10,11,12,13-Tetrahydro Derivatives of Tylosin

II. Synthesis, Antibacterial Activity and Tissue Distribution of 4′-Deoxy-10,11,12,13-tetrahydrodesmycosin

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4′-Deoxy-10,11,12,13-tetrahydrodesmycosin was prepared in six-step reactions. Antibacterial screening shows retained antibacterial spectrum of tylosin with some improvement against tylosin-sensitive *Staphylococci* and *Haemophilus influenze*. However, pharmacokinetic data demonstrated rapid distribution from blood in tissues and prolonged maintenance in all tissues, especially in the lungs, in comparison with tylosin.

10,11,12,13-Tetrahydrodesmycosin, a 16-membered macrolide antibiotic is obtained by selective catalytic hydrogenation of desmycosin in the C-10, C-11, C-12, C-13 position, or by mild acid hydrolysis of previously prepared 10,11,12,13-tetrahydrotylosin[1].

16-Membered macrolides: rosamycin[2] and mycinamicins[3] with desosamine (4′-deoxy-mycaminose) in the C-5 position instead of mycaminose, were found to be active against some strains of Gram-negative and macrolide resistent Gram-positive bacteria.

Deoxygenation of C-4′ hydroxyl group of desmycosin[4], 19-deformyl-desmycosin[5] or related 16-membered macrolide, neospiramycin[6], has already been accomplished. In preceding papers[4,5] it was shown that the 4′-deoxy derivatives of desmycosin exhibit enhanced activity in comparison to those of corresponding 4′-hydroxy compounds. Contrary to expectation C-4′ deoxygenation of neospiramycin do not contribute any enhancement in activity, however isomerisation of diene influenced on increasing of activity. Pharmacokinetic studies[7] of 4′-deoxy-19-deformyl-desmycosin show rapid distribution and prolonged maintenance in all tissues in comparison with 19-deformyl-desmycosin.

Our intention was to examine whether C-4′ deoxygenation of 10,11,12,13-tetrahydrodesmycosin, a compound with the flexible aglycon obtained through hydrogenation of diene, influences the antibacterial activity and pharmacokinetic behaviour.

In this report we wish to describe the synthesis, antibacterial activity and pharmacokinetic properties of 4′-deoxy-10,11,12,13-tetrahydrodesmycosin.

Results and Discussion

Synthesis of 4′-Deoxy-10,11,12,13-tetrahydrodesmycosin

For the purpose of C-4′ deoxygenation we started from 10,11,12,13-tetrahydrodesmycosin (**1**). At the first step the aldehyde group was protected by acetalation (i)[4], protection of concurrent hydroxyl groups at C-3, C-2′, C-4″ was performed by silylation (ii), C-4′ was activated by sulfonation (iii) followed by displacement of sulfonyloxy group with iodine (iv)[5]. After hydrolysis of the protecting groups (v), reductive deiodination (vi) was performed catalytically with palladium on charcoal[8] (Fig. 1). The synthesis of novel 10,11,12,13-tetrahydrodesmycosin derivatives **2**～**7** was followed by TLC on silica-gel and ^{1}H NMR spectra. Structure of the compounds **2** and **7** was confirmed by ^{13}C NMR spectra (Table 1).

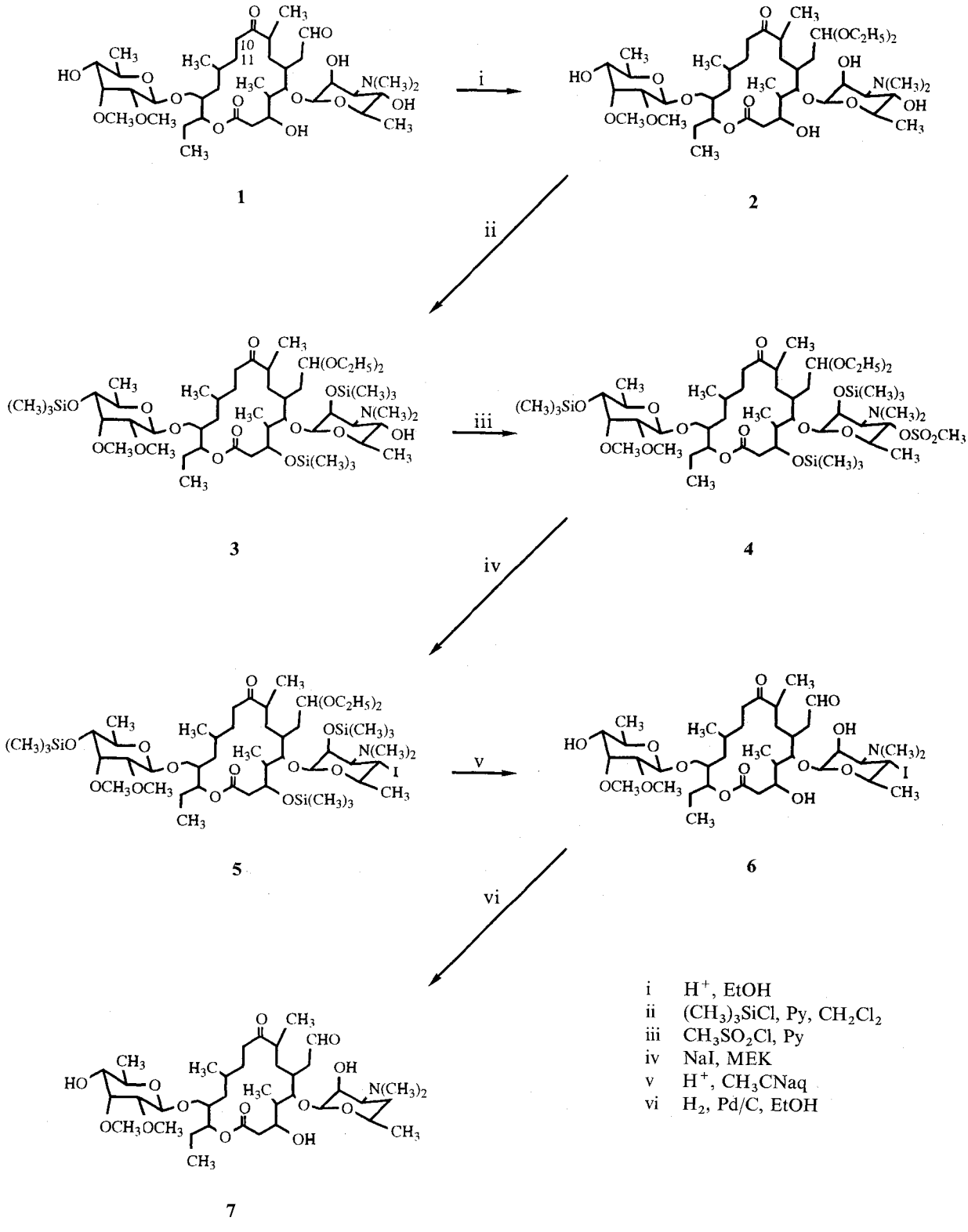
In Vitro Activity

The antimicrobial activity of 4′-deoxy-10,11,12,13-tetrahydrodesmycosin (**7**) was compared with that of 10,11,12,13-tetrahydrodesmycosin (**1**) and desmycosin. As shown in the Table 2 among macrolides tested, compound **7** showed the increased activity against tylosin-sensitive *Staphylococci*, but against tylosin-resistant *Staphylococci* and *Streptococci* there was no improvement. A significant improvement is shown against *Haemophilus influenze*, *Branhamella catarrhalis* and *Corynebacterium pyogenes*. There was no improvement against *Pasteurellas and compound* **7** is ineffective against Gram-negative bacteria such are: *Salmonella*, *Shigella* and *Escherichia*.

Tissues Distribution

Single iv doses (30 mg/kg) of the drugs (tylosin, **1** and **7**)

Fig. 1. Synthesis scheme of 4′-deoxy-10,11,12,13-tetrahydrodesmycosin.



were administered to rats for pharmacokinetic investigations. The blood and tissue samples were taken at 0.25, 0.30, 1, 2, 4, 6, 10 and 12 hours after iv application of the drug. Five rats were used at each time points. The tissue and plasma concentrations of the drug were determined by bioassay and the results are shown in Figs. 2 and 3. The plasma concentrations of **1** and **7** at the first point (15 minutes) were very low (3 mcg/ml) or undetectable, respectively. Tissues samples show rapid distribution of compound **7** to all tissues and an excellent penetration in the liver, kidney, spleen and lungs. The concentration of **7** in the kidney, spleen and liver (Fig. 2) is greater than those of tylosin, especially in the spleen. The concentration of **1** and **7** in the lungs (Fig. 3) are

Table 1. The ^{13}C NMR chemical shifts of **2** and **7** in $CDCl_3$.

Carbon	2	7	Carbon	2	7
1	172.52 s	172.47	20-O-CH_2-CH_3	61.80 t	
2	39.36 t	39.28		60.62 t	
3	71.75 d	71.61	20-O-CH_2-CH_3	15.35 q	
4	39.43 d	39.97	21	17.75 q	15.59
5	83.77 d	85.15	22	20.56 q	20.83
6	33.27 d	33.25	23	69.93 t	69.75
7	30.12 t	30.14			
8	42.76 d	41.89	1′	105.31 d	105.21
9	215.07 s	214.79	2′	70.73 d	70.51
10	35.13 t	35.11	3′	70.27 d	65.44
11	30.11 t	30.14	4′	70.73 d	28.27 t
12	29.95 d	29.97	5′	73.32 d	69.49
13	40.54 t	40.58	6′	17.79 q	21.17
14	39.41 d	39.39	$N(CH_3)_2$	41.72 q	40.21
15	76.21 d	75.67			
16	23.28 t	22.93	1″	100.62 d	100.68
17	10.34 q	10.69	2″	81.91 d	81.96
18	8.10 q	7.62	3″	79.56 d	79.60
19	33.10 t	45.25	4″	72.72 d	72.73
20	102.31 d	202.99	5″	70.37 d	69.68
			6″	17.75 q	17.76
			2″ OCH_3	59.31 q	59.34
			3″ OCH_3	61.63 q	61.70

^{13}C NMR spectra were taken at 300 MHz; chemical shifts values in d (ppm from internal TMS).

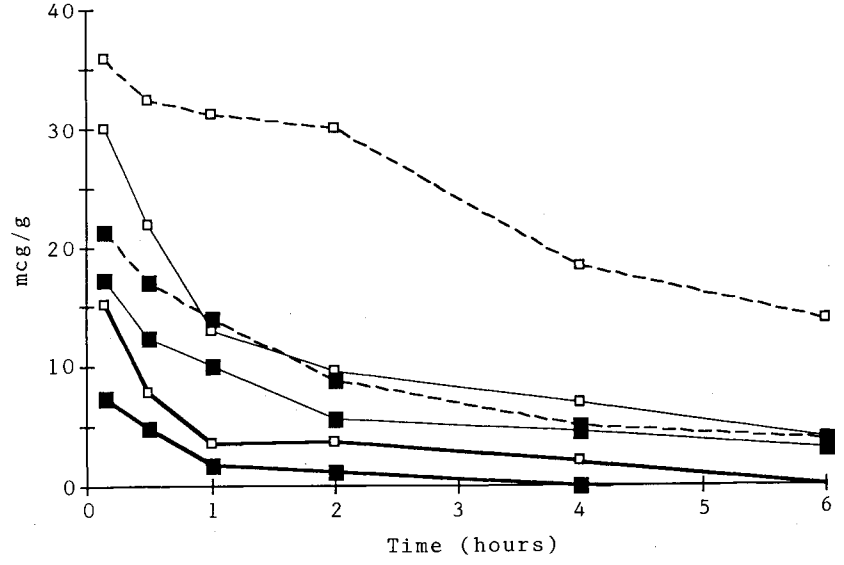
Table 2. Antimicrobial activity of 4′-deoxy-10,11,12,13,-tetrahydrodesmycosin (**7**) compared with that of 10,11,12,13-tetrahydrodesmycosin (**1**) and desmycosin.

Test organism	MIC (mcg/ml)		
	7	1	Desmycosin
Micrococcus luteus ATCC 9341	0.2	0.39	0.39
M. luteus (4)[a]	0.2	0.39	0.39
M. flavus ATCC 10420	0.78	0.78	1.56
Staphylococcus aureus ATCC 6538 P	0.2	0.78	0.78
S. aureus (13)[a]	0.78	1.56	0.78
S. aureus 6686[a]	100	100	100
S. epidermidis ATCC 12228	3.12	6.25	3.12
S. epidermidis 474 R[b]	100	100	100
Streptococcus faecalis ATCC 8043	6.25	6.25	3.12
S. pneumoniae (4)[a]	0.39	0.39	0.78
Streptococcus A (2)[a]	0.39	0.39	0.78
Streptococcus B (5)[a]	1.56	3.12	1.56
Bacillus subtilis NCTC 8236	1.56	1.56	0.78
B. cereus ATCC 11778	0.78	1.56	1.56
Pasteurella haemolitica L-314	25	25	25
P. multocida L-315	12.5	12.5	12.5
Haemophilus influenze (5)[a]	0.39	0.78	1.56
Corynebacterium pyogenes (1)[a]	0.39	0.78	1.56
Branhamella catarrhalis (4)[a]	0.1	0.2	0.39
Brucella abortus VB[b]	1.56	3.12	1.56
B. suis VB[b]	12.5	25	6.25
B. melitensis VB[b]	12.5	12.5	12.5
Escherichia coli ATCC 10596	100	100	100
Shigella sonnei 34 Z[b]	100	100	100
Salmonella enteritidis 5 Z	100	100	100
Klebsiella pneumoniae P[b]	100	100	100

( )[a] Number of clinical isolates.
[b] Strains from PLIVA culture collection.

Fig. 2. Tissue distribution[a] of 4′-deoxy-10,11,12,13-tetrahydrodesmycosin (7) in comparison with tylosin.

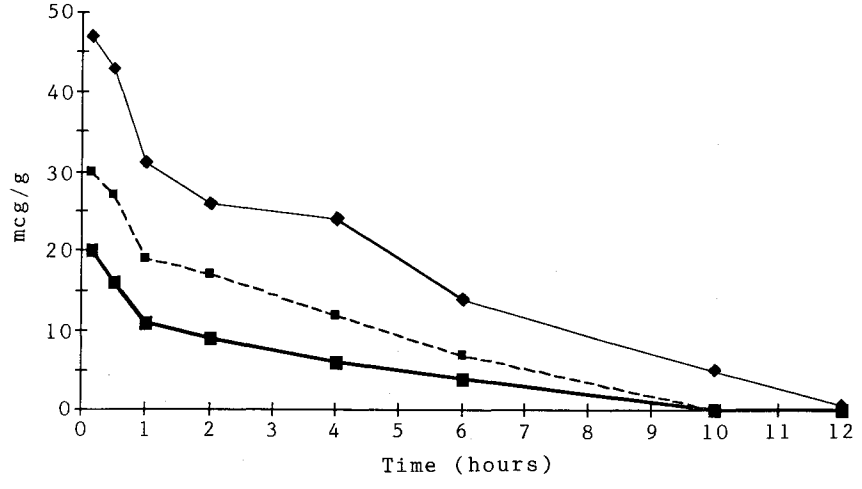
—■— Liver, tylosin; —□— liver, 7; —■— kidney, tylosin; —□— kidney, 7; --■-- spleen, tylosin; --□-- spleen, 7.



[a] Bioassay *Micrococcus luteus* ATCC 9341.

Fig. 3. The concentration[a] of 4′-deoxy-10,11,12,13-tetrahydrodesmycosin (7) in lungs in comparison with 10,11,12,13-tetrahydrodesmycosin (1) and tylosin.

—■— Tylosin, --■-- 1, —◆— 7.



[a] Bioassay *Micrococcus luteus* ATCC 9341.

about 50～150% greater than those of tylosin. The high concentration of **7** in lung tissue is maintened until the fourth hour, it decreases in the sixth and still is detectable in the twelfth hour. The elimination from the liver and kidney is rather fast ($T_{1/2}$ 30 and 45 minutes respectively), whereas in the spleen and especially in the lungs $T_{1/2}$ is prolonged (4 hours).

As 4′-deoxy-desmycosin[7] is rapidly distributed into all tissues after iv administration and its elimination is faster than that of compounds **1** and **7**, our pharmacokinetic data suggest that not only the C-4′ deoxygenation, but also hydrogenation of diene influences on the prolonged maintenance of **7** in tissues.

Experimental

Physico-chemical Determination and Chromatography

^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ on JEOL 90Q and VARIAN GEMINI 300 spectrometers. 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 toluene-acetone (4 : 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 coloumn 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 Tests for Bacteria that grow Aerobically (Second ed.) Document M7-A2 Vol. 10, No. 8, April 1990.

Pharmacokinetic Investigation

A solution (3 mg/ml) of each drug for iv infusion was obtained by dissolving 30 mg of the drug as the tartarate salt in 10 ml of sterile water. Single iv doses (30 mg/kg) were administered to Fisher male rats (260~310 g). The bioactivity in the samples of blood and tissues was determined by an agar-well method using *Micrococcus luteus* ATCC 9341 as the test organism.

10,11,12,13-Tetrahydrodesmycosin Diethylacetal (**2**)

10,11,12,13-Tetrahydrodesmycosin (50 g, 64.4 mmol) was dissolved in ethanol (500 ml), *p*-toluensulfonic acid monohydrate (12.5 g, 65 mmol) was added thereto. Upon stirring for 2 hours at room temperature, triethylamine (6 ml) was added; ethanol was evaporated at reduced pressure to one quarter of the volume, then there was added a saturated solution of sodium bicarbonate (700 ml) and it was extracted with chloroform (2×100 ml portions). Combined extracts were dried (K_2CO_3) and evaporated to dryness to yield 50.9 g (93%) of 10,11,12,13-tetrahydrodesmycosin diethylacetal.

Rf_A 0.30. ^1H NMR (δ): 3.61 (3H, 3″-OCH_3), 3.56 (2H, 20-OCH_2–), 3.50 (3H, 2″-OCH_3), 3.45 (2H, 20-OCH_2–), 2.49 (6H, $N(CH_3)_2$).

3,2′,4″-Tri-*O*-trimethylsilyl-10,11,12,13-tetrahydrodesmycosin Diethylacetal (**3**)

The compound **2** (10 g, 11.7 mmol) was dissolved in dry methylene chloride (200 ml) and pyridine (7.8 ml, 96.6 mmol). The solution was cooled to 0°C and chlorotrimethylsilane (9 ml, 71.2 mmol) was added dropwise. Upon stirring for 2 hours at 5°C it was poured into ice-water (400 ml), adjusted to pH 9 and extracted with chloroform (2×100 ml). Extracts were washed with brine, dried (K_2CO_3) and evaporated to dryness to yield 12.0 g (96%) of 3,2′,4″-tri-*O*-trimethylsilyl-10,11,12,13-tetrahydrodesmycosin diethylacetal.

Rf_A 0.75. ^1H NMR (δ): 3.59 (5H, 3″-OCH_3, 20-OCH_2–), 3.51 (5H, 2″-OCH_3, 20-OCH_2–), 2.52 (6H, $N(CH_3)_2$), 0.17 (27H, 3×$Si(CH_3)_3$)

4′-Methanesulfonyl-3,2′,4″-tri-*O*-trimethylsilyl-10,11,12,13-tetrahydrodesmycosin Diethylacetal (**4**)

The compound **3** (12 g, 11.25 mmol) was dissolved in pyridine (100 ml), into the cooled solution methanesulfonyl chloride (5.2 ml, 67 mmol) was added and it was kept stirring under cooling for 4 hours. The reaction solution was poured into ice-water (1,500 ml) and adjusted to pH 9. After 30 minutes the precipitate was separated by filtration, immediately dissolved in chloroform (100 ml), washed with brine, dried (K_2CO_3) and evaporated to dryness to yield 12.1 g (94%) of 4′-methanesulfonyl-3,2′,4″-tri-*O*-trimethylsilyl-10,11,12,13-tetrahydrodesmycosin diethylacetal.

Rf_A 0.90 ^1H NMR (δ): 3.59 (5H, 3″-OCH_3, 20-OCH_2–), 3.51 (5H, 2″-OCH_3, 20-OCH_2–), 3.15 (3H, –SO_2–CH_3), 2.54 (3H, N–CH_3), 2.49 (3H, N–CH_3), 0.16 (27H, 3×$Si(CH_3)_3$)

4′-Deoxy-4′-iodo-3,2′,4″-tri-*O*-trimethylsilyl-10,11,12,13-tetrahydrodesmycosin Diethylacetal (**5**)

The compound **4** (12 g, 10.5 mmol) was dissolved in methylethylketone (120 ml), sodium iodide (7.8 g, 52 mmol) was added and it was heated under mild reflux for 1 hour. The solvent was evaporated, chloroform (100 ml) and water (200 ml) were added, adjusted to pH 9 and the layers were separated. The organic layer was washed with 10% solution of sodium thiosulfate (3×100 ml), dried (K_2CO_3) and evaporated to dryness to yield 11.22 g (90.9%) of 4′-deoxy-4′-iodo-3,2′,4″-tri-*O*-trimethylsilyl-10,11,12,13-tetrahydrodesmycosin diethylacetal.

Rf_A 0.95, Rf_B 0.85. ^1H NMR (δ): 3.39 (5H, 3″-OCH_3, 20-OCH_2–), 3.50 (5H, 2″-OCH_3, 20-OCH_2–), 2.54 (3H, N–CH_3), 2.49 (3H, N–CH_3), 0.16 (27H, 3×$Si(CH_3)_3$)

4′-Deoxy-4′-iodo-10,11,12,13-tetrahydrodesmycosin (**6**)

The compound **5** (11 g, 9.3 mmol) was dissolved in mixture of acetonitrile-0.2 N HCl (200 ml) (1:1) and stirred at room temperature for 2 hours. Upon addition of solid sodium bicarbonate up to pH 9, it was extracted with chloroform (2×60 ml), washed with saturated solution of sodium bicarbonate and evaporated to dryness. The crude product (7.3 g) was purified on silica-gel in solvent system A. Evaporation of fractions Rf_B 0.33 yielded 3.44 (41.5%) of 4′-deoxy-4′-iodo-10,11,12,13-tetrahydrodesmycosin.

Rf_A 0.85, Rf_B 0.33. ^1H NMR (δ): 9.67 (1H, CHO), 3.61 (3H, 3″-OCH_3), 3.49 (3H, 2″-OCH_3), 2.58 (3H, N–CH_3), 2.56 (3H, N–CH_3).

4′-Deoxy-10,11,12,13-tetrahydrodesmycosin (**7**)

The compound **6** (3 g, 3.4 mmol) was dissolved in dry ethanol (150 ml). Upon addition of 10% Pd on charcoal (0.6 g), it was hydrogenated for 2 hours at 2 atm. The catalyst was separated, ethanol evaporated, the crude product dissolved in chloroform (50 ml), washed with saturated solution of sodium bicarbonate (2×100 ml), dried (K_2CO_3) and evaporated at reduced pressure to yield 2.1 g (81.7%) of 4′-deoxy-10,11,12,13-tetrahydrodesmycosin.

Rf_A 0.35 ^1H NMR (δ): 9.67 (1H, CHO), 3.62 (3H, 3″-OCH_3), 3.50 (3H, 2″-OCH_3), 2.26 (6H, $N(CH_3)_2$)

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