

The Formylation of Amino Acids with Acetic Formic Anhydride

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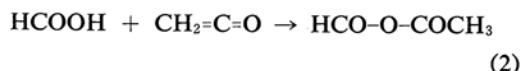
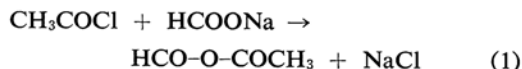
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The formyl group has been used as a blocking substituent of the amino moiety of amino acids in peptide syntheses^{1,2)} or in optical resolutions of amino acids. In these procedures, *N*-formylamino acids have been prepared with formic acid³⁾ or with formic acid and acetic anhydride.^{2,4,5)} The latter method has been widely used, since the formylation gives good yield without any accompanying racemization.

These methods, however, have the common defect that too much of the reagents are necessary compared with the amount of amino acids employed. It has been presumed that, in the formylation of amino acids with formic acid and acetic anhydride, acetic formic anhydride or formic anhydride may be formed first and that then the anhydride may react with the amino groups to yield *N*-formylamino acids. Therefore, the present authors have tried to formylate amino acids directly with pure acetic formic anhydride. In addition to the ketene-formic acid method,⁶⁾ this anhydride has been successfully and conveniently prepared by the reaction of acetyl chloride with sodium formate. In many instances, it has been found that the formylation of amino acids by the present method can be performed in a very simple way under mild conditions, and that only a 2 to 3-molar excess of the reagent is required.

Results

Acetic formic anhydride was prepared by the following reactions:



In method 1, although the purity of acetyl chloride and atmospheric humidity exerted a

great influence upon the yield, the anhydride was generally obtained in a 75–91% yield. In method 2,⁶⁾ the anhydride was constantly obtained in a 80–90% yield. The refractive indices and the infrared spectra of the anhydrides prepared by the two methods are in agreement with each other (Fig. 1). This anhydride is stable in a refrigerator, but it is decomposed with the evolution of carbon monoxide above about 60°C, above room temperature in formic acid, or even at 0°C in the presence of a small amount of pyridine.

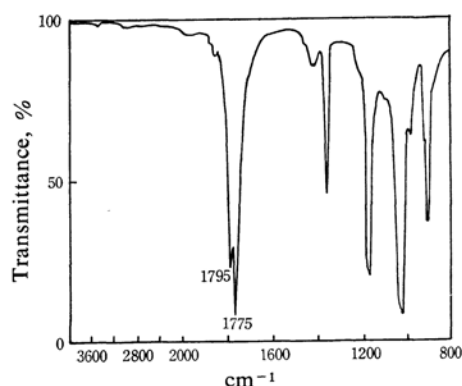


Fig. 1. Infrared absorption spectrum of acetic formic anhydride in carbon tetrachloride.

The formylation of amino acids with acetic formic anhydride was carried out using formic acid as a solvent. The temperature of the reaction was maintained below 10°C during the exothermic period in the reaction by means of an ice bath. After the stirring had been continued subsequently for two hours at room temperature, the mixture was concentrated to a solid mass or syrup under reduced pressure, and the residue was crystallized from a suitable solvent. Even when acetic acid was used as a solvent, formylation took place but acetylation did not. Mono-amino monocarboxylic acids (glycine, DL-alanine, L-valine, L-leucine, L-methionine, L-phenylalanine, L-tyrosine and β -alanine) were generally formylated in good yields (70–94%), as was the alanine ester, and their optical activities were retained. The formylation of

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glutamic acid by the present method was difficult to carry out with a good yield, unlike that of the amino acids mentioned above. The formylation of other amino acids with three or more functional groups was not attempted in our studies. Special methods^{7,8)} may be required for the monoformylation of basic amino acids.

Experimental⁹⁾

Acetic Formic Anhydride.—*Method 1.*—Into a solution of 445 ml. of acetyl chloride in 250 ml. of dry ether, 500 g. of sodium formate was added in several portions in such a way that the temperature did not exceed 27°C. The mixture was kept from moisture and vigorously shaken. After the shaking had been continued for 5.5 hr. at 25–27°C, the salt was filtered off. The filtrate was then evaporated under water-pump pressure below 20°C (bath temperature) in order to remove the ether and the remaining reagent. The residual liquid weighed 502 g. (91%). A small amount of free acid was found, by a study of the infrared spectrum, to be present in the anhydride prepared in this way, but the crude anhydride was successfully used in the formylation of amino acids without further purification. When impure acetyl chloride was used, the product decomposed gradually, with forming, during the above procedure.

Method 2.—Ketene was prepared from acetone in a ketene lamp which delivered about 0.5 mol. of ketene per hour. The solution of 2 mol. of formic acid (98–100%, E. Merck AG.) in 300 ml. of ether was saturated with ketene in an ice bath. After the evaporation of ether, the residue was fractionated under reduced pressure, and the fractions boiling between 30°C/24 mmHg (n_D^{20} 1.386) and 24°C/9 mmHg (n_D^{20} 1.388) were collected; yield, 163 g. (93%). These fractions were successfully employed for the formylation of amino acids. From the infrared spectra of the distillates, it was found that the first runnings contained only a trace of free acid, while the last ones a small amount of acetic anhydride. The main fraction boiled at 30°C/19 mmHg (n_D^{20} 1.387) (in the lit.,⁶⁾ b. p., 27–28°C/10 mmHg, n_D^{20} 1.388; its infrared spectrum is shown in Fig. 1. This spectrum shows absorption bands at 1795, 1775, 1380, 1178, 1037 and 924 cm^{-1} (in carbon tetrachloride). It is very interesting that the difference (20 cm^{-1}) between the wave numbers of the two carbonyl bands (1795 and 1775 cm^{-1}) of acetic formic anhydride is less than those of ordinary acid anhydrides (about 60 cm^{-1}).¹⁰⁾

N-Formylglycine.—To a solution of 3.76 g. (50 mmol.) of glycine in 20 ml. of formic acid (90%), 13.2 g. (150 mmol.) of acetic formic anhydride was added. The reaction mixture was stirred at a temperature maintained below 10°C by means of an ice bath. After 40 min., the bath was removed. Stirring was continued for a further 80 min., and then the solution was concentrated to dryness under reduced pressure. The solid residue was twice recrystallized from a mixture of ethyl acetate (75 ml.) and ethanol (25 ml.). *N*-Formylglycine was obtained as white crystals; 4.39 g. (85%); m. p. 151–153°C (lit.³⁾ m. p. 153–154°C). (Found: N, 13.41%).

When acetic acid was used as the solvent instead of formic acid in the above instance, *N*-formylglycine was also obtained in a 76% yield.

N-Formyl-DL-alanine.—After 3.57 g. (40 mmol.) of DL-alanine had been acylated with 10.57 g. (120 mmol.) of acetic formic anhydride by a method similar that of to the formylation of glycine in formic acid, the product was recrystallized from ethyl acetate-ethanol to yield 4.41 g. (94%) of pure crystals (m. p. 145–148.5°C; lit.¹¹⁾ m. p. 147–148°C). (Found: C, 40.93; H, 6.17; N, 11.97%).

N-Formyl-L-valine.—After 3.52 g. (30 mmol.) of L-valine had been treated with 10 g. (113 mmol.) of the anhydride in a similar way, the product was twice recrystallized from ethanol to yield 3.65 g. (84%) of pure crystals. M. p. 150–151°C, $[\alpha]_D^{25} +11.7^\circ$ (c 3.97, ethanol); lit.¹²⁾ m. p. 156°C, $[\alpha]_D^{25} +13.1^\circ$ (c 9.0, ethanol). (Found: C, 49.21; H, 7.81; N, 9.59%).

N-Formyl-L-leucine.—L-Leucine (3.94 g., 30 mmol.) was formylated with the anhydride (7.93 g., 90 mmol.) in 20 ml. of formic acid. The recrystallization of the product from ethyl acetate gave 3.35 g. (70%) of crystals; m. p. 139–141°C. The analytical sample was prepared by further recrystallization from the same solvent; m. p. 140.5–142°C, $[\alpha]_D^{25} -17.7^\circ$ (c 4.97, ethanol); lit.¹³⁾ $[\alpha]_D^{25} -18.4^\circ$ (c 10, ethanol). (Found: C, 52.76; H, 8.30; N, 8.86%).

N-Formyl-L-methionine.—L-Methionine (3.0 g., 20 mmol.) was treated with the anhydride (5.4 g., 60 mmol.) in 4 ml. of formic acid. The crude product obtained in a similar manner was twice recrystallized from ethyl acetate-petroleum ether to give 2.73 g. (77%) of pure crystals; m. p. 86–87°C, $[\alpha]_D^{25} -10.0^\circ$ (c 2.41, water); lit. m. p. 86–87°C,¹⁴⁾ $[\alpha]_D^{25} -10.0^\circ$ (c 0.8, water).¹⁴⁾

N-Formyl-L-phenylalanine.—L-Phenylalanine (2.65 g., 16 mmol.) was formylated with the anhydride (2.7 g., 30 mmol.) in 4 ml. of formic acid. The first crop of *N*-formyl-L-phenylalanine was taken out by filtration from the reaction mixture, while the second crop was obtained from the mother liquor. They were then washed with water, dried, and combined. Yield, 2.58 g. (83%); m. p. 165–166°C. The analytical sample was obtained by recrystallization from water. M. p. 165.5–166°C,

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$[\alpha]_D^{20} +72.5^\circ$ (c 1.58, ethanol); lit.¹⁵⁾ m. p. 167°C , $[\alpha]_D^{20} +75.2^\circ$ (c 4, ethanol). (Found: N, 7.43%).

N-Formyl-L-tyrosine.—To a suspension of 7.25 g. (40 mmol.) of L-tyrosine in 10 ml. of formic acid, 10.5 g. (120 mmol.) of the anhydride was added. The mixture was allowed to react for 2 hr., water was added thereto, and the solution was concentrated to dryness under reduced pressure. An ice cold N-hydrochloric acid was then added to the residue in order to dissolve the remaining tyrosine. The product was collected by filtration and washed with water. 7.05 g. (84%) of N-formyl-L-tyrosine was obtained; it melted at 166°C . The recrystallization from ethanol gave an anhydrous compound; m. p. 167°C , $[\alpha]_D^{20} +89.7^\circ$ (c 1.78, ethanol), $[\alpha]_D^{20} +56.3^\circ$ (c 1.33, water); lit.¹⁶⁾ m. p. $170\text{--}171^\circ\text{C}$, $[\alpha]_D^{19} +89.4^\circ$ (c 1.74, ethanol).

Found: C, 57.29; H, 5.51; N, 7.11. Calcd. for $\text{C}_{10}\text{H}_{11}\text{NO}_4$: C, 57.41; H, 5.30; N, 6.70%.

The recrystallization from water generally gave a hydrated compound;¹⁷⁻¹⁹⁾ m. p. 166°C (sinters at 90°C), $[\alpha]_D^{20} +53.0^\circ$ (c 1.52, water).

Found: H_2O , 7.88% (dried in vacuo at 100°C for 3 hr.). Calcd. for $\text{C}_{10}\text{H}_{11}\text{NO}_4 \cdot \text{H}_2\text{O}$; H_2O , 7.92%.

N-Formyl-L-glutamic Acid.—L-Glutamic acid (4.42 g., 30 mmol.) in 6.5 ml. of formic acid was formylated with the anhydride (3.36 g., 38 mmol.). The crude product was recrystallized from ethyl acetate to yield pure crystals of N-formyl-L-glutamic acid (1.85 g., 35%). M. p. $110\text{--}112^\circ\text{C}$, $[\alpha]_D^{20.5} -7.4^\circ$ (c 2.02, water); lit.²⁰⁾ m. p. $112\text{--}113^\circ\text{C}$, $[\alpha]_D^{20} -7.25^\circ$ (c 2, water). Better yields have been

reported in the literature^{20,21)} from the usual formic acid-acetic anhydride method.

N-Formyl-β-alanine.—β-Alanine (3.1 g., 35 mmol.) was formylated with acetic formic anhydride (11.2 g., 130 mmol.) in a similar manner. The crystallization of the product from ethyl acetate yielded 3.24 g. (79%) of crystals (m. p. $73\text{--}74^\circ\text{C}$; lit.²²⁾ m. p. $75\text{--}76^\circ\text{C}$).

N-Formyl-DL-alanine Ethyl Ester.²³⁾—Into a solution of 91 g. of DL-alanine ethyl ester in 91 ml. of formic acid, 118 ml. of acetic formic anhydride was added with stirring in an ice bath. After the mixture had been stirred for 3 hr. at room temperature, the mixture was distilled under reduced pressure to afford the formylated ester; yield, 95.3 g. (85%); b. p. $98^\circ\text{C}/1\text{ mmHg}$ (lit.²⁴⁾ b. p. $100^\circ\text{C}/1\text{ mmHg}$).

Summary

The formylation of amino acids with acetic formic anhydride has been studied. This anhydride is prepared by the reaction of acetyl chloride with sodium formate or, preferably, from ketene and formic acid. When various monoamino monocarboxylic acids are treated with acetic formic anhydride by a simple procedure and under mild conditions, N-formyl-amino acids were obtained in satisfactory yields.

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