Hydroxyl Deformation Frequencies as a Probe of Hydrogen Bonding in Lasalocid A (X-537A) and Its Sodium Complex

George D. J. Phillies*[1a] and H. Eugene Stanley[1b]

Contribution from the Harvard-Massachusetts Institute of Technology Program in Health Sciences and Technology, Cambridge, Massachusetts 02139. *Received October 10, 1975*

Abstract: The hydroxyl deformation frequencies (900–1500 cm^{-1}) of lasalocid A, its sodium complex, and their deuterated derivatives were studied with Raman spectroscopy. The secondary and tertiary hydroxyl groups of lasalocid A give rise to spectral lines near 1425, 1303, 1172, and 738 cm^{-1}, typical of hydrogen-bonded hydroxyl groups. In the lasalocid A–sodium complex, these are replaced by peaks at 1382, 1200–1250 cm^{-1}, which are not typical of either free or hydrogen-bonded secondary or tertiary hydroxyl residues. The lack of splitting of the 1382-cm^{-1} line is interpreted to indicate the absence of hydrogen bonding of the aliphatic hydroxyl groups in the lasalocid A–sodium complex, implying that cation–oxygen interactions rather than hydrogen bonds are primarily responsible for stabilizing the observed molecular conformations. This result is not consistent with the conventional interpretation of x-ray diffraction data of Johnson et al. on the Ag^+ and Ba^{2+} complexes of lasalocid A, which have been interpreted as indicating that in these complexes the lasalocid A hydroxyl groups are hydrogen bonded.

I. Introduction

Lasalocid A or X-537A[2a] (Figure 1a) is a carboxylic ionophorous antibiotic related to nigericin and monensin. Such antibiotics owe their biological activity to an ability to bind cations, thereby forming electrostatically neutral lipid-soluble complexes. This electrostatic neutrality is in contrast to the macrocyclic antibiotics (e.g., enniatin B, nonactin, valinomycin) which are innately neutral and thus form charged cation complexes.[2b]

The crystalline structures of lasalocid A[3] and its Ag^+ and Ba^{2+} complexes[4,5] have been determined by means of x-ray crystallography. Lasalocid A is composed of a salicylic acid residue (which ionizes during complex formation) and a long hydrocarbon backbone containing ketone, hydroxyl, and ether groups. Comparison of the structures of lasalocid A and derivatives with those of other members of the nigericin family reveals several common features (Figure 1b):[2]

(1) The carboxylic antibiotics and their complexes are linear chains bent into loops. The x-ray structures have been interpreted as indicating that hydrogen bonds connect the two ends of the molecule (Figure 1a, 1b), thereby stabilizing the molecule in the "loop" conformation.

(2) The cation is found in the center of the antibiotic molecule, liganded to the oxygen atoms of the antibiotic. The cation is thus located in a hydrophilic environment, shielded from contact with solvent by the hydrophobic exterior of the antibiotic. However, because of the long-range nature of electrostatic interactions, the antibiotic molecule will reduce but not prevent interactions between a bound cation and a polar solvent. The analysis of Friedman and Degani[6] of thermodynamic data on alkali ion complexes of lasalocid A in hexane and in methanol indicate that such an interaction is present.

(3) The stoichiometry of the crystal unit depends on the cation. For Ba^{2+}, $Ba(X\text{-}537A)_2$ is found, while for Ag^+, $Ag_2(X\text{-}537A)_2$ was reported.[4,5]

The presence of hydrogen bonds in the crystal was inferred from close approaches seen in the x-ray data between neighboring oxygen atoms. Johnson et al.[5] point out that the ^{31}O–^{26}O and ^{40}O–^{27}O hydrogen bonds of the Ba^{2+} complex are unusual in that they are along edges of a polyhedron of oxygen atoms which are liganded to a central cation, hydrogen bonds not generally being formed in such positions.[7]

Degani and Friedman[6] have used circular dichroism and fluorescence spectroscopy to study stoichiometries, formation constants, and conformations of lasalocid A and many of its cation complexes in a variety of solvents. These workers found (for lasalocid A concentrations of 10^{-3}–10^{-5} M) that for an ion M^+ the dominant complex was MX, where XH and X^- denote lasalocid A in the free acid and ionized forms, respectively. They also obtained evidence for conformations of the form MXH^+.

We have used Raman spectroscopy to study hydrogen bonding in crystalline lasalocid A and its sodium complex. A complementary study on CCl_4 and CH_3OH solutions gives information on the conformation of lasalocid A in polar and nonpolar solvents. Measurements focus on the region 900–1800 cm^{-1}, which contains the hydroxyl deformation frequencies. Our interpretation of these frequencies is based primarily on the infrared studies of Stuart and Sutherland[8] and Krimm et al.,[9] who studied a variety of primary, secondary, and tertiary alcohols as pure liquids, in dilute, nonpolar solution, and in the gas phase.

II. Methods and Materials

Raman spectra were measured using a SPEX Ramalog 4 double grating monochromator with cryogenically cooled GaAs photomultiplier tube; a Spectra-Physics Model 164-03 argon ion laser was used to illuminate the sample. Laser plasma lines were eliminated with a Claasen filter. The polarization vector of the incident light was perpendicular to the scattering plane: a polarization scrambler was permanently mounted in front of the entrance slit of the monochromator. Most measurements were made using the 4579-Å laser line (maximum power, 100 mW); checks were made using the 4880- and 5145-Å lines. Samples were held in Kimax glass capillaries (inner diameter, 1.7 mm) mounted perpendicular to the scattering plane.

The samples of lasalocid A were the generous gifts of Dr. Julius Berger (Hoffmann-La Roche, Inc., Nutley, N.J.), Professor Harold Friedman (State University of New York at Stony Brook), and Dr. Dinshaw Patel (Bell Telephone Laboratories, Murray Hill, N.J.).

Partial deuterations of lasalocid A and its Na^+ complex were accomplished by dissolution in CH_3OD, followed by equilibration for 48 h at room temperature and slow recrystallization at 4 °C. Salicylic acid derivatives were prepared from reagant grade salicylic acid and lithium hydroxide. Lithium salicylate was deuterated by exchange against D_2O; deuterated salicylic acid was prepared by precipitation of the lithium salicylate from D_2O with DCl, followed by rinse of the precipitate with D_2O. Concentrations for the solution studies were: lasolocid A/CCl_4: ~0.08 M; lasalocid A/CH_3OH: saturated solution (<0.1 M); Na^+ lasalocid A/CCl_4: saturated solution (<0.1 M); Na^+ lasalocid A/CH_3OH: ~0.08 M.

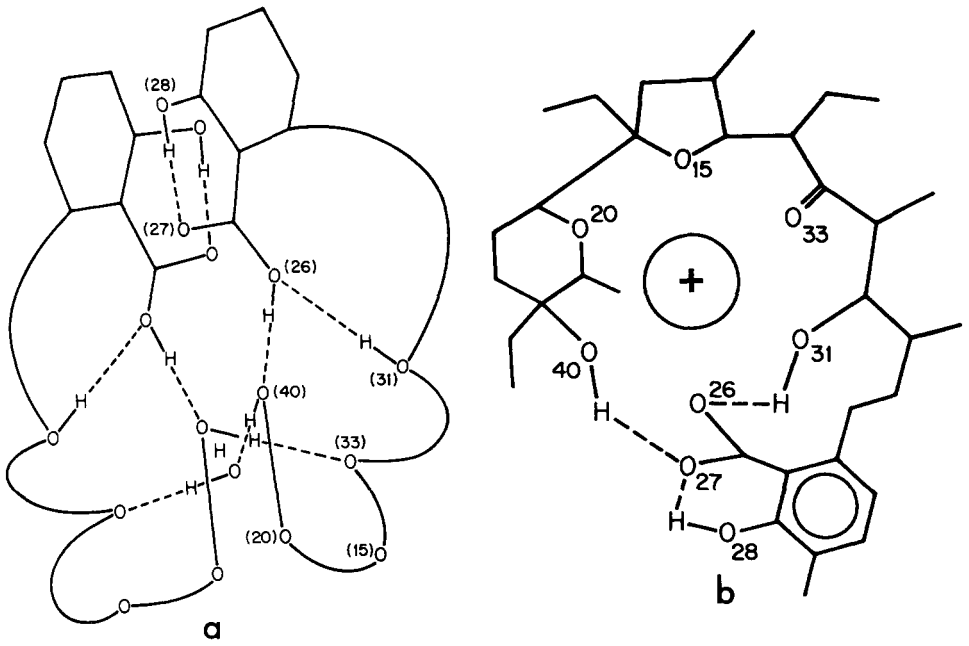

Figure 1. Chemical structure of (a) lasalocid A and (b) its cation complex (as inferred from ref 4-6), showing (dashed lines) the hydrogen bonding patterns inferred from the x-ray diffraction data (redrawn from ref 2).

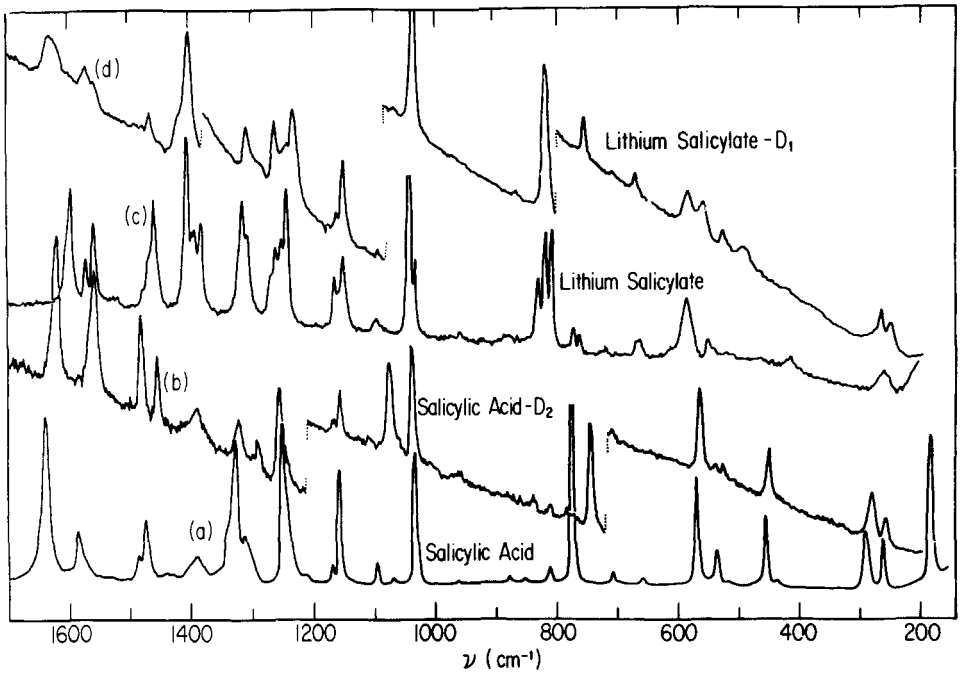

Figure 2. Raman spectra of salicylic acid, deuterated salicylic acid, lithium salicylate, and deuterated lithium salicylate in the range 200-1700 cm^{-1} at a resolution of 5 cm^{-1}. Spectra were obtained using 20-50 mW of laser power with a scanning speed of 0.5 cm^{-1}/s.

III. Interpretation of Spectra

A. General Remarks. Figures 2-4 compare spectra of lasalocid A, its Na^+ complex, and several salicylic acid derivatives with their deuterated analogues; line frequencies and our assignments are presented in Tables I and II. Following Degani and Friedman, the pure acid form of lasalocid A, its deuterated analogue, the sodium complex of lasalocid A, and its deuterated analogue will henceforth be referred to as XH, DXD, NaX, and NaDX, respectively.

Some spectral lines may be identified immediately by comparison with known group frequencies. The 1712-cm^{-1} line of lasalocid A may be assigned to the ketone carbonyl stretch mode. Similarly, the intense lines in the 1440-1470-cm^{-1} region are readily assigned to methylene bending modes.

Identification of many spectral lines of lasalocid A, especially those localized in its salicylic acid residue, is greatly facilitated by comparison with pure salicylic acid and its derivatives. The peaks in the 1550-1660-cm^{-1} region of the salicylic acid derivatives (Figure 2) and of the lasalocid A derivatives can be identified with carboxyl stretch and aromatic ring vibrations. The 1652-cm^{-1} peak of XH shifts to 1643 cm^{-1} in DXD and disappears entirely in the Na^+ complex; it corresponds to carboxylic acid C=O stretch. The 1638-cm^{-1} peak of the salicylic acid derivatives is similarly reduced in frequency by deuteration and also disappears completely in the lithium salt. Weltner[10] has previously observed that the carbonyl stretch frequencies of simple organic acids are not affected by deuteration if they are in the monomer form. That the 1652-cm^{-1} peak is sensitive to deuteration indicates that

Table I. Raman Lines and Their Assignments for the 900–1800-cm^{-1} Region of the Spectrum of Lasalocid A and Its Derivatives[a]

Acid form	Deuterated acid	Sodium salt	Deuterated sodium salt	
906 sat	908	909	908	
	937 sat	935	935	
		949	948	
946				CO_2H out of plane deformation
960				
	959	959	958	
986 sh		974	970	
1009 sh		1009 sat	1008 sh	
1025 sh		1022 sh	1018	
			1038 sh	
1043	1043 brd	1046	1048	
		1073 sh	1077 sat	
		1084 sh	1084	Aliphatic secondary or tertiary OH stretch
1095 asym	1092 asym	1097	1097	
			1106 sat	
1129	(1128)	(1136)	1134	
		1142		
(1146 sh)	(1150)		1150	
(1157 sh)		1158	1158	
	1163			
1172				−OH···O deformation
1183	1182	1183 sh	1181	
	1196 sat	1191	1199	
		1208		
	1240			OD···O or CO_2D deformation
		1238 sh		OH (free?) deformation
		1250 brd		
1249	1251		1242	
			1257	
	1263 sat			CO_2D or OD···O deformation
		1282	1284	
	1299	1295	1297	
	1319	1321 asym	1321	
1303				CO_2H and OH···O deformation
1322				
		1348 wk	1346	
		1366 sh	1369 brd	CO_2 antisym stretch
		1382		OH(free?) deformation
1379	1382			
	1402		(1397)	
1425 sat				−OH···O deformation
1447 sh	1445 sh	1442	1442	
			1451	CH_2 bend
1464	1460	1461	1460	
		1598	1597	CO_2 sym stretch
1617	1600	1622		salicylate ring deformation
	1643			CO_2H deformation
1652				
1712	1713	1713	1713	Ketone carbonyl stretch

[a] Abbreviations include: sh, shoulder; sat, satellite; brd, broad; wk, weak; sym, symmetric; asym, asymmetric.

the carboxylic acid groups of XH are hydrogen bonded (though not necessarily to each other). In NaX and NaDX, peaks are seen near 1622 and 1590 cm^{-1}; the 1590-cm^{-1} peak is at an appropriate frequency for the carboxylate anion asymmetric stretch vibration. The peaks of lasalocid A and salicylic acid derivatives near 1620 and 1560–1585 cm^{-1}, respectively, may reasonably be interpreted as aromatic ring vibrations.[11] The observed difference in frequency presumably arises from changes in the loading of the aromatic ring in the different compounds.

B. Hydroxyl and Carboxyl Deformation Frequencies. Identification of the carboxyl deformation vibrations (1300–1450 cm^{-1}) of salicylic acid greatly facilitates identification of the corresponding lines of lasalocid A. Salicylic acid shows a doublet at 1326, 1340 cm^{-1} which disappears both in lithium salicylate and in deuterated salicylic acid. New doublets appear near 1250 and 1390 cm^{-1} in lithium salicylate, while new lines appear at 1290 and 1075 cm^{-1} in deuterated salicylic acid. The 1326, 1340 cm^{-1} peaks of salicylic acid, thus, presumably represent the CO_2H deformation. (If the carboxyl groups in adjoining salicylic acid molecules formed head-to-head dimers, the deformation vibration might be expected to be seen as a doublet near 1390, 1320 cm^{-1}, as is seen in other organic acids in dimer form.[12] The 1390-cm^{-1} peak of lithium salicylate represents carboxylate anion symmetric stretch; the 1290, 1075-cm^{-1} lines of deuterated salicylic acid are comparable in frequency with the 1320–1390- and 1055-cm^{-1} lines observed in other deuterated acids in monomer form.[12]

By comparison with the salicylic acid derivatives, in which

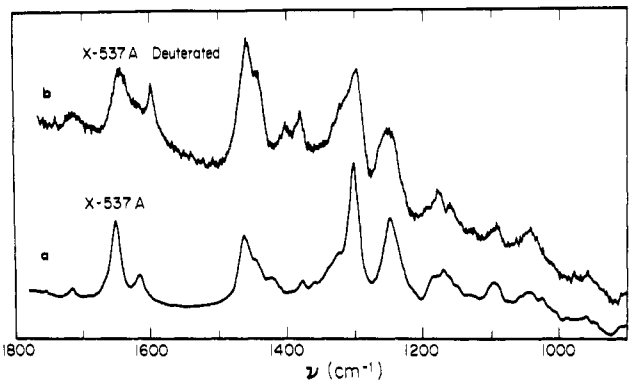

Figure 3. Raman spectra of (a) lasalocid A and (b) its deuterated derivative in the range 900–1800 cm^{-1} at a resolution of (a) 5 cm^{-1}, (b) 5 cm^{-1} using a scanning speed of (a) 0.5 cm^{-1}/s, (b) 0.5 cm^{-1}/s and an (a) 40 mW 4579 Å, (b) 70 mW 4579 Å exciting line.

Table II. Raman Spectral Lines of Lasalocid A and Its Sodium Salt in Methanol and Carbon Tetrachloride, for Frequencies in the Ranges 900–1800 and (for the Free Acid Form in CCl_4) 3400–3700 cm^{-1} [a]

Free acid		Sodium salt	
CCl_4	CH_3OH	CCl_4	CH_3OH
	901		900 brd
941			958
962			
991 wk			
1010			
1042 brd		1050	
1094		1095	
1130			
1147 sh		1159	
1178 brd	1175	(1178)	1180
		1194	(1191)
1246	1250	1240	1242
		1256	1256
		1287	1285
1305	1303		
1327			1323
		1373	1371
1383	1382	1384	1387
1421			
1444		1445	
1461		1455	
1616	1617		
1656	1655		
3593			
3647			

[a] Abbreviations and line assignments as per Table I. Gaps in table reflect obscurations by solvent lines and limits on lasalocid A line intensities due to solubility limits.

the carboxyl deformation frequency was found at 1326, 1340 cm^{-1}, the 1300–1327-cm^{-1} structure of XH is interpreted to include the carboxyl in-plane deformation vibration. While the intensity of the 1303-cm^{-1} peak is reduced approximately 50% by deuteration, substantial activity remains in this region, which disappears on formation of the Na^+ complex.

Comparison of XH, DXD, and NaX spectra allow identification of the 940–961-cm^{-1} peaks with the carboxylic acid out-of-plane deformation vibration. That activity remains near 941–960 and 1303–1327 cm^{-1} after deuteration, but disappears from these regions when the sodium complex is formed, may indicate that the carboxylic acid protons of lasalocid A only exchange partially against CH_3OD under our conditions. It is noteworthy that deuteration of XH creates no new peaks

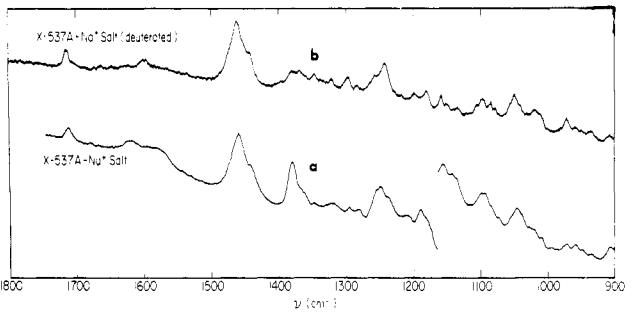

Figure 4. Raman spectra of (a) lasalocid A-sodium salt and (b) its deuterated derivative at a resolution of (a) 5.0 cm^{-1}, (b) 2.5 cm^{-1} using a scanning speed of (a) 0.1 cm^{-1}/s, (b) 0.5 cm^{-1}/s and an (a) 40 mW 4579 Å, (b) 60 mW 4880 Å exciting line.

in the 1050–1100-cm^{-1}-region, even though deuteration of salicylic acid produces a new peak near 1075 cm^{-1}.

The hydroxyl deformation frequencies of XH are readily identified by comparison with DXD and NaX. Deuteration of XH eliminates the 738-, 1172-, 1425-cm^{-1} peaks and decreases the intensity of the 1303-cm^{-1} peak by 50%; in the deuterated spectra new peaks appear near 460, 1263, 1382, and 1402 cm^{-1}. Stuart and Sutherland[8] and Krimm et al.[9] have shown that hydrogen-bonded primary and secondary alcohols have peaks near 670, 1110, 1330, and 1410 cm^{-1}, which on deuteration are replaced by lines near 470, 930–960, and 1225 cm^{-1}. Hydrogen-bonded tertiary alcohols give lines near 670, 1155–1195, and 1410 cm^{-1}. Comparison of our results with those of ref 8 and 9 indicates that the 738-, 1172-, 1303-, and 1425-cm^{-1} peaks of XH include vibrations of hydrogen-bonded secondary and tertiary hydroxyl groups (presumably those at ^{31}O, ^{40}O). The salicylic hydroxyl group deformation line is apparently too weak to be seen as a separate line.

Comparison of XH with its sodium salt shows an extensive series of differences in the 700–1500-cm^{-1} region. The 738-, 1172-, and 1425-cm^{-1} peaks of XH disappear, as does the intense activity near 1303–1320 cm^{-1}, while new peaks appear in the 1190–1250-cm^{-1} region and near 1366, 1382 cm^{-1}. Deuteration of the Na^+ complex abolishes the 1382-cm^{-1} peak and part of the 1190–1250-cm^{-1} band. The 1366-cm^{-1} peak of NaDX (seen as a shoulder in NaX) is reasonably identified as the carboxylate anion symmetric stretch. From their response to deuteration, the 1382- and 1190–1250-cm^{-1} bands of NaX may be identified with hydroxyl deformation frequencies.

Stuart et al.[8] and Krimm et al.[9] do not indicate 1250 or 1382 cm^{-1} as typical frequencies for either free or hydrogen-bonded aliphatic secondary or tertiary alcohols. The 1382-cm^{-1} line is midway between frequencies appropriate for free (1315–1330 cm^{-1}) or hydrogen-bonded (1410 cm^{-1}) alcohol groups. However, Stuart and Sutherland[8] found that a free secondary hydroxyl group gives a single line in this region, while a hydrogen-bonded secondary hydroxyl group gives rise to a pair of lines. That the deformation line is not split (one frequency at 1382 cm^{-1} rather than a pair near 1320, 1410 cm^{-1}) suggests that normal hydrogen bonds are not present. Indeed, the interpretation of the data of ref 8 and 9 by Colthup et al.[11] is that 1382 cm^{-1} should be taken as a standard group frequency for the deformation vibration of a *free* aliphatic hydroxyl group. Coordination of the ^{33}O and ^{40}O by the Na^+ cation could explain why the observed frequencies (1252, 1382 cm^{-1}) may not entirely match those usually found for hydroxyl deformation vibrations.

The 3300–3500-cm^{-1} region of both the free acid and sodium complex was examined for O–H···O stretch vibrations, as these would provide more direct evidence for the presence or absence of hydrogen bonds. However, while the O–H···O

stretch line is very strong in the infrared, it is only weakly Raman active; this weakness combined with the substantial width expected for this line and the high level of background luminescence prevented observation of this line.

C. Solution Spectra. Spectra of XH and NaX dissolved in CH_3OH and CCl_4 are remarkably similar to the crystal spectra; solubility limits (especially for XH in CH_3OH and for NaX in CCl_4) reduced the visibility of weaker peaks. The ketone stretch peak near 1712 cm^{-1} shifts by a few cm^{-1} in these solvents, indicating that there is some interaction between ketone group and solvent molecules (perhaps only a long-range interaction depending on the dielectric constant of the solvent). The XH ketone group evidently does not form hydrogen bonds with the methanol molecules, since such bonds would reduce the C=O stretch frequency to approximately 1695 cm^{-1}. Further evidence that the hydrogen bonding pattern of XH and NaX is retained in solution is provided by the continued presence of the 1178-, 1303-, 1327-, and 1421-cm^{-1} peaks of XH and the 1386-cm^{-1} line of NaX in solution. Indeed, by comparison with the 1461-cm^{-1} peak, the relative intensities of the 1178-, 1303-, 1327-, and 1421-cm^{-1} peaks of XH in CCl_4 are enhanced over their intensities in the crystal.

A 0.04 M solution of XH in CCl_4 shows features at 3593, 3647 cm^{-1}, indicating the presence of some free hydroxyl groups. Crystalline XH does not have spectral lines at these frequencies. Therefore, in the CCl_4 solution some of the hydrogen bonds of XH must be broken, implying either a dimer–monomer equilibrium or a partial unfolding of the crystalline dimer structure. The continued presence of the hydroxyl deformation frequencies near 1178, 1303, 1327, and 1421 cm^{-1} shows that many of the hydroxyl groups are still hydrogen-bonded, suggesting that the free hydroxyl groups represent a minority conformation. Since some hydrogen bonds must be broken in order for the reaction XH → NaX to proceed, this minority conformation may still be of appreciable chemical importance.

IV. Conclusions

Raman spectra of the crystalline free acid form of lasalocid A support the hydrogen-bonding pattern predicted from x-ray diffraction data. In the nonpolar solvent CCl_4, this pattern is basically retained. However, weak spectral features near 3600 cm^{-1} indicate the presence of some free hydroxyl groups, as might arise from the presence in CCl_4 solution of partially unfolded dimers. The spectrum of lasalocid A dissolved in the polar solvent methanol is little changed, so far as can be seen, from the crystal. The methanol hydroxyl peak obscures the 3600-cm^{-1} region. This lack of change suggests that the dimer structure is still retained in this solvent, since in monomeric form the ketone group (^{33}O) might be able to accept hydrogen bonds, which would substantially change its stretch frequency. Further, the monomer → dimer transition might be expected to change the hydrogen bonding pattern, but no changes are seen in the sensitive hydroxyl deformation frequencies.

Degani and Friedman[6] interpreted their circular dichroism spectra of XH and NaX in terms of three conformations I, II, and II. They found conformation III to be the dominant conformation of XH in hexane, III and I (in that order) to be the most important conformations of NaX in methanol, and I and II (in that order) to be the important conformations of XH in methanol. They proposed that I, II, and III differ in the degree of hydrogen bonding, with I being an open-chain conformation with one internal hydrogen bond (^{27}O–$H^{28}O$), II being a conformation with two internal hydrogen bonds (^{27}O–$H^{28}O$, ^{26}O–$H^{31}O$), and III being the ring conformation with a head-to-tail (^{27}O–$H^{40}O$) hydrogen bond in addition to the other two hydrogen bonds. However, this particular interpretation of the different conformations was not central to their identification of the specific conformations seen in different solvents.

The apparent lack of consistency of our results and those of Degani and Friedman should not be overstated. Free hydroxyl groups gives rise to a spectral line (albeit a weak one) in an elsewise vacant region of the spectrum; with a favorable signal-to-noise ratio, it is probably easier to use Raman spectroscopy to identify small numbers of free hydroxyl groups than it would be to identify small amounts of conformations I or II in the circular dichroism spectra. That we see primarily the same conformation (presumably III) in methanol that we see in CCl_4 and in the solid state, while Degani and Friedman report that new conformations (I, II) are present, may be caused by the much higher concentrations (10^{-1} M as opposed to 10^{-4} M) which we used. Our higher concentrations would favor formation of the dimer $(XH)_2$, while their lower concentrations would favor formation of the monomer XH.

The hydrogen bonds proposed for the Ag^+ and Ba^{2+} complexes on the basis of close approaches between neighboring oxygen atoms were previously known to be unusual.[4,5] Our study of the Na^+ complex of lasalocid A indicates that the $O^{31}H$ and $O^{40}H$ groups do not form normal hydrogen bonds; indeed, the lack of splitting of the 1382-cm^{-1} hydroxyl deformation line of the Na^+ complex is more consistent with an absence of hydrogen bonds. The absence of hydrogen bonding would be contrary to the conventional explanation of the conformation of lasalocid A–cation complexes, in which hydrogen bonds between the two ends of the lasalocid A molecule play a significant part in bringing the two ends of the molecule together.[2b] An absence of hydrogen bonding would imply that cation–oxygen interactions are sufficient to stabilize the molecule in its observed conformations. The strong interactions between the oxygen atoms and the central cation may be responsible for the close oxygen–oxygen approaches seen in the x-ray structure.

The hydroxyl deformation frequencies of the sodium complex are virtually unshifted in methanol solution, implying retention of the hydrogen bonding pattern of the crystal in this solvent. Furthermore, the hydroxyl groups must be relatively shielded from the solvent, as they are in models of the crystal structure, since otherwise they would be more influenced by donated hydrogen bonds. Since the proposed hydrogen bonds of NaX are all intramolecular, this result does not indicate whether NaX or $(NaX)_2$ is the dominant species in methanol. If NaX be dominant, it is disappointing that partial exposure of the cation to the solvent has no substantial effect on the spectrum. Degani and Friedman[6] report that for the Na^+ complex in methanol, conformation III is dominant, but that some conformation I is also present. We see only one conformation (apparently identical with the crystalline conformation) in methanol solution; however, because of noise in our spectra due to sample luminescence, we do not rule out the presence of limited amounts of NaX in some second conformation.

Acknowledgments. We are especially indebted to Professor Harold Friedman, Professor Berton Pressman, and Dr. Dinshaw Patel for encouraging us to investigate lasalocid A. This research was only possible because of generous gifts of lasalocid A from Dr. Julius Berger (Hoffmann-La Roche), Professor Harold Friedman (State University of New York at Stony Brook), and Dr. Dinshaw Patel (Bell Telephone Laboratories, Murray Hill, New Jersey). We should also like to thank Dr. Irvin Asher and Dr. Kenneth Rothschild for their comments and suggestions. This research was supported in part by grants from the National Science Foundation and the National Heart and Lung Institute (HL14322-03, R. W. Mann, Principal Investigator).

References and Notes

(1) (a) Author to whom correspondence should be addressed: Department of Chemistry, University of California, Los Angeles, California 90024. (b) Department of Physics, Massachusetts Institute of Technology.
(2) (a) J. Berger, A. I. Rachlin, W. E. Scott, L. H. Sternbach, and M. W. Goldberg, *J. Am. Chem. Soc.* **73**, 5295 (1951). (b) Yu. A. Ovchinnikov, V. T. Ivanov, and A. M. Shkrob, "Membrane-Active Complexones", Elsevier, Amsterdam, 1974, pp 77, 78.
(3) E. C. Bissel and I. C. Paul, *Chem. Commun.*, 967 (1972).
(4) C. A. Maier and I. C. Paul, *Chem. Commun.*, 181 (1971).
(5) S. M. Johnson, J. Herrin, S. J. Lin, and I. C. Paul, *Chem. Commun.* 72 (1970); S. M. Johnson, J. Herrin, S. J. Lin, and I. C. Paul, *J. Am. Chem. Soc.*, **92**, 4228 (1970).
(6) H. Degani and H. Friedman, *Biochemistry*, **13**, 5022 (1974).
(7) J. R. Clark, *Rev. Pure Appl. Chem.*, **13**, 50 (1963); D. H. Templeton, *Acta Crystallogr.*, **13**, 684 (1960); as cited in ref 6.
(8) A. V. Stuart and G. B. B. M. Sutherland, *J. Chem. Phys*, **24**, 559 (1956).
(9) S. Krimm, C. Y. Liang, and G. B. B. M. Sutherland, *J. Chem. Phys.*, **24**, 778 (1956).
(10) W. Weltner, Jr., *J. Am. Chem. Soc.*, **77**, 3941 (1955).
(11) N. B. Colthup, L. H. Daley, and S. E. Wiberley, "Introduction to Infrared and Raman Spectroscopy", Academic Press, New York, N.Y., 1964, pp 269–277.
(12) M. Avram and G. Mateescu, "Infrared Spectroscopy", Wiley-Interscience, New York, N.Y., 1972, pp 250–289, 384–415.

Wurster's Blue as a Fluorescence Quencher for Anthracene, Perylene, and Fluoranthene

Geoffrey W. Kinka and Larry R. Faulkner*

Contribution from the Department of Chemistry, University of Illinois, Urbana, Illinois 61801.

Received October 6, 1975

Abstract: The Wurster's Blue cation radical ($TMPD^+$, where TMPD = *N,N,N′,N′*-tetramethyl-*p*-phenylenediamine) strongly quenches fluorescence from anthracene, perylene, and fluoranthene in acetonitrile. Apparent stationary second-order rate constants from fluorescence yield data are 4.9×10^{10}, 8.6×10^{10}, and 3.1×10^{10} M^{-1} s^{-1}, respectively. Such large values indicate a long-range interaction and support Förster's transfer as the mechanism. Quantitative comparisons have been made with the Yokota–Tanimoto treatment of resonance transfer in a diffusing system. For these tests, all necessary parameters, viz., fluorescence yields (Φ_f), lifetimes (τ_0), diffusion coefficients (D), and critical transfer radii (R_0), were evaluated experimentally.

Radicals and radical ions have received interest as quenchers of aromatic singlets[1-10] because they may exert important limitations on emission yields from chemiluminescent systems, liquid scintillators, or other samples subjected to radiation damage. The interaction itself is also interesting from a mechanistic standpoint because several possible quenching modes exist. These species are often easily reduced or oxidized; thus reversible charge transfer quenching of the type demonstrated first by Leonhardt and Weller[11-16] is conceivable. Alternatively, Förster's transfer according to

$$^1A^* + {}^2Q \rightarrow A + {}^2Q^* \qquad (1)$$

is allowed, so that a long-range interaction might occur.[17,19] In addition, the paramagnetism in the quenching radical may cause exchange-induced intersystem crossing to be important.[20]

Van Duyne studied the quenching of several long-lived aromatic hydrocarbon singlets by the cation radical of *N,N,N′,N′*-tetramethyl-*p*-phenylenediamine ($TMPD^+$, the Wurster's Blue cation) in acetonitrile.[9] He reported rate constants up to 3.0×10^{10} l./mol·s, which is significantly above the diffusion-controlled limit for collisional quenching (about 2.0×10^{10} l./mol·s).[9,15,16] Furthermore, there was a correlation between the quenching rate constant and the spectral overlap for singlet emission and $TMPD^+$ absorption. From this evidence he suggested that Förster's transfer was a significant element in the quenching process.

Independently, Lisovskaya et al.[10] showed that diphenylpicrylhydrazyl free radicals can quench singlets even in solid matrices. Their work has given strong support to the idea of efficient long-range singlet–doublet resonance transfer.

We report here some studies of the quenching of anthracene, perylene, and fluoranthene fluorescence by $TMPD^+$ in acetonitrile. The rate constants are very large, and there is a strong correlation with the product of the spectral overlap integral and the fluorescer's oscillator strength; hence the Förster mechanism appears to operate. Several theories have been advanced to describe resonance energy transfer in diffusing systems,[21-29] and we have chosen the widely cited one by Yokota and Tanimoto[27] for comparison with our data. Strictly quantitative tests are possible in these cases, because all parameters required by the theory, including diffusion coefficients, have been evaluated experimentally.

Experimental Section

Blue-violet fluorescence grade anthracene (Eastman or Aldrich) or Prinz quality material (Princeton Organics, 99.999%, zone refined) was used without further purification. There were no noticeable differences in behavior. Fluoranthene (Eastman, White Label) and perylene (Aldrich, Gold Label) were also used as received.

Wurster's Blue perchlorate ($TMPD^+ClO_4^-$) was synthesized by an adaptation of the procedure of Michaelis and Granick.[30] The starting material was *N,N,N′,N′*-tetramethyl-*p*-phenylenediamine dihydrochloride (Eastman), but it was half-neutralized with NaOH upon dissolution into the water–methanol reaction solvent. Oxidation to $TMPD^+$ was accomplished with a stoichiometric amount of bromine generated at reaction time by the addition of a slight excess of H_2SO_4 to an aqueous solution of NaBr and $NaBrO_3$. This solution was added dropwise with stirring to the cold TMPD solution. Good purity in the product could be obtained only when the reaction temperature was at or below −10°. The precipitated $TMPD^+ClO_4^-$ was filtered, washed, and recrystallized from methanol.

The purity of this product was monitored by its absorbance maximum at 566 nm as measured on a Cary Model 14 or a Coleman Model 124 spectrophotometer. Beer's law plots were linear and showed $\epsilon = (1.29 \pm 0.02) \times 10^4$ for acetonitrile solutions. This figure is comparable to the value of 1.26×10^4 for 568 nm reported by Van Duyne for coulometrically generated $TMPD^+$.[9]

Spectroquality acetonitrile from Aldrich, Eastman, Burdick and Jackson, and Matheson Coleman and Bell were used without further purification and without noticeable differences in behavior. Solutions of $TMPD^+$ in these solvents showed stable absorbances for at least