STRUCTURE-ACTIVITY RELATIONSHIP AMONG POLYHYDRO DERIVATIVES OF TYLOSIN

AMALIA NARANDJA*, BOŽIDAR ŠUŠKOVIĆ, ŽELJKO KELNERIĆ and SLOBODAN DJOKIĆ

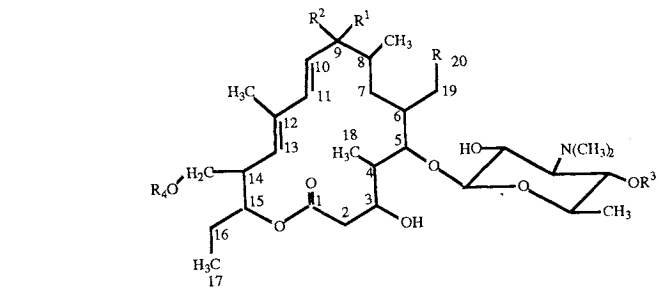
PLIVA-Pharmaceutical, Chemical, Food and Cosmetic Industry, Research Institute, Prilaz baruna Filipovića 89, 41000 Zagreb, Croatia

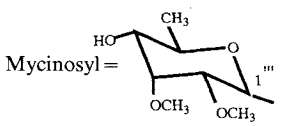
(Received for publication November 8, 1993)

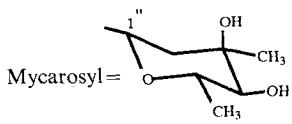
Tetra-, hexa- and octahydro derivatives of tylosin were prepared by the reduction of the conjugated double bond and carbonyl groups. Hydrogenation of the diene did not change the *in vitro* antimicrobial activity of compounds, while reduction of carbonyls causes small or complete loss of activity.

Several macrolide antibiotics related to tylosin have been reported which differ only in the degree of reduction[1~4]. Catalytic hydrogenation of the conjugated double bond and reduction of both carbonyl groups with metal hydrides has already been reported. Thus catalytic hydrogenation of 5-*O*-mycaminosyl tylonolide (OMT), protected at C-20 position by acetalation[1], and 19-deformyl-4'-demycarosyltylosin (DFDES)[2] (Fig. 1) yielded 10,11,12,13-tetrahydro derivatives thereof. The different tetrahydro derivative 9,20-dideoxo-9,20-dihydroxytylosin has been prepared by reduction of both carbonyl groups with metal hydride[3]. Depending on the reaction conditions it is possible to reduce separately keto or aldehyde group, yielding dihydro products: 9-deoxo-9-hydroxytylosin (9H-T)[1] and 20-deoxo-20-hydroxytylosin (relomycin)[4].

Fig. 1. Structure of tylosin and derivatives.



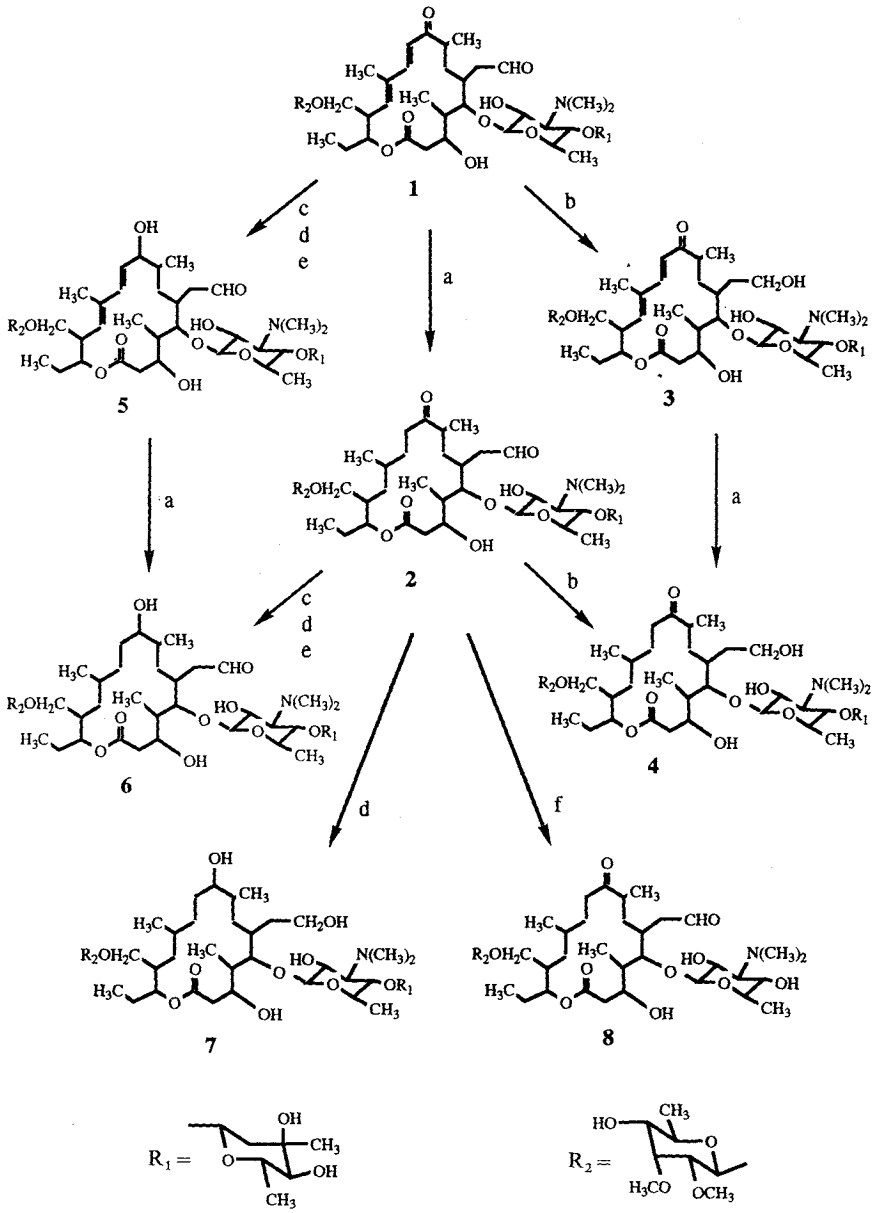




Tylosin	R=CHO	R_1, R_2 = =O	R_3=mycarosyl	R_4=mycinosyl
Relomycin	R=CH_2OH	R_1, R_2 = =O	R_3=mycarosyl	R_4=mycinosyl
9HT	R=CHO	R_1=H, R_2=OH	R_3=mycarosyl	R_4=mycinosyl
DFDES	R=H	R_1, R_2 = =O	R_3=H	R_4=mycinosyl
OMT	R=CHO	R_1, R_2 = =O	R_3=H	R_4=H

For a long time it was considered that the aldehyde group is important for antibiotic activity[4,5,10]. Recent reports suggests enhanced activity for aldehyde modified tylosin derivatives[6~8]. The keto group at C-9 does not appear to be important so long as the aldehyde group remains intact[3]. Hydrogenation of conjugated diene of related 16-membered macrolides cause no remarkable change in the activity[5,9].

Fig. 2. Synthesis scheme of polyhydro derivatives of tylosin.



a H_2/Pd, EtOH
b $NaBH_4$, phosphate buffer, MeOH
c H^+, MeOH
d $NaBH_4$, EtOH
e H^+, CH_3CN
f 0.2 N HCl

There was no data about 10,11,12,13-tetrahydrotylosin (**2**) (Fig. 2), their derivatives and antibacterial activity. It was uncertain whether the change in the aglycone moiety, the transformation of relatively rigid diene structure into more flexibile molecule, influences the antibacterial activity of 10,11,12,13-tetrahydrotylosin derivatives. In this paper we report the preparation, antimicrobial evaluation and structure-activity relationship of tetra-, hexa- and octahydro derivatives of tylosin; compounds with 10,11,12,13-tetrahydro structure.

Results and Discussion

Selective catalytic hydrogenation of the conjugated double bond of tylosin (**1**) was performed in ethanol in the presence of palladium on charcoal at a hydrogen pressure of 2～5 atm at a room temperature for 2～6 hours giving 10,11,12,13-tetrahydrotylosin (**2**) (Fig. 2). No changes at the keto or the aldehyde groups were detected. Relomycin (**3**) and 9-deoxo-9-hydroxytylosin (**5**) were hydrogenated on the same manner as tylosin with increased quantity of catalyst giving hexahydro compounds: 10,11,12,13-tetrahydrorelomycin (**4**) and 9-deoxo-9-hydroxy-10,11,12,13-tetrahydrotylosin (**6**). Hexahydro derivatives **4** and **6** were also prepared by selective reduction of the aldehyde or keto group of compound **2** by known procedure. The octahydro compound 9,20-dideoxo-9,20-dihydroxy-10,11,12,13-tetrahydrotylosin (**7**) was obtained by simultaneous reduction of both carbonyl groups of 10,11,12,13-tetrahydrotylosin (**2**).

All polyhydro derivatives of tylosin (**2**, **4**, **6**, **7**) were converted to the corresponding 4'-demycarosyl product. Thus starting from **2**, the 10,11,12,13-tetrahydrodesmycosin (**8**) was prepared.

Elucidation of Structure

The structure of tetra-, hexa- and octahydro derivatives of tylosin were assigned by UV, IR and NMR spectroscopy.

The disappearance of dienone chromophore was confirmed by UV and IR spectra. 10,11,12,13-Tetrahydro compounds have no absorption in UV spectra; in IR spectra characteristic signal at 1600 cm^{-1}

Table 1. Characteristic ^{13}C NMR chemical shifts for polyhydro derivatives of tylosin[a].

	1	**2**	**4**	**6**	**7**	**8**
C-1	173.86	172.10	172.14	172.68	172.74	172.16
C-8	44.71	42.80	42.81	39.35	39.37	42.65
C-9	203.18	214.64	215.18	76.11	76.06	214.81
C-10	118.45	34.96	34.14	32.62	32.59	35.03
C-11	148.14	30.07	30.25	30.49	30.52	30.09
C-12	134.83	29.78	30.10	29.23	29.26	29.83
C-13	142.41	40.66	39.98	41.82	41.87	40.54
C-14	45.00	39.31	39.16	39.79	39.81	39.36
C-19	43.68	45.24	33.63	45.11	33.45	45.30
C-20	203.08	202.85	60.22	202.90	60.92	203.14
C-22	12.97	20.50	20.43	19.95	19.69	20.59

[a] Chemical shifts values in δ (ppm from internal TMS); ^{13}C NMR spectra were taken in $CDCl_3$ at 300 MHz.

1 Tylosin.
2 10,11,12,13-Tetrahydrotylosin.
4 10,11,12,13-Tetrahydrorelomycin.
6 9-Deoxo-9-hydroxy-10,11,12,13-tetrahydrotylosin.
7 9-Deoxo-9-hydroxy-10,11,12,13-tetrahydrorelomycin.
8 10,11,12,13-Tetrahydrodesmycosin.

Table 2. Antimicrobial *in vitro* activity of polyhydro derivatives of tylosin.

Organism	MIC (μg/ml)					
	1	**2**	**4**	**6**	**7**	**8**
Micrococcus luteus ATCC 9341	0.2	0.2	25	0.2	25	0.39
M. luteus (4)[b]	0.39	0.39	6.25	0.39	25	0.39
M. flavus ATCC 10420	0.78	0.78	25	1.56	25	0.78
Staphylococcus aureus ATCC 6538P	0.39	0.39	12.5	0.78	25	0.78
S. epidermidis ATCC 12228	1.56	3.12	25	6.25	50	6.25
S. epidermidis 474R[a]	100	100	100	100	100	100
S. epidermidis 322 R[a]	3.12	3.12	50	3.12	50	1.56
S. aureus 500 R[a]	1.56	1.56	50	3.12	50	3.12
S. aureus 6686 R[a]	100	100	100	100	100	100
S. aureus (13)[b]	0.78	1.56	25	6.25	50	1.56
S. saprophiticus (6)[b]	1.56	1.56	50	6.25	50	3.12
Bacillus subtilis NCTC 8236	0.78	0.78	12.5	1.56	25	1.56
B. cereus ATCC 11778	0.78	1.56	6.25	3.12	12.5	1.56
B. pumilus NCTC 8241	0.78	0.39	6.25	0.78	25	0.78
Streptococcus faecalis ATCC 8043	1.56	3.12	25	6.25	50	6.25
Streptococcus A P[a]	0.39	0.39	12.5	0.78	50	0.39
Streptococcus B P[a]	0.39	0.78	25	3.12	50	3.12
Streptococcus D 8M[a]	1.56	3.12	50	6.25	50	6.25
Streptococcus G 7Z[a]	0.78	0.78	50	3.12	100	0.78
Streptococcus pneumoniae (3)[b]	0.39	0.39	100	0.39	100	0.39
S. agalactiae (2)[b]	0.39	0.78	50	1.56	50	1.56
S. suis SS-23[a]	100	100	100	100	100	100
S. suis SS-27[a]	1.56	1.56	50	3.12	50	6.25
S. suis SS-48[a]	0.78	0.78	50	1.56	50	3.12
Pasteurella haemolitica L-314	50	50	50	50	100	100
P. multocida L-315	25	25	25	25	50	12.5
P. multocida 22 B[a]	25	12.5	100	12.5	100	12.5
P. multocida S-83[a]	50	50	100	25	100	25
Escherichia coli (2)[b]	100	100	100	100	100	100.
Enterobacter aeruginosa (2)[b]	100	100	100	100	100	100

[a] Strains from PLIVA culture collection.
( )[b] Number of fresh clinical isolates.
1 Tylosin.
2 10,11,12,13-Tetrahydrotylosin.
4 10,11,12,13-Tetrahydrorelomycin.
6 9-Deoxo-9-hydroxy-10,11,12,13-tetrahydrotylosin.
7 9-Deoxo-9-hydroxy-10,11,12,13-tetrahydrorelomycin.
8 10,11,12,13-Tetrahydrodesmycosin.

is missing. In the ^{1}H NMR spectra, hydrogenation of diene causes a great upfield shifts of coressponding signals. In the ^{13}C NMR spectra there are no more signals in the range of 110~150 ppm, but four additional resonances appeared in the 29~40 ppm. Multiplicity of signals was determined by DEPT technique. The doublet at 29~30 ppm was assigned to C-12; three triplets were attributed to three methylene at C-10, C-11 and C-13 positions. The changeable signal in the range of 35 ppm for compounds **2**, **4**, **8** or 32 ppm for compounds **6** and **7** is assigned as C-10; remaining signals at 30 and 40 ppm are attributed to C-11 and C-13.

Table 3. The *in vitro* biological potency[a].

Compound	(IU/mg)
Tylosin (**1**)	983
10,11,12,13-Tetrahydrotylosin (**2**)	1,138
10,11,12,13-Tetrahydrodesmycosin (**8**)	1,118

[a] Determined on *Micrococcus luteus* ATCC 9341.

In the ^1H NMR spetra, signals at 5.5~5.7 ppm, characteristic for diene, and sharp singlet at 1.81 ppm attributed to methyl at C-12 are missing. New doublet appearing at 0.98 ppm is now attributed to C-12 methyl. The most characteristic chemical shifts in ^{13}C NMR spectra of new polyhydro tylosin derivatives are presented in the Table 1.

Structure-activity Relationship

The antibacterial activity (MIC) of tetrahydro (**2**, **8**) hexahydro (**4**, **6**) and octahydro (**7**) derivatives of tylosin was compared with that of tylosin. All compounds were tested against tylosin-sensitive and tylosin-resistant Gram-positive and Gram-negative bacteria including veterinary pathogens (Table 2).

The high activity of tetrahydro compounds **2** and **8** demonstrated that the conjugated double bond was not essential for antimicrobial activity. The same conclusions were reached previously for related 16-membered macrolides leucomycin A3 and niddamycin[3,5,9]. In fact, the activity against *Micrococcus luteus* ATCC 9341 (Table 3) showed somewhat increased activity of tetrahydro compounds **2** and **8** in comparison with tylosin.

Compound **8** showed almost the same activity as **2** indicating that the presence of mycarose in hydrogenated compounds is not essential for the activity as reported for desmycosin[10]. However there is a great difference in antimicrobial activity between two hexahydro compounds. Compound **6** showed about the same or somewhat decreased activity compared with **2**, whereas **4** possesses negligible antimicrobial activity. It is obvious that the contribution of 9-keto group in the 10,11,12,13-tetrahydro structures is not essential for antimicrobial activity. It is in agreement with tylosin and 9-hydroxytylosin relationship or related macrolides leucomycin A_3 - magnamycin B3.

The octahydro compound **7** is inactive, indicating that the reduction of both carbonyl groups results in the loss of activity.

New polyhydro compounds did not improve antimicrobial activity against some Gram-negative bacteria and some tylosin-resistant Gram-positive bacteria and they retained antimicrobial spectrum of tylosin and their derivatives, respectively.

Experimental

Physico-chemical Determination and Chromatography

^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ on JEOL 90 Q and VARIAN GEMINI 300 spectrometers. UV spectra were measured in ethanol solutions on SP8-100 PYE-UNICAM spectrometer. IR spectra were recorded in $CHCl_3$ solution on PERKIN-ELMER 256 G spectrometer.

Thin layer chromatography (TLC) was performed using E. Merck plates of Silica gel 60 with fluorescent indicator in: methylene chloride - methanol - ammonium hydroxide (90 : 9 : 1.5) (System A) and chloroform - methanol - ammonium hydroxide (60 : 1 : 0.1) (System B); visualisation was effected by spraying plates with 5% H_2SO_4 in ethanol, followed by heating at 120~140°C.

Product purification for NMR spectra was carried out by column chromatography on Silica gel 60 (70~230 mesh, E. Merck).

In Vitro Evaluation

Antibiotic susceptibility data given in Table 2 were obtained by micro dilution methodology recommended by National Committee for Clinical Laboratory Standards (NCCLS); Methods for Dilution Antimicrobial Susceptibility Test for Bacteria that Grow Aerobically (Second ed.) Document M7-A2, Vol. 10, No. 8, April 1990.

10,11,12,13-Tetrahydrotylosin (**2**)

Tylosin (10 g, 10.9 mmol) was dissolved in ethanol (200 ml), 10% palladium on charcoal (0.25 g) was

added and it was hydrogenated at a hydrogen pressure of 2 atm for 6 hours at room temperature. The completion of reaction was determined by TLC (system A). The catalyst was separated by filtration, the ethanol evaporated to yield 9.4 g (93.5%) of 10,11,12,13-tetrahydrotylosin.

Rf_A 0.39; UV no absorption.

^1H NMR (δ): 9.69 (H, s, 20-H), 3.61 (3H, s, 3'''-OCH_3), 3.50 (3H, s, 2'''-OCH_3), 2.49 (6H, s, $N(CH_3)_2$).

^{13}C NMR (δ): 214.64 (s, C-9), 202.85 (d, C-20), 172.10 (s, C-1), 103.58 (d, C-1'), 100.89 (d, C-1'''), 95.98 (d, C-1''), 40.66 (t, C-13), 34.96 (t, C-10), 30.07 (t, C-11), 29.78 (d, C-12).

10,11,12,13-Tetrahydrorelomycin (**4**)

Method A

Relomycin (4 g, 4.4 mmol) was dissolved in ethanol (100 ml), 10% palladium on charcoal (0.8 g) was added and hydrogenated at a hydrogen pressure of 5 atm for 3 hours at room temperature. Isolation was performed as described above to yield 3.7 g (79.6%) of 10,11,12,13-tetrahydrorelomycin.

Rf_A 0.42; UV no absorption.

^1H NMR (δ): 3.61 (3H, s, 3'''-OCH_3), 3.51 (3H, s, 2'''-OCH_3), 2.48 (6H, s, $N(CH_3)_2$).

^{13}C NMR (δ): 215.18 (s, C-9), 172.14 (s, C-1), 103.58 (d, C-1'), 100.88 (d, C-1'''), 95.97 (d, C-1''), 60.22 (t, C-20), 39.98 (t, C-13), 34.14 (t, C-10), 30.25 (t, C-11), 30.10 (d, C-12).

Method B

10,11,12,13-Tetrahydrotylosin (5 g, 5.4 mmol) was dissolved in the mixture of methanol (75 ml) and phosphate buffer pH 7.5 (15 ml), sodium borohydride (0.2 g, 5.4 mmol) was added and reaction mixture was stirred for 2 hours at room temperature: methanol was evaporated, water (75 ml) was added and extracted with chloroform (3 × 25 ml portions). Combined extracts were washed with brine, dried (K_2CO_3), evaporated to dryness to yield 4.1 g (82%) of the product identical with that according to process of method A.

9-Deoxo-9-hydroxy-10,11,12,13-tetrahydrotylosin (**6**)

Method A

9-Deoxo-9-hydroxytylosin (4 g, 4.4 mmol) was dissolved in ethanol (200 ml), 10% palladium on charcoal (1.0 g) was added and mixture was hydrogenated at a hydrogen pressure of 5 atm for 3 hours at room temperature. The isolation was performed on the manner as for compound **2** to yield 3.6 g (89.6%) of the 9-deoxo-9-hydroxy-10,11,12,13-tetrahydrotylosin.

Rf_A 0.37; UV no absorption.

^1H NMR (δ): 9.67 (H, s, 20-H), 3.61 (3H, s, 3'''-OCH_3), 3.51 (3H, s, 2'''-OCH_3), 2.49 (6H, s, $N(CH_3)_2$).

^{13}C NMR (δ): 202.90 (d, C-20), 172.68 (s, C-1), 103.58 (d, C-1'), 100.88 (d, C-1'''), 96.02 (d, C-1''), 76.11 (d, C-9), 41.82 (t, C-13), 32.62 (t, C-10), 30.49 (t, C-11), 29.23 (d, C-12).

Method B

10,11,12,13-Tetrahydrotylosin (5 g, 5.4 mmol) was dissolved in dry methanol (100 ml), trifluoroacetic acid (1 ml) was added. After standing 20 hours at room temperature, there was added saturated solution of sodium bicarbonate (60 ml) in order to adjust pH of the solution to 8.5, followed by evaporation of methanol and extraction with chloroform (3 × 15 ml portions). After washing with brine and drying (K_2CO_3) there was obtained 4.75 g of crude product, which after column chromatography (system B) gave 2.85 g of 10,11,12,13-tetrahydrotylosin dimethylacetal, Rf_A 0.70. So protected 10,11,12,13-tetrahydrotylosin was dissolved in dry ethanol (45 ml), sodium borohydride (0.15 g, 4 mmol) was added and stirred for 10 hours at room temperature. The mixture was reduced to 1/3 of its volume, 30 ml of water was added and then extracted with chloroform. After washing with brine, drying and evaporation, 2.3 g (80.4%) of 9-deoxo-9-hydroxy-10,11,12,13-tetrahydrotylosin dimethylacetal was obtained., Rf_A 0.60.

The 9-hydroxy compound underwent to hydrolysis of acetal group in the mixture of acetonitrile - 0.5% aqueous trifluoroacetic acid (1 : 1), (100 ml) for 2 hours at room temperature. The reaction solution was adjusted to pH 8.5 ($NaHCO_3$), extracted with chloroform to yield 1.7 g of product, which after purification on column chromatography (system A) gave 1.3 g (26%) of 9-deoxo-9-hydroxy-10,11,12,13-tetrahydrotylosin, with the same physico-chemical characteristics as the product obtained according to the

process of method A.

9-Deoxo-9-hydroxy-10,11,12,13-tetrahydrorelomycin (7)

10,11,12,13-Tetrahydrotylosin (5 g, 5.4 mmol) was dissolved in dry ethanol (100 ml), there was added sodium borohydride (0.41 g, 10.8 mmol) and was stirred 10 hours at room temperature. The reaction mixture was concentrated to 1/3 volume, water (100 ml) was added and extracted with chloroform. Combined extracts were washed with brine, dried (K_2CO_3) and evaporated to obtained 4.6 g (91%) of 9-deoxo-9-hydroxy-10,11,12,13-tetrahydrorelomycin.

Rf_A 0.35; UV no absorption.

^{1}H NMR (δ): 3.61 (3H, s, 3‴-OCH_3), 3.51 (3H, s, 2‴-OCH_3), 2.49 (6H, s, $N(CH_3)_2$).

^{13}C NMR (δ): 172.74 (s, C-1), 103.58 (d, C-1′), 100.89 (d, C-1‴), 96.22 (d, C-1″), 76.06 (d, C-9), 60.92 (t, C-20), 41.87 (t, C-13), 32.59 (t, C-10), 30.52 (t, C-11), 29.26 (d, C-12).

4′-Demycarosyl-10,11,12,13-tetrahydrotylosin (10,11,12,13-Tetrahydrodesmycosin) (8)

10,11,12,13-Tetrahydrotylosin (5 g, 5.4 mmol) was dissolved in 0.2 N HCl (100 ml). After stirring for 4 hours solution was alkalised to pH 8.5, extracted with chloroform (2 × 50 ml portions), washed with brine, dried and evaporated giving 3.9 g (92%) of 10,11,12,13-tetrahydrodesmycosin.

Rf_A 0.31; UV no absorption.

^{1}H NMR (δ): 9.67 (H, s, C-20), 3.61 (3H, s, 3‴-OCH_3), 3.51 (3H, s, 2‴-OCH_3), 2.48 (6H, s, $N(CH_3)_2$).

^{13}C NMR (δ): 214.81 (s, C-9), 203.14 (d, C-20), 172.16 (s, C-1), 103.55 (d, C-1′), 100.89 (d, C-1‴), 70.22 (d, C-4′), 40.54 (t, C-13), 35.03 (t, C-10), 30.09 (t, C-11), 29.83 (d, C-12).

Acknowledgments

We acknowledge to Dr. T. TAMBIĆ, General Hospital "Sv. Duh" Zagreb for providing the *in vitro* data of clinical isolates.

This work was supported in part by Grant-in-Aid from Ministry of Science, Technology and Informatics of Republic Croatia (1-07-035).

References

1) ŌMURA, S.; A. NAKAGAWA, M. MACHIDA & H. IMAI: Evidence for configurational identity between leucomycin and tylosin. Tetrahedron Lett. 1977: 1045~1048, 1977
2) SAKAKIBARA, H.; T. FUJIWARA, O. OKEGAWA, E. HONDA, S. WATANABE & T. MATSUDA: Deformyltylosin derivatives. U.S. 4.345.069 Aug. 17, 1982
3) ŌMURA, S.; M. TISHLER, A. NAKAGAWA, Y. HIRONAKA & T. HATA: Relationship of structures and microbiological activities of the 16-membered macrolides. J. Med. Chem. 15: 1011~1015, 1972
4) WHALEY, H. A.; E. L. PATTERSON, A. C. DORNBUSH, E. J. BACKUS & N. BOHONOS: Isolation and characterisation of relomycin a new antibiotic. Antimicrob. Agents Chemother. -1963: 45~48, 1964
5) ŌMURA, S.; M. KATAGIRI, I. UMEZAWA, K. KOMIYAMA, T. MAEKAWA, K. SEKIKAWA, A. MATSUMAE & T. HATA: Structure-biological activities relationships among leucomycins and their derivatives. J. Antibiotics 21: 532~538, 1968
6) KIRST, H. A.; J. E. TOTH, M. DEBONO, K. E. WILLARD, B. A. TREUDELL, J. L. OTT, F. T. COUNTER, A. M. FELTY & R. S. PEKAREK: Synthesis and evaluation of tylosin related macrolides at the aldehyde function: A new series of orally effective antibiotics. J. Med. Chem. 31: 1631~1641, 1988
7) FUJIWARA, T.; H. WATANABE, Y. KOGAMI, Y. SHIRITANI & H. SAKAKIBARA: 19-Deformyl-4′-deoxy desmycosin (TMC-016): Synthesis and biological properties of a unique 16-membered macrolide antibiotic. J. Antibiotics 42: 903~912, 1989
8) KIRST, H. A.; K. E. WILLARD, M. DEBONO, J. E. TOTH, B. A. TRUEDELL, J. P. LEEDS, J. L. OTT, A. M. FELTY-DUCKWORTH & F. T. COUNTER: Structure-activity studies of 20-deoxo-20-amino derivatives of tylosin related macrolides. J. Antibiotics 42: 1673~1683, 1989
9) MARING, C. J.; L. A. FREIBERG, P. A. LARTEY, D. J. GRAMPOVNIK, C. M. EDWARDS, D. J. HARDY, J. S. WANSON & P. B. FERNANDES: Synthesis and antimicrobial activities of 9(*S*)-*N*,*N*-dimethylamino-9-deoxo-10,11,12,13-tetrahydroniddamycin. J. Antibiotics 44: 448~450, 1991
10) MATSUBARA, H.; K. MIYANO, A. NAKAGAWA & S. ŌMURA: Chemical transformation of tylosin, a 16-membered macrolide and its structure-activity relationship. Chem. Pharm. Bull. 30: 97~110, 1982