Tetrahedron. Vol. 26, pp. 1859 to 1867. Pergamon Press 1970. Printed in Great Britain

EXTRACTIVES OF *APHANAMIXIS POLYSTACHYA* WALL (PARKER)

THE STRUCTURES AND STEREOCHEMISTRY OF APHANAMIXIN AND APHANAMIXININ

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(*Received in the UK 8 September* 1969; *Accepted for publication 5 January* 1970)

Abstract—The structures of aphanamixin (XVI) and aphanamixinin (XXIX) have been derived from the studies of spectroscopic and chemical properties and subsequently confirmed by their correlation with compounds of known structure and established stereochemistry.

THE occurrence of a large number of terpenoids[1–3] of diverse structural skeleta in the *Meliaceae* family, is well documented. This induced us to examine *Aphanamixis polystachya* wall (Parker) (Syn: *Amoora rohituka*), commonly known as "tiktaraj" in Bengal. Its bark, leaves and fruits taste bitter and are widely used in folk medicine.

A systematic chemical investigation of the fruit-shell, bark and leaves yielded a number of interesting terpenoids viz. aphanamixin,[4] aphanamixinin,[5] eperu-13ene-8β, 15 diol,[6] aphanamixin lactone* and aphanamixolide*. The present communication concerns the isolation and structure-elucidation of aphanamixin and aphanamixinin.

The petrol-extract of the fruit-shell furnished a new triterpene designated as aphanamixin. The elemental analysis and the mass spectrometrically derived mol wt (M^+ 514) establish its molecular formula as $C_{32}H_{50}O_5$, m.p. 232–234°, $[\alpha]_D$ −45° ($CHCl_3$). It contains an OAc group (1720 and 1260 cm^{-1}) and a OH (3380 cm^{-1}) which forms an acetate I (21, 23*R*, 24*R*, 25-diepoxytirucall-7-ene-3β, 21β diol diacetate), $C_{34}H_{54}O_6$, m.p. 128–130°. The NMR spectrum of aphanamixin indicates the presence of seven tertiary Me groups [singlets at δ 1·31 (6H), 0·78–1·00 (15H)], one OAc [singlet at δ 2·06 (3H)] and a OH function [singlet at δ 3·24 (1H)] which disappears when refluxed with D_2O. The positive tetranitromethane colour reaction, the IR spectral band at 1640 and 825 cm^{-1} and the olefinic proton signal at δ 5·28 suggest the presence of a tri-substituted double bond ($\rangle C{=}CH-$). The latter is endocyclic as alphamixin does not afford any detachable fragment on ozonolysis. Alphamixin upon oxidation with chromium trioxide in pyridine yields a γ-lactone II (3β-

* Details of these were presented at the 56th session of the Indian Science Congress Association held in Bombay, 1969.

acetoxy-24, 25-epoxy-23 hydroxytirucall-7-ene-21-oic acid 23,21 lactone), $C_{32}H_{48}O_5$ (1785 cm^{-1} and no OH absorption). On alkaline hydrolysis aphanamixin furnishes a deacetyl compound, $C_{30}H_{48}O_4$ III (21, 23R, 24R, 25-diepoxytirucall-7-ene-3β, 21β diol), which on reduction with sodium borohydride produces a triol IV (24, 25 epoxytirucall-7-ene-3β, 21, 23 triol) characterized as its triacetate V (24, 25-epoxytirucall-7-ene-3β, 21, 23 triol triacetate). Two of the hydroxyls in the triol arise from the reductive cleavage of a 5-membered hemiacetal present in aphanamixin.

Besides the occurrence of an OAc group and a hemiacetal in aphanamixin, the presence of an epoxide has also been observed. It exhibits the characteristic signal in its NMR spectrum (one proton doublet at δ 2·85 and a six proton singlet at δ 1·31) showing thereby the presence of an α-oxirane moiety (), chemical evidence of which was obtained by the formation of acetone during oxidation with chromic acid in acetic acid. The above functionalities and the molecular formula $C_{32}H_{50}O_5$ for aphanamixin demand that it must be tetracyclic. In conformity with this view it gives 1,2,8-trimethyl phenanthrene on selenium dehydrogenation.

To determine the relative positions of the functional groups, deacetylaphanamixin (III) was oxidized with chromium trioxide in pyridine when a hydroxyketone VI (21, 23R, 24R, 25-diepoxy-21β-hydroxytirucall-7-ene-3-one) and a ketolactone VII (24, 25 epoxy-23 hydroxy-3-oxotirucall-7-ene-21-oic acid 23, 21 lactone) were obtained. The latter which shows a band at 1700 cm^{-1} for a 6-membered saturated CO function is formed by oxidation of the secondary alcohol arising from the hydrolysis of the OAc group in aphanamixin. The OAc group in aphanamixin is, therefore, attached directly to a 6-membered ring in its molecule. Further, the appearance of a one proton multiplet at δ 5·28 (J = 12 c/s) for a methine proton of 3β-acetoxyterpenoids[7] suggests the location of the OAc function at the biogenetically preferred C-3 position.

Aphanamixintriol triacetate (V) with mercuric acetate affords a compound VIII (24, 25 epoxytirucall-7, 9(11) diene-3β, 21, 23 triol triacetate), the UV spectrum [λ_{max} 231, 239 and 249 mμ (ε, 9500, 10342 and 5700)] of which is typical of a 7, 9 (11)-heteroannular diene of the euphane series thus allocating the double bond in aphanamixin at Δ^7. This reaction also establishes the stereochemistry of ABCD ring juncture as that of the euphane series.

Since aphanamixin which occurs in a meliaceous species is a tetracyclic triterpenoid and contains a 5-membered hemiacetal with an α-oxirane system, it may be presumed on biogenetic ground that aphanamixin is a close structural analogue of flindissol[8] IX (21, 23*R*-epoxy tirucall-7, 24*R*-diene-3α,21α diol), turraeanthin[7] X (21, 23R, 24R, 25-diepoxytirucall-7-ene-3β, 21α diol-3β monoacetate), melianone[9] XI (21, 23*R*, 24*R*, 25-diepoxy-21α hydroxytirucall-7-ene-3 one) and melianol[9] XII (21, 23*R*, 24*R*, 25-diepoxytirucall-7-ene-3β, 21α diol). This view could be substantiated by the conversion of aphanamixin to deoxyepi-flindissol. Deacetyl aphanamixin (III) on reduction with LAH furnishes deacetyldeoxydihydroaphanamixin XIII (21, 23*R*-epoxy-24, 25-dihydrotirucall-7-ene-3β, 25 diol) which upon acetylation forms anhydroacetyl derivative XIV (21, 23 epoxytirucall-7, 24 diene-3β-ol monoacetate). Alkaline hydrolysis of the latter afforded a compound whose physical properties are

comparable to those reported for deoxy-3-epi flindissol* XV (21, 23 epoxytirucall-7, 24 diene-3β-ol). On the basis of the above spectral data and chemical reactions, structure of aphanamixin could be completely elaborated as XVI (21, 23*R*, 24*R*, 25-diepoxytirucall-7-ene-3β, 21β diol-3β monoacetate).

The structure of aphanamixin (XVI) thus derived suggests that it should be either identical with turraeanthin (X) or one of its epimer at C-21 or C-23 or at both centres. But their nonidentity has been settled from a comparison of their physical properties. However, Sarett oxidation of aphanamixin and turraeanthin furnish the same lactone (II) which observation infers aphanamixin as C-21 epimer of turraeanthin.

The stereochemistry at C-21 and C-23 of aphanamixin has finally been established by its correlation with melianone (XI).

Deacetylaphanamixin (III) was oxidized with chromium trioxide in pyridine yielding two products viz. (i) 3-deacetoxy-3-ketoaphanamixin (VI) and (ii) 3-deacetoxy-3-ketoaphanamixin lactone (VII). The nonidentity of VI and III with melianone (XI) and melianol (XII) and the identity of VII with melianone lactone (prepared by Sarett oxidation of melianone) firmly establish the structure and stereochemistry of aphanamixin as XVI.

The second new triterpenoid, aphanamixinin, $C_{27}H_{34}O_7$ (M^+ 470), m.p. 208°, $[\alpha]_D$ −120° ($CHCl_3$), isolated from the petrol extract of the dried bark shows bands in its IR spectrum at 1510 and 875 cm^{-1} (furan ring), a broad band in the region 1715–1740 cm^{-1} (carbonyl, ester and/or lactone functions) but no peak absorption for OH. The NMR spectrum shows signals at δ 6·35 (1H) and 7·38 (2H) corresponding to one β-furanoproton and two α-furanoprotons respectively, a singlet at δ 3·75 (3H) for a methoxycarbonyl group, four protons associated with singlets (δ 5·65, 5·10, 4·90 and 3·82) and four tertiary C-Me groups (δ 1·15, 1·10, 1·05 and 0·90).

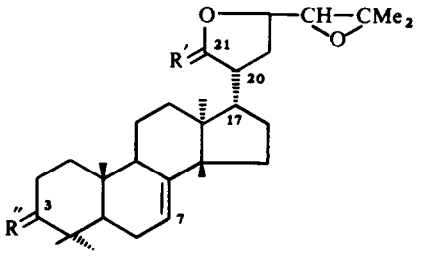
Aphanamixinin is a lactone and consumes one equivalent of alkali in the cold and is regenerated from the alkaline solution upon acidification. Alkaline hydrolysis of aphanamixinin with alcoholic alkali produces aphanamixinic acid (XVII), $C_{26}H_{32}O_7$ which with diazomethane regenerates the parent compound, thus proving aphanamixinin as the methyl ester of aphanamixinic acid.

Hydrogenation of the compound in acetic acid with 10% Pd-C furnishes a tetrahydro derivative (XVIII) $C_{27}H_{38}O_7$ but when the hydrogenation is carried out with Adams' catalyst, a hexahydroacid (XIX) $C_{27}H_{40}O_7$, is formed. The furan ring in both the compounds is saturated (absence of furan bands in the IR spectra). This behaviour is reminiscent of the β-substituted furanolactone as observed in columbin,[10] limonin[11] and swietenolide.[12]

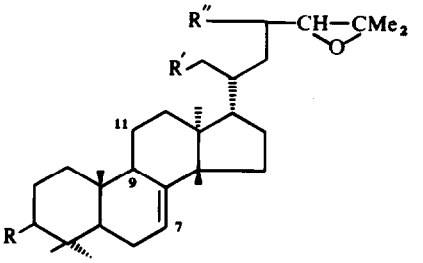
Now the comparison of the NMR spectrum of aphanamixinin (two singlets at δ 5·65 and 3·82) with that of 1,2-dihydrogedunin XX (singlets at δ 5·60 and 3·52 for C-17 and C-15 protons) suggests the presence of similarly environed C-17 and C-15 protons in the former. This is in keeping with the observation that the signal of the C-17 proton disappears in the NMR spectrum of hexahydroacid monomethyl ester (XIX). Other two singlets at δ 5·10 and 4·90 in the NMR spectrum of aphanamixinin are assigned to the protons of an exocyclic methylene group, supportive evidence of which has been provided by IR spectrum (absorption at 910 cm^{-1}) as well as by the

* In a recent publication Halsall *et al.* [*Chem. Comm.* 48 (1969)] have made a comment that aphanamixin is probably 3-epi-turraeanthin which possibility has been excluded from its spectral data and chemical reactions reported in this communication.

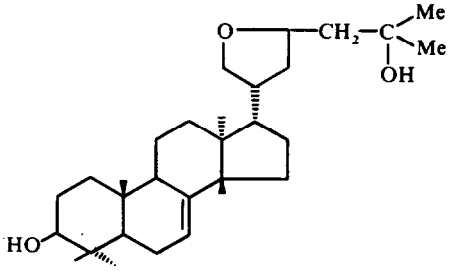
isolation of formaldehyde during ozonolysis of the compound. In conformity the proton signals attributed to this group have disappeared in the NMR spectrum of dihydroaphanamixinin (XXI) which shows the presence of an additional C-methyl peak (δ 1·02, 1·02, 1·75, 0·98, 0·88) as expected.



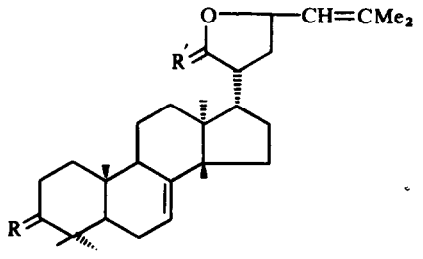
I : R′ = R″ = βOAc, H
II : R′ = O; R″ = βOAc, H
III : R′ = R″ = βOH, H
VI : R′ = βOH, H; R″ = O
VII : R′ = R″ = O
X : R′ = αOH, H; R″ = βOAc, H
XI : R′ = αOH, H; R″ = O
XII : R′ = αOH, H; R″ = βOH, H
XVI : R′ = βOH, H; R″ = βOAc, H



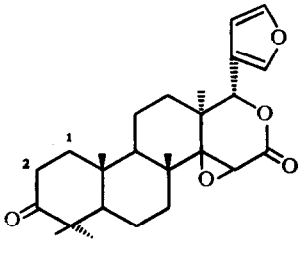
IV : R = βOH; R′ = R″ = OH
V : R = βOAc; R′ = R″ = OAc
VIII : R = R′ = R″ = OAc; 9(11) dehydro



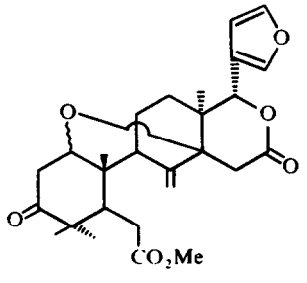
XII



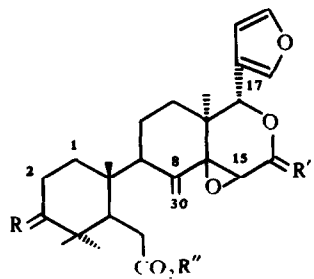
IX : R = R′ = αOH, H
XIV : R = βOAc, H; R′ = H_2
XV : R = βOH, H; R′ = H_2



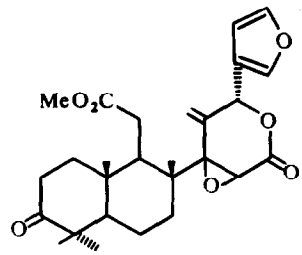
XX α-OAc at C-7
XXVII : 1, 2 dehydro-(XX)



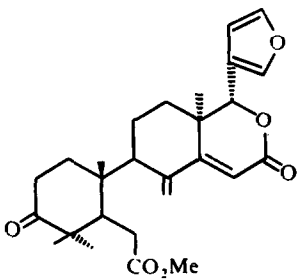
XXVI



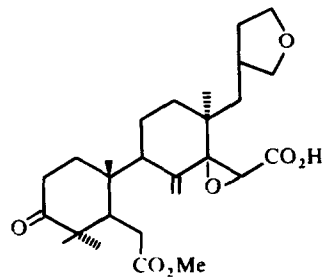
XXIX: R = R′ = O; R″ = Me
XXI : 8,30 dihydro-(XXIX)
XVII : R = R′ = O; R″ = H
XVIII: R = R′ = O; R″ = Me; furan saturated
XXIV: R = R′ = OH, H; R″ = Me
XXV : R = R′ = OAc, H; R″ = Me
XXX : 1,2 dehydro-(XXIX)



XXVIII



XXII
XXIII: 1,2 dehydro-(XXII)



XIX

Aphanamixinin undergoes facile reduction with chromous chloride to furnish deoxyaphanamixinin (XXII) $C_{27}H_{34}O_6$, m.p. 170–185°. The subtraction of the UV spectrum of aphanamixinin from that of deoxyaphanamixinin (XXII) shows an absorption maximum at 257 mμ (ε, 7900). This chromophoric characteristic is strikingly similar to those of a transformation product of limonin and deoxyandirobin (XXIII) which suggests the presence of a γδ-location of the vinyl double bond with respect to the αβ-epoxy-δ-lactone ring as observed in limonin[11] and andirobin.[13]

Aphanamixinin contains a ketomethylene group as indicated from the positive Zimmermann colour reaction. On reduction with sodium borohydride the compound yields a diol (XXIV), $C_{27}H_{38}O_7$, characterized as its diacetate (XXV). The IR spectrum of the diol lacks peak absorption for cyclic ketone and lactonic carbonyl and instead exhibits two new bands at 3380 and 3500 cm^{-1} attributable to the hydroxyl groups. The diol can be easily reoxidised to aphanamixinin. Such behaviour which finds a parallel in the chemistry of methylangolensate[14, 15] (XXVI) and gedunin[1] (XXVII) would signify that the formation of aphanamixinin diol (XXIV) involves the reduction of a 6-membered ketone to a secondary alcohol and that of the δ-lactone to a hemiacetal. This would explain the shifting of the C-17 proton to upfield (δ 5·65 to δ 4·65) in the diol. Since the latter also exhibits the characteristic chemical shift for C-3 proton at δ 3·35 (typical of α and axial proton at C-3 in triterpenoids), one of the hydroxyls in the diol and hence the ketonic carbonyl in aphanamixinin occupies C-3

in ring A. The aforesaid observations lead to biogenetically attractive formulations XXVIII or XXIX for aphanamixinin. But XXIX where ring B is cleaved is preferred for the following reasons:

(i) the spectroscopic behaviour of deoxyaphanamixinin; (ii) the stability of deoxyaphanamixinin under acid conditions and (iii) the comparison of the chemical shift of the C-17 proton in aphanamixinin with that of andirobin (XXX), methylangolensate (XXVI) and gedunin (XXVII) where C-17 proton was not situated allylic with respect to the exocyclic methylene group as in XXVIII.

The decisive proof for the structure and stereochemistry of aphanamixinin as XXIX has been provided by its chemical conversion to deoxyandirobin (XXIII). Deoxyaphanamixinin (XXII) prepared by chromous chloride reduction of aphanamixinin was treated with bromine in acetic acid at room temperature when a non-separable mixture of several products was obtained. The mixture was dehydrobrominated with lithium chloride in dimethylformamide.[16] One of the isolable products analysing for $C_{27}H_{32}O_6$ has been identified (m.p., m.m.p., and superimposable IR spectra) as deoxyandirobin (XXIII). This correlates aphanamixinin with andirobin as its 1:2 dihydroderivative.

The co-occurrence of aphanamixin (XVI) and aphanamixinin (XXIX) in the same plant lends circumstantial evidence to the current views of biogenesis of triterpenoids.[17]

EXPERIMENTAL

All m.ps are uncorrected. Petrol refers to light petroleum b.p. 60–80°. The UV spectra were measured with a Carl Zeiss Universal Spectrophotometer (Model VSU-1) using 95% aldehyde-free EtOH and IR spectra with Perkin–Elmer Spectrophotometer using nujol mull or chloroform as stated. NMR spectra were determined with a Varian A-60 instrument. Unless otherwise stated, the analytical samples were routinely dried at 80° over P_2O_5 for 24 hr *in vacuo*.

Isolation of aphanamixin (XVI). Air dried powdered fruitshell of *Aphanamixis polystachya* (1·8 kg) was exhaustively extracted with petrol (b.p. 60–80°). The reddish petrol extract (4 lit) was concentrated (100 ml) and chromatographed over Brockmann alumina (800 g). Upon washing the column with benzene–chloroform (1:1), XVI (100 mg) migrated out and was crystallized from MeOH in the form of stellets m.p. 232–234°, $[\alpha]_D$ −45° ($CHCl_3$), (M^+ 514), R_f 0·50 (MeOH:$CHCl_3$ = 1:9), ν_{max} (Nujol) 3380 (OH), 1720 and 1260 ($OCOCH_3$), 1285 (epoxide) cm^{-1}; NMR signals ($CDCl_3$) at δ 5·28 (2H, m, C-7 and C-21), 4·66 (1H, t, C-3), 3·86 (1H, m, C-23), 3·24 (1H, s, C-21-OH), 2·85 (1H, d, C-24, J = 8·5 c/s), 2·06 (3H, s, OAc), 1·31 (6H, s, C-26 and C-27) and 0·78–1·00 (15H, singlets, five C-methyls, C-18, C-19, C-28, C-29 and C-30). (Found: C, 74·5; H, 9·7; O, 15·2; OAc, 7·2. $C_{32}H_{50}O_5$ requires: C, 74·7; H, 9·8; O, 15·5 and OAc, 8·4%).

Acetylation of aphanamixin. To a soln of aphanamixin (0·15 g) in pyridine (2·5 ml) was added Ac_2O (4 ml) and the mixture was kept overnight. Working up in the usual way afforded I (0·12 g), m.p. (from MeOH) 128–130°; ν_{max} (Nujol) 1740 and 1248 ($OCOCH_3$) cm^{-1}. (Found: C, 73·5; H, 9·3; O, 17·0. $C_{34}H_{52}O_6$ requires: C, 73·3; H, 9·4; O, 17·3%).

Oxidation of aphanamixin. Aphanamixin (100 mg) in pure dry pyridine (2·5 ml) was added to CrO_3 (0·3 g) in dry pyridine (3 ml) and the mixture was left overnight, The reaction product upon extraction with ether afforded II (70 mg), m.p. (from MeOH) 217–218°, ν_{max} (Nujol) 1785 (saturated 5-membered ring lactone) cm^{-1}. (Found: C, 75·1; H, 9·4; O, 15·3. $C_{32}H_{48}O_5$ requires: C, 75·0; H, 9·4; O, 15·6%).

Selenium dehydrogenation of aphanamixin. A mixture of aphanamixin (500 mg) and selenium (1 g) was heated in a sealed tube at 320° for 48 hr. The product was extracted with benzene and then chromatographed over Brockmann alumina. Elution of the column with light petrol-benzene (9:1) mixture afforded 1,2,8-trimethyl phenanthrene (50 mg), m.p. (from ethanol) 144–146°, characterized as its picrate, m.p. 164–166°. (Found: C, 93·02; H, 7·10. $C_{17}H_{16}$ requires: C, 92·68; H, 7·32%).

Alkali hydrolysis of aphanamixin. Aphanamixin (100 mg) in MeOH (5 ml) containing NaOH (0·1 N, 10 ml) was refluxed for 2 hr. The product upon usual working up afforded III (65 mg) m.p. (from MeOH)

189–190°, ν_{max} (Nujol) 3400 (OH) cm^{-1} (no band for acetyl group). (Found: C, 74·5; H, 9·7. $C_{30}H_{48}O_4$, $\frac{1}{2}H_2O$ requires: C, 74·8; H, 10·2%).

Conversion of deacetylaphanamixin to its epoxytriol. Deacetylaphanamixin (150 mg) in MeOH (5 ml) was treated with $NaBH_4$ (150 mg) and NaOMe (100 mg). The reaction mixture was stirred for 10 hr. and the product on chromatography afforded IV (100 mg), m.p. (from MeOH) 183–185°, $[\alpha]_D$ −40° ($CHCl_3$), (M^+ 474). (Found: C, 75·6; H, 10·3. $C_{30}H_{50}O_4$ requires: C, 75·9; H, 10·8%).

Epoxytriacetate from epoxytriol (IV) *and its conversion to diene by oxidation with mercuric acetate.* Epoxytriol (75 mg) in pyridine (1·5 ml) was treated with Ac_2O (2 ml) and the mixture was kept overnight. Working up in the usual way afforded V, (60 mg), m.p. (from MeOH) 192–194°, $[\alpha]_D$ −7° ($CHCl_3$). (Found: C, 72·2; H, 9·0. $C_{36}H_{56}O_7$ requires: C, 71·9; H, 9·4%). The triol triacetate prepared as above was dehydrogenated with mercuric acetate following the method of Ruyle *et al.*[17] The reaction product on chromatography over deactivated alumina and subsequent elution with petrol–benzene (1:1) afforded VIII (20 mg) m.p. (from MeOH) 165–166°, $[\alpha]_D$ −80° ($CHCl_3$), λ_{max}^{EtOH} 231, 239 and 249 mμ (ε, 9500, 10342, 5700). (Found: C, 71·9; H, 8·8. $C_{36}H_{54}O_7$ requires: C, 72·2; H, 9·1%).

Oxidation of deacetylaphanamixin. Deacetylaphanamixin (125 mg) in pyridine (5 ml) was added to a slurry of CrO_3 (80 mg) and pyridine (12 ml) at 0°. The reaction product upon dilution and extraction with ether afforded a gummy mass (100 mg). The extract was chromatographed over 5% deactivated alumina (10 g) and eluted with benzene which furnished VII (60 mg), m.p. (from MeOH) 168–170°, $[\alpha]_D$ −58° ($CHCl_3$), (M^+ 468), ν_{max} ($CHCl_3$), 1700 (6-membered ring ketone) and 1770 (γ-lactone) cm^{-1}. (Found: C, 76·5; H, 9·2. $C_{30}H_{44}O_4$ requires: C, 76·9; H, 9·5%). Further elution with benzene–ether (4:1) gave VI (40 mg), m.p. (from MeOH) 198–200°, ν_{max} ($CHCl_3$) 3390 (OH) and 1770 (cyclohexanone) cm^{-1}. (Found: C, 76·1; H, 9·6. $C_{30}H_{46}O_4$ requires: C, 76·5; H, 9·8%).

Oxidation of aphanamixin with chromic acid-acetic acid. Aphanamixin (100 mg) in glacial AcOH (7·5 ml) was cooled to 0°. To this CrO_3 (100 mg) in 50% aqueous AcOH (3 ml) was added and kept at room temp for 2 days. The reaction mixture was neutralized and distilled. The distillate (10 ml) which contained acetone was characterized as its DNPH derivative, m.p. 125–126°. (Found: C, 45·43; H, 4·30; N, 23·18. $C_9H_{10}O_4N_4$ requires: C, 45·38; H, 4·25; N, 23·52%).

Reduction of deacetylaphanamixin with LAH. Deacetylaphanamixin (200 mg) in dry THF (25 ml) was refluxed with LAH (100 mg) for 5 hr. Working up in the usual way afforded XIII (160 mg), m.p. (from MeOH) 172–174°. (Found: C, 78·45; H, 10·54. $C_{30}H_{50}O_3$ requires: C, 78·60; H, 10·92%).

Acetylation of XIII. Deoxydeacetyldihydroaphanamixin (150 mg) in pyridine (2 ml) was treated with Ac_2O (4 ml) in pyridine (1 ml) and the mixture warmed for 30 min. After usual work-up, a crystalline solid (XIV, 120 mg), m.p. (from EtOAc) 158–162°, was obtained.

Hydrolysis of XIV *with 4% alcoholic* KOH. The above acetylated product (100 mg) was refluxed with 4% ethanolic KOH for 30 min. Removal of the solvent and extraction into $CHCl_3$ afforded fine needles of XV (60 mg), m.p. (from EtOAc) 131–133°, $[\alpha]_D$ −46° ($CHCl_3$). (Found: C, 81·92; H, 10·78. $C_{30}H_{48}O_2$ requires: C, 81·80; H, 11·00%).

Isolation of aphanamixinin (XXIX). Air-dried bark of *Aphanamixis polystachya* (10 kg) was exhaustively extracted (35 hr) with petrol when a crude yellow solid (800 mg) deposited in the boiling flask of soxhlet apparatus. The benzene soln of the crude solid was chromatographed over Brockmann alumina (30 gm). Upon washing the chromatogram with benzene–$CHCl_3$ (1:1), aphanamixinin (100 mg) migrated out and was crystallized from MeOH in prisms, m.p. 208°, $[\alpha]_D$ −120° ($CHCl_3$), (M^+ 470), R_f 0·47 (MeOH–EtOAc 1:3), ν_{max} (Nujol) 1735 (δ-lactone), 1715 (cyclohexanone), 1725 (CO_2Me), 1510 and 875 (furan), 910 (exocyclic methylene group) cm^{-1}; NMR signals ($CDCl_3$) at δ 7·38 and 6·35 (2α and 1β furanoprotons), 3·75 (3H, s, CO_2Me), 5·10 and 4·90 (2H, s, $>C=CH_2$), 3·82 (1H, diffused s, C-15), 5·65 (1H, s, C-17). (Found: C, 69·02; H, 7·22; O, 23·76; OMe, 6·32. $C_{27}H_{34}O_7$ requires: C, 68·92; H, 7·28; O, 23·80; OMe, 6·60%).

Alkaline hydrolysis of aphanamixinin. Aphanamixinin (60 mg) in MeOH (5 ml) containing NaOH (0·1N, 10 ml) was refluxed for 5 hr. The product obtained by usual work-up afforded XVII (40 mg), m.p. (MeOH: EtOAc = 1:1), NMR signals at δ 7·63 and 6·40 (2H, m and 1H, m; 2α and 1β furano protons), 5·55 (1H, s, C-17), 3·99 (1H, diffused, s, C-15), 5·0 and 5·10 (2H, s, $>C=CH_2$). (Found: C, 69·20; H, 7·10; OMe–Nil. $C_{26}H_{32}O_7$ requires: C, 68·47; H, 7·06%; OMe–Nil).

Esterification of aphanamixinic acid to aphanamixinin. To aphanamixinic acid (10 mg) in dry ether (5 ml) was added ethereal diazomethane (5 ml) at 0°, slowly with stirring. The reaction product upon chromatography over alumina furnished aphanamixinin (8 mg) from the benzene–$CHCl_3$ (1:1) eluates, m.p. (from MeOH) 208° (Found: C, 68·54; H, 7·62; OMe, 6·24. $C_{27}H_{34}O_7$ requires: C, 68·92; H, 7·28; OMe, 6·60%).

Preparation of aphanamixinin diol (XXIV). To aphanamixinin (100 mg) in dry MeOH (7 ml) was added $NaBH_4$ (70 mg) slowly at 0° with stirring and then refluxed for 2–3 hr on steam bath. Working up in the usual way afforded aphanamixinin diol (65 mg), m.p. (from ethylacetate) 212°, ν_{max} (Nujol) 3380 and 3600 (OH), 1505 and 875 (furan), and 1725 (CO_2Me) cm^{-1}. (Found: C, 68·20; H, 7·98; $C_{27}H_{38}O_7$ requires: C, 68·33; H, 8·07%).

Acetylation of diol XXIV. The diol (XXIV, 10 mg) was dissolved in pyridine (0·8 ml) and Ac_2O (1 ml) and kept at 25° overnight. The reaction mixture was worked by dilution and extraction into chloroform. Evaporation of the solvent furnished glistening needles of XXV (7 mg), m.p. (from EtOAc) 180°. (Found: C, 66·3; H, 7·7; Acetyl 7·15. $C_{31}H_{42}O_9$ requires: C, 66·9; H, 7·22; Acetyl 7·7%).

Oxidation of diol. To the diol (15 mg) in pyridine (0·5 ml) was added anhyd CrO_3 (15 mg) in pyridine (2 ml) in the cold with stirring and left overnight. The reaction product upon dilution and extraction into ether afforded aphanamixinin (7 mg), m.p. (from MeOH) 208°. (Found: C, 68·60; H, 7·68. $C_{27}H_{34}O_7$ requires: C, 68·92; H, 7·28%).

Preparation dihydroaphanamixinin (XXI). Aphanamixinin (75 mg) in dry MeOH (6 ml) was hydrogenated with 5% Pd-C (100 mg) for 1 hr (1 mole absorbed). Working up in the usual way afforded XXI (45 mg), m.p. (from EtOAc) 184°; ν_{max} (Nujol) 1720 (CO), 1725 (CO_2Me), 1740 (δ-lactone), 1512 and 878 (furan) cm^{-1}; NMR signals (DMSO) at δ 7·66, 6·43 (2H, 1H, m, 2α and 1β furano protons), 3·66 (3H, s, CO_2Me), 3·65 (1H, s, C-15), 5·63 (1H, s, C-17), 1·75 (3H, d, C-30 Me), 0·88–1·10 (12H, s, four C-Me's). (Found: C, 68·25; H, 7·60. $C_{27}H_{36}O_7$ requires: C, 68·62; H, 7·68%).

Tetrahydro aphanamixinin (XVIII) *from aphanamixinin.* Aphanamixinin (30 mg) was dissolved in glacial AcOH (7 ml) and was hydrogenated using 10% Pd-C (60 mg) for 5 hr. Working up in the usual way afforded tetrahydro aphanamixinin (15 mg), m.p. 175–180°; (negative furan test and absence of furan bands in the IR spectrum). (Found: C, 67·28; H, 7·20; $C_{27}H_{38}O_7$ requires: C, 68·33; H, 8·07%).

Hexahydro acid monomethylester from aphanamixinin. Aphanamixinin (75 mg) dissolved in glacial AcOH (10 ml) was hydrogenated with Adams' catalyst for 7 hr. Working up in the usual way afforded XIX (40 mg), m.p. (from ether) 194°; NMR signals (DMSO) at δ 3·70 (3H, s, CO_2Me), 4·90–5·02 (2H, s, $\diagdown C{=}CH_2$), (absence of furano protons and C-17 proton). (Found: C, 67·65; H, 7·92. $C_{27}H_{40}O_7$ requires: C, 68·04; H, 8·46%).

Ozonolysis of aphanamixinin. Aphanamixinin (50 mg) was dissolved in EtOAc (7 ml) and ozonized at −10° for 45 min. The reaction product was heated on a steam-bath and a stream of N_2 was passed. The issuing gas was led into an aqueous soln of dimedone when a flocculent ppt was obtained. It was crystallised from aqueous alcohol m.p. 187°. This was identified as the dimedone derivative of formaldehyde. The non-volatile portion was a noncrystallizable gummy mass.

Chromous chloride reduction of aphanamixinin. Excess of $CrCl_2$ in N-HCl was added to a soln of aphanamixinin (200 mg) in acetone (8 ml) and AcOH (10 ml). The mixture was stored in CO_2 for 4 days. The product was extracted with $CHCl_3$. Evaporation of the solvent left an amorphous residue XXII (120 mg), m.p. 170–185°, which failed to crystallize. (Found: C, 70·46; H, 7·92; O, 21·62. $C_{27}H_{34}O_6$ requires: C, 71·32; H, 7·54; O, 21·12%).

Bromination of deoxyaphanamixinin and its conversion to deoxyandirobin. A soln of Br_2 (3·5 ml) in AcOH (5 ml) was added to a soln of deoxyaphanamixinin (100 mg) in a mixture of $CHCl_3$ (5 ml) and AcOH (5 ml) at 0° during a period of 1 hr. It was then stirred for an additional 1 hr at room temp. Working up gave a brown noncrystallizable gummy mass (80 mg). To this product anhydrous LiCl (80 mg) and freshly distilled DMF (7 ml) was added and refluxed for 6 hr in an atmosphere of N_2 with stirring. The product was cooled and extracted with $CHCl_3$. Evaporation of the solvent furnished an oily product (70 mg). This was treated with ether and the ether insoluble residue furnished XXIII (10 mg), m.p. (from MeOH) 168–170°; ν_{max} ($CHCl_3$) 1514 and 878 (furan), 1680 (cyclohexenone), 1722 (unsaturated δ-lactone) cm^{-1}. (Found: C, 70·98; H, 7·22. $C_{27}H_{32}O_6$ requires: C, 71·68; H, 7·08%). The major ether soluble gum failed to crystallise from any solvent.

Acknowledgements—The authors express their sincere thanks to Dr. B. C. Das, Gif-Sur-Yvette, France, for Mass and NMR spectra. Thanks are also due to Professor C. W. L. Bevan and Dr. T. G. Halsall for supplying the authentic sample of turraeanthin, and to Professor W. D. Ollis for the superimposable IR spectra of deoxyandirobin with an authentic specimen. Financial support from ICMR (A.B.K.), and CSIR (S.C.), India is also gratefully acknowledged.

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