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Synthesis of New Reagents for the Detection of Fingerprints and Amino Acids

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Synthesis of New Reagents for the Detection of Fingerprints and Amino Acids

Björn Borup

Advisor: Dr. Forrest J. Frank

Chemistry 499

Illinois Wesleyan University

May 13, 1992

To:

my sister

Birthe Borup,

and two friends

Kelly Jean Luckey,

Kürstan Lyn Hurd.

Approval Page

Synthesis of New Reagents for the Detection of Fingerprints and Amino Acids

by Björn Borup

A PAPER SUBMITTED IN PARTIAL FULFILLMENT OF THE
REQUIREMENTS FOR CHEMISTRY 499 AND HONORS IN CHEMISTRY

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1992

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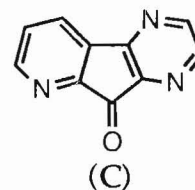
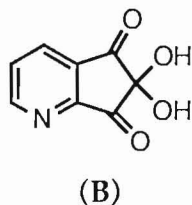
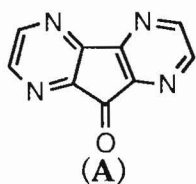
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Abstract

DFO (1,8-Diazafluoren-9-one) is a new reagent for the detection of latent fingerprints. It reacts with amino acids present in fingerprints to give a fluorescent product, and is an improvement over ninhydrin which has been used in forensic laboratories for years. The object of this work was to synthesize new analogs of ninhydrin and DFO. The preparation of 9H-cyclopenta[1,2-b:3,4-b']dipyrazine-9-one (**A**) was attempted but was not successful. Currently the synthesis of 7H-cyclopenta[b]pyridine-5,6,7-trione (**B**) is being completed. The compound 9H-cyclopenta[1,2-b]pyrazine[3,4-b]pyridine-9-one (**C**) will then be made from (**B**), an analog of ninhydrin.



Introduction

Amino acids are a fundamental chemical unit of life. Two areas in which they are routinely analyzed are biochemistry and forensic science. For analysis, proteins are hydrolyzed to amino acids and the identity and amount of each amino acid is determined. In forensic science latent fingerprints are often made visible by a reaction with the amino acids present in sweat and oils on the skin.

It has been discovered that fingerprints are an apparently infallible means of human identification, since no two fingers have ever been found that have the same print. Also individuals' prints do not change during their lifetimes.¹ Fingerprint classification systems have been developed which make the identification of an individual easy, especially now with the advent of computers that can match partial prints.¹

Thus fingerprints taken at a crime scene are an important piece of evidence that can lead to the uncovering of a criminal's identity. Although fingerprints are usually invisible, they can be made visible by a variety of methods. One of the most important methods is by spraying or dipping the piece of evidence having the fingerprint into a solution of some compound which reacts with the fingerprint, making it visible. The trace amounts of amino acids in the fingerprint react with some compound to form a colored complex. The most widely used compound today is 3H-indan-1,2,3-trione (ninhydrin, NIN). A more recently discovered compound called 9H-cyclopenta[1,2-b:4,3-b']dipyridine-9-one (1,8-diazafluoren-9-one, DFO) has now been introduced as a more sensitive alternative, but it is still too early to tell if the crime labs of the police departments will accept it.

Background

The red colored compounds that form when ninhydrin and DFO react with amino acids in fingerprints is often used to make fingerprints visible. The difference in absorbance of light between the surface and the fingerprint can be used to take a photograph, which can then be used, for example in a court of law. For weak fingerprints this method does not work quite as well, since the difference in absorbance is very small. The small amount of red color produced by the reaction cannot be detected very easily, sometimes making it impossible to detect fingerprints.

However a different technique can be used for the detection of fingerprints using DFO, taking advantage of the fact that DFO forms a luminescent product with amino acids. Small amounts of emitted light can be very easily detected. This makes luminescence an ideal technique for the detection of fingerprints. DFO forms a luminescent product when it reacts with amino acids, whereas the product from ninhydrin and amino acids is not luminescent. Thus, DFO is more sensitive than ninhydrin.

Ninhydrin is also used in biochemistry in the study of proteins. Ninhydrin reacts reproducibly with amino acids and thus is used for the quantitative determination of proteins in amino acid analyzers.² It has not yet been established that DFO reacts reproducibly. To do so is important, since any new reagent will have limited usefulness for biochemists unless it is shown to react reproducibly.

There is a further problem associated with the use of both DFO and ninhydrin as fingerprinting reagents on paper. Ninhydrin has limitations because it does not form a luminescent product. Although the reaction product of DFO and amino acids luminesces, the light is yellow which is the same color at which brighteners in paper luminesce³. This creates a large

amount of interference, sometimes preventing the detection of the fingerprint. Research is therefore being conducted to find new reagents that react with amino acids.^{4,5}

Since luminescence is almost a prerequisite for the detection of weak fingerprints, the luminescence properties of new compounds are being studied⁶. This is being done in order to find a reagent that has a luminescent reaction product with amino acids. The reagent should not luminesce on its own, since that would interfere with the detection of the fingerprints.

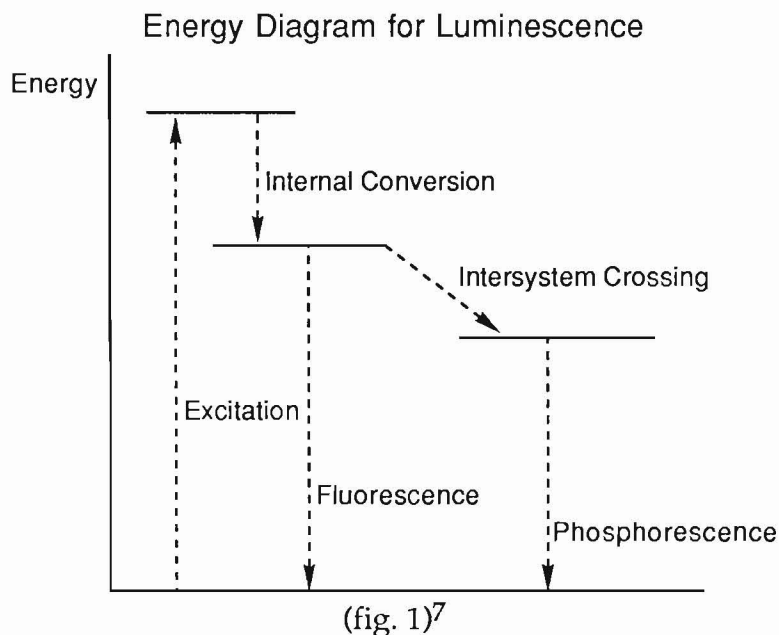
Luminescence

When light of a certain wavelength hits a molecule it will go into an excited state (the singlet state). Usually when the electron returns to the ground state light is given off, a process called luminescence, which includes both fluorescence and phosphorescence.

A molecule that is in the singlet state will first deexcite vibrationally to a lower vibrational level (see fig.1), a process called internal conversion. From this lower vibrational level the molecule can deexcite to the ground state in three ways:

- It can come back down to the ground state and give off a photon. This process is called fluorescence and takes a few nanoseconds (see fig. 1).
- It can undergo intersystem crossing, the deexcitation to another electronic state (the triplet state) and then come back to the original ground state giving off a photon. This process is called phosphorescence and it takes a few microseconds. The reason for this delay time between the excitation and the emission is because the triplet to ground state transition is forbidden. The transition does take place, since the transition is not entirely forbidden (see fig. 1).

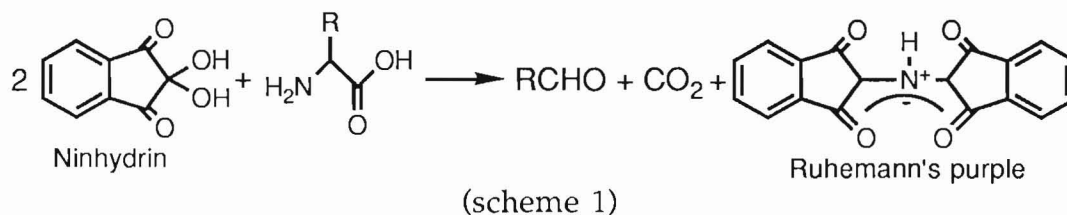
- It can undergo further internal conversion, also called nonradiative decay, to the ground state. In this case the molecule deexcites without giving off a photon (not shown).



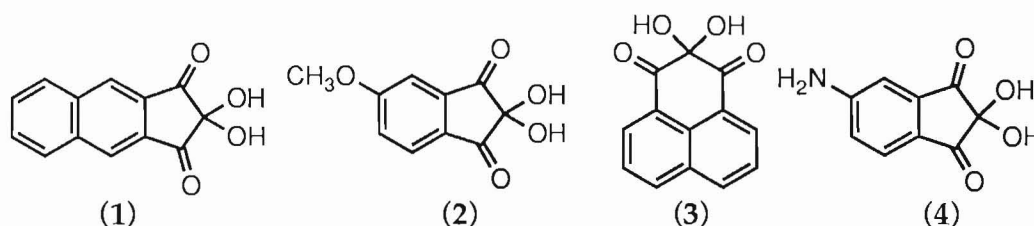
Certain characteristics are associated with the light emitted by molecules. First, the light emitted is of lower energy. Since the molecule first deexcites to lower vibrational levels the emission wavelength is longer (and thus of lower energy) than the excitation wavelength. Second, the difference between the excitation wavelength and the emission wavelength is always constant for the same molecule under the same conditions. Third, the excitation wavelength which results in the largest luminescence is different for each molecule. Finally, different groups on the molecule can shift the emission wavelength to higher or lower values. For example, more conjugation or conjugated groups will shift the excitation wavelength toward longer wavelengths.

Ninhydrin and its Analogs

Ninhydrin makes fingerprints visible by reacting with amino acids (scheme 1) present in the sweat and oils left behind by the fingers. It forms a purple complex called Ruhemann's purple which was discovered 1910⁸. Unfortunately the complex does not fluoresce.



It is known that other triketones also give colored reaction products with amino acids. In 1982 Joseph Almog et al.⁹ synthesized the first ninhydrin analogs and tested them for their ability to detect fingerprints. The one that worked best was compound (1). Later, other substituted ninhydrins were synthesized. An example is 3H-5-methoxyindan-1,2,3-trione (5-methoxyninhydrin, 2).¹⁰ Also triones that do not include a five membered ring react with amino acids. An example is (perinaphthoninhydrin, 3).¹⁰ A newer addition to the range of ninhydrin analogs is 3H-5-aminoindan-1,2,3-trione (5-aminoninhydrin, 4).



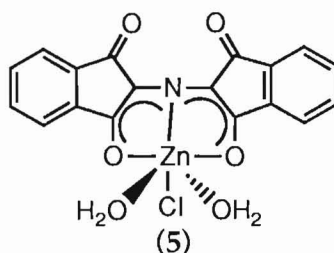
These compounds are referred to as triones. Since under most circumstances the central carbonyl is hydrated it is drawn as such.

The reaction of ninhydrin (scheme 1) and its analogs with amino acids at room temperature on paper is quite slow and takes a day or more

depending on what analog is reacted. For instance for full development of a fingerprint it takes ninhydrin 1 day, compound (2) 3 to 4 days, and compound (4) about 7 days.¹¹

Ninhydrin and Luminescence

Since luminescence is almost a prerequisite for the detection of weak fingerprints, researchers have tried for years to make Ruhemann's purple luminesce by reacting it with some kind of reagent. In the early eighties Herod and Menzel¹² discovered that ninhydrin luminesces when treated with zinc(II) chloride or cadmium(II) chloride, resulting in the planar complex (5).¹³



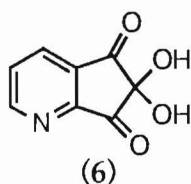
The luminescence has been explained. In organometallic complexes nonbonding electrons of the heteroatom, in the case of molecule (5) the nitrogen, associate with the metal ion. This association stabilizes the nonbonding electrons and thus lowers them in energy.¹⁴ The lowest energy transition often changes from $n \rightarrow \pi$ to $\pi \rightarrow \pi^*$.¹⁴ Internal conversion to the ground state or intersystem is rarely observed for $\pi^* \rightarrow \pi$ transitions, and thus luminescence occurs.

Also, the metal halide prevents the two ninhydrin molecules from twisting away from the planar form giving it the ability to luminesce. This maximizes the complex's π -orbital overlap maximizing its ability to luminesce. If the two ninhydrin molecules were to twist out of the planar

form, as they do in Ruhemann's purple with an angle of about 20° ,¹⁵ the molecule can undergo internal conversion preventing luminescence.¹⁶ Ninhydrin luminesces when it is treated with zinc(II) chloride or cadmium(II) chloride and cooled to 77 K with liquid nitrogen. The cadmium complex does not luminesce with as large an intensity as the zinc complex does.¹⁷

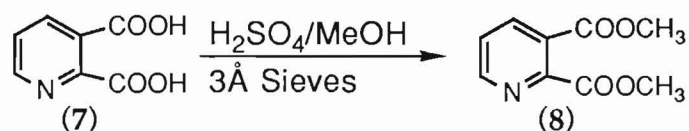
Before 1991, 3H-5-methoxyindan-1,2,3-trione (2) was the only ninhydrin analog that showed luminescence in the absence of zinc(II) chloride. The more recently discovered compound 3H-5-aminoindan-1,2,3-trione (4) also shows luminescence. The advantage of compound (4) is that it luminesces at room temperature, making it easier to develop fingerprints. Therefore liquid nitrogen does not have to be used as coolant.¹¹ Also at lower temperatures more things luminescence resulting in a larger background light from paper which makes detection more difficult.

The main thrust in the past for the synthesis of new reagents has been to change the groups that are attached to the ninhydrin molecule. The introduction of heteroatoms into the ring has so far not been accomplished, but is still being tried. Several methods have been unsuccessfully tried for the synthesis of 7H-cyclopenta[b]pyridine-5,6,7-trione (6).¹⁸



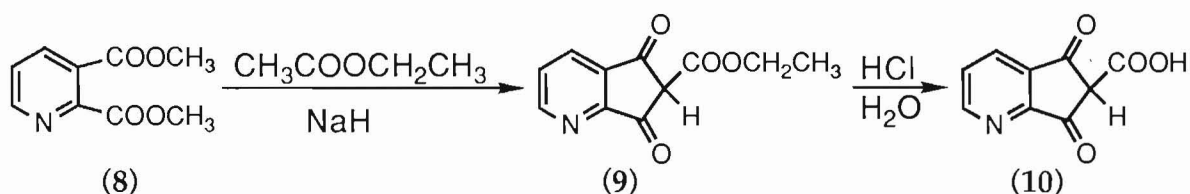
Synthesis of a Ninhydrin Analog

A ninhydrin analog that has a heteroatom included in its skeleton is 7H-cyclopenta[b]pyridine-5,6,7-trione (6). Previous attempts (schemes 2 - 6) to prepare the compound during the summer of 1990 did not succeed.¹⁸ Both attempts used dimethyl 2,3-pyridinedicarboxylate (8) as starting material, which can be easily made from 2,3-pyridinedicarboxylic acid (7) a readily available material (scheme 2).

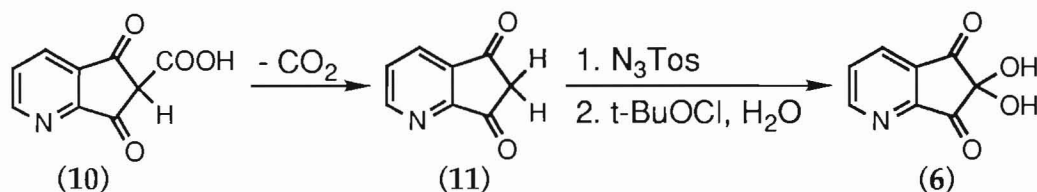


(scheme 2)

The first approach involves ring closure using ethyl ethanoate and sodium hydride and then oxidation of the α -carbon to a carbonyl in several steps (schemes 3 and 4).

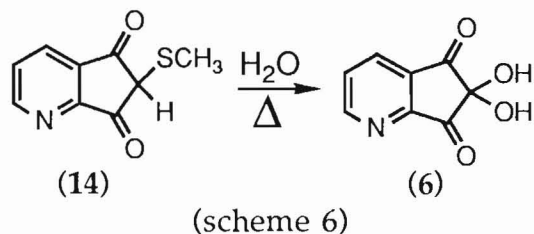
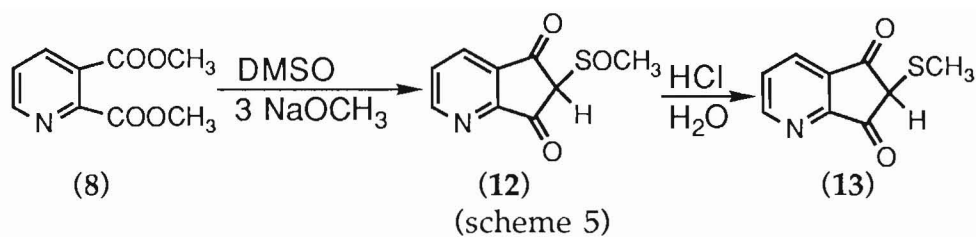


(scheme 3)



(scheme 4)

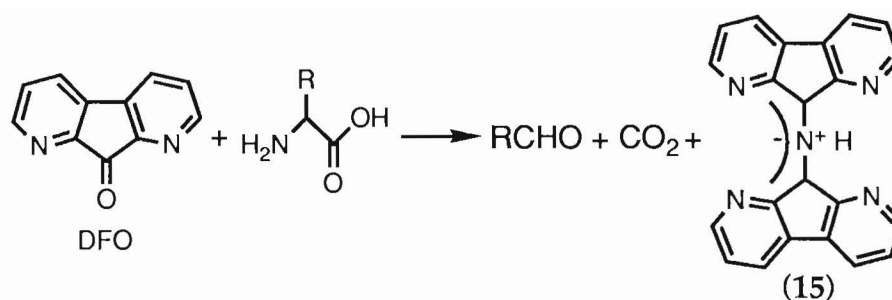
In the second approach the ring is closed using dimethyl sulfoxide and sodium methoxide. The α -carbon is then again oxidized to a carbonyl giving the desired triketone (6) after several steps (schemes 5 and 6).



Only recently has (6) been prepared elsewhere¹⁹ and since it did not show luminescence it was not pursued further. Unfortunately the fluorescence properties with zinc(II) chloride were not investigated, thus the compound should be prepared again and tested. Also, compound (6) is an intermediate in the preparation of other possible amino acid detection reagents. The proposed method of preparing the compound is in the section entitled "Proposed Method of Preparation of 7H-Cyclopenta[b]pyridine-5,6,7-trione (6)".

DFO and its Analogs

In 1990 a new compound was found to react with amino acids in fingerprints to form a colored compound that fluoresces (scheme 7).²⁰ It is not yet certain how DFO reacts with amino acids, but it seems as if it reacts with amino acids in the same way that ninhydrin does (scheme 1), giving a red product that is assumed to have a similar structure (15) to that of Ruhemann's purple. The structure was proposed from ¹HNMR and MS data.^{21,22}

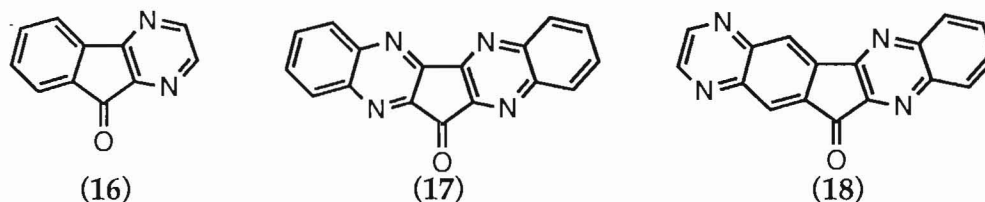


Since DFO reacts similarly to ninhydrin, a structural similarity can be inferred. Both ninhydrin and DFO have a central carbonyl that is flanked by two groups on either side that are electron withdrawing. In ninhydrin the carbonyl serves that function, whereas in DFO it is the nitrogen atoms included in the pyridine rings. It is thus probable that the nitrogens in the rings adjacent to the carbonyl perform the same function as the two carbonyls adjacent to the central carbonyl in ninhydrin and its analogs.

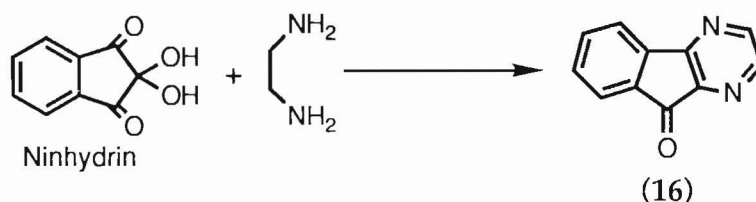
The red reaction product from DFO is not quite as intensely colored as Ruhemann's purple, but it luminesces at room temperature in the yellow region. As previously stated, brighteners in paper unfortunately also luminesce in the yellow region, making it difficult to detect fingerprints on paper with DFO.³ It would be better to find an analog to DFO that would keep its highly luminescent properties, but move the emission wavelength toward the red part of the spectrum.

In order to move the luminescence toward the red part of the spectrum, either more nitrogens have to be introduced into the ring, or additional benzene rings or substituents added onto the molecule. The intensity of fluorescence might also be changed by changing the position of the nitrogen in the molecule. A few analogs of DFO have been made so far, but have not yet been studied sufficiently. Compound (16)²³ only has the nitrogens in a different position. Compounds (17)²³ and (18)²¹ have more

nitrogens and additional benzene rings. Compound (17) is unfortunately very insoluble making it impractical to use as a fingerprint reagent, since solutions of the reagent are needed for the development of fingerprints.²³



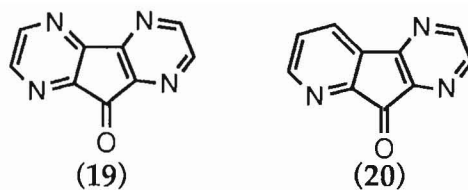
These compounds were made due to their ease of synthesis. Pyrazine rings can be made easily by a reaction of a 1,2-diamino substituted compound and a trione. This well studied reaction yields a molecule containing a pyrazine ring.^{24,25,26} Scheme 8 shows the reaction of ninhydrin with 1,2-diaminoethane to yield the compound (16),²⁷ an analog of DFO that has both nitrogens in the same ring. This reaction can also be useful for the synthesis of other compounds containing a pyrazine ring.



(scheme 8)

Since none of the reagents prepared and studied so far {e.g. (16), (17), and (18)} is useful under all circumstances, others are being synthesized and investigated. Research is therefore still being undertaken to find a reagent for weaker fingerprints on paper and other highly fluorescent surfaces.

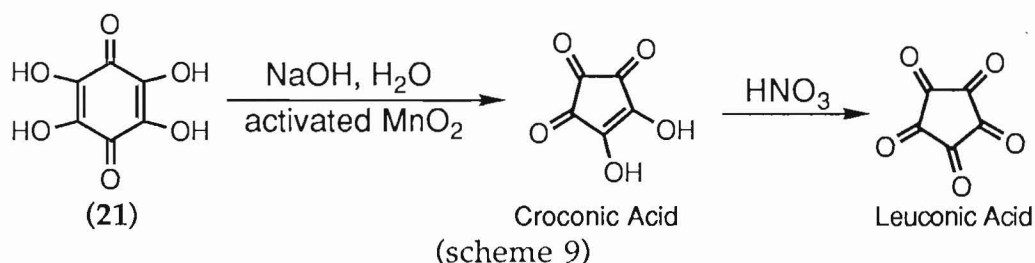
Two analogs of DFO that have been proposed are 9H-cyclopenta[1,2-b:3,4-b']dipyrzine-9-one (19), and 9H-cyclopenta[1,2-b]pyridine[3,4-b]pyrazine-9-one (20).



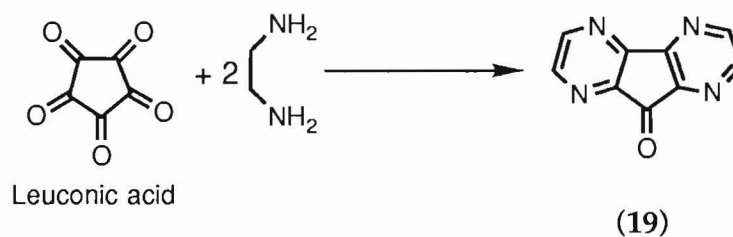
These compounds have been selected for three reasons. First, the additional nitrogens in the rings should shift the luminescence toward the red part of the spectrum. Second, these compounds should be reasonably easy to make. Finally, they should react with amino acids, since they have the nitrogens adjacent to the carbonyl.

Proposed Methods of Synthesis for 9H-Cyclopenta[1,2-b:3,4-b']dipyrazine-9-one (19)

For the synthesis of compound (19), it seems that two similar approaches can be used. Both methods use croconic acid as starting material, which can be easily prepared from tetrahydroxy-1,4-quinone (scheme 9).²⁸

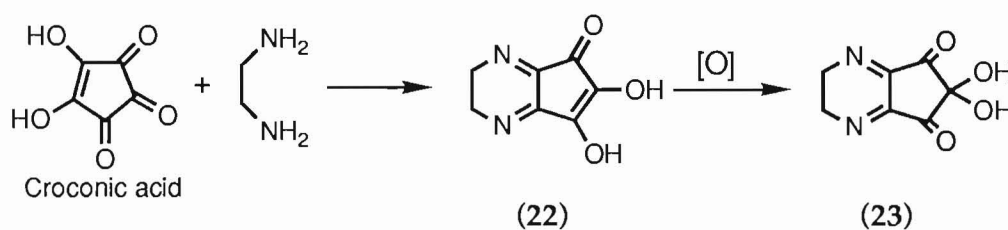


In the first method, the croconic acid is oxidized to leuconic acid (scheme 9)²⁸ which is then reacted with 1,2-diaminoethane (scheme 10), a reaction that has been modeled after scheme 8. This reaction should result in compound (19), an analog of DFO.



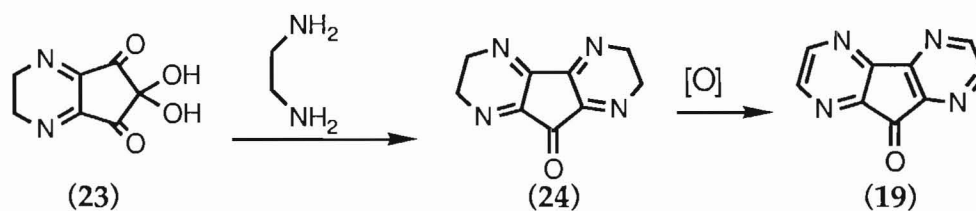
(scheme 10)

Also a more elaborate approach can be taken, first adding 1,2-diaminoethane to croconic acid to form the diol (22) which after oxidation should form the trione (23) (scheme 11), an analog of ninhydrin.



(scheme 11)

The reaction of another molecule of 1,2-diaminoethane with (23) and further oxidation of the resulting product (24) should give the desired DFO analog (19).

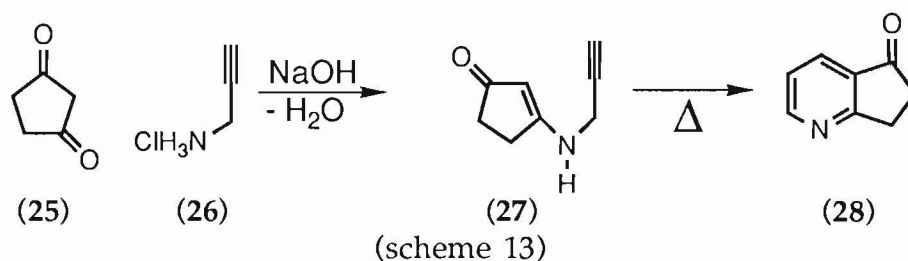


(scheme 12)

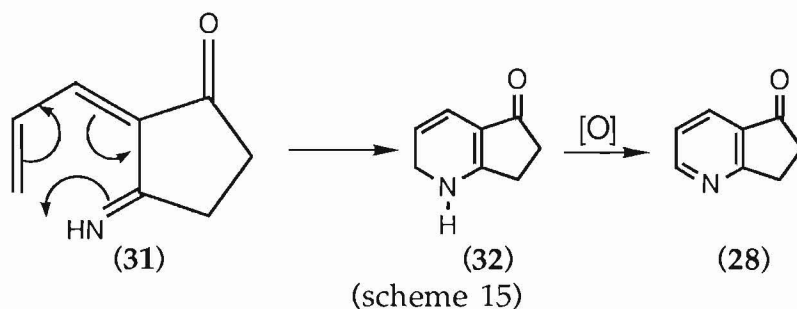
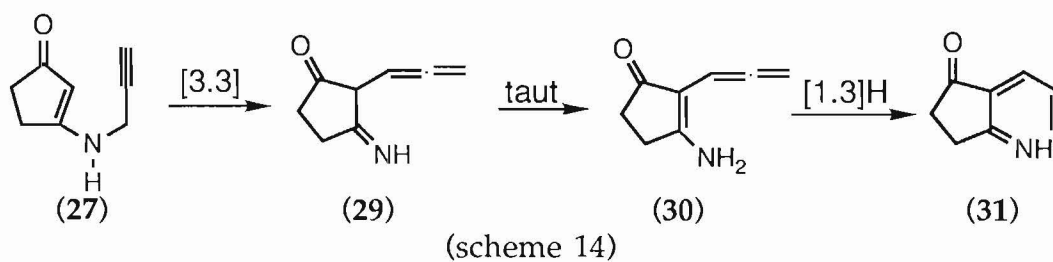
Proposed Methods of Synthesis of 7H-Cyclopenta[b]pyridine-5,6,7-trione (6)

Another possible fingerprint reagent is (6). Previously discussed syntheses of the compound (schemes 3 to 6) did not work. Two other possible methods were therefore found. The two methods are unrelated and thus will be discussed separately.

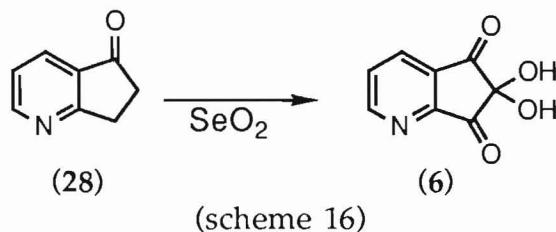
The first method (schemes 13 and 16) involves the condensation of cyclopentane-1,3-dione with 3-amino-1-propyne hydrochloride to form 3-(2-propynylamino)-2-cyclopenten-1-one (27). Compound (27) then undergoes rearrangement at high temperature to yield the product (28).²⁹



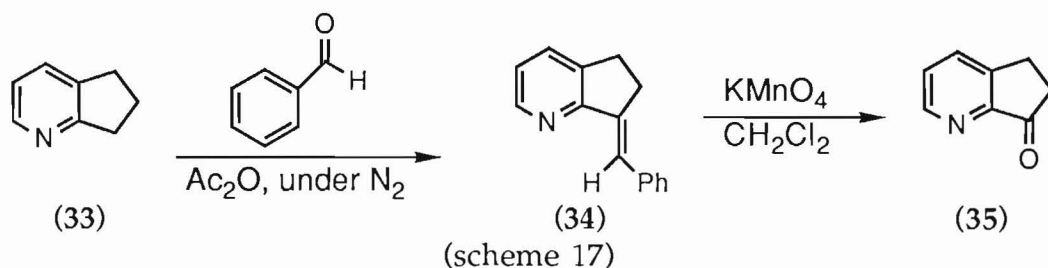
The mechanism (schemes 14 and 15)²⁹ proposed for this reaction corresponds with other similar reactions that have been analyzed³⁰. A Claisen rearrangement of the original compound is followed by a tautomerisation. A [1.5] sigmatropic hydrogen shift is then possible to yield the intermediate (31), which undergoes ring closure to form the monoketone (32). The final product (28) is formed by air oxidation of the monoketone (32) as shown in scheme 15.



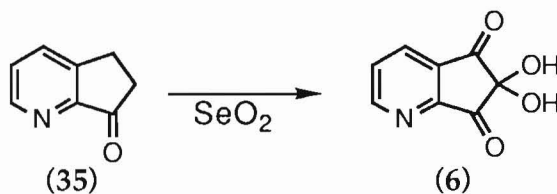
Selenium dioxide has been used in the past to oxidize the α -carbon of a ketone to diketone and triketone^{31,19} Thus a possible oxidizing agent for the oxidation of (28) to the triketone (6) is selenium dioxide (scheme 16).



Another method starts with 7H-5,6-dihydrocyclopenta[b]pyridine (33) which can be condensed with benzaldehyde to form the benzilidine adduct (34) (scheme 17). The double bond can then be cleaved using ozone to form (35).^{19,32,33} Unfortunately Wesleyan does not have ozone readily available and thus another oxidation method is needed. A procedure has recently been published for the mild cleavage of double bonds using potassium permanganate in dichloromethane solvent.^{34,35,36} This method will be tried in this synthesis to form the monoketone (35) (scheme 17).



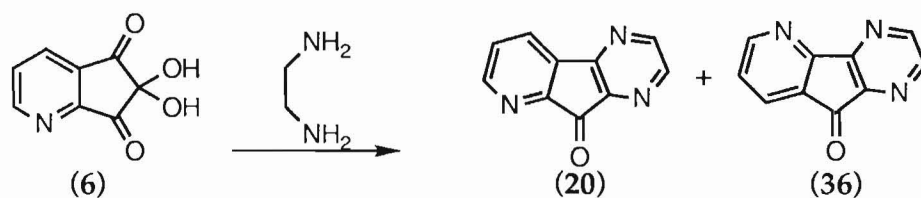
The monoketone (35) can then be oxidized using selenium dioxide to form the final product (6) (scheme 18).



(scheme 18)

Proposed Method of Synthesis of 9H-Cyclopenta[1,2-b]pyridine[3,4-b]pyrazine-9-one (20) and 9H-Cyclopenta[1,2-b]pyridine[4,5-b]pyrazine-9-one (36)

Once the ninhydrin analog (6) has been made, the next logical step is the reaction with 1,2-diaminoethane to yield the DFO analog (20). The reaction is very similar to scheme 8, where ninhydrin is reacted. Unfortunately this reaction can also form another isomer (36). It should be possible to select for one or the other by changing the experimental conditions.



(scheme 19)

Experimental

Instrumentation

All infrared spectra were measured on a Perkin Elmer Model 398 Infrared Spectrophotometer. All samples were run using potassium bromide pellets (dried in oven before use), prepared using a minipress. Eight minute scan times were used. Spectra were run using the narrow slit.

Ultraviolet spectra were measured on the Perkin Elmer Model 559 Ultraviolet and Visible Spectrophotometer. The samples were at ambient temperature and in quartz cells.

A Varian Anaspec Nuclear Magnetic Resonance Spectrometer Model 360 EM was used to run all ^1H NMR spectra. The solvent used is indicated, and unless otherwise specified the samples contained tetramethylsilane and thus were run locked at 0 ppm.

Preparation of Croconic Acid

The procedure described is an adaptation from Fatiadi et al.²⁸, and has been previously used for the preparation of croconic acid in aqueous solution²³.

Into a 100 mL round bottom flask, .5 g (2.9 mmol, Aldrich) of tetrahydroxy-1,4-quinone hydrate and 1.7 g (19.5 mmol, Aldrich) of activated manganese dioxide were added to a solution of 3 g (75 mmol) of sodium hydroxide in 65 mL of water. The solution was refluxed with stirring for 60 min, allowed to cool and then filtered leaving the manganese dioxide in the filter. The manganese dioxide was washed with 100 mL of hot water. The water was combined with the rest of the solution. The manganese dioxide was discarded. About 10 mL (120 mmol) of concentrated hydrochloric acid

was added after which the solution turned bright yellow. Repeated preparations of the croconic acid solution worked just as well. UV(1M HCl): λ_{max} : 228, 295, 315(shoulder) nm [lit(1M HCl): 231, 298, 315(shoulder) nm](see Appendix).

To the above solution, 3.5 g (1.8 mmol) of barium chloride in 15 mL of water were added. The solution was slowly heated to 85 °C and then left to cool in the air for 30 min. The yellow precipitate was filtered and washed with 10 mL water and 15 mL of ethanol. The crystals were dried and added to 10 mL of a 10 % sulfuric acid solution. This resulted in a yellow precipitate from which the supernatant was decanted. The solvent was evaporated with the rotary evaporator, leaving a brown oil. After one night in the vacuum oven at 80 °C some crystals formed. After a few hours in the air the crystals disappeared, leaving the same brown oil. The compound was too hygroscopic to obtain an IR spectrum. One other trial to get croconic acid crystals had the same result.

Attempted Preparation of Leuconic Acid

This has also been adapted from Fatiadi et al.²⁸.

Some croconic acid solution, as prepared in the previous section, was evaporated to give 2 g of yellow sludge mostly consisting of croconic acid and sodium chloride. The yellow solid was slowly added to 12 mL of concentrated nitric acid held at 0 °C with an ice bath. The reaction was quite vigorous and resulted in a white solid. About 3 mL of ice cold methanol were added to precipitate more solid. But this did not occur, probably leaving the leuconic acid in solution. The white precipitate that had formed was presumed to be sodium nitrate, since no peaks were present on the UV

spectrum at the concentration given in the literature (3 mg/mL). Another trial resulted in the same result.

Attempted Preparation of 7H-5,6-Dihydroxycyclopenta[b]pyrazine-7-one (22) at room temperature

To 5 mL of croconic acid solution as prepared above (.1 mmol croconic acid), contained in a 25 mL erlenmeyer flask, .250 mL (.225 g, 3.74 mmol, Aldrich) of 1,2-diaminoethane were added. About 1.5 mL of 6 M sodium hydroxide was added since no color change had taken place after 10 minutes. The solution was heated for 10 minutes. TLC's were obtained for both the reactant and the product solutions. Immediately after mixing, an aliquot of the reaction solution was put on a TLC plate. The TLC was developed with methanol solvent. The spot on this TLC did not move, whereas a TLC (methanol solvent) of the product solution had a line from $R_f = .00$ to $R_f = .30$, indicating that a reaction had taken place. After ten hours at room temperature the color of the solution had turned orange from yellow. The solution was neutralized with 6 M hydrochloric acid to $\text{pH} = 7.00$ as indicated by short range pH paper. White-yellow crystals formed at this pH. Filtration of the solution failed due to the small size of the particles, and extraction with ether also did not get the crystals into solution. After another 5 hours at room temperature an orange solid also formed and precipitated. Some of the liquid was first removed with a pasteur pipet, then a Craig recrystallization tube was used to filter the solid from the solution. This turned out to be a good method of separation for the two solids. The orange solid stayed behind in the Craig recrystallization tube, whereas the yellow solid followed the solution into the centrifuge tube. The water was evaporated from the yellow solid. The orange solid was soluble in methanol but not in acetone or

toluene. The yellow solid was not soluble in toluene, acetone, or methanol, but did dissolve in 1 M sodium hydroxide. Neither solid melted below 230 °C but both did turn black keeping their crystalline structure. This indicated that most of the solid was some kind of salt. A later repeat of the same procedure did not produce any precipitate.

Attempted Preparation of 7H-5,6-Dihydroxycyclopenta[b]pyrazine-7-one (22) under Reflux

About 10 mL of the croconic acid solution prepared above (.2 mmol of croconic acid) was refluxed for four hours with 50 μ L (.045 g, .75 mmol, Aldrich) of 1,2-diaminoethane. UV spectra (figures 4 to 9) were obtained at about 30 minute intervals. They indicated a change in the composition of the mixture. The solution was then left standing for about three days after which a very small amount of black solid had crystallized. The fine powder could not be filtered since it went through the filter paper. A small amount of solution was taken and dissolved in 1 M HCl. The UV spectrum contains peaks of both the solution and the solid, since some of the solid was also in the aliquot. The solid dissolved in the 1 M HCl. UV(1 M HCl): λ_{max} = 388, 357, 235 nm (figure 9). The UV spectrum of tetrahydroxy-1,4-quinone (figure 3) has a peak at 312 nm and the spectrum of croconic acid (figure 2) has peaks at 350, 315, 295, 228, and 220 nm. Both of these spectra are different from that of the solution, indicating that all of the croconic acid had disappeared.

Preparation of 3-(2-Propynylamino)-2-cyclopenten-1-one (27)

This preparation is an adaptation from Berg-Nielson and Skuttebøl.²⁹ All literature values were taken from this reference.

Into a 25 mL RB flask 1.00 g (10.9 mmol, Aldrich) of 3-amino-1-propene hydrochloride (**26**) was placed along with .401 g (10.0 mmol) of sodium hydroxide and 1.3 mL of water. About 12 mL of benzene were added followed by 1.02 g (10.4 mmol, Aldrich) of 1,3-cyclopentanedione (**25**). The solution was refluxed for 6 hours. Thereafter the hot benzene layer was poured off from the solid material at the bottom of the reaction flask into a 25 mL round bottom flask. Solid crystalized out of solution as soon as it cooled down in the round bottom flask. The benzene was evaporated with a rotary evaporator yielding 70 mg of material. More benzene was added to the reaction flask. The reaction flask was heated and the mixture was refluxed for 20 minutes. Again the benzene was poured into a round bottom flask while still hot. Successive 20 minute refluxes of more benzene in the reaction flask resulted in a total of 237 mg (17 %) of product (mp 123-6 °C [lit: 125-7 °C]) after evaporation of the benzene. Most of the remaining solid was dissolved in 5 mL of dichloromethane. The solution was poured off from the insoluble inorganic salt and then boiled down to 1 mL, after which the solution was cooled down by putting it into an ice bath. About 1 mL of cyclohexane was added and 592 mg (43 %) of solid was collected (mp 118-24 °C). The total yield was 829 mg (6.05 mmol, 61 % [lit: 76%]). TLC(2-propanone): R_f (brown spot) = .35. IR(KBr): 3200(s), 3050(s), 2930(m), 2140(w), 1655(s), 1600-1500(s, broad), 1435, 1250(s), 1200(s), 1080(s), 995(w), 940(w), 870(w), 830(m), 810(m), 725(m), 640(s) cm^{-1} (figure 10)[lit(KBr): 3220(s), 3190(s), 3040(s), 2910(m), 2125(m), 1648(s), 1575(s, broad), 1440(s), 1281(s), 1208(s) cm^{-1}]. ^1H NMR(CDCl_3): 6.6-6.1(1H, broad), 5.5(1H,s), 4.3(2H,dd), 3.2-2.6(6H,complex) δ (figure 12) [lit(CDCl_3): 7.65-7.15(1H, broad), 5.15(1H,s), 3.97(2H,dd), 2.75(2H,complex), 2.45(2H, complex), 2.45(2H, t) δ].

Attempted Preparation of 5H-6,7-Dihydrocyclopenta[b]pyridine-5-one (28)

This preparation was adapted from Berg-Nielson and Skuttebøl.²⁹ The use of the copper(I) chloride as catalyst was taken from Barmettler and Hansen.³⁷

About 325 mg (2.4 mmol) of 3-(2-propynylamino)-2-cyclopenten-1-one (27) were dissolved in 20 mL of nitrobenzene contained in a 25 mL erlenmeyer flask. The mixture was stirred and heated to between 170 and 180 °C. The mixture was then kept at that temperature for two hours. The solution was left to cool for 20 minutes after which it was extracted once with 70 mL of 2 M hydrochloric acid. A polymeric substance had formed at the interface. The aqueous layer was extracted three times with 20 mL of diethyl ether to remove the nitrobenzene. A pH meter was used to adjust the pH of the aqueous layer to 10.7 with 50% sodium hydroxide. A pH meter was used. The somewhat cloudy solution was extracted three times with 20 mL each of diethyl ether. After evaporation in the hood, a brown oil was left behind. An IR spectrum of the oil was indistinguishable from the IR spectrum of nitrobenzene. A trial using 20 mg of copper(I) chloride as catalyst for the rearrangement reaction also did not produce any product.

Preparation of 7-Benzylidene-5,6-dihydro-7H-cyclopenta[b]pyridine (34)

This procedure is an adaptation from Thummel et al.³³ and Almog.¹⁹

A pasteur pipet was filled half way with alumina. About 7.8 mL (8.2 g, 77 mmol, Mallinckrodt, reagent) of benzaldehyde was purified by letting it run through the column. While nitrogen was flushing the 50 mL round bottom flask having a thermometer attachment, 7.8 mL (8.4 g, 83 mmol, Fisher Scientific, certified) of acetic anhydride, the column purified benzaldehyde and then 4.6 mL (4.7 g, 39 mmol, Lancaster Synthesis) of 7H-5,6-

dihydrocyclopenta[b]pyridine (33) were added and mixed. A septum with a needle through it was put onto the condenser to prevent the entrance of oxygen into the system. The solution was left to reflux overnight (17 hours) at a temperature of about 145 to 155 °C, after which the solution was left to cool for 20 minutes. A vacuum distillation was performed using an aspirator to generate the vacuum. About 7 mL of acetic anhydride and unreacted benzaldehyde were collected at a temperature of 98 to 118 °C. About 40 mL of water were added to the brown liquid in the reaction pot. At the bottom of the flask a brown mud remained. It was not water soluble and did not dissolve even when 10 drops of 50% sodium hydroxide were added to the mixture. All material in the flask dissolved once 15 mL of dichloromethane had been added. The layers were separated and the aqueous layer was extracted twice more with 15 mL of dichloromethane. All extracts were combined and dried with anhydrous sodium sulfate. A rotary evaporator was used to evaporate the solvent. This resulted in 5 mL of brown syrup.

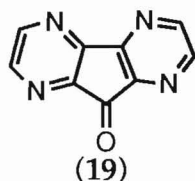
About 1.5 mL of the the syrup was chromatographed on a 5 cm long column of silica gel initially using 5% ethyl ethanoate in ligroin as mobile phase. The ethyl ethanoate concentration was slowly increased. A yellow layer was separated from an orange layer which came off the column at 20% ethyl ethanoate in ligroin. A brown mud seemed to stay stuck in the column even using ethanol as the mobile phase. The yellow layer was evaporated and 1.2 g of yellow product resulted which was further purified by squeezing it against filter paper. mp. 64-74°C [lit: 74-5 °C]³³. TLC(1:3 ethyl ethanoate:ligroin): R_f = .45 (blue fluorescence at 365 nm)[lit(1:3 ethyl ethanoate:hexane): R_f = .3]¹⁹. IR(KBr): 3050(w), 3000(w), 2920(w), 1750(w), 1575(m), 1490(m), 1435(m), 1420(m), 1270(m), 1205(w), 1170(w), 1110(w), 1010(w), 925(m), 890(m), 808(m), 780(m), 760(s), 745(m), 700(s), 550(m), 520(s)

cm⁻¹ (figure 11). ¹HNMR(CDCl₃): 8.55(1H,d), 7.7-7.0(8H, complex), 3.1(4H,s) δ (figure 13).

Off-white crystals had formed in the rest of the brown mud after two days at room temperature. The crystals were put on filter paper to drain the brown liquid. Most of the brown liquid was soaked up by the filter paper leaving behind 2.2 g of off-white crystals having a brown coating.

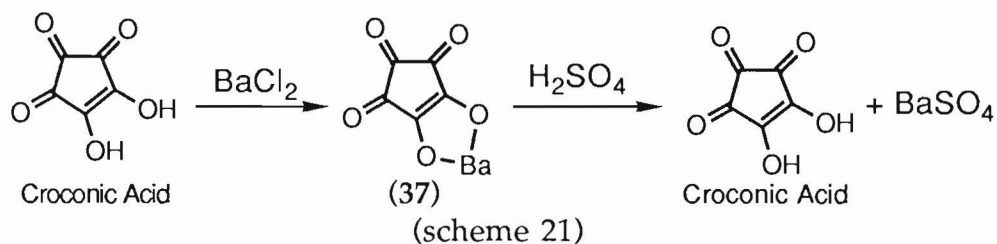
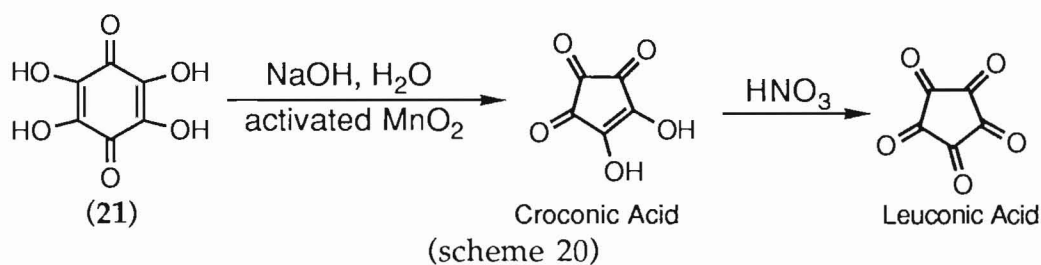
Results and Discussion

Attempted Synthesis of 9H-Cyclopenta[1,2-b:3,4-b']dipyrazine-9-one (19)



The synthesis of the starting material, croconic acid, from tetrahydroxy-1,4-quinone worked (scheme 20) as previously shown.²³ The experimental UV spectrum (figure 2) corresponds to the literature²⁸ spectrum in 1 M HCl (table 1).

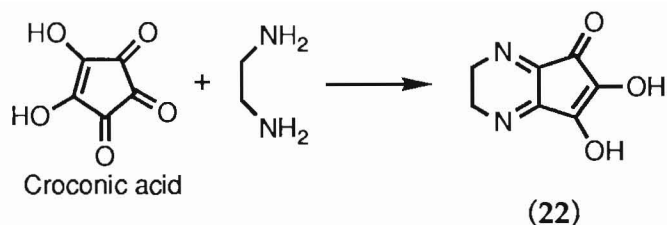
Table 1: UV Peaks of Croconic Acid	
Experiment: λ /nm	Literature ²⁸ λ /nm
228	231
295	298
315 (shoulder)	315 (shoulder)



Tetrahydroxy-1,4-quinone (21) yields sodium croconate under reflux in basic solution. This is converted into croconic acid by the addition of hydrochloric acid. The second product that is formed is sodium chloride. Both of these precipitate from aqueous solution with the addition of organic solvents such as methanol or acetone. This makes purification of the croconic acid difficult, since it cannot be separated from the inorganic salt.

The purification of the croconic acid described in the literature²⁸ was somewhat elaborate (scheme 21). It has been found to be unnecessary for other reactions previously reported²³ that use croconic acid as starting material. But since the preparation of leuconic acid failed, the purification of croconic acid (scheme 21) was tried. The croconic acid was precipitated from the solution by the addition of barium chloride, leaving the sodium chloride in solution. The aqueous solution of sodium chloride was removed from the barium croconate (37) precipitate. Croconic acid was recovered by the addition of sulfuric acid which precipitates the water insoluble barium sulfate and leaves the croconic acid in solution. Unfortunately when the solvent was evaporated, the same brown oil remained that had been observed in previous purifications of croconic acid³⁸. The compound was highly hygroscopic, since it would only solidify after more than 10 hours in a vacuum oven at 80 °C. Also it turned to a brown oil again when left in the air for a few hours. This was probably due to the high humidity in the laboratory during the summer and the fall.

Leuconic acid never precipitated from the solution. The precipitate that formed instead was sodium nitrate. Since the purification of croconic acid did not succeed, the preparation of leuconic acid was abandoned. A more elaborate method of preparation of 9H-cyclopenta[1,2-b:3,4-b']dipyrazine-9-one (19) was used.



(scheme 22)

Since the above approach failed, the alternative method was tried using croconic acid as starting material. Croconic acid was reacted with 1,2-diaminoethane (scheme 22) resulting in some kind of product. This is indicated by a change in color and different properties on the TLC plate. The melting point indicated that the product was mostly a salt, since the crystals became black, but kept their crystalline structure. In neutral solution, a yellow precipitate resulted that was soluble only in polar solvents such as water and methanol. The most likely interpretation of this is that an amine salt had formed. This was not a wanted product. A repeat of the experiment did not result in a precipitate. Since the reaction was inconsistent in the formation of product, the reaction was studied more carefully.

Monitoring this reaction by UV spectroscopy (figures 2 to 9) indicated that the reaction is very slow. Table 2 shows the relative sizes of peaks at different time intervals during the reflux of croconic acid and 1,2-diaminoethane. A comparison of peaks at different times and of the starting materials is given in table 2.

Table 2: UV Peaks of Starting Materials and Reaction Solution						
solution measured in 1M HCl	reaction conditions	λ /nm (relative intensity)				
tetrahydroxy-1,4-quinone	-	-	-	312 (100)	-	-
croconic acid solution	-	350 (50)	315 (90) shoulder	-	295 (100)	228 (55) 220 (55)
reaction mixture	10 min, 30 °C	383 (90)	359 (90)	-	297 (100)	231 (80)
reaction mixture	30 min 50 °C	385 (90)	362 (90)	-	297 (100)	231 (80)
reaction mixture	45 min 65 °C	378 (100)	362 (100)	315 (80)	299 (85)	231 (75)
reaction mixture	85 min 95 °C	383 (100)	362 (100)	318 (70)	302 (70)	232 (70)
reaction mixture	105 min 95 °C	383 (100)	363 (95)	319 (70)	301 (75)	232 (70)
reaction mixture	72 hours at 25 °C	388 (100)	357 (70) shoulder	-	-	235 (40)

It is quite obvious that the croconic acid solution does not contain measurable amounts of tetrahydroxy-1,4-quinone. Also a reaction occurred even though the solution had a yellow color until it had been left for 72 hours after which a small amount of precipitate appeared.

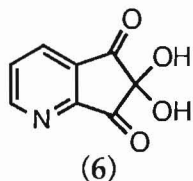
The reaction seems quite complicated, since there is evidence for two intermediates. During the course of the reaction a peak appears at about 318 nm. It later disappears. Another peak at 360 nm is equal in size to the peak at 388 nm during most of the reaction. It later declines in size relative to the peak at 388 nm, indicating another intermediate. The reaction should probably have been refluxed for a while longer to get more product for analysis. The product's spectrum probably has a λ_{max} at 385 nm.

A range of problems was encountered with the synthesis of these compounds. It was impossible to complete the syntheses and analyze the

products. The main problem with the above methods is that the compounds and products are very water soluble and thus impossible to isolate and purify, as they were usually mixtures of organic products and inorganic impurities. The solids were also very hygroscopic which made the isolation of dry compounds an impossibility. Another problem was determining if a reaction had taken place. The only methods that worked reasonably well at differentiating between reactants and products was ultraviolet spectroscopy. Thin layer chromatography did not work well, because the spots did not move even when methanol was used as solvent. Paper chromatography also did not work.

Better methods of synthesis for the above compounds are being investigated. These would include replacing the two protons of croconic acid with large organic groups, which would decrease the water solubility of the reactants and products. A probable candidate could be a large organic ester (such as phenyl ester) which would be easy to remove and would decrease the compound's water solubility so that it can be separated from inorganic salts. Unfortunately the area of croconic acid ester derivatives has not yet been explored and thus syntheses of these must be developed if they are to be used.

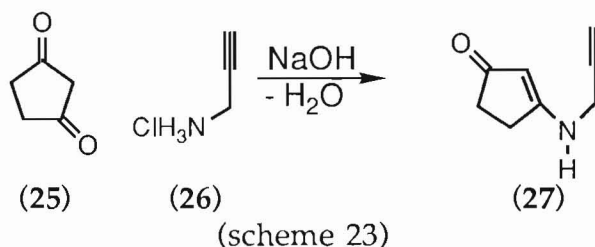
Attempted Synthesis of 7H-Cyclopenta[b]pyridine-5,6,7-trione (6)



Two unrelated methods were tried for the synthesis of (6). They will be discussed separately. The first method was not successful and thus was discarded. The second is still in progress and shows promise.

Method 1:

The condensation of the amine with the ketone to form 3-(2-propynylamino)-2-cyclopenten-1-one (**27**) (scheme 23) worked as described by Berg-Nielson and Skuttebøl²⁹ and with relatively high yield (60.6%)[lit: 76%]. Also the melting point (123-6 °C) of the compound is consistent with the literature value (125-7 °C).



On the other hand the IR spectrum (figure 10) is a little more problematic. All of the peaks found in the product are present in the literature spectrum. One product peak (3200 cm^{-1}) is better resolved in the literature and is indicated as two in the literature (3200 cm^{-1} and 3190 cm^{-1}). All peaks are somewhat shifted from the literature. Above about 1500 cm^{-1} the product peaks are at higher wavenumbers (about 10 to 20 cm^{-1}), whereas below 1500 cm^{-1} the literature values are higher (about 5 to 15 cm^{-1}). This would suggest that one of the instruments was not tuned. The instrument was thus checked with polyethylene and test peak was exactly at 1601 cm^{-1} , the expected value. This would indicate the linearity of one of the instruments was suspect.

Even more problematic is the ^1H NMR spectrum (figure 12) which casts doubts on the other results. The proposed structure in the literature has an empirical formula of $\text{C}_8\text{H}_9\text{NO}$ which has a formula weight of 135. The paper shows the results of the mass spectrometer as containing a molecular ion

peak at 135, which is not surprising. Unfortunately the integration in the paper that was done on the ^1H NMR accounts for 10 hydrogens which is inconsistent with the proposed structure and the MS results given. It would be easy to just discard the literature spectrum as an error, were it not for our experimental results and the integration that proves to be consistent with the literature. Table 3 shows the literature and experimental integrations. The experimental integrations are given for both 9 and 10 hydrogens.

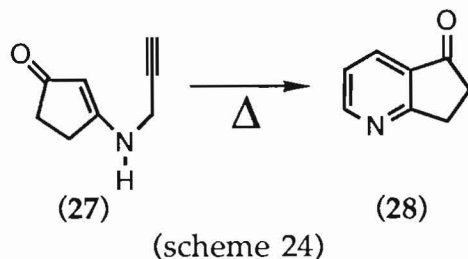
Table 3: ^1H NMR Spectrum Integrations for (27)				
literature spectrum ²⁹		experimental spectrum		
chemical shift / δ	integration/H's	chemical shift / δ	integration/H's (for 10 H's)	integration/H's (for 9 H's)
7.65-7.15	1	6.6-6.1	1 (.96)	1 (.87)
5.15	1	5.5	1 (1.08)	1 (.98)
3.97	2	4.3	2 (1.93)	2 (1.7)
2.75	2	3.2-2.6	6 (6.02)	5 (5.4)
2.45	4			
Total:	10 hydrogens		10 hydrogens	9 hydrogens

As can be seen, the integration for ten hydrogen atoms works better than the integration for nine. Thus it seems possible that the literature spectrum is correct, even though this poses a problem for the proposed structure. At this point the source of this inconsistency is uncertain.

The chemical shift for most peaks is about .3 δ lower in the literature than in the experiment. This is probably due to improper locking of the tetramethylsilane signal at 0 δ . As for the large difference between the literature and the observed shift at the 6.6-6.1 δ , that is harder if not impossible to explain, especially since the same ^1H NMR solvent was used (CDCl_3).

On the other hand the next step in which the compound is heated in nitrobenzene did not yield the product, 5H-6,7-dihydrocyclopenta[b]pyridine-

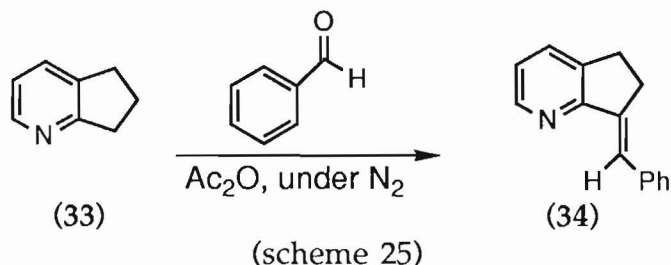
5-one (28) (scheme 24). A likely reason for the small yield in this reaction is polymerization during extraction.



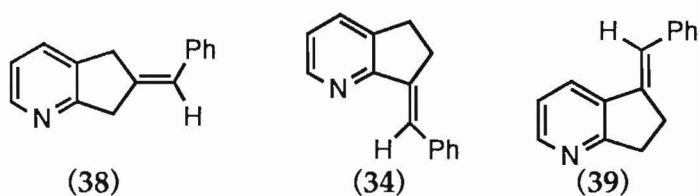
Similar pericyclic rearrangements have been performed using copper(I) chloride as catalyst^{30,37}. When this procedure was applied to this reaction, a polymeric material still formed during the extraction and no product resulted. The oil resulting after extraction must be nitrobenzene, since the IR spectrum was indistinguishable from the IR spectrum of nitrobenzene. As the procedure did not work it was dropped in favour of the next method.

Method 2:

The condensation of 7H-cyclopenta[b]pyridine with benzaldehyde formed a product whose melting point (64-74 °C) seems to correspond with the literature (74-5 °C)³³, even though it is somewhat lower indicating that it is not quite as pure as the literature sample. Unfortunately the paper does not give IR or ¹HNMR spectra or peaks.



The literature is not very specific about the ^1H NMR having the sentence "the NMR was consistent with the structure"¹⁹. The structure proposed in the literature is (34) having the E configuration around the double bond. Other structures that are possible from the condensation reaction are (38) and (39). All compounds are shown in the E form, but the respective Z enantiomer is also a likely product. The ^1H NMR peaks obtained are: 8.55 (1H, d), 7.7-7.0 (8H, complex), and 3.1 (4H, s) δ .



An explanation for the ^1H NMR spectrum (figure 13) obtained is a little more elusive. The peak at 8.55 δ is probably the hydrogen next to the nitrogen in the pyridine ring. The 8 hydrogens at 7.7 to 7.0 δ are probably due to the other aromatic hydrogens and the vinyl proton. The problem arises in trying to explain the singlet at 3.1 δ . It is not a doublet and therefore the four hydrogens are either equivalent or are not adjacent to each other. This would fit the structure (38) better than (34). Structures (34) and (39) have two sets of two hydrogens in the cyclopentane ring that are clearly different and are next to each other, which would give rise to a doublet in the spectrum. On the other hand in structure (38) the hydrogens are not next to each other and thus would show up as a singlet in the spectrum. The peak is somewhat broad which can be explained by the fact that the two sets of hydrogens are not quite equal since the nitrogen in the pyridine ring is closer to one of them than to the other. Also there should be a mixture of E and Z enantiomers in the

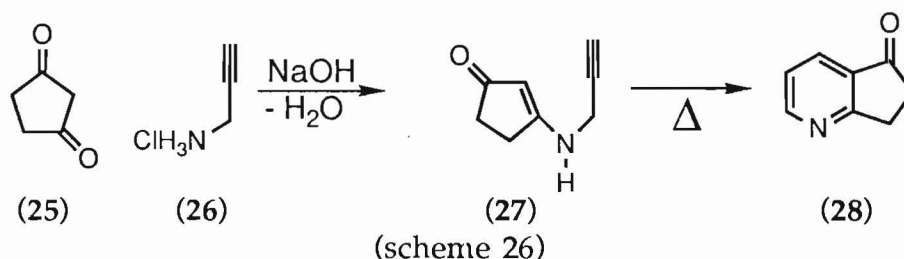
product, which could give rise to a broadening of the peak, since this would change the environment of the protons somewhat.

The location of the double bond should not matter for the next step as long as a ketone can be made. The potassium permanganate is not selective and thus will cleave the bond at any position. Once the ketone has been made, the selenium dioxide should still oxidize the compound, since it as well is not selective.

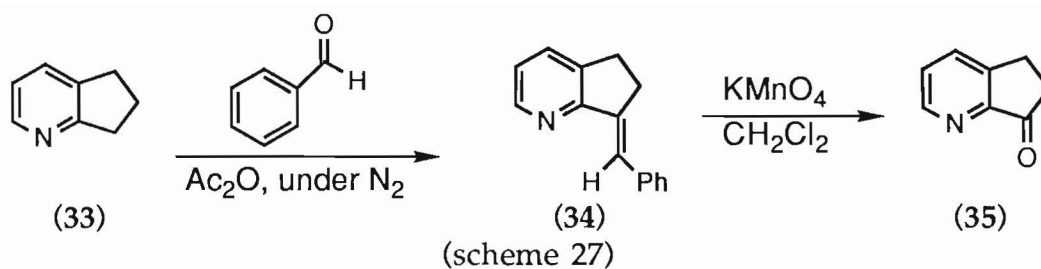
Conclusion

The research conducted can be split up into three different categories. The work completed and the problems associated with each project have been summarized below.

The synthesis of 9H-cyclopenta[1,2-b:3,4-b']dipyrazine-9-one (**19**) using croconic acid did not work, mostly due to the high solubility of croconic acid and its products in water. This prevented the isolation and purification of the compounds. For the synthesis of (**19**) to succeed, the solubility of the compounds in water must be decreased. One method to do this is to replace the hydrogens on the croconic acid with a large organic group. The best candidate for this would be a large organic ester such as a phenyl ester.



The synthesis of 7H-cyclopenta[b]pyridine-5,6,7-trione (**6**) via method 1 (scheme 26) did not work. The first step yielded a product for which the IR, ¹HNMR, and melting point corresponded to the literature. However the literature is self-contradictory. The structure of the compound proposed has 9 hydrogens, but it integrated the ¹HNMR for 10. Since the next step did not yield any product, this method was abandoned.

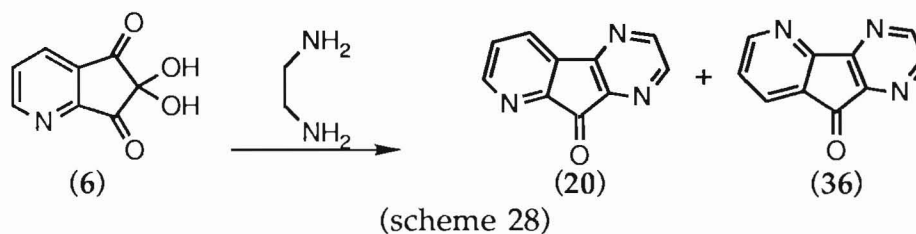


The synthesis of 7H-cyclopenta[b]pyridine-5,6,7-trione (**6**) via method 2 (scheme 27) has worked so far. Currently compound (35) is being made, but the product has not yet been investigated. The ^1H NMR spectrum of (34) casts doubt on the configuration suggested by the literature. Since this approach has worked so far, it should be continued.

Future Work

As a first priority the synthesis of 7H-cyclopenta[b]pyridine-5,6,7-trione (6) using method 2 should be continued. The compound can then be tested for luminescence once it reacts with amino acids. Since the luminescence of the zinc(II) chloride complex have not been tested, they should be investigated.

Compound (6) can then be used as starting material for the synthesis of two different DFO analogs. They are: 9H-cyclopenta[1,2-b]pyridine[3,4-b]pyrazine-9-one (20) and 9H-cyclopenta[1,2-b]pyridine[4,5-b]pyrazine-9-one (36).



Another promising venture would be the synthesis of 9H-cyclopenta[1,2-b:3,4-b']dipyrazine-9-one (19) using a croconic acid derivative that is not as water soluble.

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Acknowledgements

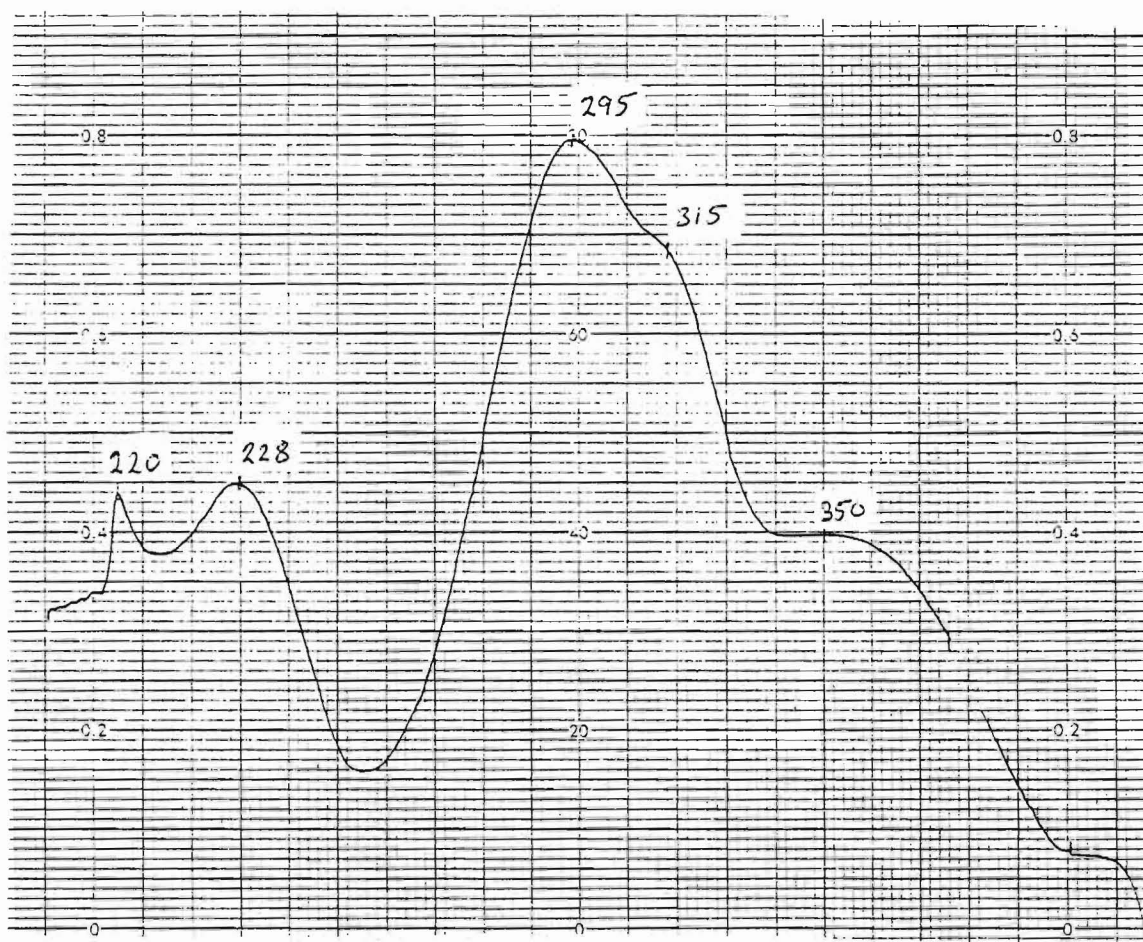
I would like to thank Illinois Wesleyan University and the Chemistry Department for financial support of this project.

Appendix

UV Spectra

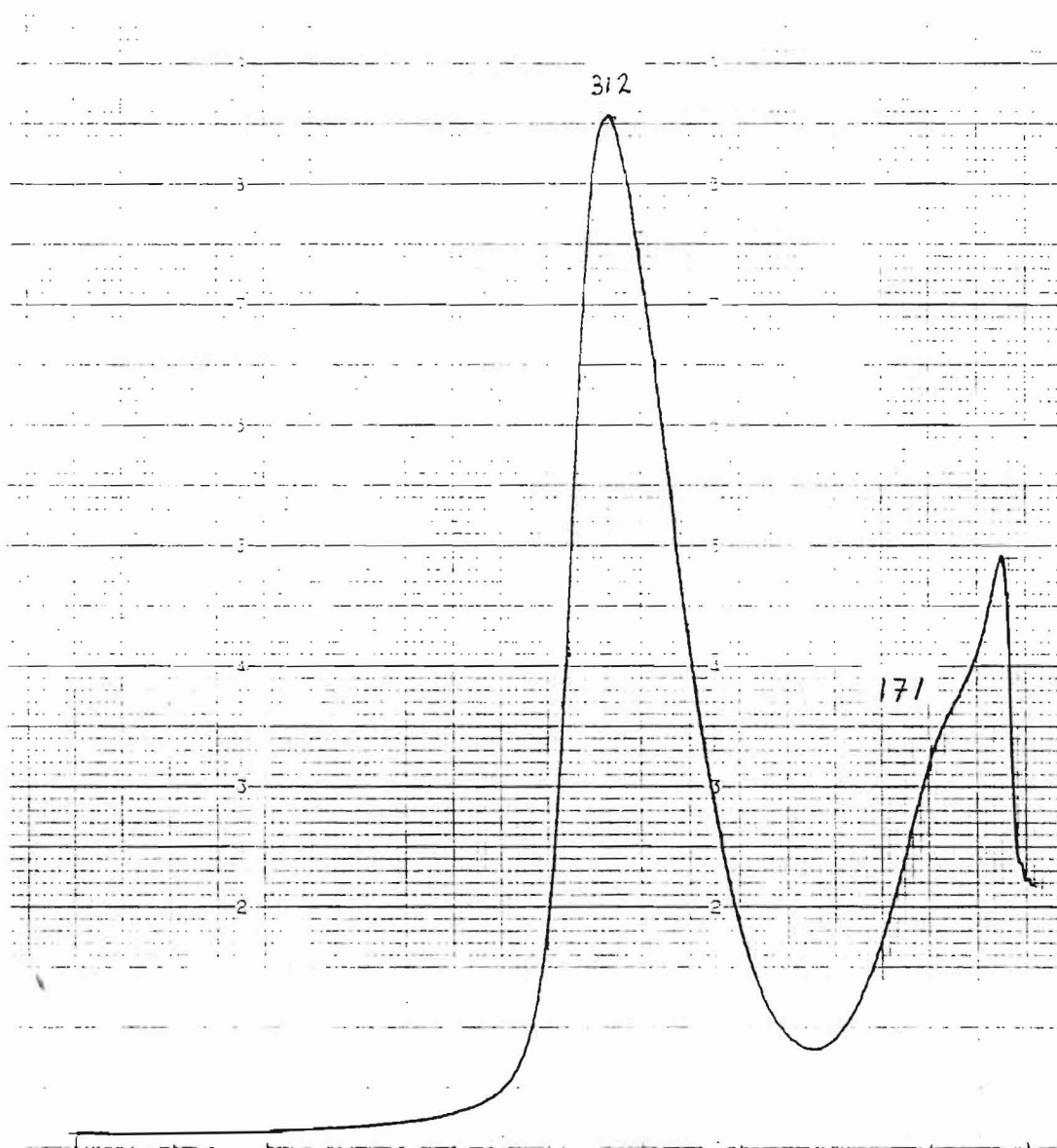
UV Spectrum of Croconic Acid

in 1 M HCl (figure 2)



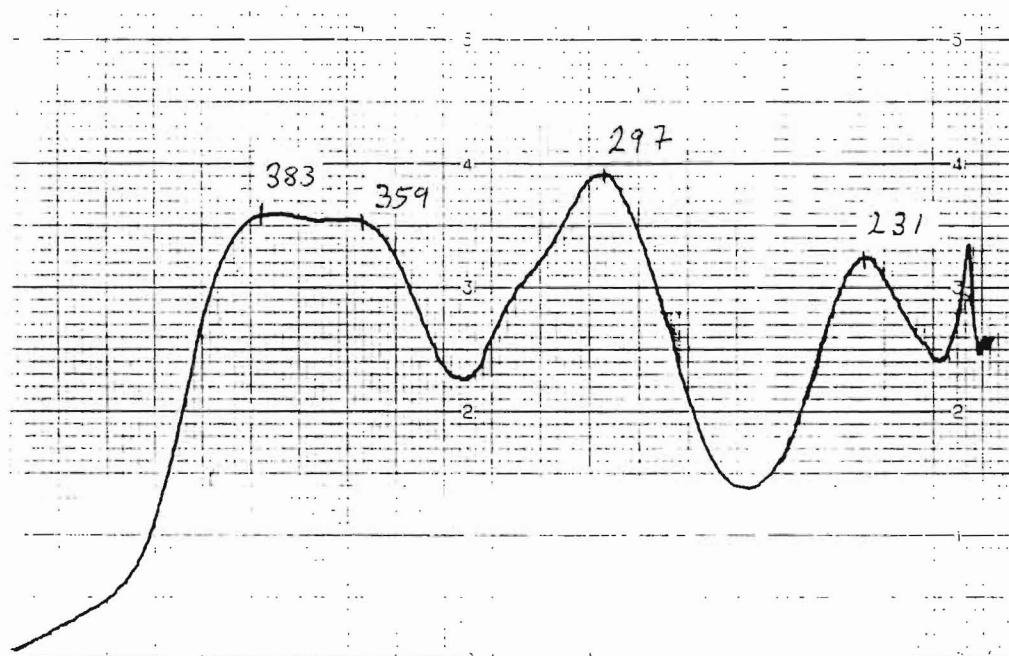
UV Spectrum of 1,4-Tetrahydroxyquinone (19)

in 1 M HCl (figure 3)



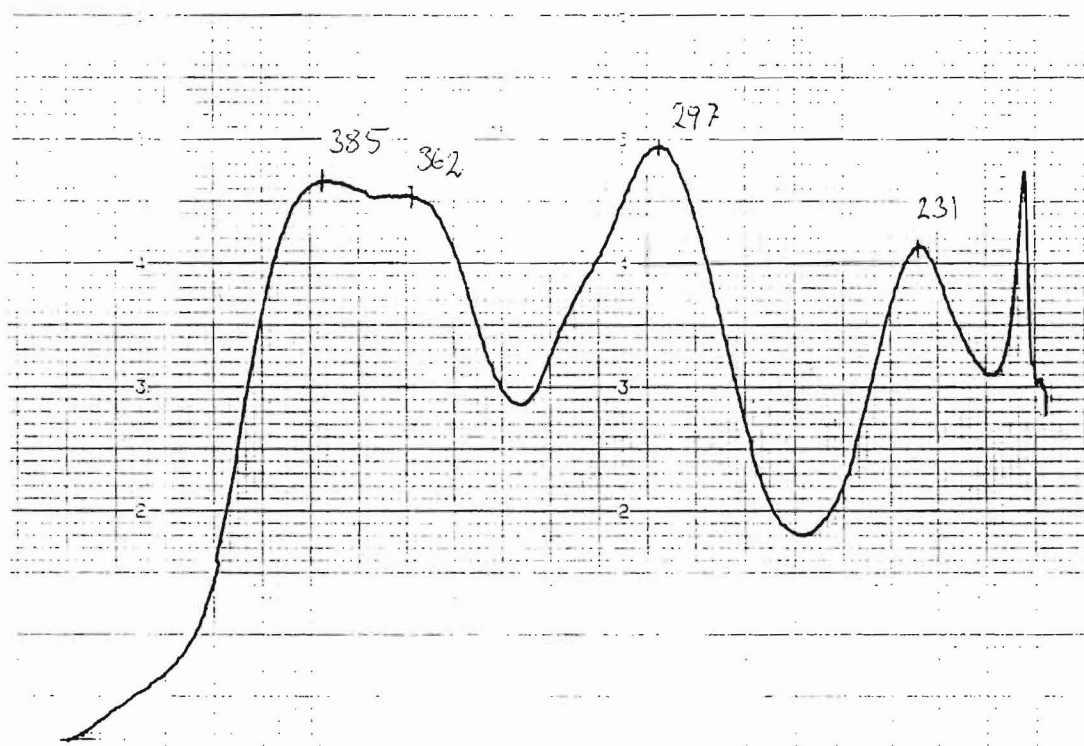
UV Spectrum of the Reaction of Croconic Acid and 1,2-Diaminoethane
in 1 M HCl (figure 4)

10 min/ 30 °C



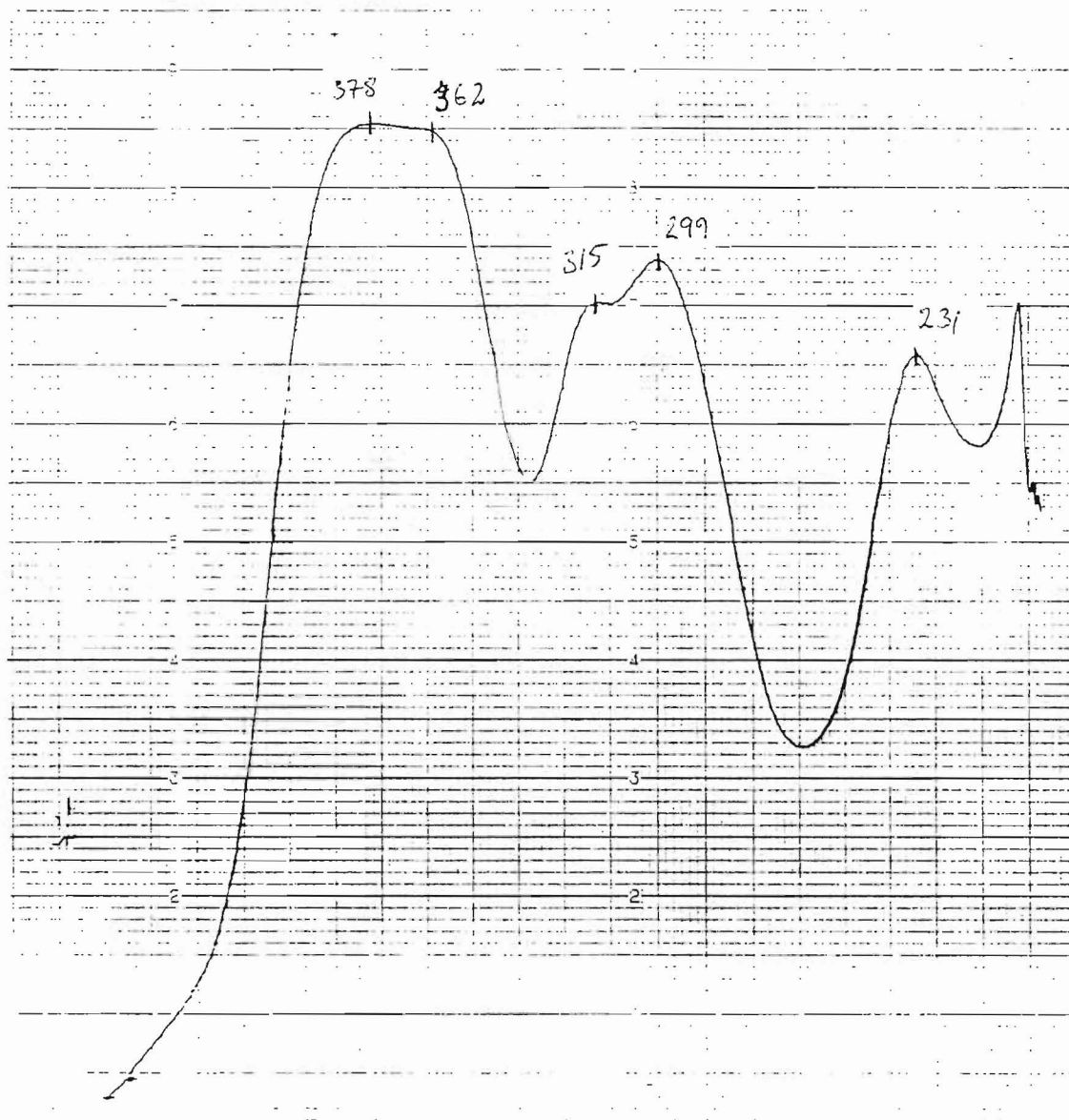
UV Spectrum of the Reaction of Croconic Acid and 1,2-Diaminoethane
in 1 M HCl (figure 5)

30 min/ 50 °C



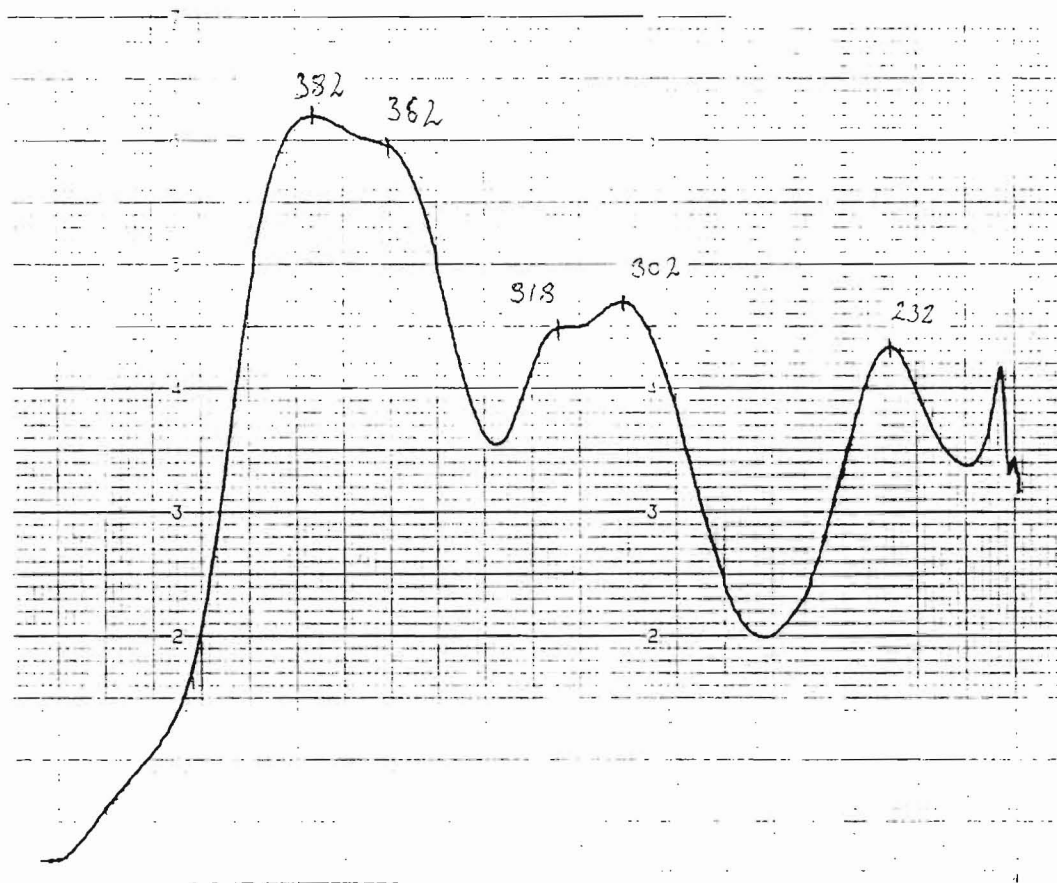
UV Spectrum of the Reaction of Croconic Acid and 1,2-Diaminoethane
in 1 M HCl (figure 6)

45 min/ 65 °C



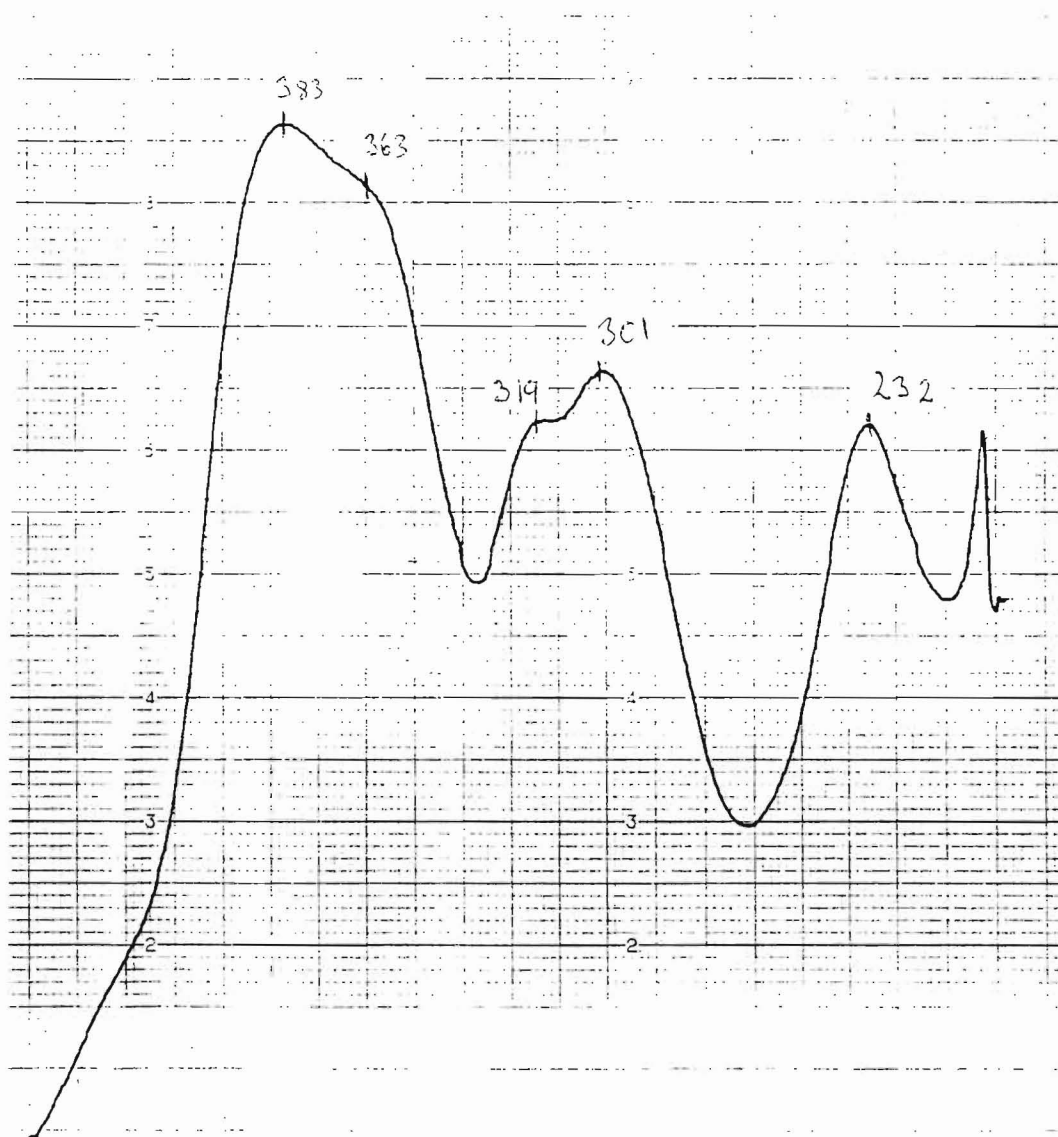
UV Spectrum of the Reaction of Croconic Acid and 1,2-Diaminoethane
in 1 M HCl (figure 7)

85 min/ 95 °C



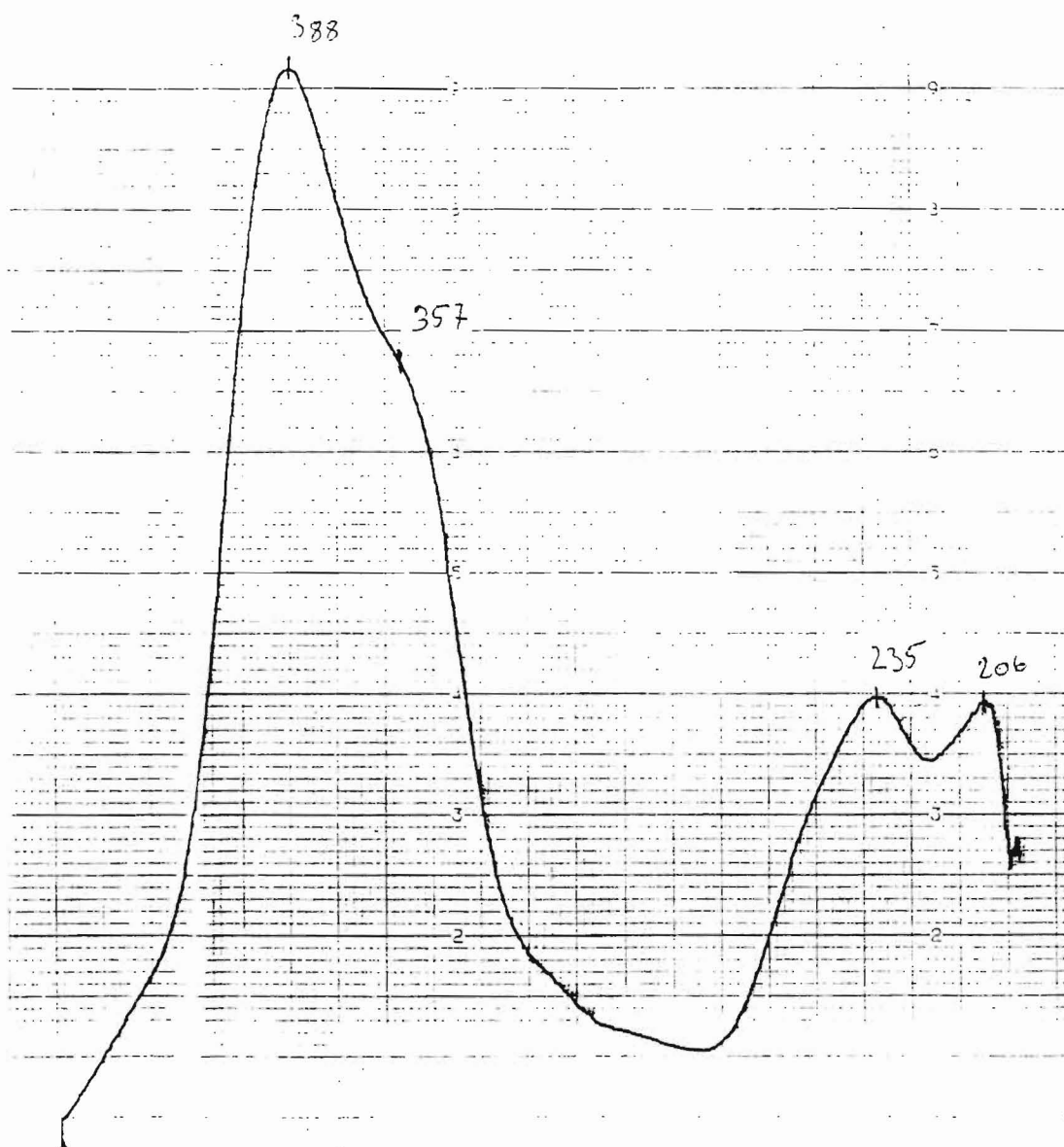
UV Spectrum of the Reaction of Croconic Acid and 1,2-Diaminoethane
in 1 M HCl (figure 8)

105 min/ 95 °C



UV Spectrum of the Reaction of Croconic Acid and 1,2-Diaminoethane
in 1 M HCl (figure 9)

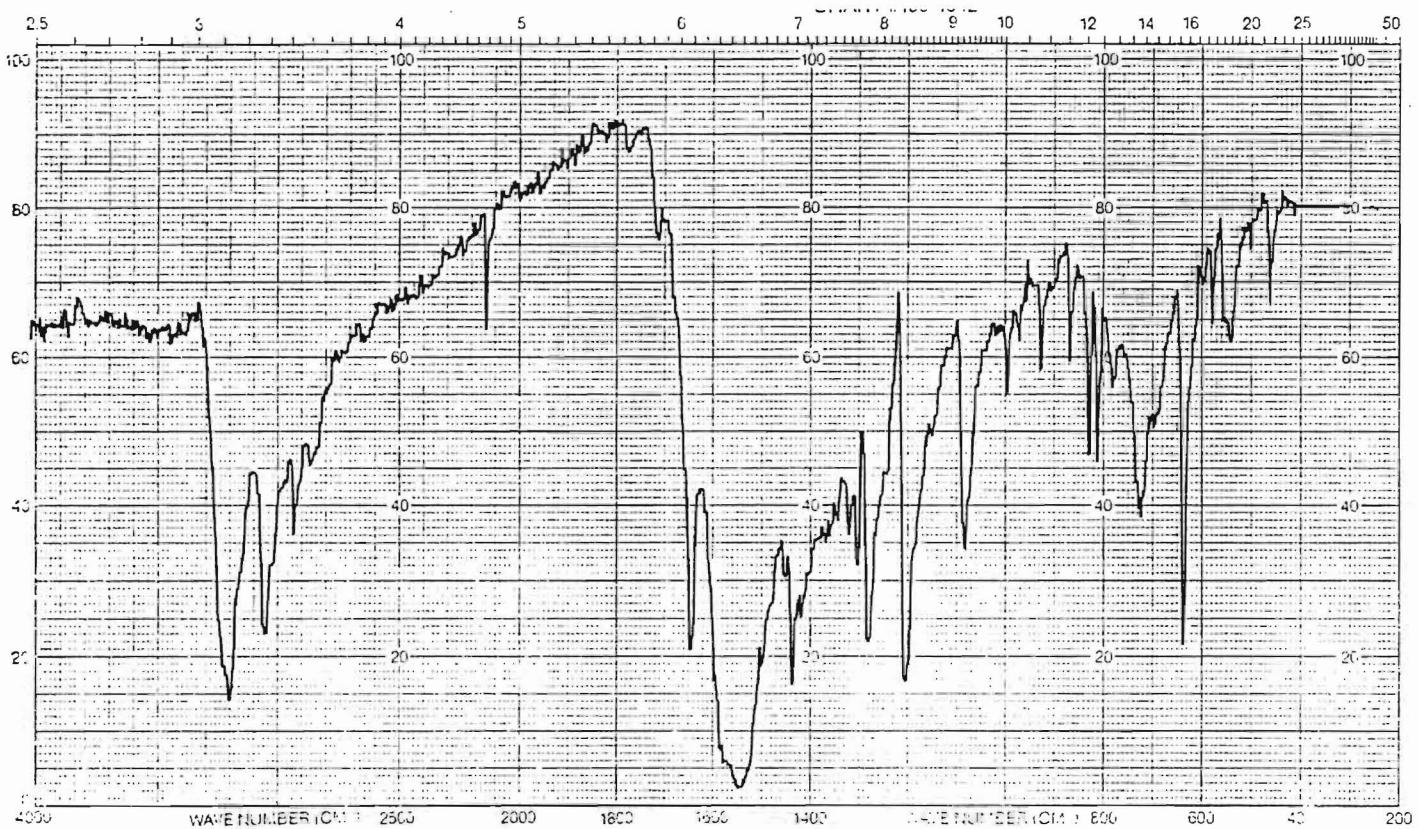
72 hours/ 25 °C



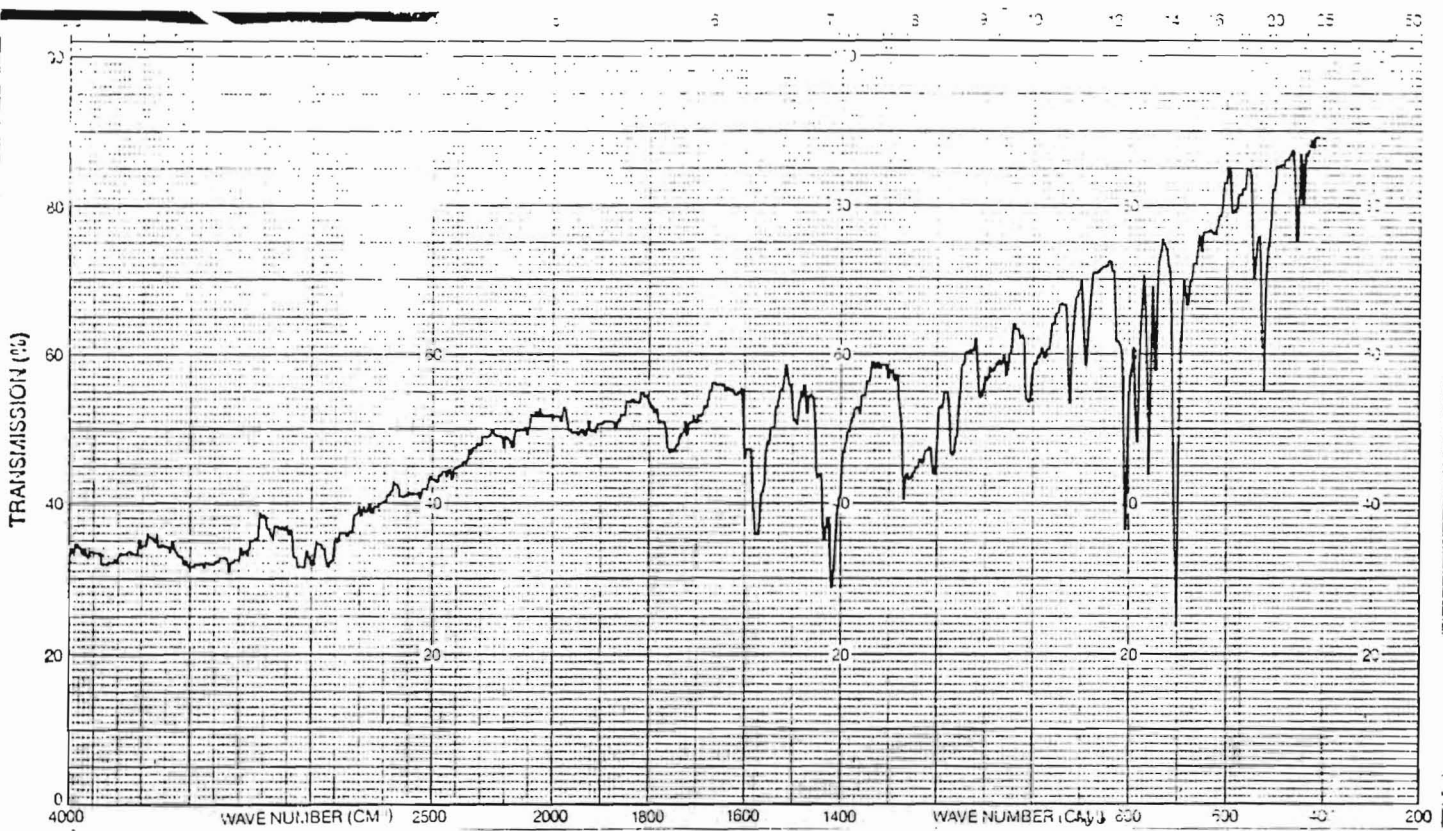
Infrared Spectra

Infrared Spectrum of 3-(2-Propynyl)-2-cyclopenten-1-one (27)

KBr pellet (figure 10)



Infrared Spectrum of 7-Benzylidene-5,6-dihydro-7H-cyclopenta[b]pyridine (34)
KBr pellet (figure 11)



Proton Nuclear Magnetic Resonance Spectra

¹H NMR Spectrum of 3-(2-Propynyl)-2-cyclopenten-1-one (27)

Solvent: CDCl₃ (figure 12)



¹H NMR Spectrum of 7-Benzylidene-5,6-dihydro-7H-cyclopenta[b]pyridine (34)

Solvent: CDCl₃ (figure 13)

