Bis-steroids as Potential Enzyme Models: Perylene Solubilisation and Dye Spectral Changes with Aqueous Solutions of Some Derivatives of Conessine and Cholic Acid

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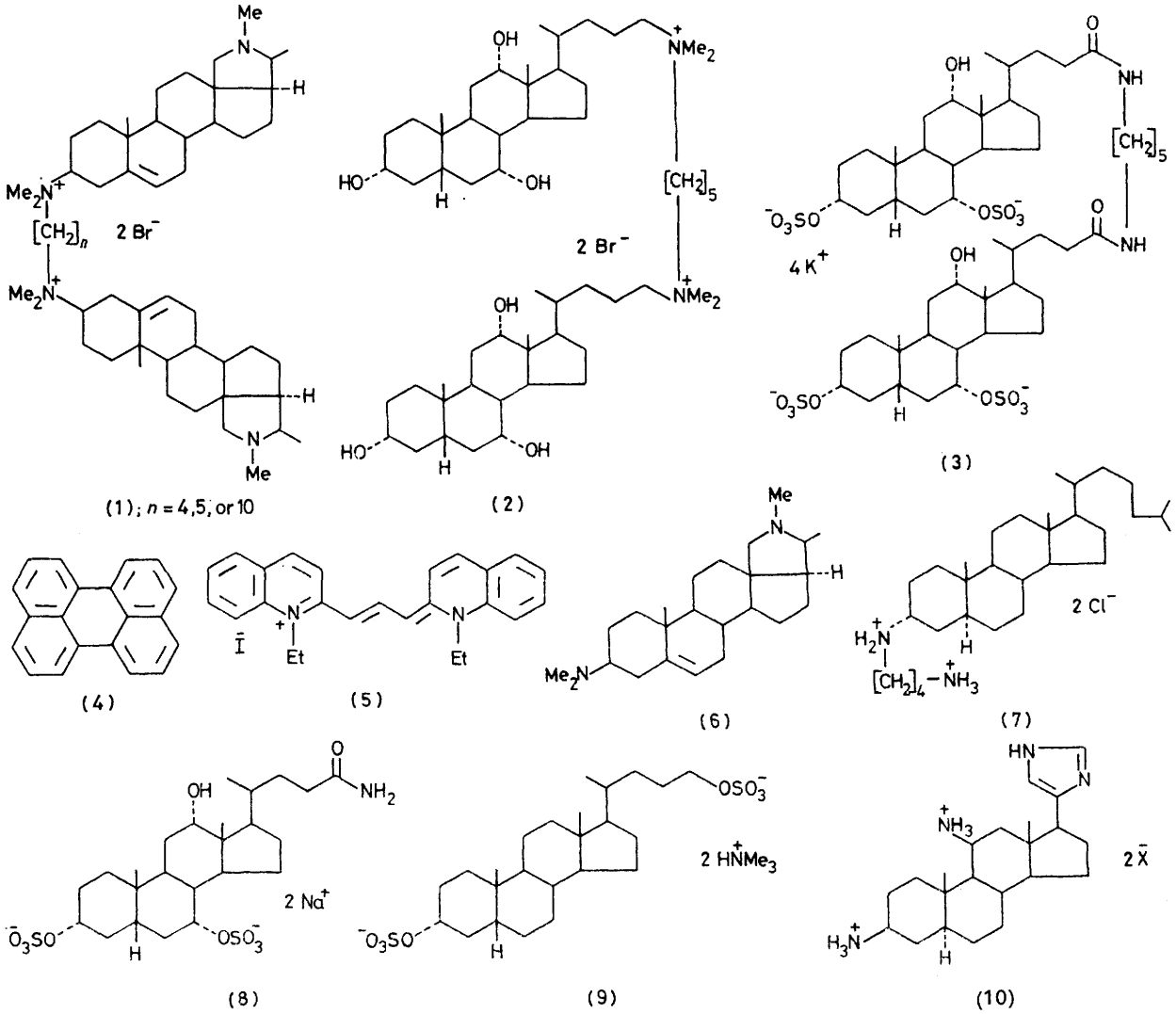
Summary Several bis-steroids derived from conessine or cholic acid solubilise perylene into aqueous solution without evidence of micelle formation, and cause spectral changes in aqueous pinacyanol iodide; mono-steroids examined show these effects (characteristic of hydrophobic interaction) only on micellisation or not at all.

ENZYME models reflect various aspects of enzyme chemistry; our specification is for large chiral molecules capable of hydrophobic interactions[1] with smaller 'substrates' in aqueous solution, so modifying the rates and selectivities of their chemical reactions. Two or more units derived from naturally occurring polycyclic compounds such as steroids or alkaloids linked by one or more hinges or bridges and with appropriate water-solubilising and functional groups appear to offer substantial advantages in development of models of novel type, and we present here some physical evidence on the strong hydrophobic interactions of some such compounds [(**1**)—(**3**)] with substrates both water-insoluble [perylene[2] (**4**)] and water-soluble [pinacyanol iodide[3] (**5**)]. Most importantly, unlike other compounds which show similar effects with these substrates, the bis-steroids exhibit no evidence of micellar inclusion.

Compounds (**1**) were synthesised from conessine (**6**), and (**2**) and (**3**) from cholic acid; these and other novel compounds (**7**), (**8**), and (**9**) have been fully characterised. Aqueous solutions of each bis-steroid [(**1**)—(**3**)] take up perylene (solubility in water[4] $< 2 \times 10^{-9}$ M) to give clear solutions in which the hydrocarbon concentrations may be determined by absorption (440 nm; extinction taken as for alcohol solutions at λ_{max} 435 nm) and fluorescence spectrophotometry (excitation at 416 nm, emission intensity measured at 473 nm). The saturation concentration of

perylene is in each case linear in relation to steroid concentration down to the limit of reliable observation of fluorescence intensity (varies with sample: typically *ca.* 10^{-4} M steroid); the characteristic kinks exhibited in cases of micellar inclusion were absent. We did observe micellar inclusion of perylene in aqueous solutions of (**7**) (synthesised from cholesterol), (**8**) (from cholic acid), and (**9**) (from lithocholic acid) as well as for sodium cholate, sodium taurocholate, sodium lauryl sulphate (NaLS), and cetyltrimethylammonium bromide (CTAB) which were examined for comparison. The critical micellar concentration (c.m.c.) of compound (**7**) is unusually low at $4{\cdot}5 \times 10^{-4}$ M.

experiments with the dihydrochlorides of the bis-conessine derivatives (**1**; $n = 5$ or 10) showed rather surprisingly that the solubilising ability of the proton salts differed only slightly from that of the free bases. Solubilisation by the other compounds examined was negligible at 10^{-2} M; even at 5×10^{-2} M, a concentration greater than any of the c.m.c.'s, perylene concentrations range only from 5×10^{-7} M [compound (**8**)] to $6{\cdot}7 \times 10^{-6}$ M (sodium cholate). The impressively strong hydrophobic effects at convenient concentrations shown by the bis-steroids, the ease of functionalisation of steroids as required for particular reactivity studies, the freedom from complexities in inter-



The strong solubilising effect of the bis-steroids (**1**)—(**3**) may be demonstrated by reference to saturated perylene concentrations in aqueous solutions of the various solubilisers all at 10^{-2} M, a concentration above the c.m.c.'s of (**7**), CTAB, and NaLS. The reducing order (perylene concentrations in brackets) is (**1**) ($n = 4$; $10{\cdot}5 \times 10^{-6}$ M), (**7**) ($8{\cdot}2 \times 10^{-6}$ M), (**1**) ($n = 10$; $6{\cdot}6 \times 10^{-6}$ M), CTAB ($6{\cdot}5 \times 10^{-6}$ M), (**1**) ($n = 5$; $4{\cdot}7 \times 10^{-6}$ M), (**2**) ($3{\cdot}3 \times 10^{-6}$ M), (**3**) ($1{\cdot}5 \times 10^{-6}$ M), and NaLS ($0{\cdot}8 \times 10^{-6}$ M). Some pretation[5] associated with micellar effects anticipated from these preliminary results, and the attractive possibility that the large molecules in V-shaped conformations may be enfolding the substrates, enzyme-like, in their clefts combine to suggest that bis-steroids and their analogues will be excellent enzyme models.

Conessine (**6**) dihydrochloride did not solubilise perylene in aqueous solution even at 6×10^{-2} M; apparently micelles are not readily formed and hydrophobic interactions are too

weak for a concentration of 1 : 1 complex with the hydrocarbon high enough to be detected by fluorescence. However, the water-soluble steroid (**10**) which is (apart from the imidazole grouping) somewhat analogous to conessine dihydrochloride, has been effectively employed as an enzyme model in reactivity studies.[6]

Pinacyanol iodide (**5**) (10^{-5} M) in water has absorption bands at 550 and 597 nm; in acetone these lie at 560 and 606 nm. Aqueous solutions of any of the compounds employed in our work as perylene solubilisers (except CTAB[7]) containing the dye (**5**) at 10^{-5} M showed band shifts to 558—564 and 604—609 nm; the exact positions of the bands depended on the compound examined, which was *ca.* 5×10^{-3} M or higher if necessary to exceed the c.m.c. These shifts imply interaction between dye and the other solute, most significantly for the non-aggregating bis-steroids (**1**)—(**3**). Conessine dimethiodide showed no similar effect, demonstrating again the potential advantages of bis- relative to mono-steroids in the design of enzyme models.

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