Interaction of Povidone with Aromatic Compounds III: Thermodynamics of the Binding Equilibria and Interaction Forces in Buffer Solutions at Varying pH Values and Varying Dielectric Constant

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Abstract □ The complex formation of a series of aromatic compounds with povidone was studied in buffer solutions and organic solvent mixtures by equilibrium dialysis. For all the ligand molecules studied, a linear relationship was found between r, the number of moles of bound ligand per mole of povidone, and the free ligand concentration. The binding constants and the free energies of binding ($-\Delta F$), were greater for compounds in the nonionic state and increased with the number of hydroxyl groups which were capable of forming hydrogen bonds. They decreased with temperature elevation. The thermodynamic data showed entropy gains during the binding process accompanied by small negative enthalpy values. The increased ability to form hydrogen bonds and the increase in ionization of the ligand molecule was reflected in more negative ΔH and decreasing ΔS values. (The thermodynamic values were interpreted on the basis of the "iceberg" concept of water structure.) From these entropy and enthalpy changes, hydrogen and hydrophobic bondings appeared to be the most important types of binding. In organic solvent mixtures, the association constants lowered with increasing ethanol or propylene glycol concentration; a line relationship between the free energy and the dielectric constant of the solvent mixtures was observed.

Keyphrases □ Povidone—interaction with aromatic compounds, thermodynamic considerations □ Complexation—evaluation of povidone–aromatic compound interaction, thermodynamic considerations □ Thermodynamics—interaction of povidone with aromatic compounds

Two types of binding appear to be important in the complexing of ligand molecules onto povidone. It was suggested (1) that hydrogen bonding plays a role, since substituted groups capable of hydrogen bonding enhanced complex formation. However, ligand molecules in the nonionic form, showed a greater tendency to form complexes than when in the ionic state (2–4), indicating the importance of hydrophobic binding. The thermodynamics of binding (5–10) and the thermodynamic properties of the interaction between povidone and small molecules have been reported previously (2, 4, 11–17).

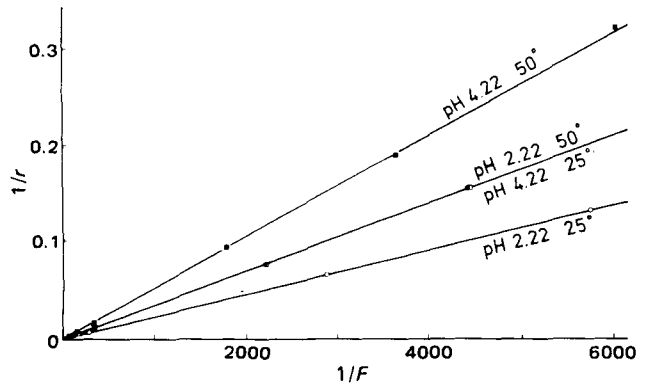

Figure 1—*Influence of temperature on complex formation of 4-hydroxysalicylic acid with povidone. The 4-hydroxysalicylic acid concentration was* 2.5×10^{-4}–2.00×10^{-2} M, *the povidone concentration was 6.00% (*8.57×10^{-5} M*).*

However, the influence of the dissociation of the ligand molecule on the thermodynamic constants has not been studied. To achieve more insight into the binding process, this study evaluated how the thermodynamic constants are affected by the degree of ionization of the ligand molecules and by the dielectric constant of the solvent.

EXPERIMENTAL

Materials—Povidone[1] (molecular weight 700,000) was used as the macromolecule.

The following ligand molecules were studied: benzoic acid[2], nicotinic acid[3] (niacin), isonicotinic acid[2] (isoniazid), salicylic acid[4], salicylamide[2], salicylic acid hydrazide[5], 4-hydroxysalicylic acid[6], 5-hydroxysalicylic acid[4] (gentisic acid), and 5-nitrosalicylic acid[6].

The following buffer solutions were used: hydrochloric acid–potassium chloride buffer (18), pH 1.30 and 1.93; McIllvaine buffers (19), pH 2.20–5.60; a phosphate buffer (18), pH 7.00; a boric acid–sodium tetraborate buffer (20), pH 7.20; and a boric acid–sodium hydroxide buffer (18), pH 9.20 with sodium chloride. The solutions were brought to the ionic strength given in Table I. Sodium chloride showed no tendency to complex with povidone (21). The pH of the solutions was always checked potentiometrically[7] and adjusted, if necessary.

To control the influence of the dielectric constant on complex formation, water–ethanol and water–propylene glycol mixtures were prepared in various proportions, providing a range of dielectric constants. The dielectric constants of the mixtures were measured with a meter[8] at 25.0 and 35.0°.

Equilibrium Dialysis—The ligand–macromolecule interactions were investigated using the method of equilibrium dialysis described previously (22–26).

Dialysis cells[9] similar to those described by Neuhoff (27) were used. The regenerated cellulose membranes were freed of soluble material by leaching in frequently refreshed water and were then immersed in the respective buffers. The two compartments of the dialysis cells were filled, one with solution, the other with the same ligand solution containing the macromolecule.

The cells were rotated at 5 rpm in a constant temperature bath until equilibrium was reached. The povidone-free compartment was analyzed for free ligand. Experiments were carried out at 25.0 and 50.0° with the buffer solutions, and at 25.0 and 35.0° with the ethanol–water and propylene glycol–water mixtures.

Spectrophotometric Analysis—The ligand molecules, with UV absorption bands between 224 and 320 nm, were spectrophotometrically[10] determined in the samples from the dialysis experiments.

RESULTS AND DISCUSSION

The polymer concentration, as well as the range of cosolute concentration, are indicated in Table I.

[1] Povidone (Kollidon K 90), BASF, Brussels, Belgium.
[2] UCB, Brussels, Belgium.
[3] BHD, Poole, England.
[4] Merck, Darmstadt, West Germany.
[5] Aldrich, Beerse, Belgium.
[6] Merck-Schuchardt, München, West Germany.
[7] Radiometer, Copenhagen, Denmark.
[8] Deka meter, DK 300 WTW.
[9] Kontron Diapack.
[10] Perkin-Elmer, model 124.

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Table I—Association Constants, $nk = k_1$ (liter/mole) for Povidone–Cosolute Systems in Buffer Solutions at Varying pH

Ligand Molecule	pKa of Ligand	Ionic Strength	Ligand Concentration	Povidone Concentration, $M \times 10^5$	pH	$nk = k_1$ ($\times 10^{-3}$), liter/mole 25.0°	50.0°
Benzoic acid	4.2[a]	0.25	$1.00.10^{-4}$–$2.00.10^{-2}$	8.57	3.40[b]	7.8	8.5
Benzoic acid	4.2[a]	0.25	$1.00.10^{-4}$–$2.00.10^{-2}$	8.57	5.00[b]	2.5	2.0
Nicotinic acid	4.83[a]	0.32	$5.00.10^{-4}$–$1.00.10^{-2}$	7.14	5.60[b]	1.1	0.7
Isonicotinic acid	4.84[a]	0.32	$5.00.10^{-4}$–$1.00.10^{-2}$	7.14	5.60[b]	1.5	1.2
Salicylic acid	2.97[a]	0.15	$2.50.10^{-4}$–$1.00.10^{-2}$	8.57	2.20[b]	16.2	15.4
Salicylic acid	2.97[a]	0.15	$2.50.10^{-4}$–$1.00.10^{-2}$	8.57	3.80[b]	13.1	9.9
Salicylamide	8.20[c]	0.25	$2.50.10^{-4}$–$5.00.10^{-3}$	8.57	5.00[b]	9.3	8.9[d]
Salicylamide	8.20[c]	0.25	$2.50.10^{-4}$–$5.00.10^{-3}$	8.57	5.00[b]	9.3	8.5
Salicylamide	8.20[c]	0.15	$2.50.10^{-4}$–$1.00.10^{-2}$	8.57	7.20[e]	8.6	6.5
Salicylamide	8.20[c]	0.15	$2.50.10^{-4}$–$1.00.10^{-2}$	8.57	9.20[f]	2.1	1.4
Salicylic acid hydrazide	—	0.25	$1.00.10^{-4}$–$5.00.10^{-3}$	8.57	7.00[g]	9.0	7.7
4-OH-Salicylic acid	3.22[a]	0.25	$2.50.10^{-4}$–$2.00.10^{-2}$	8.57	2.22[b]	44.1	29.7
4-OH-Salicylic acid	3.22[a]	0.25	$2.50.10^{-4}$–$2.00.10^{-2}$	8.57	4.22[b]	28.5	17.5
5-OH-Salicylic acid	2.93[a]	0.25	$2.50.10^{-4}$–$1.00.10^{-2}$	7.14	1.93[h]	24.3	19.2
5-OH-Salicylic acid	2.93[a]	0.25	$2.50.10^{-4}$–$1.00.10^{-2}$	7.14	3.93[b]	18.4	10.8
5-NO_2-Salicylic acid	2.30[a]	0.15	$1.00.10^{-4}$–$2.00.10^{-3}$	7.14	1.30[h]	6.0	4.6
5-NO_2-Salicylic acid	2.30[a]	0.25	$1.00.10^{-4}$–$2.00.10^{-3}$	7.14	3.30[b]	10.8	8.5

[a] pKa of the carboxyl function. [b] McIllvaine buffer. [c] pKa of the hydroxyl function. [d] 40.0°. [e] Boric acid–sodium tetraborate buffer. [f] Boric acid–sodium hydroxide buffer. [g] Phosphate buffer. [h] Hydrochloric acid–potassium chloride buffer.

Preliminary experiments indicated that the binding constants were independent of povidone concentration. For the experiments carried out on the solvent mixtures, a Donnan correction (28) was taken into account.

Theory of Multiple Equilibria—The principles and concepts, fundamental to an understanding of macromolecular binding, have been reported (22–26, 29–30). If no interaction phenomena take place (25–26, 30–33), reversible binding can be described by the equation:

$$r = \frac{nkF}{1 + kF} \qquad \text{(Eq. 1)}$$

where r is the number of moles of cosolute bound per mole of polymer, n is the number of binding sites per mole of macromolecule, F is the molar concentration of free ligand at equilibrium, and k is the intrinsic binding constant (in liters per mole) for the cosolute on the binding sites.

The data were evaluated using a rearrangement of Eq. 1 in a double reciprocal plot, as suggested by Klotz (34):

$$\frac{1}{r} = \frac{1}{nkF} + \frac{1}{n} \qquad \text{(Eq. 2)}$$

The results could also be described by the Freundlich isotherm:

$$r = nkF^{1/m} \qquad \text{(Eq. 3)}$$

where $m = 1$.

Association Constants and Thermodynamics in Buffer Solutions—A characteristic set of results is shown in Fig. 1, where $1/r$ was plotted against $1/F$; the slope of this plot was $1/nk$, and the intercept on the ordinate was $1/n$. Straight lines nearly intersecting the origin were obtained; the other derivatives under investigation behaved similarly. From Eq. 1 it can be seen that this implies $kF \ll 1$, which is equivalent to Eq. 3 where $m = 1$. From Eq. 2, it is observed $1/n$ equals zero, or n is infinite, indicating a large number of adsorption sites. The fraction of the drug bound to povidone did not vary significantly with drug concentration and the binding process appeared nonsaturable.

The results were in accordance with other sources (2, 4, 14, 17, 35, 36) reporting on the complex formation between povidone and ligand molecules. It has been found (2, 14, 25) that the y-axis intercept values ($1/n$) are often determined with great uncertainty, implying it has no physical meaning. In this case, it is preferable to use the values of $nk = k_1$ (25) since it is a measure of the strength of the binding, while n only indicates the binding capacity.

The association constants, nk, deduced from the slopes for povidone–cosolute systems in buffer solutions at different temperatures, are listed in Table I. The thermodynamic functions could be compared with each other because parameters such as the nature of buffer ions and ionic strength had no influence on the binding tendency (1) and consequently, on the thermodynamic functions.

The association constants, for all the derivatives under investigation, were largest at 25.0°, except for benzoic acid in the undissociated form (pH 3.40) (Table I).

The increase in the number of hydroxyl functions substituted on the benzene ring involved higher association constants (4-hydroxysalicylic acid > salicylic acid > benzoic acid), suggesting the importance of hydrogen bonding.

However, the compounds generally interacted to a lesser degree in the ionic than in the nonionic state, suggesting the importance of the hydrophobicity of the substances in the binding process. An exception was 5-nitrosalicylic acid, which showed an increasing binding tendency at higher pH values.

From the association constants, $nk = k_1$ (see Table I) and its temperature dependence, thermodynamic functions for the binding of one mole of ligand with one mole of povidone could be obtained (Table II). For all the ligand molecules, the largest free energy ($-\Delta F$) values were obtained at 50.0° (Table II). From the thermodynamic data listed in Table II, it was clear that the binding process involved a gain in entropy ($+\Delta S$) and that the enthalpies varied from small positive values (benzoic acid, pH 3.40) to small negative values. The standard free energy of binding could be computed from the thermodynamic relation:

$$\Delta F^\circ = -RT \ln k_1 \qquad \text{(Eq. 4)}$$

where $k_1 = nk$, R is the gas constant, and T the absolute temperature.

Assuming no significant temperature dependence of enthalpy change occurring within the temperature range used, the standard enthalpy change ΔH° for the association of one mole of ligand with one mole of macromolecule was estimated from:

$$\ln \frac{nk_{T_1}}{nk_{T_2}} = \frac{-\Delta H^\circ}{R}\left(\frac{1}{T_1} - \frac{1}{T_2}\right) \qquad \text{(Eq. 5)}$$

Table II—Thermodynamic Data for the Binding of One Mole of Ligand by One Mole of Povidone in Buffer Solutions at Varying pH

Ligand Molecule	pH	ΔF_1°, kcal/mole 25.0°	ΔF_2°, kcal/mole 50.0°	ΔH°, kcal/mole	ΔS°, cal/mole degree
Benzoic acid	3.40[a]	−5.3	−5.8	+0.7	20.1
Benzoic acid	5.00[a]	−4.6	−4.9	−1.5	10.4
Nicotinic acid	5.60[a]	−4.2	−4.3	−3.0	4.0
Isonicotinic acid	5.60[a]	−4.4	−4.5	−2.1	7.5
Salicylic acid	2.20[a]	−5.7	−6.2	−0.4	18.0
Salicylic acid	3.80[a]	−4.3	−5.9	−2.1	11.8
Salicylamide	5.00[a]	−5.4	−5.7[b]	−0.5	16.5
Salicylamide	5.00[a]	−5.4	−5.8	−0.7	15.8
Salicylamide	7.20[c]	−5.4	−5.6	−2.1	10.9
Salicylamide	9.20[d]	−4.5	−4.6	−3.2	4.6
Salicylic acid hydrazide	7.00[e]	−5.4	−5.8	−0.9	15.0
4-Hydroxysalicylic acid	2.22[a]	−6.3	−6.6	−3.0	11.1
4-Hydroxysalicylic acid	4.22[a]	−6.1	−6.3	−3.8	7.8
5-Hydroxysalicylic acid	1.93[f]	−6.0	−6.3	−1.8	14.0
5-Hydroxysalicylic acid	3.93[a]	−5.8	−6.0	−4.9	5.8
5-Nitrosalicylic acid	1.30[f]	−5.2	−5.4	−1.9	10.9
5-Nitrosalicylic acid	3.30[a]	−5.5	−5.8	−1.8	12.4

[a] McIllvaine buffer. [b] 40.0°. [c] Boric acid–sodium tetraborate buffer. [d] Boric acid–sodium hydroxide buffer. [e] Phosphate buffer. [f] Hydrochloric acid–potassium chloride buffer.

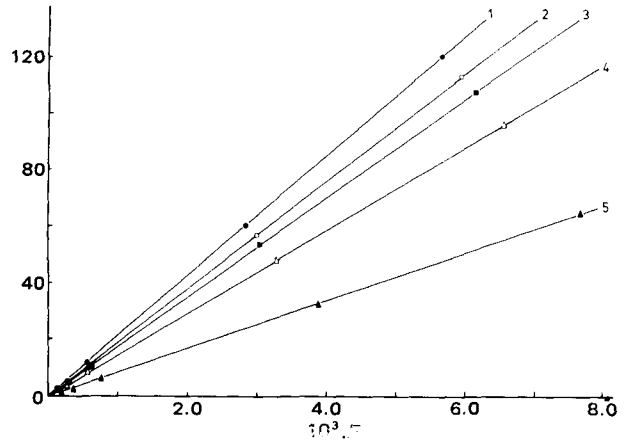

Figure 2—*Influence of solvent mixtures on complex formation. Complexing of 5-hydroxysalicylic acid with povidone. The 5-hydroxysalicylic acid concentration was 2.50 × 10⁻⁴–1.00 × 10⁻² M, the povidone concentration was 5.00% (7.14 × 10⁻⁵ M). Key: 1, water; 2, 5% (v/v) propylene glycol; 3, 10% propylene glycol; 4, 20% (v/v) propylene glycol; 5, 50% (v/v) propylene glycol.*

where T_1 and T_2 represent the absolute temperatures under investigation. The relationship:

$$\Delta F^\circ = \Delta H^\circ - T\Delta S^\circ \qquad \text{(Eq. 6)}$$

permitted calculation of the entropy of binding.

The thermodynamic functions calculated from $nk = k_1$ do not necessarily refer to a single site on the macromolecule. However, their values are useful in considering the nature of the binding, in comparing the binding of different ligand molecules by the same macromolecule, or in analyzing the effect of factors such as pH and ionic strength on the binding (25).

For the ligand molecules investigated, both ΔH° and ΔS° decreased while the degree of dissociation increased. An exception was 5-nitrosalicylic acid, where ΔH° and ΔS° increased with an increase in the degree of dissociation.

From the four types of binding, which could be considered in complex formation of ligands with povidone, *i.e.*, ion–dipole, dipole–dipole, hydrogen, and hydrophobic bonding, the latter appeared to play an important role. Indeed, if complex formation was only due to hydrogen bonding, both ΔH° and ΔS° should be negative (37). However, ΔS° showed a positive value which is characteristic of not only hydrophobic (5, 38–40), but also electrostatic bonding (5–6). Although the ligand molecules also were investigated as ions, this last type of bonding was unlikely to occur since povidone has no ionizable groups (41). The results ruled out the possibility of electrostatic bonding because of the positive influence of the dielectric constant (D) of the solution and the ionic strength (1) on the binding (5). On the other hand, positive enthalpies

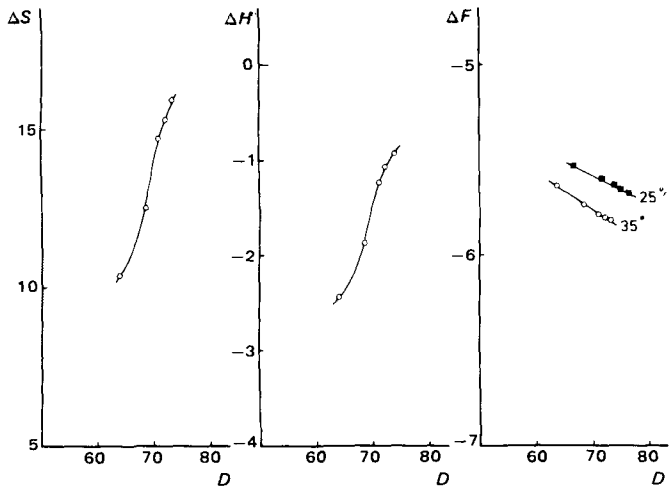

Figure 3—*Complex formation between salicylic acid and povidone. Change of free energy (ΔF°), enthalpy (ΔH°), and entropy (ΔS°) as a function of the ethanol concentration and dielectric constant.*

Table III—Thermodynamic Data (liter/mole) for Povidone–Cosolute Systems as a Function of Solvent Mixtures and Corresponding Dielectric Constant (Ligand Concentration: 2.50 × 10⁻⁴ *M*–1.00 × 10⁻² *M*)

Ligand	Solvent Mixtures, % (v/v)	Dielectric Constant		$nk = k_1$ (×10⁻³), liter/mole 25.0°	$nk = k_1$ (×10⁻³), liter/mole 35.0°
	Ethanol	25.0°	35.0°		
Salicylic acid	0.0	76.4	73.3	14.3	13.6
	2.5	75.2	72.1	13.8	13.0
	5.0	73.9	70.9	13.5	12.6
	10.0	71.5	68.6	12.8	11.6
	20.0	66.6	63.8	11.4	9.9
4-Hydroxy-salicylic acid	0.0	76.4	73.3	39.4	36.2
	2.5	75.2	72.1	37.9	33.9
	5.0	73.9	70.9	35.9	32.1
	10.0	71.5	68.6	33.9	28.9
	20.0	66.6	63.8	29.4	24.6
5-Hydroxy-salicylic acid	0.0	76.4	73.3	21.2	18.6
	2.5	75.2	72.1	20.5	18.0
	5.0	73.9	70.9	19.4	17.1
	10.0	71.5	68.6	18.0	16.0
	20.0	66.6	63.8	15.9	14.1
	Propylene glycol				
5-Nitrosalicylic acid	0.0	76.4	73.3	21.2	18.6
	5.0	74.1	71.1	19.1	16.8
	10.0	71.8	68.9	17.6	15.5
	20.0	67.2	64.5	14.7	12.9
	50.0	53.4	51.2	8.4	7.3

(9) and large positive entropies are characteristic of hydrophobic bondings. It is believed that entropy plays a dominant role in this process (38). From the results [*i.e.*, negative enthalpies (Table II) and the decrease of complex formation with increasing temperature] it could be deduced that the binding process was not only due to hydrophobic bondings (5, 42), but also to exothermic reactions, such as Van der Waals forces or hydrogen bondings.

Positive entropies are associated with many reactions involving the binding of ligand molecules onto povidone. They were generally attributed to the formation of hydrophobic bonds (2, 4, 11, 12, 15, 17) although hydrogen bonding cannot be overlooked (2, 12, 14, 15).

The results are interpreted on the basis of the "iceberg" concept (43) which assumes that hydrocarbon groups, present both in the polymer and in the aromatic cosolutes, are surrounded in aqueous solution with one or more layers of water molecules which are more highly ordered than the molecules in ordinary liquid water. Those layers are referred to as icebergs. In polar groups, such as carboxylic groups and anions, there will also be true hydration about the substituent group.

The entropy changes occurring during the binding process are solely due to the disordering of the icebergs that accompany both the polymer and the cosolute molecules (5). The complex formed will be accompanied by an iceberg which is less ordered compared to the icebergs of the two separate entities which results in a proportional gain in entropy. In studies concerning the interaction of povidone with aromatic compounds, it was calculated (12) that the net enthalpy change was associated with: (*a*) the heat needed to overcome any specific interactions (*i.e.*, true hydration) between water and the macromolecule and between water and the ligand molecule; (*b*) exothermic interaction between the dehydrated entities; and (*c*) exothermic interaction between the water and the bound system (*i.e.*, reformation of true hydration), and is essentially constant with a value of −5 kcal/mole. This value represented the interaction of the polar groups of the polymer with the π-electron system of the aromatic cosolutes (12). By subtracting this value from ΔH° in Table II, the net enthalpy changes associated with the disordering of the water molecules in the icebergs around the polymer and the cosolute can be computed before the binding and the reformation of hydrogen bonds in the icebergs around the complex. These positive enthalpies, and the entropy values are responsible for the hydrophobic bonds.

Ionic groups or substituents capable of hydrogen bond formation may also contribute to the enthalpy values of bonding. Furthermore, hydrogen bonds are able to weaken hydrophobic bonds (5). The results (Table II)

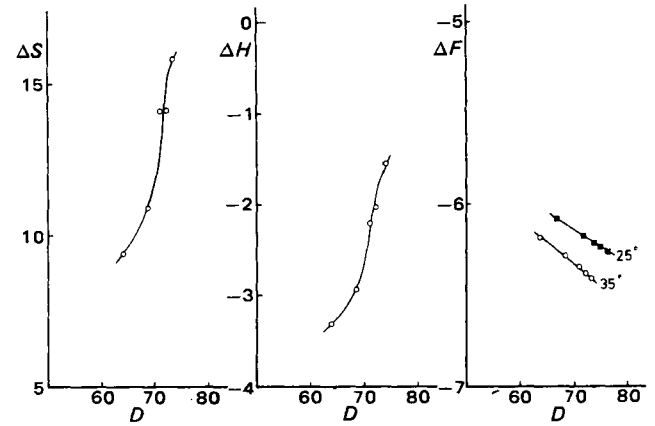

Figure 4—*Complex formation between 4-hydroxysalicylic acid and povidone. Change in free energy (ΔF°), enthalpy (ΔH°), and entropy (ΔS°) as a function of the ethanol concentration and the dielectric constant.*

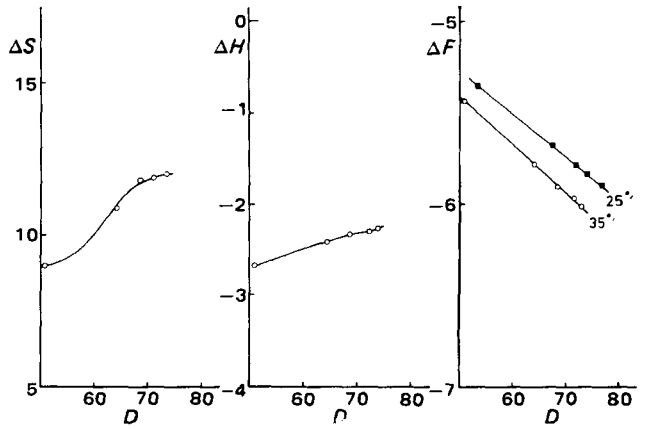

Figure 6—*Complex formation between 5-hydroxysalicylic acid and povidone. Change of free energy (ΔF°), enthalpy (ΔH°), and entropy (ΔS°) as a function of the propylene glycol concentration and dielectric constant.*

agreed with this iceberg concept in that the increase in the ability to form hydrogen bonds (from benzoic acid over salicylic acid to 4- and 5-hydroxysalicylic acid) and the increase in ionization of the ligand molecules is reflected in more negative enthalpy and decreasing entropy values. Therefore, hydrogen bonding was associated with higher binding constants while hydrophobic bonding was associated with the lower constants. One exception was 5-nitrosalicylic acid which, in the case of the bulky nitro group, steric hindrance would be a significant factor (2). The thermodynamic constants obtained for a series of aromatic compounds (12) can be interpreted similarly. From positive enthalpies and high positive entropy values for benzene, both thermodynamic constants diminish more and more with an increasing number of hydroxyl functions substituted on the benzene ring (more possibilities for hydrogen bonding) and are smallest for ionized ligand molecules. It is concluded that both types of bondings, hydrophobic and hydrogen, must play a role in the binding process.

Povidone contains hydrophilic (pyrrolidine ring) and hydrophobic (vinyl chain) groups. A molecular model of the povidone macromolecule shows that the pyrrolidine ring and the paraffin backbone are accessible for the ligand molecules (16). Therefore, it is assumed that the hydrophilic segment is responsible for hydrogen bonding, the paraffin backbone for hydrophobic bonding.

Thermodynamics in Solvent Mixtures—For three ligand molecules, isotherms were determined in a series of ethanol–water mixtures and for one ligand molecule in propylene glycol–water mixtures. A characteristic set of experimental data is shown in Fig. 2, where r is plotted as a function of the free ligand concentration F. In these solvent mixtures, the shape of the isotherms are the same as in the buffer solutions.

From the isotherms, the association constants were determined and summarized in Table III with the measured dielectric constants of the different solvent mixtures. With the aid of the association constants obtained at two temperatures, the thermodynamic functions were computed and represented as a function of the dielectric constants in Figs. 3–6.

From Table III it is noted that the association constants and therefore, the free energies ($-\Delta F$), diminish with increasing ethanol or propylene glycol concentration. For these same concentrations, the association constants are largest at 25.0°; for all the solvent mixtures, the reactions are exothermic ($\Delta H°$ negative), accompanied by high positive entropy values (Figs. 3–6). For the three ligand molecules under investigation, a linear relationship is found between the free energy ($-\Delta F$) and the dielectric constant of the solvent.

This relationship can be written as:

$$-\Delta F° = cst + k'D \qquad \text{(Eq. 7)}$$

or

$$\ln nk = cst + k'D \qquad \text{(Eq. 8)}$$

The shape of the $\Delta H°$ and $\Delta S°$ curves indicated that enthalpy and entropy are connected.

A decrease in bond formation with a decrease in dielectric constant is generally attributed to hydrophobic bonds (4, 5, 44, 45). However, the decrease in dielectric constant influenced the solubility of the ligand molecule; therefore, changes in the solubility of ligand are responsible for the change in the binding tendency.

The correlation between degree of binding, solubility, and dielectric constant of solvent mixtures is being investigated.

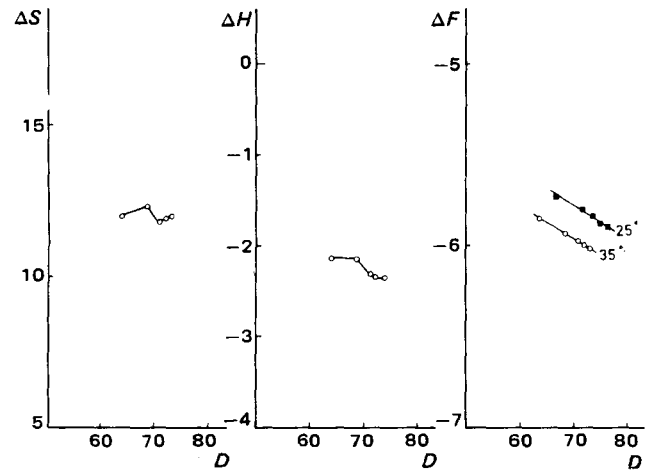

Figure 5—*Complex formation between 5-hydroxysalicylic acid and povidone. Change of free energy (ΔF°), enthalpy (ΔH°), and entropy (ΔS°) as a function of the ethanol concentration and dielectric constant.*

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Nitro- *para*- and *meta*-Substituted 2-Phenylindolizines as Potential Antimicrobial Agents

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Abstract □ Some *para*- and *meta*-substituted nitro-2-phenylindolizines were prepared and tested as potential antimicrobial agents. The syntheses were accomplished *via* the Chichibabin–Stepanow synthesis, using the properly substituted α-picoline and phenacyl bromides followed by direct nitration.

Keyphrases □ Antimicrobial activity—potential, nitro *para*- and *meta*-substituted 2-phenylindolizines □ 2-Phenylindolizines—potential antimicrobials, nitro *para*- and *meta*-substituted, synthesis □ Heterocycles—*para*- and *meta*-substituted 2-phenylindolizines, preparation, potential antimicrobials

Earlier reports (1–4) have generated considerable interest in the fundamental chemistry of the indolizine heterocyclic system (I). However, there have been few reports of the biological activity of indolizines (5–7), and no systematic study has been reported.

BACKGROUND

One report (8) considered 2-(4-fluoro-2-methylphenyl)indolizine and 2-(4-fluoro-2-methylphenyl)-7-methylindolizine as carcinogens, but failed to mention whether these compounds were actually tested for carcinogenic properties. 2-(4-Cyclohexylphenyl)indolizine was reported to be noncarcinogenic when painted on the skin of experimental animals (9).

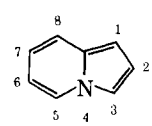
Another report (10) considered 1-indolizinealanine to be a tryptophan antimetabolite. Preliminary tests (11) reported that indolizine-1-acetic acid, the structural analog of indole-3-acetic acid(heteroauxin), showed some auxinlike activity.

1-Diethylaminomethyl-3-methyl-2-phenylindolizine reportedly possessed central nervous system (CNS) depressant activity (12). No useful activity was found for some 1-aminoalkyl-2-phenylindolizines, which were screened for their effects on the CNS in mice and in cats (13). The compounds were stimulants at low doses, depressants at higher doses, and caused death by convulsions.

The 2,3-bis(*p*-methoxyphenyl)indolizines were reported to possess antiexudative activity (14). It was previously reported (15) that 2-(4-fluoro-3-methylphenyl)indolizine decreased the duration of paralysis caused by the drug zoxazolamine when tested in rats. No anti-inflammatory activity for 2-(*p*-methylphenyl)-1-phenylindolizine and 2-(*p*-bromophenyl)-1-(*p*-methoxyphenyl)indolizine was found (16) relative to the reference indoxole.

Rosseels *et al.* (17) found that 3-acetyl-2-alkyl-1-nicotinoylindolizines showed anti-inflammatory activities equivalent to acetylsalicylic acid, and 2-ethyl and 2-*n*-propyl-1-nicotinoylindolizines possessed analgesic activities greater than that of antipyrine.

Two earlier reports (18, 19) stated that N^1-substituted hydrazides of indolizine-2-carboxylic acid were more active than iproniazid in the inhibition of monoamine oxidase. Antonini *et al.* (20) also found that 3-(3-aminopropyl)-2-methylindolizine possessed antiserotonin antihis-



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