SYNTHESIS OF THE ISOAMYL ESTER OF 7-N-ACETYL-11'-HYDROXY-3'-DEMETHYLLAVENDAMYCIN

An Honors Thesis (HONRS 499)

by

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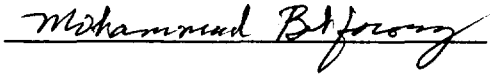

Ball State University

Muncie, Indiana

April 27, 1992

Date of Graduation: May 2, 1992

PURPOSE OF THESIS

The purpose of this project is to synthesize the isoamyl ester of 7-N-acetyl-11'-hydroxy-3'demethyllavendamycin which may potentially be an antitumor drug. Similar compounds have been previously made in our laboratory, but this new compound has two distinctly different groups attached to the N-acetyllavendamycin backbone. These groups will hopefully increase the solubility of the compound in water, thus making it a more practical drug for use in humans, as well as increasing its selectivity in destroying the cancer cells relative to the normal cells. The new compound will be sent to Eli Lilly for testing first in vitro, and then in animals to determine its selectivity and toxicity. Lavendamycin-based drugs offer some hope in treating certain kinds of human cancers for which there are no drugs at the present time.

ACKNOWLEDGEMENTS

I would like to thank Dr. Mohammad Behforouz for being an excellent instructor, as well as a patient supervisor. He always made sure that I understood what I was doing!

Wen Cai gave me more help than I ever could ask for. I appreciated her help, and I am especially grateful to her for "keeping me on my toes". This synthesis was carried out much easier with her perfectionist style.

Thanks also to Sandy, Mohammad, and Charmaine for helping me with some of those very necessary "day to day" tasks. Also, thanks to David Bir for your help on the nmr and mass spectroscopy analyses.

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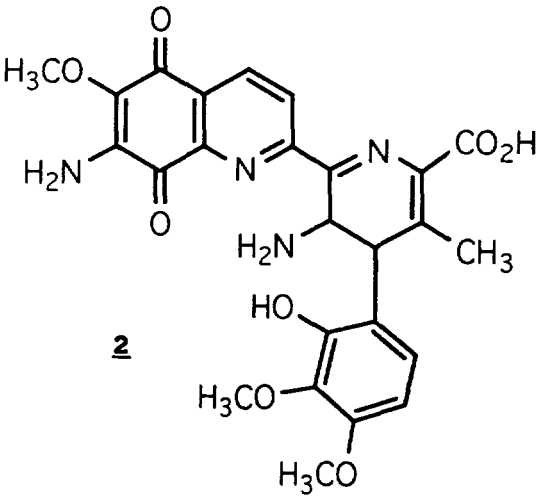
HISTORICAL

LAVENDAMYCIN

I. BACKGROUND

The antitumor, antibiotic Lavendamycin (1a) bears a close resemblance to the potent antitumor, antibiotic Streptonigrin (2). Lavendamycin was first isolated in 1981 from Streptomyces lavendulae strain c22030. Lavendamycin was obtained as a dark, red solid, mp > 300°, with only limited solubility in organic solvents.[1] As an antibiotic, Lavendamycin is generally less potent than Streponigrin. The exceptions lie in the lavendamycin inhibition of Trichophton and Microsporum.



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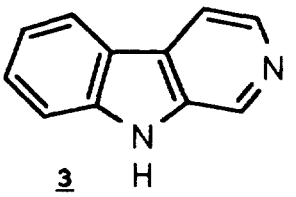

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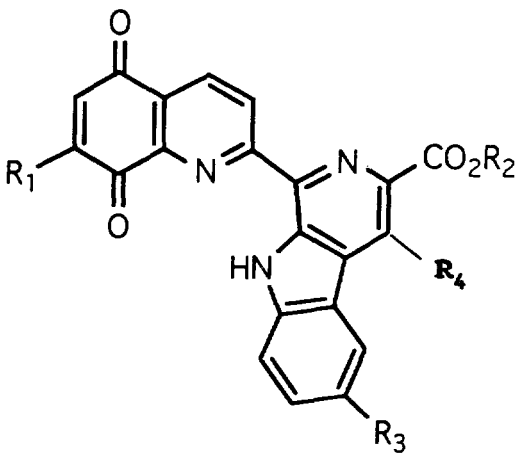


Compound	R_1	R_2	R_3	R_4
1a	NH_2	H	H	CH_3
1b	NH_2	CH_3	H	CH_3
4	NHAc	CH_3	H	CH_3
5	NHAc	$(CH_2)_2CH(CH_3)_2$	OH	H

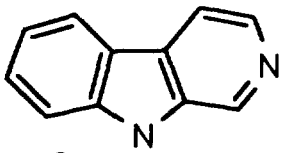
Table 1[2]

minimum inhibitory concentration (μg/ml)

Organism	strain	Lavendamycin	Streptonigrin
Trichophyton rubrum	WW	0.5	8
Trichophyton mentagrophytes	A-9870	0.5	4
Microsporum canis	A-9872	0.5	16
Microsporum canis	A-22494	0.25	4

The potential clinical usage of both Lavendamycin and Streptonigrin as antitumor drugs has been unsuccessful due to their toxicity.[2]

Lavendamycin has a ß-carboline backbone(3). ß-carbolines and their derivatives possess a number of behavioral and neurochemical activities.[3]



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II. PHARMACOLOGICAL STUDIES

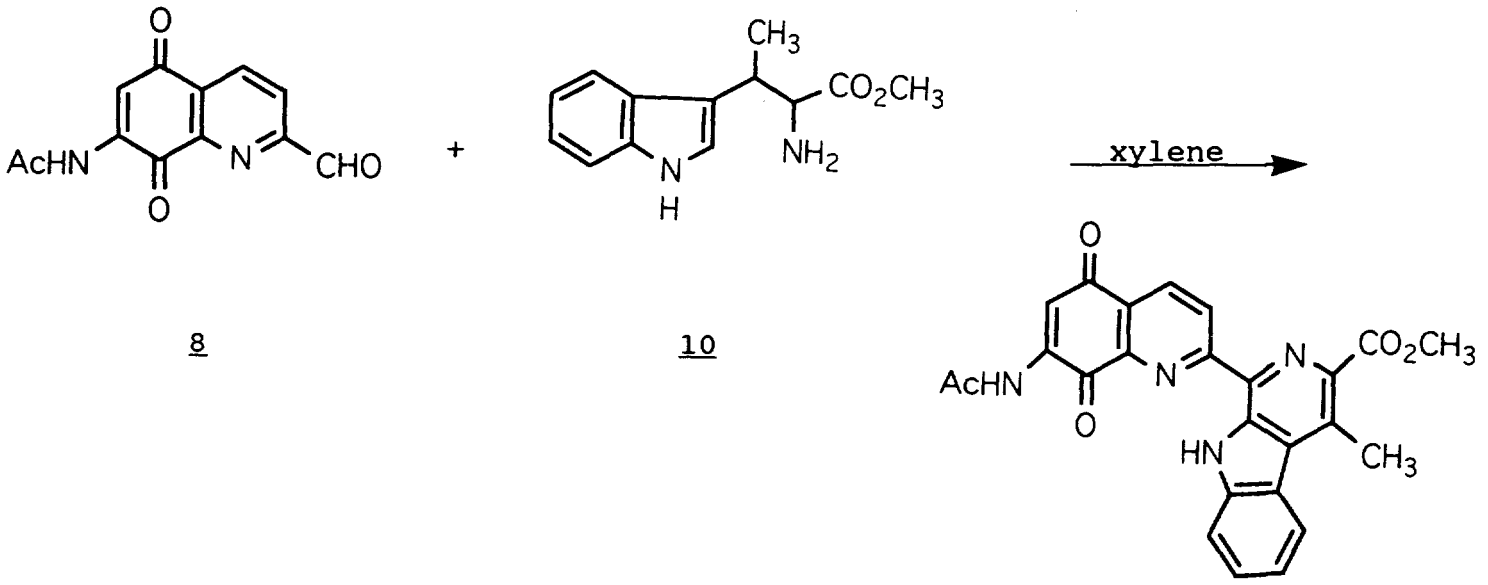
In an *in vitro* drug screen, N-acetyllavendamycin methyl ester(4) had shown high selective cytotoxicity for *ras*^{k} transformed epithelial cells, compared to the normal cells. The *ras*^{k} oncogene is associated with 90% of pancreatic solid tumors in humans, 60% of colon tumors, and 30% of breast cancer tumors. Compound (4) selective cytotoxicity is unprecedented and marks an exciting advancement, as there is currently no drug that cures these tumors.[4]

III. SYNTHESIS

In 1984, the first total synthesis of Lavendamycin methyl ester(1b) was reported by a team at the University of Rochester.[5] The precursor used was ß-methyl tryptophan. Recently Behforouz and co-workers have found an efficient route for the synthesis of lavendamycin methyl ester(1b).[6]

A. Synthesis of Lavendamycin methyl ester(1b) and its N-Acetyl Derivative(4)

The new and efficient route referred to above was carried out by the Pictet-Spengler condensation of aldehyde(8) with ß-methyltryptophan methyl ester(10) in refluxing xylene to give N-Acetyllavendamycin methyl ester(4) in 79% yield. Hydrolysis of (4) in a mixture of trifluoroacetic acid and water produced lavendamycin methyl ester(1b) in 91% yield (Scheme I).[3]



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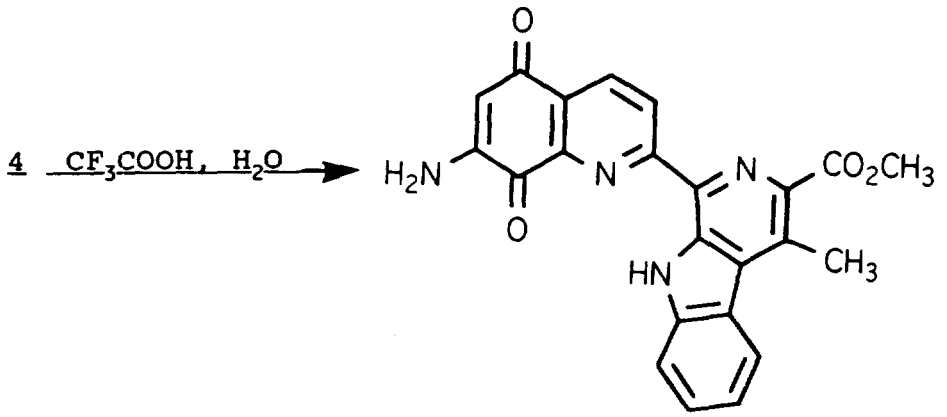
SCHEME I



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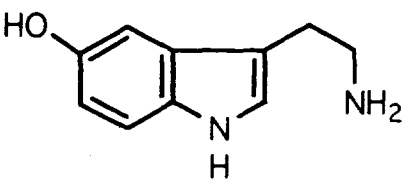
IV. PROPOSAL FOR THE SYNTHESIS OF THE NEW COMPOUND 7-N-ACETYL-11'-HYDROXY-3'-DEMETHYLLAVENDAMYCIN ISOAMYL ESTER(5)

Lavendamycin methyl ester is a very insoluble compound and consequently, it is difficult to be introduced into animals for screening tests. More soluble analogs of this compound are desired. The addition of a hydroxyl group to the lavendamycin backbone will hopefully increase the solubility of the compound in water, making it more suitable for introduction into animals. The location of the hydroxyl group also makes the compound similar to the naturally occurring Serotonin(6) found in the human brain. The isoamyl group at the C-2' position will hopefully increase the selective cytotoxicity. The antitumor activity of compound (5) and a number of other lavendamycin analogs prepared by other members of our research group will be studied, and the results will be compared. Based on this study, the minimum potent structure will be determined, and possibly the mechanism of the action of lavendamycin against cancer cells will be clarified.



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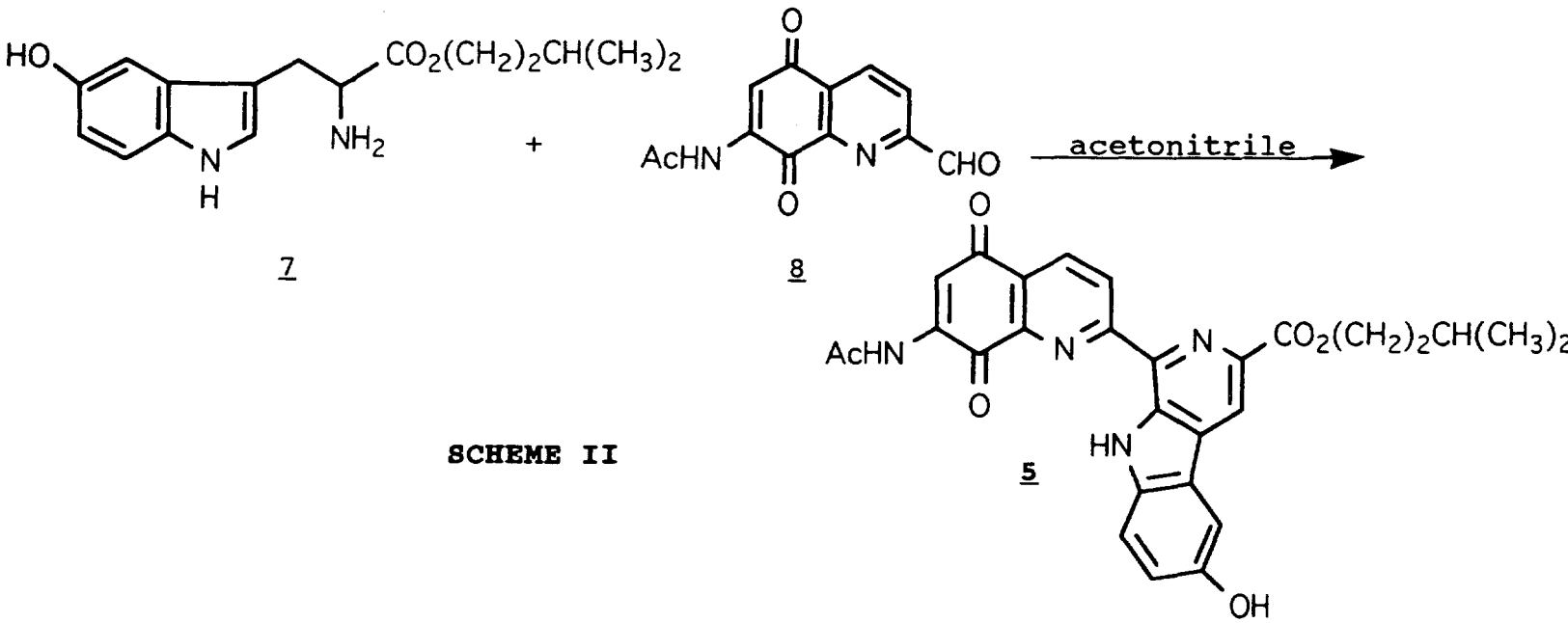
RESULTS AND DISCUSSION

SNTHESIS OF THE ISOAMYL ESTER OF 7-N-ACETYL-11'-HYDROXY-3'-DEMETHYLLAVENDAMYCIN

The major objective of this research was the synthesis of the isoamyl ester of 7-N-acetyl-11'-hydroxy-3'-demethyllavendamycin(5). This compound was produced through the Pictet-Spengler condensation of 5-Hydroxytryptophan isoamyl ester(7) with 7-Acetamido-2-formylquinoline-5,8-dione(8) in refluxing dry acetonitrile for 19 hours. This procedure gave a 6.9% yield of a red solid material according to the following scheme (scheme II).



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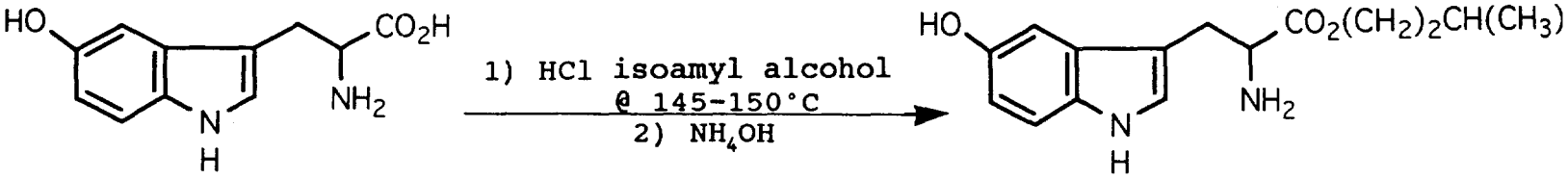
SCHEME II

Thin Layer Chromatography (silica gel, CH_2Cl_2 - MeOH, 100:1) showed the compound to be pure (Rf=0.4). The structure of 7-N-acetyl-11'-hydroxy-3'-demethyllavendamycin isoamyl ester was confirmed by nmr. The nmr showed traces of unreacted ester, however.

SYNTHESIS OF 5-HYDROXYTRYPTOPHAN ISOAMYL ESTER

5-Hydroxytryptophan isoamyl ester(7) was prepared according to a similar method described for the synthesis of ß-methyltryptophan methyl ester.[7] The following scheme was used (scheme III).



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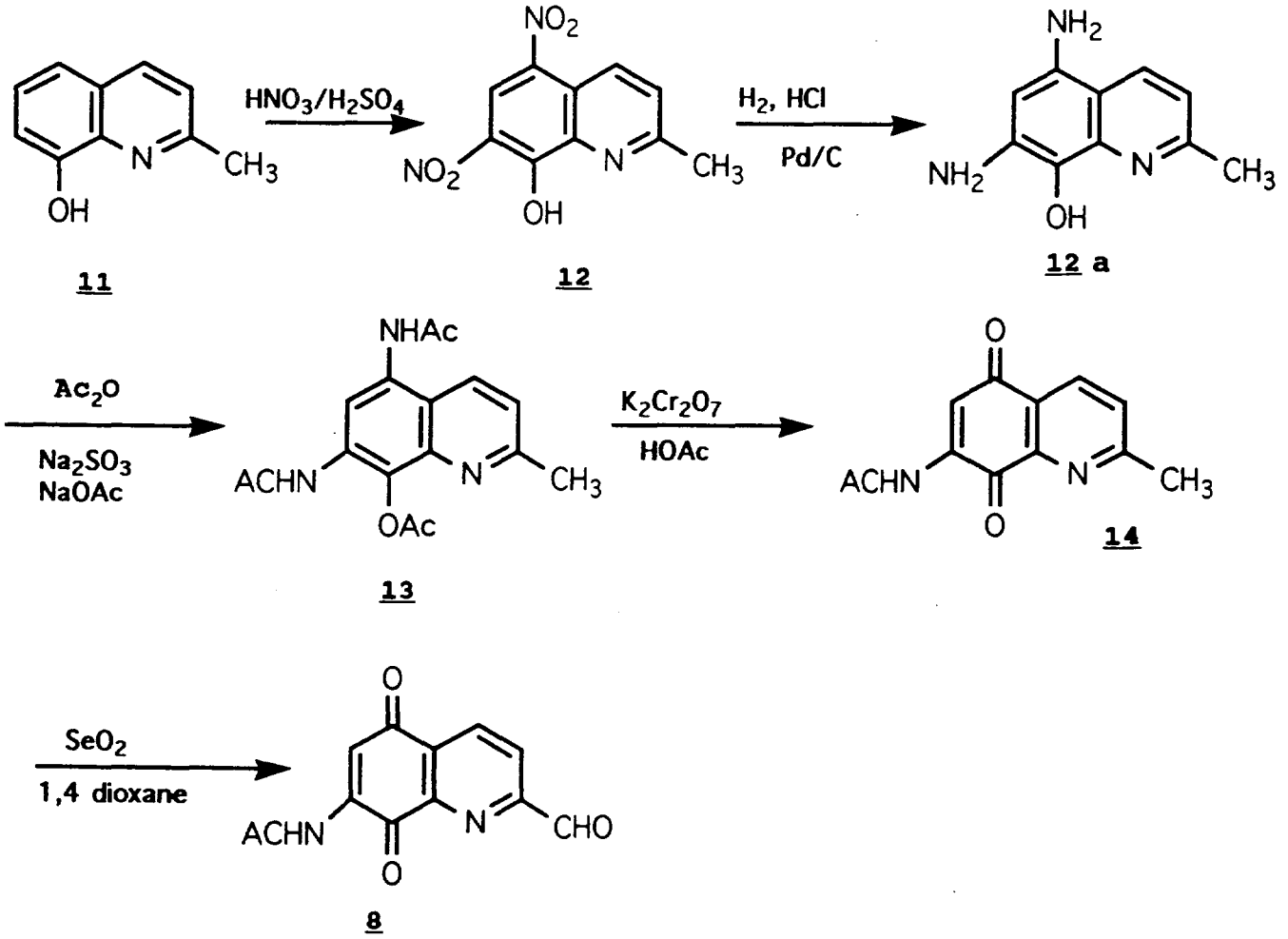
SCHEME III

Condensation of 9 with an excess of isoamyl alcohol in the presence of dry hydrogen chloride at 145-150°C for 24 hours gave ester 7 in 80% yield as a dark brown semisolid. Thin layer chromatography (silica gel, EtOAc/MeOH 9:1) showed the compound to be pure (Rf=0.51). The structure was confirmed by nmr, ir, and mass spectroscopy.

PREPARATION OF 7-ACETAMIDO-2-FORMYLQUINOLINE-5,8-DIONE(8)

7-Acetamino-2-formylquDinoline-5,8-dione(8) was prepared according to the following scheme (scheme IV). The procedure was similar to that described by A.G. Richardson.[8]



(SCHEME IV)

Compound 11 was treated with 70% HNO_3-H_2SO_4 at 0-5°C for 90 minutes to give a 58% yield of compound 12 as a bright yellow solid.

Reduction of 12 with molecular hydrogen in the presence of 5% Palladium on charcoal at 25°C for four hours gave a dark red mixture. The resulting mixture was filtered off and the filtrate was treated with excess acetic anhydride in the presence of sodium sulfite and sodium acetate to give an 80% yield of compound 13 as a white solid (m.p. 255°C).

Oxidation of compound 13 in acetic acid by potassium dichromate for 20 hours at 25°C gave a 56% yield of compound 14 as an orange/yellow solid.

Oxidation of compound 14 in 1,4-dioxane by selenium oxide for 24 hours at reflux gave a 73% yield of compound 8 as a yellow solid.

EXPERIMENTAL:

I. GENERAL INFORMATION

REAGENTS: 8-Hydroxyquinalidine, palladium on charcoal (5%), 5-Hydroxytryptophan

SOLVENTS: Acetonitrile, 1,4-Dioxane, and Isoamyl alcohol were dried and distilled before use. Chloroform, ethyl acetate, ethyl ether, dichloromethane, 95% ethanol, and methanol were used without distillation.

MELTING POINTS: Melting points were performed with a Thomas Hoover capillary melting point apparatus, and are not corrected.

NUCLEAR MAGNETIC RESONANCE SPECTRA (^{1}H NMR): NMR spectra were recorded on a Varian Gemini 200 in deuterated chloroform ($CDCl_3$) with TMS as the internal standard. The chemical shifts are reported in δ values.

THIN LAYER CHROMATOGRAPHY: TLC's were performed on Kodak silica gel sheets containing a fluorescent indicator.

INFRARED SPECTROSCOPY: IR's were performed on a Nicolet 5ZDX FT-IR spectrometer.

MASS SPECTROSCOPY: Mass spectra were recorded on an Extrel ELQ 400 mass spectrometer.

PLATE CHROMATOGRAPHY: Plate chromatographies were performed on Analtech alumina gel thin layer chromatography plates.

II. PROCEDURE

A. PREPARATION OF 5,7- DINITRO-8-HYDROXY-2-METHYLQUINOLINE(12)

In a 500 ml erlenmeyer flask (Note 1), equipped with a magnetic stirring device, was placed 100 ml of a 70%(v/v) solution of conc. nitric acid-sulfuric acid. The solution was stirred and cooled in an ice bath for five minutes. To this solution, **8-Hydroxyquinaldine** (10 g, 62.9 mmol) was added in small portions over a five minute period. The addition of the 8-Hydroxyquinaldine caused a brownish gas to evolve (Note 2). The mixture was allowed to stir in the ice bath for 90 minutes. The mixture appeared black with a hint of red. The mixture was then poured into a 1000 ml beaker which contained 600 ml of distilled water, and stirred with a glass rod. A bright yellow precipitate formed upon the addition of the mixture to the water. The precipitate was then either vacuum filtered and collected, or it was allowed to stand overnight and the supernate was carefully vacuum pipetted out with a pipet attached to the aspirator, and followed by a short vacuum filtration. The solid material was added to 150 ml of 95% ethanol. The precipitate tended to cling to the filter paper, so the filter paper was rinsed with 50 ml of 95% ethanol and added to the ethanolic mixture in a 500 ml erlenmeyer flask (Note 3). The mixture was magnetically stirred for 15 minutes (Note 4). This solution was again filtered, and the solid on the funnel was washed with ethyl ether (150 ml), with the aspirator off (Note 5). The solid 5,7-Dinitro-8-hydroxy-2-methylquinoline was left on the paper

to dry overnight in the hood. The compound was a very bright yellow. A greenish tint shows impurity.

Note 1: The use of a 500 ml flask, instead of a 250 ml flask, will greatly lessen the chances of an intense evolution of gas, and an overflowing of the mixture during the next procedure.

Note 2: Be careful! If too much compound is added at once, the mixture may overflow. Perform this procedure under a hood only.

Note 3: The cleaner the filter paper, after rinsing, the higher the yield. Get the filter paper as clean as possible, sometimes "dipping" the paper in ethanol helped to get the compound off of the paper. This compound is very "messy," latex gloves and a lab coat are highly recommended.

Note 4: Additional ethanol may have to be added while stirring to wash the compound efficiently. The amount of ethanol used here is not crucial, since the solution will be filtered later.

Note 5: The addition of the ether to the solid on the funnel caused the paper to be raised up, and the ether seeped under the paper, taking some of the product with it. To avoid this, make sure the filter paper is secure and tight on the funnel, with **no bubbles**, before beginning the filtration. If unsure of the status of the filter paper, add the ether **slowly**, and do not allow it to build up and flow around the edges.

Yield: 57.5% (9.0 grams of product)

Yields of 77% were reported by A.G.Richardson[8]

B. PREPARATION OF 5,7-DIACETAMIDO-2-METHYL-8-ACETOXYQUINOLINE(13)

Into a 500 ml hydrogenation bottle, 5,7-Dinitro-8-hydroxy-2-methylquinoline (6.03 g, 24.2 mmol), water (100 ml), and conc. HCl (13 ml) were added. To this suspension, 5% Palladium on Charcoal (2.00 g) was added as a catalyst. This mixture was hydrogenated at 40 psi overnight (Note 1). The solution was then carefully vacuum filtered to remove the Pd/C (Note 2) and the filtrate was transferred to a 500 ml beaker. To the filtrate, sodium sulfite (2.85 g), sodium acetate (3.58 g), and acetic anhydride (2.5 ml) were added, using a magnetic stirrer. Generally, no precipitate formed. After 30 minutes, the same amount of the above three compounds were added again. The slow addition of acetic anhydride (7.5 ml) caused a orange/white precipitate to form (Note 3). The solution was then vacuum filtered and the precipitate was washed three times with water (100 ml). This process washed the orange color out of the precipitate, and left a white solid on the filter paper. The filtrate was evaporated down to one-quarter of its volume. Any precipitate that formed was filtered and washed, as above. The filtrate was treated with sodium acetate and acetic anhydride again until no more precipitate was formed (Note 4). All washed precipitates were combined and allowed to dry overnight under the hood, or on a vacuum pump.

Note 1: The hydrogenation should take place until no more of the hydrogen gas is "used" by the reaction. Therefore, do not stop the hydrogenation until the rate of pressure decrease falls below 0.5 psi/hour. This takes approximately four hours.

Note 2: **Do Not** rinse the Pd/C that is on the filter paper with water! This will dilute the solution, which is concentration sensitive. The filtrate should take up, at most, 130 ml.

Note 3: If after this point, no precipitate has formed, then perform the following procedure:

Using a rotary evaporator and heat, remove the solvent until a small amount of solution is obtained. Add the three compounds (sodium sulfite, sodium acetate, and acetic anhydride) in small quantities (approximately one-quarter of that called for in the procedure), and then wait 15 minutes. Next add 5 ml acetic anhydride and stir **vigorously** (use a magnetic stirrer, cover the beaker with parafilm, and set the stirrer on maximum). Add small amounts of sodium sulfite and sodium acetate while vigorously stirring for 10 minutes. Stop the stirring and allow the mixture to stand, the precipitate should then form. Problems like this can be avoided by following the prescribed procedure <u>exactly</u> as written.

Note 4: The addition of the acetic anhydride is what generally causes more precipitate to form.

Yield: 80% (6.05 grams of product [m.p. 255° C])

Yields of 88% were reported by A.G.Richardson[8]

C. PREPARATION OF 7-ACETAMIDO-2-METHYLQUINOLINE-5,8-DIONE(14)

Into a 1000 ml erlenmeyer flask, 240 ml of glacial acetic acid, and 5,7-Diacetamido-2-methyl-8-acetoxyquinoline (6.3 g, 2.0 mmol) were added. To the resulting suspension, a solution of potassium dichromate (17.64 g) in 200 ml of water was added. This black solution was magnetically stirred at room temperature for 20-24 hours. The solution was then poured into 900 ml of water and extracted with dichloromethane (5 x 200 ml). The organic extracts were washed with a solution of 5% sodium carbonate in a saturated salt solution (3 x 300 ml). The organic layer was dried overnight with anhydrous magnesium sulfate (Note 1). The magnesium sulfate was filtered off, and the solvent was evaporated to leave a orange/yellow solid. The solid 7-Acetamino-2-methylquinoline-5,8-dione was dried overnight on the vacuum pump.

Note 1: The organic layer is more efficiently dried if the flask is tightly stoppered, sealed with parafilm, and placed in a cold room.

Yield: 55.65% (2.56 g of product)

Yields of 53% were reported by A.G. Richardson[8]

D. PREPARATION OF 7-ACETAMIDO-2-FORMYLQUINOLINE-5,8-DIONE(8)

In a dry 100 ml round bottomed flask, equipped with a magnetic bar, 2.30 g (10 mmol) of 7-acetamido-2-methylquinoline-5,8-dione and 1.388 g (12.5 mmol) of selenium (IV) oxide (note 1) was added to 35.0 ml of dried 1,4-dioxane (note 2) and 1.25 ml of water. The reaction mixture was heated **slowly** to reflux over a two hour period. The mixture was then refluxed for 24 hours. This reaction was carried out under an argon balloon. The completion of the reaction was tested by TLC (note 13). To the mixture 35 ml of dioxane was added and was allowed to reflux for fifteen more minutes. The solution was then hot filtered to remove the black selenium metal. The flask was rinsed with approximately 10 ml of dichloromethane and poured over the solid on the filter paper. The filtrate was then rotary evaporated to dryness. The solid was extracted with 2500 ml of dichloromethane. The filtrate was then rotary evaporated to obtain the yellow-tan aldehyde (7-acetamino-2-formylquinoline-5,8-dione). The product was dried on the vacuum pump overnight and a TLC was performed (note 3). When dried, the product was yellow in color.

Note 1: The purity and quality of the selenium (IV) oxide will severely effect the yield. Be sure the selenium (IV) oxide is pure! If it is, it will appear as orange crystals.

Note 2: Dioxane was refluxed over argon, adding KOH until no more black resin appeared. This took approximately 4-6 hours. The resin was filtered out and the dioxane was refluxed with sodium spheres until they appeared shiny. The dioxane was then distilled. Dioxane was kept in an argon atmosphere in a bottle equipped with a septum, and removed with a sterile syringe, using an argon balloon to regulate the pressure.

Note 3: Use a solvent system of 100% EtOAc. An Rf value of approx. 0.51-0.56 indicates completion. There should be one spot.

Yield: 72.95% (356 mg of product)

Yields of 80% were reported by A.G. Richardson[8]

E. SYNTHESIS OF 5-HYDROXYTRYPTOPHAN ISOAMYL ESTER (7)

Into a dry 250 ml round bottomed flask was added 60 ml of dried and distilled isoamyl alcohol (note 1). Dry HCl gas was bubbled through the isoamyl alcohol for 15 minutes. The HCl tank was opened so that it bubbled into the isoamyl alcohol through a pipett in a side neck of a three necked round bottomed flask. The isoamyl alcohol was kept under an argon stream flowing throug a 'T' tube at the top of the condenser leading to a concentrated solution of sodium hydroxide. To the saturated HCl solution was added 5-Hydroxytryptophan (9 1 gram, 4.54 mmol). This created a suspension. The reaction mixture was heated in an oil bath to 145 - 150° C. The colorless solution was refluxed for 24 hours (note 2). The solution was then allowed to cool at room temperature. The mixture was evaporated to "dryness" under vacuum to give the hydrochloride salt. The oil material was dissolved in 40 ml EtOAc and then was treated with 10 - 15 ml of conc. NH_4OH. The pH of the solution was recorded to be ≈ 9. This mixture was magnetically stirred for 10 minutes. The organic layer was separated and the aqueous layer was extracted with EtOAc (2 x 10 ml). The combined organic extracts were washed with 25 ml of a saturated NaCl solution. The organic layer was separated and dried over anhydrous $MgSO_4$ for 2 hours or overnight in the cold room. The mixture was filtered, and the solution was evaporated to dryness under vacuum. Small portions of ethyl ether were added several times to the oil and then evaporated to help the removal of the EtOAc. The TLC with

a solvent system of 9:1 EtOAc/MeOH showed one spot (Rf=0.51), but the nmr showed traces of EtOAc. To remove the EtOAc, the oil was dried overnight on the vacuum pump at 70°C. A subsequent nmr showed the product to be pure. The 5-Hydroxytryptophan isoamyl ester was a dark brown, highly viscous oil, and was stored in the cold room.[9]

Yield: approx. 80% (approx. 1 gram of product)

^{1}H NMR ($CDCl_3$, ppm) 8.03 (1H, s, NH), 7.265 (1H, s, C-8 H), 7.20 (1H, d, J=4.4 Hz, C-6 H), 7.013 (1H, s, C-4 H), 6.75 (1H, d, J=0.91 Hz, C-7 H), 4.135 (2H, t, J=7 Hz, CO_2CH_2-), 3.8 (1H, t, J=6 Hz, C-2 H), 2.95-3.25 (2H, m, HaHb style, C-3 H), 1.54-1.73 (1H, m, $-CH_2-$), 1.47-1.53 (1H, m, -CH'-), 0.896 (6H, d, J=3.1 Hz, C-$(CH_3)_2$); IR (Neat) ν_{max} 3420(O-H); 3359,3299(N-H_2); 3206(N-H); 3054(aromatic C-H); 2959,2929,2872(aliphatic C-H); 1729(C=O); 1667,1626,1582(aromatic C=C); 1468($-CH_2-$ bending); 1386,1366(C-$(CH_3)_2$ bending); 1208(Ar-O-); 1052($-CH_2-O-$); EIMS, m/e (relative intensity) 290 (M^+, 6.3), 274 (1.5), 216 (7.4), 173 (1.6), 162 (1.3), 158 (1.1), 146 (100), 117 (1.9).

Note 1: The isoamyl alcohol (3-methyl-1-butanol) was dried over anhydrous $CuSO_4$ and distilled. The alcohol turned brown upon the addition of the $CuSO_4$. The boiling range of the isoamyl alcohol is 130.5 - 131.5°C.

Note 2: When the oil bath reached 145°C, the 5-Hydroxytryptophan dissolved in the alcohol.

F. SYNTHESIS OF THE ISOAMYL ESTER OF 7-N-ACETYL-11'-HYDROXY-3'-DEMETHYLLAVENDAMYCIN(5)

Into a dry 150 ml round bottomed flask was added 75 ml of dried and distilled acetonitrile (Note 1). To this was added 5-Hydroxytryptophan isoamyl ester (7, 56 mg, 0.2 mmol), and 7-Acetamido-2-formylquinoline-5,8-dione (8, 48.8 mg, 0.2 mmol). The reaction mixture was heated in an oil bath to 82°C. The lemon yellow solution was refluxed for 19 hours. The mixture was then evaporated to dryness under vacuum to give a brown solid. The solid was then dissloved in a minimal amount of Chloroform and the compound was separated by plate chromatography (Rf=0.23) to give the red solid lavendamycin derivative. Thin layer chromatography (silica gel, CH_2Cl_2 - MeOH, 100:1) showed the compound to be pure (Rf=0.4). The nmr showed traces of unreacted ester, the lavendamycin derivative peaks are listed below (Note 2).

Yield: 6.9%

^1H NMR ($CDCl_3$, ppm) 11.6(1H, s, NH), 9.16(1H, d, J=4.14 Hz, C-4 H), 8.89(1H, s, C-6 H), 8.47 (1H, d, J=13.2 Hz, C-3 H), 8.4(1H, s, AcNH), 7.97(1H, s, C-3' H), 7.65(1H, d, J=0.91 Hz, C-10' H), 7.6(1H, s, C-12' H), 7.3(1H, d, J=7.4 Hz, C-9' H), 4.5(2H, t, J=6 Hz, CO_2CH_2), 2.0-2.15 (2H, m, C-CH_2-), 1.82(1H, q, J=6 Hz, -CH'-), 1.1(6H, d, J=6 Hz, C-$(CH_3)_2$.

Note 1: Acetonitrile was dried by distilling over calcium hydride.

Note 2: The reaction was done twelve times and no pure compound was made. The starting materials MUST be pure, and the water should be removed effectively from the reaction flask.

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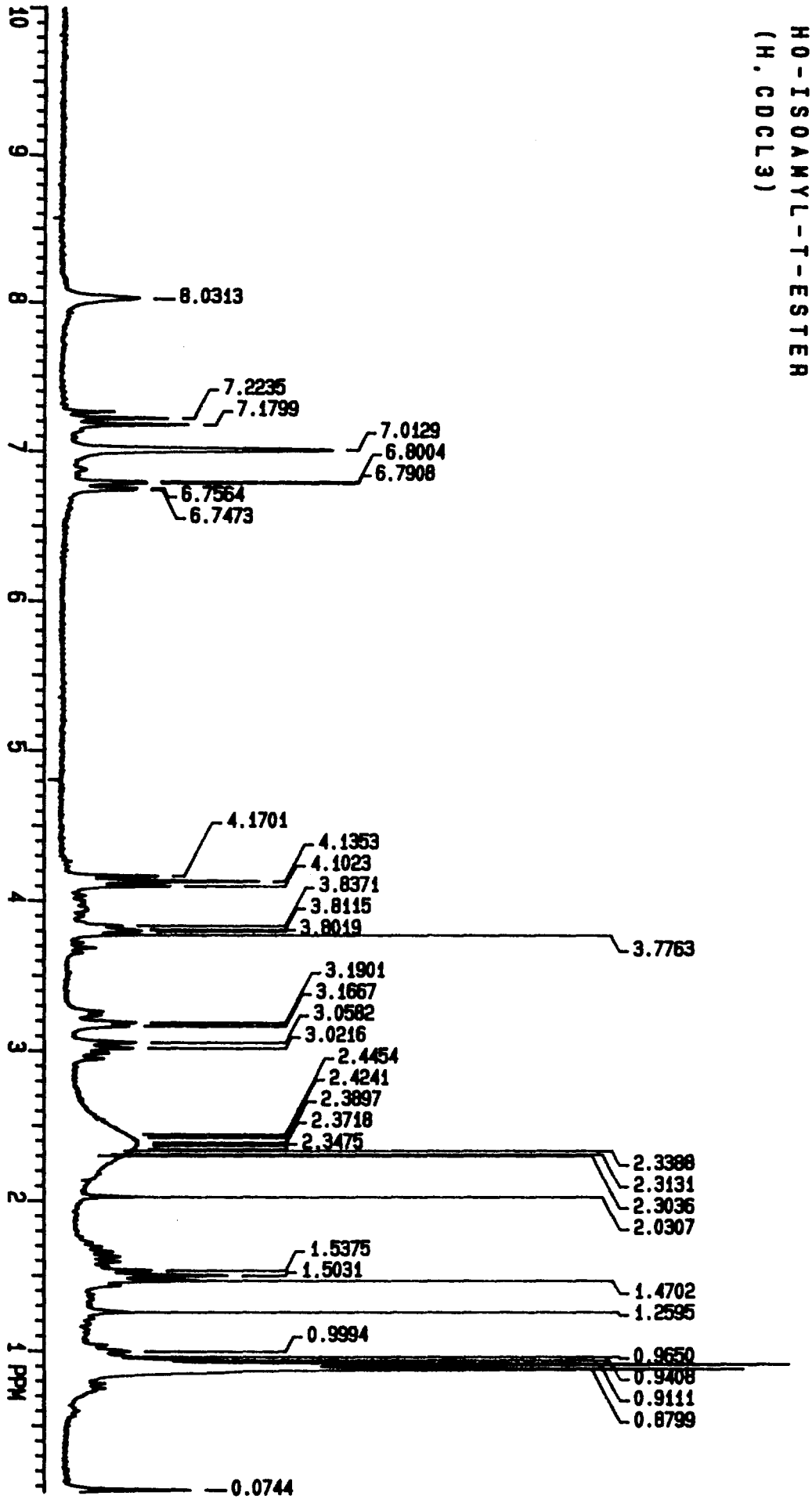
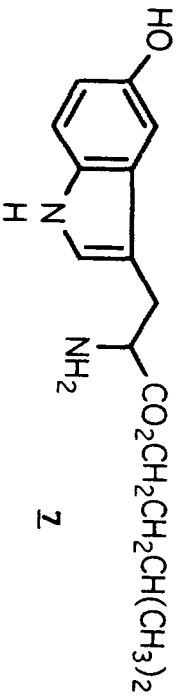
9 Cai, W., Synthesis of Tryptophan isoamyl methyl ester, Ball State University Chemistry Dept. research notebook, 1991.

APPENDIX:

The following pages contain nmr, ir, and mass spectra for compound 7, and an nmr spectrum of compound 5.

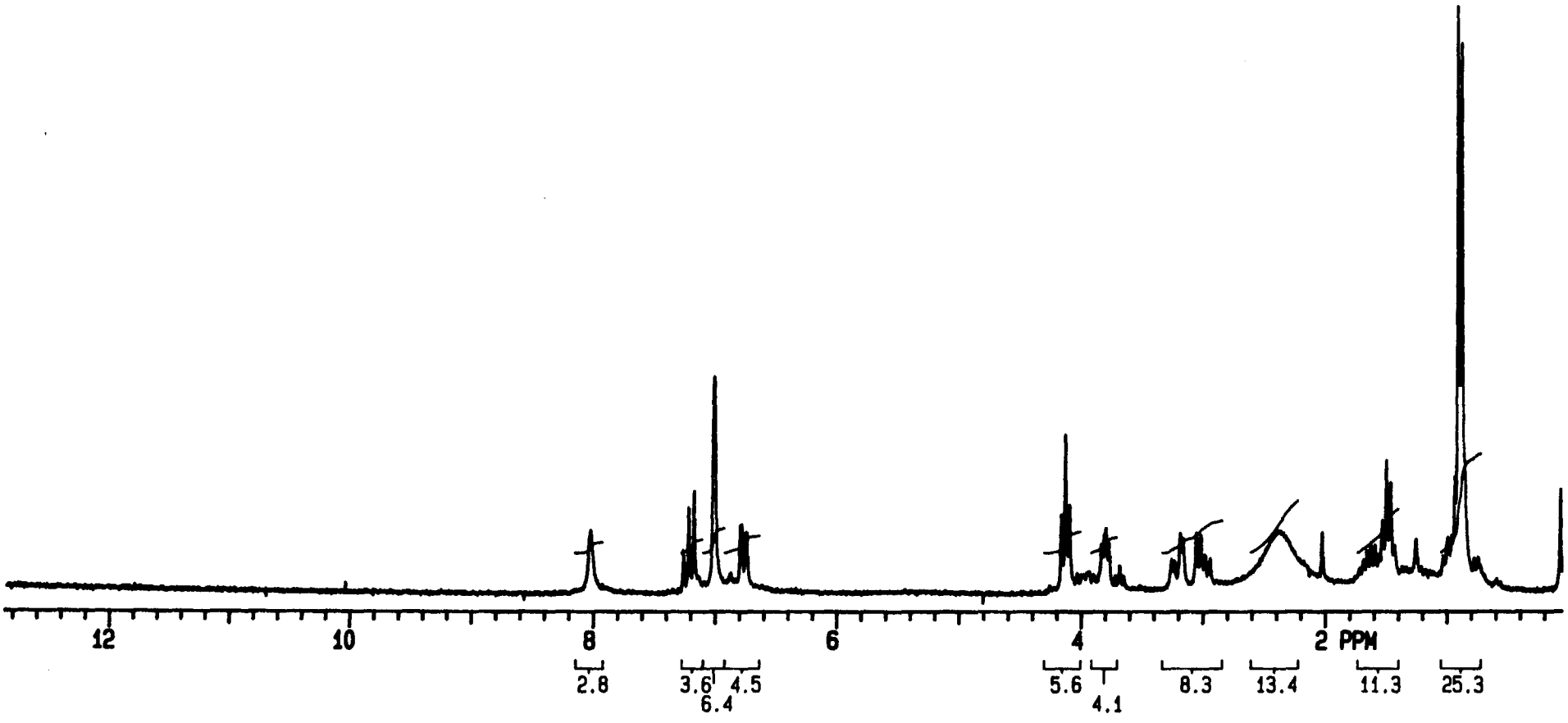
HO-ISOAMYL-T-ESTER
(H, CDCL3)

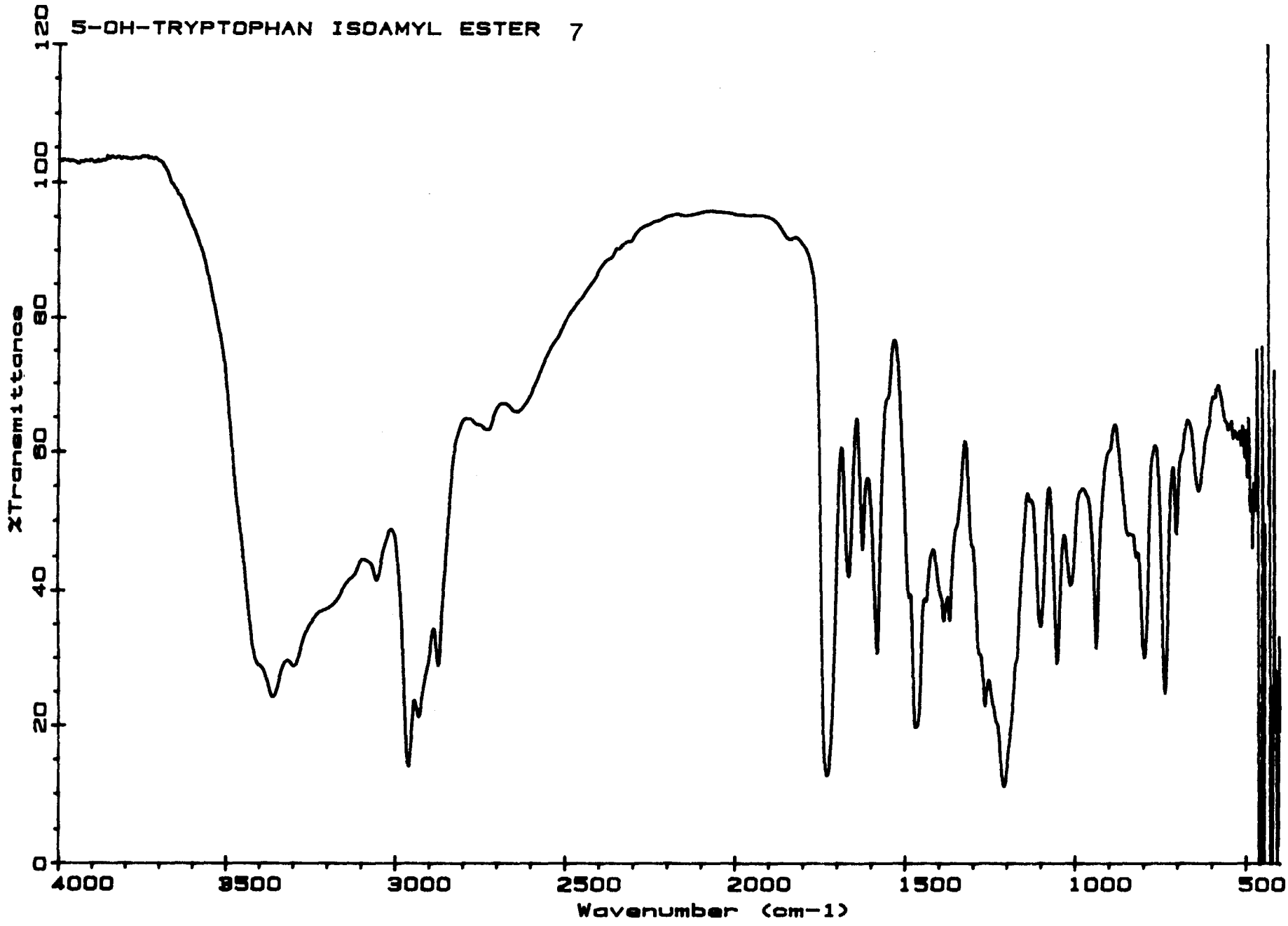


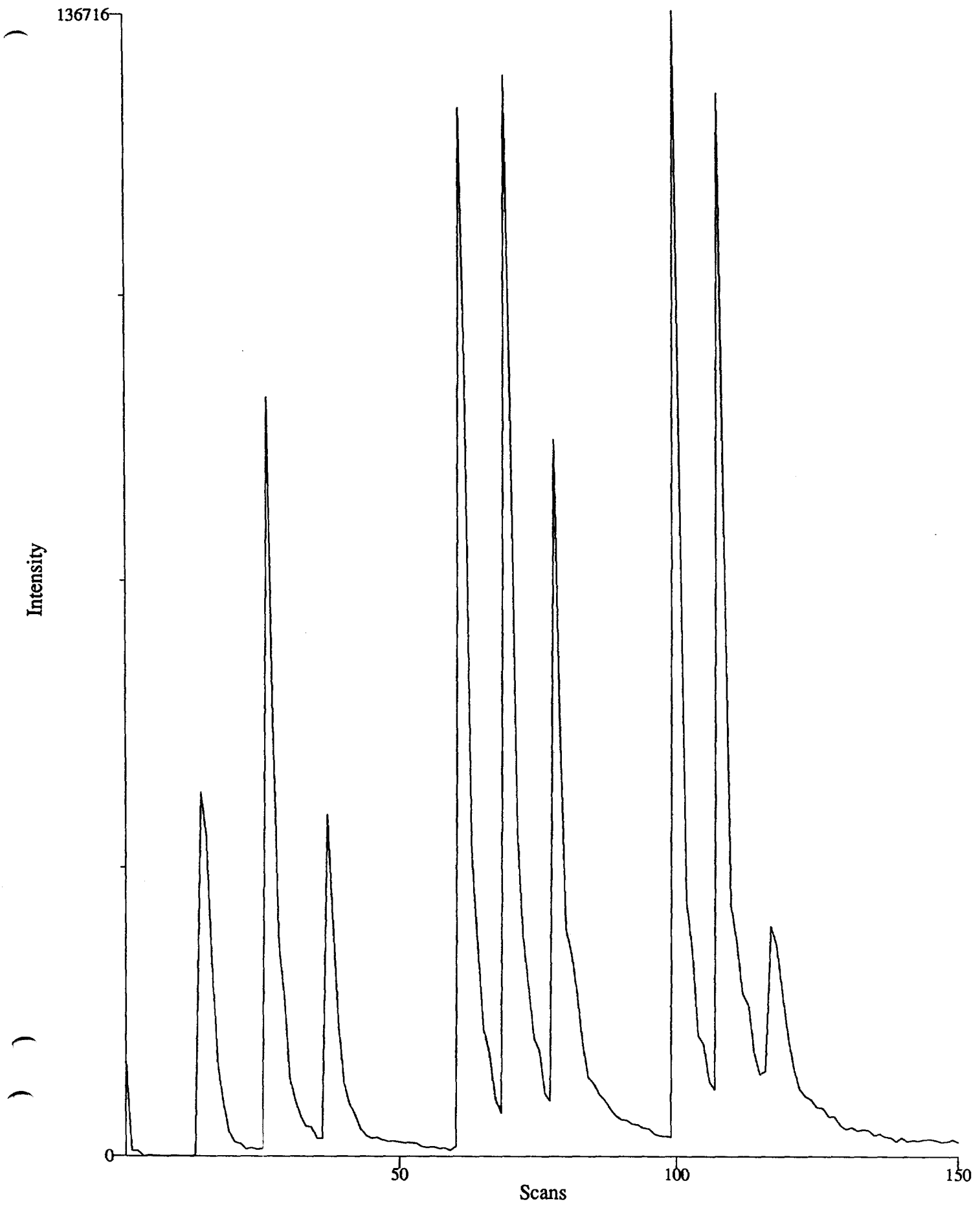


Z

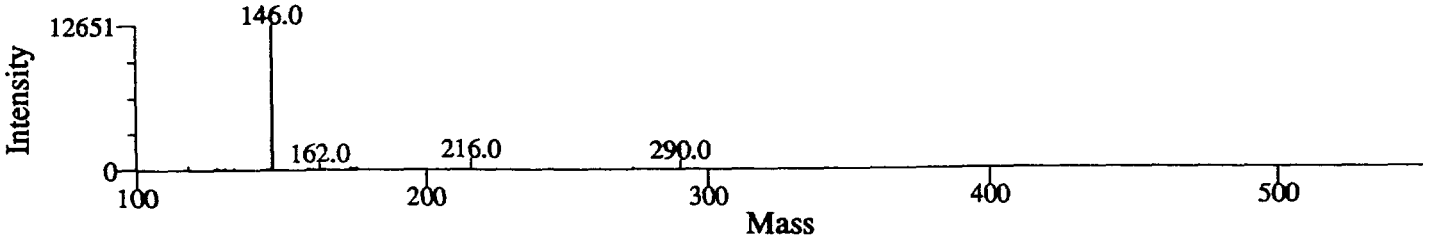
7



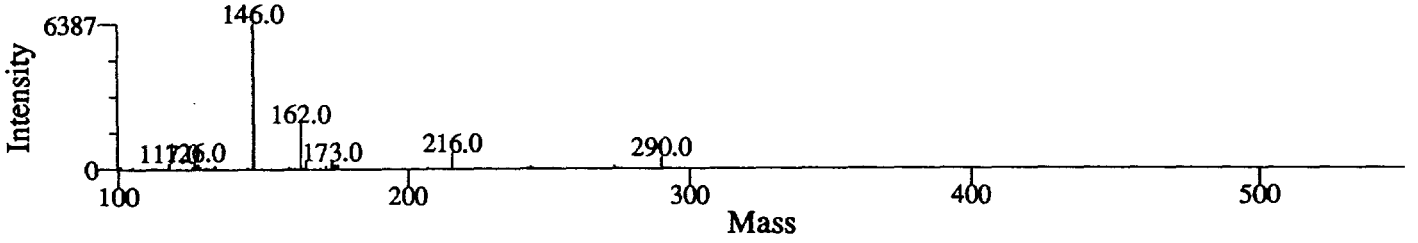

5-OH-TRYPTOPHAN ISOAMYL ESTER 7
%Transmittance
120
100
80
60
40
20
0
4000
3500
3000
2500
2000
1500
1000
500
Wavenumber (cm-1)


Tom Eads Tryp-Ester EI 4-10-92.scan TIC
136716
Intensity
0
50
100
150
Scans

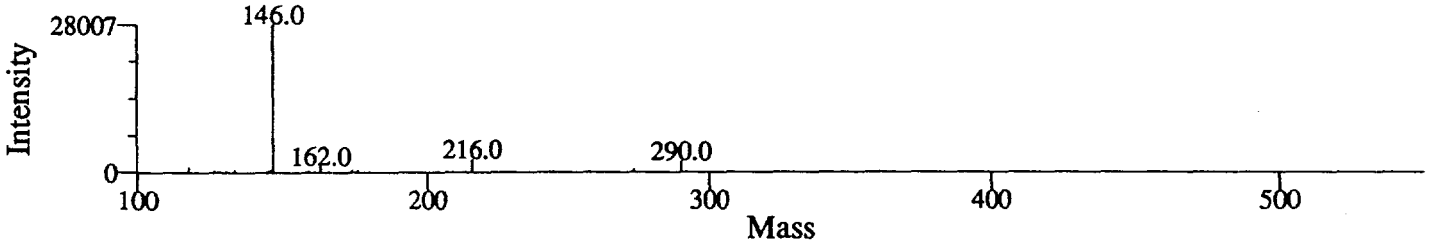
Tom Eads Tryp-Ester EI 4-10-92.scan scan 16 - 126



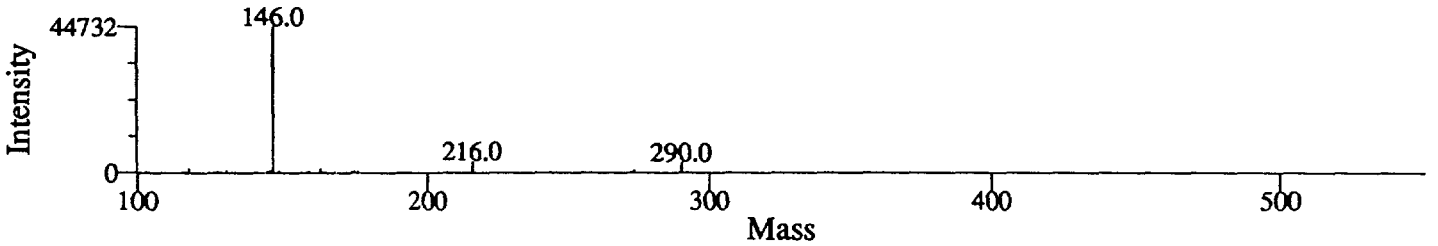
Tom Eads Tryp-Ester EI 4-10-92.scan scan 12 - 20



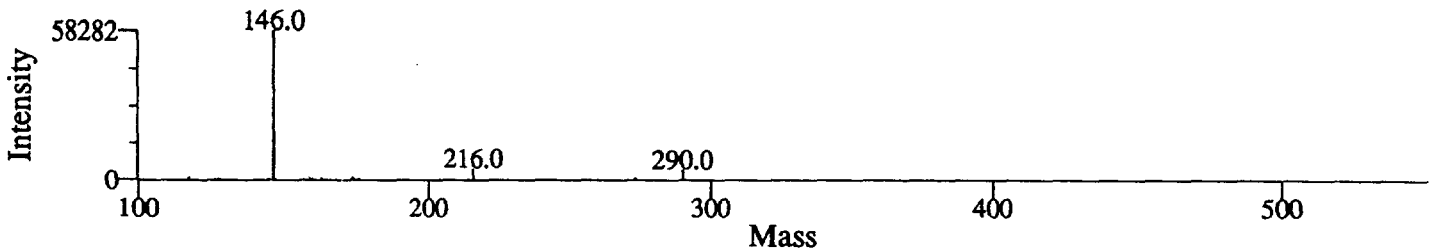
Tom Eads Tryp-Ester EI 4-10-92.scan scan 59 - 65



Tom Eads Tryp-Ester EI 4-10-92.scan scan 68 - 71



Tom Eads Tryp-Ester EI 4-10-92.scan scan 100 - 102



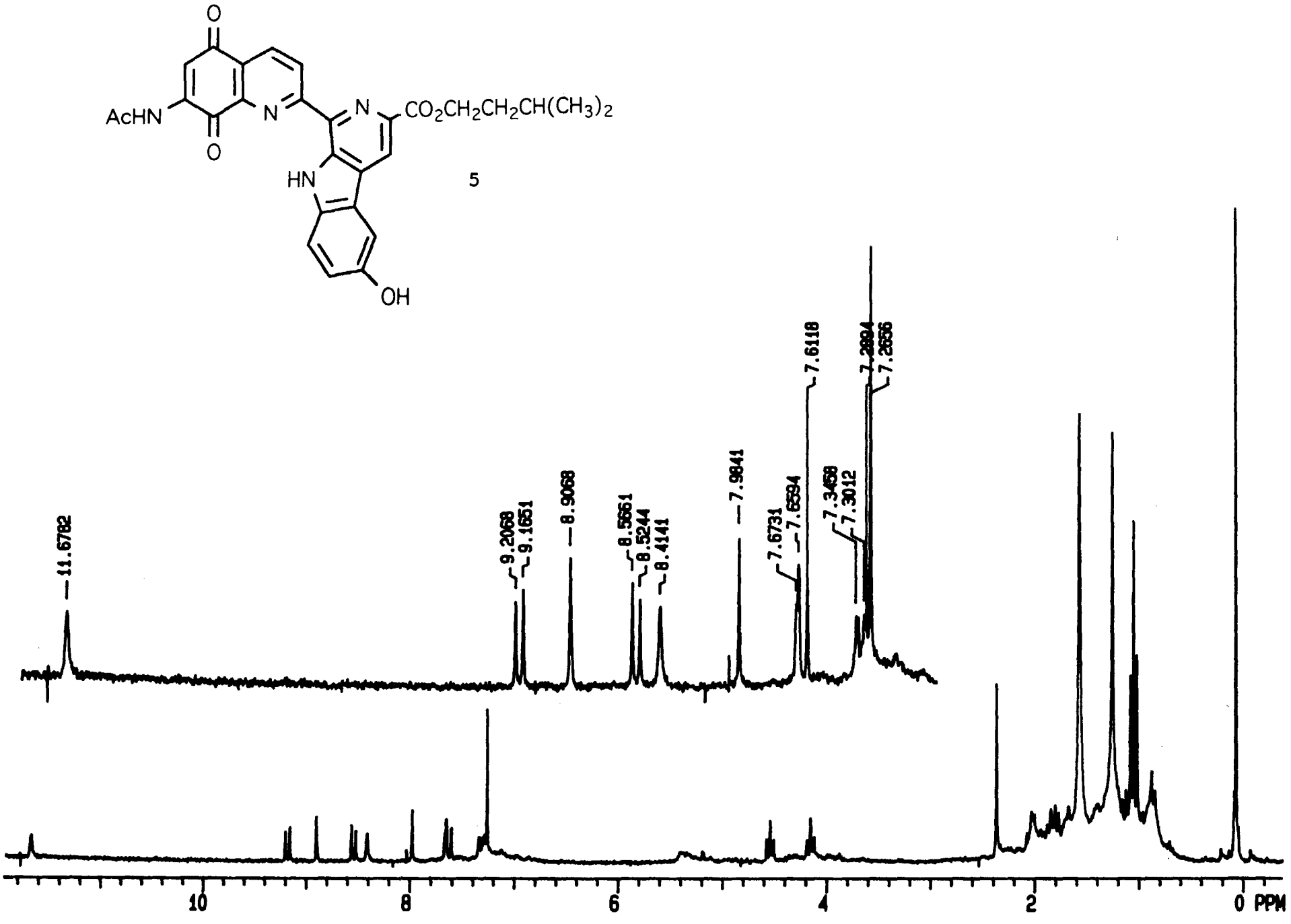
Tom Eads Tryp-Ester EI 4-10-92.scan scan 100 - 102

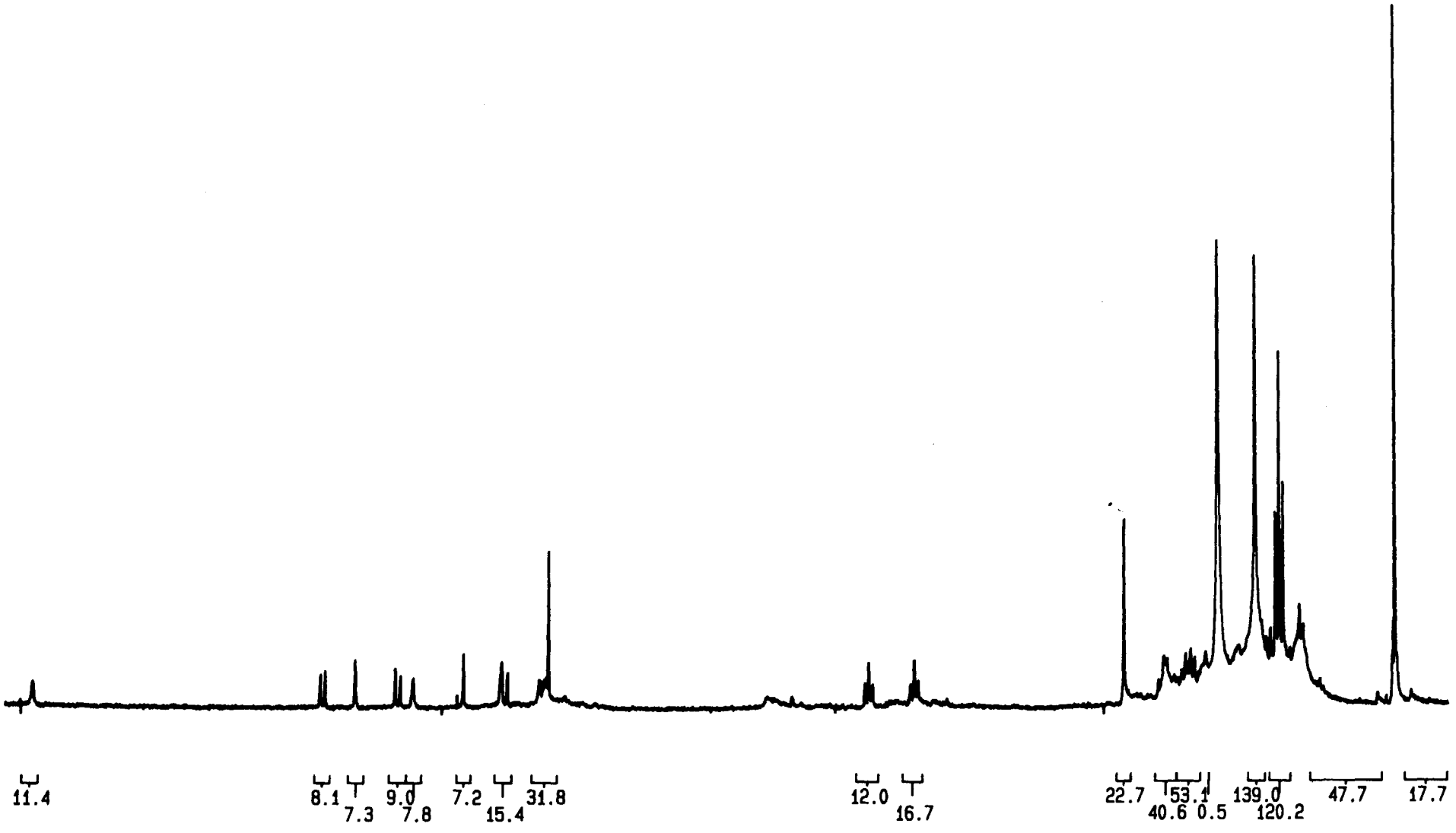
Fri Apr 10 11:11:11 1992

Mass	Intensity	I%
101.0	238	0.4
102.0	238	0.4
103.0	358	0.6
104.0	206	0.4
105.0	136	0.2
106.0	55	0.1
107.0	66	0.1
108.0	117	0.2
109.0	120	0.2
110.0	22	0.0
111.0	35	0.1
112.0	104	0.2
114.0	14	0.0
115.0	70	0.1
116.0	362	0.6
117.0	1123	1.9
120.0	61	0.1
121.0	41	0.1
122.0	20	0.0
123.0	61	0.1
124.0	6	0.0
125.0	70	0.1
126.0	173	0.3
127.0	404	0.7
128.0	272	0.5
129.0	101	0.2
130.0	359	0.6
131.0	136	0.2
133.0	324	0.6
134.0	79	0.1
136.0	17	0.0
140.0	23	0.0
141.0	48	0.1
142.0	50	0.1
143.0	61	0.1
144.0	525	0.9
145.0	336	0.6
146.0	58282	100.0
150.0	9	0.0
151.0	6	0.0
153.0	21	0.0
154.0	12	0.0
155.0	125	0.2
156.0	168	0.3
158.0	668	1.1
159.0	481	0.8
160.0	256	0.4
161.0	21	0.0
162.0	780	1.3
163.0	164	0.3
164.0	236	0.4
165.0	152	0.3
167.0	146	0.3
168.0	9	0.0
169.0	15	0.0
170.0	31	0.1
171.0	124	0.2

172.0	252	0.4
173.0	916	1.6
174.0	334	0.6
175.0	453	0.8
178.0	15	0.0
182.0	6	0.0
185.0	36	0.1
186.0	63	0.1
188.0	48	0.1
190.0	18	0.0
191.0	53	0.1
194.0	16	0.0
195.0	11	0.0
198.0	12	0.0
199.0	21	0.0
202.0	34	0.1
203.0	68	0.1
204.0	275	0.5
206.0	10	0.0
207.0	278	0.5
208.0	19	0.0
213.0	12	0.0
214.0	173	0.3
215.0	43	0.1
216.0	4314	7.4
219.0	41	0.1
222.0	8	0.0
224.0	10	0.0
228.0	8	0.0
229.0	23	0.0
231.0	62	0.1
239.0	17	0.0
242.0	5	0.0
244.0	188	0.3
245.0	212	0.4
246.0	42	0.1
252.0	31	0.1
254.0	10	0.0
258.0	126	0.2
259.0	24	0.0
261.0	11	0.0
266.0	4	0.0
269.0	3	0.0
270.0	4	0.0
271.0	6	0.0
272.0	73	0.1
274.0	858	1.5
275.0	61	0.1
276.0	13	0.0
281.0	31	0.1
284.0	3	0.0
285.0	15	0.0
286.0	9	0.0
288.0	95	0.2
289.0	102	0.2
290.0	3696	6.3
292.0	26	0.0
301.0	97	0.2
302.0	27	0.0
305.0	5	0.0
315.0	22	0.0
330.0	16	0.0
343.0	9	0.0
344.0	30	0.1
357.0	4	0.0

358.0	13	0.0
360.0	105	0.2
361.0	141	0.2
362.0	47	0.1
434.0	4	0.0
436.0	3	0.0


AcHN
N
N
CO2CH2CH2CH(CH3)2
HN
OH
5
11.6782
9.2068
9.1651
8.9068
8.5661
8.5244
8.4141
7.9841
7.6731
7.6394
7.6118
7.3458
7.3012
7.2894
7.2656
10
8
6
4
2
0 PPM


11.4
8.1
7.3
9.0
7.8
7.2
15.4
31.8
12.0
16.7
22.7
40.6
53.1
0.5
139.0
120.2
47.7
17.7