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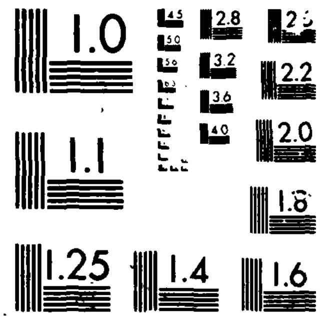
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Construction of New Potential Reactivators of Phosphonylated Acetylcholinesterase: Substitution of F for H in the Nucleus of Pyridinecarboxaldehyde Oximes

Annual and Final Report

Jerome McLick and Ernest Kun

November 1983

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21701-5012

Contract No. DAMD17-81-C-1160

Cardiovascular Research Institute
University of California
San Francisco, CA 94143

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REPORT DOCUMENTATION PAGE

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1. REPORT NUMBER

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4. TITLE (and Subtitle)
Construction of New Potential Reactivators of Phosphonylated Acetylcholinesterase: Substitution of F for H in the Nucleus of Pyridinecarboxaldehyde Oximes.

5. TYPE OF REPORT & PERIOD COVERED
Annual (1 Sep 82 - 30 Nov 83
Final (1 Sep 81 - 30 Nov 83)

6. PERFORMING ORG. REPORT NUMBER

7. AUTHOR(s)
Jerome McLick and Ernest Kun

8. CONTRACT OR GRANT NUMBER(s)
DAMD17-81-C-1160

9. PERFORMING ORGANIZATION NAME AND ADDRESS
Cardiovascular Research Institute
University of California
San Francisco, CA 94143

10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS
62772A.3S162772A875.AA087

11. CONTROLLING OFFICE NAME AND ADDRESS
U.S. Army Medical Research and Development Command
Fort Detrick, Frederick, MD 21701 -5012

12. REPORT DATE
November 1983

13. NUMBER OF PAGES
44

14. MONITORING AGENCY NAME & ADDRESS(if different from Controlling Office)

15. SECURITY CLASS. (of this report)
Unclassified

15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

Approved for public release; disribution unlimited

18. SUPPLEMENTARY NOTES

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

Acetylcholinesterase reactivator; chemical synthesis; Fluoro-substitution; 3-F-2-PAM; 5-F-2-PAM; NMR spectra; oxime; PAM compounds; pyridinecarboxaldehyde oxime; reactivator; pKa; UV spectra

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

Two new Fluoro-pyridinecarboxaldehyde oxime methiodide (F-PAM) compounds have been synthesized: 3-F-2-PAM and 5-F-2-PAM. These compounds and their unquaternized oxime precursors have been rigorously characterized by elemental analysis, mass spectroscopy, NMR, UV, and pKa determination. Detailed spectroscopic interpretations are provided and correlated with analogous data for other PAM compounds. Both F-isomers of 2-PAM are obtained in low yield, 3-F-2-PAM is unstable during titration with NaOH, while 5-F-2-PAM is stable but its pKa is only 0.15 pKa units lower than that of 2-PAM.

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Note to reader: The proton NMR spectra reproduced in this report include the operator's handwritten notations of instrumental settings and other incidental notations at the righthand side of each spectra. Upon photographic reduction of the spectra to conventional page size, some of these notations may be difficult to read or illegible. However, they are not essential to the content of the report and in all cases NMR data subjected to interpretation in this report have been entered in typography to insure legibility.

LIST OF FIGURES

SUMMARY

Work performed during this period was a continuation and conclusion of our project to synthesize and chemically characterize new quaternary pyridine carboxaldehyde oxime halides (PAM compounds) where F is substituted for H at various positions in the pyridine ring. The rationale for this project is that introduction of F (to form F-PAM's) will electronically modify the parent PAM compounds. Current empirical knowledge of the reactivation of organophosphorus-poisoned acetylcholinesterase by PAM compounds indicates that molecular electronics (which, for example, finds expression in the pKa of the oxime group) is one determinant of reactivator performance. However, the degree to which this parameter is critical relative to other molecular parameters, especially structural stereochemistry and enzyme affinity, is in need of clarification. While introducing electronic modifications, F-substitution should only minimally alter the stereochemistry and enzyme-binding character of known PAM compounds. Therefore F-substitution has the potential of contributing to a clarification with less ambiguity than other substitutions on the PAM molecule. Toward the goal of designing reactivators of higher performance than those currently available such a clarification is highly desirable and possibly necessary.

In the First Quarter synthetic precursors to 3-F-2-PAM were prepared on a small scale. Additionally, physical studies relating to the 4-isomer (namely 3-F-4-PAM, synthesized during the previous year) were performed. The pKa, proton NMR, and UV absorption behavior of TMB-4 and 3-F-4-PAM were compared.

In the Second Quarter reactions in the synthetic pathway to 3-F-2-PAM were scaled-up. Low yields were encountered for the specific nitration isomer in this pathway which placed a limitation on the amount of 3-F-2-PAM synthetically practicable.

In the Third Quarter 3-F-2-pyridinecarboxaldehyde oxime was synthetically achieved by way of the Markovac-Stevens-Ash-Hackley reaction, and the compound was characterized by its mass spectrum, NMR spectrum, and UV spectrum. The compound was quaternized to 3-F-2-PAM and satisfactorily characterized by elemental analysis and NMR spectroscopy. Contrary to expectation, in the NMR spectrum of 3-F-2-PAM the aldehydic proton resonance is less chemically shifted than in 2-PAM. A possible explanation is that in 3-F-2-PAM the F atom, in combination with the N-Methyl group sterically disfavors coplanarity between the oxime group and the pyridine ring. Thus the electron-withdrawing (and deshielding) effect of the F atom may be minimized. Determination of the pKa of 3-F-2-PAM by potentiometric titration was pursued. However this compound displayed aberrant titration behavior apparently undergoing decomposition during addition of NaOH to give unstable pH readings.

In the Fourth Quarter , after preparation of necessary precursors, synthesis of 5-F-2-pyridinecarboxaldehyde oxime was achieved by the Markovac-Stevens-Ash-Hackley reaction. However only a very low yield (4.3%) was obtained, this being a possible correlate of the observation of Markovac, et.al., that the iodine/dimethylsulfoxide reaction fails if there is a hydroxy group in the 5-position. The amount of 5-F-2-pyridinecarboxaldehyde oxime obtained was sufficient for rigorous structure proof by high resolution mass spectroscopy and NMR spectroscopy. Quaternization proceeded in low yield (8.6%), and the product 5-F-2-PAM was characterized by proton NMR, UV, and SIMS ("Sputtering Ion Mass Spectroscopy"). In the NMR spectrum the aldehydic proton is chemically shifted essentially to the same extent as 2-PAM, thus the electron-withdrawing effect

of the F atom in the 5-position appears to express itself only weakly at the 2-oxime group. Spectroscopic determination of the pKa showed that 5-F-2-PAM has a pKa which is 0.15 pKa units lower than that of 2-PAM.

As an overview, two new F-PAM compounds have been synthesized and physically characterized (3-F-2-PAM and 5-F-2-PAM). Unlike the isomer prepared during the previous year (3-F-4-PAM) which was obtained in relatively good yields, 3-F-2-PAM and 5-F-2-PAM afforded only low yields. Because of that fact together with the observations that 3-F-2-PAM is unstable during titration and 5-F-2-PAM displays only a slightly lower pKa than 2-PAM, these F-analogs of 2-PAM do not offer impetus to biological studies as reactivators of poisoned acetylcholinesterase. However the systematic physical studies which the current research has generated should add to the fundamental data base pertaining to PAM compounds and assist in the selection of new approaches to high-performance acetylcholinesterase reactivators.

Section I

First Quarter

(September 1 - November 30, 1982)

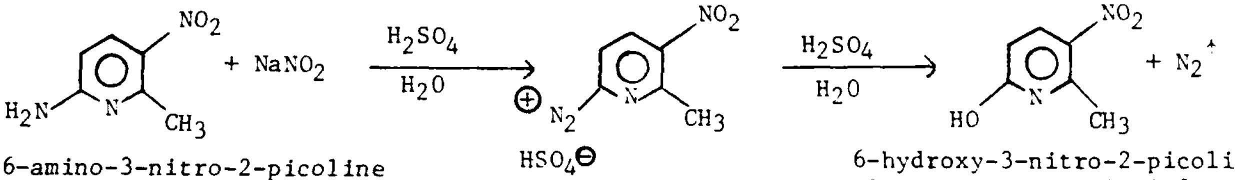
Work performed in this quarter was principally focused on isomer separation and chemical reactions in the synthetic pathway to 3-Fluoro-2-PAM and was secondarily focused on physical and spectroscopic measurements as part of our systematic studies on PAM compounds.

As mentioned in our Annual Report (dated November 1982, Section IV), synthesis of one of the F-PAM isomers named in this contract, 4-F-3-PAM, is hampered by virtual inoperation of the diazotization-Schiemann reaction (fluorination) at the 4-position of the pyridine ring, and we have not been able to remedy this situation using sodium nitrite or ethyl nitrite as the diazotization agent. As a pragmatic result it is necessary for us to designate 4-F-3-PAM as unattainable in our present work.

By contrast, as already demonstrated in the synthesis of 3-F-4-PAM, the diazotization-Schiemann reaction operates excellently at the 3-position and thus there is no impediment to 3-F-2-PAM. As noted in Annual Report, November 1982, Section III, the synthetic reaction sequence for this latter compound commences with nitration of commercial 6-amino-2-picoline, which gives a mixture of two nitration isomers. During the present quarter we received the electric steam generator necessary for separation of these isomers and we successfully isolated the desired structure (6-amino-3-nitro-2-picoline). This isomer is not volatile with steam but requires passage of considerable amounts of steam to remove the other isomer (6-amino-5-nitro-2-picoline). This latter isomer is indeed steam-volatile but its vapor pressure and hence its mole fraction in the steam distillate is apparently quite low, accounting for the relatively high steam volume required for complete distillation.

Once we obtained purified 6-amino-3-nitro-2-picoline (m.p. 188-190°C, lit. m.p. 188°C, (Ref. 1), we carried out pilot reactions to verify succeeding steps in the proposed synthesis of 3-F-2-PAM. The first of these reactions was conversion to 6-hydroxy-3-nitro-2-picoline via diazotization in the presence of aqueous H_2SO_4 (eq.1).

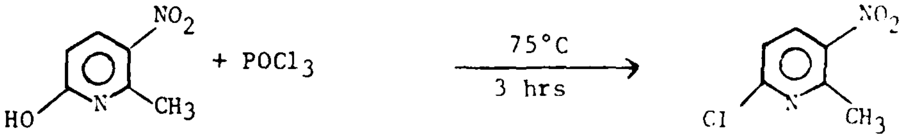
(eq.1)



6-amino-3-nitro-2-picoline
m.p. 188-190°C
4.00 g.
26.1 mMoles

6-hydroxy-3-nitro-2-picoline
3.22 g., m.p. 228-230°C
80.1% yield

The next reaction was conversion of the hydroxy compound to the corresponding chloro compound (eq.2).

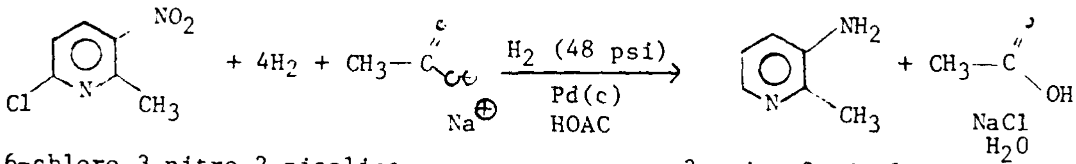


(eq.2)

6-hydroxy-3-nitro-2-picoline
3.08 g.
20.0 mMoles

6-chloro-3-nitro-2-picoline
2.70 g., m.p. 52-54°C
78.5% yield

Finally in this quarter we catalytically reduced the chloro compound to 3-amino-2-picoline in the Parr apparatus (eq.3).



(eq.3)

6-chloro-3-nitro-2-picoline
1.72 g.
10.0 mMoles

3-amino-2-picoline
1.00 g. m.p. 114-117°C
(Lit. m.p. 112-114°C)
92.5% yield

The product 3-amino-2-picoline was previously prepared in the literature by reductive dechlorination using zinc and H_2SO_4, but only in 50% yield.[2]

During the next quarter we intend to (a) scale up the above reactions, (b) convert 3-amino-2-picoline to 3-F-2-picoline via the diazotization-Schiemann reaction, and (c) convert 3-F-2-picoline to 3-F-2-pyridinecarboxaldehyde oxime via the Markovac-Stevens-Ash-Hackley reaction. As part of our broader, comparative studies on PAM compounds, during the present quarter we carried out several physical measurements on the duplex-(4-PAM) structure, TMB-4, a sample of which was supplied to us by the Department of Medicinal Chemistry, Division of Experimental Therapeutics, Walter Reed Army Institute of Research. Firstly we determined the pKa value of 8.2 reported by Poziomek, Hackley, and Steinberg[3], as well as by Wilson and Ginsburg[4]. Concurrently we re-measured the pKa of 3-F-4-PAM and obtained a value of 8.21. Our previous values for 3-F-4-PAM were 8.18 and 8.12; in the literature we shall report a value of 8.2 (rounded off to one decimal place).

Regardless of the absolute accuracy of our pKa [illegible] 3-F-4-PAM has a pKa close to, but slightly lower than, that [illegible] means that any difference in the ultimate reactivator performance [illegible] *versus* 3-F-4-PAM cannot be ascribed to substantive pKa differences [illegible] to structural differences (duplex structure *versus* monomer structure [illegible] since the pKa of 3-F-4-PAM (unlike 4-PAM) is not greatly disparate from [illegible] of 2-PAM (pKa 8.0), reactivator performance of 3-F-4-PAM *versus* 2-PAM [illegible] constitute a test of the importance of the 4-oxime configuration *versus* the 2-oxime configuration.

In addition to the pKa value of TMB-4 we also measured the proton NMR spectrum of this compound (see Fig.1). Analogous to the other PAM compounds studied, the NMR spectrum was obtained in D_2O solvent with DSS internal standard. Spectrum interpretation is provided in Fig.1, and for our present purposes the datum of special interest is the chemical shift of the aldehydic proton, which is observed to be 8.39 ppm from DSS. This chemical shift is slightly greater than that of 4-PAM (8.37 ppm), but is less than that of both 3-F-4-PAM (8.45 ppm) and 2-PAM (8.69 ppm). It is of interest to summarize these data together with the respective pKa values for the same compounds:

Compound	pKa	Chemical Shift (aldehydic proton)
4-PAM	8.5(8.6)	8.37 ppm
TMB-4	8.3(8.2)	8.39
3-F-4-PAM	8.2	8.45
2-PAM	8.0(8.0)	8.69

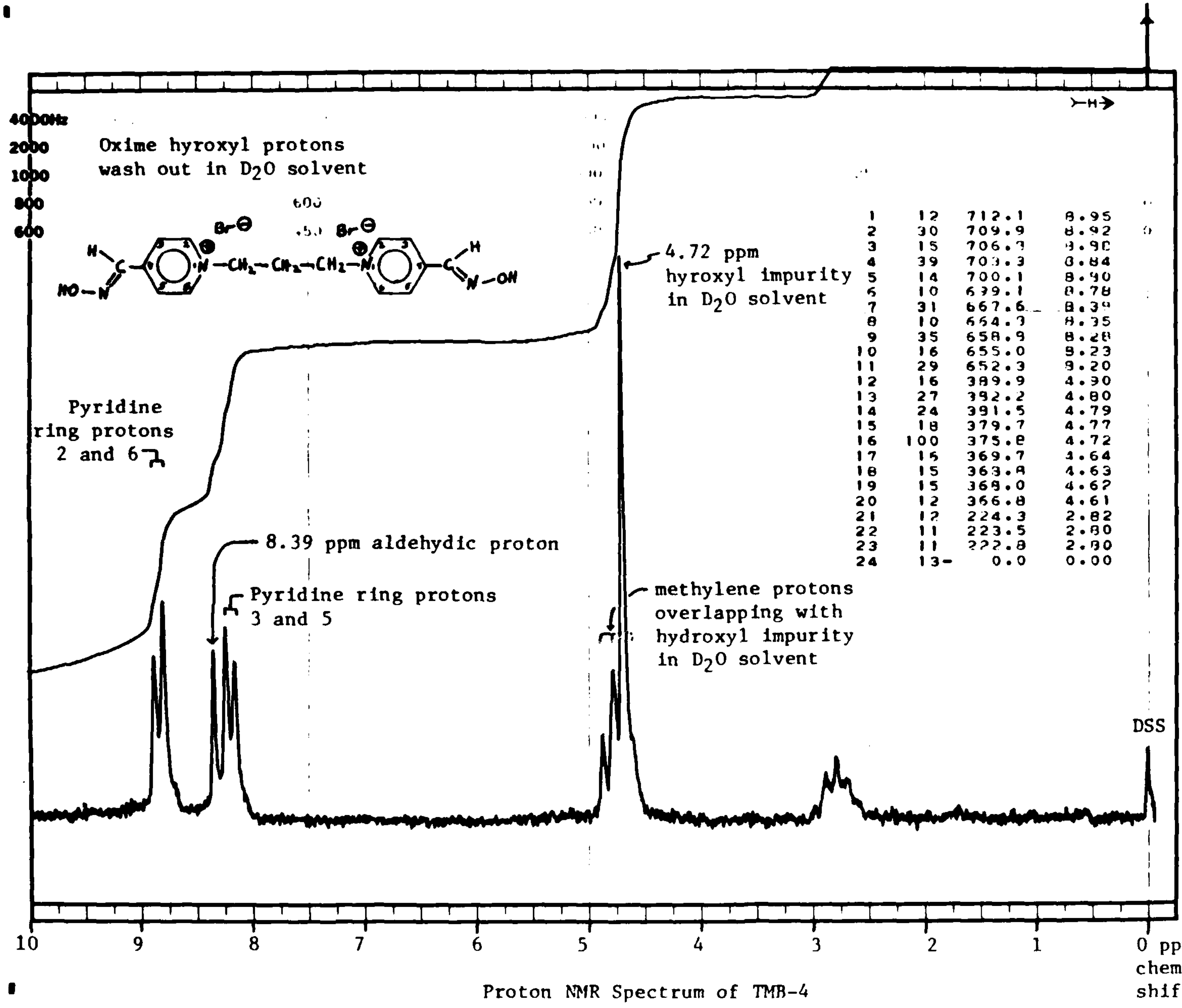
(Note: the pKa value in parentheses refer to values previously reported in the literature by other workers).

It is seen from the above tabulation that with increasing acidity (decreasing pKa) there is an increasing chemical shift of the aldehydic proton. This is as expected, since those electronic factors (electron withdrawal) which increase the acidity of the oxime group should concomitantly deshield the aldehydic proton and thus increase its chemical shift. Additionally it should be noted that these chemical shift data reinforce our pKa measurements, e.g., they are consistent with our finding that the pKa of 3-F-4-PAM is lower than that of TMB-4.

To complement our previous UV absorption spectral study of 2-PAM, 4-PAM, and 3-F-4-PAM (Annual Report, November 1982, Section IV) we additionally measured the UV absorption of TMB-4. The resultant spectrum is displayed in Figure 2 together with that of 3-F-4-PAM for comparison. As noted in our previous discussion of UV spectra (Annual Report, November 1982, Section IV) the mono-PAM compounds each display four absorption bands (Bands 1,2,3 and 4), as exemplified by 3-F-4-PAM. Curiously, TMB-4 displays a complete absence of Band 3 (see Figure 2) which at the present time we cannot explain. However, nearly superimposable curves for Band 4 and similar curves for Bands 1 and 2 serve to verify that for the most part in TMB-4 and 3-F-4-PAM strictly analogous electronic transitions inhere. It is worthy to note in Figure 2 that the Band 1 absorption of 3-F-4-PAM is somewhat stronger than that of TMB-4, which is consistent with a larger population of anionic 3-F-4-PAM molecules than anionic TMB-4 molecules, which in turn is consistent with a lower pKa value for 3-F-4-PAM than for TMB-4. This observation is complemented by the stronger Band 2 absorption of TMB-4 compared to 3-F-4-PAM. As explained in the previous discussion cited above, Band 2 absorption is attributable to the population of

PAM molecules whose oxime groups have not dissociated from their protons.

Finally, in the present quarter, using TMB-4 we tested Hagedorn's[5] nucleophilic reactivity test for potential acetylcholinesterase reactivators. As found in the previous quarter for 2-PAM, 3-F-4-PAM, and 4-PAM, in the presence of a 1000-fold excess of triethylphosphate at pH 7.40, TMB-4 reacts with triethylphosphate at best only very slowly and even after 20 hours (see Figure 3) the change in the UV spectrum is not large enough to afford useful data.



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 6   10   679.1   8.78
 7   31   667.6   8.39
 8   10   664.3   8.35
 9   35   658.9   8.28
10   16   655.0   8.23
11   29   652.3   8.20
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13   27   392.2   4.80
14   24   391.5   4.79
15   18   379.7   4.77
16  100   375.8   4.72
17   15   369.7   4.64
18   15   363.8   4.63
19   15   368.0   4.62
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TMB-4

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Proton NMR Spectrum of TMB-4

Fig. 1

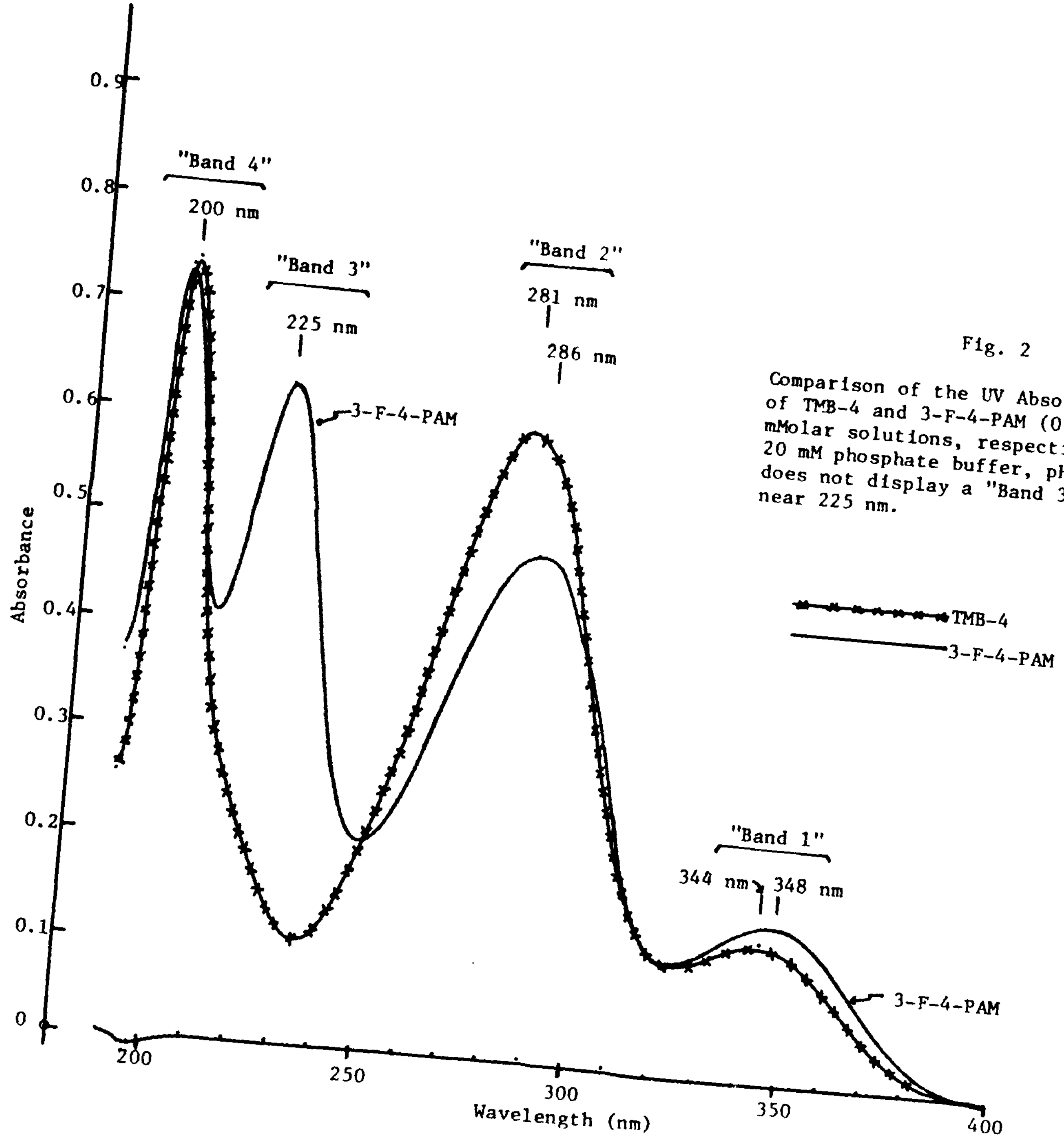


Fig. 2

Fig. 2

Comparison of the UV Absorption Spectra of TMB-4 and 3-F-4-PAM (0.020 and 0.040 mMolar solutions, respectively, each in 20 mM phosphate buffer, pH 7.40). TMB-4 does not display a "Band 3" absorption near 225 nm.

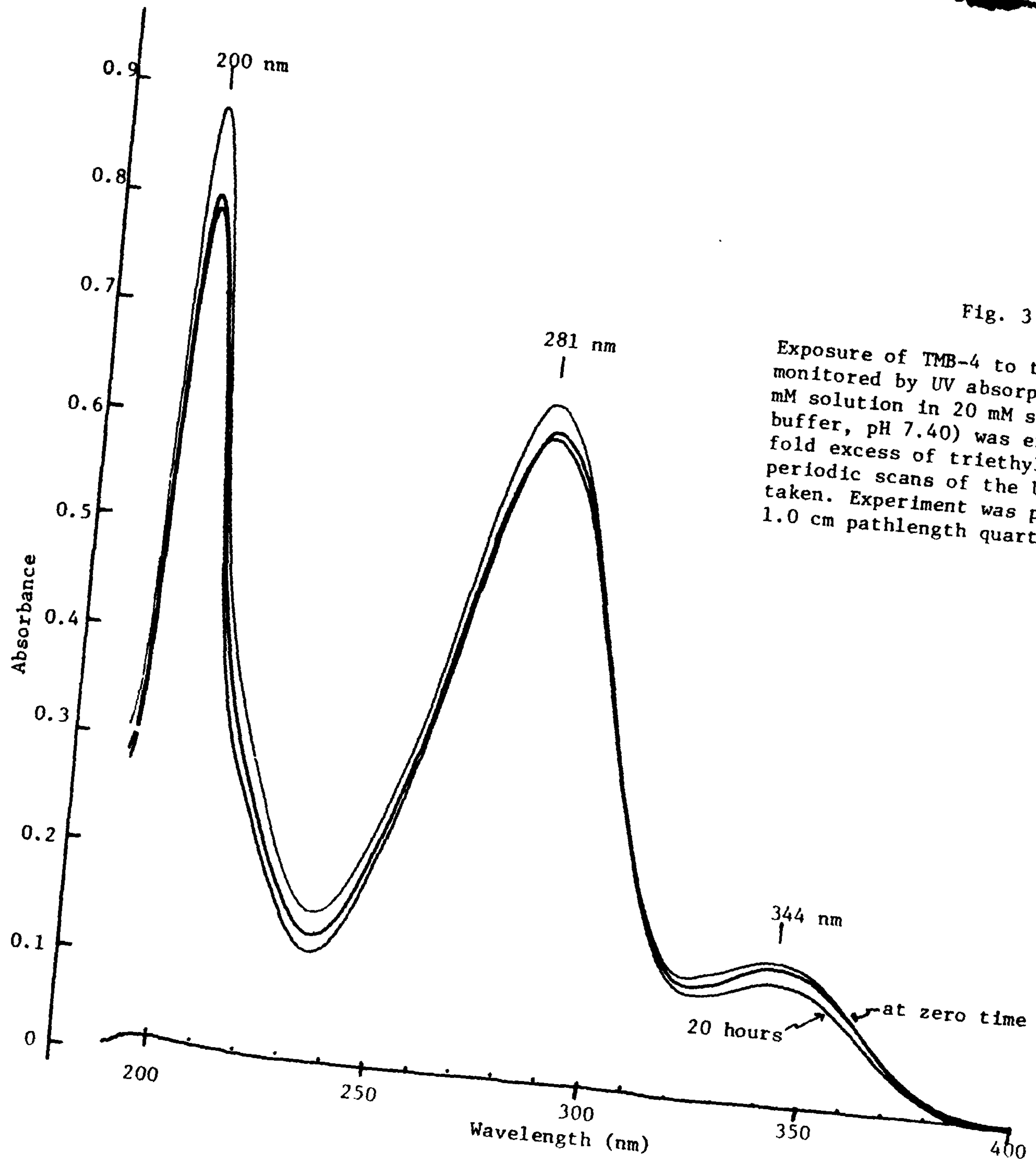


Fig. 3

Fig. 3

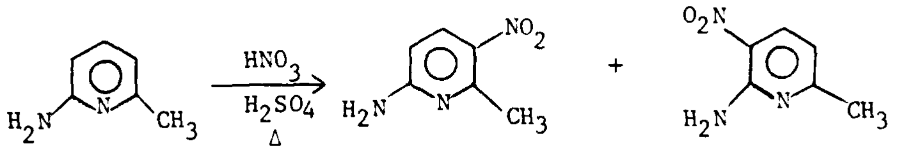
Exposure of TMB-4 to triethyl phosphate monitored by UV absorption. TMB-4 (0.020 mM solution in 20 mM sodium phosphate buffer, pH 7.40) was exposed to a 1000-fold excess of triethyl phosphate and periodic scans of the UV spectrum were taken. Experiment was performed in a 1.0 cm pathlength quartz cuvette.

SECTION II

Second Quarter

(December 1, 1982 - February 28, 1983)

Work in this quarter was aimed at scaling up the chemical reactions in the synthetic pathway to 3-F-2-PAM, as projected in Section I, First Quarter. During the present quarter, it was found that the method of nitration as reported in the literature[1,6] gave unexpectedly low yields of 3-nitro-6-amino-2-picoline (Isomer A) in the initial synthetic step (eq. 1). Consequently the choice had to be made either to improve the yield by modifying the synthesis or scaling up yet further the existing method.



(eq. 1)

Isomer A

Isomer B

6-amino-2-picoline
(Aldrich)

3-nitro-6-amino-2-picoline
m.p. 188-190°C
(lit. 188°C)

5-nitro-6-amino-2-picoline
(steam-volatile)
m.p. 153-155°C
(lit. 150-153°C; also 141°C)

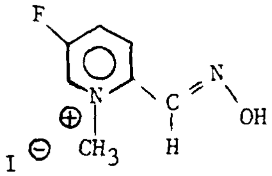
Previously, from a portion of our first nitration mixture, we successfully isolated Isomer A from the dark residue remaining after the more volatile Isomer B was steam-distilled off. The resultant quantity of A (4 g.) was utilized in pilot reactions outlined in Section I, First Quarter.

In the present quarter we ran two additional nitration batches (eq. 1), each starting with 125 g. of 6-amino-2-picoline, following the procedure of Parker & Shive plus modifications by Baumgarten & Su. It is necessary to mention that in this nitration, employing 125 g. of starting material per batch, the work-up and steam-distillation steps are considerably time-consuming. For example, due to the large amounts of concentrated acids utilized (730 ml per batch), neutralization with concentrated base must be carried out slowly (because highly exothermic), requiring almost a day. Additionally, a large quantity of sodium sulfate is generated which co-precipitates with the nitration products and which takes up space in the steam distillation flask. A modification proposed by Baumgarten & Su (op. cit., extraction of the nitration products with Ethanol) is in turn time-consuming because it requires large volumes (4-5 litres) of Ethanol, prolonged mixing, and subsequent removal of the Ethanol before steam distillation, all of which adds 2-3 days to the procedure. In our final nitration batch this quarter we tried rapid collection of the precipitates to minimize the amount of sodium sulfate co-precipitate, as alternatively suggested by Baumgarten & Su to save time. However, this technique has been reported by the same authors to reduce yields of the nitration product and this may account in part for a low yield in our second batch. Also, as explained in more detail in Section I, the steam-distillation step is quite inefficient and it requires about 6 gallons of steam-condensate to be collected per batch (where a batch is based on 125 g. of starting material). Using a 2-litre distillation flask and a manageable rate of steam input, about 1 gallon of steam-condensate is generated per work day.

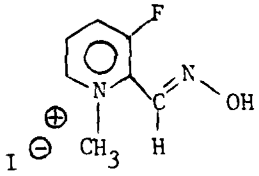
We obtained 9.3 g. and 9.2 g. (5% yields) of Isomer A per batch. We do not understand the discrepancy between these yields and those reported by Parker & Shive and by Baumgarten & Su (54-58%) since we followed as closely as possible their (somewhat vague) procedures, and resolution of this problem may require development of new synthetic procedures. In addition to Isomer A we isolated Isomer B (the steam-volatile isomer) in a yield of 16%, compared to 25% reported by Parker & Shive and 33% reported by Baumgarten & Su. We have also isolated a third crystalline nitration product (m.p. 220-225°C) in approximately 3% yield. This product was not mentioned by the above-cited previous workers and its identity needs to be established. Therefore this is a new result. Additionally, we have noted that the m.p. which we obtain for Isomer B (153-155°C) disagrees with that reported by Baumgarten & Su for the same isomer (141°C) but agrees with that of Parker & Shive (150-153°C). NMR analysis will be carried out to settle this question.

Our low yields so far for Isomer A is of concern because the amount of that isomer currently available is probably only enough to carry out the synthesis of approximately 0.5-1.0 g. of 3-F-2-PAM. This will be sufficient to physically characterize 3-F-2-PAM, but insufficient for biological studies. Therefore in the first month of the next quarter we intend to further investigate the synthesis of Isomer A in an attempt to increase its yield. In the succeeding months of the quarter we intend to carry the synthesis of 3-F-2-PAM to completion and physically characterize that compound, including a pKa determination.

It should be noted that our synthesis work above has yielded Isomer B (the steam-volatile isomer) in greater amounts than Isomer A. Isomer B can serve as the starting material for another F-PAM isomer, specifically 5-F-2-PAM, where the F atom is located at a position sterically remote from the oxime group. If the F atom in this position nevertheless electronically perturbs the pKa of the oxime group, then the reactivator behavior of this F-analog <u>versus</u> 2-PAM will constitute a clear test of the importance of pKa in 2-PAM without the steric ambiguities engendered by substitution adjacent to the oxime group.



5-F-2-PAM



3-F-2-PAM

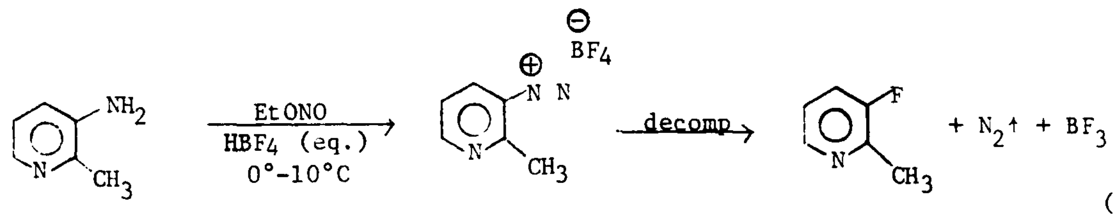
SECTION III

Third Quarter

(March 1 - May 31, 1983)

Work in this quarter focused on the synthesis and physical characterization of 3-Fluoro-2-PAM. As explained in Section II, Second Quarter, synthesis of this compound was impeded by unexpectedly low yields of the required nitration isomer ("Isomer A") in the initial synthetic step. Due to constraints in the amount of time investable in each F-PAM isomer in this contract, during the current quarter we decided to carry to completion the synthesis of 3-F-2-PAM regardless of low yield. This would at least allow us to perform some fundamental physical measurements on 3-F-2-PAM important to our systemmatic studies.

As outlined in detail in Section I, First Quarter, Isomer A (6-amino-3-nitro-2-picoline) is converted to 3-amino-2-picoline by way of 3 additional reaction steps, each of which in our hands proceeds in good yield (78-92%) The next step is conversion of 3-amino-2-picoline to 3-F-2-picoline via the diazotization-Schiemann reactions, which during this quarter we achieved in a 70.7% yield:

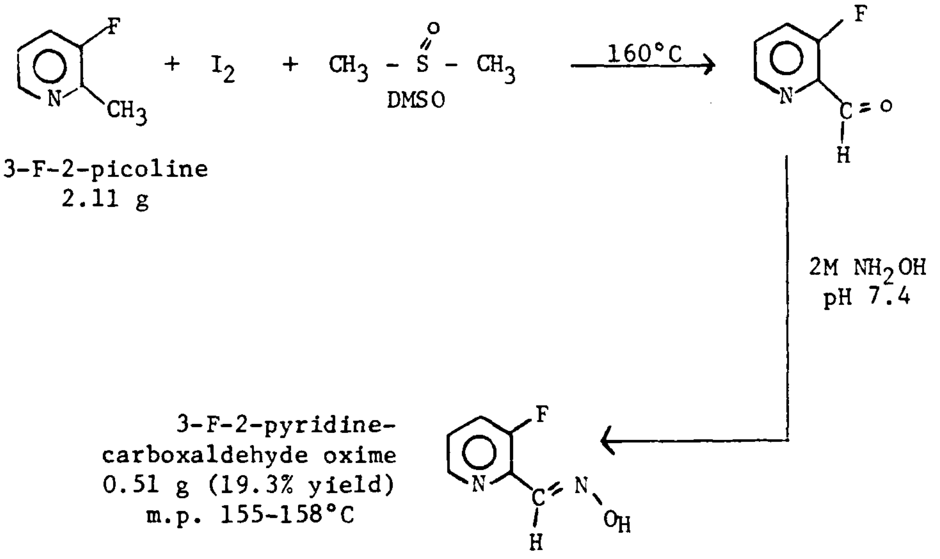


(Eq. 1)

3-amino-2-picoline
2.94 g

3-F-2-picoline
2.13 g
(70.7% yield)

Eq. 1 was followed by conversion of 3-F-2-picoline to 3-F-2-pyridinecarboxaldehyde oxime via application of the Markovac-Stevens-Ash-Hackley reaction:



(Eq. 2)

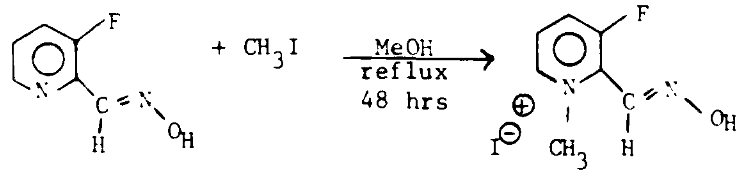
3-F-2-picoline
2.11 g

3-F-2-pyridine-
carboxaldehyde oxime
0.51 g (19.3% yield)
m.p. 155-158°C

The 3-F-2-pyridinecarboxaldehyde oxime, which we assume to be of the <u>syn</u>-configuration, is crystallized from aqueous ethanol and gives a satisfactory mass spectrum (Figure 4), including high resolution data. Specifically, for the M^+ peak, calculated for $C_6H_5N_2OF$: 140.038594; found: 140.038516; deviation = 0.56 ppm. For the $(M-H_2O)^+$ peak, calculated for $C_6H_3N_2F$: 122.028027; found: 122.028760; deviation = 0.27 ppm. These data are sufficient to prove the elemental composition of the above F-oxime product.

The proton NMR spectrum of 3-F-2-pyridinecarboxaldehyde oxime was obtained in $CDCl_3$ solvent (Figure 5) and in D_2O solvent (Figure 6). Spectral interpretations are included in the Figures. In $CDCl_3$ solvent the oxime hydroxyl proton gives a broad resonance around 8.99 ppm (Figure 5) which is washed out by deuterium exchange in D_2O solvent (Figure 6). The resonance of the aldehydic proton is located at 8.42 ppm (Figure 5). The corresponding aldehydic proton of the non-fluorinated parent compound (2-pyridinecarboxaldehyde oxime) is at 8.29 ppm (Annual Report, November 1982, Section III) and thus the presence of the F atom in the 3-position electronically deshields (shifts to downfield) the aldehydic proton as expected.

After the above structural evidence for 3-F-2-pyridinecarboxaldehyde oxime was obtained we quarternized the material by refluxing it with iodomethane in methanol:



(Eq. 3)

3-F-2-pyridine-
carboxaldehyde oxime

3-F-2-PAM, m.p. 178-180°C
0.32 g (38.7% yield)

Compared to quaternization of the 3-F-4-oxime under the same conditions (91% yield), this quaternization occurred in a relatively low yield (38%), possibly due to some decomposition of the quaternary product. The desired 3-F-2-PAM crystallizes satisfactorily from absolute ethanol, giving dull gold platelets, m.p. 178-180°C. A test sample of this material became discolored (darkened) upon being left out on the benchtop for a couple of days. However, the material is stable if stored in screw cap vials.

Elemental analysis of this material, performed by Schwarzkopf Microanalytical Laboratory, is satisfactory for 3-F-2-PAM: Calculated for $C_7H_8N_2OFI$: C,29.81; H,2.86; N,9.93; F,6.74; I,44.99. Found: C,30.06; H,3.00; N,10.00; F,6.46; I, 44.84.

The proton NMR spectrum of the material in D_2O solvent is consistent with the 3-F-2-PAM structure (Figure 7). Spectral interpretation is included in the Figure, the salient features of which are the intense singlet at 4.47 ppm due to the methyl group on the pyridine ring nitrogen, and the singlet at 8.59 ppm due to the aldehydic proton. Regarding the pyridine ring protons, which are all nonequivalent, they give distinct multiplets, each of whose location and splitting pattern accrues from a unique position on the pyridine ring.

An important datum from the NMR spectrum of 3-F-2-PAM is the chemical shift of the aldehydic proton, which is at 8.59 ppm from the DSS internal standard. This affords a direct comparison with 2-PAM as well as with the other PAM compounds measured in our systemmatic study:

Compound	Chemical Shift (aldehydic proton)
4-PAM	8.37 ppm
TMB-4	8.39
3-F-4-PAM	8.45
3-F-2-PAM	8.59
2-PAM	8.69

As is expected, the chemical shifts displayed by 3-F-2-PAM and 2-PAM are greater than those of the 4-PAM derivatives (4-PAM, TMB-4, and 3-F-4-PAM), due to the greater proximity of the aldehydic groups in the 2-position to the positively charged ring nitrogen atom (1-position). However, the fact that the chemical shift of 3-F-2-PAM is <u>less</u> than that of 2-PAM is contrary to our initial expectation that placement of an F atom in the 3-position of 2-PAM would increase the chemical shift via an electron-withdrawing effect of the F atom. The observed result is all the more unexpected since, as noted earlier above, the F atom in the nonquaternized precursor does shift the aldehydic proton downfield relative to that in the nonfluorinated parent compound. Upon quaternization, however, the chemical shift of the nonfluorinated compound (2-PAM) surpasses that of the F-compound. At the present time the simplest speculative explanation for this finding is that quaternization (n-methylation) has a differential steric effect on the two compounds, specifically with respect to the torsional angle between the methylated pyridine ring and the oxime group. If ring-oxime coplanarity is less favored in the F-compound than in the nonfluorinated compound this could ramify itself to a decreased chemical shift.

In this connection it was of interest to compare the pKa of 3-F-2-PAM with that of 2-PAM, since so far in our studies we have found a positive correlation between aldehydic proton chemical shift and oxime acidity. Unfortunately, we have discovered that 3-F-2-PAM behaves in an unstable manner during pKa determination (titration with 0.10 N NaOH). Upon incremental additions of NaOH the pH rises as expected for partial neutralization but then quickly falls as if an acid is being generated in situ. Titration of 2-PAM during the same experiment displayed normal behavior. Due to the limited amount of 3-F-2-PAM in our possession, we have not as yet investigated its aberrant titration behavior.

Finally, we measured the UV absorption spectrum of 3-F-2-PAM, which is shown in Figure 8 together with that of 2-PAM for comparison. As discussed previously, (Annual Report, November 1982, Section IV), PAM compounds characteristically display four absorption bands (Bands 1,2,3 and 4) and these are readily discernable in Figure 8. With respect to the two short wavelength bands (Bands 3 and 4, at 225 and 199 nm, respectively) the absorption curves of 3-F-2-PAM and 2-PAM are virtually superimposable. However, with respect to the two longer wavelength bands (Bands 1 and 2), those of 3-F-2-PAM are somewhat broadened and red-shifted relative those of 2-PAM. The intensity of Band 1 of 3-F-2-PAM (350 nm) is slightly greater than that of 2-PAM (335 nm), suggesting that the acidity of 3-F-2-PAM equals or slightly exceeds that of 2-PAM.

During the next quarter (Section IV, Fourth Quarter), we intend to synthesize another F-PAM compound in our series, 5-F-2-PAM. The requisite nitration isomer (Isomer B, 5-nitro-6-amino-2-picoline) was previously isolated by steam-distillation and is available in sufficient quantities to support a larger-scale synthesis (grams) of the target compound.

ID: MS9/APR29,MASS,6799,11
LABEL: ARMY-3, MCLICK UCSF

NP(I): 180982 BP(I): 180496 TI: 1976928
NP(M): 83 BP(M): 83.029

Mass Spectrum of 3-F-2-pyridinecarboxaldehyde oxime

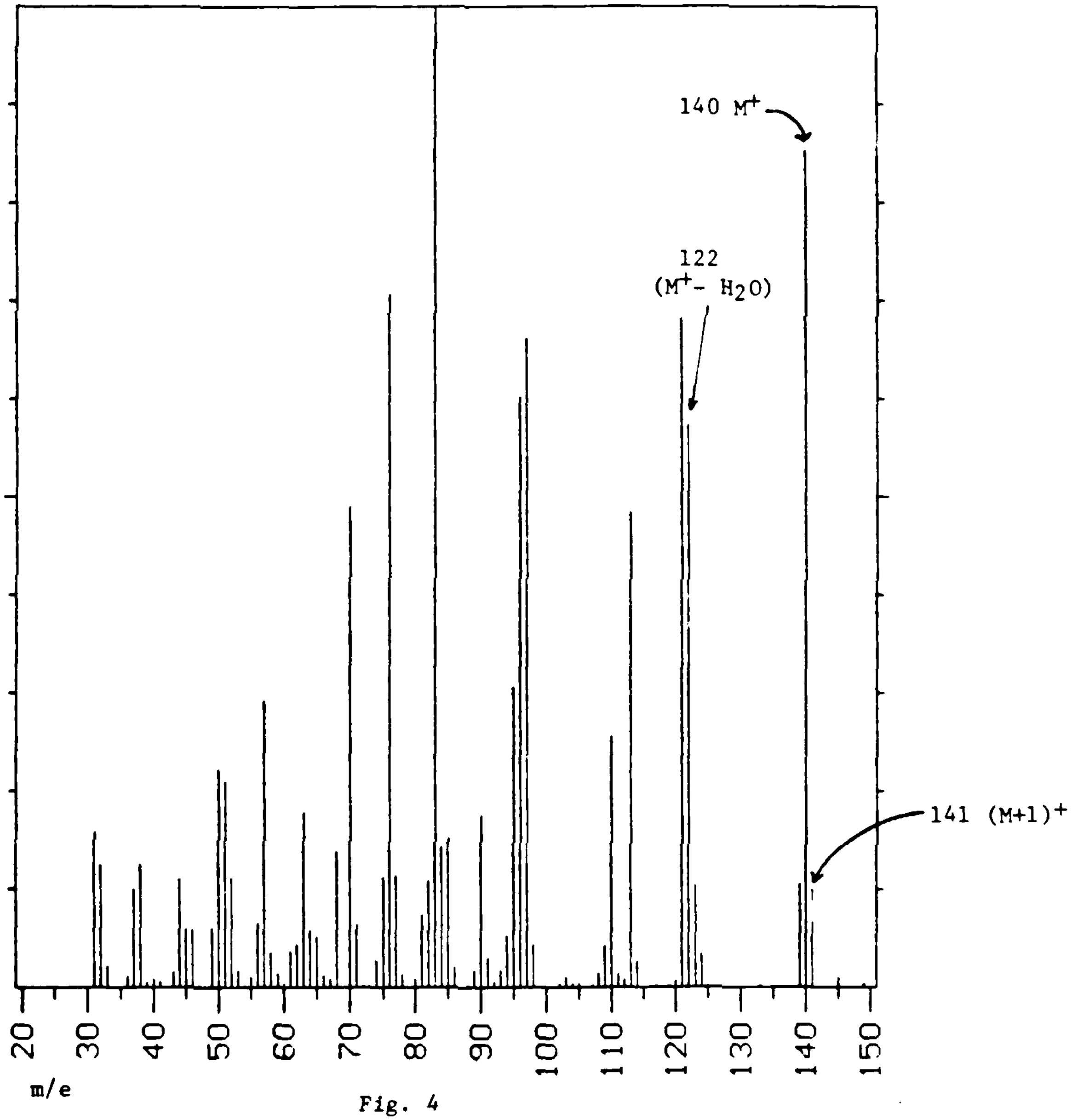


Fig. 4

Proton NMR Spectrum of 3-F-2-pyridinecarboxaldehyde oxime in $CDCl_3$ solvent

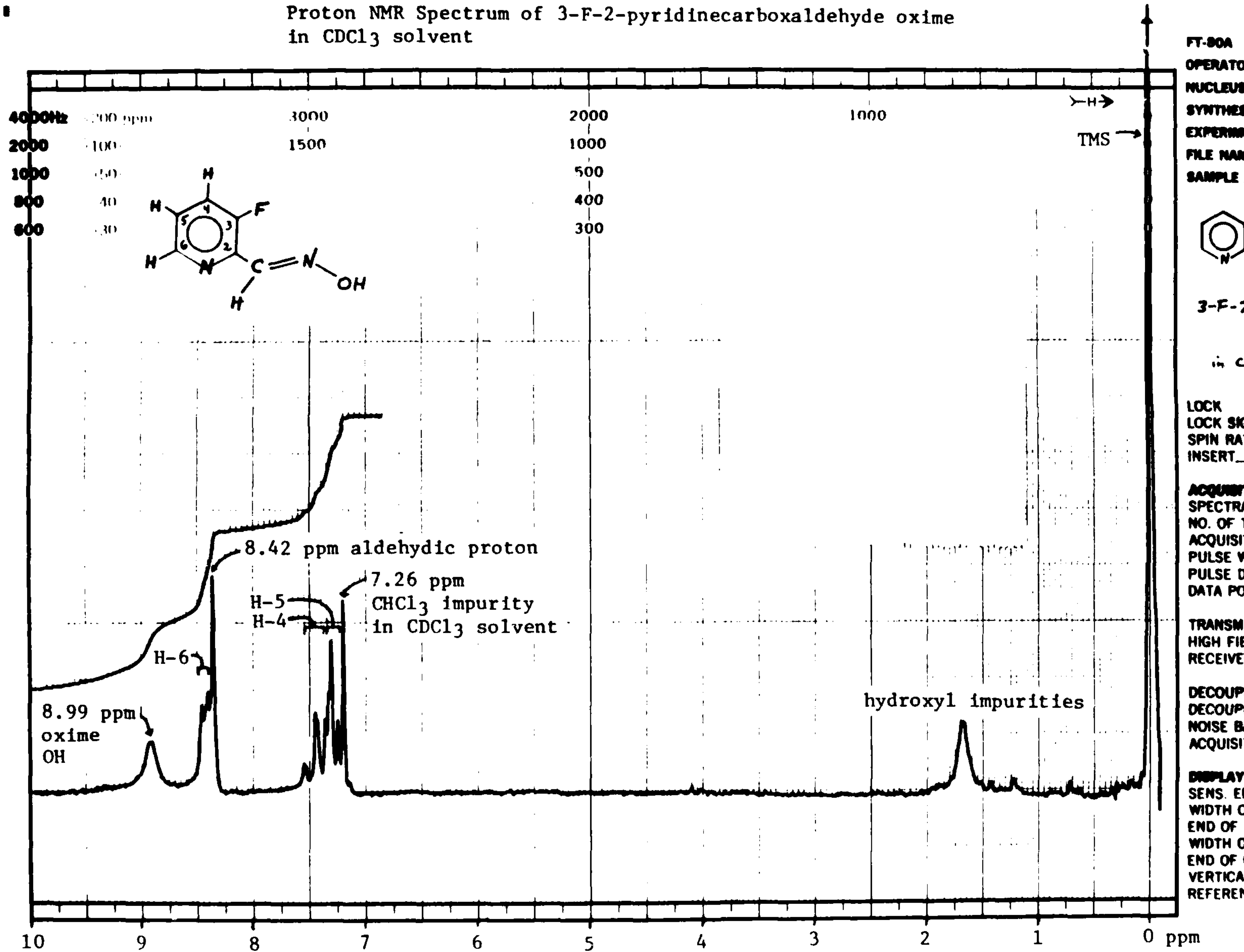
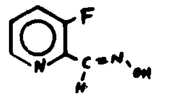


Fig.5

FT-80A SPECTRUM NO. J-24
OPERATOR J. McLick DATE 4/13/83
NUCLEUS 1H FREQUENCY 80 MHz
SYNTHESIZER SETTING –
EXPERIMENT NAME –
FILE NAME –
SAMPLE 3-F-2-Pyr carboxaldehyde oxime



in $CDCl_3$ solvent

3-F-2-Pyridine carboxaldehyde Oxime (0.040 M)

in $CDCl_3$ solvent (TMS int std)

LOCK ☑ INTERNAL ☐ EXTERNAL
LOCK SIGNAL $CDCl_3$
SPIN RATE 6 rps. TEMP amb °C
INSERT 5 mm

ACQUISITION
SPECTRAL WIDTH (SW) 1000 Hz
NO. OF TRANSIENTS (NT) 50
ACQUISITION TIME (AT) 4.095 sec.
PULSE WIDTH (PW) 4.5 μsec.
PULSE DELAY (PD) 1.0 sec.
DATA POINTS (DP) 8192

TRANSMITTER OFFSET (TO) 44
HIGH FIELD ✓ LOW FIELD
RECEIVER GAIN (RG) 8

DECOUPLER MODE (DM) –
DECOUPLER OFFSET (DO) –
NOISE BANDWIDTH (NB) – kHz
ACQUISITION MODE (AM) –

DISPLAY
SENS. ENHANCEMENT (SE) -1.5 sec.
WIDTH OF PLOT (WP) 850 Hz
END OF PLOT (EP) -50 Hz
WIDTH OF CHART (WC) 800 Hz
END OF CHART (EC) 0 Hz
VERTICAL SCALE (VS) 500
REFERENCE LINE (RL) 0.00 (TMS)

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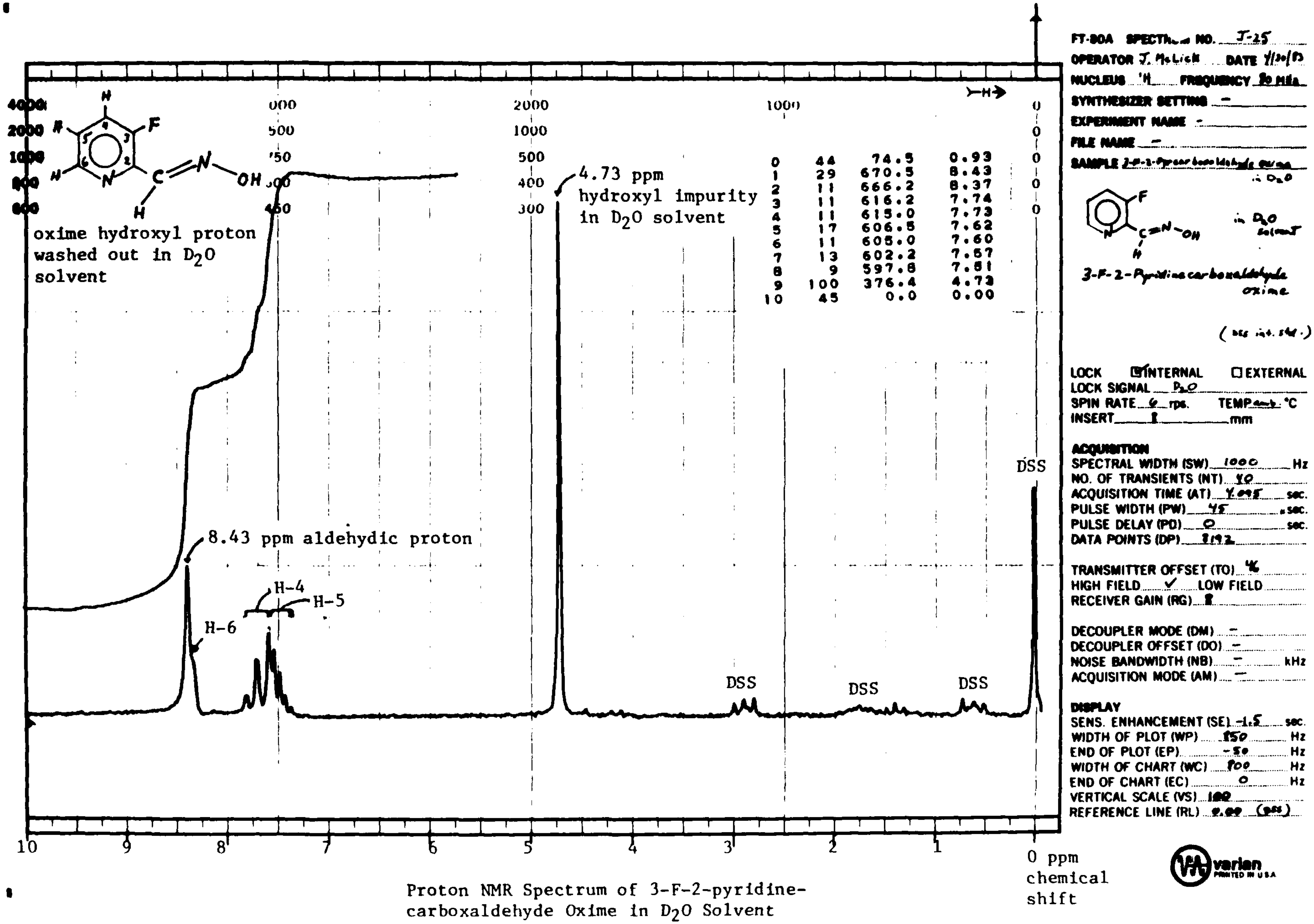
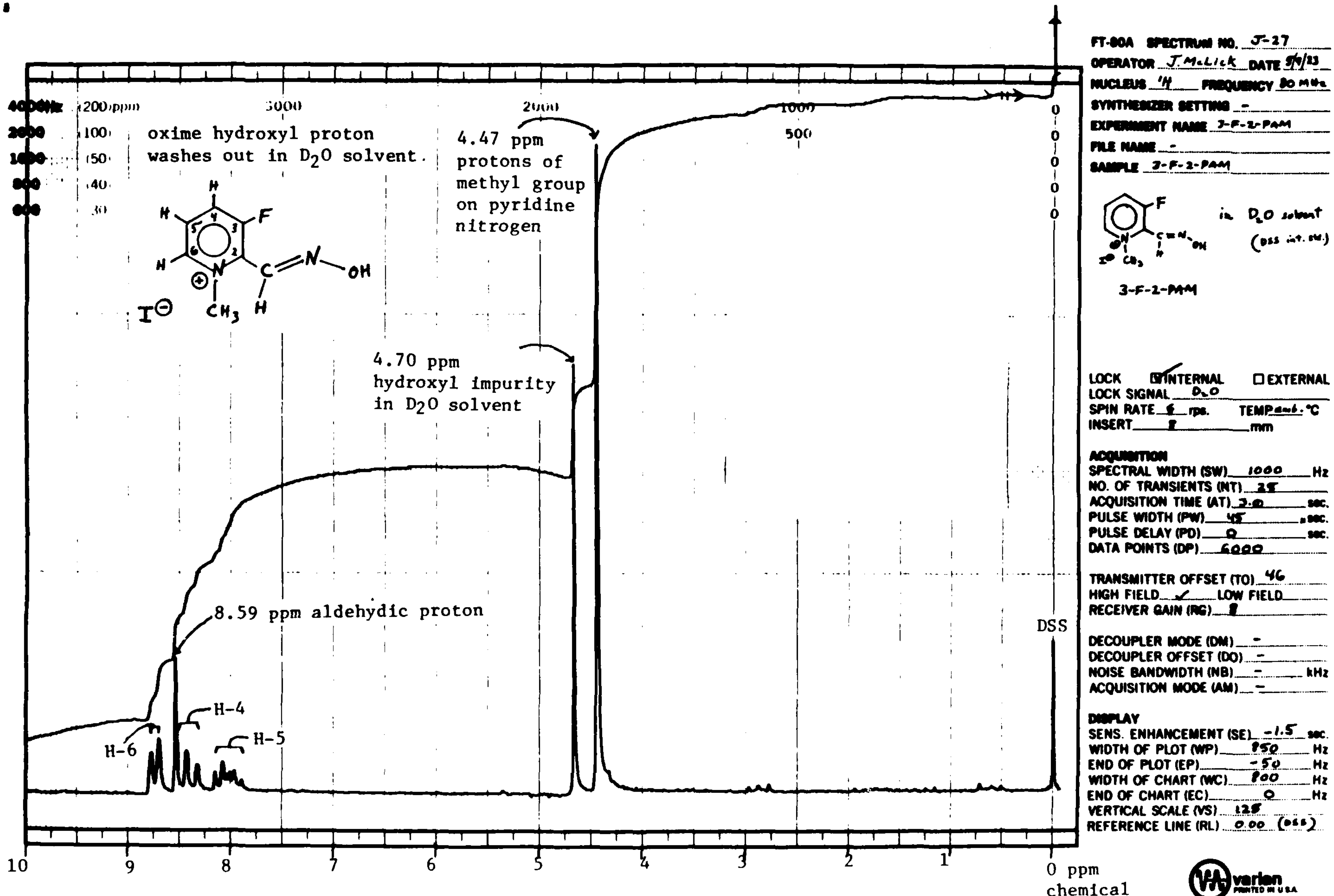


Proton NMR Spectrum of 3-F-2-pyridine-carboxaldehyde Oxime in D_2O Solvent

Fig. 6



FT-80A SPECTRUM NO. J-27
OPERATOR J. McLick DATE 5/9/83
NUCLEUS ¹H FREQUENCY 80 MHz
SYNTHESIZER SETTING -
EXPERIMENT NAME 3-F-2-PAM
FILE NAME -
SAMPLE 3-F-2-PAM

in D_2O solvent (DSS int. std.)

LOCK ☑ INTERNAL ☐ EXTERNAL
LOCK SIGNAL D_2O
SPIN RATE 6 rps. TEMP amb. °C
INSERT 8 mm

ACQUISITION
SPECTRAL WIDTH (SW) 1000 Hz
NO. OF TRANSIENTS (NT) 25
ACQUISITION TIME (AT) 3.0 sec.
PULSE WIDTH (PW) 45 μsec.
PULSE DELAY (PD) 0 sec.
DATA POINTS (DP) 6000

TRANSMITTER OFFSET (TO) 46
HIGH FIELD ✓ LOW FIELD
RECEIVER GAIN (RG) 8

DECOUPLER MODE (DM) -
DECOUPLER OFFSET (DO) -
NOISE BANDWIDTH (NB) - kHz
ACQUISITION MODE (AM) -

DISPLAY
SENS. ENHANCEMENT (SE) -1.5 sec.
WIDTH OF PLOT (WP) 850 Hz
END OF PLOT (EP) -50 Hz
WIDTH OF CHART (WC) 800 Hz
END OF CHART (EC) 0 Hz
VERTICAL SCALE (VS) 125
REFERENCE LINE (RL) 0.00 (DSS)

Proton NMR Spectrum of 3-F-2-PAM

Fig. 7

Fig. 8

Comparison of the UV Absorption Spectra of 2-PAM and 3-F-2-PAM (0.04 mMolar solutions in 20 mM phosphate buffer, pH 7.40).

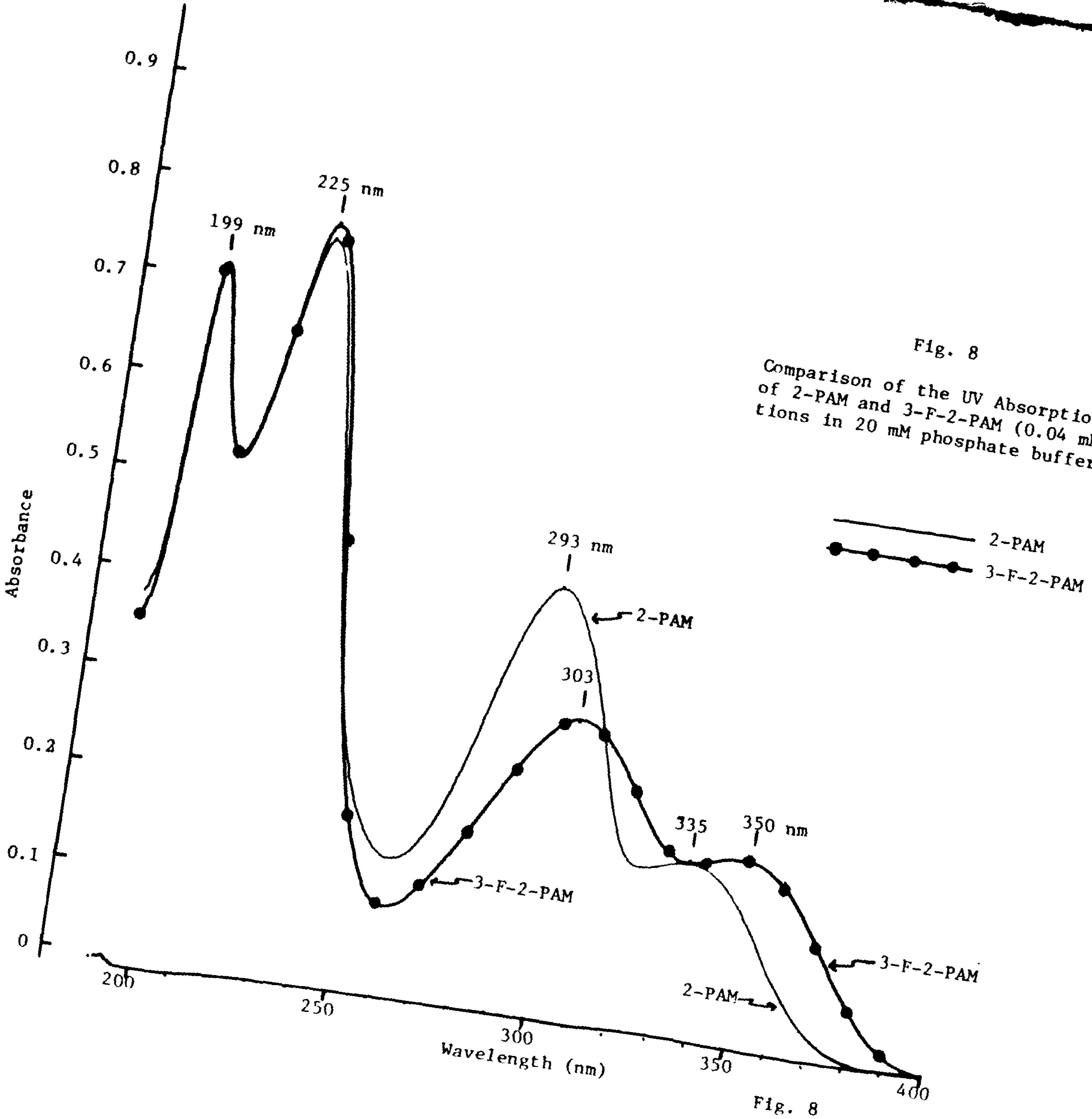


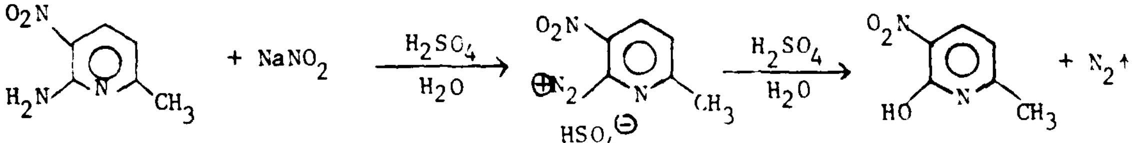
Fig. 8

Section IV

Fourth Quarter

June 1 - August 31, 1983

During this quarter synthesis of 5-F-2-PAM was carried out, thereby obtaining the last of the F-PAM isomers currently proposed in our studies. As mentioned in Sections II and III, the requisite amino-picoline nitration isomer ("Isomer B", 6-amino-5-nitro-2-picoline) was isolated by steam distillation in larger amounts than its companion nitration isomer, 6-amino-3-nitro-2-picoline. In the present quarter the 5-nitro compound was converted to 6-hydroxy-5-nitro-2-picoline in 64% yield.

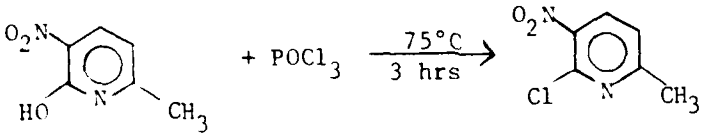


(eq.1)

6-amino-5-nitro-2-picoline
29.9 g
195 mMoles

6-hydroxy-5-nitro-2-picoline
19.3 g (64% yield)
m.p. 222-224°C

The hydroxy compound was then converted to 6-chloro-5-nitro-2-picoline using phosphorus oxychloride:

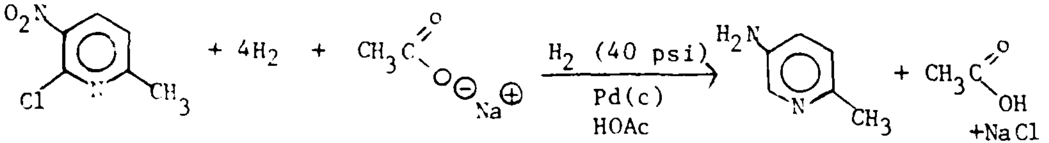


(Eq. 2)

19.25 g
(x2 batches)

6-chloro-5-nitro-2-picoline
14.13 g (65% yield) x 2 batches
m.p. 69-72°C

Subsequently the 6-chloro-5-nitro-2-picoline was reduced to 5-amino-2-picoline in the Parr apparatus:



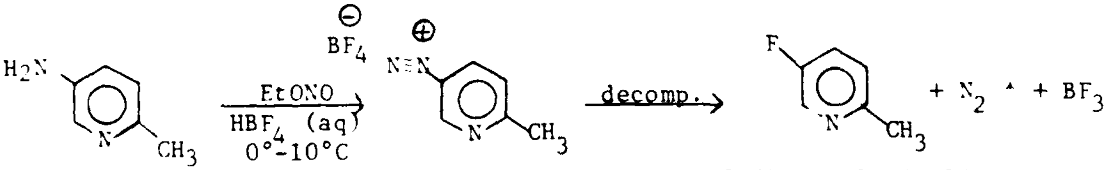
(Eq. 3)

+NaCl
+H_2O

11.0 g (x2 batches)

5-amino-2-picoline
11.02 g (total of 2 batches)
(79.9% yield)
m.p. 95-98°C

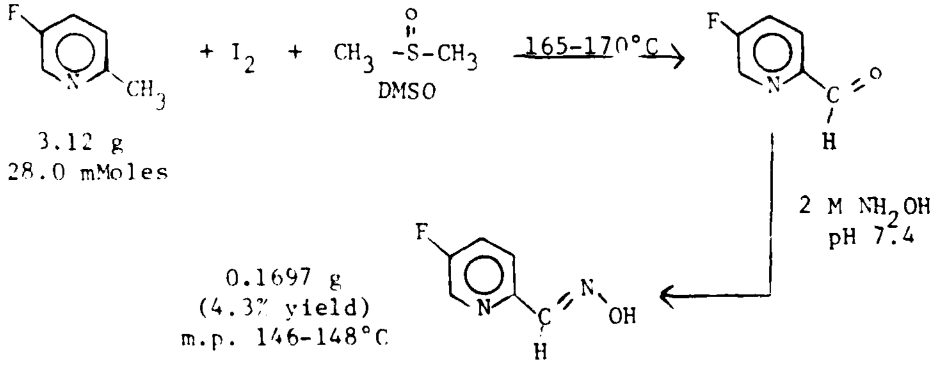
The 5-amino-2-picoline was then converted to 5-fluoro-2-picoline by way of the Schiemann reaction, followed by conversion to 5-F-2-pyridinecarboxaldehyde oxime using the Markovac-Stevens-Ash-Hackley method.



(Eq. 4)

5.40 g (x2 batches)

5-fluoro-2-picoline
3.12 g (total yield, 28.1%)



(Eq. 5)

3.12 g
28.0 mMoles

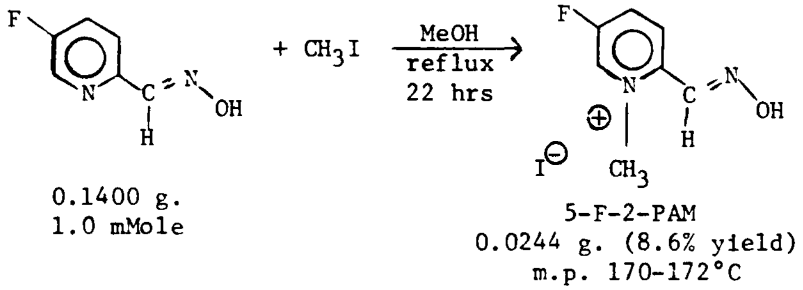
0.1697 g
(4.3% yield)
m.p. 146-148°C

5-F-2-pyridinecarboxaldehyde oxime

As can be seen, the yield of the carboxaldehyde oxime is very poor (4.3%). It was noticed that the thermal reaction of the 5-F-2-picoline-I_2 complex in DMSO was less vigorous (including evolution of dimethylsulfide) than in the case of previous F-picolines and required somewhat hotter temperatures (165-170°C). During workup the test for aldehyde product (dinithrophenylhydrazine reagent) was positive as expected, but it was noticeably weaker than in the case of previous Markovac-Stevens-Ash-Hackley reactions. That the reaction in the case of 5-F-2-picoline is markedly less efficient than the other 2-picolines may be related to the observation of Markovac-Stevens-Ash-Hackley[7] that the reaction fails where there is a hydroxy or dimethylamino group as a 5-substituent in the 2-picoline substrate. (However, 5-chloro-2-picoline reacts normally). This effect seems to be related to the presence of relatively high electron-withdrawing groups in the 5-position.

Although a low yield of the aldehyde (and subsequently its oxime derivative) was obtained, it was sufficient to crystallize the oxime from aqueous ethanol (yellow crystals, 0.1697 g., m.p. 146-148°C). A sample of this material has been submitted for high resolution mass spectral analysis and the results are given on p. 38 of this report. The proton NMR spectrum of this material is shown in Figure 9. A spectral interpretation is included in the Figure. In this spectrum (in $CDCl_3$ solvent) the oxime hydroxyl proton gives a broad resonance around 7.98 ppm (which is washed out by deuterium exchange in D_2O solvent, spectrum not shown), and the aldehydic proton gives a sharp singlet at 8.23 ppm. The corresponding aldehydic proton of the nonfluorinated parent compound (2-pyridinecarboxaldehyde oxime) is at 8.29 ppm (Annual Report, November 1982, Section III), and thus the presence of the F atom in the 5-position does not have any deshielding effect on the aldehydic proton. (By contrast, in 3-F-2-pyridinecarboxaldehyde oxime (reported in Section III, Third Quarter), the aldehydic proton resonates at 8.42 ppm, and thus the F atom in the 3-position deshields the aldehydic proton).

After the above NMR spectrum was obtained, the 5-F-2-pyridinecarboxaldehyde oxime was quaternized by refluxing it with iodomethane in methanol:



(Eq. 6)

Curiously, this reaction also gave a poor yield (8.6%). The quaternized species was crystallized from ethanol/ether solvent to give an olive-drab product. A sample of this material has been submitted for high resolution mass spectral analysis and the results are given on p. 38 of this report. The proton NMR spectrum of this material in D_2O solvent is given in Figure 10. Compared to its nonquaternized precursor (Figure 9) the resonances of the pyridine ring protons of 5-F-2-PAM (Figure 10) have lost much of their resolution. However, the expected methyl group singlet is clearly present (at 4.42 ppm) as well as the singlet due to the aldehydic proton (at 8.66 ppm). It is useful to compare the latter value with the analogous chemical shifts of the aldehydic protons of 3-F-2-PAM (Section III, Third Quarter) and 2-PAM. These values are tabulated as follows:

Compound	Chemical Shift (aldehydic proton)
2-PAM	8.69 ppm
3-F-2-PAM	8.59
5-F-2-PAM	8.66

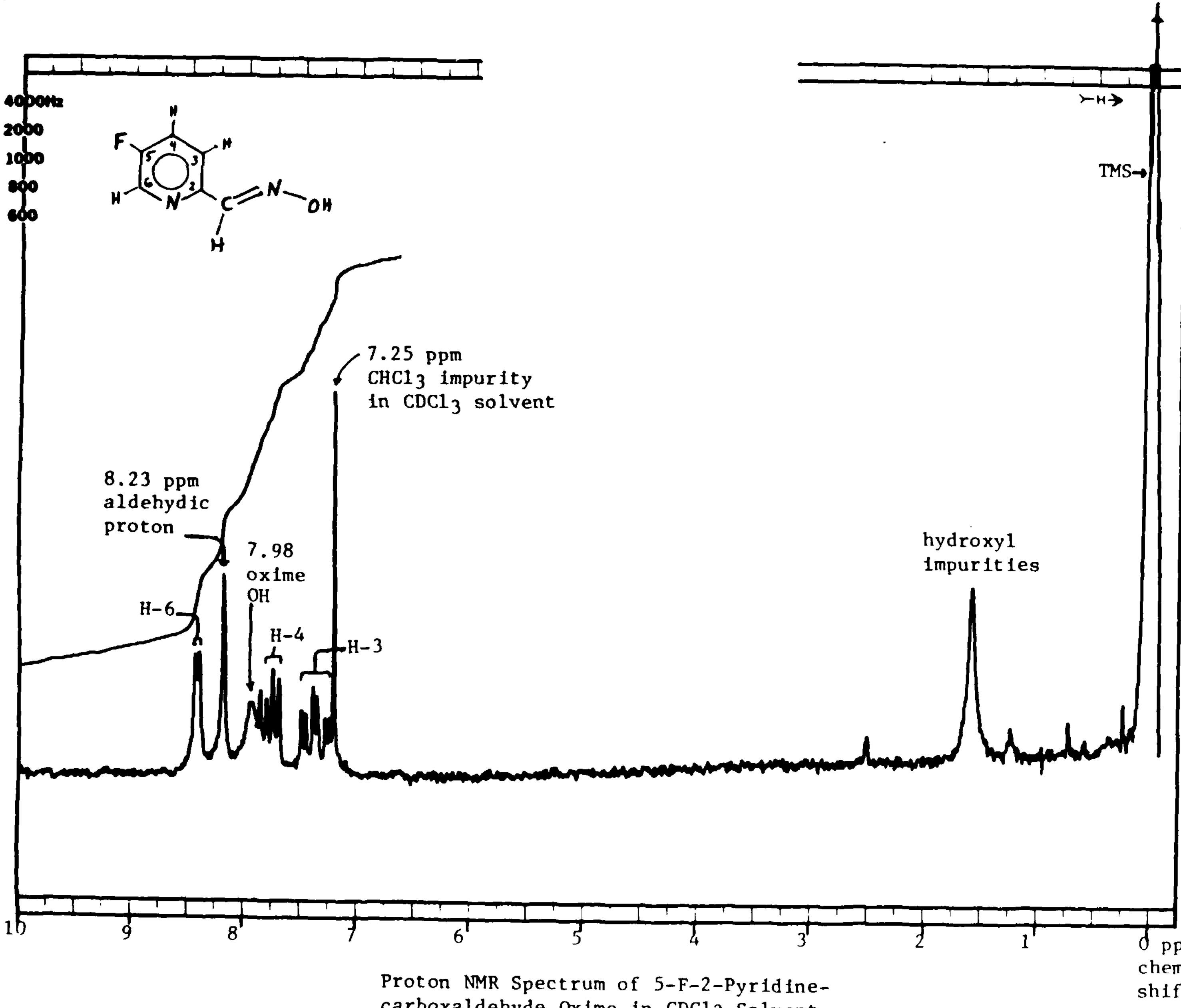
It is seen that the chemical shift of 5-F-2-PAM is essentially the same as that of 2-PAM, and thus the F atom does not exert any significant deshielding effect on the aldehydic proton. This is unexpected but is consistent with the earlier observation (above) that in the nonquaternized precursor (5-F-2-pyridinecarboxaldehyde oxime) the F atom does not effectively deshield the aldehydic proton. Concerning the other F-derivative of 2-PAM (3-F-2-PAM), it was speculated in Section III, Third Quarter, that upon quaternization (N-methylation) when F is in the 3-position it sterically causes the oxime group to be twisted out of coplanarity with the pyridine ring. Such twisting could decrease the electron-withdrawing and deshielding effect.

In studying further the properties of 5-F-2-PAM and 2-PAM, it was desirable to compare the pKa values of these compounds. Because such a small amount of 5-F-2-PAM was available it was necessary to determine the pKa values by UV spectrometry rather than by potentiometric titration. As a preliminary requirement, the UV absorption spectrum of 5-F-2-PAM was determined and compared with that of 2-PAM. These spectra are shown in Figure 11. As with the other mono-PAM compounds studied, 5-F-2-PAM displays four separate absorption bands, and with the F substituent present the first two bands (Band 1 at 342 nm and Band 2 at 300 nm) are somewhat red-shifted relative to the analogous bands of 2-PAM (335 nm and Band 2 at 294 nm). The other pair of bands (Band 3 at 225 nm and Band 4 at 199 nm) occur at essentially the same wavelength in both compounds independent of the presence or absence of the F atom.

Band 1 constitutes the UV absorption of the oxime group in the dissociated (anionic) form and Band 2 constitutes the absorption of the oxime group in the undissociated (neutral) form. For spectrometric determination of pKa we chose Band 1 as the analytical band (344 nm for 5-F-2-PAM and 336 nm for 2-PAM), and measured the absorbance of a series of 0.040 mMolar solutions of the PAM compounds over a range of pH's, using Tris-phosphate buffers. (The absorbance of the PAM compounds with their populations completely in the anionic form was determined in 0.10 M KOH solution (pH 12.5) and with their populations completely in the undissociated form in 0.10 M HCl (pH 2.1). Copies of the spectrometric worksheets for these pKa determinations are included in this report (Figure 12 and 13) for their informational content and are not intended for formal publication since they follow already established pKa measurement techniques.

For 2-PAM we obtained a pKa value of 7.77 ± 0.06 and for 5-F-2-PAM we obtained a pKa value of 7.62 ± 0.05. (Note: the value for 2-PAM is lower than that reported in the literature by other workers as well as by us using potentiometric titration (pKa 8.00); by contrast Hagedorn, Stark and Lorenz report a value of 7.68 for 2-PAM). These data indicate, contrary to the NMR data which show no significant electronic deshielding (electron-withdrawal) effect by the F atom, that the F atom does lower the pKa of the 2-oxime. However, the extent of this lowering is only 0.15 pKa units.

Due to poor synthetic yields, a quantity of 5-F-2-PAM sufficient to carry out biochemical tests is currently not available. However it is hoped that the purely physical chemical studies reported in this quarter will be helpful in choosing or eliminating future research ideas as well as extending systematic studies.



FT-80A SPECTRL). J-28
OPERATOR J McLick DATE 1/11/83
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EXPERIMENT NAME -
FILE NAME -
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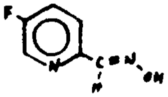

in CDCl3 solvent

5-F-2-pyridinecarboxaldehyde oxime (0.040 M) in CDCl3 (TMS int std)

LOCK ☑ INTERNAL ☐ EXTERNAL
LOCK SIGNAL CDCl3
SPIN RATE 6 rps. TEMP amb. °C
INSERT 8 mm

ACQUISITION
SPECTRAL WIDTH (SW) 1000 Hz
NO. OF TRANSIENTS (NT) 75
ACQUISITION TIME (AT) 4.095 sec.
PULSE WIDTH (PW) 4.5 μsec.
PULSE DELAY (PD) 1.00 sec.
DATA POINTS (DP) 8192

TRANSMITTER OFFSET (TO) 44
HIGH FIELD ✓ LOW FIELD
RECEIVER GAIN (RG) 8

DECOUPLER MODE (DM) -
DECOUPLER OFFSET (DO) -
NOISE BANDWIDTH (NB) - kHz
ACQUISITION MODE (AM) -

DISPLAY
SENS ENHANCEMENT (SE) -1.5 sec
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END OF PLOT (EP) -50 Hz
WIDTH OF CHART (WC) 800 Hz
END OF CHART (EC) 0 Hz
VERTICAL SCALE (VS) 2000
REFERENCE LINE (RL) 0.00 (TMS)

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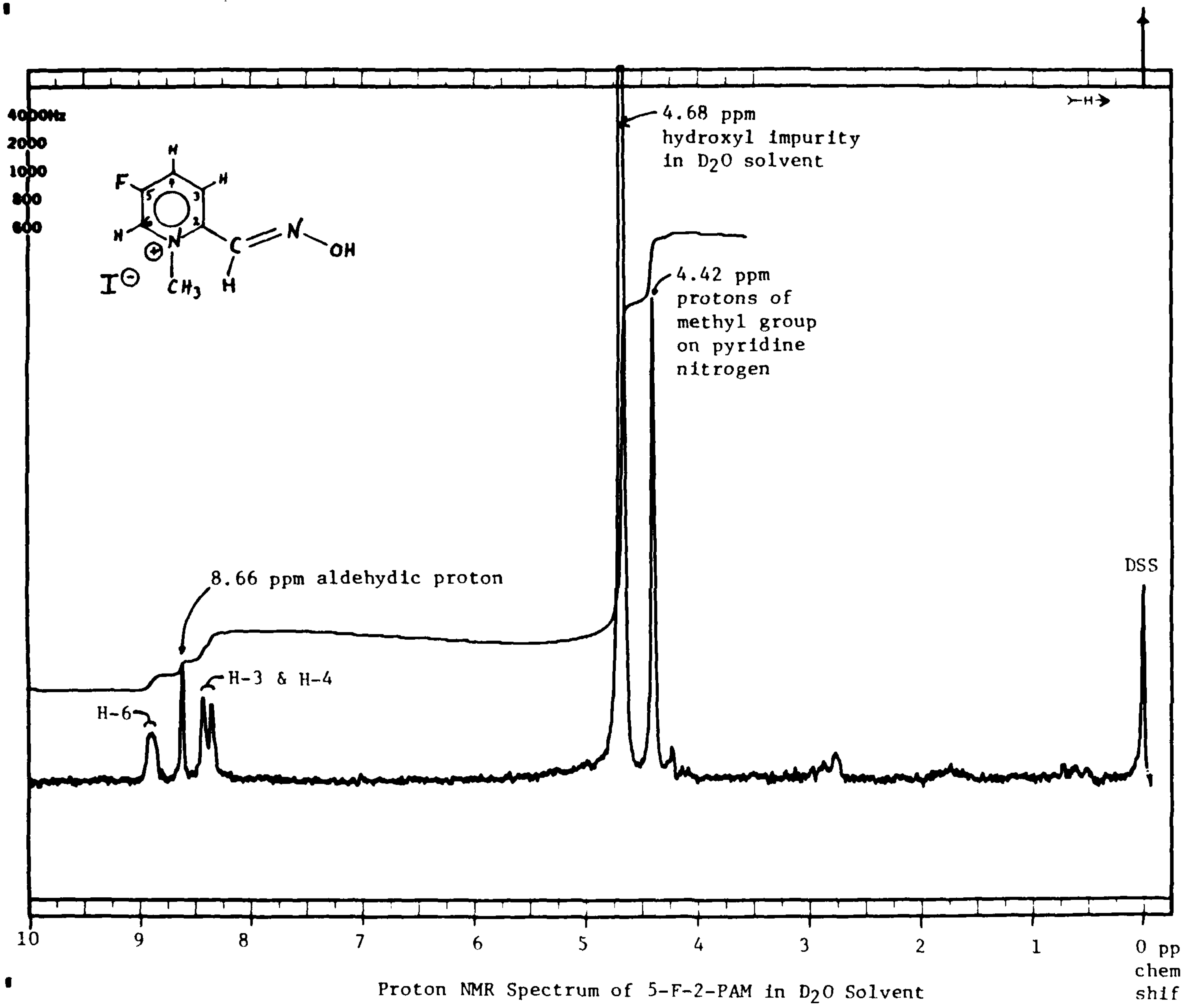
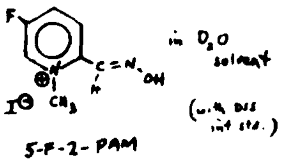
Proton NMR Spectrum of 5-F-2-Pyridinecarboxaldehyde Oxime in CDCl3 Solvent

Fig. 9



FT-80A SPECTRUM J-31
OPERATOR J. McLeca DATE 1/31/78
NUCLEUS ¹H FREQUENCY 80 MHz
SYNTHESIZER SETTING -
EXPERIMENT NAME -
FILE NAME -
SAMPLE 5-F-2-PAM



in D_2O solvent
(with DSS int std.)

5-F-2-PAM

LOCK ☑ INTERNAL ☐ EXTERNAL
LOCK SIGNAL D_2O
SPIN RATE 6 rps. TEMP °C
INSERT 8 mm

ACQUISITION
SPECTRAL WIDTH (SW) 1000 Hz
NO OF TRANSIENTS (NT) 100
ACQUISITION TIME (AT) 4.095 sec.
PULSE WIDTH (PW) 4.5 μsec.
PULSE DELAY (PD) 0 sec.
DATA POINTS (DP) 8192

TRANSMITTER OFFSET (TO) 46
HIGH FIELD ✓ LOW FIELD
RECEIVER GAIN (RG) 8

DECOUPLER MODE (DM) -
DECOUPLER OFFSET (DO) -
NOISE BANDWIDTH (NB) - kHz
ACQUISITION MODE (AM) -

DISPLAY
SENS ENHANCEMENT (SE) -4.5 sec
WIDTH OF PLOT (WP) 150 Hz
END OF PLOT (EP) -50 Hz
WIDTH OF CHART (WC) 100 Hz
END OF CHART (EC) 0 Hz
VERTICAL SCALE (VS) 500
REFERENCE LINE (RL) 0.00 (DSS)

varian PRINTED IN U.S.A.

Proton NMR Spectrum of 5-F-2-PAM in D_2O Solvent

Fig. 10

Fig. 11

Comparison of the UV Absorption Spectra of 2-PAM and 5-F-2-PAM (0.040 mMolar solutions in 20 mM phosphate buffer, pH 7.40).

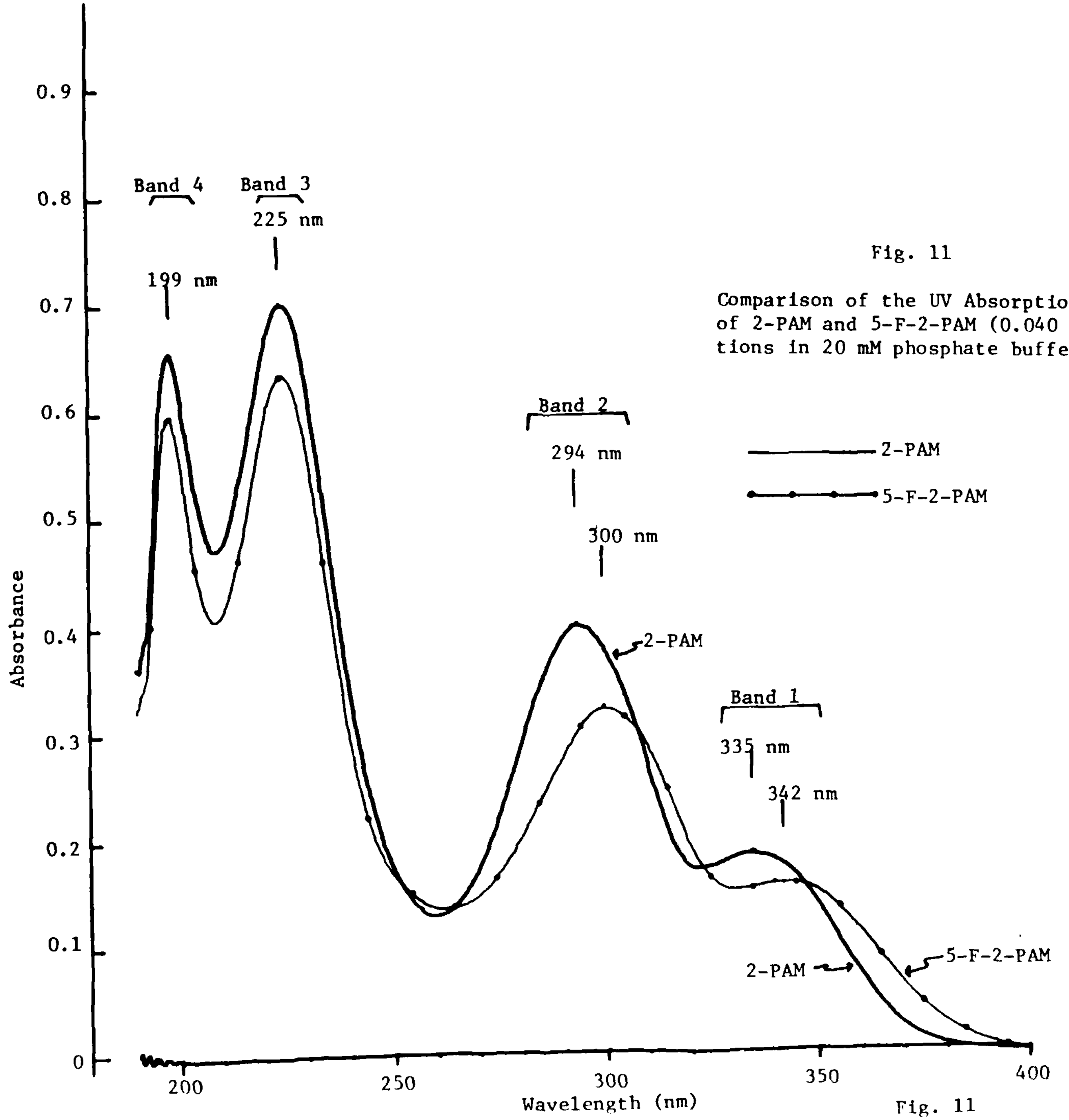
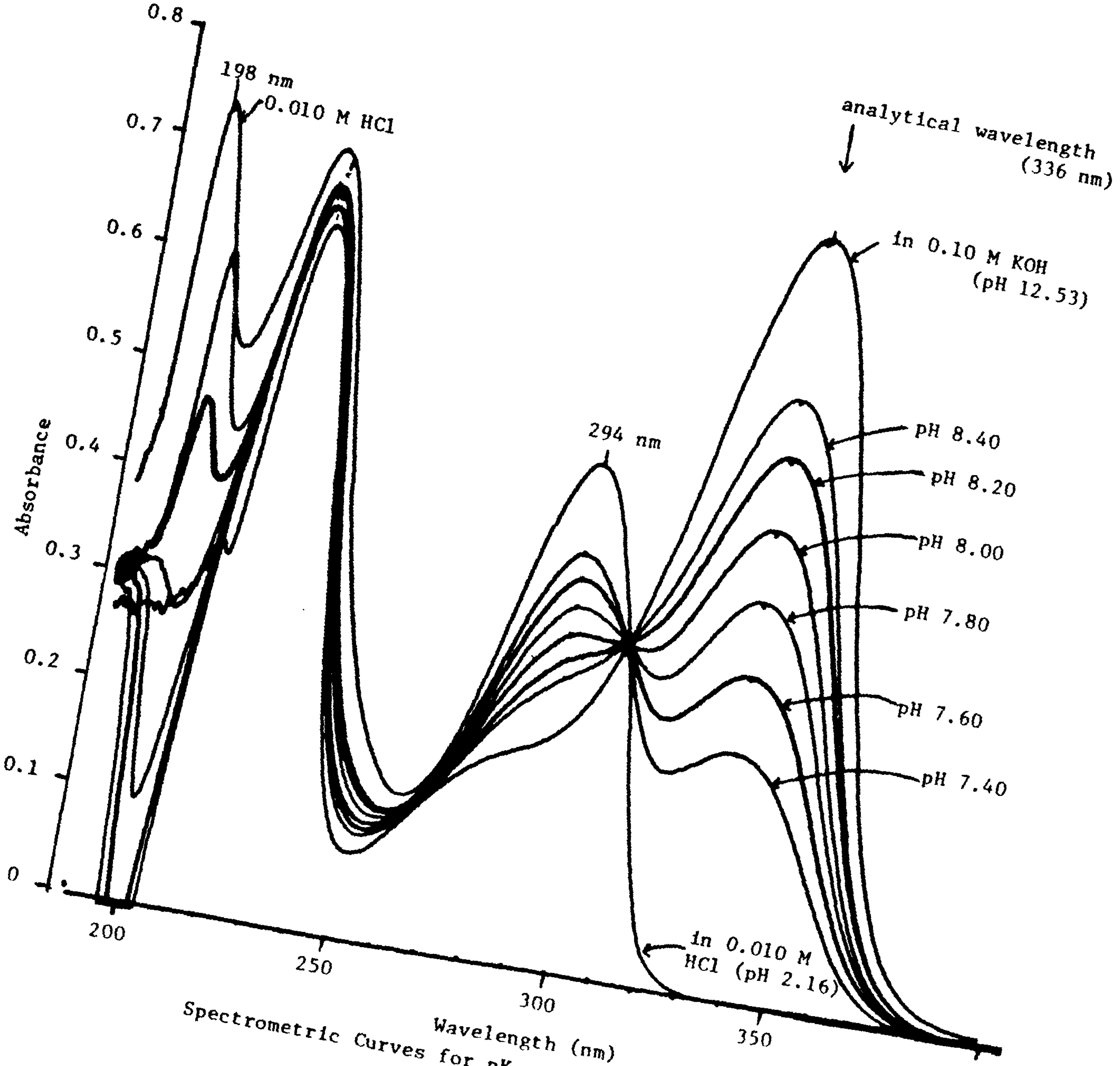
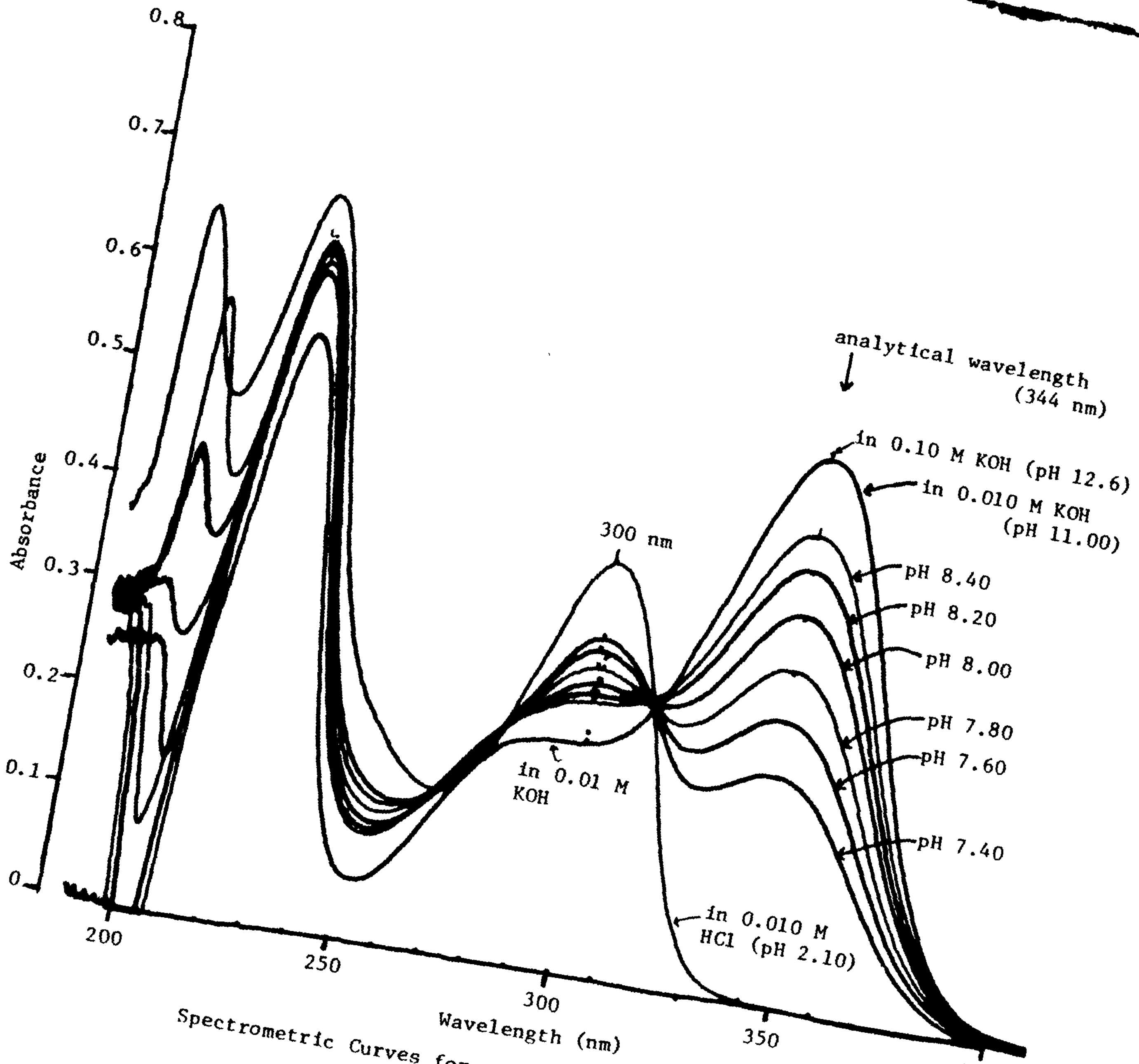


Fig. 11



Spectrometric Curves for pKa Determination of 2-PAM

Fig. 12



Spectrometric Curves for pKa Determination of 5-F-2-PAM

Fig. 13

Addendum

Mass spectral results for 5-F-2-pyridinecarboxaldehyde oxime and for 5-F-2-PAM.

The low-resolution mass spectrum obtained for 5-F-2-pyridinecarboxaldehyde oxime is shown in Figure 14. The base peak of the spectrum corresponds to the oxime molecular ion (M^+) at m/e 140 and the expected water loss peak ($M^+ -H_2O$) also is evident (m/e 122). High-resolution data for this compound: M^+ peak, calculated for $C_6H_5N_2OF$: 140.038594; found: 140.037996 (deviation = 4.28 ppm); ($M^+ -H_2O$) peak, calculated for $C_6H_3N_2F$: 122.028027; found: 122.027967 (deviation = 0.51 ppm). These data are sufficient to prove the elemental composition of 5-F-2-pyridinecarboxaldehyde oxime.

The low-resolution mass spectrum obtained for 5-F-2-PAM is shown in Figure 15. For this compound "sputtering ion mass spectrometry" (SIMS, alternatively called "fast atom bombardment" (FAB) was employed. This relatively new spectroscopy technique is utilized for relatively nonvolatile compounds. The sample is suspended in a glycerol matrix and bombarded with fast atoms which displace the sample molecules into the gas phase. Only a low resolution spectrum was carried out for 5-F-2-PAM. This spectrum shows an intense peak at m/e 155 which corresponds to 5-F-2-PAM whose iodide ion has been lost, i.e., the peak represents the positively charged quaternary pyridinium ion. This spectrum does not rigorously prove the 5-F-2-PAM structure but strongly supports it.

ID: SEP27,MASS,3072,1
LABEL: #3512 AF1H-4 HREI MOLID [illegible]

NP(I): 102483 BP(I): 102483 TI: 418002
NP(M): 140 BP(M): 140.039

Low-Resolution Mass Spectrum of 5-F-2-Pyridinecarboxaldehyde Oxime

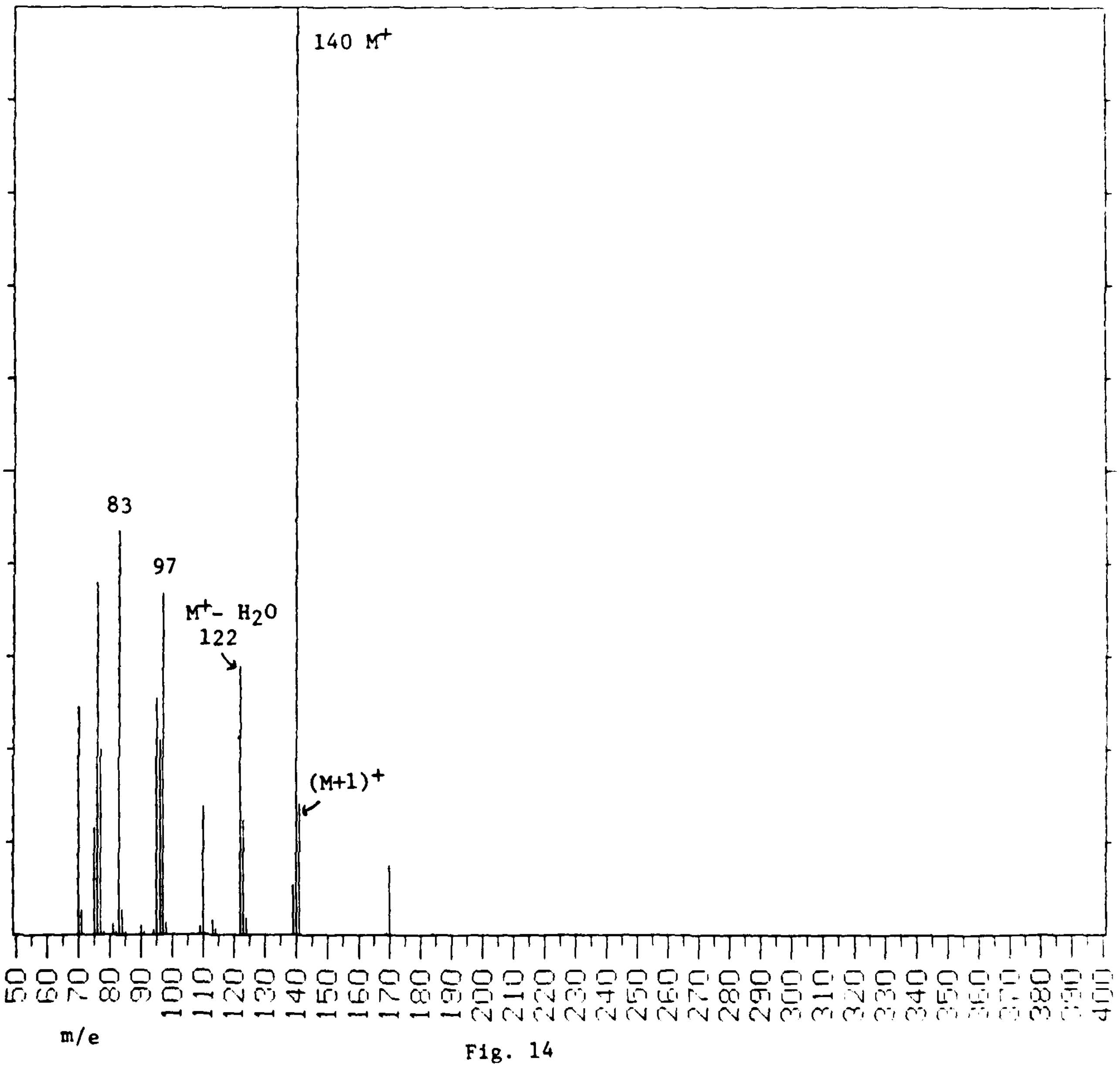
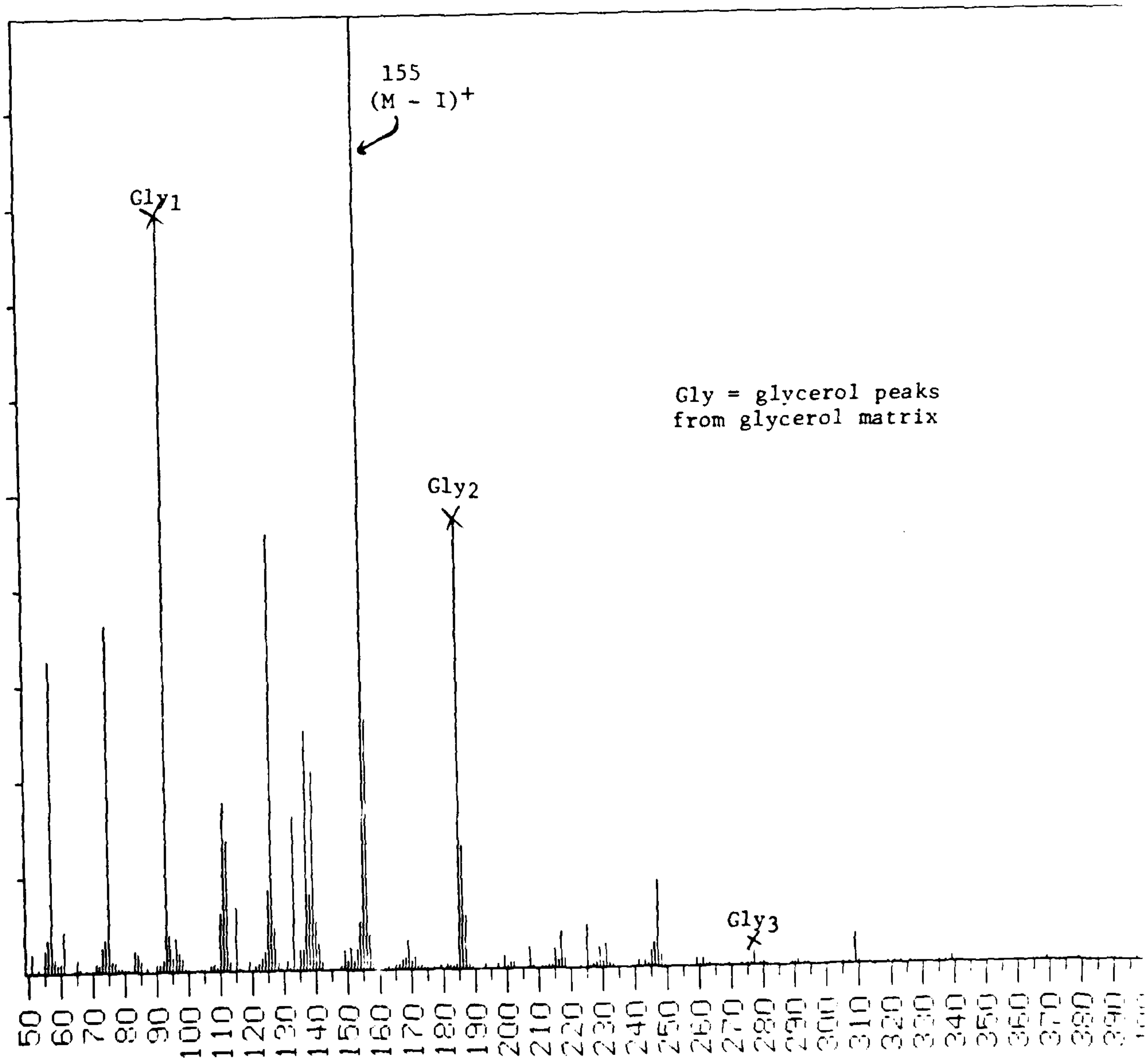


Fig. 14

```
   ID: SEP29,MASS,3075,8
LABEL: =8520 AR 1Y-5, SIMS FDS

NP(I): 620452          BP(I): 618142          TI: 4743375
NP(M): 155             BP(M): 155.035
```

Low-Resolution SIMS Spectrum of 5-F-2-PAM



Gly = glycerol peaks
from glycerol matrix

m/e

Fig. 15

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DAT
FILM