Binding Site of Naphthalene to Bile Salt Micelles as Determined by ^{1}H Nuclear Magnetic Resonance

By FREDRIC M. MENGER,* JUNG-UNG RHEE, and LEON MANDELL
(*Department of Chemistry, Emory University, Atlanta, Georgia* 30322)

Summary Naphthalene is shown to bind in the core of bile salt micelles near the C-19 angular methyl groups.

BILE salts are steroidal surfactants which are emptied into the upper intestines for the purpose of binding and solubilising dietary lipids. The principal bile salts are cholanic acid derivatives bearing hydroxy-substituents at one or more of the 3α-, 7α-, and 12α-positions (see Figure). When dissolved in water above a critical concentration, a bile salt forms micellar aggregates. The interior of these micelles is comprised of the hydrophobic β sides of the steroids lying back-to-back.[1] This arrangement exposes the α-faces with their hydroxy-groups to the water. Thus, there are three possible adsorption sites for a guest molecule: the hydrophobic micelle interior, the micelle surface studded with hydroxy-groups, and a polar region near the carboxylates and their counterions. We report here the first ^{1}H n.m.r. study of the binding site of bile salt micelles.[2]

N.m.r. spectra were obtained for solutions of 0·20M glycodeoxycholic acid (pH = 8·00, I = 0·30) containing varying amounts of naphthalene (0·005—0·02M). Since the presence of organic solutes can modify micellar structure,[3] the naphthalene concentration was kept as low as possible.† Certainly, virtually all the naphthalene was micelle-bound because the concentrations greatly exceeded the naphthalene solubility in pure water. As shown in the Figure, the chemical shifts of both the C-18 and C-19 methyl groups were found to move upfield upon addition of the guest molecule, whereas the shift of the 'methylene envelope' was hardly affected. The fact that two peaks were not observed for each angular methyl group at concentrations much less than one naphthalene per micelle proves that transfer of naphthalene among the micelles is fast on the n.m.r. time scale.

Two important conclusions arise from the Figure. First, the binding site for naphthalene can be identified as

† The articles in ref. 2 employ molar ratios of guest to bile salt as high as 2.

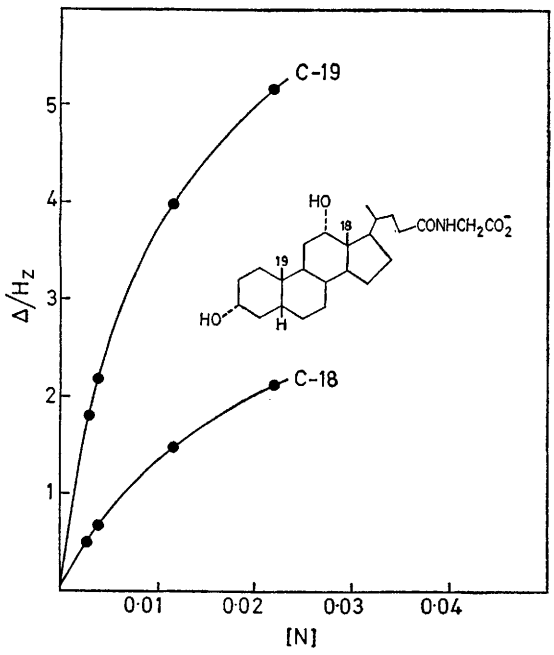

FIGURE. Upfield shift (Δ) (100 MHz data) for the C-18 and C-19 angular methyl protons of glycodeoxycholic acid as a function of naphthalene concentration [N]. Bile salt concentration is 0·2M, pH 8·00.

the micelle interior (as opposed to the micelle surface) because the angular methyl groups are located in this region. Second, the greater perturbation of the C-19 methyl signal relative to that of the C-18 methyl signal points to preferential adsorption near the A/B ring junctures away from the ionic side chains of the steroids. The upfield shifts very likely represent only net anisotropy effects from many orientations at the binding sites of the micelles.

The above conclusions are reinforced by n.m.r. spectra of glycodeoxycholic acid and glycocholic acid with 1-phenylhexane. Although the shifts were not as large as in the naphthalene system, the C-19 signal moved upfield much more than did the C-18 signal for both bile salts.

Control experiments were carried out to show that the effects of naphthalene on the n.m.r. spectra of glycodeoxycholic acid are due to specific adsorption. Thus, binding of a non-aromatic hydrocarbon, cyclohexane, did not modify the chemical shifts of the angular methyl groups. Moreover, addition of 0·02M-naphthalene to 0·2M-bile salt in methanol (a solvent in which micelles do not form[4]) caused the C-18 and C-19 methyl group signals to shift by an identical and insignificant amount (0·2 Hz).‡

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‡ Spectra were obtained with the aid of a Jeol JNM-MH-100 spectrometer using an internal lock.

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[2] A ^{13}C n.m.r. study of bile salt complexes has been recently reported by D. Leibfritz and J. D. Roberts, *J. Amer. Chem. Soc.*, 1973, **95**, 4996. This work could not provide the information we report here since anisotropy effects are relatively insignificant in ^{13}C n.m.r. (see G. C. Levy and G. L. Nelson, 'Carbon-13 Nuclear Magnetic Resonance for Organic Chemists,' Wiley-Interscience, New York, 1972); Related work using synthetic surfactants is described by J. C. Eriksson and G. Gillberg, *Acta Chem. Scand.*, 1966, **20**, 2019.

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[4] D. M. Small, S. A. Penkett, and D. Chapman, *Biochim. Biophys. Acta*, 1969, **176**, 178.