Use of Ethoxyacetylene for the Synthesis of *N*-Protected Amino-acids

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Ethoxyacetylene has been used to form phthalimido- and substituted phthalimido-derivatives of amino-acids and their esters both in anhydrous and in aqueous solution. It was not found possible to make analogous maleimido-derivatives in this manner.

ETHOXYACETYLENE has been used to form peptide links without isolation of the intermediate 1-ethoxyvinyl esters (I; R = R'CO),[1] although a compound of this type, (I; R = phthalimidoacetyl), was isolated by Sheehan and Hlavka.[2] General methods developed to prepare 1-ethoxyvinyl esters [3,4] were of limited value when the phthaloyl protecting group was not present,[5]

$H_2C{=}C(OEt){\cdot}OR$ (I)

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[1] L. Heslinga and J. F. Arens, *Rec. Trav. chim.*, 1957, **76**, 982.

[2] J. C. Sheehan and J. Hlavka, *J. Org. Chem.*, 1958, **23**, 635.

[3] H. H. Wasserman and P. S. Wharton, *J. Amer. Chem. Soc.*, 1960, **82**, 661.

[4] H. H. Wasserman and D. Cohen, *J. Amer. Chem. Soc.*, 1960, **82**, 4435; *J. Org. Chem.*, 1964, **29**, 1817.

[5] G. Tadema, E. Harryvan, J. J. Panneman, and J. F. Arens, *Rec. Trav. chim.*, 1964, **83**, 345.

although when a large excess of ethoxyacetylene was used it was possible to isolate such a compound (I; R = gly-L-phe).[6] We have now investigated the action of ethoxyacetylene on 1,2-dicarboxylic acids alone and in the presence of amino-acids and their esters. Although it proved possible to isolate di-1-ethoxyvinyl terephthalate in high yield, all attempts to prepare the corresponding di-1-ethoxyvinyl phthalate resulted in the formation of phthalic anhydride. When phthalic acid reacts with ethoxyacetylene in the presence of amino-acids or amino-acid esters, the phthaloyl derivatives are obtained smoothly and in good yield. This reaction proceeds quite well in aqueous solution and promises to be not only a good preparative method for protected amino-acids but also a useful *N*-terminal labelling technique for peptide fragments.

In this manner we have synthesised *N*-phthaloylglycine and its ethyl ester, *N*-phthaloyl-L-phenylalanine methyl ester, and *N*-phthaloyl-DL-alanine ethyl ester.

Similarly, using 4-nitrophthalic acid, we obtained the *N*-4-nitrophthaloyl derivative of glycine methyl ester. This derivative was not sufficiently deeply coloured to act as an easily visible spot on paper chromatography. It seems clear, however, that suitably substituted phthalic acids could be used for this purpose.

In contrast with the behaviour of phthalic acid, maleic acid reacts with amines in the presence of ethoxyacetylene to yield the corresponding maleamic acid. This is in accord with the results of earlier workers,[7] who were unable to obtain substituted maleimides by cyclisation using standard methods.

EXPERIMENTAL

Ultraviolet spectra were measured for solutions in 95% ethanol on a Unicam S.P. 800 spectrophotometer, infrared spectra on Perkin-Elmer Infracord, 221, or 257G spectrometers, and n.m.r. spectra on a Perkin-Elmer 60 Mc./sec. spectrometer. M. p.s were determined on a Kofler hot-stage apparatus.

Ethoxyacetylene was prepared using the synthesis described by Nazarov, Krasnaia, and Vinogradov (modified by Cohen and Springall [6]).

Reaction of Terephthalic Acid with Ethoxyacetylene.—A suspension of anhydrous terephthalic acid (4·16 g., 0·03 mole) in dry methylene dichloride (50 ml.) was added during 30 min. to magnetically stirred ethoxyacetylene (14·14 g., 0·20 mole) and mercuric acetate (200 mg.) in tetrahydrofuran (50 ml.). After stirring for 2 days the suspension had disappeared, a greenish solution resulting. The excess of ethoxyacetylene and solvents was removed by evaporation under reduced pressure, and a brown crystalline compound remained. This was recrystallised three times from dry methylene dichloride–hexane, to yield *di-1-ethoxyvinyl terephthalate* (3·5 g., 50%), m. p. 82·5—83·5° (Found: C, 62·6; H, 6·1. $C_{16}H_{18}O_6$ requires C, 62·7; H, 5·9%), $\nu_{max.}$ 1740, 1675 cm.$^{-1}$ (1-ethoxyvinyl esters), n.m.r. τ 1·98 (singlet), 6·10 (complex splitting), 8·65 (triplet), integrating as 4 : 8 : 6.

The N-Protection of Amino-acids by Use of 1,2-Dicarboxylic Acid and Ethoxyacetylene.—(a) *Preparation of N-phthaloylglycine ethyl ester.* (i) In anhydrous solvent. Glycine ethyl ester hydrochloride was converted into the free base, to yield ethyl glycinate (2·23 g.). The glycine ester (2·23 g., 0·02 mole) was run into a cold (0°) solution of ethoxyacetylene (18·2 g., 0·26 mole) in dry methylene dichloride (50 ml.), and the solution stirred magnetically. Anhydrous phthalic acid (3·59 g., 0·02 mole) in tetrahydrofuran (30 ml.) was added to this solution during 1 hr., stirring being continued for a further 6 hr. at room temperature. After removal of solvents and excess of ethoxyacetylene, a brown gum remained from which transparent needles crystallised on trituration with aqueous ethanol. These were *N*-phthaloylglycine ethyl ester (4·08 g., 80%), m. p. 112·5—113·5° (lit., 111—113°) (Found: C, 62·0; H, 4·8; N, 5·7. Calc. for $C_{12}H_{11}NO_4$: C, 61·8; H, 4·8; N, 6·0%). The infrared spectrum was as expected, and the n.m.r. spectrum consisted of peaks at τ 2·17 (complex splitting), 5·57 (unsplit), 5·78 (quartet), 8·70 (triplet), integrating in the ratio 4 : 2 : 2 : 3.

(ii) In aqueous solution. Phthalic acid (1·66 g., 0·01 mole) and pyridine (2·3 g., 0·03 mole) in water (30 ml.) were allowed to drip into an aqueous solution of glycine ethyl ester hydrochloride (1·39 g., 0·01 mole) and ethoxyacetylene (5·42 g., 0·08 mole) cooled in an ice–water bath. The solution was stirred for 8 hr., and the needles which had formed were filtered off, washed with cold 95% ethanol, and dried. *N*-Phthaloylglycine ethyl ester, m. p. 114—114·5° (2·16 g., 93%), was identified by its infrared and n.m.r. spectra, and analysis.

(b) *Preparation of N-phthaloylglycine (in aqueous solution).* A solution of glycine (0·75 g., 0·01 mole) and phthalic acid (1·66 g., 0·01 mole) in pyridine (2 ml.) and water (15 ml.) was added during 1 hr. to rapidly stirred ethoxyacetylene (5·42 g., 0·08 mole); the reaction was markedly exothermic. After stirring for 6 hr., the two-phase solution was evaporated to a yellow oil which was triturated with absolute ethanol; a fine white solid was precipitated. This was filtered off and washed with ethanol, to give glycine (122 mg.), m. p. 258—260°, mixed m. p. 260—262°. Evaporation of the filtrate yielded a further crop (78 mg.). The filtrate was evaporated to dryness under reduced pressure, and phthalic anhydride (78 mg.) sublimed round the neck of the flask, m. p. 133—134°, mixed m. p. 132·5—134°. The solid residue in the flask was dissolved in a small amount of 95% ethanol and triturated with benzene–light petroleum; *N*-phthaloylglycine crystallised (1·1 g., 54%), m. p. 192—194° (lit., 192°) (Found: C, 58·2; H, 3·5; N, 7·1. Calc. for $C_{10}H_7NO_4$: C, 58·5; H, 3·4; N, 6·8%). The infrared and n.m.r. spectra were as expected.

(c) *Preparation of N-4-nitrophthaloylglycine methyl ester (in aqueous solution).* 4-Nitrophthalic acid (4·22 g., 0·02 mole) and pyridine (2·0 g., 0·03 mole) in water (20 ml.) were added during 1 hr. to glycine methyl ester hydrochloride (2·52 g., 0·02 mole) and ethoxyacetylene (9·6 g., 0·14 mole) in water (20 ml.), cooled in an ice–water bath. The mixture was stirred and allowed to warm to room temperature after the addition had been completed. After 8 hr. stirring a yellow oil had separated. Solvents were evaporated under reduced pressure, leaving an oily solid which yielded a pale yellow powder (5·0 g.), m. p. 121—125°, on trituration with methanol. The methanol solution was left at 0° for 2 days, after which a further 1·49 g. of the pale

[6] D. Cohen and H. D. Springall, "Fifth European Peptide Symposium," ed. G. T. Young, Pergamon, 1964, p. 73.

[7] F. E. King, J. W. Clark-Lewis, R. Wade, and W. A. Swindon, *J. Chem. Soc.*, 1957, 873.

yellow solid had been precipitated. A sample of this solid was recrystallised twice from methanol (charcoal), to give a very pale yellow solid, m. p. 124—125°, which was identified as N-4-*nitrophthaloylglycine methyl ester* (crude yield 6·59 g., 62%) (Found: C, 50·3; H, 2·9; N, 10·5. $C_{11}H_8N_2O_6$ requires C, 50·0; H, 3·05; N, 10·6%). The n.m.r. spectrum consisted of peaks at τ 1·23, 1·25, 1·78, 1·92 (complex splitting); 5·45 (unsplit) and 6·16 (unsplit) and integrated in the ratio 2·9 : 2·0 : 3·1. The infrared spectrum was as expected, and the ultraviolet spectrum in 95% ethanol showed $\lambda_{max.}$ 236 mμ (ε 1·89 × 10^4).

(d) *Preparation of* N-*phthaloyl*-L-*phenylalanine methyl ester* (*in aqueous solution*). Phthalic acid (1·26 g., 0·01 mole) in pyridine (2·0 g., 0·03 mole) and water (20 ml.) was allowed to drip during 30 min. into L-phenylalanine methyl ester hydrochloride (1·64 g., 0·01 mole), ethoxyacetylene (13·8 g., 0·20 mole), and water (5 ml.). The solution became quite hot; it was stirred for a further 2 hr., and evaporated *in vacuo* to a brown oil, from which N-phthaloyl-L-phenylalanine methyl ester crystallised on trituration with benzene–light petroleum (2·1 g., 84%), m. p. 113—114° (Found: C, 70·2; H, 5·1; N, 4·7. Calc. for $C_{18}H_{15}NO_4$: C, 69·9; H, 4·9; N, 4·5%).

(e) *Preparation of* N-*phthaloyl*-DL-*alanine ethyl ester* (*in aqueous solution*). Phthalic acid (0·83 g., 0·005 mole) in pyridine (1·0 g., 0·13 mole) and water (10 ml.) was allowed to drip during 1 hr. into a magnetically stirred solution of DL-alanine ethyl ester hydrochloride (0·77 g., 0·005 mole) and ethoxyacetylene (4·0 g., 0·06 mole) in water (5 ml.). The reaction was exothermic and the mixture was cooled in an ice-bath. After stirring overnight, the mixture was evaporated to a brown oil, extracted with ethyl acetate, and the extract reduced to an oil, which was triturated with ethyl acetate–light petroleum, to give a fine white precipitate. On recrystallisation from ethyl acetate–light petroleum, the product (0·73 g., 59%) had m. p. 65° (lit., 65, 61—63°) (Found: C, 63·3; H, 4·95; N, 5·4. Calc. for $C_{13}H_{13}NO_4$: C, 63·15; H, 5·3; N, 5·65%).

(f) *Reaction of aniline with maleic acid in the presence of ethoxyacetylene.* Ethoxyacetylene (3·20 g., 0·05 mole) was added to a homogeneous mixture of maleic acid (1·16 g., 0·01 mole) and redistilled aniline (1·00 g., 0·01 mole). After about 15 min. the mixture became warm and an oily yellow solid began to separate. Stirring was continued for 3 hr., and volatile components were removed by evaporation under reduced pressure. The yellow powder remaining was recrystallised three times from 95% ethanol and identified as maleanilic acid (1·70 g., 98%), m. p. 187—199° (lit., 187—187·5, 198° [7]) (Found: C, 62·9; H, 4·6; N, 7·3. Calc. for $C_{10}H_9NO_3$: C, 62·8; H, 4·8; N, 7·3%).

We are grateful to the S.R.C. and to Keele University for Studentships (to G. R. B. and J. A. G. T.).

[6/1062 *Received, August 22nd,* 1966]