

Anal. Calcd. for $C_{15}H_{13}BrO$: C, 62.29; H, 4.52. Found: C, 62.39; H, 4.39.

Distillation of the liquid residue revealed that it consisted of a complex mixture of by-products.

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p-Nitroacetophenone in the Mannich Reaction

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Of the three nuclear nitroacetophenones, only the *ortho*¹ and *meta*^{1,2} have been reported as participants in the Mannich reaction. Investigation of the chemistry of *p*-nitroacetophenone has shown that the Mannich reaction proceeds quite satisfactorily, giving the expected β -(di)-alkylamino-*p*-nitropropiofenones, generally in fair yields.

secondary Mannich base as the only isolable product.

A few reactions of these Mannich bases suggest normal reactivity for this type of compound. Catalytic reduction over palladium-on-carbon gave the *p*-aminoketones; over platinum an amount of hydrogen corresponding to reduction of nitro to amino and ketone to alcohol was absorbed, although no crystalline products could be isolated. In the latter case the absorption of the first three moles of hydrogen was approximately ten times as fast as that of the fourth mole. In the one example tried, the expected pyrazoline formed on treatment of the Mannich base with phenylhydrazine.

Experimental

β -(Di)-alkylamino-*p*-nitropropiofenone Hydrochlorides (See Table I).—Mannich reactions with *p*-nitroacetophenone were carried out in a manner similar to that described for acetophenone.³ Method A refers to experiments in which the amine hydrochloride was used, method B to those in

TABLE I

NR_2^a	Yield, %	Method ^b	M.p., °C. (cor.)	Recryst. solvent	Formula	Analyses, %			
						Carbon Calcd.	Carbon Found	Hydrogen Calcd.	Hydrogen Found
$N(CH_3)_2$	64	A	187.5–188.5	EtOH	$C_{11}H_{14}N_2O_3 \cdot HCl$	51.1	51.2	5.8	5.9
$N(C_2H_5)_2$	60	A	147–152	EtOH	$C_{13}H_{18}N_2O_3 \cdot HCl$	54.4	54.8	6.7	6.8
NC_4H_9	37	B	182.5–185	<i>n</i> -BuOH	$C_{13}H_{18}N_2O_3 \cdot HCl$	54.8	55.1	6.0	6.2
NC_5H_{10}	62	B	198–200	<i>i</i> -PrOH–Me ₂ CO	$C_{14}H_{18}N_2O_3 \cdot HCl$	56.3	56.4	6.4	6.5
NC_4H_9O	51	A	207–209.5	EtOH–H ₂ O	$C_{17}H_{16}N_2O_4 \cdot HCl$	52.1	52.4	5.7	5.8
$N(n-C_4H_9)_2$	53	B	150–210 dec.	<i>i</i> -PrOH–H ₂ O	$C_{17}H_{26}N_2O_3 \cdot HCl$	59.5	59.5	7.9	7.9
$N(C_2H_4OH)_2$	58	B	138–140	EtOH	$C_{13}H_{18}N_2O_5 \cdot HCl$	49.0	49.1	6.0	5.9
$N(CH_2-CH=CH_2)_2$	8	B	138–140.5	<i>i</i> -PrOH–H ₂ O	$C_{17}H_{22}N_2O_3 \cdot HCl$	60.2	60.7	6.6	6.8
$N \begin{matrix} < CH_3 \\ < CH_2C_6H_5 \end{matrix}$	70	A	186–187	MeOH	$C_{17}H_{18}N_2O_3 \cdot HCl$	61.0	61.1	5.7	6.0
$N \begin{matrix} < C_2H_5 \\ < C_2H_4OH \end{matrix}$	28	B	124.5–127	MeOH	$C_{13}H_{18}N_2O_4 \cdot HCl$	51.6	51.8	6.3	6.7
$NHCH_2C_6H_5$	68	A	219.5–221 dec.	EtOH–Me ₂ CO	$C_{16}H_{16}N_2O_3 \cdot HCl$	59.9	60.2	5.3	5.6
$NHCH(CH_3)_2$	10	B	207–209 dec.	MeOH–Me ₂ CO	$C_{12}H_{16}N_2O_3 \cdot HCl$	52.8	53.1	6.3	6.5
NHC_6H_{11}	52	B	191–195 dec.	MeOH– <i>i</i> -PrOH	$C_{15}H_{20}N_2O_3 \cdot HCl$	57.6	58.0	6.8	6.8
$NHC_8H_{17}(t)$	57	A	208–209	MeOH	$C_{17}H_{26}N_2O_3 \cdot HCl$	59.6	59.7	7.9	7.9
$NHCH_2CO_2C_2H_5$	33	A	177–179	<i>i</i> -PrOH–H ₂ O	$C_{13}H_{16}N_2O_5 \cdot HCl$	49.3	49.9	5.4	5.7

^a NC_4H_9 = 1-pyrrolidyl; NC_5H_{10} = 1-piperidyl; NC_4H_9O = 4-morpholinyl; C_6H_{11} = cyclohexyl; C_8H_{17-t} = 1,1,3,3-tetramethylbutyl. ^b See Experimental.

In Table I are recorded the results of a number of reactions involving primary and secondary amines with formaldehyde and *p*-nitroacetophenone. The general procedure was that described by Maxwell³ for acetophenone. In some cases the amine hydrochloride was employed, in others the amine hydrochloride was prepared *in situ* by addition of one equivalent of concentrated hydrochloric acid to the amine. The method of choice appears to be dictated by the availability of the amine or its hydrochloride. Under the conditions used, diisopropylamine and β, β' -iminodipropionitrile failed to react, and isopropylamine and dimethylamine gave only poor yields. Methylamine yielded the tertiary amine by reaction of two moles of ketone and two moles of formaldehyde; the other primary amines yielded the

which the amine plus one equivalent of concentrated hydrochloric acid was used.

β, β' -Methyliminodi-(*p*-nitropropiofenone)-Hydrochloride.—A solution of 165 g. (1.0 mole) of *p*-nitroacetophenone, 30 g. (1.0 mole) of paraformaldehyde and 33.9 g. (0.5 mole) of methylamine hydrochloride in 160 ml. of 95% ethanol was refluxed for three hours. The clear hot solution was poured into 800 ml. of acetone; crystals formed on cooling. Filtration gave 38 g. of solid, m.p. 195–200°. Evaporation of the filtrate and crystallization of the residue from methanol gave an additional 66 g. of solid, m.p. 195–200° (total yield, 49%). Recrystallization from dimethylformamide entailed a large loss, but with little improvement in melting point; an analytical sample thus prepared melted at 198–201° dec.

Anal. Calcd. for $C_{13}H_{19}N_3O_6 \cdot HCl$: C, 54.1; H, 4.8. Found: C, 54.2; H, 5.4.

Unreacted *p*-nitroacetophenone (42 g., 25%) was recovered from the methanol mother liquors.

p-Amino- β -dimethylaminopropiofenone Hydrochloride.— β -Dimethylamino-*p*-nitropropiofenone hydrochloride (19.4 g., 0.075 mole) was hydrogenated in 200 ml. of methanol over 0.5 g. of 5% palladium-on-carbon. At three atmospheres, hydrogen absorption was 89% complete (for reduction of the nitro group only) in two hours. The reduc-

(1) C. Mannich and M. Dannehl, *Arch. Pharm.*, **276**, 206 (1938).

(2) H. B. Wright and M. Freifelder, *This Journal*, **71**, 1513 (1949).

(3) C. F. Maxwell, *Org. Syntheses*, **23**, 30 (1943).

tion mixture was filtered hot, concentrated and cooled. The precipitated solid was collected by filtration; 15.0 g. (87% yield) was obtained, which decomposed unsharply at about 200°.

Anal. Calcd. for $C_{11}H_{16}N_2O \cdot HCl$: C, 57.8; H, 7.5. Found: C, 58.1; H, 7.4.

***p*-Amino- β -diethylaminopropiophenone Hydrochloride.**—Reduction of β -diethylamino-*p*-nitropropiophenone hydrochloride exactly as described above was rapid and exothermic, being complete in 15 minutes. The product was isolated by diluting the filtered reaction mixture with ether. Recrystallization of the precipitated solid from isopropyl alcohol gave material melting at 134.5–136.0° dec.

Anal. Calcd. for $C_{13}H_{20}N_2O \cdot HCl$: C, 60.8; H, 8.3. Found: C, 60.5; H, 8.5.

Hydrogenation over Platinum Oxide.—Both β -dimethylamino- and β -diethylamino-*p*-nitropropiophenone hydrochlorides were hydrogenated under similar conditions except that the catalyst was platinum oxide. Four moles of hy-

drogen were absorbed, the first three within 15 minutes, the fourth in about two hours. The products were obtained as orange gums, which resisted all attempts to crystallize them.

Reaction of β -Diethylamino-*p*-nitropropiophenone and Phenylhydrazine.—A mixture of 5 g. of β -diethylamino-*p*-nitropropiophenone hydrochloride, 5 g. of phenylhydrazine and 2 g. of anhydrous sodium acetate in 50 ml. of ethanol was heated on the steam-bath for two hours. A dark gummy solid was collected by filtration and washed on the filter with water. After several recrystallizations from pyridine-methanol, 2.2 g. of dark red crystals was obtained, m.p. 152.0–152.5°. Analytical data are in agreement with those calculated for 3-(*p*-nitrophenyl)-1-phenylpyrazoline.

Anal. Calcd. for $C_{15}H_{13}N_3O_2$: C, 67.4; H, 4.9. Found: C, 67.6; H, 5.0.

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COMMUNICATIONS TO THE EDITOR

A LINK BETWEEN FATTY ACYL CoA DEHYDROGENASE AND CYTOCHROME *c*: A NEW FLAVIN ENZYME¹

Sir:

A green (G)¹ and yellow (Y)² flavoprotein catalyze the dehydrogenation of fatty acyl derivatives of CoA.³ A preparation of Y from pig liver showed four peaks on electrophoresis.⁴ The proteins constituting two of these peaks were identified as G⁵ and Y, respectively. Although these sampled components were immediately reducible by substrate, the reduced forms were not oxidizable by electron acceptors. Catalytic activity with indophenol³ could be restored when G or Y was supplemented with the additional protein components separated during electrophoresis. These latter components were not reducible by substrate nor did they catalyze the oxidation of substrate by indophenol. Only certain fractions of this factor (DRF)⁶ which mediates the reoxidation of reduced G and Y by indophenol are able to catalyze the interaction with Cyt as well. This Cyt reducing factor (CRF) was isolated from pig liver and was found to be a flavoprotein with the following characteristics: (1) extinction maxima at 270, 375 and 440 m μ , minima at 310 and 400 m μ ($E_{270}:E_{310}:E_{375}:E_{440} = 6.5:0.3:0.9:1.0$); (2) a characteristic shoulder at 450–460 m μ ; (3) a riboflavin content

(1) D. E. Green, S. Mii, H. R. Mahler and R. M. Bock, *J. Biol. Chem.*, **206**, 1 (1954); H. R. Mahler, *ibid.*, **206**, 13 (1954).

(2) H. Beinert and F. L. Crane, *Fed. Proc.*, **13**, 181 (1954); D. E. Green, *Biol. Revs.*, **29**, 330 (1954). The relationship of G or Y to a similar enzyme reported by W. Seubert and F. Lynen, *THIS JOURNAL*, **75**, 2787 (1953), cannot be evaluated on the basis of available data.

(3) CoA = coenzyme A; indophenol = 2,6-dichlorophenolindophenol; Cyt = cytochrome *c*.

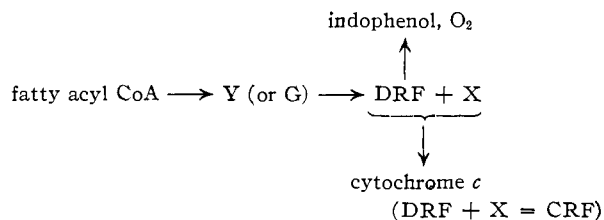
(4) Kindly carried out for us by Dr. R. M. Bock.

(5) The amount of G present was about 5% of that of Y.

(6) DRF = dye reducing factor; CRF = cytochrome *c* reducing factor. DRF and CRF also catalyze the auto-oxidation of G or Y; DRF and CRF are heat labile.

of 0.45%; (4) the released flavin was identified as FAD by its spectrum and by paper chromatography; it replaces FAD quantitatively with *d*-amino acid apo δ oxidase; (5) the flavin is not reducible by a fatty acyl CoA except in presence of catalytic mounts of Y (25% of $E_{440m\mu}$ remaining). The purest CRF fractions are likewise the most active DRF preparations, the ratio of CRF to DRF activity being about 1. Such preparations lose CRF activity within a few days without decline of DRF activity or perceptible change in the spectrum. This relationship of CRF to DRF is reminiscent of that between Cyt reductase and diaphorase.⁷ CRF seems to be a more complex form of DRF, which retains only DRF activity after some degradative process. So far a metal could not be implicated in this phenomenon.

No metal has been found in Y in an amount comparable to that of copper in G¹. Nonetheless, G requires CRF for optimal interaction with Cyt. The following scheme illustrates the path of electrons, indicated by the arrows, as it appears now in view of the reported work:



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(7) H. R. Mahler and D. G. Elowe, *THIS JOURNAL*, **75**, 5769 (1953).

(8) Postdoctoral trainee of the National Heart Institute, National Institutes of Health.