Relative Biological Activities of Individual Streptovaricins and Streptovaricin Acetates†

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ABSTRACT: The isolation of eight streptovaricins, A–G and J, is described. Structural variations reside within their aliphatic *ansa* chains. The preparation of streptovaricin G triacetate (streptovaricin A diacetate) and of streptovaricin C tri- and tetraacetates (streptovaricins B and J di- and triacetates) is also described. These eight streptovaricins and three synthetic acetates have been assayed for their antibacterial spectra and activity against *E. coli* DNA dependent RNA polymerase. The streptovaricin order of activity in these assays is A, G > B, C > D, J > E ≫ F, while the acetates are totally inactive. The antibiotics and the three synthetic acetates have also been tested against the reverse transcriptase of Rauscher leukemia virus and the degree of inhibition is in the order: streptovaricin A diacetate and streptovaricin C tri- and tetraacetates ≃ streptovaricin D > streptovaricins J, C, G, and B > streptovaricins E, A, and F. Since the reverse transcriptase inhibition pattern is quite different from those for bacterial RNA polymerase and bacterial growth, it is possible to separate these activities from one another.

Streptovaricin, a complex of antibiotics isolated in 1957 (Siminoff *et al.*, 1957), originally elicited interest for its antituberculosis activity (Rhuland *et al.*, 1957), but for some years biological interest in the compound waned. However, following the identification of its mode of action as an antibacterial agent, in inhibiting DNA-dependent RNA polymerase (Yamazaki *et al.*, 1968; Mizuno *et al.*, 1968), the complex has become the subject of renewed interest, heightened in the past 2 years by the observation (Brockman *et al.*, 1971) that streptovaricin is a potent inhibitor of the recently discovered reverse transcriptase (RT,[1] RNA-dependent DNA polymerase) (Temin and Mizutani, 1970; Baltimore, 1970), present in oncogenic RNA virus. In the course of our studies (Rinehart, 1972) on the streptovaricins, during which the structures of the components were assigned, extensive comparisons of the relative activities of the individual streptovaricins have been carried out. We wish to describe here procedures used to isolate the individual components (Figure 1), evidence which assigns the structure of the hitherto unreported streptovaricin J (SvJ),[1,2] and procedures used for the preparation of streptovaricin acetates. We also report here the results of comparative biological studies and draw certain conclusions regarding the structural features important for these activities. Detailed accounts of the chemistry of these intriguing compounds will be published elsewhere.

Experimental Section

General. Melting points are uncorrected and were determined on a Kofler micro hot-stage apparatus. Infrared spectra (KBr pellets or $CHCl_3$ solutions) were obtained on a Perkin-Elmer spectrophotometer, Model 21, ultraviolet spectra on a Perkin-Elmer spectrophotometer, Model 202, or Beckman spectrophotometer, Model DB. Nuclear magnetic resonance spectra were determined in appropriate solvents on Varian Associates spectrometers, Models HA-100 and HR 220; chemical shift values (δ) are reported in ppm from internal tetramethylsilane. Mass spectra were obtained on Atlas CH-4 and CH-5 mass spectrometers with heated direct inlet systems. Molecular weights were established by mass spectrometry, employing low resolution except where high resolution (HRMS) is noted. Microanalyses were obtained at the University of Illinois.

Column chromatographic separations were performed with Merck silica gel (0.05–0.20 mm). Separation of the streptovaricin components was followed by thin-layer chromatography (tlc) and mass spectrometric analysis. The components were observed on tlc by their orange color and fluorescence under ultraviolet light. Thin-layer chromatographic plates (0.25 mm) were prepared with silica gel GF 254 (E. Merck), preparative tlc plates (1 mm) with silica gel PF 254 (E. Merck).

Separation of Streptovaricin Complex. *n*-Hexane (87 ml) was added to a solution of 20 g of streptovaricin complex (Upjohn, 11560-3) in 100 ml of dioxane and the mixture was allowed to stand at room temperature overnight. The orange precipitate (fraction 1, 3.05 g) contained mainly streptovaricin A, with smaller amounts of streptovaricins B and C. The filtrate was diluted with a large amount of *n*-hexane to give an orange-yellow precipitate (fraction 2, 16.20 g) containing a mixture of all the streptovaricins. The mother liquor was concentrated *in vacuo* below 25°, then diluted with *n*-hexane to give another orange-yellow precipitate (fraction 3, 0.55 g)

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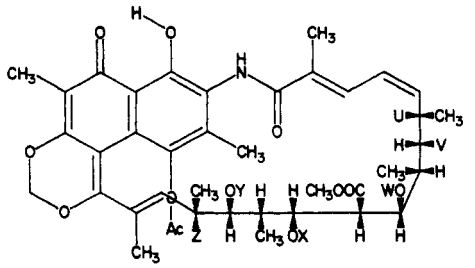
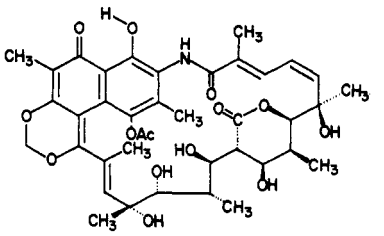
[1] Abbreviations used are: RT, reverse transcriptase or RNA-dependent DNA polymerase; RLV, Rauscher leukemia virus; SvA, SvB, SvC, SvD, SvE, SvF, SvG, and SvJ, streptovaricins A–G and J, respectively.

[2] Streptovaricins D and J are separated chromatographically with great difficulty and our earliest report of the properties of "SvD" actually described those of SvJ (Rinehart *et al.*, 1966). However, to avoid confusion, we retain the name SvD for that compound whose structure was assigned later (Rinehart *et al.*, 1971) and which has been discussed in a recent review (Rinehart, 1972).





STREPTOVARICIN F

STREPTOVARICIN	U	V	W	X	Y	Z
A	OH	OH	H	Ac	H	OH
B	H	OH	H	Ac	H	OH
C	H	OH	H	H	H	OH
D	H	OH	H	H	H	H
E	H	=O	H	H	H	OH
G	OH	OH	H	H	H	OH
J	H	OAc	H	H	H	OH
C-Ac_3(B-Ac_2, J-Ac_2)	H	OAc	Ac	Ac	H	OH
C-Ac_4(B-Ac_3, J-Ac_3)	H	OAc	Ac	Ac	Ac	OH
G-Ac_3(A-Ac_2)	OH	OAc	Ac	Ac	H	OH

FIGURE 1: Structures of streptovaricins and their acetates discussed in the present report.

containing a mixture of the less polar streptovaricins. The total recovery was thus 99%.

A portion of fraction 2 (8.00 g) was chromatographed over silica gel, employing 2% methanol in chloroform as solvent; 10-ml fractions were collected, analyzed for composition on tlc employing 4% methanol in chloroform, and combined into appropriate larger fractions, as summarized in Table I. Considerable decomposition occurred on this column, but the order of elution is typical. For further purification several runs were pooled.

Isolation of Streptovaricins A and B. Fraction 1 obtained in the preceding section (5.000 g) was chromatographed over silica gel employing 3% methanol in chloroform to give two well-separated fractions. Fraction 1-2 contained 495 mg of streptovaricin B (pure by tlc) and fraction 1-3 contained 3.300 g of streptovaricin A (tlc).

Streptovaricin A had mp 200–201° after precipitation from 1-chlorobutane (mp 233–243° after drying for 25 hr over phosphorus pentoxide in high vacuum), $[\alpha]_D^{25}$ +610° (*c* 0.118, $CHCl_3$).

TABLE I: Silica Gel Column Chromatograph of Streptovaricin Complex.

Fraction	Composition[a]	Amount (g)
2-1	Pigment	Trace
2-2	SvE (D,C)	0.02
2-3	SvD (E,C)	0.08
2-4	SvC (D)	0.10
2-5	SvC	1.20
2-6	SvC (B,G)	0.20
2-7	SvG	0.55
2-8	SvG + pigment	0.16
2-9	SvG (A)	0.20
2-10	SvA + more polar material	0.50
2-11	Yellow-brown material	0.10
Total recovery		3.11

[a] Streptovaricins in parentheses are minor components of the fractions indicated.

Anal. Calcd for $C_{42}H_{53}NO_{16}$: C, 60.93; H, 6.45; N, 1.69; O, 30.92; OCH_3, 3.75; $(OAc)_2$, 10.36; $(CCH_3)_{10}$, 18.01; mol wt, 827.3364. Found: C, 60.76; H, 6.55; N, 1.62; O, 30.68; OCH_3, 3.88; OAc, 9.89; CCH_3, 16.91; mol wt, 827.3364 (HRMS).

Streptovaricin B had mp 187–189° after precipitation from 1-chlorobutane, $[\alpha]_D^{27}$ +576° (*c* 0.191, $CHCl_3$).

Anal. Calcd for $C_{42}H_{53}NO_{15}$: C, 62.13; H, 6.59; N, 1.73; O, 29.56; OCH_3, 3.83; $(CCH_3)_{10}$, 18.52; $(OAc)_2$, 10.62; mol wt, 811. Found: C, 62.25; H, 6.55; N, 1.62; O, 29.15; OCH_3, 3.97; C-CH_3, 16.54; OAc, 9.6; mol wt, 811 (mass spectrum).

Isolation of Streptovaricin C. Fraction 2-5 of the initial separation (100 mg) was rechromatographed over silica gel employing 2% methanol in chloroform to give 70 mg of streptovaricin C, mp 189–191°, after precipitation from 1-chlorobutane, $[\alpha]_D^{27}$ +602° (*c* 0.14, $CHCl_3$), pure by tlc.

Anal. Calcd for $C_{40}H_{51}NO_{14}$: C, 62.41; H, 6.69; N, 1.82; O, 29.13; OCH_3, 4.04; OAc, 5.59; mol wt, 769.3309. Found: C, 62.17; H, 6.65; N, 1.88; O, 28.60; OCH_3, 4.01; OAc, 5.45; mol wt, 769.3253 (HRMS).

Isolation of Streptovaricin D. Fraction 2-3 from the original separation of streptovaricin complex was repeatedly purified by preparative tlc, employing 4% methanol in chloroform and benzene–ethyl acetate (1:1). It was then precipitated from chloroform by addition of *n*-hexane, mp 172–175°, $[\alpha]_D^{27}$ +590.3° (*c* 0.155, $CHCl_3$).

Anal. Calcd for $C_{40}H_{51}NO_{13}$: C, 63.72; H, 6.82; N, 1.86; mol wt, 753.3360. Found: C, 63.62; H, 6.93; N, 1.68; mol wt, 753.3374 (HRMS).

Isolation of Streptovaricin E. Fraction 2-2 from the original separation of streptovaricin complex was purified by two-dimensional preparative tlc, employing 3% methanol in chloroform and benzene–ethyl acetate (1:1) as developing solvents. The streptovaricin E thus obtained was crystallized first from 1-chlorobutane, then from ether, mp 198–202°, $[\alpha]_D^{27.2}$ +412.3° (*c* 0.123, $CHCl_3$).

Anal. Calcd for $C_{40}H_{49}NO_{14}$: C, 62.58; H, 6.38; N, 1.81; mol wt, 767. Found: C, 62.34; H, 6.45; N, 1.80; mol wt, 767 (mass spectrum).

Isolation of Streptovaricin F. Fraction 2-10 from the original separation of streptovaricin complex was chromatographed over silica gel employing methanol–chloroform, then subjected

to repeated preparative tlc (10% methanol in chloroform) to give streptovaricin F, which was crystallized from ethyl acetate–methanol to give fine, hair-like crystals, mp 222–224°.

Anal. Calcd for $C_{39}H_{47}NO_{14}$: C, 62.14; H, 6.28; N, 1.86; mol wt, 753. Found: C, 61.86; H, 6.53; N, 1.57; mol wt, 753 (mass spectrum).

Isolation of Streptovaricin G. Fraction 2-7 from the initial separation of streptovaricin complex (1.17 g) was chromatographed repeatedly over silica gel employing 2% methanol in chloroform to give 470 mg of streptovaricin G, mp 190–192°, after precipitation from 1-chlorobutane, $[\alpha]_D^{31}$ +473° (*c* 0.074, $CHCl_3$), pure by tlc.

Anal. Calcd for $C_{40}H_{51}NO_{15}$: C, 61.13; H, 6.54; N, 1.78; O, 30.54; OCH_3, 3.95; $(CCH_3)_9$, 17.2; OAc, 5.50; mol wt, 785. Found: C, 61.45; H, 6.64; N, 1.85; O, 30.01; OCH_3, 3.91; CCH_3, 16.11; OAc, 5.72; mol wt, 785 (mass spectrum).

Isolation of Streptovaricin J. Twenty grams of a streptovaricin mixture (Upjohn, 10347-WJH-123-8) was subjected to separation in an all glass 100-tube (25-ml per phase) counter double current distribution apparatus (Post and Craig, 1963) employing the solvent system cyclohexane–ethyl acetate–95% ethanol–water (1:1:1:1). The material was dissolved in 175 ml of upper phase and put in tubes 21–27 from the end of the machine at which the lower phase portion of the apparatus was numbered as 1. After distribution, the fractions were analyzed by means of antibacterial activity against *Bacillus subtilis*, by weight, and by tlc. Fractions 25–70 were combined and evaporated to dryness under reduced pressure to give 1.2 g of impure SvJ. A portion of the material (120 mg) was applied to two 20 cm × 20 cm preparative tlc plates (PF 254, 1 mm), which were developed five times using ether–acetone (94:6). The biggest orange band was removed and eluted with ethyl acetate to give 49 mg of a yellow glass, which was precipitated from ether to give a solid, mp 177–180°, $[\alpha]_D$ +326° (*c* 0.25, in EtOH), $[\alpha]_D^{26}$ +436° (*c* 0.094, $CHCl_3$).

Anal. Calcd for $C_{42}H_{53}NO_{15}$: C, 62.13; H, 6.59; N, 1.73; O, 29.56; $(CCH_3)_{10}$, 18.52; $(OAc)_2$, 10.62; mol wt, 811. Found: C, 62.12; H, 6.58; N, 1.68; O, 28.95; CCH_3, 16.34; OAc, 11.75; mol wt, 811 (mass spectrum).

On GF 254 silica gel plates using ether–acetone (9:1), SvJ moved slower than SvD; on Eastman Chromogram sheets using the same solvent, SvJ was the faster moving. The mass spectrum of SvJ contained a molecular ion at *m/e* 811; pertinent peaks from its proton magnetic resonance spectrum are found in Figure 2.

Preparation of Streptovaricin C Triacetate and Tetraacetate. A solution of 3.0 g (3.9 mmol) of streptovaricin C, 20 ml of pyridine, and 120 ml of acetic anhydride was stirred at room temperature for 25 hr, then was poured into ice–water and extracted with ethyl acetate. The organic extract was washed with water and saturated sodium chloride solution, dried, filtered, and concentrated *in vacuo* to 3.4 g of an orange-red solid. The product was chromatographed over 250 g of silica gel, gradient eluting with acetone in benzene. Elution with benzene–acetone (4:1) yielded 1.65 g of streptovaricin C triacetate, which was crystallized from methylene chloride–ether; yield, 1.28 g (32%); mp 228.5–229.5°.

Anal. Calcd for $C_{46}H_{57}NO_{17}$: C, 61.67; H, 6.41; N, 1.56; $(OAc)_4$, 19.22; mol wt, 895. Found: C, 61.39; H, 6.39; N, 1.60; OAc, 18.46; mol wt, 895 (mass spectrum).

Continued elution with benzene–acetone (3:1) yielded 498 mg of slightly impure streptovaricin C tetraacetate, which was purified by precipitation from ethyl acetate with *n*-hexane to yield 396 mg (11%) of an orange amorphous solid, mp 183–185°.

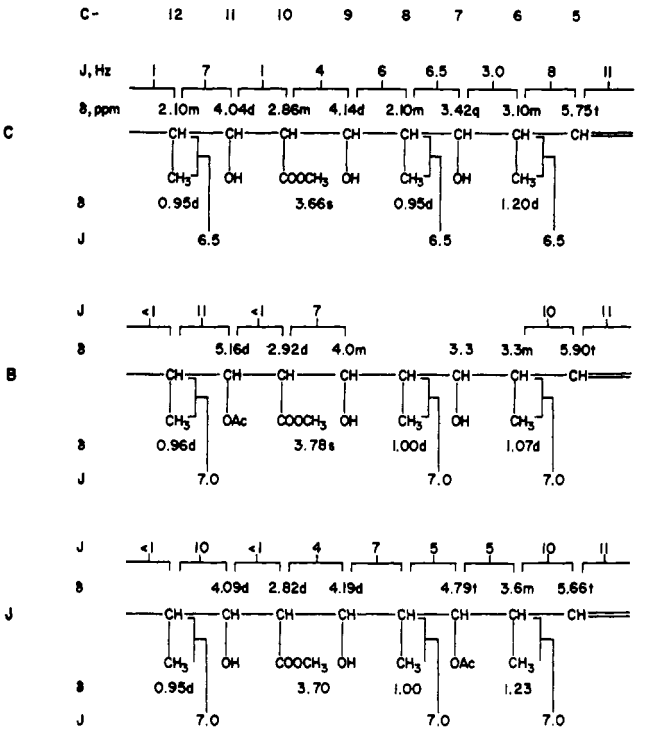


FIGURE 2: Nmr chemical shifts of protons in comparable regions of streptovaricins C, B, and J.

Anal. Calcd for $C_{48}H_{59}NO_{18}$: C, 61.46; H, 6.34; N, 1.49; $(CCH_3)_{13}$, 20.84; $(OAc)_5$, 22.95; mol wt, 937. Found: C, 61.25; H, 6.48; N, 1.55; CCH_3, 21.25; OAc, 16.43; mol wt, 937 (mass spectrum).

Preparation of Streptovaricin B Diacetate. A solution of 240 mg of streptovaricin B, 5 ml of pyridine, and 10 ml of acetic anhydride was allowed to stand at room temperature for 24 hr, then was poured into ice–water, stirred for 1 hr, and extracted with ethyl acetate. The extract was washed with water and sodium chloride, dried, and evaporated to dryness under reduced pressure to an orange-red oil. The oil was chromatographed over silica gel with 1% methanol in chloroform. Rechromatography of appropriate fractions with 2% methanol in chloroform gave an orange oil (single spot on tlc) which crystallized from ethyl acetate and *n*-hexane as red crystals (84 mg, 32%), mp 230–231°; ir and nmr spectra identical with those of streptovaricin C triacetate. A mixture melting point was undepressed, 230°.

The more polar chromatographic fractions yielded 30 mg (11%) of a second product, corresponding to streptovaricin C tetraacetate by tlc.

Preparation of Streptovaricin J Diacetate. A solution of 272 mg (0.335 mmol) of streptovaricin J, 2 ml of pyridine, and 5 ml of acetic anhydride was stirred at room temperature for 24 hr, then worked up as described for streptovaricin C tri- and tetraacetates above. Chromatography over 25 g of silica gel, gradient eluting with benzene–acetone (9:1 to 4:1), gave streptovaricin J diacetate. Crystallization from ethyl acetate–hexane yielded 35 mg (12%), mp 220.5–222°. This material was identical with streptovaricin C triacetate, mp 221.5–223°, mmp 220–222°.

Streptovaricin G Triacetate. A solution of streptovaricin G (76.5 mg) in 3 ml of pyridine and 10 ml of acetic anhydride was allowed to stand at room temperature for 24 hr, then

worked up as described for streptovaricin B diacetate. Chromatography over silica gel with 2% methanol in chloroform gave a yellow powder (pure by tlc) after precipitation from ethyl acetate and *n*-hexane, mp 202–204°, $[\alpha]_D^{27}$ +498° (*c* 0.24, $CHCl_3$).

Anal. Calcd for $C_{46}H_{57}NO_{18}$: C, 60.59; H, 6.26; N, 1.54; $(CCH_3)_{12}$, 19.8; $(OAc)_4$, 18.9; mol wt, 911. Found: C, 60.16; H, 6.39; N, 1.61; CCH_3, 20.0; OAc, 17.0; mol wt, 911 (mass spectrum).

Streptovaricin A Diacetate. A solution of 500 mg of streptovaricin A in 10 ml of pyridine and 30 ml of acetic anhydride stood at room temperature for 24 hr, then was worked up as described for streptovaricin B diacetate. Before chromatography, the red residue was dissolved in 1-chlorobutane and precipitated with *n*-hexane, giving a yellow-orange powder which contained (tlc) one major product, slightly less polar than streptovaricin A, with a trace of less polar materials. The yellow powder was chromatographed over silica gel with 2% methanol in chloroform, yielding 295 mg (54%) of streptovaricin A diacetate, mp 201–203°, mixture melting point with streptovaricin G triacetate, 202–204°. The ir and nmr spectra were identical with those of SvG triacetate.

Antibacterial Spectra. The antibacterial spectra were determined by the agar diffusion method. Solutions containing 1 mg/ml of each compound were prepared in methanol. Aliquots of 0.02 ml were applied on filter discs of 0.25 in. diameter (20 μg/disc). The discs were layered on agar trays seeded with the test organisms. After incubation of the trays overnight the diameters of the resulting zones of inhibition were measured.

DNA and RNA Polymerase Assays. The methods used for the isolation of the DNA and RNA polymerases from *Escherichia coli* cells and their assay have been described (Reusser, 1971).

Reverse Transcriptase Assay. Rauscher leukemia virus (RLV) supplied by Electro-Nucleonics Laboratories, Inc., Bethesda, Md., was used as the RT source. The virus preparation (between 10^{11} and 10^{12} particles per ml) was mixed with glycerol (final concentration was 8%), then divided into 50-μl fractions and stored at −10° for at least 2 weeks without significant loss of RT activity.

The assay procedure for RT was a modification of that described earlier (Brockman *et al.*, 1971). The reaction was carried out in a 1-ml polystyrene centrifuge tube. To stop the reaction 50 μl of carrier DNA (4 mg/ml) and 0.5 ml of 10% CCl_3COOH were added to each tube. After standing in ice for about 20 min, the precipitate was collected on a glass fiber paper disk (Whatman GF/C, 2.4 cm presoaked in saturated phosphate) supported on a porcelain Hirsch funnel, and washed exhaustively with 10% CCl_3COOH and other organic solvents. The paper disk was individually placed in a polyethylene counting vial (Packard Instrument Company, Inc., Downers Grove, Ill.). DNA and RNA were hydrolyzed on the paper disk with 0.5 ml of 0.5 N $HClO_4$ at 70° for 40 min. The radioactivity was measured in a Packard TriCarb liquid scintillation counter by adding 15 ml of BBS-3 counting solution.

Results

Streptovaricins and Acetates. Partial separations of the streptovaricin complex have been reported earlier, employing countercurrent distribution (Whitfield *et al.*, 1957) and paper chromatography (Siminoff *et al.*, 1957; Sokolski *et al.*, 1958). The complex contains many streptovaricin components, as well as other materials, and differs in composition from one lot to another. No single method suffices to separate all the streptovaricins, but silica gel chromatography proves effective in isolating most of them, though at considerable loss of material. A combination of techniques gives adequate preparations of streptovaricins A–G and J.

Structures have been assigned elsewhere to streptovaricins A–G (Rinehart *et al.*, 1971) and are shown in Figure 1. One of the key reactions in interrelating the structures of the streptovaricins is acetylation. Since streptovarcins A and G both give the same compound, SvG triacetate (SvA diacetate), on acetylation and SvA has one more acetate group than SvG, SvA is a monoacetate of SvG. Similarly, SvC and SvB give the same compounds (SvC triacetate and SvC tetraacetate) on acetylation, and SvB has one more acetate group than SvC. Thus, SvB is a monoacetate of SvC.

Streptovaricin J, like streptovaricins B and C, is converted to SvC triacetate. Streptovaricin J has the molecular formula $C_{42}H_{51}NO_{18}$, established by microanalyses and mass spectrometry, and is thus isomeric with SvB, a monoacetate of SvC. The nuclear magnetic resonance spectrum of SvJ is very similar to that of SvB, containing ten methyl groups, six in the $CH_3C=$ region (δ 2.0–2.3) and four in the CH_3C region (δ 0.9–1.2). Location of the acetate group of streptovaricin J at C-7 was made according to the following argument. Since acetylation of SvJ gives SvC triacetate, the acetate group of SvJ must be on C-7, C-9, or C-11. It cannot be on C-11, since the C-11 acetate of SvC is SvB. The nmr spectrum of SvJ (see Figure 2) contains a carbinyl acetate proton at δ 4.79 (t, J = 5 Hz), which is not coupled to the C-10 proton at δ 2.82 (d, J = 2 Hz). Thus, the acetate of SvJ must be on C-7. In confirmation of this assignment, the usual C-7 carbinol proton absorption of the other streptovaricins (A-D, G), found in the δ 3.3–3.5 region, is missing. These nmr data thus clearly assign the structure of SvJ as that shown in Figure 1.

In Vitro Antibacterial Spectra. The streptovaricins have been reported to be active against both gram-positive and gram-negative organisms, but especially against *Mycobacterium tuberculosis* (Siminoff *et al.*, 1957). However, the activity of the individual streptovaricins in inhibiting the growth of several organisms is by no means uniform, as shown in Table II, which presents the *in vitro* antibacterial spectra of the known streptovaricins. Streptovaricins A, B, C, and G showed broad spectrum activity against the test organisms used, although the gram-positive strains were generally much more susceptible to streptovaricin inhibition than the gram-negative organisms.

Streptovaricins A and G were the most active compounds, followed by streptovaricins B and C. The potency of streptovaricins D and J was not greatly inferior as far as the gram-positive organisms are concerned but only one gram-negative organism (*K. pneumoniae*) was inhibited. Streptovaricin E was less active in inhibiting gram-positive strains than streptovaricins A–D, G, and J and had no gram-negative activity. Streptovaricin F, the least active, showed activity (moderate) only against *M. avium*, all the other test organisms being insensitive to this compound. The breadth of activity, as measured by the number of organisms inhibited, is generally paralleled by the degree of activity, as measured by the size of the zones of inhibition. By a combination of these two criteria the order of activity is: streptovaricins A, G > B, C > D, J > E ≫ F. The acetylated streptovaricins (SvG triacetate, SvC tri- and tetraacetates) are completely inactive in the antibacterial assay.

E. coli RNA Polymerase Activity. The mode of action of

TABLE II: *In Vitro* Antibacterial Spectra of Streptovaricins and Their Acetates.

Organism	Zones of Inhibition[a]										
	Streptovaricins								Streptovaricin Acetates		
	A	B	C	G	D	J	E	F	C-Ac_3	C-Ac_4	G-Ac_3
B. subtilis UC 564	22	21	22	22	21	20	21	0	0	0	0
S. aureus UC 80	25	26	26	26	24	24	15	0	0	0	0
S. lutea UC 130	35	34	33	33	30	28	23	0	0	0	0
K. pneumoniae UC 57	21	20	21	20	12	17	0	0	0	0	0
E. coli UC 51	18	14	13	15	0	0	0	0	0	0	0
S. schottmuelleri UC 126	14	11	10	13	0	0	0	0	0	0	0
P. vulgaris UC 93	18	10	8	14	0	0	0	0	0	0	0
M. avium UC 159	24	17	15	22	20	20	22	14	0	0	0
L. casei UC 236	28	26	25	21	26		16	0			

[a] Zones of inhibition, expressed in mm of diameters, were measured after 16-hr incubations.

streptovaricin (complex) in inhibiting the growth of *E. coli* was shown some time ago to involve inhibition of the DNA-dependent RNA polymerase of that organism (Mizuno *et al.*, 1968; Yamazaki *et al.*, 1968). In this, streptovaricin resembles rifamycin and its derivative rifampicin. With the successful separation of the individual components of the streptovaricin complex it was of interest to assess their relative activities *vs.* bacterial RNA polymerase. The relative activity of the streptovaricins in inhibiting growth of *E. coli* was shown in Table II to follow the order: A > G > B > C ≫ D, J, E, F, with the last four being essentially inactive. In view of the mode of action of the antibiotic complex one might expect that the relative activities of the individual streptovaricins in inhibiting *E. coli* RNA polymerase would follow a similar order. The extent of inhibition of *E. coli* polymerase by the individual streptovaricins is shown in Table III. Streptovaricin A proved to be the most potent component of the complex in the RNA polymerase test system. The reaction was inhibited to an extent of 90% by 0.1 μmol of antibiotic/ml; a concentration of 0.01 μmol/ml caused 83% inhibition. Streptovaricins B, C, and G were somewhat less inhibitory than A and caused inhibitions of 72–78% at 0.1 μmol/ml and 64–69% at 0.01 μmol/ml. Streptovaricin D was slightly less active (54% inhibition at 0.01 μmol/ml), followed by streptovaricins J (44%), E (33%), and F (0%). From these data one can arrange the streptovaricins' activity in the following order: streptovaricin A > G, C > B > J > E ≫ F. This is almost precisely the same order as that found for the whole cell bacterial inhibition seen in Table II. The acetylated streptovaricins show no activity in inhibiting this enzyme, just as they show no antibacterial activity.

Effect on DNA Polymerase I. None of the streptovaricins described inhibited the activity of DNA polymerase I isolated from *E. coli*. Acetates were not tested in this system.

Effect on Reverse Transcriptase. The eight streptovaricins have also been tested against RT of RLV and the results are summarized in Table IV. On an equimolar basis (0.25 μmol/ml), SvD was found to be the most active of the components. RT activity was inhibited to an extent of about 70% by this streptovaricin. Streptovaricins J, C, and G were less inhibitory than D, SvB was less active than streptovaricins J, C, and G, streptovaricins A and E were still less active, and SvF was essentially inactive under our assay conditions.

Streptovaricin C tri- and tetraacetates and SvG triacetate were also tested against RT of RLV. They were considerably more active than unacetylated streptovaricins C and G; SvC tetraacetate and SvG triacetate were as active as the most active streptovaricin, SvD.

Discussion

All of the streptovaricins studied have the same basic carbon skeleton, and the aromatic portion of the molecule is identical for all. As seen in Figure 1, the molecular region in

TABLE III: Effect of Streptovaricins and Their Acetates on DNA-Dependent RNA Polymerase from *E. coli*.[a]

Streptovaricin	Concentration (μmol/ml)	Incorporation (cpm)		% Inhibition
		Sample	Control	
A	0.1	390	4070	90
	0.01	700	4070	83
B	0.1	930	3360	72
	0.01	1210	3360	64
C	0.1	910	4070	78
	0.01	1390	4070	66
D	0.1	530	1800	70
	0.01	830	1800	54
E	0.1	1910	4070	53
	0.01	2740	4070	33
F	0.1	3280	4070	19
	0.01	4190	4070	0
G	0.1	730	3360	73
	0.01	1050	3360	69
J	0.1	1400	3360	58
	0.01	1870	3360	44
C triacetate	0.1	2300	1920	0
C tetraacetate	0.1	2280	1920	0
G triacetate	0.1	2090	1920	0

[a] Assay mixtures contained in a total volume of 0.25 ml: Tris-hydrochloride buffer (pH 7.9), 5 μmol; $MgCl_2$, 1 μmol; mercaptoethanol, 3 μmol; $MnCl_2$, 0.25 μmol; GTP, CTP, UTP, 0.1 μmol each; ATP-8-^{14}C, 0.1 μmol containing 0.05 μCi; dAT, 0.2 μ; polymerase, ~35 μg. The reaction mixtures were incubated at 30° for 15 min. The results shown are averages of experiments.

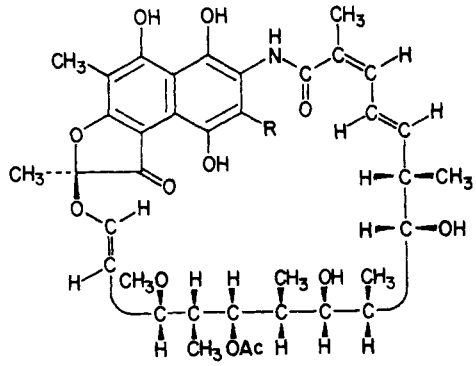
TABLE IV: Effect of Streptovaricins and Their Acetates on Reverse Transcriptase of Rauscher Leukemia Virus.[a]

Streptovaricin[b]	[^{3}H]TMP Incorporation (cpm)		% Inhibition[c]
	Sample	Control	Range
A	2965	2922	0–19
B	1814	2922	27–38
C	1376	2922	31–53
D	956	2922	67–74
E	2347	2922	20–23
F	2701	2922	8
G	2067	2922	29–46
J	1576	2922	46–48
C triacetate	975	2081	53
C tetraacetate	534	2081	74
G triacetate	707	2081	66

[a] The reactions were carried out at 37° for 120 min. The assay mixtures contained in a total volume of 0.05 ml: Tris-HCl buffer (pH 7.8), 25 μmol; NaCl, 30 μmol; $MgCl_2$, 0.25 μmol; dithiothreitol, 0.1 μmol; Triton X-100, 0.015%; dATP, dCTP, and dGTP, 0.04 μmol each; TTP-^3H (25 Ci/mM), 0.1 μmol; and virion, 5 μl (*ca.* 1×10^{10} particles/ml). [b] Concentration, 200 μg/ml in 2% Me_2SO; control, 2% Me_2SO. [c] Results of three experiments, except where a single value is shown.

which they differ is a portion of the aliphatic *ansa* bridge, in particular the portion from C-6 through C-14.

The relative *in vitro* antibacterial activities of the individual streptovaricins parallel their relative potencies as inhibitors of bacterial DNA-dependent RNA polymerase. Some are quite active, others are relatively inactive. From these relationships some conclusions can be drawn regarding the structure–activity relationship. The most striking facet is the nearly complete loss of antibacterial activity and of RNA polymerase activity on formation of a δ-lactone involving the C-10 carboxyl and the C-7 hydroxyl, as found in SvF. One could interpret this as a consequence of the removal of the C-7 hydroxyl group. Wehrli and Staehelin (1971) have argued that a hydroxyl group at C-7 is essential for antibacterial activity in rifamycin derivatives such as rifampicin. This argument does not appear to apply rigorously to the streptovaricins. Streptovaricin E has a keto group at C-7, yet it is moderately active against gram-positive bacteria, and SvJ, with an acetate at C-7, is more active than SvE, about as active as SvD. Both possess reasonable activity in inhibiting *E. coli* RNA polymerase, refuting any argument that the keto group of SvE or the acetate group of SvJ is converted *in vivo* to a hydroxyl group (as in SvC) before inhibiting this enzyme. The nearly total loss of activity by SvF seems more likely a consequence of a severe change of conformation than a simple loss of the C-7 hydroxyl. Nonetheless, streptovaricins E and J are somewhat less active than the others and a C-7 hydroxyl group appears to be a desirable, if unnecessary, feature for antibacterial activity.



RIFAMPICIN : R = —CH=N—N⟨ ⟩N—CH_3

Other positions at which variations in substitution can be judged are C-6, C-11, and C-14. Streptovaricins A and G, which have hydroxyl groups at C-6, are slightly more active than their counterparts, streptovaricins B and C, which are unsubstituted at C-6, both in overall antibacterial activity and in RNA polymerase inhibition. Streptovaricin A is 11-*O*-acetylstreptovaricin G and is somewhat more active than the latter in both RNA polymerase inhibition and antibacterial spectra; on the other hand, SvB, which is 11-*O*-acetylstreptovaricin C, possesses the same antibacterial spectrum and is slightly less active in inhibiting RNA polymerase than SvC. The only difference between streptovaricins C and D is a hydroxyl group at C-14 in the former. Streptovaricin C has a considerably broader antibacterial spectrum and is a somewhat more potent inhibitor of *E. coli* RNA polymerase than SvD; thus, a hydroxyl group at C-14 seems advantageous.

It is also of interest to examine the antibacterial and RNA polymerase activities of the acetates of streptovaricins C and G. In SvC tetraacetate all the hydroxyl groups are acetylated except the tertiary and phenolic hydroxyls. The compound is totally inactive in both assays. Clearly one or more of the hydroxyls at C-7, C-9, C-11, or C-13 is required for activity. Streptovaricin C triacetate, with the C-7, C-9, and C-11 hydroxyls acetylated, is also inactive in both assays. Thus, the 13-hydroxyl appears to be insufficient and is probably unimportant for activity. Of the three remaining hydroxyls, we have seen that acetylation at the 11-hydroxyl appears to enhance somewhat the activity (SvB *vs.* SvC) and acetylation at the 7-hydroxyl does not greatly diminish the activity (SvJ *vs.* SvC). A related argument can be made for SvG triacetate; *i.e.*, acetylation at C-11 enhances activity, thus acetylation at C-7 or C-9 must destroy it. From this one could conclude that the C-9 hydroxyl is the most important in terms of antibacterial and RNA polymerase activity. However, one cannot *per se* exclude a synergistic or buttressing effect of acetylating all the secondary hydroxyl groups.

In summary, with the possible exception of acetylation at C-9, lactone formation at C-7 provides the only dramatic change in activity, leading to the conclusion that the basic conformation of the antibiotic is a very important parameter, as might be expected for a molecule whose mode of action involves reversible complex formation with bacterial RNA polymerase (Mizuno *et al.*, 1968).

The mode of inhibition of RT by ansamycin antibiotics is much less well understood at present and the significance of the relative inhibitory activities of the individual streptovaricins is also less clear. Inhibition of RT by streptovaricins is less complete and requires higher concentrations than inhibitions of *E. coli* RNA polymerase. The order of inhibition of RT by individual streptovaricins is also different from that for RNA polymerase: SvD is the most potent inhibitor of RT but only a moderately strong inhibitor of bacterial RNA polymerase, while SvA, which is the most potent inhibitor of RNA poly-

merase, is a very weak inhibitor of RT. A similar, but slightly different order of inhibition of RT's by individual streptovaricins has been obtained with RT systems in other laboratories (Carter *et al.*, 1972). In view of the relatively low activity of the compounds as RT inhibitors, these conclusions should be regarded as tentative. The somewhat greater RT inhibitory activity of the streptovaricin acetates (SvG triacetate, SvC tri- and tetraacetates) *vis-à-vis* the unacetylated compounds is encouraging, since acetates tested are derived in one step from the major components of the complex, SvC, SvG, and SvA, and are thus available in larger quantity than SvD, a minor component of the complex.

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Interaction of Concanavalin A with the Capsular Polysaccharide of Pneumococcus Type XII and Isolation of Kojibiose from the Polysaccharide†

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ABSTRACT: The disaccharide kojibiose (2-*O*-α-D-glucopyranosyl-D-glucose) was isolated from a partial acid hydrolysate of the capsular polysaccharide of type XII pneumococcus. Acetolysis of the polysaccharide provided α-kojibiose octaacetate. These findings confirm several decades of immunochemical support for the presence of kojibiosyl residues in type XII pneumococcal polysaccharide. A precipitin curve is generated when the Jack bean lectin, concanavalin A, interacts with SXII, but no precipitate forms with periodate oxidized, borohydride-reduced SXII. Since oxidation by periodate destroys kojibiosyl residues and this oligosaccharide was shown to be a good inhibitor of the reaction of SXII with concanavalin A, it is probable that this disaccharide forms the basis for the interaction of SXII with concanavalin A.

A previous paper (Cifonelli *et al.*, 1966) described the purification of the specific polysaccharide (SXII) of type XII pneumococcus. Hydrolysis of the polysaccharide gave rise to neutral (D-glucose and D-galactose) and amino (D-galactosamine and L-fucosamine) sugars. One of the procedures employed for the purification of SXII involved complex formation with concanavalin A (*cf.* Goldstein and Iyer, 1966), the phytohemagglutinin of the Jack bean (Sumner and Howell, 1936). Of the component sugars present in SXII, only D-glucose is capable of interacting with the combining sites of concanavalin A (Goldstein *et al.*, 1965; Smith and Goldstein, 1967; So and Goldstein, 1967b; Poretz and Goldstein, 1970). Furthermore, there are substantial immunochemical data indicating the occurrence of kojibiosyl residues (*O*-α-D-glucopyranosyl-(1→2)-D-glucose) in SXII (Goodman and Kabat, 1960; Suzuki and Hehre, 1964). This paper reports the interaction of concanavalin A with SXII and the isolation of kojibiose from a controlled acid hydrolysis of the polysaccharide.

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Experimental Section

SXII polysaccharide and periodate-oxidized, borohydride-reduced SXII were prepared as described earlier (Cifonelli