STRUCTURAL FEATURE
OF ANTIBIOTIC A-396-I

Sir:

We have previously reported\textsuperscript{1} the isolation
of a new water-soluble basic antibiotic A-
396-I from Streptoverticillium eurocidicus
A-396-I, which also produces in nearly equal
amounts hygromycin B. The molecular
formula C\(_{19}H\_{35}O\_{13}N_3\) and the IR spectrum
indicated close similarity of this antibiotic
to destomycin A\textsuperscript{2,3} and hygromycin B\textsuperscript{5,6}.

The antibiotic A-396-I (100 mg) was hy-
drolyzed with 4 N HCl at 105°C for 10 hours.
The hydrolysate was passed through an XE-
64 (Na\textsuperscript{+}) column and eluted with 14 % am-
onium hydroxide. The eluate, exhibiting
positive ninhydrin reaction, was concentrated
to dryness to give colorless crystals (16 mg).

By paper chromatographic examination [sol-
vent 1: \(\pi\)-propanol - pyridine - acetic acid
- water (15: 10: 3: 12); solvent 2: \(\pi\)-butanol
- acetic acid - water (4: 1: 2)] the compound
showed somewhat solwer mobility and a
different ninhydrin color in comparison with
a sample of (+) N-methyl-2-deoxystrept-
amine \(\left[\alpha\right]_D^{20} +38.6\pm 0.9^\circ\) (c 0.889, H\(_2\)O),
prepared from a hydrolysate of hygromycin
B). However, quite similar mobility and
color to those of an authentic specimen of
2-deoxystreptamine (Rf 0.40 and 0.20 in the
solvents 1 and 2) was observed. Confirma-
tion of the identity of this compound with
2-deoxystreptamine was made by direct
comparison of the NMR spectra and GLC
using the authentic specimen. Samples were
trimethylsilylated with 25 % bis-(trimethyl-
silyl)-acetamide in pyridine by heating at
75°C for 20 minutes, and analyzed by a
Perkin-Elmer Gas Chromatograph Model
881, on a 6 ft glass column packed with
3.0 % SE-30 coated Chromosorb P, carrier
gas: N\(_2\), temperature programming: 120~
250°C (4°C/minutes). Both samples gave a
peak with retention time of 15.0 minutes.

A ninhydrin-negative effluent fraction
from the chromatography on the resin was
treated with 0.1 N sodium hydroxide for 20
hours at room temperature and gave a nin-
hydrin-positive tests. This was thought to
mean a conversion of destomycin lactam,
contained in this fraction, to its acid form\textsuperscript{3}.

A-396-I was peracetylated with acetic an-
hydride in pyridine and subjected to mass
spectrometry, and the spectrum compared
with that of peracetylated hygromycin B.
In the spectrum of peracetylated A-396-I,
molecular ion peak was not observed. How-
ever, many peaks corresponding to the
fragment ion peaks in the peracetylated

Another portion of A-396-I (20 mg) was
hydrolyzed with 0.5 N H\(_2\)SO\(_4\) at 100°C for 2
hours. The hydrolysate was successively
passed through a Dowex 50 (H\textsuperscript{+}) and then
Dowex I (OH\textsuperscript{−}) columns. Lyophilization of the
effluent gave a colorless powder (1.5 mg).

A-396-I was peracetylated with acetic an-
hydride in pyridine and subjected to mass
spectrometry, and the spectrum compared
with that of peracetylated hygromycin B.

The NMR spectra of A-396-I and hygro-
mycin B hydrochlorides measured in D\(_2\)O
(Figs. 1 and 2) are quite similar except that
a signal (\(-\text{NCH}_3\), 2.81 ppm, 3H) given in
hygromycin B, was not observed in A-396-
I; an anomeric proton (5.29 ppm, J=3 cps)
was observed in the spectra of the both
antibiotics.

A-396-I was peracetylated with acetic an-
hydride in pyridine and subjected to mass
spectrometry, and the spectrum compared
with that of peracetylated hygromycin B.
In the spectrum of peracetylated A-396-I,
molecular ion peak was not observed. How-
ever, many peaks corresponding to the
fragment ion peaks in the peracetylated
hygromycin B with difference of mass number 14 were regularly observed: 916 (930)* (M-CH₃CO₂), 915 (929) (M-CH₃CO₂H), 856 (870) (M-CH₃CO₂H-CH₂CO₂), 855 (869) (M-2CH₃CO₂H), 813 (827) (M-CH₃CO₂H-CH₃CO₂-CH₂CO₂), 796 (810) (M-2CH₃CO₂H-CH₂CO₂), 771 (785) (M-2CH₃CO₂H-2CH₂CO₂), 736 (750) (M-3CH₃CO₂H-CH₂CO₂), 711 (725) (M-CH₃CO₂H-2CH₂CO₂-2CH₂CO₂), 669 (683) (M-3CH₃CO₂H-3CH₂CO₂), 651 (665) (M-2CH₂CO₂H-2CH₂CO₂-2CH₂CO₂), 609 (623) (M-CH₃CO₂H-3CH₂CO₂-3CH₂CO₂). A few common peaks in both spectra were also observed in the lower mass number region: 586 (B-CH₃CO₂), 544 (A-2CH₃CO₂), 543 (A-CH₃CO₂H-CH₂CO₂), 526 (B-2CH₂CO₂H), 483 (A-2CH₃CO₂H-CH₂CO₂), 358 ([C+O, H]-CH₃CO₂H), 298 ([C+O, H]-2CH₂CO₂H). These tentative assignments (described in parentheses) are considered to be reasonable, if the assumed structure of peracylated A-396-I is as shown in Fig. 3. Thus, the sequence of the three moieties of A-396-I is suggested to be the same one as in hygromycin B.

The partial structure of hygromycin B has been elucidated by Wiley et al. Elucidation of the structure of destomycin A has been completed by Kondo et al. More recently Neuss et al. have shown that the structural difference between both antibiotics consists in the presence in hygromycin B of (+)-N-methyl-2-deoxystreptamine in the

* Peaks observed with peracylated hygromycin B.
locus of (−)N-methyl-2-deoxystreptamine in destomycin A. From the above data, the structure of A-396-I is strongly suggested in relation to the established structures of the related antibiotics, except for its stereochemistry.

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