XXXVII.—N-*Acyl Derivatives of Alanine. The Resolution of Externally Compensated* m-*Nitrobenzoylalanine.*

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IN addition to extending our knowledge of *N*-acyl derivatives of alanine so as to secure the most convenient method of identifying and isolating this important amino-acid, the nitroacyl derivatives may serve as convenient starting materials for preparing arsinic acids, of which the simplest is $AsO(OH)_2 \cdot C_6H_4 \cdot CO \cdot NH \cdot CHMe \cdot CO_2H$. Such compounds, and especially their carboxyamides, containing an asymmetric carbon atom, should on stereochemical investigation afford further information concerning the relative chemotherapeutic activities of externally compensated arsinic acids and their optically active components (compare Gibson, Johnson, and Levin, J., 1929, 479; Gibson and Levin, *ibid.*, p. 2759). These compounds should be capable of being prepared by means of the Bart reaction from the reduced nitro-derivatives.

Since it and its derivatives should lead to the production of *p*-arsinic acids, the most important compound, and the simplest, is *dl-p*-nitrobenzoylalanine, the optical resolution of which proved to be unusually difficult (Colles and Gibson, J., 1928, 99). The corresponding externally compensated *m*-compound, on the other hand, is now shown to be easily resolved into its optically active components by the half molecule method. By means of quinine, the *quinine* salt of l-m-*nitrobenzoylalanine* is precipitated; when the acid from the mother-liquor, after separation of this salt, is treated with the requisite quantity of brucine, the *brucine* salt of d-m-*nitrobenzoylalanine* is readily obtained; from these two salts the pure l- and d-m-*nitrobenzoylalanines* are easily isolated by the

usual method. If one molecular proportion of dl-m-*nitrobenzoylalanine* is dissolved in water with half a molecular proportion of brucine and an equivalent quantity of sodium hydroxide, no resolution is effected, the partially racemic brucine salt being obtained. Cinchonidine used in the same way is not satisfactory, the acid remaining in the mother-liquor after separation of the cinchonidine salt being only feebly *dextro*-rotatory. A feebly *lævo*-rotatory acid is obtained from the mother-liquor after separation of the cinchonine salt obtained under similar conditions.

As in the case of the optically active α-naphthalenesulphonylalanines (Colles and Gibson, J., 1924, **125**, 2505), the rotatory powers of the optically active *m*-nitrobenzoylalanines in ethyl-alcoholic solution are of opposite sign to those of the corresponding salts in aqueous solution. Also the rotatory powers of the optically active esters in ethyl-alcoholic solution are of opposite sign to those of the corresponding salts in aqueous or alcoholic solution. This was proved by observing the rotatory powers of the ethyl ester of *l-m*-nitrobenzoylalanine undergoing hydrolysis in ethyl-alcoholic solution containing a slight excess of potassium hydroxide, the sign of the rotatory power changing during the process (compare Colles and Gibson, *loc. cit.*, p. 108). The optically active amide, on the other hand, has a rotatory power in ethyl alcohol of the opposite sign to that of the methyl ester and of the same sign as that of the acid from which it is derived.

The following externally compensated compounds are also described in the present paper: o-*nitrobenzoylalanine*, p-*toluoylalanine*, m-*nitrobenzenesulphonylalanine*, *cinnamoylalanine*, 4-*nitrotoluene*-2-*sulphonylalanine*. Evidence has been obtained that *dl-o*-nitrobenzoylalanine can be resolved by means of strychnine by the half molecule method, whereas the partially racemic salt is precipitated when brucine is employed. Preliminary experiments indicating a suitable method of reduction of *o*- and *m*-nitrobenzoylalanines have been carried out.

The relative rates of hydrolysis of the *dl-N*-acyl derivatives of alanine which have now been investigated have been approximately determined. The method consisted in (i) hydrolysing the *N*-acyl derivative with constant-boiling hydrochloric acid, (ii) neutralising the hydrolysis mixture to $p_H = 8$ (approx.) (determined by the green tint of the " B. D. H. Universal Indicator "), and (iii) titrating the liberated alanine by Sörensen's method to $p_H = 10$ (approx.) (determined by the violet-purple change of the above indicator). The above procedure was adopted after careful experiments with all available indicators and an accuracy of only $\pm$ 1% is generally claimed, this being adequate for the purpose in view (compare

Shonle and Mitchell, *J. Amer. Chem. Soc.*, 1920, **42**, 1265; Sörensen and Katschioni-Walther, *Z. physiol. Chem.*, 1928, **174**, 251).

The hydrolysis was carried out under identical conditions in all cases by gently boiling the mixture for 3 hours (except where stated otherwise), and standard sodium hydroxide free from carbonate was employed. Except *dl*-phthalylalanine (Andreasch, *Monatsh.*, 1904, **25**, 774), the alanine derivatives have been described by Gibson and co-workers (J., 1912, **101**, 939; 1915, **107**, 798; 1924, **125**, 2505; 1928, 99; 1929, 2754). The results are summarised below and attention is directed to the stability of the sulphonyl derivatives and especially of β-naphthalenesulphonylalanine, frequently isolated in the detection of alanine.

dl-Compound.	% Hydrolysis.	*dl*-Compound.	% Hydrolysis.
Benzoylalanine	96	Benzenesulphonylalanine	14
p-Toluoylalanine	95	α-Naphthalenesulphonylalanine	66
Cinnamoylalanine	92	β-Naphthalenesulphonylalanine	18 (45% after 9½ hours)
Phthalylalanine	76	*p*-Toluenesulphonylalanine	17
Nitrobenzoylalanines:		*m*-Nitrobenzenesulphonylalanine	21
ortho	47	4-Nitrotoluene-2-sulphonylalanine	2 (in 4 hours)
meta	91		
para	97		

EXPERIMENTAL.

dl-m- *and* dl-o-*Nitrobenzoylalanines*, $NO_2 \cdot C_6H_4 \cdot CO \cdot NH \cdot CHMe \cdot CO_2H$.—*dl*-Alanine and *m*-nitrobenzoyl chloride and *o*-nitrobenzoyl chloride, respectively, being used, these compounds were prepared and purified in exactly the same way as *dl*-*p*-nitrobenzoylalanine (Colles and Gibson, *loc. cit.*), an 85—90% yield being obtained in each case.

dl-m-*Nitrobenzoylalanine* crystallises from water in almost colourless needles, m. p. 163—164° (Found: C, 50·2; H, 4·3. $C_{10}H_{10}O_5N_2$ requires C, 50·4; H, 4·2%). At 20°, 0·38 g. dissolves in 100 c.c. of water, 0·37 g. in 100 c.c. of ether, and 0·43 g. in 100 c.c. of chloroform. The *silver* salt crystallises in colourless needles (Found: Ag, 31·2, 31·2. $C_{10}H_9O_5N_2Ag$ requires Ag, 31·3%).

dl-o-*Nitrobenzoylalanine* crystallises from water in almost colourless needles, m. p. 165—166°. It is slightly less soluble in water and ether than the above *m*-compound (Found: C, 50·5; H, 4·3%).

Possible conditions for the reduction of these nitro-compounds have been kindly investigated by Professor J. L. Simonsen.

dl-m-*Aminobenzoylalanine Hydrochloride*, $CO_2H \cdot CHMe \cdot NH \cdot CO \cdot C_6H_4 \cdot NH_2,HCl$.—To a solution of *dl*-*m*-nitrobenzoylalanine (11 g.) in barium hydroxide solution [$Ba(OH)_2,8H_2O$, 110 g., in water, 1000 c.c.],

ferrous sulphate ($FeSO_4,7H_2O$, 85 g., in water, 150 c.c.) was added. After the reaction was complete, carbon dioxide was passed into the solution until the latter was neutral. The evaporated filtrate was treated with just sufficient sulphuric acid to precipitate all the barium and, after filtration, was evaporated to dryness on the water-bath. The reddish-brown residue was dissolved in dilute hydrochloric acid, from which it separated on cooling in colourless prismatic needles, m. p. 150—152° (decomp.) after sintering at 145° (Found : Cl, 14·4. $C_{10}H_{13}O_3N_2Cl$ requires Cl, 14·5%).

dl-o-*Aminobenzoylalanine*.—To a solution of *dl-o*-nitrobenzoylalanine (2 g.) in barium hydroxide [$Ba(OH)_2,8H_2O$, 24 g., in water, 300 c.c.], ferrous sulphate ($FeSO_4,7H_2O$, 17 g., in water, 30 c.c.) was added. After the reduction was complete, the product was worked up as before, the final residue, however, being recrystallised from water and not converted into the hydrochloride (yield, 1·1 g.). It crystallised in small plates which do not melt at 270° (Found : C, 57·1 ; H, 5·8. $C_{10}H_{12}O_3N_2$ requires C, 57·7 ; H, 5·8%).

The *ethyl* ester of *dl-m*-nitrobenzoylalanine was prepared by saturating in the cold with hydrogen chloride a solution of the acid (2 g.) in ethyl alcohol (35 c.c.), heating the mixture for 1 hour on the water-bath, and pouring the product on ice (yield, 2 g.). It was recrystallised from aqueous alcohol and obtained in colourless needles, m. p. 89° (Found : C, 54·5 ; H, 5·5. $C_{12}H_{14}O_5N_2$ requires C, 54·2 ; H, 5·3%).

Methyl Ester of dl-m-*Nitrobenzoylalanine*.—After a mixture of the acid (2 g.) in methyl alcohol (20 c.c.) containing sulphuric acid (1 c.c.) had been heated on the water-bath for 2 hours, the product was poured on ice, and the *ester* (yield, quantitative) recrystallised from aqueous methyl alcohol. It was obtained in colourless needles, m. p. 110—111° (Found : C, 52·9, 52·6 ; H, 4·8, 5·0. $C_{11}H_{12}O_5N_2$ requires C, 52·4 ; H, 4·8%).

On treatment of this ester in the usual way with concentrated ammonia solution, the *amide* was obtained ; recrystallised from aqueous alcohol, it formed colourless needles, m. p. 189—190° (Found : C, 50·5 ; H, 4·4. $C_{10}H_{11}O_4N_3$ requires C, 50·6 ; H, 4·6%).

dl-p-*Toluoylalanine* was prepared from *dl*-alanine and *p*-toluoyl chloride in a similar manner to that used for the preparation of *dl*-benzoylalanine (Fischer, *Ber.*, 1899, **32**, 2454 ; Pope and Gibson, J., 1912, **101**, 940), a 95% yield being obtained. It crystallised from hot water in small colourless needles, m. p. 188—189° (Found : C, 63·5 ; H, 6·0. $C_{11}H_{13}O_3N$ requires C, 63·7 ; H, 6·3%).

dl-m-*Nitrobenzenesulphonylalanine*,

$$CO_2H{\cdot}CHMe{\cdot}NH{\cdot}SO_2{\cdot}C_6H_4{\cdot}NO_2.$$

—*dl*-Alanine (5 g.) was dissolved in *N*-potassium hydroxide

solution (56 c.c.) and shaken during 3 hours at room temperature while *m*-nitrobenzenesulphonyl chloride (12·5 g.), dissolved in benzene (50 c.c.), and *N*-potassium hydroxide solution (56 c.c.) were added at regular intervals. The benzene layer was separated, and the turbid aqueous solution filtered and made strongly acid with concentrated hydrochloric acid. The crystalline precipitate (13·2 g., 89% yield) was recrystallised from boiling water (charcoal) and obtained in fine yellow needles, m. p. 158·5—159° (Found : C, 39·6; H, 3·6. $C_9H_{10}O_6N_2S$ requires C, 39·4; H, 3·65%).

dl-4-*Nitrotoluene*-2-*sulphonylalanine*,

$$NO_2{\cdot}C_6H_3Me{\cdot}SO_2{\cdot}NH{\cdot}CHMe{\cdot}CO_2H,H_2O.$$

—This substance was prepared exactly as described by Siegfried (*Z. physiol. Chem.*, 1904, **43**, 70). After recrystallisation from hot water and drying in air, it had m. p. 91—97° (Found : C, 38·7, 39·2; H, 4·75, 4·6; N, 8·8, 9·0, 9·4; H_2O, 5·9, 5·85. $C_{10}H_{12}O_6N_2S,H_2O$ requires C, 39·2; H, 4·6; N, 9·15; H_2O, 6·0%). When the fused substance was allowed to solidify, the melting point was higher. The air-dried substance lost 0·5% of moisture when left over sulphuric acid in an evacuated desiccator for 6 days, the melting point being slightly raised (108—109°). When the air-dried substance was heated to constant weight at 85° and 95°, an anhydrous product was obtained, m. p. 125·5—126·5°. This anhydrous material is not hygroscopic, its melting point remaining unchanged during 6 years. These observations are somewhat different from those described by Siegfried.

dl-*Cinnamoylalanine* was prepared by shaking *dl*-alanine (2·5 g.), dissolved in the equivalent quantity of *N*-potassium hydroxide (28 c.c.), for 2 hours at the ordinary temperature during the gradual addition of cinnamoyl chloride (4·5 g. in benzene, 30 c.c.) and *N*-potassium hydroxide in small quantities. After separation of the benzene layer, the filtered aqueous solution was acidified with hydrochloric acid, and the somewhat pasty solid extracted twice with boiling ether. The residue was recrystallised from water and then from aqueous alcohol and obtained in colourless needles (yield, 30%), m. p. 196—197° (Found : C, 66·0; H, 6·05. $C_{12}H_{13}O_3N$ requires C, 65·8; H, 5·9%).

The compound is very sparingly soluble in cold water and in boiling ether. In alkaline solution, it rapidly decolorises potassium permanganate and from the resulting solution, after treatment with hydrochloric acid, benzaldehyde is obtained.

Resolution of dl-m-*Nitrobenzoylalanine*.—To a boiling solution containing the *dl*-acid (15 g.), sodium hydroxide (0·976*N*-solution, 32·4 c.c.) and water (1000 c.c.), quinine (11·92 g.) was added gradually and finally alcohol (10 c.c.). The clear solution was separated

from a small quantity of insoluble gummy material and left to crystallise during 16 hours. The crystalline air-dried material (17 g.; theoretical wt., 19·5 g.), *l*A*l*B salt, was recrystallised three times from hot water (10 g. in 600 c.c.), but the rotatory power of the anhydrous salt in ethyl alcohol at 20° did not change after the first crystallisation: *c*, 1·829; *l*, 4; $\alpha_{5461} = -10{\cdot}03°$; $[\alpha]_{5461} = -137{\cdot}1°$.

The *quinine* salt of *l*-*m*-nitrobenzoylalanine crystallises from water in long soft needles containing $2H_2O$, which it loses in a vacuum over sulphuric acid in 5—6 days (Found in air-dried material: C, 60·6; H, 6·4; H_2O, 5·9, 6·0, 6·0. $C_{30}H_{34}O_7N_4,2H_2O$ requires C, 60·2; H, 6·35; H_2O, 6·0%). The anhydrous salt has no definite melting point, being converted into a glass-like mass at about 125°.

The mother-liquor after separation of the above salt was treated with excess of concentrated ammonia solution, and the alkaloid extracted by means of chloroform. The acid obtained by acidification of the evaporated aqueous solution was recrystallised from the minimum quantity of water; it had (as ammonium salt in water) $[\alpha]_{5461} = +36{\cdot}6°$ and contained 90·4% of the *d*-acid. This crude *d*-acid (23·4 g.) with brucine (38·3 g.) and sodium hydroxide (0·38 g.) was boiled with water (250 c.c.), complete solution being effected. The *d*A*l*B salt (58·2 g.) crystallised and was separated after some 16 hours. It was recrystallised three times from water (250 c.c.), its rotatory power being constant after the first crystallisation.

The *brucine* salt of *d*-*m*-nitrobenzoylalanine crystallises from water in colourless plates which turn yellow on exposure to light. It is sparingly soluble in ethyl alcohol but more soluble in acetone. In ethyl alcohol at 20° it has $[\alpha]_{5461} = -9{\cdot}1°$ (*c*, 0·5511; *l*, 4; α_{5461} − 0·20°). The air-dried salt appears to contain $3\frac{1}{2}H_2O$, which is lost in 7 days in a vacuum over sulphuric acid (Found in air-dried salt: C, 56·5; H, 6·1; H_2O, 8·95. $C_{33}H_{36}O_9N_4,3\frac{1}{2}H_2O$ requires C, 56·9; H, 6·2; H_2O, 9·1%). When the air-dried salt is heated at 110°, slight decomposition takes place, the loss in weight being 9·2%.

l-m-*Nitrobenzoylalanine*, obtained from the above quinine salt in the usual way, was recrystallised twice from water, in which it is more soluble than the *dl*-acid (Found: C, 50·8; H, 4·3. $C_{10}H_{10}O_5N_2$ requires C, 50·4; H, 4·2%). d-m-*Nitrobenzoylalanine*, similarly isolated from the above brucine salt, was also recrystallised from water (Found: N, 11·65. $C_{10}H_{10}O_5N_2$ requires N, 11·8%). These two acids both crystallise in long, almost colourless needles, m. p. 158°. The melting point of either is depressed by admixture with a small quantity of the other and since the *dl*-acid has m. p. 163—164°, the latter must be a racemic compound. The following

determinations of rotatory powers were made at 20° in 4 dm. tubes.

l-m-Nitrobenzoylalanine,	*d-m*-Nitrobenzoylalanine,
as ammonium salt in water.	
c, 1·342; α_{5461}, − 2·40°; $[\alpha]_{5461}$, − 44·7°	*c*, 1·358; α_{5461}, + 2·43°; $[\alpha]_{5461}$, + 44·7°
in ethyl alcohol.	
c, 1·361; α_{5461}, + 0·32°; $[\alpha]_{5461}$, + 5·87°	*c*, 1·335; α_{5461}, − 0·30°; $[\alpha]_{5461}$, − 5·62°

The corresponding rotatory powers of the optically active *p*-nitrobenzoylalanines are $[\alpha]_{5461} = \pm 51{\cdot}65°$ and $\pm 15{\cdot}8°$ respectively (Gibson and Colles, *loc. cit.*).

The *ethyl* ester of *l-m*-nitrobenzoylalanine, prepared in a similar manner to that employed for the *dl*-compound, crystallised from aqueous ethyl alcohol in colourless needles, m. p. 104—105° (Found: N, 10·8. $C_{12}H_{14}O_5N_2$ requires N, 10·6%). It had $[\alpha]_{5461} = +6{\cdot}91°$ in ethyl alcohol at 20° (*c*, 1·465; *l*, 4; α_{5461} +0·405°), whereas the ethyl ester of *d-p*-nitrobenzoylalanine has $[\alpha]_{5461} = +1{\cdot}32°$ under similar conditions.

This ethyl ester (0·4103 g.) was made up to 50 c.c. with ethyl alcohol containing 0·0882 g. of potassium hydroxide. The rotatory power of the solution after 5 minutes was $\alpha_{5461} = +0{\cdot}17°$; this changed to −0·31° after 26 minutes and attained a constant value of − 1·05° after 17 hours at 20°.

The *methyl* ester of *d-m*-nitrobenzoylalanine, prepared in quantitative yield and recrystallised as in the case of the corresponding *dl*-compound, separated from aqueous methyl alcohol in colourless needles, m. p. 126° (Found: N, 11·3. $C_{11}H_{12}O_5N_2$ requires N, 11·1%). It had $[\alpha]_{5461} = -12{\cdot}7°$ in ethyl alcohol at 20° (*c*, 1·429; *l*, 4; α_{5461}, − 0·72°).

The *amide* of *d-m*-nitrobenzoylalanine, prepared from the above methyl ester in the usual way, crystallised from ethyl alcohol in colourless leaflets, m. p. 193—194° (Found: N, 17·4. $C_{10}H_{11}O_4N_3$ requires N, 17·7%). It had $[\alpha]_{5461} = +24{\cdot}2°$ in ethyl alcohol at 20° (*c*, 0·238; *l*, 4; α_{5461}, + 0·23°).

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