DRUG SOLUBILIZATION AND INTERACTION

IN BILE SALT SYSTEMS

by

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ABSTRACT

Drug Solubilization and Interaction

in Bile Salt Systems (April 1979)

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The bile salts, which are naturally occurring steroidal surfac-
tants, aid in the digestion, emulsification, and absorption of dietary
lipids and the transport of drugs across the intestinal wall. Drug-
bile salt interactions in aqueous and nonaqueous solvent systems have
been investigated using nmr spectroscopic techniques. The compounds
selected for this investigation were chosen because of their structural
diversity and are representative of a wide variety of drug types. Nmr
provides a nondestructive, nonperturbing and sensitive method of
probing the microenvironment, structure, and binding sites of inter-
active molecular assemblies as well as their reactivity with additives.
The effects of varying the concentration of the bile salt and of the
drug upon the ^{1}H and ^{13}C chemical shifts have been determined. The
nuclei which show the greatest changes with increasing concentration
are those which interact to the greatest extent and consequently are
those which are located at or near the binding site(s) of the
solubilized drug. Both the interactions and the reactivity of the
drugs are explicable in terms of their structural geometry and that
of the associated, or "micellar," bile salt.

DEDICATION

To my parents, Mr. and Mrs. Harry Shimotsu, for their patience, love, and moral support.

ACKNOWLEDGMENTS

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INTRODUCTION

A surfactant, which is a condensation of the term surface active agent, is an amphiphilic substance that lowers the surface and interfacial tension of a liquid.[1] Amphiphiles are molecules which have regions of hydrophobic and hydrophilic character. Among the naturally occurring surfactants which form aggregates in solution are lipids, such as fatty acids and phosphotidyl choline, and bile salts, which include cholic and deoxycholic acid. The physical and chemical behavior of surfactants in aqueous and non-aqueous solutions have been treated extensively in books and reviews.[2-4]

Micellar Structure in Aqueous Solutions

Micellization, the formation of aggregates or micelles[5] by amphiphiles, which occurs at amphiphilic concentrations above a critical micelle concentration (CMC), is the basis of surfactant organization. Micelles begin to appear over a narrow amphiphilic concentration range rather than at a precise point.[2]

Micellar structures in aqueous solutions are generally "normal" in type, usually involving micelles of average radii 12-30A, which contain 20-100 monomers, and which are spherical in shape (see Figure 1).[2] In normal structures, the hydrophobic, nonpolar ends of the monomers which make up the micelle are oriented toward the center, or core, of the aggregate. The hydrophilic, polar ends are oriented

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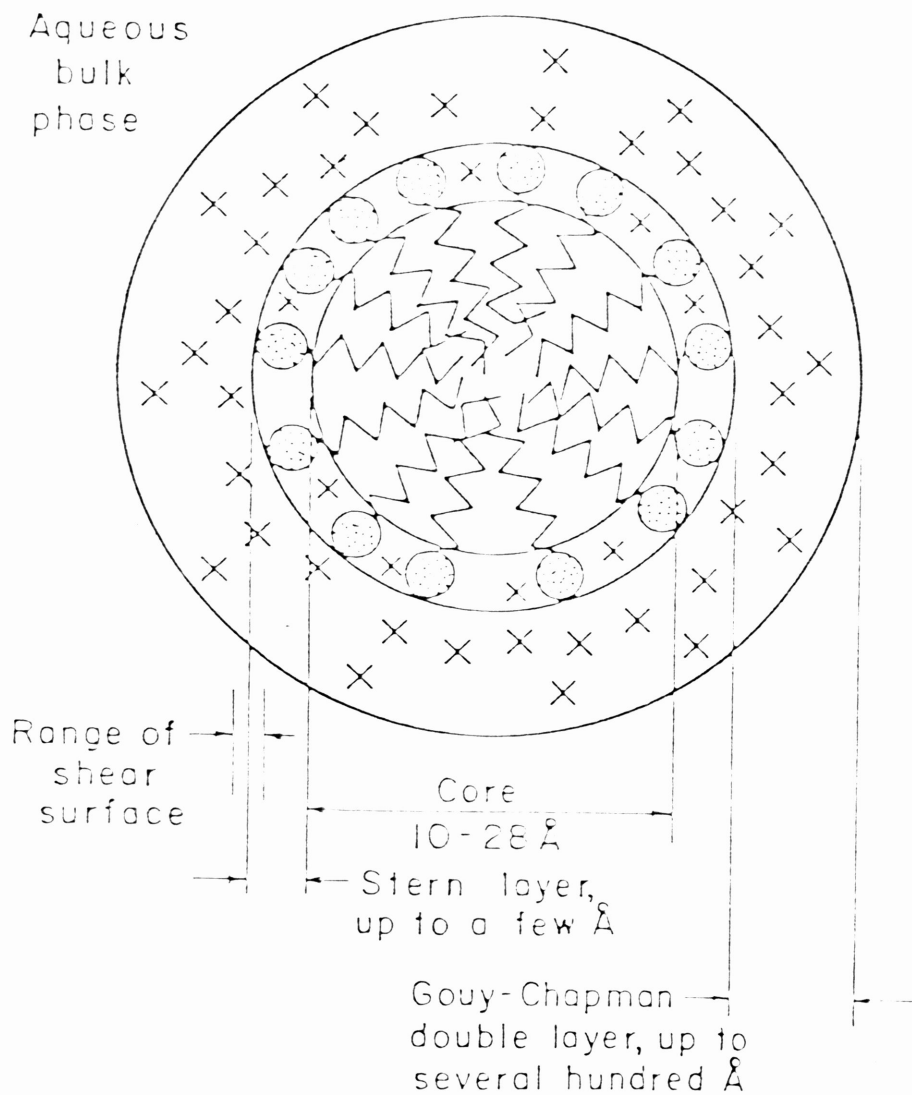
Figure 1. A two-dimensional schematic representation of the regions
of a spherical ionic micelle. The counterions (X), the head groups ( ),
and the hydrocarbon chains (   ) are schematically indicated to denote
their relative locations but not their number, distribution, or
configuration.

toward the outer edges of the aggregate sphere. Therefore, the polar groups are in contact with and are hydrated by water molecules at the water-micelle interface as illustrated in Figure 1.

Water molecules may be trapped within the micelle, and part of the hydrocarbon chain may extend into the aqueous phase.[6] However, water is considered, at present, to penetrate the micellar surface only up to distances of approximately 3 to 6 carbon atoms.[6] The interior, or core, of the micelle is hydrocarbon-like in character.[7]

Micellar Solubilization

McBain has defined the process of micellar solubilization as the spontaneous dissolving of water insoluble molecules, which are called solubilizates, by an aqueous solution or surfactant, called a solubilizer or solubilizing agent, and the formation of the thermodynamically stable isotropic solution.[8]

The site of incorporation of solubilized molecules depends on their hydrophilic tendencies.[2,7,10] Recent studies indicate, in many cases, that the solubilizate is, on the average, uniformly distributed in the micellar interior.[2] Solubilization is a dynamic equilibrium process and the solubilizate is relatively mobile.

Three types of micellar solubilization in aqueous media have been described:[2,8,11,12] (1) nonpolar (non-specific) solubilization - the solubilizate is incorporated in the hydrophobic core of the micelle and separated from the polar head groups, (2) polar-nonpolar (specific) solubilization - the solubilizate is incorporated by penetration into the pallisade layer of the micelle with the solubili-

zate molecule oriented in a similar fashion to that of the surfactant molecule in the micelle, and (3) adsorption solubilization - in this case the solubilizate is located on or near the polar micellar surface.

Micellar Structure in Non-Aqueous Solutions

Aggregate or micelle formation occurs not only in aqueous solutions, but in nonpolar solvents as well. However, information available on micelle formation and structure in non-aqueous solutions is more limited than that for aqueous systems.

In nonpolar solutions, the hydrophilic head groups of the monomers which make up the aggregates are oriented toward the center and make up the core of the aggregate. The hydrophobic moiety of the monomer groups are directed toward and are in contact with the non-aqueous apolar solvent.[2] This micellar organization is the exact inverse of that found in aqueous polar solutions and is termed "reversed" or "inverted" (see Figure 2).

Aggregation of surfactants in nonaqueous solutions differs from that in aqueous solutions. Recent studies indicate that monomers, dimers, trimers, tetramers, and some higher oligamers coexist in organic solvents and that the surfactant aggregates by a stepwise building of small n-mers instead of by the monomer-n-mer equilibria of surfactants in aqueous solutions. This smooth variation of physical properties renders the CMC value useless with no physical meaning.[12] Another difference is seen in the fact that aggregation in non-aqueous solvents generally begins at much lower concentrations than in aqueous solvents.
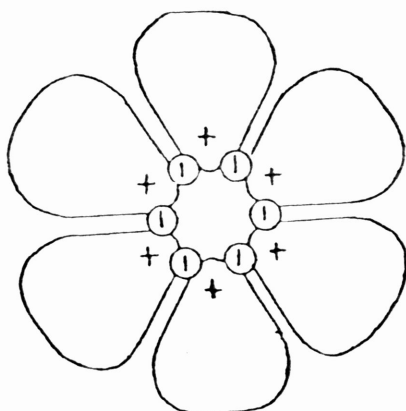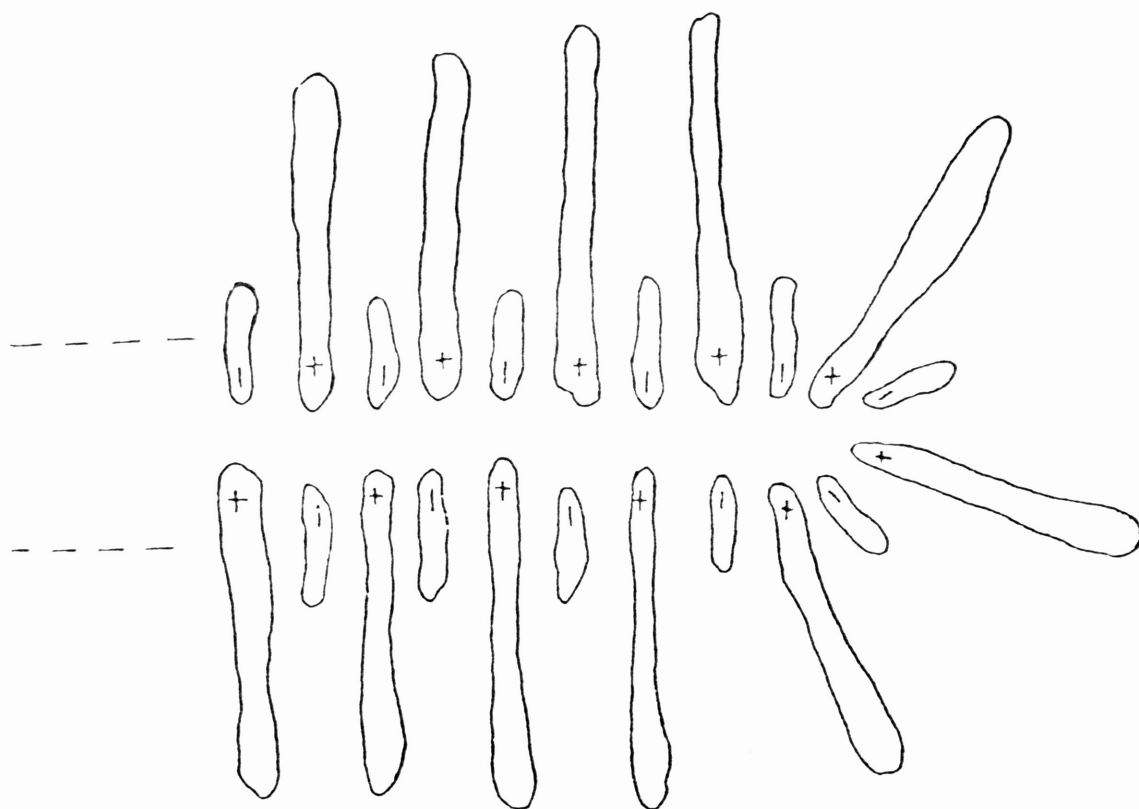
Figure 2. Schematic representations of reversed micelles of surfactants composed of a long hydrocarbon chain cation and a short-chain anion with small (on left) and relatively large (on right) aggregation numbers.

Bile Salts

Bile salts are naturally occurring surfactants which form aggregates or micelles in both aqueous[2,3,13,14] and non-aqueous solutions.[2-4] The bile salts, the most abundant constituent secreted in bile by the liver cells, are among the most common naturally occurring surfactants. These bile acids, synthesized from cholesterol in the liver, comprise a group of compounds which basically differ only in the position and number of hydroxyl groups on the steroid nucleus:
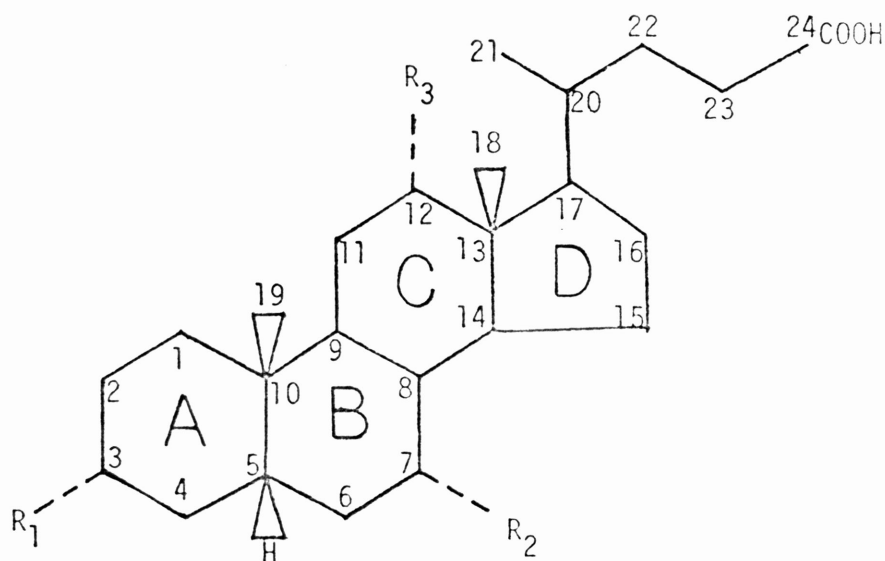
$R_1=R_2=R_3=H$ Cholanic acid

$R_1=R_2=R_3=OH$ Cholic acid (3α, 7α, 12α-trihydroxycholanic acid)

$R_1=R_2=OH$, $R_3=H$ Chenodeoxycholic acid (3α, 7α-dihydroxycholanoic acid)

$R_1=R_3=OH$, $R_2=H$ Deoxycholic acid (3α, 12α-dihydroxycholanoic acid)

$R_1=OH$, $R_2=R_3=H$ Lithocholic acid (3α-hydroxycholanoic acid)

In man, and most higher vertebrates, the bile salts are mainly com-
comprised of the sodium and potassium salts of the glycine ($H_2NCH_2COO^-$)
and taurine ($H_2NCH_2CH_2SO_3^-$) conjugates of cholic, chenodeoxycholic,
and deoxycholic acids with a cis A/B ring juncture (5β-hydrogen), but
also contain small amounts of the monohydroxy compound, salts of
lithocholic acid.[13,14]

In the small intestine of the gastrointestinal tract, the bile
acids are involved in the solubilization of fats, fat-soluble vitamins,
phospholipids, and cholesterol. They are also able to cause changes
in the permeability of the gastrointestinal membranes and are known to
be involved in the transport of a variety of drugs across the
intestinal wall.

As evidenced in their structures (Figure 3), bile salt molecules
exhibit a hydrophobic, highly fat-soluble steroid nucleus and a hydro-
philic polar group which gives them the propensity to form micelles.[2]
The nonpolar portion of the steroid is oriented toward the center of
the aggregate and the polar groups project to the outside in water.
In the aqueous microenvironment of the gastrointestinal tract, these
aggregates can co-micellize or form mixed aggregated with lipids. The
bile salt-lipid micelles solubilize and aid in the transport of a
variety of compounds ingested, especially those which are water
insoluble, e.g., water insoluble drugs.

Drug Solubility

Drugs require membrane absorption in order to achieve their
desired effect. The drug literature contains a number of reports

Figure 3. Perspective structural formula, Stuart-Briegleb space filling model, and longitudinal and transverse shorthand representations of a molecule of cholic acid.

COOH
OH
OH
OH

3   7   12

7   12
3

|— 10 Å —|
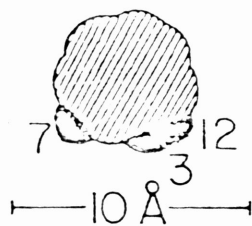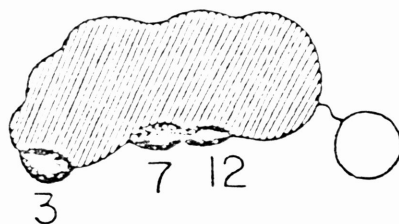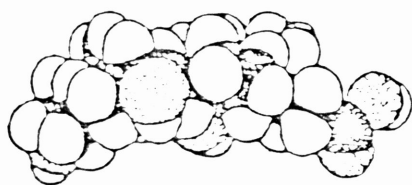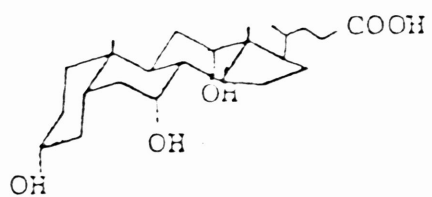
which clearly demonstrate that surface-active agents, surfactants, can influence the rate and the extent of absorption of certain drugs. However, enhancement as well as inhibition of the absorption and pharmacologic activity of drugs has been observed in the presence of surfactants.[15-18] A critical determinant of the extent and rate of drug absorption is drug solubilities and dissolution rates.[15]

According to the Noyes-Whitney equation, the enhanced solubility of a drug in a micellar solution of surfactant should result in a proportional increase in the dissolution rate.[19]

Dissolution rates have been reviewed by Levy[20] and by Fincher.[21] The dissolution rate of a drug, regardless of dissolution mechanism, is always directly proportional to the effective surface area of the drug, i.e., the surface area of drug available to the dissolution fluids.[15]

The effective surface area of drugs is much less than that found in artificial, in vitro conditions. However, many drugs, whose solubility characteristics could be improved by an increase in surface area or a particle size reduction, are hydrophobic and resist wetting by gastrointestinal fluids. The surface area of these hydrophobic drug particles can often be increased with the addition of a surfactant.[15] In the gastrointestinal tract, the surfactants would be the naturally-occurring bile salts.

Among the studies which have attempted to quantitate the relationship between drug solbuility in micellar solutions and dissolution rates is the work by Bates,[22] who reported substantial increases in the dissolution rates of griseofulvin and hexestrol in micellar solu-

tions of bile salts. Weintraub and Gibaldi have also found a positive correlation between surface tension lowering and the dissolution rate of aspirin from a tablet.[23]

Feldman studied the influences of sodium deoxycholate, an unconjugated bile salt, on the absorption of phenol red in a rat and found that the bile salt markedly enhances the absorption or transfer rate of phenol red across the gastrointestinal membranes.[15,24]

Interactions of solubilizates in micellar systems has been investigated by proton resonance (nmr) spectroscopy. Chemical shifts (nmr frequencies) and line width are dependent on the molecular environment of the nuclei, so changes in these properties for solubilizates and surfactants as a function of concentration can provide precise information on the location of a solubilizate with respect to the micellar nuclei as well as on the mode of micellization.[2]

In a study made by Novak and Swift, ^{1}H, ^{31}P, and ^{13}C magnetic resonance was employed to investigate the nature and strength of barbiturate-phospholipid interactions as a model for possible drug-membrane interactions.[25] Through the ^{1}H nmr studies, note was made of shifts and line broadening in characteristic signals of the utilized barbiturates in various solvents and at various temperatures. The potential importance of observation of barbiturate-phospholipid interaction is evident through this study.[25]

Since the rate-limiting step in the absorption process of a drug through membranes is the dissolution step, any factor influencing the rate of solution must also influence the rate of absorption.[2] Because of the role played by bile salts in drug dissolution and

transport, and because the lack of information on the physico-chemical properties of bile salts and drug solubilization, interactions, and reactivity in the presence of bile salts, I have studied drug interactions and reactivity, particularly binding and interaction sites, in the presence of bile salts.

EXPERIMENTAL

Reagents and Materials

The drugs used in this study were reagent grade materials and were used without further purification. The purity of the compounds was confirmed by the melting points and the absence of impurities in their nuclear magnetic resonance (nmr) spectrum. The drugs used include amygdalin, hexistrol, 5-flourouracil, thymidine (Sigma), anti-pyrine (Eastman), and acetylsalicylic acid (Aldrich).

The sodium cholate (NaC, Sigma) was monitored for impurities using IR and NMR spectroscopy and for water using a Photovolt Aquatest.

Dimethylsulfoxide-d_6, (DMSO-d_6, 99.5% D, Stohler), was used as the solvent for the ^{1}H nmr determinations.

Spectroscopic Techniques

Generally, two different types of serial solutions were used for the ^{1}H nmr spectral study of bile salt and drug interactions:

1) series differing in bile salt concentration but invariant in drug concentration and

2) series differing in drug concentration but invariant in bile salt concentration.

Stock solutions A, 0.10 molar drug and 0.50 molar sodium cholate, and B, 0.10 molar drug, used for spectral studies were prepared by accurately weighing out a dry, solid sample of the appropriate compound and diluting it to volume with dry DMSO-d_6 in a volumetric flask. All solutions were made-up immediately prior to use. Variance in bile salt concentration series was achieved by volumetrically adding

different volumes of stock solution A to stock solution B, as shown in Table I.

The HA-100 nmr spectrum were obtained at 100 MHz on a modified Varian HA-100 spectrometer with a Hewlett-Packard model 200-ABR audio oscillator and frequency counter. All spectra were determined on freshly prepared solutions and were measured to neat tetramethyl-silane (TMS) in a Wilmad 520-2 internal coaxial tube. A downfield chemical shift difference of 47.5 Hz at 100 MHz was observed for the chloroform signal (10% V/V in CCl_4) between the "external" neat TMS in the coaxial tube and the "internal" 10% volume TMS in the same solution. Each spectrum was recorded after equilibrium to the ambient probe temperature of $31.0\pm0.5°C$. Chemical shifts, with the exception of the downfield resonances, were obtained from spectra recorded at 500 Hz (100 MHz) sweep widths and are given on the δ scale in ppm relative to TMS (δ = 0 ppm). Individual measurements are accurate to ±0.002 ppm at 100 MHz.

The solubilities of the drugs were determined in DMSO-d$_6$ and found to be appreciably greater than those in its absence.

Table I. Serial Solutions of Drugs and Sodium Cholate in DMSO-d_6.

Sample No.	A^a (ml)	B^b (ml)	[Drug], M	[NaC], M
1	0	5.00	0.10	0
2	1.00	4.00	0.10	0.10
3	2.00	3.00	0.10	0.20
4	3.00	2.00	0.10	0.30
5	4.00	1.00	0.10	0.40
6	5.00	0	0.10	0.50

[a] Stock solution A containing 0.10 M drug and 0.50 M NaC.

[b] Stock solution B containing 0.10 M drug.

RESULTS AND DISCUSSION

The interactions of acetylsalicylic acid (aspirin), amygdalin, antipyrine, 5-flourouracil, hexistrol, and thymidine have been investigated as a function of increasing sodium cholate concentraton in DMSO-d_6 solution, using ^{1}H nmr spectroscopy. The structures and primary physiological functions are given in Figures 4-9 and their proton nmr spectra are illustrated in Figures 10-14. Dependence of the discrete chemical shifts of these drugs as a function of increasing sodium cholate concentration is exemplified by the hexestrol spectra (Figures 15-19). It is apparent that they generally shift linearly as a function of increasing bile salt concentrations (Figures 20-25). It is notable, however, that two of the resonances for acetylsalicylic acid exhibit curvilinear behavior (Figure 20).

The nmr data has been treated assuming a monomer-n-mer type of self-association (equation 1) using equation 2,[15]

$$M + S \quad \overset{K_{MS}}{\longrightarrow} \quad MS \tag{1}$$

$$\frac{1}{\delta - \delta_o} = \frac{1}{K_{MS}(\delta_{MS} - \delta_o)} \frac{1}{[M]} + \frac{1}{\delta_{MS} - \delta_o} \tag{2}$$

where δ is the observed chemical shift, δ_o and δ_{MS} are the chemical shifts of the free solubilizate and bound solubilizate, respectively, K_{MS} is the equilibrium binding constant of equation 1, and $[M]$ is the micellary concentration. Treatment of the data for the drugs according to equation 2, where $[M]$ was equal to the total bile salt concentration,
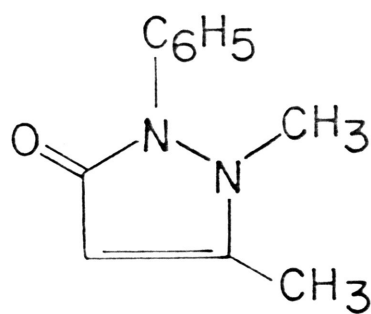
COOH

OOCCH₃
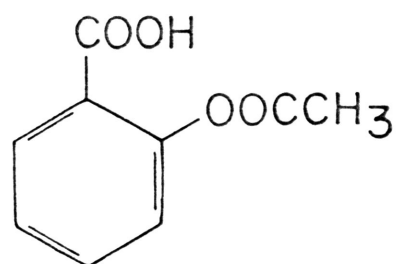
ACETYLSALICYCLIC ACID (ASPIRIN)
Analgesic, Antipyretic, Anti-inflammatory Activity

Figure 4. Structure and primary functions of acetylsalicylic acid (aspirin).
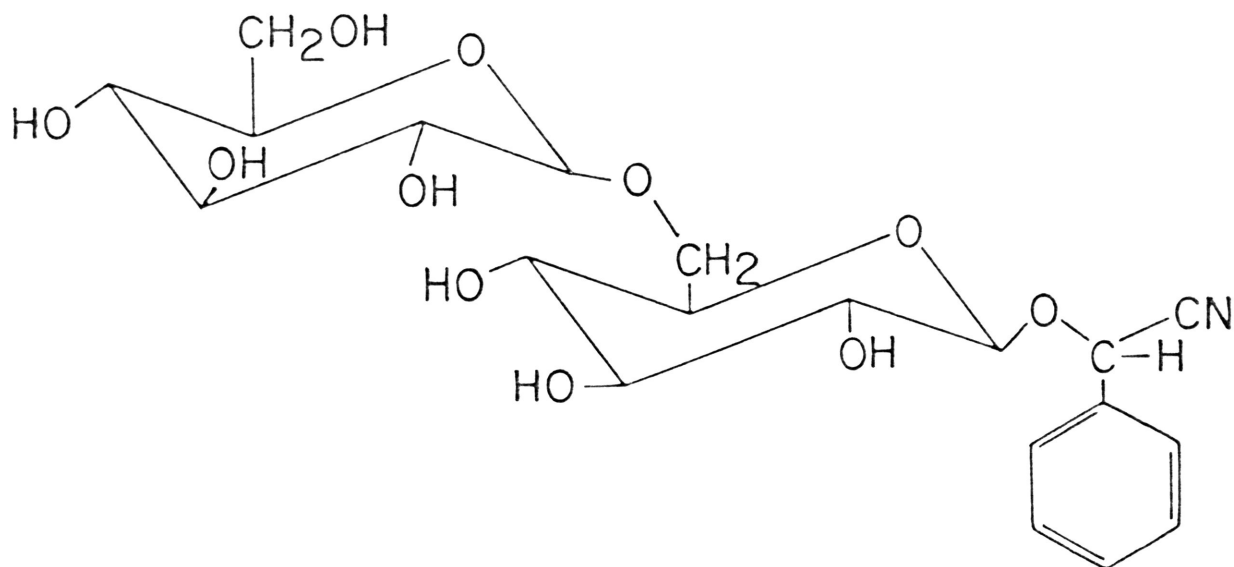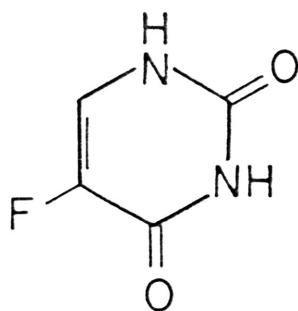
AMYGDALIN
Mutagenic Activity

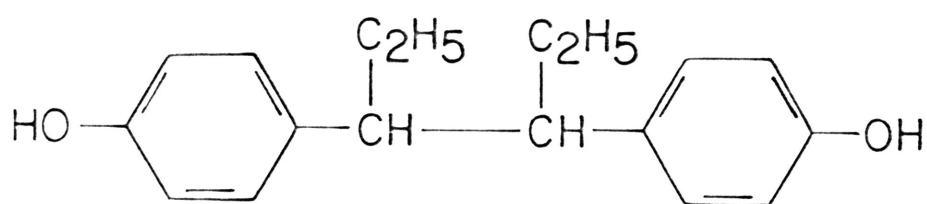Figure 5. Structure and primary function of amygdalin.

ANTIPYRINE
Analgesic Activity
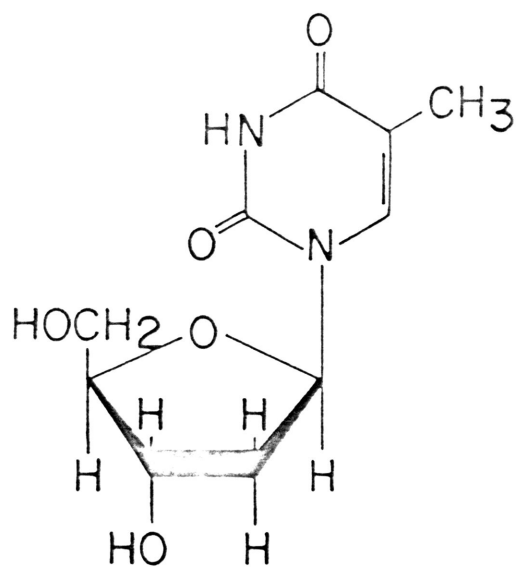
Figure 6. Structure and primary function of antipyrine.

5-FLOUROURACIL
Antineoplastic Activity

Figure 7. Structure and primary function of 5-flourouracil.

HEXESTROL
Estrogenic Activity

Figure 8. Structure and primary function of hexestrol.

THYMIDINE
Anticarcinogenic Activity

Figure 9. Structure and primary function of thymidine.
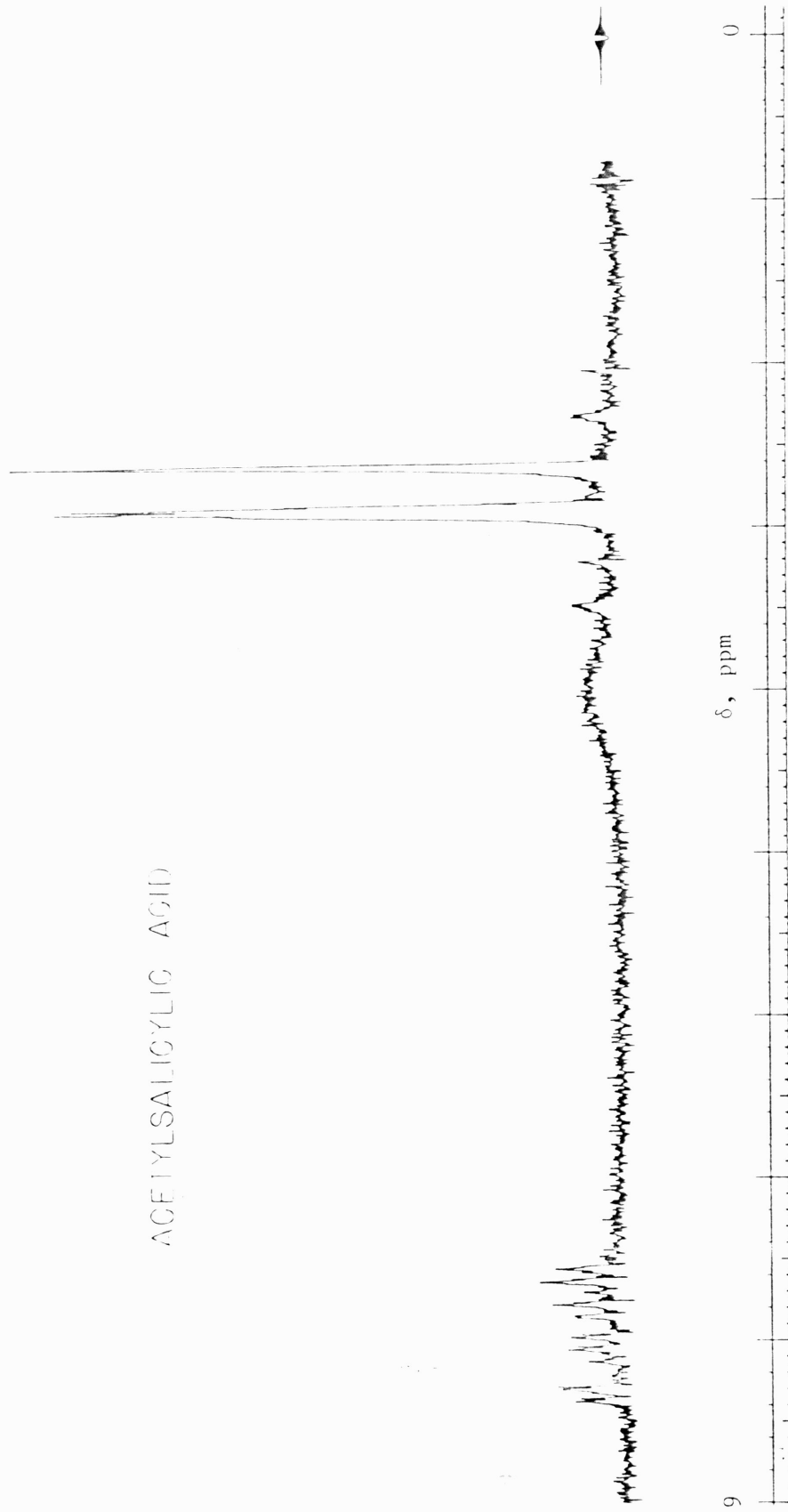
ACETYLSALICYLIC ACID

δ, ppm

Figure 10. ^{1}H nmr spectrum of acetylsalicylic acid in DMSO-d$_6$ at 100 MHz and ambient probe temperature 31.0±0.5°C.

AMYGDALIN

δ, ppm
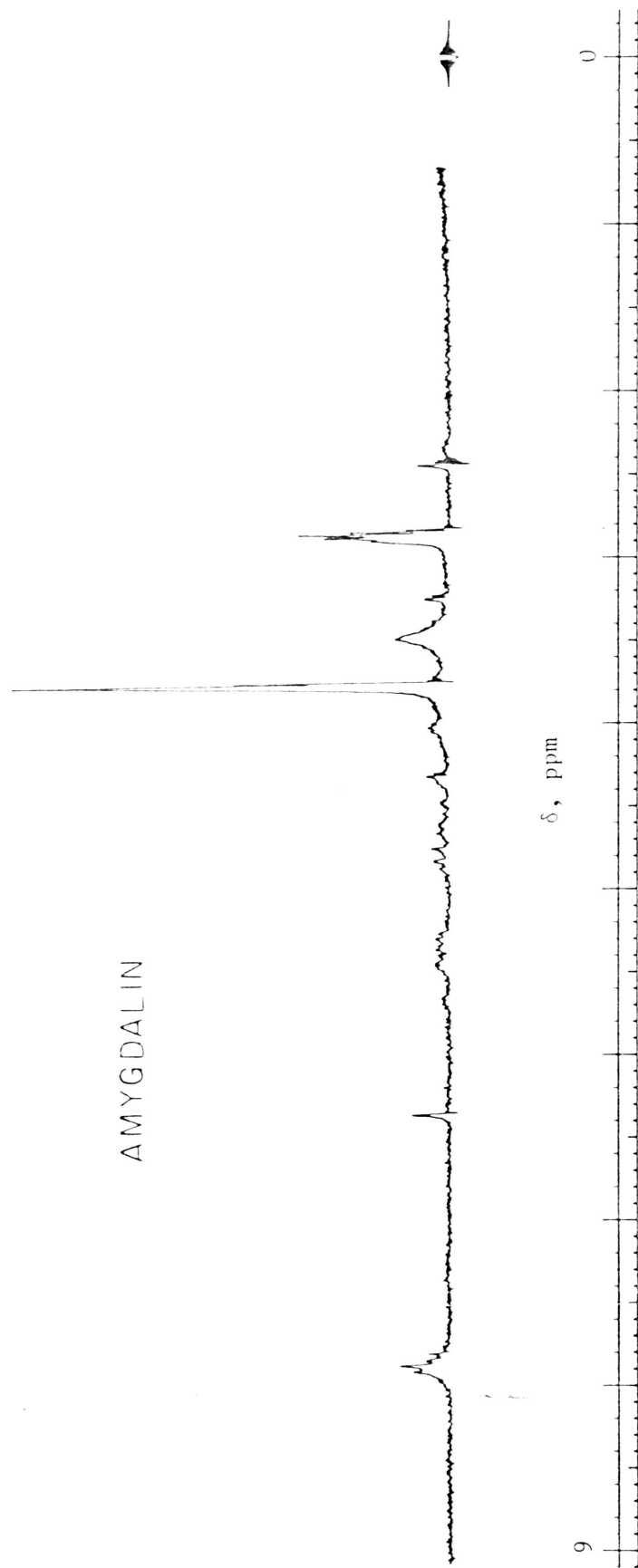
Figure 11. ^{1}H nmr spectrum of amygdalin in DMSO-d$_6$ at 100 MHz and ambient probe temperature 31.0±0.5°C.

ANTIPYRINE

δ, ppm

9   0
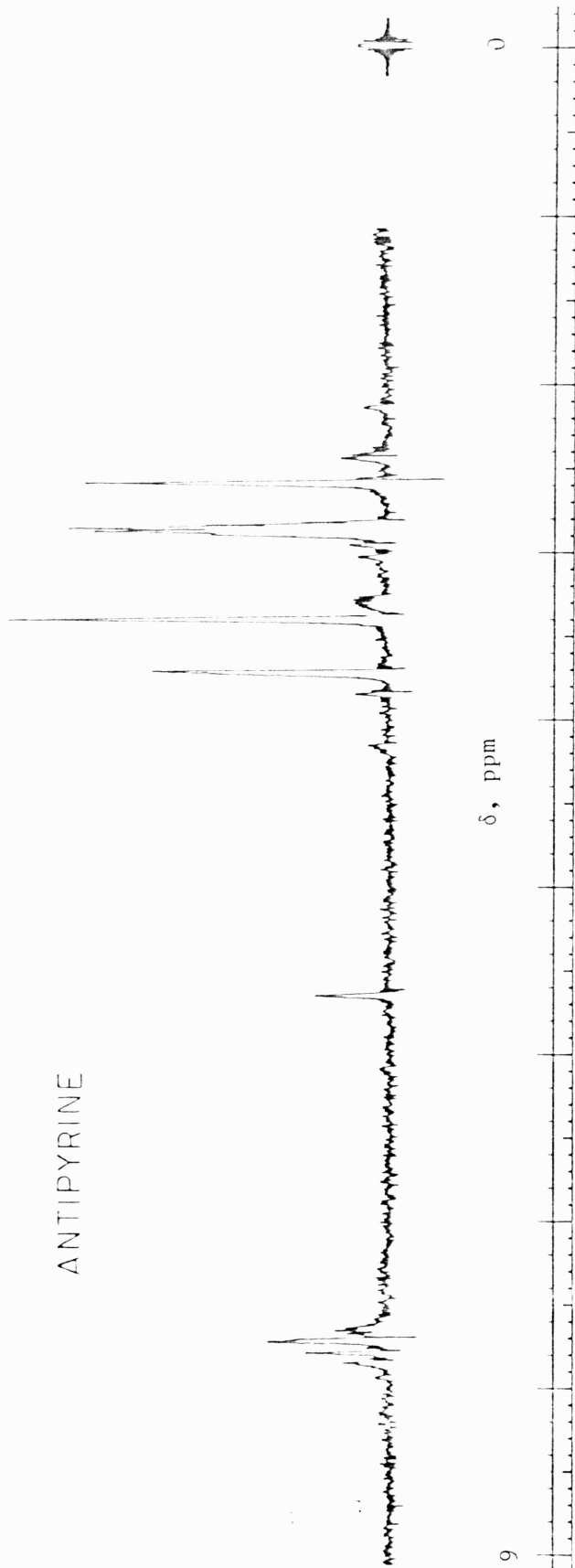
Figure 12. ^{1}H nmr spectrum of antipyrine in DMSO-d_6 at 100 MHz and ambient probe temperature 31.0±0.5°C.
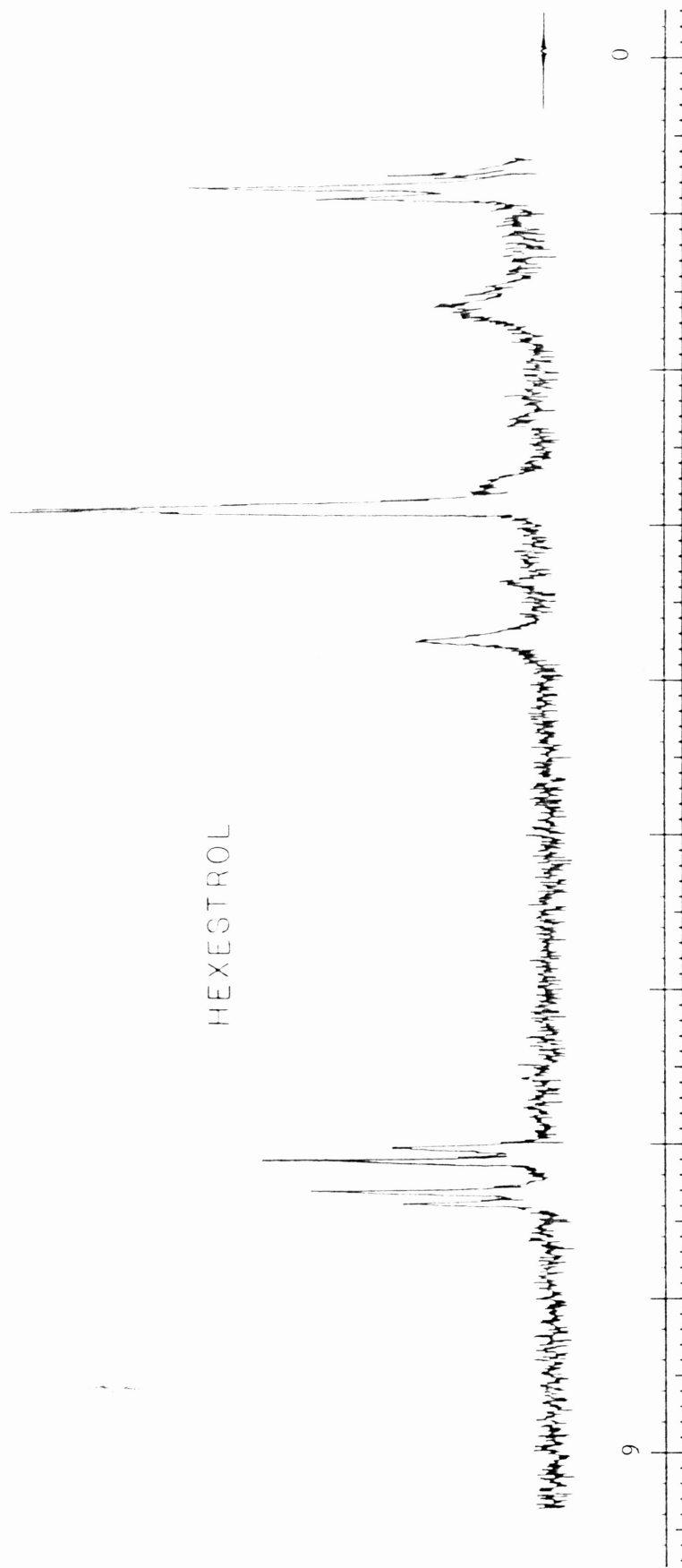
HEXESTROL

Figure 13. ^{1}H nmr spectrum of hexestrol in DMSO-d_6 at 100 MHz and ambient probe temperature 31.0±0.5°C.

THYMIDINE

δ, ppm
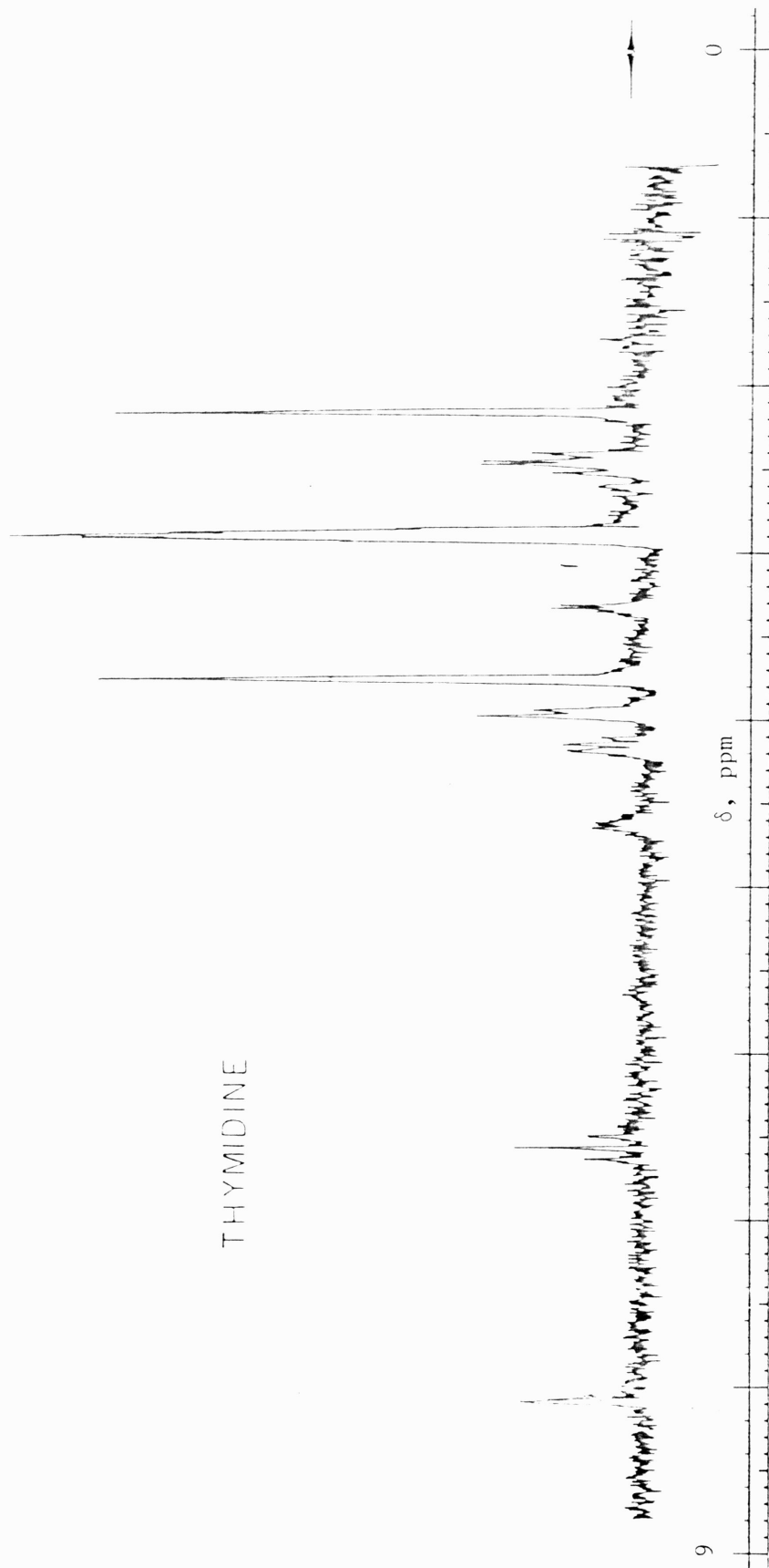
Figure 14. ^{1}H nmr spectrum of thymidine in DMSO-d$_6$ at 100 MHz and ambient probe temperature 31.0±0.5°C.
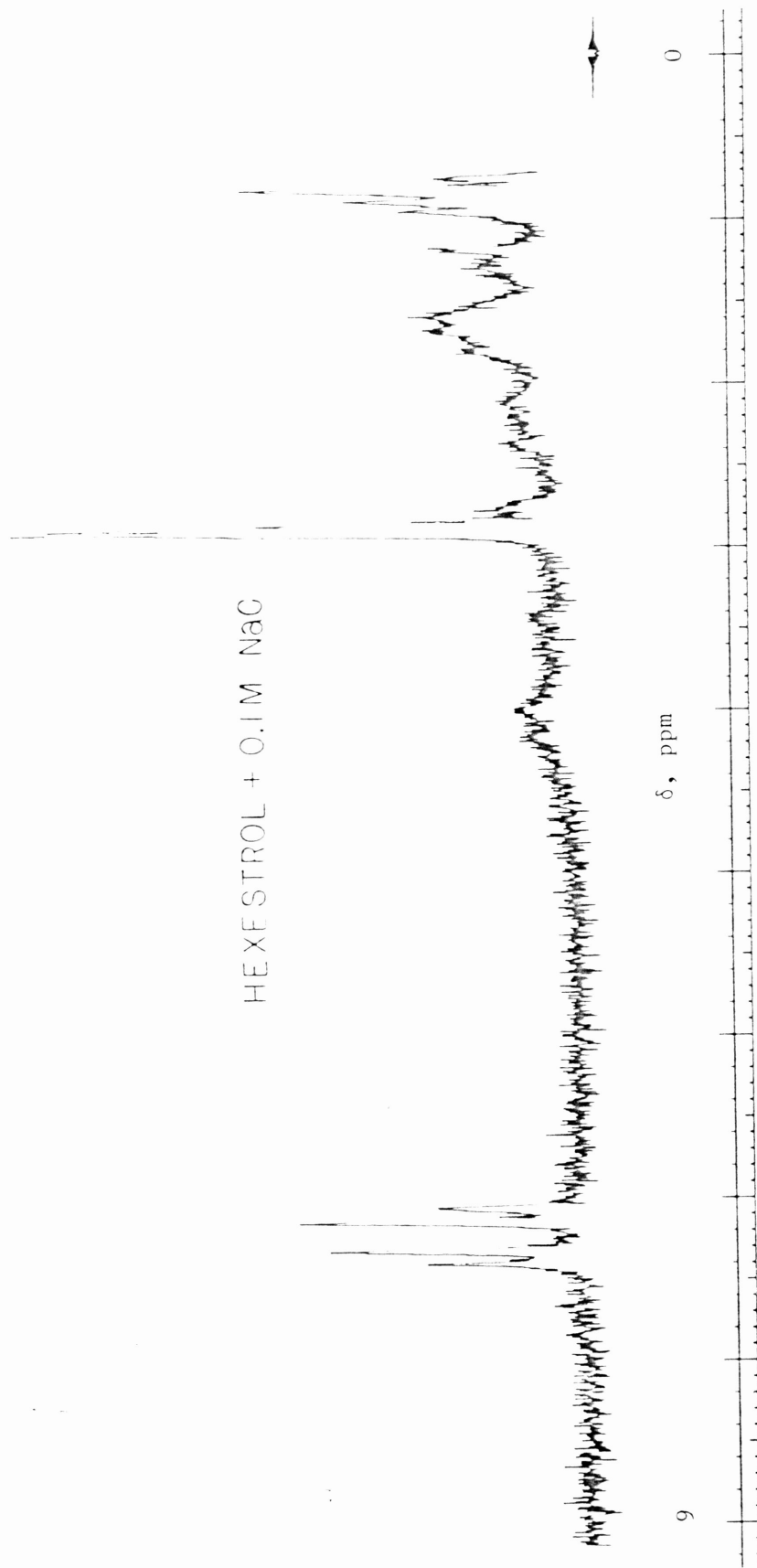
HEXESTROL + 0.1M NaC

δ, ppm

Figure 15. ^{1}H nmr spectrum of 0.01 M hexestrol and 0.10 M NaC solution in DMSO-d_6 at 100 MHz and ambient probe temperature 31.0±0.5°C.
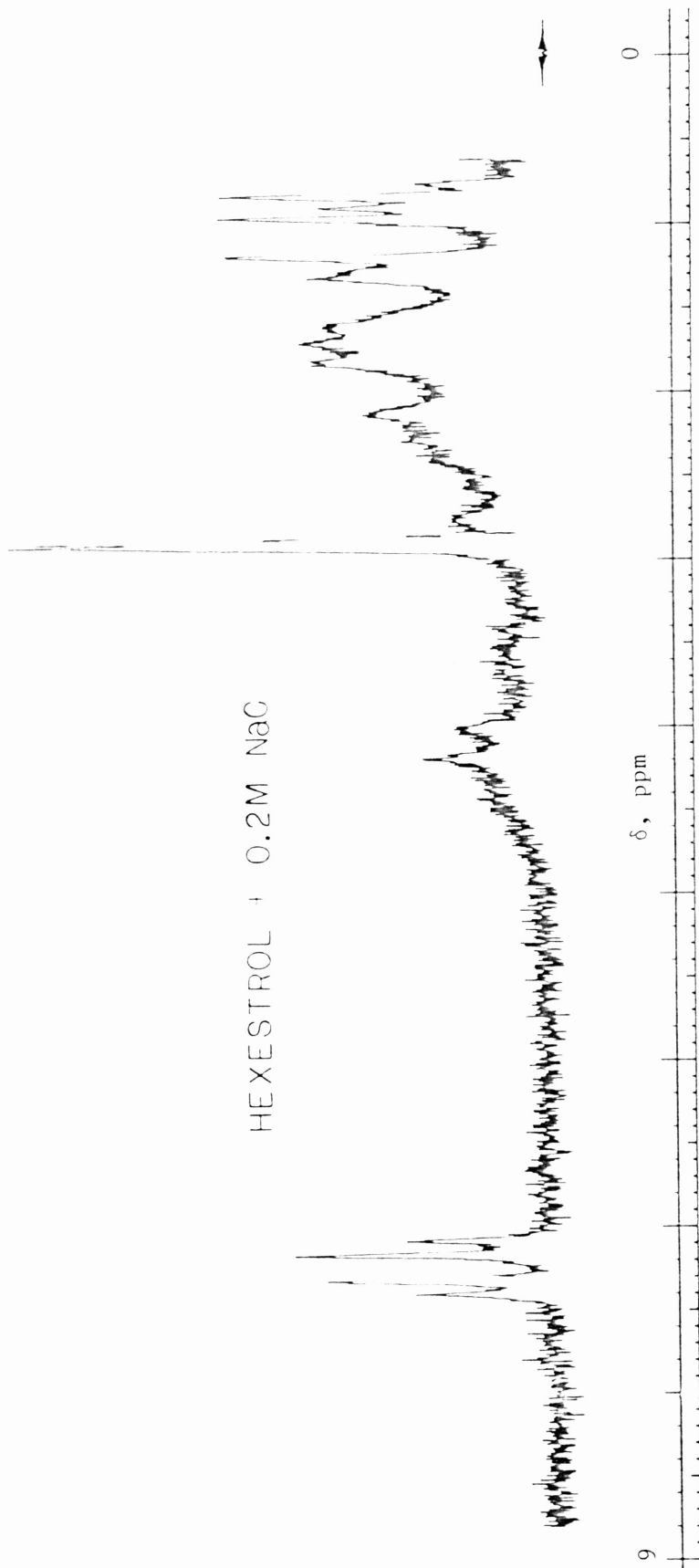
HEXESTROL + 0.2M NaC

δ, ppm

0

9

Figure 16. ^1H nmr spectrum of 0.10 M hexestrol and 0.20 M NaC solution in DMSO-d_6 at ambient probe temperature 31.0±0.5°C.

HEXESTROL + 0.3M NaC

δ, ppm

Figure 17. ^{1}H nmr spectrum of 0.10 M hexestrol and 0.30 M NaC solution in DMSO-d$_6$ at ambient probe temperature 31.0±0.5°C.

HEXESTROL + 0.4M NaC

δ, ppm

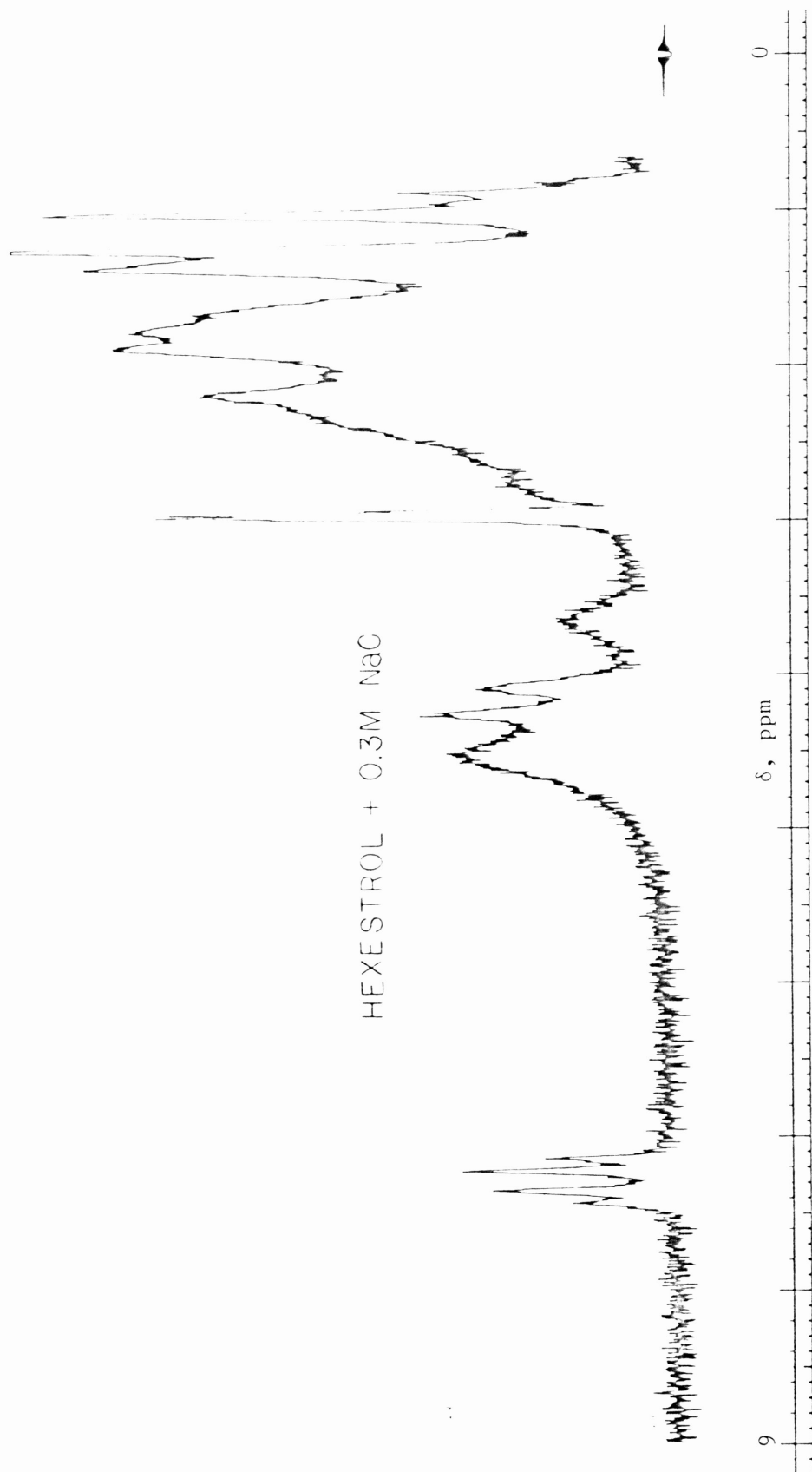Fiugre 18. ^{1}H nmr spectrum of 0.10 M hexestrol and 0.40 M NaC solution in DMSO-d$_6$ at ambient probe temperature 31.0±0.5°C.
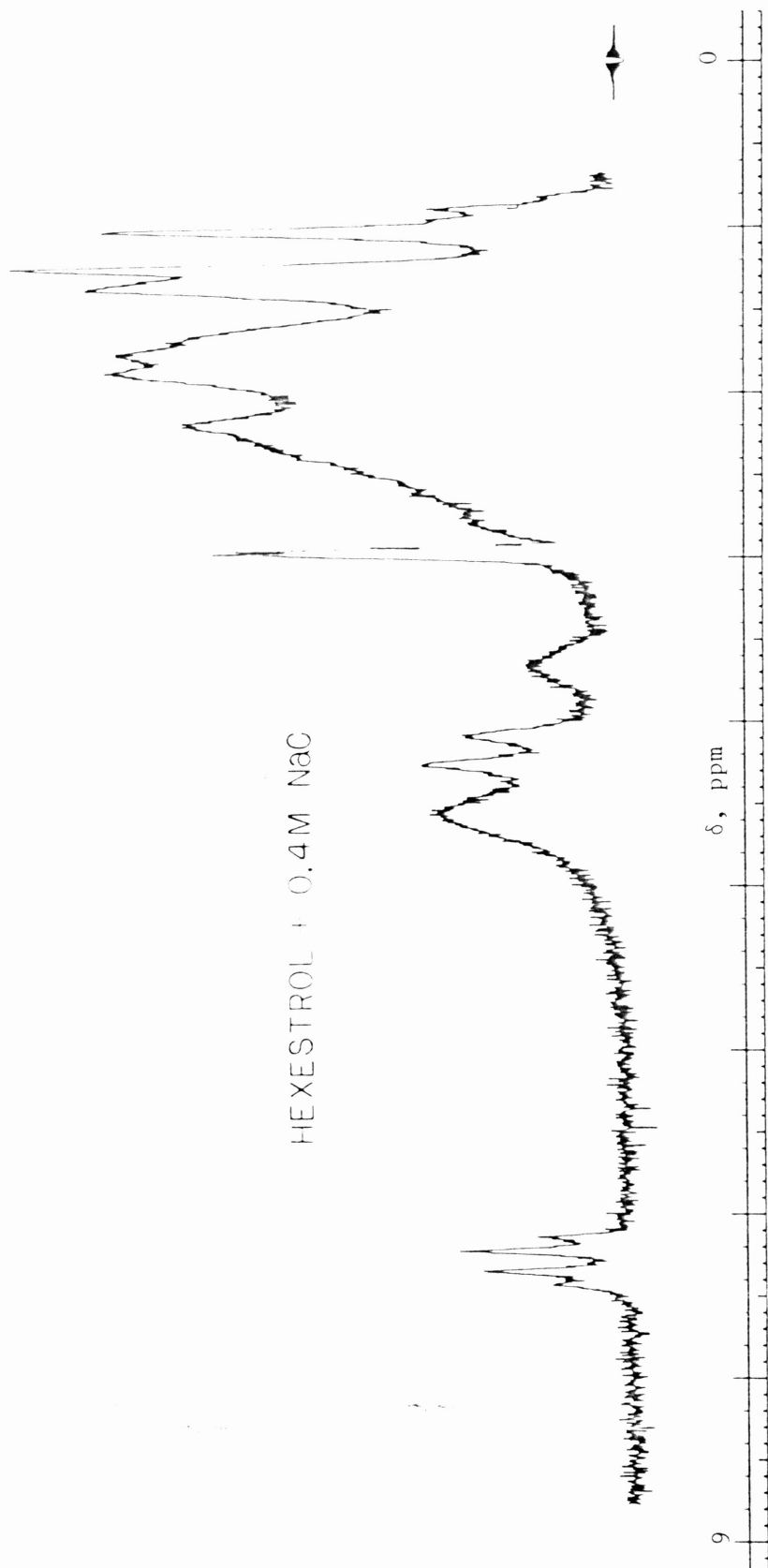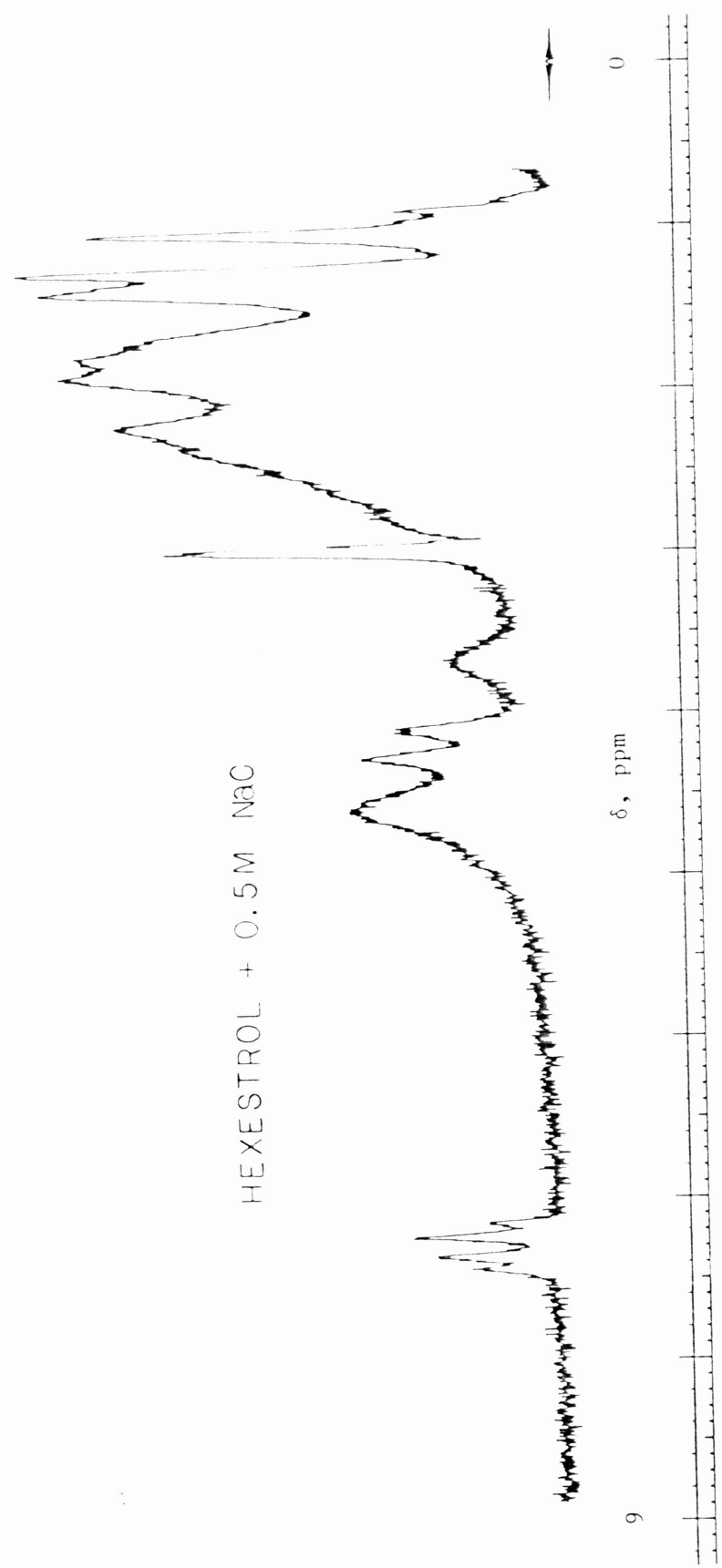
Figure 19.. ^1H nmr spectrum of 0.10 M hexestrol and 0.50 M NaC solution in DMSO-d_6 at ambient probe temperature 31.0±0.5°C.

Figure 20. Chemical shifts of acetylsalicylic acid protons as a function of concentration of NaC in DMSO-d_6.

Figure 21. Chemcial shifts of amygdalin protons as a function

of concentration of NaC in DMSO-d$_6$.

Figure 22. Chemical shifts of antipyrine protons as a function of concentration of NaC in DMSO-d$_6$.

Figure 23. Chemical shifts of 5-flourouracil protons as a function of concentration of NaC in DMSO-d$_6$.

Figure 24. Chemical shifts of hexestrol protons as a function of concentration of NaC in DMSO-d_6.
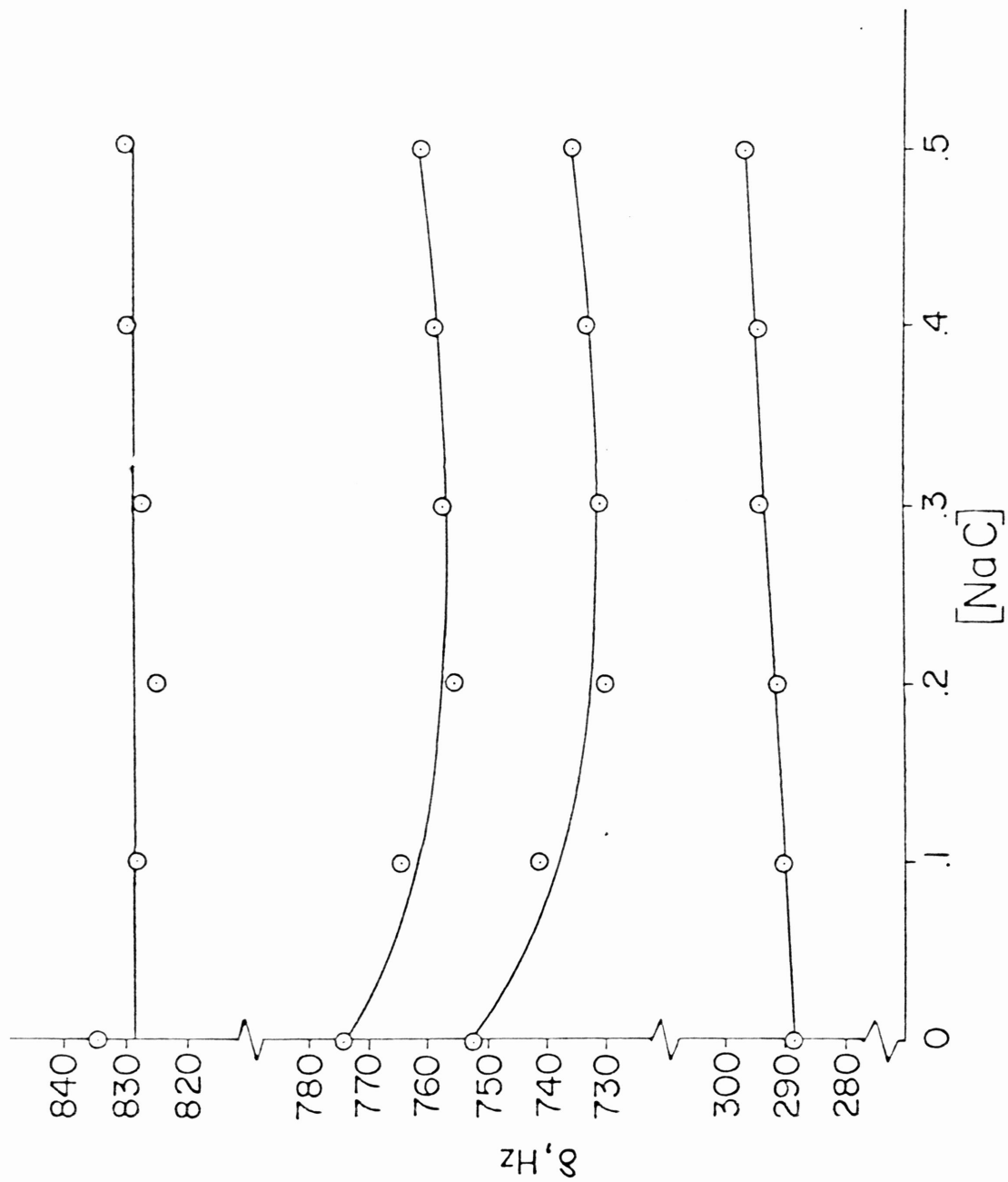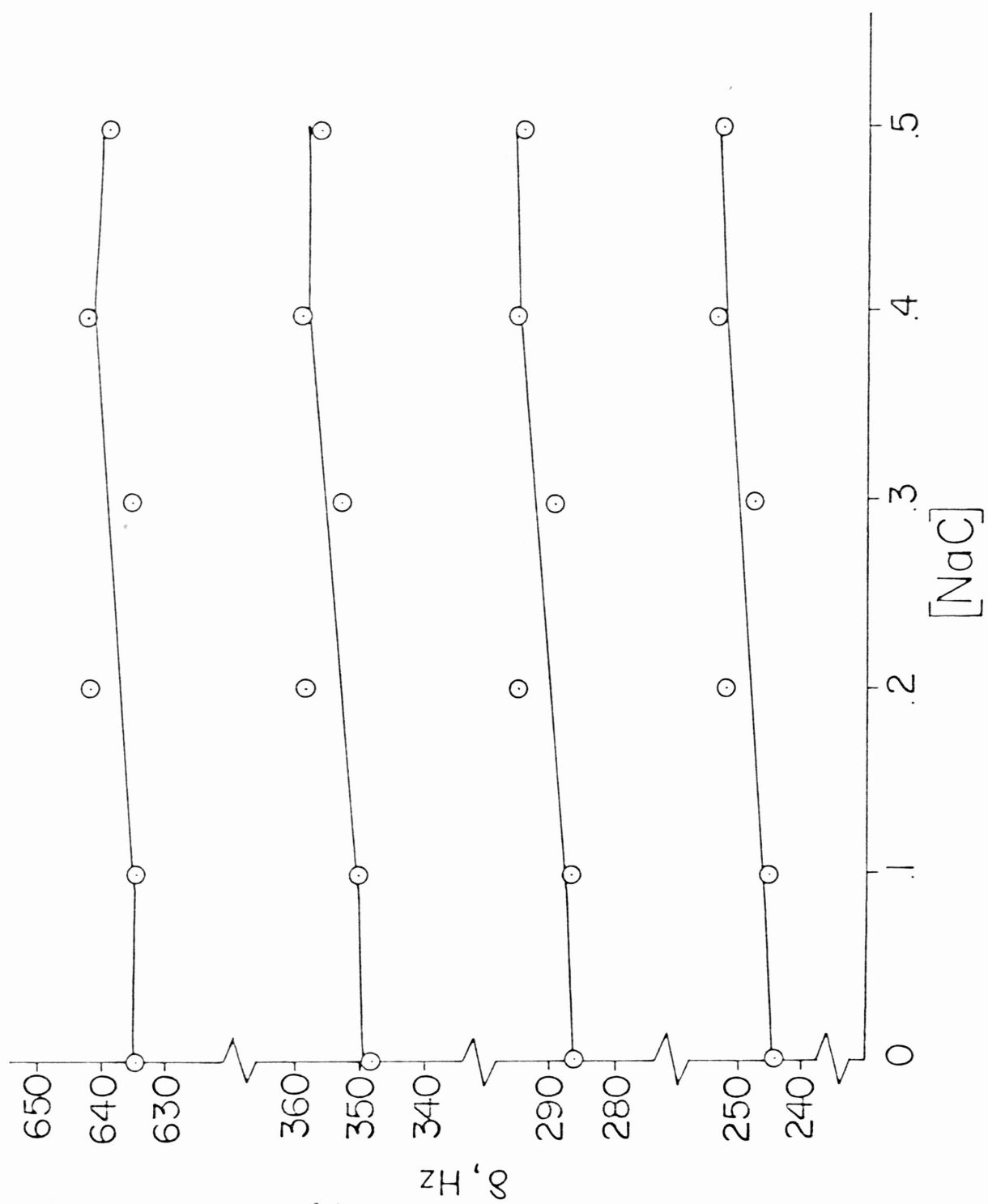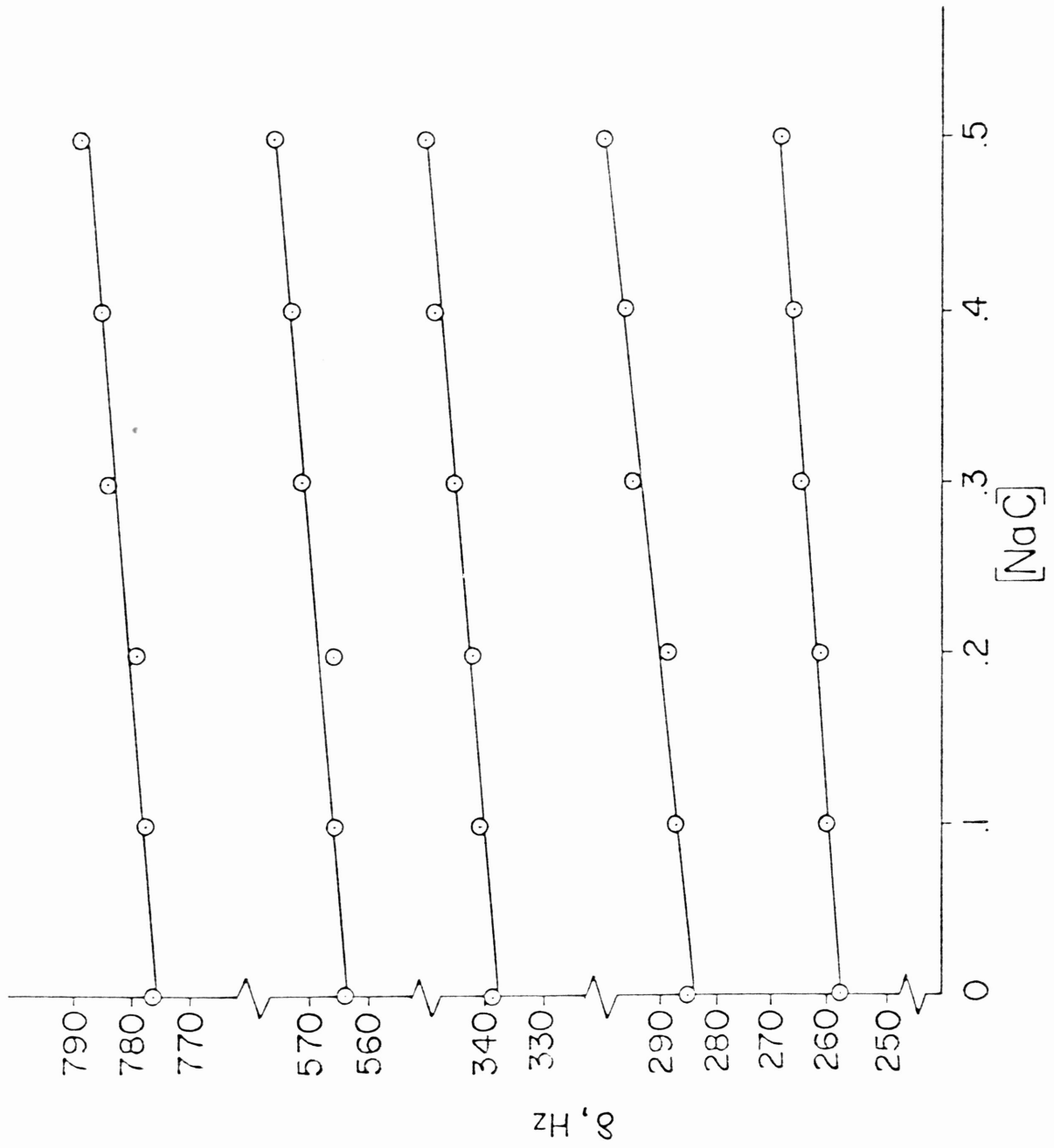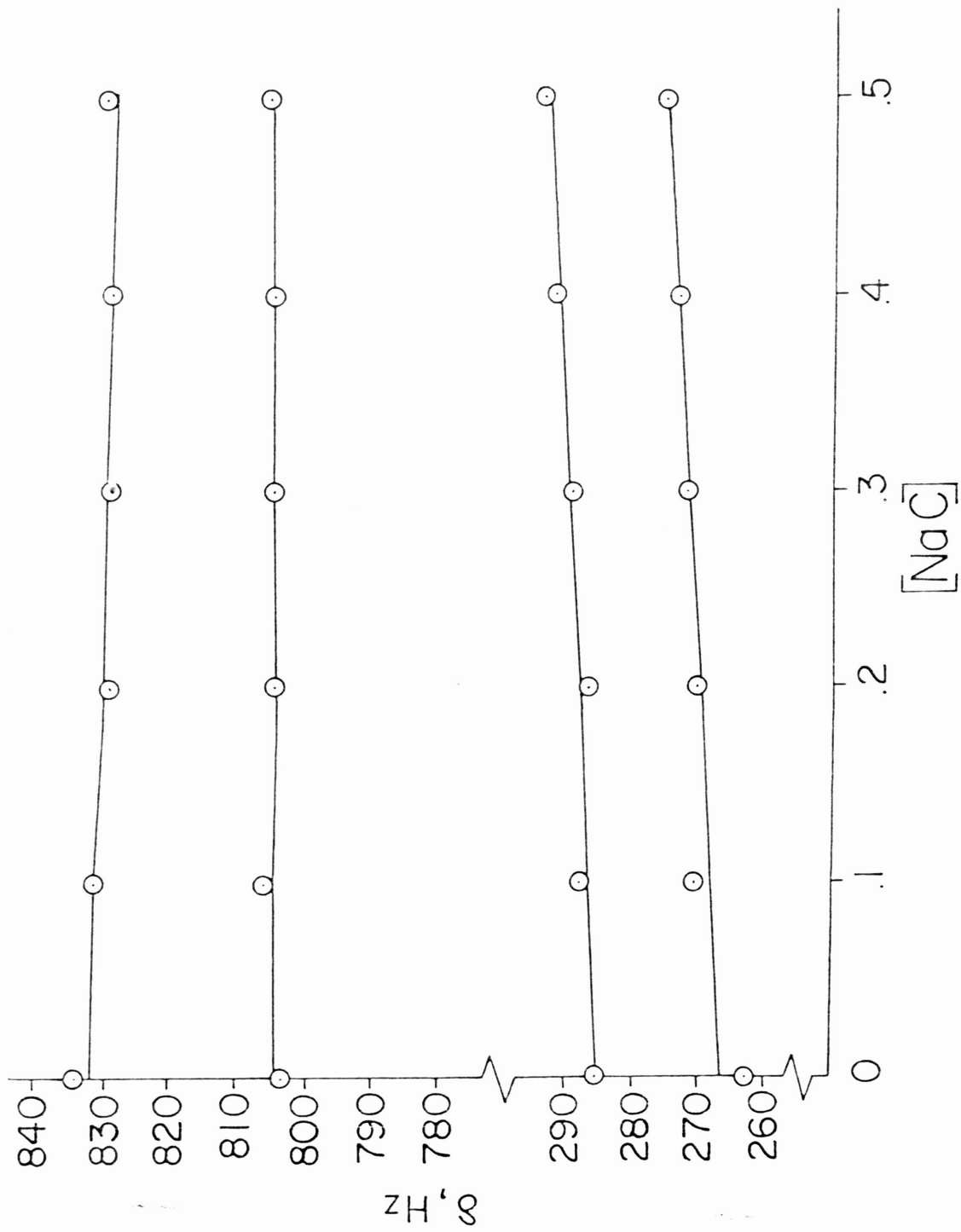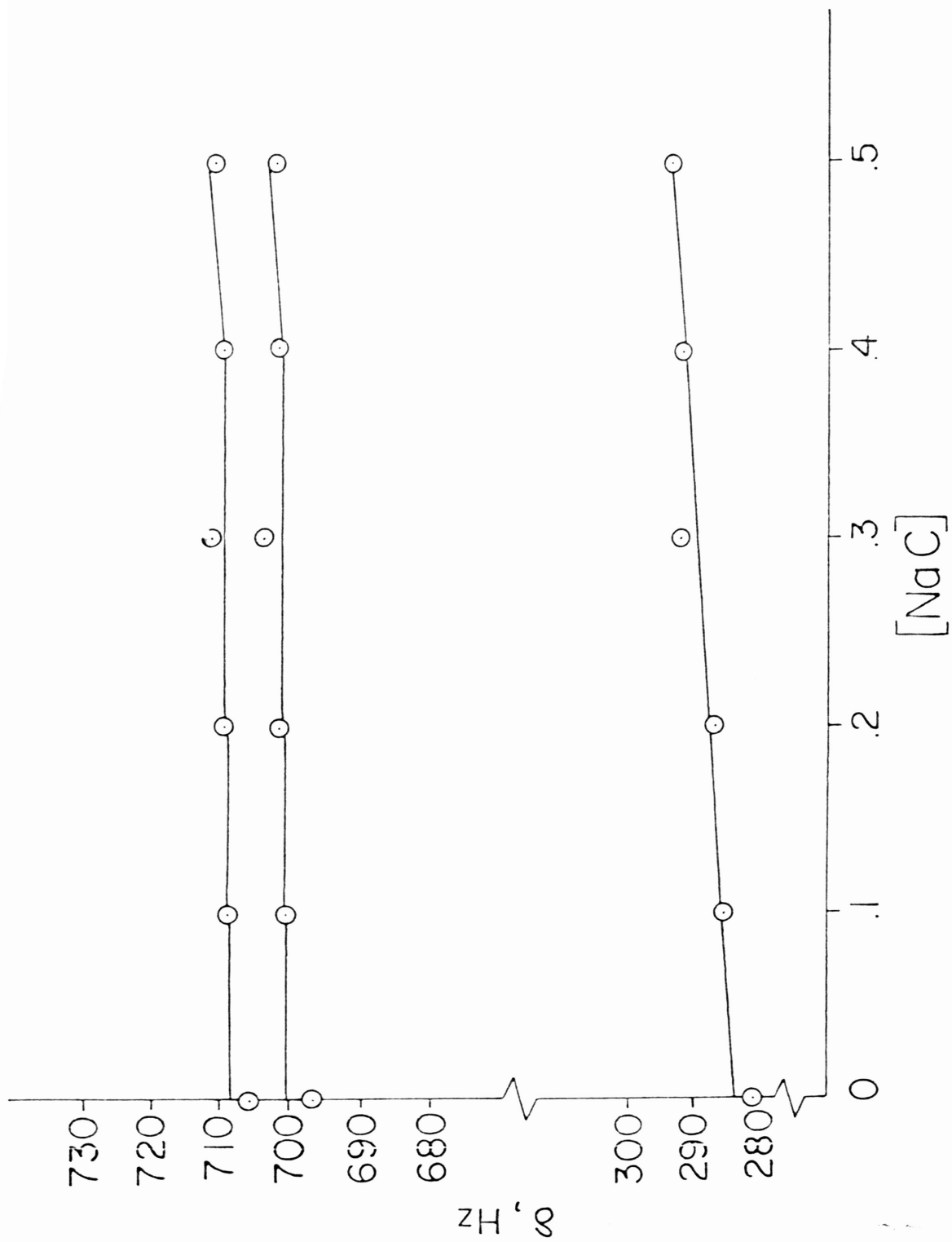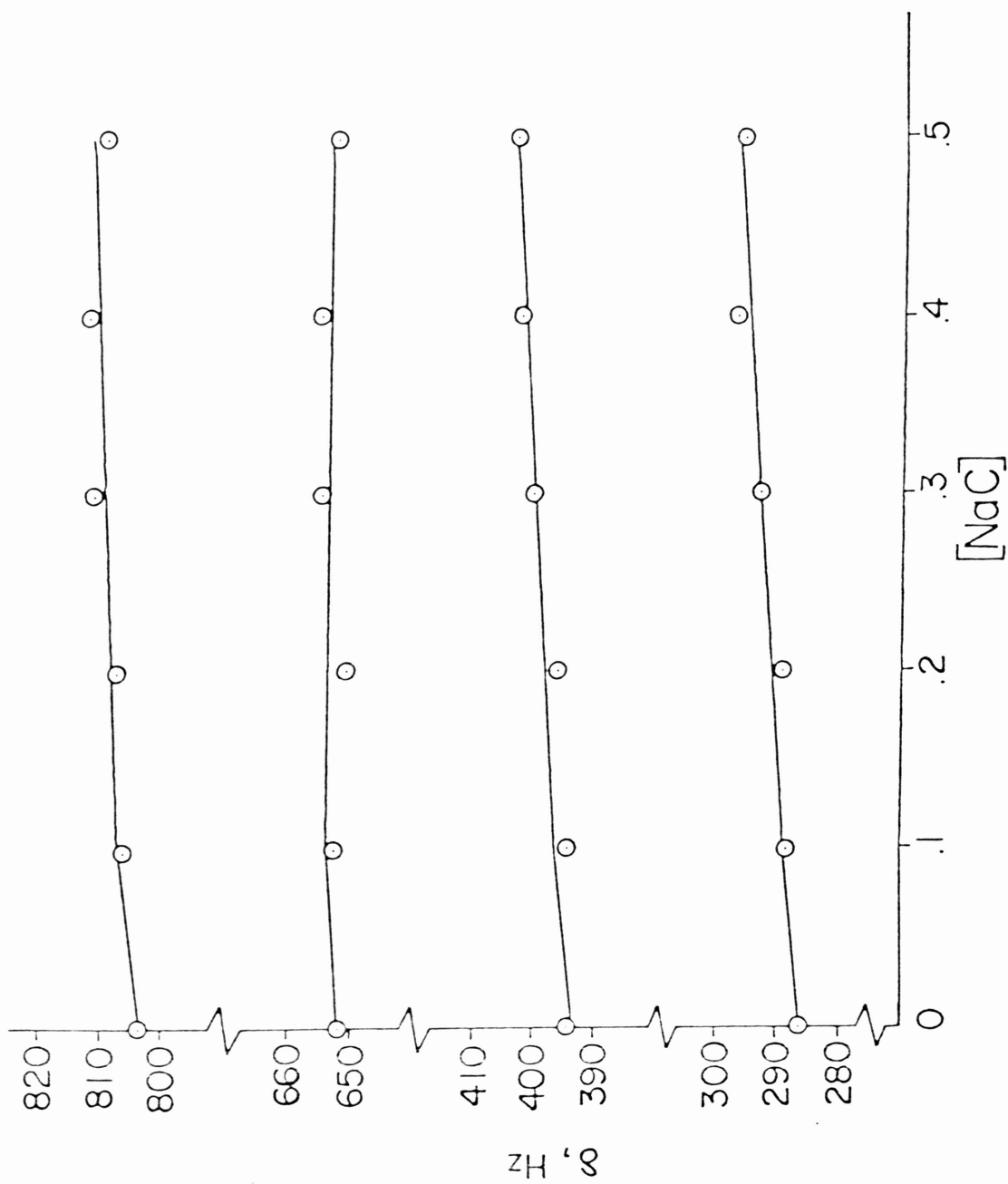
Figure 25. Chemical shifts of thymidine protons as a function of concentration of NaC in DMSO-d_6.

gave a linear dependency with the exception of acetylsalicylic acid (Figure 20) and hexestrol (Figure 24). For hexestrol, the data given in Table II, treated using equation 2, is illustrated in Figure 26, from which a value for K_{MS} was found to be 3.31 M. However, for the drugs other than hexestrol a negative K_{MS}, or K_{MS} so small that it approximated zero, was found.

Clearly, these results are incompatible with the very appreciable increase in solubility of the drugs as a function of increasing sodium cholate concentration as evidenced in Figures 20-25. The assumption involved in this derivation, that of equating the concentration of micelles to the total bile salt surfactant concentration, has been shown to be a clearly inappropriate data treatment by the resultant negative and insignificant K_{MS} values. Additionally, sodium cholate associates by a definite or indefinite type of self-association in DMSO-d_6 and, consequently, the drugs can be solubilized by sodium cholate aggregates containing different numbers of monomeric sodium-cholate molecules. Each of these aggregates would result in discrete chemical shifts of which the observed chemical shift is an average. It is evident from the chemical shift behavior and the type of the aromatic resonance lines of the drugs (Figures 4-9) that they interact strongly via hydrogen bonding and hydrophobic interactions with aggregated sodium cholate. This evidence is in direct conflict with the results obtained from this type of treatment of the nmr data.

Table II. ^{1}H Nmr Chemical Shifts of Hexesterol as a Function of NaC Concentration in DMSO-d_6.

[NaC], M	δ, Hz[a]	$\delta - \delta_o$, Hz[b]
0.00	281.2	0
0.10	285.9	4.7
0.20	287.7	6.5
0.30	293.9	12.7
0.40	294.1	12.9
0.50	295.9	14.7

[a]Chemical shift of the 281.2 Hz resonance frequency at 100 M ambient probe temperature.

[b]δ_o = 281.2 Hz.

Figure 26. Typical binding constant plot for hexestrol

according to equation 2.

The y-axis is labeled $\dfrac{1}{\delta - \delta_0}$ with gridlines at .05, .10, .15.

The x-axis is labeled $\dfrac{1}{[NaC]}$ with values 0, 2, 4, 6, 8, 10.
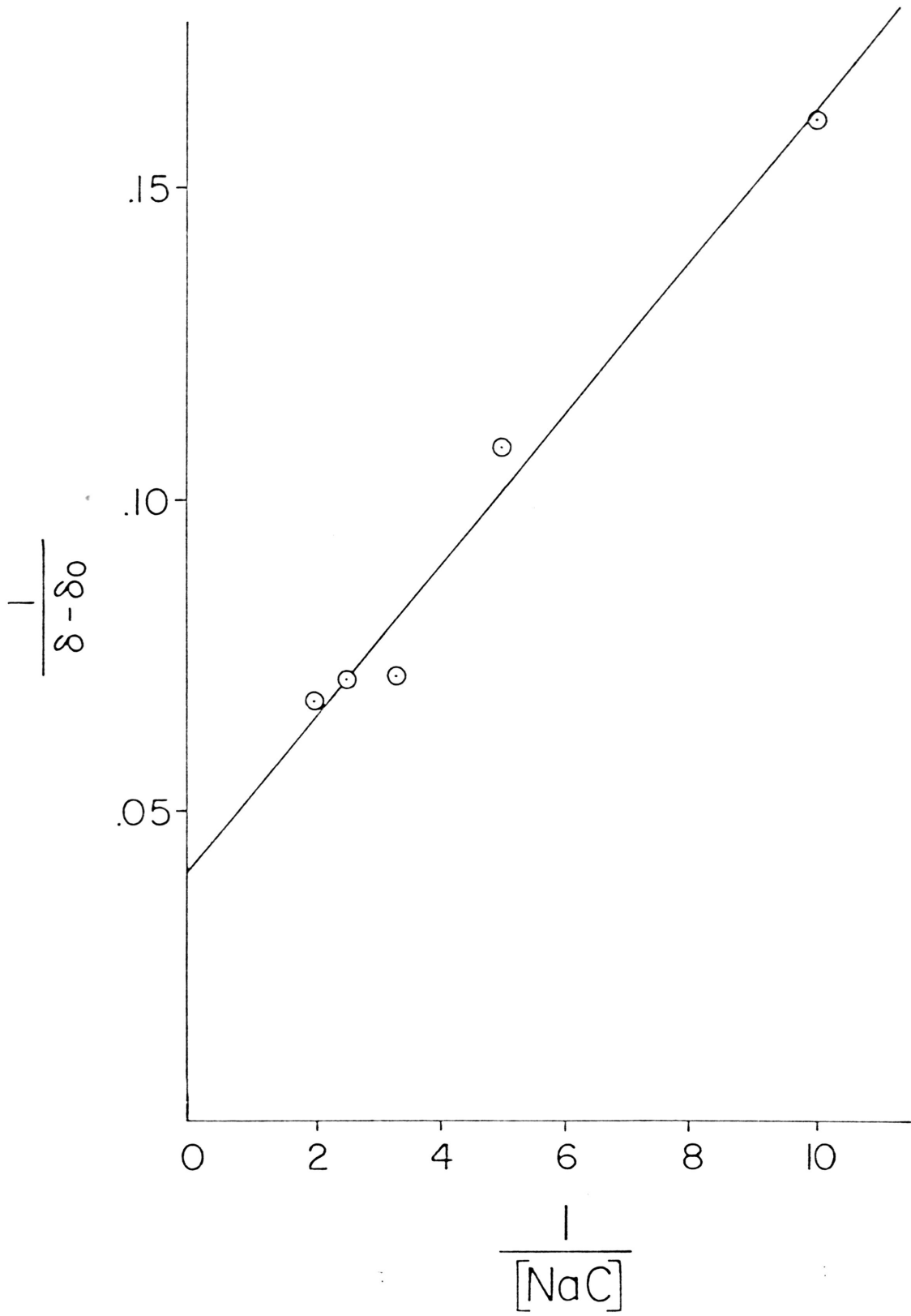
CONCLUSION

From the conflicting results obtained, it is apparent that thermo-
dynamic and hydrodynamic techniques can compliment the data obtained
using spectroscopy. The former (e.g., vapor pressure osmometry,
sedimentation equilibrium, gel filtration, partition, and gas layer
chromatography), can be used to obtain data on the extent and temperature
dependence of drug-surfactant binding, whereas the latter (e.g., nmr
spectroscopy) provides information on the site and nature of the inter-
actions involved. Moreover, thermodynamic techniques are superior due
to the independence of the data obtained from the type of association
involved, be it monomer-n-mer or otherwise, which often invalidates
data obtained through treatment of nmr spectroscopic data.

The drug moiety which interacts most strongly with the sodium
cholate is evidenced by the magnitude of the chemical shift differences
of the proton nmr peaks as a function of bile salt concentration
(Figures 15-19). These results are in agreement with the observed
solubility behavior for the same systems (e.g., hexesterol in NaC).
As exemplified by acetylsalicylic acid, the aromatic ring hydrophobi-
cally interacts and the carboxylic acid and ester groups hydrogen bond
to the bile salt as evidenced by the curvilinear behavior and chemical
shift differences (Figure 20).

It is obvious from these results and those of other workers that
a more "sophisticated" mathematical treatment of spectroscopic data
is needed. These results should be compared and combined with those
obtained from a variety of other physical techniques - in a compliment-
ary fashion, of course.

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