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Anion Recognition by Alkyl Cholates: Neutral Anionophores Closely Related to a Natural Product

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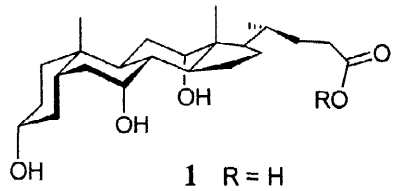
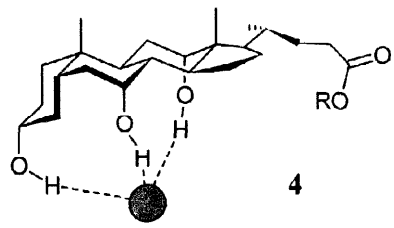
Abstract: Alkyl cholates **2** and **3** have been shown to bind tridentate oxoanions using ^{1}H NMR and solubilisation measurements in C_6D_6 and benzene-hexane mixtures respectively. Recognition is presumed to occur through hydrogen bonding to the three hydroxyl groups on the α face of the steroid nucleus.

The bile acids, such as cholic acid (**1**), might be said to play divergent rôles in biology and in supramolecular chemistry. While nature exploits the hydrophobic face of **1** to bind and solubilise *non-polar* molecules in aqueous media,[1] supramolecular chemists have often used its functionality to synthesize more or less elaborate structures aimed at the recognition of *polar* species, usually in non-polar media.[2] As part of our programme on the design and synthesis of neutral anionophores based on **1**,[3,4] we were curious to establish whether even the native steroid unit might exhibit "inverse" recognition properties if removed from its natural, aqueous environment. Specifically, it seemed possible that the three hydroxyl groups in cholate esters, such as **2** or **3**, might cooperate to bind an anionic substrate by H-bond donation as in **4** (shaded sphere = anionic substrate).



1 R = H
2 R = Me
3 R = n-C_8H_{17}



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The three preorganised H-bond donor groups in **1-3** suggested complementarity to tridentate anionic substrates such as sulfonates. Indeed, modelling of the complex between **2** and methanesulfonate anion (MsO^-) yielded plausible structures with 3 nearly linear hydrogen bonds (e. g. Figure 1).[5] Accordingly, we investigated the interaction of tetrabutylammonium *p*-toluenesulfonate (TBA^+TsO^-) with **2** by NMR in deuterated organic solvents. Addition of TBA^+TsO^- to solutions of **2** in $(CD_3)_2SO$ or $CDCl_3$ did not result in substantial changes to the ^{1}H NMR spectrum of the cholate, but on changing to C_6D_6 as solvent, clear signs of complex formation were observed. Analysis of the hydroxyl protons was hampered by their

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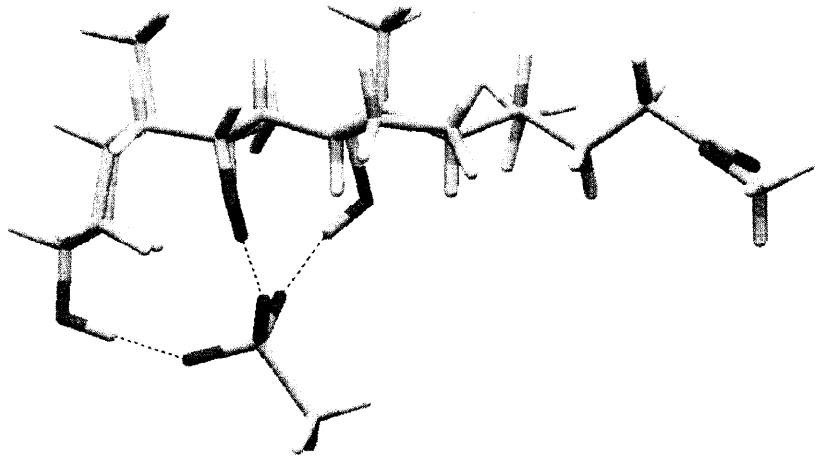


Figure 1: Energy-minimised structure for the complex between methyl cholate (**2**) and $MeSO_3^-$.[5] The three intermolecular hydrogen bonds (1.60 - 1.65 Å) are represented as broken lines.

exchangeability and was not attempted. However, we have previously shown that anion receptors derived from **1** exhibit significant changes in C*H* chemical shifts ($\Delta\delta$) on complex formation.[3] In this case the simplicity and (commercial) availability of receptor **2** allowed a detailed analysis of the phenomenon. While most of the signals in **2** moved downfield by between 0 and 0.1 p.p.m., a limited subset showed much larger $\Delta\delta$. As illustrated in Table 1, these protons were spread across the lower, functionalised face of the steroid nucleus, suggesting that all three hydroxyl groups were involved in recognition. The signal due to the 4α proton, which showed the greatest sensitivity to complex formation, was used in attempts to quantify the process. Titrations performed at [**2**] = 8 - 11 mM[6] gave curves which clearly showed saturation behaviour but could not be fitted accurately to a 1:1 binding model. On the basis of a Job plot[7] which peaked at [**2**]/[TsO^-] $\approx$ 55%, the data was reanalysed for simultaneous 1:1 and 2:1 binding, using the HOSTEST programme.[8] The calculation yielded K_a = 220 M^{-1} for the 1:1 interaction and 50 M^{-1} for the equilibrium between 1:1 and 2:1 stoichiometries.[9]

Other substrates investigated were TBA^+MsO^- and tetrabutylammonium hydrogen phenylphosphonate [$TBA^+PhP(OH)O_2^-$]. The former yielded much the same pattern of $\Delta\delta$ as TBA^+TsO^-, implying that the aromatic ring in the latter is held away from the steroid in the complex. In the case of the phosphonate, analysis by NMR titration was precluded by the insolubility of the salt in benzene. However, complex formation was clearly evident from the increased solubility of $TBA^+PhP(OH)O_2^-$ in the presence of **2**, and the resulting changes to the ^1H NMR spectrum of the latter. Again, downfield motions of the steroidal C*H* signals were observed, concentrated especially on the α-face of the steroid (Table 1). The somewhat different pattern of $\Delta\delta$ in this case presumably reflects the change from anions with three H-bond acceptor centres to one containing an H-bond donor.

The solubilisation of $TBA^+PhP(OH)O_2^-$ allowed a straightforward comparison of the binding potencies of a range of bile acid esters. Experiments were done in benzene-hexane mixtures in which the phosphonate was found to have negligible solubility. **2** was also insoluble in these systems, but could be replaced by the more lipophilic **3**.[10] As shown in Table 2, cholate **3** proved able to solubilise a full equivalent

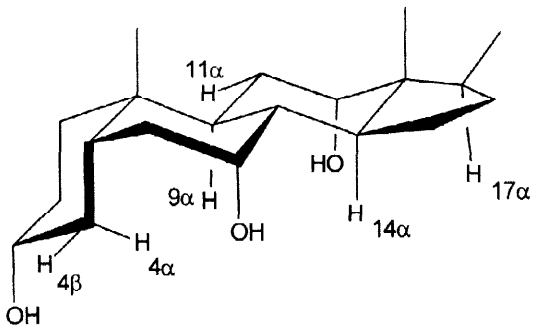
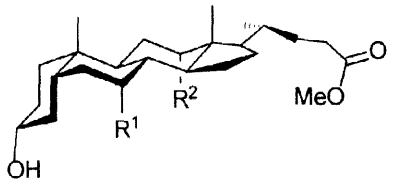


Table 1: ^{1}H NMR chemical shift changes (Δδ, p.p.m., C_6D_6 solvent) of steroidal protons on formation of complexes between anions and methyl cholate (**2**).

Proton[a]	*4α*	*4β*	*9*	*11α*	*14*	*17*
Δδ in **2**.TsO$^-$ [b]	0.48	0.27	0.34	0.2	0.2	0.2
Δδ in **2**.PhP(OH)O_2^- [c]	0.55	0.2	0.7	0.25	0.4	0.2

[a] Assignments based on ^{1}H-^{1}H and ^{1}H-^{13}C COSY spectroscopy, supported by comparisons with earlier work from this[13] and other[14] laboratories. [b] Extrapolated to 100% complexation based on the HOSTEST binding analysis. [c] Measured directly on solutions from solid phase extraction experiments.

of phosphonate in benzene-hexane (1:1) while, at the other end of the scale, methyl lithocholate (**7**) was completely ineffective. Methyl deoxycholate (**5**) and chenodeoxycholate (**6**) were both partially successful, the former being somewhat more efficient. Increasing the proportion of hexane lowered the degrees of solubilisation without greatly altering the relative extraction abilities. The results confirm that, as expected, all three hydroxyl groups in **2** and **3** contribute to the binding process. In a separate experiment the alkene **8**[11] was shown to be roughly as effective as **2**,[12] militating against the ester carbonyl as an H-bond acceptor for substrate P-O*H*.



5 R^1 = H, R^2 = OH
6 R^1 = OH, R^2 = H
7 R^1 = R^2 = H

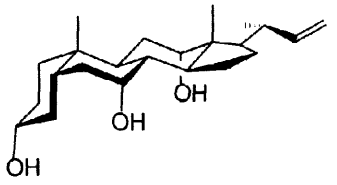


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Table 2: Extraction of TBA$^+$PhP(OH)O_2^- into benzene-hexane mixtures by bile acid esters (%).[a]

Ester	**3**	**5**	**6**	**7**
Benzene-hexane (1:1)	100	25	9	0
Benzene-hexane (3:7)	78	17	5	-[b]

[a] Phosphonate/steroid molar ratios expressed as percentages. Solutions of the steroids were stirred for 3 h at ~293 K with an excess of the phosphonate, filtered, evaporated, and analysed by ^{1}H NMR in $CDCl_3$. [b] Experiment not attempted.

In conclusion, we have demonstrated that cholates **2** and **3** can act as receptors for tridentate oxoanions in hydrocarbon solvents. Our measurements suggest that they operate through hydrogen bonding involving the three hydroxyl groups on the α-face of the steroid nucleus. Although the affinities are limited by the modest H-bond donor power of the OH groups, it is interesting (and probably unprecedented) to find such effects in a neutral, lipophilic system so closely related to an important natural product.

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References and Footnotes

1. Haslewood, G. A. D. *The Biological Importance of Bile Salts*; North-Holland: Amsterdam, 1978. *Sterols and Bile Acids*; Danielsson, H., Sjövall, J., Eds.; Elsevier: Amsterdam, 1985.
2. Leading refs.: Davis, A. P. *Chem. Soc. Rev.* **1993**, *22*, 243. Davis, A. P.; Bonar-Law, R. P.; Sanders, J. K. M. *Comprehensive Supramolecular Chemistry*; Murakami, Y., Ed.; Pergamon: Oxford, 1996; Vol. 4, p 257. Li, Y. X.; Dias, J. R. *Chem. Rev.* **1997**, *97*, 283. Walliman, P.; Marti, T.; Furer, A.; Diederich, F. *Chem. Rev.* **1997**, *97*, 1567.
3. (a) Davis, A. P.; Gilmer, J. F.; Perry, J. J. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 1312. (b) Davis, A. P.; Perry, J. J.; Williams, R. P. *J. Am. Chem. Soc.* **1997**, *119*, 1793.
4. For general discussions of anion recognition see: Schmidtchen, F. P.; Berger, M. *Chem. Rev.* **1997**, *97*, 1609; Dietrich, B. *Pure and Appl. Chem.* **1993**, *65*, 1457. For examples of neutral organic anionophores from other laboratories, see: Valiyaveettil, S.; Engbersen, J. F. J.; Verboom, W.; Reinhoudt, D. N. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 900. Morzherin, Y.; Rudkevich, D. M.; Verboom, W.; Reinhoudt, D. N. *J. Org. Chem.* **1993**, *58*, 7602. Raposo, C.; Pérez, N.; Almaraz, M.; Mussons, M. L.; Caballero, M. C.; Morán, J. R. *Tetrahedron Lett.* **1995**, *36*, 3255. Ishida, H.; Suga, M.; Donowaki, K.; Ohkubo, K. *J. Org. Chem.* **1995**, *60*, 5374. Wilcox, C. S.; Kim, E.; Romano, D.; Kuo, L. H.; Burt, A. L.; Curran, D. P. *Tetrahedron* **1995**, *51*, 621. Gale, P. A.; Sessler, J. L.; Král, V.; Lynch, V. *J. Am. Chem. Soc.* **1996**, *118*, 5140. Berger, M.; Schmidtchen, F. P. *J. Am. Chem. Soc.* **1996**, *118*, 8947. Kavallieratos, K.; deGala, S. R.; Austin, D. J.; Crabtree, R. H. *J. Am. Chem. Soc.* **1997**, *119*, 2325. Buhlmann, P.; Nishizawa, S.; Xiao, K. P.; Umezawa, Y. *Tetrahedron* **1997**, *53*, 1647.
5. Monte Carlo molecular mechanics search employing MacroModel V5.5, OPLS force field, $CHCl_3$ solvation; see Mohamadi, F.; Richards, N. G. J.; Guida, W. C.; Liskamp, R.; Caufield, C.; Chang, G.; Hendrickson, T.; Still, W. C. *J. Comput. Chem.* **1990**, *11*, 440. Alternative geometries were predicted by other force fields which, however, often gave unrealistic (non-C_3-symmetrical) structures for the anion.
6. Guest concentrations in the range 0 - 24 mM.
7. Connors, K. A. *Binding Constants*; Wiley: New York, 1987, pp. 24-28.
8. Wilcox, C. S. *Frontiers in Supramolecular Chemistry and Photochemistry*; Schneider, H.-J. and Durr, H., Eds.; VCH: Weinheim, 1990, p 123. Version 5.0 was used for the present work.
9. The error analysis in HOSTEST gave a standard deviation of 56 for the 1:1 binding constant. In view of the modest fit and the low value for the second binding constant, use of the 1:1 + 2:1 binding model must be seen as tentative. The intrusion of other stoichiometries can certainly not be excluded.
10. Prepared from cholic acid and octanol under catalysis by conc. HCl aq.
11. Synthesized by hydrolysis of the corresponding triformate: see Carlson, G. L.; Belobaba, D. T. E.; Hofmann, A. F.; Wedmid, Y. *Steroids* **1977**, *30*, 787.
12. This comparison was made in benzene, in which $TBA^+PhP(OH)O_2^-$ has appreciable solubility. In parallel experiments, **8** and **2** were both accompanied by 1.2 equivalents of phenylphosphonate after filtration.
13. Bonar-Law, R. P.; Davis, A. P. *Tetrahedron* **1993**, *49*, 9845.
14. Waterhous, D. V.; Barnes, S.; Muccio, D. D. *Journal of Lipid Research* **1985**, *26*, 1068.