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New Derivatives of Tylosin: Chemical and Electrochemical Oxidation Products of Desmycosin

Z. MANDIĆ*, A. NARANĐA, P. NOVAK, K. BRAJŠA and M. ĐEREK

PLIVA Research Division, Pharm. Ind. Inc.,
Prilaz b. Filipovića 25, HR-10000 Zagreb, Croatia

D. IVEKOVIĆ

Department of Chemistry and Biochemistry, Faculty of Food Technology and Biotechnology, University of Zagreb,
HR-10000 Zagreb, Croatia

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Chemical and electrochemical oxidation of dienonic moiety of desmycosin were carried out. Successive chemical oxidation of desmycosin with *m*-chloroperbenzoic acid afforded a family of 12,13-dihydro-12,13-dihydroxy derivatives. Indirect electrochemical oxidation *via* hypobromite as an intermediate gave rise to the new bicyclo derivative of desmycosin, 13-hydroxy-3-dehydroxy-3,12-oxa-desmycosin.

The dienonic moiety of naturally occurring 16-membered macrolide compounds is a convenient target for the chemical modifications hopefully leading to a novel synthetic antibiotics. There have been several attempts to accomplish a desired chemical modifications on this moiety.

The family of macrolides with an epoxyenone partial structure in the macrolactone was enlarged by synthetically prepared 12,13-epoxy derivatives.[1,2] The attempts to cleave the oxirane ring of various epoxyenones resulted in different products depending on the method used. Reductive opening of the oxirane ring of maridomycin II by catalytic hydrogenation was accomplished together with the reduction of the C_{10}–C_{11} double bond giving 13-hydroxy-10,11,12,13-tetrahydro compound.[3] The cleavage of the oxirane ring of rosamycin by catalytic hydrogenation gave, contrary to maridomycin II, only 10,11-dihydro derivative with preserved 12,13-epoxy structure.[4] Reductive opening of the oxirane of naturally occurring macrolides such as deltamycin or angolamycin was performed by microbial deepoxidation[5] and with dissolving metals[6] giving enol type of derivatives at the C-11, C-12 position, which spontaneously were converted to geometric isomers. The oxirane ring in desmycosin was cleaved by dissolved metals[7] and by electrochemical reduction[8] in both cases leading to 10,13-dihydro-13-hydroxy-desmycosin.

Apart from expoxydation of C_{12}–C_{13} double bond by *m*-chloroperbenzoic acid[1,2] there has been little attempt to oxidize the dienonic moiety by other methods or to obtain higher oxidation states from the epoxy derivatives itself.

In the case of tylosin and its derivatives, various reductions of the conjugated double bond at position C-10 to C-13, ketone at position C-9, and aldehyde at position C-20 have been described in the literature[9~12]. Reduction of both carbonyl groups can be performed by metal hydride and 9,20-dideoxo-9,20-dihydroxytylosin has been prepared in this way[9]. Depending on the reaction conditions it is possible to reduce separately keto or aldehyde group, yielding 9-deoxo-9-hydroxytylosin[10] and 20-deoxo-20-hydroxytylosin (relomycin)[11]. Tetrahydro derivative of tylosin, 10,11,12,13-tetrahydrotylosin, can be prepared by selective catalytic hydrogenation of the conjugated double bond in the presence of palladium[12].

In contrast to the wide variety of chemical methods employed for structural modifications of macrolide antibiotics, electrochemical methods are rarely used to

* Corresponding author: Zoran.Mandic@pliva.hr

perform desired synthetic tasks. In our previous paper[13] we described the electrochemical reduction of desmycosin. The electrochemical reduction results in 10,11-dihydro-desmycosin and symmetric dimer at position C-13.

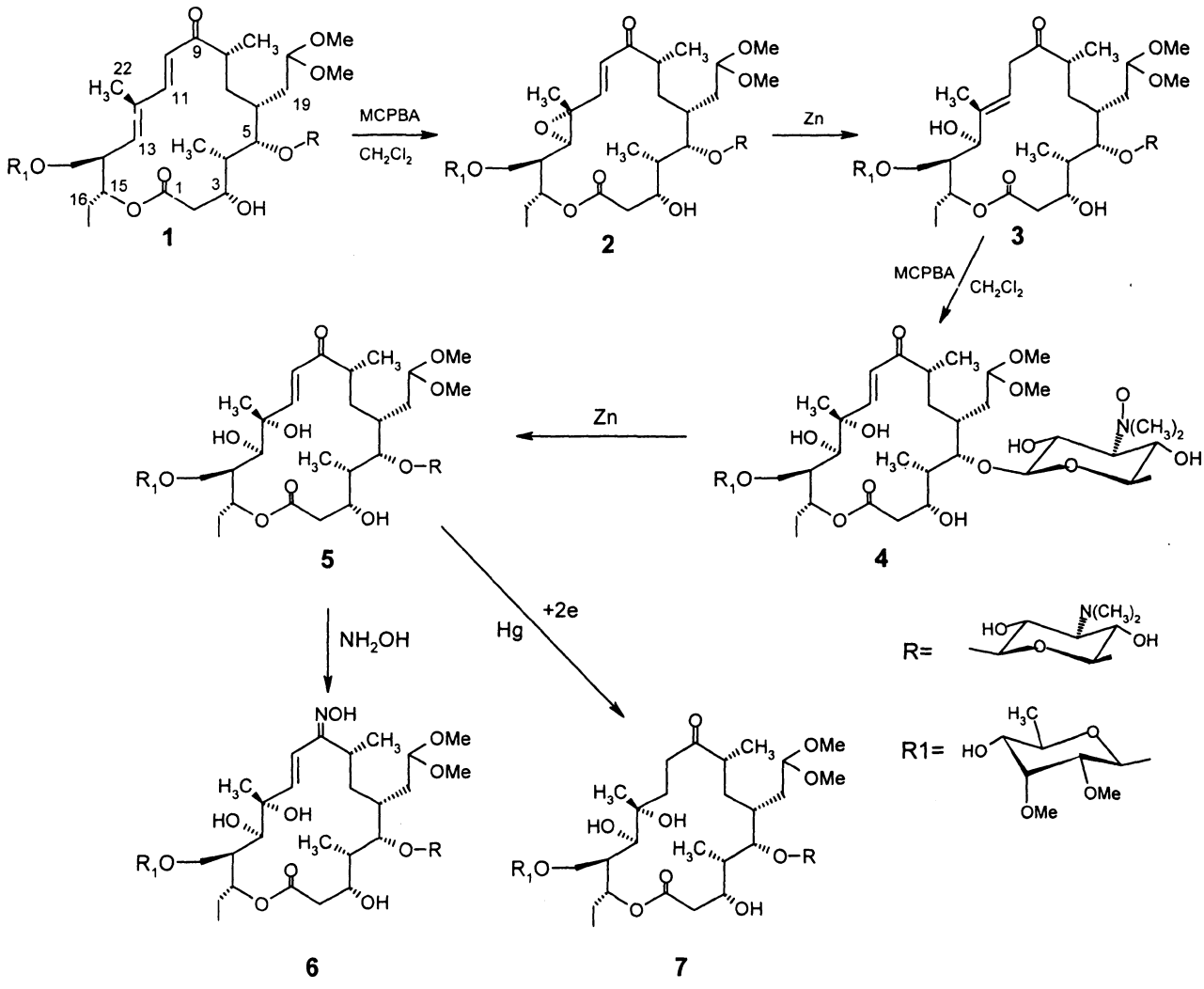
We herein report our investigations on further chemical and electrochemical oxidations of desmycosin and 10,13-dihydro-13-hydroxy-desmycosin, the structural and antimicrobial evaluation of the resulting products and structure-activity relationship.

Results and Discussion

Chemical oxidation of desmycosin dimethylacetal (**1**) resulting in 12,13-epoxydesmycosin dimethylacetal (**2**) was carried out according to the known procedures.[7] The values of proton–proton coupling constants, $J_{13,14}$ (9.7 Hz) and $J_{14,15}$ (9.9 Hz), indicated *trans* configuration of H-13 and H-14. Reductive cleavage of the oxirane ring was performed with Zn powder yielding 10,13-dihydro-13-hydroxy desmycosin (**3**). The ^{13}C-NMR spectrum of **3** with a singlet at 211.4 ppm, attributed to C-9 with an adjacent methylene group, a new doublet at 76.5 ppm attributed to C-13 prove the cleavage of oxirane ring. A singlet at 139.7 ppm and doublet at 117.3, attributed to the C_{11}–C_{12} double bond, confirmed that cleavage of the oxirane ring had occurred with an allylic rearrangement.

Further chemical oxidation of **3** with *m*-chloroperbenzoic acid in methylene chloride afforded 12,13-dihydro-12,13-dihydroxy desmycosin 3′-*N*-oxide (**4**). The latter was converted into the free base (**5**) by selective reduction with Zn powder at pH=5. Molecular ion peaks (*m/z* 868 and *m/z* 852 respectively), downfield shifts of H-10 (δ 6.42) and H-11 (δ 6.80) in ^{1}H-NMR spectra and C-12 appearing as

Fig. 1. The chemical pathway for the synthesis of 12,13-dihydroxy derivatives of desmycosin.



a singlet at 76.2 ppm in ^{13}C-NMR spectra confirmed the proposed structure of **4** and **5** as given in Figure 1.

Oximation of **5** gave a mixture of *E*- and *Z*-oximes (**6**) in good yield and their structures were elucidated on the basis of its MS, ^{1}H-NMR and ^{13}C-NMR spectra. The increase of molecular ion peak by 15 amu in comparison to that of the parent ketone is in agreement with the replacement of the C-9 keto group with a hydroxyimino one. In the ^{1}H NMR (DMSO-d_6) spectrum compound **6** showed N–OH absorption exchangeable with D_2O at 10.77 and 10.49 ppm.

10,11,12,13-Tetrahydro-12,13-dihydroxy desmycosin (**7**) was prepared by electrochemical reduction of **5** at mercury electrode. The disappearance of enone absorption at 234 nm in the UV spectrum indicated reduction of the C_{10}–C_{11} double bond. Further evidence was obtained by NMR and mass spectra. In the ^{13}C-NMR spectra C-9 shifted downfield to 212.4 ppm while C-10 and C-11 shifted upfield which confirmed hydrogenation of C_{10}–C_{11} double bond. Molecular ion peak at *m/z* 854 confirmed the addition of 1 mol of hydrogen. The same tetrahydro derivative was prepared also by catalytic hydrogenation at palladium on charcoal in excellent yield.

Alternative route for the oxidative functionalization of olefins involves electrochemical generation of halonium ions capable of forming a halohydrin or cyclic halonium intermediate. Cyclic halonium intermediate is a very reactive species that reacts with a suitable nucleophile to give a halofunctionalized or bifunctionalized derivatives of olefin.

Thus, electrochemical oxidation of **1** was carried out in aqueous solution of KBr at pH=7 at Pt electrode with constant current density of 1 mA/cm^2. After the passage of 4 Faradays two products, **8** and **9**, were isolated (Figure 2) with molecular ion peaks *m/z* (MH^+) at 834 and 820, respectively. The fragmentation of the obtained mass spectra for these two compounds as well as integration of methyl protons at 3′-*N* in ^{1}H-NMR spectra show that one and two methyl groups are attached to this nitrogen atom in **9** and **8**, respectively. This proves that the electrochemical oxidation was accompanied by demethylation of tertiary 3′-amino group. ^{1}H and ^{13}C chemical shifts observed for other atoms in **8** and **9** were very similar reflecting the same structure of the rest of the molecules.

Since anodic oxidation of alkenes with bromide ions as mediators might give bromohydrin as intermediate which is a subject to the nucleophilic attack, 12,13-dihydroxy compound (**5**) might be expected as a result of the electrochemical oxidation after the substitution of bromine with hydroxide. However, the difference of molecular ion peaks between **5** and **8** is 18 indicating that **8** was formed after the loss of water from **5**.

Table 1. The ^{13}C-NMR chemical shifts of **5** and **9** in comparison to starting compound (**1**)*.

C	1	5	9
1	173.5	172.8	171.0
2	39.2	40.4	41.0
3	67.2	74.1	70.6
4	40.2	35.8	39.8
5	81.1	87.0	81.7
6	31.8	37.4	34.8
7	32.5	34.5	35.1
8	44.6	42.2	41.8
9	203.1	204.3	204.0
10	118.5	127.9	124.4
11	148.1	148.8	146.6
12	134.9	76.2	84.9
13	142.3	75.5	81.1
14	45.0	42.5	46.4
15	75.1	77.1	80.7
16	25.3	22.3	27.5
17	9.6	9.9	9.6
18	9.0	6.5	8.1
19	44.7	32.1	32.6
20	202.9	104.8	103.1
21	17.3	14.7	16.8
22	12.9	23.7	26.9
23	69.0	66.6	66.1

* There are no significant changes in chemical shifts of sugar moieties, except for the C-3′ in **9** where demethylation of nitrogen induces ^{13}C upfield chemical shift from 70.1 ppm to 64.8 ppm. Chemical shifts of the compound **8** resemble those of compound **9**.

Having this information, a comparison of their ^{1}H and ^{13}C NMR chemical shift values with those obtained for 12,13-dihidroxy derivative (**5**), pointed towards bridged structures of these two compounds (Table 1, Figure 2). Namely, the largest change in C-13 chemical shifts were observed for atoms C-12 (8.7 ppm), C-13 (5.6 ppm), C-3 (3.5 ppm) and C-5 (5.3 ppm). 2D ROESY spectra provided further insight into the structures of **8** and **9**. Among others, particularly interested ROE contacts were those involving atoms H-3, H-11, H-13 and H-1′. The bridging between C-12 and C-3 *via* ether linkage should impose significant conformational changes in the lower macrocyclic region and hence affect proton–proton spatial proxymities. Indeed, additional ROE contacts were observed for the above mentioned protons in **8** and **9** with respect to **5**. These includes H_3-H_{11}, H_3-H_7, H_{13}-H_{17}, H_{11}-H_{16}, $H_{1'}$-H_2 and

Fig. 2. The products obtained after electrochemical oxidation of desmycosin.

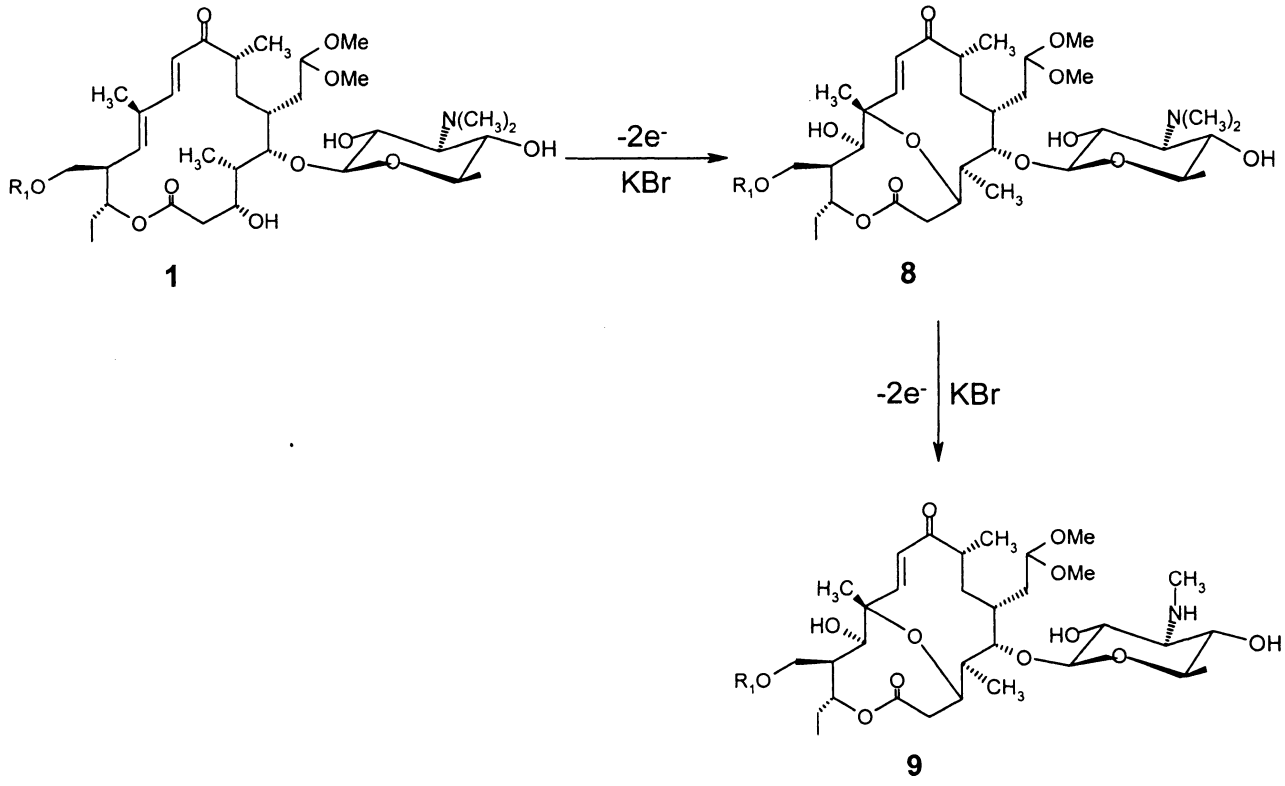


Table 2. Antibacterial *in vitro* activity of oxidation products of desmycosin.

	MIC (μg/ml)								
	Des.	**1**	**3**	**4**	**5**	**6**	**8**	**9**	tylosin
Staphylococcus aureus B 0329	0.5	0.25	1	2	>64	>64	>64	>64	0.5
Staphylococcus aureus B 0538 (iMLS)[a]	1	1	16	4	>64	>64	>64	>64	1
Staphylococcus aureus B 0330 (cMLS)[b]	>64	>64	>64	>64	>64	>64	>64	>64	>64
Streptococcus pneumoniae B0541	≤ 0.125	32	>64	1	>64	>64	>64>	>64	≤ 0.125
Streptococcus pneumoniae B0326 (M)[c]	≤ 0.125	>64	>64	2	>64	>64	>64	>64	≤ 0.125
Streptococcus pneumoniae B0328 (cMLS)	>64	>64	>64	>64	>64	>64	>64	>64	>64
Streptococcus pyogenes B0542	≤ 0.125	0.25	0.5	≤ 0.125	>64	>64	>64	>64	≤ 0.125
Streptococcus pyogenes B0543 (iMLS)	≤ 0.125	2	2	0.5	>64	>64	>64	>64	0.25
Streptococcus pyogenes B0544 (cMLS)	>64	>64	>64	>64	>64	>64	>64	>64	>64
Escherichia coli B0001	>64	>64	>64	>64	>64	>64	>64	>64	>64
Haemophilus influenzae B0529	4	>64	64	16	>64	>64	>64	>64	16

[a] iMLS: inducible resistance to macrolide, lincosamide, and streptogramin (MLS) antibiotics
[b] cMLS: constitutive MLS resistance
[c] M: efflux mediated macrolide resistance

$H_{1'}$-H_{18} contacts. The other chemical shifts (Table 1) are consistent with the assigned structures.

In Vitro Activity

The antibacterial activities of new compounds **4**, **5**, **6**, **8**, and **9** were compared with that of desmycosin (**1**) and its 13-hydroxy derivative (**3**) (Table 2).

MIC (minimal inhibitory concentrations) levels for all compound were determinated on a panel of susceptible and resistant Gram (+) strains, like *Staphylococcus aureus*, *Streptococcus pneumoniae* and *Streptococcus pyogenes*. Also, two Gram (−) strains were tested (*Escherichia coli* and *Haemophilus influenzae*). In all tests control substance was tylosin.

Only the substance **4** had 1- to 2-fold higher MIC values comparing to desmycosin. All other derivates were non-active on all strains. The substance **4** was also non-active on constitutive resistant strains.

Experimental

Physico-chemical Determination and Chromatography

NMR spectra were recorded on a Bruker Avance DRX500 spectrometer operating at 500.13 MHz for ^1H, equipped with a 5 mm diameter inverse detection probe and z-gradient accessory.

UV spectra were measured in methanol solution on a VARIAN Carry 100 spectrometer. Mass spectra (MS) were determined using the fast atom bombardment method with an Auto-Spec Q (VG Analytical) mass spectrometer. Product purification was carried out by column chromatography using Silica-gel 60, 230~400 mesh (Merck), and a CH_2Cl_2-MeOH-NH_4OH (90 : 9 : 1.5) mixture as eluent (system A). Thin layer chromatography (TLC) was performed on Silica-gel 60 F_{254} (Merck) in system A.

Antibiotic Susceptibility Test

Antibiotic susceptibility data given in Table 1 were obtained by microdilution test in Mueller-Hinton media.

Test substances and standards are dissolved in DMF (Merck) at concentration 5 mg/ml. Solutions of the substances were prepared in Mueller-Hinton broth media, final concentration from 64 to 0.125 μg/ml. After 24 hours incubation, optical density was detected by measuring absorbance at 600 nm. Minimal inhibitory concentration (MIC) is defined as the concentration which shows 90% growth inhibition. All screening procedures were done on TECAN Genesis 150 robot unit.

Preparation of 12,13-Dihydro-12,13-dihydroxy-desmycosin(3′-*N*-oxide)-20-dimethyl Acetal (**4**)

10,13-Dihydro-13-hydroxy-desmycosin-20-dimethyl acetal (10 g, 12 mmole) was dissolved in methylene chloride (150 ml), *m*-chloroperbenzoic acid 71% (11.6 g, 48 mmole) was added and it was stirred at room temperature for 8 hours. The reaction solution was poured into 400 ml of water, alkalized to a pH value of 8.5 by the addition of 20% NaOH, stirred for 30 minutes and, subsequently, after the removal of the organic layer, extracted once more by a mixture CH_2Cl_2 and *i*-propanol (5 : 1). The combined extracts were washed with a saturated $NaHCO_3$ solution, dried and evaporated to a dry residue. The crude product (6.92 g) was purified by chromatography on a column (system E).

Obtained: 4.5 g (43.3%) Rf (E) 0.35, MH^+ 868; UV (EtOH) λ_{max} 230 nm, log ε 3.88; ^1H-NMR ($CDCl_3$) δ ppm 6.84 (1H, d, H-11), 6.41 (1H, d, H-10), 4.52 (1H, d, 1″), 4.50 (1H, m, H-20), 4.34 (1H, d, 1′), 3.60 (3H, s, 3″OMe), 3.44 (6H, s, N-Me, 2″OMe), 3.34 (3H, s, 20-OMe), 3.29 (3H, s, 20-OMe), 3.25 (3H, s, N-Me); ^{13}C-NMR ($CDCl_3$) δ ppm 204.4 (s, C-9), 172.2 (s, C-1), 148.7 (d, C-11), 127.9 (d, C-10), 104.6 (d, C-1′, C-20), 99.7 (d, C-1″), 76.2 (s, C-12), 75.4 (d, C-13), 61.3 (q, 3″OMe, N-Me), 57.4 (q, 2″OMe), 54.0 (q, 20-OMe, N-Me), 51.8 (q, 20-OMe).

Preparation of 12,13-Dihydro-12,13-dihydroxy-desmycosin-20-dimethyl Acetal (**5**)

Compound **4** (1 g, 1.15 mmole) was dissolved in 35% ethanol (60 ml), 3.1 g of NH_4Cl and stepwise 1 g of Zn under maintaining the pH value of 5.0~5.5 were added. It was stirred at room temperature for 5 hours, whereupon Zn was separated by filtration and EtOH was removed by evaporation at reduced pressure. The aqueous solution was alkalized to a pH value of 8.5, followed by extraction with chloroform. The extracts were dried and evaporated to a dry residue.

Obtained: 0.83 g (84.6%) Rf (E) 0.48, Rf (E1) 0.43, MH^+ 852; UV (EtOH) λ_{max} 230 nm, log ε 3.91; ^1H-NMR ($CDCl_3$) δ ppm 6.83 (1H, d, H-11), 6.39 (1H, d, H-10), 4.51 (1H, d, 1″), 4.49 (1H, m, H-20), 4.35 (1H, d, 1′), 3.60 (3H, s, 3″OMe), 3.44 (3H, s, 2″OMe), 3.34 (3H, s, 20-OMe), 3.29 (3H, s, 20-OMe), 2.50 (6H, s, $N\text{-}Me_2$); ^{13}C-NMR ($CDCl_3$) δ ppm 204.5 (s, C-9), 172.3 (s, C-1), 148.9 (d, C-11), 127.9 (d, C-10), 104.6 (d, C-1′, C-20), 99.8 (d, C-1″), 76.4 (s, C-12), 75.4 (d, C-13), 61.3 (q, 3″OMe), 57.4 (q, 2″OMe), 54.0 (q, 20-OMe), 51.8 (q, 20-OMe), 40.1 (q, NMe_2).

Preparation of 12,13-Dihydro-12,13-dihydroxy-desmycosin-9($E+Z$) Oxime-20-dimethyl Acetal (**6**)

Compound **5** (2 g, 2.34 mmole) was dissolved in dry pyridine (25 ml), hydroxylamine hydrochloride (1.9 g, 27.6 mmole) was added and it was stirred in a nitrogen stream for 6 hours at room temperature. The reaction mixture was poured into 150 ml of water, alkalized to a pH value of 9, whereupon pyridine was removed by azeotropic distillation. An extraction with a mixture of $CHCl_3$ and *i*-propanol (5 : 1) was performed and the combined extracts were dried and evaporated to a dry residue. The crude product (1.75 g) was subjected to chromatography on a slilica gel column (system AJ).

Obtained: 1.14 g (55.9%) Rf (E1) 0.39, MH^+ 867; UV (EtOH) λ_{max} 230 nm, log ε 3.97; ^{1}H-NMR (DMSO-d_6) δ ppm 10.77, 10.49 (1H, s, 9-NOH), disappear by agitation with D_2O; ^{1}H-NMR ($CDCl_3$) δ ppm 6.23 (1H, d, H-11), 6.09 (1H, d, H-10), 4.52 (1H, d, 1″), 4.50 (1H, m, H-20), 4.33 (1H, d, 1′), 3.61 (3H, s, 3″OMe), 3.45 (3H, s, 2″OMe), 3.36 (3H, s, 20-OMe), 3.32 (3H, s, 20-OMe), 2.50 (6H, s, N-Me_2); ^{13}C-NMR ($CDCl_3$) δ ppm 171.9 (s, C-1), 163.6, 159.2 (s, C-9), 148.7, 143.8 (d, C-11), 123.6, 116.0 (d, C-10), 104.7 (d, C-1′, C-20), 99.6 (d, C-1″), 76.6 (s, C-12), 75.5 (d, C-13), 61.3 (q, 3″OMe), 57.4 (q, 2″OMe), 54.0 (q, 20-OMe), 51.8 (q, 20-OMe), 40.3 (q, NMe_2).

Preparation of 10,11,12,13-Tetrahydro-12,13-dihydroxy-desmycosin-20-dimethyl Acetal (**7**)

Compound **5** (0.2 g, 0.23 mmole) was dissolved in 50 ml of phosphate buffer (pH=5.4) and then it was transferred into an electrochemical cell having separate anode and cathode compartments. The Hg pool served as a cathode, whereas graphite was used as a counter electrode and Ag/AgCl as a reference electrode. The electroreduction was carried out under constant potential of −1.4 V at room temperature. The reduction was finished in 40 minutes with the charge consumption of 50°C. The reaction solution was alkalized to a pH-value of 8.5 and extracted with chloroform. The extract was washed with a saturated $NaHCO_3$ solution and evaporated to a dry residue.

Obtained: 0.16 g (81.6%) Rf (E) 0.45, Rf (E1) 0.43, MH^+ 854; ^{1}H-NMR ($CDCl_3$) δ ppm 4.55 (1H, d, 1″), 4.52 (1H, m, H-20), 4.35 (1H, d, 1′), 3.60 (3H, s, 3″OMe), 3.44 (3H, s, 2″OMe), 3.34 (3H, s, 20-OMe), 3.29 (3H, s, 20-OMe), 2.50 (6H, s, N-Me_2); ^{13}C-NMR ($CDCl_3$) δ ppm 212.4 (s, C-9), 173.0 (s, C-1), 104.6 (d, C-1′, C-20), 99.7 (d, C-1″), 61.3 (q, 3″OMe), 57.4 (q, 2″OMe), 54.0 (q, 20-OMe), 51.8 (q, 20-OMe), 40.1 (q, NMe_2).

Preparation of 13-Hydroxy-3-dehydroxy-3,12-oxa-desmycosin-20-dimethyl Acetal (**8**) and 13-Hydroxy-3-dehydroxy-3,12-oxa-desmycosin(3′-*N*-demethyl)-20-dimethyl Acetal (**9**)

Compound **1** (200 mg, 0.23 mmole) was dissolved in 50 ml phosphate buffer (pH=7.0) and then KBr (595 mg, 5 mmole) was added. The solution was transferred into the anodic compartment of the conventional, H-type electrochemical cell. Pt-sheet served as anode whereas graphite served as a cathode. Anodic and cathodic compartments were separated with the glass frit. The solution was electrolyzed with the constant current density of 1 mA/cm^2 until the passage of 4F. The reaction solution was alkalized to a pH-value of 8.5 and extracted with chloroform. The extract was washed with a saturated $NaHCO_3$ solution and evaporated to a dry residue. The crude product (170 mg) was subjected to chromatography on a slilica gel column (system AJ).

Obtained: 55 mg (27.5%) of **8**. MH^+ 834; ^{1}H-NMR ($CDCl_3$) δ ppm 4.00 (1H, q, H-3), 1.71 (1H, t, H-4), 6.55 (1H, d, H-10), 6.74 (1H, d, H-11), 3.80 (1H, d, H-13), 2.52 (6H, s, 3′-$NHCH_3$); ^{13}C-NMR ($CDCl_3$) δ ppm 40.8 (d, C-2), 70.5 (d, C-3), 204.0 (s, C-9), 124.4 (d, C-10), 146.6 (d, C-11), 84.9 (s, C-12), 81.1 (d, C-13), 46.4 (d, C-14), 70.1 (d, C-3′).

Obtained: 120 mg (60%) of **9**. MH^+ 820; ^{1}H-NMR ($CDCl_3$) δ ppm 4.02 (1H, q, H-3), 1.69 (1H, t, H-4), 6.52 (1H, d, H-10), 6.73 (1H, d, H-11), 3.80 (1H, d, H-13), 2.49 (3H, s, 3′-$NHCH_3$); ^{13}C-NMR ($CDCl_3$) δ ppm 41.0 (d, C-2), 70.6 (d, C-3), 204.0 (s, C-9), 124.4 (d, C-10), 146.6 (d, C-11), 84.9 (s, C-12), 81.1 (d, C-13), 46.4 (d, C-14), 64.8 (d, C-3′).

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