MULTISTEP BIOCONVERSION OF 20-DEOXO-20-DIHYDRO-12,13-DEEPOXY-12,13-DEHYDROROSARANOLIDE TO 22-HYDROXY-23-*O*-MYCINOSYL-20-DEOXO-20-DIHYDRO-12,13-DEEPOXY-ROSARAMICIN

Sir:

We set out to mutasynthesize hybridized antibiotics encampassing the biological activity of rosaramicin[1~3] and oral absorption characteristics of AR-5 antibiotics (mycinamicins)[4,5]. The results of our efforts are reported here.

Non-antibiotics producing mutant GS-9001 was isolated from a population of mycinamicin producing *Micromonospora polytrota* (NRRL 12066, ATCC 31584)[6,7] after treatment with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (NTG). The mutant is blocked in the biosynthesis of mycinamicins, but capable of converting exogenously added 20-deoxo-20-dihydro-12,13-deepoxy-12,13-dehydrorosaranolide[3] (**I**) to 20-deoxo-20-dihydrodeepoxyrosaramicin (**II**), [23-*O*-(2'',3''-didemethylmycinosyl)]-20-deoxo-20-dihydrodeepoxyrosaramicin (**III**), [23-*O*-(3''-demethylmycinosyl)]-20-deoxo-20-dihydrodeepoxyrosaramicin (**IV**), 23-*O*-mycinosyl-20-deoxo-20-dihydrodeepoxyrosaramicin (**V**), and 22-hydroxy-23-*O*-mycinosyl-20-deoxo-20-dihydrodeepoxyrosaramicin (**VI**).

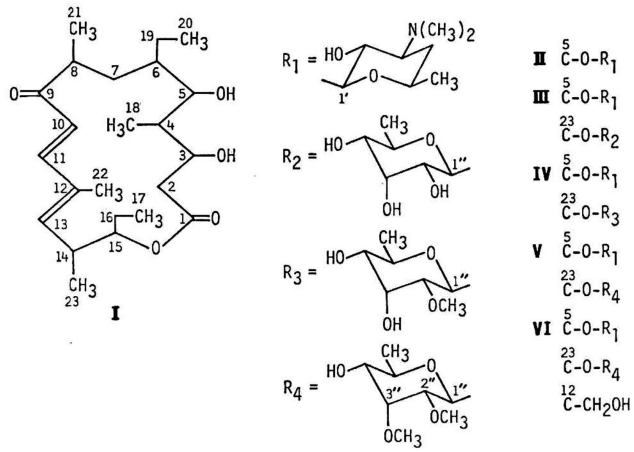
For preparative fermentation, the mutant GS-9001 was fermented at 30°C for 120 hours in a 75-liter fermentor containing 50 liters medium consisting of 50 g starch, 7.5 g distillers soluble, 5 g Pharmamedia, 5 g Cerelose and 1 ml antifoam-1 in tap water. Compound **I** (2.5 g) was added at 24 hours, and the fermentation continued for additional 96 hours to convert the substrate. Fermentation broth was harvested and extracted with 100 liters of ethyl acetate at pH 9.5. The organic extract was concentrated, and the resulting oily residue was re-dissolved in a small volume of acetone and the antibiotic complex (360 mg) was precipitated with a mixture of ethyl ether - hexane (6: 4). Compounds **II**, **III**, **IV**, **V** and **VI** were isolated by silica gel column chromatography of the crude antibiotic complex using a mixture of toluene - methylene chloride - methanol - methanol saturated with ammonia (16: 4: 1: 1) for elution.

In order to determine the sequence of the bioconversion of compound **I** to the other compounds, we performed feeding experiments in tubes using mutant GS-9001. The tube fermentation results were: Compound **II** was converted to **III**, **V** and **VI**; and compound **III** or **IV** was transformed to **V** and **VI**. We did not have enough material to test compound **V** for its conversion to **VI**. Compound **VI** did not convert to any new antibiotic. Compound **IV** was not detectable in the incubation mixtures of the mutant with compound **II** or **III**. Probably, compound **IV** was formed from conversion of **III**. But the subsequent conversions of **IV** to **V** and **VI** may have been too rapid to be detectable under the conditions we examined. Based on these data, it was likely that compound **I** converted in a sequential order to compounds **II**, **III**, **IV**, **V** and **VI** (Fig. 1). The structures of new isolates were based upon spectroscopic data. The structures of **I** and **II**, a cofermentation product of rosaramicin, have previously been reported.[6]

Compound **III** ($C_{37}H_{63}O_{12}N$, *m/z* 713) indicated the presence of proton resonances due to methyl groups at δ 0.8~1.38, 1.80 (broad doublet) and 2.24 (s, $N(CH_3)_2$), methine resonances at δ 4.22 (d, 6.0, H-1'), 4.52 (d, 8.0, H-1'') and 4.88 (*m*, CH–O–C=O), and olefinic protons at δ 5.84 (dd, 10.0, 1.0), 6.22 (d, 16.0) and 7.22 (d, 16.0). Mass spectroscopic data indicated diagnostic ions at 713 (M^+), 567 (H-aglycone-*O*-desosamine), 393 (H-*O*-aglycone), 376 (aglycone), 174 (desosamine-*O*), 158 (desosamine, base peak), 163 (mycinose-*O*) and 147 (mycinose lacking two CH_3 groups). ^{13}C NMR spectrum was also consistent with the assigned structure.*

^1H NMR spectrum of compound **IV** ($C_{35}H_{65}O_{12}N$, *m/z* 727) indicated the presence of methyl groups at δ 0.86 (t), 0.92 (t), 1.12~1.22 (d), 1.80 (d, 1.0), 2.38 (s, $N(CH_3)_2$) and 3.50 (s, OCH_3), methine protons at δ 4.22 (d, 6.0, H-1'), 4.56 (d, 8.0, H-1'') and 4.98 (m, CH–O–C=O) and olefinic protons at δ 5.90 (dd, 10.0, 1.0), 6.24 (d, 15.0) and 7.30 (d, 15.0). Mass spectrometric data indicated diagnostic ions at 727 (M^+), 377 (H-aglycone), 376 (aglycone), 309 ($M^+-161-157$), 177 (mycinose-*O*, lacks CH_3), 174 (desosamine-*O*), 161 (mycinose lacking one CH_3), 158 (desosamine, base peak) and 74 (HO–CH=CH–OCH_3). Compound **V** ($C_{39}H_{67}O_{12}N$, *m/z* 741) indicated

* The stereochemistry of mycinose sugar is assumed as in mycinamicin antibiotics.



the presence of methyl groups at δ 0.84 (t), 1.10~1.24 (d), 1.78 (d, 1.0), 2.28 (s, $N(CH_3)_2$), 3.48 (s, OCH_3) and 3.62 (s, OCH_3), methine protons at δ 4.28 (d, 6.0, H-1'), 4.60 (d, 8.0, H-1'') and 4.96 (m, CH–O–C=O) and olefinic protons at δ 5.86 (broad doublet, 10.0, 1.0), 6.26 (d, 15.0) and 7.26 (d, 15.0). Mass spectrometric data indicated diagnostic ions at 741 (M^+), 376 (aglycone), 175 (mycinose), 174 (desosamine-*O*), 158 (desosamine) and 88 (CH_3O–CH=CH–OCH_3).

^1H NMR spectrum of VI ($C_{39}H_{67}O_{18}N$, *m/z* 757) indicated the presence of methyl groups at δ 0.86 (t), 0.9 (t), 1.10~1.24 (d), 2.30 (s, $N(CH_3)_2$), 3.48 (s, OCH_3) and 3.61 (s, OCH_3), methine protons at δ 4.28 (d, 6.0, H-1'), 4.60 (d, 8.0, H-1'') and 4.96 (m, CH–O–C=O), and olefinic protons at δ 5.93 (dd, 10.0, 1.0), 6.58 (d, 15.0) and 7.22 (d, 15.0). The CH_2OH function appeared at δ 4.30 (broad). Mass spectroscopic studies indicated ions at 757 (M^+), 393 (H-aglycone), 392 (aglycone), 175 (mycinose), 174 (desosamine-*O*), 158 (desosamine, base peak) and 88 (CH_3O–CH=CH–OCH_3).

The antimicrobial activities of some of these compounds, rosaramicin and mycinamicins were compared for large groups of macrolide sensitive strains. Based upon geometric mean MICs against Gram-positive bacteria, the new macrolides were about two fold less active than rosaramicin and mycinamicins.

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BONG K. LEE
MOHINDAR S. PUAR
MAHESH PATEL
PETER BARTNER
JASON LOTVIN
HANAN MUNAYYER
J. ALLAN WAITZ

Research Division,
Schering-Plough Corporation
Bloomfield, New Jersey 07003, U.S.A.

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