INVESTIGATIONS OF TOXIC PLANTS: *ALBIZIA JULIBRISSIN* AND *ASCLEPIAS SUBVERTICILLATA*

By

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PREFACE

This thesis comprises three parts each of which encompasses one component of the research conducted between 1995 and the present. Chapter I briefly defines secondary plant compounds and describes their possible roles and uses. I have included this chapter to show commonality in my investigations of *Albizia julibrissin* and *Asclepias subverticillata*, and how the study of secondary plant metabolites brings together the many disciplines of organic chemistry, biochemistry, pharmacology, toxicology, plant taxonomy, and ecology. Chapter II describes the investigation of a neurotoxic alkaloid found in *Albizia julibrissin* (mimosa). Chapter III describes the investigation of the neurotoxic compounds present in *Asclepias subverticillata* (western-whorled milkweed).

Chapters II and III will be submitted for publication in *Poisonous Plants, Proceedings of the 5th International Symposium on Poisonous Plants* and the format of each is that required for submission. The format of Chapter I follows that of Chapters II and III in order to have continuity within the thesis.

Sincere appreciation is expressed to Dr. Ronald J. Tyrl, my major advisor, for his loyal, patient dedication to the advancement of knowledge. His guidance, understanding, and

friendship have greatly assisted me in achieving my academic goals. Special gratitude also is expressed to committee members Dr. George E. Burrows and Dr. Elizabeth M. Holt. Their assistance, patience, and forthrightness have been greatly appreciated. Sincere appreciation also is conveyed to Dr. A. Daniel Jones, Director of the Facility for Advanced Instrumentation at the University of California, Davis, for his time and spectrometric expertise on *Asclepias subverticillata*. Finally, an enormous thank you to my husband, Randy Robinson, for his advice, support, and patience during this exploratory period of my life.

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CHAPTER I

INTRODUCTION

Plant metabolites are classified as either primary or secondary (1,2,3,4,5,6). Primary ones, such as common sugars, low-molecular weight carboxylic acids, and amino acids, are required for a plant's basic metabolism. They are universal in distribution and provide the starting material for syntheses of secondary compounds. Secondary compounds are diverse, complex structures which are more restricted in distribution and generally are not required for basic growth and development. Examples of commonly studied secondary compounds are alkaloids, terpenoids, and flavonoids (1,4,7,8). Their chemical classification is usually based on the biosynthetic pathway from which they are derived (1,6,8). However, this is not always the case. For example, all alkaloids do not share a common biosynthesis, but do share other chemical characteristics (8,9).

Secondary compounds may represent metabolic by-products, storage products, competition inhibitors, pollinator attractants, or herbivore and microbial toxins (3,4,5,10). There is strong evidence to support the hypothesis that secondary compounds play a key role in insect-plant coevolution (4,7,9,10,11). Despite the voracious appetite of

herbivorous insects, a rich diversity of angiosperms dominates earth (4,7,11), and it is believed that the development of toxic secondary compounds was, and remains, a crucial defense mechanism of plants in this evolutionary struggle (11).

Toxic secondary compounds also can have an effect on large herbivores, typically domesticated animals such as cattle and sheep, and rarely large, wild herbivores (10). This is due to a wild animal's evolution in its natural environment in contrast to the domesticated animal's introduction into environments different from that in which it originated (4,12). For example, arid regions of the western U.S. include extensive rangelands, and the conditions which livestock must endure to survive can be quite harsh (12,14). Though typically unpalatable, toxic plants will be consumed when more desirable vegetation is scarce (12,13). As a result, the livestock industry has suffered sporadic, sometimes severe, losses (12,14,15,16).

Despite the ill-effects caused by plant toxins, many provide relief for human diseases. Classic examples include curare, a composite of alkaloids isolated from species of *Strychnos* of the Loganiaceae and *Chondrodendron*, *Cissampelos*, and other genera of the Menispermaceae and digitoxin, a cardenolide isolated from *Digitalis purpurea* of the Schrophulariaceae (9,17,18,19,20). The elucidation of the pharmacological actions of these toxins have provided relief for spastic disorders and congestive heart failure in humans

as well as providing invaluable tools for studies of cell membrane transport proteins (17,18).

Secondary compounds not only rouse scientific interest because of their physiological properties but also for their potential value in the discipline of plant taxonomy (1,21, 22,23,24,25). Many secondary compounds are characteristic of and restricted to specific genera or families (1,8), and when used in conjunction with other plant attributes may provide clues to phylogenetic relationships within the kingdom Plantae (21,23).

On a personal note, I find the study of secondary compounds to be a vast and greatly interesting subject. However, more research is needed to unlock the secrets of their importance and implications for society. I hope that the studies embodied in this thesis provide clues to assist scientists in understanding some of nature's mysteries about plant toxins.

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EVALUATION OF THE TOXIC EFFECTS OF THE LEGUMES OF *ALBIZIA JULIBRISSIN* (MIMOSA) AND IDENTIFICATION OF THE TOXICANT

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ABSTRACT

Albizia julibrissin, known as mimosa or silk-tree, is a common ornamental tree. In the United States, it is cultivated primarily in the southern states and has become widely naturalized. Preliminary studies by other researchers have indicated a potential for its legumes to cause neurotoxic effects. In addition, species of *Albizia* in South Africa are reported to cause neurotoxic effects via pyridoxine antagonists. Thus both the potential for and mechanism of intoxication were evaluated. Dried, ground legumes of *A. julibrissin* caused severe neurotoxic effects in sheep at a single intraruminal dosage of 1-1.5% of body weight and contained the same neurotoxic alkaloid as the South African species *A. tanganyicensis*. Pyridoxine HCl administered intramuscular and/or subcutaneously concurrently with the legumes prevented the occurrence of adverse effects, and also appeared to be an effective antidote when administered intravenously after the onset of signs of intoxication.

INTRODUCTION

For three decades, occurrences of *Albizia* poisoning in livestock have been reported in South Africa, Zambia, and Zimbabwe (1,2,3). Neurologic effects and death are caused by ingestion of legumes from *A. tanganyicensis* Baker f. and *A. versicolor* Welw. ex Oliver. Severe episodes of livestock intoxication, including mortalities, spurred South African researchers to pursue identification of *Albizia*'s neurotoxins

in an effort to provide a remedy for its adverse effects. This culminated in a 1987 report by Steyn and coworkers (4) attributing the problems to two neurotoxic alkaloids (Figure 1A, 1B). Based on the similarity of 4-methoxypyridoxine to that of pyridoxine or vitamin B_6 (Figure 1C) and previous work on pyridoxine antagonists (5,6), they hypothesized (4) that the *Albizia* alkaloids acted as pyridoxine antagonists and that their effects could be counteracted by dosing with pyridoxine. It and pyridoxine HCl subsequently were shown to be effective therapeutic agents for guinea-pigs and sheep intoxicated by the legumes of *A.versicolor* (7,8).

Introduced into North America as an ornamental, *A. julibrissin* Durazz is a member of Mimosaceae and commonly known as mimosa or silk-tree. It is a small tree native to tropical Asia and is widely cultivated in the southeastern United States where it has escaped and naturalized (9,10,11). Trees are 3-6 m tall and have a light tan, smooth bark. The flowers bloom from May to August, are pink in color, and occur in globose clusters 2.5-5 cm in diameter in corymbose racemes. The mature legumes are oblong, flat, 12-20 cm long, and 1.5-2.5 cm wide (9,10).

Although there are few reports of intoxications in North America, experimental administration of the legumes of this common ornamental tree to sheep produced clinical signs of neurointoxication followed by death (12). Because of its toxic potential and increasing abundance in the southern portion of the United States, *A. julibrissin* warranted study.

The objectives of this investigation were to confirm its neurotoxic potential, to determine if its legumes have alkaloids identical or similar to those present in the South African species, and to evaluate the effects of pyridoxine HCl as a preventive and antagonist of intoxication.

MATERIALS AND METHODS

Well developed legumes, most green and 12-15 cm long, were collected in the vicinity of Stillwater, Oklahoma in August and September 1994 and frozen until used for either sheep toxicity studies or chemical extraction. In addition, green legumes stored at room temperature for approximately 1 year and more mature, brown legumes, also stored at room temperature for 1 year, were fed to 3 animals.

Physiologic Response

Six mature, cross-bred, white-faced, female sheep ranging in weight from 42-74 kg were used in the experiments. The sheep were prepared with a ruminal fistula and maintained on chopped corn, commercial pelleted feed, and alfalfa cubes (Stillwater Milling Co, Stillwater, Oklahoma). The sheep were housed indoors at a constant temperature and allowed free access to water.

The legumes were thawed, dried, ground, and stored thereafter either at room temperature or refrozen until administered to the sheep. The sheep were given varying dosages ranging from 5-25 g/kg of body weight (b.w.) (0.5-2.5% b.w.) of the ground legumes administered directly into the rumen and followed by 3 liters of tap water. Blood

samples for evaluation of serum chemistry were taken before, and 24 and 48 hr after the legumes were given. The sheep were monitored by visual observation. Because of the small number of experimental animals, some were used more than once, but at least 2 weeks elapsed between exposures.

Pyridoxine HCl was administered intramuscularly (i.m.) and/or subcutaneously (s.c.) simultaneously with the ground legumes at a dosage of 20 mg/kg b.w. or intravenously (i.v.) after the onset of seizures at a dosage of 10-15 mg/kg.

Chemistry

Extraction and isolation procedures were similar to those previously reported (4). The legumes (19 kg) were ground to a coarse pulp in a Waring blender with ethyl acetate and extracted at room temperature for approximately 20 hr. The extract was concentrated under reduced pressure to produce a thick, black syrup which was extracted with hot petroleum ether (40-50 °C). The insoluble residue was extracted with $CHCl_3$, and the $CHCl_3$ soluble portion subjected to silica gel chromatography (grade 12, 28-200 mesh 0.22; 35.5 cm x 8.9 cm). The column was eluted consecutively with $CHCl_3$, $CHCl_3$-MeOH (90:10, volume/volume, $CHCl_3$-MeOH (50:50), and MeOH. The eluates from $CHCl_3$-MeOH (90:10) were rechromatographed on silica gel (50.8 cm x 2.5 cm) using solvents of increasing polarity from $CHCl_3$ to MeOH. Eluates were collected in 356, 10 ml fractions and characterized using GC/EIMS. Samples of the pure alkaloids isolated from *A. tanganyicensis* (4) were obtained from South African

researcher Robert Vleggaar for comparative purposes, and were similarly characterized using GC/EIMS.

RESULTS

Physiologic Response

Results of administration of varying dosages of the legumes are shown in Table 1. The lethal dose was $\geq$15 g/kg b.w. and the toxic dose was in the range of 10-15 g/kg. Typically, signs of intoxication were apparent 12-14 hr after administration of a toxic dose. The first sign was hyperesthesia. There was an exaggerated response to tactile, auditory, and visual stimuli. Following this, there was muscular twitching, which lasted either briefly or for several minutes. Temperature increased slightly in some animals. More severe signs included convulsive seizures with deep, labored respiration, excessive salivation, tremors or shaking, backing-up or turning, torticollis, opisthotonus, collapse, outstretched forelimbs and paddling hindlimbs. Seizures lasted about 2 minutes, followed by quiescence in the lateral and then sternal positions. In mild cases, the seizures were infrequent, at intervals of an hour or more. In contrast, the seizures occurred every few minutes in severe cases. Two sheep given either 15 g/kg or 20 g/kg of the green legumes stored at room temperature for approximately 1 year exhibited similar signs and died, as did an animal given 25 g/kg of the brown legumes stored at room temperature for 1 year.

One sheep given 15 g/kg of legumes in combination with

20 mg/kg of pyridoxine HCl, divided half i.m. and half s.c., failed to develop any signs of intoxication. A second animal simultaneously given 20 g/kg of legumes and 20 mg/kg of pyridoxine HCl s.c. also failed to develop signs. Two weeks later, these sheep were given the identical dosage of legumes alone, with fatal results. Two other sheep developed severe seizures when given 15 g/kg of legumes but prompt relief was apparent following administration of 10-15 mg/kg of pyridoxine HCl i.v. However, the animals remained depressed for 1-2 days.

Serum chemistry evaluations performed in animals which did not die within the first 24 hr, showed no significant or consistent alterations from baseline values. The evaluated parameters included glucose, urea nitrogen, creatinine, total protein, albumin, sodium, potassium, chloride, calcium, phosphorus, cholesterol, total and direct bilirubin, alkaline phosphatase, lactic dehydrogenase (LDH), creatine phosphokinase (CPK), aspartate transaminase (AST), and γ-glutamyl transaminase (GGT).

Chemistry

GC/MS revealed a compound in fraction 267 ($CHCl_3$-MeOH; 85:15) that possessed a fragmentation pattern and retention time which matched that of the alkaloid 5-acetoxymethyl-3-hydroxy-4-methoxymethyl-2-methylpyridine found in *A. tanganyicensis* (Figure 2).

DISCUSSION

The legumes of *A. julibrissin* are clearly toxic. Although in some instances a substantial amount of material must be ingested, the availability of large amounts of the legumes on the pendant branches of these small, low growing trees presents a significant risk. Abruptly appearing several hours after ingestion, the signs are clearly neurologic; tremors and seizures. The legumes are toxic whether fresh or dried, and their toxicity is not appreciably reduced when stored at room temperature instead of frozen.

A compound present in the legumes of *A. julibrissin* displays the same fragmentation pattern and retention time of an alkaloid present in *A. tanganyicensis;* therefore, is identified as the same alkaloid. It has a molecular weight of 225, a molecular formula of $C_{11}H_{15}NO_4$, and its toxicity has been confirmed in weaned guinea-pigs (4).

It has been shown previously (7,8) that the toxins present in the legumes of *A. versicolor* act as pyridoxine antagonists and their neurotoxic effects are readily prevented or counteracted by administration of pyridoxine or pyridoxine HCl. Likewise, in this study when 10-15 mg/kg of pyridoxine HCl was given, there was prompt relief of seizure activity albeit the animals remained depressed for one to two days. When administration of legumes was accompanied by parenteral pyridoxine HCl, no signs of intoxication were observed. Thus, the protective effects of 10-15 mg/kg b.w. of pyridoxine HCl given either s.c., i.m., or i.v. were

confirmed, providing additional evidence of toxicant identity and its mechanism of action.

The toxicity of *A. julibrissin* seems to be less than the South African species (8), approximately 15 g/kg b.w. versus 5 g/kg respectively. However, the mode of action involving pyridoxine seems to be similar for both. The specific mechanism of the toxic effects is not fully understood, but pyridoxine antagonists such as 4-deoxypyridoxol and 4-methoxymethylpyridoxol are well-recognized causes of seizures in laboratory animals (13,14,15). Pyridoxine serves as a cofactor with glutamic acid decarboxylase in the formation of γ-aminobutyric acid (GABA) and with GABA transaminase in the breakdown of GABA to succinic acid (16). These roles suggest that the seizures may be due to impairment of synthesis of this inhibitory pathway neurotransmitter (17). In mice, administration of 4-deoxypyridoxine resulted in a decrease in glutamic acid decarboxylase and GABA in the brain. There also was a decrease in L-DOPA decarboxylase associated with a decrease in DOPA formation. Furthermore, 4-deoxypyridoxine causes a decrease in GABA transport, an effect which is counteracted by pyridoxal phosphate (18). The antagonists also impaired activity of GABA transaminase and the degradation of GABA. However, it is of interest that while the effects of the toxicants in the South African species of *Albizia* are ameliorated by pyridoxine, they are not by pyridoxal (7).

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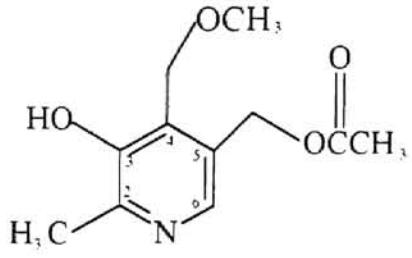
TABLE 1
Results of administration of various dosages of dried, ground legumes of *Albizia julibrissin* to sheep.

Dosage g/kg	Number of Animals	Results Observed
5	2	no signs of intoxication
10	1	no signs of intoxication
10	1	mild seizures
15	2	death
15	2	seizures, but recovery when given pyridoxine HCl i.v.
15	1	no signs when given pyridoxine HCl i.m. and s.c. simultaneously
20	2	death
20	1	no signs when given pyridoxine HCl s.c. simultaneously
25	2	death



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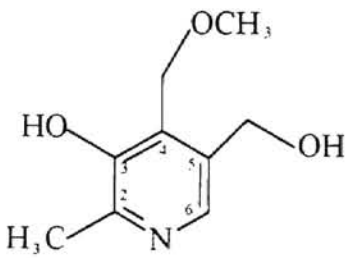

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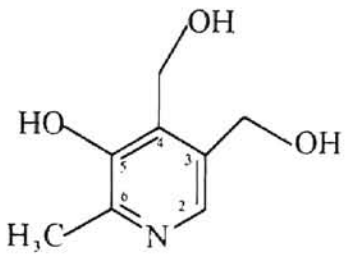
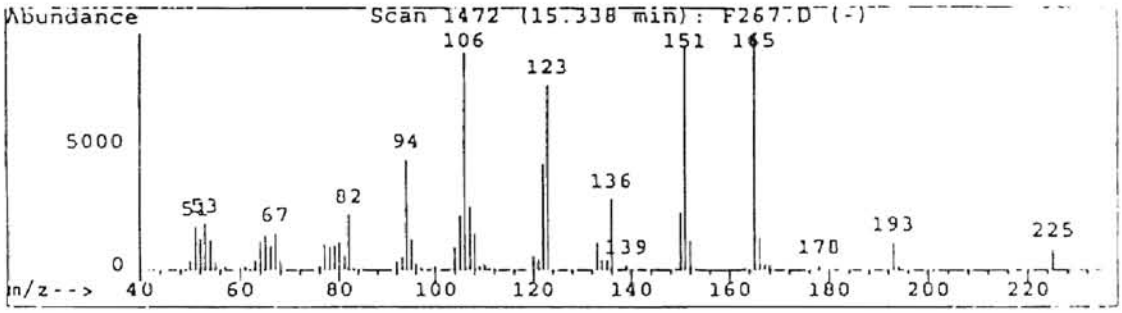


(C)

FIGURE 1
Chemical structures of neurotoxins in *Albizia tanganyicensis* and pyridoxine (Vitamin B_6).
(A) 5-acetoxymethyl-3-hydroxy-4-methoxymethyl-2-methylpyridine; (B) 3-hydroxy-5-hydroxymethyl-4-methoxymethyl-2-methylpyridine (= 4-methoxypyridoxine); (C) 5-hydroxy-6-methyl-3,4-pyridinedimethanol (= pyridoxine).



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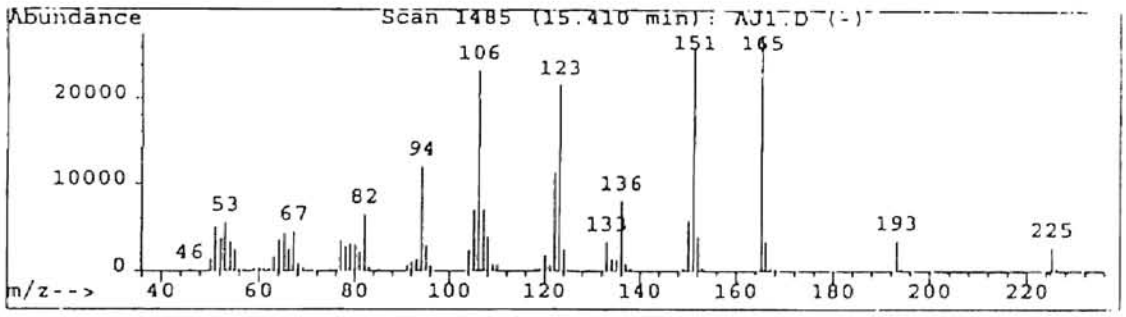

(B)

FIGURE 2
Mass spectra of compounds extracted from species of *Albizia*. (A) Mass spectrum of compound at retention time 15.338 minutes in fraction 267 from North American *A. julibrissin*. (B) Mass spectrum of compound at retention time 15.410 minutes from South African *A. tanganyicensis*.

INVESTIGATION OF THE NEUROTOXIC COMPOUNDS IN *ASCLEPIAS SUBVERTICILLATA* (WESTERN-WHORLED MILKWEED)

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ABSTRACT

Species of *Asclepias*, commonly known as milkweeds or silkweeds, are toxic plants with many cases of livestock poisoning reported. Previous investigations indicated the presence of multiple secondary metabolites that produce cardiotoxic-gastrointestinal and/or neurotoxic syndromes. Toxicants producing the former are cardenolides or cardiac glycosides. Those producing the neurotoxic effects were unknown, but recognized to occur only in the verticillate-leaved species of the genus. It is hypothesized that these verticillate-leaved species are neurotoxic due to the presence of a toxicant(s) unique to the group. The neurotoxic extract obtained from the dried, aerial portions of *Asclepias subverticillata* (western-whorled milkweed) appeared to contain a cinnamate-containing cardenolide. Toxicity was tested using chickens and structural studies were made via UV spectroscopy, low resolution ESMS, MS/MS, GC/EIMS and NMR.

INTRODUCTION

Since the early 1900's, species of *Asclepias*, members of the Asclepiadaceae or milkweed family and commonly known as milkweeds or silkweeds, have caused devastating losses to livestock in the western portions of the United States. Sheep, cattle, goats, horses, turkeys, and chickens have been affected by their toxic constituents (1,2,3,4,5,6,7,8,9). These highly toxic plants are not normally eaten, but may be consumed by very hungry animals (2,8). Drying seems to

increase palatability and does not diminish their toxicity. All plant parts are considered toxic (10,11).

Initial toxicity studies led toxicologists to divide the species of *Asclepias* into a narrow-leaved group and a broad-leaved group with blades greater than 3.5 cm wide (8,11,12). Based on clinical observations, it was believed that the broad-leaved group was cardiotoxic and the narrow-leaved group neurotoxic and more deadly (8,10,11). However, Ogden and coworkers (13,14,15) discovered that several narrow-leaved species produced cardiotoxic effects and that only verticillate-leaved species produced neurotoxic effects. These verticillate-leaved species were classified by Woodson (16) in his monograph of the genus, in the subgenus *Asclepias* and series *Incarnatae*. This series comprises 16 species, five of which are distinctly verticillate-leaved: *A. subverticillata* (western-whorled milkweed), *A. fascicularis* (narrow-leaved milkweed), *A. verticillata* (eastern-whorled milkweed), *A. pumila* (plains-whorled milkweed), and *A. mexicana* (mexican-whorled milkweed); and one which appears nearly so, *A. incarnata* (swamp milkweed). These six taxa form a complex of intergrading species across the western half of the United States and Mexico, and are hypothesized by Woodson (16) to be derived from the eastern-most *A. incarnata*.

Asclepias toxicants producing the cardiotoxic effects are cardenolides or cardiac glycosides and have been extensively studied (17,18,19,20,21,22). The basic

cardenolide aglycone is a C_{23} steroidal genin with a five-membered, singly unsaturated lactone ring at C-17, a hydroxyl group at C-14, and methyl groups at C-10 and C-13 (Figure 1A). Glycosidic linkage usually occurs at C-3 to one or more sugar moieties, but can also involve C-2, creating a cyclic bridge to a single sugar. Additional methyl, hydroxyl, and carbonyl groups can be attached at other carbons of the genin, their presence further influencing lipid solubility as well as protein binding. Other cardenolide containing genera of the Asclepiadaceae include *Calotropis*, *Cryptostegia*, *Gomphocarpus*, and *Pergularia* (19,21,23).

Cardenolides inhibit Na^+,K^+-ATPase by binding to the extracellular side of the α subunit. The resulting increase in intracellular Na^+ diminishes the exchange between extracellular Na^+ and intracellular Ca^{2+}, creating increased intracellular Ca^{2+} concentrations. It has been suggested (24) that the amino acid composition of transmembrane and extracellular domains, as well as an extracellular loop, determines affinity of the α subunit which varies interspecifically and intraspecifically. The structure-function relationship is believed to reside in the unsaturated lactone ring at C-17 and a hydroxyl group at C-14 (21,24).

Although the toxicants producing the neurotoxic effects of *Asclepias* had not been identified, a few investigations had provided insight as to their character. In 1920, Marsh

and coworkers (3) conducted a partial chemical analysis of *A. subverticillata*. They extracted alkaloids, glycosides, and a benzol-soluble resin. In guinea pigs, the alkaloids were nontoxic, the glycosides produced narcosis, and the resin produced neurotoxic symptoms. Additional work by Ogden and coworkers (13,14,15), using sheep and chickens, suggested several toxins or neurotoxic cardenolides may be responsible for the neurologic effects. Interestingly, these same studies described symptoms similar to those produced by *Cynanchum africanum* R.Br., a South African species of the family (25,26). This plant synthesizes neurotoxic pregnane glycosides which are responsible for a disorder called cynanchosis in domestic ruminants (25,27). Further studies (28,29,30,31,32,33,34,35,36) in South Africa, China, India, and Japan on species of *Cynanchum* (sandvine), as well as those of *Sarcostemma* (waxy twinevine), *Dregea* (dregea), *Marsdenia* (marsdenia), and *Periploca* (silkvine), other genera of the Asclepiadaceae, likewise identified pregnane glycosides.

Pregnanes, the putative biological precursors to cardenolide genins, are believed to be derived from splitting of the cholesterol side chain to pregnenolone (37,38). Their basic structure is a C_{21} steroid with methyl groups at C-10 and C-13, and a 2 carbon side-chain at C-17 (37,39) (Figure 1B). Commonly, pregnane glycosides, as well as cardenolides, of the Asclepiadaceae have rare 2,6-dideoxysugars such as, but not limited to, cymarose, oleandrose, and diginose

attached directly to the genin at C-3, followed by more 2,6-dideoxysugars or 6-deoxysugars, and then terminated by glucose (19,20,30,36,40). However, 6-deoxysugars also have been found to be attached directly to the genin at C-3, and glucose may not always be the terminal sugar (19,20,22,27,28,30,34). More intriguing is the discovery of a pregnane glycoside in *Periploca sepium* where two separate sugar moieties are attached to the genin at C-3 and C-20 (36).

The study reported here was designed as one step in the series to test the hypothesis that a unique neurotoxicant(s) occurs in *Asclepias* and is restricted in distribution to the verticillate-leaved species. We limited our investigation to *A. subverticillata*, commonly known as western-whorled or horsetail milkweed, for our initial studies. It has caused heavy livestock losses and has been recognized as one of the most poisonous plants in the United States (1,2,3,6,41). Early publications refer to it as *A. galioides* (16). It is an erect, herbaceous perennial 15-120 cm tall. Its stems may bear small, sterile branches. The leaves are petiolate, 1-4 mm wide, and whorled with 3-5 at each node. Usually solitary at the upper nodes, the umbellate inflorescenses are several to many flowered. The flowers are small with a white or sometimes lightly tinged greenish purple corolla. Horns arch over the anther heads and are longer than the corona. The fruit is a follicle, borne on a short, erect pedicel (16). Flowering is from June to August, and it is found in Arizona,

Colorado, Mexico, New Mexico, Oklahoma, Texas, and Utah (10,16). Plants of *A. subverticillata* occur on sandy, rocky plains and flats, and its stout, woody rootstalk allows it to spread rapidly in waterways, irrigation ditches, and damp pastures, where it often forms dense stands (11,12,16). This growth form readily lends itself to invasive growth in hay fields, especially alfalfa. The objectives of this investigation were to extract and identify its toxic fractions and to determine the structure of the neurotoxicant(s).

MATERIALS AND METHODS

Plant Collection

Fresh, whole plants including stems, leaves, flowers, and fruits were collected in the summer from the vicinities of Rocky Ford, CO (1995), Tucumcari, NM (1995), New Harmony, UT (1989,1995) and Kingman, AZ (1988). The plants were air-dried, ground in a Wiley mill, and refrigerated until used for either toxicity studies or chemical extractions. One specimen from each site was preserved and deposited in the Oklahoma State University Herbarium (OKLA) as a voucher.

Bioassay

Dried, ground plant material, crude extracts, and their fractions were placed into individual No.00 gel capsules and fed to chickens, a bioassay model previously developed by Ogden and coworkers (13,15). Male and female white leghorn chickens, weighing 0.4-2.6 kg, were housed indoors, individually or in pairs, in wire cages at a constant

temperature, and allowed free access to commercial pelleted feed and water. The chickens were observed at periodic intervals during the tests. When signs of intoxication appeared and became severe, birds were euthanized by cervical dislocation.

Dried, ground plant material, crude extracts, and the petroleum ether layer of the MeOH extract were administered at an approximate dosage of 10 mg/g body weight (b.w.) (1.0% b.w.). After the initial test, the dried, ground plant material was administered at approximately 7.5 mg/g b.w. (0.75% b.w.). The fractions derived from silica gel chromatography which did not produce neurotoxic symptoms within 24 hr were considered non-toxic at the following approximate dosages; 0.0725 mg petroleum ether-insoluble portion of CH_2Cl_2 extract/g b.w. (0.0073% b.w.), 0.2257 mg petroleum ether-soluble portion of CH_2Cl_2 extract/g b.w. (0.0226% b.w.), and 0.2400 mg MeOH layer of MeOH extract/g b.w. (0.0240% b.w.) for fractions obtained from the first two columns or 0.1584 mg/g b.w. (0.0158% b.w.) for fractions obtained from the third and fourth columns. These dosages were determined by multiplying the amount of initial crude extract received from 1 g of dried plant material by the dried plant material dosage of 0.75% b.w.

Extraction, Isolation, and Structural Determination

Extraction procedures were similar to those previously performed during preliminary studies at Oklahoma State University on *Asclepias subverticillata* (M. Khan, personal

communication) and on species of *Cynanchum* and *Sarcostemma* at the Veterinary Research Institute, Onderstepoort, South Africa (G.L. Erasmus, personal communication). The dried plant material was first extracted with distilled CH_2Cl_2, then air-dried and re-extracted with MeOH. Crude extracts and their fractions were kept under N_2(g) at room temperature and in darkness until used for toxicity studies or further chemical analyses.

Methylene Chloride. Dried plant material (3 kg in 500 g increments = approximately 17 kg fresh material) was extracted with distilled CH_2Cl_2 (2.5 L x 6) at room temperature for 30 minutes and again (1.25 L x 6) at room temperature for 1 hr. A dark blackish green tar, obtained from the filtrates by concentration under reduced pressure, was extracted with hot petroleum ether (33-40 °C) (7.30 L). The insoluble residue was subjected to a series of silica gel chromatography (28-200 mesh 22 Å), always proceeding in the series with the most toxic fraction (Table 1).

Prior to loading fraction 25A onto the sixth silica gel column (Table 1), approximately 20 mg was removed for characterization using low res ESMS, and purified further on a separate silica gel column (30 g/g extract) using a step-wise elution with cyclohexane-CH_2Cl_2 (40:60/30:70/20:80/15:85/10:90), CH_2Cl_2, CH_2Cl_2-MeOH (50:50), and MeOH. The CH_2Cl_2-MeOH and MeOH eluates were combined and analyzed using ^1H, ^1H COSY, and ^{13}C NMR and RP-HPLC coupled with UV diode array detector.

A small portion of fraction 16A (Table 1) was used for structural studies using RP-HPLC coupled with diode array UV detector, low res ESMS, MS/MS, GC/EIMS, ^{1}H NMR, and ^{1}H COSY NMR. GC/EIMS was made possible by derivatizing with BSTFA/TMCS following strong methanolysis. Methanolysis was performed using 0.2N H_2SO_4 with MeOH and heating to 60 °C. Barium hydroxide was then added along with CH_2Cl_2 and H_2O. The mixture was centrifuged and the supernatant contained the aglycone moieties.

Methanol. Plant material was air-dried after extraction with CH_2Cl_2 then re-extracted with MeOH (6.00 L x 3) at room temperature for 12 hr. A dark, green tar, obtained from the filtrates by concentration, was dissolved in MeOH (1.70 L) and partitioned with petroleum ether (13.95 L). The MeOH layer was concentrated to produce a brownish green tar which was subjected to a series of silica gel chromatography, always proceeding in the series with the most toxic fraction (Table 2).

RESULTS

Bioassay

The symptoms of intoxication in chickens fed the dried plant material resembled those in chickens fed the plant extracts. Typically, intoxication was apparent 6-12 hr after administration of the plant material or crude extracts. Later fractions produced intoxication within 2-4 hr. Neurotoxic signs included ataxia, excitement, head tremors, intermittent seizures, torticollis, depression, and sometimes

death. These signs were consistent with those previously described by Ogden and coworkers (13,15).

Extraction, Isolation, and Structural Determination

Methylene Chloride. Petroleum ether-soluble (90 g) and insoluble (29 g) portions were toxic. From the insoluble portion (Table 1), fractions 11A (100% petroleum ether to 80:20 petroleum ether-chloroform), 21A, 31A (100% chloroform), 22A, 23A, 24A, 25A, and 16A (100% toluene) were toxic. Fraction 16A appeared to exhibit reduced toxicity compared to fraction 25A.

As noted in the materials and methods, the 20 mg of fraction 25A retained for characterization and subjected to further purification by silica gel chromatography, most eluted in the more polar fractions. The positive ion spectrum of these eluates revealed a major component with a molecular weight of 1067. The ^1H NMR spectrum at 500 MHz in $CDCl_3$ showed a pair of doublets in the olefinic region suggesting two different cinnamoyl olefinic hydrogen atoms. Other signals originating from the genin moiety appeared at δ1.15 (C-19 Me), 1.53 (C-18 Me), and 5.35 (C-6 olefinic proton). UV was consistent with a cinnamoyl group ($\lambda_{max,CH3CN}$=277 nm) and lactone ring ($\lambda_{max,CH3CN}$=220 nm) (42).

For the small portion of fraction 16A taken for structural studies, UV indicated the presence of a cinnamoyl group ($\lambda_{max,CH3CN}$=277 nm) and lactone ring ($\lambda_{max,CH3CN}$=220 nm). The low resolution ESMS positive ion spectrum of analytes with the greatest UV absorbance intensity at 277 nm

and 220 nm, showed a dominant peak at 1067. Daughters of 1067 closely corresponded to the loss of rhamnose (m/z 921), thevetose (m/z 761), cymarose (m/z 617), and cinnamoyl group (m/z 471). GC/EIMS spectra agreed with the presence of a cinnamoyl group and genin moiety. The ^{1}H NMR spectrum at 500 MHz in $CDCl_3$ confirmed the cinnamoyl group at δ6.08 (d, J=15.8 Hz) and 7.39 (d, J=15.8). Other signals originating from the genin moiety appeared at δ1.14 (C-19 Me), 1.52 (C-18 Me), and 5.38 (C-6 olefinic proton) (42).

The chemical data obtained on fractions 25A and 16A were consistent with the presence of genin glycosides; genin and carbohydrate moieties of approximately the same molecular weight. Complexity of the various spectra indicated the analytes were still a mixture of several compounds, some which were probably similar glycosides. Composition of the 2 fractions was similar, however, fraction 16A appeared to contain fewer polar compounds.

Methanol. The petroleum ether layer (46 g) was non-toxic and the MeOH layer (96 g) was toxic. From the MeOH layer (Table 2), fractions 2 and 3 of the first and second columns, respectively, were toxic. From the 6 fractions obtained from the third column, fractions 2 (55:45 petroleum ether-ethyl acetate to 80:20 ethyl acetate-petroleum ether), 3 (80:20 ethyl acetate-petroleum ether to 100% ethyl acetate), and 5 were toxic. Fractions 2 and 3 were combined and retained for future studies. Fraction 5 lost toxicity after further fractionation by silica gel chromatography.

DISCUSSION

Our study suggests the presence of a cinnamate-containing cardenolide in the neurotoxic CH_2Cl_2 extract of *A. subverticillata*. The proposed structure (Figure 2) has a molecular weight of 1066 and a molecular formula of $C_{57}H_{78}O_{19}$. The position of the tigloyl group at C-8 was determined by difference in molecular weight and labile character in acid. Tigloyl groups have been found in pregnane glycosides isolated from other genera of the Asclepiadaceae (28,39). Although cinnamoyl groups occur in pregnane glycosides isolated from species of *Cynanchum*, *Dregea*, and *Marsdenia* (28,43,44) and in a cardenolide isolated from *Asclepias asperula* (45), this is the first report of a cinnamate-containing cardenolide with the cinnamoyl group attached directly to the genin moiety.

The toxic fractions obtained from the MeOH extract may contain similar glycosides as those extracted via CH_2Cl_2. Future investigators should consider extracting the plant material in smaller increments or with a greater quantity of CH_2Cl_2 and extracting the MeOH extract with CH_2Cl_2.

In addition to a cinnamate-containing cardenolide, the toxic, petroleum ether-soluble portion of the CH_2Cl_2 extract contains a compound(s) that may play a role in the neurotoxicity of *Asclepias subverticillata*. It could be a cyclic triterpene similar to those isolated from *Cynanchum hancokianum* (Maxim.) Al. Iljinski, a Mongolian plant believed to possess antitumor activity (46). Petroleum ether-soluble

and insoluble portions derived from the EtOH extract of this plant have produced cyclic triterpenes and pregnane glycosides, respectively (29,46). It is hoped that future studies will provide insight into the character of the petroleum ether-soluble compounds of *Asclepias subverticillata*.

Our evidence of a cinnamate-containing cardenolide in the aerial portions of *A. subverticillata* supports the hypothesis that a unique neurotoxicant(s) occurs in a verticillate-leaved species. This finding is consistent with Ogden and coworkers' studies (13,14,15) describing myocardial infiltrates in sheep and chickens fed *A. subverticillata* and *A. verticillata*. Our work provides a basis for future studies to determine the nature and mode of action of the neurotoxins present in the other verticillate-leaved species. Identification of the neurotoxic constituents in the six verticillate-leaved species will provide additional evidence for Woodson's (16) interpretation that these taxa form a complex of intergrading species which probably originated from *A. incarnata*.

Elucidating the mode of action of intoxication may permit the development of an antidote. It also may enhance our pharmacopoeia against human diseases. For example, differentiation-inducing activity towards mouse myeloid leukemia (M1) cells using acyl-containing pregnane glycosides from *Marsdenia cundurango* Reich. showed cinnamoyl groups were the most potent inducers (47). In addition, folk remedies in

South America and parts of Asia have used plants of the Asclepiadaceae as treatments for syphilis and cancer, and as emetics, antifebriles, tonics, diuretics, antitussives, and expectorants (28,29,30,31,35,43,48). In order to fully understand the neurotoxins of the verticillate-leaved species of *Asclepias*, continued research is required.

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TABLE 1
Silica gel chromatography series of petroleum ether-insoluble portion received from CH_2Cl_2 extract.

Column Number	Solvent System (Total Liters)	Total Number of Fractions	Fraction Rechrom.*	Solvent System**	g silica gel/ g extract+
1	pet. ether to $CHCl_3$ to MeOH (25.40 L)	5	21A	50:50 pet. ether-$CHCl_3$	33
2	$CHCl_3$ to MeOH (1.75 L)	7	22A	100% $CHCl_3$	54
3	CH_2Cl_2-$CHCl_3$ 50:50 to MeOH (0.98 L)	6	23A	50:50 CH_2Cl_2-$CHCl_3$	41
4	CH_2Cl_2 to $CHCl_3$ to MeOH (1.00 L)	5	24A	100% CH_2Cl_2	41.5
5	benzene to $CHCl_3$ to MeOH (1.00 L)	4	25A	100% benzene	45
6	toluene to $CHCl_3$ to MeOH (1.40 L)	5	NA	NA	121

* Fraction rechromatographed based on neurotoxic symptoms observed in chicken bioassay; first digit=fraction number, second digit=column number, A=CH_2Cl_2

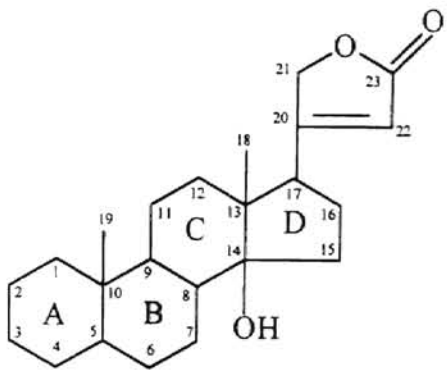
** Solvent system in which fraction to be rechromatographed eluted

\+ g silica gel used per g of extract for the column

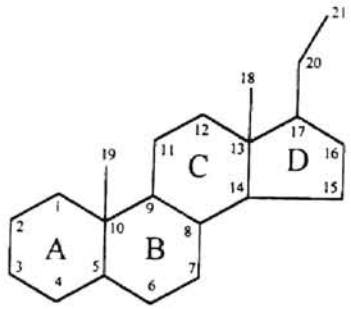
TABLE 2
Silica gel chromatography series of MeOH layer received from MeOH extract.

Column Number▲	Solvent System (Total Liters)	Total Number of Fractions	Fraction Rechrom.*	Solvent System**	g silica gel/ g extract+
1	pet. ether to ethyl acetate to acetone to MeOH (16.35 L)	3	2	70:30 pet. ether-ethyl acetate to 50:50 ethyl acetate-acetone	++
2	as above (5.34 L)	10	3	100% ethyl acetate	10
3▲	as above (7.30 L)	6	5	90:10 ethyl acetate-acetone to 100% acetone	41.5
4▲	as above (3.35 L)	7	NA	NA	180

▲ Pressure used
* Fraction rechromatographed based on neurotoxic symptoms observed in chicken bioassay
** Solvent system in which fraction to be rechromatographed eluted
\+ g silica gel used per g of extract for the column
++ g silica gel per g extract not available; silica gel layer was 20.3 cm x 8.3 cm



(A)



(B)

FIGURE 1
(A) Basic cardenolide genin characteristic of members of the Asclepiadaceae. (B) Basic plant pregnane characteristic of members of the Asclepiadaceae.

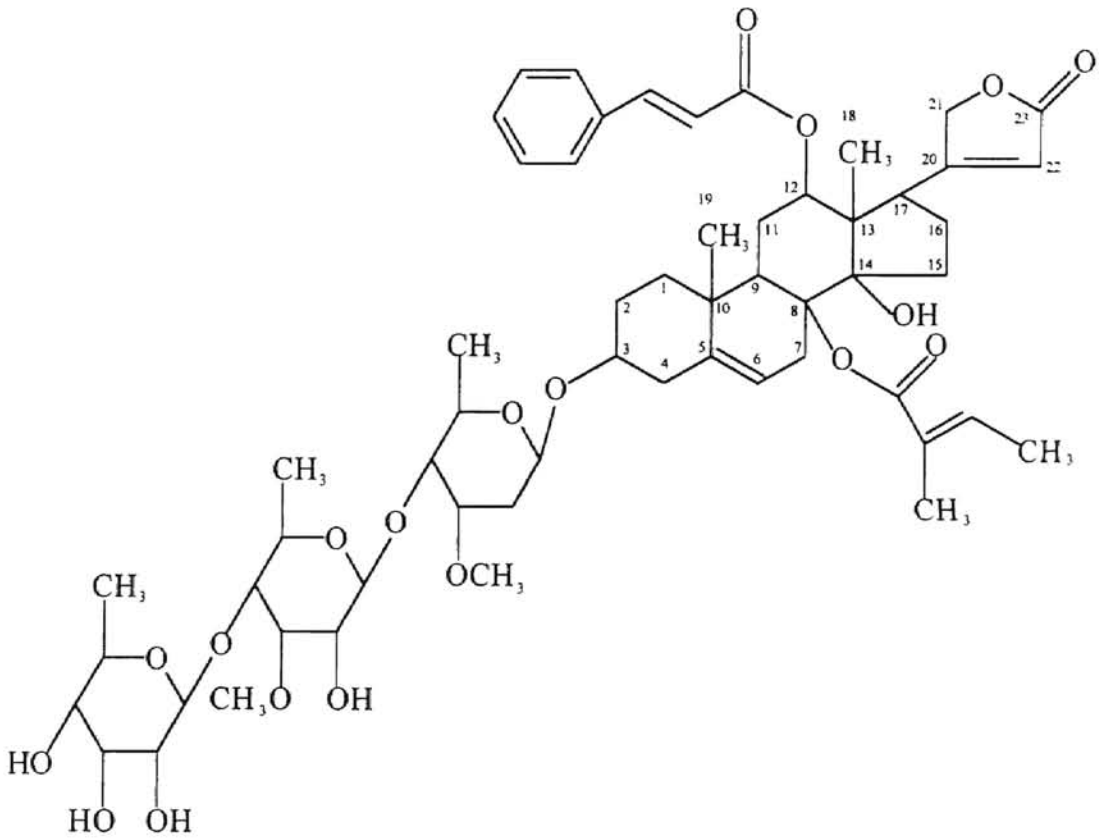
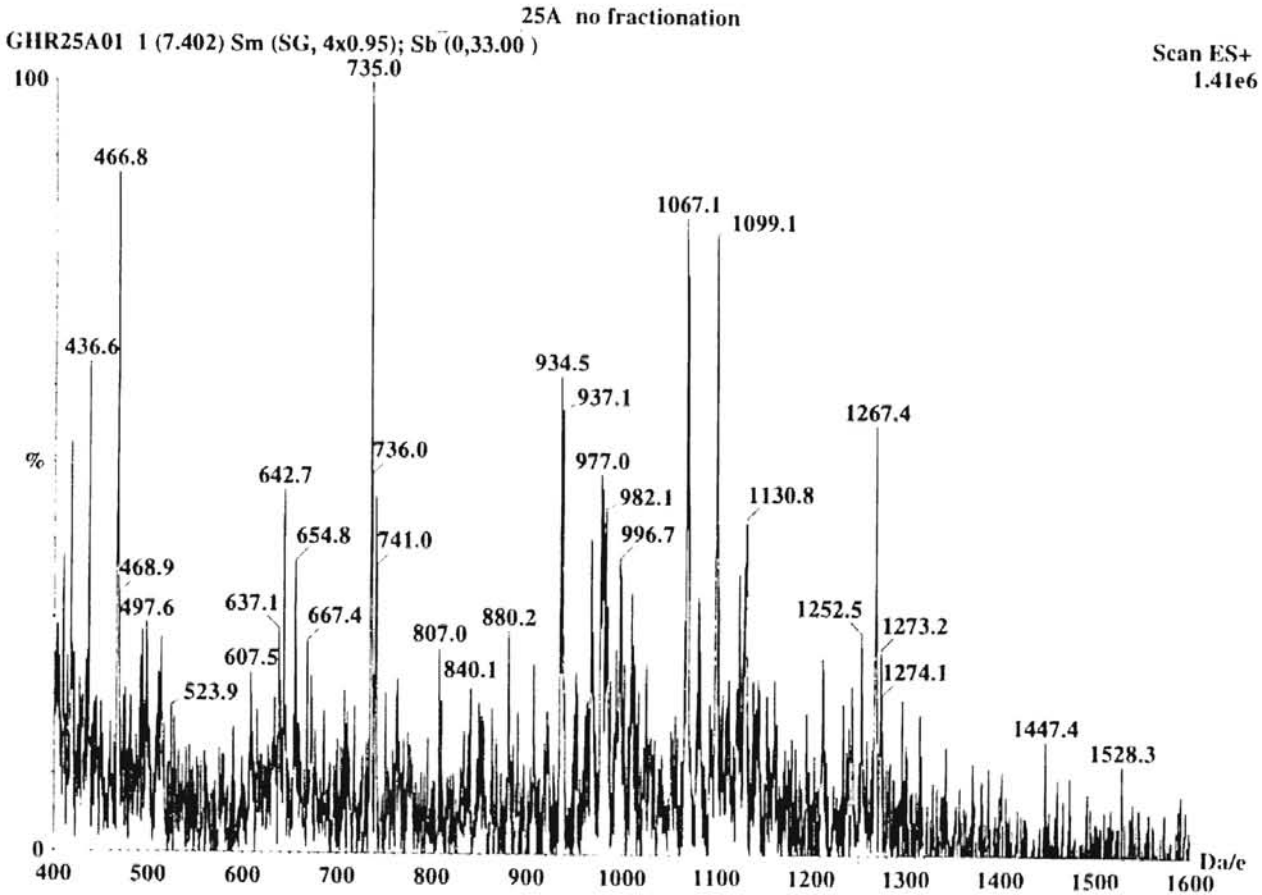


FIGURE 2
Proposed cardenolide in the neurotoxic extract of *Asclepias subverticillata*.

APPENDICES

APPENDIX A

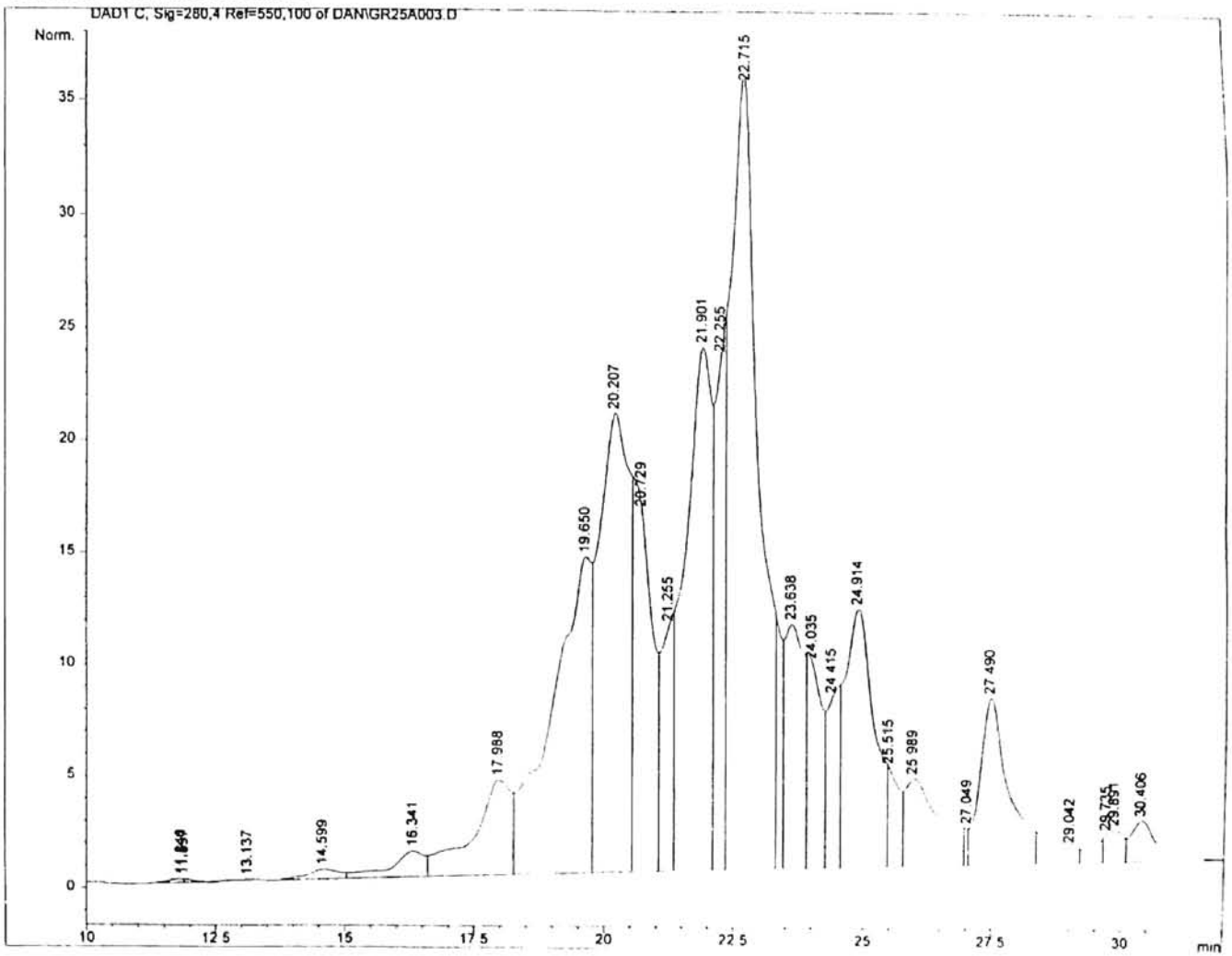
MASS SPECTRUM FROM LOW RESOLUTION ELECTROSPRAY IONIZATION MASS SPECTROMETRY ON FRACTION 25A


25A no fractionation
GHR25A01 1 (7.402) Sm (SG, 4x0.95); Sb (0,33.00)
Scan ES+
1.41e6
100
%
0
735.0
466.8
1067.1
1099.1
436.6
934.5
937.1
1267.4
736.0
977.0
642.7
982.1
1130.8
654.8
741.0
996.7
468.9
497.6
637.1
667.4
880.2
1252.5
1273.2
807.0
607.5
840.1
1274.1
523.9
1447.4
1528.3
400
500
600
700
800
900
1000
1100
1200
1300
1400
1500
1600
D:a/e

APPENDIX B

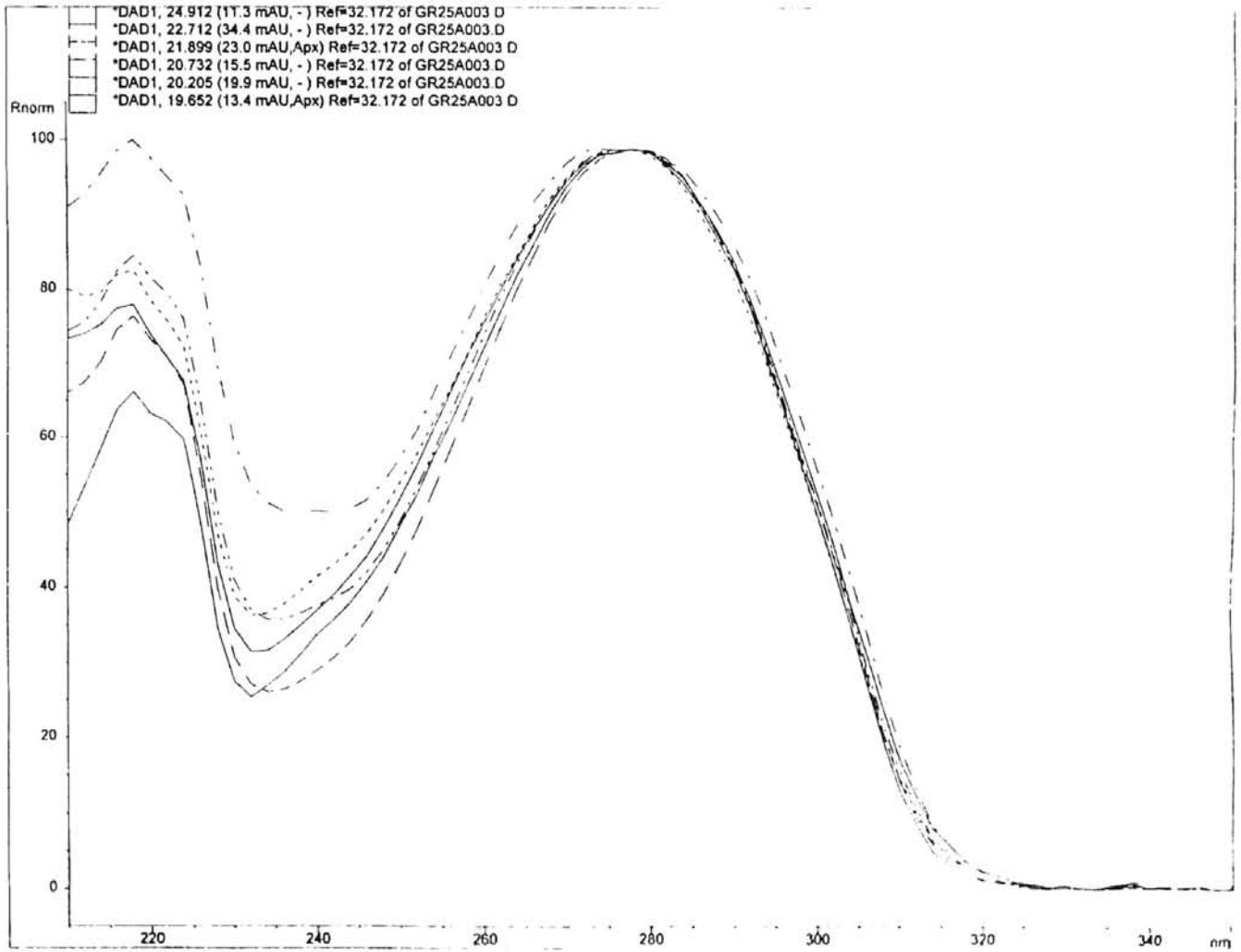
CHROMATOGRAM AND SPECTRUM FROM REVERSE PHASE HPLC COUPLED WITH DIODE ARRAY DETECTOR ON FRACTION 25A

Current Chromatogram(s)



Diode Array 1/25/97 10:40:04 PM A. D. Jones

UV Apex spectrum of Peak 19.65 of GR25A003.D

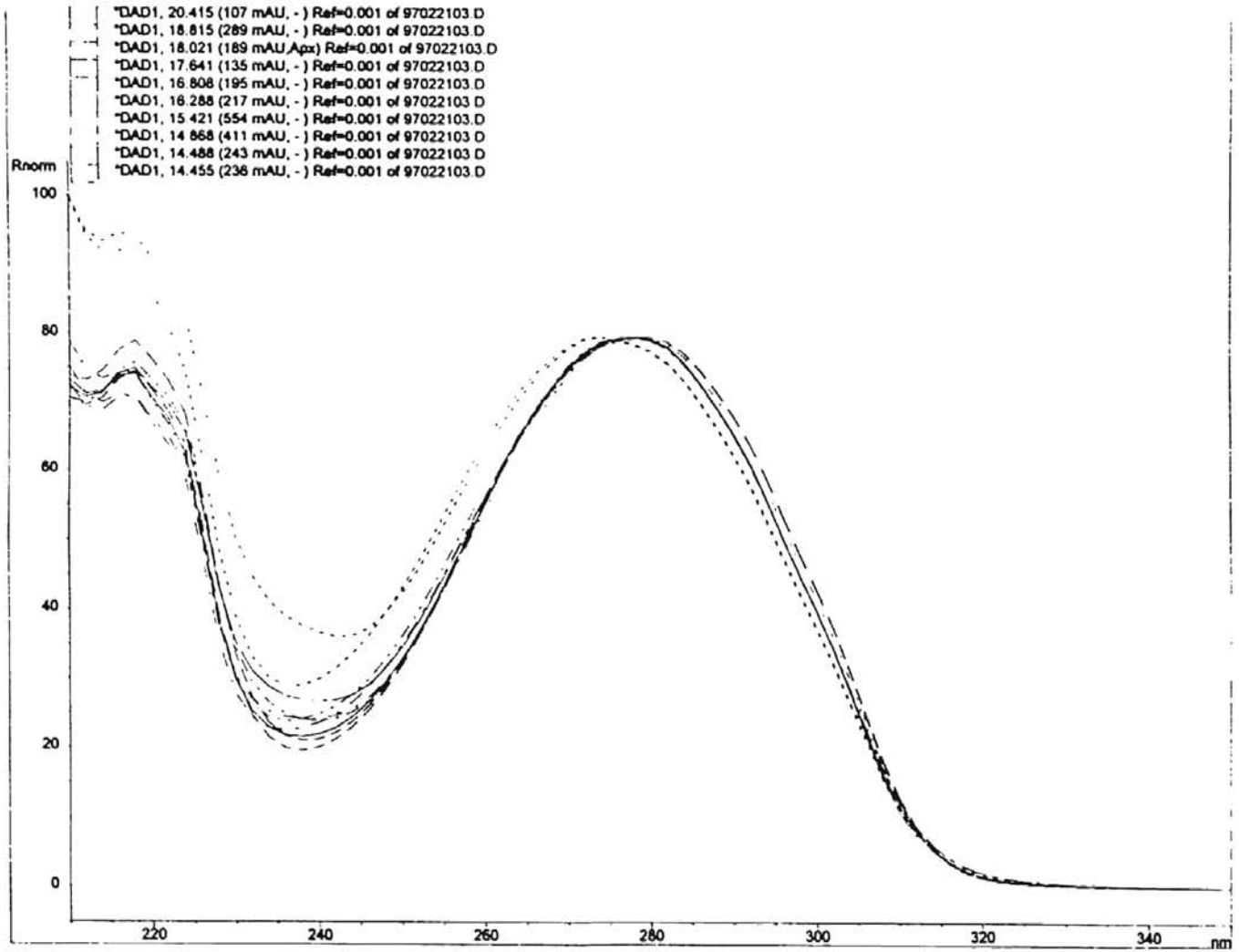


Diode Array 1/25/97 10:29:54 PM A. D. Jones

APPENDIX C

SPECTRUM FROM REVERSE PHASE HPLC COUPLED WITH DIODE ARRAY DETECTOR ON FRACTION 16A

DAD1, 14.455 (236 mAU, -) Ref=0.001 of 97022103.D

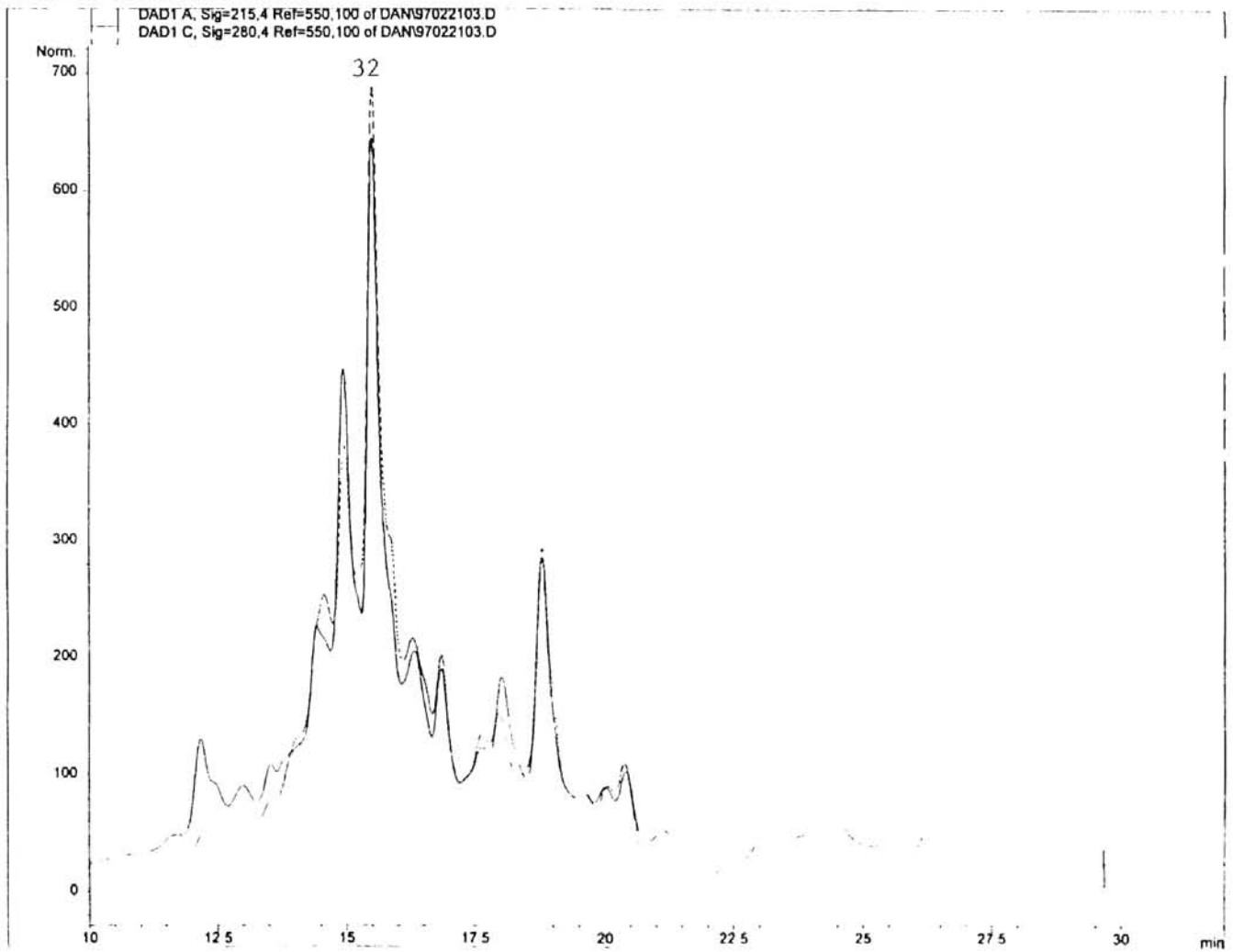


Diode Array 2/21/97 4:30:43 PM gregg

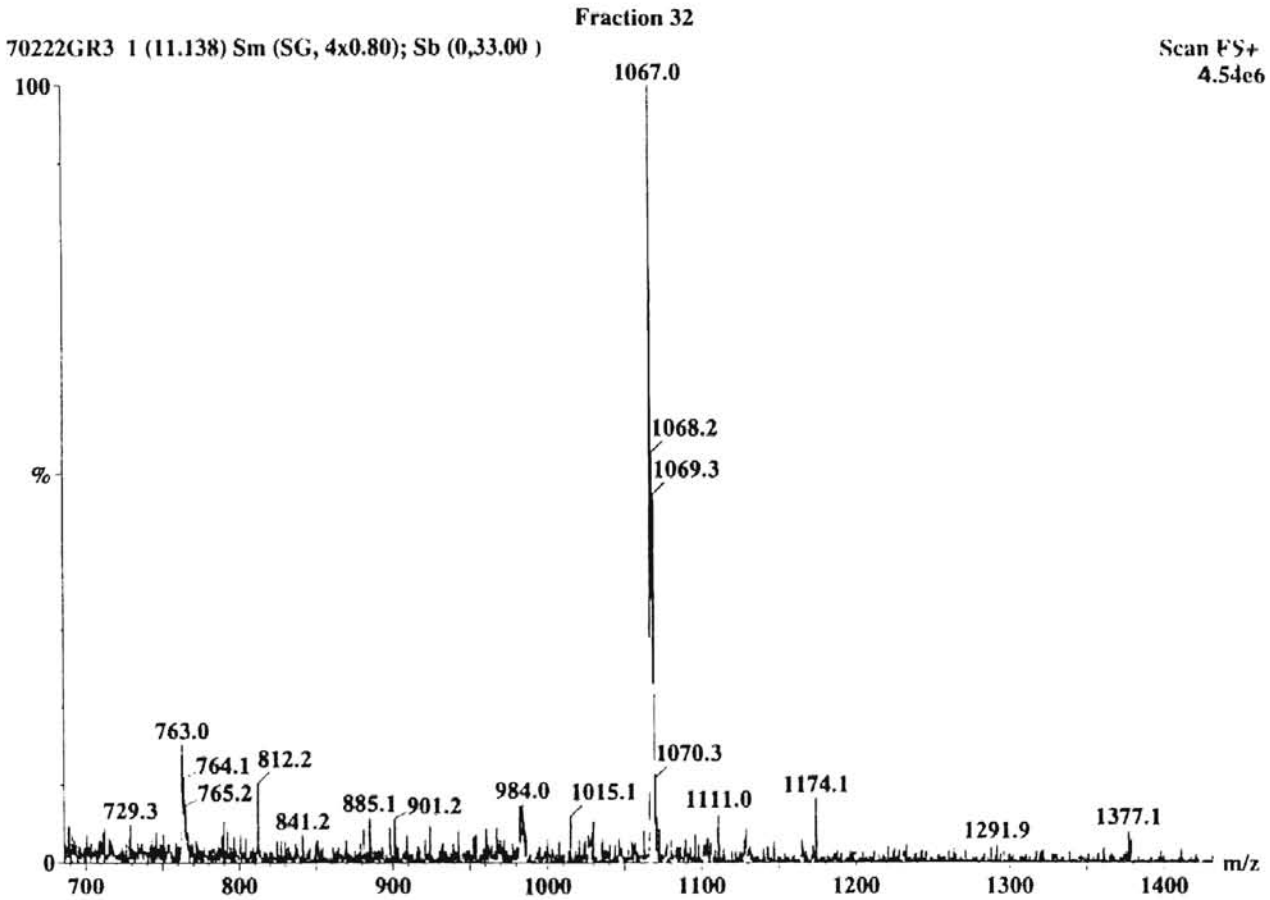
APPENDIX D

CHROMATOGRAM FROM REVERSE PHASE HPLC, SPECTRUM FROM LOW RESOLUTION ELECTROSPRAY IONIZATION MASS SPECTROMETRY, AND SPECTRUM FROM TANDEM MASS SPECTROMETRY ON FRACTION 16A

Current Chromatogram(s)



Diode Array 2/21/97 3:56:22 PM gregg

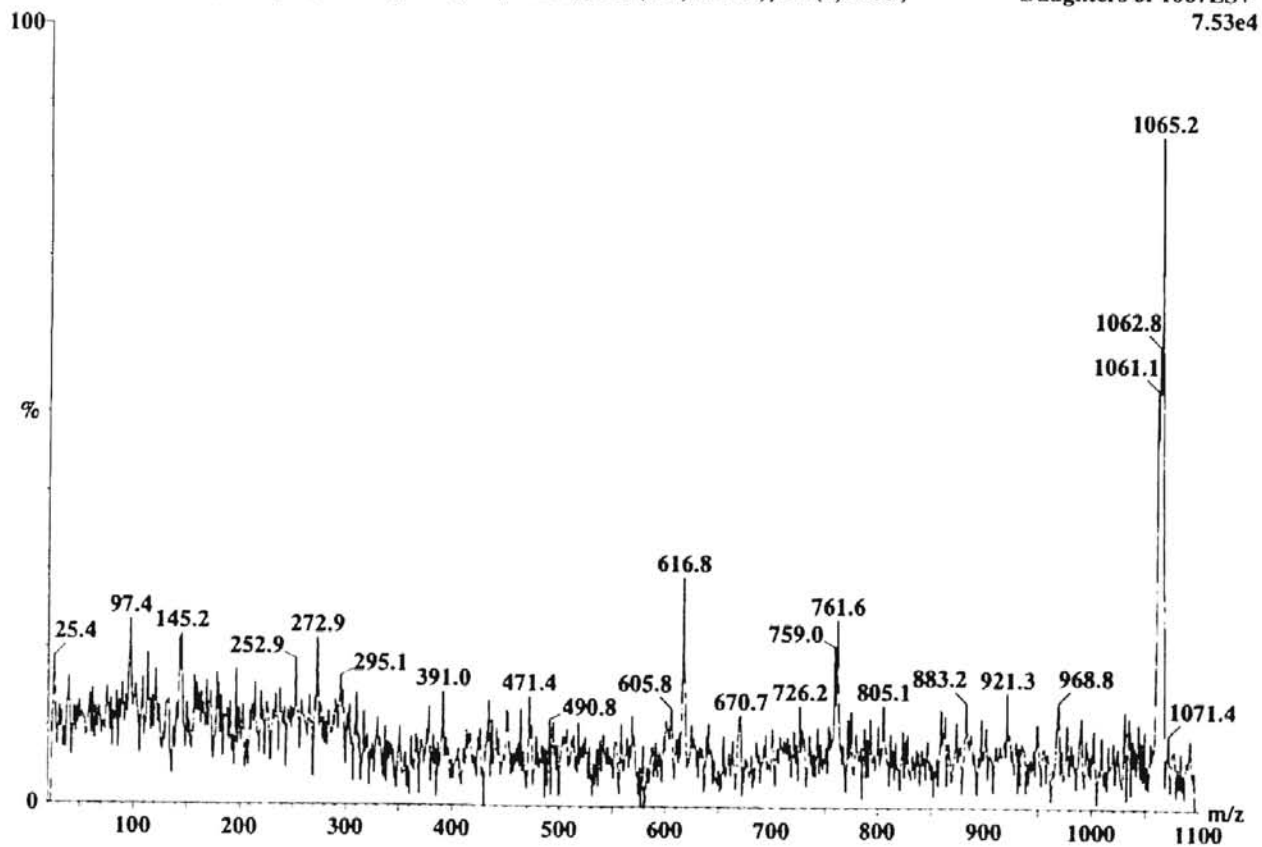

Fraction 32
70222GR3 1 (11.138) Sm (SG, 4x0.80); Sb (0,33.00)
Scan ES+
4.54e6
100
%
0
1067.0
1068.2
1069.3
1070.3
763.0
764.1
765.2
812.2
729.3
841.2
885.1
901.2
984.0
1015.1
1111.0
1174.1
1291.9
1377.1
m/z
700
800
900
1000
1100
1200
1300
1400

Sample 16A Fraction 32 , products of 1067 20 eV

7222GR17 1 (16.482) Sm (Mn, 4x1.50); Sm (Mn, 4x1.00); Sm (Mn, 4x1.00); Sb (0,20.00)

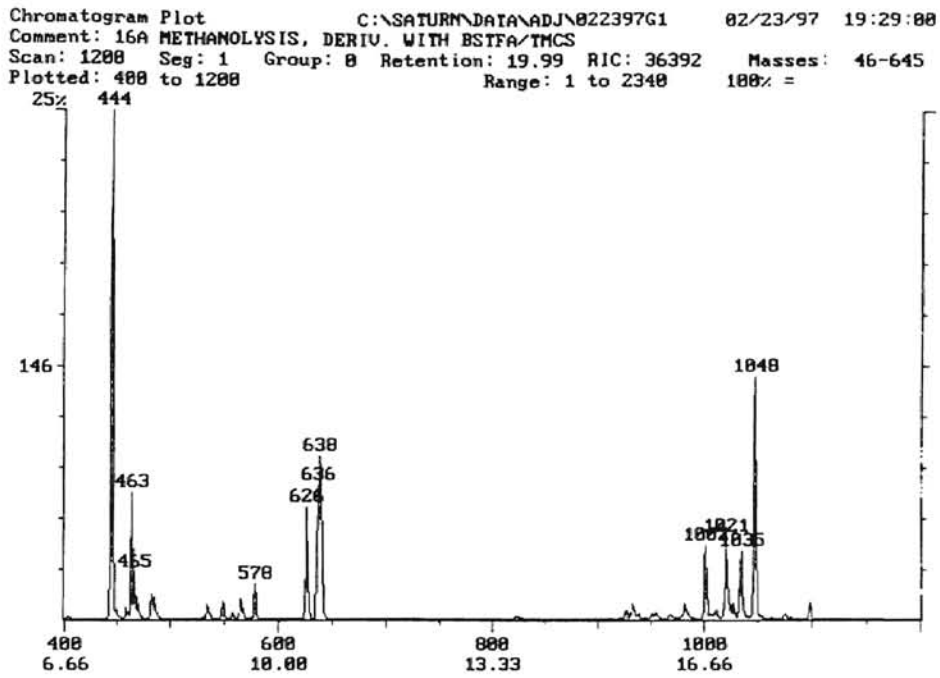
Daughters of 1067ES+

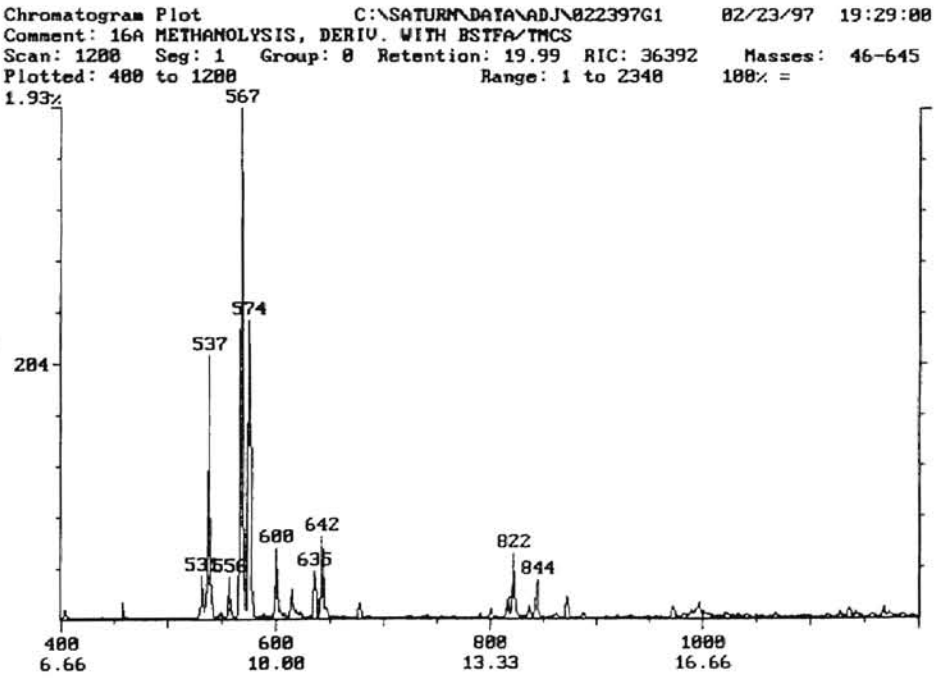
7.53e4

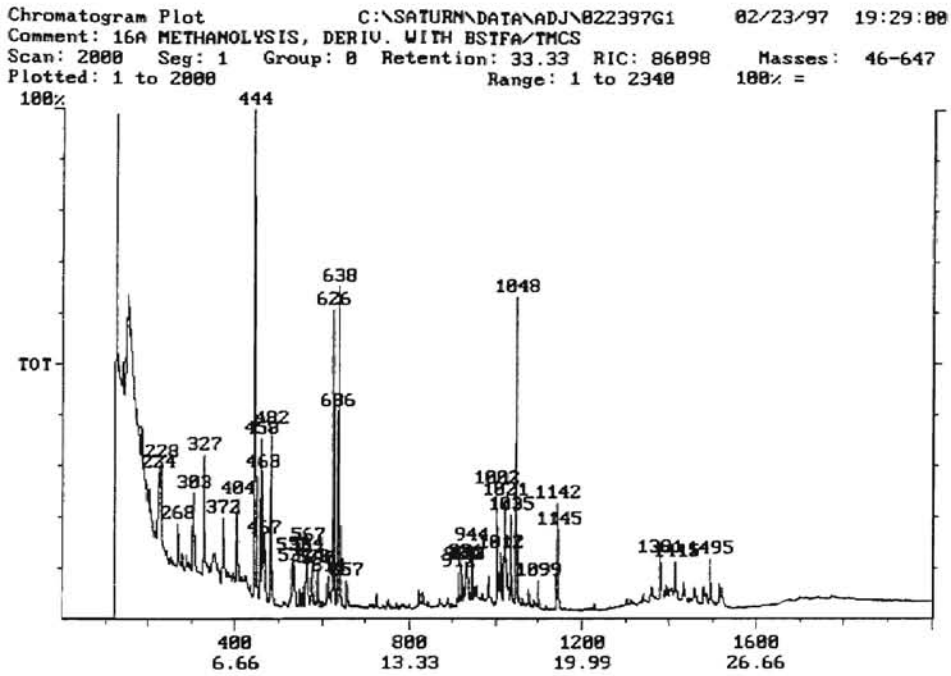


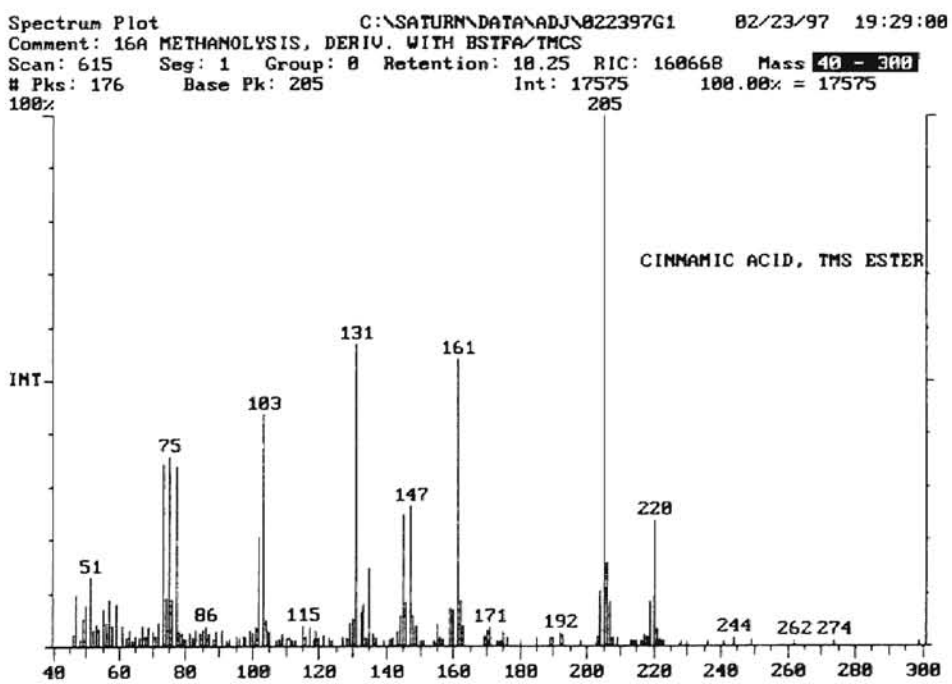
APPENDIX E

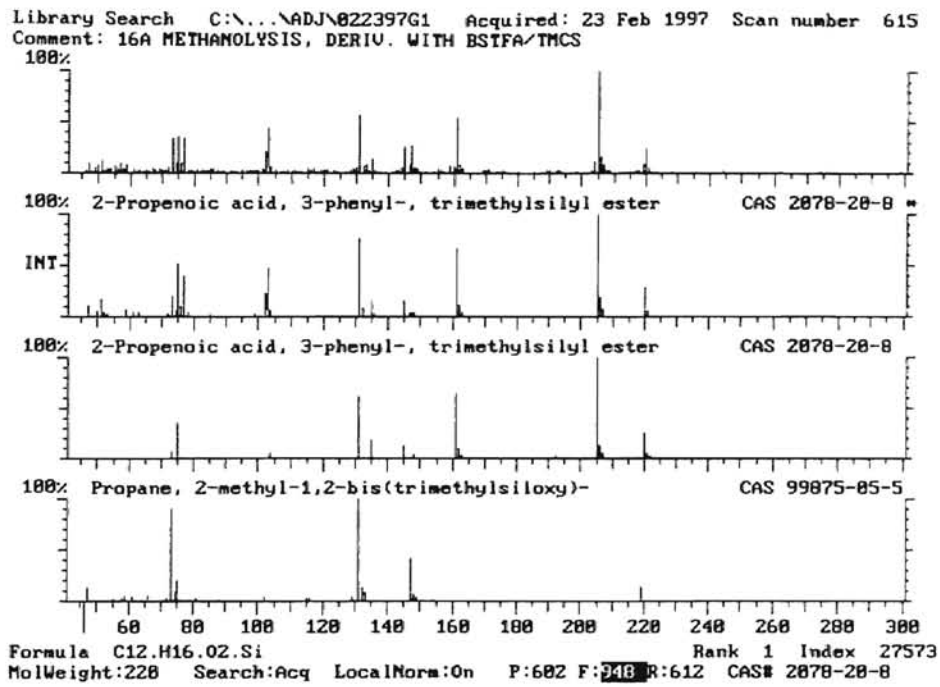
CHROMATOGRAMS AND SPECTRA FROM GAS CHROMATOGRAPHY COUPLED WITH ELECTRON IMPACT MASS SPECTROMETRY ON FRACTION 16A

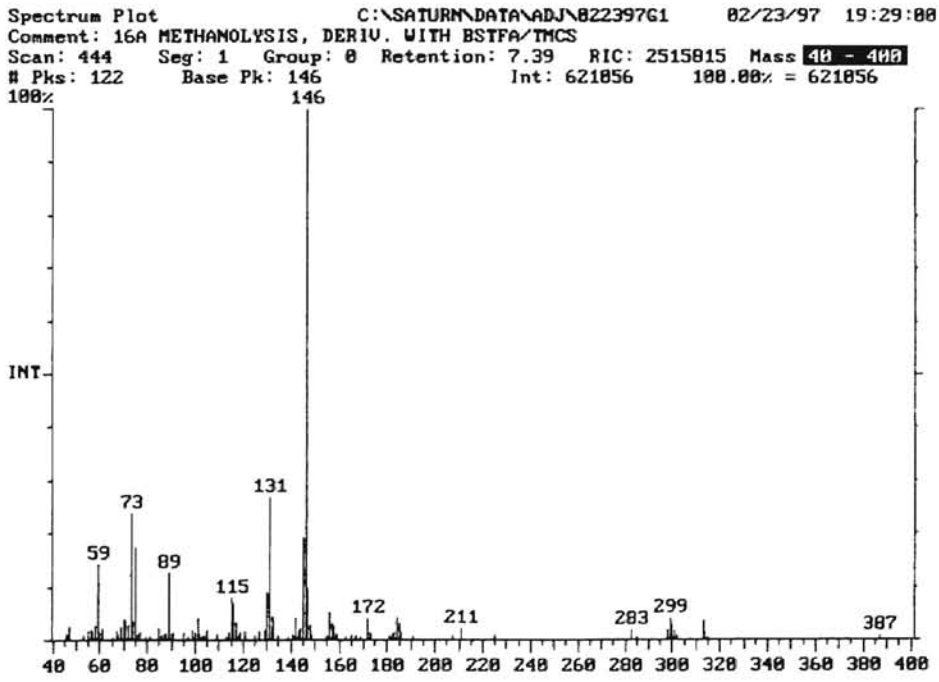

Chromatogram Plot C:\SATURN\DATA\ADJ\022397G1 02/23/97 19:29:00
Comment: 16A METHANOLYSIS, DERIV. WITH BSTFA/TMCS
Scan: 1200 Seg: 1 Group: 0 Retention: 19.99 RIC: 36392 Masses: 46-645
Plotted: 400 to 1200 Range: 1 to 2340 100% =
25% 444
146
1048
638
636
626
463
465
578
400
6.66
600
10.00
800
13.33
1000
16.66

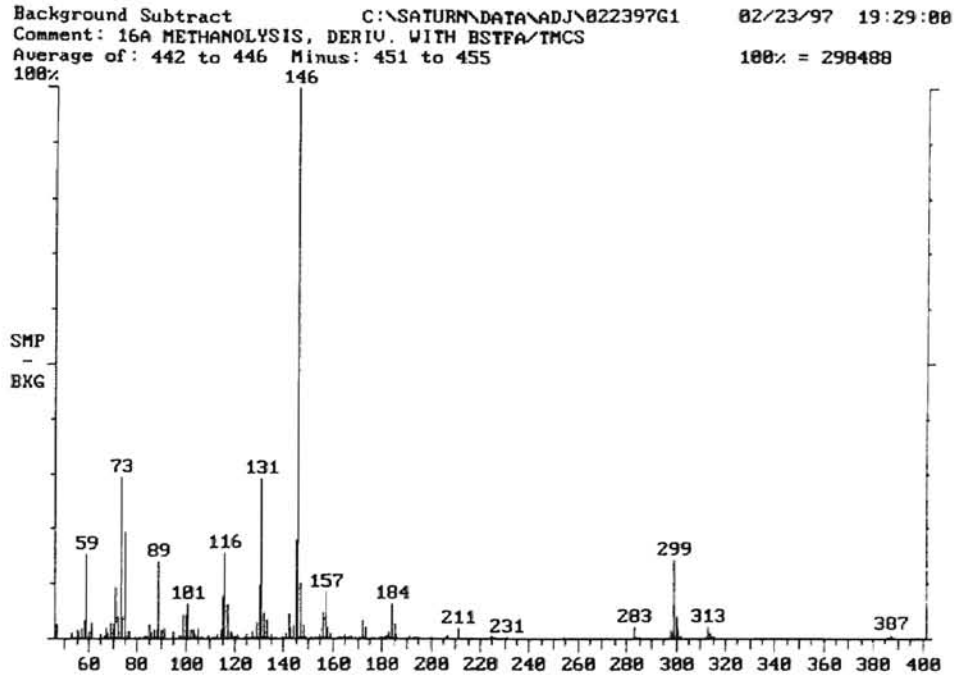

Chromatogram Plot C:\SATURN\DATA\ADJ\022397G1 02/23/97 19:29:00
Comment: 16A METHANOLYSIS, DERIV. WITH BSTFA/TMCS
Scan: 1200 Seg: 1 Group: 0 Retention: 19.99 RIC: 36392 Masses: 46-645
Plotted: 400 to 1200 Range: 1 to 2340 100% =
1.93% 567
204
574
537
642
600
635
822
844
400
6.66
600
10.00
800
13.33
1000
16.66

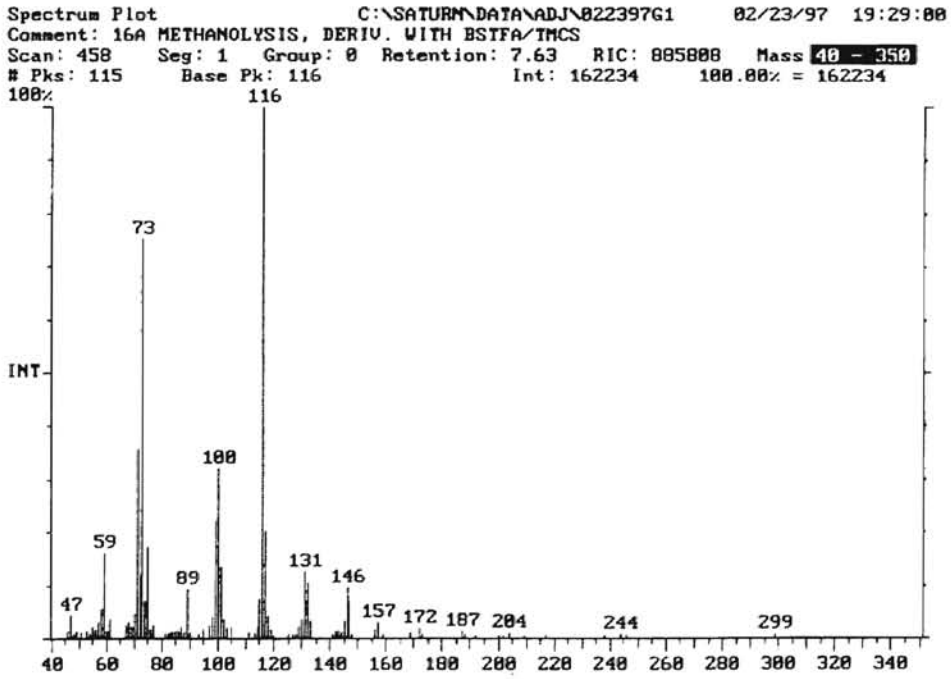

Chromatogram Plot C:\SATURN\DATA\ADJ\022397G1 02/23/97 19:29:00
Comment: 16A METHANOLYSIS, DERIV. WITH BSTFA/TMCS
Scan: 2000 Seg: 1 Group: 0 Retention: 33.33 RIC: 86098 Masses: 46-647
Plotted: 1 to 2000 Range: 1 to 2340 100% =
100%
TOT
444
638
626
1048
686
482
458
468
327
228
224
308
404
268
372
457
567
1142
1145
1099
1495
400
6.66
800
13.33
1200
19.99
1600
26.66

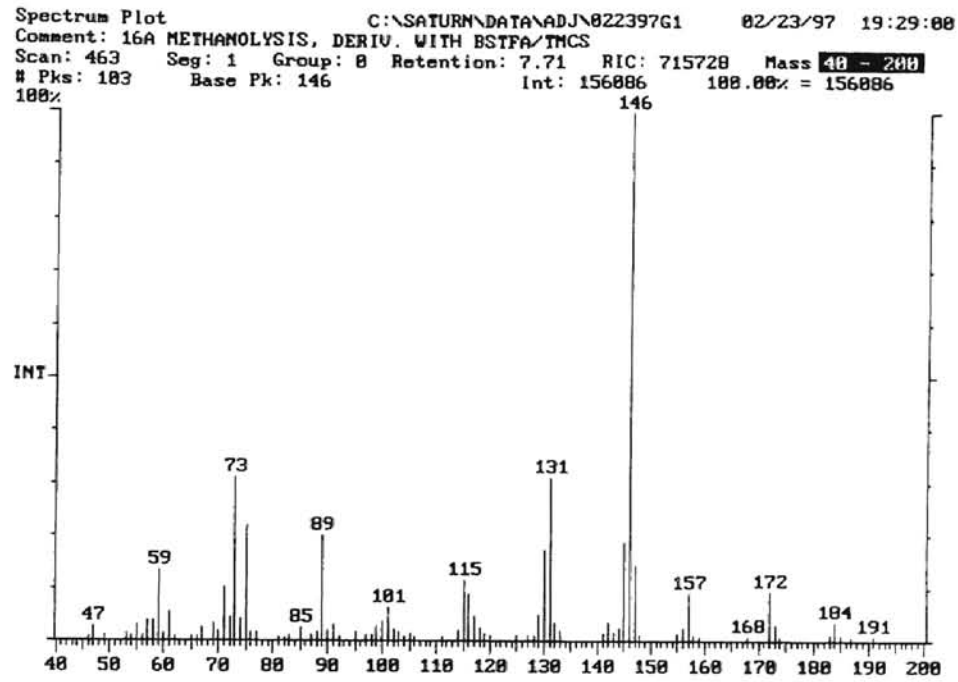

Spectrum Plot C:\SATURN\DATA\ADJ\022397G1 02/23/97 19:29:00
Comment: 16A METHANOLYSIS, DERIV. WITH BSTFA/TMCS
Scan: 615 Seg: 1 Group: 0 Retention: 10.25 RIC: 160668 Mass 40 - 300
Pks: 176 Base Pk: 205 Int: 17575 100.00% = 17575
100%
205
CINNAMIC ACID, TMS ESTER
INT
131
161
103
75
147
220
51
86
115
171
192
244
262
274
40
60
80
100
120
140
160
180
200
220
240
260
280
300

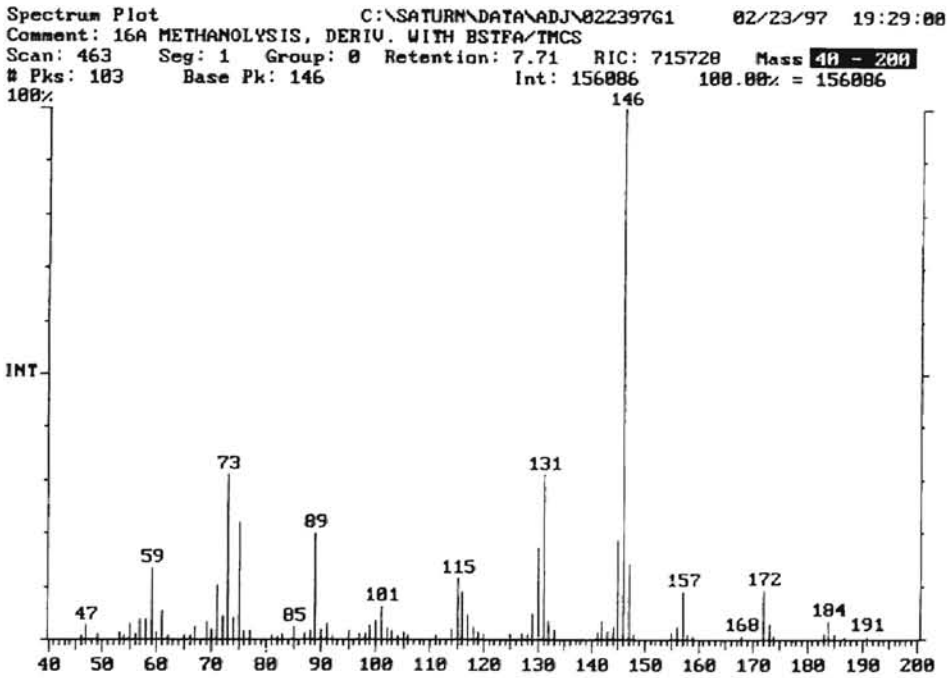

Library Search C:\...\ADJ\022397G1 Acquired: 23 Feb 1997 Scan number 615
Comment: 16A METHANOLYSIS, DERIV. WITH BSTFA/TMCS
100%
100% 2-Propenoic acid, 3-phenyl-, trimethylsilyl ester CAS 2078-20-8
INT
100% 2-Propenoic acid, 3-phenyl-, trimethylsilyl ester CAS 2078-20-8
100% Propane, 2-methyl-1,2-bis(trimethylsiloxy)- CAS 99875-85-5
60 80 100 120 140 160 180 200 220 240 260 280 300
Formula C12.H16.O2.Si Rank 1 Index 27573
MolWeight:220 Search:Acq LocalNorm:On P:602 F:348 R:612 CAS# 2078-20-8

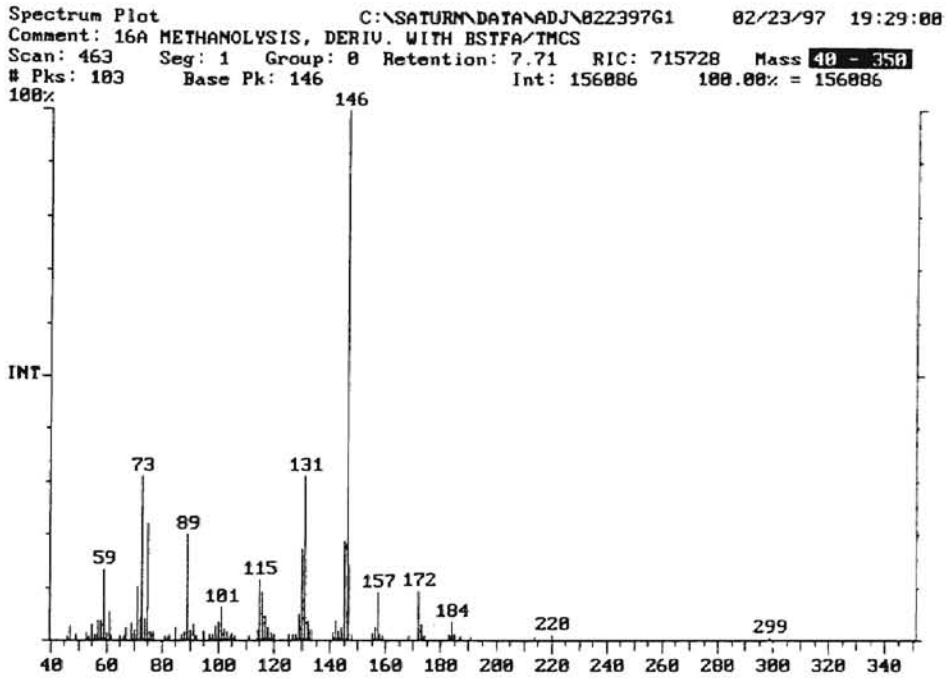

Spectrum Plot C:\SATURN\DATA\ADJ\022397G1 02/23/97 19:29:08
Comment: 16A METHANOLYSIS, DERIV. WITH BSTFA/TMCS
Scan: 444 Seg: 1 Group: 0 Retention: 7.39 RIC: 2515815 Mass 40 - 400
Pks: 122 Base Pk: 146 Int: 621856 100.00% = 621856
100%
146
INT
131
73
59
89
115
172
211
283
299
387
40 60 80 100 120 140 160 180 200 220 240 260 280 300 320 340 360 380 400

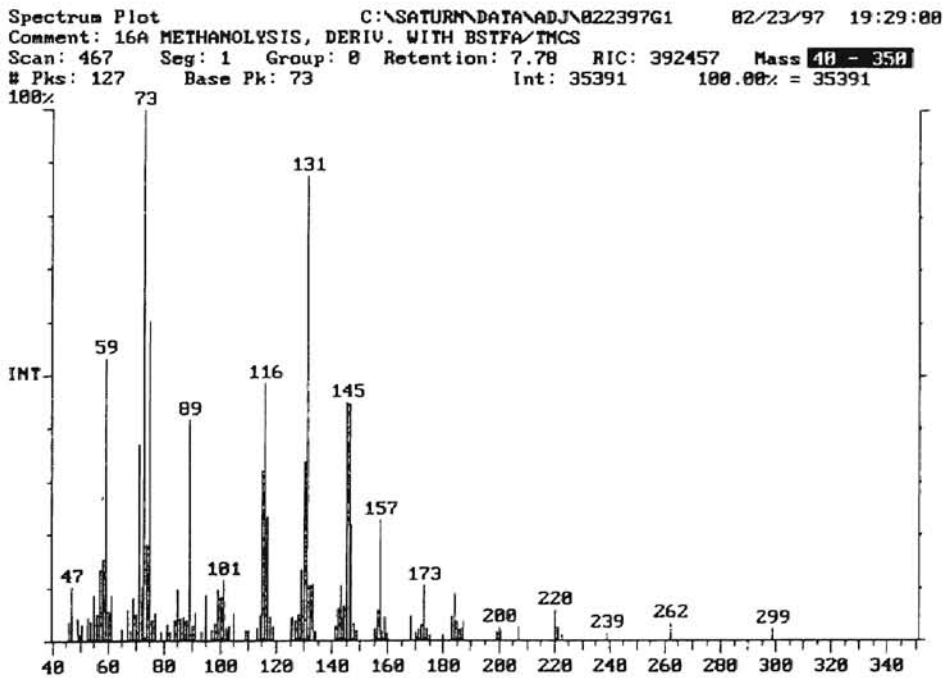

Background Subtract C:\SATURN\DATA\ADJ\022397G1 02/23/97 19:29:00
Comment: 16A METHANOLYSIS, DERIV. WITH BSTFA/TMCS
Average of: 442 to 446 Minus: 451 to 455 100% = 298488
100%
SMP
-
BKG
146
73
131
59
89
116
157
184
101
211
231
283
299
313
387
60 80 100 120 140 160 180 200 220 240 260 280 300 320 340 360 380 400

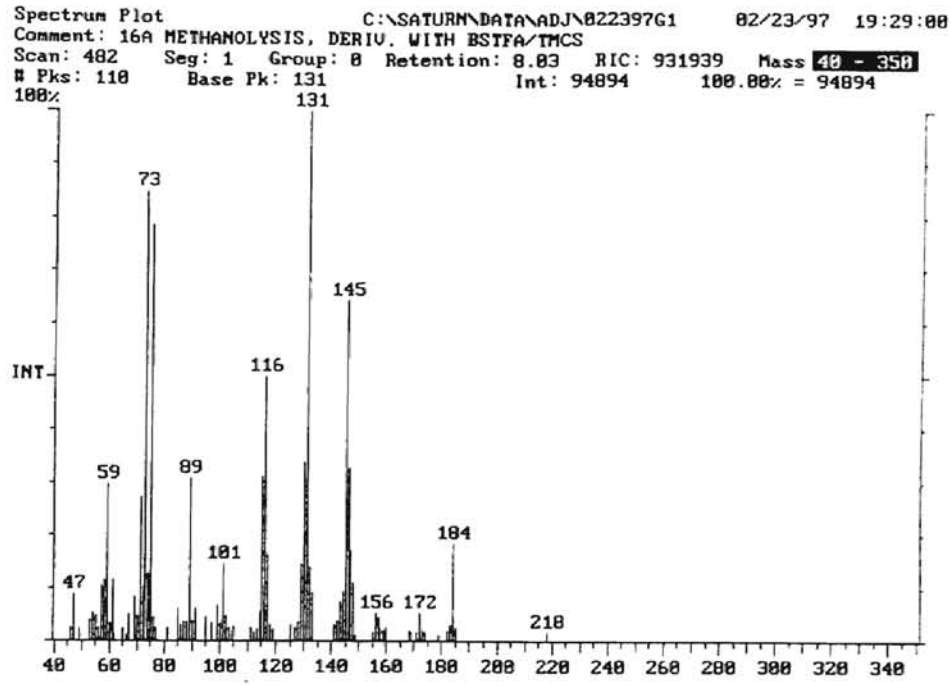

Spectrum Plot C:\SATURN\DATA\ADJ\022397G1 02/23/97 19:29:00
Comment: 16A METHANOLYSIS, DERIV. WITH BSTFA/TMCS
Scan: 458 Seg: 1 Group: 0 Retention: 7.63 RIC: 885808 Mass 40 - 350
Pks: 115 Base Pk: 116 Int: 162234 100.00% = 162234
100%
INT
116
73
100
59
131
89
146
47
157
172
187
204
244
299
40 60 80 100 120 140 160 180 200 220 240 260 280 300 320 340

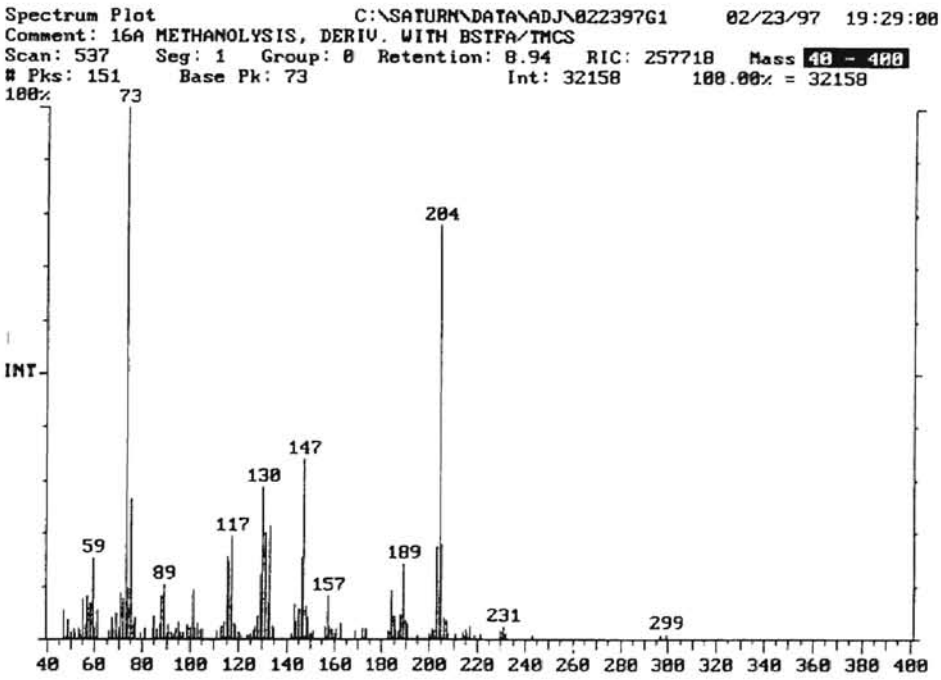

Spectrum Plot C:\SATURN\DATA\ADJ\022397G1 02/23/97 19:29:00
Comment: 16A METHANOLYSIS, DERIV. WITH BSTFA/TMCS
Scan: 463 Seg: 1 Group: 0 Retention: 7.71 RIC: 715728 Mass 40 - 200
Pks: 103 Base Pk: 146 Int: 156086 100.00% = 156086
100%
INT
146
73
131
89
59
115
157
172
101
47
85
168
184
191
40 50 60 70 80 90 100 110 120 130 140 150 160 170 180 190 200

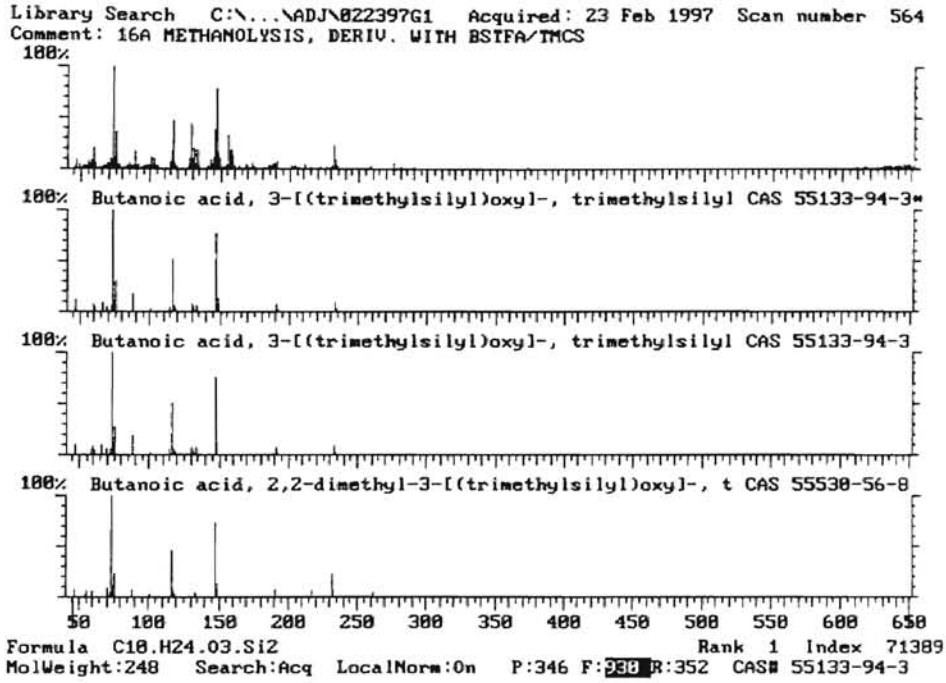

Spectrum Plot C:\SATURN\DATA\ADJ\022397G1 02/23/97 19:29:00
Comment: 16A METHANOLYSIS, DERIV. WITH BSTFA/TMCS
Scan: 463 Seg: 1 Group: 0 Retention: 7.71 RIC: 715728 Mass 40 - 200
Pks: 103 Base Pk: 146 Int: 156086 100.00% = 156086
100%
INT
146
73
131
89
59
115
157
172
101
47
85
168
184
191
40 50 60 70 80 90 100 110 120 130 140 150 160 170 180 190 200

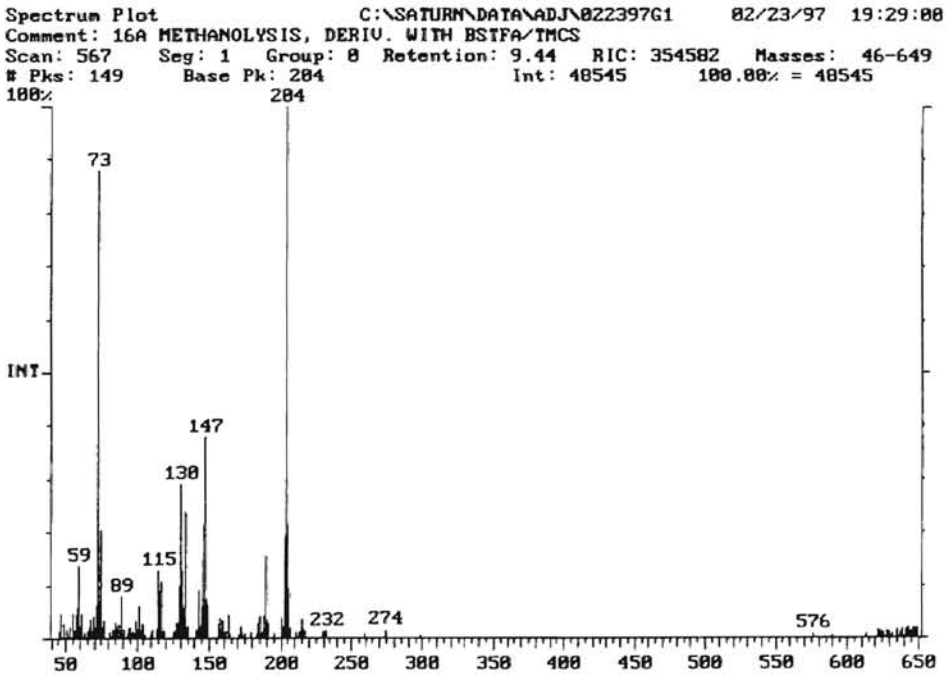

Spectrum Plot C:\SATURN\DATA\ADJ\822397G1 02/23/97 19:29:08
Comment: 16A METHANOLYSIS, DERIV. WITH BSTFA/TMCS
Scan: 463 Seg: 1 Group: 0 Retention: 7.71 RIC: 715728 Mass 40 - 350
Pks: 103 Base Pk: 146 Int: 156086 100.00% = 156086
100%
INT
146
73
131
89
59
115
157
172
184
101
220
299
40 60 80 100 120 140 160 180 200 220 240 260 280 300 320 340

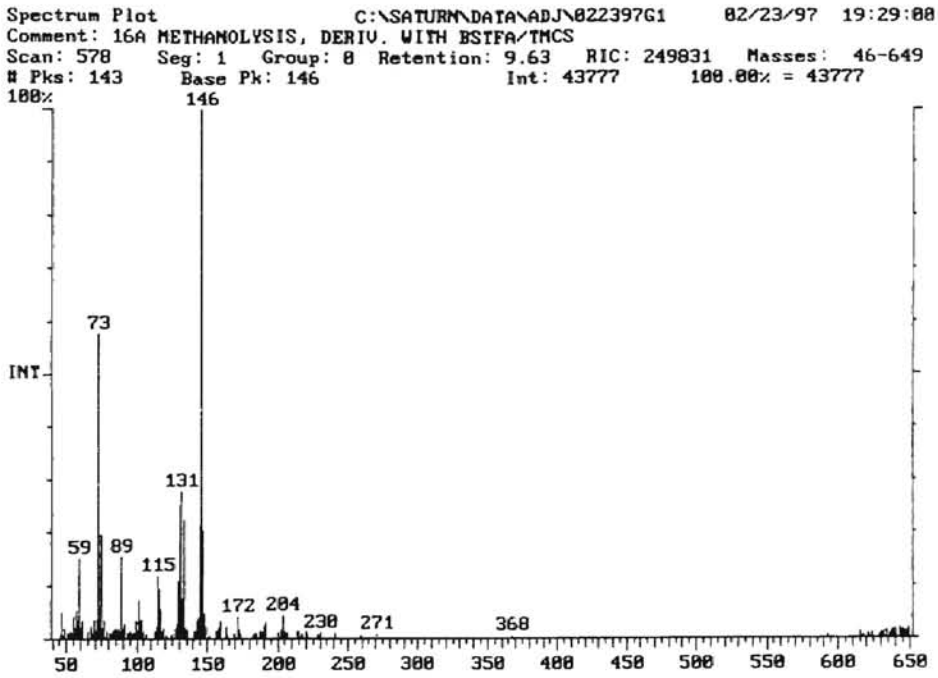

Spectrum Plot C:\SATURN\DATA\ADJ\822397G1 02/23/97 19:29:08
Comment: 16A METHANOLYSIS, DERIV. WITH BSTFA/TMCS
Scan: 467 Seg: 1 Group: 0 Retention: 7.78 RIC: 392457 Mass 40 - 350
Pks: 127 Base Pk: 73 Int: 35391 100.00% = 35391
100%
INT
73
131
59
116
145
89
157
47
101
173
200
220
239
262
299
40 60 80 100 120 140 160 180 200 220 240 260 280 300 320 340

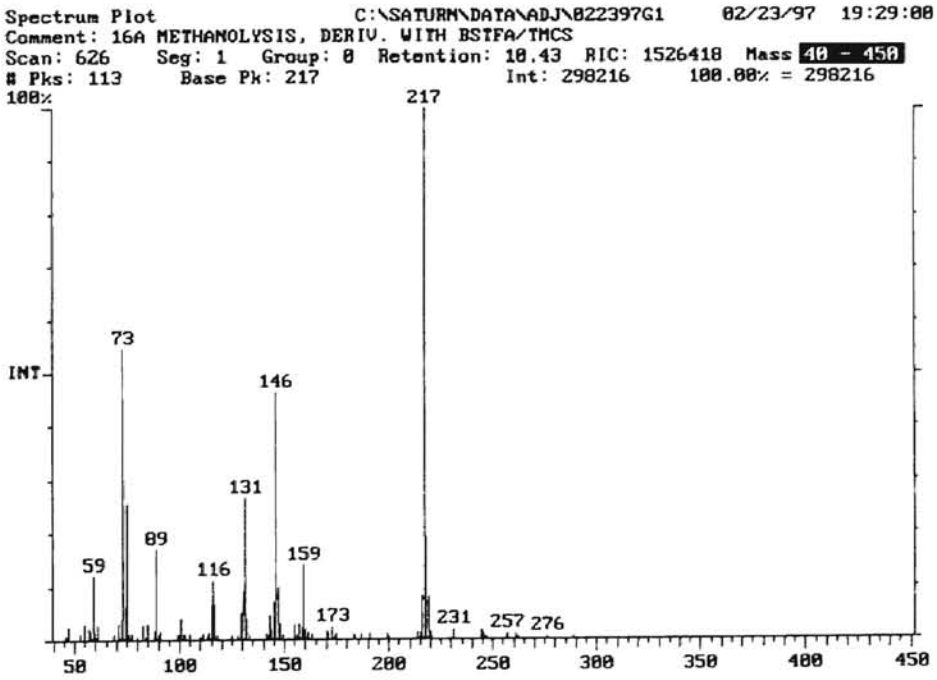

Spectrum Plot C:\SATURN\DATA\ADJ\022397G1 02/23/97 19:29:00
Comment: 16A METHANOLYSIS, DERIV. WITH BSTFA/TMCS
Scan: 482 Seg: 1 Group: 0 Retention: 8.03 RIC: 931939 Mass 40 - 350
Pks: 110 Base Pk: 131 Int: 94894 100.00% = 94894
100%
INT
131
73
145
116
59
89
184
101
47
156 172
218
40 60 80 100 120 140 160 180 200 220 240 260 280 300 320 340

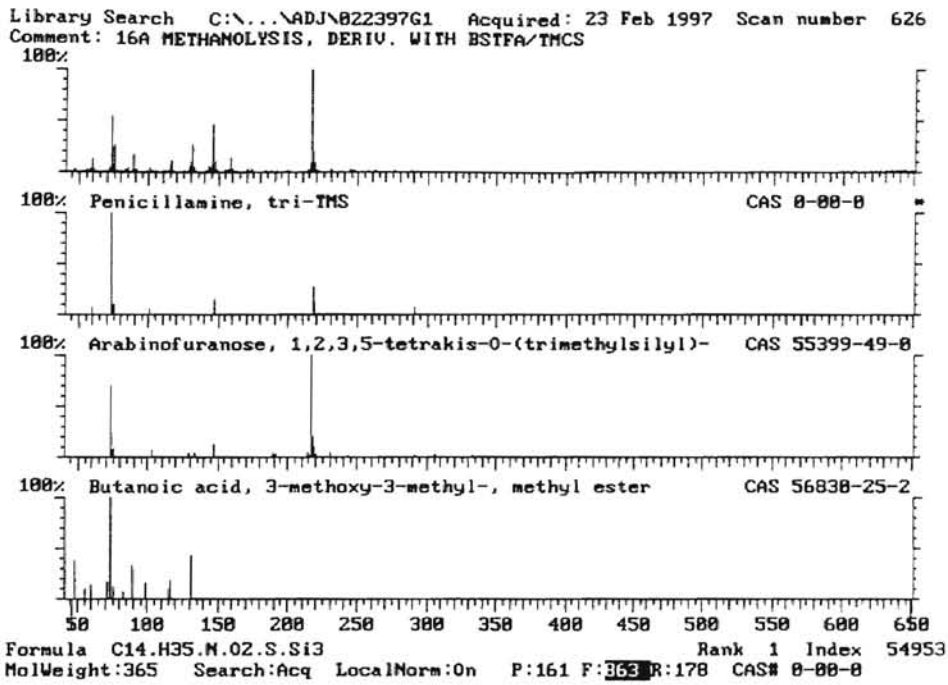

Spectrum Plot C:\SATURN\DATA\ADJ\022397G1 02/23/97 19:29:00
Comment: 16A METHANOLYSIS, DERIV. WITH BSTFA/TMCS
Scan: 537 Seg: 1 Group: 0 Retention: 8.94 RIC: 257718 Mass 40 - 400
Pks: 151 Base Pk: 73 Int: 32158 100.00% = 32158
100%
INT
73
204
147
130
117
59
189
89
157
231
299
40 60 80 100 120 140 160 180 200 220 240 260 280 300 320 340 360 380 400

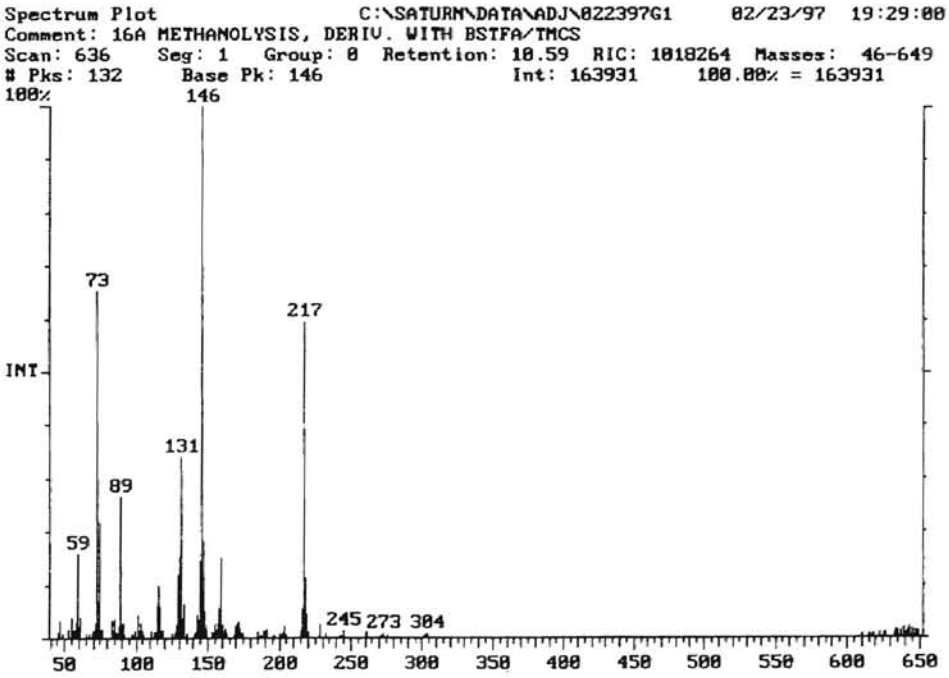

Library Search C:\...\ADJ\022397G1 Acquired: 23 Feb 1997 Scan number 564
Comment: 16A METHANOLYSIS, DERIV. WITH BSTFA/TMCS
100%
100% Butanoic acid, 3-[(trimethylsilyl)oxy]-, trimethylsilyl CAS 55133-94-3
100% Butanoic acid, 3-[(trimethylsilyl)oxy]-, trimethylsilyl CAS 55133-94-3
100% Butanoic acid, 2,2-dimethyl-3-[(trimethylsilyl)oxy]-, t CAS 55530-56-8
50 100 150 200 250 300 350 400 450 500 550 600 650
Formula C18.H24.03.Si2 Rank 1 Index 71389
MolWeight:248 Search:Acq LocalNorm:On P:346 F:330 R:352 CAS# 55133-94-3

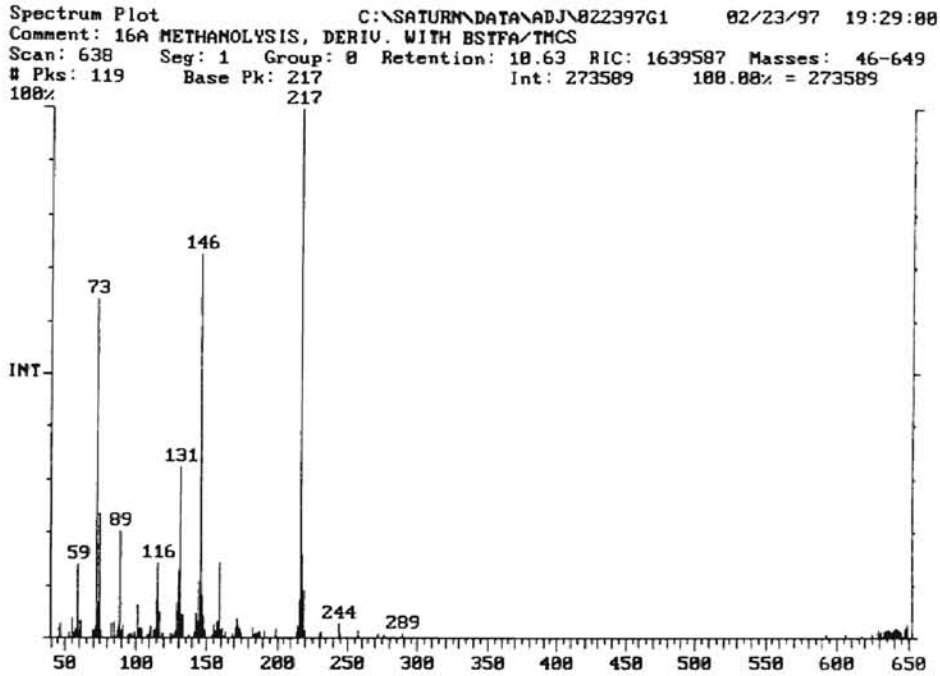

Spectrum Plot C:\SATURN\DATA\ADJ\022397G1 02/23/97 19:29:00
Comment: 16A METHANOLYSIS, DERIV. WITH BSTFA/TMCS
Scan: 567 Seg: 1 Group: 0 Retention: 9.44 RIC: 354582 Masses: 46-649
Pks: 149 Base Pk: 204 Int: 40545 100.00% = 40545
100%
204
73
INT
147
130
59
115
89
232
274
576
50 100 150 200 250 300 350 400 450 500 550 600 650

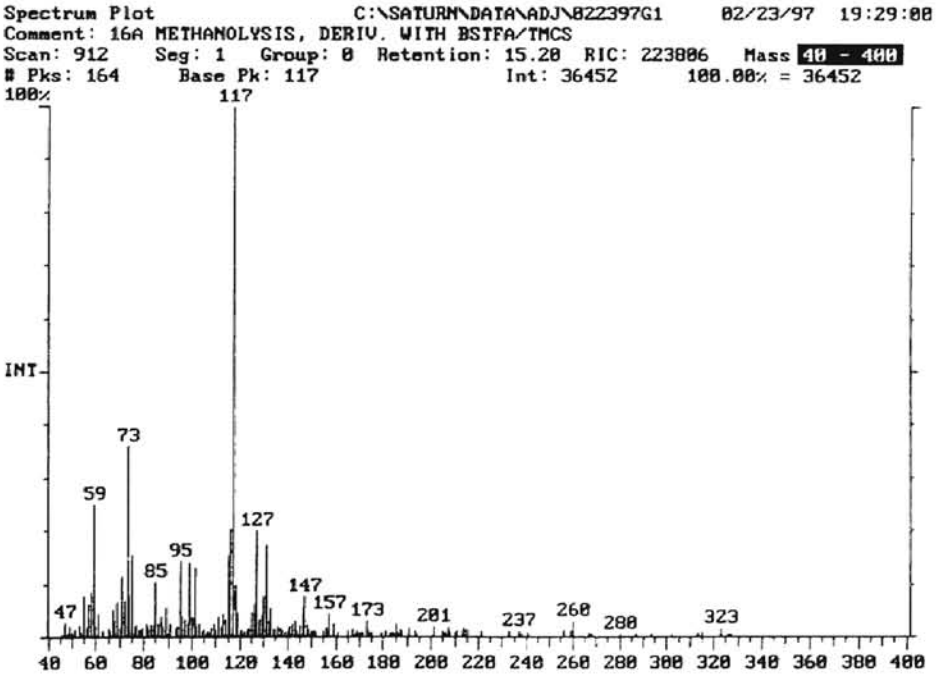

Spectrum Plot C:\SATURN\DATA\ADJ\022397G1 02/23/97 19:29:00
Comment: 16A METHANOLYSIS, DERIV. WITH BSTFA/TMCS
Scan: 578 Seg: 1 Group: 0 Retention: 9.63 RIC: 249831 Masses: 46-649
Pks: 143 Base Pk: 146 Int: 43777 100.00% = 43777
100%
INT
146
73
131
59
89
115
172
204
230
271
368
50 100 150 200 250 300 350 400 450 500 550 600 650

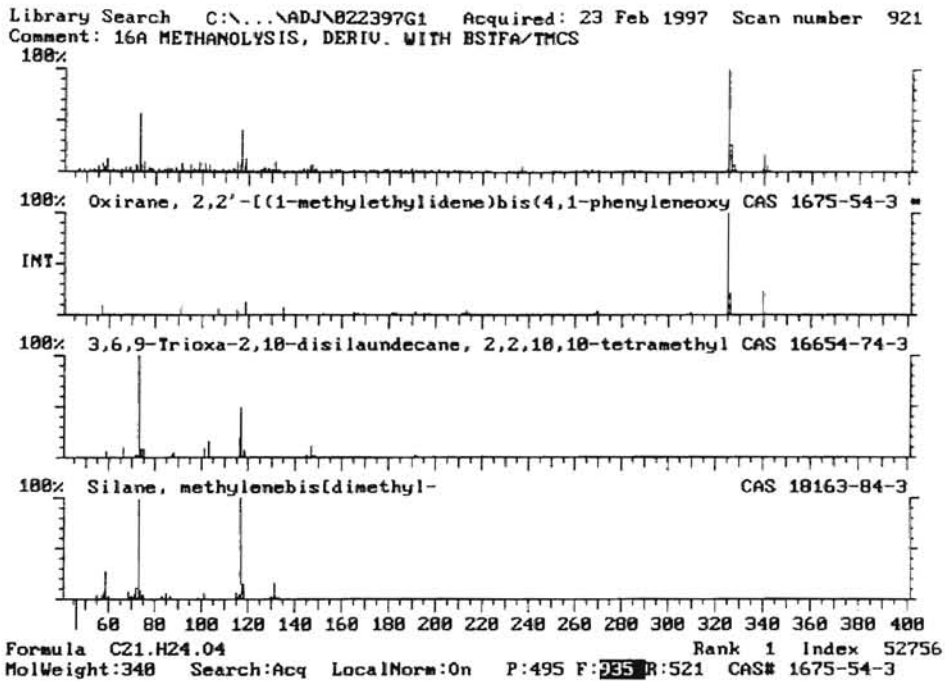

Spectrum Plot C:\SATURN\DATA\ADJ\022397G1 02/23/97 19:29:00
Comment: 16A METHANOLYSIS, DERIV. WITH BSTFA/TMCS
Scan: 626 Seg: 1 Group: 0 Retention: 10.43 RIC: 1526418 Mass 40 - 450
Pks: 113 Base Pk: 217 Int: 298216 100.00% = 298216
100%
INT
217
73
146
131
89
59
116
159
173
231
257
276
50 100 150 200 250 300 350 400 450

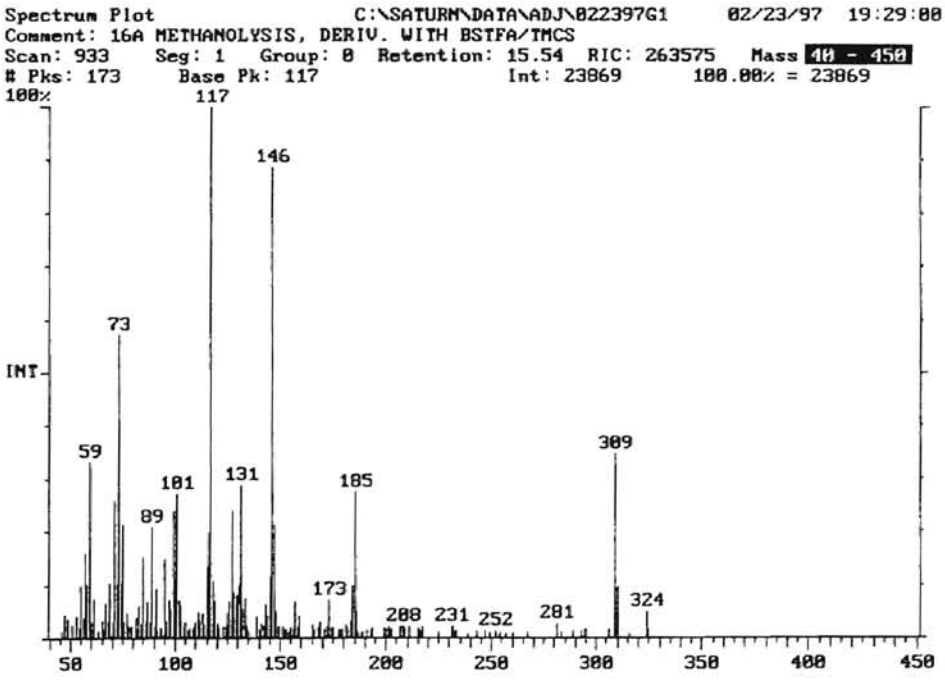

Library Search C:\...\ADJ\822397G1 Acquired: 23 Feb 1997 Scan number 626
Comment: 16A METHANOLYSIS, DERIV. WITH BSTFA/TMCS
100%
100% Penicillamine, tri-TMS CAS 0-00-0
100% Arabinofuranose, 1,2,3,5-tetrakis-O-(trimethylsilyl)- CAS 55399-49-0
100% Butanoic acid, 3-methoxy-3-methyl-, methyl ester CAS 56838-25-2
50 100 150 200 250 300 350 400 450 500 550 600 650
Formula C14.H35.N.O2.S.Si3 Rank 1 Index 54953
MolWeight:365 Search:Acq LocalNorm:On P:161 F:363 R:178 CAS# 0-00-0

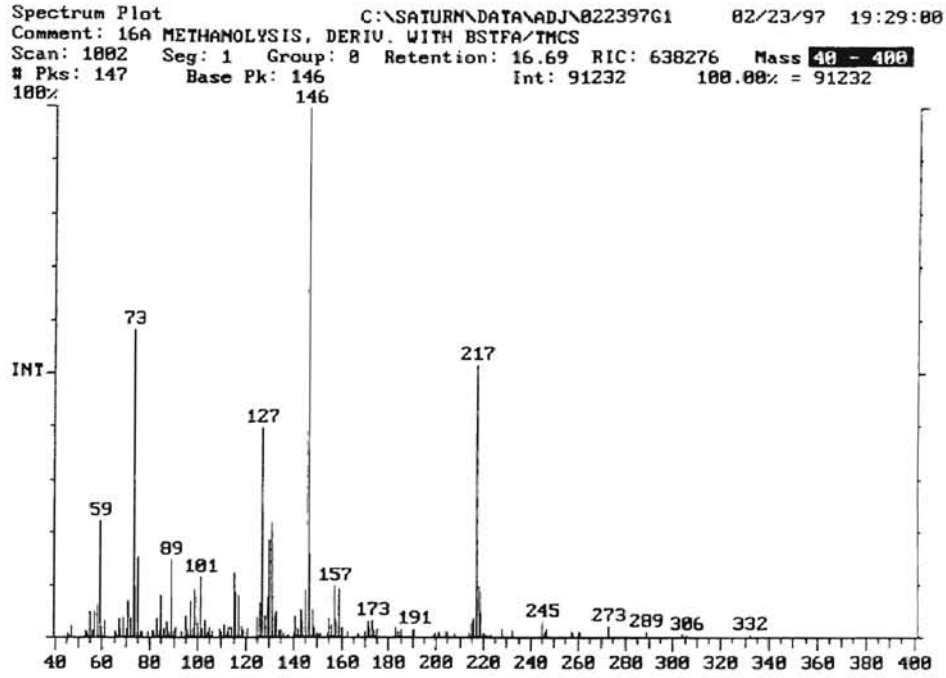

Spectrum Plot C:\SATURN\DATA\ADJ\822397G1 02/23/97 19:29:00
Comment: 16A METHANOLYSIS, DERIV. WITH BSTFA/TMCS
Scan: 636 Seg: 1 Group: 0 Retention: 10.59 RIC: 1018264 Masses: 46-649
Pks: 132 Base Pk: 146 Int: 163931 100.00% = 163931
100%
146
73
217
INT
131
89
59
245 273 304
50 100 150 200 250 300 350 400 450 500 550 600 650

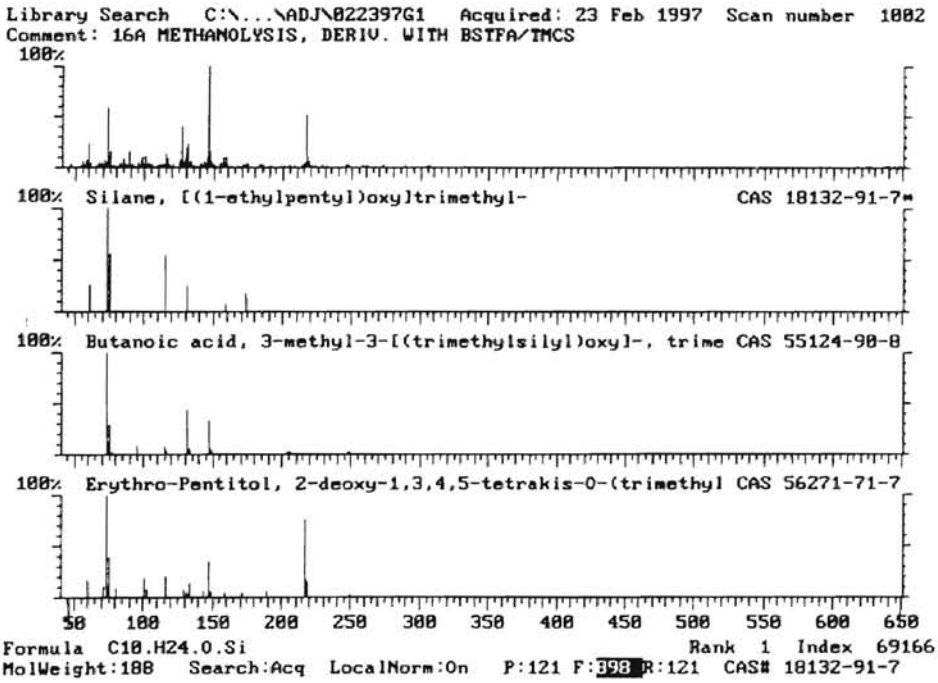

Spectrum Plot C:\SATURN\DATA\ADJ\822397G1 02/23/97 19:29:00
Comment: 16A METHANOLYSIS, DERIV. WITH BSTFA/TMCS
Scan: 638 Seg: 1 Group: 0 Retention: 10.63 RIC: 1639587 Masses: 46-649
Pks: 119 Base Pk: 217 Int: 273589 100.00% = 273589
100%
INT
217
146
73
131
89
59
116
244
289
50 100 150 200 250 300 350 400 450 500 550 600 650

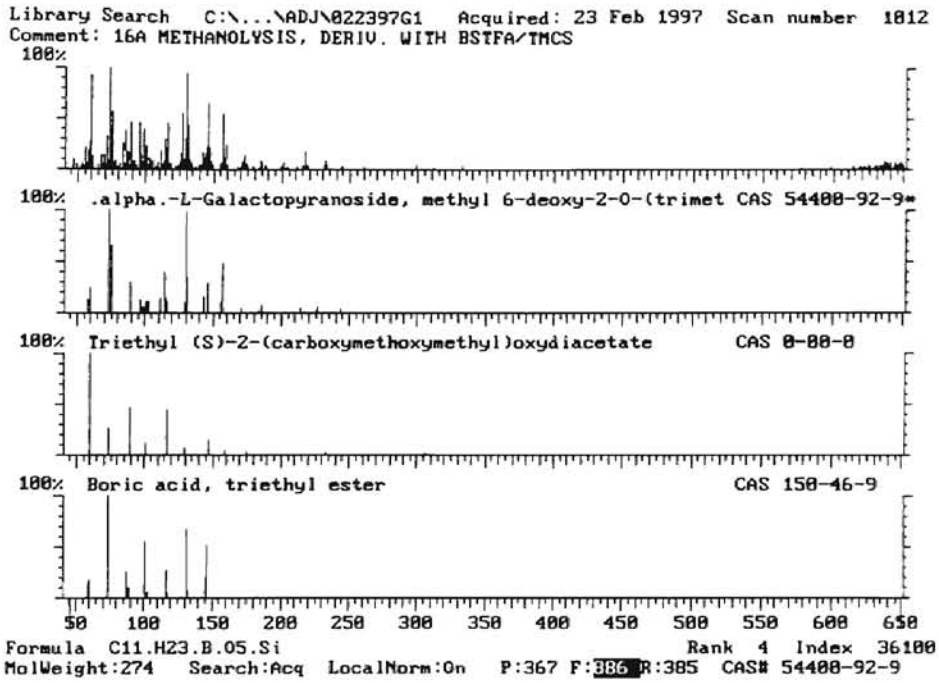

Spectrum Plot C:\SATURN\DATA\ADJ\822397G1 02/23/97 19:29:00
Comment: 16A METHANOLYSIS, DERIV. WITH BSTFA/TMCS
Scan: 912 Seg: 1 Group: 0 Retention: 15.20 RIC: 223806 Mass 40 - 400
Pks: 164 Base Pk: 117 Int: 36452 100.00% = 36452
100%
INT
117
73
59
127
95
85
147
157
47
173
201
237
260
280
323
40 60 80 100 120 140 160 180 200 220 240 260 280 300 320 340 360 380 400

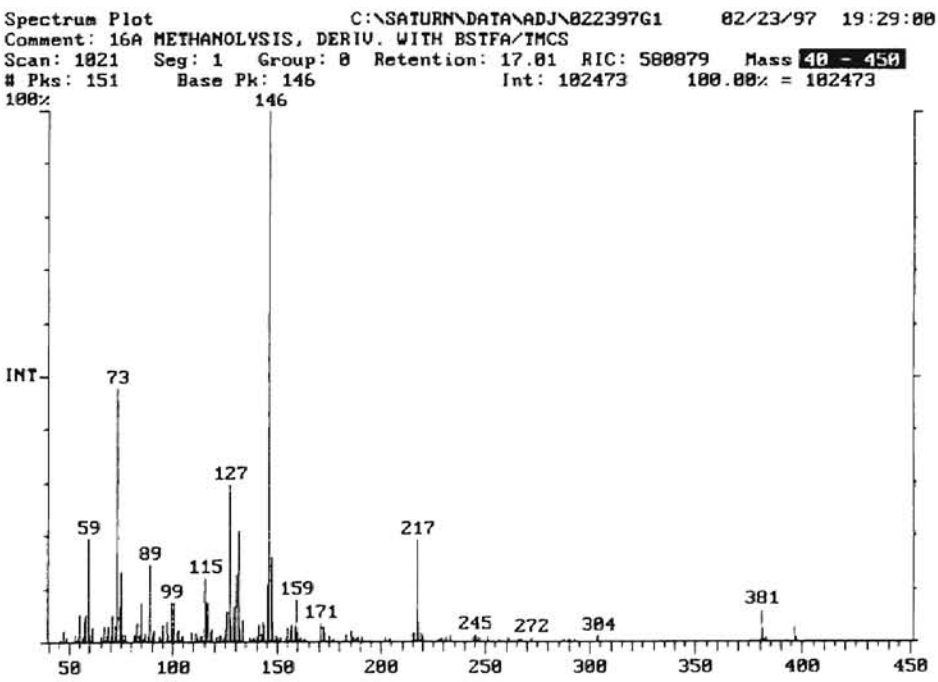

Library Search C:\...\ADJ\022397G1 Acquired: 23 Feb 1997 Scan number 921
Comment: 16A METHANOLYSIS, DERIV. WITH BSTFA/TMCS
100%
100% Oxirane, 2,2'-[(1-methylethylidene)bis(4,1-phenyleneoxy CAS 1675-54-3
INT
100% 3,6,9-Trioxa-2,10-disilaundecane, 2,2,10,10-tetramethyl CAS 16654-74-3
100% Silane, methylenebis[dimethyl- CAS 18163-84-3
60 80 100 120 140 160 180 200 220 240 260 280 300 320 340 360 380 400
Formula C21.H24.04 Rank 1 Index 52756
MolWeight:340 Search:Acq LocalNorm:On P:495 F:335 R:521 CAS# 1675-54-3

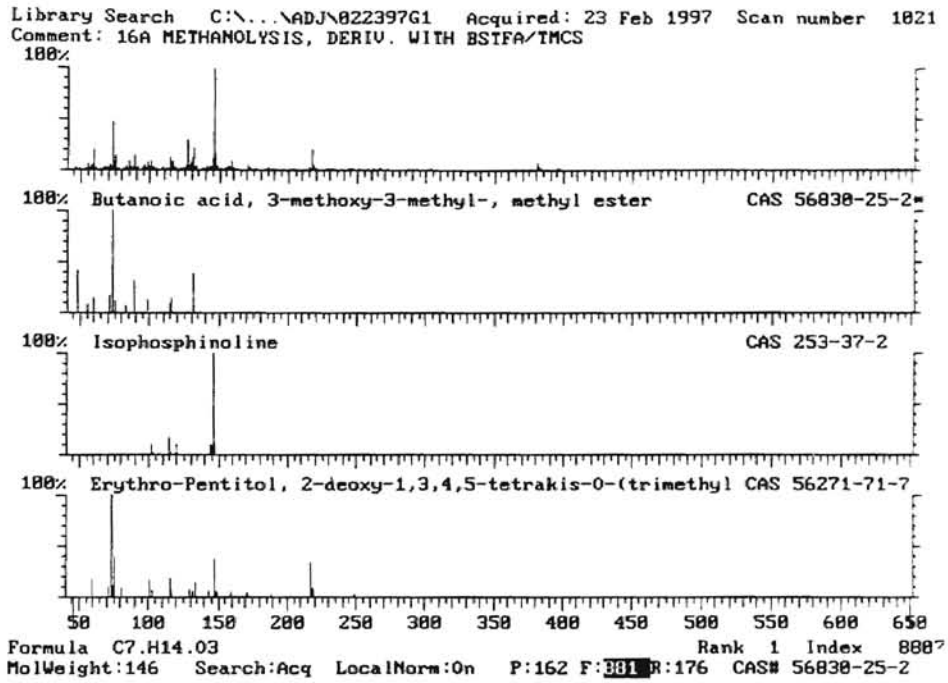

Spectrum Plot C:\SATURN\DATA\ADJ\022397G1 02/23/97 19:29:00
Comment: 16A METHANOLYSIS, DERIV. WITH BSTFA/TMCS
Scan: 933 Seg: 1 Group: 0 Retention: 15.54 RIC: 263575 Mass 40 - 450
Pks: 173 Base Pk: 117 Int: 23869 100.00% = 23869
100%
INT
117
146
73
59
101
131
89
185
309
173
324
208 231 252 281
50 100 150 200 250 300 350 400 450

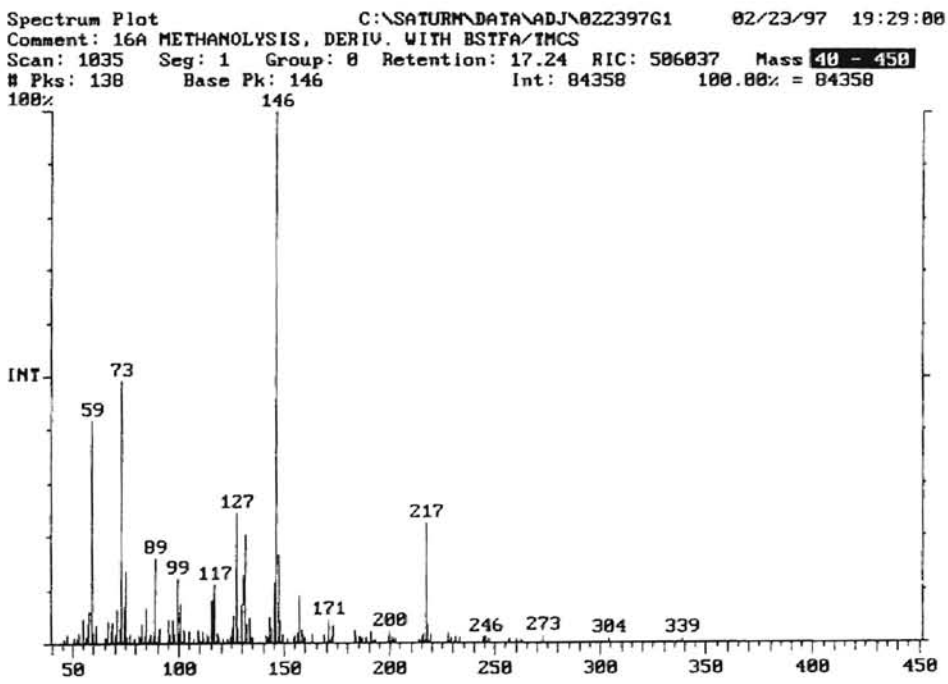

Spectrum Plot C:\SATURN\DATA\ADJ\022397G1 02/23/97 19:29:00
Comment: 16A METHANOLYSIS, DERIV. WITH BSTFA/TMCS
Scan: 1002 Seg: 1 Group: 0 Retention: 16.69 RIC: 638276 Mass 40 - 400
Pks: 147 Base Pk: 146 Int: 91232 100.00% = 91232
100%
INT
146
73
217
127
59
89
101
157
173
191
245
273
289
306
332
40 60 80 100 120 140 160 180 200 220 240 260 280 300 320 340 360 380 400

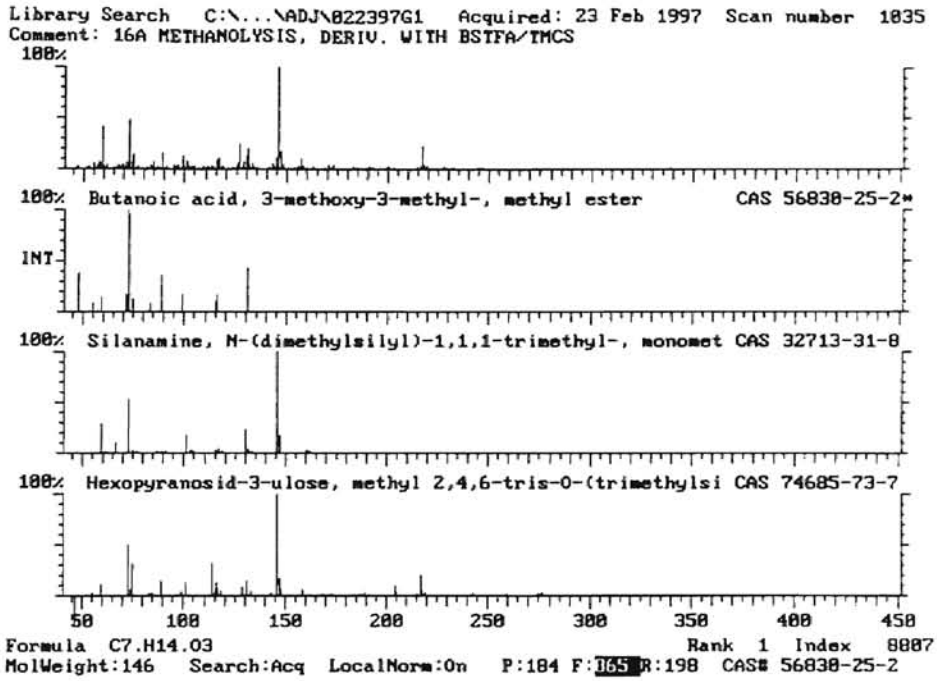

Library Search C:\...\ADJ\022397G1 Acquired: 23 Feb 1997 Scan number 1002
Comment: 16A METHANOLYSIS, DERIV. WITH BSTFA/TMCS
100%
100% Silane, [(1-ethylpentyl)oxy]trimethyl- CAS 18132-91-7
100% Butanoic acid, 3-methyl-3-[(trimethylsilyl)oxy]-, trime CAS 55124-90-8
100% Erythro-Pentitol, 2-deoxy-1,3,4,5-tetrakis-O-(trimethyl CAS 56271-71-7
50 100 150 200 250 300 350 400 450 500 550 600 650
Formula C10.H24.O.Si Rank 1 Index 69166
MolWeight:188 Search:Acq LocalNorm:On P:121 F:398 R:121 CAS# 18132-91-7

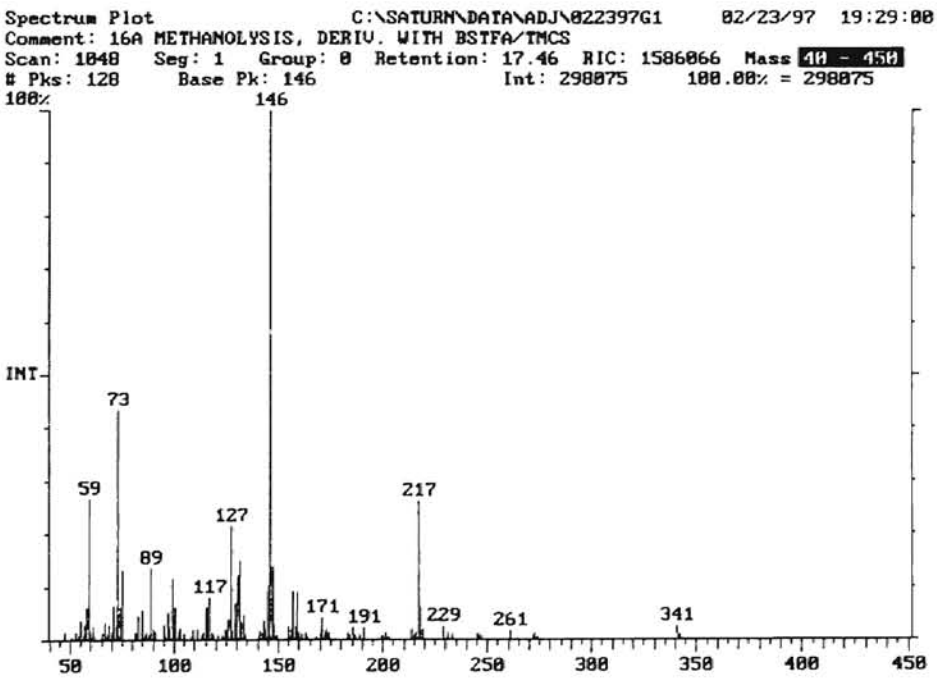

Library Search C:\...\ADJ\022397G1 Acquired: 23 Feb 1997 Scan number 1812
Comment: 16A METHANOLYSIS, DERIV. WITH BSTFA/TMCS
100%
100% .alpha.-L-Galactopyranoside, methyl 6-deoxy-2-O-(trimet CAS 54400-92-9
100% Triethyl (S)-2-(carboxymethoxymethyl)oxydiacetate CAS 0-00-0
100% Boric acid, triethyl ester CAS 150-46-9
50 100 150 200 250 300 350 400 450 500 550 600 650
Formula C11.H23.B.O5.Si Rank 4 Index 36100
MolWeight:274 Search:Acq LocalNorm:On P:367 F:386 R:385 CAS# 54400-92-9

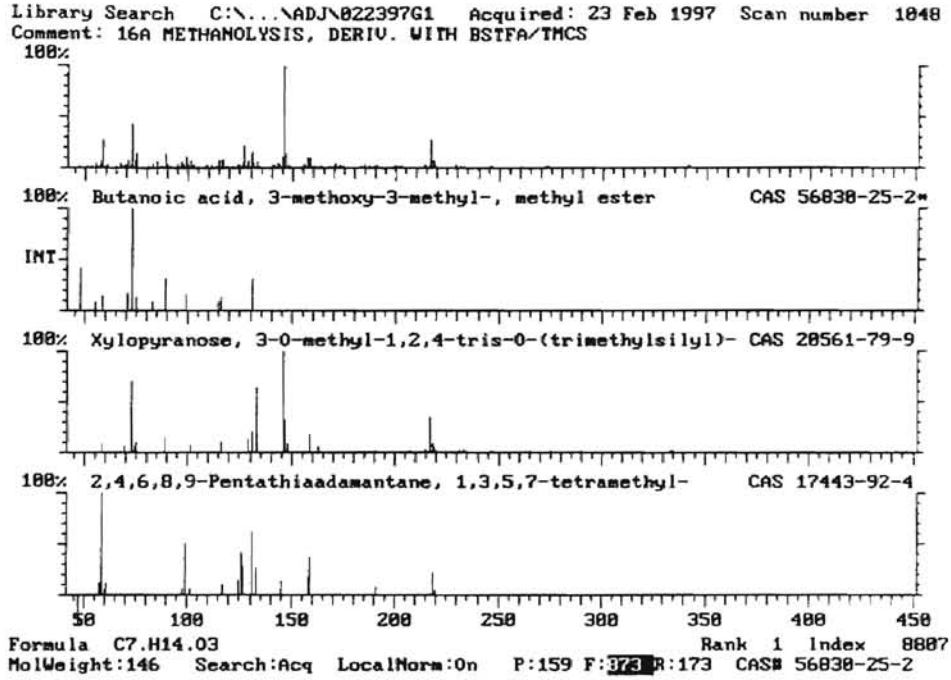

Spectrum Plot C:\SATURN\DATA\ADJ\022397G1 02/23/97 19:29:00
Comment: 16A METHANOLYSIS, DERIV. WITH BSTFA/TMCS
Scan: 1021 Seg: 1 Group: 0 Retention: 17.01 RIC: 580879 Mass 40 - 450
Pks: 151 Base Pk: 146 Int: 102473 100.00% = 102473
100%
INT
146
73
127
59
89
115
99
159
171
217
245
272
304
381
50 100 150 200 250 300 350 400 450

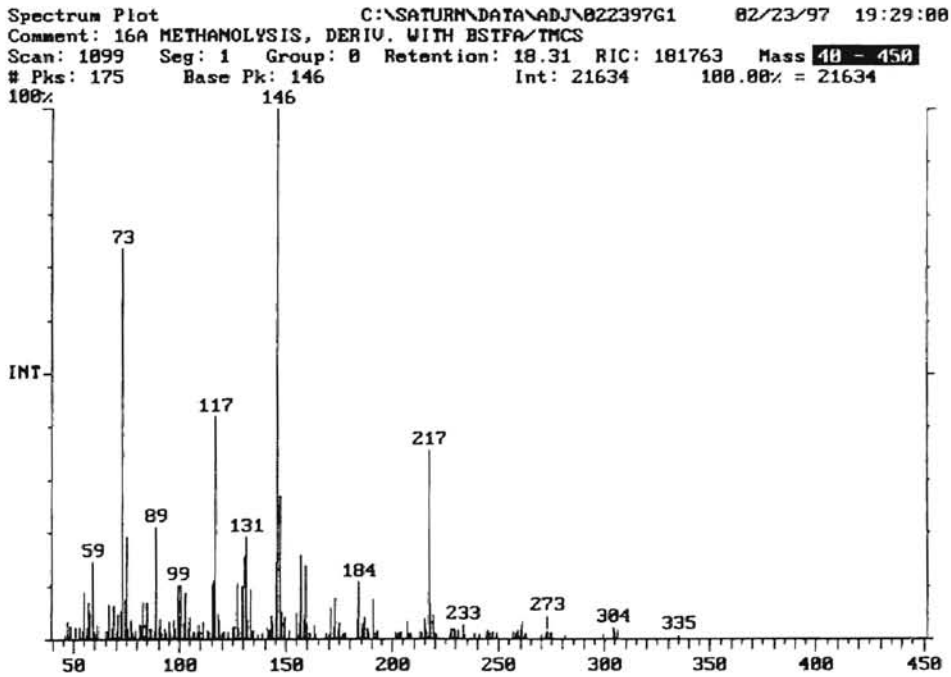

Library Search C:\...\ADJ\022397G1 Acquired: 23 Feb 1997 Scan number 1021
Comment: 16A METHANOLYSIS, DERIV. WITH BSTFA/TMCS
100%
100% Butanoic acid, 3-methoxy-3-methyl-, methyl ester CAS 56830-25-2
100% Isophosphinoline CAS 253-37-2
100% Erythro-Pentitol, 2-deoxy-1,3,4,5-tetrakis-O-(trimethyl CAS 56271-71-7
50 100 150 200 250 300 350 400 450 500 550 600 650
Formula C7.H14.O3 Rank 1 Index 880
MolWeight:146 Search:Acq LocalNorm:On P:162 F:381 R:176 CAS# 56830-25-2

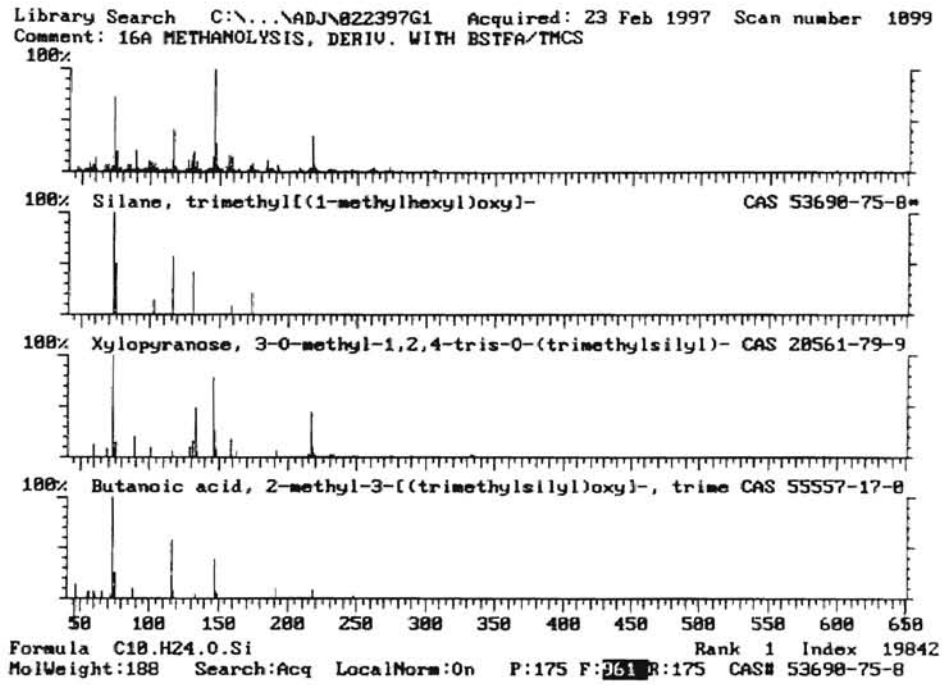

Spectrum Plot C:\SATURN\DATA\ADJ\022397G1 02/23/97 19:29:00
Comment: 16A METHANOLYSIS, DERIV. WITH BSTFA/TMCS
Scan: 1035 Seg: 1 Group: 0 Retention: 17.24 RIC: 506037 Mass 40 - 450
Pks: 138 Base Pk: 146 Int: 84358 100.00% = 84358
100%
INT
146
73
59
127
217
89
99
117
171
200
246
273
304
339
50 100 150 200 250 300 350 400 450

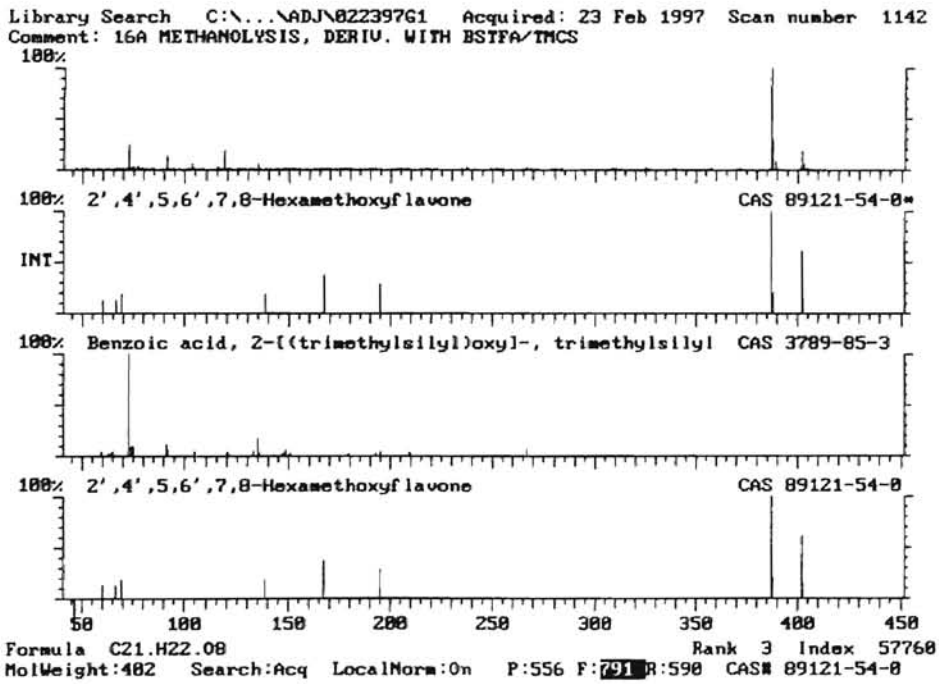

Library Search C:\...\ADJ\022397G1 Acquired: 23 Feb 1997 Scan number 1035
Comment: 16A METHANOLYSIS, DERIV. WITH BSTFA/TMCS
100%
100% Butanoic acid, 3-methoxy-3-methyl-, methyl ester CAS 56830-25-2
INT
100% Silanamine, N-(dimethylsilyl)-1,1,1-trimethyl-, monomet CAS 32713-31-8
100% Hexopyranosid-3-ulose, methyl 2,4,6-tris-O-(trimethylsi CAS 74685-73-7
50 100 150 200 250 300 350 400 450
Formula C7.H14.O3 Rank 1 Index 8887
MolWeight:146 Search:Acq LocalNorm:On P:184 F:165 R:198 CAS# 56830-25-2

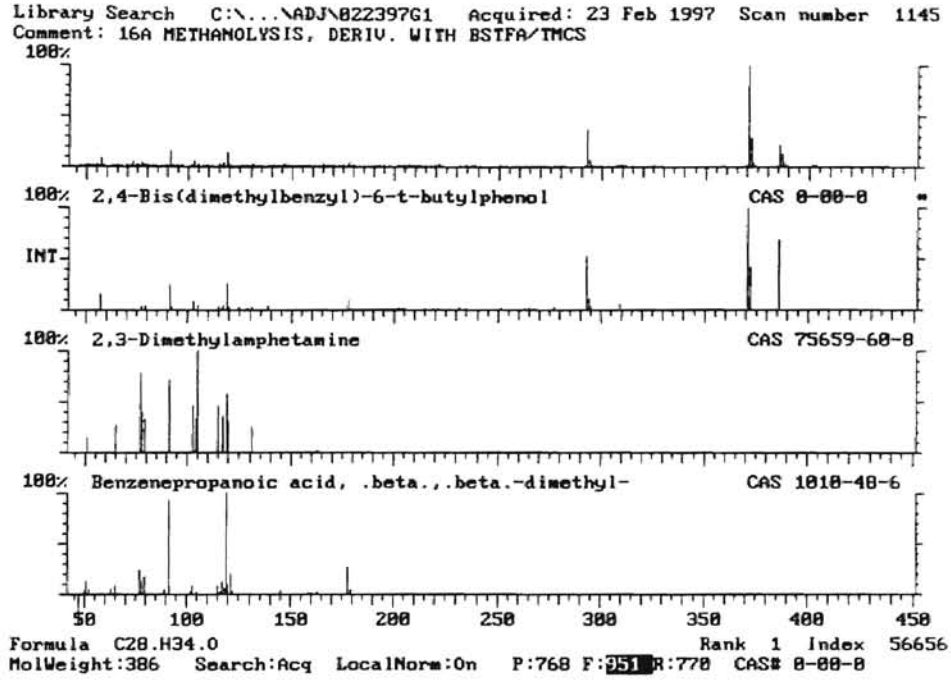

Spectrum Plot C:\SATURN\DATA\ADJ\022397G1 02/23/97 19:29:00
Comment: 16A METHANOLYSIS, DERIV. WITH BSTFA/TMCS
Scan: 1048 Seg: 1 Group: 0 Retention: 17.46 RIC: 1586066 Mass 40 - 450
Pks: 128 Base Pk: 146 Int: 298075 100.00% = 298075
100%
146
INT
73
59
217
127
89
117
171
191
229
261
341
50 100 150 200 250 300 350 400 450

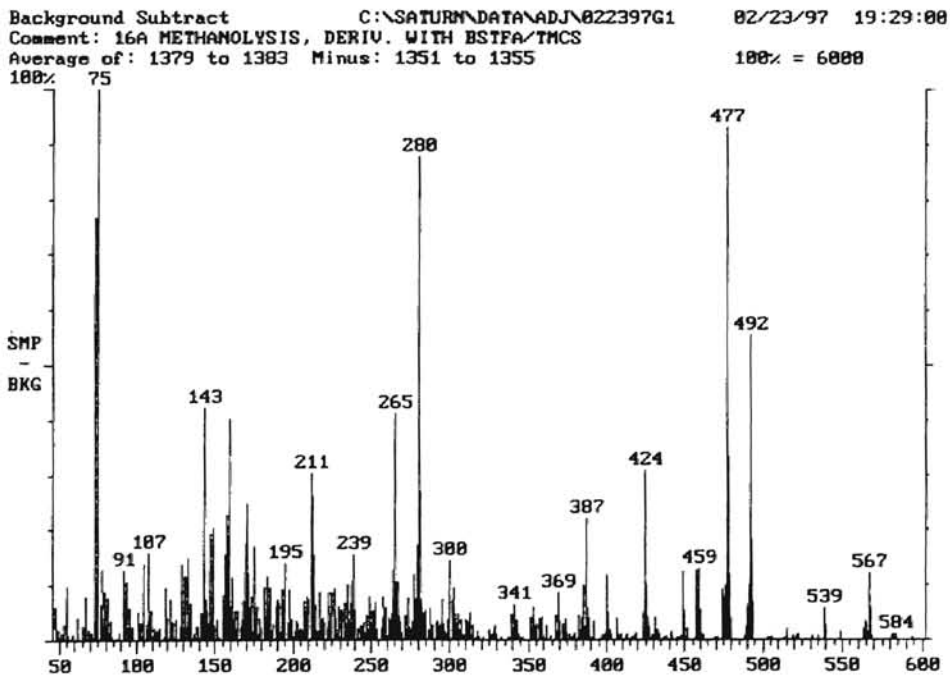

Library Search C:\...\ADJ\022397G1 Acquired: 23 Feb 1997 Scan number 1048
Comment: 16A METHANOLYSIS, DERIV. WITH BSTFA/TMCS
100%
100% Butanoic acid, 3-methoxy-3-methyl-, methyl ester CAS 56830-25-2
INT
100% Xylopyranose, 3-O-methyl-1,2,4-tris-O-(trimethylsilyl)- CAS 28561-79-9
100% 2,4,6,8,9-Pentathiaadamantane, 1,3,5,7-tetramethyl- CAS 17443-92-4
50 100 150 200 250 300 350 400 450
Formula C7.H14.03 Rank 1 Index 8887
MolWeight:146 Search:Acq LocalNorm:On P:159 F:373 R:173 CAS# 56830-25-2

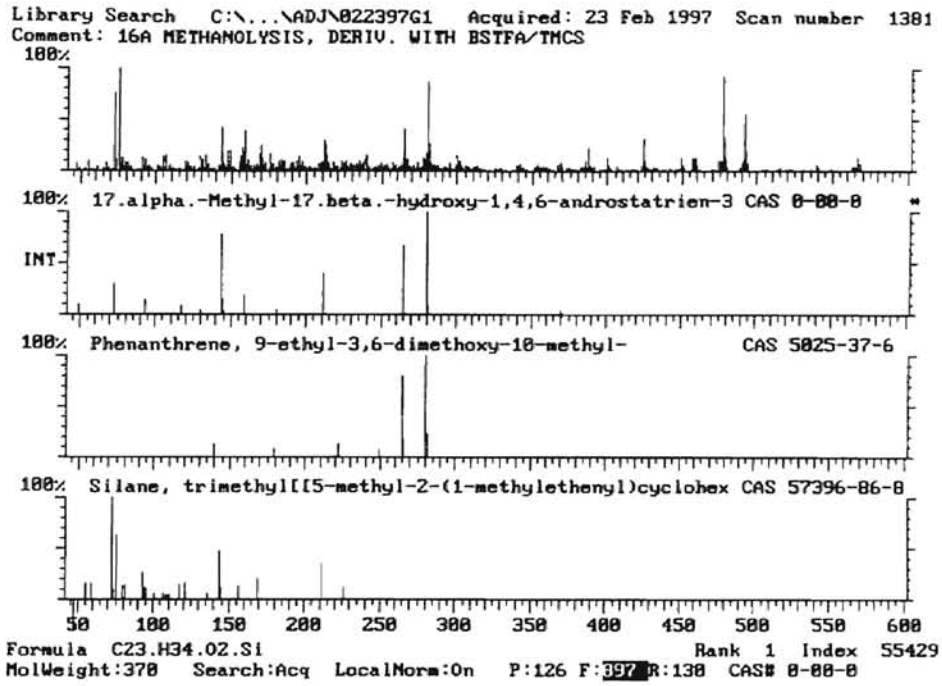

Spectrum Plot C:\SATURN\DATA\ADJ\022397G1 02/23/97 19:29:00
Comment: 16A METHANOLYSIS, DERIV. WITH BSTFA/TMCS
Scan: 1099 Seg: 1 Group: 0 Retention: 18.31 RIC: 181763 Mass 40 - 450
Pks: 175 Base Pk: 146 Int: 21634 100.00% = 21634
100%
146
73
INT
117
217
89
131
59
99
184
233
273
304
335
50 100 150 200 250 300 350 400 450


Library Search C:\...\ADJ\022397G1 Acquired: 23 Feb 1997 Scan number 1099
Comment: 16A METHANOLYSIS, DERIV. WITH BSTFA/TMCS
100%
100% Silane, trimethyl[(1-methylhexyl)oxy]- CAS 53690-75-8
100% Xylopyranose, 3-O-methyl-1,2,4-tris-O-(trimethylsilyl)- CAS 28561-79-9
100% Butanoic acid, 2-methyl-3-[(trimethylsilyl)oxy]-, trime CAS 55557-17-8
50 100 150 200 250 300 350 400 450 500 550 600 650
Formula C18.H24.O.Si Rank 1 Index 19842
MolWeight:188 Search:Acq LocalNorm:On P:175 F:761 R:175 CAS# 53690-75-8


Library Search C:\...\ADJ\022397G1 Acquired: 23 Feb 1997 Scan number 1142
Comment: 16A METHANOLYSIS, DERIV. WITH BSTFA/TMCS
100%
100% 2',4',5,6',7,8-Hexamethoxyflavone CAS 89121-54-0
INT
100% Benzoic acid, 2-[(trimethylsilyl)oxy]-, trimethylsilyl CAS 3789-85-3
100% 2',4',5,6',7,8-Hexamethoxyflavone CAS 89121-54-0
50 100 150 200 250 300 350 400 450
Formula C21.H22.O8 Rank 3 Index 57760
MolWeight:402 Search:Acq LocalNorm:On P:556 F:791 R:590 CAS# 89121-54-0


Library Search C:\...\ADJ\022397G1 Acquired: 23 Feb 1997 Scan number 1145
Comment: 16A METHANOLYSIS, DERIV. WITH BSTFA/TMCS
100%
100% 2,4-Bis(dimethylbenzyl)-6-t-butylphenol CAS 0-00-0
INT
100% 2,3-Dimethylamphetamine CAS 75659-60-8
100% Benzenepropanoic acid, .beta.,.beta.-dimethyl- CAS 1010-48-6
50 100 150 200 250 300 350 400 450
Formula C28.H34.0 Rank 1 Index 56656
MolWeight:386 Search:Acq LocalNorm:On P:768 F:351 R:770 CAS# 0-00-0


Background Subtract C:\SATURN\DATA\ADJ\022397G1 02/23/97 19:29:00
Comment: 16A METHANOLYSIS, DERIV. WITH BSTFA/TMCS
Average of: 1379 to 1383 Minus: 1351 to 1355 100% = 6000
100%
SMP
-
BKG
75
91
107
143
195
211
239
265
280
300
341
369
387
424
459
477
492
539
567
584
50 100 150 200 250 300 350 400 450 500 550 600



Search Results Datafile:022397G1 Spectrum:1381 Libr:NIST92 Range:Acq

(1) 17.alpha.-Methyl-17.beta.-hydroxy-1,4,6-androstatrien-3-one, mono-TMS
55429 Purity: 126 Fit: 897 Rfit: 130 CAS# 0-00-0
Formula: C23.H34.O2.Si Mol Wt: 370

(2) Phenanthrene, 9-ethyl-3,6-dimethoxy-10-methyl-
46088 Purity: 83 Fit: 891 Rfit: 91 CAS# 5025-37-6
Formula: C19.H20.O2 Mol Wt: 280

(3) Silane, trimethyl[[5-methyl-2-(1-methylethenyl)cyclohexyl]oxy]-, [1R-(1.
29142 Purity: 71 Fit: 876 Rfit: 79 CAS# 57396-86-8
Formula: C13.H26.O.Si Mol Wt: 226

(4) 3H-Pyrazol-3-one, 4-chloro-1,2-dihydro-5-methyl-2-phenyl-1-(trimethylsil
46007 Purity: 123 Fit: 823 Rfit: 135 CAS# 57396-96-8
Formula: C13.H17.Cl.N2.O.Si Mol Wt: 280

(5) Acetic acid, [[[(17.beta.)-17-methyl-17-[(trimethylsilyl)oxy]androsta-1,
60863 Purity: 138 Fit: 800 Rfit: 159 CAS# 74299-10-8
Formula: C26.H41.N.O4.Si Mol Wt: 459

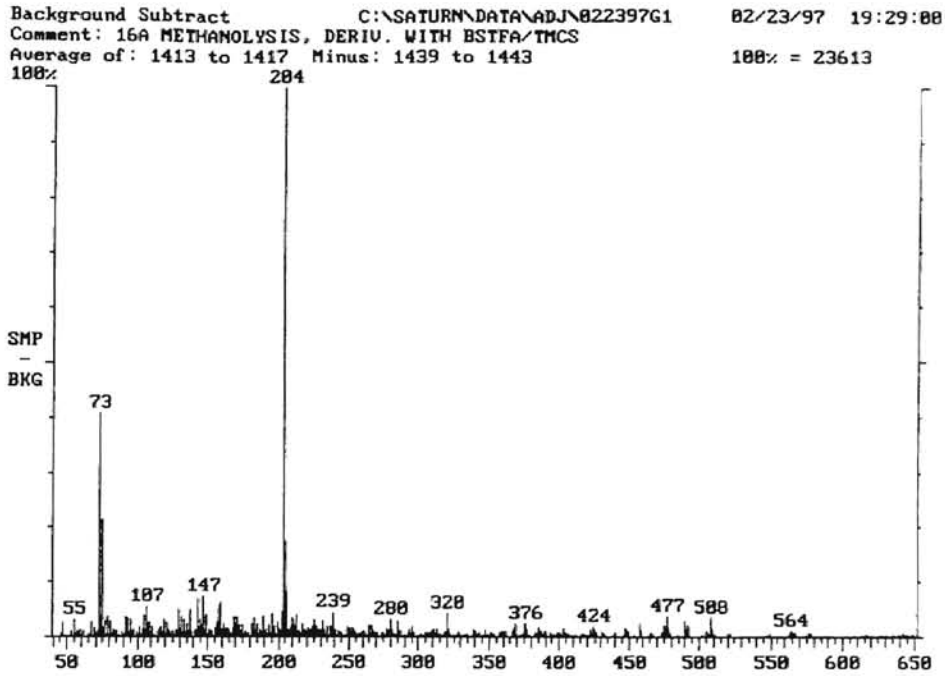
(6) 9H-Purine, 9-(trimethylsilyl)-6-[(trimethylsilyl)oxy]-
45993 Purity: 108 Fit: 782 Rfit: 115 CAS# 17962-89-9
Formula: C11.H20.N4.O.Si2 Mol Wt: 280

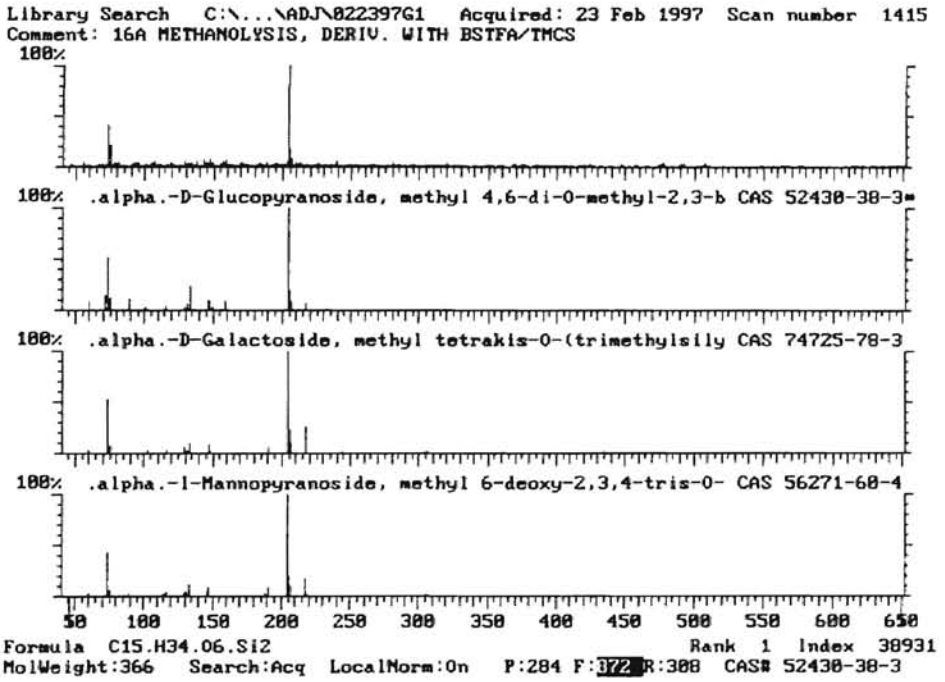
(7) 5.alpha.Androst-16-ol, 17-ethylidene-3,5-dedihydro-6-methoxy-,
58544 Purity: 122 Fit: 782 Rfit: 143 CAS# 0-00-0
Formula: C27.H42.O3 Mol Wt: 414

(8) Pyridoxine-TMS
56483 Purity: 89 Fit: 765 Rfit: 94 CAS# 0-00-0
Formula: C17.H35.N.O3.Si3 Mol Wt: 385

(9) Pregnan-20-one, 3,21-bis[(trimethylsilyl)oxy]-, O-(phenylmethyl)oxime, (
63789 Purity: 163 Fit: 743 Rfit: 205 CAS# 57325-89-0
Formula: C34.H57.N.O3.Si2 Mol Wt: 583

(10) 2-Isopropyl-3-keto-trimethylsilylbutyrate
26658 Purity: 58 Fit: 709 Rfit: 75 CAS# 0-00-0
Formula: C10.H20.O3.Si Mol Wt: 216


Background Subtract C:\SATURN\DATA\ADJ\022397G1 02/23/97 19:29:00
Comment: 16A METHANOLYSIS, DERIV. WITH BSTFA/TMCS
Average of: 1413 to 1417 Minus: 1439 to 1443 100% = 23613
100%
204
SMP
BKG
73
55
107
147
239
280
320
376
424
477
508
564
50 100 150 200 250 300 350 400 450 500 550 600 650


Library Search C:\...\ADJ\022397G1 Acquired: 23 Feb 1997 Scan number 1415
Comment: 16A METHANOLYSIS, DERIV. WITH BSTFA/TMCS
100%
100% .alpha.-D-Glucopyranoside, methyl 4,6-di-O-methyl-2,3-b CAS 52430-38-3
100% .alpha.-D-Galactoside, methyl tetrakis-O-(trimethylsily CAS 74725-78-3
100% .alpha.-l-Mannopyranoside, methyl 6-deoxy-2,3,4-tris-O- CAS 56271-60-4
50 100 150 200 250 300 350 400 450 500 550 600 650
Formula C15.H34.06.Si2 Rank 1 Index 38931
MolWeight:366 Search:Acq LocalNorm:On P:284 F:372 R:308 CAS# 52430-38-3

Search Results Datafile:022397G1 Spectrum:1415 Libr:NIST92 Range:Acq

(1) .alpha.-D-Glucopyranoside, methyl 4,6-di-O-methyl-2,3-bis-O-(trimethylsi
  # 38931 Purity: 284 Fit: 872 Rfit: 308 CAS# 52430-38-3
  Formula: C15.H34.06.Si2 Mol Wt: 366

(2) .alpha.-D-Galactoside, methyl tetrakis-O-(trimethylsilyl)-
  # 39730 Purity: 271 Fit: 872 Rfit: 285 CAS# 74725-78-3
  Formula: C19.H46.06.Si4 Mol Wt: 482

(3) .alpha.-l-Mannopyranoside, methyl 6-deoxy-2,3,4-tris-O-(trimethylsilyl)-
  # 39273 Purity: 271 Fit: 860 Rfit: 296 CAS# 56271-60-4
  Formula: C16.H38.05.Si3 Mol Wt: 394

(4) .alpha.-D-Glucopyranoside, methyl 2,3,4,6-tetrakis-O-(trimethylsilyl)-
  # 72263 Purity: 251 Fit: 846 Rfit: 272 CAS# 2641-79-4
  Formula: C19.H46.06.Si4 Mol Wt: 482

(5) .alpha.-D-Galactopyranoside, methyl 2,3,4,6-tetrakis-O-(trimethylsilyl)-
  # 39725 Purity: 263 Fit: 834 Rfit: 281 CAS# 4133-45-3
  Formula: C19.H46.06.Si4 Mol Wt: 482

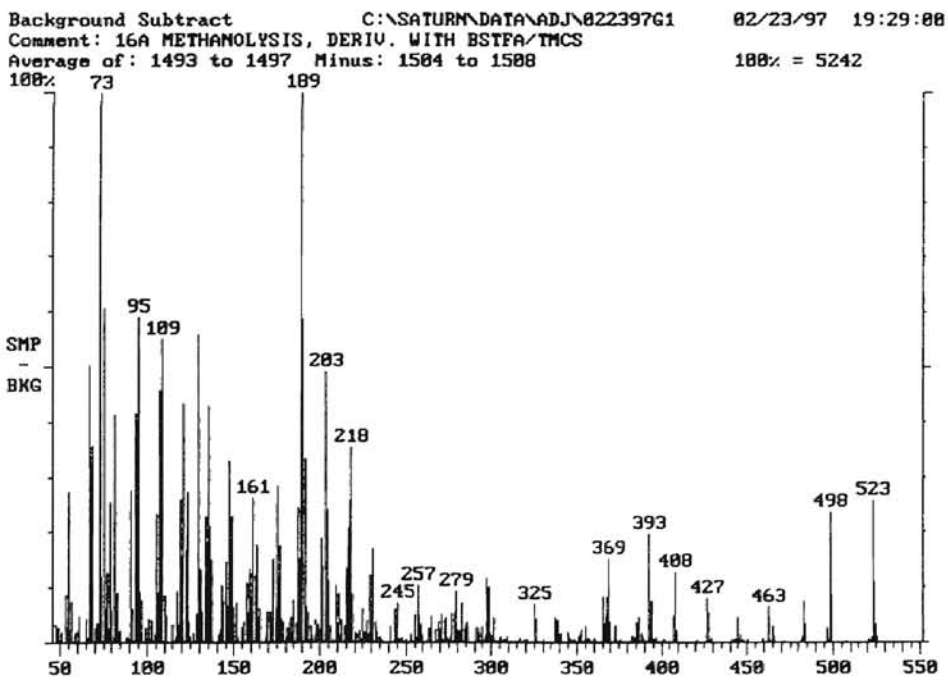
(6) .beta.-D-Galactopyranoside, methyl 2,3,6-tris-O-(trimethylsilyl)-, aceta
  # 60513 Purity: 280 Fit: 833 Rfit: 318 CAS# 52419-51-9
  Formula: C18.H40.07.Si3 Mol Wt: 452

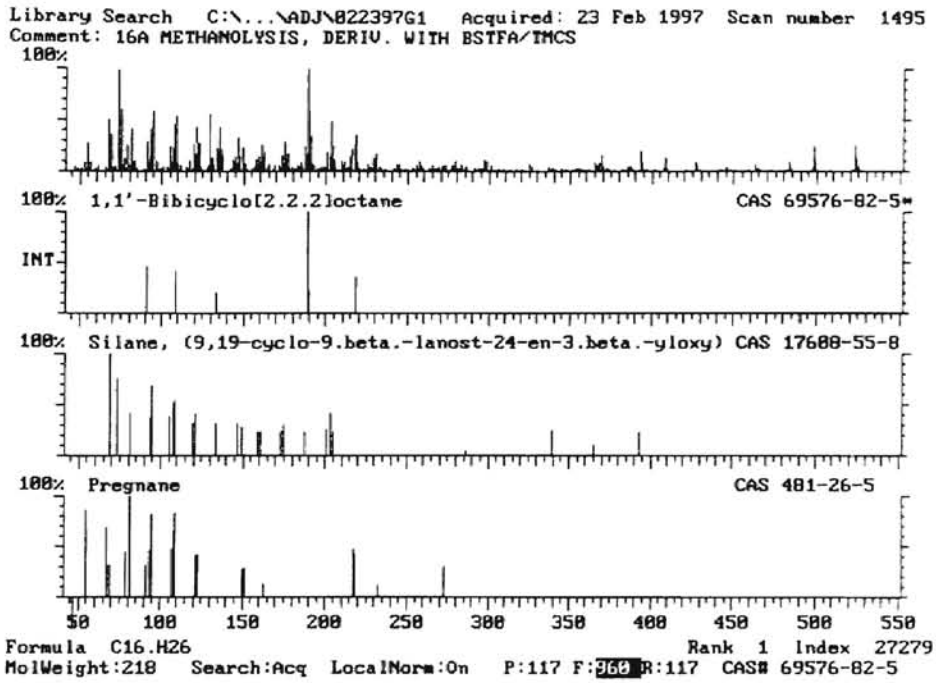
(7) 1-Cyclohexene-1-carboxylic acid, 3,4,5-tris[(trimethylsilyl)oxy]-, trime
  # 60948 Purity: 278 Fit: 833 Rfit: 323 CAS# 55520-78-0
  Formula: C19.H42.05.Si4 Mol Wt: 462

(8) .alpha.-D-Glucopyranoside, methyl 2,3-bis-O-(trimethylsilyl)-, cyclic bu
  # 39360 Purity: 319 Fit: 829 Rfit: 361 CAS# 56211-14-4
  Formula: C17.H37.B.06.Si2 Mol Wt: 404

(9) .alpha.-L-Galactopyranoside, methyl 6-deoxy-2,3,4-tris-O-(trimethylsilyl
  # 57151 Purity: 269 Fit: 828 Rfit: 294 CAS# 56271-58-0
  Formula: C16.H38.05.Si3 Mol Wt: 394

(10) .beta.-l-Galactopyranoside, methyl 6-deoxy-2,3,4-tris-O-(trimethylsilyl)
  # 39272 Purity: 279 Fit: 822 Rfit: 303 CAS# 56271-59-1
  Formula: C16.H38.05.Si3 Mol Wt: 394





Search Results Datafile:822397G1 Spectrum:1495 Libr:NIST92 Range:Acq

(1) 1,1'-Bibicyclo[2.2.2]octane
27279 Purity: 117 Fit: 960 Rfit: 117 CAS# 69576-82-5
Formula: C16.H26 Mol Wt: 218

(2) Silane, (9,19-cyclo-9.beta.-lanost-24-en-3.beta.-yloxy)trimethyl-
62172 Purity: 242 Fit: 956 Rfit: 242 CAS# 17608-55-8
Formula: C33.H58.0.Si Mol Wt: 498

(3) Pregnane
47153 Purity: 162 Fit: 921 Rfit: 165 CAS# 481-26-5
Formula: C21.H36 Mol Wt: 288

(4) D-Norandrostane-16-methanol, (5.alpha.,16.beta.)-
45675 Purity: 221 Fit: 915 Rfit: 227 CAS# 54411-68-8
Formula: C19.H32.0 Mol Wt: 276

(5) Tricyclo[4.3.0.07,9]nonane, 2,2,5,5,8,8-hexamethyl-, (1.alpha.,6.beta.,7
24458 Purity: 170 Fit: 906 Rfit: 180 CAS# 54832-82-5
Formula: C15.H26 Mol Wt: 206

(6) Silane, [[(3.beta.)-lanosta-9(11),24-dien-3-yl]oxy]trimethyl-
62170 Purity: 258 Fit: 905 Rfit: 262 CAS# 55538-95-9
Formula: C33.H58.0.Si Mol Wt: 498

(7) .beta.-Amyrin trimethylsilyl ether
39765 Purity: 256 Fit: 887 Rfit: 256 CAS# 1721-67-1
Formula: C33.H58.0.Si Mol Wt: 498

(8) 1H-Indene-2-ethanol, octahydro-2-(hydroxymethyl)-3a,4-dimethyl-
29180 Purity: 252 Fit: 875 Rfit: 259 CAS# 54833-42-8
Formula: C14.H26.02 Mol Wt: 226

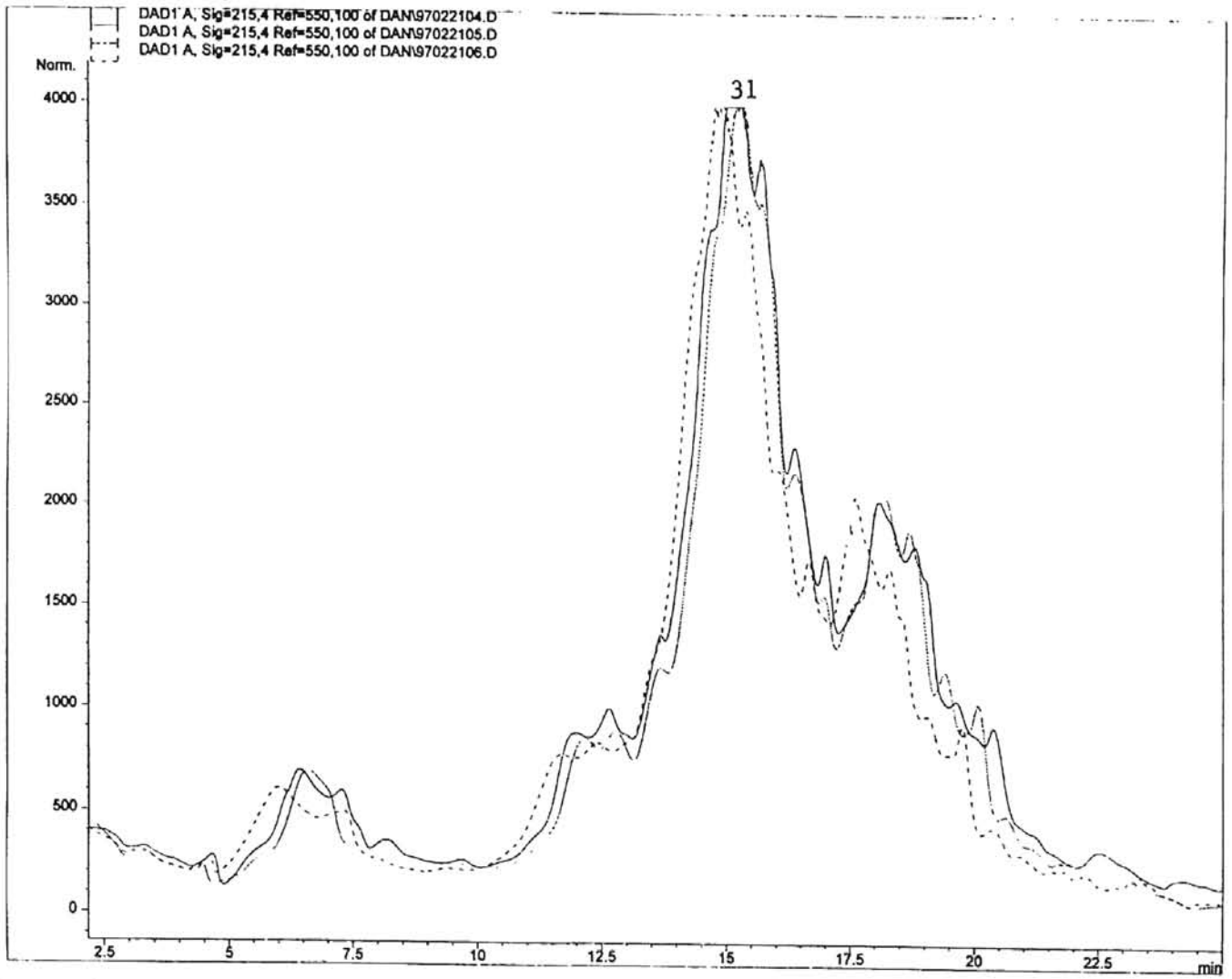
(9) 5H-3,5a-Epoxynaphth[2,1-c]oxepin, dodecahydro-3,8,8,11a-tetramethyl-, [3
36338 Purity: 262 Fit: 874 Rfit: 287 CAS# 1153-35-1
Formula: C18.H30.02 Mol Wt: 278

(10) 3,4-Heptadien-2-one, 3,5-dicyclopentyl-6-methyl-
43741 Purity: 196 Fit: 862 Rfit: 213 CAS# 63922-51-8
Formula: C18.H28.0 Mol Wt: 260

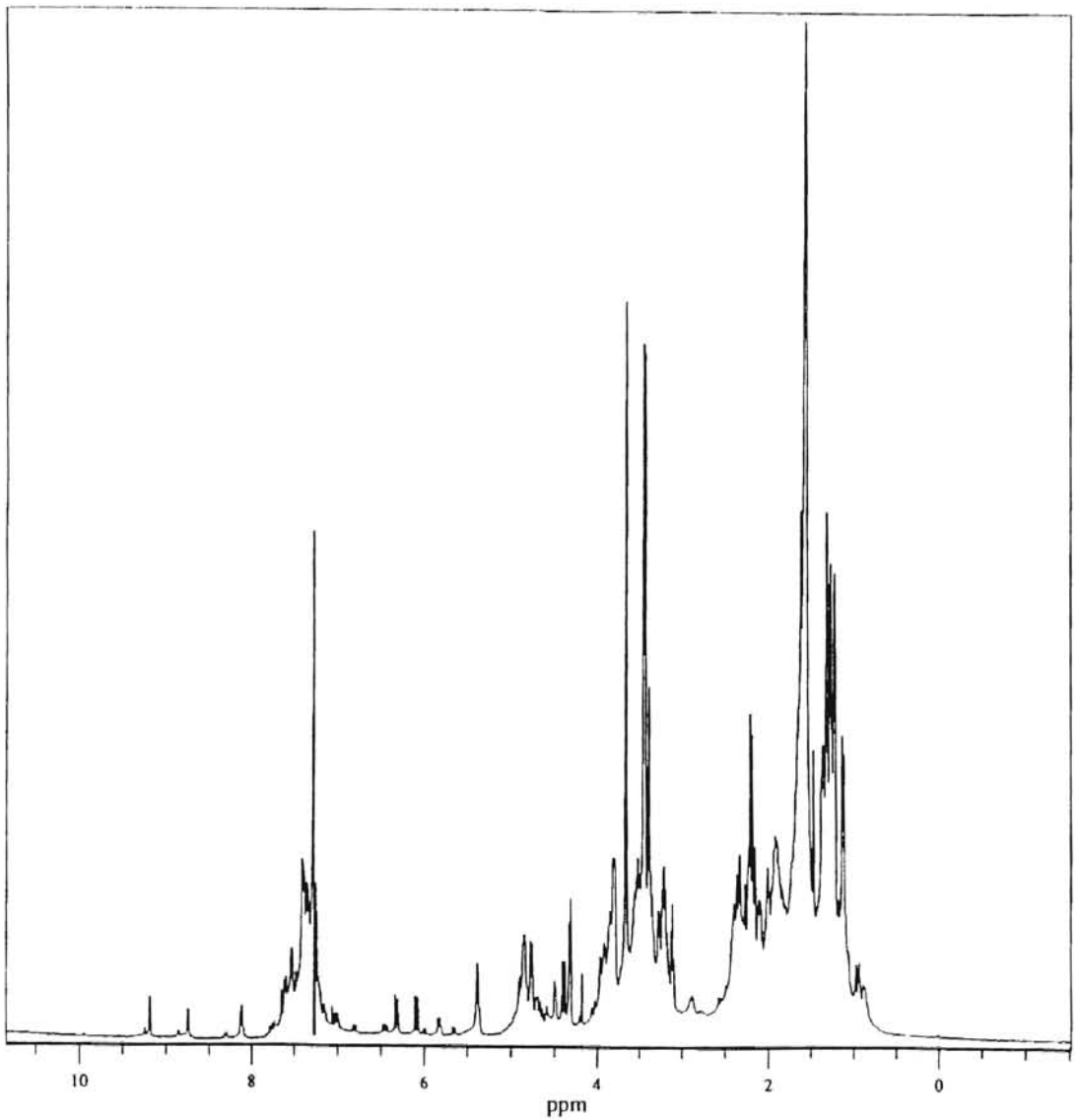
APPENDIX F

CHROMATOGRAM FROM REVERSE PHASE HPLC AND SPECTRUM FROM ^{1}H NMR SPECTROSCOPY ON FRACTION 16A

Current Chromatogram(s)



Diode Array 2/23/97 6:47:42 PM Gregg



GE NMR OMEGA

...home/fiji1/wanda/acq.data

Date: Feb 24 10:13:16.2 1997

OPERATOR: ********

ACQ TIME = 1.33 sec
DATA SIZE = 8192
NUM OF BLKS = 1
NUM OF SCANS = 64

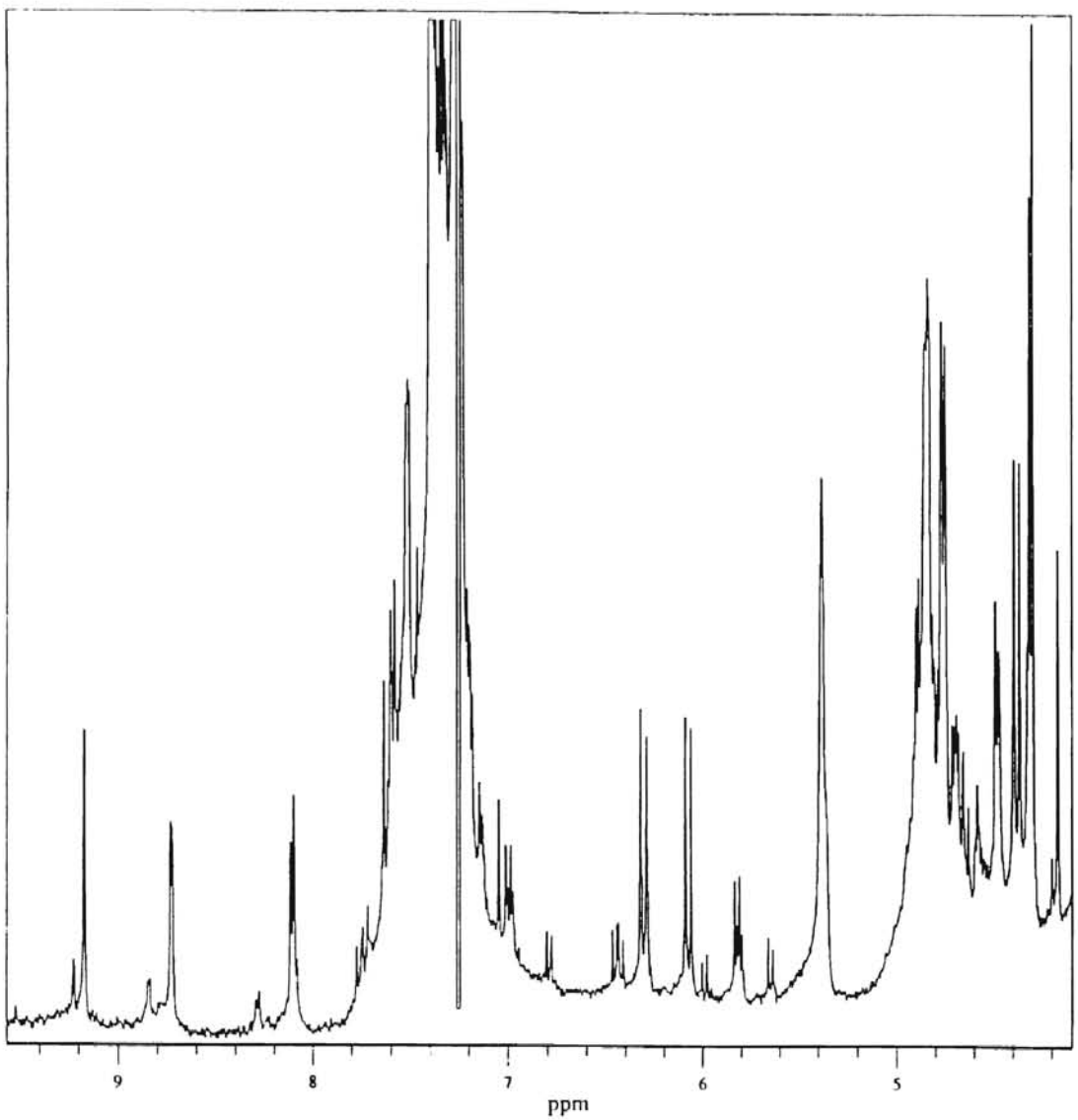
PULSE SEQUENCE:
SEQUENCE NAME = flpresat.s

OBSERVE:
f1 FREQ = 500.1351150 MHz
SPEC WIDTH = 6172.84 Hz
SPEC OFFSET = 2325.60 Hz
GAIN = 200.0
POWER LEVEL = 60
LOW POWER = ON

DECOUPLER:
f2 FREQUENCY = 500.1352900 MHz
f2 POWER = 0 db
f2 MODULATION = CW
f3 FREQUENCY = 125.7713594 MHz
f3 POWER = 0 db
f3 MODULATION = CW

PROCESSING:
PHASE A = 338.61
PHASE B = -12.05

PLOT RANGE:
X From 10.82 TO 1.52 ppm



GE NMR OMEGA

...home/fiji1/wanda/acq.data

Date: Feb 24 10:13:16.2 1997

OPERATOR: ********

ACQ TIME = 1.33 sec
DATA SIZE = 8192
NUM OF BLKS = 1
NUM OF SCANS = 64

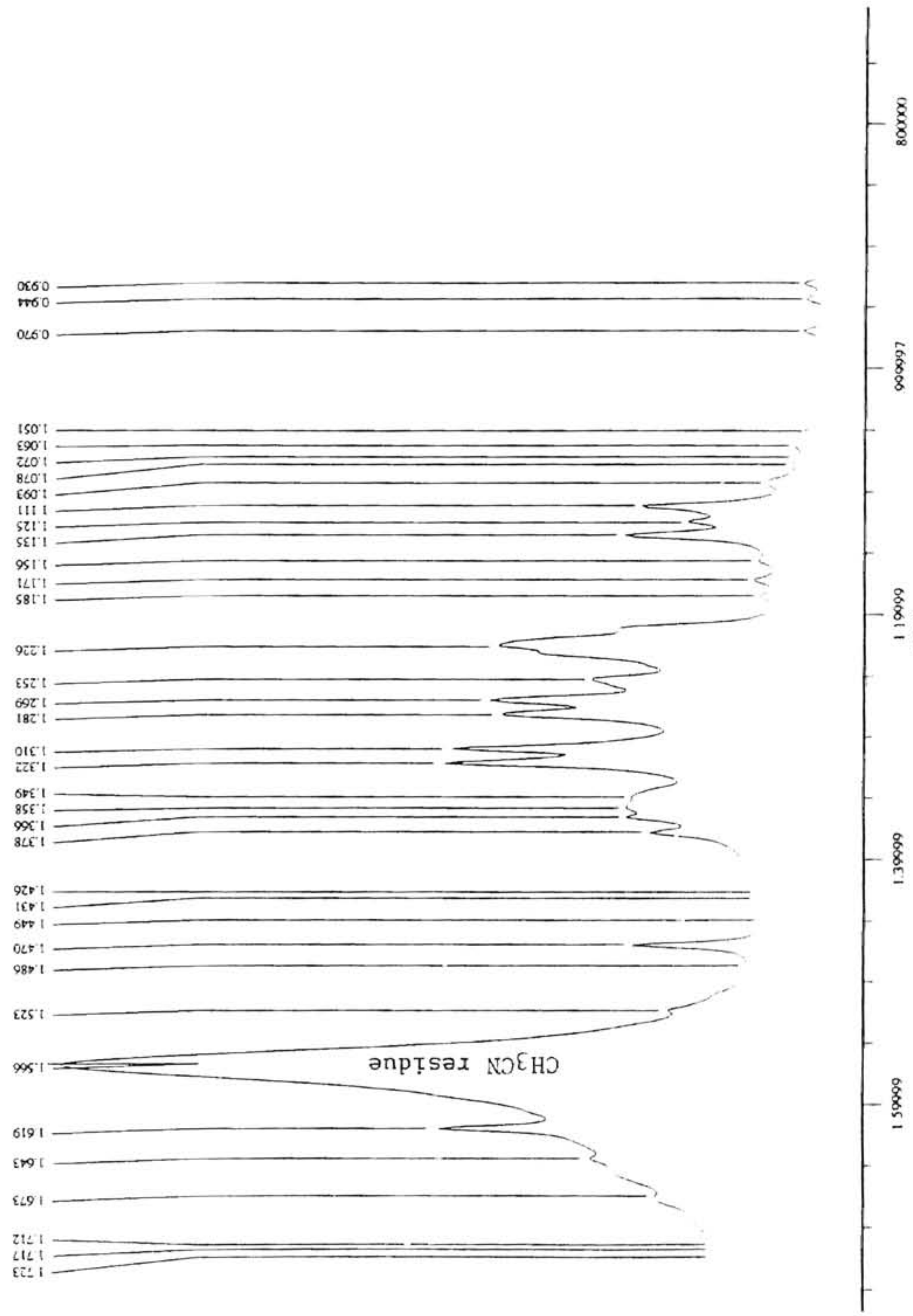
PULSE SEQUENCE:
SEQUENCE NAME = f1presat.s

OBSERVE:
f1 FREQ = 500.1351150 MHz
SPEC WIDTH = 6172.84 Hz
SPEC OFFSET = 2325.60 Hz
GAIN = 200.0
POWER LEVEL = 60
LOW POWER = ON

DECOUPLER:
f2 FREQUENCY = 500.1352900 MHz
f2 POWER = 0 db
f2 MODULATION = CW
f3 FREQUENCY = 125.7713594 MHz
f3 POWER = 0 db
f3 MODULATION = CW

PROCESSING:
PHASE A = 338.61
PHASE B = -12.05

PLOT RANGE:
X From 9.57 TO 4.09 ppm


CH3CN residue
ppm

Filename: /home/wanda/data/djplot

Threshold: 5.80
Scale: 100.00
Scale fit for largest peak: none
Scope: global
Mode: positive peaks only
Search region: 884.32 Hz to 352.27 Hz
1.768 ppm to 0.704 ppm

#	height (uncorr)	freq (Hz)	freq (ppm)
1	17.95	861.71	1.723
2	18.15	858.69	1.717
3	18.07	856.43	1.712
4	25.08	836.84	1.673
5	33.32	821.77	1.643
6	52.61	809.71	1.619
7	100.00	783.33	1.566
8	23.65	761.48	1.523
9	14.02	743.39	1.486
10	28.03	735.10	1.470
11	11.85	724.55	1.449
12	12.74	715.51	1.431
13	12.50	713.25	1.426
14	25.91	689.13	1.378
15	28.95	683.10	1.366
16	28.89	679.33	1.358
17	28.15	674.81	1.349
18	51.85	661.25	1.322
19	50.83	655.22	1.310
20	44.49	640.90	1.281
21	46.14	634.87	1.269
22	33.21	626.58	1.253
23	44.98	613.02	1.226
24	12.20	592.67	1.185
25	12.97	585.89	1.171
26	12.43	578.35	1.156
27	29.21	567.80	1.135
28	21.35	562.52	1.125
29	26.95	555.74	1.111
30	11.46	546.70	1.093
31	8.25	539.16	1.078
32	7.83	536.15	1.072
33	7.92	531.63	1.063
34	6.63	525.60	1.051
35	6.83	484.90	0.970
36	6.48	472.09	0.944
37	6.97	465.31	0.930

2.99998
2.79998
2.59999
2.39999
2.19999
2.00000
1.80000
ppm
2.922
2.918
2.900
2.889
2.879
2.870
2.858
2.840
2.598
2.592
2.588
2.585
2.577
2.562
2.555
2.543
2.538
2.528
2.517
2.508
2.478
2.466
2.412
2.387
2.378
2.354
2.324
2.288
2.259
2.214
2.202
2.180
2.152
2.148
2.124
2.110
2.101
2.083
2.060
2.050
2.027
1.997
1.982
1.954
1.928
1.914
1.901
1.895
1.874
1.837
1.831
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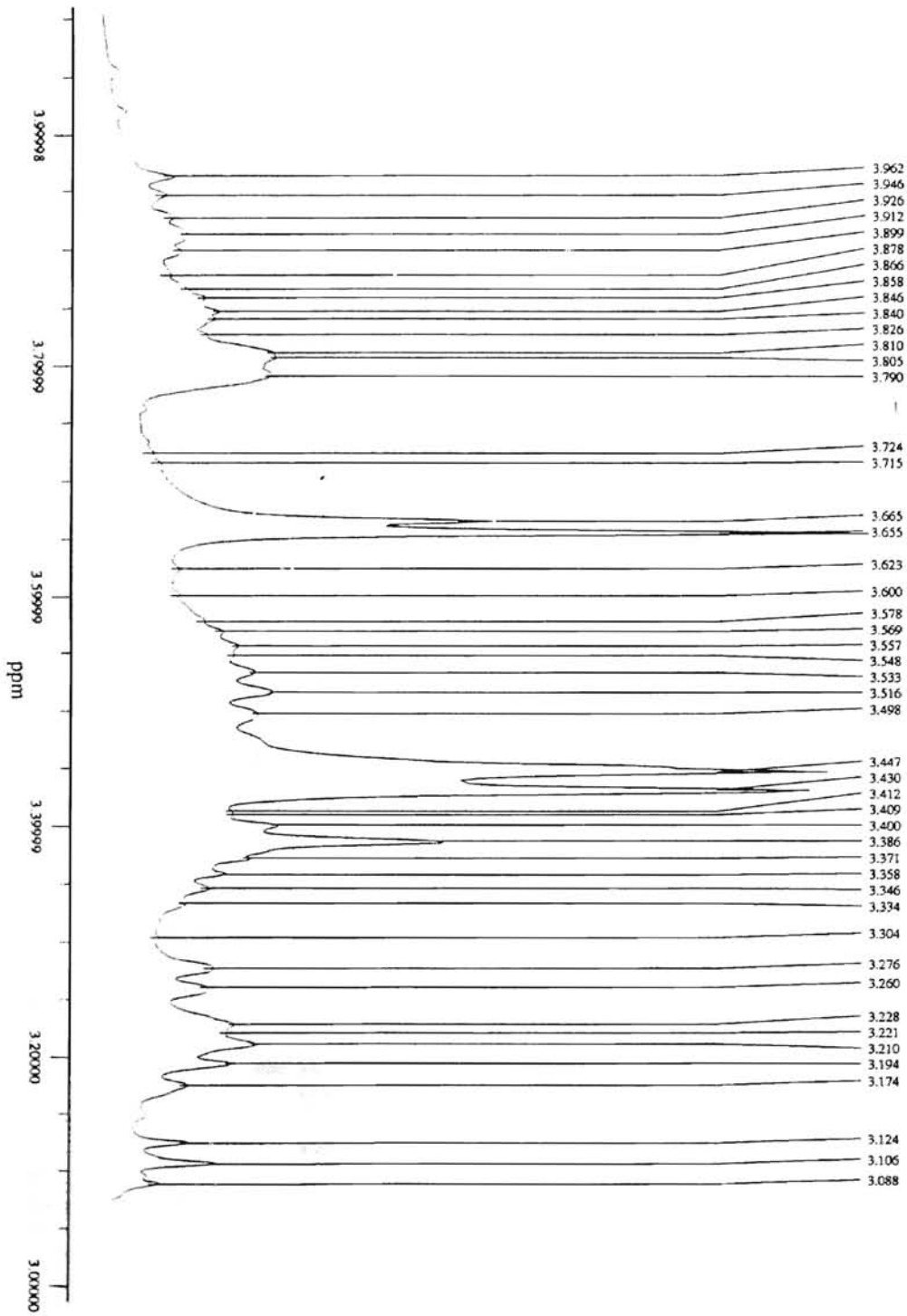
1.812
1.798
1.795
1.785
1.777

Filename: /home/wanda/data/djplot

Threshold: 9.40
Scale: 100.00
Scale fit for largest peak: none
Scope: local
Mode: positive peaks only
Search region: 1511.32 Hz to 887.33 Hz
3.022 ppm to 1.774 ppm

#	height (uncorr)	freq (Hz)	freq (ppm)
1	9.84	1461.58	2.922
2	9.85	1459.32	2.918
3	12.08	1450.28	2.900
4	12.99	1445.00	2.889
5	12.90	1439.73	2.879
6	13.38	1435.21	2.870
7	12.77	1429.18	2.858
8	9.81	1420.13	2.840
9	9.90	1299.56	2.598
10	10.17	1296.54	2.592
11	10.22	1294.28	2.588
12	10.27	1292.77	2.585
13	10.87	1289.01	2.577
14	13.90	1281.47	2.562
15	13.65	1277.70	2.555
16	11.96	1271.67	2.543
17	12.10	1269.41	2.538
18	13.67	1264.14	2.528
19	13.18	1258.86	2.517
20	13.76	1254.34	2.508
21	17.86	1239.27	2.478
22	16.76	1233.24	2.466
23	32.53	1206.11	2.412
24	43.16	1194.05	2.387
25	39.32	1189.53	2.378
26	51.89	1177.47	2.354
27	57.60	1162.40	2.324
28	38.42	1144.31	2.288
29	48.73	1129.99	2.259
30	59.72	1107.39	2.214
31	100.00	1101.36	2.202
32	93.42	1090.05	2.180
33	56.91	1076.49	2.152
34	58.87	1074.23	2.148
35	35.02	1062.17	2.124
36	42.00	1055.39	2.110
37	43.30	1050.86	2.101
38	43.13	1041.82	2.083
39	30.72	1030.52	2.060
40	28.46	1025.24	2.050
41	34.43	1013.94	2.027
42	53.46	998.87	1.997
43	46.06	991.33	1.982
44	47.72	977.01	1.954
45	58.61	964.20	1.928
46	63.22	957.42	1.914
47	60.59	950.63	1.901
48	61.36	947.62	1.895
49	53.51	937.07	1.874
50	45.71	918.98	1.837
51	46.82	915.97	1.831
52	44.19	912.20	1.824
53	41.31	906.17	1.812
54	42.24	899.39	1.798
55	42.26	897.88	1.795
56	39.69	892.61	1.785
57	39.34	888.84	1.777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3.600
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3.412
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3.228
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3.210
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3.124
3.106
3.088

Filename: /home/wanda/data/djplot

Threshold: 9.30
Scale: 100.00
Scale fit for largest peak: none
Scope: local
Mode: positive peaks only
Search region: 2055.43 Hz to 1493.24 Hz
4.110 ppm to 2.986 ppm

#	height (uncorr)	freq (Hz)	freq (ppm)
1	12.14	1981.58	3.962
2	11.08	1973.29	3.946
3	11.94	1963.49	3.926
4	13.96	1956.71	3.912
5	13.08	1949.92	3.899
6	11.70	1939.37	3.878
7	14.14	1933.34	3.866
8	16.34	1929.58	3.858
9	18.17	1923.55	3.846
10	17.63	1920.53	3.840
11	16.45	1913.75	3.826
12	25.16	1905.46	3.810
13	25.33	1903.20	3.805
14	24.82	1895.66	3.790
15	9.57	1862.51	3.724
16	10.54	1857.98	3.715
17	53.51	1833.11	3.665
18	100.00	1827.84	3.655
19	13.05	1812.01	3.623
20	13.03	1800.71	3.600
21	16.24	1789.40	3.578
22	18.73	1784.88	3.569
23	20.74	1778.85	3.557
24	20.11	1774.33	3.548
25	22.80	1766.80	3.533
26	25.22	1758.51	3.516
27	23.14	1749.46	3.498
28	94.77	1723.84	3.447
29	92.69	1715.55	3.430
30	19.93	1706.51	3.412
31	19.96	1705.00	3.409
32	25.86	1700.48	3.400
33	47.20	1693.70	3.386
34	22.23	1686.16	3.371
35	19.21	1679.38	3.358
36	17.02	1673.35	3.346
37	14.22	1667.32	3.334
38	10.46	1652.25	3.304
39	17.44	1638.68	3.276
40	16.92	1630.39	3.260
41	20.37	1614.57	3.228
42	19.45	1610.80	3.221
43	23.27	1605.52	3.210
44	20.08	1597.23	3.194
45	14.32	1587.44	3.174
46	14.64	1562.57	3.124
47	18.10	1553.52	3.106
48	10.68	1544.48	3.088

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4.809
4.788
4.770
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4.729
4.723
4.714
4.700
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4.574
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4.542
4.530
4.520
4.517
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4.197
4.169
4.087

Filename: /home/wanda/data/djplot

Threshold: 15.60
Scale: 100.00
Scale fit for largest peak: none
Scope: local
Mode: positive peaks only
Search region: 2741.22 Hz to 2041.86 Hz
5.481 ppm to 4.083 ppm

#	height (uncorr)	freq (Hz)	freq (ppm)
1	54.57	2691.48	5.382
2	17.18	2479.71	4.958
3	19.54	2475.19	4.949
4	22.41	2464.64	4.928
5	28.84	2457.11	4.913
6	43.30	2451.08	4.901
7	46.05	2445.05	4.889
8	39.75	2438.27	4.875
9	69.60	2428.47	4.856
10	70.27	2425.45	4.850
11	75.08	2420.93	4.841
12	40.55	2409.63	4.818
13	36.45	2405.11	4.809
14	36.00	2394.56	4.788
15	70.88	2385.51	4.770
16	67.17	2375.72	4.750
17	19.50	2365.16	4.729
18	20.65	2362.15	4.723
19	29.83	2357.63	4.714
20	29.67	2350.85	4.700
21	30.63	2345.57	4.690
22	29.03	2341.80	4.682
23	27.08	2328.99	4.657
24	16.59	2322.21	4.643
25	21.88	2315.43	4.630
26	19.25	2297.34	4.593
27	24.51	2292.06	4.583
28	20.19	2287.54	4.574
29	18.18	2280.76	4.560
30	17.10	2275.48	4.550
31	16.86	2271.72	4.542
32	16.72	2265.69	4.530
33	15.78	2260.41	4.520
34	15.99	2258.91	4.517
35	18.43	2253.63	4.506
36	43.19	2245.34	4.489
37	37.70	2237.05	4.473
38	15.78	2214.44	4.428
39	15.92	2212.18	4.423
40	56.99	2197.11	4.393
41	20.35	2190.33	4.379
42	57.05	2183.54	4.366
43	18.90	2174.50	4.348
44	39.43	2161.69	4.322
45	83.51	2157.17	4.313
46	100.00	2148.88	4.297
47	17.89	2099.14	4.197
48	48.16	2084.82	4.169
49	15.68	2044.13	4.087

7.013
7.003
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6.979
6.947
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6.781
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6.438
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6.325
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6.091
6.060
6.002
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5.847
5.834
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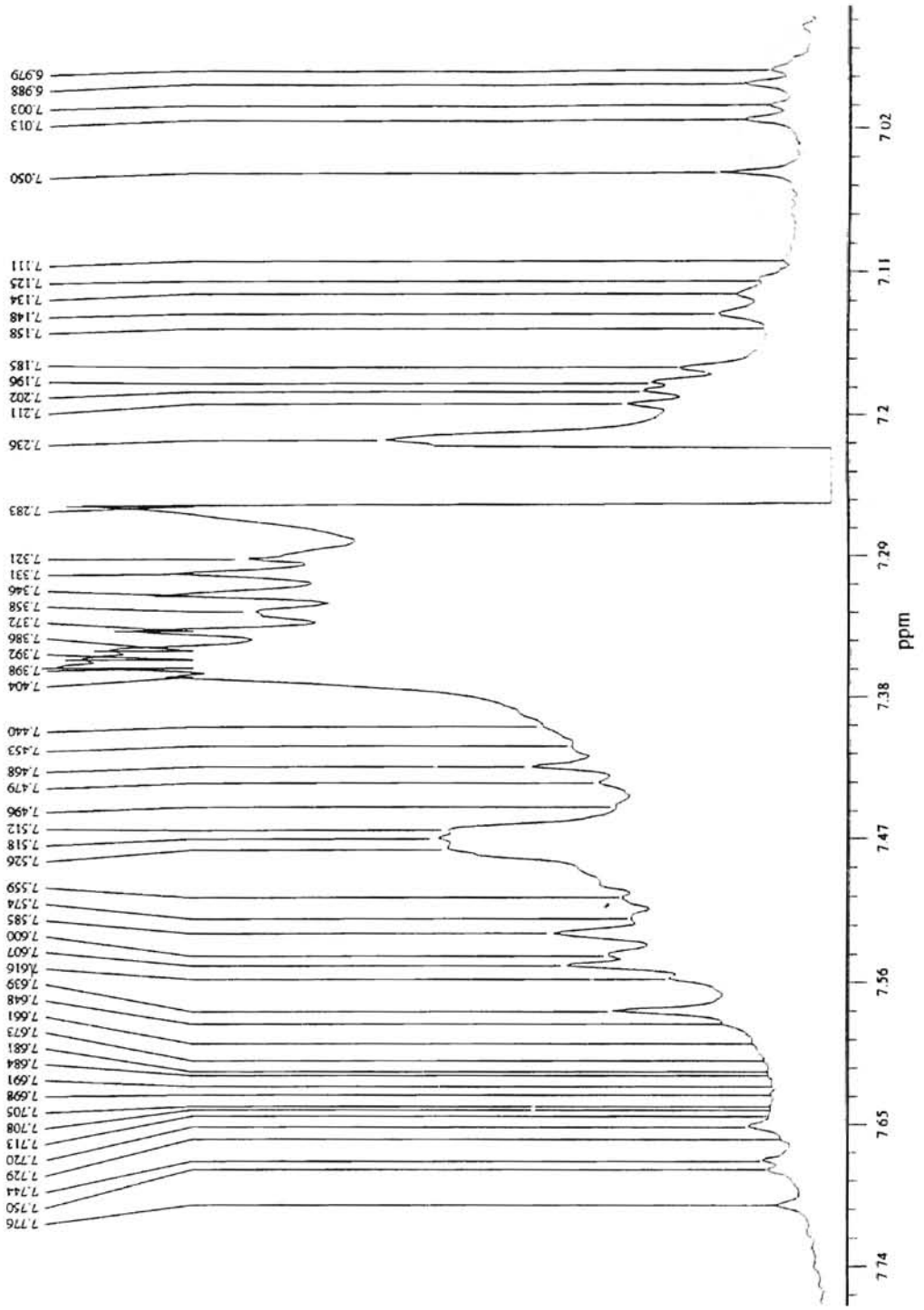
6.19999
5.99999
5.80000
5.60000
ppm

Filename: /home/wanda/data/djplot

Threshold: 14.30
Scale: 100.00
Scale fit for largest peak: none
Scope: local
Mode: positive peaks only
Search region: 3514.42 Hz to 2789.45 Hz
7.027 ppm to 5.577 ppm

#	height (uncorr)	freq (Hz)	freq (ppm)
1	48.94	3507.64	7.013
2	34.82	3502.37	7.003
3	48.59	3494.83	6.988
4	33.97	3490.31	6.979
5	16.82	3474.48	6.947
6	24.58	3403.64	6.805
7	23.33	3391.58	6.781
8	28.20	3235.59	6.469
9	29.93	3224.28	6.447
10	30.40	3219.76	6.438
11	24.37	3207.70	6.414
12	14.88	3179.82	6.358
13	14.69	3176.81	6.352
14	17.14	3173.04	6.344
15	17.76	3170.78	6.340
16	100.00	3163.24	6.325
17	44.79	3159.47	6.317
18	20.22	3155.70	6.310
19	25.75	3152.69	6.304
20	90.85	3147.41	6.293
21	38.99	3143.65	6.286
22	15.69	3136.86	6.272
23	14.96	3061.50	6.121
24	15.31	3059.24	6.117
25	14.40	3056.98	6.112
26	16.35	3054.72	6.108
27	96.31	3046.43	6.091
28	92.66	3030.60	6.060
29	18.82	3001.97	6.002
30	21.55	2989.91	5.978
31	14.46	2924.35	5.847
32	45.91	2917.56	5.834
33	31.81	2911.53	5.822
34	47.19	2905.50	5.809
35	28.19	2898.72	5.796
36	16.69	2895.71	5.790
37	28.41	2830.14	5.659
38	25.10	2818.09	5.635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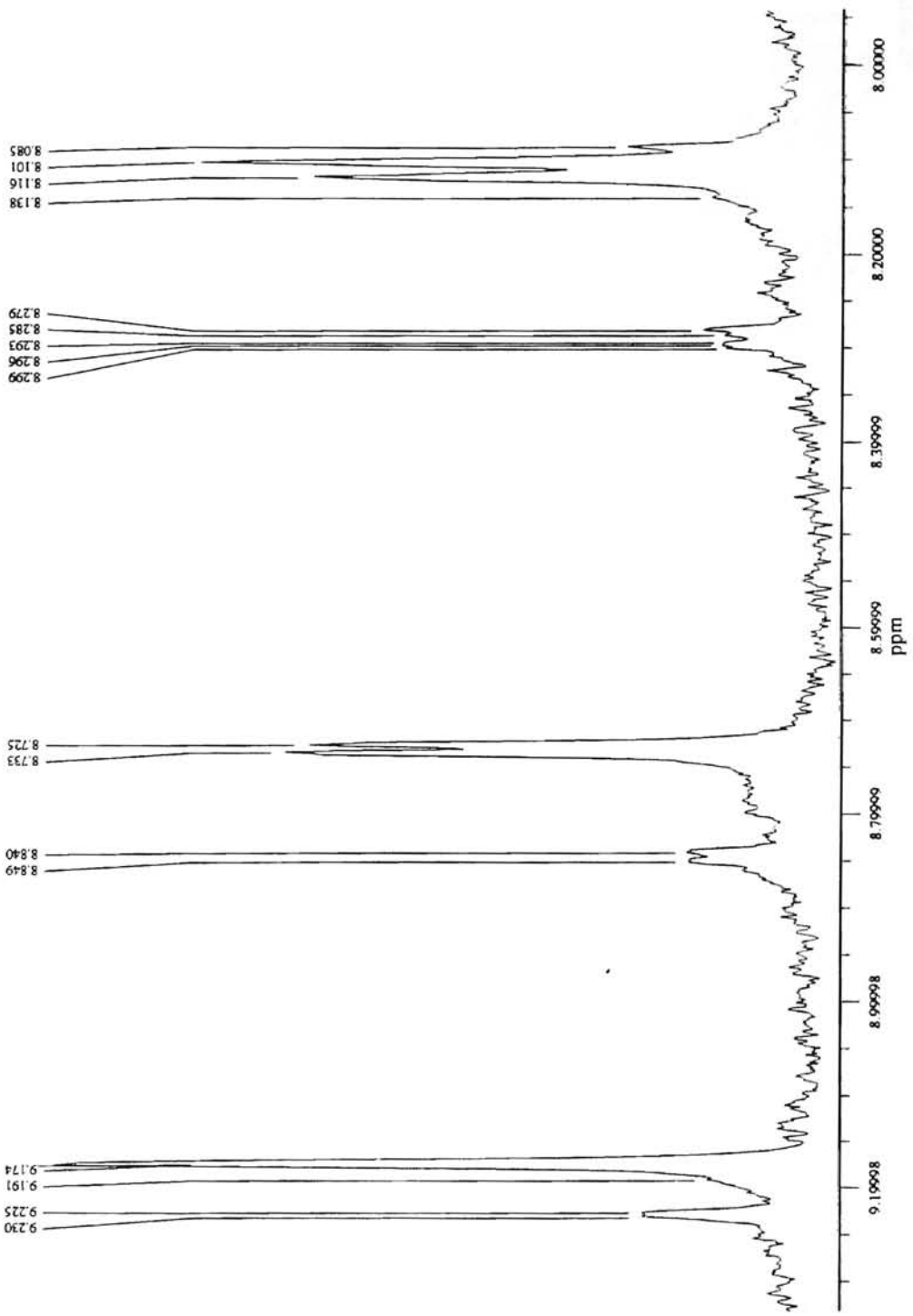
7.38
7.29
7.2
7.11
7.02
ppm

Filename: /home/wanda/data/djplot

Threshold: 6.30
Scale: 100.00
Scale fit for largest peak: none
Scope: local
Mode: positive peaks only
Search region: 3924.39 Hz to 3467.70 Hz
7.847 ppm to 6.934 ppm

#	height (uncorr)	freq (Hz)	freq (ppm)
1	7.08	3888.97	7.776
2	7.98	3876.16	7.750
3	8.71	3873.14	7.744
4	6.46	3865.61	7.729
5	10.50	3861.09	7.720
6	8.01	3857.32	7.713
7	7.72	3855.06	7.708
8	7.72	3853.55	7.705
9	7.66	3849.78	7.698
10	7.54	3846.77	7.691
11	8.07	3843.00	7.684
12	8.06	3841.49	7.681
13	8.61	3837.72	7.673
14	10.10	3831.69	7.661
15	14.06	3824.91	7.648
16	28.02	3820.39	7.639
17	20.77	3809.09	7.616
18	33.72	3804.56	7.607
19	28.56	3800.80	7.600
20	35.55	3793.26	7.585
21	25.53	3787.99	7.574
22	26.71	3780.45	7.559
23	49.23	3763.87	7.526
24	50.60	3760.10	7.518
25	49.19	3757.09	7.512
26	27.56	3748.80	7.496
27	29.83	3740.51	7.479
28	38.39	3735.23	7.468
29	33.27	3727.70	7.453
30	37.03	3720.91	7.440
31	84.34	3702.83	7.404
32	100.00	3699.81	7.398
33	96.75	3696.80	7.392
34	93.20	3693.78	7.386
35	90.57	3687.00	7.372
36	74.35	3680.22	7.358
37	85.61	3674.19	7.346
38	85.16	3666.65	7.331
39	75.30	3661.38	7.321
40	96.82	3642.54	7.283
41	57.53	3619.18	7.236
42	26.14	3606.36	7.211
43	24.15	3601.84	7.202
44	23.05	3598.83	7.196
45	19.49	3593.55	7.185
46	8.75	3579.99	7.158
47	14.48	3574.71	7.148
48	12.20	3567.93	7.134
49	9.29	3563.41	7.125
50	6.31	3556.63	7.111
51	14.59	3525.73	7.050
52	11.51	3507.64	7.013
53	8.19	3502.37	7.003
54	11.43	3494.83	6.988
55	7.99	3490.31	6.979


9.230
9.225
9.191
9.174
8.849
8.840
8.733
8.725
8.299
8.296
8.293
8.285
8.279
8.138
8.116
8.101
8.085
9.19998
8.99998
8.79999
8.59999
8.39999
8.20000
8.00000
ppm

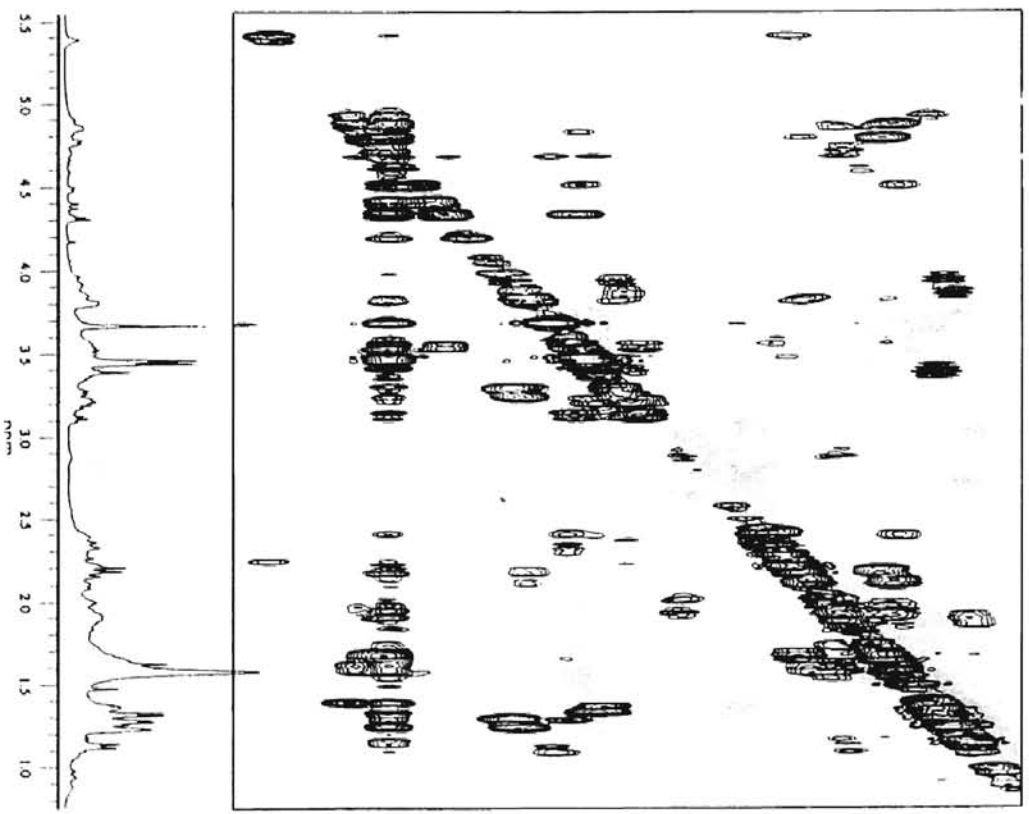
Filename: /home/wanda/data/djplot

Threshold: 7.90
Scale: 100.00
Scale fit for largest peak: none
Scope: local
Mode: positive peaks only
Search region: 4666.70 Hz to 3970.36 Hz
9.331 ppm to 7.939 ppm

#	height (uncorr)	freq (Hz)	freq (ppm)
1	19.74	4616.21	9.230
2	19.70	4613.94	9.225
3	10.83	4596.61	9.191
4	100.00	4588.32	9.174
5	13.46	4425.54	8.849
6	13.70	4421.02	8.840
7	68.82	4367.51	8.733
8	65.64	4363.75	8.725
9	8.42	4150.47	8.299
10	9.07	4148.97	8.296
11	8.77	4147.46	8.293
12	8.70	4143.69	8.285
13	11.60	4140.68	8.279
14	10.49	4069.84	8.138
15	65.20	4059.29	8.116
16	80.58	4051.75	8.101
17	22.21	4043.46	8.085

APPENDIX G

SPECTRUM FROM ^{1}H COSY NMR SPECTROSCOPY ON FRACTION 16A



GE NMR OMEGA

...nda/data/dj3116A_cosy_pro

Date: Feb 24 12:29:42.6 1997

OPERATOR: ********

ACQ TIME = 0.17 sec
DATA SIZE = 1024 * 1024
NUM OF BLKS = 128
NUM OF SCANS = 64

PULSE SEQUENCE:
SEQUENCE NAME = fl cosy_ntypa60 s

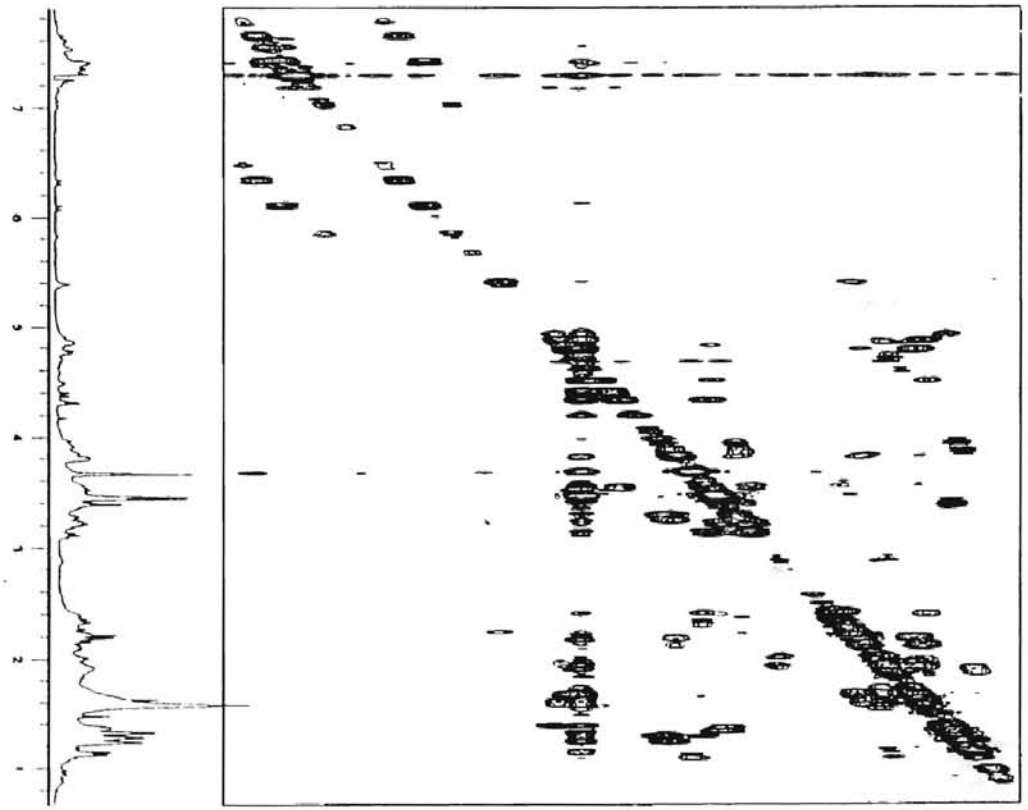
OBSERVE:
f1 FREQ = 500.1351150 MHz
SPEC WIDTH = 6172.84 Hz
SPEC OFFSET = 2325.60 Hz
V. FREQ = 500.1327894 MHz
V. SPEC WD = 6172.84 Hz
V. SPEC OF = 2325.60 Hz
GAIN = 160.0
POWER LEVEL = 60
LOW POWER = ON

DECOUPLER:
f2 FREQUENCY = 500.1352900 MHz
f2 POWER = 0 db
f2 MODULATION = CW
f3 FREQUENCY = 125.7713594 MHz
f3 POWER = 0 db
f3 MODULATION = CW

PROCESSING:
PHASE A = 0.00
PHASE B = 0.00

PLOT RANGE:
X From 5.54 TO 0.75 ppm
Y From 5.58 TO 0.78 ppm

CONTOURING:
LEVELS = 13
SPACING = LOG
POLARITY = +
SCALING = GLOBAL
FLOOR = 0.2920 %
CEILING = 17.7600 %



GE NMR OMEGA

...nda/data/dj3116A_cosy_pro

Date: Feb 24 12:29:42.6 1997

OPERATOR: ********

ACQ TIME = 0.17 sec
DATA SIZE = 1024 * 1024
NUM OF BLKS = 128
NUM OF SCANS = 64

PULSE SEQUENCE:
SEQUENCE NAME = ffcosy_ntype60 s

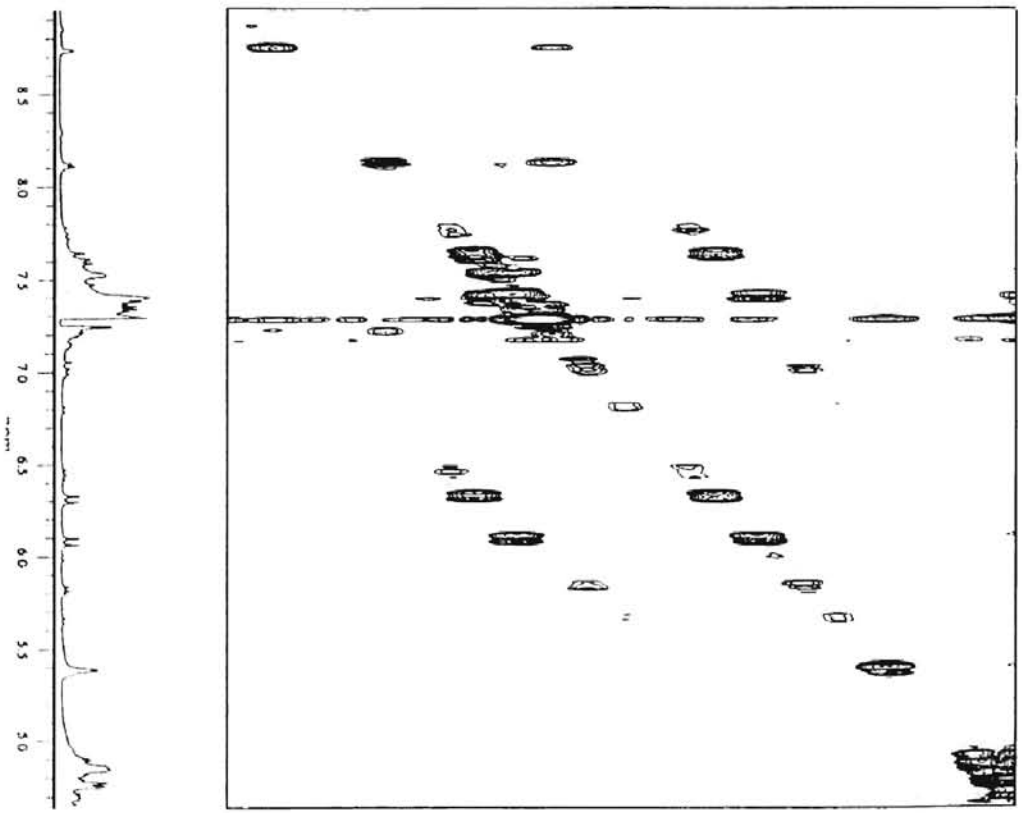
OBSERVE:
f1 FREQ = 500.1351150 MHz
SPEC WIDTH = 6172.84 Hz
SPEC OFFSET = 2325.60 Hz
V. FREQ = 500.1327894 MHz
V. SPEC WD = 6172.84 Hz
V. SPEC OF = 2325.60 Hz
GAIN = 160 0
POWER LEVEL = 60
LOW POWER = ON

DECOUPLER:
f2 FREQUENCY = 500.1352900 MHz
f2 POWER = 0 db
f2 MODULATION = CW
f3 FREQUENCY = 125.7713594 MHz
f3 POWER = 0 db
f3 MODULATION = CW

PROCESSING:
PHASE A = 0 00
PHASE B = 0 00

PLOT RANGE:
X From 7 89 TO 0 66 ppm
Y From 7 91 TO 0 69 ppm

CONTOURING:
LEVELS = 13
SPACING = LOG
POLARITY = +
SCALING = GLOBAL
FLOOR = 0 2920 %
CEILING = 17 7600 %



GE NMR OMEGA

...nda/data/dj3116A_cosy_pro

Date: Feb 24 12:29:42.6 1997

OPERATOR: ********

ACQ TIME = 0.17 sec
DATA SIZE = 1024 * 1024
NUM OF BLKS = 128
NUM OF SCANS = 64

PULSE SEQUENCE:
SEQUENCE NAME = fl cosy_mypa60.s

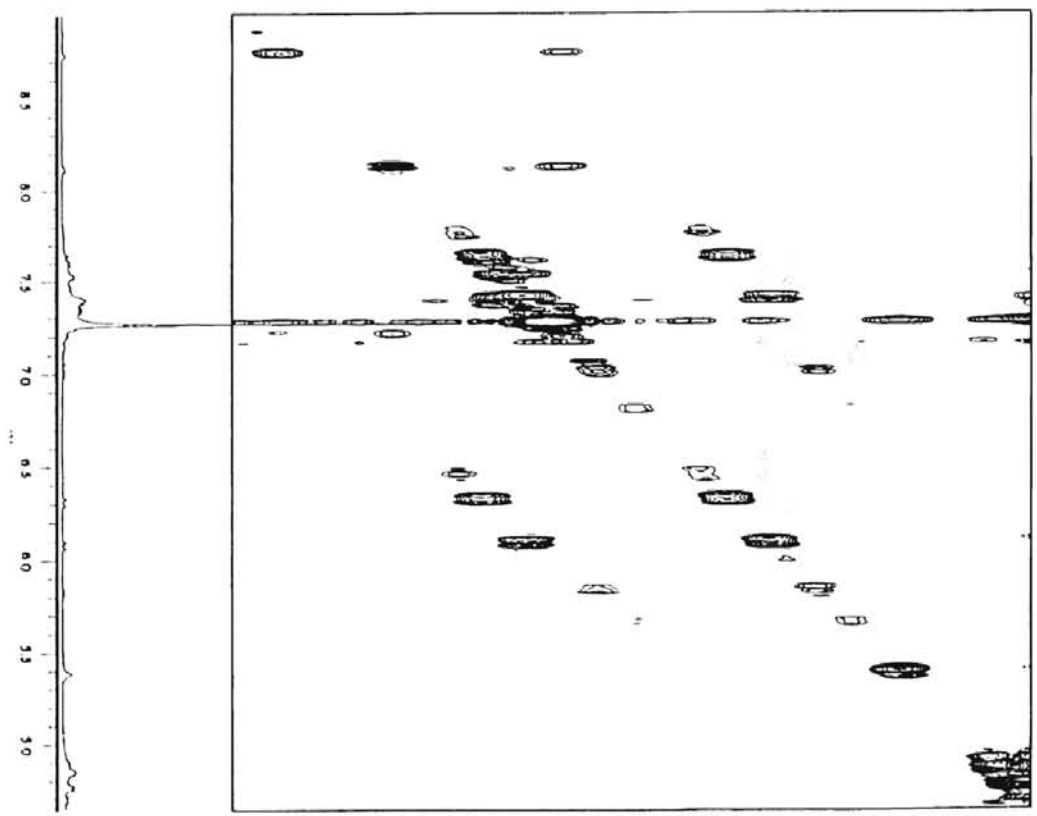
OBSERVE:
f1 FREQ = 500.1351150 MHz
SPEC WIDTH = 6172.84 Hz
SPEC OFFSET = 2325.60 Hz
V. FREQ = 500.1327894 MHz
V. SPEC WD = 6172.84 Hz
V. SPEC OF = 2325.60 Hz
GAIN = 160.0
POWER LEVEL = 60
LOW POWER = ON

DECOUPLER:
f2 FREQUENCY = 500.1352900 MHz
f2 POWER = 0 db
f2 MODULATION = CW
f3 FREQUENCY = 125.7713594 MHz
f3 POWER = 0 db
f3 MODULATION = CW

PROCESSING:
PHASE A = 0.00
PHASE B = 0.00

PLOT RANGE:
X From 8.95 TO 4.64 ppm
Y From 8.96 TO 4.67 ppm

CONTOURING:
LEVELS = 13
SPACING = LOG
POLARITY = +
SCALING = GLOBAL
FLOOR = 0.2820 %
CEILING = 17.7600 %



GE NMR OMEGA

...nda/data/dj3116A_cosy_pro

Date: Feb 24 12:29:42.6 1997

OPERATOR: ********

ACQ TIME = 0.17 sec
DATA SIZE = 1024 * 1024
NUM OF BLKS = 128
NUM OF SCANS = 64

PULSE SEQUENCE:
SEQUENCE NAME = f1cosy_ntypo60.s

OBSERVE:
f1 FREQ = 500.1351150 MHz
SPEC WIDTH = 6172.84 Hz
SPEC OFFSET = 2325.60 Hz
V. FREQ = 500.1327894 MHz
V. SPEC WD = 6172.84 Hz
V. SPEC OF = 2325.60 Hz
GAIN = 160
POWER LEVEL = 60
LOW POWER = ON

DECOUPLER:
f2 FREQUENCY = 500.1352900 MHz
f2 POWER = 0 db
f2 MODULATION = CW
f3 FREQUENCY = 125.7713594 MHz
f3 POWER = 0 db
f3 MODULATION = CW

PROCESSING:
PHASE A = 0.00
PHASE B = 0.00

PLOT RANGE:
X From 8.95 TO 4.64 ppm
Y From 8.96 TO 4.67 ppm

CONTOURING:
LEVELS = 13
SPACING = LOG
POLARITY = +
SCALING = GLOBAL
FLOOR = 0.2820 %
CEILING = 17.7600 %

APPENDIX H

CHROMATOGRAM FROM REVERSE PHASE HPLC ON FRACTIONS 25A AND 16A

Column: Vydac 3 micron 4.6 x 100 mm 90 Angstrom C18

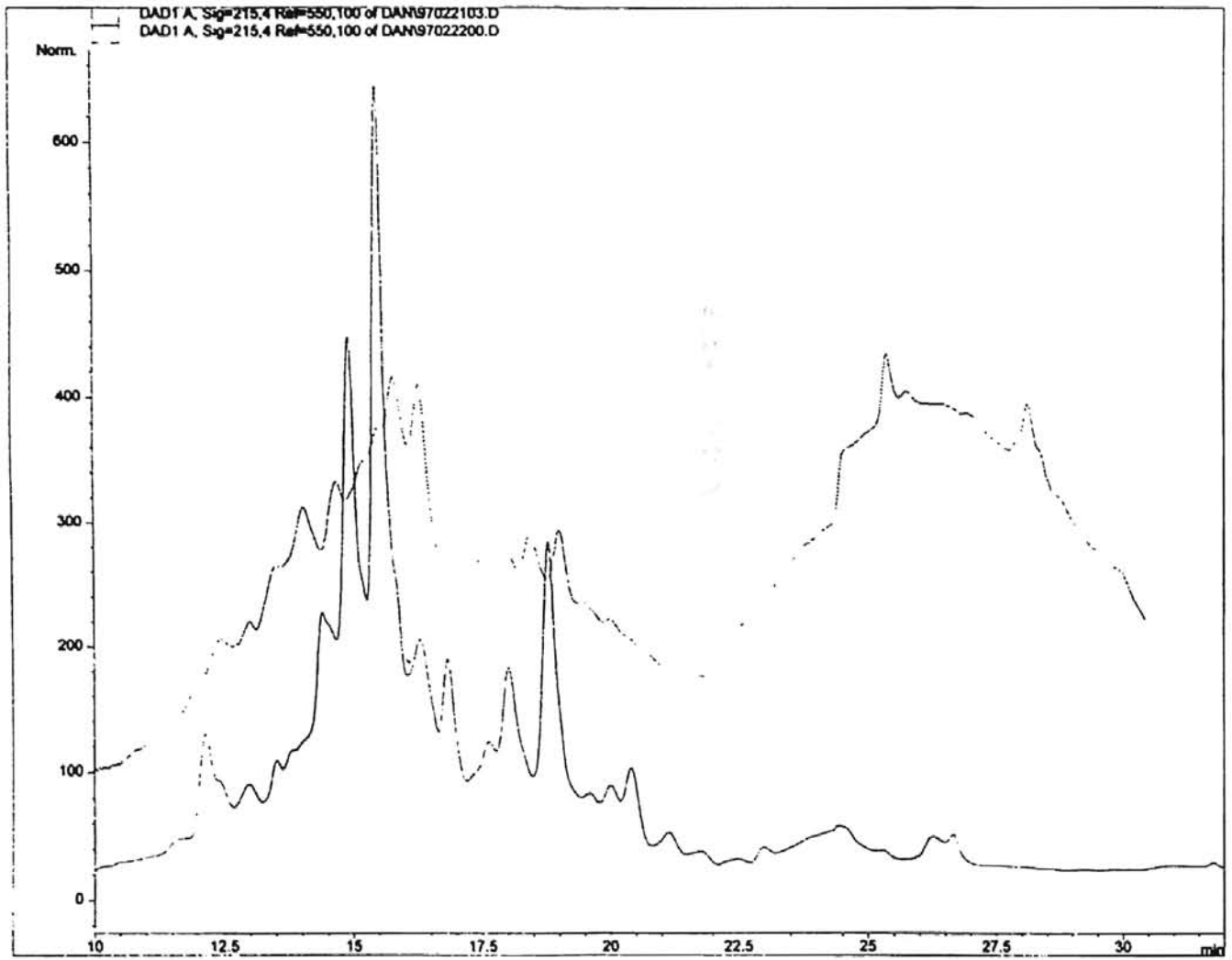
Conditions: Solvent A = H_2O Solvent B = Acetonitrile

0-5 minutes 60% A to 25% A at 25 minutes to
0% A at 40 minutes hold 0% A until 45 minutes

Flow rate: 1 ml per minute

Fraction
Collection: 30 seconds

Current Chromatogram(s)



Diode Array 2/22/97 5:32:47 PM gregg

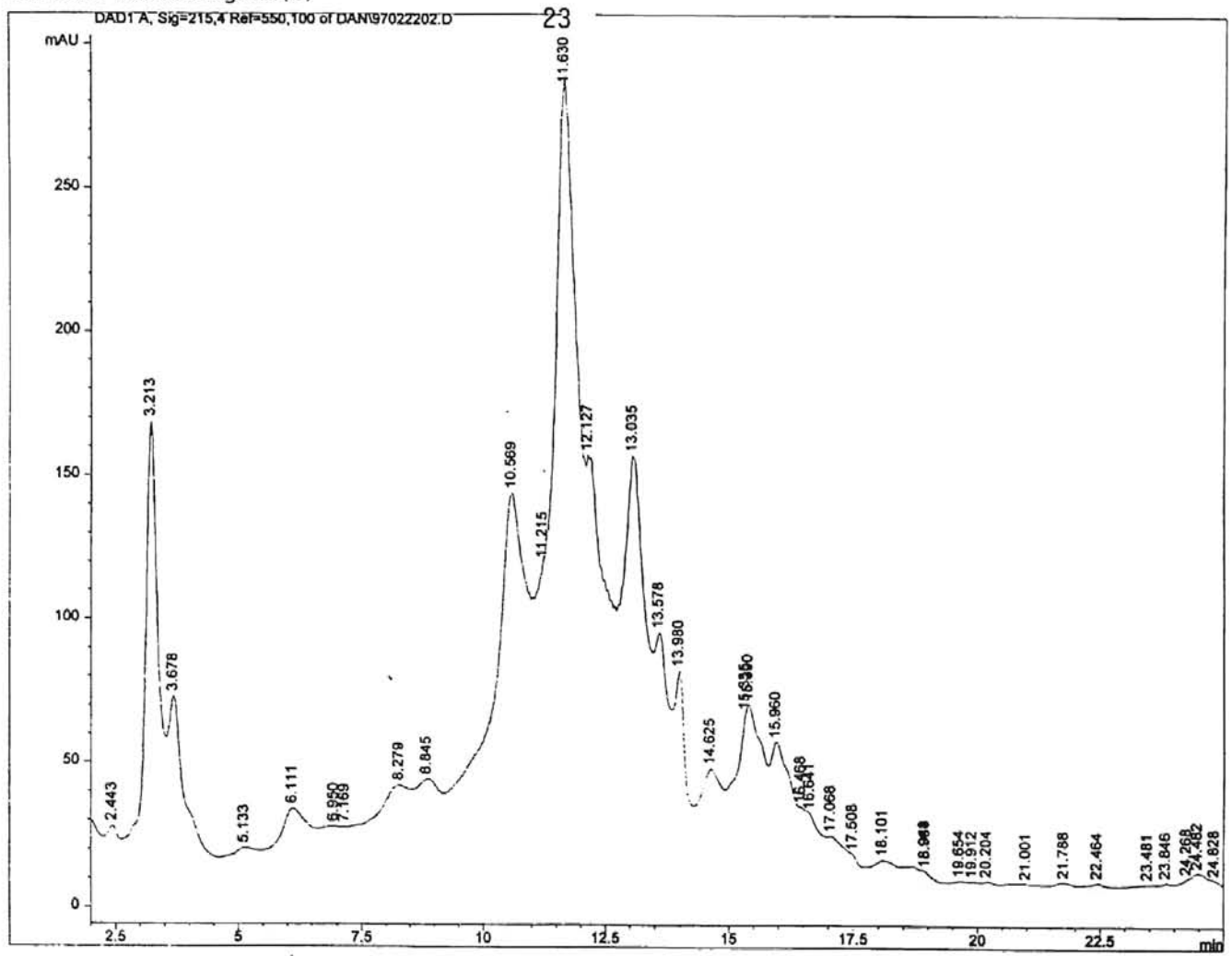
APPENDIX I

REVERSE PHASE HPLC AND LOW RESOLUTION ELECTROSPRAY MASS SPECTROMETRY ON FRACTION 16A

AFTER MILD METHANOLYSIS
(AGLYCONES ONLY)

Print of window 38: Current Chromatogram(s)

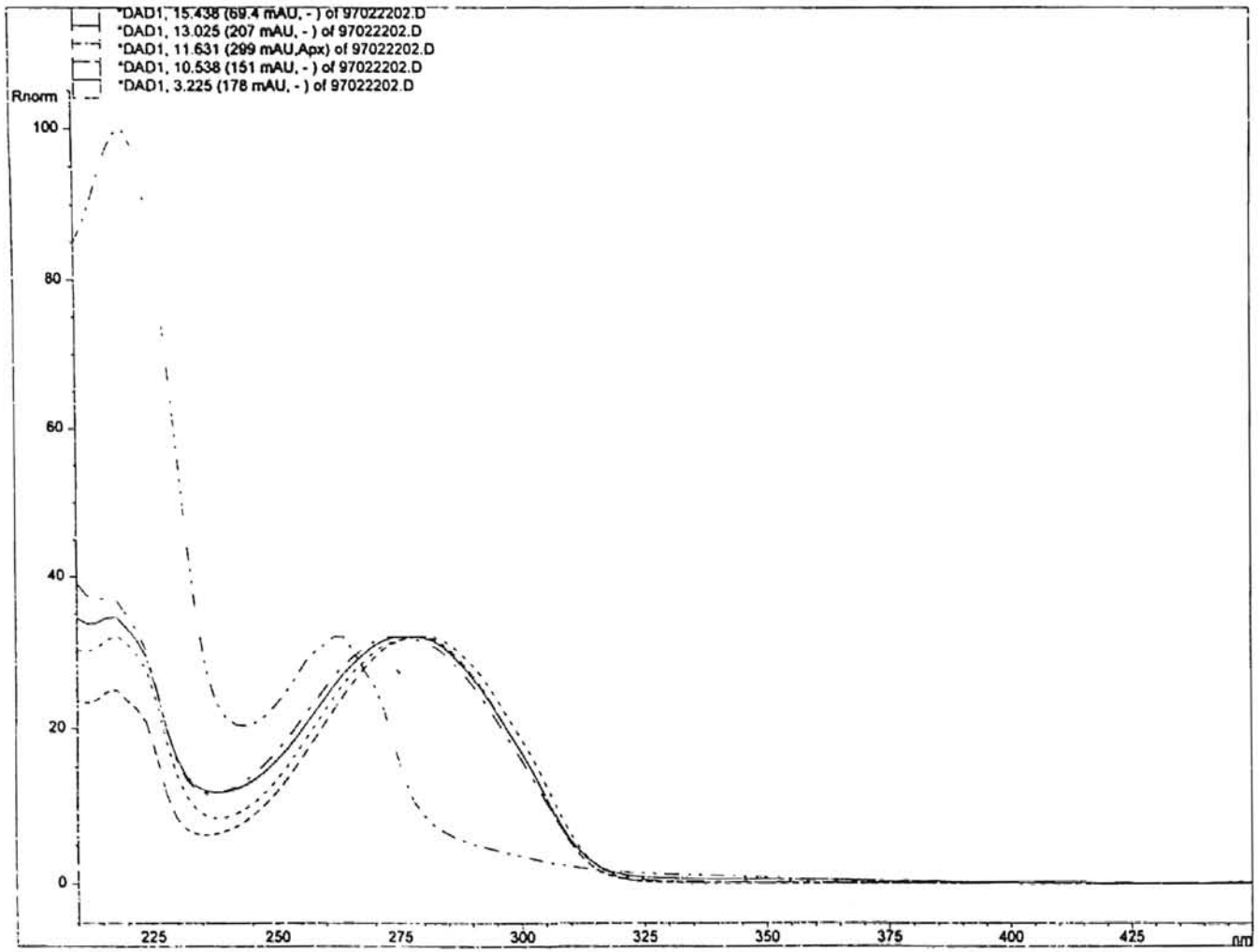
Current Chromatogram(s)



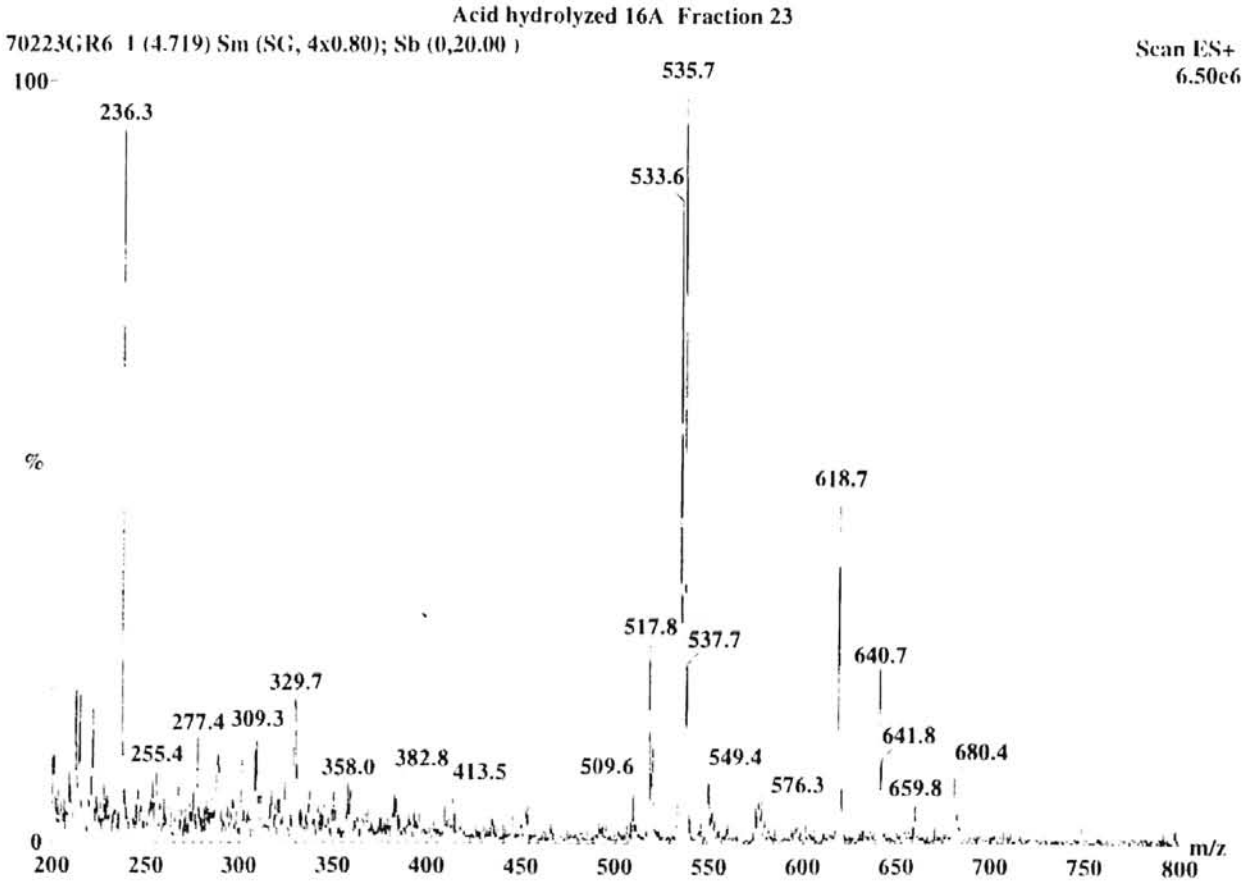
Diode Array 2/22/97 9:10:32 PM gregg

Print of window 39: DAD1, 3.225 (178 mAU, -) of 97022202.D

DAD1, 3.225 (178 mAU, -) of 97022202.D

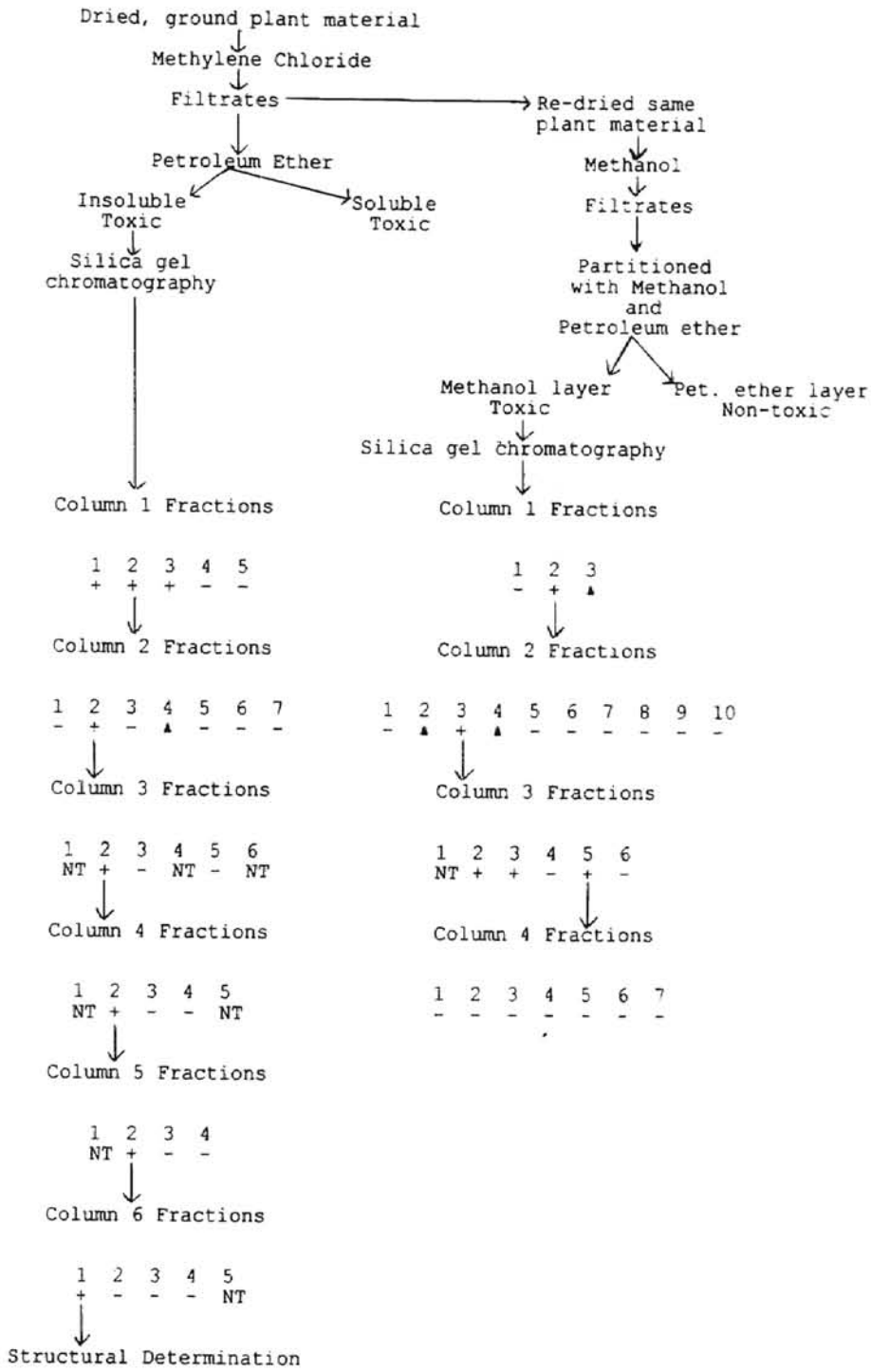


Diode Array 2/22/97 9:09:05 PM gregg


Acid hydrolyzed 16A Fraction 23
70223GR6 1 (4.719) Sm (SG, 4x0.80); Sb (0,20.00)
Scan ES+
6.50e6
100
%
0
236.3
535.7
533.6
618.7
517.8
537.7
640.7
329.7
277.4
309.3
641.8
680.4
255.4
358.0
382.8
413.5
509.6
549.4
576.3
659.8
m/z
200
250
300
350
400
450
500
550
600
650
700
750
800

APPENDIX J

FLOW CHART ON BIOASSAY RESULTS



+ = Toxic
- = Non-toxic
▲ = Slightly toxic
NT = Not tested due to insufficient amount for bioassay

VITA

Gregg Lea Humpert Robinson

Candidate for the Degree of

Master of Science

Thesis: INVESTIGATIONS OF TOXIC PLANTS: *ALBIZIA JULIBRISSIN* AND *ASCLEPIAS SUBVERTICILLATA*

Major Field: Botany

Biographical:

Education: Graduated from Broken Arrow High School, Broken Arrow, Oklahoma in May 1979; Tulsa Community College, Tulsa, Oklahoma; Oklahoma City Community College, Oklahoma City, Oklahoma; Oklahoma State University-Oklahoma City, Oklahoma City, Oklahoma; Northern Oklahoma College, Tonkawa, Oklahoma; University of Oklahoma Biological Station, Kingston, Oklahoma; received Bachelor of Science degree in Botany from Oklahoma State University, Stillwater, Oklahoma in December 1994. Completed the requirements for the Master of Science degree with a major in Botany at Oklahoma State University in May 1997.

Experience: Employed by Employer's Insurance of Wausau as a Commercial Service Office Correspondent in Oklahoma City, Oklahoma, 1980 to 1983; Ben Kennedy & Associates as a Commercial Property and Casualty Underwriting in Oklahoma City, Oklahoma, 1983 to 1985; Meyers-Reynolds & Associates as an Account Executive in Oklahoma City, Oklahoma, 1985 to 1991; Oklahoma State University, Department of Botany, as an Undergraduate Teaching Assistant, 1994 and as a Graduate Teaching Assistant, 1995 to present.

Professional Memberships: Phi Kappa Phi, Golden Key National Honor Society, and Oklahoma Native Plant Society.

Grants: James K. McPherson Memorial Fund, $225 for research in 1995, $210 for research in 1996, and $200 for out-of-state symposium registration fee in 1997; Oklahoma Agricultural Experiment Station, $4,000 for research in 1996/1997 with G.E. Burrows and R.J. Tyrl.

Papers Presented: Vitamin B_6 antagonist in *Albizia julibrissin* (mimosa) legumes. 85th annual meeting of the Oklahoma Academy of Science, November 1996.

Investigation of the neurotoxic compounds in *Asclepias subverticillata* (western-whorled milkweed). Facility for Advanced Instrumentation, University of California at Davis, February 1997.

Papers to be Presented: Investigation of the neurotoxic compounds in *Asclepias subverticillata* (western-whorled milkweed). 5th International Symposium on Poisonous Plants, May 1997.

Evaluation of the toxic effects of the legumes of *Albizia julibrissin* (mimosa) and identification of the toxicant. 5th International Symposium on Poisonous Plants, May 1997.