Thallium-205 and Proton Nuclear Magnetic Resonance Investigation of the Complexation of Thallium by the Ionophores Monensin and Nigericin†

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ABSTRACT: A thallium-205 and proton nuclear magnetic resonance study of the ionophores monensin and nigericin is reported. Evidence is presented for the simultaneous existence of two forms of monensin free acid in chloroform solution, one containing a water molecule in the central cavity and one with a water molecule on the periphery, linking the ends of the monensin molecule by hydrogen bonding. There appear to be three structurally different monensin complexes of thallium, two in the salt form and one in the acid form. In free nigericin acid, both terminal hydroxyl moieties hydrogen bond to the terminal carboxylic acid oxygens; in the Na^+ salt, the binding of one of the carboxylate oxygens to the metal ion allows but one of the terminal OH's to hydrogen bond strongly. Thallium-205 chemical shifts and spin–lattice relaxation times in the title complexes are discussed and compared to those in ionophores studied previously.

The antibiotics monensin (A-3823A) and nigericin (A-51, in some earlier papers often called polyetherin A) are members of the carboxylic antiobiotics, which inhibit mitochondrial respiration and reverse the translocation of cations induced by antibiotics such as valinomycin or the actins (Pressman, 1968; Haney and Hoehn, 1968; Estrada-O. et al., 1968). This family of antibiotics includes dianemycin, grisorixin, lasalocid (X-537A), lysocellin, X-206, A204A, lenoremycin (Ro 21-6150 or A-130A), lonomycin, salinomycin, septamycin, carriomycin, and alborixin. Monensin and nigericin are isolated from cultures of *Streptomyces cinnamonensis* and *Streptomyces hygroscopicus*, respectively.

X-ray crystallography studies have provided structures for monensin acid (Lutz et al., 1971b), its sodium bromide complex (Ward et al., 1978), and its silver, sodium, potassium, and thallium salt complexes (Smith et al., 1977b; Agtarap et al., 1967; Pinkerton and Steinrauf, 1970), as well as for the silver (Steinrauf et al., 1968, 1971; Kubota et al., 1968, Shiro and Koyama, 1970) and potassium (Geddes et al., 1974) salts of nigericin. The cation complexation specificity has been found to be $K^+ > Rb^+ > Na^+ > Cs^+ > Li^+$ for nigericin (Pressman, 1968; Lardy et al., 1967; Ashton and Steinrauf, 1970; Henderson et al., 1969; Lutz et al., 1970; 1971a) and $Ag^+ > Na^+ > K^+ > (Li^+) > Rb^+$, $Tl^+ > Cs^+ > (Li^+)$, NH_4^+ for monensin (Pressman, 1968; Lardy et al., 1967; Ashton and Steinrauf, 1970; Henderson et al., 1969; Lutz et al., 1970; 1971a; Cornelius et al., 1974; Gertenbach and Popov, 1975). There is disagreement as to the exact placement of lithium in the monensin series, depending upon the systems studied and the technique employed for stability constant determination. Computer simulations of the antibiotics have recently been reported (Steinrauf and Sabesan, 1977; Smith and Duax, 1977a).

In addition to ^1H NMR[1] studies of monensin (Agtarap and Chamberlin, 1968; Gorman et al., 1968; Anteunis and Rodios, 1978; Anteunis, 1977), including ones in which analyses yielded solution conformations of monensin acid (Anteunis and Rodios, 1978) and its sodium salt (Anteunis, 1977), ^{23}Na NMR of the sodium salt complex (Haynes et al., 1971) and ^{13}C, ^1H, ^7Li, and ^{23}Na NMR of the acid and its lithium and sodium salts (Gertenbach and Popov, 1975) have been reported. Nuclear magnetic resonance studies of nigericin are fewer in number, including a ^1H study and conformational analysis of both the acid and the sodium salt (Rodios and Anteunis, 1977) and a ^{23}Na NMR study of the sodium salt (Haynes et al., 1971).

In this paper we report the results of ^{205}Tl NMR and ^1H NMR investigations of monensin and nigericin and their metal complexes. Previous results of ^{205}Tl NMR investigations of the neutral, cyclic ionophores valinomycin (Briggs and Hinton, 1978b), nonactin, monactin, and dinactin (Briggs and Hinton, 1978a) indicated that characteristic, widely separated ^{205}Tl chemical-shift regions might serve to diagnose the antibiotics as to number, type, and spatial arrangement of ligand atoms. In this respect, it was desirable to see what effect complexation of Tl^+ by acyclic, carboxylic acid antibiotics, such as monensin and nigericin, might have on the ^{205}Tl chemical shift and spin–lattice relaxation time.

If studies of ^{205}Tl chemical shifts and T_1 values in these relatively well-characterized species do reveal information about the number and geometry of ligand atoms and/or the type of ligands, as was the case for Tl^+ solvated by various solvents (Hinton and Briggs, 1975), ^{205}Tl NMR could prove to be a very useful method for studying the chemistry of monovalent ions in biological systems. Features of Na^+ and K^+ ion transport across biomembranes should be amenable to a thallium probe, as Tl^+ is remarkably similar to alkali metal ions—especially potassium—in its chemical and biological behavior.

Experimental Procedure

Materials. Monensin and nigericin were received as their sodium salts through the generosity of Eli-Lilly and Co. All metal salts were reagent grade. Fisher spectrophotometry grade $CHCl_3$ and Stohler Isotope Chemicals $CDCl_3$ (99.8% ^2H) were dried over freshly activated Linde 3-Å molecular sieves.

Methods. The acid forms of the antibiotics were prepared by extraction of a $CHCl_3$ solution of the sodium salt with an

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[1] Abbreviations used: NMR, nuclear magnetic resonance; T_1, spin–lattice relaxation time; FFT, fast Fourier transform; o.d., outside diameter; IR, infrared; CSA, chemical-shift anisotropy; SR, spin rotation.

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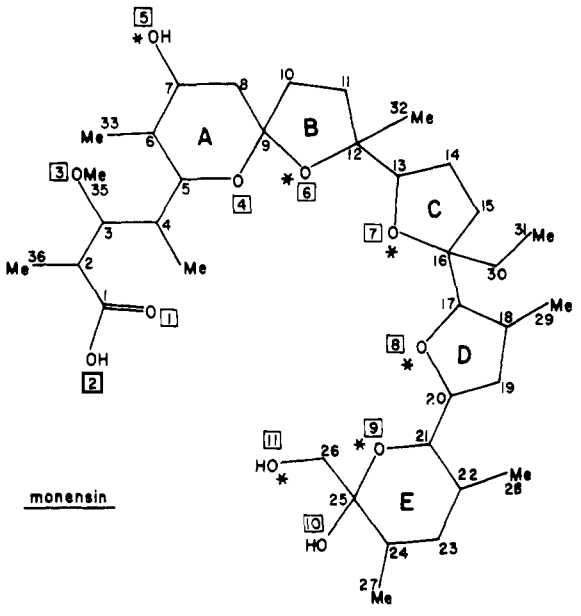

FIGURE 1: Monensin acid structure. Asterisks indicate oxygen atoms which participate in metal binding.

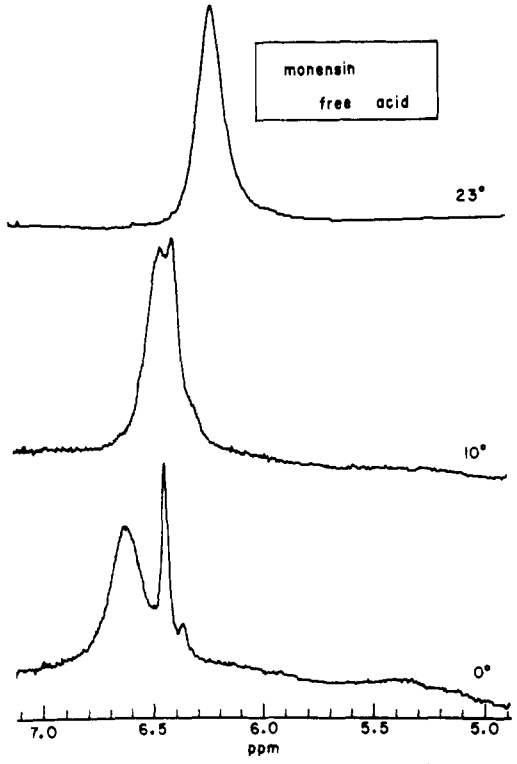

FIGURE 2: Temperature dependence of the 90-MHz ^{1}H NMR signals of the CO_2H, H_2O, and OH-10 and -11 protons of monensin acid in chloroform (concentration ca. 0.1 M).

excess of 0.1 M aqueous $HClO_4$. After evaporation of solvent from the organic phase, the acidified antibiotic was dissolved in $CDCl_3$ for accumulation of ^{1}H spectra. Thallium salts of monensin and nigericin were prepared by repeated passage of a chloroform solution of the acid form of the ionophores over a column of Tl_2CO_3 or by stirring of excess Tl_2CO_3 in the chloroform solution followed by filtration.

The thallium complex of monensin acid was prepared by adding a tenfold excess of $TlClO_4$ in methanol to the acid, stirring vigorously for about 1 h, evaporating the methanol, dissolving the complex in chloroform, and filtering to remove excess $TlClO_4$. After ^{1}H spectra of the thallium complexes in $CDCl_3$ were recorded, the solvent was evaporated and $CHCl_3$ was added to provide a ^{1}H lock signal for accumulation of ^{205}Tl spectra.

Degassed samples for T_1 experiments were prepared by application of six or seven consecutive freeze–pump–thaw cycles, admission of ca. 0.8 atm of dry nitrogen gas, and sealing. T_1 values were obtained using the inversion–recovery method.

Both ^{1}H and ^{205}Tl NMR spectra were obtained using the pulsed FFT mode on a Bruker HFX-90 operating at 2.114 T (21.14 kG). Nicolet Corp. pulse timers, NMR-80 minicomputer, and disk system were used for control of data acquisition parameters and storage of spectra. The ^{205}Tl system, employing a homemade probe, transmitter, and receiver, also included a Model 3100L ENI broad-band amplifier. Ambient probe temperature was 23 ± 1 °C. Samples were contained in 5-mm o.d. NMR tubes; concentrations were 0.10–0.15 M.

Results and Discussion

Monensin Acid. The structure of monensin acid is shown in Figure 1. The asterisks denote those oxygen atoms which participate in metal bonding. Proton NMR spectra of the acid have previously been published (Gertenbach and Popov, 1975; Anteunis and Rodios, 1978) and analyzed to yield chemical shifts and coupling constants (Anteunis and Rodios, 1978). One of the more interesting characteristics of the molecule is the hydrogen bonding between the head and tail, which "buttons" the chain together to form a quasicyclic structure both in the solid (Lutz et al., 1971b) and in solution (Gertenbach and Popov, 1975; Anteunis and Rodios, 1978). This head to tail buttoning also occurs in the cation complexes. Another interesting feature is the role of the water molecule associated with the antibiotic. The X-ray structure of the crystalline solid (Lutz et al., 1971b) shows a water molecule to be bound in the center of the cavity, hydrogen bonded to O-7 of the furan ring C, the hydroxyl proton OH-11 of the primary alcohol function, and O-1 of the carboxylate group. Other investigators (Gertenbach and Popov, 1975; Anteunis and Rodios, 1978) have reported that a broad ^{1}H NMR peak at ca. 6 ppm separates at low temperatures into a broad downfield peak and a sharper upfield signal. One group (Gertenbach and Popov, 1975) attributed the broad signal to enclathrated water trapped in the antibiotic cavity and the narrow signal to associated, exchangeable water bridging the terminal carboxylic and alcoholic functions. The other group (Anteunis and Rodios, 1978) assigned the broad peak to the carboxylic acid proton, the primary alcohol proton, and water, while the narrow peak was assigned to the tertiary alcohol proton OH-10. Their assignments were made from the measurement of peak areas attributable to four protons and one proton, respectively, and detection of coupling of the narrow signal to the H-24 proton ($J \leq 1.5$ Hz). They were not able to definitely assign the water molecule to either the cavity (enclathrated) position or the peripheral (buttoning) position.

We also performed the low-temperature experiments, including measurement of peak areas, which showed the broad peak to be comprised of four protons and the narrow peak, one proton, corroborating Anteunis' assignment. However, as depicted in Figure 2, at lower temperatures *two* narrow signals could be resolved, as well as an additional very broad, low-intensity signal at ca. 5.4 ppm. We interpret this to reflect the simultaneous presence of both forms of hydrated antibiotic. If one compares the proposed hydrogen-bonding configuration of monensin with enclathrated H_2O [Rodios and Anteunis

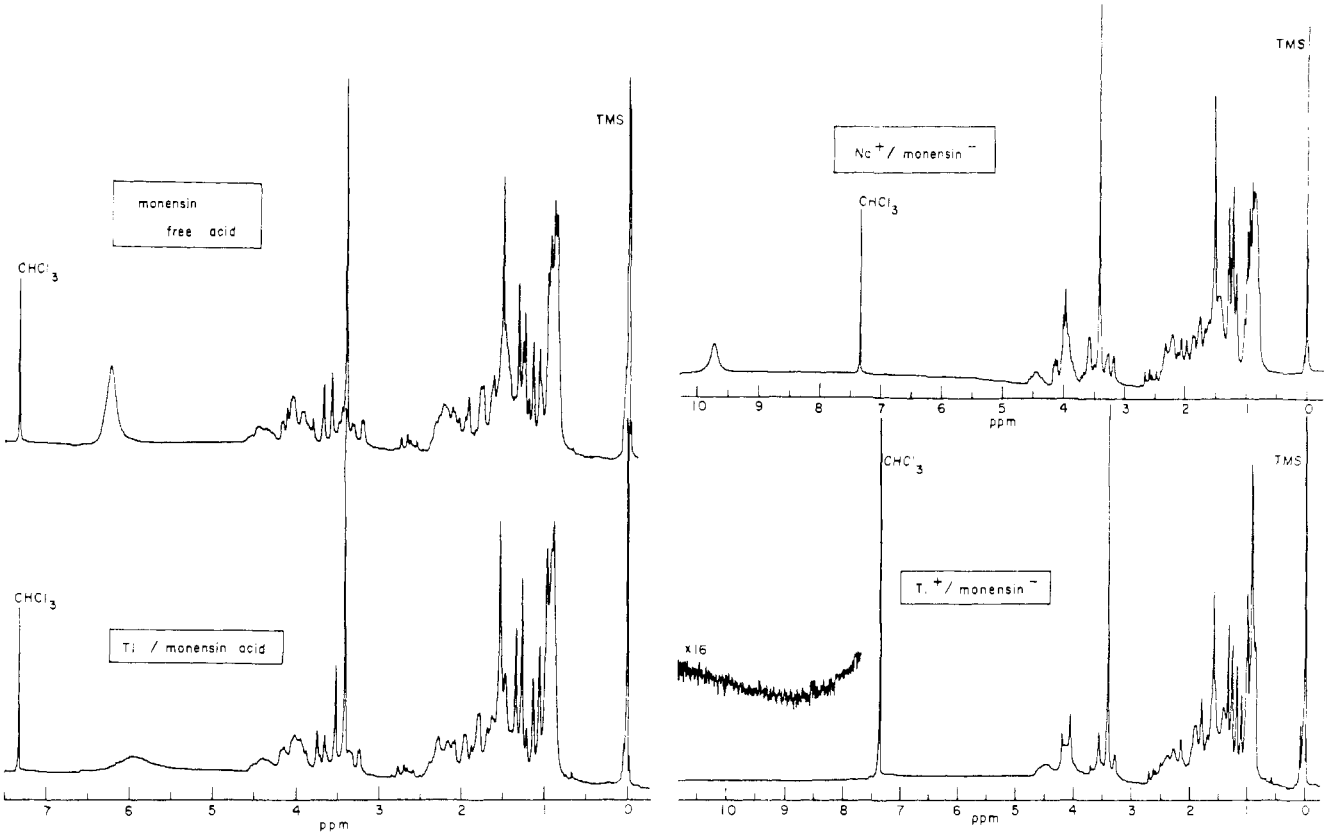

FIGURE 3: The 90-MHz ^1H NMR spectra of monensin acid, the thallium-monensin acid complex, and the sodium and thallium salts of monensin. Concentrations were 0.1 M in chloroform; the temperature was 23 °C.

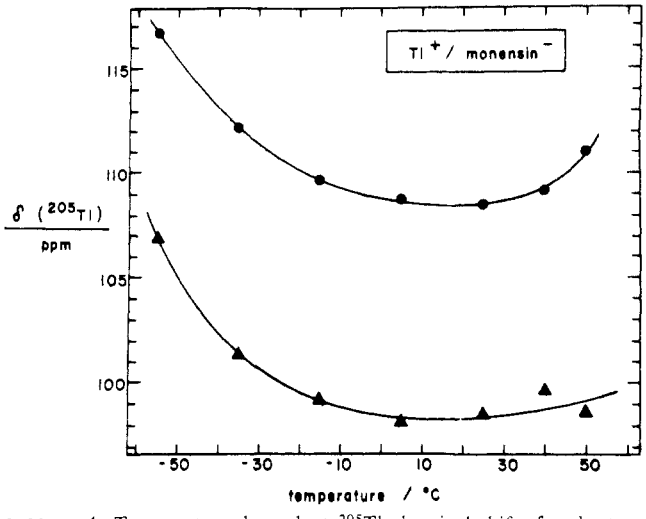

FIGURE 4: Temperature-dependent ^{205}Tl chemical shifts for the two forms of the Tl^+/monensin$^-$ complex in chloroform. Concentration was 0.15 M.

(1977), Figure 5] with that of monensin with peripheral H_2O [Anteunis and Rodios (1978), Figure 2], one of the most apparent differences is the role played by OH-10. In the clathrate structure, OH-10 hydrogen bonds only to O-5; in the peripheral structure, O-10 also hydrogen bonds to the peripheral water molecule. Thus, if exchange rates are sufficiently slowed, two OH-10 signals should be resolved, one for each hydrate species, as is observed. At the appropriate temperature conditions, two water peaks would also be expected to be observed. Both models permit the observed rapid exchange of the CO_2H, H_2O, and OH-11 protons.

A definite assignment of the two sets of peaks is not possible at present. The ratio of the downfield pair at 6.62 (broad) and 6.43 ppm (narrow, OH-10) to the upfield pair at 5.4 (broad) and 6.36 ppm (narrow, OH-10) is about 4 to 1.

Monensin Acid-Tl Complex. The ^1H spectrum of the Tl-monensin acid complex differed noticeably from that of the free acid (Figure 3), especially the downfield peak which shifted from 6.2 to 5.9 ppm and broadened from 11.5 to 40 Hz upon complexation. In the complexation of Na^+ by monensin acid, Gertenbach and Popov (1975) noted a similar upfield shift, and that, although H^+ is lost as Na^+ is complexed, the 1704-cm^{-1} carboxylic acid IR stretch remained unchanged and no 1563 cm^{-1} carboxylate band appeared. The structure of the NaBr complex of monensin acid has recently been published (Ward et al., 1978) showing that the carboxyl group is also protonated in the crystal, with the bromide ion hydrogen bonded between OH-5 and -10. The neutral sodium complex (Ward et al., 1978) is structurally very similar to the dihydrated silver salt (Pinkerton and Steinrauf, 1970), with silver replaced by sodium, water-2 replaced by bromide, and water-1 vacant. Thallium-205 chemical-shift data, shown in Table I, indicate that the neutral thallium complex and the thallium salt are structurally different, probably because of the carboxyl protonation and the presence of the ClO_4^- ion in the former.

Monensin-Metal Salt Complexes. The ^1H spectrum of the Tl^+-monensin$^-$ complex differs markedly from that of either the free acid or the acid complex (Figure 3). The only indication of the hydroxyl protons, OH-10 and -11, was an extremely broad band at ca. 14 ppm (which, in Figure 3, is folded from the high-frequency side of the carrier frequency and, thus, appears inverted). The ^1H spectrum of the thallium-monensin$^-$ complex was also different from that of the sodium-monensin$^-$ complex, the latter showing a broad absorption ($\Delta\nu_{1/2} \approx 11.5$ Hz) at 9.64 ppm which integrated for two pro-

TABLE I: Thallium-205 Chemical Shifts and Spin-Lattice Relaxation Times of Some Ionophore Complexes of Tl^+ in $CHCl_3$.[a]

ionophore	δ (^{205}Tl)(ppm)[b]	nondegassed T_1(s)[c]	degassed T_1(s)[c]	temp (°C)
monensin (acid)	134.1	0.15 ± 0.05	0.40 ± 0.10	23
monensin$^-$			0.47	−20
	106.7, 96.6	0.14 ± 0.03	0.51	23
			0.52 ± 0.07	45
nigericin$^-$	126.6	0.08 ± 0.01	0.33 ± 0.06	23
nonactin[d]	−261.5	2.05	2.45	24
monactin[d]			1.91	0
	−261.7	2.00	2.15	24
			2.12	40
			1.94	50
dinactin[d]	−262.0	2.07	2.08	24
valinomycin[e]			1.90	0
	−540.0	0.40	2.26	23
			2.72	38

[a] Concentrations were 0.11 ± 0.03 M. [b] Chemical shifts with respect to extrapolated infinite-dilution shift of aqueous $TlNO_3$; positive shifts denote high frequency. [c] ±10%, except as listed. [d] Briggs and Hinton (1978a). [e] Briggs and Hinton (1978b).

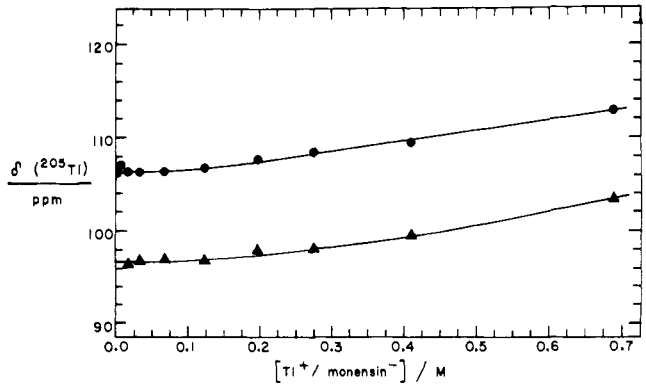

FIGURE 5: Concentration-dependent ^{205}Tl chemical shifts for the two forms of the Tl^+/monensin$^-$ complex in chloroform (23 °C).

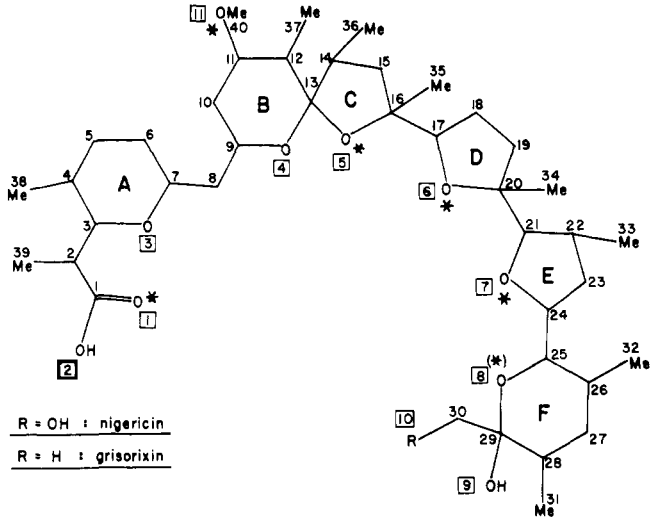

FIGURE 6: Structure of the acid forms of nigericin and grisorixin. Oxygen atoms which participate in metal binding are denoted by asterisks.

tons and which we ascribe to OH-10 and -11, both strongly hydrogen bonded to the carboxylate tail. Anteunis (1977) is the only investigator to have previously reported such a downfield peak, which he attributed to OH-10 (no OH-11 peak was observed). Although his reported shift and line width were 9.60 ppm and 45 Hz, he employed a lower temperature (19 °C) and a higher field strength (70.47 kG).

Although crystallographic studies (Agtarap et al., 1967; Pinkerton and Steinrauf, 1970) of monensin salts show two associated water molecules, we, like Anteunis (1977), see no evidence for their presence in chloroform solution. Even in the solid, it was postulated that the water molecules were cocrystallized as a result of the packing of the unit cell, rather than being an integral part of the molecular structure of the complex (Pinkerton and Steinrauf, 1970).

The ^{205}Tl spectrum of the Tl^+–monensin$^-$ complex showed that two types of complexes were present, with the downfield species being predominant by a factor of about six at −55 °C. Neither of these two species corresponds to the complex formed by monensin acid (see Table I). Both the temperature dependence (Figure 4) and the concentration dependence (Figure 5) of the two Tl^+/monensin$^-$ species were monitored to verify this.

Two different crystal forms of monensin salt complexes have been isolated for X-ray studies (Pinkerton and Steinrauf, 1970; Smith and Duax, 1977b), type Na (sodium, silver) and type K (potassium, sodium, silver, thallium). As the *b* axis for type K crystals was about twice that for type Na crystals, while the *a* axes and the *c* axes were nearly the same for both types, it was felt that the two types resulted merely from differences in packing arrangements and not from differences in molecular structure (Pinkerton and Steinrauf, 1970). Our ^{205}Tl data suggest that the two molecular conformations do indeed exist in chloroform solution, even though only one crystal form was observed for Tl^+–monensin$^-$. Two conformations for Tl^+ complexes of nonactin and monactin were also inferred from ^{205}Tl temperature-dependent shift data, and two thallium-205 peaks were observed concurrently for the Tl^+–dinactin system at low temperature (Briggs and Hinton, 1978a). Two crystalline forms, type Na (sodium, potassium) and type Rb (potassium, rubidium), of the tetranactin complex have been shown to be structurally different (Iitaka et al., 1972; Nawata et al., 1974).

Nigericin Acid. Figure 6 shows the structure of nigericin acid, with asterisks signifying oxygen atoms which participate in binding the cation. Its 300-MHz ^{1}H NMR spectrum in $CDCl_3$ has been previously analyzed in another laboratory (Rodios and Anteunis, 1977). They reported a broad peak of greater than 300-Hz line width at about 5.3 ppm, which resulted from about three protons (CO_2H, OH-9, OH-10). We also observed the broad three-proton signal, but at about 6.0 ppm and with a line width of about 80 Hz. We attribute this

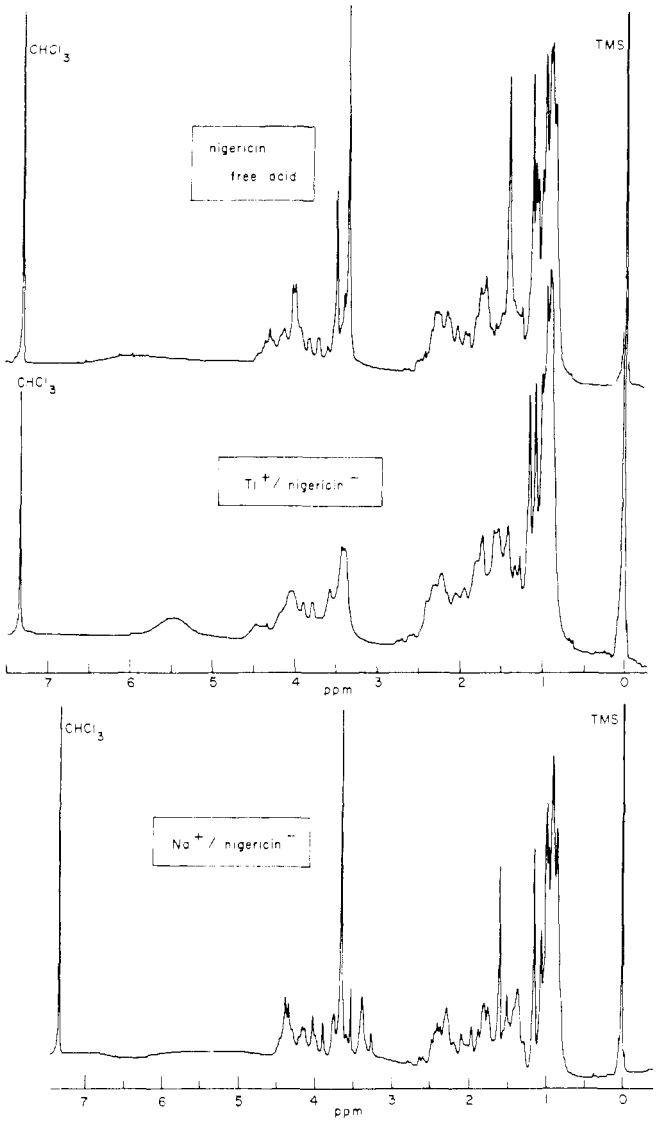

FIGURE 7: The 90-MHz ^1H NMR spectra of nigericin acid and its thallium and sodium salts. Concentrations were 0.1 M in chloroform; the temperature was 23 °C.

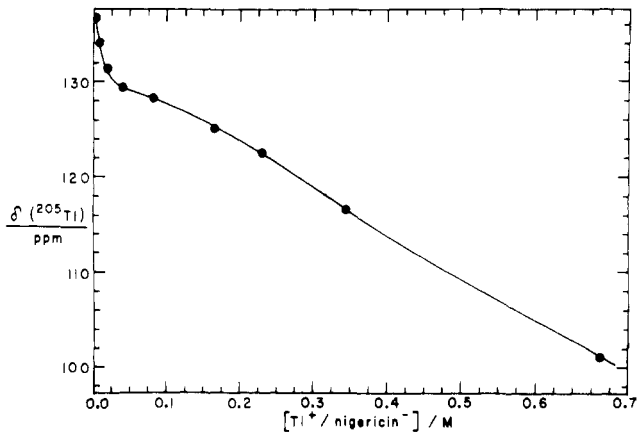

FIGURE 8: Concentration-dependent ^{205}Tl chemical shift of the Tl^+/nigericin$^-$ complex in chloroform (23 °C).

difference to the difference in field strength and the fact that our spectra were obtained at 23 °C rather than 19 °C. No water signal was observed in any of the nigericin acid or salt spectra, in accord with their results.

Nigericin–Metal Salt Complexes. The proton spectra of the Na^+–nigericin$^-$ and Tl^+–nigericin$^-$ complexes were markedly different from those of the free acid (Figure 7). We observed only one broad ($\Delta\nu_{1/2} \approx 50$ Hz) hydroxyl proton band, at about 9.54 ppm, for the sodium salt. Rodios and Anteunis (1977) observed two bands, one at 5.54 ppm (OH-10) and one at 10.29 ppm (OH-9), which exchanged slowly and collapsed with increasing temperature. They proposed that in the acid both OH-9 and -10 could hydrogen bond with the carboxylic acid oxygens in "buttoning" the ends of nigericin together, while in the sodium salt, where one of the carboxylate oxygens is bound to the sodium ion, only one hydroxyl group, OH-9, could effectively hydrogen bond with the remaining carboxylate oxygen.

The ^1H spectrum of the thallium–nigericin salt differs in three significant respects from that of the sodium salt. First, the sharp methoxy (OMe-40) singlet, which appears at 3.63 ppm in the sodium salt, is less intense in the thallium salt. Second, the sharp Me-35 (or Me-34) singlet at 1.59 ppm in the sodium salt spectrum is also less intense in the thallium salt spectrum. The smaller intensities are probably due to couplings with the thallium nuclei. Thallium–proton couplings have previously been reported for thallium complexed by the actins (Briggs and Hinton, 1978a) and by the cryptand C-222 (Lehn et al., 1970). An appreciable coupling is especially likely for the methoxy moiety, whose oxygen is a ligand for the entrapped metal ion (Steinrauf et al., 1968, 1971; Kubota et al., 1968; Shiro and Koyama, 1970; Rodios and Anteunis, 1977; Geddes et al., 1974). Third, only one hydroxyl proton band is observed for the thallium complex, at 5.47 ppm, with a line width of 35 Hz. This suggests that in the case of the thallium salt *both* hydroxyl protons are involved in buttoning together the ends of nigericin, much as they were in the acid form. This leads to the conclusion that the metal to carboxylate oxygen binding is much weaker in the thallium complex than in the sodium complex.

It has been speculated (Steinrauf et al., 1971) that when nigericin complexes larger cations, the carboxylate group would be forced away from the central cavity, making both of its oxygens available for hydrogen bonding. Concurrently, as the hydroxyl functions rotate outward to follow the carboxylate, pyran ring F would twist its oxygen inward toward the metal ion, and O-8 could then either supplant or supplement the carboxylate oxygen as ligand. An X-ray diffraction study of the potassium salt (Geddes et al., 1974) compared to results for the silver salt (Steinrauf et al., 1968, 1971; Kubota et al., 1968; Shiro and Koyama, 1970) supports this speculation: the metal–oxygen distance for O-1 increased from 2.23 Å in the Ag^+ salt to 2.58 Å in the K^+ salt, while the distance from the cation to oxygen-8 and -9 decreases when K^+ replaces Ag^+ from 3.40 to 3.09 Å and from 3.77 to 3.06 Å, respectively. (The ionic radii are 1.26, 1.33, and 1.40 Å for Ag^+, K^+, and Tl^+, respectively.)

The concentration dependence of the ^{205}Tl chemical shift (Figure 8) suggests that these metal–oxygen distances might be dependent upon concentration as well. Whereas the concentration dependence of the thallium signal(s) in monensin is less than 7.5 ppm (vide supra), in nigericin it is more than 35 ppm. More significantly, as the concentration decreases below 0.02 M, there is a significant change in the shift, much as would be expected for an ion-pairing process. We attribute this to an inward movement of the carboxylate moiety, accompanied by O-8 twisting away from the thallium ion. This might reasonably be expected if one assumes that aggregation of 1:1 complexes, through interactions of their polar head to tail regions, occurs at high concentrations. Then, as the con-

centration decreases aggregation should diminish, leaving the polar regions of the isolated complexes exposed to the hydrophobic solvent. Such an unfavorable interaction might stimulate the conformational change, in which part of the polar hydrogen-bonding region of nigericin is rotated toward the polar ion cavity and away from the hostile solvent environment. Such a conformational change might also be expected as the nigericin complex begins its journey through the lipid portion of a membrane after the complexation of the cation at the polar membrane boundary.

Support for this explanation is afforded by comparison of the thallium shifts in the monensin salt complex, in which there is sixfold coordination (asterisks, Figure 1), with those of the nigericin complex, in which coordination is either five- or sixfold (asterisks, Figure 6). At high concentrations, where the nigericin coordination is proposed to be sixfold and the thallium interaction with the carboxylate oxygen ligand is proposed to be relatively weak, the shift is about 100 ppm, nearly the same as the monensin value. At low concentrations, where we suggest that the nigericin coordination is fivefold and the carboxylate oxygen interaction with the thallium ion is relatively strong, the shift is >135 ppm, reflecting the increased electronic anisotropy and the resultant larger parametric shift of the thallium ion.

^{205}Tl Spin-Lattice Relaxation. The spin-lattice relaxation times for ^{205}Tl in the ionophore complexes (Table I) also indicate the inherent asymmetry of the ligand atoms of monensin and nigericin. In valinomycin (Briggs and Hinton, 1978b) and the actins (Briggs and Hinton, 1978a), which are characterized by a symmetrical ligand geometry, the ^{205}Tl T_1 values in degassed solutions are relatively long, ca. 2.2 s. In contrast, the T_1 values for monensin and nigericin are about 0.5 and 0.33 s, respectively. In valinomycin, the relaxation was about 90% chemical-shift anisotropy (CSA) and 10% dipolar; in the actins, relaxation was approximately 50% spin rotational (SR), 40% dipolar, and 10% CSA. Although the relaxation mechanisms for monensin and nigericin were not quantitatively determined, the near invariance of the T_1 in the monensin complex from −20 to 45 °C indicates that at 21.14 kG spin rotation contributes about 50% of the total relaxation rate. The absence of crystallographic data on the hydrogen positions of monensin and nigericin precluded a quantitative estimation of a dipolar relaxation rate, which, because of participation of OH and OMe oxygens in metal binding, would be expected to be somewhat larger than for the actins (Briggs and Hinton, 1978a). This would mean that CSA would be the second most efficient relaxation mechanism for monensin (and probably nigericin, also). Relative CSA relaxation rates for ^{205}Tl appear to decrease in the same order as the observed ^{23}Na quadrupole coupling constants in the sodium complexes (Haynes et al., 1971): nigericin > monensin > valinomycin ≫ actins. This is expected, since both mirror the degree of electronic asymmetry about the metal ion. Thallium-205 SR relaxation rates apparently decrease in the order nigericin ≈ monensin > actins ≫ valinomycin. One would expect that dipolar relaxation rates, reflecting the proximity of hydrogen atoms to the ion cavity, would decrease in the order monensin ≈ nigericin ≳ actins > valinomycin.

Monensin and nigericin, like valinomycin but unlike the actins, do not completely enshroud the metal ion from its environment, as shown by the dependence of the ^{205}Tl T_1 on dissolved oxygen. Proximate interaction of Tl^+ and a paramagnetic species, such as would occur in a collision of dissolved oxygen with Tl^+, provides an extremely efficient relaxation mechanism. In solutions of Tl^+ in various solvents and in the thallium complexes with valinomycin (Briggs and Hinton, 1978b), monensin, and nigericin, the T_1 in nondegassed solutions is much shorter than it is in degassed solutions. However, in the actin complexes (Briggs and Hinton, 1978a) the T_1 values are nearly identical for both degassed and nondegassed solutions, implying that the actin framework surrounds the thallium ion in such a manner as to prevent collisional access by dissolved oxygen molecules.

Thallium-205 Chemical Shifts. The large chemical-shift range (nearly 700 ppm) of thallium in the ionophore complexes suggests that ^{205}Tl NMR could possibly be a sensitive probe for determining the ligand environment in biological membranes. At present, the experimental data are too sparse, and the factors affecting the ^{205}Tl chemical shift are not understood well enough to allow quantitative interpretation. From the data in Table I it is obvious that the shifts are divided into distinct regions, depending on the type and number of ligands.

An excellent correlation between the ^{23}Na chemical shift and solvent basicity has been found for solvation of Na^+ by a wide range of solvents (Erlich and Popov, 1971; Erlich et al., 1970; Greenberg et al., 1973). A similar correlation was found for Tl^+ (Hinton and Briggs, 1975). Unlike the shifts for ^{23}Na in sodium-antibiotic complexes (Haynes et al., 1971), the shifts of ^{205}Tl seem to parallel the Lewis basicity of the ligand atoms, moving to low frequency as the basicity decreases in the order carboxylate > alcohol > ether > carbonyl. This indicates the possible importance of covalent forces in the thallium–ligand interactions in these complexes, which is consistent with the fact that thallium–carbon and thallium–hydrogen couplings have been observed in some of the complexes (Briggs and Hinton, 1978a,b; Lehn et al., 1970; Bystrov et al., 1977). Thallium, being larger and more polarizable, would be more capable of covalent interactions than would sodium. Sodium–ligand interactions in antibiotic complexes of sodium were deemed almost entirely electrostatic, since the shift/basicity relationship was poor (Haynes et al., 1971). However, there is no reason to expect that the shift/basicity trend observed for Na^+ and Tl^+ in solvents need be followed in the ionophore complexes. X-ray crystallographic studies of cation–ionophore complexes have shown that ligand–cation distances can vary up to 0.2 Å for the same cation–ligand type combination. Differences in ligand–cation interactions between an optimally fitting complex and a poorly fitting one, especially if the ionophore cavity is larger than the cation, might then be sufficient to cause deviations from the shift/basicity correlation.

In addition to the covalent contribution to the chemical shift, repulsive overlap between the orbitals of the ligand atoms and the orbitals of the metal ion could be significant. The latter contribution depends upon the geometrical arrangement of the ligands and the "tightness" with which they encapsulate the ion. The data obtained thus far, however, seem to indicate that the covalent contribution is more important for ^{205}Tl.

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Oxidation of Heme Proteins by Alkyl Halides: A Probe for Axial Inner Sphere Redox Capacity in Solution and in Whole Cells†

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ABSTRACT: Iron(II) porphyrins in homogeneous solution, in heme proteins, and in intact human erythrocytes and lysed cells are oxidized by certain alkyl halides to the corresponding iron(III) complexes at room temperature. The mechanism established for the oxidation of hemes in homogeneous solution operates at all levels of biological integrity. It is an axial inner sphere process. Deoxyhemoglobin has about the same reactivity within and without cells. The speed of the reaction with the proteins is primarily governed by the steric accessibility to iron. The reactivity of an array of iron(II) proteins accords well with theoretical prediction. In contrast the reactivity of cytochrome b_5 does not. An examination of the oxidation and reduction of this protein with additional mechanistically defined reagents (trinitrobenzene and hydroquinone) shows it to be in the G rather than C conformation. The unusual redox characteristics of this protein can be rationalized on this basis.

The redox capacity of a heme protein may be altered by the microenvironment associated with its location in its native biological matrix. Indeed a sorting out of the reactivity parameters in whole cells at a defined molecular level for any transformation is difficult. To probe this problem, we have attempted to develop a series of mechanistically defined organic reagents with good permeation characteristics which will allow a direct comparison of the homogeneous redox chemistry of an iron porphyrin with that of this same highly ordered array in its native protein matrix and in whole cells. Our general

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