10.1; neut. equiv., 88. Found: C, 47.5; H, 6.4; Cl, 9.0; neut. equiv., 88.

After drying for 15 hr. at 60° and 5 mm., the neut. equiv. had fallen to 80.7; further drying at 80° and 2 mm. for 24 hr. caused the N.E. to drop to 78.1 and the material was reanalyzed.

Anal. Calcd. for $C_{10}H_{16}O_6$: C, 51.7; H, 6.9; neut. equiv., 77.4. Found: C, 51.9; H, 6.9; neut. equiv. 78.1. The melting point of the solvent-free acid was 83–84°.

A two gram sample of acid (m.p. 64–67°) lost the theoretical amount of chloroform when heated for 2 hr. at 120° and 1 mm. The same sample, when heated further at 180° and 1 mm. pressure for 6 hr. lost an additional weight corresponding to the loss of 1 molecule of water. The crude *anhydride* thus obtained melted at 81–83°; after crystallization from benzene-petroleum ether, it melted at 80–81°.

Anal. Calcd. for $C_{10}H_{14}O_5$: C, 56.1; H, 6.6; sapon. equiv., 72. Found: C, 56.3; H, 6.8; sapon. equiv., 73.

The infrared spectrum (KBr pressed plate) exhibited absorptions at 5.35 and 5.61μ; succinic anhydride absorption occurs at 5.36 and 5.61μ.[4]

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(4) H. M. Randall, R. G. Fowler, N. Fuson, and J. R. Dangl, *Infrared Determination of Organic Structures*, D. Van Nostrand Co., Inc., New York, 1949, p. 163.

p-Nitrophenylsemicarbazones of Trioses and Carbonyl Compounds of Biochemical Interest

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The derivatives of *p*-nitrophenylsemicarbazide with carbonyl compounds of biological origin are of interest in that they may offer a means of isolation and identification of the latter substances. It was of particular interest to us to secure a tool for the analysis of trioses. Earlier we have reported the preparation of *p*-phenylazophenylsemicarbazones of trioses and biologically related carbonyl compounds.[1] These derivatives were found unsatisfactory as analytical tools. In a search for a quantitative precipitant for glucose, Barré and Piché[2,3] prepared 4-substituted semicarbazides and obtained *p*-nitrophenylsemicarbazones of acetone, pyruvic acid, glyoxylic acid, and glucose among others. The presence of a nitrophenyl group in the *p*-nitrophenylsemicarbazones should provide an intense coloration by its derivatives in alkaline solution. This property of possible analytical application merited expoloration; consequently, *p*-nitrophenylsemicarbazones of the above mentioned trioses and several carbonyl compounds of biochemical interest were synthesized.

The one-step synthesis of *p*-nitrophenylsemicarbazide from the commercially available *p*-nitrophenyl isocyanate and the preparation of *p*-nitrophenylsemicarbazones are described in this report. Barré and Piché have obtained *p*-nitrophenylsemicarbazide by different methods.[2] However, the simple conversion of isocyanate into semicarbazide with anhydrous hydrazine was not attempted. This conversion has been carried out with good yield in this laboratory. The *p*-nitrophenylsemicarbazide thus obtained was converted into its hydrochloride to increase its solubility in water. Mixing of a saturated solution of the semicarbazide hydrochloride in 0.1*N* hydrochloric acid with an aqueous solution of the carbonyl compound yields the corresponding semicarbazone at room temperature. Crude semicarbazones are purified by recrystallization from ethanol or acetic acid.

All except one of the *p*-nitrophenylsemicarbazones prepared decompose at melting temperatures; therefore, these derivatives appear to be of little value for the identification of carbonyl compounds by melting point. Paper chromatography of the *p*-nitrophenylsemicarbazones was investigated for possible analytical application and the results will be reported elsewhere. The high extinction coefficients of the solutions of these derivatives in the ultraviolet range and the intense coloration on the addition of alkali to these solutions indicate that a promising reagent for the analysis of trioses and the carbonyl compounds of biochemical interest has been found.

EXPERIMENTAL

p-Nitrophenyl isocyanate, acetaldehyde, and ethyl acetoacetate were obtained from the Distillation Products Industries; acetaldehyde was redistilled just before use. Other starting material from various sources were used without further purification. Dihydroxyacetone was obtained from Dougherty Chemical Co. Glyceraldehyde, *alpha*-ketobutyric acid, *alpha*-ketoglutaric acid, and sodium *beta*-phenylpyruvate were from Sigma Chemical Co. Crude pyruvaldehyde (30% aqueous solution) was from Bios Laboratories, Inc. Oxalacetic acid and crude barium oxalosuccinate were from California Foundation for Biochemical Research.

Melting points are corrected. Absorption maxima, λ_{max}, and molar extinction coefficients, ϵ, were determined in ethanol unless otherwise indicated; a 0.001% solution was used for the determinations with a Beckman DU spectrophotometer.

p-Nitrophenylsemicarbazide. Commercial *p*-nitrophenyl isocyanate was partially purified by filtration in its molten state through a filter paper using a heated funnel. In 250 ml. of anhydrous toluene, 29 g. of the isocyanate was dissolved and the solution was directly filtered into a 1 l. 3-neck flask to remove the small amount of insoluble residue. A suspension of 9 ml. of anhydrous hydrazine in 350 ml. of anhydrous toluene was added dropwise into the isocyanate solution in 45 min. while vigorous stirring of the latter was maintained. Heat was generated by the reaction and immediate precipitation of an orange colored product was observed. The reaction flask was stoppered and allowed to stand overnight. The precipitate was collected on a funnel and washed with toluene, followed with petroleum ether (30–60°). The solvent was removed under vacuum in a desiccator. The crude product (33.7 g.) was recrystallized from boiling absolute ethanol yielding 22.0 g. (63%) of yel-

(1) M. U. Tsao and E. Van Dyke, *J. Am. Chem. Soc.*, **77**, 6693 (1955).

(2) R. Barré and L. Piché, *Can. J. Research*, **19B**, 158 (1941).

(3) R. Barré and L. Piché, *Can. J. Research*, **20B**, 17 (1942).

low needle-like crystals, m.p. 185–188° (reported 191° dec.[2]).

p-Nitrophenylsemicarbazide hydrochloride. This compound was prepared by dissolving 2 g. of *p*-nitrophenylsemicarbazide in 100 ml. of boiling absolute ethanol and adding 1 ml. of concentrated hydrochloric acid. Crystalline product in quantitative yield was obtained on cooling, m.p. 239° dec. (reported 265° dec.[2]).

p-Nitrophenylsemicarbazones. The general procedure for the preparation is described here. A saturated solution of *p*-nitrophenylsemicarbazide hydrochloride in 0.1*N* hydrochloric acid containing 1 or 2 millimole of the reagent was mixed with 1 millimole of the carbonyl compound dissolved in minimal amount of water or 0.1*N* hydrochloric acid. The precipitate thus obtained was collected on a glass funnel and washed with water. The crude product was dried and purified by recrystallization from ethanol, 50% ethanol, glacial acetic acid, or 50% acetic acid as indicated in Table I.

TABLE I

p-NITROPHENYLSEMICARBAZONES R=N—NH—C_6H_4—NO_2

	R	Yield, %	M.P.	Solvent for Recrystallization
1.	CH_3CH=	62	200° dec.	Ethanol
2.	$(CH_2OH)_2C$=	62	192° dec.	Ethanol
3.	CH_2OH—CHOH—CH=	52	196° dec.	Ethanol
4.	CH_3C= HC=	20	246° dec.	Acetic acid
5.	C_2H_5C= COOH	57	205° dec.	Acetic acid
6.	HOOC$(CH_2)_2C$= COOH	85	181° dec.	50% Ethanol
7.	(C_2H_5)OOCCH_2C= CH_3	69	174–175°	Ethanol
8.	HOOCCH_2C= COOH	65	219° dec.	50% Acetic acid
9.	HOOCC= HOOCCH HOOCCH_2	7	150° dec.	Acetic acid
10.	HOOCC= $CH_2.C_6H_5$	66	197° dec.	Acetic acid

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Isolation of Two Sodium Methazonates

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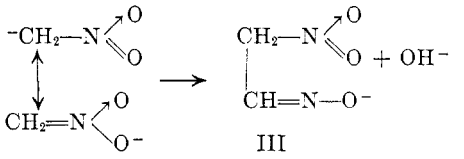
Methazonic acid is usually prepared by the action of concentrated sodium hydroxide solution on nitromethane.[1]

Various attempts have been made to formulate a mechanism for the reaction but with little success. Levy and Rose[2] stated that an intermediate monosodium compound I is formed by the interaction of nitromethane and its sodium salt. This is then dehydrated to form monosodium methazonate II.

$$O_2N—CH_2—CH_2—N(OH)—ONa \longrightarrow$$
I

$$HO—N=CH—CH=NO_2Na$$
II

Drew, McNesby, and Gordon,[3] however, who studied the reaction spectrophotometrically, stated that the methazonate anion III is formed by the reaction of two nitromethane anions, although the structure III would involve the formation of hydroxyl ions in a strongly alkaline medium.



III

The work reported in this paper shows that the reaction product is a disodium salt IV which is probably monohydrated. This then forms a hexahydrate which can be readily isolated from the solution.

TABLE II

p-NITROPHENYLSEMICARBAZONES R=N—NH—C_6H_4—NO_2

	Analysis							
	Carbon, %		Hydrogen, %		Nitrogen, %			
R	Calcd.	Found	Calcd.	Found	Calcd.	Found	λ_{max}	ϵ
1.	48.65	48.69	4.54	4.89	25.22	25.55	322	186,000
2.	44.77	44.64	4.51	4.25	20.89	20.76	322	213,000
3.	44.77	45.01	4.51	4.35	20.89	20.21	323	183,000
4.	47.66	47.30	3.77	3.81	26.16	26.86	331[a]	640,000[a]
5.	47.14	47.06	4.32	4.25	19.99	19.40	319	228,000
6.	40.00[b]	40.02	4.48[b]	4.40	15.55[b]	15.66	317	201,000
7.	50.65	50.83	5.23	5.65	18.18	17.94	322	197,000
8.	42.59	42.90	3.25	3.54	18.06	18.02	318	216,000
9.	42.40	42.96	3.29	3.42	15.22	15.09	319	208,000
10.	56.14	55.96	4.12	4.15	16.37	16.22	318	216,000

[a] In glacial acetic acid. [b] On basis of two moles of water of crystallization.

$$^-O—N=CH—CH=NO_2^-$$
IV

The formation of a disodium salt supports Drew's

(1) W. Meister, *Ber.*, **40**, 3435 (1907).
(2) N. Levy and J. D. Rose, *Quart. Revs.* (*London*), **1**, 358 (1947).
(3) C. M. Drew, J. R. McNesby, and A. S. Gordon, *J. Am. Chem. Soc.*, **77**, 2622 (1955).