TRANSFORMATION OF STREPTONIGRIN INTO STREPTONIGRONE; SYNTHESIS AND BIOLOGICAL EVALUATION OF ANTIBIOTICS STREPTONIGRIN AND STREPTONIGRONE ALKYL ETHERS

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A method of synthesis of antibiotic streptonigrin 8′-*O*-alkyl ethers by alkylation of streptonigrin diphenylmethyl ester and consequent deprotection of carboxylic group with CF_3COOH is developed. An attempt to deblock carboxylic group of 8′-*O*-methylstreptonigrin diphenylmethyl ester by hydrogenation over Pd produced 8′-*O*-methylstreptonigrone. Similarly streptonigrin was transformed into streptonigrone over Pd-black in H_2 stream. Methylation of streptonigrone afforded 5′,5′-*N*-dimethyl-2′,8′-*O*-dimethylstreptonigrone and 1′,5′,5′-tri-*N*-trimethyl-8′-*O*-methylstreptonigrone. Alkyl streptonigrin ethers demonstrated lower antibacterial activity *in vitro* than the parent antibiotic.

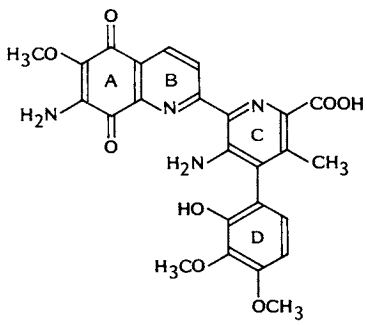
Streptonigrin (**1**), a nonintercalative antitumor antibiotic, induces mammalian topoisomerase II dependent DNA cleavage[1], it is a potent inhibitor of viral reverse transcriptases[2]. Severe side effects of **1** resulted in discontinuation of its clinical application as an antitumor drug. Streptonigrone (**2**) is a minor component in culture broth of *Streptomyces* 114[3] or *Streptomyces albus* var. *bruneomycini*[4], producing streptonigrin. **2** has lower cytostatic and antibacterial activities than **1**. Until now total synthesis of streptonigrone has not been described. The search for new methods of **1** and **2** modification and biological evaluation of new antibiotic derivatives represent a part of screening program for novel antitumor or antiretroviral semisynthetic antibiotics of streptonigrin series.

Though streptonigrin C-2′-carboxylate is very important for observable biological activity the main direction of **1** modification was derivatization of this group what led to different streptonigrin amides, esters, hydrazides and other derivatives (*e.g.*[2,5~8]).

Other positions of streptonigrin molecule are less available for modification; the only exception being preparation of methyl ester of 8′-*O*-methylstreptonigrin (**3**) by methylation of **1** with $(CH_3)_2SO_4$ in the presence of K_2CO_3[9]. Besides this several quinone-imine type derivatives of **1** were described[10,11].

We now report an unusual transformation of streptonigrin (**1**) into streptonigrone (**2**) and the synthesis and antibacterial evaluation of 8′-*O*-alkyl derivatives of **1** and **2**. The interest to *O*-alkyl ethers of streptonigrin is stimulated by the analysis of structure-activity relationships in the series of antitumor anthracycline antibiotics which showed that the compounds with unsubstituted phenolic group and their analogs with alkylated OH group differ in their cytotoxic and antitumor properties (*e.g.* carminomycin-daunorubicin)[12], the *O*-substituted compounds having higher pharmaceutical index than their *O*-unsubstituted congeners.

The initial step of transformation was carboxylic group protection. The interaction of **1** with diphenyldiazomethane in dioxane yielded diphenylmethyl ester of streptonigrin (**4**) in 72.7% yield. Alkylation of **4** with methyl, ethyl or *n*-propyl iodide in DMSO in the presence of *tert*-BuOK afforded corresponding 8′-*O*-alkyl derivatives **5a**, **5b** and **5c** in 58.5%, 30.3% and 31% yields, respectively. Deblocking was accomplished with CF_3COOH in CH_2Cl_2 to give 8′-*O*-methyl (**6a**), 8′-*O*-ethyl (**6b**) and 8′-*O*-*n*-propyl



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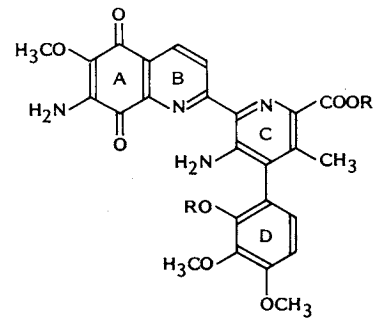
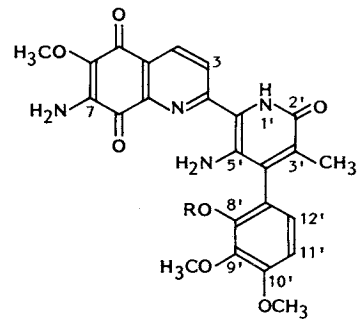


3 R=R'=CH_3
4 R=H R'=$CHPh_2$
5a R=CH_3 R'=$CHPh_2$
5b R=Et R'=$CHPh_2$
5c R=*n*-Pr R'=$CHPh_2$
6a R=CH_3 R'=H
6b R=Et R'=H
6c R=*n*-Pr R'=H
10 R=H R'=CH_3

1 or 4 ⟶

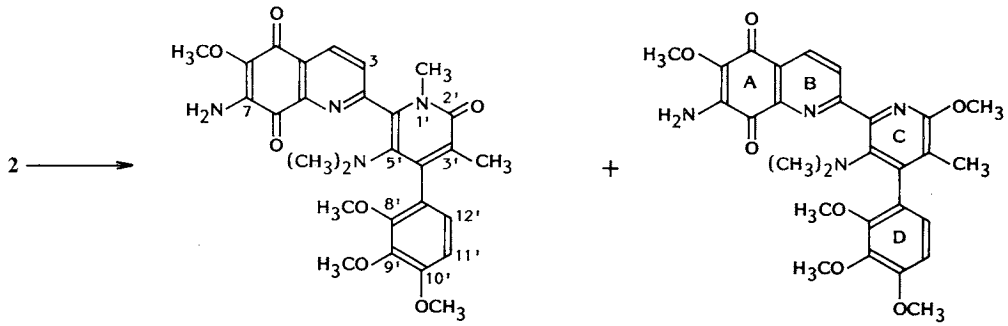


2 R=H or
7 R=CH_3

(**6c**) ethers in 79~83% yields. Their structures were confirmed by ^{1}H NMR, IR and UV spectroscopies and mass-spectrometry.

An attempt to deprotect diphenylmethyl ester **5a** by hydrogenation over Pd-black gave 8'-*O*-methylstreptonigrone (**7**).

This promoted us to investigate the transformation of **1** in similar conditions. We have transformed **1** into **2** over Pd-C or Pd-black in a yield of 20% in the stream of H_2 at room temperature. The obtained streptonigrone is identical with the natural compound (HPLC, UV, IR and ^{1}H NMR data)[3,4]. Though the mechanism of streptonigrin transformation into streptonigrone still has to be clarified, decarbonylation over Pd could be assumed to be a path of streptonigrone formation. The extremely easy conversion of **1** or diphenylmethyl ester **4** into **2** or its methyl ether **7** are rather unusual and intriguing examples of decarbonylation reaction. Decarbonylation of carboxylic acids has not been described previously; however decarbonylation of acyl chlorides and aldehydes over Pd and its salts is well known[13]. As 8'-*O*-methylstreptonigrin is not formed in these conditions it suggests that the limiting stage of the reaction is deblocking of carboxylic group while the transformation of carboxylic group into hydroxy group is very fast.



8 9

Earlier it has been supposed that the biosynthetic conversion of **1** or a precursor to **2** would involve decarboxylation and oxidation steps or alternatively covalent hydration of 1′,2′ bond of the pyridine ring followed by oxidative decarboxylation of the resulting α-hydroxylic acid[3]. These pathways of streptonigrone formation over Pd in H_2 stream at room temperature seem to be less probable than the decarbonylation reaction.

Two new derivatives **8** and **9** were obtained by methylation of **2** with Me_2SO_4 in dioxane in the presence of K_2CO_3. They were separated by chromatography method and isolated in 26% (**8**) and 20% (**9**) yields, respectively. ^1H NMR spectra showed that **8** and **9** are tetramethyl derivatives of **2**. Once it is known that alkylation of 2-pyridone yields *N*- and 2-*O*-alkyl derivatives[14], we supposed that the compounds **8** and **9** differ in the position of Me group in C ring. Methylation of **1** by Me_2SO_4 in the presence of K_2CO_3 was described to give the dimethyl derivative **3**, amino groups being not touched in these conditions[9]. As in **2** the AB rings are similar to AB rings of **1** it suggests that methylation of **2** is directed also towards 8′-OH and 5′-NH_2 groups.

For the compound **8** the structure of tri-1′,5′,5′-*N*,*N*,*N*-mono-8′-*O*-methylstreptonigrone was proposed, for the compound **9** the structure of di-2′,8′-*O*-di-5′,5′-*N*,*N*-methylstreptonigrone. Both compounds move in paper electrophoresis at pH 2 (E_8 0.10, E_9 0.13), the E values being close to that of streptonigrone (E_2 0.09) and its 8′-*O*-methyl derivative **7** (E_7 0.07); **1** does not move in paper electrophoresis in these conditions. As in **2** the substituents in C ring are different from substituents in C ring of **1** it suggests that the mobilities of **2**, **7**, **8** and **9** in electrophoresis at pH 2 depend on basicity of amino group in C ring.

The direction of streptonigrone methylation in **8** and **9** was unambiguously confirmed by 2D ^{13}C-^1H chemical shift correlation spectroscopy *via* long-range couplings using standard pulse sequence HETCOR[15]. The HETCOR spectra of **8** and **9** showed the following relevant correlations: protons of 5′-NMe and carbon C-5′, protons 12′-H, 8′-OMe and carbon C-8′. In the HETCOR-spectrum of **8** connectivities of protons 3′-Me and 1′-NMe with C-2′ was observed, while in the spectrum of **9** C-2′ exhibits cross peaks to protons 3′-Me and 2′-OMe (see the Table 1).

Analysis of conformation of streptonigrin and its derivatives using Desktop Molecular Modelling Programme (Oxford Electronic Publishing, 1988)[16] demonstrates the planar chirality of **1**, depending on D ring being out of the plane of AB and C rings, while in the compounds **8** (Fig. 1) and **9** (Fig. 2) all three ring systems-AB, C and D are noncoplanar and the rotation around $N_{5'}$-C bond is restricted.

Antimicrobial Activity

Antibacterial activity of 8′-ethers of streptonigrin (**6a**, **6b**, **6c**) in comparison with the parent antibiotic

Table 1. ^{13}C assignments of compounds (**8**) and (**9**) in C_6D_6 (128.00 ppm) with ^1J- and ^nJ-heteronuclear carbon-proton shift correlation methods.

Carbon No.	**8**			**9**		
	δ_C (ppm)	δ_H (ppm) of correlation peaks		δ_C (ppm)	δ_H (ppm) of correlation peaks	
		via ^1J	*via* ^nJ		*via* ^1J	*via* ^nJ
2	146.61		8.09	146.06		8.27
3	128.89	6.70		128.63	7.95	
4	133.56	8.09		133.71	8.27	
4a	128.2[a]		6.70	127.8[a]		7.95
5	176.64		8.09	177.22		8.27
6	137.38		4.37 (7-NH_2)/3.78	137.19		3.63
7	139.60			139.31		
8	179.46		4.37 (7-NH_2)	180.13		
8a	157.99		8.09	162.79		
2′	161.98		3.19/2.31	158.46		3.86/2.21
3′	148.39		2.31	121.89		2.21
4′	129.40		2.31	150.41		2.21
5′	131.09		2.15 (5′-NMe_2)	128.2[a]		2.68
6′	141.98		3.19	139.31		
7′	125.23		6.34	124.92		6.40
8′	151.63		6.60/3.69	151.70		6.64
9′	143.31		6.34/3.72	143.02		6.40/3.77
10′	154.43		6.60/3.35	154.02		
11′	107.70	6.34		107.31	6.40	
12′	124.34	6.60		124.77	6.64	
6-OMe	59.97	3.78		59.95	3.63	
8′-OMe	60.55	3.69		60.54[b]	3.78[b]	
9′-OMe	60.49	3.72		60.52[b]	3.76[b]	
10′-OMe	55.84	3.35		55.51	3.36	
3′-Me	15.62	2.31		15.54	2.21	
1′-Me	33.62	3.19				
5′-NMe_2	43.72[c]			44.01	2.68	
2′-OMe				53.28	3.86	

[a] Data were obtained from long-range C/H-correlation spectra.
[b] Signal assignments may be interchanged.
[c] Broad signal.

1, its esters **3** and **10** as well as antibacterial activity of streptonigrone (**2**) and its derivatives **7**, **8**, **9** against *Bacillus subtilis* and *Bacillus micoides* was investigated on agar medium by the paper-disk method (10 mg/disk) (see the Table 2). Among all tested new compounds **6a**, **6b** and **6c** demonstrated the most pronounced activity which is lower than that of **1**. Streptonigrone derivative with fixed pyridone structure (**8**) is devoid of activity, whereas derivatives **2**, **7**, **8**, **9** demonstrate some antimicrobial properties.

Experimental

Physico-chemical determinations were made on the following instruments: NMR; VXR-400 (Varian, U.S.A.), EI-MS; Varian-MAT-112 spectrometer at 210~230°C ion source temperature and 70 eV electron energy, samples being introduced by direct insertion, IR; spectrophotometer SP-1100 (Pye Unicam, England), UV; Beckman instrument UV-5260 (Austria), electrophoretic mobilities (E) were measured in HCOOH - CH_3COOH - water (20 : 24 : 56) (pH 2) at 300 V during 2 hours on FN15 paper.

Analytical TLC was carried out on Silufol plates UV-254 (Kavalier, Czeco-Slovakia) in chloroform (A) or chloroform - acetone - methanol (8 : 1 : 1) (B). For preparative chromatography plates (20 ×

Fig. 1. The predominant conformation of **8**.

(A) The projection on the plane perpendicular to the plane of D ring. (B) The projection on the plane perpendicular to the plane of C ring.

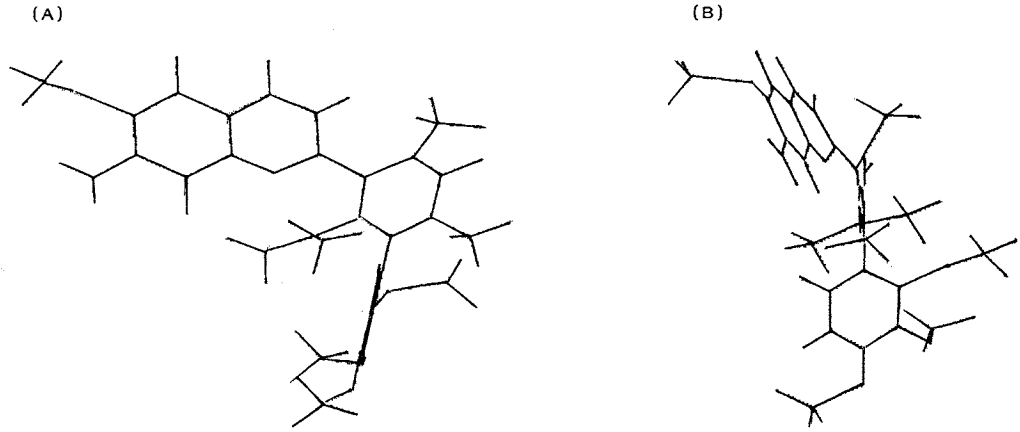
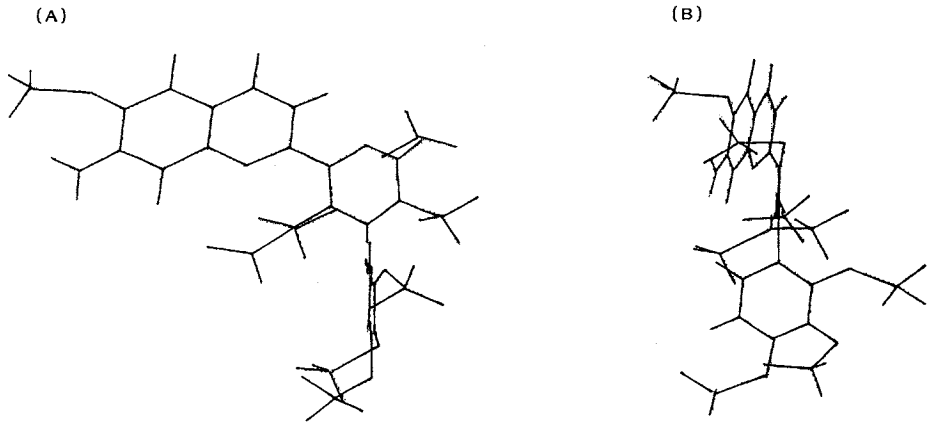


Fig. 2. The predominant conformation of **9**.

(A) The projection on the plane perpendicular to the plane of D ring. (B) The projection on the plane perpendicular to the plane of C ring.



20 cm) covered with nonfixed Kieselgel-60PF_{254} (Merck) and for column chromatography Kieselgel-60 (0.040 ~ 0.063 mm, Merck, BRG) were employed.

Diphenylmethyl Ester of Streptonigrin (**4**)

To the solution of diphenyldiazomethane prepared from 260 mg (1.3 mM) of benzophenone hydrazone by method[17] streptonigrin (139 mg, 0.28 mmol) in 4 ml of dry dioxane was added. The mixture was stirred for 72 hours at room temperature, the solvents were removed under reduced pressure and the solid residue was dissolved in minimal amount of $CHCl_3$ and was chromatographed on silica gel. The fraction eluted with chloroform - hexane mixture (1 : 1) gave 90.3 mg (73%) of **4**. Rf 0.38 (A). UV λ_{max}^{MeOH} nm (ε) 292

Table 2. Antibacterial activity of compounds **1**, **2**, **3**, **6a**, **6b**, **6c**, **7**, **8**, **9** and **10** in agar assay.

Compound	*Bacillus subtilis*	*Bacillus mycoides*
	Diameter of inhibition zones (mm)	
1	40	43
2	21	27
3	19	20
6a	27	30
6b	26	31
6c	27	31
7	10	12
8	0	0
9	11	15
10	18	20

Agar media No. 7[18], 24 hours incubation at 37°C, compound 10 mg/disk (diameter 6 mm).

(15,000), 375 (13,000). IR ν_{max} ($CHCl_3$) cm^{-1} 3590~3290 (NH, OH), 2980 (CH), 1620 (CO). ^1H NMR (400 MHz, $CDCl_3$) δ (ppm), J (Hz): 9.09 (1H, d, $J_{3,4}$=8.5, 4-H), 8.42 (1H, d, 3-H), 7.58~7.33 (10H, m, $2C_6H_5$), 6.79 (1H, d, $J_{11',12'}$=8.6, 11'-H), 6.63 (1H, d, 12'-H), 5.87 (1H, OH), 5.08 (2H, NH_2), 4.07, 3.96, 3.92 (3H, 3H, 3H, s, s, s, $3OCH_3$), 3.66 (1H, s, CH), 2.27 (3H, s, CH_3).

Diphenylmethyl Ester of 8'-Methylstreptonigrin (**5a**)

To a solution of **4** (10 mg, 0.015 mM) in DMSO (3 ml) *tert*-BuOK (obtained from 0.58 mg, 0.015 mM of K) and methyl iodide (0.1 ml, 1.25 mM) were added. The reaction was stirred for 1 hour, ethyl acetate (30 ml) was added and the mixture was washed with water (10 ml × 3). Organic phase was concentrated to dryness under reduced pressure, the residue was dissolved in minimal amount of chloroform and then was purified by column chromatography in chloroform - hexane (1 : 2) mixture to give 6.0 mg (58.5%) of **5a**, Rf 0.63 (A). ^1H NMR (400 MHz, C_6D_6) δ (ppm), J (Hz): 9.19 (1H, d, $J_{3,4}$=2.1, 4-H), 8.25 (1H, d, 3-H), 7.63~7.15 (10H, m, C_6H_5), 6.51 (1H, d, $J_{11',12'}$=2.1, 11'-H), 6.37 (1H, d, 12'-H), 4.24 (2H, s, NH_2), 3.77, 3.71, 3.46, 3.36 (3H, 3H, 3H, 3H, s, s, s, s, $4OCH_3$), 3.28 (1H, s, CH), 2.51 (3H, s, CH_3).

Diphenylmethyl Ester of 8'-*O*-Ethylstreptonigrin (**5b**)

5b (11.1 mg, 30.2%) was obtained from 35 mg (0.052 mM) of **4**, *tert*-BuOK (from 2.2 mg, 0.052 mM of K) and excess of EtI (0.5 ml) in a procedure analogous to **5a**. Rf 0.66 (A). ^1H NMR (400 MHz, C_6D_6) δ (ppm), J (Hz): 9.19 (1H, d, J=2.2, 4-H), 8.25 (1H, d, 3-H), 7.63~7.15 (10H, m, C_6H_5), 6.51 (1H, d, J=2.1, 11'-H), 6.37 (1H, d, 12'-H), 4.24 (2H, s, NH_2), 3.77, 3.71, 3.36 (3H, 3H, 3H, s, s, s, $3OCH_3$), 3.28 (1H, s, CH), 3.09 (2H, q, OCH_3), 2.51 (3H, s, CH_3), 1.12 (3H, t, CH_3).

Diphenylmethyl Ester of 8'-*O*-*n*-Propylstreptonigrin (**5c**)

5c (12.3 mg, 31.1%) was obtained from **4** (37 mg, 0.055 mM), *tert*-BuOK (from 2 mg, 0.05 mM of K) and excess (0.5 ml) of *n*-PrI. Rf 0.68 (A). ^1H NMR (400 MHz, C_6D_5) δ (ppm), J (Hz): 9.19 (1H, d, J=2.2, 4-H), 8.25 (1H, d, 3-H), 7.63~7.15 (10H, m, C_6H_5), 6.51 (1H, d, J=2.1, 11'-H), 6.37 (1H, d, 12'-H), 4.24 (2H, s, NH_2), 3.77, 3.71, 3.46 (3H, 3H, 3H, s, s, s, $3OCH_3$), 3.22 (2H, t, OCH_2), 2.51 (3H, s, CH_3), 1.26 (2H, m, CH_2), 0.52 (3H, t, CH_3).

8'-*O*-Methylstreptonigrin (**6a**)

5a (6.0 mg) was dissolved in CH_2Cl_2 (1 ml), the mixture was cooled to 0°C and 0.5 ml of CF_3COOH - CH_2Cl_2 mixture (1 : 1) cooled to 0°C was added. The reaction was warmed to room temperature, stirred for 20 minutes and concentrated to dryness under reduced pressure. **6a** was purified on silica gel in A system to give 4.15 mg (81%), mp 259~261°C. Rf 0.23 (A). UV λ_{max}^{MeOH} nm (ε) 290 (17,000), 376 (19,000). IR ν_{max} ($CHCl_3$) cm^{-1} 3580~3290 (NH, OH), 2980, 2880 (CH), 1760 (COOH), 1620 (CO). ^1H NMR (400 MHz, $CDCl_3$) δ (ppm), J (Hz): 8.69 (1H, d, J=8.5, 4-H), 8.47 (1H, d, 3-H), 6.85 (1H, d, J=8.5, 11'-H), 6.78 (1H, d, 12'-H), 5.11 (2H, s, NH_2), 4.09 (3H, s, OCH_3), 3.94, 3.93, 3.72 (3H, 3H, 3H, s, s, s, $3OCH_3$), 2.47 (3H, s, CH_3). EI-MS *m/z* 520 ($M^{+\cdot}$, $C_{26}H_{24}N_4O_8$).

8'-*O*-Ethylstreptonigrin (**6b**)

Deblocking of 11.1 mg of **5b** afforded 9.13 mg (83%) of **6b**, mp 210~211°C, Rf 0.25 (A). UV λ_{max}^{MeOH} nm (ε) 287 (12,000), 380 (13,000). IR ν ($CHCl_3$) cm^{-1} 3580~3290 (NH, OH), 2980, 2880 (CH), 1760 (COOH), 1620 (CO). ^1H NMR (400 MHz, $CDCl_3$) δ (ppm), J (Hz): 8.70 (1H, d, J=8.5, 4-H), 8.47 (1H, d, 3-H), 6.84 (1H, d, J=8.63, 11'-H), 6.68 (1H, d, 12'-H), 5.11 (2H, s, NH_2), 4.10, 3.93, 3.92 (3H, 3H, 3H, s, s, s, OCH_3), 3.72 (2H, q, OCH_2), 2.16 (3H, s, CH_3), 1.23 (3H, t, CH_3). EI-MS *m/z* 534 ($M^{+\cdot}$, $C_{27}H_{26}N_4O_8$).

8'-*O*-Propylstreptonigrin (**6c**)

Deblocking of 12.3 mg of **5c** was proceeded in a procedure analogous to **5a**. **6c** was obtained in 79.5% (11.8 mg) yield. mp 247~248°C. Rf 0.28 (A). UV λ_{max}^{MeOH} nm (ε) 282 (10,000), 378 (11,000). IR ν_{max} ($CHCl_3$) cm^{-1} 3580~3290 (NH, OH), 2980, 2880 (CH), 1760 (COOH), 1620 (CO). ^1H NMR (400 MHz, $CDCl_3$) δ (ppm), J (Hz): 8.69 (1H, d, J=8.5, 4-H), 8.48 (1H, d, 3-H), 6.83 (1H, d, J=8.5, 11'-H), 6.78 (1H, d, 12'-H), 5.11 (2H, s, NH_2), 4.09, 3.93, 3.91 (3H, 3H, 3H, s, s, s, OCH_3), 3.77 (2H, t, OCH_2), 2.16 (3H, s, CH_3), 1.43 (2H, m, CH_2), 0.61 (3H, t, CH_3). EI-MS *m/z* 548 ($M^{+\cdot}$, $C_{28}H_{28}N_4O_8$).

8′-*O*-Methylstreptonigrone (**7**)

10 mg of **5a** was dissolved in ethyl acetate - methanol mixture (1 : 1), Pd-black (10 mg) was added and the reaction mixture was stirred in H_2 stream for 6 hours. Then the mixture was filtered, evaporated *in vacuo*, the dry residue was dissolved in $CHCl_3$ and purified on silica gel layer in A system. **7**(2.8 mg, 28%) was obtained after eluation with $CHCl_3$. Rf 0.32 (B), mp 277～278°C, UV λ_{max}^{MeOH} nm (ε) 415 (14,000); MS *m/z* 492 ($M^{+\cdot}$, $C_{25}H_{24}N_4O_7$). ^1H NMR (400 MHz, $CDCl_3$) δ (ppm), *J* (Hz): 8.48 (1H, d, $J_{3,4}=9.4$, 3-H), 8.34 (1H, d, 4-H), 6.82 (2H, s, 11′-H, 12′-H), 5.05 (2H, s, 7-NH_2), 4.06, 3.93, 3.75 (3H, 3H, 3H, s, s, s, 6, 9′ and 10′-OCH_3), 3.94 (3H, s, 8′-OCH_3), 2.01 (3H, s, 3′-CH_3). EI-MS *m/z* 492 ($M^{+\cdot}$, $C_{25}H_{24}N_4O_7$).

Transformation of Streptonigrin (**1**) into Streptonigrone (**2**)

30 mg of **1** was dissolved in ethyl acetate - methanol mixture (1 : 1), Pd-black (30 mg) was added and the reaction mixture was stirred in H_2 stream at room temperature for 6 hours. The mixture was filtered, the filtrate was evaporated *in vacuo*, dissolved in chloroform and purified on silica gel in B system to give 6.5 mg (20%) of **2**. Rf 0.58 (B). mp >300°C (dec), UV λ_{max}^{MeOH} nm (ε) 340 (13,000) (pH <7), 420 (14,000) (pH 7), ^1H NMR spectrum and HPLC data are identical to the spectrum and HPLC data of the natural compound obtained by the method[4].

1′,5′,5′-*N*-Trimethyl-8′-*O*-methylstreptonigrone (**8**) and 5′,5′-*N*-Dimethyl-2′,8′-*O*-dimethylstreptonigrone (**9**)

Streptonigrone (**2**) (24 mg, 0.05 mmol) was dissolved in dioxane, 0.071 ml (0.7 mmol) of Me_2SO_4 and 77.6 mg of dry K_2CO_3 (0.71 mmol) were added. The reaction mixture was stirred during 72 hours at room temperature, then was filtered, evaporated *in vacuo*, the dry residue was dissolved in $CHCl_3$ and the obtained compounds were chromatographed on silica gel in B system to afford the compounds **8** (mp 154～155°C, UV λ_{max}^{MeOH} nm (ε) 270 (21,000), 320 (13,000); EI-MS *m/z* 534 ($M^{+\cdot}$, $C_{28}H_{30}N_4O_7$), 519 ($M-CH_3$)) and **9** (mp 137～139°C, UV λ_{max}^{MeOH} nm (ε) 230 (23,000), 303 (17,000); EI-MS *m/z* 534 ($M^{+\cdot}$, $C_{28}H_{30}N_4O_7$), 503 ($M-CH_3O$)) in 26% and 20% yields, correspondingly. ^1H NMR (400 MHz, C_6D_6) of **8** δ (ppm), *J* (Hz): 6.70 (1H, d, $J_{3,4}=8.0$, 3-H), 8.09 (1H, d, 4-H), 6.34 (1H, d, $J_{11',12'}=8.5$, 11′-H), 6.60 (1H, d, 12′-H), 4.37 (2H, s, 7-NH_2), 3.78 (3H, s, 6-OCH_3), 3.72 (3H, s, 9′-OCH_3), 3.35 (3H, s, 10′-OCH_3), 2.31 (3H, s, 3′-CH_3), 3.69 (3H, br s, 8′-OCH_3), 3.19 (3H, s, 1′-NCH_3), 2.15 (6H, br s, 5′-$(CH_3)_2N$). ^1H NMR (400 MHz, C_6D_6) of **9** δ (ppm), *J* (Hz): 7.95 (1H, d, $J_{3,4}=8.1$, 3-H), 8.27 (1H, d, 4-H), 6.40 (1H, d, $J_{11',12'}=8.5$, 11′-H), 6.64 (1H, d, 12′-H), 4.29 (2H, s, 7-NH_2), 3.63 (3H, s, 6-OCH_3), 3.76 (3H, s, 9′ or 8′-OCH_3), 3.36 (3H, s, 10′-OCH_3), 2.21 (3H, s, 3′-CH_3), 3.78 (3H, s, 8′ or 9′-OCH_3), 2.68 (6H, s, 5′-$(CH_3)_2N$), 3.86 (3H, s, 2′-OCH_3).

References

1) YAMASHITA, Y.; S. KAWADA, N. FUJII & H. NAKANO: Induction of mammalian DNA topoisomerase II dependent DNA cleavage by antitumor antibiotic streptonigrin. Cancer Res. 50: 5841～5844, 1990
2) OKADA, H.; H. MUKAI, Y. INOUYE & S. NAKAMURA: Biological properties of streptonigrin derivatives. II. Inhibition of reverse transcriptase activity. J. Antibiotics 39: 306～308, 1986
3) HERLT, A. J.; R. W. RICKARDS & J.-P. WU: The structure of streptonigrone, and a comment on the biosynthesis of the streptonigrin antibiotics. J. Antibiotics 38: 516～518, 1985
4) KOZLOVA, N. V.; N. A. LVOVA, O. A. LAPCHINSKAYA, E. B. DOKUCHAEVA, L. M. RUBASHEVA, B. V. ROZYNOV & M. N. PREOBRAZHENSKAYA: Streptonigrone a minor component of bruneomycin complex. Antibiotiki i Khimioterapia 35: 13～16, 1990
5) MIYASAKA, T.; S. HIBINO, Y. INOUYE & S. NAKAMURA: Synthesis of novel streptonigrin 2-amide derivatives with 3,3′(phenylphosphonyl)bis(1,3-thiazolidine-2-thione). J. Chem. Soc. Perkin Trans. I 1986: 479～482, 1986
6) TOLSTIKOV, V. V.; N. V. KOZLOVA, I. V. YARTSEVA, Y. V. DOBRYNIN, E. A. SINYAGINA, T. G. NIKOLAEVA, V. E. FINKO, A. A. ARUTYUNIAN, R. G. MELIK-OGANDZANIAN & M. N. PREOBRAZHENSKAYA: Synthesis of amides and esters of antibiotic bruneomycin and evaluation of their cytotoxic and antiretroviral activities. Khim. Pharm Zh. (Russ.) 23: 130～132, 1990
7) TOLSTIKOV, V. V.; N. V. KOZLOVA, I. V. YARTSEVA & M. N. PREOBRAZHENSKAYA: Chimeric antibiotics daunorubicin and its congeners, *N*-acylated with bruneomycin (streptonigrin). Bioorg. Khim. (Russ.) 15: 277～280, 1989
8) TAKE, Y.; Y. INOUYE, S. NAKAMURA, H. S. ALLAUDEEN & A. KUBO: Comparative studies of the inhibitory properties of antibiotics on human immunodeficiency virus and avian myeloblastosis virus reverse transcriptase and cellular

DNA polymerases. J. Antibiotics 42: 107~115, 1989

9) RAO, K. V.; K. BIEMANN & R. B. WOODWARD: The structure of streptonigrin. J. Am. Chem. Soc. 85: 2532~2533, 1963
10) RAO, K. V.: Quinone natural products; streptonigrin and lapachol. Structure-activity relationships. Cancer Chemother. Rep. Part II 4: 11~17, 1974
11) CHIRIGOS, M.; J. PEARSON, T. PAPAS, W. WOODS, H. WOOD & S. SPAHN: Effect of streptonigrin and analogs on oncornavirus replication and DNA polymerize activity. Cancer Chemother. Rep. Part I 57: 305~310, 1973
12) PREOBRAZHENSKAYA, M. N.; E. V. BAKINA, L. S. POVAROV, E. I. LAZHKO, L. G. ALEKSANDROVA, J. BALZARINI & E. DE CLERCQ: Synthesis and cytostatic properties of daunorubicin derivatives, containing *N*-phenylthiourea or *N*-ethylthiourea moieties in the 3'-position. J. Antibiotics 44: 192~199, 1991
13) TSUJI, J. & K. OHNO: Organic syntheses by means of noble metal compounds. XXXIV. Carbonylation and decarbonylation reactions catalyzed by palladium. J. Am. Chem. Soc. 90: 94~98, 1968
14) SMITH, D.: Pyridines. Hydroxy-, amino- and mercapto derivatives. *In* Comprehensive Organic Chemistry. The Synthesis and Reactions of Organic Compounds. Vol. 4. Heterocyclic Compounds. *Ed.*, P. SAMMES. pp. 30~40, Pergamon Press, 1985
15) BAX, A. & G. MORRIS: An improved method for heteronuclear chemical shift correlation by two-dimensional NMR. J. Magn. Reson. 42: 501~505, 1981
16) VINTER, J.; A. DAVIS & M. SAUNDERS: Strategic approaches to drug design. I. An integrated software framework for molecular modelling. J. Computer Aided Molecular Design 1: 31~51, 1987
17) EISTER, B.; M. REGITZ, G. HECK & H. SCHWALL: Methoden zur Herstellung und Umwandlung von aliphatischen Diazoverbindungen. *In* Methoden der Organischen Chemie (Houben Weyl), Vol. 10 (IV). *Ed.*, E. MÜLLER, p. 569, Georg Thieme Verlag, 1968
18) State Pharmacopeia of the USSR (Russ.) Moscow. Medizina, p. 948, 1968