PERGAMON

Phytochemistry 50 (1999) 647–651

PHYTOCHEMISTRY

Withanolides from *Physalis peruviana*

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Revised 4 August 1998

Abstract

Two new withanolides isolated from the whole plant material of *Physalis peruviana* (Solanaceae) have been characterized as (20*R*,22*R*)-5α,6β,14α,20,27-pentahydroxy-1-oxowith-24-enolide, and (20*S*,22*R*)-5β,6β-epoxy-4β,14β,15α-trihydroxy-1-oxowith-2,24-dienolide in addition to the known withanolides, withaphysanolide and viscosalactone B on the basis of spectroscopic techniques and chemical transformations.

Keywords: *Physalis peruviana*; Solanaceae; Withanolides; Withaphysanolide; Viscosalactone B; (20*R*,22*R*)-5α,6β,14α,20,27-pentahydroxy-1-oxowith-24-enolide; (20*S*,22*R*)-5β,6β-epoxy-4β,14β,15α-trihydroxy-1-oxowith-2,24-dienolide

1. Introduction

The broad spectrum of biological properties exhibited by withanolides is responsible for the undiminishing interest in them (Ray & Gupta, 1994). The genus *Physalis* is reputed for elaborating C-28 steroids with structural diversity. It includes about 120 species growing mainly in South and North America, while a small number of species have also been recorded in Europe and in the countries of Southeastern and Central Asia (Shishkin & Bobrov, 1955). Three species of *Physalis* are found in Pakistan (Nasir & Ali, 1985). *Physalis peruviana* Linn., commonly known as 'Capegooseberry', is a tropical hairy plant with fuzzy, slender-pointed, heart-shaped leaves, bearing yellowish flowers and orange edible fruits (Nasir & Ali, 1985; Bailey, 1977). The plant is diuretic and juice of its leaves is given in worm and bowel complaints, while heated leaves are applied as a poultice (Publication and Information Directorate, 1969). An extract of the leaves shows antibiotic activity against *Staphylococcus* (Perry & Metzger, 1980). The presence of a number of withanolides in different parts of this plant has been reported (Ray & Gupta, 1994). In the present paper, we wish to describe the isolation and characterization of two new withanolides **1** and **2** along with two reported withanolides, withaphysanolide (Abdullaev, Maslennikova, Tursunova & Abukakrov, 1984) and viscosalactone B (Pelletier, Gebeyehu, Nowacki & Mody, 1981).

2. Results and discussion

Compound **1**, $C_{28}H_{42}O_8$, isolated as an amorphous solid, showed UV absorption maximum at 215 nm, characteristic of an α,β-unsaturated lactone chromophore. Its IR spectrum displayed the presence of a hydroxyl (3450 cm^{-1}), an α,β-unsaturated δ-lactone (1715 cm^{-1}), and cyclohexanone (1705 cm^{-1}) moieties. The HRMS displayed characteristic peaks at *m*/*z* 506.2875 $[M]^+$, 488.2770 $[M-H_2O]^+$, 470.2662 $[M-2H_2O]^+$, 321.2063 [M–side chain]$^+$, 303.1962 [M–side chain–H_2O]$^+$, and the fragment at *m*/*z* 185.0816 of composition $C_9H_{13}O_4$, resulting by the cleavage of C-17/C-20 bond, indicated the presence of a hydroxyl group at C-20 while the peak at *m*/*z* 141.0553 of composition $C_7H_9O_3$ was due to a hydroxy-substituted α,β-unsaturated δ-lactone which originated by cleavage of the C-20/C-22 bond (Ramaiah, Lavie, Budhiraja, Sudhir & Garg, 1984). The molecular formula

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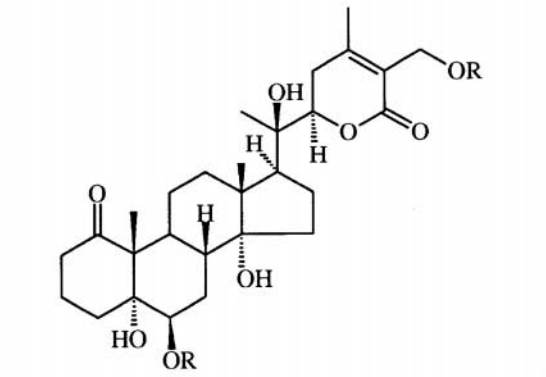
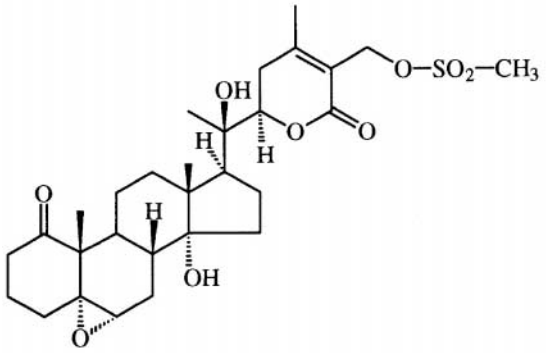
0031-9422/98 $ - see front matter
PII: S0031-9422(98)00567-6

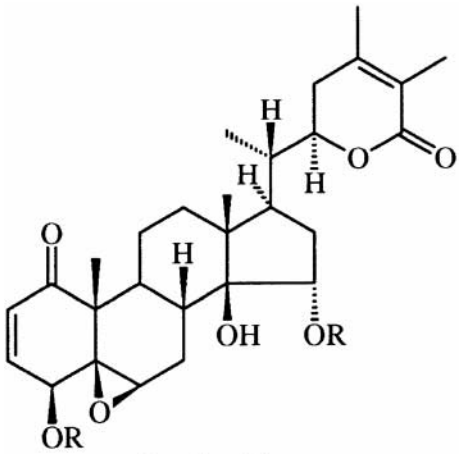


1 R = H
1a R = Ac



1b

(obtained from HRMS) indicated eight double bond equivalents which were in accordance with the assigned structure. The ^{1}H NMR spectrum of **1** did not show any olefinic signal and consequently the most common enone system in ring A of withanolides was absent (Chiu-Ming, Zong-Tsi, Chiu-Hsiang, Wen Sen & Say-Yee, 1990). It showed four methyl singlets assignable to three tertiary methyls (δ 1.30, 1.29, 1.05) and one vinylic methyl (δ 1.94). The missing vinylic methyl signal and the appearance of two AB doublets at δ 4.12 and 4.26, which moved downfield to δ 4.81 and 4.89 in the corresponding diacetate derivative (**1a**) suggested that C-27 was present as a hydroxymethyl. Acetylation also results in downfield shift of signal at δ 3.52 to 4.60 indicating the presence of one secondary hydroxyl group. The IR spectrum of **1a** still showed the hydroxyl absorption at 3500 cm^{-1}. Since all the rings equivalents have already been accounted for, the remaining three oxygen atoms must be present as tertiary hydroxyl groups. The secondary hydroxyl group was assigned to C-6 in β-configuration because of an appreciable downfield shift of 0.26 ppm suffered by the C(10)Me when ^{1}H NMR spectrum was repeated in pyridine-d_5 instead of $CDCl_3$ (Fajardo, Podesta, Shamma & Freyer, 1991). One of the tertiary hydroxyl groups was assigned to C-5 in α-configuration as the resonances of hydrogens/carbons of ring A and B were in complete agreement to those of withametelin C, a withanolide with a 5α,6β-dihydroxy-1-one moiety (Gupta, Manickam, Sinha, Bagchi & Ray, 1992). Further confirmation of the *trans*-fusion of the A/B ring system was provided by the ^{13}C NMR spectrum. The presence of a β-hydroxy function at C-5 causes an upfield shift of C(10)Me to around δ 8.0–9.6 because of *gauche*-interaction. However, no such upfield shift of C(10)Me was observed and its signal was observed at δ 19.1, thereby confirming *trans*-A/B ring junction (Fajardo et al., 1991). Mesylation followed by alkaline treatment resulted in the corresponding 5α,6α-epoxide derivative (**1b**) (Gupta et al., 1992), providing further evidence for 5α,6β-dihydroxy groupings in ring B. A downfield double doublet at δ 4.38 (J = 13.3, 3.4 Hz), assignable to H-22, showed ^{1}H–^{1}H COSY couplings to the vicinal protons at δ 2.45 and 2.55 (H_a-23 and H_b-23) further supporting the presence of a hydroxyl group at C-20. Another significant COSY interaction was observed between proton at δ 3.52 (H-6) with both protons at δ 1.75 (H_2-7). Of the two possible locations of the remaining tertiary hydroxyl group, the position C-17 is excluded because it would cause significant deshielding of C-20 (Glotter, Sahai, Kirson & Gottlieb, 1985) than actually found. Its location at C-14 in α-configuration was confirmed by comparing the ^{13}C NMR spectral data of compound **1** with corresponding 14β-hydroxy derivatives (Glotter et al., 1985). The 14α-hydroxy group deshields C-8 through a β-effect and shields C-7, C-9 and C-12 through γ-effect (Chiu-Ming et al., 1990; Gottlieb, Cojocaru, Sinha, Sahai, Bagchi et al., 1987), which is actually observed in the ^{13}C NMR spectrum of compound **1**. The position of hydroxyl at C-14 was further confirmed by HMBC experiment which showed a ^{2}J correlation of C-18 methyl protons (δ 1.05) to C-13 (δ 47.8) and ^{3}J to C-12 (δ 32.6), C-14 (δ 83.8) and C-17 (δ 50.3). The



2 R = H
2a R = Ac

C-21 methyl protons (δ 1.30) also showed ^2J correlation to C-20 (δ 76.9) and ^3J correlations to C-17 (δ 50.3) and C-22 (δ 81.1). The stereochemistry at C-20 and C-22 was assigned by comparison of chemical shifts with the related withanolides (Pelletier et al., 1981; Velde & Lavie, 1982; Vasina, Maslennikova, Abdullaev & Abubakirov, 1986).

The ^{13}C NMR spectrum of **1** showed signals for 28 carbons and their shift values corroborated the proposed structure. All the assignments were confirmed by HMQC and HMBC experiments and comparison with related withanolides (Pelletier et al., 1981; Gupta et al., 1992; Velde & Lavie, 1982; Vasina et al., 1986). Based on this evidence compound **1** was assigned the structure (20*R*,22*R*)-5α,6β,14α,20,27-pentahydroxy-1-oxowith-24-enolide.

Compound **2**, $C_{28}H_{38}O_7$, fine needles, mp 142–144°C, showed UV absorption at 224 nm. Its IR spectrum showed the presence of hydroxyl (3450 cm^{-1}), an α,β-unsaturated δ-lactone (1710 cm^{-1}) and α,β-unsaturated ketone (1690 cm^{-1}) functions. The high resolution mass spectrum displayed characteristic peaks at *m/z* 486.2613 $[M]^+$, 468.2510 $[M–H_2O]^+$, 361.2018 [M–δ-lactone]$^+$, 333.1707 [M–side chain]$^+$, 315.1591 [M–side chain–H_2O]$^+$, 153 [side chain]$^+$ and the base peak at *m/z* 125 due to cleavage of the C-20/C-22 bond which is a common characteristic peak for withanolides possessing the unsaturated δ-lactone system. The ^{1}H NMR spectrum exhibited signals due to two tertiary methyls (δ 1.36, 1.10), a secondary methyl (δ 1.01, *d*, *J* = 6.5 Hz) and two vinyl methyls (δ 1.95, 1.86). In addition signals at δ 6.19 (1H, *d*, *J* = 10.2 Hz, H-2) and δ 6.97 (1H, *dd*, *J* = 10.2, 5.6 Hz, H-3), could be attributed to the most common enone system of withanolides (Chiu-Ming et al., 1990). Moreover, signals of four oxymethine protons were observed at δ 4.32 (1H, *dt*, *J* = 12.5, 3.6 Hz, H-22), δ 3.96 (1H, *d*, *J* = 4.4 Hz, H-15), δ 3.76 (1H, *d*, *J* = 5.6 Hz, H-4) and δ 3.35 (1H, *m*, H-6). In view of its molecular formula, compound **2** must possess an additional tertiary hydroxyl which was confirmed through IR of its diacetate (**2a**) which still showed hydroxyl absorption at 3450 cm^{-1}. It was assigned to C-14 through HMBC experiment which revealed ^2J correlation of C-18 methyl protons (δ 1.10) to C-13 (δ 45.5) and ^3J to C-14 (δ 84.7) and C-17 (δ 51.17).

The ^{1}H NMR spectrum of **2a** showed two singlets at δ 2.00 and 2.06 due to two acetoxy groups and the expected downfield shifts of protons geminal to secondary hydroxyl groups. The diacetate was found to be identical to physapubenolide monoacetate confirming the presence of secondary hydroxyl groups at C-4 and C-15 in β and α-configurations, respectively. The *trans*-disposition of hydroxyls at C-14 and C-15 was further confirmed by non formation of acetonide under usual conditions. The compound **2** was therefore assigned structure (20*S*,22*R*)-5β,6β-epoxy-4β,14β,15α-trihydroxy-1-oxowith-2,24-dienolide (15-desacetylphysabubenolide). The ^{13}C NMR spectrum of compound **2** confirmed the presence of 28 carbons and their chemical shifts were in complete conformity to the assigned structure.

3. Experimental

3.1. General

Optical rotations were measured on a JASCO DIP-360 polarimeter. IR and UV spectra were recorded on a JASCO 302-A and on a Hitachi U 3200 spectrophotometers, respectively. EI, FAB and HRMS were recorded on JMS HX 110 with a data system and on JMS-DA 500 mass spectrometers. The ^{1}H, ^{13}C NMR, COSY, HMQC and HMBC spectra were recorded on Bruker spectrometers operating at 500 and 400 MHz. The chemical shift values are reported in ppm (δ) units and the coupling constants (*J*) are in Hz.

3.2. Chromatographic conditions

For TLC precoated aluminium sheets silica gel 60 F-254 (20×20 cm, 0.2 mm thick) (E. Merck) were used. Visualization of the TLC plates was achieved under UV at 254 and 366 nm and by spraying with saturated $CHCl_3$ soln of $SbCl_3$ (with heating) or Dragendorff's reagent. Solvent systems; *1*. $CHCl_3$–MeOH (9.5:0.5). *2*. $CHCl_3$–C_6H_6–MeOH (5:6:1).

3.3. Plant material

Physalis peruviana, whole plant, collected from District Chitral, N.W.F.P (Pakistan) in May, 1996 was identified by Mr Iftikhar Shah, Plant Taxonomist, Department of Pharmacy, Gomal University, D.I.Khan, where a voucher specimen is deposited in the Herbarium.

3.4. Extraction and isolation

The air-dried ground plant (30 kg) was exhaustively extracted with 90% EtOH at room temp. The extract was concentrated and the residue (1.5 kg) was dissolved in MeOH and defatted with petrol. The defatted MeOH extract was evaporated and partitioned between $CHCl_3$ and H_2O. The $CHCl_3$ extract was loaded on a silica gel column and eluted with *n*-hexane, *n*-hexane–$CHCl_3$, $CHCl_3$–MeOH mixtures gradually increasing polarity. The fractions obtained in $CHCl_3$–MeOH 9:1 were subjected to flash chromatography (fcc) on silica gel using EtOAc and increasing the polarity with MeOH. Fractions obtained from

EtOAc–MeOH (95:5) were combined and further purified through medium pressure liquid chromatography (mplc) using Lobar (LiChroprep Si 60, MERCK) column and $CHCl_3$–MeOH (97:3) as mobile phase. Final purification of the resulting fractions by TLC in solvent systems 1 and 2 afforded pure compounds **1** (40 mg), **2** (25 mg), withaphysanolide (20 mg) and viscosalactone B (24 mg).

3.5. Compound *1*

Amorphous powder (40 mg); $[\alpha]_D$ +72.5° ($c = 0.23$, MeOH); UV $\lambda_{max}^{(MeOH)}$ nm (log ϵ): 215 (3.99). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3450, 1715, 1705. (+)HRFABMS: Found, $[M + H]^+$ m/z 507.2954; Calcd. for $C_{28}H_{43}O_8$, [M + H] 507.2957; EIMS: m/z (rel. int.): M^+ 506 (3), 488 (4), 470 (6), 365 (4), 452 (7), 321 (9), 303 (11), 285 (4), 185 (39), 167 (37), 141 (35), 124 (100). ^1H NMR (500 MHz, $CDCl_3$): δ 4.38 (1H, *d* $J = 13.3$ and 3.4 Hz, H-22), 4.26 (1H, *d*, $J = 12.0$ Hz, H_a-27), 4.12 (1H, *d*, $J = 12.0$, Hz, H_b-27), 3.52 (1H, *s*, H-6), 1.94 (3H, *s*, Me-28), 1.30 (3H, *s*, Me-21), 1.29 (3H, *s*, Me-19),1.05 (3H, *s*, Me-18). ^{13}C NMR(125 MHz, $CDCl_3$): δ 214.7 (*s*, C-1), 164.2 (*s*, C-26), 156.5 (*s*, C-24), 121.9 (*s*, C-25), 83.8 (*s*, C-14), 81.1 (*d*, C-22), 76.9 (*s*, C-20), 78.7 (*s*, C-5), 75.5 (*d*, C-6), 57.9 (*t*, C-27), 50.3 (*d*, C-17), 52.3 (*s*, C-10), 47.8 (*s*, C-13), 40.8 (*t*, C-7), 39.9 (*d*, C-9), 34.2 (*d*, C-8), 34.1 (*t*, C-4), 32.8 (*t*, C-23), 32.6 (*t*, C-12), 32.0 (*t*, C-15), 21.3 (*t*, C-16), 30.4 (*t*, C-2), 27.2 (*t*, C-3), 23.1 (*t*, C-11), 20.8 (*q*, C-28), 20.0 (*q*, C-21), 19.1 (*q*, C-19), 18.3 (*q*, C-18).

3.6. Acetylation of *1*

Compound **1** (20 mg) was acetylated with Ac_2O (2 ml) in pyridine (2 ml) at room temp for 24 hr. Solvent was removed under vacuo and prep. TLC of the residue afforded **1a** (10 mg); $[\alpha]_D$ +60° ($c = 0.30$, MeOH); UV $\lambda_{max}^{(MeOH)}$ nm (log ϵ): 220 (3.98). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3500, 1730, 1710, 1690 cm^{-1}. (–)HRFABMS: Found, $[M-H]^-$ m/z 589.3013; Calcd. for $C_{32}H_{45}O_{10}$, [M-H] 589.3012; EIMS: m/z (rel. int.) M^+ 590 (2), 572 (1), 512 (2), 494 (4), 434 (3), 406 (2), 371 (3), 363 (1), 227 (4), 209 (5), 183 (25), 149 (2), 124 (100). ^1H NMR (400 MHz, $CDCl_3$): δ 4.87 (1H, *d*, $J = 12.0$ Hz, H_a-27), 4.83 (1H, *d*, $J = 12.0$ Hz, H_b-27), 4.60 (1H, *br s*, H-6), 4.22 (1H, *dd*, $J = 13.1$ and 3.3 Hz, H-22), 2.06, 2.04 (3H, *s* each, 2×OAc), 2.03 (3H, *s*, Me-28), 1.25 (3H, *s*, Me-21), 1.08 (3H, *s*, Me-19), 1.05 (3H, *s*, Me-18).

3.7. Epoxidation of *1*

It was achieved by treating compound **1** (10 mg) in pyridine (1 ml) with mesyl chloride (8 drops) and the mixture left overnight under anhydrous conditions. It was treated with methanolic alkali (2% 1.5 ml), diluted with H_2O and extracted with $CHCl_3$. Prep. TLC of the solvent free residue afforded **1b** (6 mg); EIMS m/z: 470 (M^+–$MeSO_3H$). ^1H NMR (400 MHz, $CDCl_3$): δ 4.94 (1H, *d*, $J = 11.5$ Hz, H_a-27), 4.90 (1H, *d*, $J = 11.5$ Hz, H_b-27), 4.32 (1H, *dd*, $J = 13.2$ and 3.4 Hz, H-22), 3.04 (1H, *d*, $J = 6$ Hz, H-6), 3.02 (3H, *s*, Me–SO_3–), 2.04 (3H, *s*, Me-28), 1.27 (3H, *s*, Me-21), 1.06 (3H, *s*, Me-19), 1.03 (3H, *s*, Me-18).

3.8. Compound *2*

Colourless needles (25 mg), $[\alpha]_D$ +68° ($c = 0.2$, MeOH); UV $\lambda_{max}^{(MeOH)}$ nm (log ϵ): 224 (4.15). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3500–3200, 1710, 1690 and 1020 cm^{-1}. (+)HRFABMS: Found, $[M + H]^+$ m/z 487.2685; Calcd. for $C_{28}H_{39}O_7$ [M + H] 487.2695; EIMS m/z (rel. int.) M^+ 486 (4),468 (8), 360 (6), 333 (12), 315 (16), 153 (28), 125 (100); ^1H NMR (500 MHz, $CDCl_3$): δ 6.97 (1H, *dd*, $J = 10.2$ and 5.6 Hz, H-3), 6.19 (1H, *d*, $J = 10.2$ Hz, H-2), 4.32 (1H, *dt*, $J = 12.5$ and 3.6 Hz, H-22), 3.96 (1H, *d*, $J = 4.4$ Hz, H-15), 3.76 (1H, *d*, $J = 5.6$ Hz, H-4), 3.35 (1H, *m*, H-6), 1.95 (3H, *s*, Me-28), 1.86 (3H, *s*, Me-27), 1.36 (3H, *s*, Me-19), 1.10 (3H, *s*, Me-18), 1.01 (3H, *d*, $J = 6.5$ Hz, Me-21). ^{13}C NMR(100 MHz, $CDCl_3$): δ 204.2 (*s*, C-1), 167.7 (*s*, C-26), 152.3 (*s*, C-24), 121.3 (*s*, C-25), 142.6 (*d*, C-3), 130.7 (*d*, C-2), 84.7 (*s*, C-14), 78.8 (*d*, C-15), 78.4 (*d*, C-22), 70.4 (*d*, C-4), 64.4 (*s*, C-5), 60.9 (*d*, C-6), 51.7 (*d*, C-17), 48.5 (*s*, C-10), 45.5 (*s*, C-13), 41.6 (*t*, C-12), 39.1 (*d*, C-8), 38.1 (*d*, C-20), 36.8 (*d*, C-9), 36.1 (*t*, C-16), 31.4 (*t*, C-23), 26.6 (*t*, C-7), 21.4 (*t*, C-11), 20.4 (*q*, C-28), 17.3 (*q*, C-21), 15.4 (*q*, C-19), 14.5 (*q*, C-18), 12.6 (*q*, C-27).

3.9. Acetylation of *2*

Acetylation was carried out (as described for **1**) to obtain the diacetate derivative **2a**, an amorphous solid, $[\alpha]_D$ +84°($c = 0.2$, $CHCl_3$). It was identified by comparison of its physical and spectroscopic data with that of physapubenolide monoacetate (Glotter et al., 1985).

References

Abdullaev, N. D., Maslennikova, V. A., Tursunova, R. N., & Abukakrov, N. K. (1984). *Khim. Prir. Soedin., 20,* 197.

Bailey, L. H. (1977) *Manual of cultivated plants* (p. 871). New York: Macmillan.

Chiu-Ming, C., Zong-Tsi, C., Chiu-Hsiang, H., Wen Sen, L., & Say-Yee, W. (1990). *Heterocycles, 31,* 1371.

Fajardo, V., Podesta, F., Shamma, M., & Freyer, A. J. (1991). *J. Nat. Prod., 54,* 554.

Glotter, E., Sahai, M., Kirson, I., & Gottlieb, H. E. (1985). *J. Chem. Soc. Perkin. Trans., I,* 2241.

Gottlieb, H. E., Cojocaru, M., Sinha, S. C., Sahai, M., Bagchi, A., Ali, A., & Ray, A. B. (1987). *Phytochemistry, 26,* 1801.

Gupta, M., Manickam, M., Sinha, S. C., Bagchi, A. S., & Ray, A. B. (1992). *Phytochemistry, 31,* 2423.

Pelletier, S. W., Gebeyehu, G., Nowacki, J., & Mody, N. V. (1981). *Heterocycles, 15,* 317.

Perry, L. M., & Metzger, J. (1980) *Medicinal plants of East and Southeast Asia* (p. 393). Cambridge, Massachusetts and London, England: The MIT Press.

Publication and Information Directorate (1969). *The wealth of India, vol. VII* (p. 38). New Delhi: CSIR.

Nasir, E., & Ali, S. I. (1985). *Flora of West Pakistan, vol. 168* (p. 24). Department of Botany, University of Karachi.

Ramaiah, P. A., Lavie, D., Budhiraja, R. D., Sudhir, S., & Garg, K. N. (1984). *Phytochemistry, 23,* 143.

Ray, A. B., & Gupta, M. (1994). *Prog. Chem. Org. Nat. Prod., 63,* 1–106.

Shishkin, B. K., & Bobrov, E. G. (1955). *Flora of the USSR, vol. 22* (p. 62). Moscow.

Vasina, O. E., Maslennikova, V. A., Abdullaev, N. D., & Abubakirov, N. K. (1986). *Khim. Prir. Soedin, 20,* 596.

Velde, V. V., & Lavie, D. (1982). *Phytochemistry, 21,* 731.