of *serine hydroxymethylase*[9] and glycine, hFH_4 is converted to FH_4 (equation 2)

$$\text{Glycine} + hFH_4 \rightleftharpoons \text{Serine} + FH_4 \qquad (2)$$

Treatment of hFH_4 with *hFH_4 dehydrogenase*[9] and TPN produces equivalent amounts of "active formyl" and TPNH according to equation 3

$$hFH_4 + TPN^+ \rightleftharpoons fFH_4 + TPNH + H^+ \qquad (3)$$

The results of two typical experiments may be summarized as follows: (a) 0.070 μmole of hFH_4 produced 0.066 μmole of TPNH; and (b) 0.024 μmole of hFH_4 was converted to 0.026 μmole of fFH_4 (measured as $f^{5-10}FH_4$).

hFH_4 is decomposed by treatment with hydroxylamine or acetylacetone[10]; the latter reagent can be used to estimate the bound HCHO in hFH_4. The stability constant ($K_{diss} \cong 10^{-3}$ for reaction 1 in reverse) of hFH_4, and the resistance of the compound to air-oxidation, suggests that hFH_4 is the N^5,N^{10}-hydroxymethyl[11] bridge compound ($h^{5-10}FH_4$) rather than h^5FH_4 or $h^{10}FH_4$. This conclusion has been reached also by other investigators,[4,5] and is consistent with our previous finding,[12] that with purified preparations of hFH_4 dehydrogenase, $f^{5-10}FH_4$, rather than f^5FH_4 or $f^{10}FH_4$, is the reaction product in equation 3.

(9) Y. Hatefi, M. J. Osborn, L. D. Kay and F. M. Huennekens, *J. Biol. Chem.*, **227**, 637 (1957).

(10) T. Nash, *Biochem. J.*, **55**, 416 (1953).

(11) Because the term "active hydroxymethyl" is already established in the literature, it is retained here even though the structure $h^{5-10}FH_4$ represents a methylene, rather than a hydroxymethyl, derivative of FH_4.

(12) M. J. Osborn and F. M. Huennekens, *Biochim. Biophys. Acta*, in press.

(13) Research Fellow of the National Institute of Arthritis and Metabolic Diseases, Public Health Service.

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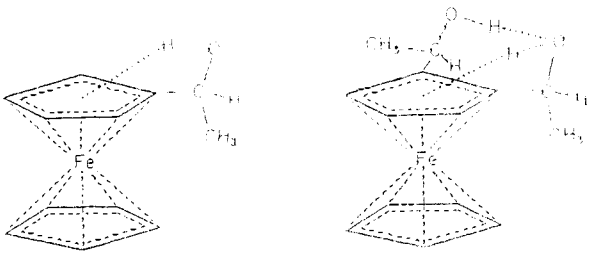
INTRAMOLECULAR AROMATIC RING–HYDROGEN BONDING[1]

Sir:

Evidence has been available indicating the occurrence of intermolecular hydrogen bonding between alcohol solutes and benzene aromatic solvents through observation in the overtone[2] and fundamental[3] spectral region of the greater shift of the hydroxyl stretching frequency in these solvents with respect to the vapor state than in CCl_4 solvent. However, there has not yet been reported any instance of the important general case of an intramolecular structure involving hydrogen bonding to an aromatic ring. Such an instance would additionally provide a clarifying distinction between the previously combined roles of the aromatic system as both solvent and reactant.

Interesting examples of this type of intramolecular hydrogen bonding to the π-electron systems of both ferrocene and benzene aromatic rings have now been observed in the case of several ferrocenyl and phenyl alcohols through infrared absorption studies using a Perkin–Elmer Model 21 spectrometer with LiF optics. For example, the spectrum of α-hydroxyethylferrocene in CCl_4 exhibits both the free hydroxyl absorption band at 3617 cm.$^{-1}$ and another concentration-independent, but temperature-dependent, hydroxyl absorption band of greater intensity at 3574 cm.$^{-1}$ corresponding to that fraction of the molecules at equilibrium having a ring–hydrogen bonded structure of the probable representation I. Similarly, β-phenylethanol exhibits the same corresponding absorptions at 3630 and 3601 cm.$^{-1}$,[4] respectively, but with reversed intensities.



I II

The homoannular-diacetylferrocene first prepared by Rosenblum and Woodward[5] and presumed to have the 1,3-structure was shown here to have the 1,2-structure[6] on the basis of an intramolecular hydrogen bond between the two hydroxyl functions of the corresponding diol, 1,2-di-α-hydroxyethylferrocene. In the present connection, however, the point of interest is that in addition to the intra-hydroxyl band at 3457 cm.$^{-1}$, the only stretching frequency of the second hydroxyl group observed is at 3588 cm.$^{-1}$, *i.e.*, in the ring–hydrogen bonded state (II). Similar free hydroxyl approach for interaction with the aromatic ring is permitted here as in I, while in the contrasting parallel case of the intramolecularly hydrogen bonded 1,1′-di-α-hydroxyethylferrocene, the structure does not permit close approach of the free hydroxyl to either of the two aromatic rings resulting in a characteristic free band at 3621 cm.$^{-1}$.[7]

The hydrogen bonding role of the ferrocene ring also was demonstrated intermolecularly in CCl_4 with a solution 0.1 *M* in both ferrocene and *n*-butanol through the appearance of a new, additional weak hydroxyl absorption at 3597 cm.$^{-1}$.

With the magnitude of $\Delta\nu$ as a measure of the strength of a hydrogen bond,[8,9] it is clear from the $\Delta\nu$ value for α-hydroxyethylferrocene (43 cm.$^{-1}$) compared to the value for β-phenylethanol (29 cm.$^{-1}$) that the π-electron system of the ferrocene

(1) This work was supported in part by the Army and Navy under Signal Corps Contract No. DA-036-39SC-70154.

(2) L. H. Jones and R. M. Badger, THIS JOURNAL, **73**, 3132 (1951).

(3) M. Tamres, *ibid.*, **74**, 3375 (1952).

(4) H. Kwart, private communication, also has informed us of his observation of these two bands.

(5) M. Rosenblum, Ph.D. Thesis, Harvard University, 1953.

(6) J. H. Richards and T. J. Curphey, *Chemistry and Industry*, 1456 (1956), have independently made this same 1,2-structure assignment *via* anhydride formation from the corresponding diacid.

(7) Other additionally interesting aspects of the spectra associated with the hydroxyl groups of these diols together with a more detailed and quantitative account of these over-all results will be presented subsequently.

(8) R. M. Badger, *J. Chem. Phys.*, **8**, 288 (1940).

(9) L. P. Kuhn, THIS JOURNAL, **74**, 2492 (1952).

ring produces a substantially stronger hydrogen bond than that of the benzene ring. An interesting contrast in the steric requirements of the π-electron systems of the two aromatic rings is provided by the spectrum of α-phenylethanol, the phenyl analog of I, which shows only the single stretching frequency of the free hydroxyl at 3617 cm.$^{-1}$. In both of these α-substituted compounds the hydroxyl hydrogen is only above the outer periphery of the ring and the observed difference under these geometric conditions may reflect the greater contribution of delocalization energy to the total energy of the hydrogen bond[10] in the case of the ferrocene ring, consistent with other evidence of its unusually high π-electron lability.[11] Derivatives of varying side chain lengths are presently being studied in this connection.

(10) C. A. Coulson, *Research*, **10**, 149 (1957).

(11) D. S. Trifan, P. T. Huang and J. W. Herrick, Abstracts of Papers, 131st Meeting, Am. Chem. Soc., Miami, Florida, April 7–12, 1957, p. 5-S.

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EVIDENCE FOR THE OCCURRENCE OF A METABOLITE OF ALDOSTERONE IN URINE[1]

Sir:

This report describes the isolation of a new α-ketol (I) from human urine which yielded on oxidation with HIO_4 a monohydroxy-γ-lactone (II). The evidence suggested that I is a tetrahydro derivative of aldosterone[2] with a primary hydroxyl group at C_{18}.

Neutral extracts of urine[3] which had been hydrolyzed with β-glucuronidase[4] were chromatographed on paper and appropriate eluates which reduced blue tetrazolium were oxidized with HIO_4. The neutral oxidation products were treated with hydroxylamine to convert γ-lactones to hydroxamic acid derivatives which were quantitatively estimated by measurement of radioactivity of the hydroxamic acid–Fe^{59} complexes after extraction into *n*-butanol. Substance I, detected by this procedure, migrated with THE[5] in $CHCl_3$–formamide[6] paper chromatograms, and was separated from THE in the $EtCl_2$–formamide system, in which I migrated with an R_{THE}[7] = 0.72 ± 0.02.

Eluates from the $EtCl_2$–formamide paper chromatograms were purified by two partition chromatograms: one with MeOH–H_2O (1:1) on Celite with benzene–ethyl acetate[8] (9:1) as mobile phase and the other with MeOH–H_2O (7:3) on Celite with $EtCl_2$[9] as mobile phase. This procedure yielded 0.5 mg. of amorphous I which was homogeneous in the following paper chromatograms: ethylene glycol–$EtCl_2$:toluene (1:1) (R_{THE} = 1.0); formamide–$EtCl_2$:toluene (1:1) (R_{THE} = 0.70); *t*-butyl alcohol:H_2O:isoöctane[10] (R_{THE} = 0.80); and MeOH:H_2O (1:1)–benzene[11] (R_{THE} = 0.85). I exhibited no absorption in the ultraviolet and a 1705 cm.$^{-1}$ absorption band ($CHCl_3$) in the infrared. HIO_4 oxidation of 0.13 mg.[12] of I yielded 0.014 mg. of HCHO[13] (theor. = 0.011 mg.) and 0.11 mg.[14] of a neutral product II which possessed absorption bands in the infrared (CS_2) characteristic of hydroxyl (3580, 3400 cm.$^{-1}$), γ-lactone (1775 cm.$^{-1}$) and unconjugated carbonyl (1705 cm.$^{-1}$) functions.

In an alternate method for the isolation of II, unhydrolyzed urine was treated with HIO_4 to accomplish the simultaneous oxidative cleavage of glucuronides[15] and α-ketols. The neutral ether-extractable fraction was then hydrolyzed with NaOH and the saponifiable fraction so obtained was lactonized with acid. The neutral lactone fraction was chromatographed on alumina and yielded II[16] in the benzene–CH_2Cl_2 (1:1) eluates.

In order to determine the number of acetylatable hydroxyl groups per mole,[2] the acetates of I and II (I Ac, II Ac) were prepared from acetic anhydride-1-C^{14} in pyridine and chromatographed on paper and on alumina until constant isotope content was achieved. When desoxycorticosterone and THE were treated with the same acetylation mixture, they yielded acetates with specific activities of 30 ± 1 × 10^3 c.p.m./μmole[17] for each acetyl group. I Ac, purified by chromatography on paper (R_{DOCA}[18] = 1.0) and on alumina (eluted with benzene) gave 92,000 c.p.m./μmole[17] and therefore was a triacetate. Its infrared spectrum (CS_2) showed absorption bands at 1750 and 1730 cm.$^{-1}$, characteristic of 21-acetoxy-20-ketosteroids,[19] and gave no evidence of a free hydroxyl group.

II Ac was chromatographed on paper (R_{DOCA}[18] = 0.52) and on alumina. Fractions of constant specific activity were eluted with benzene:ligroin (4:1)

(1) This work was supported by a grant (PHS-A110) from the National Institute of Arthritis and Metabolic Diseases of the National Institutes of Health, Education and Welfare. The capable technical assistance of Annabelle Long is gratefully acknowledged.

(2) S. A. Simpson, J. F. Tait, A. Wettstein, R. Neher, J. v. Euw, O. Schindler and T. Reichstein, *Helv. Chim. Acta*, **37**, 1163 (1954).

(3) Urine from patients with edema and increased excretion of aldosterone was the best source of substance I. We are indebted to Dr. Herbert C. Stoerk of the Merck Institute for Therapeutic Research for bioassays of aldosterone.

(4) Ketodase, generously furnished by the Warner-Chilcott Laboratories.

(5) Abbreviations: THE = 3α,17α-21-trihydroxypregnane-11,20-dione. $EtCl_2$ = 1,2-dichloroethane.

(6) A. Zaffaroni and R. B. Burton, *J. Biol. Chem.*, **193**, 749 (1951).

(7) R_{THE}, distance traveled by sample relative to THE on paper chromatograms scanned with blue tetrazolium.

(8) In this system I had the same R_f as 3α,17α,11β,21-tetrahydroxypregnane-20-one.

(9) In this system I had the same R_f as THE.

(10) E_2B system. W. R. Eberlein and A. M. Bongiovanni, *Arch. Biochem. Biophys.*, **59**, 90 (1955).

(11) B_4 system. I. E. Bush, *Biochem. J.*, **50**, 370 (1951).

(12) Determined by the blue tetrazolium test with THE as the standard. R. O. Recknagel and M. Litteria, *J. Lab. Clin. Med.*, **48**, 463 (1956).

(13) Determined colorimetrically with chromotropic acid. D. A. MacFadyen, *J. Biol. Chem.*, **158**, 107 (1945).

(14) Determined by lactone test (hydroxamic acid–Fe^{59} complex).

(15) C. F. Huebner, R. Lohmar, R. J. Dimler, S. Moore and K. P. Link, *J. Biol. Chem.*, **159**, 503 (1945).

(16) Samples of II obtained by both methods of isolation were identical by infrared analysis of the free compounds and their acetates, and by the R_{DOCA} values of their acetates.

(17) Counts per minute of C^{14} relative to the density of formazan color formed from one μmole of desoxycorticosterone acetate (or THE diacetate) in the blue tetrazolium test.

(18) R_{DOCA}, distance traveled by sample relative to desoxycorticosterone acetate on paper chromatograms in the methylcyclohexane–formamide system scanned by radioautography.

(19) R. N. Jones, P. Humphries, F. Herling and K. Dobriner, THIS JOURNAL, **74**, 2820 (1952).