# Structure of Avoparcin Components[1c]

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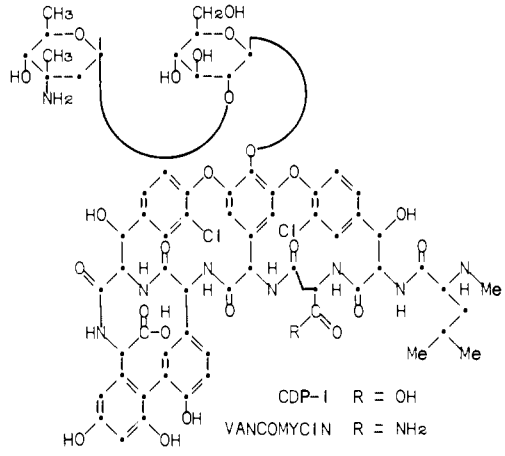
**Abstract:** The structures of avoparcin $\alpha$ and $\beta$ disclosed in this paper are based on extensive hydrolytic studies and the identification of the isolated fragments by spectral and, where possible, X-ray analysis. The amino acid sequence is suggested in part by Edman degradation work, by spectral similarities between avoparcin and vancomycin, and by the similarity between the isolated subunits of these antibiotics. The structure of vancomycin was recently elucidated by X-ray crystallography. In our deduced structures there are two uncertainties, one with regard to the position of the covalent chlorine on the triphenyl ether moiety, and the other involving the interchangeable positions of the single sugar fragments, ristosamine and mannose.

## Introduction

Avoparcin, vancomycin, actinoidin, ristocetin, ristomycin, and compound A35512B are a class of water-soluble glycopeptide antibiotics active against Gram-positive bacteria.[2] Although vancomycin was first isolated over 20 years ago, it is only recently that Williams et al. of Cambridge obtained modified vancomycin (CDP-1) which was suitable for single-crystal X-ray work, thus obtaining an unequivocal structure for this material.[3] Prior to that in a brilliant series of wet chemical studies he had obtained the major fragments of the aglycone of vancomycin, namely, the triphenyl ether and biphenyl moieties.[4] In addition, using $^1H$ and $^{13}C$ magnetic resonance data, he had essentially proposed the correct structure before the X-ray work.[5]

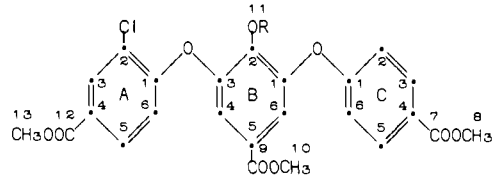



Avoparcin is a complex of antibiotics composed primarily of two main components, $\alpha$ and $\beta$, which account for the bulk of the UV area present in the LC profile. In our laboratory-prepared material, the $\beta$ component predominates about 3:1 over the $\alpha$ metabolite. There is evidence of other very minor components in the LC profile. A sample of pure $\beta$ and 90% pure $\alpha$ together with a mixture of two minor components has been isolated with some difficulty for spectral studies. These data, primarily $^{13}C$ NMR, show that $\alpha$ and $\beta$ are identical except for one aromatic chlorine atom extra in $\beta$ while the two minor components are identical with $\alpha$ and $\beta$ except for the absence of a ristosamine sugar moiety. The two desugar derivatives are also obtained from $\alpha$ and $\beta$ by mild acid hydrolysis. Thus, the sample employed for most of the degradative studies was not separated into its individual components but was a mixture principally of avoparcin $\alpha$ and $\beta$.

Much of our hydrolytic work was modeled on that of Williams et al., while the Edman degradation study was an extension of the work of Hlavka et al.[6] Studies on the structures of actinoidin, ristocetin, and ristomycin are in progress in Russia, Hungary, and the United States.[7]

## Alkaline Degradation of Avoparcin and Its Aglycone

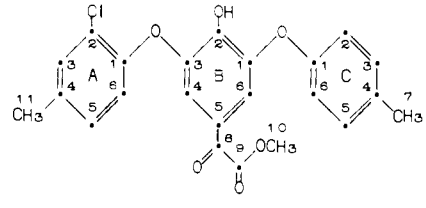
Alkaline degradations of avoparcin or its aglycone were carried out under oxidizing, normal, and reducing conditions to give a variety of interesting derivatives. The aglycone of avoparcin was prepared by a slight modification of the method of Marshall[8] to yield a product which by TLC on cellulose overnight in the system *n*-BuOH- pyridine- acetic acid-$H_2O$ (15:10:1:12) was a 4:1 mixture of two components. This aglycone mixture was methylated in methanol using methyl iodide and potassium carbonate by overnight reflux. The resultant product was subjected to oxidation with permanganate in the presence of ammonia. After esterification with diazomethane, compound I was isolated by silica gel column chromatography followed by thick layer chromatography using an acetone-hexane system.



I R = $CH_3$
Ia R = $C_2H_5$

This compound corresponds to the dichlorotriphenyl ether fragment isolated by Williams et al. from vancomycin. Compound Ia will be discussed a little later on in the text in our discussion of free phenolic groups of avoparcin. The numbering system used in this and other compounds of this report is purely arbitrary and is included to facilitate spectral assignments in the Experimental Section.

Drastic alkaline hydrolysis (reflux in 50% NaOH solution) of the intact antibiotic under positive nitrogen pressure gave compound II among the more interesting fragments isolated following derivatization and repeated chromatography.

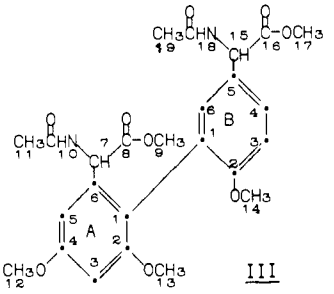


II

0002-7863/80/1502-1671$01.00/0 

Compound II gave rise to clean, readily interpretable spectral data.

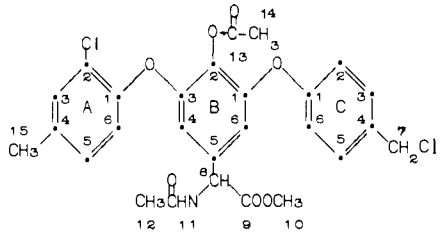
The methylated aglycone mixture from avoparcin was also subjected to basic alkaline hydrolysis in the presence of excess sodium borohydride. Following overnight reflux in 50% NaOH solution, the cooled reaction mixture was neutralized with concentrated hydrochloric acid and evaporated to dryness. The resultant solid was allowed to sit overnight in methanol saturated with dry HCl and the solid obtained from this was acetylated in pyridine-acetic anhydride. The ethyl acetate extract was then subjected to intensive chromatography and thick layer purification to provide the crystalline compound III, mp 199-201 °C.



III

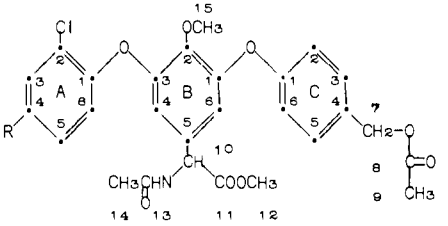
Single-crystal X-ray analysis of III put the proposed structure ($^1$H and $^{13}$C NMR) beyond doubt. It is worth noting at this stage that, when methylated avoparcin was refluxed in 2 N hydrochloric acid and the resultant derivatized fragments were subjected to intensive chromatography, III was also isolated.

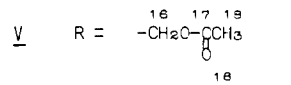
Basic hydrolysis of the aglycone of avoparcin in the presence of a limited amount of sodium borohydride, followed by the workup procedures already mentioned, yielded the fragment IV.



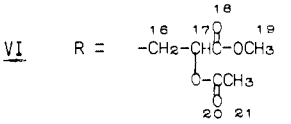
IV

Similar drastic basic hydrolysis of the aglycone in still another experiment in the presence of a limited amount of sodium borohydride yielded after derivatization and intensive chromatography three interesting fragments: V, VI, and VII. Fragment VI contains three carbon atoms in the form of a lactate attached to the triphenyl ether moiety.

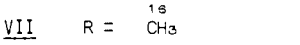




V R =

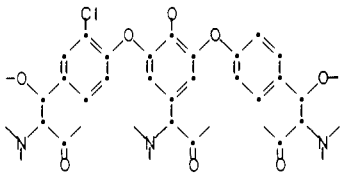


VI R =



VII R = $CH_3$

The presence of the aromatic *C*-methyls in II was initially baffling since $^1$H and $^{13}$C NMR studies rule out their presence in the intact antibiotics. However, their formation along with the benzylic chloride in IV, the substituted benzylic alcohol in V, VI, and VII, and the lactate side chain in VI suggests a common origin for all the groupings.



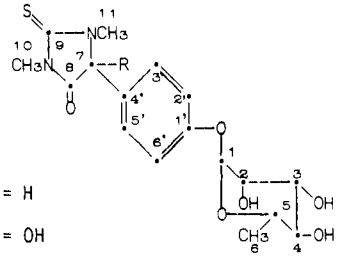
On base treatment of avoparcin, elimination of the $\beta$ oxygen functions could yield an enamide which is in turn hydrolyzed to a keto acid. Reduction with borohydride would then provide the lactate derivative (e.g., VI), whereas deoxalation would give the corresponding methylbenzene. The benzyl alcohol moieties no doubt arise by dealdolization followed by borohydride reduction of the intermediate aldehyde similar to the chemistry of vancomycin (see scheme below). The benzyl chloride unit in IV must arise from the corresponding alcohol by acidification with hydrochloric acid during workup of the crude reaction mixture.[9,10]

## Application of the Edman Method to the Avoparcin Complex

Initially in our Edman work, we used the reagent phenyl isothiocyanate, but later used methyl isothiocyanate, since this reagent is less likely to give abnormal adducts.[11]

In the first stage of the normal Edman degradation, the N-terminal peptide is stirred in aqueous pyridine with the pH adjusted to 9.0[12] using sodium hydroxide. In the second stage, hydrolysis of the coupled urea to a thiazolone in refluxing acid is followed by rearrangement to the thiohydantoin.

When avoparcin is subjected to the coupling stage of the Edman procedure, the solvent may be evaporated off and TLC of the resulting solid shows two UV-positive, isolable materials already present at this stage of the reaction. The two materials are extractable in ethyl acetate, and after suitable chromatography we recovered VIII and IX.

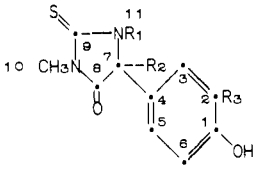


VIII R = H

IX R = OH

The corresponding phenylthiohydantoin of VIII was first reported by Hlavka et al.[6] while the corresponding phenyl compound of IX was isolated by Cosulich.[13] To our knowledge, there are no prior published reports on the occurrence of an oxidized thiohydantoin as a coproduct during Edman degradation.

This remarkable ease of cleavage and cyclization in an Edman reaction had been previously observed by Elmore[14] in his work on the amide of sarcosine. The implication of this phenomenon with avoparcin is that, at the end of a normal first stage of an Edman degradation, in effect, one and one-half actual Edman cycles have occurred in part at least. When a normal two-stage Edman cycle was carried out on avoparcin, thiohydantoins X and XI were recovered. In our hands, at the end of two actual complete Edman cycles on avoparcin, two more thiohydantoins (XII and XIII) were recovered in about a 4:1 ratio.



| | | | |
|---|---|---|---|
| X | $R_1 = CH_3$, | $R_2 = H$, | $R_3 = H$ |
| XI | $R_1 = CH_3$, | $R_2 = OH$, | $R_3 = H$ |
| XII | $R_1 = H$, | $R_2 = H$, | $R_3 = Cl$ |
| XIII | $R_1 = H$, | $R_2 = H$, | $R_3 = H$ |

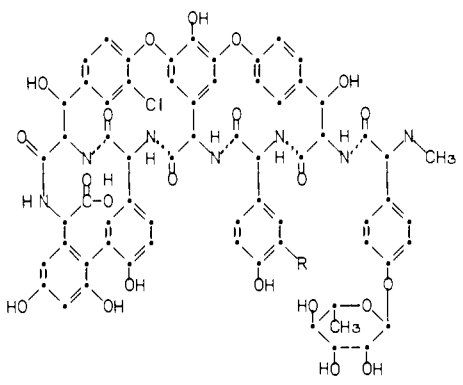
Compound XII, mp 268-270 °C, was a perfectly stable compound contrary to the experience of Hlavka[6] but in agreement with the observations of Lomakina.[7a] By TLC on silica gel using a chloroform-methanol developing system, XII showed a normal $R_f$ depending on the amount of methanol used. In addition, if the developed thin layer was allowed to sit on the bench top for about 4 h or more, the spot developed in a fashion analogous to the appearance of an amino acid spot which had been subjected to ninhydrin spray reagent. This characteristic of the material proved a useful marker for its detection. If our logic is correct, the small amounts of XII and XIII that were isolated are the end products of three effective Edman cycles on avoparcin. This fact is of significance when we come to our proposed overall structure.

The isolation of XII and XIII in the ratio of about 4:1 most likely means that the major $\beta$ component of the aglycone observed by TLC contains the chlorinated hydroxyphenylglycine compared to hydroxyphenylglycine in the minor $\alpha$ component.

We detected no trace of the corresponding oxidized thiohydantoins of XII and XIII. From this, we are forced to conclude that the important structural factor both in the ease of cleavage followed by cyclization and oxidation of thiohydantoins VIII and X is solely connected with the presence of the *N*-methyl group.

It should be pointed out that the application of the Edman procedure to avoparcin is anything but a clean, straightforward reaction. In any one step, the reaction goes to just a few percentile of the theoretical amount. Even under the most exhaustive conditions, the rhamnoside thiohydantoin was isolated in only about 25% of the expected yield. We also felt that work on the thiohydantoin derivatives would be facilitated if the free phenolic groups of the amino acid were blocked as methyl ethers. Consequently, avoparcin was methylated using methyl iodide only to find that the product failed to react with the thiocyanate reagent probably owing to further methylation of the *N*-methyl grouping.

On the basis of the work described so far, the subunits of the rhamnoside of the aglycone of avoparcin may be set down and by biogenetic analogy with vancomycin (see below) it is logical



RHAMNOSIDE OF AVOPARCIN α AGLYCONE R = H

RHAMNOSIDE OF AVOPARCIN β AGLYCONE R = Cl

to include the broken lines indicating how these fragments are connected.

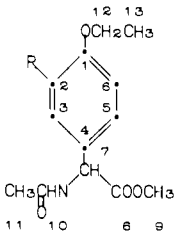
If the Edman degradation sequence is carried beyond three complete cycles, it is to be expected that at some point between the third and fifth cycles the dithiohydantoin of the biphenylamino acid will be released.

We did in fact isolate a few milligrams of a material which had the expected IR and $^1H$ NMR characteristics of such a compound. The material failed, understandably so, to give a molecular ion by electron impact mass spectrometry.

### Attempts to Determine the Free Phenolic Groups of Avoparcin

The avoparcin complex was methylated by refluxing in methanol in the presence of methyl iodide and potassium carbonate. The methylated product was then converted to the aglycone by refluxing in methanol saturated with dry HCl. The aglycone obtained from this was again methylated, this time using deuterated methyl iodide. The product from this reaction was subjected to drastic alkaline hydrolysis in the presence of a limited amount of sodium borohydride. The fragments of hydrolysis were esterified by the Fischer method and acetylated and isolated in the usual fashion. From this experiment, compound III was recovered. The isolation of III from this experiment means that all three phenolic groups of the biphenyl moiety are free in the antibiotic complex.

Avoparcin was ethylated and processed in a manner analogous to that just described except that, in the esterification step of the hydrolytic fragments, diazomethane was used instead of the Fischer procedure. After N-acetylation followed by adsorption chromatography and repetitive thick layer work, the crystalline XIV was isolated. Crystals of XIV were suitable for X-ray crystallography. The mother liquor from which XIV was obtained yielded a gummy solid which spectral data indicated was a mixture of XIV and XV.



XIV R = Cl
XV R = H

These data indicate that the phenolic group of the chlorophenylglycine of avoparcin is free. In addition, they support the notion that the minor $\alpha$ component of the aglycone mixture mentioned previously probably contains *p*-hydroxyphenylglycine in place of the corresponding chloro amino acid of the major component, again with the phenolic group free.

The remaining phenolic group is at position 2 of the B ring in the triphenyl ether moiety. Avoparcin was treated overnight with excess diazomethane, then hydrolyzed to the aglycone, which was subjected to diazoethane, and the product was oxidized with permanganate in the presence of ammonia. After esterification with diazomethane, the compound Ia was isolated, which proves that this group is glycosylated in the intact antibiotic.

### Carbohydrates of Avoparcin

In prior degradation studies on avoparcin,[6] a 2,3,6-trideoxy-3-amino sugar was isolated as the phenyl isothiocyanate derivative and the presence of glucose, mannose, and rhamnose had been detected in acidic and enzymatic hydrolysates.[13] We identified ristosamine as the amino sugar in avoparcin by methanolysis of the antibiotic and subsequent conversion of the methyl glycoside to methyl *N,O*-diacetylristosaminide which is identical with that isolated by Bognar et al. from ristomycin.[15] The presence of glucose, mannose, and rhamnose

Table I

| compd | composition of products | molar ratios |
|---|---|---|
| ristocetin | rhamnitol, arabitol, ristosaminitol, mannitol, glucitol, peracetates | 1:1:1:2:1 |
| avoparcin CDP-I | mannitol, ristosaminitol, peracetates | 1:1 |
| β-avoparcin | mannitol, ristosaminitol, rhamnitol, glucitol, peracetates | 1:2:1:1 |

was reconfirmed by methanolysis work which yielded the methyl glycosides of D-glucose, D-mannose, and L-rhamnose as shown by TLC and spectral comparisons with authentic samples.

Hakomori permethylation of avoparcin followed by chromatographic resolution of the products from methanolysis of the permethylated material yielded methyl 3,4,6-trimethylglucoside, methyl 2,3,4,6-tetramethylmannoside, and methyl 2,3,4-trimethylrhamnoside. No methylated derivative of ristosamine was isolated. These results indicate that glucose is the only nonterminal neutral sugar and that it is linked glycosidically to another sugar through the C-2 hydroxyl group.

Very mild acid hydrolysis of avoparcin yielded a microcrystalline derivative which we designate avoparcin CDP-I·HCl which still contains mannose and ristosamine as indicated by further hydrolysis. We have carried out a comparative quantitative study of the monosaccharides produced by hydrolysis of β-avoparcin, avoparcin CDP-I, and ristocetin with 2 N hydrochloric acid.[16] The carbohydrate products were reduced with sodium borohydride to the corresponding alditols, which were then peracetylated and subjected to quantitative GLC analysis. The standard compounds required for this analysis were prepared by similar treatment of glucose, mannose, arabinose, and rhamnose. Since ristosamine was not available for this study, the ristosaminitol peracetate was recovered from avoparcin degradation by thick layer chromatography. The results of this work are summarized in Table I.

## Discussion of the $^{13}C$ NMR Spectra of Avoparcin Components

Purified samples of α and β for $^{13}C$ NMR studies were prepared from the original complex by careful ion-exchange chromatography and by preparative LC on a μ-Bondapak C-18 reverse-phase column[17] (Waters Associates). The $^{13}C$ spectra of these materials (70 °C, $D_2O$, 25.5 MHz) are complex, especially in the 50-80- and 115-140-ppm regions, due to overlapping signals as well as line broadening from perhaps aggregation phenomena.[18,19] However, the spectra of the α and β components are almost identical except for slight differences in the aromatic and carbonyl regions of the curves. The spectra show three sharp *C*-methyl signals at ca. 18 ppm and are assigned to the *C*-methyls of rhamnose and ristosamine. That two of these signals are attributable to ristosamine is suggested by the double signals at 31 and 50 ppm assigned to the C-2 and C-3 carbons, respectively. These chemical shifts match closely the corresponding signals in ristocetin which also contains ristosamine.[20] A strong *N*-methyl signal is observed at 33 ppm.

Although complex, the 50-85-ppm regions include seven signals typical of amino acid α-CH's (50-55 ppm), a strong signal at 62 ppm attributable to hydroxymethyl groups, and an isolated signal at 80.5 ppm assigned to the glycosylated C-2 of glucose.

The 90-110-ppm region is best interpreted as showing four signals attributable to aromatic carbons ortho and para to oxygen substituents: two from the triphenyl and two from the biphenyl units based on the vancomycin assignments. Also visible are five signals assigned to anomeric carbons consistent with 1 mol each of glucose, mannose, and rhamnose and 2 mol of ristosamine. One of these anomeric signals resonates at 94 ppm, which is relatively high for a glycosylated anomeric carbon. We assign this signal to the C-1 of a ristosamine linked to a benzylic hydroxyl based on arguments to be discussed below in addition to $^{13}C$ NMR comparison studies with ristocetin.[7b,20]

Table II. Glycine Produced by Alkaline Hydrolysis of Glycopeptide Antibiotics (4 M NaOH)

| compd | mol wt | glycine, μmol from 1 μmol |
|---|---|---|
| avoparcin β | 1942 | 0.55 |
| vancomycin | 1560 | 1.65 |
| avoparcin CDP-I | 1504 | 0.50 |
| ristocetin | 2066 | 1.01 |

The aromatic region between 120 and 140 ppm is extremely complex and no really useful information was obtained.

The aromatic oxy carbon region at 150-160 ppm, however, is very clear, especially in the β spectrum, where nine sharp signals are seen consistent with the aglycone subunits which require the presence of nine aromatic carbons bound to oxygen not including the C-2 in the 1,2,3-trioxygenated aromatic ring of the triphenyl ether unit. This latter signal has been shown to resonate upfield in the 135-140-ppm region.[5a]

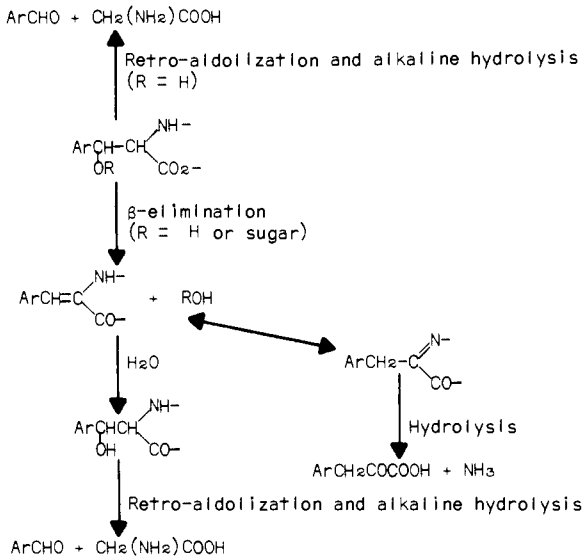
Six distinct carbonyl signals are observed between 168 and 173 ppm. In addition, there is a broader and less intense resonance at 177 ppm which we have assigned to a carboxylate anion similar to that in vancomycin. This sample was prepared by freeze-drying a pH 5.5 solution of β in which the carboxyl grouping is in the anionic form. Samples prepared from more acidic solutions (e.g., pH 2) gave less clear spectra (owing to some cleavage of glycosidic linkages) in which the chemical shift of the carboxyl carbon was moved upfield ca. 3-5 ppm consistent with this grouping being fully protonated.[21]

A $^{13}C$ spectrum of avoparcin CDP-I ($Me_2SO$-$d_6$, pH 3.5, 70 °C, 25.5 MHz) shows clearly the *C*-methyl signal of ristosamine at 18.5 ppm, a 60.9-ppm signal for the $CH_2OH$ of mannose, and six signals (all doublets in the off-resonance spectrum) at 92.5, 99.0, 103.0, 103.5, 106.0, and 107.5 ppm consistent with this compound being the aglycone to which is still attached 1 mol each of mannose and ristosamine. In addition, nine well-defined signals between 147.5 and 153.3 ppm are again assigned to the aromatic carbons carrying oxygen substituents. Six sharp carbonyl carbon signals resonate between 167.1 and 170.8 ppm in addition to two less intense signals at 169.4 and 169.9 ppm. No signal at 177 ppm is observed. The absence of a signal between 75.2 and 85.0 ppm compared with the presence of a signal at 80.3 ppm in the intact antibiotics[22] proves the presence of ristosaminylglucose disaccharide in avoparcin. Biogenetic analogy with vancomycin leads us to the conclusion that this disaccharide is linked glycosidically to the C-2 oxygen of the 1,2,3-trioxygenated ring of the aglycone.

We have carried out a study on the amounts of glycine produced on alkaline hydrolysis of vancomycin, avoparcin, ristocetin, and avoparcin CDP-I. The hydrolyses were effected with 4 N sodium hydroxide and the glycine produced in each case was estimated quantitatively using the amino acid analyzer. The results are given in Table II.

The purpose of this was to get some insight into the presence of free or glycosylated β-hydroxyphenylalanine residues in the respective compounds. It had originally been assumed that glycine would arise from retro-aldolization of the free β-hydroxyphenylalanine residues as had been demonstrated for vancomycin[4a] but that glycosylated residues would not give rise to glycine. However, the situation is more complex than

**Scheme I**




this, since $\beta$-elimination could also occur, whether or not the $\beta$ oxygen was hydroxyl or *O*-glycoside, to give an $\alpha$-aminocinnamic acid residue. This could then be hydrolyzed to ammonia and the corresponding phenylpyruvic acid or alternatively it could fission to give glycine by hydration and subsequent retro-aldolization (see Scheme I). It has been shown[23] that a glycopeptide which contains an *O*-glycosylthreonine residue gives both glycine and $\alpha$-oxobutyric acid on alkaline hydrolysis, both presumably arising from 2-aminobut-2-enoic acid by a $\beta$-elimination mechanism analogous to that shown in Scheme I. In view of this result, it would appear that, since both avoparcin and CDP-I produce some glycine on hydrolysis, they are likely to contain two $\beta$-glycosyloxyphenylalanine residues. However, the amounts are small compared with vancomycin, which has two free $\beta$-hydroxyphenylalanine residues.

The difficulty of removal of one mannose and one ristosamine from avoparcin further supports the conclusion that they are on the benzylic oxygens. Hence we are left with the ristosaminylglucose disaccharide which must be located as already suggested on the phenolic group of the middle ring of the triphenyl ether which we have shown is glycosylated in the intact antibiotic.

Our data also supports Williams' contention[24] based on NOE studies that in ristocetin one benzylic oxygen is free and one is glycosylated.

## $^{13}$C-Labeling Experiments

In order to assign the $^{13}$C NMR signals of the amino acid $\alpha$-CH's belonging to the $\beta$-hydroxytyrosine units of the triphenyl moiety as well as to provide information as to the biogenetic origin of the phenylglycine carboxyl carbons, D,L-[2-$^{13}$C]tyrosine was fed to shaker flask fermentations of avoparcin.[25] The $^{13}$C NMR spectrum of the isolated antibiotic (mixture of $\alpha$ and $\beta$) showed a methine signal at 59 ppm, the intensity of which was ca. twice that of the corresponding signal in a normal spectrum. This is consistent with the direct incorporation of tyrosine into the two flanking subunits of the triphenyl moiety.

Although the carbonyl region of the spectrum was not as clear as had been hoped, most but not all the carbonyl signal intensities were ca. twice those of unlabeled carbonyl carbons consistent with most of the phenylglycine carboxyl carbons originating from the C-2 of tyrosine. These results are in agreement with labeling experiments on nocardicin[26] and etamycin,[27] antibiotics which also contain phenylglycine subunits.

In a similar manner to the above labeling experiment with $^{13}$C-enriched tyrosine, L-[$^{13}$Me]methionine was fed to flask fermentations. As expected, the $^{13}$C NMR spectrum of the isolated avoparcin showed the *N*-methyl signal at 32.5 ppm to be greatly enhanced (ca. three times) over that of the corresponding signal in a spectrum of unlabeled avoparcin. No other signals were affected.

## Comparison of Avoparcin NMR Spectra and Structural Subunits to Those of Vancomycin

Although there are obvious differences as expected between the $^{13}$C NMR spectra of avoparcin and vancomycin, the general pattern of resonances is similar, especially with respect to the anomeric, aromatic carbon bearing oxygen, and carbonyl carbon areas, indicating that these antibiotics are structurally related. Further evidence for this relationship comes from $^1$H NMR comparison studies at 270 MHz in $Me_2SO$-$d_6$ (courtesy of Walter Krol, Yale University). Even though the spectra are complex, the two meta-substituted protons on the tetrasubstituted ring of the biphenyl unit have unique chemical shifts at $\delta$ 6.30 and 6.44 in the vancomycin[5b] spectrum and $\delta$ 6.31 and 6.43 in that of avoparcin. The spectrum of avoparcin shows, in addition, three sharp *C*-methyl doublets ($J$ = 6 Hz) at $\delta$ 1.12, 1.17, and 1.24 consistent with the presence of 2 mol of ristosamine and 1 mol of rhamnose. A broad 4 H multiplet at $\delta$ 1.18-2.3 is assigned to the C-2 of the two ristosamines.

On consideration of the very closely related structural subunits in avoparcin, vancomycin, ristocetin, and antibiotic A35512B, it seems clear that all of these antibiotics have a common biogenetic origin. All have oligosaccharide appendages on the peptide aglycone usually including glucose, mannose, or rhamnose and a 2,3,6-trideoxy-3-aminohexose. With regard to the aglycone, all of these antibiotics contain the same biphenyl unit and the same basic triphenyl ether moiety which varies as to the number of chlorine substituents. Although vancomycin contains aspartic and *N*-methylleucine units, avoparcin has instead two *p*-hydroxyphenylglycine units. Ristocetin and A35512B, on the other hand, contain diphenyl ethers composed of the two phenylglycine units and no aliphatic amino acids. Indeed, it seems likely that these diphenyl ether units correspond biogenetically to the isolated *p*-hydroxyphenylglycines in avoparcin.

Because of the structural similarity between the isolated subunits from avoparcin and vancomycin, the peptide sequence as indicated by the Edman studies, and the placement of mannose, ristosamine, and the ristosaminylglucose as described above, the avoparcin components have the structures shown below for $\alpha$ and $\beta$.

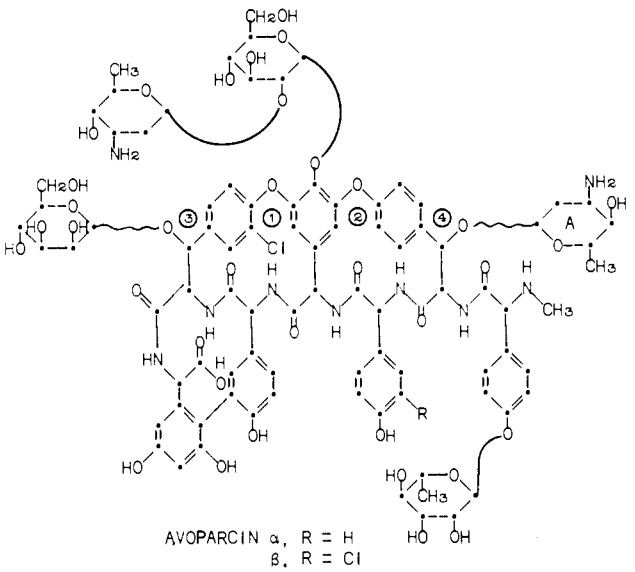


**Table III**

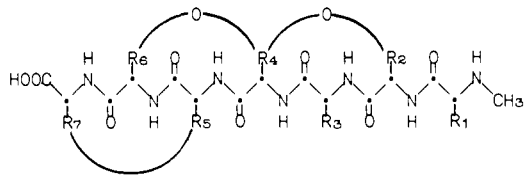
| position | A $^{13}C$ | A $^{1}H$ | A $J$ | B $^{13}C$ | B $^{1}H$ | B $J$ | C $^{13}C$ | C $^{1}H$ | C $J$ |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 161.2 | | | 148.6 | | | 156.4 | | |
| 2 | 124.1 | | | 147.9 | | | 116.5 | 6.97 d | 9 |
| 3 | 132.4 | 8.14 d | 2.5 | 148.4 | | | 131.9 | 8.00 d | 9 |
| 4 | 126.0 | | | 117.0 | 7.53 d | 2.5 | 125.2 | | |
| 5 | 129.6 | 7.86 dd | 2.5, 9 | 126.3 | | | 131.9 | 8.00 d | 9 |
| 6 | 120.2 | 6.83 d | 9 | 119.0 | 7.60 d | 2.5 | 116.5 | 6.97 d | 9 |

| position | $^{13}C$ | $^{1}H$ | position | $^{13}C$ | $^{1}H$ |
|---|---|---|---|---|---|
| 7 | 165.4 | | 11 | 61.4 | χ |
| 8 | 52.4 | χ | 12 | 165.2 | |
| 9 | 166.4 | | 13 | 52.1 | χ |
| 10 | 52.4 | χ | | | |

In the preceding structures, the chlorine drawn at position 1 may just possibly be at 2 but not at both positions. Also, the mannose and ristosamine moieties at positions 3 and 4 may be interchanged.

As mentioned in the Introduction, we have isolated a small quantity of two of the very minor components as a mixture. The retention times and peak heights of these components by LC indicate that they have the same relationship to each other as $\alpha$ and $\beta$. In other words, they differ by a covalent chlorine. By hydrolysis, these components contain glucose, mannose, rhamnose, and ristosamine. A $^{13}C$ NMR of the mixture clearly shows that only one ristosamine is present (sugar A in diagram). Only two *C*-methyl signals are seen in the 18-ppm region and the strengths of the C-2 and C-3 ristosamine signals at 31.6 and 49.7 ppm, respectively, are half those of the corresponding signals in the $\alpha$ and $\beta$ spectra. Equally definitive is the absence of the anomeric carbon signal at 99.3 ppm present in $\alpha$ and $\beta$ as well as the absence of the 80.7-ppm signal ascribed to the glycosylated C-2 of glucose.

The structures shown are formally that of a peptide chain with R groups representing seven aromatic amino acid residues possibly six of which are derived from tyrosine, three of which are cross-linked via hydroxyl groups ($R_2$, $R_4$, and $R_6$ to give the triphenyl ether) and two of which are linked aromatic carbon to aromatic carbon ($R_5$ and $R_7$ to give biphenyl).



Plasma desorption mass spectroscopy[28] (courtesy of Professor R. D. MacFarlane at Texas A&M) on the $\alpha$ component showed a strong peak at 1931 ± 2. This is the quasi-molecular ion ($M + Na^+$) consistent with the molecular formula $C_{89}H_{101}O_{36}N_9Cl$ (1907) for this component as suggested by the chemical degradation studies. A similar determination on the $\beta$ component proved more difficult; nevertheless, as mentioned in our communication,[29] a value for a quasi-molecular ion ($M + Na^+$) was observed at 1965 ± 2 which is consistent with the formula $C_{89}H_{100}O_{36}N_9Cl_2$ (1942). The ($M + Na^+$) value for avoparcin CDP-I was observed at 1529 ± 2, which matches $C_{71}H_{69}O_{25}N_8Cl_2$ (1504).

## Experimental Section

All degradative work was carried out on laboratory-prepared avoparcin. Adsorption chromatography was carried out on SilicAr CC-7 (Mallinckrodt) unless otherwise stated. Silica gel thin and thick layer plates were purchased from Brinkmann. IR curves were run in a Perkin-Elmer 21 or a Nicolet 7199 FT IR spectrophotometer. Electron impact mass spectral data were obtained on a high-resolution direct inlet AE1 MS9 spectrometer. $^{1}H$ NMR curves were made on a Varian A-100 MH2 spectrometer and $^{13}C$ curves were obtained on a Varian XL-100 instrument. Uncorrected melting points were taken on a Fisher-Johns hot stage. Solvents and solutions were usually dried using anhydrous $MgSO_4$.

**Table IV**[a]

| position | A $^{1}H$ | A $J$ | B $^{1}H$ | B $J$ | C $^{1}H$ | C $J$ |
|---|---|---|---|---|---|---|
| 1 | | | | | | |
| 2 | | | | | 6.97 d | 9.0 |
| 3 | 8.17 d | 2.5 | | | 8.03 d | 9.0 |
| 4 | | | 7.60 | 2.0 | | |
| 5 | 7.88 dd | 2.5,8.5 | | | 8.03 d | 9.0 |
| 6 | 6.82 d | 8.5 | 7.08 d | 2.0 | 6.97 d | 9.0 |

[a] Me of Et 1.05 t ($J$ = 7.0 Hz), $CH_2$ of Et 4.10 q ($J$ = 7.0 Hz), $OCH_3$ at 3.85 s, 3.89 s, and 2.91 s.

**Preparation of Aglycone of Avoparcin.** Approximately 20 g of avoparcin was heated on a steam bath for 1 h in 200 mL of 1 N HCl. After cooling, the pH was adjusted to 6.0 with 4 N NaOH. The precipitate was filtered off and dried to 15 g of solid. If spotted on cellulose, a solution of this solid showed major and minor spots (about 4:1) when developed overnight in the system *n*-BuOH-pyridine-acetic acid-water (15:10:1:12). The spots were readily detected using a spray reagent of *p*-nitrobenzenediazonium fluoroborate in aqueous solution.

A salt-free preparation of the aglycone could be obtained by adjusting the pH of the acidic hydrolysate with resin IR-45 ($OH^-$ form) instead of NaOH; however, large amounts of resin were required.

**Methylation of the Aglycone.** About 10 g of aglycone was refluxed overnight in 200 mL of MeOH in the presence of 10 g of $K_2CO_3$ and 10 mL of $CH_3I$. Except for a small amount of $K_2CO_3$, everything dissolved. The reaction mixture was concentrated to 50 mL and filtered. The filtrate was concentrated to a solid, yielding about 10 g of product.

**Isolation of I.** About 2.5 g of the methylated aglycone of avoparcin was slurried in 100 mL of $H_2O$. To this was added a suspension of 14 g of $KMnO_4$ in 200 mL of $H_2O$ to which was added 40 mL of 2 N $NH_4OH$. The reaction mixture was heated on a steam bath until a filter-paper test showed that all the $KMnO_4$ was used up. The mixture was filtered and the filtrate acidified with 4 N HCl, which caused a color change from red to colorless, The solution was extracted with EtOAc twice. The dried extracts were evaporated to 800 mg of orange solid which was taken up in 10 mL of MeOH and treated with ethereal $CH_2N_2$ for 1 h. The solvent was evaporated to a yellow oil which was subjected to partition chromatography over 200 g of diatomaceous earth using the system hexane-EtOAc-MeOH-$H_2O$ (85:15:15:6). Fraction volumes of 20 mL were collected. Fractions 1 and 2 yielded 250 mg of an oil which was charged to two thick layer plates and developed using the system hexane-acetone (4:1). Three UV-absorbing bands were noted. The least polar band was recovered. The batchwise elution with 50 mL of 4:1 EtOAc-MeOH yielded 70 mg of white crystals, mp 93–94 °C. A $^{1}H$ NMR spectrum in $CDCl_3$ revealed this material to be 3-chloro-4-methoxymethyl benzoate.

The methoxy singlets were at $\delta$ 3.94 and 3.88, $H_5$ doublet at 6.94 ($J$ = 9 Hz), $H_6$ quartet at 7.02 ($J$ = 9 and 2.5 Hz), and $H_2$ at 8.05 d ($J$ = 2.5 Hz). Mass spectrum: $M^+$ *m/e* 200 with 3:1 peak ratios in-

**Table V**

| position | A $^{13}C$ | A $^1H$ | A $J$ | B $^{13}C$ | B $^1H$ | B $J$ | C $^{13}C$ | C $^1H$ | C $J$ |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 153.8 | | | 145.4 | | | 148.9 | | |
| 2 | 125.3 | | | 144.8 | 5.94 bs | for OH | 118.3 | 6.95 d | 8.5 |
| 3 | 131.4 | 7.27 d | 2.5 | 145.4 | | | 130.5 | 7.14 d | 8.5 |
| 4 | 136.1 | | | 114.2 | 7.23 d | 2.5 | 133.9 | | |
| 5 | 128.8 | 7.0 m | | 124.3 | | | 130.5 | 7.14 d | 8.5 |
| 6 | 120.6 | 7.0 m | | 116.2 | 7.35 d | 2.5 | 118.3 | 6.95 d | 8.5 |

| position | $^{13}C$ | $^1H$ | position | $^{13}C$ | $^1H$ |
|---|---|---|---|---|---|
| 7, 11 | 20.6, 20.7 | 2.33 s | 8 | 183.6 | |
| | | | 9 | 163.7 | |
| | | | 10 | 52.6 | 3.82 s |

**Table VI**

| A ring | $^{13}C$ | $^1H$ | B ring | $^{13}C$ | $^1H$ |
|---|---|---|---|---|---|
| 1 | 121.5 | | 1 | 126.7 | |
| 2 | 160.1 | | 2 | 159.4 | |
| 3 | 99.8 | 6.58 s | 3 | 112.2 | 7.0 m |
| 4 | 162.1 | | 4 | 133.1 | 7.31 m |
| 5 | 104.8 | 6.58 s | 5 | 128.8 | |
| 6 | 137.4 | | 6 | 129.9 | 7.04 m |

| position | $^{13}C$ | $^1H$ |
|---|---|---|
| 7 | 55.2 and 55.4 | 4.98 m |
| 15 | 57.9 | 5.35 bd |
| 8, 16 | 173.0, 173.2 | |
| 9, 17 | 52.9 | 3.64 s |
| 10, 18 | 160.1, 162.1 | |
| 11, 19 | 22.0, 22.2 | 1.90 and 1.92 d; 1.81 s |
| 12, 13, 14 | 55.9, 56.0, 56.3 | 3.52, 3.54, 3.81 all s |

dicating one chlorine present for a molecular formula of $C_9H_9O_3Cl$.

The middle band of the three bands from the thick layers when eluted batchwise with EtOAc-MeOH yielded 70 mg of an oil, I. High-resolution mass spectroscopy on I indicated a formula of $C_{25}H_{21}O_9Cl$, calcd, 500.0873; found, 500.0882. UV max in MeOH: 253 nm ($\epsilon$ 4150).

IR taken on a smear showed bands at ($cm^{-1}$) 2950, 1730 (s), 1600, 1575, 1503, 1430, 1420, 1332, 1280, 1250, 1230, 1210, 1165, 1112, 1060, 1033, 1000, 908, 863, 848, 763, 733, and 694. NMR curves were taken in $CDCl_3$ with $Me_4Si$ internal standard. Chemical-shift values are in parts per million and coupling constants in hertz. For interpretation of the $^{13}C$ NMR spectra of I and most of the other aromatic hydrolytic fragments, the Varian curves for benzyl acetate (no. 345) and *N*-acetylphenylalanine methyl ester (no. 435) were useful.[30] By making adjustments based on Stother's shielding values for substituted benzenes,[31] we were able to calculate chemical-shift values (Table III) and match them with observed values.

$\chi$ is one of the values 3.80 s (3 H), 3.84 s (3 H), or 3.90 s (6 H). It is noteworthy that the chemical-shift values of carbons $B_4$ and $B_6$ are not identical owing to lowering of the $B_4$ value by proximity to chlorine at $A_2$.

**Isolation of Ia.** Avoparcin (2 g) in 100 mL of EtOH-$H_2O$ (1:1) was methylated with ethereal methanolic $CH_2N_2$. The clear solution was evaporated to a powder (2.1 g) which was refluxed with 100 mL of 2 N HCl for 45 min. On cooling, the insoluble aglycone was filtered off and dried to 0.9 g, which was ethylated in 60 mL of EtOH with a solution of $C_2H_4N_2$ (from 40 g of nitrosoethylurea) overnight at room temperature. The clear yellow solution was concentrated to a solid (0.85 g). About 0.8 g of this solid suspended in 40 mL of $H_2O$ was treated with a solution of 4.8 g of $KMnO_4$ in 90 mL of $H_2O$ and 14 mL of 2 N $NH_4OH$ at 75 °C with stirring until the $KMnO_4$ color was no longer diluted. The mixture was filtered, acidified with 2 N HCl, and extracted with EtOAc and the extract was dried over $Na_2SO_4$ and concentrated to a gum. The gum was treated overnight with ethereal $CH_2N_2$. Preparative thick layer chromatography on the product yielded three fractions (A, B, and C) (Table IV).

Fraction A (9 mg) had mp 89 °C and was 3-chloro-4-methoxybenzoate. Fraction B on GLC examination consisted of two components which were identified as methyl *p*-methoxybenzoate and methyl *p*-ethoxybenzoate in a ratio of 1:3. Fraction C (8 mg) had *m/e* 516, 514, 500, 486 and is assigned structure Ia.

**Recovery of II.** Approximately 80 g of avoparcin was refluxed under positive $N_2$ pressure for 20 h in 1 L of 4 N NaOH. The hydrolysate was acidified with concentrated HCl and the resultant mixture extracted three times with 500 mL of EtOAc. The combined dried EtOAc extracts were concentrated to 6 g of dark gum which was taken up in 25 mL of MeOH and treated with ethereal $CH_2N_2$ for 1 h. The solvent was evaporated to a dark gum which was triturated with ether. The ether filtrate yielded 4 g of black gum. Half of this material was chromatographed over 60 g of silica gel slurried in $CH_2Cl_2$ (bed depth 26 in.). Elution was carried out using a stepwise gradient of 0, 0.5, 1.0, and 2.0% MeOH in $CH_2Cl_2$. The first significant material off the column amounted to 160 mg of yellow oil which we labeled A. This was followed closely by 170 mg of slightly darker oil which on standing overnight yielded white crystals, mp 103–107 °C, which single-crystal X-ray crystallography showed to be 3-chloro-4-hydroxyphenyl glyoxylic methyl ester. The material labeled A was put on one thick layer plate and developed for five passes using hexane-acetone (4:1) to show two just barely resolved bands. The more polar band was a pure yellow color and fluoresced. Batchwise recovery yielded 56 mg (B). The less polar band was faintly yellow and showed normal UV absorption and upon batchwise recovery yielded 57 mg (C). This material C had essentially the same structure as B except that the grouping -CO-$COOCH_3$ was replaced by -CHO at $B_4$. Preparation B yielded better spectral data and is designated II. The mass spectrum showed a molecular ion at $M^+$ *m/e* 426 for a formula of $C_{23}H_{19}O_6Cl$. The IR taken on a smear showed peaks at 3080, 2980, 1725, 1675, 1570, 1495, and 1440 $cm^{-1}$ among others. The NMR in $CDCl_3/Me_4Si$ is given in Table V.

**Preparation and Isolation of III.** About 5 g of methylated aglycone of avoparcin was refluxed overnight in 100 mL of 50% w/w NaOH solution with 10 g of powdered $NaBH_4$ added. With great care and over an extended period of time, the reaction mixture was adjusted to pH 2.5 with concentrated HCl and the resulting mixture carefully evaporated to a solid. The solid was triturated several times with EtOAc. The resultant solid was allowed to sit in MeOH saturated with dry HCl overnight. Again the solvent was evaporated off until the odor of HCl was nearly absent and this solid was then stirred for 4 h in 30 mL of pyridine-$Ac_2O$ (1:1). Workup of this reaction mixture yielded 1.3 g of solid which was chromatographed over 60 g of silica gel (bed depth 40 in.), slurried in $CH_2Cl_2$. Development was made with a stepwise gradient of 0–5% MeOH in $CH_2Cl_2$. At 3% MeOH in $CH_2Cl_2$, about 120 mg of gummy, yellow material was eluted off. Trituration of this with EtOAc and subsequent filtration and scratching yielded 40 mg of white crystals of III: mp 199–201 °C;

**Table VII**

| position | A $^{13}C$ | A $^{1}H$ | A $J$ | B $^{13}C$ | B $^{1}H$ | B $J$ | C $^{13}C$ | C $^{1}H$ | C $J$ |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 156.7 | | | 150.1 | | | 149.2 | | |
| 2 | 125.2 | | | 135.2 | | | 118.6 | 6.99 d | 9 |
| 3 | 131.1 | 7.2 d | 2.5 | 150.6 | | | 130.3 | 7.32 d | 9 |
| 4 | 135.6 | | | 113.3 | 6.72 d | 2.5 | 133.0 | | |
| 5 | 128.7 | 7.01 dd | 2.5,9 | 132.8 | | | 130.3 | 7.32 d | 9 |
| 6 | 120.6 | 6.92 d | 9 | 112.1 | 6.59 d | 2.5 | 118.6 | 6.99 d | 9 |

| position | $^{13}C$ | $^{1}H$ | position | $^{13}C$ | $^{1}H$ |
|---|---|---|---|---|---|
| 7 | 45.7 | 4.58 s | 12 | 22.9 | 1.97 s |
| 8 | 55.7 | 5.41 d 8 | 13 | 168.0 | |
| 9 | 170.7 | | 14 | 20.1 | 2.12 s |
| 10 | 53.0 | 3.68 s | 15 | 20.6 | 2.34 s |
| 11 | 169.3 | | NH | | 6.35 d 8 |

**Table VIII**

| position | A $^{13}C$ | A $^{1}H$ | A $J$ | B $^{13}C$ | B $^{1}H$ | B $J$ | C $^{13}C$ | C $^{1}H$ | C $J$ |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 157.4 | | | 150.1 | | | 149.8 | | |
| 2 | 122.1 | | | 143.3 | | | 117.4 | 6.96 d | 9 |
| 3 | 130.7 | 7.48 d | 2.5 | 152.6 | | | 130.1 | 7.32 d | 9 |
| 4 | 132.6 | | | 116.0 | 6.84 d | 2.5 | 132.4 | | |
| 5 | 128.0 | 7.20 dd | 2.5,9 | 130.8 | | | 130.1 | 7.32 d | 9 |
| 6 | 118.6 | 6.9? | unclear | 114.9 | 6.76 | | 117.4 | 6.96 d | 9 |

| position | $^{13}C$ | $^{1}H$ | position | $^{13}C$ | $^{1}H$ |
|---|---|---|---|---|---|
| 7, 16 | 65.8, 65.0 | 5.06 ss | 10 | 55.6 | 5.44 d 8[a] |
| | | | 11, 13 | 169.4 | |
| 8, 17 | 170.9 | | 12 | 52.9 | 3.72 s |
| 9, 18 | 21.0 | 2.10, 2.12 ss | 14 | 23.0 | 2.00 s |
| | | | 15 | 61.3 | 3.80 s |

[a] By adding a few drops of $CD_3OD$, this doublet becomes a singlet.

$[\alpha]^{25}_D$ 0°; mass spectrum $M^+$ *m/e* 502, indicating a formula $C_{25}H_{30}N_2O_9$; IR (KBr) peaks at 3295, 2990, 1755, 1655, and 1205 $cm^{-1}$. The NMR in $CD_3OD/Me_4Si$ is given in Table VI. One of the amide methyl signals is split in the proton spectrum ($\delta$ 1.90 and 1.92). We attribute this to hindered rotation in the biphenyl. The amide protons appear as broad doublets at $\delta$ 8.33 and 8.61. The signal at $\delta$ 8.61 is cleaner and hence is associated with the $CH_3$ at $\delta$ 1.81.

**Compound IV.** About 10 g of the aglycone of avoparcin was slurried in 20 mL of 50% w/w NaOH solution, a suspension of 300 mg of $NaBH_4$ in 20 mL of Cellosolve added, and the mixture refluxed for 3 h. The reaction mixture was diluted to 180 mL with $H_2O$ and extracted several times with ether to remove the Cellosolve. The aqueous phase was then acidified to pH 1.5 with concentrated HCl solution and again extracted with ether. The aqueous phase was then extracted three times with EtOAc. The dried EtOAc extracts yielded 450 mg of solid which was treated with dry HCl gas in MeOH. After 1 h, the solvent was evaporated to dryness several times using MeOH reconstitution to eliminate HCl. The resultant gum was acetylated in a mixture of 20 mL of $Ac_2O$ and 5 mL of pyridine. Upon elimination of these solvents, the product was chromatographed over 55 g of silica gel. Elution was carried out using a stepwise gradient of 0–3% MeOH in $CH_2Cl_2$. Fraction volumes collected were about 19–20 mL. Fractions 34–45 were concentrated to 100 mg of yellow solid which after Darco treatment gave 67 mg of white solid labeled IV, mass spectrum $M^+$ *m/e* 545 for $C_{27}H_{25}NO_7Cl_2$. The NMR in $CDCl_3/Me_4Si$ is given in Table VII.

**Resolution of the Fragments V, VI, and VII.** About 7.5 g of the aglycone of avoparcin was slurried in 15 mL of 50% w/w NaOH solution together with 1.5 g of $NaBH_4$ which had been slurried in 15 mL of methyl Cellosolve and the mixture refluxed for 1.5 h to get a reddish biphasic solution. This was diluted with $H_2O$ to 100 mL and extracted three times with ether to eliminate the Cellosolve. The aqueous phase was then acidified to pH 1.9 with concentrated HCl and the mixture was then extracted four times with EtOAc. The EtOAc extracts were dried and concentrated to 2 g of yellow gum. $CH_2N_2$ was generated from 8 g of Diazald in tetrahydrofuran (THF) and allowed to condense into the 2 g of material dissolved in a minimum of MeOH. After 2 h, the solvent was evaporated off to 1.9 g, which was stirred overnight in 40 mL of 1:1 $Ac_2O$–pyridine. Evaporation of the resultant solution yielded 2.3 g of gum which was chromatographed over 200 mL of silica gel slurried in $CH_2Cl_2$. Fraction volumes were 85–90 mL. Fractions 44–49 were combined to give 100 mg of yellow gum labeled A. Fraction 50–56 yielded 230 mg of similar gum labeled B while 57–70 gave a further 280 mg of gum labeled C. Preparation B was put on two thick layers and developed using 1:1 hexane–EtOAc without further resolution. The elongated main band was recovered batchwise to yield 150 mg of yellow gum which was chromatographed on one thick layer using 2:1 hexane–acetone to get partial resolution. The most polar portion of the main band was recovered batchwise to give 64 mg of yellow gum labeled V. The mass spectrum showed a peak at $M^+$ *m/e* 599 which matched the formula $C_{30}H_{30}NO_{10}Cl$. The spectrum also contained a weak signal at *m/e* 685. This was peak matched to find 685.1864 vs. a value of 685.1926 for the formula $C_{34}H_{36}NO_{12}Cl$. The NMR is given in Table VIII. Preparation V contained between 10 and 15% of an impurity to which the minor unassigned $^{13}C$ NMR signals at 20.6, 21.9, 32.9, 36.4, 52.3, 59.3, 72.5, 124.4, 128.8, and 131.6 ppm belong. Many of these signals would fit VI. However, the mass spectrum showed no sign of a signal at *m/e* 671 which judging from the mass spectrum of VI should be clearly visible.

Preparation C was charged to two thick layers and developed using 99:1 $CHCl_3$–MeOH for two passes. This failed to resolve the suspected two components present so the bands were combined and recovered batchwise using 20:30 MeOH–EtOAc to obtain 180 mg of gummy solid. This in turn was chromatographed on one thick layer plate using the system EtOAc–hexane (50:50). Once again resolution eluded us. Recovery yielded 85 mg which was again developed on one thick layer for three passes using hexane–acetone (2:1) to get, just barely, resolution of two bands. The more polar major band yielded on recovery 24 mg of gum labeled VI. The molecular ion was observed at *m/e* 671

**Table IX**

| position | A $^{13}C$ | A $^{1}H$ | B $^{13}C$ | B $^{1}H$ | C $^{13}C$ | C $^{1}H$ | C $J$ |
|---|---|---|---|---|---|---|---|
| 1 | 157.3 | | 150.1 | | 150.1 | | |
| 2 | 124.5 | | 145.0 | | 117.4 | 6.94 d | 9 |
| 3 | 131.6 | 7.30 m | 151.7 | | 130.1 | 7.31 d | 9 |
| 4 | 132.6 | | 115.7 | 6.72 m | 132.6 | | |
| 5 | 128.8 | 7.1 m | 130.8 | | 130.1 | 7.31 d | 9 |
| 6 | 119.1 | unclear | 114.8 | 6.64 m | 117.4 | 6.94 d | 9 |

| position | $^{13}C$ | $^{1}H$ | position | $^{13}C$ | $^{1}H$[a] |
|---|---|---|---|---|---|
| 7 | 65.8 | 5.06 s | 12 | 52.9 | χ s |
| 8, 11, 18, 20 | 170.9 | | 13 | 169.4 | |
| | | | 14 | 23.0 | 2.00 s |
| | | | 15 | 61.2 | χ s |
| | | | 16 | 36.4 | 3.12 m |
| 9, 21 | 21.0, 20.6 | 2.10, 2.12 ss | 17 | 72.6 | 5.22 m |
| | | | 19 | 52.4 | χ s |
| 10 | 55.6 | 5.44 d 8 | | | |

[a] χ is any one of the values 3.71, 3.75, or 3.80.

**Table X**

| position | A $^{13}C$ | A $^{1}H$ | A $J$ | B $^{13}C$ | B $^{1}H$ | B $J$ | C $^{13}C$ | C $^{1}H$ | C $J$ |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 157.5 | | | 150.1 | | | 149.9 | | |
| 2 | 124.6 | | | 142.8 | | | 117.4 | 6.92 d | 9 |
| 3 | 131.2 | 7.30 m | | 150.8 | | | 130.2 | 7.32 d | 9 |
| 4 | 135.9 | | | 115.2 | 6.78 d | 2.5 | 132.4 | | |
| 5 | 128.7 | 7.10 dd | 2.5,9 | 134.9 | | | 130.2 | 7.32 d | 9 |
| 6 | 119.5 | unclear | | 113.7 | 6.64 d | 2.5 | 117.4 | 6.92 d | 9 |

| position | $^{13}C$ | $^{1}H$ | position | $^{13}C$ | $^{1}H$ |
|---|---|---|---|---|---|
| 7 | 65.9 | 5.06 s | 14 | 23.0 | 1.98 s |
| 8, 11 | 170.0 (2) | | 15 | 61.3 | 3.82 s |
| | | | 16 | 20.6 | 2.34 s |
| 13 | 169.5 | | | | |
| 12 | 53.0 | 3.70 s | | | |
| 9 | 21.0 | 2.10 s | | | |
| 10 | 55.7 | 5.41 d 8 | | | |

**Table XI**

| position | $^{13}C$ | $^{1}H$ |
|---|---|---|
| 1 | 98.4 | 5.40 s hint of splitting $J$ = 0.5 |
| 2, 3, 4, 5 | 71.7, 70.7, 70.1, 69.6 | lumpy signal between 3 and 4 |
| 6 | 17.8 | 1.12 d $J$ = 6 |
| 1′ | 156.8 | |
| 2′, 6′, 3′, 5′ | 117.0, 129.1 | all aromatic H's essentially s at 7.13 |
| 4′ | 135.7 | |
| 7 | 66.6 | 5.32 s |
| 8 | 172.3 | |
| 9 | 182.3 | |
| 10, 11 | 28.1, 32.1 | 3.07, 3.17 ss |

(7% rel abundance). Major fragment ions were observed at *m/e* 639 (M − $CH_3OH$, 3%), 628 (M − $CH_3$ − CO, 11%), 611 (M − $CH_3COOH$, 44%), 569 (M − $CH_3COOH$ − $CH_2CO$, 100%), 510 (M − $CH_3COOH$ − $CH_3CO$ − $CH_3COO$, 26%). Peak matching *m/e* 611 gave 611.1551 found, calcd for $C_{31}H_{30}NO_{10}Cl$ 611.1558, which means that VI has the formula $C_{33}H_{34}NO_{12}Cl$. The NMR ($CDCl_3$/$Me_4Si$) is given in Table IX. Minor unassigned $^{13}C$ NMR peaks due to impurities were observed at 65.1, 114.0, 114.6, 115.9, 118.4, 118.7, and 128.0 ppm.

Preparation A was developed on one thick layer plate using the system hexane–acetone (2:1). Once again the main UV band was barely resolved for a less polar fluorescent-type band. A portion of the main band was processed batchwise to obtain 40 mg of labeled VII. Mass spectrum showed a molecular ion at $M^+$ *m/e* 541 which matched the formula $C_{28}H_{28}NO_8Cl$. IR on a smear showed peaks at 1755, 1665, 1245, 1220, 1064, and 1036 $cm^{-1}$. The NMR is given in Table X. Minor $^{13}C$ NMR peaks at 21.2, 22.0, 32.9, 52.3, 59.4, 122.1, and 130.8 ppm are unaccounted for.

**Edman Degradation of Avoparcin. Compounds VIII and IX.** The free base of avoparcin was prepared by adjusting the pH of an aqueous solution of the sulfate to 9.0 with hot $Ba(OH)_2$ solution followed by filtration and lyophilization. About 9 g of the free base was stirred in 80 mL of $H_2O$ and then 80 mL of pyridine was added. After 12 mL of methyl isothiocyanate (MITC) was added, the pH was kept at about 9.0 by adding 1 N NaOH solution. After 2 h, the reaction solution was evaporated to dryness and the resultant solid was triturated several times with ether. The solid was then triturated with a few milliliters of MeOH and the slurry diluted to 150 mL with EtOAc. After stirring, the mixture was filtered and the filtrate concentrated to 1.3 g of solid which was chromatographed over 150 g of silica gel slurried in $CH_2Cl_2$ and developed using a stepwise gradient of 0, 0.5, 1.0, 2.0, and 3.0% MeOH in $CH_2Cl_2$. Fraction volume was about 90 mL and fractions 50–77 gave on concentration 580 mg of solid which by TLC on silica gel using 9:1 $CHCl_3$–MeOH was a single spot at $R_f$ 0.3. In our hands it did not crystallize. After Darco treatment in EtOAc and evaporation, it was a nice white solid labeled VIII, $[\alpha]^{25}D$ −101 ± 1° (*c* 0.94, MeOH). The mass spectrum $M^+$ *m/e* 382 answers the formula $C_{17}H_{22}N_2O_6S$. IR (KBr): peaks at 3440, 1749, 1515, 1330, 1235, 1138, 1062, 1018, 980, and 838 $cm^{-1}$. The NMR ($Me_2SO$-$d_6$/$Me_4Si$) is given in Table XI.

The α configuration at $C_1$ is assigned on the basis of the chemical shift of $C_5$ (normally 70–73 ppm) to α and 75–77 ppm for methyl α-L-rhamnopyranoside. Application of Hudson's rule strongly bolsters this assignment.[32]

Fractions 82–97 yielded 290 mg which was also after treatment a pure, single-spot product which did not crystallize, $[\alpha]^{25}D$ −93 ± 1°

**Table XII**

| position | $^{13}C$ | $^{1}H$ |
|---|---|---|
| 1 | 98.4 | 5.36 hint of splitting $J \sim 0.5$ |
| 2, 3 | 71.7, 70.4 | lumpy signal between 3 and 4 |
| 4, 5 | 70.1, 69.6 | |
| 6 | 17.8 | 1.10 d $J = 6$ |
| 1′ | 156.8 | |
| 2′, 6′ | 116.5 | 7.06 d $J = 9$ |
| 3′, 5′ | 127.1 | 7.20 d $J = 9$ |
| 4′ | 128.1 | |
| 7 | 87.5 | 7.66 exch s (OH) |
| 8 | 172.4 | |
| 9 | 181.8 | |
| 10 | 27.7, 28.7 | 2.96, 3.18 ss |

**Table XIII**

| position | $^{13}C$ | $^{1}H$ |
|---|---|---|
| 1 | 158.2 | |
| 2,6 | 115.4 | 6.80 d $J = 9$ |
| 3,5 | 126.9 | 7.12 d $J = 9$ |
| 4 | 125.0 | |
| 7 | 87.6 | 9.0 exch OH |
| 8 | 172.5 | |
| 9 | 181.7 | |
| 10, 11 | 27.7, 28.7 | 3.22, 3.26 ss |

($c$ 0.71, MeOH). Mass spectrum $M^+$ $m/e$ 398 for a formula $C_{17}H_{22}N_2O_7S$. The material was labeled IX. The IR in KBr of IX was virtually identical with that of VIII except for the strongly enhanced intensity of the peak at 1062 $cm^{-1}$. In addition, a minor peak present at 1100 $cm^{-1}$ in VIII was absent in IX. The NMR ($Me_2SO$-$d_6$/$Me_4Si$) is given in Table XII. The three sugar OH's appear as sharp exchangeable doublets at $\delta$ 4.58, 4.74, and 4.94 ($J \simeq 4.5$ Hz) in the proton spectrum.

**Compounds X, XI, XII, and XIII.** About 15 g of the aglycone of avoparcin was stirred in 200 mL of 1:1 pyridine-$H_2O$ with 15 mL of MITC added and the pH was kept at 9.0 by addition of 1 N NaOH solution. After 1.5 h, the reaction mixture was stripped of MITC by ether extraction and concentrated to 13 g of solid which was stirred for 2 h in 200 mL of HOAc which had been saturated with dry HCl gas. The suspension was filtered to obtain 11 g after drying of solid labeled A and the filtrate was evaporated to 1.9 g of gum labeled B.

Preparation B was chromatographed over 60 g of silica gel slurried in $CH_2Cl_2$ using 500-mL aliquots of 0, 1, 1.5, and 2% MeOH in $CH_2Cl_2$. Fraction volumes were in the range 30-50 mL. Fractions 19-24 yielded 290 mg of solid which after Darco treatment was crystallized from EtOAc-hexane to give 120 mg of labeled X: mp 218-219 °C; $[\alpha]^{25}_D$ $-1 \pm 1°$ ($c$ 1.05, MeOH); mass spectrum $M^+$ $m/e$ 236 for a formula $C_{11}H_{12}O_2N_2S$; IR (KBr) 3319, 1720, 1520, 1323, and 840 $cm^{-1}$; $^1H$ NMR ($Me_2SO$-$d_6$/$Me_4Si$) 9.55 exch s, phenolic OH, 6.99 (d, $J = 9$ Hz, $H_3$ and $H_5$), 6.77 (d, $J = 9$ Hz, $H_2$ and $H_6$), 5.20 s $H_7$, 3.16 and 3.04 ppm both s for 2 $NCH_3$.

Fractions 29-32 yielded on concentration 120 mg which upon processing afforded 80 mg of white solid labeled XI. Mass spectrum: $M^+$ $m/e$ 252, which matches a formula $C_{11}H_{12}O_3N_2S$. IR (KBr) had peaks at 3420, 1735, 1515, 1325, 1060, and 838 $cm^{-1}$. $^{13}C$ NMR ($Me_2SO$-$d_6$/$Me_4Si$) and $^1H$ NMR in $CDCl_3$ are given in Table XIII.

Peptide A was subjected to another Edman first stage by stirring in 200 mL of pyridine-$H_2O$ (1:1) with 12 mL of MITC and keeping the pH at 9.0. This was worked up after 2 h and subjected to the Edman second stage by stirring for 2 h in 200 mL of HOAc saturated with dry HCl gas. The insoluble peptide from this reaction weighed 8.4 g (C) and the HOAc filtrate yielded 2 g of labeled D. TLC on D did not look promising, so D and C were combined and subjected to another Edman cycle in pyridine-$H_2O$ with MITC. From this stage, 9.5 g of solid was obtained which was stirred in 200 mL of HOAc saturated with dry HCl for 2 h. Filtration yielded 8 g of solid E and evaporation of filtrate yielded 1.5 g of yellow solid which was refluxed in 60 mL of 1 N HCl solution for 3 h. On cooling, precipitation occurred and the suspension was extracted with EtOAc. The dried EtOAc extract on evaporation yielded 380 mg of a yellow solid F which was chromatographed over 120 mL of silica gel slurried in $CH_2Cl_2$ and developed using aliquots of 0, 0.5, 0.6, 0.7, and 1% MeOH in $CH_2Cl_2$. Fraction volumes were 40-60 mL and fractions 79-86 gave on evaporation 90 mg of solid from which by suitable workup 20 mg of white crystals were obtained labeled XII, mp 268-270 °C, material melted to a red liquid. $R_f$ in TLC using 9:1 $CHCl_3$-MeOH was 0.55. If the developed thin layer was allowed to sit for a few hours, color developed exactly as an amino acid spot after ninhydrin treatment. In addition, a solution of this compound in MeOH took on a blue coloration. Mass spectrum: $M^+$ $m/e$ 256 for a formula $C_{10}H_9N_2O_2ClS$. IR (KBr) had peaks at 3395, 3188, 1740, 1525, 1495, 1290, 1183, 1113, 728, and 695 $cm^{-1}$. $^{13}C$ NMR in $Me_2SO$-$d_6$/$Me_4Si$ and $^1H$ NMR in $CDCl_3$ with drop of $Me_2SO$-$d_6$ added are given in Table XIV.

**Table XIV**

| position | $^{13}C$ | $^{1}H$ |
|---|---|---|
| 1 | 153.4 | 9.46 exch s (OH) |
| 2 | 119.8 | |
| 3 | 128.4 | 7.22 d $J = 2$ |
| 4 | 125.8 | |
| 5 | 126.7 | 7.02 dd $J = 2.5, 9$ |
| 6 | 116.9 | 6.98 d $J = 9$ |
| 7 | 61.1 | 4.99 s |
| 8 | 173.1 | |
| 9 | 183.1 | |
| 10 | 27.0 | 3.20 s |

Fractions 101-114 on evaporation yielded 32 mg of yellow solid. Attempts to obtain this material crystalline failed. After some effort, 10 mg of faintly reddish solid was obtained labeled XIII. Mass spectrum: $M^+$ $m/e$ 222 for a formula $C_{10}H_{10}N_2O_2S$. IR (KBr) had peaks at 3280, 1722, 1320, 1230, 1118, and 835 $cm^{-1}$. $^1H$ NMR in $Me_2SO$-$d_6$/$Me_4Si$ $\delta$ 9.46 s, exch phenolic OH, 7.04 (d, $J = 9$ Hz, $H_2$ and $H_6$), 6.78 (d, $J = 9$ Hz, $H_3$ and $H_5$), 5.22 (s, $H_7$), 3.12 (s, $CH_3$).

**Successive Edman Degradation on the Aglycone of Avoparcin. First Edman Cycle.** About 8 g of aglycone of avoparcin was reacted with 12 mL of MITC at pH 9.0 in pyridine-$H_2O$. Workup yielded 9 g of labeled A. TLC showed that A contained X. In accordance with Hlavka's suggestion,[6] A was refluxed overnight in MeOH and then concentrated to a solid B. TLC showed a decided enhancement of X in B over A. Preparation B was then refluxed for 2 h in 180 mL of 1 N HCl. The cooled mixture was extracted three times with EtOAc. The EtOAc dried extracts were evaporated to 1.5 g of labeled C. The aqueous phase was evaporated to 8.0 g of brown solid D.

**Second Edman Cycle.** Preparation D was treated with 7 mL of MITC in 160 mL of pyridine-$H_2O$ (1:1) at pH 9.0 for 1.25 h. The reaction mixture was processed to a solid which was refluxed in 180 mL of 1 N HCl for 2 h. The reaction mixture was then cooled and extracted with EtOAc. The dried EtOAc extracts yielded 240 mg of gummy solid E. TLC clearly showed the presence of XII in E. The aqueous phase upon evaporation gave 8.0 g of brown solid F.

**Third Edman Cycle.** Preparation F was treated with 10 mL of MITC in 120 mL of pyridine-$H_2O$ (1:1) at pH 9.0 for 1.5 h. The product from this stage was refluxed for 2 h in 180 mL of 1 N HCl and extracted with EtOAc. The EtOAc extract amounted to 570 mg of yellow solid G and the aqueous phase came to 8 g of dark brown solid H.

**Fourth Edman Cycle.** The fourth Edman cycle was carried out on H to give after two stages 560 mg of labeled J by EtOAc extraction and 10 g of solid from the aqueous phase K.

Preparations E and G were combined because of similarities by TLC, passed over 150 mL of silica gel slurried in $CH_2Cl_2$, and developed using a stepwise gradient of 1-L aliquots of 0.5, 1, 2, and 3% MeOH in $CH_2Cl_2$. Fraction volumes were 15-18 mL. Fractions 53-65 gave 150 mg of yellow solid labeled L, fractions 91-102 gave 35 mg of M, fractions 103-113 gave 60 mg of N, fractions 165-170 gave 25 mg of P, and fractions 175-185 gave 70 mg of Q.

Preparation L was treated with Darco in EtOAc, filtered, and concentrated to yield 90 mg of off-white solid XII. Preparations M and N were combined and worked up to get 35 mg of off-white solid XIII. Preparation P, handled similarly, gave a greenish gum which yielded no useful information.

**Table XV**

| $C_{13}H_{16}NO_4Cl$ (XIV) | | | $C_{13}H_{17}NO_4$ (XV) | | |
|---|---|---|---|---|---|
| mass | % RA | fragment | mass | % RA | fragment |
| 285 | 11 | $M^+$ | 251 | 10 | $M^+$ |
| 253 | 46 | $M - CH_3OH$ | 219 | 43 | $M - CH_3OH$ |
| 242 | 36 | $M - CH_3CO$ | 208 | 27 | $M - CH_3CO$ |
| 226 | 27 | $M - CH_3COO$ | 192 | 35 | $M - CH_3COO$ |
| 180 | 100 | $M - CH_3COO - CH_2CO$ | 150 | 100 | $M - CH_3COO - CH_3CO$ |

Preparation Q upon similar workup yielded 40 mg of off-white solid R, which melted above 250 °C to a reddish-brown liquid with gas evolution. Preparation R moved with an $R_f$ of 0.15 using 9:1 $CHCl_3$-MeOH on TLC. After overnight standing, the spot turned gray. A proton spectrum in $CDCl_3$ with a few drops of $Me_2SO$-$d_6$ added indicated that this material was the dithiohydantoin of the biphenyl amino acid. The two $NCH_3$ groups are singlets at $\delta$ 3.00 and 3.20 and the benzylic protons are doublets at $\delta$ 4.78 and 5.00 ($J$ = 4 Hz). The two aromatic protons of the tetrasubstituted aromatic ring are singlets at $\delta$ 6.20 and 6.38. The three aromatic protons of the other ring are essentially two singlets at $\delta$ 6.90 and 7.10. There are five exchangeable protons lumped between $\delta$ 8.5 and 9.5. These represent the two NH's and the three phenolic protons. IR (KBr) shows peaks at 3300, 1725, 1500, 1310, 1118, and 845 $cm^{-1}$. No molecular ion matching $m/e$ 458 was detected in the mass spectrum.

**Determination of Free Phenolic Groups in Avoparcin.** About 10 g of avoparcin was methylated by refluxing overnight in 150 mL of MeOH with 10 g of $K_2CO_3$ and 30 mL of $CH_3I$ added. The solvent was evaporated to 50 mL and the resultant slurry diluted to 100 mL with cold MeOH and filtered. The residue was discarded and the filtrate concentrated to a solid which was refluxed overnight in 250 mL of MeOH saturated with dry HCl gas. The mixture was filtered and the filtrate concentrated to 39 g of brown solid which was subjected to overnight reflux in the presence of 60 mL of MeOH, 4 g of $K_2CO_3$, and 10 mL of $CD_3I$. The suspension was filtered and the filtrate concentrated to a solid which was refluxed in 30 mL of 50% w/w NaOH in the presence of 4 g of powdered $NaBH_4$ for 4 h. The reaction mixture was neutralized carefully with concentrated HCl and evaporated to a solid. This solid was triturated with MeOH and the salt filtered off. The filtrate was saturated with dry HCl. More salt precipitated. This was filtered off and saturation with HCl gas was repeated. After a short time, the solvent was evaporated to dryness, worked to eliminate most of HCl, and then stirred overnight in 50 mL of $Ac_2O$-pyridine (1:1). This solvent system was evaporated thoroughly and the gummy residue triturated with 2-3 mL of MeOH and then diluted with 100 mL of EtOAc. The filtrate from this processing was evaporated to 3 g of gum which was chromatographed over 100 mL of silica gel slurried in $CH_2Cl_2$. Development was made using 1-L aliquots of 0, 1, 2, and 3% MeOH in $CH_2Cl_2$. Fraction volumes were around 80-85 mL. Fractions 46-54 yielded 70 mg of a brown gum which was charged to a thick layer and developed using 9:1 $CHCl_3$-MeOH. About six bands were visible on the developed plate. The fifth band in order of decreasing polarity was recovered in the usual fashion to obtain after Darco treatment 18 mg of faintly yellow solid. TLC and IR, MS, and NMR spectral data showed this material to be III which proves that the three phenolic groups of the biphenyl entity are free in avoparcin.

**Identification of XIV and XV.** About 20 g of avoparcin was ethylated by overnight reflux in 200 mL of MeOH with 10 g of $K_2CO_3$ and 20 mL of $C_2H_5I$ added. Workup in the usual manner gave 28 g of residue which was refluxed overnight in MeOH saturated with dry HCl gas. After evaporation of solvent, the solid was triturated with $H_2O$. After filtration, the residue was in turn triturated with MeOH. The MeOH extract was concentrated to 8 g of dry solid which was subjected to drastic basic hydrolysis by refluxing for 1.5 h in 15 mL of 50% w/w NaOH solution with 1.5 g of $NaBH_4$ slurried in 15 mL of Cellosolve added. The resultant dark red solution mixture was diluted to 100 mL with $H_2O$ and extracted several times with ether. The aqueous phase was then acidified with concentrated HCl to pH 2.0 and extracted several times with EtOAc. The aqueous phase was then evaporated to 27 g of turnip-red solid which was suspended in MeOH, stirred, and filtered. The filtrate was concentrated to 6 g of solid which was redissolved in a minimum amount of MeOH and subjected to excess $CH_2N_2$ generated from Diazald in THF. This reaction mixture was left standing over the weekend. The suspension was then filtered and the filtrate evaporated to a gum which was acetylated in 1:1 pyridine-$Ac_2O$ for 3 h. Workup yielded 5 g of brown, gummy residue which was chromatographed over 300 mL of silica gel slurried in $CH_2Cl_2$. The column was developed stepwise using 1-L aliquots of 0, 0.5, 1, and 1.5% MeOH in $CH_2Cl_2$. Fractions 83-88 gave 400 mg of yellow gum. About 1/3 of this preparation was charged to a thick layer and developed using 60:40 hexane-EtOAc. The main UV-absorbing band was eluted batchwise with 50 mL of acetone to yield an oil which upon scratching and standing overnight gave 38 mg of white crystals, mp 61-62 °C, which were suitable for X-ray work. This material was labeled XIV. X-ray indicated a formula $C_{13}H_{16}NO_4Cl$. The material from which the crystals were removed was gummy. A $^{13}C$ spectrum on this gum clearly indicated that it was an 80:20 mixture of XIV and XV. The mass spectrum curve was interpreted as shown in Table XV. The gum had sharp IR bands (KBr) at 3260, 1755, 1650, 1540, 1258, 1210, and 1175 $cm^{-1}$. The NMR in $CDCl_3/Me_4Si$ is given in Table XVI.

**Table XVI**

| position | $^{13}C$ XIV | $^{13}C$ XV | $^1H$ XIV | $J$ |
|---|---|---|---|---|
| 1 | 154.7 | 159.2 | | |
| 2 | 123.3 | 115.0 | | |
| 3 | 129.0 | 128.5 | 7.52 d | 2.5 |
| 4 | 129.6 | unclear | | |
| 5 | 126.9 | 128.5 | 7.18 dd | 2.5, 9 |
| 6 | 113.5 | 115.0 | 6.84 d | 9 |
| 7 | 55.5 | 55.9 | 5.48 d | 8 |
| 8 | 169.4 | unclear | | |
| 9 | 52.9 | 52.7 | 3.70 s | |
| 10 | 171.3 | unclear | | |
| 11 | 23.0 | 23.0 | 2.00 s | |
| 12 | 64.9 | 63.6 | 4.06 q | 6 |
| 13 | 14.7 | 14.8 | 1.42 t | |

Avoparcin, CDP-I, and ristocetin (5 mg each) were hydrolyzed with aqueous 4 M NaOH (2 mL) in a Teflon-lined brass tube at 100 °C for 48 h. The solution was acidified (HCl), evaporated, and examined for glycine on a JEOL JLC-6AM amino acid analyzer, precalibrated for quantitative work. The yields of glycine were as indicated in Table II. When the hydrolysis was carried out under more forcing conditions with 6 M NaOH (2 mL), the yield of glycine from vancomycin was 2.2 as against 0.54 molar equiv for avoparcin.

**Carbohydrate Studies. Preparation of Methyl *N,O*-Diacetylristosaminide by the Procedure of Bognar et al.[15]** Avoparcin (20 g, dried over $P_2O_5$) was dissolved in 200 mL of absolute MeOH previously reacted with 20 mL of $CH_3COCl$. The acidic MeOH solution of avoparcin was refluxed for 4 h and the MeOH was removed in vacuo to give a syrupy residue. This residue was dissolved in 30 mL of MeOH and poured into a suspension of 200 mL of Dowex 1-X4 ($HCO_3^-$) and 300 mL of $H_2O$. The suspension was filtered to give a clear filtrate. The resin was washed with 200 mL of $H_2O$ and the combined wash and filtrate was chromatographed on Dowex 50-X 4 ($Na^+$).

The resin was then washed with 100 mL of $H_2O$ and eluted with 2% $NH_4OH$. The bulk of the $NH_3$ was removed in vacuo and the concentrate was freeze-dried to give a tan material weighing 891 mg.

The residue from freeze-drying was dissolved in 9 mL of dry pyridine and 9 mL of $Ac_2O$. The acetylation was allowed to proceed for 24 h at room temperature and then MeOH (50 mL) was added to destroy unreacted $Ac_2O$.

The crude acetylation products were dissolved in 15 mL of $C_6H_6$-MeOH (9:1), applied to a column of silica gel (1 × 18 in.), and eluted using the same solvent. Fraction volumes were about 10 mL. A spot from each fraction was applied to a plate of TLC silica gel and the plate charred by $H_2SO_4$. On this basis, fractions 52-61 were

Table XVII. $R_f$'s of Permethylated Neutral Sugars

| $R_f$ | compd | name | wt, mg |
|---|---|---|---|
| 0.06 | A | methyl 3,4,6-trimethylglucoside | 519 |
| 0.26 | B | methyl 2,3,4,6-tetramethylmannoside | 248 |
| 0.50 | C | methyl 2,3,4-trimethylrhamnoside | 234 |

combined and concentrated in vacuo to a residue. Residual pyridine acetate was removed azeotropically using toluene and the remaining residue was dried over $P_2O_5$ in vacuo at room temperature to give a clear, syrupy residue. The dried residue was triturated with diethyl ether (30 mL) and petroleum ether (60 mL). The resulting suspension was filtered to remove a solid. The filtrate was then concentrated in vacuo to give a syrup weighing 704 mg. This syrup was allowed to stand at 4 °C for several weeks. Crystals appeared in a syrupy material. The round-bottom flask was turned so that the syrup flowed away from the crystals and the crystals were triturated with 5 mL of cold petroleum ether–diethyl ether (1:1). The suspension was filtered to give 193 mg of crystalline solid melting at 50–52 °C: $[\alpha]^{20}_D$ −126° (*c* 1.27, $CHCl_3$) (lit.[15] 50–52 °C and $[\alpha]^{20}_D$ −134° (*c* 0.5, $CHCl_3$)); $^1H$ NMR (100 MHz, $CDCl_3$) δ 1.18 (3 H, d, *J* = 7 Hz, C-6), 1.89–2.09 (2 H, broad multiplet, C-2), 1.98 (3 H, s, $NHCOCH_3$), 2.00 (3 H, s, $OCOCH_3$), 3.41 (3 H, s, $OCH_3$), 3.98 (1 H, m, H-5), 4.57 (1 H, m, H-4), 4.66 (1 H, m, H-3), and 4.79 (1 H, d, H-1); mass spectrum $M^+$ at *m/e* 245 (not seen), M − $OCH_3$ (214), M − $CH_3COOH$ (185), M − $CH_3COOH$ − $CH_3OH$ (153), M − $CH_3COOH$ − $CH_2C{=}O$ (143).

**Methyl Glycosides of Glucose, Mannose, and Rhamnose from the Methanolysis of Avoparcin.** A solution of avoparcin (40 g) in 500 mL of MeOH made acidic by the prior addition of 30 mL of $CH_3COCl$ was refluxed for 18 h. The MeOH was removed in vacuo to give a syrupy residue. This material was dissolved in 800 mL of $H_2O$, neutralized with IR-45 ($OH^-$) resin, filtered, and freeze-dried to give a crude mixture weighing 12.8 g.

The above crude mixture was purified by chromatography on cellulose powder (Whatman CF-11) using the partition system cyclohexane–EtOAc–2-propanol–$H_2O$ (1:16:9:9). This gave in the order of elution (a) methyl α-L-rhamnoside (1.36 g); (b) methyl α-D-mannoside (1.44 g); (c) methyl α-D-glucoside (2.20 g) as shown by TLC silica gel, IR, and optical rotation comparisons with preparations made from authentic sugars.

For TLC, the silica gel plates were developed with EtOAc–IPA–$H_2O$ (10:6:4) and the zones were detected by charring with $H_2SO_4$ spray.

**Preparation of Methyl 3,4,6-Trimethylglucoside from Vancomycin Using the Method of Hakomori.**[33] Both vancomycin and the $Me_2SO$ were dried over $P_2O_5$ at room temperature for at least 3 days prior to use. All reactions were conducted under an atmosphere of $N_2$.

NaH (12 g) was added to 150 mL of dry $Me_2SO$ to give a suspension. This was allowed to stand for 1 h and then a solution of vancomycin (6 g) in 125 mL of $Me_2SO$ was added along with 200 mL of $CH_3I$. The reaction was allowed to proceed for 2 h and then $H_2O$ (300 mL) and $CHCl_3$ (300 mL) were added to give a two-phase system. The $CHCl_3$ layer was separated and washed with an equal volume of 2 N $Na_2S_2O_3$. The washed $CHCl_3$ layer was dried over $Na_2SO_4$ for 3 h. The $Na_2SO_4$ was removed by filtration and the $CHCl_3$ was removed in vacuo to give a syrup.

The syrupy residue in 250 mL of MeOH made acidic by the prior addition of 20 mL of $CH_3COCl$ was refluxed overnight. The MeOH was removed in vacuo to give a residue which was triturated with 50 mL of $CHCl_3$ and neutralized by the addition of solid $Na_2HPO_4$. The neutralized $CHCl_3$ was decanted and concentrated in vacuo to give a second residue.

This second residue was purified using chromatography on silica gel (1.25 × 16 in.). The charge was dissolved in a minimal quantity of $CHCl_3$ and allowed to seep into the column of silica gel. The column was developed first with 1.5 L of $CHCl_3$ and this was collected as one fraction and discarded. The column was then developed with $CHCl_3$–EtOAc (1:1 by volume) with collection of 15-mL fractions. The fractions were checked for the presence of desirable material using TLC on silica gel. The chromatograms were developed with EtOAc with detection of zones by $H_2SO_4$ charring. Fractions 31–52 ($R_f$ 0.41) were combined and concentrated in vacuo to give a syrupy residue of methyl 3,4,6-trimethylglucoside, weighing 733 mg: $^{13}C$ NMR ($CDCl_3/Me_4Si$) 55.2, 59.2, 60.3, 60.8, 70.3, 71.1, 72.4, 79.4, 84.5, and 99.5 ppm; $[\alpha]^{20}_D$ +123.9° (*c* 3.51, $CHCl_3$).

Table XVIII

| position | ppm |
|---|---|
| H-1 | 4.08 (2 H) |
| H-2 | 1.70 m (1 H), the other masked by H-6 |
| H-3 | 4.34 m (1 H) |
| H-4 | 4.95 dd (1 H) *J* = 4.4 and 6.8 |
| H-5 | 5.03 (1 H) |
| H-6 | 1.22 d (3 H) *J* = 6.5 |
| $CH_3CO$'s | 1.99 s, 2.01 s, 2.11 s |
| NH | 5.78 bd |

**Permethylation of Avoparcin Using the Method of Hakomori and Isolation of Methyl 3,4,6-Trimethylglucoside, Methyl 2,3,4-Trimethylrhamnoside, and Methyl 2,3,4,6-Tetramethylmannoside.** Dried avoparcin, 20 g, was dissolved in 125 mL of dry $Me_2SO$. A suspension of 22 g of NaH in 150 mL of dry $Me_2SO$ was allowed to stand for 1 h and then the solution of avoparcin was added. $CH_3I$ (300 mL) was added and the reactants were allowed to react for 2 h. Care was taken to keep a $N_2$ atmosphere above all reactions. Water (750 mL) and $CHCl_3$ (750 mL) were added and the $CHCl_3$ layer was separated. The $CHCl_3$ layer was washed with an equal volume of 2 N $Na_2S_2O_3$, dried over $Na_2SO_4$, and concentrated in vacuo to give a syrup. The syrup was triturated with ether and filtered to give an air-dried solid weighing 24 g.

A solution of the permethylated avoparcin (15 g in 250 mL of methanol, made acidic by the prior addition of 15 mL of acetyl chloride) was refluxed for 7 h. The methanol was then removed in vacuo to give a syrup. This residue was triturated with 400 mL of $CHCl_3$–ether (1:1 by volume) to give a clear decantate. The $CHCl_3$ was removed in vacuo to give a material suitable for chromatographic separation.

The material to be chromatographed was dissolved in a minimal quantity of $CHCl_3$ and allowed to seep into a cylinder of silica gel (1.5 × 30 in.). The column was first developed with chloroform (2 L) and finally $CHCl_3$–EtOAc (4:1). Fractions with a volume of 17 mL each were collected. A spot from each cut was chromatographed on TLC silica gel using $CHCl_3$–EtOAc (4:1 by volume) as developing agent and $H_2SO_4$ charring for detection. Three main substances were observed: compound A, $R_f$ 0.06; compound B, $R_f$ 0.26; compound C, $R_f$ 0.50. The fractions comprising the respective substances were combined using TLC as a guide and concentrated separately to give a series of syrups. Compound A weighed 943 mg, compound B weighed 299 mg, and compound C weighed 519 mg. The three substances were rechromatographed using the same procedure to give the amounts of material shown in Table XVII. Each component was a single spot by TLC.

**Methyl 3,4,6-Trimethylglucoside (A).** This material was identical with that isolated from vancomycin described under the previous entry (TLC, $^{13}C$ NMR).

$^{13}C$ NMR (25.5 MHz, $CDCl_3$): 55.2, 59.2, 60.2, 60.8, 70.3, 71.1, 72.3, 79.5, 84.5, and 99.5 ppm. $^1H$ NMR (100 MHz, $CDCl_3$): δ 3.2–3.9 (17 H, m), 4.7 (1 H, d, $J_{1,2}$ = 6 Hz). Mass spectrum: $M^+$ *m/e* 236 (not seen), $M^+$ − $CH_3OH$ (204), M − $CH_3OH$ − $CH_3O$ (173).[34] $[\alpha]^{20}_D$: +119° (*c* 4.52, $CHCl_3$).

**Methyl 2,3,4,6-Tetramethylmannoside (B).** $^{13}C$ NMR (25.5 MHz, $CDCl_3$): 54.9, 57.7, 59.0, 59.2, 60.6, 71.3, 71.8, 76.5, 77.2, 81.4, and 98.1 ppm. $^1H$ NMR (100 MHz, $CDCl_3$): 3.2–3.8 (21 H, m), 4.80 (1 H, bs, $J_{1,2}$ = 1 Hz). Mass spectrum: $M^+$ *m/e* 250 (not seen), $M^+$ − $OCH_3$ (219), $M^+$ − $OCH_3$ − $CH_3OH$ (198).[34] $[\alpha]^{20}_D$: +66.6° (*c* 4.20, $CHCl_3$).

**Methyl 2,3,4-Trimethylrhamnoside (C).** $^{13}C$ NMR (25.5 MHz, $CDCl_3$): 17.8, 54.7, 59.9, 60.8, 67.8, 77.5, 81.3, 82.2, and 98.8 ppm. $^1H$ NMR (100 MHz, $CDCl_3$): δ 1.25 (3 H, d), 3.2–3.7 (16 H, m), 4.7 (1 H, bs, $J_{1,2}$ = 1 Hz). Mass spectrum: $M^+$ (*m/e* 220), $M^+$ − $OCH_3$ (189), $CH_3O^+{=}CHCH{=}OCH_3$ (101), $CH_3OCH{=}CHOCH_3^+$ (88), $CH_3O^+{=}CHOCH_3$ (75).[34] $[\alpha]^{20}_D$: 45.1° (*c* 3.28, $CHCl_3$).

**Formation of a Crystalline Decomposition Product (Avoparcin CDP-I).** Avoparcin, 50 g, was dissolved in 500 mL of 0.14 N HCl and the solution refluxed for 2 h and allowed to cool to room temperature. The cooled acidic solution was adjusted to pH 7.0 using the basic resin, IR-4B ($OH^-$). The resin and other insoluble material were collected on a filter, washed with water, and stirred in the funnel with 800 mL of MeOH. Acetone (600 mL) was added to the MeOH-rich filtrate

**Table XIX.** Crystal Data and Reliability Indices

| | III | XIV | XVI |
|---|---|---|---|
| formula | $C_{25}H_{30}N_2O_9$ | $C_{13}H_{16}NO_4Cl$ | $C_9H_7O_4Cl$ |
| mol wt | 502 | 285 | 214 |
| space group | triclinic $P\bar{1}$ | monoclinic $P2_1/c$ | monoclinic $P2_1/c$ |
| $a$ | 13.800 (25) Å | 8.444 (3) Å | 8.957 (3) Å |
| $b$ | 12.088 (9) Å | 19.083 (7) Å | 15.114 (5) Å |
| $c$ | 10.010 (5) Å | 9.177 (3) Å | 7.940 (2) Å |
| $\alpha$ | 129.51 (5)° | 90.0° | 90.0° |
| $\beta$ | 98.55 (8)° | 109.00 (2)° | 123.27 (2)° |
| $\gamma$ | 92.27 (10)° | 90.0° | 90.0° |
| cell vol | 1254 Å$^3$ | 1398 Å$^3$ | 899 Å$^3$ |
| $Z$ | 2 | 4 | 4 |
| $\rho_{calcd}$ | 1.330 | 1.354 | 1.581 |
| $\rho_{obsd}$[a] | 1.322 g cm$^{-3}$ | 1.315 g cm$^{-3}$ | 1.562 g cm$^{-3}$ |
| reflections measd | 3784 | 1955 | 1157 |
| unobsd $I < 2.0\sigma(I)$ | 2237 | 414 | 345 |
| crystal size | 220 × 120 × 100 μm | 250 × 150 × 750 μm | 400 × 250 × 150 μm |
| reliability index $R$[b] | 0.159 | 0.064 | 0.059 |

[a] By flotation in $CCl_4$-hexane mixture. [b] $R = [\Sigma||F_o| - |F_c||/\Sigma|F_o|]$ for observed reflections.

and the resulting solution was stored at 4 °C for 48 h. The microcrystalline solid, CDP-I, was collected on a filter and washed with 200 mL of acetone-MeOH (1:1 by volume) and finally with acetone. The washed solid was dried in vacuo over $P_2O_5$ at room temperature to give 8.92 g of dry CDP-I.

**Preparation of Avoparcin CDP-I Hydrochloride Salt.** CDP-I, 1 g, was dissolved in 50 mL of MeOH previously reacted with 5 mL of $CH_3COCl$. Three volumes of EtOAc were added and the flocculent solid was collected on a filter and washed with fresh EtOAc. The washed solid was dried in vacuo over $P_2O_5$ at room temperature to give CDP-I HCl salt weighing 789 mg, $[\alpha]^{25}_D$ −48° (*c* 1.1, DMF); CDP-I had antibiotic activity.[35]

**Isolation of Ristosaminitol Peracetate.** Avoparcin was refluxed in 2 N HCl for 30 min. The cooled solution was filtered, neutralized with 1 N NaOH to pH 7.0, and treated overnight with 0.2 g of $NaBH_4$. Excess $NaBH_4$ was decomposed with 1 M HCl to pH 7.0. The solution was concentrated to a solid and acetylated with $Ac_2O$-pyridine after removal of boric acid. The solid thus obtained was extracted with ether and about 0.3 g of the concentrate subjected to preparative TLC using EtOAc.[36] The band with $R_f$ 0.4 was eluted to yield 10 mg of 1,5-di-*O*-acetyl-3-acetamido-2,3,6-trideoxy-L-*ribo*-hexitol: $[\alpha]^{25}_D$ −17.7° (*c* 0.45, $CHCl_3$); MS $M^+$ *m/e* 318, 274, 258. The 220-MHz $^1H$ NMR in $CDCl_3$ is given in Table XVIII.

**Acid Hydrolysis of β-Avoparcin, CDP-I, and Ristocetin.** About 10 mg of each of these was refluxed in 3 mL of 2 N HCl for 35 min. The cooled solutions were neutralized with 1 N NaOH, treated with 20 mg each of $NaBH_4$ overnight, and acetylated ($Ac_2O$-pyridine) to give mixtures of alditol acetates (TLC in ether) which were examined by GLC (3% OV 225 on Gas Chrom Q) to observe the ratios outlined in Table I.

**X-ray Structure Determinations.** Crystals of III, XIV, and 3-chloro-4-hydroxyphenyl glyoxylic methyl ester or XVI were deemed suitable for X-ray crystallography studies. Intensities for crystals of III, XIV, and XVI were measured in the range $3° < \theta < 60°$ using the $\theta/2\theta$ scan method on an Enraf-Nonius CAD-3 computer-controlled diffractometer. Nickel-filtered Cu K$\alpha$ radiation from a fine focus tube was used with pulse height analysis of the diffracted beam to provide further wavelength discrimination. After correction for Lorentz and polarization effects, normalized structure factors $E(hkl)$ were computed for each compound. The structures were solved by the direct phase determination method, using MULTAN.[37] Crystal data and final reliability indices for observed reflections are listed in Table XIX. In all three structures, nonhydrogen atoms were refined anisotropically; hydrogen atoms were found, included, and refined isotropically for compounds XIV and XVI. The high reliability index for III as compared with the values for the other structures reflects the poor quality of the crystals available in this case.[38] Attempts to obtain a better crystal from the small amount of material available were not successful. All refinement calculations were made using the X-ray System (1976) programs;[39] atomic scattering factors were taken from the International Tables for X-ray Crystallography.[40] Full details of the crystallographic structures are available from one of the authors (F.M.L.).

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# Reactions of Vitamin $B_{12r}$ with Organic Halides

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**Abstract:** Vitamin $B_{12r}$ was found to react with certain organic halides (RX) according to the stoichiometry $2B_{12r} + RX + H_2O$ (or $CH_3OH$) $\rightarrow B_{12a} + R\text{-}B_{12} + X^-$ (where $B_{12a} = (H_2O)B_{12}^+$ + or $(CH_3OH)B_{12}^+$). For a variety of organic halides in methanol and of organic chlorides and bromides in water (0.5 M $KH_2PO_4$/NaOH, pH 7.0) the kinetics conformed to the second-order rate law $-d[B_{12r}]/dt = 2k_7[B_{12r}][RX]$. The results are interpreted in terms of a stepwise atom-transfer mechanism: $B_{12r} + RX \rightarrow X\text{-}B_{12} + R\cdot$ (rate determining); $B_{12r} + R\cdot \rightarrow R\text{-}B_{12}$ (fast); $X\text{-}B_{12} + H_2O$ (or $CH_3OH$) $\rightarrow B_{12a} + X^-$ (fast); in some cases, $R\text{-}B_{12}$ (or $R\cdot + B_{12r}$) $\rightarrow B_{12a}$ + unidentified products. Trends in the kinetic data, notably the dependence of $k_7$ on RX, are discussed. It is concluded that, contrary to earlier views, vitamin $B_{12r}$ does react directly with organic halides. In aqueous solution, the reactions of vitamin $B_{12r}$ with organic iodides, while conforming to the same stoichiometry, exhibited different kinetic behavior, corresponding to the third-order rate law $-d[B_{12r}]/dt = 2k_9[B_{12r}]^2[RX]$. No inhibition by vitamin $B_{12a}$ was observed. The mechanism proposed for these reactions is $B_{12r} + RI \rightleftarrows [B_{12r}\cdot RI]$ (rapid equilibrium); $[B_{12r}\cdot RI] + B_{12r} \rightarrow R\text{-}B_{12} + B_{12a} + I^-$ (rate determining).

## Introduction

Previous work in this laboratory has demonstrated that organic halides react with pentacyanocobaltate(II) and with cobalt(II) complexes of dimethylglyoxime and of Schiff's bases according to the stoichiometry and mechanism depicted by eq 1-3.[1-5] Since such low-spin cobalt complexes have been widely invoked as "models" or analogues of the corresponding vitamin $B_{12}$ derivatives,[6,7] it was clearly of some interest to compare these systems to the reactions of vitamin $B_{12r}$, i.e., cob(II)alamin,[8] with organic halides.

$$L_5Co^{II} + RX \xrightarrow{k_1} L_5Co^{III}X + R\cdot \quad \text{(rate-determining)} \quad (1)$$

$$\underline{L_5Co^{II} + R\cdot \rightarrow L_5Co^{III}R \quad \text{(fast)}} \quad (2)$$

$$2L_5Co^{II} + RX \rightarrow L_5Co^{III}X + L_5Co^{III}R \quad (3)$$

Several prior studies have led to the conclusion (or have assumed) that, in contrast to the behavior described above for other low-spin cobalt(II) complexes, vitamin $B_{12r}$ does not react directly with organic halides.[9-11] The most extensive such study was described by Yamada et al.,[11] who confirmed the stoichiometry of eq 6, but concluded from indirect evidence (notably the influence of electrolytes on the rate) that the mechanism was that depicted by eq 4 and 5, i.e., disproportionation of vitamin $B_{12r}$ to $B_{12s}$ and $B_{12a}$, followed by the well-known alkylation of $B_{12s}$ by the organic halide to form $R\text{-}B_{12}$.[12] This surprising discrepancy between the behavior of vitamin $B_{12r}$ and other low-spin cobalt(II) compounds, which have been widely accepted as $B_{12r}$ "models", suggested that a more thorough investigation of the reactions of vitamin $B_{12r}$ with organic halides was warranted. The results of such an investigation of the products and kinetics of the reactions of vitamin $B_{12r}$ with a variety of organic halides in methanol and aqueous solutions are reported in this paper.

$$2B_{12r} \underset{k_{-4}}{\overset{k_4}{\rightleftarrows}} B_{12s} + B_{12a} \quad (4)$$

$$\underline{B_{12s} + RX \xrightarrow{k_5} R\text{-}B_{12} + X^-} \quad (5)$$

$$2B_{12r} + RX \rightarrow R\text{-}B_{12} + B_{12a} + X^- \quad (6)$$

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