8.85 (br s, 1 H, NH); MS (CI) m/z 273 $[M + H]^+$.

N-[4-[N-[(2,4-Dichloroquinolin-6-yl)methyl]-N-prop-2-ynylamino]-2-fluorobenzoyl]-L-glutamic Acid (2dd). Method S. The diester **3dd** (0.18 g, 0.279 mmol) was stirred for 15 min in trifluoroacetic acid (1.8 mL). The trifluoroacetic acid was evaporated and the residue dissolved in 1 N aqueous NaOH (5 mL), filtered, and acidified to pH 3 with 2 N HCl. The resulting precipitate was isolated by filtration, washed with H_2O (20 mL), and dried to give **2dd** as a white amorphous solid: 107 mg (72%); mp 88–94 °C; NMR δ (Me_2SO-d_6) 2.00 (m, 2 H, CH_2), 2.31 (t, J = 7.5 Hz, 2 H, CH_2CO_2H), 3.28 (br s, 1 H, C≡CH), 4.37 (m, 1 H, NHC*H*), 4.41 (d, J = 1.5 Hz, 2 H, $CH_2C{\equiv}CH$), 4.96 (s, 2 H, CH_2N), 6.65 (m, 2 H, Ar 3′-H and 5′-H), 7.54 (t, J = 9 Hz, 1 H, Ar 6′-H), 7.85 (dd, J = 8.5 and 1.5 Hz, 1 H, Ar 7-H), 7.93 (s, 1 H, Ar 3-H), 8.01 (d, J = 8.5 Hz, 1 H, Ar 8-H), 8.12 (d, J = 1.5 Hz, 1 H, Ar 5-H); MS (FAB) m/z 530 $[M - H]^-$. Anal. ($C_{25}H_{20}Cl_2FN_3O_5 \cdot H_2O$) C, H, N.

This procedure was repeated with diesters **3u** and **3v** to give **2u** and **2v** as amorphous solids. The compounds had correct elemental analyses (C, H, N) for the formulae listed (Table I) and NMR and mass spectra consistent with the assigned structures.

Registry No. 1, 76849-19-9; **2a**, 123637-02-5; **2b**, 123636-84-0; **2c**, 123637-07-0; **2d**, 123637-10-5; **2e**, 123636-86-2; **2f**, 123637-00-3; **2g**, 123636-98-6; **2h**, 123636-89-5; **2i**, 141848-56-8; **2j**, 123636-91-9; **2k**, 123636-90-8; **2l**, 123636-88-4; **2m**, 123636-97-5; **2n**, 123637-13-8; **2o**, 123636-71-5; **2p**, 123637-06-9; **2q**, 141848-57-9; **2r**, 123637-11-6; **2s**, 123637-05-8; **2t**, 123636-76-0; **2u**, 123637-26-3; **2v**, 123636-69-1; **2w**, 123637-16-1; **2x**, 123637-09-2; **2y**, 123636-72-6; **2z**, 123651-23-0; **2aa**, 123637-22-9; **2bb**, 123636-66-8; **2cc**, 123636-67-9; **2dd**, 123636-65-7; **2ee**, 123636-77-1; **3t**, 141848-58-0; **3bb**, 123637-34-3; **3dd**, 141848-59-1; **4**, 76858-72-5; **5**, 70280-71-6; **6**, 2378-95-2; **7**, 13726-52-8; **8a**, 123637-49-0; **8b**, 141848-60-4; **8e**, 141848-61-5; **8g**, 123637-46-7; **8h**, 123637-33-2; **8l**, 141848-62-6; **8m**, 123637-43-4; **8n**, 123637-71-8; **8o**, 123637-51-4; **8q**, 141848-63-7; **8s**, 123637-54-7; **8v**, 123651-25-2; **8w**, 123637-77-4; **8x**, 123637-60-5; **8v**, 123637-55-8; **9a**, 123638-03-9; **10c**, 75896-58-1; **10d**, 123637-61-6; **10f**, 123637-47-8; **10g**, 123637-45-6; **10h**, 6270-08-2; **10n**, 123637-70-7; **10p**, 123637-58-1; **10q**, 123637-66-1; **10r**, 123637-63-8; **10t**, 123651-26-3; **10u**, 123637-82-1; **10x**, 3913-18-6; **10z**, 123637-72-9; **10aa**, 123637-85-4; **11**, 25428-07-3; **12**, 123637-44-5; **13**, 123638-04-0; **14**, 141848-64-8; **15**, 90033-68-4; **17**, 1677-44-7; **18**, 4053-34-3; **19**, 123638-05-1; **20**, 23947-37-7; **21**, 106585-57-3; **22**, 112888-47-8; **23**, 123637-80-9; thymidylate synthase, 9031-61-2; 6-(hydroxymethyl)-2-methyl-4-quinolone, 123651-24-1; cinnamoyl chloride, 102-92-1; *p*-toluidine, 106-49-0; pivaolyl chloride, 3282-30-2.

Inhibition of Collagenase by Aranciamycin and Aranciamycin Derivatives

Mikael Bols*

Department of Organic Chemistry, The Technical University of Denmark, Building 201, DK-2800 Lyngby, Denmark

Lise Binderup, Jytte Hansen, and Poul Rasmussen

Leo Pharmaceutical Products, DK-2750 Ballerup, Denmark. *Received January 21, 1992*

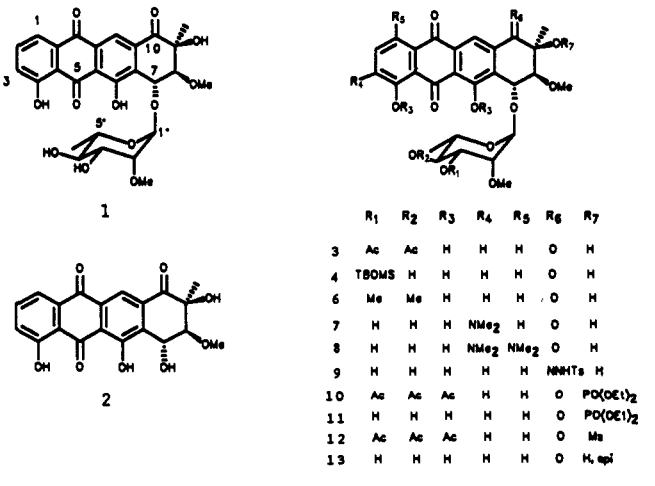
Aranciamycin (1), an anthracycline antibiotic, was found to be an inhibitor of *Clostridium histolyticum* collagenase, with an $IC_{50} = 3.7 \times 10^{-7}$ M. Elastase and trypsin were not inhibited at concentrations $\leq 10^{-6}$ M. A number of aranciamycin derivatives **2–13** were prepared and tested for collagenase inhibition. While loss of activity was found for derivatives modified in the sugar ring or rings B and D of the aglycone, increased potency was found when the tertiary alcohol at C-9 was esterified. All compounds **1–13** were found to inhibit DNA synthesis of Yoshida sarcoma tumor cells.

Introduction

Collagenase, the metalloprotease that cleaves collagen, plays an important role in the organism by "tidying up" dead or defective connective tissue.[1] However uncontrolled high levels of collagenase are suspected to be a major destructive instrument of several diseases such as arthritis[2] or tumor metastasis.[3] Thus, there is an increasing interest in collagenase inhibitors as therapeutic agents.[4]

The classes of compounds that have been found to inhibit collagenase include mostly peptide analogs,[5] but recently tetracyclines[6] and anthraquinones[7] have been found to be moderate inhibitors.

Scheme I



In this paper we report the inhibition of collagenase by the naturally occuring antibiotic aranciamycin (**1**, Scheme I), the preparation of a number of derivatives of **1**, and our biological findings regarding these derivatives.

(1) Ramachandran, G. N.; Reddi, A. H. *Biochemistry of Collagen*; Plenum Press: New York, 1976.
(2) Krane, S. M.; Conca, W.; Stephenson, M.; Amento, E. P.; Goldring, M. B. Mechanisms of matrix degradation in rheumatoid arthritis. *Ann. N.Y. Acad. Sci.* **1990**, *580*, 340–354.
(3) Reich, R.; Stratford, B.; Klein, K.; Martin, G. R.; Mueller, R. A.; Fuller, G. C. Inhibitors of collagenase IV and cell adhesion reduce the invasive activity of malignant tumor cells. *Ciba Found. Symp.* **1988**, *141*, 193–210.
(4) Johnson, W. H.; Roberts, N. A.; Borkakoti, N. Collagenase inhibitors: their design and potential therapeutic use. *J. Enzyme Inhib.* **1987**, *2*, 1–22.
(5) Bartlett, P. A.; Marlowe, C. K.; Giannousis, P. P.; Hanson, J. E. Phosphorus-containing peptide analogs as peptidase inhibitors. *Cold Spring Harbor Symp. Quant. Biol.* **1987**, *52*, 83–90.
(6) Golub, L. M.; McNamara, T. F.; D'Angelo, G.; Greenwald, R. A.; Ramamurthy, N.S. A non-antibacterial chemically-modified tetracycline inhibits mammalian collagenase activity. *J. Dent. Res.* **1987**, *66*, 1310–1314.
(7) Tanaka, T.; Metori, K.; Mineo, S.; Matsumoto, H.; Satoh, T. Studies on collagenase inhibitors. II. Inhibitory effects of anthraquinones on bacterial collagenase. *J. Pharm. Soc. Jpn.* **1990**, *110*, 688–692.

0022-2623/92/1835-2768$03.00/0

Table I. ^{1}H-NMR Chemical Shifts (δ) of Aranciamycin Derivatives

	1	3	4	6	7	8[d]	9	11	13
H-11	8.41	8.41	8.44	8.35	8.41	8.24	8.53	8.44	8.49
H-1	7.89	7.89	7.90	7.84	7.89		7.88	7.89	7.90
H-2	7.76	7.76	7.76	7.73	7.17	6.78	7.75	7.75	7.76
H-3	7.35	7.47	7.36	7.32			7.32	7.35	7.36
H-7	5.22	5.22	5.25	5.21	5.21	5.22	5.28	5.25	5.26
H-8	3.79	3.78	3.80	3.80	3.79	3.78	3.56	4.5	3.76
9-Me	1.54	1.61	1.56	1.56	1.54	1.50	1.57	1.86	1.51
H-1′	5.66	5.62	5.89	5.62	5.66	5.61	5.60	5.65	5.62
H-2′	3.5–6	3.73	3.40	3.66	3.5–6	3.5–6	3.4–5	3.4–6	3.5–6
H-3′	3.5–6	5.03	3.6–8	3.35	3.5–6	3.5–6	3.4–5	3.4–6	3.5–6
H-4′	3.5–6	5.22	3.6–8	3.23	3.47	3.46	3.4–5	3.4–6	3.46
H-5′	3.84	4.03	3.85	3.80	3.83	3.84	3.73	3.92	3.84
H-6′	1.41	1.31	1.35	1.41	1.41	1.36	1.39	1.45	1.42
other		2.06	0.89	3.57	3.13	3.40	8.00	4.1–3	
		2.06	0.05	3.44	(MeN)	3.17	7.39	1.3–4	
		(OAc)	(TBDM)	(OMe)		(MeN)	(Ar)	(EtOP)	
$J_{7,8}$ (Hz)	2.4	2.5	2.5	2.4	2.4	2.5	2.7	2.6	3.6

[a] All spectra were recorded in $CDCl_3$ except 8 which was recorded in d_6-acetone.

Microbiology

In the course of our screening program for new compounds with enzyme inhibiting properties we detected collagenase inhibition in the growth medium of a *Streptomyces* sp. isolated from a soil sample collected at Taman Nagara in Malaysia. The microorganism was identified at the German Collection of Microorganism (DSM) in Braunschweig as a *Streptomyces griseoflavus*. The biological activity was proven to be due to aranciamycin production. Formerly aranciamycin was stated as a product of *Streptomyces echinatus*.[8] The microorganism was maintained on oatmeal agar.

Chemistry

Aranciamycinone (2) was prepared from aranciamycin (1) according to the literature procedure;[8] test compounds **3**,**4**, **6**–**9**, 11, and 13 were prepared from 1 as described below. The structures of the new compounds were determined by ^{1}H-NMR spectroscopy (Table I) and ^{13}C-NMR where appropriate. The chemical shift assignments were made with assistance of previous assignments of 8-demethoxyaranciamycin derivatives[9] and 2-*O*-methylrhamnosides.[10]

Acetylation of 1 gives the 4,6,3′,4′-tetraacetate;[8] we found however that the phenolic acetates in this compound hydrolyzed readily during aqueous workup to give the 3′,4′-diacetate **3**. Similarly silylation of 1 with *tert*-butyldimethylsilyl chloride and aqueous workup gave the 3′-monosilylated derivative **4**. The location of the silyl group was seen by the upfield β-shift of H-2′ (Table I). Alkylated derivative **6** could not be prepared in this manner; therefore it was synthesized by trimethylsilyl triflate-catalyzed glycosidation of **2** with 1-*O*-acetyl-6-deoxy-2,3,4-tri-*O*-methyl-α-L-mannopyranose (**5**). Compound **5** was available by acetylation of known 2,3,4-tri-*O*-methyl-L-rhamnose.[11] The α-configuration of **6** was confirmed by the chemical shift of H-1′ (Table I), being identical to the H-1′ in 1. It has previously been shown that there is a large (0.3 ppm) difference in the chemical shift of H-1 of methyl 2-*O*-methyl α- and β-L-rhamnoside diacetates.[10]

Introduction of amino groups in the D-ring was done by using dimethylamine/MeCN in the presence of air. Reaction of 1 with Me_2NH for 4 days gave three products: Unreacted 1, monoamino derivative **7**, and diamino derivative **8**. The location of the amino group in **7** was determined by a COLOC-experiment showing a three-bond distance between the aminated carbon and a phenolic proton. Tosylhydrazine on the contrary reacted with 1 to form hydrazone exclusively with the ketone of ring A. The ^{1}H-NMR spectrum of the product **9** showed no changes in the chemical shifts of the aromatic protons in ring D, but the singlet aromatic proton H-11 had moved 0.1 ppm downfield compared to 1. The ^{13}C-NMR spectrum also showed changes. While 1 had three signals at 198, 192, and 180 ppm for the three ketones, **9** only had two signals at 191 and 181 ppm and an additional signal in the aromatic region (120–140 ppm). This meant that the less conjugated ketone (C-10) had formed hydrazone. Reaction for 10 h at 100 °C gave **9** in 58% yield.

Protection of 1 as the tetraacetate[8] made it possible to do selective reactions on the tertiary alcohol. Reaction with PCl_5 followed by treatment with EtOH gave the tetraacetylated 9-diethyl phosphate **10**. Deacetylation of **10** using K_2CO_3/EtOH gave 9-(diethoxyphosphoryl)aranciamycin (**11**). Similarly the tetraacetate could be reacted with CH_3SO_2Cl/pyridine to give the mesylate **12**. However **12** reacted with K_2CO_3/MeOH to give mainly a product **13** with a chemical composition identical to 1, but the coupling constant between H-7 and H-8 has increased dramatically (Table I). This meant a conformational change in ring A, and could only be due to a change of stereochemistry at C-9. It has been shown for 8-demethoxyaranciamycin derivatives,[9] that when the configuration of C-9 is inverted to the unnatural configuration, the coupling constants between H-7 and H-8, and H-7 and H-8a increase from 2.0 Hz and 4.8 Hz to 6.0 Hz and 7.0 Hz. On the basis of this we concluded that **13** had to be the 9-epimer of 1.

Biological Results

The effects of 1 and three well-established anthracycline drugs (Daunomycin, Adriamycin, and Aclacinomycin) on collagenase and tumor cell DNA synthesis are listed in Table II. The compounds were tested for inhibition of collagenase acting on a synthetic substrate or intact collagen, and for inhibition of the incorporation of labeled thymidine in the Yoshida sarcoma cells. Compound 1 was

(8) Keller-Schierlein, W.; Sauerbier, J.; Vogler, U.; Zähner, H. Stoffwechselprodukte von Mikroorganismen. Aranciamycin. *Helv. Chim. Acta* **1970**, *53*, 779–789.
(9) Krohn, K.; Broser, E. Synthetic Anthracyclinones. 25. An improved Route to 8-Demethoxyaranciamycinone and Synthesis of the α-L-Daunosamine Glycosides. *J. Org. Chem.* **1984**, *49*, 3766–3771.
(10) Keller-Schierlein, W.; Müller, A. The Sugar Component of Aranciamycin: 2-*O*-Methyl-L-rhamnose. *Experientia* **1970**, *26*, 929–930.
(11) Kuhn, R.; Löw, I.; Trischmann, H. Die Konstitution des α-Chaconins. *Chem. Ber.* **1955**, *88*, 1690–1693.

Table II. Collagenase and Tumor Cell Inhibition of Aranciamycin Derivatives

compd	collagenase inhibn:[a] IC_{50}, μM	inhibn of [^{3}H]Tdr incorp:[b] IC_{50}, μM
1	0.37	2.2
2	19	0.6
3	>100	1.3
4	17	1.4
6	0.67	4.7
7	2.0	0.63
8	>100	5.0
9	0.42	3.9
11	0.22	0.63
13	3.0	1.0
Daunomycin	1.9	<0.02
Adriamycin	1.1	<0.02
Aclacinomycin	9.9	<0.02

[a] Synthetic substrate. [b] Inhibition in Yoshida sarcoma cells.

a 5–30 times more potent inhibitor of collagenase acting on the synthetic substrate than the reference anthracycline drugs. This was even more pronounced for collagenase acting on intact collagen. Aranciamycin had an $IC_{50} = 1.1 \times 10^{-6}$ M, while the three drugs showed inhibition lower than 30% at 10^{-5} M. On the other hand, 1 was at least a 100 times weaker inhibitor of thymidine incorporation in Yoshida tumor cells than the three other compounds.

The compounds were also tested for inhibition of elastase and trypsin. None showed inhibition at 10^{-5} M against these proteases.

The effects of synthetic compounds 2–9, 11, and 13 on collagenase and DNA synthesis are also listed in Table II.

Discussion

Our aim was to produce a potent collagenase inhibitor ideally with a high degree of specificity. Aranciamycin (1), we found, was a potent collagenase inhibitor that did not inhibit the proteases elastase and trypsin. However, the antiproliferative effects exerted by 1 limited its potential clinical usefulness in nonmalignant diseases. The anthracycline antitumor antibiotics Daunomycin, Adriamycin, and Aclacinomycin used in tumor therapy also inhibited collagenase; compared to 1 they were 5–30 times less potent collagenase inhibitors but more than 100 times more potent against tumor cells. Thus these two activities seemed unrelated. To attempt separating the activities or optimizing collagenase inhibition the synthetic derivatives of 1 were studied. As 2 had a 50-fold lower collagenase activity, the sugar seemed to be important. This was emphasized by derivatives 3 and 4, in which blocking of one or both hydroxy groups in the sugar with bulky substituents lowered the activity. Interestingly, 6 had only a slight decrease in biological activity, indicating that the sugar hydroxy groups played a role as hydrogen bond acceptors. The increased bulk and electron donation of dimethylamino groups in the D-ring resulted in loss of activity in compounds 7 and 8. However steric bulk in the A-ring in the form of a tosylhydrazone did not play a role, as the activity of 9 was almost identical to that of 1. However a diethyl phosphate in the 9 position in compound 11 increased the activity 2-fold. Apparently a more lipophilic substituent in this region increased binding. Inversion of configuration at C-9 resulted in an 8-fold loss of activity in 13 compared to 1. So the 9-hydroxy group was however not irrelevant, or the change of conformation in the A-ring affected activity directly. The latter explanation was probably the most likely since conformational change will affect the position of the sugar, relative to the ring system, which has already been established to be important for activity. The fluctuations in tumor inhibition were considerably smaller: From a 6-fold increase to a 2-fold decrease. A slight increase in collagenase/tumor cell inhibition ratio compared to 1 was observed for compounds 6 and 9.

In conclusion we have shown that 1 is not only a potent inhibitor of Clostridium collagenase, but also a tumor cell DNA synthesis inhibitor. Slightly increased collagenase inhibition could be obtained when the tertiary alcohol was esterified; however, convincing separation of collagenase and tumor cell inhibition was not obtained. A slight increase in the collagenase/DNA synthesis ratio was obtained for compounds 6 and 9.

Experimental Section

For the production of aranciamycin, at first the spores of *Streptomyces griseoflavus* are transferred to a propagation medium consisting of: glucose 1%, glycerol 0.75%, corn steep liquor 0.25%, soybean meal 1.5%, sodium chloride 0.4%, magnesium sulfate 0.005%, potassium dihydrogen phosphate 0.006%, ferrous sulfate 0.0005%, cupric sulfate 0.0004%. After four days of incubation at 26 °C on a reciprocating shaker, the culture is transferred to a fermentation tank of 2000 L. The inoculum amounts to 1–2%. The production medium consists of glucose 2%, soybean meal 1%, calcium carbonate 0.02%, and cobalt chloride 0.0001%. The fermentation is performed at 26 °C, at an aeration rate of 0.3 vol/vol per min and with a stirring speed of 100 rpm. After 3–8 days of fermentation, the culture is harvested and the aranciamycin is obtained by extraction.

A. Chemistry. Melting points are uncorrected. NMR spectra were recorded on a Bruker AC-300 instrument with Me_4Si as internal reference. Optical rotations were measured on a Perkin-Elmer PE241 apparatus. TLC was performed on silica gel 60 F_{254} plates (Merck). Elemental analyses were performed by the Microanalytical Laboratory, Leo Pharmaceutical Products.

3′,4′-Di-*O*-acetylaranciamycin (3). Aranciamycin (1, 476 mg), pyridine (15 mL), and acetic anhydride (15 mL) were stirred at 25 °C for 18 h. H_2O (60 mL) was carefully added, and the mixture was extracted with CH_2Cl_2 (3 × 25 mL). The combined organic layers were dried ($MgSO_4$) and concentrated to give a yellow oil (0.55 g). Crystallization from $CHCl_3$–hexanes gave the desired product 3 as orange crystals (289 mg, 53%): mp 135–142 °C; $[\alpha]^{20}_D$ +133° (*c* 1, $CHCl_3$). Anal. ($C_{31}H_{32}O_{14}\cdot H_2O$) C, H.

3′-*O*-(*tert*-Butyldimethylsilyl)aranciamycin (4). Aranciamycin (1, 100 mg), *tert*-butyldimethylsilyl chloride (200 mg), and pyridine (1 mL) were stirred for 3 d at 25 °C. H_2O (0.5 mL) was added, and the mixture was stirred for 1 h. $CHCl_3$ (50 mL) was added, and the organic layer was washed with HCl (1 N, 2 × 20 mL), H_2O (20 mL), $NaHCO_3$ (saturated 2 × 20 mL), and H_2O (20 mL). Drying ($MgSO_4$) and concentration left a yellow residue. Addition of Et_2O–hexanes gave 4 as orange crystals (68 mg, 56%): mp 191–193 °C, $[\alpha]^{20}_D$ +140° (*c* 0.8, $CHCl_3$). Anal. ($C_{33}H_{42}O_{12}Si\cdot H_2O$) C, H.

1-*O*-Acetyl-6-deoxy-2,3,4-tri-*O*-methyl-α-L-mannopyranose (5). 2,3,4-Tri-*O*-methyl-L-rhamnose[11] (0.85 g) was acetylated with pyridine (5 mL) and acetic anhydride (1 mL) for 18 h at 25 °C. H_2O (50 mL) was added over 30 min., and the mixture was extracted with EtOAc (3 × 50 mL). The combined organic phases were dried (Na_2SO_4) and concentrated to a residue that was purified by flash chromatography (EtOAc–pentane 1:1) to give the desired product 5 as a clear syrup (0.88 g, 86%). ^{1}H-NMR ($CDCl_3$) δ 1.30 (d, 3 H, J = 6.2 Hz, CH_3-6), 2.11 (s, 3 H, OAc), 3.4–3.7 (m, 13 H) and 6.16 (d, 1 H, J = 2.0 Hz, H-1). Anal. ($C_{11}H_{20}O_6$) C, H.

3′,4′-Di-*O*-methylaranciamycin (6). Aranciamycinone (2)[8] (200 mg) and 5 (277 mg) were dissolved in dry CH_2Cl_2 (5 mL) and trimethylsilyl triflate (0.16 mL) was added. The mixture was stirred under N_2 at 25 °C for 2 h. CH_2Cl_2 (50 mL) was added, and the organic layer was washed with H_2O (3 × 50 mL), dried ($MgSO_4$), and concentrated to leave a residue (391 mg) containing the desired glycoside 6, 2, and 5. Flash chromatography (EtOAc–pentane–HCOOH 66:33:1) afforded 6 as a slower moving fraction (147 mg). Crystallization from MeOH–CH_2Cl_2 gave orange crystals (130 mg, 44%): mp 116–120 °C, $[\alpha]^{20}_D$ +138° (*c* 0.4, $CHCl_3$). Anal. ($C_{29}H_{32}O_{12}\cdot 2H_2O$) C, H.

3-(Dimethylamino)aranciamycin (7) and 1,3-Bis(dimethylamino)aranciamycin (8). A solution of aranciamycin

(1, 200 mg) in CH_3CN (5 mL) was saturated with Me_2NH (g) and kept for 24 h at 25 °C. Air was bubbled through the solution for 5 min, and the solution was again saturated with Me_2NH (g) and kept for another 24 h at 25 °C. This process was repeated twice. The solvent was evaporated, $CHCl_3$ (50 mL) was added, and the solution extracted with HCl (concentrated, 50 mL). The water layer was washed with $CHCl_3$ (2 × 50 mL) and carefully neutralized with $NaHCO_3$ solution (2 M). The water layer was extracted with $CHCl_3$ (3 × 50 mL), and the combined extracts were dried ($MgSO_4$) and concentrated. The resulting residue was chromatographed (EtOAc–MeOH–HCOOH 83:16:1) to give a faster fraction of **7** as a grey amorphous solid (30 mg, 14%. Anal. ($C_{29}H_{33}NO_{12}$·1.5MeOH), C, H, N.) and a slower fraction of deep blue **8** (70 mg, 30%, mp 220 °C. Anal. ($C_{31}H_{38}N_2O_{12}$·3.5H_2O) C, H, N.).

Aranciamycin 10-(*p*-Tolylsulfonyl)hydrazone (9). A solution of aranciamycin (1, 500 mg) and *p*-toluenesulfonohydrazide (500 mg) in 1,4-dioxane (5 mL) was refluxed for 10 h. Concentration left a red oil, that on addition of MeOH, Et_2O, and pentane gave the desired hydrazone **9** (382 mg, 58%) as a red solid: mp 164–166 °C; $[\alpha]^{20}_D$ +411° (*c* 0.02, MeOH). Anal. ($C_{34}H_{36}N_2O_{13}S$·1.5H_2O) C, H, N, S.

4,6,3′,4′-Tetra-*O*-acetyl-9-*O*-(diethoxyphosphoryl)aranciamycin (10). A solution of 4,6,3′,4′-tetra-*O*-acetylaranciamycin[8] (2.0 g) in CH_2Cl_2 (20 mL) was stirred with $CaCO_3$ (2.2 g) in CH_2Cl_2 at 0 °C, and PCl_5 (4.0 g) was slowly added. After stirring 1 h at 25 °C, $CHCl_3$ (20 mL) and EtOH (20 mL) were added. The mixture was neutralized by addition of $CaCO_3$ (2.2 g), filtered, and concentrated to a yellow oil that was dissolved in toluene–Et_2O 5:1 (120 mL) and washed with H_2O (3 × 300 mL). The organic layer was dried ($MgSO_4$) and concentrated to a residue that was purified by flash chromatography (EtOAc–$CHCl_3$ 1:2) to give the desired product **10** (1.69 g 63%) as an amorphous solid: ^{1}H-NMR ($CDCl_3$) δ 1.3–1.45 (m, 6 H, $(EtO)_2P$), 1.93 (s, 3 H, 9-Me), 4.15–4.3 (m, 4 H, $(EtO)_2P$), 4.60 ppm (d, 1 H, H-8, $J_{7,8}$ = 3.1 Hz); MS (FAB) 871 ($M + Na^+$), 849 ($M + H^+$). Anal. ($C_{39}H_{45}O_{19}P$·0.5H_2O) C, H.

9-*O*-(Diethoxyphosphoryl)aranciamycin (11). A solution of **10** (1.69 g) in EtOH (170 mL) was stirred with K_2CO_3 (4.2 g) at 25 °C for 18 h. Hydrochloric acid (36%) was added to color change, and the mixture was filtered and concentrated. Purification by flash chromatography (EtOAc–HCOOH 99:1) gave the desired product (0.87 g, 64%). Addition of EtOH gave orange crystals (0.39 g): mp 175–178 °C, $[\alpha]^{20}_D$ +89° (*c* 1, $CHCl_3$); MS (FAB) 703 ($M + Na^+$), 681 ($M + H^+$). Anal. ($C_{31}H_{37}O_{15}P$·2H_2O) C, H, P.

4,6,3′,4′-Tetra-*O*-acetyl-9-*O*-(methylsulfonyl)aranciamycin (12). To a solution of 4,6,3′,4′-tetra-*O*-acetylaranciamycin[8] (0.5 g) in pyridine (5 mL) was added methanesulfonyl chloride (0.5 mL) at 0 °C. The mixture was stirred at 25 °C for 2.25 h. $CHCl_3$ (50 mL) was added, and the solution was washed with H_2O (5 × 50 mL). The organic layer was dried, filtered, and concentrated to a residue that was purified by flash chromatography (EtOAc–$CHCl_3$ 1:2) to afford the desired product as a yellow amorphous substance (0.40 g, 73%): ^{1}H-NMR ($CDCl_3$) δ 2.03, 2.07, 2.09 (3s, 9 H, 2Ac and 9-Me), 2.45 (Ac), 2.56 (Ac), 3.24 (CH_3SO_2). Anal. ($C_{36}H_{38}O_{18}S$·0.5H_2O) C, H.

9-Epiaranciamycin (13). A solution of **12** (1.1 g) in MeOH (100 mL) was stirred with K_2CO_3 (2.5 g) for 4 d and filtered. To the filtrate was added H_2O (100 mL) and HCl (4 M) to color change. The solution was extracted with $CHCl_3$ (3 × 30 mL). The combined organic layers were dried ($MgSO_4$) and concentrated to a yellow oil (0.63 g). Flash chromatography (EtOAc–pentane–HCOOH 66:33:1) gave the desired product as a yellow crystalline solid (0.19 g, 26%): mp 256–258 °C, $[\alpha]^{20}_D$ +111° (*c* 0.1, acetone). ($C_{27}H_{28}O_{12}$·0.33H_2O) C, H.

B. Biologal Methods. Inhibition of Tumor Cell DNA Synthesis. Yoshida sarcoma cells were passaged weekly by intraperitoneal administration to Lewis rats. For in vitro assays, cells were collected from the peritoneal cavity, washed with Hank's Balanced Salt Solution (BSS), suspended in growth medium (RPMI 1640 (Gibco), supplemented with 10% Fetal Calf Serum, 100 μg/mL penicillin, 100 units/mL streptomycin, and 2 mM L-glutamine) and incubated at 37 °C in an atmosphere of 5% CO_2 in air. The nonadherent tumor cells were collected after 2 h, adjusted to 10^5 cells/mL in growth medium and incubated for 24 h in the presence or absence of test compounds (10^{-8}–10^{-4} M). [^{3}H]thymidine ([^{3}H]TdR, 1 μCi/mL, 5 Ci/mmol, Amersham, U.K.) was added during the last 4 h of culture. Assessment of [^{3}H]TdR incorporation into DNA was measured as previously described.[12] The concentration resulting in 50% inhibition of [^{3}H]TdR incorporation (IC_{50}) was calculated from the dose–response curve.

Inhibition of Collagenase Activity. The assay using native collagen was performed according to Mandl et al.[11] Otherwise, collagenase isolated from *Clostridium histolyticum* (Boehringer-Mannheim) was dissolved at 0.5 mg/mL in 0.1 M Tris-HCl, pH 7.1. The substrate [[[4-(phenylazo)benzyl]oxy]carbonyl]-L-prolyl-L-leucyl-glycyl-L-prolyl-D-arginine (Sigma) was dissolved at 5 mg/mL in methanol. Prior to use, it was diluted 10× with 0.1 M Tris-HCl, pH 7.1. Substrate solution (0.5 mL) was incubated with 0.1 mL 0.1 M $CaCl_2$ for 5 min at 37 °C, in the presence or absence of test compounds (10^{-8}–10^{-4} M). Collagenase solution (50 μL) was added, and the reaction was allowed to proceed for 30 min at 37 °C. The reaction was stopped with citric acid, 25 nM, pH 3.5. After extraction with ethyl acetate, the organic phase was dried with anhydrous sodium sulfate and measured at 320 nM.

Inhibition of Elastase Activity. Elastase, isolated from porcine pancreas, in suspension (Sigma) was diluted to 2 mg/mL with phosphate-buffered saline (PBS), pH 7.5. The substrate was *N*-acetyl-L-alanyl-L-alanyl-L-alanine-*p*-nitroanilide (Sigma), 0.2 mM in PBS containing 5% DMSO. Substrate solution (2.0 mL) was incubated for 5 min at 37 °C in the presence or absence of test compounds (10^{-8}–10^{-4}). Elastase solution (50 μL) was added, and the reaction was followed spectrophotometrically (410 nm) for 5 min.

Inhibition of Trypsin Activity. Trypsin (Sigma) was dissolved at 1 mg/mL in PBS, pH 7.5. The substrate was *N*-α-benzoyl-L-arginine-*p*-nitroanilide (Sigma) 0.2 mM in PBS containing 5% DMSO. Substrate solution (20 mL) was incubated for 5 min at 37 °C in the presence or absence of test compounds (10^{-8}–10^{-4}). Trypsin solution (50 μL) was added, and the reaction was followed spectrophotometrically (410 nm) for 5 min.

Compounds. Test compounds were dissolved in DMSO. Control cultures and samples received DMSO, at a maximum concentration of 0.5%.

Registry No. 1, 72389-06-1; 1 tetraacetate, 141958-46-5; **2**, 95722-75-1; **3**, 141958-38-5; **4**, 141958-39-6; **5**, 142035-26-5; **6**, 132111-78-5; **7**, 141958-40-9; **8**, 141958-41-0; **9**, 141958-42-1; **10**, 141958-43-2; 11, 141958-44-3; **12**, 141958-45-4; **13**, 142035-27-6; collagenase, 9001-12-1; 2,3,4-tri-*O*-methyl-L-rhamnose, 7439-05-6; elastase, 9004-06-2; trypsin, 9002-07-7.

(12) Binderup, L.; Bramm, E.; Arrigoni-Martelli, E. Peritoneal macrophages from adjuvant arthritic rats enhance tumor cell growth in vitro. *Experientia* **1979**, *35*, 1230–1231.

(13) Mandl, I.; MacLennan, J. D.; Howes, E. L.; DeBellis, R. H.; Sohler, A. Isolation and characterization of proteinase and collagenase from Clostridium histolyticum. *J. Clin. Invest.* **1953**, *32*, 1323–1329.