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THE MECHANISM OF OSAZONE FORMATION

M. M. SHEMYAKIN
Institute for Chemistry of Natural Products, USSR Academy of Sciences,
Moscow, USSR

V. I. MAIMIND, K. M. ERMOLAEV and E. M. BAMDAS
Institute of Biological and Medical Chemistry, USSR Academy of Medical Sciences,
Moscow, USSR

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Abstract—It has been shown by means of ^{15}N that the *p*-nitrophenylhydrazones of benzoin, cyclohexanolone and D-fructose form osazones according to Weygand's Scheme A. The intermediate monoimino-α-diketones postulated in this mechanism have been isolated in the form of their N-acyl derivatives.

DESPITE repeated attempts to elucidate osazone formation from α-hydroxycarbonyl compounds and arylhydrazines,* the mechanism of this interesting reaction still remains obscure. It may be considered as established that the first stage in the reaction is the formation of arylhydrazones from one mole each of the arylhydrazine and the α-hydroxycarbonyl compound.[4,5] The further conversion of the hydrazones into osazones according to Fischer[4] involves the oxidation of the hydrazone by a second mole of arylhydrazine into a hydrazone of the ozone, which condenses with a third mole of the arylhydrazine (see Scheme). In 1940, Weygand[6] proposed two other mechanisms (Scheme A and Scheme B) differing from Fischer's hypothesis in that the oxidation-reduction processes responsible for the osazone formation occur intramolecularly. Schemes suggested by other authors* do not differ in principle from the ones mentioned.

In recent years Weygand *et al.*,[3,7,8] on the basis of their investigations, in particular, with the aid of deuterium and tritium, came to the conclusion that formation of the osazones proceeds simultaneously by both mechanisms A and B.

Contrary to this view, the results of an investigation which is summarized in this paper, have led to the inference that the osazone formation can be described by means of only Scheme A without resort to Scheme B.

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* For references to the literature on this problem see our previous publications[1,2] as well as the paper by Weygand et al.[3]

[1] M. M. Shemyakin and V. I. Maimind, *Dokl. Akad. Nauk USSR* **102,** 1147 (1955).

[2] M. M. Shemyakin, V. I. Maimind, K. M. Ermolaev and E. M. Bamdas, *Dokl. Akad. Nauk USSR* **128,** 564 (1959); *Chem. & Ind.* 1195 (1959).

[3] F. Weygand, H. Simon and J. F. Klebe, *Chem. Ber.* **91,** 1567 (1958).

[4] E. Fischer, *Ber. Dtsch. Chem. Ges.* **20,** 821 (1887); *Ibid.* **23,** 2114 (1890).

[5] V. Wolf, *Chem. Ber.* **86,** 840 (1953).

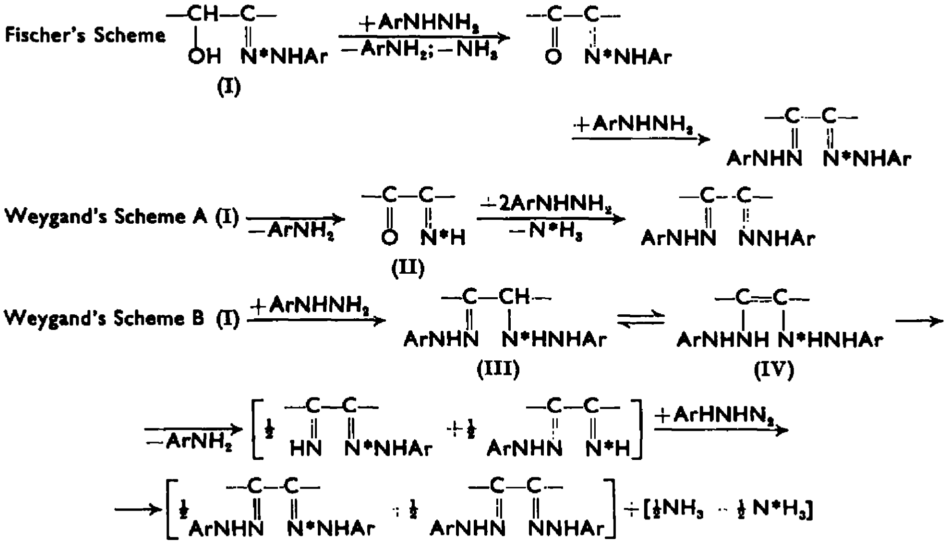
[6] F. Weygand, *Ber. Dtsch. Chem. Ges.* **73,** 1284 (1940).

* See footnote on p. 1.

[7] H. Simon, K. D. Keil and F. Weygand, *Chem. Ber.* **95,** 17 (1962).

[8] H. Simon, H. D. Dorrer and A. Trebst, *Chem. Ber.* **96,** 1285 (1963).

The first proof of the validity of Scheme A was obtained with the aid of ^{15}N. If β-^{15}N-arylhydrazones and unlabelled arylhydrazine are used as reactants, then, as one can readily see from the schemes presented (^{15}N is designated by N*) the question of whether the oxidoreduction reaction occurs intramolecularly at the arylhydrazone (I) stage (Weygand's Scheme A), or the hydrazone–hydrazide (III $\rightleftharpoons$ IV) stage (Weygand's Scheme B), or whether it occurs intermolecularly (Fischer's Scheme) can be resolved on the basis of a study of the isotopic composition of the end products (osazone and ammonia).



Indeed, if Fischer's Scheme holds then ammonia, in this case resulting from reduction of the unlabelled arylhydrazine, should not contain the excess ^{15}N, which is then to be found completely in the osazone. On the other hand, if it is Weygand's Scheme A that obtains the ammonia, stemming in this case solely from the labelled hydrazone should contain all the excess ^{15}N, with none present in osazone. Finally, in the case of Scheme B both ammonia and the osazone should each contain half of the excess ^{15}N present in the initial labelled hydrazone, because formation of these two end products is preceded by the symmetric tautomeric form (IV), owing to which the formation of labelled and unlabelled ammonia and osazone molecules are equally probable.

Unfortunately, owing to possible dilution of the label through exchange of the labelled hydrazine residue in the hydrazone or osazone for the unlabelled residue by hydrolysis or direct substitution, a ^{15}N-tracer study of osazone formation meets with considerable difficulties. The ^{15}N content in the end products and in the unreacted starting materials can thereby fluctuate within wide limits (Table 1).

Although the reaction conditions are restricted such an approach is still valid provided the exchange does not assume appreciable proportions.

The β-^{15}N-*p*-nitrophenylhydrazones of various α-hydroxy-ketones, namely benzoin cyclohexanolone and D-fructose, were selected for investigation, their conversion to the osazones being carried out either in boiling isoamyl alcohol or in glacial acetic acid

TABLE 1

Reaction mechanism	^{15}N Content in % to Initial							
	Ammonia		Osazone		Unreacted hydrazone		Unreacted hydrazine	
	No exchange	Complete exchange	No exchange	Complete exchange	No exchange	Complete exchange	No exchange	Complete exchange
Fischer's Scheme	0	33	100	33	100	33	0	33
Weygand's A Scheme	100	33	0	33	100	33	0	33
Weygand's B Scheme	50	33	50	33	100	33	0	33

at 50°. In order to exclude exchange through hydrolysis, non-aqueous solvents were used, while in the runs with isoamyl alcohol the water formed was distilled off in the course of the reaction. Moreover, the reaction was usually discontinued after a certain time in order that the accompanying exchange should not reach too high a value and so distort the true picture of the process. A device was also provided for by means of which in a number of cases the unreacted arylhydrazone and arylhydrazine, whose isotopic composition could be utilized as control of the degree of exchange, could be isolated. When the reaction was carried out in isoamyl alcohol, the ammonia formed was immediately distilled off. The isolation and separation of the osazone, arylhydrazone and arylhydrazine at the end of the heating period was carried out as rapidly as possible and under conditions inhibiting isotopic exchange.

The data presented in Table 2 allow a quite definite choice of the reaction mechanism to be made, since exchange of hydrazine residues in all cases was only to an extent that did not appreciably influence the result. (The ^{15}N content of the β-N-atom of unreacted *p*-nitrophenylhydrazone was always more and in *p*-nitrophenylhydrazine less than 33% of the initial value). It should also be pointed out that the decrease in ^{15}N content in the unreacted arylhydrazone is compensated for by the appearance of ^{15}N in the unreacted arylhydrazine, indicating the absence of all other side reactions

TABLE 2

No of run	*p*-Nitrophenyl-hydrazone (I) (39·6 At% ^{15}N Referred to β-N-Atom)	Medium	Time min.	^{15}N Content							
				Ammonia		β-N-Atoms of osazone		β-N-Atom of unreacted hydrazone		β-N-Atom of unreacted hydrazine	
				At%	% of initial I	At%	% of initial I	At%	% of initial I	At%	% of initial I
1	D-Fructose	i-AmOH	10	35·6	90			32·2	81	3·6	9·1
2	Cyclo-hexanolone	i-AmOH	5	23·4	59·1			23·8	60	7·9	20
3	Benzoin	i-AmOH	360	39·2	98·5			39·4	99·5	0·7	1·7
4	Benzoin	AcOH	330	20·1	50·8	21·1	53·2			7·9	20

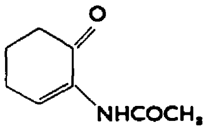
other than exchange. For example, under the given reaction conditions no decomposition into aniline, ammonia, nitrogen and benzene, as observed by some authors[9,10] on the example of phenylhydrazine, took place (with the exception of run 3; see Experimental).

The results obtained (runs 1–3) showed that ammonia evolved in the beginning of the reaction always contained more than 50% of the ^{15}N contained in the initial arylhydrazone. Even without accounting for exchange of the hydrazine residues this means that Fischer's Scheme is invalid, and that Weygand's Scheme B cannot play any significant part in osazone formation. If a correction be made for the exchange which, judging from the ^{15}N content in the arylhydrazone and arylhydrazine equals 20, 40 and about 2%, respectively, the content of the label in the ammonia practically coincides with the initial content. It thus follows that osazone formation proceeds almost exclusively according to Weygand's Scheme A, the contribution by the other mechanisms (including Weygand's Scheme B) not exceeding a few percent.

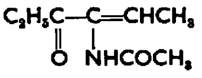
In run 4, the ^{15}N content of ammonia and of the osazone was found to be about equal to that required by Weygand's B Scheme.* However, the high content of ^{15}N found later in the unreacted *p*-nitrophenylhydrazine (isolated in the form of the acetyl derivative) indicates that under these conditions intensive exchange occurs, so that this run cannot be considered as indicative of the operation of Scheme B.

The results of the ^{15}N study of osazone formation made possible another approach to the proof of the reaction mechanism. Since, in contrast to the other two schemes the first stage of osazone formation according to Weygand's A Scheme proceeds without participation of arylhydrazine, an attempt was made to isolate the intermediate ketimines (II) formed in the course of this reaction. On heating cyclohexanolone-*p*-nitrophenylhydrazone in glacial acetic acid containing 2 moles of acetic anhydride we were able to obtain in high yield the N-acetyl derivative of cyclohexane-1,2-dione monoimine in addition to *p*-nitroacetanilide. A study of the IR spectrum (the presence of amide and conjugated keto group) showed that in the crystalline state the compound has an enamine structure and is 2-acetylaminocyclohexen-2-one (V). Its *p*-nitrophenyl hydrazone has a similar structure and its heating with *p*-nitrophenylhydrazine in aqueous solution in the presence of acid (necessary to split off the N-acetyl group) led to the corresponding osazone in high yield. These reactions thus provide straightforward proof that ketimines of type II are intermediates in osazone formation.

Propioin-*p*-nitrophenylhydrazone like the arylhydrazone of cyclohexanolone decomposes under similar conditions and with the equal ease into *p*-nitroacetanilide and ketimine, whose N-acetyl derivative also exists in the enamine form (VI).



V



$$C_2H_5\underset{\overset{\|}{O}}{C}-\underset{\overset{|}{NHCOCH_3}}{C}=CHCH_3$$

VI

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* It was because of results such as these that the erroneous conclusion that Scheme B holds was made in the preliminary communication.[1]

[9] B. Glassmann and Rochworger-Walbe, *Ber. Dtsch. Chem. Ges.* **61**, 1444 (1928).

[10] F. Micheel and J. Dijong, *Liebig's Ann.* **669**, 136 (1963).

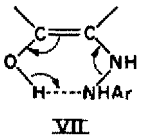
The arylhydrazones of benzoin and fructose also undergo similar splitting and although the corresponding ketimines were not isolated, their formation was proved in the following way.

Prolonged heating of benzoin-*p*-nitrophenylhydrazone in acetic acid does not yield the osazone, but if the solution is cooled and two moles of *p*-nitrophenylhydrazine are added in the cold (i.e. under conditions when the osazone is not formed at any appreciable rate from the benzoin-*p*-nitrophenylhydrazone) the osazone rapidly crystallizes out and one can easily isolate *p*-nitroaniline and ammonia from the filtrate. When a similar experiment is carried out with benzoin-β-^{15}N-*p*-nitrophenylhydrazone and unlabelled *p*-nitrophenylhydrazine (run 5 in Table 3) the ammonia contains all of the original ^{15}N and the osazone is found to be unlabelled. It thus follows that at the moment of arylhydrazine addition the solution already contains instead of the original arylhydrazone, the corresponding ketimine of type (II), forming the osazone by reaction with the arylhydrazine under milder conditions than in the case of the arylhydrazone itself. Similar heating of D-fructose-β-^{15}N-*p*-nitrophenylhydrazone in acetic acid in the presence of acetic anhydride also leads to the formation in quantitative yield of unlabelled *p*-nitroacetanilide and of ammonia containing almost the entire initial ^{15}N (run 7 in Table 3).

TABLE 3

No of run	*p*-Nitrophenyl-hydrazone (I) (39·6 At% ^{15}N referred to β-N-atom	Reaction time	^{15}N Content: Ammonia: At%	Ammonia: % of initial I	β-N-Atoms of osazone: At%	β-N-Atoms of osazone: % of initial I
5	Benzoin	8 hr	39·0	98·5		
6	Benzoin	48 hr	35·8	90·4	2·7	6·9
7	D-Fructose	10 min	38·3	96·0		

The N—N bond rupture we have observed in the α-hydroxy-ketone arylhydrazones is apparently common to all compounds of this class and proceeds intramolecularly in the 6-membered complex (VII).



VII

It can thus be seen that osazone formation proceeds almost entirely according to Weygand's A Scheme.

EXPERIMENTAL

Reactants

β-^{15}N-*p-Nitrophenylhydrazine* was obtained from 6·9 g $Na^{15}NO_2$[11] under conditions as described for the unlabelled compound[12]; yield 70%, m.p. 157° (from toluene), ^{15}N content of the β-N-atom 39·6 At%.

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[11] V. I. Maimind, B. V. Tokarev, E. Gomes, R. G. Vdovina, K. M. Ermolaev and M. M. Shemyakin, *Zh. Obshch Khim.* **26**, 1962 (1956).

[12] H. E. Fierz-David and L. Blangey *Grundlagende Operationen der Farbchemie.* Springer-Verlag, Wien (1952).

β-^{15}N-p-Nitrophenylhydrazones of D*-fructose and benzoin* were prepared by refluxing in MeOH similar to the synthesis of the unlabelled compounds.[13] The ^{15}N content corresponded to the initial β-^{15}N-*p*-nitrophenylhydrazine.

β-^{15}N-p-Nitrophenylhydrazone of cyclohexanolone was prepared by mixing freshly distilled cyclohexanolone and a warm (50°) solution of β-^{15}N-*p*-nitrophenylhydrazine in MeOH. The mixture was immediately cooled and kept at $-20°$ for 2–3 hr. The yield was 80%, m.p. 172° (MeOH) (cf.[14]).

Propioin-p-nitrophenylhydrazone was prepared by stirring a methanolic solution of the reactants for 40 min; the m.p. of the product, after crystallization from EtOH and then from toluene, was 134°; yield 80%.

Study of osazone formation

Runs 1–3. The reaction was carried out in a dual-chambered vessel furnished with a N_2 inlet and a condenser with a Kjeldahl head connected to a receiver containing standardized HCl to absorb the ammonia formed. In the compartments, stoichiometric amounts of β-^{15}N-*p*-nitrophenylhydrazone and unlabelled *p*-nitrophenylhydrazine were placed and followed by isoamyl alcohol in amounts of 1·25 ml for each 0·001 mole of *p*-nitrophenylhydrazine, 2·5 ml for each 0·001 mole of cyclohexanolone and benzoin-*p*-nitrophenylhydrazones and 5 ml in the case of fructose-*p*-nitrophenylhydrazone. The contents of each chamber was heated to boiling while N_2 was passed through, until the solid had completely dissolved, following which the solutions were mixed together by a turn of the vessel, with continuation of the boiling in a current of N_2. After some time (Table 2) the heating was stopped and the vessel immediately cooled by a mixture of ice and salt.

In the case of cyclohexanolone-arylhydrazone the resultant precipitate was immediately separated and treated with 10 ml of 5% HCl, the undissolved osazone was filtered off, washed with water and crystallized from dioxan. The *p*-nitrophenylhydrazine was precipitated from the acid solution by sodium acetate and crystallized from toluene. The primary alcoholic filtrate was kept in the cooling mixture for 2 hr, the precipitated arylhydrazone filtered off, washed with cooled MeOH and ether and then with 5% HCl and water, following which it is crystallized from chlorobenzene.

In the case of the fructose and benzoin-arylhydrazones the precipitate formed on cooling contained a mixture of osazone, hydrazone and hydrazine. This was quickly filtered off, washed with cooled alcohol and ether and then repeatedly extracted with boiling ether. The arylhydrazine obtained after distilling off the ether was crystallized from toluene. From the residual mixture the arylhydrazone was extracted with hot alcohol and then crystallized from MeOH in the case of benzoin and from EtOH in the presence of activated carbon in the case of fructose. The benzoin osazone which remained was crystallized from dioxan while the fructose osazone was precipitated from pyridine with isobutyl ether.

The results of the mass spectrometric determinations are presented in Table 2.*

Run 4. Benzoin-β-^{15}N-*p*-nitrophenylhydrazone (0·001 mole) and 0·002 mole of unlabelled *p*-nitrophenylhydrazine were heated with 1·4 ml anhydrous acetic acid in a sealed test tube at 50° for 5·5 hr. The precipitated osazone was filtered off, washed with boiling alcohol and crystallized from dioxan. The acetic acid filtrate was diluted with water and filtered; HCl was added and the filtrate evaporated to dryness. The residue was made alkaline and ammonia driven off in the cold in a current of N_2.

Runs 5 *and* 6. Benzoin-β^{15}-N-*p*-nitrophenylhydrazone (0·001 mole) and 2 ml anhydrous acetic acid were heated in a sealed test tube at 50° for the times indicated in Table 3. After cooling, a solution of 0·002 moles unlabelled *p*-nitrophenylhydrazine in 2 ml acetic acid was added. The rapidly precipitated osazone was filtered off and then treated as in run 4.

Run 7. Fructose-β-^{15}N-*p*-nitrophenylhydrazone (0·001 mole), 4 ml glacial acetic acid and 1 ml acetic anhydride were heated at 100° for 10 min. The solution was then poured into water and kept

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* In the case of benzoin, owing to the length of the run, part of the ammonia was due to decomposition of *p*-nitrophenylhydrazine (cf.[9]). The amount of ammonia due to the side reaction was determined in a control run which differed only in the absence of arylhydrazone. This run was also repeated with labelled *p*-nitrophenylhydrazine.

[13] W. Teilacker and P. Tröster, *Liebig's Ann.* **572,** 144 (1951).

[14] R. Willstätter and E. Sonnenfeld, *Ber. Dtsch. Chem. Ges.* **46,** 2957 (1913).

at 0° for 15–20 hr. The precipitated *p*-nitroacetanilide was filtered off and the filtrate evaporated; the residue was triturated with water, then kept again at 0° and the additional *p*-nitroacetanilide precipitated, filtered off (yield ca. 100%). After making the filtrate alkaline the ammonia was distilled off in the cold in a current of N_2.

The results of the mass spectrometric measurements in runs 5–7 are given in Table 3.

Isolation of acetylketimines

2-N-*Acetylaminocyclohexen*-2-*one* (V). A mixture of 25 ml acetic anhydride and 50 ml glacial acetic acid was added to 0·1 mole cyclohexanolone-*p*-nitrophenylhydrazone and the flask immersed in a bath heated to 100°. After 2–3 min a clear solution was formed, and the temp quickly reached 115–130°. The heating was stopped when the temp of the mixture again became equal to the bath temp (ca. 5 min). After cooling, the *p*-nitroacetanilide was filtered off, washed with abs. ether and the filtrate evaporated *in vacuo* (1–2 mm), the bath temp being gradually raised to 100°. The residue was mixed with 100 ml ether and kept at 0° for 24 hr. On removing the additionally precipitated *p*-nitroacetanilide (over-all yield 80%) the solvent was distilled off and the residue distilled *in vacuo* (0·01–0·1 mm) gradually raising the bath temp to 180–190°. The oily distillate readily crystallized. The substance was recrystallized from ether (with activated carbon); yield 80%, m.p. 64°. (Found: C, 62·64; H, 7·39; N, 9·20. Calc. for $C_8H_{11}O_2N$: C, 62·72; H, 7·24; N, 9·15%.) IR spectrum: 3294 cm^{-1} (NH); 1689, 1670 cm^{-1} (conjugated keto-group and amide carbonyl); 1505 cm^{-1} (amide II).

The 2-N-*acetylaminocyclohexen*-2-*one*-p-*nitrophenylhydrazone* was obtained in aqueous solution, the precipitate being filtered off after keeping the mixture at 0° for 10–12 hr, yield 80%, m.p. 221° from MeOH. (Found: C, 58·35; H, 5·67; N, 19·38. Calc. for $C_{14}H_{16}O_3N_4$: C, 58·32; H, 5·59; N, 19·44%.)

Cyclohexanolone-p-*nitrophenylosazone* was obtained by refluxing 0·0005 mole 2-N-acetylamino-cyclohexen-2-one-*p*-nitrophenylhydrazone with 0·0005 mole *p*-nitrophenylhydrazine in 5 ml alcohol and 2 ml 1 N HCl (30 min); yield 83%, m.p. 250° from dioxan.

3-N-*Acetylaminohexen*-2-*one*-4 (VI) was prepared using the conditions described above for V; yield 46%, m.p. 52–53°, the *p*-nitroacetanilide simultaneously formed was isolated in 80% yield. (Found: C, 61·85; H, 8·50; N, 8·84. Calc. for $C_8H_{13}O_2N$: C, 61·91; H, 8·44; N, 9·03%.) IR spectrum: 3290 cm^{-1} (NH); 1675, 1645 cm^{-1} (conjugated keto group and amide carbonyl); 1535 cm^{-1} (amide II).