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

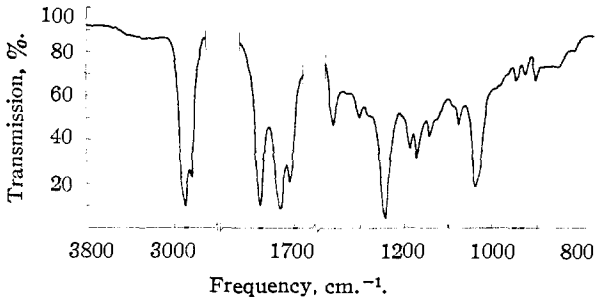

Fig. 1.—Infrared spectrum of monoacetoxy derivative of γ-lactone (II Ac) in carbon disulfide.

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.

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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).

In an alternate preparation of this diamine, a solution containing ethyl β-cyanobutyrate[3] (12 g.; b.p. 66° at 1 mm., $n^{25}D$ 1.4195), dimethylamine (16 g.) and ethanol (100 ml.) was heated in a bomb tube at 105° for 5 days. The N,N-dimethyl-β-cyanobutyramide (7.25 g., b.p. 77–78° at 5 mm;. $n^{25}D$ 1.4620) so prepared was reduced with excess lithium aluminum hydride (10 g.) in ether (600 ml.). 1-Amino-4-dimethylamino-2-methylbutane (3 g., b.p. 106–108° at 100 mm.) was isolated.[4] Its dioxalate (m.p. 175–176°) was the same as that of the diamine derived from the pyridazinium bromide (infrared spectra identical).

The ion ($R_2N{=}NH^+$) is probably the intermediate that reacts with the isoprene rather than the neutral species ($R_2N^+{=}N^-$) obtained when the reaction mixture is neutralized. The above pyridazinium salt was obtained (84% yield) directly from the acidic reaction mixture. When an acid solution from oxidation of 1,1-dimethylhydrazine and concentrated base were added simultaneously to a solution of isoprene in methanol at such rates that the reaction mixture remained just basic to phenolphthalein, tetramethyltetrazene (84% yield) was obtained.

Evidence that this intermediate reacts with aniline, phenol, styrene and vinyl *n*-butyl ether has been gained from experiments in which acid solutions of it were mixed with each of these substances (1–2 hour reaction times), and little or no tetramethyltetrazene was found by spectroscopic analysis after the reaction mixtures were made basic. The products here formed and further possible reactions are under investigation.

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(4) L. K. Amundsen and L. S. Nelson, THIS JOURNAL, **73**, 242 (1951).

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RECEIVED NOVEMBER 2, 1957

ISOLATION AND SYNTHESIS OF A NEW STEROL FROM RAT FECES[1,2]

Sir:

In previous reports,[3,4] the presence of a new sterol (I) in rat feces was announced. Separation from the less polar coprostanyl *p*-phenyl azobenzoate was effected by chromatography of the mixed esters on silicic acid:Celite (2:1)[5] columns using petroleum ether (b.p., 90–95°). Saponification of (I) *p*-phenyl azobenzoate, and crystallization of the ensuing sterol (I) from absolute ethanol yielded thin needles; m.p. 146–147°, $[\alpha]^{25}D$ 0.00 in chloroform (*Anal.* Calcd. for $C_{28}H_{48}O$: C, 84.00; H, 12.00. Found: C, 83.35; H, 12.11). The infrared spectrum indicated absorptions at all the wave lengths believed to be characteristic of a Δ^7-stenol,[6] and the rate of color development with the Liebermann–Burchard reagent was similar to that of Δ^7-stenols.[7] The behavior of the acetate of the new sterol (II), m.p. 93–94°, to hydrogenation in glacial acetic acid over Adams catalyst (no hydrogen uptake) was also characteristic of Δ^7- and $\Delta^{8(9)}$-stenols,[8] and resulted in isomerization. The reaction product, m.p. 76–77°, absorbed one mole of hydrogen upon forced hydrogenation in the presence of HCl at 60°[9] to yield a saturated sterol acetate, m.p. 99–101°. The alcohol, m.p. 153–155°, resulting from the hydrolysis of the acetate failed to respond to the Liebermann–Burchard reagent, and its infrared spectrum indicated the absence of double bonds. On Kuhn-Roth C-methyl determination,[10] II liberated 3.48 ± 0.27 equivalents of acetic acid (calcd. as 4-methyl-Δ^7-cholestenyl acetate) or 3.60 ± 0.26 (calcd. as 4,4-dimethyl-Δ^7-cholestenyl acetate). Synthetic 4,4-dimethylcholesteryl acetate,[11] cholesteryl acetate and Δ^7-cholestenyl acetate gave 3.60 ± 0.31, 2.90 ± 0.03, and 3.00 ± 0.22 equivalents of acetic acid, respectively. Thus II possesses approximately one more C-methyl group than the C_{27} sterol acetates (C-methyl = *ca.* 0.6 mole of acetic acid). From chemical and biogenetic considerations, either 4(α or β)-methyl-Δ^7-cholesten-3β-ol or 4,4-dimethyl-Δ^7-cholesten-3β-ol became attractive plausible structures for I. Δ^7-Cholesten-3β-ol was subjected to Oppenauer oxidation to give Δ^7-cholesten-3-one, m.p. 143–145°. This ketone upon treatment with potassium, *t*-butyl alcohol, and methyl iodide, gave a mixture which was reduced with $LiAlH_4$. Purification of the resulting alcohols through the digitonides[12] followed by silicic acid chromatography of the β-sterols afforded two main compounds of m.p. 122–124° and m.p. 146–147°, respectively. The former compound was identical to Δ^7-cholesten-3β-ol, and the latter sterol (less polar) was identical to I (*Anal.* Calcd. for $C_{28}H_{48}O$: C, 84.00; H, 12.00. Found: C, 83.22; H, 12.01) by m.m.p. and infrared spectrum. Evidence has been obtained which tentatively rules out 4,4-dimethyl-Δ^7-cholesten-3β-ol. Future publication of the data will be made. The possibility of the 2(α or β)-methyl isomers cannot as yet be eliminated, but is unlikely on biological grounds. Others[13,14] have reported the monoalkylation of Δ^4-3-one steroids at position 4 using conditions similar to ours. Since these conditions favor the more stable equatorial configuration, it appears likely that I is 4α-methyl-Δ^7-cholesten-3β-ol. The origin of I suggests the possibility that this sterol arises from the

(1) This work was supported by a research grant (H-2458 C, CS) from the National Institutes of Health, U. S. Public Health Services.

(2) Some of the properties of this sterol were studied in collaboration with D. L. Coleman and C. A. Baumann, Department of Biochemistry, University of Wisconsin.

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(7) D. R. Idler and C. A. Baumann, *J. Biol. Chem.*, **203**, 389 (1953).

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(13) F. Sondheimer and Y. Mazur, THIS JOURNAL, **79**, 2906 (1957).

(14) N. W. Atwater, *ibid.*, **79**, 5315 (1957).