PREPARATION OF AMIDES AND ESTERS OF THE ANTIBIOTIC BRUNEOMYCIN AND EXAMINATION OF THEIR CYTOTOXIC AND ANTIRETROVIRAL ACTIVITY

V. V. Tolstikov, N. V. Kozlova,
I. V. Yartseva, Ya. V. Dobrynin,
E. A. Sinyagina, T. G. Nikolaeva,
V. E. Fin'ko, A. A. Arutyunyan,
R. G. Melik-Ogandzhanyan, and M. N. Preobrazhenskaya

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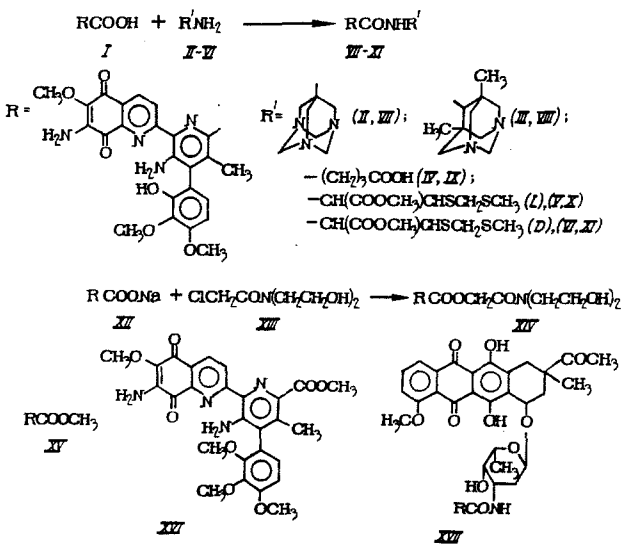
The antibiotic bruneomycin (I) (streptonigrin), and some of its amides and esters, in addition to possessing antitumor activity, are able to inhibit reverse viral transcriptase of retroviruses, in particular human immunodeficiency virus [7, 9, 11], and are of interest as potential drugs for the treatment of AIDS. For this reason, a search for new amides and esters of bruneomycin with improved cytotoxic and antiretroviral activity is justified.

The starting materials chosen for the preparation of amides of bruneomycin were amino-diaza- (and triaza-)adamantanes, together with aminoacids, which have not hitherto been used to obtain amides of bruneomycin.

7-Amino-1,3,5-triazaadamantane (II) and 5,7-dimethyl-6-amino-1,3-diazaadamantane (III) are of interest in possessing antiviral activity [3], and furthermore they are polyamines, enabling their derivatives to be obtained as salts.

Aminoacids can facilitate the transport of the antibiotic through the cell membranes. In addition, the free carboxy groups of the aminoacids can form salts, which is of some im-



portance for increasing the solubility of the drug. Amides were obtained from (I) and 4-aminobutyric acid (IV), and (D)- and (L)-S-methylthiomethylcysteine (V), (VI) [6]. The configuration of (V) is the same as that of a fragment of the naturally occurring antibiotic sparsomycin [2].

The condensing agent used to obtained the amides [8] (VII-XI) was dicyclohexylcarbodiimide. The condensations were carried out at ambient temperature using equimolar amounts of the reactants (or a 20% excess in the case of the aminoacids). Bearing in mind our experience in the use of dicyclohexyl-carbodiimide [4], N-hydroxysuccinimide was added in equimolar amounts.

Research Institute for New Antibiotics, Academy of Medical Sciences of the USSR. VONTs, Academy of Medical Sciences of the USSR, Moscow. Mndzhoyan Institute of Fine Organic Chemistry, Academy of Sciences of the Armenian SSR, Erevan. Translated from Khimiko-farmatsevticheskii Zhurnal, Vol. 24, No. 2, pp. 130-132, February, 1990. Original article submitted March 30, 1989.

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TABLE 1. Comparison of the Biological Activity of Bruneomycin Derivatives

Compound	Antibacterial activity		CaOv cytotoxicity, μg/ml	Minimum cytotoxic concentration (C-127 mouse cells), μg/ml
	B. mycoides	B. subtilis		
XII	++	++	0,03	0,02—0,2
VII	—	—	50,00	20,00
VIII	—	—	...	20,00
IX	±	±	0,70	...
X	—	—	>200,00	...
XI	—	—	>200,0	...
XIV	+	+	0,50	2,00
XV	+	+	200,00	...
XVI	+	+	1000,00	...
XVII	—	—	...	200,00

Note. Minimum concentrations inhibiting the transformation of MSV (μg/ml): (XII) 0.002-0.02, (XIV) 0.02-0.2, (XVII) 2-20.

The carboxy group of the acid (IV) was protected by trimethylsilylation, by first treating (IV) with a twofold excess of N,O-bis(trimethylsilyl)trifluoroacetamide in boiling acetonitrile.

The reaction products were isolated by preparative TLC on silica gel. Their structures were confirmed by PMR spectroscopy. Interestingly, the spectra of the amides of (I) with the optically active antipodes (V) and (VI) (amides X and XI) were identical. This was most unexpected, since the antibiotic molecule is unsymmetrical as a result of rotational isomerism around the 4'–1' bond, so that (X) and (XI) are diastereoisomers, and their PMR spectra would be expected to differ.

Another interesting group of bruneomycin derivatives comprises the esters. It has recently been reported that some of these, which have poor antitumor and antibacterial activity, inhibit human immunodeficiency virus reverse transcriptase, although they are virtually inactive against avian myeloblastoma virus reverse transcriptase [11]. In addition, esters of N,N-disubstituted glycollic acid amides have been described [5] as potential depot forms of drugs containing a carboxyl group. Esters undergo enzymatic cleavage in vivo to the free acids.

The ester of glycollic acid diethanolamide and (I) (XIV) was obtained by heating the sodium salt of bruneomycin (XII) with chloroacet-N-di(2-hydroxyethyl)amide (XIII) [5] in DMF at 80°C, the product being isolated by column chromatography on silica gel.

For comparison of their biological properties, the previously reported [10, 12] mono- (XV) and dimethyl esters (XVI) of bruneomycin were prepared. The dimethyl ester (XVI) was obtained by prolonged treatment of (I) with dimethyl sulfate in dioxane. The biological activity of the previously prepared amide of bruneomycin with the anthracycline antibiotic daunorubicin (XVII) [4] was also examined.

Antibacterial activity was assessed by the disc method using test cultures of the Gram-positive microorganism B. mycoides and B. subtilis. The cytotoxic activity of the test compounds was assessed in tests using cultured cells of human ovarian carcinoma (strain CaOv). The criterion of activity was inhibition of the incorporation of ^{3}H-thymidine into the cellular DNA. Using the results obtained, a plot was constructed from which was found the semi-effective dose (SE_{50}) at the 95% confidence level. The method has been described [1].

The presence of antiretroviral activity in the test antibiotics was detected by their ability to inhibit the transformationally degradative effects of mouse sarcoma virus (MSV) in infected mouse cells. Since low multiples of infection were used in the tests (approximately 0.01 parts of the 50% transforming doses (TD_{50}) per cell), inhibition of the transformationally degradative effect indicates suppression of multiplication of the virus.

The biological test results for the bruneomycin derivatives are shown in Table 1. It will be seen that only (XIV) and bruneomycinyl-N-4-aminobutyric acid (IX) retain high cyto-

TABLE 2. Properties of Compounds Obtained

Compound	Yield, %	mp., °C	R_f (system)	Empirical formula
VII	61,5	>300	0,2 (B)	$C_{32}H_{34}N_8O_7$
VIII	58,0	>300	0,28 (B)	$C_{35}H_{39}N_7O_7$
IX	76,0	194—196	0,25 (A)	$C_{28}H_{29}N_5O_9$
X*	71,6	117—118	0,51 (C)	$C_{31}H_{33}N_5O_{10}S$
XI**	68,7	118—119	0,51 (C)	$C_{31}H_{33}N_5O_{10}S$
XIV	53,0	139—141	0,37 (A)	$C_{31}H_{33}N_5O_{11}$

*$[\alpha]_D^{20}$ −64° (c 0.25 MeOH).
**$[\alpha]_D^{20}$ + 62.5° (c 0.16 MeOH).

toxic and antiviral activity. The absence of antitumor and antiviral activity in the di- (and tri-)azaadamantanes (VII, VIII) is probably due to steric hindrance of the amide bond, analogous amides of bruneomycin and anthracycline antibiotics likewise failing to show cytotoxic or antiviral activity [4]. There have been reports [3] that di- (and tri-)azaadamantanes display biological activity only in vivo, so that further examination of compounds of this type is desirable.

EXPERIMENTAL (CHEMISTRY)

TLC was carried out on Silufol UV-254 plates (Czech SSR) in the systems: chloroform—acetone—methanol, 8:1:1 (A), butanol—acetic acid—water, 13:5:5 (B), and chloroform—methanol, 45:5 (C). Preparative separation was effected on a column of Kieselgel-60 (0.040-0.063 μ), or on glass plates with layers of Kieselgel-60 PF_{254} (Merck, West Germany). Melting points were obtained on a Buchi SMP-20 instrument (Switzerland), and ^1H NMR spectra on a Bruker HW-360 spectrometer (West Germany) in $CDCl_3$.

The elemental analyses were in agreement with the calculated values.

N-Bruneomycinyl-7-amino-1,3,5-triazaadamantane (VII). To a solution of 30 g of (I) in 8 ml of dioxane was added 5.93 mg (0.059 mmole) of N-hydroxysuccinimide, and when this had dissolved, 12.21 mg (0.059 mole) of dicyclohexylcarbodiimide. The mixture was stirred for 1 h at 20°C, then 9.13 g (0.059 mmole) of (II) was added. After stirring for 72 h at 20°C, the mixture was evaporated to dryness under reduced pressure, and the residue dissolved in the minimum volume of chloroform and purified by preparative TLC in system A. The product was eluted with a mixture of chloroform and methanol (1:1), evaporated, and the residue washed with dry ether and dried in vacuo to give 24 mg of (VII) (Table 2). ^1H NMR spectrum, δ, ppm., J, Hz: 2.44 s (3H, 3^1-CH_3), 3.83 s (6H, N—CH_2—N), 3.95 s (3H, OCH_3), 3.98 s (3H, OCH_3), 4.10 s (3H, OCH_3), 4.27 d (3H), 4.53 d (3H), 5.08 s (2H, 5-H_2), 6.66 d (1H, H-5^{II}), 6.77 d (1H, J_{6II5II} 8.7, H-6^{II}), 7.58 s (1H, 2^I-CONH), 8.44 d (1H, H-4^I), 8.61 d (1H, J_{3I4I} 8.3, H-3^I).

N-Bruneomycinyl-6-amino-5,7-dimethyl-1,3-diazaadamantane (VIII). Obtained as for (VII), from 30 mg (0.059 mmole) of (I) and 10.79 mg (0.059 mmole) of (III). There was isolated 23.66 mg of (VIII) (Table 2). ^1H NMR spectrum, δ, ppm., J, Hz: 0.95 s (6H, CH_3), 2.80-3.04 m (4H), 2.46 s (3H, 3-CH_3), 3.95 s (3H, OCH_3), 3.99 s (3H, OCH_3), 4.09 s (3H, OCH_3), 4.29-3.90 m (4H), 4.31 d (1H), 5.08 s (2H, 5-NH_2), 5.91 s (1H, OH), 6.66 d (1H, H-5^{II}), 6.79 d (1H, J_{6II5II} 8.6, H-6^{II}), 8.18 d (1H, 2-CONH), 8.42 d (1H, H-4^I), 8.58 d (1H, J_{3I4I} 8.4, H-3).

N-Bruneomycinyl-4-aminobutyric Acid (IX). To 12.5 mg (0.12 mmole) of 4-aminobutyric acid in 3 ml of acetonitrile was added 0.055 ml of N,O-bistrimethylsilyltrifluoroacetamide. The mixture was boiled for 2 h until the solid had dissolved, cooled, and a mixture obtained by adding 11.1 mg (0.1 mmole) of N-hydroxysuccinimide and 20.6 mg (0.1 mmole) of dicyclohexylcarbodiimide in 5 ml of dichloromethane to 50.6 mg (0.1 mmole) of (I) was added. This mixture was stirred for 48 h at 20°C, then 1.5 ml of methanol was added, and stirring continued for another 2 h. After evaporation under reduced pressure, the residue was dissolved in chloroform, and purified by TLC in system A to give 48 mg of (IX). ^1H NMR spectrum, δ, ppm., J, Hz: 2.02 m (2H, 3-CH_2), 2.42 s (3H, 3^I-CH_3), 2.51 m (2H, 2-CH_2), 3.58 m (2H, 4-CH_2), 3.97 s (3H, OCH_3), 4.04 s (3H, OCH_3), 4.08 s (3H, OCH_3), 5.11 s (2H, 5-H_2), 6.63 d (1H, H^{II}-5), 6.77 d (1H, J_{6II5II} 8.6; H-6^{II}), 8.15 t (1H, 2-CONH), 8.38 d (1H, H-4^I), 8.73 d (1H, J_{3I4I} 8.4, H-3^I).

N-Bruneomycinyl-L-S-methylthiomethycysteine Methyl Ester (X). To 5.06 mg (0.01 mmole) of (I) in 1 ml of chloroform were added 1.11 mg (0.01 mmole) of N-hydroxysuccinimide and 2.06 mg (0.01 mmole) of dicyclohexylcarbodiimide, and the mixture stirred for 30 min at 20°C. After adding 2.54 mg (0.011 mmole) of (V) hydrochloride and 1.11 mg (0.011 mmole) of N-methylmorpholine in 1 ml of chloroform, the mixture was stirred for 24 h at 20°C. The product was isolated by preparative TLC in system A to give 4.9 mg of (X). ^{1}H NMR spectrum, δ, ppm., J, Hz: 2.13 s (3H, $COOCH_3$), 2.45 s (3H, 3^I-CH_3), 3.25 d.d (1H, J_{vic} 5.7; S-CH_2), 3.33 d.d, (1H, J_{vic} 4.9, J_{gem} 13.9; S-CH_2), 3.72 s (2H, S-CH_2-S), 3.84 s (3H, SCH_3), 3.94 s (3H, OCH_3), 3.97 s (3H, OCH_3), 4.06 s (3H, OCH_3), 5.01 m (1H, NH-CH), 5.11 br. s (2H, 5-NH_2), 6.66 d (1H, H-5^{II}), 6.79 d (1H, $J_{5^{II}6^{II}}$ 8.6; H-6^{II}), 8.41 d (1H, H^I-4), 8.71 d (1H, J_{NH-CH} 7.9; 2-CONH), 8.84 d (1H, $J_{3^I4^I}$ 8.6; H-3^I).

N-Bruneomycinyl-D-S-methylthiomethylcysteine Methyl Ester (XI). Obtained as for (X), from 5.06 mg of (I) and 2.54 mg of (VI) hydrochloride, yield 4.7 mg. ^{1}H NMR spectrum, δ, ppm, 2, Hz: 2.14 s (3H, $COOCH_3$), 2.45 s (3H, 3^I-CH_3), 3.26 d.d (1H, S-CH_2), 3.32 d.d (1H, S-CH_2), 3.72 s (2H, S-CH_2-S), 3.84 s (3H, S-CH_3), 3.94 s (1H, OCH_3), 3.98 s (1H, OCH_3), 4.07 s (3H, OCH_3), 5.01 m (1H, NH-CH), 5.10 br. s (2H, 5-NH_2), 6.66 d (1H, H-5^{II}), 6.79 d (1H, $J_{5^I6^{II}}$ 8.6; H-6^{II}), 8.43 d (1H, H-4), 8.74 d (1H, J_{NH-CH} 7.8; 2-CONH), 8.87 d (1H, $J_{3^I4^I}$ 8.4; H-3^I).

Bis-(2-hydroxyethyl)aminocarbonylmethyl Ester of Bruneomycin (XIV). A mixture of 50 mg (0.095 mmole) of (XII) and 17.6 mg (0.095 mmole) of (XIII) in 0.8 ml of DMF was heated at 80°C for 1.5 h, cooled, and 10 ml of ether added. The solid which separated was air-dried and purified on a column of silica gel. The product was eluted with a mixture of chloroform and methanol (96:4), yield 33 mg, and recrystallized from chloroform. ^{1}H NMR spectrum, δ, ppm., J, Hz: 2.34 s (3H, 3^I-CH_3), 3.48-3.64 m (4H), 3.75-3.90 m (4H), 3.94 s (3H, OCH_3), 3.97 s (3H, OCH_3), 4.07 s (3H, OCH_3), 5.02 s (2H, OCH_2CO), 5.09 s (2H, 5-NH_2), 6.04 br. s (1H, OH), 6.65 d (1H, H-5^{II}), 6.79 d (1H, H-6^{II}), 8.37 d (1H, H-4^I), 8.95 d (1H, H-3^I).

EXPERIMENTAL (BIOLOGY)

Activity against the Moloney sarcoma virus was examined. Transplanted cells of C127 mice, grown for 24 h in 96-hole planchets (Flow-Lab), were treated with DEAE-dextran (2.5 μg/ml, 30 min, room temperature), then infected with Moloney strain MSV (approximately 300 TD_{50} per hole). After adsorption of the virus for 1 h at 37°C, growth medium containing the test antibiotic in the appropriate concentration was added to the test cultures. The cultures were observed for five days, the transformationally degradative and cytostatic activity of the drug being recorded.

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