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_8 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).

and yielded 2.0 mg. of an amorphous solid. Its infrared spectrum (Fig. 1) indicated the presence of

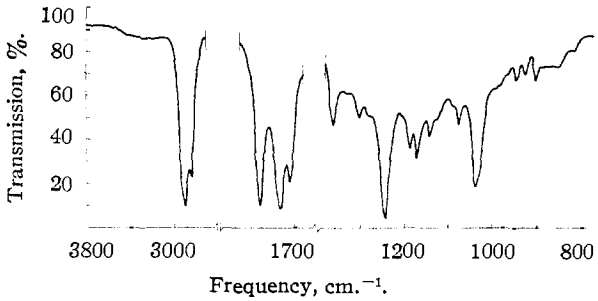

Fig. 1.—Infrared spectrum of monoacetoxy derivative of γ-lactone (II Ac) in carbon disulfide.

γ-lactone (1775 cm.$^{-1}$), acetoxy (1735 cm.$^{-1}$) and unconjugated carbonyl (1705 cm.$^{-1}$) groups and the absence of a free hydroxyl group. The single absorption band at 1242 cm.$^{-1}$ was characteristic of steroid acetates with axial conformations in ring A[20] but did not distinguish between a 3α,5β- and a 3β,5α-isomer. II Ac had a specific activity of 77,500 c.p.m./mg. which indicated an approximate molecular weight of 385 for a monoacetate.[21] Quantitative saponification of lactone and acetoxy groups in II Ac confirmed that it was a lactone monoacetate; a sample which contained 1.07 ± 0.03 μmole of C^{14}-acetate consumed 1.9 ± 0.1 μeq. NaOH per μmole of acetate. 1.800 mg. consumed 9.78 μeq. of NaOH to give a saponification equivalent of 184 (theor. for a lactone monoacetate, $C_{20}H_{27}O_4{\cdot}CH_3CO$ = 374/2 = 187).

These observations are consistent with the formulation of I as a $C_{21}O_5$-pregnane derivative possessing three hydroxyl groups at C_3, C_{18} and C_{21}, one carbonyl group at C_{20} and another probably at C_{11}. A definitive characterization and assignment of structure, however, must await the isolation of larger amounts of I. Direct evidence that I is a metabolite of aldosterone has been obtained by the isolation of tritium-labeled I from urine after the administration of tritium-labeled aldosterone to a human subject.

(20) R. N. Jones and F. Herling, THIS JOURNAL, **78**, 1152 (1956).

(21) 30 ± 1 × 10^3 counts per minute/μmole ÷ 77,500 counts per minute/mg. = 385 ± 15 μg./μmole of acetate.

(22) Public Health Service Research Fellow of the National Institute of Arthritis and Metabolic Diseases, 1954–1956; American Heart Association Research Fellow, 1956–1957.

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NOVEL ORGANIC REACTIONS OF THE INTERMEDIATE FROM THE TWO-ELECTRON OXIDATION OF 1,1-DIALKYLHYDRAZINES IN ACID

Sir:

Evidence has been presented[1] for the formation of an ionic intermediate ($R_2N{=}NH^+$) of surprising stability in the two-electron oxidation of 1,1-dialkylhydrazines in acid solution: (1) no tetraalkyltetrazene is detected by spectrographic analysis in such oxidized solutions; but when they are made alkaline, high yields of the tetrazenes are obtained; (2) quantitative yields of the 1,1-dialkylhydrazines are obtained upon reduction with stannous chloride, a reagent which does not reduce tetraalkyltetrazenes under the conditions used; and (3) the perchlorate salt of the intermediate, $(CH_3)_2N{=}NH^+ClO_4^-$, has been isolated. The discovery of a series of new organic reactions and additional evidence for the intermediate has resulted from a study of its reactions with reactive organic substances.

To a stirred solution of 1,1-dimethylhydrazine (12 g., 0.20 mole) in hydrobromic acid (3.25 *N*, 170 ml.) held at 0°, a solution of bromine (32 g., 0.20 mole) in hydrobromic acid (3.94 *N*, 200 ml.) was added dropwise. Then, isoprene (40 g., 0.20 mole) was added and the mixture was stirred vigorously for two hours at 0°. Unreacted isoprene was removed under vacuum (25 g. recovered), and the remaining solution was made basic (*p*H 7.5) with concentrated sodium hydroxide solution. Work-up of an ether extract of this solution gave the dimethylhydrazone of tiglic aldehyde (3.0 g., b.p. 58–63° at 25 mm., 12% yield). Its picrate (m.p 98°) was prepared.

Anal. Calcd. for $C_{13}H_{17}N_5O_7$: C, 43.94; H, 4.82; N, 19.7. Found: C, 44.35; H, 4.94; N, 19.7.

The oil product obtained by the acid hydrolysis of the hydrazone gave the known 2,4-dinitrophenylhydrazone of tiglic aldehyde (m.p. 215–216°; m.p. of mixture with authentic sample[2] 215–216°). When this hydrolysis mixture was made alkaline and distilled, 1,1-dimethylhydrazine (picrate, m.p. 150°) was obtained.

Water was removed from the original reaction mixture in an evaporator, and extraction of the remaining salts with hot propanol-2 gave sodium bromide (162.8 g.). Evaporation of the propanol-2 solution gave a salt, presumed to be 1,1,4-trimethyltetrahydro-Δ^4-pyridazinium bromide (32.7 g., 79% yield, m.p., after recrystallization from propanol-2, 151–153°).

Anal. Calcd. for $C_7H_{15}N_2Br$: C, 40.6; H, 7.3; N, 13.5. Found: C, 40.3; H, 7.6; N, 13.4.

This product (34 g., 0.165 mole) was hydrogenated over Adams catalyst in ethanol (7.4 l. S.C. of H_2 absorbed; theory for 2 moles per mole of salt, 7.4 l.). When the gummy salt that remained after the ethanol was evaporated was treated with concentrated sodium hydroxide solution, 1-amino-4-dimethylamino-2-methylbutane separated. It gave a dioxalate salt (m.p. 175–176°; "mixed melting point" with an authentic sample, no depression).

Anal. Calcd. for $C_{11}H_{22}N_2O_8$: C, 42.6; H, 7.2; N, 9.1. Found: C, 42.4; H, 7.1; N, 9.0.

In another hydrogenation experiment in which hydrochloric acid was added to the ethanol solution after the hydrogenation, the mixed hydrobromide–hydrochloride salt of the diamine (m.p. 202–203°) precipitated as the ethanol was removed.

Anal. Calcd. for $C_7H_{20}N_2BrCl$: C, 33.95; H, 8.14; N, 11.31; Ag equiv., 123.8. Found: C, 34.1; H, 8.3; N, 11.1; Ag equiv., 124.

(1) W. R. McBride and H. W. Kruse, THIS JOURNAL, **79**, 572 (1957).

(2) K. Bernauer and I. Skudrzyk, *J. prakt. Chem.*, **155**, 310 (1940).