solution (to avoid peptization), with a small portion of alcohol, and again with salt solution, dissolving in sulfuric acid, reducing with hydrogen sulfide and titrating with permanganate.

Magnetic measurements were performed both with the solid, finely powdered, and with aqueous solutions. The method was the one recently described,[4] a modified Gouy method using the double vessel of Freed and Kasper, and reading the lines of deflection on a microscopic scale at the pointer of a damped semi-micro balance. Calibration for the particular vessel used, with the pole distance used (1.39 cm.), showed that for 10 amperes one line of deflection corresponds to an increment of susceptibility $= 4.37 \times 10^{-10}$ cgs. The actual measurements were performed with lower current intensities so as to bring about conveniently measurable deflections. They are recalculated for 10 amperes. This recalculation is based on the agreeable property of the magnet used, that for pole gaps not smaller than 1 cm. the deflection is strictly proportional to the square of the amperage, at least up to 10 amperes.

One experiment will be described in detail. A solution (almost saturated) containing 3.063% of the substance, was filled into the upper compartment of the double vessel, the lower compartment being permanently filled with a 1% agar gel. The deflection measured at 6.90 amperes and recalculated to 10 amperes, was +185 lines. Water gave, under equal conditions, −87 lines. The difference, +272, corresponds to an increment of volume susceptibility, due to the solute, of $+1.19 \times 10^{-6}$ c. g. s. The correction for the difference of the diamagnetic contribution of the solute (according to Pascal) and of the water displaced by it is too small to be worthy of consideration. The result is: the susceptibility per gram-atom of Fe, $10^6\chi_{Fe} = 1970$, at 24°, and the effective moment, calculated according to the simple Curie law, is found to be 2.10 BM.

The same method, for potassium ferricyanide, in many experiments with the solid and with solutions down to $^1/_{300}$ molar at slightly varied room temperatures, gave values for μ_{eff} all in the range from 2.33 to 2.40, in agreement with acknowledged values. Table I shows the result of several experiments carried out with samples of the substance prepared independently.

For the solid, the values given in Table II were obtained.

(4) L. Michaelis, THIS JOURNAL, 63, 2446 (1941)

TABLE I

MEASUREMENT OF SOLUTIONS

Preparation	Concn., %	Temp., °C.	Susceptibility per gram atom of Fe, $\chi_{Fe} \times 10^6$	Effective moment, μ_{eff}
1	3.063	24	1970	2.17
2	2.900	24	1995	2.18
3	2.961	23	1810	2.08

TABLE II

MEASUREMENT OF THE SOLID COMPOUND

Preparation	Temp., °C.	$10^6 \times \chi_{Fe}$ Uncorrected	$10^6 \times \chi_{Fe}$ Corrected for diamagnetism	μ_{eff}.
1	24	2100	2380	2.38
2	24	2200	2480	2.42
3	23	2230	2510	2.44

A slightly higher value is obtained for the solid than for the dissolved state. This difference appears to be greater than the limits of error.

At any rate, no values in the neighborhood of those reported by the authors quoted were obtained. Our values do not suggest any Fe–Fe interaction of appreciable magnitude but lend themselves to the same interpretation as for ferricyanide ion, which, according to Pauling, may be conceived as an octahedral complex with six covalent d^2sp^3 bonds, allowing for the magnetic effect of one unpaired electron slightly increased by incompletely quenched orbital contributions. In the phenanthroline complex, two octahedra share an edge, of which the two corners are represented by oxygen atoms. In $Fe_2(CO)_9$, two octahedra share a face, so that the distance of the two central Fe atoms is smaller than in the case of a shared edge. Perhaps this is the reason why Fe–Fe interaction is strong in the carbonyl compound, but not obvious in the phenanthroline complex.

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The Synthesis of Aldehydes from Acylhydrazides

BY CARL NIEMANN AND JOHN T. HAYS[1]

The reaction

$$RCONHNHSO_2Ar + OH^- \longrightarrow RCHO + ArSO_2^- + N_2 + H_2O$$

which has been used for the preparation of a number of substituted benzaldehydes[2] and hetero-

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(2) (a) McFadyen and Stevens, *J. Chem. Soc.*, 584 (1936); (b) Hill and Short, *ibid.*, 260 (1937); (c) Harington and Pitt-Rivers, *ibid.*, 1101 (1940); (d) Natelson and Gottfried, THIS JOURNAL, 63, 487 (1941); (e) Ungnade, *ibid.*, 63, 2091 (1941); (f) Niemann, Benson and Mead, *ibid.*, 63, 2204 (1941).

cyclic aldehydes[3] is known to be limited to those cases where R is an aromatic, but not necessarily benzenoid, radical.[2,3] A further limitation of the above reaction arises from the fact that it is not applicable for the synthesis of *p*-nitrobenzaldehyde[2a] and isonicotinic aldehyde.[3e] The unexpected conversion of benzenesulfonylpicolinic hydrazide to picolinic aldehyde[3e] led us to investigate the behavior of benzenesulfonyl-*o*-nitrobenzhydrazide. We now find that *p*-nitrobenzaldehyde cannot be prepared by the above reaction and we are necessarily led to the conclusion that the conversion of benzenesulfonylpicolinic hydrazide to picolinic aldehyde constitutes an exception to the generalization that the reaction proceeds as given above except in those cases where the aromatic radical R contains a strongly meta directing group ortho or para to the hydrazide side-chain.

If the conditions set forth above are not satisfied it appears, on the basis of the single case that we have studied, that the arylsulfonylacylhydrazide can decompose via an alternative route involving the following reactions[4]

$$2\ RCONHNHSO_2Ar + 2\ OH^- \longrightarrow RCONHNHCOR + 2\ ArSO_2^- + N_2 + H_2O$$

$$RCONHNHCOR + 2\ OH^- \longrightarrow 2\ RCO_2^- + NH_2NH_2$$

Thus depending upon the nature of the radical R one can expect either the aldehyde RCHO or the acid RCO_2H and it is not unlikely that in some instances the two reactions proceed simultaneously giving as final products both acid and aldehyde. This explanation appears to account for the low yields observed in the synthesis of heterocyclic aldehydes[3] as compared with the substituted benzaldehydes.[2]

An alternative procedure of preparing aldehydes from acylhydrazides is that of Kalb and Gross.[5] In this synthesis, which is based on the earlier work of Darapsky[6] and of Curtius,[7] the acylhydrazide is converted into the aldehyde by the reaction

$$RCONHNH_2 + 2OH^- - 2e \longrightarrow RCHO + N_2 + 2H_2O$$

As with the McFadyen–Stevens reaction[2a] this reaction cannot be used for the preparation of aliphatic aldehydes and it is well established that arylacylhydrazides with a strongly meta directing group ortho or para to the hydrazide side-chain are transformed not into the corresponding aldehydes[5,7b] but into the symmetrical diacylhydrazides[5] according to the reaction

$$2RCONHNH_2 + 4OH^- - 4e \longrightarrow RCONHNHCOR + N_2 + 4H_2O$$

The similarity between the base catalyzed thermal decomposition of arylsulfonylacylhydrazides and the base catalyzed oxidation of acylhydrazides suggests that both of these reactions are governed by a common mechanism, and for preparative purposes are essentially equivalent. The question of whether one or the other should be used for the preparation of a given aldehyde can be answered only by considering the possibility of secondary reactions in which the aldehyde is the principal reactant.

Experimental[8]

Benzenesulfonyl-*o*-nitrobenzhydrazide.—Eighteen ml. of benzenesulfonyl chloride was added at 10° to a well stirred solution of 21.5 g. of *o*-nitrobenzhydrazide, m. p. 118–119°,[7b,9] in 150 ml. of pyridine. The pyridine was removed *in vacuo*, the sirupy residue titrated with water, the aqueous phase discarded and ether added to the residue to bring about its crystallization. The crude product (36 g.) was dried at 100° and recrystallized from 95% ethanol to give 28 g. of benzenesulfonyl-*o*-nitrobenzhydrazide, m. p. 184–184.5°.

Anal. Calcd. for $C_{13}H_{11}O_5N_3S$ (321.3): C, 48.6; H, 3.5; N, 13.1. Found: C, 48.5; H, 3.6; N, 12.8.

A solution of 27.5 g. of benzenesulfonyl-*o*-nitrobenzhydrazide in 100 ml. of ethylene glycol was heated to 160°, 24 g. of anhydrous sodium carbonate added, the vigorous exothermic reaction allowed to go to completion, 150 ml. of water added to the cooled reaction mixture, the solution saturated with sodium nitrate, filtered and the filtrate repeatedly extracted with ether. Distillation of the dried ethereal extract gave no indication of a fraction of b. p. 153° (23 mm.)[10] and qualitative tests on the fractions that were obtained showed no indication of the presence of *o*-nitrobenzaldehyde.

Benzenesulfonyl-*p*-nitrobenzhydrazide.[2a]—The product obtained by the reaction of *p*-nitrobenzhydrazide, m. p. 211–212°,[9] with benzenesulfonyl chloride was recrystallized from 95% ethanol to give benzenesulfonyl-*p*-nitrobenzhydrazide, buff colored prisms, m. p. 201–202°.

Anal. Calcd. for $C_{13}H_{11}O_5N_3S$ (321.3): C, 48.6; H, 3.5; N, 13.1. Found: C, 48.8; H, 3.4; N, 13.0.

(3) (a) Buchman and Richardson, THIS JOURNAL, **61**, 891 (1939); (b) Price, May and Pickel, *ibid.*, **62**, 2813 (1940); (c) Tamamusi, *J. Pharm. Soc. Japan*, **60**, 184 (1940); *C. A.*, **34**, 5447 (1940); (d) Panizzon, *Helv. Chim. Acta*, **24**, 24 (1941); (e) Niemann, Lewis and Hays, THIS JOURNAL, **64**, 1678 (1942).

(4) This formulation is based upon the demonstration of the presence of $ArSO_2^-$ and RCO_2^- in the reaction mixture and upon the observation that nitrogen and a fourth reaction product of high melting point are also formed. It appears that this latter substance is the *sym*-dihydrazide.

(5) Kalb and Gross, *Ber.*, **59**, 727 (1926).

(6) Darapsky, *J. prakt. Chem.*, **76**, 464 (1907); *Ber.*, **40**, 3033 (1907).

(7) (a) Curtius, *Ber.*, **33**, 2559 (1900); (b) Curtius and Melsbach, *J. prakt. Chem.*, **81**, 501 (1910).

(8) Microanalyses by Dr. G. Oppenheimer and Mr. G. A. Swinehart.

(9) Curtius and Trachmann, *J. prakt. Chem.*, **51**, 165 (1895).

(10) Camps, *Arch. Pharm.*, **240**, 18 (1902).

A solution of 20 g. of benzenesulfonyl-*p*-nitrobenzhydrazide in 100 ml. of ethylene glycol was heated to 160°, 20 g. of anhydrous sodium carbonate added, the vigorous exothermic reaction allowed to go to completion, 250 ml. of water added to the cooled reaction mixture, the latter acidified with 5 *N* hydrochloric acid, the precipitate collected, and solid *p*-benzoquinone carefully added to the filtrate. The crystalline product thus formed was collected and recrystallized from aqueous ethanol to give 2,5-dioxodiphenylsulfone, orange rhombs, m. p. 202–203°.[11]

Anal. Calcd. for $C_{12}H_8O_4S$ (248.2): C, 58.1; H, 3.2. Found: C, 57.9; H, 3.6.

The precipitate obtained by acidification of the reaction mixture was dissolved in hot ethanol, the solution filtered, the precipitate discarded, and an equal volume of ether added to the filtrate. The precipitate was discarded, the filtrate evaporated to dryness, the residue taken up in ethanol, an equal volume of water added, the precipitate collected and dried to give 6 g. of crude *p*-nitrobenzoic acid. Recrystallization of the crude product from hot water gave *p*-nitrobenzoic acid, pale yellow irregular platelets, soluble in aqueous sodium bicarbonate, m. p. 238–239°.

Anal. Calcd. for $C_7H_5O_4N$ (167.1): C, 50.3; H, 3.0; N, 8.4. Found: C, 50.5; H, 3.1; N, 8.5.

(11) Hinsberg, *Ber.*, **27**, 3259 (1894).

GATES AND CRELLIN LABORATORIES OF CHEMISTRY
CALIFORNIA INSTITUTE OF TECHNOLOGY
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The Absence of β-Alanine in Proteins

BY MAXWELL A. POLLACK[1]

Although β-amino acids have never been isolated from proteins, there is no evidence which rules out the possibility that they might be present in amounts too small to be detected in the ordinary procedures. Of all the possible β-amino acids, β-alanine is the simplest and at the same time the most likely to be present in proteins, in view of the fact that peptides of it, namely, carnosine and anserine, as well as pantothenic acid, have already been isolated from natural extracts.

It is possible to test for the presence of β-alanine by virtue of its strong growth-promoting action for yeast.[2,3] This phenomenon can be employed in the direct assay of hydrolysis products from proteins, for none of the other known amino acids will produce this growth response. The method as described below will show the presence of one β-alanyl unit in a protein of one million molecular weight.

Using this test, the hydrolysis products from silk fibroin, horse hemoglobin, egg albumin, gelatin, casein and lactoglobulin were assayed and found to be substantially free from β-alanine. It is very unlikely that this result can be accounted for by *destruction of β-alanine during the hydrolysis* of the proteins, for the amino acid was tested and found to be stable under these conditions, and it is not probable that peptides of β-alanine would be much more unstable than the amino acid.

The results indicate that β-alanine is not a general constituent of proteins. However, the possibility of its occurrence in special proteins still remains.

Experimental

Protein Hydrolyzates.—The lactoglobulin, egg albumin, and horse hemoglobin were all thrice-recrystallized materials. The silk fibroin had a nitrogen content of 18.97% (dry weight basis), while the gelatin was the commercial product "Knox U.S.P. Plain Sparkling Gelatine," and the casein was the Difco vitamin-free brand. These proteins were dried *in vacuo* over anhydrous calcium chloride before use.

The hydrolyzates were kindly furnished by Mr. J. R. McMahan, who used the following procedure in their preparation. One cc. of 10% hydrochloric acid was added to 100 mg. of the protein in a test-tube which was sealed and heated in an autoclave under a pressure of 15 pounds of steam for ten hours. The tube was opened, the contents rinsed out and brought to a *p*H of 6.6–7.0 by adding sodium hydroxide, and finally adjusted to the desired concentration. Pure β-alanine was put through this procedure without loss of growth-promoting potency.

Assay Method.—Varying amounts of the above solutions (filtered to remove humin) were added to short, wide-mouthed test-tubes so that the amounts in the tubes corresponded with 0.2, 1.0, 2.0, and 4.0 mg. of the original proteins. One sequence of tubes contained 0.1, 0.3, 0.5, and 1.0 microgram of pure β-alanine to serve as a standard. The volumes were adjusted to 2.0 ml. by the addition of distilled water where necessary.

The tubes were sterilized by heating in steam for ten minutes and were then cooled. To each tube was then added five ml. of medium containing ten micrograms of suspended Gebrüder Mayer (G.M.) yeast cells. The medium was the same as that of Snell, Eakin, and Williams,[4] except that the β-alanine was omitted and 0.2 microgram of biotin was added per liter of medium. The tubes were incubated at 30° for sixteen hours, following which the yeast productions were determined by shaking the tubes and measuring the turbidities of the resulting suspensions.[5]

Whereas the tubes containing added pure β-alanine showed a striking production of yeast, the turbidities of all the other tubes were approximately equal to those of the blanks, except in the case of the hydrolyzate from hemoglobin, which showed a slightly higher turbidity. The original solution in this case had been colored light

(1) Present address, E. F. Drew and Co., Inc., Boonton, N. J.

(2) R. J. Williams and E. Rohrmann, THIS JOURNAL, **58**, 695 (1936).

(3) E. E. Snell, *J. Biol. Chem.*, **141**, 121 (1941).

(4) E. E. Snell, R. E. Eakin and R. J. Williams, THIS JOURNAL, **62**, 175 (1940).

(5) R. J. Williams, E. D. McAlister and R. R. Roehm, *J. Biol. Chem.*, **83**, 315 (1929).