XV.—*The Resolution of Externally Compensated* p-*Nitrobenzoylalanine.*†

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The resolution of those externally compensated *N*-acyl derivatives of alanine which have so far been studied in this series of investigations (Pope and Gibson, J., 1912, **101**, 939; Gibson and Simonsen, J., 1915, **107**, 798; Colles and Gibson, J., 1924, **125**, 2505) has, in

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* In this and similar experiments, the boiling points of the distillates are to be regarded, not as the physical constants of the pure substances, but as the temperatures at which the various decomposition products—often superheated—passed over.

† Waser and Brauchli (*Helv. Chim. Acta,* 1924, **7**, 740) have described a convenient and sensitive test for amino-acids (except glycine and cystine) : in sodium carbonate solution they give an intense dark red colour with *p*-nitrobenzoyl chloride. We have found this test more sensitive when the sodium carbonate is replaced by pyridine (compare Ingersoll and Adams, *J. Amer. Chem. Soc.,* 1922, **44**, 2930).

each case, proved fairly straightforward; but the resolution of *dl-p*-nitrobenzoylalanine has been attended with complications hardly to be anticipated with simple substances of this type.

dl-p-Nitrobenzoylalanine has a marked tendency to form partial racemates or series of mixed crystals of the diastereoisomeric salts with various alkaloids. With brucine and cinchonidine it forms partial racemates unaffected by recrystallisation. When strychnine (0·5 mol.) was used to resolve the *dl*-acid under the conditions used for the resolution of *dl*-benzoylalanine (J., 1912, **101**, 939) a mixture of the *d*A*l*B salt (colourless, soft needles) and markedly phototropic (turning deep yellow on exposure to light), long, hard needles or prisms of what approximates to the partial racemate crystallised. The *d*A*l*B salt is less soluble than the *l*A*l*B salt in water, but the above mixture had to be recrystallised four times in order to obtain the pure *d*A*l*B salt; the yield was only 39% and the acid obtained from the original mother-liquor contained only 60% of the lævo-isomeride. Although the *d*A*l*B salt was obtained pure by this method (the acid obtained from it being compared with that prepared from pure *d*-alanine), it was impossible to obtain the pure *l*A*l*B salt from aqueous solution owing to the separation of mixed crystals.

The *l*A*l*B salt is more soluble than the *d*A*l*B salt both in water and in alcohol at the ordinary temperature, but it is less soluble in alcohol at higher temperatures; the ratio of their solubilities is 1 : 1·36 at 50°. This difference in solubility led to the isolation of the pure diastereoisomeric salts, equivalent proportions of *dl*-acid and strychnine being used. The crude *l*A*l*B salt which separated at 50° was recrystallised from absolute alcohol at 50° until pure. The crude *d*A*l*B salt obtained from the original mother-liquor needed only one crystallisation from absolute alcohol at the ordinary temperature for complete purification. This resolution of *dl-p*-nitrobenzoylalanine recalls the result obtained by Ladenburg and Fischl (*Ber.*, 1907, **40**, 2279), who found that whereas the brucine hydrogen racemate separated from aqueous solution below 50°, the brucine hydrogen *l*-tartrate separated above this temperature.

The foregoing resolution of *dl-p*-nitrobenzoylalanine was extremely laborious. Frequently the salt separating consisted chiefly of the phototropic crystals referred to above (the pure diastereoisomeric salts are not phototropic). When once the pure, or nearly pure, *l*A*l*B salt had been obtained and the homogeneous solution at 50° was seeded with this, a salt reasonably rich in the *l*A*l*B constituent was deposited unless the seeding was delayed. The difficulties of the resolution are due not only to the small differences in solubility of the diastereoisomeric salts in alcohol and in water, but also to the marked tendency to the formation of a series of mixed crystals which are unaffected by crystallisation at the ordinary temperature and do not markedly differ in solubility from the pure diastereoisomerides.

Externally compensated *p*-nitrobenzoylalanine is a racemic substance. From the melting points of mixtures of the optically active acids it is evident that the melting-point curve is of the type depicted in Roozeboom's Fig. 7 (*Z. physikal. Chem.*, 1899, **28**, 508).

The rotatory powers of the optically active *p*-nitrobenzoylalanines in ethyl-alcoholic solution are much lower than those of the corresponding ammonium salts in aqueous solution, but they are of the same sign. (The rotatory powers of the optically active α-naphthalenesulphonylalanines in ethyl-alcoholic solution are of the opposite sign to those of the corresponding salts in aqueous solution : Colles and Gibson, *loc. cit.*) The *ethyl* ester of *d*-*p*-nitrobenzoylalanine has a very much lower rotatory power, but of the same sign, in ethyl-alcoholic solution than the corresponding acid in the same solvent. When this ester is hydrolysed with sodium hydroxide at the ordinary temperature, the rotatory power of the solution rises rapidly to a constant value. For comparison, the *ethyl* ester of *d*-α-naphthalenesulphonylalanine was prepared : its rotatory power in ethyl-alcoholic solution, like that of the acid in the same solvent, is of the opposite sign to that of the salts of the corresponding acid in water. When the ester is hydrolysed with sodium hydroxide at the ordinary temperature the rotatory power changes slowly, finally reaching a constant value of the opposite sign.

EXPERIMENTAL.

dl-p-*Nitrobenzoylalanine*, $CHMe(NH\cdot CO\cdot C_6H_4\cdot NO_2)\cdot CO_2H$.—*dl*-Alanine (1 mol.; 25 g.) dissolved in *N*-potassium hydroxide (1 mol.; 281 c.c.) is cooled to 7°, and benzene (25 c.c.) added. *p*-Nitrobenzoyl chloride (rather less than 1 mol.; 50 g.), dissolved in benzene (150 c.c.), and *N*-potassium hydroxide (1 mol.) cooled to 7° are gradually added during 75 minutes with rapid stirring. The temperature should not rise above 10°, neither should the potassium hydroxide be added too rapidly, otherwise a pink coloration, changing to claret-red, develops (Waser and Brauchli, *loc. cit.*). The mixture is stirred for a further 15 minutes, the benzene layer separated, and the aqueous solution acidified with concentrated hydrochloric acid (150 c.c.). The crude product, a microcrystalline mass (yield, 58—60 g.; 88—90% of the theoretical), m. p. 191—193°, is freed from a small quantity of *p*-nitrobenzoic acid by a short extraction with ether and recrystallised from boiling water (20 g. per litre), the pure substance being thus obtained (yield, 75—79%) in very pale yellow needles, m. p. 194°. The solubility of the acid per 100 g. of solvent is 0·27 in water at 26° and 19 in alcohol at 78°. It is very sparingly soluble in ether, chloroform, and benzene and moderately easily soluble in acetone. The acid is readily hydrolysed by *N*-alkalis at the water-bath temperature, deep red solutions being obtained [Found: C, 50·8, 50·8; H, 4·4, 4·1; N, 11·8; *M*, by titration with 0·1*N*-$Ba(OH)_2$, 238. $C_{10}H_{10}O_5N_2$ requires C, 50·4; H, 4·2; N, 11·8%; *M*, 238]. The *silver* salt is a white, granular substance, sparingly soluble in water (Found: Ag, 31·5. $C_{10}H_9O_5N_2Ag$ requires Ag, 31·3%).

The acid is a racemic compound, since its melting point is depressed by admixture with either of the optically active isomerides, and conversely.

Resolution of dl-p-*Nitrobenzoylalanine*.—In a preliminary experiment with strychnine and *N*-sodium hydroxide (0·5 mol. of each) and the *dl*-acid (1 mol.), an acid, m. p. 167°, [α] + 13·52° * in alcoholic solution, was obtained from the salt that separated on cooling and had been once recrystallised from water. This salt, therefore, was the *dAlB* salt.

When brucine was used instead of strychnine, a readily soluble salt separated from the deep orange solution after considerable concentration at the ordinary temperature. The salt was recrystallised from hot water, and the acid liberated from a portion in the usual way. The acid had m. p. 194° and was optically inactive. The *brucine* salt of dl-p-*nitrobenzoylalanine* crystallises in thin, yellow plates which are readily soluble in water and in alcohol and appear to contain $4H_2O$ (Found: N, 8·3; H_2O, 10·8. $C_{33}H_{36}O_9N_4,4H_2O$ requires N, 7·95; H_2O, 10·2%).

By means of cinchonidine an almost insoluble salt (10·8 g. instead of 11·2 g.) was obtained which proved also to be the partial racemate. The *cinchonidine* salt of dl-p-*nitrobenzoylalanine* was recrystallised from water and obtained in colourless, fine needles containing $3H_2O$ (Found: N, 9·5; H_2O, 7·6. $C_{29}H_{32}O_6N_4,3H_2O$ requires N, 9·6; H_2O, 7·3%). The acids obtained from the recrystallised salt and from the mother-liquor were optically inactive.

The salt obtained under similar conditions by means of cinchonine was also a partial racemate, and that obtained from quinine was an inseparable mixture of the two diastereoisomerides.

In the large-scale resolution, the *dl*-acid (100 g.; 1 mol.) and *N*-sodium hydroxide (210 c.c.; 0·5 mol.) were boiled with water (500 c.c.), strychnine (70·2 g.; 0·5 mol.) being added gradually, until complete solution was effected. The crystalline strychnine

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* All rotatory powers were determined at 20° for the mercury-green (5461) line.

salt (123 g.) was filtered off after standing in the ice-chest for 16 hours. The mother-liquor (A) was worked up later.

The salt obtained was a mixture, a part of which was phototropic, turning deep yellow on exposure to daylight. The salt was recrystallised from 700 c.c. of boiling water and 111·4 g. crystallised. This in alcoholic solution had [α] − 11·19° (*c* = 1·4092). The rotatory power gradually diminished on recrystallisation and became constant after four recrystallisations under similar conditions. This constant value was confirmed after a fifth crystallisation; the rotatory power of the salt which crystallised was then identical with that of the salt obtained from the mother-liquor. Further crystallisation from absolute alcohol caused no alteration in the rotatory power. 47·7 G. of the pure strychnine salt of the *d*-acid were obtained.

The *strychnine* salt of d-p-*nitrobenzoylalanine*,

$$CHMe(NH{\cdot}CO{\cdot}C_6H_4{\cdot}NO_2){\cdot}CO_2H,C_{21}H_{22}O_2N_2,1\tfrac{1}{2}H_2O,$$

crystallises from hot water (solubility at 20°, 0·4; at 100°, 4) in colourless needles which are not phototropic (Found: H_2O, 4·1. Required for 1½ H_2O: H_2O, 4·5%. Found in anhydrous material: N, 9·9. $C_{31}H_{32}O_7N_4$ requires N, 9·8%). Its rotatory power in ethyl alcohol was [α] − 1·90° (*c* = 1·5018, *l* = 4, α = − 0·114°). Other values obtained under similar conditions were [α] − 2·00° and − 1·83°. That the salt was pure was proved by the rotatory power of the acid obtained from it (see below). A solution of the anhydrous salt in absolute alcohol slowly deposits large, colourless prisms and the solubility of the salt in alcohol diminishes from 3·7 to 0·54 at the ordinary temperature. The prisms, kept in a vacuum desiccator or exposed to air, rapidly effloresce.

The acid liberated from the mother-liquor (A) in the usual way was recrystallised from 2400 c.c. of water. It then weighed 44·1 g. and had m. p. 189—190° (softening at 168°). In aqueous solution as the ammonium salt, it had [α] − 10·35° (*c* = 2·028, *l* = 4). It contained only 60% of the *l*-acid.

The mother-liquors from the first four crystallisations of the above strychnine salt were concentrated in two portions, and the strychnine salts separating on cooling collected. After repeated recrystallisation from boiling water, decolorisation with charcoal, and recrystallisation from absolute alcohol, 9·2 and 13·3 g., respectively, of samples of strychnine salt of identical and definitely constant rotatory power were obtained; [α] − 40·16°, − 40·71°, − 40·42° (*c* = 0·9130, 0·9828, 1·0222; *l* = 4 in ethyl alcohol). Although at the time it was believed that this was pure strychnine *l*-acid salt, subsequent work showed that it still contained *dAlB* salt.

In view of the difficulties indicated above, an approximate determination of the solubilities of the two diastereoisomeric salts in absolute alcohol was made. The *l*A*l*B salt was the less soluble in warm alcohol. At 50°, the solubilities in alcohol were : 2·68 for the *l*A*l*B and 3·64 for the *d*A*l*B salt. This difference in solubility, although small, indicated a method for the isolation of the pure salts.

The following conditions are typical of a series of experiments : To a solution of the *dl*-acid (10 g.; 1 mol.) in boiling alcohol (200 c.c.), strychnine (14·04 g.; 1 mol.) was added gradually. When the alkaloid had dissolved, the solution was placed in a thermostat at 50° and seeded with the purest strychnine *l*-acid salt available. A nodular deposit of radiating tufts of needles formed rapidly and the deposition was complete in 5 hours. The mother-liquor (B) was poured off and the deposit was washed with warm absolute alcohol and dried as rapidly as possible by suction (yield, 10—12 g.). The rotatory power in ethyl alcohol was $[\alpha] - 36{\cdot}2°$ ($c = 0{\cdot}7916$, $l = 4$). The mother-liquor (B) was treated as described later.

The purification of the above crude strychnine *l*-acid salt was very difficult. Successive fractional crystallisations from absolute alcohol at 50° was the only effective method; recrystallisation from water or from alcohol at the ordinary temperature was useless. The method was naturally wasteful of material. The acid liberated from a salt which had reached a rotatory power of $[\alpha] - 45{\cdot}0°$ had $[\alpha] - 47{\cdot}8°$ (as ammonium salt in water; $c = 1{\cdot}5040$, $l = 4$) and contained 96·5% of the *l*-acid. A specimen of a salt which had a rotatory power of $[\alpha] - 46{\cdot}6°$ gave an acid having $[\alpha] - 48{\cdot}35°$ under similar conditions and containing 97·0% of *l*-acid. Finally, 42·5 g. of strychnine *l*-acid salt having $[\alpha] - 40{\cdot}0°$, after four fractional crystallisations at 50° from absolute alcohol, gave 9·0 g. of pure strychnine *l*-acid salt having a rotatory power identical with that of the salt remaining in the mother-liquor.

The *strychnine* salt of l-p-*nitrobenzoylalanine*,

$$CHMe(NH{\cdot}CO{\cdot}C_6H_4{\cdot}NO_2){\cdot}CO_2H,C_{21}H_{22}O_2N_2,C_2H_5{\cdot}OH,$$

crystallises from absolute alcohol in colourless needles which are not phototropic (Found : $C_2H_5{\cdot}OH$, 7·15. $C_{31}H_{32}O_7N_4,C_2H_5{\cdot}OH$ requires $C_2H_5{\cdot}OH$, 7·3%. Found in " anhydrous " substance : N, 9·95. $C_{31}H_{32}O_7N_4$ requires N, 9·8%). The " anhydrous " material in ethyl alcohol at 20° gave $\alpha - 1{\cdot}433°$ ($c = 0{\cdot}7340$, $l = 4$); whence $[\alpha] - 48{\cdot}8°$.

The resolution described above was frequently complicated by the separation, along with the nodular deposit, of large prisms (partial racemate) which were strongly phototropic. This invariably occurred when the solution was not seeded at once. When it did occur, the salt was redissolved and the experiment repeated. This explanation was proved correct by the following experiments: (a) 10 G. of the *dl*-acid were treated exactly as described above and the solution in the thermostat at 50° was seeded with the above-mentioned phototropic partial racemate. The crystalline deposit consisted of needles and prisms (no nodules), formed during 5 hours. The markedly phototropic crystals were separated as previously described. The rotatory power in ethyl alcohol ($c = 0{\cdot}8570$, $l = 4$) was $[\alpha] - 26{\cdot}6°$. (b) 10 G. of the *dl*-acid were treated with the equivalent quantity of strychnine in double the quantity of alcohol and the solution was allowed to cool to the ordinary temperature. After 8 hours, 76% of the salt had crystallised and consisted of small, well-formed, phototropic, efflorescing prisms and needles. The rotatory power in ethyl alcohol ($c = 1{\cdot}2438$, $l = 4$) was $[\alpha] - 24{\cdot}06°$. The deposit obtained from the mother-liquor on standing had $[\alpha] - 21{\cdot}8°$ under the same conditions.

From the mother-liquor (B), allowed to cool to the ordinary temperature, a crystalline mixture of phototropic needles and non-phototropic, efflorescing prisms was deposited (8—10 g.). The rotatory power of an average specimen of this mixture was $[\alpha] - 15°$ (in alcohol). This salt therefore contained the greater proportion of the *dAlB* salt. Under the conditions of the first resolution, this would have required five successive recrystallisations from water for complete purification. The solubilities of the *dAlB* and the *lAlB* salt in alcohol at 20° being 0·69 and 0·91, respectively, this difference was used to obtain pure *dAlB* salt from the mixture. The mixture was dissolved in sufficient boiling absolute alcohol to make a 1·5% solution, and this was seeded with pure *dAlB* salt and kept for at least 24 hours. The crystalline deposit (45—50% yield) had an average rotatory power of $[\alpha] - 3°$ (in alcohol). One recrystallisation of this from boiling water was sufficient for complete purification. The mother-liquor was concentrated, and the crystalline salt which separated (30—35%) had an average rotatory power of $[\alpha] - 40°$ (in alcohol) and therefore contained an excess of the *lAlB* salt.

Optically Active p-*Nitrobenzoylalanines.*—d-p-*Nitrobenzoylalanine* was isolated from the pure strychnine *d*-acid salt by grinding it with a slight excess of dilute aqueous ammonia, filtering off the strychnine, and acidifying the filtrate, after thorough extraction with chloroform, with an excess of hydrochloric acid. The acid was recrystallised from boiling water (10 g. in 110 c.c.) and obtained in long, very pale yellow needles, m. p. 168·5—169° (Found: C, 50·8; H, 4·2; N, 11·8. $C_{10}H_{10}O_5N_2$ requires C, 50·4; H, 4·2; N, 11·8%).

The rotatory power was (as ammonium salt) [α] + 51·40° in water (c = 1·7940, l = 4, α = + 3·688°) and [α] + 15·76° in ethyl alcohol (c = 1·9882, l = 4, α = + 1·254°).

The optical purity of this compound was proved by comparison with that of the *p*-nitrobenzoyl derivative of pure *d*-alanine (kindly supplied by Sir William Pope, F.R.S.) prepared by the method already described. This preparation (as ammonium salt) had [α] + 51·32° in water (c = 1·5964, l = 4, α = + 3·277°). The m. p. was 168·7—169° and was not affected by admixture with the above compound.

d-*p*-Nitrobenzoylalanine has a solubility in water at 15° of 0·26 and is easily soluble in alcohol. A mixture with a small quantity of pure *dl*-acid had m. p. 162—167°, and with a larger quantity 191·5°. A specimen having [α] + 15·15° (as ammonium salt in water) and therefore containing 64·7% of the pure *d*-acid softened at 165° and melted at 188·5—189·5°. Another specimen having [α] + 45·82° (as ammonium salt in water) and containing 94·6% of the pure *d*-acid had m. p. 167°.

l-p-*Nitrobenzoylalanine* was obtained in the same manner from the pure strychnine *l*-acid salt. It crystallised from water in long, pale yellow needles, m. p. 167·5—168° (Found: *M*, by titration, 238·1. $C_{10}H_{10}O_5N_2$ requires *M*, 238). As ammonium salt, it had [α] — 51·66° in water (c = 1·6462, l = 4, α = — 3·402°) and [α] — 15·81° in ethyl alcohol (c = 0·9960, l = 4, α = — 0·628°). A specimen of acid having [α] — 47·8° (as ammonium salt in water) and containing 96·5% of *l*-acid had m. p. 165·5—166·5°. Another specimen having [α] — 48·35° (as ammonium salt in water) and containing 97·03% of *l*-acid had m. p. 166—167°. A third specimen having [α] — 44·99° (as ammonium salt in water) and containing 93·7% of *l*-acid had m. p. 162·5—163·5° (? eutectic point).

Owing to the difficulty of obtaining the pure strychnine *l*-acid salt, attempts were made to purify the optically impure acid by various methods. On recrystallisation from water, the *l*-acid content only changed from 79·5% to 78·7%. Although the solubility of the optically active acid in alcohol is markedly greater than that of the racemic compound, recrystallisation from alcohol was not completely successful. 10·75 G. of a specimen of the acid containing 78·7% of the *l*-acid, dissolved in 50 c.c. of boiling alcohol, yielded after 48 hours 6 g. of prismatic needles which contained 69·7% of *l*-acid. The residue recovered from the mother-liquor, after recrystallisation from water, weighed 2·75 g. and contained 93·7% of *l*-acid (? eutectic).

The method of purifying optically impure *l*-α-naphthalenesulphonylalanine (Colles and Gibson, *loc. cit.*) was tried, cinchonidine being used (see above). 10 G. of acid containing 60% of *l*-acid were dissolved in water, and sufficient cinchonidine to combine with the racemic acid present and sufficient standard sodium hydroxide to combine with the excess of *l*-acid were added. The whole was brought into solution on boiling and the crystalline salt separated on cooling. The acid recovered from the filtrate was optically inactive, showing that the cinchonidine *l*-acid salt has the same solubility as the partial racemate (see above). A similar experiment was attempted, sufficient strychnine being used to combine with the *d*-acid salt, which is the less soluble in water. The acid recovered from the mother-liquor contained 77% of *l*-acid, whereas the acid used contained 60%.

The *ethyl* ester of dl-p-*nitrobenzoylalanine*,

$$CHMe(NH{\cdot}CO{\cdot}C_6H_4{\cdot}NO_2){\cdot}CO_2Et,$$

is easily prepared by the alcohol–hydrogen chloride method in 75% yield, and crystallises from benzene–ligroin in waxy needles, m. p. 117·5—118°. It is readily soluble in alcohol and chloroform and sparingly soluble in ether (Found: N, 10·6. $C_{12}H_{14}O_5N_2$ requires N, 10·5%). The amide is not obtained by passing dry ammonia into a benzene–ether solution of the ester.

The *ethyl* ester of d-p-*nitrobenzoylalanine* is prepared from *d-p*-nitrobenzoylalanine by the same method in 77% yield. By recrystallising from the same mixture of solvents it is obtained in colourless needles, m. p. 121—121·5°, which retain benzene very obstinately. Its solubility is rather greater than that of the externally compensated ester (Found: N, 10·5%). Its rotatory power in ethyl alcohol was very small; $c = 1{\cdot}416$, $l = 4$, $\alpha = 0{\cdot}075°$, whence $[\alpha] + 1{\cdot}32°$.

1·1328 G. of the ester were dissolved in 90% ethyl alcohol, rather more than the calculated quantity of *N*/2-sodium hydroxide was added, and the whole made up to 100 c.c. The rotatory power was observed immediately after mixing in a 4-dcm. tube and showed that hydrolysis had commenced; $\alpha = + 1{\cdot}45°$ (the corresponding value for the unchanged ester in alcoholic solution would have been $+ 0{\cdot}06°$) and after 10 minutes, $\alpha = + 2{\cdot}07°$. After 2½ hours a constant value of $\alpha = + 2{\cdot}43°$ was observed. The *d*-acid recovered from the solution and after recrystallisation had m. p. 168·5°, alone or mixed with pure *d-p*-nitrobenzoylalanine.

An attempt was made to purify an optically impure specimen of *d-p*-nitrobenzoylalanine (containing 64·7% of the pure *d*-acid) by converting it into its ethyl ester. The optically active ester, being the more soluble, remained in the mother-liquor together with any impurities on recrystallisation. The low rotatory power rendered criteria of purity almost impossible.*

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