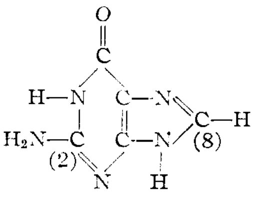
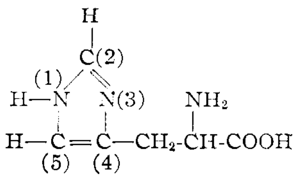


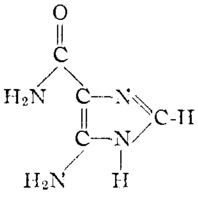
Guanine



Histidine

The source of the nitrogen of histidine was investigated by growing the organism on glucose in a medium whose only nitrogenous constituents were 12 mg. of unlabelled guanine and 400 mg. of $(N^{15}H_4)_2SO_4$ (60 atom % excess) per liter. The N^{15} content of the histidine isolated from the bacterial protein (determined[3] after dilution with a known amount of carrier) was found to be 38.5 atom % excess, 64% that of the exogenous ammonium sulfate, indicating that only two of its three nitrogen atoms had been derived from the latter. The histidine was hydrolyzed enzymatically with dried cells of histidine-grown *Aerobacter aerogenes* to a mixture of ammonia, glutamic acid, and formamide.[4] The three nitrogenous compounds were separated by consecutive passage of the mixture over columns of Permutite (retaining ammonia) and Dowex-2-chloride (retaining glutamic acid) and analyzed for N^{15} with the following results (expressed in per cent. of the atom % excess of the ammonium sulfate): ammonia (amino group of histidine) 94%, glutamic acid (imidazole-nitrogen 3) 97%, formamide (imidazole-nitrogen 1) 0.8%. It appears therefore that guanine is not only the source of carbon 2,[2] but also of the adjacent nitrogen 1 of the imidazole ring of histidine.

In another experiment the mutant was grown in the glucose–ammonium sulfate medium supplemented with 50 mg. of guanine-8-C^{14} per liter. The ribotide and riboside of 4-amino-5-imidazole carboxamide were found to accumulate in the culture fluid and were isolated by adsorption to charcoal, elution with a mixture of ethanol, ammonia and water, followed by chromatography and electrophoresis on filter paper.[5] The radioactivity (in counts per micromole) of the carboxamide obtained by the acid hydrolysis of its derivatives was equal to that of the exogenous guanine, and the amount accumulated, 7 mg. per liter, was roughly equivalent to the amount of histidine in the cells.



4-Amino-5-imidazole carboxamide

The results suggest that guanine is converted to the ribotide of 4-amino-5-imidazole carboxamide by the loss of a C–N unit which eventually becomes the nitrogen 1–carbon 2 portion of the imidazole ring of histidine.

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[3] I am indebted to Dr. D. Elwyn for help with the analysis.

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[5] A. R. Greenberg and E. L. Spilman, *ibid.*, **219**, 411 (1956).

EVIDENCE FOR THE PRESENCE OF COBALT HYDROCARBONYL UNDER CONDITIONS OF THE OXO REACTION

Sir:

Recent kinetic studies of the oxo reaction[1,2] purport to indicate that the cobalt catalyst is present as dicobalt octacarbonyl and that the first step in the reaction consists of olefin attack on this catalyst. Although cobalt hydrocarbonyl has been postulated repeatedly to be present during the reaction,[3] no conclusive evidence for its presence (or absence) has been presented, owing to its instability.[4]

In some early work, not concerned with the oxo reaction, it was reported that treatment of dicobalt octacarbonyl at 165° for 18 hours with H_2:CO, gave a small (unspecified) quantity of cobalt hydrocarbonyl.[5] We have now found that dicobalt octacarbonyl under carbon monoxide pressure is rapidly converted by hydrogen at 110° to the hydrocarbonyl. The hydrocarbonyl was isolated (as the anion) by rapid cooling (−50°) of the pressure vessel. However, if an olefin is present at the time the vessel is cooled, no hydrocarbonyl can be isolated. If the conventional oxo reaction is allowed to proceed until the olefin is consumed, the hydrocarbonyl again appears uncombined. The results listed in Table I also show that the partial pressure of hydrogen affects the carbonyl conversion. These results strongly suggest olefin–hydrocarbonyl rather than olefin–octacarbonyl interaction as the step in the oxo synthesis.

TABLE I

CONVERSION OF $[Co(CO)_4]_2$ to $HCo(CO)_4$

1-Hexene, mole	$[Co(CO)_4]_2$ mmoles	Synthesis gas, p.s.i. at 112° Start	Finish	Time (min.)[a]	Per cent. Co as $HCo(CO)_4$
0	4.38	3120	3120	10	50[b]
0	4.59	2600	2600	10	27
0.40	4.00	3200	3000	10	0[b]
.04	4.00	3200	3100	10	29
.226	4.52	3400	2300	77	34
.226	4.52	4000	2850	100	62

[a] After addition of hydrogen to twice the carbon monoxide pressure. [b] Duplicate experiments.

An additional experiment using the technique of rapid cooling showed that the standard procedure[6] for the preparation of $[Co(CO)_4]_2$ results in the formation of $HCo(CO)_4$. The former is isolated as

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[1] A. R. Martin, *Chem. Ind.*, 1536 (1954).

[2] G. Natta, R. Ercoli, S. Castellano and F. H. Barbieri, THIS JOURNAL, **76**, 4049 (1954).

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[5] W. Hieber, H. Schulten and R. Marin, *Z. anorg. Chem.*, **240**, 261 (1939).

[6] I. Wender, H. Greenfield and M. Orchin, THIS JOURNAL, **73**, 2656 (1951).

the product only because the hydrocarbonyl decomposes to the octacarbonyl during the pressure let-down at room temperature.

Experimental.—In a typical experiment a solution of 0.78 g. (4.57 mmole) of dicobalt octacarbonyl in 100 ml. of pure hexane in a 250 ml. "Magnedash" autoclave was pressured with 85 atm. of carbon monoxide. The autoclave was heated with agitation to a temperature of 110° (70 min.) at which time the pressure was 110 atm. Hydrogen was then added until the total pressure was 220 atm. The heater was immediately removed and the autoclave cooled with a Dry Ice-bath. After the pressure no longer dropped (30 min.), the autoclave was vented, and opened. The contents were poured into a solution of approximately 2.5 mmole of nickel *o*-phenanthroline chloride in 50 ml. of water and the cold mixture shaken until warmed to room temperature. The flocculent precipitate was filtered, and dissolved in pyridine. Approximately 200 ml. of carbon monoxide (S.T.P.) was liberated from this solution on treatment with iodine according to the known procedure.[7] This quantity corresponds to 2.23 mmole of $Co(CO)_4$ or a 48.6% yield based on starting dicobalt octacarbonyl.

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[7] H. Sternberg, I. Wender and M. Orchin, *Anal. Chem.*, **24**, 174 (1952).

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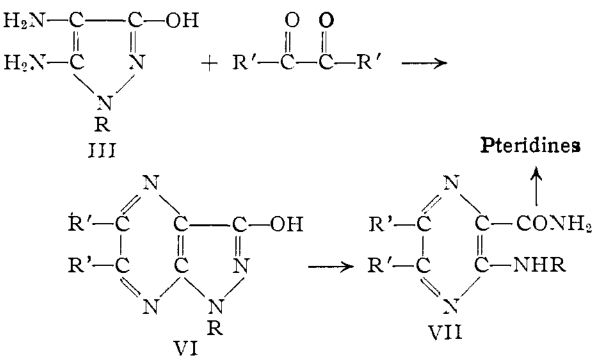
A NEW SYNTHETIC APPROACH TO PTERIDINES*

Sir:

The conventional and most widely-employed synthetic route to pteridines involves the condensation of a 4,5-diaminopyrimidine with an α,β-dicarbonyl compound, an α-halocarbonyl compound, an α-keto alcohol or related derivatives of such intermediates, but this approach suffers from several inescapable limitations.[1] An alternative general synthetic approach to pteridines *via* the ring closure of pyrazines has received recent attention,[1–8] although the method suffers from the relative inaccessibility of the requisite intermediates, which have previously been prepared rather circuitously by the ring cleavage of other pteridines.[7,9] We now wish to describe a new general route to these pyrazine intermediates which makes possible for the first time the ready synthesis of pteridines substituted in position 1.

Condensation of ethyl phenylazocyanoacetate with hydrazine or hydrazine hydrate in ethanol solution gave 3-hydroxy-4-phenylazo-5-aminopyrazole (I, R = $-H$, m.p. 256° dec. *Anal.* Calcd. for $C_9H_9ON_5$: C, 53.2; H, 4.5; N, 34.5. Found: C, 53.2; H, 4.3; N, 34.3). Reduction of I (R = $-H$) with hydrogen in 98% formic acid using 10% palladium-on-charcoal as catalyst afforded 3-hydroxy-4,5-diformylaminopyrazole (II, R = $-H$, m.p. 212–213° dec. *Anal.* Calcd. for $C_5H_6O_3N_4$: C, 35.3; H, 3.5; N, 32.9. Found: C, 35.4; H, 3.2; N, 32.4). Treatment of II (R = $-H$) with 50% sulfuric acid resulted in cleavage of the formyl groups to give crystalline 3-hydroxy-4,5-diaminopyrazole[10] sulfate (III, R = $-H$). III (R = $-H$) was alternatively prepared by cyclization of the hydrazine salt of nitrosocyanoacetohydrazide[11] (IV) with 40% sodium hydroxide at room temperature to give 3-hydroxy-4-nitroso-5-aminopyrazole[10] (V, R = $-H$), followed by catalytic reduction. Repetition of the above reactions using methylhydrazine yielded III (R = $-CH_3$, m.p. > 250°. *Anal.* Calcd. for $C_4H_8ON_4.H_2SO_4$: C, 21.2; H, 4.5; N, 24.8; S, 14.2. Found: C, 21.3; H, 4.7; N, 25.2; S, 14.2).

Condensation of III with glyoxal, biacetyl and benzil yielded 3-hydroxy-1-pyrazolo[b]pyrazines (VI, R = R′ = $-H$, m.p. 314–315° dec. *Anal.* Calcd. for $C_5H_4ON_4$: C, 44.1; H, 3.0; N, 41.2. Found: C, 44.4; H, 3.0; N, 41.2. VI, R = $-H$, R′ = $-CH_3$, m.p. 325° dec. *Anal.* Calcd. for $C_7H_8ON_4$: C, 51.2; H, 4.9; N, 34.1. Found: C, 50.9; H, 4.7; N, 34.4. VI, R = $-H$, R′ = $-C_6H_5$, m.p. 269° dec. *Anal.* Calcd. for $C_{17}H_{12}ON_4$: C, 70.8; H, 4.2; N, 19.4. Found: C, 70.8; H, 4.0; N, 19.4. VI, R = $-CH_3$, R′ = $-H$, m.p. 242–243°. *Anal.* Calcd. for $C_6H_6ON_4$: C, 48.0; H, 4.0; N, 37.3. Found: C, 48.1; H, 4.2; N, 37.1. VI, R = R′ = $-CH_3$, m.p. 267–268°. *Anal.* Calcd. for $C_8H_{10}ON_4$: C, 53.9; H, 5.7; N, 31.5. Found: C, 54.1; H, 5.7; N, 31.6). Treatment of these 3-hydroxy-1-pyrazolo[b]pyrazines (VI) with Raney nickel according to the method of Ainsworth[12] cleaved the pyrazole ring at the hydrazine N–N linkage to give 2-aminopyrazine-3-carboxamides (VII). For example, treatment



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* This investigation was aided by a grant from the American Cancer Society.

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