REFERENCES

- Adamson, D. W. (1939). *J. chem. Soc.* p. 1564.
 Catch, J. R., Jones, T. G. S. & Wilkinson, S. (1949). *Ann. N.Y. Acad. Sci.* **51**, 917.
 Coleman, R. G. (1958). *Nature, Lond.*, **181**, 776.
 Consden, R., Gordon, A. H. & Martin, A. J. P. (1944). *Biochem. J.* **38**, 224.
 Dent, C. E. (1947). *Biochem. J.* **41**, 327.
 Fowden, L. (1955). *Nature, Lond.*, **176**, 347.
 Fowden, L. (1956). *Biochem. J.* **64**, 323.
 Fowden, L. & Steward, F. C. (1957). *Ann. Bot., N.S.*, **21**, 53.
 Grobbelaar, N. & Steward, F. C. (1953). *J. Amer. chem. Soc.* **75**, 4341.
 Kasting, R. & Delwiche, C. C. (1957). *Plant Physiol.* **32**, 471.
 Lowy, P. H. (1953). *Arch. Biochem. Biophys.* **47**, 228.
 Miettinen, J. K., Kari, S., Moisio, T., Alfthan, M. & Virtanen, A. I. (1953). *Suomen Kemistilehti (Acta chem. fenn.)*, B2, 26.
 Oda, T. & Ueda, F. (1954). *J. pharm. Soc. Japan*, **74**, 1246.
 Partridge, S. M. (1948). *Biochem. J.* **42**, 238.
 Peterson, D. H. & Reineke, L. M. (1949). *J. biol. Chem.* **181**, 95.
 Virtanen, A. I. (1955a). *Nature, Lond.*, **176**, 984.
 Virtanen, A. I. (1955b). *Angew. Chem.* **67**, 381.
 Vogel, H. J. (1955). In *Amino Acid Metabolism*, p. 335. Ed. by McElroy, W. D. & Glass, B. Baltimore: Johns Hopkins Press.
 Watanabe, Y., Konishi, S. & Shimura, K. (1955). *J. Biochem., Tokyo*, **42**, 837.
 Zacharius, R. M., Cathey, H. M. & Steward, F. C. (1957). *Ann. Bot., N.S.*, **21**, 193.
 Zacharius, R. M., Pollard, J. K. & Steward, F. C. (1955). *Ann. Acad. Sci. fenn. (Chem.)*, A II, **60**, 321.

Some Observations on a Hydroxypipicollic Acid from Thrift (*Armeria maritima*)

By L. FOWDEN

Department of Botany, University College, Gower Street, London, W.C. 1

(Received 5 May 1958)

In recent years, knowledge of the free amino and imino acid components of plants has expanded rapidly (for reviews, see Virtanen, 1956, 1957; Steward & Pollard, 1957; Fowden, 1958). More than thirty new acids, additional to the twenty or so present in proteins, are known as products of higher-plant metabolism. Imino acids are among these newer compounds, and several contain a piperidine ring. Pipecolic acid (piperidine-2-carboxylic acid) is the most commonly found member of this group, being particularly characteristic of the legumes (Zacharius, Thompson & Steward, 1954), although it occurs in members of many other families (Morrison, 1953). Baikiain ($\Delta^{4:5}$ -dehydropipecolic acid), which occurs far less frequently in plants than pipecolic acid, was first isolated from *Baikiaea plurijuga* (Rhodesian teak), in which it occurs in relatively large amounts in both the seeds (Grobbelaar, Pollard & Steward, 1955) and the wood (King & Warwick, 1950). Hyde (1955) considers it to be a constituent of the developing pea seed. 5-Hydroxypipicollic acid was isolated from a palm (*Rhapis flabelliformis*) by Virtanen & Kari (1954), and from *Baikiaea* by Grobbelaar *et al.* (1955). It is also present in the fruit of the date palm, and accompanies pipecolic acid in several legume seeds, including the pea (Hyde, 1955). Another hydroxypipicollic acid, considered to be 4-hydroxypipicollic acid, was isolated from *Acacia pentadena* by Virtanen &

Kari (1955). In *Acacia* the two hydroxypipicollic acids co-existed, but in *Albizzia lophantha* only the presumed 4-hydroxy compound was present.

The present investigation reports the isolation of a hydroxypipicollic acid from thrift (*Armeria maritima*), a member of the family Plumbaginaceae. This substance is identical with that isolated from *Acacia*. However, evidence will be presented to suggest that these compounds may be 3-hydroxypipicollic acid, and not the 4-hydroxy derivative as suggested by Virtanen & Kari (1955).

EXPERIMENTAL

All melting points are corrected.

Paper chromatography. The method and solvent mixtures were as described by Fowden & Bryant (1958).

Synthesis of hydroxypipicollic acids

4-Hydroxypipicollic acid. This was obtained in small yield by catalytic hydrogenation of 4-hydroxypicolinic acid (4-hydroxypyridine-2-carboxylic acid), m.p. 263° (decomp.), which was prepared by the method of Meyer & Graf (1928), who gave m.p. 254–255° (decomp.). 4-Hydroxypicolinic acid (1.1 g.) was dissolved in 20 ml. of water and converted into its hydrochloride by the addition of the calculated amount of HCl. PtO (0.2 g.) was added and a slow stream of H₂ was passed through the mixture in a water bath at 90° for 12 hr. After 6 hr. the catalyst was replaced by a further 0.2 g. of PtO. After final removal of the Pt, the mixture was evaporated to about 5 ml., when much unchanged 4-hydroxypicolinic acid hydrochloride

crystallized out and was removed. The filtrate was evaporated to about 2 ml. and then 1 ml. was applied to each of two washed sheets of filter paper. The chromatograms were developed in butanol-acetic acid-water for 48 hr.; good separation of 4-hydroxypipicolinic acid from pipicolinic acid was achieved. 4-Hydroxypipicolinic acid was eluted from the appropriate areas of the chromatograms and obtained as a crystalline residue on evaporation. Yield about 40 mg. It did not melt on heating (Found: N, 9.4. $C_6H_{11}O_3N$ requires 9.7%).

3-Hydroxypipicolinic acid. This was obtained after catalytic hydrogenation of 3-hydroxypicolinic acid, which was prepared by the method of Sucharda (1925). From 10 g. of 8-hydroxyquinoline 3.4 g. of the imide of quinolinic acid was produced. In the conversion of quinolinic acid imide into 3-aminopipicolinic acid, Br_2 and NaOH were used instead of the Cl_2 and NaOH recommended by Sucharda (1925). The imide (3 g.) gave 1.1 g. of 3-aminopipicolinic acid, m.p. 242° (decomp.), and about 0.3 g. of 2-aminonicotinic acid. Diazotization of 1 g. of 3-aminopipicolinic acid was carried out in dil. H_2SO_4 solution with 2 g. of $Ba(NO_3)_2$ according to the procedure of Kirpal (1908) for the preparation of 3-hydroxypipicolinic acid. Yield of 3-hydroxypipicolinic acid, 0.6 g., m.p. 223° (Kirpal reported 215°). Catalytic hydrogenation proceeded more readily than with the 4-hydroxy derivative. 3-Hydroxypipicolinic acid (0.4 g.) was dissolved in 25 ml. of aq. $N-NH_3$ and 0.1 g. of PtO was added. Hydrogenation at 5 atm. H_2 pressure and room temp. was continued for 10 hr. The two main ninhydrin-reactive products were pipicolinic acid and 3-hydroxypipicolinic acid, present in about equal amounts. The Pt was removed and the filtrate evaporated to dryness. 3-Hydroxypipicolinic acid was recovered free from other ninhydrin-reactive substances after chromatography on papers developed in butanol-acetic acid-water. Yield 115 mg. It was recrystallized from 80% (v/v) ethanol and did not melt on heating (Found: N, 9.5. $C_6H_{11}O_3N$ requires 9.7%).

Hydroxypipicolinic acid from thrift

The hydroxypipicolinic acid was first encountered as an unidentified spot present on two-dimensional chromatograms prepared from an aqueous-ethanol extract of thrift leaves during a survey of the nitrogenous components of maritime plants. The substance reacted with ninhydrin, to give a yellow spot which gradually changed to blue when kept. Its position on a two-dimensional chromatogram, relative to those occupied by the other amino acids present in the extract, is shown in Fig. 1 (spot A). Another substance designated B is not a common plant constituent and remains uncharacterized.

Isolation of substance A. Fresh *Armeria* leaves (10 lb.) were chopped and then frozen at -20° for 2 days. After thawing, they were macerated with twice their weight of water in an Atomix blender (M.S.E. Ltd., London). The mush was expressed through fine muslin, and the extract immediately adjusted to pH 4.4 and warmed to 60° for 10–15 min. to coagulate the proteins and inactivate the enzymes. The precipitate was removed in a continuous-action Delaval rotary separator and 8.5 l. of clear extract were obtained. This extract was applied to a Zeo-Karb 215 column (resin 60–80 mesh), length 75 cm., diameter 6.5 cm.

The extract was applied at the rate of 1 l./hr., all the amino acids being absorbed. The column was washed with 20 l. of water and the amino acids were eluted with aq.

0.5N- NH_3 (5 l.) in 20 ml. fractions. The earliest fractions containing aspartic acid, and certain later fractions containing γ -aminobutyric acid and the basic amino acids, were discarded. The middle fractions containing A and many other amino acids were pooled and evaporated to 1 l. in a continuous rotary vacuum evaporator (Flash evaporator, Lab. Glass and Instruments Corp., New York, 31) at less than 50° . This concentrate was applied to a Dowex 50-X4 column (resin 100–200 mesh; length 45 cm., diameter 2 cm.) at the rate of 100 ml./hr. The column was washed with 2.5 l. of water, and elution of amino acids effected with aq. 0.2N- NH_3 . Elution proceeded at 50 ml./hr. and 105×10 ml. fractions were collected once the amino acids started to emerge from the column.

Substance A was present in fractions 8–29, which were combined. Other amino acids present in these pooled fractions were aspartic acid, glutamic acid, serine, threonine, homoserine and proline. The residue (3.5 g.) obtained on evaporation to dryness *in vacuo* was dissolved in water, to give 6 ml. of solution, and 1 ml. was streaked across the top edge of each of six sheets of filter paper (washed Whatman no. 3MM, 24 in. $\times$ 24 in.). The chromatograms were developed in the phenol-aq. NH_3 and the areas of paper between R_F 0.7 and R_F 0.9 were cut out and eluted. In this way a mixture (0.58 g.) of proline and A, free of the other amino acids, was obtained. This was dissolved in water to give 2 ml. of solution and 1 ml. was applied to each of two sheets of washed paper. The chromatograms were developed in the butanol-acetic acid-water for 3 days. Complete separation of A from proline was achieved, and A was eluted from the appropriate areas of the chromatograms. Crude A (217 mg.) was obtained on evaporation. After two recrystallizations from 90% (v/v) ethanol, 115 mg. of pure A was obtained. The substance did not melt but gradually darkened in colour on heating above

Fig. 1. Two-dimensional chromatogram of the amino acids present in a 70% (v/v) ethanol extract of the leaves of *Armeria maritima*. 1, aspartic acid; 2, glutamic acid; 3, serine; 4, glycine; 5, asparagine; 6, threonine; 7, homoserine; 8, glutamine; 9, alanine; 10, β -alanine; 11, arginine; 12, proline; 13, γ -aminobutyric acid; 14, valine; 15, leucine or isoleucine, or both. A is the isolated hydroxypipicolinic acid and B is an uncharacterized ninhydrin-reactive substance.

about 200° (Found: C, 49.4; H, 7.7; N, 9.5%; mol.wt. 140. $C_8H_{11}O_3N$ requires C, 49.6; H, 7.6; N, 9.7%; mol.wt. 145). The molecular weight was determined by the method of Barger (1904) with aq. hydroxyproline solutions as comparative standards. Substance *A* was readily soluble in water but virtually insoluble in absolute ethanol. When it was treated with the nitroprusside-acetaldehyde reagent of Feigl (1954) a strong blue colour, characteristic of imino compounds, developed rapidly. Proline and pipecolic acid tested under the same conditions reacted slightly more sensitively.

Reduction with hydrogen iodide and red phosphorus. A few milligrams of *A* were heated with 0.5 ml. of aq. 70% (w/v) HI and a trace of red P in a sealed ampoule at 140–150° for 4 hr. After HI had been removed by repeated evaporation *in vacuo*, a portion of the reaction mixture was run on a two-dimensional chromatogram mixed in phenol-aq. NH_3 and butanol-acetic acid-water. Three reaction products were detected on the chromatogram together with some unchanged *A*. One gave a bright-blue spot after treatment with ninhydrin, and was inseparable from added pipecolic acid. The other two products had relatively high R_F values in both solvents, and gave yellowish brown spots with ninhydrin. They were assumed to be iodinated derivatives of pipecolic acid. The sample of the hydroxypipecolic acid isolated from *Acacia* by Virtanen & Kari (1955) gave identical products after this treatment.

Comparison with synthetic hydroxypipecolic acids

Chromatography. The isolate *A* and each synthetic hydroxypipecolic acid were run separately and as a mixture on paper chromatograms. The isolate was separable from 4-hydroxypipecolic acid in butanol-acetic acid-water, 70% propanol and ethyl acetate-pyridine-water. No separation was effected in phenol-ammonia in which the substances had high R_F values (about 0.84). Whereas *A* gave with ninhydrin a bright-yellow colour initially which changed only slowly to blue, 4-hydroxypipecolic acid gave first an orange-brown colour, which rapidly changed to purple. When *A* was run against 3-hydroxypipecolic acid, no separation of the substances was apparent in any of the four solvents. In 70% propanol and in ethyl acetate-pyridine-water, 3-hydroxypipecolic acid gave a dumb-bell-shaped spot, which may have been due to the partial separation of diastereoisomers (the diastereoisomers of 5-hydroxypipecolic acid have been separated on paper chromatograms by Cohen, Irreverre, Piez, Witkop & Wolff, 1956). The position of the natural compound always matched precisely that of the faster portion of the dumb-bell spot, and their reactivity with ninhydrin was similar. Substance *A* was chromatographically distinct from 5-hydroxypipecolic acid (kindly provided by Professor A. I. Virtanen).

The isolate of thrift was compared with that obtained from *Acacia* by Virtanen & Kari (1955) in the same four solvents. The two substances behaved identically in both their positions on the chromatograms and their colour reactions with ninhydrin.

Oxidation with acid permanganate. A few milligrams of the imino acid were dissolved in 0.1 ml. of 10% (w/v) H_2SO_4 solution, and warmed to 60° in a water bath. A 1% (w/v) $KMnO_4$ solution in 10% (w/v) H_2SO_4 was then added dropwise until a permanent pink colour remained. The mixture was diluted to about 3 ml. and excess of solid

$BaCO_3$ added. When evolution of CO_2 ceased, the solids were removed by centrifuging and the clear supernatant was evaporated to dryness. The residue was redissolved in a small volume of water and small portions were used for two-dimensional chromatograms. β -Alanine, glycine, γ -aminobutyric acid and aspartic acid were produced from the isolate *A* in approximate proportions by wt. 55:25:15:5 respectively. Similar proportions were obtained from the *Acacia* isolate. Synthetic 3-hydroxypipecolic acid gave the same four amino acids in the proportions 30:10:55:5, while 4-hydroxypipecolic acid gave only β -alanine, glycine and aspartic acid (30:40:30). The identity of all amino acids was checked by co-chromatography with authentic specimens. Repetition of the oxidation at higher temperatures up to 100° and in 20% (w/v) H_2SO_4 solution did not alter the nature of the oxidation products nor bring about any significant change in their relative concentration.

α -Aminomalonic acid, a possible primary oxidation product of 3-hydroxypipecolic acid, was prepared by the method of Piloty & Finckh (1904). The acidic conditions used in the oxidation caused complete decarboxylation of this acid to glycine and no trace of it was found in the oxidation mixture.

DISCUSSION

It seems certain that the hydroxypipecolic acid isolated from *Acacia* by Virtanen & Kari (1955) is identical with that now isolated from thrift (*Armeria maritima*). The presence of the pipecolic acid ring system is also not in doubt. Virtanen allotted the hydroxyl group to C-4 after a study of the products of acidic permanganate oxidation of the imino acid. He identified aspartic acid and a smaller amount of glycine among these products, together with two other substances not identified, and argued that aspartic acid could arise only if the hydroxyl group was present on C-4. Oxidative splitting of the 4:5-linkage would occur first, followed by a 1:6-split, to give aspartic acid, or a 1:2-split, to give glycine, thus:

Re-examination of the products resulting from acidic permanganate oxidation of *Acacia* and *Armeria* isolates, performed simultaneously under conditions similar to those used by Virtanen & Kari (1955) (personal communication from Professor A. I. Virtanen), indicated that β -alanine and γ -aminobutyric acid were formed in addition to aspartic acid and glycine. Of the four amino acids present β -alanine was the largest, and aspartic acid the smallest, component.

These results suggested that 3-hydroxypipecolic acid should be considered as an alternative structure. The synthetic routes leading to 3- and 4-hydroxypipecolic acid were such that the structures

of the final products were not in doubt. The overall yields were, however, insufficient to warrant attempts at resolving the mixtures of diastereoisomers that would necessarily be formed. All comparisons were performed with an isolate that was probably a single stereoisomer and synthetic substances that probably consisted of mixtures of two pairs of diastereoisomers. Strict identity of behaviour of the natural substance with any one synthetic compound could not then be expected. The conclusions reached concerning the structure of the isolated material must therefore be regarded as tentative.

The chromatographic comparison of the two synthetic compounds and the isolate suggested that the isolate was 3-hydroxypipicolinic acid. It was different from 4-hydroxypipicolinic acid in three of the four solvent systems used. Unless one makes the unlikely assumption that the synthesis leads entirely to the formation of only one of the two possible pairs of diastereoisomers, i.e. to the alternative type to that found in the natural compound, the chromatographic evidence indicates that the compound is not 4-hydroxypipicolinic acid. In contrast, the isolate was never completely separated from synthetic 3-hydroxypipicolinic acid in any of the four solvents. Partial separation of diastereoisomers present in the synthetic preparation seemed to occur in two of the solvents, but the natural substance always matched, in position and colour reaction with ninhydrin, the faster-flowing component of the 3-hydroxypipicolinic acid.

The exact mechanism of oxidation and the linkages undergoing fission are unknown, but it is clear from the products obtained that fission must be possible at several points in the ring. Oxidation of isolate *A* gave the same four products as did 3-hydroxypipicolinic acid (β -alanine, glycine, γ -aminobutyric acid and aspartic acid) although the relative proportions were somewhat different. It seems reasonable, however, to attribute this quantitative difference to the different stereoisomeric character of the two compounds. Oxidation of 4-hydroxypipicolinic acid yielded glycine, aspartic acid and β -alanine, but not γ -aminobutyric acid. The amount of aspartic acid obtained from the isolate and 3-hydroxypipicolinic acid was so small as to raise the question of the possibility of its formation by conversion of the 3- into the 4-hydroxypipicolinic acid by successive dehydration and rehydration across the 3:4-linkage (the intermediate involved would be $\Delta^{3:4}$ -dehydropipicolinic acid), but it does not seem probable that this interconversion would occur at a sulphuric acid concentration of only 10% (w/v), since when synthetic 3-hydroxypipicolinic acid was treated with 10% (w/v) sulphuric acid at 100° for several hours no 4-hydroxypipicolinic acid was produced. If interconversion

did occur, some production of γ -aminobutyric acid on permanganate oxidation of the 4-hydroxy compound might be expected.

The isolate and the synthetic substances could be compared by other reactions, but none is likely to furnish conclusive evidence of identity, for all will be affected in greater or less degree by the different stereoisomeric characters of the reactants. Only by complete resolution of the four isomers present in the synthetic products could an unequivocal comparison be made. This would require much larger quantities of the synthetic compounds than have been prepared.

Why Virtanen & Kari (1955) identified only aspartic acid and glycine after oxidation of their *Acacia* isolate remains unknown. Whenever the oxidation was repeated together with a sample of *Armeria* isolate, both substances gave identical mixtures of products, of which β -alanine was the most important quantitatively. Virtanen & Kari's (1955) finding of aspartic acid as the principal oxidation product was the cornerstone of their evidence in favour of 4-hydroxypipicolinic acid.

The distribution of this new imino acid in plants seems sporadic. Virtanen & Kari (1955) found it in *Acacia*, *Albizia* and *Strelitzia*. Now it has been isolated from a member of the Plumbaginaceae. The imino acid is not, however, a characteristic constituent of members of this family, since it could not be detected in leaf extracts of four other species representative of the genera *Ceratostigma*, *Limonium*, *Plumbago* and *Acantholimon*, which, together with *Armeria*, constitute half the total number of genera assigned to this family.

The concentration at which the hydroxypipicolinic acid occurs in *Armeria* seems to be dependent on the conditions of growth of the plant. The plants used for extraction were grown at an inland location (Egham, Surrey) on a loam soil, and the imino acid represented one of the minor amino acid constituents of the leaves. In leaf extracts of plants collected from maritime locations, the imino acid generally contributed a higher proportion of the total soluble nitrogen fraction of the leaves; in some plants growing on the upper levels of a salt marsh, it formed the predominant spot on a two-dimensional chromatogram prepared from a leaf extract. Plants collected from the same location in different years have also shown a wide variation in their content of the imino acid.

SUMMARY

1. A hydroxypipicolinic acid has been isolated from thrift (*Armeria maritima*) and shown to be identical with a substance isolated from *Acacia* by Virtanen & Kari (1955), who provisionally characterized it as 4-hydroxypipicolinic acid.

2. Comparisons with synthetic 3-hydroxy- and 4-hydroxy-pipecolic acids indicated that the isolated imino acid may not be 4-hydroxypipecolic acid, but 3-hydroxypipecolic acid. Strictly identical behaviour of natural and synthetic 3-hydroxypipecolic acid could not be demonstrated since the two substances differed in stereoisomeric composition.

3. 3-Hydroxy- and 4-hydroxy-pipecolic acids have been synthesized.

I would like to express my thanks to Professor A. I. Virtanen (Biochemical Institute, Helsinki), who generously placed the facilities of his Laboratory at my disposal for some two months during this investigation and provided a sample of his *Acacia* isolate. I would also like to acknowledge grants from the Central Research Fund of the University of London which enabled me to visit Helsinki and to purchase the continuous rotary vacuum evaporator. Plants were supplied by the University of London Botanical Supply Unit, Egham, Surrey.

REFERENCES

- Barger, G. (1904). *J. chem. Soc.* p. 286.
 Cohen, L. A., Irreverre, F., Piez, K. A., Witkop, B. & Wolff, H. L. (1956). *Science*, **123**, 842.
 Feigl, F. (1954). *Spot Tests II. Organic Applications*, p. 189. New York: Elsevier Publishing Co.
 Fowden, L. (1958). *Biol. Rev.* (In the Press.)
 Fowden, L. & Bryant, M. (1958). *Biochem. J.* **70**, 626.
 Grobbelaar, N., Pollard, J. K. & Steward, F. C. (1955). *Nature, Lond.*, **175**, 703.
 Hyde, T. G. (1955). *Proc. Roy. Soc. Edinb.* **B65**, 299.
 King, F. E., King, T. J. & Warwick, A. J. (1950). *J. chem. Soc.* p. 3590.
 Kirpal, A. (1908). *Mh. Chem.* **29**, 227.
 Meyer, H. & Graf, R. (1928). *Ber. dtsh. chem. Ges.* **61**, 2202.
 Morrison, R. I. (1953). *Biochem. J.* **53**, 474.
 Piloty, O. & Finckh, C. (1904). *Liebigs Ann.* **333**, 71.
 Steward, F. C. & Pollard, J. K. (1957). *Annu. Rev. Pl. Physiol.* **8**, 65.
 Sucharda, E. (1925). *Ber. dtsh. chem. Ges.* **58**, 1727.
 Virtanen, A. I. (1956). *Angew. chem.* **67**, 381.
 Virtanen, A. I. (1957). *Festschrift Arthur Stoll*, p. 563. Basel: Sandoz A.G.
 Virtanen, A. I. & Kari, S. (1954). *Acta chem. scand.* **8**, 1290.
 Virtanen, A. I. & Kari, S. (1955). *Acta chem. scand.* **9**, 170.
 Zacharius, R. M., Thompson, J. F. & Steward, F. C. (1954). *J. Amer. chem. Soc.* **76**, 2908.

Enzymic Phosphorylation of Creatine by 1:3-Diphosphoglyceric Acid

By O. CORI, AÍDA TRAVERSO-CORI, MAGDALENA LAGARRIGUE AND F. MARCUS
Department of Biological Chemistry, School of Chemistry and Pharmacy, and Institute of Physiology, University of Chile, Santiago

(Received 27 January 1958)

Anaerobic glycolytic processes lead to the formation of phosphocreatine in skeletal muscle (Nachmansohn, 1928; Lundsgaard, 1931). High-energy phosphate compounds (1:3-diphosphoglyceric acid and phosphoenolpyruvic acid) are formed during glycolysis and phosphate is transferred from these substances to creatine, through the mediation of the adenylic nucleotides (Needham & van Heyningen, 1935; Needham & Pillai, 1937; Meyerhof, Schulz & Schuster, 1937; Innes, 1937) and creatine phosphokinase (Lohmann, 1934).

Needham & van Heyningen (1935) obtained direct evidence for the absolute requirement of adenylic nucleotides in the synthesis of phosphocreatine from phosphoenolpyruvate. However, when 1:3-diphosphoglyceric acid is the phosphate source, this requirement for adenosine diphosphate has only been assumed.

With the existing evidence, it could be assumed that phosphocreatine synthesis occurs in two ways: (a) as a two-stage reaction (reactions 1 and 2) connected by the adenylic system, or (b) as a single-

stage reaction (reaction 3) not requiring adenine nucleotides.

The apparent equilibrium constants of reactions (1) and (2) (Ogston & Smithies, 1948) are 5.1×10^3 and 2.3×10^{-3} . The equilibrium constant, either for the sum of both reactions or for reaction (3), may be calculated to be about 12, thus favouring the synthesis of phosphocreatine.

It is noteworthy that although myocardial tissue possesses a more efficient aerobic mechanism for the synthesis of adenosine triphosphate than does skeletal muscle, the molar concentrations of phosphocreatine and adenosine triphosphate in heart