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

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

## By Harold H. Strain

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.  $240-242^\circ$ .

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, 1706, 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**.<sup>1</sup>—Although markedly stable in acid solutions, 2,4-dinitrophenylhydrazine is quite unstable in the presence of alkalies. 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  $190-192^{\circ}$ .

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<sup>1b,1e</sup> it was observed that the presence of acetic acid led to the formation of acetyl-2,4dinitrophenylhydrazine 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, 52, 2955 (1930); and numerous papers by Neuberg in the Biochemische Zeitschrift.

### April, 1935

### TABLE I

## 3-Nitrobenzohydrazide Derivatives of Carbonyl Compounds

The abbreviations used in this and Table II are: EtOH, ethanol; MeOH, methanol; PhNO<sub>2</sub>, 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.

$ \begin{array}{llllllllllllllllllllllllllllllllllll$	Carbonyl compound	Prepd.	Recryst.	Color	Appro	x. soly.	at 20°	M. p.,	Formula	N Analy Found	ses, %
Acetophenone         EIGH EtOHPHNO,         W sl.s.         v.s.         110 0 -191         CaHuGAN, 16.13         110.19         14.8           Acetophenone         EIGH EtOHPNO,         W sl.s.         v.s.         119 -191         CaHuGAN, 17.34         17.14         17.14         17.14         17.14         17.14         17.14         17.14         17.14         17.14         17.14         17.14         17.14         17.14         17.14         17.14         17.14         17.14         17.14         17.15         17.40,0N, 18.01         17.35         17.16         17.17         17.16         17.16         17.16         17.16         17.16         17.16         17.16         17.16         17.16         17.16         17.16         17.16         17.16         17.16         17.16         17.16         17.16         17.16         17	Acetone	EtOH	EtOH	W	sis	s	V S	147 5-149	C.H.O.N.	10 13	10 00
Acetylacetone*       EIOH	Acetophenone	EtOH	EtOHPhNO	w	sis.	s.	vs	190 -191	CuHnO.N.	15 10	14 84
AcroleinEtoHWelkS.v.s.10.211.22ClapHoON11.14AnisaldehydeEtOHPiNO2LYv.sl.s.s.v.s.169.5-17.1.5ClapHoON19.3019.11AnisaldehydeEtOHPiNO2LYv.sl.s.s.v.s.169.5-17.1.5ClapHoON14.01BenzilEtOHEtOHW.v.sl.s.s.v.s.135.5-137ClapHoON18.0119.95CarvoneEtOHEtOHW.v.sl.s.s.v.s.160.5-17.1.5ClapHoON13.2913.44Cinnanic aldehydeEtOHPiNO2W.v.sl.s.sl.s.v.s.100.713.3613.33Crotonic aldehydeEtOHMcOHW.sl.s.s.v.s.184.5ClapHoON13.7913.36GyclopetnanoneEtOHEtOHW.sl.s.s.v.s.184.5ClapHoON13.7913.36OyclopetnanoneEtOHEtOHW.sl.s.s.v.s.140.5-141.5ClapHoON13.7913.38Dicarvelone <sup>b</sup> EtOHEtOHW.sl.s.s.v.s.140.5-141.5ClapHoON13.3913.88P-DimethylaminobenzaldehydeEtOHW.sl.s.s.v.s.180.5-140.5ClapHoON13.3913.88P-DimethylaminobenzaldehydeEtOHPNO2W.v.sl.s.sl.s.v.s.180.5-140.5ClapHoON14.0114.45GlyoxalHgOW.v.sl.s.s.v.s.180.5-140.5ClapHoON<	Acetylacetone <sup>a</sup>	EtOH	EtOH	w	sl s	s.	v.s.	119 -121	CuHuON.	17 34	17 14
AnisaldehydeEtoriPhNO, EtoriU y will, s. sl.s.100100, 100, 100, 100, 100, 100, 100, 100,	Acrolein	EtOH	MeOH	w	51.5 sis	s.	v.s.	169 5-171 5	CtoHeO.No	10.30	10 18
BenzilEtchEtchEtchFirst and to the construction232.5-235.5CasHapONA to 11.0011.100 $n$ -ButyraldehydeEtchEtchWsl.s.s.v.s.135.5-137Cu <sub>1</sub> H <sub>10</sub> ONA to 11.7.87CarvoneEtchEtchWsl.s.s.v.s.162.5-137Cu <sub>1</sub> H <sub>10</sub> ONA 	Anisaldehyde	EtOH	PhNO	LV	vsis	ુ. કાર	s	194 5-196	C.H.O.N.	14 01	14 05
<i>n</i> -Butyraldehyde       Eforth Eform MrVg W       Form MrVg W       Fo	Benzil	EtOH	EtOHPhNO.	w	v.si.s.	S1.3.	з. с	232 5-235 5	CasHasOaNa	15 05	15 67
$ \begin{array}{c} \text{Leven} \mbode \mbode$	<i>n</i> -Butyraldehyde	EtOH	EtOH	w	<pre>sl.sl.sl.sl.sl.sl.sl.sl.sl.sl.sl.sl.sl.s</pre>	5. S	5. V S	135 5-137	$C_{28}H_{20}O_{6}N_{6}$	18 01	17 87
$ \begin{array}{c} \mbox{Linvariant} \begin{tabular}{l l l l l l l l l l l l l l l l l l l $	Carvone	EtOH	EtOH	w	s1.5.	а. С	v.s.	162 - 163	CurHusOnN.	13 70	13 44
Citral (comml.)EtoHFinkleyWFinkleyWFinkleyWFinkleyWFinkleyWFinkleyWFinkleyWFinkleyWFinkley<	Cinnamic aldehyde	EtOH	PhNO	w	vels	s. ele	¢.5.	206 - 207 5	CuHuON.	14 35	14 93
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	Citral (comml.)	FtOH	EtOH	w	<pre> •</pre>	S1	vs	100 -101	CurHarOaNa	13 36	13 33
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	Crotonic aldehyde	EtOH	MeOH	w	51.5. el e	з. с	V.S.	172 5-173 5	$C_1/H_2/O_3N_3$	18.07	18.03
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	β-Cyclocitral	EtOH	EtOH	w	el e	с. с	v.s. v.e	184 5	C.H.O.N.	13 70	13 33
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	Cyclohexanone	EtOH	EtOH	w	s1.5.	s.	v.s.	140 5-141 5	$C_1/H_{21}O_3N_3$	16 76	16.00
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	Cyclopentanone	EtOH	EtOHMeOH	w	sis.	s.	•	148 5-149 5	$C_{13}H_{15}O_{3}N_{3}$	17 31	17 00
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	Diacetvi	H.O	PhNO	w	vele	el e	s hot	320 -321	CuHuO.N.	20.36	20.30
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	Dicarvelone <sup>b</sup>	EtOH	EtOH	w	el e	S1.5.	vs	95 -100	C.H.O.N.	13 30	13 38
PuraldelydeEtOHEtOHEtOHWittSite $1000000000000000000000000000000000000$	<i>p</i> -Dimethylaminobenzaldebyde <sup>c</sup>	EtOH	PhNO	RO	vsls	5. sis	s	219 5-221	CuHuON.	17 01	17 95
Initial of the field of the	Furaldehyde	EtOH	EtOH	w	ৰা হ	ms	s.	195 197	CiaHiaO.Na	16 35	16 15
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	Glyoxal	H <sub>0</sub>	Lion	w	vsis.	wsis	si s hot	339 5-340 5	CuHuON	21 88	21 87
Arpmenting atEffortWith the first in the first intermediation of the first intermetiation of the first inte	Heptaldehyde	EtOH	MeOH	w	vsls.	¢.51.5.	v s	108 5-110 5	CuHunOn No.	14 44	15 16
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	α-Ionone	EtOH	MeOH	w	si s	s.	v.s.	149 5-150 5	CacHarOa Na	12 10	11 83
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	β-Ionone	EtOH	EtOH	w	sis.	s.	vs.	161 - 162 5	Calles OaNs	12.10	11.83
Levulinic acidH2OPhNO2Ws.s.V.S.Ph V.S.Ph V.S.<	Isobutyraldebyde	FtOH	EtOH	w	si.s.	з. с	V.S.	141 5-142 5	C.H.O.N.	18 10	17.87
Even into and $H_2O$ Europe $H_2O$ Europe $H_2O$ $H_1O_2$ $W$ $m.s.$ $m.s.$ $s.$ $181.5-182.5$ $C_{12}H_{18}O_8N_3$ $15.00$ $15.05$ Levulinic aldehyde $H_2O$ $EtOAc$ $PhNO_2$ $W$ $v.sl.s.$ $s.$ $155.5-156$ $C_{19}H_{18}O_8N_6$ $18.91$ $19.72$ Menthone $EtOH$ $EtOH$ $W$ $v.sl.s.$ $s.$ $v.s.$ $105$ $-107$ $C_{17}H_{29}O_3N_3$ $13.12$ $13.25$ Mesityl oxide <sup>d</sup> $EtOH$ $EtOH$ $W$ $v.sl.s.$ $s.$ $v.s.$ $120$ $-140$ $C_{18}H_{18}O_8N_8$ $15.52$ $16.09$ $o$ -Methylcyclohexanone $EtOH$ $EtOH$ $W$ $v.sl.s.$ $s.$ $v.s.$ $115.5-118$ $C_{14}H_{17}O_8N_8$ $15.22$ $16.09$ $o$ -Methylcyclohexanone $EtOH$ $EtOH$ $W$ $v.sl.s.$ $s.$ $v.s.$ $190$ $C_{18}H_{19}O_8N_8$ $15.02$ $14.53$ Methyl $p$ -tolyl ketone $EtOH$ $EtOH$ $W$ $v.sl.s.$ $s.$ $v.s.$ $99$ $-100$ $C_{18}H_{10}O_8N_8$ $13.71$ $14.30$ Nonylaldehyde $EtOH$ $PhNO_2$ $W$ $v.sl.s.$ $s.$ $v.s.$ $87$ $C_{16}H_{20}O_8N_8$ $13.47$ $13.42$ Propionaldehyde $EtOH$ $EtOH$ $W$ $v.sl.s.$ $s.$ $v.s.$ $156.5-158.5$ $C_{10}H_{10}O_8N_8$ $19.28$ $19.00$ Pyruvic acid <sup>1</sup> $H_2O$ $PhNO_2$ $W$ $v.sl.s.$ $s.$ $185.5-186.5$ <	Levulinic acid	H <sub>0</sub> O	PhNO		51.5.	5.	v.s.	141.0 142.0	C111113O3143	10.19	11.01
Levulinic aldehydeH2OEtOAc PhNO2H3.5H3.5H3.5H3.6H3.	ber unite usia	1120	EtOAc	w	ms	ms	c	181 5-182 5	C.H.O.N.	15.00	15 05
Phone <th< td=""><td>Levulinic aldehyde</td><td>HO</td><td>EtOAc</td><td>••</td><td><b>m</b>.s.</td><td><b>m</b>.5<b>.</b></td><td>5.</td><td>101.0 102.0</td><td>012111805143</td><td>10.00</td><td>10.00</td></th<>	Levulinic aldehyde	HO	EtOAc	••	<b>m</b> .s.	<b>m</b> .5 <b>.</b>	5.	101.0 102.0	012111805143	10.00	10.00
MenthoneEt OHWV.sl.s. sl.h.100.0 $100.0$ $10.01$		1120	PhNO	w	vsls	ণ হ	e	155 5-156	CuHON	18 01	10.72
Mesityl oxide <sup>d</sup> EtoliEtoliWf.kis, s.v.s.100101 $C_{11}H_{22}o_{3}N_{3}$ 15.2216.1216.12 $o$ -MethylcyclohexanoneEtOHEtOHWsl.s.s.v.s.120 $-140$ $C_{13}H_{16}O_{3}N_{3}$ 15.5216.09 $o$ -MethylcyclohexanoneEtOHEtOHWsl.s.s.v.s.115.5-118 $C_{14}H_{17}O_{3}N_{3}$ 15.4415.27Methyl petonone <sup>6</sup> EtOHEtOHWsl.s.s.v.s.99 $-100$ $C_{13}H_{19}O_{3}N_{3}$ 15.0214.53Methyl p-tolyl ketoneEtOHEtOHWsl.s.s.v.s.99 $-100$ $C_{16}H_{19}O_{3}N_{3}$ 13.7814.14m-NitrobenzaldehydeEtOHEtOHWv.sl.s.sl.s.s.253 $-255$ $C_{14}H_{10}O_{5}N_{4}$ 17.8517.83NonylaldehydeEtOHEtOHWsl.s.s.v.s.87 $C_{16}H_{23}O_{3}N_{3}$ 13.4713.42PropionaldehydeEtOHPhNO2LYv.sl.s.sl.s.s.229 $-230.5$ $C_{16}H_{10}O_{5}N_{3}$ 16.3616.73Pyruvic acid <sup>1</sup> H_2OMeOHEtOAcpet. et.Wm.s.s.s.185.5-186.5 $C_{10}H_{9}O_{5}N_{3}$ 16.3616.73Pyruvic acid <sup>1</sup> H_2OPhNO2Wv.sl.s.v.sl.s.sl.to185.5 $C_{11}H_{10}O_{5}N_{3}$ 16.3616.73Pyruvic acid <sup>1</sup> H_2OPhNO2W <td>Menthone</td> <td>EtOH</td> <td>EtOH</td> <td>w</td> <td>vsle</td> <td>s</td> <td>vs</td> <td>105 - 107</td> <td>CurHanOoNo</td> <td>13 19</td> <td>13 25</td>	Menthone	EtOH	EtOH	w	vsle	s	vs	105 - 107	CurHanOoNo	13 19	13 25
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	Mesityl oxide $^{d}$	EtOH	EtOH	w	si s	s.	V.S.	120 - 140	CuHuO.No	15.52	16.00
Methylheptenone*EtOHEtOHWsl.s.sl.r.s.1010 $C_{14}H_{1}O_{3}A_{3}$ 10.1410.21Methylheptenone*EtOHEtOHWsl.s.sl.sl.99-100 $C_{13}H_{19}O_{3}N_{3}$ 15.0214.53Methyl p-tolyl ketoneEtOHEtOHWv.sl.s.sl.s.sl.99-100 $C_{13}H_{19}O_{3}N_{3}$ 13.7814.14 <i>m</i> -NitrobenzaldehydeEtOHEtOHWv.sl.s.sl.s.sl.193-194 $C_{16}H_{13}O_{3}N_{3}$ 13.7814.14 <i>m</i> -NitrobenzaldehydeEtOHPhNO2Wv.sl.s.sl.s.sl.255 $C_{14}H_{10}O_{5}N_{4}$ 17.8517.83NonylaldehydeEtOHEtOHWsl.s.s.v.sl.87 $C_{16}H_{23}O_{3}N_{3}$ 13.1714.30PiperonalEtOHPhNO2LYv.sl.s.sl.s.s.229-230.5 $C_{16}H_{11}O_{5}N_{3}$ 13.4713.42PropionaldehydeEtOHEtOHWsl.s.s.v.s.156.5-158.5 $C_{10}H_{11}O_{5}N_{3}$ 19.2819.00Pyruvic acid'H_2OMeOHEtOAcpet. et.Wm.s.s.s.185.5-186.5 $C_{10}H_{9}O_{5}N_{3}$ 16.3616.73Pyruvic aldehyde*H_2OPhNO2Wv.sl.s.v.sl.s.shot288.5 $C_{17}H_{14}O_{5}N_{6}$ 20.7521.10ThujoneEtOHEtOHWsl.s.s.v.sl.s.sl.s. <td>o-Methylcyclohexanone</td> <td>EtOH</td> <td>EtOH</td> <td>w</td> <td>sis.</td> <td>s.</td> <td>v.s.</td> <td>115 5-118</td> <td>C.H.D.N.</td> <td>15 44</td> <td>15 27</td>	o-Methylcyclohexanone	EtOH	EtOH	w	sis.	s.	v.s.	115 5-118	C.H.D.N.	15 44	15 27
InterpretationInterpretationInterpretationInterpretationInterpretationMethyl p-tolyl ketoneEtOHEtOHWv.sl.s. sl.s.v.sl.103 $-194$ $C_{16}H_{16}O_{3}N_{3}$ $13.78$ $14.14$ m-NitrobenzaldehydeEtOHPhNO2Wv.sl.s.sl.s.sl.s. $253$ $-255$ $C_{14}H_{10}O_{5}N_{4}$ $17.85$ $17.83$ NonylaldehydeEtOHEtOHWsl.s.s. $253$ $-255$ $C_{14}H_{10}O_{5}N_{4}$ $17.85$ $17.83$ PiperonalEtOHEtOHWsl.s.s.v.s. $87$ $C_{16}H_{28}O_{3}N_{3}$ $13.71$ $14.30$ PropionaldehydeEtOHEtOHWsl.s.s. $229$ $-230.5$ $C_{16}H_{11}O_{5}N_{3}$ $13.47$ $13.42$ PropionaldehydeEtOHEtOHWsl.s.s. $v.s.$ $156.5-158.5$ $C_{10}H_{11}O_{5}N_{3}$ $19.28$ $19.00$ Pyruvic acid <sup>1</sup> H_2OMeOHEtOAcpet. et.Wm.s.s. $s.$ $185.5-186.5$ $C_{10}H_{9}O_{5}N_{3}$ $16.36$ $16.73$ Pyruvic aldehyde <sup>a</sup> H_2OPhNO2Wv.sl.s.v.sl.s.shot $288.5$ $C_{17}H_{14}O_{6}N_{6}$ $20.75$ $21.10$ ThujoneEtOHEtOHWsl.s.s. $v.s.$ $156$ $-156.3$ $C_{17}H_{21}O_{3}N_{3}$ $13.65$ $13.13$ <i>p</i> -TolualdehydeEtOHPhNO2Wv.sl.s. $sl.s.$ $s.$ $238$ $-239$ <	Methvlheptenone <sup>e</sup>	EtOH	EtOH	w	sis.	s.	v.s.	99	CuH.O.N.	15.02	14 53
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	Methyl <i>p</i> -tolyl ketone	EtOH	EtOH	w	vels	ु. डो.ड	¢.3.	103 104	CuHuON.	13.78	14.00
NonylaldehydeEtOHEtOHEtOHWsl.s. <t< td=""><td><i>m</i>-Nitrobenzaldehyde</td><td>EtOH</td><td>PhNO</td><td>w</td><td>vsls</td><td>sis.</td><td>s. s</td><td>253 -255</td><td>C.H.O.N.</td><td>17 85</td><td>17.83</td></t<>	<i>m</i> -Nitrobenzaldehyde	EtOH	PhNO	w	vsls	sis.	s. s	253 -255	C.H.O.N.	17 85	17.83
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$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	Piperonal	EtOH	PhNO	τv	vsls	୍. ବାବ	e	229 -230 5	C.H.O.N.	13 47	13 42
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	Propionaldehvde	EtOH	EtOH	w	si s	s	vs	156 5-158 5	CheHuO.N.	10.27	10.42
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	Pyruvic acid <sup>7</sup>	H <sub>0</sub> O	MeOHEtOAd		51.5.	0.	•	100.0 100.0	C10111) 03143	10.20	10.00
$\begin{array}{cccccccccccccccccccccccccccccccccccc$		2+	pet. et.	w	m.s.	S.	S.	185 5-186 5	C.H.O.N.	16 36	16 73
Thujone       EtOH       EtOH       W       sl.s.       v.s.       156 $-156.3$ $C_{11}H_{20}O_{16}V_{16}$ $20.46$ $21.16$ <i>p</i> -Tolualdehyde       EtOH       Holos       W       sl.s.       s.       v.s.       156 $-156.3$ $C_{11}H_{21}O_{3}N_{5}$ $13.65$ $13.13$ <i>p</i> -Tolualdehyde       EtOH       PhNO2       W       v.sl.s.       sl.s. $189$ $-190.5$ $C_{14}H_{16}O_{3}N_{5}$ $14.57$ $14.84$ <i>2</i> ,4,6-Trinitrobenzaldehyde       EtOH       Dioxane       v.       v.sl.s.       sl.s.       v.s $238$ $-239$ $C_{14}H_{5}O_{9}N_{6}$ $19.28$ $20.29$ EtOH       LY       with dec.	Pvruvic aldehvde"	H₀O	PhNO	w	vsis	ਪ ਵੀ ਵ	s hot	288 5	C.H.O.N.	20.75	21 10
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	Thujone	EtOH	EtOH	w	si s	\$ .51.5.	vs	156 -156 3	CurHarO.N.	13 65	13 13
2,4,6-Trinitrobenzaldehyde EtOH Dioxane v. v.sl.s. sl.s. v.s $238 -239 C_{14}H_8O_9N_6$ 19.28 20.29 EtOH LY with dec.	<i>p</i> -Tolualdehvde	EtOH	PhNO <sub>2</sub>	w	vsis.	ୁ. ବାବ	s	189 -190 5	CuHuON.	14 57	14 84
EtOH LY with dec.	2.4.6-Trinitrobenzaldehvde	EtOH	Dioxane	v.	vsl.s.	sis.	vs	238 -239	CuHoON	10.28	20.20
			EtOH	LV		-1.01		with dec	C14112 C9148	10.40	-0.20
Vanillin EtOH EtOH W-	Vanillin	EtOH	EtOH	w-				ucc.			
<b>PhNO</b> <sub>2</sub> LY sLs s. s. $213 - 215$ CuHurOrNa 13 53 13 33			PhNO <sub>2</sub>	LY	sl.s	s.	s.	213 -215	C18H19OrNo	13 53	13 33
Veratraldehyde EtOH PhNO <sub>2</sub> Y v.sl.s. sl.s. s. $225.5-227.5 C_{10}H_{15}O_{5}N_{2}$ 12.78 12.77	Veratraldehyde	EtOH	PhNO <sub>2</sub>	Y	v.sl.s.	sl.s.	s.	225.5-227.5	C16H15O5N2	12.78	12.77

<sup>a</sup> The analysis is calculated on the basis of the formula for 3-nitrobenzo-dimethyldihydropyrazole. <sup>b</sup> The dicarvelone was prepared from carvone with aluminum amalgam and undoubtedly consisted of a mixture of isomers. <sup>c</sup> The dimethylaminobenzaldehyde derivative crystallizes from nitrobenzene as glistening brick-red crystals which become redorange 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. <sup>d</sup> The mesityl oxide derivative was very difficult to crystallize. <sup>e</sup> Prepared from citral. <sup>f</sup> Dried over phosphorus pentoxide at 76° and 1 mm. <sup>g</sup> 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

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#### TABLE II

	Derivatives									
Carbonyl compound	Prepd. in	Recryst. from	Color	Appro Water	x. soly. Ethano	at 20° 1 Nitrobz.	M. p., corr. °C.	Formula	N Anal Found	yses, % Calcd.
Acetaldehyde	$H_2O$	EtOH	0	v.sl.s.	s.	v.s.	163.5-164.5 C	C <sub>8</sub> H <sub>8</sub> O <sub>4</sub> N <sub>4</sub>	25.11	<b>25</b> .00
Acetoacetic acid ethyl ester	EtOH	EtOH	OY	v.sl.s.	s.	• •	96 C	$C_{12}H_{14}O_6N_4$		
Acetol	$H_2O$	EtOH	0	v.sl.s.	s.	s.	127.5-129.5 0	$C_9H_{10}O_5N_4$	22.11	22.05
Azelaic acid half aldehyde	$H_2O$	EtOH	Y	v.sl.s.	s.	v.s.	114 -115 C	$C_{15}H_{20}O_6N_4$	15.56	15.90
Citral (comml.)	EtOH	EtOH pet. et.	0	v.sl.s.	s.	v.s.	99 -115 C	$C_{16}H_{20}O_4N_4$	16.87	16.87
$\beta$ -Cyclocitral	EtOH	Acetone								
		pet. et.	0	v.sl.s.	s.	v.s.	171 –172 C	$C_{16}H_{20}O_4N_4$	16.70	16.87
Cyclopentanoue	EtOH	AcOH	0	sl.s.	s.	v.s.	145.5-146.5 C	$C_{11}H_{12}O_4N_4$	21.50	21.21
Diacetyl	$H_2O$	$PhNO_2$	RO	v.sl.s.	sl.s.	m.s.	314 –315 C	$C_{16}H_{14}O_8N_8$	24.45	25.11
<i>p</i> -Dimethylaminobenzaldehyde	EtOH	EtOHPhNO <sub>2</sub>	Bk	v.sl.s.	sl.s.	s. 324	4.5-326 dec. C	$C_{1\delta}H_{1\delta}O_4N_5$	21.23	21.28
Geronic acid	$H_2O$	AcOH	OY	sl.s.	<b>S</b> .	v.s.	135.5–137 C	$C_{15}H_{20}O_6N_4$	16.02	15.91
Glyoxal	$H_2O$	$PhNO_2$	RO	v.sl.s.	v.sl.s.	sl.s.	326 –328 C	$C_{14}H_{10}O_8N_8$	26.67	26.79
α-Ionone	EtOH	Et <sub>2</sub> O pet. et.	0	sl.s.	s.	v.s.	147.5-149.5 C	$C_{19}H_{24}O_4N_4$	15.16	15.05
β-Ionone	EtOH	AcOH Crin	nson	v.sl.s.	s.	v.s.	128 –129 C	$C_{19}H_{24}O_4N_4$	15.00	15.05
Isogeronic acid	$H_2O$	AcOH	OY	sl.s.	s.	v.s.	140 –141 C	$C_{15}H_{20}O_6N_4$	15.90	15.91
Levulinic acid	$H_2O$	AcOH	OY	v.sl.s.	s.		206 C	$C_{11}H_{12}O_6N_4$	18.94	18.92
Levulinic acid ethyl ester	EtOH	∫ Dioxane	OY	v.sl.s.	s.	v.s.	101 -102 C	$C_{13}H_{16}O_6N_4$	17.40	17.28
Levulinic acid methyl ester	MeOH	[ ] EtOH	YO	v.sl.s.	s.	v.s.	141.5-142.5 C	$C_{12}H_{14}O_6N_4$	18.13	18.06
Levulinic acid <i>n</i> -propyl ester	PrOH	PrOH	OY	v.sl.s.	s.	v.s.	67 -68 C	$C_{14}H_{18}O_6N_4$	16.57	16.57
Levulinic acid isopropyl ester	i-PrOI	H j-PrOH	OY	v.sl.s.	s.	v.s.	8889 C	$C_{14}H_{18}O_6N_4$	16.62	16.57
Levulinic aldehyde	$H_2O$	PhNO <sub>2</sub> EtOH	OY	v.sl.s.	sl.s.	s.	235.5-236.5 0	$C_{17}H_{16}O_8N_8$	24.33	24.35
o-Methylcyclohexanone	EtOH	AcOH	OY	v.sl.s.	s.	<b>S</b> .	135.5–137 C	$C_{13}H_{16}O_4N_4$	19.33	19.18
Nonylaldehyde	EtOH	EtOH	Y	v.sl.s.	s.	v.s.	100 C	$C_{15}H_{22}O_4N_4$	17.59	17.39
Pyruvic acid	$H_2O$	AcOH	$\mathbf{Y}$	sl.s.	s.	v.s.	218 C	$C_9H_8O_6N_4$	20.94	20.89
Pyruvic acid ethyl ester	EtOH	Dioxane								
		EtOH	Y	v.sl.s.	s.	v.s.	154.5-155 C	$C_{11}H_{12}O_6N_4$	18.79	18.92
Pyruvic acid methyl ester	MeOE	I Dioxane								
		MeOH	Υ	v.sl.s.	s.	v.s.	186.5-187.5 0	$C_{10}H_{10}O_6N_4$	19.81	19.86
Pyruvic acid <i>n</i> -propyl ester	PrOH	PrOH	Y	v.sl.s.	s.	<b>v.</b> s.	119 –120 C	$C_{12}H_{14}O_6N_4$	18.11	18.06
Pyruvic acid isopropyl ester	i-PrOI	H i-PrOH	Y	v.sl.s.	s.	v.s.	160.5-161.5 C	$C_{12}H_{14}O_6N_4$	18.07	18.06
Pyruvic aldehyde	$H_2O$	$PhNO_2$	RO	v.sl.s.	v.sl.s.	sl.s.	299 –300 C	$C_{15}H_{12}O_8N_8$	26.19	25.93
Thujone	EtOH	i-PrOH	0	v.sl.s.	s.	<b>v</b> .s.	106 -107.5 C	$C_{16}H_{20}O_4N_4$	16.89	16.87
<i>p</i> -Tolualdehyde	EtOH	EtOHPhNO <sub>2</sub>	OY	v.sl.s.	sl.s.	s.	232.5-234.5 (	$C_{14}H_{12}O_4N_4$	18.51	18.67
Veratraldehyde	EtOH	$PhNO_2$	0	v.sl.s.	sl.s.	s.	261 –263 C	$C_{15}H_{14}O_6N_4$	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 2,4dinitrophenylhydrazine, 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 2,4dinitrophenylhydrazine 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 alkalies which has been reported characteristic of the pyruvic aldehyde derivative.<sup>2</sup>

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

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

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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.<sup>3</sup> 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 phosphate, 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.

The writer is indebted to Dr. H. A. Spoehr for many helpful suggestions and to Mr. Harold W. Milner for some of the nitrogen determinations.

#### Summary

New hydrazones prepared from carbonyl compounds with 3-nitrobenzohydrazide and 2,4dinitrophenylhydrazine 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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<sup>(3)</sup> Winterstein in Klein "Handbuch der Pflanzen Analyse," Vol. IV, Second part. Wien, 1933, p. 1403; Strain, J. Biol. Chem., 105, 523 (1934).