dergo allylic isomerization to the 2,4-dimethyl-3-penten-2-yl radical. The 1,1,3-trimethylallylcarbinyl radical V exists in a symmetric conformation about the C_α–C_β bond similar to the *tert*-amyl radical. The pronounced out-of-phase broadening observed in the esr spectrum of V is attributed to hindered internal rotation about the C_β–C_γ bond. A barrier of 3.5 kcal mol^{-1} has been calculated from the line shapes using the relaxation matrix theory and compared to other literature values. Difficulties in applying the temperature dependence of a_β to obtain barrier heights for alkyl radicals are pointed out.

Experimental Section

Esr Measurements. The modified Varian X-band spectrometer, microwave frequency measurements, light source, and sample tubes are as described previously.[11,20]

To minimize the error in the *g*-value determinations, all measurements were made on spectra recorded on the same day for increasing magnetic field. Perylene cation radical (g = 2.00258)[27] was used as a standard in the configuration employed. The accuracy of the measurements is estimated as ±0.00003. The temperature in the tube was calibrated with a thermocouple and accurate to ±5°.

Materials. 1,1,2,2-Tetramethylcyclopropane obtained from the Chemical Samples Co. was prepared by zinc dust reduction of 2,4-dibromo-2,4-dimethylpentane and contained less than 1% 2,3,3-trimethyl-1-butene as the only detectable impurity by gas chromatography. The nmr spectrum of tetramethylcyclopropane in CCl_4 showed two singlet resonances at δ 0.23 and 1.21 ppm with the relative intensity ratio of 1 : 6, respectively.

4-Bromo-2,4-dimethyl-1-pentene was prepared from 20 g of 4-hydroxy-2,4-dimethyl-1-butene (Chemical Samples Co.) and 8 ml of pyridine in 60 ml of absolute ether. A solution of 6.1 ml of phosphorus tribromide in 20 ml of ether was added dropwise with stirring. The mixture was stirred at room temperature for 4 hr, and then poured onto ice and worked up to afford 12.5 g (40%) of 4-bromo-2,4-dimethyl-1-pentene, bp 30–31° (23 mm).[28]

2,2,3,3-Tetramethylcyclopropanecarboxylic acid was prepared from the copper-catalyzed reaction of tetramethylethylene and ethyl diazoacetate[29] and converted to the *tert*-butyl perester *via* the acid chloride.[30]

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Optical Activity and Conformation of the Cation Carrier X537A

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Abstract: The polycyclic polyether antibiotic X537A exhibits strong optical activity in the presence of several amines and monovalent and divalent metals but weak optical activity when free in aqueous ethanol. In lipid solvents like heptane, X537A again shows optical activity similar to that seen in the presence of a metal ion. Since the two chromophores of particular interest, salicylic acid (~245 and 308 nm) and a ketone (~290 nm), are themselves symmetric, the induced optical activity associated with these absorption bands reflects changes in asymmetry arising from the repositioning of neighboring asymmetric centers. These changes in optical activity can be correlated quite well with the presence of a disordered structure in aqueous ethanol and a cyclic conformation in heptane and in the presence of a complexing cation. Thermodynamic arguments are presented to explain the temperature dependence of the circular dichroism. The changes in circular dichroism of free X537A in heptane seen with increasing temperature are consistent again with the unfolding of a cyclic conformation. The circular dichroism of cation complexes in absolute ethanol become more intense with increasing temperature, the opposite of that seen with free X537A in heptane. This behavior appears to indicate that competition of polar solvent for the polar interior of the cyclic structure diminishes with increasing temperature, thereby leaving a tighter metal ion complex.

Measurements of circular dichroism have facilitated resolution of the solution conformation of a number of cation carriers including gramicidin A,[2,3] enniatin,[4] valinomycin,[4] and antamanide.[5] In each of these molecules changes in the intensities of optically active amide transitions accompanied the complexation of predominantly monovalent cations. In an attempt to extend our knowledge of these lipid soluble ion carriers we investigated the optical activity of the polyether antibiotic X537A, which, unlike the above molecules, also strongly complexes divalent cations.[6] Crystalline structures of the barium and silver salts of X537A are

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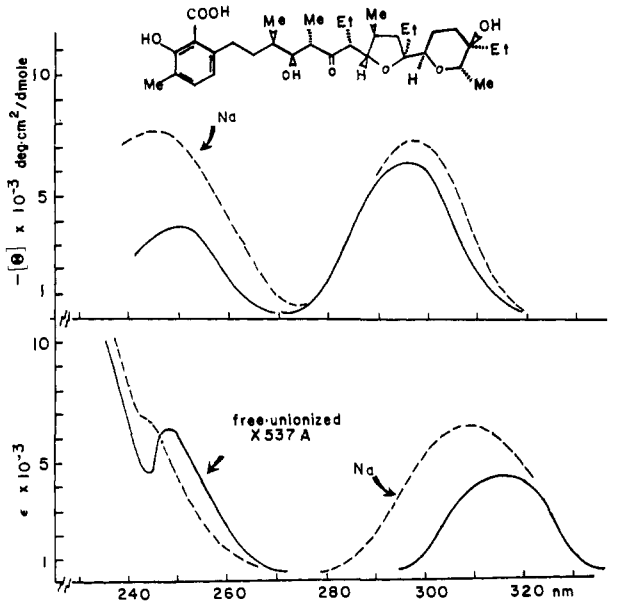

Figure 1. Ultraviolet circular dichroism and absorption of free (protonated) X537A and the sodium complex. Spectra were measured in absolute ethanol. The concentration of Na ion is 2 equiv relative to the amount of X537A. Solid lines denote free X537A; dashed lines are the Na complex. Sodium is unusual in that 2 equiv/equiv of X537A is required to reach maximum ellipticity in the 245- and 295-nm bands. The structure of X537A is given above the spectra. Note that the ordinate in all figures indicating circular dichroism is the negative ellipticity.

known,[7] but we are not aware of any spectroscopic data on the structure and properties of these complexes in solution.

X537A has the primary structure[8] shown in Figure 1. The molecule contains two near-ultraviolet chromophores, the methyl-substituted salicylic acid end group and a ketone. Both chromophores are symmetric. One might expect, therefore, weak optical activity if in solution the molecule is freely flexible. However, X537A and its salts have considerable optical activity in most common solvents. What contribution to the optical activity is the result of a rigid tertiary conformation and what part is due to induced local asymmetry of the chromophores are the subjects of this article. Analysis of these contributions can be expected to give insight into the biological properties associated with the ability of X537A to chelate cations.

Experimental Section

All solvents were spectral grade. Ultraviolet absorption was measured on a Cary 15 spectrophotometer. Measurements of circular dichroism were performed on a Cary 60 spectropolarimeter with a CD attachment. The instrument contained a thermostated cell chamber which was heated and cooled by an external Forma 2095 circulator bath for the temperature-regulated studies. The spectropolarimeter was standardized with an aqueous solution of *d*-10-camphorsulfonic acid (K and K Laboratories, Planview, N. Y.; batch No. 4829), giving $\epsilon_L - \epsilon_R = 2.20 \pm 0.05$ at 290 nm.

Metal salts of X537 were generated by mixing equivalent amounts of metal hydroxide to a solution of X537A in absolute ethanol and were crystallized repeatedly by slow cooling of the saturated solution. Salt-free X537A itself came from a single stock, obtained by extraction of the sodium salt in benzene with 0.1 *N* sulfuric acid. The benzene was then washed repeatedly with doubly distilled water and rotoevaporated; the product was recrystallized from absolute ethanol and vacuum dried. Thus all temperature-regulated studies and metal and amine titrations were made on the same batch of salt-free material.

Alkylammonium hydroxide was purified by two-phase partitioning between water and methylene chloride to remove alkylamines. The aqueous fraction was tapped and partitioned a second time in the same solvents.

Examples of standard deviation of the mean (standard error) for ellipticities of X537A in ethanol and heptane are in Chart I (number of scans in parentheses).

Chart I

	295 nm band	245 nm band
Ethanol	6,070 ± 110 (13)	1,700 ± 300 (13)
Heptane	12,900 ± 130 (9)	17,200 ± 440 (9)

Results

Figure 1 depicts the uv absorption and circular dichroism (CD) of free X537A in ethanol. Effects of other solvents on the CD of free X537A and three salts are listed in Table I.

Table I. Molar Ellipticities of X537A and Salts in Different Solvents[a]

Solvent	295 nm	245 nm
$CHCl_3$	−12,900	−13,200
Heptane	−12,500	−18,000
DMSO	<800	*b*
EtOH (abs)	−6,500	−3,400
EtOH (90%, pH 1.7)	−6,500	0
EtOH (90%)	−7,300	−2,400
EtOH (77%)	−8,850	0
	Na^+ Salt	
Heptane	−20,000	−24,000
EtOH	−8,100	−6,750
	Cs^+ Salt	
Heptane	−24,000	−36,000
EtOH	−12,000	−18,800
	Ba^{2+} Salt	
Heptane	−5,500	−16,000
EtOH	−4,400	−5,500

[a] Measurements at 23°. [b] Solvent extinction too large to measure ellipticity.

The optical activity of either X537A alone or its salts in pure dimethyl sulfoxide (DMSO) exhibits peculiarities not seen with any of the other solvents examined including heptane, ethanol, methanol, and chloroform in that the spectra do not obey Beer's law over the spectral range 310 to 275 nm. This behavior is not surprising, because the greatest deviation from Beer's law is in the region of large changes in DMSO extinction. Large changes in extinction are a common source of spectrophotometric error. We therefore chose to study the effect of DMSO on X537A by serial dilution of an ethanolic solution of X537A with DMSO. The addition of increasing fractions of DMSO to the ethanolic solution of X537A drastically diminishes the molar ellipticity, until at 50:50 DMSO in ethanol the peak ellipticity at 295 nm is less than 10% of that in pure ethanol. Whereas the spectra in pure DMSO do not obey Beer's law, the DMSO:ethanol mixtures do obey Beer's law up to 50:50 DMSO in ethanol. This behavior was shown by free X537A, as well as the Ba, Cs, and Na salts. Even though large ellipticities can be induced in X537A with large excesses of metal ion, free X537A and its salts with 1 equiv of metal ion show

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negligible limiting optical activity, as the DMSO concentration approaches 100%. We therefore consider DMSO unique among the solvents studied in that its effect on X537A optical activity is generally to abolish it. This effect is observed also with the salts of X537A.

In ethanol, free X537A exhibits a band at 295 nm and a weaker and relatively noisy band at 245 nm. The salts of X537A, on the other hand, have a much more intense band at 245 nm. For both X537A and its salts, the peak heights are greater in heptane than in ethanol. Spectra of the salts and the free form of X537A in heptane are very similar to those of the salts of X537A in ethanol.

Since X537A is an acidic chelator, the problem must be considered of distinguishing effects of metal complexation from the effects of deprotonation on the parameter one uses to monitor the extent of complexation. Ionization of the salicylic acid moiety produces shifts in the uv $\pi-\pi^*$ transitions of the ring. Therefore changes in the intrinsic electronic absorption upon deprotonation were measured to assess first of all the magnitude of these ionization effects and to isolate the nonconformational contributions to the optical activity.

In Table II are given the uv intensities of X537A and its salts in various solvents. Clearly, the uv absorption intensities are not strongly dependent either on solvent or on the cation complexed. CD band intensity is strongly dependent on solvent and cation. This observation argues against the possibility that CD changes with complexation result simply from deprotonation of the carboxyl group. The changes that exist in the uv absorption are neither large enough nor consistently in the proper direction to explain the observed CD changes with cation or solvent. The two uv bands of X537A are very similar to those of free salicylic acid (ϵ_{237} 9000 and ϵ_{303} 3600 in 0.1 *N* HCl), and the wavelength shift from 318 to 308 nm, observed on deprotonation, is characteristic of such *o*-hydroxybenzoic acids.

Table II. Uv Molar Extinctions of Maxima of Free X537A and Its Salts[a]

Cation	Solvent	1	1	2	2
H^+	EtOH	318	4440	249	6,430
Cs^+	EtOH	307	3440	243	4,300
Ba^{2+}	EtOH	308	4180	240	4,900
Na^+	EtOH	307	6370	243	6,750
Na^+	Heptane	308	4000	244	3,980
H^+	Heptane	318	4300	249	6,420
Ba^{2+}	Heptane	311	3700	246	4,400
Cs^+	Heptane	310	3270	244	3,160
Cs^+	DMSO-EtOH	308	3350		
Ba^{2+}	DMSO-EtOH	308	4120		

[a] Measured at 23°.

However, the uv absorption maximum occurs at 308 nm, while the corresponding CD band is centered near 295 nm. That the ketone chromophore also contributes to the optical activity near 280 nm with the same sign (−) as the salicylic acid band at 308 nm probably accounts for the observed position of the long-wavelength CD band at 295 nm. This conclusion is supported by our attempts to determine the stoichiometry of metal-carrier complexes. The CD spectrum of the free form of X537A was titrated with soluble cation salts (K^+, Cs^+, Rb^+, Na^+, Ba^{2+}, Mg^{2+}, and Ca^{2+} thiocyanates). Figure 2 depicts the titration plots. The 245- and 295-nm bands increase in parallel for most ions. This behavior probably reflects the significant contribution of the salicylic acid group to the 295-nm peak and the relatively fixed spatial configuration and environment of the ketone moiety under conditions of complexation. Of the solvents studied, DMSO is the one exception to this simple scheme, probably because this solvent unravels the backbone of X537A and permits no complex formation for either free X537A or its salt. Some variation in the simplicity of this interpretation can be appreciated from the observations on the small divalent cations Mg^{2+}, Ca^{2+}, and Sr^{2+} given in Figure 3. These highly charged species appear to interact more strongly with the ring than other cations and to perturb the 295-nm component of the ketone optical activity. Titration with Mg^{2+} causes the ketone band to increase without a concomitant rise in the salicylate peak. Titration with calcium causes the ketone contribution to change sign and become positive, together with a small decrease in the 245-nm band. Whether in this case the isolated salicylate component is also reduced in absolute intensity or is relatively diminished by the oppositely polarized ketone band is not yet clear. Finally, Sr^{2+} causes a large increase in the ketone band with only a small increase in the salicylate band. As indicated by the abrupt plateau of the titration curves, the association constants were so large that the K_A of the cation complexes could not be calculated at the X537A concentrations needed to measure CD accurately.

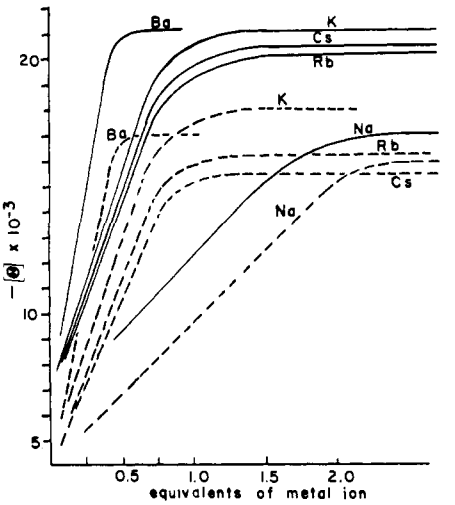

Figure 2. Titration curves of metal ions. Solid lines indicate changes in ellipticity of the 245-nm band. Broken lines denote changes in ellipticity of the 295-nm band. The barium complex reaches a limiting ellipticity near the point of 2 equiv of X537A/equiv of Ba ion. Except for sodium, other monovalent metals approached limiting ellipticities at 1:1 metal ion:X537A.

For the ions K^+, Rb^+, and Cs^+, the titration data show a one to one complex at saturation while Ba^{2+} shows a 2:1 ionophore:ion ratio. The ions Na^+, Sr^{2+}, Mg^{2+}, and Ca^{2+} exhibit nonintegral values of complexation. It is probably significant that these ions represent the lower extreme in size. Whether this observation relates only to their size or not we cannot yet say.

Regarding different X537A conformers in solution, there exists the ambiguity of whether the observed spectra are due to low concentrations of a high specific ellipticity conformer or a high concentration of a less

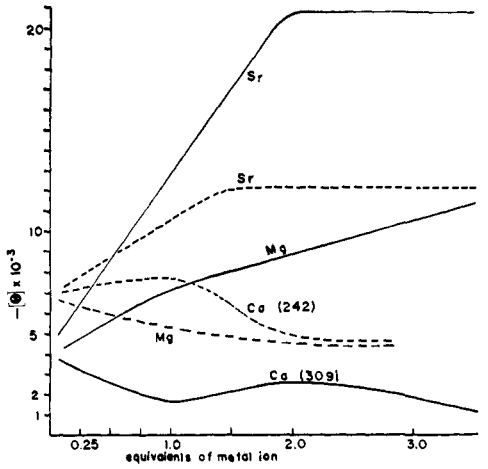

Figure 3. Titration curves of X537A in ethanol with metal ions whose behavior is irregular. Strontium does not form a 1:1 complex. Whether it forms a 2:1 metal ion:X537A complex is uncertain; the behavior of the 295-nm band suggests that a 2:1 metal ion complex is formed, whereas the 245-nm band indicates a point of saturation between 1:1 and 2:1. These differences may represent the presence of multiple species of complexes. Part of the difficulty with the Ca complex is the shape of its spectrum. All metal and amine complexes showed wholly negative CD bands in the 295-nm region except the Ca complex; it showed a biphasic curve. If this curve was resolved, the behavior of the resolved bands might be consistent with the metals of Figure 1. It is not apparent that this is the case, and for the time being we treat Ca as an aberrant complex. At least for the Ca complex the maximum at 242 nm and minimum at 309 nm occur at a 1:1 metal ion:X537A ratio, suggesting that a 1:1 equivalence in the complex is possible but not limiting. Similar ambiguities are apparent in the Mg titrations.

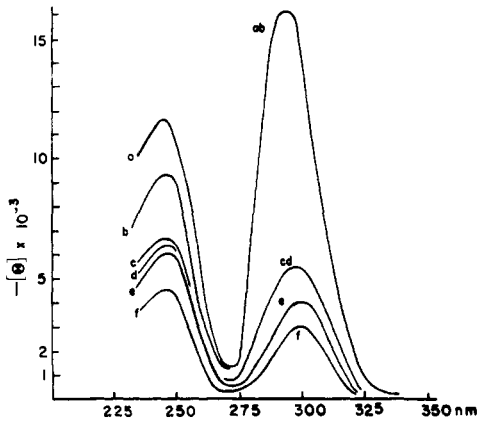

Figure 4. Limiting ellipticities of X537A after saturation with several amines in ethanol: (a) NH_3, (b) tris(hydroxymethyl)aminomethane, (c) tetramethylammonium hydroxide, (d) tetraethylammonium hydroxide, (e) triethylamine, and (f) triethanolamine. Compared with free (protonated) X537A, the 245-nm band appears to indicate varying degrees of complexation in the presence of these amines, since it is altogether abolished in aqueous ethanol and still small in heptane.

active conformer. In an attempt to resolve this issue, as well as determine some of the thermodynamic factors affecting complexation, the circular dichroism of each of the several complexes was studied as a function of solution temperature.

For free X537A in ethanol, the circular dichroism of the 245-nm band diminishes from -2910 ± 610 at $-25°$ to -2500 ± 330 at room temperature and further to -556 ± 290 at 55°. It can be seen in Table

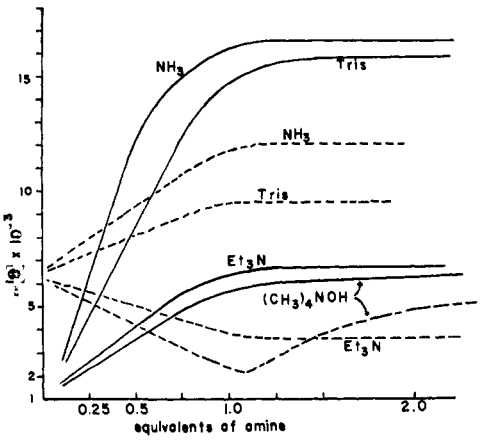

Figure 5. Titration curves of X537A with amines in ethanol. The solid line indicates ellipticities of the 245-nm band. Broken lines represent the 295-nm band. Limiting ellipticities are reached at 1 equiv of amine/equiv of X537A for all cases except tetramethylammonium hydroxide, which exhibits a biphasic curve in the 295-nm band. Even for this case, however, a minimum occurs at 1:1 metal ion:X537A, suggesting that a 1:1 equivalence in the complex is possible but not limiting.

Table III. Temperature Dependence of the CD Spectra of the Salts of X537A in Ethanol

Salt	Na^{+a}	Cs^{+a}	Sr^{+b}	Ba^{2+a}	TEA^{+c}
Ratio[d]	1.37	1.59	2.44	3.00	1.90

[a] Recrystallized salt. [b] Saturated solution with $Sr(ClO_4)_2$. [c] No crystallization, 1 equiv of TEA^+OH^- added to X537A in ethanol. [d] Ratio of the molar ellipticity of the 245-nm peak at 55° to that at −25°. Ratio for uncomplexed X537A is 0.15.

III that the opposite behavior occurs for the cation salts of X537A. The circular dichroism for these cases increases significantly in magnitude as the temperature rises. All of the cation salts measured show this increase with temperature. The largest changes are associated with the ion (Ba) with the most charge. In the Discussion we will adduce the arguments which support the conclusion that solvent entropy is the major driving force for complexation consistent with this behavior.

Considering the suggested entropic driving force for complexation, we can imagine that any positive ion in solution, provided it had enough charge to orient the surrounding solvent molecules, ought to complex with X537A. Several representative amines were titrated into an ethanolic solution of free X537A to test this hypothesis. It can be seen in Figure 4 that these amines caused spectral changes quite like those seen in the titration with inorganic cations.

Ammonia, being small in size and symmetrical, appears to be the best complexing amine. Both bands increase in intensity, until ellipticity levels off at $-11{,}700$ for the 295-nm band and $-16{,}280$ for the 245-nm band, Figure 4. Again, as can be seen from the titration curves, Figure 5, ammonia appears to be a tightly bound substrate. Trishydroxymethylaminomethane forms another very tight complex. Both bands increase to final ellipticity values at $-9{,}500$ and $-15{,}820$ at 295 and 245 nm, respectively, as shown in Figure 4.

To investigate the effect of steric hindrance about the amine, the tertiary amine triethylamine was employed. Again the titration curves, Figure 5, indicate that the

complex is strong. However, the final ellipticity values (−3900 at 295 nm and −6200 at 245 nm) are not large in relation to the primary amines, Figure 5. Similar limiting values were found for triethanolamine.

The sterically hindered tetramethylammonium ion appears also to perturb the ketone region upon complexation, but here the spectra become somewhat more complex. At 1 equiv of ammonium ion per equivalent of X537A the 295-nm band reaches a low value of −2400. With the addition of more tetramethylammonium hydroxide, however, the 295-nm band rises to a saturation value of −5470. The 245-nm band behaves like the case of triethylamine except the terramethylammonium titration rises more gradually, indicating a weaker complex. Ellipticities at final saturation with tetraethylammonium ion are similar: −5240 at 295 nm and −6470 at 245 nm.

In an attempt to determine whether cations other than those from the first two columns of the periodic table could complex with X537A, several transition-metal ions were investigated. It was hoped that if complexation occurred, induced optical activity could be measured in the long-wavelength electronic transitions of the cation itself. It is known that optically active transitions arise in the visible region for the cupric and cobalt ion complexes of α-amino acids. The ions investigated were Cu^{2+}, Ni^{+}, Er^{3+}, Dy^{3+}, Ti^{+}, Mn^{2+}, Na^{+}, Be^{2+}, Co^{2+}, and Ag^{+}. The visible absorption bands associated with these metals were not measurably optically active. Also the signal to noise ratio of the 245-nm band was generally too small to quantitate with any acceptable accuracy. Except for thallium (whose 295-nm band in absolute ethanol has an ellipticity of 11,500 deg cm^2/dmol) the 295-nm bands of X537A were not affected by these ions.

Discussion

The most striking aspect of the CD spectra of X537A is their solvent dependence. It is apparent from Table I that a wide range of spectroscopic and presumably molecular structures can be induced either by suitably chosen cations or solvents. Thus the appearance of the spectrum for free X537A in heptane is very similar to that of the cesium salt of X537A in ethanol. Also, the use of solvents containing greater than ~50% DMSO causes such extensive unraveling of the backbone that there is negligible optical activity in the accessible regions of the spectra for either free X537A or its salts.

There exists an ambiguity in assignment of the CD bands, because none of the chromophores present possesses intrinsic asymmetry. Further, the minima of the CD bands are shifted considerably relative to the maxima of the ultraviolet absorption bands (respectively, 295 *vs.* 308 nm). Such shifts due to vibrational contributions have been reported in other systems but are usually smaller than we see here.[9]

A more likely explanation is that a weak uv band of the ketone group probably contributes optical activity near 280 nm with the same sign (−) as the salicylic band at 308 nm. The π–π^* transitions of doubly substituted ketones occur at 281 nm.[10] The combination of these two bands results in the observed position of the long-wavelength peak at 295 nm. The following observations support this view. Most importantly, the CD of X537A, in which the ketone has been reduced to an alcohol, shows a negative peak at much longer wavelengths, near 311 nm, corresponding to the uv absorption of the salicylic acid group. With some agents (*e.g.*, Ca^{2+}) the CD intensity near 284 nm reverses sign, while maintaining the original negative contributions at 245 nm and above 295 nm. These findings clearly show the existence of two independent bands within the 295-nm band. There are many examples of structurally rigid systems containing ketones which show optical activity in their π–π^* transitions.[11]

We conclude, therefore, that contributions to the 295-nm band arise both from the salicylic acid and ketone groups. The 245-nm band, where ketones have no absorption, appears to reflect purely the asymmetry of the environment of the salicylic acid groups.

Space-filling models show that, when attempting to fold a molecule of X537A, no smaller cyclic conformation is sterically available than a simple head-to-tail loop. This type of head-to-tail cyclization has been observed in studies of dianemycin,[12] monensin,[13] and nigericin.[14] It results in a stable hydrogen bond between the carboxyl and hydroxyl groups at the chain termini. This bonding is most prevalent in nonpolar, nonhydrogen-bonding solvents. The proximity of the salicylic acid chromophore to the asymmetrically disposed oxygen dipoles in the opposite end of the molecule could then adequately explain the induced dichroism in the 245-nm band.

Supporting evidence for head-to-tail cyclization is found in the great sensitivity of the 245 nm to hydrogen-bonding solvents, a feature not seen in the 295-nm band. Hydrogen bonding to solvent in ethanol *vs.* internal hydrogen bonding in heptane would account for a diminished attraction between the two ends of the chain in the former solvent. Correspondingly, the circular dichroism of the 245-nm peak goes from −1700 in absolute ethanol to −17,200 in heptane. It vanishes in 77% aqueous ethanol. The intramolecular hydrogen bond is not between two X537A molecules, because there is no concentration dependence (*i.e.*, no Beer's law deviation) to the circular dichroism and the spectrum is not altered by the addition of excess benzoic acid. When a model is made of this cyclic conformation there is an easily formed ring of oxygen atoms on the interior of the loop. The structural image is that of a basket whose inner edge is lined with upward and inward projecting oxygens and whose outside is very hydrophobic. The hydrophobic exterior and hydrophilic interior have been depicted in ionophores like valinomycin.[15] However, in the special case of X537A the complexed ion is wholly encapsulated but only covered or shielded in one aspect. This partial flexibility and one-sided chelation helps to account for the relatively broad range of cations capable of complexing with X537A.

Along with the data for solvent and temperature

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effects the CD spectrum of protonated (free) X537A with its relatively small 245-nm band and that of the salts whose 245-nm bands are considerably more intense appear to indicate an equilibrium of three conformations, the first of which is a random chain, the second is a head-to-tail loop, and the third, which, though also cyclic, has the entire salicylic acid group directed out of the loop and into the solvent. Thus, in DMSO the optical activity of X537A and its salts is made very weak. In nonpolar solvents like heptane one promotes internal (head-to-tail) hydrogen bonding and stabilization of the carboxyl group with a concomitant formation of the fully cyclic structure. And in ethanol a stronger interaction between the salicylic acid moiety and ethanol draws the salicylic acid out of the cyclic structure, as evidenced by the greatly diminished 245-nm CD band.

The head-to-tail conformation of free X537A is an ordered structure relative to the random coil. One would expect less of this conformation and more of the uncyclized species (and less dichroism) with increasing temperature. As can be seen from Table III, the CD of free X537A does indeed decrease with increasing temperature. However, for the cation salts of X537A the opposite effect occurs, increasing circular dichroism with increasing temperature.

The phenomenon can be explained by the following reasoning. When the cation undergoes complexation, the solvating molecules (ethanol), which interact *via* a charge-dipole mechanism, are replaced by the dipoles of X537A, which also originate from oxygen atoms bound to aliphatic groups and are of the same order of magnitude. The difference in being solvated by ethanolic dipoles on one side of the cation and by the five oxygen dipoles of X537A in the complex is probably very small relative to the internal energy of the system. Thus, although the sign of ΔH or enthalpy of complexation is most probably positive, it is small in magnitude. The entropy term for complexation, on the other hand, ought to be large and positive. When the cation is solvated by ethanol, the solvent molecules in the first hydration sphere, and to a lesser extent farther out, are spatially limited in their motions. Upon complexation these molecules are freed and can then randomize. Thus, for a small and positive ΔH term we have for the complexation constant

$$K = \frac{[\text{complex}]}{[\text{uncomplexed}]} = \exp(-\Delta G/RT) = \exp[(-\Delta H + T\Delta S)/RT]$$

K is greater than 1 ($T\Delta S > \Delta H$) and increases with increasing temperature. Said another way, the energy of interaction of complexed X537A with the cation remains unaffected by increasing temperature, while the solvent is driven to more disorder, as does uncomplexed X537A.

Complexation with Amines

Considering the half-shell nature proposed for the solution conformation of X537A and the suggested entropic driving force for complexation, we anticipated that any positive ion in solution, provided it had strong enough charge density to orient the surrounding solvent molecules, ought to complex with X537A. Amines, which are cationic at neutral pH, were used to test this hypothesis, and as can be seen from Figure 4 these amines caused spectral changes quite like those occurring with the titration of cations. Ammonia, being small in size and symmetrical, is the ablest complexing amine, Figure 5. Further, since it is proposed that X537A covers only one side of the cation being complexed, one alkyl substituent on the amine should not obstruct its complexation. An example of a primary amine with steric bulk on one side of the amine is "Tris" (tris(hydroxymethyl)aminomethane). Here again, the complex is very tight and both bands increase (Figure 5).

The tertiary amine, triethylamine, was used to investigate the effect of steric hindrance about the amine. Here again the titration curve indicates a relatively strong complex but not as strong as the primary amines (Figure 5), probably because the steric bulk of triethylamine does not allow the salicyclic acid group to approach the hydroxyl tail. There is also a perturbation of the mid-chain conformation, as can be seen from the decrease of the 295-nm band. Similar limiting values were found for triethanolamine ($N(CH_2CH_2OH)_3$).

The sterically hindered tetramethylammonium ion also appears to perturb the loop structure, but here the spectra are more complicated. The 245-nm band behaves like the triethylamine case, with a somewhat more gradual increase after the addition of 1 equiv. Like the triethylamine case, a final value for the 245-nm band of only -6800 suggests that the steric bulk of tetramethylammonium ion prevents full loop formation. Distortion of the midchain conformation (as measured by diminution of ketone optical activity at 295 nm) might also be expected to be extensive in this sterically hindered amine. The 295-nm CD band reaches a low value of -2400 at 1 equiv of tetramethylammonium ion, Figure 5.

The driving force for complexation of these alkylammonium groups in ethanol is more than charge-dipole stabilization of the cation complex. It probably involves release of ordered solvent during complexation. The tetraalkylammonium ions are already solvated in solution, and the same diminution of charge-dipole interaction between those ions and the X537A oxygens, because of steric bulk, is also present in the solvation in ethanol of the alkylammonium ion itself. Since the driving force for complexation is due to the difference in charge-dipole stabilization and solvent disorder in going from solvent solvation to X537A complexation, steric bulk ought to affect the ability of both systems to form charge dipoles similarly with little resultant effect on the equilibrium constant. Nevertheless, the entropic driving force persists and explains the favorable equilibrium toward tetraalkylammonium complexes.

Analysis of the effects of binding tetramethylammonium ion depends partially on the fact that deprotonation of X537A does not cause marked CD or absorption changes (other than a 10-Å shift of the long-wavelength uv absorption band from 318 to 308 nm). Other expeiments reinforce this view. Addition of 0.2 equiv of tetramethylammonium hydroxide[16] to X537A in heptane *reduces* both bands, the 295 band by 8% and the 245 band by 16%; a similar drop occurs in the 245-nm band of X537A in chloroform upon addition of excess tetramethylammonium hydroxide (from $-15,200$ to $-8,450$). This behavior would be consistent with our previous interpretation that the salicylic acid group and

(16) No more could be added due to solubility problems.

the other end of the molecule are drawn closer together by hydrogen bonding in the free form than when complexing the sterically hindered tetramethylammonium ion. For deprotonation alone to explain the effect of addition of tetramethylammonium hydroxide to an ethanol solution of X537A, one would have expected a rise in the 245-nm peak in nonpolar solvents as is observed when ammonia is added to X537A in heptane.

Addition of excess pyridine did not affect the ellipticity of X537A in ethanol. However, based on the relative pK's, an equilibrium between pyridine and X537A ought to favor deprotonation of the carboxyl and formation of the pyridine ion by a factor of at least 100. Whatever the reason, there is no apparent complexation. This appears to be a case of deprotonation without complexation and there is no detectable change in the circular dichroism.

It must be remembered that though tetramethylammonium hydroxide complexes with X537A, the point of equilibrium ought to be driven forward in ethanol, by the stability of the by-product, water. The converse can hold as well; if in ethanol one adds the stable salt, tetramethylammonium thiocyanate,[17] to X537A, no evidence of complexation occurs. The combination of HSCN and $CO_2^-TMA^+$ must have a higher free energy than CO_2H and TMA^+SCN^-. In general, then, complexation with X537A as we have discussed up to now has two aspects: (1) ionization of salicylic acid and (2) liganding to one or more carbonyl or ether groups. Complex formation, per se, apart from the additional phenomenon of carboxylate formation, could therefore be better assessed by titrating the metal ion in the presence of 1 equiv of tetramethylammonium hydroxide in order to remove any spectral contribution associated with carboxyl ionization or with changes in the cation equilibrium. It is for this reason and the insolubility of metal hydroxide that the metal titrations were done with metal thiocyanates on solutions of X537A which were neutralized with 1 equiv of tetramethylammonium hydroxide, Table IV.

As noted in the Discussion, attempts to observe optical activity of transition metal ions in the visible region failed. Outside the case of thallium, mentioned in the Results, one other exceptional case is that of the silver salt, which in heptane shows the values of −8200 and −7750, whereas free X537A has values of −13,000 and −16,000. The crystal structure of the silver salt of X537A is known to be quite unlike that of the barium salt in conformation and contains direct aromatic π-electron interactions with silver.

In summary, the optical activity of free X537A is abolished by polar solvents and enhanced by lipid solvents like heptane. Moreover, the enhanced optical activity in heptane appears to reflect folding of the chain into a cyclic conformation, wherein intrachain hydrogen bonding contributes to stabilization of the cyclic structure concomitantly showing a hydrophobic exterior to the solvent. A similar conformation is likely in the presence of mono- and divalent metal ion, amines, and alkylammonium ion even in the presence of polar solvents like ethanol, because ion complexation contributes to stabilization of the circular structure by liganding with carbonyl moieties distributed along the chain. This is reinforced by the high ellipticities in solvents where only intrachain hydrogen bonding is possible along with the absence of significant optical activity in disruptive solvents like DMSO. Further, the dependence of the circular dichroism with temperature is strong evidence for the existence of a particular cyclic structure, and it is that dependence which suggests the thermodynamic agruments for this model. The decrease in circular dichroism of free X537A in absolute ethanol, seen with increasing temperature, is consistent with the unfolding of a cyclic conformation. The CD of cation complexes in absolute ethanol intensifies with increasing temperature, the opposite of that seen with free X537A in absolute ethanol. This behavior indicates a competition between polar solvent and the polar interior of the cyclic structure for the cation, a competition which diminishes with the increasing temperature, thereby leaving a tighter cation complex and enhanced optical activity.

Table IV. Molar Ellipticities of X537A and Cation in Ethanol in the Presence of 1 Equiv of TMA^+OH^- [a]

Salt	295 nm	245 nm	284 nm
Na^+	−16,900	−16,900	
K^+	−18,200	−20,300	
Cs^+	−14,400	−20,300	
Rb^+	−16,900	−20,300	
Ca^{2+}	−300	−4,300	+3,100
Ba^{2+}	−17,100	−20,600	
Sr^{2+}	−21,000	−11,900	
Mg^{2+}	−23,000	−4,500	

[a] Measured at 23°.

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(17) Since metal hydroxides were not adequately soluble in ethanol even in the presence of X537A, the metal thiocyanates were used. To be consistent we also used tetramethylammonium thiocyanate.