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DIRECT ACYLATION OF α-AMINO ACIDS AND OF α-HYDROXY ACID DERIVATIVES

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Received June 2, 1952

N-Chloroacetylated amino acids. The usual method used for the synthesis of these compounds is the Schotten-Baumann procedure using chloroacetyl chloride as the acylating agent. Though high yields have been reported by use of this method, experience has shown that yields vary considerably for each individual reaction. In fact, it was found that the method frequently failed to give any product, despite rigorous adherence to the detailed procedures available in the literature. Similar difficulties in the preparation of these compounds have recently been reported by Birnbaum, Levintow, Kingsley, and Greenstein (1). These authors resorted to the use of chloroacetic anhydride as the acylating agent in a Schotten-Baumann reaction type in the synthesis of the chloroacetylated derivatives of methionine, leucine, and lysine, and have found this reagent to be superior to the acid chloride in these cases. However, work in this laboratory on other amino acids, using chloroacetic anhydride in a Schotten-Baumann reaction type, proved unsuccessful.[3]

In the present work, undertaken to provide substrates for an investigation of the activity of beef, pancreatic carboxypeptidase,[3, 4] it was decided to find a more suitable method for the synthesis of N-chloroacetylated derivatives of amino acids. The approach resorted to was a direct acylation. The only other illustrations of this type of acylation, known to the author, are those of Knoop and Blanco (2) and of Feiser and Martin (3).

An advantage of the method is the lack of the necessity to watch or tend to the reaction mixture during the progress of the reaction. At the end of the heating period, the product, which is many-fold more soluble in the organic solvent than is the amino acid, is separated by filtration. The unreacted acid chloride in the filtrate can be drawn off together with the excess solvent, leaving nearly pure acylated amino acid products. These compounds are then subjected to final purification, generally by recrystallization from suitable solvents. Table I contains the list of the N-chloroacetylated amino acids synthesized by the direct method.

The direct method did not prove successful with all amino acids. Despite numerous attempts, the N-chloroacetyl derivatives of DL-serine, DL-threonine, DL- and L-glutamic acid, DL-tryptophan, β-alanine, L-asparagine, L-cystine, L-histidine, and *p*-aminobenzoic acid could not be obtained. At first hand, it appears that no racemization is caused by the procedure.

[1] This research was done during the tenure of a Life Insurance Medical Research Predoctoral Fellowship.

[2] Excerpted from the dissertation presented to the University of California in fulfillment of the requirements for the Degree of Doctor of Philosophy, 1952.

[3] This work was performed at approximately the same time as that of Birnbaum, *et al.* (1).

[4] The results of the biochemical research will be presented elsewhere.

N-Trichloroacetylated amino acids. As a result of the success obtained in the monochloroacetylation of amino acids, the direct method was resorted to in the

TABLE I

DATA ON N-CHLOROACETYLATED AMINO ACIDS SYNTHESIZED BY DIRECT ACYLATION

AMINO ACID MOIETY	YIELD, % (PURE PRODUCT)	M.P., °C.	LIT. M.P., °C.	REFLUX TIME (HOURS)	DESCRIPTION OF FILTRATE RESIDUE	SOLVENT USED FOR PURIFICATION[a]
DL-Valine[b]	24[c]	132	132 (4)	0.75	Col. crys.	Ethyl ether
DL-Aspartic[d] acid	58	150–151	149 (4)	12	Yellowish-brown oil	[e]
DL-Isoleucine	76[f]	111[g]	117 (4)	0.25	Col. crys.	Ethyl acetate
DL-Norvaline	56	102	101 (5)	2	White crys.	Washed with $CHCl_3$
DL-Norleucine	72	115–116	116 (5)	0.5	White crys.	Washed with $CHCl_3$
α-Amino-iso-butyric acid	100[h]	147	[i]	14	White crys.	Benzene
DL-Alanine	54	124–125	126 (1)	1	White crys.	Extracted with ethyl ether
Glycine	25	100	100 (6)	4[j]	Yellow oil	Ethyl ether
DL-Leucine	45	142	142 (7)	1[k]	White crys.	Ethyl acetate
L-Leucine	63	136	136 (7a)	0.75[l]	White crys.	Washed with $CHCl_3$-hexane
DL-Tyrosine	46	158	158 (8)	4[m]	Yellow oil	[e]
DL-Phenylalanine	30	114[n]	108–109 (9) 131 (10) 128 (6)	1	Yellow oil	[e]
L-Phenylalanine[o]	21	125	125 (1)	1.5	Yellow oil	[e]
DL-Methionine	48	92–93	92[p] (1)	3	White crys.	Ethyl acetate-acetone-ethanol

[a] Unless noted otherwise the solvent was employed in recrystallization procedures. [b] Attempts to synthesize the compound by the direct method using benzene and toluene as reaction solvents proved unfruitful. [c] Yield from Schotten-Baumann reaction, 25%. [d] An earlier attempt to use benzene as the reaction solvent was unsuccessful. [e] See Experimental section. [f] Yield from Schotten-Baumann reaction, 18%. [g] The same melting point was observed on this product from both reaction types despite several recrystallizations. This may be due to a contamination of the original DL-isoleucine preparation with some of the allo-form. [h] Based on that part of the amino acid which reacted. [i] *Anal.* Calc'd for $C_6H_{10}ClNO_3$: N, 7.9. Found: N, 7.8. [j] In the first attempt, a twenty-hour heating period was used, but this appeared to destroy the product. [k] The reaction using a three-hour reflux period failed to yield a product. [l] $[\alpha]_D^{22}$ −14.95° (7.04% in abs. alcohol); $[\alpha]_D^{22}$ −14.4° (7.1% in abs. alcohol) (7a). [m] The reaction appeared to proceed somewhat violently at the start, subsiding later. [n] As found by Snoke and Neurath (9), the melting point of this compound could not be altered after repeated recrystallizations. [o] $[\alpha]_D^{25}$ +44.8° (3.63% in 95% ethanol); $[\alpha]_D^{22}$ +51.8° (2.92% in alcohol) (11). [p] At the time of synthesis, the recently published work of Birnbaum, *et al.* (1) was not known to the author. *Anal.* Calc'd for $C_7H_{12}ClNO_3S$: N, 6.2. Found: N, 6.0.

preparation of N-trichloroacetylated amino acids. The compounds reported here do not appear to have been previously synthesized.

In general, the yields obtained with the direct method in the trichloroacetyla-

tions were lower than those obtained in the monochloroacetylations. This is in part attributable to the presence of a thermal decomposition of the trichloro-

TABLE II

DATA ON N-TRICHLOROACETYLATED AMINO ACIDS SYNTHESIZED BY DIRECT ACYLATION

AMINO ACID MOIETY	YIELD, % (PURE PRODUCT)	M.P., °C.	REFLUX TIME (HOURS)	DESCRIPTION OF FILTRATE RESIDUE	SOLVENT USED FOR PURIFICATION[a]	NITROGEN	
						Calc'd	Found
DL-Isoleucine	22	117	4.5	Yellow oil[b]	Ethyl ether	5.1	4.9
DL-Norleucine	35	118–119	1.5	Col. needles	CCl_4	5.1	4.9
L-Leucine[c, d]	13	118	18	Yellow oil[e]	Washed with $CHCl_3$	5.1	4.9
α-Amino-isobutyric acid	67[f]	180–181	5	White crys.	Ethyl ether	5.6	5.4
DL-Tyrosine	16	202–204	1	Yellow oil[g]	Acetone	4.3	4.3
DL-Aspartic acid	5	176–177	12	White crys.	Ethanol-ethyl ether	5.0	4.8
DL-Alanine	13	159	1.75	White crys.	Ethyl ether	6.0	6.0
DL-Valine	8	203–204 dec.	1.5	White crys.	Ethanol-ethyl ether	5.3	5.2
DL-Methionine	16	86–87	0.5	Yellow oil[h]	H_2O[i]	4.8	4.8

[a] Unless otherwise indicated the solvent was employed in recrystallization procedures. [b] The oil rapidly crystallized upon standing at −15°. [c] An attempt to use acetone as the solvent in the reaction mixture proved unfruitful. [d] $[\alpha]_D^{26.5}$ −14.45° (1.91% in 95% ethanol). [e] The oil spontaneously crystallized by standing in a vacuum desiccator for an hour. [f] Based on that part of the amino acid which reacted. [g] The oil was mixed with a small quantity of ethyl ether and crystallized from the mixture after a night in the refrigerator. [h] The oil formed a highly viscous, clear mass after a day at −15°. Addition of 1 ml. of petroleum ether accompanied by scratching caused the mass to crystallize to a white solid. [i] The precipitate was a white, viscous mass at first: however, after two days in the refrigerator the compound took the form of small, colorless needles.

acetyl chloride (I), during the reflux period, into carbon tetrachloride (II) and carbon monoxide (III)(12).

$$\underset{\text{I}}{CCl_3\overset{\overset{O}{\|}}{C}Cl} \longrightarrow \underset{\text{II}}{CCl_4} + \underset{\text{III}}{CO}$$

The L-glutamic acid derivative of this series could not be prepared by this method. A probable explanation lies in the tendency for the glutamic acid to form the pyrrolidine ring upon heating. Also, the trichloroacetyl derivatives of L-phenylalanine and DL-tryptophan could not be made.

Table II contains a compendium of the trichloroacetyl derivatives of amino acids which were synthesized.

N-Hippurylated amino acids and O-hippurylated hydroxy acid derivatives. Several amino acid representatives from this series have been previously prepared (9, 13). The method used in these syntheses was that originated by Curtius (13) which is basically a Schotten-Baumann reaction using the acyl azide as the acylat-

ing agent. The method frequently gives good yields: however, several steps are involved in the preparation of the acyl azide.

In view of the ease with which "hippuryl chloride"[5] can be prepared (14) and the success obtained in the synthesis of mono- and tri-chloroacetyl derivatives of amino acids, the direct method of acylation appeared to be most attractive.

Five amino acid derivatives of this series were prepared during the course of this research. With the exception of hippuryl-DL-valine, the synthesis of these compounds had not been previously reported in the literature. However, it ap-

TABLE III

DATA ON THE SYNTHESIS OF N-HIPPURYL AMINO ACIDS AND O-HIPPURYL HYDROXY-ACID DERIVATIVES BY DIRECT ACYLATION

AMINO ACID MOIETY OR RELATED ALCOHOLS	YIELD, % (PURE PRODUCT)	M.P., °C.	REFLUX TIME (HOURS)	DESCRIPTION OF FILTRATE RESIDUE	SOLVENT USED FOR PURIFICATION[a]	NITROGEN	
						Calc'd	Found
DL-Methionine	38	171–172	2	Yellow oil[b]	Washed with acetone	9.1	9.1
DL-Valine	7	144[c]	12	Yellow oil[d]	Water	10.0	10.0
DL-Norleucine	38	194	2	White crys.	Washed with ethyl ether	9.6	9.6
DL-Leucine	34	155	3	White crys.	Ethanol-water	9.6	9.5
L-Tyrosine[e]	26	198–199	12	Yellow oil	Toluene[f]	8.2	8.2
dl-Lactamide	27	168–169	1.5	Reddish oil[g]	Ethanol	11.2	11.2
Ethyl *dl*-lactate	20[h]	106–109[i]	12[j]	White crys.	Toluene-washed with CCl_4	5.0	5.0

[a] Unless otherwise indicated the solvent was employed in recrystallization procedures. [b] The oil crystallized after standing overnight at −15°. [c] Ref. 15 gives m.p. 135–136°. [d] Addition of ethyl ether caused the oil to crystallize. [e] $[\alpha]_D^{20}$ +51.8° (1.76% in 95% ethanol). [f] An attempted recrystallization of a portion of the product from water was unsuccessful. [g] The oil crystallized upon standing at −15°. [h] Botvinnik and Severin (16) reported a 19% yield. These authors refluxed the reactants in absolute ether for 10 hours. See footnote *i* of this table. [i] Ref. 16 gives m.p. 106°. [j] The reactants were shaken at room temperature in a tightly stoppered flask. Benzene (60 ml.) was used as the reaction solvent.

pears that their preparation, by another method,[6] had been successfully performed, at approximately the same time, at the laboratories of Fox. An earlier synthesis of hippuryl-DL-valine by the benzoylation of glycyl-DL-valine was reported by Abderhalden, *et al.* (15).

Efforts to prepare the N-hippuryl derivatives of DL-phenylalanine, DL-isoleucine, and DL-aspartic acid by direct acylation proved unfruitful.

Attempts to prepare O-hippuryl derivatives of mandelic and malic acids failed to yield the desired products. However, the O-hippuryl derivatives of ethyl

[5] The author is aware of the azlactone hydrochloride structure of this compound.

[6] Private communication from Dr. Sidney W. Fox, Professor of Chemistry, Iowa State College, Ames, Iowa. The valine compound was prepared in the same manner.

lactate and lactamide were synthesized. The latter has not been previously reported in the literature.

In the few cases where comparisons are available, the range of yields, using the direct method of acylation, was higher in the hippuryl group than in the trichloroacetyl series, but lower than in the chloroacetyl group.

Table III contains a description and appropriate comments on the hippuryl derivatives that were prepared.

EXPERIMENTAL

Acylating agents. Chloroacetyl chloride and trichloroacetyl chloride were prepared by the method of Brown (17). "Hippuryl chloride" was synthesized as described by Fischer (14). Attempts to prepare hippuryl chloride using CCl_4 as the solvent, as directed by Botvinnik and Severin (16), were unsuccessful.

Lactic acid derivatives. Ethyl *dl*-lactate was prepared according to the directions of Filachione, *et al.* (18, 19) and 96 ml. (41%, based on the original quantity of lactic acid employed) of the product, b.p. 63° (24 mm.) was obtained. *dl*-Lactamide was prepared by treatment of ethyl *dl*-lactate with NH_3 and 45 g. (80%) of the product, m.p. 76–77° (Lit. 74°) (20), resulted.

General procedure in direct acylation. A suitable quantity of amino acid or α-hydroxy acid derivative (usually 1 to 5 g.) was suspended in 50 to 100 ml. of ethyl acetate and refluxed under anhydrous conditions with 1 to 2 equivalents of acylating agent for varying periods of time as indicated in Tables I, II, and III. At the end of the heating period, the unreacted amino acid residues were filtered off. These residues can be used again. Then the excess solvent (and those acid chlorides which are volatile) was removed from the filtrate by the aid of a current of air. This usually left a solid or readily crystallizing oil. The solids were then recrystallized from suitable solvents (Tables I, II, and III).

In the following are offered pertinent comments concerning variations in individual procedures:

*N-Chloroacetyl-*DL*-aspartic acid.* The filtrate residue was swirled in 20 ml. of petroleum ether and put into the refrigerator. After three days the oil had completely crystallized. It was washed with ethyl ether and filtered off as a pure product.

*N-Chloroacetyl-*DL*-tyrosine.* The filtrate residue was shaken with 10 ml. of petroleum ether and the mixture was placed in the refrigerator. After three days, the oil became whitish, but gummy. Recrystallization from ethyl ether yielded a pure product.

*N-Chloroacetyl-*DL*-phenylalanine.* The filtrate residue was washed out with 10 ml. of ethyl ether and soon crystallized spontaneously to colorless needles. The product was recrystallized from ethyl acetate and then from water. After two more recrystallizations from ethyl alcohol, the melting point of the compound was still not raised, see Table I.

Anal. Calc'd for $C_{11}H_{12}ClNO_3$: N, 5.8. Found: N, 5.8.

*N-Chloroacetyl-*L*-phenylalanine.* The oily residue from the filtrate was mixed with 10 ml. of petroleum ether and, after 10 minutes in the deep freeze, it crystallized spontaneously.

Acknowledgement. The author wishes to express his gratitude to Dr. David M. Greenberg for his support of this work.

SUMMARY

Difficulties frequently incurred in the synthesis of N-chloroacetylated amino acids by the Schotten-Baumann procedure are eliminated by the use of a direct method of acylation. The direct method is simpler and requires little effort. Further, the procedure appears to respect the optical purity of the product. Fourteen N-chloroacetylated amino acids were prepared in this way.

The direct method of acylation has also been employed to synthesize nine N-trichloroacetylated amino acids which have not been previously reported in the literature. In addition, five N-hippuryl derivatives of amino acids, four of which are new, and the O-hippuryl derivatives of ethyl *dl*-lactate and *dl*-lactamide are reported.

The method is not applicable to all amino acids.

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