vertebrates, the isoelectric point of hemoglobin being 6.7 and that of *Arenicola* and *Lumbricus* erythrocruorin being 4.56 and 5.3, respectively.

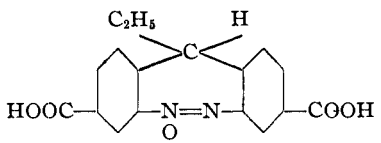
UPSALA, SWEDEN RECEIVED JANUARY 23, 1933
PUBLISHED JULY 6, 1933

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE UNIVERSITY OF ILLINOIS]

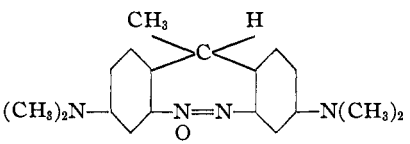
A Proof of the Unsymmetrical Structure of the Azoxy Group[1]

BY TSE-TSING CHU[2] AND C. S. MARVEL

The isolation of optically active isomers of certain types of azoxy compounds would furnish evidence to establish the unsymmetrical structure of the azoxy group which was advanced by Angeli.[3] The first compound selected for resolution in order to obtain this evidence was prepared by the reduction of an *o,o'*-dinitrodiphenylmethane derivative.[4] In view of previous work[5] on closely related compounds, the substance was assigned a cyclic structure (I). King[6] has undertaken to solve the problem in a similar fashion and for this purpose has prepared a related compound to which he has assigned formula II.



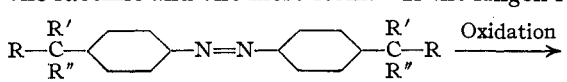
I



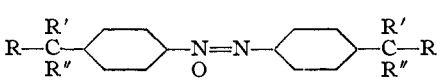
II

The azoxy compound (I) which was prepared in this Laboratory was not successfully resolved. A further study of this substance has shown that it is really not a simple cyclic compound since it has a molecular weight of 2000–3000. Obviously such a polymeric type of molecule is not suited to the work at hand and a new attack on the problem was undertaken.

An azo compound such as is represented in formula III should exist in one racemic and one meso form. If the Angeli formula of the azoxy group



III



IV

---

(1) This communication is a portion of a thesis submitted by Tse-Tsing Chu in partial fulfilment of the requirements for the Degree of Doctor of Philosophy in Chemistry at the University of Illinois.

(2) Research fellow of the China Foundation for the Promotion of Education and Culture, 1932–1933.

(3) Angeli, *Gazz. chim. ital.*, **46**, II, 67 (1916).

(4) Hussey, Marvel and Hager, THIS JOURNAL, **52**, 1122 (1930).

(5) Täuber, *Ber.*, **24**, 3083 (1891); Ullmann and Dieterle, *ibid.*, **37**, 24 (1904); Duval, *Compt. rend.*, **141**, 198 (1905); *Bull. soc. chim.*, [4] **7**, 527, 681 (1910); King, *J. Chem. Soc.*, **117**, 988 (1920).

(6) King, *J. Soc. Chem. Ind.*, **49**, 281 (1930).

is the correct one, the meso form of such an azo compound should yield a racemic azoxy compound (IV). On the other hand, if the older symmetrical structure of the azoxy group —N—N— is the correct one, the meso azo compound should yield a meso azoxy compound.

The meso and racemic forms of an azo compound of the type indicated (III) have been prepared and characterized. On oxidation these yielded two distinct racemic modifications of the azoxy compound (IV). These experiments furnish a new type of evidence to support the unsymmetrical structure of the azoxy group.

The compounds which were actually prepared, the methods of synthesis and the important properties of isomers which were obtained are outlined in the chart.

It should be pointed out that Kenner[7] has shown that the azoxy group itself will not produce molecular asymmetry. The isolation of an unstable isomeric form of such a simple substance as azoxybenzene[8] does not affect the conclusions which have been deduced from the consideration of the number of stable isomers which were obtained in this work.

The characterization of the four stable optical isomers of α-*p*-azoxyphenylbutyric acid, herein described, furnishes conclusive proof of the unsymmetrical nature of the azoxy group.

Experimental

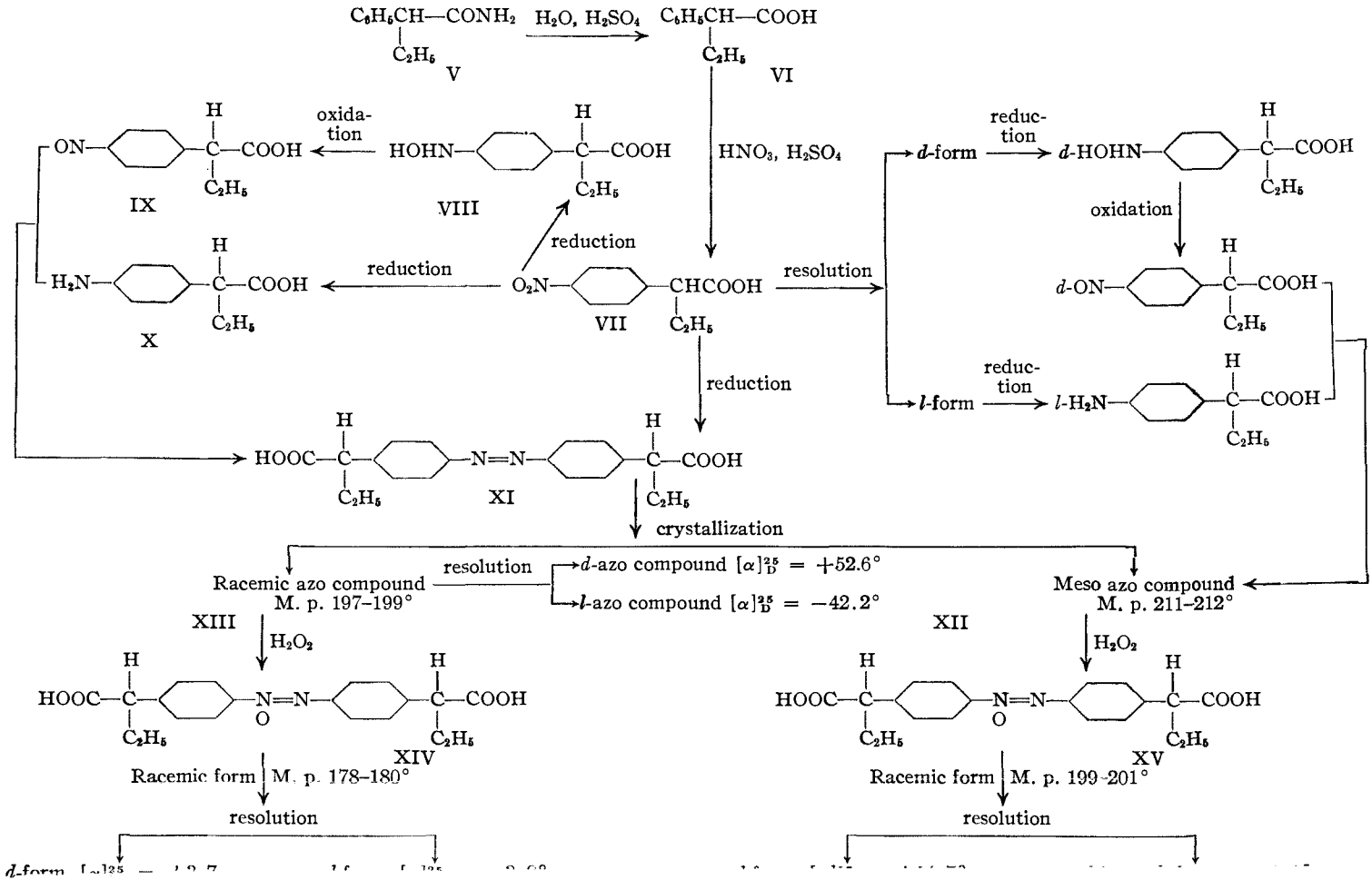
Properties of the Compound Described as Ethyl-2,2'-azoxydiphenylmethane-4,4'-dicarboxylic Acid (I).[4]—The compound previously thought to be a cyclic azoxy compound was studied further and the following properties indicate that it is a polymeric product; amorphous character; no crystals could be detected even under the microscope; the melting point as previously reported was not sharp; the compound was extremely insoluble in all solvents except pyridine; the molecular weight in camphor (Rast method)[9] and in pinene hydrochloride (Pirsch method)[10] was found to be about 2000-3000.

α-Phenylbutyramide (V).—This material was kindly furnished by the Mallinckrodt Chemical Works.

α-Phenylbutyric Acid (VI).—A mixture of 600 g. of V, 1 liter of water and 400 cc. of concentrated sulfuric acid was vigorously stirred and boiled with a reflux for two hours. Another liter of water was added and the mixture was cooled. The oily layer was dissolved in 12% sodium hydroxide and the acid reprecipitated with 30% sulfuric acid, separated and distilled under reduced pressure. The yield was 530–554 g. (88–90% of the theoretical amount) of b. p. 136–138° (3 mm.) and m. p. 42°.

α-*p*-Nitrophenylbutyric Acid (VII).—A mixture of 60 cc. of fuming nitric acid (sp. gr. 1.6) and 70 cc. of concentrated sulfuric acid was dropped during about two hours into a well-stirred solution of 164 g. of VI in 600 cc. of concd. sulfuric acid kept below 10°. The nitration mixture was stirred for two hours longer at 10° and then allowed to stand at room temperature overnight before pouring onto about 2 kg. of cracked

(7) Kenner, *J. Soc. Chem. Ind.*, **49**, 397 (1930).
(8) Reissert, *Ber.*, **42**, 1364 (1909); Müller, *Ann.*, **493**, 166; **495**, 132 (1932).
(9) Rast, *Ber.*, **55**, 1053, 3727 (1922).
(10) Pirsch, *ibid.*, **65**, 862 (1932).


C6H5CH—CONH2
C2H5
V
H2O, H2SO4
C6H5CH—COOH
C2H5
VI
HNO3, H2SO4
oxidation
reduction
ON—
HOHN—
H2N—
O2N—
—COOH
—CHCOOH
IX
VIII
X
VII
resolution
d-form
l-form
d-HOHN—
d-ON—
l-H2N—
oxidation
reduction
reduction
HOOC—
—N=N—
XI
crystallization
Racemic azo compound
M. p. 197–199°
resolution
d-azo compound [α]D25 = +52.6°
l-azo compound [α]D25 = −42.2°
Meso azo compound
M. p. 211–212°
XIII
XII
H2O2
O
XIV
XV
Racemic form
M. p. 178–180°
M. p. 199–201°
resolution
resolution

ice. The nitro acid separated as a gummy material and solidified into lumps. After collecting the crude acid on a Buchner funnel and washing it thoroughly with water, it was dissolved in about 3500 cc. of boiling 30% acetic acid. On cooling the solution, a red cake formed at the bottom but crystals separated in the main part of the solution. By separating the crystals and re-treating the red cake with solvent, 110–125 g. of crude nitro acid was obtained. This was then dissolved in 1500 cc. of boiling 50% acetic acid; the solution was treated with decolorizing carbon, filtered and diluted with an equal volume of water. The yield was 105–109 g. (50–52% of the theoretical amount) of a product of m. p. 118–120°.

Anal. Calcd. for $C_{10}H_{11}NO_4$: C, 57.41; H, 5.26; neutral equivalent 209. Found: C, 57.21; H, 5.28; neutral equivalent 211.

The position of the nitro group was established by oxidizing a sample with chromic acid to yield *p*-nitrobenzoic acid.

Conversion of α-*p*-Nitrophenylbutyric Acid to the Mixture of Isomers of α-*p*-Azophenylbutyric Acid (XI).—To a solution of 52 g. of the nitro acid in 500 cc. of 95% alcohol was added a solution of 66 g. of potassium hydroxide in 200 cc. of water. This solution was heated to about 55° and with vigorous stirring 65 g. of zinc dust was added rapidly enough to keep the solution refluxing gently. The addition required about ten minutes. At the end of about one hour the spontaneous reaction slowed down and the mixture was then heated to gentle boiling for about four hours until the solution was practically colorless. The hot solution was filtered. The solution immediately turned red. The precipitate was washed with about 250 cc. of water and the washings were added to the alcoholic filtrate.

Air was bubbled through this filtrate for about ten hours. The blood-red solution was evaporated to about 300–400 cc. and 150 cc. of water was added. The solution was cooled and poured into a mixture of 400 cc. of concentrated sulfuric acid, 300 g. of ice and 100 cc. of water. Orange-red crystals of the azo acid separated. This product was collected on a suction filter and dried. After one recrystallization from about 1700 cc. of 50% alcohol and working up the mother liquors, 28 g. (64% of the theoretical amount) of a product melting at 204–207° (dec.) was obtained. This was a mixture of the meso and racemic azo acids.

Anal. Calcd. for $C_{20}H_{22}N_2O_4$: C, 68.18; H, 6.25; neutral equivalent 176. Found: C, 67.9; H, 6.24; neutral equivalent 177.

Separation of the Meso (XII) and Racemic (XIII) α-*p*-Azophenylbutyric Acids.—A solution of 8 g. of the mixed azo compounds in 2100 cc. of 95% alcohol and 1400 cc. of water was heated to boiling and then cooled to room temperature and allowed to stand for two days. Crystals separated and were collected on a filter. The fraction of material thus obtained weighed 2.59 g. and melted at 209–211° (dec.). On recrystallization from a mixture of 400 cc. of acetone and 300 cc. of water, 1.8 g. of product melting at 211–212° (dec.) was obtained. Further purification did not change the melting point.

The alcoholic mother liquors from the first crop of azo compound were allowed to stand for another two days and 0.5 g. of crystals melting at 205–207° (dec.) was obtained. The solution was concentrated to about one-half the original volume, cooled and filtered. The crystals thus obtained weighed 3.5 g. and melted at 202–205° (dec.). The filtrate from this third fraction was evaporated to one-half volume and cooled for about twelve hours. The crystals thus obtained were recrystallized from 100 cc. of equal parts of acetone and water. There was thus obtained 0.8 g. of crystals which melted at 197–199° (dec.).

Ten 8-g. portions of the mixture of azo compounds were crystallized by this procedure and the total yield of azo compound, m. p. 211–212° (decomp.) was 25.5 g.

Anal.[11] (Micro Dumas). Calcd. for $C_{20}H_{22}N_2O_4$: N, 7.93. Found: N, 7.93.

The more soluble lower melting fractions were recrystallized from 30% alcohol and finally from 50% acetone. The total yield of the azo compound of m. p. 197–199° (dec.) was 8.5 g.

Anal.[11] Calcd. for $C_{20}H_{22}N_2O_4$: N, 7.93. Found: N, 8.1.

Resolution of *dl*-α-*p*-Azophenylbutyric Acid (XIII).—Salts of the low melting azo acid were made with brucine, strychnine, quinine, cinchonine, cinchonidine, nicotine, morphine and *d*-α-phenylethylamine and these salts were crystallized from various solvents. The most satisfactory resolution was obtained when the strychnine salt was fractionally crystallized from a mixture of methyl alcohol and ethyl acetate.

To 3 g. of the low-melting azo acid dissolved in 200 cc. of dry ethyl acetate was added a solution of 5.68 g. of strychnine in a mixture of 300 cc. of absolute methyl alcohol and 300 cc. of dry ethyl acetate. The mixture was boiled to get a clear solution. No material separated on cooling. The solution was concentrated to about 70–80 cc. On cooling and filtering 1.8 g. of crystals was obtained. This material was found to contain some strychnine.

The mother liquors from the first crop of crystals were concentrated to about 15–20 cc. On cooling a viscous, reddish liquid was obtained. This viscous material was heated with 50 cc. of dry ethyl acetate. The material gradually crystallized, giving 5 g. of fine orange crystals. The product was dried at 80–90°. It softened at 190° and melted at 195–197°. *Rotation.* 0.2 g. subs., in 25 cc. of pyridine; α, −1.506°; *t*, 25°; *l*, 2 dcm.; $[\alpha]_D^{25}$ −94.12°.

This salt was recrystallized from dry ethyl acetate three or four times. The least soluble portions were separated at one end of the series and the more soluble at the other end of the series.

The least soluble fraction melted at 197–199°. *Rotation.* 0.215 g. subs., in 25 cc. of pyridine; α, −1.37°; *t*, 25°; *l*, 2 dcm.; $[\alpha]_D^{25}$ −79.6°.

Anal.[12] Calcd. for $C_{62}H_{66}N_6O_8$: N, 8.21. Found: N, 8.21.

The more soluble material was isolated by evaporating the mother liquors and washing the sticky red material with petroleum ether (b. p. 30–60°). After drying at 70–80°, this material softened at 115° and melted at 130–136°. It was dissolved in dry ethyl acetate and on slow evaporation of the solution a small amount of crystalline material which softened at 130° and melted at 140–146° was obtained. *Rotation.* 0.215 g. subs., in 25 cc. of pyridine; α, −1.63°; *t*, 25°; *l*, 2 dcm.; $[\alpha]_D^{25}$ −94.7°.

Anal.[12] Calcd. for $C_{62}H_{66}N_6O_8$: N, 8.21. Found: N, 8.16.

The active azo acid was prepared from each salt by dissolving it in a mixture of equal parts of pyridine and water and then acidifying the solution with hydrochloric acid at 0°. The free acids were recrystallized from dilute alcohol.

The acid from the least soluble salt melted at 195–196° with some decomposition. *Rotation.* 0.1760 g. subs., in 25 cc. of absolute alcohol; α, +0.741°; *t*, 25°; *l*, 2 dcm.; $[\alpha]_D^{25}$ +52.6°. From a slightly less pure salt a dextro acid with a rotation of +50.5° was obtained.

Anal.[11] Calcd. for $C_{20}H_{22}N_2O_4$: N, 7.93. Found: N, 7.92.

The acid from the more soluble salt melted at 194–196° with some decomposition. *Rotation.* 0.2320 g. subs., in 25 cc. of absolute alcohol; α, −0.784°; *t*, 25°; *l*, 2 dcm.; $[\alpha]_D^{25}$ −42.2°.

Anal.[11] Calcd. for $C_{20}H_{22}N_2O_4$: N, 7.93. Found: N, 7.89.

(11) This analysis was made by Mr. K. Eder.

(12) This analysis was made by Mr. J. M. Fulton.

Meso-α-*p*-azophenylbutyric Acid (XII).—The identification of the high melting compound as the meso form was made by two methods. This azo compound could not be resolved, and it was synthesized by combining *d*-α-*p*-nitrosophenylbutyric acid with *l*-α-*p*-aminophenylbutyric acid.

The strychnine salt of the meso form was prepared by mixing 3 g. of the azo acid and 5.68 g. of strychnine in 600 cc. of a mixture of equal parts of absolute methyl alcohol and dry ethyl acetate. The salt was isolated in several portions, each fraction melted at 148–150° and the rotations were practically identical. The least soluble fraction had a rotation of −83.5° and the most soluble fraction had a rotation of −83.4°.

Anal.[12] Calcd. for $C_{62}H_{66}N_6O_6$: N, 8.21. Found: N, 8.29.

The free azo acids isolated from the two salts showed no rotation when dissolved in absolute alcohol.

Resolution of α-*p*-Nitrophenylbutyric Acid (VII).—To a solution of 52.2 g. of α-*p*-nitrophenylbutyric acid in 3.5 liters of dry methyl alcohol was added a solution of 40.5 g. of anhydrous quinine in 500 cc. of dry methyl alcohol. After this solution had stood for twenty-four hours at 0°, it was filtered. The crystals thus obtained weighed 29–32 g. (fraction I). The salt was recrystallized four times from methyl alcohol and then had a constant rotation and melted at 183–185°. The yield was 10–11 g. *Rotation.* 0.3800 g. subs., in 20 cc. of pyridine; α, −1.605°; *t*, 25°; *l*, 2 dcm.; $[\alpha]_D^{20}$ −42.2°.

Anal.[11] Calcd. for $C_{30}H_{35}N_3O_6$: N, 7.88. Found: N, 7.74.

All of the mother liquors from the original filtration and the recrystallization of this first salt were combined and a solution of 40.5 g. of anhydrous quinine in 500 cc. of dry methyl alcohol was added. The solution was allowed to stand for three hours and filtered. This fraction of crystals weighed 34–42 g. After recrystallization four times from dry methyl alcohol, 9–12 g. of salt melting at 183–185° with a rotation of −42.7° was obtained.

The original mother liquor and the solution from the purification of this salt were combined and concentrated to about 1500 cc. The solution was cooled to room temperature and allowed to stand for three hours. The crystals thus obtained weighed 30–45 g. and after six recrystallizations gave 9–10 g. of the salt melting at 183–185°, $[\alpha]_D^{25}$ −42.2°.

The mother liquors were combined and again evaporated to 1500 cc. About 40–50 g. of salt was obtained. It was a mixture and could not be purified to give either pure *d*- or pure *l*-salt. This material was discarded. The mother liquors were concentrated to 1000 cc. and 20–30 g. of mixed salt was obtained. The solution was next concentrated to 500 cc. and 16–25 g. of mixed salt was obtained. This was recrystallized from methyl alcohol and the mother liquors were added to the original filtrate. The solution was concentrated to 250 cc. and on cooling 5–10 g. of the salt was obtained. This was crystallized and the solution combined with the filtrate. The solution was evaporated to 150 cc. and cooled at 0° for twelve hours. The salt thus obtained weighed 4–6 g. On recrystallization from methyl alcohol this gave 3–4 g. of a salt which melted at 180–182°. *Rotation.* 0.1830 g. subs., in 20 cc. of pyridine; α, −0.984°; *t*, 25°; *l*, 2 dcm.; $[\alpha]_D^{25}$ −53.8°.

Anal.[11] Calcd. for $C_{30}H_{35}N_3O_6$: N, 7.88. Found: N, 8.05.

The filtrate was evaporated to dryness and the residue was washed with acetone to remove some colored impurities and then recrystallized from a little methyl alcohol. Another 4–6 g. of the same salt was obtained; $[\alpha]_D^{25}$ −54°.

The less soluble fraction proved to be the quinine salt of the *d*-acid and the more soluble fraction was the *l*-salt. Many different salts and different conditions were tried in an effort to obtain a more satisfactory resolution but the above procedure was the best separation that could be obtained.

The pure *d*-acid was obtained by treating 60 g. of the less soluble salt ($[\alpha]_D^{25}$ −42.2°) with 30 cc. of concentrated hydrochloric acid at 0°. This mixture was then treated with 300 cc. of water at 0° and another 10 cc. of hydrochloric acid was added. The acid separated and was collected on a Buchner funnel. The yield was 22 g. After recrystallization from 2500 cc. of hot water, 20 g. of pure *d*-acid of m. p. 120–122° was obtained. *Rotation.* 0.2845 g. subs., in 20 cc. of ethyl acetate; α, +0.503°; *t*, 25°; *l*, 2 dcm.; $[\alpha]_D^{25}$ +17.7°.

Anal.[11] Calcd. for $C_{10}H_{11}NO_4$: N, 6.7; neutral equivalent, 209. Found: N, 6.7; neutral equivalent, 211.

In a similar fashion 24 g. of the more soluble salt gave 8 g. of pure *l*-acid m. p. 120–122°. *Rotation.* 0.2945 g. subs., in 20 cc. of ethyl acetate; α, −0.528°; *t*, 25°; *l*, 2 dcm.; $[\alpha]_D^{25}$ −17.8°.

Anal.[11] Calcd. for $C_{10}H_{11}NO_4$: N, 6.7; neutral equivalent, 209. Found: N, 6.99; neutral equivalent, 208.

The active nitro acids racemized readily in hot 50% acetic acid and in boiling water some racemization occurred in two to three hours.

α-*p*-Aminophenylbutyric Acid (X).—A solution of 15 g. of the nitro acid in 100 cc. of methyl alcohol was reduced by the method of Adams and Shriner[13] using 0.2 g. of platinum catalyst. The reduction was complete in thirty to forty-five minutes. After filtering off the catalyst, the solution was evaporated and the amino acid was recrystallized from benzene. The yield was 10–12 g. of a product melting at 142–143°.

Anal. Calcd. for $C_{10}H_{13}NO_2$: N, 7.82; neutral equivalent, 179. Found: N, 7.93; neutral equivalent, 180.

In a similar manner, *l*-α-*p*-aminophenylbutyric acid was obtained from the *l*-nitro acid. *Rotation.* 0.5025 g. subs., in 20 cc. of absolute alcohol; α, −0.621°; *t*, 25°; *l*, 2 dcm.; $[\alpha]_D^{25}$ −12.4°.

Anal.[11] Calcd. for $C_{10}H_{13}NO_2$: N, 7.82. Found: N, 7.83.

d-α-Aminophenylbutyric acid, m. p. 138–140°, $[\alpha]_D^{25}$ +12.2°, was obtained from the *d*-nitro compound.

Anal.[11] Calcd. for $C_{10}H_{13}NO_2$: N, 7.82. Found: N, 7.85.

α-*p*-Hydroxylaminophenylbutyric Acid (VIII).—The hydroxylamine derivative was prepared by a method devised from one described by Bamberger and Pyman[14] for the preparation of *o*-hydroxylaminobenzoic acid.

A mixture of 26.1 g. of α-*p*-nitrophenylbutyric acid, 210 cc. of water and 19.6 g. of powdered barium hydroxide was stirred vigorously for about fifteen minutes until all of the acid was converted to the barium salt and the solution was alkaline to phenolphthalein. Then 10 g. of ammonium chloride was added and while the solution was vigorously stirred 20 g. of zinc dust was added over a period of about fifteen minutes. The reduction started slowly but after a second fifteen minutes the temperature was about 40–45°. The temperature was held at this point by external cooling. The solution turned dark red when the zinc was added and then gradually faded through a pale orange to a grayish white. Better results were obtained when the temperature was carefully controlled and air was excluded by carrying out the reduction in an atmosphere of nitrogen. The stirring was continued for about a half hour after the temperature had dropped to about 20°. The solution was then rapidly filtered with suction and the precipitate was washed with 50 cc. of cold water. The yellow filtrate was at once poured into a well-stirred mixture of 20 cc. of concentrated hydrochloric acid and 20 g. of cracked ice. The mixture was held at 0 to −5° for one hour, and then filtered with suction. The colorless

(13) Adams and Shriner, "Organic Syntheses," **8**, 66 (1928).

(14) Bamberger and Pyman, *Ber.*, **42**, 2306 (1909).

crystals were dried in a vacuum desiccator. The yield was 15 g. An additional amount of material (1 g.) could be obtained by extracting the filtrate with ether. The product could be purified by careful crystallization from a mixture of one part of alcohol and three parts of chloroform. The pure material melted at 112–113° with some evolution of gas and then resolidified to an orange-colored substance which melted at 166–168°. The substance was unstable to air or light and decomposed within a short time. In the absence of air, light caused the substance to darken. In air the finely powdered crystals first turned yellow and then green. In alcoholic or alkaline solution, air oxidized the substance rapidly to give red-colored solutions.

Anal. Calcd. for $C_{10}H_{13}NO_3$: N, 7.18. Found: N, 7.15.

d-α-*p*-Hydroxylaminophenylbutyric acid was obtained in a similar manner from the *d*-nitro acid. *Rotation.* 0.4865 g. subs., in 20 cc. of absolute alcohol; α, +1.85°; *t*, 25°; *l*, 2 dcm.; $[\alpha]_D^{25}$ +38°.

Anal.[11] Calcd. for $C_{10}H_{13}NO_3$: N, 7.18. Found: N, 7.25.

α-*p*-Nitrosophenylbutyric Acid (IX).—The method of Bamberger and Pyman[14] was used for the oxidation of the hydroxylamine derivative to the nitroso compound. A cold (0°) solution of 10 g. of the freshly prepared hydroxylamine derivative in 100 cc. of ethyl alcohol was quickly poured into a mixture of 25 g. of ferric chloride (hydrated) in 250 cc. of water and 150 g. of cracked ice. The best results were obtained when these operations were carried out in an atmosphere of nitrogen. The solution became emerald green and the nitroso compound began to separate in a few minutes. When no more crystals seemed to be forming, the mixture was filtered with suction in an atmosphere of nitrogen. The crystals were colorless at first but turned to an amorphous yellow powder very quickly. The yield was 4–5 g. of the crude product which melted at 182–186° with decomposition. No satisfactory method of purification could be devised and the crude compound was not analyzed. It was used directly in the condensation reactions described below.

Crude *d*-α-*p*-nitrosophenylbutyric acid was prepared from the active hydroxylamine compound. *Rotation.* 0.3370 g. subs., in 20 cc. of absolute alcohol (green solution); α, +1.42°; *t*, 25°; *l*, 2 dcm.; $[\alpha]_D^{25}$ +42.1°.

α-*p*-Azophenylbutyric Acid (XI) from α-*p*-Nitrosophenylbutyric Acid (IX) and α-*p*-Aminophenylbutyric Acid (X).—The procedure for this condensation was devised from similar reactions reported by Mills[15] and Angeli.[16]

To a solution of 5 g. of the amino acid in a mixture of 55 cc. of glacial acetic acid and 20 cc. of absolute alcohol was added 5 g. of the freshly prepared nitroso compound. The mixture was heated in a water-bath at 70–85° for thirty to forty minutes and then allowed to stand for another thirty minutes at room temperature. The fine orange crystals were collected on a Buchner funnel and recrystallized from 300 cc. of 50% ethyl alcohol. The yield was 5 g. of a product melting at 199–201°. An additional gram of the azo compound was obtained by diluting the mother liquors with water. The product thus obtained was a mixture of the meso and racemic azo acids almost identical in composition with that prepared by the reduction of the nitro compound reported above.

A similar condensation of 5 g. of *l*-α-*p*-aminophenylbutyric acid with 5 g. of freshly prepared *d*-α-*p*-nitrosophenylbutyric acid gave 5.5 g. of azo compound, m. p. 202–204°. This product had a slight dextro rotation. Recrystallization of 4 g. of this product from 1600 cc. of 65% alcohol gave 2 g. of the azo compound (XII) melting at 211–212°. This product had no rotation and agreed in all of its properties with the unresolvable azo compound obtained from the direct reduction of the nitro compound.

Anal.[11] Calcd. for $C_{20}H_{22}N_2O_4$: N, 7.9. Found: N, 7.92.

(15) Mills, *J. Chem. Soc.*, **67**, 925 (1895).

(16) Angeli, *Atti Accad. Lincei*, [V] **23**, ii, 30 (1914).

To a solution of 1 g. of the *d*-amino acid in a mixture of 14 cc. of glacial acetic acid and 14 cc. of absolute alcohol was added 1 g. of freshly prepared *d*-nitroso acid. The mixture was warmed only four or five minutes to avoid racemization. The azo compound thus obtained was recrystallized from 50% alcohol. The yield was 0.8 g.; m. p. 192–194°. *Rotation.* 0.3890 g. subs., in 20 cc. of absolute alcohol; α, +1.65°; *t*, 25°; *l*, 2 dcm.: $[\alpha]_D^{25}$ +42.4°. The rotation of the *d*-azo compound obtained by resolution was +52.6°.

Anal.[11] Calcd. for $C_{20}H_{22}N_2O_4$: N, 7.9. Found: N, 8.05.

Oxidation of *dl*-α-*p*-Azophenylbutyric Acid (XIII) to *dl*-α-*p*-Azoxyphenylbutyric Acid (XIV).—The method used for the oxidation was adapted from the general method of Angeli.[17] A solution of 4 g. of the racemic azo acid and 160 cc. of 30% hydrogen peroxide in 350 cc. of glacial acetic acid was heated under a reflux condenser on a steam cone for four to five hours. The solution was cooled to room temperature and poured into 1500 cc. of water. The mixture was allowed to stand overnight at room temperature and then filtered on a Buchner funnel. The crystals thus obtained were recrystallized three or four times from 30% methyl alcohol until the melting point was constant. The yield was 2–2.3 g. of a slightly yellow product; m. p. 178–180°.

Anal.[11] Calcd. for $C_{20}H_{22}N_2O_5$: N, 7.57; neutral equivalent, 185. Found: N, 7.62; neutral equivalent, 188.

Oxidation of Meso-α-*p*-azophenylbutyric Acid (XII) to α-*p*-Azoxyphenylbutyric Acid (XV).—In the same manner 3.5–4 g. of an azoxy acid (m. p. 199–201°) was obtained from 6 g. of the meso azo acid, 200 cc. of 30% hydrogen peroxide and 450 cc. of glacial acetic acid.

Anal.[11] Calcd. for $C_{20}H_{22}N_2O_5$: N, 7.57; neutral equivalent, 185. Found: N, 7.45; neutral equivalent, 183.

Resolution of α-*p*-Azoxyphenylbutyric Acid (XV) Prepared from the Meso Azo Acid.—The resolution of the azoxy acid was not satisfactorily accomplished by use of strychnine, brucine, quinine or cinchonine using as solvents, ethyl alcohol, methyl alcohol, acetone, ethyl acetate and chloroform. *d*-α-Phenylethylamine gave a neutral salt which separated into two fractions when ethyl acetate was used as a solvent.

To a solution of 4 g. of the high melting azoxy acid (m. p. 199–201°) in 2 liters of dry ethyl acetate was added a solution of 2.6 g. of *d*-α-phenylethylamine in 700 cc. of the same solvent. Crystals separated at once. The solution was heated to boiling until these redissolved. This solution was allowed to stand overnight and the first fraction of crystals was collected on a suction filter. The yield was 4.8 g. of a product which sintered at 196° and melted at 205–208°. This material was recrystallized from 2400 cc. of ethyl acetate and then twice more from a mixture of 400–500 cc. of chloroform and 20–40 cc. of methyl alcohol. This gave 1.5 g. of a product which sintered at 205° and melted at 207–210°. Further crystallization did not change the melting point or the optical rotation. *Rotation.* 0.313 g. subs., in 25 cc. of absolute methyl alcohol; α, +0.21°; *t*, 25°; *l*, 2 dcm.; $[\alpha]_D^{25}$, +8.3°.

Anal.[12] Calcd. for $C_{36}H_{44}N_4O_5$: N, 9.15. Found: N, 9.27.

The filtrate from the first fraction was concentrated to 500 cc. and allowed to stand overnight. A second fraction of salt weighing 1.2 g. was obtained. This fraction was recrystallized from ethyl acetate until it had a constant melting point and rotation; m. p. 192–196°. *Rotation.* 0.1800 g. subs., in 25 cc. of methyl alcohol; α, +0.15°; *t*, 25°; *l*, 2 dcm.; $[\alpha]_D^{25}$, +9.7°.

Anal.[12] Calcd. for $C_{36}H_{44}N_4O_5$: N, 9.15. Found: N, 9.16.

The filtrate from the second fraction was evaporated to dryness and another 0.5 g. of less pure salt with a rotation of +9.1° was obtained.

(17) Angeli, *Atti Accad. Lincei*, [5] **19**, i, 793 (1910); **20**, i, 896 (1911).

The free *l*-azoxy acid was obtained by treating a solution of 1.6 g. of the less soluble salt in 180 cc. of methyl alcohol at 0° with ice-cold concentrated hydrochloric acid and diluting with 250 cc. of water. The acid was collected on a suction filter and recrystallized from 30% methyl alcohol. The product thus obtained weighed 0.92 g.; m. p. 206–208°. *Rotation.* 0.4420 g. subs., in 25 cc. of absolute methyl alcohol; α, −0.47°; *t*, 25°; *l*, 2 dcm.; $[\alpha]_D^{25}$ −13.3°.

Anal.[12] Calcd. for $C_{20}H_{22}N_2O_5$: N, 7.57. Found: N, 7.75.

In the same manner 0.8 g. of the more soluble salt gave 0.45 g. of *d*-azoxy acid; m. p. 206–208°; rotation, 0.4040 g. subs., in 25 cc. of methyl alcohol; α, +0.478°; *t*, 25°; *l*, 2 dcm.; $[\alpha]_D^{25}$ +14.7°.

Anal.[12] Calcd. for $C_{20}H_{22}N_2O_5$: N, 7.57. Found: N, 7.58.

The last fraction of the salt obtained above gave a sample of azoxy acid which melted at 195–199° and had a rotation of +11°.

Resolution of the Low Melting *dl*-α-*p*-Azoxyphenylbutyric Acid (XIV).—The resolution of the low melting (178–182°) azoxy acid was accomplished in essentially the same manner. The salt was prepared from 1.5 g. of the azoxy acid and 0.98 g. of *d*-α-phenylethylamine in 2 liters of ethyl acetate. The first fraction which separated weighed 1.1 g. and after recrystallization to constant melting point and rotation gave 0.42 g. of a product; m. p. 204–206°. *Rotation.* 0.3510 g. subs., in 25 cc. of methyl alcohol; α, +0.22°; *t*, 25°; *l*, 2 dcm.; $[\alpha]_D^{25}$ +7.8°.

Anal.[12] Calcd. for $C_{36}H_{44}O_5$: N, 9.15. Found: N, 9.16.

Evaporation of the original mother liquor to 450 cc., cooling and filtering gave 0.75 g. of product; m. p. 200–203°. After crystallization to constant melting point, the yield was 0.41 g.; m. p. 201–203°. *Rotation.* 0.3180 g. subs., in 25 cc. of methyl alcohol; α, +0.14°; *t*, 25°; *l*, 2 dcm.; $[\alpha]_D^{25}$ +5.5°.

Anal.[12] Calcd. for $C_{36}H_{44}N_4O_5$: N, 9.15. Found: N, 9.15.

Another less pure fraction with a rotation of +5.1° was obtained from the further concentration of the mother liquors.

The *l*-azoxy acid was obtained by the procedure described before; 0.38 g. of the less soluble salt yielded 0.21 g. of acid; m. p. 182–183°. *Rotation.* 0.2000 g. subs., in 25 cc. of methyl alcohol; α, −0.061°; *t*, 25°; *l*, 2 dcm.: $[\alpha]_D^{25}$ −3.8°.

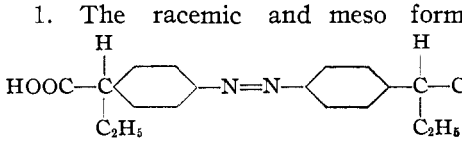
Anal.[12] Calcd. for $C_{20}H_{22}N_2O_5$: N, 7.57. Found: N, 7.72.

In the same manner 0.38 g. of the more soluble salt yielded 0.2 g. of product; m. p. 182–184°. *Rotation.* 0.2000 g. subs., in 25 cc. of methyl alcohol; α, +0.06°; *t*, 25°; *l*, 2 dcm.; $[\alpha]_D^{25}$ +3.7°.

Anal.[12] Calcd. for $C_{20}H_{22}N_2O_5$: N, 7.57. Found: N, 7.72.

Summary

1. The racemic and meso forms of α-*p*-azophenylbutyric acid



have been prepared and characterized by resolution and synthesis, respectively.

2. Meso-α-*p*-azophenylbutyric acid has been oxidized to give a resolvable form of α-*p*-azoxyphenylbutyric acid. This has furnished a new type of evidence to confirm the unsymmetrical structure of the azoxy group.

URBANA, ILLINOIS RECEIVED JANUARY 23, 1933
PUBLISHED JULY 6, 1933