DEVELOPMENT OF SELECTIVE FUNCTIONAL GROUP ANALYTICAL METHODS: ACYLATION OF AMINES WITH trans-CINNAMOYL DERIVATIVES

by

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TO

my wife,

my parents,

and to

my children

Jack and Lynda

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I. INTRODUCTION

The wide distribution of amines in nature, their importance in industry as raw materials, intermediates, and finished products, their use in the laboratory, and the pharmaceutical significance of many biologically active amines lead to continued interest in their analysis. Owing to the presence of a basic amino group (or groups), nearly all amines can potentially be analyzed through this functional group. However, functional group methods of organic analysis tend to be relatively non-specific and insensitive, and these disadvantages have led many analysts to discount the wet chemical functional group approach as a viable contributor to organic analysis. We believe that these disadvantages can be overcome by suitable experimental design, and that the advantages of this approach, namely simplicity and economy, make it an important area for analytical development. Much can be gained, even with conventional functional group methods, by choosing optimal reaction conditions on the basis of a firm knowledge of the chemistry of the reactions, rather than by empirical "cut-and-try" methods. The great body of reactions that can generally be classed as acylation reactions provides opportunities for the development of improved, and new, functional group methods of analysis.

A. Spectrophotometric Methods for Amines

Methods of amine determination have been reviewed by many authors (1-5). Spectrophotometric methods are widely used, simply because they usually offer certain advantages for quantitative analysis such as speed, accuracy, sensitivity and often preservation of the sample. Direct spectrophotometric determination of amines in the ultraviolet and infrared regions of the spectrum is sometimes possible (6-8). However, many indirect light absorption methods have been introduced to achieve greater sensitivity and specificity. The most widely applicable of these methods are based upon the reaction types summarized in Table I. Further discussion on the sensitivity, specificity and reproducibility of the analytical methods can be found in the references. In general, most of these methods are fairly sensitive, being suitable for microanalysis.

B. Acylation Methods for Amines

Acylation methods are applied widely for the determination of both alcoholic and phenolic hydroxyl groups as well as amino groups. Mehlenbacher (42) has reviewed acylation methods to 1952, and Mathur (43), in 1966, reviewed methods that have appeared since 1955. Recent books by Siggia (3) and Critchfield (2) have included many methods that have appeared since 1952.

Clearly, all types of primary and secondary amines, including aliphatic, aromatic, and alicyclic, are subject

Table I

Methods for the Spectrophotometric Determination of Amines

Reaction type	Reagent	Amine analyzed	Reference
Imine formation	Salicylaldehyde	Primary amines	9
	$CuCl_2$, Salicylaldehyde, bis-(2-hydroxyethyl)-dithiocarbamic acid	Primary aliphatic amines	10
	2-Ethylhexanal	Primary aliphatic amines	11
	Vanillin	Primary aromatic amines	12,13
Salt and complex formation	Methylene blue, pinacyanol, bromophenol blue, etc.	Quaternary ammonium salts	14
	Bromophenol blue	Long chain amines	15
	Salicylaldehyde, bromcresol green	Secondary aliphatic amines	16
	Methyl orange	Primary, secondary, and tertiary fatty amines	17
	$CuCl_2$ in ethanol	Primary, secondary, and tertiary aliphatic amines	18
	CS_2, Cu (II)	Secondary aliphatic amines	19

Table I - Cont.

Reaction type	Reagent	Amine analyzed	Reference
Salt and complex formation	Copper-EDTA	Primary amines	20
	Acetic anhydride, tetracyanoethylene	Tertiary aromatic amines	21
	Aconitic anhydride	Tertiary and quaternary amines	22
	Pinacolyl pyridinium bromide[a]	All amines	23
Acylation	Acetic anhydride	Primary and secondary aromatic amines	24
Alkylation or arylation	1-Fluoro-2,4-dinitrobenzene	Primary and secondary amines	25,26
	9-Chloroacridine	Primary aromatic amines	27
	p-Nitrobenzyl halide[a]	Primary and secondary amines	28
	1,2-Naphthoquinone-4-sulfonate	Primary amines	29,30,31
	Ethanolic p-quinone	Hexylamine	32
Diazo dye formation	N-(1-naphthyl)-ethylene diamine	Primary aromatic amines	33,34, 35,36
	1-Naphthylamine	Primary aromatic amines	37
	Chicago acid	Primary aromatic amines	38
	Azobenzene-4-diazonium fluoborate[a]	Primary aromatic amines	39

Table I - Cont.

Reaction type	Reagent	Amine analyzed	Reference
Diazo dye formation	3-Methyl-2-benzothiazolone hydrazone	Primary, secondary, and tertiary aromatic amines	40
Miscellaneous reactions	Chloranil	Tertiary amines	22
	Diphenylpicryl-hydrazyl	Aromatic amines	41

[a]For qualitative analysis only.

to analysis by the acylation method. Tertiary amines, which possess no replaceable hydrogen, obviously do not interfere in the analysis, but they do form 1:1 complexes with some acylating agents, for example, triethylamine forms a complex with benzoyl chloride. The stability of these complexes is not large due to the steric interference of the branched alkyl groups (44).

Carboxylic acid anhydrides are the usual acylating agents, having moderate reactivity with few side reactions. The usual methods involve reaction of the corresponding anhydrides with the amine (or hydroxyl compound). The unreacted anhydride is hydrolyzed to the corresponding carboxylic acid which is then titrated with standard alcoholic sodium hydroxide. A blank is run in the same manner, omitting only the sample. The difference between the blank and sample titrations is equivalent to the amount of sample present. Mitchell, Hawkins, and Smith (45) devised an acylation method using acetic anhydride whereby the excess anhydride is determined by adding excess water and titrating the unreacted water with Karl Fisher reagent. Angelescu and Burbulescu (46) determined methylaniline by measuring the heat generated when the sample was acylated. Olson and Feldman (47) used acetyl chloride for the determination of amines. The results show that simple aromatic and secondary amines are acylated to the extent of from 80 to 100%, while halogen or nitro substituted and

aliphatic amines are acetylated to a much smaller extent. Perez (48) reported the first acid-catalyzed method for hydroxyl and amine groups. His reagent was 0.15 M p-toluenesulfonic acid catalyst and 1 M propionic anhydride in glacial acetic acid solvent. Elving and Warshowsky (49) studied the phthalation of simple alcohols, phenols, and amines. These authors found that phthalic anhydride does not react stoichiometrically with primary amines. It gives high results with aniline and ethylenediamine, probably because of phthalimide formation. Siggia, Hanna and Culmo reported (50,51) that pyromellitic dianhydride (PMDA) offers definite advantages over acetic anhydride and phthalic anhydride as a reagent for the determination of alcohols and amines. It is more reactive and it reacts stoichiometrically with both primary and secondary amines. Schenk, Wines, and Mojzis (52) found that triethylenediamine [1,4-diaza-bicyclo(2.2.2)octane] is a better catalyst than pyridine for the acetylation of hydroxyl and amino groups. The bridgehead nitrogens of this amine are almost as free from steric hindrance in their reactions as the nitrogen of pyridine. Most amines react at room temperature within 15 minutes if this catalyst is employed. Schenk and Fritz (53) have also extended the perchloric acid-catalyzed acetylation methods to the determination of phenols, thiols, and amines. Quantitative reaction was achieved in 5 minutes at room temperature. The "finish"

of these methods mentioned above is usually titrimetric, and so these methods are mainly applicable on the semi-micro and macro scales, though Belcher (54) has extended the titrimetric method to the submicro range for many classes of compounds.

Greater sensitivity in routine analyses, however, can be expected when the final measurement is spectrophotometric, and recently some acylation procedures for hydroxyl compounds have been reported, in which spectrophotometric finishes were employed. For example, alcohols can be quantitatively acetylated, the esters converted to the hydroxamic acids, and a color developed with ferric ion (55). Scoggins (56) reacted alcohols with p-nitrobenzoyl chloride and measured the esters spectrophotometrically. Reynolds, et al. (24), have described the photometric titration of aromatic amines with acetic anhydride. The absorbance of the amine in the ultraviolet region of the spectrum is measured as a function of the volume of standard acylating agent.

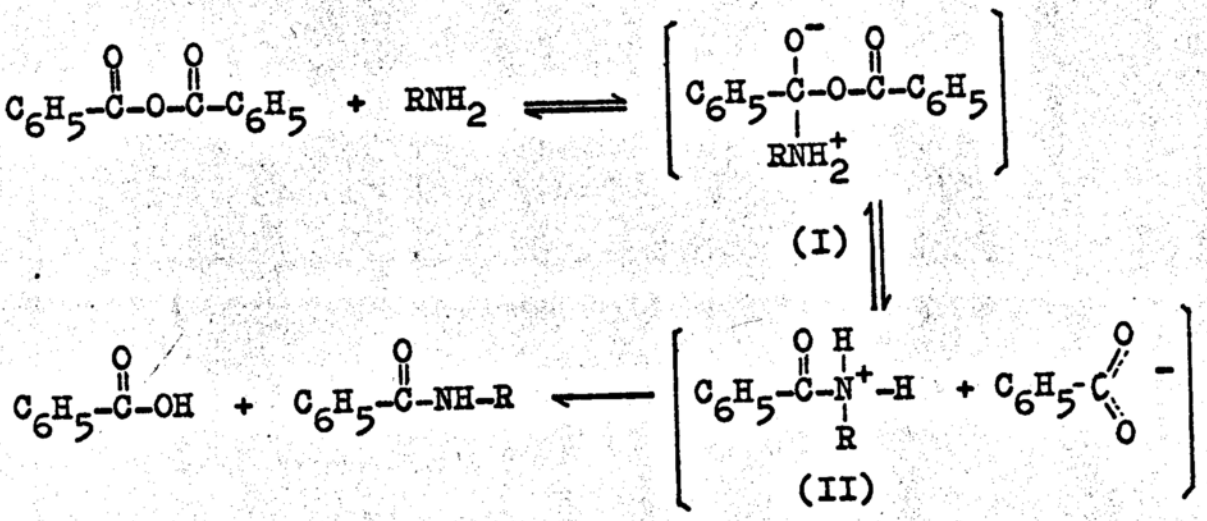
C. Acylation Mechanisms (Carboxylic Acid Anhydrides)

Primary and secondary amines react with many carboxylic compounds to form amides. In these reactions the normal order of carbonyl reactivity is: acid halides > acid anhydrides > esters > acids and amides. Tertiary amines are generally non-reactive, although salt-like addition products have been isolated from

reaction with acid chlorides.

Acylation of amines by acid anhydrides has not been subjected to extensive mechanistic investigation. However, it seems probable that the mechanism is similar to that of anhydride hydrolysis, which brings the reaction in line with the mechanism of amine acylation by other reagents. Substituents that increase the positive charge on the carbonyl carbon increase the rate of reaction. The reaction is also assisted by increasing the nucleophilic character of the nitrogen atom in the amine, and electron donating substituents in the amine increase the rate of acylation.

Following oxygen-18 studies, Denney and Greenbaum (58) have suggested the following scheme for the reaction between amines and benzoic anhydride. The tetrahedral intermediate (I) is expected, the second equilibrium is



not, but is necessitated by the experimental results. If a single carbonyl oxygen in the anhydride is labelled

with oxygen-18, then, on reaction with aniline in ether, the products give benzanilide containing 33% of the label and benzoic acid containing 67%, a result which can only arise if all of the oxygens in the anhydride become equivalent during the reaction. In this case, reversion to starting material from (II) could lead to equilibration of the oxygens. With ammonia (in the absence of ether as the solvent), the products, benzamide and benzoic acid, each contain 50% of the label, indicating that decomposition of (II) to products is much faster than return to starting materials.

Loucheux and Banderet (59) found that the reaction of various amines with acid anhydrides in benzene was catalyzed by the monomeric form[a] of the acid produced. The catalytic activity of carboxylic acids and their sulfur containing analogs in the acylation of aromatic amines was also studied by Litvinenko, et al. (60-63). Litvenenko, et al. (60) have suggested that the catalytic effect is due to complex formation (III or IV) and the catalytic power of the carboxylic acid increases with acid strength until protonation of the amine occurs. The evidence in favor of the formation of the complexes of this type is tenuous and without any

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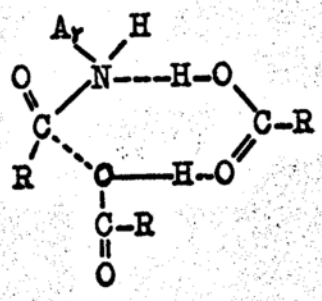
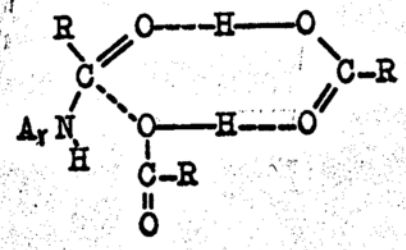
[a]In benzene solution carboxylic acids exist in a monomer $\rightleftharpoons$ dimer equilibrium and this situation leads to complex kinetics.



(III)



(IV)

significant precedent. Recent studies by Bruckenstein and Saito (64) indicate extensive formation of ion pairs and other ionic aggregates between carboxylic acids and amines in benzene, which may be a factor influencing the rate of acylation.

Fritz and Schenk (53,65) introduced an acylation procedure utilizing perchloric acid as the catalyst. The mechanism is believed to involve the formation of the acylium ion, which possesses great reactivity.

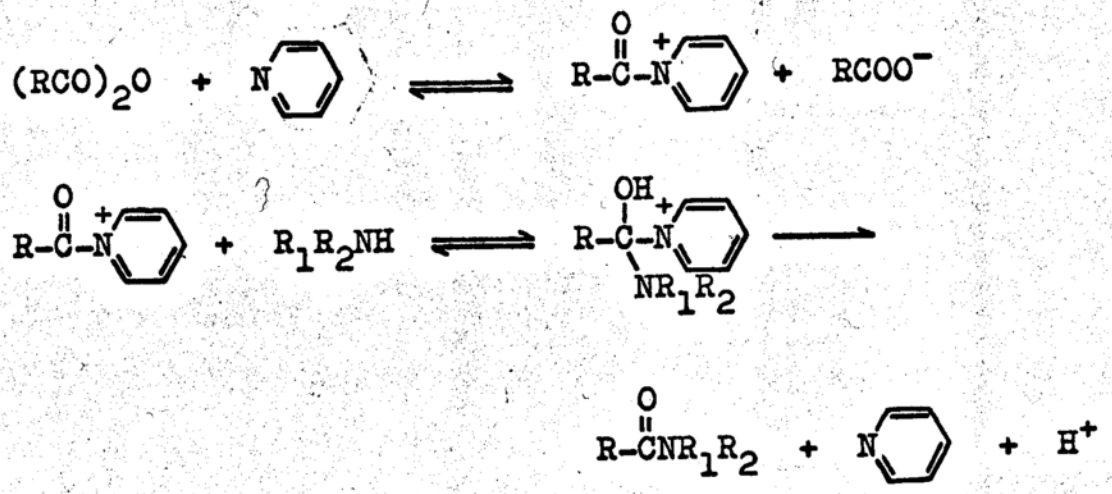
$$(Ac)_2O + HClO_4 \rightleftharpoons (Ac)_2OH^+ + ClO_4^-$$

$$(Ac)_2OH^+ \rightleftharpoons Ac^+ + HOAc$$

$$Ac^+ + R_1R_2NH \rightleftharpoons AcNR_1R_2 + H^+$$

Pyridine has also been applied as a catalyst for the determination of amines by the acylation method (1). In this case, pyridine has two roles: it reacts with the acidic product of the acylation, thus displacing the

equilibrium in favor of the product, and it acts as a catalyst. Its catalytic effect probably is exerted according to the following mechanism (125)



In mixtures of acetic anhydride and acetic acid, acylation of various nitroanilines reaches a maximum value of rate constant at 45% anhydride (67) which is also the mixture with maximum electrolytic conductivity (68). This suggests that ions participate in the reaction. Since nitroanilines are unprotonated in this solvent, the ion must be the acylium ion. Because the rate of reaction depends on the structure of the amine, ionization cannot be the rate-determining step, which must be attack of amine by the acylium ion.

The reaction of amines with mixed anhydrides has been investigated by Emery and Gold (69). In the acylation of aniline and 2,4-dinitroaniline by acetic chloroacetic anhydride in benzene, chloroacetylation predominates (86%), as expected on electronic grounds.

However, the product contains progressively larger amounts of the acetyl derivative as more polar solvents are employed; in 50% acetone-water 72% of the product is the acetanilide. The effect of further substitution in the anhydride moiety upon the rate of amine acylation is complicated by steric factors.

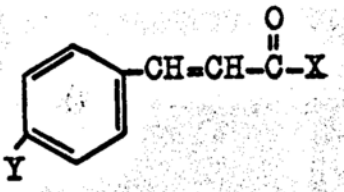
D. Research Plan

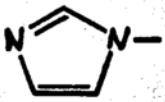
In determining amines spectrophotometrically after acylation, both sensitivity and selectivity can be influenced by selecting the acyl group for its spectral properties. A series of acyl groups, with their wavelengths of maximal absorption extending throughout the range of electronic absorption, would be desirable as a source from which the analyst could select the group suitable to his sample. The trans-cinnamoyl group, $C_6H_5CH=CH-\overset{O}{\overset{\|}{C}}-$, which possesses powerful light absorption in the ultraviolet region ($\epsilon_{max} \sim 2.2 \times 10^4$ for most compounds containing this function) does meet this requirement. Table II shows a variety of acylating agents with this function, which were chosen for this investigation. All of them represent some alteration either in the acyl group or in the "leaving group", through which it was hoped that some selectivity could be achieved.

Aung, et al. (70) introduced a kinetic approach to the identification of aliphatic amines by measuring the

Table II

Acylating Agents Related to trans-Cinnamic Acid



X	Y	Name[a]
C_6H_5-CH=CHCOO-	H	cinnamic anhydride
p-CH_3-C_6H_4CH=CHCOO-	CH_3	p,p'-dimethylcinnamic anhydride
p-CH_3O-C_6H_4CH=CHCOO-	CH_3O	p,p'-dimethoxylcinnamic anhydride
p-Cl-C_6H_4CH=CHCOO-	Cl	p,p'-dichlorocinnamic anhydride
	H	N-cinnamoylimidazole
Cl	H	cinnamoyl chloride
ClO_4	H	cinnamoyl perchlorate
α-chymotrypsin	H	cinnamoyl chymotrypsin

[a]All have the trans geometry about the double bond.

first-order dissociation in acidic solution of the complex between iron (II) and the Schiff base $RN{=}CHC_5H_5N$, formed by the test amine with 2-pyridine-carboxaldehyde. Recently, Robinson and his coworkers described the identification of alcohols from rates of alkaline hydrolysis of their 3,5-dinitrobenzoate esters (71) and of sugars from their rates of oxime formation (72). Apparently, kinetic studies provide a powerful approach to the characterization of organic compounds because of marked sensitivity of the reaction rate to structure of the reactants. Because of its potential for distinguishing between closely related compounds, and its advantages of sensitivity, simplicity and speed, rate measurement (actually rate constant measurement) cast a light on the feasibility of identifying aliphatic amines from the rate of cinnamoylation. In the meantime, such studies could also provide information to elucidate the reaction mechanism of cinnamoylation.

II. EXPERIMENTAL

A. Materials

trans-Cinnamic anhydride (J. T. Baker Chemical Co.) was recrystallized three times from benzene; mp 134-6° (lit. 136° (73)). The molar absorptivity at 294 mμ (the band maximum) was 4.32×10^4 in acetonitrile.

p,p'-Dimethoxy-, p,p'-dimethyl-, and p,p'-dichlorocinnamic anhydrides were synthesized as follows (74): 5×10^{-2} moles of p-substituted cinnamic acid and 10 g of thionyl chloride were mixed in a 50 ml glass-jointed round bottom flask which was fitted with a reflux condenser carrying a Drierite guard tube. The flask was heated on a water bath with occasional shaking for one hour. The excess of thionyl chloride was distilled off (bp 77°) slowly and the distillation continued until the temperature rose rapidly to about 120°; this ensured that all the thionyl chloride was removed. Then the residual acid chloride was distilled under reduced pressure. p-Methoxycinnamoyl chloride, pale yellow crystals; b_1 143-4° (lit. b_{4-5} 158-9°, mp 49-50° (75); yield, 7.6 g. p-Methylcinnamoyl chloride, yellow crystals; b_4 147°; mp 67-8° (76); yield, 7.6 g. p-Chlorocinnamoyl chloride, pale yellow crystals; b_6 145°; mp 78-9° (lit. 77-9° (75), 79-79.5° (77), 79-81° (78)).

A p-substituted cinnamoyl chloride (4×10^{-2} moles) obtained from above was added to 20 ml dry pyridine in a loosely stoppered 125 ml flask and was warmed on a steam bath for about 5 minutes. The reaction mixture was poured on 50 g of crushed ice and 20 ml of concentrated hydrochloric acid. The anhydride separated out at once. When the ice had melted sufficiently, the mixture was filtered by suction. The solid was washed with cold methanol and then with dry benzene. The crude anhydride was further recrystallized from benzene-cyclohexane, using Norit as a decolorizing adsorbent.

p-p'-Dimethoxycinnamic anhydride, white needles; mp 101-2°C; yield, 1.3 g. Anal. calcd. for $C_{20}H_{18}O_5$: C, 70.99; H, 5.36. Found: C, 70.92; H, 5.24.

p,p'-Dimethylcinnamic anhydride, colorless needles; mp 116-7° C; yield , 3.2 g. Anal. calcd. for $C_{20}H_{18}O_3$: C, 78.41; H, 5.92. Found: C, 78.05; H, 5.96.

p,p'-Dichlorocinnamic anhydride, pale brownish scale; mp 182-3°; yield, 1.0 g. Anal. calcd. for $C_{18}H_{12}O_3Cl_2$: C, 62.27; H, 3.48; Cl, 20.43. Found: C, 61.94; H, 3.48; Cl, 20.38.

trans-Cinnamic acid (Eastman Organic Chemicals) was recrystallized from 1% HCl using Norit as a decolorizing adsorbant; mp 132-3° (lit. 134-5°, (79)). p-Methoxy-, p-methyl-, and p-chlorocinnamic acids (Aldrich and Eastman products) were used without further purification.

trans-Cinnamoyl chloride (Eastman Organic Chemicals) was distilled under reduced pressure, colorless crystals; b_{13} 135°; mp 34.5-35.8° (lit. 35-6° (80)). The molar absorptivity at 299 mμ (the band maximum) was 2.43×10^4 in acetonitrile. N-trans-cinnamoylimidazole was prepared from trans-cinnamoyl chloride and imidazole by the method of Schonbaum, et al. (81). The crude product was further recrystallized from benzene-cyclohexane mixture, giving shiny colorless needles; mp 133-134.5° (lit. 133-133.5° (81)); ϵ_{300} 2.45×10^4 in acetonitrile (lit. ϵ_{310} 2.38×10^4, solvent, 0.1 M (total) acetate buffer containing 3.2% CH_3CN, pH = 5.05 (81)). Imidazole (Aldrich Chemical Co.) was recrystallized twice from benzene using Norit, mp 89-90° (lit. 89-90° (82)). Silver perchlorate, anhydrous (The G. Frederick Smith Chemical Co.) was dried at 110° for at least three hours and then dried under vacuum overnight. α-Chymotrypsin, three-time recrystallized, was a Worthington product.

N-substituted cinnamamides and N-substituted cinnamanilides were prepared as follows: 0.01 mole of trans-cinnamoyl chloride in 20 ml of dry benzene was added dropwise to 0.016 mole of amine dissolved in 20 ml of benzene. After two hours at room temperature, the solution was washed successively with water, 5% HCl, 0.1 N NaOH, and water. The solvent was evaporated under reduced pressure and the residue was recrystallized either from acetone-petroleum ether or acetone-

cyclohexane mixture. Norit was used whenever necessary. The melting points and elemental analyses are listed in Tables III and IV.

Acetonitrile (Fisher Scientific Co., Catalog No. A-21) was refluxed over phosphorus pentoxide and then distilled from P_2O_5 through a packed column (83); bp 81-81.5°. Methanol, chloroform, pyridine, perchloric acid, glacial acetic acid, ethyl acetate, sodium hydroxide, were of reagent grade and were used without further purification. Isooctane (2,2,4-trimethylpentane) (Matheson Coleman and Bell practical grade) was purified as described by Mollica and Connors (84). Cyclohexane was redistilled, bp 81° (lit. 80.7° (85)). Amines (Aldrich and Eastman products) were purified by distillation to constant boiling point.

Cinnamic anhydride reagent solution: 5 mg per ml in acetonitrile, freshly prepared.

Cinnamoyl chloride reagent solution: 3-4 mg per ml in acetonitrile, freshly prepared.

0.02 M cinnamic anhydride titrant was freshly prepared by dissolving 55.66 mg of trans-cinnamic anhydride in enough acetonitrile to make 10 ml.

Buffer chemicals were of reagent grade. Water was redistilled from alkaline permanganate in an all-glass system. Standard buffers were prepared according to Bates (86) using freshly boiled redistilled water.

Table III

N-Substituted Cinnamamides

Cinnamamide	mp(°C)	lit. mp(°C)
N-Propyl	88.5-89.5	
N-Butyl	80-80.5	85 (89)
N-t-Butyl	148-149	143 (90), 152-4 (91)
N-Hexyl	43-44	
N-Octyl	79-80	
N-Dodecyl	75-76	74-74.5 (92)
N-Allyl	91-92	90-2 (93)
N-Benzyl	111-111.5	103-4 (94), 111-111.5 (95), 94-6 (95)
N-β-phenethyl	127-128	
N-Piperidyl	118-119	122 (97)
N,N-Diethyl	69.5-71	72 (97), 65-66 (98)
N,N-Dibenzyl	131.5-132.5	
N-Morpholyl	92-93	94 (97)
N-α- Naphthyl	220-221	217 (99)
N-β-Naphthyl	181-182	
N-Butyl-p-methoxy	83-84	
N-Butyl-p-methyl	94-95	
N-Butyl-p-chloro	120.5-121.5	

[a]Elemental analyses by Spang Microanalytical Laboratory, Ann Arbor, Michigan 48106.

Table IV

Substituted Cinnamanilides

Cinnamanilide	mp(°C)	lit. mp(°C)
Cinnamanilide	154-5	152-4 (100), 150 (101)
N-Methyl	234-5/14 mmHg[b]	231/15 mmHg (102)[b]
N-Ethyl	238-9/15 mmHg[b]	234/15 mmHg (102)[b]
N-Isopropyl	100.5-101	
p'-Chloro	185-6	185-6 (103), 185.5 (104)
p'-Bromo	194-5	191 (105)
p'-Iodo	204.5-206	190 (106)
p'-Methoxy	153-154.5	149 (107), 152-3 (108)
m'-Methoxy	123-4	115 (108), 121-2 (109), 124 (110)
o'-Methoxy	117-118	136-8 (109), 116 (111)

[a]Elemental analyses by Spang Microanalytical Laboratory, Ann Arbor, Michigan 48106.

[b]Boiling point.

Other buffers were prepared according to published formulas (87,88).

Tables V and VI list useful spectrophotometric data for some of the materials described in this section.

B. Apparatus

Spectral measurements were made with either a Cary Model 14 or Model 15 recording spectrophotometer fitted with a thermostated cell compartment that maintained temperature constant to $\pm 0.1°$.

Spectrophotometric titrations were done in a 50-ml photometric titration flask (112), a flow-through cylindrical 1-cm cuvette being connected to the circulation flask with glass tubing and short lengths of rubber tubing. Titrant was delivered from a 2-ml micrometer buret (Roger Gilmont Instruments), the tip of which passed through a rubber stopper in the mouth of the titration flask.

pH was measured with a Sargent pH meter model DR equipped with a Sargent-pH Combination Electrode (either S-30070-10 or S-30072-15). The meter-electrode system was standardized against the basic standard buffers recommended by Bates (86).

Water bath temperatures were maintained to $\pm 0.1°$ with Brownwill Scientific Thermoregulator. Thermometers were checked against a thermometer carrying a National Bureau of Standards certificate.

Table V

Ultraviolet Spectrophotometric Data for N-Substituted trans-Cinnamamides

Cinnamamide	In Chloroform		In Methanol	
	λ_{max}	$10^{-4} \epsilon_{max}$ [a]	λ_{max}	$10^{-4} \epsilon_{max}$ [b]
N-Propyl	274	2.27	273	2.45
N-Butyl	274	2.22	273	2.54
N-t-Butyl	273	2.18	272.5	2.54
N-Hexyl	274	2.21	273	2.54
N-Octyl	274	2.27	273	2.50
N-Dodecyl	274	2.27	273	2.57
N-Allyl	274	2.27	273.5	2.49
N-Benzyl	275	2.44	275	2.68
N-β-phenethyl	274	2.31	273	2.62
N-Piperidyl	277.5	2.18	278.5	2.42
N,N-Diethyl	277	2.14	279	2.48
N,N-Dibenzyl	283	2.36	284	2.60
N-Morpholyl	280	2.17	281	2.40
N-α-Naphthyl	283	2.11	283	2.45
N-β-Naphthyl	285	3.18	283.5	3.55
N-Butyl-p-methoxy	292	2.21	291	2.62
N-Butyl-p-methyl	285	2.35	284	2.72
N-Butyl-p-chloro	280	2.51	277	2.85

[a] Beer's law is followed by all cinnamamides in chloroform solution.

[b] Based on one concentration.

Table VI

Ultraviolet Spectrophotometric Data for trans-Cinnamanilides

Cinnamanilide	In Chloroform		In Methanol	
	λ_{max}	$10^{-4}\,\epsilon_{max}$ [a]	λ_{max}	$10^{-4}\,\epsilon_{max}$ [b]
Cinnamanilide	295	2.58	294	2.82
N-Methyl-	285	2.09	286	2.29
N-Ethyl-	285	2.24	286	2.33
N-Isopropyl-	285	2.18	285	2.41
p'-Chloro-	298	2.88	297.5	3.16
p'-Bromo-	300	2.93	302	3.26
p'-Iodo-	304	3.03	303.5	3.37
p'-Methoxy-	293	2.23	292.5	2.31
m'-Methoxy-	295	2.48	291	2.68
o'-Methoxy-	307	2.09	285	2.28

[a] Beer's law is followed by all cinnamanilides in chloroform solution.

[b] Based on one concentration.

Melting points were determined on a Thomas-Hoover Capillary Melting-Point apparatus.

C. Spectrophotometric Determination of Aliphatic Amines

Transfer 1.0 ml of sample solution containing 0.7-4.2 μmole of amine (dissolved in acetonitrile) to a 50-ml volumetric flask. Add 1.0 ml of cinnamic anhydride solution (or p,p'-disubstituted anhydride solution) and one drop of 0.1 M tri-n-butylamine (in acetonitrile). Mix the solution well and allow it to stand at room temperature until acylation is complete (see Results section). Add 2 ml of 0.1 N aqueous sodium hydroxide, mix well, and then add 0.1 N NaOH approximately to volume. Allow to stand 10 minutes to hydrolyze the excess reagent. Quantitatively transfer the solution, with the aid of 15 ml of chloroform, to a 125-ml separatory funnel fitted with a stopcock made of Teflon (Dupont). (If a glass-stopcock separatory funnel is employed, a dry-film lubricant, e.g., Fluo-Kem, should be used instead of a stopcock grease.) Extract with three portions of chloroform (15, 15, and 10 ml, the first two portions having been used in the transfer). Combine the chloroform extracts in a separatory funnel and wash with 20 ml of water; filter the extract through filter paper into a 50-ml volumetric flask. Rinse the filter paper with chloroform, adding the rinsings to the flask. Dilute to volume with chloroform. Measure the

absorbance of this solution against chloroform in a 1-cm cell at the wavelength of maximum absorption for the amide. Prepare a blank solution by omitting the amine, carrying it through the entire procedure, and measure its absorbance.

Calculate the number of micromoles of amine contained in the 1 ml of initial sample as shown:

$$\underset{\text{amine}}{\mu\text{moles of}} = \frac{5 \times 10^4 \, (A_s - A_b)}{b \, \epsilon_{max}}$$

where A_s and A_b are the absorbances of the sample and blank final solutions, b is the cell path length, and ϵ_{max} is the molar absorptivity of the amide. Beer's law is followed by all cinnamamides in chloroform solution as shown in Figure 1.

Some other acylating agents, namely a saturated solution of N-trans-cinnamoylimidazole in acetonitrile and cinnamoyl perchlorate solution, were also used in the analysis by the procedure described above.

A modified procedure was used when trans-cinnamoyl-α-chymotrypsin was studied as an acylating agent:

Transfer 4.0 ml of phosphate buffer (pH 8.02) into a 25-ml volumetric flask containing about 45 mg of α-chymotrypsin. Shake until solution is complete and add 0.1 ml cinnamoylimidazole solution (3 mg per milliliter in acetonitrile). Mix the solution well, and

allow to stand at room temperature for one minute. Add 1 ml of sample solution containing approximately 1 μmole of amine (in water). Mix well and allow to stand for another 10 minutes, then quantitatively transfer the solution, with the aid of 20 ml of chloroform, to a 125-ml separatory funnel fitted with a stopcock made of Teflon (Dupont). Extract with two portions of chloroform (20 and 20 ml all having been used in the transfer). Then follow the same procedure as described in C.

D. Spectrophotometric Determination of Aromatic Amines

Transfer 1.0 ml of sample solution containing 0.5-2.0 μmoles of aromatic amine (dissolved in acetonitrile) to a 50-ml volumetric flask. Add 1.0 ml of cinnamoyl chloride solution and one drop of 0.1 M tri-n-butylamine (in acetonitrile). Mix the solution well and allow it to stand at room temperature for about 15 minutes. Add 2 ml of 0.01 N aqueous sodium hydroxide, mix well, and then add 0.01 N NaOH approximately to volume. Follow the same procedure thereafter as described in C.

E. Spectrophotometric Titration of Aliphatic Amines

Acetonitrile (24 ml) was delivered into the titration flask and the cuvette was firmly positioned in the light path. A 1.0 ml aliquot of an amine sample solution, containing about 20 μmoles of amine per ml (in

acetonitrile) was added to the flask. Titrant was added and the solution was stirred magnetically; stirring ensures homogeneity, but is also necessary to circulate the solution to the cuvette. Absorbance was monitored, at a fixed wavelength, until it assumed a constant value, when a reading was taken. Further titrant was then added. Usually three absorbance readings were taken before the end-point and three after the end-point. A titration plot was made of absorbance against titrant volume; the end-point is marked by the intersection of the two straight line segments.

F. Amine Acylation Kinetics

The kinetics of the acylation reactions were studied by following the disappearance of absorption of acylating agent at a suitable wavelength when acetonitrile solutions of the acylating agent and amine were mixed. Many of the reactions were very fast for conventional measurements, and the rates were decreased to accessible values by working at low reactant concentrations. All reactions were studied at 25°±0.1°C. The reaction between N-trans-cinnamoylimidazole and amine was also studied by following the rate of formation of amide with the extraction method.

a) Kinetics between cinnamic anhydride (also its p,p'-disubstituted anhydrides) and amines.

Acetonitrile solutions of cinnamic anhydride (approximately 4×10^{-6} M) and of an amine (5.0×10^{-5} to

2.5×10^{-2} M, depending upon reactivity) were equilibrated at 25.0±0.1°. Fifteen milliliters of each solution was mixed in a 50-ml beaker and rapidly transferred to a 10-cm cylindrical cell. The absorbance of the reaction mixture at 305 mμ, 320 mμ and 330 mμ, for cinnamic anhydride, p,p'-dimethylcinnamic anhydride and p,p'-dimethoxycinnamic anhydride, respectively, was followed with time as a continuous trace on the recorder charts.

b) Kinetics between N-trans-cinnamoylimidazole and amines.

1) Direct spectrophotometric method.

Reactions were initiated by mixing 2 ml of amine solution (0.1 to 2.5 M, depending upon reactivity) and 2 ml of N-trans-cinnamoylimidazole solution (9.0×10^{-5} M) and quickly transferring to a 1-cm cuvette. The change in absorbance was followed at 310 mμ.

2) Extraction method.

15 ml of amine solution, containing 1.5-3.0 μmoles of amine per milliliter in acetonitrile, was transferred to a 50-ml volumetric flask. 15 ml of saturated N-trans-cinnamoylimidazole solution (in acetonitrile) was added. The mixture was shaken and allowed to stand in a constant temperature bath (25±0.1°C). At suitable time intervals, two ml of the reaction mixture were withdrawn and transferred to a 50-ml volumetric flask.

0.1 N aqueous sodium hydroxide is added to volume. The amount of amide formed was determined as described in C.

In the studies of acid-catalyzed acylation, the acid, such as trans-cinnamic acid, benzoic acid, and acetic acid, was incorporated into the solution of acylating agent.

G. Anhydride Hydrolysis Kinetics

Two ml of 1.2×10^{-4} M cinnamic anhydride solution in acetonitrile was poured into 50 ml of aqueous borate buffer. The mixed solution was transferred to a 10-cm cylindrical cuvette and its absorbance was monitored at 305 mμ.

H. Anilide Hydrolysis Kinetics

25 ml of water (borate buffer or aqueous sodium hydroxide solution) and 1 ml of cinnamanilide solution (approximately 1.6×10^{-4} M in acetonitrile) were mixed in a 50-ml beaker and rapidly transferred to a 5-cm cylindrical cuvette. The rate of disappearance of anilide was then followed at the wavelength corresponding to the maximum absorption.

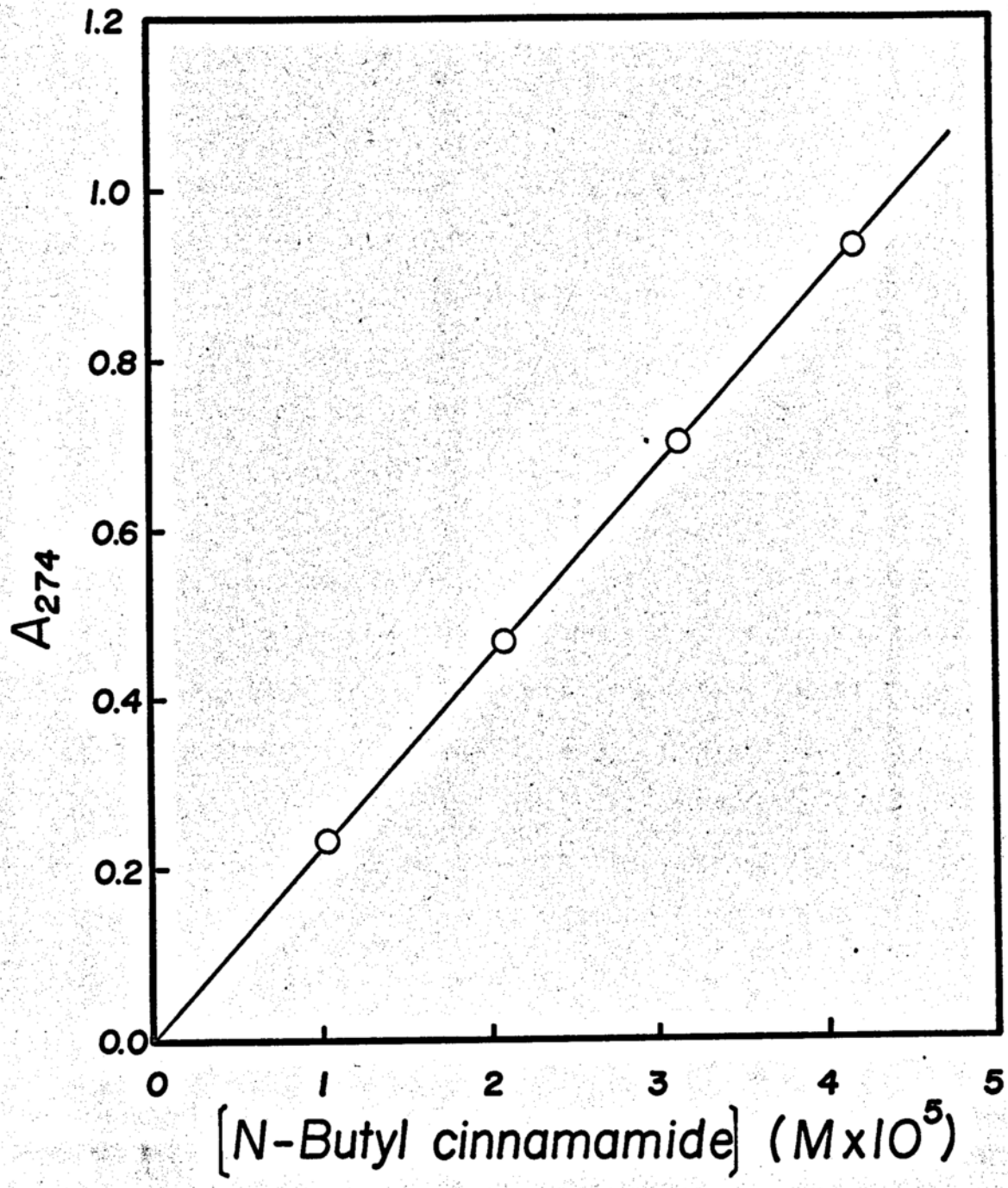


Figure 1. Beer's law plot of N-butyl cinnamamide in chloroform.

III. RESULTS

A. trans-Cinnamic Anhydride and p,p'-Disubstituted Cinnamic Anhydrides

The analytical results by the trans-cinnamic anhydride method are presented in Tables VII to X.

Table VII lists the analytical data for twelve typical aliphatic amines, with the mean and standard deviation expressed in terms of per cent purity. As a comparison analysis, the perchloric acid-catalyzed acetylation method of Fritz and Schenk (65) was applied to the same samples, and these results are included in Table VII. The results by the two methods agree well, especially so considering that the cinnamoylation method is carried out on the micromole scale while the acetylation procedure used millimole samples. The standard deviation of the new procedure is approximately 0.5-1.0% (relative).

The initial ratio of trans-cinnamic anhydride to amine was varied from 1.2 to 24.3 (five different amines being studied) without significantly altering the extent of conversion to amide (Table VIII).

The effect of water and methanol on the analysis was investigated with the results shown in Table IX. Even when the amine sample solution contains as much as 60% water, no interference is observed. No interference is observed too from methanol in the sample, if the hydrolysis time in the alkali is increased to permit

Table VII

Analysis of Amines by the trans-Cinnamic Anhydride Method

Amine	Cinnamic anhydride method, % purity mean[a]	std. dev.	Comparison method[b] % purity mean[a]	std. dev.
n-Propyl	94.7	0.14	96.3	0.64
n-Butyl	97.2	1.13	97.4	0.22
t-Butyl	99.1	0.56	95.3	1.16
n-Hexyl	96.4	0.61	98.5	0.16
n-Octyl	98.3	0.83	96.4	1.45
n-Dodecyl	97.5	0.19	96.3	0.46
Allyl	97.1	0.58	97.2	0.61
Benzyl	95.7	0.28	97.7	0.17
β-Phenethyl	97.9	0.23	98.1	1.20
Piperidine	95.3	0.67	96.0	0.81
Diethyl	96.4	1.08	96.4	1.43

[a]mean of 4 to 16 determinations.

[b]ref. (65).

[c]mean of 3 determinations.

Table VIII

Determination of Aliphatic Amines at Various Ratios of *trans*-Cinnamic Anhydride to Amine

Amine	[Anhydride]/[Amine]	% Recovery mean[a]	% Recovery std. dev.
n-Butyl	4.47	97.0	0.20
	7.45	98.6	0.93
	9.14	97.7[b]	--
n-Hexyl	4.4	96.5	0.32
	5.5	96.8	0.21
	7.3	96.5	0.21
	10.9	95.5	0.61
β-Phenethyl	1.2	97.9	0.70
	7.0	97.9	0.23
Diethyl	4.9	96.0	0
	8.1	95.8	0.07
	12.2	97.1	0.29
	24.3	96.5	1.77
Dibenzyl	6.1	95.4	0.17
	8.1	94.9	0.10
	12.2	95.7	0.52
	24.3	95.1	0.41

[a]mean of 3 to 6 determinations unless otherwise noted.

[b]mean of 2 determinations.

Table IX

Determination of Amines in the Presence of Water and Methanol

Amine, A	Interference, I	(I)/(A)	% Recovery mean[a,b]	% Recovery std.dev.
Allyl	Water (60%)[d]	9,300	96.3 (97.1)	0.34
β-Phenethyl	Water (60%)[d]	13,000	97.8 (97.9)	0.74
n-Butyl[c]	Methanol (20%)[d]	1,500	97.0 (97.2)	0.08

[a]mean of 4 determinations.

[b]The value in parentheses is that obtained in the absence of I.

[c]hydrolysis time, 20 minutes.

[d]volume per cent of I in the initial amine sample solution.

preferential hydrolysis of the ester.

A series of p,p'-disubstituted cinnamic anhydrides has also been studied for the analysis of n-butylamine, and the results are shown in Table X, which shows satisfactory recoveries except with p,p'-dichloro cinnamic anhydride, which gave low results. This may be due to its low solubility in acetonitrile.

Table X

Analysis of n-Butylamine by p,p'-Disubstituted Cinnamic Anhydrides

Acylating agent	% Recovery mean[a]	std. dev.
Cinnamic anhydride	97.20	1.13
p,p'-Dimethoxy cinnamic anhydride	97.7	1.01
p,p'-Dimethyl cinnamic anhydride	98.9	0.63
p,p'-Dichloro cinnamic anhydride	59.6	0;15

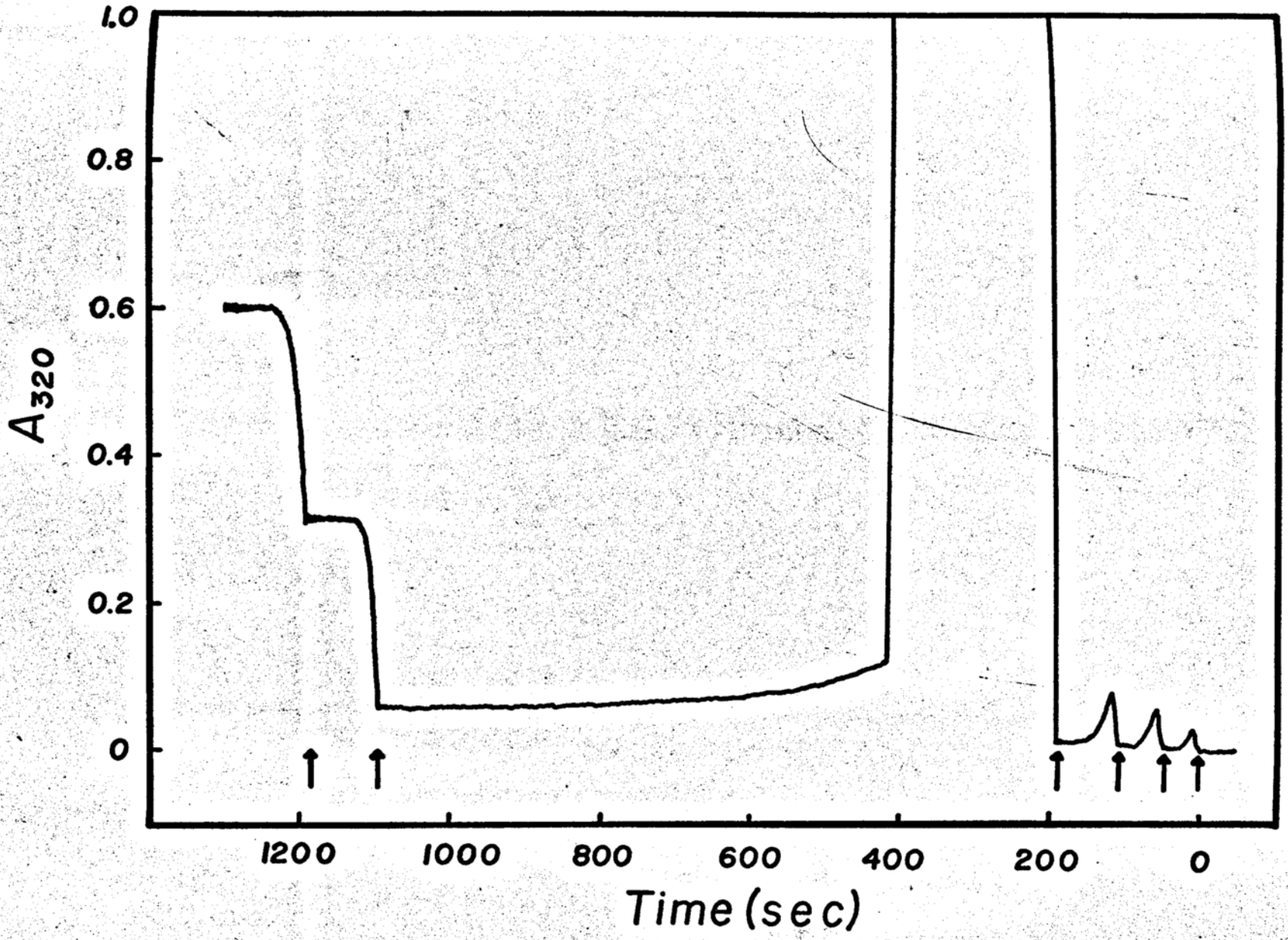
[a]mean of 3 to 16 determinations.

The difference in spectral properties between trans-cinnamic anhydride and cinnamamides suggested that the separation step of the analysis might be eliminated by carrying out a direct titration of the amine with the anhydride, the progress of the titration being followed

spectrophotometrically. Figure 2 shows the absorbance tracing in a typical titration. Although the second-order rate constants for the reaction between amines and trans-cinnamic anhydride are quite large (Table XII), the reaction rates are not high because the reactant concentrations are quite low in these systems. The decay in absorbance after each titrant addition is clearly visible in the figure. After the fourth addition of titrant, the reaction is at its slowest, because with the slight excess of anhydride the amine concentration is driven to a very low value. The approach to a constant absorbance after the fifth and sixth additions is controlled by the circulation rate in the titration assembly. The appearance of the titration plot is determined by the wavelength at which measurements are made. Figure 3 shows a titration plot of data taken at 320 mμ.

Table XI lists analytical results for the spectrophotometric titration of seven amines with trans-cinnamic anhydride. The slow-reacting amines tended to give slightly higher results in the photometric titration method as compared with those obtained from the previous extraction method.

The rates of acylation of amines by trans-cinnamic anhydride in acetonitrile solution were studied spectrophotometrically. The amine concentration was in


1.0
0.8
0.6
0.4
0.2
0
A_{320}
1200
1000
800
600
400
200
0
Time (sec)

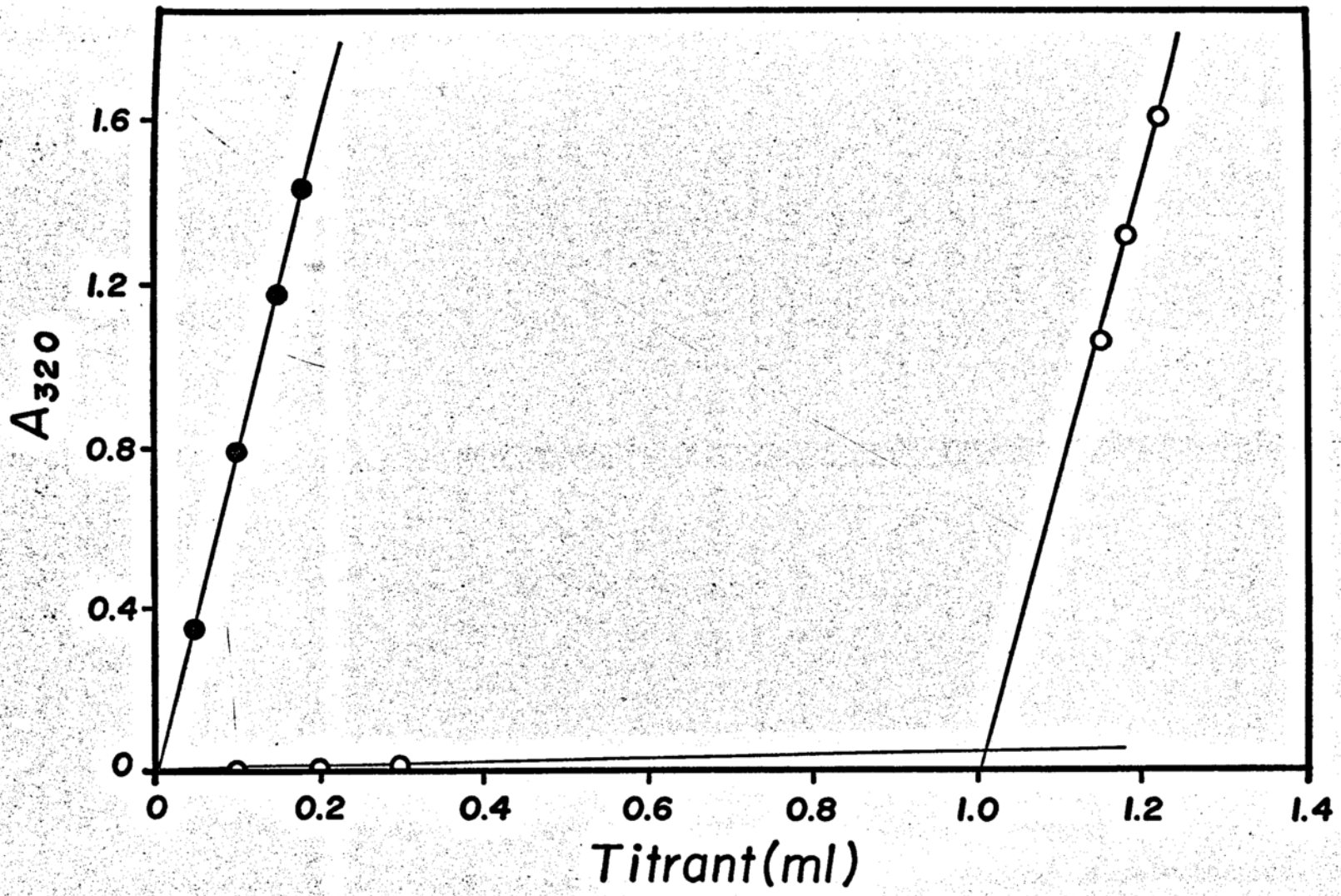


Figure 3. Spectrophotometric titration of 20 μmoles of n-butylamine. (●) blank titration; (o) sample titration.

Table XI

Spectrophotometric Titration of Amines with trans-Cinnamic Anhydride[a]

Amine	% Purity mean[b]	std. dev.
n-Butylamine	96.7	0.56
n-Hexylamine	97.9	0.10
n-Octylamine	99.1	0
Benzylamine	99.8	1.90
β-Phenethylamine	99.6	0.10
Diethylamine[c]	101.0	1.91
Piperidine[c]	98.8	0.35

[a] at 320 mμ unless otherwise noted.

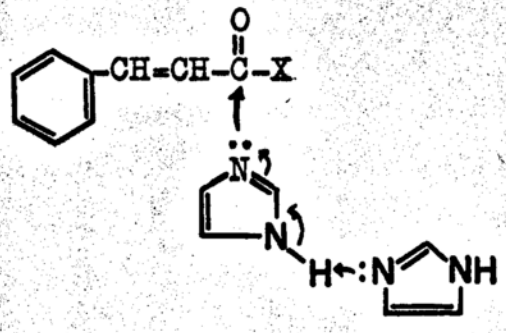
[b] mean of 4–6 determinations.

[c] at 325 mμ.

excess, and the change in absorbance as the anhydride was converted to products was followed at 305 mμ. The data gave linear first-order plots (Figure 4), showing that the reaction is first-order with respect to the anhydride. From the slopes of these plots the apparent first-order rate constants were evaluated. Variation in amine concentration led to a change in the first-order constant that was directly proportional to the change in the amine concentration; the reaction is therefore

first-order with respect to the amine. The second-order rate constants are given in Table XII. The reaction kinetics of a series of p,p'-disubstituted cinnamic anhydrides with n-butylamine, sec-butylamine, iso-butylamine and tert-butylamine have also been studied, with the results shown in Table XIII.

There is, however, a significant difference in the acylation of imidazole by trans-cinnamic anhydride. In this reaction, the rate expression has two terms, one being first-order in imidazole and the other being second-order in imidazole. It is probable that the term that is second-order in imidazole is due to the general base-catalysis of the addition of imidazole to the carbonyl carbon (113).



The mechanism for the reaction between trans-cinnamic anhydride and imidazole can be shown as follows:

$$CAn + Im \xrightarrow{k_1} p + c$$

$$CAn + 2Im \xrightarrow{k_2} p + c + Im$$

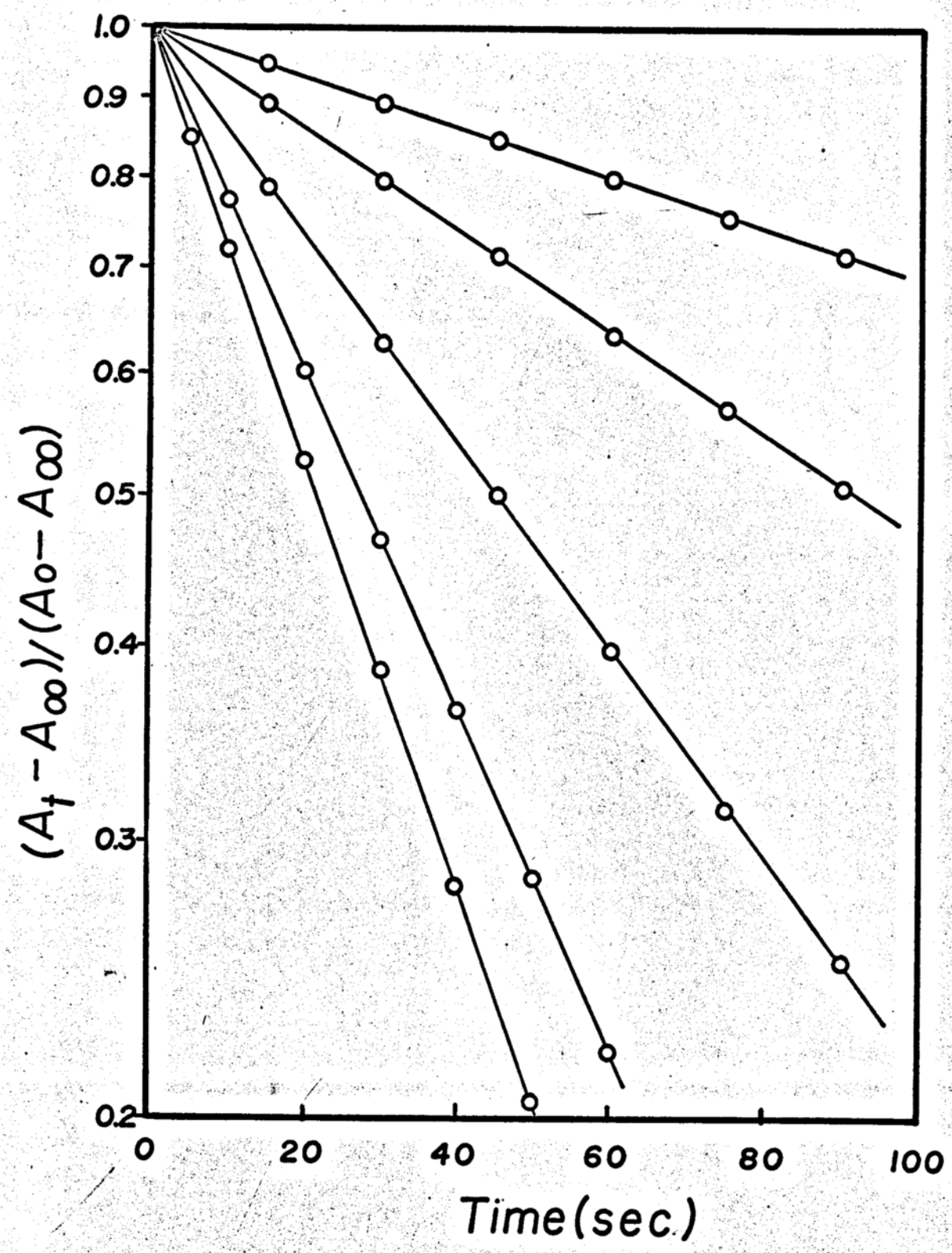

1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0
20
40
60
80
100
$(A_t - A_\infty)/(A_o - A_\infty)$
Time(sec.)

Table XII

Acylation Rate Constants for Aliphatic Amines by *trans*-Cinnamic Anhydride in Acetonitrile at 25°C

Amine	bp, °C[a]	k (M^{-1} sec^{-1})[b] Mean[c]	Av.dev.[d]	pK_a
Isopropyl-	33 (32-33)	21.42	0.09	10.63
tert-Butyl-	46 (44-45)	0.436	0.001	10.82
n-Propyl-	49 (47)	134.5	3.6	10.53
Diethyl-	55 (54)	30.3	0.6	10.98
Allyl-	56 (53)	33.8	0.5	9.69
sec-Butyl-	63 (62)	16.95	0.07	10.56
Isobutyl-	69 (66-66.3)	115.3	0.0	10.72
n-Butyl-	77 (76)	158.3	0.4	10.60
Isoamyl-	95 (94-95)	156.8	5.9	10.6
n-Amyl-	104 (103-105)	160.8	4.5	10.64
Cyclopentyl-	105 (104-106)	46.0	0.8	9.95
Piperidine	109 (104-105)	1139	17	11.22
Di-*n*-propyl-	110 (109)	23.8	0.1	11.00
n-Hexyl-	128 (129-130)	168.8	5.7	10.64
Morpholine	130 (130-131)	151.6	4.1	8.70
Cyclohexyl-	134 (131-131.2)	25.9	0.3	10.64
Diisobutyl-	139 (137-139)	6.76	0.27	10.68
N-Methylcyclohexyl-	146 (146.5-147.5)	17.37	0.01	10.9
N-Ethyl-cyclohexyl-	164 (163-164)	0.629	0.006	11.3
N-Isopropyl-cyclohexyl-	174 (171-172)	0.107	0.004	11.3
n-Octyl-	180 (178-180)	164.4	3.7	10.65
Benzyl-	184 (181-183)	25.97	0.02	9.34
Diisoamyl-	187 (186-189)	3.69	0.05	10.94

Table XII - Cont.

Amine	bp, °C[a]	k (M^{-1} sec^{-1})[b] Mean[c]	Av.dev.[d]	pK_a
β-Phenethyl-	198 (197-198)	58.3	0.5	9.83
Diisooctyl-	--- (276)	8.34	0.03	--
Dibenzyl-	300 (285)	1.08	0.02	--

[a]Values in parentheses are the author's values.

[b]At 25° in acetonitrile solution.

[c]Mean of 2 to 5 determinations.

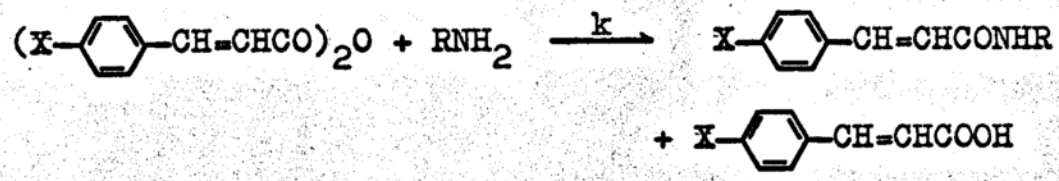
[d]Average deviation = $\Sigma|X_i - \bar{X}|/(n-1)$.

Table XIII

Acylation Rate Constants for Aliphatic Amines by p,p'-Disubstituted Cinnamic Anhydride in Acetonitrile at 25°C

X[a]	k (M^{-1} sec^{-1})[b]			
	n-$BuNH_2$	i-$BuNH_2$	sec-$BuNH_2$	t-$BuNH_2$
CH_3O	39.6	30.9	4.60	0.127
CH_3	80.0	60.8	9.54	0.262
H	158.3	115.3	16.95	0.436

[a]In the following reaction:



[b]mean of 3 to 4 determinations.

Here, CAn represents the trans-cinnamic anhydride and Im, the imidazole; p is the product, trans-cinnamoyl-imidazole, and c, trans-cinnamic acid. Since the reaction was followed by the disappearance of CAn, therefore,

$$\frac{-d[CAn]}{dt} = k_1[CAn][Im] + k_2[CAn][Im]^2$$

$$= \left\{ k_1[Im] + k_2[Im]^2 \right\} [CAn]$$

$$= k_{obs}[CAn]$$

where,

$$k_{obs} = k_1[Im] + k_2[Im]^2$$

Figure 5 shows a plot of k_{obs} divided by imidazole concentration, $k_{obs}/[Im]$, vs. imidazole concentration, [Im], with an intercept (k_1) of 9.27×10^{-2} M^{-1} sec^{-1} and a slope (k_2) of 5.18×10^{-1} M^{-2} sec^{-1}.

The alkaline hydrolysis of trans-cinnamic anhydride is first-order in anhydride and in hydroxide (Figure 6). The apparent first-order rate constants are listed in Table XIV. A plot of log k_{obs} against pH is slightly curved at the lower pH end (Figure 7), indicating marked incursion of the uncatalyzed "water" reaction, so that the apparent first-order rate constant can be related to hydroxide activity by: $k_{obs} = k_{H_2O} + k_{OH}a_{OH}$ (though it is possible that the buffer components may be involved

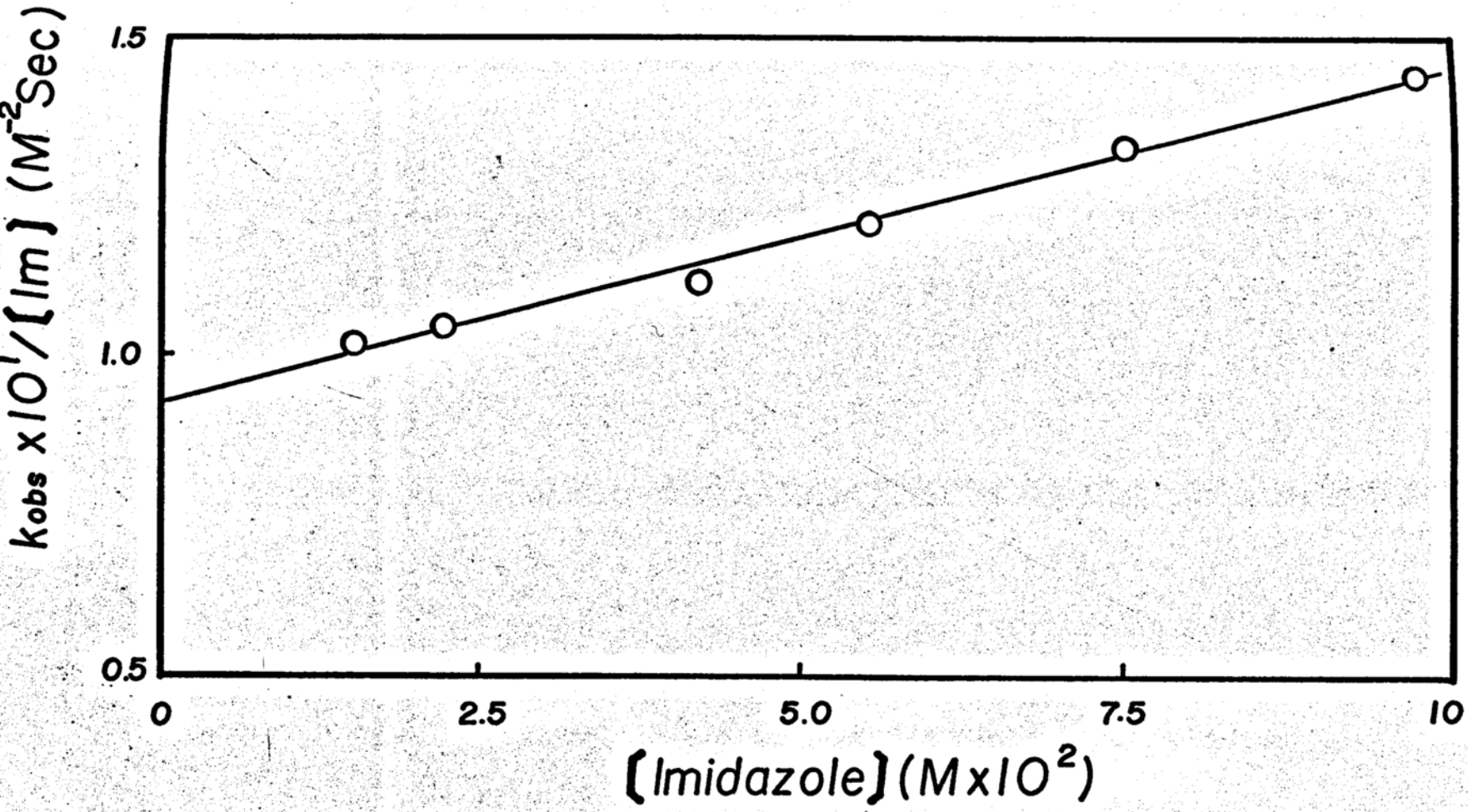


Figure 5. A plot of k_{obs}/[imidazole] against [imidazole] for the reaction of <u>trans</u>-cinnamic anhydride and [imidazole] in acetonitrile.

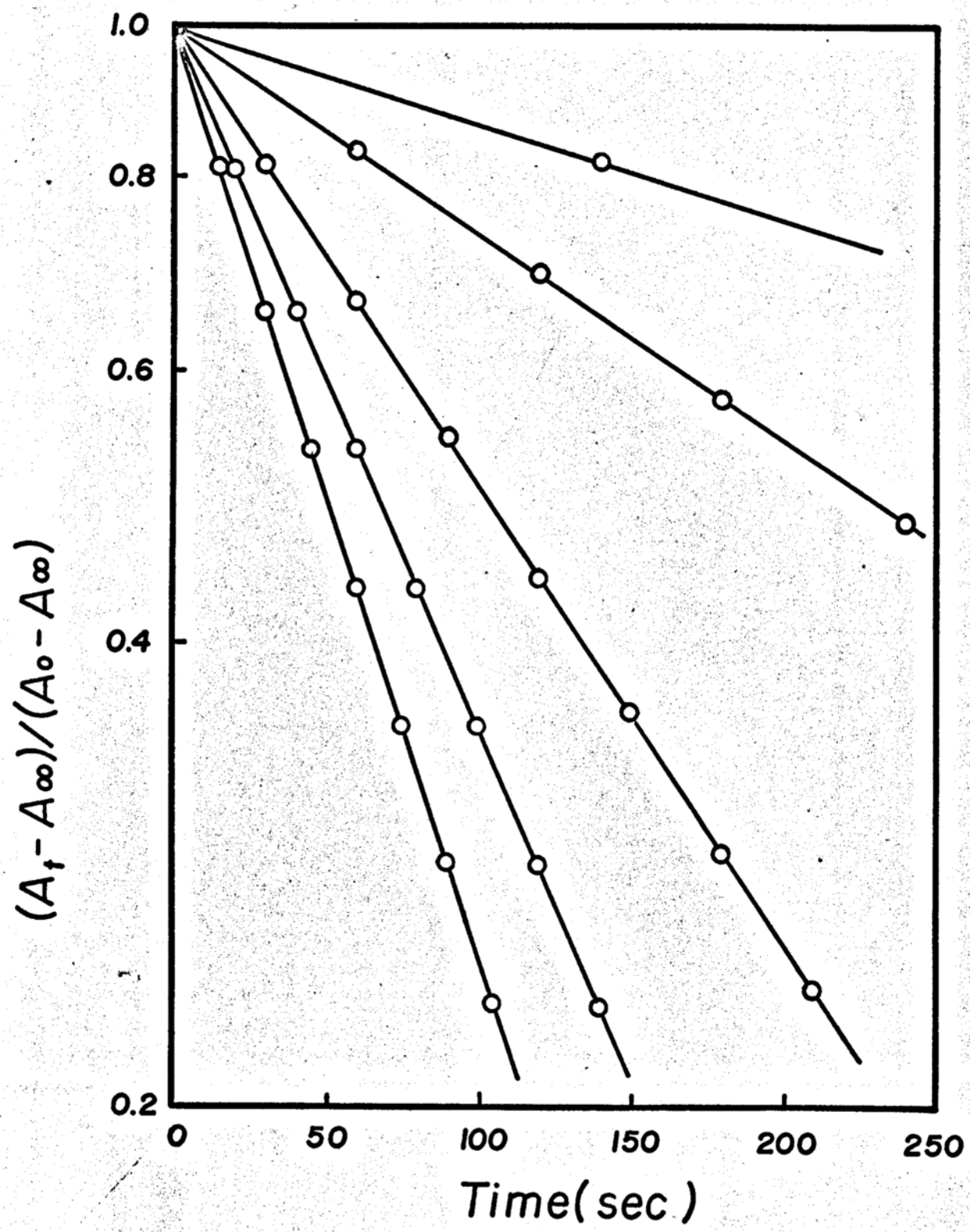


Figure 6. First-order plots for the alkaline hydrolysis of trans-cinnamic anhydride in borate buffer (total borate concentration 0.0125 M). From top to bottom: pH 8.72; pH 9.16; pH 9.64; pH 9.84; pH 9.95.

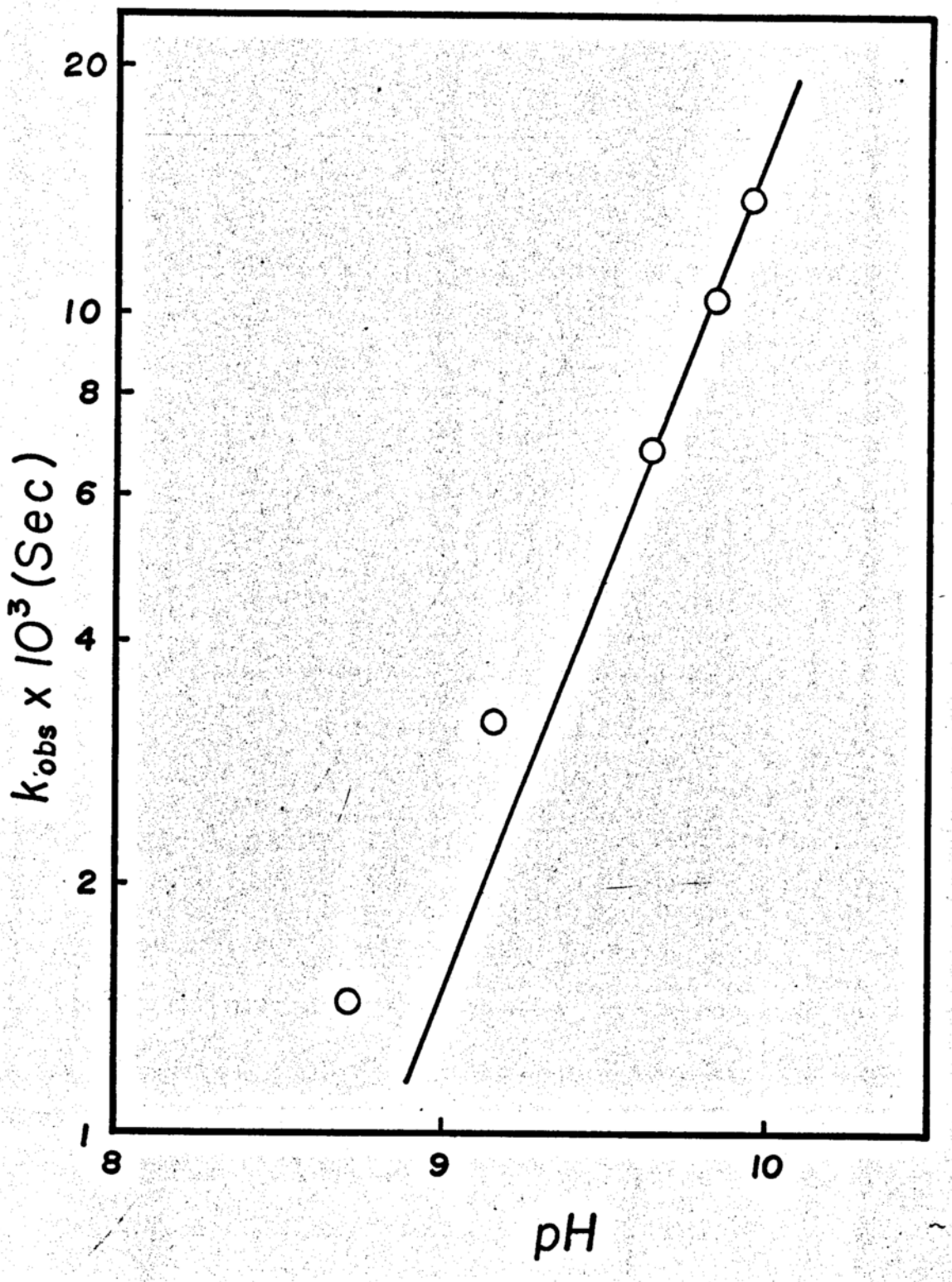


Figure 7. pH rate profile of alkaline hydrolysis of <u>trans</u>-cinnamic anhydride in borate buffer (total borate concentration 0.0125 M).

in the rate equation). k_{OH} has the value 155 M^{-1} sec^{-1}.

Table XIV

Kinetics of Hydrolysis of trans-Cinnamic Anhydride at 25°C

pH	10^3 k_{obs} $(sec^{-1})^a$
8.72	1.44
9.16	3.09
9.64	6.87
9.84	10.49
9.95	13.87

[a]In aqueous borate buffers containing 4% (v/v) acetonitrile. Total borate concentration 0.0125 M; ionic strength ranged from 0.042 to 0.025.

B. trans-Cinnamoyl Chloride

Generally, the acid chlorides are more reactive acylating agents than the corresponding anhydrides, which usually do not react quantitatively with aromatic amines. trans-Cinnamoyl chloride has been satisfactorily applied to the analysis of some aromatic amines with the results shown in Table XV. For comparison, the pyridine-catalyzed acetylation method of Ogg, Porter and Willits (114) was applied to the same samples, and these results are also included in Table XV. The results indicate that

Table XV

Analysis of Aromatic Amines by the *trans*-Cinnamoyl Chloride Method

Amine	Cinnamoyl chloride method, % purity		Comparison method[b] % purity	
	Mean[a]	Std.dev.	Mean[c]	Std.dev.
Aniline	98.4	0.25	99.8	0.06
N-Methylaniline	96.3	1.89	101.6	1.30
N-Ethylaniline	94.4	0.34	100.3	0.46
N-Isopropylaniline	67.5[d]	0.45	39.2	0.50
p-Chloroaniline	96.0	0.64	100.7	0.13
p-Bromoaniline	95.8	0.43	102.7	0.34
p-Iodoaniline	95.5	0.48	103.2	0.61
p-Methoxyaniline	94.3	0.71	101.2	0.19
m-Methoxyaniline	93.5	0.45	98.9	0.78
o-Methoxyaniline	96.5	0.09	100.4	0.18
α-Naphthylamine[e]	96.3	0.33	100.8	0.48
β-Naphthylamine[e]	97.7	0.21	101.2	0.86

[a] mean of 3 to 9 determinations.

[b] Ref. (114).

[c] mean of 3 determinations.

[d] The per cent recovery could be increased to 89% if the reaction time was extended to about 19 hours.

[e] used without further purification.

the cinnamoylation method gives lower per cent recovery of the amine than the pyridine-catalyzed acetylation. Analysis of sterically hindered aromatic amine, N-isopropylaniline, failed by both methods.

The acylation time employed for all amines studied was fifteen minutes at room temperature. The p-iodoaniline and m-anisidine gave essentially the same results for ten minutes acylation as for fifteen minutes reaction time, but N-isopropylaniline did not. Although the reaction time could be shortened by increasing the concentration of trans-cinnamoyl chloride, it would result in a slower separation of chloroform layer from aqueous layer during the extraction. Eventually, it might extend the total time required for the analysis.

Table XVI shows the extractability of cinnamanilide under experimental conditions of the analysis, indicating that the anilide can be quantitatively extracted into chloroform if it is formed in the system. Figure 8 shows the relationship between the absorbance of the final solution of p'-iodocinnamanilide and the amount of p-iodoaniline (originally used for the analysis) with the same dilution.

The kinetics of alkaline hydrolysis of a series of p-substituted acetanilides was investigated by Bender and Thomas (115) who reported the first-order hydrolytic rate constant for p-chloroacetanilide at 0.239 M hydroxide

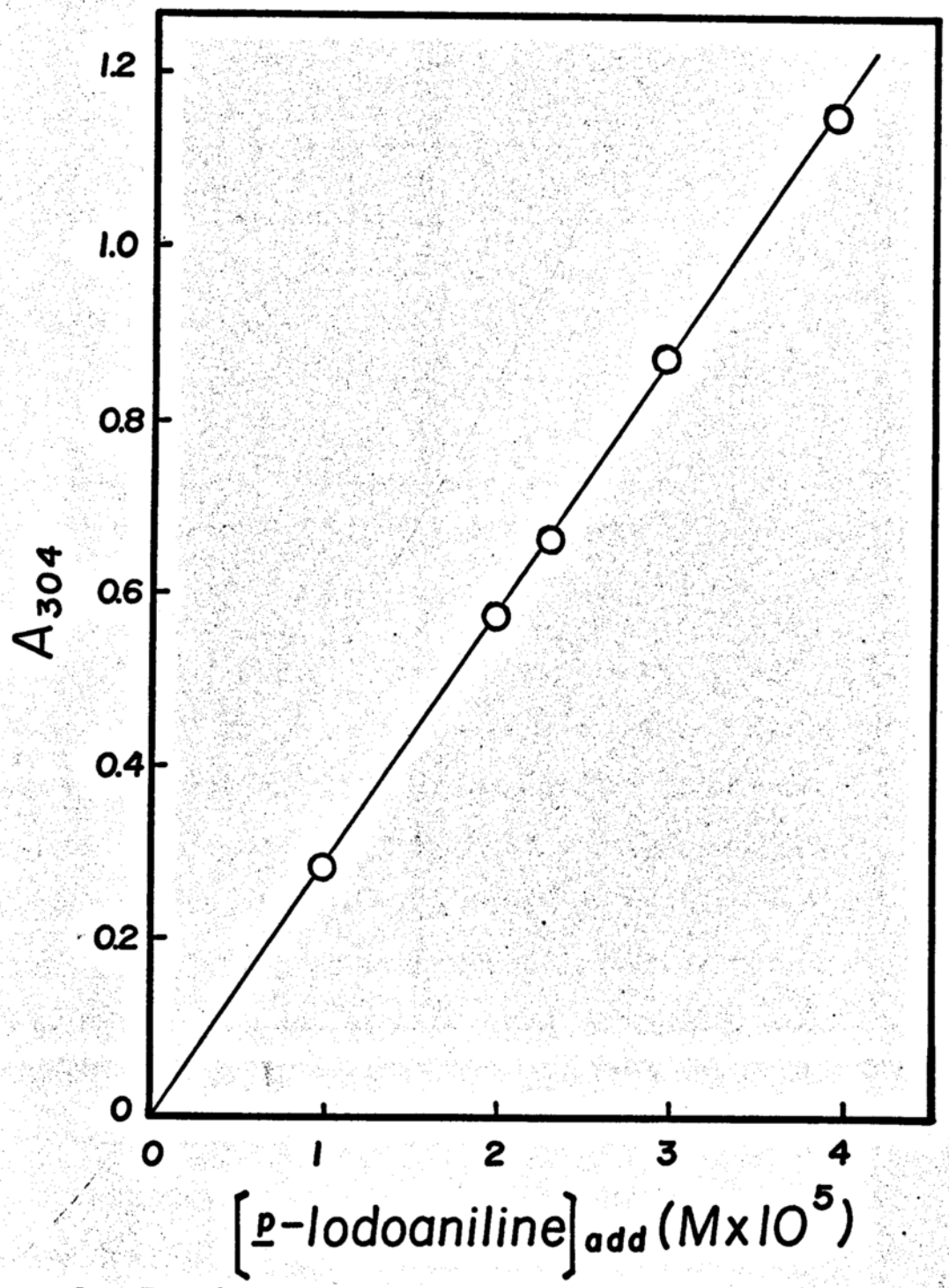


Figure 8. Beer's law plot for the acylation of p-iodoaniline by trans-cinnamoyl chloride.

Table XVI

Extractability of Cinnamanilide under Experimental Conditions of the Analysis

Anilide taken (μmole)	Found (μmole)	
	in 0.01 N NaOH[a]	Cin Cl + 0.01 N NaOH
0.63	0.63	0.63
1.26	1.26	1.28
1.89	1.88	1.87
2.52	2.49	2.52

[a]without cinnamoyl chloride in the system.

concentration was 0.00729 hr^{-1} (this corresponds to $t_{1/2}$ = 95 hrs). Apparently, greater stability would be expected for the cinnamanilides because the carbonyl group is able to conjugate with the ring through a α,β-unsaturated linkage and attack by nucleophilic reagents is thereby greatly reduced. Since the present study was carried out at much lower hydroxide ion concentrations, the loss of cinnamanilides due to hydrolysis is not a serious problem.

However, some interesting phenomena were observed during the study of the hydrolysis of p-substituted cinnamanilides and these are summarized as follows:

a) Cinnamanilide and N-isopropyl cinnamanilide are relatively stable in 0.01 N aqueous sodium hydroxide, i.e., no spectral change was observed in one hour.

b) p-Chloro-, p-bromo-, and p-iodocinnamanilides show an initial fast reaction, followed by a second slow reaction.

c) The rate of the initial fast reaction is not markedly pH dependent in the case of p-bromo- and p-iodocinnamanilides; but the rate increases as hydroxide ion concentration increases for p-chlorocinnamanilide.

d) The rate of the second slow reaction is also not markedly pH dependent.

e) Neither the initial fast reaction nor the second slow reaction corresponds completely to the acyl nitrogen fission.

These observations imply that more than one intermediate might exist in the hydrolysis of p-halogenocinnamanilides.

C. N-trans-Cinnamoylimidazole[a]

N-trans-Cinnamoylimidazole is a mild acylating agent which was first introduced for the determination of -chymotrypsin by Bender and his coworkers (81). In this study, this reagent was utilized for the analyses of various amines, and the analytical results are given in Tables XVII and XVIII.

[a]The author would like to thank Dr. Jurgen von Bredow for his help in preliminary experiments.

Table XVII

Determination of Amines with N-trans-Cinnamoylimidazole

Amine	Sample size (μmole)	% Recovery[a]	Std.dev.	# Determination
n-Hexyl	0.5-2.7	85.1	0.83	19
n-Octyl	0.5-2.1	72.6	4.50	12
β-Phenethyl	0.5-2.7	90.5	1.48	15
Piperidine	0.6-3.0	68.4	6.40	16

[a]based on 12 to 15 hours reaction time.

The data of Table XVII indicate that the formation of cinnamamide is not quantitative and the reproducibility of the results is relatively poor.

Tables XIX and XX demonstrate the relationship of % yield of amide to the concentration of imidazole added and to the concentration of N-trans-cinnamoylimidazole added. Clearly, an increase in imidazole concentration or a decrease in N-trans-cinnamoylimidazole concentration results in a lower per cent recovery of the cinnamamide, suggesting that the reaction is an equilibrium limiting process as follows:

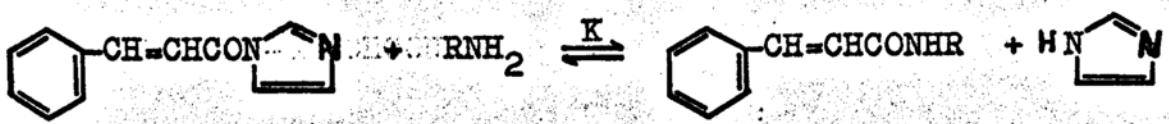


Table XVIII

Analysis of Some Aliphatic Amines with N-*trans*-Cinnamoylimidazole in the Presence of Other Substances[a]

Sample amine (S)[b]	Interfering substance (I)	(I)/(S)	% Recovery of S[c]
n-Hexylamine	--	0	84.7
"	Di-n-hexylamine	4.7×10^{-2}	84.3
"	"	9.4×10^{-2}	85.0
"	"	2.34×10^{-1}	86.5
"	"	4.7×10^{-1}	89.0
n-Hexylamine	--	0	77.6
"	t-Butylamine	4.36×10^{-2}	78.7
"	"	8.72×10^{-2}	78.9
"	"	2.18×10^{-1}	78.6
"	"	4.36×10^{-1}	80.0
β-Phenethylamine	Aniline	4.19×10^{-2}	89.2
"	"	8.38×10^{-2}	89.9
"	"	2.09×10^{-1}	90.7
"	"	4.18×10^{-1}	91.9
n-Hexylamine	--	0	86.0
"	n-Propanol	0.95	86.7
"	"	1.90	86.5
"	"	4.76	86.2
"	"	9.50	85.3
β-Phenethylamine	--	0	84.5
"	β-Phenethylalcohol	1.04	84.0
"	"	2.08	83.8
"	"	5.20	86.6
"	"	10.40	123.4

[a] based on 12 to 15 hours reaction time.

[b] sample size was 1.5-2.0 μmoles.

[c] each value is the average of two determinations.

Table XIX

Relationship of Per Cent Yield of Amide to the Concentration of Imidazole Added for the Reaction Between N-*trans*-Cinnamoylimidazole and Aliphatic Amines

[CIm][a] (M)	[RNH_2][a] (M)	$C_6H_5CH{=}CHCONHR$ (%)	[Im] added (M)
1.13×10^{-1}	1.115×10^{-3}[b]	85.8	0
"	"	84.6	1.195×10^{-2}
"	"	82.8	2.99×10^{-2}
"	"	73.1	6.00×10^{-2}
"	"	63.7	8.95×10^{-2}
1.105×10^{-1}	1.04×10^{-3}[c]	82.2	0
"	"	81.5	5.88×10^{-3}
"	"	76.9	2.96×10^{-2}
"	"	73.3	5.88×10^{-2}
1.145×10^{-1}	1.093×10^{-3}[d]	92.0	0
"	"	91.1	5.88×10^{-3}
"	"	88.5	2.94×10^{-2}
"	"	80.7	5.88×10^{-2}

[a]Initial concentration.

[b]Reaction between N-*trans*-cinnamoylimidazole and *n*-butylamine for 72 hrs.

[c]Reaction between N-*trans*-cinnamoylimidazole and *n*-octylamine for 48 hrs.

[d]Reaction between N-*trans*-cinnamoylimidazole and benzylamine for 48 hrs.

Table XX

Relationship of Per Cent Yield of Amide to Concentration of N-trans-Cinnamoylimidazole Added in the Presence of Imidazole

[CIm][a] (M)	[RNH_2][a] (M)	$C_6H_5CH=CHCONHR$ (%)	[Im] added (M)
1.13×10^{-1}	1.115×10^{-3}[b]	83.4	2.99×10^{-2}
6.80×10^{-2}	1.115×10^{-3}	78.3	2.99×10^{-2}
4.52×10^{-2}	1.115×10^{-3}	74.9	2.99×10^{-2}
1.125×10^{-1}	7.28×10^{-4}[c]	86.6	2.94×10^{-2}
6.75×10^{-2}	7.28×10^{-4}	83.5	2.94×10^{-2}
4.50×10^{-2}	7.28×10^{-4}	79.7	2.94×10^{-2}
2.25×10^{-2}	7.28×10^{-4}	67.7	2.94×10^{-2}

[a]Initial concentration.

[b]Reaction between N-trans-cinnamoylimidazole and n-butylamine for 72 hrs.

[c]Reaction between N-trans-cinnamoylimidazole and benzylamine for 71.5 hrs.

However, if this reaction condition is reversed, i.e., the amine is taken in large excess, only 35% of cinnamamide was formed[a] (based on N-trans-cinnamoyl-imidazole) instead of about 86% for the reaction between N-trans-cinnamoylimidazole and n-butylamine seen in Table XIX. This implies that some reaction other than the one mentioned above is involved. It is conceivable that a side reaction, addition of amine across the double bond (116,117), might occur, resulting in a significant decrease in absorption due to the loss of the conjugated system.

The acylation reaction is very slow. The reaction time employed for this analysis was 12 to 15 hours at room temperature. In spite of these disadvantages, however, some selectivity is obtained, which enables us to analyze amine mixtures or amines in the presence of other substances by taking advantage of differences in the rates of reaction of the components (Table XVIII). tert-Butylamine does not react with N-trans-cinnamoyl-imidazole, probably because of its high degree of steric hindrance, which depresses its nucleophilicity (118). Aromatic amines are much weaker bases than aliphatic amines. Therefore, no interference would be expected in

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[a]The reaction essentially gave the same results for 3 hours reaction as for 24 hours reaction time.

these analyses. The high per cent recovery of β-phenethylamine when the ratio of β-phenethylalcohol to amine is increased up to 10.4 can be ascribed to the insufficient hydrolysis of the corresponding ester formed in the system. Hence, the increase in time for hydrolysis after acylation is recommended when the ratio is very high.[a]

Table XXI shows the extractability of N-butylcinnamamide under the experimental conditions of analysis, showing that the amide can be quantitatively extracted into chloroform. Figure 9 shows the relationship between the absorbance of the final solution of N-hexylcinnamamide and the amount of n-hexylamine (originally used for the analysis) with the same dilution. The relationship has been examined for other amines as well--namely, n-octylamine and β-phenethylamine. In every case, a linear relationship was observed. As would be expected, the slope of the line differed slightly from one amine to another.

The rates of acylation of amine by N-trans-cinnamoylimidazole in acetonitrile were studied by either following the rate of formation of the corresponding cinnamamide (by extraction) or following the disappearance of N-trans-cinnamoylimidazole at a suitable, fixed

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[a]The amide did not show any significant loss after being in contact with 0.1 N aqueous sodium hydroxide for 2 hrs.

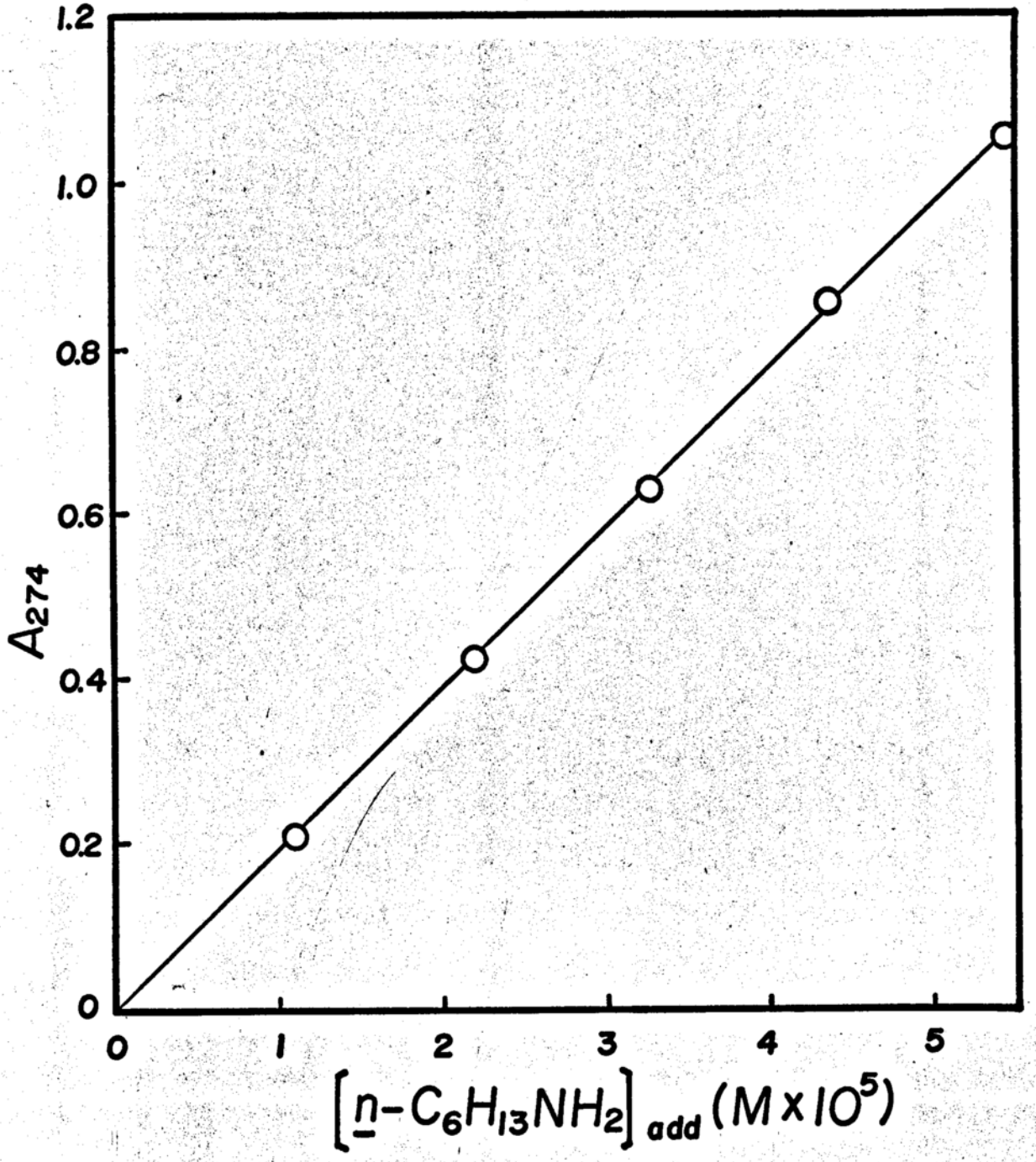


Figure 9. Beer's law plot for the acylation of n-hexylamine by N-trans-cinnamoylimidazole.

Table XXI

Extractability of N-Butylcinnamamide under Experimental Conditions of the Analysis

Cinnamamide taken (μmole)	Found (μmole)	
	in 0.1 N NaOH[a]	$C_{in\ Im}$ + 0.1 N NaOH
0.64	0.64	0.65
1.28	1.30	1.29
1.92	1.95	1.92
2.56	2.59	2.58

[a]without N-trans-cinnamoylimidazole.

wavelength: a) In the former, the N-trans-cinnamoylimidazole concentration was in excess, and after acylation, the excess N-trans-cinnamoylimidazole was hydrolyzed. The amide was then extracted into chloroform and the ultraviolet absorption of this solution was measured at a suitable wavelength (usually at maximum absorption). The reaction kinetics followed a pseudo-first-order equation in all cases for the first two or three half-life periods, the data yielding a straight line when plotted as $\log (r_\infty - r_t)/r_\infty$ vs. time, where r_∞ is the per cent recovery of cinnamamide at equilibrium and r_t, the per cent recovery of cinnamamide at time t. Some typical plots for n-propylamine, allylamine, diethylamine, and dibenzylamine are shown in Figure 10.

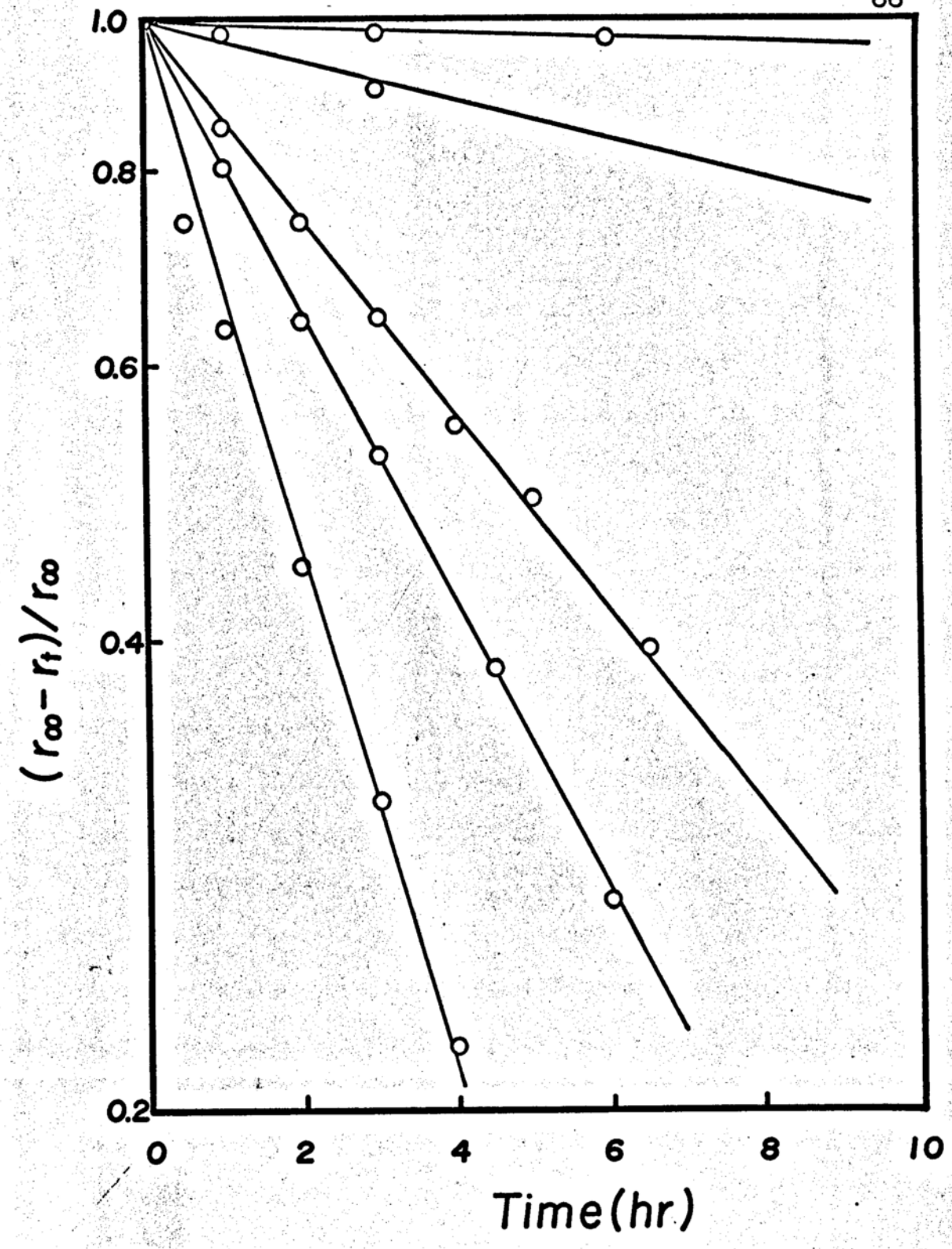


Figure 10. First-order plots for the formation of N-substituted cinnamamides in acetonitrile. From top to bottom: N,N-dibenzyl cinnamamide; N,N-diethyl cinnamamide; N-β-phenethyl cinnamamide; N-allyl cinnamamide; N-propyl cinnamamide.

In the presence of added trans-cinnamic acid, a non-linear first-order plot was obtained as shown in Figure 11. trans-Cinnamic acid appears to catalyze the acylation. Similar effects were observed with benzoic acid and acetic acid (Figure 12).

b) In the second method, the amine was in excess, and the disappearance of N-trans-cinnamoylimidazole was followed at 310 mμ spectrophotometrically. The results indicate that the acylation kinetics depend on the attacking nucleophile. For example, Figure 13 shows that the reaction is first-order with respect to the N-trans-cinnamoylimidazole. Variation in amine concentration led to a change in the first-order rate constant that was not directly proportional to the change in the amine concentration. However, a linear relationship was obtained if the apparent first-order rate constant divided by the amine concentration, $k_{obs}/[n\text{-}BuNH_2]$, was plotted against the amine concentration, $[n\text{-}BuNH_2]$, as shown in Figure 14, indicating that a term second-order in amine was included. It is possible that this second-order term in n-butylamine is due to the general base-catalysis of the addition of n-butylamine to the carbonyl carbon.

Figures 15 and 16 show the first-order plots of acylation of sec-butylamine and piperidine by N-trans-cinnamoylimidazole in acetonitrile, respectively. Apparently, an initial fast reaction occurred, followed by a slow pseudo-first-order reaction in both cases,

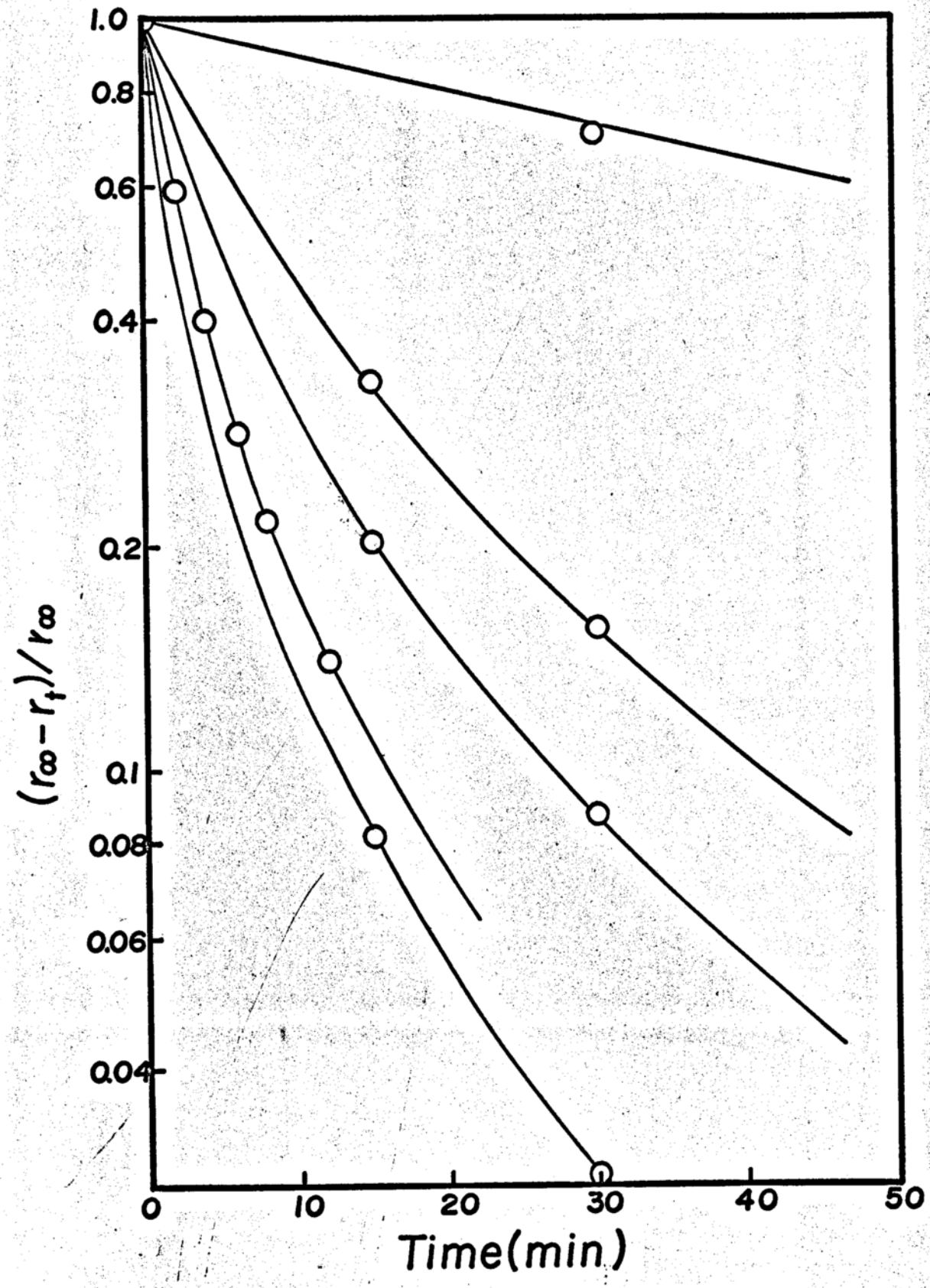
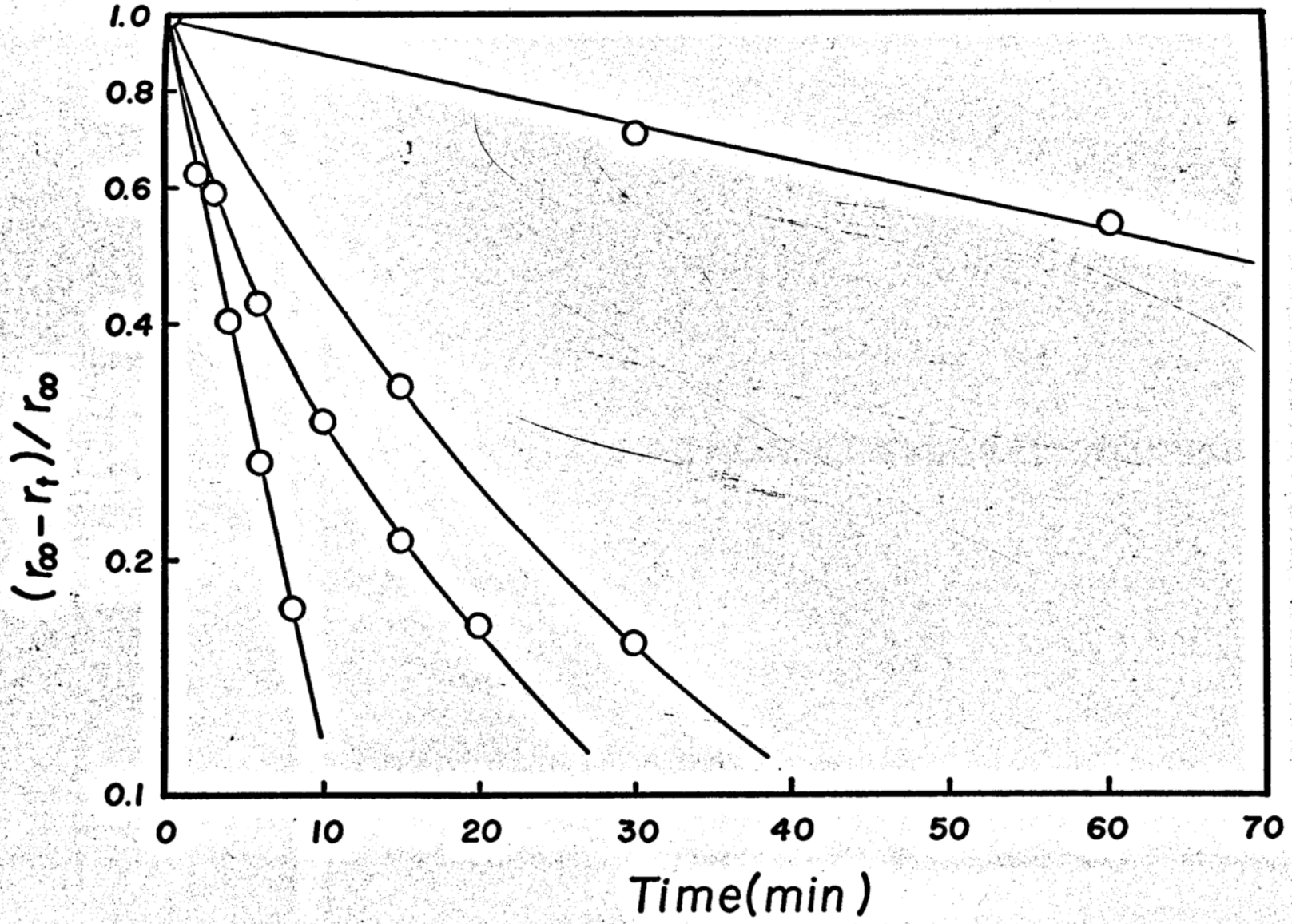
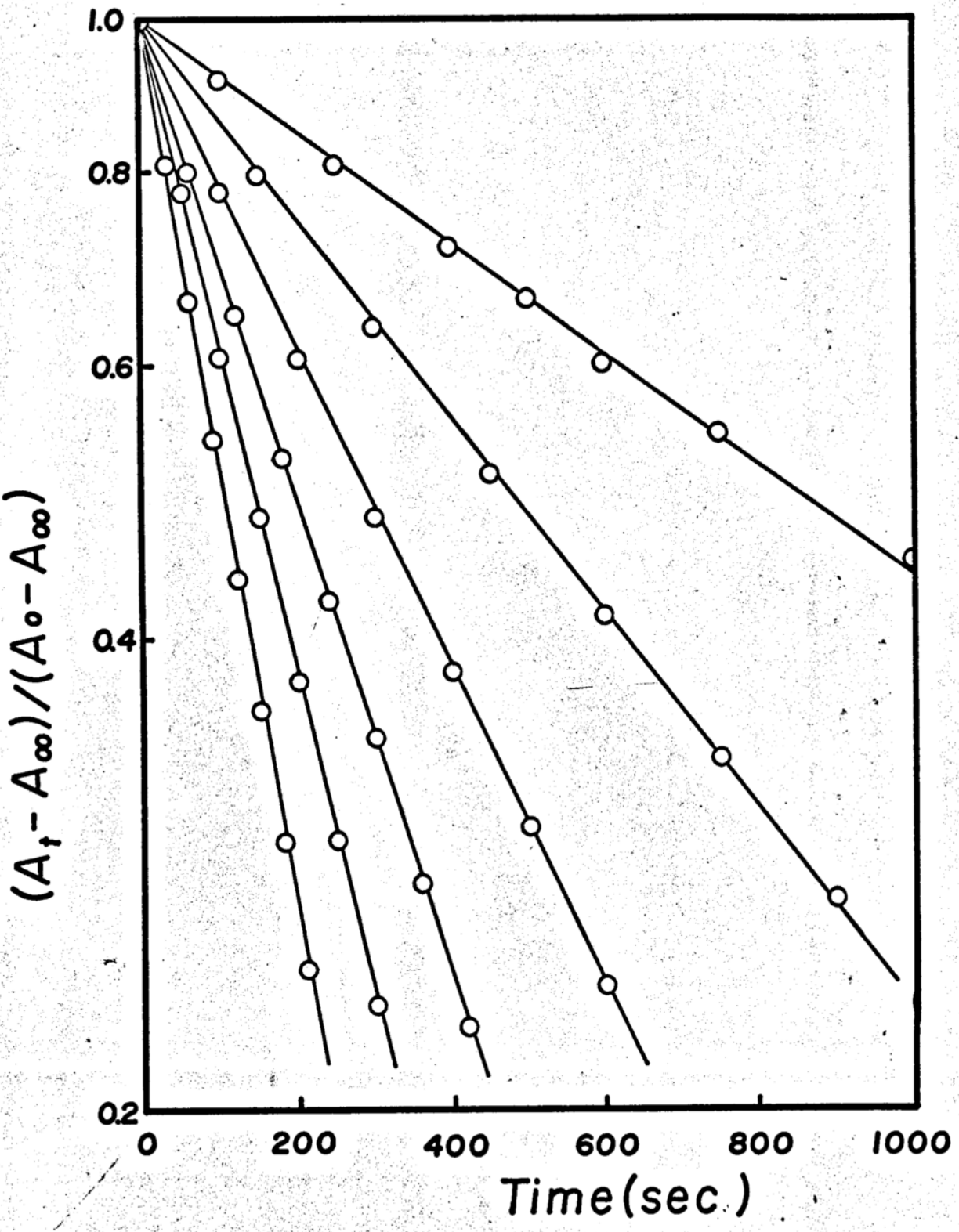


Figure 11. First-order plots of N-butyl cinnamamide formation catalyzed by <u>trans</u>-cinnamic acid. From top to bottom: <u>trans</u>-cinnamic acid added; none; 0.53 umoles; 1.06 umoles; 2.24 umoles; 3.17 umoles.


1.0
0.8
0.6
0.4
0.2
0.1
$(r_8 - r_t)/r_8$
0
10
20
30
40
50
60
70
Time(min)


1.0
0.8
0.6
0.4
0.2
0
200
400
600
800
1000
Time (sec.)
$(A_t - A_\infty)/(A_0 - A_\infty)$

(13)

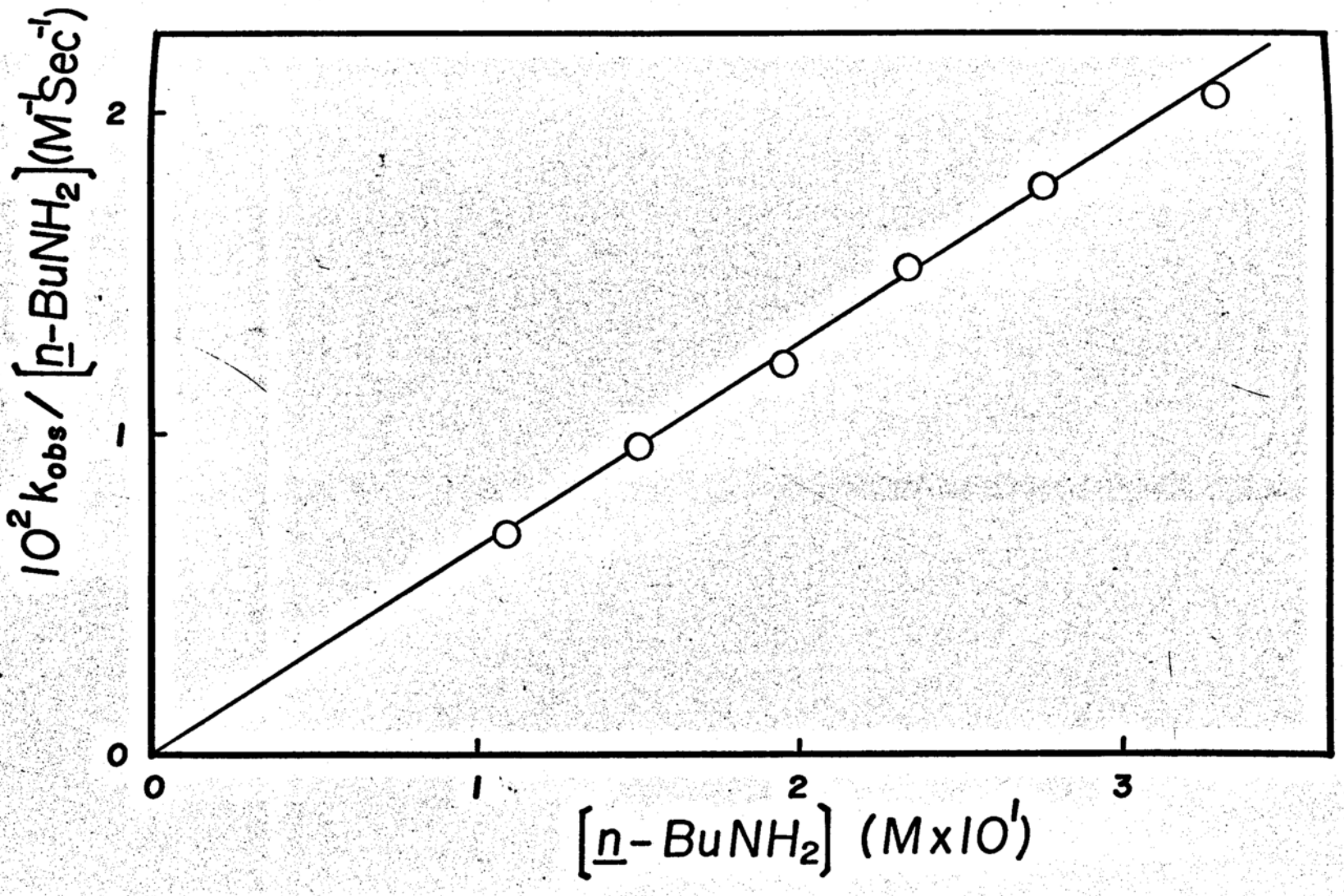
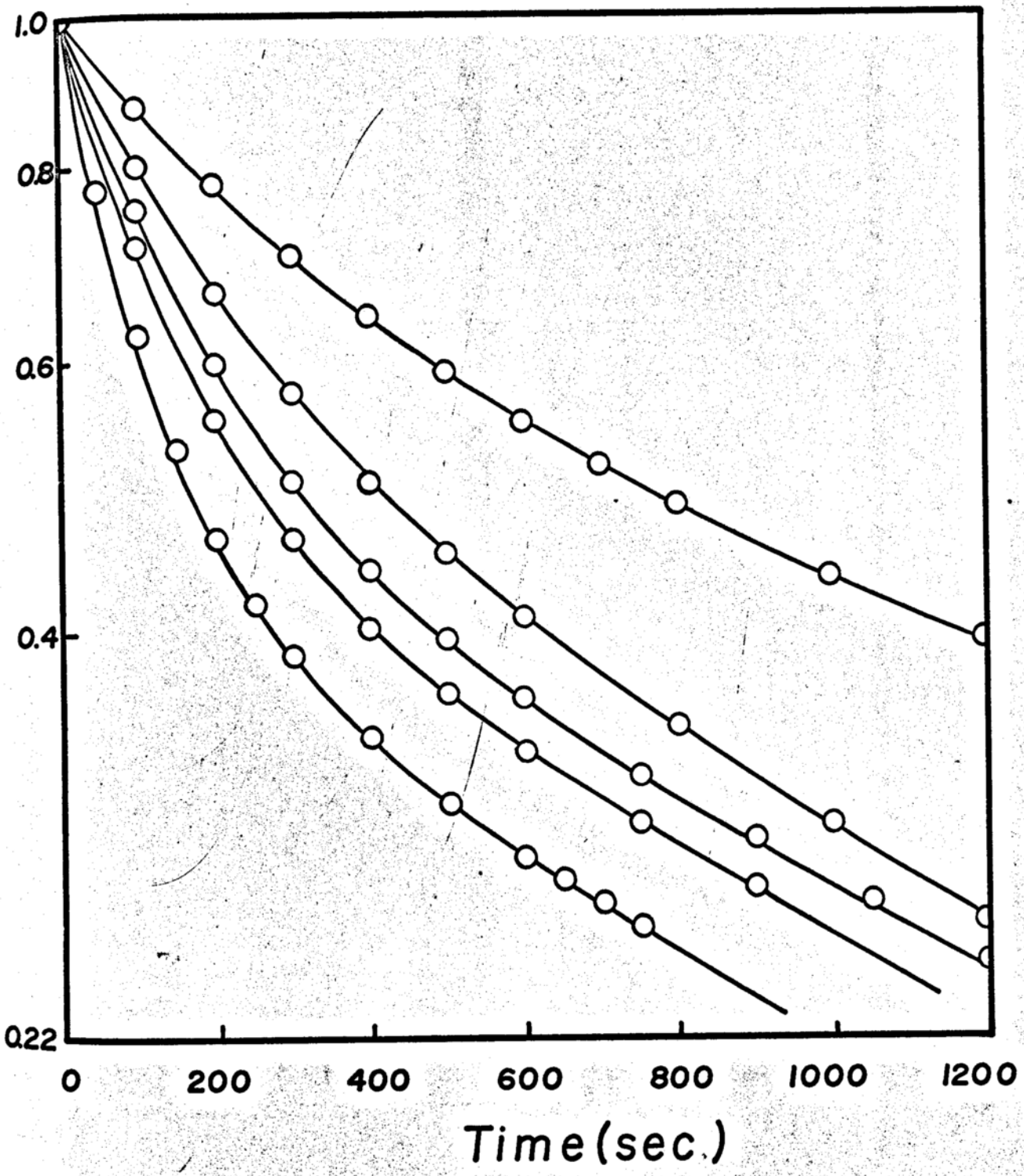
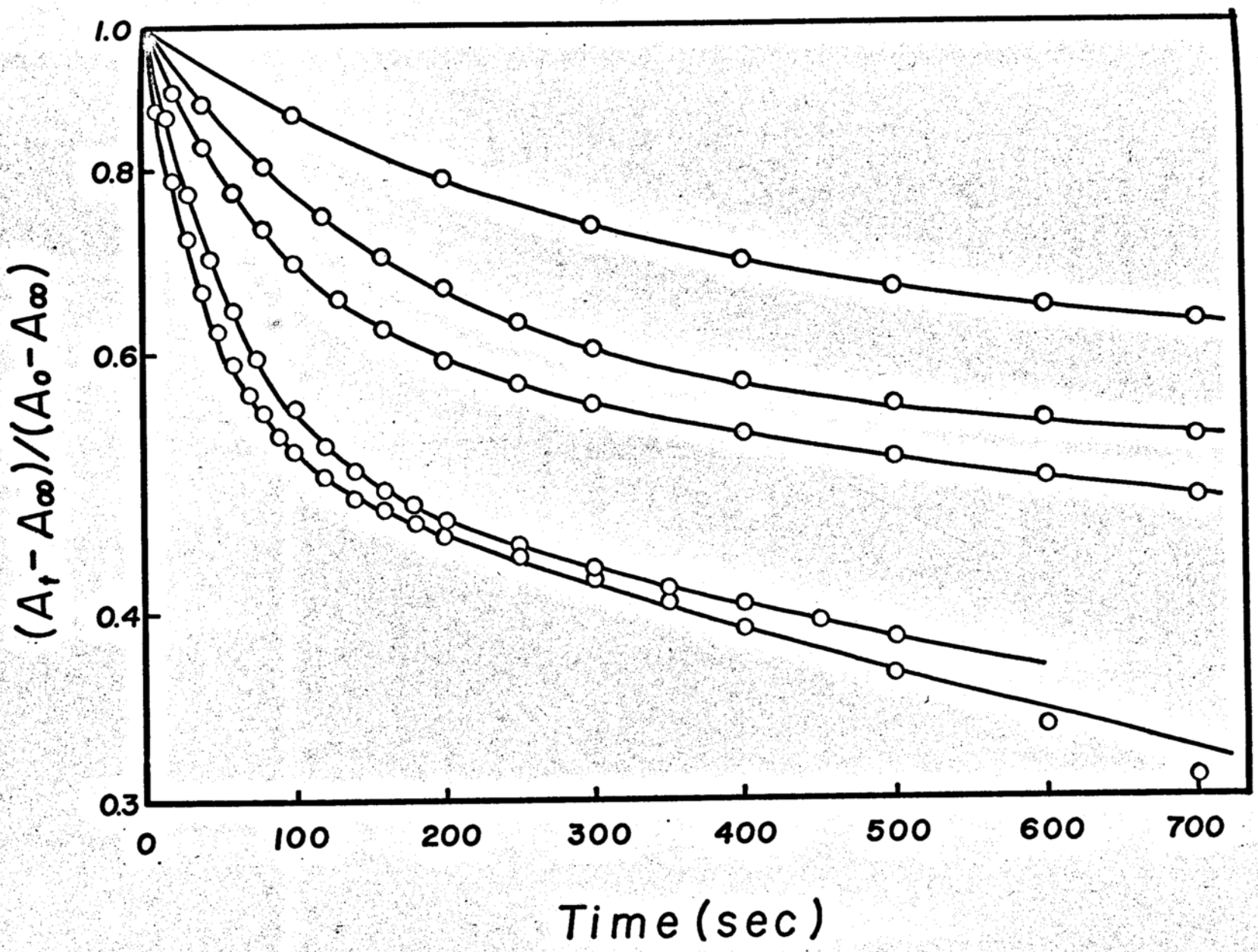


Figure 14. A plot of $k_{obs}/[\underline{n}\text{-}BuNH_2]$ vs. $[\underline{n}\text{-}BuNH_2]$ for the reaction of N-_trans_-cinnamoylimidazole and _n_-butylamine in acetonitrile.


1.0
0.8
0.6
0.4
0.22
0
200
400
600
800
1000
1200
Time (sec.)


1.0
0.8
0.6
0.4
0.3
0
100
200
300
400
500
600
700
$(A_t - A_\infty)/(A_0 - A_\infty)$
Time (sec)

16

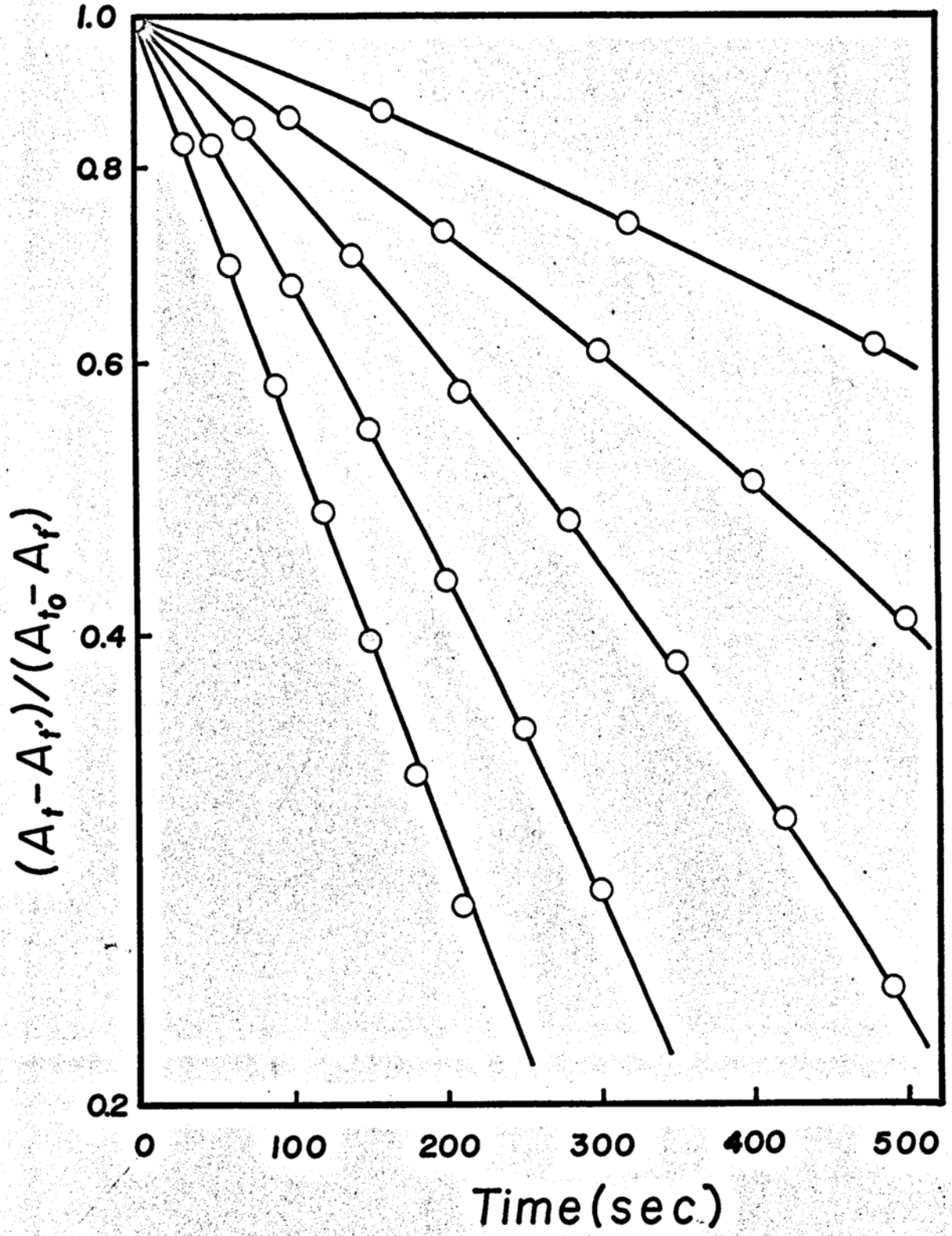
suggesting that an intermediate is formed. However, isobutylamine behaves in a different fashion as shown in Figure 17. The rate increases as the reaction proceeds, then approaches pseudo-first-order kinetics when the amine concentration is high.

Since pseudo-first-order kinetics could be achieved for the acylation of n-butylamine by N-trans-cinnamoyl-imidazole in acetonitrile, its reaction mechanism was further studied. Figure 18 shows the first-order plots for the acylation of n-butylamine by N-trans-cinnamoyl-imidazole in the presence of various amounts of trans-cinnamic acid. The kinetic data are given in Table XXII.

D. trans-Cinnamoyl Perchlorate

In view of the hazards associated with many organic perchlorates (119), no attempt has been made to isolate trans-cinnamoyl perchlorate. However, the reagent can easily be prepared by the addition of trans-cinnamoyl chloride to a solution of silver perchlorate in acetonitrile.

Ethanol was the only alcohol subjected to the analysis by using this reagent and the per cent purity of ethanol turns out to be 98.2% (standard deviation 0.97%) with a sample size of approximately 2 μmoles. Since the analysis involved a tedious chromatographic separation, an extraction technique was applied for the analysis of n-butylamine. Nevertheless, satisfactory results were not obtained, and the reagent was abandoned.


1.0
0.8
0.6
0.4
0.2
0
100
200
300
400
500
Time (sec)
$(A_t - A_f)/(A_{to} - A_f)$

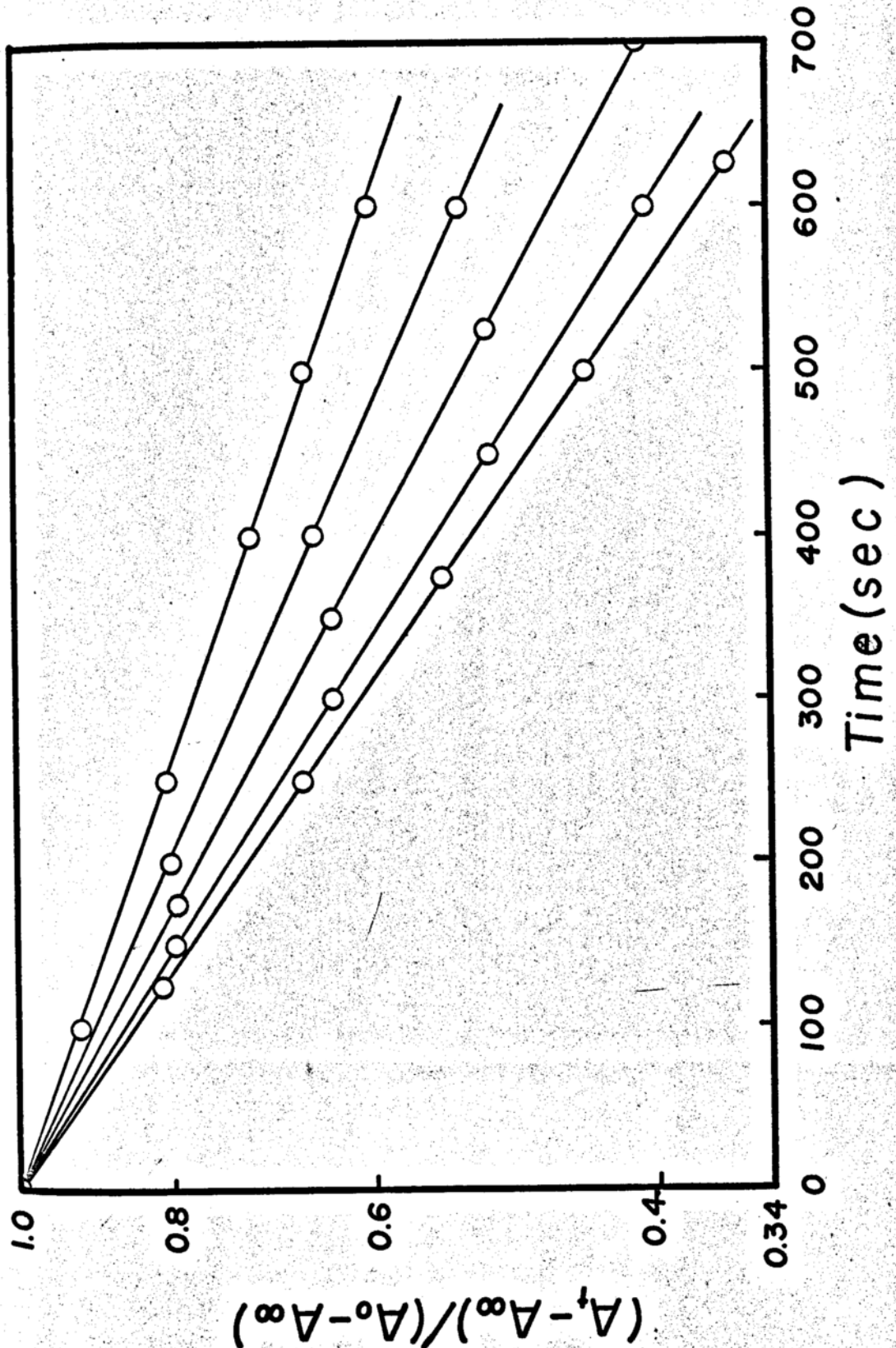

$(A_t - A_\infty)/(A_o - A_\infty)$
1.0
0.8
0.6
0.4
0.34
0
100
200
300
400
500
600
700
Time (sec)

Table XXII

Kinetic Data for the Acylation of n-Butylamine by N-trans-Cinnamoylimidazole Catalyzed by trans-Cinnamic Acid

[n-$BuNH_2$] (M)	10^3 k_{obs} (sec^{-1})[b] at given concentration of trans-cinnamic acid				
	0	1.998×10^{-4} M	3.995×10^{-4} M	5.99×10^{-4} M	7.99×10^{-4} M
1.099×10^{-1}	0.768	1.059	1.281	1.502	1.629
1.507×10^{-1}	1.439	1.795	2.062	2.357	2.511
1.952×10^{-1}	2.372	2.858	3.114	3.376	3.626
2.340×10^{-1}	3.555	3.815	4.161	4.359	4.770
2.751×10^{-1}	4.880	5.110	5.585	5.930	6.237
3.277×10^{-1}	6.738	7.198	7.493	8.274	8.428

[a] acylation was carried out in acetonitrile.

[b] mean of 1 to 3 determinations.

E. trans-Cinnamoyl-α-chymotrypsin

The reagent was freshly prepared by mixing equimolar quantity of N-trans-cinnamoylimidazole (in acetonitrile) and α-chymotrypsin (in phosphate buffer, pH 8.02); then an aqueous solution of amine sample (n-butylamine or morpholine), containing approximately 1 μmole per ml, was added. No amide was formed in any case studied (even though the amine sample was increased to about 320 μmoles), indicating that the competing water hydrolysis is dominant.

IV. DISCUSSION

A. Validity of the Analytical Methods

(1) trans-Cinnamic anhydride method.

trans-Cinnamic anhydride has been successfully used as an acylating agent for the determination of primary and secondary aliphatic amines. The method is simple and sensitive, being suitable for the determination of amine samples in the 1-5 μmole range. Larger samples can be easily handled by dilution. Somewhat smaller samples could be analyzed by increasing the cell path length, but of course, this results also in an increased blank absorption. With the procedure as described, the blank absorbance A_b should fall in the range of 0.02-0.04.

The acylation reaction is extremely rapid for most amines. A reaction time of about two minutes should suffice for complete acylation unless the amine is sterically hindered. Prolongation of the reaction time appears to be without deleterious effect, and times of 15-60 minutes have been used successfully. The time required for hydrolysis of excess reagent is very short, and the ten minutes allotted in the detailed procedure may seem excessive. It normally is so, but occasionally it happens that upon addition of the aqueous alkali some of the reagent comes out of solution; then its hydrolysis

rate is controlled by its rate of dissolution. The prolonged hydrolysis time is therefore recommended for safety, but it may not usually be necessary. The maximum acylation reaction time is readily calculated with data in Table XII and the rate equation, which is rate = k_2[anhydride][amine]. The initial anhydride concentration in the analytical procedure is about 0.009 M. While this is not always a large excess over the amine concentration, it often will be, and it will be assumed that pseudo-first-order conditions applied. Then, roughly, taking n-butylamine as an example, k_{obs} = (158.3 $M^{-1}sec^{-1}$)(0.009 M) = 1.42 sec^{-1}, so $t_{½}$ = 0.693/k_{obs} = 0.5 sec. Reaction is essentially complete--i.e., 99.9%--in ten half-lives, or about five seconds. A considerable latitude is then granted to allow for thorough mixing of the solutions, as well as for the expected rate decrease if the anhydride concentration decreases significantly during reaction. A tertiary aliphatic amine, tri-n-butylamine, is included in the reaction mixture primarily because it seems to result in more reproducible blank values; the original reason for including it had been to ensure that the reaction equilibrium is completely displaced in favor of the products.

No interference is observed from water and methanol. Evidently, the amine is so much more powerful a nucleophile than is water that its rate of acylation

permits the amide to be quantitatively formed before the reagent is hydrolyzed. Alcohols might be expected to interfere in two ways: they can consume reagent, and they produce cinnamate esters, which are extractable into chloroform. No interference is observed, however, if the hydrolysis time in the alkali is increased to permit preferential hydrolysis of the ester. This is possible because of the much greater susceptibility of esters, compared with amides, to alkaline hydrolysis. Thus the second-order rate constant for alkaline hydrolysis of methylcinnamate (84) is 0.0618 $M^{-1}sec^{-1}$, while for N-butylcinnamamide this constant is approximately 2×10^{-6} $M^{-1}sec^{-1}$.

Predetermined molar absorptivities, rather than concurrently established working curves, are employed in the calculation. This can be done because the reaction system is sufficiently well understood and controlled that empirical corrections do not have to be applied to compensate for systematic errors. This may be considered an unusual and important feature of the method.

p,p'-Disubstituted cinnamic anhydrides also gave satisfactory results, except for p,p'-dichlorocinnamic anhydride, which gave low recoveries. This may be due to its low solubility in acetonitrile. Some selectivity could be obtained, however, by choosing a suitable acylating agent. For example, with p,p'-dimethoxycinnamic anhydride, the ultraviolet absorption measurement could be

made at 305 mu instead of 274 mμ.

The direct spectrophotometric titration of aliphatic amine with trans-cinnamic anhydride is a simple technique applicable to small samples. Titration of 20 μmole samples (corresponding to 8×10^{-4} M in the titration solution) gives standard deviations usually in the range of 0.5-2.0%. Reduction of the sample size to 5 μmole (2×10^{-4} M) is feasible, with some loss in precision. The total titration time ranges from 15 minutes (for the highly reactive piperidine) to 70 minutes (for the very slow benzylamine); for most amines a titration takes about 25 minutes. In an effort to reduce this time, several substances were tested as catalysts. Perchloric acid appears to catalyze the reaction, but it seems to lead to a reaction between anhydride and the solvent. Trichloroacetic acid, diphenylphosphate, and pyridine (Table XXIII) did not give useful catalytic effects, and indeed, diphenylphosphate reduces the reaction rate markedly. Reaction in dioxane is much slower than in acetonitrile.

Some selectivity is achieved because of differences in rates of acylation. Thus n-butylamine could be titrated in the presence of an equal quantity of tert-butylamine without interference. A 10-fold excess of aniline did not significantly interfere in the titration of n-butylamine. Similarly, a 4000-fold excess of methanol caused no interference. Some reaction of these

Table XXIII

Effect of Pyridine on the Acylation of n-Hexylamine With trans-Cinnamic Anhydride in Acetonitrile at 25°C[a]

[Pyridine] (M)	$10^2 k_{obs}$[b] (sec^{-1})
0	1.39
4.65×10^{-6}	1.41
2.33×10^{-5}	1.41
7.30×10^{-4}	1.44
6.97×10^{-3}	1.44

[a]The rate of reaction was followed by the disappearance of trans-cinnamic anhydride at 305 mμ.

[b]In all cases, pseudo-first-order kinetics were obtained.

potential interfering substances with excess of reagent does of course occur after the end-point, and the proper absorbance reading was obtained by extrapolating the absorbance back to the time of titrant addition. Water interferes significantly.

(2) trans-Cinnamoyl chloride method.

Acid chlorides are commonly used for synthetic acylations, but are less often applied in quantitative work because their solutions are not very stable. Although Olson and Feldman (47) used acetyl chloride for the determination of amines, it has no advantages over

acetic anhydride. There is serious interference if water is present in the samples, and with primary amines the acid chlorides may even form the diacyl derivatives under relatively mild conditions (121). Generally, however, the formation of diacyl derivatives is not a serious problem since in many cases the conversion of these compounds to the monoacyl derivatives can be easily accomplished by partial hydrolysis. The analytical data show slightly lower results (94–98%) for the cinnamoyl chloride method developed here (Table XV) than those obtained from the pyridine-catalyzed acetylation method. These low per cent recoveries cannot be attributed to the hydrolysis of cinnamanilides in alkaline medium, because cinnamanilides can be quantitatively recovered as shown in Table XVI. The method is about 20 times as sensitive as the acetic anhydride method developed by Reynolds, et al. (24). Nevertheless, it shows lower sensitivity if compared with the Bratton-Marshall coupling method (33), which is specific for the determination of primary aromatic amines, and which has an accuracy of about ±2% (3, 38). The molar absorptivity for the azo dyes fall in the range of 4.70×10^4 to 5.50×10^4 (35, 36), while cinnamanilides have ϵ_{max} 2.10×10^4 to 3.0×10^4 in chloroform, suggesting that twice the sensitivity could be expected for the Bratton-Marshall coupling method. However, its applicability can be somewhat reduced by the complexity

of the coupling reaction. Dux, et al. (35) found that the intensity of the color of the azo dye depends upon the pH of the solution. Turner (37) found that, although 4-coupling predominates with α-naphthylamine, some 2-coupling occurs and the reaction mixture often yields small amounts of other colored products. If the Bratton-Marshall reagent similarly undergoes coupling to give a mixture of 4- and 2-coupling products, this might lead to variations in reproducibility and sensitivity, depending upon the reaction conditions. The cinnamoyl chloride method may find application in cases where the Bratton-Marshall coupling method cannot be applied, that is, for p-substituted anilines and some secondary aromatic amines.

(3) N-trans-Cinnamoylimidazole method.

The practical usefulness of N-trans-cinnamoylimidazole as an acylating agent is reduced by its slowness and incompleteness of reaction and the poor reproducibility (Table XVII). The incomplete reaction can be accounted for in terms of an equilibrium limiting process and also the presence of a side reaction, namely, addition of amine across the double bond of N-trans-cinnamoylimidazole (this addition does not occur to N-alkylcinnamamides, the main product.) Although the acylation reaction is relatively slow, the conditions for acylation are rather mild, which contributes a certain level of selectivity to the method. Thus, n-hexylamine

could be determined in the presence of about 20%[a] of di-n-hexylamine or tert-butylamine, where the steric effect may be responsible for the specificity. About 40%[a] of aniline did not cause significant interference in the analysis of β-phenethylamine, because aromatic amines are much weaker bases than aliphatic amines. Similarly, a ten-fold excess of n-propanol and a five-fold excess of β-phenethylalcohol did not significantly interfere in the determination of n-hexylamine and β-phenethylamine, respectively. The high per cent recovery of β-phenethylamine when the ratio of alcohol to amine is increased up to 10.4 can be ascribed to the insufficient hydrolysis of the corresponding ester formed in the system. Therefore, an increase in hydrolysis time for the analysis is recommended when the ratio is high.

B. Structural Effects on Reactivity

Acylation reactions are polar reactions, in which an electron-rich species attacks an electron-deficient site. Obviously, the rate of reaction is strongly influenced by the structural effects within the reactants. These structural effects may be polar (or inductive) effects, resonance effects, and steric effects; and the manner in which these effects will influence reactivity is dependent upon the reaction mechanism. In considering the

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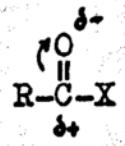
[a]Based on 100% of test amine.

structural effects on reactivity, it is convenient to divide the discussion into two parts; namely, structural effects on the acylating agent and on the nucleophile.

(1) Acylating agent.

An acylating agent, RCOX, acts as an electrophilic entity, and the site of attack is at the carbonyl carbon atom. This is undoubtedly partly due to the electron displacement in the direction indicated as follows (V),



(V)

For acylation to occur, the group X must be displaced. Clearly, one important factor governing the effectiveness of an acylating agent is the nature and strength of the C-X bond. It will be seen that, in general, the greater the approach of C-X to the structure $C^{+}X^{-}$, the more reactive the acylating agent will be, and thus less selective. Another way to view this is in terms of the stability of the leaving group, X^{-}. Therefore, the expected order of reactivity of the acylating agents is trans-cinnamoyl perchlorate > trans-cinnamoyl chloride > trans-cinnamic anhydride > N-trans-cinnamoylimidazole.

The problem of a quantitative evaluation of the effect of various substituents on the reactivity of

molecules has interested chemists for many years. For simplicity, consider a substituent that only exerts its influence by donating or withdrawing electrons. This restriction means that the substituent must not be so close to the reaction site that it sterically interferes with the progress of the reaction. Figure 19 shows a Hammett-type plot, a plot of the logarithm of the second-order acylation rate constants, log k_2, against σ_p for several p,p'-disubstituted cinnamic anhydrides. The slopes of these lines are essentially the same, with $\rho = 2$, indicating that the effects of the para-substituents are due only to the polar effects. The greater the electron donating effect of the substituent, the greater the decrease in the rate of acylation.

(2) Nucleophiles.

The ease with which many amines react with acylating agents may often be related to the greater basicity (really the nucleophilicity) of nitrogen compounds compared with those of oxygen. Nevertheless, comparisons of this sort should be made with care, because the nucleophilicity of the substance that reacts with the acylating agent is of greater importance than its basicity. In general, when a series of related nitrogen bases is being compared, it is permissible to use basicity as a measure of nucleophilicity, but it is possible that the order in basicity may vary with the solvent.

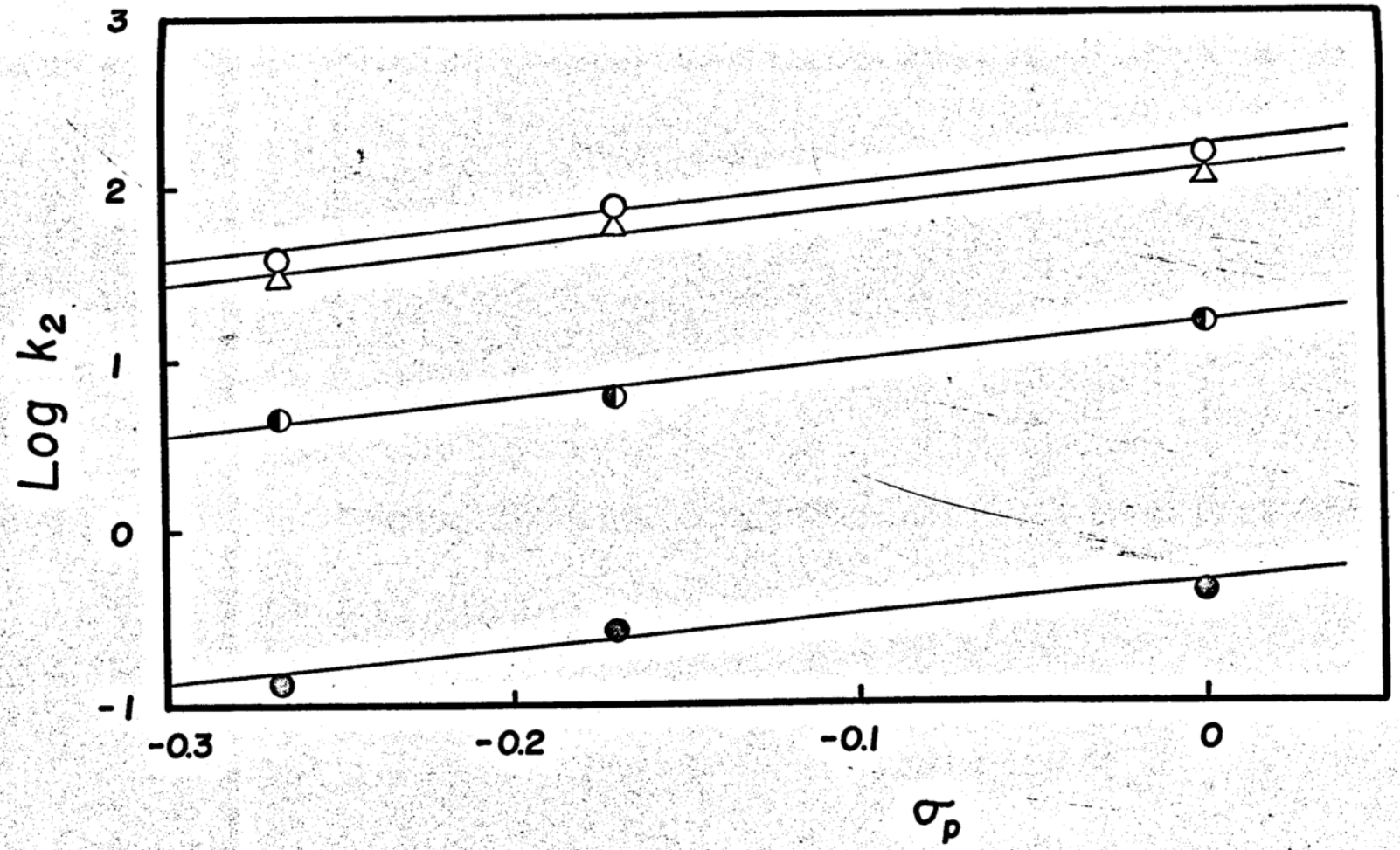


Figure 19. Plots of log k_2 against σ_p. (o) n-butylamine; (Δ) i-butylamine; (◐) sec-butylamine; (●) tert-butylamine.

Figure 20 is a plot of the logarithm of the second-order cinnamoylation rate constants (anhydride), log k_2, against the aqueous pK_a valuesfor the conjugate acids of the amines. The primary amines without a α- or β-substituent fall on a straight line with a slope of 0.78 and the secondary amines fall on another straight line with a slope of 1.91, (this is formally analogous to a Brönsted type plot (122,123)), illustrating that the greater the basicity of the amine, the greater the rate of acylation. This empirical relationship then permits an estimate of k_2 to be made for other aliphatic amines, and thus allows prediction of feasibility of analysis. For example, it is apparent that a typical primary amine with pK_a greater than about 8 should be susceptible to analysis by this method. On the other hand, aromatic amines are too weakly basic to be quantitatively acylated by trans-cinnamic anhydride; thus aniline (pK_a 4.63) gave only 7.9% conversion to cinnamanilide with a reaction time of 60 minutes.

The deviations from the line in Figure 20 are interesting. The amines whose points fall below the line have bulky substituents on the nitrogen atom, and the negative deviation in rate (relative to the line) may be ascribed to steric hindrance. The two secondary amines lying above the line, piperidine and morpholine, are alicyclic; their behavior suggests that they are much more nucleophilic than their basicity constants would

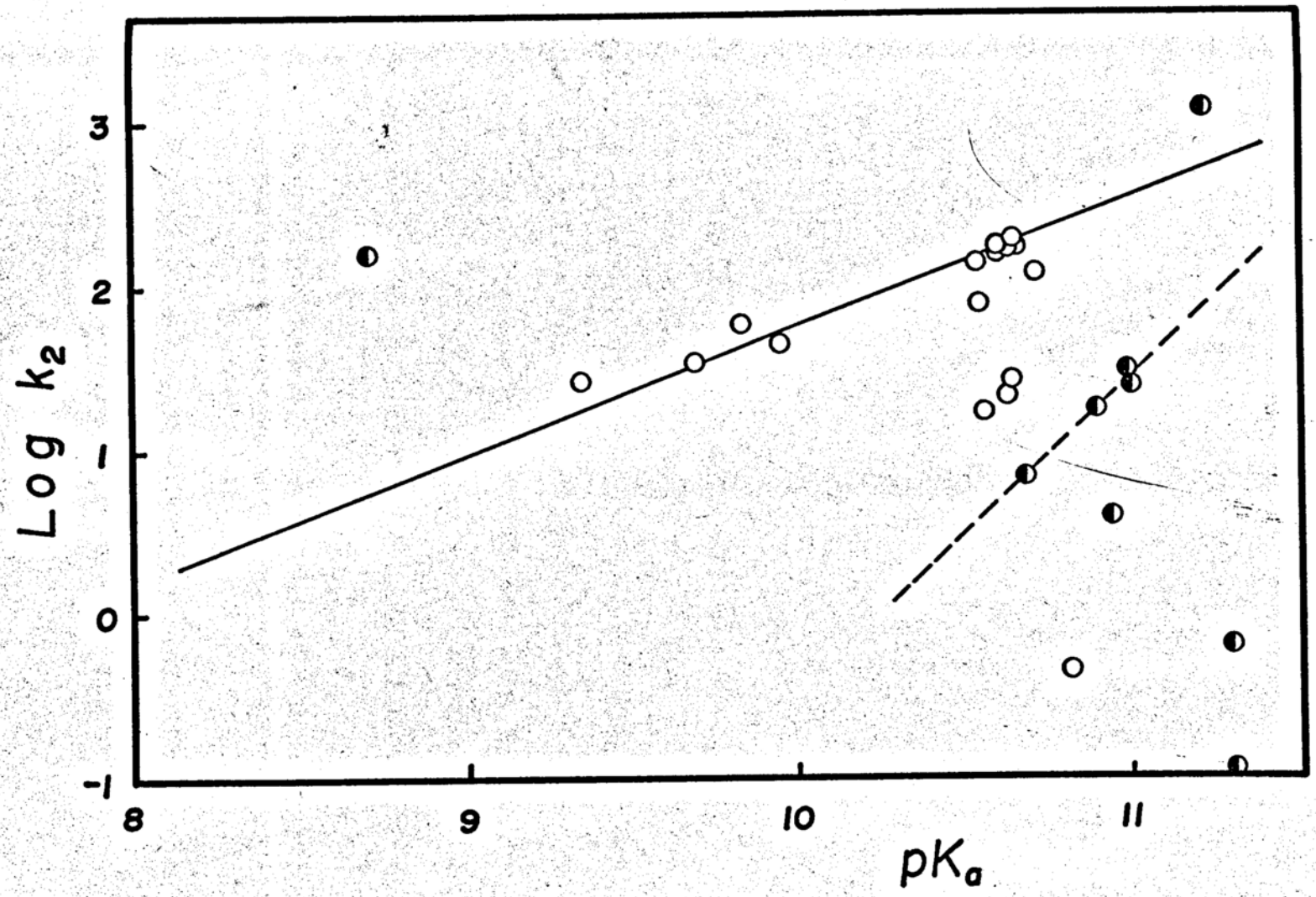


Figure 20. Plot of log k_2 against pK_a for the acylation of aliphatic amines by trans-cinnamic anhydride. (o) primary aliphatic amine; (o) secondary aliphatic amine.

indicate. Fedor, et al. (124) studied the reactions of phenylacetate with aziridine (VI) and 3,3-disubstituted azetidine (VII) in water and rationalized that the enhanced nucleophilicity of the cyclic amines is attributed to C-N-C bond angle constraint.

(VI)

(VII)

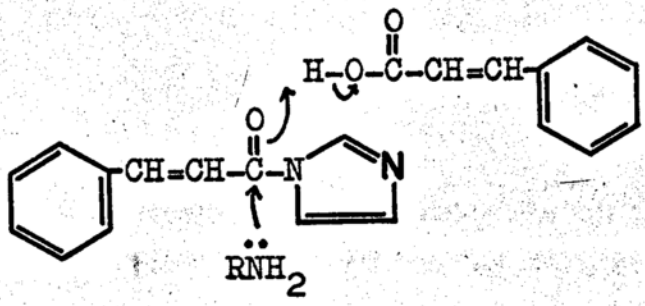
C. Cinnamoylation Kinetics and Their Application

Numerous analytical techniques for the identification and determination of closely related compounds are based upon the difference of reaction rates of the components with a common reagent, provided that the reactions follow simple kinetics. Complicated kinetics are undesirable, because the reactions are usually not easily controlled.

As far as the acylation of aliphatic amines with trans-cinnamic anhydride is concerned, the reaction follows overall second-order kinetics, i.e., first-order with respect to the anhydride and first-order with respect to the amine. The kinetic data given in Table XII show that the cinnamoylation rate constant is a very discriminating criterion of identity, the maximum range in relative rate being about 10^4. (Aromatic amines are

much less reactive than aliphatic amines). In fact, this rate constant alone would permit one to identify most of the amines listed. By taking both boiling point and rate constant, positive identification could be made of every amine studied, except perhaps diethylamine and allylamine (and these can be distinguished by their pK_a values).

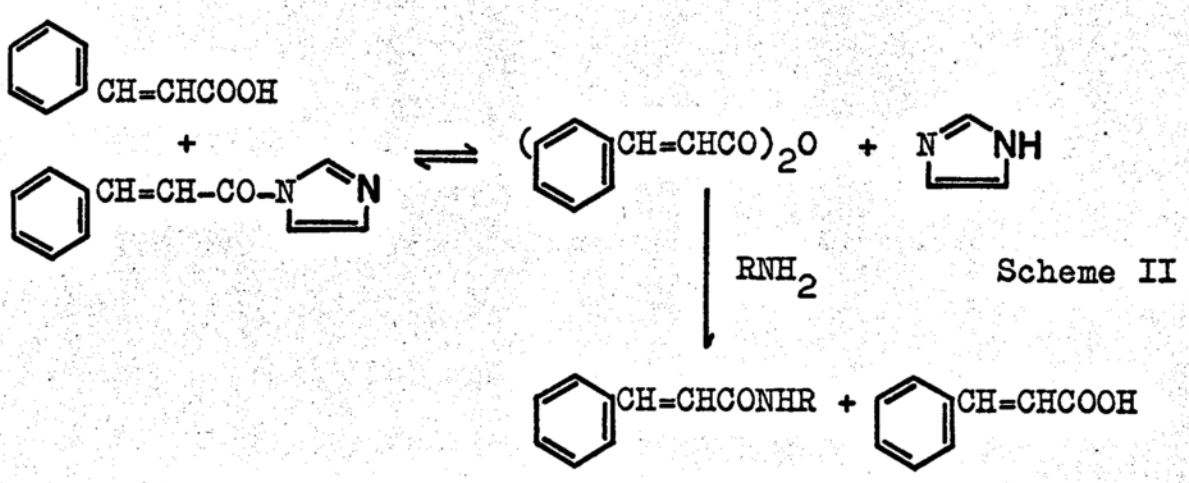
The reaction kinetics between N-trans-cinnamoyl-imidazole and aliphatic amines show much more complicated behavior than was expected. It is found that the reaction is catalyzed by trans-cinnamic acid[a]; the amide formation is not only faster, but the per cent yield of the amide is also increased. Two reasonable mechanisms may be proposed for this catalytic effect (I). trans-Cinnamic acid acts as a general acid to facilitate the attack of the amine molecule on the carbonyl carbon,



Scheme I

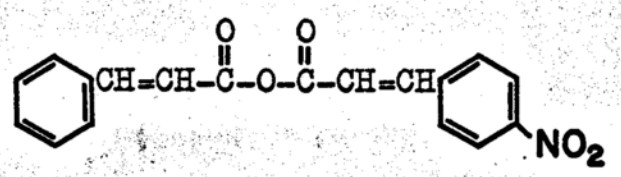
[a]Free carboxylic acids can sometimes be used for making amides. It is usually necessary to use vigorous conditions to promote the amide formation. However, no amide is formed from trans-cinnamic acid and n-butylamine under our experimental conditions. Certain acids and amines form amides in mild conditions. See the series of papers by T. Higuchi, et al. (57,66,120).

or (II). It reacts with trans-cinnamoylimidazole to form an intermediate, trans-cinnamic anhydride, which possesses greater reactivity.



Scheme II

Since the kinetic studies were carried out in acetonitrile, it is believed that the catalysis is mainly due to the participation of the undissociated trans-cinnamic acid. It is also found that p-nitrocinnamic acid (as well as acetic and benzoic acids) catalyze the reaction between N-trans-cinnamoylimidazole and n-butylamine. If the anhydride intermediate mechanism is correct, a mixed anhydride (VIII) should be formed.

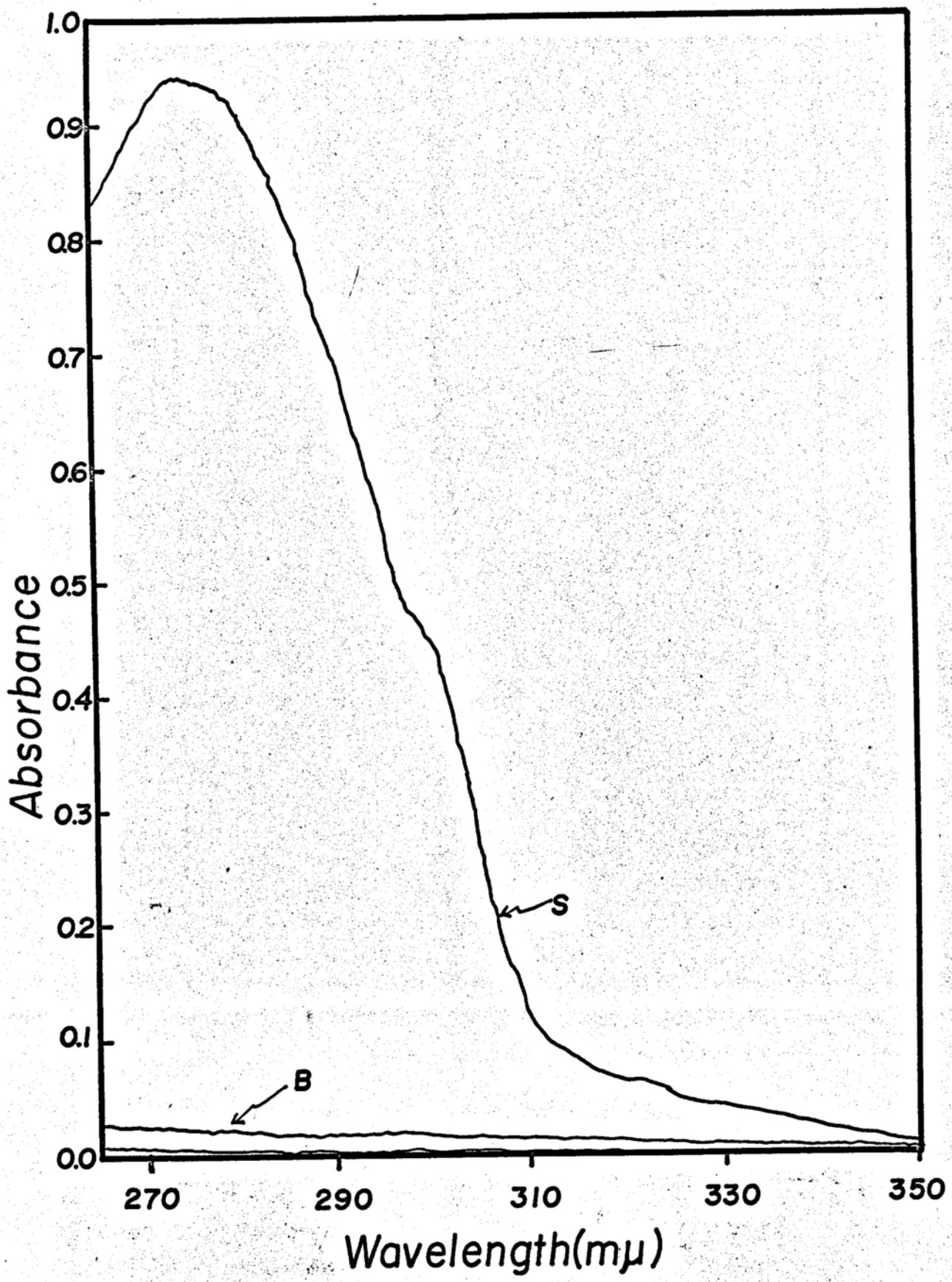


(VIII)

The attack of an amine molecule on the carbonyl carbon of the p-nitrocinnamoyl group would be much more favorable due to the electron withdrawing effect of the

p-nitro group. Therefore, a spectral difference in the product would undoubtedly be anticipated because of formation of some p-nitrocinnamamide. Figure 21 shows the ultraviolet spectra of the final solution for the analysis of n-butylamine with N-trans-cinnamoylimidazole in the presence of p-nitrocinnamic acid. Apparently, as increasing absorption is observed in the region from 350-320 mμ, which is not observed if the acylation reaction is carried out in the absence of p-nitrocinnamic acid. It is, therefore, reasonable to suspect that this increasing absorption comes from N-butyl-p-nitrocinnamamide, which has a maximum absorption at 310 mμ with a molar absorptivity of 2.2×10^4 in chloroform (Figure 22). This spectral change is further verified by taking the ultraviolet spectra of N-butylcinnamamide in the presence of N-butyl-p-nitrocinnamamide in chloroform as shown in Figure 23. The product ratio can then be estimated from the absorbance in region of 330-320 mμ and 274 mμ; from Figure 21, it is found that approximately 82% of n-butylamine is converted to N-butylcinnamamide while 6% is converted to N-butyl-p-nitrocinnamamide.

A tentative reaction mechanism for the acylation of n-butylamine by trans-cinnamoylimidazole in acetonitrile may be written as follows:


1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Absorbance
S
B
270
290
310
330
350
Wavelength(mμ)

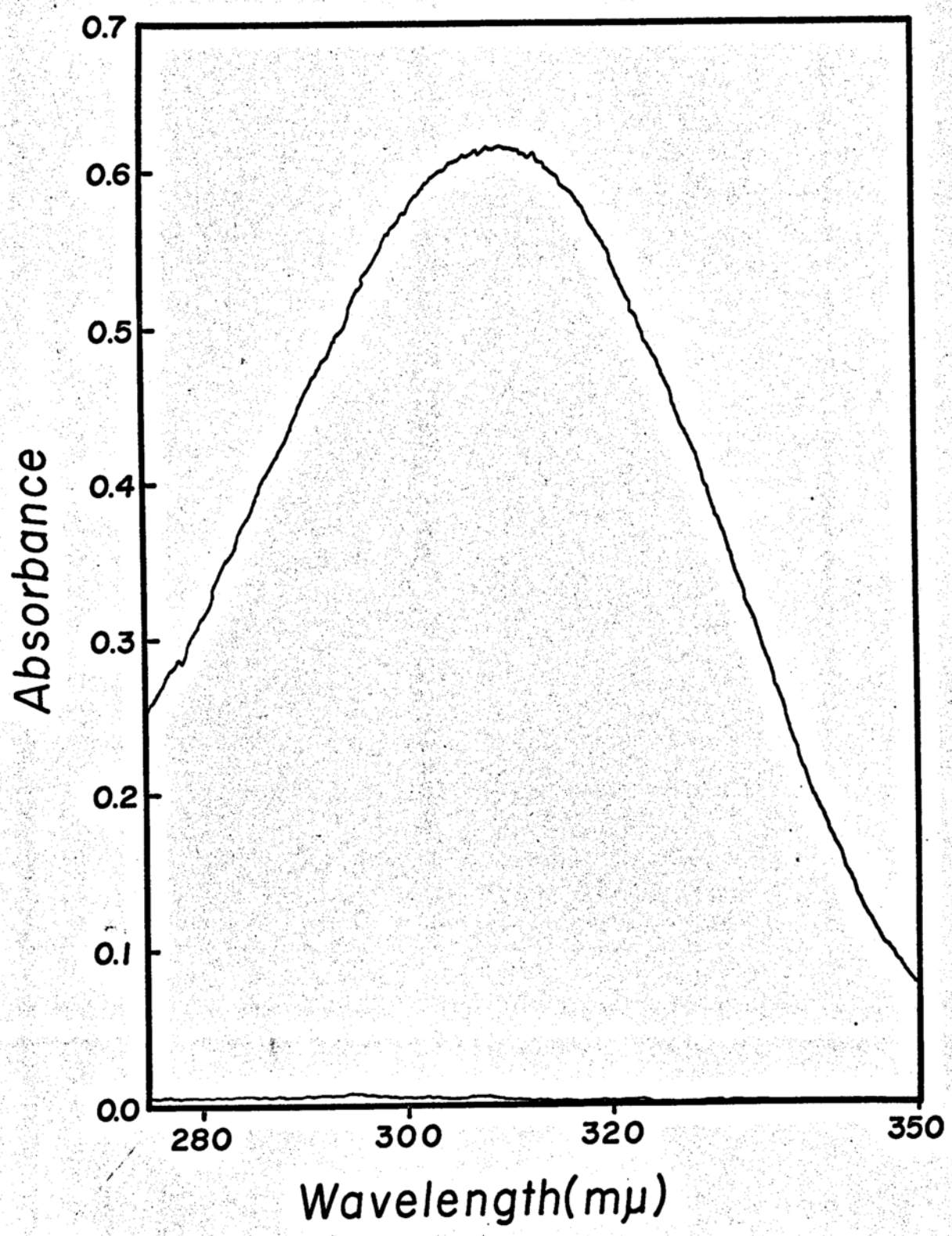
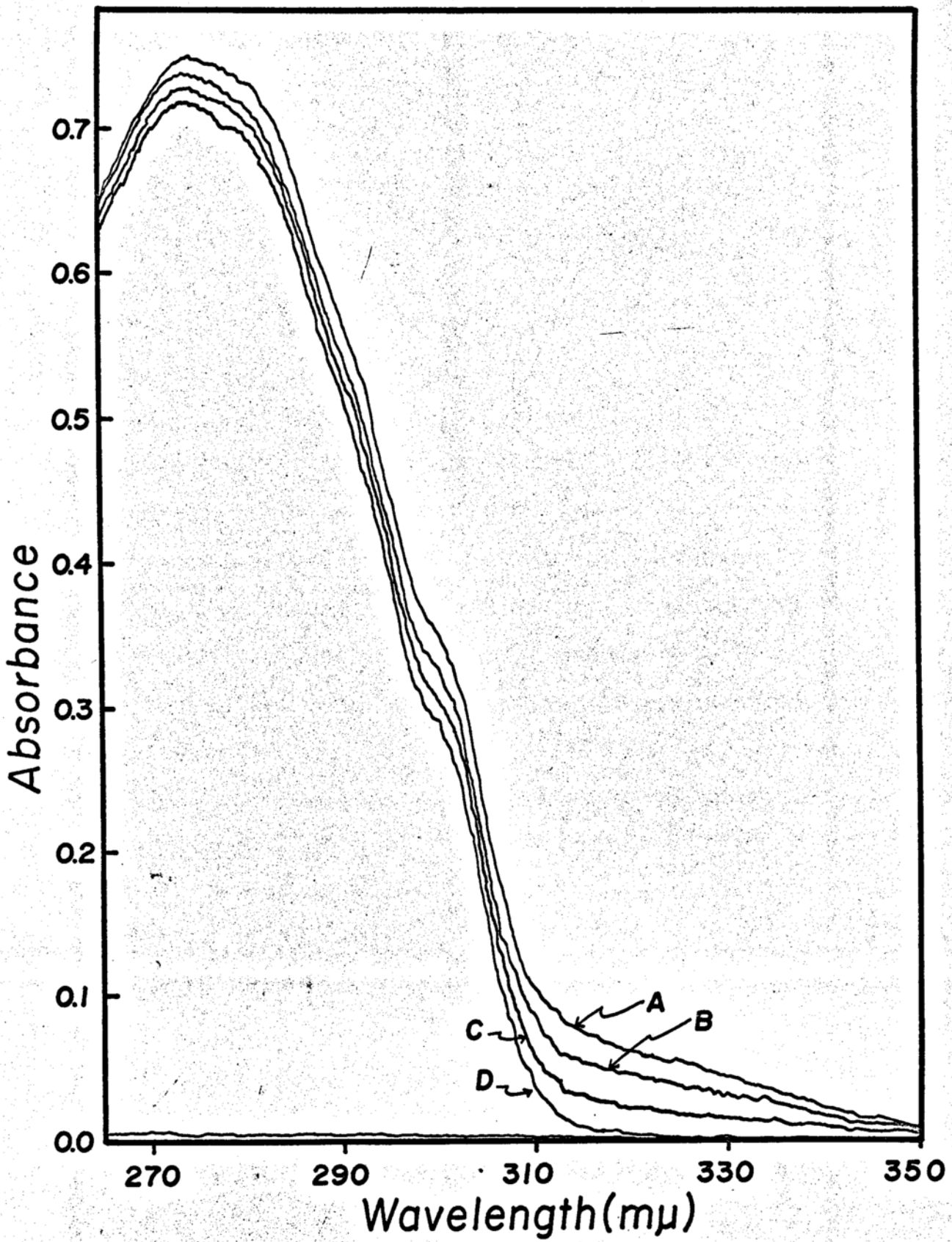


Figure 22. Ultraviolet spectrum of N-butyl-p-nitrocinnamamide in chloroform (2.797×10^{-5} M).


0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Absorbance
A
B
C
D
270
290
310
330
350
Wavelength(mμ)

$$CIm + RNH_2 \xrightarrow{k_1} p + Im \qquad (1)$$

$$CIm + 2\,RNH_2 \xrightarrow{k_2} p + Im + RNH_2 \qquad (2)$$

$$CIm + RNH_2 \xrightarrow{k_3} p' \qquad (3)$$

$$CIm + 2RNH_2 \xrightarrow{k_4} p' + RNH_2 \qquad (4)$$

$$CIm + RNH_2 + C \xrightarrow{k_5} p + Im + C \qquad (5)$$

$$CIm + RNH_2 + C \xrightarrow{k_6} p' + C \qquad (6)$$

$$CIm + C \underset{k_{-7}}{\overset{k_7}{\rightleftharpoons}} CAn + Im \qquad (7)$$

$$CAn + RNH_2 \xrightarrow{k_8} p + C \qquad (8)$$

Here, CIm represents N-trans-cinnamoylimidazole and C, trans-cinnamic acid; CAn is the intermediate, trans-cinnamic anhydride, and Im, imidazole; p represents the corresponding cinnamamide, and p', the product obtained from the addition of amine across the double bond; k_1 to k_8 are the rate constants for the eight processes listed above.

Since the reaction was followed by the disappearance of CIm,

$$-\frac{d[CIm]}{dt} = k_1[CIm][RNH_2] + k_2[CIm][RNH_2]^2 + k_3[CIm][RNH_2] + k_4[CIm][RNH_2]^2 + k_5[CIm][RNH_2][C] + k_6[CIm][RNH_2][C] + k_7[CIm][C] - k_{-7}[CAn][Im] \qquad (9)$$

It is known that the reaction between trans-cinnamic anhydride and n-butylamine occurs sufficiently rapidly (Table XII) so that the concentration of CAn is very

small, and the steady-state treatment can therefore be applied. The steady-state expression for [CAn] is

$$\frac{d[CAn]}{dt} = k_7[CIm][C] - k_{-7}[CAn][Im] - k_8[CAn][RNH_2] = 0$$

or

$$[CAn] = \frac{k_7[CIm][C]}{k_{-7}[Im] + k_8[RNH_2]} \qquad (10)$$

Insertion of equation 10 in equation 9 gives, after rearrangement,

$$\frac{-d[CIm]}{dt} = k_{obs}[CIm] \qquad (11)$$

where

$$k_{obs} = (k_1 + k_3)[RNH_2] + (k_2 + k_4)[RNH_2]^2 + \left\{(k_5 + k_6)[RNH_2] + k_7 - \frac{k_7 k_{-7}[Im]}{k_{-7}[Im] + k_8[RNH_2]}\right\} \times [C] \qquad (12)$$

Here, it is convenient to consider two special cases by manipulating the experimental conditions.

Case 1. In the absence of added trans-cinnamic acid, [C] = 0, equation 12 therefore becomes

$$k_{obs} = (k_1 + k_3)[RNH_2] + (k_2 + k_4)[RNH_2]^2 \qquad (13)$$

or

$$\frac{k_{obs}}{[RNH_2]} = (k_1 + k_3) + (k_2 + k_4)[RNH_2] \qquad (14)$$

The apparent rate constant divided by amine concentration, $k_{obs}/[RNH_2]$, now varies linearly with the concentration of the substrate, $[RNH_2]$, i.e., a plot of $k_{obs}/[RNH_2]$ against $[RNH_2]$ will give a straight line with an intercept of $(k_1 + k_3)$ and a slope of $(k_2 + k_4)$. This relationship is demonstrated in Figure 14, giving an intercept of 1.80×10^{-5} $M^{-1}sec^{-1}$ and a slope of 6.35×10^{-2} $M^{-2}sec^{-1}$.

Case 2. In the presence of added trans-cinnamic acid, Since $k_8[RNH_2] \gg k_{-7}[Im]$ ($k_8 = 158.3$ $M^{-1}sec^{-1}$; $k_{-7} = 9.27 \times 10^{-2}$ $M^{-1}sec^{-1}$ and $[RNH_2] \gg [Im]$ under experimental conditions) and if $k_8[RNH_2] \gg k_7k_{-7}[Im]$ (actually this is the case under experimental conditions), it follows that

$$\frac{k_7k_{-7}[Im]}{k_{-7}[Im] + k_8[RNH_2]} \cong 0$$

Equation 12 therefore approximates to

$$k_{obs} = (k_1 + k_3)[RNH_2] + (k_2 + k_4)[RNH_2]^2 + \left\{(k_5 + k_6)[RNH_2] + k_7\right\}[C] \qquad (15)$$

This equation suggests a linear relationship between the apparent first-order rate constant, k_{obs}, and the concentration of trans-cinnamic acid, [C]. As shown in Figure 24, the plots are linear. The values of the intercepts and slopes are summarized in Table XXIV.

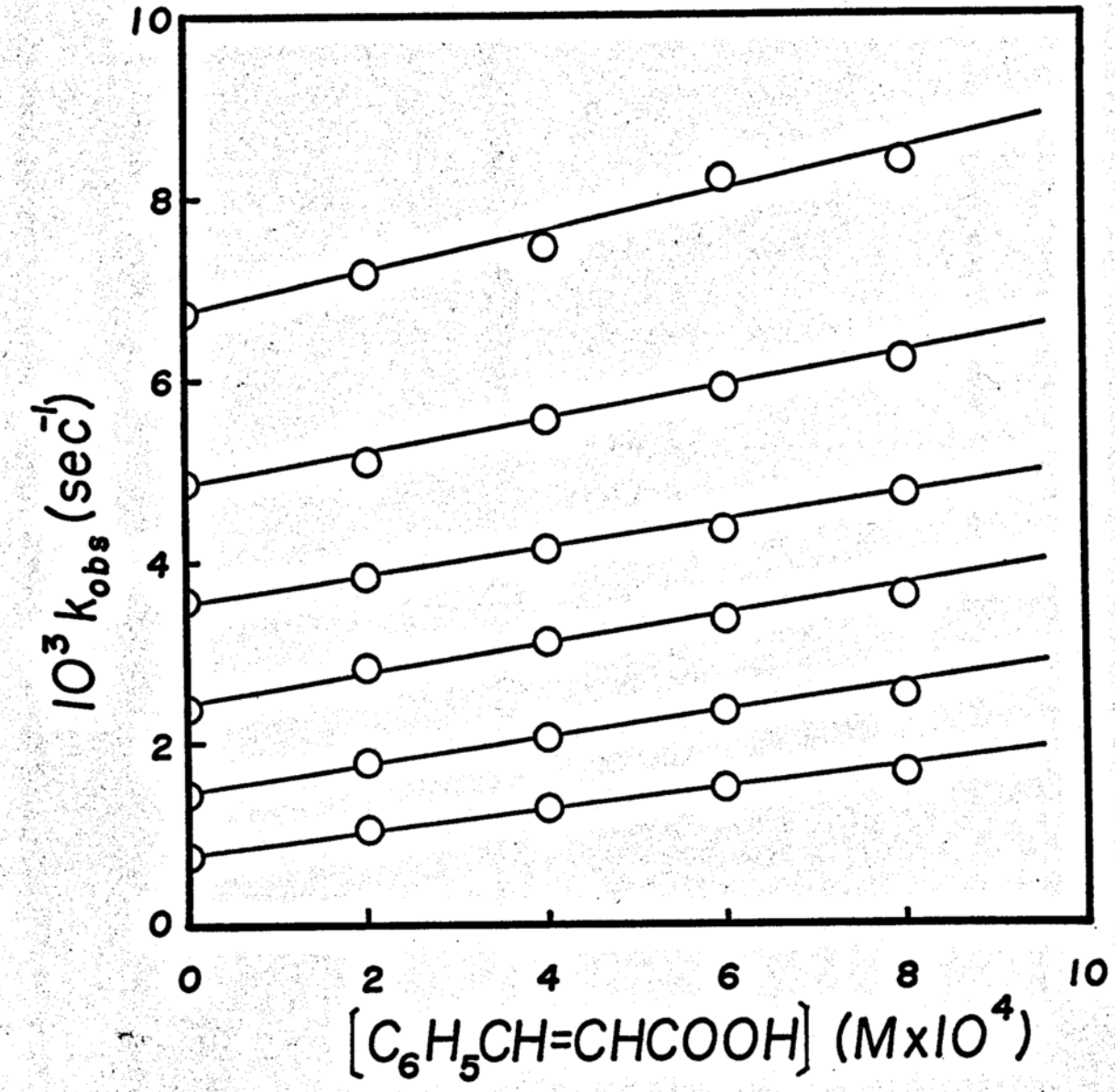


Figure 24. Plots of k_{obs} vs. trans-cinnamic acid concentration at various concentrations of n-butylamine. From top to bottom: n-butylamine 3.277×10^{-1} M; 2.751×10^{-1} M; 2.340×10^{-1} M; 1.952×10^{-1} M; 1.507×10^{-1} M; 1.099×10^{-1} M.

Table XXIV

[n-$BuNH_2$] M	10^3 Intercept[a] (sec^{-1})	Slope[a] ($M^{-1}sec^{-1}$)
1.099×10^{-1}	0.815	1.084
1.507×10^{-1}	1.492	1.355
1.952×10^{-1}	2.464	1.515
2.340×10^{-1}	3.537	1.489
2.751×10^{-1}	4.841	1.771
3.277×10^{-1}	6.735	2.232

[a]The values are obtained by least-square treatment.

Since intercept = $(k_1 + k_3)[RNH_2] + (k_2 + k_4)[RNH_2]^2$, a plot of the intercept divided by amine concentration, Intercept/$[RNH_2]$, vs. amine concentration, $[RNH_2]$, will be a straight line with an intercept equal to $(k_1 + k_3)$ and a slope equal to $(k_2 + k_4)$. Figure 25 demonstrates this relationship, giving an intercept of 7.77×10^{-4} $M^{-1}sec^{-1}$ and a slope of 6.08×10^{-2} $M^{-2}sec^{-1}$.

Similarly, from equation 15, slope = $k_7 + (k_5 + k_6)[RNH_2]$, suggesting that there exists a linear relationship between the slope and the amine concentration. This plot as shown in Figure 26 gives an intercept of 5.74×10^{-1} $M^{-1}sec^{-1}$ and a slope of 4.80 $M^{-1}sec^{-1}$.

The kinetic data, obtained from the above treatment and summarized in Table XXV indicate that the general acid-catalyzed nucleophilic reactions $(k_5 + k_6)$, general

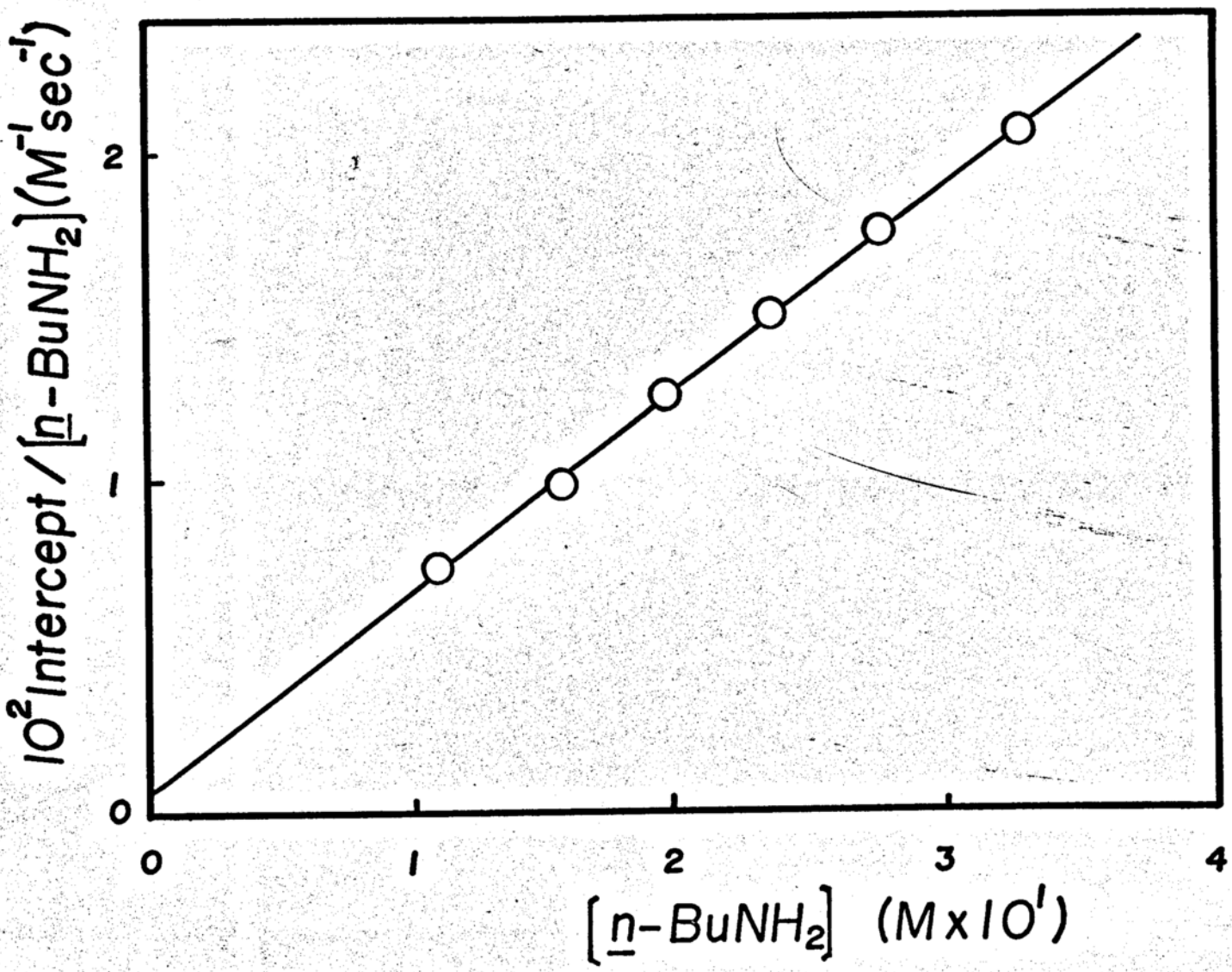


Figure 25. A plot of Intercept/[n-butylamine] vs. [n-butylamine].

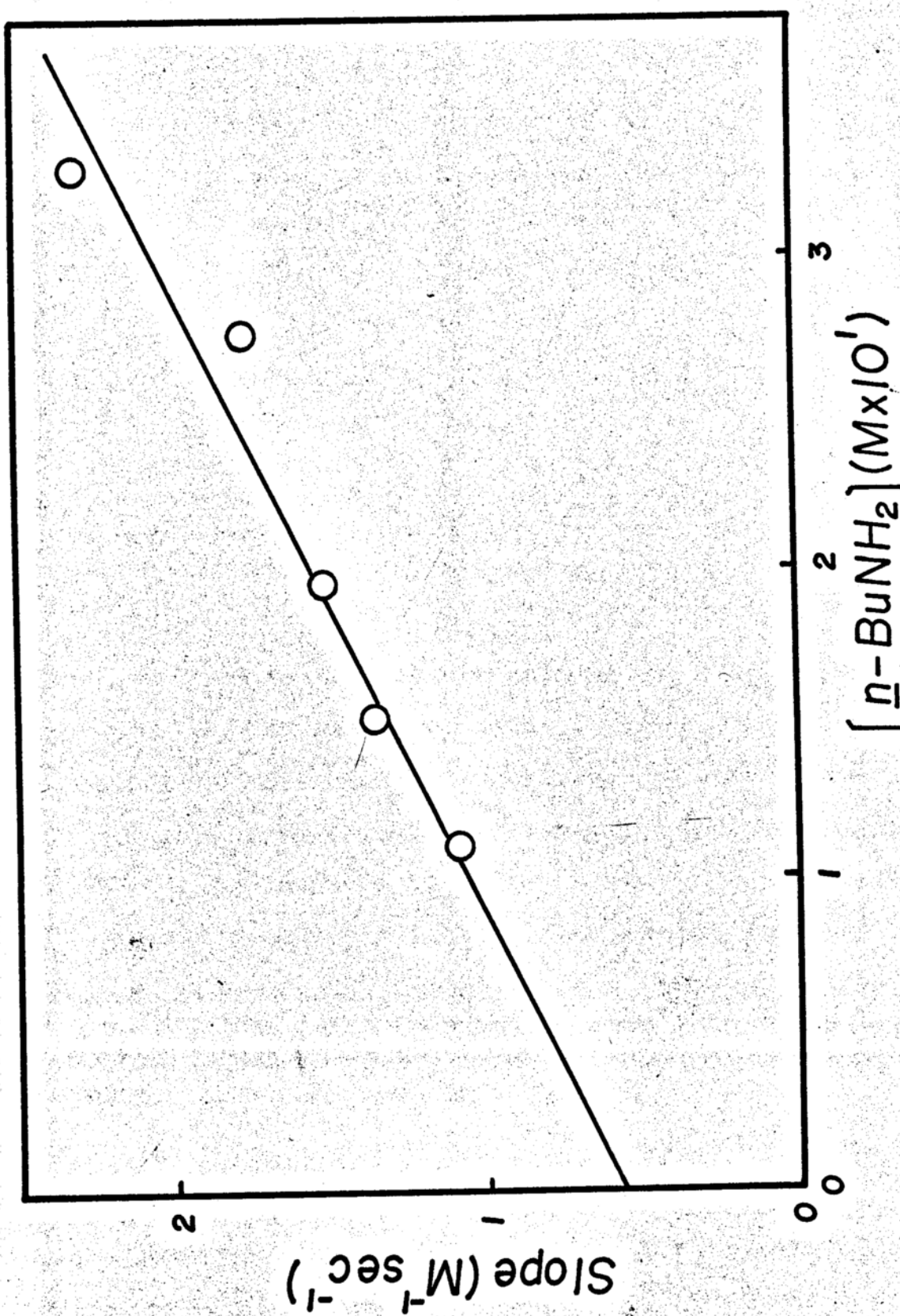


Figure 26. A plot of Slope vs. [n-butylamine].

Table XXV

Kinetic Data for the Acylation of n-Butylamine with N-trans-Cinnamoylimidazole in Acetonitrile at 25°C

$k_1 + k_3$:	1.8×10^{-5} $M^{-1}sec^{-1}$; 7.77×10^{-4} $M^{-1}sec^{-1}$
$k_2 + k_4$:	6.35×10^{-2} $M^{-2}sec^{-1}$; 6.08×10^{-2} $M^{-2}sec^{-1}$
$k_5 + k_6$:	4.80 $M^{-2}sec^{-1}$
k_7:	5.74×10^{-1} $M^{-1}sec^{-1}$
k_{-7}[a]:	9.27×10^{-2} $M^{-1}sec^{-1}$
k_8[a]:	158.3 ± 0.4 $M^{-1}sec^{-1}$

[a]obtained from independent study.

base-catalyzed nucleophilic reactions ($k_2 + k_4$), and the anhydride intermediate mechanism (k_7 and k_8) are of importance. The uncatalyzed nucleophilic reactions, (k_1 and k_3), can be considered insignificant as compared to all other reactions. k_{-7} and k_8 were obtained from independent studies of the reactions between trans-cinnamic anhydride and imidazole, and amines. Although not all the individual rate constants could be resolved from the above treatment, some features of the reaction mechanism appeared to be clear. The kinetics of the reactions between N-trans-cinnamoylimidazole and i-butylamine, sec-butylamine, and piperidine behave differently from that of n-butylamine. In all cases studied, no pseudo-first-order kinetics was obtained by

following the disappearance of N-trans-cinnamoyl-imidazole at 310 mμ (Figures 15, 16 and 17), suggesting that different mechanisms may be operating. These systems are not understood.

V. SUMMARY

Several acylating agents, possessing the trans-cinnamoyl function ($\epsilon_{max} \sim 2.2 \times 10^4$ for most compounds containing this function), namely, trans-cinnamic anhydride, p,p'-disubstituted-trans-cinnamic anhydrides, trans-cinnamoyl chloride, and N-trans-cinnamoyl-imidazole, have been used for the micro-determination of primary and secondary amines by acylation. The method is based upon the conversion of amines to the corresponding N-substituted cinnamamides. After acylation of the amine in acetonitrile solution, the excess reagent is hydrolyzed, the amide is extracted into chloroform, and the amide is measured spectrophotometrically. The method is sensitive, being applicable to amine samples in the 1-5 μmole range. The validity of the analytical method, including sensitivity, selectivity, and interferences, is discussed. trans-Cinnamoyl perchlorate and trans-cinnamoyl-α-chymotrypsin have also been investigated as potential acylating agents. The analytical results for these reagents, however, were unsatisfactory and the reagents were abandoned.

The difference in spectral properties between trans-cinnamic anhydride and N-substituted cinnamamides provides a simple method of spectrophotometric titration

of aliphatic amines with trans-cinnamic anhydride. Its principal advantages are its sensitivity and, in part, its selectivity.

The kinetics of the acylation reaction between trans-cinnamic anhydride (or its p,p'-disubstituted derivatives) and aliphatic amines follow over-all second-order kinetics, that is, first-order with respect to the anhydride and first-order with respect to the amine. It is found that this cinnamoylation rate constant is a very discriminating criterion of identity, the maximum range in relative rate being about 10^4 for the twenty-six aliphatic amines studied. This offers an approach to the characterization of aliphatic amines (primary and secondary) because of the marked sensitivity of the reaction rate to structure of the reactants. The second-order cinnamoylation rate constants were further correlated with the aqueous pK_a values for the conjugate acids of the amines; in general, the cinnamoylation rate constants are in parallel with the basicities of the amines. Sterically hindered amines react more slowly than would be anticipated from their basicity constants while alicylic amines react much faster.

The kinetics of the acylation reaction between N-trans-cinnamoylimidazole and aliphatic amines, however, show much more complicated behavior than was expected. It is believed that a side reaction, addition of amine across the α,β-unsaturated linkage, occurs. A kinetic

scheme has been developed for the acylation of n-butylamine with N-trans-cinnamoylimidazole, which accords well with the experimental results. Several other amines, in this system, give more complicated kinetics.

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APPENDIX A

SPECTROPHOTOMETRIC DETERMINATION OF ALIPHATIC AMINES BY ACYLATION WITH CINNAMIC ANHYDRIDE