

Comparison of the Solubility in Supercritical Fluids of the Polycyclic Ether Antibiotics: Lasalocid, Monensin, Narasin, and Salinomycin[†]

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Solubility measurements for the polycyclic ethers (lasalocid, monensin, narasin, and salinomycin) were determined as sodium salts in pure CO₂ and with CO₂ modified with methanol and water at temperatures of 60–80 °C and 140–400 bar using an SFE instrument with a recirculating-loop fluid system. Wide variations in solubility within this antibiotic class were observed. Narasin and salinomycin were found to have almost identical solubilities in supercritical CO₂, 1.35×10^{-3} mol/L (80 °C and 390 bar) while monensin was much lower in solubility, 2.34×10^{-4} mol/L (80 °C and 390 bar). Lasalocid showed no measurable solubility under similar experimental conditions. Similar differences were observed when solubilities were determined in CO₂ modified with methanol and water. These variations in measured solubilities were attributed, in part, to dissimilarities among the conformations of the polycyclic ether/cation complexes in solution.

Keywords: antibiotic, ionophore, polycyclic ether, supercritical solubility

INTRODUCTION

Antibiotics of various structural types are fed to cattle and poultry for both prophylactic and therapeutic purposes. Dosage limits are set by the Food and Drug Administration (FDA), which also establishes permissible residue levels for these compounds in tissue after slaughter.¹ Currently, wet chemical methods of analysis are used to measure drug residues in edible tissue to determine whether they exceed permissible levels. In many instances, the analytical procedures used are time consuming and are tedious to perform. To overcome their inherent problems, efforts have been initiated by the Food Safety Inspection Service (FSIS) of the USDA to develop alternatives to many of the drug isolation methods currently used in their laboratories, so as to improve regulatory efficiency. In response to FSIS needs, supercritical-fluid extraction is now being investigated in our laboratory for its potential application in drug residue isolation from animal tissue samples. Prior to conducting experiments with antibiotic spiked or incurred tissues, it was first necessary to measure the solubilities of the antibiotics of in-

terest in supercritical media. To date, few studies have described the solubility of veterinary antibiotics^{2–5} in supercritical fluids or their extraction from tissue,⁶ and no report has described the comparison of solubility behavior within an antibiotic class.

The purpose of this study was to determine the solubilities of one veterinary antibiotic class, the polycyclic ethers, in pure and modified carbon dioxide with the aim of discerning trends in solubility as a function of molecular structure. Results from these investigations could be used in attempts to predict which of these antibiotics might be extractable at prescribed regulatory levels (ppm–ppb). The members of this class — lasalocid, monensin, narasin, and salinomycin — are used only as veterinary antibiotics and are generally added to medicated feeds as their sodium salts at levels from 60–100 ppm. They are administered to prevent coccidiosis in poultry and to improve feed efficiency in ruminants.⁷ The polycyclic ethers are characterized by a number of cyclic ether functionalities within their structures, which accounts for their cyclic conformations in solution and their ability to complex metal cations. Because of their unusual solution properties, this antibiotic class affords the opportunity to study

conformational effects on solubility behavior in supercritical fluids.

EXPERIMENTAL

Equipment and Reagents. Solubilities of the polycyclic ether antibiotics were determined using a SPATM supercritical fluid extractor^{**} (SFE), (LDC Analytical, Riviera Beach, FL). Prior to its use for solubility measurements, certain design modifications, which have been reported in detail elsewhere,⁸ were made to this instrument to insure reproducible off-line solute recovery. Carbon dioxide and 1% methanol in CO₂ (SFE grade) were obtained from Scott Specialty Gases (Plumsteadville, PA). All of the solvents used in this investigation were HPLC grade and were from Burdick and Jackson (Muskegan, WI).

The four polycyclic ether antibiotics were gifts from their manufacturers: lasalocid sodium (100%) from Hoffman-LaRoche (Nutley, NJ), monensin sodium (96%) and narasin sodium (94%) from Eli Lilly and Co. (Indianapolis, IN), and salinomycin sodium (97%) from A.H. Robins Co. (Richmond, VA).

Solubility Measurement Procedure. The SPATM recirculation system consists of an extraction vessel, an in-line UV/Vis variable-wavelength monitor, two air-activated injection valves, and a magnetically driven recirculation pump, all of which are contained in a temperature-controlled oven. All of these devices are part of a continuous loop where constant fluid flow is maintained by means of the recirculation pump. The automated valves in the loop system are configured to direct an aliquot of the total volume of the recirculation loop to the mobile phase stream of a high-performance liquid chromatograph (HPLC), or the aliquot can be collected off-line by directing the resultant expanded gas stream into a receiver using a modification to the instrument design developed in this laboratory.⁸ In experiments with the polycyclic ethers, all aliquots were collected using the off-line modification.

In a typical experiment, an antibiotic sample (300 mg ± 0.01 mg) was placed in a 2-mL extraction vessel fitted at each end with 2- μ m frits. The sample then was hand mixed thoroughly with sufficient glass beads (0.25–0.32 mm o.d.) to fill the total volume of the 2-mL vessel. The extraction vessel then was inserted into the extractor and sealed. The system was pressurized with CO₂ or CO₂ with modifier to the initial set pressure and the oven was heated to the set temperature. (Experiments with water-saturated CO₂ were carried out by adding water to the well in the housing of the chamber which holds the extraction vessel.) After set point conditions were obtained, the recirculation pump was activated. The supercritical fluid was continuously circulated through the

sample in the extraction vessel until equilibrium solubility was achieved. This condition was determined in one of two ways depending upon the nature of the compound under study: (a) if the polycyclic ether had a chromophoric group (lasalocid), its concentration in solution was monitored by means of the in-line UV/Vis monitor in the SPATM recirculation loop; or (b) if the ionophore had no chromophore, (monensin, narasin, and salinomycin) its concentration in solution was determined by removing a measured aliquot of the solution through an in-line sample loop on the air-activated injection valve and then subsequently determining the solute concentration off-line. Aliquots were removed for analysis until a steady concentration was established at a specified temperature and pressure. Then, while maintaining constant temperature, the pressure was programmed to the next higher set point and aliquots again were removed until equilibrium again was established. This process was repeated until samples had been removed for all of the pressure points selected for the experiment.

Off-Line Determination of Analyte Concentration. Two analytical techniques were used to quantitate the recovered solute in each aliquot — UV absorption or refractive index detection. For lasalocid sodium, which has a chromophoric group, its UV absorbance was used to measure the amount of recovered solute. The linearity of response was calculated by preparing standard curves from serially diluted standards whose UV absorbances were measured using a Beckmann DU-70 Spectrophotometer. Each recovered aliquot from the SFE then was diluted to a fixed volume with an appropriate solvent and its absorbance measured. Plots of concentration vs. pressure were made from this data to determine equilibrium solubilities. Analytes having no UV chromophoric groups, monensin, narasin, and salinomycin, were analyzed by HPLC with refractive index detection. Serially-diluted standards of each polycyclic ether were prepared and were injected into an HPLC system comprised of a Beckmann 114A pump with a C18 (25 cm × 4.6 mm) 10- μ m column, connected to a Hewlett-Packard 1037A refractive index detector. The mobile phase was methanol, water, and acetic acid (940:59:1). The refractive index responses for each set of standards were plotted against concentration and were linear for the three compounds over the pressure range tested. Solute concentrations of recovered aliquots from the SFE were determined from calibration standards as above.

RESULTS AND DISCUSSION

The SPATM extractor used in these studies was designed for on-line HPLC analyte take-off or off-line recovery through a restrictor orifice. Neither option was useful for obtaining the results needed in this study due to limitations in the original instrument design. Therefore, extensive changes were made to the flow system of this instrument, so that reproducible, quantitative recovery of

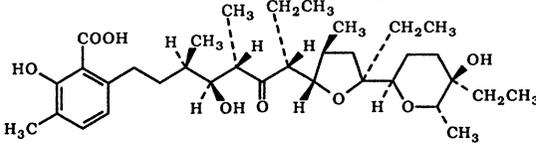
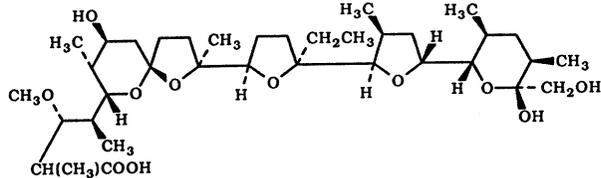
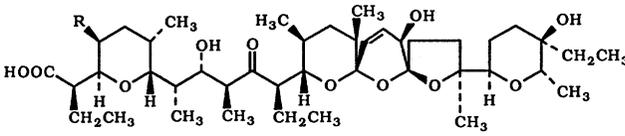
	Molecular Weight Sodium Salt	Equilibrium Solubility (mol/L) at 80°C, 400 bar
 Lasalocid	$C_{34}H_{53}O_8$ Na-612	2.3×10^{-4} (CO ₂ /1% MeOH)
 Monensin	$C_{36}H_{61}O_{11}$ Na-692	5.7×10^{-5}
 R = CH ₃ , Narasin	$C_{43}H_{71}O_{11}$ Na-786	1.4×10^{-3}
R = H, Salinomycin	$C_{42}H_{69}O_{11}$ Na-772	7.4×10^{-3}

Figure 1. The structures of the four polycyclic ether antibiotics and their equilibrium solubilities at 80 °C in pure and modified CO₂.

analyte could be obtained.⁸ For instance, in experiments where this modified apparatus was used to measure the solubility of *o*-anisic acid, the reproducibility of quadruplicate aliquots recovered at one pressure level was 0.0094 ± 0.0007 mol/L. With these modifications in place, rapid quantitative off-line solute recovery from the polycyclic ethers was possible using the SPATM extraction apparatus.

The compounds, whose solubilities we measured with the modified SPATM extractor were the sodium salts of the polycyclic ether antibiotics — lasalocid, monensin, narasin, and salinomycin. Their chemical structures, molecular weights, and measured equilibrium solubilities at 80 °C are shown in Figure 1. The conditions used for these determinations were temperatures from 60 to 80 °C over a pressure range of 140–400 bar. The limit of 80 °C was set for these studies because this temperature generally is the highest used for solute extractions from animal tissue in supercritical fluids.^{10,11} Solubility experiments were carried out in CO₂, water-saturated CO₂, and CO₂ containing 1% methanol. The solubility parameters obtained for the four polycyclic ethers under the experimental conditions employed are given in Table I. The solubilities in the table are displayed in two units, as mol/L (mol solute/L CO₂) and as mole fraction, while all of curves shown in the figures were calculated using mol/L. The estimated densities for the mixtures CO₂/methanol and CO₂/water were calculated using the modified Peng-

Robinson equation-of-state.⁹

Solubility data obtained for monensin sodium under selected conditions are shown in Figure 2 and Table I. The highest solubility for this analyte was obtained at 80 °C and 185–402 bar (7.6×10^{-6} to 2.1×10^{-4} mol/L) using CO₂ with 1% methanol. Solubilities of monensin at 80 °C and 201–408 bar in pure CO₂ were lower (1.2×10^{-5} to 5.7×10^{-5} mol/L) than when methanol was present and still lower when the CO₂ was saturated with water (7.7×10^{-6} to 2.5×10^{-5} mol/L). Attempts were made to measure the solubility of monensin in CO₂ at 60 °C however, its solubility at this temperature was too low to measure with the experimental apparatus employed.

The solubility data for narasin sodium appears in Table I and Figure 3. (Because of their structural similarities, narasin and salinomycin gave similar results; therefore only the data for narasin is presented in Figure 3 and is representative for both polycyclic ethers.) The solubilities of these antibiotics were measured at 60, 70, and 80 °C in CO₂ and at 60 °C in water-saturated CO₂. No solubility measurements were made with methanol in CO₂ with these two compounds because of the high solubility that narasin and salinomycin exhibited in CO₂ and water-saturated CO₂. The isotherms for narasin at the three temperatures studied were similar. For example, at 60 °C and 195–401 bar, the solubility of narasin in CO₂ was

TABLE I
Solubility Parameters for the Polycyclic Ether Antibiotics in Modified and Pure Carbon Dioxide

Pressure (bar)	CO ₂ Density (mol/L)	Solubility	
		Mol/L	Mole Fraction
Lasalocid			
CO ₂ + 1% MeOH/80 °C			
152	10.07	7.00×10^{-5}	6.95×10^{-6}
207	13.60	5.00×10^{-5}	3.68×10^{-6}
279	16.44	6.55×10^{-5}	3.98×10^{-6}
344	18.17	1.17×10^{-4}	6.44×10^{-6}
382	18.93	1.37×10^{-4}	7.22×10^{-6}
390	19.08	2.34×10^{-4}	1.23×10^{-5}
Monensin			
CO ₂ /80 °C			
201	13.60	1.23×10^{-5}	9.04×10^{-7}
277	16.40	1.57×10^{-5}	9.57×10^{-7}
309	17.15	3.39×10^{-5}	19.77×10^{-7}
408	18.85	5.71×10^{-5}	30.29×10^{-7}
CO ₂ + 1% MeOH/80 °C			
185	12.36	7.59×10^{-6}	6.14×10^{-7}
243	15.22	4.35×10^{-5}	2.86×10^{-6}
316	17.48	1.20×10^{-4}	6.87×10^{-6}
402	19.30	2.08×10^{-4}	1.08×10^{-5}
CO ₂ + H ₂ O ¹ /80 °C			
182	12.01	7.68×10^{-6}	6.35×10^{-7}
242	14.49	8.02×10^{-6}	5.55×10^{-7}
316	16.30	1.43×10^{-5}	8.77×10^{-7}
401	17.71	2.54×10^{-5}	1.43×10^{-6}
Narasin			
CO ₂ /60 °C			
195	16.30	2.6×10^{-4}	1.59×10^{-5}
206	16.65	3.3×10^{-4}	1.98×10^{-5}
264	18.20	4.3×10^{-4}	2.36×10^{-5}
274	18.40	4.4×10^{-4}	2.39×10^{-5}
332	19.35	7.0×10^{-4}	3.62×10^{-5}
401	20.25	1.12×10^{-3}	5.53×10^{-5}
CO ₂ /70 °C			
175	13.65	3.10×10^{-4}	2.27×10^{-5}
241	16.48	4.80×10^{-4}	2.91×10^{-5}
314	18.18	8.40×10^{-4}	4.62×10^{-5}
405	19.55	1.26×10^{-3}	6.44×10^{-5}
CO ₂ /80 °C			
141	8.80	1.61×10^{-4}	1.82×10^{-5}
217	14.35	2.69×10^{-4}	1.87×10^{-5}
276	16.35	5.31×10^{-4}	3.24×10^{-5}
364	18.15	1.35×10^{-3}	7.43×10^{-5}
CO ₂ + H ₂ O ¹ /60 °C			
181	14.81	3.9×10^{-4}	2.63×10^{-5}
184	14.94	5.7×10^{-4}	3.82×10^{-5}
243	16.88	2.23×10^{-3}	1.32×10^{-4}
311	18.34	4.48×10^{-3}	2.44×10^{-4}
401	19.72	6.29×10^{-3}	3.19×10^{-4}

TABLE I
(continued)

Pressure (bar)	CO ₂ Density (mol/L)	Mol/L	Solubility	Mole Fraction
Salinomycin				
CO ₂ /70 °C				
185	14.23	2.42×10^{-4}		1.70×10^{-5}
249	16.73	9.86×10^{-4}		5.39×10^{-5}
318	18.25	2.37×10^{-3}		1.30×10^{-4}
391	19.38	2.92×10^{-3}		1.51×10^{-4}
CO ₂ /80 °C				
142	8.90	2.32×10^{-4}		2.61×10^{-5}
202	13.60	6.35×10^{-4}		4.67×10^{-5}
256	15.80	1.77×10^{-3}		1.12×10^{-4}
273	16.30	2.05×10^{-3}		1.26×10^{-4}
318	17.35	4.71×10^{-3}		2.71×10^{-4}
395	18.65	7.36×10^{-3}		3.95×10^{-4}
CO ₂ + H ₂ O ¹ /60 °C				
175	14.53	2.57×10^{-4}		1.77×10^{-5}
184	14.94	5.7×10^{-4}		3.82×10^{-5}
243	16.88	2.23×10^{-3}		1.32×10^{-4}
313	18.38	3.24×10^{-3}		1.76×10^{-4}
326	18.60	5.74×10^{-3}		3.09×10^{-4}
404	19.75	5.82×10^{-3}		2.95×10^{-4}

¹Water-saturated CO₂ (Experimental).

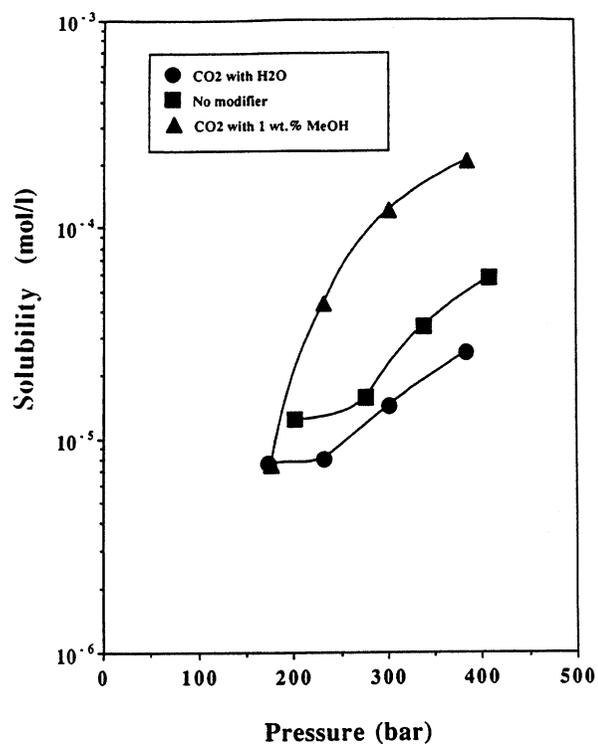


Figure 2. Solubility (mol/L) of monensin at 80 °C in modified and pure CO₂.

2.6×10^{-4} to 1.1×10^{-3} mol/L; at 70 °C and 175–405 bar, the range was 3.1×10^{-4} to 1.3×10^{-3} mol/L; and at

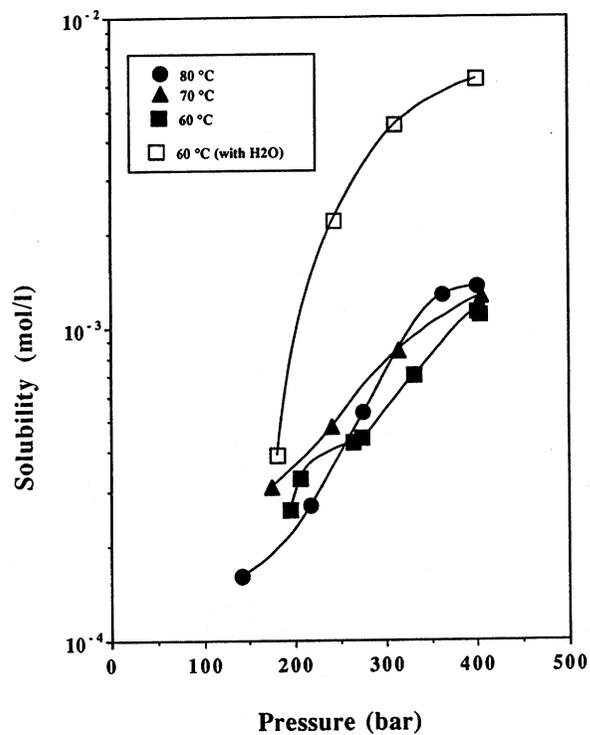


Figure 3. Solubility (mol/L) of narasin at 60–80 °C in modified and pure CO₂.

80 °C and 141–364 bar, the range was 1.6×10^{-4} to 1.4×10^{-3} mol/L (Figure 3).

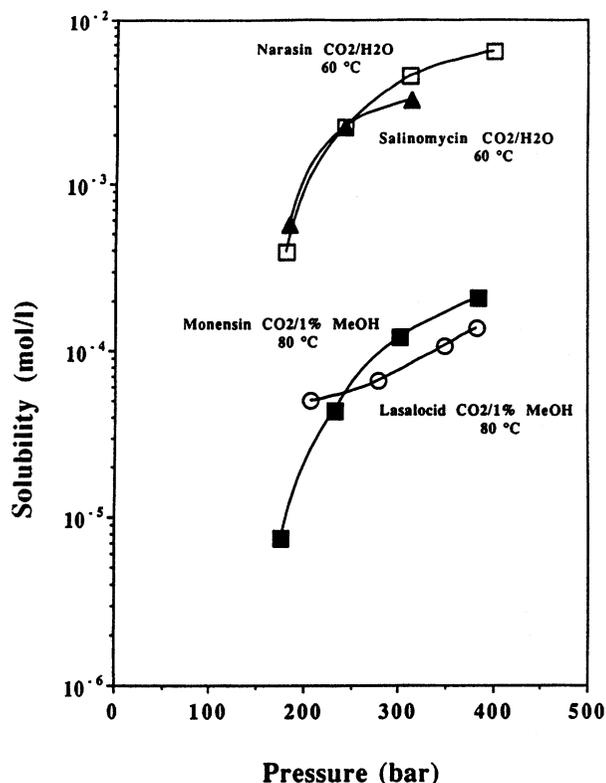


Figure 4. Optimum equilibrium solubility conditions (mol/L) for the polycyclic ether antibiotics.

Figure 4 shows the solubility curves for the four polycyclic ethers at their optimum solubility conditions found in this study. Their individual solubilities at 400 bar and 80 °C are given in Figure 1. The molecular structures of narasin and salinomycin (Figure 1) differ only by one methyl group, therefore, it is not surprising that the solubility curves for these two compounds in CO₂/water are similar. (The CO₂/water system was studied to approximate the environment of a matrix such as wet tissue.) Of the four polycyclic ethers measured, narasin and salinomycin display the highest solubilities in this medium. The solubility of narasin in CO₂/water at 60 °C ranged from 3.9×10^{-4} to 6.3×10^{-3} mol/L while that of salinomycin under the same conditions ranged from 2.6×10^{-4} to 5.8×10^{-3} mol/L (Table I). Lasalocid, on the other hand, was insoluble in CO₂ at both 60 and 80 °C. It was slightly soluble in CO₂/1% MeOH at 80 °C with values ranging from 7.0×10^{-5} to 2.3×10^{-4} mol/L over the pressure range evaluated (Table I and Figure 4). A similar curve (Figure 4) was obtained for monensin when its equilibrium solubility was measured at 80 °C in CO₂/1% MeOH (7.6×10^{-6} to 2.1×10^{-4} mol/L) over the same pressure range.

Comparison of the molecular structures of these four compounds does not immediately suggest an explanation for the wide differences in observed solubilities. Each of the four members of this class studied has an end carboxylic acid group and a tertiary hydroxyl group at-

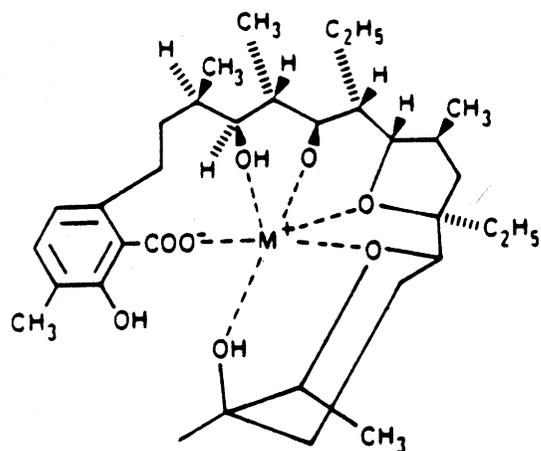


Figure 5. Structure of the lasalocid-cation complex.⁷ (© 1983 American Chemical Society, Reprinted with permission.)

tached to the tetrahydropyran group at the other terminus. In between these two groups are a variety of four- and five-membered cyclic ethers and numerous alkyl groups at various points of attachment (Figure 1). However, an inspection of their solution conformations does offer one possible explanation for their behavior in supercritical media, since both the free acids and the sodium salts are reported to assume cyclic conformations in solution.⁷ The solution conformation of the lasalocid-cation complex, measured in water and organic solvents, is representative of this antibiotic class and is shown in Figure 5. In assuming the cyclic conformation, it should be noted that the polar oxygenated functionalities of this molecule are directed toward the central cation, while the branched alkyl groups are spread over the outer surface, rendering the complex lipid-like in character. The sodium salt of lasalocid exhibits limited solubility in water and hexane,⁷ behavior which is similar to that observed for this compound in supercritical CO₂. Although monensin would be expected to assume a similar conformation in solution, its observed solubility in CO₂ was found to be greater than that of lasalocid (Figure 1). One structural difference between these two compounds is the presence of a benzene ring at the terminus of the lasalocid backbone, which, in part, must contribute to a decrease in the hydrophobic-like properties of its outer sphere. Similarly, the higher molecular weights of narasin and salinomycin are due to the inclusion of additional hydrocarbon groups in their backbones adding to the hydrophobicity of their outer spheres and which partially may account for their enhanced solubility in CO₂.

King and Friedrich have reported several examples where a solute's molecular structure can be correlated with its solubility in supercritical fluids using the concept of the reduced solubility parameter.¹² The reduced solubility parameter may be computed by using the group contribution method described by Fedor.¹³ This method estimates solubility by summing the contributions from all of the

individual functional groups contained in a molecule. Such an approach was tried with the polycyclic ethers to determine whether the derived parameters are in agreement with the experimental results of this study. Using the group contribution method the solubility parameter (δ , $\text{cal}^{1/2}/\text{cm}^{3/2}$) for the four solutes were — lasalocid ($\delta = 11.2$), monensin ($\delta = 11.1$), narasin ($\delta = 11.1$), and salinomycin ($\delta = 11.2$). These values were derived for the free acids rather than the sodium salts since values for group contributions from cations have not been calculated. Since these values are nearly identical for all four compounds, this method cannot be used to estimate the solubility of the polycyclic ether antibiotics in supercritical fluids. Although the solubility parameter concept is very useful in many instances, it cannot easily be used for salts because of the lack of data for charged functional groups such as cations.

CONCLUSIONS

Wide variations in solubility behavior were observed for the four polycyclic ether antibiotic salts in supercritical fluids. Solubilities were influenced by the presence of modifiers such as methanol or water, and by changes in temperature and pressure. It is thought that these salts assume conformations in supercritical fluids similar to those conformations reported in liquids. The solubility parameter concept could not be used to calculate solubilities for the polycyclic ether antibiotic salts because critical individual group contributions for cations have not been calculated to date.

ACKNOWLEDGMENT

The authors wish to thank Dr. Mark R. Coleman, Eli Lilly and Co., for the gifts of monensin sodium and narasin sodium; Dr. Alexander MacDonald, Hoffman-LaRoche, for the gift of lasalocid sodium and Dr. Lawrence F. Sancilio, A. H. Robins Co., for the gift of salinomycin sodium. They also wish to thank Dr. Jerry King NCAUR/USDA, Peoria, IL, for many helpful discussions and for calculating the solubility parameters.

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