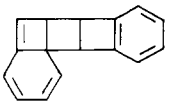
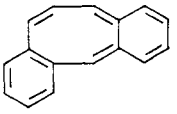
1 proton), 6.47 (multiplet, 1 proton),[14] and 7.10 (doublet, 2 protons). No evidence for the formation of the other dimeric products mentioned above was obtained.

The formation of the dimer VII may be rationalized in a manner similar to that postulated for the formation of V.[10] Both reactions could lead to an intermediate VIII but having different configurations of the central four-membered ring. The *cis* isomer, presumed to be formed in the present instance, could rearrange to



VIII



IX

1,2,4,5-dibenzocyclooctatetraene (IX) which then undergoes an internal Diels–Alder reaction to give VII. Whatever the mechanism of the dimerization, it is apparent that the presence of transition metals has a profound effect upon the course of these reactions.

The scope of the reactions of 3,4-dihalocyclobutenes with $Fe_2(CO)_9$ and further reactions of cyclobutadiene–iron tricarbonyl complexes will be reported in later papers.

Acknowledgment. We wish to thank the National Science Foundation and the Robert A. Welch Foundation for financial assistance. We are also grateful to Antara Chemicals for a gift of iron pentacarbonyl.

(14) Models suggest that this nonbenzylic proton of the three-membered ring lies almost in the plane of each of the two benzene rings; this would explain its absorption at an abnormally low τ-value.

(15) University of Texas Socony-Mobil Fellow, 1964–1965.

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Hydrophobic Bonding. Its Detection by Nuclear Magnetic Resonance Spectroscopy and Its Effect on the Chemical Shifts of Internal Standards

Sir:

There has been a great deal of recent interest in hydrophobic bonding[1] (the formation of inter- or intramolecular aggregates of the nonpolar portions of molecules surrounded by highly ordered solvent molecules in aqueous medium) due to the recognition that this phenomenon plays an important role in the behavior of proteins.[2] We now wish to report the utilization of nuclear magnetic resonance spectroscopy for the study of hydrophobic bonding and to point out the implications of this phenomenon with regard to the chemical shifts of internal standards in aqueous solution.

When 0.8 *m* (1.5 mole %) aqueous solutions of 1,3-naphthalenediol (I), naphthalene-2-sulfonic acid (II), or phenanthrene-3-sulfonic acid (III) are diluted, their n.m.r. spectra shift to lower field (relative to external TMS). Since the appearance of the spectra also changes markedly upon dilution, it is evident that the chemical shifts of different protons of the same substance are affected to different extents. For example, a fourfold dilution of the 0.8 *m* solution of I results in downfield shifts of *ca.* 10 c.p.s. for the peaks due to the AB system of H-2 and H-4, and of *ca.* 20 c.p.s. for the multiplet due to the remaining protons. Since the water peak does not shift within experimental error (relative to external TMS) during these dilutions, bulk diamagnetic susceptibility changes are probably not large at these concentrations.[3] Similar dilution of a solution of I in acetone or methanol, solvents in which hydrophobic bonding cannot occur, causes a small (3 c.p.s.) shift of the aromatic spectrum in the opposite direction (upfield).

These results can be explained by the existence of a concentration controlled association of the organic molecules in aqueous solution, but not in the organic solvents. Such an association would cause the observed effect since the proximity of an aromatic molecule is known to cause protons of another molecule to experience a net shielding effect.[4,5]

If hydrophobic bonding, rather than a more specific attractive interaction among the aromatic molecules, is responsible for this aggregation, unfavorable interactions of the solvent water with other organic compounds should lead to coaggregation of these and the aromatic species. The proton chemical shifts of such compounds in the presence of the aromatics should therefore also be displaced to higher field. In Table I

Table I. N.m.r. Shifts of Aliphatic Protons in the Presence of Aromatics[a]

	I[b,e]	I[c,e]	II[c,e]	III[c,e]	IV[c,e]
CH_3OH		5			4.5
$(HOCH_2)_2$		9	9	8.5	5
CH_3CH_2OH	−4	10.5	10.5	13.5	3.5
CH_3CH_2OH	−4.5	11	11	13.5	3.5
$(CH_3)_3CCH_2OH$	−2.5	25	19.5	34	7
$(CH_3)_3CCH_2OH$	−5.5	23.5	19	30.5	7.5
CH_3CN		28.5	18	22	8
DSS[d]		48	21	31	9.5

[a] Shifts in c.p.s. of aliphatic protons in the presence of 0.8 *m* aromatics relative to their absorption in solvent alone. [b] In acetone. [c] In water. [d] Sodium salt of 4,4-dimethyl-4-silapentane-1-sulfonic acid. [e] I, 1,3-naphthalenediol; II, naphthalene-2-sulfonic acid; III, phenanthrene-3-sulfonic acid; IV, resorcinol.

are listed the shifts observed for a number of aliphatic compounds (1.5–3.0 wt. %) when the medium is changed from pure water to water which is 0.8 *m* in the aromatic

(1) W. Kauzmann, *Advan. Protein Chemistry*, **14**, 1 (1959); G. Némethy and H. A. Scheraga, *J. Phys. Chem.*, **66**, 1773 (1962); E. E. Schrier, M. Pottle, and H. A. Scheraga, *J. Am. Chem. Soc.*, **86**, 3444 (1964).

(2) Hydrophobic bonding almost certainly has considerable implications also for organic chemistry. Note, for example, the decrease in the rate of reaction of hydroxide ion with imido ester caused by the addition of various amines to the aqueous reaction medium: E. S. Hand and W. P. Jencks, *J. Am. Chem. Soc.*, **84**, 3505 (1962), or the selective terminal epoxidation of squalene in highly polar medium: E. E. van Tamelen, Abstracts, 148th National Meeting of the American Chemical Society, Chicago, Ill., Sept. 1964, p. 10F.

(3) The water peak does shift upon dilution of solutions of II or III, but these shifts are in the opposite direction from those of the aromatic protons. These shifts are primarily due to the change which dilution causes in the acidity of the solutions, since the same shifts for water are observed upon dilution of a sulfuric acid solution of corresponding normality.

(4) J. A. Pople, W. G. Schneider, and H. J. Bernstein, "High-Resolution Nuclear Magnetic Resonance," McGraw-Hill Book Co., Inc., New York, N. Y., 1959, pp. 424–428.

(5) Benzene, when added to micellar solutions, has been reported to cause shielding of the protons of the soap: J. C. Eriksson, *Acta Chem. Scand.*, 17, 1478 (1963).

components I, II, III, and resorcinol (IV). As indicated, (1) the protons of aliphatic compounds do experience considerable upfield shifts in the presence of aromatics in aqueous solution; (2) this effect is absent in acetone solution; (3) there is a qualitative correspondence between the nonpolar character of the aliphatics and the magnitude of the anomalous shifts; (4) resorcinol (IV), which is more polar (*i.e.*, interacts more favorably with the solvent water) and has a smaller diamagnetic anisotropy than I, is much less effective than I in causing the anomalous shift; and (5) the two different types of protons of a given aliphatic component (*e.g.*, neopentyl or ethyl alcohol)[6] are shifted to about the same extent, probably indicating the nonspecific nature of the hydrophobic bonding between the aromatic and aliphatic moieties.

Dilution of the aqueous solutions leads to less shielding,[7] and when the concentration of the aliphatic component is increased at constant aromatic concentration slight downfield shifts are observed for both the aliphatic and aromatic protons. All of these observations are consistent with the formation of aggregates.

A further indication of the considerable effect of hydrophobic bonding is the 13-fold increase in solubility of neopentyl alcohol in a 2.5 *m* solution of III as compared to pure water.

Finally, it may be noted that one of the aliphatic substances, DSS (the sodium salt of 4,4-dimethyl-4-silapentane-1-sulfonic acid) has been recommended[8] as an internal standard for aqueous solutions. Since the peak due to the methyl protons of DSS does exhibit a particularly large shift upon addition of the aromatics, clearly there are circumstances in which the use of this salt as an internal standard should be avoided and in which water itself could serve the purpose more satisfactorily.

(6) This is also true for propionic acid and for the α- and ω-protons of 1-butanol.

(7) A plot of the chemical shift *vs.* concentration of the organic component gives a curve, the initial steep slope of which levels off at higher concentrations. A series of these curves will be presented and discussed in the complete paper.

(8) G. V. D. Tiers, Abstracts, 137th National Meeting of the American Chemical Society, Cleveland, Ohio, April 1960, p. 17R.

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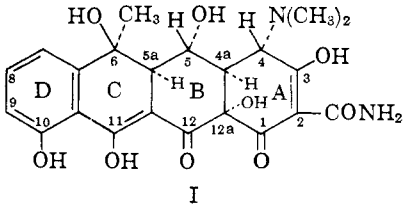
The Stereochemistry at C-5 in Oxytetracycline

Sir:

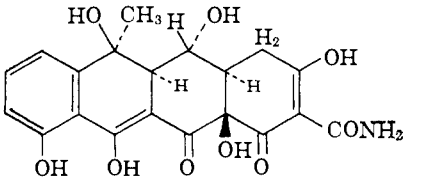
While the structure of oxytetracycline (I) has been known for many years,[1] its configuration has been the subject of several investigations. The early[1] assignments at C-4a, C-5a, C-6, and C-12a have been confirmed subsequently by X-ray data[2,3] which also showed conclusively that the stereochemistry at C-4 differed from that suggested originally. The configuration at



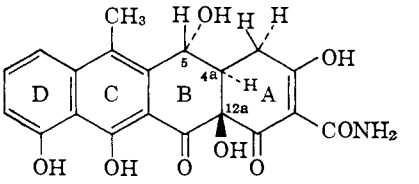
I

C-5, however, has not yet been determined unequivocally, since interpretations of the X-ray data led to conflicting conclusions. We wish to present evidence obtained by n.m.r. spectroscopy that the configuration of oxytetracycline is as shown by structure I. The hydroxyl group at C-5 is *trans* to that at C-6.

For the purpose of a configurational assignment at C-5 by means of n.m.r. spectroscopy oxytetracycline (I) and most of its derivatives are not suitable, since they may assume a variety of conformations. 12a-*epi*-Dedimethylaminoanhydrooxytetracycline (III), however, can exist in one conformation only, determined by the *trans* junction of the A–B rings. In this molecule the proton at C-5 has to be either in a *trans* diaxial or in an equatorial–axial relationship to the proton at C-4a, depending upon the configuration at C-5. The conclusive stereochemical assignment became possible when the n.m.r. spectrum (pyridine) of III showed the signal for the C-5 proton to be a doublet (5.7 p.p.m. from TMS) with an apparent coupling constant of $J = 8$ c.p.s. The protons at C-5 and C-4a thus are *trans* diaxial and the hydroxyl group at C-5 is *cis* to the hydrogen atom at C-4a.



II



III

trans-Junction of the A–B rings in the oxytetracycline skeleton was achieved in the following manner. Treatment of dedimethylamino-12a-deoxyoxytetracycline[1] with *m*-chloroperbenzoic acid in chloroform yielded (75%) dedimethylamino-12a-*epi*-oxytetracycline (II) in its C-11,C-12 diketonic tautomeric form. *Anal.* Calcd. for $C_{20}H_{19}O_9N$: C, 57.55; H, 4.59; N, 3.36. Found: C, 57.12; H, 4.68; N, 3.51. Spectral data showed: λ_{max} (KBr or dioxane) 5.8 μ; $\lambda_{max}^{MeOH-0.01NHCl}$ 262 and 335 mμ (log ϵ 4.37 and 3.67); $\lambda_{max}^{MeOH-0.01NNaOH}$ 249, 260, and 379 mμ (log ϵ 4.21, 4.23, and 4.16). Reacidification of a basic solution showed $\lambda_{max}^{MeOH-HCl}$ 262 and 357 mμ (log ϵ 4.22 and 4.20).

Treatment of II with refluxing 0.8 *N* hydrochloric acid in aqueous acetone yields III (95%). *Anal.* Calcd. for $C_{20}H_{17}O_8N$: C, 60.16; H, 4.29; N, 3.51. Found: C, 59.73; H, 4.64; N, 3.48. Spectral data showed $\lambda_{max}^{MeOH-0.01NHCl}$ 224, 268, and 413 mμ (log ϵ

(1) F. A. Hochstein, C. R. Stephens, L. H. Conover, P. P. Regna, R. Pasternack, P. N. Gordon, F. J. Pilgrim, K. J. Brunings, and R. B. Woodward, *J. Am. Chem. Soc.*, 75, 5455 (1953).

(2) (a) S. Hirokawa, Y. Okaya, F. M. Lovell, and R. Pepinsky, *Acta Cryst.*, 12, 811 (1959); (b) *Z. Krist.*, 112, 439 (1959); (c) Y. Takeuchi and M. T. Buerger, *Proc. Natl. Acad. Sci. U. S.*, **46**, 1366 (1960).

(3) J. Donohue, J. D. Dunitz, K. N. Trueblood, and M. S. Webster, *J. Am. Chem. Soc.*, 85, 851 (1963).