

tophane do not favor the amyloclastic activity of this enzyme as do the mono-amino acids tested.

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OPTICALLY ACTIVE DYES. I

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The nature of the action by which dyes are absorbed and more or less permanently held by animal or vegetable fibers, has been the subject of a large number of investigations. The first work recorded was during the eighteenth century and since then the experiments in this field have been very extensive.² From results so far obtained, no definite conclusions can be drawn as to whether there is involved a physical or chemical phenomenon or both. Scores of articles have been published by many authors, some of whom are advocates of a physical, some of a chemical explanation.

An excellent method which should throw light upon this subject is the study of the action of optically active dyes upon fibers. Each of a pair of mirror images would be expected to have the same dyeing properties, if the absorption by the fibers is purely a physical process. On the other hand, if a chemical reaction of any sort is taking place when the dye is absorbed, a different degree or rate of absorption of the *d* and *l* forms might take place on account of the optical activity of the substances of which the fibers are composed. In this connection, however, it must be considered that even though the dyeing process may be a chemical one, the *d* and *l* forms of every pair of dyes would not necessarily be absorbed to a different degree, sufficient to be measured. Only after the study of a large number of such pairs could a convincing conclusion be drawn.

Very little work upon the selective absorption of one active compound as compared with its enantiomorph has been carried out and no pairs of optically-active dyes have been made. In 1904, Willstaetter³ discussed the possibility of selective absorption by wool of one active component of a racemic mixture. He carried out experiments upon solutions of

¹ This communication is an abstract of a thesis submitted by A. W. Ingersoll in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Chemistry at the University of Illinois.

² An excellent review of the articles on the theory of dyeing from the earliest times may be found in the following books: Georgievics, "Chemistry of Dyestuffs," Scott, Greenwood and Co., 1903; Dreaper, "Chemistry and Physics of Dyeing," P. Blakiston's Son and Co., 1906; Knecht, Rawson and Loewenthal, "A Manual of Dyeing," Charles Griffin and Co., 1910; Wood, "Chemistry of Dyeing," D. Van Nostrand Co., 1913.

³ Willstaetter, *Ber.*, 37, 3758 (1904).

racemic alkaloids, but in no case was evidence of selective absorption found. Unsuccessful attempts to prepare optically active dyes are reported by Mayer,⁴ Meisenheimer⁵ and Longobardi.⁶

An interesting paper by Porter and Hirst⁷ has appeared in which a number of dyes containing an asymmetric carbon atom are described. These were made by the diazotization and coupling of aminodiphenyl alkyl carbinols. No experiments are described on the selective absorption of a single form by wool although the authors state that certain qualitative results serve to indicate selective absorption. The resolution of the dyes was not accomplished.

In studying the mechanism of dyeing by this general method, there are many reasons why it would be a distinct advantage to work with the pure *d* and *l* forms of various dyes in preference to the racemic modifications. With the latter, selective absorption of one form could be found only by determination of the rotation of the resulting solution after dyeing had taken place. Not only does the determination of the rotation of a deeply colored solution present difficulties, but if, as it might happen in many cases, the difference in the rate of absorption of one form was only slightly different from that of the other form, the rotation of the resulting solution might be so small that it could not be determined. This would be especially true if the rotation of the pure dyes was small, a possibility which must not be overlooked. If, however, the pure *d* and *l* forms of the dye were available, a comparison of two equivalent solutions as regards absorption could be made by taking a colorimeter reading on the partially exhausted *d* and *l* dye solutions and comparing them with the original solutions. Very accurate data could thus be obtained. The rotation of the partially exhausted racemic dye solutions could be used merely as a method of checking the other results.

In considering a procedure for obtaining *d* and *l* dyes in a pure state, the methods first thought of are: (1) preparation of a dye containing an asymmetric carbon atom and in addition a group which will allow the effecting of its resolution; (2) the resolution of an intermediate containing an asymmetric carbon atom and, in addition, a group through which it could be converted into dyes. The first method seems less promising because of the probable difficulties in resolution. Even the second method would probably require extensive experiments before the active forms were obtained; moreover, the chances are that only one form could be obtained pure. Without both forms of the dyes in a pure state, results from the dyeing experiments would be of little value.

The better method of approach for the preparation of numerous pairs of

⁴ Mayer, *Atti accad. Lincei* [V] **23**, 353 (1914).

⁵ Meisenheimer, *Ann.*, **423**, 105 (1921).

⁶ Longobardi, *Anales^s soc. quim. Argentina*, **8**, 153^s (1920); *C. A.*, **15**, 3984 (1921).

⁷ Porter and Hirst, *This^s JOURNAL*, **41**, 1264 (1919).

of dyes, of which some are basic and some acid, are being prepared. The absorption spectra, rotation, and other constants are now being accurately determined. A discussion of the conclusions as to whether the evidence is for chemical combination or adsorption is being reserved till more accurate experimental data are available.

Experimental

***dl*-Phenyl-amino-acetic Acid.**—The phenyl-amino-acetic acid was made by the method of C. S. Marvel and W. A. Noyes⁸ by the interaction of sodium cyanide, ammonium chloride and benzaldehyde. A few slight changes were made as follows: ethyl alcohol in place of methyl alcohol was used as a medium for the initial condensation; the time of hydrolysis of the nitrile was increased from 2 to 8 hours; the crude phenyl-amino-acetic acid after air drying was heated on a steam-bath for 15 minutes with approximately an equal weight of benzene. This treatment caused the water to separate as a layer under the benzene and at the same time dissolved a large amount of dark-colored impurity. After filtration and drying, the phenyl-amino-acetic acid was pure enough for use in this investigation.

***d*- and *l*-Phenyl-amino-acetic Acid.**—The phenyl-amino-acetic acid was resolved to yield pure *l*-product and impure *d*-product by the method of Betti and Mayer,⁹ the fractional crystallization of the *d*-camphor sulfonate of the racemic modification. This process is a very convenient one. From 151 g. of pure *dl*-phenyl-amino-acetic acid and 245 g. of pure *d*-camphor sulfonic acid in 800 cc. of boiling water, 150 g. of pure *l*-phenyl-amino-acetic acid *d*-camphor sulfonate crystallized upon slow cooling of the solution. By concentration of the filtrate from these crystals and repeated fractionation, the yield of pure product was increased to over 90%. The sirupy mother liquors contained impure *d*-phenyl-amino-acetic acid *d*-camphor sulfonate. By hydrolysis of these salts with ammonia pure *l*-phenyl-amino-acetic acid was obtained and *d*-phenyl-amino-acetic acid of about 90% purity. The *d* form was completely purified by the method of Fischer and Weickhold.¹⁰ The rotations of the *d* and *l* forms used in the preparation work were as follows.

***l*-Phenyl-amino-acetic Acid.** Subs., 3.8364: made up to 50 cc. with a mixture of 34.54 cc. of *N* HCl and 15.46 cc. of H₂O at 20° gave a rotation of -22.75° in a 2dcm. tube with sodium light; $[\alpha]_D^{20} = -157.5^\circ$.

***d*-Phenyl-amino-acetic Acid.** Subs., 1.6161: made up to 50 cc. with a mixture of 14.55 cc. of *N* HCl and 6.51 cc. of H₂O at 20° gave a rotation of $+22.84^\circ$ in a 2dcm. tube with sodium light; $[\alpha]_D^{20} = +158.0^\circ$.

***p*-Nitrobenzoyl Chloride.**—*p*-Nitrobenzoyl chloride was made from *p*-nitrobenzoic acid and phosphorus pentachloride.¹¹

***dl*-Phenyl(*p*-nitrobenzoylamino)acetic Acid.**—HO₂CC(C₆H₅)HNHCOC₆H₄NO₂.—A solution of 30.2 g. (1 mole) of pure phenyl-amino-acetic acid was dissolved in 240 cc. of 10% sodium hydroxide solution and then made up to a total volume of 500 cc. with water. Thirty-two g. (2 mole) of sodium bicarbonate was then added, thus causing a part of the amino acid to precipitate. Finally, 40 g. (1.1 mole) of pure, finely powdered *p*-nitrobenzoyl chloride was added and the mixture vigorously agitated with a mechanical stirrer for 40 to 50 minutes. During this procedure the mixture was kept below 20° by cooling, since the condensation product is rather easily decomposed by

⁸ Marvel and Noyes, THIS JOURNAL, 42, 2264 (1920).

⁹ Betti and Mayer, Ber., 41, 2071 (1908).

¹⁰ Fischer and Weickhold, *ibid.*, 41, 1286 (1908).

¹¹ "Organic Syntheses," II, John Wiley and Sons.

warm alkali. At the end of this time the amino acid and practically all of the *p*-nitrobenzoyl chloride had gone into solution and a deep purple color had developed. The solution was filtered, thus removing any undissolved material, and an excess of 10% hydrochloric acid was cautiously added. The purple color was discharged and a pink, rather sticky solid was precipitated. Without filtering, this solid product was allowed to stand and stirred occasionally with a glass rod until after several hours it became flocculent and nearly white. It was then filtered from the solution. The product weighed when dry 54 to 58 g. (70 to 75%). After three crystallizations from 95% alcohol it was absolutely pure and formed pale yellow needles melting at 184° (corr.).

From the alcoholic mother liquors from the crystallization, it was possible to recover a certain amount of the condensation product. There always was found, however, in these mother liquors a certain amount of the corresponding ester which formed during the crystallization. Consequently all product recovered in this way should be carefully tested for impurities before using.

Analysis. Subs. 0.2000: 18.52 cc. of 0.07139 *N* HCl. Calc. for $C_{15}H_{12}O_5N_2$: N, 9.33. Found: 9.26.

dl-Ethyl-phenyl(*p*-nitrobenzoylamino) Acetate, $C_2H_5O_2CC(C_6H_5)HNHCOC_6H_4NO_2$.—This substance was obtained in the mother liquors from the crystallization from alcohol of the above acid. It was also obtained by esterification of the acid with absolute ethyl alcohol and dry hydrogen chloride. The substance was purified by crystallizing from alcohol, after which it formed pale yellow needles melting at 140° (corr.).

Analysis. Subs., 0.3000: 25.5 cc. of 0.07138 *N* HCl. Calc. for $C_{17}H_{16}O_5N_2$: N, 8.5. Found: 8.5.

dl-Phenyl(*p*-aminobenzoylamino)acetic Acid, $HO_2CC(C_6H_5)HNHCOC_6H_4NH_2$.—A solution of 20 g. of *dl*-phenyl(*p*-nitrobenzoylamino)acetic acid was dissolved in a slight excess of dil. ammonium hydroxide and the solution added to a hot solution of 120 g. (6.5 mole) of pure hydrated ferrous sulfate crystals in 300 cc. of water. To this hot mixture was then added in 20cc. portions during 10 minutes, 100 cc. of conc. ammonium hydroxide and the whole mixture heated for a half an hour on a steam-bath. The reduction proceeded rapidly, the dark green ferrous hydroxide being changed to the brown ferric hydroxide after each addition of ammonium hydroxide. After the reduction was complete the mixture was filtered while hot, first with suction and then through a fluted filter. The filtrate was concentrated on a steam-bath under diminished pressure until a volume of 200 cc. remained. This was cooled and carefully acidified with the quantity of dil. hydrochloric acid calculated to liberate the free base. After standing for about an hour, the precipitated amino acid was filtered, washed with a little cold water and crystallized from about 600 cc. of boiling water. A small amount of yellow insoluble oil was almost invariably present but was readily removed by filtration of the hot solution. The yield of product after one crystallization amounted to 12–14 g. The substance at this point was almost always slightly yellow and two additional crystallizations using bone black were necessary in order to leave the substance pure white. The compound melted at 152° (corr.).

Analyses. Subs., 0.3000, 0.3000: 30.48, 30.48 cc. of 0.07138 *N* HCl. Calc. for $C_{16}H_{14}O_3N_2$: N, 10.4. Found: 10.2, 10.2.

dl-Phenyl(*p*-aminobenzoylamino)acetic Acid Hydrochloride, $HO_2CC(C_6H_5)HNHCOC_6H_4NH_2.HCl$.—The hydrochloride of the amine was prepared by dissolving the base obtained after one crystallization in hot 1:1 hydrochloric acid and warming with bone black. The hot solution was filtered and cooled, whereupon white, hard needles precipitated. They melted with decomposition when heated slowly between 190° and 200° but when immersed in a bath already heated, the decomposition was fairly sharp at 215°.

Analyses. Subs., 0.5989, 0.6225: AgCl, 0.2760, 0.2875. Calc. for $C_{15}H_{15}O_3N_2Cl$: Cl, 11.56. Found: 11.40, 11.43.

l-Phenyl(*p*-nitrobenzoylamino)acetic Acid, $HO_2CC(C_6H_5)HNHCOC_6H_4NO_2$.—This substance was prepared under exactly the same conditions as the corresponding racemic acid, using *p*-nitrobenzoyl chloride and *l*-phenyl-amino-acetic acid. The condensation product, however, was much more soluble in alcohol than the racemic modification and it was, therefore, necessary to crystallize from ethyl acetate in order to get the best results. When pure, the substance formed yellow needles melting at 163° (corr.). The yield of crude product was more than 90%. During crystallization of this compound, excessive concentration of the mother liquor in order to obtain a further yield of product was avoided since the small amount of alcohol which was present in the ethyl acetate readily caused esterification of the acid and this ester was always present in the mother liquors.

Analyses. Subs., 2.0000: made up to 25 cc. in abs. ethyl alcohol at 20° gave rotation of -13.85° in 2dcm. tube with sodium light; $[\alpha]_D^{20} = -86.56^\circ$. Subs., 0.2000: 18.56 cc. of 0.07138 *N* HCl. Calc. for $C_{15}H_{12}O_3N_2$: N, 9.3. Found: 9.3.

l-Ethyl(*p*-nitrobenzoylamino)acetic Acid, $C_2H_5O_2CC(C_6H_5)HNHCOC_6H_4NO_2$.—This ester was obtained from the mother liquors in the crystallization of the corresponding acid just mentioned above.

It was also prepared by esterifying the acid with absolute alcohol and dry hydrogen chloride. After crystallizing from alcohol, the product formed yellowish needles which melted sharply at 155° (corr.).

Analyses. Subs., 1.0150: made up to 25 cc. in $CH_3CO_2C_2H_5$ (U. S. P.) at 20° gave rotation of -5.50° in 2dcm. tube with sodium light; $[\alpha]_D^{20} = -67.7^\circ$. Subs., 0.2000, 0.2000: 17.07, 17.05 cc. of 0.01738 *N* HCl. Calc. for $C_{17}H_{18}O_3N_2$: N, 8.54. Found: 8.53.

l-Phenyl(*p*-aminobenzoylamino)acetic Acid, $HO_2CC(C_6H_5)HNHCOC_6H_4NH_2$.—This substance was prepared by the reduction of the corresponding *l*-nitro compound under exactly the same conditions as used for the racemic isomer. It was best purified by crystallizing from water in which it was slightly more soluble than the racemic modification. The yield was 65 to 70% after one crystallization, and it was essentially pure. The second and third crystallizations took the last traces of color from the compound but did not essentially change the melting point or rotation. The product formed large needles which melted sharply at $168-169^\circ$ (corr.).

Analyses. Subs., 2.0000: made up to 25 cc. in *N* HCl at 20° gave rotation of -15.00° in 2dcm. tube with sodium light; $[\alpha]_D^{20} = -93.75^\circ$. Subs., 0.2000: 20.60 cc. of 0.07138 *N* HCl. Calc. for $C_{15}H_{14}O_3N_2$: N, 10.4. Found: 10.3.

l-Phenyl(*p*-aminobenzoylamino)acetic Acid Hydrochloride, $HO_2CC(C_6H_5)HNHCOC_6H_4NH_2 \cdot HCl$.—The hydrochloride was prepared from the base by cooling a hot solution of the amino compound in strong hydrochloric acid. White crystals were thus obtained which did not melt sharply, but decomposed when plunged into a melting-point bath held between 220° and 222° .

Analyses. Subs., 0.5005, 0.5019: 14.76, 14.74 cc. of 0.1096 *N* AgNO₃. Calc. for $C_{15}H_{15}O_3N_2Cl$: Cl, 11.56. Found: 11.47, 11.41.

d-Phenyl(*p*-nitrobenzoylamino)acetic Acid, $HO_2CC(C_6H_5)HNHCOC_6H_4NO_2$.—This substance was obtained in exactly the same manner as the corresponding *l*-derivative, by condensing *p*-nitrobenzoyl chloride and *d*-phenyl-amino-acetic acid. The product had the expected physical and chemical properties which were identical with the *l*-compound. The same precaution was taken not to evaporate the mother liquors too far when crystallizing in order to avoid the contamination with ethyl ester. The substance melted at 163° (corr.).

Analyses. Subs., 2.0000: made up to 25 cc. in abs. ethyl alcohol gave rotation of $+13.78^\circ$ in 2dcm. tube with sodium light; $[\alpha]_D^{20} = +86.13^\circ$. Subs., 0.2000: 18.41 cc. of 0.07138 *N* HCl. Calc. for $C_{15}H_{12}O_3N_2$: N, 9.33. Found: 9.20.

d-Ethyl(*p*-nitrobenzoylamino)acetic Acid, $C_2H_5O_2CC(C_6H_5)HNHCOC_6H_4NH_2$.—This substance was obtained from the mother liquors from the crystallization of the *d*-acid and also by esterification by means of alcoholic hydrogen chloride. It melted at 155° (corr.).

Analyses. Subs., 1.1207: made up to 25 cc. in $CH_3CO_2C_2H_5$ (U. S. P.) at 20° gave rotation of $+6.04^\circ$ in 2dcm. tube with sodium light; $[\alpha]_D^{20} = +67.4^\circ$. Subs., 0.3000, 0.3000: 25.85, 25.65 cc. of 0.07138 *N* HCl. Calc. for $C_{17}H_{16}O_5N_2$: N, 8.54. Found: 8.62, 8.55.

d-Phenyl(*p*-aminobenzoylamino)acetic Acid, $HO_2CC(C_6H_5)HNHCOC_6H_4NH_2$.—This substance was prepared by the reduction of the *d*-nitro acid, as in the preparation of the corresponding *l*-compound. After two crystallizations from water the product was practically white and melted at 168 – 169° (corr.).

Analyses. Subs., 2.0000: made up to 25 cc. in *N* HCl at 20° gave rotation of $+14.98^\circ$ in 2dcm. tube with sodium light; $[\alpha]_D^{20} = +93.63^\circ$. Subs., 0.2000: 20.62 cc. of 0.07138 *N* HCl. Calc. for $C_{15}H_{14}O_3N_2$: N, 10.38. Found: 10.31.

d-Phenyl(*p*-aminobenzoylamino)acetic Acid Hydrochloride, $HO_2CC(C_6H_5)HNHCOC_6H_4NH_2 \cdot HCl$.—By warming the base with conc. hydrochloric acid and then cooling, the hydrochloride separated in hard, white needles. These melted with decomposition fairly sharply when dipped into a bath heated at 220° .

Analyses. Subs., 0.5041, 0.5015: 14.85, 14.80 cc. of 0.1096 *N* $AgNO_3$. Calc. for $C_{15}H_{15}O_3N_2Cl$: Cl, 11.56. Found: 11.45, 11.47.

Diazotization of *dl*-Phenyl(*p*-aminobenzoylamino)acetic Acid and Coupling with β -Naphthol, $HO_2CC(C_6H_5)HNHCOC_6H_4N_2C_{10}H_8OH$.—The base was dissolved in dil. hydrochloric acid, diazotized and coupled with β -naphthol in potassium hydroxide solution in the usual way. After standing for about an hour at room temperature, the red jelly-like mass was strongly acidified with hydrochloric acid and the dye which precipitated was filtered and dried. It was purified by crystallizing from boiling glacial acetic acid, in which it was soluble to the extent of about 2 g. in a liter. The yield was practically quantitative. The substance when pure formed small orange-red needles which melted sharply at 252° . It is readily soluble in alkalis and ammonium hydroxide but insoluble in water and organic solvents.

Analyses. Subs., 0.1843: N_2 , 17.3 cc. (33° , 748 mm.). Calc. for $C_{25}H_{19}O_4N_2$: N, 9.9. Found: 10.1.

Diazotization of *l*-Phenyl(*p*-aminobenzoylamino)acetic Acid and Coupling with β -Naphthol, $HO_2CC(C_6H_5)HNHCOC_6H_4N_2C_{10}H_8OH$.—The crude dye was washed a few times with cold glacial acetic acid in order to remove most of the water before crystallization. The product was nearly 10 times as soluble in glacial acetic acid as the racemic compound, and consequently was crystallized fairly readily. It formed orange-red crystals melting at 238° . The yield was 8.7 g.

Analyses. Subs., 1.0000: made up to 50 cc. in water containing 1.1 equivalents of NaOH at 20° gave rotation of -1.09° with sunlight; $[\alpha]^{25} = -27.25^\circ$. Subs., 0.2417: N_2 , 23.2 cc. (36° , 748.5 mm.). Calc. for $C_{25}H_{19}O_4N_2$: N, 9.9. Found: 10.1.

Diazotization of *d*-Phenyl(*p*-aminobenzoylamino)acetic Acid and Coupling with β -Naphthol, $HO_2CC(C_6H_5)HNHCOC_6H_4N_2C_{10}H_8OH$.—The product looked and acted in identically the same way as the *l*-compound and melted sharply at 238° (corr.).

Analyses. Subs., 1.0000: made up to 50 cc. in water containing 1.1 equivalents of NaOH at 20° gave a rotation of $+1.14^\circ$ in 2dcm. tube with sunlight; $[\alpha]^{25} = +28.50^\circ$.

Subs., 0.2036, 0.1310: N_2 , 19.7 cc. (32° , 748 mm.), 12.1 cc. (30° , 747 mm.). Calc. for $C_{23}H_{19}O_4N_3$: N, 9.9. Found: 10.3, 10.0.

Diazotization of *l*-Phenyl(*p*-aminobenzoylamino)acetic Acid and Coupling with Dimethylaniline, $HO_2CC(C_6H_5)HNHCOC_6H_4N_2C_6H_4N(CH_3)_2$.—The base was diazotized and coupled with dimethylaniline in the usual way. To the resulting solution, with mechanical stirring, 25 g. of crystallized sodium acetate was added in small portions, thus reducing the acidity to a point where coupling occurred and the dye separated from solution as a yellow-brown precipitate. It was filtered and crystallized from 1 liter of 50% alcohol. After three crystallizations the product, which formed red-brown needles, was pure and melted at 189 – 190° .

An attempt was made to determine the rotation in absolute alcohol using sunlight but no readings could be obtained with this light.

Analysis. Subs., 0.1994: N_2 , 23.3 cc. (33° , 751 mm.). Calc. for $C_{23}H_{22}O_3N_4$: N, 13.92. Found: 13.80.

Diazotization of *d*-Phenyl(*p*-aminobenzoylamino)acetic Acid and Coupling with Dimethylaniline, $HO_2CC(C_6H_5)HNHCOC_6H_4N_2C_6H_4N(CH_3)_2$.—The method of preparation of this dye is identical with that for the *l*-isomer. It melted when pure at 188 – 189° . No rotation could be observed by the use of sunlight.

Analysis. Subs., 0.3438: N_2 , 43.6 cc. (24° , 741 mm.). Calc. for $C_{23}H_{22}O_3N_4$: N, 13.92. Found: 13.80.

The preparation of the *dl*-phenyl(*p*-aminobenzoylamino)acetic acid from *dl*-phenyl-amino-acetic acid presented difficulties. The product was not easy to crystallize and different melting points on different samples were obtained. Further study of this compound is being made.

Summary

1. Suitable methods for the preparation of various pairs of optically active dyes are discussed.

2. A very convenient specific method is given for the preparation of optically-active dyes. It consists in starting with amino acids which have first been resolved by known methods and converting to dyes by the following series of reactions.



3. Two pairs of optically active dyes have been prepared, namely, those starting with *d*- and *l*-phenyl-amino-acetic acid, and after carrying out the series of reactions just described, coupling with β -naphthol and dimethylaniline, respectively. These pairs of dyes have been studied in only a preliminary way. The results tend to show that the β -naphthol dyes are absorbed in equal amounts by wool, but that the dimethylaniline dyes are absorbed in different amounts during the same period of time.

URBANA, ILLINOIS