[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, IOWA STATE COLLEGE]

DIRECT ACYLATION OF α-AMINO ACIDS AND DIPEPTIDES

EDWARD RONWIN[1,2]

Received April 30, 1953

This work was undertaken to explore further the utility of the recently reported direct method of acylation of amino acids and allied compounds (1) and to provide substrates for a continuing investigation of the activity of beef pancreatic carboxypeptidase.

The direct method consists essentially of the acylation of an amino acid or allied compound with an acid chloride, in the absence of added base, by refluxing for various time periods in a suitable anhydrous solvent. As a result of the high insolubility of amino acids in organic solvents, only the reacted amino acid enters the solution during the reaction. At the end of the reflux period, the unreacted amino acid is removed by filtration. Removal of the filtrate solvent yields the crude product, which is generally purified by recrystallization procedures. This method was resorted to because of previously discussed difficulties (1) encountered in the chloroacetylation of amino acids by the Schotten-Baumann method.

N-Dichloroacetylated amino acids. Table I contains a listing of these amino acid derivatives. Despite the decomposition of the acylating agent to carbon monoxide and chloroform (2, 3) (analogous to the decomposition of trichloroacetyl chloride to carbon monoxide and carbon tetrachloride), the yields in this series approach those previously reported for monochloroacetylations and are much higher than those previously observed in trichloroacetylation. Except for the glycine and phenylserine derivatives, these compounds have not been hitherto described in the literature.

N-Chloroacetylated amino acids. Several new amino acid representatives of this group are listed in Table II in addition to others previously prepared by different methods.

N-Cinnamoylated amino acids. The satisfactory use of a solid acylating agent in the direct method had been described in the previous communication (1); however, the particular agent employed (hippuryl chloride) possesses an azlactone structure. It became of interest, therefore, to employ a solid acylating agent of true acid chloride structure. This gave rise to the series of compounds listed in Table III. Except for the glycine and phenylalanine derivatives, these compounds have not been previously described.

The very low yields in this group are more probably attributable to the poor quality of the acylating agent (the practical grade of Eastman Kodak's cinnamoyl chloride was employed without expenditure of purification efforts), rather than to a low level of reaction between the acid chloride and the amino acids. It is interesting and surprising that, whereas in the previous and present

[1] Fellow of the National Foundation for Infantile Paralysis.

[2] Journal paper No. J-2304 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 1111.

descriptions of the reactions between tyrosine and leucine with various acylating agents good yields of product and smooth, rapid reactions were noted, the cinnamoyl derivatives of the L-forms of these two acids could not be prepared by the direct method.

N-Phenoxyacetylated amino acids. Table IV presents a compendium of pertinent data on a new series of N-phenoxyacetyl derivatives of amino acids. The yields

TABLE I

N-DICHLOROACETYLATED AMINO ACIDS

AMINO ACID MOIETY	M.P., °C. corr.	YIELD, %	REFLUX TIME (hr.)	DESCRIPTION OF FILTRATE RESIDUE	SOLVENT USED FOR PURIFICATION[a]	NITROGEN	
						Calc'd	Found
DL-Norleucine	125	26	2	Col. crys.	Ethanol-cyclohexane washed with CCl_4	5.8	5.8
L-Leucine[b]	122	28	2	Col. crys.	Ethyl acetate-hexane	5.8	5.9
L-Tyrosine[c]	173	59	1.5	Creamy powder	Acetone-hexane	4.8	4.8
DL-Isoleucine	150	43	2.5	Col. plates	Acetone-hexane washed with $CHCl_3$	5.8	5.7
DL-α-Amino-*n*-butyric acid	117	29	2.5	White crys.	Acetone-hexane	6.5	6.5
p-Methoxy-L-phenylalanine[d]	110	41	1.5	White needles	Acetone-hexane	4.6	4.6
DL-Phenylglycine	159	29	3	White crys.	Ethyl ether-CCl_4	5.3	5.2
DL-Phenylserine	164[e]	33	3	White crys.	H_2O	4.8	4.7
DL-Valine	155	47	3	Col. crys.	Washed with ethyl ether	6.1	6.2
DL-Alanine	164	42	2.5	White crys.	Washed with ethyl ether	7.0	7.0
DL-Methionine	125	37	1.5	White crys.	Acetone-ethyl ether	5.4	5.3
α-Aminoisobutyric acid	230	10	2.5	White crys.	Washed with toluene-hexane	6.5	6.3
Glycine	124[f]	20	2.5	Col. crys.	Acetone-ethyl ether-CCl_4	7.5	7.5
3,5-Diiodo-L-tyrosine[g]	205	42	2.5	White crys.	Acetone-hexane washed with $HCCl_3$	2.6	2.5
DL-Phenylalanine	140	30	2.5	White crys.	Acetone-ethyl ether	5.1	5.2
DL-Norvaline	103	15	2	White crys.	Xylene-ethyl ether-hexane	6.1	6.0

[a] Unless otherwise indicated the solvent was employed in recrystallization procedures. [b] $[\alpha]_D^{25}$ −20.7° (2.02% in 95% ethanol). [c] $[\alpha]_D^{25}$ +58.4° (2.00% in 95% ethanol). [d] $[\alpha]_D^{25}$ +58.7° (2.06% in 95% ethanol). [e] M.p. 164° (4); m.p. 170° (5). [f] M.p. 125–126° (6). [g] $[\alpha]_D^{25}$ +45.8° (1.97% in 95% ethanol).

in this group are excellent and exceed those found with the other acid chlorides thus far employed. This acid chloride is a high-boiling oil, and, as with benzoyl chloride, is not removed by taking the ethyl acetate filtrate to dryness with the aid of a current of air. It is, however, easily separated from the product during the recrystallization process.

N-Benzoylated amino acids. The representatives of this group that were prepared by direct acylation are presented in Table V.

In the previous communication (1) it had been demonstrated that the optical activity of the product was conserved by the method. Though no literature studies are available, the magnitude and direction of the specific rotations of the optically active dichloroacetyl, chloroacetyl, and phenoxyacetyl derivatives reported here would indicate that in these series optical activity is also preserved. A different situation is present in direct benzoylation. It will be noted in the footnotes to Table V that the rotation of *N*-benzoyl-*p*-methoxy-"D"-phenylalanine is almost equal and *opposite* to that quoted for the L-form by two

TABLE II

N-CHLOROACETYLATED AMINO ACIDS

AMINO ACID MOIETY	M.P., °C. corr.	YIELD, %	REFLUX TIME (hr.)	DESCRIPTION OF FILTRATE RESIDUE	SOLVENT USED FOR PURIFICATION[a]	NITROGEN	
						Calc'd	Found
DL-Phenylglycine	124	30	1.5	Yellowish solid	Acetone-hexane	6.2	6.4
L-3-Nitro-4-hydroxy-phenylalanine[b]	165	41	2	Yellow crys.	Acetone-hexane	9.2	9.3
DL-Phenylserine	167[c]	23	1.5	Yellow oil[d]	H_2O	5.4	5.2
α-Methyl-α-amino-DL-*n*-butyric acid	159	30	12	White crys.	CCl_4-$CHCl_3$	7.2	7.3
DL-α-Amino-*n*-butyric acid	126[e]	24	6	Yellow oil[f]	Ethyl ether		
3,5-Diiodo-L-tyrosine[g]	221d.[h]	85	1.25	White crys.	EtOH-H_2O		
p-Methoxy-L-phenyl-alanine[i]	125	43	1.5	White needles	Ethyl ether-hexane	5.2	5.1
L-Tyrosine[j]	155–156[k]	60	0.5	Yellow oil[l]	$HCCl_3$-acetone washed with ethyl ether		

[a] Unless otherwise indicated the solvent was employed in recrystallization procedures. [b] $[\alpha]_D^{23.5}$ +49.2° (2.00% in 95% ethanol). [c] M.p. 167° (7); m.p. 155–157° (8). [d] The oil crystallized upon treatment with hexane. [e] M.p. 128° (9). [f] The oil crystallized upon treatment with ethyl ether. [g] $[\alpha]_D^{25.5}$ +37.9° (2.00% in 95% ethanol). [h] M.p. 221°d. (10). [i] $[\alpha]_D^{23.5}$ +56.9° (1.98% in 95% ethanol). [j] $[\alpha]_D^{26}$ +59.2° (1.99% in 95% ethanol). [k] M.p. 155–156° (11). [l] The oil crystallized by stirring in a mixture of CCl_4-hexane.

different sources (14, 18); however, the compound was synthesized by the direct benzoylation of pure *p*-methoxy-L-phenylalanine. Apparently, then, an inversion, judging from the magnitude of the optical activity, of almost 85% had occurred as a result of the reaction. It is also to be noted that benzoyl-"DL"-leucine and benzoyl-"DL"-phenylalanine, which were prepared by the direct benzoylation of the L-forms of the amino acids, are pure racemic products, as judged by nitrogen analysis and melting point; however, these products are optically impure to the extent that they contain 23% and 19%, respectively, of the L-isomers, as judged by the specific rotation shown by these products (coupled with the assumption that the effect of the concentration of the solute upon the specific rotation is negligible with these compounds). In these cases, then, benzoylation had resulted

in partial racemization. The benzoylated γ-methylglutamic acid ester has been listed as the L-form since no previous synthesis of either the racemate or the optical isomers are reported for this compound and, further, the pure L-form of the amino acid was employed. However, as a result of the effects discussed above, it seems doubtful that this represents the pure L-form of the benzoylated amino acid. Where DL-pairs of amino acids are used, as in the case of DL-aspartic acid and DL-α-amino-*n*-butyric acid (Table V), the resultant product is the pure benzoylated racemate.

TABLE III

N-CINNAMOYLATED AMINO ACIDS

AMINO ACID MOIETY	M.P., °C. corr.	YIELD, %	REFLUX TIME (hr.)	DESCRIPTION OF FILTRATE RESIDUE	SOLVENT USED FOR PURIFICATION[a]	NITROGEN	
						Calc'd	Found
DL-Norleucine	169	15	3	White crys.	Acetone-hexane	5.3	5.3
DL-Isoleucine	160	17	3	White crys.	Acetone-hexane	5.3	5.4
p-Methoxy-L-phenylalanine[b]	177	14	2	Yellowish crys.	Acetone-hexane washed in ethyl ether	4.3	4.3
DL-Phenylglycine	176	21	2.5	Yellowish crys.	Acetone-hexane washed with ethyl ether	5.0	4.9
DL-Phenylserine	168–170	16	2.5	White crys.	Acetone-hexane	4.5	4.4
DL-Valine	188	16	3	White crys.	Acetone	5.6	5.7
Glycine[c]	193[d]	5	1.5	White crys.	H_2O-ethanol		
DL-Alanine	191	20	1.5	White crys.	Acetone-hexane	6.4	6.4
DL-Methionine	171	36	1.5	White crys.	Acetone-hexane	5.0	5.0
DL-Phenylalanine	199[e]	25	1.5	White crys.	Acetone-hexane		
DL-Norvaline	168	9	3	Yellow crys.	Acetone-hexane washed with $HCCl_3$-ethyl ether	5.6	5.6

[a] Unless otherwise indicated the solvent was employed in recrystallization procedures. [b] $[\alpha]_D^{25}$ −18.2° (2.01% in 95% ethanol). [c] This compound was also prepared, but only in 3% yield, by the fusion of cinnamic anhydride and glycine at 150° for 15 minutes. [d] M.p. 193° (12). [e] M.p. 198–199° (13).

It may be concluded that benzoylation by the direct method does not conserve the optical purity of the product under the conditions employed. It is suggested that the substitution of mechanical shaking at room or lower temperatures for the refluxing periods employed in the present procedure and, possibly, the use of other reaction solvents may eliminate the undesirable optical effects.

The tendency of benzoylated amino acids to undergo racemization is well known (19). However, it is believed that the type of inversion reported here has not been previously described.

Acylated dipeptides. The three acylated glycyl dipeptides that were prepared are described in Table VI. Their successful synthesis in fair yield by the direct method suggests that other dipeptides, higher peptides and possibly, too, proteins may undergo this type of acylation.

TABLE IV

N-PHENOXYACETYLATED AMINO ACIDS

AMINO ACID MOIETY	M.P., °C. corr.	YIELD, %	REFLUX TIME (hrs.)	DESCRIPTION OF FILTRATE RESIDUE	SOLVENT USED FOR PURIFICATION[a]	NITROGEN	
						Calc'd	Found
L-Tyrosine[b]	92–93	58	2.5	Yellow oil	Washed with xylene-ethyl ether[c]	4.4	4.1
L-Leucine[d]	108–109	82	2.5	Col. crys.	Ethanol (40%)	5.3	5.5
DL-Phenylalanine	134	74	2.5	White crys.	Ethanol (40%)	4.7	4.6
DL-Methionine	97	49	2.5	Yellow oil[e]	Acetone-hexane-ethyl ether	4.9	4.8
DL-Isoleucine	88	62	0.66	White crys.	Ethanol (40%)	5.3	5.4
DL-Valine	112	72	2.5	White crys.	Acetone-hexane	5.6	5.5
DL-Alanine	166	40	2.5	White crys.	Acetone-ethyl ether	6.3	6.2

[a] Unless otherwise indicated the solvent was employed in recrystallization procedures. [b] $[\alpha]_D^{25}$ +22.0° (2.00% in 95% ethanol). [c] This compound is extremely resistant to crystallization. *It oils out of the crystallization solutions and does not crystallize until* all the solvent evaporates and then only after several days of standing accompanied by considerable scratching. [d] $[\alpha]_D^{23.5}$ −2.68° (1.73% in 95% ethanol). [e] Crystallizes readily on scratching in ethyl ether.

TABLE V

N-BENZOYLATED AMINO ACIDS

AMINO ACID MOIETY	M.P., °C, (corr.)	LIT.		YIELD, % (Pure product)	REFLUX TIME (hours)	DESCRIPTION OF FILTRATE RESIDUE	SOLVENT USED FOR PURIFICATION[a]
		m.p., °C.	Ref.				
p-Methoxy-"D"-phenylalanine[b,c]	135	136–137	(14)	16	3	Yellow oil[d]	Acetone-hexane
DL-α-Amino-*n*-butyric acid[e]	144–145	145–146	(15)	33	4.5	White crys.	H_2O
DL-Aspartic acid[f]	172	165	(16)	19	5	White crys.	Acetone-hexane
γ-Methyl-"L"-glutamic acid ester[g,h]	107			24	5	Yellow oil[i]	Acetone-hexane, washed with ethyl ether
"DL"-Lucine[j,k]	141	137–141	(17)	33	2.5	Col. oil	Acetone-CCl_4
"DL"-Phenylalanine[l,m]	185	187–188	(15)	34	1.5	White crys.	H_2O-ethanol

[a] Unless otherwise indicated the solvent was employed in recrystallization procedures. [b] $[\alpha]_D^{23.5}$ +3.14° (both 2.04% and 1.5% in 95% ethanol); For the L-form: $[\alpha]_D^{20}$ −3.7° (1.5% in alcohol) (14); $[\alpha]_D^{20}$ −3.8° (4.0% in ethanol) (18). [c] *Anal.* Calc'd for $C_{17}H_{17}NO_4$: N, 4.7. Found: N, 4.6. [d] Crystallizes by scratching in ethyl ether. [e] No rotation observed (7.94% soln. in H_2O cont'g 1 equiv. of NaOH). For values on optically active isomers see ref. 15. [f] No rotation observed (8.7% soln. in H_2O cont'g 2 equiv. of KOH). For values on optically active isomers see ref. 16. [g] $[\alpha]_D^{23.5}$ −7.15° (2.01% in 95% ethanol). [h] *Anal.* Calc'd for $C_{13}H_{15}NO_5$: N, 5.3. Found: N, 5.5. [i] Crystallizes spontaneously after several hours in the refrigerator. [j] $[\alpha]_D^{23}$ +1.25° (8.7% in 0.5 *N* KOH); For the L-form: $[\alpha]_D^{20}$ +6.59° (8.8% in 0.5 *N* KOH) (17). [k] *Anal.* Calc'd for $C_{13}H_{17}NO_3$: N, 5.9. Found: N, 5.9. [l] $[\alpha]_D^{23}$ +3.96° (6.3% in 0.25 *N* KOH); for the D-form: $[\alpha]_D^{20}$ −17.1° (6.35% in 0.25 *N* KOH) (15). [m] *Anal.* Calc'd for $C_{16}H_{15}NO_3$: N, 5.2. Found: N, 5.1.

Unsuccessful attempts. As expected from previous observations (1), L-asparagine and L-glutamic acid failed to react with all acylating agents employed here. With the exception of the successful synthesis of N-benzoyl-γ-methyl-"L"-glutamic acid ester, the γ-methyl and γ-ethyl esters of L-glutamic acid failed to react with mono- and di-chloroacetyl chloride, benzoyl chloride, and cinnamoyl chloride. In addition, L-citrulline and L-aspartic acid did not yield the desired crystalline products with dichloroacetyl chloride.

General comments. The results obtained with phenoxyacetyl chloride and benzoyl chloride demonstrate the feasibility of using the direct method for preparing derivatives of high-boiling acid chlorides. However, it was found that all attempts to prepare N-acetylated amino acids using acetyl chloride were unsuccessful. Apparently, this acid chloride, which boils some twenty degrees below

TABLE VI

ACYLATED DIPEPTIDES

COMPOUND	M.P., °C. corr.	LIT.		YIELD, %	REFLUX TIME (hrs.)	DESCRIPTION OF FILTRATE RESIDUE	SOLVENT USED FOR PURIFICATION[a]
		m.p., °C.	Ref.				
N-Phenoxyacetylglycyl-DL-phenylalanine[b]	175			31	1.25	White crys.	Ethyl acetate
N-Benzoylglycyl-DL-phenylalanine	172	172	(20)	34	1.25	White crys.	Acetone-H_2O
N-Benzoylglycyl-DL-valine	143	144	(1)	25	2	Yellow oil[c]	Acetone-hexane washed with CCl_4

[a] Unless otherwise indicated, the solvent was employed in recrystallization procedures [b] *Anal.* Calc'd for $C_{19}H_{20}N_2O_5$: N, 7.9. Found: N, 8.0. [c] Crystallizes by scratching in acetone-hexane.

ethyl acetate, is removed before any reaction can take place. Consequently, the boiling point of the reaction solvent sets a lower limit on the liquid acid chlorides which can be used.

Attention is drawn to the fact that acylations of compounds, such as tyrosine or phenylserine, which possess more than one group capable of being acylated, yield in all cases thus far examined only the N-acyl derivative.

It will be noted that in many cases considerably greater than 50% yields (based on the starting quantity of amino acid) result. It might be thought that half of the amino acid present would act as a base by combining with the HCl released from the reacted portion of the amino acid, thereby setting an upper limit of 50% on yields. However, during the reaction, the effusion of HCl gas is frequently noted, as attested by its reaction to litmus paper and smell. Apparently, then, the formation of the amino acid hydrochlorides, while known to readily occur in this solvent at lower temperatures (unpublished results of the author), is not greatly favored at the reflux temperature, which is high enough to expel some or all of the HCl gas formed during the reaction thereby permitting greater than 50% yields.

EXPERIMENTAL

Acylating agents. The cinnamoyl chloride, phenoxyacetyl chloride, and chloroacetyl chloride were products of the Eastman Kodak Co. and were employed without further purification. The benzoyl chloride was a product of Merck and Co. Dichloroacetyl chloride was prepared in 56% yield by the method of Brown (3).

Amino acids and dipeptides. Glycyl-DL-valine was synthesized by the treatment of chloroacetyl-DL-valine with conc'd aq. ammonia. It was obtained in 71% yield, m.p. 237° (Lit. 240°) (6).

γ-Methyl-L-glutamic acid ester was synthesized in 60% yield as described by Hanby, Waley, and Watson (21), m.p. 182° dec. (Lit. 182° dec.).

Glycyl-DL-phenylalanine, *p*-methoxy-L-phenylalanine, DL-phenylserine,[3] and L-3-nitro-4-hydroxyphenylalanine were available in pure condition at this laboratory. All the other amino acids were commercial products.

Acylation procedure. The method employed is essentially that previously described by Ronwin (1). Caution to maintain anhydrous conditions is urged, since the presence of water yields only the HCl salts of the corresponding amino acids as products. In recrystallization, as for example from acetone-hexane, the compound was dissolved in the acetone, filtered and the volume of the solution was reduced; then the hexane was filtered into the solution until the appearance of turbidity.

Acknowledgement. The author wishes to express his gratitude to Dr. Sidney W. Fox for his interest and support of this work and for his criticism of this paper. Thanks are due to Miss Onnalee Underwood for her nitrogen analyses by the Kjeldahl method.

SUMMARY

The scope of the direct method of acylation of amino acids and allied compounds has been extended to include acylations with dichloroacetyl chloride, benzoyl chloride, cinnamoyl chloride, and phenoxyacetyl chloride. The failure of acetyl chloride to react is noted indicating that, to be useful in this method, liquid acid chlorides should boil higher than the reaction solvent. Glycyl dipeptides also permit acylation in this manner. It has been found that the direct benzoylation of optically pure isomers of amino acids results either in partial racemization or in inversion. The necessity of anhydrous conditions and the purity of the acid chlorides for maximum yields is stressed. Some 36 new compounds are characterized.

AMES, IOWA

REFERENCES

(1) RONWIN, *J. Org. Chem.*, **18,** 127 (1953).
(2) BOESEKEN, *Rec. trav. chim.*, **29,** 85 (1910).
(3) BROWN, *J. Am. Chem. Soc.*, **60,** 1326 (1938).
(4) WOOLLEY, *J. Biol. Chem.*, **185,** 293 (1950).
(5) BILLET, *Compt. rend.* **231,** 295 (1950).
(6) ABDERHALDEN, RINDTORFF, AND SCHMITZ, *Fermentforschung,* **10,** 220 (1929).
(7) BERGMANN, SCHMITT, AND MIEKELEY, *Z. physiol. Chem.*, **187,** 264 (1930).
(8) ABDERHALDEN AND BUADZE, *Fermentforschung,* **8,** 487 (1926).
(9) GREENSTEIN, GILBERT, AND FODOR, *J. Biol. Chem.*, **182,** 451 (1950).
(10) ABDERHALDEN AND GUGGENHEIM, *Ber.*, **41,** 1237 (1908).

[3] As used throughout this paper, this term refers to the β-, *threo* form of this compound.

(11) FISCHER, *Ber.*, **37**, 2494 (1904).
(12) KLAGES, *J. prakt. Chem.*, [2] **65**, 192 (1902).
(13) FISCHER, *Ber.*, **37**, 3069 (1904).
(14) BEHR AND CLARKE, *J. Am. Chem. Soc.*, **54**, 1630 (1932).
(15) FISCHER AND MOUNEYRAT, *Ber.*, **33**, 2388 (1900).
(16) FISCHER, *Ber.*, **32**, 2460 (1899).
(17) FISCHER, *Ber.*, **33**, 2377 (1900).
(18) FOX AND PETTINGA, *Arch. Biochem.*, **25**, 13 (1950).
(19) CARTER AND HINMAN, *J. Biol. Chem.*, **178**, 403 (1949).
(20) CURTIUS AND MULLER, *J. prakt. Chem.*, [2] **70**, 226 (1904).
(21) HANBY, WALEY, AND WATSON, *J. Chem. Soc.*, 3239 (1950).