

Withametelin H and Withafastuosin C, Two New Withanolides from the Leaves of *Datura* Species[#]

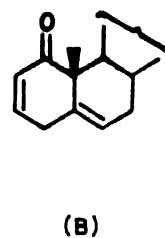
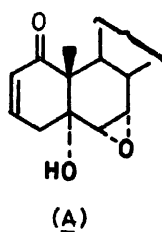
M. MANICKAM, S. KUMAR^a, A. SINHA-BAGCHI^a, S. C. SINHA^a and A. B. RAY^{*}

Department of Medicinal Chemistry, I M S., Banaras Hindu University, Varanasi-221 005

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Two new withanolides, withametelin H and withafastuosin C, isolated respectively, from *Datura metel* and *Datura fastuosa*, were fully characterised by chemical and spectroscopic methods. Daturametelin B (as its acetate) and withametelin F were also isolated from *D. fastuosa*. The results establish a chemotaxonomic relationship between the two *Datura* species.

Withasteroids, a group of steroidal lactones built on ergostane framework, are elaborated almost exclusively by the plants of the family Solanaceae. Though the abundance and variety of oxygen functions in these molecules have led to many structural variants through the formation and cleavage of C–C and C–O bonds, the majority of the compounds are built on 22-hydroxy-ergosta-26-oic acid 26,22-lactone skeleton (I) and these are known as withanolides¹⁻³. *Datura* is one of the few genera of the family Solanaceae that are known to yield withanolides and about 32 compounds of this class have so far been reported from different *Datura* species^{3,4}. While the substitution pattern of AB rings of the withanolides of *D. quercifolia*, *D. stramonium* and *D. ferox* are the same and known to bear 5 α -hydroxy-6 α ,7 α -epoxy-2-en-1-one system (A), a 2,5-dien-1-one system (B) and an oxygen function at C-21 are the common features of the withanolides of *D. metel*. In fact, oxygenation at C-21 is a unique feature of the withanolides of *D. metel*. Very recently, however, *D. fastuosa* has also been shown to yield 21-oxygenated withanolides⁵, which in turn, establishes its chemotaxonomic relationship with *D. metel*.



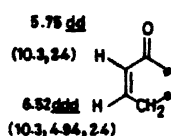
In continuation of our investigation on the withanolides of *Datura* species⁵⁻¹¹, we have isolated two new withanolides from the leaves of *D. metel* and *D. fastuosa* and these have been named respectively, as withametelin H and withafastuosin C.

Withametelin H, C₂₉H₄₂O₇, (*M*⁺, *m/z* 502), m.p. 275–78° showed a uv absorption maximum at 227 nm (ϵ 12 696), characteristic of withanolides having an enone and an α,β -unsaturated- δ -lactone system. Its ir spectrum showed, in addition to the bands (1700sh, 1670 cm⁻¹) for the above functionalities, a broad band in the hydroxyl region (3380 cm⁻¹) and ¹H nmr spectral data of the compound indicated the presence of the moieties (C) and (D), as have been observed in secowithametelin.

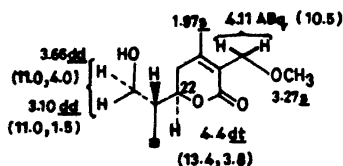
Acetylation of withametelin H with Ac₂O-pyridine at room temperature gave a diacetate

[#]Part 25 in the series on withanolides. For part 24 see Ref 5.

^aPresent address: The Scripps Research Institute, 10666 North Torrey Pines Road, La Jolla, CA 92037, U.S.A

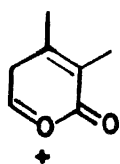
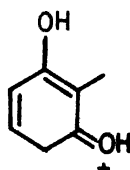


(c)



(d)

(2a) which still showed a band in the hydroxyl region (3474 cm^{-1}) of its ir spectrum. ^1H nmr spectrum of 2a showed three carbinyl hydrogen signals at a field lower than those observed in the spectrum of the parent molecule, indicating that withametelin H bears in its molecule a secondary and a tertiary hydroxyl group, in addition to a primary alcoholic group shown in the expression (D). The probable sites for the secondary and tertiary hydroxyl groups were settled from the following observations. Like typical withanolides, withametelin H showed a significant ion peak at m/z 125 in its mass spectrum but as the genesis of ion *a* is unlikely from a secowithametelin-type withanolide, it was logically considered to be due to ion *b* formed from ring A of the molecule by initial McLafferty rearrangement followed by 5–6 bond cleavage¹². The tertiary hydroxyl group in withametelin H was therefore, settled to be at C-5.

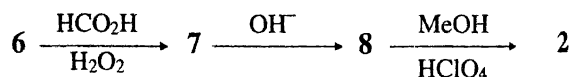
ion *a*ion *b*

m/z 125

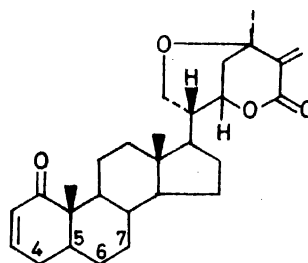
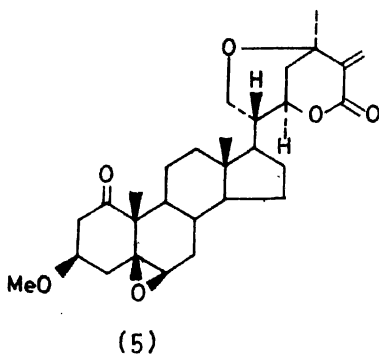
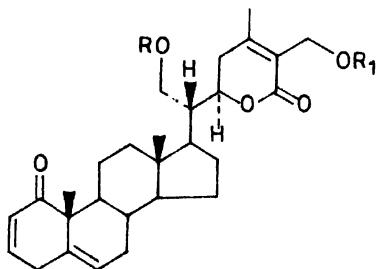
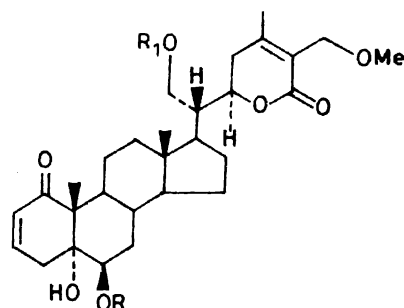
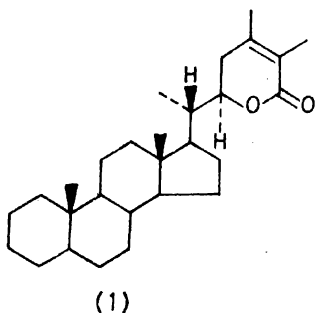
^{13}C nmr spectral comparison with secowithametelin (3) revealed the identity of CD rings and side-chain of the two molecules, and indicated a difference only in the resonance signals of the carbons of AB rings. Withametelin H contains two oxygen atoms and two oxycarbons (δ_c 77.7 and 74.9) more, and two sp^2 carbons less than those of secowithametelin (3) which led to the obvious speculation that the former possesses

a vicinal diol system in place of an isolated double bond of the latter. The structure of withametelin H was thus advanced as 2, the orientation of the vicinal hydroxyls being based on the chemical shifts of C-19 (δ_c 16.1) and H-6 (δ 3.45, 1H, br). A comparison of ^{13}C nmr data of the AB ring carbons of withametelin H with those of withaminimin¹³ also corroborated this assignment.

Chemical evidence in support of this structure came from the conversion of withametelin (6) to withametelin H (2) via withametelin G (8)¹¹. Treatment of withametelin (6) with performic acid gave a crystalline compound which was characterised as withametelin G menofomate (7) by spectral analysis. Alkaline hydrolysis of 7 yielded withametelin G (8) which on methanolysis with methanol-perchloric acid yielded a compound, indistinguishable from withametelin H (2). The *trans*-fusion of AB rings of withametelin G is based on the observed negative Cotton effect at 348 nm and its conversion to withametelin H obviously confirms the identical geometry of AB rings of withametelin H.



The isolation of two new withanolides, withafastuosins A and B, were earlier reported from the methanolic extract of the leaves of *D. fastuosa*⁵. Our continued investigation with the plant led to the isolation of a third new withanolide, withafastuosin C (5). Withafastuosin C, $\text{C}_{29}\text{H}_{40}\text{O}_6$ (M^+ , m/z 484), m.p. 235–37°, was recognised to be a withanolide-type C_{28} -steroidal lactone like its congeners, from its spectral data. The extra carbon atom present in the molecule was proved to be due to a methoxyl group from the observed 3H singlet at δ 3.27 in its ^1H nmr spectrum. The uv spectral maximum at 225 nm (ϵ 4 438) with a low molar absorptivity, and its ir spectral bands at 1718 and 1698 cm^{-1} suggested the presence of a keto carbonyl and α,β -unsaturated- δ -lactone and the probable absence of an enone



system which was corroborated from the absence of characteristic enone hydrogen signals in its ^1H nmr spectrum. Withafastuosin C failed to exhibit the diagnostic mass spectral peak of a typical withanolide, formed by fission across 20, 22 bond. The diagnostic double triplet or double doublet splitting pattern of H-22 which is normally discernible around δ 4.50 and regarded as the withanolide 'fingerprint' was also not observed in its ^1H nmr spectrum. It, however, showed a broad 1H singlet at δ 4.62, a feature

that has so far been witnessed only in the ^1H nmr spectra of withanolides having a withametelin-type bicyclic side-chain. That withafastoucine C bears a side-chain as well as C/D rings, same as that of withametelin became evident from nmr data; the hydrogens and carbons associated with this part of the molecule, showed nmr parameters virtually identical with those of withametelin (Chart 1). The difference in the two molecules was, therefore, proved to be in the AB rings which accommodated the extra

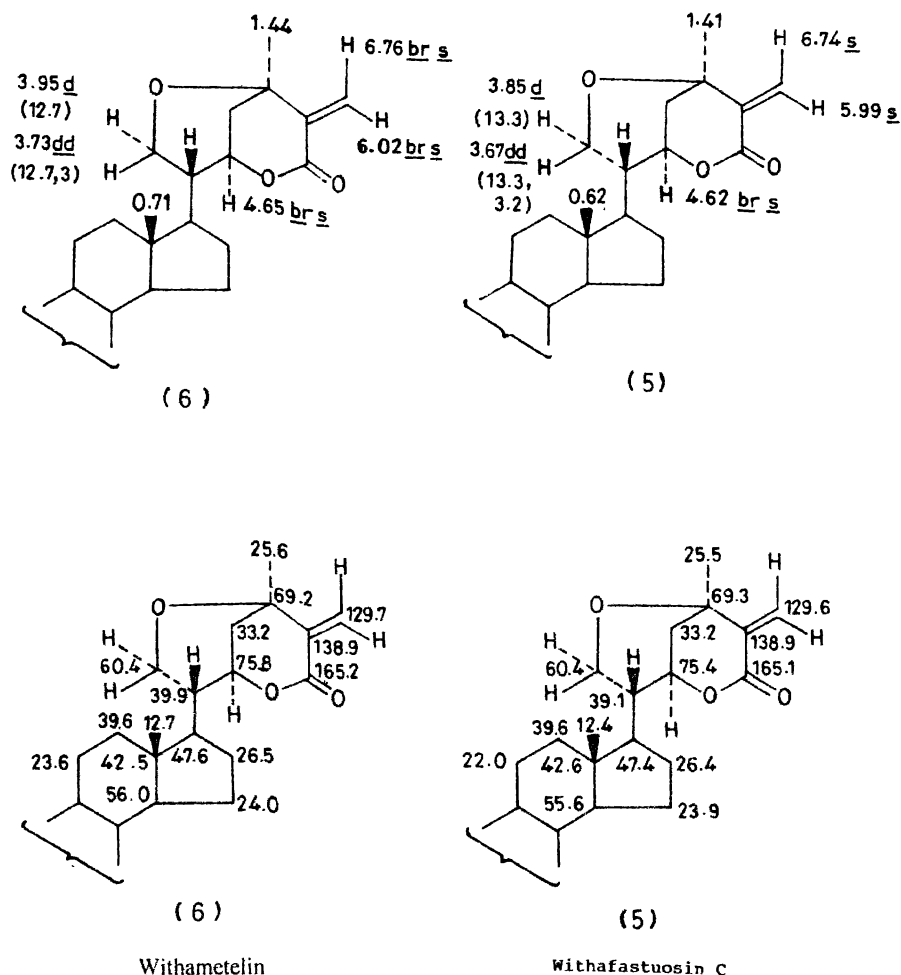
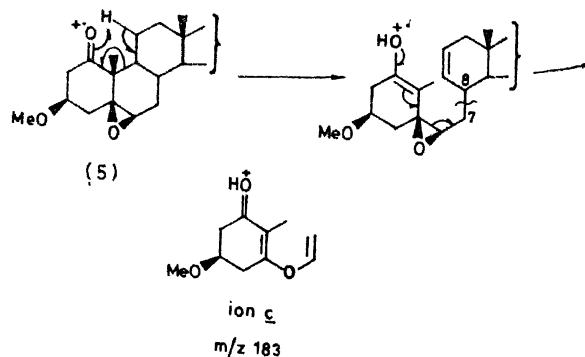


Chart 1

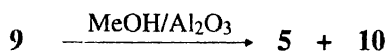
methoxyl group and two other oxygen atoms. While one of these oxygen atoms was associated with the carbonyl function at C-1 (δ_c 210.4), the other was considered to be present as 5 β ,6 β -epoxide (δ_c 61.1, 62.1 and δ 3.20 br s), the only logical and most common site for a trisubstituted oxirane in AB rings of a withanolide. The site of the methoxyl group was fixed at 3 β -position from the observed broad multiplet at δ 3.75, partly obscured by H-21 hydrogen signals in its ^1H nmr spectrum. Withafastuosin C was thus formulated as 5, the orientation of the epoxide function being based on ^1H nmr parameters of H-6 and biogenetic ground. The structure also fits with the mass peak of m/z 183 (ion c), formed by initial McLafferty

rearrangement and subsequent cleavage of 7, 8 bond as shown below :



Conversion of withametelin F (9) to withafastuosin C (5) provided the desired chemical evidence

in support of the structure of the latter. The conversion was achieved by smooth Michael addition of methanol across the enone double bond of withametelin F, effected on a bed of alumina. Several methods for such a Michael addition have been reported in the literature¹⁴⁻¹⁶ and reagents like TsOH, Ba(OMe)₂ and DBU have been used but our method was proved to be relatively simple. However, the formation of withafastuosin C was accompanied by a side-product which was characterised as withametelin B (10)⁹,



Michael addition of methanol to the enone system is known but withafastuosin C was regarded as a natural product, rather than an artefact, because a methanolic solution of withametelin F, on being refluxed for 24 h or being kept on silica gel column for 72 h, did not show any spot corresponding to withafastuosin C on thin layer chromatograms.

The known withanolides, withametelin F (9) and daturametelin B acetate (4) were characterised by comparison of their spectral data with those reported in the literature^{11,18}. The structural similarity of the withanolides of two *Datura* species speaks of their taxonomic relationship.

Experimental

M.ps. were taken on a Toshniwal apparatus and are uncorrected. Ir spectra (KBr) were recorded on a FT IR-5300 spectrophotometer, uv spectra (MeOH) on a JASCO-7800 spectrophotometer and nmr spectra (TMS as internal standard; in CDCl₃) on Bruker AC-E 200 and AMX-400 spectrometers. Samples were dried over P₂O₅ at 80–140°. Column and thin layer chromatography were carried out respectively, over silica gel (60–120 mesh) and silica gel G (Qualigens Fine Chemicals).

Plant material : Leaves of *D. metel*¹⁰ were supplied by M/s. United Chemicals and Allied

Products, Calcutta, and leaves of *D. fastuosa*⁵ were collected from the plants, growing in the University campus, and identified by Professor G. N. Choudhri, Department of Botany, Banaras Hindu University, Varanasi. Specimen samples are being preserved in the department.

Extraction and isolation of withanolides : Dried and powdered leaves (4 kg) of *D. metel* were extracted with MeOH in a Soxhlet apparatus and the extract was concentrated to a thick syrup (370 g), diluted with water (350 ml), and the aqueous suspension was sequentially extracted with petroleum ether-benzene mixture (1 : 1) and CHCl₃. The CHCl₃-soluble fractions were freed from solvent, chromatographed over silica gel and the column was eluted first with C₆H₆ and then with C₆H₆-EtOAc* mixture of increasing polarity. C₆H₆-EtOAc (1:3) eluates afforded withametelin H (0.49 g) which was crystallised from EtOAc as white microcrystalline powder; *withametelin H* (2): C₂₉H₄₂O₇. m.p. 275–78° (Found: C, 68.7, H, 8.05. C₂₉H₄₂O₇ requires : C, 69.22, H, 8.36%); λ_{max} (MeOH) 227 nm (ε 12 696); ν_{max} (KBr) 3 380br, 1 700sh, 1 670 cm⁻¹; ¹H nmr (200 MHz) δ 6.52 (1H, ddd, *J* 10.3, 4.9, 2.4 Hz), 5.75 (1H, dd, *J* 10.3, 2.4 Hz), 4.40 (1H, dt, *J* 13.4, 3.8 Hz), 4.11 (2H, ABq, *J* 10.5 Hz), 3.65 (1H, dd, *J* 11.0, 4.0 Hz), 3.87 (1H, dd, *J* 11.0, 1.5 Hz), 3.45 (1H, br s), 3.27 (3H, s), 3.17 (1H, dt, *J* 14.0, 2.0 Hz), 2.90 (1H, dd, *J* 14.0, 5.0 Hz), 1.97, 1.20 and 0.66 (3H, s each); *m/z* 502 (*M*⁺), 484 (*M*⁺-H₂O), 471, 470, (*M*⁺-MeOH), 452 (*M*⁺-H₂O-MeOH), 414, 382, 334, 316, 291, 235, 232; ¹³C nmr (C₅D₅N) δ_c 205.0, 129.1, 142.1, 36.7, 77.7, 74.9, 27.3, 38.4, 34.4, 52.7, 24.0, 40.1, 42.2, 57.8, 24.6, 47.5, 46.2, 12.8, 16.1, 43.3, 59.0, 78.2, 31.1, 157.5, 123.5, 165.8, 66.1, 20.2 (C-1-C-28), 56.2 (OMe).

Withametelin H diacetate (2a) : A mixture of withametelin H (40 mg), freshly distilled Ac₂O (0.5 ml) and C₅H₅N (0.2 ml) was kept overnight at ambient temperature under anhydrous condition and the reaction mixture was freed

from reagents under vacuum over boiling C_6H_6 ; ν_{max} (KBr) 3470, 1700, 1687 cm^{-1} ; ^1H nmr (200 MHz) δ 6.55 (1H, ddd, J 10.2, 5.0, 3.0 Hz), 5.88 (1H, dd, J 10.2, 3.0 Hz), 4.45 (1H, dt, J 13.4, 4.0 Hz), 4.22 (2H, ABq, J 15.2 Hz, H-27), 4.20 (2H, dd partly obscured by H-27), 4.77 (1H, br s), 3.35 (3H, s), 2.09, 2.03 (3H, s each), 2.0, 1.2 and 0.73 (3H, s each); m/z 587 (MH^+).

Conversion of withametelin (6) to withametelin H (2) : A solution of withametelin (0.2 g) in $HCOOH$ (3 ml) was stirred with 30% H_2O_2 (0.2 ml) for 1 h, and then kept overnight in a refrigerator. The reaction mixture was diluted with hot water and extracted with EtOAc. The residue from EtOAc extract was chromatographed over silica gel to yield **7** from hexane-EtOAc (3 : 1) eluate; m/z 498 (M^+), 452 ($M^+ - HCOOH$), 434 ($M^+ - HCOOH - H_2O$), 225, 197, and 125 (ion *b*); ^1H nmr (500 MHz) δ 8.12 (1H, s CHO), 6.74 and 6.02 (1H, s each), 6.54 (1H, ddd, J 10.0, 5.0, 2.4 Hz), 5.90 (1H, dd, J 10.0, 2.4 Hz), 4.92 (1H, t, J 2.5 Hz), 4.64 (1H, br s), 3.88 (1H, d, J 13.1 Hz), 3.66 (1H, dd, J 13.1, 3.1 Hz), 1.42, 1.25, 0.67 (3H, s each).

To a methanolic solution of **7** (25 mg) was added aqueous NaOH (25%; 2 ml) and the mixture was heated on a water-bath for 15 min. The reaction mixture was then diluted with H_2O , acidified with dilute HCl and extracted with EtOAc. The residue from the EtOAc extract was chromatographed over a short bed of silica gel and eluted with hexane-EtOAc (1 : 1) to yield a compound (10 mg) which was indistinguishable (^1H nmr, m.m.p., co-tlc) from naturally occurring withametelin **G** (**8**).

To a methanolic solution of **8** (50 mg in 5 ml) was added a few drops of $HClO_4$ (70%) and left overnight at ambient temperature. Addition of water separated a solid which was filtered, dried and chromatographed over silica gel. Elution with C_6H_6 -EtOAc (1 : 1) removed unreacted **8** while elution with EtOAc afforded a white

amorphous powder (25 mg), identical with withametelin **H** (**2**) (co-tlc, ^1H nmr).

Withafastuosin C (5) : Dried and powdered leaves (3 kg) of *D. fastuosa* were extracted with MeOH, and the extract was fractionated following a procedure same as that employed for the isolation of **2**. The $CHCl_3$ -soluble fraction adsorbed on a bed of silica gel column was eluted first with C_6H_6 (Fraction A), then with EtOAc and finally with EtOAc-MeOH (19 : 1) mixture (Fraction B). Fraction A (20 g) on rechromatography over silica gel furnished withametelin **F** (0.5 g) from early fractions and withafastuosin **C** (125 mg) from later fractions of petrol-EtOAc (3 : 1) eluates : **5**, white crystals m.p. 235–37°, $C_{29}H_{40}O_6$ (M^+ , m/z 484); λ_{max} (MeOH) 225 nm (ϵ 4438); ν_{max} (KBr) 1718, 1698 cm^{-1} ; ^1H nmr (400 MHz) δ 6.74 (1H, br s), 5.99 (1H, br s), 4.62 (1H, br s), 3.84 (1H, d, J 13.3 Hz), 3.67 (1H, dd, J 13.3, 3.2 Hz), 3.75 (1H, m), 3.29 (3H, s), 3.20 (1H, br s), 1.41, 1.14 and 0.62 (3H, s, each); m/z 485 (MH^+), 467 ($MH^+ - H_2O$), 183 (ion *c*).

Daturametelin B acetate (4) :

Fraction B was chromatographed over silica gel to yield a greenish yellow amorphous solid (2 g) from EtOAc-MeOH (9 : 1) eluate. Acetylation ($Ac_2O - C_5H_5N$) of the solid at ambient temperature and purification by column chromatography yielded white crystals (200 mg), m.p. 148–50°, FAB MS, m/z 595 ($M^+ + Cs$). ^1H and ^{13}C nmr parameters of the acetate were in perfect agreement with the reported data of daturametelin **B** acetate^{11,18}.

Conversion of withametelin F (9) to withafastuosin C (5) and withametelin B (10)⁹ : A solution of withametelin **F** (0.1 g) in MeOH (5 ml) was adsorbed on a small bed of alumina (3 g) and left at ambient temperature for 72 h, after which the column was washed with MeOH. The washing was freed from solvent and chromatographed over silica gel in a column

which was eluted first with petroleum ether and then with petroleum ether–EtOAc mixture of increasing polarity. Elution with petrol– EtOAc (3 : 1) and crystallisation from the eluting solvent yielded withafastuosin C (10 mg). Elution with petrol–EtOAc (1 : 1) afforded a compound (7 mg), λ_{max} (MeOH) 206 and 314 nm (ϵ , 10 241, 4 430) which was identified as withametelin B (10) by direct comparison (m.m.p., co-tlc, ^1H nmr).

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