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WITHAPERUVIN E AND NICANDRIN B, WITHANOLIDES FROM *PHYSALIS PERUVIANA* AND *NICANDRA PHYSALOIDES**

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Key Word Index—*Physalis peruviana*; *Nicandra physaloides*; Solanaceae; withanolides; withaperuvin E; nicandrin B.

Abstract—Novel withanolides, withaperuvin E and nicandrin B, isolated respectively, from *Physalis peruviana* and *Nicandra physaloides*, were fully characterized by chemical and spectroscopic means.

INTRODUCTION

Physalis peruviana Linné and *Nicandra physaloides* Gaertn. (Solanaceae) are noted for elaborating a variety of C_{28}-steroidal lactones based upon the ergostane skeleton [1]. In view of the unique structural features of this class of compounds and the significant antineoplastic activity of some of these compounds [2], we have continued our search for newer compounds from these two sources. We now report the isolation of two new withanolides, withaperuvin E from the roots of *P. peruviana* and nicandrin B from the seeds of *N. physaloides*. The structures of these two compounds were established on the basis of detailed spectral analysis and chemical transformations to compounds of known structure.

RESULTS AND DISCUSSION

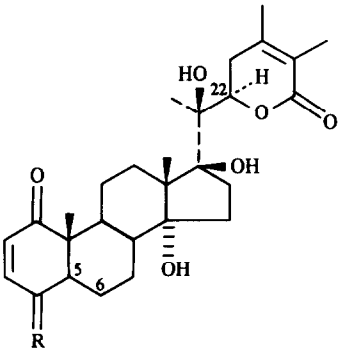
Withaperuvin E (**1**), $C_{28}H_{36}O_8$ was recognised as a 14,17,20-tri-hydroxywithanolide like the congeneric withaperuvins [3]. The IR spectrum of withaperuvin E indicated the presence of an α,β-unsaturated δ-lactone (1686 cm^{-1}), an α,β-unsaturated ketone (1676 cm^{-1}) and hydroxyls (3400 cm^{-1}). The ^1H NMR spectrum exhibited not only signals due to three tertiary methyls (δ1.19, 1.35 and 1.51, 3H each, *s*) and two vinylic methyls (δ1.88, 6H, *s*) but also the signal for H-22 (δ4.95, 1H, *dd*) which is regarded as a withanolide fingerprint. Moreover, its mass spectrum showed the base peak at *m/z* 169, in addition to an intense peak at *m/z* 125, formed by fission across the C-17 to C-20 and C-20 to C-22 bonds, respectively. The complete identity of the side chain at C-17 and the C and D rings of withaperuvin E with those of withaperuvin (**1a**) [3] was established by the observation that the chemical shifts and splitting patterns of the resonance signals for the carbon atoms associated with this part of the molecule were virtually identical in both the compounds.

However, withaperuvin E, unlike other known withanolides from *Physalis peruviana*, showed three carbonyl carbon resonances (δ166.7, 194.1 and 202.1) in its ^{13}C NMR spectrum and a two-proton singlet at δ6.88 characteristic of Δ^2-1,4-dione system [4] in the ^1H NMR spectrum. The yellow colour of withaperuvin E in contrast to the colourlessness of common withanolides and the UV maximum at 225.5 nm in withanolide E implied the existence of a *cis*-Δ^2-1,4-dione system. The accumulated data demonstrated the presence of an enedione moiety in the A ring of withaperuvin E.

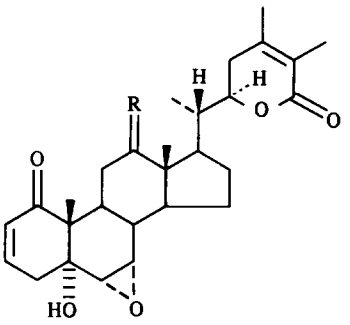
The presence of an enedione, a δ-lactone and three tertiary hydroxyl groups at C-14, C-17 and C-20 account for seven oxygen atoms and the remaining one oxygen atom was considered to be present as an oxide ring from calculation of double bond equivalence as well as from the fact that though withaperuvin E is not amenable to acetylation it showed a low field carbinyl hydrogen signal at δ3.47, whose chemical shift and splitting pattern were in perfect agreement with those of the 6-H signal of 4β-hydroxywithanolide E (**1b**) which bears a 5β,6β-epoxy system. Withaperuvin E was thus considered to be 4-oxowithanolide E (**1**) which was confirmed by manganese dioxide oxidation of **1b** to **1**.

Chromatographic resolution of the ether soluble fraction of the alcoholic extract of defatted seeds of *N. physaloides* yielded the known 12-oxowithanolide, withanicandrin (**2**) [5] and a new C_{28}-steroidal lactone designated as nicandrin B (**2a**). Nicandrin B, $C_{28}H_{38}O_6$, showed characteristic IR bands at 1682 and 1690 cm^{-1} and a UV maximum at 226 nm, indicating the presence of α,β-unsaturated ketone and α,β-unsaturated-δ-lactone [6]. Most of the parameters of the ^1H NMR spectral signals of nicandrin B were similar to those of the corresponding hydrogen signals in daturalactone B [1, 7] and 5α,12α-dihydroxy-1-oxo-6α,7α-epoxy-with-24-enolide, a reaction product of daturalactone B with a Zn–Cu couple [7], pointing to a close relationship between these compounds. Thus, two vinylic hydrogen signals at δ5.83 (1H, *dd*, J = 10, 2.5 Hz) and 6.61 (1H, *ddd*, J = 10, 5, 2.3 Hz) were attributable to the C-2 and C-3 hydrogens respectively, in a steroidal Δ^2-1-one system and two carbinyl hydrogen signals at δ3.04 (1H, *d*, J = 4 Hz) and 3.37 (1H, *dd*, J = 4, 1.7 Hz) were assignable to the C-6 and C-7 hydrogens in a 5α-hydroxy-6α,7α-epoxy steroid. The negative Cotton effect at 338 nm in the CD spectrum indicated the 5α-configuration [8]. Signals due to the hydrogens in the side chain at C-17 were discernible at δ1.14 (3H, *d*, J = 6 Hz), 1.88 (3H, *s*), 1.98 (3H, *s*) and 4.40

*Part 33 in the Tohoku University series on Steroids.



1 R = O, 5β, 6β – epoxy
1a R = β – OH, α – H, 5β, 6α – di – OH
1b R = β – OH, α – H, 5β, 6β – epoxy



2 R = O
2a R = α – OH, β – H

(1H, *m*). In addition, a characteristic signal appeared at δ4.02 (1H, *m*) for a carbinyl hydrogen and its band width at a half height ($W_{\frac{1}{2}}$ = 7 Hz) suggested it to be in an equatorial orientation. Similarity of the chemical shifts of the methyl hydrogen signals at C-18 and C-21 in nicandrin B to those of the corresponding hydrogen signals in 5α,12α-dihydroxy-1-oxo-6α,7α-epoxywith-24-enolide indicated the presence of a 12α-hydroxyl group in nicandrin B. In the CD spectrum the positive Cotton effect at 250 nm demonstrated the absolute configuration of C-22 to be *R* [9].

From the above data nicandrin B was assumed to have the structure **2a**. Conclusive evidence in support of this structure (**2a**) for nicandrin B came from its conversion to withanicandrin (**2**) by Jones oxidation. The structure **2a** is also confirmed by comparing its ^{13}C NMR data with those of withanicandrin (**2**), which were not reported earlier.

EXPERIMENTAL

Isolation of withaperuvin E. General directions and work-up of plant material have been given before [3]. Chromatography of the ethereal extract (200 g) from the air dried roots (8.5 kg) of *P. peruviana* over silica gel and elution with C_6H_6–EtOAc (3:2) yielded physalolactone [10]. The mother liquor of physalolactone on chromatography over silica gel and elution with C_6H_6–EtOAc (3:2) furnished withaperuvin E (**1**) (260 mg) as pale yellow crystals from Me_2CO, mp 248–250°. $[\alpha]_D$ −72.7 (*c* 0.715, MeCN). CD (MeOH): $\Delta\varepsilon_{345}$ −0.71, $\Delta\varepsilon_{245}$ +7.24. UV λ_{max}^{MeOH} nm: 225.5 (ε10 740). IR ν_{max}^{KBr} cm^{-1}: 3400, 1696, 1676. ^1H NMR [100 MHz, $C_5D_5N + (CD_3)_2CO + CD_3OD$]: δ6.88 (*s*, H-2 and H-3), 4.95 (*dd*, *J* = 13 and 5 Hz, H-22), 3.47 (*s*, H-6, $W_{\frac{1}{2}}$ = 4 Hz), 1.88 (*s*, H_3-27 and H_3-28), 1.51 (*s*, H_3-21), 1.53 (*s*, H_3-19) and 1.19 (*s*, H_3-18). MS *m/z*: 482 $[M - H_2O]^+$, 170, 169, 152, 125.

Manganese dioxide oxidation of 4β-hydroxy withanolide E. A soln of **1b** (50 mg) in Me_2CO (20 ml) was stirred with activated MnO_2 (100 mg) for 40 hr. The reaction mixture was then filtered through a short bed of alumina and from the filtrate pale yellow needles (from Me_2CO) were obtained which were identical with withaperuvin E (**1**).

Isolation of nicandrin B. Silica gel chromatography of the Et_2O soluble fraction of the alcoholic extract of the defatted seeds

Table 1. ^{13}C NMR spectral assignments of compounds **1**, **1a**, **2** and **2a**

Carbon assigned	1*	1a*	2†	2a†
1	202.1	202.4	201.2	203.5
2	139.3	127.2	129.0	129.0
3	141.6	146.7	139.8	140.1
4	194.1	67.9	36.8	36.8
5	64.5	79.8	73.3	73.3
6	64.8	74.7	56.3	56.2
7	26.2	32.9	57.0	57.1
8	34.5	37.8	35.7	36.2
9	36.6	38.5	37.7	30.1
10	50.1	56.2	51.6	50.7
11	23.5	23.5	42.9	28.8
12	35.1	35.0	212.1	72.4
13	54.8	55.2	57.8	43.9
14	81.7	82.2	38.4	42.9
15	30.9	30.9	23.7	23.1
16	37.1	37.2	27.2	26.6
17	88.2	88.0	52.9	47.1
18	21.2	21.4	13.6	12.5
19	19.1	10.3	14.8	14.6
20	79.3	79.1	40.0	39.2
21	19.6	19.6	11.5	12.0
22	81.5	81.5	77.3	79.8
23	33.2	33.3	30.1	29.9
24	‡	151.0	149.3	149.4
25	121.4	121.3	122.1	122.1
26	166.7	166.8	166.9	167.3
27	12.5	12.5	12.5	12.5
28	20.2	20.3	20.5	20.5

*In C_5D_5N.
†In $CDCl_3$.
‡Not detected.

(2 kg) of *N. physaloides* and elution with C_6H_6–EtOAc (3:1) yielded withanicandrin (**2**), (0.2 g), mp 267–269°, and nicandrin B (**2a**) (0.1 g), as white needles (from MeOH), mp 246–248°. $[\alpha]_D$ +110.7° (*c*, 0.24, $CHCl_3$). CD (MeOH): $\Delta\varepsilon_{338}$ −1.95, $\Delta\varepsilon_{250}$ +3.83. UV λ_{max}^{MeOH} nm: 226 (ε13 080). IR ν_{max}^{KBr} cm^{-1}: 3500, 1690, 1682. ^1H NMR (270 MHz, $CDCl_3 + D_2O$): δ6.61 (*ddd*, *J* = 10, 5, 2.3 Hz, H-3), 5.83 (*dd*, *J* = 10.0, 2.5 Hz, H-2), 4.40 (*m*, H-22), 4.02 (*m*, H-12), 3.37 (*dd*, *J* = 4, 1.7 Hz, H-7), 3.04 (*d*, *J* = 4 Hz, H-6), 1.94 (*s*, H_3-27), 1.88 (*s*, H_3-28), 1.17 (*s*, H_3-19), 1.09 (*d*, *J* = 6 Hz, H_3-21), 0.747 (*s*, H_3-18). MS *m/z*: 470 $[M]^+$, 450, 434, 125.

Jones oxidation of nicandrin B. A soln of **2a** (20 mg) in Me_2CO (5 ml) was titrated with Jones reagent (0.05 ml) at room temp (35°). The reaction mixture was left for 30 min, when the green chromium salt settled. The supernatant liquid was decanted off, mixed with the Me_2CO washings and evaporated to dryness at room temp and needles obtained by crystallisation from EtOAc. These were identified as withanicandrin (**2**) by ^1H NMR and co-TLC.

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