

Antibiotics with Ansa Rings

KENNETH L. RINEHART, JR.

Department of Chemistry, University of Illinois, Urbana, Illinois 61801

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Often when an antibiotic of a new structural type appears on the chemotherapeutic scene it is soon joined by additional representatives of the same type, so that one can begin to speak of a class of antibiotics. This is the case with a new class of antibiotics containing an aliphatic ansa bridge,¹ a bridge connecting two nonadjacent positions of an aromatic nucleus, for which the term "ansamycins" has been suggested by Prelog.

Thus far four representatives of this class of antibiotics have been identified—the rifamycins, produced by *Streptomyces mediterranei*,² the streptovaricins, from *Streptomyces spectabilis*,³ the tolypomycins, from *Streptomyces tolypophorus*,⁴ and geldanamycin, from *Streptomyces hygroscopicus* var. *geldanus* var. *nova*.⁵ The first three exist as complexes of closely related components; more than a dozen individual compounds have been assigned to the class. Moreover, some of the antibiotics have been isolated by more than one group; the nancimycins^{6a} have been reported^{6b} to be the same as the rifamycins, and the streptovaricins are apparently identical with antimicrobial substance B44P⁷ as well as the austmycins.⁸

Structures assigned to components of the rifamycin complex, rifamycins B,^{9,10} L,¹¹ and Y,^{12,13} are shown in Figure 1, those assigned to the streptovaricins, streptovaricins A, B, C, D, E, G, and F,^{14,15} in Figure 2, that of tolypomycin Y¹⁶ in Figure 3, and that of geldanamycin in Figure 4.^{17,18} Rifamycins O²¹ and SV,^{22a} originally obtained as degradation products of rifamycin B (Figure 5), have been isolated from *Streptomyces* 4107A2 and a mutant of *Streptomyces mediterranei*, respectively.

Biological Activity

The ansamycins are notably active^{22b} against gram-positive bacteria. Some broad spectrum activity has been noted for certain rifamycin derivatives²⁰ and some activity against gram-negative bacteria for the streptovaricins.³ The streptovaricins repress *Mycobacterium tuberculosis*²³ and murine leprosy,²⁴ and rifamycin derivatives^{25,26} are reported to be clinically useful, especially in the treatment of tuberculosis. Geldanamycin differs from the other ansamycins in

that its principal activity is against protozoa rather than against bacteria.⁵

Curiously, the antibacterial activity of the ansamycin antibiotics withstands considerable variation in structure. Several of the streptovaricins have antibacterial activity,^{3,27} and all the rifamycins (A, B, C,

(1) The term *ansa* compounds was originally proposed by Lüttringhaus [A. Lüttringhaus and H. Gralheer, *Justus Liebig's Ann. Chem.*, **550**, 67 (1942)] for compounds of this general structural type, usually meta- and para-bridged benzenes.

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(12) J. Leitich, V. Prelog, and P. Sensi, *Experientia*, **23**, 505 (1967).

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(18) In addition to the components shown in Figures 1 and 2, rifamycins A, C, D, and E¹⁹ and streptovaricins H and I²⁰ have been reported. Both relative and absolute stereochemistry of rifamycins B and Y has been assigned, based on X-ray data^{10,13} and degradation products.^{9b} The relative stereochemistry of tolypomycin Y has also been determined by X-ray crystallography.^{16b} The relative stereochemistry shown for the streptovaricins has very recently been established for a streptovaricin C derivative by X-ray crystallography.^{14b} It is of interest that the stereochemistry of rifamycins B and Y, of tolypomycin Y, and of streptovaricin C is the same at all the asymmetric carbon atoms. Stereochemical assignments have not yet been made for geldanamycin; the pattern shown would accord (in part) with the patterns of the other ansamycins.

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K. L. Rinehart, Jr., is Professor of Chemistry at the University of Illinois, Urbana, where he has been employed since receiving his doctorate (with James Cason) at the University of California, Berkeley, in 1954. His present research interests center around bioorganic chemistry, especially structural, biosynthetic, and synthetic aspects of antibiotics, and mass spectrometry as applied to biologically interesting compounds. Former research efforts have included forays into ferrocene and cycloprene chemistry.

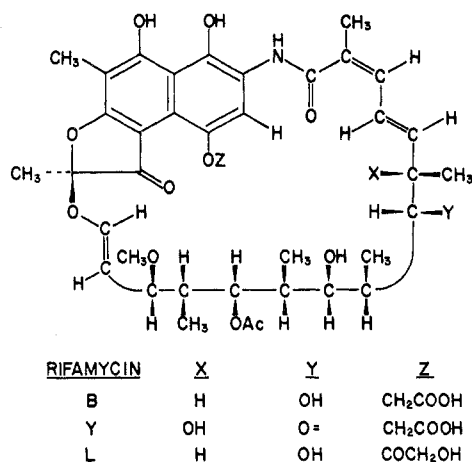


Figure 1. Structures assigned to rifamycins.

D, E,) are biologically active.^{22,28} Moreover, a number of the derivatives of rifamycin B are more active than the antibiotic itself. The biological activity of rifamycin B is retained through the sequence (Figure 5) of oxidation to rifamycin O (which forms the lactone), hydrolysis to rifamycin S (which removes the glycolic acid), and reduction to rifamycin SV (which reforms the hydroquinone).

Acyl derivatives of rifamycins B, O, S, and SV with the phenolic hydroxyl substituted show activity.²⁹ Amides of the glycolic acid acid group of rifamycin B, such as rifamide,³⁰ are also active. Condensation of rifamycin O (at the less hindered quinone carbonyl of rifamycin S) with various amines gave active derivatives, of which the aminoguanidine derivative was reported to be the most active.^{29,31} Condensations with *o*-diamino aromatic compounds gave active derivatives such as rifazine.³² An even more remarkable transformation with retention of biological activity involves a Mannich reaction at the unsubstituted hydroquinone position and oxidation to the formyl derivative, followed by condensation of the formyl group with amines (Figure 6). In these derivatives, such as rifampicin, the antibacterial activity of the antibiotic is considerably enhanced.³³

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(28) Under some conditions of fermentation, notably on addition of diethylbarbituric acid to the medium, the normally occurring complex of rifamycins (predominantly rifamycins C and D) is replaced by what is mostly a single entity, rifamycin B, differing from the other rifamycins in solubility properties.¹⁹ Rifamycin B is acidic, whereas the other rifamycins are essentially neutral. This ability to obtain one component, and the most stable component at that, aided considerably in the structural investigations of the rifamycins, since the tedious separation, preliminary to the structural investigations of the streptovaricins, for example, was obviated.

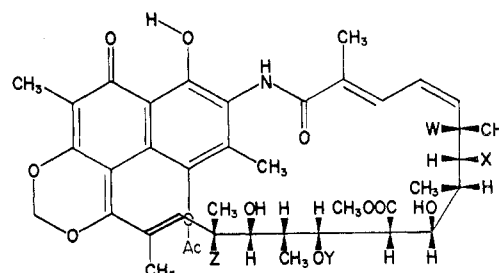
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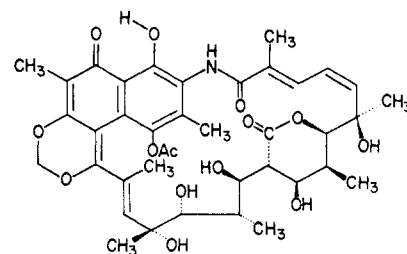
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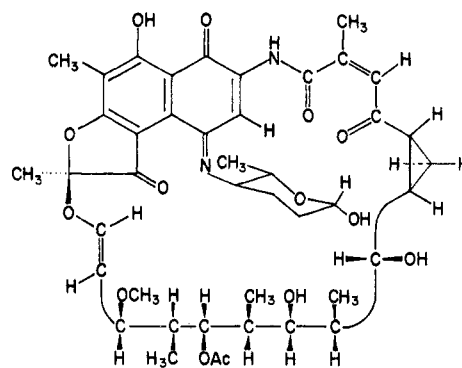


STREPTOVARICIN	W	X	Y	Z
A	OH	OH	Ac	OH
B	H	OH	Ac	OH
C	H	OH	H	OH
D	H	OH	H	H
E	H	O=	H	OH
G	OH	OH	H	OH



STREPTOVARICIN F

Figure 2. Structures assigned to streptovaricins.



TOLYPOMYCIN Y

Figure 3. Structure assigned to tolypomycin Y.

The modes of action of rifamycin^{34,35} and streptovaricin^{36,37} as antibacterial agents are very similar. Both antibiotics inhibit DNA-directed RNA polymerase in bacterial cells more strongly than any other known agent.³⁸ It seems likely that both antibiotics act at a similar site, since *E. coli* resistant to one of the antibiotics is also resistant to the other.³⁹ Specific

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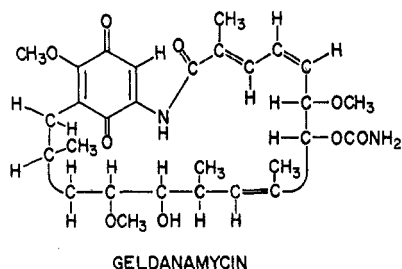


Figure 4. Structure assigned to geldanamycin.

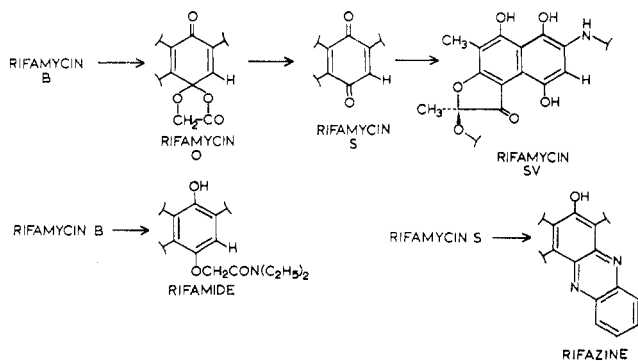


Figure 5. Conversion of rifamycin B to related biologically active compounds.

structural requirements needed to ensure activity have not been ascertained. In view of the wide variety of rifamycin derivatives which retain antibacterial activity, the principal requisite feature may be the ansa bridge. Unfortunately, reports of the mode of action of tolypomycin and geldanamycin are not available.

The effect of rifamycin and streptovaricin in inhibiting RNA polymerase recalls the similar activity of actinomycin; however, the mode of action appears to differ. Whereas actinomycin interacts with the DNA, rifamycin appears to interact directly with the polymerase itself.⁴⁰ In the case of rifamycin, the antibiotic and enzyme form a very stable complex.⁴¹ As expected, RNA polymerase from rifamycin-resistant mutants does not form a similar complex.⁴¹ The action of chromomycin is the same as that of actinomycin.⁴² In keeping with this difference in mode of action between streptovaricin-rifamycin and actinomycin-chromomycin: (1) the former antibiotics do not interfere with DNA-dependent DNA synthesis,^{35a} (2) their effect on RNA synthesis is independent of the base composition of the DNA template,^{35a} (3) they must be added prior^{37,42} to the initiation of the polymerization reaction since their specific action lies in blocking initiation of the RNA synthesis,^{42,43} and (4) the activity is much greater against bacterial RNA polymerase than against mammalian⁴⁰ or tumor³⁷ RNA polymerase.

In bacterial polymerase studies rifampicin (Figure 6) usually is used, although rifamycins B and SV (Figures

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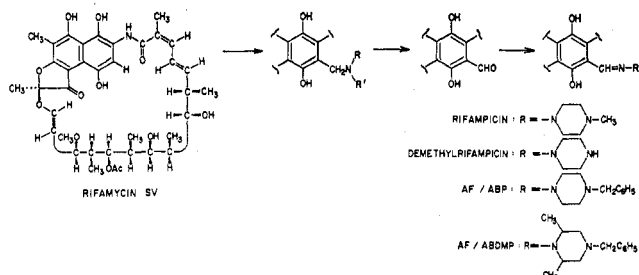


Figure 6. Biologically important compounds derived from rifamycin SV.

1 and 5) also appear to be effective. Other organisms are inhibited by rifamycin derivatives as well. Growth of *Anacystis montana* (a blue-green algae representative) was completely blocked by rifamycins B and S and rifampicin, while growth of *Chlorella pyrenoidosa* (green algae) was unaffected.⁴⁴ On the other hand, development of trachoma agent was markedly inhibited by rifampicin and rifamycin SV but not by rifamycin B.⁴⁵

The ansamycins are also antiviral agents; the activity of rifampicin has been tested with human volunteers.⁴⁶ Rifampicin (Figure 4) inhibits replication of vaccinia poxvirus,⁴⁷⁻⁴⁹ and the same effect is manifested by at least one (but not by all) of the streptovaricin components.⁵⁰ More recently *N*-demethylrifampicin and AF/ABDMP (2,6-dimethyl-*N*-demethyl-*N*-benzylrifampicin), other derivatives of formylrifamycin SV (Figure 6) have been shown to be much more potent than rifampicin in inhibiting vaccinia virus, *Herpes simplex*, and pseudorabies.⁵¹ The mode of action of rifampicin in this inhibition of viral replication appears to be different^{49,52} from that described in the preceding paragraphs for bacteria. The gross effect is inhibition of virus particle formation, and this appears to proceed by prevention of the conversion of one polypeptide to another.⁵²

Most dramatic (and most recent) of the activities of the ansamycins is that as potential antitumor agents. A number of derivatives of formylrifamycin SV (Figure 6) have been shown⁵³ to inhibit the RNA-dependent DNA polymerase activity^{54,55} of several RNA tumor viruses. In this system rifampicin was without effect, but *N*-demethylrifampicin, AF/ABP (*N*-demethyl-

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N-benzylrifampicin), and AF/ABDMP (2,6-dimethyl-*N*-demethyl-*N*-benzylrifampicin) were highly active.⁵³ For inhibition of RNA-dependent DNA polymerase activity in murine leukemia virus, the streptovaricin complex is reported to be highly active, considerably more so than purified streptovarinins A or C, or rifamycin SV, which in turn are reportedly more active than rifampicin.⁵⁶ The clinical utility of these ansamycins as antitumor agents remains unknown.

Structural Studies on Streptovarinins

Studies leading to the assignment of structure to rifamycin B have been reviewed elsewhere.^{9,10,57} Similarly, evidence for the structures of rifamycin Y^{12,13} and tolypomycin Y^{4,16,17} can be found in other sources. The structure assigned to geldanamycin is based nearly entirely on interpretation of the nmr spectra of geldanamycin and simple derivatives or degradation products.¹⁸ Consequently, we shall here exercise an author's prerogative and concentrate our attention on our own studies of the streptovarinins.

The streptovaricin complex was initially characterized in work at the Upjohn Company, where the antibiotic was demonstrated to consist of a mixture of related components⁵⁸ separable by countercurrent distribution, partition chromatography, and paper chromatography. In our work, we have made extensive use of thin (and thick) layer chromatography in separating these orange antibiotics.⁵⁹ In the normal fermentation products streptovarinins A and C are the principal components, while a number of other components can be identified in concentrates from which most of the A or C components have been removed.

Difficulty was initially encountered in establishing molecular formulas for the streptovarinins due to their formation of stable complexes with solvents. However, mass spectrometry, combined with elemental analyses of samples from which solvent had been scrupulously removed under high vacuum, allowed assignment of molecular formulas. The molecular formulas indicated immediately that the streptovarinins are closely related, being either C₄₀ or C₄₂ compounds with varying numbers of oxygen atoms. Significantly, the C₄₂ compounds contain ten *C*-methyl groups (including two acetate groups), while the C₄₀ compounds contain nine *C*-methyl groups (including one acetate group). Accordingly, it was felt that the streptovarinins all contain an identical carbon skeleton and that they differ from one another in the degree of oxygenation and degree of acetylation.⁵⁹ This supposition was supported by the fact that the ultraviolet spectra of the streptovarinins⁵⁸ are nearly identical.

Because the compounds share a common chromo-

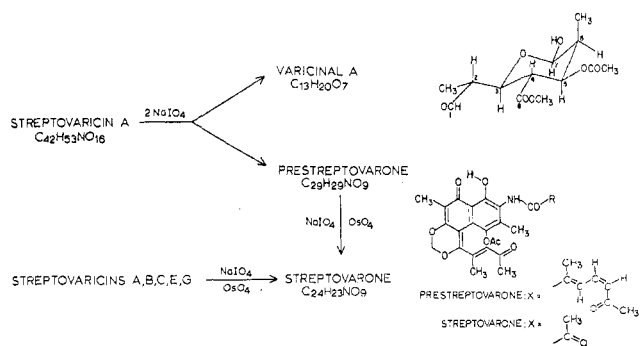


Figure 7. Degradation products derived from the streptovarinins.

phoric system, initial attempts centered on degrading the streptovarinins to a structural entity in which the chromophore was retained. However, attempts to degrade the compound by standard hydrolytic techniques were singularly unsuccessful. The failure to hydrolyze contrasts with the successful hydrolyses of rifamycin B⁹ and tolypomycin Y⁴ and can be attributed to the replacement of the enol ether function of the rifamycins and tolypomycins by a carbon-carbon bond in the streptovarinins.

Initial attempts at oxidative degradation of streptovarinins also yielded little information, but ultimately streptovaricin A was shown (Figure 7) to react with 2 moles of sodium periodate to give a colorless water-soluble product, varicinal A,^{60,61} and a water-insoluble orange product, prestreptovarone.^{15,62} Of the other streptovarinins, only streptovaricin G gave prestreptovarone on treatment with periodate. However, all the streptovarinins except streptovaricin D, on treatment with a mixture of sodium periodate and osmium tetroxide, gave streptovarone, a somewhat smaller orange compound, which was shown to be related to prestreptovarone by conversion of the latter compound to the former on oxidation with periodate-osmium tetroxide. Since both compounds contain the chromophore of the streptovarinins,⁶³ attention was directed initially to the smaller compound, streptovarone.^{62,64}

On mild acidic hydrolysis, streptovarone was converted to a deep red compound lacking nitrogen, and other hydrolyses of streptovarone yielded formaldehyde and pyruvic acid, isolated as suitable derivatives (Figure 8).⁶² A third group of streptovarone absent in the deep red compound was an enol acetate function, identified by infrared and nmr spectra. Accordingly, the new aromatic compound was referred to as dapmavarone (*deacetyldepyruvyl demethylenedioxy streptovarone*).

Dapmavarone in turn was further degraded, by a serendipitous Dakin reaction, to 3,7-dimethyl-2,5,6,8-tetrahydroxynaphthoquinone and 2-methyl-4-oxo-

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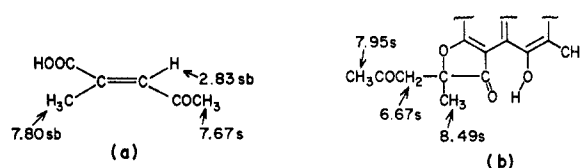
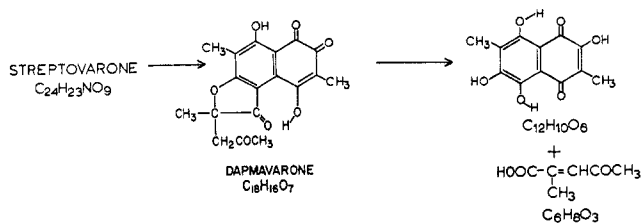


Figure 8. Degradation products derived from streptovarone.

2-pentenoic acid. The hydroxyl and methyl groups in the quinone were located by consideration of its nmr and mass spectra⁶² and by comparison of these spectral properties with those of similar compounds prepared in the extensive studies of Scheuer and Moore on spinochromes.⁶⁵ The keto unsaturated acid was initially identified from its nmr spectrum⁶² and subsequently compared to a synthetic sample, prepared by a known route.⁶⁶

With all the carbon atoms in streptovarone identified, there remained only the problem of putting the pieces together again. Since the quinone and the keto unsaturated acid had been isolated from a Dakin reaction, they could be assumed to be formed by the introduction of 1 mole of hydrogen peroxide between an aromatic ring and a carbonyl group of dapmavarone. Because the quinone has a center of symmetry, only three positions needed to be considered for attaching the carbonyl group. Ultimately, the decision rested on the nmr spectrum of dapmavarone. Indeed, many of the structural assignments from this point on rest on the shifting positions and splitting patterns of certain protons observed initially in the nmr spectrum of the keto unsaturated acid (a, Figure 9).

The olefinic proton of the unsaturated acid is lacking in the nmr spectrum of dapmavarone (b, Figure 9), being replaced by absorption of a new methylene group. Moreover, the olefinic methyl group has been shifted to a position more appropriate to a methyl group on a quaternary aliphatic carbon. These changes imply addition of an oxygen function across the carbon-carbon double bond to give an additional ring, an implication supported by the appearance of a saturated ketone's infrared absorption at 1725 cm^{-1} . The very low frequency of the aromatic ketone band (below 1635 cm^{-1}) indicates hydrogen bonding and requires the aromatic carbonyl (removed in the Dakin reaction) to be located in the central position (for hydrogen bonding) among the three hydroxyl groups of the quinone (Figure 8). Comparison of the properties of dapmavarone to those of synthetic model compounds confirms the structure shown for dapmavarone in Figure 8.⁶⁷

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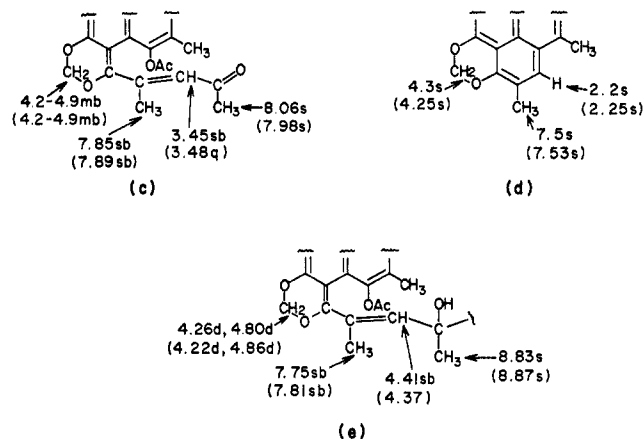
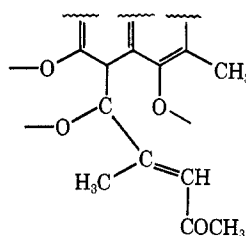


Figure 9. Chemical shifts of characteristic protons of streptovaricins and their degradation products: (a) 3-oxo-*trans*-2-pentenoic acid, (b) dapmavarone, (c) streptovarone (prestreptovarone), (d) photostreptovarone (deptovarone), (e) streptovaricin A (C).

In streptovarone (c, Figure 9), the olefinic hydrogen and an olefinic methyl group are found again. The ketone group in the side chain was confirmed by the formation of a dioxime of streptovarone, the other ketone being in the pyruvamide group.⁶⁴ The functional group in streptovarone which is hydrolyzed to formaldehyde was identified as a methylenedioxy group by its appearance at low field in the nmr spectrum, with the methylene protons being deshielded by two oxygen atoms. The amide function was identified by the nitrogen atom's neutrality and hydrogen exchange and located by comparison of its properties to those of 2-aminonaphthoquinone and 8-hydroxynaphthoquinone.⁶²

Although the partial structure in the enolic portion of the molecule obviously involved a methylenedioxy group and enol acetate, as shown here, it was not clear



which two of the three oxygens the methylene group was spanning or, by difference, which oxygen was involved in the enol acetate group. This was ultimately assigned by consideration of the structure of photostreptovarone, discussed below, which was first observed as a yellow spot appearing on thin layer plates during chromatography of the orange streptovarone.

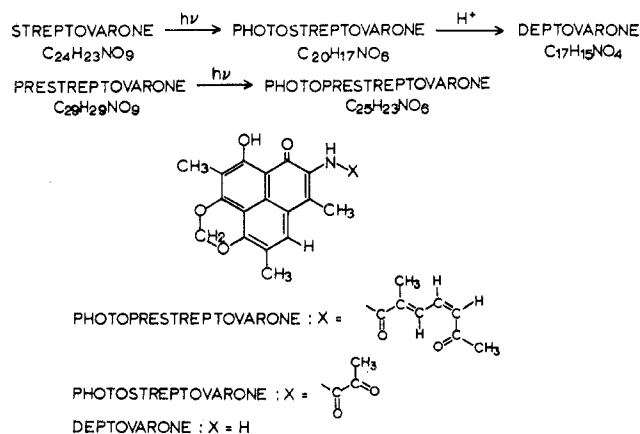
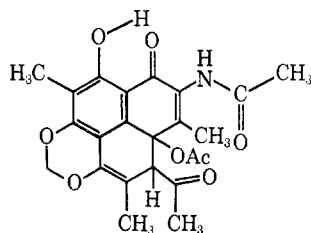


Figure 10. Structures of photostreptovarone and related products.

More careful radiation studies in solution allowed the isolation of photostreptovarone.⁶⁸ The molecular formula of photostreptovarone indicated loss of $\text{C}_4\text{H}_6\text{O}_3$ (the elements of acetic anhydride) from streptovarone and, indeed, acetic anhydride could be identified as a product of the photolysis. The yellow compound itself was shown, by comparison of its ultraviolet spectrum to those of related compounds, to be an 8-hydroxyphenalenenone (Figure 10), which would be formed *via* the intermediate dihydrophenalenone shown. A hy-



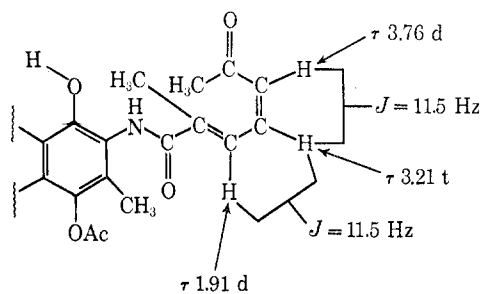
drolysis product, deptovarone (*depyruvylphotostreptovarone*), was also prepared. The methyl and hydrogen groups employed in assigning the structures of dapmavarone and streptovarone appear at the appropriate aromatic positions in photostreptovarone (d, Figure 9). The isolation of photostreptovarone and its assigned structure define the locations of the methylenedioxy and enol acetate groups in streptovarone. Taken together, these lines of argument allowed assignment of the structure of streptovarone.^{62,64}

The structure of the immediate precursor of streptovarone, prestreptovarone, was apparent once the structure of streptovarone was established, since the nmr spectrum of prestreptovarone indicated an additional unsaturated methyl group and three olefinic hydrogens. The chemical shifts and coupling constants of these hydrogens indicated the stereochemistry shown here.^{15,69,70} Photoprestreptovarone, with structure analogous to photostreptovarone, was ob-

(68) R. J. Schacht and K. L. Rinehart, Jr., *J. Amer. Chem. Soc.*, **89**, 2239 (1967).

(69) K. L. Rinehart, Jr., H. H. Mathur, K. Sasaki, P. K. Martin, and C. E. Coverdale, *ibid.*, **90**, 6241 (1968).

(70) The stereochemistry of an early sample of prestreptovarone was assigned⁶² as *trans,trans* instead of the *trans,cis* stereochemistry shown which is also that of the streptovaricins.⁶⁹ Apparently the dienamide system isomerizes readily.



tained from photolysis of prestreptovarone (Figure 10).

With the structure of prestreptovarone in hand, attention was directed toward the colorless water-soluble product from periodate oxidation of streptovaricin A, varicinal A.^{60,61} Although the electron-impact-produced mass spectrum of varicinal A did not give a molecular ion, its molecular formula could be assigned from the occurrence of peaks at $M - 31$ and $M - 18$ whose juxtaposition indicated them to be fragment ions rather than molecular ions. A field-emission mass spectrum of varicinal A gave a strong molecular ion and confirmed the molecular formula.⁷¹

The structure of varicinal A (Figure 7) rests on its nmr spectrum and those of related compounds derived from streptovaricin C.⁶¹ Individual peaks in the spectra occur at appropriate positions, and spin decoupling (Figure 11) indicates which of the protons are on adjacent carbon atoms. From the nmr spectrum of varicinal A relative stereochemistry at the atoms in the six-membered ring can be assigned (Figure 7); it is the same as that found in the rifamycins (Figure 1).

From the structures of prestreptovarone and varicinal A one can develop the structure of streptovaricins A and C,^{15,64,69} remembering that 2 moles of periodate is required to cleave streptovaricin A to prestreptovarone and varicinal A. The nmr spectra of the streptovaricins indicate that all the oxygens of the streptovaricins which are found in varicinal A are present in the streptovaricins as hydroxyl, acetate, or carbomethoxy groups, leaving as the only positions available for attachment to the prestreptovarone part of the molecule the terminal carbons, as shown in Figure 11. One point of attachment must be the ϵ carbon of the oxodienamide unit in prestreptovarone. The carbonyl group at that position must have existed as a tertiary alcohol in streptovaricin A. In principle, that carbon could have been attached to either aldehyde carbon of varicinal A. However, spin-decoupling experiments carried out on streptovaricins A and C and related compounds (Figure 11)⁶⁹ completed the linking of the carbon atoms of varicinal A with those of the prestreptovarone dienamide unit.

The second point of attachment (and the second point of periodate cleavage) was more difficult to locate. It is particularly significant that one of the olefinic methyl groups of streptovarone appears in streptovaricin A (e, Figure 9) as a deshielded aliphatic methyl

(71) E. M. Chait, T. W. Shannon, J. W. Amy, and F. W. McLafferty, *Anal. Chem.*, **40**, 835 (1968).

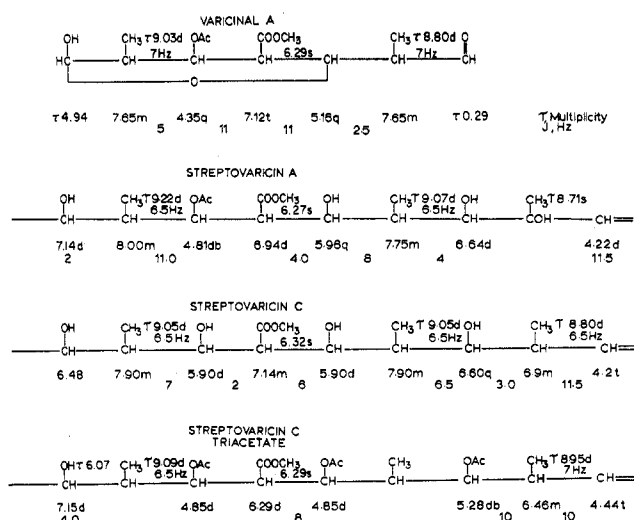
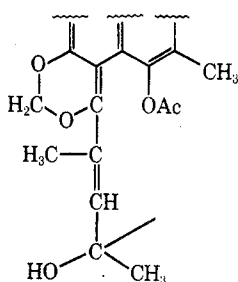


Figure 11. Chemical shifts and coupling of varicinal A protons and related protons in streptovaricins.

group, indicating the tetragonal nature of the carbon to which it is attached and suggesting an oxygen substituent. The latter point was confirmed by the appearance of the quaternary carbon at 82.9 ppm from tetramethylsilane in the ^{13}C nmr spectrum of streptovaricin C, a region appropriate for a hydroxyl-substituted carbon atom.⁶⁴ The only carbon in the diene side chain of streptovarone which is appropriate for a $>\text{C}(\text{CH}_3)\text{O}-$ group is C-4', as shown in the partial structure here. The methylenedioxy group appears



as an AB quartet. In the spectrum of streptovarone that group appeared as a very broad absorption which could be sharpened to a singlet on heating or split into an AB quartet on cooling. In photostreptovarone, the peak occurs as a singlet (d, Figure 9). Thus the structures of streptovaricins A and C are assigned as shown in Figure 2.

With the structures of streptovaricins A and C in hand, we turn to the structures of the other streptovaricins. As mentioned, these compounds must all be closely related in light of their similar molecular formulas—most likely by a common carbon skeleton differing in degree of oxygenation and acetylation.

A useful tool in assigning structures to the streptovaricins was mass spectrometry. Mass spectra of nearly all the streptovaricins contain strong peaks in the lower mass region at m/e 390, 324, 297, and 269. These peaks were initially a source of perplexity, even after determination of high-resolution spectra and assignment of structures to streptovaricins A and C,

in that there is no contiguous set of atoms in streptovaricins A and C which could lead to fragment ions having this composition. However, the perplexity was resolved with the observations that prestreptovarone and photoprestreptovarone give intense ions at m/e 390, 297, and 269, that streptovarone and photostreptovarone give peaks at m/e 324, 297, and 269, and that deptovarone gives peaks at m/e 297 and 269.^{15,72} These results indicate a process occurring in the mass spectrometer akin to the photolytic process observed in solution (Figure 10) and the streptovaricin fragmentation joins the growing list of mass spectral-photochemical analogies.⁷³ Indeed, a metastable ion has been found indicating the one-step loss of $\text{C}_4\text{H}_6\text{O}_3$ (acetic anhydride) from the $M - 43$ ion (m/e 426) of streptovarone.⁷² Since not only streptovaricins A and C but streptovaricins B, E, F, and G all give the m/e 390, 324, 297, and 269 peaks, the other streptovaricins must also contain the structural unit of streptovaricins A and C giving rise to the peaks.

A second useful tool was periodate oxidation, both in assigning the amount of periodate consumed and in establishing the formation of prestreptovarone or the lack thereof. Thus, only streptovaricin G besides streptovaricin A gives prestreptovarone on treatment with sodium periodate, consuming 2 moles of oxidant. This cleavage indicates a hydroxyl in the ϵ position of the dienamide for streptovaricin G. Lacking an acetate of streptovaricin A, it is assigned the structure in Figure 2. Streptovaricins B, C, E, and F do not give prestreptovarone on cleavage with periodate but react with 1 mole of periodate to give compounds which retain both the chromophoric unit and the complete side chain. Streptovaricins B, C, and E do not have a hydroxyl at C-6 of the macrolide ring; F has a protected hydroxyl at C-7 of the ring.

In assigning structures to the other streptovaricins, 100- and 220-MHz nmr spectroscopy were employed throughout, and essentially every proton could be located and assigned. The spectrum of streptovaricin C (Figure 11), as the most abundant streptovaricin, has been studied most extensively and has been compared carefully to those of the other streptovaricins. Structural parameters deduced by these nmr spectral comparisons have been confirmed by chemical evidence. Streptovaricin B has one acetate unit lacking in streptovaricin C; it is located at the same position as in streptovaricin A. Acetylation of streptovaricins B and C gives a common product, as does acetylation of streptovaricins A and G.¹⁵ Streptovaricin E has a molecular formula two hydrogen atoms less than that of streptovaricin C and its infrared spectrum contains an extra carbonyl group, which its nmr spectrum locates

(72) R. J. Schacht, Ph.D. Thesis, University of Illinois, 1969.

(73) *Inter alia*: A. L. Burlingame, C. Fenselau, W. J. Richter, W. G. Dauben, G. W. Shaffer, and N. D. Vietmeyer, *J. Amer. Chem. Soc.*, **89**, 3346 (1967); N. J. Turro, D. W. Weiss, W. F. Haddon, and F. W. McLafferty, *ibid.*, **89**, 3370 (1967); R. C. Dougherty, *ibid.*, **90**, 5780 (1968); M. K. Hoffmann, M. M. Bursey, and R. E. K. Winter, *ibid.*, **92**, 727 (1970); C. Fenselau, G. W. Shaffer, and W. G. Dauben, *Org. Mass Spectrom.*, **3**, 1 (1970).

at C-7 of the side chain. On reduction with sodium borohydride streptovaricin E gives streptovaricin C.¹⁵

The most distinctly different streptovaricins are streptovaricins D and F. Streptovaricin D has one oxygen atom less than streptovaricin C and one of the aliphatic singlet methyl groups in the nmr spectra of the other streptovaricins is replaced by a methyl doublet at higher field. Streptovaricin D is not cleaved by sodium periodate at all, in accord with its lack of hydroxyl groups at C-6 and C-4'. The mass spectrum of streptovaricin D lacks the characteristic peaks at m/e 390, 324, 297, and 269 found in the spectra of the other streptovaricins and its structure is assigned as shown in Figure 2.¹⁵

The molecular formula of streptovaricin F suggests it to be related to streptovaricin G but lacking the elements of methanol. This is confirmed by their respective nmr spectra in that the methoxyl group absorption of the other streptovaricins is absent in the spectrum of streptovaricin F and the C-7 proton is shifted downfield (due to acylation of the C-7 hydroxyl) relative to the spectra of other streptovaricins.¹⁵

Biosynthesis

Little work has been reported dealing with the biosynthesis and bioconversion of the ansamycins. Inspection of their formulas suggests the highly branched side chains are likely formed from acetate and propionate (malonate and methylmalonate) units,⁷⁴ or, possibly, from acetate with subsequent methylation of the polyacetate chain by methionine or another one-carbon unit. Preliminary results concerning the primary source of the carbon atoms of rifamycin B indicate that propionate is a good precursor but that methionine is incorporated only in the methoxyl group.⁷⁴⁻⁷⁶ The naphthoquinone rings may also be derived from acetate-propionate or from acetate with subsequent methylation.⁷⁴ However, an alternative pathway to

naphthoquinone rings could be involved, such as that involving shikimic and glutamic acids.^{77,78}

Bioconversions of some of the rifamycins have been reported. Labeled rifamycin B added to the fermentation medium has been shown to be converted to rifamycin Y, by hydroxylation and oxidation.⁷⁹ Rifamycin B itself is derived from rifamycin S, as is rifamycin L, the isomer of rifamycin B.¹¹ The latter two components appear to interconvert, but not directly, since aromatic ring-labeled rifamycin L gives labeled rifamycin B but label in the glycolate unit of rifamycin L is not retained in rifamycin B. Rifamycin O has been proposed to be an intermediate for both.¹¹

Future Directions

The ansamycins represent some of the most complex antibiotics thus far isolated, and further members of this family will presumably be reported. The very high activity of the compounds promises continuing interest in them, both as tools of molecular biology through study of their mode of action and as potentially useful chemotherapeutic agents. In the latter capacity, it seems likely that the remarkable latitude allowed in structural modification with retention of biological activity will prompt further investigation along these lines. Moreover, the demonstrated ability to direct *Streptomyces mediterranei* toward production of one selected component of the rifamycins suggests the promising nature of continuing efforts toward biological modification of all these antibiotics.

It is a pleasure to express here my sincere appreciation to my collaborators whose work is discussed here—F. J. Antosz, C. E. Coverdale, M. L. Maheshwari, P. K. Martin, H. H. Mathur, K. Sasaki, R. J. Schacht, and D. C. Schlegel; to the Upjohn Co. for streptovaricin samples; and to the National Institute of Allergy and Infectious Diseases for their grants (AI 01278 and AI 04769) in support of this work.

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(76) V. Prelog, personal communication.

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(78) D. J. Robbins, I. M. Campbell, and R. Bentley, *ibid.*, **39**, 1081 (1970), and earlier papers.

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