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COMPLEXATION AND SOLUBILIZATION OF ACRONINE WITH ALKYLGENTISATES

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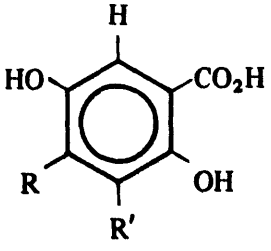
SUMMARY

The complexation of acronine with gentisate, and 3-methyl, 4-methyl, 3-ethyl, and 4-ethyl-substituted gentisates was studied by solubility techniques in aqueous solutions at 25°C. In all cases both 1 : 1 and 1 : 2 (acronine : gentisate) complexes were found and apparent increases in the solubility of acronine were observed with 3-methyl-gentisate bringing about the largest increase. Both the nature of the substituent and its position were important in the complexation and the effects appear to be due to opposing steric and hydrophobic contributions.

INTRODUCTION

Gentisic acid (Ia) has been reported to form complexes



I

(a) R=R′=H; gentisic acid
(b) R=H, R′=CH_3; 3-methyl-gentisic acid
(c) R=H, R′=CH_2CH_3; 3-ethyl-gentisic acid
(d) R=CH_3, R′=H; 4-methyl-gentisic acid
(e) R=CH_2CH_3, R′=H; 4-ethyl-gentisic acid

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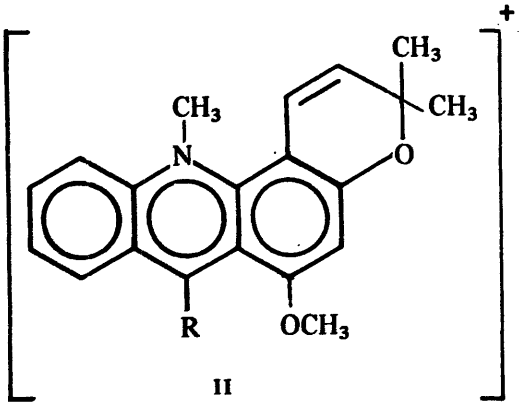
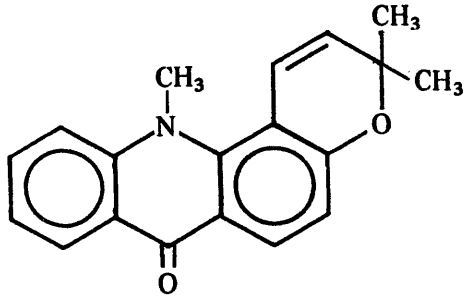
with several substrates (Higuchi and Connors, 1965; Kreilgård, et al., 1975). It has been observed for numerous systems which undergo complexation, that the apparent properties of both the substrate and the ligand may exhibit significant changes such as substantially altered solubility and/or stability (Kreilgård et al., 1975; Huang et al., 1976; Higuchi and Lachman, 1955; Lachman et al., 1956; Lachman and Higuchi, 1957; Lachman et al., 1957; Higuchi and Pitman, 1973).

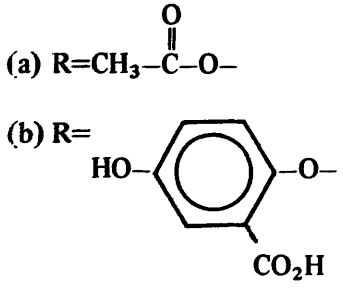
In previous works (Huang et al., 1976; Huang, 1975) the use of gentisic acid has been suggested for the stabilization



II



III Acronine



against hydrolysis of 7-acetylacroninum ion (IIa), a pro-drug ester of acronine (III) an experimental anti-tumor agent. Gentisic acid was also observed to have apparent solubilizing effects on both the pro-drug and acronine itself (Huang et al., 1976). Huang (1975) had shown that a variety of gentisic acid-related compounds influenced the apparent stability of the pro-drug in aqueous solutions and it was felt that they might also associate with acronine to varying degrees.

Recent work by Rasadi (1978), Jain (1978), and Repta et al. (1977) has led us to revise the interpretation of the mechanism by which gentisate apparently stabilized by acetylacroninum ion. It now appears that the apparent stabilizing influence of gentisic acid and related compounds on the hydrolysis of the acetylacroninum ion is actually a rapid reaction involving displacement of the acyl group by a phenolic group of gentisic acid (and chemically related compounds) (Rasadi, 1978) to yield a species such as IIb

which then hydrolyzes more slowly than the acetylacroninum ion.

In light of these recent developments and the high reactivity of the acetylacroninum ion, the formulation based on a stabilized pro-drug (Huang et al., 1976) is not viable and we sought possible alternate approaches for solubilizing acronine itself. Complexation was one of the apparent alternatives, and since gentisic acid has been found to complex with acronine (Huang et al., 1976) we undertook a study of the comparative properties of gentisic acid and closely related alkyl gentisates as complexing agents for acronine.

The objectives of this study were to enhance the solubility of acronine and to establish the importance of position and chain length of alkyl substituents on the solubility and association constants of the various gentisate–acronine complexes.

MATERIALS AND METHODS

Gentisic acid (Aldrich) was recrystallized from water. The product, after air-drying, melted at 204–205°C [lit. (Merck Index, 1976) mp = 205°C]. The 3- and 4-methyl-gentisic acids were synthesized by the procedures of Nudenberg et al. (1943) and the 3- and 4-ethyl-gentisic acids were synthesized according to the methods reported by Renz (1947). All were recrystallized from water and air-dried. The nature of the products was confirmed by NMR and elemental analysis. The melting points of the compounds were: 3-methyl-gentisic acid, 220–222°C [lit. (Nudenberg et al., 1943) mp = 221–223°C]; 4-methyl-gentisic acid 245–246°C [lit. (Nudenberg et al., 1943) mp = 203–204°C] [1]; 3-ethyl-gentisic acid 213–214°C [lit. (Renz, 1947) mp = 188°C] [1], 4-ethyl-gentisic acid 216–218°C [lit. (Renz, 1947) mp = 209–214°C] [1].

The water used was deionized (1 μmbo) and passed through a charcoal filter prior to use. All other chemicals used were of reagent grade and were used without further purification.

The solubility studies were carried out by equilibrating excess solid acronine with a buffered aqueous solution containing various amounts of the chosen gentisic acid species. The buffer solution contained 0.1 M succinic acid, 0.4 M sodium nitrate, and concentrations of the gentisate from 0.0 to 0.5 M and sufficient sodium hydroxide (~0.9 M) to yield pH = 5.5. The ionic strength ranged from 0.6 to 1.1 M.

Aliquots (5 ml) of the above solutions containing the various gentisates species at concentrations of 0–0.5 M were placed in 4 dram screw-cap vials containing acronine (~10 mg) and 4–5 glass beads (5 mm diameter). The vials were tightly closed and equilibrated with agitation at 25°C for 72 h. In separate experiments it was determined that there was no change in the solution composition beyond 72 h. After 72 h, agitation was halted, the precipitate allowed to settle, and the liquid layer was removed and passed through a membrane filter (5 μm pores). An aliquot (1 ml) of the filtrate was vortexed with an equal volume of chloroform. The layers were separated and 0.5 ml of the chloroform layer was removed by pipette and diluted with methanol to a total volume of

[1] The reasons for the discrepancies in melting points for the 4-methyl- and the 3- and 4-ethyl-gentisic acids prepared here and those reported in the literature are unknown. It may be assumed that the materials first synthesized were either very impure and/or a lower-melting crystal modification.

2 ml (in a 2 ml volumetric flask) and an aliquot (25 μl) was analyzed directly by high performance liquid chromatography (HPLC).

In separate experiments it was demonstrated that even in the presence of the alkyl gentisates >95% of the acronine was recovered using the above extraction procedure.

Chromatography. The HPLC system used consisted of Waters Model 440 absorbance detector (at 280 nm), U6K injector, an M6000 A solvent delivery system, and a μBondapak C_{18}-column. The mobile phase was methanol–water (70 : 30) and the flow-rate was 2 ml/min. The temperature was ambient. At a detector attenuation of 0.02 AUFS, 25 μl injections of solution containing acronine at $\geq 1 \times 10^{-6}$ M were easily detected and a linear relationship between peak height and concentration was obtained with R = 0.999, slope = 1.144×10^{6} and a zero intercept. The retention volume of acronine was approximately 19 ml.

The data obtained in the solubility studies were used to calculate the association constants according to the method of Higuchi and Kristiansen (1970).

RESULTS AND DISCUSSION

The complexation of acronine with gentisic acid and the 3-methyl-, 3-ethyl-, 4-methyl- and 4-ethyl-substituted gentisic acids was studied by phase solubility methods (Higuchi and Connors, 1965; Kreilgård, 1975) at 25°C and pH = 5.5. Under such conditions the carboxyl groups of the acids should be ionized to an extent of >99% and acronine ($pK_a \sim 1.6$) (Bourne et al., 1977) would be essentially unionized.

The phase solubility diagrams obtained are shown in Fig. 1 and it is apparent that in all cases complexation occurs. The non-linear relationship between acronine in solution and added ligand indicates the formation of at least 2 complexes with differing stoichiometry suggesting the presence of both 1 : 1 and 1 : 2 (substrate : ligand) complexes (1).

Under the conditions studied, the complexes almost certainly involve the gentisate ion and thus the complexes would be anionic in nature. This undoubtedly is responsible for the large increases in solubility which were achieved. In none of the systems studied was there any evidence of the formation of a precipitate of the complexed species.

When the data points in Fig. 1 were curve-fitted according to published methods (Higuchi and Kristiansen, 1970), the solid lines in Fig. 1 were obtained and they described the experimental data quite well. The values of $k_{1:1}$ and $k_{1:2}$ which are found to yield the solid lines are presented in Table 1.

The two important observations from the data are that: (a) the 3-alkyl-substituted gentisates interact with acronine to a much greater extent than the 4-alkyl-gentisates (Id and Ie) or gentisate itself (Ia); and (b) while a methyl substituent at either the 3 or 4 position resulted in increased complexation (relative to the unsubstituted gentisate) a further increase in chain length produced marked decreases in the extent of complexation. These results suggest that the stability of the complex is greatly influenced by steric factors involving both position and total bulk. At the time it appears that there is a significant hydrophobic contribution to the stability constant in that the 3-alkyl-substituted species exhibit much stronger complexation than gentisate. Thus in the systems studied, it appears that the steric and hydrophobic interactions have opposing effects.

Regardless of the physicochemical details involved in the complex formation, one

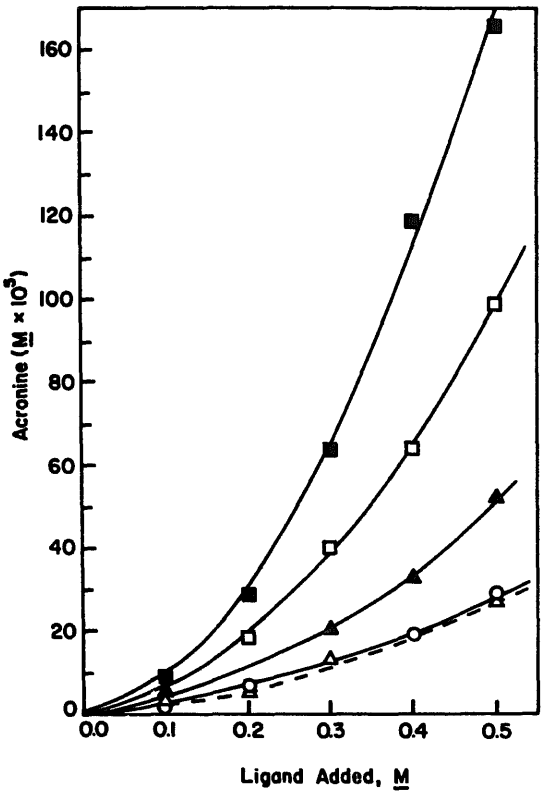


Fig. 1. Plot of the apparent solubility of acronine at 25°C as a function of the concentration of various gentisic acid ligands in aqueous buffer (0.1 M succinate, pH 5.5, ionic strength ~0.6 M with sodium nitrate). The solubility of acronine in the absence of ligands is 8.5×10^{-6} M. The smooth curves were calculated using the constants in Table 1. Key: gentisic acid (○, ———); 3-ethyl-gentisic acid (□, ———); 4-ethyl-gentisic acid (△, — — —); 3-methyl-gentisic acid (■, ———); 4-methyl-gentisic acid (▲, ———).

TABLE 1

ASSOCIATION CONSTANTS FOR FORMATION OF COMPLEXES BETWEEN ACRONINE AND VARIOUS GENTISIC ACID DERIVED SPECIES IN AQUEOUS SOLUTION AT pH = 5.5 AND 25°C. SOLUTIONS CONTAINED 0.1 M SUCCINATE AND 0.4 M SODIUM NITRATE.

Ligand	$k_{1:1}$ (M^{-1})	$k_{1:2}$ (M^{-1})
Gentisate	8.9	12.7
4-ethyl-gentisate	18.7	4.8
4-methyl-gentisate	13.3	16.0
3-ethyl-gentisate	25.3	16.4
3-methyl-gentisate	35.2	20.9

important fact emerges and it is that by using 3-methyl-gentisic acid, one can achieve a relatively high concentration of acronine in solution. At a 0.5 M concentration of Ic, an apparent acronine solubility of ~0.53 mg/ml was observed which is about 330-fold greater than the solubility of acronine (~2.7 μg) in the absence of the ligand. Furthermore, we were able to prepare 0.8 M solutions of Ic at 5.5, and although we did not study the complexation under such conditions, calculations based on the values of $k_{1:1}$ and $k_{1:2}$ in Table 1 yield an apparent total solubility for acronine of 1.16 mg/ml under such circumstances. This would represent more than a 400-fold increase in the apparent solubility of acronine.

A parenteral formulation of acronine utilizing Ic (to increase the solubility of acronine) would be quite stable and it would release the drug immediately upon dilution. Whether or not the rapid release would result in precipitation in the blood stream would be dependent upon the dynamics of administration and subsequent dilution as well as any protein binding which might occur.

The suitability of 3-methyl-gentisate (Ic) for intravenous use has not been reported. Previously, the vasotoxicity of aqueous gentisate solutions at pH = 3.5 and 4.5 were studied in rabbits (Cradock, personal communication). The concentration of gentisate ranged up to 0.3 M. Only mild vaso-irritation was observed after 5 consecutive daily injections of up to 25 mg of gentisate. These results suggest that gentisate may be suitable for parenteral use. In the light of the above results, samples of 3-methyl-gentisate (sodium salt) have been furnished to the National Cancer Institute for intravenous toxicity studies. However, the results are not yet available.

In summary it does appear that complexation of acronine with 3-methyl-gentisate and perhaps other alkylgentisates may provide a basis for a parenteral formulation of this experimental cytotoxic agent. In addition, the alkylgentisates may prove valuable in the complexation of other planar aromatic compounds whose inherent solubility and/or stability characteristics differ from those desired.

ACKNOWLEDGEMENTS

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