Solution Chemistry of Monensin and Its Alkali Metal Ion Complexes. Potentiometric and Spectroscopic Studies

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Abstract: Sodium complex of an antibiotic drug, monensin, was investigated by potentiometric, infrared, and ^{23}Na, ^{7}Li, ^{13}C, and ^{1}H NMR techniques in several nonaqueous solvents. The acidity constant of monensin and the stability constant for the monensin–sodium ion complex were determined in methanol solutions. Complexation titrations with other cations show that the selectivity of monensin varies in the order $Ag^+ > Na^+ > K^+ > Rb^+ > Cs^+ > Li^+ \sim NH_4^+$. Spectroscopic measurements indicate that monensin forms two sodium complexes of different configuration and stability. These measurements also indicate that water molecules can bind to the monensin molecule at two different sites. Addition of the sodium ion results in water exchange between these two sites and the free water in the solvent.

The isolation of the monocarboxylic acid antibiotic monensin (Figure 1) from culture filtrates of *Streptomyces cinnamonensis* was first reported by Agtarap et al.[1] in 1967. Some chemical properties of the molecule, then called monensic acid, and of its sodium and silver salts were discussed. More details of its isolation, of its chemistry, and of its biological activity were given later by Haney and Hoehn.[2] During this same period, other papers appeared dealing with investigation of structural properties using proton magnetic resonance and mass spectrometry[3] and with studies of monensin effects on alkali metal ion transport in mitochondria.[4] Thin-layer chromatography[2,5] showed the existence of four closely related factors of monensin referred to as monensin A, B, C, and D. The components were found to be very nearly identical, differing by no more than one $-CH_2-$ unit. At the present time the name, monensin, usually refers to the major factor, A.

After this period of discovery and initial chemical characterization many workers began to study the chemistry of the molecule by a variety of experimental techniques. Pressman examined the complexing ability of monensin and reported a marked specificity for the sodium ion.[6] He also commented on the pH dependency of molecular conformation and of the complexation phenomena.[7] Crystallographic studies revealed the crystal structure of the silver, sodium, potassium, and thallium salts[8] of monensin. This compound was also shown to be an active ion transporter in model membrane systems.[9] A complete study of the mass spectrometry of the molecule and its factors and salts was also made.[10] A Swiss group led by Simon contributed infrared and electrochemical studies which showed monensin to be cyclic in solution and allowed estimates of alkali metal ion complex formation constants.[11] Further X-ray crystallographic studies compared the monensin structure–specificity relationship to that of related antibiotics[12] and also showed interesting differences between the salt and the acid structures.[13] Computerized microcalorimetric measurements produced estimates of various thermodynamic parameters for the interaction of monensin with sodium and potassium ions in basic methanol solution.[14,15] The results indicate that the formation constant for the sodium–monensin complex is $\sim 10^6$. A high affinity of the monensin sodium salt for sodium ion in methanol and chloroform solutions was confirmed by a sodium-23 NMR study.[16] Simple membranes, constructed to incorporate monensin sodium salt, showed that a pH gradient was capable of causing selective sodium ion countertransport[17-19] and model calculations regarding monensin sodium specific complexing behavior were published by the Swiss group.[20] Another contribution by Simon[21] summarized the specificity of monensin and other complexing agents in membrane systems.

We are currently involved in the study of other weak and strong alkali metal ion complexing agents such as tetrazoles, glutarimides, crowns, and cryptands by spectroscopic techniques. In this paper we report some new information concerning the soluion chemistry of the monensin molecule and its alkali metal ion complexes.

Experimental Section

Materials. A. Reagents. Tetrabutylammonium hydroxide (TBAH) (Eastman Kodak) was used as received as were deuterium oxide (Columbia Organic, 99.5%) and tetramethylsilane (Aldrich). Benzoic acid (Matheson Coleman and Bell) was dried at 90° for 48 hr. Lithium perchlorate (Fisher) was dried at 190° for several days. Sodium perchlorate (G. F. Smith), potassium chloride (Matheson Coleman and Bell), rubidium iodide (Alfa), cesium iodide (Alfa), ammonium perchlorate (G. F. Smith), and silver perchlorate (G. F. Smith) were all dried at 110° for at least 72 hr; $Eu(fod)_3$ (Alfa) was used as received.

B. Solvents. Methanol (Baker-analyzed reagent) was refluxed over granulated calcium hydride (Baker) for 24 hr and fractionally distilled. Water content was found by Karl Fischer titration to be less than 70 ppm. Chloroform-d_1 (Norell, 99.8%), tetrahydrofuran-d_8 (Norell, 99%), acetone-d_6 (Diaprep-Aldrich, 99%), and methanol-d_4 (Diaprep-Aldrich, 99.5%) were used as received and solutions involving the use of these solvents were prepared in a drybox. Chloroform (Mallinckrodt), acetone (Mallinckrodt), methanol (Matheson Coleman and Bell), ethyl ether (Mallinckrodt), hydrochloric acid (Baker-analyzed reagent), and petroleum ether (Aldrich) were used in drug purification and preparation as received.

C. Monensin. Monensin was received as the sodium salt (QA166H Lot 910 AD3, hereafter, MonNa) through the generous gift of Eli Lilly and Co. The salt was dissolved in boiling methanol, filtered, and then reprecipitated with water from the filtrate after cooling. This procedure was repeated twice and eliminated most of the brown impurity first noticed as a straw yellow color in solution. The salt was further purified by recrystallization from a 1:1 ether–petroleum ether mixture and dried at 110° for 48 hr.

The acid form of the molecule (hereafter, MonH) was prepared as follows. A concentrated solution of the salt was prepared in chloroform and treated with an equal volume of aqueous 0.1 *M* hydrochloric acid. The chloroform phase was evaporated to dryness at room temperature. The product was then dissolved in acetone and reprecipitated with water. The white solid was dried for 24 hr at 30° under vacuum and then dissolved in boiling methanol. The solution was filtered and MonH was reprecipitated from the cooled filtrate by the addition of water. After drying, the acid was recrystallized from 1:1 ethyl ether–petroleum ether mixture and again dried under vacuum for 24 hr at 30°.

Elemental analyses of the sodium salt and of the acid form gave the following results. Anal. Calcd for $C_{36}H_{61}O_{11}Na$: C, 62.70; H,

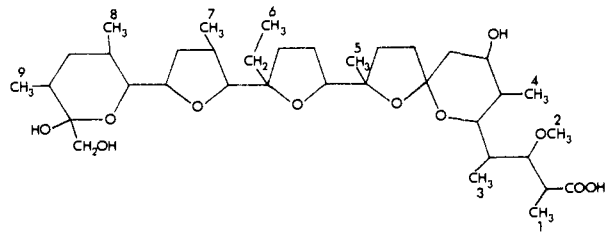

Figure 1. Monensin.

8.87. Found: C, 61.93; H, 8.88. Anal. Calcd for $C_{36}H_{62}O_{11}\cdot H_2O$: C, 62.77; H, 9.36. Found: C, 62.63; H, 9.28. Melting points of 269 and 117° for the salt and acid, respectively, agreed with reported values of 267–269°[2] and 117–122°.[13] Mass spectra of the two solids showed fragmentation patterns similar to those previously observed.[10] Infrared spectra taken in chloroform solutions agreed with the available literature spectra[2] and showed a diagnostic shift of the carbonyl band from 1563 to 1704 cm^{-1} in the salt to acid transformation. Carbon-13 NMR spectra, shown in Figure 2, revealed a dramatic variation in carbon environments and were especially useful in showing a change from 188.2 to 177.8 ppm in the carbonyl chemical shift, which is the characteristic difference between a salt and a free carboxylic acid. Proton NMR spectra in chloroform-d_1 (Figure 3) also showed many differences between the acid and the sodium salt, the most obvious one being the appearance of a previously unreported broad water peak at approximately 6 ppm (all ^1H chemical shifts are given downfield from Me_4Si) in the spectrum of the acid which is totally absent in the spectrum of the salt.

On the basis of these measurements, it was concluded that the pure free acid had been prepared. No attempt was made to separate the four related factors of either the sodium salt or the acid. The acid was found to be stable in solid form but quite susceptible to degradation in solution.

Measurements. A. Potentiometric. Potentiometric measurements were made using an airtight cell constructed to accommodate a Beckman 41263 glass pH electrode, a Sargent Welch S-30080-150 saturated calomel reference electrode, and the extended tip of a 50-ml delivery burette. The electrodes were initially soaked in methanol for 48 hr and at least for 5 hr between runs. In some experiments somewhat more stable potentials were obtained by replacing the aqueous saturated potassium chloride solution in the SCE by a saturated methanolic solution of this salt. Measurements were taken at ambient temperature with a Heath EU-302A servodigital pH/voltmeter allowing sufficient time between readings for the stabilization of the potential. A constant pressure of nitrogen was maintained in the cell at all times and a magnetic stirrer was used for mixing but stopped during measurements. Voltage drift and reading error combined to give potential readings with an uncertainty of ±0.002 V and volumes were read with an uncertainty of ±0.02 ml. A CDC-6500 computer was used for data processing in conjunction with a revised version of a program initially developed by Briggs and Steuhr[22] for the determination of equivalence points and pK values or the FORTRAN IV KINFIT program.[23]

B. Spectroscopic. Perkin-Elmer 237B and 457 grating infrared spectrophotometers were used to obtain infrared spectra. A Barnes Engineering fixed path length (0.109 mm) potassium bromide solution cell was used for all infrared studies. The frequencies were calibrated by using polystyrene reference peaks.

Lithium-7 NMR spectra were obtained on a Varian Associates DA-60 spectrometer operating at 23.3 MHz with a field of 14.09 kG. The instrument was frequency locked to a 4.0 M aqueous solution of lithium perchlorate held by Teflon spacers in a 1-mm o.d. melting point capillary positioned in a Wilmad 506 pp 5-mm o.d. polished NMR tube. Chemical shifts were measured at probe temperature with respect to lock with a Hewlett Packard 5245L frequency counter. Sodium-23 NMR spectra were obtained at ambient probe temperature on a highly modified Nuclear Magnetic Resonance Specialties MP-1000 spectrometer operating at 60 MHz with a field of 53.3 kG. A Nicolet 1083 computer was used for time averaging and to drive the frequency synthesizer. Chemical shifts were measured with respect to a 3.0 M aqueous solution of sodium chloride in a Wilmad 520-2 coaxial reference tube inserted in either a Wilmad 506 pp 5-mm o.d. or 513A-5 pp 8-mm

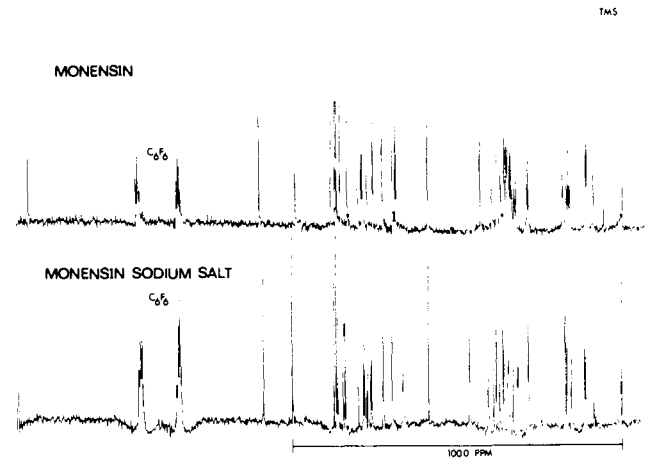

Figure 2. Carbon-13 nuclear magnetic resonance spectra of MonH and MonNa.

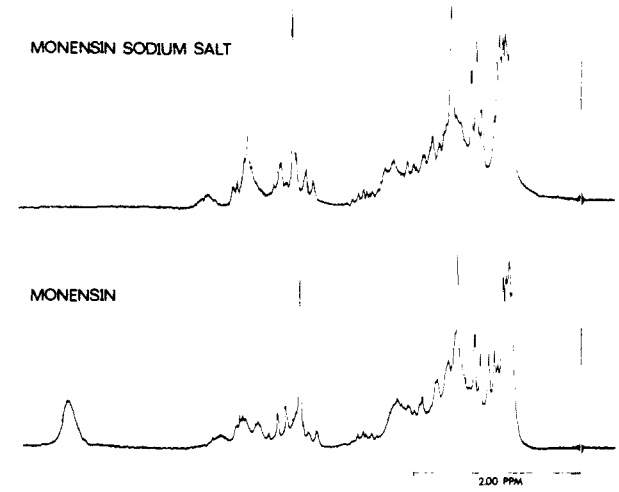

Figure 3. Proton nuclear magnetic resonance spectra of MonNa and MonH at 100 MHz.

o.d. polished NMR tube. Uncertainties in chemical shift values were estimated individually for each spectrum and varied with sweep width and signal line width. Proton NMR spectra were run on one of three instruments. The Varian T-60 spectrometer was used in the normal mode and also for decoupling experiments. The Varian A56/60D with temperature controller was also used with calibrated chart paper and side-band calibrated sweep widths. The Varian HA-100 spectrometer was used for precision work with either a tetramethylsilane or chloroform frequency lock. All chemical shifts were ultimately referenced to Me_4Si. Carbon-13 NMR spectra were obtained using the Bruker HFX-10 spectrometer operating at 22.6 MHz with a field of 21.1 kG. The instrument operated in the normal FT mode under control of Nicolet 1083 and Nicolet 290 computers with complete proton decoupling at 90 and 84 MHz hexafluorobenzene internal lock and Me_4Si internal reference. Samples were run in Wilmad 513-3 pp 10-mm o.d. polished NMR tubes.

Mass spectra were run on the Hitachi Perkin-Elmer RMU-6 spectrometer. Analysis for water was accomplished with a Photovolt Aquatest II automatic Karl Fischer titration apparatus.

Results and Discussion

Potentiometric Studies. A. Acid Titration. Neither sodium salt nor the acid form of monensin is appreciably water soluble; consequently all studies have been carried out in nonaqueous solvent systems. Methanol was chosen as a solvent for the study of the MonH acid behavior because of previous work done with the drug in this solvent and also due to its suitability for potentiometric measurements. Sodium methoxide is often chosen as a basic titrant in methanol but cannot be used in this case due to the complexation

of the cation and the introduction of equilibria other than the acid–base reaction. Therefore tetrabutylammonium hydroxide (TBAH) whose cation is too large for complexation was used as the titrant.

Approximately 0.04 M stock solutions of TBAH were prepared in methanol and were standardized by triplicate titrations of benzoic acid. Benzoic acid was also used to test the method of pK_a determination and gave smooth titration curves in methanol. Potentials observed far past the equivalence points were assumed to arise only from excess base in solution thus allowing the establishment of a voltage to pH relationship. The electrodes were assumed to have a Nernstian response and calculations gave pH values in the titration buffer region. Neglecting solvent autoprotolysis, these values were used to calculate K_a according to the method of Meites and Thomas.[24] The acidity constant is given by the expression

$$K_a = [H^+]\frac{fC_a\frac{V_a}{V_a + V_b} + [H^+]}{(1 - f)C_a\frac{V_a}{V_a + V_b} - [H^+]} \quad (1)$$

where C_a is the analytical acid concentration, V_a is the initial volume of acidic solution, V_b is the volume of base titrated into solution, C_b is the base concentration, and $f = V_bC_b/V_aC_a$ the fraction of the equivalent volume of base added. A more precise calculation was accomplished using the revised Steuhr[22] program which determined the pK_a using a linear form of the exact equation for acid dissociation. Using these methods, a pK_a of 9.25 ± 0.05 for benzoic acid in methanol was calculated. This value has been corrected for a small amount of basic impurity in the methanol and is in good agreement with a previously published pK_a value of 9.27.[25]

The same technique applied to MonH gave a pK_a value of 10.15 ± 0.05. The only previously determined value was that of 6.65 in a 66% dimethylformamide–water mixture.[1] This lower value would be expected in a solvent mixture of higher dielectric constant. Our results indicate that MonH is a relatively weak acid in methanol, and in neutral solution it is mainly in the associated form. Consequently the Mon^- anion is a relatively strong base in methanol solutions.

B. Complexometric Titrations. The knowledge of the MonH acidity constant made it possible to investigate quantitatively the complexation reaction with the sodium ion. Neglecting solvent autoprotolysis and temporarily disregarding the role of the water molecule of the acid, the following equilibria (eq 2–4) represent the complexation of an

$$MonH \rightleftharpoons Mon^- + H^+ \quad K_a \quad (2)$$

$$Mon^- + M^+ \rightleftharpoons MonM \quad K_f \quad (3)$$

$$MonH + M^+ \rightleftharpoons MonM + H^+ \quad K = K_aK_f \quad (4)$$

alkali metal ion by the acid in a neutral solution. It has been postulated[13] that the deprotonation of the acid is accompanied by loss of a water molecule and the removal of an H_3O^+ ion from MonH. It will be shown below that this postulate agrees with our observations. By using the approximation that the concentration of Mon^- in solution is negligible, the following expression for K is obtained if activity corrections are neglected

$$K = \frac{[MonM][H^+]}{[MonH][M^+]} \cong \frac{[H^+]^2}{(C_a - [H^+])(C_{M^+} - [H^+])} \quad (5)$$

where C_a and C_{M^+} are the analytical concentrations of the acid and of the metal ion, respectively. Titration of MonH in methanol with sodium perchlorate solutions of various concentrations gave the potential vs. volume curves displayed in Figure 4 which indicate a rapid increase in hydrogen ion concentration with sodium ion addition. A computer fit of these data to eq 5, allowing variation of the intercept of a Nernstian pH vs. voltage plot in each case, gives the solid lines also shown in Figure 4. Table I presents the values of K, the equilibrium constant for formation of a monensin–sodium complex in neutral solution calculated using these titration data. The small value of K indicates that the tendency of MonH to form complexes in neutral methanol solution is really quite small and the protonated form is a poorer complexing agent than the anion by several orders of magnitude.

Table I. Equilibrium Constant for the Reaction MonH + $Na^+ \rightleftarrows$ MonNa + H^+ in Methanol Solution at 25°

MonH, mmol	$NaClO_4$, M	K
0.0975	0.00502	0.22 ± 0.07
0.195	0.0100	0.79 ± 0.04
0.488	0.0400	0.53 ± 0.11
0.975	0.0400	0.17 ± 0.07

All former studies of MonH complexation ability have been done by first adding excess base to MonH solutions to ensure complete deprotonation. With all of MonH in the Mon^- form, the equilibrium in expression 3 was studied and K_f values of about 10^6 for sodium ion complex methanol have been found.[14,15] Using the previously determined K_a and the calculated K values, we should be able to estimate K_f from the K/K_a ratio. These calculations, however, give K_f values of about 10^9 which are considerably higher than the reported value of 10.[6] This fact gave the first indication of the possibility that the complex formed in neutral solution is not identical with that obtained under the basic conditions. Later experiments supported this assumption (vide infra).

The titration experiment can also be extended to other cations as shown in Figure 5. Solubility requirements dictated the use of other anions, but experiments with several sodium salts showed little or no dependence of the observed potential on the choice of the anion. These data have not as yet been analyzed according to eq 5 but do reveal a complexation selectivity order of $Ag^+ > Na^+ > K^+ > Rb^+ > Cs^+ > Li^+ \approx NH_4^+$. This scheme is in some agreement with results found in basic solutions and correlates extremely well with ionic size parameters, with the lithium ion too small and the ammonium ion too large for coordination in the monensin cavity. Evidence for MonH complexation of alkaline earth and other ions has also been obtained in this manner.

Spectroscopic Studies. A. Infrared. It was desired to monitor the gradual disappearance of the 1704-cm^{-1} band of the carboxylic acid and appearance of the 1563-cm^{-1} carboxylate anion band during the deprotonation observed in the titration experiments described above. This frequency shift was easily observed by the addition of excess base to MonH solutions and the opposite change could also be induced by acidifying a solution of the salt. However, infrared samples obtained in the course of the titration of MonH with sodium perchlorate *failed to show any change in the position or intensity of the 1704-cm^{-1}* band during the entire experiment.

It was found that monensin is capable of solubilizing sodium perchlorate in deuteriochloroform up to 1:1 mole ratios. We monitored the 1704-cm^{-1} MonH band in chloroform-d_1 solutions with increasing sodium perchlorate concentration. Again, even at 1:1 sodium ion to MonH mole ratios, no change in the intensity or position of the 1704-cm^{-1} band was observed nor did any trace of the 1563-cm^{-1} band

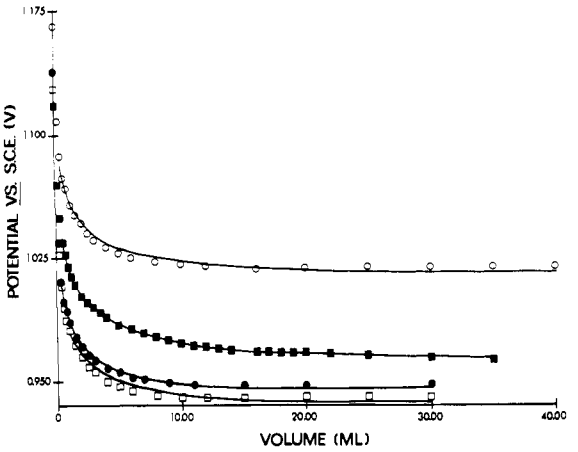

Figure 4. Titration of various amounts of MonH with varying concentration of sodium perchlorate in several initial volumes of methanol: (○) 0.0975 mmol, 0.00502 *M*, 50.0 ml; (■) 0.195 mmol, 0.0100 *M*, 70.0 ml; (●) 0.488 mmol, 0.0400 *M*, 50.0 ml; (□) 0.975 mmol, 0.0400 *M*, 50.0 ml. Solid lines are the computer-fitted data.

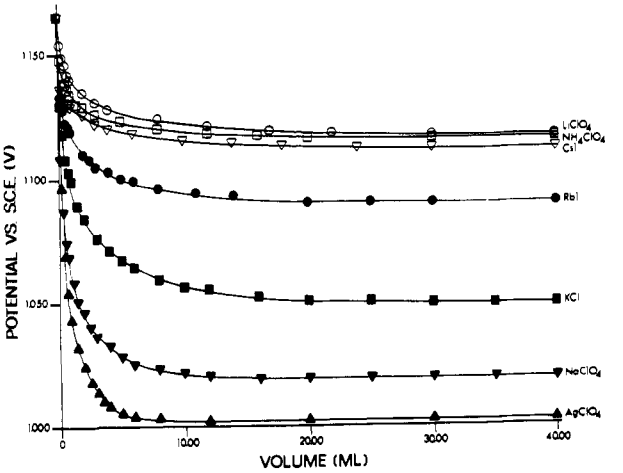

Figure 5. Titrations of 1.00 mmol of MonH with various 0.00500 *M* salts in methanol.

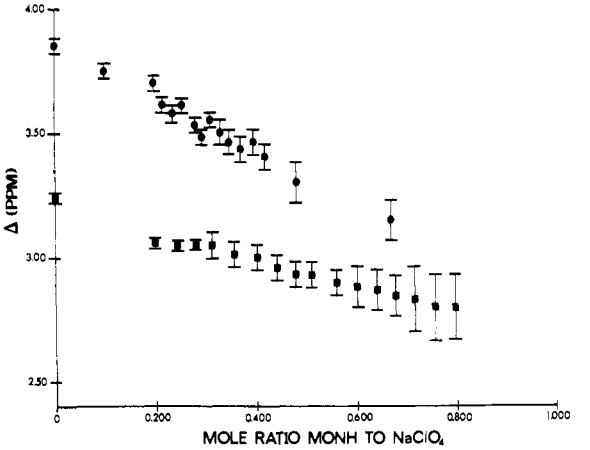

Figure 6. Sodium-23 nuclear magnetic resonance study in methanol: 0.500 *M* $NaClO_4$ (●) and 0.250 *M* $NaClO_4$ (■).

appear. It was, therefore, concluded that if the carboxylic proton is lost by the acid molecule, some other proton from the hydrogen-bonded pattern near this area takes its place. This is to say, that in the addition of an alkali metal ion to monensin in neutral solution, some other proton of the molecule must be more acidic than the one on the carboxylic group. This more acidic hydrogen ion might well belong to one of the several hydroxyl groups. Consequently the complex may have a lower energy state in which the negatively charged oxygen is somehow closer to the positive alkali metal ion than the carbonyl oxygen (see Figure 9). The resultant zwitterionic structure may reflect a charge-stabilized complex, which would give the molecule a more hydrophobic exterior. In any case, this behavior is a second indication that the acid-sodium complex formed in neutral solution cannot be of the same structure as the ordinary salt.

Other areas of the spectrum monitored during the sodium perchlorate addition in chloroform-d_1 gave further evidence of a complexation mechanism different from the one described by expression 4. In the O-H stretching region, MonH showed a broad band at about 3300 cm^{-1} and a sharper band on its shoulder at 3520 cm^{-1}. As sodium concentration was increased, the sharper band gradually disappeared and the 3300-cm^{-1} band increased slightly in intensity. Again, the 1:1 spectrum bore little resemblance to the MonNa infrared spectrum in chloroform-d_1. These spectral changes must result from the removal of the water molecule from the center of the monensin ring. Also noted in the above experiment was the gradual appearance of two new bands at 2025 and 2220 cm^{-1} which were also present in the spectrum of the pure MonNa salt. Their growth was such that at 1:1 sodium perchlorate to MonH mole ratios, this area of the spectrum was identical with the spectrum of salt in chloroform-d_1.

In summary, these rather qualitative results gave evidence that the acid-sodium complex formed in neutral solution has a structure and stability which is definitely different than the salt. This fact explains the discrepancy between the K_f value obtained by us in neutral solution and the one previously determined in basic solution.

Lithium-7 Nuclear Magnetic Resonance Studies. Solutions of lithium perchlorate were made up in 0.02 *M* methanol solutions with increasing concentrations of MonH ranging from 0.02 to 0.20 *M*. Even with the drug to lithium mole ratio of 10 to 1, a shift of only about 2 Hz from the normal 0.02 *M* lithium perchlorate position had been induced. This value, while experimentally significant, indicates an exceedingly weak interaction when compared to lithium ion interaction with other complexing agents such as various cryptands.[26] This result correlates with the data obtained in the potentiometric titration and does confirm the evidence which shows that the lithium ion is only weakly complexed by MonH probably because of ionic size considerations.

Sodium-23 Nuclear Magnetic Resonance Studies. Preliminary measurements were made on a saturated solution of MonNa in methanol solution. A very broad peak was obtained with a width of about 700 Hz at half-height. Because of its line width, it was impossible to determine the ^{23}Na chemical shift.

A more direct and interesting approach to the problem involved the use of two constant sodium perchlorate concentrations of 0.500 and 0.250 *M* with gradual addition of MonH out to the line broadening limit of about 0.75 to 1. The data shown in Figure 6 indicate marked shifts due only to drug addition. The observation of potentially larger shifts is precluded by line broadening, which also makes it impossible to determine the complexation equilibrium constant as was accomplished for other drugs.[27] The ^{23}Na NMR study definitely confirms the potentiometric and infrared results indicating a fairly strong MonH-sodium ion interaction in methanol.

Proton Magnetic Resonance Studies. The entire ^1H NMR spectrum of MonNa in chloroform-d_1 was tentatively assigned using spectra obtained at 60 and 100 MHz, nuclear magnetic double resonance, and a shift reagent. We

Table II. Frequencies of the MonNa Methyl Groups at 100 MHz in $CDCl_3$ vs. Me_4Si

Methyl (Figure 1)	Frequency, Hz
1	116, 123
2	340
3	112, 128
4	91, 98
5	152
6	88, 95, 102
7–9	79, 85 80, 86 81, 87

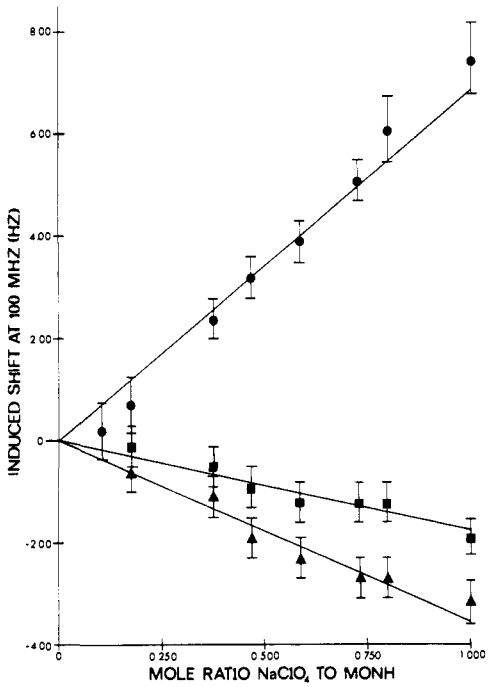

Figure 7. Proton nuclear magnetic resonance study of 0.25 *M* MonH in chloroform-d_1: methyl 3 (●), methyl 1 (■), and methyl 5 (▲).

will limit our discussion to phenomena involving several of the methyl groups and the water molecule of MonH. The methyl groups as numbered in Figure 1 were assigned as shown in Table II. The positions in the MonH spectrum are not identical with some changes in coupling constant values. The previously unreported broad peak at about 6 ppm in the MonH spectrum in chloroform-d_1 was assigned to the bound water molecule of the acid. This assignment was confirmed by an experiment in which MonH in concentrated methanol solution was reprecipitated twice with deuterium oxide. The product gave a ^1H NMR spectrum in which the water peak lost 50% of its intensity. Likewise, the infrared spectrum of this compound showed that the intensities of the 3300- and 3520-cm^{-1} bands also decreased by about 50% and new bands, characteristic of deuterium oxide, appeared at 2450 and 2600 cm^{-1}.

As shown in Figure 7, the 1, 3, and 5 methyl signals undergo induced shifts in 0.25 *M* MonH as sodium perchlorate is added in chloroform-d_1 solutions. Approximately linear shifts are observed as the complex is formed, but different slopes are observed indicating possible molecular conformation changes as MonH and the sodium ion interact. The limiting shifts at 1:1 MonH to sodium perchlorate mole ratios are not in the positions of the same signals in the MonNa ^1H NMR spectrum (Table III). Carbon-13 NMR and ^1H NMR had already predicted conformational differences between acid and salt forms. This evidence indicates that similar changes also occur in the formation of the sodium complex with MonH in neutral solution.

Table III. Frequencies (Hz) of Several MonNa Methyl Groups at 100 MHz in $CDCl_3$ vs. Me_4Si as Compared to Solutions of 1:1 MonH to $NaClO_4$

Methyl	MonNa frequency	1:1 MonH–$NaClO_4$ frequency
1	116, 123	105, 112
2	340	341
3	112, 128	131, 138
5	152	147

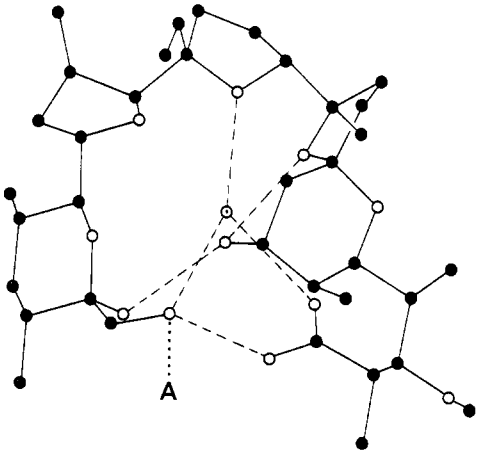

Figure 8. The monensin crystalline structure.[13] Closed circles are carbons, open circles are oxygens with the water oxygen dotted, dashed lines indicate hydrogen bonds, and A is a possible position for associated exchangeable water.

The position of the bound water signal has been found to be solvent dependent, even though, as shown in Figure 8, the water molecule itself would seem to be isolated somewhat from the solvent environment. Shifts of 6.03 ppm in chloroform-d_1, 4.83 ppm in tetrahydrofuran-d_8, and 4.98 ppm in acetone-d_6 were observed for 0.20 *M* MonH solutions. Attempts to obtain a reasonable ^1H NMR spectrum in methanol-d_4 were unsuccessful due to the interference of the normal methanol hydroxyl peak. The deuterium oxide experiment did indicate that the water molecule was labile in methanol which explains the interference of the OH signal as the OD exchange of methanol-d_4 with the water available from MonH. The variation in peak position in the other three solvents is also due to exchange of the hydrogen-bonded water molecule with free water in the solvent. All three peaks are in a position downfield of the shift of pure free water. In tetrahydrofuran the peak is closest to the free water position of 4.72 ppm,[28] with the bound water signal in acetone-d_6 slightly further and chloroform-d_1 very distant from the free position.[29] This would tend to support a simple model of free and bound exchange based primarily on water solubility in each solvent.

Lowering the temperature produces a broadening and downfield shift of the water peak in all solvents (Figure 9). In addition, the peak splits to give a lower field broader line and an upfield sharper peak. Both lines migrate further downfield at approximately constant separation with further decrease in temperature. This splitting seems to indicate that the water molecule can be attached to MonH at two different sites. The separations and temperatures of coalescence, shown in Figure 9, indicate that the exchange between these sites is the easiest in tetrahydrofuran-d_8 and most difficult in chloroform-d_1. The further downfield shift reaffirms the previous model of exchange with water in the solvent in that the decrease in temperature seems to favor

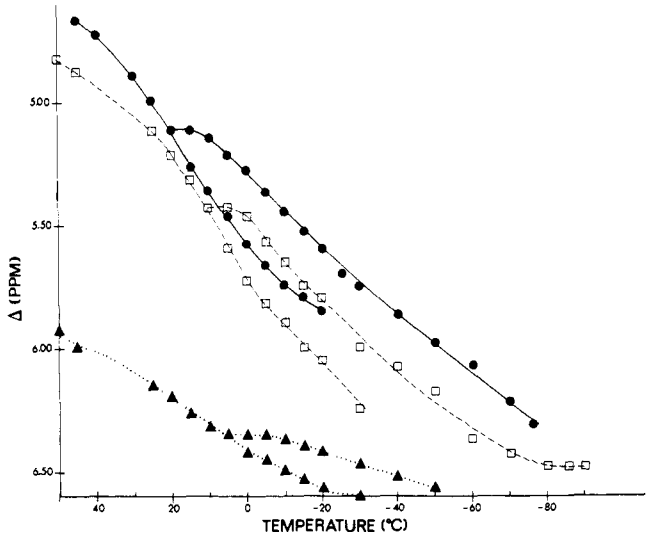

Figure 9. Proton nuclear magnetic resonance study of 0.25 *M* MonH in tetrahydrofuran-d_8 (●), acetone-d_6 (□), and chloroform-d_1 (▲). Signal separations and coalescence temperatures are 18 Hz, 20°; 15 Hz, 10°; and 8 Hz, 0°, respectively.

an equilibrium toward the bound water molecules with fewer and fewer free water molecules in the solvent. There is little indication of the signals reaching a frequency characteristic of completely bound water molecules before broadening and solvent freezing make measurements impossible.

Figure 10 shows the behavior of the bound water peak in acetone-d_6 and chloroform-d_1 at ambient temperature as sodium perchlorate is gradually added to the solutions. In both cases a steady decrease in peak intensity is observed as the 1:1 ratio is approached, but in acetone a downfield shift is noted while in chloroform the peak moves upfield. A simple displacement of the water molecule in the monensin cavity by the sodium ion fails to account for the observed behavior since in this case only increasing amounts of free water would be observed. Rather it may be necessary to invoke a model in which water is exchanged into the solvent by way of two sites, each site being affected differently by interaction of MonH with the sodium ion. The sharper signal, due to the more loosely hydrogen-bonded molecule, is thought to arise from a water in position A illustrated in Figure 8 rather than in the center of the MonH species. The broad signal may come from the water molecule in the center of the MonH ring. The water molecule at A would be easier to release to the solvent since only one hydrogen bond would need to be broken. It could also remain on its site even when a sodium ion occupies the monensin cavity. Further differential behavior between acetone-d_6 and chloroform-d_1 is noted when sodium perchlorate addition experiments are conducted at temperatures below the coalescence temperature of the two water signals. In chloroform-d_1, as sodium ion is added, the sharper peak, due to associated water, gradually decreases in intensity without change in position. The bound water peak after a small shift at low sodium concentrations shows the same behavior. As mentioned above, the sharp peak in the infrared spectrum also slowly decreased with sodium ion addition. In the acetone-d_6 case, however, the sharp ^{1}H NMR peak immediately disappears even at very low sodium perchlorate concentration and the broader peak stays in position and gradually loses intensity as 1:1 mole ratios are reached.

Further analysis is definitely required, but the data obtained above and below the coalescence temperature seem to warrant several speculative conclusions. At the higher temperatures addition of sodium in acetone-d_6 causes an increase in the population of the more tightly held water molecules, perhaps by way of depleting the associated molecules which causes an increase in the bound population as equilibrium between associated and bound is reestablished and free available water is taken up. At lower temperatures, however, this pathway is blocked by the lack of exchange between bound and associated positions and only a sudden drop in the associated peak with no shift is observed. In chloroform-d_1 at higher temperatures, sodium addition causes an increase in some freer water population, perhaps by simple gradual displacement of water from the cavity into the bulk solvent. Low-temperature results confirm this idea in that associated and bound peaks shrink at approximately the same rate.

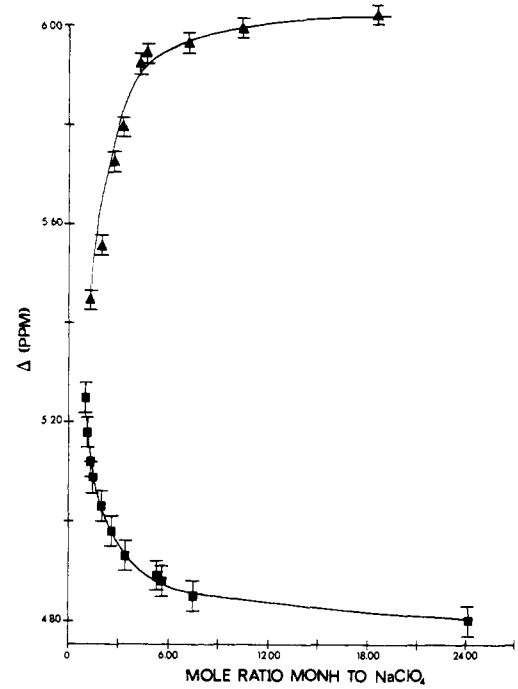

Figure 10. Proton nuclear magnetic resonance study of 0.25 *M* MonH in chloroform-d_1 (▲) and acetone-d_6 (■).

The model of three-site water exchange, complicated by monensin complexation of both water and sodium ion, anomalous deprotonation, and solvent and temperature dependent phenomena, is far from being fully understood at this point and deserves further study. However, the definite existence of three sites for the water molecule and the dramatic differences observed with sodium ion interaction in a hydrophilic and a hydrophobic solvent indicate that the behavior of monensin in various solvents is much more complex than expected from previous studies.

Conclusion

In summary, we have shown that monensin is a very weak acid in methanol solution, existing in primarily the associated form, but is still capable of selectively forming alkali metal ion complexes. It appears that the complex formed in this manner has a structure dissimilar to that of the normal salt prepared in basic solution. Efforts to isolate and more fully characterize this new complex are presently underway. In addition, some of the chemistry of the neglected monensin water molecule has been revealed and a case of solvent dependency, site exchange, and sodium ion interaction has been found. Further solution studies may provide a clearer model for monensin selective ion complexation and transport and may also assist in explanations of its biological activity.

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