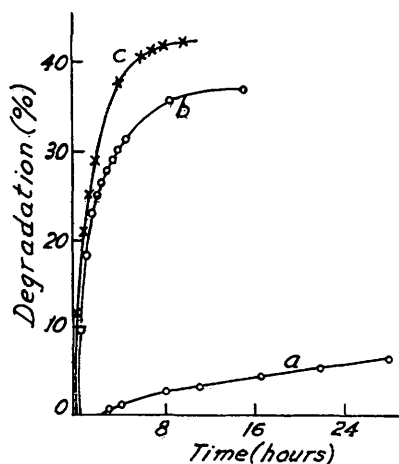


power of the groups R and R' (cf. Tables 1—5 and Figure). The last influence is verified by Herbst's finding (*J. Amer. Chem. Soc.*, 1936, **58**, 2239) that the rate of evolution of carbon dioxide during the reaction of pyruvic acid with certain α -amino-acids was highest with phenylglycine and lowest with α -aminoisobutyric acid.

The relative rates at which a proton is taken up at the two positions in the mesomeric anion (II) appear to be the most important factor in determining the proportions of the tautomerides (III) and (IV) formed. Thus, in the azomethine anion (V), where R is an electron-attracting group, R' is an alkyl group, and R'' is hydrogen or an alkyl group, the electron density will be higher on the γ - than on the α -carbon atom, thus leading to the preferential recombination of the proton at the former, to give the tautomeride (VI). However, the formation of this tautomeride is less probable than that of (VII), since the latter is stabilised by the conjugation of the $-\text{CH}:\text{N}-$ with the phenyl group, whereas (VI) is stabilised by the less effective hyperconjugation.

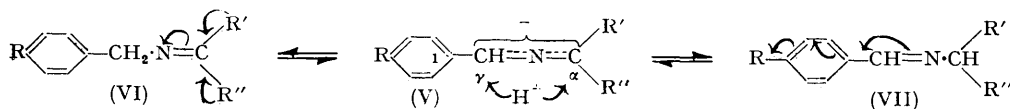
When the α -carbon atom is attached to a phenyl or a *m*-nitrophenyl group (V; R' = Ph or *m*-NO₂C₆H₄, R'' = H), the two tautomerides will be nearly equally stabilised by conjugation. The presence of electron-attracting groups explains why the amount of



The rate of degradation of alanine with *p*-nitrobenzaldehyde heated with (a) 50% glycerol, (b) 75% pyridine, on a boiling-water bath, and (c) 75% pyridine on an oil-bath at $120^{\circ} \pm 2^{\circ}$.

degradation of aromatic α -amino-acids such as phenyl- and *m*-nitrophenyl-glycine was always greater than that of aliphatic acids such as alanine, α -aminoisobutyric acid, and 1-aminocyclohexanecarboxylic acid.

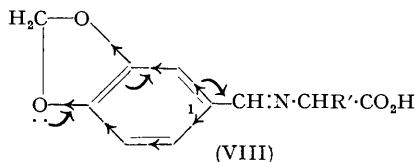
The experimental values showed that the amount of degradation of α -amino-acids with carbonyl compounds, *i.e.*, the proportion of the tautomeride (III), decreased with decrease



in the electron-attracting property of the group R in R·CHO. *E.g.*, it decreased from 2 : 4 : 6-trinitro- to *m*-nitro-, and from 2 : 4 : 6-trichloro- to *p*-chloro-benzaldehyde. The fact that *p*- was a weaker degrading agent than *o*- or *m*-chlorobenzaldehyde proved Baddar's previous assumption (*J.*, 1950, 136) that the chlorine atom in chlorobenzaldehydes operates, in this reaction, mainly by its inductive effect ($-I$). Moubasher's criticism (*J.*, 1951, 231) of Baddar's explanation (*loc. cit.*) of the high degrading power of *s*-dichloroacetone in comparison with the chlorobenzaldehydes is not valid: formation of hydrochloric acid by hydrolysis of dichloroacetone would decrease rather than increase the amount of acetaldehyde liberated from alanine (cf. Herbst and Engel, *J. Biol. Chem.*, 1934, **107**, 505; Herbst, *loc. cit.*), as it will inhibit the condensation of dichloroacetone with alanine (cf. Gulland and Mead, *loc. cit.*) and retard the prototropic change (cf. Ingold and

Shopee, *J.*, 1929, 1199). Experimentally, no acetaldehyde was liberated from alanine when it was refluxed with dichloroacetone in acidified water. Dichloroacetone was also capable of degrading alanine in dry dioxan at 100°; the reaction mixture gave practically no precipitate with silver nitrate acidified with nitric acid, *i.e.*, no hydrolysis took place (unpublished work).

The degrading power of hydroxybenzaldehydes was very low, and it decreased in the order $m- > o- > p-$. This is in agreement with the known facts that the *o*- and *p*-hydroxy-groups increase the electron density on $C_{(1)}$ (V ; $R = OH$) by their $+T$ effect.



Similarly, piperonaldehyde was a slightly stronger degrading agent than *p*-hydroxybenzaldehyde, since the methylenedioxy-group occupies both the *m*- and the *p*-position to $C_{(1)}$ (see VIII); this leaves that atom with less electron density than the corresponding atom in *p*-hydroxybenzaldehyde.

However, the degrading power of piperonaldehyde was found to be much less than that of *m*-nitrobenzaldehyde under similar conditions (cf. Moubasher, *loc. cit.*). The fact that glucose has a higher degrading power than the hydroxybenzaldehydes is not unexpected, since the hydroxyl groups in the former case operate by their $-I$ effect.

Steric factors appear not to play an important role in this degradation, especially in the case of *o*-monosubstituted benzaldehydes, since, in most cases, both *o*- and *p*-nitrobenzaldehyde degraded α -amino-acids to the same extent. Similarly, 2 : 4 : 6-trinitro- and 2 : 4 : 6-trichloro-benzaldehyde degraded most of the α -amino-acids to a greater extent than 2 : 4-dinitro- and 2 : 5-dichloro-benzaldehyde, respectively.

It is, therefore, evident from the present investigation that a carbonyl compound which fails to degrade a certain α -amino-acid under mild conditions may degrade it under more drastic conditions, *e.g.*, at a higher temperature, with longer heating, or with a stronger nucleophilic reagent. The results showed also that a given carbonyl compound, such as *p*-hydroxybenzaldehyde, could fail to degrade a certain α -amino-acid (*e.g.*, alanine), but could nevertheless degrade another, such as phenylglycine.

Finally, it may be mentioned that α -amino-acids (*e.g.*, alanine and phenylglycine) can be degraded by oxidising agents containing no carbonyl groups, such as bromine water.

EXPERIMENTAL

1-Aminocyclohexanecarboxylic Acid.—To aqueous ammonium chloride (40 g. in 100 c.c.), stirred at 0°, cyclohexanone (58.5 g.) in ether (100 c.c.) was added during 15 min., followed by, dropwise, aqueous sodium cyanide (32 g. in 70 c.c.), the temperature being kept below 12°. Stirring was continued for a further 1.5 hr., and the mixture was left overnight. The aqueous layer was extracted with ether (3×100 c.c.). The residue left on the evaporation of the combined ethereal extracts was dissolved in methyl alcohol (160 c.c.), cooled in ice, and saturated with dry ammonia, then kept for 4 days at room temperature. The excess of ammonia was removed by a current of air, and the methyl alcohol on the water-bath. The remaining liquid was treated with 48% hydrobromic acid (200 g.) and water (30 c.c.), the mixture extracted with ether, and the acid layer was refluxed for 3 hr., left overnight, and worked up as described for α -aminoisobutyric acid in *Org. Synth.*, 11, 5. The crystalline 1-aminocyclohexanecarboxylic acid (10 g.) was filtered off and recrystallised from water in colourless plates, m. p. 350° (Found: C, 58.6; H, 9.3; N, 10.0, 9.5. Calc. for $C_7H_{13}O_2N$: C, 58.7; H, 9.15; N, 9.8%) (cf. Cocker, Lapworth, and Peters, *J.*, 1931, 1391; Bucherer and Brandt, *J. pr. Chem.*, 1934, 140, 142).

Degradation of Alanine.—A mixture of alanine (0.294 g., 0.00337 mole) and the degrading agent (0.00337 mole) in 50% (v/v) aqueous glycerol (20 c.c.) or 75% (v/v) aqueous pyridine (20 c.c.) was heated on a boiling-water bath, sand-bath, or oil-bath in a carbon dioxide atmosphere (the rate of bubbling was kept nearly constant) for 3 hr. in an apparatus similar to that used by Schönberg *et al.* and Baddar (*loc. cit.*). The liberated acetaldehyde was estimated as its 2 : 4-dinitrophenylhydrazone, and identified by m. p. and mixed m. p. The greater the degrading power of the carbonyl compound, the faster the insoluble reactants went into solution.

The efficiency of the apparatus towards acetaldehyde was determined by refluxing a known weight of pure paraldehyde with dilute hydrochloric acid in a stream of carbon dioxide, the liberated acetaldehyde being collected as its 2 : 4-dinitrophenylhydrazone. In two experiments recovery of acetaldehyde from paraldehyde was 83.8 and 82.8% (mean correction factor 0.833). Although the results are not of absolute quantitative significance they show that the groups behave in the expected order. The results are in Table 1.

TABLE 1.

Degrading agent	Degradation (%)			
	50% G (H ₂ O-bath)	50% G * (B. p.)	75% P † (H ₂ O-bath)	75% P ‡ (120° ± 2°)
Isatin	40.0	60.0	36.8	55.0
2 : 4 : 6-Trinitrobenzaldehyde	7.0	46.4	36.0	48.8
2 : 4-Dinitrobenzaldehyde	7.0	42.4	29.6	47.5
<i>p</i> -Nitrobenzaldehyde	0.6 ¹	16.0 ²	27.2 ³	40.3 ⁴
<i>o</i> -Nitrobenzaldehyde	0.6	6.4 ^{2a}	25.6	40.8
<i>m</i> -Nitrobenzaldehyde	—	3.2 ^{2a}	9.6 ^{2a}	23.5
2-Chloro-5-nitrobenzaldehyde	0	12.8	24.0	35.0
2 : 4 : 6-Trichlorobenzaldehyde	—	9.0	28.0	45.0
2 : 5-Dichlorobenzaldehyde	—	3.2	20.9	30.0
<i>o</i> -Chlorobenzaldehyde	—	ca. 1.6 ⁵	14.4 ⁵	24.3
<i>m</i> -Chlorobenzaldehyde	—	0	10.4 ⁵	17.0
<i>p</i> -Chlorobenzaldehyde	—	0	3.7	9.3
Piperonaldehyde	—	0	0	Trace
<i>m</i> -Hydroxybenzaldehyde	—	—	—	4.8
Salicylaldehyde	—	—	—	3.0
<i>p</i> -Hydroxybenzaldehyde	—	0	—	0 ⁶
Glucose	—	4.8	4.8	18.6
<i>s</i> -Dichloroacetone	—	ca. 8.0 ⁷	—	—
1 : 3 : 5-Trinitrobenzene	—	—	—	2.0

G = glycerol; P = pyridine. The results were the mean of at least two experiments, and were reproducible within ±5%.

*, †, ‡ The temperatures of the reaction mixtures were 117–120°, 90–92°, and 97–100°, respectively. Although the b. p. of 75% pyridine was less than 100°, the temperature of the bath should be kept around 120° in order to maintain the solution refluxing regularly.

¹, ³, ⁴ These experiments were repeated and the heating was continued for a long time or until no more acetaldehyde was liberated. The 2 : 4-dinitrophenylhydrazone was collected at intervals. The results are shown in the Figure (curves *a*, *b*, and *c*, respectively). ² (*a*) Calc. on the basis of Baddar's results (*J.*, 1949, S 163). (*b*) When alanine (0.294 g., 1 mol.) was heated with *p*-nitrobenzaldehyde (0.5 g., 1 mol.) in a mixture of glycerol (10 c.c.), water (5 c.c.), and concentrated hydrochloric acid (5 c.c.), degradation amounted to about 4.8%. ³ Two experiments were carried out: in one, alanine (0.294 g., 1 mol.) was heated with *p*-nitrobenzaldehyde (1.0 g., 2 mols.); in the second, alanine (0.588 g., 2 mols.) was heated with *p*-nitrobenzaldehyde (0.5 g., 1 mol.). The amounts of degradation were slightly higher (32.0 and 31.1%, respectively). ⁵ Calc. on the basis of Baddar's results (*J.*, 1950, 136). ⁶ With 5 hours' refluxing, traces of acetaldehyde 2 : 4-dinitrophenylhydrazone were precipitated. ⁷ When a mixture of alanine (0.588 g., 1 mol.), *s*-dichloroacetone (0.5 g., 1 mol.), concentrated hydrochloric acid (0.5 c.c.), and water (20 c.c.) was refluxed for 3 hr., no acetaldehyde was liberated.

Degradation of α-Aminoisobutyric Acid.—A mixture of α-aminoisobutyric acid (0.218 g., 1 mol.) and the carbonyl compound (1 mol.) in 75% (v/v) pyridine (10 c.c.) was refluxed on an oil-bath at 120° ± 2° (temp. of the reaction mixture = 97–100°) for 3 hr. in a stream of carbon dioxide. The liberated acetone was collected as its 2 : 4-dinitrophenylhydrazone and identified by m. p. and mixed m. p.

In a control experiment carried out to determine the correction factor, a known weight of pure acetone was heated in the same apparatus and under identical conditions, and the recovered acetone was determined. In two experiments (78.1 and 80.1%). The mean was used in the calculation of the values in Table 2, which were reproducible within ±5% (based on the weight of the 2 : 4-dinitrophenylhydrazone).

Degradation of 1-Aminocyclohexanecarboxylic Acid.—This α-amino-acid (0.3 g.) was heated as in the preceding experiments. The reaction mixture was acidified with 3*N*-hydrochloric acid, refluxed for 20 min., and distilled in a stream of carbon dioxide until the distillate gave no precipitate with 2 : 4-dinitrophenylhydrazine reagent, or until the m. p. of the precipitated hydrazone indicated the absence of the cyclohexanone derivative. The precipitated hydrazone was filtered off, dried, and weighed. For determining its composition, and calculating the percentage of degradation, the following control experiments were carried out :

(i) A known weight of cyclohexanone was mixed with 75% aqueous pyridine and treated as

above. Recovery of *cyclohexanone* in two experiments was 88.3 and 90.7%, giving the required correction factor. With 2 : 4 : 6-trinitrobenzaldehyde, the correction factor was 0.859.

TABLE 2.

Carbonyl compound	Degradation (%)	Carbonyl compound	Degradation (%)
Isatin	57.6	2-Chloro-5-nitrobenzaldehyde ...	34.3
2 : 4 : 6-Trinitrobenzaldehyde ...	56.4	2 : 4 : 6-Trichlorobenzaldehyde	32.6
2 : 4-Dinitrobenzaldehyde	53.9	2 : 5-Dichlorobenzaldehyde	22.6
<i>p</i> -Nitrobenzaldehyde	43.9	<i>o</i> -Chlorobenzaldehyde	16.3
<i>o</i> -Nitrobenzaldehyde	47.6	<i>m</i> -Chlorobenzaldehyde	9.8
<i>m</i> -Nitrobenzaldehyde	19.0	<i>p</i> -Chlorobenzaldehyde	0

(ii) Since *cyclohexanone* 2 : 4-dinitrophenylhydrazone was soluble in hot aqueous alcohol, whereas those of the degrading agents were practically insoluble, the two hydrazones could be separated by extraction with hot aqueous alcohol. A mixture containing a known weight of *cyclohexanone* and *m*-nitro- or *p*-chloro-benzaldehyde 2 : 4-dinitrophenylhydrazone was extracted with a known volume of hot alcohol, filtered while hot, and washed with hot aqueous alcohol. The filtrate was diluted with water and allowed to crystallise. The weight of recovered *cyclohexanone* 2 : 4-dinitrophenylhydrazone was found to be 93.3 and 93.7% of the original (correction factor = 0.935).

For carbonyl compounds which were not volatile in steam, such as isatin, 2 : 4 : 6-trinitro-, 2 : 4-dinitro-, and *p*-hydroxy-benzaldehyde, correction factor (i) was used. For carbonyl compounds which were volatile in steam, both correction factors (i) and (ii) had to be taken into consideration.

For the chlorobenzaldehydes, the composition of the mixed hydrazones was determined by both extraction with alcohol and chlorine content. Each experiment was repeated twice and was found to be reproducible within $\pm 5\%$. The values in Table 3 represent the results.

TABLE 3.

Carbonyl compounds	Degradation (%)			
	By wt. of hydrazone *	By EtOH extract	By Cl content	By N content
Isatin	65.1	—	—	—
2 : 4 : 6-Trinitrobenzaldehyde...	54.0	—	—	—
2 : 4-Dinitrobenzaldehyde	62.3	—	—	—
<i>p</i> -Nitrobenzaldehyde	—	59.4	—	—
<i>o</i> -Nitrobenzaldehyde	—	59.4	—	—
<i>m</i> -Nitrobenzaldehyde	—	25.0	—	—
2-Chloro-5-nitrobenzaldehyde...	—	55.3	—	—
2 : 4 : 6-Trichlorobenzaldehyde	—	59.4	57.3	—
2 : 5-Dichlorobenzaldehyde.....	—	52.1	—	50.5
<i>o</i> -Chlorobenzaldehyde	—	33.9	33.9	—
<i>m</i> -Chlorobenzaldehyde	—	18.4	17.2	—
<i>p</i> -Chlorobenzaldehyde	—	12.7	12.5	—
Piperonaldehyde	—	3.9	—	—
<i>p</i> -Hydroxybenzaldehyde	2.9	—	—	—

* The precipitated derivative was found to be pure *cyclohexanone* 2 : 4-dinitrophenylhydrazone by m. p. and mixed m. p.

Degradation of Phenylglycine.—Phenylglycine (0.32 g., 1 mol.) was heated for 3 hr. under carbon dioxide with the carbonyl compound (1 mol.) in 75% pyridine (10 c.c.) at $120^\circ \pm 2^\circ$ (oil-bath), or on a boiling-water bath, with 50% glycerol at $145^\circ \pm 5^\circ$ (oil-bath) or on a boiling-water bath. For the estimation of benzaldehyde produced (Table 4), the following procedures were adopted.

(i) For degrading agents which were not volatile in steam. The mixture was treated with concentrated hydrochloric acid (35 c.c.) and water (5 c.c.), and refluxed for 20 min. Water (100 c.c.) was added, and the mixture distilled in a stream of carbon dioxide. The distillate was treated with phenylhydrazine hydrochloride solution, and the precipitated hydrazone was filtered off. In a control experiment recovery of benzaldehyde was only 78% [this correction factor was used for procedure (i)]; with 2 : 4 : 6-trinitrobenzaldehyde, recovery of benzaldehyde was 72%.

(ii) For nitrobenzaldehydes. The mixture was treated with concentrated hydrochloric acid (30 c.c.), a 10% solution of stannous chloride in concentrated hydrochloric acid (20 c.c.), and water (5 c.c.), and heated on a boiling-water bath for 30 min. in a stream of carbon dioxide. Water (100 c.c.) was added, the mixture steam-distilled, and the benzaldehyde estimated as its

phenylhydrazone. In a control experiment with benzaldehyde and *p*-nitrobenzaldehyde recovery was 82.5% (correction factor). With 2 : 4 : 6-trinitrobenzaldehyde it was 74.4%.

(iii) For degrading agents which were volatile in steam. (a) The mixture was refluxed with concentrated hydrochloric acid (30 c.c.) and water (5 c.c.), and treated with few c.c. of alcohol (aldehyde-free), warmed on a water-bath until clear, then mixed with excess of 2 : 4-dinitrophenylhydrazine hydrochloride. The precipitated hydrazone was filtered off. Its weight was calculated, by difference, from the chlorine content of the mixture. This method was adopted in the case of chlorobenzaldehydes.

TABLE 4.

Carbonyl compound	Degradation (%)			Procedure
	75% P at 120°	75% P at 100°	50% G at 145°	
Isatin	85.3	83.9	—	(i)
2 : 4 : 6-Trinitrobenzaldehyde	77.7	—	—	(ii)
2 : 4-Dinitrobenzaldehyde	70.6	—	—	(ii)
<i>p</i> -Nitrobenzaldehyde	66.2	65.3	—	(ii)
<i>o</i> -Nitrobenzaldehyde	66.2	—	—	(ii)
<i>m</i> -Nitrobenzaldehyde	32.3	—	—	(ii)
2-Chloro-5-nitrobenzaldehyde	64.7	—	—	(ii)
2 : 4 : 6-Trichlorobenzaldehyde	62.4	—	—	(iii, b)
2 : 5-Dichlorobenzaldehyde.....	59.2 ¹	—	—	(iii, a, b)
<i>o</i> -Chlorobenzaldehyde	40.4 ²	—	—	"
<i>m</i> -Chlorobenzaldehyde	31.8 ³	—	—	"
<i>p</i> -Chlorobenzaldehyde	19.3 ⁴	—	—	"
Piperonaldehyde	8.2	—	2.0	(iii, b) ⁵
<i>m</i> -Hydroxybenzaldehyde	12.1	—	—	(i)
<i>p</i> -Hydroxybenzaldehyde	5.5	—	0 ⁶	(i)

P = pyridine; G = glycerol.

¹ Mean of 57.7 and 60.7%. ² Mean of 41.6 and 39.1%. ³ Mean of 29.3 and 34.3%. ⁴ Mean of 18.6 and 20.1%. ⁵ The two derivatives were separated by extraction with hot alcohol in which the benzaldehyde derivative was slightly soluble, and that of piperonaldehyde was insoluble. ⁶ In glycerol in an oil-bath at 165° ± 5°, the degradation was 9.0%.

Control experiments with benzaldehyde gave 87.9 and 88.1% recovery (correction factor).

(b) The mixture was heated with hydrochloric acid as in (a), and then steam-distilled. The distillate was treated with few c.c. of alcohol (aldehyde-free), heated on a water-bath until clear, and mixed with excess of 2 : 4-dinitrophenylhydrazine hydrochloride. The precipitate was analysed for chlorine, from which the amount of benzaldehyde in the mixture was calculated. Control experiments with benzaldehyde gave recoveries of 77.8 and 76.1%.

Degradation of m-Nitrophenylglycine.—*m*-Nitrophenylglycine (0.42 g., 1 mol.) (Langenbeck *et al.*, *Annalen*, 1931, 485, 59) and chlorobenzaldehyde (1 mol.) in 75% (v/v) pyridine (10 c.c.) were refluxed under carbon dioxide in an oil-bath heated at 120° ± 2° for 3 hr. The insoluble reactants dissolved faster than in parallel experiments with the other α -amino-acids. When

TABLE 5.

Carbonyl compound	Degradation (%)		
	Procedure ii	Procedure iii, b	
		Cl content	N content
2 : 5-Dichlorobenzaldehyde	70.5	70.8	—
<i>o</i> -Chlorobenzaldehyde	55.7	56.6	57.05
<i>m</i> -Chlorobenzaldehyde	50.0	49.8	—
<i>p</i> -Chlorobenzaldehyde	42.3	41.5	—

the mixture was acidified with dilute hydrochloric acid and distilled, the chlorobenzaldehyde, being much more volatile in steam than *m*-nitrobenzaldehyde, distilled in the first fractions. The last fractions contained the degradation product, *m*-nitrobenzaldehyde, which was identified as its 2 : 4-dinitrophenylhydrazone. Under similar conditions, *m*-nitrophenylglycine was not degraded in absence of carbonyl compounds. However, this method could not be used for the quantitative estimation of *m*-nitrobenzaldehyde owing to its low volatility in steam. Its estimation was, therefore, achieved indirectly by determining the amount of the chlorobenzaldehyde. Since the decrease in the original amount of this aldehyde would be equivalent to the amount of *m*-nitrobenzaldehyde produced in the reaction, the amount of degradation could be thus determined (Table 5).

The amount of the chlorobenzaldehyde was estimated by the following methods :

(1) The mixture was treated according to procedure (ii) for phenylglycine, then the chlorobenzaldehyde in the distillate was precipitated as its 2 : 4-dinitrophenylhydrazone. A control experiment gave the correction factor.

(2) The mixture was treated according to procedure (iii, *b*), and the amount of the chlorobenzaldehyde in the mixture of the 2 : 4-dinitrophenylhydrazones was estimated from the chlorine or nitrogen content. Control experiments gave the correction factors.

p-Hydroxybenzaldehyde degraded *m*-nitrophenylglycine in boiling 75% pyridine or 50% glycerol, but it was difficult to estimate the degradation quantitatively.

Degradation of α -Amino-acids with Oxidising Agents.—When alanine or phenylglycine was shaken with bromine water for 15—20 min., acetaldehyde and benzaldehyde were produced, being identified as their 2 : 4-dinitrophenylhydrazone and phenylhydrazone, respectively.

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