Withacoagin, a New Withanolide from *Withania coagulans* Roots

Partha NEOGI, Masao KAWAI, Yasuo BUTSUGAN,* Yuji MORI,† and Makoto SUZUKI†
Department of Applied Chemistry, Nagoya Institute of Technology, Showa-ku, Nagoya 466
†Faculty of Pharmacy, Meijo University, Tenpaku-ku, Nagoya 468
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Synopsis. The structure of withacoagin, a new withanolide isolated from *Withania coagulans*, has been elucidated as (20*R*,22*R*)-5α,20-dihydroxy-1-oxowitha-2,6,24-trienolide by spectral analysis and chemical transformation to a known compound. This is the second report of a Δ^6-withanolide. Isolation of three known compounds, (20*R*,22*R*)-6α,7α-epoxy-5α,20-dihydroxy-1-oxowitha-2,24-dienolide, (20*S*, 22*R*)-6α,7α-epoxy-5α-hydroxy-1-oxowitha-2,24-dienolide, and withaferin A, are also reported.

Withanolides[1] are a group of ergostanolides, generally having a δ-lactone in the side chain and a 2-en-1-one system in the A ring. These are exclusive to the family *Solanaceae*[2-6] and withaferin A,[1] the first withanolide, was isolated from *Withania somnifera* L. Dun. Withanolides are interesting because they show antitumorous[2,5] antibacterial,[7] antiinflammatory,[8] and immunosupressive[9] activities.

W. coagulans Dunal, which grows widely in India and West Pakistan, is used in the Indian indigenous system of medicine[10] and fruits of the plant have milk coagulating properties.[11] In addition to a biogenetically important precursor of withanolides,[12] four withanolides from the roots,[13] two from the leaves,[14] and two from the fruits[12,15] of this plant were reported. Here we will describe the isolation from the roots of *W. coagulans* and the structure elucidation of a new withanolide (**1**), named withacoagin, as well as the isolation and characterization of three known withanolides.

Dried roots of *W. coagulans* were defatted with hexane and extracted with methanol. The extract was subjected to silica gel column chromatography and the major component **2** was characterized as the known (20*R*,22*R*)-6α,7α-epoxy-5α,20-dihydroxy-1-oxowitha-2,24-dienolide[13a] by ^{1}H and ^{13}C NMR spectra (Tables 1 and 2).

Withacoagin (**1**), also isolated from the methanol extract as colorless crystals (mp 230—232 °C), showed a molecular peak at *m/z* 454. The M^+-H_2O peak in the high-resolution mass spectrum established its molecular formula as $C_{28}H_{38}O_5$. The IR spectrum showed

Table 1. ^{1}H NMR Data of Compounds **1**, **2**, and **3** in $CDCl_3$ Solution (Chemical shift in δ and multiplicity with coupling constants in Hz in parentheses)

Assignment	1	2	3
2	5.89, dd (10.0, 2.4)	5.85, dd (10.1, 2.3)	5.85, dd (10.1, 2.7)
3	6.58, ddd (10.0, 5.2, 2.2)	6.59, ddd (10.1, 5.1, 2.2)	6.59, ddd (10.1, 5.1, 2.2)
4α	2.36, dd (19.0, 5.2)	2.53, dd (19.0, 5.1)	2.53, dd (19.0, 5.1)
4β	2.63, dt (19.0, ca. 2.3)	2.68, br d (19.0)	2.69, br d (19.0)
6	5.73, dd (9.9, 1.7)	3.04, d (3.7)	3.04, d (3.7)
7	5.57, dd (9.9, 2.7)	3.32, m	3.31, m
18	0.95, s	0.96, s	0.77s
19	1.20, s	1.18, s	1.18s
21	1.32, s	1.32, s	1.02, d (6.6)
22	4.21, dd (13.3, 3.5)	4.21, dd (13.3, 3.5)	4.38, dt (13.2, 3.4)
23α	2.10, dd (16.8, 3.5)	2.12, dd (17.1, 3.0)	1.97 (overlapped)
23β	2.40, br t (ca. 15)	2.39, br t (ca. 15)	2.45, br t (ca. 15)
27	1.89, s	1.89, s	1.89, s
28	1.95, s	1.95, s	1.95, s

Table 2. ^{13}C NMR Data (δ) of Compounds **1**, **2**, and **3** in $CDCl_3$ Solution[a,b]

Assignment	1	2	3	Assignment	1	2	3
1	203.8	203.1	203.1	15	23.6	23.3	23.6
2	129.6[a1]	129.1	129.1	16	21.9	21.9[a2]	27.3
3	140.4	139.6	139.6	17	54.7[a3]	54.6	51.5[a2]
4	36.5	36.8	36.8	18	13.8	13.8	12.1
5	74.9[a2]	73.3	73.3	19	14.6	14.8	14.7
6	133.3	56.4	56.4	20	75.2[a2]	75.1	39.0
7	129.4[a1]	57.3	57.3	21	21.0	21.2	13.3
8	37.5	35.2[a1]	35.8[a1]	22	81.0	81.0	78.3
9	41.5	35.7[a1]	35.6[a1]	23	31.7	31.7	29.7
10	51.4	51.1	51.0	24	148.7	148.8	149.1
11	22.4	21.9[a2]	21.9	25	122.1	122.1	122.0
12	40.7	40.5	39.9	26	166.0	166.0	167.0
13	44.1	43.9	43.5	27	12.5	12.5	12.5
14	54.3[a3]	50.2	51.9[a2]	28	20.5	20.5	20.5

a) Superscripts a1, a2, and a3 refer to interchangeable data. b) Assignments are based on Ref. 23.

absorption bands for hydroxyl (3435 cm^{-1}), cyclohexenone (1672 cm^{-1}), and α,β-unsaturated δ-lactone (1716 cm^{-1}). The ultraviolet spectrum showed absorption maximum at 225 nm (ε 12000) characteristic of summation of absorptions of the conjugated enone and the conjugated δ-lactone chromophores[16] which are commonly present in withanolides. The fragment ion peaks at m/z 169 and 125 in its mass spectrum suggested that **1** is a 20-hydroxywithanolide.[17]

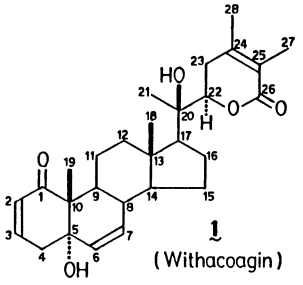
The ^1H NMR spectrum of **1** showed three singlets at δ 0.95, 1.20, and 1.32 which were assigned to C(18)-, C(19)-, and C(21)-methyl groups, respectively. The chemical shift of the deshielded C(21)-methyl singlet and the appearance of the C(22)-methine signal (δ 4.21) as a double doublet (J=13.3 and 3.5 Hz) also supported the presence of an OH group at C(20)-position. The two vinylic methyl singlets at δ 1.89 and 1.95 were assigned to the methyl groups attached to the conjugated lactone group in the side chain. The ^1H signals assignable to the hydrogens at C(18), C(21), C(22), C(27), and C(28) of **1** resembled closely to those of **2** as shown in Table 1, which suggested the identity of substitution pattern of the D ring and the side chain in **1** and **2**. Dependence of the chemical shifts of the methyl peaks upon the stereochemistry of the hydroxyl group at C(20) was already documented.[18]

The α- and β-hydrogen signals of the conjugated enone system at the A ring were observed at δ 5.89 (dd, J=10.0 and 2.4 Hz) and 6.58 (ddd, J=10.0, 5.2, and 2.2 Hz), respectively. The allylic methylene signals at δ 2.36 (dd, J=19.0 and 5.2 Hz) and 2.63 (dt, J=19.0 and 2.3 Hz) indicated that C(4) is unsubstituted and that C(5) bears no hydrogen atom. Thus, hydroxyl group can be located at the C(5)-position. Unlike the common withanolides it showed an additional set of mutually coupled olefinic proton signals at δ 5.57 (dd, J=9.9 and 2.7 Hz) and 5.73 (dd, J=9.9 and 1.7 Hz) indicating the presence of a cis-disubstituted double bond. The cis-double bond can be located either at C(6)-C(7), at C(11)-C(12), or at C(15)-C(16) position. The comparison of ^{13}C NMR spectral data of **1** and **2** given in Table 2 revealed their close resemblance except the resonances assignable to C(6) and C(7). Thus, the Δ^6-structure has been assigned to **1**. All of the 28 carbon resonance signals of **1** are consistent with the 5,20-dihydroxy-Δ^6-withanolide structure.

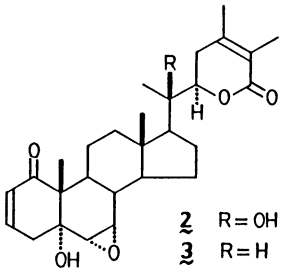
Stereochemistry of **1** was studied by the CD spectrum measured in methanol solution. A negative Cotton effect ([θ] −5200) at 333 nm observed for **1** revealed the trans juncture of the A/B ring moiety,[19] and a positive Cotton effect ([θ] +7000) at 250 nm suggested the R-configuration at C(22).[20]

Withacoagin (**1**) has been found to differ from **2** only at the moiety of C(6) and C(7), i.e., while a double bond is present in the former, the latter possesses an α-epoxide group in the corresponding position. So a chemical transformation of **1** to **2** was then attempted to prove the structure of **1**. Epoxidation of **1** with m-choloroperbenzoic acid yielded an epoxide which was identical in all respects with the naturally occuring **2**. Thus, structure of **1** was established unambiguously as (20R,22R)-5α,20-dihydroxy-1-oxowitha-2,6,24-trienolide. This compound constitutes the second example of a Δ^6-withanolide.[17]

The third compound (**3**) isolated from the roots, mp 254—255 °C, was found to be isomeric with **1** and showed close resemblance in spectral properties to its congener **2**. Detailed analysis of the ^1H and ^{13}C NMR spectral data revealed that **3** is a 20-dehydroxy analog of **2**. The compound **3**, namely (20S,22R)-6α,7α-epoxy-5α-hydroxy-1-oxowitha-2,24-dienolide, is known as lycium substance B, isolated earlier from *Lycium chinense* and *Datura* species,[21,22] but this is the first report of the isolation of **3** from a *Withania*



1 (Withacoagin)



2 R=OH
3 R=H

species. The study of ^{13}C NMR spectra with DEPT method indicated that the assignments of C(10) and C(17) resonances in the original paper[21a] should be interchanged as shown in Table 2.

In addition to the three withanolides **1**, **2**, and **3** described above, withaferin A was also isolated, though only in minor quantity, from the extracts of *W. coagulans* and was characterized by ^1H NMR spectroscopy.

Withanolides are significant for displaying antitumour activity. Withacoagin (**1**), compound **2**, and lycium substance B (**3**) were studied for their cytotoxicity against HeLa cells. All the three compounds, however, did not show significant antitumour activity, i.e., inhibitory concentrations of **1**, **2**, and **3** to the proliferation of HeLa cells were 84, 87, and >100 $\mu g\,ml^{-1}$, respectively. While these impotent withanolides contain a 5α-hydroxyl and/or a 6α,7α-epoxy group, the cytotoxic withanolides possess a 5α,6β-epoxy group.[2,5] In the case of physalins, cytotoxic steroids from *Physalis* plants having highly oxygenated ergostane skeleton, it was proposed that α-hydroxyl and α-epoxy groups at ring A or ring B markedly decrease the cytotoxicity and that β-hydroxyl and β-epoxy groups increase or do not affect the activity.[24]

Experimental

Melting points are uncorrected. Optical rotation was measured using a JASCO DIP-4 digital polarimeter. IR

spectra were recorded as KBr discs on a JASCO IRA-1 spectrometer and FT-IR on a JEOL JIR-100 instrument using a diffuse reflectance accessory. NMR spectra were run on a JEOL JNM-GX 400 spectrometer at 400 MHz for ^{1}H NMR and at 100 MHz for ^{13}C NMR with $CDCl_3$ solutions. UV, CD, and mass spectra were measured on HITACHI 124, JASCO J-40C, and HITACHI M 2000 spectrometers, respectively. Column chromatography was performed using SiO_2 (Merck, Silicagel 60, 7734). Precoated SiO_2 plates (Merck, Silica Gel 60 F_{254}) were used for TLC analysis and R_f values with $CHCl_3$-MeOH (19 : 1) system are given.

Plant Material. The roots of *W. coagulans* Dunal were purchased from United Chemicals and Allied Products, Calcutta, India and a specimen sample is being preserved.

Isolation. The air-dried roots (3.0 kg) were powdered, defatted with hexane, and extracted twice with cold MeOH. The MeOH extract was chromatographed over SiO_2 and the column was eluted with hexane-AcOEt (1 : 0, 3 : 1, 1 : 1, 1 : 2, and 0 : 1). The eluates with hexane-AcOEt (1 : 1 and 1 : 2) were pooled together and subjected to repeated chromatography using $CHCl_3$-MeOH as eluent to yield **1** (62 mg), **2** (650 mg), **3** (40 mg), and withaferin A (43 mg).

Withacoagin (1). Colorless needles from $CHCl_3$; mp 230—232 °C; R_f 0.29; $[\alpha]_D^{24}$ +114° (*c* 0.19, $CHCl_3$); IR (KBr) 3435, 1716, and 1672 cm^{-1}; UV (MeOH) 225 nm (ε 12000); MS (70 eV) *m/z* (rel intensity) 454 (M+; 0.2), 436 (M^+-H_2O; 5), 421 (2), 311 (57), 269 (15), 171 (25), 169 (16), 148 (16), 134 (18), 125 (100), 85 (15), and 69 (12); CD (MeOH) $[\theta]_{333}$ −5200, $[\theta]_{268}$ 0, and $[\theta]_{251}$ +7000. Found: *m/z* 436.2576. Calcd for $C_{28}H_{36}O_4$: M−H_2O, 436.2611.

Compound 2. Colorless needles from $CHCl_3$; mp 282—285 °C (decomp) (lit,[13] mp 282—284 °C); R_f 0.36.

Compound 3. Colorless plates from $CHCl_3$; mp 252—254 °C (lit,[21b] mp 260—65 °C); R_f 0.55.

Withaferin A. Colorless needles from $CHCl_3$; mp 241—243 °C (lit,[1] mp 243—245 °C); R_f 0.35.

Epoxidation of 1. To a solution of **1** (10 mg) in $CHCl_3$ was added *m*-chloroperbenzoic acid (10 mg) and stirred at room temperature for 26 h. Usual workup and SiO_2 column chromatography ($CHCl_3$) yielded a solid (7.6 mg), which crystallized from $CHCl_3$ as fine needles, mp 286—289 °C; FT-IR (KBr) 3456, 3429, 1716, 1683, 1373, and 1344 cm^{-1}. The FT-IR spectrum of this epoxide was found to be indistinguishable with that of **2**.

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