above. Five minutes were allowed for the establishment of thermal equilibrium, and the length of the emergent stem of the floating hydrometer was then measured. The density of the liquid fluorine, calculated from a two-constant interpolation formula, was 1.56 g./cc. The reading did not change with time, nor after agitation. As a further verification of the accuracy of the hydrometer, it was removed from the apparatus and placed in a phosphoric acid solution of such concentration that the hydrometer floated at the same level as in the liquid fluorine; the density of this solution as determined in a pycnometer was 1.554 g./cc. The sensitivity of the hydrometer in the density range of interest was determined to be 0.008 g./cc. for a millimeter change in emergent stem length.

After the application of a 1% correction for the difference between the surface tensions of the fluorine and the calibration liquids, and assuming a possible uncertainty in the cathetometer reading of ±1 mm., the density of liquid fluorine at −196° was computed to be 1.54 g./cc. with an estimated uncertainty of 0.02 g./cc.

A second sample of fluorine from a cylinder which had been stored for approximately one year, and hence presumably was not from the same lot, was analyzed as 98.6% pure by the method of Kimball and Tufts.[3] This sample gave values of the density identical with that reported above.

A third sample of fluorine was condensed directly from an electrolytic cell after passage over potassium fluoride pellets to remove the major portion of the hydrogen fluoride. The density, measured with a smaller hydrometer and with less precision than before, was found, after a 2% correction for surface tension effects, to be 1.51 ± 0.04 g./cc.

It was observed that a white flocculent precipitate was present in all the samples of liquefied fluorine. This material was volatile at room temperature, hence it may have been hydrogen fluoride. The flocculent solid had no apparent effect upon the hydrometer readings inasmuch as the same readings were obtained before and after careful stirring to dislodge precipitate which had settled on the hydrometer bulb.

Acknowledgment.—The financial support of this work by the Office of Naval Research is gratefully acknowledged. The authors also wish to thank Mr. J. B. Hatcher of the Jet Propulsion Laboratory, California Institute of Technology, for assistance with portions of this work.

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Preparation of 2-Methyl-5-*t*-butyl-3-carbethoxyfuran during the Hydrolysis of Ethyl α-Pinacolonylacetoacetate

BY NICHOLAS MESSINA AND ELLIS V. BROWN

During the course of our investigation of the Friedel–Crafts reaction in the thiophene series,[1] it became necessary to prepare 6,6-dimethylheptanedione-2,5 by the hydrolysis and decarboxylation of ethyl α-pinacolonylacetoacetate. The hydrolysis of this substituted ethyl acetoacetate gave some unexpected results when the hydrolyzing agent was boiling 20% potassium carbonate solution. Youtz and Perkins[2] have shown that the above-mentioned reagent could be used to prepare 3-methylacetonylacetone from ethyl α,β-diacetylbutyric ester. Hughes and co-workers[3] employed a similar procedure to prepare heptanedione-2,5 from ethyl α-acetyl-β-propionylpropionate. Rodionov[4] prepared acetonylacetone from ethyl α-acetylacetoacetate by this method. When 20% potassium carbonate solution was used in the hydrolysis of ethyl α-pinacolonylacetoacetate, 2-methyl-5-*t*-butyl-3-carbethoxyfuran was produced. The structure of this ester was established by alkylating the known 2-methyl-3-carbethoxyfuran[5] with *t*-butyl chloride according to the method of Gilman.[6] The resulting ester and that obtained by ring closure with potassium carbonate were hydrolyzed to the corresponding acid (2-methyl-5-*t*-butyl-3-furancarboxylic acid). A mixture of the two samples showed no depression of melting point.

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Experimental

2-Methyl-5-*t*-butyl-3-carbethoxyfuran.—Ninety-nine grams (0.43 mole) of ethyl α-pinacolonylacetoacetate was refluxed for six hours with a solution containing 80 g. of potassium carbonate in 320 ml. of water. After cooling, the reaction mixture was extracted with ether, washed with water and dried over anhydrous sodium sulfate. Rectification yielded 54 g. (60%) of 2-methyl-5-*t*-butyl-3-carbethoxyfuran, b.p. 75–77° (1 mm.).

Anal. Calcd. for $C_{12}H_{18}O_3$: C, 68.57; H, 8.57. Found: C, 68.41; H, 8.69.

Hydrolysis of the furan ester with 15% aqueous potassium hydroxide resulted in 2-methyl-5-*t*-butyl-3-furancarboxylic acid, m.p. 95°, after crystallization from alcohol–water.

Anal. Calcd. for $C_{10}H_{14}O_3$: C, 65.93; H, 7.69. Found: C, 66.14; H, 7.84.

2-Methyl-5-*t*-butyl-3-furancarboxylic Acid by Friedel–Crafts Reaction.—2-Methyl-3-carbethoxyfuran, 15.4 g. (0.1 mole) and 13.7 g. (0.1 mole) of *t*-butyl bromide were added dropwise over a 25-minute period to 19.1 g. (0.15 mole) of aluminum chloride and 200 ml. of carbon disulfide contained in a three-necked flask cooled by an ice-bath. A dark viscous mass separated making stirring impossible. When the addition was complete, the ice-bath was removed and the mixture allowed to stand for 24 hours. The reaction product was poured on cracked ice with vigorous stirring, the solvent separated, and the aqueous layer extracted with ether. The ether and carbon disulfide solutions were combined, washed with water, 10% sodium carbonate and dried over sodium sulfate. Fractionation yielded 13.4 g. (54%) of 2-methyl-5-*t*-butyl-3-carbethoxyfuran, b.p. 80–81° (2 mm.).

Ten grams (0.04 mole) of 2-methyl-5-*t*-butyl-3-carbethoxyfuran was refluxed with a 15% aqueous solution of potassium hydroxide. Isolation of the acid in the usual manner yielded, after recrystallization from an alcohol–water mixture, 7.5 g. (85%) of 2-methyl-5-*t*-butylfuran-3-carboxylic acid, m.p. 95°. A mixed melting point with a sample obtained from the hydrolysis of the ester derived from ethyl α-pinacolonylacetoacetate showed no depression.

Anal. Calcd. for $C_{10}H_4O_3$: C, 65.93; H, 7.69. Found: C, 66.20; H, 7.65.

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Additional *o*-Phenylenediamines Tested for Vitamin B_{12} Activity

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Since the discovery of the presence of 5,6-dimethylbenzimidazole in acid hydrolysates of

vitamin B_{12},[1,2] it has been shown that certain substituted benzimidazoles and *o*-phenylenediamines possess vitamin B_{12}-like activity. The vitamin B_{12}-like activities of 5,6-dimethylbenzimidazole and 4,5-dimethyl-1,2-diaminobenzene have been assayed by Emerson and her co-workers[3] through measurement of their growth-eliciting responses in vitamin B_{12}-deficient rats. One other substituted benzimidazole of those tested, 5-methylbenzimidazole, had significant activity.

The purpose of this investigation was to assay three newly prepared substituted *o*-phenylenediamines: 4-ethyl-1,2-diaminobenzene, 4-chloro-5-methyl-1,2-diaminobenzene and 4,5-diethyl-1,2-diaminobenzene for vitamin B_{12}-like activity. The syntheses of the first two compounds are described below. One other compound, 4-methyl-1,2-diaminobenzene, was tested because its corresponding benzimidazole, 5-methylbenzimidazole, was shown to have activity.[3] It was of interest to determine whether these compounds would show activity either in promoting or inhibiting growth in vitamin B_{12}-deficient rats.

Experimental

Preparation of Compounds.[4]—The preparation of 4-methyl-1,2-diaminobenzene and 4,5-diethyl-1,2-diaminobenzene has been described.[5]

4-Ethyl-1,2-diaminobenzene.—*p*-Ethylaniline,[6] 30 g., was carbethoxylated and nitrated to 2-nitro-4-ethylcarbethoxyanilide,[7] 32.6 g. (58%). The urethan, 10 g., was hydrolyzed as described[5] except that immediately following digestion, 100 ml. of water was added and the mixture placed in the refrigerator. Following recrystallization from *n*-hexane the 2-nitro-4-ethylaniline, 5.4 g., (77%) was obtained as orange prisms, m.p. 46–48°.[8] The 2-nitro-4-ethylaniline, 2.0 g., was reduced in 60 ml. of ethyl alcohol with 0.05 g. of platinum oxide at initial hydrogen pressure of 55 p.s.i. The solvent was removed under an atmosphere of hydrogen and the diamine recrystallized from *n*-hexane to produce 1.3 g. (79%) of white plates, m.p. 59–60°. The material sublimes at 115° and above at atmospheric pressure.

Anal. Calcd. for $C_8H_{12}N_2$: C, 70.5; H, 8.9; N, 20.6. Found: C, 70.8; H, 9.0; N, 20.6.

4-Chloro-5-methyl-1,2-diaminobenzene (a) From *o*-Chlorotoluene.—*o*-Chlorotoluene, 254 g. (2.0 moles), was nitrated[9] to yield 368 g. (95%) of material which distilled at 124–127° (11 mm.). The nitration product, 86 g., was reduced in 750 ml. of concentrated hydrochloric acid by the portionwise addition of 85 g. of iron filings. Two such batches were combined, made strongly alkaline, and steam distilled. The product which distilled 114–122° (15 mm.) weighed 128–136 g. (90–96%). Recrystallization from *n*-hexane produced only 42–45 g. (29–32%) of pure material m.p. 83°, which distilled 133–136° (23 mm.). The 3-methyl-4-chloroaniline, 167 g. (m.p. 83°), produced 170 g. (78%) of the acetanilide m.p. 90–92°. The acetanilide, 92 g., was nitrated[10] to produce 44 g. (39%) of 2-nitro-4-chloro-5-methylacetanilide, m.p. 114–115°. This material, 20 g., was hydrolyzed,[10] and the nitroaniline recrystallized from *n*-hexane to yield 14.2 g. (87%) of orange prisms, m.p. 160.5°. The nitroaniline, 2.0 g., was reduced catalytically as above, recrystallized from *n*-hexane to produce 1.3 g. (78%) of light tan plates m.p. 145°. The diamine sublimes at 78° at 3 mm. to produce a slightly lighter colored product m.p. 146.5°.

Anal. Calcd. for $C_7H_9N_2Cl$: C, 53.7; H, 5.8; N, 17.9; Cl, 22.6. Found: C, 54.0; H, 6.0; N, 18.2; Cl, 22.3.

(b) **From 2-Chloro-4-nitrotoluene.**—The crude 2-chloro-4-nitrotoluene prepared as described[11] from 274 g. (2.0 moles) of *p*-nitrotoluene was dried and distilled at 122–124° (13 mm.) (260° (750 mm.)) to produce 337 g. (96%) of the product m.p. 65° after recrystallization from dilute alcohol. The 2-chloro-4-nitrotoluene, 85.5 g., was reduced with iron filings in 22% hydrochloric acid to yield 63 g. (89%) of 3-chloro-4-methylaniline which distilled 113–114° (12 mm.). The aniline, 61.5 g., yielded 64.7 g. (81%) of acetanilide m.p. 85° when recrystallized from dilute alcohol and 104–105° when *n*-hexane was used.[12] The acetanilide, 55.2 g. (0.3 mole), was nitrated as above to yield 45.2 g. (66%) of 2-nitro-4-methyl-5-chloroacetanilide, m.p. 116°. The nitroacetanilide, 10 g., was hydrolyzed as above, recrystallized from *n*-hexane to yield 7.4 g. (91%) of 2-nitro-4-methyl-5-chloroaniline m.p. 167–168° as orange prisms. The nitroaniline, 0.80 g., reduced catalytically as above yielded 0.60 g. (89%) of 4-chloro-5-methyl-1,2-diaminobenzene in the form of light tan plates m.p. 143–144°. This material when mixed with that prepared by procedure (a), m.p. 144°.

Biological Assay.—Female weanling rats of the Wistar strain, with an average weight of 44 g., were placed on a soybean meal diet the same as Emerson's diet 282[13,14] except that sucrose was used instead of dextrose. The amounts of micronutrients[15] were also the same as those used by Emerson.

At the end of the depletion period the animals were divided into six groups to give in each group an even distribution of animals according to the deviation of weight gain of each from the mean value.

The crystalline vitamin B_{12}[15] and *o*-phenylenediamines were administered by stomach tube in 0.5 ml. of 6% gum acacia solution. The animals in the negative control group were also given 0.5 ml. of gum acacia solution daily. It was found that these four *o*-phenylenediamines began to undergo air oxidation on standing several minutes in solution or suspension and, therefore, ascorbic acid at the level of 1.0 mg./ml. of gum acacia solution was introduced as an antioxidant. Fresh solution of the *o*-phenylenediamines had to be prepared daily, however.

TABLE I

Substance	No. of rats	Quantity fed daily	Wt. gain, g./15 days	*p* value
Negative controls	7	.....	3	....
Vitamin B_{12}	10	0.126 mcg.	23	<0.001
4-Methyl-1,2-diaminobenzene	9	2.0 mg.	10	.11
4-Ethyl-1,2-diaminobenzene	10	2.0 mg.	7	.30
4,5-Diethyl-1,2-diaminobenzene	9	2.0 mg.	7	.35
4-Chloro-5-methyl-1,2-diaminobenzene	10	2.0 mg.	10	.11

The weight gains for these animals during the supplementation period (Table I) were less than those reported by Emerson. This may have been due to the difference in the strain and sex.[16] The groups receiving 4-ethyl-1,2-diaminobenzene and 4,5-diethyl-1,2-diaminobenzene showed weight gains of over twice that of the negative controls, while those groups receiving 4-methyl-1,2-diaminobenzene and 4-chloro-5-methyl-1,2-diaminobenzene showed weight

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(15) The vitamin K_1 and vitamin B_{12} were generous gifts of Merck and Co.

(16) References 3 and 13 did not record the strain of these animals.

gains of three times that of the controls. The weight gain of the vitamin B_{12} group was almost eight times that of the negative control group. An analysis of the data showed that the increased weight gain of the B_{12} control over that of the negative control group was highly significant, but that the increased weight gains of the experimental groups over that of the negative control group could not be considered significant due to the wide variation in responses of the individual animals to their particular regimen. None of the *o*-phenylenediamines investigated here were found to possess vitamin B_{12} activity or inhibitor properties.

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The Deamination of Crystalline Egg Albumin. IV. Side Reactions

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It is well known that during the deamination of proteins by nitrous acid other reactions occur in addition to the removal of free amino groups. In the reports from this Laboratory on the deamination of crystalline egg albumin (Ea)[2-4] discussion of the side reactions other than oxidation of -SH to -S-S- was inadvertently omitted. While conditions may be chosen, as in this study,[2] so that oxidation and the introduction into the tyrosine and phenylalanine residues of chromophoric -NO and diazo groups are minimal[5-7] attempts were made to arrive at information on the extent of these reactions under the conditions chosen.

Results and Discussion

(1) **-SH and -S-S- Groups.**—Except in instances in which it was specifically desired to study the -S-S- form of the deaminated protein, all products were routinely reduced with thioglycolic acid.[2] The effects of thioglycolic acid on the viscosity and nitroprusside tests of the partially deaminated fraction were discussed in the previous papers.

(2) **Folin Colors.**—The Folin-Ciocalteau phenol reagent[8] was employed to detect changes in the reducing power of the deaminated Ea fractions. The introduction of -NO and diazo groups often alters the Folin color, but such effects as are noted are difficult to evaluate quantitatively.[6,7] In the case of the deaminated Ea fractions no detectable alteration in Folin color was observed (Fig. 1).

(3) **Color of Deaminated Preparations.**—Deaminated proteins in which substitution has occurred have colors ranging from light yellow to yellow-red depending upon the extent of the secondary reactions.[6,7,9,10] The deaminated Ea preparations described in the previous papers were straw yellow in color.

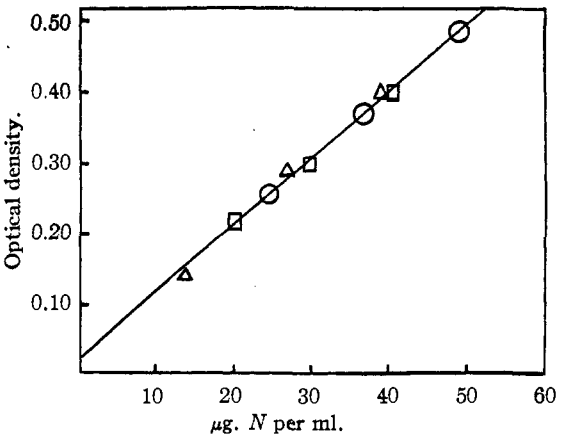

Fig. 1.—Folin colors: O, Ea; □, 3A; Δ, 3B.

For comparison tyrosine was deaminated as was crystalline Ea in acetate buffer at *p*H 4.0 with 1 *M* $NaNO_2$ at 0° for 8 hours.[2] The deaminated and partially substituted tyrosine, which was deep yellow in color, gave the absorption indicated in Fig. 2 when measured at 25° with a Beckman model DU spectrophotometer. Not only had the absorption peak shifted from 285 to 265 mμ, but there was a decrease in absorption at 285 mμ and appearance of a new absorption peak at 400 mμ.[6]

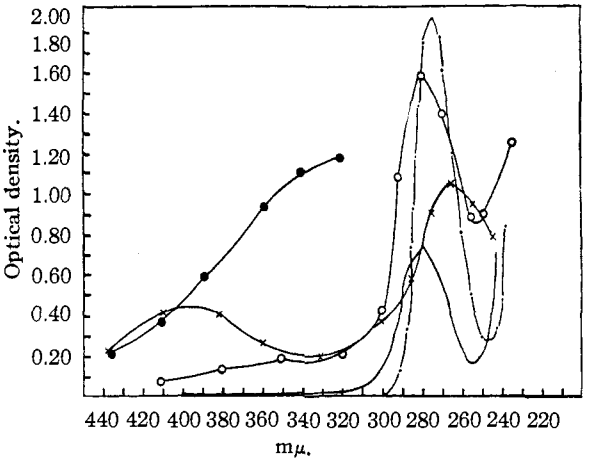

Fig. 2.—Absorption spectra: —·—·— tyrosine (0.25 mg./ml.); —x—x—, deaminated tyrosine (0.05 mg./ml.); —o—o—, deaminated Ea 3B (0.39 mg. N/ml.); —●—●—, deaminated Ea 3B (1.86 mg. N/ml.); ———, Ea (0.15 mg. N/ml.).

Deaminated Ea 3B had its maximum absorption at 280 mμ as did Ea itself. However, from 300 to 440 mμ there was general absorption due to the introduction of chromophoric nitroso, azo and/or diazo groups into the aromatic acid residues.[5] The absorption at 411.5 mμ has been used as a measure of the extent of this reaction,[5,11,12] although the validity of any quantitative comparisons may be legitimately questioned. If one assumes 4% of tyrosine and 15.5% of nitrogen in Ea, it is evident that the deaminated Ea 3B exhibits the same color intensity as deaminated tyrosine at nine times its concentration. Therefore the second-

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