Journal of Natural Products
Vol. 53, No. 5, pp. 1342-1344, Sep-Oct 1990

A NEW PENTACYCLIC TRITERPENE FROM *ABUTILON PAKISTANICUM*

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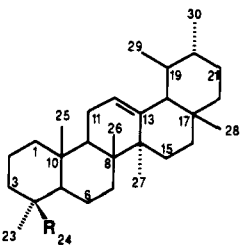
ABSTRACT.—A new ursene type triterpene, pakistanol [**1**], was isolated from *Abutilon pakistanicum*. Its structure has been assigned as urs-12(13)-en-24β-ol on the basis of chemical and spectral studies. Taraxasterol, β-sitosterol, and α-amyrin were also isolated.

The genus *Abutilon* (Malvaceae) comprises about 120 species and is distributed throughout the tropical and subtropical regions. A large number of these are used in indigenous medicine for the treatment of rheumatism and as demulcents, emollients, and diuretics (1). Some are also prescribed for fevers as cooling medicines (1). This has prompted us to carry out phytochemical studies on *Abutilon pakistanicum* Jafri and Ali (syn. *Abutilon cornutum* Cooke) which is endemic to Pakistan (2). The only reference available in the literature on this species describes the isolation of ceryl alcohol, α-sitosterol, and a mixture of α- and β-amyrins and chromatographic detection of phenolic acids, carbohydrates, and amino acids (3). This manuscript describes the isolation of taraxasterol, β-sitosterol, α-amyrin, and a new triterpene of the ursene series named pakistanol. All were isolated from the hexane-soluble fraction of the total plant extract according to the procedure described in the Experimental section.

RESULTS AND DISCUSSION

Pakistanol [**1**] crystallized as colorless needles from a mixture of $CHCl_3$ and MeOH, mp 112-114°; $[\alpha]^{20}D$ 32.96° ($c = 0.34$, $CHCl_3$). Its ir spectrum showed absorptions at 3450 cm^{-1} (OH group), 3050, 1650, and 810 cm^{-1} (trisubstituted double bond). The hrms gave a molecular ion peak at *m/z* 426.3827 consistent with the molecular formula $C_{30}H_{50}O$ (calcd 426.3849), indicating six double bond equivalents in the molecule. The molecular ion peak was also confirmed by fd ms. The presence of a primary alcoholic group was shown by the acetylation of **1** to a monoacetate **2** and oxidation with Jones' reagent to an aldehyde **3**. The ^{1}H-nmr spectrum ($CDCl_3$, 400 MHz) showed signals for a proton on a double bond (dd at δ 5.36, $J = 11.78$ Hz and $J = 6.53$ Hz), two secondary methyl groups (doublets at 0.89, $J = 7.00$ Hz and δ 1.01, $J = 6.00$ Hz), five tertiary methyls (singlets at δ 0.83, 0.87, 0.98, 1.02, and 1.06), and a methylene group (δ 3.62, 2H, ABq, $J = 13.21$ Hz) of a primary alcohol with no proton on the adjacent carbon atom. This was further confirmed by the ^{1}H-nmr spectrum of **2**; it similarly showed a two-proton quartet, but that was downfield at δ 3.95. The ^{13}C-nmr spectrum showed 30 carbon atoms; the multiplicities of these were determined by using DEPT experiments (4,5), which revealed the presence of 7 methyl, 11 methylene, and 6 methine carbon atoms.

The mass spectrum of **1** was characteristic of pentacyclic triterpenes of the Δ^{12}-unsaturated ursene series. The



1 R=CH_2OH
2 R=CH_2OAc
3 R=CHO

genesis of various fragments was confirmed by link-scan measurements. The characteristic ions [a] *m/z* 218.2050 ($C_{16}H_{26}$) and [b] *m/z* 208.2057 ($C_{14}H_{24}O$) were generated by a retro-Diels-Alder reaction in ring C (6). Further ions [c] and [d] at *m/z* 203.1795 ($C_{15}H_{23}$) and 204.1860 ($C_{15}H_{24}$) originated from ion [a]. The ion [e] at *m/z* 205.1941 ($C_{15}H_{25}$) resulted directly from the molecular ion whereas the ion [f] at *m/z* 133.0190 ($C_{10}H_{13}$) originated from ion [c]. The ion [g] at *m/z* 207.1739 ($C_{14}H_{23}O$) resulted from the molecular ion peak, and further loss of a H_2O molecule from this ion gave fragment [h] at *m/z* 189.1628 ($C_{14}H_{21}$). All of these fragments have compositions similar to those observed for the corresponding ions of α-amyrin (7), showing that pakistanol is an isomer of α-amyrin, the former differing from the latter in having a primary alcoholic function on rings A or B. This is further supported by chemical shifts of various carbon atoms of rings C, D, and E, which showed close agreement to those of α-amyrin. The absence of species at $[M-31]^+$ for **1** and $[M-73]^+$ for **2** showed that the primary hydroxyl group in **1** is not at an angular position. It must therefore be assigned to C-23 or C-24. The latter was proven to be correct by the ^{1}H-nmr data for the methylene groups in **1** and **2**, which agreed with those for axial-CH_2OH and -CH_2OAc, respectively (8). The ^{1}H-nmr spectrum of **3** showed the signal of an aldehydic proton at δ 9.71 corresponding to axial orientation (9). The long range ^{1}H-^{13}C correlated spectrum (COLOC) showed a cross peak between the aldehydic carbon and the methyl protons of C-23, confirming the presence of the aldehydic group in **3** and hence the hydroxymethyl group in **1** in β and axial orientation.

To the best of our knowledge this is the first instance of a natural occurrence of a monooxygenated triterpene with a primary hydroxyl group. The related compounds reported in the literature carry additional hydroxy or other functional groups (10,11).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—The ir spectra were recorded on a JASCO A-302 spectrometer. Hrms and fdms were recorded 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 in $CDCl_3$ with TMS as internal reference. The DEPT experiments were carried out with the last pulse angle θ = 45°, 90°, and 135°. The quaternary carbons were determined by subtraction of these spectra from the broad-band ^{13}C-nmr spectrum.

PLANT MATERIAL.—The plant material was collected in Karachi, Pakistan, and was identified by the plant taxonomist, Department of Botany, University of Karachi, where a voucher specimen is deposited (voucher no. 697 KUH).

ISOLATION OF THE COMPOUNDS.—The freshly collected plant material (10 kg) was chopped into small pieces and then extracted thrice with MeOH (40 liters). The combined MeOH extract was evaporated under reduced pressure, and the resulting residue was partitioned with H_2O and hexane. The hexane fraction (16 g) was chromatographed over Si gel (600 g) and successively eluted with increasing polarities of a mixture of hexane and $CHCl_3$. The hexane-$CHCl_3$ (9:1 and 8:2) eluents yielded crystalline residues, which on repeated crystallization from hexane/$CHCl_3$ provided β-sitosterol and α-amyrin, respectively. These were identified through comparison of physical and spectral data with those reported in the literature (12,13). The eluent obtained from hexane-$CHCl_3$ (3:7) was found to be a binary mixture of triterpenes, which could be separated through flash chromatography over Si gel hexane-$CHCl_3$ (4:6) as eluent. One of these melted at 224–226° and showed $[\alpha]^{20}D$ 94.8° (*c* = 0.18, $CHCl_3$). It was identified as taraxasterol by comparing the spectra with data reported in literature (14).

The other compound, pakistanol [**1**], crystallized from Et_2O/MeOH showing $[\alpha]^{20}D$ 32.96 (*c* = 0.34, $CHCl_3$); ir ($CHCl_3$) ν max cm^{-1} 3450, 3050, 1650, 810; fdms *m/z* 426; ms *m/z* (rel. int. %) $[M]^+$ 426 (12), 218 (65), 208 (25), 207 (15), 205 (4), 204 (3), 203 (20), 189 (12), 133 (15); ^{1}H nmr ($CDCl_3$, 400 MHz) δ 5.36 (1H, dd, *J* = 11.78 Hz, and *J* = 6.53 Hz, H-12), 3.62 (2H, ABq, *J* = 13.21 Hz, H_2-24), 1.06 (3H, s, Me-27), 1.02 (3H, s, Me-26), 1.01 (3H, d, *J* = 6.00 Hz, Me-29), 0.98 (3H, s, Me-23), 0.89 (3H, d, *J* = 7.00 Hz, Me-30), 0.87 (3H, s, Me-25), 0.83 (3H, s, Me-28); ^{13}C nmr ($CDCl_3$, 100.61 MHz) δ 37.47 (C-1), 25.10 (C-2), 24.50

(C-3), 38.10 (C-4), 49.21 (C-5), 22.67 (C-6), 32.86 (C-7), 41.51 (C-8), 47.50 (C-9), 35.51 (C-10), 24.82 (C-11), 123.13 (C-12), 140.20 (C-13), 42.80 (C-14), 25.78 (C-15), 25.19 (C-16), 33.18 (C-17), 59.44 (C-18), 42.06 (C-19), 39.42 (C-20), 31.95 (C-21), 39.90 (C-22), 24.78 (C-23), 63.18 (C-24), 14.12 (C-25), 16.20 (C-26), 19.77 (C-27), 28.01 (C-28), 22.73 (C-29), 22.64 (C-30). The assignments were made through comparison with published ^{13}C-nmr spectra of α-amyrin and related compounds (15) and confirmed by ^{1}H-^{13}C heteronuclear chemical shift correlated spectroscopy (Hetero-COSY).

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 **2**: hrms *m/z* 468.3931 ($C_{32}H_{52}O_2$ requires 468.3954); ms *m/z* (rel. int. %) $[M]^+$ 468 (20), 453 (12), 409 (45); ^{1}H-nmr ($CDCl_3$, 400 MHz) δ 5.38 (H-12), 3.95 (2H, ABq, J = 13.21 Hz, H_2-24), 2.11 (3H, s, OAc).

OXIDATION OF **1**.—Compound **1** (7.0 mg) was dissolved in $CHCl_3$ and treated with 2.5 ml Jones reagent (16) (prepared by dissolving 5.0 mg chromic oxide in 1.0 ml H_2SO_4 and diluting with 2.0 ml H_2O). The reaction mixture was stirred at room temperature for 24 h. It was then diluted with H_2O and extracted with $CHCl_3$. The removal of solvent from the $CHCl_3$ extract yielded **3** as colorless needles from MeOH: mp 144–146°; hrms *m/z* 424.3669 ($C_{30}H_{48}O$ requires 424.3693); ms *m/z* (rel. int. %) $[M]^+$ 424 (14), 396 (20); ir ($CHCl_3$) ν max cm^{-1} 2850, 1710; ^{1}H nmr ($CDCl_3$, 400 MHz) δ 9.71 (1H, s, *CH*O), 5.38 (1H, dd, J = 11.78 Hz and J = 6.53 Hz, H-12), 1.07 (3H, s, Me-27), 1.03 (3H, s, Me-26), 1.02 (3H, d, J = 6.00 Hz, Me-29), 1.00 (3H, s, Me-23), 0.90 (3H, d, J = 7.01 Hz, Me-30), 0.88 (3H, s, Me-25), 0.84 (3H, s, Me-28).

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Received 16 October 1989