A New Withanolide Glycoside from *Physalis peruviana*

Saeed Ahmad,[†] Abdul Malik,*[,†] Nighat Afza,[‡] and Rehana Yasmin[§]

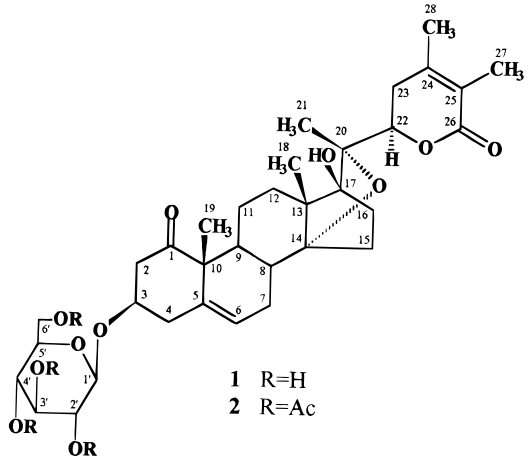
International Centre for Chemical Sciences, H.E.J. Research Institute of Chemistry, University of Karachi, Karachi-75270, Pakistan, Pharmaceutical Fine Chemical Research Centre, PCSIR Laboratories Complex, Karachi-75280, Pakistan, and Department of Chemistry, Gomal University, Dera Ismail Khan, N.W.F.P, Pakistan

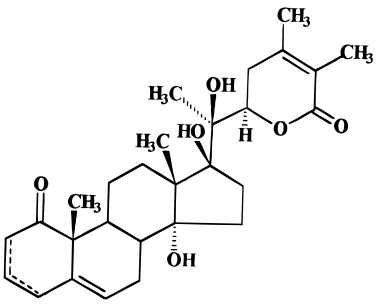
Received June 3, 1998

A new withanolide glycoside, 17β-hydroxy-14,20-epoxy-1-oxo-[22*R*]-3β-[*O*-β-D-glucopyranosyl]-witha-5,24-dienolide (**1**), has been isolated from the whole plant of *Physalis peruviana*. Its identity was determined using a combination of spectroscopic data including 2D NMR techniques and chemical transformations.

Withanolides are natural steroidal lactones produced mainly by plants in the *Solanaceae*. Such substances often have antimicrobial, antitumor, antiinflammatory, hepatoprotective, or immunomodulatory activity and insect antifeedent properties.[1] Due to our interest in biological properties of the glycosidic derivatives of these substances, we carried out bioassay-directed isolation of withanolides from *Physalis peruviana* L. These studies resulted in the isolation of a new withanolide glycoside with novel features. In the present paper, we report the isolation and characterization of this new withanolide glycoside (**1**).

Withanolide glycoside (**1**) was isolated from the aqueous methanolic fraction of an ethanolic extract of the whole plant by a combination of MPLC and preparative chromatography. The positive HRFABMS showed an $[M + H]^+$ peak corresponding to the formula $C_{34}H_{49}O_{11}$. Further peaks were observed at *m*/*z* 615 and 471 due to the loss of water and hexose moieties, respectively, from the parent ion. The UV spectrum was characteristic of an α,β-unsaturated δ-lactone with an intense band at 222 nm.[2] This was supported by the IR spectrum, which showed absorption bands at 1698, 1710, and 3500 cm^{-1} for a six-membered cyclic ketone, an α,β-unsaturated δ-lactone, and hydroxyl groups, respectively.[3] Acetylation of **1** provided a tetraacetate (**2**), which still showed hydroxyl absorption at 3300 cm^{-1} in the IR spectrum, confirming the presence of a tertiary hydroxyl group in **1**. Acid hydrolysis of **1** yielded glucose and an aglycon mixture composed of withanolide F (**3**)[4,5] and Δ^3-isowithanolide F (**4**).[4,5]





The ^1H NMR spectrum of **1** showed close resemblance to 3β-hydroxy-2,3-dihydrowithanolide F[4], indicating the same substitution in rings A and B and the C-17 side chain. However, instead of a hydroxyl group at C-3, a β-glycosidic linkage was evident from the signal at δ 4.23 (J = 7.7 Hz). This was supported by the signal at 101.2 (CH) in ^{13}C NMR. The ^1H and ^{13}C NMR data have been summarized in Table 1. All the assignments were made on the basis of COSY 45°, HMQC, and HMBC experiments and by comparison with similar withanolides.[4,6−9]

From the molecular formula $C_{34}H_{48}O_{11}$ (11 double-bond equivalents); mass spectral peaks at *m*/*z* 125.0601 ($C_7H_9O_2$), 152.0834 ($C_9H_{12}O_2$), and 327.1931 ($C_{21}H_{27}O_3$), and ^{13}C NMR signals at δ 78.1 (C), 81.3 (C), and 87.3 (C), an ether linkage was inferred between either C-14/C-17 or C-14/C-20. The Drieding model of **1** confirmed that an ether linkage is only possible between C-14 and C-20 (joining of C-14 with C-17 would afford a four-membered cyclic ether within a five-membered ring, which would be too strained to exist). Chemical evidence for an ether linkage between C-14 and C-20 and the tertiary hydroxyl at C-17 was provided by treatment of acetylated derivative **2** (in situ, in the NMR tube) with trichloroacetyl isocyanate, which gave a monocarbamate derivative (NH at δ 10.32). Moreover, the reaction took 72 h for completion, indicating the presence of a hindered hydroxyl group as reported earlier by Kirson et al.[8] in a similar type of withanolide. The stereochemistry at various asymmetric centers of compound **1** was assigned on the basis of the known aglycons (**3** and **4**), which were formed as a result of acid hydrolysis.

* To whom correspondence should be addressed. Tel.: 92-21-4968733. Fax: 92-21-4963373, 49631241.
[†] H. E. J. Research Institute of Chemistry.
[‡] PCSIR Karachi.
[§] Gomal University.

10.1021/np980228o CCC: $18.00
Published on Web 02/05/1999

Table 1. NMR Data[a] (DMSO-d_6) for Compound (**1**)

position	^{13}C	^{1}H (*J* Hz)	position	^{13}C	^{1}H (*J* Hz)
1	210.7 (s)		18	19.1 (q)	0.98 s
2	45.7 (t)	2.50 H-α 2.61 H-*β*	19	18.2 (q)	1.10 s
3	74.5 (d)	3.80 m	20	81.3 (s)	
4	37.5 (t)	2.46 H-α 2.59 H-*β*	21	20.1 (q)	1.24 s
5	134.5 (s)		22	80.8 (d)	4.64 dd (12.5, 3.4)
6	125.6 (d)	5.61 m	23	34.2 (t)	2.32 H-α 2.42 H-*β*
7	35.8 (t)	1.70 H-α 2.08 H-*β*	24	150.8 (s)	
8	35.0 (d)		25	120.0 (s)	
9	35.3 (d)		26	166.0 (s)	
10	52.2 (s)		27	12.0 (q)	1.73 s
11	21.6 (t)		28	20.0 (q)	1.87 s
12	25.3 (t)		1′	101.2 (d)	4.23 d (7.7)
13	53.9 (s)		2′	73.3 (d)	3.95 t (7.9)
14	87.3 (s)		3′	76.5 (d)	4.19 m
15	29.8 (t)		4′	70.1 (d)	4.17 m
16	31.8 (t)	1.21 H-α 1.52 H-*β*	5′	76.5 (d)	3.98 m
17	78.1 (s)		6′	61.12 (t)	4.55 dd (10.0, 2.6) 4.31 m

[a] ^{13}C NMR (125 MHz), ^{1}H NMR (400 MHz), and chemical shifts are given in δ units.

Experimental Section

General Experimental Procedures. Melting points were determined on a micro-melting point apparatus and are uncorrected. Optical rotations were measured in $CHCl_3$ and MeOH solutions on a JASCO DIP-360 polarimeter. IR and UV spectra were measured on JASCO 302-A and on Hitachi U3200 spectrophotometers, respectively. HREIMS and HRFABMS were recorded on JMSHX 110 with data system and on JMS-DA 500 mass spectrometers. The ^{1}H,^{13}C NMR, COSY, HMQC, and HMBC spectra were recorded on Bruker AM 400 and AM 500 spectrometers in DMSO-d_6 containing TMS as internal standard.

Plant Material. The whole plant of *Physalis peruviana* Linn. was collected from Chitral, N. W. F. P., Pakistan, in May 1996. It was identified by Mr. Iftikhar Shah, Plant Taxanomist, Gomal University. A voucher specimen (P-14696) is deposited in the Herbarium of the Department of Pharmacy, Gomal University, D. I. Khan, Pakistan.

Extraction and Isolation. The air-dried plant was crushed and ground into a fine powder, which was exhaustively extracted with EtOH (90%) at room temperature. The extract was concentrated under reduced pressure to yield a viscous greenish mass (1.5 kg). This material was partitioned between H_2O and $CHCl_3$. The insoluble middle layer was separately collected and further partitioned between petroleum ether and MeOH (90%). The aqueous MeOH fraction was freed of solvent and loaded on a Si gel (230−400 mesh) column and eluted with *n*-hexane, *n*-hexane−$CHCl_3$, $CHCl_3$−MeOH mixtures. The fraction obtained in $CHCl_3$−MeOH (84:16) was again chromatographed on Si gel, eluting with EtOAc. The last few fractions showing similar TLC profiles were combined and further separated through MPLC using $CHCl_3$−MeOH (98:2). The last few fractions were purified by TLC to afford compound **1** (45 mg), 4*β*,5,17*β*,20*R*-tetrahydroxy-3α,6α-epoxy-1-oxo-[5*β*,22*R*]-witha-14,20-dienolide (withaperuvin F) (23 mg) and 17*β*,27-dihydroxy-14,20-epoxy-1-oxo-[22*R*]-witha-3,5,24-trienolide (coagulin) (20 mg). The latter compounds were identified through comparison of their physical and spectral data with those reported in the literature.[9,10]

17*β*-Hydroxy-14,20-epoxy-1-oxo-[22*R*]-3*β*-[*O*-*β*-D-glucopyranosyl]-witha-5,24-dienolide (1): white amorphous solid; mp 210−211 °C; $[\alpha]^{25}_D$ + 78° (*c* 0.0077, MeOH); UV (MeOH) λ_{max} (log ϵ) 222 (3.98) nm; IR (KBr) ν_{max} 3500, 1710, 1698 cm^{-1}; HRFABMS *m*/*z* 633.3274 (calcd for $C_{34}H_{49}O_{11}$, 633.3271); EIMS *m*/*z* (rel int %) 452 (7), 434 (8), 283 (80), 152 (100), 125 (70); ^{1}H and ^{13}C NMR, see Table 1.

Acetylation of Compound (1). A solution of **1** (20 mg) in pyridine−Ac_2O (1:1, v/v, 4 mL) was kept at room temperature overnight, and the product was subjected to preparative TLC (*n*-hexane−EtOAc 2:3, v/v), which afforded the tetraacetate **2** (9.2 mg): white amorphous powder; $[\alpha]^{25}_D$ +73° (*c* 0.008, $CHCl_3$); positive FABMS *m*/*z*, 801 $[M + H]^+$; 783 $[M - H_2O + H]$; 471 $[M - GLC (Ac) + H]^+$; UV (MeOH) λ_{max} (log ϵ) 226 (3.96) nm; IR (KBr) ν_{max} 3300 (OH), 1740 (ester), 1710 (α,*β*-unsaturated *δ*-lactone), 1670 cm^{-1} (cyclic ketone); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 1.05 (3H, s, H-19), 1.20 (3H, s, H-18), 1.24 (3H, s, H-21), 1.74 (3H, s, H-27), 1.87 (3H, s, H-28), 2.00, 2.02, 2.03, 2.09 (each 3H, s, −COOCH_3 × 4), 3.82 (1H, m, H-3), 4.53 (1H, dd, $J_{22\alpha,23\alpha}$ = 12.0, $J_{22\alpha,23\beta}$ = 4.5 Hz, H-22), 5.52 (1H, d, *J* = 5.52 Hz, H-6).

Acid Hydrolysis of Compound (1). Compound **1** (20 mg) was refluxed for 4 h. with 1N methanolic HCl (5 mL). The solution was concentrated under reduced pressure and diluted with 5 mL of H_2O. It was extracted with EtOAc, and the residue from the organic phase was subjected to preparative TLC, which afforded **3** and **4.** These were identified as withanolide F and Δ^3-isowithanolide F, respectively, by comparison of their physical constants and spectral data with those reported in the literature.[4,5] The aqueous phase was concentrated and glucose was identified by PC using Schleicher & Schuell 2043b chromatographic paper and solvent system *n*-BuOH−HOAc−H_2O (4:1:5); detection was with aniline−phthalic acid. It was further confirmed by comparing retention time of its TMS ether with a standard sample in GC.[11]

Reaction of 2 with Trichloroacetylisocyanate (TAI). Because compound **1** was not soluble in $CHCl_3$, its corresponding acetylated derivative **2** was used. It was dissolved in $CDCl_3$ in the NMR tube and the ^{1}H NMR spectrum recorded. After dropwise addition of TAI, the NMR spectrum was again recorded; no change was immediately observed. However, the signal of a carbamate proton started to appear after 24 h. The reaction was complete after 72 h, when the peak of carbamate proton was observed at δ 10.32.

Coagulin: white crystalline solid; $[\alpha]^{25}_D$ −11° (*c* 0.007, $CHCl_3$ + MeOH). The spectral data of this compound was consistent with those reported in the literature.[9]

Withaperuvin F: colorless needles; mp 174−176 °C; $[\alpha]^{25}_D$ +39.75° (*c* 0.26, $CHCl_3$). The spectral data showed complete agreement with the published data.[10]

References and Notes

(1) Gil, R. R.; Misico, R. I.; Sotes, I. R.; Oberti, J. C. *J. Nat. Prod.* **1997**, *60*, 568−572.
(2) Scott, A. I. *Interpretation of UV Spectra of Natural Products*; Pergamon: Oxford, 1964, p 82.
(3) Pavia, D. L.; Lampan, G. M.; Kriz, G. S. *Introduction to Spectroscopy*; Saunders College Publishing: West Washington Square, Philadelphia, 1979, pp 41−59.
(4) Vande Velde, V.; Lavie, D.; Budhiraja, R. D.; Sudhir, S.; Garg, K. N. *Phytochemistry* **1983**, *22*, 2253−2257.
(5) Glotter, E.; Abraham, A.; Gunzberg, G.; Kirson, I. *J. Chem. Soc., Perkin Trans 1* **1977**, 341−346.
(6) Glotter, E. *Nat. Prod. Rep.* **1991**, 415−440.
(7) Ray, A. B.; Gupta, M. *Prog. Chem. Org. Nat. Prod.* **1994**, *63*, 1−106.
(8) Kirson, I.; Gunzberg, G.; Glotter, E. *J. Chem. Soc., Perkin Trans 1* **1980**, 531−534.
(9) Atta-ur-Rahman; Abbas, S.; Dur-E-Shahwar; Jamal, S. A.; Choudhary, M. I. *J. Nat. Prod.* **1993**, *56*, 1000−1006.
(10) Neogi, P.; Sahai, M.; Ray, A. B. *Phytochemistry* **1987**, *26*, 243−247.
(11) Markham, K. R. *Techniques of Flavonoids Identification*; Academic: London, 1982, p 52.

NP980228O