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Solubilization and Reaction Studies of Polar Substrates in the Presence of Apolar Octahydroxy Reverse Micelles

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Abstract. Octahydroxy tetrameric cyclophane **1** containing four cetyl chains was synthesized from resorcinol and cetanal under acidic conditions and was characterized by ^{1}HNMR, ^{13}CNMR, and elemental analysis. Cyclophane **1** forms aggregates with CMC 7.1×10^{-5} M in a nonpolar solvent such as CCl_4. The smaller molecule glycine is more solubilized than the bigger lysine, demonstrating size discrimination. The highly polar compounds aspartic acid and glutamic acid, however, required 1.5 mm of **1** for solubilization at the detectable limit. Kinetic study of the oxidation of glycine by ninhydrin in the presence of **1** revealed that the rate enhancement compares to that in an aqueous environment, whereas the reduction of crystal violet by Hanzchester shows a decrease in the rate over that in aqueous solution.

Key words: Cyclophanes, aggregates, amino acids, molecular interaction.

1. Introduction

Resorcinol-aldehyde tetramers with different aldehydes have been synthesised and studied for host–guest complexation [1, 2]. These tetrameric cyclophanes containing eight hydroxyl groups were soluble in basic aqueous solutions. The solubility of these tetramers changed with the chain length of the bridging aldehyde portion. Cyclophanes containing more than six carbons were soluble in organic solvents and dodecanal-resorcinol tetramer has been shown to form reverse micellar aggregates in CCl_4. Reverse micelles obtained from CTAB (cetyl trimethyl ammonium bromide), lecithin, and water Arosol-OT interact with cholesterol, protein and enzymes [2–5] mimicking biological membranes. These types of compounds have been used to solubilize polar monosaccharides in apolar solvents [6] and are also of interest as models to study the types of interaction in an isolated environment, like the active site of enzymes. Even enzymes have shown selectivity changes while dissolved in a reverse micellar system [3, 5]. The present study is devoted to a new kind of tetrameric molecule, **1**, that forms aggregates in CCl_4, helps in solubilising polar amino acids in CCl_4, and interacts with dissolved molecules, such as amino acids and crystal violet to affect the kinetics of their oxidation and reduction reactions.

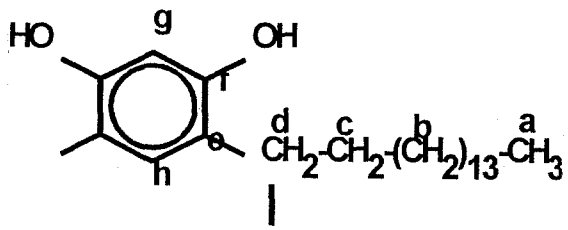


Figure 1.

2. Experimental

2.1. Preparation of 2, 8, 14, 20-tetracetylpentacyclo ($1^{9.3}$, $1^{3,7}$, $1^{9,13}$, $1^{15,19}$)-octacosa-1(25), 3, 5, 7(28), 9, 11, 13(27), 15, 17, 19(26), 21, 23-dodecaene-4, 6, 10, 12, 16, 22, 24-octol (**1**)

To a solution of resorcinol (19.8 g, 0.18 M) in ethanol–hydrochloric acid (4 : 1) at 20 °C was added a solution of cetanal (43.2 g, 0.18 M) in 95% ethanol (50 mL) dropwise, followed by stirring for 2 h. The resulting solution was allowed to warm slowly to 25 °C and then heated to 75 °C for 24 h. The precipitate that separated was washed repeatedly with methanol and dried. Recrystallization twice from methanol gave the product **1**. *Yield* 70%. *Elemental analysis* for C H O. *Calculated:* C = 79.50%, H = 10.84%. *Found* C = 79.70%, H = 11.10%. ^{1}H-NMR ($CDCl_3$): δ 0.89 (t,12H,CH), 1.23 (brs, 104 H, 52(CH_2O, 2.02(m, 8H, CH_2—CH Res_2) 11, 4.09 (t, 4H, methine), 4.5 (brs, 4 OH), 6.84(s, 8H, Ar), 8.88(s,4 OH). ^{13}C-NMR (Figure 1): δ 14.1 (q, CH_3), 20.4 to 31.8 (overlapping t, CH_2), 37.1 (d, CH methine), 96.0(d, C_g), 124 (d, C_h),150 (s, C_f). INEPT 20.4–31.8 (CH_2) negative.

2.2. General procedure for solubilization of amino acids in CCl_4 with **1**

Amino acid (250 mg) was shaken with CCl_4 (10 mL) for 1 h and filtered. To an aliquot portion (5 mL) was added ninhydrin (1 mL, 1 M) followed by heating to 60 °C for 5 min. The violet colored solution was extracted with doubly distilled water (5 mL). The concentration was calculated from the absorption at 550 nm. Similar experiments were repeated with **1** in CCl_4 (0.5 mM) (Table I).

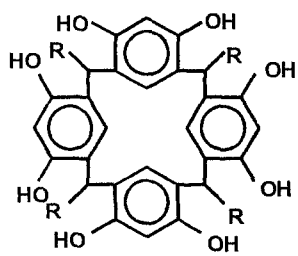
2.3. Kinetic study of oxidation of glycine by ninhydrin (general procedure, Scheme 1)

Compound **3** (250 mg) was shaken with CCl_4 (10 mL) for 1 h. The amount dissolved was allowed to react with ninhydrin (1 mL, 0.1 M). The rate of formation of the violet complex was followed by observing the rise in absorption at 540 nm every 3 min. for a period of 2 h and the rate was calculated. The rate was similarly calculated for the reaction in CCl_4 (0.5 mM) (Table II).

Table I. Solubilization of amino acids with 1 in CCl_4 (0.5 mM).

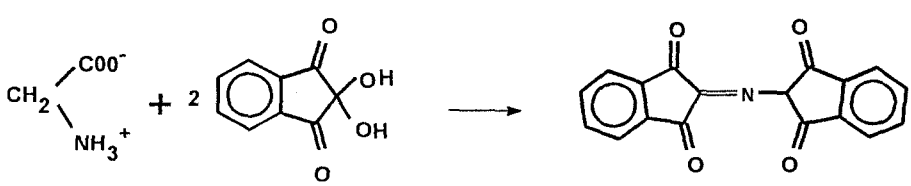
Amino acids	Solubility in CCl_4 $ML^{-1} \times 10^{-3}(S_1)$	Solubility in CCl_4 with $ML^{-1} \times 10^{-3}(S_2)$	S_2/S_1
Leucine	7.10	9.00	1.77
isoLeucine	1.10	5.60	1.96
Lysine	1.52	2.66	1.75
Serine	4.40	6.34	1.44
Threonine	1.58	3.94	2.49
Alanine	0.28	0.70	2.50
Glycine	0.90	2.44	2.71
Glutamic acid*	0.00	0.22	–
Aspartic acid*	0.00	0.58	–

* 1.5 mM of **1** was used when these amino acids are insoluble in CCl_4 with **1** (0.05 mM).



1 $R=(CH_2)_{10}\text{-}CH_3$

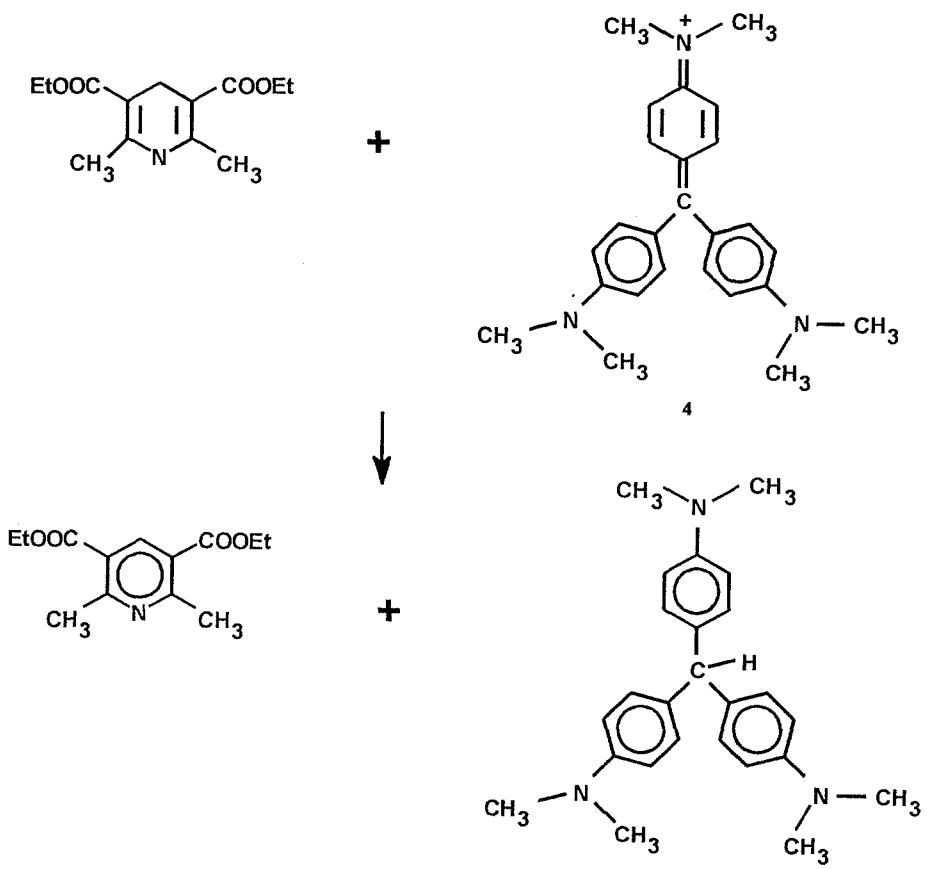
2 $R=(CH_2)_{14}\text{-}CH_3$



Scheme 1.

Table II. Rate of decarboxylation of glycine with ninhydrin (0.01 M).

Solvent system	k_{obs} s^{-1}
H_2O	2.9×10^{-3}
CCl_4 with **1**	1.6×10^{-2}
CCl_4	3.0×10^{-2}



Scheme 2.

2.4. SOLUBILIZATION OF CRYSTAL VIOLET (4) AND ITS REDUCTION WITH HANZCHESTER WITH CCl_4 (SCHEME 2)

Compound **4** (250 mg) was shaken with CCl_4 (10 mL) for 30 min, filtered twice, and diluted 20 times, and its concentration observed by noting the absorption at 580 nm. The experiment was repeated with **1** in CCl_4 (0.5 mM).

Hanzchester (0.1 M) was added to **4** dissolved in CCl_4 and in CCl_4 with **1** (0.5 mM) and its reduction was studied by following the decrease in absorption at 580 nm.

3. Results and Discussion

Resorcinol-cetanal tetramer **1** was obtained under acidic conditions. The ^{1}H-NMR spectrum shows a triplet at 4.09 (methine CH) and eight hydroxyl protons similar to those of **2** at δ 4.5 and 8.8, establishing the tetrameric structure of **1**. Similarly, the ^{13}C-NMR spectrum indicates a bridging methine at δ 37.1 (Figure 1) and an inept experiment clarifies CH_2 signals appearing from δ 20.5–31.8. Cyclophane **2** forms aggregates, as shown by vapour pressure osmometry in CCl_4 [6]. Similarly, **1** aggregates with CMC 7.1×10^{-5} M [7].

The octahydroxy end of **1** gives a unique cyclic hydrophilic site for polar solutes and helps in their solubilization in apolar CCl_4. Many amino acids were shaken with **1** in CCl_4 (0.5 mM), and checked for their increased solubilities by converting into the violet ninhydrin complex, followed by electronic absorption spectroscopy (Table I). Smaller amino acids like glycine showed a larger increase in solubility (*viz.* 2.71-fold), whereas the larger lysine was only 1.75 times more soluble than in pure CCl_4. The role of **1** in increasing solubility was clear from the fact that dicarboxylic amino acids such as glutamic and aspartic acid were not at all soluble in pure CCl_4 and even with **1** in CCl_4 (0.5 mM), but did solubilize on increasing the concentration of **1** to 1.5 mM. Compound **2** has been shown [6] to solubilize different sugars in CCl_4 where ribose is bound in the α-pyranose form to a pair of hydrogen-bonded OH groups on the adjacent benzene rings in **2**, providing the essential binding site for the guest OH groups, as discussed below. The kinetic study shows that there is a definite effect due to the presence of **1** in the reaction medium; the exact points of interactions of aminoacids and crystal violet with **1** are not clear in micellar form.

3.1. Kinetic studies in the presence of 1

The interaction of **1** with the dissolved molecules was clear from the effect of **1** on the rate of reaction of dissolved compounds. Oxidation of glycine **3** and reduction of crystal violet **4** were studied in three different environments: in water, in CCl_4 and in CCl_4 with **1**. The apparent rates were compared to discern the effect of **1**. Both reactions were carried out under quasi-first-order conditions using one reactant in excess.

Decarboxylation of glycine in the presence of **1** by ninhydrin was observed by following the absorption of the product at 540 nm. The rate is slowest in water and fastest in CCl_4 (Table II) due to desolvation of reactants, whereas the rate in CCl_4 with **1** is 1.87 times slower than in CCl_4 and 5.71 times faster than in water, clearly establishing an interaction of **1** with **3**. Compound **3** might enter into the cavity of

Table III. Rate of reduction of crystal violet with Hanzchester (0.1 M).

Solvent system	k_{obs} s^{-1}
H_2O	3.1×10^{-2}
CCl_4 with **1**	1.6×10^{-2}
CCl_4	9.2×10^{-2}

1, as indicated by CPK models, preventing a free attack of ninhydrin on glycine and hence causing the rate to slow down, but the exact nature of interactions between **1** and **3** have to be established by further experiments.

Crystal violet **4** was 5.71 times more soluble in CCl_4, in the presence of **1**. The cationic dye showed a strong interaction with electron-rich **1** in CCl_4 as indicated by its reduction with Hanzchester (0.1 M). The reaction was followed by the decrease in intensity at 580 nm in three different systems, as for **3** (Table III). Interestingly, this study indicated that the rate was slowest in CCl_4. Desolvation causes a rate enhancement over that in water. The slowest rate shows an even stronger interaction of **1** with **4** in CCl_4 than the interaction of water molecules with **4**.

Acknowledgement

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