Anal. Found: C, 29.35, 29.45; H, 6.86, 6.92; N (Dumas), 9.21; $SO_4^=$ (as barium sulfate), 28.26, 28.44.

Acknowledgment.—We wish to express our appreciation to Dr. Means' group for the microanalyses, to Mr. Kersey for the biological assays and to Mr. Carboni for certain technical assistance.

CONTRIBUTION FROM
RESEARCH LABORATORIES
CHAS. PFIZER AND CO., INC. PETER P. REGNA
BROOKLYN, NEW YORK FRANCIS X. MURPHY

RECEIVED DECEMBER 9, 1949

STEROIDS. II.[1] A METHOD FOR THE CONVERSION OF *ALLO*-STEROIDS INTO Δ^4-3-KETOSTEROIDS

Sir:

The current interest in corticosteroids as possible therapeutic agents in arthritis has made the availability of starting materials extremely important. Since the majority of the *abundant* steroidal plant sapogenins, representing a potentially unlimited source for 20-keto-pregnanes, either belong to the *allo* series or possess a Δ^5-3-hydroxy grouping, which in turn is convertible in nearly quantitative yield into the 3-keto*allo*steroid system (I), it has become an urgent matter to develop a general procedure for the transformation of I into the essential Δ^4-3-keto moiety.

We have observed that while 3-keto-4-bromosteroids of the *normal* series (rings A/B *cis*) do not react with sodium iodide in acetone solution, 2-bromo-3-keto*allo*steroids (rings A/B *trans*) readily react to yield the corresponding iodo derivatives, which on treatment with zinc dust in ethanol, chromous chloride in acetone, or even short *boiling with collidine* regenerate the saturated 3-keto*allo*steroids. When applied to 2,4-dibromo-3-keto*allo*steroids (II), obtainable in high yield from I, short boiling with sodium iodide affords a 2-iodo-4-bromo-3-keto*allo*steroid (*e. g.*, 2-iodo-4-bromoandrostan-17-ol-3-one 17-hexahydrobenzoate, m. p. 146–149°. Calcd. for $C_{26}H_{38}O_3BrI$: C, 51.58; H, 6.33. Found: C, 51.71; H, 6.35), which on refluxing with collidine suffers simultaneous dehydrobromination and deiodination to lead directly to the required Δ^4-3-ketosteroid. Even more strikingly, if II is refluxed with sodium iodide in acetone solution for twenty hours, there is obtained in good yield a 2-iodo-Δ^4-3-ketosteroid (III), which is smoothly transformed to the Δ^4-3-ketosteroid. The generality of this method has already been demonstrated in five diverse instances in this Laboratory and will be exemplified here by the preparation of the important adrenal hormone 17α-hydroxyprogesterone.

N-Bromoacetamide oxidation of *allo*pregnane-3β,17α-diol-20-one[2] gave a high yield of *allo*pregnan-17α-ol-3,20-dione (IV) (m. p. 251–253°, $[\alpha]^{20}D$ + 24°), which on dibromination in acetic acid led to the 2,4-dibromo derivative (V) (m. p. 183–185° $[\alpha]^{20}D$ 0°. Calcd. for $C_{21}H_{30}O_3Br_2$: C, 51.44; H, 6.17. Found: C, 51.64; H, 5.88). Twenty hours of refluxing with sodium iodide in acetone yielded 2-iodo-17α-hydroxyprogesterone (m. p. 112–115°, $[\alpha]^{20}D$ + 71°, maximum 244 mμ (log E 4.15). Calcd. for $C_{21}H_{29}O_3I$: I, 27.81. Found: I, 28.32), which without isolation on deiodination afforded 17α-hydroxyprogesterone (VI) (m. p. 220–222°, $[\alpha]^{20}D$ + 103° (acetone), maximum 241 mμ (log E 4.30)). The present method, in addition to ready availability of starting materials and good yields, has the marked advantage over the corresponding *normal* ketones in that from each saturated 3-keto*allo*steroid (*e. g.*, IV), three unsaturated ketones of interest for clinical trial can be prepared: in addition to VI, V on collidine treatment afforded the interesting $\Delta^{1,4}$-pregnadien-17α-ol-3,20-dione (m. p. 232–234°, $[\alpha]^{20}D$ + 38.5°, maximum 244 mμ (log E 4.14). Calcd. for $C_{21}H_{28}O_3$. C, 76.79; H, 8.59. Found: C, 76.96; H, 8.21), while the monobromination product of IV on dehydrobromination yielded the Δ^1-isomer of VI, Δ^1-allopregnen-17α-ol-3,20-dione (m. p. 254–257°, $[\alpha]^{20}D$ + 71°, maximum 230 mμ (log E 4.05). Calcd. for $C_{21}H_{30}O_3$: C, 76.32; H, 9.15. Found: C, 76.49; H, 9.33).

Details, applications and extension of this method to other cortical hormones and analogs will be reported shortly.

RESEARCH LABORATORIES G. ROSENKRANZ
SYNTEX, S. A. ST. KAUFMANN
MEXICO CITY, D. F. J. PATAKI
CARL DJERASSI

RECEIVED DECEMBER 5, 1949

(1) Paper I, THIS JOURNAL, **71**, 3689 (1949).

(2) Kritchevsky and Gallagher, *J. Biol. Chem.*, **179**, 507 (1949).

NITROGEN FIXATION IN AN ULTRASONIC FIELD

Sir:

Our observations on the oxidative fixation of molecular nitrogen in ultrasonic field have led to results which are of special interest in the connection of biological nitrogen fixation.

The experiments were carried out in water solution at ordinary pressure; radiating surface of the vessel was 42 mm. in diameter; radiation intensity in the radiation point was ~10 W/sq. cm., frequency 300 kc./sec. The hydrogen and nitrogen gases were led to the other side of the solution at the rate of about 1 l./min., carbon monoxide gas 0.4 l./min. Thus, oxygen was present in the solution in each experiment.

The nitrogen fixation in ultrasonic field does not, at least essentially, depend on the hydrogen ion concentration of the solution as far as the total amount of fixed nitrogen, nitrite *plus* nitrate N (other N-compounds have not been found), is considered. On the other hand, the mutual relation of nitrite and nitrate N is decided by the *p*H of the solution (Figs. 1 and 2). These results explain the observation of Loiseleur[1] on the rapid

(1) Loiseleur, *Compt. rend.*, **218**, 876 (1944).

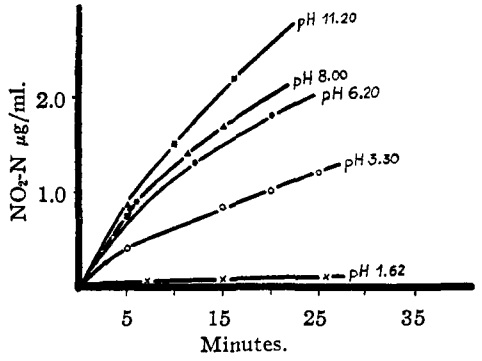


Fig. 1.—Formation of NO_2-N from nitrogen in ultrasonic field.

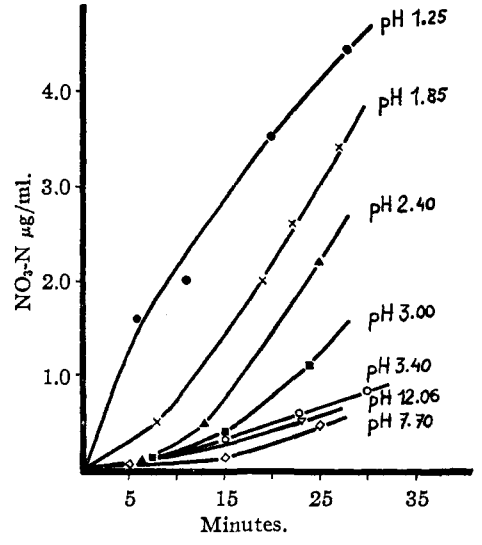


Fig. 2.—Formation of NO_3-N from nitrogen in ultrasonic field.

lessening of the formation of hydrogen peroxide below *p*H 4. The question is then not about the diminishing of the formation of hydrogen peroxide but about the consumption of hydrogen peroxide to the oxidation of nitrite to nitrate. This idea could be proved correct when we found the inhibitory effect of hydrogen and carbon monoxide on the nitrogen fixation (Fig. 3). In the presence of hydrogen (formation of nitrite-N inhibited) hydrogen peroxide is formed in almost equal quantities at *p*H 1.85 and 6.85.

The inhibitory effect of hydrogen on the nitrogen fixation in ultrasonic field is probably due to the competition of hydrogen and nitrogen for oxygen. The aerobic fixation of nitrogen in ultrasonic fields leads to a nitrogen oxide, *e. g.*, NO or possibly N_2O. Which in fact is the first oxide formed is not known.

In the biological nitrogen fixation by Azotobacter and leguminous root nodules Wilson and collaborators[2] found the inhibitory effect of hydrogen. Virtanen[3] has proposed that the first phase in *aerobic* biological nitrogen fixation is oxidative, and has explained this inhibition similarly to the present paper as due to the corresponding inhibition of the nitrogen fixation in an ultrasonic field. *Anaerobic* biological nitrogen fixation is not prevented by hydrogen, and Virtanen, *et al.*,[4] consider the reaction then to be a pure reduction.

(2) Wilson and Umbreit, *Arch. Microbiol.*, **8**, 440 (1937); Wilson, *Ergeb. Enzymforsch.*, **8**, 13 (1939); Wyss and Wilson, *J. Bact.*, **41**, 186 (1941).

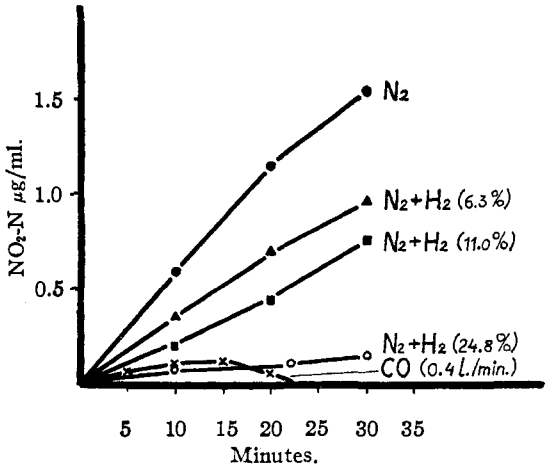


Fig. 3.—Inhibitory effect of hydrogen and carbon monoxide on the fixation of nitrogen at *p*H 6.8.

A detailed account will be published in *Acta Chemica Scandinavica*.

(3) Virtanen, *Ann. Rev. Microbiol.*, **2**, 485 (1948).
(4) Virtanen and Hakala, *Acta Chem. Scand.*, in press.

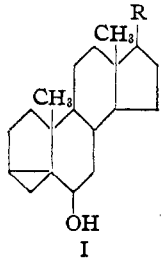
LABORATORY OF THE FOUNDATION FOR CHEMICAL RESEARCH
BIOCHEMICAL INSTITUTE ARTTURI I. VIRTANEN
HELSINKI, FINLAND NILS ELLFOLK

RECEIVED NOVEMBER 25, 1949

MOLECULAR REARRANGEMENTS IN THE STEROLS. VI. THE PREPARATION OF *Epi-i*-CHOLESTEROL AND ITS ACID REARRANGEMENT PRODUCT

Sir:

The present accepted structure of *i*-cholesterol (I) was first proposed by Wallis, *et al.*,[1] and later confirmed by him and his co-workers.[2] The



I

formation of these *i*-steroids has since been shown[3]

(1) Wallis, Fernholz and Gephart, THIS JOURNAL, **59**, 137 (1937).
(2) Ford, Chakravorty and Wallis, *ibid.*, **59**, 1415 (1937); **60**, 413 (1938); Ladenburg, Chakravorty and Wallis, *ibid.*, **61**, 3483 (1939).
(3) Henry Gilman, "Treatise of Organic Chemistry," 2nd ed., Vol. 2, 1943, p. 1383.