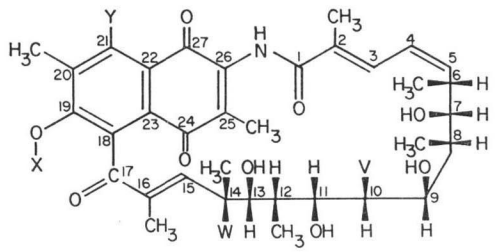
STREPTOVARICIN U, AN ACYCLIC ANSAMYCIN

Sir:

The ansamycins[1] constitute an important class of antibiotics which includes the clinically useful rifamycins and numerous other compounds as well as the streptovaricins, the subject of the present report. We have described earlier our extensive investigations of the components of streptovaricin complex, including the protostreptovaricins (**1**~**5**)[2] and damavaricins (**6**, **7**)[3], presumed biosynthetic precursors of the streptovaricins. We describe here a novel member of this class, streptovaricin U, in which the characteristic *ansa* chain of the ansamycins has been cleaved.



1: V=CH_3; W=X=Y=H
2: V=X=CH_3; W=Y=H
3: V=CH_3; W=OH; X=Y=H
4: V=X=CH_3; W=OH; Y=H
5: V=W=X=Y=H
6: V=$COOCH_3$; W=X=H; Y=OH
7: V=$COOCH_3$; W=Y=OH; X=H

Streptovaricin complex was chromatographed four times over silica gel, employing various mixtures of chloroform - methanol - water and ethyl acetate - ethanol - water as eluants, and finally subjected to countercurrent distribution (C_6H_{12} - EtOAC - 95% EtOH - H_2O = 1: 1: 1: 1, 500 transfers) to give streptovaricin U [0.6% yield from complex, $C_{36}H_{49}NO_{10}$*, orange powder, mp 115~140°C, $[\alpha]_D$ −110.2° (*c* 1, MeOH)], whose molecular formula corresponds to that of a hydrate of protostreptovaricin I (**1**).

Hydrolysis of streptovaricin U in refluxing methanolic sodium hydroxide followed by purification on Biosil A and silica gel and sublimation gave 2-amino-3,7-dimethyl-6-hydroxy-1,4-naphthoquinone (**8**, $C_{12}H_{11}NO_3$**, red needles subliming 150~260°C), which is decolorized by sodium hydrosulfite, whose principal ultraviolet maxima (λ_{max} 274, 281; ϵ 17,000, 18,000) are in the same region as those of 2-amino-1,4-naphthoquinone (λ_{max} 270, log ϵ 4.3)[4] and 6-hydroxy-1,4-naphthoquinone (λ_{max} 270, log ϵ 4.4)[5], and whose ^{1}H NMR spectrum indicates one olefinic methyl and one aromatic methyl group (1.86 ppm and 2.19 ppm, singlets) and two aromatic protons (7.31 ppm, singlet, and 7.65 ppm, broad singlet) as well as an NH_2 group (6.57 ppm, 2 H). Methylation with diazomethane gave **9** ($C_{13}H_{13}NO_3$***, red needles, mp 208~215°C), establishing the phenolic hydroxyl. The identity of the substituents on the aromatic ring was established by the presence of a major C_7H_6O*** ion in the EI mass spectrum of **8**, arising from loss of the quinone ring[6], and the aromatic protons were located at C-5 and C-8 by their lack of splitting. The aromatic methyl was located at C-6 by analogy to the streptovaricins, leaving the hydroxyl for C-7.

The benzenoid structural unit of **8** is found in streptovaricin U: The aromatic methyl and H-5 absorptions in the ^{1}H NMR spectra of **8** (Me_2SO-d_6), protostreptovaricin I (**1**, $CDCl_3$) and streptovaricin U (Me_2SO-d_6) are at nearly identical positions (2.19, 2.21 and 2.25; 7.65, 7.89 and 7.77, respectively) and the H-8 aromatic proton of **8** (7.31 ppm) is found in the ^{1}H NMR spectrum of streptovaricin U at 7.40 ppm. Moreover, the quinone unit of **8** was confirmed in streptovaricin U itself by reductive acetylation of the latter with zinc-acetic anhydride to a colorless hydroquinone triacetate ($C_{42}H_{57}NO_{13}$****, mp 122~128°C, IR 1770 cm^{-1}).

Spin decoupling of the ^{1}H NMR spectrum (C_5D_5N) of streptovaricin U showed the *ansa* side chain to be substituted as in protostreptovaricin I, from C-2 through C-16, as was con-

* Microanalyses, high resolution electron impact mass spectral data and field desorption mass spectral data are in agreement with the molecular formula assigned.

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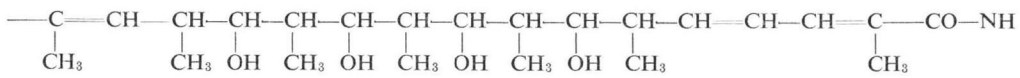
**** Field desorption mass spectral data are in agreement with the molecular formula assigned.

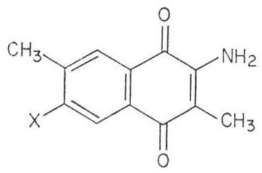
Fig. 1. ^{13}C NMR signals (pyridine solution) for streptovaricin U and protostreptovaricin I (values in brackets).

C-	16	15	14	13	12	11	10	9	8	7	6	5	4	3	2	1
[PSvI]	[132.8	144.5	37.2	70.5	37.4	74.0	34.9	82.0	38.7	83.7	40.7	138.9	125.9	128.8	135.3	167.3]
SvU	130.6	146.2	35.2	71.1	37.2	72.8	34.0	79.2	38.5	82.1	39.2	140.1	*	129.8	132.4	166.8

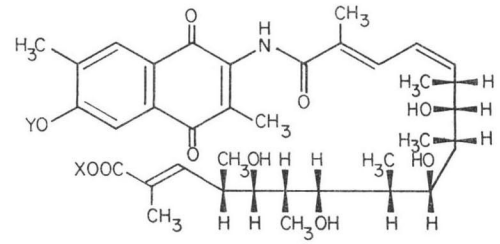


SvU	12.3		12.4		10.6		16.5		13.2		18.4				12.3	
[PSvI]	[12.6		14.6		10.9		17.2		15.0		21.6				12.1]	

* Obscured by solvent line.



8: X=OH
9: X=OCH_3



10: X=Y=H
11: X=CH_3; Y=H
12: X=Y=CH_3

firmed by the correspondence of ^{13}C NMR signals shown in Fig. 1. The nature of the molecular formula of streptovaricin U (that of a hydrate of protostreptovaricin I, **1**) and the replacement of the acyl group at C-18 of **1** by a proton in **8** suggests the addition of a hydroxyl group to the C-17 carbonyl, *i.e.*, that C-17 is in a carboxyl group in streptovaricin U, which then is assigned structure **10**. That assignment is confirmed (1) by the presence of a conjugated carboxyl carbon at 169.9 ppm in the ^{13}C NMR spectrum of streptovaricin U, replacing the conjugated ketone carbon (C-18) of **1**, which is found at 198.0 ppm, and (2) by the conversion of streptovaricin U by diazomethane to its methyl ester (**11**) and 19-O-methyl methyl ester (**12**), both of which give a new infrared band at 1710 cm^{-1} (unsaturated ester).

Identification of streptovaricin U (**10**) as hydrolyzed protostreptovaricin I (**1**) raises the question of whether the *ansa* ring is cleaved enzymatically by *Streptomyces spectabilis* or during isolation by a β-diketone cleavage, *i.e.*, whether streptovaricin U is an artifact of the work-up procedure. Although this point has not been definitively resolved, we favor enzymatic hydrolysis since isolation of streptovaricin complex does not involve base and when protostreptovaricin I (**1**) was treated with methanolic hydroxide (under conditions which give **8** from **10**), neither **8** nor **10** was formed. Streptovaricin U, the first open-chain "ansamycin",* has no antimicrobial or L1210 inhibitory activity but it shows 40% inhibition of RLV RDDP (RAUSCHER leukemia virus RNA-dependent DNA polymerase) at 200 μg/ml, which demonstrates again[7] that the *ansa* ring is unnecessary for reverse transcriptase inhibition.

Acknowledgment

This work was carried out during the period from June 2~December 31, 1975, under Contract NIH-NCI-C-72-3208 from the Division of Cancer Treatment, National Cancer Institute. High resolution and field desorption mass spectra were obtained on a mass spectrometer provided by grants from the National Cancer Institute (CA 11,388) and National Institute of General Medical Sciences (GM 16864). ^{13}C NMR spectra were obtained on a spectrometer purchased with an instrumentation grant from the National Science Foundation.

* Similar open-chain analogs in the rifamycin series have also been reported quite recently[8].

WALTRAUT M. J. KNÖLL
KENNETH L. RINEHART, Jr.

Roger Adams Laboratory,
University of Illinois
Urbana, Illinois 61801

PAUL F. WILEY
LI H. LI

The Upjohn Company
Kalamazoo, Michigan 49001
U.S.A.

(Received November 5, 1979)

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