A STUDY OF GEOMETRIC ISOMERISM IN THE THIOSEMICARBAZONES OF SOME PYRIDINECARBOXALDEHYDES

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CHAPTER I

INTRODUCTION

Since organisms show preference in their metabolism for specific isomers of certain types of molecules, e.g. D-isomers of sugars and L-isomers of amino acids for carbohydrate and protein synthesis respectively, it seems reasonable to suppose that if a drug which shows some type of physiological activity can exist in isomeric forms, the biological system in which the drug is active might show a preference for one isomer over the other, i.e. one isomer might be more active than the other. Thiosemicarbazones have been receiving increasing interest in recent years due to their physiological activities, especially their antifungal, antiviral and anticancer activities. Since the carbon-nitrogen double bond allows the possibility of geometric isomers and since such isomerism does exist in other azomethines it was decided to investigate the stereochemistry of some thiosemicarbazones to detect the existence of such isomerism and possibly isolate the individual Z and E isomers of some of the drugs.

If individual isomers can be isolated, it must then be ascertained if they are stable enough to permit testing of their anticancer activity. If the isomers interconvert rapidly then no purpose would be served in testing the activity of individual isomers. Therefore, if isomers can be isolated, their stability under various conditions must be investigated, including those encountered in a physiological

system. Since the thiosemicarbazones selected for investigation are excellent ligands for some metal ions one of the factors to be investigated is the effect of metal ions on the isomers when at physiological pH.

The goal of the investigation then is threefold:

1. isolation of E and Z geometric isomers of selected thiosemicarbazones and proof of their configuration;
2. investigation of the interconversion of the isomers under conditions which are found in a biological system, i.e. aqueous solution, pH 7.0, and in the presence of metal ions; and
3. preparation of samples for testing.

If one isomer of a given drug is more active than the other, the anticancer activity of the drug might be greatly improved by administering the more active isomer only. In this way the actual dose could be maintained at a "safe" low-toxicity level while the effective dose of the active isomer could be increased.

CHAPTER II

HISTORICAL

PHYSIOLOGICAL ACTIVITY OF THIOSEMICARBAZONES

In 1956 Brockman et al.[1] observed that the thiosemicarbazone of pyridine-2-carboxaldehyde showed mild but definite antileukemic activity. This observation was verified by French and Freedlander.[2] In 1966 French and Blanz reported the carcinostatic activity of the thiosemicarbazones of 43 heterocyclic aldehydes and ketones.[3] Notable compounds in this list were the thiosemicarbazones of 3-hydroxypyridine-2-carboxaldehyde and isoquinoline-1-carboxaldehyde. In this paper French and Blanz hypothesized that the chemotherapeutic activities of the compounds were due to their functioning as tridentate ligands with certain divalent transition metal ions. Isoquinoline-1-carboxaldehyde thiosemicarbazone had previously been discussed individually.[4] Sartorelli and several other workers have worked extensively with isoquinoline-1-carboxaldehyde thiosemicarbazone (1-IQ).[5,6,7]

In 1958 French and Blanz[8] reported on the carcinostatic activity of 5-hydroxypyridine-2-carboxaldehyde thiosemicarbazone (5-HP) which is more active than 1-IQ or 3-hydroxypyridine-2-carboxaldehyde thiosemicarbazone (3-HP) in some tumor test systems, especially L-1210 leukemia and ascitic sarcoma S-180 of the mouse.[9]

Suggested Modes of Action

Some of the published work on these thiosemicarbazones refer to their ability to complex certain metals. This idea of metal ion involvement in the physiological activity of a thiosemicarbazone is not unique. The *in vivo* activity of kethoxal bis(thiosemicarbazone) against Walker 256 carcinosarcoma is enhanced by the presence of copper and zinc ions.[10,11] The antitumor activity of the copper chelate of pyruvaldehyde bis(thiosemicarbazone) is much greater than that of the ligand itself.[12]

A direct relation was found between the antifungal activity of certain thiosemicarbazones and the extent of their reaction with copper ions. The N^2-methyl derivatives do not form complexes and have little antifungal activity.[13]

French *et al.* suggest that a Fe(II)-enzyme-thiosemicarbazone complex is responsible for the anticancer activity of some thiosemicarbazones.[7]

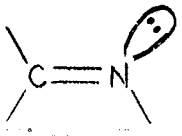
In 1967 Sartorelli[14] reported that 1-IQ caused greater than 10% in vivo inhibition of DNA synthesis as indicated by the incorporation of thymidine-^{3}H into the thymine of DNA. Use of other labeled precursors of DNA showed that the retarded incorporation of labeled thymidine was indeed due to inhibition of DNA synthesis and not a unique characteristic of thymidine-^{3}H. In the same report Sartorelli suggests that the inhibition of DNA synthesis may be due to blockage of the enzymatic reduction of ribonucleotides to deoxyribonucleotides. This proposal is made attractive because the inhibition of DNA synthesis

by 1-IQ is partially reversed by the administration of ferrous sulfate to sarcoma 180 tumor-bearing mice subsequent to treatment by 1-IQ. This fact is important because of the observation by Moore and Reichard that more than a 2-fold stimulation of dCDP formation occurred on addition of ferric chloride to the cytidine diphosphate reductase system isolated from Novikoff rat hepatoma.[15] The requirement for iron in the CDP-reductase system was further demonstrated by the inhibition of reductase activity upon addition of EDTA to the system. Addition of ferric chloride to the reaction mixture reversed the inhibition. Sartorelli and Booth[16] in 1968 showed a positive correlation between tumor-inhibitory potency and inhibition of the synthesis of DNA. Also in 1968 Sartorelli et al.[17] reported that 1-IQ inhibited the reduction of CDP by the purified CDP-reductase system from Novikoff rat tumor. Brockman et al.[18] reported that the antiviral activity of 5-HP, 1-IQ, and several other thiosemicarbazones against Herpes simplex, strain HF virus and human cytomegalovirus, strain Casazza appears to be due to an interference with an intracellular process necessary for viral replication. The results imply that the antiviral activity is due at least in part to inhibition of ribonucleotide reductase and consequently DNA synthesis.

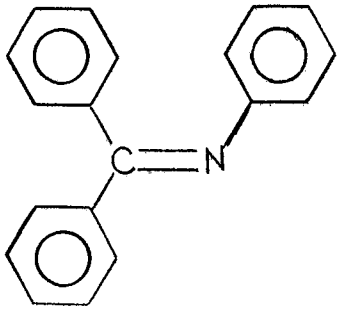
STEREOCHEMISTRY OF THE CARBON-NITROGEN DOUBLE BOND

The azomethine bond ($\diagdown\!\!\diagup C{=}N-$) allows the existence of isomers analogous to cis and trans isomers in alkenes. The bond is considered to have both carbon and nitrogen bonds trigonally hybridized, with each contributing a π-electron and the nitrogen atom having a lone pair orbital in the plane of the other groups. The valence angles are

close to 120°. [19] The bond is represented as follows:



Early reports of the isolation of syn and anti isomers were disputed as merely examples of polymorphism. Usually the evidence given for the presence of different isomers was different crystalline structure, different melting points, or different colors. The argument most frequently used in attempts to refute this evidence was that polymorphism can exist in some cases where stereoisomerism is not possible, e.g. benzophenone phenylimine (1) which exists in two crystalline forms even though no stereoisomerism is possible.[20]

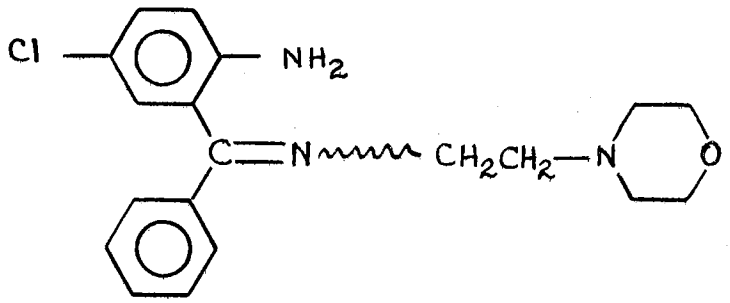


1

Preparation and Identification of Isomers

Two excellent reviews of the isolation of geometric isomers of various oximes, phenylhydrazones and other imines are those by Wettermark[21] and by McCarty.[22]

Isolation of syn and anti isomers of an imine is reported for 2.[23] Bell shows by spectroscopic differences and by unequivocal synthesis that the two forms are indeed isolated.



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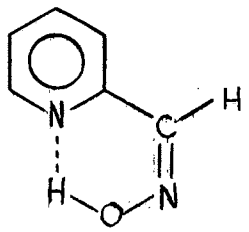
Raffauf[24] distinguishes isomeric furaldoximes on the basis of their ultraviolet spectra. The syn isomer displays an absorption maximum in water at 270 nm while the anti absorbs at 265 nm.

Poziomek et al.[26,27] identify the syn and anti isomers of 4-formyl-1-methylpyridinium iodide oxime on the basis of their NMR spectra. Poziomek also reports two methods of preparing syn and anti isomers of pyridine-2-carboxaldehyde oxime. One preparation consists of separating a mixture of the isomers on a Florisil column. Under the conditions given and using chloroform as solvent the anti isomer (3) passes through the column more rapidly than the syn. The second method consists of irradiating a concentrated acetone solution of the syn oxime, which can be purchased. Irradiation at 0-5° with a 253.7 nm source for 17 hours results in precipitation of the crude anti oxime. Identification is based on the presence of an intramolecular hydrogen bond indicated by the spectrum of the anti oxime.



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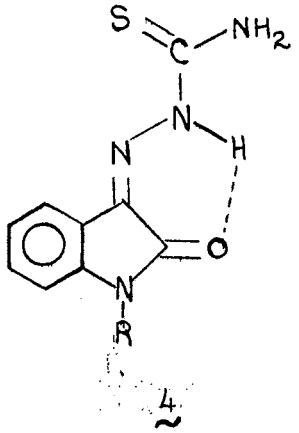
Schnekenburger[28] reports that the syn isomers of 2- and 4-pyridinecarboxaldehyde-1-oxide oxime are more acidic than the anti isomers.

Isatin 3-thiosemicarbazone, according to infrared and NMR spectroscopy, in the solid state exists in the syn form (4) with an intramolecular hydrogen bond.[29]



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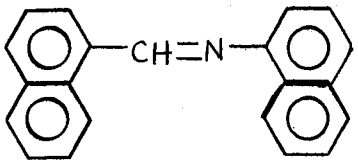
Interconversion of Isomers

Part of the difficulty in showing the existence of isomerism in azomethines lay in the ease with which the isomers could interconvert. With the advent of more modern detection and measuring equipment and techniques the study of these isomers has become easier and consequently their interconversion has been studied with increasing frequency.

Fisher and Frei[30] present what appears to be the first study of the kinetics of syn-anti isomerization at the azomethine group in compounds 5 and 6. When methylcyclohexane solutions of 5 and 6 (and related compounds) were irradiated at -100° pronounced changes occurred in the ultraviolet spectrum of each compound. Upon slow



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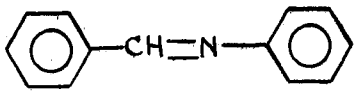

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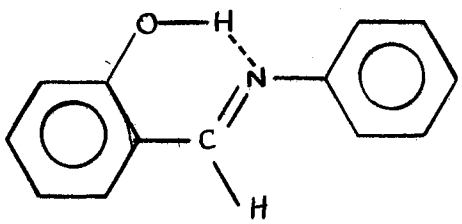
warming of the solutions a temperature was reached at which the spectra began to revert to their original form. This reversion is reported to follow first order kinetics and is considered to be related to interconversion of the isomers.

Wettermark[31] reports that flash-photolyzing the Schiff base prepared from salicylaldehyde and aniline (7) produces two transient species as indicated by absorption maxima at 470 nm and 385 nm. The species indicated by the maximum at 470 nm disappears with a lifetime in the millisecond region. The disappearance shows a dependence on pH and is believed to involve hydrogen transfer from the hydroxyl group ortho to the nitrogen atom. The absorption peak at 385 nm decays with a half-life of approximately 1 second. The decay is first-order and independent of pH; the absorption is attributed to the unstable syn form, which is converted back to the stable anti form. The anti form is regarded as more stable because it is planar and has an internal hydrogen bond.



7

These experiments were extended to other anils formed from o- and p-hydroxybenzaldehyde.[32] Again the transient absorbances near 385 nm are attributed to the syn isomer, which has been formed by flash photolysis and which isomerizes to the more stable anti isomer. Again the reaction is first-order with a half-life of about one second.

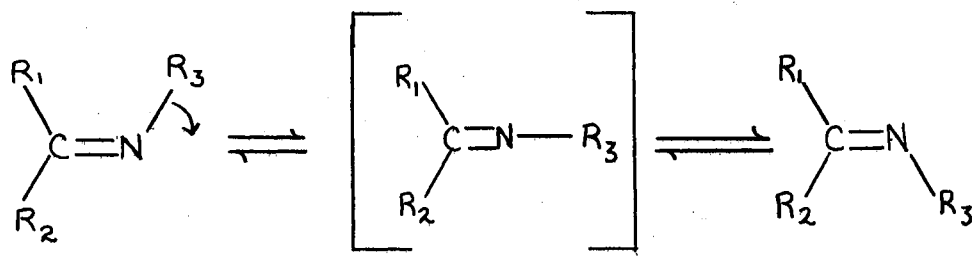
Curtin et al.[33] have investigated the syn and anti isomers of several imines by NMR and ultraviolet spectroscopy and report the isomerizations to be first-order, with rate constants ranging from 1 to 12.4 sec.$^{-1}$ depending on temperature. They also mention the surprising configurational stability of the O-methyl ethers of syn- and anti-p-chlorobenzophenone oxime.

Karabatsos et al. utilized NMR spectroscopy to investigate solutions of syn and anti isomers of different azomethines and report per cent composition of the isomers in solution.[35,36,37,38]

The reviews by Wettermark[21] and Walker[22] should be consulted for further references to the syn and anti isomers of azomethines.

Mechanisms of Isomerization

Of the several mechanisms which have been suggested for the isomerization of syn to anti forms the lateral shift seems most likely under neutral conditions.[22] This first-order mechanism is analogous to



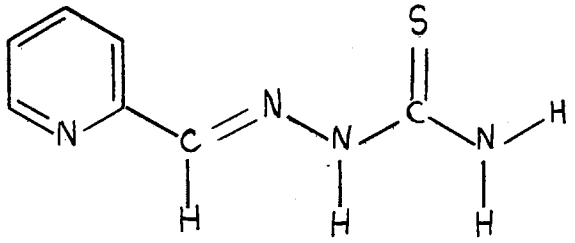
inversion at the nitrogen atom which occurs in amines. Support for this mechanism is given by Kessler[34], who investigated several arylamines by NMR and reports that inversion at the nitrogen atom is the mechanism for syn-anti interconversion.

CHAPTER III

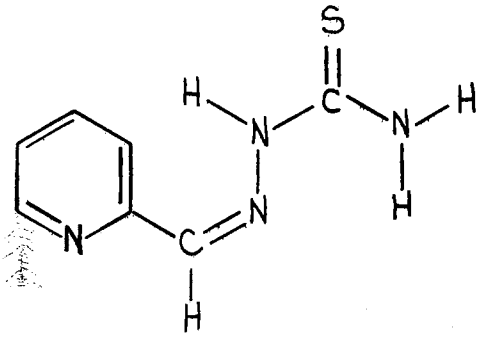
DISCUSSION OF THE PROBLEM

The thiosemicarbazones are condensation products of aldehydes or ketones and thiosemicarbazide. This condensation results in the formation of the carbon-nitrogen double bond which is characteristic of azomethines. The terms *syn* and *anti* which have been used to describe the stereochemistry around the double bond are being replaced by a new symbolism which uses the letters *Z* (zusammen) and *E* (entgegen). To assign the prefixes *Z* and/or *E* the sequence-rule-preferred atom or group attached to one of a doubly bonded pair of atoms is compared to the sequence-rule-preferred atom or group attached to the other of that doubly bonded pair of atoms. If the selected pair are on the same side of the reference plane, i.e. the double bond, an italic capital letter *Z* prefix is used; if the pair are on opposite sides an italic capital letter *E* prefix is used. The prefixes normally precede the whole name and are placed in parentheses and followed by a hyphen.

The prefixes *Z* and *E* will be used to designate the geometric isomers of the thiosemicarbazones. If the thiocarbamido group ($-NHC(S)NH_2$) is on the opposite side as the substitute or unsubstituted pyridine ring the isomer is the *E* isomer (8-a). The *Z* isomer is one in which the pyridine ring and thiocarbamido moiety are on the same side of the double bond (8-b).

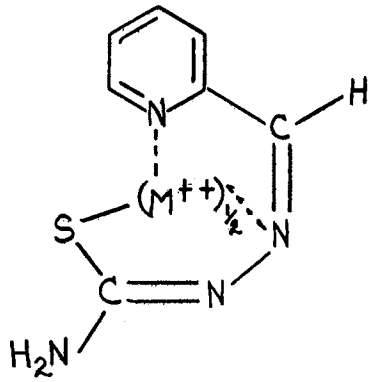


8-a



8-b

It has already been suggested by French[3] that the active thiosemicarbazones in his list of 43 are active because they function as tridentate ligands. In order to do so they must exist in the Z configuration and the complex will be represented as in 9. Thus



9

possibly the Z configuration of the thiosemicarbazones is the active form of the drugs because of this ability to complex certain metals.

As already indicated, the thiosemicarbazones can slow the synthesis of DNA by inhibiting the enzymatic reduction of ribonucleotides to deoxyribonucleotides. This fact increases the plausibility of the statement that the Z isomer may be the active form of the drugs because enzymes can exhibit extreme selectivity for substrates that have the required steric configuration. The most common specificity has already been mentioned, the use of L-amino acids and D-sugars for protein and carbohydrate synthesis respectively. Because of this selectivity of enzymes and because the inhibition of DNA synthesis is inhibition of enzymatic reactions, it seems logical that the inhibited reductase system would be sensitive to steric modifications in its inhibitor.

The suggestion by French of complex involvement implies a stereochemical consideration for activity and intuition also says that the stereochemistry of the inhibitor is important in inhibition of DNA synthesis. It is logical therefore to carry the investigation of the anticancer activity of the thiosemicarbazones further and attempt to discover if indeed one form is more active than the other. Since it has been shown that other compounds displaying the carbon-nitrogen double bond can exist as E and Z isomers, the first approach to the problem should be to determine if the anticancer thiosemicarbazones can exist as E and Z isomers. If these can exist then the next step will be development of usable techniques by which the isomers may be prepared or separated in quantities sufficient to study.

Of course the structures must be identified. This type of therapy has been shown to be effective in at least two published reports.

Poziomek et al.[25] reports that the E and Z isomers of 4-formyl-1-methylpyridinium iodide oxime were tested for reactivation of inhibited acetylcholinesterase. The Z isomer was the more active reactivator.

Byrne and O'Sullivan[39] report the antibacterial and antitumor activities of some mixed hydrazone derivatives of methylglyoxal. The monoarylhydrazones of methylglyoxal were prepared and the thiosemicarbazones were prepared from these. Different methods of preparation gave different isomeric products which were identified on the basis of an intramolecular hydrogen bond. The isomeric derivatives were tested against a number of microorganisms and in every case the E isomer gave the more active material. The E isomer of one thiosemicarbazone in particular was active against sarcoma 180 in mice. The Z isomer showed no significant activity.

This preliminary work then indicates that this therapy can be effective and very desirable. A primary reason for the desirability of this type of study is the general toxicity of the thiosemicarbazones. Heretofore only the mixtures resulting from synthesis have been tested for activity. If it is found that one isomer is more active than the other then therapy could consist of administration of the active isomer only. In this way the actual dose of thiosemicarbazone could be maintained but since only the active isomer is given the therapeutic effect might be increased. It is possible that the therapeutic value of a drug might be increased without exceeding toxicity limits.

It is evident that if this type of therapy is to be pursued then the first step in such a problem is to determine if the thiosemicarbazones being studied do actually exist as *syn* and *anti* isomers. This was determined by chromatographing samples of 2-FP on prepared thin-layer chromatography sheets.

Chromatography of 2-FP

Our original supply of 2-FP was obtained from Frederic A. French of the Mount Zion Hospital and Research Center of San Francisco, California. A sample of this compound was dissolved in absolute ethanol, diluted with water, and spotted on a 1" x 3" strip of prepared thin-layer chromatography (TLC) sheet. The chromatogram was developed in a 99:1 solution of ethyl ether : *N*,*N*-dimethylformamide. When viewed under short-wave ultraviolet light the chromatogram revealed two spots, one which migrated and one in the original location which did not migrate. A new supply of 2-FP was synthesized and the experiment was repeated on this product with the same results.

One of two alternative conclusions may be drawn: the two spots are characteristic of the thiosemicarbazone, or else the thiosemicarbazone decomposes in solution. To ascertain which of these conclusions is correct a TLC strip was prepared on which were spotted samples of 2-FP from French, freshly prepared 2-FP, pyridine-2-carboxaldehyde, and thiosemicarbazide. Development of this strip revealed that the two samples of 2-FP behaved identically and neither component corresponded to the aldehyde or thiosemicarbazide.

Since the two components are characteristic of 2-FP obtained from French and also of a freshly prepared supply of 2-FP, they are

characteristic of the thiosemicarbazone itself. Since the thiosemicarbazone possesses a carbon-nitrogen double bond the logical conclusion is that these two spots represent the geometric E and Z isomers of 2-FP.

The experiments were duplicated with different solvents and solvent mixtures. The mixed solvent which gave the best separation of components ($R_f \doteq .56$) consisted of an 80:20 mixture of chloroform and diethyl ether.

Chromatography of 3-HP

When 3-HP was chromatographed two components were found which showed the same migration characteristics as 2-FP, i.e. one component migrated and the other did not.

The stability of the isomers at physiological pH should be determined. Administration of a pure isomer would have no value if it equilibrated to a mixture of two isomers within a few seconds. Also, since the compound will encounter metal ions in the body fluids, the stability of the isomers should be determined in the presence of those divalent metal ions which form complexes with the thiosemicarbazones and also are most likely to be encountered in the body. The ions selected are magnesium(II), manganese(II), calcium(II), iron(II), copper(II), zinc(II), and cobalt(II).

In summary, the problem consists of three major portions as already mentioned: (1) isolation and identification of isomers, (2) investigation of the stabilities of the isomers, and (3) testing for the activities of the isomers. This work will be performed on pyridine-2-carboxaldehyde thiosemicarbazone and 3-hydroxypyridine-2-carbox-

aldehyde thiosemicarbazone which have different antitumor activities. 2-FP is not very active while 3-HP is quite active in some tumor systems. The goal of the project will be to increase the therapeutic value of these drugs and to develop a method to improve the value of other similar drugs.

CHAPTER IV

EXPERIMENTAL

Materials

Acetic acid (Du Pont). Reagent grade used without further purification.

Acetone (Fisher Scientific). Certified grade used without further purification.

Activated charcoal (Nuchar). Nuchar C-115-N vegetable carbon.

n-Butyl acetate (Fisher Scientific). Reagent grade used without further purification.

Calcium acetate (J.T. Baker). Reagent grade used without further purification.

Chloroform (Fisher Scientific). Spectral grade redistilled before use.

Cobalt chloride (J.T. Baker). Reagent grade used without further purification.

Cupric sulfate (J.T. Baker). Reagent grade used without further purification.

Diethyl ether (Fisher Scientific). Anhydrous ether used without further purification.

Dimethylformamide (Fisher Scientific). Certified reagent grade used without further purification.

Ethyl alcohol (U.S.I.). Absolute Pure U.S.P.-N.F. grade used without further purification.

Ferrous sulfate (J.T. Baker). Reagent grade used without further purification.

Formaldehyde (Merck). Reagent grade formalin used without further purification.

Hexanes (Fisher Scientific). Certified grade, a mixture of isomers used without further purification.

Hydrochloric acid (Fisher Scientific). Certified grade 0.1 N standard reagent.

Hydrogen chloride (Matheson). A lecture bottle was used.

3-Hydroxypyridine (Aldrich). White-label grade used without further purification.

Magnesium chloride (Fisher Scientific). Certified grade, used without further purification.

Manganous carbonate (Baker and Adamson). Reagent grade used without further purification.

Manganous sulfate (J.T. Baker). Reagent grade used without further purification.

Methylhydrazine (Eastman). White-label grade used without further purification.

Potassium bromide (International Crystal Laboratories). Spectrographic grade was used.

Potassium thiocyanate (Baker and Adamson). N.F. grade was used without further purification.

Pyridine-2-carboxaldehyde (Aldrich). Distilled under reduced pressure before use.

Silica gel PF-254 (E.Merck).

(di) Sodium phosphate (Fisher). Certified A.C.S. grade used without further purification.

(mono) Sodium phosphate (Fisher). Certified A.C.S. grade used without further purification.

Thiosemicarbazide (Aldrich). White-label grade used without further purification.

Zinc acetate (Fisher Scientific). Certified reagent used without further purification.

Instrumentation

Ultraviolet spectra were taken on a model 14 Cary recording spectrophotometer. Infrared spectra were obtained using a IR5-A Beckman spectrophotometer. The samples were prepared in potassium bromide pellets.

Apparatus

The tanks used for developing the thin layer chromatography (TLC) plates were obtained form Brinkman Instruments, of Westbury, New York. The developing jars for TLC slides and small strips were small screw-cap jars with filter paper placed around the walls to insure that the atmosphere in the jar is saturated with solvent. Prepared TLC sheets were Eastment Chromagram Sheets, Type K 301R (silica gel). The silica gel is impregnated with an indicator which fluoresces under ultraviolet light. Organic materials on the

sheets which absorb in the ultraviolet appear as dark spots on a bright background when observed under ultraviolet light (254 nm). The same viewing technique was utilized for the plates prepared from the Merck PF-254 silica gel which also contains a fluorescent dye.

Preparation of Preparative TLC Plates

Glass plates, 20 x 20 cm., were prepared by washing with scouring powder, rinsing with tap water and with 95% ethanol and air drying. Strips of masking tape were placed on two opposite edges of the plates six layers thick. A slurry was prepared from 30 g of silica gel and 50-60 ml of water (depending on the consistency desired) shaken vigorously in a screw-cap bottle. The slurry was poured onto two plates placed end-to-end and spread evenly by drawing a glass rod smoothly across the plates as shown in Figure 1. The excess slurry was pushed off the ends of the plates. The plates were allowed to air-dry for one hour, the tape was removed, and the plates were activated by placing them in an oven at 120° for 2 hours.

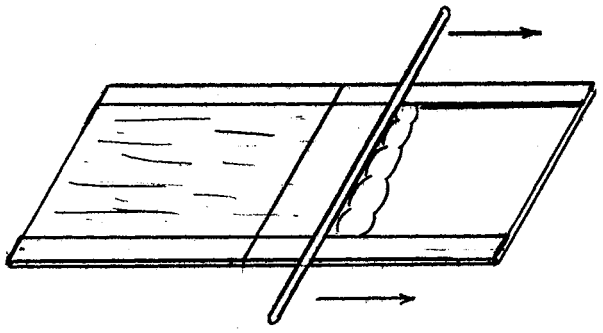


Figure 1. Preparation of TLC Plates

Separation and Recovery of Isomers

A solution (7 to 8%) of a thiosemicarbazone in absolute ethanol was repeatedly streaked over the same narrow band at one end of the plate and dried after each application until 4-5 ml had been applied.

The plate was then placed in the developing tank having approximately 3/8 of an inch of solvent in the bottom. The tank had been previously prepared by standing sheets of filter paper the height of the tank by each of the four inner walls of the tank. Sufficient solvent was put in the tank to saturate the filter paper and still maintain approximately 3/8 inch of solvent in the bottom.

The solvent front was allowed to rise to approximately three-fourths of the distance of the plate and the plate was removed from the tank and dried. The locations of the two bands were found under ultraviolet light and scraped off the plate, each band being kept separate from the other. The silica gel from each band was independently washed with successive 10-ml portions of absolute alcohol and the mixture filtered between washings until the filtrate was colorless.

The filtrates were put individually into round-bottom flasks and the solvent removed under reduced pressure. The residue (which was one of the isomers) was scraped off the walls of the flask and saved.

Synthesis of 2-FP

Thiosemicarbazide (9.11 g, 0.1 mole) was added to 80 ml of distilled water plus 3 ml of glacial acetic acid in a 3-necked round-bottom reaction flask. The flask was heated with an electric heating mantle and the slurry was stirred magnetically until the thiosemi-

carbazide dissolved in the hot water. To this solution was added over a period of 15 minutes 10.71 g (0.1 mole) of pyridine-2-carboxaldehyde and the resulting yellow solution was refluxed for 30 minutes. The solution was allowed to cool whereupon a yellow crystalline product precipitated. This solid was recrystallized from ethanol; the product melted at 211-213°. Literature, 210°.[61]

Preparation of 3-Hydroxy-2-hydroxymethylpyridine Hydrochloride

The procedure of Heinert and Martell[40] was followed to prepare 3-hydroxypyridine-2-carboxaldehyde thiosemicarbazone (3-HP).

To a solution of 3-hydroxypyridine (95 g, 1 mole) and sodium hydroxide (40 g) in water (400 ml), 38% formaldehyde solution (80 ml, 1 mole) was added. The clear mixture was warmed for 3 hours at 90°, cooled to room temperature and acetic acid (60 g., 1 mole) was added. The water was removed under reduced pressure, the remaining viscous oil (or pale yellow solid) was stirred with acetone (1 l), and the precipitated sodium acetate was filtered off. The solid was extracted with warm acetone (two 500-ml portions) and the combined extracts were further diluted with acetone (1 l). The additional precipitate was removed by filtration and the solution was concentrated under reduced pressure (to 1 l). Hydrogen chloride gas was introduced at 0°, whereupon a colorless crystalline precipitate immediately formed. As soon as formation of the precipitate ceased, it was filtered off, washed with acetone (100 ml) and stirred with a saturated solution of hydrogen chloride in ethanol at 0° to dissolve unreacted hydroxypyridine. Filtration and washing with ethanol resulted in the isolation of a nearly colorless solid. The product was purified by dis-

solving it in a minimum amount of warm water, treating with Nuchar (activated charcoal), and adding a large volume of acetone at room temperature. The colorless crystals obtained darkened above 180° and turned black at about 200°. Literature 180–200°.[40]

Preparation of 3-Hydroxypyridine-2-carboxaldehyde Thiosemicarbazone

3-Hydroxy-2-hydroxymethylpyridine bydrochloride (8.1 g, 0.05 mole) was oxidized by heating in refluxing absolute alcohol (100 ml) in which 4.35 g amorphous MnO_2 were placed. Concentrated sulfuric acid (5.1 g, 0.1 mole) in ethanol (30 ml) was added over a period of 30 minutes. After additional heating under reflux for 1 hour the black solid turned brown and the pH of the solution had risen to 6. The reaction mixture was cooled to 40° and filtered. The dark yellow solution was diluted with water (200 ml) and manganous carbonate was precipitated by adding excess sodium bicarbonate. The filtrate was extracted with ether (one 400-ml and two 150-ml-portions) and the combined ether extracts were extracted with 3.7% hydrochloric acid (four 25-ml portions containing 0.1 mole of HCl). The acidic extracts were neutralized with 8.4 g (0.1 mole) of sodium bicarbonate. A small excess was added beyond the 8.4 g to insure complete removal of acid. Thiosemicarbazide (4.5 g, 0.05 mole) was dissolved in 100 ml of boiling water; 5 ml glacial acetic acid was added to the solution. The aldehyde solution was added over a period of 15 minutes. The solution was refluxed 30 minutes, during which time a dark yellow precipitate formed. The mixture was filtered while hot to avoid contaminating the product with thiosemicarbazide. The precipitate was washed several times with deionized water.

The overall process (oxidation and condensation) yielded 1.38 g of the thiosemicarbazone (14.1%), m. 225-230° (dec. with darkening). Literature: 225-235 (dec.).[40]

Preparation of N^2-Methylthiosemicarbazide

This procedure follows closely that described by Jensen.[41] Methylhydrazine (4.6 g, 0.1 mole), was dissolved in 100 ml of 1 N HCl (0.1 mole) and to this solution was added 9.8 g. (0.101 mole) of potassium thiocyanate. Ethanol (100 ml) was added to precipitate potassium chloride and the solution was filtered and evaporated to dryness. Xylene (35 ml) was added and the mixture refluxed for 5 minutes. After cooling, the solid was filtered, dried, and recrystallized twice from water. Yield, 2.05 g. (20%) of N^2-methylthiosemicarbazide which melted at 174-175° Lit. 173-174°.[41]

Preparation of Pyridine-2-carboxaldehyde N^2-methylthiosemicarbazone

The methyl-substituted thiosemicarbazone was prepared by the procedure previously described for the unsubstituted thiosemicarbazone. A yellow product was obtained which displayed the characteristic infrared absorptions of a thiosemicarbazone.

Preparation of pH 7.0 Phosphate Buffer

Monosodium phosphate (0.4265 g) and disodium phosphate (o.5115 g) were dissolved in 500 ml of distilled, deionized (by passing through a Deeminac filter) water. The pH of the solution was measured with a pH meter (it usually is around 6.7-6.8 using the quantities of phosphates listed) and then adjusted by carefully adding 10% sodium

hydroxide until the pH reached 7.0. The solution was then diluted in a volumetric flask to a volume of 1000 ml. This procedure gives 0.005 M pH 7.0 phosphate buffer.

Preparation of pH 12.0 Phosphate Buffer

Disodium phosphate (1.3404 g, 0.005 M) was dissolved in 500 ml of distilled deionized water. The pH of the resulting solution was measured and then monitored with a pH meter as 10% sodium hydroxide was added slowly to adjust the solution to pH 12.0. The solution was then diluted to 1000 ml in a 1-liter volumetric flask. The resulting solution was 0.005 M pH 12.0 phosphate buffer. The buffer was stored in a polyethylene bottle which had been fitted with a drying tube filled with soda lime to prevent the entrance of carbon dioxide.

Preparation of Metal Solutions

The metal solutions (0.01 M) were prepared by dissolving 0.005 mole of the chloride, sulfate, or acetate in 100 ml of water and then diluting this solution to 500 ml in a volumetric flask. The sample of metal salt used was weighed accurately to 0.1 mg. The solutions were not analyzed for metal because the techniques by which the solutions of metal complexes were prepared for determination of ultraviolet spectra introduced errors so that the actual concentraction of the metal in the complex could not be known accurately but only closely approximated. The water used was doubly-distilled water which had then been passed through a Deeminac ion-exchange filter.

Determination of Ultraviolet Spectra

A sample of one isomeric form of the thiosemicarbazone (3-5 mg) was weighed accurately to 0.1 mg. When ready for use, the sample was dissolved in 10.0 ml of absolute ethanol. An aliquot was removed and diluted to 10.0 ml with the proper buffer. The volume of alcoholic solution of thiosemicarbazone was sufficient to make the final buffer solution approximately 6×10^{-5} molar.

A 3-ml aliquot of the solution was pipetted into a cuvette and spectra of the solution were taken at 2-30 min. intervals for periods up to three hours. The cuvettes which were used for determination of the spectra were Perkin-Elmer Spectrosil cells with a 1 cm path length.

Determination of Spectra of Metal Complexes

A fresh solution of the thiosemicarbazone was prepared exactly as above. A 3-ml sample was pipetted into the sample cell and the cell placed in the spectrophotometer. To this was added 10 µl of metal solution, the sample stirred and a spectrum taken immediately. This procedure was performed as rapidly as possible. Spectra were taken at intervals for periods up to three hours. Samples prepared in this manner were about 6×10^{-5} M in ligand and 3.3×10^{-5} M in metal with a ligand-metal ratio of approximately 2:1.

Preparation of E Isomers

The thiosemicarbazone was dissolved in a minimum amount of boiling water and the solution refluxed for 15 minutes. The solution

was allowed to cool slowly and those crystals which precipitated while the solution was still hot were filtered out. These crystals were analyzed by thin-layer chromatography and were shown to consist of only one component which did not migrate. Infrared and ultraviolet spectra indicated that this was the E isomer.

Preparation of the Z Isomers

The thiosemicarbazone was mixed with sufficient n-butyl acetate so that the solid completely dissolved in the refluxing ester. The solution was heated at reflux temperatures for 1 hour, after which the boiling solution was poured rapidly into a slurry prepared from crushed Dry ice and hexane (petroleum ether may be substituted for the hexane). The precipitated thiosemicarbazone was removed with a fritted glass filter containing crushed Dry ice to insure that the isomer would remain cold until filtration was complete and the product could be dried.

This procedure yielded a product which was identified by its infrared and ultraviolet spectra as the Z isomeric form of the thiosemicarbazone. When analyzed by TLC the product was shown to consist of two components, one which migrated and one which did not. The component which migrated is identified as the Z isomer and it shows up much more intensely on the TLC chromatogram than on similar chromatograms of untreated thiosemicarbazones obtained from French or those synthesized in this laboratory. This procedure increases substantially the amount of Z isomer in the mixture but does not give a pure isomer.

Determination of Isomerization Rate of (E) -2-FP by TLC

Samples of the E isomer were dissolved in 1 ml of absolute ethanol; 2 ml of H_2O was added to the solution. Aliquots of 5 µl were withdrawn at 5-minute intervals and spotted on TLC plates which had been prepared from microscope slides dipped in a slurry made from chloroform and silica gel HF_{254} (Brinkman) and activated at 120° after air drying. The slides were developed in a Chloroform-ether mixture and observed under short wave ultraviolet light. When the top spot (the Z isomer which migrates) first became visible the reaction was stopped. The procedure was repeated on another sample at a different concentration. The experiments were repeated with the thiosemicarbazone dissolved in 3 ml of absolute alcohol instead of the alcohol-water mixture.

Titration Spectrum of 3-HP

In order to follow the change in the spectrum of 3-HP with pH more closely than allowed by use of the two buffers (pH = 7.0 and 12.0) and HCl (pH = 1), a solution of (Z)-3-HP was prepared in pH 12.0 buffer as described previously. A micropipette was used to add 2 µl of 12 N HCl to 10 ml of solution. The pH was tested with indicator paper (three different kinds); comparison of the colors of the papers gave the approximate pH of the solution. The spectrum of this solution was taken. This procedure was repeated and spectra were obtained for pH values differing by approximately 1 unit from pH 12 to pH 3.

Preparation of TLC Solvents

All of the mixed solvents which were used to develop the TLC chromatograms were prepared by volume measurements rather than by weight.

CHAPTER V

RESULTS AND DISCUSSION

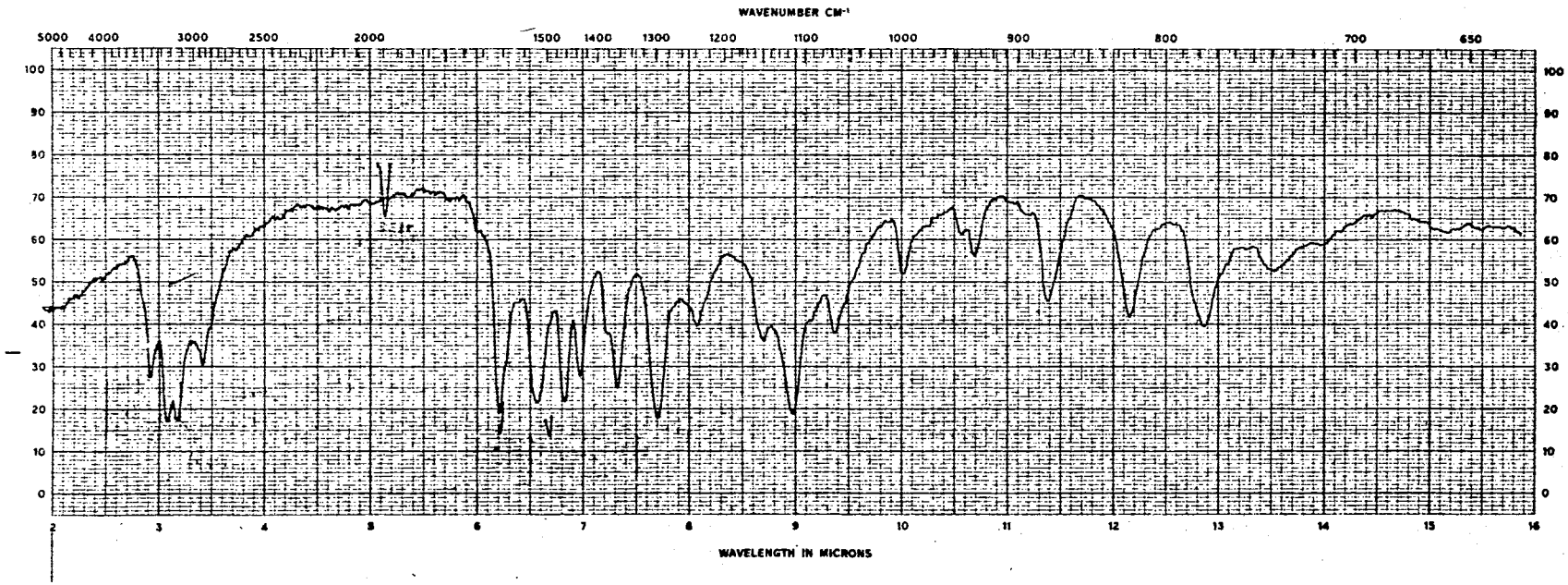
INFRARED SPECTRA OF (E)- AND (Z)-2-FP

Potassium bromide pellets were prepared for the two isomers of 2-FP which were separated by TLC and infrared spectra were taken of them. In the discussion of these spectra the term "top spot" refers to the component which migrated when the thiosemicarbazone was chromatographed. The term "bottom spot" refers to the component which did not migrate when the thiosemicarbazone was chromatographed. The spectra of the two components were identical in every respect but two

Both spectra (Plates I and II) exhibit three strong absorbances in the region of 2.9-3.3 μ. These peaks are attributed to N-H stretch of the molecule. Both the bottom and top components exhibit absorbance at 3.08 μ (3247 cm^{-1}) and 3.18 μ (3145 cm^{-1}) of approximately equal intensity. The bottom spot exhibits a third absorbance at 2.92 μ (3424 cm^{-1}), which takes the form of a sharp peak of medium intensity as compared to the other two N-H stretching frequencies. In the spectrum of the top spot the intensity of the absorbance at 2.94 μ (3401 cm^{-1}) approaches the intensities of the two N-H frequencies and is considerably broader than the peak at 2.92 μ seen in the spectrum of the bottom component.

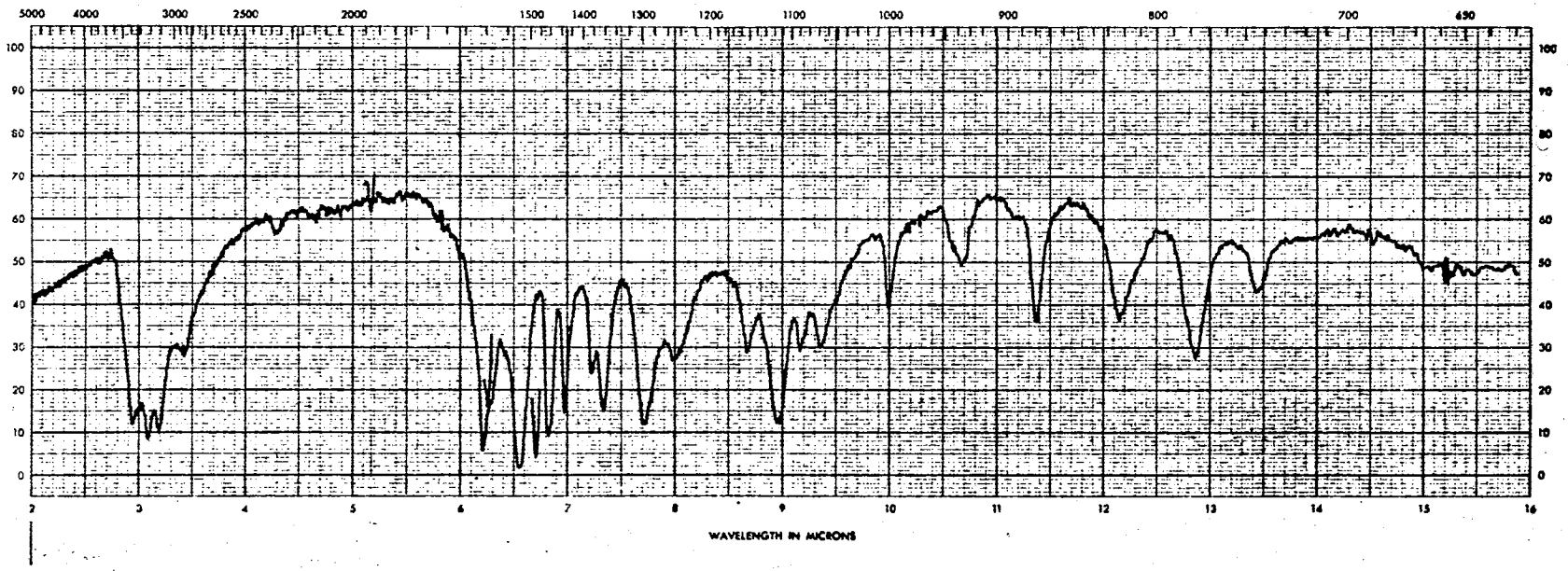
The absorptions at 3.08 μ and at 3.18 μ are interpreted as asymmetric and symmetric N-H stretching frequencies of the terminal

Plate I



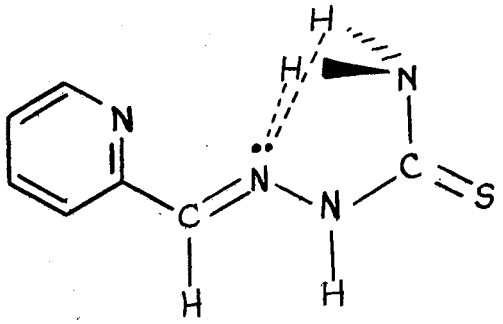
Infrared Spectrum of (E)-2-FP Prepared by TLC

Plate II

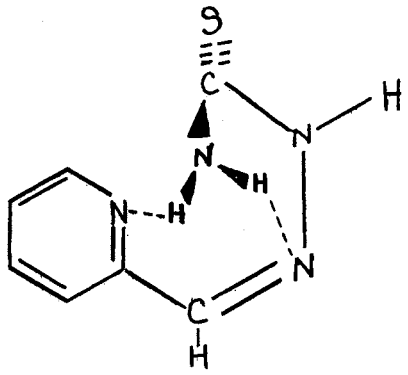


Infrared Spectrum of (Z)-2-FP Prepared by TLC

NH_2 group of the molecule. Conley[42] reports that intramolecular N-H hydrogen bonding occurs in the region 2.86-3.13 μ (3500-3200 cm^{-1}). Molecular scale models of the isomers of the compound showed that both can form intramolecular bonds between the terminal NH_2 group and another nitrogen atom as shown in the following illustration.



10



11

The terminal NH_2 group of the thiosemicarbazone in the E form (10) can form hydrogen bonds intramolecularly with the imino nitrogen and this should result in an infrared absorbance characteristic of a bonded N-H stretching frequency in addition to the free N-H stretching frequencies. Models show that the Z isomer (11) can form hydrogen bonds not only with the imino nitrogen but also with the nitrogen in the pyridine ring. Evidence for this increased hydrogen bonding should be found in the infrared spectrum of the isomer. The only variation of the infrared spectra of the "top spot" and the "bottom spot" is in the region 2.92-2.94 μ. The absorbance at 2.94 μ in the spectrum of the "top spot" is more intense and broader than the absorbance at 2.92 μ in the spectrum of the "bottom spot". The increased intensity and the broadening of the absorbance at 2.94 μ indicate a difference

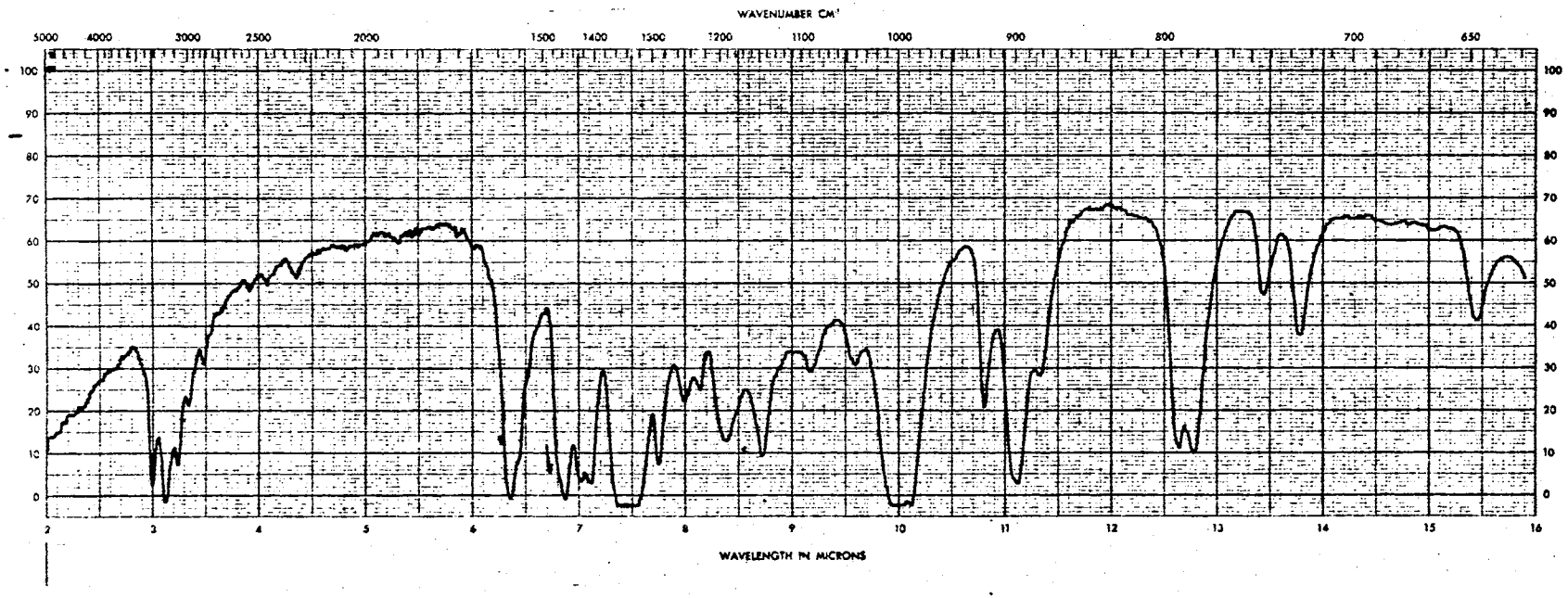
in the two components which must involve the N-H bonds of the molecules. If the absorbances at 2.92 μ and 2.94 μ are assumed to be characteristic of intramolecular hydrogen bonding in the "bottom spot" and the "top spot" respectively then the increased intensity of the absorption at 2.94 μ would indicate an increase in the hydrogen bonding of the molecule. The broadened absorbance at 2.94 μ would also indicate increased hydrogen bonding. Thus the spectrum of the "top spot" possibly indicates an increase in hydrogen bonding over the "bottom spot".

It is possible that the absorbances at 2.92 μ and 2.94 μ are characteristic of the N-H stretching frequency of the N^2 hydrogen. To investigate this possibility the potassium salt of 2-FP and the $\underline{N}^2$-methylthiosemicarbazone of pyridine-2-carboxaldehyde were prepared, both of which have the N^2 hydrogen replaced. The salt retains a sharp absorbance at 2.91 μ and the methylthiosemicarbazone absorbs at 2.95 μ. The retention of absorption in the region 2.91 - 2.95 μ indicates that these peaks are not characteristic of the N-H stretching frequency of the N^2 hydrogen. The infrared spectra of the methylthiosemicarbazone and the salt are shown in Plates III and IV respectively.

If the absorptions in the region 2.92 μ - 2.94 μ are characteristic of hydrogen bonding then the increased hydrogen bonding of the "top spot" indicates that the component which migrates when 2-FP is chromatographed is the Z isomer, and the component which does not migrate is the E isomer.

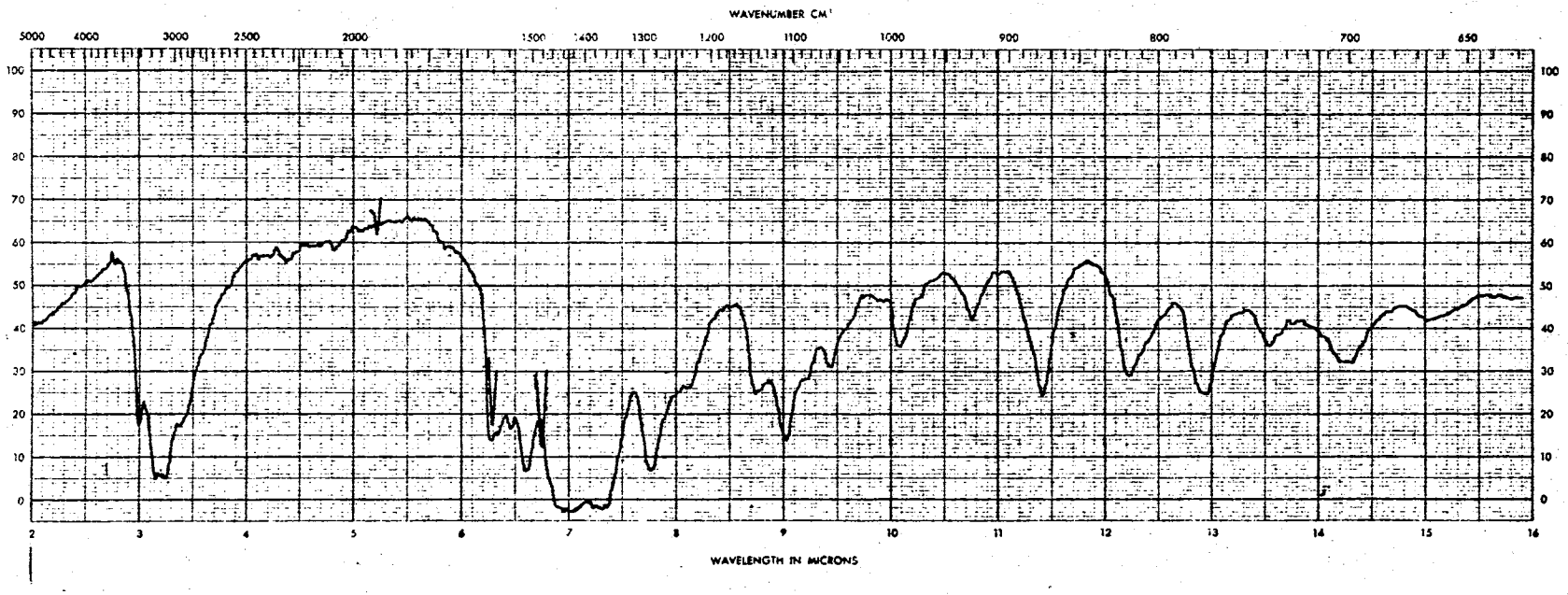
One sample of 2-FP was recrystallized from hot water and an infrared spectrum was taken of the sample. Another sample of 2-FP

Plate III



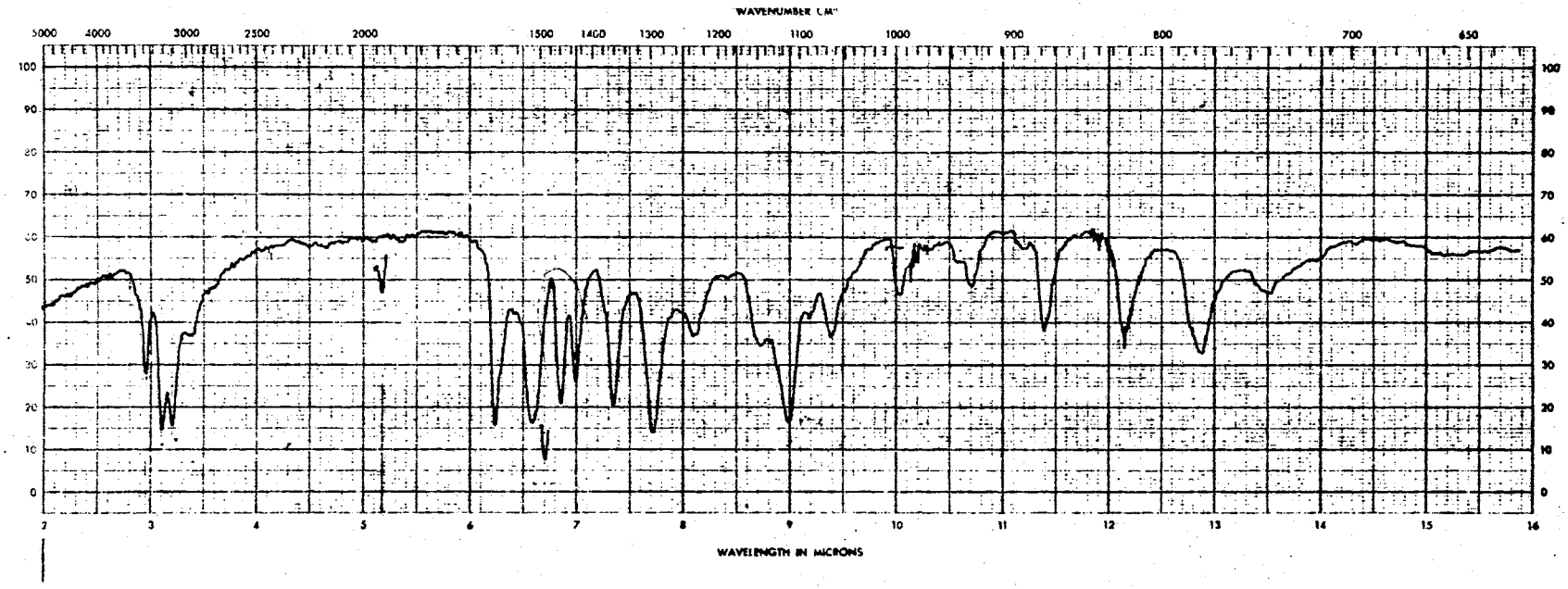
Infrared Spectrum of Pyridine-2-Carboxaldehyde $\underline{N}^2$- Methylthiosemicarbazone

Plate IV



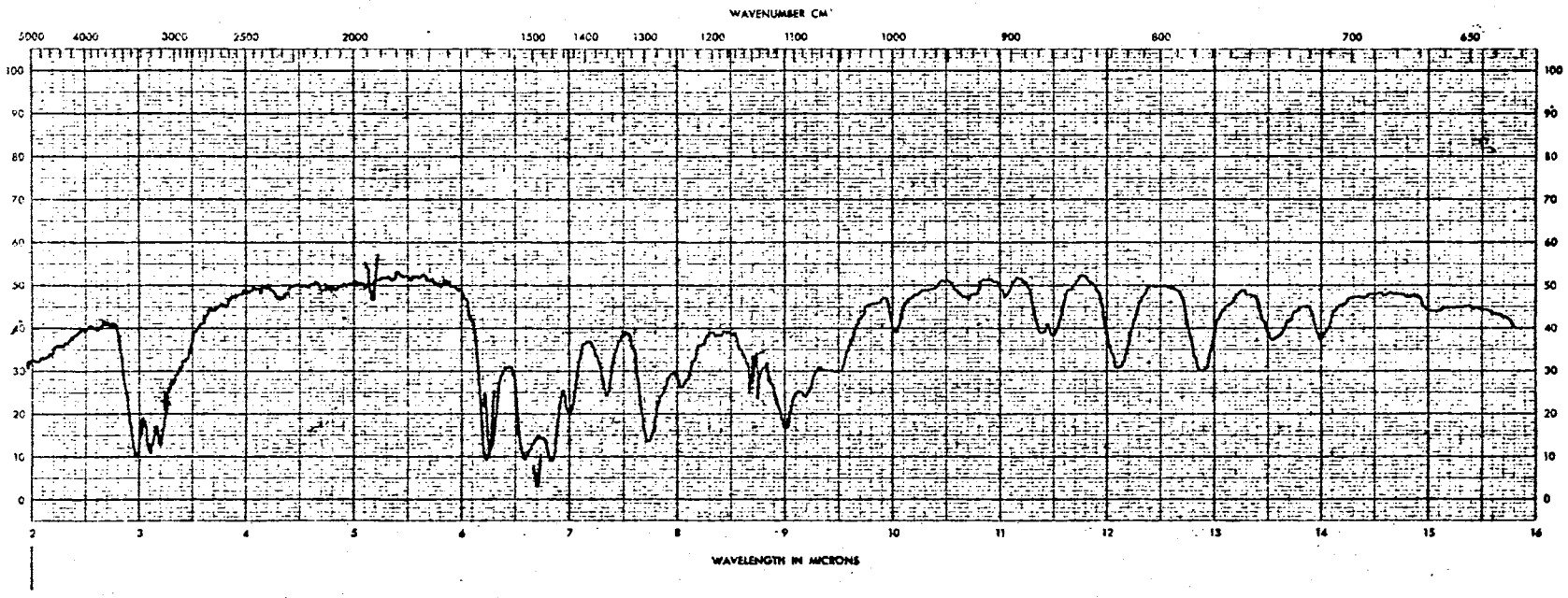
Infrared Spectrum of the Potassium Salt of 2-FP

Plate V



Infrared Spectrum of (E)-2-FP Prepared from Water

Plate VI



Infrared Spectrum of (Z)-2-FP Prepared from n-Butyl Acetate

was heated in refluxing n-butyl acetate and the solution poured over Dry-ice/petroleum ether. An infrared spectrum of this sample was also prepared.

The infrared spectrum of 2-FP recrystallized from water (shown in Plate V) was the same as the spectrum of the "bottom spot"; the infrared spectrum of 2-FP heated in n-butyl acetate (shown in Plate VI) was the same as the spectrum of the "top spot". If the "top spot" is the Z isomer and the "bottom spot" is the E isomer it appears that recrystallizing 2-FP from water gives the E isomer and heating 2-FP in n-butyl acetate gives the Z isomer.

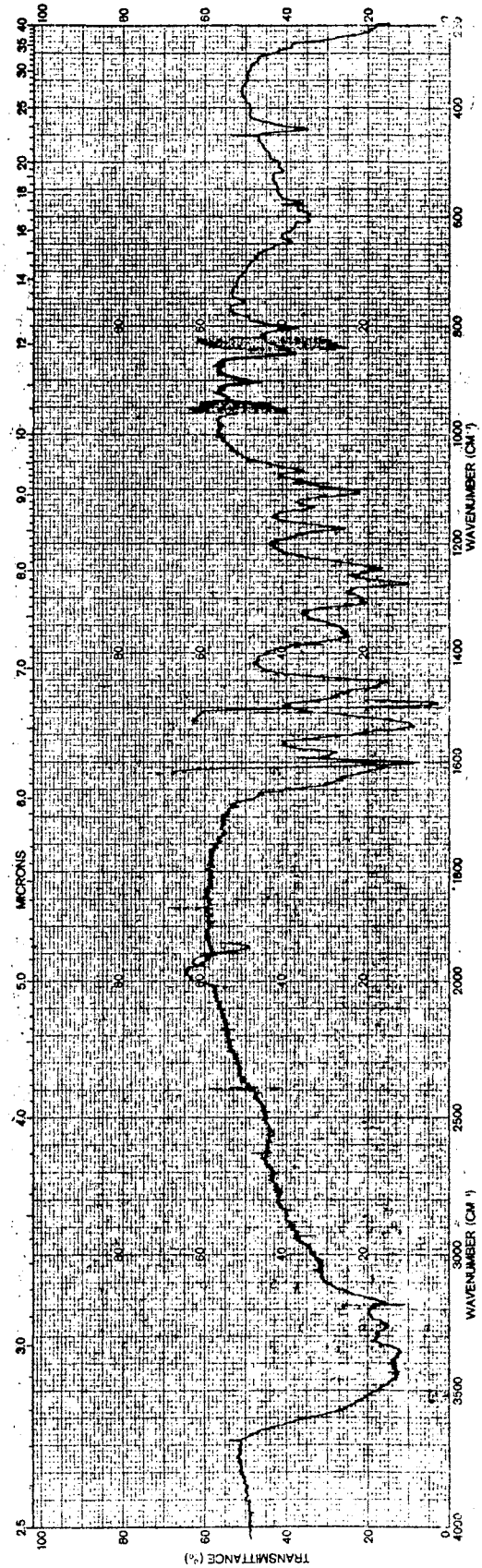
INFRARED SPECTRA OF (E)- AND (Z)-3-HP

Samples of 3-HP were prepared by recrystallization from water and by heating in refluxing n-butyl acetate. The samples prepared in this manner were tentatively called the E isomer and the Z isomer of 3-HP respectively. Assignment of the Z and the E configurations were made since treating 2-FP with hot water and n-butyl acetate apparently gave the E and Z isomers and an analogous treatment of 3-HP would hopefully give the same respective isomers.

The N-H and O-H stretching regions of the spectra of 3-HP were more complex than in the case of 2-FP, because of the presence of the OH group in the former. The sample tentatively identified as the E isomer showed more pronounced absorptions at 2.90 μ (3448 cm^{-1}), 2.97 μ (3363 cm^{-1}), 305 μ (3273 cm^{-1}), and 3.14 μ (3183 cm^{-1}). The Z isomer showed its main absorptions at 2.90 μ (3428 cm^{-1}), 2.98 μ (3358 cm^{-1}), 305 μ (3277 cm^{-1}), and 3.14 μ (3186 cm^{-1}). These four peaks are the same for each isomer except in the region of 2.90 μ -

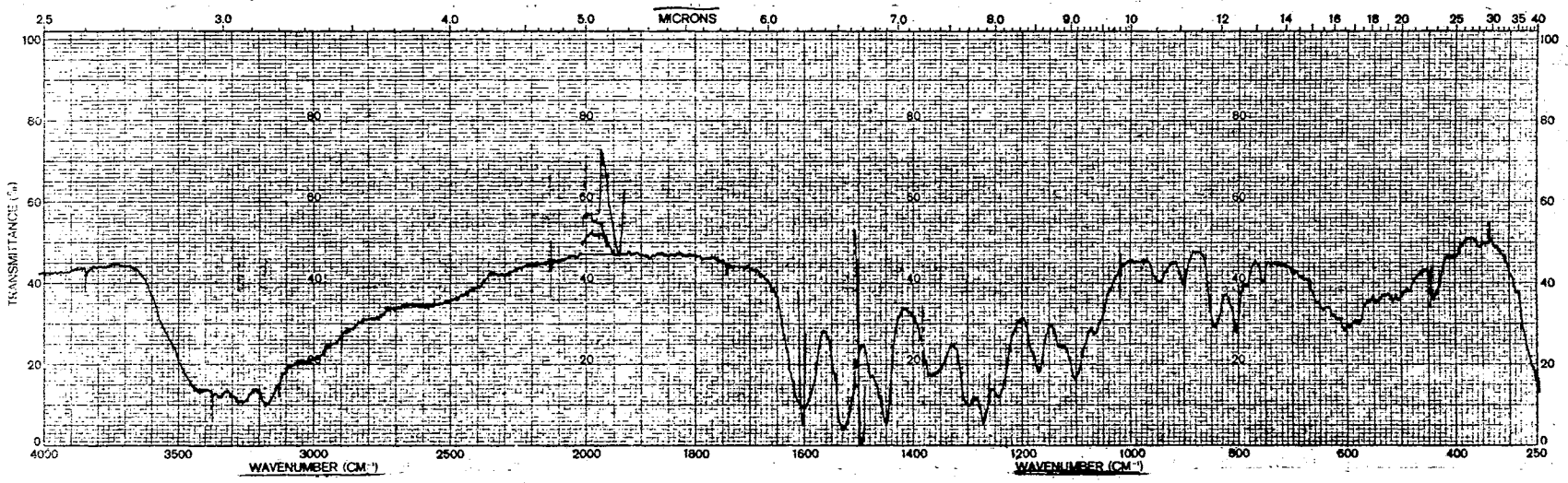
2.92 μ where the absorption for the E isomer occurs at 2.90 μ and the absorption for the Z isomer occurs at 2.92 μ. As was the case in the spectra of 2-FP, these absorptions are proposed to be due to intramolecular hydrogen bonding and, as was the case in the spectra of 2-FP, the absorbance of the E isomer in this region occurs at a shorter wavelength than the absorbance of the Z isomer in the same region. On the basis of arguments of hydrogen bonding which were applied for 2-FP it appears that the two isomers of 2-HP have been prepared and that preparation from water and from n-butyl acetate yielded the E and Z isomers respectively for both compounds.

Plate VII



Infrared Spectrum of (E)-3-HP

Plate VIII



Infrared Spectrum of (Z)-3-HP

RATIONALE FOR METHOD OF Z ISOMER PREPARATION

Models of the two isomers of 2-FP show that the E isomer should be favored sterically since it is fairly planar with little crowding among neighboring atoms. The molecule should be heavily solvated in polar solvents and any intramolecular hydrogen bonding should be decreased in polar solvents. In non-polar solvents, however, there should be little solvation of the molecule thus the intramolecular bonding would be of considerable greater importance. Intramolecular hydrogen bonding should make the Z isomer more stable in non-polar solvents than in polar solvents since intramolecular hydrogen bonding will provide a stabilizing effect on the Z isomer. This stabilizing effect should partially compensate for the unfavorability of the sterically hindered Z isomer and in non-polar solvents might provide a driving force to enhance E $\longrightarrow$ Z isomerization.

It was hoped that by heating the compound in a non-polar solvent it might be changed thermally to the Z isomer and then if the isomer were not allowed time to re-isomerize to the sterically favored E configuration, but were rapidly cooled, it might be "trapped" in the Z configuration. The product obtained by this method proved to be the same as the component which migrated when 2-FP was chromatographed by TLC, i.e. the Z isomer; thus the conversion was successful.

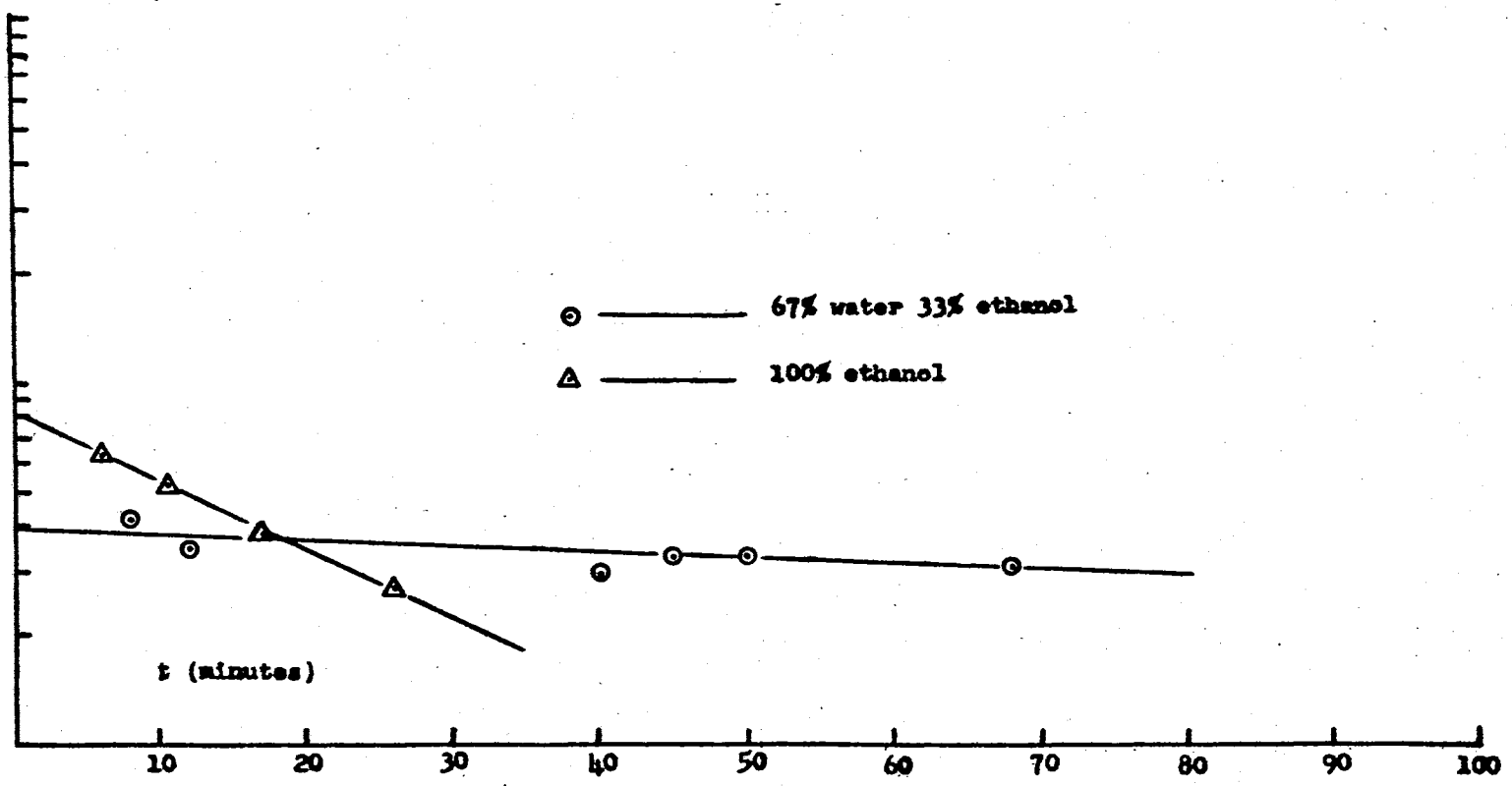
TLC STUDIES OF THE ISOMERIZATION OF (E)-2-FP

When solutions of (E)-2-FP were chromatographed at various intervals of time the Z isomer would eventually appear. The time required for the Z isomer to become visible was dependent on the original concentration of the E isomer. By preparing solutions of several concentrations and chromatographing samples at various intervals sufficient data were collected to prepare first-order rate plots of the isomerization. The experiment was performed in 100% ethanol and in 67% water-33% ethanol. Straight lines were obtained in each experiment when the logarithm of C_o (the original concentration of the E isomer) was plotted against t (the time required for the Z isomer to reach sufficient concentration, to be detected by TLC). These plots are shown in Plate IX.

The half-time of approach to equilibrium for the E isomer in 100% ethanol is 15.5 minutes. In 67% water-33% ethanol the E isomer is halfway to equilibrium in 170 minutes.

Since the Z isomer of 2-FP was not pure but was contaminated with E isomer, this experiment could not be performed on the Z isomer because the technique requires that the appearance of one component be monitored as the other component isomerizes. Since both components are visible on TLC of the Z preparation, the technique could not be used.

Plate IX



Rate Plots of the Approach to Equilibrium by (E)-2-FP as Determined by TLC

SPECTRA OF (E)- AND (Z)-2-FP IN VARIOUS SOLUTIONS

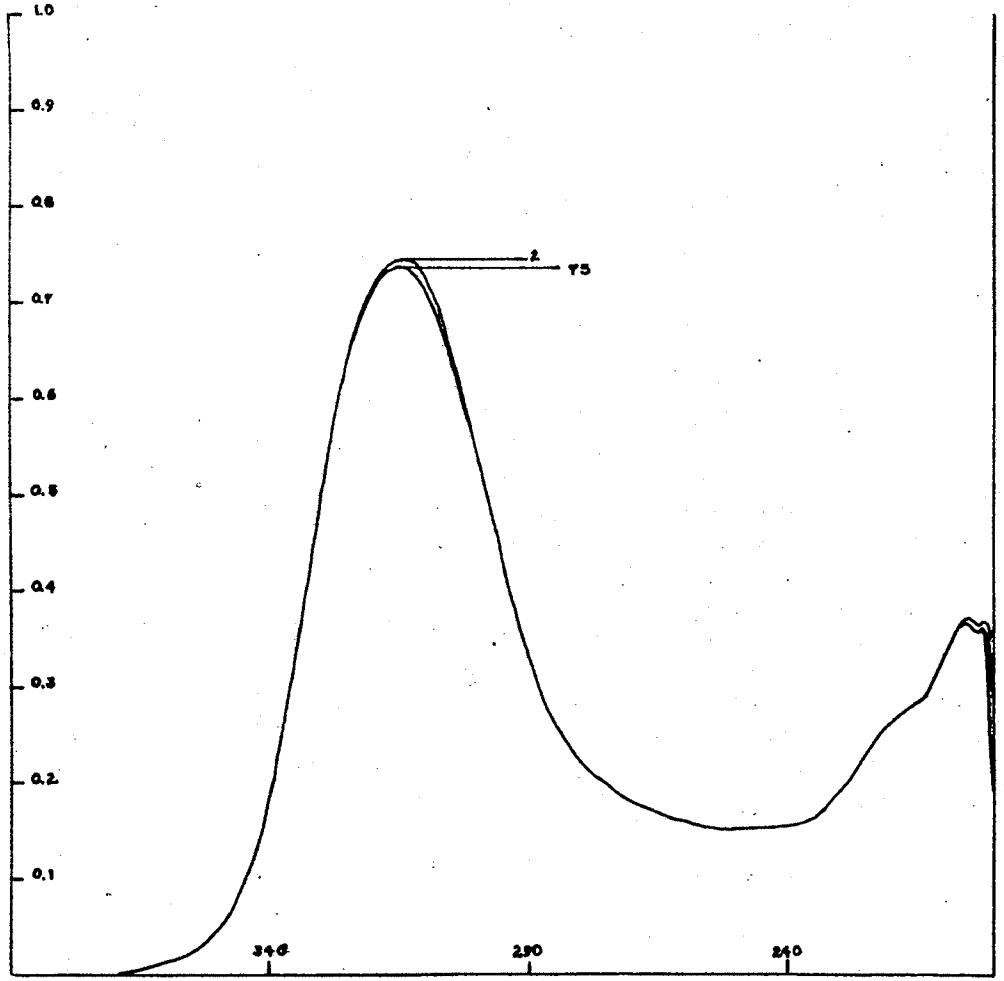
Ultraviolet spectra were studied in 100% ethanol, pH 7.0 buffer, pH 12.0 buffer, and 0.1 N HCl. Before the isomerization studies were performed on the isomers of 2-FP and 3-HP, the compounds were dissolved in pH 7.0 buffer and allowed to stand for periods up to eight days to determine the stability of the compounds toward hydrolysis. When allowed to stand for two days 3-HP showed no alteration in its ultraviolet spectrum that would indicate hydrolysis; 2-FP is unchanged after eight days.

Spectra of (E)- and (Z)-2-FP in pH 7.0 Buffer

The ultraviolet spectra of (E)- and (Z)-2-FP in pH 7.0 buffer are shown in Plates X and XI. The E isomer showed a maximum at 313 nm at pH 7.0. The Z isomer showed maxima at 317 and 265 nm. The major maximum of the E isomer is at a shorter wavelength than the maximum for the Z isomer. A slight hypochromic effect is observed at 313 nm and a bathochromic (red) shift toward 315 nm occurs when the E isomer is allowed to stand 75 minutes. On standing 40 minutes the Z isomer exhibits a substantial hyperchromic effect at 317 nm. over the same 40 minutes the absorption at 265 nm decreased to the extent that not even an inflection was seen. These compounds which are suppose to be opposite isomers exhibit opposite behavior in their ultraviolet spectra with time.

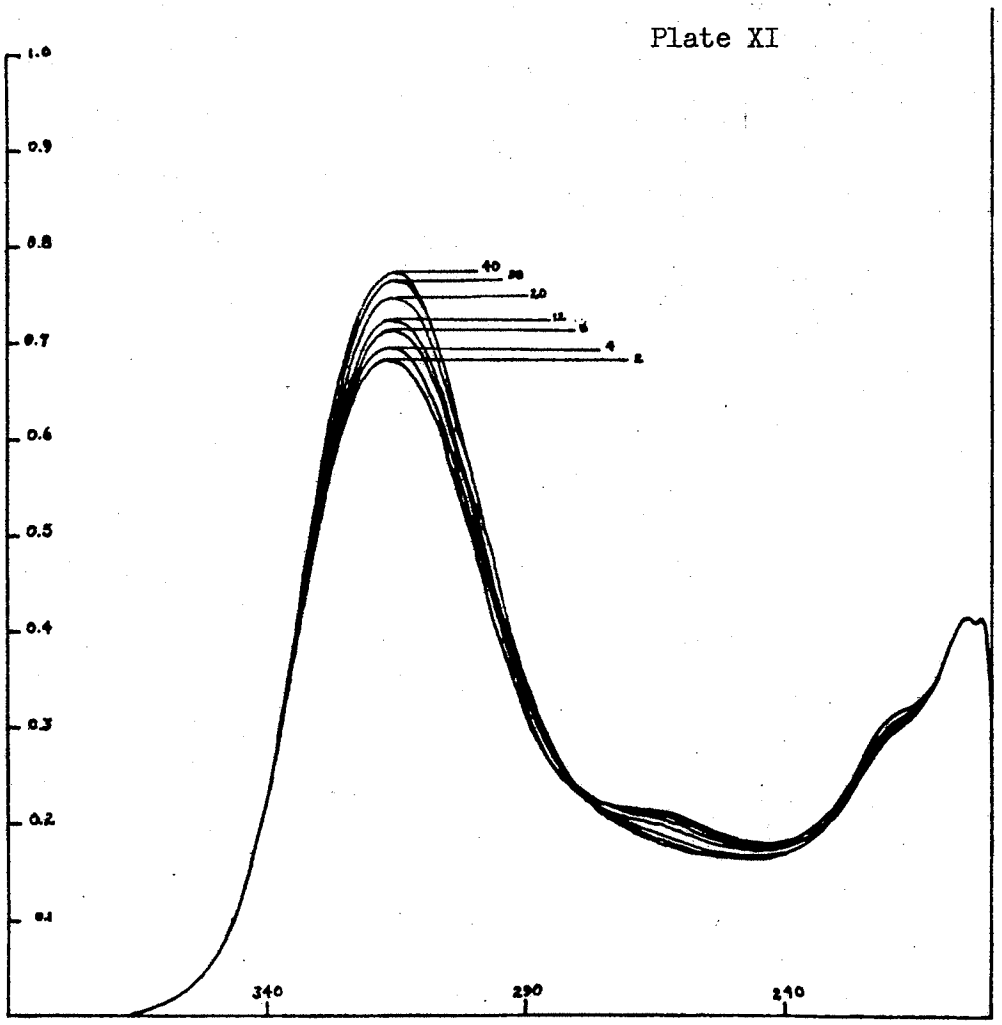
The intensities ($\log \epsilon = 4.4$) of the bands from 313 to 328 nm indicate that these bands, i.e. $\pi \longrightarrow \pi^*$ transitions of conjugated π systems. Gillam and Stern[46] attribute the absorption maxima

Plate X



Ultraviolet Spectrum of (E)2-FP in pH 7.0

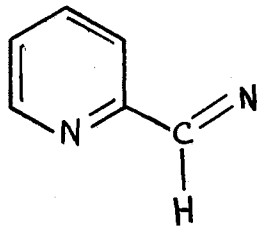
Plate XI



t = 2 min ⟶ t = 40 min.
λmax = 317 nm
pH = 7.0

Ultraviolet spectrum of (Z)-2-FP in pH 7.0

of thiosemicarbazones around 300 nm to the C=C-C=N chromophore of the derivative. In the system under consideration, the pyridine ring is in conjugation with the imino moiety. The α,β-unsaturated chromophore may be written as:



As discussed earlier, the E isomer can form intramolecular hydrogen bonds between the terminal NH_2 hydrogen atoms and the imino nitrogen. This bonding should exert a polarizing effect upon the -system and lower the electron density of the conjugated system. The Z isomer can form hydrogen bonds with both the imino nitrogen and the non-bonding electrons of the pyridine nitrogen. This isomer therefore has twice the capacity for hydrogen bonding that the E isomer does. Since hydrogen bonding is more extensive in the Z isomer the electron density of the α,β-conjugated system of the Z isomer should be less than the α,β-conjugated system of the E isomer. Thus the absorbance of the α,β-conjugated system should be a measure of the electron density of the system which is dependent on the intramolecular hydrogen bonding in the system.

In the spectrum of the Z isomer the absorbance at 317 nm, characteristic of the α,β-conjugated system of the Z isomer, increases in intensity with a concurrent hypsochromic shift to 315 nm as the Z isomer is converted to the E form with loss of hydrogen bonding. In the spectrum of the E isomer a hypochromic effect is seen at

313 nm (which is characteristic of the α,β-conjugated system of the E isomer) which indicates an increase in hydrogen bonding as the E isomer is converted to the Z isomer. A bathochromic shift of the absorbance at 313 nm to 315 nm is also observed in the spectrum of the E isomer.

Hydrogen bonding by solvent molecules with the lone electron pair of pyridine produces a hyperchromic effect on λmax in the range 250-255 nm.[47,48] This may be attributed to a localizing effect of the hydrogen on the lone pair.

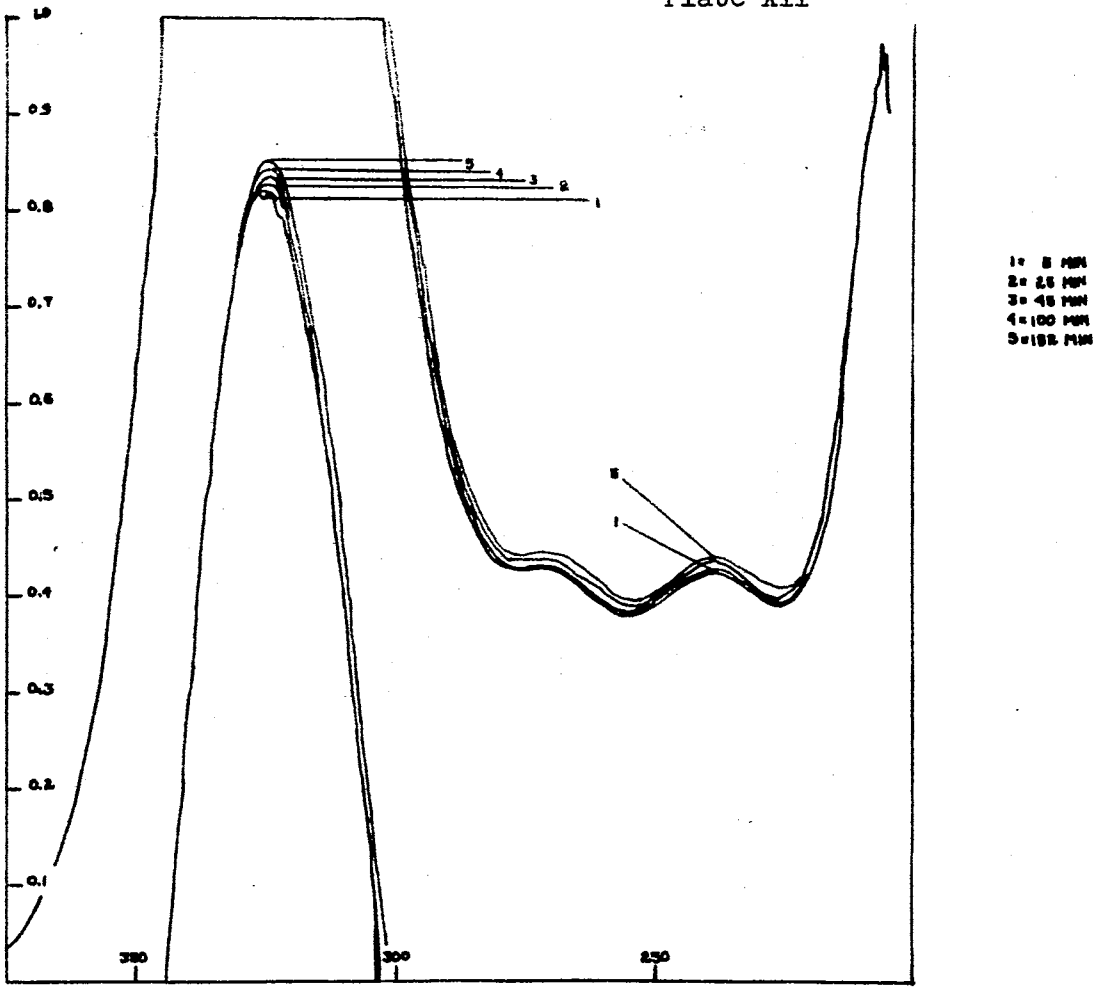
The Z isomer displays a peak of medium intensity at 265 nm which decreases in intensity with time. This hypochromic effect may be due to the Z form isomerizing to the E form with a decrease in intramolecular hydrogen bonding to the pair of electrons of the nitrogen on the pyridine ring. The electrons can thus be more delocalized throughout the conjugated π system; such delocalization would cause the hyperchromic effect at 317 nm due to increase of the electron density of the α,β-conjugated system.

The E isomer reveals a minimum absorbance at 265 nm with no evidence of a peak. After 75 minutes, however, a slight increase of intensity is observed at 265 nm, indicating hydrogen bonding with the pyridine nitrogen. As mentioned before, absorbance at 313 nm decreases over the same period, indicating a decrease of electron density of the conjugated system.

Spectra of (E)- and (Z)-2-FP in 100% Ethanol

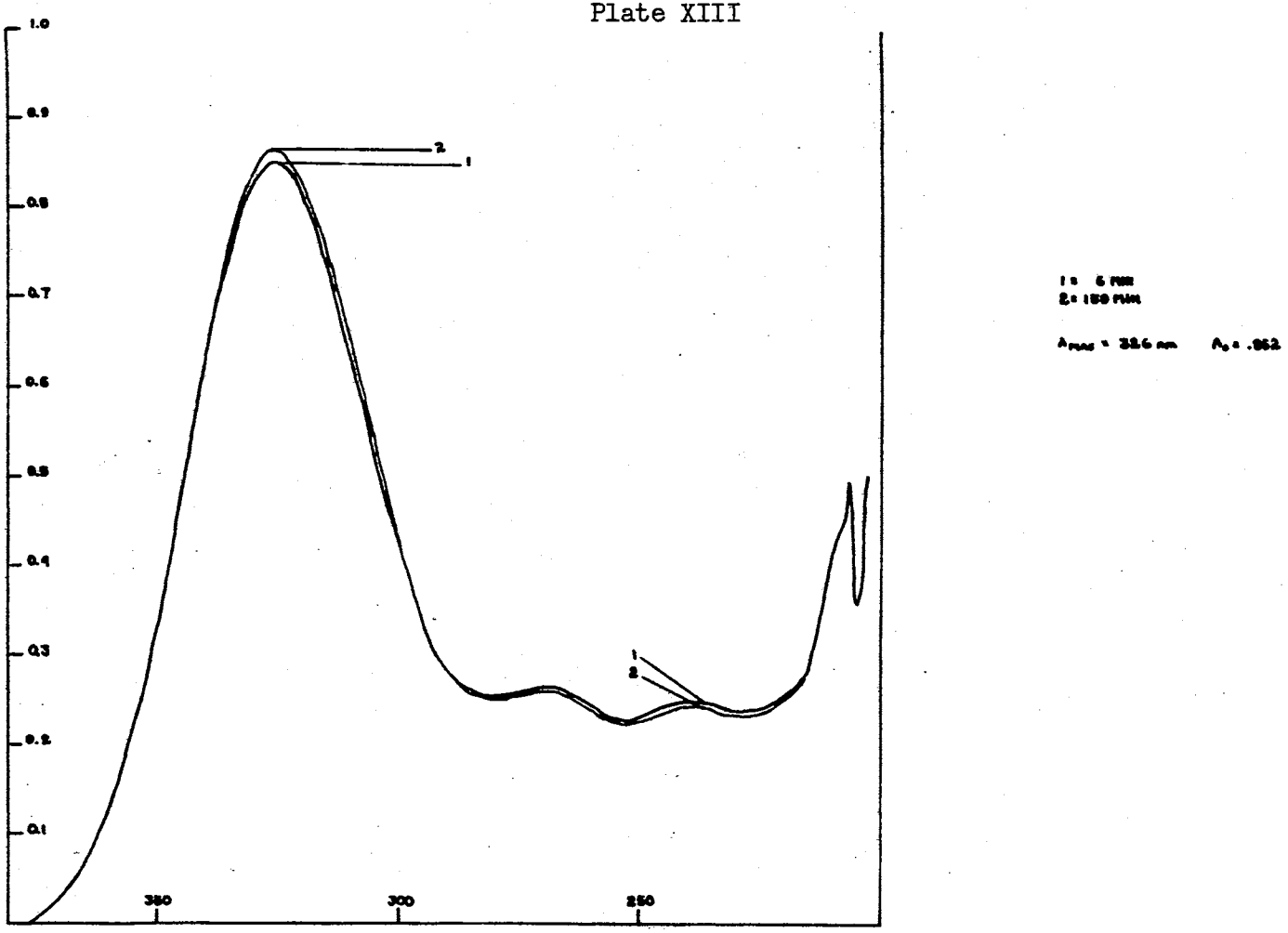
The spectra of (E)- and (Z)-2-FP in ethanol are shown in Plates XII and XIII. The E isomer of 2-FP in absolute ethanol displays ab-

Plate XII



Ultraviolet Spectrum of (E)-2-FP in Absolute Ethanol

Plate XIII



Ultraviolet Spectrum of (Z)-2-FP in Absolute Ethanol

sorption maxima at 324, 269, and 238 nm. When the solution is allowed to stand for 180 minutes the absorption at 269 nm, characteristic of localization of the non-bonded electrons on the pyridine nitrogen, increases in intensity.

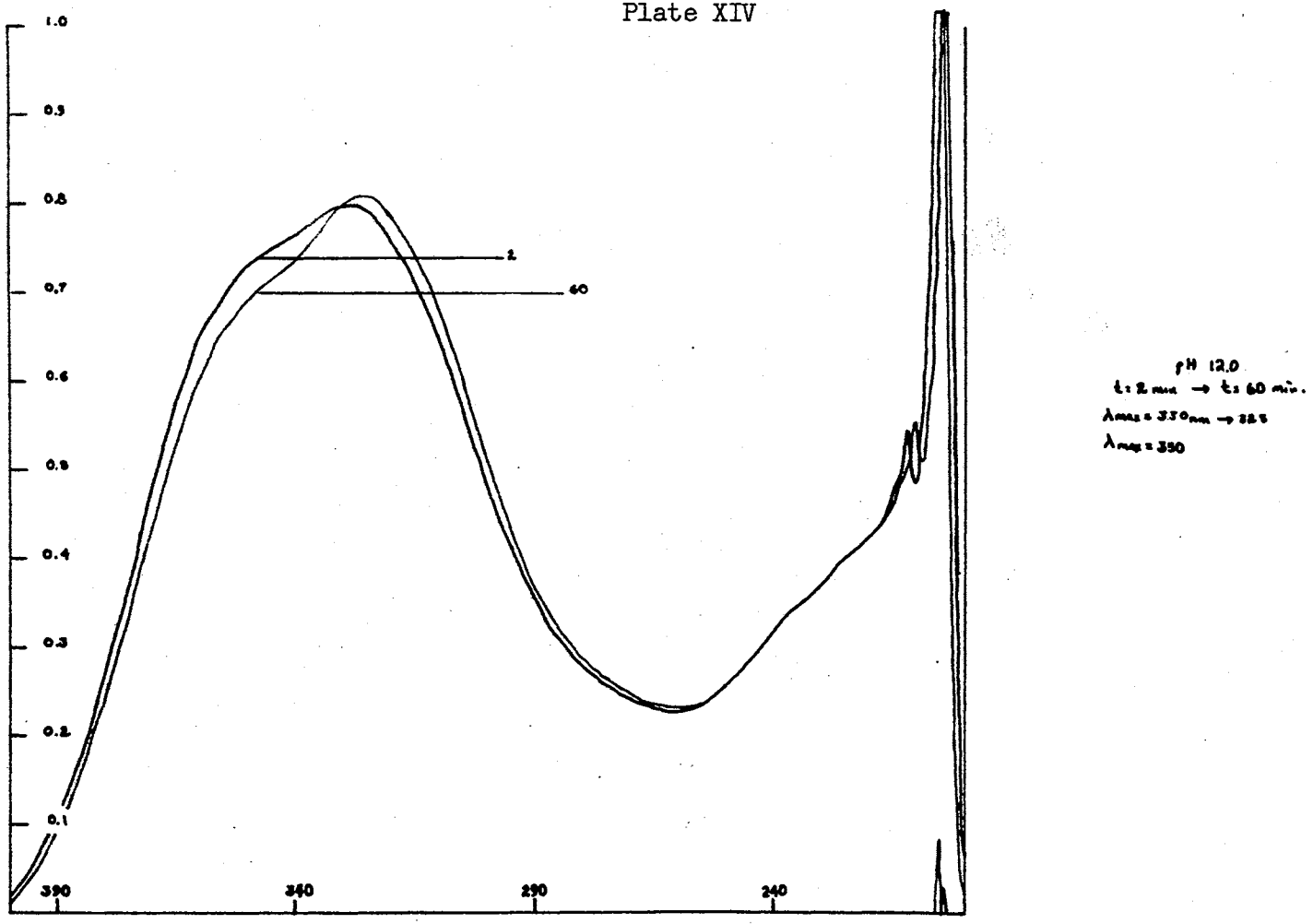
The Z isomer of 2-FP in absolute ethanol displays absorption maxima at 327, 272, and 240 nm. The peaks are similar to those of the E isomer but they are each shifted toward longer wavelengths. The absorbance at 272 nm shows a slight hypochromic effect when the E isomer stands for 150 minuts.

The isomers display opposite behavior in the region 269 to 272 nm which is the region characteristic of the ring nitrogen. The absorbance of the E isomer increases with time indicating that the isomer is being converted to the Z isomer with increase in intramolecular hydrogen bonding at the ring nitrogen. Conversely, the absorption of the Z isomer decreases in intensity in this region indicating that the Z isomer is converted to the E isomer with loss of hydrogen bonding involving the ring nitrogen.

Spectra of (E)- and (Z)-2-FP in pH 12.0 Buffer

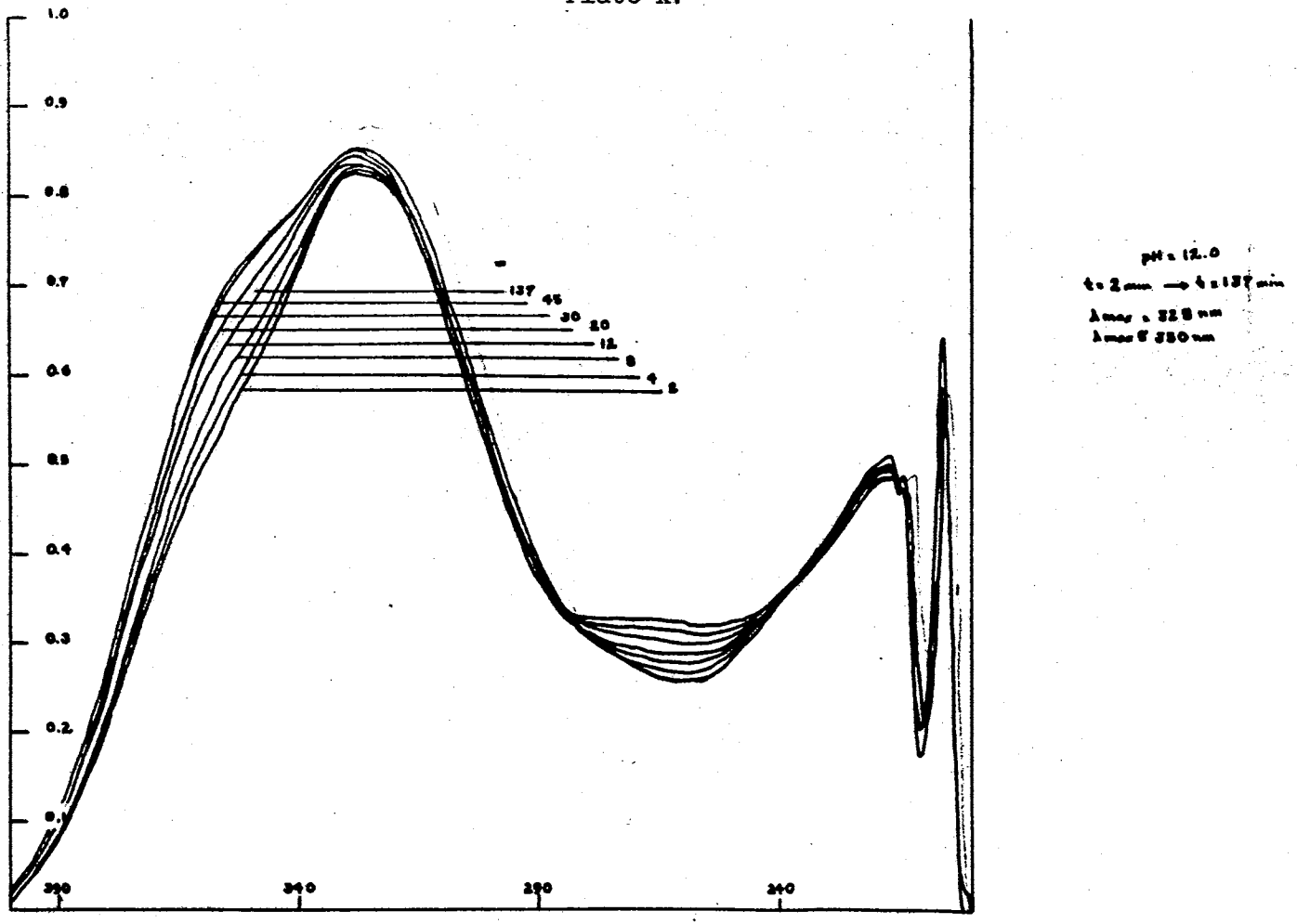
The spectra of (E)- and (Z)-2-FP at pH 12.0 are shown in Plates XIV and XV. (E)-2-FP exhibits a maximum at 330 nm, which undergoes a hypsochromic shift to 325 nm and shows a slight hypochromic effect when observed for 60 minutes. A minimum in the region 265-275 nm shows a slight hyperchromic effect over the same 60 minutes. This behavior is attributed to conversion of the E form to the Z form. The E isomer also exhibits a strong shoulder in the region 350 to 355 nm, which may be characteristic of the conjugated system in the mercapto-

Plate XIV



Ultraviolet Spectrum of (E)-2-FP in pH 12.0 Buffer

Plate XV

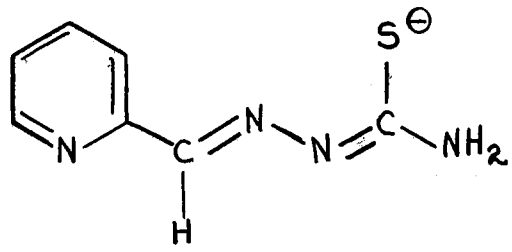


Ultraviolet Spectrum of (Z)-2-FP in pH 12.0 Buffer

imine formed by action of the basic buffer on the thiosemicarbazone. Since the E isomer is shown by models to be a relatively planar molecule, this tautomerism to form the thiol form can occur quite easily because of the resulting extensive orbital overlap and the absorption characteristic of the new conjugated structure should be more evident in the spectrum of the E isomer than that of the Z.

(Z)-2-FP has maxima at 328 nm and 265-275 nm, the latter, a broad absorbance of medium intensity. When freshly prepared, solutions of the isomer show no absorbance in the region around 350-355 nm. After 45 minutes, however, the band at 265-275 nm has diminished to the extent that it is not even an inflection which is once again characteristic of loss of intramolecular hydrogen bonding with the ring nitrogen as the (Z) form isomerizes to the (E) form. After the same 45 minutes a strong absorbance has appeared in the region 350-360 nm which indicates that the thiol structure has been formed.

The thiol structure has already been discussed in the interpretation of the infrared spectra of 2-FP. It is represented at pH 12.0 as:



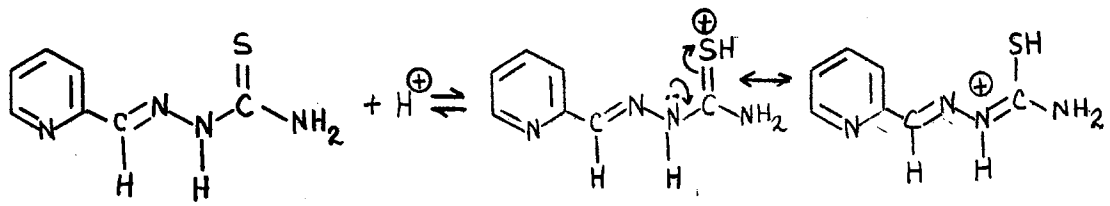
Spectra of (E)- and (Z)-2-FP in 0.1 N HCl

(E)-2-FP in 0.1 N HCl exhibits maxima at 348, 267, and 213 nm. The maximum at 348 nm increases slightly in intensity for the first 20 minutes of observation and then decreases steadily. The other maxima increase continuously.

(Z)-2-FP exhibits maxima at 351, 267, and 213 nm. When the solution is observed for a period of 150 minutes the maximum at 351 nm decreases in intensity while those at 267 nm and 213 nm increase. This behavior is the same as that observed in the spectra of the E isomer. The disappearance of the maximum at 348-351 nm indicates the loss of the conjugated structure and is probably due to hydrolysis of the molecule.

The E isomer appears to begin isomerization in the acidic medium but hydrolysis is a competing reaction and soon becomes the predominant observable reaction of the E as well as the Z isomer.

The spectra of (E)- and (Z)-2-FP in 0.1 N HCl bear a resemblance to those taken in pH 12.0 buffer, particularly in the appearance of an absorbance at 350-360 nm. As before this is attributed to the highly conjugated tautomeric thiol structure of the thiosemicarbazone. This structure can be produced by protonation at the sulfur as indicated:



TITRATION SPECTRUM OF 3-HP

In pH 12.0 buffer 3-HP exhibits maxima at 383-385, 278-280, and 211-213 nm.

Gillam and Stern[49] report that phenol absorbs at 210.5 nm and 3-hydroxypyridine absorbs at 230 nm in alkali.[50] The band displayed by 3-HP at 213 nm is tentatively identified as the phenolic absorbance of the hydroxypyridine ring. At pH 12.0 the phenol will exist as the phenolate anion with the electrons participating in resonance of the pyridine ring. As the pH decreases to 9 a hyperchromic effect is seen. This is interpreted as a localization of the electrons on the oxygen, i.e. the increased H^+ concentration causes the electrons to participate less in ring resonance and to localize on the oxygen.

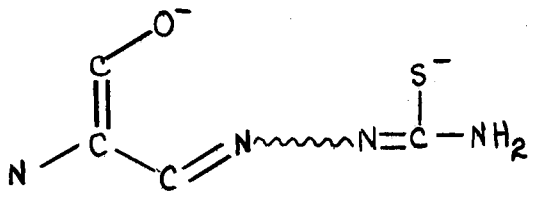
A hypochromic effect is noticed at pH $\cong$ 9-7. Since the pKa of the phenolic OH is 7.98[51] this hypochromic effect is probably due to protonation of the O^- anion to OH.

At pH $\cong$ 6-7 protonation of the O^- should be extensive and in this neutral region the spectrum changes drastically. The spectrum of 3-HP at pH 7.0 will be discussed later.

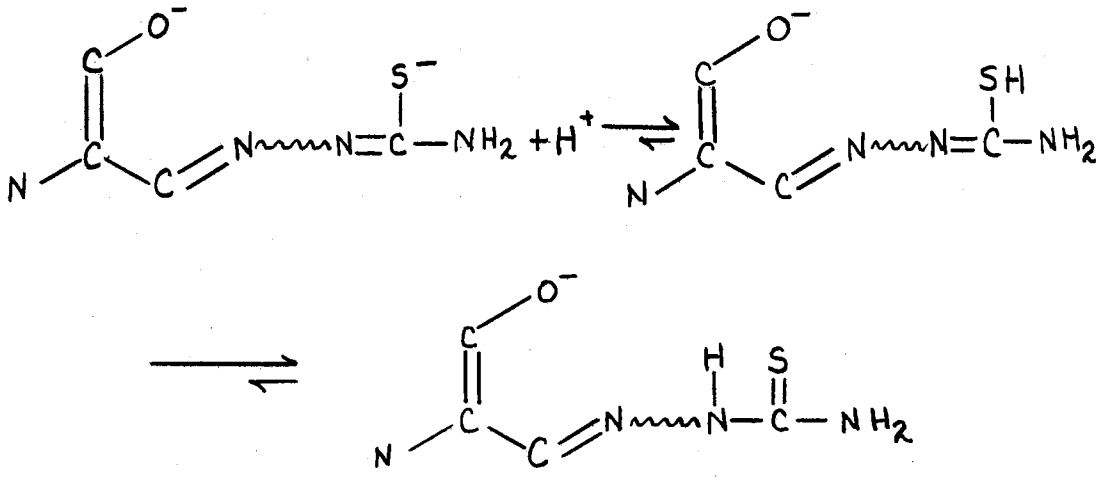
At pH 12.0 3-HP displays a band at 265-285 nm which is tentatively attributed to the ring nitrogen on the basis of work performed on 2-FP which has previously been discussed. This absorption remains fairly constant in the pH range 12.0-7.5. Below pH 7.5 a hypochromic effect is seen which may be because of a decrease in overall electron density of the ring due to protonation of the phenolate anion. In the pH range 7.0 - 3.0 a hyperchromic effect is observed which can be interpreted as due to localization of the non-bonding electrons of the

pyridine ring by protonation.

At pH 12.0 3-HP displays a high-intensity peak at about 370-385 nm which is probably absorbance by the conjugated azomethine structure shown:



As the pH decreases the pH decreases the S^- becomes protonated and the extent of conjugation decreases as shown by the following reactions.



This is an explanation for the hypochromic effect observed at 370-385 nm as the pH goes from 12.0 to ~ 7.5. At pH 6-7 the absorbance around 380 nm is replaced by 3 others at 417, 397, and 342-345 nm. These will be discussed later.

In 0.1 N HCl the spectrum once again displays the characteristics similar to those above pH ~7.5 - 8.

The titration spectrum of 3-HP is shown in Plate XVI.

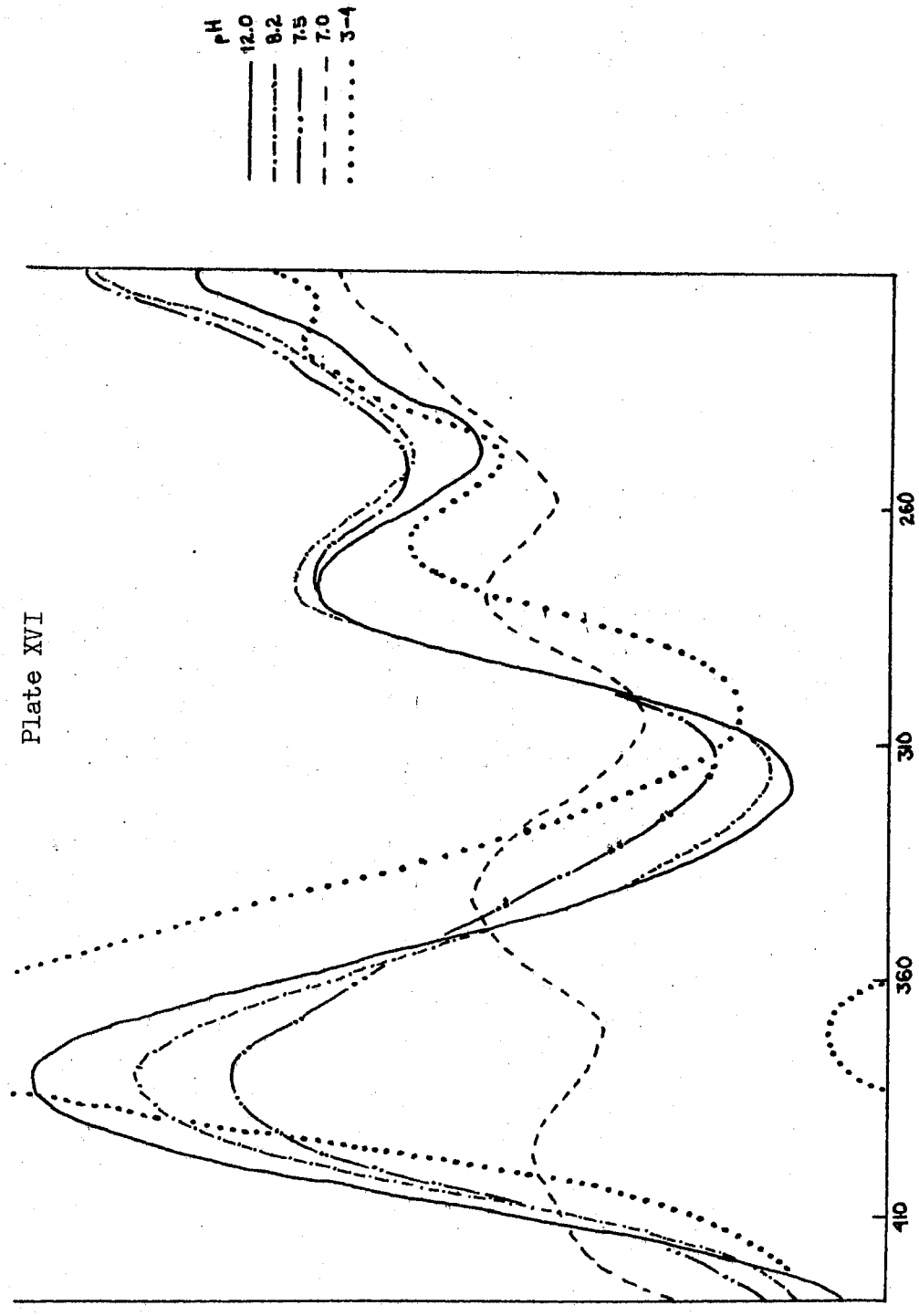
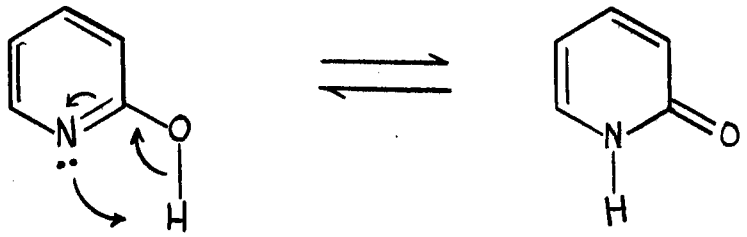


Plate XVI

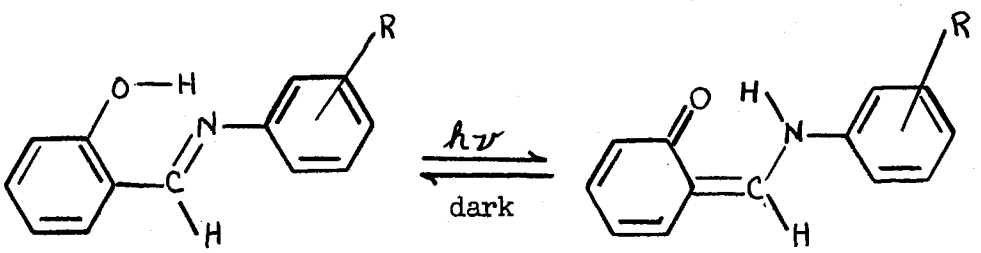
Titration Spectrum of 3-HP

TAUTOMERISM OF 3-HP

Hydroxy- substituted pyridines exhibit keto-enol tautomerism[51,52,53] such as that illustrated:



Azomethines prepared from substituted anilines with o- and p-substituted benzaldehydes undergo a photoinduced tautomerism such as that illustrated:[31,32,54]

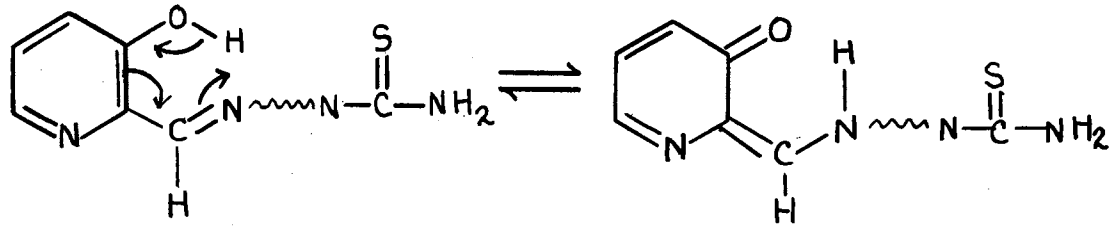


Ledbetter[55,56,57] discusses this type of tautomerism and attributes certain low-intensity bands above 400 nm to the quinonoid structure. He also suggests that hydrogen transfer from the o-hydroxyl to the nitrogen occurs when the molecule is in the E configuration.

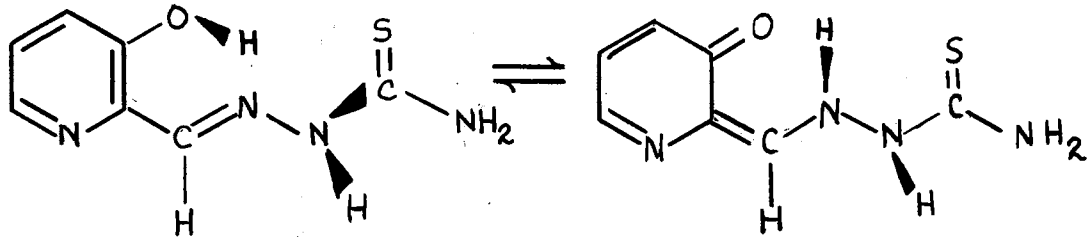
A more thorough review of tautomerism of anils can be found on pages 54 and 55 in the review by Sandorfy.[19]

The absorption maxima have already been presented for 3-HP at pH 7.0 (Plate XVI). On the basis of the spectra discussed by Ledbetter[55,56,57] the maxima of 3-HP at 397 and 417 nm are attributed to

a quinonoid structure which results from an enol imine–keto enamine tautomerism:



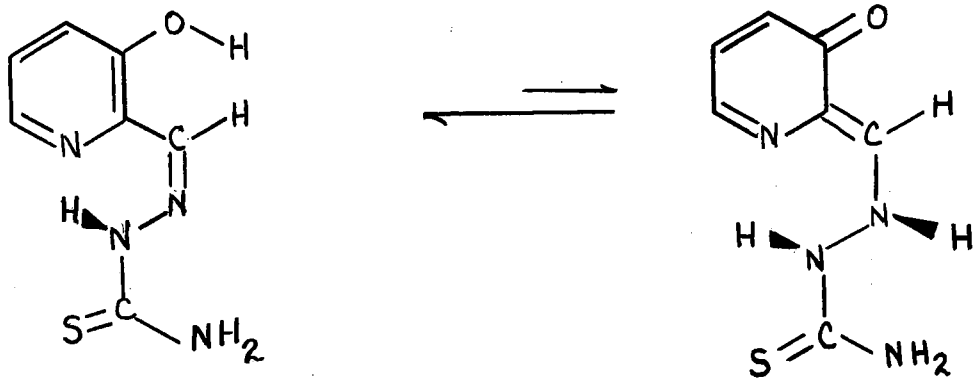
Models demonstrate that the phenolic proton and imino nitrogen of (E)-3-HP (12-a) are in very close proximity and assume a configuration which is very nearly co-planar with the pyridine ring; thus the tautomeric keto enamine structure (12-b) should be easily formed as shown in the following reaction. The Z isomer (13-a), on the other hand



12-a 12-b

cannot assume a planar configuration which will allow close proximity between the phenolic proton and the imino nitrogen. The bond angles and the congestion about the imino nitrogen of the Z configuration do not allow for a planar structure. The more favored configuration of the Z isomer is one which allows increased intramolecular bonding. This configuration does not allow the imino nitrogen and the phenolic

hydrogen to approach each other in a planar configuration. It is thus expected that the quinonoid tautomer (13-b) should not form as extensively with the Z isomer as it does with the E isomer. The following equation illustrates the shift of the equilibrium away from the quinonoid form:

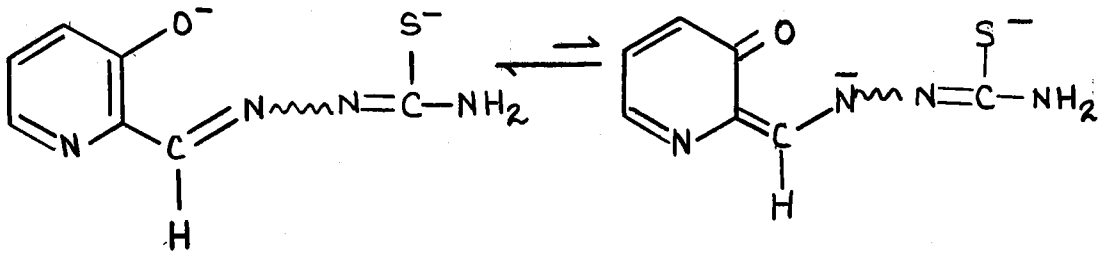


13-a 13-b

The tautomeric equilibrium of 3-HP also appears to be shifted to increase the concentration of the quinonoid form relative to the phenolic form in ethanol. The spectrum of (E)-3-HP in 100% ethanol shows a shoulder at 398 nm previously suggested to be characteristic of the quinonoid tautomer. An absorption at 207 nm is attributed to the hydroxy group on the pyridine ring.

The ultraviolet absorptions which appear in pH 7.0 buffer and in 100% ethanol and which are characteristic of the quinonoid tautomer of 3-HP are not present in the ultraviolet spectra of 3-HP at pH 12 and pH 1. The absence of the quinonoid tautomer indicates that the enol imine $\rightleftharpoons$ keto enamine equilibrium has been shifted so that the compound exists almost exclusively as the phenolic (enol imine) form. Since the shift away from the keto form of 3-HP does not

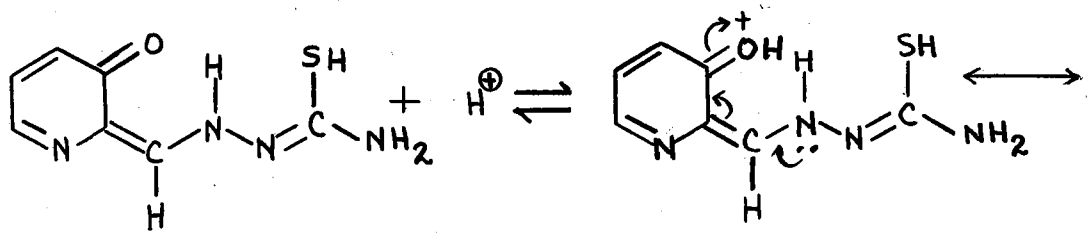
occur at neutral pH but does occur in both acid and base, the shift must be sensitive to pH. The absence of significant levels of the quinonoid tautomer in base and in acid has the following explanation.

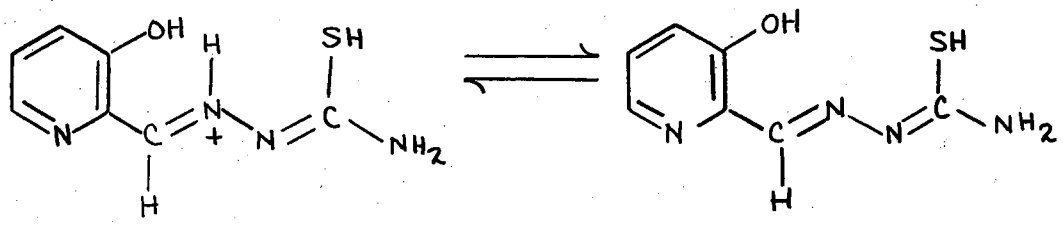


14-a 14-b

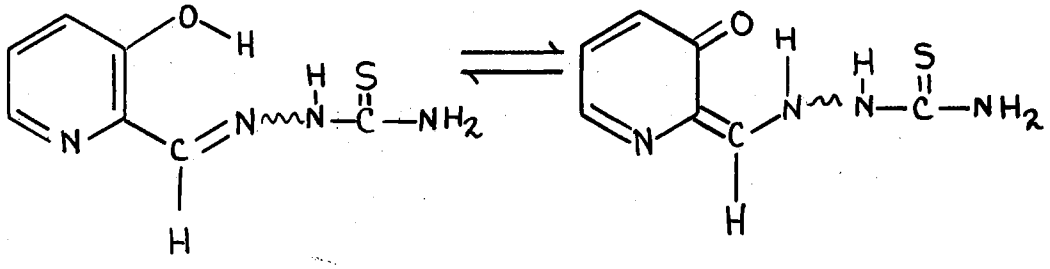
In base the molecule exists as the di-anion. While a tautomeric equilibrium between 14-a and 14-b may exist, it must lie overwhelmingly in favor of 14-a for two reasons: (1) upon formation of the quinonoid form the conjugated system 14-a is disrupted and (2) the negative charge in 14-b is localized on the nitrogen atom. Both of these points contribute to the unfavorability of the keto form 14-b. Loss of conjugation would be unfavorable but perhaps tolerable. However, since oxygen is more electronegative than nitrogen it seems reasonable that the tautomeric equilibrium would strongly favor the form which allows the more electronegative oxygen atom to accomodate the negative charge.

In acid the carbonyl is assumed to be protonated and the equilibrium shifts as shown:





In neutral solution the absence of charge on the thiosemicarbazone permits the position of equilibrium to be shifted toward the keto-enamine form.



15-a 15-b

BEHAVIOR OF (E)- AND (Z)-3-HP IN SOLUTION

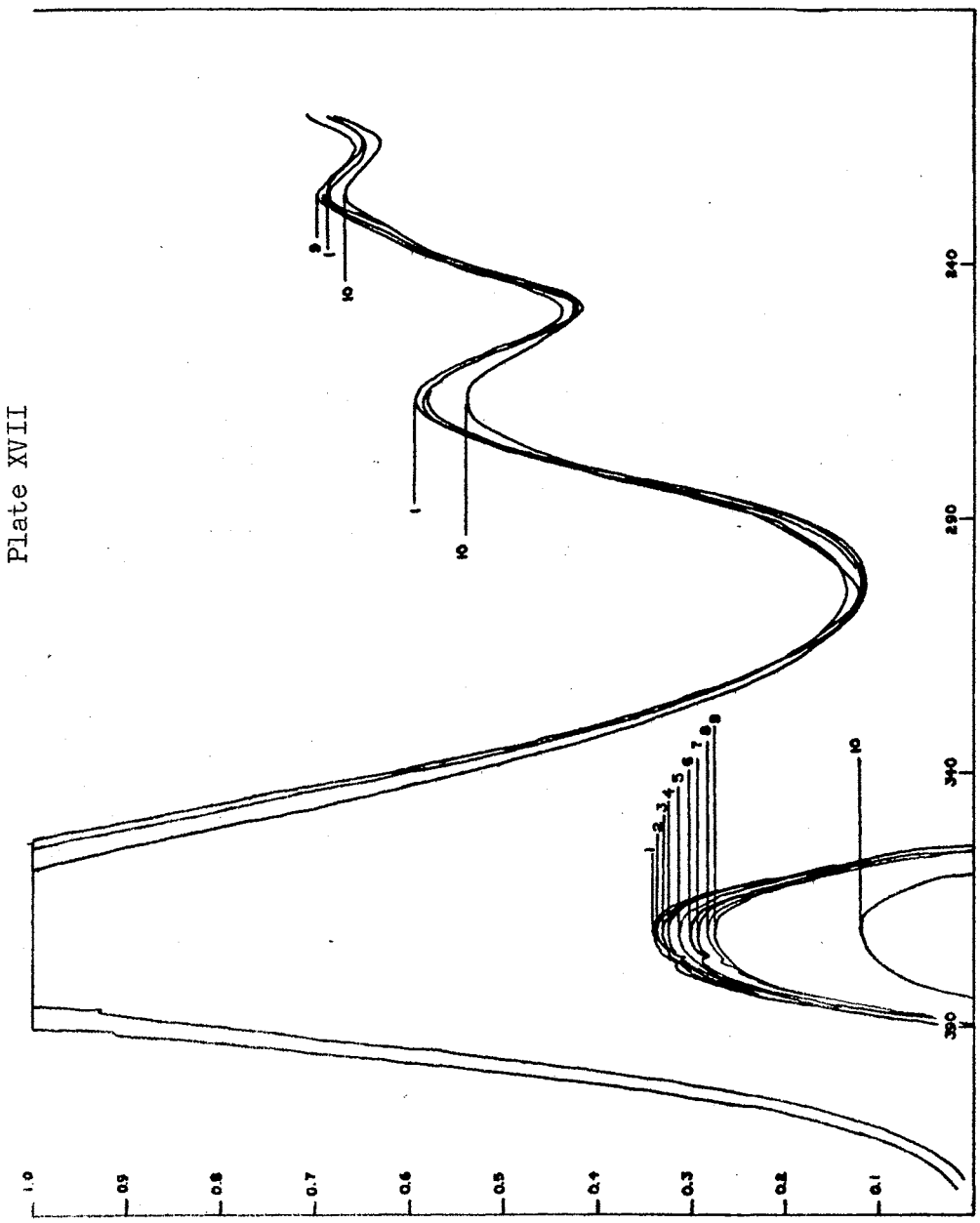
(E)-3-HP in 0.1 N HCl

(E)-3-HP in 0.1 N HCl shows maxima at 372, 267, and 227 nm. After the solution stands for 14.5 hours the maximum at 372 nm shows a substantial decrease in intensity.

(Z)-3-HP in 0.1 N HCl

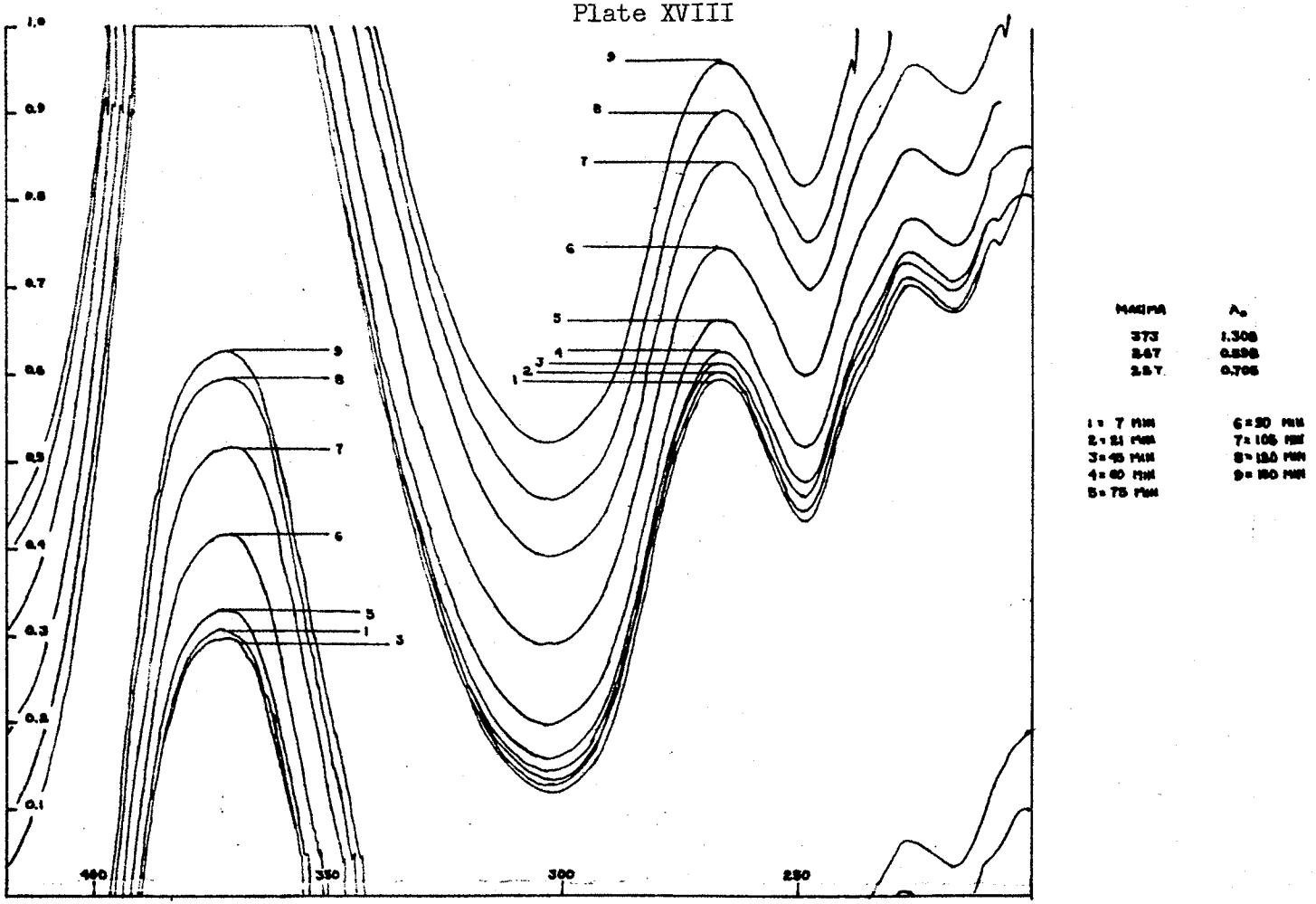
(Z)-3-HP in 0.1 N HCl shows maxima at 373, 267, and 227 nm. After 150 minutes the entire spectrum has increased in intensity.

Plate XVII



Ultraviolet Spectrum of (E)-3-HP in 0.1 N HCl

Plate XVIII



Ultraviolet Spectrum of (Z)-3-HP in 0.1 N HCl

Thus the behaviors of the two isomers are opposite in 0.1 N HCl. The spectra for the two isomers in 0.1 N HCl are shown in Plates XVII and XVIII.

(E)-3-HP in pH 7.0 Buffer

(E)-3-HP in pH 7.0 buffer exhibits maxima at 217, 280, 344, 397, and 417 nm (shoulder). With the exception of the absorbance at 217 nm, which undergoes a slight hyperchromic effect, the spectrum of (E)-3-HP in 7.0 buffer remains essentially unchanged when observed for a period of 180 minutes.

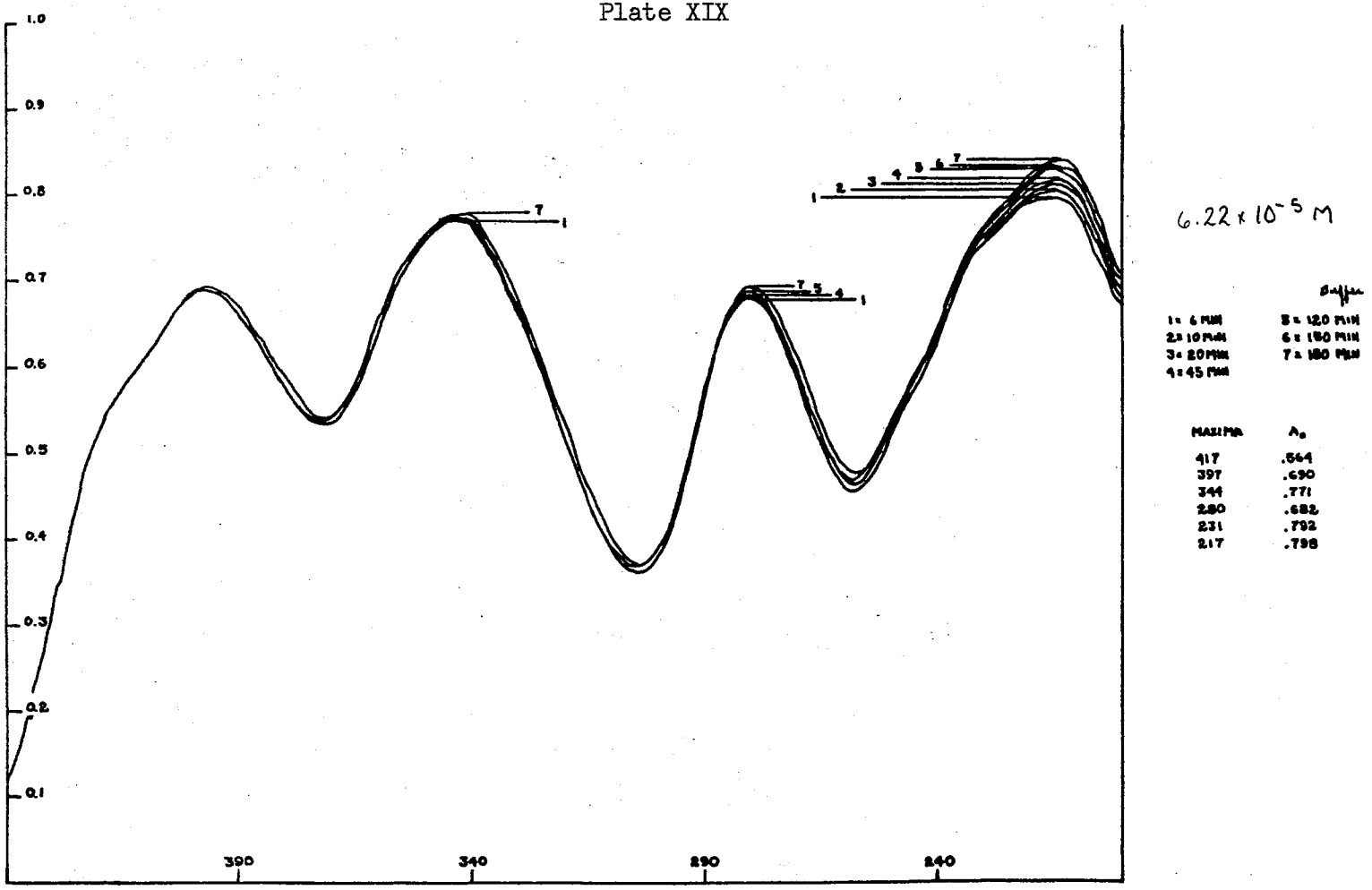
(Z)-3-HP in pH 7.0 Buffer

(Z)-3-HP in pH 7.0 buffer exhibits maxima at 213, 281, 343, 397, and 417 nm (shoulder). The entire spectrum demonstrates a substantial hyperchromic effect when observed for 185 minutes.

The fact that the spectrum of a solution of the E isomer does not change when observed for 180 minutes indicates that the compound does not isomerize. At pH 7.0 the quinonoid form of 3-HP does not have the carbon-nitrogen double bond, but does have a carbon-nitrogen single bond. The E isomer can equilibrate easily with the Z isomer by rotation around the carbon-nitrogen bond and no apparent isomerization would be seen in the ultraviolet spectrum.

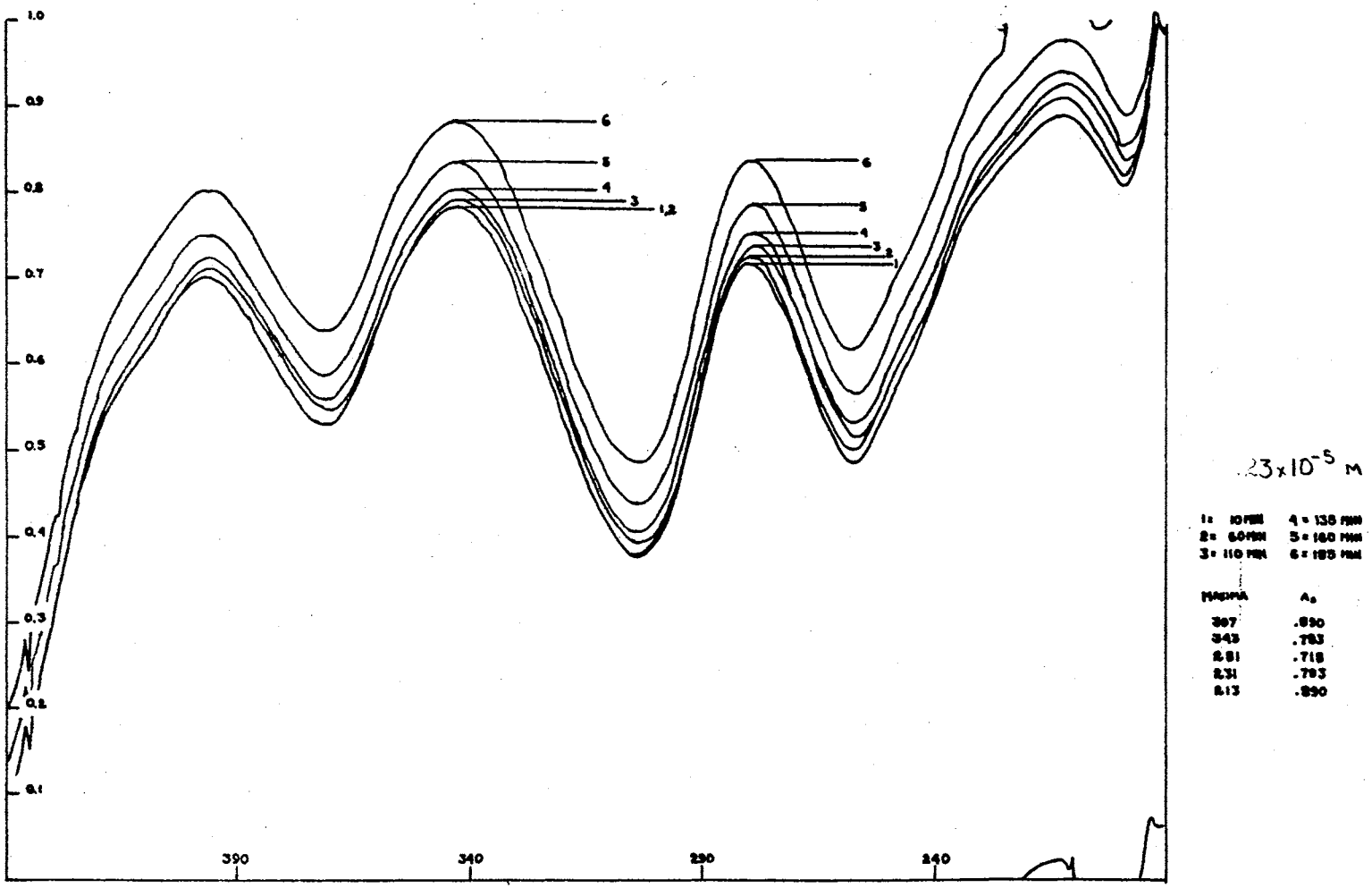
The Z isomer probably does not tautomerize as readily as the E isomer, as indicated by the ultraviolet spectra. The Z isomer is converted to the E isomer which then tautomerizes to the quinonoid form. This conversion of Z to E is observed as a hyperchromic effect of the spectrum of the tautomer. Spectra of the two isomers are

Plate XIX



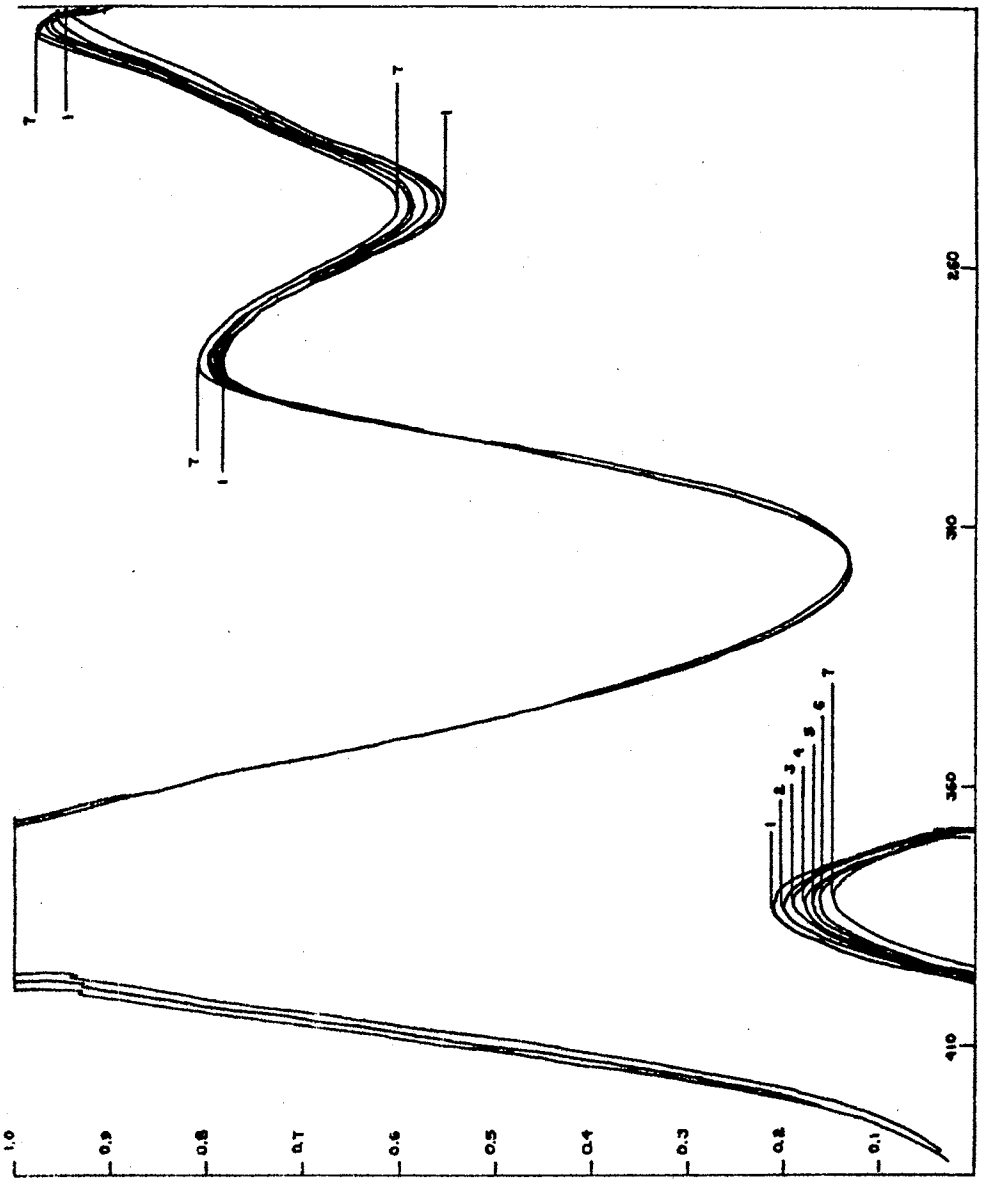
Ultraviolet Spectrum of (E)-3-HP in pH 7.0 Buffer

Plate XX



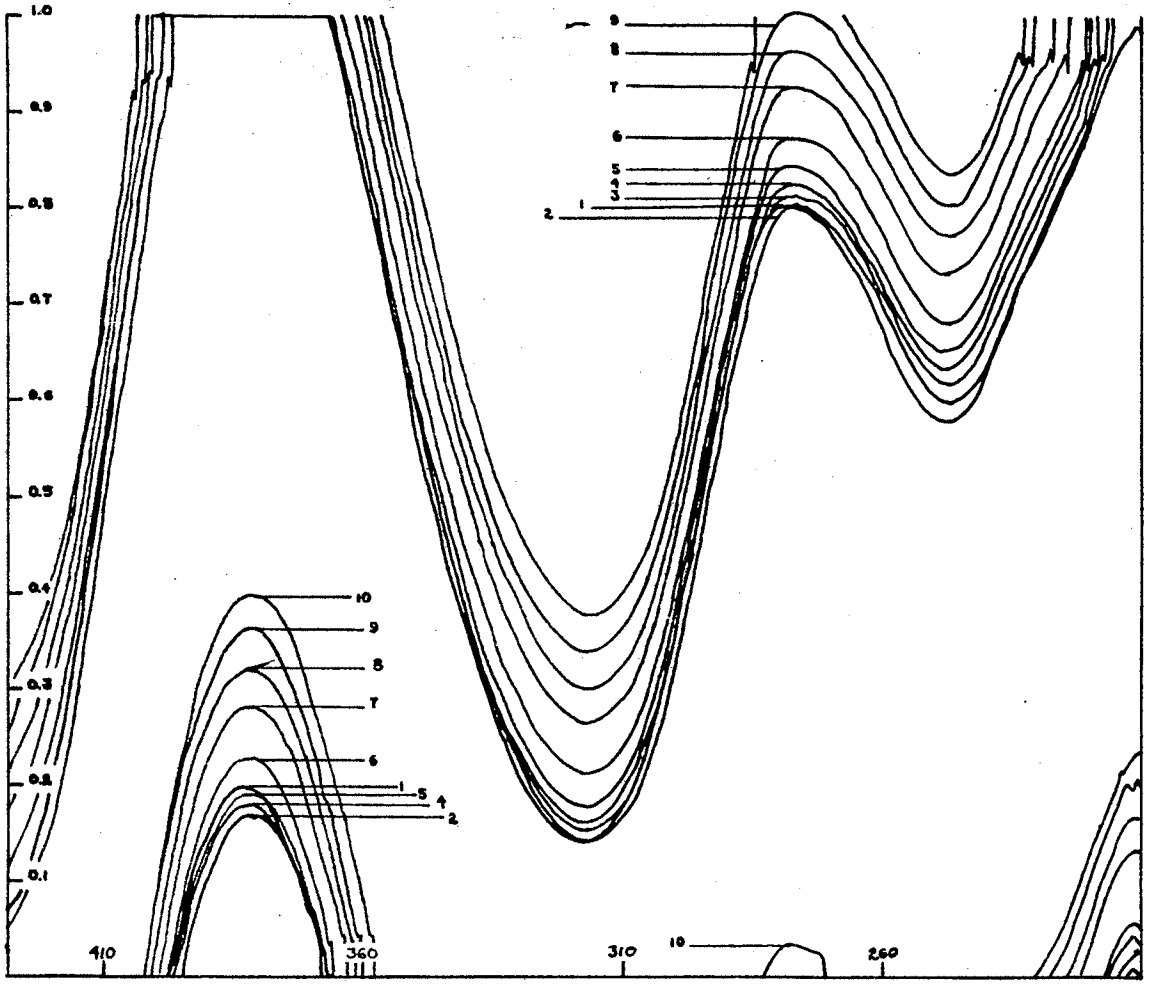
Ultraviolet Spectrum of (Z)-3-HP in pH 7.0 Buffer

Plate XXI



Ultraviolet Spectrum of (E)-3-HP in pH 12.0 Buffer

Plate XXII



Ultraviolet Spectrum of (Z)-3-HP in pH 12.0 Buffer

shown in Plates XIX and XX.

(E)-3-HP in pH 12.0 Buffer

(E)-3-HP in pH 12.0 buffer exhibits maxima at 211, 278, and 383 nm. When observed for 194 minutes the maximum at 383 nm shows a hypochromic effect while those at 278 and 211 nm show a slight hyperchromic effect.

(Z)-3-HP in pH 12.0 Buffer

The Z isomer of 3-HP when observed in pH 12.0 buffer exhibits maxima at 211, 280, and 385 nm. When observed for 180 minutes the entire spectrum increases greatly in intensity. The spectra of the two isomers are shown in Plates XXI and XXII.

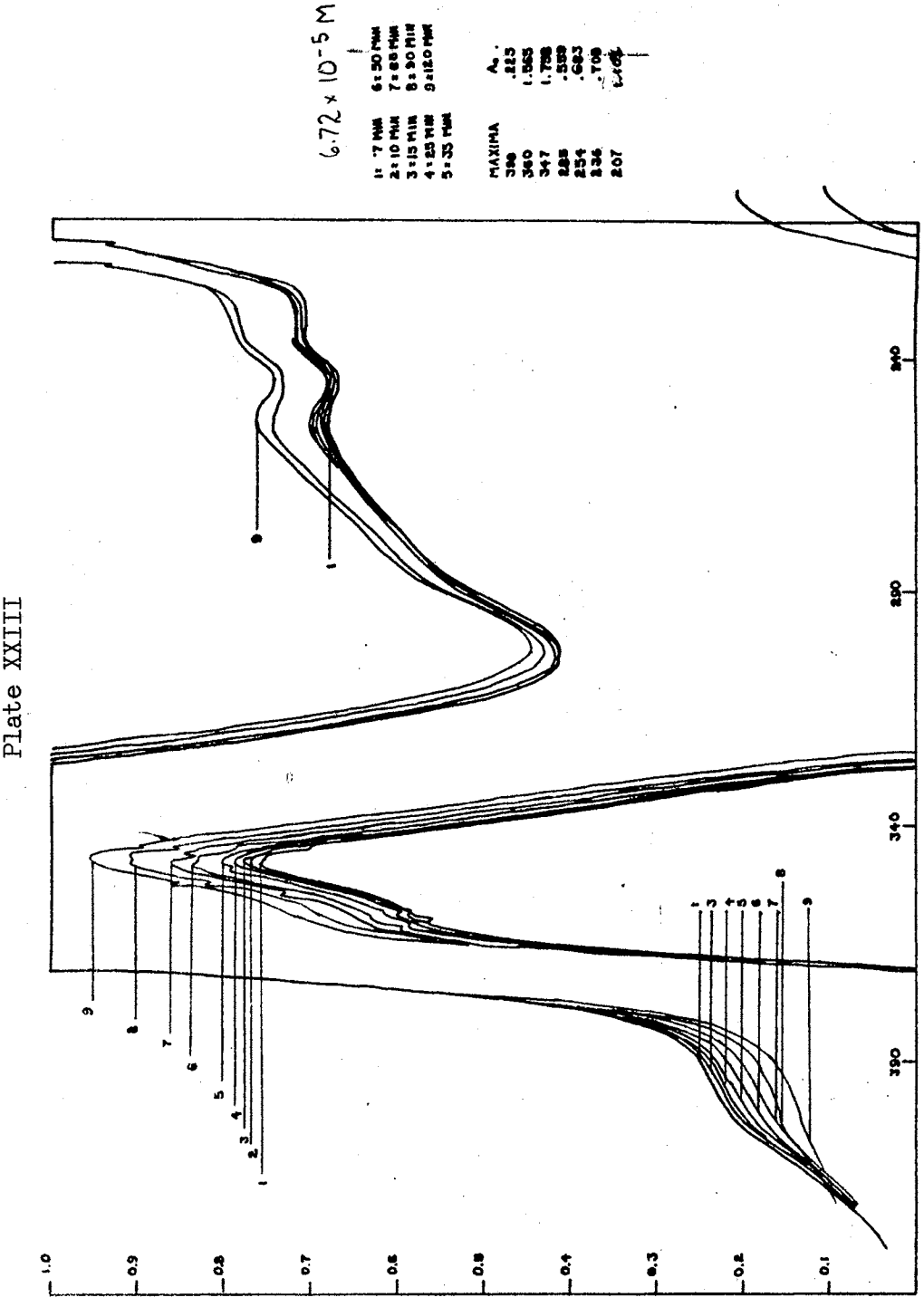
(E)-3-HP in 100% Ethanol

(E)-3-HP in ethanol exhibits maxima at 207, 254, 347, and 398 nm. When observed for 120 minutes the shoulder at 398 nm decreases in intensity. The E isomer isomerizes to the Z isomer; the quinonoid isomer as indicated by the shoulder at 398 nm will not be as abundant and absorbance due to it will decrease.

(Z)-3-HP in 100% Ethanol

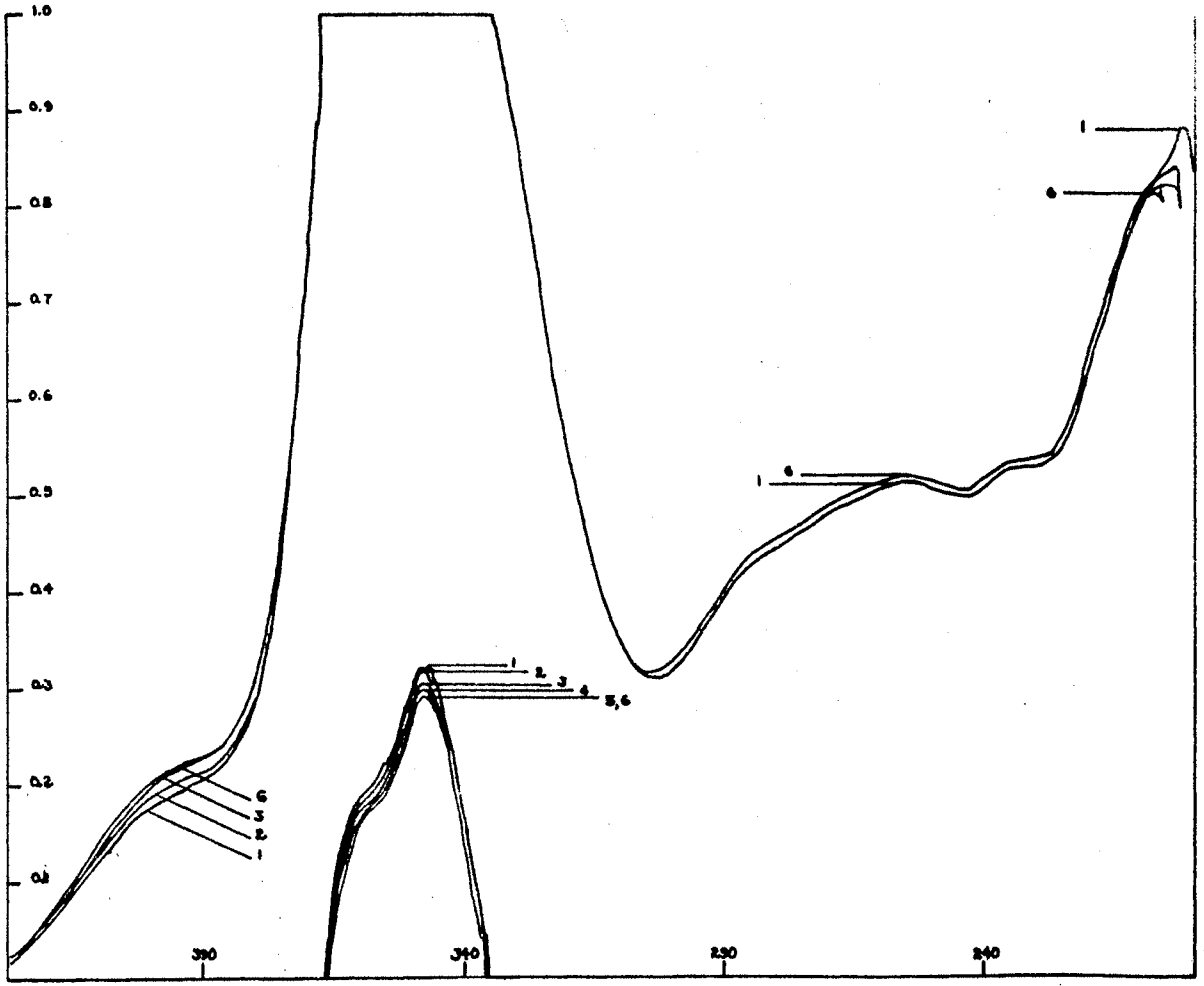
(Z)-3-HP in ethanol exhibits maxima at 203, 255, 347, and 398 nm. When observed for 180 minutes the maxima at 203 and 347 nm show a hypochromic effect while the maximum at 398 nm increases in intensity. The spectra of the two isomers are shown in Plates XXIII and XIV.

Plate XXIII



Ultraviolet Spectrum of (E)-3-HP in 100% Ethanol

Plate XXIV



6.16×10^{-5} M

1 = 30 MIN
2 = 30 MIN
3 = 60 MIN
4 = 90 MIN
5 = 135 MIN
6 = 180 MIN

MAXIMA	A_0
398	0.189
358	1.194
341	1.323
280	0.450
255	0.816
236	0.832
203	0.885

Ultraviolet Spectrum of (Z)-3-HP in 100% Ethanol

DETERMINATION OF E ⇌ Z RATES OF INTERCONVERSION

Frost and Pearson[63] discuss the reversible first-order reaction $X \underset{k_2}{\overset{k_1}{\rightleftharpoons}} Y$ and write a rate expression for the reaction as:

$$\ln \frac{A_o - A_e}{A - A_e} = (k_1 + k_2)t$$

The derivation of Frost and Pearson may be altered slightly (see Appendix A) to arrive at the rate expression written as:

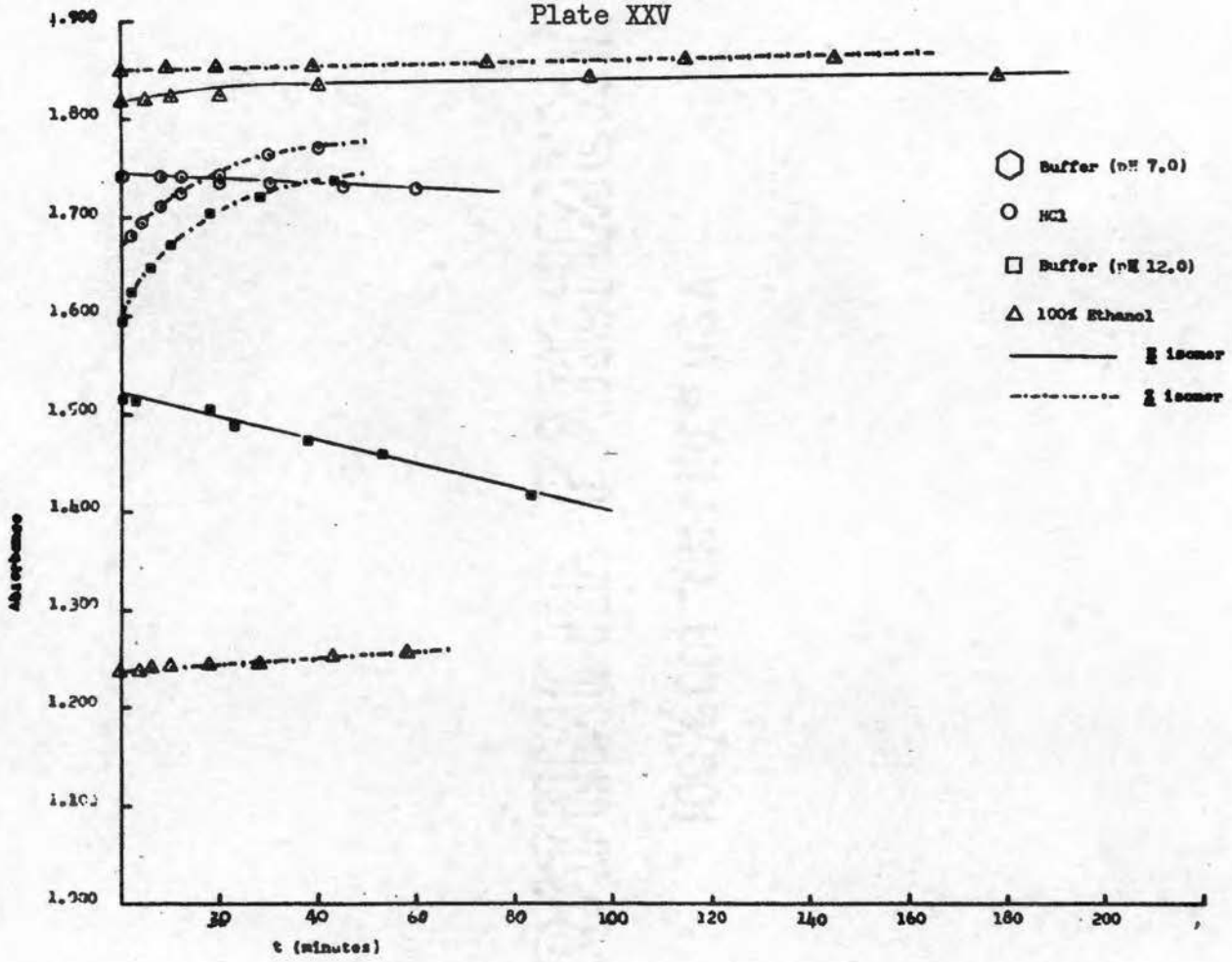
$$\ln \frac{[Y]_e - [Y]_1}{[Y]_e - [Y]_{1+n}} = (k_1 + k_2)\,(t_{1+n} - t_1),\ n = 1,2,3,\ldots$$

where $[Y]_1$, $[Y]_{1+n}$ and $[Y]_e$ are the concentrations of Y at time = t_1, t_{1+n} and at equilibrium, and the sum $(k_1 + k_2)$ is defined as k_{eff}, the effective rate constant for the approach to equilibrium. Plotting $\log \frac{[Y]_e - [Y]_1}{[Y]_e - [Y]_{1+n}}$ against $(t_{1+n} - t_1)$ for different values of n results in a straight line with slope = $k_{eff}/2.303$. The terms $[Y]_e - [Y]_1$ and $[Y]_e - [Y]_{1+n}$ may be conveniently evaluated by spectroscopic methods (see Appendix B).

Rates of Equilibration of (E)- and (Z)-2-FP

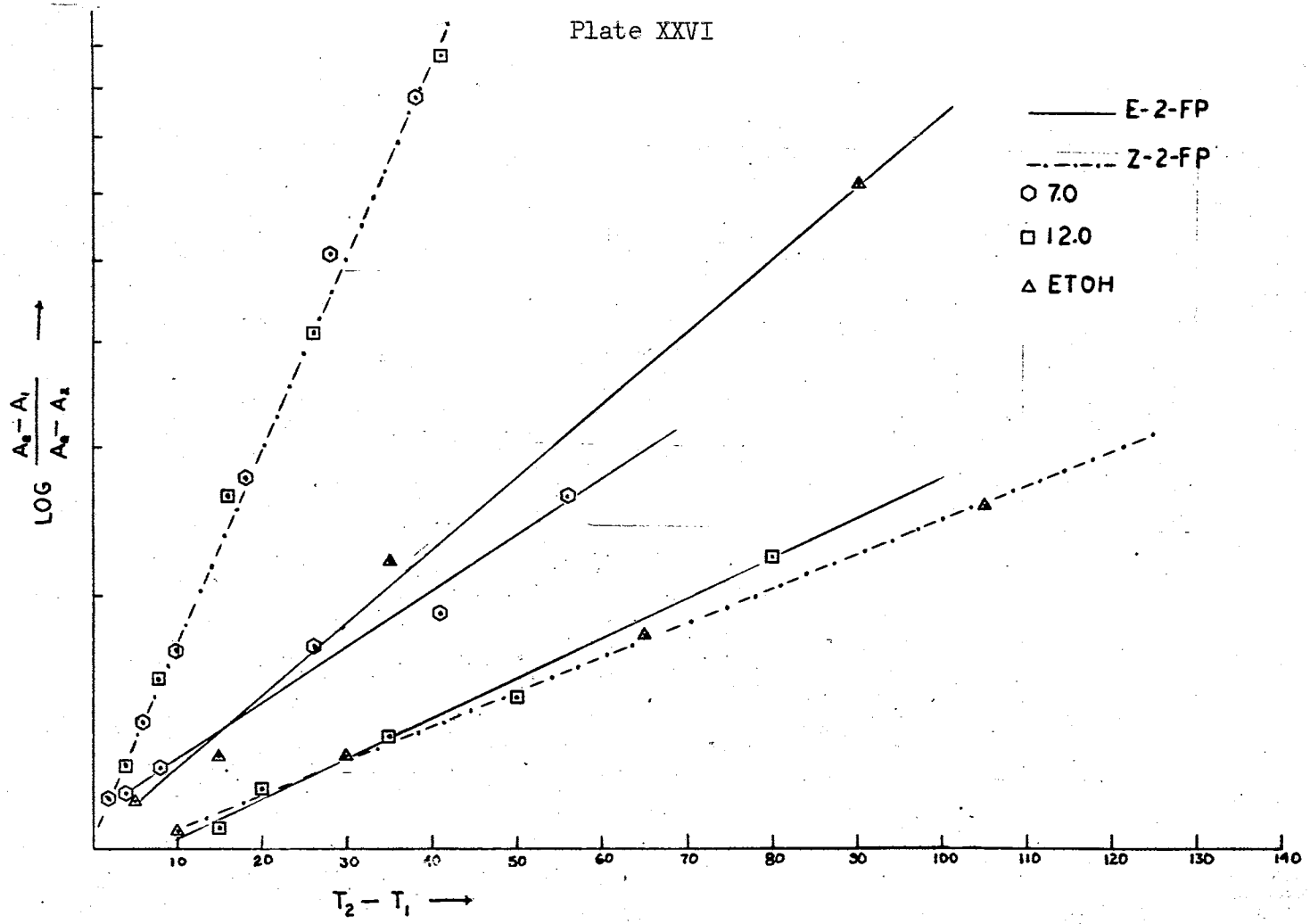
The spectra of the E and Z isomers are compared for each solvent. For each pair of spectra, e.g. (E)-2-FP in ethanol and (Z)-2-FP in ethanol, an absorbance is chosen which was representative of the isomerization. The absorbance was plotted against time for each individual isomer and, if necessary, the plot was extrapolated to equilibrium. The approach of the absorbance to this equilibrium is analyzed by plotting $\log \frac{A_e - A_1}{A_e - A_{1+n}}$ against time. The terms A_1, A_{1+n}, and A_e

Plate XXV



Absorption Curves for (E)- and (Z)-2-FP in Various Solvents

Plate XXVI



Rate Plots for the Approach to Equilibrium by (E)- and (Z)-2-FP in Various Solvents

are the absorbances at the selected wavelength at t_1, t_{1+n} and at equilibrium. Straight lines result which indicate that the process being observed, i.e. approach to equilibrium, is first-order. The slopes of these lines are measured and the effective rate constants for approach to equilibrium were calculated. These rate constants are summarized in Table I.

TABLE I

EFFECTIVE RATE CONSTANTS FOR APPROACH TO EQUILIBRIUM BY (E)-2-FP AND (Z)-2-FP

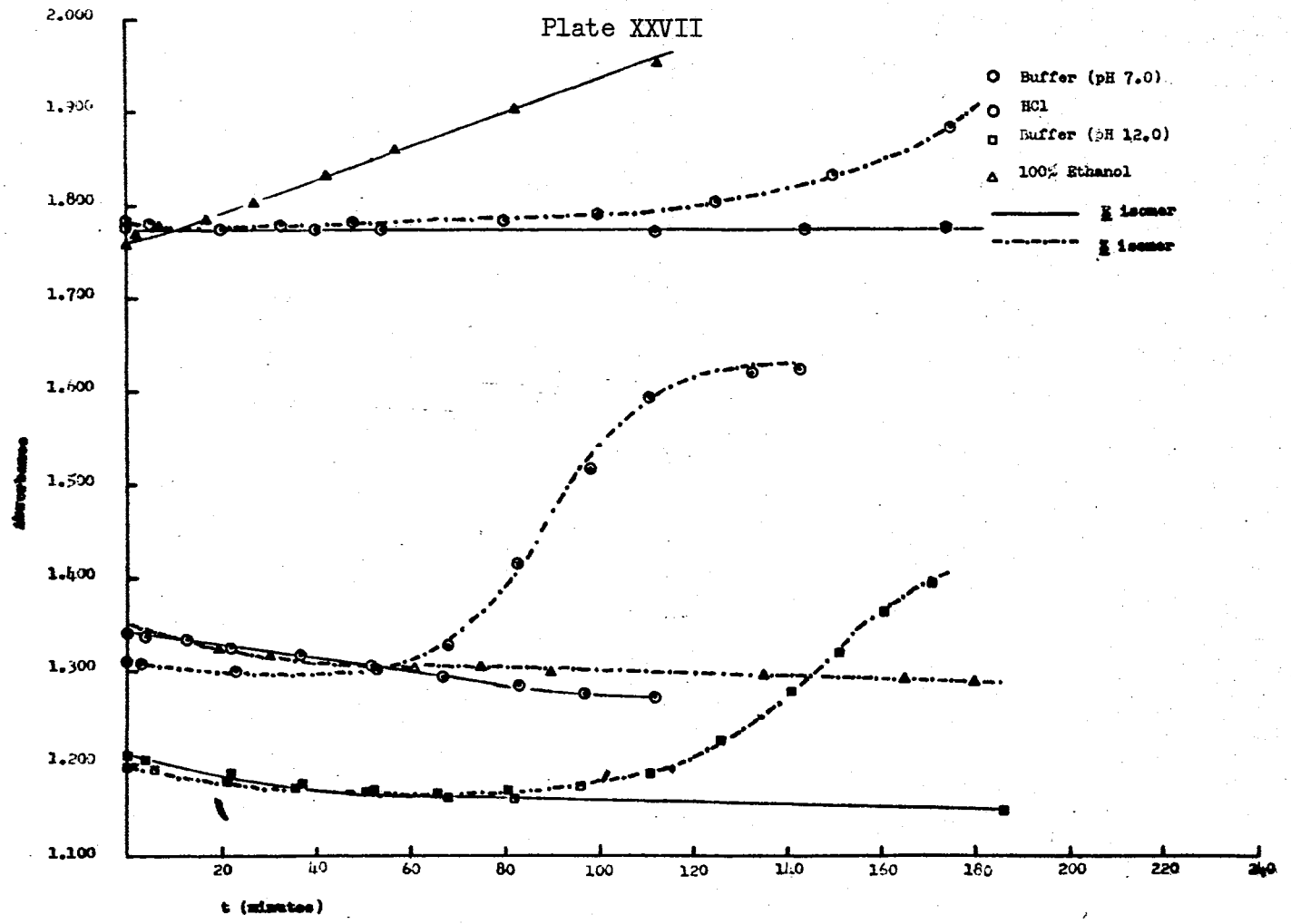
Solvent	k_{eff} for (E)-2-FP min^{-1}	k_{eff} for (Z)-2-FP min^{-1}
Aqueous, pH 1	isomer hydrolyzes	isomer hydrolyzes
Aqueous, pH 7	1.55×10^{-2}	[illegible]
Aqueous, pH 12	1.21×10^{-2}	[illegible]
100% Ethanol	2.09×10^{-2}	1.04×10^{-2}

The absorption curves and the rate plots for (E)- and (Z)-2-FP in various solvents are presented in Plates XXV and XXVI respectively.

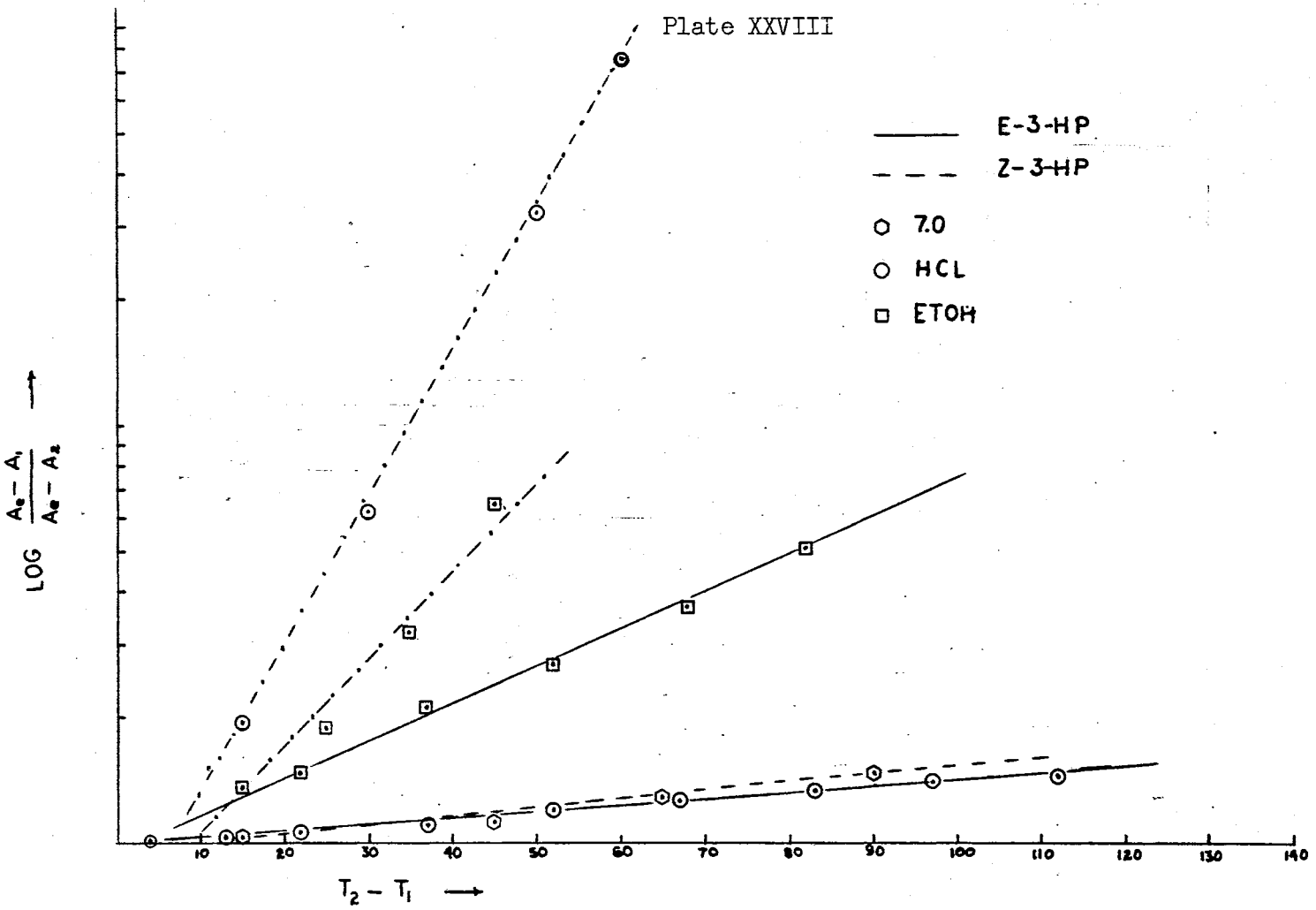
Rates of Equilibration of (E)- and (Z)-3-HP

The rate constants for the approach to equilibrium of the E and Z isomers of 3-HP were calculated by the same method and are summarized in Table II.

Plate XXVII



Absorption Curves for (E)- and (Z)-3-HP in Various Solvents



Rate Plots for the Approach to Equilibrium By (E)- and (Z)-3-HP in Various Solvents

TABLE II

EFFECTIVE RATE CONSTANTS FOR APPROACH TO EQUILIBRIUM BY (E)-3-HP AND (Z)-3-HP

Solvent	k_{eff} for (E)-3-HP min^{-1}	k_{eff} for (Z)-3-HP min^{-1}
Aqueous pH 1	3.93×10^{-3}	7.37×10^{-2}
Aqueous pH 7	too fast to measure	5.53×10^{-3}
Aqueous pH 12	2.08×10^{-2}	1.64×10^{-2}
100% Ethanol	**	**

** a straight line was not obtained from the rate plots.

The absorption curves and the rate plots for (E)- and (Z)-3-HP are presented in Plates XXVII and XXVIII respectively.

Discussion of the Reactions

Comparison of the effective rate constants for the approach to equilibrium in water by the Z isomers of 2-FP and 3-HP and also for the approach to equilibrium by the E isomers of 2-FP and 3-HP illustrates two points concerning the approach to equilibrium by the E and the Z isomers:

1. the approach is first-order for the Z isomers and the E isomers, and is independent of pH from 7.0 to 12.0.
2. the E isomers and the Z isomers each apparently approach equilibrium by the same mechanism.

The approach to equilibrium of the Z isomers is faster than the approach to equilibrium by the E isomers in water and the approach to equilibrium of the E isomers is faster than the approach to equilibrium by

the Z isomers in ethanol. The absence of solvation enhances the formation of intramolecular hydrogen bonds which serve as a driving force for the conversion of E to Z.

Since the effective rate constant (k_{eff}) is defined as equal to ($k_1 + k_2$), the constant should have the same value regardless of whether the approach to equilibrium is measured starting with the E isomer or the Z isomer (under the same conditions). Allowing for experimental error, it is seen that the effective rate constants for (E) and (Z)-2-FP and 3-HP come close to meeting this condition. In those reactions where k_{eff} does not remain constant for two isomers under the same conditions, e.g., (E) and (Z)-3-HP in 0.1 N HCl, other reactions such as hydrolysis or more than one mechanism for isomerization may be the cause for the deviation.

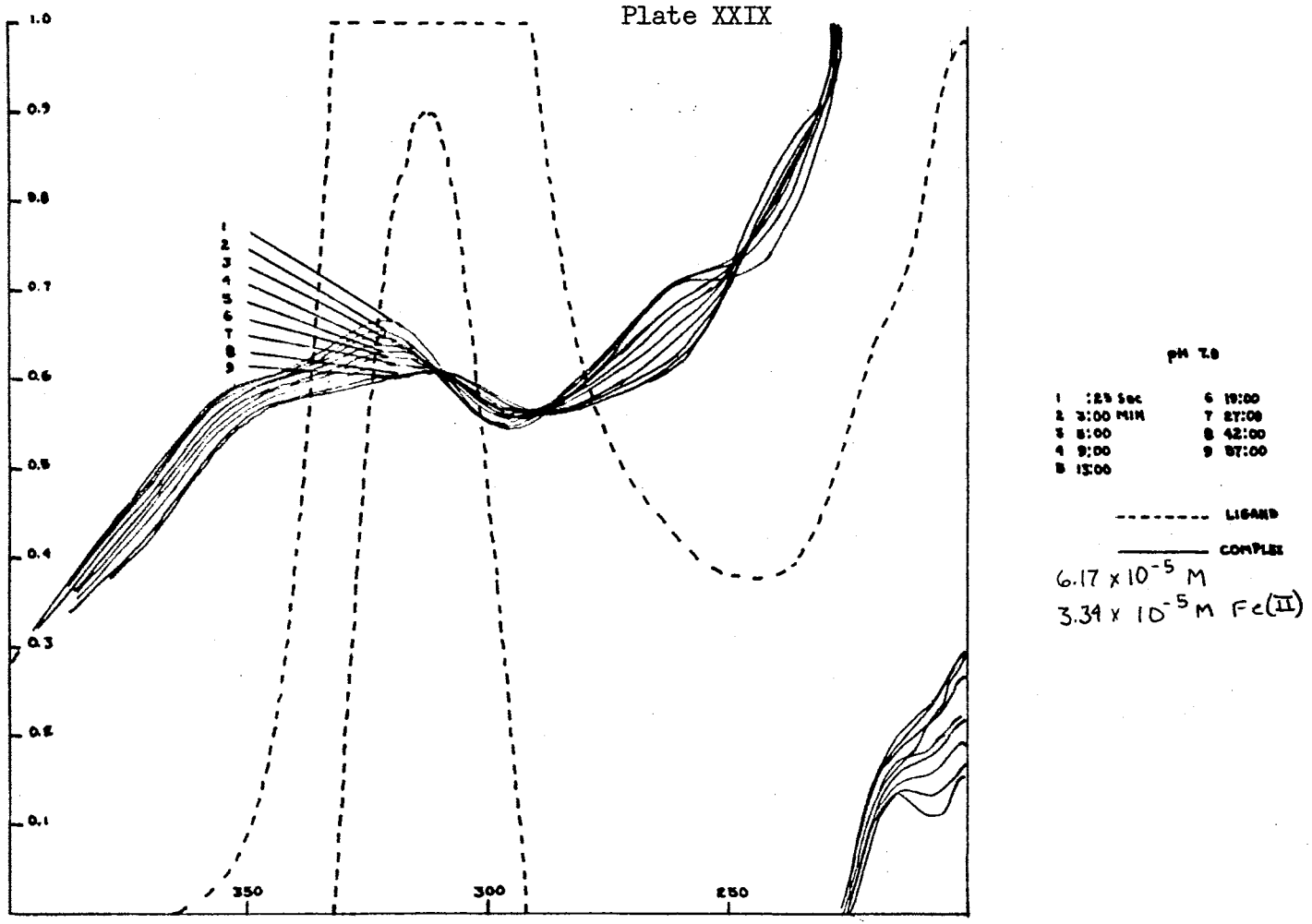
EFFECT OF METAL IONS ON THE APPROACH TO EQUILIBRIUM

The spectra were taken as previously discussed except that they were taken only in pH 7.0 in phosphate buffer since the purpose was to approximate conditions found in biological systems. The metals used in this portion of the experiments were the divalent ions of calcium, magnesium, manganese, iron, cobalt, copper, and zinc.

For those spectra which exhibited some change over the period observed, the absorbance of a single wavelength was plotted against time. The curve (if equilibrium had not already been reached) was extrapolated to a reasonable equilibrium and the spectral data were treated exactly as the isomerization data in the absence of metal ions.

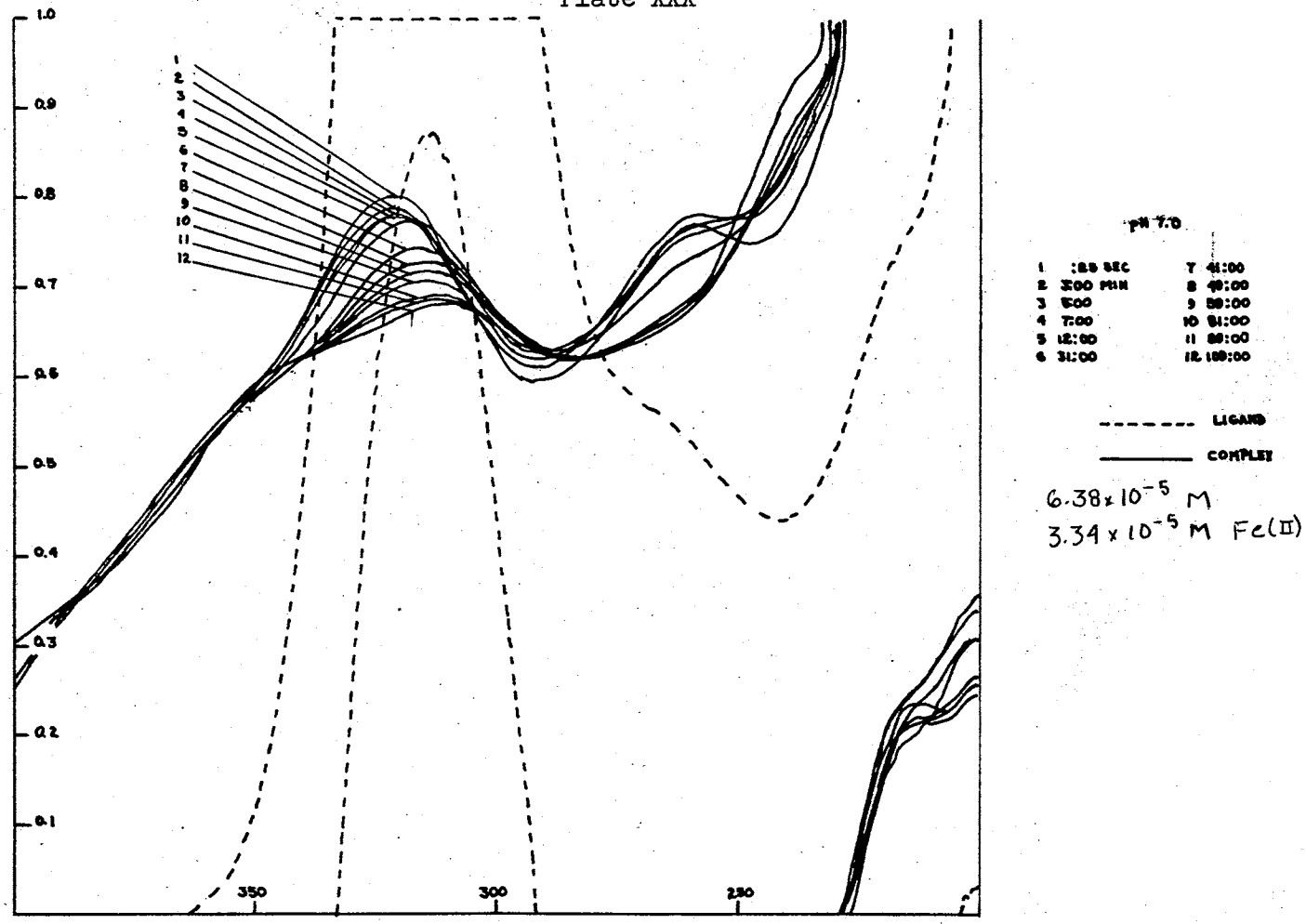
Neither 2-FP nor 3-HP complexed with calcium (II), magnesium (II), or manganses (II) so it seems reasonable that these ions will have no effect on the isomerization of the molecules.

Plate XXIX



Ultraviolet Spectrum of the Fe(II) Complex of (E)-2-FP

Plate XXX



Ultraviolet Spectrum of the Fe(II) Complex of (Z)-2-FP

(E)-2-FP-Fe(II) Complex

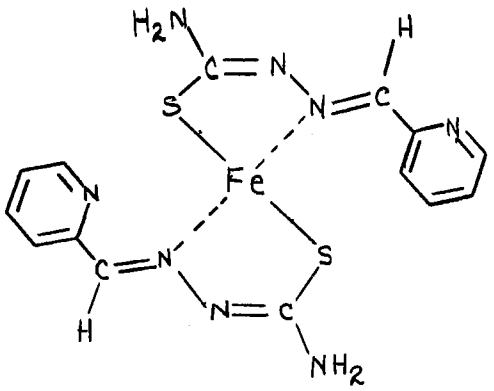
The spectrum was observed for 60 minutes during which numerous changes were observed in the spectrum. The presence of three isosbestic points (see Plate XXIX) indicates the presence of at least two absorbing species. The only absorbance change exhibited by the E complex for which there is no identical change in the spectrum of the Z complex occurs around 357 nm. Since all other changes occur in the spectra of both the E and Z complexes, the oxidation of Fe(II) to Fe(III) is assumed to be responsible to some extent for them. Since the hypochromic effect at 357 nm occurs only with the E complex, some phenomenon is occurring which does not occur with the Z. The hypochromic effect is attributed to the isomerization of E to Z.

The logarithm of the approach to equilibrium was plotted against time and a straight line resulted whose slope yielded an effective rate constant of 3.98×10^{-2} min.$^{-1}$.

(Z)-2-FP-Fe(II) Complex

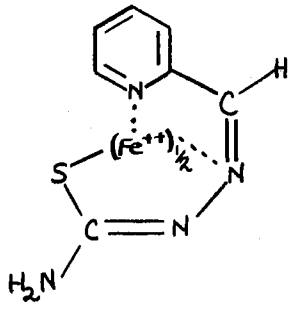
The major difference between Z and E isomers of 2-FP is around 357 nm where no change is observed over 110 minutes with the Z isomer but a hypochromic effect is witnessed in the spectrum of the E isomer.

The appearance of an absorbance around 357 nm in the spectra of the complexes indicates the formation of the thiol of the thiosemicarbazone and indicates that formation of the complex probably involves the sulfur. One possible structure of the complex is 16. As the (E)-complex is converted to the Z, the complex can involve the ring



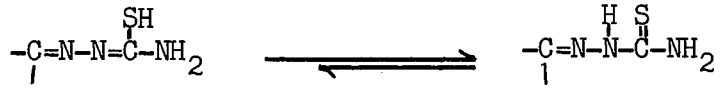
16

nitrogen as 17 illustrates. This conversion of the (E)- complex to



17

the (Z)-complex will cause the +2 charge on the Fe (II) to be dissipated not only by the imino nitrogen and sulfur but also by the ring nitrogen. The result of this could be less localization of electrons on the sulfur which would shift the thiol form of the thiosemicarbazone toward the thione form:



This would explain the hypochromic effect seen at 357 nm during the isomerization of E to Z. The spectra of the Fe(II) complexes of the

isomers of 2-FP are shown in Plates XXIX and XXX.

(E)-2-FP-Cu(II) Complex

The complex exhibits maxima at 260-265 nm, 314 nm, and 376 nm. Over a period of 104 minutes only a slight hypochromic effect was seen at 314 nm which indicates no isomerization.

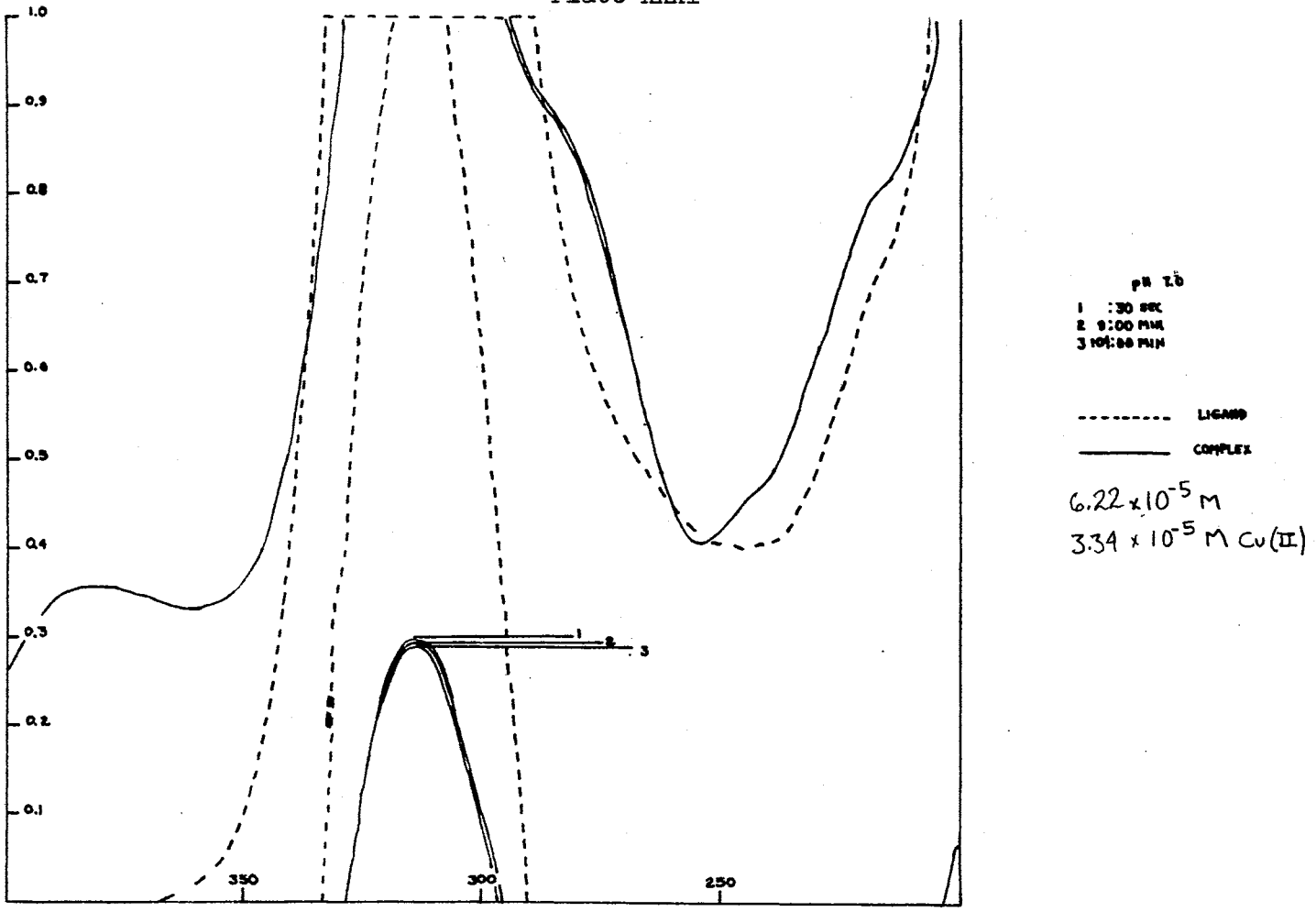
(Z)-2-FP-Cu(II) Complex

The complex exhibits maxima at: 280-285 nm which increases in intensity over 125 minutes; and 376 nm. Approach to equilibrium is followed by following the change in absorbance at 317 nm. The change is first-order with a rate constant of 7.97×10^{-2} $min.^{-1}$.

The E complex is the more stable than the Z complex. The E complex involves the ring nitrogen and the imino nitrogen as indicated by the localization of electrons on the ring and decrease in electron density of the azomethine bond as evidenced in the spectrum of the complex. The sulfur is not involved significantly in formation of the complex. The Z complex is structurally similar to the E but the sulfur probably is involved more in the complex due to its close proximity to the metal. The sulfur is not complexed enough to "lock" the ligand in the Z conformation necessary for the ligand to be tridentate, instead the complex isomerizes to the more favorable E form.

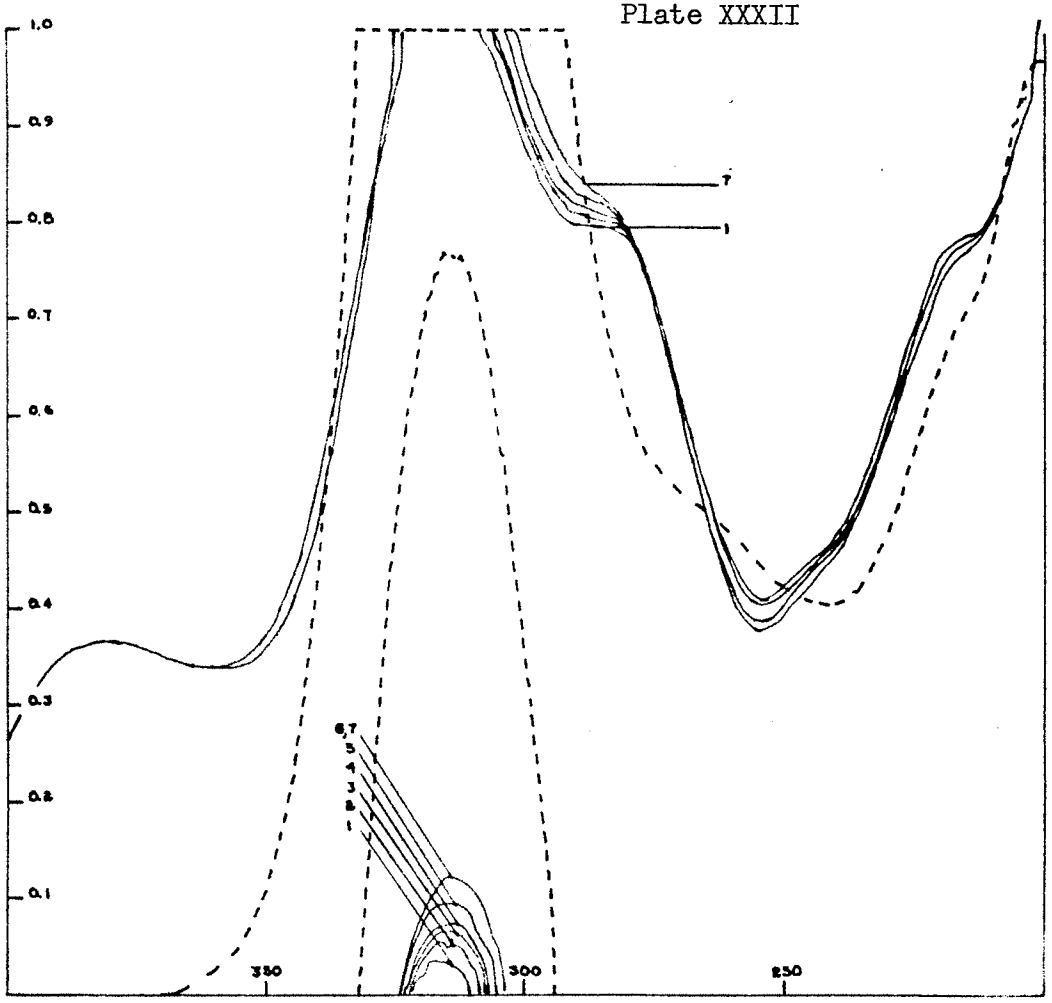
The E and Z complexes are represented at 18 and 19 respectively. Spectra of these complexes are shown in Plates XXXI and XXXII.

Plate XXXI



Ultraviolet Spectrum of the Cu(II) Complex of (E)-2-FP in pH 7.0 Buffer

Plate XXXII



Ultraviolet Spectrum of the Cu(II) Complex of (Z)-2-FP in pH 7.0 Buffer

(E)-2-FP-Zn(II) Complex

The Zn(II) complex of (E)-2-FP exhibits maxima at 280-283 nm; 314 nm which shows a very slight hypochromic effect after 85 minutes; and 365 nm which also shows a very small hypochromic effect over 85 minutes. The spectrum shows very little if any indication of isomerization which suggests that the E complex is stable.

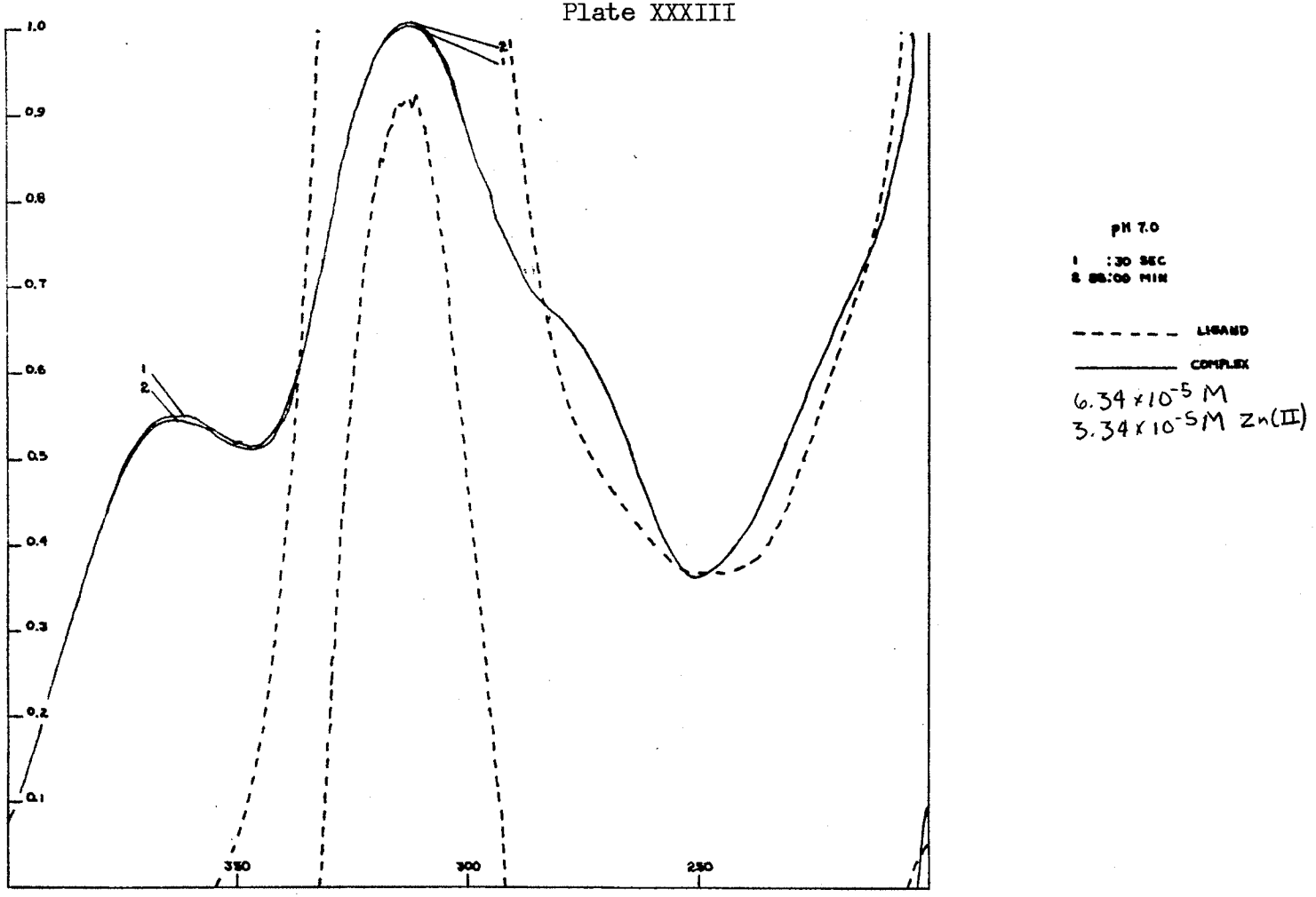
(Z)-2-FP-Zn(II) Complex

The Z complex shows maxima at: 365 nm which increases in intensity over 2 hours; 316 nm which shows both a hyperchromic effect and a hypsochromic shift to 314 nm over a period of 2 hours; and 280-283 nm. The approach to equilibrium is followed and a rate constant is calculated as 6.36×10^{-2} min.$^{-1}$.

The variance of intensities of absorbances at 365 nm and 316 nm indicates that the isomerization involves the sulfur and the imino nitrogen. If the tridentate Z complex is represented as $\underset{\sim}{2}0$ and the bidentate E as $\underset{\sim}{2}1$ interpretation of the isomerization follows the same logic as that of the Fe(II) complex. The tridentate ligand dissipates the +2 charge of the Zn(II) ion more than the bidentate ligand does. Consequently the Zn(II) is able to localize the thiol and azomethine electrons to a greater degree in the case of the bidentate E complex. The increased localization would increase the intensity of absorbance due to the thiol and azomethine chromophores.

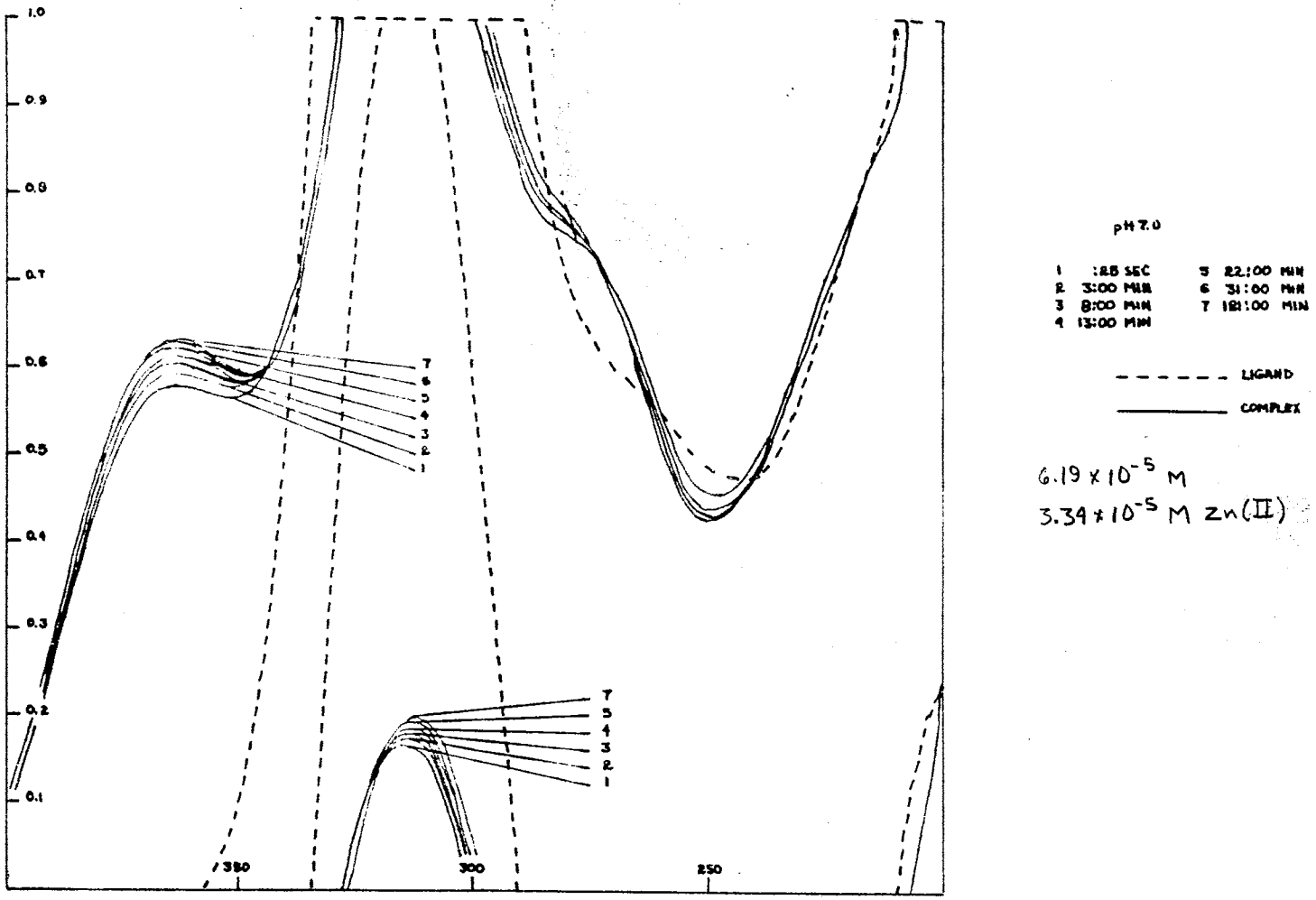
Jones[58] states that "---ions with an external electron shell of 18 or 18 plus 2 electrons generally form stable complexes with ligands containing divalent sulfur---.". The Zn(II) ion fits this description.

Plate XXXIII

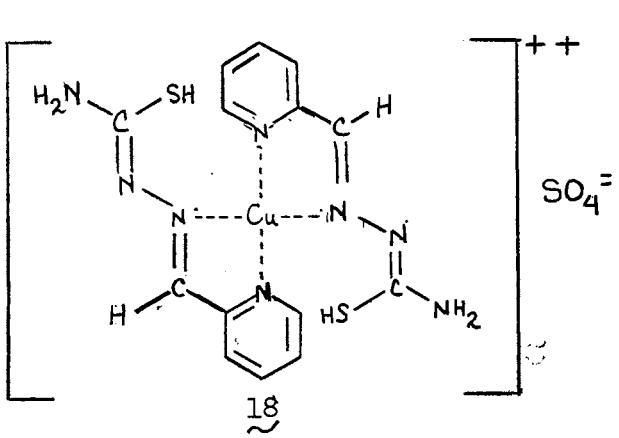


Ultraviolet Spectrum of the Zn(II) Complex of (E)-2-FP in pH 7.0 Buffer

Plate XXXIV



Ultraviolet Spectrum of the Zn(II) Complex of (Z)-2-FP in pH 7.0 Buffer



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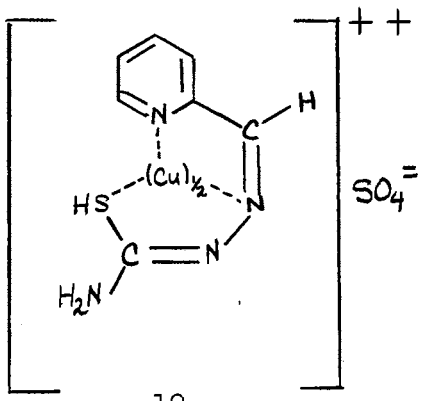
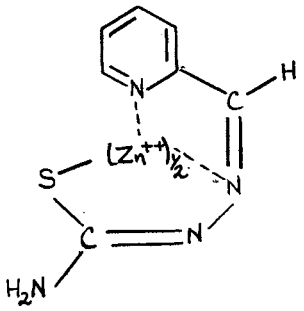


19



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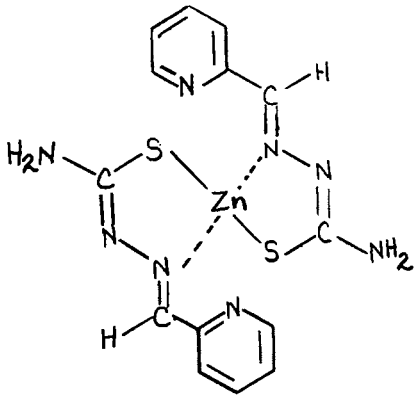


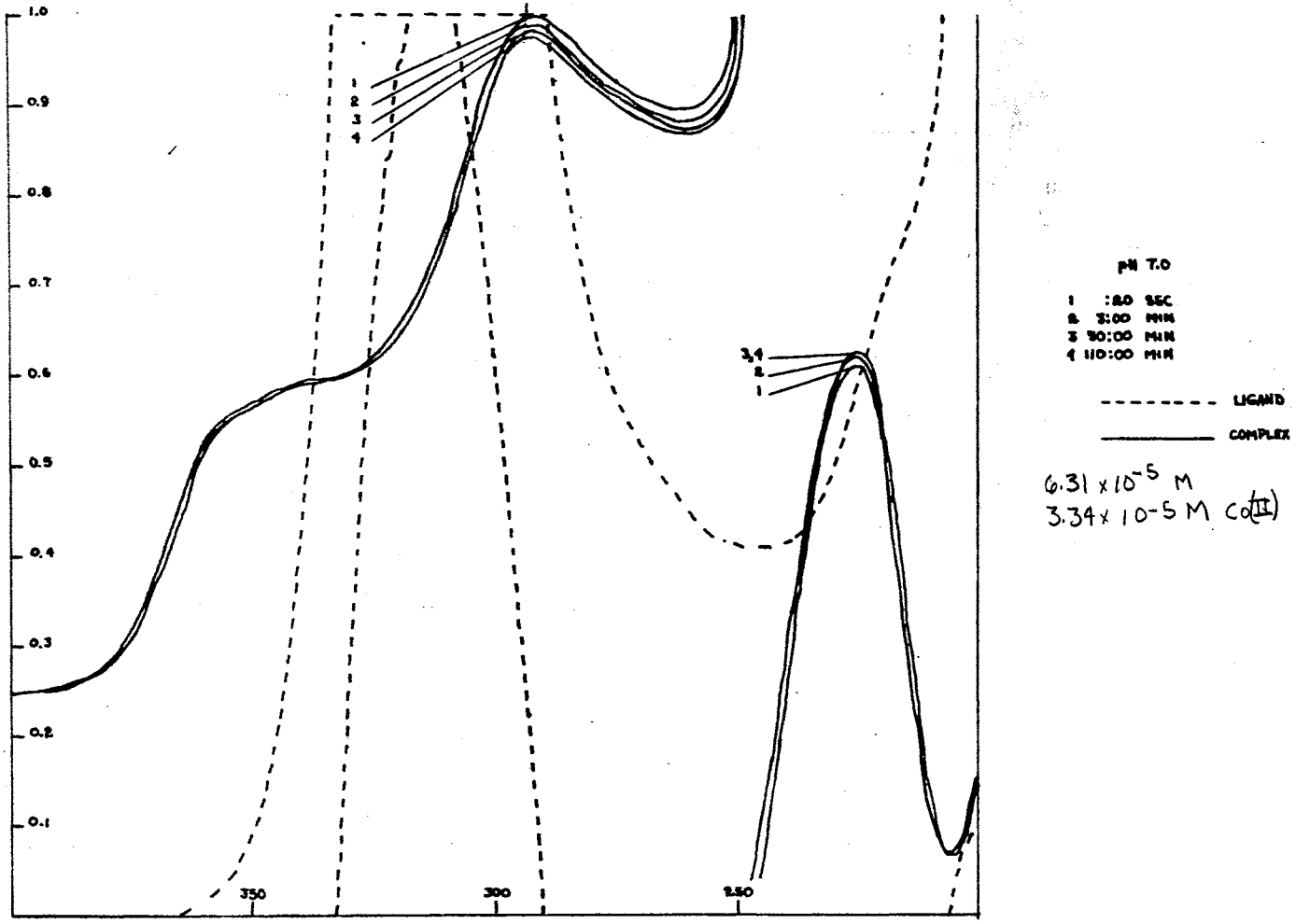
21

He also states that Zn(II) can form stable nitrogen chelates. On this basis and considering the behavior of the spectra, 20 and 21 seem reasonable. The spectra of the complexes are shown in Plates XXXIII and XXXIV.

(E)-2-FP-Co(II) Complex

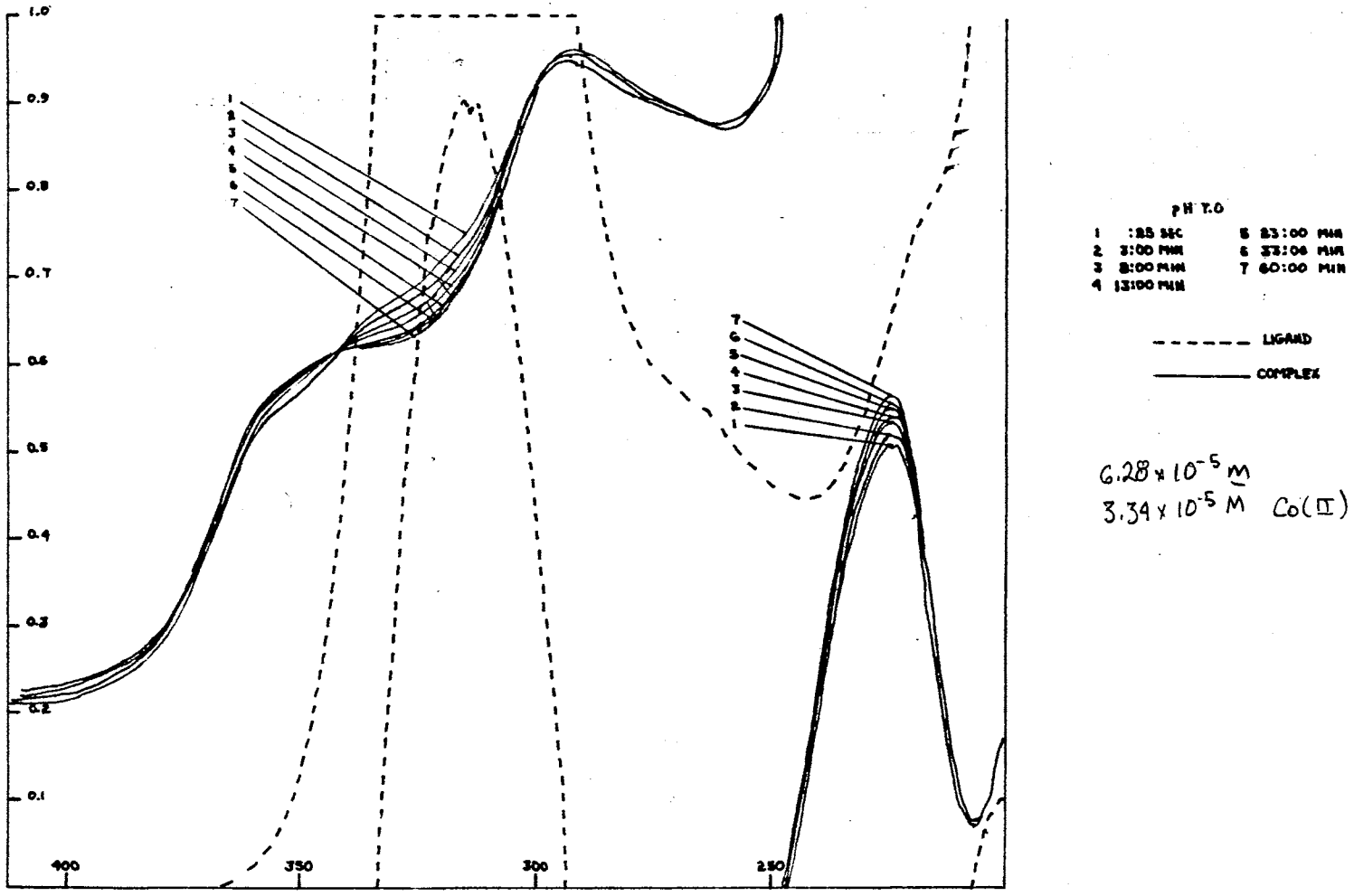
The E complex exhibits maxima at: 226 nm which increases in intensity very slightly over 110 minutes; 292 nm which decreases somewhat in intensity over the same 110 minutes; and 350-355 nm which remains relatively unchanged over the same 110 minutes.

Plate XXXV



Ultraviolet Spectrum of the Co(II) Complex of (E)-2-FP in pH 7.0 Buffer

Plate XXXVI



Ultraviolet Spectrum of the Co(II) Complex of (Z)-2-FP in pH 7.0 Buffer

(Z)-2-FP-Co(II) Complex

The Z complex displays maxima at: 224 nm which increases substantially and undergoes a bathochromic shift to 226 nm in a period of 60 minutes; 294 nm which increases slightly and shifts to 292 nm in 60 minutes; 332 nm (inflection) which decreases in 60 minutes; and 350-360 nm which increases slightly over the 60 minutes. The approach to equilibrium based on the change at 332 nm was measured and the rate constant calculated as 6.42×10^{-2} min.$^{-1}$.

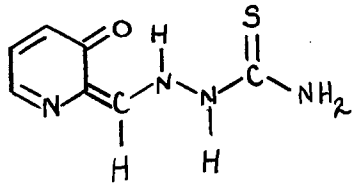
Since the spectra of the Co(II) complexes are somewhat different from those of the other three metals they are interpreted with difficulty. However, isomerization does occur as evidenced by the shift of the absorbances at 224 and and 294 nm which are characteristic of the Z complex to 226 and 292 nm which are characteristic of the E complex. No structures are suggested. The spectra of the complexes of the isomers are shown in Plates XXXV and XXXVI.

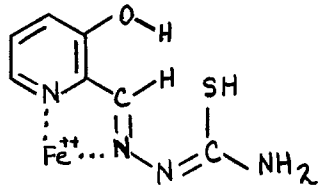
(E)-3-HP-Fe(II) Complex

Upon addition of Fe(II) to the buffer solution of (E)-3-HP the absorbance at 343 nm disappears completely while that at 397 nm displays a drastic hypochromic effect but does not disappear. This probably indicates a loss of quinonoid structure upon formation of the complex. At the same time there is a strong hyperchromic effect at 212 nm indicating an increase of phenolic structure of the molecule as shown:

The absorbance at 281 nm increases in intensity upon complexation with a slight bathochromic shift to 283 nm indicating localization of electrons on the heterocyclic nitrogen. As the complex stands, the absorbances at 283 and 212 nm increase in intensity indicating a change in the complexation with accompanying loss of quinonoid structure.

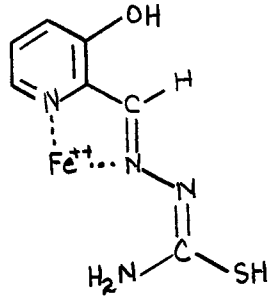
Based on the hyperchromic effect at 283 nm, complex formation is assumed to involve the nitrogen atom of the ring. Examination of scale models reveals that the quinonoid form of (E)-3-HP cannot participate easily in a complex structure involving both the heteroaroaromatic nitrogen and the N^1 (imino) nitrogen. If the quinonoid structure is lost, however, the imino nitrogen can easily participate in complexation and the resulting structure then conforms with the spectra. Thus it seems reasonable that 22 is the complex formed between (E)-3-HP and Fe(II). The hyperchromic effect seen at 212 nm would indicate a fur-

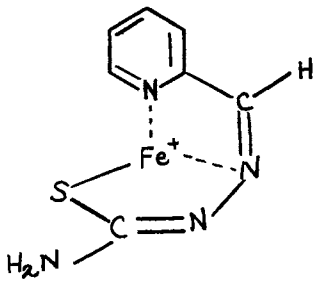




22

ther loss in quinonoid structure with increase in -OH. This indicates a shift from E to Z as shown. Since pyridine-2-carboxaldehyde thio-





23

semicarbazone behaves as a tridentate ligand with Fe(II)[59] the tridentate structure (23) probably also exists in the case of 3-HP.

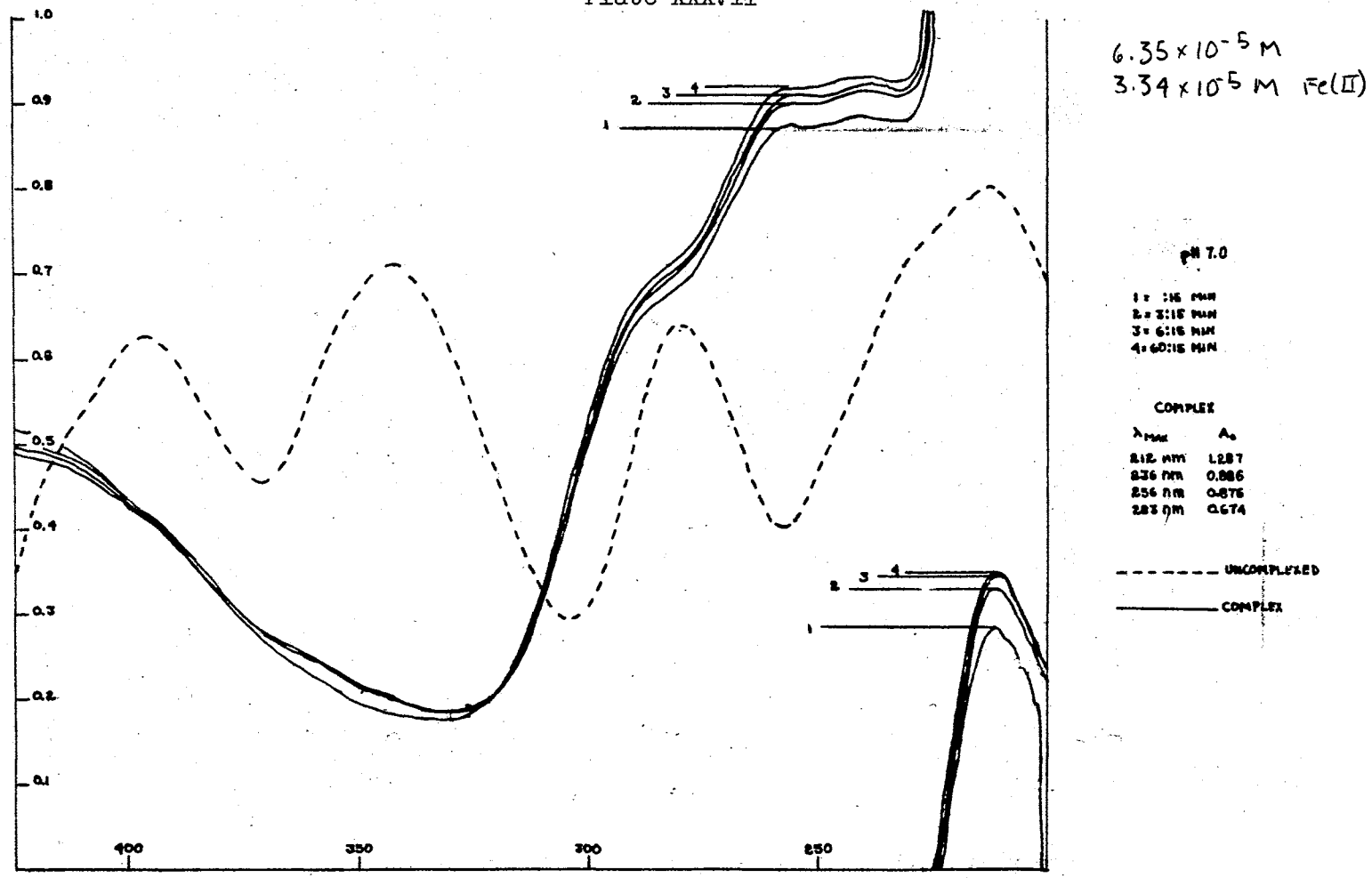
When the hyperchromic effect at 212 nm is used to measure the approach to equilibrium the rate constant is calculated as 2.11×10^{-1} $min.^{-1}$.

(Z)-3-HP-Fe(II) Complex

When (Z)-3-HP is mixed with Fe(II) the same spectral changes are seen that occurred with the E isomer except that the absorbance at 212 nm decreases when allowed to stand whereas in the spectrum of the E complex it increased. This decrease indicates a loss of phenolic form and formation of the quinonoid form, indicating a conversion from Z to E. When the hypochromic effect at 212 nm is followed as a measure of approach to equilibrium a rate constant is calculated as 9.49×10^{-2} $min.^{-1}$.

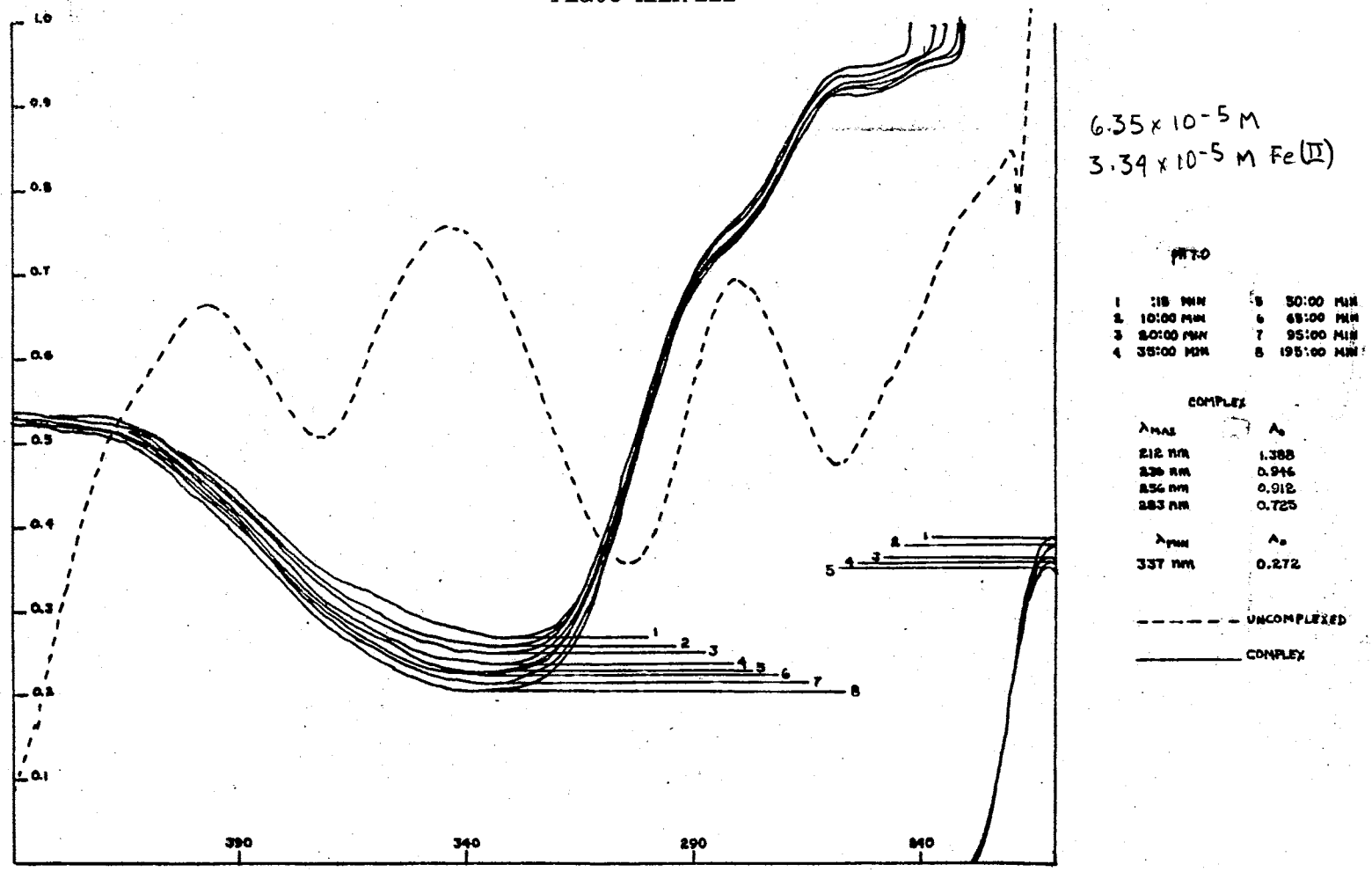
It is interesting to note that when allowed to react completely, the Fe(II) complex of each isomer reaches an equilibrium with the other as evidenced by the fact that each complex reaches an equilibrium extinction coefficient (λ = 212 nm) of 21,200 ($\log \epsilon = 4.327$). This would indicate that the complex formed between 3-HP and Fe(II) does not rigidly "lock" the ligand in one particular form, rather, the complex is fairly labile. Since the pKa of the enolic SH group is reported at 11.23 ($11.228 \pm .093$)[50] it is likely that this lability exists at the SH. The SH is not appreciably ionized at pH 7.0 and it is unlikely that the Fe(II) will be very successful in complexing with the sulfur.

Plate XXXVII



Ultraviolet Spectrum of the Fe(II) Complex of (E)-3-HP in pH 7.0 Buffer

Plate XXXVIII



Ultraviolet Spectrum of the Fe(II) Complex of (Z)-3-HP in pH 7.0 Buffer

Thus it appears that, since the isomerization of E to Z has essentially reached equilibrium in 10 minutes, the Fe(II) provides an impetus to accelerate the isomerization of E to Z at pH 7.0. However, since the Fe(II) complex of the Z isomer isomerizes to the same equilibrium as the E, the Fe(II) ion evidently does not form a strong enough complex with the SH to maintain the molecule in a particular conformation. The spectra of the Fe(II) complexes of 3-HP are shown in Plates XXXVII and XXXVIII.

(E)-3-HP-Cu(II) Complex

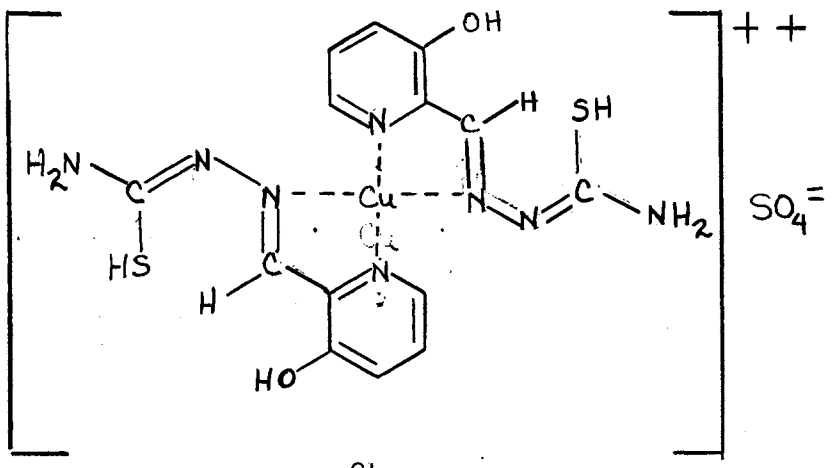
The spectrum of (E)-3-HP plus Cu(II) reveals strong hyperchromic effects on the absorbance at 212 nm and 281 nm with accompanying bathochromic and hypsochromic shifts to 214 and 277 nm respectively. The absorbances at 344 and 397 nm showed hypochromic effects upon formation of the complex but dod not disappear completely. This indicates that the quinonoid structure remains to some extent in the complex. The following deductions can be made concerning the complex:

(a) complexation shifts the quinonoid form toward the phenolic form as indicated by increase of phenolic absorbance at 212-214 nm.

(b) complexation involves the heterocyclic nitrogen atom as indicated by an increase of absorbance at 277 nm.

A structure which agrees with these conditions is 24.

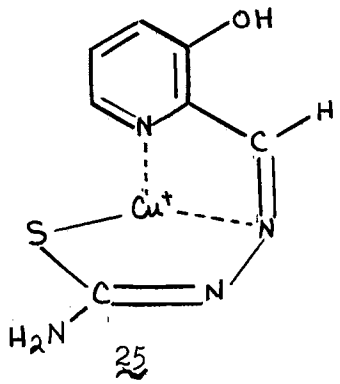
The slight hypochromic effect seen at 214 nm will be discussed after discussion of the Cu(II)-(Z)-3-HP complex.



24

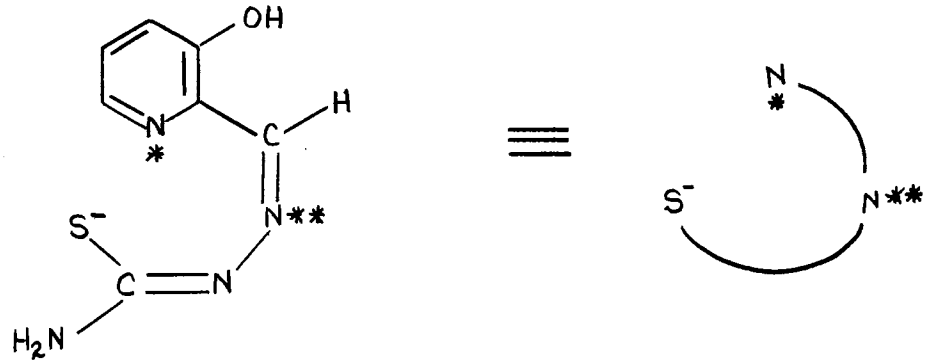
(Z)-3-HP-Cu(II) Complex

The absorbances seen in the spectrum of the Cu(II) complex of (Z)-3-HP are identical to those of the E complex, i.e. upon formation of the complex hyperchromic effects are seen at 214 and 277 nm with maintenance of some quinonoid evidence. One major difference was noticed. Although both solutions (E and Z) had the same concentration (6.23×10^{-5} M and 6.24×10^{-5} M), the spectrum of the E complex remained nearly unchanged for a period of 1 hour but the entire spectrum of the Z complex over the same period of time showed hypochromic effect. Examination of the sample cell revealed that the complex had precipitated. The hypochromic effect of the spectra of the Z complex is the result of the precipitation of the complex. This indicates that the E complex is a soluble complex and the Z is an insoluble complex. A possible representation of 1:1 complex of (Z)-3-HP and Cu(II) is 25 wherein the ionized enolic sulfhydryl neutralizes half of the Cu(II) ion. For simplicity the ligand is represented schematically as shown below with those atoms which are suggested as points of attachment with

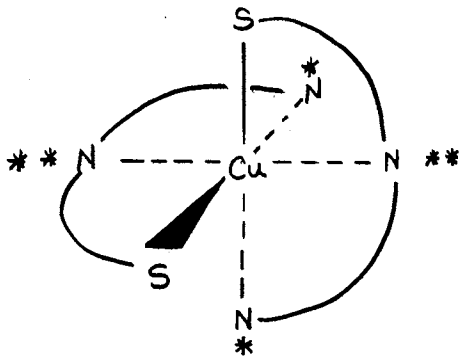


25

the metal ion approximately placed on the representation. Using this



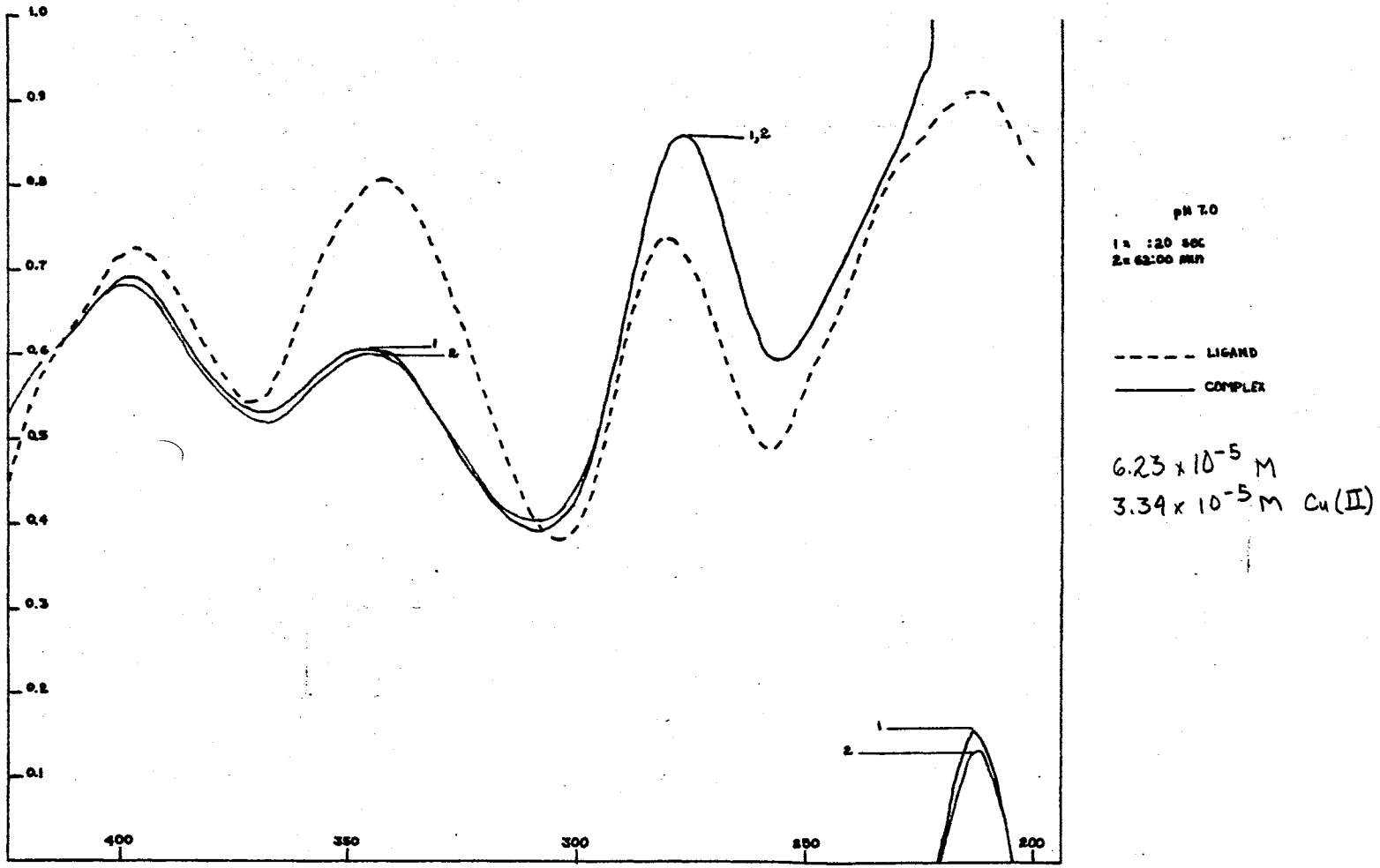
shorthand representation the 2:1 complex may be represented as 26.



26

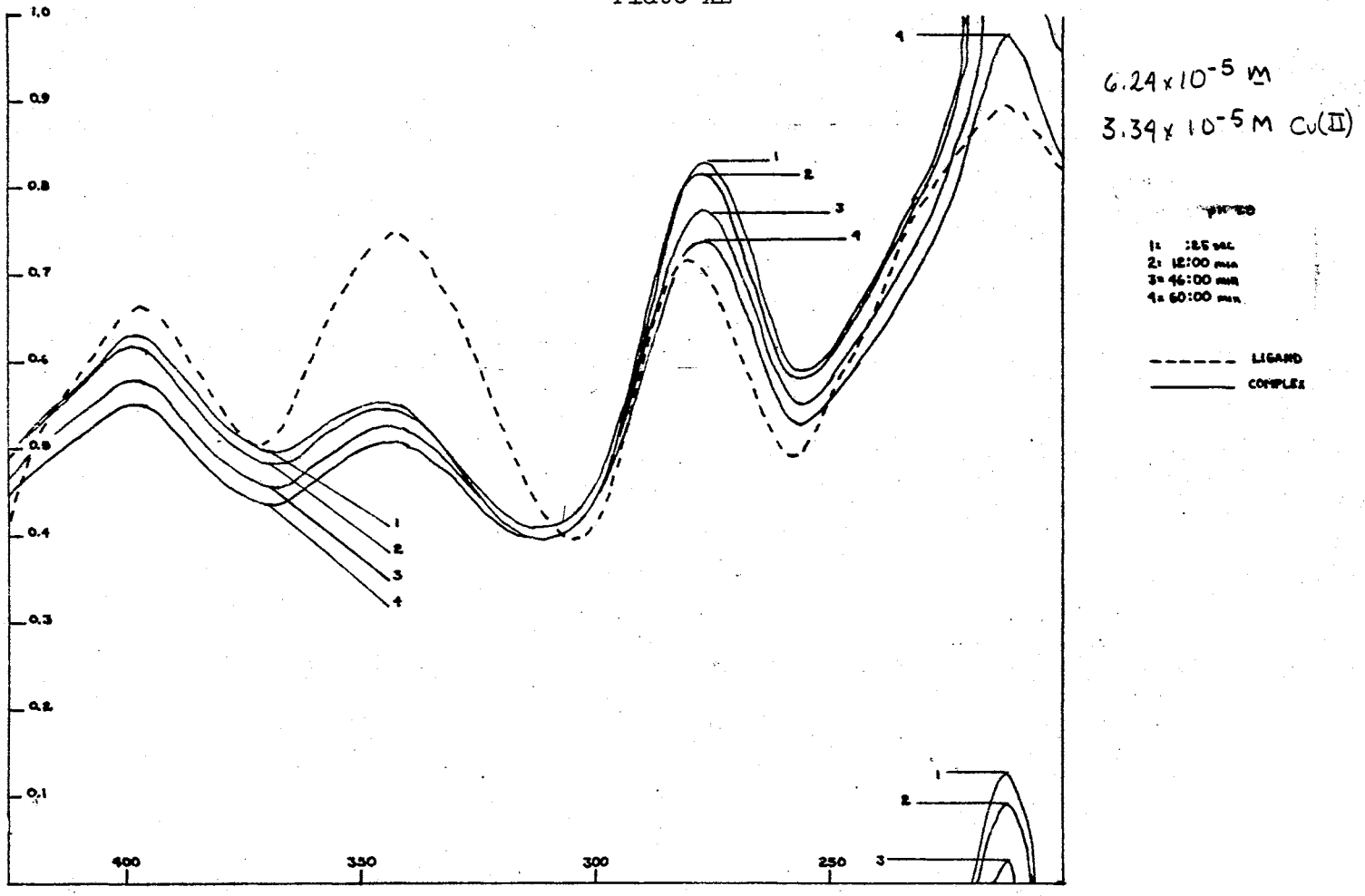
It is thus possible to represent the (Z)-Cu(II) complex (2:1) as a neutral molecule which is water insoluble. The E complex is re-

Plate XXXIX



Ultraviolet Spectrum of the Cu(II) Complex of (E)-3-HP in pH 7.0 Buffer

Plate XL



Ultraviolet Spectrum of the Cu(II) Complex of (Z)-3-HP in pH 7.0 Buffer

presented as the sulfate salt which is soluble. These complex structures would satisfactorily explain the changes in their spectra.

It is now easier to explain the slight hypochromic effect seen at 212 nm in the spectrum of the E complex. The effect is caused by the isomerization of the E complex to the Z complex which then precipitates.

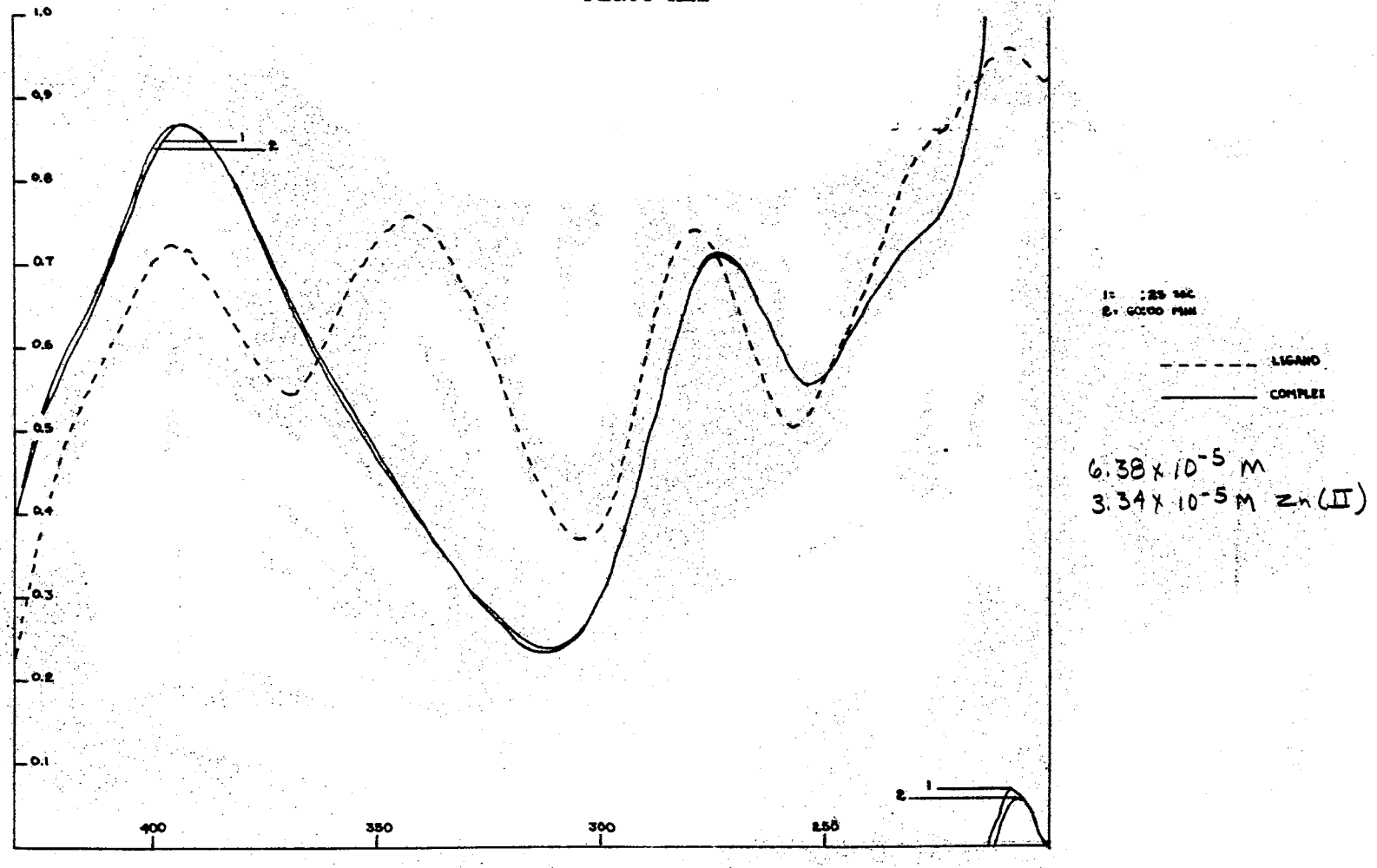
It thus appears that $CuSO_4$ does not accelerate the isomerization of 3-HP to any degree. The isomerization of the E complex is too slow to measure and the Z complex precipitates. The Cu(II) spectra of the isomers are shown in Plates XXXIX and XL.

(E)- and (Z)-3-HP-Zn(II) Complexes

The spectra of the E and Z complexes are superimposable. They exhibit maxima at: 393 nm which remain unchanged over periods of 60-100 minutes; 274 nm which decreases only very slightly over 60-100 minutes and which is also exhibited with the same intensity in the spectra of the free ligands; and 208 nm which decreases slightly in 60-100 minutes.

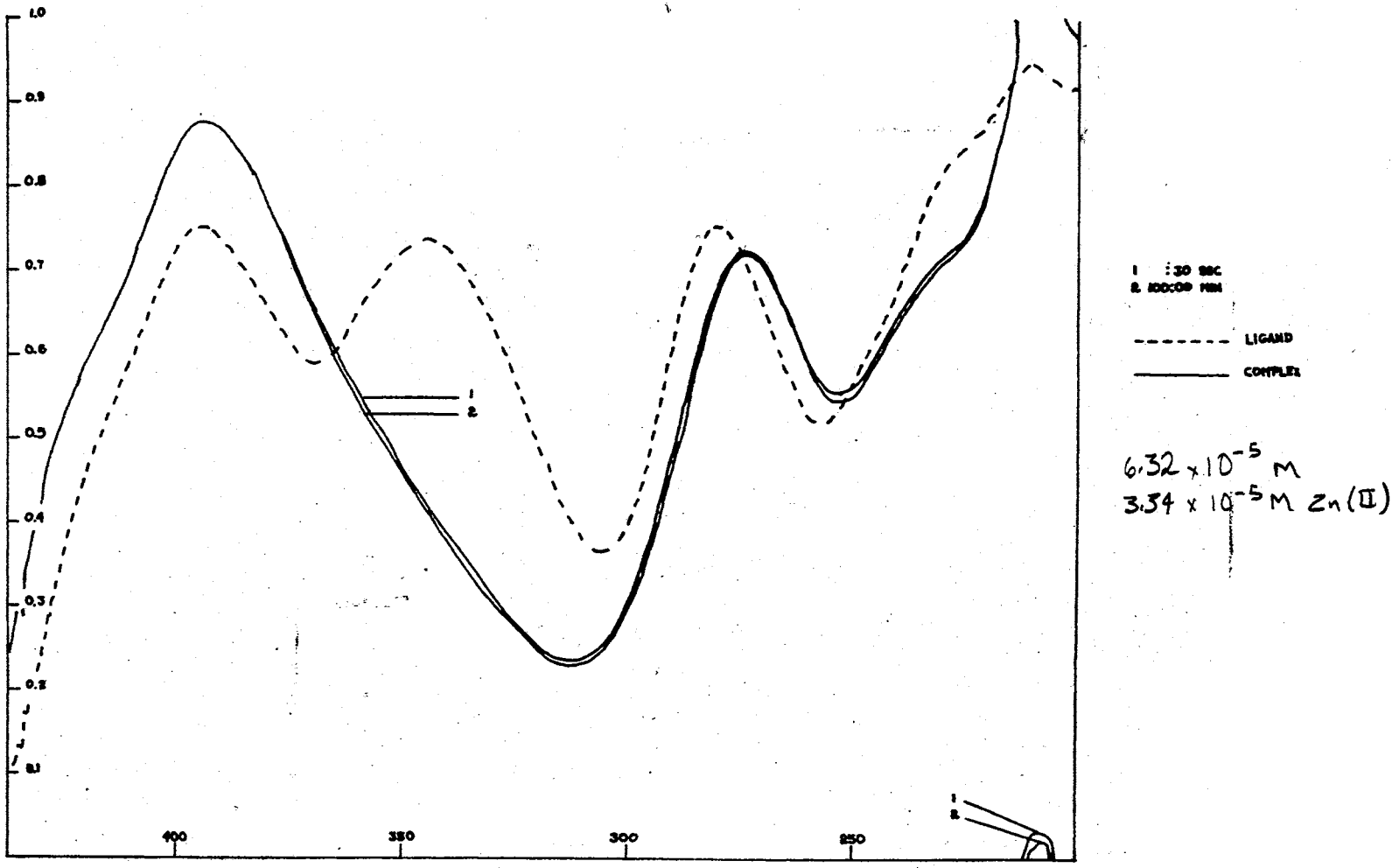
The strong maximum at 393 nm along with the nearly complete loss of absorption at 343 nm upon formation of the complex indicate that the molecule exists almost completely in the quinonoid form. The lack of change upon complexation at 274 nm indicates that the ring nitrogen is not involved in the complex. The slight hypochromic effects at 208 and 274 nm are reminiscent of the (Z)-3-HP-Cu(II) complex. These concentrations probably border on the solubility limits of the complex and slight precipitation or coagulation has occurred.

Plate XLI



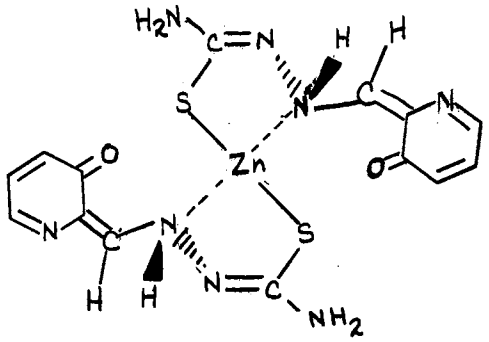
Ultraviolet Spectrum of the Zn(II) Complex of (Z)-3-HP in pH 7.0 Buffer

Plate XLII



Ultraviolet Spectrum of the Zn(II) Complex of (Z)-3-HP in pH 7.0 Buffer

Since it appears that the ligand is in the keto form, the E and Z isomers both form the same complex (27). The spectra of the Zn(II) complexes are shown in Plates XLI and XLII.



27

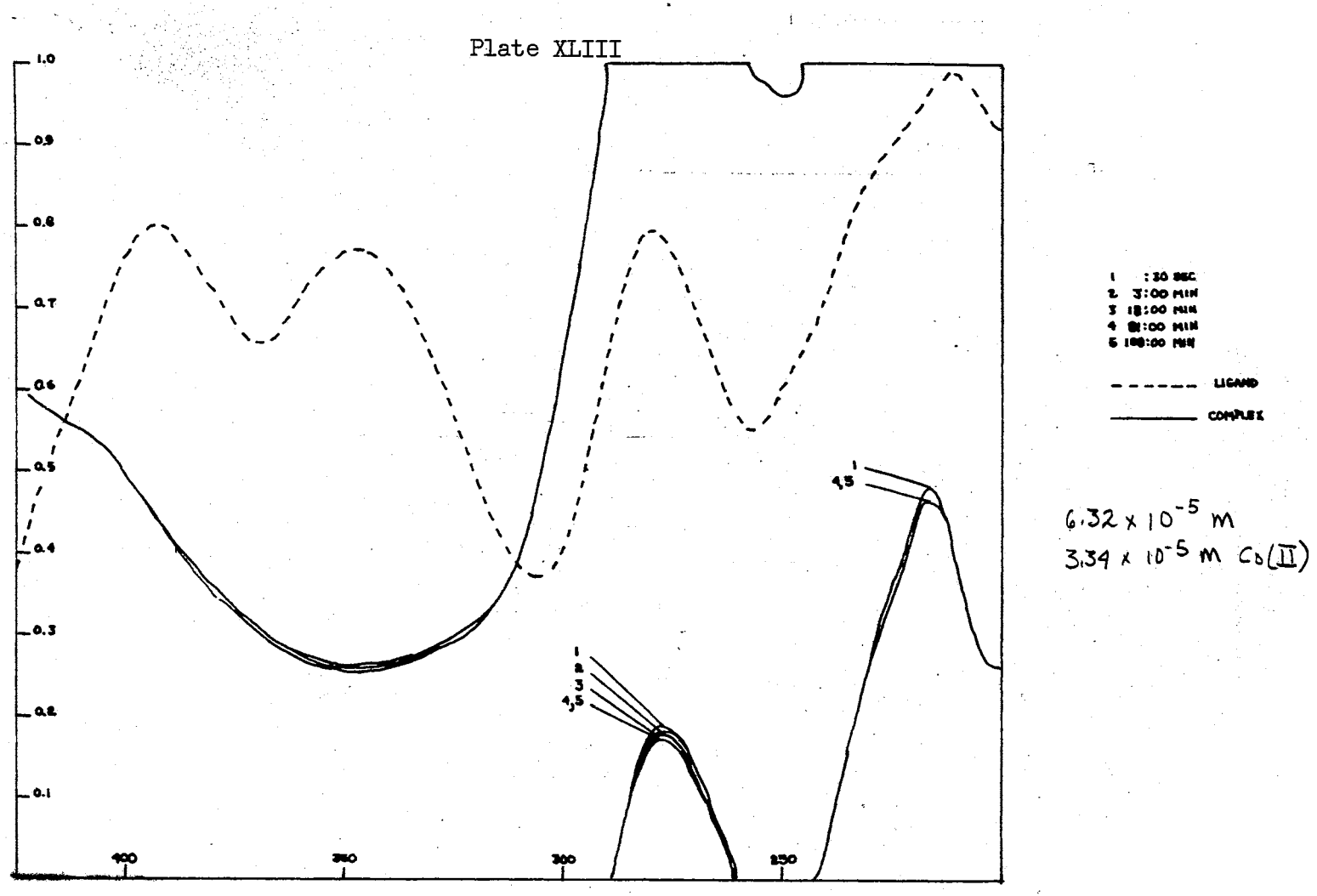
(E)- and (Z)-3-HP-Co(II) Complexes

The spectra of the E and Z complexes are superimposable. They exhibit maxima at 217 nm and 278 nm, neither of which change significantly in 80-100 minutes. No effective rate constant for approach to equilibrium was calculable.

The lack of absorbance in the 360-370 nm range indicates that the sulfur is not involved in the complex; loss of absorbance at 400-410 nm when the complex is formed indicates that the quinonoid form is not a major contributor to the complex. The increase at 217 nm characteristic of an increase in the abundance of OH also indicates loss of the quinonoid structure when the complex is formed.

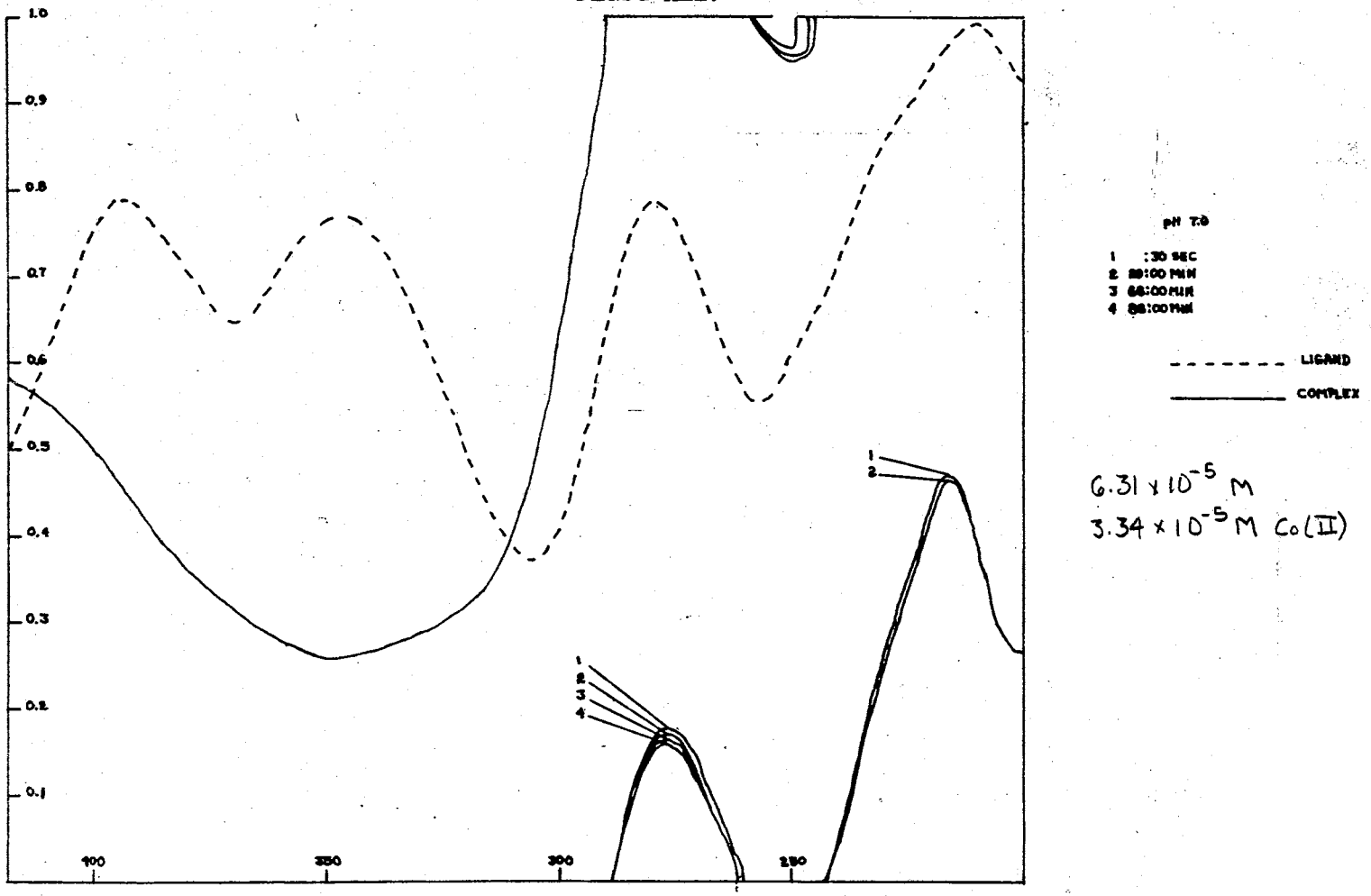
The superimposability of the spectra, the absence of change, and the absence of the quinoid tautomer all indicate that both the E and Z form the same complex via the quinonoid tautomer, i.e. the tautomeric forms of both (E)- and (Z)-3-HP are identical to each other and

Plate XLIII



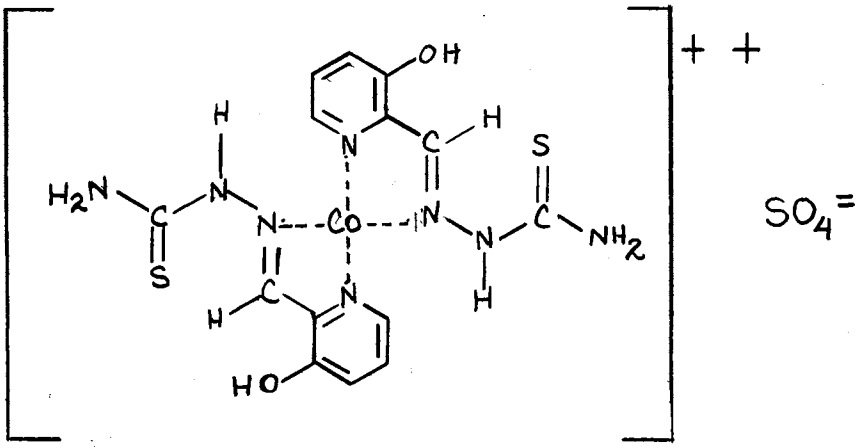
Ultraviolet Spectrum of the Co(II) Complex of (E)-3-HP in pH 7.0 Buffer

Plate XLIV



Ultraviolet Spectrum of the Co(II) Complex of (Z)-3-HP in pH 7.0 Buffer

consequently will rapidly form the same complex. This complex must be either a bidentate E complex or a tridentate Z complex. Since the spectra indicate that the sulfur does not participate in complexation, and since the structure would be favored for steric reasons the bidentate E complex (28) is suggested as the predominant form of the complex. Spectra of the Co(II) complexes of the isomers are shown in Plates XLIII and XLIV.

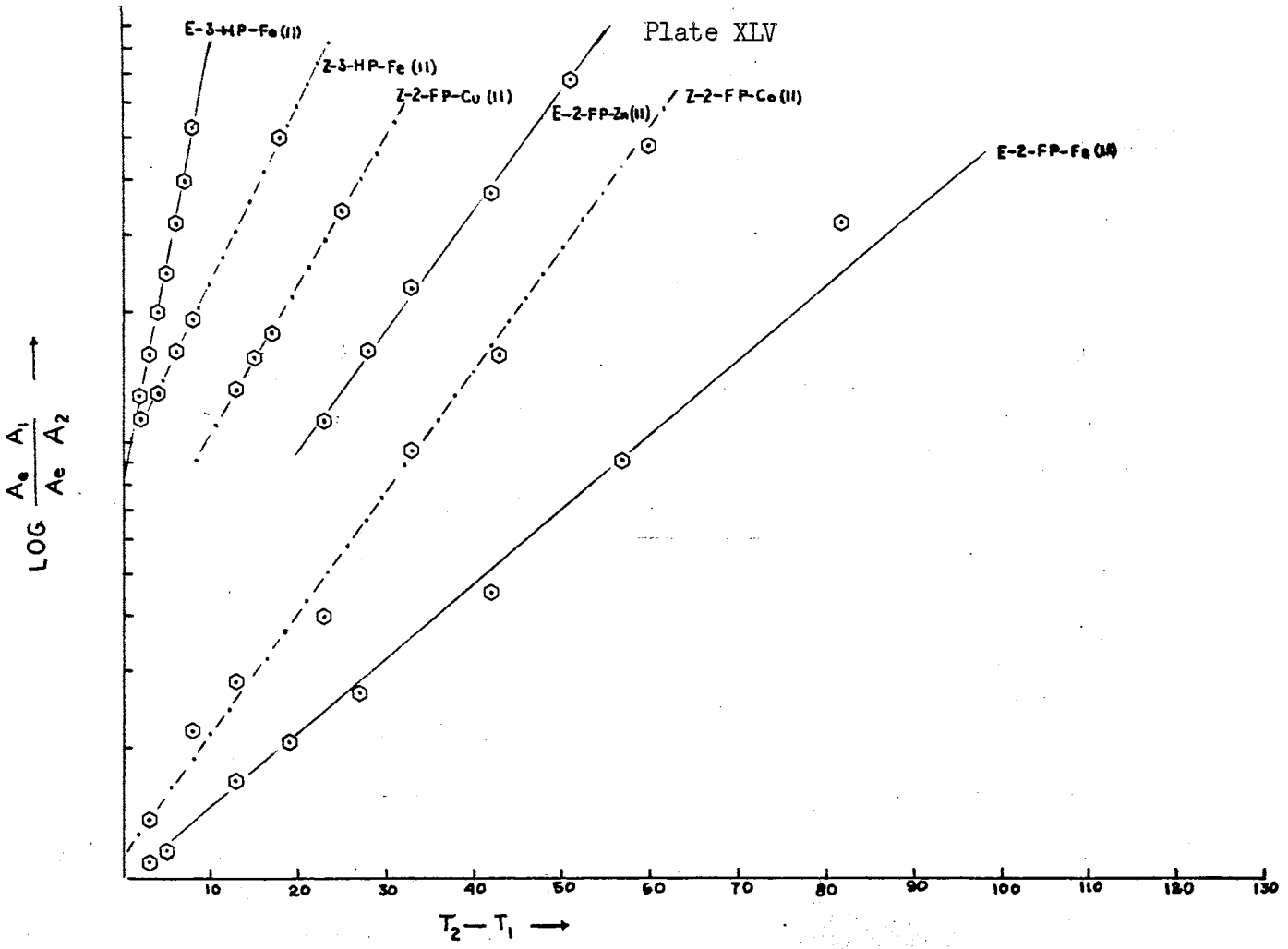


28

SUMMARY

Table III summarizes the changes of the various metal complexes in 0.005 M pH 7.0 phosphate buffer; where possible the effective rate constants of the approach to equilibrium are reported. The rate plots for the approach to equilibrium by the complexes are shown in Plate XLV.

It thus appears that 2-FP forms a stable E complex with each metal except Fe(II). Since 2-FP behaves as a tridentate ligand with Fe(II) it is necessary that the ligand be in the Z form to be tridentate.



Rate Plots for the Approach to Equilibrium by the Metal Complexes of 3-HP and 2-FP

TABLE III

REACTIONS OF THE E AND Z ISOMERS OF 2-FP AND 3-HP WITH METALS IN pH 7.0, 0.005 M PHOSPHATE BUFFER

Metal	k_{eff} for (E)-2-FP, min^{-1}	k_{eff} for (Z)-2-FP, min^{-1}	k_{eff} for (E)-3-HP, min^{-1}	k_{eff} for (Z)-3-HP, min^{-1}
Fe^{++}	3.98×10^{-2}	No apparent isomerization	2.11×10^{-1}	9.49×10^{-2}
Cu^{++}	Too slow to measure	7.97×10^{-2}	Too slow to measure	Precipitated
Zn^{++}	Too slow to measure	6.36×10^{-2}	No isomerization	No isomerization
Co^{++}	Too slow to measure	6.42×10^{-2}	No isomerization	Isomerizes rapidly
Ca^{++}	No complex No effect	No complex No effect	No complex No effect	No complex No effect
Mg^{++}	No complex No effect	No complex No effect	No complex No effect	No complex No effect
Mn^{++}	No complex No effect	No complex No effect	No complex No effect	No complex No effect

The effective rate constants of the (Z)-Cu(II), Zn(II), and Co(II) complexes suggest two major points:

(a) The fact that the rate constants are indeed "constant" i.e. the same magnitude for all the complexes of 2-FP, suggests that the same mechanism is possibly responsible for all the isomerizations of this particular ligand;

(b) using the relationship $.693 = kt_{\frac{1}{2}}$ and using 6×10^{-2} as a representative effective rate constant, the half-life of the approach to equilibrium by the Z complexes is about 11-12 minutes which, within experimental error, is the same as the 12-13 minute half-life of the

uncomplexed Z ligand. It appears therefore that complexation does not accelerate the isomerization of (Z)-2-FP. (As mentioned before, however, the isomerization of the E isomer to the Z isomer is accelerated by Fe(II).) This means that metal ions will not interfere with the stereochemistry of the isomeric forms of 2-FP if they are administered in physiological tests.

No generalization can be made concerning complexes of 3-HP isomers. Each complex must be considered individually and it is possible that certain metal ions will alter the configuration of an isomer of 3-HP immediately after administering the drug. But these ions (Zn^{++} and Co^{++}) are in low concentrations in serum so administration of high levels of an isomer should preclude rapid equilibration of all the isomer.

It therefore should be feasible to achieve a difference of therapeutic effectiveness by administering the individual isomers of 2-FP and 3-HP, assuming that the "active site" at which the drug is effective reacts with one isomer preferentially over the other, and the next logical step is testing of the isomers individually, to see if there is a therapeutic difference.

A SELECTED BIBLIOGRAPHY

1. Brockman, R. W., Thomson, J. R., Bell, M. J., and Skipper, H. P., Cancer Res., 16, 167 (1956).

2. French, F. A., and Freedlander, B. L., ibid., 18, 129.0 (1958).

3. French, F. A., and Blanz, E. J., Jr., J. Med. Chem., 9 (4), 585 (1966).

4. French, F. A., and Blanz, E. J., Jr., Cancer Res., 25, 1454 (1965).

5. Agrawal, K. C., Booth, B. A., and Sartorelli, A. C., J. Med. Chem., 11, 700 (1968).

6. Agrawal, K. C., Cushley, Robert J., McMurray, Walter J., and Sartorelli, A. C., J. Med. Chem., 13 (3), 431 (1970).

7. French, F. A., et al., J. Med. Chem., 13 (6), 1117 (1970).

8. Blanz, E. J., Jr., and French, F. A., Cancer Res., 28, 2419 (1968).

9. Blanz, E. J., Jr., J. Med. Chem., 13 (6), 1124 (1970).

10. Petering, H. G., Buskirk, H. H., Crim, J. A., and Van Giessen, G. J., Pharmacologist, 5, 271 (1963).

11. Petering, H. G., Buskirk, H. H., and Kupiecki, F. P., Fed. Proc., 24, 454 (1965).

12. Cappucino, J. G., Proc. Am. Assoc. Cancer Res., 41 (1966).

13. Gingras, B. A., Colin, G., and Bayley, C. H., J. Pharm. Sci., 54 (11), 1674 (1965).

14. Sartorelli, A. C., Biochem. Biophys, Res. Comm., 27 (1), 26 (1967).

15. Moore, E. C., and Reichard, P., J. Biol. Chem., 239 (10), 3453 (1964).

16. Sartorelli, A. C., and Booth, B. A., Proc. Am. Assoc. Cancer Res., 9, 61 (1968).

17. Sartorelli, A. C., Zedeck, M.S., Agrawal, K. C., and Moore, E. C., Federation Proc., 27, 650 (1968).

18. Brockman, R. W., Sidwell, R. W., Arnett, G., Shaddix, S., P. Soc. Exp. M., 133, 609 (1970).

19. C. Sandorfy, General and Theoretical Aspects, The Chemistry of the Carbon-Nitrogen Double Bond, ed. Saul Patai, John Wiley and Sons Ltd., New York, 1970, p. 8.

20. Knoevenagel, E., J. Prakt. Chem., 89, 38 (1914).

21. Wettermark, G., Svensk Kemisk Tidsk., 79 (4), 249 (1967).

22. C.G. McCarty, Syn - Anti Isomerizations and Rearrangements, The Chemistry of the Carbon-Nitrogen Double Bond, ed. Saul Patai, John Wiley and Sons Ltd., New York, 1970, p. 363.

23. Bell, S. C., Conklin, G. L., and Childress, S. J., J. Am. Chem. Soc., 85, 2868 (1963).

24. Raffauf, R. F., J. Am. Chem. Soc., 68, 1765 (1946).

25. Poziomek, E. J., Kramer, D. N., Mosher, W. A., Michel, H. O., J. Am. Chem. Soc., 83, 3916 (1961).

26. Poziomek, E. J., J. Pharm. Sci., 54, 333 (1965).

27. Poziomek, E. J., and Vaughan, L. C., ibid., 54, 811 (1965).

28. Schnekenburger, Joerg, Arch. Pharm., 302 (7), 494-504 (1969); Chem. Abstr., 71, 91225g (1969).

29. Barz, P.; Fritz, Heinz P., Z. Naturforsch, 25 (2), 199-204 (1970), C. A. 72 (18), 96148q (1970).

30. Fischer, E., and Frei, Y., J. Chem. Phys., 27, 808 (1957).

31. Wettermark, G., and Dogliotti, Libera, J. Chem. Phys., 40 (6), 1486 (1964).

32. Anderson, D. G., and Wettermark, G., J. Am. Chem. Soc., 87 (7), 1433 (1965).

33. Curtin, D. Y., Grubbs, E. J., and McCarty, C. G., ibid., 88, 2775 (1966).

34. Kessler, H., Tetrahedron Lett., 17, 1423 (1970).

35. Karabatsos, G. J., Graham, J. D., and Vane, F. M., J. Am. Chem. Soc., 84, 753 (1962).

36. Karabatsos, G. J., et al., ibid., 85, 2784 (1963).

37. Karabatsos, G. J., Taller, R. A., Vane, F. M., ibid, 85, 2326 (1963).

38. Karabatsos, G. J., Taller, R. A., ibid., 85, 3624 (1963).

39. Byrne, J., and O'Sullivan, J. F., Proc. Roy. Irish Acad., Sect. B, 69 (8), 179-83 (1970).

40. Keinert, D., and Martell, D. E., Tetrahedron, 3, 49 (1958).

41. Jensen., K. A., et al, Acta. Chem. Scand., 22 (1), 33 (1968).

42. Conley, Robert T, Infrared Spectroscopy, p. 135, Allyn and Bacon Inc., Boston, 1966.

43. Sweeny, et al., J. Amer. Chem. Soc., 77, 6521 (1955).

44. Phillips, John P., Spectra - Structure Correlation, p. 117, Academic Press, New York, 1964.

45. Bogornolov, S. G., and Pastovskii, I. Ya., Daklady Akad. Nauk. S. S. S. R., 91, 1111 (1953); Chem. Abstr., 48, 450g (1954).

46. Gillam, A. E., and Stern, E. S., An Introduction to Electronic Absorption Spectroscopy in Organic Chemistry, 2nd ed., Edward Arnold, London, 1957, p. 118.

47. Jaffe', H. H. and Orchin, Milton, Theory and Application of Ultraviolet Spectroscopy, John Wiley and Sons Inc., New York, 1962, p. 363.

48. Silverstein, Robert M., and Bassler, G. Clayton, Spectrometric Identification of Organic Compounds, John Wiley and Sons, New York, 1967, p. 166.

49. Gillam, A. E., and Stern, E. S., p. 141.

50. Gillam A. E., and Stern, E. S., p. 157.

51. Mooney, P. D., M. S. Thesis, Oklahoma State University, 1969, p. 42.

52. Rao, C. N. R., Ultraviolet and Visible Spectroscopy, Butterworth Inc., Washington D. C., 1961, p. 55.

53. Jaffe', H. H., and Orchin, Milton, p. 377.

54. Gillam, A. E., and Stern, E. S., p. 156.

55. Becker, R. S. and Richey, W. F., J. Am. Chem. Soc., 89 (6), 1298 (1967).

56. Ledbetter, John W. Jr., J. Phys. Chem., 70, 2245 (1966).

57. Ibid., 71, 2351 (1967).

58. Ibid., 72, 4111 (1968).

59. Jones, Mark M., Elementary Coordination Chemistry, Englewood Cliffs, N. J., Prentice Hall Inc., 1964, p. 40.

60. Ablov, A. V., Belichuk, N. I., Zh. Neorg. Khim., 14 (1), 179 (1969); via C. A. 70, 83798 K (1969).

61. J. Org. Chem., 35 (9), 2849 (1970).

62. Supniewski, J., Bany, T., and Krupinska, J., Bull. Acad. Polon. Sci., Classe II, 3, 55 (1955); C. A. 50, 7800 (1956).

63. Frost, H. H., and Pearson, R. G., Kinetics and Mechanism, John Wiley and Sons, Inc., New York, 1953, p. 172.

RATE EXPRESSION DERIVATION FOR THE REACTION $X \rightleftharpoons Y$

For the reversible first-order reaction $X \underset{k_2}{\overset{k_1}{\rightleftharpoons}} Y$ the rate of formation of Y is $\frac{d[Y]}{dt} = k_1 [X] - k_2 [Y]$. (Eq. 1) If at initiation of the reaction ($t = 0$), $[X] = [X]_o$ and $[Y] = [Y]_o$ then at any time t, $[X] = [X]_o + [Y]_o - [Y]$ where $[Y]$ is the concentration of Y at time = t. Equation 1 may then be rewritten:

$$\frac{d[Y]}{dt} = k_1([X]_o + [Y]_o - [Y]) - k_2[Y]$$

$$= k_1([X]_o = [Y]_o) - (k_1 + k_2)[Y] \quad \text{(Eq. 2)}$$

At equilibrium $\frac{d[Y]}{dt} = 0$.

$$\frac{d[Y]}{dt} = 0 + k_1([X]_o + [Y]_o) - [Y]_e(k_1 + k_2);$$

and $([X]_o + [Y]_o) = \frac{(k_1 + k_2)}{k_1}[Y]_e$. (Eq. 3)

Substitution for $([X]_o + [Y]_o)$ in equation 2 yields:

$$\frac{d[Y]}{dt} = (k_1 + k_2)[Y]_e - (k_1 + k_2)[Y]$$

which may then be integrated,

$$\int_{Y_1}^{Y_{1+n}} \frac{d[Y]}{([Y]_e - [Y])} = \int_{t_1}^{t_{1+n}} (k_1 + k_2)\, dt$$

and after integration becomes:

$$-\ln ([Y]_e - [Y]) \Big]_{[Y]_1}^{[Y]_{1+n}} = (k_1 + k_2)t \Big]_{t_1}^{t_{1+n}} \quad n = 1,2,3,\ldots$$

$$-\ln ([Y]_e - [Y]_{1+n}) + \ln ([Y]_e - [Y]_1) = (k_1 + k_2)\ (t_{1+n} - t_1)$$

$$\ln \frac{([Y]_e - [Y]_1)}{([Y]_e - [Y]_{1+n}} = (k_1 + k_2)\ (t_{1+n} - t_1)$$

APPENDIX B

EVALUATION OF RATE EQUATION

The reactions in this thesis which are followed by ultraviolet spectroscopy are assumed to be the isomerization of either the E isomer to the Z isomer or the Z isomer to the E isomer. It is assumed that there are no other competing reactions and thus, at any given time t, the only absorbing species are the E and the Z isomers which are interconverting as a reversible first-order reaction $X \underset{k_2}{\overset{k_1}{\rightleftharpoons}} Y$. Any alteration in the spectrum of a particular isomer as that isomer is observed for a period of time is assumed to be due to the gain or loss of that particular isomer. Using these assumptions, it is possible to evaluate the term $\frac{[Y]_e - [Y]_1}{[Y]_e - [Y]_{1+n}}$ $(n= 1,2,3,...)$ by showing that the change in absorbance of an isomer at a particular wavelength is equal to the change in concentration of that isomer. Since the absorbances of the E and Z isomers are only separated by a few nanometers and consequently most maxima are due to absorbance by both E and Z isomers, it is necessary to prove also that the change in absorbance is due only to the change in concentration of the isomer of interest and any absorbance by the other isomer may be disregarded. It is thus necessary to prove that $\frac{[Y]_e - [Y]_1}{[Y]_e - [Y]_{1+n}} = \frac{A_e - A_1}{A_e - A_{1+n}}$ $(n = 1,2,3,...)$ where A_1, A_{1+n}, and A_e are absorbances at time = t_1, t_{1+n}, and at equilibrium. The proof is as follows for n = 1 and may be shown to be the same for all values of n:

$$A_1 = \epsilon_x [X]_1 + \epsilon_y [Y]_1$$

$$A_2 = \epsilon_x [X]_2 + \epsilon_y [Y]_2$$

$$A_e = \epsilon x [X]_e + \epsilon_y [Y]_e$$

where $[X]_1$, $[X]_2$, $[X]_e$, $[Y]_1$, $[Y]_2$, and $[Y]_e$ are concentrations of X and Y at times t_1, t_2, and at equilibrium.

$$\frac{A_e - A_1}{A_e - A_2} = \frac{\epsilon_x [X]_e + \epsilon_y [Y]_e - \epsilon_x [X]_1 + \epsilon_y [Y]_1}{\epsilon_x [X]_e + \epsilon_y [Y]_e - \epsilon_x [X]_2 + \epsilon_y [Y]_2}$$

$$= \frac{\epsilon_x ([X]_e - [X]_1) + \epsilon_y ([Y]_e - [Y]_1)}{\epsilon x ([X]_e - [X]_2) + \epsilon_y ([Y]_e - [Y]_2)} \quad \text{(Eq. 1)}$$

Assuming that X reacts to give Y only, then the term $[X]_1 - [X]_e$ is a measure of the quantity of X which reacts to give Y over the time $t_e - t_1$. It therefore is also a measure of the quantity of Y formed over the same time, i.e. $[X]_1 - [X]_e = [Y]_e - [Y]_1$. Therefore, $[Y]_1 - [Y]_e = [X]_e - [X]_1$. (Eq.2) Substitution of $[Y]_1 - [Y]_e$ and $[Y]_2 - [Y]_e$ for the terms $[X]_e - [X]_1$ and $[X]_e - [X]_2$ in equation 1 gives:

$$A_e - A_1 = \frac{\epsilon_x ([Y]_1 - [Y]_e) + \epsilon_y ([Y]_e - [Y]_1)}{\epsilon_x ([Y]_2 - [Y]_e) + \epsilon_y ([Y]_e - [Y]_2)} \quad \text{(Eq. 3)}$$

Expansion of equation 3 gives:

$$\frac{A_e - A_1}{A_e - A_2} = \frac{\epsilon_x [Y]_1 - \epsilon_x [Y]_e + \epsilon_y [Y]_e - \epsilon_y [Y]_1}{\epsilon_x [Y]_2 - \epsilon_x [Y]_e + \epsilon_y [Y]_e - \epsilon_y [Y]_2}$$

$$= \frac{(\epsilon_y - \epsilon_x) [Y]_e - (\epsilon_y - \epsilon_x) [Y]_1}{(\epsilon_y - \epsilon_x) [Y]_e - (\epsilon_y - \epsilon_x) [Y]_2}$$

$$= \frac{[Y]_e - [Y]_1}{[Y]_e - [Y]_2}$$

VITA[3]

Paul David Mooney

Candidate for the Degree of

Doctor of Philosophy

Thesis: A STUDY OF GEOMETRIC ISOMERISM IN THE THIOSEMICARBAZONES OF SOME PYRIDINECARBOXALDEHYDES

Major Field: Chemistry

Biographical:

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