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

Optical Activity and the Direct Method of Acylation

EDWARD RONWIN[1,2]

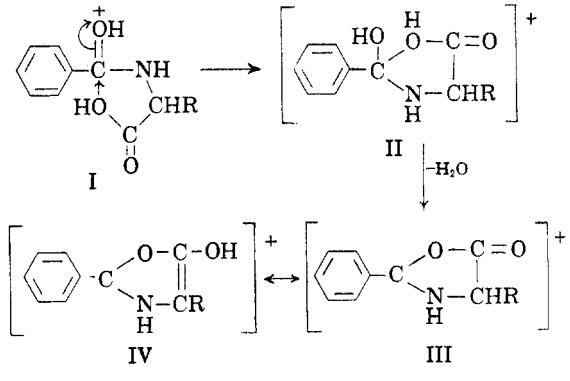
Received December 16, 1953

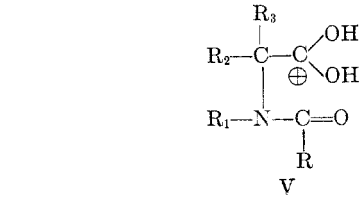
The product of the direct acylation of amino acids is optically pure in every instance except where benzoyl chloride is employed. This is not the case when the Schotten-Baumann procedure is used. Several nitrobenzoylated amino acids are characterized.

The occurrence of both partial racemization and inversion in the direct benzoylation of optically pure amino acids has been noted previously;[3] other acylating agents did not yield these effects.[3,4] Karrer and Keller[5] reported a complete racemization of the acylated leucine resulting from the Schotten-Baumann reaction between L-leucine and *p*-nitrobenzoyl chloride, which they attributed to the formation of an azlactone-alkali salt intermediate that they were able to isolate as the non-salt form (4-isobutyl-2-*p*-nitrophenyl oxazolone-5). In view of the above, it was desirable to determine whether or not direct acylation with *p*-nitrobenzoyl chloride and 3,5-dinitrobenzoyl chloride would preserve the optical purity of the amino acid moiety of the product. By comparisons of the rotation of *p*-nitrobenzoyl-L-leucine prepared by direct acylation with the optically pure product described by Karrer and Kehl,[6] and of the rotation of 3,5-dinitrobenzoyl-L-leucine prepared by direct acylation with one made by the Schotten-Baumann method, it has been established that the corresponding acylating agents do not cause racemization in direct acylation. It was noted that increased concentrations of base in the Schotten-Baumann reaction reduced the degree of racemization.

Because no prior synthesis of γ-methyl-N-benzoyl-"L"-glutamic acid ester was known, and because benzoylation by direct acylation causes racemization, no statement of the optical purity of the compound could be made. More recently attempts to synthesize the compound by the Schotten-Baumann method have met with failure. However, the degree of optical purity of the compound was determined by hydrolysis to the corresponding *N*-benzoyl-"L"-glutamic acid and comparison of the optical activity of this product with that of an authentic optically pure sample of *N*-benzoyl-L-glutamic acid that had been prepared by the Schotten-Baumann method. While the possibility exists that the γ-methyl ester was partially racemized as a consequence of the hydrolytic procedure, the data indicate that such racemization or the original racemized state of the ester was no greater than 14%. Table I contains a summary of the data on the compounds of stereochemical interest.

Since no base or metallic cation is present in the direct method, the azlactone-alkali salt intermediates suggested by Karrer and Keller[5] to explain racemization in the Schotten-Baumann medium would not be expected to form. Further, the reaction medium is somewhat on the acid side due to the HCl released in the amide formation. This suggests that the racemization which has been observed during direct benzoylation[3] might be due to the formation of an oxazolonium ion (I to IV) similar to the proposal of O'Brien and Niemann[8] to explain the cyclization of α-acylamino acids in absolute sulfuric acid or acetic anhydride. I to IV differ somewhat from the suggested intermediates of these authors, since their entity (V) would not be expected to form in the slightly acidic medium of the direct method.





(1) Present address: Dept. of Pharmacology, Univ. of Southern California, Los Angeles 7, Calif.

(2) Aided by a Fellowship from the National Foundation for Infantile Paralysis, Journal paper No. J-2066 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 1111.

(3) E. Ronwin, *J. Org. Chem.*, **18**, 1546 (1953).

(4) E. Ronwin, *J. Org. Chem.*, **18**, 127 (1953).

(5) P. Karrer and R. Keller, *Helv. Chim. Acta*, **26**, 50 (1943).

(6) P. Karrer and W. Kehl, *Helv. Chim. Acta*, **13**, 50 (1930).

(7) S. W. Fox and H. Wax, *J. Am. Chem. Soc.*, **72**, 5087 (1950).

(8) J. L. O'Brien and C. Niemann, *J. Am. Chem. Soc.*, **72**, 5348 (1950).

TABLE I
COMPOUNDS OF STEREOCHEMICAL INTEREST[a]

Compound	M.P., °C (corr.)	Lit., M.P., °C.	Synthesis,[b] Method	Specific Rotation[c]	Racemization, %
γ-Methyl *N*-Benzoyl-L-glutamic acid ester	119	107[3,d]	D	−7.0° (22°) (2% in 95% ethanol)[e]	≦14
N-Benzoyl-L-glutamic acid	136–137		O[f]	+15.7° (22°) (4% in N KOH)	≦14
N-Benzoyl-L-glutamic acid	139–140		SB[7]	+18.2° (28°) (5% in N KOH)	None
N-*p*-Nitrobenzoyl-L-leucine	228–229		D	−9.15° (28°) (2.8% in 95% ethanol)	None
N-*p*-Nitrobenzoyl-L-leucine	219–220		O[g,6]	−8.87° (20°) (2.8% in alcohol)	None
N-*p*-Nitrobenzoyl-L-leucine	222–223		SB[h,5]	Inactive	100
N-*p*-Nitrobenzoyl-L-leucine	228		SB[i]	−4.96° (26°) (2.9% in 95% ethanol)	46
N-3,5-Dinitrobenzoyl-L-leucine	184–185	186–187[9,j] 188[10,j]	D	−10.4° (28°) (2.9% in 95% ethanol)	None
N-3,5-Dinitrobenzoyl-L-leucine	188	186–187[9,j] 188[10,j]	SB	−10.5° (28°) (2.9% in 95% ethanol)	None

[a] A further description of the compounds prepared in this work can be found in Table II or under the Experimental section. [b] D = direct method of acylation; SB = Schotten-Baumann procedure; O = other method which is described by an additional footnote. Unless a reference is provided, the synthesis was performed by the author. [c] All rotations were reported at the D line of sodium. The figure in parenthesis is the temperature in degrees centigrade. [d] This discrepancy may be due to an impurity in the previous product. [e] $[\alpha]_D^{23.5}$ −7.15° (2% in 95% ethanol). [f] By hydrolysis of the corresponding γ-methyl ester; see Experimental section. [g] By hydrolysis of the corresponding methyl ester. [h] One equivalent of sodium hydroxide. [i] Two equivalents of sodium hydroxide. [j] Compound prepared by the Schotten-Baumann method; no rotation quoted.

Further, the presence of the *p*-nitro or the 3,5-dinitro groups on the benzene nucleus should act to suppress ring closure (I to IV) by their electron attraction which deters the oxygen atom of the amide carbonyl group from adding a proton. This notion is compatible with the experimental observations (Table I).

Saunders[9] and Town[10] raised considerable doubt concerning the identity of several of the 3,5-dinitrobenzoylated amino acids which they describe. In fact, Town labeled his norvaline and norleucine derivatives with question marks. These authors differed on melting points and, as they both employed alcohol-water mixtures for recrystallization, several of their products contained water of hydration. Also, they did not report the rotation of their optically active products.

In this work several new 3,5-dinitrobenzoylated, *p*-nitrobenzoylated, and *m*-nitrobenzoylated amino acids are characterized in addition to a number of previously reported members of each series prepared for the first time by direct acylation (Table II). As found by Town and by Saunders for the Schotten-Baumann procedure, the direct method failed to yield 3,5-dinitrobenzoylated tyrosine; similarly 3,5-dinitrobenzoyl-DL-aspartic acid could not be obtained. The difficulty which Town reported in separating the product from the 3,5-dinitrobenzoic acid was overcome by using acetone-CCl_4 or acetone-$CHCl_3$ mixtures which, additionally, avoid formation of hydrated products.

(9) B. C. Saunders, *Biochem. J.*, **28**, 580 (1934).
(10) B. W. Town, *Biochem. J.*, **35**, 578 (1941).
(11) P. Karrer and C. Christoffel, *Helv. Chim. Acta*, **27**, 622 (1944).
(12) W. S. Fones and M. Lee, *J. Biol. Chem.*, **201**, 847 (1953).

EXPERIMENTAL

Reagents. The acid chlorides were products of Eastman Kodak. Anhydrous ethyl acetate was obtained from Eastern Chem. Co. All amino acids were commercial products. γ-Methyl-L-glutamate was synthesized in 60% yield by the procedure of Hanby, Waley, and Watson,[13] m.p. 182° dec. (Lit. 182° dec.).

Direct acylation procedure. This procedure has been adequately described on two previous occasions.[3,4]

Synthesis of γ-methyl N-benzoyl-"L"-glutamic acid ester by the direct method. Three grams (0.019 mole) of the amino acid was suspended in 60 ml. of anhydrous ethyl acetate and refluxed under anhydrous conditions with 3.7 g. (0.026 mole) of benzoyl chloride. After 3 hr., the reaction mixture was filtered and the filtrate was taken to dryness with the aid of a current of air. The remaining crystalline product was washed with cyclohexane and recrystallized from acetone-hexane. This yielded 1.4 g. (28%) of material, m.p. 119° (corr.). A mixed melting point with benzoic acid gave an erratic result over a 30° range. For the optical rotation of this product see Table 1. (The product was a mixture of 93% of the L-form and 7% of the D-form.)

Anal. Calcd. for $C_{13}H_{15}NO_5$: N, 5.3. Found: N, 5.1.

Conversion of the ester to N-benzoyl-"L"-glutamic acid. One gram (0.004 mole) of γ-methyl-*N*-benzoyl-"L"-glutamic acid ester was refluxed with 7.6 ml. of 2*N* NaOH (4 equivalents) for 0.75 hr. The solution was cooled and acidified to Congo red with 4*N* HCl. No precipitate was observed. The solution was filtered and the filtrate was taken to dryness, *in vacuo*. The remaining residue was extracted with acetone. The acetone extract was filtered, reduced in volume, refiltered, and treated with hexane until turbidity. A colorless oil precipitated which upon considerable scratching crystallized. Six-tenths gram of a hard, white compound was obtained. Recrystallization from acetone-cyclohexane was hastened by scratching. This yielded 0.55 g. (58%) of a hard, white crystalline product, m.p. 136–137° (Lit. 139–140°).[7] The optical rotation of this product (Table 1) indicates that the compound is a mixture of 93% of the L-form and 7% of the D-form.

(13) W. E. Hanby, S. G. Waley, and J. Watson, *J. Chem. Soc.*, 3239 (1950).

TABLE II
NITROBENZOYLATED AMINO ACIDS

Amino Acid Moiety	M.P., °C. (Corr.)	Lit M.P., °C.	Yield, %	Reflux Time, Hr.	Description of Filtrate Residue	Solvent Used for Purification[a]	Nitrogen[b] Calcd.	Found
N-3,5-Dinitrobenzoylated Amino Acids								
L-Leucine[c]	184–185	186–187[9] 188[10]	28	3	White crys.	Acetone-CCl_4	12.9	13.0
DL-Methionine	160		39	3	White crys.	Acetone-$CHCl_3$	12.3	12.2
DL-Phenylalanine	167	161[10]	25	1.5	Col. crys.	Acetone-CCl_4	11.7	11.6
DL-Alanine	177	177[9,10]	26	1.75	White crys.	Acetone-CCl_4	14.8	14.8
DL-Valine	211–212	211.4[10]	45	2	White crys.	Acetone-$CHCl_3$	13.5	13.6
DL-Norleucine	208	163[10,d]	30	1.75	White crys.	Acetone-$CHCl_3$	12.9	12.7
DL-Isoleucine	182–183	170.4[10]	52	1.75	White crys.	Acetone-$CHCl_3$	12.9	12.8
DL-α-Amino-*n*-butyric acid	213		23	2.5	White crys.	Acetone-$CHCl_3$	14.1	14.1
DL-Norvaline	234–235	182[d,10]	33	2.5	White crys.	Ethyl acetate-$CHCl_3$	13.5	13.5
L-3-Nitro-4-hydroxyphenylalanine[e]	188–189		19	2	Yellow crys.	Acetone-CCl_4	13.3	13.2
N-*p*-Nitrobenzoylated Amino Acids								
DL-Methionine	191–192		40	2.5	White crys.	Acetone-CCl_4	9.4	9.3
L-Tyrosine[f]	236–237		8	1.5	White crys.	CCl_4	8.5	8.5
L-Leucine[c]	228–229	219–220[6]	39	1.5	White crys.	Acetone-CCl_4	10.0	10.0
DL-Phenylalanine[g]	168	168.5[11]	31	2	White crys.	Washed with ethyl ether; acetone-CCl_4	8.9	8.9
N-*m*-Nitrobenzoylated Amino Acids								
DL-Methionine	128–129		73	1	White crys.	Acetone-CCl_4	9.4	8.9
DL-Phenylglycine	169		99	3.5	White crys.	Acetone-CCl_4	9.3	8.9
DL-Alanine	163	162–164[12]	56	3.5	White crys.	Acetone-CCl_4	11.8	11.8
DL-α-Amino-*n*-butyric acid	174		69	4	White crys.	Acetone-CCl_4	11.1	10.9
DL-Aspartic Acid	133–134		42	3	White crys.	Acetone-CCl_4	9.9	9.9
DL-Norleucine	148–149		72	2	White crys.	Acetone-CCl_4	10.0	9.9
DL-Isoleucine	120		36	2	White crys.	Acetone-CCl_4	10.0	9.8
DL-Valine	151–152		83	3	White crys.	Acetone-CCl_4	10.5	10.8
DL-Phenylalanine	138–139		67	3	White crys.	Acetone-CCl_4	8.9	8.8

[a] Unless otherwise indicated the solvent was employed in recrystallization procedures. [b] Analyses were performed by the reductive Kjeldahl method using $Na_2S_2O_3$ as reducing agent. [See report of H. A. Davis, *J. Assoc. Offic. Agr. Chemists*, *37*, 359 (1954)]. [c] For optical rotation see Table I. [d] Labeled by Town[10] with a question mark. [e] $[\alpha]_D^{28}$ −30.7° (2.02% in 1:1 acetone:95% ethanol). [f] $[\alpha]_D^{28}$ + 2.59° (2.09% in 95% ethanol). [g] Erroneously labeled *N*-*p*-nitrobenzoyl-DL-alanine in the paper by Karrer and Christoffel.[11]

Anal. Calcd. for $C_{12}H_{13}NO_5$: N, 5.6. Found: N, 5.4.

Synthesis of N-p-nitrobenzoyl-"L"-leucine by the Schotten-Baumann procedure. Two grams (0.015 mole) of the amino acid were dissolved in 15 ml. of 2*N* NaOH (2 equivalents) and the solution was diluted to 40 ml. Then a solution of 2.8 g. (0.015 mole) of the acid chloride in 40 ml. of ethyl ether was slowly dropped during 0.5 hr. while the reaction medium was mechanically stirred at room temperature. The aqueous layer took on a characteristic purple hue. At the end of the dropping period the solution was acidified with 25% acetic acid. An orange precipitate formed which was filtered off and air dried. Three recrystallizations from acetone-carbon tetrachloride yielded 0.9 g. (21%) of a powdery, white crystalline product, melting at 228° (corr.) to a clear slightly reddish liquid [lit. 219–220°[6], 228–229° (Table 11)]. The optical rotation of this product (Table I) indicates that this compound is a mixture of 77% of the L-form and 23% of the D-form.

Anal. Calcd. for $C_{13}H_{16}N_2O_5$: N, 10.0. Found: N, 9.8.

Synthesis of N-3,5-dinitrobenzoyl-L-leucine by the Schotten-Baumann procedure. Two grams (0.015 mole) of the amino acid were dissolved in 15 ml. of 2*N* NaOH (2 equivalents) and 3.47 g. (0.015 mole) of the acid chloride were added at one time. The acid chloride immediately dissolved and the solution became purple. The solution was mechanically shaken for 0.5 hr. At the end of the reaction period, the solution was acidified with 25% acetic acid. The purple color disappeared and a pale yellow compound precipitated. It was filtered off. The filtrate yielded another crystalline crop after a night of refrigeration. Recrystallization of the combined fractions from acetone-chloroform yielded 1.4 g. (28%) of a pale yellow product, m.p. 188° (corr.) [lit. 186–187;[9] 188°:[10] 184–185° (Table II)]. The optical rotation (Table I) of this compound is in excellent agreement with that found for the product synthesized by the direct method of acylation and therefore presumed to be optically pure.

Anal. Calcd. for $C_{13}H_{15}N_3O_7$: N, 12.9. Found: N, 13.0.

AMES, IOWA