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Mohammad Ataullah Khan, Shaikh Sirajuddin Nizami, Muhammad Najmul Islam Khan, Syed Wasif Azeem, and Zaheer Ahmed

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NEW TRITERPENES FROM *MANGIFERA INDICA*

MOHAMMAD ATAULLAH KHAN,* SHAIKH SIRAJUDDIN NIZAMI,
MUHAMMAD NAJMUL ISLAM KHAN, SYED WASIF AZEEM,
and ZAHEER AHMED

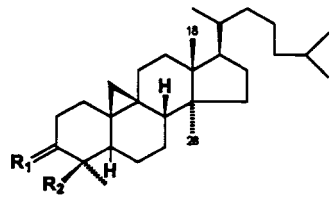
Department of Chemistry, University of Karachi, Karachi-75270, Pakistan

ABSTRACT.—Two new cycloartane-type triterpenes have been isolated from the roots of *Mangifera indica*. Their structures were determined as cycloartan-3β,30-diol and cycloartan-30-ol, respectively, on the basis of chemical and spectroscopic methods.

Earlier investigations of *Mangifera indica* L. (Anacardiaceae) have led to the isolation of alkylgallates, amino acids, sugars, biflavones, and saponins (1–5). The present study on the roots of this species has yielded two new triterpenes, **1** and **2**.

Compound **1** showed an $[M]^+$ peak at *m/z* 444.3928 (hrms), which corresponded to the molecular formula $C_{30}H_{52}O_2$ (calcd 444.3954) and indicated that **1** was a triterpene with five double-bond equivalents. The ir spectrum revealed the presence of an OH group (3460 and 3430 cm^{-1}), a cyclopropane ring (3040 cm^{-1}), and a *gem*-dimethyl group (1380 cm^{-1}). Additional bands between 1180 and 930 cm^{-1} agreed with a 3β-OH, 5α-structure (6).

The ^{1}H-nmr spectrum ($CDCl_3$, 300 MHz) of **1** showed signals due to three secondary methyl groups (doublets at δ 0.90, J=7.18 Hz and δ 0.87, J=6.72 Hz), three tertiary methyl groups (singlets at δ 0.95, 0.92, and 0.88) and characteristic doublets at δ 0.56 (J=4.18 Hz) and δ 0.33 (J=4.26 Hz) for non-equivalent protons of a cyclopropyl methylene group. In addition, there were signals due to a methine proton attached to a carbon bearing a hydroxyl group (δ 3.27, 1H, dd, $J_{a,a}$=11.21 Hz and $J_{a,e}$=4.32 Hz) and a methylene group of a primary alcohol with no proton on the adjacent carbon atom (δ 3.64, 2H, ABq, J=12.78 Hz). The ^{13}C-nmr spectrum showed 30 carbon atoms. The multiplicity of each carbon atom was determined using DEPT experiments (7,8) which revealed 6 methyl, 13 methylene, and 6 methine carbon atoms. The number of quaternary carbons was determined by subtracting these from the broad-band ^{13}C-nmr spectrum.



1 R_1=αH, βOH R_2=CH_2OH
1a R_1=αH, βOAc R_2=CH_2OAc
1b R_1=O R_2=CHO

Both the ^{1}H- and ^{13}C-nmr spectra indicated the presence of one primary and one secondary hydroxyl group in **1**, which were confirmed by acetylation of **1** to a diacetate **1a** and oxidation with Jones reagent to **1b** bearing both aldehydic and ketonic groups.

The mass spectrum of **1** was characteristic of cycloartane-type triterpenes (9,10). The genesis of various fragments was confirmed by link-scan measurements. The strain imposed on ring B was relieved by opening of the 9,10-bond followed by cleavage of the 5,6-bond and McLafferty rearrangement. The characteristic ion at *m/z* 288.2798 ($C_{21}H_{36}$) represented the fragmentation inducted by a cyclopropane ring in 9,19-cyclosterols and related tetracyclic triterpenes (11). Loss of the side-chain from this ion gave another fragment at *m/z* 175.14161 ($C_{13}H_{19}$). On the other hand, the direct loss of the side-chain from the parent ion

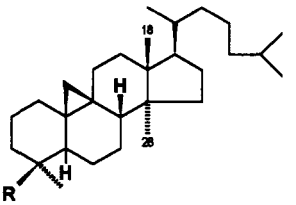
gave a fragment at *m/z* 331.2601 ($C_{22}H_{35}O_2$). Further ions at *m/z* 429.3689 ($C_{29}H_{49}O_2$), 426.3837 ($C_{30}H_{50}O$), and 411.3597 ($C_{29}H_{47}O$) originated by the loss of a methyl radical, H_2O and methyl plus H_2O, respectively, from the molecular ion. It was observed that all of these fragment ions were formed through the same route in cycloartanol (9,10), which helped to show that compound **1** differs from cycloartanol only in having one additional hydroxyl group.

The ions at *m/z* 331.2601 and 175.1461 suggested that both hydroxyl groups are present in rings A and B. This conclusion was further supported by nmr chemical shifts of various carbon atoms of rings C and D and the side-chain, which were in close agreement with those of cycloartanol.

On biogenetic grounds and by ^{1}H-^{1}H-correlated nmr spectroscopy, the secondary hydroxyl group was assigned to C-3. The carbinylic proton at δ 3.27 showed cross-peaks with two other protons limiting it to either position 1 or 3. However, the chemical shifts of C-1 through C-3 showed close correlations with those of cycloartanol providing evidence for the hydroxyl group being affixed to C-3 rather than C-1. The chemical shift and coupling constants of the carbinylic proton are in accord with its axial and α-orientation.

The remaining question was the position of the primary hydroxyl group. Careful comparison of the ms and ^{13}C-nmr spectrum of **1** with those of cycloartanol indicated that the primary hydroxyl group could be assigned to either C-29 or C-30. The latter proved to be correct by interpretation of ^{1}H-nmr data for the methylene group in **1** and **1a** which agreed with those for axially oriented-CH_2OH and -CH_2OAc groups, respectively (12). The ^{1}H-nmr spectrum of **1b** also showed a signal characteristic of an aldehydic proton at δ 9.71, corresponding to an axial orientation (13). The long-range ^{1}H-^{13}C-correlated spectrum (COLOC) of **1b** showed a cross-peak between the aldehydic carbon and the methyl protons of C-29. Hence the hydroxymethyl group in **1** was assigned to C-30 in a β- and axial orientation. The stereostructure of this compound is therefore represented as **1**.

Compound **2** showed an $[M]^+$ peak at *m/z* 428.3977 (hrms) corresponding to a molecular formula of $C_{30}H_{52}O$ (calcd 428.4005), indicating five double-bond equivalents in the molecule. The ir spectrum showed an OH group (3420 cm^{-1}), a cyclopropane ring (3040 cm^{-1}), and a *gem*-dimethyl group (1375 cm^{-1}).



2 R=CH_2OH
2a R=CH_2OAc
2b R=CHO

The ^{1}H-nmr spectrum of **2** showed signals for three secondary methyl groups (doublets at δ 0.90, J=6.98 Hz and δ 0.86, J=6.72 Hz) three tertiary methyl groups (singlets at δ 0.94, 0.91, and 0.88) besides the characteristic doublets at δ 0.56 (J=4.21 Hz) and δ 0.33 (J=4.28 Hz) for a cyclopropane ring. In addition, there was a signal due to a methylene group for a primary alcohol with no proton on the adjacent carbon atom (δ 3.62, 2H, ABq, J=12.18 Hz). The ^{13}C-nmr spectrum showed 30 carbon atoms whose multiplicities were determined by DEPT experiments (7,8), which revealed the presence of 6 methyl, 14 methylene, and 5 methine carbon atoms. Both the ^{1}H- and ^{13}C-nmr spectra showed one primary hydroxyl group in **2** which was confirmed by acetylation of **2** to a monoacetate **2a** and oxidation with Jones reagent to **2b**.

The mass spectrum of **2** was also characteristic of cycloartane-type triterpenes (9,10). The diagnostic ions at *m/z* 288.2796 ($C_{21}H_{36}$) and 175.1462 ($C_{13}H_{19}$) indicated that compound **2** is a tetracyclic triterpene (11). Further ions at *m/z* 315.2651 ($C_{22}H_{35}O$), 413.3738 ($C_{29}H_{49}O$), 410.3886 ($C_{30}H_{50}$) and 395.3645 ($C_{29}H_{47}$) were generated by the loss of side-chain, methyl radical, H_2O and methyl plus H_2O, respectively, from the molecular ion. All of these fragments have compositions similar to those observed for the corresponding ions of cycloartanol, demonstrating that compound **2** is an isomer of cycloartanol, the former differing from the latter in having a primary alcoholic group in ring A. This was further supported by chemical shifts of various carbon atoms of rings B, C, D, and the side-chain, which showed close agreement with those of cycloartanol.

On the basis of ms and ^{13}C-nmr data it was concluded that the hydroxymethyl group in **2** is at C-29 or C-30. The latter was proved to be correct by analysis of the ^{1}H-nmr data of **2** and **2a**, which agreed with the presence of an axial -CH_2OH and -CH_2OAc group, respectively (12). The aldehydic proton at δ 9.72 in **2b** also confirmed axial orientation (13). The COLOC experiment revealed a cross-peak between carbon and methyl protons of C-29. The hydroxymethyl group in **2** was therefore assigned to C-30 in a β-axial orientation and the stereostructure of compound **2** is as shown in **2**.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—The ir spectra were recorded in $CHCl_3$. DEPT nmr experiments were carried out with θ=45°, 90°, and 135°, with quaternary carbons being determined by subtraction of these spectra from the respective broad-band ^{13}C-nmr spectra. The hrms were recorded on a double-focusing instrument coupled to a PDP 11/34 computer system. The ^{1}H-nmr spectra ($CDCl_3$) were recorded at 300 MHz with TMS as internal reference. The 2D COSY-45 experiments were acquired at 300 MHz with a sweep width of 4000 Hz (2K data points in w_2) and 2000 Hz (256 t_1 values zero-filled to 1K) in w_1. The heteronuclear two-dimensional ^{1}H-^{13}C chemical shift correlation experiments were carried out at 300 MHz with a sweep width of 12820 Hz (2K data point in w_2) and 1024 Hz (256 t_1 values zero-filled to 2 K) in w_1. In both the 2D nmr experiments a 2 sec relaxation delay was used and 16 transients were performed for each t_1 value.

PLANT MATERIAL.—*Mangifera indica* roots were collected from the Karachi region and were identified by the Department of Botany, University of Karachi. A voucher specimen has been deposited in the herbarium of the Department of Botany, University of Karachi (voucher No. KUH 4378).

EXTRACTION AND ISOLATION.—The air-dried roots (2 kg) were crushed into small pieces and then extracted three times with MeOH (total 5 liters). The combined MeOH extract was evaporated under reduced pressure to afford a gummy residue which was partitioned between hexane and H_2O. The residue recovered from the hexane fraction was chromatographed over activated Si gel and elution was carried out with solvent gradients of increasing polarity consisting of hexane, hexane/$CHCl_3$, $CHCl_3$, $CHCl_3$/MeOH, and MeOH. The hexane-$CHCl_3$ eluents (4.5:5.5 and 4:6) yielded a crystalline residue which, on repeated crystallization from a mixture of $CHCl_3$ and MeOH, provided colorless fine needles of compound **2** (16 mg) and compound **1** (18 mg), respectively.

Cycloartan-3β,30-diol [**1**].—Mp 196–198°; $[\alpha]_D$ +34.9° (*c*=0.18, $CHCl_3$); ir, see text; ^{1}H nmr ($CDCl_3$, 300 MHz) δ 3.64 (2H, ABq, *J*=12.78 Hz, H_2-30), 3.27 (1H, dd $J_{a,a}$=11.21 Hz $J_{a,e}$=4.32 Hz, H-3), 0.95 (3H, s, Me-18), 0.92 (3H, s, Me-29), 0.90 (6H, d, *J*=7.18 Hz, Me-26 and -27), 0.88 (3H, s, Me-28), 0.87 (3H, d *J*=6.72 Hz, Me-21), 0.56 and 0.33 (2H, dd, *J*=4.18 and 4.26 Hz, H_2-19); ^{13}C nmr ($CDCl_3$, 75.43 MHz) δ 31.91 (C-1), 30.33 (C-2), 78.60 (C-3), 39.61 (C-4), 47.08 (C-5), 21.00 (C-6), 28.08 (C-7), 47.84 (C-8), 20.10 (C-9), 26.20 (C-10), 26.01 (C-11), 35.62 (C-12), 45.13 (C-13), 48.79 (C-14), 32.71 (C-15), 26.51 (C-16), 52.21 (C-17), 17.91 (C-18), 29.81 (C-19), 36.01 (C-20), 18.30 (C-21), 36.42 (C-22), 24.01 (C-23), 39.41 (C-24), 28.21 (C-25), 22.51 (C-26), 22.71 (C-27), 19.32 (C-28), 22.01 (C-29), and 63.14 (C-30); ms *m/z* 444, 429, 426, 411, 331, 288, 175.

ACETYLATION OF **1**.—Compound **1** (5 mg) was refluxed with Ac_2O (2.5 ml) in pyridine (1 ml) for 30 min. The reaction mixture was worked up in the usual manner to yield acetate **1a** (3.7 mg) that was crystallized from $CHCl_3$/MeOH, mp 212–214°; $[\alpha]_D$ +31.2° (*c*=0.12, $CHCl_3$); ir ν max 3040, 1720, 1380, and 1220 cm^{-1}; ^{1}H nmr ($CDCl_3$, 300 MHz) δ 3.94 (2H, ABq, *J*=12.76 Hz, H-30), 3.91 (1H, dd, $J_{a,a}$=11.28 Hz, and $J_{a,e}$=4.18 Hz, H-3), 2.13 (3H, s, OAc), 2.10 (3H, s, OAc), 0.95 (3H, s, Me-18), 0.93 (3H, s, Me-29),

0.90 (6H, d, J=7.12 Hz, Me-26 and -27), 0.88 (3H, s, Me-28), 0.87 (3H, d, J=6.81 Hz, Me-21), 0.57 and 0.34 (2H, dd, J=4.19 and 4.24 Hz, H-19); ms *m/z* 532, 517, 472, 412, 288, 175.

OXIDATION OF **1**.—Compound **1** (7 mg) was dissolved in $CHCl_3$ and then treated with Jones reagent (2.5 ml) (prepared by dissolving 5 mg CrO_3 in 1 ml H_2SO_4 and diluting with 2 ml H_2O). The reaction mixture was stirred at room temperature for 24 h, diluted with H_2O, and extracted with $CHCl_3$. Removal of solvent yielded **1b**, mp 220–222°; $[\alpha]_D$ +28.17° (c=0.14, $CHCl_3$); ir ν max 3040, 2850, 2750, 1720–1695, and 1380 cm^{-1}; ^1H nmr ($CDCl_3$, 300 MHz) δ 9.71 (1H, s, -CHO), 0.98 (3H, s, Me-29), 0.95 (3H, s, Me-18), 0.90 (6H, d, J=7.18 Hz, Me-26 and -27), 0.88 (3H, s, Me-28), 0.86 (3H, d, J=6.95 Hz, Me-21), 0.57 and 0.34 (2H, dd, J=4.21 and 4.26 Hz, H_2-19); ms *m/z* 440, 425, 288, 175.

Cycloartan-30-ol [**2**].—Mp 180–182°; $[\alpha]_D$ +27.38° (c=0.21, $CHCl_3$); ir, see text; ^1H nmr ($CDCl_3$, 300 MHz) δ 3.62 (2H, ABq, J=12.18 Hz, H_2-30), 0.94 (3H, s, Me-18), 0.91 (3H, s, Me-29), 0.90 (6H, d, J=6.98 Hz, Me-26 and -27), 0.88 (3H, s, Me-28), 0.86 (3H, d, J=6.72 Hz, Me-21), 0.56 and 0.33 (2H, dd, J=4.21 and 4.28 Hz, H_2-19); ^{13}C nmr ($CDCl_3$, 75.43 MHz) δ 31.91 (C-1), 30.34 (C-2), 29.84 (C-3), 39.80 (C-4), 47.09 (C-5), 21.01 (C-6), 28.10 (C-7), 47.84 (C-8), 20.10 (C-9), 26.21 (C-10), 26.02 (C-11), 35.62 (C-12), 45.13 (C-13), 48.79 (C-14), 32.71 (C-15), 26.52 (C-16), 52.21 (C-17), 17.91 (C-18), 29.81 (C-19), 36.02 (C-20), 18.30 (C-21), 36.42 (C-22), 24.01 (C-23), 39.42 (C-24), 28.21 (C-25), 22.51 (C-26), 22.71 (C-27), 19.33 (C-28), 22.03 (C-29), and 62.98 (C-30); ms *m/z* 428, 413, 410, 395, 315, 288, 175.

ACETYLATION OF **2**.—Compound **2** (5 mg) was refluxed with Ac_2O (2.5 ml) in pyridine (1 ml) for 30 min. The reaction mixture was worked up in the usual manner to yield acetate **2a** (3.5 mg) which was crystallized from $CHCl_3$/MeOH, mp 198–200°; $[\alpha]_D$ +24.2° (c=0.14, $CHCl_3$); ir ν max 3045, 1710, 1375, and 1220 cm^{-1}; ^1H nmr ($CDCl_3$, 300 MHz) δ 3.24 (2H, ABq, J=12.78 Hz, H_2-30), 2.12 (3H, s, OAc), 0.95 (3H, s, Me-18), 0.92 (3H, s, Me-29), 0.90 (6H, d, J=6.99 Hz, Me-26 and -27), 0.87 (3H, s, Me-28), 0.86 (3H, d, J=6.81 Hz, H_2-19); ms *m/z* 470, 410, 455, 288, 175.

OXIDATION OF **2**.—Compound **2** (7 mg) was dissolved in $CHCl_3$ and then treated with Jones reagent (2.5 ml). The reaction mixture was stirred at room temperature for 24 h, diluted with H_2O, and extracted with $CHCl_3$. Removal of solvent yielded **2b**, as colorless needles, mp 204–206°; $[\alpha]_D$ +21.46° (c=0.17, $CHCl_3$); ir ν max 3040, 2850, 2750, 1710, and 1375 cm^{-1}; ^1H nmr ($CDCl_3$, 300 MHz) δ 9.72 (1H, s, -CHO), 0.97 (3H, s, Me-29), 0.95 (3H, s, Me-18), 0.90 (6H, d, J=7.18 Hz, Me-26 and -27), 0.88 (3H, s, Me-28), 0.87 (3H, d, J=7.02 Hz, Me-21), 0.52 and 0.33 (2H, dd, J=4.22 and 4.28 Hz, H_2-19); ms *m/z* 426, 398, 411, 288, 175.

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