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Acylative Decarboxylation with Particular Reference to the Acetyltative Decarboxylation of the 2:4-Dinitrophenyl Derivatives of some Amino Acids

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In studies on glycine metabolism in normal human subjects (Watts & Crawhall, 1959) and in patients with hyperoxaluria (Watts, Scowen & Crawhall, 1958) we needed to degrade 2:4-dinitrophenylglycine to yield the ^{13}C -labelled carboxyl-group carbon atom selectively as carbon dioxide for mass spectrometric analysis. We attempted to achieve this by oxidative means but were unable to limit the reaction to the carboxyl group. However, base-catalysed acetyltative decarboxylation was satisfactory, and the present paper describes the procedure together with the result of an attempt to identify the reaction product other than carbon dioxide. The application of this reaction to the decarboxylation of hippuric acid and of some other dinitrophenyl-amino acids has also been studied. A small amount of carbon dioxide is formed when acetic anhydride and a basic catalyst are heated together in the absence of any other reactant, and we have investigated the time course of this reaction and the relative amounts of carbon dioxide which are evolved when different catalysts are used in order to reduce this to a minimum. The apparent acetyltative decarboxylation of the dinitrophenyl derivatives of secondary amino acids is of some theoretical interest, for it is difficult to see how such compounds can form the intramolecular ring structures which have been postulated for the intermediates in this type of reaction (see, for example, Cornforth & Elliott, 1950). The rates of

carbon dioxide evolution from dinitrophenyl-sarcosine and dinitrophenylglycine are not appreciably different and the possible significance of this observation will be discussed.

The acetyltative decarboxylation reactions which we have observed may be considered to have an analogy with the biological synthesis of δ -amino-laevulinic acid from glycine and succinyl-coenzyme A (cf. Gibson, Laver & Neuberger, 1958; and Kikuchi, Kumar, Talmage & Shemin, 1958). We therefore sought evidence for the succinylative decarboxylation of dinitrophenylglycine and of the related compound glyoxylic acid-2:4-dinitrophenylhydrazine. The latter reaction was of some further interest in view of the possibility of reducing the theoretical product (4-oxoglutaraldehyde-2:4-dinitrophenylhydrazine) to δ -aminolaevulinic acid.

EXPERIMENTAL

Melting points were determined by the standard method, except where otherwise stated, and are uncorrected. Micro-analyses were performed by Weiler and Strauss, Oxford. [$1\text{-}^{13}\text{C}$]Glycine was synthesized as described previously (Watts & Crawhall, 1959).

Dinitrophenyl derivatives. The dinitrophenyl (DNP) derivatives of alanine, serine, cystine, phenylalanine, tyrosine, leucine and valine were a generous gift from Dr D. F. Elliott. DNP-sarcosine, DNP-proline, DNP- α -aminoisobutyric acid, DNP-aspartic acid and DNP-[$1\text{-}^{13}\text{C}$]glycine were prepared by dissolving the amino acid in

a saturated aqueous solution of sodium hydrogen carbonate and shaking with an excess of 1 fluoro-2,4-dinitrobenzene in methanol for 24 hr. at room temperature. DNP-sarcosine was crystallized from ethyl acetate-cyclohexane (1:9), m.p. 177° (Dalglish, Johnson, Todd & Vining, 1950, give m.p. 178°). DNP-proline crystallized from ethyl acetate-cyclohexane (1:9) had m.p. 186° (Dalglish *et al.* give m.p. 189°). DNP- α -aminoisobutyric acid was crystallized from ethyl acetate-cyclohexane (1:9) and acetone-light petroleum (b.p. 40–60°), m.p. 173° (Found: C, 44.0; H, 4.1; N, 15.6. $C_{10}H_{11}O_6N_3$ requires C, 44.6; H, 4.1; N, 15.6%). DNP- δ -aminolaevulinic acid was prepared in the same way as the other DNP-amino acids except that 0.25 M-sodium phosphate buffer (pH 7.0) was substituted for the saturated sodium hydrogen carbonate solution. DNP- δ -aminolaevulinic acid was crystallized from ethyl acetate-cyclohexane (1:9) and had m.p. 156° (decomp.) (Found: C, 45.1; H, 4.0; N, 13.1. $C_{11}H_{11}O_7N_3$ requires C, 44.4; H, 3.7; N, 14.1%).

Acetylation decarboxylation of dinitrophenyl-[1- ^{13}C]glycine and purification of the evolved $^{13}CO_2$ for mass-spectrometric analysis. DNP-[1- ^{13}C]glycine (approx. 15 mg.; 0.063 m-mole) on a watch glass was dissolved in an equivalent amount of 0.1 N-NaOH and the water removed by evaporation *in vacuo*. The dry sodium salt was transferred to a pear-shaped flask (10 ml.) and suspended in redistilled acetic anhydride (1 ml., 10.7 m-moles). Dry helium was passed down a tube to the bottom of the flask, which was connected by a small reflux condenser to a vacuum manifold line. The gas was passed through the flask and condenser and out to the atmosphere by an additional tap A for 10 min. before heat was applied to the acetic anhydride. Then tap A was closed and the tap connecting the vacuum line to the reaction flask was gently opened. This tap had a groove cut in the barrel (Glascock, 1954) to enable a gentle flow of gas (20 ml./min. at atmospheric pressure) to be maintained. The flask was then heated so that the acetic anhydride refluxed gently. The helium and evolved CO_2 were drawn through three spiral traps (1, 2 and 3) which were cooled in liquid O_2 . The reaction mixture changed from a deep yellow to a straw colour and finally became dark brown, with the formation of a small white precipitate. After 0.5 hr. the grooved tap was closed and the pressure in the manifold line reduced to 0.005 mm. Hg. Tap B, between the first and second of the liquid O_2 -cooled traps, was then closed, as was the main tap connecting the vacuum line to the pumping system (two-stage rotary oil pump, model no. 2SC20, Edwards and Co. Ltd., Crawley, Sussex). The liquid O_2 surrounding the first trap was then replaced by a solid CO_2 -ether mixture. Tap B was opened for 2 min. and CO_2 allowed to pass from trap 1 to traps 2 and 3. This procedure was designed to separate the CO_2 from any acetic anhydride that had passed over from the reaction vessel into trap 1. The liquid O_2 surrounding traps 2 and 3 was removed and the CO_2 transferred to the cooling limb of a manometer. The tap of the manometer was closed, the CO_2 allowed to vaporize and its volume calculated from the observed pressure change. The pressure in the manometer was again reduced to 0.005 mm. Hg by immersing its cooling limb in liquid O_2 ; the Dewar flask containing liquid O_2 was replaced by one containing a solid CO_2 -ether mixture and the gas transferred to an evacuated flask (250 ml.) which was also fitted with a cooling limb and containing a copper coil (22 gauge, 6 ft. coiled on $\frac{1}{4}$ in.

former). The coil was raised to dull-red heat for 10 sec. by the current from a 12 v accumulator. The gas was finally transferred to the mass spectrometer (Consolidated Nier Mass Spectrometer model 21–201) and the ^{13}C -abundance measured.

Isolation of the product other than carbon dioxide from the acetylation decarboxylation of dinitrophenylglycine. DNP-glycine (150 mg., 0.62 m-mole) was converted into its anhydrous sodium salt, which was heated under reflux with redistilled acetic anhydride (10 ml., 107 m-moles) for 1.5 hr. The acetic anhydride was removed by steam-distillation and a yellow crystalline solid (77 mg.) separated from the cooled aqueous solution. This was recrystallized from water. The melting point of this derivative could not be satisfactorily determined because it sublimed at 250° in the hot-stage melting-point apparatus, and this may be why the microanalyses have been unsatisfactory (Found: C, 42.7; H, 3.5; N, 16.6. $C_9H_9O_6N_3$ requires C, 42.5; H, 3.5; N, 16.5%). The expected DNP-aminoacetone, $C_9H_9O_5N_3$, requires C, 45.2; H, 3.7; N, 17.6%). The infrared-absorption spectrum was determined and showed a strong peak at 1720 μ suggestive of a carbonyl group, but after attempts to make a semicarbazone the reactants were recovered unchanged.

Acetylation decarboxylation of other dinitrophenylamino acids and of N-dimethylglycine. The reaction was carried out essentially as described for DNP-glycine. The yield of CO_2 was calculated from the volume of gas which was present in the manometer before the hot-copper-wire treatment.

Acetylation decarboxylation of [1- ^{13}C]hippuric acid. Hippuric acid was dissolved in 1.5 mol.prop. of 0.1 N-NaOH and the reaction continued as described for DNP-glycine.

Comparison of the rates of acetylation decarboxylation of dinitrophenylglycine and dinitrophenylsarcosine. The appropriate DNP derivative (0.5 m-mole) on a watch glass was dissolved in 2.5 ml. of 0.2 N-NaOH, and the water removed by evaporation *in vacuo*. The dry residue was then transferred to a pear-shaped flask (25 ml.) and redistilled acetic anhydride (10 ml., 107 m-moles) was added. The flask was fitted with an inlet for passing helium beneath the surface of the acetic anhydride. After helium had been passed through the apparatus for about 10 min. the acetic anhydride was brought to the boil. The effluent gas flowed through a small water-cooled reflux condenser, then passed a cold-finger cooled with solid CO_2 -ether and finally via a three-way tap through two traps arranged in series and containing aq. $Ba(OH)_2$ (0.05 N, 10 ml. under light petroleum, b.p. 100–120°), the second of these was isolated from the atmosphere by a soda-lime guard tube. The other limb of the three-way tap was connected to a second identical set of traps with aq. $Ba(OH)_2$. Every 10 min. the flow of gas was changed from one set of traps to the other. The contents of the first set of traps were titrated with 0.1 N-HCl (with phenolphthalein as indicator). The traps were then washed, refilled and replaced ready for use at the end of the subsequent 10 min. period.

Evolution of carbon dioxide from acetic anhydride and basic catalysts. These experiments were carried out in the apparatus described in the previous experiment. Acetic anhydride (10 ml., 107 m-moles) was heated under reflux with anhydrous sodium acetate (250 mg., 3.05 m-moles), pyridine (0.5 ml., 6.2 m-moles) or β -picoline (0.5 ml., 5.2 m-moles), the CO_2 being collected in aq. $Ba(OH)_2$ and determined titrimetrically.

Evolution of carbon dioxide from succinic anhydride containing sodium succinate as catalyst. The apparatus used was the same as that employed to study the rate of CO_2 evolution during the acetylation decarboxylation of DNP-glycine and DNP-sarcosine, except that the solid CO_2 -ether-cooled cold-finger was omitted. Succinic anhydride (50 mg., 0.5 m-mole) was dissolved in 0.5 ml. of 0.1N-NaOH and the water removed by evaporation *in vacuo*. The dry sodium salt was then mixed with more succinic anhydride (1 g., 10 m-moles) and transferred to the reaction flask, which was heated in an oil bath at 160° . Evolution of CO_2 commenced after 15 min. After 1 hr. the mixture began to darken and after 2 hr. it was jet black.

Reaction of succinic anhydride with dinitrophenylglycine.

(a) DNP-glycine (116 mg., 0.48 m-mole) was converted into its sodium salt, mixed with succinic anhydride and heated to 160° as in the previous experiment. At the end of 3 hr. the reaction mixture, which had become jet black, was extracted with hot water (10 ml.) and the water-insoluble residue was dissolved in ethanol (40 ml.). Samples from both solutions were submitted to chromatography on buffered Whatman no. 1 paper with the 2-methylbutan-2-ol (*tert.*-pentanol) system of Blackburn & Lowther (1951). The water-insoluble material gave a faint yellow spot (R_F 0.36). No yellow derivatives were present in the water-soluble material. Control spots of DNP-glycine and DNP- δ -aminolaevulinic acid had R_F values of 0.38 and 0.63 respectively.

(b) Four samples of DNP-glycine (12 mg., 0.05 m-mole) were converted into the sodium salt. Succinic anhydride (100 mg.) was added to each and the mixture stirred. They were then transferred to an oil bath at 160° and removed from the bath after 15, 30, 45 and 60 min. respectively. The mixtures were then extracted with hot water (10 ml. each) and the insoluble residues, 7.2, 9.0, 7.8 and 5.8 mg. respectively, were dissolved in acetone. Samples of the four aqueous and four acetone solutions were submitted to chromatography as described in (a) above.

The aqueous solutions showed only DNP-glycine in quantities which decreased with increasing time of heating. The acetone-soluble material showed a brown spot at the origin and a trace of DNP-glycine. No spot corresponding to the marker DNP- δ -aminolaevulinic acid (R_F 0.70) was observed.

Reaction of succinic anhydride with glyoxylic acid-2:4-dinitrophenylhydrazone. (a) Glyoxylic acid-2:4-dinitrophenylhydrazone (127 mg., 0.5 m-mole) was converted into its sodium salt and mixed with succinic anhydride (1 g.). The mixture was heated in an oil bath to 160° and the rate of evolution of CO_2 studied as described above.

(b) Glyoxylic acid-2:4-dinitrophenylhydrazone (46.7 mg., 0.18 m-mole) was converted into its sodium salt, mixed with powdered succinic anhydride (300 mg.) and heated at 160° for 1 hr. The mixture was cooled, suspended in 5 ml. of hot water and then cooled to 2° . The precipitate was removed by filtration (31.8 mg.). Chromatography of this product in butan-1-ol-ethanol-aq. 0.5N- NH_3 soln. (70:10:20, by vol.) showed three yellow spots, R_F values 0.59, 0.76 and 0.86. Catalytic reduction of the product at room temperature and atmospheric pressure in water with PtO as catalyst gave a colourless solution which rapidly turned green on exposure to air. (A control reduction of the 2:4-dinitrophenylhydrazone of glyoxylic acid showed that 7 mol.prop. of hydrogen was absorbed.) No free δ -aminolaevulinic acid could be detected after chromatography in

butan-1-ol-acetic acid-water (60:15:25, by vol.) of the reduced solution with the reagents described by Shuster (1956).

RESULTS

In our first attempts to degrade DNP-[1- ^{13}C]-glycine by acetylation decarboxylation we heated 15 mg. of the amino acid derivative with 4 ml. of acetic anhydride and 100 mg. of anhydrous sodium acetate, and used nitrogen as the carrier gas. The carbon dioxide was collected in spiral traps which were cooled in liquid oxygen, and its volume was measured manometrically before it was transferred to the mass spectrometer without any further purification. This procedure yielded approximately 100% of the theoretical volume of carbon dioxide, but the values which were obtained for the isotope enrichment of replicate samples of known ^{13}C -abundance did not agree with one another, and were too low. If the carbon dioxide was converted into barium carbonate and reliberated with sulphuric acid, approximately 100% of the theoretical

Fig. 1. Evolution of carbon dioxide on boiling acetic anhydride (10 ml., 107 m-moles) with basic catalysts. ●, Anhydrous sodium acetate (250 mg., 3.05 m-moles); ○, β -picoline (0.5 ml., 5.2 m-moles); ×, pyridine (0.5 ml., 6.2 m-moles).

volume of carbon dioxide was still obtained, but the results of the ^{13}C -determination although reproducible were still approximately 10% too low. The observation that the amount of carbon dioxide which was obtained from DNP-alanine under these circumstances was equivalent to 110% of the theoretical value suggested that the apparent 100% yield of carbon dioxide which we had obtained from DNP-glycine might have been due to the formation of extraneous carbon dioxide by the reagents. It was shown that anhydrous sodium acetate, pyridine and β -picoline all caused the evolution of some carbon dioxide when boiled with acetic anhydride and this occurred most extensively with anhydrous sodium acetate (Fig. 1). However, β -picoline and pyridine appeared to be less efficient catalysts than anhydrous sodium acetate for the acylative decarboxylation of DNP-glycine, because, although DNP-alanine yielded 90% of the theoretical volume of carbon dioxide when these reagents were used, the yield from DNP-glycine was only 60%. In order to utilize the greater catalytic potency of sodium acetate without introducing more than the minimum amount of extraneous carbon dioxide from the reagents, we prepared the anhydrous sodium salts of the DNP-amino acids and treated these with the smallest convenient volume (1 ml.) of acetic anhydride. A further trace of extraneous carbon dioxide was eliminated by using pure helium (supplied for anaesthetic purposes) instead of 'industrial' nitrogen as the inert carrier gas. The yield of carbon dioxide under these conditions was reproducible, but if the barium carbonate step was omitted, the apparent ^{13}C -enrichment of DNP-[1- ^{13}C]glycine prepared from [1- ^{13}C]glycine of known isotope content was still variable, and it appeared that there might be some impurity in the gas which was removed when the carbon dioxide was precipitated as barium carbonate. Purification of the gas in this way doubled the length of time required for each analysis and was very difficult to carry out on such a small scale without the ingress of traces of atmospheric carbon dioxide. The carbon dioxide which was obtained from the acylative decarboxylation of DNP-amino acids, and which had not been purified by precipitation as barium carbonate, yielded an ion of mass 43 in the mass spectrometer as well as the ions of masses 44 and 45 due to $^{12}\text{CO}_2$ and $^{13}\text{CO}_2$ respectively. The peak at mass 43 was variable in height but was sometimes as great as that at mass 45 due to $^{13}\text{CO}_2$. When the gas was submitted to the barium carbonate treatment, the peak at mass 43 disappeared. The nature of the impurity responsible for the production of an ion of mass 43, and the reason for its apparent interference, is unknown. The free radical ($^{12}\text{CH}_3 \cdot ^{12}\text{CO} \cdot$) is an obvious possible impurity of mass 43 and

might have been formed in the ionizing beam of the mass spectrometer from, for example, acetone or acetic anhydride. However, when the gas was purified by allowing the previously frozen gas to evaporate from a container cooled in solid carbon dioxide-ether, the peak due to this ion did not disappear though it sometimes decreased. The other anomaly is that the height of the mass 43 peak was never more than 1% of that at mass 44 (due to $^{12}\text{CO}_2$) so that the isotope enrichment might have been expected to have been decreased by 1% at the most if this peak was interfering, but in fact the apparent isotope enrichment was always increased when it was present, suggesting that some additional impurity of mass 45 was also present. This could not be confirmed because a peak of mass 45 is always present in carbon dioxide owing to the natural abundance of the ^{13}C isotope. Nevertheless, it was found empirically that this impurity or its precursor could be removed by exposing the gas to a copper wire electrically heated to dull-red heat for 10 sec., and it is presumed that pyrolysis of the impurity took place as after this treatment no peak of mass 43 was detectable. The exact quantity of extraneous carbon dioxide evolved by the reagents cannot be assessed directly because the catalytic activity of the sodium salt of DNP-glycine cannot be determined, but it was found that under the conditions which we describe the isotopic enrichment of the carbon dioxide obtained was always 6% less than that expected from the known isotopic enrichment of ^{13}C in the glycine from which the DNP-glycine was prepared (Table 1), and this correction is always applied in routine analyses.

Various other DNP-amino acids were submitted to this reaction (Table 2). The DNP derivatives of aliphatic amino acids other than glycine gave yields of carbon dioxide between 85 and 95%. The decarboxylation of DNP-serine was not very satisfactory, possibly because the elements of water can be removed, leaving an unsaturated derivative which can undergo alternative side reactions. DNP-aspartic acid yielded more than 100% of the maximum possible yield of carbon dioxide, and

Table 1. *Isotope enrichment of carbon dioxide obtained by combustion of [1- ^{13}C]glycine and by acylative decarboxylation of dinitrophenyl-[1- ^{13}C]glycine and [1- ^{13}C]hippuric acid made from it*

Substance	Observed isotope enrichment in sample (atoms % excess)	Isotope enrichment after correction for dilution (atoms % excess)
[1- ^{13}C]Glycine	0.548 $\pm$ 1%	49.8
DNP-[1- ^{13}C]glycine	1.029 $\pm$ 1%	46.7
[1- ^{13}C]Hippuric acid	1.026 $\pm$ 1%	46.3

some decarboxylation from the β -carboxyl group may have taken place. The sodium salt of DNP-tyrosine was only sparingly soluble in water, so the lower yield of carbon dioxide may have been caused by incomplete conversion of the acid into its sodium salt. The yield of carbon dioxide from the sodium salt of hippuric acid was increased from 35 to 50% of the theoretical volume by the use of 1.5 instead of 1.0 mol.prop. of sodium hydroxide. The isotopic enrichment of the carbon dioxide obtained from $[1-^{13}\text{C}]$ hippuric acid by this method was also 6% less than the theoretical value, and with

the application of a suitable correction factor, isotopic analyses of $[1-^{13}\text{C}]$ hippuric acid could be carried out as a routine procedure.

The rate of evolution of carbon dioxide by the action of acetic anhydride on the sodium salts of DNP-glycine and DNP-sarcosine were compared under identical conditions, and the results (Fig. 2) indicated that carbon dioxide was evolved at similar rates from the two substances.

An investigation was also made into the possibility of using succinic anhydride as a reagent for the acylative decarboxylation of DNP-glycine because of the similarity of such an acylation to the biological synthesis of δ -aminolaevulinic acid. The results of these experiments are rather difficult to assess because the action of heat on succinic anhydride containing sodium succinate alone yielded an amount of carbon dioxide equivalent to 40% of that to be expected if an equivalent quantity of DNP-glycine sodium salt had been present (Fig. 3). When succinic anhydride was treated with the sodium salt of DNP-glycine a yield of 115% of the expected amount of carbon dioxide was obtained. No isotopic label was used in these experiments so it was not known how much of this

Table 2. Yields of carbon dioxide obtained from the acylative decarboxylation of some dinitrophenyl-amino acids and related compounds

	Yield of CO_2 (%)
DNP-glycine	80
DNP-alanine	95
DNP-leucine	87
DNP-valine	97
DNP-serine	40
DNP-cystine	84
DNP-aspartic acid	120
DNP-tyrosine	64
DNP-phenylalanine	77
DNP-sarcosine	80
DNP-proline	95
N-Dimethylglycine	86
DNP- α -aminoisobutyric acid	10
Hippuric acid	50

Fig. 2. Evolution of carbon dioxide during the acylative decarboxylation of the sodium salts of $\times$, DNP-glycine and $\circ$, DNP-sarcosine [0.5 m-mole of the amino acid derivative and 10 ml. (107 m-moles) of acetic anhydride were used].

Fig. 3. Evolution of carbon dioxide when succinic anhydride (1 g., 10 m-moles) was heated with: $\bullet$, sodium succinate (0.5 m-mole); $\circ$, the sodium salt of DNP-glycine (0.48 m-mole); $\times$, sodium glyoxylate-2:4-dinitrophenylhydrazone (0.5 m-mole).

carbon dioxide came from the DNP-glycine and how much from the succinic anhydride, but the results did suggest that decarboxylation of the DNP-glycine was taking place.

In another series of experiments in which the time of heating of the reactants was varied, chromatographic analysis of the products showed that the amount of free DNP-glycine decreased as the length of heating increased but it was not replaced by any other DNP derivative. In an attempt to synthesize δ -aminolaevalic acid by the succinylation procedure, succinic anhydride was treated with the sodium salt of the 2:4-dinitrophenylhydrazone of glyoxylic acid. Carbon dioxide was generated in this reaction (Fig. 3), but if the reaction was continued for 3 hr. the mixture became black and no 2:4-dinitrophenylhydrazone could be identified in the product. At the end of 1 hr. it could be shown by paper chromatography that at least three 2:4-dinitrophenylhydrazones were present. If one of these had been the expected 2:4-dinitrophenylhydrazone of 4-oxoglutaraldehyde, reduction of the mixed products should have given δ -aminolaevalic acid (Meister & Abenschein, 1956; Smith, 1958). The electrolytic-reduction method of Smith (1958) rapidly destroyed free δ -aminolaevalic acid. By the method of Meister & Abenschein (1956) no δ -aminolaevalic acid could be identified chromatographically.

DISCUSSION

The reaction of an amino acid with acetic anhydride in the presence of anhydrous sodium acetate as a catalyst to yield an acylamido ketone and carbon dioxide was first described by Dakin & West (1928), and since then many workers have investigated this reaction and some have studied the rate and quantity of carbon dioxide evolution. It is therefore surprising that the original observation of Perkin (1886) that anhydrous sodium acetate and acetic anhydride evolve carbon dioxide on heating has only recently received any attention. Rondstedt, Manning & Tabibian (1950) carried out the Dakin & West reaction on $[1-^{14}\text{C}]$ alanine and obtained a lower proportion of $^{14}\text{CO}_2$ in the evolved gas than they had expected, but they attributed this to an ingress of atmospheric carbon dioxide. Fahey (1957) investigated the reaction of $[1-^{14}\text{C}]$ -acetic anhydride on *o*-chlorophenylacetic acid in the presence of anhydrous sodium acetate and found that three reactions can occur simultaneously. The main reaction is the acetylation decarboxylation of *o*-chlorophenylacetic acid with the formation of *o*-chlorophenylacetone. A very minor reaction is that of the mixed anhydride of *o*-chlorophenylacetic acid and acetic acid with $[1-^{14}\text{C}]$ acetic anhydride, with the formation of *o*-

chlorophenylacetone and $^{14}\text{CO}_2$ derived from the carbonyl group of acetic anhydride. A third reaction is the intermolecular reaction of acetic anhydride molecules with the evolution of $^{14}\text{CO}_2$. Results which we have obtained (Fig. 2) also demonstrate that carbon dioxide is evolved when acetic anhydride is heated with various basic catalysts. In order to reduce the amount of extraneous carbon dioxide which is formed to a minimum, we have converted the DNP-glycine into its anhydrous sodium salt. Even under these conditions it appears that carbon dioxide is evolved from the reagents because the mass-spectrometric analysis of the evolved carbon dioxide derived from DNP- $[1-^{13}\text{C}]$ glycine is 6% lower than the theoretical value (Table 1). This additional carbon dioxide may have arisen from either the second or third reaction described by Fahey (1957).

Wiley (1950), Cornforth & Elliott (1950) and Buchanan, Reid, Thomson & Wood (1957) have discussed the possibility of formation of cyclic intermediates when secondary amino acids are submitted to the Dakin & West reaction. Wiley (1950) suggested that decarboxylation can precede acetylation in the reaction of acetic anhydride with sarcosine. We have found that DNP-aliphatic amino acids, with the exception of DNP- α -aminoisobutyric acid, which possesses no free α -hydrogen atom, readily decarboxylate under our conditions. If decarboxylation preceded acetylation it is difficult to see why DNP- α -aminoisobutyric acid should not decarboxylate as readily as DNP-alanine unless considerable steric hindrance from the α -methyl group is postulated. The fact that a small quantity of carbon dioxide is evolved (Table 2) can be explained by Fahey's (1957) second type of reaction, that is that the mixed anhydride of the DNP-amino acid and acetic acid can react with the reagents with the formation of carbon dioxide. As this DNP-amino acid does not undergo acylative decarboxylation, it will remain for a longer period in the reaction mixture, enabling this second type of reaction to occur to a greater extent. Buchanan *et al.* (1957) found that *N*-phenylglycine and *N*-phenyl-*N*-acetyl glycine were decarboxylated under the Dakin & West (1928) conditions, but that *N*-phenyl-*N*-formyl glycine was not. On the basis of this observation they questioned whether the current theories are adequate to explain this result, although we are not aware of any experimental evidence that *N*-formyl amino acids can form oxazolones.

DNP-sarcosine readily yields carbon dioxide on treatment of its sodium salt with acetic anhydride and the rate of evolution was similar to that observed with DNP-glycine (Fig. 2 and Table 2). It is difficult to see how any intramolecular cyclization can take place with DNP-sarcosine because it

possesses a tertiary nitrogen atom. It can therefore be deduced from the results with DNP-sarcosine and DNP-proline that the formation of an intramolecular cyclic structure is not obligatory for the acylative decarboxylation of all substituted amino acids. It is possible that the presence of a 2:4-dinitrophenylmethylamino group attached to the α -carbon atom causes sufficient activation to permit attack by acetic anhydride at the α -position and that the reaction is therefore analogous to the acylative decarboxylation of *o*-chlorophenylacetic acid by acetic anhydride (Fahey, 1957). The similarity between the rates of acetylation of DNP-sarcosine and DNP-glycine raises the possibility that this latter reaction may also proceed without the formation of an intramolecular cyclic intermediate.

Kikuchi *et al.* (1958) have shown in a biological system that, in the reaction of succinyl-coenzyme A with glycine in the presence of pyridoxal phosphate and magnesium ions as catalysts, decarboxylation does not precede succinylation, and a similar type of condensation has been postulated to explain the synthesis of aminoacetone by washed suspensions of *Staphylococcus aureus* (Elliott, 1959). It therefore seemed that there might be a similarity between the biological synthesis of δ -aminolaevulinic acid and the acylative decarboxylation of DNP-glycine which we have investigated. Succinic anhydride has not previously been described as a reagent for acylative decarboxylation, though Fittig (1897) showed that acetic anhydride reacted with succinic acid to form laevulinic acid. We found that heating DNP-glycine and the 2:4-dinitrophenylhydrazone of glyoxylic acid with succinic anhydride and a basic catalyst caused the reactants to evolve carbon dioxide, but a simple succinylative decarboxylation did not take place.

SUMMARY

1. The acetylation of 2:4-dinitrophenylglycine has been investigated with particular reference to the degradation of dinitrophenyl-[1-¹³C]glycine to yield the carboxyl-group carbon atom in the form of carbon dioxide for mass-spectrometric analysis. An 80–85% yield of carbon dioxide was obtained from the carboxyl group of dinitrophenylglycine and the observed ¹³C enrichment of this gas has to be multiplied by a factor of 1.06 to allow for a small but constant amount of extraneous carbon dioxide which is formed as a result of unavoidable side reactions. The procedure has been used with minor modifications for the similar degradation of [1-¹³C]hippuric acid.

2. The yield of carbon dioxide from the acetyl-

ative decarboxylation of the dinitrophenyl derivatives of other primary and secondary amino acids has been investigated.

3. The decarboxylation of dinitrophenylsarcosine and dinitrophenylproline indicates that the formation of intramolecular cyclic intermediates may not be universally essential for acetylation of substituted amino acids.

4. Carbon dioxide was evolved when succinic anhydride was heated with the sodium salts of dinitrophenylglycine and the 2:4-dinitrophenylhydrazone of glyoxylic acid but no evidence for the formation of the respective derivatives of δ -aminolaevulinic acid was obtained.

5. The experimental findings are discussed with particular reference to the postulated mechanisms of acylative decarboxylation reactions.

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