

[CONTRIBUTION FROM THE DIVISION OF PLANT BIOLOGY, CARNEGIE INSTITUTION OF WASHINGTON]

3-Nitrobenzohydrazones, 2,4-Dinitrophenylhydrazones and the Separation of Hydrazones by Adsorption

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A number of new 3-nitrobenzohydrazones and 2,4-dinitrophenylhydrazones which are suitable derivatives for the identification of carbonyl compounds have been prepared. Descriptions of these derivatives and of some properties of the hydrazones which may facilitate the preparation of pure derivatives are recorded in this paper. Where discrepancies exist in the properties of known derivatives the properties of those prepared in this Laboratory are also reported.

The Tswett adsorption method, developed for the separation of leaf pigments, has been applied to the separation of colored hydrazones. By means of this method, very small quantities of mixtures of closely related, colorless carbonyl compounds may be separated preliminary to the identification of the components of the mixture.

Experimental

Properties of 3-Nitrobenzohydrazide.—3-Nitrobenzohydrazide is moderately soluble in water (approximately 0.6 g. per 100 ml. at room temperature) and is somewhat more soluble in dilute sulfuric, phosphoric, acetic and nitric acids. It is soluble in ethanol and glacial acetic acid but is only slightly soluble in hydrocarbons.

Aqueous solutions of 3-nitrobenzohydrazide are stable over long periods of time. In acid solution (aqueous, alcoholic or acetic acid) particularly in the presence of strong acids at elevated temperatures, 3-nitrobenzohydrazide is slowly converted into *N,N'*-bis-3-nitrobenzohydrazide of m. p. 240–242°.

Anal. Calcd. for $C_{14}H_{10}O_6N_4$: C, 50.91; H, 3.03; N, 16.97. Found: C, 51.30, 51.36; H, 2.87, 3.00; N, 17.06, 17.16.

In alkaline solution 3-nitrobenzohydrazide is rapidly decomposed, yielding unknown products.

As compared to the aryl hydrazines, 3-nitrobenzohydrazide is an extremely weak oxidizing agent. Thus, it does not oxidize acetol, the triose or other sugars.

Derivatives of 3-Nitrobenzohydrazide.—3-Nitrobenzohydrazide does not form insoluble or slightly soluble derivatives with aqueous solutions of acetaldehyde, acetoacetic acid, acetol, dimethylhydroresorcinol, dihydroxyacetone, glyceraldehyde, glycolic aldehyde, hydroquinone, and keto and aldehyde acids such as glyoxylic, pyruvic, levulinic, geronic and isogeronic acids. When heated with 3-nitrobenzohydrazide in alcoholic solution the following compounds failed to form satisfactory derivatives: benzalacetone, benzophenone, *d*- and *d,l*-camphor, ethyl methyl ketone, fenchone and umbellulone; benzoin was slowly oxidized to benzil; chloral, dimethylhydroresorcinol

and formaldehyde formed white crystals of indefinite composition.

The new derivatives of 3-nitrobenzohydrazide and some of their properties are recorded in Table I. The nitrogen content of the derivatives was determined by Pregl's modification of the Dumas method. The melting points were determined in a Berl block and are corrected.

Properties of 2,4-Dinitrophenylhydrazine.¹—Although markedly stable in acid solutions, 2,4-dinitrophenylhydrazine is quite unstable in the presence of alkalis. This hydrazine dissolves in hot sodium carbonate solution. When the solution is acidified, light brown crystals are obtained. The crystals are soluble in glacial acetic acid, ethanol and most organic solvents. They decompose with explosive violence at 190–192°.

Anal. Found: N, 27.14.

Application of heat to a solution of 2,4-dinitrophenylhydrazine in sodium hydroxide solution (6 *N*) results in the formation of a white, slightly soluble precipitate. This precipitate was recrystallized from pyridine by the addition of water and ethanol; m. p. 145.5–147.5°.

Anal. Found: C, 50.50, 50.40; H, 3.07, 3.00; N, 19.54, 19.47.

Upon preparing derivatives of 2,4-dinitrophenylhydrazine in alcoholic sulfuric acid^{1b,1c} it was observed that the presence of acetic acid led to the formation of acetyl-2,4-dinitrophenylhydrazine which subsequently contaminated the derivatives. 2,4-Dinitrophenylhydrazine (1 g.) was dissolved in concentrated sulfuric acid (3 ml.) which was diluted with ethanol (10 ml.). Acetic acid (1 ml.) was added and after warming and cooling, petroleum ether (20 ml.) was added. Placed in the ice box for twenty-four hours, the solution deposited large yellow crystals (0.25 g.) which melted at 199–201°. Mixed with an authentic sample of acetyl-2,4-dinitrophenylhydrazine (m. p. 201°) prepared from 2,4-dinitrophenylhydrazine and acetic anhydride, the melting point of the crystals was unchanged.

Anal. Calcd. for $C_8H_8O_6N_4$: N, 23.33. Found: N, 23.63, 23.56.

Keto acids heated with 2,4-dinitrophenylhydrazine, mineral acids and alcohol formed derivatives of the esters of the acids as well as derivatives of the acids. The derivatives of the esters of the keto acids may be freed of the derivatives of the keto acids by dissolving the latter in alkaline solutions. The insoluble esters are then recrystallized from organic solvents. These reactions may be made the basis of a method for the identification of alcohols. (See derivatives of esters of levulinic and pyruvic acids in Table II.)

(1) About forty papers describing the preparation of new derivatives of 2,4-dinitrophenylhydrazine have already been published. The most important of these are: (a) Brady and Elsmie, *Analyst*, **51**, 77 (1926); (b) Brady, *J. Chem. Soc.*, **133**, 756 (1931); (c) Allen, *THIS JOURNAL*, **53**, 2955 (1930); and numerous papers by Neuberg in the *Biochemische Zeitschrift*.

TABLE I
 3-NITROBENZOHYDRAZIDE DERIVATIVES OF CARBONYL COMPOUNDS

The abbreviations used in this and Table II are: EtOH, ethanol; MeOH, methanol; PhNO₂, nitrobenzene; EtOAc, ethyl acetate; pet. et., petroleum ether; W, white; Y, yellow; O, orange; R, red; Bk, black; L, light; s, soluble; sl, slightly; v, very; m, moderately.

Carbonyl compound	Prepd. in	Recryst. from	Color	Derivatives			M. p., corr. °C.	Formula	N Analyses, %	
				Approx. soly. at 20°	Water	EtOH			Nitrobz.	Found
Acetone	EtOH	EtOH	W	sl.s.	s.	v.s.	147.5-149	C ₁₀ H ₁₁ O ₃ N ₃	19.13	19.00
Acetophenone	EtOH	EtOHPhNO ₂	W	sl.s.	s.	v.s.	190 -191	C ₁₆ H ₁₃ O ₃ N ₃	15.19	14.84
Acetylacetone ^a	EtOH	EtOH	W	sl.s.	s.	v.s.	119 -121	C ₁₂ H ₁₁ O ₃ N ₃	17.34	17.14
Acrolein	EtOH	MeOH	W	sl.s.	s.	v.s.	169.5-171.5	C ₁₀ H ₉ O ₃ N ₃	19.30	19.18
Anisaldehyde	EtOH	PhNO ₂	LY	v.sl.s.	sl.s.	s.	194.5-196	C ₁₅ H ₁₃ O ₃ N ₃	14.01	14.05
Benzil	EtOH	EtOHPhNO ₂	W	v.sl.s.	s.	s.	232.5-235.5	C ₂₃ H ₂₀ O ₃ N ₃	15.95	15.67
<i>n</i> -Butyraldehyde	EtOH	EtOH	W	sl.s.	s.	v.s.	135.5-137	C ₁₁ H ₁₃ O ₃ N ₃	18.01	17.87
Carvone	EtOH	EtOH	W	sl.s.	s.	v.s.	162 -163	C ₁₇ H ₁₉ O ₃ N ₃	13.79	13.44
Cinnamic aldehyde	EtOH	PhNO ₂	W	v.sl.s.	sl.s.	s.	206 -207.5	C ₁₆ H ₁₃ O ₃ N ₃	14.35	14.23
Citral (comml.)	EtOH	EtOH	W	sl.s.	s.	v.s.	100 -101	C ₁₇ H ₂₁ O ₃ N ₃	13.36	13.33
Crotonic aldehyde	EtOH	MeOH	W	sl.s.	s.	v.s.	172.5-173.5	C ₁₁ H ₁₁ O ₃ N ₃	18.07	18.03
β -Cyclocitral	EtOH	EtOH	W	sl.s.	s.	v.s.	184.5	C ₁₇ H ₂₁ O ₃ N ₃	13.79	13.33
Cyclohexanone	EtOH	EtOH	W	sl.s.	s.	v.s.	140.5-141.5	C ₁₃ H ₁₅ O ₃ N ₃	16.76	16.09
Cyclopentanone	EtOH	EtOHMeOH	W	sl.s.	s.	s.	148.5-149.5	C ₁₂ H ₁₃ O ₃ N ₃	17.31	17.00
Diacetyl	H ₂ O	PhNO ₂	W	v.sl.s.	sl.s.	s.hot	320 -321	C ₁₈ H ₁₆ O ₆ N ₆	20.36	20.39
Dicarvelone ^b	EtOH	EtOH	W	sl.s.	s.	v.s.	95 -100	C ₃₄ H ₄₀ O ₆ N ₆	13.39	13.38
<i>p</i> -Dimethylaminobenzaldehyde ^c	EtOH	PhNO ₂	RO	v.sl.s.	sl.s.	s.	219.5-221	C ₁₆ H ₁₆ O ₃ N ₄	17.91	17.95
Furaldehyde	EtOH	EtOH	W	sl.s.	m.s.	s.	195 -197	C ₁₂ H ₁₀ O ₄ N ₃	16.35	16.15
Glyoxal	H ₂ O	W	v.sl.s.	v.sl.s.	sl.s.hot	339.5-340.5	C ₁₆ H ₁₂ O ₆ N ₆	21.88	21.87
Heptaldehyde	EtOH	MeOH	W	v.sl.s.	s.	v.s.	108.5-110.5	C ₁₄ H ₁₉ O ₃ N ₃	14.44	15.16
α -Ionone	EtOH	MeOH	W	sl.s.	s.	v.s.	149.5-150.5	C ₂₀ H ₂₅ O ₃ N ₃	12.10	11.83
β -Ionone	EtOH	EtOH	W	sl.s.	s.	v.s.	161 -162.5	C ₂₀ H ₂₅ O ₃ N ₃	12.00	11.83
Isobutyraldehyde	EtOH	EtOH	W	sl.s.	s.	v.s.	141.5-142.5	C ₁₁ H ₁₃ O ₃ N ₃	18.19	17.87
Levulinic acid	H ₂ O	PhNO ₂								
		EtOAc	W	m.s.	m.s.	s.	181.5-182.5	C ₁₂ H ₁₈ O ₃ N ₃	15.00	15.05
Levulinic aldehyde	H ₂ O	EtOAc								
		PhNO ₂	W	v.sl.s.	sl.s.	s.	155.5-156	C ₁₉ H ₁₅ O ₆ N ₆	18.91	19.72
Menthone	EtOH	EtOH	W	v.sl.s.	s.	v.s.	105 -107	C ₁₇ H ₂₃ O ₃ N ₃	13.12	13.25
Mesityl oxide ^d	EtOH	EtOH	W	sl.s.	s.	v.s.	120 -140	C ₁₃ H ₁₅ O ₃ N ₃	15.52	16.09
<i>o</i> -Methylcyclohexanone	EtOH	EtOH	W	sl.s.	s.	v.s.	115.5-118	C ₁₄ H ₁₇ O ₃ N ₃	15.44	15.27
Methylheptenone ^e	EtOH	EtOH	W	sl.s.	s.	v.s.	99 -100	C ₁₅ H ₁₉ O ₃ N ₃	15.02	14.53
Methyl <i>p</i> -tolyl ketone	EtOH	EtOH	W	v.sl.s.	sl.s.	s.	193 -194	C ₁₆ H ₁₅ O ₃ N ₃	13.78	14.14
<i>m</i> -Nitrobenzaldehyde	EtOH	PhNO ₂	W	v.sl.s.	sl.s.	s.	253 -255	C ₁₄ H ₁₀ O ₃ N ₄	17.85	17.83
Nonylaldehyde	EtOH	EtOH	W	sl.s.	s.	v.s.	87	C ₁₈ H ₂₃ O ₃ N ₃	13.71	14.30
Piperonal	EtOH	PhNO ₂	LY	v.sl.s.	sl.s.	s.	229 -230.5	C ₁₅ H ₁₁ O ₃ N ₃	13.47	13.42
Propionaldehyde	EtOH	EtOH	W	sl.s.	s.	v.s.	156.5-158.5	C ₁₀ H ₁₁ O ₃ N ₃	19.28	19.00
Pyruvic acid ^f	H ₂ O	MeOHEtOAc								
		pet. et.	W	m.s.	s.	s.	185.5-186.5	C ₁₀ H ₉ O ₃ N ₃	16.36	16.73
Pyruvic aldehyde ^g	H ₂ O	PhNO ₂	W	v.sl.s.	v.sl.s.	s.hot	288.5	C ₁₇ H ₁₄ O ₆ N ₆	20.75	21.10
Thujone	EtOH	EtOH	W	sl.s.	s.	v.s.	156 -156.3	C ₁₇ H ₂₁ O ₃ N ₃	13.65	13.13
<i>p</i> -Tolualdehyde	EtOH	PhNO ₂	W	v.sl.s.	sl.s.	s.	189 -190.5	C ₁₆ H ₁₅ O ₃ N ₃	14.57	14.84
2,4,6-Trinitrobenzaldehyde	EtOH	Dioxane	v.	v.sl.s.	sl.s.	v.s.	238 -239	C ₁₄ H ₃ O ₉ N ₆	19.28	20.29
		EtOH	LY				with dec.			
Vanillin	EtOH	EtOH	W							
		PhNO ₂	LY	sl.s.	s.	s.	213 -215	C ₁₅ H ₁₃ O ₃ N ₃	13.53	13.33
Veratraldehyde	EtOH	PhNO ₂	Y	v.sl.s.	sl.s.	s.	225.5-227.5	C ₁₆ H ₁₅ O ₃ N ₃	12.78	12.77

^a The analysis is calculated on the basis of the formula for 3-nitrobenzo-dimethyldihydropyrazole. ^b The dicarvelone was prepared from carvone with aluminum amalgam and undoubtedly consisted of a mixture of isomers. ^c The dimethylaminobenzaldehyde derivative crystallizes from nitrobenzene as glistening brick-red crystals which become red-orange when heated to near their melting point or when dried at 100° and 1 mm. The nitrogen analysis of the air-dried compound indicated the presence of 0.5 mole of nitrobenzene. ^d The mesityl oxide derivative was very difficult to crystallize. ^e Prepared from citral. ^f Dried over phosphorus pentoxide at 76° and 1 mm. ^g The melting point of 280° [*J. Biol. Chem.*, 89, 527 (1930)] was uncorrected.

The 2,4-dinitrophenylhydrazones of carbonyl acids are more stable in aqueous sodium bicarbonate than in more alkaline solutions. Hence, when it is desired to separate the 2,4-dinitrophenylhydrazones to keto or aldehyde acids

TABLE II
 2,4-DINITROPHENYLHYDRAZINE DERIVATIVES OF CARBONYL COMPOUNDS

Carbonyl compound	Prepd. in	Recryst. from	Color	Derivatives			M. p., corr. °C.	Formula	N Analyses, %			
				Approx. soly. at 20°	Water	Ethanol			Nitrobz.	Found	Calcd.	
Acetaldehyde	H ₂ O	EtOH	O	v.s.l.s.	s.	v.s.	163.5-164.5	C ₃ H ₅ O ₄ N ₄	25.11	25.00		
Acetoacetic acid ethyl ester	EtOH	EtOH	OY	v.s.l.s.	s.	..	96	C ₁₂ H ₁₄ O ₆ N ₄		
Acetol	H ₂ O	EtOH	O	v.s.l.s.	s.	s.	127.5-129.5	C ₉ H ₁₀ O ₆ N ₄	22.11	22.05		
Azelaic acid half aldehyde	H ₂ O	EtOH	Y	v.s.l.s.	s.	v.s.	114	-115	C ₁₃ H ₂₀ O ₆ N ₄	15.56	15.90	
Citral (comml.)	EtOH	EtOH	pet. et.	O	v.s.l.s.	s.	v.s.	99	-115	C ₁₅ H ₂₀ O ₄ N ₄	16.87	16.87
β-Cyclocitral	EtOH	Acetone										
		pet. et.	O	v.s.l.s.	s.	v.s.	171	-172	C ₁₅ H ₂₀ O ₄ N ₄	16.70	16.87	
Cyclopentanone	EtOH	AcOH	O	sl.s.	s.	v.s.	145.5-146.5	C ₁₁ H ₁₂ O ₄ N ₄	21.50	21.21		
Diacetyl	H ₂ O	PhNO ₂	RO	v.s.l.s.	sl.s.	m.s.	314	-315	C ₁₆ H ₁₄ O ₈ N ₈	24.45	25.11	
p-Dimethylaminobenzaldehyde	EtOH	EtOHPhNO ₂	Bk	v.s.l.s.	sl.s.	s.	324.5-326	dec.	C ₁₉ H ₁₉ O ₄ N ₅	21.23	21.28	
Geronic acid	H ₂ O	AcOH	OY	sl.s.	s.	v.s.	135.5-137		C ₁₈ H ₂₀ O ₆ N ₄	16.02	15.91	
Glyoxal	H ₂ O	PhNO ₂	RO	v.s.l.s.	v.s.l.s.	sl.s.	326	-328	C ₁₄ H ₁₀ O ₈ N ₈	26.67	26.79	
α-Ionone	EtOH	Et ₂ O	pet. et.	O	sl.s.	s.	147.5-149.5		C ₁₉ H ₂₄ O ₄ N ₄	15.16	15.05	
β-Ionone	EtOH	AcOH	Crimson	v.s.l.s.	s.	v.s.	128	-129	C ₁₉ H ₂₄ O ₄ N ₄	15.00	15.05	
Isogeronic acid	H ₂ O	AcOH	OY	sl.s.	s.	v.s.	140	-141	C ₁₈ H ₂₀ O ₆ N ₄	15.90	15.91	
Levulinic acid	H ₂ O	AcOH	OY	v.s.l.s.	s.	..	206		C ₁₁ H ₁₂ O ₆ N ₄	18.94	18.92	
Levulinic acid ethyl ester	EtOH	Dioxane EtOH	OY	v.s.l.s.	s.	v.s.	101	-102	C ₁₃ H ₁₆ O ₆ N ₄	17.40	17.28	
Levulinic acid methyl ester	MeOH		YO	v.s.l.s.	s.	v.s.	141.5-142.5		C ₁₂ H ₁₄ O ₆ N ₄	18.13	18.06	
Levulinic acid n-propyl ester	PrOH	PrOH	OY	v.s.l.s.	s.	v.s.	67	-68	C ₁₄ H ₁₈ O ₆ N ₄	16.57	16.57	
Levulinic acid isopropyl ester	i-PrOH	i-PrOH	OY	v.s.l.s.	s.	v.s.	88	-89	C ₁₄ H ₁₈ O ₆ N ₄	16.62	16.57	
Levulinic aldehyde	H ₂ O	PhNO ₂ EtOH	OY	v.s.l.s.	sl.s.	s.	235.5-236.5		C ₁₇ H ₁₈ O ₈ N ₈	24.33	24.35	
o-Methylcyclohexanone	EtOH	AcOH	OY	v.s.l.s.	s.	s.	135.5-137		C ₁₃ H ₁₆ O ₄ N ₄	19.33	19.18	
Nonylaldehyde	EtOH	EtOH	Y	v.s.l.s.	s.	v.s.	100		C ₁₅ H ₂₂ O ₄ N ₄	17.59	17.39	
Pyruvic acid	H ₂ O	AcOH	Y	sl.s.	s.	v.s.	218		C ₉ H ₈ O ₆ N ₄	20.94	20.89	
Pyruvic acid ethyl ester	EtOH	Dioxane										
		EtOH	Y	v.s.l.s.	s.	v.s.	154.5-155		C ₁₁ H ₁₂ O ₆ N ₄	18.79	18.92	
Pyruvic acid methyl ester	MeOH	Dioxane										
		MeOH	Y	v.s.l.s.	s.	v.s.	186.5-187.5		C ₁₀ H ₁₀ O ₆ N ₄	19.81	19.86	
Pyruvic acid n-propyl ester	PrOH	PrOH	Y	v.s.l.s.	s.	v.s.	119	-120	C ₁₂ H ₁₄ O ₆ N ₄	18.11	18.06	
Pyruvic acid isopropyl ester	i-PrOH	i-PrOH	Y	v.s.l.s.	s.	v.s.	160.5-161.5		C ₁₂ H ₁₄ O ₆ N ₄	18.07	18.06	
Pyruvic aldehyde	H ₂ O	PhNO ₂	RO	v.s.l.s.	v.s.l.s.	sl.s.	299	-300	C ₁₈ H ₁₂ O ₈ N ₈	26.19	25.93	
Thujone	EtOH	i-PrOH	O	v.s.l.s.	s.	v.s.	106	-107.5	C ₁₆ H ₂₀ O ₄ N ₄	16.89	16.87	
p-Tolualdehyde	EtOH	EtOHPhNO ₂	OY	v.s.l.s.	sl.s.	s.	232.5-234.5		C ₁₄ H ₁₂ O ₄ N ₄	18.51	18.67	
Veratraldehyde	EtOH	PhNO ₂	O	v.s.l.s.	sl.s.	s.	261	-263	C ₁₅ H ₁₄ O ₆ N ₄	16.20	16.28	

from the 2,4-dinitrophenylhydrazones of other carbonyl compounds it is preferable to dissolve the former in sodium bicarbonate solutions.

In aqueous solutions of mineral acids and 2,4-dinitrophenylhydrazine, pinacol rearranged to pinacolone which formed the characteristic, orange-yellow derivative, m. p. 125.5-126.5°. Hexoses and lactose did not form insoluble compounds with 2,4-dinitrophenylhydrazine. Upon the addition of phenylhydrazine to solutions of hexoses and 2,4-dinitrophenylhydrazines, a red, flocculent precipitate, which was apparently a 2,4-dinitrophenylhexosazone, separated. Quinone and hydroquinone formed red to black derivatives of indefinite composition.

The following substances failed to form derivatives when treated with a solution of 2,4-dinitrophenylhydrazine in alcoholic sulfuric acid, indicating that no oxidation or ketone formation

by rearrangement had taken place: carvacrol, furoic acid, lactic acid, menthol, propandiol-1,2, thymol, urea and uric acid.

The color of 2,4-dinitrophenylhydrazones in the crystalline state depends to some extent upon the size of the crystals and upon the solvent from which the crystals were obtained. Examination of solutions of 2,4-dinitrophenylhydrazine and its derivatives in the spectroscope revealed that these substances have a broad absorption band in the violet portion of the spectrum. The bis-2,4-dinitrophenylhydrazones of glyoxal and diacetyl exhibit the dark blue color when treated with alkalis which has been reported characteristic of the pyruvic aldehyde derivative.²

The regeneration of carbonyl compounds from the hydrazine derivatives may be effected

(2) Barronscheen and Dregus, *Biochem. Z.*, **233**, 305 (1931); Neuberger and Kobel, *ibid.*, **203**, 463 (1928).

by treating the latter with dicarbonyl compounds such as glyoxal, methylglyoxal and diacetyl. The reaction may be carried out in water, aqueous acids or in glacial acetic acid.

Separation of Carbonyl Compounds.—By taking advantage of the differences in properties of 3-nitrobenzohydrazide and 2,4-dinitrophenylhydrazine and their derivatives it is possible to separate and identify many closely related carbonyl compounds. Thus, with 3-nitrobenzohydrazide, glyoxal, methylglyoxal and diacetyl may be precipitated quantitatively and without contamination by other derivatives even in the presence of acetaldehyde, acetol, triose and other sugars. The methylglyoxal (pyruvic aldehyde) and diacetyl derivatives may be separated from the glyoxal derivative by dissolving the former in boiling nitrobenzene. With 2,4-dinitrophenylhydrazine, many other carbonyl compounds may be separated from mixtures as will be evident from an inspection of Tables I and II.

Separation of 2,4-Dinitrophenylhydrazones by Adsorption.—One of the most efficacious methods for the separation of closely related organic compounds is the use of the Tswett adsorption column.³ When attempts are made to separate colorless substances by this method it is difficult to section the column at the proper places. To obviate this difficulty it is now proposed that colorless substances be converted into colored derivatives which can be separated on the Tswett column in the usual way. The individual compounds may then be recovered and identified or reconverted into the original colorless substances. For example, many closely related carbonyl compounds may be converted into the 2,4-dinitrophenylhydrazones and separated by adsorption.

It has been found that many substances may be used as adsorbents for 2,4-dinitrophenylhydrazones, the most suitable being talc, fibrous alumina, Hydralo (alumina), aluminum phos-

phate, tribasic magnesium phosphate and fuller's earth. Magnesium oxide and other basic adsorbents caused decomposition of the 2,4-dinitrophenylhydrazones. (A solution of *p*-tolualdehyde 2,4-dinitrophenylhydrazone in dichloromethane passed over a column of magnesium oxide gave rise to a periodic formation of black rings.)

By the general procedure outlined above, a mixture of beta ionone and camphor 2,4-dinitrophenylhydrazones dissolved in petroleum ether was separated by adsorption on a column of talc and the individual components recovered by elution with ethanol. The camphor derivative formed the lower or least readily adsorbed band.

A mixture of geronic acid and levulinic acid 2,4-dinitrophenylhydrazones dissolved in benzene was separated by adsorption on a column of talc and the components recovered by elution with ethanol. The geronic acid 2,4-dinitrophenylhydrazone formed the lower or least readily adsorbed band.

This method has already been extended by Dr. H. M. Leicester to the separation of other types of colored derivatives such as the picrates of tetraethyl- and tetramethylammonium hydroxides.

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Summary

New hydrazones prepared from carbonyl compounds with 3-nitrobenzohydrazide and 2,4-dinitrophenylhydrazine are described.

Methods for the separation and identification of many carbonyl compounds are suggested.

Colorless compounds may be separated on Tswett adsorption columns by converting the former into colored derivatives which may then be adsorbed in the usual manner. This procedure shows promise of application to a great variety of chemical substances.

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(3) Winterstein in Klein "Handbuch der Pflanzen Analyse," Vol. IV, Second part. Wien, 1933, p. 1403; Strain, *J. Biol. Chem.*, **105**, 523 (1934).