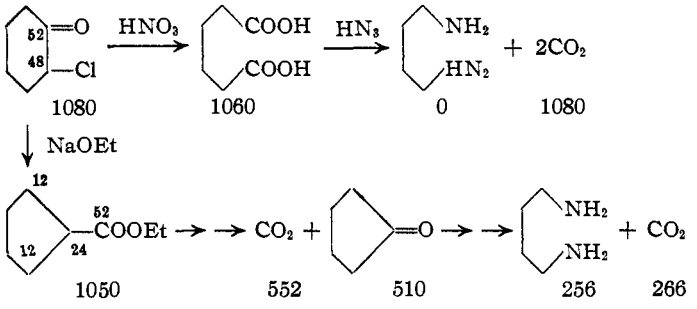
bearing carbon atom of the 2-chloro-1-cyclohexanone I is different depending on the path followed. In the first case it becomes exclusively the α-carbon atom of the cyclopentanecarboxylic acid III; in the second case it appears in both the α- and β-positions of III. Hence it should be possible to select between the two mechanisms using chlorocyclohexanone appropriately tagged with C-14.

Chlorocyclohexanone labeled as shown below was prepared by an unambiguous synthesis. To establish that no chlorine had migrated from the tagged to the untagged α-carbon atom during synthesis, the tagged chlorocyclohexanone was oxidized with nitric acid to adipic acid. The adipic acid was degraded to putrescin and carbon dioxide by the Schmidt reaction. The absence of radioactivity in the putrescin proved that only carbon atoms 1 and 2 of the chlorocyclohexanone were labeled.

The tagged chloroketone reacted with sodium ethoxide in ethanol to yield the ester which was hydrolyzed to cyclopentanecarboxylic acid. The acid was brominated, then hydrolyzed to the corresponding α-hydroxy acid which was oxidized smoothly with potassium permanganate to cyclopentanone and carbon dioxide. The cyclopentanone on successive Schmidt reactions yielded putrescin and carbon dioxide. The figures on the carbon atoms indicate percentages of total radioactivity which are consistent with the radioactivities observed in the degradation products (figures below the formulas are observed counts per minute per one-tenth millimole as barium carbonate).



It is apparent that the present results support strongly the cyclopropanone mechanism or some other mechanism in which carbon atoms 2 and 6 are at some time equivalent. It must be admitted that there is a possibility of the chlorine migrating from the labeled to the unlabeled α-carbon atom of I, perhaps by nucleophilic displacement on chlorine by the carbanion IV. Such migration, proceeding very rapidly compared to the Faworskii rearrangement, would account for our results. Experiments are in progress to check this point.

It is also apparent that the cyclopropanone mechanism cannot be applied to the rearrangement of all α-halo ketones. Thus although 1-benzoyl-1-chlorocyclohexane can give no anion corresponding to IV, it does rearrange slowly to 1-phenylcyclohexane-1-carboxylic acid.[4] However, it seems possible that the rearrangement of α-halo ketones proceeds through the cyclopropanone intermediate whenever possible.

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RECEIVED NOVEMBER 16, 1949

(4) Tchoubar, *Compt. rend.*, **208**, 1020 (1939).

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REARRANGEMENTS AND REVISIONS IN THE TETRAHYDROCARBAZOLE SERIES

Sir:

Recent mention in the literature[1,2] of 11-hydroxytetrahydrocarbazolenine[3] prompts us to report some results in this field.

It was shown previously[4] that the action of alkali on 9-acetyl-10,11-dihydroxyhexahydrocarbazole (I) does not yield 11-hydroxytetrahydrocarbazolenine (II), but spiro-[cyclopentane-1,2′-pseudoindoxyl] (III). By catalytic oxidation of tetrahydrocarbazole in ethyl acetate over platinum and subsequent gentle hydrogenation[5] we have now prepared authentic II in 75% yield (big, colorless prisms, m. p. 159°. *Anal.* Calcd. for $C_{12}H_{13}ON$: C, 77.0; H, 6.95; N, 7.42. Found: C, 77.21; H, 7.08; N, 7.48. Absorption spectra: ultraviolet, $\lambda_{max.}$ (log ϵ): 260 (3.42); $\lambda_{min.}$ (log ϵ): 235 (3.20); infrared; 3.02 μ (OH), 6.25 μ (Ph—N=C<)). II, treated with alkali, undergoes a benzilic acid type of rearrangement to III, and is the intermediate in the conversion of I to III. Either acid, heat or acetic anhydride likewise caused rearrangement to III (yield 85%) evidently by a different route, but in addition two more products were isolated of which one formed colorless plates (m. p. 255° and, after resolidification, 313°. *Anal.* Calcd. for $C_{24}H_{18}N_2$: C, 85.25; H, 6.51; N, 8.3; mol. wt., 338. Found: C, 85.15; H, 6.89; N, 8.40; mol. wt. (Rast), 325). We have found II and the colorless high melting product to be identical, respectively, with the "10-hydroxy-1,2,3,10-tetrahydrocarbazole" and the "2,3-dihydrocarbazole" previously reported.[6]

I, because of the N-acetyl group, with acetic anhydride shows a Wagner–Meerwein shift to give V (formerly thought to be IV[3]) which, after deacetylation and nitration, can be methylated to yield VII.[3] We have synthesized VI by condensing N-methyloxindole with tetramethylene bromide (colorless hexagons, m. p. 63°. *Anal.* Calcd. for $C_{13}H_{15}ON$: C, 77.61; H, 7.96. Found:

(1) Beer, McGrath, Robertson and Woodier, *Nature*, **164**, 362 (1949).

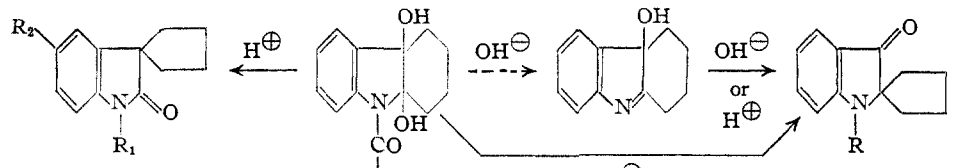
(2) Barnes, Pausacker and Schubert, *J. Chem. Soc.*, 1381 (1949).

(3) Perkin and Plant, *ibid.*, **123**, 688 (1923).

(4) Witkop, THIS JOURNAL, **71**, 614 (1950).

(5) The method has been previously employed with natural products containing the indole system (Witkop, to be published).

(6) Plant and Tomlinson, *J. Chem. Soc.*, **298** (1933).



V, R_1 = CH_3CO—, R_2 = H
VI, R_1 = CH_3, R_2 = H
VII, R_1 = CH_3, R_2 = NO_2

I

II

III, R = H
IV, R = CH_3CO—

C, 77.81; H, 7.67). The smooth nitration furnished VII, identical with the product from I (pale yellow prisms, m. p. 142°. *Anal.* Calcd. for $C_{13}H_{14}O_3N_2$: C, 63.41; H, 5.7. Found: C, 63.57; H, 5.96).

Further reactions and rearrangements in this series will be reported shortly.

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RECEIVED NOVEMBER 13, 1949.

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FORMATION OF 4-AMINO-5-CARBOXAMIDOIMIDAZOLE DURING GROWTH OF *ESCHERICHIA COLI* IN THE PRESENCE OF 4-AMINOPTEROYLGLUTAMIC ACID

Sir:

When *Escherichia coli* is grown in the presence of amounts of 4-aminopteroylglutamic acid just sufficient to inhibit multiplication slightly, 4-amino-5-carboxamidoimidazole accumulates in the medium, and has been isolated from it. This is the same substance which was found by Stetten and Fox[1] when this and other bacteria were grown in the presence of sulfadiazine or sulfapyridine. It was identified by Shive, *et al.*,[2] and recognized as the probable precursor in the biosynthesis of hypoxanthine.

The accumulation of the imidazole through the intervention of the antimetabolite of folic acid is of importance in consideration of the mode of action of sulfonamide drugs and of folic acid. Thus, inhibition analysis has led to the conclusion that *p*-aminobenzoic acid participates in several reactions, of which the first to be affected by sulfanilamide derivatives is the formation of methionine, the next is concerned with purine formation,[3] and less sensitive processes, presumably the synthesis of folic acid,[4] are then retarded. On the other hand, Woods[5] has concluded that the primary action of the sulfonamides is the inhibition of folic acid formation, and that synthesis of purines and of methionine are secondary events in which that vitamin participates. The present finding would favor the latter view. Since the folic acid antagonist leads to the accumulation of the same imidazole as do the *p*-aminobenzoic acid antimetabolites, the latter presumably act by creating a deficiency of folic acid, which in turn is responsible for the failure in purine formation.

The demonstration was conducted as follows: *E. coli* was grown in the manner of Stetten and Fox[1] except that sulfadiazine was omitted and 0.2 mg. per cc. of 4-aminopteroylglutamic acid[6] was added. Judged colorimetrically, about the same amount of diazotizable amine accumulated as when sulfadiazine was the inhibitor. Isolation of the base was accomplished as in[1] except that 2.5 times as much mercury salt was used and the ether extraction was omitted. Final separation was made on paper strips with butanol–diethylene glycol–water solvent, in an atmosphere containing ammonia,[7] in which the imidazole showed R_F of 0.5. Identity of the isolated substance with synthetic 4-amino-5-carboxamidoimidazole[8] was established by comparison of (a) the R_F in the solvent just mentioned, (b) the absorption spectra in the ultraviolet region at *p*H 2.0 and 11.0, and (c) the melting points (with decomposition) of the picrate. In every case the behavior of the known and the unknown was the same.

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RECEIVED DECEMBER 7, 1949

(1) M. R. Stetten and C. L. Fox, *J. Biol. Chem.*, **161**, 333 (1945).
(2) W. Shive, W. W. Ackermann, M. Gordon and M. E. Getzendaner, THIS JOURNAL, **69**, 725 (1947).
(3) W. Shive and E. C. Roberts, *J. Biol. Chem.*, **162**, 463 (1946).
(4) K. C. Winkler and P. G. de Haan, *Arch. Biochem.*, **18**, 97 (1948).
(5) D. D. Woods, *Bull. soc. chim. biol.*, **30**, 730 (1948).
(6) Kindly made available by Dr. T. H. Jukes of Lederle Laboratories.
(7) E. Vischer and E. Chargaff, *J. Biol. Chem.*, **176**, 703 (1948).
(8) E. Shaw and D. W. Woolley, *ibid.*, **181**, 89 (1949).
(9) Fellow of the National Institutes of Health.

SPECIFICITY OF UREASE ACTION

Sir:

Urease has been repeatedly cited[1] as a strictly specific enzyme which hydrolyzes only urea. In the course of experiments with substances related to urea we have observed a hydrolysis of biuret $H_2N—\overset{\overset{O}{\|}}{C}—NH—\overset{\overset{O}{\|}}{C}—NH_2$ by urease preparations.

As much as 33% of nitrogen initially contained in solutions of biuret was identified as ammonia (by Nessler technique[2]) after prolonged enzy-

(1) Sumner and Sommers, "Enzymes," Academic Press, New York, N. Y., p. 156.
(2) Ambrose, Kistiakowsky and Kridl, THIS JOURNAL, **72**, 317 (1950).