crystallization from methanol (IV, X = F) was obtained as thin colorless prisms, m.p. 107–108°, yield 0.8 g. (70%).

Anal. Calcd. for $C_{16}H_{13}FO_3$: C, 70.6; H, 4.8; F, 7.0. Found: C, 70.5; H, 4.8; F, 6.8.

Methyl 3-chloro-6-methoxyfluorene-9-carboxylate (IV, X = Cl), was obtained analogously from 3-chloro-6-methoxy-9-trifluoromethylfluorene (III, X = Cl, 1.2 g.). It formed thin colorless prisms of m.p. 129–130°, yield 0.92 g. (79%).

Anal. Calcd. for $C_{16}H_{13}ClO_3$: C, 66.6; H, 4.5. Found: C, 65.9; H, 4.8.

3-Fluoro-6-methoxyfluorenone (V, X = F).—A well-stirred suspension of methyl 3-fluoro-6-methoxyfluorene-9-carboxylate (IV, X = F, 0.4 g.) in 5 *N* sodium hydroxide solution (5 ml.) was heated on a boiling water-bath. To this was added, drop by drop, 30% hydrogen peroxide (2 ml.). When the reaction had subsided, water (10 ml.) was added and the mixture extracted with ether. By evaporation of the solvent and recrystallization of the residue from a benzene–petroleum ether mixture, there were obtained bright yellow prisms, which dissolve in concentrated sulfuric acid with a deep purple color; m.p. 152–153°, yield 0.1 g. (30%).

Anal. Calcd. for $C_{14}H_9FO_2$: C, 73.7; H, 4.0. Found: C, 74.0; H, 3.8.

3-Chloro-6-methoxyfluorenone (V, X = Cl).—Methyl 3-chloro-6-methoxyfluorene-9-carboxylate (IV, X = Cl, 0.5 g.), when treated as above, gave 0.12 g. (28%) of bright yellow prisms, giving the same color reaction with concentrated sulfuric acid as the fluorine analog, m.p. 181–182°.

Anal. Calcd. for $C_{14}H_9ClO_2$: C, 68.7; H, 3.7. Found: C, 68.6; H, 3.8.

The oxime formed small yellowish prisms, which, after recrystallization from alcohol, melted at 229–230° dec., yield 66%.

Anal. Calcd. for $C_{14}H_{10}ClNO_2$: C, 64.7; H, 3.9. Found: C, 65.0; H, 4.0.

Synthesis of 3-Chloro-6-methoxyfluorenone (V, X = Cl).—(a) To a hot solution of pure[21] 4-chloroanthranilic acid[22] (9 g.) in 20% sodium carbonate solution (45 ml.), there was added, in small portions, *p*-toluenesulfonyl chloride (9 g.). Decolorizing charcoal was then added and the mixture held at 70–80° for 10 minutes, filtered while still hot and, after cooling, acidified with excess hydrochloric acid. The precipitated N-*p*-toluenesulfonyl-4-chloroanthranilic acid was filtered off and recrystallized successively from 70% ethanol and benzene; m.p. 223–225°, yield 8.5 g. (50%).

Anal. Calcd. for $C_{14}H_{12}ClNO_4S$: C, 51.6; H, 3.7. Found: C, 50.6; H, 4.0.

(b) The foregoing compound (7.5 g.) was refluxed with phosphorus pentachloride (5.5 g.) in carbon disulfide (150 ml.) for 45 minutes. The solution was cooled in ice-water, and anisole (8 g.) and finely powdered aluminum chloride (6 g.) added. The reaction mixture was then refluxed for 2 hours, with occasional shaking, decomposed with a mixture of ice and excess hydrochloric acid, and extracted with ether. By removal of the solvent and recrystallization of the crude product from methanol, **4-chloro-4′-methoxy-2-*p*-tosylaminobenzophenone** (VII) was obtained as long colorless needles, m.p. 107–108°, yield 5.4 g. (56%).

Anal. Calcd. for $C_{21}H_{18}ClNO_4S$: C, 60.7; H, 4.4. Found: C, 61.0; H, 4.3.

(c) The ketone VII (5 g.) was heated with a mixture of acetic acid (10 ml.) and concentrated sulfuric acid (10 ml.) on a water-bath for 30 minutes. Water (20 ml.) was added and the reaction mixture, which crystallized partly, cooled to 0–5° and diazotized by the slow addition of a solution of sodium nitrite (0.8 g. in 15 ml. of water). The reaction mixture was then heated on a boiling water-bath for 45 minutes, cooled and extracted with ether. The ether extracts were washed with 10% sodium hydroxide solution, dried over anhydrous sodium sulfate and evaporated to dryness. The residue was recrystallized from a mixture of benzene and petroleum ether, and gave bright yellow crystals of 3-chloro-6-methoxyfluorenone (V, X = Cl), melting at 181–182°. A mixture with the product described above showed no depression of the melting point; yield 1.2 g. (41%).

TEL-AVIV, ISRAEL

(21) M.p. 232–234°; the purity is very important, as otherwise difficulties are encountered in the purification of the reaction products.

(22) E. B. Hunn, THIS JOURNAL, **45**, 1024 (1923).

[CONTRIBUTION FROM AVERY LABORATORY, UNIVERSITY OF NEBRASKA]

Synthesis of 3-Amino- and 3-Nitro-2-arylquinolines[1]

BY HENRY E. BAUMGARTEN AND JOHN L. SAYLOR

RECEIVED JULY 16, 1956

Condensation of *o*-aminobenzaldehyde with ω-nitroacetophenone gave 3-nitro-2-phenylquinoline, while condensation of *p*-chloro-, *p*-methyl- and *p*-methoxy-ω-nitroacetophenone and of *o*,ω-dinitroacetophenone with *o*-aminobenzaldehyde yielded 3-nitro-2-(*p*-chlorophenyl)-quinoline, 3-nitro-2-(*p*-tolyl)-quinoline, 3-nitro-2-(*p*-anisyl)-quinoline and 3-nitro-2-(*o*-nitrophenyl)-quinoline, respectively. All but the last-named 3-nitro-2-arylquinoline were reduced with iron and acetic acid to the corresponding 3-amino-2-arylquinolines.

For other studies being carried out in this Laboratory a ready source of variously substituted 3-amino- and 3-nitro-2-phenylquinolines was required. This communication describes a reaction sequence that we found to be convenient for the preparation of 3-amino-2-phenylquinoline (VIIa) and 3-nitro-2-phenylquinoline (VIa) and a number of their derivatives.

The various methods available for the preparation of 3-nitroquinolines have been reviewed.[2] Of these methods, the one involving the condensation of methazonic acid (II, R_2 = H, Y = N — OH) with *o*-amino carbonyl compounds[2–4] appeared to be the most promising for the purpose at hand. This reaction can be regarded as a specific application of the general sequence illustrated in I → III. According to this sequence, the condensation of *o*-aminobenzaldehyde (IV) with ω-nitroacetophenones (V) should lead to the desired 3-nitro-2-arylquinolines (VI).

The required ω-nitroacetophenones were prepared by the base-catalyzed condensation of an aromatic aldehyde with nitromethane followed by oxidation of the intermediate α-aryl-β-nitroeth-

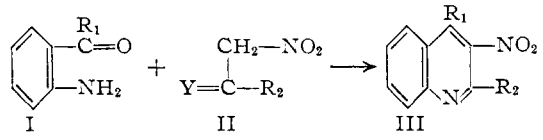
(1) This work was supported in part by grant G-1090 of the National Science Foundation.

(2) K. Schofield and R. S. Theobald, *J. Chem. Soc.*, 395 (1950).

(3) K. Schofield and R. S. Theobald, *ibid.*, 2992 (1951).

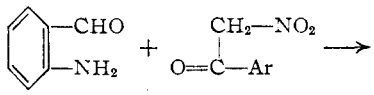
(4) D. W. Ockenden and K. Schofield, *ibid.*, 1915, 3914 (1953).

anol.[5-8] Several attempts to prepare *o*-chloro-ω-nitroacetophenone by this route gave none of the desired product.



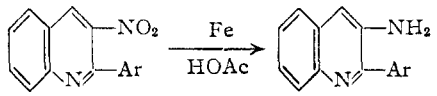
I II III

R = H, alkyl, aryl, OH, CO_2H; R_2 = H, alkyl, aryl; Y = O, N-OH



IV

Va, Ar = C_6H_5
Vb, Ar = *p*-ClC_6H_4
Vc, Ar = *p*-MeC_6H_4
Vd, Ar = *p*-$MeOC_6H_4$
Ve, Ar = *o*-$NO_2C_6H_4$



VIa, Ar = C_6H_5
VIb, Ar = *p*-ClC_6H_4
VIc, Ar = *p*-MeC_6H_4
VId, Ar = *p*-$MeOC_6H_4$
VIe, Ar = *o*-$NO_2C_6H_4$

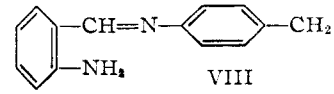
VIIa, Ar = C_6H_5
VIIb, Ar = *p*-ClC_6H_4
VIIc, Ar = *p*-MeC_6H_4
VIId, Ar = *p*-$MeOC_6H_4$

Because Va[7] was readily and cheaply prepared, the reaction between Va and IV was examined first and most thoroughly. When approximately equimolar amounts of IV and Va in ethanol solution were heated in the absence of any added catalyst, VIa slowly precipitated from the hot solution. Addition of small amounts of potassium hydroxide, potassium carbonate, sodium methoxide or piperidine not only failed to facilitate the reaction but also lowered the yield considerably and complicated the working up of the product. In the "uncatalyzed" reaction it is possible that traces of ammonia remaining in the crude IV used may have acted as the catalyst for the reaction. Inasmuch as it was generally difficult to determine the exact amount of IV being used as starting material, slight excesses of Va undoubtedly were employed and often the product was contaminated with the unreacted material (usually indicated by a low nitrogen analysis). Although removal of even traces of Va by recrystallization was only partially successful, heating the crude product with dilute aqueous sodium bicarbonate readily removed the contaminant. Presumably, in this treatment Va was cleaved to sodium benzoate and other fragments.

Under conditions similar to those described above the condensation of *p*-chloro- (Vb), *p*-methyl- (Vc) and *p*-methoxy-ω-nitroacetophenone (Vd) and of *o*,ω-dinitroacetophenone (Ve) with IV gave 3-nitro-2-(*p*-chlorophenyl)-quinoline (VIb), 3-nitro-2-(*p*-tolyl)-quinoline (VIc), 3-nitro-2-(*p*-anisyl)-quinoline (VId) and 3-nitro-2-(*o*-nitrophenyl)-quinoline (VIe) in 25–58% yields (Table I).

(5) L. Canonica, *Gazz. chim. ital.*, **79**, 192 (1949).
(6) L. Canonica and C. Cardani, *ibid.*, 262.
(7) L. M. Long and H. D. Troutman, THIS JOURNAL, **71**, 2469 (1949).
(8) O. Dann, H. Ulrich and E. Moller, *Z. Naturforsch.*, **71**, 344 (1952).

Borsche[9] has shown that in numerous examples the classical Friedlander synthesis (of which the sequence IV → VI is an example) may be modified to advantage by replacing IV by *o*-aminobenzaltoluidine (VIII). The Borsche modification is especially valuable where certain substituted derivatives of IV are involved, for these are often prepared *via* derivatives of VIII.[10,11] To establish the feasibility of using the Borsche modification in the present work, the reaction of VIII with Va and Vb



VIII

was studied. From the condensation of VIII with Va (again in the absence of added catalyst) VIa was obtained in 47–56% yield and from Vb, VIb was obtained in 41% yield. These results, together with those described above, suggest that the Friedlander reaction and the Borsche modification should be generally applicable to derivatives of V. Other examples of the general reaction I → III are being studied.

By reduction with iron and acetic acid, compounds VIa, VIb, VIc and VId were converted into the corresponding amines, VIIa, 3-amino-2-(*p*-chlorophenyl)-quinoline (VIIb), 3-amino-2-(*p*-tolyl)-quinoline (VIIc) and 3-amino-2-(*p*-anisyl)-quinoline, in 45–95% yields (Table II). The results of the present study, together with the simplicity of the operations involved, may recommend the use of the sequence IV → VII over those previously employed for the preparation of VII,[12-17] especially in those instances where both IV and V are readily available.

Experimental[18]

3-Nitro-2-arylquinolines (VI). (a) From *o*-Aminobenzaldehyde.—The general procedure used is illustrated by the preparation of 3-nitro-2-phenylquinoline. Deviations from the general procedure are described below.

A mixture of 38 g. (0.30 mole estimated) of freshly prepared *o*-aminobenzaldehyde[19] (from two 30-g. (0.20-mole) batches of *o*-nitrobenzaldehyde) and 52 g. (0.31 mole) of ω-nitroacetophenone[7] in 250 ml. of 95% ethanol was heated under reflux on the steam-bath for four hours. During the heating period the solids dissolved, the solution turned dark red and crystals began to collect on the bottom of the flask. The mixture was filtered hot, giving 43 g. (55%) of nearly pure 3-nitro-2-phenylquinoline, m.p. 155–156°. Recrys-

(9) W. Borsche, W. Doeller and M. Wagner-Roemich, *Ber.*, **76**, 1099 (1943); W. Borsche and J. Barthenhier, *Ann.*, **548**, 50 (1941); W. Borsche, M. Wagner-Roemich and J. Barthenhier, *ibid.*, **550**, 165 (1942); W. Borsche and W. Ried, *ibid.*, **554**, 269 (1943).
(10) H. E. Baumgarten and A. L. Krieger, THIS JOURNAL, **77**, 2438 (1955).
(11) H. E. Baumgarten and K. E. Cook, *J. Org. Chem.*, in press.
(12) G. Gargellini and S. Berlingozzi, *Gazz. chim. ital.*, **53**, 3 (1923).
(13) S. Berlingozzi and C. Napolitano, *ibid.*, **53**, 369 (1923).
(14) H. Johns and H. Ottawa, *J. prakt. Chem.*, **131**, 346 (1931).
(15) French Patent 789,068; *C. A.*, **30**, 1809 (1936).
(16) H. de Diesbach and E. Moser, *Helv. Chim. Acta*, **20**, 132 (1937).
(17) V. A. Petrov, M. V. Stack and W. R. Wragg, *J. Chem. Soc.*, 316 (1943).
(18) Melting points are corrected. Analyses by Clark Microanalytical Laboratory, Urbana, Ill., and by Micro-Tech Laboratories, Skokie, Ill.
(19) L. I. Smith and J. W. Opie, "Organic Syntheses," Coll. Vol. III, John Wiley and Sons, Inc., New York, N. Y., 1954, p. 56. This material was used without further purification and was probably contaminated with water, sodium chloride and ammonium hydroxide. To afford a basis for calculation, in these experiments it was assumed that the yield of *o*-aminobenzaldehyde was 75%.

TABLE I

3-NITRO-2-ARYLQUINOLINES

Compd.	Starting ketone[a]	Crude yield, % (m.p., °C.)	Final yield,[b] %	Final m.p., °C.	Formula	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Nitrogen, % Calcd.	Nitrogen, % Found
VIa[c]	Va(71)[7]	68(155–156)	54–61	156.5–157.5	$C_{15}H_{10}N_2O_2$	71.99	71.95	4.03	4.08	11.20	11.31
VIb[d]	Vb(85)[8]	66(162–164)	55	164–165	$C_{15}H_9N_2O_2Cl$	63.28	63.50	3.19	2.92	9.84	9.65
VIc[e]	Vc(73)	62(108–123)	27	124–124.5	$C_{16}H_{12}N_2O_2$	72.71	72.78	4.58	4.38	10.60	10.35
VId[d]	Vd(20)[5]	69(129–145)	25	157–157.5	$C_{16}H_{12}N_2O_3$	68.56	68.82	4.32	4.38	10.00	9.97
VIe[d]	Ve(90)[6,f]	68(160–166)	38	163–164	$C_{15}H_9N_3O_4$	61.02	61.49	3.07	3.27	14.23	13.88

[a] Figure in parentheses indicates maximum yield obtained following procedure of ref. indicated. [b] Carried to analytical purity. [c] Bright yellow plates. [d] Bright yellow needles. [e] Pale yellow needles. [f] In following the procedure of reference 6 it was important to chill thoroughly the acetic acid solution of chromic acid before adding the 1-(*o*-nitrophenyl)-2-nitroethanol. In one experiment in which the alcohol and chromic acid were added to the acetic acid at a temperature somewhat below room temperature, the temperature rose rapidly to 90° and flames leaped out of the reaction flask before the reaction could be brought under control.

TABLE II

3-NITRO-2-AMINOQUINOLINES

Compd.	Crude yield, % (m.p., °C.)	Final yield,[a] %	Final m.p., °C.	Formula	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Nitrogen, % Calcd.	Nitrogen, % Found
VIIa[b]	95(118–119)	85–95	118–119[d]							
VIIb[b]	83(*ca.* 173)	50	171–172	$C_{15}H_{11}N_2Cl$	70.73	70.47	4.35	4.19	11.00	10.93
VIIc[b]	96(*ca.* 135)	45	135–135.5	$C_{16}H_{14}N_2$	82.02	82.35	6.06	5.84	11.96	12.13
VIId[c]	94(139–146)	48	140–141	$C_{16}H_{14}N_2O$	76.78	77.06	5.64	5.52	11.19	11.29

[a] Carried to analytical purity. [b] Nearly colorless needles. [c] Pale buff plates. [d] Lit. m.p. 115–116°,[12] 121–122°.[17]

tallization of this material from 1.5 l. of absolute ethanol gave 31.2 g. of 3-nitro-2-phenylquinoline, m.p. 156.5–157.5°, as large, flat, yellow plates. The original reaction mixture was allowed to stand overnight (or chilled in ice) and a second crop (10 g.) of crystals was collected. This material was contaminated with ω-nitroacetophenone. The latter was destroyed by heating a suspension of the solid in 100 ml. of 5% aqueous sodium bicarbonate on the steam-bath for one hour. The undissolved residue was filtered off and dissolved (with heating) in the filtrate from the recrystallization above. The filtrate was concentrated to a volume of 500 ml., giving 11 g. of additional 3-nitro-2-phenylquinoline (total yield 42.2 g. (54%)[20]). In a large number of experiments using one-half of the above quantities, the yields varied from 54–61%. For analysis a small sample was treated with hot aqueous bicarbonate and recrystallized from ethanol with no perceptible change in melting point.[21]

The procedures used for the other 3-nitro-2-arylquinolines (Table I) differed from the above only in the quantity of ethanol used as a reaction solvent and in the working up of the product. For 0.15 mole of the appropriate ω-nitroacetophenone, the following quantities of solvent were most satisfactory: for Vb, 350 ml.; for Vc, 700 ml.; for Vd, 225 ml.; for Ve, 250 ml. The reaction mixtures containing Vb-Ve were worked up by chilling the reaction mixture, collecting the entire crude product, treating the crude product with hot aqueous sodium bicarbonate, and recrystallizing the bicarbonate-insoluble residue from absolute ethanol.

(b) **From *o*-Aminobenzaltoluidine (VIII).**—A mixture of 3.0 g. (0.02 mole) of *o*-nitrobenzaldehyde and 2.2 g. (0.02 mole) of *p*-toluidine in 20 ml. of absolute ethanol was heated under reflux for two hours on the steam-bath. To the hot solution was added a solution of 7.3 g. (0.042 mole) of sodium sulfide pentahydrate in 5 ml. of ethanol and 5 ml. of water. A vigorous reaction took place and the tan solution turned dark brown. On chilling in ice and diluting the reaction mixture with about one-half its volume of water, the pale yellow product was precipitated. It was collected by filtration, washed with 50% ethanol and air-dried, giving 2.33 g. (56%) of crude *o*-aminobenzaltoluidine, which was used without further purification.

A mixture of 210 mg. (0.001 mole) of *o*-aminobenzal toluidine and 170 mg. (0.001 mole) of ω-nitroacetophenone in 2 ml. of ethanol was heated under reflux on the steam-bath overnight. The mixture was filtered hot, giving 175 mg. of crude product. The latter was recrystallized from 7 ml. of absolute ethanol, giving 140 mg. (56%) of 3-nitro-2-phenylquinoline, m.p. 156–157.5°. In one experiment using ten times the above quantities the yield was 47%.

A mixture of 1.13 g. (0.0054 mole) of *o*-aminobenzaltoluidine and 1.10 g. (0.0055 mole) of *p*-chloro-ω-nitroacetophenone in 50 ml. of absolute ethanol was heated under reflux for ten hours on the steam-bath. The mixture was cooled in ice and filtered, giving 0.80 g. of crude product. Recrystallization of the product from 30 ml. of 95% ethanol gave 0.62 g. (41%) of 3-nitro-2-(*p*-chlorophenyl)-quinoline, m.p. 164–165°.

3-Amino-2-arylquinolines (VII).—The general procedure used is illustrated by the preparation of 3-amino-2-phenylquinoline.

To a hot suspension of 5.0 g. (0.02 mole) of 3-nitro-2-phenylquinoline in 40 ml. of acetic acid and 20 ml. of water was added over a period of about five minutes 2.8 g. (0.05 mole) of iron powder (J. T. Baker reduced). The mixture was well shaken during the addition until a vigorous reaction began. The mixture was heated under reflux on the steam-bath for one hour with frequent shaking. The hot solution was poured onto *ca.* 200 g. of crushed ice and water and was neutralized with 150 g. of cold 33% potassium hydroxide solution. The amorphous precipitate (mixture of amine and black iron oxide) was collected and suspended in 300 ml. of absolute ethanol. The mixture was heated to boiling on the steam-bath and filtered hot. The hot solution was treated with charcoal, refiltered and evaporated to half-volume. Water was added to incipient precipitation; the solution was clarified by heating and allowed to stand overnight. The product was collected and air-dried, giving 4.2 g. (95%) of 3-amino-2-phenylquinoline, m.p. 118–119° (lit. m.p. 115–116°,[12] 121–122°[17]). The ultraviolet spectrum of a 10^{-5} *M* solution of the product in 95% ethanol had maxima at 254 mμ (log ε 4.54) and 355 mμ (log ε 3.87) and minima at 230 mμ (log ε 4.39) and 315 mμ (log ε 3.44). In other experiments, including some using three times the above quantities, the yields varied from 85–95%.

The other 3-amino-2-arylquinolines (Table II) were prepared by essentially the same procedure. Absolute ethanol was used for their recrystallization.

***p*-Methyl-ω-nitroacetophenone (Vc)** was prepared from *p*-tolualdehyde using the procedure described by Long and Troutman[7] for the preparation of ω-nitroacetophenone. From 60 g. (0.50 mole) of *p*-tolualdehyde, 65 g. (73%) of nearly colorless *p*-methyl-ω-nitroacetophenone, m.p. 142.5–144°, was obtained. This material was sufficiently pure

(20) Because of the assumptions made concerning the *o*-aminobenzaldehyde,[19] the over-all yield (based on *o*-nitrobenzaldehyde) would be three-fourths of the value stated here.

(21) In one experiment purification by recrystallization alone gave a product with the analysis: C, 71.79; H, 3.95; N, 10.75, 10.52.

for the preparation of VIc without further treatment. For analysis a small sample was recrystallized from benzene. The colorless plates melted at 145–147°.

Anal. Calcd. for $C_9H_9NO_3$: C, 60.32; H, 5.06; N, 7.82. Found: C, 60.25; H, 5.05; N, 7.78.

LINCOLN 8, NEBRASKA

COMMUNICATIONS TO THE EDITOR

THE ROLE OF ADENOSINE TRIPHOSPHATE IN THE ENZYMATIC ACTIVATION OF CARBON DIOXIDE[1]

Sir:

Although many compounds are known to undergo chemical modification or "activation" prior to entrance into biochemical reactions, carbon dioxide has generally been considered to participate simply as such or as bicarbonate ion in a variety of carboxylations in living cells. As recently proposed,[2] however, the reaction of carbon dioxide with ATP[3] may furnish a reactive intermediate capable of carboxylating HIV CoA (an intermediate in leucine metabolism). Further study of this system has led to the discovery of the bicarbonate- and hydroxylamine-dependent cleavage of ATP to AMP and pyrophosphate, catalyzed by extracts of bacteria, yeast and various animal tissues. The reactions shown are proposed to account for this finding and for the mechanism of carbon dioxide activation

$$CO_2 + ATP \rightleftharpoons AMP\text{-}CO_2 + \text{pyrophosphate} \quad (1)$$

$$AMP\text{-}CO_2 + NH_2OH \longrightarrow AMP + \text{(carbonohydroxamic acid?)} \quad (2)$$

$$AMP\text{-}CO_2 + \text{HIV CoA} \rightleftharpoons AMP + \text{HMG CoA} \quad (3)$$

$$\text{HMG CoA} \rightleftharpoons \text{acetoacetate} + \text{acetyl CoA} \quad (4)$$

The incubation of hydroxylamine and bicarbonate with a heart preparation free of myokinase and pyrophosphatase but containing the carbon dioxide-activating enzyme (H enzyme) results in the degradation of ATP to approximately equimolar amounts of adenylic acid[4] and pyrophosphate (Table I). These products would be expected if this enzyme catalyzes the formation of $AMP\text{-}CO_2$ (the mixed anhydride of adenylic and carbonic acids) according to Reaction 1, and the intermediate decomposes non-enzymatically according to Reaction 2. Attempts to detect carbonohydroxamic acid have so far proved unsuccessful. However, hydroxylamine has been shown to convert synthetically prepared $AMP\text{-}CO_2$[8] to AMP, and synthetically prepared $AMP\text{-}CO_2$ ethyl ester to AMP and a heat-labile hydroxamic acid chromatographically indistinguishable from authentic carbonohydroxamic acid ethyl ester[9] (R_f 0.72 in water-saturated butanol). The H enzyme has been completely separated in this laboratory from fluorokinase (F enzyme), the enzyme in heart extracts which has been shown by Flavin, Castro-Mendoza, and Ochoa[10] to catalyze the fluoride- and bicarbonate-dependent cleavage of ATP to ADP and fluorophosphate.[11] These investigators have proposed[10] that the F enzyme may activate carbon dioxide for propionyl CoA carboxylation.[12] As indicated in Table I, the H enzyme has no signi-

TABLE I

SEPARATION OF H AND F ENZYMES CATALYZING THE BICARBONATE-DEPENDENT CLEAVAGE OF ATP

The test system contained 500 μmoles of potassium bicarbonate, 10 μmoles of ATP, 2 μmoles of Versene, 50 μmoles of magnesium chloride, and, where indicated, crystalline pyrophosphatase[5] (0.3 mg. of protein), 10-fold purified H enzyme (2.3 mg. of protein) or 30-fold purified F enzyme (1.0 mg. of protein), and 200 μmoles of neutralized hydroxylamine or 300 μmoles of potassium fluoride, in a final volume of 3.0 ml. Incubation, 30 minutes at 38°. In control experiments, 500 μmoles of tris-(hydroxymethyl)-aminomethane buffer, *p*H 8.1, was substituted for bicarbonate.

Additions	μMoles orthophosphate formed[6]	μMoles pyrophosphate formed[7]
H enzyme + NH_2OH	0.04	0.76
H enzyme + NH_2OH + pyrophosphatase	1.50	0.02
H enzyme + KF	0	0
F enzyme + NH_2OH	0	0
F enzyme + KF	(1.97 μmoles of fluorophosphate[a])	

[a] Estimated colorimetrically[7] using an authentic sample of fluorophosphate as a standard. The absence of pyrophosphate was demonstrated by paper chromatography.

(1) Supported by grants from the United States Public Health Service (Grant No. A-993C) and the Michigan Memorial-Phoenix Project of the University of Michigan.

(2) M. J. Coon, *Federation Proc.*, **14**, 762 (1955).

(3) Abbreviations: adenosine triphosphate, ATP; adenosine diphosphate, ADP; adenylic acid, AMP; adenosine phosphoryl carbonate, $AMP\text{-}CO_2$; β-hydroxyisovaleryl coenzyme A, HIV CoA; β-hydroxy-β-methylglutaryl coenzyme A, HMG CoA.

(4) Assayed by the action of adenylic deaminase according to H. M. Kalckar, *J. Biol. Chem.*, **167**, 429 (1947).

(5) Kindly furnished by Dr. M. Kunitz.

(6) Estimated according to C. H. Fiske and Y. SubbaRow, *J. Biol. Chem.*, **66**, 375 (1925).

(7) Estimated according to R. M. Flynn, M. E. Jones and F. Lipmann, *J. Biol. Chem.*, **211**, 791 (1954).

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(9) L. W. Jones, *Am. Chem. J.*, **20**, 1 (1898).

(10) M. Flavin, H. Castro-Mendoza and S. Ochoa, *Biochim. et biophys. acta*, **20**, 591 (1956).

(11) This compound was incorrectly identified as pyrophosphate in an earlier report.[2] Fluorophosphate and pyrophosphate are readily distinguishable from orthophosphate but not from each other by colorimetric test[7] and by paper electrophoresis in citrate buffer, *p*H 9.0.

(12) M. Flavin, P. J. Ortiz and S. Ochoa, *Nature*, **176**, 823 (1955).