A Study of the Reaction of the Ascorbic-Dehydroascorbic Acid System and Related Model Compounds with Amphetamine

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On the basis of a report[1] that ascorbic-dehydroascorbic acid was capable of deaminating amphetamine, the reaction has been studied *in vitro* using model compounds analogous to ascorbic acid and dehydroascorbic acid. With benzil and biacetyl, mono-Schiff base condensation products were obtained which did not undergo isomerization and hydrolysis to produce ammonia and benzyl methyl ketone. Benzoin and acetoin reacted with amphetamine to give rearranged α-N-alkylamino ketones. In buffered aqueous solution, contrary to the previous report, no evidence was obtained for the formation of ammonia or benzyl methyl ketone from amphetamine and the ascorbic acid–dehydroascorbic acid system.

Some years ago, Beyer[1] reported that the ascorbic–dehydroascorbic acid system was capable of performing the non-enzymatic destruction of amphetamine and related sympathomimetic bases (Fig. 1), both *in vivo* and *in vitro*. This report led us to speculate on possible chemical mechanisms for these transformations, and experiments to establish one of these, involving tautomerism of a Schiff base, resulted in the work reported here.

Analogous mechanisms have been proposed for the ascorbic acid deamination of certain amino acids with the production of ammonia, carbon dioxide, and the corresponding aldehydes.[2] Dicarbonyl compounds of the type $-CO(CH{=}CH)_nCO-$ also were found to deaminate α-amino acids[3] and on this basis a general mechanism was proposed[3] for the Strecker degradation[4] of α-aminoacids by carbonyl compounds. This mechanism involved formation of a Schiff base which then was decarboxylated and isomerized to give a new Schiff base which on hydrolysis gave the carbonyl compound as shown in Fig. 2. Tautomerism of Schiff bases was also observed in

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(1) (a) K. H. Beyer, *J. Pharmacol. Exptl. Therap.*, **71**, 394 (1941); (b) **76**, 119 (1942).

(2) E. Abderhalden, *Wien. Klin. Wochschr.*, **50**, 815 (1937).

(3) A. Schönberg, R. Moubasher, and A. Mostafa, *J. Chem. Soc.*, 176 (1948).

(4) A. Strecker, *Ann.*, **123**, 363 (1862).

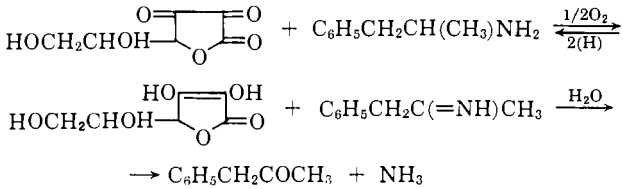

Fig. 1.—Beyer's scheme for the deamination of amphetamine by dehydroascorbic acid.

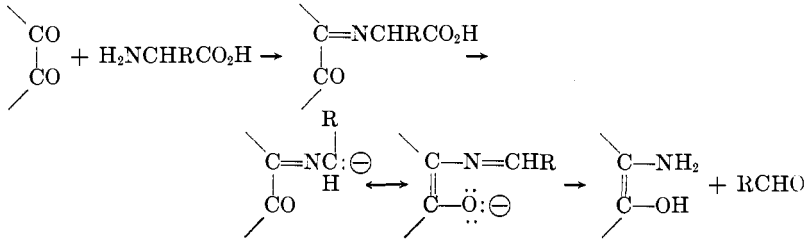

Fig. 2.—Deamination of amino acids by α-diketones.

strongly basic solution for compounds of the type aryl[1] –CHRN=C(aryl[2])$_2$.[5]

This type of reaction appeared attractive as an explanation for Beyer's deamination scheme and, accordingly, experiments were undertaken to isolate Schiff base intermediates from amphetamine and the ascorbic–dehydroascorbic acid system, as well as simpler model compounds. Evidence that such a mechanism was involved would indicate an important role for vitamin C not only in the metabolism of sympathomimetic amines but possibly of amines in general in the animal system. Also, chemical evidence for the mechanism would provide the basis for the synthesis of substances even more effective than vitamin C in controlling the metabolism of sympathomimetic amines.

Since the initial experiments with either ascorbic acid or dehydroascorbic acid and amphetamine appeared to give complex mixtures, attention was turned to simpler model systems. Benzil and biacetyl were chosen as model α-diketones since both exhibited the same type of activity as dehydroascorbic acid in the Strecker degradation of α-amino acids.[3] When benzil was heated with amphetamine (I) one molar equivalent of water was produced and benzilmono-(β-phenylisopropyl)-imine (II, R = C_6H_5) was obtained in 59% yield. Biacetyl

(5) C. K. Ingold and C. L. Wilson, *J. Chem. Soc.*, 1493 (1933); 93 (1934); S. K. Hsii, C. K. Ingold, and C. L. Wilson, *ibid.*, 1778 (1935).

and amphetamine reacted to give 3-(β-phenylisopropylimino)-butanone (II, R = CH_3) in 33% yield. As a proof of structure both azomethine compounds were reduced with lithium aluminum hydride to the corresponding amino alcohols III, which were characterized by their infrared spectra and elemental analysis.

No shift of the carbon–nitrogen double bond took place in aqueous acid, but hydrolysis to the starting diketone and amphetamine occurred and no benzyl methyl ketone could be detected. When the benzil azomethine II (R = C_6H_5) was treated with either methanolic sodium methoxide or aqueous ethanolic sodium hydroxide, only starting material could be isolated. With the biacetyl azomethine (II) (R = CH_3) only polymeric material was obtained, and no benzyl methyl ketone could be isolated in either case, thus indicating that no shift of the double bond, necessary for deamination, had occurred. Similar results were obtained by Baddar and Iskander,[6] who were unable to isomerize azomethines of the type RCH=NR^1 where R = substituted phenyl and R^1 = ethyl, isopropyl, or cyclohexyl. Presumably these compounds and the Schiff bases derived from benzil and biacetyl failed to undergo isomerization because the driving force supplied by loss of a carboxyl group (amino acids)[3] or conjugation with a phenyl group[5] was absent. Although the model systems analogous to dehydroascorbic acid failed to deaminate amphetamine, the possibility still existed that ascorbic acid and its analogs might form Schiff bases with amphetamine, which could then undergo isomerization and deamination by subsequent hydrolysis. To study this possibility benzoin and acetoin were chosen as model compounds.

When benzoin and amphetamine were heated together in toluene, water and a compound whose properties corresponded to a keto-amine (IV, R = C_6H_5) were formed (Fig. 3). Lutz and co-workers[7] had shown previously that the condensation of benzoin with primary amines led to keto-amines through a Voight condensation and rearrangement,[8] and accordingly the benzoin-amphetamine product would be α-(N-β-phenylisopropylamino)-desoxybenzoin (IV, R = C_6H_5). The melting point behavior of the hydrochloride salt indicated that the product was a mixture of stereoisomers. Sodium borohydride reduction of the salt gave what appeared to be a mixture of stereoisomeric amino alcohols, which on recrystallization gave a single sharp melting compound III (R = C_6H_5) whose infrared spec-

(6) F. G. Baddar and Z. Iskander, *J. Chem. Soc.*, 203, 209 (1954).

(7) R. E. Lutz, J. A. Freck, and R. S. Murphy, *J. Am. Chem. Soc.*, **70**, 2015 (1948); R. E. Lutz and J. W. Baker, *J. Org. Chem.*, **21**, 49 (1956).

(8) K. Voight, *J. prakt. Chem.*, **142**, 1 (1886).

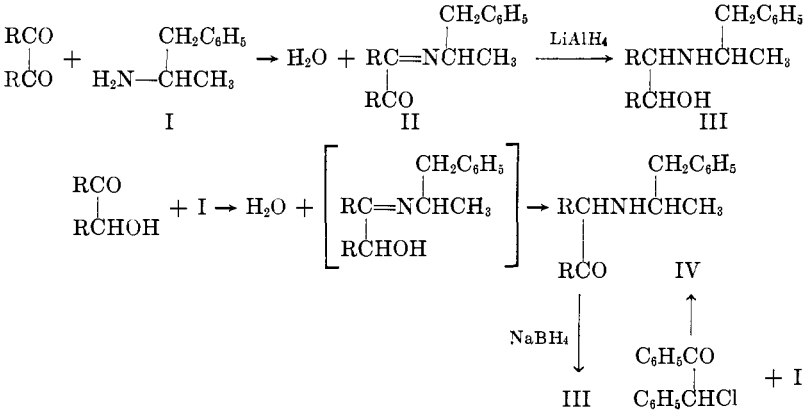

Fig. 3.—Reactions of amphetamine with ascorbic acid–dehydroascorbic acid analogs.

trum was identical with that of the product III (R = C_6H_5) obtained by the lithium aluminum hydride reduction of benzilmono-(β-phenylisopropyl)-imine (II, R = C_6H_5), but whose melting point was 40° higher, indicating that it was a stereoisomer.

The structure of the amino alcohol was further proven by treating desyl chloride with amphetamine to give α-(N-β-phenylisopropylamino)-desoxybenzoin hydrochloride (IV, R = C_6H_5), whose infrared spectrum was identical with that of the benzoin–amphetamine rearrangement product, but whose melting point was different. Reduction of the salt with sodium borohydride gave an amino alcohol (III, R = C_6H_5) identical in all respects with the one obtained from the rearrangement product.

Although no reports were found of the reaction of aliphatic amines and alkyl acyloins to form the rearranged α-N-alkylaminoketones, it seemed probable that they would behave in the same manner as the aromatic acyloins. Accordingly, acetoin was treated with amphetamine in benzene and 3-(N-β-phenylisopropylamino)-butanone (IV, R = CH_3) was formed. This was converted to the hydrochloride salt which on reduction with sodium borohydride gave an amino alcohol (III, R = CH_3) identical with the one formed from the lithium aluminum hydride reduction of 3-(β-phenylisopropylimino)-butanone (II, R = CH_3). It is of interest to note that ketohexoses, such as fructose, react with cyclohexyl, isopropyl, and dodecylamines, for example, to give 2-alkylamino-2-deoxyaldohexoses by the same type of condensation and rearrangement.[9]

(9) J. F. Carson, *J. Am. Chem. Soc.*, **77**, 1881 (1955); J. G. Erickson, *ibid.*, **77**, 2839 (1955).

All of these reactions establish that these model systems do not lead to products which can result in deamination of amphetamine to produce benzyl methyl ketone and ammonia. Since, however, it is not certain how closely the model compounds resemble ascorbic and dehydroascorbic acid, and since Beyer did report the deamination of amphetamine *in vitro*, studies were made of the reaction of ascorbic acid and of dehydroascorbic acid with amphetamine, including repetition of Beyer's original experiments.

When ascorbic acid was treated with amphetamine in aqueous 2-propanol, the only product which could be isolated was amphetamine oxalate. This is readily explainable in view of the fact that ascorbic acid readily undergoes air oxidation in aqueous base to form oxalic acid derivatives.[10] When the reaction was attempted under a nitrogen atmosphere no amphetamine oxalate was formed, but when the reaction mixture was subsequently exposed to air, or better, when oxygen was added, the oxalate was formed. Attempts were made to obtain a condensation product by treating amphetamine and ascorbic acid in absolute ethanol but the only product isolated was amphetamine ascorbate.

Beyer's experiments[1] using a buffered aqueous solution and admitting oxygen at pH 7.0 were repeated. No evidence for the formation of ammonia or benzyl methyl ketone could be obtained in our hands.[11]

Dehydroascorbic acid and amphetamine in anhydrous 2-propanol or anhydrous ethanol formed amphetamine ascorbate, and no condensation product, nor deamination product, could be isolated.

On the basis of these experiments, it would appear that the role (if any)[12] of ascorbic acid or dehydroascorbic acid in the metabolism of amphetamine and related sympathomimetic amines is more complex than previously believed, and does not involve simple Schiff base formation and deamination. Metabolic studies (which are beyond the scope of this investigation) should shed further light on the problem.

(10) A. Weissberger, J. E. LuValle, and D. S. Thomas, Jr., *J. Am. Chem. Soc.*, **65**, 1934 (1943); J. Parrod, *Bull. Soc. Chim. France*, **5**, 938 (1938).

(11) Small amounts of a volatile base were obtained in the Kjeldahl determination. The fact that the base was also obtained when ascorbic acid was left out of the solution (Table I) suggests that the volatile base was amphetamine. This would also account for the fact that Beyer obtained no volatile base when a hydroxyl group was present on the benzene ring of the sympathomimetic amine, since it would be present in the basic solution as the sodium salt, which would not be volatile with steam.

(12) S. C. Harris, L. M. Searle, and A. C. Ivy, *J. Pharmacol. Exptl. Therap.*, **89**, 92 (1947), were unable to detect any influence on the blood level of ascorbic acid in humans when fed amphetamine.

Experimental[13]

Amphetamine Derivatives.—Two new derivatives of amphetamine were prepared for use in this investigation.

(a) **Phenylthiourea.**—Equimolar quantities of amphetamine and phenyl isothiocyanate were heated in ether on a steam bath until all of the ether had evaporated. The residue was recrystallized from ethanol and the analytical sample had m.p. 134–135°.

Anal. Calcd. for $C_{16}H_{18}N_2S$: C, 71.08; H, 6.71; N, 10.36. Found: C, 70.70; H, 6.64; N, 10.15.

(b) **Diamphetamine Oxalate.**—To a solution of amphetamine in anhydrous ether was added dropwise a saturated solution of anhydrous oxalic acid in anhydrous ether. The precipitate was recrystallized from ethanol and the analytical sample had m.p. 237–238° (dec.), λ_{max}^{Nujol} 6.28–6.67μ (broad).

Anal. Calcd. for $C_{20}H_{28}N_2O_4$: C, 66.63; H, 7.83; N, 7.77. Found: C, 66.79; H, 7.70; N, 7.43.

Benzilmono-(β-phenylisopropyl)-imine (II, R = C_6H_5).—A solution of 21 g. (0.10 mole) of benzil and 13.5 g. (0.10 mole) of amphetamine in 150 ml. of benzene was boiled under reflux with a constant water separator for 16 hr. The solvent was then evaporated at room temperature under reduced pressure and the residue was recrystallized from ethanol to give 19.2 g. (59%) of product, m.p. 84–89°. The white crystals slowly turned bright yellow in light and the transformation was more rapid in direct sunlight. An analytical sample was obtained by recrystallization from ether and had m.p. 84–86.5°, $\lambda_{max}^{CCl_4}$ 3.19, 3.34, 5.98, 6.17, 6.25 and 6.71 μ.

Anal. Calcd. for $C_{23}H_{21}NO$: C, 84.36; H, 6.47; N, 4.25. Found: C, 84.32; H, 6.57; N, 4.44.

2-(N-β-Phenylisopropylamino)-1,2-diphenylethanol (III, R = C_6H_5).—To a stirred slurry of 1.5 g. of lithium aluminum hydride in 150 ml. of anhydrous ether was added dropwise a solution of 8.2 g. (0.025 mole) of benzil mono-(β-phenylisopropyl)-imine (II, R = C_6H_5) in 70 ml. of anhydrous ether, and stirring was continued for 1 hr. after the addition was complete. Then 50 ml. of ethyl acetate was added, followed by 50 ml. of water. The liquid phase was decanted from the aluminates, which were then washed with three 50 ml. portions of ether. The washings and decanted layer were combined and evaporated to dryness at room temperature under reduced pressure. The residue was triturated with 10 ml. of hexane and the insoluble material was collected to give 1.0 g. (12%) of crude product, m.p. 104–106°. An analytical sample was obtained by recrystallization from hexane and had m.p. 104.5–106°, $\lambda_{max}^{CHCl_3}$ 2.76, 2.94, 3.72, 6.22 and 6.85 μ.

Anal. Calcd. for $C_{23}H_{25}NO$: C, 83.34; H, 7.60; N, 4.23. Found: C, 83.25; H, 7.69; N, 4.27.

The hexane filtrate from which the crude amino alcohol was obtained was dried over anhydrous magnesium sulfate and evaporated to dryness under reduced pressure. The residue was taken up in hexane, a little ether was added, and the solution was cooled to 0°. The solid which formed, 0.7 g. (8%) had m.p. 85–100° and could not be recrystallized to constant m.p. However, all fractions gave

(13) Melting points are corrected and were determined with a Hershberg apparatus. Analytical samples were recrystallized to constant melting point unless stated otherwise. Analyses by Dr. S. M. Nagy and associates, Microchemical Laboratory, Massachusetts Inst. of Technology.

infrared spectra which were identical with that of the constant melting amino alcohol, and presumably the 85–100° material was a mixture of diastereomers.

Acid Hydrolysis of Benzilmono-(β-phenylisopropyl)-imine.—A mixture of 2.0 g. (0.006 mole) of benzilmono-(β-phenylisopropyl)-imine in 30 ml. of 15% sulfuric acid was boiled under reflux for 30 min. Then the mixture was extracted with two 15 ml. portions of ether. Evaporation of the ether extract yielded 1.12 g. (84%) of benzil, m.p. 95–97°, which did not depress the m.p. of pure benzil. The acidic aqueous layer was made basic, 1.4 g. of phenyl isothiocyanate was added, and the mixture was heated on a steam bath for 30 min. On cooling, 1.3 g. (78%) of 1-amphetamine-3-phenylthiourea was obtained, m.p. 131–132°, which did not depress the m.p. of an authentic sample.

Attempted Alkaline Isomerization of Benzilmono-(β-phenylisopropyl)-imine (a) With Sodium Methoxide in Methanol.—A solution of 1.0 g. of crude benzilmonoimine, (II, R = C_6H_5) in 50 ml. of 0.1 *N* sodium methoxide in methanol was boiled under reflux for 2.5 hr. and then poured into 50 ml. of water. The precipitate was collected, recrystallized from ethanol, and then had m.p. 86–91.5°, undepressed when admixed with starting material. Acidic hydrolysis of this product as described above yielded only benzil and amphetamine.

(b) With Sodium Hydroxide in Aqueous Ethanol.—To a solution of 2.0 g. of the crude monoimine in the minimum amount of ethanol was added 22 ml. of 2 *N* sodium hydroxide solution and the mixture was heated on a steam bath for 30 min. The reddish-brown mixture was concentrated to one half of the original volume under reduced pressure at room temperature and the precipitate was collected and recrystallized from ethanol to yield 1.0 g. (50%) of starting imine, m.p. 86–89°; there was no depression in melting point on admixture with authentic starting material. The basic filtrate was extracted with two 25-ml. portions of ether, the ether extract was then extracted with two 25-ml. portions of 1 *M* hydrochloric acid and when this acidic extract was made basic no amine was found. The ether extract was evaporated under reduced pressure and the residue was taken up in 10 ml. of ethanol and 2 ml. of water. To this solution 10 ml. of 2,4-dinitrophenylhydrazine reagent[14] was added and the mixture was heated on a steam bath for 15 min. and then cooled. Only 2,4-dinitrophenylhydrazine could be obtained crystalline, and none of the derivative of benzyl methyl ketone could be isolated.

α-(N-β-Phenylisopropylamino)-desoxybenzoin (IV, R = C_6H_5) from Benzoin and Amphetamine.—A solution of 10.6 g. (0.05 mole) of benzoin and 6.8 g. (0.05 mole) of amphetamine in 85 ml. of toluene (dried over sodium) was boiled under reflux with a constant water separator for 6.5 hr., during which time 0.9 ml. of water was collected. Most of the solvent was then removed by withdrawal through the extractor and the remainder by distillation at 100° under reduced pressure. The product was a viscous yellow oil, λ_{max}^{film} 3.14, 3.39, 5.92, 6.25 and 6.71 μ. It was taken up in 100 ml. of anhydrous ether and excess ethanolic hydrogen chloride was added dropwise. After cooling the mixture overnight at 0° the hydrochloride was collected to give 11.5 g. (63%). Several recrystallizations from ethanol did not give a sharp melting product, indicating the presence of diastereomers. The analytical sample had m.p. 204–210° dec., λ_{max}^{Nujol} 3.56, 5.88, 6.21 and 6.67 μ.

(14) R. L. Shriner, R. C. Fuson, and D. Y. Curtin, "The Systematic Identification of Organic Compounds," John Wiley and Sons, Inc., New York, N. Y., 4th ed., 1956, p. 219.

Anal. Calcd. for $C_{23}H_{24}ClNO$: C, 75.50; H, 6.62; N, 3.83. Found: C, 75.48; H, 6.70; N, 4.30.

2-(N-β-Phenylisopropylamino)-1,2-diphenylethanol (III, R = C_6H_5): (a) by Sodium Borohydride Reduction of α-(N-β-Phenylisopropylamino)-desoxybenzoin (IV, R = C_6H_5).—To a stirred solution of 9.2 g. (0.025 mole) of α-(N-β-phenylisopropylamino)-desoxybenzoin hydrochloride in 100 ml. of methanol was added in portions 5 g. of sodium borohydride. The product precipitated during the exothermic reduction and the mixture was stirred for 3 hr. after the addition was complete and then was poured into an ice and water mixture. The collected product was dissolved in ether, the solution was dried over anhydrous magnesium sulfate, the ether was evaporated, and the residue was washed with hexane to yield 5.9 g. (71%), $\lambda_{max}^{CHCl_3}$ 2.76, 2.94, 3.22, 6.22 and 6.85 μ, identical with the infrared spectrum of the amino alcohol III (R = C_6H_5) from the reduction of benzil mono-(β-phenylisopropyl)-imine. The analytical sample was prepared by recrystallization from ether and had m.p. 145–146.5°.

Anal. Calcd. for $C_{23}H_{25}NO$: C, 83.34; H, 7.60; N, 4.23. Found: C, 83.44; H, 7.83; N, 4.28.

(b) By Reaction of Desyl Chloride with Amphetamine.—A solution of 4.5 g. (0.02 mole) of desyl chloride and 5.4 g. (0.04 mole) of amphetamine in 50 ml. of ethanol was heated on a steam bath for 15 min. and then allowed to stand at room temperature for 37 hr. The reaction mixture was poured into 100 ml. of water and this mixture was extracted with two 25-ml. portions of ether. The ether extract was then extracted with four 25-ml. portions of 15% hydrochloric acid and this extract on standing gave 1.0 g. of white solid, which was recrystallized from ethanol and then had m.p. 218–230° dec., infrared spectrum identical with that of α-(N-β-phenylisopropylamino)-desoxybenzoin from the condensation of benzoin and amphetamine.

This product (1.0 g.) was reduced with sodium borohydride as described above for IV (R = C_6H_5). The reduction product, after two recrystallizations from ether, had m.p. 141–147°, not depressed when mixed with 2-(N-β-phenylisopropylamino)-1,2-diphenylethanol prepared above.

3-(β-Phenylisopropylimino)-butanone (II, R = CH_3).—To a solution of 9.10 g. (0.015 mole) of biacetyl in 25 ml. of anhydrous ether was added a solution of 13.5 g. (0.10 mole) of amphetamine in 15 ml. of anhydrous ether. After the exothermic reaction had subsided the cloudy solution was boiled under reflux for 30 min. and then the water formed was removed by means of a separatory funnel. The ether solution was dried over anhydrous magnesium sulfate overnight under nitrogen and then the solvent was removed under reduced pressure to give 18.4 g. of a viscous reddish-brown oil. This was distilled through an 18 cm. Vigreux column and three fractions were collected: frac. I, 1.4 g., b.p. up to 98° (0.40 mm.); frac. II, 5.0 g., b.p. 98–100° ((0.40 mm.); frac. III, 2.0 g., b.p. 100–103° (0.40 mm.). There was extensive decomposition during the distillation and a better yield was obtained by using a flame rather than an oil bath. Fractions I and III were combined and redistilled to give 1.6 g. of liquid b.p. 92–96° (0.30 mm.), which was combined with fraction II to give a total of 6.6 g. (32%) of 3-(β-phenylisopropylimino)-butanone, $n^{25}D$ 1.5095, λ_{max}^{film} 3.29, 5.88, 6.07, 6.21, and 6.71 μ. An analytical sample was prepared by redistillation through an 18 cm. Vigreux column and had b.p. 95–96° (0.30 mm.).

Anal. Calcd. for $C_{13}H_{17}NO$: C, 76.81; H, 8.43; N, 6.89. Found: C, 77.10; H, 8.50; N, 6.96.

3-(N-β-Phenylisopropylamino)-2-butanol (III, R = CH_3).—2-(β-Phenylisopropylimino)-butanone, (II, R = CH_3) (11.4 g., 0.056 mole), was reduced with lithium aluminum hydride, as described above for benzilmono-(β-phenylisopropyl)-imine, to give 7.6 g. (66%) of 3-(N-β-phenylisopropylamino)-2-butanol, m.p. 65–69°. An 0.33 g. sample of this material was sublimed at 50° (0.5 mm.) to give 0.29 g. of pure material, m.p. 71–72.5°. Mixture melting points with analytically pure samples of the same compound, m.p. 70.5–71.5°, prepared as described below, using varying proportions of each product, gave m.p. 70–71° to 72.5–74.5°. The infrared spectrum, $\lambda_{max}^{CHCl_3}$ 2.81, 3.11, 3.29, 6.21, and 6.71 μ was identical with that of the analytically pure material.

3-(N-β-Phenylisopropylamino)-butanone (IV, R = CH_3).—A solution of 8.8 g. (0.10 mole) of acetoin in 50 ml. of benzene was boiled under reflux with a constant water separator until no more water was collected. Then 13.6 g. (0.10 mole) of amphetamine was added and the solution was boiled under reflux for 2.5 hr. Removal of the solvent under reduced pressure left 17.6 g. of viscous reddish-brown oil, which was distilled through an 18 cm. Vigreux column to give 3 fractions: frac. I (1.48 g.), b.p. up to 100° (0.5 mm.); frac. II (8.83 g.), b.p. 100–104° (0.5 mm.) (most of the fraction boiled at 103.5°); frac. III (4.40 g.), 100–110° (0.5 mm.).

Fraction II corresponded to 43% of 3-(N-β-phenylisopropylamino)-butanone and had λ_{max}^{film} 2.84, 3.29, 5.82, 6.21 and 6.70 μ. It was dissolved in anhydrous ether and absolute ethanol (4:1 vols.) and ethanolic hydrogen chloride was added dropwise with stirring. The resulting solution was kept overnight at 0° and then diluted with a large volume of anhydrous ether. The precipitated hydrochloride salt weighed 8.6 g. (36% based on acetoin) and was recrystallized from acetone and ethanol (10:1 vols.) to give an analytical sample, m.p. 183–184° dec., $\lambda_{max}^{CHCl_3}$ 5.74, 6.43 and 6.89 μ.

Anal. Calcd. for $C_{13}H_{21}NO$: C, 75.31; H, 10.21; N, 6.76. Found: C, 75.85; H, 10.29; N, 6.82.

Acid Hydrolysis of 3-(β-Phenylisopropylimino)-butanone (II, R = CH_3).—A mixture of 2.0 g. (0.01 mole) of II (R = CH_3), b.p. 112–122° (0.45 mm.) and 30 ml. of 15% sulfuric acid was heated on a steam bath for 30 min., cooled, and extracted with four 15 ml. portions of ether. The ether solution was distilled at room temperature under reduced pressure to give 0.7 g. (82%) of crude biacetyl, infrared spectrum λ_{max}^{film} 2.78, 3.25 and 5.82 μ, identical with that of a pure sample. The aqueous layer was made basic, the insoluble material was taken up in ether. phenyl isothiocyanate was added to the ether solution and this solution was heated on a steam bath until the ether had evaporated. The residue was recrystallized from ethanol and 1.82 g. (68%) of 1-amphetamine-3-phenylthiourea was obtained. m.p. 133–135°; no depression when mixed with an authentic material.

Attempted Alkaline Isomerizations of 3-(β-Phenylisopropylimino)-butanone.—The procedures used were the same as those described for benzil mono-β-phenylisopropylimine (II, R = C_6H_5) and only polymeric products were obtained. No evidence could be obtained for the presence of benzyl methyl ketone in the ether extracts using 2,4-dinitrophenylhydrazine.

Reaction of Ascorbic Acid with Amphetamine: (a) In Water–2-Propanol.—A solution of 4.4 g. (0.025 mole) of ascorbic acid and 6.8 g. (0.050 mole) of amphetamine in 50 ml. of a one to one mixture of 2-propanol and water was kept in a stoppered flask at 0° for 2 weeks. The bright yellow solution contained a small amount of a solid which was collected, recrystallized from ethanol, and then had

m.p. 237–238° dec., not depressed when mixed with an authentic sample of amphetamine oxalate. The infrared spectra were identical.

When the same experiment was conducted under an atmosphere of nitrogen no oxalate was formed even after 1 month. However, on subsequent exposure of the yellow solution to air for 12 hr., a small amount of the oxalate was formed. When oxygen was bubbled through a freshly prepared solution, it rapidly turned reddish-brown and after 6 hr. 0.5 g. (20%) of the oxalate was obtained.

(b) **In Absolute Ethanol; Formation of Amphetamine Ascorbate.**—A solution of 4.4 g. (0.025 mole) of ascorbic acid and 4.0 g. (0.38 mole) of amphetamine in 100 ml. of commercial absolute ethanol was kept in a stoppered flask at room temperature for 8 hr. The orange-colored solution was then evaporated to dryness under reduced pressure, the residue was washed by decantation with three 50 ml. portions of anhydrous ether, and then was dried over calcium chloride. The resulting yellow powder (8 g.) was stable at 0° but slowly turned bright red when kept at room temperature. A 1.0 g. sample was dissolved in 25 ml. of anhydrous 2-propanol at room temperature and the solution was cooled to 0° The solid which formed was collected by decantation, washed several times with anhydrous ether, and dried over calcium chloride at reduced pressure. The dried solid was very hygroscopic and had m.p. 80–120° dec., $[\alpha]^{27}D$ + 57° (1% in water), + 107° when calculated on the basis of the ascorbate ion concentration. For comparison, calcium ascorbate had $[\alpha]^{27}D$ + 95° (1% in water), [(reported[15] $[\alpha]D$ + 91° (0.3% in water)], + 106° when calculated on the basis of the ascorbate ion concentration. Spectral data: λ_{max}^{Nujol} 5.77 and 6.32 μ, λ_{max}^{KBr} 2.83, 5.75 and 6.26 μ, $\lambda_{max}^{2\text{-propanol}}$ 252 mμ (ϵ = 5,088). It was not possible to obtain reproducible analytical values, but they corresponded most closely to amphetamine ascorbate monohydrate.

Anal. Calcd. for $C_{15}H_{23}NO_6 \cdot H_2O$: C, 54.70; H, 7.04; N, 4.25. Found: C, 54.97, 55.61; H, 6.58, 6.83; N, 3.96.

(c) **In Aqueous Buffered Solutions; Test for Ammonia.**—Beyer's procedure[1] for the oxygenation of the solution of amphetamine and ascorbic acid was followed. The test for ammonia was performed by the following method. The solution, after oxygenation for 24 hr., was made up to 500 ml. and 50 ml. aliquots were withdrawn, made basic, and heated in a Kjeldahl flask at 80° for 3 hr. while CO_2 was bubbled through. The distillate was collected in a standard sulfuric acid solution, which was then back-titrated with standard potassium hydroxide solution. Test experiments, using a standard ammonium sulfate solution, resulted in 99.68 and 99.72% recovery of ammonia after 3 hr. of distillation. The results for the ascorbic acid-amphetamine solutions are shown in Table I.

(d) **In Aqueous Buffered Solution; Test for Benzyl Methyl Ketone.**—Beyer's procedure for the oxygenation of the solution was followed, using 1.5 g. each of amphetamine hydrochloride and ascorbic acid, and 400 ml. of the freshly oxygenated solution was acidified and distilled into a solution of 0.5 g. of 2,4-dinitrophenylhydrazine in 30 ml. of concd. hydrochloric acid and 50 ml. of water. No 2,4-dinitrophenylhydrazone could be isolated.

The remaining 500 ml. of oxygenated solution was added to an 0.1 *N* hydrochloric acid solution saturated with 2,4-dinitrophenylhydrazine. The solution became cloudy after 5 min., was allowed to stand overnight, and then filtered. A small amount of solid was obtained, a part of which was soluble in ethanol.

(15) R. W. Herbert, E. L. Hirst, E. G. V. Percival, R. J. W. Reynolds, and F. Smith, *J. Chem. Soc.*, 1270 (1933).

TABLE I

AMINE BASE DETERMINATIONS ON OXIDIZED SOLUTIONS OF ASCORBIC ACID AND AMPHETAMINE

Expt.	Amine-HCl, mmoles	Ascorbic acid, mmoles	Det.	Distilled amine base, mmoles[a]
1	5.03	5.02	1	0.309
			2	0.429
2	5.00	0.00	1	0.731
			2	0.784
3	8.00	5.01	1	0.400
			2	0.472
4	8.00	0.00	1	0.972
			2	1.070

[a] Total amine base calculated as 10 times the value for a 50 ml. aliquot of the 500 ml. reaction solution.

The insoluble part did not melt when heated to 400°. Evaporation of the ethanol solution gave a dark resinous material which was not the 2,4-dinitrophenylhydrazone of benzyl methyl ketone.

Reactions of Dehydroascorbic Acid with Amphetamine (a) in 2-Propanol.—To a solution of 1.0 g. (0.0048 mole) of dehydroascorbic acid–methanol complex[16] in 10 ml. of anhydrous 2-propanol was added a solution of 1.3 g. (0.0096 mole) of amphetamine in 5 ml. of 2-propanol. After 12 hr. at room temperature the dark brown solution was diluted with 50 ml. of anhydrous ether. The light-tan precipitate was collected by decantation, washed with another 50 ml. of ether, and dried over calcium chloride. The infrared spectrum was identical with that of amphetamine ascorbate. The ether solution yielded a dark syrupy residue on evaporation of the ether.

The reaction was also run using a 1:1 ratio of amine and methanol complex in 0.0024 mole quantities in 10 ml. of anhydrous 2-propanol for 4 days at 0°. The yellow precipitate was dried over calcium chloride under reduced pressure and had an infrared spectrum identical with that of amphetamine ascorbate.

(b) **In Anhydrous Ethanol.**—A solution of 0.50 g. (0.0024 mole) of dehydroascorbic acid–methanol complex and 0.34 g. (0.0024 mole) of amphetamine in 10 ml. of anhydrous ethanol was kept at 0° for 24 hr. The solvent was then evaporated at room temperature under reduced pressure and the residue was washed with three 25 ml. portions of anhydrous ether and dried over calcium chloride under reduced presure to give a yellow powder whose infrared spectrum was identical with that of amphetamine ascorbate.

The experiment was repeated but with 2.06 g. (0.01 mole) of dehydroascorbic acid and 2.7 g. (0.02 mole) of amphetamine in 20 ml. of anhydrous methanol. After removal of the solvent, 80 ml. of 2 *M* sulfuric acid was added to the syrupy residue and the mixture was continuously extracted with ether for 24 hr. The ether extract was evaporated and 25 ml. of 95% ethanol was added to the gummy residue. A gummy solid formed which was dissolved in boiling ethanol and treated with 2,4-dinitrophenylhydrazine. No hydrazone could be isolated. The

(16) Dehydroascorbic acid was used in all experiments in the form of its methanol complex because of the greater solubility. The material was obtained from Nutritional Biochemicals Corporation, and its preparation and properties are described by B. Pecherer, *J. Am. Chem. Soc.* **73**, 3827 (1951). The complex gives all of the normal reactions of dehydroascorbic acid.

ethanol filtrate was treated with 2,4-dinitrophenylhydrazine and a hydrazone was obtained. It was recrystallized from acetone and had m.p. 306–307° dec. Reported for benzyl methyl ketone 2,4-dinitrophenylhydrazone, m.p. 156°.[17]

(c) **In Aqueous Buffered Solution.**—A solution of 2.0 g. (0.012 mole) of amphetamine hydrochloride in 500 ml. of 0.0625 *M* potassium dihydrogen phosphate was adjusted to pH 7.0 by the addition of potassium hydroxide solution, 1.0 g. (0.0048 mole) of dehydroascorbic acid–methanol complex in 20 ml. of buffer was added, and the pH was again brought up to 7.0. The solution was then kept at room temperature for 36 hr., during which time it turned dark amber. Then 25 ml. of concd. sulfuric acid was added and the solution was distilled into a saturated aqueous hydrochloric acid solution of 2,4-dinitrophenylhydrazine. No hydrazone derivative could be isolated.

(17) S. M. McElvain, "The Characterization of Organic Compounds," The Macmillan Co., New York, N. Y., 1953, p. 256.

Acetylhydrazine as an Intermediate in the Metabolism of Aroylhydrazines[1,2]

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The oral administration of benzhydrazide or 1-acetyl-2-benzoylhydrazine leads in the rabbit to the urinary excretion of 1,2-diacetylhydrazine. 1-Acetyl-C^{14}-2-benzoylhydrazine, prepared from benzhydrazide and acetic anhydride-1-C^{14}, in the rat similarly led to the urinary excretion of 1,2-diacetylhydrazine, which had a specific activity of approximately 80% of the administered compound. 1,2-Diacetylhydrazine-C^{14} itself was converted only slightly (0.1–0.3%) to respiratory carbon dioxide. Acetylhydrazine in the dog was converted in part to hydrazine. These data are consistent with previous experiments on isoniazid and its metabolite acetylisoniazid. They suggest that one of the routes for the metabolism of certain aroylhydrazines in man, the rabbit, and the rat is: aroylhydrazine → 1-acetyl-2-aroylhydrazine → acetylhydrazine → 1,2-diacetylhydrazine.

Previous studies[3,4] were designed to test a hypothetical route[5] in which the metabolism of isonicotinic acid hydrazide leads to the

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(2) Aided by a research grant (RG-5337) from the National Institutes of Health, U. S. Public Health Service.

(3) A. S. Yard and H. McKennis, Jr., *Federation Proc.*, **17**, 421 (1958).

(4) A. S. Yard and H. McKennis, Jr., *J. Med. Pharm. Chem.*, **5**, 196 (1962).

(5) H. McKennis, Jr., A. S. Yard, J. H. Weatherby, and J. A. Hagy, *J. Pharmacol. Exptl. Therap.*, **126**, 109 (1959).