 5.59 for two olefinic protons was resolved into two separate signals by the 2D *J*-resolved spectrum at δ 5.59 (dt, $J = 6.5$, 14.8 Hz) and 5.60 (dd, $J = 7.1$, 14.8 Hz), which can only be accommodated for a C-22 double bond (12,13). It was further supported by a strong fragment ion peaks at *m/z* 315.2702 ($C_{22}H_{35}O$) and 342.2957 ($C_{24}H_{38}O$) in the mass spectrum of **1**. These ion peaks arise from allylic cleavage at the C-17–C-20 bond and vinylic cleavage of the C-20–C-22 bond together with a hydrogen transfer, which is characteristic of a Δ^{22}-sterol (14). This parent-daughter relationship (*m/z* 442↦342, 315) was established by a linked scan experiment (15).

The structure of **1** was fully supported by extensive 2D nmr experiments. A ^{1}H-^{13}C heteronuclear chemical shifts correlation spectrum (hetero-COSY) (16) was recorded to locate the chemical shifts of various protons. The signals of C-3, C-20, C-21, C-22, C-23, C-24, C-25, C-26, and C-27 in the ^{13}C-nmr spectrum could easily be correlated with the chemical shifts of their respective protons in the ^{1}H-nmr spectrum.

The position of the double bond at C-22 was finally confirmed by 2D ^{1}H-^{1}H homonuclear chemical shift correlation spectroscopy (COSY-45°) (16), which showed the connectivity of H-20 (δ 1.75) to both H-21 (δ 0.84) and H-22 (δ 5.60). On the other hand, H-23 (δ 5.59) showed cross peaks for H-22 (δ 5.60) and H-24 (δ 2.03). Finally, H-24 (δ 2.03) showed a cross peak for only H-23, as C-25 is substituted with a tertiary hydroxyl group. On the basis of the above evidence, the structure of **1** was concluded to be cycloart-22-ene-3α,25-diol [**1**], and it is therefore an isomer of cycloart-23-ene-3β,25-diol isolated by Djerassi and McCrindle (17) from *Tillandsia usneoides* L.

Cycloeucalenol, mp 143°, $[\alpha]_D$ +44.3° ($CHCl_3$), analyzed for $C_{30}H_{50}O$ (hrms 426.3833). The mass and ^{1}H-nmr

spectra of this triterpene showed characteristic features of the 31-nor cycloartane type triterpenes with an exocyclic double bond at C-24 (18). The triterpene was identified as cycloeucalenol by direct comparison of its spectral and physical data with those reported in the literature (10,11,18).

24-Methylenecycloartanol, mp 221°, $[\alpha]_D$ +48.5° ($CHCl_3$), was found to have the formula $C_{31}H_{52}O$ (hrms 440.7560). The mass spectrum was very similar to the cycloartane type of triterpenes. It showed a fragment ion peak at *m/z* 353 corresponding to the elimination of a 69 mass unit from the $[M-18]^+$ ion peak. This process is typical for 4,4,dimethyl-9:19-cycloartane (10,11). These findings together with ^{1}H-nmr spectral data led us to identify this triterpene as 24-methylenecycloartanol (10,11,19).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mp's are uncorrected. Ir spectra were recorded in $CHCl_3$ on JASCO-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 Bruker AM-300 spectrometer with TMS as internal lock. The DEPT experiments were carried out with $\theta = 45°$, 90°, and 135°. The quaternary carbons were determined by substraction of these spectra from the broad band ^{13}C-nmr spectrum.

The 2D COSY-45° experiment was performed at 300 MHz with 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 hetero-COSY experiments were carried out at 300 MHz with sweep width of 12820 Hz (2K data points in ω_2) and 1024 Hz (256 t_1 values zero-filled in 1K) in ω_1. In both the 2D experiments a relaxation delay of 2 sec was used, and 16 transients were performed for each t_1, value.

PLANT MATERIAL.—The plant material was collected from the Karachi region and was identified by Prof. S.I. Ali, Department of Botany, University of Karachi, where a voucher specimen has been deposited.

ISOLATION PROCEDURES.—The freshly collected plant material (40 kg) was extracted 4 times with MeOH at room temperature, and the bulk extract was concentrated in vacuum. The residue was further subjected to partitioning between H_2O and hexane, and the hexane-soluble fraction was evaporated to dryness in vacuum and subjected to cc over Si gel. The elution was carried out with a solvent gradient of increasing order of polarity. The eluate obtained in hexane-$CHCl_3$ (6:4) yielded a binary mixture of triterpenes which resolved in pure state by preparative tlc impregnated with $AgNO_3$ to provide cycloeucalenol and 24-methylenecycloartanol. These known compounds were identified through comparison of their physical and spectral data with those reported in the literature (11,12,18,19). The eluate obtained in hexane-$CHCl_3$ (2:8) was further purified through preparative layer chromatography using a solvent system of $CHCl_3$-MeOH (9.5:0.5) to yield compound **1**.

CYCLOART-22-ENE-3α,25-DIOL [**1**].—Obtained as colorless needles from Me_2CO-MeOH (1:1) (82 mg): mp 188°; $[\alpha]_D$ +38.5° (*c* = 0.198, $CHCl_3$); ir 3590, 3440, 3045 cm^{-1}; hrms *m/z* (rel. int. %) $[M]^+$ 442.3870 ($C_{30}H_{50}O_2$) (31), $[M-H_2O]^+$ 424.3714 ($C_{30}H_{48}O$) (55), $[M-H_2O-Me]^+$ 409.3500 ($C_{29}H_{45}O$) (48), $[M-2H_2O]^+$ 406.3615 ($C_{30}H_{46}$) (18), $[M-2H_2O-Me]^+$ 391.3387 ($C_{29}H_{43}$) (18), $[M-C_3H_9O]^+$ 381.3168 ($C_{27}H_{41}O$) (14), $[M-C_3H_9O-H_2O]^+$ 363.3071 ($C_{27}H_{39}$) (6), $[M-H_2O-C_5H_9]^+$ 355.300 ($C_{25}H_{41}O$) (14), $[M-C_6H_{12}O]^+$ 342.2957 ($C_{24}H_{38}O$) (10), $[M-C_8H_{15}O]^+$ 315.2702 ($C_{22}H_{35}O$) (11), $[M-C_8H_{17}O]^+$ 313.2564 ($C_{22}H_{33}O$) (10), $[M-C_9H_{16}O]^+$ 302.2650 ($C_{21}H_{34}O$) (38), $[M-C_8H_{17}O-H_2O]^+$ 295.2425 ($C_{22}H_{31}$) (12), $[M-C_9H_{16}O-H_2O]^+$ 284.2525 ($C_{21}H_{32}$) (11), $[M-C_9H_{16}O-H_2O-Me]^+$ 269.2275 ($C_{20}H_{29}$) (9), $[M-C_9H_{16}O-C_8H_{17}O]^+$ 175.1489 ($C_{13}H_{19}$) (31); ^{1}H-nmr ($CDCl_3$) δ 5.60 (dd, *J* = 7.1, 15.1 Hz, H-22), 5.59 (dt, *J* = 6.5, 14.8 Hz, H-23), 3.34 (t, *J* = 2.6 Hz, H-3), 1.30 and 1.31 (s, H_3-26 and H_3-27), 0.97 (s, H_3-30), 0.96 (s, H_3-18), 0.88 (s, H_3-28), 0.86 (s, H_3-29), 0.84 (d, *J* = 6.4 Hz, H_3-21), 0.30 and 0.50 (AB quartet, *J* = 4.5 Hz, H_2-19); ^{13}C-nmr ($CDCl_3$) C-1 (26.46), C-2 (28.09), C-3 (76.88), C-4 (40.51), C-5 (47.13), C-6 (21.12), C-7 (27.41), C-8 (47.98), C-9 (20.01), C-10 (26.13), C-11 (26.01), C-12 (35.60), C-13 (45.34), C-14 (48.86), C-15 (32.81), C-16 (31.98), C-17 (52.04), C-18 (18.08), C-19 (29.88), C-20 (37.41), C-21 (18.29), C-22 (125.67), C-23 (139.44), C-24 (39.06), C-25 (70.77), C-26 (29.88), C-27 (29.99), C-28 (19.30), C-29, (19.00), C-30 (25.45).

ACETYLATION OF **1**.—Compound **1** (25 mg) was refluxed with Ac_2O (10 ml) in pyridine (5 ml) for 45 min. Usual workup provided diacetate **2** (21.8 mg), which was recrystallized from EtOAc-MeOH (1:1): mp 155°; $[\alpha]_D$ +34.5° (*c* = 0.127, $CHCl_3$); ir ($CHCl_3$) 1735–1720 cm^{-1} (broad

band); ms *m/z* (rel. int.) $[M]^+$ 526, $[M-HOAc]^+$ 466 (23), $[M-HOAc-Me]^+$ 451 (12), $[M-2HOAc]^+$ 406 (28), $[M-2HOAc-Me]^+$ 391 (15), $[M-C_{10}H_{17}O_2+2H]^+$ 355 (13), $[M-C_{10}H_{17}O_2]^+$ 357 (15), $[M-C_{11}H_{18}O_2]^+$ 344 (15), $[M-C_{11}H_{18}O_2-HOAc]^+$ 284 (10), $[M-C_{11}H_{18}O_2-HOAc-Me]^+$ 269 (18), $[M-C_{10}H_{19}O_2-C_{11}H_{18}O_2]^+$ 175 (50).

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