LXXI.—*Compounds of the Tryparsamide Type. Part I. Resolution of* N-*Phenylalanine*-4-*arsinic Acid and of its Amide.*

By CHARLES STANLEY GIBSON, JOHN DOBNEY ANDREW JOHNSON, and BARNETT LEVIN.

N-PHENYLGLYCINEAMIDE-4-ARSINIC acid (" tryparsamide ") was first prepared by Jacobs and Heidelberger (*J. Amer. Chem. Soc.*, 1919, **41**, 1589) and, along with numerous derivatives, examined

therapeutically by (Miss) Pearce and Brown (*J. Exp. Med.*, 1921, **33**, 193). Tryparsamide has been described as the most effective organic arsenical for the treatment of human sleeping sickness due to infection by *Tr. gambiense*.

In attempting to extend our knowledge of organic arsenicals belonging to this series, it was decided to investigate in the first place those externally compensated compounds which can be resolved into their optically active components, so that the therapeutic value of the optical isomerides might be compared as well as that of the externally compensated compound itself. Further, it is hoped to ascertain whether the marked differences in pharmacological and therapeutical action found among other analogous groups of optical isomerides exist in this series also (compare Cushny, " Biological Relations of Optically Active Substances," 1926).*

dl-N-*Phenylalanine*-4-*arsinic acid* (I) was prepared by the condensation of sodium *p*-arsanilate (" atoxyl ") with α-bromopropionic acid in aqueous solution (compare D.-R.P. 204,664 of 1908; Meister, Lucius, and Brüning), and preliminary investigation indicates that it has some therapeutic value. It is essentially a dibasic acid, although titration against sodium hydroxide and phenolphthalein indicates a slightly greater basicity than two in dilute solutions. The *methyl* and the *ethyl* ester—only the carboxyl group being esterified—of the acid were prepared, and from the former, but not from the latter, the corresponding amide-acid, *dl-N*-phenylalanine-amide-4-arsinic acid (II), was obtained by the action of aqueous ammonia. This amide-acid has been prepared by Jacobs and Heidelberger [*loc. cit.*, p. 1597; who name it *N*-(phenyl-4-arsonic acid)-α-amino-propionamide] and by Fourneau and Nicolitch (*loc. cit.*, p. 1211) by the action of α-bromopropionamide on *p*-arsanilic acid. The decomposition point of this *dl*-amide-acid recorded by Jacobs and Heidelberger (darkening at 255° and decomposing at

* The present work was begun some two years ago. It was almost completed when Fourneau and Nicolitch announced the successful resolution of *dl-N*-phenylalanineamide-4-arsinic acid (phenylmethylglycineamidearsinic acid) and the application of the optically active enantiomorphs to the resolution of synthetic ephedrine. In correspondence, M. Fourneau encouraged us to continue our work and sent us a copy of the thesis of M. Nicolitch. This has since been published (Fourneau and Nicolitch, *Bull. Soc. chim.*, 1928, **34**, 1232).

262—263·5°) is somewhat higher than that recorded by us (never higher than 244°), but too much stress should not be laid on this, since the actual temperature at which the profound decomposition sets in has been found to depend almost entirely on the rate of heating and it is not easy to record the same decomposition point on identical specimens.

The resolution of *dl*-*N*-phenylalanine-4-arsinic acid was effected in aqueous solution, 2 mols. of the dibasic acid, 2 mols. of sodium hydroxide, and 2 mols. of brucine being used. The *normal brucine salt* of the *d*-acid separated under the conditions described on p. 484 and was optically pure after one recrystallisation from aqueous solution. From this pure brucine salt the pure d-*acid* was easily isolated. The pure l-*acid* was obtained by crystallising three times from water the crude acid liberated from the mother-liquor from which the above brucine salt had been removed, the optically active acids being appreciably less soluble in water than the *dl*-acid. The optically active *monoethyl* and *monomethyl esters* were prepared, their rotatory powers being of the same sign as those of the optically active acids from which they were obtained. They have very much higher melting and decomposition points than the inactive esters and their lower solubility, especially of the methyl esters in water, is very marked. The corresponding amide-acids were made by dissolving the optically active methyl esters in aqueous ammonia: their rotatory powers were of opposite sign to those of the esters (and of the optically active acids) from which they were prepared.

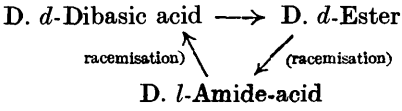
When a solution of the monosodium salt of the optically active amide-acid was boiled with an excess of sodium hydroxide until ammonia ceased to be evolved, the rotatory power of the remaining aqueous solution was of the opposite sign to that of the amide-acid taken, but it was very much lower in value than that of the sodium salt of the pure acid in equivalent concentration. This was confirmed in the case of both isomerides, and it was evident that considerable racemisation had taken place during the cycle of operations *d*-dibasic acid ⟶ *d*-methyl ester ⟶ *d*-amide-acid (*lævo*rotatory) ⟶ *d*-dibasic acid.

There was no evidence in the experimental work to indicate that racemisation had taken place during the conversion of the optically active acid into the corresponding methyl ester: the latter only needed to be freed from mechanical impurities (one crystallisation) to be optically pure. It was possible, however, that some racemisation had occurred during amide-acid formation (compare Wren, J., 1909, **95**, 1596) and possibly also during the hydrolysis of the amide-acid.

The pure optically active amide-acids were prepared by the resolution of *dl*-*N*-phenylalanineamide-4-arsinic acid, which was easily effected in aqueous solution with 2 mols. of the amide-acid and 1 mol. each of sodium hydroxide and quinine under the conditions described in the experimental portion. The quinine salt of the *l*-amide-acid needed only one crystallisation from water for complete purification, and from this pure quinine salt the pure *l*-amide-acid was isolated. The pure *d*-amide-acid was obtained by recrystallising three times from water the crude *d*-amide-acid isolated from the mother-liquor from the above quinine salt of the *l*-amide-acid. This method of resolution of the *dl*-amide-acid is somewhat different from that employed by Fourneau and Nicolitch, who used equimolecular quantities of the *dl*-amide-acid and quinine and appear to have had greater difficulties than have been encountered in the present work in obtaining the pure optically active amide-acids. Attempted resolutions, using the half-molecule method, with brucine and strychnine were unsuccessful; in the latter case a crystalline strychnine salt was obtained, but this proved to be a partial racemate.

These optically pure amide-acids had distinctly higher rotatory powers than those obtained from the optically active methyl esters by the action of ammonia. When the pure *l*-amide-acid (*dextro*-rotatory) was hydrolysed by boiling its aqueous solution with an excess of sodium hydroxide until ammonia ceased to be evolved, the resulting solution was *lævo*rotatory and the rotatory power was very much lower than that of the pure *lævorotatory* dibasic acid in equivalent concentration. This proved that in the above cycle of operations racemisation takes place, not only during the conversion of the optically active methyl ester into the optically active amide-acid of the opposite sign, but also during the hydrolysis of the optically active amide-acid to the optically active dibasic acid, again of the opposite sign.

Since the *dextro*rotatory dibasic acid has been proved to be the parent substance of the *lævo*rotatory amide-acid, it seems advisable to adopt Fischer's system of nomenclature for configuration and sign of rotation. The above cycle of operations is therefore more completely expressed as follows :



The following is a résumé of the optical rotation constants ($[\alpha]_{5461}$) of the pure compounds described in the present communication :

Brucine salt of D. *d*-dibasic acid (water)	− 10·61°
Quinine salt of D. *l*-amide-acid (water)	−123·8

	Dextro.	Laevo.
N-Phenylalanine-4-arsinic acid (disodium salt, water)	+ 56·40°	− 55·94°
N-Phenylalanine-4-arsinic acid (ethyl ester) (alcohol)	+127·9	−125·8
N-Phenylalanine-4-arsinic acid (ethyl ester) (sodium salt, water)	+103·0	−102·8
N-Phenylalanine-4-arsinic acid (methyl ester) (sodium salt, water)	+176·6	−116·3
N-Phenylalanineamide-4-arsinic acid (sodium salt, water)	− 17·88	+ 16·47

EXPERIMENTAL.

dl-N-*Phenylalanine*-4-*arsinic Acid* (I).—A solution of α-bromopropionic acid (35 g.) in water (38 c.c.) was added to a hot solution of sodium *p*-aminophenylarsinate ("atoxyl" or "soamin" containing $5H_2O$; 50 g.) in water (165 c.c.), and the mixture boiled for 8 hours. The product crystallised in somewhat yellowish needles when the resulting solution was kept for 16—40 hours in the ice-chest. dl-N-*Phenylalanine*-4-*arsinic acid*, recrystallised from a large volume of boiling water, decolorising charcoal being used to remove the small amount of colour, formed colourless needles, decomp. 207—210°. 16·75 G. were consistently obtained in every preparation (Found: As, 25·9, 26·1. $C_9H_{12}O_5NAs$ requires As, 25·9%).

The acid is soluble to the extent of about 0·5% in cold water and about 6% in boiling water. It dissolves readily in dilute mineral acids and in acetic acid; it is readily soluble in hot methyl and ethyl alcohols and moderately easily soluble in the cold solvents. It is also slightly soluble in acetone, but insoluble in benzene and ether. In aqueous solution it reduces ammoniacal silver nitrate, a silver mirror being produced, and thus behaves similarly to *o*- and *p*-tolylglycines (Staats and Ehrlich, *Ber.*, 1883, **16**, 204; Cosack, *ibid.*, 1880, **13**, 1091). The acid itself behaves on titration as slightly more than a dibasic acid (phenolphthalein); the end-point is, however, not well defined and varies with the dilution.

Ethyl Ester of dl-N-*Phenylalanine*-4-*arsinic Acid*.—A mixture of the preceding acid (5·0 g.), absolute ethyl alcohol (30 c.c.), and concentrated sulphuric acid (0·5 c.c.) was boiled for 2 hours and poured into water (100 c.c.). The *ethyl* ester, which separated on standing, crystallised from dilute ethyl alcohol (30%) in colourless, doubly-refracting prisms, m. p. 175—177° (decomp.) (Found: As, 23·6. $C_{11}H_{16}O_5NAs$ requires As, 23·6%). The ester is readily soluble in ethyl alcohol and in hot water and behaves on titration as a slightly more than monobasic acid (phenolphthalein), the end-point not being sharply defined.

Methyl ester of dl-N-*phenylalanine*-4-*arsinic acid* was prepared in an

analogous manner to the ethyl ester, pure methyl alcohol being used. After distillation of some 50% of the excess of alcohol, the reaction product was poured into a cold saturated aqueous solution of ammonium sulphate. The separated ester, containing a little ammonium sulphate, was completely extracted with pure methyl alcohol, the methyl alcohol distilled off, and the residue crystallised from the minimum quantity of hot water. It was obtained in colourless acicular needles, m. p. 181° (slight decomp.); yield, 70% (Found : As, 24·3. $C_{10}H_{14}O_5NAs$ requires As, 24·75%). This methyl ester is distinctly more soluble than the ethyl ester.

dl-N-*Phenylalanineamide*-4-*arsinic Acid* (II).—This amide was prepared in two ways :

(*a*) The methyl ester (4 g.) was added gradually to an aqueous solution of ammonia (*d* 0·880; 12 c.c.), cooled in ice. It dissolved fairly readily, and the solution was kept at the ordinary temperature for 48 hours, the excess of ammonia then being removed by leaving the mixture over sulphuric acid under reduced pressure. The residue, a thick gum, was stirred with a little water, and the amide precipitated by the addition of a slight excess of acetic acid. The product crystallised from boiling water in colourless needles, m. p. 233—240° (decomp.) (Found : N, 9·5; As, 25·5. Calc. for $C_9H_{13}O_4N_2As$: N, 9·7; As, 26·0%).

(*b*) α-Bromopropionamide (70 g.) was boiled with a solution of " atoxyl " or " soamin " (containing $5H_2O$; 115 g.) in water (325 c.c.) for 1 hour. The method of working up the product was similar to that described by Fourneau and Nicolitch (*loc. cit.*, p. 1241); 75 g. were obtained, m. p. 244° (decomp.) (Found : N, 9·4%) (compare Jacobs and Heidelberger, *loc. cit.*).

Attempted Reduction of dl-N-*Phenylalanine*-4-*arsinic Acid*.—Reduction of the arsinic acid with sodium hydrosulphite, with or without magnesium chloride, gave a yellow material, soluble in alkaline solutions, which contained much sulphur but too little arsenic for an arseno-compound. Reduction of a saturated solution of the acid in concentrated hydrochloric acid containing a trace of hydriodic acid at a low temperature with sulphur dioxide gave a crystalline product which could not be isolated on account of its ready solubility in the mixture at the ordinary temperature.

Resolution of dl-N-*Phenylalanine*-4-*arsinic Acid*.—To a boiling solution of the acid (70 g.) in 0·516*N*-sodium hydroxide (469·5 c.c.) and water (565·5 c.c.), brucine (113 g.) was added and the boiling was continued until almost the whole had dissolved. Crystallisation began almost immediately, and, after standing at the ordinary temperature for 16 hours, the crystalline material was filtered off, washed with a little water, and recrystallised from 2 litres of boiling

water. After one further recrystallisation under similar conditions, its rotatory power * in aqueous solution was constant: $c = 1{\cdot}1446$, $l = 4$, $\alpha = -0{\cdot}48°$, whence $[\alpha] = -10{\cdot}61°$. The *brucine* salt of *d*-*N*-phenylalanine-4-arsinic acid crystallises from water in large colourless plates containing $7H_2O$ (Found for air-dried material: H_2O, 10·6; N, 6·0. $C_9H_{12}O_5NAs,2C_{23}H_{26}O_4N_2,7H_2O$ requires H_2O, 10·5; N, 5·8%. Found for anhydrous material: As, 6·9. $C_9H_{12}O_5NAs,2C_{23}H_{26}O_4N_2$ requires As, 6·9%).

d- *and* l-N-*Phenylalanine*-4-*arsinic Acids*.—To obtain the d-*acid*, the above brucine salt was ground three times with small quantities of a concentrated aqueous solution of ammonia, the brucine filtered off, and the filtrate extracted thoroughly with chloroform to remove any dissolved brucine. The aqueous solution after evaporation on the water-bath to about 100 c.c. was acidified with hydrochloric acid (Congo red), and the precipitated acid filtered off from the cold mixture. After one recrystallisation from water its rotatory power was constant and it was obtained in colourless needles, m. p. 220—221° (decomp.) (Found: As, 26·0. $C_9H_{12}O_5NAs$ requires As, 25·9%). For its rotatory power determination, the acid was treated with the calculated quantity of a standard aqueous solution of sodium bicarbonate to form the normal salt, and this solution made up to volume with water: $c = 0{\cdot}8562$, $l = 4$, $\alpha = +1{\cdot}93°$, whence $[\alpha] = +56{\cdot}40°$.

The l-*acid* was isolated from the mother-liquor remaining after the separation of the above brucine salt of the *d*-acid. The solution was made alkaline with aqueous ammonia, the brucine filtered off, and the solution extracted thoroughly with chloroform and evaporated to small bulk on the water-bath. The *l*-acid was precipitated with hydrochloric acid and after three crystallisations from water its rotatory power was constant. It resembled the *d*-acid in appearance and had the same decomposition point (Found: As, 25·6%). As in the case of the *d*-acid, its rotatory power was determined in aqueous solution containing exactly sufficient sodium bicarbonate to form the disodium salt: $c = 0{\cdot}8942$, $l = 4$, $\alpha = -2{\cdot}00°$, whence $[\alpha] = -55{\cdot}94°$.

15·5 G. of the pure *d*-acid and 10·0 g. of the pure *l*-acid were obtained. The partly resolved acid recovered on working up the mother-liquors was submitted to further resolution, the quantities of brucine, etc., employed being adjusted in accordance with the rotatory power of the partly resolved acid.

Ethyl Esters of d- *and* l-N-*Phenylalanine*-4-*arsinic Acids*.—These were prepared from the corresponding optically active acids by the

* All rotatory power determinations were carried out at 20° with the mercury-green ($\lambda = 5461$) line.

method used for the preparation of the ethyl ester of the *dl*-acid. Being somewhat less soluble, they are even more easily obtained. In appearance they resemble the *dl*-ester, but their decomposition point is very much higher, 275—276° [Found : (for the d-*ester* prepared from the *d*-acid) As, 23·5; (for the l-*ester* prepared from the *l*-acid) As, 23·75. $C_{11}H_{16}O_5NAs$ requires As, 23·6%].

The following rotatory power determinations were made in pure ethyl-alcoholic solutions :

d-ester : $c = 0{\cdot}4464$, $l = 4$, $\alpha = +2{\cdot}28°$, whence $[\alpha] = +127{\cdot}9°$.
l-ester : $c = 0{\cdot}7132$, $l = 4$, $\alpha = -3{\cdot}59°$, whence $[\alpha] = -125{\cdot}8°$.

The following rotatory power determinations were made in aqueous solutions containing the exact quantity of sodium bicarbonate to form the sodium salt :

d-ester : $c = 0{\cdot}4118$, $l = 4$, $\alpha = +1{\cdot}70°$, whence $[\alpha] = +103{\cdot}0°$.
l-ester : $c = 0{\cdot}3960$, $l = 4$, $\alpha = -1{\cdot}63°$, whence $[\alpha] = -102{\cdot}8°$.

Methyl Esters of d- *and* l-N-*Phenylalanine-4-arsinic Acids.*— These were made in a similar manner to the methyl ester of *dl*-*N*-phenylalanine-4-arsinic acid. A preliminary experiment indicated that the optically active ester is very much less soluble in water than the optically inactive one; the esterification mixture therefore was poured into water instead of into a saturated solution of ammonium sulphate. After one recrystallisation from water the esters were optically pure, a yield of over 90% being obtained. The two esters crystallised in long colourless needles, m. p. 277—278° (decomp.). They are very sparingly soluble in water and are conveniently recrystallised from boiling water (6 g. to 200 c.c.) [Found : (d-*ester*) As, 25·1; (l-*ester*) As, 24·6. $C_{10}H_{14}O_5NAs$ requires As, 24·75%]. The rotatory power determinations were done in aqueous solutions containing the calculated quantity of sodium bicarbonate to form the sodium salt :

d-ester : $c = 0{\cdot}6194$, $l = 4$, $\alpha = +2{\cdot}92°$, whence $[\alpha] = +117{\cdot}6°$.
l-ester : $c = 0{\cdot}4982$, $l = 4$, $\alpha = -2{\cdot}32°$, whence $[\alpha] = -116{\cdot}3°$.

Each of these optically active methyl esters was converted into the corresponding optically active amide-acid under precisely the same conditions as those employed in the preparation of the *dl*-amide-acid from the *dl*-methyl ester. In each case the product was recrystallised once from water and obtained in colourless needles, m. p. 242—243° (decomp.) [Found : (amide-acid from *d*-methyl ester) N, 9·9; (amide-acid from *l*-methyl ester) N, 9·6. Calc. for $C_9H_{13}O_4N_2As$: N, 9·7%].

The rotatory powers were determined in aqueous solution con-

taining the exact quantity of sodium bicarbonate to form the sodium salt :

(*a*) Amide-acid from *d*-methyl ester : $c = 0{\cdot}6114$, $l = 4$, $\alpha = -0{\cdot}33°$, whence $[\alpha] = -13{\cdot}3°$.

(*b*) Amide-acid from *l*-methyl ester : $c = 0{\cdot}6536$, $l = 4$, $\alpha = +0{\cdot}36°$, whence $[\alpha] = +13{\cdot}9°$.

The solution used in (*a*) (40 c.c.) was treated with sodium hydroxide (1 g. in 2 c.c. of water) and boiled for 10 minutes; ammonia then ceased to be evolved. The resulting solution was cooled and made up to 50 c.c., and its rotatory power determined. It had $[\alpha] = +26{\cdot}6°$. The solution used in (*b*) (40 c.c.) was treated in an exactly similar manner and the final rotatory power was $[\alpha] = -29{\cdot}5°$.

Resolution of dl-*Phenylalanineamide*-4-*arsinic Acid.*—The amide-acid was prepared by the method described by Jacobs and Heidelberger (*loc. cit.*; compare Fourneau and Nicolitch, *loc. cit.*). The amide-acid (36 g.) was dissolved in boiling water (2000 c.c.) and aqueous sodium hydroxide (0·516*N*; 121 c.c.), and quinine (22·3 g., allowing 10% excess on account of water content) added. The whole was brought into solution and allowed to cool during 20 hours. The salt crystallised in soft needles, and was separated from the mother-liquor. The salt was recrystallised from boiling water (2000 c.c.), and another recrystallisation from water (1500 c.c.) failed to affect the rotatory power. The salt was anhydrous (Found : N, 9·1; As, 12·2. Calc. for $C_9H_{13}O_4N_2As,C_{20}H_{24}O_2N_2$: N, 9·15; As, 12·3%). The rotatory power was determined in aqueous solution : $c = 0{\cdot}2412$, $l = 4$, $\alpha = -1{\cdot}20°$, whence $[\alpha] = -123{\cdot}8°$.

l- *and* d-N-*Phenylalanineamide*-4-*arsinic Acids.*—The above quinine salt was decomposed by grinding with aqueous ammonia (*d* 0·880), and the liquid filtered. The separated quinine was re-extracted twice with further quantities of ammonia solution. The filtrate was acidified with concentrated hydrochloric acid (Congo red), and the precipitated amide-acid recrystallised from boiling water (decolorising charcoal); its rotatory power was then constant. The pure *l*-amide-acid crystallised in colourless needles, m. p. 247° (decomp.) (Found : N, 9·4. Calc. for $C_9H_{13}O_4N_2As$: N, 9·7%). Its rotatory power was determined in aqueous solution containing the exact quantity of sodium bicarbonate to form the sodium salt : $c = 0{\cdot}5868$, $l = 4$, $\alpha = -0{\cdot}42°$, whence $[\alpha] = -17{\cdot}88°$.

The mother-liquor after separation of the above salt was evaporated to about half its original volume, and the quinine precipitated by addition of a slight excess of an aqueous solution of ammonia.

After filtration, the liquid was acidified with concentrated hydrochloric acid (Congo red), and the amide-acid precipitated as before. This was recrystallised three times from boiling water; its rotatory power was then constant. In appearance and general behaviour the pure *d*-amide acid was similar to its enantiomorphous isomeride and it had the same melting-decomposition point (247°) (Found : N, 9·5%). Its rotatory power was determined as in the previous case : $c = 0{\cdot}7770$, $l = 4$, $\alpha = +0{\cdot}51°$, whence $[\alpha] = +16{\cdot}5°$.

Before the effect of hydrolysis on these pure optically active amide-acids was examined, the rotatory power of another specimen of the pure *d*-amide-acid was determined under the same conditions as the above : $c = 0{\cdot}7050$, $l = 4$, $\alpha = +0{\cdot}46°$, whence $[\alpha] = +16{\cdot}4°$.

This solution (40 c.c.) was treated in an exactly similar way to those in the previous experiments on the hydrolysis of the optically active amide-acids : the rotatory power of the final solution was $[\alpha] = -28{\cdot}5°$.

The authors wish to express their grateful thanks to the Government Grant Committee of the Royal Society and to Messrs. Imperial Chemical Industries Ltd. for grants which have been of great assistance in the purchase of chemicals and apparatus. One of them (B. L.) is indebted to the Department of Scientific and Industrial Research for a grant which enabled him to take part in the work.

GUY'S HOSPITAL MEDICAL SCHOOL (UNIVERSITY OF LONDON),
LONDON, S.E. 1. [*Received, January 25th*, 1929.]