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STRUCTURES OF WITHAMETELIN AND ISOWITHAMETELIN, WITHANOLIDES OF DATURA METEL LEAVES[1]

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ABSTRACT : Structures of withametelin and isowithametelin, two novel hexacyclic withanolides isolated from the leaves of Datura metel, have been established as 1 and 2 respectively, on the basis of detailed spectral and chemical evidences. The facile transformation of withametelin to a ring A aromatic withanolide and some other interesting chemical transformations are described.

The structure elucidation of withametelin, the major steroidal constituent of the dried leaves of Datura metel Linné, was reported by us almost exclusively on the basis of spectral evidence in a preliminary communication.[2] A full account of the isolation and structure determination of withametelin and its congener, isowithametelin, is presented in this paper together with some interesting chemical transformations of these molecules.

Withametelin, $C_{28}H_{36}O_4$ (M^+ 436.2426), m.p. 210°, $[\alpha]_D$ -64.4°, was proved to bear in its molecule an enone and a α,ß-unsaturated-δ-lactone moiety from its UV and IR spectral characteristics (λ_{max} 222 nm (ε 7000); ν_{max} 1718, 1655 cm^{-1}) which are comparable with those of a typical withanolide.[3] Of the five olefinic hydrogen signals discernible in its ^1H NMR spectrum, the two appearing as singlets with fine splitting at δ 6.76 and 6.02 were assigned to hydrogens of a terminal methylene conjugated with a δ-lactone and the remaining three were considered to be associated with steroidal 2,5-dien-1-one system on the basis of their chemical shifts and splitting patterns (δ 6.77, 1H ddd, J=9.8, 5.1, 2.5 Hz, 5.88, 1H dd, J=9.8, 2.5 Hz, 5.58, 1H t, J=5.8 Hz).[4] Chemical evidence to verify the presence of 2,5-dien-1-one moiety was secured by acetoxylation of withametelin with mercuric acetate which yielded a derivative (3) having 6ß-acetoxy-2,4-dien-1-one system. This chemical conversion, which can be readily recognized from a change in UV absorption characteristics (λ_{max} 310, 207 nm) and appearance of signals for three contiguous olefinic hydrogens (δ 6.94, 1H dd, J=10, 6 Hz, 6.30, 1H dd, J=6, 1 Hz, 6.04, 1H dd, J=10, 1 Hz) and an acetoxy methyl (δ 2.05, 3H s) with concomitant downfield shift of the 19-methyl signal in the ^1H NMR spectrum, has been studied in similar system[5] and considered to be a diagnostic proof for the presence of the 2,5-dien-1-one moiety. The exclusive formation of the 6ß-acetoxy

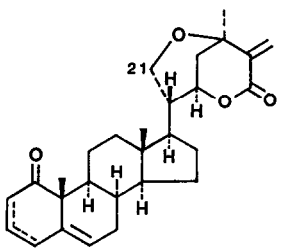
derivative (3) may be rationalized by assuming the formation of a cyclic mercuronium ion[6] by the addition of $^{+}HgOAc$ on the 5,6-double bond from the less hindered α-face of the molecule followed by the opening of the cyclic complex with concomitant generation of the 4,5-double bond and nucleophilic substitution of the 6α-mercurated compound with acetic acid. A report[7] of the exclusive formation of 5α,6α-epoxide of a withasteroid having 2,5-dien-1-one system by treatment with m-chloroperbenzoic acid lent credence to the α-attack, as envisaged in the formation of the cyclic mercuronium complex.

While the isolation of withametelin from a solanaceous plant, its molecular formula and the presence of enone and α,ß-unsaturated-δ-lactone chromophores in its molecule, suggested it to be a withanolide or a structurally related compound, it failed to exhibit diagnostic mass and ^{1}H NMR spectral features of a typical withanolide. The mass spectrum of withametelin showed no significant fragment ion peak formed by fission across C-20,22 bond which is characteristic of common withanolides. Further, the diagnostic double triplet or double doublet splitting pattern of the C-22 carbinyl hydrogen signal which normally appears around δ 4.5, and is regarded as the withanolide "finger print", was not discernible in its ^{1}H NMR spectrum and instead, a broad 1H singlet was observed at δ 4.65. Again, while a typical withanolide shows, in its ^{1}H NMR spectrum, five methyl signals including two vinylic methyl signals, only three 3H singlets appeared at δ 0.71, 1.22 and 1.44 in the ^{1}H NMR spectrum of withametelin and these were assigned respectively, to 18- and 19-methyls and to a methyl on a carbon bearing oxygen function. The absence of two methyl groups in the molecule was, however, compensated by the presence of a terminal methylene ($>C=CH_2$) and an oxymethyl ($-CH_2-O$) grouping (δ 3.95, 1H d, J=12.7 Hz, 3.73, 1H dd, J=12.7, 3 Hz). The terminal methylene conjugated to the lactone carbonyl was assigned to C-27 but the location of the oxymethyl group could not be easily settled and it was an open question which of the remaining two methyls (C-21 and C-28) of this C_{28}-steroid was oxygenated?

Of the four oxygen atoms present in withametelin, three are associated with an enone and δ-lactone function and the remaining one was considered to be an ether in view of the fact that withametelin was not amenable to acetylation under normal condition and it exhibited no hydroxyl band in the IR spectrum. Based on these observations and from valency calculation, withametelin was proved to be hexacyclic and the fourth oxygen atom associated with an oxymethyl group was considered responsible for the construction of the sixth ring. The two oxycarbon signals at δ 69.2 (s) and 60.4 (t) in the ^{13}C NMR spectrum lent support to this assumption. The foregoing data permitted advancement of two alternative structures 1 and 4 for withametelin. An inspection of these two alternative structures revealed that the cyclic ether is allylic in nature only in 1 and, therefore, it was considered possible to make a correct choice by ascertaining whether the ether oxygen in withametelin is allylic or not. With this objective, withametelin was subjected to catalytic hydrogenation to give a mixture, which on chromatographic resolution yielded a hexahydro derivative (M^{+} 442) as the major product, along with tetrahydrowithametelin (5) (M^{+} 440) in a poor yield. The tetrahydro derivative (5), formed by hydrogenolysis of the cyclic ether, showed signals for two vinylic methyls (δ 1.93 and 1.88, 3H s each) and the characteristic double triplet for H-22

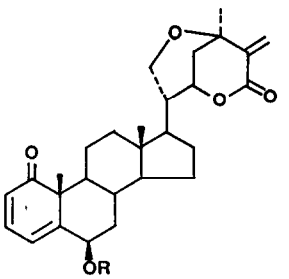
(δ 4.37, 1H dt, J=13.5, 3.6 Hz) in its ^{1}H NMR spectrum and the base peak at m/z 125 (ion a) in its mass spectrum like typical withanolide. Withametelin was thus established to have a cyclic allyl ether function and formulated as 1 which was verified by ^{13}C shift correlation two dimentional spectrum (2D INADEQUATE) and reported in the previous communication.[2]

Both tetrahydrowithametelin (5) and secowithametelin (6), the latter being obtained in near quantitative yield by treatment of withametelin with methanol in presence of perchloric acid,[8] showed positive Cotton effect around 250 nm, indicating 22R configuration. A comparative study of the ^{13}C NMR spectra of withametelin and its derivatives with that of withaferin A acetate,[9] revealed 14α and 17ß configurations in withametelin. The stereochemistry at C-20, as shown in the expression 1 is based on biogenetic arguments because all the reported withanolides unsubstituted at C-20 have the same configuration. The Dreiding stereomodel study of withametelin revealed that 28-methyl and H-22 are on the same side of the molecule.



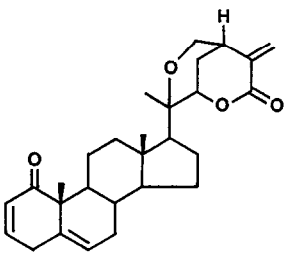
1 : Δ^2

2 : Δ^3



3 : R=Ac

3a : R=H



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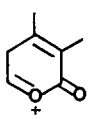
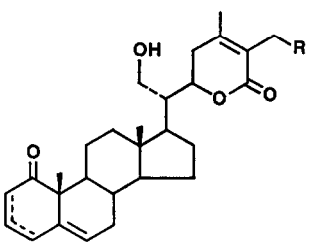


ion a
(m/z 125)



5 : R=H, 2,3,5,6-tetrahydro

6 : Δ^2, R=OMe

6a : Δ^3, R=OMe

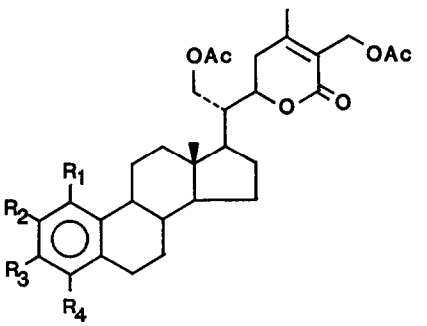
6b : Δ^2, R=OGlc

The smooth addition of methanol to withametelin in presence of perchloric acid[8] led to the assumption that withametelin, on treatment with acetic anhydride and perchloric acid, should give a diacetate but, to our surprise, the product obtained was a triacetate (7) (M^+ 580). An analysis of its spectral properties revealed significant difference in its carbocyclic moiety from that of withametelin. The ^{1}H NMR spectrum of the triacetate (7) showed signals for three acetoxy methyls (δ 2.08, 2.06 and 2.05, 3H s each), and a vinylic methyl (δ 1.98, 3H s) and the 18-methyl (δ 0.76, 3H s), while the signal for the 19-methyl was found to be missing and it was replaced by a 3H singlet at δ 2.30 assignable to an aromatic methyl group. A pair of 1H doublets at δ 7.32 and 6.80 (J=8 Hz) for two vicinal aromatic hydrogens was discernible in the ^{1}H NMR spectrum. The presence of two $-CH_2-OAc$ groupings was manifest from two sets of signals appearing as two pairs of doublets (δ 4.36, 1H dd, J=12, 1 Hz, 4.26, 1H dd, J=12, 4 Hz and δ 4.88, 2H AB q, J=15.4 Hz) assigned to 21- and 27-methylenes, respectively. The ^{1}H NMR spectrum of the triacetate (7) also showed the diagnostic double triplet at δ 4.49 (J=13, 3.5 Hz) for H-22 of withanolides unsubstituted at 20-position.

That the A ring of this triacetate (7) is aromatic in nature became evident from an inspection of its ^{13}C NMR spectrum which lacked the enone carbonyl signal of withametelin and showed six aromatic carbon signals of a tetrasubstituted benzene ring (δ 147.1 s, 138.0 s, 136.8 s, 128.0 s, 123.6 d, 118.9 d). A subtraction UV spectrum of the triacetate (7) and physalolactone B (1α-acetoxy-3β,20-dihydroxywitha-5,24-dienolide)[10] which showed an absorption maximum at 278 nm also spoke of the presence of a benzenoid chromophore. The mass spectrum of the triacetate (7) showed not only the molecular ion peak at m/z 580 but also three significant fragment ion peaks at m/z 538, 478 and 418, formed by sequential loss of a molecule of ketene and two molecules of acetic acid. The loss of ketene suggested the presence of a phenol acetate moiety which was supported by positive phosphomolybdic acid test.

The aromatization of the ring A of withametelin is assumed to take place by the well known dienone-phenol rearrangement, the dienone in this case being 2,4-dien-1-one, formed by appropriate prototropic shift under the reaction condition. While three possible structures (7, 7a and 7b) having different substitution patterns of the ring A may be conceived, the volume of literature[11] available for dienone-phenol rearrangement of related 2,4-dien-3-one steroids suggests the formulation 7 for the triacetate. In fact, that no nOe was detected on aromatic hydrogen signals on irradiation of the aromatic methyl signal at δ 2.30 rules out the substitution pattern as shown in expression 7a. Further, it was found that the ^{13}C NMR signals of the aromatic carbons of estrone acetate[12] matched well with those of the triacetate, except C-4 and C-6. The signal for the carbon (C-10) corresponding to C-4 of estrone acetate appeared at lower field and that for C-6 at higher field, demonstrating the presence of methyl substituent at C-10 position and thus confirming the correctness of the structure (7) proposed for the triacetate of withametelin. It is pertinent to mention in this connection that though aromatization of the ring A of withametelin with acetic anhydride and perchloric acid is considered to take place by dienone-phenol rearrangement, withametelin-derived 6β-acetoxy-2,4-dien-1-one (3) failed to undergo aromatization with acetic acid and perchloric-

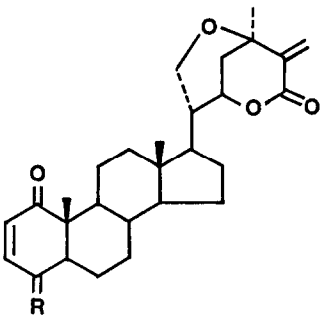
acid and yielded a conjugated trien (8). While it may be argued that the facile acid-catalyzed elimination of the allylic acetoxy function at C-6 prevents aromatization of the dienone (3), no suitable explanation is available for the failure in aromatization of the ring A of withametelin on treatment with methanol and perchloric acid. Secowithametelin (6) obtained under this reaction condition retains the 2,5-dien-1-one system. It is, therefore, quite logical to believe that acetic anhydride has an important role in the aromatization of the ring A of 2,5-dien-1-one steroids.



7 : $R_1=R_2=H$, $R_3=OAc$, $R_4=Me$

7a : $R_1=OAc$, $R_2=R_3=H$, $R_4=Me$

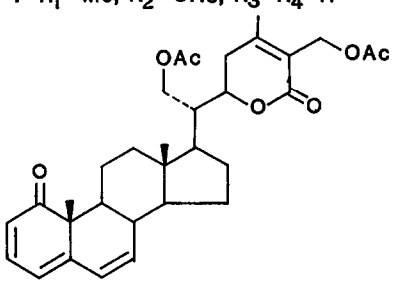
7b : $R_1=Me$, $R_2=OAc$, $R_3=R_4=H$



9 : $R=H_2$, 5α,6α-epoxide

10 : $R=H_2$, 5β,6β-epoxide

11 : R=O



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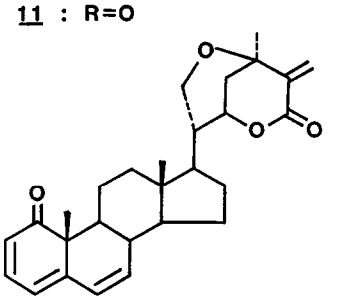


12

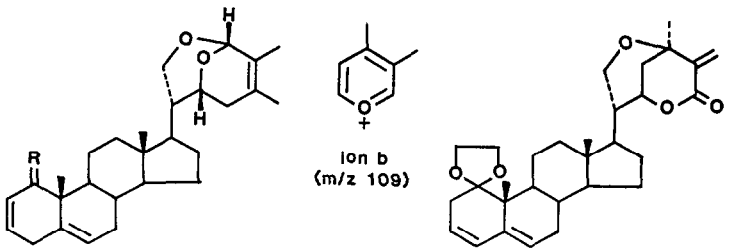
As the variety of oxygen substituents witnessed in AB rings of withanolides are known[13] to be derived from the one having 2,5-dien-1-one system, efforts were made to introduce some of these oxygen functions in view of their possible existence as natural products. Withametelin on treatment with m-chloroperbenzoic acid yielded a mixture of epoxides. The mixture was resolved by HPLC and the major product, $C_{28}H_{36}O_5$ (M^+ 452), which showed a 1H narrow triplet at δ 3.02 was recognized as withametelin-5α,6α-epoxide (9) from the negative Cotton effect at 345 nm. The minor product, isomeric with 9 showed positive

Cotton effect at 345 nm and the carbinyl hydrogen signal of the oxirane at δ 3.10[14] in its ^{1}H NMR spectrum and on this basis it was identified as withametelin-5ß,6ß-epoxide (10). In an effort to insert a hydroxyl group at the bis-allylic 4-position of withametelin, it was oxidized with selenium dioxide in aqueous acetic acid medium. The reaction mixture, on chromatographic separation yielded four compounds which were identified from detailed spectral analysis as 3, 3a, 11 and 12.

Withametelin, which bears a α-methylene-δ-lactone as well as an oxide ring in its side chain, could be transformed to yet another interesting hexacyclic withanolide (14). Reduction of withametelin with diisobutylaluminium hydride gave an allyl alcohol (13), the formation of which can be explained by hydrogenolysis of allyl ether, reduction of the δ-lactone to lactol and formation of an acetal with the participation of hydroxyl group at C-21. Oxidation of 13 with pyridinium dichromate in methylene chloride yielded 14 without affecting the bicyclic system. Mass spectra of both 13 and 14 showed a base peak at m/z 109 (ion b) and their ^{1}H NMR showed the absence of the signals for exocyclic methylene and the low field methyl attached to the carbon bearing oxygen function and instead showed two 3H singlets for two vinylic methyls and a low field 1H broad singlet for the acetal hydrogen.

Daturilin, isolated from the fresh leaves of D. metel by the Pakistani workers[15] has been assigned the same gross structure as withametelin but with 22S configuration. Though no direct comparison of withametelin with daturilin has been made, they appear to be identical and, therefore, 22S configuration assigned to this compound exclusively on the basis of nOe measurements, is not tenable.

Isowithametelin, $C_{28}H_{36}O_4$, m.p. 280°, $[\alpha]_D$ -77.7°, a congener of withametelin, showed a R_f value very close to that of the latter on TLC in a variety of solvent systems and it was found to be present in mother liquor of withametelin from which it was isolated by preparative HPLC work. The UV spectrum of isowithametelin showed twin absorption maxima at 233 and 227 nm, indicating the probable presence of a α,ß-unsaturated-δ-lactone and a heteroannular dien chromophore[14] and its IR spectrum showed a strong carbonyl absorption band at 1714 cm^{-1} but no band for the enone carbonyl or hydroxyl group. The ^{13}C NMR spectrum of isowithametelin, however, showed two carbonyl carbon signals at δ 210.7 and 165.4 assignable, respectively, to a saturated keto carbonyl and a lactone carbonyl. The ^{1}H NMR spectrum of isowithametelin showed striking resemblance with that of withametelin, the only observable difference being the chemical shifts and splitting patterns of three olefinic hydrogens of the AB rings and 19-methyl of the two molecules. Based on this finding and from the correspondance of ^{1}H NMR parameters of these three olefinic hydrogens with those of withanolide I,[16] the AB ring of isowithametelin was proved to have 3,5-dien-1-one system and the structure 2 was advanced for isowithametelin. ^{13}C NMR data of isowithametelin (Table I) is in perfect agreement with the proposed structure. Chemical evidence in support of the structure was provided by conversion of withametelin to isowithametelin. When a solution of withametelin in benzene was refluxed with ethylene glycol in presence of p-toluenesulphonic acid, the ketal (15) was obtained as the major product, which on deketalization with p-toluenesulphonic acid in aqueous acetone furnished a compound indistinguishable from

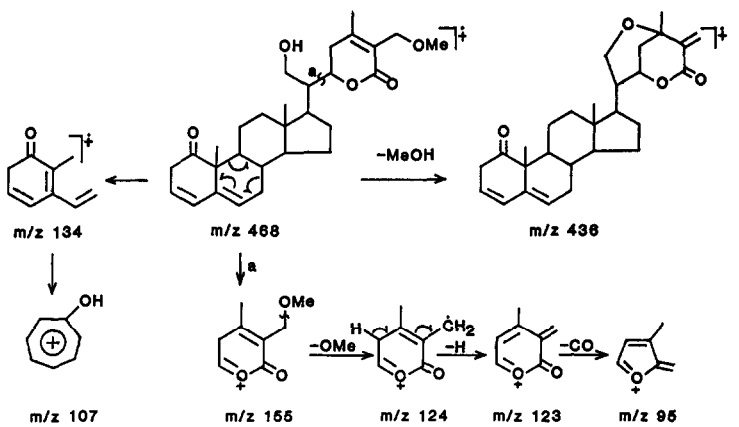


13 : R=H(OH)

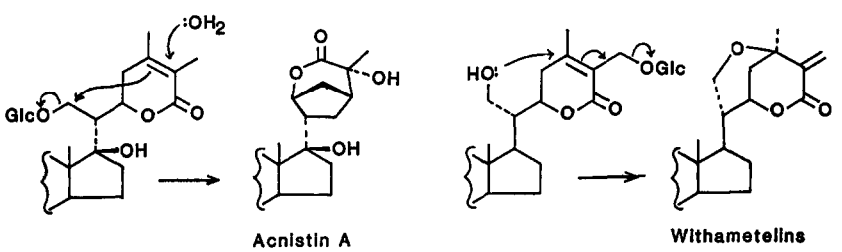
14 : R=O

15

isowithametelin in all respects. The methanol adduct (6a) of isowithametelin obtained by treatment of its methanolic solution with perchloric acid showed positive Cotton effect at 241 nm, indicating that isowithametelin also has 22R configuration like withametelin. The mass spectrum of the methanol adduct (6a) displayed significant fragment ion peaks, the genesis of which can be very well rationalized from the expression 6a (Scheme I).



Scheme I



Scheme II

Table I : Carbon-13 shieldings in withametelin and its derivatives (125 MHz, $CDCl_3$)

Carbon No.	1	2*	5	7 **	9*	10*	Carbon No.	1	2*	5	7	9*	10*
C-1	204.5 s	210.7 s	214.7 s	147.1 s	201.6 s	202.3 s	C-16	26.5 t	26.7 t	26.3 t	27.6 t	25.3 t	25.4 t
C-2	128.0 d	39.6 t	38.4 t	118.9 d	127.6 d	128.2 d	C-17	47.6 d	47.7 d	46.6 d	46 8 d	46.2 d	46.5 d
C-3	145.0 d	127.0 d	27.4 t	123.6 d	141.1 d	143.1 d	C-18	12.7 q	12.9 q	12.6 q	12.2 q	11.7 q	11.5 q
C-4	33.4 t	129.5 d	31.2 t	138.0 s	33.0 t	31.9 t	C-19	18.9 q	20.5 q	19.4 q	12.1 q	14.5 q	14.0 q
C-5	135.9 s	141.2 s	141.4 s	136.8 s	63.7 s	61.0 s	C-20	39.9 d	40.0 d	42.6 d	42.7 d	38.8 d	38.6 d
C-6	124.5 d	121.7 d	121.8 d	27.8 t	57.5 d	62.2 d	C-21	60.4 t	60.6 t	60.0 t	61.8 t	59.4 t	59.4 t
C-7	30.7 t	31.1 t	32.2 t	27.7 t	27.7 t	30.2 t	C-22	75.8 d	75.7 d	78.2 d	***	74.4 d	74.7 d
C-8	33.2 d	31.8 d	32.0 d	37.7 d	29.7 d	28.8 d	C-23	33.2 t	33.4 t	31.0 t	32.6 t	32.2 t	32.2 t
C-9	42.8 d	41.0 d	45.5 d	44.2 d	36.9 d	43.5 d	C-24	69.2 s	69.5 s	150.3 s	157 3 s	68.3 s	68.0 s
C-10	50.4 s	52.2 s	53.9 s	128.0 s	47.2 s	47.4 s	C-25	138.9 s	139.1 s	122.8 s	122.1 s	137.9 s	137.8 s
C-11	23.6 t	22.6 t	22.7 t	26.8 t	21.6 t	22.5 t	C-26	165.2 s	165.4 s	166.8 s	164.9 s	164.2 s	164.2 s
C-12	39.6 t	39.9 t	39.1 t	38.7 t	38.4 t	38.5 t	C-27	129.7 t	130.0 t	12.3 q	58.1 t	128.8 t	128.8 t
C-13	42.5 s	43.1 s	42.5 s	43.0 s	41 5 s	41.5 s	C-28	25.6 q	25.7 q	20 6 q	20.7 q	24.5 q	24.5 q
C-14	56.0 d	56.0 d	56.3 d	55.1 d	55 1 d	54.6 d	-OAc				21.2 q		
C-15	24.0 t	24.2 t	24.3 t	23 9 t	22.9 t	22 9 t					21.0 q		
											20.9 q		

* 25 MHz ** Carbon numbering is based on withametelin, i.e., carbons bearing -OAc and methyl are C-1 and C-10 respectively
*** Not detected

Beside withametelins, the only other withanolides known to bear bridged bicyclic side chains are acnistins[17] and the only difference in the side chain of these two groups of compounds is that while in acnistins C-21 and C-24 are directly linked, in withametelin they are separated by an oxygen bridge. Biogenetically, however, there exists a commonness in these two systems which are considered to be formed by S_N2' type reaction of appropriately substituted withanolide (Scheme II). Daturametelin B (6b), isolated from the methanolic extract of fresh leaves of D. metel by Japanese workers[18] might very well be the precursor of withametelin but the suggestion that withametelin is an artefact formed during chromatography over silica gel column is not tenable because withametelin was isolated from the petroleum ether extract of the plant material in a fairly good yield (0.1%) and daturametelin B (6b), a glucoside, is not likely to be extracted with this solvent. The alternative suggestion[19] that daturametelin B (6b), a constituent of fresh leaves of D. metel, is transformed to withametelin during drying of the plant material is, no doubt, a possibility but our observation based on HPLC analysis, suggests that both daturametelin B (6b) and withametelin coexist in fresh as well as in dry leaves, and that the former is the likely precursor of the latter.

EXPERIMENTAL :

M.ps were taken on a TOSHNIWAL melting point apparatus and are uncorrected. Column chromatography was carried out over silica gel (60-120 mesh, B.D.H.) and TLC over silica gel G (B.D.H.). All the samples were routinely dried over P_2O_5 at 80-140° (depending upon the m.ps of the compounds) for 24 hrs in vacuo and were tested for purity by TLC. Optical rotations were measured on a JASCO DIP-360 polarimeter and CD spectra on a JASCO A-3 instrument. IR

spectra were recorded on a SHIMADZU IR-27G spectrometer, UV spectra on a SHIMADZU UV-202 spectrophotometer, ^{1}H and ^{13}C NMR spectra on a JEOL FX-90Q, JEOL JNM FX-100 and JNM FX-500 spectrometers (TMS as internal standard). Low resolution EIMS were determined with a HITACHI M-52 and high resolution EIMS with a JEOL-01-SG-2 spectrophotometers.

Isolation of withametelin (1) and isowithametelin (2): Air dried and pulverized leaves of D. metel (600 g) were extracted with petroleum ether (80-100°) in a soxhlet apparatus for 20 hrs. The petroleum ether extract (25 g) was chromatographed over silica gel (125 g) and eluted initially with petroleum ether followed by C_6H_6. C_6H_6 fractions (4 g) on rechromatography over EtOAc washed alumina (40 g) and elution with petroleum ether-EtOAc (17:3) afforded withametelin (600 mg). Isowithametelin was isolated by HPLC of the mother liquor of withametelin over silica gel column (No. S.I.G., I.D. 22 mm). Elution of the column with n-hexane-EtOAc (3:1) at the rate of 9 ml/min afforded isowithametelin after 20 min and withametelin after 22 min.

Withametelin (1) crystallized from EtOAc as colourless needles, m.p. 210°; $[\alpha]_D$ -64.4° (c 0.45, $CHCl_3$); HREIMS m/z: 436.2426 (M^+); EIMS m/z: 436 (M^+), 421, 354, 268, 253, 225, 173, 147, 145, 131, 107, 105, 91, 67, 55; UV (MeOH) λ_{max} nm: 222 (ε 7000); IR (KBr) ν_{max} cm^{-1}: 1718, 1655; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 6.77 (1H ddd, J=9.8, 5.1, 2.5 Hz, H-3), 6.76 (1H br s, H-27), 6.02 (1H br s, H-27), 5.88 (1H dd, J=9.8, 2.5 Hz, H-2), 5.58 (1H t, J=5.8 Hz, H-6), 4.65 (1H br s, W/2=8.8 Hz, H-22), 3.95 (1H d, J=12.7 Hz, H-21), 3.73 (1H dd, J=12.7, 3.0 Hz, H-21), 3.30 (1H dt, J=20.0, 2.5 Hz, H-4β), 2.84 (1H dd, J=20.0, 5.1 Hz, H-4α), 1.44, 1.22, 0.71 (3H s each, H_3-28, -19, -18); Found: C, 76.42; H, 8.09, calc. for $C_{28}H_{36}O_4$: C, 77.03; H, 8.31%.

Isowithametelin (2), m.p. 280°; $[\alpha]_D$ -77.7° (c 0.26, $CHCl_3$); EIMS m/z: 436 (M^+); UV (MeOH) λ_{max} nm: 233, 227sh (ε 13000); IR ($CHCl_3$) ν_{max} cm^{-1}: 1720, 1714; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 6.73 (1H s, H-27), 6.02 (1H dd, J=10.0, 2.5 Hz, H-4), 5.99 (1H s, H-27), 5.62 (1H dd, J=5.0, 2.5 Hz, H-6), 5.58 (1H dt, J=10.0, 5.0 Hz, H-3), 4.63 (1H br s, W/2=10.0 Hz, H-22), 3.89 (1H d, J=13.0 Hz, H-21), 3.70 (1H dd, J=13.0, 2.0 Hz, H-21), 3.28 (1H d, J=20.0 Hz, H-2β), 2.72 (1H dd, J=20.0, 5.0 Hz, H-2α), 1.42, 1.33, 0.68 (3H s each, H_3-28, -19, -18).

Conversion of withametelin to 3 by treatment with mercuric acetate: A solution of withametelin (25 mg) in AcOH (10 ml) was treated with $Hg(OAc)_2$ at 80° for 3 hrs. The rection mixture was freed from inorganic salts by filtration and from organic solvents by evaporation under reduced pressure. The product was purified over silica gel column to yield 3 (15 mg), m.p. 259°; UV (MeOH) λ_{max} nm: 310, 207 (ε 4710, 10539); ^{1}H NMR (90 MHz, $CDCl_3$) δ· 6.94 (1H dd, J=10.0, 6.0 Hz, H-3), 6.74 (1H br s, H-27), 6.30 (1H dd, J=6.0, 1.0 Hz, H-4), 6.04 (1H dd, J=10.0, 1.0 Hz, H-2), 5.99 (1H br s, H-27), 5.50 (1H br t, H-6), 4.61 (1H br s, H-22), 3.79 (2H br q, H_2-21), 2.05 (3H s, -OAc), 1.41, 1.33, 0.77 (3H s each, H_3-28, -19, -18).

Hydrogenation of withametelin to 2,3,5,6,25,27-hexahydrowithametelin and 5: Withametelin (100 mg) was hydrogenated in methanolic solution using 5% Pd-C (30 mg) as a catalyst for 4 hrs. Usual work-up followed by purification over silica gel column yielded hexahydrowithametelin (55 mg) and 5 (10 mg). Hexahydrowithametelin, m.p. 258°; $[\alpha]_D$

-82.7° (c 0.26, $CHCl_3$); EIMS m/z: 442 (M^+), 424, 369, 351, 319, 271, 231, 147, 124; IR (KBr) λ_{max} cm^{-1}: 1725, 1690; ^1H NMR (100 MHz, $CDCl_3$) δ: 4.58 (1H br s, H-22), 3.86, 3.46 (1H br d, J=13.0 Hz each, H_2-21), 1.32 (3H d, J=7.0 Hz, H_3-27), 1.20, 1.12, 0.64 (3H s each, H_3-28, -19, -18); 5, m.p. 278°; $/\alpha/_D$ -68.4° (c 0.36, $CHCl_3$); EIMS m/z: 440 (M^+), 355, 152, 150, 125; UV (MeOH) λ_{max} nm: 228 (ε 9200); ^1H NMR (500 MHz, $CDCl_3$) δ: 5.42 (1H d, J=6.0 Hz, H-6), 4.37 (1H dt, J=13.5, 3.6 Hz, H-22), 4.00 (1H br d, J=11.8 Hz, H-21), 3.74 (1H dd, J=11.8, 5.0 Hz, H-21), 1.93, 1.88, 1.24, 0.70 (3H s each, H_3-28, -27, -19, -18); CD (MeOH): $[\theta]_{250}$ +10700.

Transformation of withametelin to secowithametelin (6): A solution of withametelin (100 mg) in MeOH (15 ml) was treated with a few drops of $HClO_4$ and left overnight at room temp. The reaction product on chromatography over silica gel furnished white plates of secowithametelin (60 mg) from petroleum ether-EtOAc (3:1) eluates, m.p. 190°; $/\alpha/_D$ +28.5° (c 0.75, $CHCl_3$); UV (MeOH) λ_{max} nm: 228 (ε 14040); IR (KBr) ν_{max} cm^{-1}: 3490, 1718, 1684; ^1H NMR (100 MHz, $CDCl_3$) δ: 6.73 (1H ddd, J=10.0, 5.5, 2.5 Hz, H-3), 5.85 (1H dd, J=10.0, 2.5 Hz, H-2), 5.53 (1H d, J=6.0 Hz, H-6), 4.49 (1H dt, J=12.0, 4.0 Hz, H-22), 4.24 (2H AB q, J=12.0 Hz, Δν 16.0 Hz, H_2-27), 3.94 (2H m, H_2-21), 3.38 (3H s, -OMe), 2.08, 1.23, 0.75 (3H s each, H_3-28, -19, -18); CD (Dioxane): $[\theta]_{253}$ +13540, $[\theta]_{320}$ -8000.

Conversion of withametelin to 7: Withametelin (100 mg) was treated with Ac_2O (5 ml) and 30% $HClO_4$ (0.4 ml) for 1 hr at room temp. Usual work-up followed by purification over silica gel column and crystallization from EtOAc yielded 7 (40 mg) as white crystals, m.p. 200°; UV (MeOH) λ_{max} nm: 212 (ε 16709); IR (nujol) ν_{max} cm^{-1}: 1760, 1740, 1700; ^1H NMR (500 MHz, $CDCl_3$) δ: 7.32 (1H d, J=8.0 Hz, H-3), 6.80 (1H d, J=8.0 Hz, H-2), 4.88 (2H AB q, J=15.4 Hz, H_2-27), 4.49 (1H dt, J=13.0, 3.5 Hz, H-22), 4.36 (1H dd, J=12.0, 1.0 Hz, H-21), 4.26 (1H dd, J=12.0, 4.0 Hz, H-21), 2.30 (3H s, H_3-19), 2.08, 2.06, 2.05 (3H s each, 3 x -OAc), 1.98, 0.76 (3H s each, H_3-28, -18).

Conversion of 3 to 8: Compound 3 (20 mg) in AcOH (1.5 ml) was treated with 30% $HClO_4$ (0.2 ml) at room temp. and left overnight. Usual work-up followed by chromatography over silica gel yielded 8 as yellow needles, UV (MeOH) λ_{max} nm: 312, 219 (ε 2509, 14200).

Epoxidation of withametelin to 9 and 10: A solution of withametelin (50 mg) in $CHCl_3$ (5 ml) was treated with m-chloroperbenzoic acid (50 mg) at room temp. for 4 hrs. Usual work-up yielded a mixture of epoxides which was resolved by HPLC to give 9 (20 mg) as a major product, m.p. 218°(dec); EIMS m/z: 452 (M^+); ^1H NMR (90 MHz, $CDCl_3$) δ: 6.75 (1H s, H-27) 6.62 (1H m, H-3), 6.01 (1H s, H-27), 5.85 (1H m, H-2), 4.64 (1H br s, H-22), 3.80 (2H AB q, J=20.0 Hz, H_2-21), 3.02 (1H t, J=6.0 Hz, H-6), 1.44, 1.33, 0.66 (3H s each, H_3-28, -19, -18); CD (Dioxane): θ_{345} -4565; 10 (7 mg) as a minor product, m.p. 228°(dec); EIMS m/z: 452 (M^+); ^1H NMR (90 MHz, $CDCl_3$) δ. 6.86 (1H ddd, J=10.0, 5.0, 2.5 Hz, H-3), 6.74 (1H s, H-27), 6.02 (1H dd, J=10.0, 2.5 Hz, H-2), 6.00 (1H s, H-27), 4.63 (1H br s, H-22), 3.10 (1H br s, H-6), 1.44, 1.24, 0.67 (3H s each, H_3-28, -19, -18); CD (Dioxane): $[\theta]_{345}$ +2983.

Selenium dioxide oxidation of withametelin to 3, 3a, 11 and 12: A solution of withametelin (250 mg) and SeO_2 (90 mg) in 3 ml C_6H_6 and 6 ml AcOH was refluxed for 8

hrs. Usual work-up followed by silica gel column chromatography of the product afforded 3 (45 mg), 3a (20 mg), 11 (20 mg) and 12 (5 mg). 3a, m.p. 278°; ^{1}H NMR (80 MHz, $CDCl_3$) δ: 6.92 (1H dd, J=9.6, 6.1 Hz, H-3), 6.75 (1H s, H-27), 6.14 (1H d, J=6.1 Hz, H-4), 6.03 (1H d, J=9.6 Hz, H-2), 6.00 (1H s, H-27), 4.62 (1H br s, H-22), 4.59 (1H br s, H-6), 3.80 (2H AB q, J=14.0 Hz, H_2-21), 1.47, 1.41, 0.76 (3H s each, H_3-19, -28, -18); 11, m.p. 328°(dec); UV (MeOH) λ_{max} nm: 207 (ε 9744); ^{1}H NMR (90 MHz, $CDCl_3$) δ: 6.75 (1H s, H-27), 6.74 (1H d, J=10.3 Hz, H-3), 6.46 (1H d, J=10.3 Hz, H-2), 6.00 (1H s, H-27), 4.62 (1H br s, H-22), 3.70 (2H AB q with fine splitting, J=12.6 Hz, H_2-21), 1.44, 1.40, 0.68 (3H s each, H_3-28, -19, -18); 12, UV (MeOH) λ_{max} nm: 355, 207 (ε 3986, 19395); ^{1}H NMR (90 MHz, $CDCl_3$) δ: 6.96 (1H dd, J=10.0, 6.0 Hz, H-3), 6.76 (1H s, H-27), 6.16 (1H d, J=10.0, 2.5 Hz, H-4), 6.00 (1H s, H-27), 6.00-5.80 (3H m, H-2, -6, -7), 4.63 (1H br s, H-22), 3.80 (2H AB q with fine splitting, J=12.6 Hz, H_2-21), 1.44, 1.24, 0.78 (3H s each, H_3-28, -19, -18).

Diisobutylaluminium hydride reduction of withametelin to 13: A solution of withametelin (27 mg) in dry tetrahydrofuran (1 ml) was treated with 0.2 ml of DIBAH (1M/l) at -30° for 1 hr. Acidic work-up (NH_4Cl+3N HCl) of the reaction mixture and purification by silica gel column yielded 13 (15 mg), EIMS m/z: 424 (M^+), 406, 109; IR ($CHCl_3$) ν_{max} cm^{-1}: 3450, 1460, 1395, 1100, 1000, ^{1}H NMR (90 MHz, $CDCl_3$) δ: 5.72-5.20 (3H m, H-2, -3, -6), 5.00 (1H s, H-26), 4.02-3.82 (2H m, H-1, -22), 3.50 (2H t, J=12.0 Hz, H_2-21), 1.75, 1.64, 0.99, 0.76 (3H s each, H_3-27, -28, -19, -18).

Pyridinium dichromate oxidation of 13 to 14: A solution of 13 (10 mg) in 1 ml CH_2Cl_2 was stirred with PDC (40 mg) for 1 hr at room temp. Reaction mixture was passed through a small bed of silica gel to afford 14 (5 mg), EIMS m/z: 422 (M^+), 355, 296, 109; ^{1}H NMR (90 MHz, $CDCl_3$) δ: 6.76 (1H ddd, J=10.0, 5.0, 2.5 Hz, H-3), 5.86 (1H ddd, J=10.0, 2.5, 1.0 Hz, H-2), 5.56 (1H br d, J=6.0 Hz, H-6), 5.00 (1H s, H-26), 3.90 (1H m, H-22), 3.52 (2H t, J=12.0 Hz, H_2-21), 1.74, 1.63, 1.22, 0.76 (3H s each, H_3-27, -28, -19, -18).

Conversion of isowithametelin to 6a: Treatment of a solution of isowithametelin (10 mg) in MeOH (1.5 ml) with 1 drop of 30% $HClO_4$ and a work-up same as that applied in case of secowithametelin (6), yielded a compound 6a (4 mg), EIMS m/z: 468 (M^+); UV (MeOH) λ_{max} nm: 227 (ε 16300); ^{1}H NMR (90 MHz, $CDCl_3$) δ: 5.97 (1H br d, J=9.2 Hz, H-4), 5.66 (1H br d, J=6.0 Hz, H-6), 5.56 (1H dd, J=9.2, 4.0 Hz, H-3), 4.41 (1H dt, J=12.6, 3.9 Hz, H-22), 4.16 (2H AB q, J=11.0 Hz, H_2-27), 3.94 (2H m, H_2-21), 3.31 (3H s, -OMe), 2.00, 1.29, 0.69 (3H s each, H_3-28, -19, -18); CD (Dioxane) $[\theta]_{241}$ +32600.

Transformation of withametelin to isowithametelin: A solution of withametelin (25 mg), ethylene glycol (0.2 ml) and p-toluenesulphonic acid (2 mg) in C_6H_6 (5 ml) was heated at 110° for 24 hrs. Usual work-up and purification gave 15 (10 mg), EIMS m/z 480 (M^+); ^{1}H NMR (90 MHz, $CDCl_3$) δ: 6.68 (1H s, H-27), 6.02 (1H s, H-27), 5.97 (1H dd, J=9.0, 2.0 Hz, H-4), 5.66 (1H br d, J=6.0 Hz, H-6), 5.48 (1H m, H-3), 4.66 (1H br s, H-22), 4.00 (4H br s, -O-CH_2-CH_2-O-), 3.84 (2H AB q, J=12.6 Hz, H_2-21), 2.28 (2H m, H_2-2), 1.44, 1.14, 0.72 (3H s each, H_3-28, -19, -18). Compound 15 (8 mg) was treated with p-toluenesulphonic acid (2 mg) in aq. acetone for 12 hrs at room temp. Work-up and purification gave 2 (5 mg) indistingui-

shable from isowithametelin in all respects (t.l.c., m.m.p., mass, IR, ^{1}H NMR).

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