[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE UNIVERSITY OF ILLINOIS]

The Use of *l*-Menthoxyacetyl Chloride for the Resolution of Amino Acids

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In recent years a large number of amino acids have become available through synthetic methods. The separation of these into their optically active forms is necessary for many investigations and the most convenient method in present use is that involving the resolution of an acyl derivative, in particular the formyl derivative, by means of an alkaloid, with subsequent removal of the acyl group. Since this method of resolution requires four operations involving the amino acid, a method has now been investigated which involves only two operations.

By the use of an optically active chloride, diastereoisomeric amides of the type RCONHCHR'-COOH are formed, separated by fractional crystallization and then hydrolyzed. *l*-Menthoxyacetyl chloride has been used as the active acid chloride, a reagent which is readily prepared from natural menthol. It reacts with amino acids in a cold alkaline solution to give in excellent yields well-crystallized amides. By fractional recrystallization the two diastereoisomers, especially the less soluble form, could readily be obtained pure. Hydrolysis of the amides in aqueous alcohol by means of hydrobromic acid yielded the pure active amino acid without difficulty and no racemization occurred in the cases studied. The absence of any menthol or menthol derivatives in the hydrolyzed products was proved by the complete inactivity of glycine which had been put through a similar procedure.

The *l*-menthoxyacetyl derivatives of synthetic glycine, alanine, valine, phenylglycine and phenylalanine were prepared, and the alanine, valine and phenylglycine successfully resolved, with the isolation of the pure *d*-forms. The derivative of phenylalanine gave an inactive amino acid on hydrolysis. The only explanation offered is the probable formation of a partial racemate of the two isomeric forms.

The diastereoisomeric amides differ widely in melting point and solubility and have high rotations. The over-all yields are comparable with those from the use of an alkaloid resolution of an acyl derivative. The method appears, therefore, to present certain advantages over the method most generally used.

Experimental

***l*-Menthoxyacetyl Chloride.**—Menthoxyacetic acid was prepared in a manner similar to that described by Frankland and O'Sullivan except that toluene was substituted for benzene as a solvent.[2] It was purified by vacuum distillation, b. p. 165–175° (7–10 mm.).

The acid chloride was produced by dissolving 90 g. of the acid in 150 cc. of thionyl chloride and warming at 50° for three hours. The excess thionyl chloride was removed by warming in a water-bath under diminished pressure and the acid chloride used directly. For identification and tests of purity a few simple derivatives were prepared.

Menthoxyacetamide.—Menthoxyacetyl chloride and concentrated aqueous ammonia gave an immediate precipitate of the amide, m. p. 94–95°. Rule[3] reported m. p. 93°.

β-Naphthyl-*l*-menthoxyacetate.—A solution of 3 g. of the acid chloride in pyridine was added to a solution of 2 g. of β-naphthol in pyridine, and the solution warmed on the steam-bath for three hours. Upon pouring into an excess of concentrated hydrochloric acid with cooling, a brown oil separated which was extracted with ether. Beautiful white crystals of the ester were obtained from alcohol; m. p. 108–109.5°.

Rotation. 0.1200 g. made up to 15 cc. with acetone gave α_D −1.35°; l = 2, $[\alpha]_D^{25}$ −84.4°.

Anal. (micro.) Calcd. for $C_{22}H_{28}O_3$: C, 77.60; H, 8.29. Found: C, 77.68; H, 8.11.

***l*-Menthoxyacetyl-*p*-nitroanilide.**—A solution of 8 g. of the acid chloride in hot benzene was added to 17 g. of *p*-nitroaniline in hot benzene. *p*-Nitroaniline hydrochloride which precipitated was extracted with dilute hydrochloric acid and the benzene layer evaporated. The product was purified from alcohol, m. p. 106°.

Rotation. 0.3392 g. made up to 15 cc. with acetone gave α_D −3.12°; l = 2, $[\alpha]_D^{25}$ −69.0°.

Anal. (micro.) Calcd. for $C_{18}H_{26}O_4N$: C, 64.63; H, 7.84; N, 8.38. Found: C, 65.03; H, 7.88; N, 8.39.

Menthoxyacetyl Amino Acids.—These were all prepared by the addition of menthoxyacetyl chloride to an equivalent amount of the amino acid dissolved in 5% sodium hydroxide solution and shaking the emulsion at room temperature for several minutes until a clear solution resulted. This solution was then poured very slowly in small portions into dilute hydrochloric acid containing cracked ice, allowing the oil which separated to solidify after each addition. In this manner a solid product was obtainable in every case, and no difficulty was encountered in procuring beautiful crystals thereafter. After filtering,

(1) Submitted as part of a thesis for the degree of Doctor of Philosophy at the University of Illinois.

(2) Frankland and O'Sullivan, *J. Chem. Soc.*, **99**, 2329 (1911).

(3) Rule and Tod, *ibid.*, 1932 (1931).

TABLE I

ROTATIONS OF AMIDE AND AMINO ACIDS

Amino acids	*l*-Menthoxyacetamino acids Made up to 15 cc. With acetone: $l = 2$ Wt., g.	α_D	$[\alpha]_D^{25}$	Amino acids $[\alpha]_D^{25}$ Found	Literature
Glycine	0.4940[a]	−3.65	− 73.7	0	
d-Alanine	0.0840	−0.74	− 50.6	+ 10.6[b]	+ 10.4[4]
l-Alanine	0.2697	−2.69	− 74.8	− 2.2[b]	− 10.3[4]
d-Valine	0.1650	−0.86	− 39.1	+ 6.3[c]	+ 6.4[5]
l-Valine	0.3731	−3.86	− 77.6	− 14.5[d]	− 29.0[5]
				− 2.7[c]	− 5.7[6]
d-Phenylglycine	0.2322	+1.02	+ 32.9	+157.5[e]	+157.8[7]
l-Phenylglycine	0.2638	−4.32	−122.7	− 67.2[e]	
				−140.6[e,f]	−157.8[7]
dl-Phenylalanine[g]	0.6055	−4.42	− 54.7	0	

[a] Made up to 10 cc. with acetone: $l = 1$. [b] Rotation of the hydrochloride in water. [c] Rotation in water. [d] Rotation in 20% hydrochloric acid. [e] Rotation in 2.6% hydrochloric acid. [f] Rotation of more soluble fraction after recrystallization from water. [g] Over half the product was obtained in large crystals of this melting point, yielding an inactive amino acid on hydrolysis, as did the residual oil from the mother liquor. Recrystallization from the solvents aqueous alcohol, petroleum ether and ethyl acetate, petroleum ether and benzene, at various temperatures, did not alter the melting point. Attempts to prepare crystalline brucine and strychnine salts failed.

TABLE II

CONSTANTS AND ANALYSES OF *l*-MENTHOXYACETAMINO ACIDS

l-Menthoxyacetyl derivative	M. p., °C.	Formula	Analyses, % Calcd. C	H	N	N. E.	Found[a] C	H	N	N. E.
Glycine	155–156	$C_{14}H_{25}O_4N$			5.16	271.2			5.42	272.8
d-Alanine	147–148	$C_{15}H_{27}O_4N$	63.11	9.54	4.92	285.2	63.61	9.28	5.17	283.6
l-Alanine	117–118	$C_{15}H_{27}O_4N$			4.92	285.2			5.09	
d-Valine	156–157.5	$C_{17}H_{31}O_4N$	65.12	9.96	4.47	313.3	65.40	10.04	4.71	313.4
l-Valine	93– 96	$C_{17}H_{31}O_4N$			4.47	313.3			4.70	
d-Phenylglycine	162	$C_{20}H_{29}O_4N$	69.20	8.42	4.03	347.2	69.20	8.40	4.07	347.3
l-Phenylglycine	113–116	$C_{20}H_{29}O_4N$			4.03	347.2			4.22	
dl-Phenylalanine	100–101	$C_{21}H_{31}O_4N$			3.88	361.3			4.12	362.3

[a] Micro-analyses. The neutral equivalents were taken on optically impure samples chosen at random from the diastereoisomeric mixtures.

the mixed diastereoisomers were separated by fractional crystallization from either 60% ethyl alcohol or a 75 : 25 mixture of high-boiling petroleum ether and ethyl acetate, the latter usually giving the more satisfactory results. The more soluble form was in each case contaminated with the less soluble and no attempt was made to obtain it in a pure state. The less soluble form was easily obtained pure after a very few crystallizations. In early experiments the amides separated frequently as oils from the reaction mixture, and thus introduced considerable difficulty. By following carefully the procedure just described, the products were obtained solid in every instance.

Hydrolysis of the Menthoxyacetyl Amino Acids.—The amino acid derivative was suspended in a small amount of 20% hydrobromic acid and just enough alcohol added to effect solution when boiling. This solution was refluxed from four to six hours, and the alcohol then removed by boiling in the open. After washing with ether, the acid solution was evaporated to dryness and the amino acid hydrobromide dissolved in absolute ethyl alcohol. The free amino acid was precipitated by making slightly ammoniacal with concentrated aqueous ammonia, filtering, and washing with hot absolute alcohol to remove traces of ammonium bromide.

Summary

A new practicable method for the resolution of amino acids is described. It involves reaction with *l*-menthoxyacetyl chloride, with separation and hydrolysis of the diastereoisomeric amides thus formed. Alanine, valine and phenylglycine were successfully resolved by this method.

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(4) Fischer, *Ber.*, **39**, 464 (1906).
(5) Fischer, *ibid.*, **39**, 2325 (1906).
(6) Ehrlich, *Biochem. Z.*, **1**, 29 (1906).
(7) Fischer and Weichhold, *Ber.*, **41**, 1291 (1908).