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THE STRUCTURE OF TETRAMYCIN, A NEW POLYENE MACROLIDE ANTIBIOTIC

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Abstract—The structure of the tetraene antifungal antibiotic tetramycin has been established by detailed studies of mass, ^1H and ^{13}C NMR spectra as well as by chemical degradation. A newly developed mass analysis has been introduced for determining the carbon skeleton of the agiycone.

Tetramycin (I) is an antifungal, tetraene macrolide antibiotic produced by *Streptomyces noursei* Hazen and Brown, 1950 var. jenesis nov. var. JA 3789. Earlier, we reported the isolation and preliminary physico-chemical characterization of this molecule.[1-3] Now we describe the complete structure of tetramycin as inferred from chemical transformations using procedures similar to those reported in the structural studies of pimaricin,[4] lucensomycin,[5] tetrins A[6] and B[7] and rimocidin,[8] as well as from detailed MS, ^1H and ^{13}C NMR spectroscopic examinations of I, its derivatives and transformation products (Fig. 1).

RESULTS AND DISCUSSION

General characterization of tetramycin. Tetramycin is closely related to pimaricin (II), lucensomycin (III), tetrins A (IV) and B (V). The respective structures are displayed in Fig. 2.

Due to the notorious thermal instability of glycosidic polyene antibiotics, neither electron impact (EI), nor electron attachment (EA) mass spectrometry (MS) could furnish the exact molecular weight for I. From its microanalysis and that of derivatives, I was tentatively assigned the molecular formula $C_{54}H_{83}NO_{14}$ (molecular weight 699).[1] However subsequent ^{13}C NMR analyses suggested that tetramycin has 35 C atoms. UV spectra disclosed the presence of a tetraene chromophore featuring spectral characteristics (λ_{max}^{MeOH} 280, 290, 304 and 318 nm, ϵ_{max} 25,200, 53,500, 85,700 and 75,000) reminiscent of related polyene antibiotics.[9] Treatment with MnO_2 leaves the chromophore unchanged, indicating the absence of any free allylic OH group. On catalytic hydrogenation, I absorbs five molar equivalents of hydrogen; four of these are accounted for by the tetraene moiety while the fifth mole is consumed by an additional double bond present in the form of an α,β-unsaturated ester group. The nature of the latter unsaturation followed from comparison of the IR spectra of I with the corresponding spectrum of decahydrotetramycin. The IR spectrum of I disclosed the

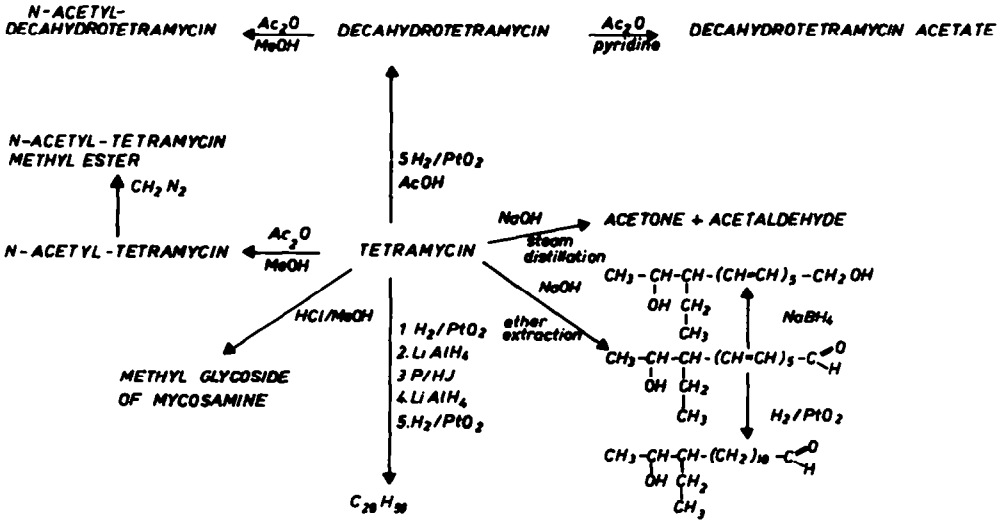


Fig. 1. Derivatives and degradation products obtained from I.

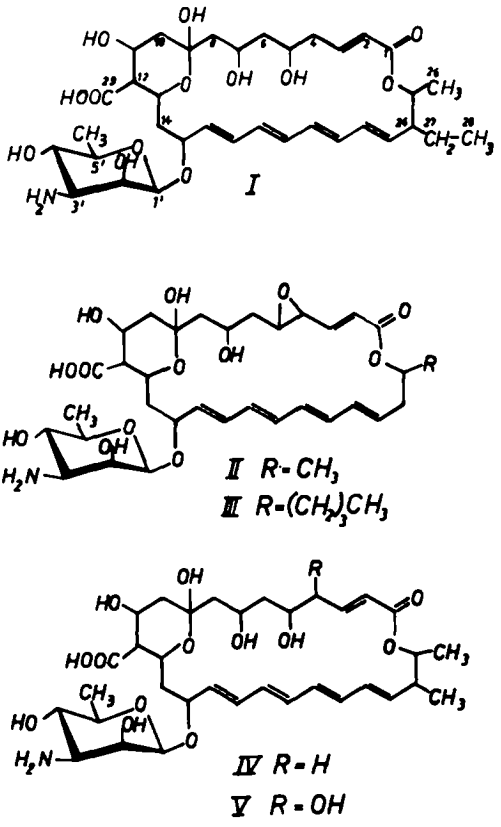


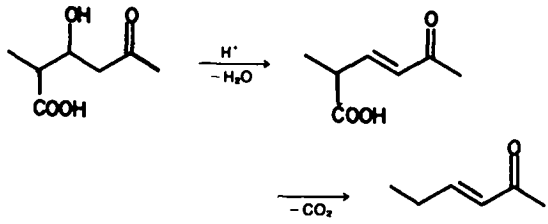
Fig. 2. The structures of tetramycin (I), pimaricin (II), lucensomycin (III), tetrin A (IV) and tetrin B (V).

presence of a conjugated ester group absorbing at 1710 cm^{-1} which, on hydrogenation, resulted in a shift to 1728 cm^{-1}. Further support is obtained by the characteristic UV maximum at 208 nm (ϵ_{max} 17,850). Conclusive evidence for this contention was provided by the ^{13}C and ^{1}H NMR spectra. The ^{13}C NMR spectrum of I features ten methine C resonances in the sp^2 region. Two of these occur at 146.34 and 124.26 ppm (DMSO-d_6) which, along with the carbonyl C resonance at 164.91 ppm, signal the presence of an α,β-unsaturated ester group. The α,β-unsaturation readily follows also from the ^{1}H chemical shifts (5.92 and 6.82 ppm, Py-d_5) of an olefinic proton pair not belonging to the polyene moiety of the molecule.

The results of the catalytic hydrogenation furthermore show that, unlike its analogs II and III, tetramycin contains no epoxide ring which should be opened through hydrogenolysis.

Acid-catalysed hydrolysis revealed the presence of a glycosidically attached mycosamine (3'-amino-3',6'-dideoxy-D-mannose) residue. Refluxing of I with methanol-hydrochloric acid gave the crystalline methyl glycoside of mycosamine identified through elemental analysis and comparison by tlc with an authentic sample obtained from acidic hydrolysis of II. In all structurally defined polyene antibiotics, the mycosamine moiety is attached to the aglycon in a position allylic to the polyene chromophore. Comparison of the ^{13}C chemical shift of mycosamine C-1' in I (95.7 ppm, DMSO-d_6) with those reported for III, IV and V (95.7 ± 0.2 ppm)[10] shows that the site of glycosidation in I should be the same.

The IR spectra disclosed the presence of an ionized carboxyl group with characteristic bands at 1580 and 1390 cm^{-1} which are absent in N-acetyltetramycin. Further support to this conclusion was provided by alcaline titration (neutralization equivalent 719 ± 25) and also by the facile decarboxylation of I and its perhydro derivative in acidic media. The ease of the decarboxylation may be rationalized by assuming the occurrence of partial structure A the transformation of which readily proceeds according to the scheme:



The same relative positions of the carboxyl and oxo groups has been established also for related polyene macrolides.

The fact that no retroaldolization and decarboxylation occur upon treatment of tetramycin with $NaBH_4$ suggests the presence of an oxo function in the molecule. The IR band at 1700 cm^{-1} is in accord with this expectation. However no resonance near 210 ppm has been detected in the ^{13}C NMR spectrum of I signalling the possibility for a masked CO function, a structural feature common for polyene macrolides with an OH group δ to the oxo function. In fact, ^{13}C NMR revealed the presence of a quaternary C resonating at 96.37 ppm (in DMSO-d_6), a value which is very close to chemical shift observed in II (97.0 ppm) and III (96.9 ppm) and assigned to the cyclic hemiketal carbonyl C.[10]

The number of acetylable functions in I has been established by ^{1}H NMR spectra of the decahydrotetramycin acetate, as well as by titration of the acetyl groups. Since the carbohydrate moiety is responsible for three acetyl groups, the formation of heptaacetyldecahydrotetramycin requires that the aglycone contains four OH groups available for acetylation.

Both tetramycin and decahydrotetramycin consume *ca.* 2 moles of periodic acid within 30 min as measured by arsenit titration[11] (Table 1) indicating thereby the presence of two 1,2- or one 1,2,3-glycol or equivalent structures. Since N-acetyl decahydrotetramycin does not react with periodate, the glycole structures cannot belong to the aglycone moiety, i.e. the macrocycle contains no vicinally located OH functions. Instead, the above finding shows that the mycosamine moiety occurs in pyranose form as it has been found with other mycosamine containing polyene macrolides. It may be noted that the periodate consumption by I did not terminate at the 2 molar equivalents limit. According to Ceder *et al.*,[12] the further uptake of periodate is explained on the basis of a periodate-induced transformation of the tetraene system into a triene structure as evidenced by characteristic changes in the UV absorption.

The Cope procedure[13] for determining the carbon skeleton of polyene antibiotics was employed to reduce I to a saturated hydrocarbon as shown in Fig. 1. The hydrocarbon has an IR spectrum characteristic of a saturated hydrocarbon. GC revealed only one high molecular weight component with a retention time just like a C-28 hydrocarbon. The molecular weight was determined by EA-MS.[14] The molecular ion of the

Table 1. Periodate uptake of I and its derivatives

	moles periodates				
	0.5 h	1 h	2 h	4 h	8 h
TETRAMYCIN	2.4	2.9	3.3	3.8	3.8
DECAHYDROTETRAMYCIN	1.74	1.70	1.83	2.22	-
N-ACETYL DECAHYDROTETRAMYCIN	0.02	-	0.01	-	-

hydrocarbon is represented by the cluster of ions at *m/e* 411 ($M^+ + OH$). This is in conformity with the molecular formula $C_{28}H_{58}$ (M = 394). Therefore, the basic skeleton of I contains 28 C atoms.

The size of the carbon skeleton of tetraene macrolide antibiotics could be established also on the basis of mass spectral data.

Mass spectral studies

Determination of the molecular formula and structural assignment of the aglycone. Recently, we reported on the mass spectral behavior of some tetraene antibiotics by means of EI-MS, EA-MS and DADI mass spectroscopy.[2] On the basis of these results, we have determined the carbon skeleton of the algycones and the number of their OH groups, using the parent compounds. The major mass spectral fragmentations of the parent compounds are relatively straightforward and the respective ions are formed by elimination of mycosamine, decarboxylation of the carboxyl group and loss of a varying number of water molecules according to the number of OH groups in the aglycone moiety (M–mycosamine–CO_2–n-H_2O). The apparent driving force for this fragmentation pattern is the energetically favorable extension of the conjugated polyene system and the production of neutral molecules. The EI- and EA-mass spectra of I show a parent peak at *m/e* 416 for which accurate mass measurement gave the composition of $C_{28}H_{32}O_3$ (Found: 416.2353. Calc: *m/e* 416.2351).

The spectra exhibit other intensive peaks at *m/e* 434 and 452, corresponding to the compositions $C_{28}H_{34}O_4$ and $C_{28}H_{36}O_5$ ($416 + 2H_2O$). Together with the carboxyl group the aglycone skeleton of I contains 29 C atoms. The carboxyl C is apparently split off by decarboxylation during the reduction to the saturated hydrocarbon. Finally, the existence of four OH groups in the aglycone of I is confirmed by the ^1H NMR spectrum of the decahydrotetramycin acetate. Hence the molecular formula of I is $C_{28}H_{32}O_3 + C_6H_{13}NO_4$ (mycosamine) $+ CO_2 + 4H_2O = C_{35}H_{53}NO_{13}$, with a molecular weight of 695. These results have been confirmed by EI-MS of perhydrotetramycin (Fig. 4). The parent peak at *m/e* 506 arises from the loss of 1 mycosamine and 2 water molecules, establishing the molecular weight of perhydrotetramycin as 705. Compared with the fragmentation pattern of I this means the uptake of 5 moles of hydrogen per molecule which is in accord with the results of the catalytic hydrogenation. The inaccuracy of analytical data from which the $C_{34}H_{53}NO_{14}$ formula was calculated is due probably to the solvation of the antibiotic crystals. The molecular weight of I has been later supported by means of field desorption mass spectroscopy.[2] This technique has been shown to be the method of choice in determining molecular weights of several antibiotics of low volatility or thermal instability.[15] The molecular ion in the FD mass spectrum of I appears at *m/e* 696 (M + H).

Retroaldol cleavage. The location of the tetraene chromophore within the macrolide skeleton was deduced from retroaldol cleavage. Treatment of I with 2 N NaOH (Fig. 5) and subsequent continuous extraction with ether at ambient temperature afforded a yellow substance assigned as 12 - ethyl - 13 - hydroxy - 2,4,6,8,10 - tetradecapentaenal (Fig. 5). Its high resolution mass spectrum showed the molecular peak at *m/e* 246.1636 ($C_{16}H_{22}O_2$). Characteristic peaks appeared at *m/e* 202.1376 ($M^+ - CH_3CHO$), 201.1293 ($M^+ - CH_3$–$\overset{+}{C}H$–OH↔CH_3–CH=$\overset{+}{O}$H) and 173.0974 ($M^+ - CH_3CHO$–CH_2CH_3). The UV spectrum exhibited a broad maximum at 377 nm characteristic of a conjugated pentaene carbonyl chromophore[9] while the IR spectrum showed OH and aldehyde CH stretching bands at 3620, 2855 and 2735 cm^{-1}, and bands at 1680 and 1590 cm^{-1} attributable, respectively, to a highly conjugated CO function and a polyene grouping. Further structural evidence was provided by the ^1H and ^{13}C NMR data (Table 2). The ^1H

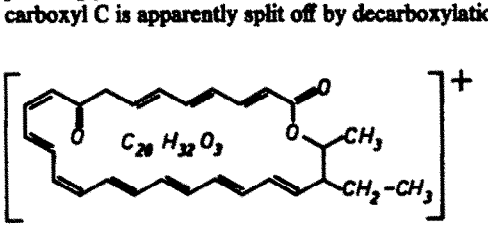


Fig. 3. Fragmentation ion of I.

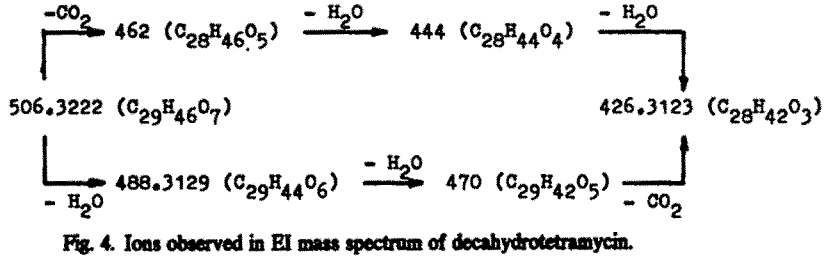


Fig. 4. Ions observed in EI mass spectrum of decahydrotetramycin.

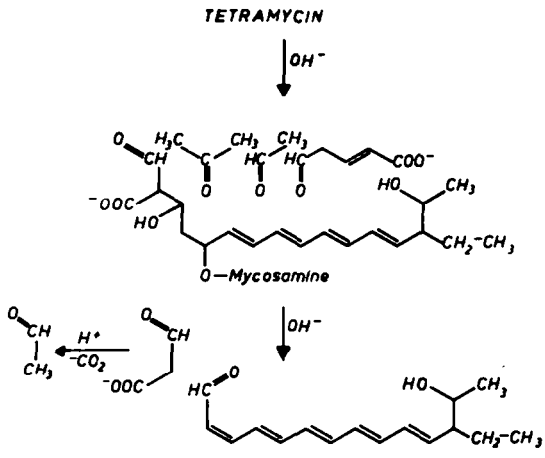


Fig. 5. Retroaldol cleavage of I.

NMR spectra disclosed the presence of an aldehyde function attached to the end of the pentaene chain having its last double bond in *cis* configuration and provided unambigous informations about the sequence of protons and proton groups in the aliphatic portion of the molecule. The number of C atoms, their chemical shift values and off-resonance multiplicities, on the other hand, were found to be in complete agreement with the assumed tetradecapentaenal structure.

Reduction of the pentaenal with sodium borohydride gave a pentaenediol (Fig. 1). Its UV spectrum (λ_{max}^{MeOH} 304, 314, 328 and 346 nm) attested the presence of an isolated pentaene chromophore.

The pentaenal is formed under conditions known to induce retroaldol reaction. The reatroaldol cleavage was found to be triggered by the oxo function: if treatment with sodium hydroxide was performed after the reduction of tetramycin with sodium borohydride, no pentaenal could be detected among the products.

Table 2. ^{1}H and ^{13}C NMR parameters of 12-ethyl-13-hydroxy-2,4,6,8,10-tetradecapentaenal[a,b]

^{1}H NMR

Chemical Shifts, Multiplicity and Coupling Constants		Proton
9.46	(d, 9 Hz)	C-13H
6.14	(d,d, 9; 8)	C-14H
7.17	(d,d, 8; 15)	C-15H
6.3 - 6.7	8H, m	C-16H - C-22H
5.64	(d,d, 9; 15)	C-23H
2.08	(d,d,t, 9; 6.5; 6.5)	C-24H
3.74	(d,q, 6.5; 6.5)	C-25H
1.14	(3H, d, 6.5)	C-26H_3
1.6	(2H, d,q, 6.5; 6.5)	C-27H_2
0.88	(3H, t, 6.5)	C-28H_3

^{13}C NMR

Chemical Shift	Carbon	Chemical Shift	Carbon
193.21	C-13	130.47	C-18
151.98	C-15	130.36	C-16
142.94	C-17	129.33	C-14
139.12	C-19	69.61	C-25
138.82	C-21	52.95	C-24
136.88	C-23	23.50	C-27
132.04	C-22	20.77	C-26
130.92	C-20	11.96	C-28

[a] Measured at ambient temperature in $CDCl_3$ at 100.1 and 25.16 MHz. Chemical shifts are in ppm relative to internal TMS.

[b] The numbering of atoms follows that of I.

In an alternative base-catalysed hydrolysis, tetramycin was treated with sodium hydroxide and then the product was steam-distilled into a solution containing 2,4-dinitrophenylhydrazine to give the 2,4-dinitrophenylhydrazones of acetaldehyde and acetone, as evidenced by high resolution mass measurements. Subsequent acidification of the residue and further steam-distillation yielded additional amounts of acetaldehyde identified again through its 2,4-dinitrophenylhydrazone. This second portion of acetaldehyde indicates the occurrence of decarboxylation of formyl acetic acid ($HOOCCH_2CHO$) as shown in IV and V. These findings are in accord with observations made on related tetraene antibiotics and indicate the occurrence of similar structural elements in I.

The proposed structure of tetramycin is in accord with biosynthetic considerations assuming polyacetate origin.[9] According to these considerations, oxygenation is expected to occur at carbons C-5, C-7, C-9, C-11, C-13 and C-15. However, alternative structures, e.g. formation of the oxo group at C-7, or hemiketal ring between C-9 and C-5, cannot be *a priori* excluded. Following the biosynthetic studies of 16-membered macrolide antibiotics,[16] formation of Et group at C-24 may be rationalized by assuming butyrate incorporation.

In connection with the present studies, we have also performed a comparative ^{13}C NMR analysis of N-acetyltetramycin methyl ester and N-acetylpimaricin methyl ester featuring higher stability and better solubilities in pyridine-d_5 and DMSO-d_6 than the underivatized antibiotics. In this way, a complete assignment of the ^{13}C resonances in I could be achieved which provided an independent support for the correctness of the proposed structure of tetramycin. These latter results, together with the stereochemical conclusions derived from the high frequency (270 MHz) ^1H NMR spectra, will be described in a forthcoming report.[17]

EXPERIMENTAL

M.ps were determined on a Kofler hot stage and are uncorr. IR spectra were recorded on Perkin–Elmer 325 and Zeiss UR 20 IR spectrophotometers, UV spectra on Zeiss spectrophotometer Specord UV VIS. ^1H NMR spectra were obtained on Varian HA-100 and XL-100 spectrometers. ^{13}C NMR spectra were recorded on Varian XL-100/15 Fourier transform instrument at 25.16 MHz. EA-MS were obtained on EA-mass spectrograph (M. v. Ardenne, Dresden). EI-MS were determined on AEI MS 902 S and Joel JMS D 100 mass spectrometers.

Tetramycin. Details of fermentation and isolation have been described by Dornberger *et al.*[1]

Decahydrotetramycin. 1 g of I was hydrogenated with 50 mg of PtO_2 in 10 ml of HOAc for 3 hr at room temp. The catalyst was removed by filtration, the solvent by freeze-drying. The residue was washed with ether, dried to give 980 mg of decahydrotetramycin (98% of theory); m.p. 250° dec.; $[\alpha]_D^{22}$ −44.8 (*c* 0.5, DMF), $[\alpha]_D^{22}$ −74.7° (*c* 0.5, pyridine); R_f 0.35 (tlc on silica gel sheets F_{254}, Merck, n-BuOH/HOAc/H_2O, 4:1:5). IR ν_{max}^{KBr} cm^{-1}: 3430, 2940, 2860, 1728, 1580, 1390. (Calc. for $C_{34}H_{63}NO_{14}$: C, 57.55; H, 8.89; N, 1.97. Calc. for $C_{35}H_{63}NO_{13}$: C, 58.71; H, 9.06; N, 2.01. Calc. for $C_{35}H_{63}NO_{13}\cdot H_2O$: C, 58.09; H, 8.99; N, 1.93. Found: C, 57.39; H, 8.90; N, 2.01%).

N-Acetyltetramycin. A mixture of 100 mg of I and 0.07 ml of HC_2O in 15 ml of abs MeOH was stirred at 0° for 2 hr while the antibiotic gradually dissolved. The resulting soln was concentrated *in vacuo* and the residue was precipitated with ether to give 80 mg of N-acetyltetramycin; m.p. 152–154° (from AcOEt); $[\alpha]_D^{22}$ +202.5° (*c* 0.49, MeOH); UV λ_{max}^{MeOH} nm: 280, 290, 304, 318; IR ν_{max}^{KBr} cm^{-1}: 3430 (OH, NH), 1720 (COOH), 1655 and 1550 cm^{-1} (amide, bands I and II); R_f 0.58 (tlc on silica gel sheets F_{254}, Merck, n-BuOH/HOAc/H_2O 4:1:5); (Calc. for $C_{36}H_{55}NO_{15}\cdot CH_3OH\cdot H_2O$: C, 56.13; H, 7.71; N, 1.76. Calc. for $C_{37}H_{55}NO_{14}\cdot CH_3OH\cdot H_2O$: C, 57.94; H, 7.75; N, 1.77. Found: C, 55.67; H, 7.47; N, 1.67%).

Heptaacetyl-decahydrotetramycin. A soln of 250 mg of decahydrotetramycin, 2.5 ml of pyridine, and 2.5 ml of Ac_2O stood at room temp. for 48 hr. The mixture was then cooled, diluted with ice water, and extracted with $CHCl_3$ three times. The combined chloroform extracts were washed with water, dried over Na_2SO_4, concentrated *in vacuo* and chromatographed on alumina (activity II, acidic). Elution with $CHCl_3$ afforded a foamy residue, which was dissolved in MeOH, treated with activated charcoal, filtered and concentrated. Addition of hexane afforded 127 mg of an amorphous solid, m.p. 105–107°; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3430 (NH), 1740 (acetate), 1675 (amide); ^1H NMR ($CDCl_3$) δ: 1.88, 1.98, 2.02 (6*H*), 2.04, 2.10 (6*H*).

Conversion of decahydrotetramycin to the parent hydrocarbon. A soln of 4 g of decahydrotetramycin in abs THF was heated under reflux for 48 hr with 4 g of $LiAlH_4$. The unchanged hydride was destroyed with AcOEt and the mixture evaporated to dryness. The residue was dissolved in dilute H_2SO_4 and the polyol extracted with n-BuOH. The combined extracts were washed with water and the n-BuOH was removed *in vacuo*. The oily residue was dissolved in a small amount of t-BuOH and freeze dried to yield 3 g of an oil.

The crude polyol was dissolved in 20 ml of HOAc and added to a refluxing suspension of 220 ml 48% HI and 6 g of red P. After 24 hr 150 ml of HI were removed by distillation, 100 ml of water were added and the mixture was extracted twice with 150 ml of $CHCl_3$. The extracts were washed with 2% $Na_2S_2O_3$, aq. then with water, and dried over Na_2SO_4. Removal of solvent gave an oil (1.53 g) which was dissolved in 55 ml of abs THF and refluxed for 24 hr with 2 g of $LiAlH_4$. The unchanged hydride was destroyed with AcOEt. After evaporation of the solvent, the inorganic material was dissolved in 20% H_2SO_4 and organic material extracted with hexane. The hexane solution, after being washed with 10% Na_2CO_3 aq. and dried over Na_2CO_3, was concentrated and hydrogenated using PtO_2 as catalyst. The catalyst was separated and the concentrated soln chromatographed on a column of neutral alumina (activity II). The first fractions eluted with hexane were combined, yielded 190 mg (9% of theory); IR ν_{max}^{film} cm^{-1}: 2920, 2850, 1465, 1380, 720; GC (260°, 1.5 m column, 2.5% fluhyzone on pyrolith) showed a single peak.

Base-catalysed hydrolysis of I

1. *Isolation of* 12 - *ethyl* - 13 - *hydroxy* - 2,4,6,8,10 - *tetradecapentaenal.* A soln of 500 mg of I in 250 ml of 2 N NaOH was extracted continuously with Et_2O for 30 hr at room temp. The Et_2O extract was washed with water, dried over Na_2SO_4 and concentrated. The yellow crude pentaenal (90 mg) was purified by preparative tlc on alumina HF_{254} (Merck) using $CHCl_3$/AcOEt (3:2) as solvent under argon atmosphere. The major yellow band was eluted with MeOH and the solvent evaporated to give the pure pentaenal: m.p. 103–105°; $[\alpha]_D$ −15.1° (*c* 0.8, MeOH); UV λ_{max}^{MeOH} 370 nm; IR ν_{max}^{KBr} cm^{-1}: 3620 (OH), 2855, 2735 (–CHO), 1680 (conjugated CO), 1590 (C=C stretching), 1008 cm^{-1} (C=C *trans*); (Calc. for $C_{16}H_{22}O_2$: mol wt 246.1620. Found: mol wt 246.1636).

2. *Isolation of acetone and acetaldehyde.* A suspension of 200 mg of I in a soln of 200 mg NaOH in 50 ml of water was steam distilled into a sat.soln of 2,4-DPNH in 2 N HCl. The distillate (1.5 l) was left overnight at 5°, then filtered, dried and identified as a mixture of the acetone and acetaldehyde 2,4-dinitrophenylhydrazones by MS (62 mg, m.p. 105–115°). (Calc. for $C_9H_{10}O_4N_4$: mol wt 238.0702; Found: mol wt 238.0709; Calc. for $C_8H_8N_4O_4$: mol wt 224.0545; Found: mol wt 224.0549). The aqueous basic residue was acidified then with HCl and steam distilled again into 2,4-DPNH-saturated 2 N HCl until no more hydrazone was formed. As before, the precipitate (3 mg) was identified as acetaldehyde 2,4-dinitrophenylhydrazone.

Preparation of 12 - *ethyl* - 2,4,6,8,10 - *tetradecapentaene* - 1,13 - *diol.* 20 mg of pentaenal was dissolved in 25 ml of MeOH at 0° and mixed up with 60 mg of $NaBH_4$. The mixture was stirred at 0° for 30 min and at room temp. for another 30 min. Then was diluted with 20 ml of water and concentrated *in vacuo* to remove

the MeOH. The aqueous solution was extracted with Et_2O (3 × 25 ml). The combined extracts was washed with water (4 × 25 ml) and dried over Na_2SO_4. Removal of solvent afforded the pentaenediol as a light yellow residue; 10.9 mg, m.p. 136–138°; UV λ_{max}^{MeOH} nm (ϵ) 347 (101.250), 329 (98.750), 314 (60.000), 301 (27.500). (Calc. for $C_{16}H_{24}O_2$: mol wt 248.1776. Found: mol wt 248.1778).

Isolation of α-methyl mycosaminide hydrochloride. 0.5 g of 1 was suspended in 10 ml of abs MeOH and 10 ml of 6 N methanolic HCl was added. After refluxing for 2 hr the soln was allowed to stand at room temp. overnight. After removal of solvent by concentration *in vacuo*, 20 ml of water was added and the aqueous soln after decanting from tarry solids, was extracted repeatedly with n-BuOH which removed most of the pigmented decomposition products. The aqueous phase was neutralised then to pH 7 with Wofatit L 150 (OH) resin, freed from BuOH by concentration *in vacuo* and lyophilized. Recrystallization from EtOH by addition of Et_2O yielded 80 mg of colorless needles (60% of theory), m.p. 194–196°; $[\alpha]_D$ + 52.9° (*c* 0.5 MeOH). (Calc. for $C_7H_{15}NO_4 \cdot HCl$: C, 39.35; H, 7.55; N, 6.56. Found: C, 39.05; H, 7.32; N, 6.80%).

Periodate oxidations were performed at room temp. as noted[11] employing standard solns of 2 M HIO_4, 0.1 N Na_2AsO_3, 20% KJ soln, 0.1 N iodine soln, starch and $NaHCO_3$.

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