column. The combined ether solutions were evaporated to give 16.7 g. (71%) of white solid, m.p. 99–111°. This material had bands in the infrared at 3.30, 4.46, 5.84 and 6.08 μ. Four recrystallizations of a 5.0-g. sample of the product, m.p. 99–111°, from 1:6 methylene chloride–ether mixture gave 2.2 g. of VII, m.p. 120–121°. The infrared spectrum of VII showed absorption in the infrared at 4.46 and 5.84 μ but none at 3.30 and 6.08 μ. The ultraviolet spectrum of VII in acetonitrile showed no maxima above 215 mμ.

Anal. Calcd. for $C_{14}H_{12}N_4$: C, 71.16; H, 5.12; N, 23.72; mol. wt., 236. Found: C, 71.33; H, 5.13; N, 23.57; mol. wt., 227.

The crude product, m.p. 99–111°, was estimated to contain 80% of VII by intensity measurements of the 5.84 μ band in the infrared using 1,2-dichloroethane solvent and solutions of known concentration of pure VII as references.

Ozonolysis of VII.—Ozone was passed into a solution of 1.0 g. of VII in 50 ml. of absolute methanol at −80° until a blue color persisted (0.5 hour). While a slow stream of nitrogen was passed through the mixture, it was allowed to warm to room temperature over the course of 0.5 hour. To the methanolic suspension of ozonide was added 150 ml. of methanol and 0.25 g. of 10% palladium-on-charcoal. This mixture was shaken under 2.5 atm. of hydrogen for 0.25 hour and then filtered to remove the catalyst. When 200 ml. of this solution was distilled into a solution of 1.6 g. of 2,4-dinitrophenylhydrazine in 8 ml. of sulfuric acid, 12 ml. of water and 40 ml. of methanol, 0.75 g. (75%) of acetone 2,4-dinitrophenylhydrazone was obtained, m.p. 123.5–124.5°, alone or mixed with an authentic sample.

WILMINGTON 98, DELA.

[CONTRIBUTION FROM THE RESEARCH DEPARTMENT, CIBA PHARMACEUTICAL PRODUCTS, INC.]

Amphenone Analogs. III. Pinacol–Pinacolone Type Rearrangement in the Pyridine Series[1]

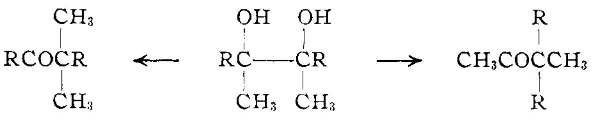
BY W. L. BENCZE AND M. J. ALLEN

RECEIVED JANUARY 12, 1959

The three isomeric acetylpyridines were reduced to the corresponding pinacols which in turn were rearranged.

During the last eight years Amphenone B, a pinacolone-type ketone, was widely investigated in numerous biological and clinical studies due to the striking endocrine effects exerted by this compound in animals as well as in man.[2]

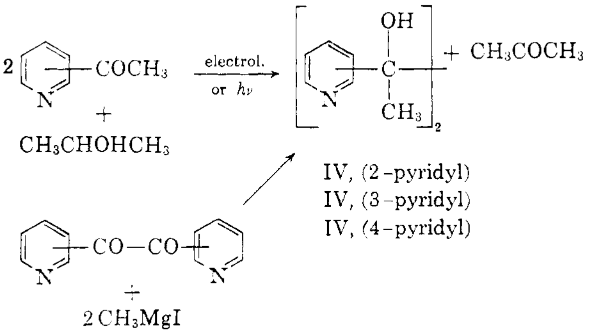
Amphenone B was prepared by subjecting the butanediol (I) (R = *p*-dimethylaminophenyl) to the pinacol–pinacolone rearrangement.[3] The *p*-aminophenyl group was observed to migrate exclusively, hence structure III recently has been assigned to Amphenone B.[4] The isomeric pinacolone II also has been prepared and was found to possess a narrower spectrum of endocrine activity.[1]



$$\underset{\text{II}}{\mathrm{RCO}\overset{\mathrm{CH_3}}{\underset{\mathrm{CH_3}}{\mathrm{CR}}}} \longleftarrow \underset{\text{I}}{\mathrm{R}\overset{\mathrm{OH}}{\underset{\mathrm{CH_3}}{\mathrm{C}}}\text{—}\overset{\mathrm{OH}}{\underset{\mathrm{CH_3}}{\mathrm{CR}}}} \longrightarrow \underset{\text{III}}{\mathrm{CH_3CO}\overset{\mathrm{R}}{\underset{\mathrm{R}}{\mathrm{C}}}\mathrm{CH_3}}$$

II I III

The present report deals with the pinacol–pinacolone rearrangement of the butanediols I (R = 2-, 3-, and 4-pyridyl) which gave the pyridyl analogs of Amphenone B. The three diols used were first prepared by bimolecular electrolytic reduction of the respective pyridyl ketones.[5] Subsequently it was found that these diols could also be prepared photochemically.



IV, (2-pyridyl)
IV, (3-pyridyl)
IV, (4-pyridyl)

The photochemical reduction[6] could be rendered more efficient by irradiating the samples with ultraviolet lamps rather than sunlight. The 2-pyridylbutanediol also was obtained by reaction of α-pyridyl with methylmagnesium iodide. Unfortunately the difficulty in obtaining the β- and γ-pyridil precluded investigation of this reaction in the 3- and 4-pyridyl-series. The 2-pyridylbutanediol thus obtained was not identical with the product obtained from the photochemical reduction of 2-acetylpyridine. No attempt has thus far been made to investigate the resolvability of these pinacols in order to assign to them the *meso* or racemic structure, respectively.

Concentrated sulfuric acid was used as the rearranging agent for all three isomeric butanediols IV. The rearrangement of IV (3-pyridyl) resulted in a binary mixture of pinacolones V and VI. Their separation was accomplished by chromatography on aluminum oxide, or by converting them to their oximes with subsequent fractional recrystallization and hydrolysis to the parent ketones. A partial separation also was achieved by distillation, whereby ketone V appeared in the first fractions followed by a distillate composed of the two com-

---

(1) Amphenone Analogs. II. W. L. Bencze, L. I. Barsky, M. J. Allen and E. Schlittler, *Helv. Chim. Acta*, **41**, 882 (1958).

(2) (a) M. J. Allen, R. Hertz and W. W. Tullner, *Proc. Soc. exp. Biol. Med.*, **74**, 632 (1950); (b) R. Hertz, W. W. Tullner, J. A. Schricker, F. G. Dhyse and L. F. Hallman, "Recent Progr. in Hormone Research," G. Pincus, ed., Academic Press, Inc., New York, N. Y., 1955, Vol. XI, p. 119; (c) O. Picinelli, *Farmaco (Pavia) Ed. Sci.*, **12**, 428 (1957); (d) H. Kless, *Arzneimittel-Forsch.*, **8**, 83 (1958).

(3) M. J. Allen and A. H. Corwin, THIS JOURNAL, **72**, 117 (1950).

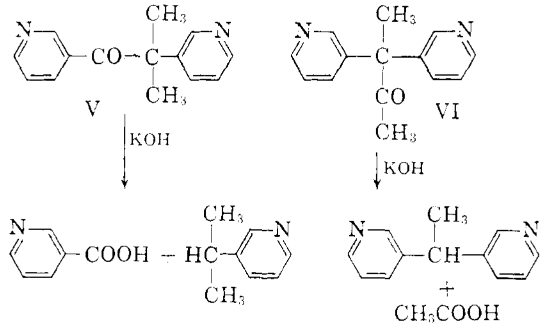
(4) (a) W. L. Bencze and M. J. Allen, *J. Org. Chem.*, **22**, 352 (1957); (b) J. Korman and E. C. Olson, *ibid.*, **22**, 870 (1957).

(5) (a) M. J. Allen, *ibid.*, **15**, 435 (1950); (b) M. J. Allen, *Can. J. Chem.*, **37**, 257 (1959); (c) M. J. Allen and H. Cohen, *J. Amer. Electrochem. Soc.*, **106**, 451 (1953).

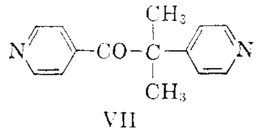
(6) W. E. Bachmann, "Organic Syntheses," Coll. Vol. II, John Wiley and Sons, Inc., New York, N. Y., 1943, p. 71.

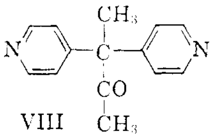
ponents V and VI in an equimolar ratio indicative of a stoichiometric azeotropic relationship of the two pinacolones in the vapor phase. Further investigation revealed that the isolation of ketone V could be accomplished readily by oxidation of the binary mixture with nitric acid, whereby ketone V remained unaffected and methyl ketone VI was oxidized.

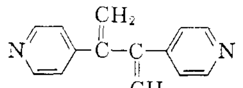
The composition of the mixture was found to be 68–72% of ketone V and 32–28% of ketone VI. Accordingly the intrinsic migratory aptitude[7] of the 3-pyridyl residue, when compared with the migration of the methyl group, was found to be 0.43 ± 0.04/1. Variation of the temperature in the range from 5 to 75° had no effect on the migratory aptitude.



Degradations of both isomeric pinacolones were undertaken to verify the assigned structures. Methanolic potassium hydroxide caused fission of pinacolone V yielding nicotinic acid and 2-(3-pyridyl)-propane. Under the same conditions pinacolone VI gave 1,1-bis-(3-pyridyl)-ethane and acetic acid.

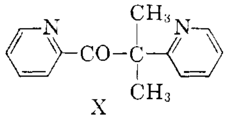

VII

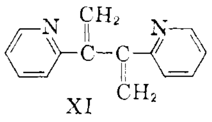

VIII

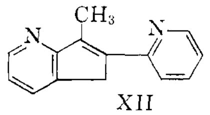

IX

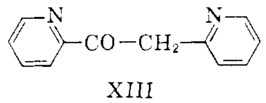
Rearrangement of the 4-pyridyl isomer of glycol IV gave ketones VII and VIII in approximately the same ratio as in the case of the 3-pyridyl isomers. In addition, the two ketones were accompanied by a third compound, which was identified as 2,3-bis-(4-pyridyl)-butadiene (IX). Catalytic hydrogenation of IX gave 2,3-bis-(4-pyridyl)-butane. Consequently, rearrangement and dehydration of the 4-pyridyl isomer of diol IV occurred simultaneously.

Rearrangement of the 2-pyridyl isomer of diol IV resulted in a complex mixture. An infrared absorption spectrum of the distilled reaction mixture indicated the absence of an unconjugated carbonyl absorption. Thus, the methyl ketone type pinacolone could not be isolated. Chromatographic separation of the reaction mixture yielded five components: the pinacolone X, and the butadiene XI, both showing ultraviolet absorption spectra comparable to the analogous 4-pyridyl isomers VII and IX. For the third compound isolated, structure XII, 6-(2-pyridyl)-7-methyl-5H-1-pyridine is proposed. Elemental analysis and identical boiling points of compounds XI and XII indicate that both of them possess the same empirical formula $C_{14}H_{12}N_2$. Ultraviolet and infrared absorption spectra are in agreement with the proposed structures of XI and XII.[8] A fourth compound was found to be identical with 2-picolyl 2-pyridyl ketone (XIII),[9a] which was synthesized according to the method of Goldberg, *et al.*[9b] In this instance the diol lost both methyl groups under the influence of concentrated sulfuric acid. The fifth compound isolated appeared to have lost one methyl group as indicated by elemental analysis. No structural formula has been assigned to this last compound.


X


XI


XII


XIII

2-Methyl-1,2-bis-(3-pyridyl)-1-propanone (V) (Su-4885), was found to possess distinctive effects on the adrenal cortex, similar to those exhibited by Amphenone B.[10]

Acknowledgment.—We wish to express our appreciation to Dr. E. Schlittler for his continued interest and encouragement throughout this project. We are grateful to Mr. L. Dorfman and his staff for the micro-analysis and the spectral data.

Experimental

General Method for the Bimolecular Pinacol Reduction.—A mixture of 10 g. of an acetylpyridine, 50 ml. of isopropyl alcohol and 0.5 ml. of acetic acid was irradiated by an ultraviolet lamp in a Pyrex test-tube at room temperature. After 2 to 4 days of irradiation the precipitated reduction product was collected and the filtrate irradiated for 2 more days. The photochemical reduction of 3-acetylpyridine requires a longer time or a more powerful light source. The solvent and unreacted acetylpyridines were removed under reduced pressure and the crystalline 2,3-di-(pyridyl)-2,3-butandiols washed with cold ethanol and ether. The compounds were

---

[7] G. W. Wheland, "Advanced Organic Chemistry," John Wiley and Sons, Inc., New York, N. Y., 1949, p. 513.

[8] Compound XII and 1,2-bis-(2-pyridyl)-ethylene showed maximal absorption at 312–313 mμ, due to a stilbene-type double bond. Compound XII showed a very strong band in its infrared absorption spectrum at 790 cm.$^{-1}$ for the aromatic out-of-plane deformation frequency, indicative of a 2,3-disubstituted pyridine nucleus, while compound XI, possessing two monosubstituted pyridyl groups, exhibited two bands of equal medium intensity at 790 and 750 cm.$^{-1}$ (compare: H. Shindo and N. Ikekava, *Pharmaceutical Bull.* (*Japan*), **4**, 192 (1956)). In addition, compound XI, a substituted butadiene, showed a strong absorption band for a $=CH_2$ group at 925 cm.$^{-1}$ and its overtone at 1850 cm.$^{-1}$ (L. J. Bellamy, "The Infrared Spectra of Complex Molecules," Methuen and Co., London, 1954, p. 31). Neither of these two bands was present in the infrared absorption spectrum of XII.

[9] (a) J. P. Wibaut and J. I. de Jong, *Rec. trav. chim.*, **68**, 485 (1949); (b) N. N. Goldberg, L. B. Barkley and R. Levine, THIS JOURNAL, **73**, 4301 (1951).

[10] J. J. Chart, H. Sheppard, M. J. Allen, W. L. Bencze and R. Gaunt, *Experientia*, **14**, 151 (1958).

recrystallized from a mixture of ethanol and water or ethylene glycol and water.

Pinacols (IV) from	2-Acetylpyridine	3-Acetylpyridine[a]	4-Acetylpyridine
M.p., °C.	140–141,[b] 136–137[c]	244–245[11]	209–211
Anal. Calcd. for $C_{14}H_{16}N_2O_2$:	C, 68.83; H, 6.60	. .	C, 68.83; H, 6.60
Found:	(b) C, 69.08; H, 6.73 (c) C, 68.87; H, 6.59		C, 69.09; H, 6.70
Yield, %	(b) 44, (c) 58	42	78

[a] Identical infrared absorption spectra were obtained from both preparations made by electrochemical[5a] or photochemical reductions. [b] This higher melting form was obtained from photochemical reduction. Mixture melting point with a sample obtained *via* electrolytic reduction (m.p. 138–139°)[5c] showed no depression. [c] This lower melting form was obtained by dropwise addition of 5 g. of α-pyridil in 35 ml. of benzene to a Grignard solution made from 7.9 g. of methyl iodide and 1.35 g. of magnesium in 50 ml. of ether. The mixture was refluxed for two hours and worked up in the usual way. The mixture melting point of the two different forms was 108–110°.

General Method for the Pinacol–Pinacolone Rearrangement.—Each experiment was carried out in the following way. The pinacol was dissolved portionwise in concentrated sulfuric acid and the acidic solution kept at temperatures for periods of time given individually for each of the pinacols. The sulfuric acid solution then was cooled to room temperature and poured onto ice. The aqueous, acidic solution was rendered slightly basic (approximately *p*H 8) with 50% aqueous sodium hydroxide and crystalline sodium carbonate. The pinacolones and other reaction products were extracted three times with ether or ethyl acetate. The combined extracts were washed twice with saturated sodium chloride solution, dried over anhydrous sodium sulfate, filtered and evaporated to dryness under reduced pressure.

Rearrangement of 2,3-Bis-(3-pyridyl)-2,3-butanediol.—The crude diol (3.43 g.) was dissolved in 25 ml. of concentrated sulfuric acid prewarmed to 50°. This solution was kept at 75–76° for 7 hours and at room temperature for an additional 15 hours. Distillation of the crude product at 140–160° (0.07 mm.) gave 1.86 g. of a straw-colored oil which evidently was a mixture of V and VI. Its infrared spectrum showed a strong carbonyl band at 1685 cm.$^{-1}$ and a weaker carbonyl peak at 1705 cm.$^{-1}$. Elemental analysis of this mixture agreed with the empirical formula $C_{14}H_{14}N_2O$ (yield 58%).

Anal. Calcd. for $C_{14}H_{14}N_2O$: C, 74.30; H, 6.25; N, 12.37. Found: C, 74.55; H, 6.38; N, 12.44.

This rearrangement also was carried out at 22° for 20 hours and at 5° for 4 days. The reaction product thus obtained was distilled and the areas under the two carbonyl peaks of the infrared spectra were measured. By the aid of similar measurements on five known mixtures of spectroscopically pure samples of ketones V and VI, the ratio of these two pinacolones as obtained from the rearrangements was found to be 68–72% of ketone V and 28–32% of ketone VI. This ratio was not influenced by the temperature in the range from 5 to 76°.

Separation of the Isomers V and VI by Oxime Formation.—An aqueous solution (60 ml.) of 12 g. of hydroxylamine sulfate buffered with 5 g. of sodium carbonate monohydrate and 10 g. of crystalline sodium acetate was added to a solution of 10.5 g. of the above distilled mixture in 100 ml. of 95% ethanol.

After refluxing the reaction mixture for 5 hours most of the ethanol was removed under reduced pressure, water added and the *p*H adjusted to approximately 8 with crystalline sodium carbonate. Extraction with ethyl acetate and concentration of the washed and dried extracts gave a crystalline precipitate, which was collected and dried. The crude material (m.p. 163–169°) weighed 5.6 g. (54%). After recrystallization from ethanol, it melted at 175–177°.

Anal. Calcd. for $C_{14}H_{15}N_3O$: C, 69.80; H, 6.28; N, 17.43. Found: C, 69.79; H, 6.39; N, 17.40.

Five grams of this oxime was refluxed in 100 ml. of 4 *N* concentrated sulfuric acid for 24 hr. The reaction mixture was rendered slightly basic and extracted three times with ether. The combined ethereal extracts yielded an oil which crystallized spontaneously. Distillation at 130–140° (0.05 mm.) and recrystallization from hexane gave 3.95 g. of ketone V, m.p. 51–52°, with infrared carbonyl peak at 1685 cm.$^{-1}$ (yield 84%).

Anal. Calcd. for $C_{14}H_{14}N_2O$: C, 74.30; H, 6.25; N, 12.37. Found: C, 74.25; H, 6.24; N, 12.39.

Proof for the structure of V was obtained in the following way. Two grams of the ketone was refluxed in 50 ml. of methanol containing 10 g. of potassium hydroxide for 30 hours. On allowing the mixture to cool to room temperature, the crystals separating were collected and washed twice with methanol and ether, wt. 760 mg. After dissolving this salt in 3 ml. of water and neutralizing with 2 *N* hydrochloric acid, the precipitated crystals were collected, washed with cold water and dried; m.p. and mixed m.p. with an authentic sample of nicotinic acid was 232–233°.

The methanol filtrate was evaporated to dryness under reduced pressure, the residue was dissolved in water and extracted five times with ether. The combined ether extracts yielded a colorless oil. This was distilled at 110–130° (100 mm.), yield 510 mg. (48%). The picrate melted at 138–139°. The picrate of 2-(3-pyridyl)-propane is reported[11] to melt at 138.1–138.6°.

Anal. Calcd. for $C_8H_{11}N \cdot C_6H_3N_3O_7$: C, 48.00; H, 4.03. Found: C, 48.03; H, 4.20.

Isomeric Ketone VI.—From the filtrate of the above oxime 4.37 g. of semi-solid material was isolated on concentration. Repeated recrystallizations from ethyl acetate and pentane or benzene gave the oxime of the isomeric ketone VI **3,3-bis-(3-pyridyl)-2-butanone oxime**, m.p. 146–148°.

Anal. Calcd. for $C_{14}H_{15}N_3O$: C, 69.80; H, 6.28; N, 17.43. Found: C, 69.51; H, 6.31; N, 17.67.

A melting point of a mixture of this oxime with the oxime first isolated was 126–140°. Hydrolysis of 5 g. of this oxime, under the same conditions as above, yielded 4.18 g. of the distilled ketone VI (89%), which showed one infrared carbonyl peak at 1705 cm.$^{-1}$ and after recrystallization from ether and pentane melted at 46–48°. A mixture m.p. of this ketone VI with ketone V (m.p. 51–52°) gave a depression of 22°.

Anal. Calcd. for $C_{14}H_{14}N_2O$: C, 74.30; H, 6.25; N, 12.37. Found: C, 74.18; H, 6.27; N, 12.11.

To develop chemical proof for the structure of VI: 2 g. of the ketone was refluxed for 25 hr. in 50 ml. of methanol containing 10 g. of potassium hydroxide. The solvent was removed under reduced pressure; the residue was dissolved in water and extracted three times with ether. The combined ether extracts yielded a colorless oil. Distillation at 110° (0.05 mm.) yielded 1.3 g. of 1,1-bis-(3-pyridyl)-ethane (yield 78%).

Anal. Calcd. for $C_{12}H_{12}N_2$: C, 78.23; H, 6.57; N, 15.21. Found: C, 77.94; H, 6.48; N, 15.44.

Rearrangement of the Pinacol, 2,3-Bis-(4-pyridyl)-2,3-butanediol.—The pinacol (23.7 g.) was rearranged as given in the description of the general method. Temperature and reaction time were 63–68° and 18 hours, respectively. A first crop of the reaction product (fraction A, 2.9 g., m.p. 155–178°) was obtained when the ethyl acetate extract was concentrated to 100 ml. A second crop (fraction B, 5.2 g., m.p. 127–134°) was obtained when the ethyl acetate residue was triturated with ether. Complete evaporation of this ether left an oil which solidified at 0° (fraction C, 7 g.).

Fraction B was recrystallized from ethyl acetate and pentane (4.32 g.) and for analysis this material was recrystallized from aqueous ethanol, m.p. 135–136°. The ultraviolet absorption max. was at 234–235 mμ (ε 18,200) which is in agreement with 2,3-bis-(4-pyridyl)-1,3-butadiene (IX).

Anal. Calcd. for $C_{14}H_{12}N_2$: C, 80.74; H, 5.82; N, 13.45. Found: C, 80.38; H, 5.75; N, 13.64.

2,3-Bis-(4-pyridyl)-butane.—The above butadiene (420 mg.) in 20 ml. of 0.5 *N* hydrochloric acid was hydrogenated at room temperature and atmospheric pressure in the presence of 200 mg. of platinum catalyst. The calculated amount of two moles of hydrogen was absorbed in 90 minutes. After the hydrogen uptake had dropped from 1 ml./min. to 0.3 ml./min., the hydrogenation was discontinued. The catalyst was filtered off and the filtrate neutralized with

---

(11) H. C. Brown and W. A. Murphey, THIS JOURNAL, **73**, 3308 (1951).

2 *N* sodium hydroxide solution. The crystalline product which separated was collected, washed with water, dried (280 mg.) and recrystallized from benzene and hexane, m.p. 135–136°. The melting point of a mixture of this hydrogenation product and the starting material was 105–124°.

Anal. Calcd. for $C_{14}H_{16}N_2$: C, 79.21; H, 7.60; N, 13.20. Found: C, 79.42; H, 7.82; N, 13.20.

Fraction C was distilled at 120–140° (0.2 mm.) and weighed 5.6 g. Half of this oily mixture was chromatographed on 120 g. of aluminum oxide (Woelm, basic, activity 3). Elution with a mixture of hexane and benzene (2:3) gave a crystalline compound, which on twice recrystallizing from benzene and pentane melted at 77.5–78.5° and was 1,2-bis-(4-pyridyl)-2-methyl-1-propanone (VII). Its infrared spectrum showed one carbonyl peak at 1685 cm.$^{-1}$.

Anal. Calcd. for $C_{14}H_{14}N_2O$: C, 74.30; H, 6.25; N, 12.37. Found: C, 74.00; H, 6.17; N, 12.51.

Further elution of the aluminum oxide column with benzene and a benzene–ether (9:1) mixture gave 400 g. of an additional crystalline compound. For analysis it was twice recrystallized from ether and pentane and subsequently distilled. It had one carbonyl peak at 1705 cm.$^{-1}$ and was the structural isomer, 3,3-bis-(4-pyridyl)-2-butanone (VIII), m.p. 76–77°.

Anal. Calcd. for $C_{14}H_{14}N_2O$: C, 74.30; H, 6.25. Found: C, 74.03; H, 6.04.

Rearrangement of the Pinacol 2,3-Bis-(2-pyridyl)-2,3-butanediol.—The pinacol was rearranged as described under the general method. Temperature and reaction time were 160° and 9 hours, respectively. In another experiment the rearrangement was carried out at 160° for 2 hours and then the temperature was raised to 230° for 2 hours. The oily mixture, obtained by evaporation of the ethyl acetate extract, turned dark at room temperature; storage at −8° prevented discoloration. In the experiment carried out at 160°, from 5.5 g. of pinacol 2.2 g. of distilled oily product (b.p. 100–140° (0.03 mm.)) was obtained. In the second experiment at 230°, from 33 g. of pinacol only 6.02 g. of distilled yellow product was isolated. Five grams of this yellow, viscous liquid was chromatographed on 200 g. of aluminum oxide (Woelm, basic, activity 3). Hexane–benzene mixtures 4:1 and 3:1 eluted a crystalline compound. This was recrystallized several times from ether and pentane and hexane alone and then melted at 88–90°. It had a carbonyl peak at 1690 cm.$^{-1}$, and was 1,2-bis-(2-pyridyl)-2-methyl-1-propanone (X).

Anal. Calcd. for $C_{14}H_{14}N_2O$: C, 74.30; H, 6.25. Found: C, 74.78; H, 6.19.

A hexane–benzene mixture 1:1 eluted a compound which was distilled at 120–130° (0.03 mm.) and recrystallized twice from benzene and pentane. It melted at 110–112° and had an ultraviolet max. at 249–250 mμ (ε 3,900). No structure has been assigned to this compound.

Anal. Calcd. for $C_{13}H_{10}N_2$: C, 80.38; H, 5.19; N, 14.42. Found: C, 80.62; H, 5.11; N, 14.43.

A benzene–ether mixture 9:1 eluted another crystalline compound from the above column, which was recrystallized twice from ether and pentane and distilled at 110–112° (0.03 mm.). It melted at 66–67°, had an ultraviolet max. at 312–313 mμ (ε 24,690) (ethanol) and the structure 6-(2-pyridyl)-7-methyl-5H-1-pyridine (XII) is proposed for it.

Anal. Calcd. for $C_{14}H_{12}N_2$: C, 80.74; H, 5.81; N, 13.45. Found: C, 80.72; H, 5.71; N, 13.58.

A benzene–ether mixture 1:1 eluted a third compound from the above column which distilled at 120–130° (0.03 mm.) and which was recrystallized from benzene. It formed yellow prisms melting at 86–87.5° and was 2-pyridyl 2-picolyl ketone (XIII).

Anal. Calcd. for $C_{12}H_{10}N_2O$: C, 72.71; H, 5.09; N, 14.12. Found: C, 73.04; H, 4.87; N, 13.82.

This compound was synthesized according to the procedure given by Goldberg, *et al.*,[9] and the two compounds were found to be identical. Their infrared absorption spectra were identical and a mixture of the two samples melted undepressed at 86–88°.

From the rearrangement experiment carried out in sulfuric acid at 160° for 9 hr. a 920-mg. sample of the distilled reaction product was chromatographed as above. Benzene alone eluted 140 mg. of a compound which on recrystallization from a mixture of benzene and hexane melted at 65–66°. The ultraviolet spectrum showed maximal absorption at 231–233 mμ (ε 17,410). The analytical data correspond with structure XI, 2,3-bis-(2-pyridyl)-1,3-butadiene.

Anal. Calcd. for $C_{14}H_{12}N_2$: C, 80.74; H, 5.81. Found: C, 80.43; H, 5.86.

SUMMIT, N. J.

[CONTRIBUTION FROM THE MALLINCKRODT LABORATORIES OF HARVARD UNIVERSITY]

Isotope Effects in the Enzymatic Decarboxylation of Oxalacetic Acid

BY STANLEY SELTZER,[1a] GORDON A. HAMILTON[1b] AND F. H. WESTHEIMER

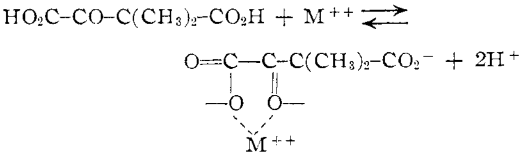
RECEIVED NOVEMBER 12, 1958

$HO_2C-CO-CH_2-C^{13}O_2H$ has been prepared and decarboxylated in the presence of metal ions and in the presence of the partially purified enzyme from *Micrococcus lysodeikticus*. The reaction catalyzed by Mn^{++} at 10° showed an isotope effect, k^{12}/k^{13}, of nearly 1.06, wheras the ratio for the enzymatic reaction was 1.00. Further, the enzymatic decarboxylation proceeds less rapidly in D_2O than in H_2O, whereas the rate for the metal ion-promoted reaction is unaffected by this change in solvent. These facts show that the carbon–carbon bond scission is the rate-controlling process for the non-enzymatic reaction but not for that promoted by the enzyme. The actual rates make it probable that the carbon–carbon bond cleavage for the enzymatic reaction exceeds that for the non-enzymatic reaction by a factor of the order of 10^8.

In 1941, Krampitz and Werkman[2] isolated from the aerobic bacteria, *Micrococcus lysodeikticus*, a thermolabile enzyme which catalyzes the decarboxylation of oxalacetic acid

$$HO_2C-CO-CH_2-CO_2H \longrightarrow HO_2C-CO-CH_3 + CO_2 \quad (1)$$

A metal ion, preferably Mn^{++} or Mg^{++}, is required[2] as cofactor. Later, Krebs[3] observed that various cations (*e.g.*, Mn^{++}, Cu^{++}, Al^{+++}) catalyze the decarboxylation in the absence of enzyme. Subsequently, the mechanism of the metal ion-catalyzed decarboxylation of dimethyloxalacetic acid was shown[4] to proceed by the steps



$$HO_2C-CO-C(CH_3)_2-CO_2H + M^{++} \rightleftarrows \underset{\underset{M^{++}}{-O\ \ O-}}{O{=}C{-}C{-}C(CH_3)_2{-}CO_2^-} + 2H^+ \quad (2)$$

---

(1) (a) Du Pont Predoctoral Fellow in Chemistry, 1956–1957. (b) Holder of National Research Council of Canada Special Scholarship, 1957–1959.

(2) L. O. Krampitz and C. H. Werkman, *Biochem. J.*, **35**, 595 (1941).

(3) H. Krebs, *ibid.*, **36**, 303 (1942).

(4) R. Steinberger and F. H. Westheimer, THIS JOURNAL, **71**, 4158 (1949); **73**, 429 (1951).