The reactions used for the conversion of the N-L-lyxityl-4,5-dimethylaniline to L-lyxoflavin are identical with those which were devised for a synthesis[6] of riboflavin.

Experimental[7]

Calcium L-Galactonate.[8]—A mixture of 100.0 g. of α-D-galacturonic acid (Eastman Kodak Co.) and 17.5 g. of calcium hydroxide was added slowly, with stirring, to 500 ml. of water. The final solution was filtered and the hydrogenation was carried out in the presence of 6 g. of Raney nickel catalyst at 90° and about 100 atmospheres. After removal of the catalyst, the solution was concentrated under reduced pressure until crystallization had begun. The mixture was chilled, and the resulting crystalline product was collected on a filter and washed with water. After drying over phosphorus pentoxide at 50°, the calcium L-galactonate pentahydrate weighed 107.7 g.; yield 88%.

L-Lyxose.—Crude, sirupy L-lyxose was prepared by the method of Hockett and Hudson.[4]

N-L-Lyxityl-4,5-dimethylaniline.—A solution of 23.7 g. of crude, sirupy L-lyxose in 100 ml. of methanol was treated with a solution of 19 g. of 3,4-xylidine in 50 ml. of methanol, and hydrogenation was carried out at about 100 atmospheres and at 90–100° in the presence of 6 g. of Raney nickel catalyst. The crystals present when the bomb was opened were dissolved by warming, and the catalyst was removed. The crystals which separated on cooling were collected on a filter and dried; weight 16.3 g.; m.p. 147–148°. A second fraction, weighing 4.1 g., raised the total yield to 51%. When the reaction is carried out with pure lyxose, the yield is almost quantitative. The yield in this experiment represents the purity of the crude L-lyxose.

Anal. Calcd. for $C_{13}H_{21}NO_4$: C, 61.15; H, 8.29; N, 5.49. Found: C, 61.43; H, 8.00; N, 5.43.

N-L-Lyxityl-2-phenylazo-4,5-dimethylaniline.—A solution of 8 g. of aniline in a mixture of 23.5 ml. of 12 *N* hydrochloric acid and 55 ml. of water was cooled to 0°. Solid sodium nitrite was added in small portions at such a rate that the temperature of the solution did not exceed 3°, until 6 g. had been added. The solution was kept at 0° for one-half hour.

A suspension of 17.7 g. of N-L-lyxityl-4,5-dimethylaniline in 140 ml. of water was treated with 23 ml. of 12 *N* hydrochloric acid and 22.8 g. of anhydrous sodium acetate, and the mixture was cooled to −5°. The solution of diazotized aniline was added to this suspension. The resulting solution was stirred at −9 to −5° for one hour and at 0° for two hours. After warming to 20°, the stirred solution was treated with a solution of 21.5 g. of anhydrous sodium acetate in 175 ml. of water at such a rate that the *p*H remained approximately 3, and the temperature 17–20°. The resulting mixture was stirred at 22–25° for 17 hours. The crude N-L-lyxityl-2-phenylazo-4,5-dimethylaniline was collected on a filter, washed with two 70-ml. portions of water, and dried to constant weight in a vacuum oven at 50–60°. The material weighed 26 g.

L-Lyxoflavin.—A solution of the N-L-lyxityl-2-phenylazo-4,5-dimethylaniline from the experiment described above in 150 ml. of *n*-butanol was added to 26.7 ml. of glacial acetic acid containing 13.8 g. of barbituric acid, and the mixture was stirred and refluxed for five hours. After cooling and stirring in an ice-bath for an hour, the mixture was filtered. The solid material was slurried in 160 ml. of water at 80° for one-half hour and, after cooling to 70°, the solid was collected on a filter and washed with water and then methanol. The dark, crude material was dissolved in a mixture of 60 ml. of concentrated hydrochloric acid and 20 ml. of water. After two extractions with ether, the aqueous solution was freed from ether by a current of air and was then treated with 7 ml. of 30% hydrogen peroxide. After standing for about ten minutes, the solution was filtered through a layer of super-cel and poured into 700 ml. of boiling water. Cooling for several hours at 5° caused the precipitation of 14.0 g. of L-lyxoflavin (54% based on 17.7 g. of N-L-lyxityl-4,5-dimethylaniline). Before drying, the material had been washed thoroughly with water and methanol. After two recrystallizations from concentrated hydrochloric acid, 30% hydrogen peroxide and water, as described above, the orange needles melted at 283–284° (dec.); $\alpha^{23}D$ −49° ± 3° (*c*, 0.26 in 0.05 *N* sodium hydroxide). The analytical sample was dried over phosphorus pentoxide at 100°.

Anal. Calcd. for $C_{17}H_{20}N_4O_6$: C, 54.25; H, 5.36; N, 14.89. Found: C, 54.38; H, 5.39; N, 15.20.

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(6) Tishler, Pfister, Babson, Ladenburg and Fleming, *ibid.*, **69**, 1487 (1947).

(7) We are indebted to Mr. Richard Boos and his associates for the microanalyses.

(8) This procedure is similar to ones described by Isbell, *J. Research Natl. Bur. Standards*, **33**, 45 (1944).

[CONTRIBUTION NO. 232 FROM THE RESEARCH LABORATORIES OF HOFFMANN-LA ROCHE, INC.]

The Preparation of Dehydro-L-ascorbic Acid and its Methanol Complex. Some Reactions of Dehydro-L-ascorbic Acid[1]

BY B. PECHERER

An improved procedure for the oxidation of L-ascorbic acid to dehydro-L-ascorbic acid by means of chlorine has been developed. A new substance, the crystalline equimolecular complex of dehydro-L-ascorbic acid and methanol, has been obtained and some of its reactions described.

Dehydro-L-ascorbic acid has been the subject of numerous studies since the elucidation of the structure of vitamin C. Since the substance defied all attempts to isolate it in crystalline form, all of the work was done on freshly prepared solutions of the oxidized vitamin. Finally in 1948, Kenyon and Munro[2] succeeded in isolating crystalline dehydro-L-ascorbic acid by the appropriate treatment[3] of a product prepared by the method of Hirst[3] and confirmed its constitution.[4]

When a large quantity of dehydro-L-ascorbic acid was needed in this laboratory, a study was made of various methods for its preparation. In the first series of experiments,[5] L-ascorbic acid was treated in aqueous solution with oxidants selected to give as

(1) Presented before the Division of Biological Chemistry at the 118th Meeting of the American Chemical Society, Chicago, Illinois, September 4, 1950.

(2) J. Kenyon and M. Munro, *J. Chem. Soc.*, 158 (1948).

(3) Unpublished work of E. L. Hirst quoted by E. M. Crook and E. J. Morgan, *Biochem. J.*, **38**, 10 (1944).

(4) For example, its reduction to ascorbic acid by hydrogen sulfide, the formation of the 2,4-dinitrophenylosazone of dehydro-L-ascorbic acid, identical with that obtained directly from ascorbic acid, and the formation of 5,6-diacetyl-dehydro-L-ascorbic acid. The extreme insolubility in cold water is surprising.

(5) These were carried out before the work of Kenyon and Munro was published and are described in the Experimental Section.

few and as simple by-products as possible. However, most of these experiments gave only intractable sirups; only the combination hydrogen peroxide and a trace of iodine gave a high yield of a solid product that occasionally assayed as high as 90% dehydro-L-ascorbic acid, but the product was characterized by very poor stability. It was finally concluded that the isolation of stable dehydro-L-ascorbic acid from an aqueous solution was extremely unlikely. The successful isolation of the desired product from a methanol solution by Kenyon and Munro confirmed this opinion.

Although the required product was now accessible by the procedure of Kenyon and Munro,[2] the iodine requirements were formidable for the amounts of material needed. A study of the published procedure showed that with some modification the yield could be increased from 23 to 55%. Despite the increased yield, attention was directed to chlorine as an oxidant in place of iodine even though there appeared to be possible complications.[6,7]

It was found that under closely controlled conditions L-ascorbic acid in absolute methanol, in the presence of excess lead carbonate, was oxidized by gaseous chlorine to the dehydro acid. After filtering off the insoluble lead salts, it was observed that, when the bulk of the solvent had been removed *in vacuo*, the residual methanolic sirup of dehydro-L-ascorbic became pale green in color and gradually deepened until frothing started, whereupon the green color faded.[8] From the dry sirup dehydro-L-ascorbic acid was isolated in 39% yield.

On the other hand, scratching and cooling the green sirup started crystallization, and in a short time, the entire sirup was converted to a mass of colorless, thin, narrow platelets quite different in appearance from the microcrystalline dehydro-L-ascorbic acid. This new product was soluble in cold water, lower alcohols, ketones and esters, and after recrystallization from the latter solvents, it melted at 103–105° to a green sirup that effervesced, gradually became colorless leaving a white solid that melted at 223–225° with decomposition. Elementary analysis of a recrystallized sample corresponded to the formula $C_7H_{10}O_7$, an equimolecular complex of dehydro-L-ascorbic acid and methanol, *i.e.*, $C_6H_6O_6 \cdot CH_3OH$. This composition was confirmed by the ultraviolet absorption spectrum,[9] which was identical with that of the unsolvated acid when corrected for the presence of methanol, the assay[10] and the equivalent weight.

When the properties of the methanol complex were elucidated, conditions were found for the chlorine oxidation of L-ascorbic acid to yield 81% of the dehydro acid as the methanol complex (72%) and the unsolvated acid (9%). Under somewhat similar conditions, bromine and iodine were found to oxidize L-ascorbic acid to the dehydro acid, which could be isolated as the methanol complex, but optimum conditions were not developed for these oxidants.

The isolated crystalline complex yielded dehydro-L-ascorbic acid of the highest purity by thermal decomposition at 100–120°.[11]

Reconversion of the unsolvated acid to the methanol complex occurred on refluxing with an excess of methanol until solution was complete, concentrating the solution *in vacuo* until a green sirup was obtained and then isolating as described below. Attempts to prepare other solvates with ethanol, isopropyl alcohol, benzyl alcohol and ethylene glycol were unsuccessful; either the original unsolvated acid was recovered, or an uncrystallizable sirup obtained.

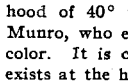
At present it is impossible to locate the methanol in the complex. A methyl ester of 2,3-diketogulonic acid is not likely in view of the ultraviolet absorption spectrum and the ready loss of methanol at the melting point. The most reasonable point of attachment seems to be at the 2- or 3-carbonyl group as a hemi-ketal. It should be noted that Drake, Smythe and King obtained spectroscopic evidence for the existence in solution of complexes between dehydro-L-ascorbic acid and sulfhydryl compounds.[12]

During these experiments it was noted that solutions of dehydro-L-ascorbic acid in contact with the skin gave pink stains that gradually turned brown and persisted for several days. Attempts to reproduce this color *in vitro* showed that the acid and the methanol complex reacted with amino acids to give pink solutions that turned a deep cherry red. It is not necessary that the amino acid be an α-amino acid. In heated solutions the color developed as rapidly as the purple color formed from amino acids and ninhydrin. While this reaction has not been studied exhaustively, it appears to take the same course as the latter reaction. Thus a warm methanol solution of an amino acid and dehydro-L-ascorbic acid turns pink and gradually deepens, carbon dioxide is evolved and, in the case of α-alanine, acetaldehyde is evolved. If heated for a sufficiently long time, the red color deepens to dark brown and an amorphous brown precipitate is obtained. Attempts to characterize these products failed.[13]

(6) W. Odling, *Chem. News*, **2**, 61 (1868), stated that moist chlorine and methanol yielded carbonic acid and hydrogen chloride. *Cf.* C. A. Jacobson, "Encyclopaedia of Chemical Reactions," Vol. II, Reinhold Publishing Co., New York, N. Y., 1948, p. 269.

(7) A. Brochet, *Ann. chim.*, [7] **10**, 294 (1897), reported that moist chlorine and methanol yielded formaldehyde and its polymers, dichloromethyl ether and hydrogen chloride among other products; *cf.* "Beilstein," IV, Vol. I, p. 277.

(8) This green color was invariably observed in over twenty oxidations employing chlorine, one with bromine and two with iodine. Its exact cause is unknown but probably resides in the chromophore



The temperature of the sirup was in the neighborhood of 40° when the green color became evident. Kenyon and Munro, who evaporated their sirup at 20°, made no mention of the color. It is conceivable that an excited state of the chromophore exists at the higher temperature. There remains the possibility that the color is due to colloidal lead salts; however, the sirups appeared perfectly clear on visual inspection and gave no tests for the presence of lead. Further evidence against this latter view is the fact that the white crystals of the complex became green at the melting point and a white residue of the unsolvated acid remains after the methanol is lost.

(9) Obtained by Mr. A. Motchane of these laboratories.

(10) *I.e.*, reduction with hydrogen sulfide and iodometric titration of the ascorbic acid thus formed.

(11) This is based on the appearance of the ultraviolet absorption spectrum.

(12) B. B. Drake, C. V. Smythe and C. G. King, *J. Biol. Chem.*, **143**, 89 (1942).

(13) E. Abderhalden, *Fermentforschung*, **15**, 285 (1937), showed that ascorbic acid, together with oxygen and traces of heavy metals, deaminated α-amino acids and gave the corresponding aldehydes with one less carbon atom. Colorless and yellow solutions were obtained in most cases, occasionally blue. In view of the complexity of the reaction of L-ascorbic acid with oxygen in the presence of heavy metals, it cannot be stated with certainty that the course of both reactions is the same.

Experimental[14]

Oxidation of Ascorbic Acid in Aqueous Solution. (1) With Iodine.—In dilute aqueous solution[15] ascorbic acid was oxidized with the stoichiometric amount of iodine. The resulting solutions were freed of hydriodic acid by treatment with the freshly regenerated and washed anion-exchangers Amberlite IR-4B[16] and Ionac A-300.[17] Although the resins effectively freed the solutions of mineral acid, they also removed the bulk of the organic matter, giving brown solutions that yielded uncrystallizable sirups on evaporation.

(2) With Hydrogen Peroxide and Ferric Ion.—In view of the deleterious effect of the resins on the product, an oxidant was sought that would give no acidic by-products. Hydrogen peroxide appeared to be such a reagent: $C_6H_8O_6 + H_2O_2 \rightarrow C_6H_6O_6 + 2H_2O$. However, ascorbic acid proved to be stable to hydrogen peroxide unless a trace of heavy metal was present. In the presence of one drop of 10% ferric chloride, 30% hydrogen peroxide reacted with ascorbic acid at 0°, but the resultant solutions darkened considerably on evaporation of the water and yielded only sirups.[18]

(3) Hydrogen Peroxide and a Trace of Iodine.—This combination over the temperature range 20–40° oxidized L-ascorbic acid smoothly to yield colorless solutions. A distinct end-point was obtained with starch–iodide paper when the stoichiometric amount of peroxide had been added. Evaporation of the resulting solutions *in vacuo* at 30–40° gave an amber sirup that could be dried to a crisp foam. Assay of the final product in the usual manner gave values which ranged from 36–88% dehydro-L-ascorbic acid in various experiments. A small amount of oxalic acid was generally present. After storage for several days, the dehydro-L-ascorbic acid assay dropped with no visible change in the material. The powdered products were converted to hard brown glasses after several months storage. A typical product, when freshly prepared, melted over the range 91–135° with decomposition while the analysis corresponded to dehydro-L-ascorbic acid containing moisture.

Anal. Calcd. for $C_6H_6O_6$: C, 41.34; H, 3.75. Found: C, 39.93; H, 3.7.

In ethanol solution this oxidizing mixture gave about 2% yield of dehydro-L-ascorbic acid which melted at 223–225° with decomposition. At this point attempts to prepare dehydro-L-ascorbic acid in aqueous solution or with aqueous reagents were abandoned.

Oxidation of L-Ascorbic Acid with Chlorine in Methanol.—In a three-liter flask equipped with a stirrer, thermometer, gas delivery tube and gas exit tube were placed 176 g. of ascorbic acid (1 mole), 520 g. of lead carbonate (1.96 moles) and 1200 ml. of dry methanol. The contents of the flask were chilled to −10° by means of an external cooling bath, and chlorine gas was passed into the stirred suspension at such a rate that the internal temperature remained below −6°. Approximately 1.5 hours were needed for the addition of 1 mole of chlorine. A colorless suspension was obtained, and the end-point detected visually or by means of starch–iodide paper. Any small excess of chlorine was removed by the addition of the requisite amount of ascorbic acid. Stirring was continued for approximately one-half hour at −6° after the end-point had been reached, during which time carbon dioxide evolution gradually ceased. At this point the *p*H of the solution should be 5–6. The flask was removed from the cooling bath, and the suspended salts were removed by filtration on an eight-inch Buchner funnel precoated with a thin layer of filter aid. Three 150-ml. portions of absolute methanol cooled to −5° were used to wash the filter cake.

The clear, colorless solution was treated with a slow stream of hydrogen sulfide for not more than 5 seconds. Excess hydrogen sulfide was removed by blowing a brisk stream of air through the suspension of lead sulfide. Five grams of filter aid was then added, and the suspension filtered through a thin bed of filter aid, washing the flask and filter with small amounts of cold methanol.

The clear, colorless filtrate was transferred to a three-liter, round-bottom flask, and the methanol removed at 40° (bath temperature) at the water-pump. When the bulk of the methanol had been removed, the colorless sirup slowly became pale green. At this point two procedures were followed, depending on whether the desired product was dehydro-L-ascorbic acid or the methanol complex of dehydro-L-ascorbic acid.

(A) Dehydro-L-ascorbic Acid.—After the sirup had become green and no condensate was obtained, the still head and condenser were replaced by an adapter, and the volatiles drawn directly into the water pump as the bath temperature was raised to 100° over the course of 30 minutes. The green color faded, and a pale amber sirup resulted. At this point the water-pump was replaced by the mechanical pump, and the residue pumped for an hour while heating the flask directly on the steam-bath; during this operation more or less frothing occurred. After this treatment, the foamy residue was cooled *in vacuo*, dissolved in 500 ml. of anhydrous ethanol, then kept at 0° for 18 hours. A dense microcrystalline precipitate was deposited. This was filtered off, washed several times with small portions of anhydrous ethanol, and dried *in vacuo* over calcium chloride for 2 or 3 days.

From the combined filtrates and washings, pale yellow-brown in color, second and third crops of pure dehydro-L-ascorbic acid were obtained by charcoaling, followed by removal of the solvent and repetition of the baking process. Yields of 29–39% were obtained and distributed as follows: first crop, 28–32 g.; second crop, 20–28 g.; third crop, 18 g. More material could be recovered by reworking the mother liquors from the third crop.

The melting point varies with the rate of heating and the temperature at which the sample is inserted in the block. A melting point of 220–225° with decomposition is satisfactory, and such material assays 95–100% dehydro-L-ascorbic acid.

Anal. Calcd. for $C_6H_6O_6$: C, 41.34; H, 3.75. Found: C, 41.39; H, 3.47.

(B) Dehydro-L-ascorbic Acid–Methanol Complex.—The pale green sirup was dissolved in 300 ml. of methyl isobutyl ketone and chilled for 18 hours at 0°, whereupon the contents of the flask set to a mass of colorless, flat, narrow platelets. These were filtered off and washed first with cold methyl isobutyl ketone, followed by anhydrous ether, then dried *in vacuo*. A yield of 122 g. (59%) was obtained. From the mother liquor a second crop of the complex was obtained by removing the solvent *in vacuo* at 40–50°, covering the residual sirup with 100 ml. of fresh methyl isobutyl-ketone, followed by chilling. Another 26 g. of pure product was obtained; total yield 72%. Alternately, the unsolvated dehydro acid may be obtained from the filtrates as described above. In this experiment 16 g. of unsolvated acid was recovered from the mother liquors obtained from the second crop of complex, thus accounting for 81% of the ascorbic acid used.

Anal. Calcd. for $C_6H_6O_6 \cdot CH_3OH$: C, 40.7; H, 4.85. Found: C, 40.4, 40.2; H, 5.09, 4.73.

Variations in the above process showed the effect of temperature on the yield; thus at 8°, the yield was only 20%, while at higher temperatures practically no crystalline product was obtained. The sensitivity of the dehydro acid to mineral acid was shown by the very poor yield obtained when the lead carbonate was added portionwise as the oxidation proceeded.

Oxidation of L-Ascorbic Acid with Bromine.—To a stirred suspension of 88 g. of L-ascorbic acid and 260 g. of lead carbonate in 600 ml. of methanol, 80 g. of bromine was added over a period of 40 minutes while the temperature was maintained at −7 to −5°. The reaction mixture was then worked up as previously described to obtain the green sirup which was dissolved in 150 ml. of methyl isobutyl ketone. After chilling for 16 hours at 4°, there was obtained 26 g. of complex that melted at 98–101°. From the filtrate and washings there was obtained 6.8 g. of the unsolvated acid, m.p. 220–223° with decomposition.

Oxidation of L-Ascorbic Acid with Iodine.—The oxidation of 44 g. of L-ascorbic acid in 300 ml. of methanol was carried out at 5° in the presence of 130 g. of lead carbonate with 129

(14) All melting points were determined with a Berl-Kullmann block and are uncorrected.

(15) In concentrated solution the reaction does not go to completion since the reaction $C_6H_8O_6 + I_2 \longrightarrow C_6H_6O_6 + 2HI$ is reversible.

(16) Product of Rohm and Haas Company.

(17) Product of the American Cyanamid Company.

(18) Dihydropyrogallol, another ene-diol, gave a fair yield of oxidation product on treatment with hydrogen peroxide and ferric ion. *Cf.* B. Pecherer, L. M. Jampolsky and H. M. Wuest, THIS JOURNAL, **70**, 2587 (1948).

g. of iodine. From the solution of the green sirup, obtained as described above, in 75 ml. of methyl isobutyl ketone there was obtained 27 g. of the methanol complex. No attempt was made to recover further material.

Properties of the Dehydro-L-ascorbic Acid–Methanol Complex. Solubility.—The complex is readily soluble in cold water, methanol, ethanol, isopropyl alcohol, *n*-butanol, methyl, ethyl, and butyl acetate. It may be recrystallized from methyl ethyl ketone, methyl *n*-propyl ketone and methyl isobutyl ketone which is preferred. Recrystallized material melts at 103–105° to a green sirup which effervesces, losing its color and resolidifying to the unsolvated acid that melts at 223–225° with decomposition.

Titration.—Within a minute after the complex is dissolved in water, the *p*H falls from 5.7 to 4.6, indicating a rapid opening of the lactone ring to form 2,3-diketogulonic acid. Direct titration is not reproducible, but under nitrogen in oxygen-free water the sodium salt may be titrated if the operations are carried out rapidly. Under these conditions a pK of 4.0–4.2 is obtained, indicating a molecular weight of 200; theory 206.

Rotation.—A freshly prepared methanol solution of the complex shows $[\alpha]^{27}D$ 62° (*c* 1),[19] changing to 38° after 28 hours.

Thermal Decomposition of the Dehydro-L-ascorbic Acid–Methanol Complex.—In a 50-ml., round-bottom flask provided with an outlet tube which led to a trap cooled in a solid CO_2 bath, 10.3 g. of dehydro-L-ascorbic acid–methanol complex (0.05 mole) was placed. The flask was gradually heated by means of an oil-bath to 100°, whereupon a few droplets were condensed in the trap. At 102° the substance melted to a green sirup, and at 107° active gas evolution commenced, large amounts of liquid being condensed. The residue was gradually converted to a pure white solid at 118°, at which temperature no more liquid was condensed after 40 minutes. The cooled residue was crushed under anhydrous ethanol, filtered off, washed and dried. It weighed 6.90 g. (80%) and melted at 223° with decomposition.

The condensate was identified as methanol by its refractive index, $n^{25}D$ 1.3271, identical with the starting solvent and the preparation, therefrom, of methyl 3,5-dinitrobenzoate; m.p. 106.5–107°.

Conversion of Dehydro-L-ascorbic Acid to the Methanol Complex.—Dehydro-L-ascorbic acid (3.48 g.) was refluxed with 20 ml. of anhydrous methanol. The solid gradually dissolved, and the solution was complete after 50 minutes. A trace of insolubles was filtered off, and the filtrate evaporated to a sirup *in vacuo*. After seeding and scratching, crystals of the methanolate began to separate. Five ml. of methyl isobutyl ketone was added, and the mixture was refrigerated overnight. The crystalline mass was filtered off, another 2.5 ml. of cold ketone used to rinse out the flask, and the product finally washed with several portions of cold, dry ether. A yield of 1.73 g. of the methanolate, melting at 102–105°, was obtained. No attempt was made to recover material from the filtrate.

Catalytic Hydrogenation of the Complex to L-Ascorbic Acid.—A solution of 20.6 g. of the complex in 150 ml. of methanol was hydrogenated at 50 p.s.i. using methanol-washed Raney nickel catalyst. The theoretical amount of hydrogen was taken up in about 12 hours. After filtering off the catalyst, a little hydrogen sulfide was passed into the solution to remove a small amount of dissolved nickel, the solution filtered and taken to dryness. The residue, somewhat dark in color due to the presence of nickel sulfide, weighed 16 g. and melted at 178.5–179.5°, the mixed m.p. with ascorbic acid of m.p. 185–187° was 180–181°. One recrystallization from methanol raised the m.p. to 188–190°.

Reaction of Dehydro-L-ascorbic Acid with Amides and α-Amino Acids.—The first observation that dehydro-L-ascorbic acid gave pink solutions with amides was made during an attempt to obtain the ultraviolet absorption spectrum in formamide.[20] Since solutions of the acid also gave pink stains on the skin, this seemed to be a "ninhydrin type" color reaction.

It was found that glycine, α- and DL-β-alanine, DL-α-aminobutyric acid, DL-α-aminocaprylic acid, DL-α-aminocaproic acid, L-valine, L-cystine, DL-serine, DL-threonine, DL-phenylalanine, L-leucine, DL-norleucine, DL-aspartic acid, L-lysine, L-argenine, L-citrulline, DL-tryptophan, L-tyrosine and DL-methionine all gave the pink colors gradually changing to deep red with either dehydro-L-ascorbic acid or the methanol complex in aqueous or alcoholic solution. γ-Aminobutyric and γ-aminoöctanoic acids gave similar colors. γ-Aminocaproic acid gave a yellow-brown solution while DL-proline and L-hydroxyproline gave yellow colors. Red colors were also obtained with formamide, acetamide and phenylacetamide. Urea gave a very pale yellow solution. This behavior resembles that of ninhydrin which reacts with amides as well as amino acids. When a solution of dehydro-L-ascorbic acid was refluxed with glycine in water or alcohol, the pink color deepened to deep cherry red within a minute and, after 50 minutes, the solution became dark brown. Evaporation of the solvent gave an intractable brown, amorphous material. In formamide the color developed was not so intense and, on evaporation, a deep red gum was obtained. In refluxing methanol, α-alanine and the complex gave the same color reactions; considerable gas was evolved which was identified as carbon dioxide, and a strong odor of acetaldehyde was present. On evaporation of the methanol, a dark brown, amorphous product was obtained that could not be crystallized. E. Abderhalden[12] described a degradation of α-amino acids by ascorbic acid in the presence of traces of heavy metals and oxygen, but the color of the reaction mixture was either colorless, yellow, gold, or in some cases bluish. In these reactions 8–24 hours was required for complete deamination of the amino acid, whereas with preformed dehydro-L-ascorbic acid a much more rapid reaction takes place.

Acknowledgment.—Thanks are due to Mr. D. Wagner for technical assistance. Mr. N. Cohen performed the numerous ascorbic acid assays required in this work. Mr. A. Motchane obtained the ultraviolet absorption spectra, and Dr. Al Steyermark and his group provided the microanalyses.

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(19) Kenyon and Munro reported $[\alpha]^{20}D$ 56° for a freshly prepared solution of the unsolvated acid in water at *c* 1, in a phthalate buffer at *p*H 3.5.

(20) This observation was made by Mr. A. Motchane.