N-ACYLATED AMINO ACID IMINO CHLORIDES AND A QUALITATIVE STUDY OF RING CLOSURE AMONG N-ACYLAMINO ACID CHLORIDES[1]

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ABSTRACT

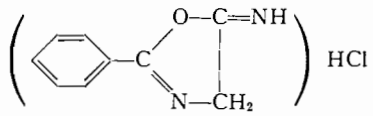
Products have been isolated from the treatment of N-acylaminoacetonitriles with dry hydrogen chloride which are either the open-chain imino acid chlorides or the dissociated salt forms. These compound types have often been postulated as reaction intermediates but never isolated with an unsubstituted nitrogen atom. In the unsubstituted condition they are analogous to *regular* or oxygen acid chlorides.

Several N-acylamino acids were treated with PCl_5 and the reaction solutions were subjected to infrared spectral analyses. The results indicate that the open-chain acid chloride, rather than the azlactone salt, is the predominant product obtained with the compounds used in this investigation.

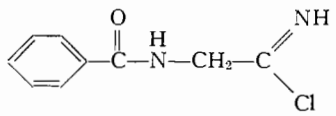
N-Acylated Amino Acid Imino Chlorides

This work is an outgrowth of attempts to prepare substrates which comply with the specificity requirements of beef pancreatic carboxypeptidase, but which contain imino ether or amidine linkages at the susceptible link.

Efforts to synthesize the hippuryl imino ether derivatives of *dl*-mandelic and *dl*-malic acids by the Pinner (6, 7) method were unfruitful. Treatment of hippurylnitrile and *dl*-mandelic acid in chloroform with dry HCl gave a product which may be hippuric iminoazlactone. HCl (I), or hippuryl imino chloride (II). The same product was obtained when *dl*-mandelic acid was omitted.



I



II

The compound yielded a positive chloride ion test, decomposed sharply at 122°, was readily soluble in concentrated nitric acid, and gave a somewhat acidic solution in water. Analyses for N and Cl were compatible with either structure I or II.

These facts, in themselves, do not permit a definite formula assignment. In the hope of resolving the structure problem, infrared spectral analysis was employed. In addition, *p*-nitrohippurylnitrile and *p*-toluenesulphonylaminoacetonitrile were prepared for a comparison of their behavior. The latter compound could not be synthesized by the Schotten-Baumann method but was obtained by acylation of the nitrile in anhydrous ethyl acetate in the presence of sodium bicarbonate. Upon HCl treatment, these compounds yielded materials having similar properties to those of the hippurylnitrile–HCl compound. All HCl addition compounds are exceedingly hygroscopic and difficult to handle.

The infrared spectra for the nitriles and their HCl reaction products are presented in Fig. 1. The spectrum for N-*p*-toluenesulphonylaminoacetonitrile (Curve A) shows a sulphonyl peak at 8.58 μ (part of another peak whose identity is uncertain). Upon treatment with HCl (Curve B), the sulphonyl band remains; however, a new peak appears at 6.06 μ. Since the sulphonyl band is unchanged, it is unlikely that this group

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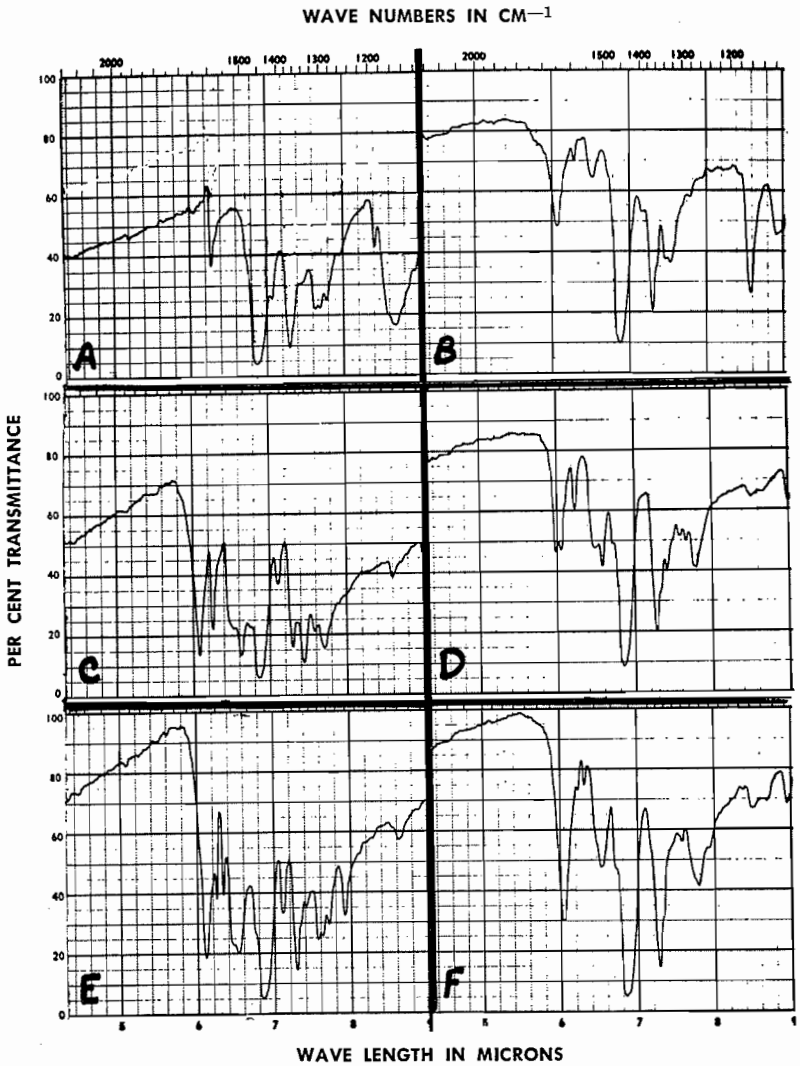
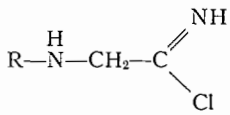


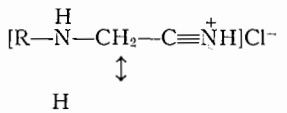
FIG. 1. Infrared spectra of N-acylated aminoacetonitriles and their HCl-treated products. Baird Associates, Model B, infrared spectrophotometer, sodium chloride prism. All run as Nujol mulls.

has been involved in ring closure. Therefore, the new strong band at 6.06 μ is ascribable to the appearance of a C=NH grouping in the molecule which points to the structure of the compound as either an open-chain imino acid chloride (IIIA) (N-*p*-toluenesulphonylglycyl imino chloride) or the dissociated relative, IVA. The data do not allow a choice between the two formulas nor do they exclude an equilibrium between them.



III

R = A—*p*-Toluenesulphonyl
B—*p*-Nitrobenzoyl
C—Benzoyl



IV

The existence of imino acid chlorides as acylating intermediates in such reactions as the Hoesch ketone synthesis (3), the Stephan aldehyde synthesis (3), the Pinner imino ether and amidine synthesis (6, 7, 8, 9), and the Walther and Grossmann amidine synthesis (11) has been often postulated. While earlier workers have reported the preparation of imino chlorides in which the nitrogen was substituted (6), this is believed to be the first report of the isolation of non-N-substituted imino acid chlorides and supports the proposed mechanisms of the above-mentioned reactions.

The curve for *p*-nitrohippurylnitrile (Curve C) shows a peak at 6.06 μ due to the amide grouping. The HCl-treated derivative has two peaks (Curve D) in the vicinity, one at 6.06 μ ascribable to the unchanged amide (hence, no ring closure took place) and the other at 5.98 μ due to the formation of a C=NH grouping which points to structure IIIB or IVB for this compound. The spectral picture for hippurylnitrile and its HCl-treated compound (Curves E and F) is much the same as for the other two nitriles and indicates that the HCl addition compound possesses either formula IIIC or IVC.

Qualitative Study of Ring Closure among N-Acylamino Acids

The infrared absorption spectra for several N-acylated amino acids were ascertained. The acids were treated with PCl_5 and samples of the reaction mixture were taken for infrared analysis. The spectral curves are presented in Fig. 2. Controls eliminated the effects observed as due to anything but reaction between the N-acylamino acid and PCl_5.

Group assignments to the various bands is based on the data compilation of Randall *et al.* (10) and by comparison with the band assignments of Carter and Hinman (1) for the free carboxyl group of benzoyl-*p*-methoxyphenyl-L-alanine (5.82 μ), the carbonyl of its azlactone ring (5.48 μ), and the carbonyl of the azlactone ring of the azlactone. HBr salt (5.35 μ).

N-*p*-Toluenesulphonylglycine (Curve A) shows a free carboxyl absorption at 5.78 μ and a sulphonyl absorption at 8.58 μ. After treatment with PCl_5 (Curve B), the sulphonyl group remains unchanged and the open-chain acid chloride carbonyl absorption appears at 5.53 μ. This result agrees well with Carter and Hinman's (1), who reported an acid chloride carbonyl band at 5.51 μ for N-*p*-toluenesulphonyl-*p*-methoxyphenyl-L-alanyl chloride. Curve C for N-carbobenzoxy-DL-valine and the product resulting from its PCl_5 treatment (Curve D) are typical of the carbobenzoxylated derivatives. The free carboxyl absorption shifts to that of an open-chain acid chloride carbonyl at 5.63 μ. The small but broad and distinct band at 5.42 μ may be due to the N-carboxy derivative or to the closed ring azlactone. The curves for N-carbobenzoxy-DL-leucine (Curves E and F) show almost identical values; those for N-carbobenzoxyglycine (Curves G and H) have bands shifted to slightly shorter wave lengths. These results are in accord with Carter and Hinman's conclusion that N-carbobenzoxy-*p*-methoxyphenyl-L-alanine yields the open-chain acid chloride which they drew as a result of the reactivity shown by their product. If the bands in the vicinity of 5.40 μ are not due to an N-carboxy derivative, then it would indicate that the reaction in this series is not wholly in one direction, but that some quantity of closed ring azlactone forms, which eluded Carter and Hinman since they isolated only the major product.

These authors noted that their acid chloride "could not be kept for more than a few hours at room temperatures before it passed to the N-carboxy anhydride". The reaction mixtures in this work were maintained at 0° for 24 hours before subjection to infrared analyses. From the shapes of the curves it would seem that the life of the N-carbobenzoxy amino acid chlorides is considerably extended at lower temperatures.

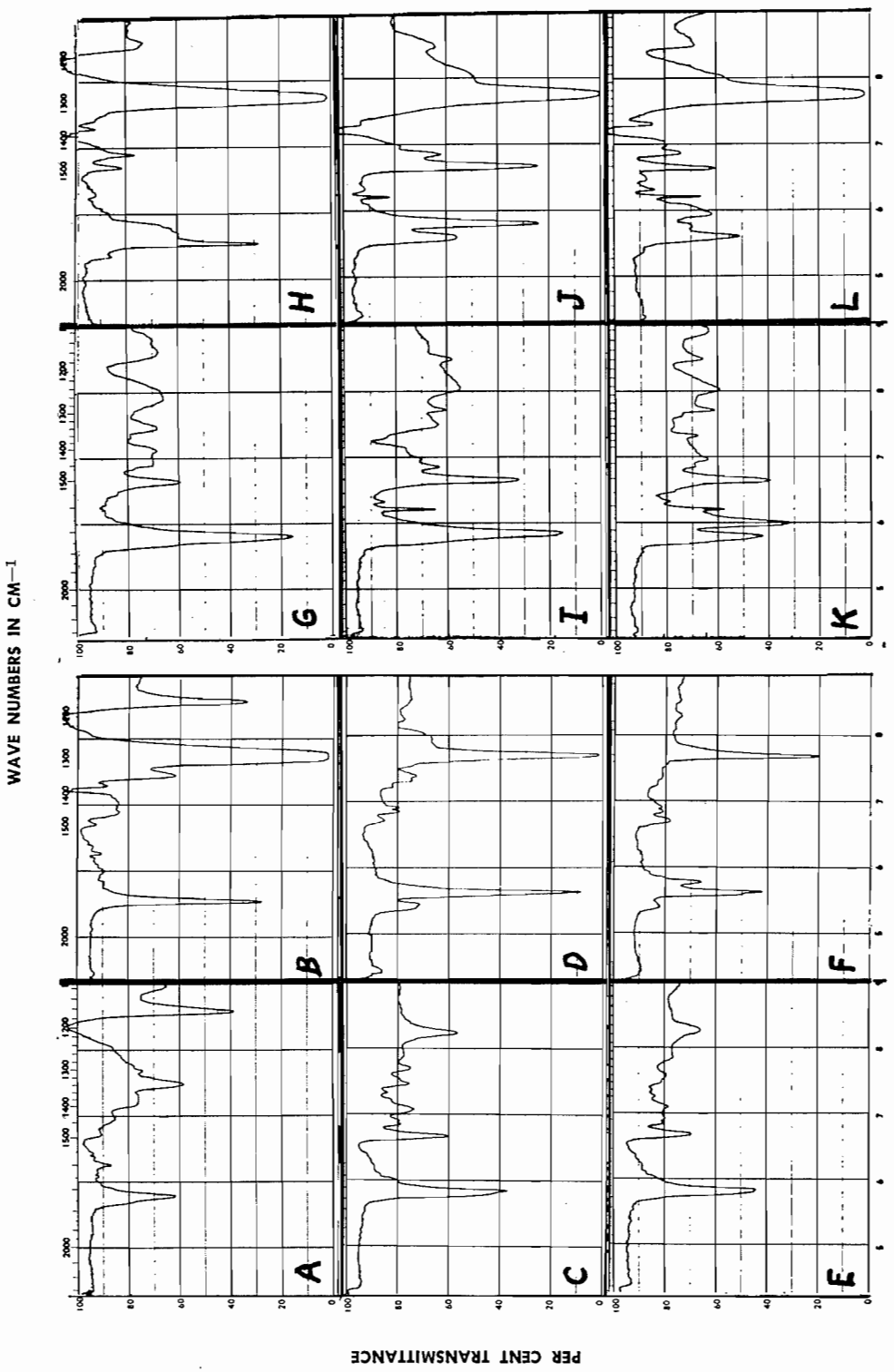


FIG. 2 (A to L).

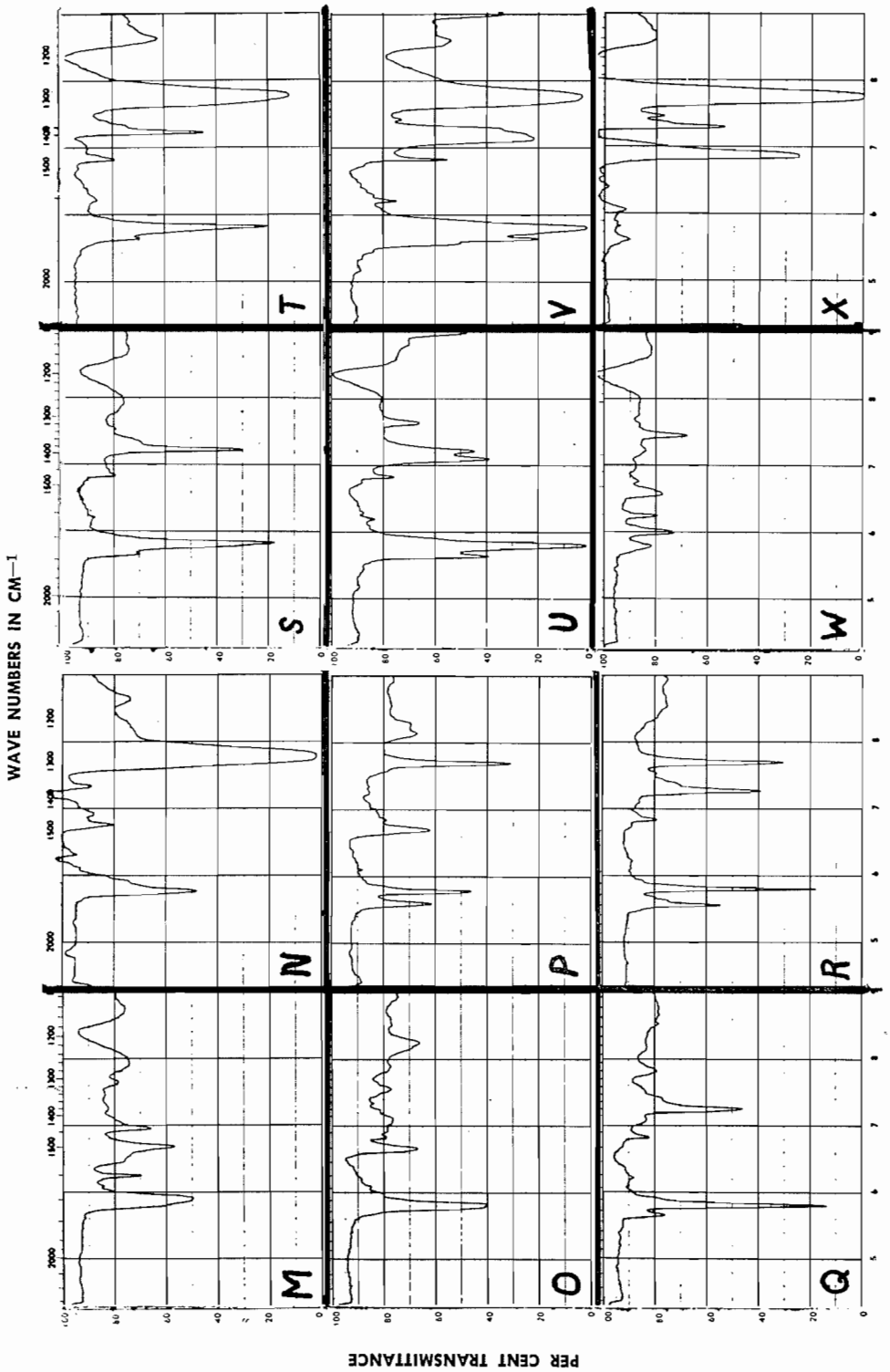


FIG. 2. Infrared spectra of N-acylated amino acids and their PCl_5-treated product mixtures. Baird Associates, Model B, infrared spectrophotometer, sodium chloride prism, 0.4 mm. cell path length.
Saturated solutions in: $CHCl_3$—A, B, G, H, I, J, K, L, M, N, S, T, U, V, W, X. CCl_4—C, D, E, F, O, P, Q, R.

The curve for N-carbobutoxy-*p*-methoxyphenyl-L-alanine (Curve I) shows free carboxyl absorption at 5.84 μ and that for N-acetyl-*p*-methoxyphenyl-L-alanine (Curve K) at 5.80 μ; both results agree well with the absorption value for this group in the benzoyl derivative (1). Upon treatment with PCl_5, the new major bands for both compounds appear at 5.60 μ (Curves J and L). These shifts are similar to the open-chain acid chlorides discussed in the preceding paragraphs and are decidedly less than anticipated for azlactone formation (1, 2). In the case of the carbobutoxy compound, there does not appear to be any indication of N-carboxy anhydride or azlactone formation as is found with the carbobenzoxy derivatives. The small, but definite, band at 5.35 μ for the acetyl derivative may be indicative of azlactone salt formation (no N-carboxy anhydride can form in this case).

N-Carboallyloxyglycine (Curve M) has a free carboxyl absorption at 5.90 μ. After treatment with PCl_5 (Curve N), the carbonyl absorption shifted to 5.76 μ, indicative of acid chloride formation. On the other hand, the free carboxyl absorption for N-carboallyoxy-DL-leucine (Curve O) occurred at 5.81 μ and its acid chloride carbonyl absorption peak was found at 5.59 μ (Curve P). The curve of neither PCl_5-treated compound bears any indication of a closed ring carbonyl group. The free acid band accompanying the leucine acid chloride indicates that an incomplete conversion or partial hydrolysis of the product had occurred.

The reaction for all three phthaloyl derivatives with PCl_5 was incomplete (Curves Q to V). The COOH band occurs at 5.80 μ accompanied by the phthalimide carbonyl absorption at about 5.67 μ. After PCl_5 treatment the acid chloride carbonyl absorption is observed in the vicinity of 5.55 μ.

The curve for N-*p*-nitrobenzoylglycine (Curve W) is weak. This was primarily due to the insolubility of the compound and its PCl_5 reaction product in chloroform. Nevertheless, its free carboxyl absorbs at 5.78 μ. Reaction with PCl_5 shifts the carbonyl band to 5.60 μ as for the other acid chlorides (Curve X).

Randall *et al.* (10) assigned a frequency of 5.2 to 5.4 μ for the acid halide carbonyl on the basis of three compounds (acetyl chloride and bromide, and phenacetyl chloride). However, the carbonyl band of benzoyl chloride comes at 5.61 μ, which variation was attributed to the conjugated quality of this carbonyl group. The average value for the acid chloride found here is 5.60 μ. This suggests that the α-carbon of N-acylated amino acid halides (or polypeptides or proteins) is involved in a bond of multiple character, possibly with the α-nitrogen atom.

EXPERIMENTAL

Hippurylnitrile and p-Nitrohippurylnitrile

These compounds were obtained by the procedure of Klages and Haack (4). A 35% yield was realized for hippurylnitrile, m.p. 144° (lit. 144°). *p*-Nitrohippurylnitrile was made in 45% yield, m.p. 144° (lit. 145°).

N-p-Toluenesulphonylaminoacetonitrile

p-Toluenesulphonyl chloride (9.4 g., 0.05 M.), the sulphate salt of aminoacetonitrile (7.7 g., 0.05 M.), and sodium bicarbonate (15.0 g., 0.17 M.) were mixed with 100 ml. of anhydrous ethyl acetate and refluxed for 1½ hours (heating beyond this point caused extensive decomposition). After being refluxed, the mixture was filtered and the filtrate was treated with hexane. A white precipitate formed, which, however, carried along a contaminant. It was found that the contaminant could be removed by dilution preci-

pitation from acetone with hexane. In this way three pure fractions weighing a total of 1.95 g. (19%), m.p. 136° (corr.), were obtained. The compound is soluble in acetone, ethanol, and ethyl acetate, but is insoluble in ether, hexane, chloroform, and water. Anal. Calc. for $C_9H_{10}N_2O_2S$: N, 13.4. Found: N, 13.6.

General Procedure for the Reaction between the Nitriles and HCl

The nitrile (1 to 10 g.) was suspended in dry, redistilled chloroform (20 to 200 ml.) and the suspension was cooled in an ice bath. Then dry HCl gas was passed in for about 30 minutes. The nitriles dissolved almost completely within 10 minutes after the start of the gassing operation. For the cases of hippurylnitrile and the nitrated derivative, the product began to precipitate after 15 to 20 minutes of gassing. No precipitate was observed in the case of *p*-toluenesulphonylaminoacetonitrile. At the end of the gassing period, the solvent was removed *in vacuo*.

Hippuryl Imino Chloride

The compound was obtained as white needles which melted with gaseous decomposition at 122° (corr.). It is highly reactive with water and quickly becomes sticky in air, but appears to be indefinitely stable when stored *in vacuo*. Anal. Calc. for $C_9H_9ClN_2O$: N, 14.3; Cl, 18.1. Found: N, 14.2; Cl, 17.9.

p-Nitrohippuryl Imino Chloride

This material appeared as white needles melting at 216–218° (corr.) with decomposition to a black liquid. Its properties are similar to hippuryl imino chloride. Anal. Calc. for $C_9H_8ClN_3O_3$: N, 17.4; Cl, 14.7. Found: N, 17.2; Cl, 14.3.

N-p-Toluenesulphonylglycylimino Chloride

This compound formed as white crystals which melted with gaseous decomposition at 110–112° (corr.). Its properties are similar to hippuryl imino chloride. Anal. Calc. for $C_9H_{11}ClN_2O_2S$: Cl, 14.4. Found: Cl, 13.6.

The water solutions of all these compounds yield positive chloride ion tests.

N-p-Toluenesulphonylglycine

This acylamino acid was prepared by the method of McChesney and Swann (5) in 37% yield, m.p. 147° (lit. 147°).

*N-Carbobutoxy-p-methoxyphenyl-*L*-alanine*

p-Methoxyphenyl-L-alanine (9.8 g., 0.05 M.) was dissolved in 50 ml. of 2 *N* NaOH. Butyl chloroformate (Pittsburgh Glass Co.) (6.8 g., 6.8 ml., 0.05 M.) was dissolved in 100 ml. of ethyl ether and added to the amino acid solution. The resulting mixture was shaken at room temperature for 1½ hours in a tightly stoppered flask. After the shaking period, the ether layer was separated and discarded. The water layer was acidified to Congo red with 4 *N* HCl. This precipitated a white oil. After a night in the refrigerator, the oil crystallized. It was filtered off and air-dried. The product was recrystallized from 1:1 ethanol:water. It first appears as an oil, which after scratching becomes crystalline. Pure material (14.8 g., 100%) was obtained, m.p. 84° (corr.), $[\alpha]_D^{25°}$ +30.4° (59.1 mg. in 3 ml. of 95% ethanol). Anal. Calc. for $C_{15}H_{21}NO_5$: N, 4.8. Found: N, 4.9.

Other N-Acylamino Acids

These derivatives were all present in pure form in this laboratory. Before use the melting points of these compounds were checked and found to compare favorably with those previously established in this laboratory or reported in the literature.

General Procedure for the Reaction between the Acylamino Acids and PCl_5

From 1 to 2 mg. of the acylamino acid was dissolved or suspended in a few milliliters of dry chloroform and approximately 2 to 3 mg. of PCl_5 was added. The test tube was tightly stoppered and left in the refrigerator for 24 hours. A sample of the chloroform solution was subjected to infrared analysis.

ACKNOWLEDGMENT

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