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# Carbon-13 Nuclear Magnetic Resonance Spectra of the Streptovaricins and Related Compounds[1,2]

Katsumi Kakinuma, Barry I. Milavetz, and Kenneth L. Rinehart, Jr.*

*School of Chemical Sciences, University of Illinois, Urbana, Illinois 61801*

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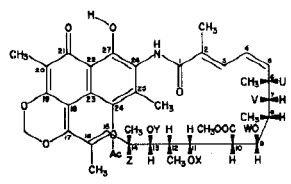
Absorptions of the 40 carbon atoms of streptovaricin C have been assigned in the carbon magnetic resonance spectrum of that ansamycin antibiotic. Carbon absorptions of other streptovaricins, including streptovaricin D, whose biosynthesis has recently been studied, have also been assigned. Methods employed in assigning the individual carbons include off-resonance decoupling, specific proton decoupling, and comparison of the streptovaricins' spectra with spectra of one another, of compounds derived from the streptovaricins, and of model compounds.

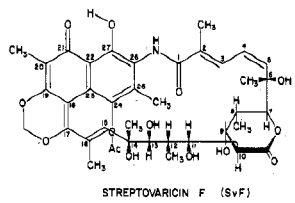
During the course of our extensive studies of the chemistry and biochemistry of the streptovaricins,[1,3] members of the ansamycin class of antibiotics,[3] it has become necessary to assign the chemical shifts of individual carbon atoms in their carbon magnetic resonance ($^{13}C$ NMR) spectra, both for studying the biosynthesis of streptovaricins[2] and for the characterization of new, related compounds. In the present paper, we report these $^{13}C$ NMR assignments.

## Discussion

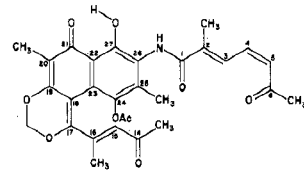
Chemical shifts for the carbon atoms of streptovaricins A–E, G, and J (SvA–SvE, SvG, and SvJ, respectively)[4] and for compounds derived from the streptovaricins (all of whose structures are shown in Figure 1) were determined on proton decoupled spectra and are summarized in Table I. Assignments were made by comparison of the spectra with proton off-resonance decoupled spectra, by single-frequency proton decoupling experiments, from standard chemical shift data, and by comparison with chemical shifts of model compounds, as discussed below. The most abundant component of the complex, SvC, was employed as the reference compound for the assignments and the spectra of other compounds were compared with that of SvC. This was especially valuable since SvC occupies a central position in the streptovaricin family (Figure 1). Thus, SvD is 14-deoxy-SvC, SvB is SvC 11-acetate, SvJ is SvC 7-acetate, SvG is 6-hydroxy-SvC, and SvE is 7-oxo-7-deoxy-SvC. Streptovaricin A is SvG 11-acetate and SvF is *O*-demethyl-SvG-7-lactone. The spectrum of SvD is of special significance, since it is the component isolated in our biosynthetic studies employing $^{13}C$-labeled precursors.[2]

Carbon atom absorptions were divided initially into the groups shown in Table I according to the number of attached hydrogen atoms (i.e., methyl, methylene, methine, quaternary carbons) by observations of the off-resonance decoupled spectra. Following this, some of the carbons—the methoxy carbon, the quaternary aliphatic C-14, the methylenedioxy carbon, and the quinonoid carbonyl carbon (C-21)[5]—were assigned unambiguously from their off-resonance multiplicities and characteristic chemical shifts[6]



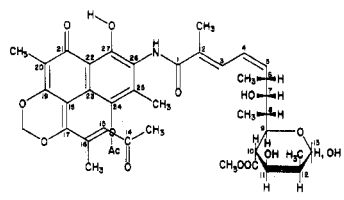


STREPTOVARICIN F (SvF)



PRESTREPTOVARONE (Pre)

| STREPTOVARICIN | U | V | W | X | Y | Z |
|---|---|---|---|---|---|---|
| A (SvA) | OH | OH | H | Ac | H | OH |
| B (SvB) | H | OH | H | Ac | H | OH |
| C (SvC) | H | OH | H | H | H | OH |
| D (SvD) | H | OH | H | H | H | H |
| E (SvE) | H | =O | H | H | H | OH |
| G (SvG) | OH | OH | H | H | H | OH |
| J (SvJ) | H | OAc | H | H | H | OH |
| C TRIACETATE (SvCAc$_3$) | H | OAc | Ac | Ac | H | OH |



STREPTOVAL C (Sval C)

**Figure 1.** Structures of streptovaricins and compounds derived from them. ASvC is atropisostreptovaricin C, in which C-15 and C-16 are *below* the aromatic acetate instead of above it as shown in the figure; SvCH$_2$ is 4,5-dihydrostreptovaricin C; Isopre is $\Delta^{4,5}$-*trans*-prestreptovarone.

as the signals at 51.9, 77.3, 89.7, and 188.5 ppm, respectively, for SvC. The remaining carbons were assigned through the more detailed analyses which follow.

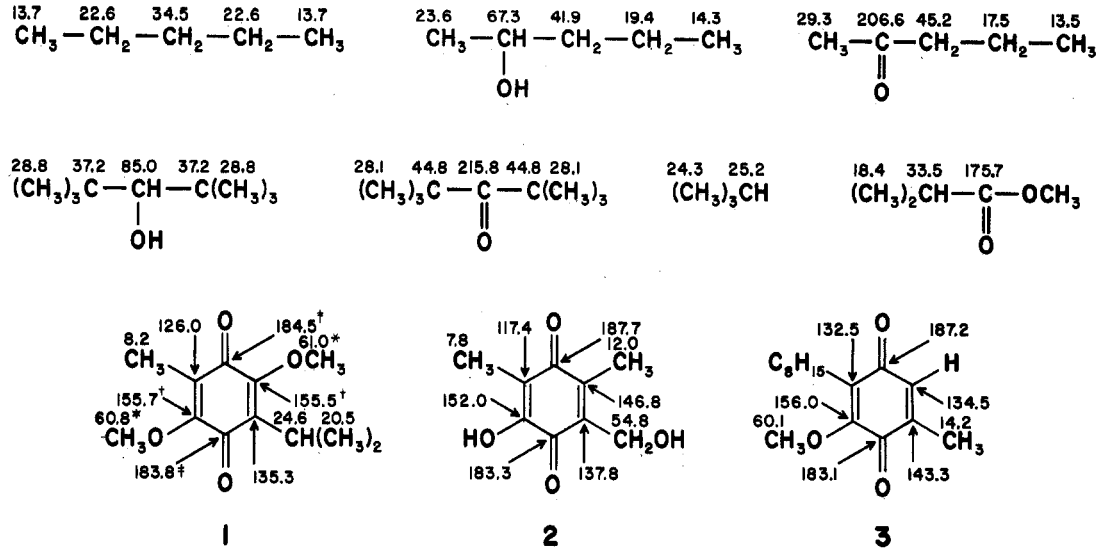
**CH$_3$ Carbons.** There are nine methyl carbons attached to other carbon atoms in SvC; these were assigned as follows. The *acetate methyl* carbon of SvC was found at 21.2 ppm and those of the other compounds between 20.9 and 21.3 ppm (see Table I), in agreement with the reported range of acetate methyl carbons (19.6–21.1 ppm).[6]

The 6-methyl, 8-methyl, and 14-methyl carbons were assigned by comparison of the chemical shifts of individual streptovaricins, whose structures are known. Chemical shifts of the methyl carbons of SvC and SvD are nearly identical, except for that at 21.9 ppm[7] in the spectrum of SvC, which appears at 15.1 ppm in the spectrum of SvD. This absorption must be due to the *14-methyl,* since SvD differs from SvC only in its lack of a 14-hydroxyl (compare pentane with 2-pentanol in Figure 2). Next, comparison of

Table I. Carbon Magnetic Resonance Assignments for Streptovaricins and Related Compounds

| Carbon | | δ, ppm[a,b,c] | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Type | No.[d] | SvA[e] | SvB[e] | SvC | SvD | SvE[e] | SvF | SvG[e] | SvJ | SvCAc$_3$ | SvalC | Pre | Isopre | SvCH$_2$ | ASvC |
| $-CH_3$ | 2-$CH_3$ | 13.3* | 13.1 | 13.1 | 12.9 | 12.9 | 13.4* | 13.3* | 13.2 | 13.4 | 13.2 | 13.3 | 13.8* | 13.3* | 13.3* |
| | 6-$CH_3$ | 25.0 | 22.4 | 22.1* | 22.2 | 18.8 | 27.8 | 25.8 | 22.3* | 19.3* | 18.7 | 31.9 | 28.0 | 20.2 | 21.9† |
| | 8-$CH_3$ | 19.0† | 19.4 | 15.8 | 15.7 | 14.0 | 16.5 | 18.8 | 15.9 | 18.6* | 13.9 | | | 14.9 | 15.6 |
| | 12-$CH_3$ | 10.8 | 10.6 | 10.4 | 9.1 | 10.4 | 12.3 | 10.4 | 10.3 | 10.7 | 10.6 | | | 10.4 | 11.1 |
| | 14-$CH_3$ | 23.4† | 19.4 | 21.9* | 15.1 | 22.0 | 25.1 | 22.1 | 21.3* | 22.8* | 32.3 | 32.2 | 32.2 | 22.2 | 21.6† |
| | 16-$CH_3$ | 12.6 | 12.4 | 12.7 | 12.7 | 12.6 | 12.9* | 12.5 | 12.5 | 12.4 | 16.9 | 16.8 | 16.8 | 12.8 | 12.9* |
| | 20-$CH_3$ | 7.5 | 7.4 | 7.4 | 7.4 | 7.4 | 7.5 | 7.4 | 7.4 | 7.5 | 7.6 | 7.6 | 7.5 | 7.5 | 7.4 |
| | 25-$CH_3$ | 13.6* | 13.4 | 13.9 | 14.2 | 13.8 | 14.1 | 13.5* | 14.1 | 13.4 | 13.9 | 13.9 | 14.1* | 13.5* | 14.5 |
| | Acetate | 21.3 | 21.2 | 21.2 | 21.3 | 21.2 | 21.0 | 21.3 | 21.3 | 20.9 | 20.8 | 20.7 | 20.7 | 21.3 | 21.3 |
| | | 21.3 | 21.2 | | | | | | 21.3 | 21.0 | | | | | |
| | | | | | | | | | | 21.3 | | | | | |
| | | | | | | | | | | 21.3 | | | | | |
| $-CH_2-$ | C-4 | | | | | | | | | | | | | 25.5 | |
| | C-5 | | | | | | | | | | | | | 29.9 | |
| >CH– | C-6 | | 38.1* | 41.6 | 41.9 | 51.6* | | | 41.6 | 37.9† | 40.9 | | | 40.3 | 41.6 |
| | C-8 | 36.9 | 37.9* | 38.9† | 38.8 | 45.6 | 40.7 | 39.0 | 38.4 | 36.9† | 38.7* | | | 37.2 | 39.0 |
| | C-10 | 46.8 | 50.6 | 47.6 | 47.4 | 46.4 | 54.9 | 46.2 | 48.8 | 50.3 | 49.3 | | | 46.6 | 48.5 |
| | C-12 | 34.9 | 36.8 | 38.7† | 38.5* | 37.7 | 30.4 | 36.8 | 37.5 | 35.9† | 36.0* | | | 38.9 | 38.5 |
| | C-14 | | | | 37.5* | | | | | | | | | | |
| $CH_3O-$ | | 52.2 | 52.2 | 51.9 | 51.9 | 51.8* | | 52.1 | 51.8 | 52.2 | 52.3 | | | 52.0 | 52.1 |
| >CHO– | C-7 | 82.1 | 81.5 | 82.7 | 83.6 | | 88.5 | 83.0 | 81.9 | 79.1 | 77.8 | | | 82.0 | 82.1 |
| | C-9 | 77.1‡ | 76.3† | 77.6 | 77.6 | 73.7 | 75.8† | 77.4 | 75.1 | 76.2 | 71.5† | | | 78.8 | 77.5 |
| | C-11 | 75.0# | 75.5‡ | 74.0‡ | 73.4† | 73.7† | 75.3† | 73.4† | 74.4† | 73.2‡ | 69.2† | | | 73.7† | 72.1‡ |
| | C-13 | 71.6# | 74.2‡ | 70.6‡ | 70.4† | 70.4† | 71.7† | 71.0† | 70.7† | 72.4‡ | | | | 70.9† | 71.3‡ |
| >CO– | C-6 | 75.0 | | | | | 78.1 | 76.4 | | | | | | | |
| | C-14 | 77.6‡ | 77.2† | 77.3 | | 77.4 | 77.3 | 77.4 | 77.4 | 77.0 | | | | 77.4 | 76.3 |
| $-OCH_2O-$ | | 89.7 | 89.9 | 89.7 | 89.8 | 89.7 | 90.9 | 89.8 | 89.8 | 89.8 | 90.1 | 89.9 | 90.0 | 89.8 | 90.0 |
| –OCHO– | C-13 | | | | | | | | | | 97.0 | | | | |
| =CH– | C-3 | 132.1 | 132.6 | 135.0 | 134.7 | 134.0 | 132.1 | 134.4 | 134.8 | 131.8 | 130.4 | 135.3 | 136.8 | 144.7 | 134.9 |
| | C-4 | 124.6 | 123.6 | 124.2 | 124.0 | 124.8 | 126.2 | 124.1 | 124.5 | 124.0 | 124.5 | 128.9* | 134.9 | | 125.6 |
| | C-5 | 142.4 | 143.1 | 143.9 | 144.1 | 139.2 | 140.0 | 144.7 | 141.5 | 140.2 | 140.4 | 128.6* | 131.9 | | 143.1 |
| | C-15 | 154.8 | 152.0 | 153.9 | 153.6 | 154.7 | 148.7 | 153.4 | 154.0 | 151.5 | 131.6 | 131.5 | 131.6 | 154.0 | 149.3 |
| =C< | C-2 | 130.8 | 129.3 | 126.7 | 127.5‡ | 127.9 | 131.2 | 129.4 | 127.1 | 130.3# | 130.7 | 140.2 | 139.6 | 127.5 | 126.1 |
| | C-16 | 132.1 | 130.8 | 130.3 | 127.9‡ | 130.0 | 134.0 | 131.1 | 130.4 | 130.7# | 144.2 | 143.9 | 144.0 | 130.9 | 127.4 |
| | C-17 | 168.5** | 168.9 | 168.8# | 169.0 | 168.3‡ | 171.1‡ | 168.9‡ | 168.4 | 168.9 | 162.6 | 162.3 | 162.6 | 169.1‡ | 169.1# |
| | C-18 | 102.3 | 102.6 | 102.0 | 101.9 | 102.0 | 102.6 | 102.2 | 102.2 | 102.6 | 106.4 | 106.3 | 106.3 | 102.2 | 101.9 |
| | C-19 | 159.3 | 159.6 | 159.4 | 159.6 | 159.5 | 161.2 | 159.4 | 159.7 | 159.6 | 159.5 | 159.2 | 159.4 | 159.7 | 159.6 |
| | C-20 | 113.4 | 113.6 | 113.3 | 113.4 | 113.6 | 114.3 | 113.7 | 113.4 | 113.6 | 113.0 | 112.9 | 113.0 | 113.6 | 114.0 |
| | C-22 | 107.3 | 107.3 | 107.2 | 106.9 | 107.1 | 107.5 | 107.4 | 107.3 | 107.3 | 109.2 | 109.0 | 109.1 | 107.4 | 107.7 |
| | C-23 | 125.4 | 125.4 | 125.4 | 125.6 | 125.5 | 126.7 | 126.4 | 125.4 | 125.6 | 125.3 | 124.8 | 124.7 | 126.0 | 126.4 |
| | C-24 | 138.3 | 136.7 | 136.6 | 136.9 | 138.3 | 137.3 | 136.5 | 136.8 | 136.8 | 137.4 | 137.2 | 137.3 | 136.7 | 136.6 |
| | C-25 | 122.3 | 122.7 | 121.7 | 121.5 | 121.5 | 123.1 | 121.1 | 122.1 | 122.5 | 123.2 | 123.2 | 122.9 | 121.6 | 122.0 |
| | C-26 | 135.9 | 135.9 | 135.0 | 134.5 | 136.6 | 137.3 | 136.0 | 135.1 | 135.8 | 135.2 | 134.9 | 135.5 | 135.8 | 135.1 |
| | C-27 | 150.5 | 155.2 | 153.5 | 153.1 | 153.1 | 156.4 | 155.2 | 153.6 | 155.2 | 154.5 | 154.3 | 154.4 | 154.4 | 154.8 |
| –C– | C-1 | 168.7** | 168.9 | 169.0# | 169.4 | 169.3‡ | 170.2‡ | 170.2‡ | 169.3‡ | 169.1** | 168.5‡ | 167.7 | 167.1 | 170.5 | 169.3# |
| O | C-6 | | | | | | | | | | | 198.4 | 198.1 | | |
| | C-7 | | | | | 178.3 | | | | | | | | | |
| | C-14 | | | | | | | | | | 199.0 | 199.0 | 198.6 | | |
| | C-21 | 188.7 | 189.0 | 188.5 | 188.7 | 188.5 | 189.7 | 188.7 | 188.8 | 188.9 | 189.1 | 188.9 | 189.0 | 188.8 | 189.0 |
| | –COOMe | 173.4 | 173.9 | 173.4 | 173.0 | 173.1 | 173.1 | 173.5 | 173.7 | 172.2 | 174.9 | | | 173.6 | 174.0 |
| | Acetate | 169.8 | 169.8 | 169.0 | 169.0 | 168.8‡ | 172.7 | 169.2 | 169.0‡ | 169.4** | 168.6‡ | 168.2 | 168.4 | 169.3‡ | 170.6 |
| | | 171.7 | 171.8 | | | | | | 170.9 | 170.3 | | | | | |
| | | | | | | | | | | 170.4 | | | | | |
| | | | | | | | | | | 171.0 | | | | | |

[a] $CD_2Cl_2$ solvent, except for SvF ($CD_3OD$ solvent). [b] For abbreviations employed, see note 4. [c] Signals marked *, †, ‡, #, ** may be interchanged in the column where they appear. [d] The numbering system is that shown in Figure 1. [e] Proton off-resonance spectra were obtained for all compounds except these.




1 2 3

**Figure 2.** Chemical shifts of carbon atoms in model compounds. Values for pentane, 2-pentanol, 2-pentanone, di-*tert*-butylcarbinol, di-*tert*-butyl ketone, isobutane, and methyl isobutyrate are from ref 6b, values for 3,6-dimethoxythymoquinone (1) from ref 6a, values for shanorellin (2) from ref 10, values for 3-*O*-methylperezone (3) from ref 12.

the spectra of SvC and SvE (which differ only in the keto group at C-7 in SvE) shows that the two methyl carbons at 15.8 and 22.1[7] ppm in the SvC spectrum are shifted upfield to 14.0 and 18.8 ppm in the SvE spectrum; no other methyl carbons are shifted more than 0.3 ppm. These carbons must then be the 6- and 8-methyl carbons (compare di-*tert*-butylcarbinol with di-*tert*-butyl ketone in Figure 2). The signal at 15.8 ppm moves to 19.4 ppm in the spectrum of SvB with its 11-acetate and is thus assigned to the 8-methyl, while the signal at 22.1 ppm shifts only slightly and is assigned to the 6-methyl. Both the 6-methyl (22.1 ppm) and the 8-methyl (15.8 ppm) absorptions are shifted downfield (to 25.8 and 18.8 ppm, respectively) in the spectrum of SvG, reflecting $\beta$ and $\delta$ effects of the 6-hydroxyl group in SvG.

Assignments of the remaining five methyl carbon absorptions can be made by comparing the $^{13}C$ NMR spectra of SvC and the oxidation products SvalC[4] (obtained from the periodate cleavage of SvC)[1b,8] and Pre[4] (obtained from the 2-mol periodate cleavage of SvA).[9] Comparison of the structures of SvC, SvalC, and Pre indicates that four methyl groups are structurally constant throughout—the acetate methyl, the 2-methyl, the 20-methyl, and the 25-methyl. Indeed, four methyl carbon absorptions do not change among the $^{13}C$ NMR spectra of SvC, SvalC, and Pre—those at 7.4, 13.1, 13.8, and 21.2 ppm. The last is the acetate methyl; thus the first three are one or another of the 2-, 20-, and 25-methyls. The methyl carbon on the quinone ring in 3,6-dimethoxythymoquinone (1, Figure 2) absorbs at 8.2 ppm[6b] and the corresponding methyl carbon in shanorellin (2) at 7.8 ppm;[10] this allows assignment of the 20-methyl carbon to the signal at 7.4 ppm. On standing, Pre isomerizes about the $\Delta^{4,5}$ double bond (cis → trans).[9] In the spectrum of the resulting isoprestreptovarone (Isopre)[4,11] the methyl signal at 13.3 ppm in the spectrum of Pre has shifted to 14.1 ppm but the methyl signal at 13.9 remains at 13.8 ppm. This allows assignment of the signal at 13.3 in Pre (13.1 in SvC) to the *2-methyl* and the signal at 13.9 in Pre (13.8 in SvC) to the *25-methyl*. It is of some interest that the cis → trans isomerization of the $\Delta^{4,5}$ double bond also shifts the 6-methyl carbon (acetyl methyl) from 31.9 ppm to 28.0 ppm, confirming the assignment of the 6-methyl in Pre.

The foregoing discussion has assigned all of the methyl carbon signals of SvC except those due to the 12- and 16-methyls, which must give the signals at 10.4 and 12.7 ppm. The $^{13}C$ NMR spectrum of SvalC contains a signal at 10.6 ppm but none near 12.7 ppm. This implies that the *16-methyl* appears at 12.7 and the *12-methyl* at 10.4 ppm in the SvC spectrum, since the carbons which are expected to shift most in SvalC vs. SvC are the 16-methyl and 14-methyl carbons. To locate the 16-methyl in the SvalC spectrum we note that the carbons which remain structurally the same in SvalC and Pre (in addition to the acetate, 2-, 20-, and 25-methyl carbons discussed in the preceding paragraph) are the 14-methyl and 16-methyl carbons, which are found at 16.9 and 32.3 ppm. Since the latter must be due to the acetyl methyl (14-methyl, see mesityl oxide in Figure 2), the 16-methyl has shifted in SvalC to 16.9 from 12.7 ppm in SvC. The assignment of the 12-methyl to the signal at 10.4 ppm is confirmed by its disappearance in the spectrum of Pre. It may be noted finally that the shift of the absorption at 21.9 ppm[7] in the SvC spectrum to 32.3 ppm in that of SvalC confirms the assignment of the 14-methyl carbon as the 21.9-ppm signal.

**>CH– Carbons.** There are four methine groups without oxygen substitution in SvC. The chemical shifts of these carbons can be assigned by comparison of the spectra of SvC, SvD, SvE, and SvG. First, a signal observed at 41.6 ppm in SvC is missing from the spectrum of SvG and must be assigned to the *C-6* carbon, which is substituted by hydrogen in SvC but by oxygen in SvG. This assignment is confirmed by the downfield shift of the 41.6-ppm signal to 51.6 ppm in SvE, where C-6 is deshielded by the carbonyl group at C-7 (compare di-*tert*-butylcarbinol and di-*tert*-butyl ketone in Figure 2). A second methine carbon signal of SvC, that at 38.9 ppm, is also shifted downfield in the spectrum of SvE, to 45.6 ppm. The signal at 38.9 ppm can then be assigned to *C-8*. Of the two remaining methine carbon signals (38.7 and 47.6 ppm) that at lower field (47.6) can be assigned to *C-10*, since that carbon is attached to a deshielding carbomethoxy group (compare isobutane and methyl isobutyrate in Figure 1). Consequently, the remaining methine carbon of SvC, *C-12*, is assigned the signal at 38.7 ppm. Streptovaricin D has one methine carbon more than SvC, *C-14;* this can be assigned to the signal at 37.5 ppm if one assumes that the signal at 38.5 ppm in the spectrum of SvD corresponds to the signal at 38.7 ppm (C-12) in the SvC spectrum. However, the absorption of the C-12 carbon is at somewhat lower field in SvC than in other

streptovaricins, being at 37.7 ppm in SvE, at 37.7 ppm in SvJ, and at 36.8 ppm in SvG. Thus, the C-14 carbon in SvD can only be assigned tentatively to the 37.5-ppm signal and the C-12 carbon to that at 38.5 ppm.

**>CHO– Carbons.** There are four methine carbons in SvC bearing hydroxyl substituents; these were assigned by comparison of spectra of streptovaricins SvC, SvE, SvJ, and $SvCAc_3$.

The signal of SvC at 82.7 ppm can be assigned to *C-7*, since it is missing in the spectrum of SvE, which has a 7-keto group. Moreover, the SvC signal at 77.6 has shifted in the spectrum of SvE to 73.7 ppm and can be assigned to *C-9*, reflecting the $\beta$ effect[6] of the carbonyl group of SvE (compare 2-pentanol with 2-pentanone in Figure 2).

The effect of acetylation on the carbinol carbons' chemical shifts is rather unpredictable and appears to depend on conformational changes as much as electronegativity. Nevertheless, C-7 shifts upfield on acetylation of SvC to SvJ (from 82.7 to 81.9 ppm) and C-7 and C-9 both shift upfield on acetylation of SvC to $SvCAc_3$ (from 82.7 to 79.1 and from 77.6 to 76.2, respectively). The third signal of SvC which shifts upfield on acetylation of SvC to $SvCAc_3$ is that at 74.0 ppm which gives either the 73.2 or 72.4 ppm signal of $SvCAc_3$. Thus *C-11* is assigned tentatively the SvC signal at 74.0 and *C-13* the SvC signal at 70.6 ppm.

**=CH Carbons.** There are four olefinic methine carbons in the streptovaricins—C-3, C-4, C-5, and C-15. Single proton decoupling of SvD assigned the first two. Irradiation of the SvD proton at 7.66 ppm (H-3) collapsed completely the $^{13}C$ NMR doublet at 134.7 ppm and partially collapsed the doublet at 124.0 ppm (due to overlap of the irradiating frequency with the H-4 frequency at 6.48 ppm in the $^1H$ NMR spectrum) but had essentially no effect on the $^{13}C$ NMR doublets at 144.1 and 153.6 ppm. Therefore, *C-3* and *C-4* of SvD are assigned to the signals at 134.7 and 124.0 ppm (135.0 and 124.2 ppm for SvC), respectively. Assignment of *C-15* was made by observing that the SvC signal at 153.9 ppm has shifted dramatically upfield in its oxidation product SvalC, to 131.6 ppm. That C-15 appears at 131.6 ppm in the latter compound follows from its appearance at the same position (131.5) in the spectrum of Pre. The fourth olefinic methine carbon of SvC (*C-5*) can then be assigned the absorption at 143.9 ppm by difference. The assignments are confirmed by the $^{13}C$ NMR spectrum of 4,5-dihydrostreptovaricin C ($SvCH_2$),[4] which retains the olefinic carbon absorption of C-15 at 154.0 ppm, which has lost the olefinic carbon absorptions for C-4 and C-5 (124.0 and 143.9 ppm), and which shows a shift of C-3 to 144.7 ppm (from 134.7 ppm in SvC). Additional confirmation of the C-15 assignment is found in the considerable shift of the C-15 absorption in the spectrum of atropisostreptovaricin C (ASvC)[1b,4] to 149.3 ppm. Atropisostreptovaricin C differs from SvC only in the absolute configuration of the helicity (P in the natural compound, M in its atropisomer) caused by steric compression of the enol acetate and $\Delta^{15,16}$ alkene groups. Thus, the spectra of ASvC and SvC should differ most in the region of steric compression. In these spectra (Table I) the acetate carbonyl carbon differs by 1.6 ppm, C-15 by 3.4 ppm (as noted above), and C-16 by 2.9 ppm (as we shall see below).

**=C< Carbons.** There are 12 quaternary olefinic or aromatic carbons in the streptovaricins. These quaternary $sp^2$ carbons can be divided further into two groups—those whose chemical environment should remain constant in the conversion SvC → SvalC (seven carbons, C-22–C-27 plus C-2) and those which should shift in that conversion (five carbons, C-16–C-20).

Instead of five, there are only four quaternary carbon peaks whose chemical shifts differ considerably (by ≥4 ppm) between the spectra of SvC and SvalC. These carbons are at 102.0, 126.7, 130.3, and 168.8 ppm in the spectrum of SvC and respectively at 106.4, 130.7, 144.2, and 162.6 ppm in the spectrum of SvalC.

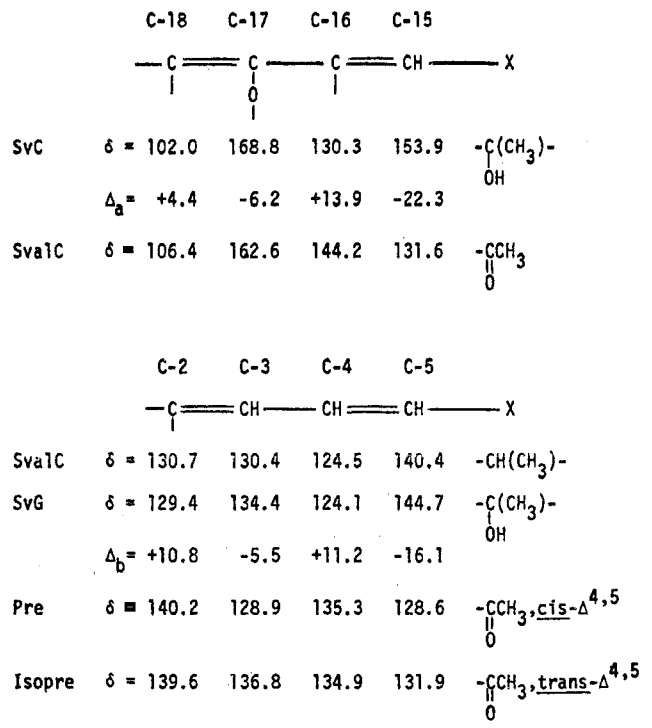



**Figure 3.** Changes in chemical shifts (ppm) of diene carbons on conversion of dienol to dienone systems; $\Delta_a = \delta_{SvC} - \delta_{SvalC}$, $\Delta_b = \delta_{Pre} - \delta_{SvG}$.

The carbons $\alpha$ and $\gamma$ to the new carbonyl group at C-14 should shift upfield.[6] We already saw that the $\alpha$ carbon, the methine C-15, shifted upfield from 153.9 to 131.6 ppm. The *C-17* carbon is then assigned to the signal at 168.8 ppm since it shifts upfield to 162.6 on conversion of C-14 to a carbonyl group ($\gamma$ effect). This assignment is in good agreement with the character of C-17, which is an enol ether and $\gamma$ to the quinone carbonyl. The carbons $\beta$ and $\delta$ to the new carbonyl group should shift downfield, the $\beta$ carbon much more than the $\delta$ (compare allyl alcohol with acrolein in Figure 2). The carbon showing the greatest downfield shift in the SvC → SvalC conversion is that at 130.3 ppm in SvC, which moves to 144.2 ppm in SvalC; it is therefore assigned from the $\beta$ effect as *C-16*. This agrees with the observation above that the signal at 130.3 ppm in SvC (C-16) is one of the two which shift most in the spectrum of ASvC due to steric compression of C-15 and C-16 by the aromatic acetate. The signal at 102.0 ppm, which shifts downfield to 106.4 ppm ($\delta$ effect), is assigned to *C-18*. The chemical shift (102.0 ppm) is appropriately upfield for the $\beta$ carbon of an enol ether.[6] The fourth quaternary carbon which shifts ≥4 ppm in the SvC → SvalC conversion absorbs at 126.7 ppm in the SvC spectrum (130.7 ppm in the SvalC spectrum), a position inappropriate for a $\beta$ carbon of an enol ether. The changes in C-15–C-18 are summarized in Figure 3.

As seen in Table I and Figure 3, a qualitatively similar, though quantitatively different, pattern is seen for the olefinic carbons near C-6 on conversion of C-6 from a carbinol carbon (SvG) to a carbonyl carbon (Pre and Isopre). Assignments of C-2 to C-5 in Pre were made by comparison of its chemical shifts to those of Isopre. From comparison of the spectra of SvalC (or SvG) and Pre it is clear that the signal at 130.7 ppm in the spectrum of SvalC (at 129.4 ppm in that of SvG) has moved downfield to 140.2 ppm in the spectrum of Pre. This must be the same quaternary carbon

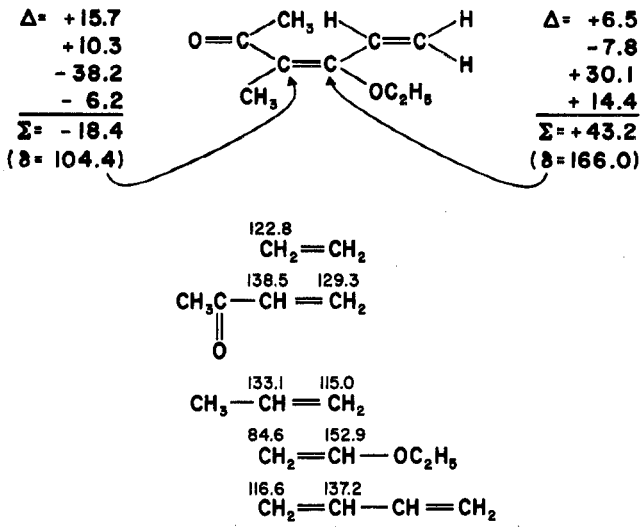



**Figure 4.** Calculated chemical shifts for the olefinic carbons of a model compound substituted like C-19 and C-20 of SvC. The substituent effects (Δ) at each olefinic carbon are listed in the order acetyl, methyl, ethoxyl, vinyl. The chemical shifts ($\delta$) for the olefinic carbons are calculated as $\delta = 122.8\ (\delta_{C_2H_4}) + \Sigma$. Chemical shifts of model compounds are shown at the bottom.

(C-2) as that giving the signal at 126.7 ppm in the spectrum of SvC. Its shift in SvalC to 130.7 must then be due to greater chromophore coplanarity in SvalC, where the constraining *ansa* bridge has been cleaved.

The other two carbons of the C-15–C-20 group (C-19 and C-20) were located by application of the substituent effects calculated in Figure 4. Starting with ethene, the effects of carbonyl group, methyl, alkoxy, and vinyl substituents can be estimated from methyl vinyl ketone, propene, ethyl vinyl ether, and butadiene, as shown, with the $\alpha$ carbon (C-20) being that attached to the carbonyl group (C-21). From these calculations *C-19* ($\beta$ carbon) would be estimated to appear near 166.0 ppm (122.8 + 43.2) and C-20 ($\alpha$ carbon) near 104.4 ppm (122.8 − 18.4). Of the signals heretofore unassigned, only that at 159.4 ppm can be considered for C-19, since that at 168.8 ppm has already been assigned to C-17 and those at 169.0 and 173.4 ppm are reserved for carbonyl carbons (see below). The signals at 107.2 and 113.3 ppm can then be considered for C-20. The signal at 113.3 ppm is slightly split in the off-resonance proton decoupled spectrum and this can be attributed to long-range coupling with a methyl group, which allows the 113.3-ppm signal to be assigned to C-20.

The six peaks which remain unassigned in the spectrum of SvC are those at 107.2, 121.7, 125.4, 135.0, 136.6, and 153.5 ppm. From simple chemical shift considerations the last three signals (but not the first three) should be due to carbons bearing oxygen or nitrogen (C-24, C-26, C-27). For example, C-1 of phenol is found at 155.6, C-1 of phenyl acetate at 151.7 and C-1 of acetanilide at 139.8 ppm.[6] On this simple basis *C-24* should give the signal at 136.6, *C-26* that at 135.0, and *C-27* that at 153.5. Of course, the chemical shift of an aromatic carbon is not determined solely by its own substituent, but by the other substituents on the ring as well. Rough chemical shifts can be calculated for the carbons of the model compound in Figure 5, based on the downfield (+) or upfield (−) shifts introduced by the six substituents shown[6] and benzene's absorption at 128.7 ppm. Although the predictions are quantitatively different, the predicted order of C-24, C-26, and C-27 is the same. In addition, the model (Figure 5) suggests the order of the final three carbons: it indicates that *C-22* should give the signal at 107.2 but cannot distinguish between C-23 and C-25 (121.7 and 125.4 ppm). Like the signal at 113.3 ppm (C-20) that at 121.7 ppm is slightly split in the proton off-resonance spectrum owing to long-range coupling to the hydrogens of an attached methyl group. Similar long-range coupling with C-20 and C-25 was observed in off-resonance decoupled spectra of SvJ, ASvC, $SvCAc_3$, and $SvCH_2$. Thus, the signal at 121.7 ppm is assigned to the methyl-bearing *C-25*, leaving the signal at 125.4 ppm for *C-23*. In connection with the latter assignment it is of some interest that the signals for both C-22 and C-23 shift downfield somewhat, by 2.0 and 1.5 ppm, respectively, in the conversion SvC → SvalC.

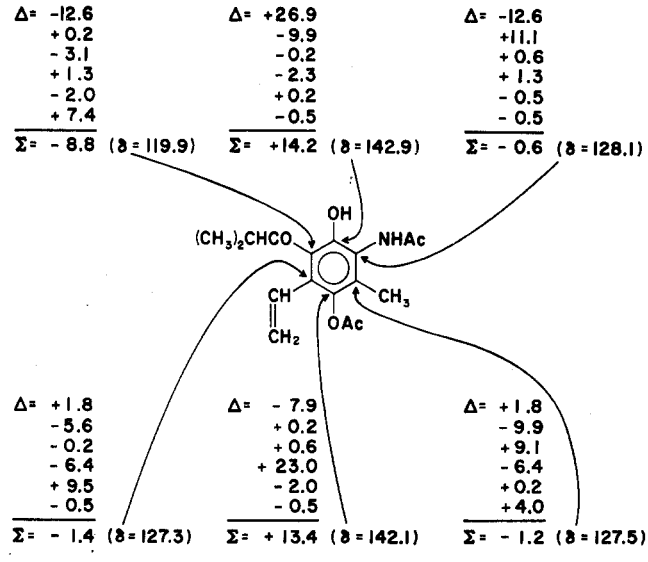



**Figure 5.** Calculated chemical shifts for a model aromatic compound substituted like SvC. The substituent effects (Δ) at each carbon are listed in the order hydroxyl, acetamido, methyl, acetoxyl, vinyl, isobutyryl. The chemical shifts ($\delta$) for individual carbons are calculated as $\delta = 128.7\ (\delta_{C_6H_6}) + \Sigma$.

**>C=O Carbons.** The carbonyl signals were assigned by comparison of the chemical shifts observed to those of model compounds. Thus, the quinone methide carbonyl carbon, *C-21*, was assigned to the signal at 188.7 ppm, since the quinones **1–3** (Figure 2) absorb in this region.[6b,10,12] The *carbomethoxy carbonyl* carbon was found at 173.3 ppm (methyl isobutyrate, 175.7 ppm),[6b] the amide carbonyl carbon (*C-1*) at 169.0 ppm (acetanilide, 169.5 ppm),[6a] and the phenolic acetate carbonyl carbon at 169.0 ppm (isopropenyl acetate, 168.9 ppm)[6c] in SvC.

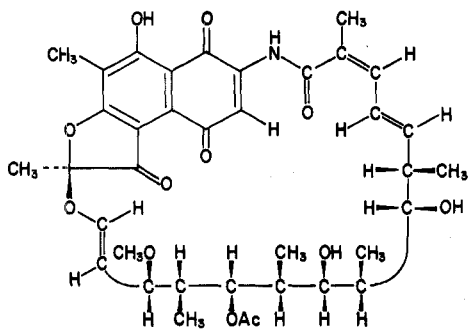
**Chemical Shifts of Other Streptovaricins.** The assignments of carbon absorptions of other streptovaricins were made by comparison of their signals with those of SvC (Table I). Some carbons shift almost none (≤1 ppm), in particular, $-OCH_2O-$, $-COOCH_3$, $-OCOCH_3$, C-1.[13] The chemical shifts for C-17–C-23 of other streptovaricins are all within 1.0 ppm of those for SvC, while those for C-2–C-5, C-15, C-16, and C-24–C-27 differ by more, but still less than 4 ppm, as shown in Table I. Similarly, the methyl carbons on C-2, C-16, C-20, and C-25 show essentially the same chemical shifts for all streptovaricins. Absorptions of other methyl carbons (those on C-6, C-8, C-12, C-14) of the ansa chain differ, however, for individual streptovaricins, mainly owing to the introduction of hydroxyl groups (SvD → SvC, SvB, SvJ → SvG, SvA); introduction of acetates (SvC → SvB → $SvCAc_3$ and SvG → SvA) has only a small effect. These methyl shifts depend mainly on the electronegativity of the attached carbons. Thus, the C-14 methyl moves upfield to 15.1 ppm in SvD (21.9 ppm in SvC) and the C-12 methyl to 9.1 ppm (10.4 ppm in SvC) owing to replacement of the C-14 hydroxyl of SvC by a hydrogen in

SvD. Similarly, the C-6 methyl is found at 22.1 ppm in SvC, but at 25.8 ppm in SvG and 25.0 ppm in SvA, and the C-8 methyl, which appears at 15.8 ppm in SvD, is also shifted downfield, to 19.0 and 18.8 ppm in SvA and SvG, respectively. All of the methyl groups are assigned, then, as shown in Table I.

Chemical shifts of the methyl-substituted methine carbons (C-6, C-8, C-10, and C-12) remain relatively constant (±1.4 ppm) for streptovaricins C, D, and J. Those carbons tend to shift together for streptovaricins with acetate groups at C-11 (SvA, SvB, $SvCAc_3$), C-6, and C-10 moving downfield by about 3 ppm, C-8 remaining constant, and C-12 moving upfield by 2–4 ppm. C-10 and C-12 of SvE remain constant but C-6 and C-8 move sharply downfield (7–10 ppm).

Methine carbons with oxygen substitution (C-7, C-9, C-11, C-13) have nearly the same chemical shift (±1.5 ppm) for all streptovaricins except SvE and SvJ, which differ by ca. 3 ppm for C-9. These assignments are again based on those of SvC as shown in Table I.

**Comparison of $^{13}C$ NMR Spectra of the Streptovaricins and Rifamycin S.**[14] The $^{13}C$ NMR spectrum of rifamycin S (4), one of the naturally occurring rifamycins,[14]



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has been assigned (and reassigned) in recent publications.[14] Since there is general structural similarity between the rifamycins and the streptovaricins, it is of interest to compare their chemical shifts. However, the rifamycins and the streptovaricins differ in a number of ways including the aromatic nuclei, an oxygen in the *ansa* chain of the rifamycins, the methylenedioxy bridge in the streptovaricins, and some stereochemical features, and it may not be meaningful to compare $^{13}C$ NMR spectra of these ansamycins in detail. Nevertheless, there are very similar chemical shifts for many carbons, including the dienamide carbons in spite of different stereochemistry ($\Delta^{2,3}$-trans, $\Delta^{4,5}$-cis double bonds in streptovaricins and $\Delta^{2,3}$-cis, $\Delta^{4,5}$-trans double bonds in rifamycin S); C-3, C-4, and C-5 are observed at 135.0, 124.2, and 143.9 ppm in SvC and at 133.8, 124.0, and 142.1 ppm in rifamycin S, respectively.

## Experimental Section[15]

**Carbon magnetic resonance spectra** were recorded by the Fourier transform technique on a Varian XL-100 spectrometer coupled to a Digilab computer. Solvent was deuterated methylene chloride, except as noted, and this was also used as deuterium lock. Chemical shifts ($\delta$) are reported as parts per million from tetramethylsilane as internal standard.

**Streptovaricin A (SvA),** purified by chromatography as described earlier,[16] was crystallized from dioxane–benzene–ether to yield deep orange needles, mp 200–203 °C (lit.[16] 200–201 °C).

**Streptovaricin B (SvB),** purified by chromatography as described earlier,[16] was crystallized twice from acetone–ether to remove small quantities of SvC, giving pure SvB, mp 186–189 °C (lit.[16] 187–189 °C).

**Streptovaricins C, D, E, and G (SvC, SvD, SvE, SvG).** The main fraction (fraction 2,[16] 108.01 g) obtained by *n*-hexane precipitation of a dioxane solution of streptovaricin complex (Upjohn 11560-3) was separated by development chromatography on 4000 g of silica gel (Brinkmann, deactivated with 10% $H_2O$) employing benzene–acetone (7:3) as solvent. After development was completed, the column was cut into appropriate fractions which were extracted to give 4.69 g of crude SvE, 4.41 g of crude SvD, 44.80 g of a mixture of SvC and SvG, and 10.60 g of a mixture of SvC, SvG, and SvA. A portion (12.01 g) of the mixture of SvC and SvG was further chromatographed on 1000 g of silica gel (Brinkmann) by elution of a column 60 × 770 mm with chloroform–methanol (98:2) to give 2.40 g of pure SvC, mp 187–190 °C (lit.[16] 189–191 °C), and 4.60 g of impure SvG which was rechromatographed over 600 g of silica gel (Brinkmann) in a column 50 × 900 mm employing benzene–acetone (4:1) as eluent. Appropriate fractions were combined, concentrated under reduced pressure, and precipitated by *n*-hexane addition to give 1.55 g of pure SvG, mp 194–196 °C (lit.[16] 190–192 °C).

A portion (4.00 g) of the SvD fraction obtained from the development chromatography column above was purified by successive chromatography over 400 g of silica gel (Brinkmann) in a column 50 × 490 mm employing benzene–acetone (9:1) as eluting solvent, over 120 g of silica gel (Brinkmann) in a column 28 × 400 mm employing chloroform–methanol (98.2) as solvent, and over 100 g of Bio-Sil A silicic acid (Bio-Rad Laboratories) in a column 28 × 350 mm employing hexane–acetone (2:1) as eluent to give 0.69 g of pure SvD, mp 183–186 °C (lit.[16] 172–175 °C).

A portion (1.68 g) of the SvE fraction obtained from the development chromatography column above was purified on 230 g of silica gel (Brinkmann) in a column 37 × 400 mm eluted with benzene–acetone (9:1). Crystallization of the SvE isolated, from 1-chlorobutane, gave 0.30 g of crystals, mp 199–202 °C (lit.[16] 198–202 °C).

**Streptovaricin F (SvF).** Crude streptovaricin F (12.40 g), obtained from streptovaricin line product (Upjohn 11560-3) by elution column chromatography on silica gel,[16] was further purified over 500 g of silica gel (Brinkmann) in a column 50 × 920 mm employing chloroform–methanol (93:7) as solvent. Appropriate fractions were combined and crystallized from ethyl acetate to give 400 mg of needles, which was recrystallized from the same solvent to yield 330 mg of pure SvF, mp 215–217 °C (lit.[16] 222–224 °C).

**Streptovaricin J (SvJ).** A sample of crude streptovaricin J[16] (3.17 g, Upjohn 10-666-JWS-36C) was purified on 200 g of silica gel (Brinkmann) in a column 40 × 500 mm employing chloroform–methanol (98:2) as eluent. Appropriate fractions were combined and evaporated to dryness under reduced pressure to give 1.80 g of pure SvJ, mp 176–180 °C (lit.[16] 177–180 °C).

**Streptovaricin C triacetate ($SvCAc_3$)** was prepared as described previously[16] and purified by chromatography over Bio-Sil A silicic acid employing chloroform–methanol (98:2) as eluent, followed by crystallization from methylene chloride–ether, mp 228–229 °C (lit.[16] 228.5–229.5 °C).

**Streptoval C (SvalC).** A mixture of 5.00 g of streptovaricin C (Upjohn 7623-WMH-59-1), 2 g of sodium metaperiodate, 300 ml of ethanol, and 40 ml of water was stirred at room temperature in a flask wrapped with aluminum foil. The reaction was followed by TLC on silica gel (Eastman Chromagram) employing chloroform–methanol (98:2) as solvent. An additional 24 g of sodium metaperiodate in 29 ml of water was added during 7 h. After reaction was complete, insoluble inorganic material was removed by filtration and the filtrate was concentrated at reduced pressure to remove ethanol. The residual aqueous suspension was extracted three times with 300-ml portions of ethyl acetate and the combined extract was dried over anhydrous sodium sulfate. The deep red residue was chromatographed on 450 g of silica gel (Brinkmann) in a column 45 × 600 mm employing chloroform–methanol (97:3) as solvent to give 0.71 g (14%) of recovered SvC and 3.76 g (75%) of SvalC, mp 140–143 °C.

Anal. Calcd for $C_{40}H_{49}NO_{14}$: C, 62.57; H, 6.43; N, 1.82; mol wt, 767. Found: C, 62.22; H, 6.59; N, 1.47; mol wt, 767 (mass spectrum).

**Prestreptovarone (Pre).** A mixture of 413 mg of streptovaricin A, 2.0 g of sodium metaperiodate, 50 ml of ethanol, and 30 ml of water was stirred at room temperature in a flask wrapped with aluminum foil. After 1.5 h, the mixture was filtered, most of the ethanol was removed from the filtrate in vacuo, and the resulting suspension was diluted with 50 ml of water and extracted three times with 50-ml portions of ethyl acetate. The extracts were combined and dried over anhydrous magnesium sulfate. The reddish-orange residue was chromatographed over 60 g of silica gel (Brinkmann) using chloroform–methanol (97:3) as eluent to give orange-red

prestreptovarone (Pre), which was crystallized from ethyl acetate to give 176 mg (84%) of Pre, mp 182.5–183.5 °C (lit.[11] 194–197 °C).

Anal. Calcd for $C_{29}H_{29}NO_9$: C, 65.03; H, 5.46; N, 2.62. Found: C, 64.01; H, 5.54; N, 2.28.

**$\Delta^{4,5}$-*trans*-Prestreptovarone (Isopre).** Prestreptovarone isomerized[3,9] on standing at room temperature in deuteriomethylene chloride solution for 26 days to give $\Delta^{4,5}$-*trans*-prestreptovarone (Isopre), whose proton NMR spectrum was identical with that of an authentic sample.[9] Isopre was used for $^{13}C$ NMR determination without isolation.

**Atropisostreptovaricin C (ASvC).** Streptovaricin C (2.35 g) was heated in 50 ml of refluxing acetonitrile for 5 h while the reaction was followed by TLC on Uniplate silica gel GF (Analtech, Inc.) using chloroform–methanol (97:3). The solution was concentrated in vacuo and the residue was chromatographed on 300 g of Bio-Sil A silicic acid in a column 40 × 600 mm employing chloroform–methanol (98:2) as eluent, with all fractions examined by TLC as above. Appropriate fractions were combined and evaporated to dryness under reduced pressure to provide 2.05 g (87%) of recovered SvC and 0.229 g (10%) of ASvC, mp 188–193 °C (lit.[1b] 188–193 °C).

**4,5-Dihydrostreptovaricin C ($SvCH_2$) and 2,3,4,5-Tetrahydrostreptovaricin C.** When streptovaricin C (5.0 g, Upjohn 7623-WMH-59-1) was hydrogenated over 1 g of 5% palladium–charcoal in 250 ml of ethanol, ca. 315 ml of hydrogen was absorbed during 5 h. The solution was filtered to remove catalyst, the filtrate was evaporated to dryness under reduced pressure, and the residue was chromatographed on 400 g of silica gel (Brinkmann) in a column 50 × 600 mm employing chloroform–methanol (98:2) as eluent to afford 0.50 g (10%) of 4,5-dihydrostreptovaricin C ($SvCH_2$), an amorphous powder, mp 163–165 °C, $[\alpha]^{25}D$ +637° (*c* 0.16, $CHCl_3$). In the $^1H$ NMR spectrum one of the olefinic protons (H-3) of SvC was shifted to 6.93 ppm (t, $J$ = 6 Hz), while the olefinic proton absorptions of SvC[9] for H-4 and H-5 were absent.

Anal. Calcd for $C_{40}H_{53}NO_{14}$: C, 62.24; H, 6.92; N, 1.81; mol wt, 771. Found: C, 61.66; H, 7.26; N, 1.81; mol wt, 771 (mass spectrum).

Continued elution of the column gave 1.20 g (24%) of a mixture of $SvCH_2$ and 2,3,4,5-tetrahydrostreptovaricin C, an amorphous powder, mp 175–179 °C. The $^1H$ NMR spectrum contained no olefinic absorption for H-3, H-4, or H-5.

Anal. Calcd for $C_{40}H_{55}NO_{14}$: C, 62.08; H 7.16; N, 1.81; mol wt, 773. Found: C, 61.26; H, 7.16; N, 1.64; mol wt, 773 (mass spectrum).

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**Registry No.**—SvA, 23344-16-3; SvB, 11031-82-6; SvC, 23344-17-4; SvD, 32164-26-4; SvE, 35413-63-9; SvF, 35512-37-9; SvG, 11031-85-9; SvJ, 52275-61-3; $SvCAc_3$, 54955-22-5; SvalC, 54955-14-5; Pre, 58074-37-6; Isopre, 58117-89-8; ASvC, 54984-97-3; $SvCH_2$, 58150-57-5; $SvCH_4$, 58074-38-7.

## References and Notes

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(4) Abbreviations: SvA = streptovaricin A; SvB = streptovaricin B; SvC = streptovaricin C, SvD = streptovaricin D; SvE = streptovaricin E; SvG = streptovaricin G; SvJ = streptovaricin J; $SvCAc_3$ = streptovaricin C triacetate; SvalC = streptoval C; Pre = prestreptovarone; Isopre = $\Delta^{4,5}$-*trans*-prestreptovarone; $SvCH_2$ = 4,5-dihydrostreptovaricin C; ASvC = atropisostreptovaricin C.
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