THE STRUCTURE OF MANUMYCIN

II. DERIVATIVES

AXEL ZEECK, KLAUS FROBEL, CHRISTA HEUSEL, KARSTEN SCHRÖDER and RALF THIERICKE

Institut für Organische Chemie, Universität Göttingen, Tammannstraße 2, D-3400 Göttingen, FRG

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Derivatives of manumycin (**1**) were obtained by acetylation and reduction, respectively, and characterized by their spectroscopic data. Structure-activity relationships of the antibiotic were discussed.

The structure of manumycin (**1**), produced by *Streptomyces parvulus*[1], was established by some key reactions in combination with a thorough spectroscopic analysis[2,3]. The still missing absolute configuration of the center of chirality will be the subject of the following paper[4]. In order to characterize this unusual antibiotic in a more chemical way and to derive structure-activity relationships, we prepared some more derivatives. Additionally, they could be regarded as further evidence confirming the structure of manumycin (**1**). In this paper we describe some chemical reactions of **1** and compare the biological activities of the derivatives.

Acetylation

Acetylation of manumycin (**1**) without an attack of the epoxide was carried out with acetic anhydride and sodium acetate. By Sephadex LH-20 column chromatography, a pale yellow acetate (Rf values see Table 1) was obtained, which, because of its instability, could only be characterized by use of spectroscopic methods. Comparing its ^{1}H NMR spectrum with that of **1**, the lack of 2″-NH and 3″-OH and the appearance of two new acetyl singlets at δ 2.21 and 2.38 gave rise to structure **2**. Based on the consequence of a tautomeric equilibrium at the C-13 amide group (**1a** and **1b**), the UV spectrum of the diacetate **2** showed no bathochromic shift as expected for a more conjugated system like **1b** which ruled out the corresponding acetate. With respect to the fully acetylated C_5N-moiety of **2**, and in order to compare its spectroscopic data, the triacetate **5** was prepared from 2-amino-3-hydroxy-2-cyclopenten-1-one (**4**)[5] by treatment with acetic anhydride at 150°C. **5** showed nearly identical spectroscopic data to that of the C_5N-moiety of **2**.

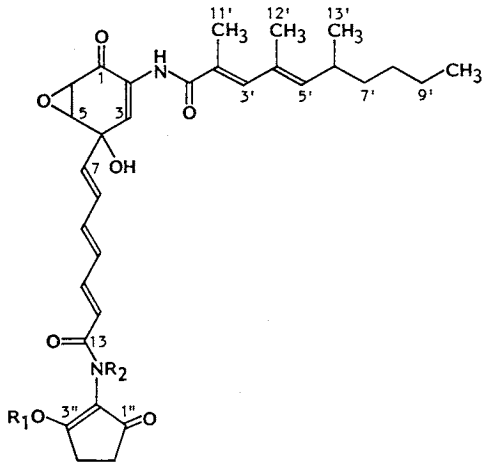
Based on the instability of manumycin diacetate (**2**), a monoacetate was obtained by stirring a

Table 1. Rf values of manumycin (**1**), its acetates **2** and **3**, the "hydromanumycins" **10**, **12** and **14**, their methanolysis products **11** and **13**, and the deoxymanumycins **7** and **8** in different solvent systems on TLC silica gel plates.

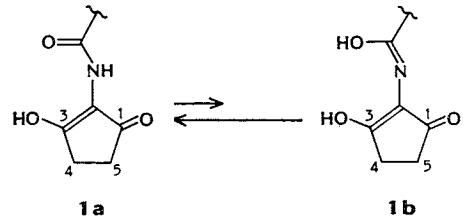
Solvent system	**1**	**2**	**3**	**7**	**8**	**10**	**11**	**12**	**13**	**14**
$CHCl_3$ - MeOH (95 : 5)	0.34	0.51	0.41	—	—	0.52	0.67	—	—	—
$CHCl_3$ - MeOH (93 : 7)	0.47	—	—	—	—	0.57	0.68	0.30	0.45	0.24
$CHCl_3$ - MeOH (9 : 1)	0.55	—	—	0.28	0.55	0.61	0.71	—	—	—

—: Not tested.

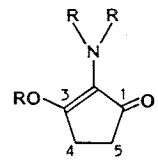
Scheme 1.



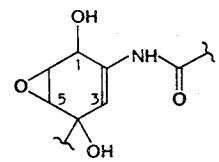
1 $R_1 = R_2 = H$
2 $R_1 = R_2 = Ac$
3 $R_1 = Ac$ $R_2 = H$



1a **1b**



4 R = H
5 R = Ac



6

(Chains as in **1**)

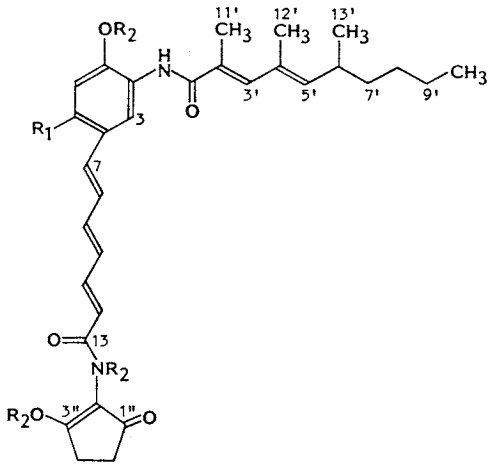
chloroform solution of **2** for two days. In the ^1H NMR spectrum of this product only one acetyl singlet at δ 2.28 was observable. The missing 3″-OH signal and the appearance of 2″-NH (δ 7.97) were in accordance with structure **3**.

Reduction of the Cyclohexenone Epoxide Moiety

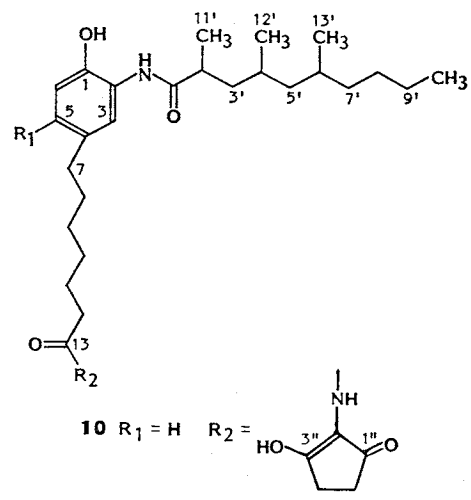
As described in the preceding paper[3], the reduction of manumycin (**1**) with sodium borohydride in aqueous methanol yielded dihydromanumycin (partial formula **6**). Using dry methanol (as a reaction solvent) a yellow product precipitated and was purified by Sephadex LH-20 column chromatography. It was less soluble than **1** and found to be deoxymanumycin (**7**). The ^1H NMR spectrum of **7** in acetone-d_6 exhibited no epoxide protons. The signals of two phenolic hydroxy groups at δ 8.88 and 9.91 close to the amide-NH (δ 8.72 and 9.09) as well as a singlet of one aromatic proton at δ 6.53 appeared additionally. The doublet of 3-H in **1** diminished to a singlet in **7** (δ 7.66); all the other protons gave identical signals in both compounds. These data located the alteration of the molecule in the cyclohexene epoxide moiety, which was changed to a tetra-substituted phenolic system. The *para*-position of the remaining aromatic protons was shown by its coupling constants, being smaller than 1 Hz. The ^1H NMR data, in connection with the molecular ion at *m/z* 534 detectable in the electron impact mass spectrum (EI-MS), as well as its elemental analysis showed the molecular formula to be $C_{31}H_{38}N_2O_6$. In comparison to **1** this indicated the lack of one oxygen. The reduction of manumycin (**1**) with sodium borohydride started at the C-1 carbonyl group, followed by an elimination of water and the regioselective opening of the epoxide. The energetically favored aromatization, which sometimes already took place during the chromatographic purification of dihydromanumycin (**6**), is probably the driving force.

Deoxymanumycin (**7**) was also produced, in lower yields, *via* reaction of **1** with potassium iodide in acetic acid. In addition, a second reduction product was isolated, which became the main product using zinc dust in acetic acid. Its physico-chemical properties showed great similarities to those of

Scheme 2.



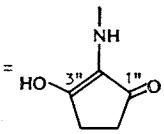
7 R_1 = OH R_2 = H
8 R_1 = R_2 = H
9 R_1 = H R_2 = Ac



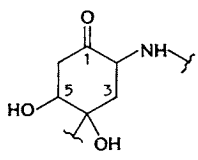
10 R_1 = H R_2 =

11 R_1 = H R_2 = OCH_3

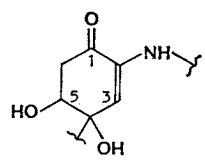
12 R_1 = OH R_2 =



13 R_1 = OH R_2 = OCH_3



14



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(Chains as in **10**)

Table 2. UV absorption bands of manumycin (**1**), decahydrodideoxymanumycin (**10**), deoxymanumycin (**7**), dideoxymanumycin (**8**) in different solvents (λ_{max} in nm (ε)).

Solvent	1	7	8	10
MeOH	314 (24,600), 278 (36,400)	378 (39,500), 317 (23,200), 256 (37,600)	358 (30,100), 260 (32,800)	278 (11,200), 256 (24,800), 210 (31,300)
MeOH - HCl	328 (32,300), 270 (31,700)	398 (41,300), 264 (26,500)	378 (32,300), 268 (27,900)	278 (9,300), 243 (19,200), 211 (29,100)
MeOH - NaOH	261 (40,200)	427 (24,900), 255 (40,500)	408 (24,700), 260 (35,100)	313 (6,000), 257 (31,800), 220 (25,800)

6. The elemental analysis pointed out the lack of a second oxygen in the molecular formula, leading to dideoxymanumycin (**8**), which was acetylated by applying acetic anhydride and sodium acetate to its triacetate **9**. The same reaction conditions converted **1** to its diacetate **2**, and the spectral data displayed the full acetylated C_5N-moiety as described in **2** and **5**. Additionally, an aromatic acetoxy group (δ 2.32) and three aromatic protons were detectable in the ^1H NMR spectrum of **9**. The epoxide protons were missing.

Hydrogenation

Depending on the reaction conditions catalytic hydrogenation of manumycin (**1**) led to three main products with varying yields. As a key substance for the structure elucidation "hydromanumycin" was isolated[2], which now is established to be decahydrodideoxymanumycin (**10**). Its molecular formula $C_{31}H_{46}N_2O_5$ was established by high resolution mass spectrometry. Important fragment ions were found at *m/z* 332, 220 and 122 by successive losses of $C_{13}H_{24}O$ (C_{13}-side chain), $C_5H_6NO_2$ (C_5N-moiety) and $C_6H_{10}O$ (C_7-side chain splitted in benzyl-position) from the molecular ion. The structure of **10** was established both by its ^{1}H NMR spectrum and the degradation product **11**, liberated by acidic methanolysis in addition to the C_5N-moiety **4**, the latter being isolated as the triacetate **5**. In acetone-d_6 the aromatic protons of **11** were well-separated, with the chemical shifts and the coupling pattern being quite the same as in the model compound 2-acetamino-4-methylphenol.

A second hydrogenation product turned out to be decahydrodeoxymanumycin (**12**). The ^{1}H NMR spectrum displayed only two singlets at δ 6.46 and 6.76, suggesting the *para* position of the aromatic protons. The methyl ester **13**, liberated by acidic methanolysis from **12**, differed from **11** only by its molecular weight. High resolution EI-MS (*m/z* 463, M^+, $C_{27}H_{45}NO_5$) indicated the presence of a further oxygen atom.

The third oily hydrogenation product sometimes appeared to be the main product. Its ^{1}H NMR spectrum showed no signals for olefinic/aromatic protons, which suggests the suppression of the favored aromatization. We assume the cyclohexene **15** to be an intermediate product and further reduction resulted in tetradecahydromanumycin (**14**). Acidic methanolysis of **14** gave **11**, which led us to the conclusion of tetradecahydromanumycin being an intermediate of the reductive pathway leading to decahydrodideoxymanumycin (**10**). We postulate **15** to be an intermediate on the way to the second aromatic reduction product, the decahydrodeoxymanumycin (**12**).

Because of varying amounts of **10**, **12** and **14** we suppose the different hydrogenation pathways of **1** will be under kinetic control. The low yield of **12** gives rise to the assumption, that the hydrogenation rate of the C-2/C-3 double bond (**15** to **14**) is faster than the elimination of water from compound **15**. The product composition strongly depends both on the quantity ratio between **14** and **15** and on the extent of the energetic barrier leading to the aromatic nucleus. The conversion from **14** to **10** is much slower than from **15** to **12**. Apparently, hydrogenation of the olefinic double bond at C-2 did not influence the attack on the cyclohexene epoxide including the regioselective opening of the epoxide. Consequently, the C_{13}-side chain's methyl-ramification caused diastereomeric mixtures of the resulting hydrogenation products **10**, **12** and **14**, which was shown by the gas chromatographic separation of the C_{13}-side chain methyl esters after alkaline hydrolysis and esterification[2].

Biological Activities and Discussion

The biological activity of manumycin (**1**) has already been described[1,3]. Acetylation of 3″-OH and 2″-NH (**2** and **3**) led to a decrease of the biological activity. Against *Bacillus subtilis* on a chemically defined medium, **3** was nearly 10-fold less active than **1** and **2**. The C_5N-moiety's importance is not clear yet, 2-acetamino-3-hydroxycyclopent-2-enone[3] itself developed no biological activity against bacteria.

Aromatization of the former cyclohexenone epoxide ring as in deoxymanumycin (**7**) and dideoxymanumycin (**8**) caused the loss of this biological activity. The chirality of the six-membered ring and the nearly planar conformation of the molecules as well as the restricted solubilities might explain these

results. Accordingly the three hydromanumycins **10**, **12** and **14** exhibited no activities against bacteria and fungi. The importance of the cyclohexenone epoxide's biological activity was evaluated additionally from dihydromanumycin (**6**)[3]. Although the main parts of the antibiotic were preserved, it showed no biological activity. This result pointed out the peculiarity of C-1 concerning its biological activity. Unfortunately, we failed to open the epoxide ring or to convert it to a C-C double bond by deoxygenation without aromatization of the six-membered ring. Thus, a differentiation between the C-1 carbonyl group and the epoxide ring was impossible.

From these results it will be difficult to enhance the biological activity of the parent antibiotic manumycin (**1**) by means of chemical transformations. Manumycin and the other manumycin group antibiotics (literature cited in ref 3) seem to be highly specific natural products.

Experimental

General and Analytical

The same equipment was used as previously described[3].

Manumycin Diacetate (2)

To a solution of 115 mg **1** in 25 ml acetic anhydride 1.05 g sodium acetate was added and stirred for 65 hours at 36°C under nitrogen. The solution was treated with ice-water for 5 hours, filtered, evaporated and dried overnight. The dark brown product was chromatographically purified on a Sephadex LH-20 column (80×2.5 cm), eluting with $CHCl_3$ to yield 71 mg pale yellow **2**: Rf see Table 1; UV λ_{max}^{EtOH} nm (ε) 328 (41,500), 293 (sh, 28,900), 258 (32,800); IR (KBr) cm^{-1} 3340, 1785, 1722 (sh), 1718 (sh), 1709, 1688, 1660, 1510; ^1H NMR (100 MHz, $CDCl_3$) δ 0.86 (t, J=7 Hz, 10′-H_3), 0.96 (d, J=7 Hz, 13′-H_3), 1.25 (br s, 7′-H_2, 8′-H_2 and 9′-H_2), 1.79 (d, J=1.5 Hz, 12′-H_3), 2.01 (d, J=1.5 Hz, 11′-H_3), 2.21 (s, 2″-$NCOCH_3$), 2.38 (s, 3″-$OCOCH_3$), 2.60 (br s, 6′-H), 2.62 (s, 5″-H_2), 3.06 (s, 4″-H_2), 3.65 (d, J=8 Hz, 6-H), 3.72 (dd, J=5 and 2.5 Hz, 5-H), 3.87 (br s, 4-OH), 5.1~7.4 (m, 9 olefinic protons), 7.98 (s, NH).

Manumycin Monoacetate (3)

89 mg **2** were dissolved in 100 ml $CHCl_3$ and stirred at room temp. After 3 days the solution was evaporated and chromatographed on a silica gel column (80×2.5 cm, $CHCl_3$) to yield 25 mg **3** as a pale yellow amorphous powder: Rf see Table 1; IR (KBr) cm^{-1} 3380, 1785, 1712 (sh), 1688 (sh), 1665, 1614, 1515; UV λ_{max}^{EtOH} nm (ε) 322 (35,300), 287 (31,900), 262 (33,400); ^1H NMR (100 MHz, $CDCl_3$) δ 0.86 (t, J=7 Hz, 10′-H_3), 0.96 (d, J=7 Hz, 13′-H_3), 1.25 (br s, 7′-H_2, 8′-H_2 and 9′-H_2), 1.80 (d, J=1.5 Hz, 12′-H_3), 2.02 (d, J=1.5 Hz, 11′-H_3), 2.28 (s, 3″-$OCOCH_3$), 2.52 (s, 5″-H_2), 2.60 (br s, 6′-H), 2.72 (s, 4″-H_2), 3.65 (d, J=4 Hz, 6-H), 3.72 (dd, J=4 and 2.5 Hz, 5-H), 4.42 (s, 4-OH), 5.2~7.4 (m, 9 olefinic protons), 7.50 (s, 2″-NH), 7.97 (s, NH).

3-Acetoxy-2-diacetylamino-2-cyclopenten-1-one (5)

The slightly acidic inorganic layer from the methanolysis of **10** (see below; description of compound **11**) was evaporated to dryness. 3.5 ml acetic anhydride were added to this dark brown oily crude product and kept in a sealed tube at 150°C for 5 hours. The reaction mixture was evaporated and chromatographed on a Sephadex LH-20 column (80×2.5 cm, $CHCl_3$) to yield 56 mg (42%) of **5** as a colorless powder: Rf 0.50 ($CHCl_3$ - MeOH, 98:2); IR (KBr) cm^{-1} 1785, 1718, 1652; UV λ_{max}^{EtOH} nm (ε) 240 (12,300), 220 (10,500); ^1H NMR (100 MHz, $CDCl_3$) δ 2.25 (s, 3-$OCOCH_3$), 2.31 (s, 2-$NCOCH_3$), 2.84 (m, 4-H_2 and 5-H_2); MS (70 eV) m/z (relative intensity) 239 (5%, M^+), 197 (8%), 155 (46%), 113 (100%).

Deoxymanumycin (7)

In small portions 61 mg sodium borohydride were added to a stirred solution of 220 mg **1** in 50 ml dried MeOH. After 15 minutes the solution was dissolved in 150 ml water, adjusted to pH 3 with 0.5 N oxalic acid and extracted with $CHCl_3$. The organic layer was dried, evaporated and

chromatographically purified on a Sephadex LH-20 column (90×2.5 cm). The column was washed with $CHCl_3$, and **7** was eluted with MeOH. This product was further purified on a Sephadex LH-20 column (90×2.5 cm, MeOH) to yield 129 mg (60%) of a yellow amorphous powder: MP 209°C; $[\alpha]_D^{20}$ −49° (*c* 0.16, DMF); Rf 0.28 ($CHCl_3$ - MeOH, 9:1); IR (KBr) cm^{-1} 3410, 3380, 3260, 2960, 2925, 1690 (sh), 1605 (sh), 1580, 1530, 1005; UV absorption bands see Table 2; ^1H NMR (200 MHz, DMSO-d_6) δ 0.86 (t, J=6.5 Hz, 10′-H_3), 0.97 (d, J=6.5 Hz, 13′-H_3), 1.26 (br s, 7′-H_2, 8′-H_2 and 9′-H_2), 1.82 (d, J=1 Hz, 12′-H_3), 2.02 (d, J=1 Hz, 11′-H_3), 2.46 (br s, 4″-H_2, 5″-H_2 and 6′-H), 5.36 (br d, J=9.5 Hz, 5′-H), 6.38~6.54 (m, 2 protons), 6.56 (s, NH or OH), 6.78~7.04 (m, 5 protons), 7.30 (dd, J=14.5 and 15 Hz, 11-H), 7.72 (s, 3-H), 7.74 (s, NH or OH), 8.86 (br s, NH or OH), 9.68 (br s, NH or OH), 9.85 (br s, NH or OH), 14.12 (br s, 3″-OH); ^{13}C NMR (50.3 MHz, DMSO-d_6) δ 168.0 (s, C-1′), 166.2 (s, C-13), 153.6 (s, C-1), 151.1 (s, C-5), 142.9 (d, C-11), 142.7 (d, C-5′), 140.6 (d, C-9), 137.6 (d, C-3′), 132.2 (d, C-7), 130.1, 129.0, 128.9 (all s, C-2, C-2′ and C-4′), 127.9 (d, C-10), 125.0 (d, C-8), 121.9 (d, C-3), 120.0 (d, C-12), 118.9 (s, C-4), 114.6 (s, C-2″), 103.2 (d, C-6), 36.5 (t, C-7′), 32.1 (d, C-6′), 29.2 (t, C-8′), 29.0 (br t, C-4″ and C-5″), 22.1 (t, C-9′), 20.7 (q, C-13′), 16.4 (q, C-12′), 14.2 (q, C-10′), 13.7 (q, C-11′); MS (70 eV) *m/z* (relative intensity) 534 (M^+, 0.3%), 431 (1.1%), 421 (2.1%), 315 (27.8%), 217 (19.5%), 193 (53.0%), 109 (59.9%), 105 (100%).

Anal Calcd for $C_{31}H_{38}N_2O_6$: C 69.64, H 7.16, N 5.24.
Found: C 69.30, H 7.31, N 5.25.

Dideoxymanumycin (**8**)

a): 500 mg **1** and 1 g potassium iodide were dissolved in 40 ml acetic acid and stirred for 5 minutes at 60°C. The dark red solution was mixed with 100 ml MeOH and chromatographed on Dowex 1-X2, eluted with MeOH. The main yellow fraction was evaporated and chromatographed on Sephadex LH-20 (80×2.5, $CHCl_3$) to yield 43 mg (9.1%) **8** and 96 mg (19.8%) **7**.

b): 500 mg **1** were dissolved in 50 ml acetic acid, stirred at room temp after adding 1 g zinc dust for 5 hours, and then filtered. The reaction mixture was dissolved in 500 ml water and the organic layer was extracted with $CHCl_3$, dried, evaporated and purified by column chromatography on silica gel (20×4.8 cm, $CHCl_3$ - MeOH, 95:5) to yield 92.1 mg (20%) **8** and 50.8 mg (11%) **7**. Dideoxymanumycin crystallized from $CHCl_3$ forming yellow needles: MP 219°C; $[\alpha]_D^{20}$ −38° (*c* 0.15, DMF); Rf 0.55 ($CHCl_3$ - MeOH, 9:1); IR (KBr) cm^{-1} 3570 (sh), 3370, 3260, 1670 (sh), 1630 (sh), 1625, 1616, 1600 (sh); UV absorption bands see Table 2.

Anal Calcd for $C_{31}H_{38}N_2O_5$: C 71.80, H 7.39, N 5.40.
Found: C 71.06, H 7.25, N 5.10.

Dideoxymanumycin Triacetate (**9**)

3 g sodium acetate, 140 mg **8** and 30 ml acetic anhydride were stirred at room temp for 84 hours, treated for 2 hours with 3 g sodium acetate in water, and extracted with $CHCl_3$. The dried organic layer was chromatographed on a Sephadex LH-20 column (100×2.5 cm, $CHCl_3$) and recrystallized from *n*-pentane to yield 76.6 mg **9** as an amorphous powder: Rf 0.78 ($CHCl_3$ - MeOH, 9:1); IR (KBr) cm^{-1} 3360, 1770, 1720 (sh), 1700, 1655, 1580; ^1H NMR (100 MHz, $CDCl_3$) δ 0.88 (t, J=6 Hz, 10′-H_3), 0.98 (d, J=6 Hz, 13′-H_3), 1.25 (br s, 7′-H_2, 8′-H_2 and 9′-H_2), 1.83 (d, J=1 Hz, 12′-H_3), 2.09 (d, J=1 Hz, 11′-H_3), 2.19 (s, 3″-$OCOCH_3$), 2.32 (s, 1-$OCOCH_3$), 2.39 (s, $NCOCH_3$), 2.62 (s, 5″-H_2), 2.60 (br s, 6′-H), 3.06 (s, 4″-H_2), 5.35 (d, J=10 Hz, 5′-H), 6.2~7.7 (m, 10 olefinic/aromatic protons), 8.42 (s, 1-NH).

Hydrogenation of Manumycin (**1**)

1.6 g 10% palladium on charcoal were added to a stirred solution of 1.0 g **1**, dissolved in 330 ml MeOH. The hydrogenation was carried out with H_2 at 21°C and 750 Torr. After 20 minutes, the solution was filtered and evaporated to yield 840 mg of a yellow oil. This was chromatographed on a silica gel column (22×7.8 cm, eluting with $CHCl_3$ - MeOH, 95:5). Three main products, decahydrodideoxymanumycin (**10**), decahydrodeoxymanumycin (**12**) and tetradecahydromanumycin (**14**), were isolated.

Decahydrodideoxymanumycin (**10**)

First, 125 mg (13%) of colorless oily **10** were eluted from the silica gel column and crystallized after leaving at room temp for a while: MP 93~95°C; Rf see Table 1; IR (KBr) cm^{-1} 3367, 3215, 3145, 1689, 1653, 1618, 1600, 819; UV see Table 2; ^1H NMR (100 MHz, $CDCl_3$) δ 0.95 (m, 9 aliphatic protons), 1~2 (m, 23 aliphatic protons), 2.5 (m, 5 protons), 2.61 (br s, 4 protons), 6.96 (br s, 2 protons), 7.02 (br s, 1 proton), 7.82 (s, NH), 8.14 (s, NH), 8.92 (s, OH), 13.39 (s, OH); MS (70 eV) *m/z* (relative intensity) 528 (28%, M^+, high resolution calcd for $C_{31}H_{48}N_2O_5$ and found: 528.3563), 510 (64%, $C_{31}H_{46}N_2O_4$, 510.3457), 388 (9%, $C_{21}H_{28}N_2O_5$, 388.1998), 332 (100%, $C_{18}H_{24}N_2O_4$, 332.1735), 314 (12%, $C_{21}H_{32}NO$, 314.2483), 220 (8%, $C_{13}H_{18}NO_2$, 220.1337), 122 (19%, C_7H_8NO, 122.0605), 114 (13%, $C_5H_8NO_2$, 114.0554).

Decahydrodeoxymanumycin (**12**)

The second product eluting from the silica gel column was the pale yellow amorphous powder **12** (112 mg, 11%): Rf value see Table 1; IR (KBr) cm^{-1} 1718 (sh), 1690 (sh), 1658 (sh), 1639 (sh), 1615 (sh), 1610, 1540, 1514 (sh); ^1H NMR (100 MHz, $CDCl_3$) δ 0.75~1.80 (m, 30 aliphatic protons), 2.45 (m, 9 protons), 6.46 (s, 6-H), 6.76 (s, 3-H), 7.08 (br s, 5-OH), 7.81 (br s, 2-NH), 8.00 (br s, 2″-NH), 9.11 (br s, 1-OH), 13.32 (br s, 3″-OH).

Tetradecahydromanumycin (**14**)

343 mg (34%) of the yellow oily tetradecahydromanumycin were obtained after hydrogenation; this compound was the last to be eluted from the silica gel column: Rf value see Table 1; IR (KBr) cm^{-1} 1723, 1660 (sh), 1648 (sh), 1639 (sh), 1615, 1540; ^1H NMR (100 MHz, $CDCl_3$) δ 0.75~1.80 (m, aliphatic protons), 2.50 (m, 9 protons), 3.0~5.1 (m, 8 protons), 8.11 (br s, 2″-NH), 13.32 (br s, 3″-OH).

Methyl 7-[4-Hydroxy-3-(2,4,6-trimethyldecanoylamino)phenyl]heptanoate (**11**)

a): 408 mg **10** were dissolved in 80 ml 1.4 N methanolic HCl and heated at 75°C in a sealed tube. After 4 hours the reaction mixture was dissolved in 200 ml $CHCl_3$ and extracted twice with water. **5** could be isolated from the inorganic layer (see above). The organic layer was dried, evaporated and purified on a silica gel column (8×1.8 cm, $CHCl_3$) to yield 180 mg (60%) of yellow oily **11**.

b): 154 mg of **14** were treated with methanolic HCl and extracted as described above. The dried organic layer was chromatographed on a silica gel column (11×1.8 cm, $CHCl_3$) to yield 62 mg (51%) of compound **11**: Rf value see Table 1; IR (KBr) cm^{-1} 1735, 1715 (sh), 1652, 1598, 1545 (sh), 1534, 1504; UV λ_{max}^{EtOH} nm (ε) 289 (5,100), 255 (5,400), 243 (8,200); $\lambda_{max}^{EtOH-HCl}$ 298 (5,100), 255 (5,400), 243 (8,200); $\lambda_{max}^{EtOH-NaOH}$ 315 (5,100), 263 (7,300), 254 (9,000); ^1H NMR (100 MHz, $CDCl_3$) δ 0.85 (m, 9 aliphatic protons), 1.0~2.0 (m, 23 aliphatic protons), 2.28 (t, J=6.5 Hz, 12-H_2), 2.48 (br t, J=6.5 Hz, 7-H_2), 2.60 (m, 2′-H), 3.67 (s, OCH_3), 6.78~6.90 (br m, 3-H, 5-H and 6-H), 7.65 (br s, 2-NH), 8.72 (s, 1-OH); MS (70 eV) *m/z* (relative intensity) 447 (5%, M^+, high resolution calcd for $C_{27}H_{45}NO_4$ and found: 447.3344), 307 (2%, $C_{17}H_{25}NO_4$), 251 (100%, $C_{14}H_{21}NO_3$), 122 (17%, C_7H_8NO).

Methyl 7-[4,6-Dihydroxy-3-(2,4,6-trimethyldecanoylamino)phenyl]heptanoate (**13**)

70 mg **12** were treated with methanolic HCl as described above. Column chromatography on silica gel (11×1.9 cm, $CHCl_3$) yielded 22 mg (38%) **13** as a colorless powder: Rf value see Table 1; IR (KBr) cm^{-1} 1738 (sh), 1715, 1639, 1605, 1540 (sh), 1515; UV λ_{max}^{EtOH} nm (ε) 294 (5,500), 250 (7,100); $\lambda_{max}^{EtOH-HCl}$ 294 (5,500), 250 (7,100); $\lambda_{max}^{EtOH-NaOH}$ 315 (8,000), 272 (sh, 7,900), 262 (9,300); ^1H NMR (100 MHz, $CDCl_3$) δ 0.7~1.8 (m, aliphatic protons), 2.19 (t, J=7 Hz, 12-H_2), 2.46 (t, J=7 Hz, 7-H_2), 3.67 (s, OCH_3), 5.43 (br s, 5-OH), 6.42 (s, 6-H), 6.63 (s, 3-H), 7.65 (br s, 2-NH), 9.06 (br s, 1-OH); MS (70 eV) *m/z* (relative intensity) 463 (54%, M^+, calcd for $C_{27}H_{45}NO_5$ and found: 463.3297), 267 (100%, $M-C_{13}H_{24}O$), 235 (17%), 138 (29%).

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