244. *The Preparation of Some Alkylamino-acids and their Electrometric Titration.*

By WESLEY COCKER and J. O. HARRIS.

The method used by Cocker and Lapworth (J., 1931, 1894) for the preparation of sarcosine, and its extension by Cocker (J., 1937, 1693) to the preparation of the simple homologues of sarcosine, have now been used to prepare *n*-amyl-, *n*-butyl-, and *iso*-butyl-aminoacetic acids and α-*n*-propyl- and α-ethyl-aminopropionic acids. The dissociation constants of these compounds and those described by Cocker (*loc. cit.*) have been investigated by electrometric titration, and evidence in favour of the " Zwitterion " theory obtained.

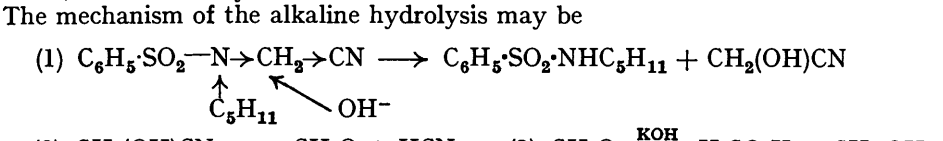
Attempts to extend the process of Cocker and Lapworth to the preparation of alkylamino-acids with larger alkyl groups than those described above were unsuccessful, owing to the difficulty of obtaining the intermediate compounds of the type $C_6H_5{\cdot}SO_2{\cdot}NR{\cdot}CH_2{\cdot}CO_2H$ where R is greater than C_5H_{11}.

THE investigation here described was undertaken with a view to the examination of alkylamino-acids with long-chain alkyl groups, the metal salts of which would possibly be of use as detergents. The method of preparation adopted was that used by Cocker (*loc. cit.*), in which the benzenesulphonyl derivative of an α-amino-acid is alkylated and the benzenesulphonyl group subsequently removed. The amino-acid is then isolated by the method developed by Cocker and Lapworth (*loc. cit.*) for the preparation of sarcosine.

It was found, however, that little or no reaction took place between benzenesulphonylglycine or -alanine and alkyl halides in which the alkyl group was larger than C_5H_{11}; for instance, benzenesulphonylglycine gives 75% and 25% yields of the benzenesulphonyl derivatives of *n*-butyl- and *n*-amyl-aminoacetic acid respectively, but negligible quantities of the derivatives of *n*-hexyl- and *n*-heptyl-aminoacetic acids. The presence of large quantities of *n*-hexyl and *n*-heptyl alcohols in the reaction mixtures indicated the preponderance of the hydrolytic over the alkylation reaction. Attempts to prepare *iso*propylaminoacetic acid from *iso*propyl iodide led largely to the production of propylene.

Better alkylation was obtained by using the ethyl ester of the benzenesulphonyl compound, but the best yields of alkyl derivative were obtained when the corresponding nitriles were used. For example, it was possible to obtain a small quantity of *n*-octylaminoacetonitrile, and this appeared to be a promising method of approach to the desired amino-acids. The hydrolysis of these nitriles, however, proved to be very difficult. Boiling concentrated hydrochloric acid gave 10% hydrolysis after prolonged action, and treatment with a cold saturated solution of hydrogen chloride in ether containing an equimolecular quantity of absolute alcohol (Pinner, *Ber.*, 1892, **25**, 352, 1643) effected no improvement. Hydrolysis with 60% sulphuric acid yielded small quantities of the required amino-acid, but in addition considerable losses took place due to fission of the nitriles in an unexpected manner. For instance, in the hydrolysis of benzenesulphonyl-*n*-amylaminoacetonitrile, acetic acid was produced, together with ammonia and amylamine. Hydrolysis with boiling solutions of potassium hydroxide of various concentrations yielded traces of the required potassium salt, large quantities of potassium cyanide and potassium formate, and some amylamine.

The mechanism of the alkaline hydrolysis may be



This course of reaction would account for large quantities of potassium cyanide and formate, and the amylamine obtained could be produced by the alkaline hydrolysis of its benzenesulphonyl derivative, which has been shown to be very slowly hydrolysed with boiling 25% potassium hydroxide. Benzenesulphonylamylamine—a colourless oil—was not however isolated from the hydrolysis mixture.

A satisfactory explanation for the course of the hydrolysis with sulphuric acid to yield acetic acid cannot yet be advanced. Both this reaction and the alkaline hydrolysis will be further studied.

Conversion of the nitriles into the corresponding amides was relatively easy by the use of sulphuric acid or syrupy phosphoric acid, but here again further hydrolysis to the corresponding acid proved to be difficult with 60% sulphuric acid, concentrated hydrochloric acid, potassium hydroxide, or nitrous acid. Small quantities of amylamine were produced when either 60% sulphuric acid or potassium hydroxide was employed, but the bulk of the amide was unchanged. Concentrated hydrochloric acid and nitrous acid gave only small yields of the desired amino-acids. All attempts to hydrolyse the amides were complicated by the low solubility of the amide in the hydrolytic agent.

It is obvious that other methods are required to obtain the desired amino-acids, but in view of present conditions, the progress of the investigation is now recorded.

The method of Cocker and Lapworth (*loc. cit.*) gave good yields of *n*-butylaminoacetic acid and α-ethylaminopropionic acid but only small yields of *iso*butyl- and *n*-amyl-aminoacetic acid and α-*n*-propylaminopropionic acid. All these amino-acids together with sarcosine but with the exception of *n*-amylaminoacetic acid have been investigated by electrometric titration. The only alkylamino-acids previously investigated in this way were sarcosine and dimethylglycine (Bjerrum, *Z. physikal. Chem.*, 1923, **104**, 147).

Titrations were performed at 25° with standard hydrochloric acid and sodium hydroxide by using the hydrogen electrode, which was found to be preferable to the quinhydrone electrode (cf. Yoshamino, *J. Biochem. Japan*, 1935, **23**, 187), the normal calomel electrode being used as reference.

The various corrections elaborated by L. J. Harris (*Proc. Roy. Soc.*, 1923, *B*, **95**, 440) and Tague (*J. Amer. Chem. Soc.*, 1920, **42**, 177) in order to determine the actual amount of acid or alkali used in the titration at any p_H value were taken into account, and graphs of p_H value plotted against quantity of standard acid or alkali used were drawn. The p_H at the mid-point (p_{K_a} or p_{K_b}) of each curve was compared with that obtained from the well-known expressions $p_{K_a} = p_H - \log(\text{salt}/\text{“ acid ”})$ for titration with alkali and $p_{K_b} = p_{K_w} - p_H - \log(\text{salt}/\text{“ base ”})$ for titration with acid, where p_{K_a} and p_{K_b} are the p_H values at the mid-points of the curves of titration, with strong base and strong acid respectively, and “ acid ” and “ base ” refer to uncombined amino-acid acting by virtue of its carboxyl and its amino-group respectively. Thus K_a and K_b are the dissociation constants of the amino-acids on the open-chain formula, and titrations with acid and base are respectively represented by the relationships

$$\overset{+}{H} + NHR\cdot CHR\cdot CO_2H \longrightarrow \overset{+}{N}H_2R\cdot CHR\cdot CO_2H \text{ and}$$

$$O\bar{H} + NHR\cdot CHR\cdot CO_2H \longrightarrow NHR\cdot CHR\cdot C\bar{O}\cdot O + H_2O$$

Alanine and glycine were similarly titrated for reference, and values of p_{K_a} and p_{Kb} were obtained which agreed with those obtained by L. J. Harris (*Proc. Roy. Soc.*, 1923, *B*, **95**, 444), *i.e.*, $p_{K_a} = 9{\cdot}75$ and $p_{K_b} = 11{\cdot}49$.

The following table gives the values of the dissociation constants (in terms of p_K) obtained for the various amino-acids by calculation and by construction of titration curves, where K_A and K_B are acidic and basic dissociation constants on the “ Zwitterion ” theory (Bjerrum, *loc. cit.*), *i.e.*, $K_A = K_w/K_b$ and $K_B = K_w/K_a$.

Amino-acid.	Calc. values of	p_{K_a}.	p_{K_b}.	p_{K_A}.	p_{K_B}.	p_H at mid-point of titration. Acid.	Alkali.
Aminoacetic acid		9·75	11·49	2·41	4·15	2·40	9·75
Methylaminoacetic acid		10·18	11·55	2·35	3·72	2·37	10·19
Ethylaminoacetic acid		10·23	11·56	2·34	3·67	2·33	10·21
n-Propylaminoacetic acid		10·19	11·55	2·35	3·71	2·36	10·20
*iso*Butylaminoacetic acid		10·12	11·55	2·35	3·78	2·34	10·11
n-Butylaminoacetic acid		10·25	11·55	2·35	3·65	2·38	10·26
α-Aminopropionic acid		9·75	11·50	2·40	4·15	2·40	9·75
α-Methylaminopropionic acid		10·19	11·68	2·22	3·71	2·21	10·18
α-Ethylaminopropionic acid		10·22	11·68	2·22	3·68	2·21	10·25
α-*n*-Propylaminopropionic acid		10·19	11·69	2·21	3·71	2·22	10·20

(p_{K_w} is taken as 13·9 at 25°.)

The values of K_a and K_b shown in the table indicate that replacement of hydrogen by alkyl in the amino-group of the various amino-acids leads to an appreciably lower acidic, and a slightly lower basic, dissociation constant. This is contrary to the usually accepted effects of alkyl groups which are well known to increase the basicity of an amino-group by replacement of a hydrogen atom. On the other hand, even if the open-chain formula be correct, there should be a slight decrease in acidity in the amino-acid due to firmer binding of the carboxyl proton as a result of the increased transmitted and direct effects of the $-NHR$ group.

The values of K_A and K_B shown in the table indicate that substitution of alkyl in the amino-group of glycine gives an appreciable increase in the basic dissociation constant and a slight increase in the acidic dissociation constant.

On the "Zwitterion" theory the structure of glycine and its derivatives is $\overset{+}{N}H_2R{\cdot}CHR{\cdot}C\overset{-}{O}O$ together with the two ions $\overset{+}{N}H_2R{\cdot}CHR{\cdot}CO_2H$ and $NHR{\cdot}CHR{\cdot}C\overset{-}{O}O$, which in turn ionise respectively to $\overset{+}{N}H_2R{\cdot}CHR{\cdot}C\overset{-}{O}O + \overset{+}{H}$ and $\overset{+}{N}H_2R{\cdot}CHR{\cdot}C\overset{-}{O}O + \overset{-}{O}H$, the latter by reaction with water. On this view it can readily be seen that where R = alkyl the amino-group will more readily abstract proton from water and so give hydroxyl ions than will be the case when R = H. In this manner the basic dissociation constant will be increased. On the other hand, two possible factors may affect the ionisation of the anion $\overset{+}{N}H_2R{\cdot}CHR{\cdot}CO_2H$. The first will be the increased electropolar effect of the alkylamino-group over the amino-group, tending to resist separation of proton and hence to lower the acidic dissociation constant, whilst an opposing effect may be steric, tending to inhibit combination of proton since the ends of the molecule are in close proximity to each other. Hence, a slight increase or decrease in acidic dissociation constant might be expected.

Again, it is noteworthy that the value of K_A for the substituted glycines is constant although the alkyl group is varied, and the same remarks apply to the substituted alanines. This might be expected with two opposing effects tending to cancel each other.

On the other hand, the value of K_B in the compounds $NHR{\cdot}CH_2{\cdot}CO_2H$ increases in the order R = H < *iso*-C_4H_9 < CH_3 < *n*-C_3H_7 < C_2H_5 < *n*-C_4H_9, and in $NHR{\cdot}CHMe{\cdot}CO_2H$ in the order R = H < (CH_3 and *n*-C_3H_7) < C_2H_5.

Hall and Sprinkle (*J. Amer. Chem. Soc.*, 1932, **54**, 3470) have shown that the basicity of primary amines (NH_2R) increases in the order R = H < *n*-C_4H_9 < *n*-C_3H_7 < *iso*-C_4H_9 < CH_3 < C_2H_5, but in the secondary amines (NHR_2) the order is R = H < CH_3 < *n*-C_4H_9 < *n*-C_3H_7 < C_2H_5 < *iso*-C_4H_9. There is reasonably good agreement between the order of basicities of the substituted amino-acids and the results obtained by Hall and Sprinkle for the secondary amines. The position of *n*-butyl and *iso*butyl is difficult to explain.

Experimental.

A. *Preparation of Materials.*—n-*Butylaminoacetic acid.* Benzenesulphonylaminoacetic acid (15 g.), dissolved in sodium hydroxide (3N, 70 c.c.) contained in a flask fitted with a reflux condenser and efficient shaker, was heated on the water-bath with *n*-butyl iodide (38·5 g.) for 40 hours. The reaction mixture was then cooled, filtered, and carefully acidified. The oil which separated soon solidified on scratching, and was collected, washed, and dried. It was then crystallised from benzene–ligroin, from which *benzenesulphonyl*-n-*butylaminoacetic acid* was obtained in flat, colourless plates (14·6 g., 75%), m. p. 101—102° (Found: N, 4·7. $C_{12}H_{17}O_4NS$ requires N, 5·1%). This compound (30 g.) was suspended in 60% sulphuric acid (28 c.c. of concentrated acid, 31·5 c.c. of water) and heated under reflux for 12 hours; the required n-*butylaminoacetic acid* was then isolated by the method of Cocker and Lapworth (*loc. cit.*). The crude material (10 g., 71%) was recrystallised from hot absolute alcohol, being obtained with little loss as flat, colourless plates, m. p. 192° (inst.) (Found: N, 10·7. $C_6H_{13}O_2N$ requires N, 10·7%). The *phenylcarbamido*-compound, obtained in the usual manner, crystallised from boiling water in long, colourless prisms or needles, m. p. 127—128° (Found: N, 11·2. $C_{13}H_{18}O_3N_2$ requires N, 11·2%).

n-*Amylaminoacetic acid.* The above preparation was repeated, but with *n*-amyl iodide (20·7 g.) for 30 hours. The cooled mixture was extracted with ether to remove excess of alkyl-

ating agent, freed from dissolved ether by a current of air, and acidified. The deposited oil solidified on freezing, and was then dried (14·2 g.) and extracted several times with boiling benzene, leaving a solid residue (8·9 g.) of unchanged starting material. From the benzene extract *benzenesulphonyl*-n-*amylaminoacetic acid* (4·9 g.) was obtained by precipitation with light petroleum and crystallised from benzene–light petroleum in colourless prisms, m. p. 84° (Found : N, 5·0. $C_{13}H_{19}O_4NS$ requires N, 4·9%). On hydrolysis with 60% sulphuric acid, 12 g. gave n-*amylaminoacetic acid* (3·6 g., 60%), which crystallised from absolute alcohol in flat, colourless plates, m. p. 201° (inst.) (Found : N, 9·6. $C_7H_{15}O_2N$ requires N, 9·7%). Its *phenylhydantoin* crystallised from boiling water in colourless prisms, m. p. 111° (Found : N, 11·2. $C_{14}H_{18}O_2N_2$ requires N, 11·4%).

iso*Butylaminoacetic acid.* Substitution of *iso*butyl iodide (19·2 g.) in the above general method led, in 48 hours, to a homogeneous mixture. When cooled, acidified, and kept in the refrigerator, this deposited a white crystalline solid (14·6 g.), which was well washed with water, dried, and extracted with benzene, from which light petroleum precipitated a white powder (6 g., m. p. 80—82°). On recrystallisation from benzene–light petroleum, *benzenesulphonyliso*butylaminoacetic acid was obtained as colourless plates, m. p. 90—91° (Found : N, 5·2. $C_{12}H_{17}O_4NS$ requires N, 5·1%). During the reaction the odour of *iso*butylene was observed.

The above compound (7·7 g.) was hydrolysed by refluxing with 60% sulphuric acid for 12 hours, and iso*butylaminoacetic acid* was obtained as a white powder, which crystallised from absolute alcohol in colourless transparent plates (3 g.), m. p. 188° (Found : N, 10·65. $C_6H_{13}O_2N$ requires N, 10·7%). Its *phenylcarbamido*-compound crystallised from hot water in long, colourless needles, m. p. 86—87° (Found : N, 11·2. $C_{13}H_{18}O_3N_2$ requires N, 11·2%).

α-*Ethylaminopropionic acid.* The following method gave consistently good yields of benzenesulphonylalanine. Alanine (9 g.), dissolved in water (80 c.c.) containing sodium hydroxide (4 g.) and sodium carbonate (2·0 g.), was vigorously shaken whilst benzenesulphonyl chloride (18 g.) was added in small quantities during 12 hours. The mixture became acid as the reaction proceeded, and 40% sodium hydroxide was added from time to time to keep it alkaline to phenolphthalein. When all the sulphonyl chloride had reacted the mixture was filtered, cooled in ice, and acidified with vigorous stirring. Almost pure benzenesulphonylalanine, m. p. 124—125° (22 g., 98%), was deposited. As the pure compound has m. p. 126°, the crude product was used in the ethylation, which was best effected with ethyl iodide. Benzenesulphonylalanine (10 g.), dissolved in sodium hydroxide (3N, 45 c.c.), was refluxed with ethyl iodide (40 g.) added in four equal quantities at long intervals. The solution was cooled and acidified, and a colourless oil was deposited which rapidly became solid on scratching; this was collected, washed with water, and recrystallised from boiling water. *Benzenesulphonyl*-α-*ethylaminopropionic acid* (6·0 g.) was obtained in flat, colourless plates, m. p. 145° (Found : N, 5·3. $C_{11}H_{15}O_4NS$ requires N, 5·4%). When it (14·5 g.) was hydrolysed during 36 hours with boiling 60% sulphuric acid, it afforded α-*ethylaminopropionic acid* (2·8 g.) in a series of crops; crystallisation from boiling alcohol afforded colourless rectangular plates, m. p. 302—303° (inst.) (Found : N, 11·9. $C_5H_{11}O_2N$ requires N, 12·0%). The benzenesulphonyl derivative was identical with the starting material.

α-n-*Propylaminopropionic acid.* Benzenesulphonylalanine (10 g.), dissolved in sodium hydroxide (3N, 45 c.c.), was heated for 15 hours under reflux with *n*-propyl iodide (15 g.). The mixture was then steam-distilled to remove excess propyl iodide, cooled, filtered, and acidified. An oil was deposited which solidified on strong cooling. It was washed with water and recrystallised from hot water (charcoal), giving *benzenesulphonyl*-α-n-*propylaminopropionic acid* as flat transparent plates, m. p. 117° (Found : N, 5·1. $C_{12}H_{17}O_4NS$ requires N, 5·1%). On hydrolysis with 60% sulphuric acid 6 g. of this compound yielded 1·7 g. of α-n-*propylaminopropionic acid*, which crystallised from alcohol in colourless plates, m. p. 302—303° (Found : N, 10·6. $C_6H_{13}O_2N$ requires N, 10·7%). It gave a benzenesulphonyl derivative identical with the starting material.

Attempts to prepare n-*Amyl*-, n-*Hexyl*-, *and* n-*Heptyl-aminoacetic Acids from Benzenesulphonylaminoacetonitrile.*—Johnson and McCollum's preparation of benzenesulphonylaminoacetonitrile (*Amer. Chem. J.*, 1906, **35**, 54) has been improved as follows. Aminoacetonitrile hydrogen sulphate (50 g.), prepared by Anslow and King's method (J., 1929, 2463), was dissolved in a minimum quantity of water and vigorously agitated with a solution of benzenesulphonyl chloride (59 g.) in benzene (200 c.c.) while a saturated solution containing sodium carbonate (52 g.) was cautiously added. When the evolution of carbon dioxide had ceased, the mixture was transferred to a pressure bottle and shaken for 2 days, during which successive crops of the required compound were obtained by filtration at intervals. The combined crops were washed

with water, dried, and recrystallised from benzene, white needles (49·5 g., 78%), m. p. 80°, being deposited.

A mixture of the above compound (7·8 g.), sodium ethoxide (from sodium, 1 g., and alcohol, 50 c.c.), and *n*-amyl iodide (11·7 g.) was refluxed until it was no longer alkaline to litmus. It was then steam-distilled to remove excess amyl iodide, basified to phenolphthalein, extracted with ether, the extract dried (sodium sulphate), and the ether removed; an oil was obtained which did not solidify after many weeks in the refrigerator. It decomposed on attempted distillation in a vacuum and was presumably the required benzenesulphonyl-*n*-amylamino-acetonitrile. This compound (10 g.), dissolved in dry ether, was kept for 4 weeks with dry ether saturated with hydrogen chloride and containing 3·2 c.c. of absolute alcohol. The ether was removed, and the residue poured into aqueous sodium hydroxide, heated on the water-bath to decompose the ester, and again extracted with ether to remove unchanged nitrile. The aqueous solution was carefully acidified, and the benzenesulphonyl-*n*-amylaminoacetic acid collected (0·8 g., 8%) and recrystallised from benzene–light petroleum (m. p. 83°). Unchanged nitrile (8·8 g.) was recovered from the ethereal extract.

Hydrolysis of the nitrile with 50%, 30%, or 15% potassium hydroxide gave little of the desired product, but only amine vapours which were collected in dilute hydrochloric acid. The solution was evaporated to dryness, extracted with absolute alcohol to remove a little ammonium chloride and the amine hydrochloride isolated and converted into the *chloroplatinate*. On recrystallisation from absolute alcohol, yellow needles were deposited of indistinct m. p. [Found: Pt, 30·2. $(C_5H_{11}NH_2)_2H_2PtCl_6$ requires Pt, 33·3%]. A little of the amine hydrochloride solution gave a strong carbylamine reaction.

Hydrolysis of the nitrile with boiling concentrated hydrochloric acid gave a little of the required compound and much starting material. In this manner *benzenesulphonyl*-n-*hexyl-aminoacetic acid* was obtained as a white powder which on crystallisation from benzene–light petroleum gave colourless plates, m. p. 85—86° (Found: N, 4·6. $C_{14}H_{21}O_4NS$ requires N, 4·7%).

Benzenesulphonyl-n-*amylaminoacetamide*. Benzenesulphonyl-*n*-amylaminoacetonitrile (10 g.) was cooled in ice and slowly treated with concentrated sulphuric acid (2 c.c.), the mixture being stirred vigorously; it was then warmed to 80°, maintained at this temperature for 20 mins., and cooled. The resulting dark syrup was carefully treated with solid sodium carbonate, and extracted with ether. From the extract, a dark solid (12 g.) was obtained which on recrystallisation from hot water (charcoal) gave colourless needles of the *amide*, m. p. 94° (Found: N, 9·85. $C_{13}H_{20}O_3N_2S$ requires N, 9·8%). Similar results were obtained by the use of syrupy phosphoric acid.

Hydrolysis of the above compound with sodium hydroxide gave varying quantities of the required benzenesulphonyl-*n*-amylaminoacetic acid according to the concentration of alkali: 15% alkali gave a 7·5% yield, and 30% alkali gave a 4·0% yield. In the former case 75% of the unchanged amide was recovered, but in the latter the recovery was smaller and quantities of amine were detected during the reaction. Hydrolyses with 60% sulphuric acid and concentrated hydrochloric acid were also unsatisfactory. Hydrolysis of the amide with nitrous acid during 24 hours at 0° gave only a 4% yield of the corresponding acid.

B. *Electrometric Titration of the Amino-acids.—Purification of materials, etc.* The amino-acids which had already given satisfactory analysis results were recrystallised twice from alcohol, or aqueous alcohol, or, for sarcosine, from distilled water alone. The compounds were then carefully dried over calcium chloride in a vacuum, and 0·1N-solutions prepared with conductivity water.

Titrations were performed with 1·12N-hydrochloric acid and 0·96N-sodium hydroxide. The latter was stored in an aspirator fitted with a syphon tube and a soda–lime trap.

The gas cell was made from a boiling-tube of 1″ diameter and $2\frac{3}{4}$″ in length. It was fitted with a Bakelite cover carrying two glass hydrogen inlet tubes of $\frac{1}{16}$″ bore, down the centres of which the electrodes of platinised platinum welded to platinum wire were carried. The hydrogen used was purified according to Tague (*loc. cit.*), and the apparatus, together with the normal calomel electrode, was kept in a thermostat at 25°. The potential differences were measured on a Pye potentiometer, a reflecting moving-coil galvanometer being used to obtain the balance point.

The amino-acid solution (5 c.c.) was placed in the gas cell, and the standard acid or alkali added from a micro-burette fitted with a soda–lime trap. The burette was graduated in 0·05 c.c. and was calibrated before use. p_H measurements were made after the addition of each 0·05 c.c. of mineral acid or alkali.

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