CHEMICAL MODIFICATION OF ANTIBIOTIC STREPTONIGRIN; SYNTHESIS AND PROPERTIES OF 2'-DECARBOXY-2'-AMINOSTREPTONIGRIN (STREPTONIGRONE-2'-IMINE)

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(Received for publication November 25, 1991)

Whereas the cytostatic properties of the antibiotic streptonigrin (**1**) and its amides and esters have been studied[1~4], structure-activity relationships for other derivatives of **1**, as well as for derivatives of antibiotic streptonigrone (**2**), which is a minor component of the streptonigrin complex[5], remain to be investigated. In this paper we describe the synthesis of 2'-decarboxy-2'-aminostreptonigrin (**8**), which can be considered as an analogue of streptonigrone.

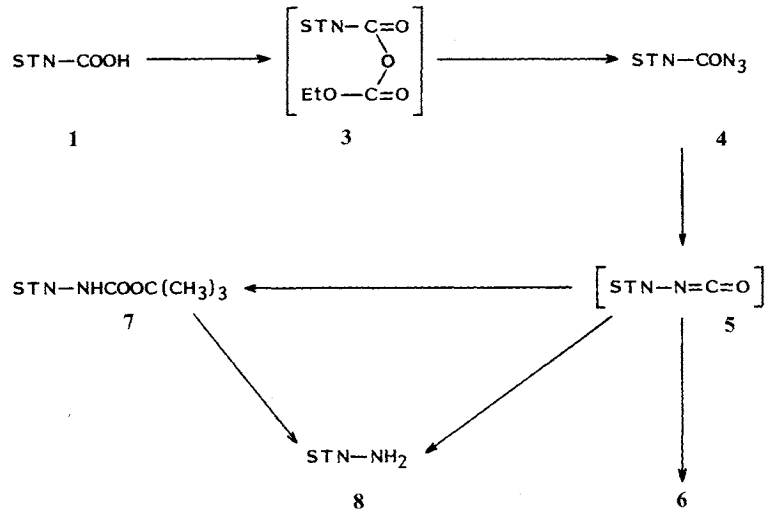
1 was converted to the mixed anhydride (**3**) (1 equiv ClCOOEt; 1.1 equiv Et_3N; THF; 0°C; 0.5 hour), which under the action of NaN_3 (3 equiv H_2O; 0°C; 1 hour) afforded, after extraction with EtOAc, the corresponding azide (**4**), (IR ν_{max} ($CHCl_3$) cm^{-1} 2250 (N_3), 1750 (C=O)), which was immediately used in the next step of conversion (see Scheme 1). All the new compounds were separated by TLC using Kieselgel 60 (Merck) plates in chloroform - acetone - methanol, 8 : 1 : 1 mixture.

Decomposition of **4** in anhydrous conditions (dry toluene, reflux for 2 hours) led *via* streptonigrin-2'-decarboxy-2'-isocyanate (**5**) to the dimer **6** (70%; MP 164~165°C; Rf 0.60; ^1H NMR (Varian VXR 400 instrument, 400 MHz, $CDCl_3$) δ 8.78 (1H, d, $J_{3,4}=8$ Hz, 4-H), 8.77 (1H, d, 4-H), 8.32 (1H, d, 3-H), 8.27 (1H, d, 3-H), 7.05 (1H, d, $J_{11',12'}=8.5$ Hz, 12'-H or 11'-H), 6.99 (1H, d, 12'-H or 11'-H), 6.72 (2H, s, 8'-OH, 2-groups), 6.70 (1H, d, 11'-H or 12'-H), 6.65 (1H, d, 11'-H or 12'-H), 6.00 (4H, br s, 7-NH_2, 2-groups), 5.10 (4H, br s, 5'-NH_2, 2-groups), 4.08 (3H, s, $-OCH_3$), 4.07 (3H, s, $-OCH_3$), 3.99 (3H, s, $-OCH_3$), 3.98 (3H, s, $-OCH_3$), 3.97 (3H, s, $-OCH_3$), 3.95 (3H, s, $-OCH_3$), 2.09 (3H, s, 3'-CH_3), 2.04 (3H, s, 3'-CH_3); CI-MS (Jeol JMS-DX 300 instrument with NH_3 as a reagent gas) *m/z* 504 ($\frac{M}{2}$+H, $C_{25}H_{22}N_5O_7$), 475 ($\frac{M}{2}$ −CO, $C_{24}H_{21}N_5O_6$); EI-MS (Varian-MAT-112 spectrometer at 210~230°C ion source and 70 eV electron energy, samples being introduced by direct insertion) *m/z* 503.1 ($(\frac{M}{2})^+$, $C_{25}H_{21}N_5O_7$). Doubling of the signals of all protons in the ^1H NMR spectrum of **6** demonstrates the absence of symmetry in the dimer structure (similar to pyridyl-2-isocyanate dimer[6]).

Decomposition of **4** in boiling *tert*-BuOH (4 hours, evaporation, isolation by preparative TLC) gave 2'-decarboxy-2'-(*tert*-butyloxycarbonylamino)-streptonigrin (**7**), amine **8** and dimer **6**. Compound **7** was obtained in 40% yield (MP 162~164°C (dec); Rf 0.85; ^1H NMR (400 MHz, DMSO-d_6) δ 8.65 (1H, d, $J_{3,4}=8$ Hz, 4-H), 8.15 (1H, d, 3-H), 6.67 (1H, d, $J_{11',12'}=8.5$ Hz, 12'-H or 11'-H), 6.45 (1H, d, 11'-H or 12'-H), 3.71 (3H, s, $-OCH_3$), 3.69 (3H, s, $-OCH_3$), 3.67 (3H, s, $-OCH_3$), 1.92 (9H, s, O–C(CH_3)$_3$), 1.86 (3H, s, 3'-CH_3)). Amine **8** was obtained in 30% yield (MP 192~194°C; Rf 0.40; ^1H NMR (400 MHz $CDCl_3$) δ 8.77 (1H, d, $J_{3,4}=8$ Hz, 4-H), 8.32 (1H, d, 3-H), 6.81 (1H, d, $J_{11',12'}=8.5$ Hz, 12'-H), 6.63 (1H, d, 11'-H), 5.04 (2H, br s, 5'-NH_2), 4.05 (3H, s, $-OCH_3$), 3.96 (3H, s, $-OCH_3$), 3.93 (3H, s, $-OCH_3$), 1.93 (3H, s, 3'-CH_3); ^{13}C NMR (Varian XL 100, 100 MHz, $CDCl_3$) δ 179.8 (C-8), 177.1 (C-5), 161.1 (C-8a), 152.6 (C-10'), 148.4 (C-2'), 147.3 (C-8'), 143.9 (C-5'), 140.3 (C-2), 137.2 (C-7), 136.9 (C-3'), 136.4 (C-9'), 132.5 (C-6), 128.3 (C-6'), 124.9 (C-4), 125.4 (C-4a), 124.3 (C-3), 121.9 (C-12'), 119.6 (C-4') 115.1 (C-7'), 103.6 (C-11'), 59.8 ($-OCH_3$), 59.1 ($-OCH_3$), 54.9 ($-OCH_3$), 13.7 (3'-CH_3); CI-MS *m/z* 477 (M, $C_{24}H_{23}N_5O_6$)). Dimer **6** was isolated in 10% yield. Treatment of **4** with a mixture of CF_3COOH - H_2O (1 : 5, steam bath; 1 hour) with subsequent evaporation and neutralization (1.1 equiv Et_3N in $CHCl_3$; 0°C; 0.5 hour), extraction with EtOAc and washing (5% aq $NaHCO_3$) afforded the amine **8** in 65% yield. Amine **8** was obtained also by cleavage of **6** in CF_3COOH - CH_2Cl_2 (1 : 1; 0°C to 20°C; 2 hours) in 80% yield. By using diphenylphosphoryl azide (DPPA)[7] for the direct conversion of **1**, we prepared amine **8** in 40% yield (1.5 equiv DPPA; 1.5 equiv Et_3N; dioxane - *tert*-BuOH (5 : 1); 80°C; 36 hours) (see Scheme 1). Amine **8**, under the action of 0.5 M HCl in MeOH, afforded the hydrochloride (**9**) (95%; MP 235°C (dec)).

The inhibitory effects of compound **8**, its hydrochloride **9** and streptonigrone **2** in comparison

Scheme 1.



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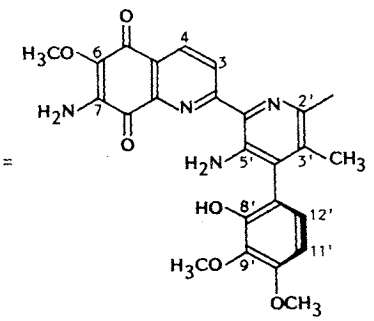
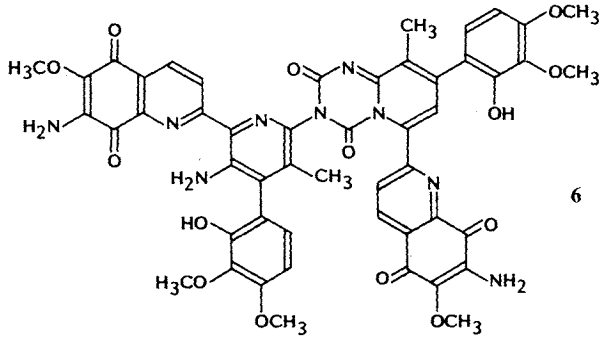
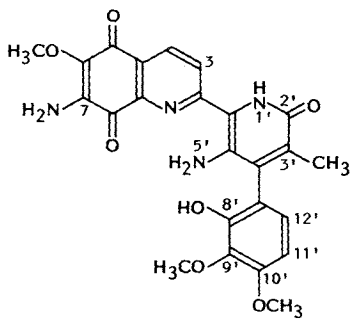




6

Scheme 2.



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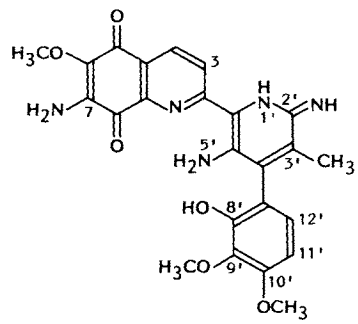

8

Table 1. Inhibitory effects of streptonigrin (**1**), streptonigrone (**2**) and derivatives **8** and **9** on the proliferation of murine leukemia (L1210), human T-lymphoblast (MOLT-4F) and human T-lymphocyte (MT-4) cells.

Compound	Inhibition of tumor cell proliferation IC_{50}* (μM/ml)		
	L1210	MOLT-4F	MT-4
1	0.044 ± 0.009	0.004 ± 0.0004	0.0002 ± 0.00001
2	2.57 ± 0.28	2.29 ± 0.46	1.83 ± 0.06
8	2.46 ± 0.24	2.29 ± 0.03	0.055 ± 0.035
9	2.91 ± 0.08	2.68 ± 0.26	0.74 ± 0.23

* 50% inhibitory concentrations, or concentrations required to inhibit cell proliferation by 50%.

with compound **1**, which can be considered as 2'-imine of streptonigrone (see Scheme 2), on the proliferation of murine leukemia (L1210), human T-lymphoblast (MOLT-4F) and human T-lymphocyte (MT-4) cells are shown in the Table 1. Compounds **8** and **9** were less cytotoxic than **1**, the IC_{50} values for **2**, **8** and **9** being similar. Compounds **8**, **9** and **2** did not prove effective against HIV-1 or HIV-2 induced cytopathogenicity in MT-4 cells at subtoxic concentrations. The assays for measuring inhibition of tumor cell growth L1210, MOLT-4F, MT-4 and anti-HIV activity in MT-4 cells were performed as previously described[8].

Acknowledgments

In the USSR this research was supported by Grant from the All Union Anti-Aids Programm, it was also supported in part by Grant from the Belgian "Nationaal Fonds voor Wetenschappelijk Onderzoek" Project No. 7.0049.90 and by the AIDS Basic Research Programme of the European Community.

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