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Quantitative Analysis of Steroids by ^{13}C NMR Spectroscopy

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QUANTITATIVE ANALYSIS OF STEROIDS BY ^{13}C NMR SPECTROSCOPY

by

Pei Wang

A Thesis
Submitted to the
Faculty of The Graduate College
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Pei Wang

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Western Michigan University, 1994

Steroids are usually analyzed quantitatively by using HPLC with ultraviolet detection and are confirmed by FTIR, GC-MS. Quantitative ^{13}C NMR method provide an alternative means to analyze steroids. Chromatographic separation and subsequent identification by match with a spectroscopic data base spectrum is not required for quantitative analysis of mixture components. Instead, equivalent information is obtained by creating subspectra from resonances with equivalent peak areas within a quantitative NMR spectrum. Techniques for eliminating Nuclear Overhauser Enhancement (NOE) effect is described.

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CHAPTER I

INTRODUCTION

Synthetic anabolic steroids were developed in 1930s to help rebuild body tissue and to prevent the breakdown of tissue during debilitating disease. Clinically, these agents are prescribed as replacement therapy in men with central or peripheral hypogonadism and in some adolescents with marked delay of pubertal development.

Anabolic steroids increase protein synthesis in skeletal muscles mass and reverse catabolic processes [1]. Because of these properties some athletes use anabolic steroids in an attempt to improve their strength, athletic performance and appearance.

Estrogenic hormones are often prescribed to hasten sexual maturation in females [2]. The widest use of estrogens is in the treatment of menopause, in which they supplement of natural estrogens. Estrogens are also used in the control of cancer of the prostate in the male. Female hormones include progesterone, estrogen and progestins, i.e., synthetic progesterone-like compounds which have no natural counterpart in the body. Their use includes a variety of conditions: functional uterine bleeding, absence of menstruation (amenorrhea) used at times with estrogens, painful menstruation (dysmenorrhea), infertility, habitual abortion in order to maintain pregnancy, and in fact, to suppress ovulation hence their use as antifertility drugs. Certain progestins such as norethindrone combined with an estrogen, are the principal components of birth control pills which suppress ovulation. Since there is no egg to fertilize, conception does not take place. Progesterones have been used as hormone

substitutes after hysterectomy and to treat dysmenorrhea, endometriosis, functional uterine bleeding, amenorrhea and habitual abortions [3].

However, these agents can cause serious side effects such as: symptoms of intolerance; disturbance of the excretory function of the liver; virilization in children and women; acceleration of skeletal maturation; antigonadotropic, antiestrogenic, or gestagenic properties of anabolic steroids in men or women; disturbances of water and electrolyte metabolism. Because of these serious side effects and questionable usefulness of products introduced in the 1960s, most of them are controlled and have been withdrawn from the U.S. market. The few remaining anabolic steroids are approved only for specific uses in treating serious illnesses, such as aplastic anemia or breast cancer, while some products are intended only for veterinary use.

In place of the limited availability of approved products, we have seen the appearance of a black market in anabolic steroids. This black market is sustained by the increasing demand for these products, which are used by athletes and body builders.

Law enforcement agencies have been attempting to stop the illegal distribution of anabolic steroids, many of which are mislabeled or improperly formulated. Investigations and legal actions require that the products be analyzed to determine their identity and potency.

Steroids

Steroids are the generic name for one of a group of substances which include the sex hormones, bile acids, saponins, vitamins, some cardiac drugs, certain constituents of the body, etc. In common usage, the steroids also include sterols, which are related substances containing the -OH group. Steroids and sterols are often found in association with fats and oils. Some of the more familiar steroids include the

following: aldosterone (a hormone of the adrenal cortex), androsterone (a male hormone), cholesterol (found in animal fats but not in vegetable fats), cortisone (used in shock and allergic conditions), ergosterol (a precursor of vitamin D), estriol (an estrogenic hormone), progesterone (a hormone produced in the ovary), and testosterone (a male sex hormone produced in the testicles) [2].

The anabolic-androgenic steroids are derivatives of testosterone, which is responsible for the anabolic and androgenic effects noted during male adolescence and adulthood. Androgenic effects are those that relate to the growth of the male reproductive tract or to the development of secondary sexual characteristics in men.

Anabolic effects are the changes that occur in the somatic or nonreproductive tract tissues and include an acceleration of linear growth that appears before bony epiphyseal closure, enlargement of the larynx and thickening of the vocal cords, the development of libido and sexual potentia, and finally an increase in muscle bulk and strength as well as a decrease in body fat. This androgen is also probably responsible for the increase in aggressive and sexual behavior, although its role in the psychological and behavioral aspects is controversial [2].

Steroids are compounds containing the cyclopentanoperhydrophenanthrene ring system, Figure 1. The three six-sided rings (A, B, C) constitute the phenanthrene nucleus to which is attached a five-sided ring (D), cyclopentane. The prefix "perhydro" refers to the fact that all the necessary hydrogen atoms have been added to the compound to make it fully saturated. This class of compounds includes such natural products as sterols (e.g., cholesterol), bile acids (e.g., cholic acid), sex hormones (e.g., estrogens, androgens), and some alkaloids (e.g., solasodine). Cholesterol will be used to illustrate the numbering system for a steroid shown in Figure 2 and Figure 3 [4]. The most descriptive name for cholesterol is Δ^5 -cholestene-3 β -ol. The symbol Δ^5 indicates the presence and position of a double bond

between carbons 5 and 6, and the term 3β -ol indicates the presence and direction of orientation of the hydroxyl group. Identification of the positions of substitution in the

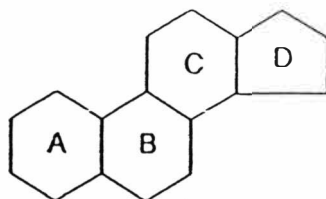


Figure 1. The Cyclopentanoperhydrophenanthrene Ring System.

sterol molecule is by means of the numbering system shown in Figure 2 for the structure of cholesterol. Figure 3 shows the conformational representation of cholesterol. In a sterol molecule, the substituents around a ring may lie above or below the plane of the ring. In the drawings, solid lines linking substituents to a ring refer to bonds above the ring plane, and dotted lines indicate substituents below the plane. In cholesterol, for example, the -OH group is above the plane; in this condition we speak of a β -oriented group (i.e., perpendicular to the plane of the rings). If the

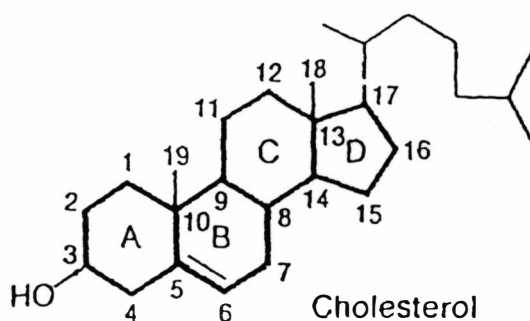


Figure 2. Ring Numbering System for Steroids.

group lies below the ring plane, it is termed α -oriented. In androgen, an unsaturated bond is at position 4 and 5, a ketone group at C-3, and a hydroxyl group (or -COOR)

in the β position at C-17. Functional groups on the β side of the molecule are denoted by solid lines; those on the α side are designated by dotted lines. Side

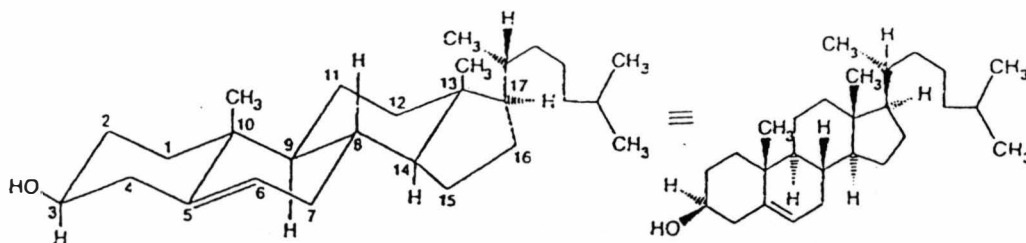


Figure 3. Conformation of Cholesterol

chains at position 17 are always β unless indicated by dotted lines or in the nomenclature of the steroid (e.g., 17 α)).

Current Analytical Method

Established analytical methods are available for the legitimate products, but are not always applicable to the wide variety of different steroids, mixtures, and dosage forms found on the illegal market. Anabolic steroid products found in the illegal market are primarily oil-based injectables or tablets and often do not contain the ingredients declared on the label.

Qualitative and quantitative analyses of these steroids in their mixtures have been carried out with both chemical method and with physical methods. (In general, analysis of organic mixtures is a two-step procedure requiring isolation of mixture components followed by identification of the pure materials. Computer-assisted methods employing chromatographic separation, followed by detection using infrared or mass spectrometers, which provide information-rich spectra for library searches of

spectral data bases, are common. Currently, high performance liquid chromatography (HPLC) with an ultraviolet (UV) or diode array detector is the most popular method for the analysis of steroids and is usually confirmed with Fourier transform infrared (FTIR) or gas chromatography (GC)/ mass spectroscopy (MS) [5], [6].

For most of anabolic steroids, this method works well. A few of the steroids, for example, oxandrolone and mesterolone, have very low UV absorptivity because of lack of conjugation in their structures and poor solubility in the 90% methanol eluant used. They can be easily overlooked by HPLC screening. Some steroids slowly react with methanol in solution, like oxymetholone and it is the reaction product rather than the free oxymetholone that is seen as a symmetrical peak in liquid chromatography (LC). The oxymetholone molecule has a polar hydroxyl group that is attracted to the silanol sites on the LC column, resulting in poor chromatography.

Identity of the steroids is usually confirmed by direct insertion probe MS or KBr microdisk FTIR. Because the extracts obtained in the sample preparation procedures often contain more than one steroid, traces of oil, preservatives, and other excipients, they are usually not suitable for direct IR or MS analysis. Before identity can be confirmed, the components of interest must be isolated and purified by a semi-prep LC cleanup that consists of injecting large portions of the sample extract and collecting eluate fractions corresponding to the peaks of interest. Usually a single LC fraction collection run is sufficient for MS, but the process may need to be repeated numerous times for enough pure component to be collected for KBr microdisk IR analysis.

Qualitative and Quantitative Analysis by ^{13}C NMR

^{13}C nuclear magnetic resonance (NMR) spectroscopy could be an alternative

method for analyzing the anabolic steroids. Qualitative analysis of mixtures may be effected via pattern recognition techniques [7], [8]. As a tool for quantitative chemical analysis, NMR spectroscopy offers several important advantages, such as not requiring a chromophoric group, ease with which multicomponent mixtures can be analyzed directly, and its nondestructive nature.

Because of the importance of carbon in chemistry, ^{13}C NMR has been developed to a level where it now is possible to obtain ^{13}C spectra from micromoles of material. ^{13}C spectra are usually measured with broad band ^1H decoupling, resulting in a series of singlets. Often a well resolved singlet for each type of carbon in the molecule is observed. One advantage of the low natural abundance of ^{13}C is that ^{13}C - ^{13}C pairs are rare and their couplings are not observed with unenriched samples. The probability that two ^{13}C nuclei exist in the same ^{13}C (unenriched) molecule is low, e.g. about 1 in 10^4 for any specified pair of positions in the molecule. These spin interactions between ^{13}C nuclei are not observed. The ^{13}C spectra obtained under heteronuclear decoupling conditions (in this case ^{13}C - ^1H) consist of singlets. For example, a proton-decoupled 25.1 MHz ^{13}C spectrum of cholestane (which contains 27 carbon atoms) exhibits 26 resolvable resonances; the chemical shift range for the resonances of these saturated carbons present in the molecule is 45 ppm. The same molecule when examined at the corresponding proton frequency (100 MHz) yields only five decipherable signals in the proton spectrum, and all proton resonances are compressed within the chemical shift range of 2 ppm. These results clearly indicate that ^{13}C NMR spectroscopy is a much more sensitive probe for studying chemical shifts. Furthermore the mixtures of steroid injectables often can be analyzed without separation simultaneously.

Principles of NMR Spectroscopy

The nuclei of certain isotopes have an intrinsic spinning motion around their axes. The spinning of these charged particles, or their circulation, generates a magnetic moment along the axis of spin, Figure 4a. If the nuclei are placed in an external magnetic field, their magnetic moment can align with or against the field. The individual nucleus spins around its axis and precesses about the force line of the applied magnetic field. These precessions are actually circular movements with respect to the force line and are restricted to a distinct number of angles between the field line and axis, Figure 4b. The field aligns the spinning nuclei against the disordering tendencies of thermal processes. However, the nuclei do not align perfectly parallel (or antiparallel) to the imposed magnetic field. Instead, their spin

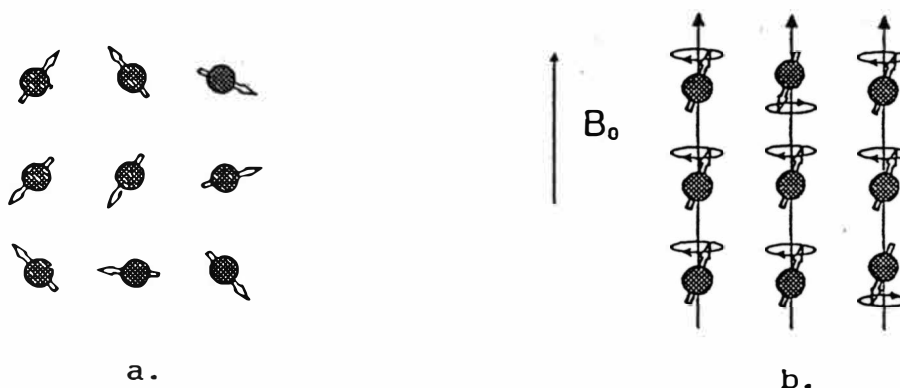


Figure 4. Nuclear Spins: (a) Without an External Magnetic Field, (b) in the Presence of an External Magnetic Field.

axes are inclined to the field and precess about the field direction, behaving like a gyroscope in a gravitational field. Each pole of the nuclear axis sweeps out a circular path in xy-plane. Increasing the strength of the field only makes the nuclei precess faster. The frequency of precession, ν_0 , is known as the Larmor frequency of the

observed nucleus.

At equilibrium the population of the various nuclear energy levels is predictable by use of a Boltzmann distribution

$$\frac{N_+}{N_-} = e^{\frac{-\Delta E}{kT}} \quad (1)$$

where N_+ is the number of nuclei populating the high-energy state; N_- is the number of nuclei populating the low-energy state; ΔE is the energy difference between the two states; k is the Boltzmann constant and T is the absolute temperature. For a magnetic nucleus of spin 1/2 in a field of 1.409 Tesla, the distribution predicts a population ratio of 0.9999904 at room temperature. The lower energy level (orientation parallel to the applied magnetic field) is favored to the extent of approximately 9.5 excess nuclei out of every million. Thus, for a sample containing approximately 10^{19} nuclei, the effective participating population will be about 10^{14} nuclei.

A radio frequency (rf) magnetic field, H_1 , is applied along the y-axis. As the frequency approaches that of the nuclear resonance frequency, there is increasing of rf field H_1 and the precessing magnetic moment. When the frequencies are identical, resonance absorption occurs and the nuclei "flip" from the lower energy level to an upper energy level, that is the spins originally precessing with H_0 flip over, and now precess against H_0 . If the frequency of the rf field is swept through the region of the resonance frequency, peak absorption of energy from the rf oscillating field will be observed at the resonance frequency. Since there is a linear relation between resonance frequency and magnetic field, H_0 , spectra may be expressed as intensity of absorption versus resonance frequency at fixed H_0 , or against H_0 at fixed resonance frequency if the rf frequency is fixed and the magnetic field swept.

Pulsed FTNMR

The conventional continuous wave (CW) NMR spectrometer scans the spectrum at a slow rate in order to avoid passing over a spectral line too rapidly since the lines are usually narrow. The spectrometer spends most of its time recording background, only occasionally does it record the desired information. Efficiency and consequently the sensitivity of such a system is far from optimum. The time required to observe a NMR spectrum by the CW method is Δ/r , where Δ is the spectral width and r is the resolution desired. For ^{13}C at 25MHz, where Δ is typically about 5 kHz and the line widths are about 1Hz, one must scan the 5-kHz region at a rate of 1 Hz/sec, or slower. This requires a minimum time of 5000 sec (or 83 min). To obtain good resolution, 100 scans are required to increase the S/N ratio. Thus, $83 \times 100 = 8300$ min = 138 hr and 20 min. In other words, more than five days. It is obvious that this is not a feasible approach to the problem.

Fortunately, an alternative method has been developed e.g. pulse NMR. If a spectrum is thought of as a large number of small increments in frequency, each increment being just large enough to contain a typical spectral line, these increments can be examined simultaneously. This removes the constraint on scanning rate, since there is no scan.

Naturally the instrumentation must be modified appropriately to accomplish the simultaneous excitation of all the spectral lines and to sort the resulting information into the conventional representation of spectral lines. This is accomplished by applying, e.g. a strong pulse of rf energy (H1) to the sample for a very short time (1-1000 μsec). Under the influence of the pulse of rf energy, the magnetic moment spirals away from the z-axis in the direction of the static field. If the pulse is of the proper strength and duration, the magnetic moment is tipped by 90° and comes to rest

in the xy-plane. After the pulse has terminated, the restoring torque of the static field, H_0 , causes a precession around the z-axis at the resonance frequency. The free precession of the nucleus under the influence of only the static field induces a decaying sinusoidal voltage in a coil of wire surrounding the sample. The voltage decays partly because the nuclear magnetic moment vector slowly spirals back up to its original position with an exponential time constant, T_1 , the spin-lattice relaxation time. After several time constants ($3T_1$ to $5T_1$), the nuclei will have regained equilibrium and a second pulse can be applied to repeat the process. Another reason for the decay of the free induction signal results from magnet inhomogeneity over the entire sample volume. Thus, some otherwise identically precessing nuclei begin to precess at slightly different rates and slowly lose phase coherence. Here we are speaking of the spin-spin relaxation time constant, T_2 . The free induction decay (impulse response) and the conventional continuous-wave display (steady state equilibrium) of the NMR spectrum form a Fourier transform pair. That is, the time response of the spins can be calculated from their frequency domain spectrum, and vice versa. The response of the entire spin system is picked up in the normal manner, amplified, and detected in the spectrometer. The free induction decay signal following each repetitive pulse is digitized by a fast analog-to-digital (ADC) converter, and the successive digitized transient signals are coherently added in the computer until an adequate signal-to-noise ratio is obtained. Using the Cooley-Tukey algorithm, a computer then performs a fast Fourier transformation to the frequency domain to plot a normal spectral presentation of the NMR absorption versus frequency in a matter of 10-20 sec. Due to chemical shielding, each nucleus may resonate within a range of Larmor frequencies, depending on the chemical environment. In order to rotate all nuclear spins within that range by the same angle, the strength of the rf pulse must meet the following requirement: the pulse width, t_p , must be much shorter than the relaxation times, that is $t_p \ll T_1, T_2$, so

that relaxation is negligible during the pulse [9].

Nuclear Overhauser Enhancement Effect (NOE)

In an ordinary ^{13}C NMR experiment, broad band decoupling technique is used for removing proton splitting from ^{13}C NMR spectra. Although decoupling increases the sensitivity of NMR experiments because the intensities of all multiplet lines in a coupled spectrum are accumulated in one singlet signal in the decoupled spectrum, the intensity of the ^{13}C signal often increases much more than expected. This effect is called Nuclear Overhauser Enhancement effect (NOE). The NOE effect arises from an intramolecular dipole-dipole relaxation mechanism. In an ^{13}C - ^1H decoupling experiment, the transitions of ^1H are irradiated while the resonance of ^{13}C nuclei are observed. Since the irradiating field is very strong, the homonuclear relaxation processes are not adequate to restore the equilibrium population of ^1H nuclei, and these nuclei transfer their energy to the ^{13}C nuclei via internuclear dipole-dipole interaction. The carbon nuclei receiving these transferred amounts of energy, behave as if they had been irradiated themselves and relax. Consequently, the population of the lower energy level of ^{13}C increases, and the intensity of the carbon signal is enhanced [10].

Quantitative Analysis

It is well known that quantitative analysis with ^{13}C NMR is difficult to perform as a result of several factors. The most important of these are differential relaxation and Nuclear Overhauser Enhancement effects. Both of these may effect carbon signal intensity, such that carbon concentrations are not accurately obtained from the spectrum. Apart from this potential problem, ^{13}C NMR spectroscopy is

ideal for quantitative analysis since chemical shifts are well resolved under proton-decoupling conditions and the shift range is large. This usually leads to a favorable situation where there are always some signals well removed from any interference.

Two methods for quantitative analysis have been suggested. The first method involves the addition of 0.1 M $\text{Cr}(\text{AcAc})_3$ or $\text{Fe}(\text{AcAc})_3$ which is called a "shiftless shift reagent" or "relaxation agent" to the solution, and using a short pulse delay and gated decoupling. The relaxation agent quenches the Nuclear Overhauser Effect for all carbons and shortens all T_1 values enough so that pulses can be repeated at about 1-sec intervals. It should be noted that any method that relies on chemical agents may yield erroneous results due to selective relaxation or shielding of the agent from the center of interest. Paramagnetic relaxation reagents, in addition to shortening the relaxation times of carbons not bonded to hydrogen, will also suppress the NOE for carbons bonded to hydrogen.

The second method involves a long pulse delay ($>5T_1$) and gated decoupling. A gated decoupling sequence can be used to eliminate the NOE while still decoupling the protons; however, this technique requires long delay times between pulses, and the process can be quite time-consuming. If the NOE is completely suppressed, quantitative integration of peaks in the ^{13}C NMR spectra can be performed.

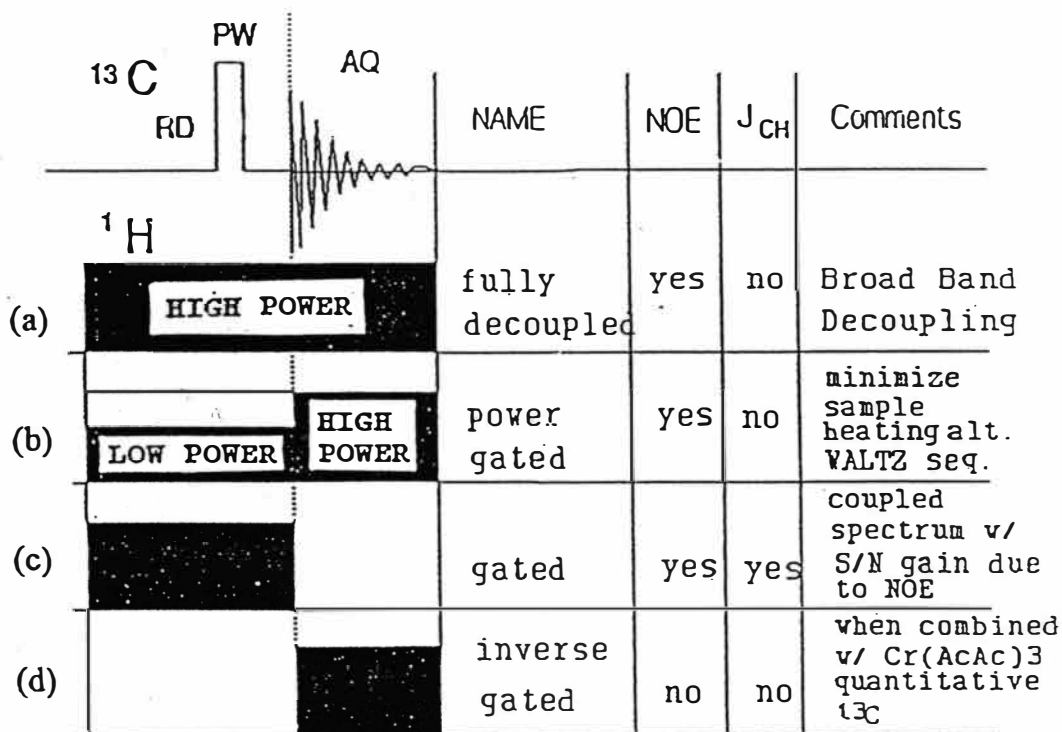
In this project both techniques for eliminating NOE effects were investigated. The use of relaxation reagents can help when one is dealing with compounds having long relaxation times. This reagent shortens relaxation times and allow faster acquisition rates. Paramagnetic (or relaxation) reagent methods employ 0.1 M chromium acetylacetonate $\text{Cr}(\text{AcAc})_3$ or $\text{Fe}(\text{AcAc})_3$ solution. This technique reduces the NOE factors of all ^{13}C nuclei uniformly toward unity and permits a shorter recycle time by shortening the T_1 values. [9]

Gated-decoupling techniques are also necessary for optimizing ^{13}C

quantitative determinations. To obtain ^{13}C NMR spectra, the following decoupling techniques are used for different purposes. The decoupling sequences are shown in Figure 5.

Broad-band ^1H - ^{13}C Decoupling

The proton decoupler is on during the whole period (Figure 5a). NOE is retained and coupling is eliminated. This technique is used for qualitative analysis.



RD = relaxation delay; PW = pulse width;
AQ = acquisition

Figure 5. NMR Decoupling Techniques.

Power gated-decoupling

The proton decoupler is on with low power during the relaxation delay and pulse period and high power level during the acquisition period (Figure 5b). The NOE is maintained and coupling is eliminated.

Gated-decoupling

In this method, the proton decoupler is on during the first period which is relaxation delay and pulse process, and is gated off during the second period-the acquisition process. Both NOE and $^{13}\text{C}\{-^1\text{H}\}$ coupling result in the spectra (Figure 5c). Power gated-decoupling and gated-decoupling techniques are used in NOE study for obtaining the structure data and are also used in 2D spectroscopy.

Inverse-gated-decoupling Technique

The proton decoupler is gated off during the relaxation delay and pulse period, and is on during the acquisition period (Figure 5d). By this method, neither NOE nor $^{13}\text{C}\{-^1\text{H}\}$ coupling are observed in the ^{13}C spectra. This is due to the fact that the multiplets collapse instantaneously and the NOE requires a time of the order of T_1 to build up. Figure 6 shows the pulse sequence employed. When combined with shiftless shift reagent ($\text{Cr}(\text{AcAc})_3$), quantitative analysis can be performed [11].

CHAPTER II

EXPERIMENTAL PROCEDURE

The NMR spectrometer used in these studies was purchased in 1987 from IBM Instruments, Inc. as a model NR/200AF. This instrument was actually an AC 200 made by Bruker Instruments. In 1988 IBM sold all their NMR instrument interests to Bruker. Thus, the instrument is now considered to be a Bruker AC 200 NMR spectrometer. This instrument was used to obtain all of the ^{13}C spectra at 50.327 MHz. The AC 200 spectrometer employs an internal deuterium lock system. The NMR samples were contained in 5-mm sample tubes. In all experiments, deuteriochloroform (CDCl_3) was used for lock stabilization and as solvent.

The ^1H -decoupled ^{13}C FT NMR experiments were performed with inverse-gated heteronuclear decoupling program provided by IBM Instruments, Inc. In this mode, the proton decoupler is on only during the acquisition period and is gated off for a much longer time interval. All experiments were completed within 13 hour. Data acquisition time was 3 seconds after each of 2500 pulses (90° pulse width) with a time delay of 3 seconds between the end of data acquisition and the beginning of the next pulse. In all experiments, the flip angles used were 90° rf pulses. A total of 2500 free induction decays were collected and time-averaged at 21°C in each ^{13}C NMR experiment. The sample tubes were sealed by glue to prevent loss of volatile components and cooling air is on to prevent the sample from overheating. The phase correction and integrations were performed with the standard software provided by Bruker Instruments. Normal ^{13}C acquisition parameters were used as shown in Table 1.

A program PRODEC listed in Table 2 was used for inverse-gated decoupling.

Table 1

NMR Spectrometer Parameters

spectrometer frequency:	50.323
synthesizer frequency:	50.323
observe frequency offset:	6534.000
decoupling frequency offset:	3154.530
data size:	32768 K (1024 points per K)
time domain:	32768 K
spectral width:	18518.518
digital resolution:	1.13
pulse width:	8
relaxation time:	3.000
receiver gain:	320

Table 2

NMR Program (PRODEC) for Inverse-Gated Decoupling for
Heteronuclear ^1H -decoupled without NOE [12]

1 ZE	Zero memory.
2 D1 BB DO	Set decoupler to BB mode, power DP gated off during D1.
3 GO=2 BB	Turn on BB decoupling simultaneously (RD=0) with PW pulse, acquire data, loop to 2 DS+NS times.
4 DO	Decoupling gated off.
5 EXIT	Exit.

Tris(acetylacetonato)chromium(III) ($\text{Cr}(\text{acac})_3$) was used as the relaxation reagent. The NMR samples were prepared by carefully weighing out quantities of the sample and $\text{Cr}(\text{acac})_3$. The mixture were then dissolved in a known amount of CDCl_3 (deuteriochloroform).

Reagents

Steroids: (a) 19-Nortestosterone 17-Decanoate (Nandrolone Decanoate), Sigma Chemical Company; (b) Testosterone (17 β -hydroxy-4-androsten-3-one 17-cyclopentylpropionate), Sigma Chemical Company; (c) 6 α -methyl-17 α -hydroxy-progesterone acetate (Depo-provera) 95%, Sigma Chemical Company; (d) Oxymetholone, U.S. Food and Drug Administration, Detroit District; and (e) Methyltestosterone, Sigma Chemical Company.

Excipients: (a) Methyl 4-Hydroxybenzoate 99%, Aldrich Chemical Company, Inc; (b) Benzyl Benzoate, Aldrich Chemical Company, Inc; (c) Benzyl Alcohol, A.C.S. grade, Aldrich Chemical Company, Inc; and (d) Propyl 4-Hydroxybenzoate 99+%, Aldrich Chemical Company, Inc.

Solvents: Chloroform-d, 99.8 Atom % D, with 0.03% (v/v) Tetramethylsilane (TMS), Aldrich Chemical Company, Inc.

Standard: Toluene, A.C.S. grade, Aldrich Chemical Company, Inc.

Preparation of Standard Solutions

Standard solutions were prepared by adding an appropriate amount of standard reagent to a series of vials containing a known amount of CDCl_3 . Solid $\text{Cr}(\text{AcAc})_3$ was also added to make the solution 0.1 M in Cr^{3+} . Toluene was also added as internal standard. For each sample the moles of toluene was approximately equal to that of the steroid added. Sample vials were stored in a refrigerator. Sample tubes were sealed before obtaining their spectra in order to prevent loss of volatile components. The composition of standard solutions are shown in Table 3.

Table 3

Composition of Standard Solutions

SOLUTION CONSTITUENTS	
Testosterone 17 β -cypionate	10.0-151 mg
Toluene	15.4-88.5 mg
CDCl ₃	1.5-2.2 g
Cr(acac) ₃	42-50.9 mg
Oxymetholone	ca. 25 mg
CDCl ₃	1-3.4 g
17 α -methyltestosterone	15-130 mg
Cr(acac) ₃ Toluene	47.1-54.2 mg
CDCl ₃	9.1-43.2 mg
	2.256-2.291 g
Medroxyprogesterone Acetate	41.7-120.1 mg
Cr(acac) ₃	52.6-53.2 mg
Toluene	13.4-31.7 mg
CDCl ₃	1-1.03 g
Nandrolone Decanoate	ca. 23.4 mg
Cr(acac) ₃	ca. 33.5 mg
CDCl ₃	0.86-3.2 g

The following procedure [12] was used to obtain the quantitative spectra and integration value for each steroid.

Exponential Multiplication (EM)

Multiplying the FID by an exponentially decaying function weighs the initial portion, which contains the most significant information, more heavily than the tailing portion, which consists mostly of noise. Exponential multiplication enhances the sensitivity (signal-to-noise ratio), but at the expense of resolution since the lines are broadened. EM is not really necessary in most ¹H measurements, because the S/N

ratio is good. With ^{13}C , the signal/noise ratio is not as high as in ^1H measurements, so EM was used for all spectra.

Fourier Transformation of the Spectrum

The FID is a combination of all of the sine waves whose frequencies and amplitudes correspond to the lines in the NMR spectrum. The time domain FID signal is converted into the frequency domain NMR spectrum by the Fourier transformation (FT).

Phase Correction

The result of the Fourier transform can be recognized as a spectrum, but the phase of lines may not be correct. The spectrum must be phase corrected. Automatic phase correction (PK) command was used for all spectra. Due to, for example, differences between sample tubes, sample compounds, concentration effects, etc., slight variations in the phase correction may occur after PK has been used. In order to optimize the automatic phase correction, an Add Phase subroutine was used in the expansion routine.

Baseline Correction

Baseline correction is critical for quantitative spectra. In order to make all spectra comparable and consistent, fully automatic baseline (FAB) command was used for baseline correction for all spectra. This command picks the peaks in the spectrum, defines the baseline, and fits a third-order polynomial function to it automatically. It then subtracts this function from the spectrum to give a more level baseline.

Integration

Integration was made manually for each peak in spectrum. In the expansion mode, each peak was expanded to fill approximately $\frac{2}{3}$ of the screen in width. The integral values were recorded accordingly in integration mode for each peak.

CHAPTER III

RESULTS AND DISCUSSION

Identification of Mixture Components

The separation of components by ^{13}C NMR depends on the equivalent response obtained for all carbon nuclei under quantitative NMR conditions. Under these conditions, peak areas for all resonances within a molecule must equal some integer multiple of the area corresponding to a single nucleus. The minimum number of components in a mixture then can be determined from the quantitative spectrum by counting the number of peak subsets with dissimilar peak intensities [13].

The well-defined relationship between ^{13}C NMR data and chemical structure is ideally suited to the development of computer-aided identification schemes. The Library search algorithms [14-16], pattern recognition techniques [7],[8] and spectral simulation [17-19] have been well developed for interpreting ^{13}C NMR spectra of mixture components. In this project, a library of spectra can be established for all of the steroids of interest. A pattern recognition program may also be developed for qualitative and quantitative analysis purposes. Since qualitative analysis was not a objective of this study, pattern recognition techniques will not be discussed.

A number of preliminary ^{13}C FT NMR experiments were carried out in order to establish the appropriate experimental conditions for this study.

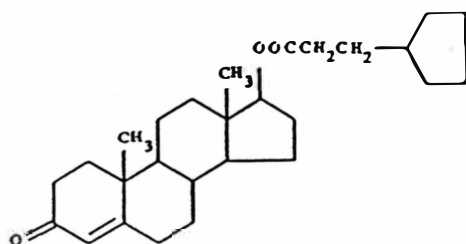
Investigation of Oil and Other Excipients

Steroids injectables are usually dissolved in oils, such as sesame oil, cottonseed

oil, and peanut oil, etc. ^{13}C spectra were obtained for thirteen oils, see appendix B. Comparison of these spectra, reveal their similarities. The peaks are ranged from 13-175 ppm. In this range, a singlet is shown at about 13.5 ppm, while a group of peaks is seen in the range from about 22-34 ppm. Two peaks are seen at 62 and 67 ppm, and another two peaks are shown at 127 and 129, and finally a singlet at 172 ppm. The characteristic peaks for other excipients include C-OH and COOR at about 65-75 ppm and 160-175 ppm respectively.

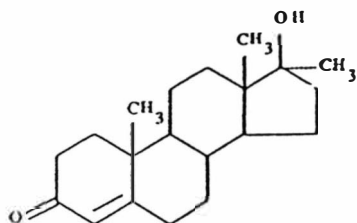
Structure of Steroids and Interpretation of Their Spectra

The structure of steroids studied and their characteristic chemical shifts are listed as follow:



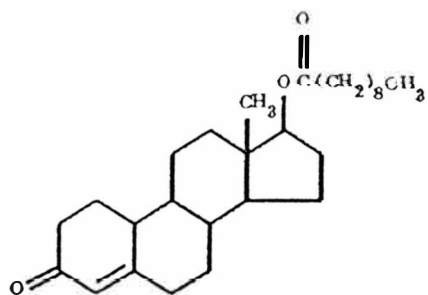
TESTOSTERONE 17-β-CYPIONATE

C-3	198	ppm
C-5	173	ppm
COOR	169.7	ppm
C-4	123	ppm
C-17	81	ppm



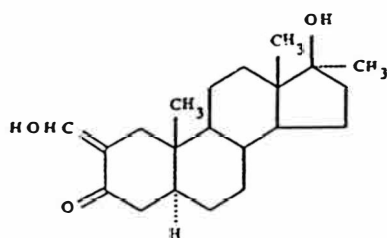
17 α- METHYLTESTOSTERONE

C-3	199	ppm
C-5	171	ppm
C-4	123	ppm
C-17	81	ppm



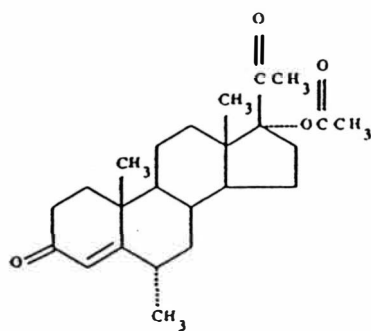
NANDROLONE DECANOATE

C-3	200	ppm
C-5	174	ppm
COOR	167	ppm
C-4	123	ppm
C-17	81	ppm



OXYMETHOLONE

C-3	188	ppm
CHOH	184	ppm
C-2	107	ppm
C-17	81	ppm



MEDROXYPROGESTERONE ACETATE

C-3	202	ppm
C-20	198	ppm
C-5	172	ppm
COOCH ₃	169	ppm
C-4	119	ppm
C-17	95	ppm

Preliminary Study

^{13}C NMR is not a particularly sensitive method when compared to many other spectroscopic techniques. The time averaging method was used for increasing precision, eg. signal-to-noise ratio (S/N), by taking advantage of a very basic and important difference between the NMR signal and noise [9]. In practice, one scan is taken and stored in a computer of average transients (CAT). The CAT stores the first spectrum; subsequent spectra are added to the sum of all previous ones. When the desired S/N is obtained, scanning is terminated, and the final spectrum is plotted on a X-Y plotter. The relationship of the S/N after n scans and S/N after one scan is described by the following equation:

$$\left(\frac{S}{N}\right)_n = \sqrt{n} \left(\frac{S}{N}\right)_1 \quad (2)$$

where S is signal voltage, N is noise voltage, n is number of scans to be added together.

Based on equation 2, the desired S/N is proportional with square n , in other words, S/N increases as the number of scans increases. Figure 6 shows the effect of the number of scans on signal-to-noise ratio or standard deviation. As the scan numbers increase from 500 to 2500, the standard deviation decreases from ca. 0.18 to 0.06. This indicates the S/N increases. The standard deviation increased when the scan number exceeded 2500. The possible reason that causes the decreased S/N is the evaporation of the solvent. The standard deviations were obtained from 5 trials. 2500 scans were used in all subsequent experiments.

Table 4

Standard Deviations of Integrations as a Function of the
Number of Pulses

Number of pulses	Standard Deviation
500	0.185
1000	0.118
2000	0.112
2500	0.065
4000	0.117

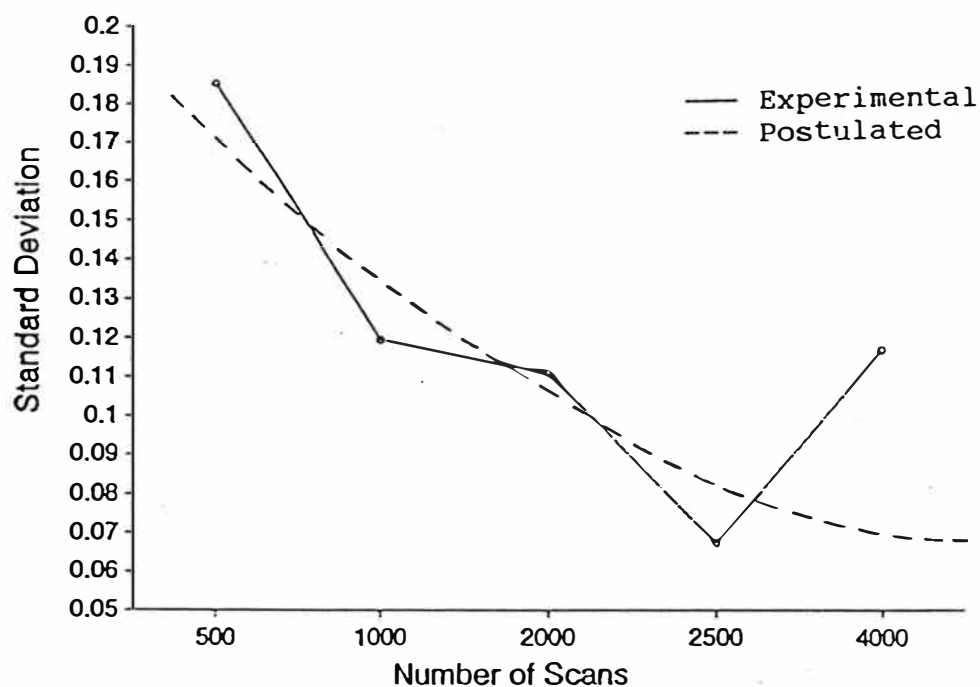


Figure 5. Standard Deviation of Integrals as a Function of the Number of Pulse.

Calibration Plots

Calibration plots were or each peak of all five steroids in order to determine

the linearity of the concentration and the peak area. These plots are shown in appendix C. The correlation coefficients are shown in Table 5. The peak areas were linearly dependent on concentration.

Table 5

Correlation Coefficient

testosterone 17 β -cypionate	oxymetholone	medroxyproge- sterone acetate	17 α -methyl- testosterone	nandrolone decanoate
δ (ppm) C.C.	δ (ppm) C.C.	δ (ppm) C.C.	δ (ppm) C.C.	δ (ppm) C.C.
198.7 0.9970	188.3 0.9783	202.4 0.9909	198.9 0.9908	200.0 0.9960
173.2 0.9980	183.9 0.9087	198.0 0.9990	170.7 0.9989	173.8 0.9960
170.0 0.9967	107.8 0.7469	172.1 0.9989	123.0 0.9967	166.7 0.9970
123.4 0.9949	81.8 0.1480	169.0 0.9999	80.6 0.9965	124.5 0.9970
81.6 0.9901	53.5 0.9823	119.9 0.9891	53.0 0.9776	82.1 0.9990
52.9 0.9881	50.6 0.9823	95.2 0.9913	49.3 0.9843	49.3 0.9990
49.5 0.9910	45.5 0.9345	51.9 0.9984	44.5 0.9974	42.5 0.9920
41.7 0.9814	40.6 0.9992	49.5 0.9747	37.9 0.9980	40.1 0.9930
38.9 0.9920	39.0 0.9963	45.3 0.9994	35.6 0.9925	36.3 0.9880
37.8 0.9862	37.5 0.9996	39.5 0.9962	34.9 0.9984	35.3 0.9960
35.9 0.9942	36.4 0.9963	37.4 0.9895	33.2 0.9998	34.4 0.9920
35.0 0.9954	35.5 0.9769	34.5 0.9826	32.0 0.9927	31.7 0.9940
34.6 0.9960	31.4 0.9814	34.0 0.9931	30.8 0.9989	30.5 0.9860
33.3 0.9957	28.1 0.9929	32.3 0.9963	25.0 0.9803	29.1 0.9840
31.9 0.9932	25.8 0.9793	29.6 0.9958	22.4 0.9952	27.3 1.0000
31.6 0.9949	23.3 0.9927	28.9 0.9958	19.8 0.9950	26.4 0.9910
30.6 0.9941	20.9 0.9998	24.9 0.9932	16.6 0.9871	25.9 0.9920
26.9 0.9914	13.9 0.9964	22.4 0.9954	13.2 0.9875	24.9 0.9950
24.4 0.9951	11.4 0.9961	19.4 0.9952		23.2 0.9950
22.7 0.9914		16.9 0.9956		22.5 0.9890
19.8 0.9922				13.9 0.9980
16.6 0.9890				11.9 0.9980

C.C.= Correlation Coefficient

Standard Addition Method

The amount of unknown present is calculated by

$$W_{unk} = W_{std} \times \frac{N_{std}}{N_{unk}} \times \frac{M_{unk}}{M_{std}} \times \frac{A_{unk}}{A_{std}} \quad (3)$$

In this expression, W_{unk} and W_{std} are the weights of the unknown to be calculated and of the standard taken, N_{unk} and N_{std} are the numbers of protons in the groups giving rise to the absorption peaks whose areas are A_{unk} and A_{std} , and M_{unk} and M_{std} are the molecular weights of the two compounds [20].

The analytical results for testosterone 17 β -cypionate, medroxyprogesterone acetate and 17 α -methyltestosterone are given in Table 6, 7&8. Four runnings were

Table 6

Analytical Results of Testosterone 17 β -cypionate by Standard Addition Method

Testosterone 17 β -cypionate δ 198.7 ppm					
Toluene δ 137 ppm					
No. of detns. (n)	added	avg. found (mg)	Std. dev.	C.V.	error (%)
1	150.9	154.7	5.0	3.2	2.5
2	131.2	139.0	7.6	5.5	5.9
3	114.0	120.9	7.1	5.9	6.0
4	100.7	113.0	4.2	3.8	12.2
5	80.0	80.6	8.3	10.3	0.8
6	40.3	46.2	10.6	22.8	14.7
7	20.7	22.8	4.3	19.0	10.3

performed for each trial. The singlet peak due to the C=O at about 200 ppm for each of the steroids was used for calculation. In this experiment, toluene was used as an internal standard. A well-resolved peak due to C-1 of the toluene at about 137 ppm was used for all of the calculations.

Table 7

Analytical Results of Medroxyprogesterone Acetate by Standard Addition Method

Medroxyprogesterone acetate		δ 202.4 ppm			
Toluene		δ 137 ppm			
No. of detns. (n)	added found (mg)	avg. Dev. (mg)	Std. dev.	C.V. dev.	error (%)
1	41.7	41.9	2.5	6.0	0.5
2	60.1	58.7	2.3	4.0	-2.2
3	80.6	88.4	2.6	3.0	9.6
4	99.8	100.1	4.8	4.8	0.3

Table 8

Analytical Results of 17 α -Methyltestosterone by
Standard Addition Method

17 α -methyltestosterone		δ 202.4 ppm			
Toluene		δ 137 ppm			
No. of detns. (n)	added (mg)	avg. found (mg)	Std. dev.	C.V. dev.	error (%)
1	129.9	131.2	5.3	4.0	1.0
2	110.0	109.8	5.1	4.7	-0.3
3	103.6	104.6	1.6	1.5	1.0
4	94.3	96.4	5.8	6.0	2.2
5	81.0	77.1	3.7	4.8	-4.8
6	50.9	49.0	2.9	6.0	-3.8
7	31.0	31.0	5.0	16.1	0.0

Table 9 shows the results calculated from all of peaks of testosterone 17 β -cypionate. In Table 9, the calculations for peaks 198.7, 173.2 and 170 ppm gave errors less than 2.5 %. Results from other peaks gave unsatisfactory high positive bias. The data shows that unprotonated carbons have better results than those of

protonated carbons. In theory, the maximum NOE factor, $\text{NOEF}=1.99$ (this theoretical limit depends on the observed and decoupled nuclear species). In molecules containing more than a few carbons (molecular weights >200) the NOE is usually

Table 9
Analytical Results of All Observed Peaks

Testosterone 17 β -cypionate

steroid added 150.9 mg
toluene added 88.5 mg peak used 137.2 ppm

chemical shift (ppm)	avg. found (mg)	stds. dev. (mg)	C.V.(%)	Error (%)
198.7	154.7	5.0	3.2	2.5
173.2	151.5	7.7	5.1	0.4
170	152.2	3.5	2.3	0.9
123.4	206.0	27.7	13.5	36.5
81.6	242.2	1.9	0.8	60.5
52.9	208.5	16.0	7.7	38.1
49.5	210.8	12.8	6.1	39.7
41.7	139.9	6.3	4.5	-7.3
38.9	162.9	6.2	3.8	8.0
37.8	143.3	11.0	7.7	-5.1
35.9	256.9	5.8	2.3	70.2
34.95	266.3	6.6	2.5	76.5
34.6	243.8	19.2	7.9	61.6
33.3	254.3	8.2	1.6	68.2
31.9	279.2	13.1	4.7	5.0
31.6	368.8	25.7	7.0	144.4
30.6	235.0	38.4	8.2	55.7
26.9	262.5	8.3	3.1	74.0
24.4	321.1	23.9	7.4	112.8
22.7	239.1	21.8	9.1	58.4
19.8	249.6	28.6	11.5	65.4
16.6	173.4	15.8	9.1	14.9
11.1	148.0	15.8	10.7	-1.9

(but not always) complete for all protonated carbons. Nonprotonated carbons achieve NOEF values of 0.8 to ~2.0. [21] In steroids, the NOEF for unprotonated carbons is about 1.3 to 1.6 which is smaller than that of protonated carbons. The NOE is probably quenched for the nonprotonated carbons. However NOE is probably not fully quenched for the protonated carbons. This may explain the high positive error for the protonated carbons.

The reproducibility of the peak integrals in a single sample has shown that the precision of the integral measurement appears to be the major limiting factor in the overall precision of the assay.

CHAPTER IV

CONCLUSION AND SUGGESTIONS FOR FURTHER WORK

The results of the studies presented here indicate that accurate qualitative and quantitative analyses for steroids by ^{13}C NMR are possible. The NOE effect for unprotonated carbons can be quenched by using shiftless shift reagent $\text{Cr}(\text{AcAc})_3$ along with the inverse gated decoupling technique. A 3-s time delay was employed between the end of each data acquisition and the beginning of the next pulse with the decoupler being turned on only during the acquisition. This condition is not satisfactory when protonated carbons are used for quantitative analysis. It should be possible to further eliminate the NOE effect of the protonated carbons with longer relaxation time. The analysis time is estimated at about 2 hours with very little sample preparation time.

An identification method is also needed to establish. A pattern recognition system of steroids of interests will be developed. Simulated libraries may also be constructed from structural data of steroids. A search program can be developed for identification.

REFERENCES

- [1] Cheever-K; House-MA, *J-Cardiovasc-Nurs.*, 1992 Jan,; 6(2); 19-30.
- [2] Foye, W. O., *Principles of Medicinal Chemistry*, 3rd Ed., Lea & Febiger, Philadelphia, London, 1989, pp. 433-443.
- [3] Weast, R. C. and Astle, M. J., *CRC Handbook of Chemistry and Physics*, CRC Press, Inc., Cleaveland, OH, 71st Ed., 1991, C738-739, C745-755.
- [4] Tietz, N. W., *Fundamentals of Clinical Chemistry*, W. B. Samnders Co., Philadelphia, PA, 1976, 481-482, 741-770.
- [5] Walters, M. J., Ayers, R. J., and Brown, D. J., *J. Assoc. Off. Anal. Chem.*, 1990, 73, No. 6, 904.
- [6] *U.S. Pharmacopeia*, 21st Rev., U.S. Pharmacopeial Convention, Rockville, MD, 1985, pp. 685, 708, 761, 983, 1025.
- [7] Wilkins, C. L. and Brunner, T. R., *Anal. Chem.* 1977, 49, 2136-2141.
- [8] Sjostrom, M. and Edlund, U. *J. Magn. Reson.* 1977, 25, 285-297.
- [9] Dybowski, C. and Lichter, R. L., *NMR Spectroscopy Techniques*, Marcel Dekker, Inc., New York, 1987, 17-25.
- [10] Deronme, A. E., *Modern NMR Techniques for Chemistry Research*, 2nd Edition, Pergamon Press, 1988, 97-127.
- [11] Shoolery, J. N., *Prog. NMR Spectrosc.*, 1977, 11, 79.
- [12] *Advanced Function (AF) Series of FT-NMR Spectrometers User's Manual*. IBM Instruments, Inc., 1985, 4-33.
- [13] Laude, D. A., Jr. and Wilkins, C. L., *Anal. Chem.*, 1986, 58, 2820-1824.
- [14] Jezi, B. A. and Dalrymple, D. L., *Anal. Chem.* 1975, 47, 203-207.
- [15] Schwarzenbach, R., Meili, J., Konitzer, H., and Clerc, J. T. *Org. Chem.*

- Reson.* 1976, 8, 11-16.
- [16] Lieth, v. d., C. W., Seil, J., Köhler, I. and Opferkuch, H. J. *Magn. Reson. Chem.* 1985, 23, 1048-1055.
- [17] Bremser, W., Klier, M. and Meyer, E. *Org. Magn. Reson.* 1975, 7, 92-105.
- [18] Small, G. W. and Jurs, P. C. *Anal. Chem.* 1983, 55, 1121-1127.
- [19] Shelly, C. A., and Munk, M. E., *Anal. Chem.* 1982, 54, 516-521.
- [20] Kasler, F., *Quantitative Analysis by NMR Spectroscopy*, Academic Press, Inc. (London) Ltd., 1973.
- [21] Levy, G. C., Lichter, R. L., and Nelson, G. L., *Carbon-13 Nuclear Magnetic Resonance Spectroscopy*, John Wiley & Sons, Inc., 1980, pp. 78-88.

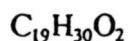
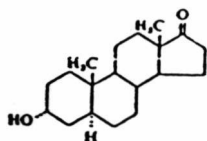
Appendix A

List of Steroid Hormones and Other Steroidal Synthetics

Androgens and Anabolic Agents

Names & synonyms: ANDROSTERONE;
 cis-androsterone;
 3 α -hydroxy-17-androstanone;
 androstane-3 α -ol-17-one.

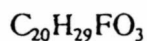
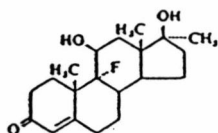
Formulae:



Molecular weight: 290.4
 Melting point(oC): 185-185.5
 Specific rotation: (α)15/D+85- +90 (150 mg. in 10 ml. dioxane)
 Absorption max:

Names & synonyms: FLUCXYMESTERONE;
 (α)-fluoro- 11 β -hydroxy-17 α -
 methyltestosterone;
 9 α -fluoro-11 β ,17 β -dihydroxy-17 α -methyl-
 4-androsten-3-one.

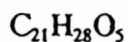
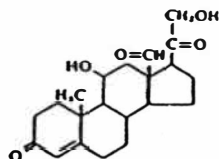
Formulae:



Molecular weight: 336.4
 Melting point(oC): 270
 Specific rotation:(α)25/D+107- +109(alcohol)
 Absorption max:240m μ (ϵ =16,700) alcohol

Names & synonyms: ALDOSTERONE; electrocortin;
 18-oxocorticosterone;
 18-formyl-11 β ,21-dihydroxy-4-pregnene-3,20-dione.

Formulae:



Molecular weight: 360.4

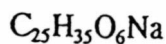
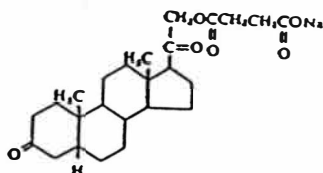
Melting point(oC): 108-112(hydra); 164(anhydrous)

Specific rotation: (α)₂₅/d +161 (10 mg. in 10 ml. chloroform)

Absorption max: 240 m μ (log ϵ =4.20 monohydr.; ϵ mol. 15,000 anhydr.)

Names & synonyms: HYDROXYDIONE SODIUM;
 21-hydroxypregane-3,20-dione-21-sodium hemisuccinate.

Formulae:



Molecular weight: 454.5

Melting point(oC): 193-203

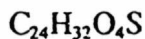
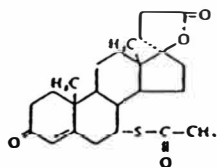
Specific rotation: (α)₂₅/D +95 (chloroform) for free acid.

Absorption max: 280 m μ (ϵ =93.2)

Names & synonyms: SPIRONOLACTONE;

3-(3-oxo-7 α -acetylthio-17 β -hydroxy-4-androsten-17 α -yl)-propionic acid
 lactone.

Formulae:



Molecular weight: 416.5

Melting point($^{\circ}\text{C}$): 135(preliminary)-202

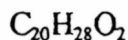
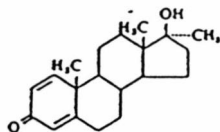
Specific rotation: (α)25/D-34 (chloroform)

Absorption max: $\epsilon^{238}=20,200$

Names & synonyms: METHANDROSTENOLONE;

17 α -methyl-17 β -hydroxy-1,4-androstadien-3-one.

Formulae:



Molecular weight: 300.4

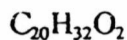
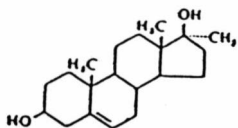
Melting point($^{\circ}\text{C}$): 166-167

Specific rotation:(α)20/D +9- +17 (100 mg. in 10 ml. alcohol)

Absorption max:

Names & synonyms: METHYLANDROSTENEDIOL;
MAD;
methandriol;
17 α -methyl-5-androsten-3 β , 17 β -diol.

Formulae:



Molecular weight: 304.4

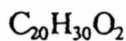
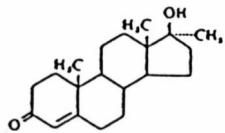
Melting point(oC): 205-207

Specific rotation: (α)₂₀/D-73(100 mg. in 10. alcohol)

Absorption max:

Names & synonyms: METHYL TESTOSTERONE;
17-methyl testosterone;
17 α -methyl- Δ 4-androsten-17 β -ol-3-one;
17(β)-hydroxy-17(α)-methyl-4-androsten-3-one.

Formulae:



Molecular weight: 302.4

Melting point(oC): 161-166

Specific rotation:(α)₂₅/D+69- +75 (100mg. in 10 ml. dioxane)

Absorption max:

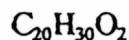
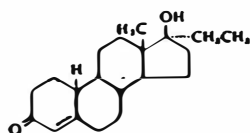
Names & synonyms: NORETHANDROLONE;

17 α -ethyl-19-nortestosterone;

17 α -ethyl-17-hydroxy-4-norandrost-3-one;

17 α -ethyl-17-hydroxy-19-norandrost-4-en-3-one.

Formulae:



Molecular weight: 302.4

Melting point($^{\circ}\text{C}$): 130-136

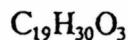
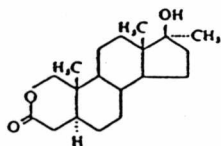
Specific rotation: (α) 25/D +21 (dioxane)

Absorption max: 240 m μ (ϵ =16,500)

Names & synonyms: OXANDROLONE;

17 β -hydroxy-17 α -methyl-2-oxa-5 α -androsterane-3-one.

Formulae:



Molecular weight: 306.4

Melting point($^{\circ}\text{C}$): 230-233.

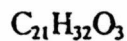
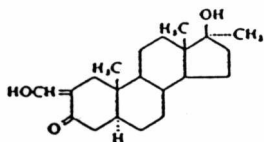
Specific rotation: (α) 25/D -21 (1% in chloroform)

Absorption max: none

Names & synonyms: OXYMETHOLONE;

17-β-hydroxy-2-hydroxymethylene-17α-methyl-3-androstanone;
2-hydroxymethylene-17-α-methyl-dihydrotestosterone.

Formulae:



Molecular weight: 332.4

Melting point(°C): 182

Specific rotation: $(\alpha)_{25/D} = +36$ (200 mg. in 10 ml. dioxane)

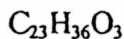
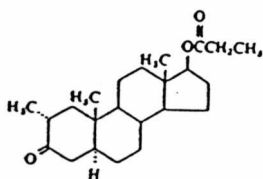
Absorption max: $E_{1\%}^{1\text{cm}} = 547$ at 315 mμ (in alkaline methanol made 0.01 N with NaOH)

Names & synonyms: PROMETHOLONE;

2α-methyl-dihydro-testosterone propionate;

2α-methyl-5α-androstane-17β-ol-3-one-propionate;

Formulae:



Molecular weight: 360.5

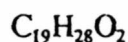
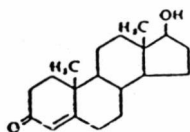
Melting point(°C): 124-130

Specific rotation: $(\alpha)_{25/D} +22- +29$ (200 mg. in 10 ml. chloroform)

Absorption max: without significant absorption from 220-300 mμ (methanol)

Names & synonyms: TESTOSTERONE;
 trans-testosterone;
 Δ^4 -androst-17 β -ol-3-one;
 17 β -hydroxy-4-androst-3-one.

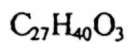
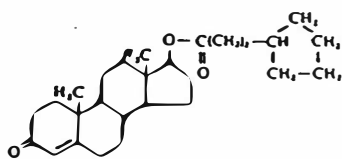
Formulae:



Molecular weight: 288.4
 Melting point($^{\circ}\text{C}$): 151-156
 Specific rotation: $(\alpha)_{\text{D}}^{25} +109$ (400 mg. in 10 ml. alcohol)
 Absorption max: 238 m μ

Names & synonyms: TESTOSTERONE CYPIONATE;
 testosterone cyclopentylpropionate;
 17 β -hydroxy-4-androst-3-one, cyclopentanepropionate.

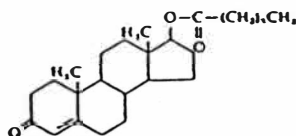
Formulae:



Molecular weight: 412.6
 Melting point($^{\circ}\text{C}$): 100-102
 Specific rotation: $(\alpha)_{\text{D}}^{25} +88.5 \pm 3.5$ (CHCl_3)
 Absorption max: $\lambda_{\text{max}} 241 \text{ m}\mu$ (ϵ 16,125)

Names & synonyms: TESTOSTERONE ENANTHATE;
 testosterone heptanoate;
 17 β -hydroxyandrost-4-en-3-one-17-enanthate.

Formulae:



Molecular weight: 400.6

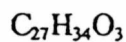
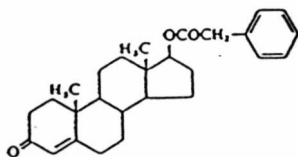
Melting point(°C): 34-39

Specific rotation: (α)₂₅/D +77- +82 (2% in dioxane)

Absorption max: 241 m μ (in ethanol)

Names & synonyms: TESTOSTERONE PHENYLACETATE;
 17 β -hydroxy-4-androsten-3-one phenyl acetate;
 testosterone α -toluate.

Formulae:



Molecular weight: 406.5

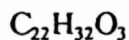
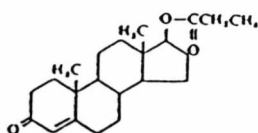
Melting point(°C): 129-131

Specific rotation: (α)₂₅/D +101 \pm 3 (1% in chloroform)

Absorption max: 241 m μ (in ethanol)

Names & synonyms: TESTOSTERONE PROPIONATE;
 Δ^4 -androstene-17- β -propionate-3-one.

Formulae:



Molecular weight: 344.4

Melting point($^{\circ}\text{C}$): 118-122

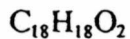
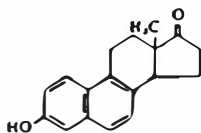
Specific rotation: (α)_{25/D} +83- +90 (100 mg. in 10 ml. dioxane)

Absorption max:

ESTROGENS

Names & synonyms: EQUILENIN;
 3-hydroxy-17-keto- $\Delta^{1,3,5-10,6,8}$ estrapentaene;
 1,3,5-10,6,8-estrapentaen-3-ol-17-one.

Formulae:



Molecular weight: 266.3

Melting point($^{\circ}\text{C}$): 258-259

Specific rotation: (α)_{25/D} +89 (dioxane)

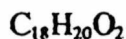
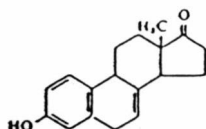
Absorption max: 231, 270, 282, 292, 325, 340 $\text{m}\mu$

Names & synonyms: EQUILIN;

3-hydroxy-17-keto- $\Delta^{1,3,5-10,7}$ estratetraene;

1,3,5,7-estratetraen-3-ol-17-one.

Formulae:



Molecular weight: 268.3

Melting point(°C): 236-240

Specific rotation: (α)_{25/D} +308 (200 mg. in 10 ml. dioxane);

+325 (200 mg. in 10 ml. alcohol).

Absorption max: 283-285 m μ

Names & synonyms: ESTRADIOL (formerly called α -estradiol);

α -estradiol;

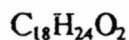
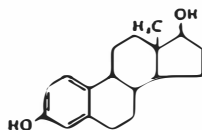
dihydrofolliculin;

dihydroxyestrin;

1,3,5-estratriene-3,17 β -diol;

3,17-epidigydroxyestratriene.

Formulae:



Molecular weight: 272.3

Melting point(°C): 173-179

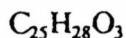
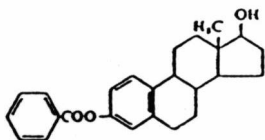
Specific rotation: (α)_{25/D} +76- +83 (100 mg. in 10 ml. dioxane)

Absorption max: 225,280 m μ

Names & synonyms: ESTRADIOL BENZOATE;

β -estradiol-3-benzoate;
estradiol monobenzoate.

Formulae:



Molecular weight: 376.4

Melting point(°C): 191-196

Specific rotation: $(\alpha)_{25/D} +58- +63$ (200 mg. in 10 ml. dioxane)

Absorption max:

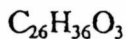
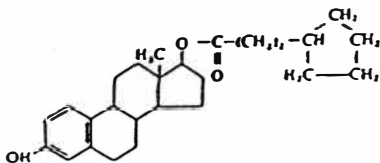
Names & synonyms: ESTRADIOL CYPIONATE;

estradiol cyclopentylpropionate;

β -estradiol 17-cyclopentanepropionate;

1,3,5(10)-estratriene-3,17 β -diol, 17-cyclopentanepropionate.

Formulae:



Molecular weight: 396.6

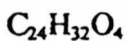
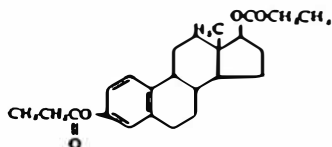
Melting point(°C): 151-154

Specific rotation: $(\alpha)_D +41.5 \pm 3.5$ (dioxane)

Absorption max: 223m μ

Names & synonyms: ESTRADIOL DIPROPIONATE;
 α -estradiol dipropionate;
 17 β -estradiol dipropionate.

Formulae:



Molecular weight: 384.5

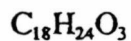
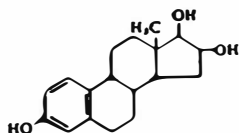
Melting point(°C): 104-109

Specific rotation: (α)_{25/D} +39 \pm 2 (1%) in dioxane)

Absorption max: 268 m μ

Names & synonyms: ESTRADIOL;
 trihydroxyestrin;
 $\Delta^{1,3,5-10}$ -estratriene-3-16-cis-17-trans-diol;
 1,3,5-estratriene-3,16 α , 17 β -triol.

Formulae:



Molecular weight: 288.3

Melting point(°C): 282

Specific rotation: (α)_{25/D} +53- +63 (40 mg. in 1 ml. dioxane)

Absorption max: 280 m μ

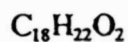
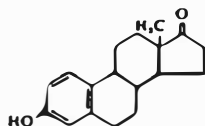
Names & synonyms: ESTRONE;

folliculin;

ketohydroxyestrin;

1,3,5-estratrien-3-ol-17-one.

Formulae:



Molecular weight: 270.3

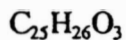
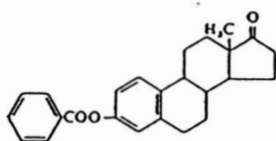
Melting point(°C): 258-262

Specific rotation: $(\alpha)_{25/D} +158$ - $+168$ (100 mg. in 10 ml. dioxane)

Absorption max: 283-285 m μ

Names & synonyms: ESTRONE BENZOATE

Formulae:



Molecular weight: 374.4

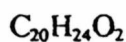
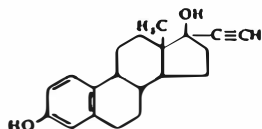
Melting point(°C): 220

Specific rotation: $(\alpha)_{25/D} +120$ (dioxane)

Absorption max:

Names & synonyms: ETHYNYL ESTRADIOL;
 17-ethinyl estradiol;
 17 α -ethynyl-1,3,5-estratriene-3,17 β -diol.

Formulae:



Molecular weight: 296.4

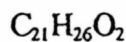
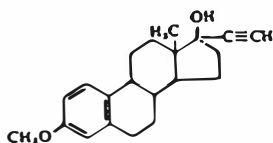
Melting point($^{\circ}\text{C}$): 141-146

Specific rotation: (α)_{25/D} +1- +10 (100 mg. in 10 ml. dioxane)

Absorption max: 248 $\text{m}\mu$

Names & synonyms: MESTRANOL;
 ethynylestradiol 3-methyl ether;
 3-methoxy-17 α -ethynyl-1,3,5(10)-estratriene-17 β -ol;
 3-methoxy-19-nor-17 α -pregna-1,3,5,10-trien-20-yn-17-ol.

Formulae:



Molecular weight: 310.4

Melting point($^{\circ}\text{C}$): 148-154

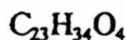
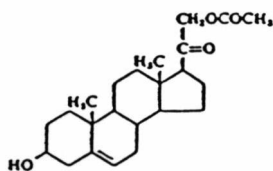
Specific rotation: (α)_{25/D} +2 to +8 (200 mg. in 10 ml. dioxane)

Absorption max: 278 to 287 $\text{m}\mu$ (methanol)

PROGESTOGENS AND PROGESTINS (INCLUDING 19-NORSTEROID COMPOUNDS)

Names & synonyms: ACETOXPREGNENOLONE;
 21-acetoxypregnenolone;
 prebediolone acetate;
 Δ^3 -pregene-3 β , 21-diol-20-one-21-monoacetate;
 21-acetoxy-5-pregnene-3-ol-20-one;
 3-hydroxy-21-acetoxy-5-pregnen-20-one.

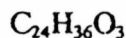
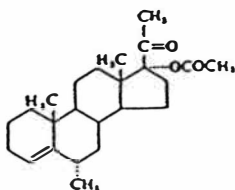
Formulae:



Molecular weight: 374.5
 Melting point(°C): 184-185
 Specific rotation: (α)_{20/D} +37-+43 (dioxane)
 Absorption max:

Names & synonyms: ANAGESTONE ACETATE;
 6α -methyl-4-pregnen-17 α -ol-20-one acetate;
 17 α -acetoxy-6 α -methylpregn-4-en-20-one;
 17 α -acetoxy-6 α -methyl-4-pregnen-20-one.

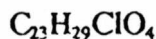
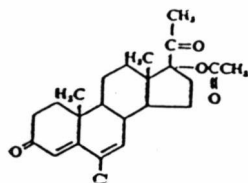
Formulae:



Molecular weight: 372.6
 Melting point(°C): 172-178
 Specific rotation: (α)_{25/D} +40 to +45 (10 mg. in 10 ml. chloroform)
 Absorption max:

Names & synonyms: CHLORMADINONE ACETATE;
 6-chloro- Δ^6 - δ -hydro-17 α -acetoxyprogesterone);
 6-chloro- $\Delta^{4,6}$ -pregnadiene-17 α -ol-3,20-dioneacetate.

Formulae:



Molecular weight: 404.9

Melting point($^{\circ}\text{C}$): 204-212

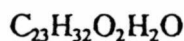
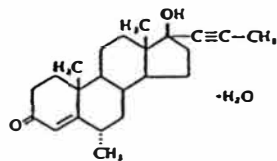
Specific rotation: (α)_{25/D} 0 to -6 (200 mg. in 10 ml. chloroform)

Absorption max: 284 m μ (methanol)

Log ϵ = 4.34 \pm 0.02

Names & synonyms: DIMETHISTERONE;
 6 α ,21-dimethylethisterone;
 6 α ,21-dimethyl-17 β -hydroxy-17 α -pregn-4-en-20-yn-3-one;
 17 β -hydroxy-6 α -methyl-17 α -(prop-1-ynyl)-androst-4-ene-3-one.

Formulae:



Molecular weight: 358.5

Melting point($^{\circ}\text{C}$): App. 100 (dec.)

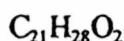
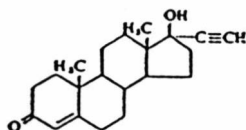
Specific rotation: (α)_{20/D} +16.5 to +18.5 (2% solution in chloroform) (calculated to the anhydrous basis)

Absorption max: App. 240 m μ (anhydrous ethanol)

$E^{1\%}_1 \text{ cm} = 443$

Names & synonyms: ETHISTERONE;
 anhydrohydroxyprogesterone;
 ethinyl testosterone;
 pregneninolone;
 17 α ethynyl testosterone;
 17 α -ethynyl-17 β -hydroxy-4-androsten-3-one.

Formulae:



Molecular weight: 312.4

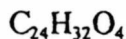
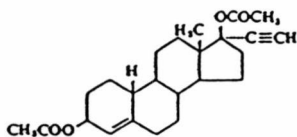
Melting point(°C): 266-273

Specific rotation: (α)25/D-32° (100 mg. in 10 ml. pyridine)

Absorption max: 241 m μ (methanol)

Names & synonyms: ETHYNODIOL DIACETATE;
 17 α -ethynyl-4-estrene-3 β , 17 β -diol-17-diacetate;
 19-nor-17 α -pregn-4-en-20-yne-3 β ,17-diol diacetate.

Formulae:



Molecular weight: 384.5

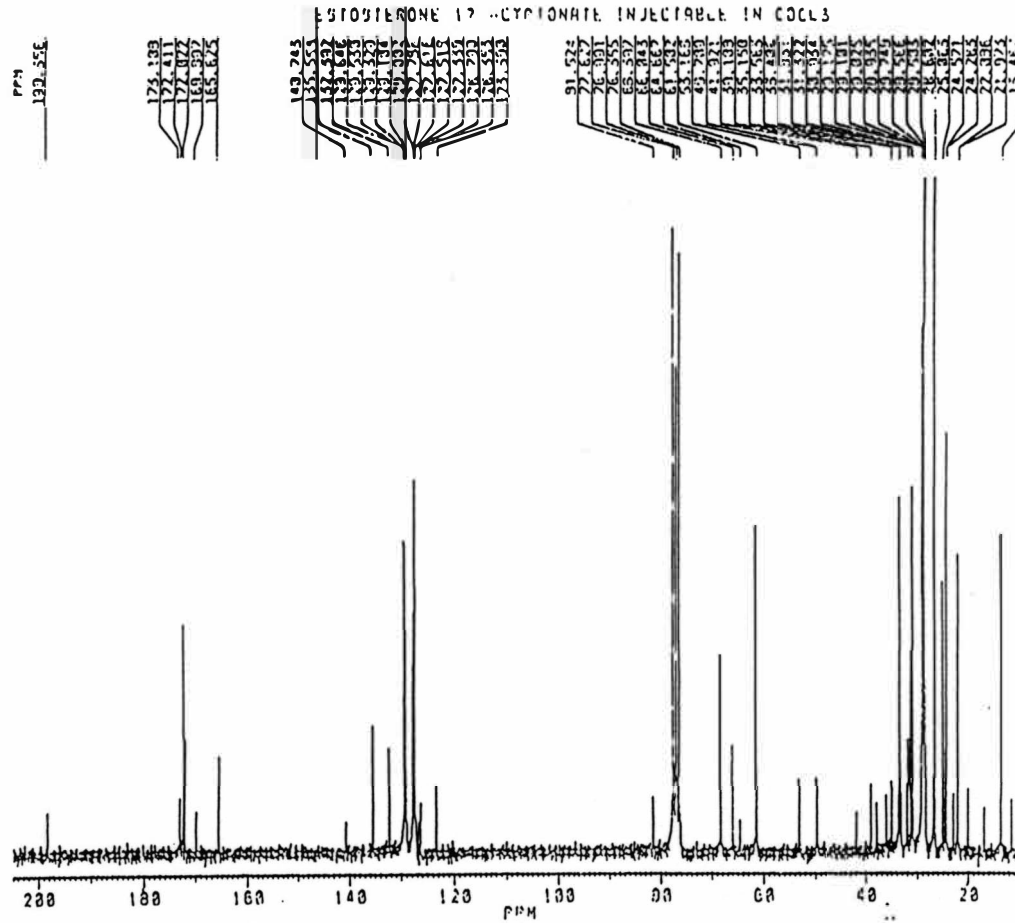
Melting point(°C): 126-132

Specific rotation: (α)25/D-74 (1% in chloroform)

Absorption max: None

Appendix B

^{13}C Spectra



~~SECRET~~

DRUG1.001
 AU PROC.
 RUN13.40
 DATE 9-5-92

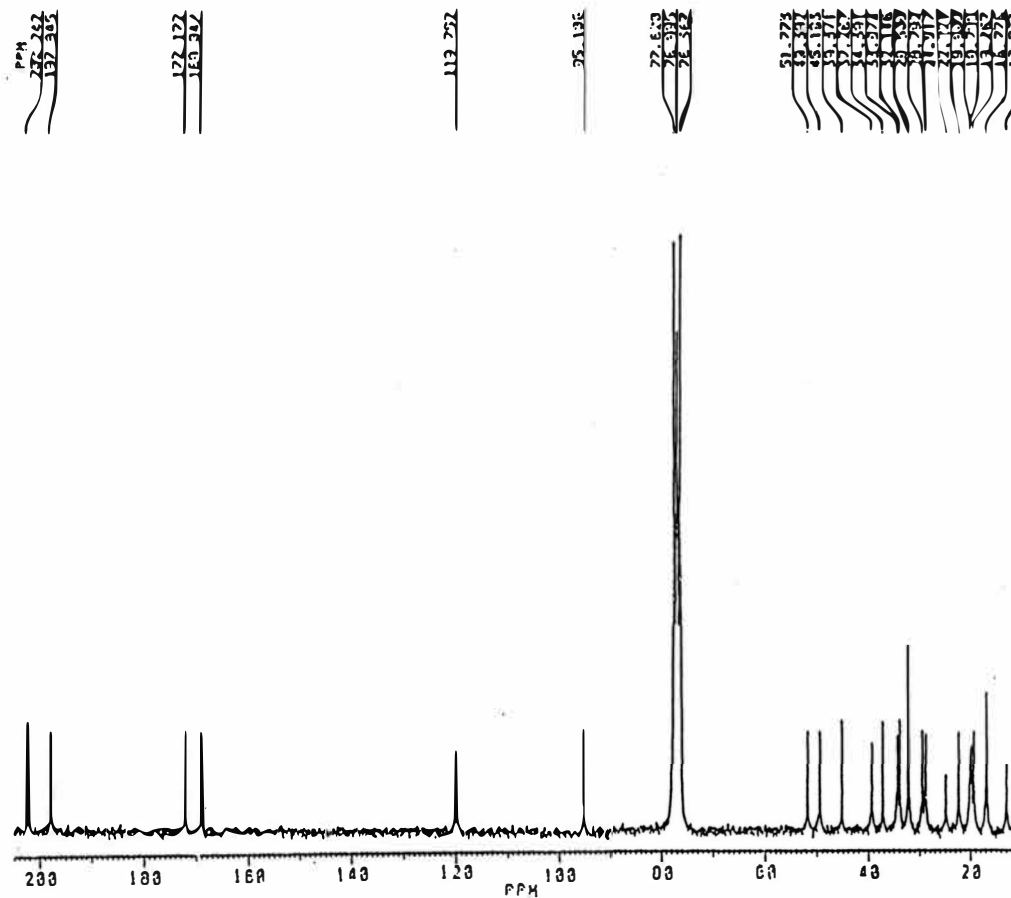
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 OI 6534.000
 SI 32260
 ID 32260
 SX 13510.510
 HZ/CM 1.132

FX 3.0
 RO 3.200
 RO 0.635
 AC 320
 HS 2500
 TE 297

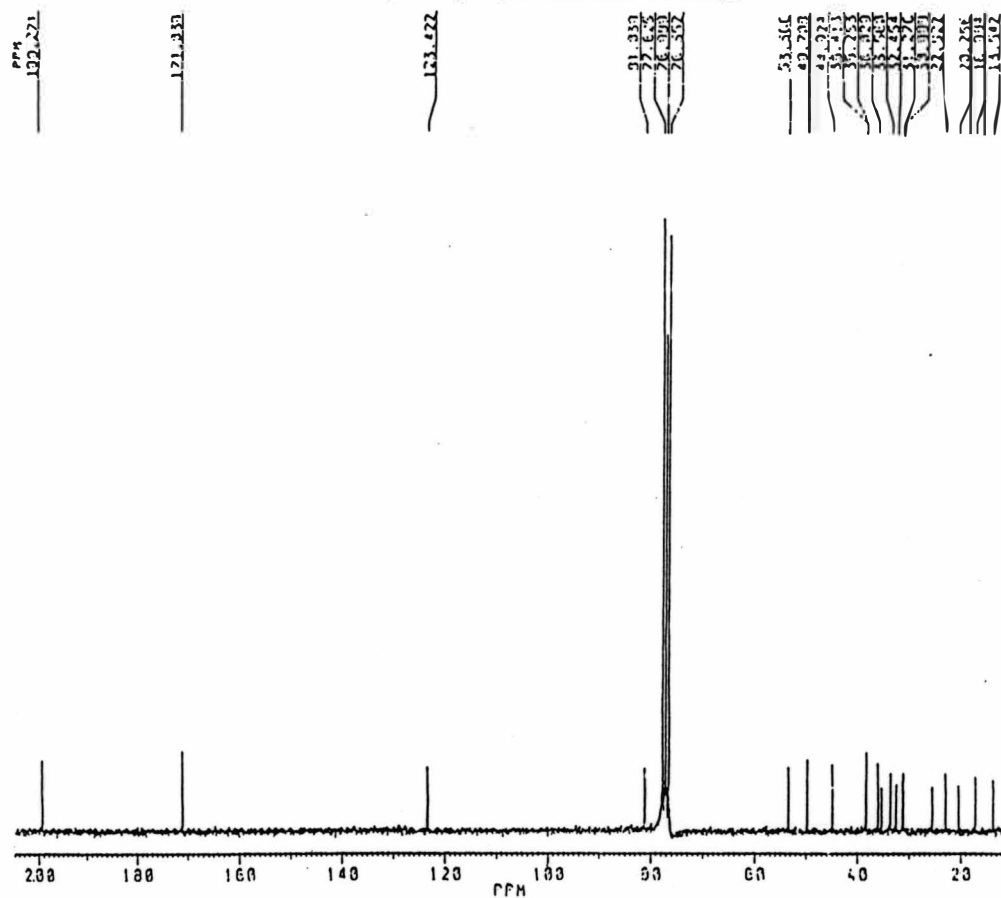
FX 23200
 OI 3154.532
 SP 136.00

F2 0.0
 F1 0.0
 F2 21.00
 F1 205.0026
 F2 13.0010
 HZ/CM 67.290
 PPM/CM 9.200
 SR 75.28

6-METHYL-17-HYDROXY-PROGESTERONE ACETATE IN CCL₄



~~XXXX~~
 PROC1.031
 AU PROD:
 PRODEC
 DATE 29-3-92
 SF 50.523
 ST 50.5232683
 OI 6534.003
 SI 32768
 TO 32768
 SW 18513.513
 HZ/P 1.133
 FW 3.3
 RO 3.030
 RO 3.030
 RG 3.23
 NS 2533
 TE 237
 FW 23203
 OI 3154.533
 OF 13L 00
 L2 1.533
 CE 0.0
 CX 21.03
 CT 0.0
 F1 205.0222
 F2 10.021
 HZ/CM 467.293
 PPM/CM 9.206
 SR 121.62

17 α -METHYLTTESTOSTERONE IN COCIS

~~SECRET~~

ETCA 542
RU PAOC:
SUM18, RU
DATE 24-5-92

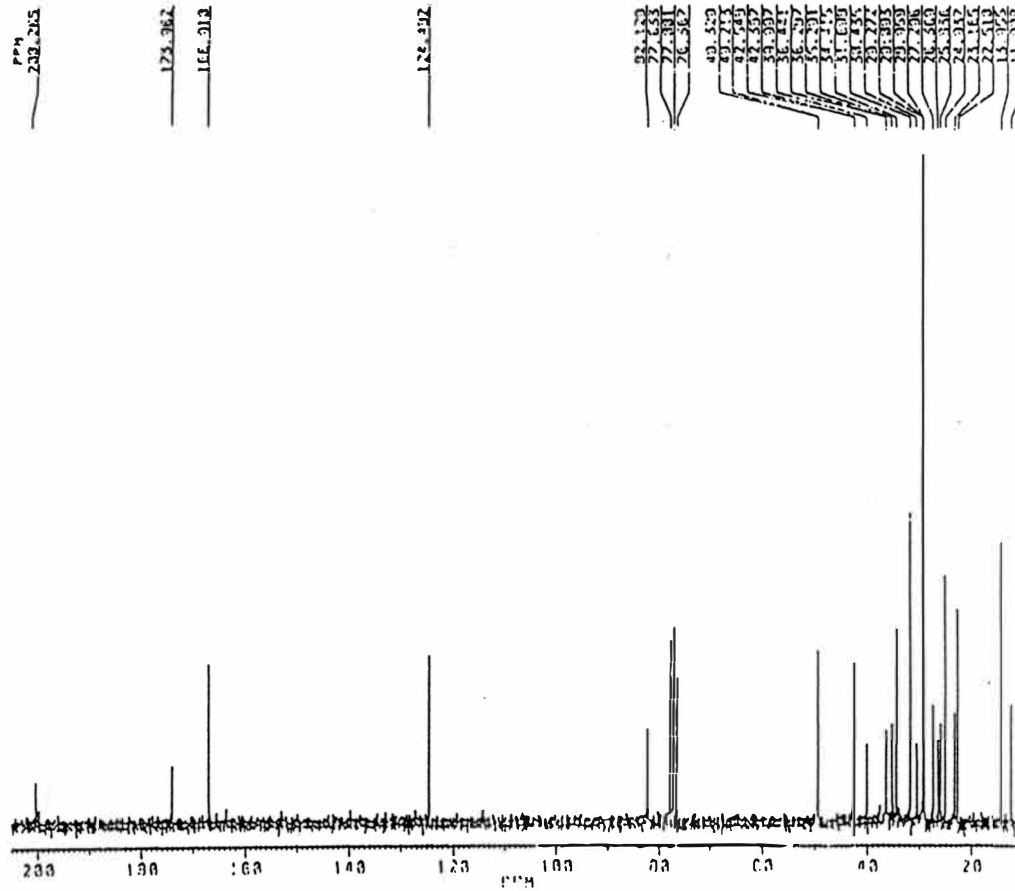
SF 50 323
ST 53 321 2058
SU 2534 380
TO 32760
-10 32769
SU 10515 519
H/P 1.138

FW 3.0
RD 3.0
RD 3.0
RU 320
NS 2508
TE 2207

FW 2303
02 3154 538
03 13L 00

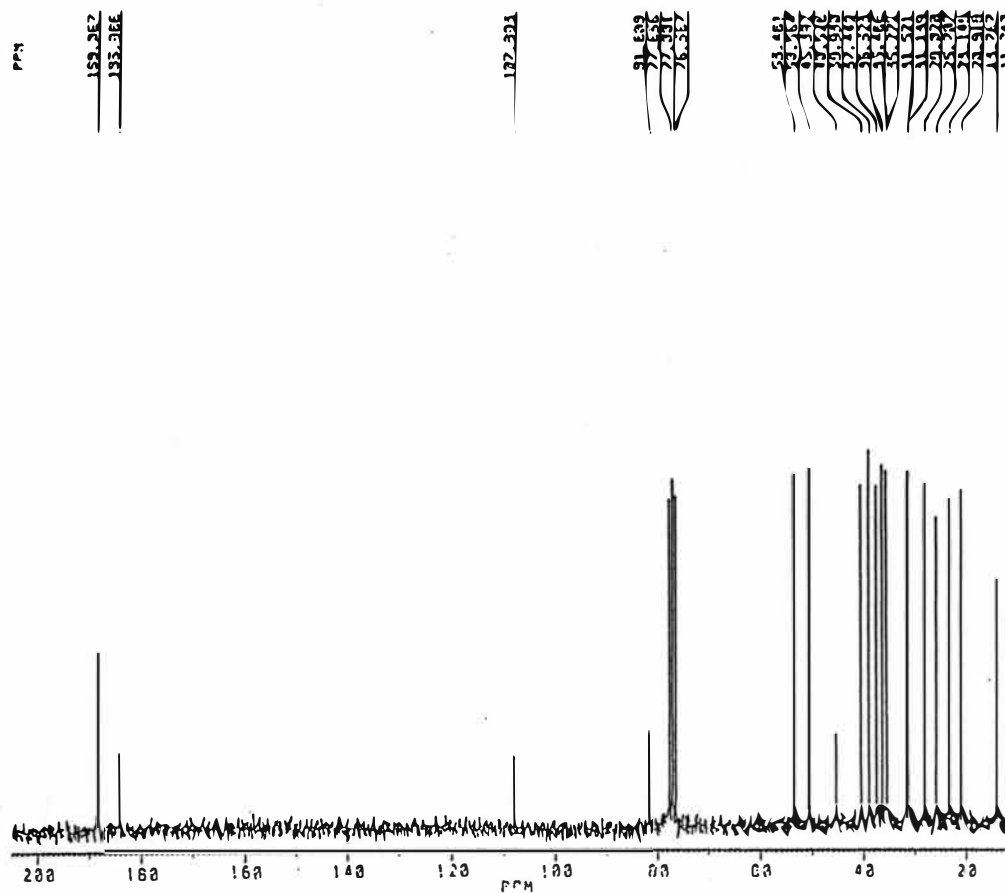
LB 0.0
CE 0.0
CX 21.00
CT 0.0
F1 205 3000
F2 10 3816
H2/CX 407 200
FM/CX 0.208
SR 69.63

19-NORTESTOSTERONE 17-DECANOATE IN CDCL3



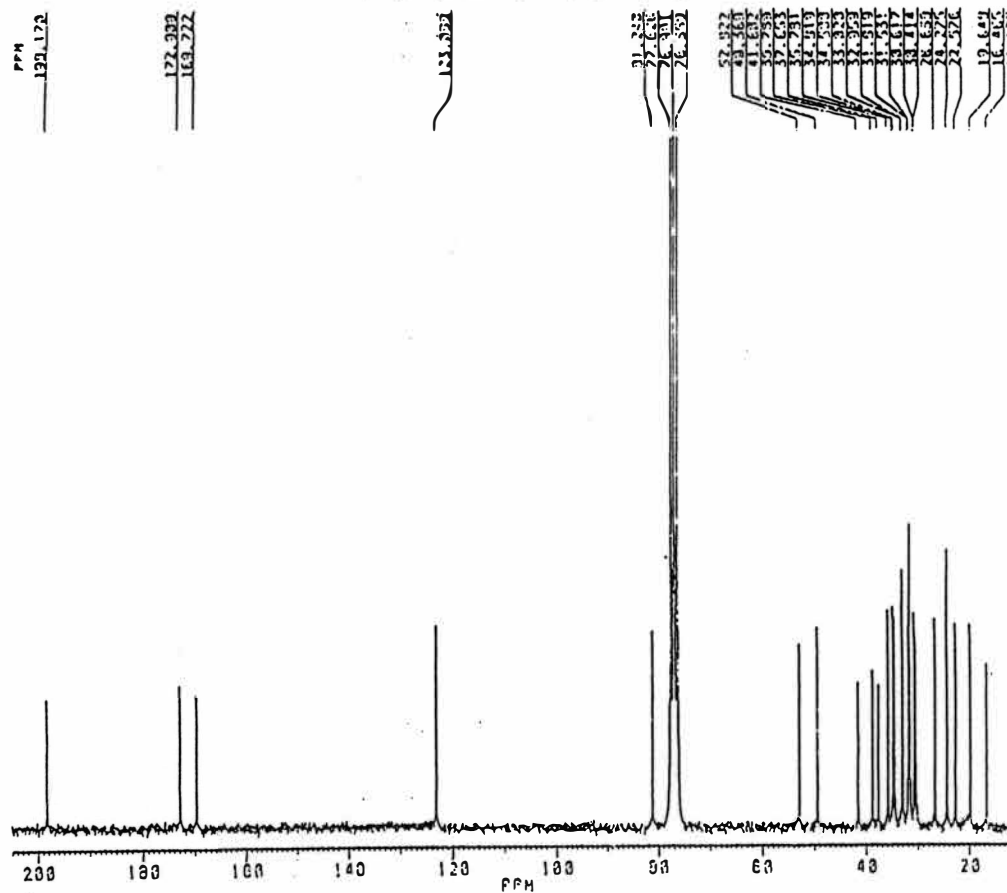
~~EXHIBIT~~
 FID: 12.001
 AU: 0.000
 SUN: 18. AU
 DATE: 7-6-92
 SF: 50.523
 SY: 50.5232693
 OI: 6334.000
 SI: 32763
 SD: 32763
 SW: 15515.519
 HZ/PT: 1.150
 PW: 0.0
 RD: 0.0
 RD: 0.05
 RC: 320
 NS: 2500
 TE: 297
 FX: 23200
 OZ: 3154.530
 OP: 13L 00
 LB: 0.0
 CB: 0.0
 CX: 21.00
 CY: 0.0
 F1: 205.0020
 F2: 10.0010
 HZ/CM: 467.250
 PPM/CM: 5.200
 SR: 51.55

OXYMETHOLONE IN CDCL3



~~OXMETHOLONE~~
 OXIMETHOLONE.002
 DATE 28-11-91
 SF 50.123
 ST 50.1232692
 DI 6534.822
 SD 32758
 SY 32758
 HZ/F 15515.519
 HZ/F 1.158
 PY 9.8
 RJ 3.888
 RQ 3.888
 RC 328
 NS 2888
 TE 297
 FY 23288
 DZ 3154.538
 DP 136.88
 LB 1.583
 CB 0.8
 CX 21.88
 CY 0.8
 FI 235.8822
 F2 18.881F
 HZ/CM 487.298
 PPM/CM 9.205
 SN 42.51

TESTOSTERONE 17-ACETONATE IN CDCL3



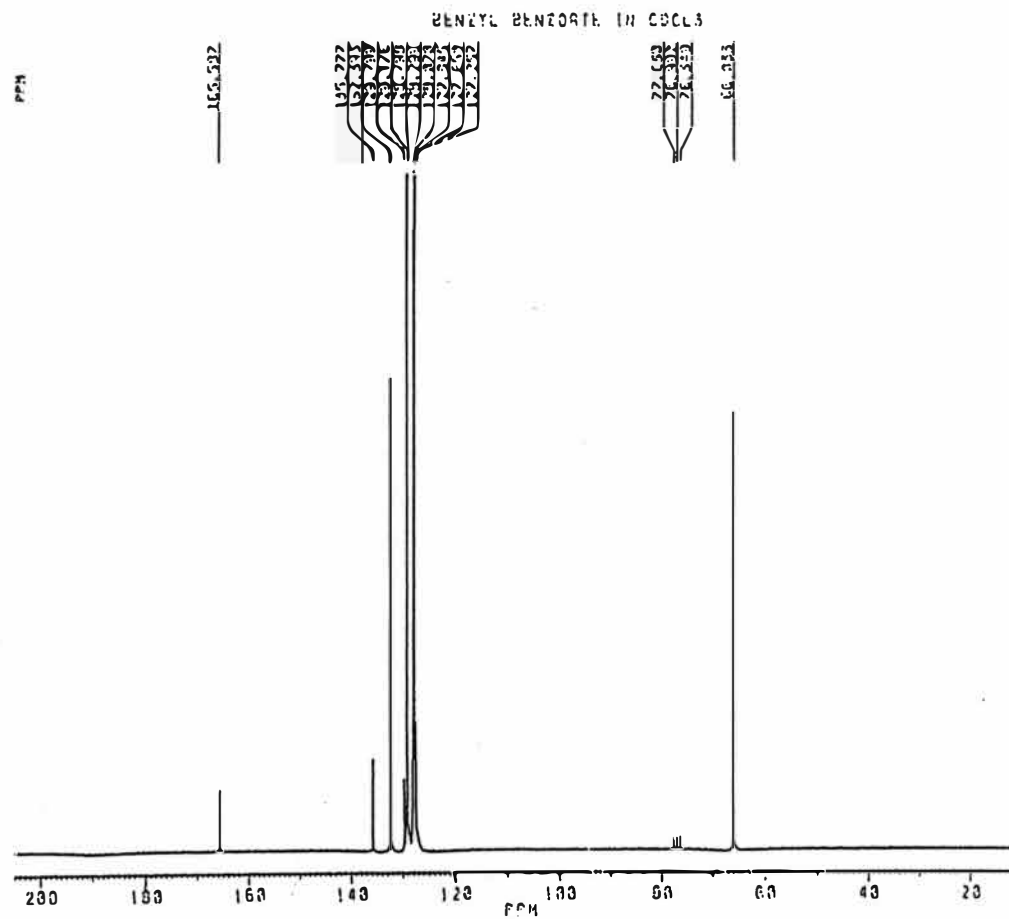
TECA 034
AU PACC
RUN 10.00
DATE 23-4-92

SF 50.323
ST 50.323
OI 50.34
SI 327.63
ID 327.63
SX 18510.518
HZ/F 1.150

FX 3.0
AD 3.030
AO 3.05
AC 3.23
NS 2883
TE 297

FX 23200
O2 3154.530
O2 13L 00

L2 1.530
O2 0.0
CX 21.03
CY 0.0
F1 285.832
F2 10.031
HZ/CH 467.290
PPM/CH 9.206
SR 92.23



~~XXXX~~

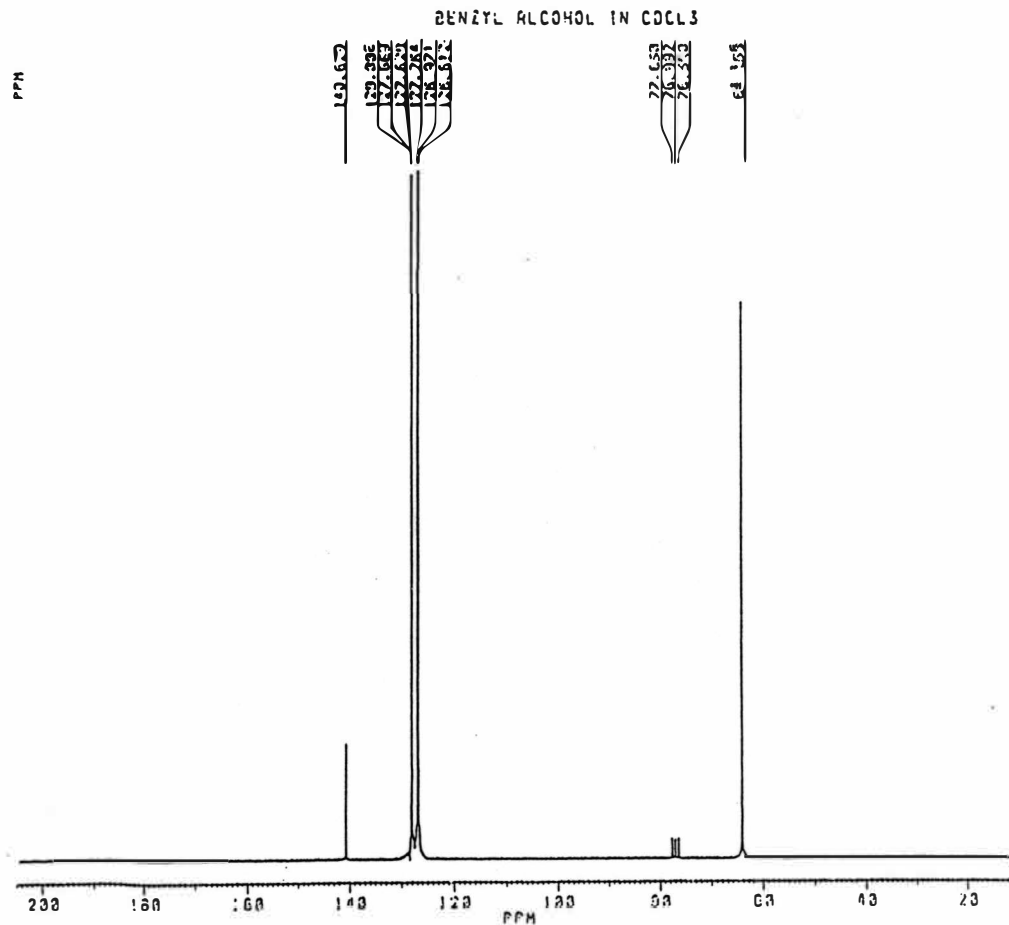
BENZ. 270
AU PROG:
PRODEC
DATE 8-5-92

SF	50.323
SY	50.3232633
O1	65.34.000
S1	32765
T0	32768
SK	13510.519
YZ/PT	1.132

FW	3.0
RO	3.000
AO	.000
RC	320
NS	14296
TE	207

FW	23203
O2	3154.530
DP	131.00

LS	1.500
CS	3.0
CX	21.00
CY	3.0
F1	235.3027
F2	10.3017
HZ/CH	467.293
PPM/CH	9.296
SR	02.93



~~CONFIDENTIAL~~

BENZ. AL
 AU PROC:
 PRODEC
 DATE 7-5-92

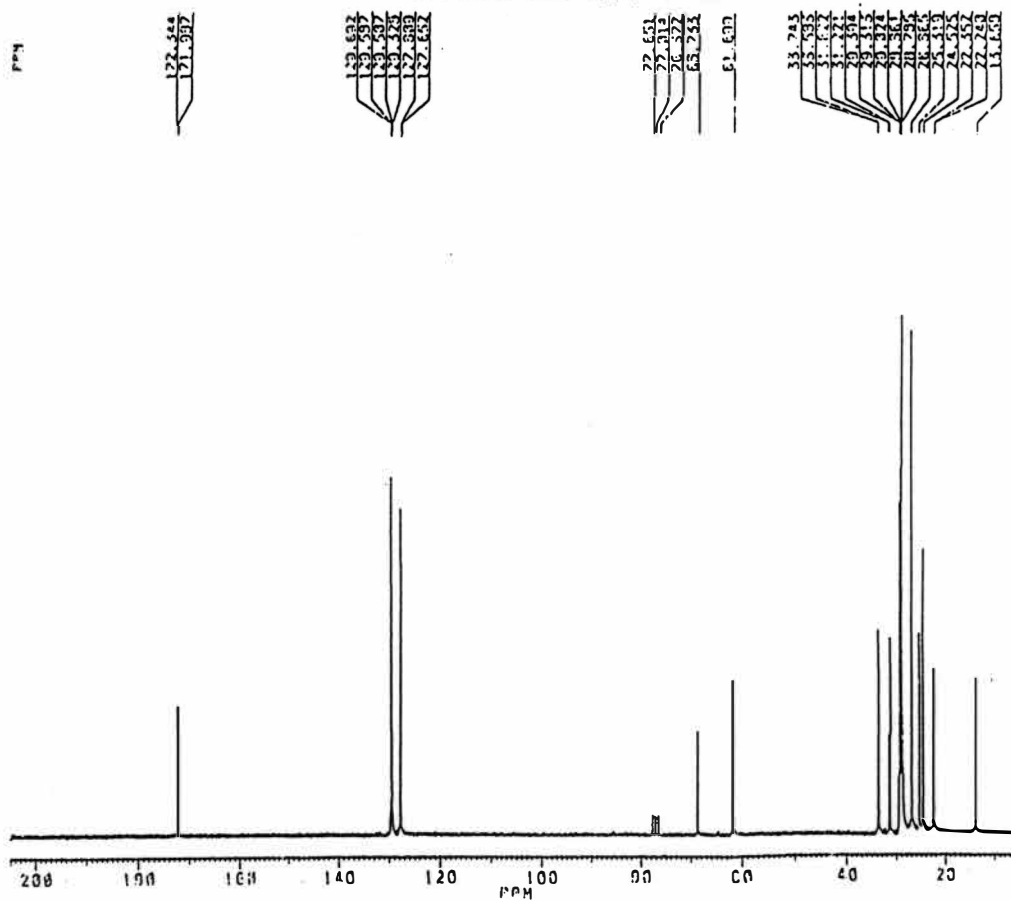
SF 50.323
 SY 50.3232680
 OI 6534.000
 SI 32768
 TO 32768
 SW 18518.519
 HZ/PT 1.130

FW 3.0
 AD 3.000
 AO 685
 RC 320
 NS 4492
 TE 297

FW 23200
 O2 3154.538
 OF 13L 00

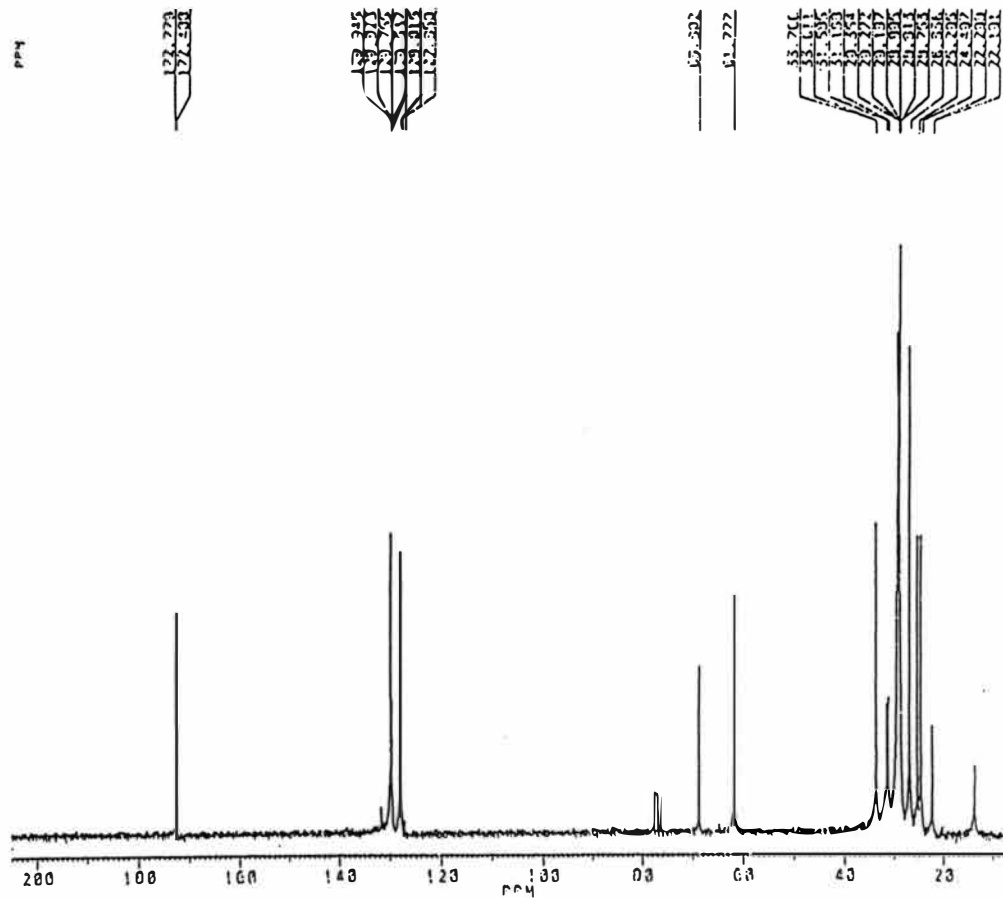
L2 1.500
 G2 0.0
 CX 21.00
 CY 0.0
 F1 205.000
 F2 10.000
 HZ/CM 467.200
 PPM/CM 9.200
 GR 76.41

SUNFLOWER SEED OIL IN COCL3



OIL
 DATE 14-5-91
 SF 50.523
 ST 50.5232690
 SI 6534.000
 SI 32768
 TD 32768
 SW 18516.510
 HZ 1.150
 FX 9.0
 AD 3.000
 AD 3.000
 AC 100
 NS 2823
 TE 207
 FX 23203
 O2 3194.532
 O2 13L 22
 CO2 0.0
 CO2 0.0
 CO2 21.00
 CO2 0.0
 F1 205.0022
 F2 5.0140
 HZ/CH 479.239
 PPM/CH 9.523
 SR 51.55

NOY 012 IN C00L3



10-5-6
3150
L10

5F	50.323
5Y	50.3232500
01	6534.000
01	32700
10	32700
5W	15510.510
42/01	1.130

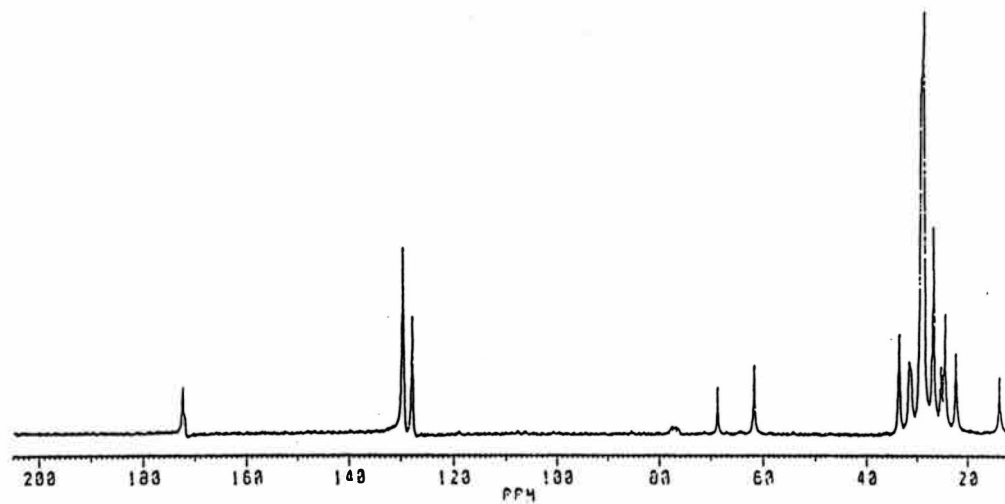
PK	2.3
RD	3.000
RD	3.005
RC	100
NS	2437
TE	207

FX 23200
02 3154.530
00 13L 28

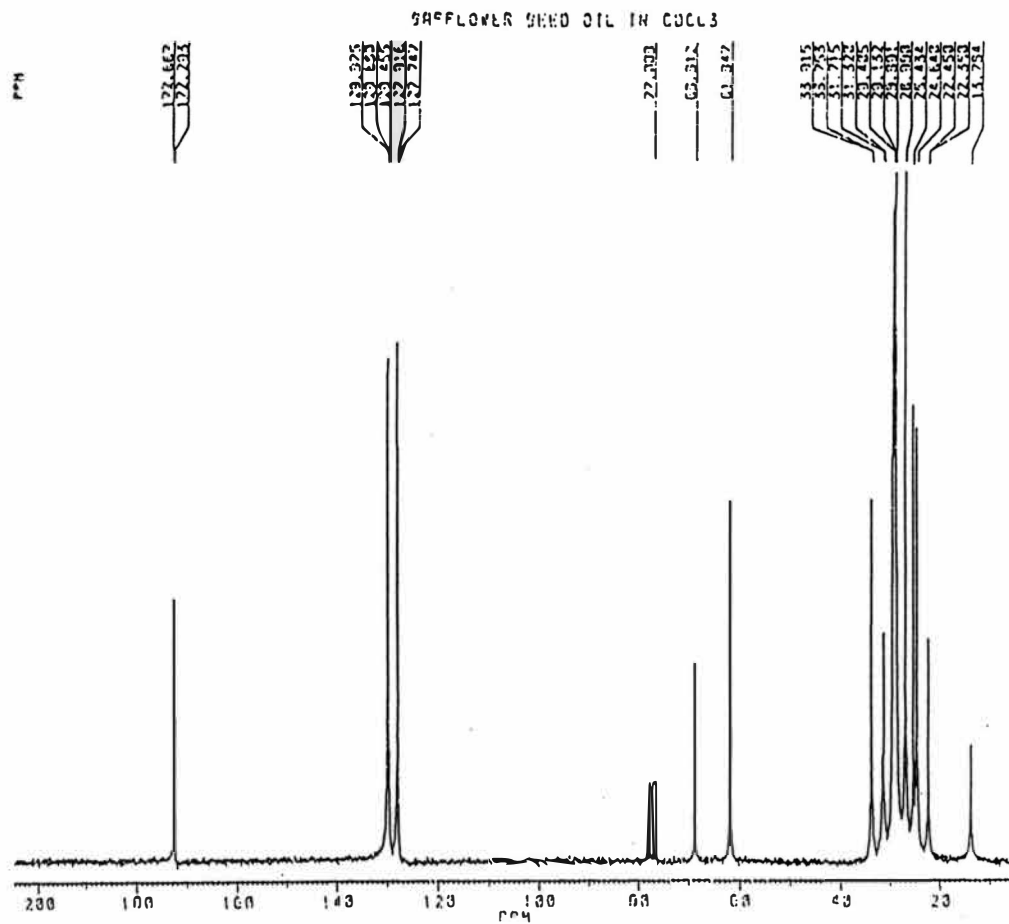
U2	3.0
C2	3.0
CX	21.00
CY	3.0
F1	205.0036
F2	5.0149
HZ/CH	479.239
FM/CH	0.523
GR	-435.57

SESAME OIL IN CCL₄

1



~~EXPER~~
 SESAME OIL
 DATE 20-8-91
 SF 50.523
 ST 50.5232500
 OI 6534.000
 SI 32768
 TO 32768
 SR 19515.515
 MZ/P 1.120
 FW 8.8
 RD 3.330
 AD 3.005
 RL 168
 NS 1005
 TL 207
 FN 23200
 O2 3154.530
 OF 132.22
 LB 1.530
 OB 2.0
 CL 21.00
 CY 3.0
 FI 205.0030
 F2 10.0000
 MZ/LH 467.200
 PPM/LH 0.200
 SR -400.10



~~EXPER~~

OIL
DATE 7-5-91

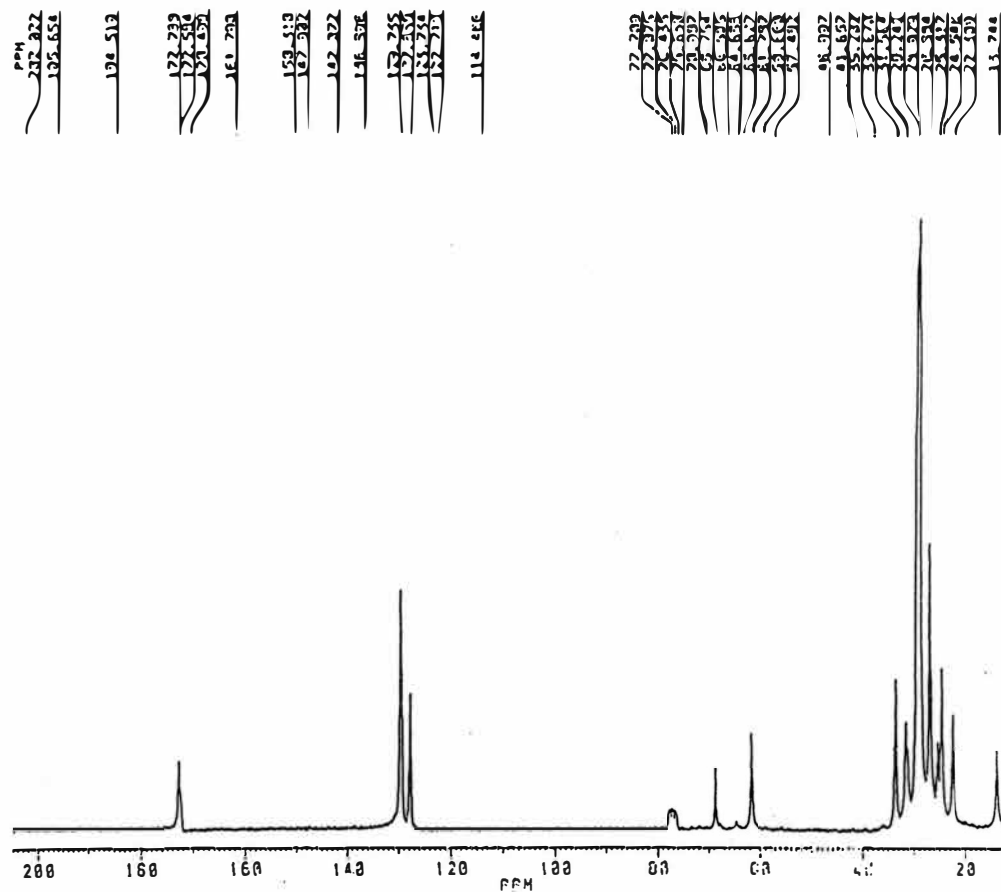
SP 50.523
ST 50.5232080
DI 6534.000
SI 32768
TD 32768
SW 13510.510
HZ/F 1.132

PW 0.0
AD 3.000
AQ 0.005
AC 1.00
NS 1150
TE 297

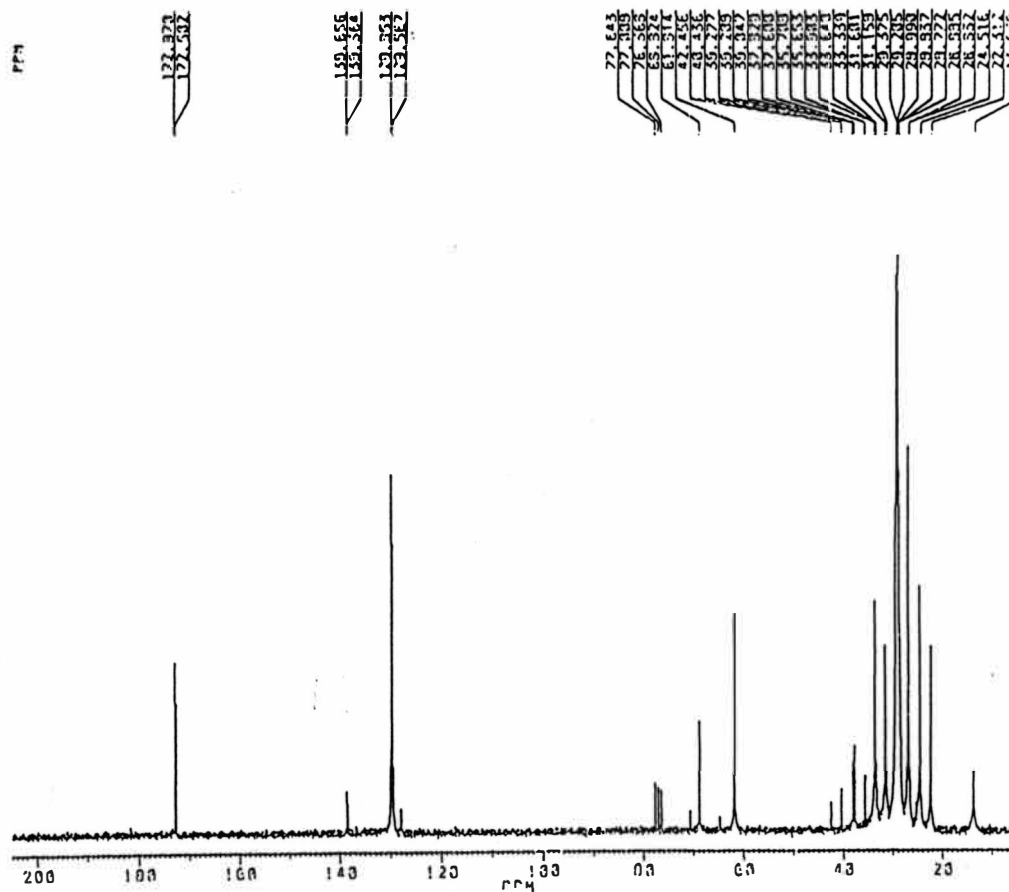
FV 23203
OZ 3194.530
OP 13L 22

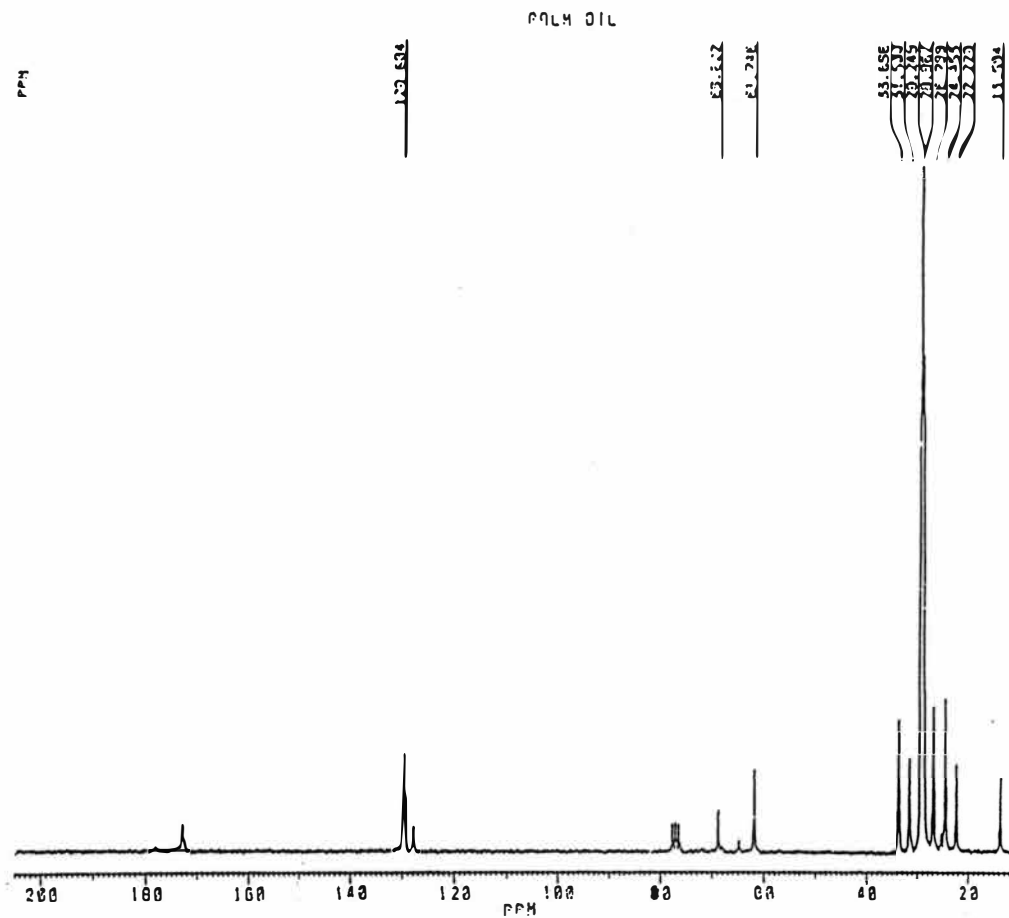
LB 1.500
CB 0.0
CX 21.00
CT 0.0
F1 205.0036
F2 5.0140
HZ/CM 479.230
PPM/CM 9.523
GR -417.40


RICE OIL IN CDCL3



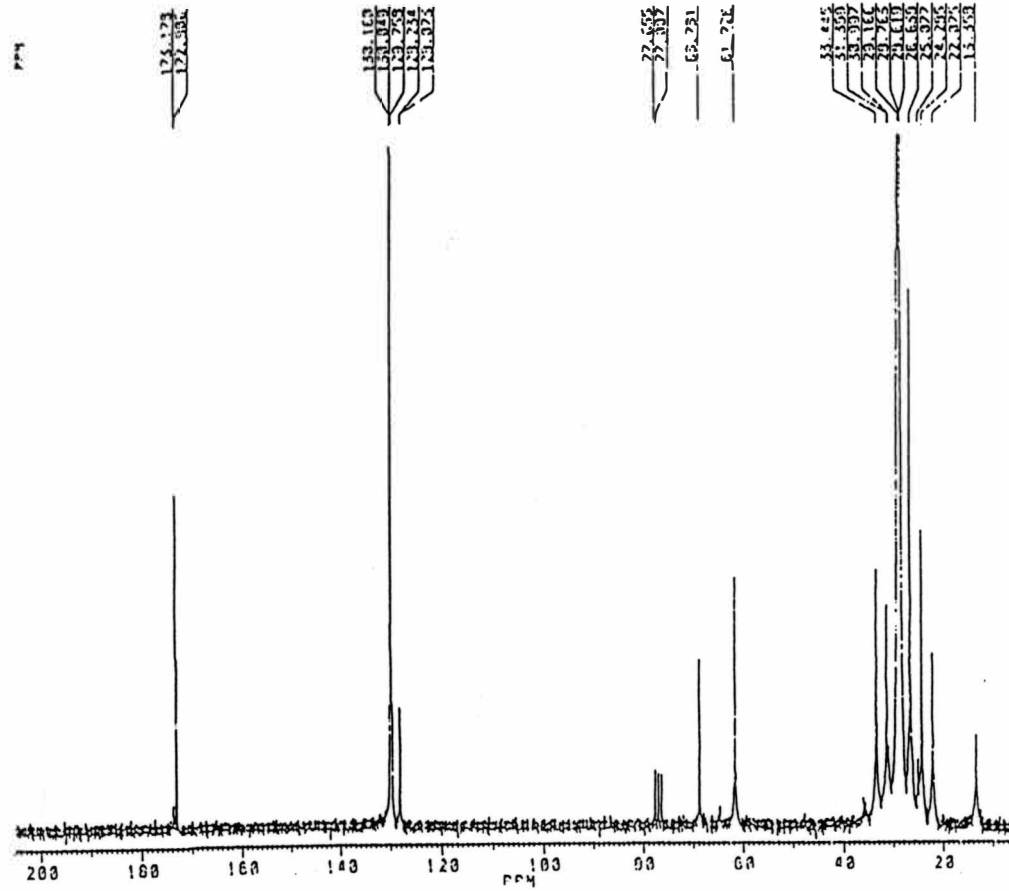
232.822	134.513	122.239	114.456	77.202	48.322	33.322	22.700
135.651		122.506		77.000	41.657	32.177	
		121.650		76.831	32.177	31.700	
		121.250		76.660	31.700	30.322	
		121.000		76.489	30.322	29.340	
		120.750		76.318	29.340	28.357	
		120.500		76.147	28.357	27.375	
		120.250		75.976	27.375	26.392	
		120.000		75.805	26.392	25.410	
		119.750		75.634	25.410	24.427	
		119.500		75.463	24.427	23.445	
		119.250		75.292	23.445	22.462	
		119.000		75.121	22.462	21.480	
		118.750		74.950	21.480	20.497	
		118.500		74.779	20.497	19.515	
		118.250		74.608	19.515	18.532	
		118.000		74.437	18.532	17.550	
		117.750		74.266	17.550	16.567	
		117.500		74.095	16.567	15.585	
		117.250		73.924	15.585	14.602	
		117.000		73.753	14.602	13.620	
		116.750		73.582	13.620	12.637	
		116.500		73.411	12.637	11.655	
		116.250		73.240	11.655	10.672	
		116.000		73.069	10.672	9.690	
		115.750		72.898	9.690	8.707	
		115.500		72.727	8.707	7.725	
		115.250		72.556	7.725	6.742	
		115.000		72.385	6.742	5.760	
		114.750		72.214	5.760	4.777	
		114.500		72.043	4.777	3.795	
		114.250		71.872	3.795	2.812	
		114.000		71.701	2.812	1.830	
		113.750		71.530	1.830	0.847	
		113.500		71.359	0.847	-0.135	
		113.250		71.188	-0.135	-1.152	
		113.000		71.017	-1.152	-2.170	
		112.750		70.846	-2.170	-3.187	
		112.500		70.675	-3.187	-4.205	
		112.250		70.504	-4.205	-5.222	
		112.000		70.333	-5.222	-6.240	
		111.750		70.162	-6.240	-7.257	
		111.500		69.991	-7.257	-8.275	
		111.250		69.820	-8.275	-9.292	
		111.000		69.649	-9.292	-10.310	
		110.750		69.478	-10.310	-11.327	
		110.500		69.307	-11.327	-12.345	
		110.250		69.136	-12.345	-13.362	
		110.000		68.965	-13.362	-14.380	
		109.750		68.794	-14.380	-15.397	
		109.500		68.623	-15.397	-16.415	
		109.250		68.452	-16.415	-17.432	
		109.000		68.281	-17.432	-18.450	
		108.750		68.110	-18.450	-19.467	
		108.500		67.939	-19.467	-20.485	
		108.250		67.768	-20.485	-21.502	
		108.000		67.597	-21.502	-22.520	
		107.750		67.426	-22.520	-23.537	
		107.500		67.255	-23.537	-24.555	
		107.250		67.084	-24.555	-25.572	
		107.000		66.913	-25.572	-26.590	
		106.750		66.742	-26.590	-27.607	
		106.500		66.571	-27.607	-28.625	
		106.250		66.400	-28.625	-29.642	
		106.000		66.229	-29.642	-30.660	
		105.750		66.058	-30.660	-31.677	
		105.500		65.887	-31.677	-32.695	
		105.250		65.716	-32.695	-33.712	
		105.000		65.545	-33.712	-34.730	
		104.750		65.374	-34.730	-35.747	
		104.500		65.203	-35.747	-36.765	
		104.250		65.032	-36.765	-37.782	
		104.000		64.861	-37.782	-38.800	
		103.750		64.690	-38.800	-39.817	
		103.500		64.519	-39.817	-40.835	
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		103.000		64.177	-41.852	-42.870	
		102.750		64.006	-42.870	-43.887	
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		102.250		63.664	-44.905	-45.922	
		102.000		63.493	-45.922	-46.940	
		101.750		63.322	-46.940	-47.957	
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		101.250		62.980	-48.975	-49.992	
		101.000		62.809	-49.992	-51.010	
		100.750		62.638	-51.010	-52.027	
		100.500		62.467	-52.027	-53.045	
		100.250		62.296	-53.045	-54.062	
		100.000		62.125	-54.062	-55.080	
		99.750		61.954	-55.080	-56.097	
		99.500		61.783	-56.097	-57.115	
		99.250		61.612	-57.115	-58.132	
		99.000		61.441	-58.132	-59.150	
		98.750		61.270	-59.150	-60.167	
		98.500		61.099	-60.167	-61.185	
		98.250		60.928	-61.185	-62.202	
		98.000		60.757	-62.202	-63.220	
		97.750		60.586	-63.220	-64.237	
		97.500		60.415	-64.237	-65.255	
		97.250		60.244	-65.255	-66.272	
		97.000		60.073	-66.272	-67.290	
		96.750		59.902	-67.290	-68.307	
		96.500		59.731	-68.307	-69.325	
		96.250		59.560	-69.325	-70.342	
		96.000		59.389	-70.342	-71.360	
		95.750		59.218	-71.360	-72.377	
		95.500		59.047	-72.377	-73.395	
		95.250		58.876	-73.395	-74.412	
		95.000		58.705	-74.412	-75.430	
		94.750		58.534	-75.430	-76.447	
		94.500		58.363	-76.447	-77.465	
		94.250		58.192	-77.465	-78.482	
		94.000		58.021	-78.482	-79.500	
		93.750		57.850	-79.500	-80.517	
		93.500		57.679	-80.517	-81.535	
		93.250		57.508	-81.535	-82.552	
		93.000		57.337	-82.552	-83.570	
		92.750		57.166	-83.570	-84.587	
		92.500		56.995	-84.587	-85.605	
		92.250		56.824	-85.605	-86.622	
		92.000		56.653	-86.622	-87.640	
		91.750		56.482	-87.640	-88.657	
		91.500		56.311	-88.657	-89.675	
		91.250		56.140	-89.675	-90.692	
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		90.500		55.627	-92.727	-93.745	
		90.250		55.456	-93.745	-94.762	
		90.000		55.285	-94.762	-95.780	
		89.750		55.114	-95.780	-96.797	
		89.500		54.943	-96.797	-97.815	
		89.250		54.772	-97.815	-98.832	
		89.000		54.601	-98.832	-99.850	
		88.750		54.430	-99.850	-100.867	
		88.500		54.259	-100.867	-101.885	
		88.250		54.088	-101.885	-102.902	
		88.000		53.917	-102.902	-103.920	
		87.750		53.746	-103.920	-104.937	
		87.500		53.575	-104.937	-105.955	
		87.250		53.404	-105.955	-106.972	
		87.000		53.233	-106.972	-107.990	
		86.750		53.062	-107.990	-109.007	
		86.500		52.891	-109.007	-110.025	
		86.250		52.720	-110.025	-111.042	
		86.000		52.549	-111.042	-112.060	
		85.750		52.378	-112.060	-113.077	
		85.500		52.207	-113.077	-114.095	
		85.250		52.036	-114.095	-115.112	
		85.000		51.865	-115.112	-116.130	
		84.750		51.694	-116.130	-117.147	
		84.500		51.523	-117.147	-118.165	
		84.250		51.352	-118.165	-119.182	
		84.000		51.181	-119.182	-120.200	
		83.750		51.010	-120.200	-121.217	
		83.500		50.839	-121.217	-122.235	
		83.250		50.668	-122.235	-123.252	
		83.000		50.497	-123.252	-124.270	
		82.750		50.326	-124.270	-125.287	
		82.500		50.155	-125.287	-126.305	
		82.250		49.984	-126.305	-127.322	
		82.000		49.813	-127.322	-128.340	
		81.750		49.642	-128.340	-129.357	
		81.500		49.471	-129.357	-130.375	
		81.250		49.300	-130.375	-131.392	
		81.000		49.129	-131.392	-132.410	
		80.750		48.958	-132.410	-133.427	
		80.500		48.787	-133.427	-134.445	
		80.250		48.616	-134.445	-135.462	
		80.000		48.445	-135.462	-136.480	
		79.750		48.274	-136.480	-137.497	
		79.500		48.103	-137.497	-138.515	
		79.250		47.932	-138.515	-139.532	
		79.000		47.761	-139.532	-140.550	
		78.750		47.590	-140.550	-141.567	
		78.500		47.419	-141.567	-142.585	
		78.250		47.248	-142.585	-143.602	
		78.000		47.077	-143.602	-144.620	
		77.750		46.906	-144.620	-145.637	
		77.500		46.735	-145.637	-146.655	
		77.250		46.564	-146.655	-147.672	
		77.000		46.393	-147.672	-148.690	
		76.750		46.222	-148.690	-149.707	
		76.500		46.051	-149.707	-150.725	
		76.250		45.880	-150.725	-151.742	
		76.000		45.709	-151.742	-152.760	
		75.750		45.538	-152.760	-153.777	
		75.500		45.367	-153.777	-154.795	
		75.250		45.196	-154.795	-155.812	
		75.000		45.025	-155.812	-	

PEANUT OIL IN CDCl_3 



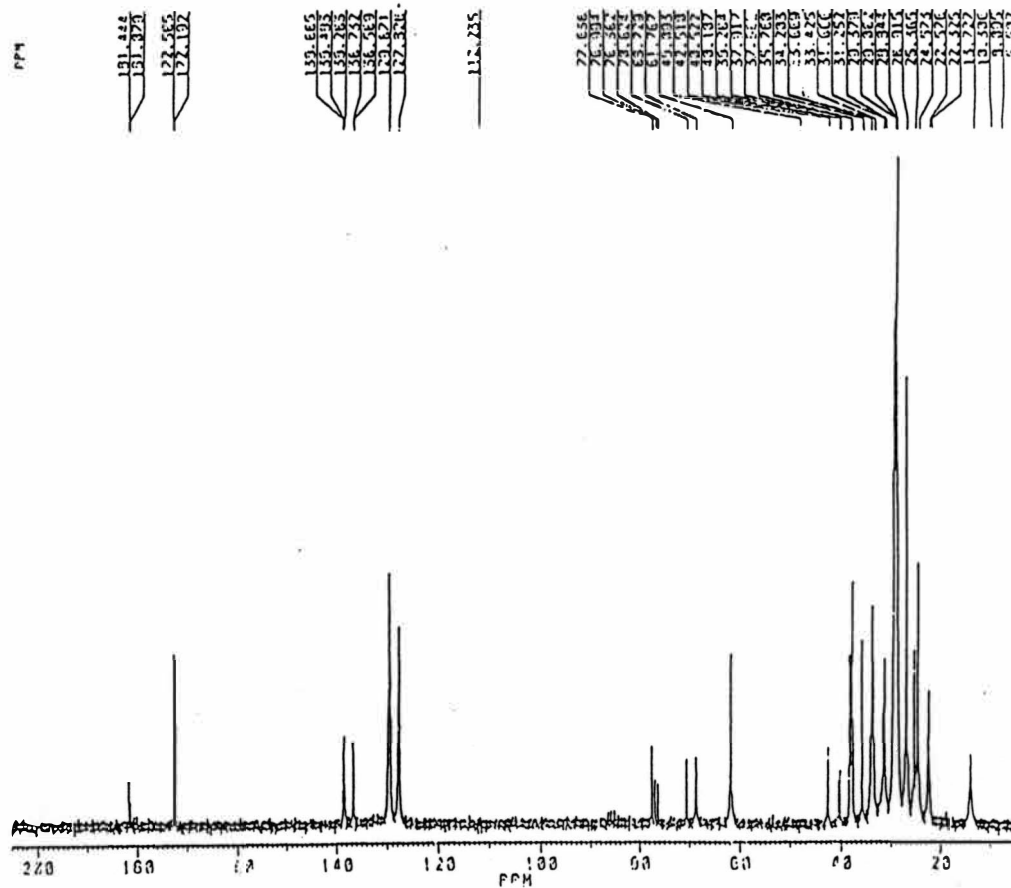

 PALM OIL
 DATE 7-0-01
 SF 50.323
 ST 50.323268
 OI 6534.222
 SI 32768
 TO 32768
 SW 13516.519
 HZ/P 1.132
 PX 2.0
 AD 3.022
 PD 3.35
 RG 200
 NS 2193.
 TE 297
 PX 23230
 DZ 3154.532
 DP 13L 22
 L2 1.523
 C2 0.0
 CX 21.00
 CT 0.0
 F1 205.002P
 F2 10.001P
 HZ/CM 467.293
 PPM/CM 9.296
 SR 52.62

OLIVE OIL IN COCL3



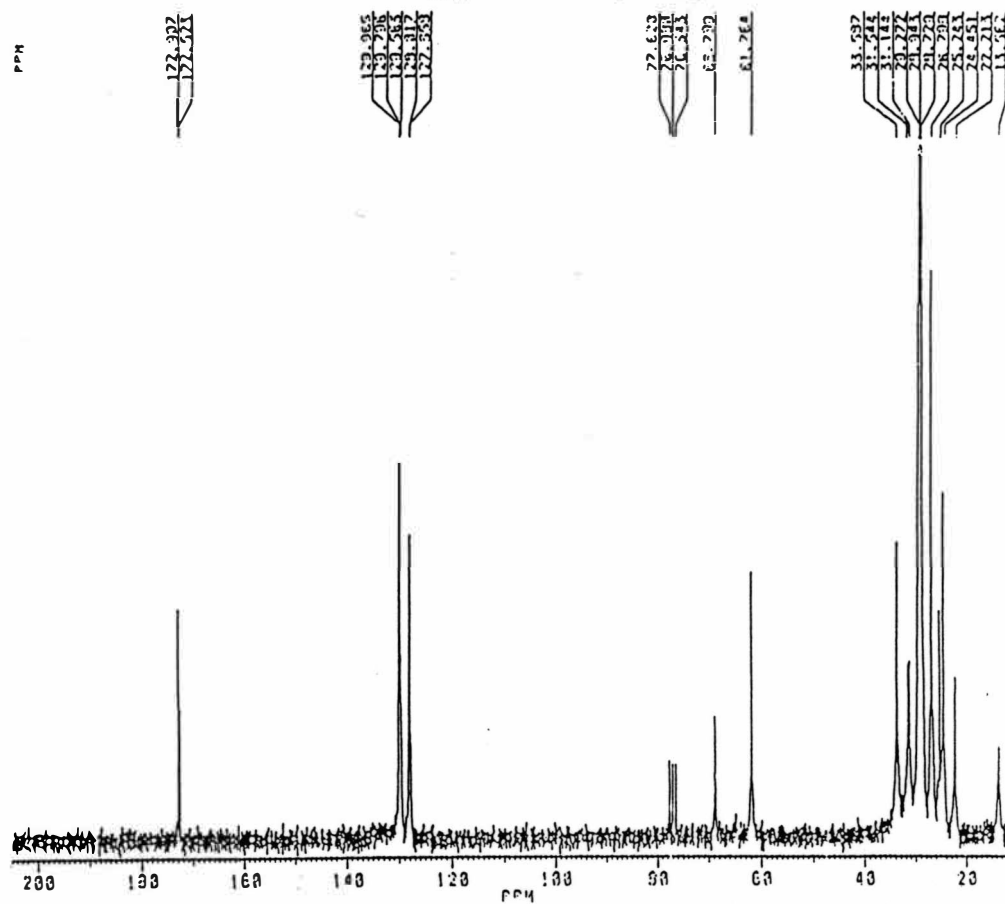
XXX
 DATE 12-5-91
 SF 50.323
 ST 50.3232650
 OI 6534.000
 SI 32768
 TO 32768
 SW 15515.510
 HZ/CM 1.130
 RX 0.0
 RO 3.020
 AD 1.905
 RC 160
 NS 2131
 TE 297
 FW 23200
 OZ 3154.532
 DP 13L 22
 L2 0.0
 CR 0.0
 CX 21.80
 CY 0.0
 F1 205.0030
 F2 5.0140
 HZ/CM 470.230
 CM/CM 0.023
 SR -415.22

MAZOLA CORN OIL IN COLLS



~~SECRET~~
 XXX
 DATE 14-5-91
 SP 53.523
 ST 58.523 468
 TO 65.54
 T-0 22768
 T-0 32768
 T-0 16510.519
 H/P 1.133
 PR 0.0
 PR 3.000
 PR 885
 PR 1.0
 HS 1235
 TE 207
 TV 23200
 ON 3154
 ON 1.1 0.8
 SN 0.0
 SN 0.0
 SN 2.0
 SN 7.0
 SN 1.0
 SN 1.0

COTTON SEED OIL IN CIGARS

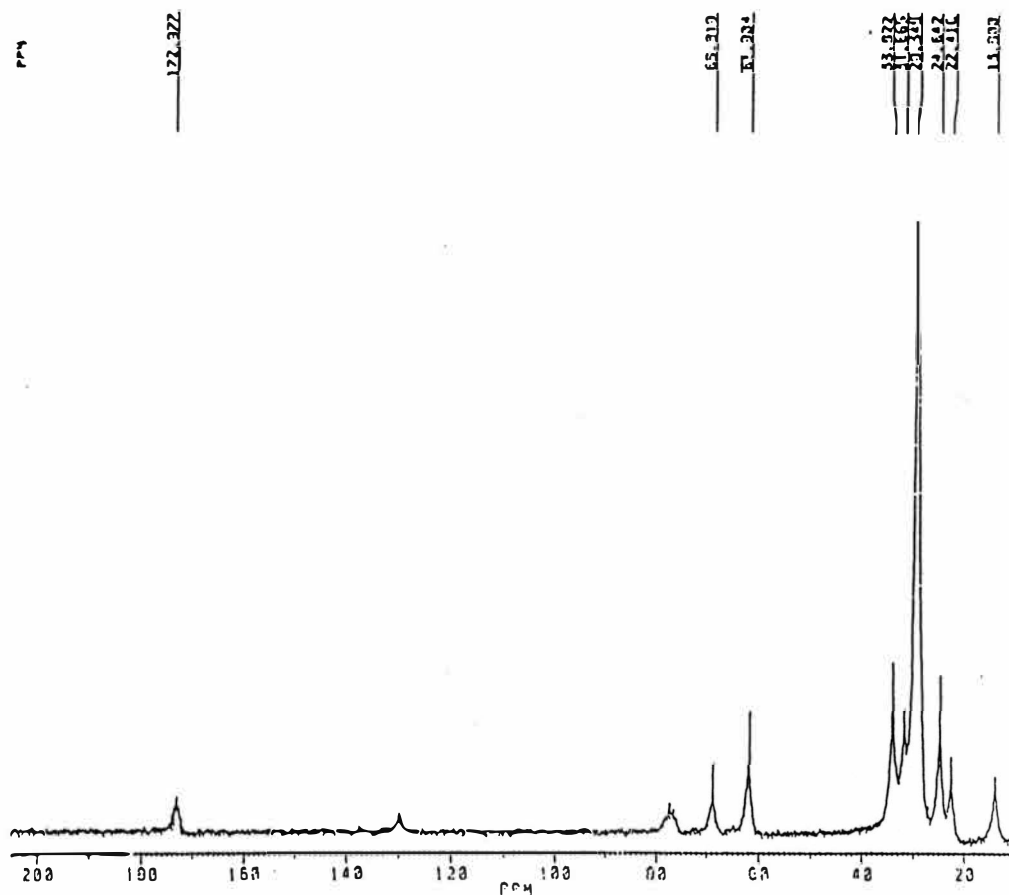


~~EXHIBIT~~

COTTON OIL
DATE 10-5-01

SF	50.323
SI	50.3232080
OI	6534.000
IO	32768
IS	32768
SW	10510.510
W/F	1.130
FW	0.0
RO	3.000
AO	0.000
SC	160
NS	1136
FE	207
FW	23200
SI	3154.530
OF	131.22
LB	0.0
GB	0.0
CX	21.00
CT	0.0
F1	205.2033P
F2	10.0000P
M/CH	467.290
PFM/CR	9.200
SR	-402.79

COCONUT OIL IN COCLS

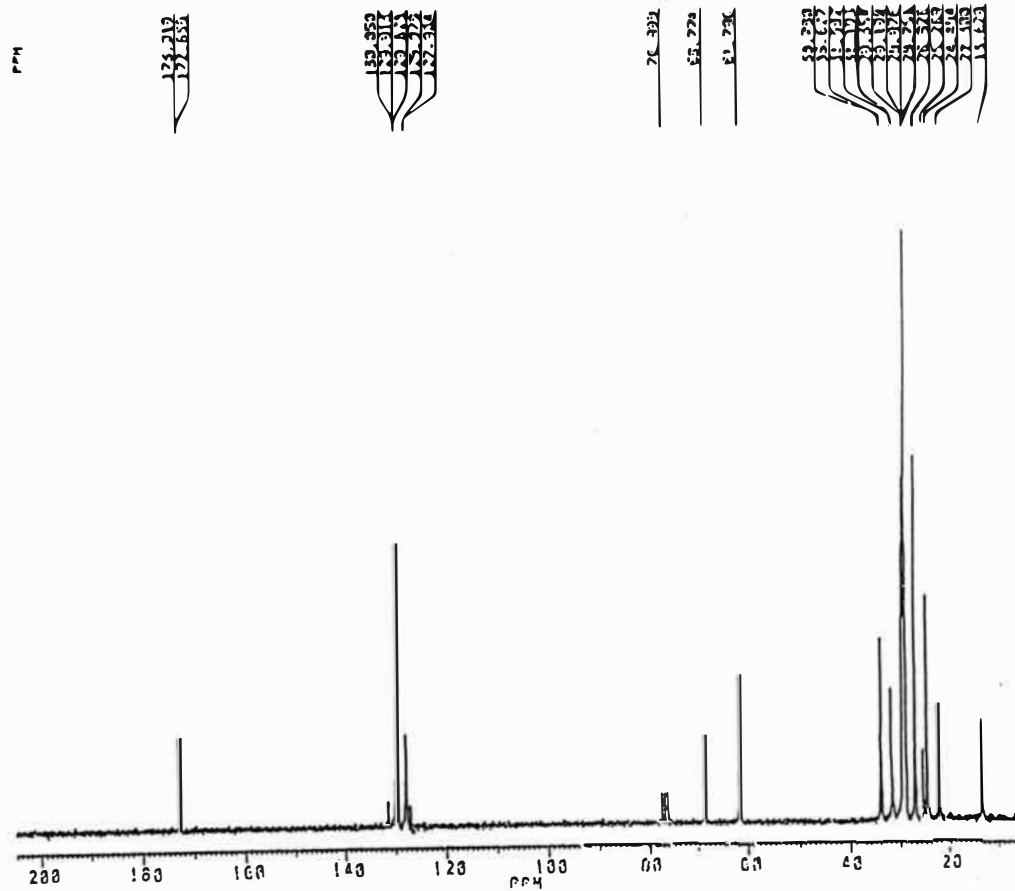


EX-100

COCONUT OIL
DATE 8-8-81

GF	52.323
GT	50.3232680
OI	65.34.000
SI	32765
TO	32765
SK	15515.510
HZ/PT	1.130
FW	0.2
AD	3.000
AD	0.00
RC	230
NS	2433
TE	207
FW	23200
OZ	3154.533
DS	13L 00
L2	1.520
CS	0.0
CK	21.00
CT	0.0
F1	205.0030
F2	10.0000
HZ/CM	407.200
FW/CM	0.220
SR	-303.75

CANOLA OIL IN COCL3



OTL
DATE 12-5-01

SF 50.323
ST 50.3232680
OI 6534.000
SI 32768
ID 32768
SM 18518.519
HZ/P 1.132

FW 0.0
RO 3.000
AO 0.000
RC 100
NS 1344
TE 237

FW 23200
OZ 3154.530
OP 131.22

LB 0.0
CB 0.0
CX 21.00
CT 0.0
F1 203.0320
F2 5.0140
HZ/CM 479.230
PPM/CM 3.523
SR 43.64

13C NMR spectrum of compound 1. The x-axis is labeled 'PPM' and ranges from 200 to 0. The spectrum shows several peaks: a small peak at ~175 ppm, a large peak at ~130 ppm, a small peak at ~80 ppm, a small peak at ~70 ppm, and a large cluster of peaks between 20 and 40 ppm. A list of peak values is provided on the right side of the spectrum.

Peak Value (PPM)
43.592
37.478
37.257
30.793
28.793
25.219
24.450
22.246
15.120

~~SECRET~~

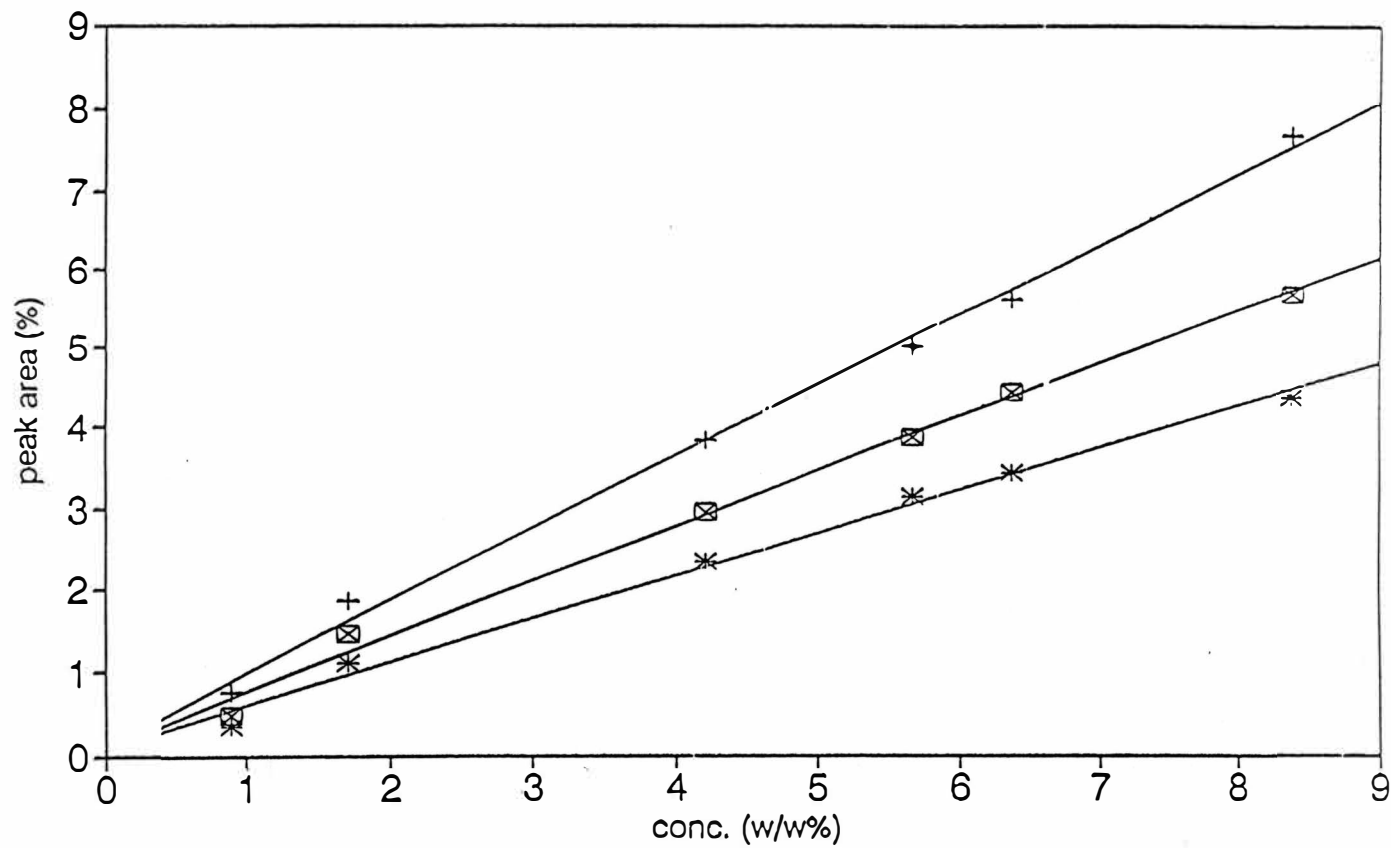
ALMOND, OIL
DATE 7-9-91

SF	50.323
SF	50.3232680
01	6534.000
S1	32760
S1	32768
SW	10519.519
H/F	1.132
FV	0.0
AO	3.000
AO	0.000
AO	100
NS	2944
FE	207
FV	23200
02	3154.532
02	3152.22
LB	1.500
CB	0.0
CA	21.00
CA	0.0
F1	205.203F
F2	10.00000
H/CM	467.200
W/CM	0.200
SR	-432.10

Appendix C

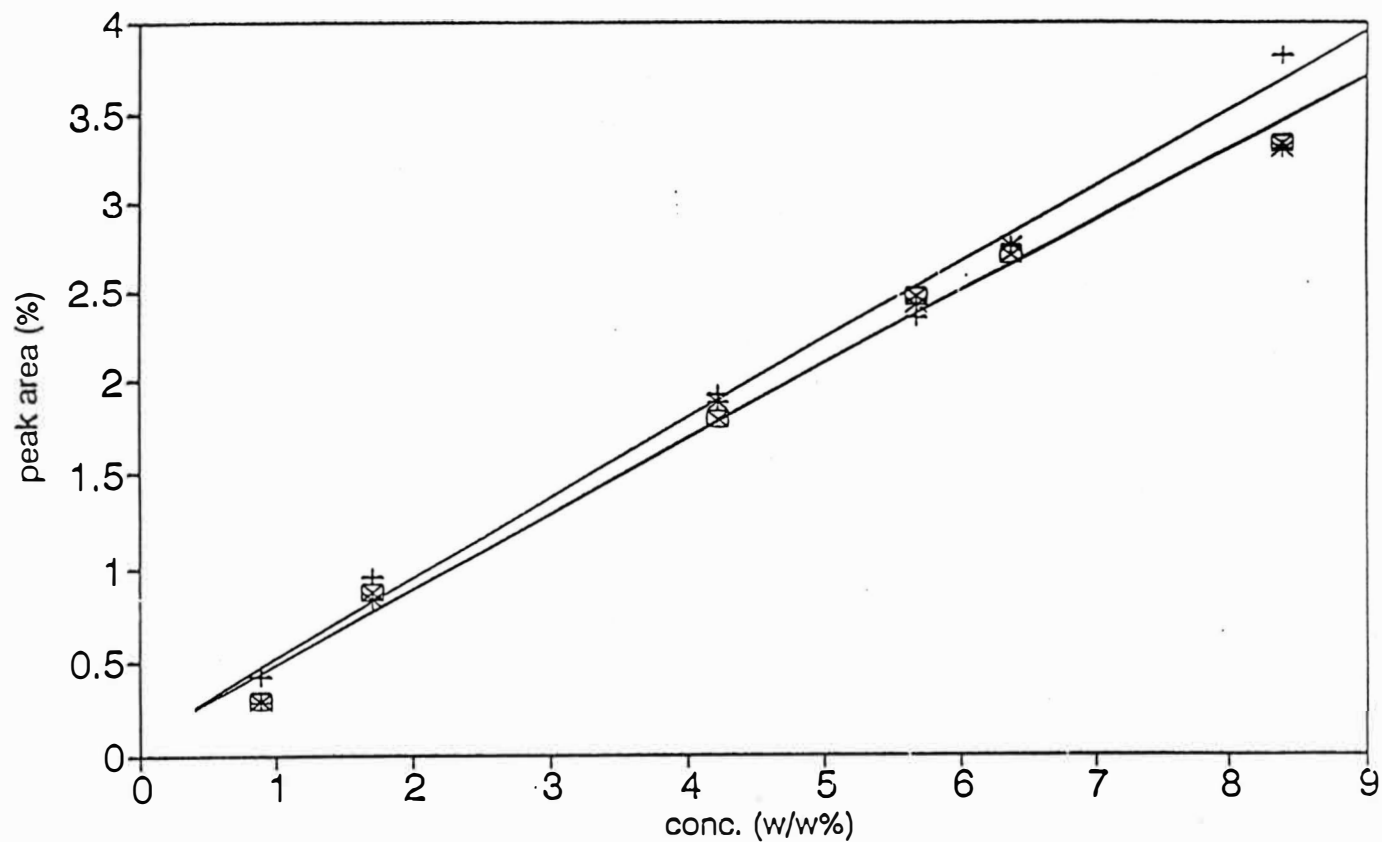
Calibration Plots

CALIBRATION PLOT FOR TESTOSTERONE



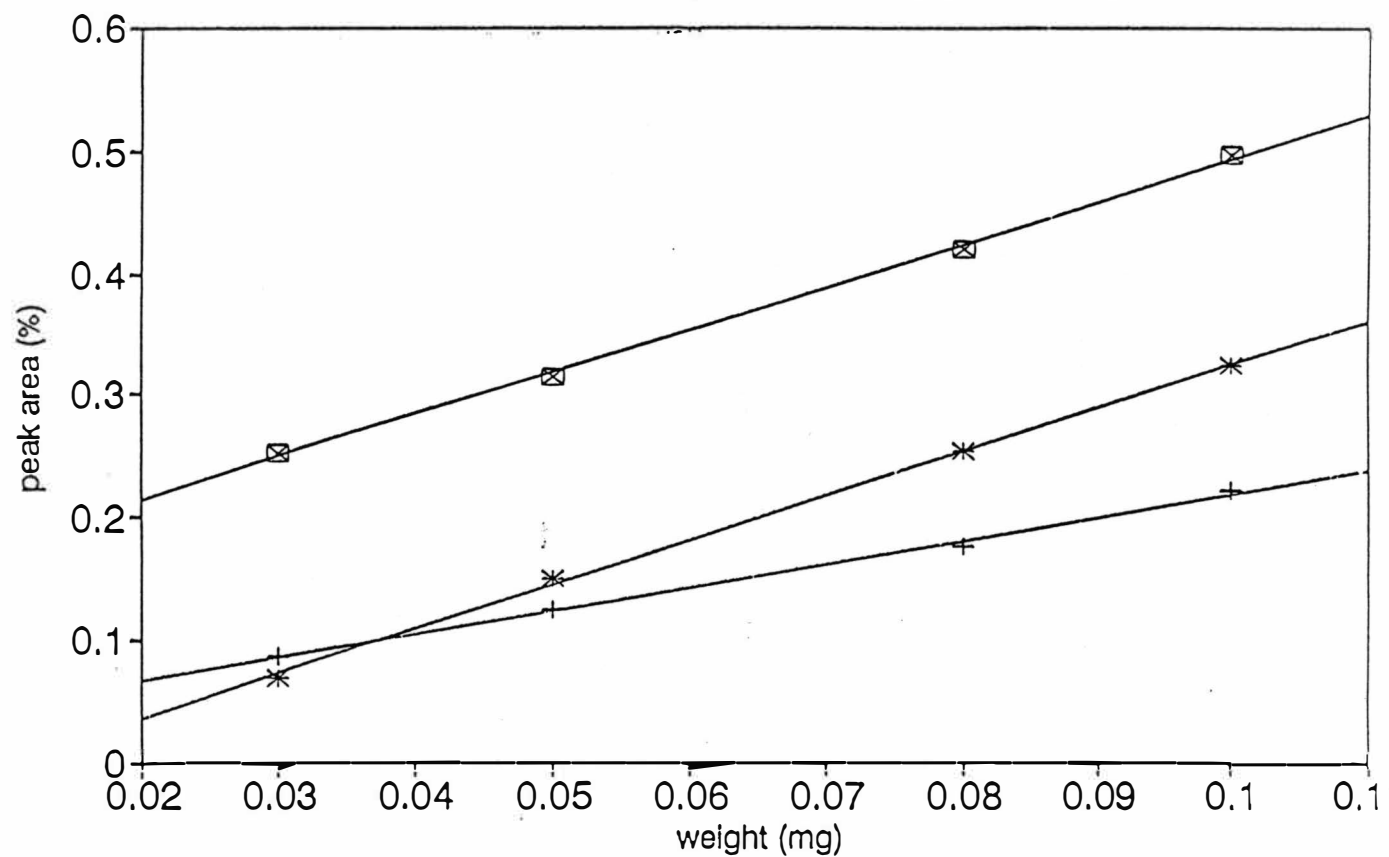
+ 198.7ppm * 173.2ppm x 123.4ppm

CALIBRATION PLOT FOR TESTOSTERONE



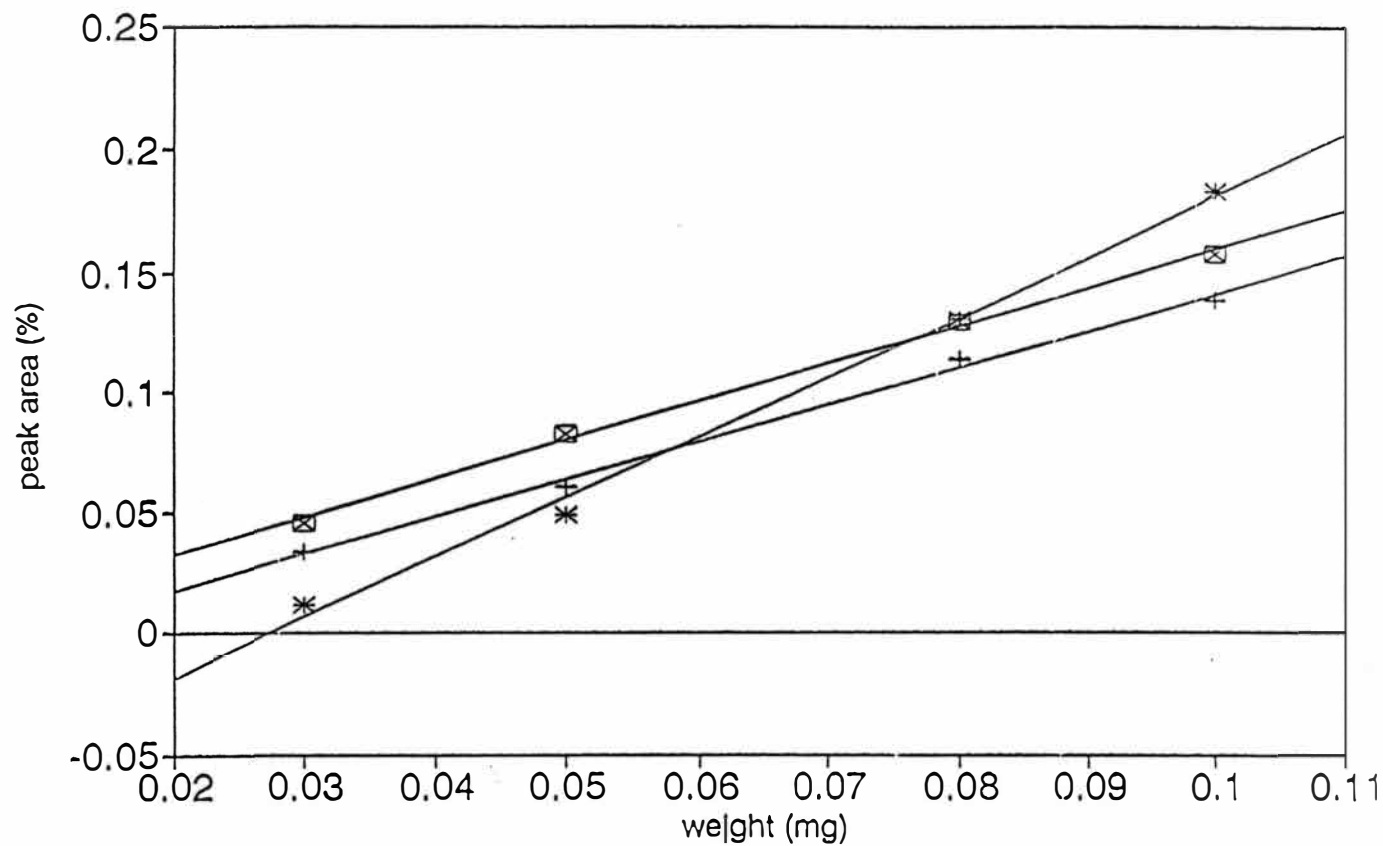
+ 81.6ppm * 52.9ppm x 49.5ppm

CALIBRATION PLOT FOR NANDROLONE DECANO



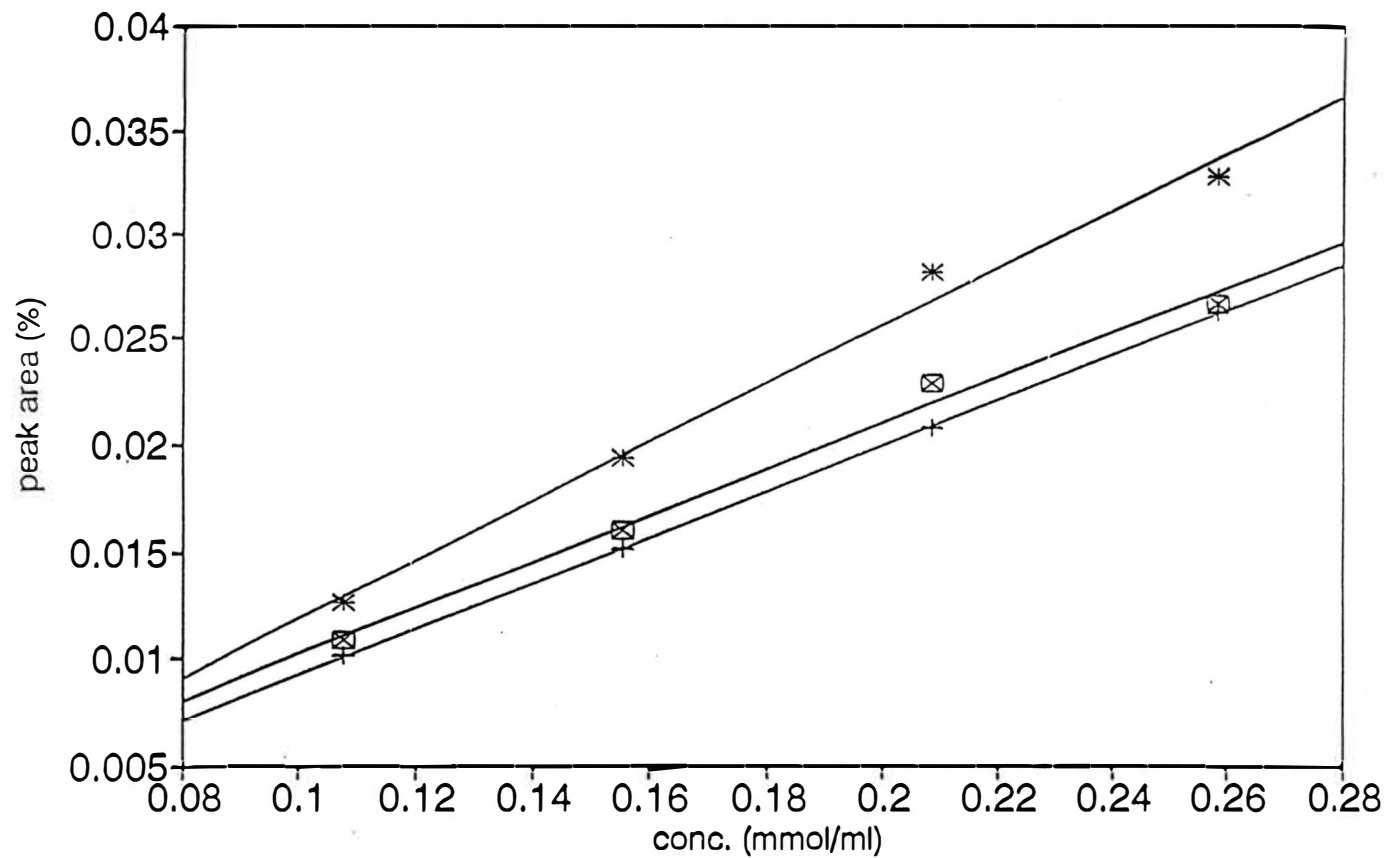
+ 124.5 ppm * 82.1434 ppm ⊠ 49.3 ppm

CALIBRATION PLOT FOR NANDROLONE DECANO



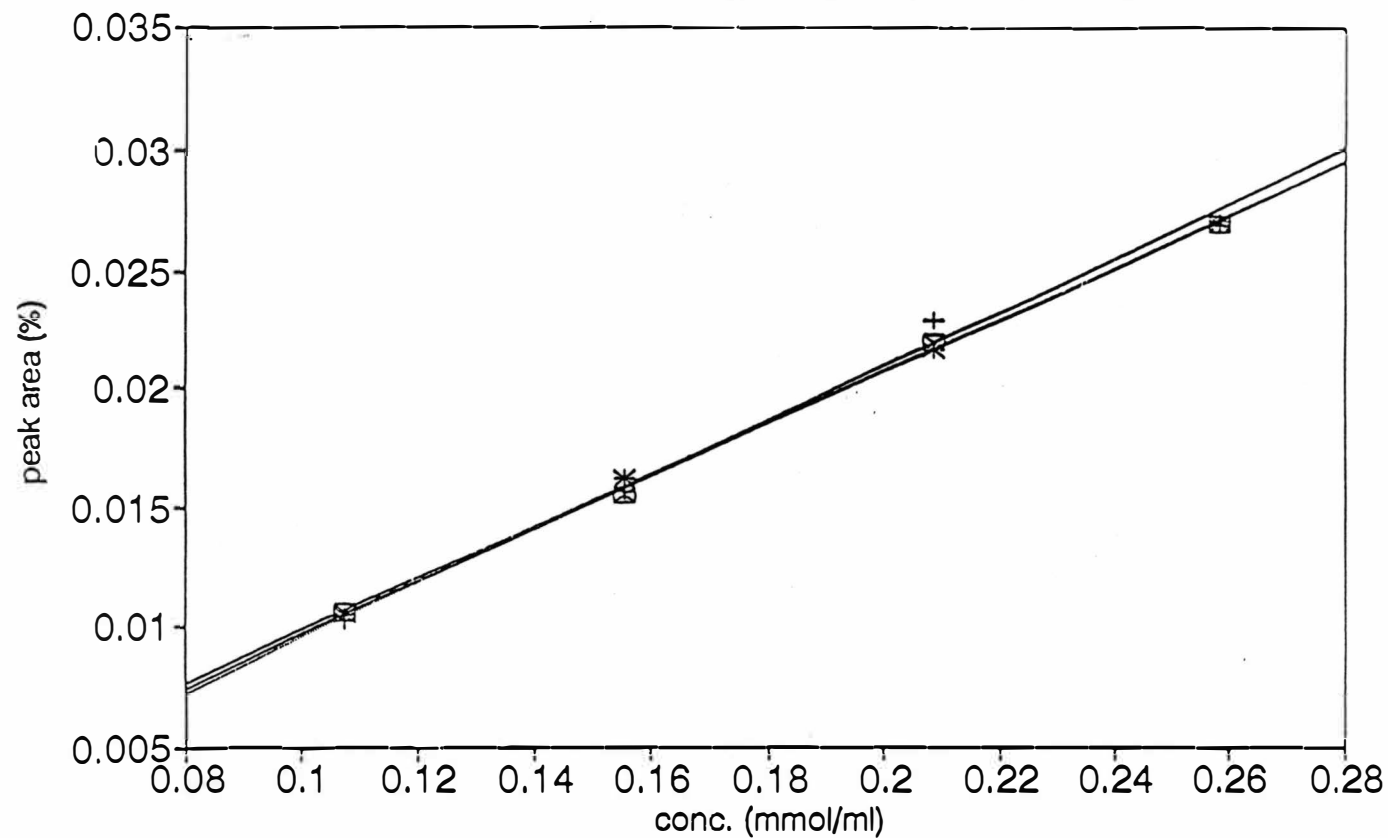
+ 200.01 ppm * 173.84 ppm x 166.66 ppm

CALIBRATION PLOT FOR PROGESTERONE



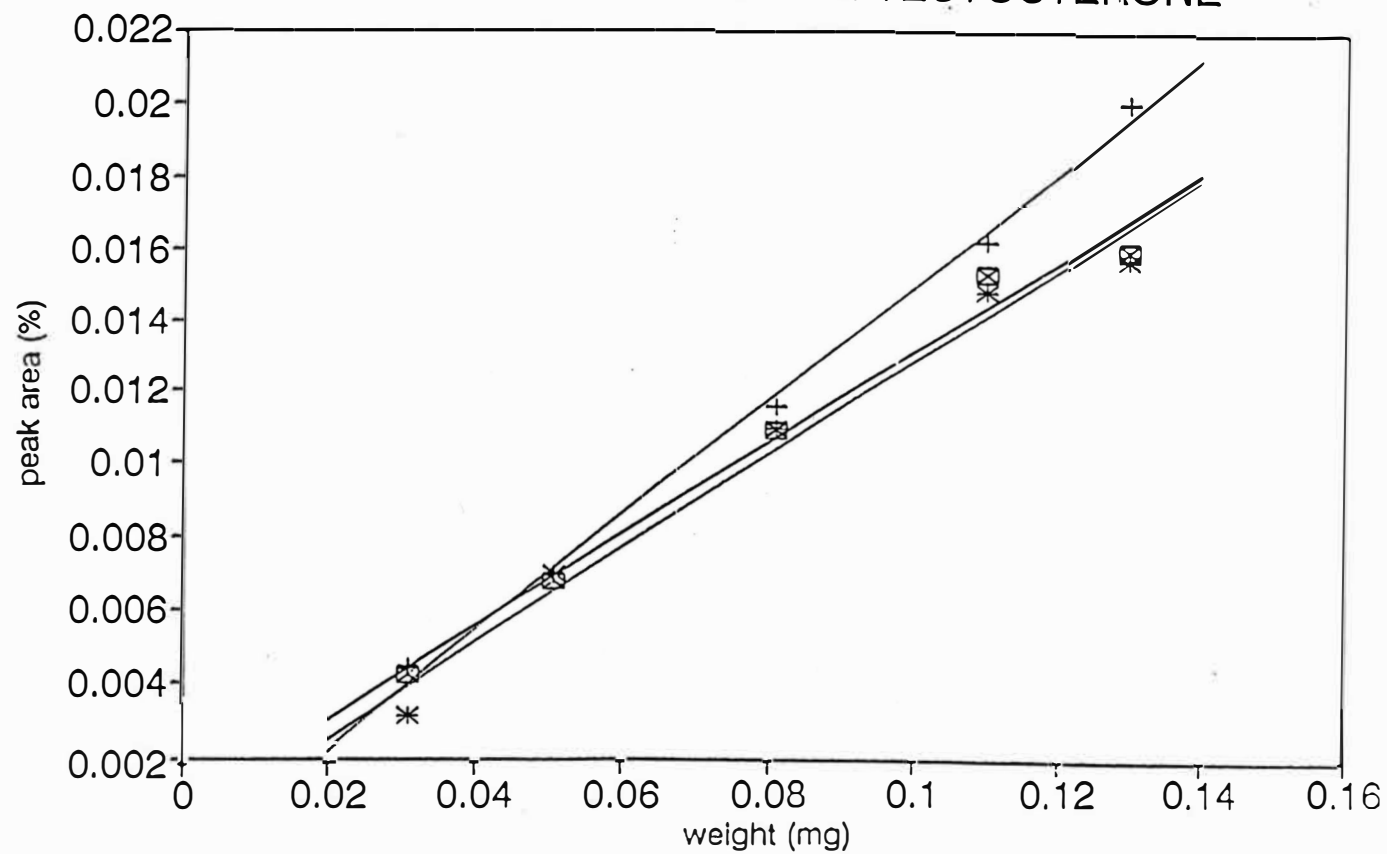
+ 169ppm * 119.9ppm ⊠ 95.2PPM

CALIBRATION PLOT FOR PROGESTERONE



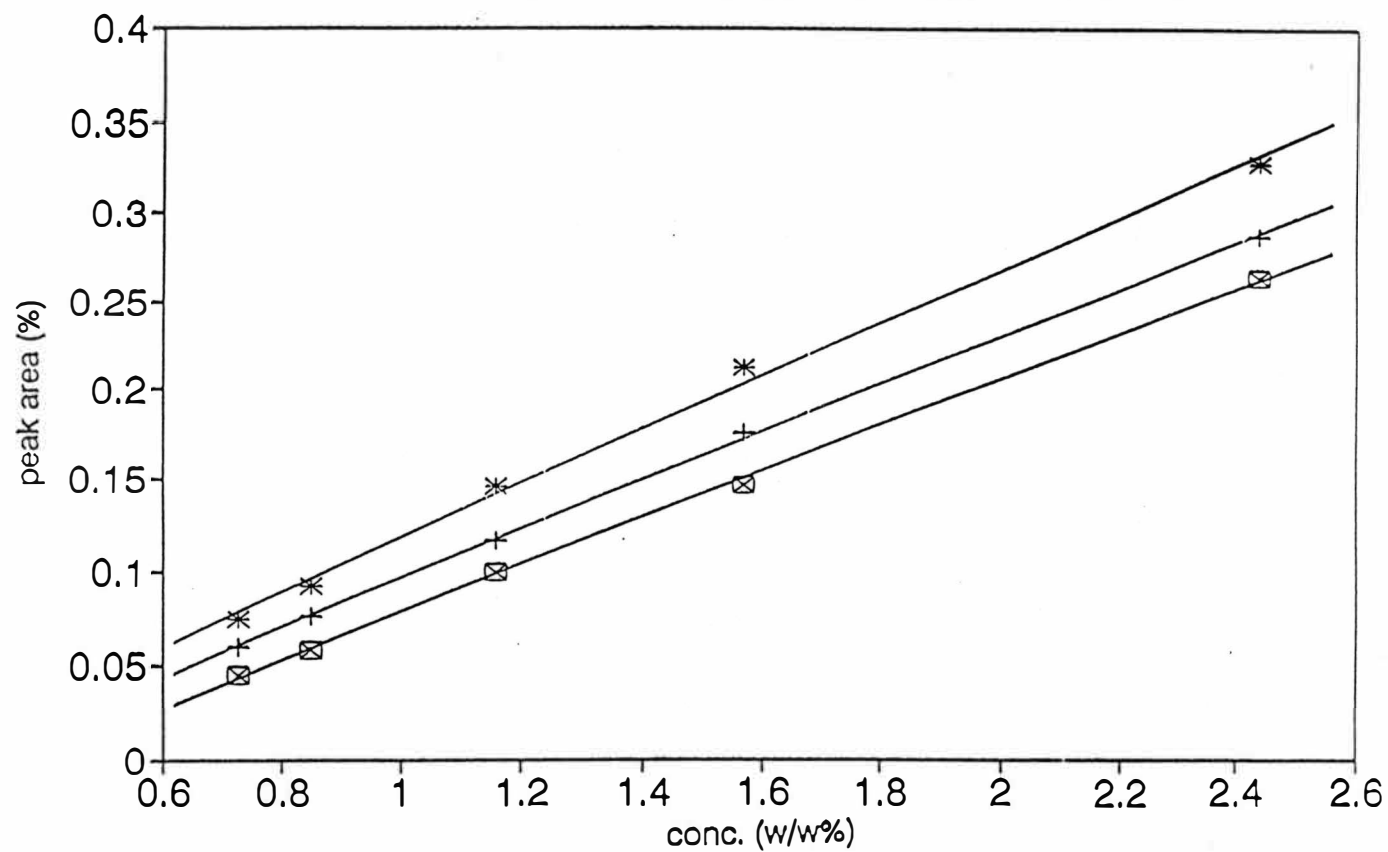
+ 202.4ppm * 198ppm ⊗ 172.07ppm

CALIBRATION PLOT FOR ME-TESTOSTERONE



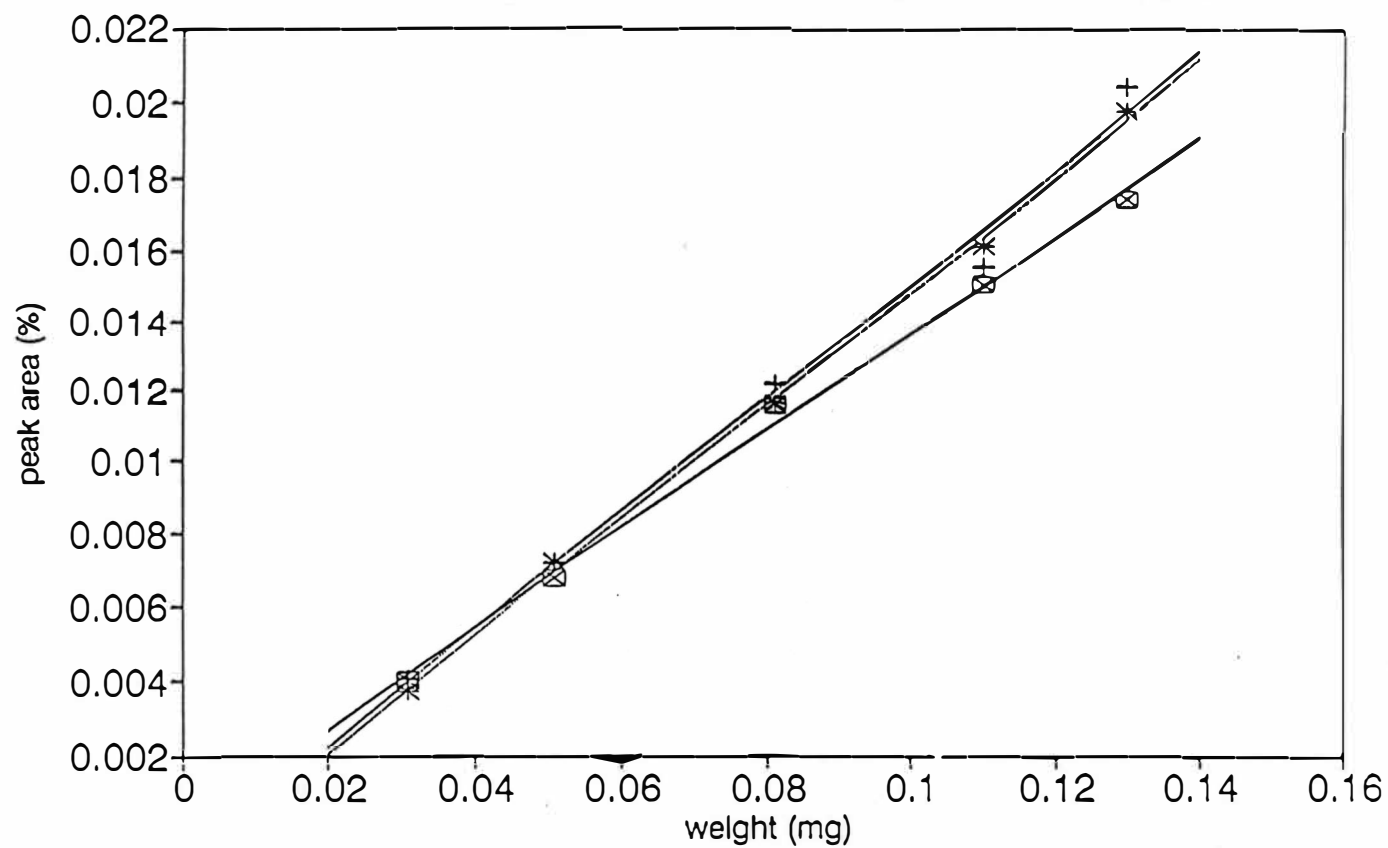
+ 80.6 ppm * 52.95 ppm x 49.3 ppm

CALIBRATION PLOT FOR OXYMETHOLONE



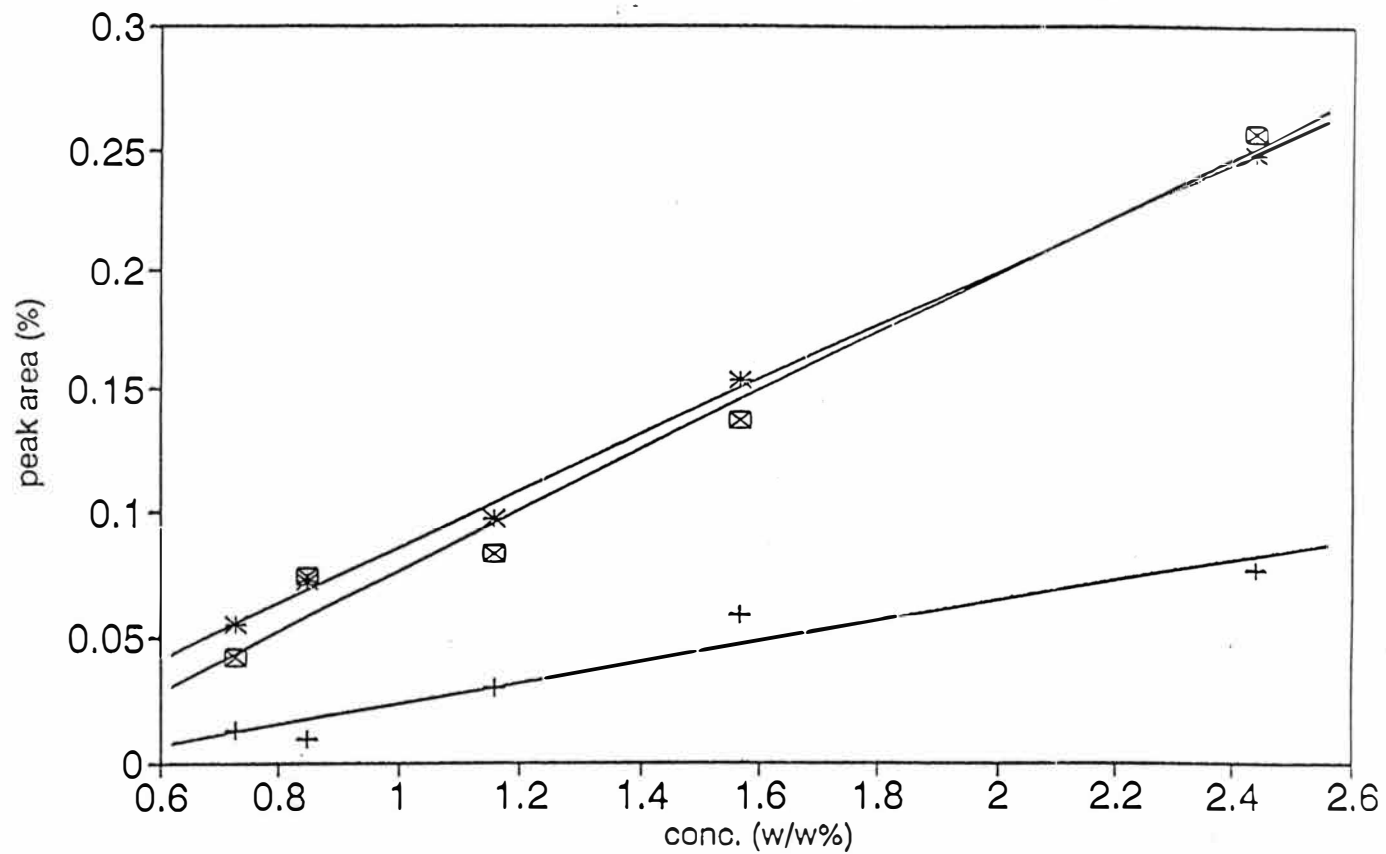
+ 37.5ppm * 39ppm x 40.57ppm

CALIBRATION PLOT FOR ME-TESTOSTERONE



+ 198.9 ppm * 170.68 ppm ⊠ 123 ppm

CALIBRATION PLOT FOR OXYMETHOLONE



+ 45.5ppm * 50.61ppm x 53.49ppm

BIBLIOGRAPHY

Advanced Function (AF) Series of FT-NMR Spectrometers User's Manual, IBM Instruments, Inc., 1985, 4-33.

Bremser, W., Klier, M. and Meyer, E. *Org. Magn. Reson.* 1975, 7, 92-105.

Cheever-K; House-MA, *J-Cardiovasc-Nurs.* 1992, Jan, 6(2), 19-30.

Deronme, A. E., *Modern NMR Techniques for Chemistry Research*, 2nd Edition, Pergamon Press, 1988, 97-127.

Dybowski, C. and Lichter, R. L., *NMR Spectroscopy Techniques*, Marcel Dekker, Inc., New York, 1987, 17-25.

Foye, W. O., *Principles of Medicinal Chemistry*, 3rd Ed., Lea & Febiger, Philadelphia, London, 1989, pp. 433-443.

Jezi, B. A. and Dalrymple, D. L. *Anal. Chem.* 1975, 47, 203-207.

Kasler, F., *Quantitative Analysis by NMR Spectroscopy*, Academic Press, Inc. (London) Ltd., 1973.

Laude, David A., Jr. and Wilkins, C. L., *Anal. Chem.* 1986, 58, 2820-1824.

Levy, G. C., Lichter, R. L., and Nelson, G. L., *Carbon-13 Nuclear Magnetic Resonance Spectroscopy*, John Wiley & Sons, Inc., 1980, pp. 78-88.

Lieth, v. d., C. W., Seil, J., Köhler, I. and Opferkuch, H. *J. Magn. Reson. Chem.* 1985, 23, 1048-1055.

Schwarzenbach, R., Meili, J., Konitzer, H. and Clerc, J. T. *Org. Chem. Reson.* 1976, 8, 11-16.

Shelly, C. A. and Munk, M. E., *Anal. Chem.* 1982, 54, 516-521.

Shoolery, J. N., *Prog. NMR Spectrosc.* 1977, 11, 79.

Sjostrom, M., Edlund, U., *J. Magn. Reson.* 1977, 25, 285-297.

Small, G. W. and Jurs, P. C., *Anal. Chem.* 1983, 55, 1121-1127.

Tietz, N. W., *Fundamentals of Clinical Chemistry*, W. B. Saunders Co., Philadelphia, PA, 1976, 481-482, 741-770.

U.S. Pharmacopeia, 21st Rev., U.S. Pharmacopeial Convention, Rockville, MD, 1985, pp. 685, 708, 761, 983, 1025.

Walters, M. J., Ayers, R. J. and Brown, D. J., *J. Assoc. Off. Anal. Chem.* 1990, 73, No. 6, 904.

Weast, R. C. and Astle, M. J., *CRC Handbook of Chemistry and Physics*, CRC Press, Inc., Cleveland, OH, 71st Ed., 1991, C738-739, C745-755.

Wilkins, C. L., Brunner, T. R., *Anal. Chem.* 1977, 49, 2136-2141.