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WITHAPHYSALIN D, A NEW WITHAPHYSALIN FROM *PHYSALIS MINIMA* LINN. VAR. *INDICA*

MAHENDRA SAHAI*

Department of Organic Chemistry, The Weizmann Institute of Science, Rehovot, Israel

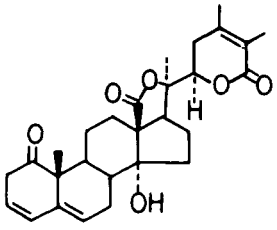
and ISAAC KIRSON

Faculty of Agriculture, The Hebrew University of Jerusalem, Rehovot, Israel

The genus *Physalis* finds an important place in the family Solanaceae for elaborating a variety of C_{28}-steroidal lactones built on the ergostane framework. The structural types that have so far been isolated from different *Physalis* species include physalins (1), withanolides (2), ixocarpalactones (3), withaphysalins (4), and the recently isolated perulactones (5,6). The variation in molecular architecture witnessed in C_{28}-steroids of this genus and the biological activity reported (7,8) for this class of compounds prompted us to extend our studies on *Physalis minima* Linn. var. *indica* C.B. Clarke, an annual herb that had thus far not been investigated for its chemical constituents. The plant has a bitter taste, and the Indian system of medicine recommends it, *inter alia* as a remedy for spleen disorders and also as a tonic, diuretic, and purgative (9).

Extraction of the whole plant and chromatographic separation of the chloroform-soluble fraction afforded two major components, one of which was readily identified as withaphysalin A (**3**), while the second compound was named withaphysalin D. Withaphysalin D is a new compound and is the fourth entry to withaphysalins (4,10). The structure of withaphysalin D was advanced as **1** on the basis of detailed spectroscopic analysis.

Withaphysalin D, $C_{28}H_{34}O_6$, (M^+, 466.2582) mp 202-203°, was found to be optically active, $[\alpha]_D$ +74.3°. Its ir spectrum contained absorption bands attributable to hydroxyl (3345), γ-lactone (1752), saturated ketone (1705), and α,β-unsaturated δ-lactone (1690 cm^{-1}). The uv spectrum (λ max 228 nm; ε, 7,8000) of withaphysalin D also spoke of the presence of an α,β-unsatu-



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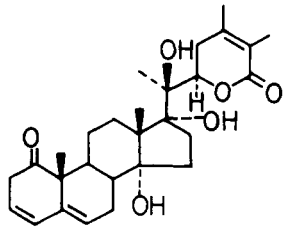

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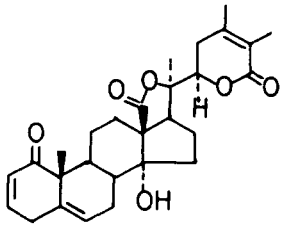

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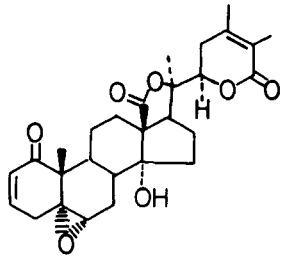

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rated δ-lactone chromophore. The signals for the olefinic hydrogens in rings A and B of withanolide K (**2**) (11) are also discerned in the pmr spectrum of withaphysalin D (**1**), indicating similar AB ring substitution patterns in the two molecules. The similarity between **1** and **2** also in the δ-lactone side chain became manifest from pmr singlets at δ 1.95 and 1.89 (3H each for two olefinic methyls) and a double doublet at δ 4.58 (C-22-H). The presence of a δ-lactone side chain in withaphysalin D is also evident from mass spectral peak at *m/z* 125, originated by the cleavage of 20-22 bond.

The appearance of 21-methyl in pmr as a singlet implies the absence of 20-H; its low-field position (δ 1.50) indicates the presence of an oxygen function at C-20. The presence of a γ-lactone carbonyl (from ir 1752 cm^{-1} and cmr δ_C 177.5) and the absence of 18-methyl in pmr suggest the presence of γ-lactone like that of the withaphysalins. The presence of a hydroxyl group at C-14 was deduced from the cmr spectrum, which exhibited three oxy-carbons at δ_C 78.2 (d), 83.7 (s), and 83.4 (s) attributable to C-22, C-14, and C-20, respectively. These assignments are based on the comparison of the cmr spectrum of **1** with that of withaphysalin A (**3**), which incidentally co-occur in the plant. Withaphysalin A was characterized from a comparison with reported data (4). We report herein, for the first time, cmr spectral data of withaphysalin A (**3**) and its 5α,6α-epoxide (**4**).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were taken on a Fisher-Johns apparatus and are uncorrected. Optical rotations were recorded with an automatic Perkin-Elmer 141 polarimeter and refer to solutions in acetonitrile. Ir spectra were recorded on a Perkin-Elmer infrared 137 spectrophotometer and refer to KBr pellets; uv spectra were recorded on a Cary 14 instrument for solutions in ethanol: pmr spectra were recorded at 270 MHz on a Brüker WH instrument, and cmr spectra were recorded at 22.63 MHz on a Brüker WH-90 instrument. The multiplicities of the signals were determined by a single-frequency off-resonance decoupled (SFORD) spectrum and were confirmed by partially-relaxed Fourier-Transform experiment. Mass spectra were determined with a Varian MAT 731 high resolution mass spectrometer.

PLANT MATERIAL.—The plant material used for this study was collected from the campus area of Banaras Hindu University (India) during September 1981, and was authenticated by Dr. S.K. Roy of the Botany Department, Banaras Hindu University, Varanasi-221 005, India.

EXTRACTION AND CHROMATOGRAPHY.—The dried and powdered whole plant (4.0 kg) was extracted with EtOH in a soxhlet for 36 h. The extract was concentrated to 4 liters and diluted with an equal volume of H_2O. The mixture was stirred mechanically and filtered. The filtrate thus obtained was extracted successively with *n*-hexane and $CHCl_3$. The $CHCl_3$ extract was washed, dried (anhydrous Na_2SO_4), and evaporated to give a gummy solid (60.5 gm).

The gummy solid was chromatographed over 2.5 kg of silica gel S and eluted with hexane-EtOAc mixtures of increasing polarity. Eluates with hexane-EtOAc (8:2) were mixed and evaporated to give a solid (6.1 g), which was found to be a mixture of three components. This fraction was rechromatographed over silica gel (mesh 230-400) (700 g) and eluted with hexane-EtOAc (7:3), and fractions of 35 ml each were collected.

WITHAPHYSALIN D (**1**).—Fractions 28-31 of the above column were mixed and removal of the solvent *in vacuo* gave a solid residue, 1.2 g (0.03%), which upon crystallization from MeOH gave colourless prism, mp 202-203°, $[\alpha]_D$ +74.3° (c, 1.005, CH_3CN); ir ν max 3345, 1752, 1705, 1690 cm^{-1}; uv λ max 228 nm (ε, 7,800); pmr δ 6.08 (1H, ddd, J=10, 5, 2.5 Hz, H-3), 5.72 (1H, ddd, J=10, 2.8, 1 Hz, H-4), 4.57 (1H, dd, J=13.2 and 4.1 Hz, 22-H), 1.95 (3H, s, H-28), 1.89 (3H, s, H-27), 1.50 (3H, s, H-21), and 1.40 (3H, s, H-19); cmr δ_c 202.8 (s, C-1), 177.5 (s, C-18), 165.0 (s, C-26), 148.7 (s, C-24), 139.9 (s, C-5), 129.2 (d, C-4), 126.9 (d, C-3), 122.6 (3, C-25), 121.5 (d, C-6), 83.8 (s, C-14 or C-20), 83.7 (s, C-20 or C-14), 78.2 (d, C-22), 60.6 (s, C-13), 57.4 (d, C-17), 53.1 (s, C-10), 39.5 (t, C-2), 38.2 (d, C-8 or C-9), 36.0 (d, C-8 or C-9), 35.4 (t, C-12), 34.7 (t, C-15), 31.6 (t, C-23), 26.9 (t, C-7), 26.9 (q, C-21), 24.8 (t, C-16), 22.5 (t, C-11), 20.6 (q, C-28), 20.2 (q, C-19), and 12.4 (q, C-27). High resolution ms found *m/z* 466.2582; $C_{28}H_{34}O_6$ requires 466.2640.

WITHAPHYSALIN A (**3**).—Fractions 50-57 were mixed according to tlc, and the solvent was removed under *vacuum*. The residue thus obtained, 1.82 g (0.045%), was crystallized from

EtOAc as needles, mp 223-224° (lit. 222-223°); $[\alpha]_D$ +42.8° (c, 0.2, CH_3CN); ir ν max 3350, 1755, 1694, 1641, cm^{-1}; uv λ max 224 nm (ϵ, 18,000); pmr δ 6.81 (1H, ddd, J=10, 5, and 2.5 Hz, H-3), 5.85 (1H, ddd, J=10, 2.8, 1 Hz, H-2), 5.60 (1H, br, J=5.5 Hz, H-6), 4.55 (1H, dd, J=13.1 and 4.2 Hz, H-22), 1.94 (3H, s, H-28), 1.88 (3H, s, H-27), 1.50 (3H, s, H-21) and 1.29 (3H, s, H-19); cmr δ_c 204.3 (s, C-1), 177.6 (s, C-18), 166.7 (s, C-26), 148.3 (s, C-24), 146.0 (d, C-23), 135.1 (s, C-5), 127.6 (d, C-2), 124.6 (d, C-6), 122.0 (s, C-25), 83.5 (s, C-14 or C-20), 83.4 (s, C-20 or C-14), 78.2 (d, C-22), 60.5 (s, C-13), 55.1 (d, C-17), 51.4 (s, C-10), 39.6 (d, C-8 or C-9), 37.8 (d, C-9 or C-8), 35.4 (t, C-12), 34.7 (t, C-15), 33.3 (t, C-4), 31.6 (t, C-23), 26.6 (q, C-21), 26.2 (t, C-7), 24.9 (t, C-16), 23.0 (t, C-11), 20.5 (q, C-25), 18.7 (q, C-19), and 12.4 (q, C-27). High resolution ms found *m/z* 466.2563; $C_{28}H_{34}O_6$ requires 466.2640.

EPOXIDATION OF WITHAPHYSALIN A (**3**).—A solution of 100 mg of **3** in $CHCl_3$ (20 ml) was stirred with *m*-chloroperbenzoic acid (50 mg) for 24 h at room temperature. The solution was then washed with dilute $NaHCO_3$ and H_2O, dried, and evaporated. The residue was purified on a preparative chromatoplate in hexane-EtOAc (2:3) to give 5α,6α-epoxywithaphysalin A (**4**) (98 mg) as a sole product, mp 229-230° (from MeOH); pmr δ 6.76 (1H, ddd, J=10, 5, and 2.6 Hz, H-3), 5.85 (1H, ddd, J=10, 2.8, and 1 Hz, H-2), 4.55 (1H, dd, J=13.1 and 4.1 Hz, H-22), 3.12 (1H, d, J=3.8 Hz, H-6), 1.95 (3H, s, H-28), 1.88 (3H, s, H-27), 1.46 (3H, s, H-21) and 1.38 (3H, s, H-19); cmr δ_c 202.8 (s, C-1), 177.4 (s, C-18), 164.7 (s, C-26), 148.4 (s, C-24), 142.9 (d, C-3), 128.7 (d, C-2), 122.0 (s, C-25), 84.1 (s, C-14 or C-20), 83.2 (s, C-20 or C-14), 78.0 (d, C-22), 64.7 (s, C-5), 60.5 (s, C-13), 58.7 (d, C-6), 57.2 (d, C-17), 49.0 (s, C-10), 36.8 (d, C-8 or C-9), 35.3 (t, C-12), 34.4 (t, C-15), 33.8 (d, C-8 or C-9), 33.4 (t, C-4), 31.6 (t, C-23), 26.6 (q, C-21), 24.7 (t, C-7 or C-16), 24.5 (t, C-16 or C-7), 22.8 (t, C-11), 20.5 (q, C-28), 15.5 (q, C-19), and 12.4 (q, C-27). M^+ at *m/z* 482.

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LITERATURE CITED

1. T. Matsuura, N. Kawai, R. Nakashima, and Y. Butsugan, *J. Chem. Soc. (C)*, 664 (1970).
2. M. Sahai, A. Ali, A.B. Ray, D.J. Slatkin, and I. Kirson, *J. Chem. Research (S)*, 152 (1983).
3. I. Kerson, A. Cohen, M. Greenberg, H.E. Gottlieb, E. Glotter, and P. Varenne, *J. Chem. Research (S)*, 103 (1979); *J. Chem. Research (M)*, 1178 (1979).
4. E. Glotter, I. Kirson, A. Abraham, S.S. Subramanian, and P.D. Sethi, *J. Chem. Soc., Perkin Trans I*, 1370 (1975).
5. H.E. Gottlieb, I. Kirson, E. Glotter, A.B. Ray, M. Sahai, and A. Ali, *J. Chem. Soc. Perkin Trans I*, 2700 (1980).
6. M. Sahai, H.E. Gottlieb, A.B. Ray, A. Ali, E. Glotter, and I. Kirson, *J. Chem. Res. (S)* 346 (1982).
7. K.R.S. Ascher, N.E. Nemny, M. Liyahu, I. Kirson, A. Abraham, and E. Glotter, *Experientia*, **36**, 998 (1980).
8. M. Suffness and J. Douros, *J. Nat. Prod.*, **45**, 1 (1982).
9. K.R. Kirtikar and B.D. Basu, "Indian Medicinal Plants," Vol. III, Delhi: Singh and Singh, 1975, p. 1769.
10. I. Kirson, Z. Zaretskii, and E. Glotter, *J. Chem. Soc. Perkin Trans I*, 1244 (1976).
11. I. Kirson and H.E. Gottlieb, *J. Chem. Res.* (S), 338, (M) 4275 (1980).

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