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# On the Mechanism of Oxidation. IX. Oxidation and Autoxidation of Hydrazones[1]

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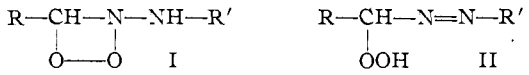
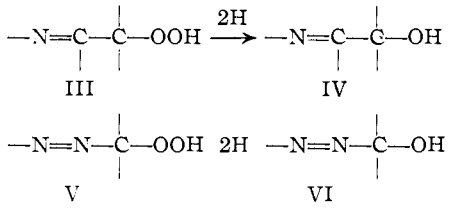
The perbenzoic acid oxidation products of aldehyde phenylhydrazones are formulated as mixed aliphatic–aromatic azoxy compounds (X) to which zwitterionic tautomers (XI) presumably contribute. The transformation of "benzaldehyde phenylhydrazone oxide" (X) to phenylbenzamidine (XVI) under the action of ethylmagnesium iodide is formulated as a Stevens rearrangement and taken as circumstantial evidence for the location of the oxygen function at the nitrogen in the center of the triad. The reduction and rearrangement of hydrazone hydroperoxides is described and discussed and forms the basis for the proposal of a hypothetical biogenetic scheme for the naturally occurring aliphatic azoxy compound macrozamin.

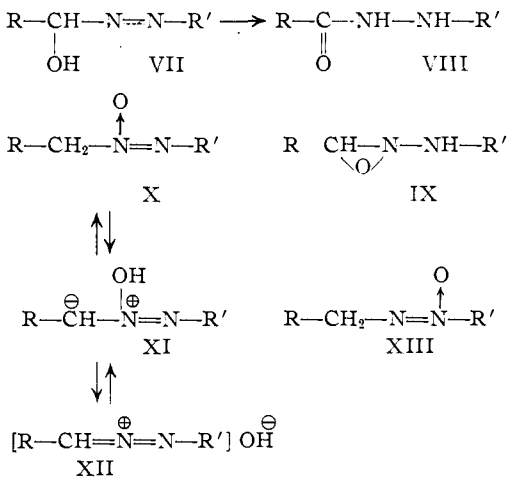
The old cyclic formulation (I) for Busch's hydrazoneperoxydes, the autoxidation products of hydrazone derivatives[3] has been corrected recently



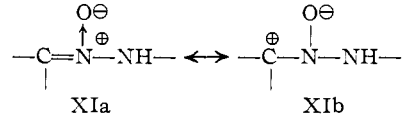
to that of a linear hydroperoxide (II).[4,5] In the same fashion as the analogously constituted *β-hydroperoxy-ψ*-indoles[6] (partial formula III) on mild hydrogenation[7] yield *β-hydroxy-ψ*-indoles (IV) one would expect the hydrazone hydroperoxides (V)



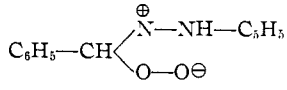
to be converted to the hydroxy compound (VI) under similar conditions. However when such mild hydrogenation experiments (using sodium iodide,[3] hyposulfite or hydrogen and palladium) were carried out with benzaldehyde phenylhydrazone hydroperoxide (II, R = R′ = $C_6H_5$), only benzoylphenylhydrazine (VIII) was isolated.[8] Apparently the intermediate hydroxy derivative VII is unstable and stabilizes itself by rearrangement to VIII. Since the action of peracids on aldehyde phenylhydrazones does *not* lead to acylated phenylhydrazines but to high-melting, neutral, salt-like, unusually stable compounds containing one more oxygen,[9] structure VII is ruled out for these "oxides," for which Bergmann proposed the three-membered ring structure IX. A reinvestigation of the spectrophotometric and chemical properties of the "oxides" derived from benzaldehyde and anisaldehyde phenylhydrazones leads us to believe that these oxides are mixed aliphatic–aromatic azoxy compounds. Of the two alternatives, X[10] and XIII, we prefer X for the following reasons: (i)



The mesionic[11] "ylide"[12] structure XI and the "cumulene" XII are salt-like tautomers of X which help to explain the high melting point, the stability and the low solubility in non-polar and polar solvents. Such structures cannot be written starting with XIII. The structure XI, *via* XIa and XIb,



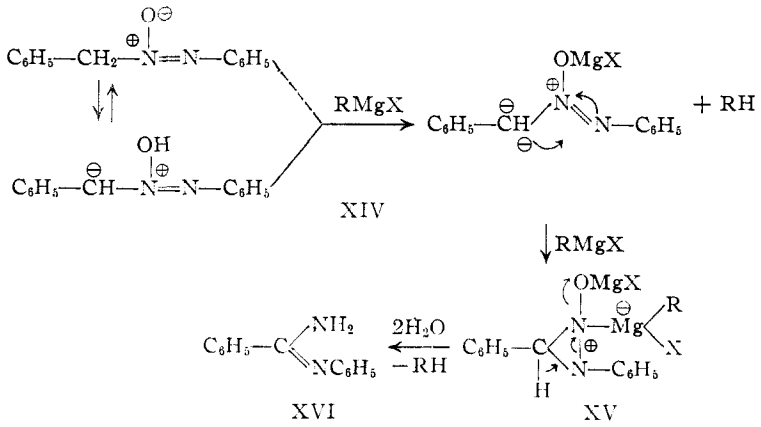
is in the same relation to Bergmann's old formulation IX, as the open hydroperoxyde structure (*cf.*



ref. 4) to the old cyclic formulation I; in the latter case, there can be no significant contribution of the ionic structure to the easily ether- and benzene-soluble hydroperoxide (II). (ii) The tautomer XI with ethylmagnesium iodide yields phenylbenzamidine (XVI) in at least 30% yield by a sequence of reactions which we feel tempted to formulate as a Stevens rearrangement.

The rearrangement, pictured here in three steps, may well be concerted. The fact that no liberation of gas (ethane, ethylene or butane) was observed, at least not at room temperature and before chang-

(1) Previous paper in this series, *cf.* THIS JOURNAL, **75**, 500 (1953).
(2) National Institute of Arthritis and Metabolic Diseases.
(3) M. Busch and W. Dietz, *Ber.*, **47**, 3277 (1914).
(4) R. Criegee and G. Lohaus, *Chem. Ber.*, **84**, 219 (1951).
(5) K. H. Pausacker, *J. Chem. Soc.*, 3478 (1950).
(6) B. Witkop and J. B. Patrick, THIS JOURNAL, **73**, 2196 (1951).
(7) B. Witkop and J. B. Patrick, *ibid.*, **73**, 2188 (1951).
(8) Catalytic hydrogenation according to a remark in the theoretical part of Criegee's paper (ref. 4) is reported to lead to the original phenylhydrazone.
(9) M. Bergmann, R. Ulpts and C. Witte, *Ber.*, **56**, 679 (1923).
(10) The condensation of benzylhydroxylamine with nitrosobenzene has not led yet to X but to the so-called bisazoxybenzyl (m.p. 210–211°) of unknown constitution: E. Bamberger and E. Renault, *ibid.*, **30**, 2278 (1897).
(11) On the use of the term "mesionic," *cf.* W. Baker, W. D. Ollis and V. D. Poole, *J. Chem. Soc.*, 1545 (1950)
(12) *Cf.* G. Wittig, *Angew. Chem.*, **68**, 15 (1951).



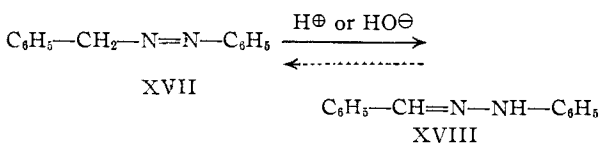
XIV



XVI XV

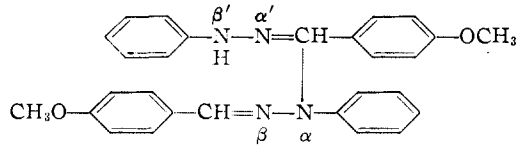
ing from ether to benzene, is not explained by the first step of this mechanism. For the same reason the elimination of oxygen from the azoxy group by Grignard reagent [13–15] has been formulated before either as a direct attack of $R^{\ominus}$ or by the formation of R–R (butane) and 2 $e^-$. An alternative pathway starts out with the known Grignard reduction of azoxy to azo compounds, which, as the tautomeric phenylhydrazone, might undergo a cleavage to benzonitrile and aniline.[16] However, the analogy for the easy combination of the latter fragments under these conditions to form an amidine is lacking.[17] Furthermore, benzaldehyde phenylhydrazone as such is reported to react with Grignard reagent to give substituted hydrazines in good yield.[18] Since there was no basic hydrazine detectable in the reaction of X with ethylmagnesium iodide, we consider the formation of phenylbenzamidine by the second route less likely. The concomitant propiophenone, isolated and identified by its dinitrophenylhydrazone, is probably formed by the action of Grignard reagent on phenylbenzamidine rather than on benzonitrile. The formation of benzonitrile was observed in the reaction of X with phenylhydrazine at 100°.[9] Interesting radical pathways are indicated for the decomposition of X with aniline (130°) and glacial acetic acid (150°) to give *p*-aminodiphenyl and phenol, respectively.[9]

The reduction of X and its *p*-methoxy analog to the phenylhydrazones of benz- and anisaldehyde with lithium aluminum hydride proceeds smoothly without indication of molecular rearrangement.[19] The reduction involves, however, a migration of a



XVII XVIII

double bond: the intermediate ω-benzeneazotoluene (phenylbenzyldiimide, XVII)[20] is the first intermediate analogous to the conversion of ordinary azoxy to azo compounds by lithium aluminum hydride. The strongly red color of the lithium aluminum hydride complex with X and the phenylhydrazones (but not the α-methylphenylhydrazones) of benz- and anisaldehyde support this view. The unstable azo compound (XVII) is apparently rearranged directly on formation in a base-catalyzed isomerization to benzalphenylhydrazone (XVIII).[21] A side product isolated in small yield in the lithium aluminum hydride "reduction" of X (R = $C_6H_5$, R′ = $C_6H_4OCH_3$), m.p. 195–197°, has the composition and the properties of $N^{\alpha},N^{\beta'}$-diphenyl-$N^{\beta}$-anisalanishydrazidine (XIX).[22]



XIX

The same "dehydroanisalphenylhydrazone" was also obtained in a very small yield by refluxing anisaldehyde phenylhydrazone with lithium aluminum hydride in ether apparently by oxidative dimerization of the salts $-\overset{\ominus}{N}-N=CH \leftrightarrow -N=N-\overset{\ominus}{C}H-$ by the action of atmospheric oxygen. The lithium aluminum hydride reduction of X (R = R′ = $C_6H_5$) as well as of benzaldehyde phenylhydrazone gave no clear evidence for the formation of "dehydrobenzalphenylhydrazone" ($N^{\alpha},N^{\beta'}$-diphenyl-$N^{\beta}$-benzalbenzhydrazidine, m.p. 207–208°).[23] The catalytic hydrogenation under various conditions led to the uptake of four to five moles of hydrogen. The isolation of two different basic compounds of complex character is described in the Experimental. The reduction with lithium phenyl[24] yielded a complex mixture of basic compounds in very small yield. The reduction with aluminum amalgam[20] in ether gave a colorless non-basic compound, m.p. 154.5–155.5°, giving a depression on admixture with benzalphenylhydrazone (m.p. 158°). 1-Benzyl-2-phenylhydrazine[20] expected in this reduction is known to be oxidized[20,25] or

(13) *Cf.* D. N. Kurssanow, A. S. Kurssanowa and A. N. Blochina, *J. Gen. Chem.* (*Russ.*), **8** [70] 1786 (1938); *C. A.*, **33**, 4979 (1939).

(14) W. H. Cumming and G. S. Ferrier, *J. Roy. Tech. Coll. Glasgow*, **2**, 49 (1929); *C. A.*, **23**, 2908 (1929).

(15) The literature on the reducing action of Grignard compounds is covered in F. Runge, "Organometallverbindungen," Stuttgart, 1944, pp. 394–404.

(16) R. Ciusa, *Gazz. chim. ital.*, **51**, II, 125 (1921); *cf.* P. Grammaticakis, *Compt. rend.*, **209**, 317, 994 (1939).

(17) *Cf.* Lottermoser, *J. prakt. Chem.*, [2] **54**, 116 (1896).

(18) P. Grammaticakis, *Compt. rend.*, **202**, 1289 (1936); **206**, 1262 (1937); **208**, 287 (1939); H. Wuyts and A. Lahourt, *Bull. Soc. Belgique*, **45**, 445 (1936).

(19) *Cf.* H. Dahn and U. Solms, *Helv. Chim. Acta*, **34**, 907 (1951).

(20) J. Thiele, *Ann.*, **376**, 267 (1910).

(21) Conversely, the formation of the "oxide" (X) with perbenzoic acid *only* in ether and hardly in chloroform goes through the unstable isomeric azo compound (XVII) which in chloroform, due to the unacid *only* in ether and hardly in chloroform goes through the unstable isomeric azo compound (XVIII) which in chloroform, due to the unavoidable presence of HCl, will immediately go back to the hydrazones. The oxidation of the azo to the azoxy compound is more often carried out with peracetic (*cf.* D. Swern, *Chem. Revs.*, **45**, 39 (1949)) than with perbenzoic acid (*cf.* K. A. Gehrckens and E. Müller, *Ann.*, **500**, 301 (1932)).

(22) G. Minunni, *Gazz. chim. ital.*, **27**, II, 244 (1897); E. Bamberger and W. Pemsel, *Ber.*, **36**, 68 (1903).

(23) G. Minunni and X. Rap, *Gazz. ital. chim.*, **26**, I, 442 (1896); *cf.* R. Ciusa, *ibid.*, **41**, I, 670 (1911).

(24) *Cf.* H. Gilman and J. C. Bailie, *J. Org. Chem.*, **2**, 84 (1937).

(25) G. H. Coleman, H. Gilman, C. E. Adams and P. E. Pratt, *ibid.*, **3**, 107 (1938).

autoxidized[26] easily to benzaldehyde phenylhydrazone. However, the analysis of this non-basic reduction product shows it to be a homolog, $C_{14}H_{14}N_{21}$, definitely different from the basic and unstable benzaldehyde benzylhydrazone (m.p. 65°).

The ultraviolet and infrared absorption data, summarized in Table I, are not in disagreement with the proposed aromatic–aliphatic azoxy structure X (R = $C_6H_5$, R′ = $C_6H_5$ and $C_6H_4OCH_3$).

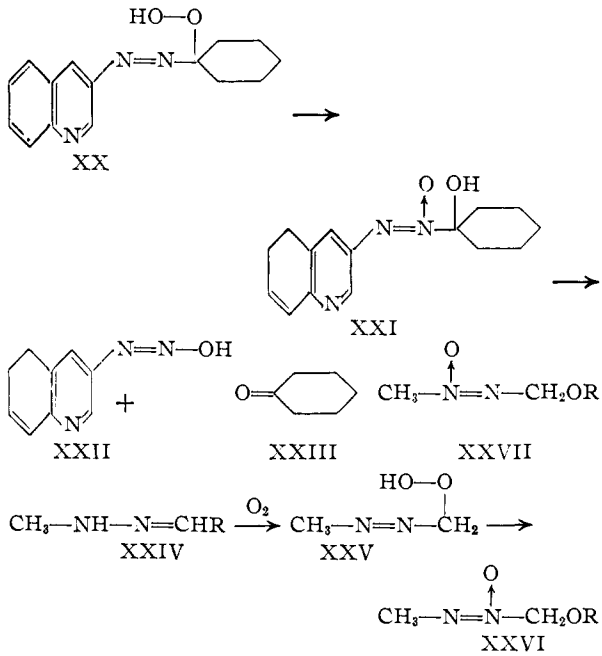
*Biochemical implications:* Whereas *molecular oxygen and peracids* attack indoles (in the form of the tautomeric indolenines) *at the same β-carbon* (labilized by the adjacent —C=N— element), phenylhydrazones (in the form of the tautomeric azo compounds) are attacked by *oxygen* at the *methylene group* next to the —N=N— group and by *perbenzoic acid*, as we tried to prove, at the *nitrogen* next to the methylene to form azoxy compounds of type X. The difference in this mode of action of the two oxidants is easy to see when one considers the first step in the autoxidation as a removal of a hydrogen radical from the terminal imino group. Addition of oxygen to this radical would lead to a nitrogenous peroxide containing the grouping —N—O—O— which is unknown in organic chemistry.[27] The mesomeric allylic radical —CH=N—N— ⟷ —CH—N=N— can either give the hydroperoxide II or dimerize with the formation of —C—C—, —C—N—[22,23,28] and N—N[29] dimers and dehydrogenation products.[30] With perbenzoic acid the reaction species, presumably $OH^{\oplus}$, combines with the most negative center in the triad, *i.e.*, the *central* nitrogen atom both in the hydrazones (XVIII) as well as in the isomeric azo compound (XVII). In the indole series the analogous reaction would lead to an indolenine oxide or nitrone, a route which has been discussed but not substantiated yet.[7,31]

TABLE I

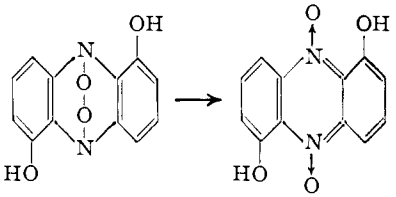
| Compound | U.v. spectrum λmax (log ε) in mμ | Infrared spectrum OH | NH | Phenyl | Ph–N or N→O[b] | Solvent |
|---|---|---|---|---|---|---|
| Benzalphenylhydrazine | 252 (3.420)[a] in EtOH | ... | ... | 6.76 | 7.62 | Nujol compensated |
| "oxide" (X, R = R′ = $C_6H_5$) | | | | | 7.68 (7.85) | |
| Anisalphenylhydrazine | 250 (4.104) | ... | ... | 6.60 | 7.84 | Nujol compensated |
| "oxide" (X, R = $C_6H_5$, R′ = $C_6H_4OCH_3$) | in ether | ... | ... | 6.61 6.77 6.72 | 7.68 7.85 7.95 | Nujol uncompensated |
| | | ... | ... | | 7.69 | Chloroform |

[a] The absorption is due to phenyl groups only. The azoxy group in macrozamin and other aliphatic azoxy compounds absorbs between 217–225 mμ.[35] [b] Azoxybenzene has a strong band (phenyl) at 6.75 μ which is also present in azobenzene; the 6.54 band of macrozamin (ref. 35), has been assigned to the aliphatic azoxy element. A less strong band in azoxybenzene at 7.85, not present in azobenzene, may be assigned to the stretching mode of an N → O element (*cf.* G. R. Clemo and A. F. Daglish, *J. Chem. Soc.*, 1483 (1940)).

The easy rearrangement of β-hydroperoxyindolenines has been discussed in connection with the problem of the primary oxidative breakdown product in the metabolism of tryptophan.[32] A similar rearrangement of a hydrazone hydroperoxide (XX)[33] has been reported by Robinson




The biogenesis of the remarkable plant product macrozamin,[34,35] as we see it, is pictured under-




Pachter and M. C. Kloetzel, THIS JOURNAL, **73**, 4958 (1951); I. J. Pachter, private communication, Nov. 29, 1951]

$$R_3\overset{\oplus}{N}-\overset{\ominus}{O} + R'CO_3H \longrightarrow R_3\overset{\oplus}{N}-O-OH \longrightarrow R_3N + O_2 + \overset{\oplus}{H}$$

The formation of a β-hydroperoxyindolenine from the alkaloid quebrachamine with perbenzoic acid may involve a similar sequence [B. Witkop, unpublished].

(26) P. Grammaticakis, *Compt. rend.*, **204**, 1263 (1937); **210**, 304 (1940).

(27) The existence of transannular peroxides in the acridine and phenazine series, containing the element -N-O-O-, though reported in one instance [K. Lehmstedt and H. Klee, *Ber.*, **69**, 1514 (1936)] has never been confirmed [*cf.* C. Dufraisse and J. Houpillart, *Bull. soc. chim.*, **5**, 626 (1938); **6**, 449 (1938)]. The biogenetic scheme for the formation of iodinine [G. R. Clemo and A. F. Daglish, *J. Chem. Soc.*, 1481 (1950)] from an unstable phenazine peroxide is still a speculation. The interesting deoxygenation of amine oxides by hydroperoxides has been formulated with the invocation of an -N-O-O- group [I. J.

(28) M. Busch and H. Kunder, *Ber.*, **49**, 2345 (1916).

(29) Formation of osazones and hydrogen peroxide in the presence of alkali, H. Biltz and A. Wienands, *Ann.*, **308**, 1 (1899); H. Biltz and O. Amme, *ibid.*, **321**, 1 (1902).

(30) E. Bamberger and J. Grob, *Ber.*, **34**, 523 (1901).

(31) B. Witkop, THIS JOURNAL, **72**, 614 (1950).

(32) A. Ek, H. Kissman, J. B. Patrick and B. Witkop, *Experientia*, **8**, 36 (1952).

(33) G. M. Robinson and R. Robinson, *J. Chem. Soc.*, **125**, 834 (1924).

(34) B. Lythgoe and N. V. Riggs, *J. Chem. Soc.*, 2716 (1949).

(35) B. W. Langley, B. Lythgoe and N. V. Riggs, *ibid.*, 2309 (1951). NOTE ADDED IN PROOF.—Dr. B. Lythgoe kindly informed us that, after initial doubts, *cf.* B. W. Langley, B. Lythgoe and L. S. Rayner, *ibid.*, 4191 (1952), he now also favors the azoxy structure X for Bergmann's hydrazone oxides. However, with regard to the structure of macrozamin, Dr. Lythgoe considers structure XXVII to be better supported by published and unpublished experimental evidence than XXVI.

neath. The autoxidation of the methylhydrazone of formaldehyde (XXIV, R = H) or its equivalent, *e.g.* glyoxylic acid (XXIV, R = COOH), should lead to a hydroperoxide (XXV), which on rearrangement and glycosidation would give macrozamin (XXVI, R = primeverosyl). This mode of formation, if correct, rules out the alternate formulation XXVII. Such a selection could not easily have been made otherwise, since the experimental methods for the differentiation between $\alpha$- and $\beta$-azoxy compounds available in the aromatic series[36] cannot be used in the aliphatic series.

These oxidation studies are being extended to open and cyclic azo and hydrazo compounds of therapeutic interest such as cardioactive tetrazols and hydrazinophthalazines.

## Experimental[37]

**Reaction of Benzaldehyde Phenylhydrazone with Perbenzoic Acid.**—Following the directions of Bergmann[10] the "oxide" X was obtained as a yellow-white crystalline powder which decomposed at 203–206°, yield 49%. This substance gave a violet chloroform solution which turned yellow on heating but changed back to violet on cooling. Addition of a few drops of ordinary ether to the colored solution brought about a discharge of the color and the precipitation of a white substance which melted with decomposition at 201–203°. Admixture of the original substance did not change the decomposition point. After this substance had been treated with ether—or with a drop of water—it did not cause the formation of a violet color in chloroform. Treatment of the chloroform with a trace of sodium methoxide prior to the addition of the compound prevented the formation of the color. The compound also gave highly colored solutions in glacial acetic acid which turned to brown on heating and did not change back to the original red-violet on cooling. The substance which had been treated with wet ether did not give colored solutions in glacial acetic acid.

*Anal.* Calcd. for $C_{13}H_{12}N_2O$: mol. wt., 212.25. Found: (the molecular weight was determined by the method of Signer[38] in chloroform; the apparatus was thermostated at 43.2°.), mol. wt., 247.3.

**Reaction of Anisaldehyde Phenylhydrazone with Perbenzoic Acid.**—To a solution of 3.30 g. (0.024 mole) of perbenzoic acid in 60 ml. of ether was added 4.52 g. (0.02 mole) of anisaldehyde phenylhydrazone. The mixture was allowed to stand in the refrigerator for three days. A starch–potassium iodide test did not reveal any active oxygen after that time. The yellow precipitate was collected and washed with ether. No other pure product could be isolated from the mother liquors. The precipitate was washed once with ether and was then recrystallized from ethyl acetate in which it was moderately soluble. There was obtained 2.6 g. (54%) of a slightly yellowish substance which decomposed at 176–177°. The reaction with phenyl isocyanate (for free OH or NH) in benzene with a trace of pyridine (refluxing for two hours) and the reaction with ethyl iodide in benzene (refluxing for two hours) gave back starting material in each case.

*Anal.* Calcd. for $C_{14}H_{14}N_2O_2$: C, 69.40; H, 5.82; N, 11.57. Found: C, 69.45; H, 5.49; N, 11.17.

**Reaction of Anisaldehyde Phenylhydrazone "Oxide" with Lithium Aluminum Hydride.**—Powdered lithium aluminum hydride (0.38 g., 0.01 mole) was added to 30 ml. of dried tetrahydrofuran. To this mixture was then added 1.2 g. of anisaldehyde phenylhydrazone "oxide." This caused the instantaneous formation of a deep orange color. The mixture was refluxed for 80 minutes after which excess lithium aluminum hydride was decomposed through the addition of a few pieces of ice. The yellow-brown solution was filtered from precipitated inorganic salts and the latter were washed with ether until most of the color had been removed. The combined organic solutions were dried over magnesium sulfate and then evaporated under vacuum. The residue crystallized upon the addition of hexane to yield 0.62 g. of a yellowish solid. Part of this material was quite soluble in methanol while a small portion of it remained undissolved. The methanol-soluble fraction upon recrystallization from that solvent and water melted at 119–120°. It did not depress the melting point of *anisalphenylhydrazine*. The infrared spectrum of this product and that of anisaldehyde phenylhydrazone (both spectra were obtained from Nujol mulls) were identical.

**N$\alpha$,N$\beta'$-Diphenyl-N$\beta$-anisalanishydrazidine ("Dehydroanisalphenylhydrazone,"[22] XIX). A. From Anisaldehyde Phenylhydrazone "Oxide" with Lithium Aluminum Hydride.**—The material which was insoluble in methanol (see above) (0.051 g.) was recrystallized several times from ethyl acetate–ethanol; m.p. 195–197°. The colorless crystals took on a reddish coloration in air and light.

*Anal.* Calcd. for $C_{28}H_{26}N_4O_2$: C, 74.64; H, 5.82; N, 12.44. Found: C, 74.76; H, 6.06; N, 12.59.

**B. From Anisalphenylhydrazine with Lithium Aluminum Hydride.**—To a solution of 0.38 g. (0.01 mole) of lithium aluminum hydride in 50 ml. of anhydrous tetrahydrofuran was added 1.13 g. (0.005 mole) of anisalphenylhydrazine. The orange solution was refluxed for two hours. Excess lithium aluminum hydride was then decomposed with ice, the precipitated inorganic salts were washed with ether, and the combined organic solutions were dried over magnesium sulfate. Evaporation of the solvents under vacuum left a yellow, gummy, residue which was treated with methanol. This left 0.08 g. of a white solid undissolved. This material was recrystallized from ethyl acetate–ethanol; m.p. 197–198°. A mixed melting point with the material obtained in the previous reaction showed them to be identical. The methanol solution on partial evaporation yielded 0.73 g. of anisalphenylhydrazine (m.p. 118–120°).

**Reaction of Benzalphenylhydrazine "Oxide" ($\omega$-Benzeneazoxytoluene, X) with Lithium Aluminum Hydride.**—Powdered lithium aluminum hydride (1.8 g., 0.05 mole) was added to 75 ml. of anhydrous ether. After the initial reaction had subsided there was added 1.21 g. (approx. 0.055 mole) of benzalphenylhydrazine "oxide" and the mixture was allowed to reflux for two hours. An intense yellow color formed as soon as the reacting components came together; the color persisted throughout the reaction. Wet ether was then added to the mixture until all excess lithium aluminum hydride had been destroyed. The precipitated inorganic salts were filtered and extracted in a Soxhlet apparatus with 100 ml. of ether for 24 hours. The combined ether solutions were dried over magnesium sulfate and then freed from solvents by evaporation under vacuum. This left 0.95 g. of brown residue; all but 0.03 g. of which was soluble in methanol. The methanol insoluble fraction decomposed at 203° undepressed on admixture with starting material. The presence of small amounts of N$\alpha$,N$\beta'$-diphenyl-N$\beta$-benzalbenzhydrazidine[23] ("dehydrobenzalphenylhydrazone," m.p. 205°) would not have been detected in this way. The methanol solution was evaporated *in vacuo* to leave a residue which dissolved only with difficulty in hot hexane. On cooling there was obtained a tan crystalline solid which after three more recrystallizations from hexane melted at 155–157°, undepressed on admixture with freshly recrystallized benzaldehyde phenylhydrazone.

**Reaction of Benzaldehyde Phenylhydrazone with Lithium Aluminum Hydride.**—Benzalphenylhydrazine (1.96 g., 0.01 mole) was added to 0.4 g. (0.01 mole) of lithium aluminum hydride in 50 ml. of dried tetrahydrofuran. The orange mixture was refluxed for three hours; it was then cooled in an ice-bath and treated with a few small pieces of ice, whereupon the color faded. The mixture was filtered free from inorganic salts and the latter were washed with a few portions of ether. The combined organic filtrates were dried over magnesium sulfate and evaporated under vacuum to leave 2 g. of oily residue. Most of the color was removed with small portions of pentane. This left 1.74 g. of a white solid which after two crystallizations from hexane melted at 155–156° and did not depress the melting point of benzaldehyde phenylhydrazone. The pentane washings were extracted with an aqueous sodium bisulfite solution which was in turn saturated with solid potassium hydroxide. This mixture was extracted with 100 ml. of ether. The ether solution was dried over magnesium sulfate and was

(36) *Cf.* E. Müller, "Die Azoxyverbindungen," Ahrens Sammlung, Neue Folge 33, Stuttgart, 1936.

(37) All melting points are corrected (Kofler block).

(38) R. Signer, *Ann.*, **478**, 246 (1930); *cf.* E. Clark, "Semimicro Quantitative Analysis," Academic Press, Inc., New York, 1943, p. 78.

then evaporated under vacuum to leave 0.08 g. of oily residue smelling strongly of benzaldehyde. No "dehydrobenzalphenylhydrazone" (m.p. 205°) was detectable in any part of the reaction mixture.

**Reaction of Benzalphenylhydrazine "Oxide" (X) with Aluminum Amalgam.**—Aluminum strips (2 g.) were amalgamated with 5% mercuric chloride,[39] washed with methanol and ether and added to a suspension of 200 mg. of benzalphenylhydrazine "oxide" in 40 ml. of ether. The mixture was stirred and refluxed for 30 minutes. When no visible reaction had taken place in that time there was added 1.5 ml. of water and stirring and refluxing were continued for 5 hours, after which the reaction mixture was allowed to stand overnight. It was filtered from aluminum salts, and the precipitate was washed with a total of 90 ml. of ether. The combined filtrates were dried over magnesium sulfate and evaporated *in vacuo* to leave 89 mg. of white solid which melted at 150–152°. It was recrystallized once from cyclohexane–methyl acetate to give colorless prisms, m.p. 153–154.5°, after another recrystallization, m.p. 154.5–155.5°. On admixture with benzaldehyde phenylhydrazone (158°) there was a large depression (135–150°). The crystalline appearance as well as the insolubility in hexane is different from benzaldehyde phenylhydrazone.

*Anal.* Calcd. for $C_{14}H_{14}N_2$: C, 79.96; H, 6.71; N, 13.32; mol. wt., 210.27. Found: C, 79.81; H, 6.93; N, 13.51.

A determination of the molecular weight according to Rast, though complicated by slight decomposition of the compound at the temperature of melting camphor, gave values rather too low (averaging around 150) for the $C_{14}$ than indicative of a $C_{28}$-compound.

An ethereal solution of this compound remained clear on addition of ethereal hydrogen chloride.

*Infrared Spectrum* (in chloroform): Imino band at 3.04 $\mu$. Aromatic region: 6.25$^s$, 6.72$^s$, 6.91$^m$, 7.43$^w$, 7.72$^m$. Benzalphenylhydrazine has a somewhat weaker imino band at 3.02 and very similar aromatic bands at 6.24$^s$, 6.68$^s$, 6.93$^m$, 7.395$^m$, 7.79$^m$ besides one *new* band 6.355$^m$.

The precipitated aluminum salts which were mixed with organic material were repeatedly washed with boiling chloroform (80 ml.). Evaporation of the chloroform *in vacuo* yielded 52 mg. of a solid residue which could be freed from traces of color by washing with ether; melting point and mixed melting point proved it to be starting material (X).

**Reaction of Benzalphenylhydrazine "Oxide" (X) with Ethylmagnesium Iodide.**—To a solution of ethylmagnesium iodide, prepared from 3.12 g. (0.02 mole) of ethyl iodide and 0.5 g. of magnesium in 50 ml. of anhydrous ether, was added a suspension of 1.86 g. (0.00875 mole) of benzaldehyde phenylhydrazone "oxide" in 70 ml. of hot benzene. The apparatus had been arranged in a manner which would allow the collection of any gas evolved during this addition. However, no gas was given off while ether was still present. The deeply colored mixture was stirred and allowed to reflux for 30 minutes; ether was then removed by distillation and refluxing was continued for another hour. Evolution of gas at this stage would not have been noticed. The cooled solution was hydrolyzed with ice and ammonium chloride and the resulting mixture was extracted with a total of 150 ml. of ether. After drying and removal of the solvent *in vacuo* there was left a colored semi-solid residue weighing 1.32 g. which was dissolved in 60 ml. of benzene. This solution was used for all subsequent tests.

Chromatography on alumina of 5 ml. of this solution showed the presence of at least five colored substances. The first two bands which were eluted with benzene did not give precipitates with ethereal hydrogen chloride. It is probable that these fractions contained the propiophenone which was isolated from the original solution (see below). The remaining fractions came off with chloroform and finally with 5% methanol in chloroform. These substances were all deeply colored and gave gummy precipitates with ethereal hydrogen chloride. There was not enough material for identification of any particular fraction.

A portion (5 ml.) of the original benzene solution was treated with 2,4-dinitrophenylhydrazine in methanol and sulfuric acid. The resulting red precipitate was recrystallized several times from cyclohexane–benzene; m.p. 187–190° with sintering at 168°, undepressed on admixture with *propiophenone 2,4-dinitrophenylhydrazone*. The identity was further confirmed by comparison of the infrared spectra.

Another portion of the original reaction mixture (20 ml.) was extracted with 1 *N* hydrochloric acid (35 ml.) and the brown acid extract was freed from color with Darco. It was cooled in ice, neutralized with sodium hydroxide (20%), and extracted with ether. Evaporation of the dried (potassium carbonate) ethereal solution *in vacuo* left 240 mg. of solid residue which after several recrystallizations from cyclohexane formed white crystals melting at 113–115°, 169 mg. This corresponds to a minimum yield of 29% in the original reaction mixture.

*Anal.* Calcd. for $C_{18}H_{12}N_2$: C, 79.56; H, 6.16; N, 14.28. Found: C, 79.95; H, 6.05; N, 14.16.

The infrared spectrum in chloroform showed the following bands: 2.81, 2.95 (primary amino groups); 6.08 (Ph—C=N—), 6.30$^m$, 6.33$^m$, 6.74$^m$, 6.91$^w$, 7.34$^m$, 8.14$^m$, 9.75$^m$, 12.02$^m$. **The hydrochloride**, prepared in ether and crystallized from ethanol–benzene, melted at 214–218°. The melting point of N-phenylbenzamidine has been reported at 111–112°[40] and 115–116°[41] and that of the hydrochloride as 221–222°.[42]

**Benzoylphenylhydrazine (VIII) by Catalytic Oxygenation and Hydrogenation of Benzaldehyde Phenylhydrazone.**—A solution of 200 mg. of benzaldehyde phenylhydrazone in 10 ml. of dry benzene was agitated in an oxygen atmosphere until 23 ml. of gas had been taken up. The solution was then added under nitrogen to 50 mg. of platinum catalyst which had been prereduced in 7 ml. of ethyl acetate. The mixture was reduced with hydrogen under atmospheric pressure until 30 ml. of hydrogen had been taken up. The solution was freed from catalyst, evaporated *in vacuo* and dissolved in pentane containing the minimum amount of ethyl acetate necessary to effect solution. Cooling and agitation caused the formation of a white crystalline compound (0.123 g.) which after one recrystallization from cyclohexane–chloroform melted at 170–171°, mixed m.p. with benzoylphenylhydrazine 169–171°. The infrared spectra of the two compounds were identical.

**Reaction of Benzaldehyde N-Methylphenylhydrazone with Lithium Aluminum Hydride.**—Benzaldehyde N-methylphenylhydrazone (1.24 g., 0.0059 mole) was added to 40 ml. of tetrahydrofuran containing 0.3 g. of lithium aluminum hydride. The solution became only slightly yellow during this process. It was refluxed for three hours and was then decomposed by the careful addition of ice. The wet precipitate was filtered and washed with 65 ml. of ether. Evaporation of the dried filtrate left 0.83 g. of a yellow oil. A portion of this substance was treated with hydrogen chloride in ether. The resulting **hydrochloride** was recrystallized from cyclohexane–benzene. It was observed that on very slow crystallization from this solvent mixture there were formed two kinds of crystals, chunky plates and long silky needles. Some of these were separated by hand; melting point determinations showed them to be identical. The plates seemed to go into the needle-form before melting. Both forms melted over quite a range (107–114°) and the melting point could not be improved by further recrystallizations. It was also found that the plate-like crystals when allowed to stand in a solvent in contact with the needles slowly changed into the latter. An infrared spectrum obtained from the free base in chloroform solution did not show the presence of an imino band. Major bands: 6.25$^s$, 6.39$^w$, 6.68$^s$, 6.88$^s$, 7.25$^m$, 7.57$^m$, 7.66$^m$, 8.43$^m$, 8.99$^s$. The paucity of the material and the difficulty of obtaining it in subsequent runs did not allow of further investigation.

**Catalytic Hydrogenation of Benzalphenylhydrazine "Oxide" (X).**—To a suspension of 1.021 g. of X (4.82 millimoles) in 100 ml. of glacial acetic acid was added 0.301 g. of palladium-on-charcoal (10%). A total of 506 ml. of hydrogen (4 equivalents = 460 ml.) was absorbed over a period of 24 hours; the starting material had gone into solution during this time. The mixture was filtered and the catalyst was washed with a few drops of glacial acetic acid. The filtrate was diluted with an equal volume of distilled water and was then carefully saturated with solid sodium

(39) V. Cherchez, *Bull. soc. chim.*, [4] **47**, 1279 (1930).

(40) H. v. Pechmann, *Ber.*, **30**, 1782 (1897).

(41) F. L. Pyman, *J. Chem. Soc.*, **123**, 3365 (1923).

(42) K. Brunner, W. Seeger and S. Dittrich, *Monatsh.*, **45**, 81 (1924).

carbonate while being cooled in an ice–salt-bath. It was then extracted with ether and the combined extracts were dried over magnesium sulfate. The aqueous layer which smelled strongly of ammonia was discarded. The ether solution was evaporated *in vacuo* and the residue was dissolved in 30 ml. of an 80% hexane–benzene mixture. This solution was poured on an alumina column (6″ × 1″). Elution with 100 ml. of 80% benzene–hexane yielded 0.0724 g. of a colorless oil which had a strong lemon-like odor (**base A**). Further elution with benzene and chloroform brought down only traces of substances which did not react with hydrogen chloride in ether. Washing with 95% ethanol (200 ml.) eluted 0.1973 g. of a white crystalline substance, **base B**.

**Base A** gave a **hydrochloride** which sublimed easily. The melting point of this salt taken in a sealed tube on the Kofler hot-stage was 213–215°. Found: C, 67.64; H, 8.34; N, 11.48. The analysis does not fit a $C_{13}$-compound; H and N would fit $C_{13}H_{20}N_2 \cdot HCl$. The substance also reacted with phenyl isothiocyanate in hexane to give a **phenylthiourea** derivative which after one sublimation melted at 144–145°. Found: C, 73.50; H, 7.03. The infrared spectrum of the hydrochloride in chloroform shows the following bands: 2.92 (very weak imino band), 3.71, 3.98 (ammonium region), 6.21$^{w}$, 6.32$^{s}$, 6.67$^{s}$, 6.75$^{m}$, 6.87$^{s}$, 7.31$^{m}$. The free liquid base A in chloroform showed: 2.95 (sharp imino band); 3.41, 3.51 (two different very characteristic C–H stretching frequencies); 6.23$^{s}$; 6.65$^{s}$, 6.83$^{s}$, 6.98$^{m}$, 7.57$^{s}$, 7.95$^{s}$, 8.50$^{m}$. A very strong band at 14.40 still indicated a monosubstituted intact benzene ring. We suspect that hydrogenolysis of the intermediate 1-benzyl-2-phenylhydrazine might have led to benzylamine and aniline; the latter might undergo further reduction analogous to the formation of dicyclohexylamine from aniline.[43]

Base B could be purified by vacuum sublimation to yield a colorless crystalline substance, recrystallized from ether, which sintered at 117° and melted at 119–120°. The mixed melting point with phenylbenzamidine (m.p. 117°) showed a large depression (87–110°).

*Anal.* Calcd. for $C_{26}H_{24}N_4O_3$: C, 70.88; H, 5.47; N, 12.73. Found: C, 70.66; H, 5.43; N, 12.64.

**Hydrochloride.**—The hydrochloride prepared in ether melted at 220–222°. An infrared spectrum obtained from solutions of varying concentration of base B in chloroform showed the following bands: 2.98, 3.05 (imino or amino bands; 5.92 (carbonyl of amid (?); 6.23$^{s}$, 6.36$^{s}$, 6.70$^{s}$, 6.88$^{s}$, 7.67$^{m}$. The presence of a monosubstituted benzene ring was shown by the typical aromatic fine structure between 5–6 $\mu$ and the strong band at 14.25 $\mu$.

**Picrate.**—The picrate, prepared in ether, proved to be almost insoluble in any organic solvent. Recrystallized from much acetone, it formed a bright-yellow crystalline powder and melted at 239–241°.

*Anal.* Calcd. for $C_{17}H_{16}N_5O_9$: C, 47.01; H, 3.72; N, 16.13. Found: C, 47.00, 46.87; H, 3.66, 3.78; N, 16.14.

The parent base of this picrate does not correspond to $C_{26}H_{24}N_4O_3$ but to $C_{11}H_{13}N_2O_2$ or $C_{22}H_{26}N_4O_4$. The nature of this discrepancy has not been investigated yet.

**Catalytic Hydrogenation of Anisalphenylhydrazine "Oxide."**—Platinum oxide (0.222 g.) was prereduced in 20 ml. of glacial acetic acid with 271 ml. of hydrogen. To the suspension was then added 1.204 g. (approx. 0.005 mole) of anisalphenylhydrazine "oxide" and the mixture was stirred at room temperature with hydrogen at atmospheric pressure. The hydrogen uptake leveled off after some 20 hours; 707 ml. of hydrogen (approx. 7 moles) had been absorbed during that time. The mixture no longer contained undissolved starting material. It was filtered from catalyst and diluted with 10 ml. of 6 *N* hydrochloric acid and 30 ml. of water. Extraction of the solution with ether and evaporation of the ether extracts showed that practically no neutral or acidic substances had been formed during the reduction. The acid solution was cooled in ice and was then saturated with solid potassium hydroxide. The resulting mixture was extracted with three 50-ml. portions of ether and the ether solution was washed once with 5 ml. of water after which it was evaporated to a volume of 40 ml. It was then extracted with 12 consecutive 2-ml. portions of 0.1 *N* hydrochloric acid and one 2-ml. portion of water. Each fraction was allowed to dry in a vacuum desiccator over potassium hydroxide. The weight of the fractions varied between 30–40 mg. Melting points of the crude hydrochlorides varied from 165–180° to 178–197° between the 1st and the 12th fraction.

The ether solution which had remained after these extractions was dried over magnesium sulfate and was evaporated *in vacuo* to leave a liquid residue of 0.4 g. This material was still basic and gave a hydrochloride melting at 210–218° (dec.).

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(43) *Cf.* R. Willstätter and D. Hatt, *Ber.*, **45**, 1476 (1912); G. S. Hiers and R. Adams, *ibid.*, **59**, 162 (1926).

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[CONTRIBUTION FROM THE CHEMICAL LABORATORY, UNIVERSITY OF CALIFORNIA, LOS ANGELES]

# The Free Amino Groups of Crystalline Bovine Plasma Albumin[1]

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Alanine, aspartic acid, histidine and methionine were identified by paper chromatography after freeing them from their thiohydantoins obtained from phenylisothiocyanated bovine plasma albumin. It may be concluded from this evidence that the $\alpha$-amino groups of these amino acids are free in this protein. The percentages of these amino acids as well as arginine, lysine and tyrosine were significantly lower in deaminized than in the native protein. This finding is explained by assuming that nitrous acid acted on the exposed side chains of these amino acids to form derivatives which, though present in a hydrolysate of the protein, were without effect on the growth of organisms used to determine the parent amino acids. The number of amino acid residues per mole of protein was shown to range from 2 for tryptophan to 100 for glutamic acid based on 66,100 the derived molecular weight. The authors' data are consistent with the view that 2 or 3 terminal $\alpha$-amino groups in bovine plasma albumin are contributed by aspartic acid, one by methionine, one by histidine and an undetermined number by alanine.

The authors' results on the free amino groups of bovine plasma albumin are presented at this time because they differ somewhat from the recent report of Van Vunakis and Brand[2] that there is only one terminal group, identified as that of aspartic acid, per mole of serum albumin. The latter workers determined aspartic acid by chromatographic analysis of its dinitrophenyl (DNP) derivative. In the present work, amino acids with free amino groups were identified by the phenyl isothiocyanate (PIC) method of Edman[3] and were determined by microbiological assay of the native and deaminized protein.

(1) Paper 72. For the preceding related paper (Paper 62) see M. S. Dunn and L. E. McClure, *J. Biol. Chem.*, **184**, 223 (1950). This work has been aided by grants from Swift and Company and the University of California.

(2) H. Van Vunakis and E. Brand, Abstracts of Papers, 119th Meeting, Amer. Chem. Soc., 28c, April, 1951.

(3) P. Edman, *Acta Chem. Scand.*, **4**, 283 (1950).