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WITHAPHYSALIN E, A WITHANOLIDE OF *PHYSALIS MINIMA* VAR. *INDICA**

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Key Word Index—*Physalis minima* var. *indica*; Solanaceae; steroidal lactone; withanolide; withaphysalin E.

Abstract—A new withanolide, withaphysalin E, was isolated from *Physalis minima* var. *indica* and its structure was determined on the basis of spectral studies.

INTRODUCTION

Physalis minima Linné var. *indica* C. B. Clarke (Solanaceae) is well known in the Indian medical system as a remedy for spleen disorder and as a tonic, diuretic and purgative [1]. Our search for new withanolides from this plant has resulted in the isolation of a new minor withanolide, designated as withaphysalin E (**1**), from the methanolic extract of the whole plant.

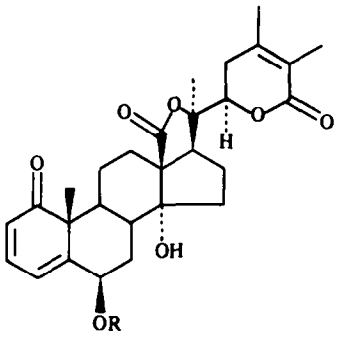
RESULTS AND DISCUSSION

Withaphysalin E (**1**), mp 311–312°C, $[\alpha]_D$ +61.4°, has been analysed as $C_{28}H_{34}O_7$ on the basis of its EI mass spectral peak at *m/z* 464 $[M-H_2O]^+$ and from its ^{13}C NMR spectrum which showed signals for 28 carbons as follows ($CH_3-\times 4$, $-CH_2-\times 6$, $>CH-\times 3$, $>C<\times 2$, $>CH-O\times 2$, $\rightarrow C-O\times 2$, $-CH=CH-\times 1$, $>C=CH-\times 1$, $>C=C<\times 1$ and $>C=O\times 3$) (Table 1). It showed IR absorption bands at 3460, 1745, 1690 and 1655 cm^{-1} assignable to hydroxyls, γ-lactone, α,β-unsaturated-δ-lactone and α,β-unsaturated ketone, respectively. The UV absorption maximum at 312 nm (ε 4800) was found to be similar to that of withaperuvin C (**2**) [311 nm (ε 4370)] [2], pointing to the presence of a 2,4-dien-1-one system in withaphysalin E (**1**). The presence of the above system in this molecule was deduced from the ^1H NMR spectrum, which exhibited signals at δ5.96, 6.93 and 6.14 assigned to three olefinic hydrogens at C-2, C-3 and C-4, respectively, in an ABC pattern (J = 9.7 and 6.0 Hz). In addition to these signals, the ^1H NMR spectrum displayed a carbinyl hydrogen signal at δ4.54 (*t*, J = 3.0 Hz) which underwent downfield shift to δ5.55 upon acetylation, and its long range coupling with the C-4 hydrogen signal at δ6.14 indicated that withaphysalin E (**1**) bears a hydroxyl group at C-6. The β-configuration of this hydroxyl group was determined from the following facts: (i) the chemical shift of the C-19 methyl signal (δ1.48) of withaphysalin E (**1**) matched with that of withaperuvin C (**2**) (δ1.48) [2] where the C-19 methyl and the C-6 hydroxyl group adopted a 1,3-diquasiaxial relationship (ii) the splitting pattern of the C-6 carbinyl hydrogen at δ4.54 (*t*, J = 3.0 Hz) in withaphysalin E (**1**) and an upfield shift (Δδ0.1 ppm) of the C-19 methyl signal from withaphysalin E (**1**) to its monoacetyl derivative (**3**) revealed the C-6 hydrogen to be quasiequatorial and (iii) a significant nuclear Overhauser effect was observed between the C-4 and C-6 hydrogens. Confirmation of the presence of the 6β-hydroxyl-2,4-dien-1-one system in

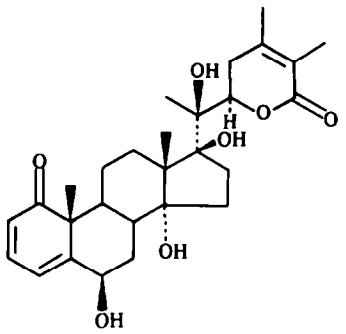
Table 1. ^{13}C NMR spectra of compounds **1**, **2** and **4** (C_5D_5N)

	1	**2**	**4**
C-1	206.1 *s*	205.4	204.3
C-2	117.5 *d*	116.4	127.6
C-3	140.4 *d*	140.7	146.0
C-4	126.0 *d*	126.0	33.3
C-5	160.0 *s*	161.0	135.1
C-6	72.8 *d*	74.2	124.6
C-7	37.1 *t*	37.2	26.2
C-8	37.8 *d*	35.5	37.8
C-9	44.7 *d*	43.7	39.6
C-10	53.8 *s*	55.2	51.4
C-11	22.7 *t*	22.1	23.0
C-12	36.3 *t*	35.2	35.4
C-13	60.0 *s*	55.1	60.5
C-14	83.1 *s*	82.9	83.5
C-15	34.8 *t*	31.1	34.7
C-16	25.7 *t*	37.9	24.9
C-17	57.7 *d*	88.0	55.1
C-18	177.3 *s*	21.4	177.6
C-19	20.0 *q*	18.7	18.7
C-20	83.9 *s*	79.2	83.4
C-21	26.3 *q*	19.6	26.6
C-22	78.4 *d*	81.6	78.2
C-23	31.9 *t*	33.3	31.6
C-24	148.7 *s*	—	148.3
C-25	121.9 *s*	121.4	122.0
C-26	165.4 *s*	166.8	166.7
C-27	12.5 *q*	12.5	12.4
C-28	20.7 *q*	20.3	20.5

*Part 15 of C_{28}-steroidal lactones from Medicinal Chemistry Department, B.H.U. and Part 34 in the Tohoku University series of Steroids.



1 R = H
3 R = Ac



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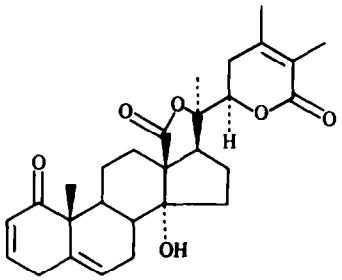

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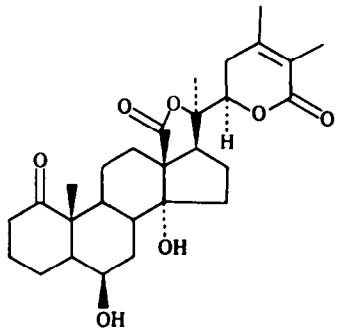

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withaphysalin E (**1**) was also made by a comparison of the ^{13}C NMR signals assigned to the A- and B-ring carbons with those of withaperuvin C (**2**) [2] (Table 1).

The mass spectral peak at *m/z* 125 and the presence of two vinylic methyl signals at δ 1.88 and 1.96 (3H, *s*, each) in the ^1H NMR spectrum of withaphysalin E (**1**) spoke that it has the same α,β-unsaturated-δ-lactone side chain like the other common withanolides [3]. The appearance of the C-21 methyl signal as a singlet at comparatively low field (δ 1.52) and the C-22 hydrogen signal as a double doublet at δ 4.60 (J = 13.4 and 3.6 Hz) indicated that, instead of a hydrogen atom, it carries an oxygen atom at C-20 which causes downfield shifts of these C-21 methyl and C-22 hydrogen signals. The lack of the C-18 methyl signal in conjunction with the presence of the IR spectral band at 1745 cm^{-1} due to a γ-lactone group and the presence of a carbonyl carbon signal at δ 177.2 led to the conclusion that withaphysalin E (**1**) contains a 18,20-γ-lactone. The unusual low field position of the C-21 methyl signal in the ^1H NMR spectrum can thus be explained in terms of deshielding by the lactone carbonyl group.

The presence of a 14α-hydroxyl group in withaphysalin E (**1**) was deduced from the perfect co-matching of the ^{13}C NMR signals of the C-, D- and E-ring carbons of withaphysalin E (**1**) with those of withaphysalin A (**4**) [4], which incidentally co-occurred in the plant. These facts also indicated that withaphysalin E (**1**) has the same relative stereochemistry as that of withaphysalin A (**4**) [4].

Withaphysalin E (**1**) was hydrogenated in the presence of 5% palladized carbon as a catalyst to afford a tetrahydro derivative (**5**) which showed a positive Cotton effect at 250 nm ([θ] 6490), determining the configuration of C-22 to be *R*.

Based on the above data the stereostructure of withaphysalin E (**1**) has been established.

EXPERIMENTAL

Mps were taken on a Toshniwal apparatus and are uncorr. Optical rotation was measured on a JASCO DIP-360 polarimeter and CD spectrum was recorded on a JASCO A-3 instrument. ^1H NMR and ^{13}C NMR spectra (TMS as internal standard) and low resolution EIMS were determined with a JEOL GX-500 spectrometer and a Hitachi M-52 mass spectrometer, respectively.

Plant material and isolation procedure. The whole plants of *Physalis minima* var. *indica* were collected from the campus area of Banaras Hindu University, Varanasi in September, 1982 and authenticated by Prof. S. K. Roy, Department of Botany of the University. Dried and pulverized plant material (4.3 kg) was first defatted and then extracted exhaustively with MeOH. The $CHCl_3$-soluble fraction of the methanolic extract was chromatographed over silica gel and eluted successively with C_6H_6 (Fraction A) and $CHCl_3$ (Fraction B). Rechromatography (silica gel) of Fraction B and elution with C_6H_6-EtOAc (2:3) furnished withaphysalin E (50 mg).

Withaphysalin E (**1**). White amorphous powder, mp 311–312°, $[\alpha]_D$ +61.4° (C_5H_5N; *c* 0.51). EIMS *m/z*: 464 $[M-H_2O]^+$; UV λ_{max}^{MeOH} nm: 228 (13700), 312 (4800); IR ν_{max}^{KBr} cm^{-1}: 3460, 1745, 1690, 1655, 1230, 1035; ^1H NMR ($CDCl_3/CD_3OD$): δ6.93

(1H, *dd*, J = 9.7 and 6.0 Hz, H-3), 6.14 (1H, *d*, J = 6.0 Hz, H-4), 5.96 (1H, *d*, J = 9.7 Hz, H-2), 4.60 (1H, *dd*, J = 13.4 and 3.6 Hz, H-22), 4.54 (1H, *t*, J = 3.0 Hz, H-6), 1.96, 1.88, 1.52, 1.48 (3H each, *s*, 28, 27, 21 and 19 Me).

Withaphysalin E monoacetate (3). A mixture of withaphysalin E (10 mg), Ac_2O (0.5 ml) and pyridine (0.5 ml) was kept under dry conditions for 24 hr. The reaction mixture was freed from organic solvents *in vacuo* and purified by column chromatography (silica gel) to yield withaphysalin E monoacetate (3) as an amorphous powder (6 mg), mp 257–259°, EIMS *m/z*: 506 $[M-H_2O]^+$; UV λ_{max}^{MeOH} nm: 226 (8300), 310 (4500); IR ν_{max}^{KBr} cm^{-1}: 3440, 1750, 1700, 1652, 1225, 1012; ^1H NMR ($CDCl_3$): δ6.93 (1H, *dd*, J = 9.2 and 6.0 Hz, H-3), 6.33 (1H, *d*, J = 6.0 Hz, H-4) 6.01 (1H, *d*, J = 9.2 Hz, H-2), 5.56 (1H, *t*, J = 3.0 Hz, H-6), 4.54 (1H, *dd*, J = 12.1 and 3.8 Hz, H-22), 2.01 (3H, *s*, $-COCH_3$), 1.96, 1.88, 1.48 and 1.38 (3H each, *s*, 28, 27, 21 and 19 Me).

Tetrahydrowithaphysalin E (5). Withaphysalin E (1) (5 mg) in MeOH (2 ml) was hydrogenated over 5% Pd–C at room temp. and atm. pres. overnight, and the reaction mixture was purified by CC (silica gel) to furnish tetrahydrowithaphysalin E (5) as an amorphous powder (1.4 mg), EIMS *m/z*: 468 $[M-H_2O]^+$; ^1H NMR ($CDCl_3$): δ4.49 (1H, *dd*, J = 12.0 and 3.4 Hz, H-22), 3.94 (1H, *br*, H-6), 1.89, 1.81, 1.41 and 1.28 (3H each, *s*, 28, 27, 21 and 19 Me); CD (MeOH): $[\theta]_{250}+6490$, $[\theta]_{290}-3720$.

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HOMOHEVEADRIDE, A CYCLONONADIENE BIS-ANHYDRIDE FROM *CLADONIA POLYCARPOIDES*

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Key Word Index—*Cladonia polycarpoides* Nyl. in Zwackh; Cladoniaceae; lichen; compound CS-1; cyclononadiene bis-anhydride; homoheveadride.

Abstract—The isolation of a novel cyclononadiene bis-anhydride, homoheveadride (8-butyl-7-pentylcyclonona-1,5-diene-1,2,5,6-tetracarboxylic dianhydride) from the lichen *Cladonia polycarpoides* Nyl. in Zwackh is reported.

INTRODUCTION

The terricolous, fruticose lichen *Cladonia polycarpoides* Nyl. in Zwackh (*Cladonia subcariosa* auct.) [1] is widely distributed in North America and Europe and also occurs in Japan, Australia and New Zealand. It contains norstictic acid and an additional compound which was tentatively identified as a fatty acid [2]. This compound was later examined by GC [3] and TLC; R_f values of the compound, referred to as CS-1, in three standard solvent systems were published [4], and the presence of the compound in the lectotype of *C. polycarpoides* has recently been confirmed (Ahti, Personal Communication). In the present work, the compound was isolated from an Australian specimen of *Cladonia polycarpoides*; by analysis of the spectroscopic data, the compound was shown to have the nonadride structure, 8-butyl-7-pentylcyclonona-1,5-diene 1,2,5,6-tetracarboxylic dianhydride (1) [5, 6]. In view of the relationship with the nonadride, heveadride (2) isolated from *Helminthosporium hevea* [7], the name homoheveadride is suggested.

RESULTS AND DISCUSSION

The molecular formula of homoheveadride was established as $C_{22}H_{28}O_6$ by high resolution mass spectrometry. The nonadride character of the substance was suggested by the high oxygen content and by the IR spectrum ν_{max}^{KBr} 1850, 1775 cm^{-1} (anhydride). The arrangement of the alkyl groups shown in (1) followed from the mass spectral fragmentation pattern and the ^1H NMR spectra. The only strong peak (base peak) in the EIMS is at *m/z* 194 ($C_{11}H_{14}O_3$), an ion which can come from both