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ADDITIONAL WITHANOLIDES OF *DATURA METEL*[1]

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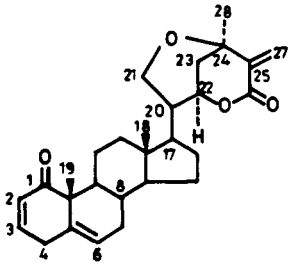
ABSTRACT.—Withametelin B [**2**], a new hexacyclic withanolide, has been isolated from the leaves of *Datura metel* collected from West Bengal, India, and its structure elucidated by chemical and spectroscopic methods. 12-Deoxywithastramonolide [**3**] and physalindicanol A [**4**] have also been isolated from this source.

The occurrence of withametelin [**1**], a novel hexacyclic withanolide with a bicyclic side chain, in *Datura metel* L. (Solanaceae) was first reported by us in a preliminary communication (1) which was followed by two other papers (2,3) providing chemical evidence in support of the structure of withametelin and two related withanolides isolated from this source. Immediately after our first publication, Pakistani and Japanese workers reported a number of structurally related withanolides from *D. metel* of their respective countries (4–9). In the present communication, we report the isolation and characterization of a new hexacyclic withanolide, withametelin B [**2**], together with 12-deoxywithastramonolide [**3**] and physalindicanol A [**4**] from the leaves of *D. metel*, collected from the eastern part of India.



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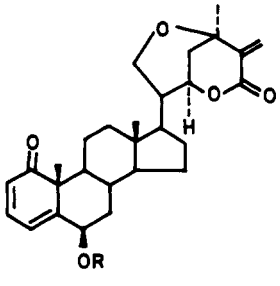

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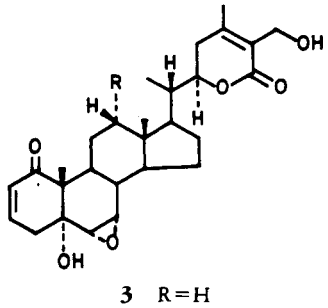

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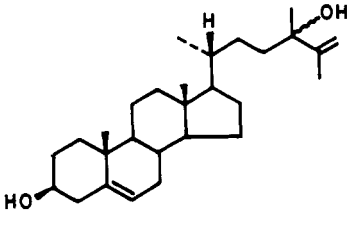

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[1]Part 21 in the series "Withanolides." For Part 20 see Y. Oshima, H. Hikino, M. Sahai, and A.B. Ray, *J. Chem. Soc., Chem. Commun.*, 628 (1989).

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Withametelin B [**2**], $C_{28}H_{36}O_5$ (*m/z* $[M]^+$ 452), mp 283–285°, $[\alpha]D$ −153.3° ($CHCl_3$), was recognized to be a close relative of withametelin [**1**] from a comparison of their spectral data. The ir spectrum of the compound showed bands for hydroxyl (3450 cm^{-1}), α,β-unsaturated-δ-lactone (1712 cm^{-1}), and conjugated carbonyl (1648 cm^{-1}) functions. Its uv absorption maxima at 206 and 314 nm (ε 10241, 4430) indicated the presence of a steroidal 2,4-dien-1-one chromophore (10), which was corroborated by the signals of three contiguous olefinic hydrogens in its ^{1}H-nmr spectrum (δ 6.93, 1H, dd, J=9.75, 5.95 Hz; 6.16, 1H, d with fine splitting, J=5.95 Hz; 6.04, 1H, d with fine splitting, J=9.75 Hz). That the side chain of withametelin B is the same as that of withametelin [**1**] became manifest from the observation that the signals associated with the hydrogens of the side chain of the latter were discernible in the ^{1}H-nmr spectrum of the former; it showed two singlets at δ 6.76 and 6.02 for the terminal methylene conjugated with the lactone carbonyl, a broad one-proton singlet at δ 4.64 for H-22, a doublet at δ 3.89 (J=13.3 Hz) and a double doublet at δ 3.72 (J=13.3, 2.45 Hz) for H-21, and a low-field methyl singlet at δ 1.48 for the Me-28 bound to C-24 bearing an oxygen function. In addition to this methyl singlet, the spectrum also showed two other methyl singlets at δ 0.77 and 1.42, respectively, for Me-18 and Me-19 groups. The chemical shift of the Me-19 signal was incidentally found to correspond to that of 2,4-dien-1-one steroids bearing a hydroxyl group at the 6β position (10), and it was formulated as illustrated in **2**. The C-6 carbinyl hydrogen appeared at δ 4.59 as a broad singlet partially overlapped by the signal for H-22. The structure of withametelin B, thus derived, was supported (2,11) by its ^{13}C-nmr data (Table 1).

Chemical evidence in support of the structure came from the observation that the acetate derivative **5** of withametelin B was indistinguishable from the product obtained by treatment of withametelin [**1**] with $Hg(OAc)_2$ in the presence of HOAc (2). One of the minor constituents of the SeO_2 oxidation products of withametelin was identified as withametelin B (2).

TABLE 1. ^{13}C-nmr Chemical Shift Assignments for **2** and **3** (in $CDCl_3$).

Carbon	Compound	
	2	**3**
C-1	206.8 s	202.2 s
C-2	119.6 d	128.9 d
C-3	141.5 d	138.6 d
C-4	128.4 d	36.8 t
C-5	159.6 s	72.2 s
C-6	77.1 d	55.2 d
C-7	41.3 t	56.2 d
C-8	32.4 d	34.5 d
C-9	51.2 d	34.7 d
C-10	55.2 s	49.9 s
C-11	23.0 t	20.8 t
C-12	42.2 t	38.7 t
C-13	44.5 s	42.4 s
C-14	57.2 d	50.7 d
C-15	25.0 t	23.1 t
C-16	28.2 t	26.6 t
C-17	49.1 d	50.4 d
C-18	14.6 q	11.0 q
C-19	20.8 q	13.2 q
C-20	41.0 d	37.8 d
C-21	62.2 t	12.0 q
C-22	75.6 d	77.7 d
C-23	34.9 t	28.7 t
C-24	71.0 s	152.0 s
C-25	140.5 s	124.0 s
C-26	166.9 s	165.9 s
C-27	131.7 t	56.4 t
C-28	27.3 q	18.9 q

The major withanolide isolated from this source was characterized by comprehensive spectral analysis as 12-deoxywithastramonolide [**3**], a compound that was first isolated as its acetate derivative from the leaves of an Indian chemotype of *Withania somnifera* and for which no trivial name was given (12). ^{13}C resonance signals of this compound (Table 1) perfectly corresponded to those reported (13) for withastramonolide [**6**],

with the expected differences due to an additional hydroxyl group at C-12 in the latter. Besides withanolides, a C_{28} sterol was also isolated from this plant material; it was identified as physalindicanol A [**4**] from spectral comparison with an authentic sample. This sterol was previously reported from two other withasteroid-bearing plants, *Withania coagulans* (14) and *Physalis minima* var. *indica* (15), and it is regarded as the precursor of withanolides and related steroidal lactones.

Withametelin [**1**], the major withanolide (yield 0.1%) of the local variety of *D. metel* (2), was isolated in a very poor yield (0.0015%) from the plant under investigation, and the 12-deoxywithastramonolide [**3**], hitherto unreported from this plant, was isolated in an appreciable yield (0.016%). The characteristic A/B ring substitution pattern discernible in all the withanolides so far reported from different *Datura* species (13,16), was not witnessed in those of *D. metel* (1–9) before the isolation of 12-deoxywithastramonolide from the plant under investigation. The presence of a typical *Datura* withanolide together with withanolides of *D. metel* in the plant makes us believe that it is a chemotype of *D. metel*, different from the local variety. The occurrence of such chemotypes in the withanolide-rich solanaceous plant *W. somnifera* has been amply demonstrated (17), and our preliminary work indicates that *D. metel*, too, may have several chemotypes. Further work is, however, necessary to establish this point; such work is in progress.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mp's were taken on a Toshniwal apparatus and are uncorrected. The uv spectra were recorded on a Shimadzu UV-260 and ir spectra on a IR-27G spectrometer. ^{1}H- and ^{13}C-nmr spectra (TMS as internal standard) were taken on JEOL JNM FX-100 and ms on Hitachi M-52 spectrometers. The ^{1}H-nmr spectrum of withametelin B was recorded on a Brucker ACE-200 spectrometer. Optical rotations were measured on a Jasco DIP-360 polarimeter and cd spectra on a Jasco A-3 instrument. Si gel (60–120 mesh) of Qualigens Fine Chemicals and Si gel G of E. Merck (India) Ltd. were used, respectively, for cc and tlc. C_6H_6-EtOAc (3:2) was used for developing tlc plates.

PLANT MATERIAL.—The plant material used in this study was purchased from M/s United Chemical & Allied Products, 10 Clive Row, Calcutta, for whom it was identified by the Botanical Survey of India, Calcutta. The plant was also identified by Prof. S.K. Roy, Department of Botany, Banaras Hindu University, Varanasi, India. An herbarium specimen of the plant is being preserved in the department.

EXTRACTION AND ISOLATION OF THE COMPOUNDS.—Powdered dried leaves (4 kg) of *D. metel* L. were extracted by percolation with 95% EtOH (20 liters), and the EtOH extract was concentrated under reduced pressure to a dark green thick syrup (360 g). This was mixed with an equal volume of H_2O and extracted successively with petroleum ether (bp 60–80°) and $CHCl_3$. The petroleum ether extract, on removal of solvent, left a dark green oily residue (110 g) that was chromatographed over Si gel and eluted first with petroleum ether and then with C_6H_6. Later fractions of the C_6H_6 eluate, showing a major Liebermann-Burchard positive spot with R_f value 0.74, were pooled together (15 g) and rechromatographed over EtOAc-washed Al_2O_3 (Neutral, Sarabhai M. Chemical). The column was first eluted with petroleum ether and then with petroleum ether/EtOAc mixtures of increasing polarity. Fractions eluted with petroleum ether-EtOAc (3:1) yielded a solid (0.15 g) which crystallized from EtOAc as white needles, mp 202°, and was *indistinguishable* (*mp*, ^{1}H *nmr*, ms, sp. rotation) from physalindicanol A [**4**], isolated from *P. minima* var. *indica* (15).

The $CHCl_3$ extract (89 g) was chromatographed over Si gel and eluted first with C_6H_6 and then with C_6H_6/EtOAc mixtures of increasing polarity. Fractions eluted with C_6H_6 and showing a major spot half-way on the chromatoplates (R_f 0.51) were pooled as Fraction A. C_6H_6-EtOAc (3:1) eluates showing a spot at R_f 0.21 were pooled as Fraction B.

WITHAMETELIN B [**2**].—Fraction A, on being freed from solvent, yielded a solid (0.13 g) that crystallized from EtOAc as a fine powder (0.07 g), indistinguishable (ir, ^{1}H nmr, co-tlc) from the 6β-hydroxy derivative of withametelin, prepared by SeO_2 oxidation of **1** in a C_6H_6/HOAc mixture (2).

Compound **2** (7 mg) was acetylated with Ac_2O (0.1 ml) in Et_3N (1 ml) overnight at room temperature. The reaction mixture after usual workup and purification by passing through a short bed of Si yielded a crystalline solid (5 mg),

mp 259°, identical in all respects (mp, mmp, ^{1}H nmr, co-tlc) with **5** prepared by treatment of withametelin [**1**] with $Hg(OAc)_2$ in HOAc (2).

12-DEOXYWITHASTRAMONOLIDE [**3**].—Fraction B crystallized from EtOAc to give a greenish white solid (1.0 g) that was recrystallized from MeOH to yield a microcrystalline solid (0.65 g): mp 292–294°, $[\alpha]_D$ +92.3° (c = 1.47, $CHCl_3$); uv λ max (MeOH) 222 nm (ε 12500); ^{1}H nmr ($CDCl_3$) δ 6.59 (1H, ddd, J = 9, 4.5, 2.2 Hz, H-3), 5.81 (1H, ddd, J = 9, 2.2, 1 Hz, H-2), 4.45 (1H, dt, J = 12.4 Hz, H-22), 4.36 (2H, s, H-27), 3.25 (1H, dd, J = 3.6, 1 Hz, H-7), 3.05 (1H, d, J = 3.6, H-6), 2.06 (3H, s, H-28), 1.20 (3H, s, H-19), 1.04 (3H, d, J = 5.8 Hz, H-21), 0.78 (3H, s, H-18); cd (dioxane), $\Delta\epsilon_{340}$ −2.9, $\Delta\epsilon_{258}$ +2.81; acetate mp 230–232° [lit. (17) mp 230–232°].

WITHAMETELIN [**1**].—Air-dried and powdered plant material (900 g) was extracted with petroleum ether (bp 60–80°) in a Soxhlet apparatus. The extract was freed from solvent to give an oily liquid (30 g) which was initially chromatographed over Si gel and then over EtOAc-washed Al_2O_3, according to the reported (2) isolation procedure, to furnish withametelin (14 mg).

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