EPIMERIZATION AND STEREOCHEMISTRY OF AVOPARCIN

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The epimerization of avoparcin entities is discussed in some detail. The absolute stereochemistry of avoparcin is now known since the *N*-methyl terminal amino acid of the aglycone has been isolated and shown to exhibit negative optical rotation and hence has the *R*-configuration. The same amino acid has been isolated from an epimerized solution of avoparcin and found to have positive rotation and hence the *S*-configuration. A comparison is made of the CD curves of β-avoparcin and *epi*-β-avoparcin. Some discussion on the effect of protonation of the terminal *N*-methyl group on the antibacterial activity of avoparcin is included.

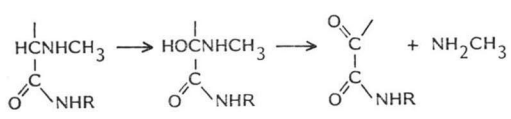
In the previous paper we outlined the isolation of most of the components of the avoparcin complex in relatively pure forms.[1] The structures of several of the minor components were also elucidated with a view towards obtaining further understanding of structure-activity relationship in this class of antibiotics. In this paper we describe in more detail the epimerization phenomenon of avoparcins. We have isolated an optically active form of the terminal *p*-hydroxyphenylsarcosine of avoparcin and the same amino acid with opposite rotation from epimerized avoparcin. Consequently we feel confident that the overall absolute configuration is as shown in Table 2. There is considerable interest in the mode of action of this glycopeptide class of antibiotics at the present time. The mechanism involves the binding of *N*-terminal ends of the antibiotics to the D-ala-D-ala terminal portions of the cross-linking pentapeptide which plays a crucial role in the cell wall formation of bacteria. The net result is the proliferation of weak spots in the cell walls thus enhancing the possibility of cell lysis. The isolation of *epi*-β-avoparcin provides another useful tool for further study of this mechanism.

Stability and Epimerization Studies

If avoparcin is allowed to stand in solution at pH 4.0 or below, there is with time a decided increase in the amount of deristosaminyl compounds present. The use of lower pH's gives rise to CDP-I and mannosyl aglycone avoparcin.[1] At pH's above 8.0 avoparcin begins to lose ammonia, probably from the benzylic ristosamine (latent β-amino aldehyde) which is liberated by β-elimination.[2]

Above pH 9.5 methylamine is excised which is not surprising since from the EDMAN work we know that the methine carbon of the terminal amino acid is readily oxidized.

All of these reactions are of course accelerated by heating. From such studies it appears that the pH range of maximum stability for the complex is between pH 4.0 and 8.0. However, if avoparcin is heated gently in aqueous solution in this pH range over a period of 3 to 18 hours, the antibacterial activity of the solution decreases dramatically.[3] If the process is monitored by HPLC, the number of peaks observed in the trace is virtually doubled and the intensities of the original peaks are greatly diminished.



When pure β-avoparcin is heated at 75°C at pH 7.0 for 18 hours, the result is an equilibrium

Fig. 1.

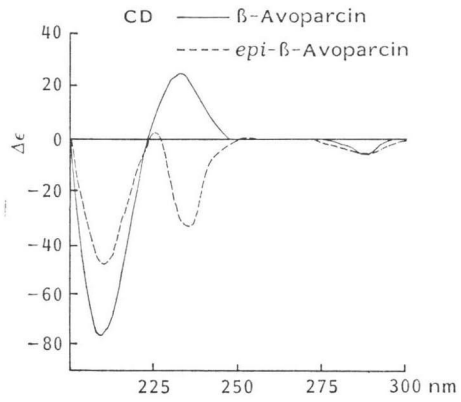


Table 1. Epimerization of avoparcin compounds

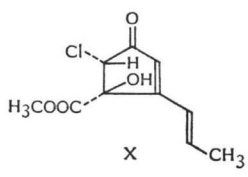
Compounds	UV area % ratio (*epi* to parent)	Ratio of retention time (*epi* to parent)
α-Avoparcin	2.3:1	1.6:1
β-Avoparcin	2.9:1	1.4:1
Galactosyl-β-avoparcin	2.0:1	1.5:1
Mannosyl aglycone of α-avoparcin	2.1:1	1.3:1
Mannosyl aglycone of β-avoparcin	2.3:1	1.5:1
α-CDP-I avoparcin	1.3:1	1.4:1
β-CDP-I avoparcin	1.5:1	1.4:1
ε-Avoparcin	0.9:1	0.9:1
Deristosaminyl-ε-avoparcin	0.9:1	0.9:1

consisting of about 30% of β-avoparcin and 70% of a diastereomer which we have called *epi*-β-avoparcin. When *epi*-β-avoparcin is subjected to the conditions described above, the same equilibrium is reached. Evidence for the structure of *epi*-β-avoparcin has already been reported[3]; other avoparcin entities were subjected to epimerization conditions and the reactions monitored by HPLC. Table 1 summarizes the results of these experiments.

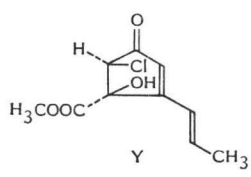
In the case of the first seven entities, each of which has a mannose appendage the equilibrium ratios as well as the retention time ratios are fairly similar. The absence of mannose in ε-avoparcin changes both ratios remarkably. Epimerization still occurs but now what is happening resembles a racemization. Apparently the mannose sugar is necessary for the extra stability of the diastereomer under equilibrium conditions. The deristosaminyl ε-avoparcin was not isolated but ε preparations were obtained where the amounts of this impurity were enhanced. The retention time of this minor component relative to that of β-avoparcin was 1.08.

We have obtained CD curves on *epi*-β-avoparcin and β-avoparcin for comparison purposes. The CD curve of β shows large peaks at 210 nm ($\Delta\varepsilon$ −76.0) and 230 nm ($\Delta\varepsilon$ +26.0) and a weak effect at 287 nm ($\Delta\varepsilon$ −4.0). The weak negative effect is attributable to a harmonic of the ^1L_b band of the aromatic chromophores. A dramatic difference is observed in the CD curve of the *epi*-β-avoparcin. The peak at 210 nm is the same as in the β-compound ($\Delta\varepsilon$ −49.2), however, the sign of the peak at 232 nm is reversed ($\Delta\varepsilon$ −35.8). The weaker higher-wavelength harmonic effect remains about the same for both compounds ($\Delta\varepsilon$ −4.5). Reproductions of the CD curves of β and *epi*-β-avoparcins are shown in Fig. 1.

A similar reversal was noted with compounds X and Y.[4] In these compounds we have an extended conjugated carbonyl chromophore and a carboxyl chromophore whose chiroptic properties are dominated by vicinal asymmetric centers, one of which is reversed in X as opposed to Y. If we regard the avoparcin molecule as consisting of one extended large chromophore A adjacent to a single aromatic chromophore B, then the analogy with X and Y seems reasonable. All the ^1H NMR data as detailed previously indicate that chromophore A is identical for both the β and *epi*-β-compounds. The only change is in the configuration of chromophore B in the *epi* compound. This change is



X



Y

reflected in the reversal of the 232 nm peak in the CD curve of the *epi* compound.

Our efforts were then directed to ascertaining the absolute configuration of the *N*-terminal phenylsarcosine amino acid. An EDMAN reaction was carried out on *p*-hydroxy-D-phenylglycine using standard conditions and the resultant thiohydantoin was optically inactive.[5] Modification of the EDMAN conditions resulted in the isolation of an optically active product. Using those milder conditions CDP-I avoparcin was subjected to the first stage of the EDMAN method and processed to yield optically inactive thiohydantoins. When these attempts failed, we resorted to the method of LOMAKINA *et al.*[6] which has also been successfully used by HARRIS *et al.* on ristocetin.[7] Using this procedure on avoparcin we recovered crystalline *N*-methyl-*p*-hydroxyphenylglycine which had a negative rotation in water. Consequently the configuration of the terminal amino acid in avoparcin is *R* in agreement with the configuration of the terminal amino acids in both vancomycin and ristocetin. *p*-Hydroxyphenylglycine from α-avoparcin and *m*-chloro-*p*-hydroxyphenylglycine from β-avoparcin were also isolated from this hydrolysate. However in the former case the material was contaminated with about 20~25% of the *p*-hydroxyphenylsarcosine and the second compound was unstable and subject to oxidation in our hands. Nevertheless both preparations exhibited good positive rotation thus confirming the assignment of the 3′ position as shown in Table 2. A comparison of the chemical shift values and splitting constants of the methine protons of the peptide backbone and benzylic positions with those of vancomycin and ristocetin indicates the same stereochemistry at these positions.[3] Thus, we are now confident of the absolute stereochemistry of β-avoparcin as depicted in Fig. 2.

Avoparcin was epimerized and the same procedures were applied to isolate the *N*-methyl terminal amino acid from the epimerized complex. Under these conditions the isolated pure *N*-methyl-*p*-hydroxyphenylglycine exhibited a positive rotation. This work confirms our epimerization postulates. Since CDP-I avoparcin was isolated from an overnight acid hydrolysis of avoparcin with no sign of epimerized material, we conclude that fully protonated avoparcin is not epimerized. Partial protonation around pH 4.5 obviously does not preclude epimerization. The topic of protonation of this family of glycopeptides relative to antibiotic activity is one of some importance. NIETRO and PERKINS first observed the binding of the model peptide Ac-L-Lys-D-Ala-D-Ala to vancomycin.[8] They indicated that binding was enhanced when the carboxylate anion of the peptide was attracted by the protonated *N*-methyl group of the antibiotic. CONVERT *et al.* agreed with these conclusions based on NMR

Table 2. Configurations of methine proton centers in avoparcin.

Methine position	Configuration
1′	*R*
2′	*R*
3′	*S*
4′	*R*
5′	*R*
6′	*S*
7′	*S**
A_{bz}	*R*
C_{bz}	*R*

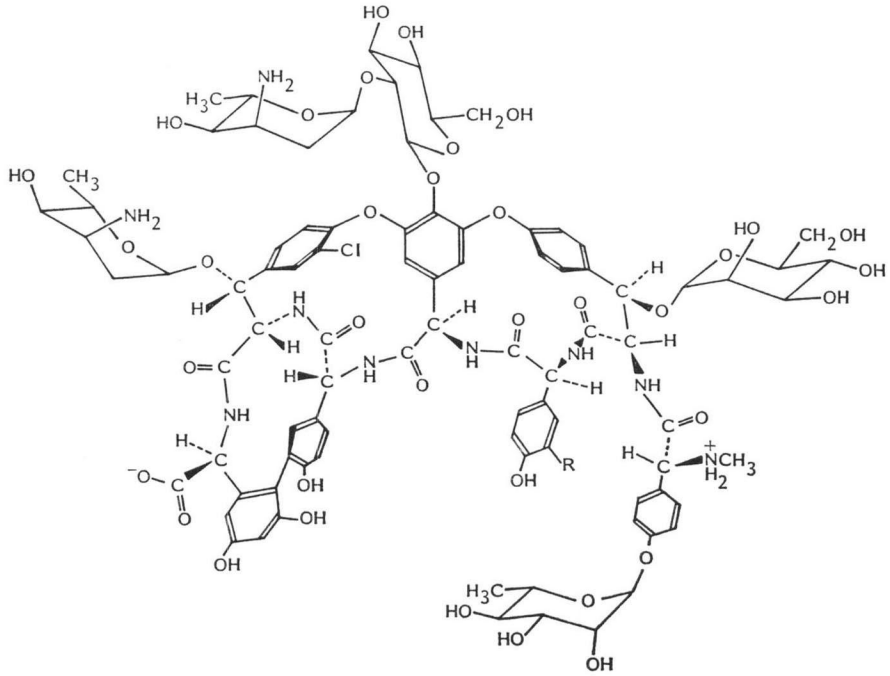
* Incorrectly depicted as *R* in ref 3.

Table 3. Comparison of activities* of β-avoparcin at pH values of 6.0, 7.0 and 8.0.

Organism	MIC of β-avoparcin (μg/ml)		
	pH 6.0	pH 7.0	pH 8.0
Staphylococcus Smith	0.5	0.5	0.25
Staphylococcus SSC BO-11	2.0	2.0	4.0
Staphylococcus SSC BO-32	4.0	4.0	4.0
Staphylococcus SSC BO-38	1.0	2.0	2.0
Staphylococcus LL #14	2.0	2.0	2.0
Staphylococcus LL #45	2.0	2.0	4.0
Staphylococcus ATCC 25923	1.0	1.0	2.0
Enterococcus SSC 80-62	0.5	0.5	1.0
Enterococcus SSC 80-63	0.25	0.25	0.5

* Mueller-Hinton agar dilution method.

Fig. 2.



Avoparcin α R=H β R=Cl

measurements while WILLIAMS *et al.* have carried the idea somewhat further, postulating a hydrophobic pocket holding the peptide carboxylate group.[9,10] In their view again the initial reaction is between the protonated antibiotic *N*-methyl amino group and the peptide carboxylate anion. Following immediately after this a conformational change occurs and the carboxylate anion falls into a newly formed pocket with hydrophobic walls. The rest of the antibiotic provides further bonding interactions with another portion of the peptide thus giving rise to the notion of antibiotic specificity. These steps namely, (a) charge-charge interaction, (b) conformational change, (c) hydrophobic pocket formation, (d) complementarity of binding sites, have already been regarded as part and parcel of enzyme-substrate interaction.

We carried out a number of experiments on avoparcin designed to examine the first of the postulated steps namely, the initial charge-charge interaction. We have shown that the ^{1}H NMR chemical shift of the *N*-methyl group of avoparcin in aqueous solution reflects the degree of protonation of that center. In a plot of these chemical shift values *versus* the pH of the antibiotic solution there is an inflection point at pH 6.5, indicating that the *pKa* of the *N*-methyl group is 6.5. Using this value a calculation involving the Henderson-Hasselbach equation shows that at pH 6.0 the *N*-methyl group is 75% protonated whereas at pH 8.0 the degree of protonation is only about 3%. Based on these values and if the proposed first step of the binding sequence is of importance, one would expect some degree of pH dependence in the observance of antibiotic activity. Nevertheless we found no significant change in the antibiotic activity of avoparcin as measured *in vitro* by the agar dilution method at pH's between 6.0 and 8.0 (Table 3). Previously vancomycin was shown to have about the same activity against *Streptococcus pyogenes* var. *aureus* in the pH range 6.5 to 8.0.

Despite this, the overall picture of hydrophobic pocket formation with subsequent locking-in of the

Ac-D-Ala-D-Ala peptide would appear to be a reasonable model of antibiotic action. The configuration of the terminal amino acid of avoparcin should then be as important as the configuration of the terminal alanine of the peptide in so far as binding is concerned. WILLIAMS *et al.* have shown that Ac-D-Ala-L-Ala peptide is not complexed or bound to vancomycin. We have shown that *epi*-β-avoparcin has very little Gram-positive antibiotic activity consequently, the Ac-D-Ala-D-Ala peptide should not be bound by the avoparcin epimer. This problem is being studied by Prof. I. ARMITAGE of Yale University. Preliminary data so far would seem to substantiate this.

Experimental

General

Analytical and preparative HPLC work was carried out as described in the previous paper. NMR spectra were run on a Nicolet NT-300 WB while mass spectra were obtained using a Varian CH 7 spectrometer with an SS-100 Data System. CD curves were recorded in water with a Jasco J-40 spectropolarimeter at concentrations between 0.01 and 0.25 mg/ml. We thank Prof. K. NAKANISHI of Columbia University for these curves.

Isolation of Partially Deuterated *epi*-β-Avoparcin and β-Avoparcin

A solution containing 1 g of pure β-avoparcin (98% by HPLC) in 35 ml of D_2O was adjusted to 6.8 using dilute NaOD solution and placed on the steam bath for 4 hours. At the end of that time HPLC analysis using a 50°C column temperature and 2.4 ml /minute pumping speed indicated that by UV area percent the solution contained 33% β-avoparcin and 46% *epi*-β-avoparcin plus other impurities.

The reaction solution was diluted in 1 liter of HSA buffer and charged to a preparative column. After equilibration the column was eluted using 14% CH_3CN in HSA buffer to obtain two nicely resolved peaks. Fractions containing the first peak were processed to the first freeze-dried stage to yield 280 mg of a solid which dissolved in MeOH.[1] Addition of 0.5 M $(TEA)_2H_2SO_4$ solution gave a precipitate which was processed to the second freeze-dried stage to yield 170 mg of white powder which was subsequently shown to be partially deuterated β-avoparcin since the 1′ methine signal normally seen at 4.09 was completely missing. $[\alpha]_D^{25}$ $-94\pm2°$ (*c* 0.55, 0.1 N HCl).

The fractions of the second peak were handled in the same fashion to obtain 180 mg of partially deuterated *epi*-β-avoparcin since the 1′ methine proton visible in normal *epi*-β-avoparcin was missing from the ^1H NMR spectrum of this preparation. The analytical HPLC trace on this material showed by UV area percent that it consisted of 83.4% *epi*-β-avoparcin and approximately 10% of other components. $[\alpha]_D^{25}$ $-110\pm2°$ (*c* 0.52, 0.1 N HCl), ^{252}Cf PDMS $[M+Na]^+$ 1,965±2*.

Preparation of ε-Avoparcin Enriched in Deristosaminyl-ε-avoparcin

The isolation of ε-avoparcin has been described in the previous paper. The heptane sulfonic acid salt of this material in concentrated aqueous solution was titrated with 10% aqueous sodium dodecyl sulfate (SDS) solution to a point just short of the end point. The ε-avoparcin salt was removed by filtration and handled as described. The filtrate was then titrated with excess SDS solution. The precipitate from this stage was washed with H_2O and taken up in methanol and the avoparcin material was precipitated using triethylamine sulfate [prepared as described in ref 1]. This solid recovered under these circumstances contained 20% of deristosaminyl-ε-avoparcin in addition to ε-avoparcin.

Epimerization Studies on Other Avoparcin Entities

Epimerization studies were frequently carried out at concentrations of 1 or 2 mg/ml in the presence of equal concentrations of NaOAc. The pH was adjusted to the range of 4.0 to 8.0 as necessary. Frequently the NaOAc was omitted and the pH was simply adjusted to the desired value. The reaction solution was placed on top of the steam bath and allowed to sit for periods of from 3 to 18 hours. For comparison purposes analytical HPLC's were run on the starting solution and on the final reaction solution. There were certain minor problems with individual samples. For example, because of solubility

* Results kindly supplied by Prof. R. D. MACFARLANE of Texas A&M University.

problems the CDP-I and mannosyl aglycone avoparcins could be run only at pH's below 5.0. On the other hand those entities with the attached disaccharide intact, if run at lower pH's (below 5.5), developed a plethora of peaks due to the generation of deristosaminyl compounds and their epimerized counterparts. Under all of these circumstances, however, there could be no mistaking the epimerization process occurring. Three individual epimerizations are discussed below in detail. A sample of α-avoparcin was heated overnight at pH 6.7 at a concentration of 2 mg/ml. After 18 hours the HPLC analytical trace showed that by UV area percent the solution contained material which was 23.3% α-avoparcin and 56.9% *epi*-α-avoparcin. When 0.25 ml of this reaction solution was added to 0.75 ml of 2 mg/ml of avoparcin, there was a decided enhancement of the minor peak (retention time 985 seconds) immediately following the major β-peak (retention time 950 seconds). Consequently we are confident that this minor peak frequently detected in fermentations is *epi*-α-avoparcin.

A 2 mg/ml solution of the mannosyl aglycone of avoparcin at pH 4.5 was heated overnight on the steam bath. The HPLC curve on the starting solution indicated that by UV area percent 17.2% of the material present was α-mannosyl aglycone and 76.2% was β-mannosyl aglycone. After overnight heating the trace on the reaction solution showed that by UV area percent the solution contained 7.3% of α-mannosyl aglycone, 9.9% of *epi*-α-mannosyl aglycone, 30.4% of β-mannosyl aglycone and 45.6% *epi*-β-mannosyl aglycone.

A 2 mg/ml solution of *epi*-β-avoparcin was adjusted to pH 8.0 using dilute NaOH solution and left on the steam bath overnight. Comparison of the HPLC traces of the starting solution and the final reaction solution could be summed up as follows. The starting solution contained 87% *epi*-β-avoparcin and 1.5% β-avoparcin plus other minor peaks. The final reaction solution contained 20.5% β-avoparcin and 54% *epi*-β-avoparcin and some other minor peaks.

EDMAN Studies on D(−)-*p*-Hydroxyphenylglycine and CDP-I Avoparcin

Standard EDMAN degradation conditions involve the formation of a ureido derivative of the terminal amino acid of a peptide by stirring the peptide with isothiocyanate in pyridine - H_2O, 50: 50 at pH 9.0. The ureido derivative is then cleaved and rearranged to a thiohydantoin by refluxing in dilute acid solution. When these conditions were applied to D(−)-*p*-hydroxyphenylglycine, we obtained a crystalline thiohydantoin, mp 228°C (melts to a red liquid), but which was optically inactive. The following modified conditions gave the optically active compound. A 1-g aliquot of D(−)-*p*-hydroxyphenylglycine was stirred in 60 ml of pyridine - H_2O, 1: 1 with the pH adjusted to 8.0. Then 2 ml of methylisothiocyanate was added and pH adjustment to 8.0 continued using 1 N NaOH solutions for 1.5 hours. The pH was then adjusted to 7.0 and the pyridine was evaporated off. The aqueous slurry was extracted with ether twice and the aqueous phase was desolventized and freeze-dried to 1.5 g of off-white solid which exhibited an $[\alpha]_D^{25}$ $-104\pm0.5°$ (*c* 1.5, MeOH). About 0.75 g of this material was stirred in 50 ml of HOAc - 6 N HCl, 4: 1 solution for two hours. The solution was diluted to 500 ml with H_2O and extracted with EtOAc. The EtOAc extract was dried and concentrated and allowed to sit at room temperature to crystallize. About 200 mg of crystals, mp 210~212°C, were obtained in the first crop. $[\alpha]_D^{25}$ $-116\pm1°$ (*c* 0.89, MeOH). MS *m/z* 222.

The first stage of the EDMAN reaction using the coupling conditions described above was applied to 2 g of CDP-I avoparcin. The pyridine was evaporated off and the excess isothiocyanate was removed using ether and the slurry was then extracted with EtOAc. The EtOAc extract upon concentration yielded 250 mg of a gum which was chromatographed on two thick-layer silica gel plates using the system $CHCl_3$ - MeOH, 9: 1. The plate exhibited major and minor UV absorbing bands very close together. The major band was processed to yield 95 mg of white crystals, mp 175~178°C, which had $[\alpha]_D^{25}$ $0\pm0.5°$ (*c* 0.66, MeOH). A mass spectrum molecular ion was observed at *m/z* 252 which corresponds to the hydroxylated thiohydantoin.[2] There was also a small peak at *m/z* 236 corresponding to the normal thiohydantoin.

Isolation of Optically Active Amino Acids from Avoparcin

About 10 g of avoparcin sulfate was refluxed for 2 hours in 500 ml of 1 N HCl solution. The reaction solution was neutralized to pH 6.5 and filtered and the residue was re-suspended in 500 ml of 2 N HCl solution and refluxed for 24 hours. The solution was neutralized to pH 6.0 using IR-45 (OH^-)

resin. After removal of resin the filtrate was percolated through a Pharmacia K15/30 column containing Dowex 50W X4 (H^+ form, 100~200 mesh). After washing with H_2O the column was eluted with a pyridine - acetate buffer (pH 4.7, 8 ml pyridine and 10 ml HOAc per liter H_2O). In our hands this column failed to resolve this amino acid mixture.

The fractions containing the amino acids (as detected by ninhydrin and *p*-nitrobenzenediazonium fluoborate sprays) were combined and freeze-dried to 0.8 g of solid which was chromatographed over 500 g of an acid-washed Celite partition column using the system EtOAc - 2-BuOH - HOAc - H_2O, 1.5: 0.75: 0.75: 1. Fractions 18 through 20 were combined and concentrated to dryness. The residue was re-constituted in H_2O, Darco treated, filtered and concentrated. Upon standing the concentrate yielded 30 mg of white crystals, mp 252~253°C. $[\alpha]_D^{25}$ $-72\pm4°$ (*c* 0.247, H_2O). This material is about 52% racemized.[12] The EI mass spectrum molecular ion at *m/z* 181 was weak. A large peak at *m/z* 136 ($M^+ - COOH$) was observed. ^1H NMR (D_2O) δ 2.59 (s, $NHCH_3$), 4.54 (s, methine proton), 6.96 and 7.3 (aromatic AB pair J=8.4, 4H). The rotation and spectroscopic data showed the material to be unequivocally *N*-CH_3-*p*-hydroxyphenylglycine (NCH_3-*p*-OH-ϕgly) of the *R* configuration.[6] The NMR curve indicated the presence of a few percent of *p*-hydroxyphenylglycine and also a percent or so of acetic acid. TLC on cellulose plates using the system 3-BuOH - HOAc - H_2O, 2: 1: 1 showed that one of the other two expected amino acids namely *p*-hydroxyphenylglycine (*p*-OH-ϕgly) was not resolved from the N-CH_3 amino acid. Nevertheless, fraction combinations were made to yield a preparation which consisted of 20~25% NCH_3-*p*-OH-ϕgly and the remainder *p*-OH-ϕgly. $[\alpha]_D^{25}$ $+27\pm2°$ (*c* 0.79, H_2O). EI mass spectrum molecular ion at *m/z* 167 was not detected. However, a large *m/z* 122 for [$M^+ - COOH$] was observed as well as a peak at one quarter the intensity at *m/z* 136 for the impurity present. There was also a large peak at *m/z* 107 for $HO\phi CH_2$. ^1H NMR (D_2O) δ 2.60 (s, NCH_3 impurity), 4.58 (1H methine of impurity), 4.73 (methine H) virtually hidden by water peak at 4.80, 6.96 and 7.23 aromatic AB pair of impurity, (these signals were about 1/4 intensity of signals at 7.00 and 7.38 (J=8 Hz) for four aromatic protons of *p*-OH-ϕgly). If we correct for the vitiating optical effect of the (−)NCH_3-*p*-OH-ϕgly, the *p*-OH-ϕgly should exhibit an $[\alpha]_D^{25}$ of +45° which is in fair agreement with the value of −43.5° cited by LOMAKINA *et al.*[6] for the other enantiomer. The 3-chloro-*p*-hydroxyphenylglycine (3-Cl-*p*-OH-ϕgly) proved fairly elusive to isolate in our hands since it oxidized so readily. A yellow freeze-dried preparation of about 20 mg was obtained which had $[\alpha]_D^{25}$ $+32\pm2°$ (*c* 0.59, H_2O). EI mass spectrum molecular ion was not visible but did show a large peak at *m/z* 156 [$M^+ - COOH$] showing the Cl isotope, also a large peak at *m/z* 141 for 3-Cl-$HO\phi CH_2^+$; ^1H NMR (D_2O) showed the presence of 50 mol % of $(CH_3)_2$-$CHCH_2OH$ and 20 mol % of HOAc; methine proton was hidden under water peak, aromatic region was clear-cut, AB pair at 7.12 and 7.52 (J=8 Hz) and proton adjacent to Cl at 7.30 (J=8, 2 Hz). If we correct for impurities, the expected $[\alpha]_D^{25}$ would be +48° which is slightly lower than LOMAKINA's value of −55° for the other enantiomer. Rf values of NCH_3-*p*-OH-ϕgly, 3-Cl-*p*-OH-ϕgly and *p*-OH-ϕgly on cellulose using system 3-BuOH - HOAc - H_2O, 2: 1: 1 are respectively 0.80, 0.75 and 0.65.

Isolation of Terminal Amino Acid from *epi*-Avoparcin

About 8 g of epimerized avoparcin sulfate was processed as described in the previous experiment. From the partition column about 60 mg of chromatographically pure NCH_3-*p*-OH-ϕgly were recovered. mp 257~258°C; $[\alpha]_D^{25}$ $+8\pm1°$ (*c* 0.98, H_2O); ^1H NMR (D_2O) δ 2.58 (NCH_3), 4.53 (methine H), 6.95 and 7.32 (J=8.4 Hz, aromatic 4H). We expected a higher rotation value but evidently the epimerization had not gone to equilibrium. Consequently there was a smaller excess of the *epi* compound. Nevertheless the data unequivocally shows that heating avoparcin overnight at 65°C causes inversion of the configuration of the terminal *p*-hydroxyphenylsarcosine.

Variation of ^1H NMR Chemical Shift of the *N*-Methyl Group of β-Avoparcin with pH

Approximately 25 mg of pure β-avoparcin was dissolved in 0.7 ml of D_2O. The N–CH_3 group of the molecule exhibited the following chemical shifts as the pH was varied. pH 6.0 δ 2.75 fairly intense peak, pH 7.0 δ 2.50 weak peak, pH 8.0 δ 2.50 broad and indistinct, pH 9.0 not visible, pH 10.0 δ 2.32 fairly intense. A plot of the above data indicates a *pKa* at about pH 6.5 for the N–CH_3 group.

Acknowledgments

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