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A New Procedure for the Pentachlorophenylation of N-Protected Amino Acids

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The pentachlorophenyl trichloroacetate and dichloroacetate which were known as a soil sterilizer or a plant growth regulator, were subjected to the ester-exchange reaction with trialkylammonium salts of acylamino acids, and it has been found that they are excellent reagents for the preparation of the pentachlorophenyl active esters of acylamino acids which are known as intermediates in peptide synthesis. Some acylpeptide esters were also synthesized by the direct method utilizing these reagent, thus, the peptide derivatives were obtained in excellent yields and satisfactory purities.

Recently Kovacs, *et al.*[2] reported an excellent method for the synthesis of polypeptides with known repeating sequence of amino acids by using pentachlorophenyl active esters. The pentachlorophenyl active ester method have many advantages, *e.g.* high reactivity of the esters, high melting point of the esters and easy availability of pentachlorophenol.

As regard to the preparation of the esters of N-protected amino acids, Kupryszewski, *et al.*[3] originally prepared the esters of carbobenzoxyglycine and leucine by the acid-chloride method, and Pless, *et al.*[4] prepared the ester of carbobenzoxyphenylalanine by the dicyclohexylcarbodiimide method.[5]

On the other hand, a new method for making various active esters of acylamino acids using the trifluoroacetate of the various phenols as a reagent was developed by Sakakibara, *et al.*[6] in 1965. In their report, a few pentachlorophenyl esters of carbobenzoxyamino acids were prepared by using pentachlorophenyl trifluoroacetate as an ester-exchange reagent in pyridine. This reagent is particularly interesting because of their high reactivity, and production of a water-soluble co-product. But this method has still some disadvantages, that is, unstability of the reagent and expensiveness of trifluoroacetic anhydride.

In the present study, it was found that pentachlorophenyl trichloroacetate (I)[7] and pentachlorophenyl dichloroacetate (II)[8] were quite useful reagents for ester exchange reactions. I and II were easily prepared as stable crystallines, which did not decompose even stored for several months at room temperature.

When I or II was subjected to the ester-exchange reaction with an acylamino acid in pyridine, the reaction was very slow at room temperature. However, they reacted smoothly and completely with trialkylammonium salts of acylamino acids within 2—5 min (I) or 10—20 min (II) in dimethylformamide at room temperature to give excellent yields of the desired esters. The results are summarized in Table I.

1) Location: *Juso, Higashiyodogawa-ku, Osaka.*
2) J. Kovacs, R. Giannotti, and A. Kapoor, *J. Am. Chem. Soc.*, **88**, 2282 (1966).
3) G. Kupryszewski, *Roczniki Chem.*, **35**, 1533 (1961).
4) J. Pless and R. A. Boissonnas, *Helv. Chim. Acta*, **46**, 1609 (1963).
5) M. Bodanszki and V. du Vigneaud, *J. Am. Chem. Soc.*, **81**, 5688 (1959).
6) S. Sakakibara and N. Inukai, *Bull. Chem. Soc. Japan*, **37**, 1231 (1964); *ibid.*, **38**, 1979 (1965).
7) F. L. Beman, U.S. Patent 2658071 (1953); G. Kupryszewski and W. Wojnowski, *Roczniki Chem.*, **36**, 359 (1962). This compound is a soil sterilizer and a plant growth regulator.
8) L. L. Baumgartner, U.S. Patent 2674527 (1954).

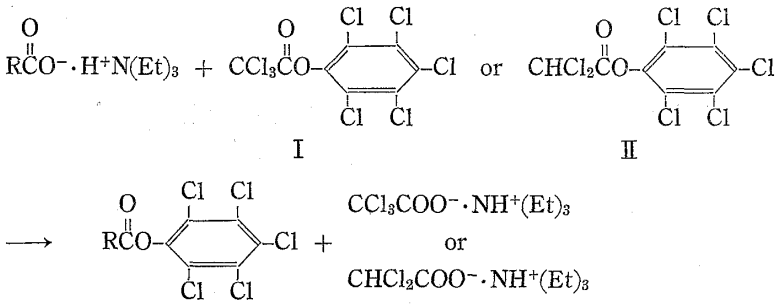

Table I. Preparation of Pentachlorophenyl Esters of Acylamino Acid by Ester-Exchange Reaction with Tri- and Di-chloroacetate Reagent

Acylamino acid	Reagent[a]	Reaction condition[b] (min)	Yield[c] (%)	mp (°C) Found/lit.	$[\alpha]_D$ $c=1$ in DMF Found/lit. (°C)	Analysis (%) (Found/Calcd.) C	H	N
Z-Ala-OH	I	3	82	175—178	−17.2 (22)	43.29	2.53	3.08
				170—171[2]	−25.1 (25)[h]	43.25	2.54	2.98
Z-Asp(OBzL) OH	I	5	80	126—132	−16.7 (22)	49.77	2.93	2.15
				155[2]		49.51	2.97	2.31
Z-Gly-OH	I	3	85	132—134		42.16	2.18	2.97
				133—134[6][d]		42.00	2.20	3.06
	II	15	84	132—134		42.15	2.15	3.11
Z-ILe-OH	I	5	74	122—123	−14.3 (22)	46.46	3.47	2.63
						46.59	3.52	2.72
Z-Len-OH	I	5	78	124—125	−21.3 (33)	46.61	3.45	2.79
				122—124[3]		46.59	3.52	2.72
	II	15	87	122—124	−21.5 (22)	46.50	3.34	2.65
di Z-Lys-OH	I	5	81	157—158	−11.8 (21)	50.82	3.70	4.22
				154—156[2]		50.73	3.80	4.23
Z-Phe-OH	I	3	83	156—157	−52.1 (22)	50.39	2.92	2.32
				158[4]	−49.4 (24)[6]	50.44	2.94	2.56
	I	N-Et-Pip.[e] 5	82	156—157	−52.0 (21)	50.36	2.74	2.47
	I	Pyridine[f] 5 hr	80	156—157	−52.7 (22)	50.81	2.93	2.36
	II	20	87	156—157	−52.1 (21)	50.19	2.67	2.53
Z-Val-OH	I	5	85	141—142	−22.7 (22)	45.76	3.07	2.59
				140—141[2]	−22.8 (24)[6]	45.67	3.23	2.80
Z-Gly-Gly-OH	I	5	82	168—170.5		42.08	2.56	5.38
						41.89	2.54	5.44
AOC-Try-OH	I	5	89	160—161.5	−55.7 (21)	49.03	3.74	4.89
						48.83	3.56	4.95
Bz-Len-OH	I	10	79	125.5—126.5[g]	0	47.36	3.48	3.01
				125—126[9]	−34.2[h] (26)	47.22	3.33	2.89

a) Reagent (I) is pentachlorophenyl trichloroacetate and (II) is pentachlorophenyl dichloroacetate.
b) The reactions were carried out at room temperature in dimethylformamide (DMF).
c) In all cases, crude yields were quantitative except for *e*) and *f*).
d) Kupryszewski[3] reported; mp 185.5—187.
e) N-Ethyl piperidine was used as the base in place of triethylamine in DMF.
f) The reaction was carried out in pyridine.
g) The product racemized during the reaction.
h) The optical rotation was determined in chloroform.

9) J. Kovacs, L. Kisfaludy, and M. Q. Ceprini, *J. Am. Chem. Soc.*, **89**, 183 (1967).

Table II. Synthesis of Dipeptide Derivatives by the Direct Method

Product	Reagent	Reaction period for peptide formation (hr)	Yield (%)	mp (°C) Found/Lit.	$[\alpha]_D$ in EtOH Found/Lit. (conc., °C)	Analysis (%) (Found/Calcd.) C	H	N
Z–Ala–Gly–OEt	I[a]	10	86	98—99 97.5—98[10]	−21.3 (1, 22) −24.4 (1, 20)[c]	58.55 58.43	6.58 6.54	9.05 9.09
Z–Gly–Gly–OBz	I	5	88	110 110[11]		64.02 64.03	5.53 5.66	7.90 7.86
Z–ILe–Gly–OEt	I	70	86	157—158 156—159[12]	−27.8 (2, 22) −27.0 (2, 20)[d]	61.83 61.70	7.45 7.48	7.96 8.00
Z–Leu–Gly–OEt	I	10	86	103.5—104.5 104—105[13]	−26.2 (5, 22) −26.4 (5, 24)	61.78 61.70	7.48 7.48	8.02 8.00
Z–Phe–Gly–OEt	I	10	83	111 111[13]	−16.8 (5, 22) −16.9 (5, 25)	65.85 65.61	6.27 6.29	7.01 7.29
	II[b]	10	86	111	−16.8 (5, 21)	65.68	6.25	7.13
Z–Val–Gly–OEt	II	70	73	166—167 166[14]	−25.8 (2, 21) −25.3 (1, 21)[15]	60.65 60.70	7.11 7.19	8.45 8.33

a) Pentachlorophenyl trichloroacetate.
b) Pentachlorophenyl dichloroacetate.
c) The same compound which was prepared in the laboratory by the *p*-nitophenyl ester method gave the following values; mp 98—99°, $[\alpha]_D^{21}$ −21.3°(*c*=1, EtOH).
d) The optical rotation was determined in methanol.

As shown in Table I, no racemization was observed during the reaction with urethane-type acylamino acids. But in the case of N-benzoyl-L-leucine, the resulting product was completely racemized. Therefore, the application of this method must be limited to the urethane-type acylamino acids or acylpeptide having glycine or proline as their C-terminus.

Incidentally, some carbobenzoxydipeptide esters were prepared by the direct method[6] without the isolation of the active ester as an intermediate for coupling with amino components. The resulting peptides were obtained in excellent yields and had satisfactory purities; the yields and physical constants of the peptides are listed in Table II.

Thus, the tri- or di-chloroacetate method should be useful not only for the preparation of pentachlorophenyl esters, but also for the stepwise synthesis of peptides in the same way as the trifluoroacetate method.[16]

Tri- and di-chloroacetate of various another phenols which are used commonly in peptide synthesis have been prepared in this laboratory. Studies of their reactivity with acylamino acids and acylpeptides will be published in subsequent papers.

Experimental[17]

Pentachlorophenyl Trichloroacetate (I)——To a solution of pentachlorophenol (266.3 g, 1.0 mole) and trichloroacetylchloride (182 g, 1.0 mole) in dry benzene (2 liter), dry pyridine (79.1 g, 1.0 mole) was added

10) M. Bergmann, L. Zervas, J. S. Fruton, F. Schneider, and H. Schleich, *J. Biol. Chem.*, **109**, 325 (1935).
11) D. Ben-Ishai, *J. Org. Chem.*, **19**, 62 (1954).
12) L. Bernardi. G. Bosisio, R. De Castiglione, O. Goffredo, and F. Chillemi, *Gazz. Chem. Ital.*, **94**, 853 (1964).
13) D. W. Clayton, J. A. Farrington, G. W. Kenner, and J. M. Turner, *J. Chem. Soc.*, **1957**, 1398.
14) W. Grassmann and E. Wünsch, *Chem. Ber.*, **91**, 449 (1956).
15) B. O. Handford, *J. Chem. Soc.*, **1965**, 6814.
16) S. Sakakibara and N. Inukai, *Bull. Chem. Soc. Japan*, **39**, 1567 (1966).
17) All melting points were taken by the capillary method and were uncorrected. Each reaction process checked by thin-layer chromatography[18] using Merck's silica gel G with chloroform: methanol : acetic acid (90:3:2 v/v).
18) K. Morita and F. Haruta, *J. Chromatog.*, **12**, 412 (1963).

dropwisely at room temperature and the reaction mixture was stirred for 2 hr. Then pyridine hydrochloride was removed by filtration, and the filtrate was concentrated to dryness under reduced pressure. The residual crystalline product was recrystallized from cyclohexane; prisms, 373 g (91%), mp 128—130° (lit.,[7] 130—130.5°). *Anal.* Calcd. for $C_8O_2Cl_8$: C, 23.30; H, 0.0; Cl, 68.93. Found : C, 23.59; H, 0.0; Cl, 67.19.

Pentachlorophenyl Dichloroacetate (II)——Pantachlorophenol (234.4 g, 0.88 mole) was allowed to react with dichloroacetylchloride (130 g, 0.88 mole) using pyridine (69.6 g, 0.88 mole) as described above, and the crude product was recrystallized from cyclohexane; Prisms, 310 g (93.3%), mp 86—88°. *Anal.* Calcd. for $C_8HO_2Cl_7$: C, 25.47; H, 0.27; Cl, 65.78. Found: C, 25.77; H, 0.23; Cl, 66.18.

Pentachlorophenyl Esters of Acylamino Acids; (A) Pentachlorophenyl Ester of Carbobenzoxyglycine(Trichloroacetate Method)——To a cooled solution of carbobenzoxylglycine (2.09 g, 0.01 mole) and 1.4 ml (0.01 mole) of triethylamine in 10 ml of dimethylformamide, (I) (4.53 g, 0.011 mole) was added with stirring at room temperature. After about 3 min, water (25 ml) was added to the reaction mixture with cooling. The formed crystals were collected by filtration and washed with water. The crude yield after drying were 4.53 g (99%), mp 127—129°. Recrystallization from ethanol gave needles, 3.09 g (85%), mp 132—134°.

Other pentachlorophenyl esters of acylamino acids and an acylpeptide which were listed in Table I, were prepared in a similar manner.

(B) Pentachlorophenyl Ester of Carbobenzoxyl-L-Phenylalanine (Dichloroacetate Method)——To a cooled solution of carbobenzoxy-L-phenylalanine (2.99 g, 0.01 mole) and triethylamine (1.4 ml, 0.01mole) in dimethyl formamide (10 ml) was added (II) (3.80 g, 0.01 mole) with cooling. After standing for 20 min at room temperature, water (15 ml) was added to the reaction mixture with cooling. The rapidly formed crystals were filtrated and washed with water, dried wt., 5.66 g (10%), mp 152—156°. Recrystallization from ethanol gave needles; 4.92 g (87%), mp 156—157°. $[\alpha]_D^{21}$ −52.1° (c=1, dimethylformamide).

Other pentachlorophenyl esters of carbobenzoxyamino acids were prepared in a similar manner (see Table I).

General Procedure for Synthesis of Carbobenzoxy-Dipeptide Esters——A solution of carbobenzoxyamino acid (0.01 mole) and triethylamine (0.01 mole) in dimethylformamide (10 ml) was treated with (I) or (II) (0.01 mole). After the reaction was completed, a solution of an amino acid ester hydrochloride or tosylate (0.01 mole) and triethylamine (0.01 mole) in dimethylformamide (10—15 ml) was added to the reaction mixture. The reaction mixture was allowed to react for 5—70 hr at room temperature, and then diluted with water(80 ml). The separated oil was extracted three times with ethyl acetate (40 ml each). The extract was washed successively with 5% aqueous sodium bicarbonate and N hydrochloric acid and then dried over anhydrous sodium sulfate. The dried solution was evaporated under reudced pressure, and the crystalline residue was recrystallized from ethyl acetate–petroleum ether. The data are given in Table II.

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