HYDROPHILIC FLAVONOID COMPONENTS OF LEAVES AND FLOWER HEADS OF POLYGONUM SENEGALENSE AND THE LARVICIDAL AND ANTIFEEDANT ACTIVITIES OF ITS HYDROPHOBIC PRINCIPLES

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BY

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A thesis submitted in partial fulfilment for the Degree of Master of Science of the University of Nairobi.

APRIL, 1991

This thesis is my original work and has not been presented for a degree in any University.

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Dedicated to

A. Waithera and Gikonyo.

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SUMMARY

Polygonum senegalense is a highland weed in eastern region of the African continent. Two forms are known to exist: forma senegalense and forma albomentosum. Both these forms are found in Kenya and are known to contain flavonoids. *P. senegalense* has two sets of flavonoids; a large number, wholly aglycones, is found on the leaf surface and on flowerheads while a lesser number is found in the inner tissues; existing mostly as glycosides.

The surface flavonoid aglycones were first removed by washing leaves and flowerheads of *P. senegalense* in acetone. The leaves were dried, macerated and cold extracted using methanol in water. The aqueous extract was washed in dichloromethane to remove any remaining aglycones before acid hydrolysis. The aqueous hydrolysate was partitioned into dichloromethane and then ethyl acetate. Chromatographic separation followed by spectroscopic (UV, IR, MS, ^{1}H NMR, ^{13}C NMR) analysis revealed that the inner tissue aglycones from the flowerheads are similar to those of the leaf, and that they are different from those on the surface. Four compounds were isolated from the dichloromethane fraction, two of which were the anthraquinones chrysophanol (2) and emodin (3), the others were

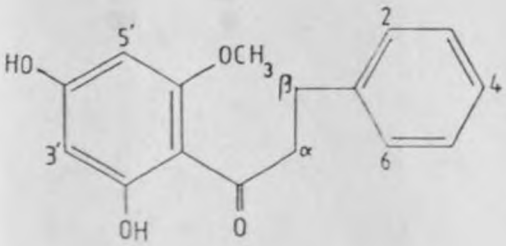
2',4'-dihydroxy-6'-methoxydihydrochalcone (1) and 3,7-dihydroxy-5,8-dimethoxyflavanol (4). These latter two are most likely remnants of surface flavonoids. The ethyl acetate fraction yielded two flavonols, 3,5,7,3',4'-pentahydroxyflavonol (5) (quercetin); and 3,5,7,4'-tetrahydroxyflavonol (6) (kaempferol). Another compound in trace amounts could not be isolated from this fraction.

Compound (7), 2',6'-dihydroxy-4'-methoxydihydrochalcone, isolated from the leaf resin, was found to have a high larvicidal activity against second instar larvae of *Aedes aegypti* and had an L_{D50} of 11.85 mg/l, 4.38 mg/l and 2.26 mg/l within 1, 4 and 7 days respectively as seen by probit analysis. For the same days as above, the acetone leaf wash (crude) had L_{D50} of 11.21 mg/l, 5.75 mg/l and 3.98 mg/l while compound (5) had L_{D50} of 16.15 mg/l, 12.27 mg/l and 8.50 mg/l for the same activity. Compound 4 and the crude ethyl acetate soluble fraction were found to have no larvicidal activity against *Aedes aegypti*.

Antifeedant activities were performed on 5th instar nymphs of the desert locust *Schistocerca gregaria* and at a concentration of 100 ug/ml of test material on a Whatman No1 filter paper, the crude acetone leaf wash was found to have a Relative Antifeedant Percentage (RAP) of 100 while the

components of the hydrolysate were found to be phagostimulatory. The crude ethyl acetate fraction of the hydrolysate had an RAP of -79.6 while the crude dichloromethane fraction of the same had an RAP of -59.1. Compound (5) had an RAP of -89.4 while compound (4) had an RAP of -45.1.

The acetate of compound (8), 2'-hydroxy-4',6'-dimethoxychalcone, had an RAP of 52.2 while the acetate of compound (9), 2',4'-dihydroxy-3',6'-dimethoxychalcone, had an RAP of 65.0. Both compounds (8) and (9) were isolated from the leaf resin and were found to have no appreciable antifeedant activities, i.e. RAP of approximately zero.



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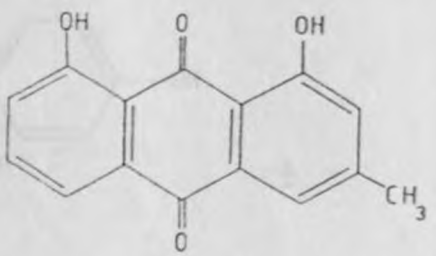

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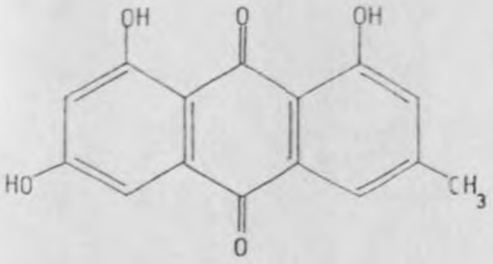

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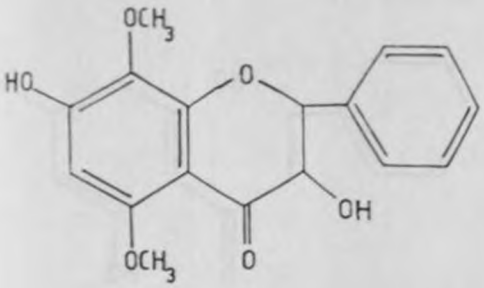

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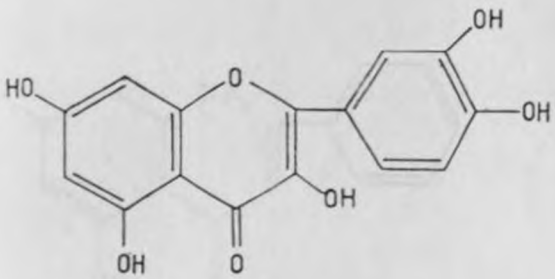

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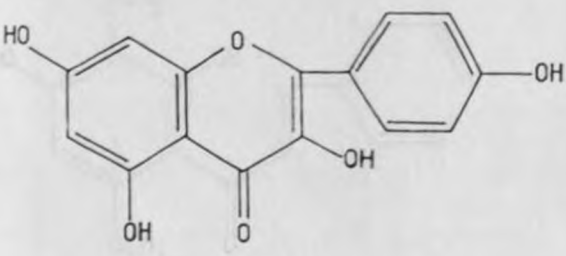

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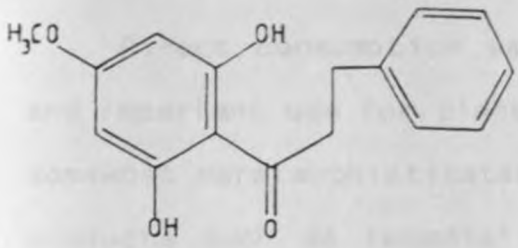

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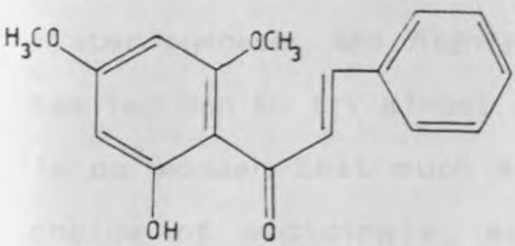

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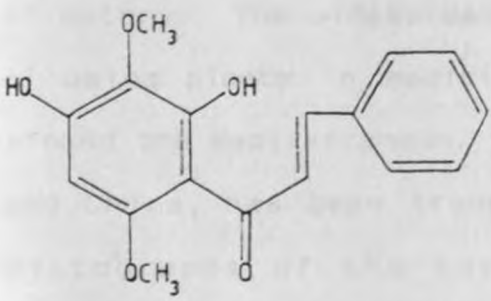

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CHAPTER 1

INTRODUCTION

Direct consumption as food is the most obvious and important use for plants, after which man turns to somewhat more sophisticated usage of plants and plant products such as remedial drugs, poisons,stimulants, pesticides etc. Mankind has been especially concerned with and awed by curatives, which until recent years were almost exclusively derived from plant sources, often from species found in the wild. The fear, mysteriousness, and highly personal nature of disease has led man to try almost any remedy offering hope. It is no wonder that much superstition surrounds the choice of medicinals, especially under primitive conditions where symptoms rather than causes must be attacked.

The history of drugs is largely the early history of botany. The widespread and long-standing practice of using plants in medicine in Eurasia, especially around the Mediterranean, the sub-continent of India , and China, has been transcribed to us through the pictographs of the Egyptians, the clay tablet ideographs of the Babylonian and the Vedic Sanskrit. About 75 B.C. the famed Dioscorides discussed in

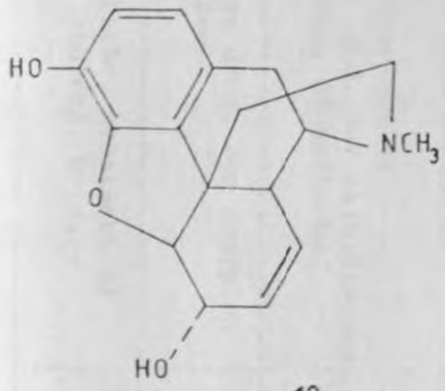
detail several thousand botanicals in the outstanding De Materia Medica; a book destined to become the authoritative reference concerning medicinal plants for the next fifteen centuries[1]. Other notable contributions to medicine were from Hippocrates (460 - 377 B.C.) who was well acquainted with oil of wintergreen (mainly methyl salicylate); recommended for many maladies, and Galen (AD 131 - 200)[2]. In the Middle Ages little new was learned, but the advent of the herbalists in the fifteenth to seventeenth centuries heralded the beginning of modern botany. The medicine men of North America and those of Africa (herbalists), and Asia (shamans), are all therapists since they treat patients with efficacious medicines, just like the formally trained therapists dealing with psychiatry in cosmopolitan or modern Western - derived medicines. Some of these local indigenous practices are organized for example the Ga Medical Association,Ghana, and the Kenya Herbalists Association, but nowhere is traditional medicine as extensive or widely accepted as in the Peoples Republic of China. There, traditional medicinal techniques uniquely involving herbology and acupuncture have been fused with cosmopolitan medicine to form the New Chinese medicine[3].

The biomedical scientist of today who does consider early practices and procedures often finds data important to modern medical therapy and practice, as might be exemplified by the neglected field of ethnomedicine. A combination of data from the empirical method and the most elaborate experimental laboratory and clinical procedures has given us a number of man's most startling and important contributions to the well-being and health of the species. For example the Chinese realized 4000 years ago that green mold applied to skin ulcers aided in their cure and stopped the festering. Pasteur, too, realized that his disease - causing bacterial cultures might be destroyed by other micro-organisms. But practical application to this ecological fact of life among micro-organisms had to await Sir Alexander Fleming's observation that a green mold (Penicillium) contaminating his bacterial cultures destroyed the bacteria[2]. From this chance observation, made in a British hospital in 1928, arose the modern concept of pitting one micro-organism against another to prevent diseases. Plant products that are harmful to man were given as much attention as those with remedial principles by the primeval man. The apple seeds contain cyanide, which may be lethal in large doses,

the alkaloid taxine from the common bedding plant English yew is rapidly absorbed and causes sudden death. The leaves and twigs of boxwood produce the alkaloid buxine, which contributes to respiratory failure in humans and domestic animals. Willows and poplars from North temperate America and Eurasia contain an aspirin like compound and were used to relieve fever and pain. Opium alkaloids also relieve pain and morphine (10) became an analgesic in cosmopolitan medicine. The curare plants were used for arrow poisons by the South American Indians and are presently used reversibly to paralyse skeletal muscles by surgeons. The treatment of glaucoma with alkaloids from the calabar been of Nigeria or leaves of Brazilian species of Pilocarpus, can prevent blindness. Over the ages man has discovered plants which can be used for pleasure. These plants having psychoactive properties may be classified as stimulants, depressants, and hallucinogens. Two of the most powerful natural stimulants are cocaine (11) from Coca (*Erythroxylum coca*), a plant native to the Andean highlands of South America, and Chat from *Catha edulis*, native from Arabia through Eastern Africa to the southern cape of Africa. The stimulating beverages include coffee, tea, cocoa, chocolate, they contain

caffeine (12), nicotine (13) and or other stimulants.[3]

For the primeval man to classify plants or any part of the plant as poisonous, curative or stimulant, he had to take great risk of poisoning. But the modern technology has made it far much easier, by first understanding the chemistry of the plants. Moreover, the modern technology has allowed us to understand the efficacy of plants used by the early man[4] as can be seen from Table 1.



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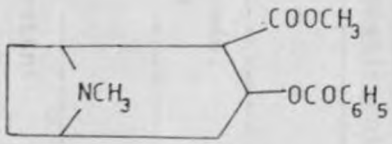

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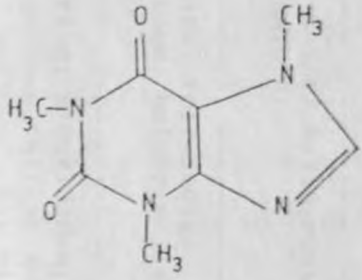

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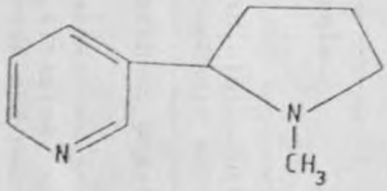

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Table 1 — Some medicinal plants, their traditional use and Chemical Constituents

Family and Species	Traditional use	Constituents
Liliaceae Aloe,vera L.	Purgative, treatment of wounds, swellings, burns and eczema	Aloe -emodin,aloesin,aloin,anthranol, dihydroxy-anthraquinone,chrysophanic acid, amino acids,etc
Zingibereae Amomium xanthioides	Antipyretic,diuretic, antiasthmatic,expectorant	Essential oils, flavones, p-methoxy-trans-ethyl cinnamate.
Bromeliaceae *Ananas comosus*	Diuretic, anthelmintic, digestant abortifacient expectorant.	Flavonoids, sterols,vitamins, aldehydes, alcohols alcohols, organic acids and esters, bromelain, amino acids etc.
Meliaceae Azadirachta indica	Antipyretic, bittertonic, antidysenteric,for skin diseases, insecticide.	fatty acids, nimbin, nimbinin,nimbidin, quercetin sitosterol, azadirachtin, nimocinolide and other terpenoids.
Bixaceae *Bixa orellana*	Colouring agent in food, for skin disease and burns,lemostatic, stomachache, antipyretic.	bixin, norbixin, methyl bixin,resin, tannin, fixed phytosterol Vitamin A.
Boraginaceae *Heliotropium indicum*	Antipyretic, as eye drop, for scorpion stings and dog bites.	Indicine, indicine, retronecine, proteins echinatine, supinine, heleurine,lasio carpine, indicine N- oxide
Piperaceae *Piper nigrum*	Carminative,stomachache, treatment of diarrhea, dysentry,cholera, Urinary calculus & headache	Enamides, feruperine, piperidines, piperoide, coumaperrine, piperines caffeic and p-coumaric acids, kaempferol and quercetin glycosides.
Verbenaceae *vitex trifolia*	expectorant, anthelmintic	Aucubin, agnuside, casticin, isorcentin, luteolin glucoside, fridalin, sitosterol, artemetin,a-pinene camphene and other terpenes, phenol, fatty acids.

1.10 PEST CONTROL

A mature civilization, with industrial complexes, the arts, and leisure, depends in large measure on man's ability to manage the ecosystem of which he is a part. Apart from his own diseases, man has to contend with plant diseases and pests notwithstanding other animal maladies. An important element in achieving increased efficiency in food production is reducing losses resulting from diseases and pest destruction. Population expansion has made pest control one of the world's most pressing problems.

All pesticides block some metabolic process. They differ, however in their composition, potency, mode of action, speed of effect, and dose requirements. Insecticide stomach poisons were once used against insects with biting mouth parts and are toxic when ingested, examples are arsenic and fluoride compounds. These are now replaced by contact poisons, which penetrate the integument of the pest and are particularly effective against insects with sucking mouth parts.

The naturally occurring contact insecticides are nicotine (13) from leaves of Nicotiana spp. (Solanaceae), pyrethroids from flowers of

Chrysanthemum cinerariifolium and other species (Asteraceae), and rotenone (14) from roots of *Derris* and *Lonchocarpus* (Fabaceae)[5]. These insecticides which are short lived are listed in Table 2 together with other natural insecticides and parasiticides used by native populations.

Synthetic contact insecticides are now the primary agents of chemical control. Some are synthetically modelled on plant extracts e.g. pyrethrins; but many stem from the development of a series of chlorinated hydrocarbons in the 1940s following the discovery of DDT as an insecticide. These include chlordane, lindane, toxaphane, aldrin, and dieldrin, most are long lasting, and their toxic effects are cummulative, therefore are being replaced by natural insecticides which have low mammalian toxicity and are biodegradable.

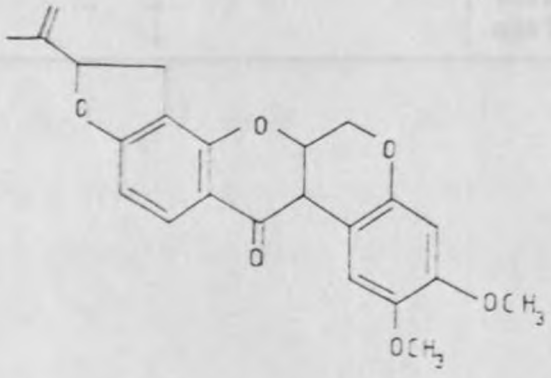

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Table 2

Plants used as insecticides and parasiticides

Angiosperm Family and Species	Locality	Remarks
APOCYNACEAE *Trachelospermum stars*	Mexico	Used to poison cockroaches
FABACEAE *Dolichos pseudopachyrrhizus*	Tropical Africa	Roots, source of rotenone
Derris elliptica, *D. uligonosa*	Old World tropics	Powdered root widely used as insecticide source of rotenone.
RUTACEAE *Clausena anisata*	Tropical Africa	Anise-scented leaves used to combat mosquitoes
ASTERACEAE *Chrysanthemum cinerariifolium*	Yugoslavia	Source of pyrethrum insect powder of dried, unexpanded flower heads containing the toxic principle pyrethron.
MELIACEAE *Melia azedarach*	Western Asia	Fruit powder an insecticide against flies.
RUBIACEAE *Adina cordifolia*	India	Sap an insecticide
Gardenia lucida	Burma	Source of combee resin, used to ward off flies.
SOLANACEAE *Nicotiana rustica*, *N. tabacum*	North America	Leaf dust containing nicotine widely used in former times by Indians against insects.

1.11 Insect Antifeedants

It is well known that special plant constituents function to establish interrelationships between insects and their food plants. An insect reaching or alighting on a plant is stimulated by attractants contained in the plant and inhibited by repellants. Feeding stimulants elicit a feeding response of insects, whereas insect feeding is inhibited by the presence of feeding deterrents or antifeedants in plants[6,7,8]. Antifeedant is defined as a feeding deterrent[9] and the first to be used in agriculture was the zinc salt of dimethyldithiocarbamic acid compound with cyclohexylamine. This was used to deter rodents and deer from feeding on the backs and twigs of trees in winter. Other compounds included chlorinated triphenyl methanes, triarylphosphines as well as a variety of triphenylphosphonium salts. Several botanical extracts have proved to have antifeedant activity for example pyrethrum which is stated to be a "gustatory repellant" against biting flies *Glossina* and *Culicoides*[10], azadirachtin from seeds of neem (*Azadirachta indica*) tree has activity against desert locust (*Schistocerca gregaria*) 5th

instar nymphs,[11,12] and *Melia volkensii* fruit kernels contain antifeedant activity against *Schistocerca gregaria* nymphs and adults.[13] Isolation and structure elucidation of insect antifeedant or feeding stimulants from plants not only provide basic knowledge about the interrelationships between insects and plants, but may also give indications for the development of agricultural chemicals to protect cultivated plants from their herbivores. Such information may also be useful for the breeding of insect-resistant varieties of crops.

1.20 CHEMICAL AND PHARMACOLOGICAL BACKGROUND FOR POLYGONUM SPECIES

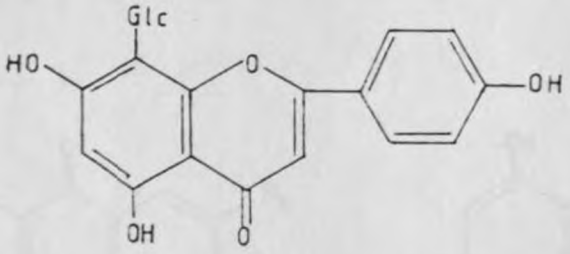
The Polygonum genus which belongs to the Polygonaceae family has been used by man in various ways ranging from food to poison. For example *Polygonum bistorta* was used to relieve toothache. Roots were mixed equally with *Anacyclus pyrethrum* (Asteraceae) and alum, beaten into paste with honey, and placed into carious teeth or held between teeth. *Polygonum punctatum* (water smartweed), containing about 7% calcium oxalate in leaves, is poisonous to man and may be fatal to livestock[14]. The seeds of *P.douglasii* were made into flour and used as food by

the Klamath and other tribes of Indians in Oregon and Washington in western North America. *P.cuspidatum* is used in Chinese Pharmacy; it contains cuspidatin and emodin and the root bark is source of a yellow dye.The roots of *P.fugax* Small are consumed by the Eskimos, while the young shoots of *P. sachalinense* are consumed as food by the Ainu. *P.aviculare*, a perenial herb has been used in some parts of Europe as a home remedy for lung complaints, hemorrhoids and rheumatism. A tea derived from it was used for asthma and bronchitis in Germany and Austria[15]. *P.salicifolium* has been a source of salt in many parts of Africa when the plants are burnt in bundles. This salt is licked as a cure for sore throats. Leaf decoction is used as a purgative, and extract from fresh leaves is used for skin troubles. The leaves of *P.pulchrum* are claimed to be used for syphilis. The leaf infusion is drunk about three times a day by the patient[16]. Recent work on polygonum plants has elucidated the chemical basis of their biological activities. For example some of the flavonoids isolated from *P. aviculare* were vitexin (15) isovitexin, luteolin (16), kaempherol -3-arabinoside (17), rhamnetin-3-galactoside (18), and quercetin-3-galactoside (19); vitexin and rhamnetin galactoside were found to inhibit aggregation of human

blood platelets under all experimental conditions, whereas luteolin and kaempherol arabinoside retarded or stimulated aggregation, depending on experimental conditions[17]. The rhizome of *P. sachalinense* has been used as a laxative, diuretics and for treatment of suppurative dermatitis, gonorrhea, favus and athletes foot. Physcion (20), emodin (21), emodin 8-O-ß-D-glucoside (22), anthraquinone derivatives and ß-sitosterol glucoside were isolated from the methanolic extract of the dried rhizome. Stilbene derivatives which have antibacterial and antifungal activities were also isolated[18]. Organic solvent extracts of whole plants of *P. cuspidatum*, *P. multiflorum*, *P. orientale* and *P. filiforme* are health food additives that decrease serum lipids and enhance metabolic activity of liver. The extracts contain the biologically active stilbenes resveratrol (23), bisade (24), and 2,3,4',5- tetrahydroxystilbene (25)[19]. From the methanol aqueous extract of the leaf of *P.glabrum* Willd; a semi aquatic Sudanese species, an anthelmintic substance was isolated. This compound (a terpenoid) also showed mulluscicidal activity against *Biomphalaria glabrata* and *Limnea truncatula* Mull[20]. Tryptanthrin (26) a specific antimicrobiol substance against dermatophytes, was isolated from

P.tinctorium[21]. From *P. nodosum* a flavonoid glycoside with molluscicidal activity was isolated. The compound was shown to be quercetin -3-ß-D- glucoside - 2"-gallate (27)[22].

The stilbene components of the roots of *P. cuspidatum*, resveratrol and piceid found to reduce triglyceride synthesis from ^{14}C-palmitate in the liver of mice, when the stilbenes were orally administered[23].



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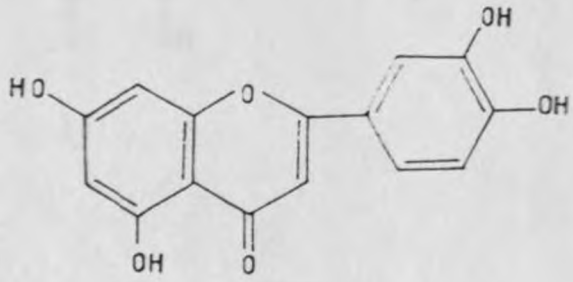

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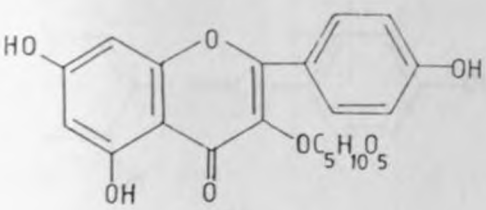

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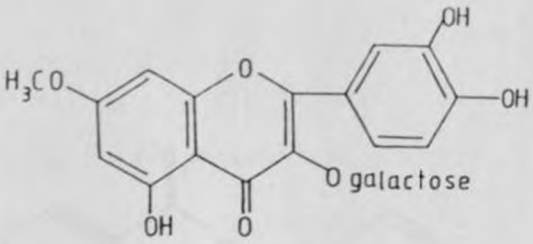

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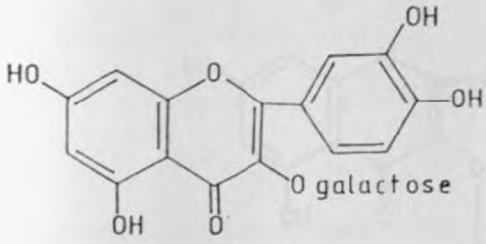

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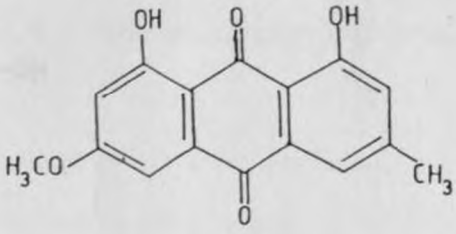

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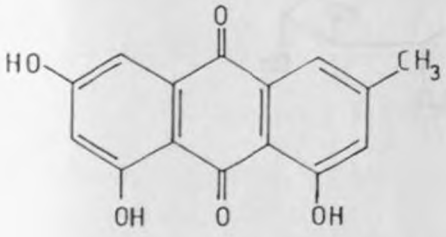

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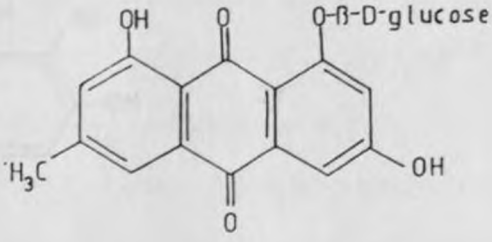

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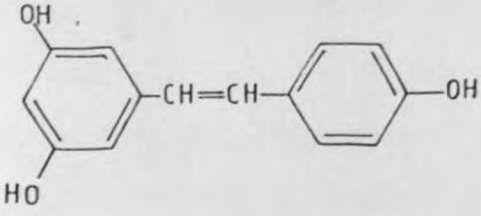

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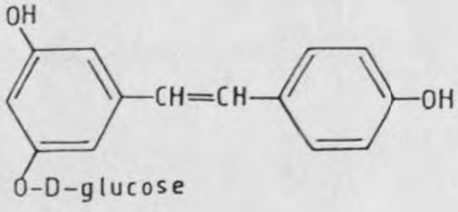

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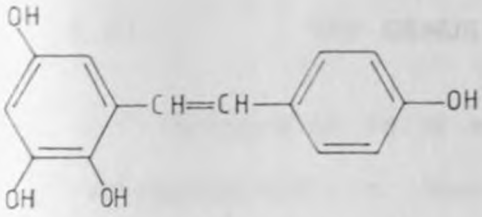

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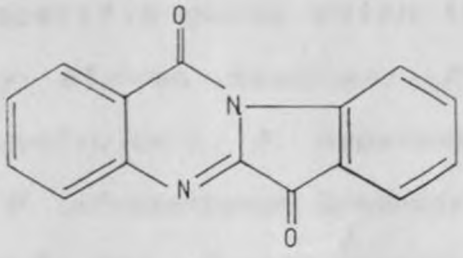

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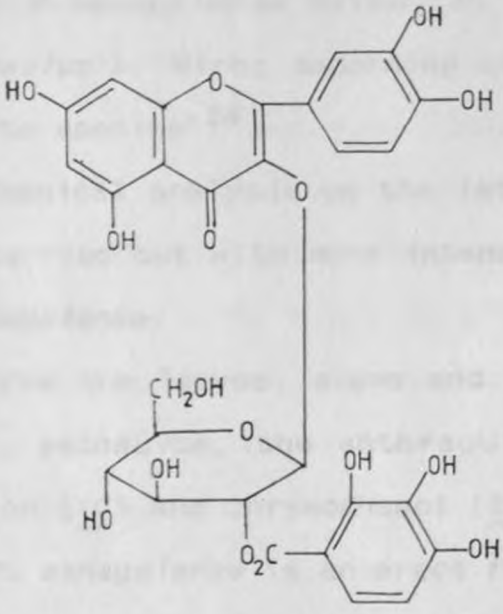

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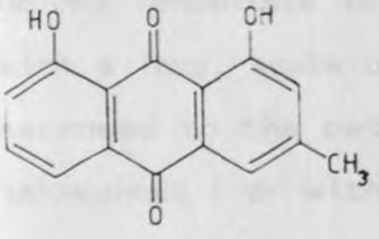

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1.21 THE GENUS POLYGONUM IN KENYA

Polygonum is a multi-specific genus which is represented in Kenya by eleven species: *P. baldschuriacum* Regel, *P. convolvulus* L, *P. nepalense* Meisn, *P. capitatum* Han, *P. afromantanum* Greenway, *P.aviculare* L, *P. strigosum* R. Br; *P. salicifolium* Willd, *P.senegalense* Meisn, *P. pulchrum* Blume and *P.setosulum* A. Rich; according to their order in the " Key to species"[24].

Chemical analysis on the latter four species has been carried out with more intense work being done on *P. senegalense.*

From the leaves, stems and roots of *P. pulchrum* and *P. setosulum*, the anthraquinones emodin (21), physcion (20) and chrysophanol (28) were isolated[25].

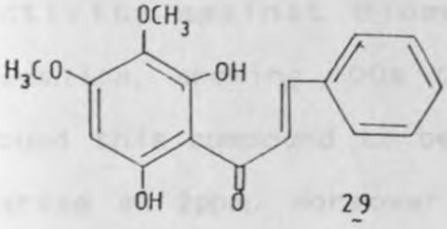
P. senegalense is an erect robust perennial plant which grows upto 3m tall. It has large petiolate, oblong lanceolate leaves which reach a size of 28x7cm with a long, acute or acuminate apex which is basally narrowed to the petiolate. Leaves may be glabrous throughout (or with short spiny bristles on the sub-surface mid - rib) to densely white on tomentose on both faces or predominantly below the undersurface covered with small yelowish glands. Petioles are upto

2.5 cm long, glabrous to tomentose. Peduncles are covered with orange glands, puberilent varying to white tomentose. The species exudes a yellow substance onto drying paper when pressed. There are two extreme forms: *forma senegalense* which has a glabrous whole plant with glabrous resinous leaves and *forma albomentosum*, with stems, leaves and peduncles thickly covered with white or yellowish tomentum: leaves are white or yellowish on both sides or more ashen above.

Intermediates between these extremes are known showing different degrees of hairiness. The leaves and flower - heads of both these forms when washed with non - polar solvent e.g. petroleum ether or acetone leads to observation of similar numerous flavonoids which are held on the leaf and flower head surfaces.

It has been surmised that the differences in leaf surface structure is moisture dependent with the tomentose leaves being observed mostly in drier habitats while the glabrous leaves (with flavonoids held in gummy material on the surface) are found under high moisture conditions[26]. The existence of flavonoids on the outer surface of the aerial parts of *P. senegalense* is intriguing but what is even more interesting is the array of compounds that have been isolated from the leaf resin. Wanjau (1989) isolated

several flavonoid aglycones from the leaf resin[27], among them: 2',6'-dihydroxy-3',4',-dimethoxy chalcone (29), 2',6',-dihydroxy -4'- methoxydihydrochalcone (30), 2',4'- dihydroxy - 3',6' - dimethoxychalcone (31), 2',6' - dihydroxy -4'- methoxy chalcone (32), 2',4' - dihydroxy -6'- methoxy chalcone (33), 2',5' - dihydroxy-3',4',6'-trimethoxy chalcone (34), 2'-hydroxy - 4',6' - dimethoxy Chalcone (35) and 5 - hydroxy - 7 -methoxy flavanone (36).



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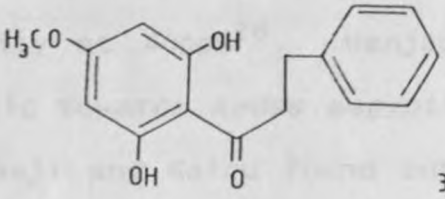

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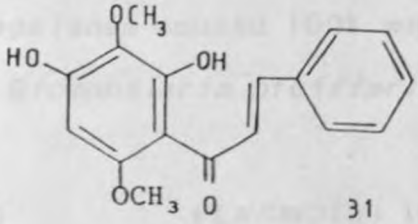

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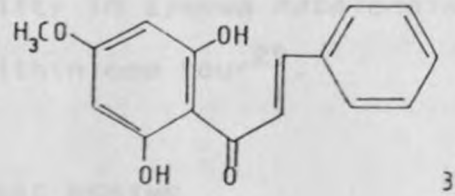

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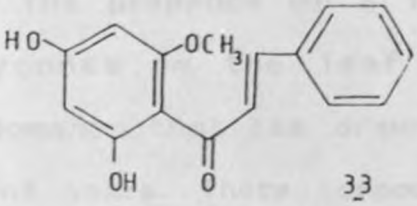

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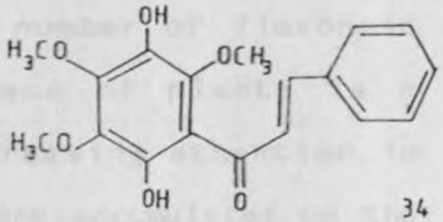

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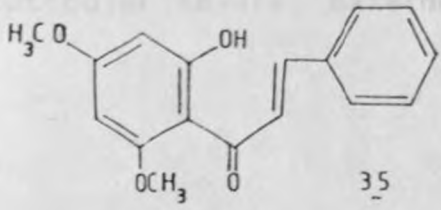

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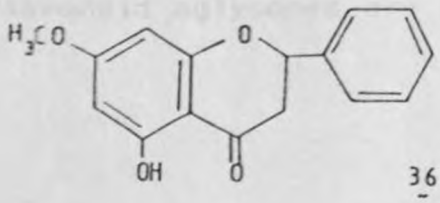

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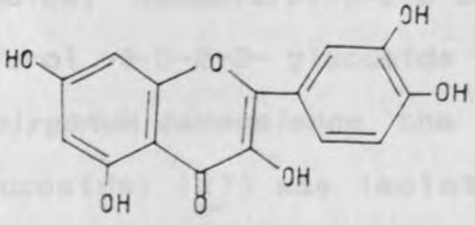
On carrying out biological activity tests on the above compounds, Wanjau found compound (30) to be toxic to the *Aedes aegypti* mosquito larvae at 0.8 ppm ; while compound (29) was found to be toxic towards *Aedes aegypti* mosquito larvae at 2 ppm. Compound (35) was found to be phago-stimulatory to *Schistocerca gregaria*. Compound (31) (2',4'-dihydroxy -3'-6'-dimethoxy Chalcone) was first reported by Marudufu (1978) from *P. senegalense* as having molluscicidal activity against *Biomphalaria pfeifferi* and *B. sudanica*, showing 100% toxicity at 40ppm[28]. Wanjau found this compound to be toxic towards *Aedes aegypti* larvae at 2ppm. Moreover Dossaji and Kairu found out that the crude undiluted aqueous extract of *P. senegalense* caused 100% mortality in *Lymnea natalensis* and *Biomphalaria pfeifferi* within one hour[29].

1.30 FLAVONOIDS IN LEAF RESINS

The presence of a large number of flavonoid aglycones on the leaf surface of plants is a phenomenon that has drawn increasing attention in recent years. These compounds are accumulated on the plant surface as constituents of leaf resins or thin epicuticular layers. External flavonoid aglycones are

found in various families throughout the higher plants,but appear to be most abudant in the *Asteraceae*[30]. Typically they exist on the leaf at 2-3% dry weight and are common amongst plants that would have originated from arid or semi-arid habitats. E. Wollenweber has done a great deal of work with the *Asteraceae*, he has isolated upto 40 different compounds from seven Baccharis species. He has worked with five species of *Ambrosia*, from Arizona, and isolated a series of flavones, flavonols and flavanones[31]. The structural diversity of the exudate flavonoids has been used for chemotaxonomy in the six species forming the *Achillea nobilis* group[32].

This surface phenomenon is not unique to the leaf; Wanjau (1989) found similar flavonoids covering the outer surface of flower heads of *P. senegalense* as on the leaf. In a much more rigorous work, Wiermann (1983) found high accumulation of flavonoids on the outer pollen wall of *Corylus orellana* [33].



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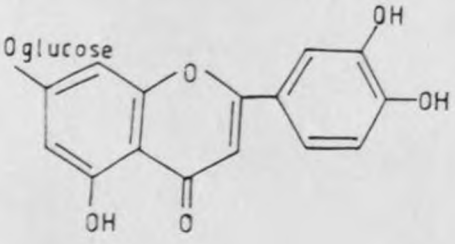


3.8

1.31 FLAVONOID GLYCOSIDES

Normally, the surface flavonoids are different from inner tissue flavonoids with greater variety usually on the surface[34]. The inner tissue flavonoids exist as glycosides dissolved in the cell sap that is located in the cell vacoule.

Flavonol glycosides are widely distributed in the polygonum species. Isobe (1987) working with the native polygonum species of Japan found out that quercetin glycosides were common in the species[35]. Kawasaki (1986), working with 28 species of Polygonaceae also found the quercetin glycosides quite frequent with 3-O-rhamnoside and 3-O- glucuronide widely distributed [36]. From *Polygonum lapothifolium*, Kulpina (1986) with his colleagues isolated 14 flavonoid glycosides[37] six of which were identified as: quercetin -3-O-D- glucopyranoside, quercetin-3-O-ß - D - galactopyranoside, quercetin - 3 - O - ß - D - glucoside - 2" - gallate, quercetin - 3 - O -α- L - arabofuranoside, kaempferol-3-O-ß-D-galactopyranoside and kaempferol -3-O-ß-D- glucoside - 2" - gallate.

For *Polygonum senegalense* the quercetin -3- (2"-galloyl glucoside) (27) was isolated by Dossaji[38]. Working with the Egyptian *P. senegalense*, Abdel-Gawad

and El-Zait[39], have reported observation of luteolin (16), quercetin (37), luteolin -7-O-glucoside (38) and quercetin -3-O-glucoside (19).

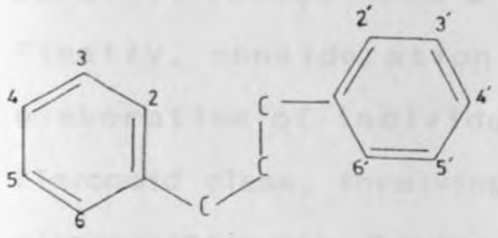
These compounds have not been observed on the leaf surface and may be constituents of inner milieu. In the isolation work with *Pericome caudata*, it was surprisingly observed that only four flavonol aglycones were accumulated on the aerial surfaces while the inner tissue yielded eight flavonols[40]. Therefore even inner tissue flavonoids may also yield desired variation. More importantly, flavonoid glycosides have also been shown by Dossaji and Kubo to possess molluscicidal activity against 3 species of snails, *Lymnae natalensis*, *Biomphalaria pfeifferi* and *B. glabratus*[41].

We have found it necessary to investigate the hydrophilic inner tissue flavonoids of the Kenyan *P. senegalense* more deeply so as to see the extent of variation. We will subject the compounds thereby isolated to biological activities especially larvicidal action against *Aedes aegypti* and antifeedant activity against nymphs of *Schistocerca gregaria* and *Locusta migratoria*. We wish also to determine the exact activity levels of some major hydrophobic chalcones through aquisation of activity curves.

1.32 BIOSYNTHESIS OF FLAVONOIDS

The flavonoids are polyphenolic compounds possessing 15 carbon atoms; two benzene rings are joined by a linear three carbon chain. This skeleton,shown in formula (39) can also be represented as the C_6- C_3-C_6 System. The flavonoids are thus 1,3-diarylpropanes. Isoflavonoids are 1,2-diarylpropanes, whilst the neoflavonoids are 1,1-diarylpropanes. The term 'flavonoid', assigned to this large class of natural substances derives from the most common groups of compounds, the flavones, such as (16); an oxygen bridge between the ortho-position of ring A and the benzylic carbon atom adjacent to ring-B forms new 4-pyrone type ring. Such heterocycles, at different oxidation levels are present in most plants[42]. The flavane structure (40) corresponds to the lowest oxidation level of the ring C, and is taken as the parent structure for the rational nomenclature of this group of compounds. An oxgyen bridge involving the central carbon atom of the C_3-chain occurs in a rather limited number of cases, where the resulting heterocycle is of the furan type, the C_{15}- skeleton of 2- benzyl - coumarane (41). The aurones, such as (42)

belong to this structural group. The oxygen bridge is absent in chalcones (43) which always exist in nature as glycosides. Besides the carbon atom link, the flavonoids also have typical oxygenation patterns in their benzene rings. The substituents can be -OH, -OCH_3, -O-CH_2-O-,or -O-glycosides.



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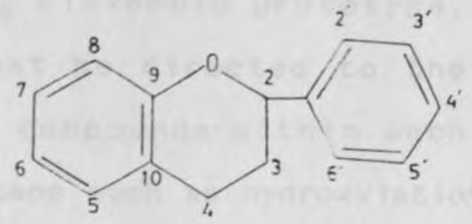

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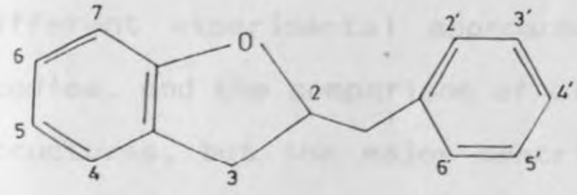

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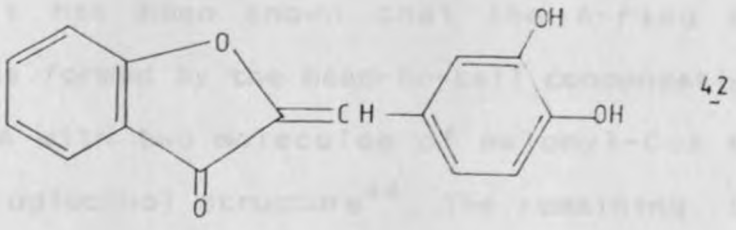

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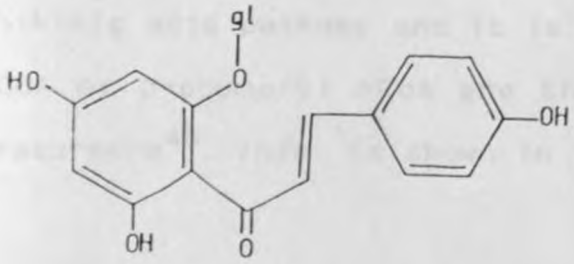

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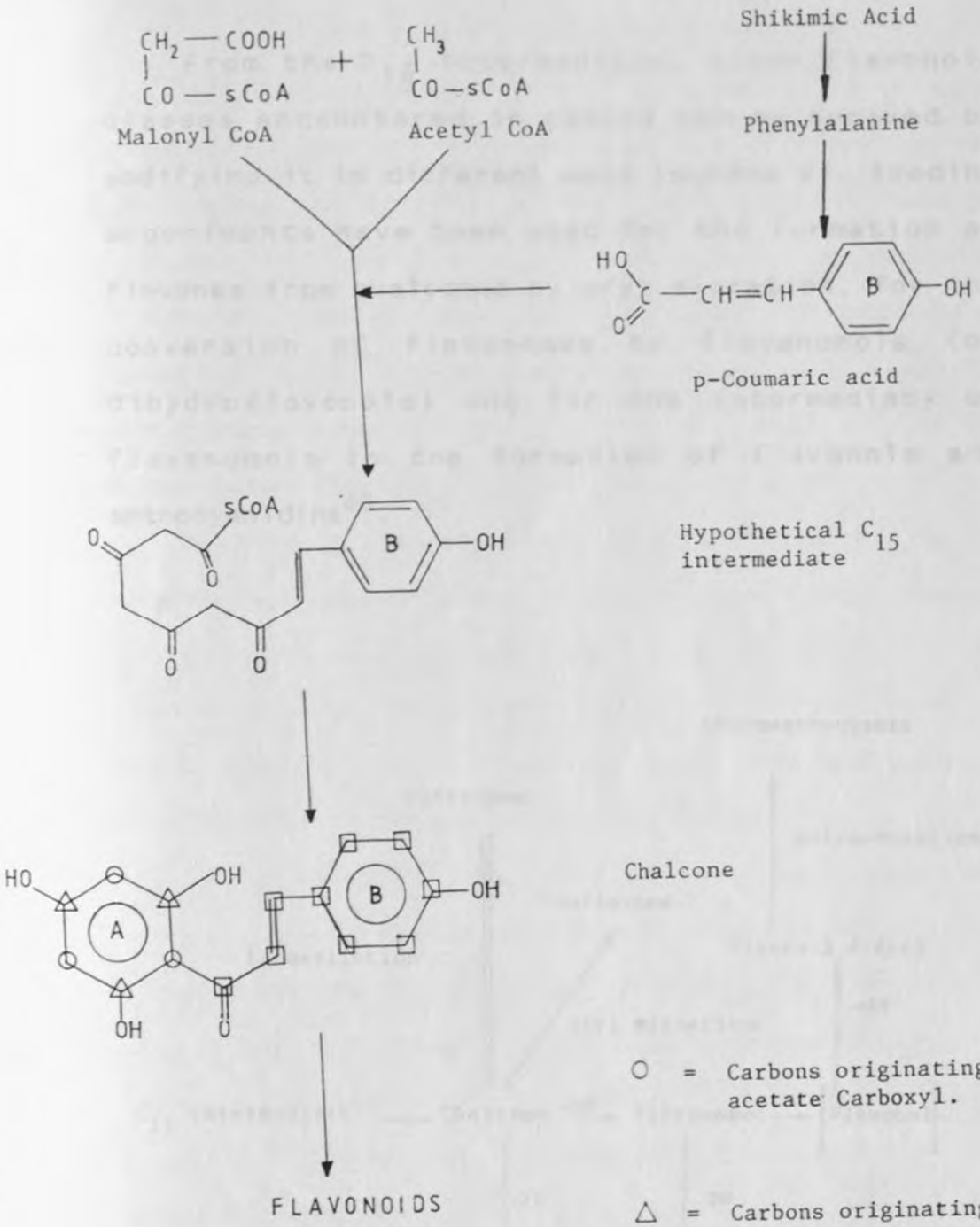
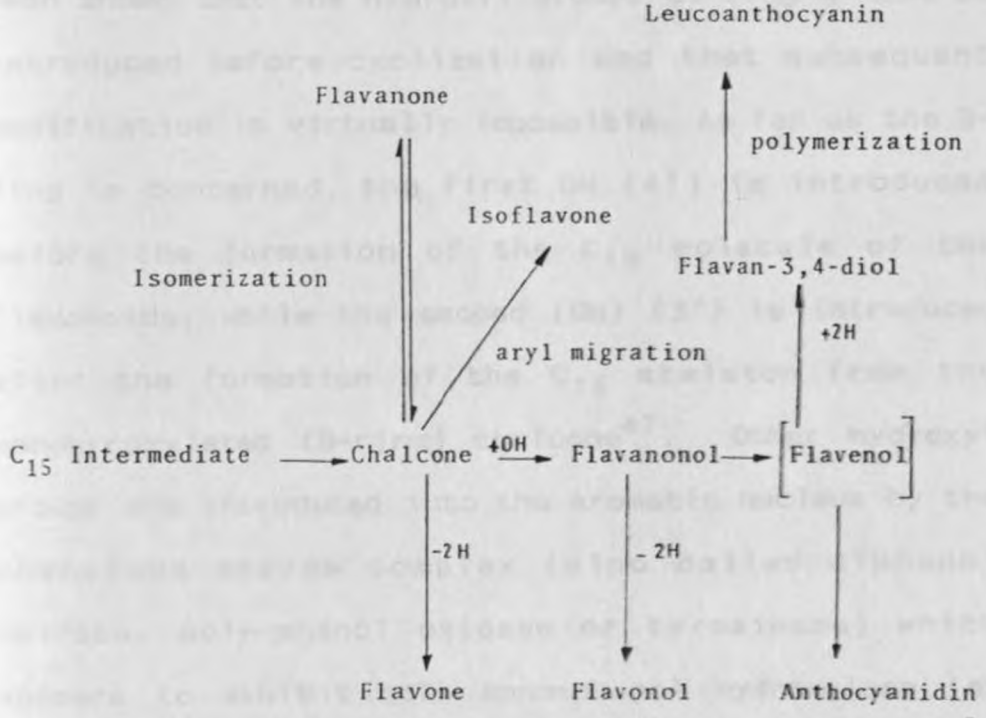
Flavonoid biosynthesis can be considered in three stages[43]. The first concerns the formation of the basic C_6 C_3 C_6 skeleton from a combination of the acetate - malonate and shikimic acid pathways to aromatic compounds. The next stage is concerned with the ways by which the different classes of flavonoids are synthesized by a combination of sequential and parallel routes from a C_{15} flavonoid prototype. Finally, consideration must be directed to the elaboration of individual compounds within each flavonoid class, involving steps such as hydroxylation, glycosylation etc. Evidence for the biosynthetic routes leading to the flavonoids has come from a number of different experimental approaches including genetic studies, and the comparison of closely related chemical structures, but the major contribution has come from tracer studies. By feeding plants with ^{14}C - labelled precursors it has been shown that the A-ring of flavonoids was formed by the head-to-tail condensation of acetyl-CoA with two molecules of malonyl-CoA to yield a phloroglucinol structure[44]. The remaining C_6 - C_3 phenylpropane unit is derived entirely from the shikimic acid pathway and it is thought that cinnamyl -CoA or p-coumaryl -CoA are the immediate C_6 - C_3 precursors[45]. This is shown in scheme 1.



Scheme 1

Proposed biosynthetic route to flavonoids.

From the C_{15} intermediate, other flavonoid classes encountered in nature can be derived by modifying it in different ways (scheme 2). Feeding experiments have been used for the formation of flavones from chalcones by aryl migration, for the conversion of flavanones to flavanonols (or dihydroflavonols) and for the intermediacy of flavanonols in the formation of flavonols and anthocyanidins[46].



Scheme 2

Interconversion of Flavonoid Compounds.

1.33 BIOSYNTHESIS OF SECONDARY FEATURES

The myriad of individual compounds within each flavonoid class are distinguished one from another by the number and orientation of hydroxyl groups and by the degree these groups are modified by methyl, isoprenyl, and glycosyl substituents. In addition, substitution by these groups may also take place at the carbon atoms of the ring giving rise to the C-substituted flavonoid compounds.

Hydroxylation of existing flavonoids certainly depends on whether it is in the A or B rings (Scheme 1). Using labelled chalcones as precursors, it has been shown that the hydroxyl groups of ring A must be introduced before cyclization and that subsequent modification is virtually impossible. As far as the B-ring is concerned, the first OH (4') is introduced before the formation of the C_{15} molecule of the flavonoids, while the second (OH) (3') is introduced after the formation of the C_{15} skeleton from the monohyroxylated (B-ring) chalcone[47]. Other hydroxyl groups are introduced into the aromatic nucleus by the phenolase enzyme complex (also called diphenol oxidase, poly-phenol oxidase or tyrosinase) which appears to exhibit both mono-phenol hydroxylase (at the 3 - position) and O - diphenol oxidase activity.

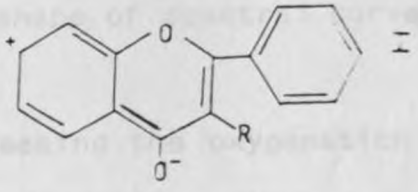
Methylation of the B-ring hydroxyl group may be effected by O-methyl transferases which use S-adenosyl - methionine as CH_3 donor[45].

1.34 IDENTIFICATION OF FLAVONOIDS

Qualitative identification of an unknown flavonoid compound can often be done by a study of preliminary chromatograms. It is possible to determine the type of flavonoid, whether or not it is a glycoside and the approximate number of hydroxyl groups, from the colour reactions and the RF values in selected solvents. By means of the colours with and without ammonia one can easily classify spots on analytical thin layer or paper chromatograms as either (1) anthocyanin, (2) flavone or flavonol, (3) anthochlor (aurone or chalcone) or (4) flavanone, isoflavone, leucoanthocyanin, or catechin as these four groups differ markedly .

One of the most valuable methods of identification of flavonoids is the use of Ultra Violet - visible (UV -VIS) spectrometry. The limiting structures involved in the mesomeric forms of the flavones (R = H) and flavonols (R=OH) are represented by (I), (II), and (III). They correspond with

different localisations of the free electrons. These bodies absorb in two regions of the UV spectrum, the first between 300 and 380 nm (band 1) and the second between 240 - and 280 nm (band2). Band 1 corresponds with the absorption of form (II) which involves the conjugation of the CO group with the B-ring, cinnamoyl system (IV), band2 being due to structure (I) which is due to conjugation of the CO group with the A -ring, benzoyl system[48].



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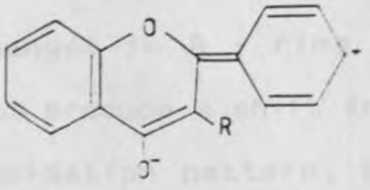
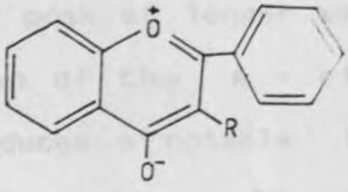
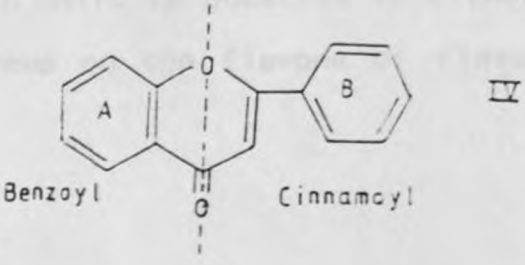


II



III



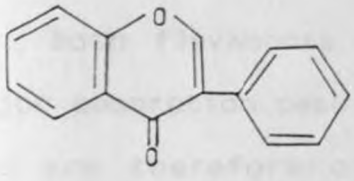
IV

The methanol spectrum, particularly the position of band 1, provides information about the type of flavonoid as well as its oxidation pattern, and especially distinguishes between flavones and 3 - hydroxyflavones (flavonols). Band 1 of flavones occurs in the range 304 - 350 nm whereas band 1 of flavonols appears at a longer wavelenghth (352 - 385nm). However, in flavonols with a substituted 3 - hyroxyl group (methylated or glycosylated), band 1 (328 - 357 nm) overlaps the region for band 1 in flavones, and the general shape of spectral curves approach those of flavones[49].

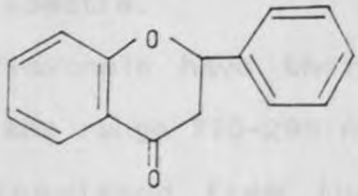
On increasing the oxygenation of the B - ring in flavones and flavonols, about a 3 - 8 nm bathochromic shift in band 1 occurs with each additional oxygen function. Changes in B - ring oxygenation pattern usually do not produce a shift in band 2. Depending on the B-ring oxidation pattern, band 2 may appear as either one or two peaks (designated IIa and IIb with IIa being the peak at longer wavelength). Increasing hydroxylation of the A - ring in flavones and flavonols produces a notable bathochromic shift in band 2 and a smaller effect on band 1. A hypsochromic shift is observed if either 3-,5-,or 4'-hydroxyl group on the flavone or flavonol nucleus is

methylated or glycosylated. Acetylation of a phenolic hydroxyl group nullifies the effect of that group on the UV spectrum [50].

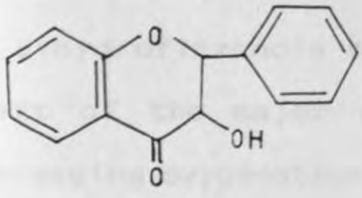
Isoflavones (V), Flavanones (VI) and Dihydroflavonols (VII) all give similar UV spectra as a result of their having little or no conjugation between the A- and B- rings.They are all readily distinguished from flavones and flavonols by their UV spectra, which typically exhibit an intense band 2 absorption with only a shoulder or low intensity peak representing band 1.



V Isoflavone skeleton



VI Flavanone skeleton



VII Dihydroflavonol skeleton

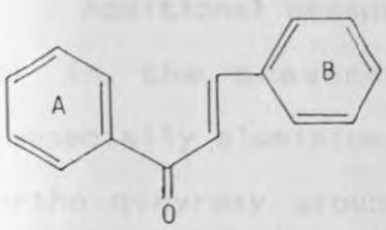
The band 2 absorption of isoflavones in methanol usually occurs in the region 245 - 270 nm, and is relatively unaffected by increasing hydroxylation of

the B-ring. Band 2 is however shifted bathochromically by increased oxygenation in the A-ring. Methylation or glycosylation of either 7- or 4'- hydroxyl groups in isoflavones has little or no effect on the UV spectrum while substitution of the 5-hydroxyl groups causes a 5-10 nm hypsochromic shift of band 2.

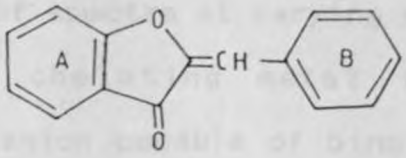
Using methanol as solvent, the UV spectra of dihydroflavonols are almost identical with those obtained for the equivalent flavanones; thus the presence or absence of the C-3 hydroxyl group in flavonoids which do not have a C_2 -C_3 double bond makes little difference to the UV spectra.

Both flavanones and dihydroflavonols have their major absorption peak (band 2) in the range 270-295 nm and are therefore clearly distinguished from the spectra of isoflavones (which have their band 2 peaks between 245 and 270 nm).

Removal of the 5-hydroxyl group from a flavanone or dihydroflavonols causes a 10-15 nm hypsochromic shift of the major absorption band, ie. band 2. Increasing oxygenation in the B-ring of flavanones and dihydroflavonols has no noticeable effect on their UV spectra[51]. The UV spectra of both chalcones (VIII) and aurones (IX) are characterized by an intense band 1 and a diminishing band 2 absorption.



VIII Chalcone skeleton



IX Aurone skeleton

Using methanol as solvent, the major absorption band in chalcones (band 1) usually occurs in the range 340-390 nm, although chalcones lacking B-ring oxygenation may have their band 1 absorption at shorter wavelengths. Band 2 is usually a minor peak in the 220 - 270 nm region. As with flavones and flavonols, increased oxygenation of either the A- or B-ring usually results in bathochromic shifts in band 1. The long wavelength absorption band in aurones is usually found in the 370-430 nm region. The position of the main absorption peak in naturally occurring aurones, however, ranges from 388 nm to 412 nm.

Methylation or glycosylation of hydroxyl groups on the aurone nucleus has little effect on the UV spectrum. Therefore, the spectra of natural aurone

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glycosides closely resemble those of their aglycones[52].

Additional measurements of spectra at varying pH, or in the presence of a chelating metal ion (especially aluminium) or an anion capable of binding ortho dihyroxy groups, gives a lot of information towards identification of the compound. The addition of sufficient base to allow all the free phenolic hydroxyl groups to ionize gives in every case a shift of all peaks to a longer wavelength[53].

Sodium methoxide (NaOMe) is a strong base and ionizes to some extent all hydroxyl groups on the flavonoid nucleus. The addition of NaOMe to flavones and flavonols in methanol usually produces bathochromic shifts in all absorption bands. However, a large bathochromic shift of band 1 of about 45-65 nm, without decrease in intensity is diagnostic for the presence of a free 3- hydroxyl group. Flavonols lacking a free 4'-hydroxyl but having a free 3 - hydroxyl group also give a 50 -60 nm bathochromic shift in band 1, however there is usually a decrease in intensity of the peak.

Flavonols which have free hyroxyl groups at both the 3 - and 4' - positions are unstable in NaOMe and the absorption peaks in the NaOMe spectrum degenerate

in a few minutes. Flavonols which contain a 3,3',4'-trihydroxyl system decompose even faster than those having the 3',4',-dihydroxylation pattern.

In the presence of NaOMe , the spectra of isoflavones containing A-ring hydroxyl groups usually show bathochromic shifts of both band 1 and band 2. Moreover, the peaks in the UV spectra of a solution of 3',4',-dihydroxy isoflavones with added NaOMe show reduced intensity within a few minutes.

Flavanones and dihydroflavonols with A-ring hydroxylation show bathochromic shifts for band 2 with NaOMe in their UV spectra. Spectra of 5,7 -dihydroxy-dihydroflavonols exhibit a consistent 35-40 nm shift of the major absorption peak, whereas 5,7 -dihydroxyflavanones exhibit a bathochromic shift of band 2 of about 35nm and 60nm for 7-hydroxyflavanones in the presence of NaOMe. Flavanones with 5,6,7,or 6,7,8,hydroxylation patterns decompose in the presence of NaOMe and therefore the UV spectra degenerate[50].

Chalcones containing a free 4-hydroxyl group or either a free 2-or 4'-hydroxyl group give, in the presence of NaOMe, a 60-100nm bathochromic shift of band 1.

Aurones with a free 4'- hydroxyl group also exhibit a large (80-95nm) diagnostic bathochromic

shift of band 1 in the presence of NaOMe. Aurones which contain a free 6 -hydroxyl group give a smaller (Ca.70nm) shift with NaOMe. However, if both 6-and 4'-hydroxyl groups are present, these bathochromic shifts may be considerably reduced[52].

The UV spectra of flavones and flavonols containing free 7- hyrdroxyl groups exhibit a diagnostic 5-20nm bathochromic shift of band 2 in the presence of NaOAc. When H_3 BO_3 is added to the NaOAc solution, a 12-30 nm bathochromic shift of band 1 occurs to those flavones and flavonols containing a B-ring ortho-dihydroxyl group.

NaOAc causes band 2 of the UV spectrum of a 7-hydroxyisoflavone to shift 6-20 nm bathochromically while the presence or absence of a free 7- hydroxyl group in flavanones and dihydroflavonols may readily be determined from their UV spectra by comparing the positions of the major absorption peak (band 2) in the methanol spectrum with that of the same peak in the NaOAc spectrum. The shift for 5,7-dihydroxyflavanones and 5,7-dihydroflavanols is about 35nm and for their 5-deoxy -equivalents is bout 60nm.

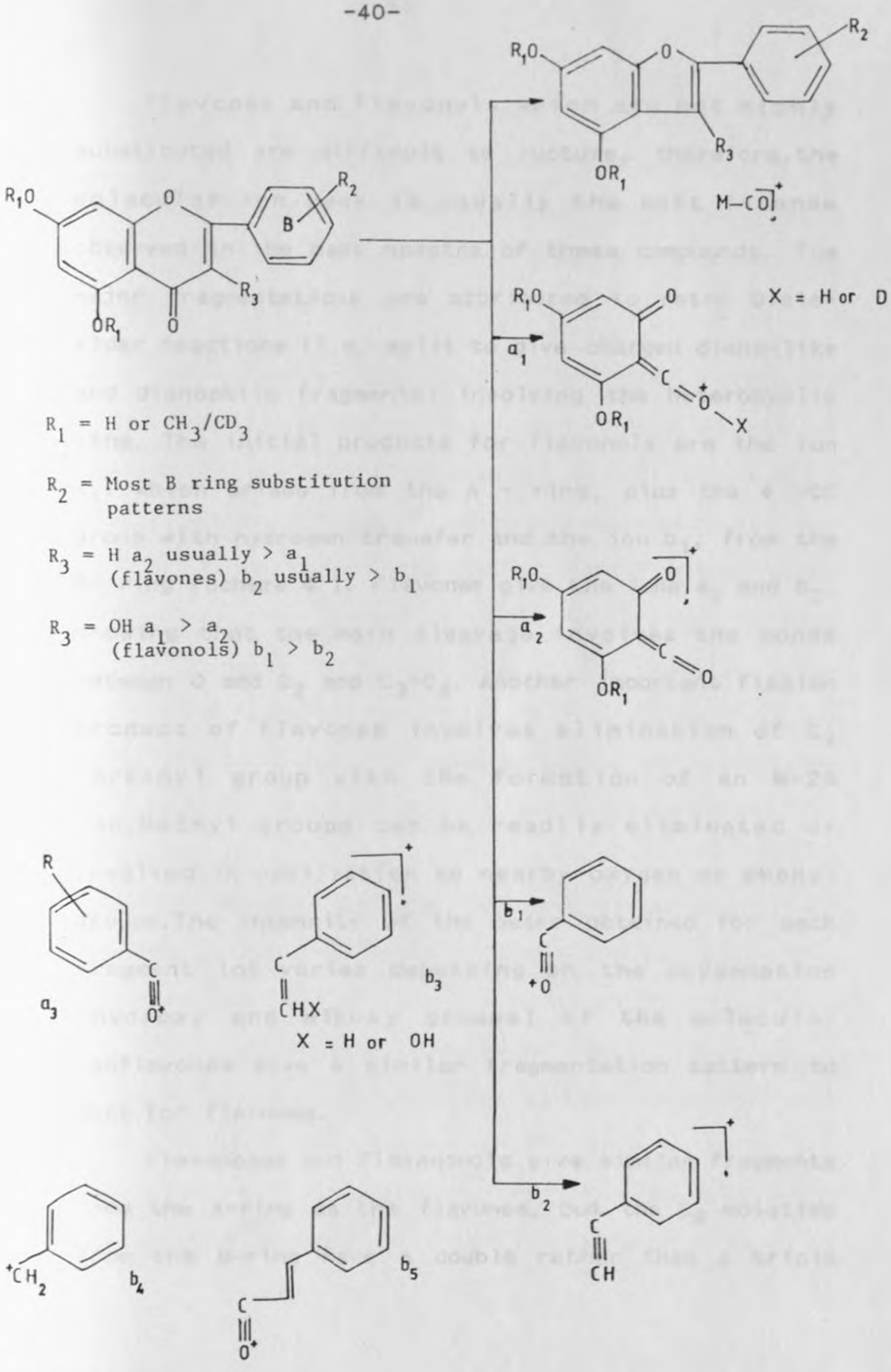
In the presence of NaOAc, a bathochromic shift of band 1 on the UV spectra, can be related to the presence in chalcones of a free 4'- and/or 4-

hydroxyl group, and in aurones to a free 4'-and/or 6-hydroxyl group. On the addition of $NaOAc/H_3BO_3$, B-ring ortho-dihydroxyl group are readily detected by the 28-36nm bathochromic shift observed in band 1 of the UV spectra of chalcones and aurones.

The presence of an ortho-dihydroxyl group in the B-ring of flavones and flavonols can be detected by a comparison of the spectrum of the flavonoid in the presence of $AlCl_3$ and with that obtained in $AlCl_3/HCl$. The hypsochromic shift (about 30-40 nm) observed in band 1 of the $AlCl_3$ spectrum on the addition of acid results from the decomposition of the complex of $AlCl_3$ with the ortho-dihydroxy group.

The presence of B-ring ortho-dihydroxyl groups in both chalcones and aurones can be detected by a 40-70nm bathochromic shift to band 1 (relative to the band 1 position in the $AlCl_3/HCl$ UV spectrum) on the addition of $AlCl_3$[52].

Structure elucidation of flavonoids as well as other compounds requires a combination of spectroscopic analysis. Mass spectroscopy is an important technique, which for flavonoids, was recently introduced especially for the determination of the molecular weight.



R_1 = H or CH_3/CD_3

R_2 = Most B ring substitution patterns

R_3 = H a_2 usually > a_1 (flavones) b_2 usually > b_1

R_3 = OH a_1 > a_2 (flavonols) b_1 > b_2

Scheme 4

Fragmentation of flavone and flavonols.

Flavones and flavonols which are not highly substituted are difficult to rupture, therefore,the molecular ion peak is usually the most intense observed in the mass spectra of these compounds. The major fragmentations are attributed to retro Diels-Alder reactions (i.e. split to give charged diene-like and dienophile fragments) involving the heterocyclic ring. The initial products for flavonols are the ion a_1, which arises from the A - ring, plus the 4 -CO group with hydrogen transfer and the ion b_1, from the B- ring (Scheme 4). Flavones give the ions a_2 and b_2, showing that the main cleavage involves the bonds between O and C_2 and C_3-C_4. Another important fission product of flavones involves elimination of C_4 carbonyl group with the formation of an M-28 ion.Methyl groups can be readily eliminated or involved in cyclization to nearby oxygen or phenyl groups.The intensity of the peaks obtained for each fragment ion varies depending on the oxygenation (hydroxy and alkoxy groups) of the molecule. Isoflavones give a similar fragmentation pattern to that for flavones.

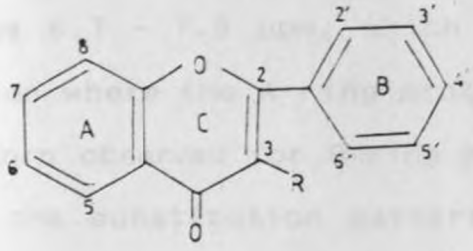
Flavanones and flavanonols give similar fragments from the A-ring as the flavones, but the C_8 moieties from the B-ring have a double rather than a triple

bond in between C_2 and C_3 and the oxygen of the flavanonols is retained. In addition to giving similar fragmentation patterns to flavones, chalcones undergo fission between the carbonyl group and the A-ring to give the 9-carbon b_5 ion[53].

The application of nuclear magnetic resonance (NMR) spectroscopy to the structure analysis of flavonoids is well established. Almost all modern investigations of new flavonoid compounds quote NMR spectral data in support of their structural assignment. The advent of machines with higher signal-to-noise ratios and the use of repeated scans with time averaging computer and pulse fourier transform techniques has enabled much lower quantities of samples (ca.250 ug or less) to be used for analysis.

The solvents used for NMR analysis of flavonoids include deuteriochloroform ($CDCl_3$) which dissolves Isoflavones and highly methylated flavones and flavonols, hexadeuteriodimethylsulfoxide (DMSO $-d_6$). Trimethylsilyl (TMS) ether derivatives have been used for obtaining NMR spectra of flavonoids which are otherwise insoluble in $CDCl_3$. Proton signals obtained in the NMR spectra of trimethylsilylated flavonoids

generally occur in the range 0 - 9 ppm (δ -scale) relative to TMS. The A-ring (X) protons at C-6 and C-8 of flavones, flavonols and isoflavones which contain the common 5,7-dihydroxy substitution pattern give rise to two doublets (J=2.5 Hz) in the range 6.0 - 6.5 ppm. The H-6 doublet occurs consistently at higher field than the signal for the H-8. When a sugar a attached to the oxygen at C-7 the signals for both H-8 and H-6 are shifted downfield.



R = H for Flavones

R = OH for Flavonols

X

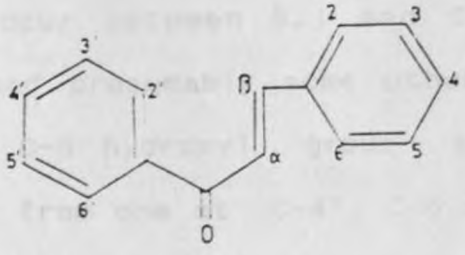
The C-3 proton gives a signal in the same region of the NMR spectrum as those of the C-6 and C-8 protons and appears as a singlet near 6.3 ppm. In flavones with the common 5,7- dihydroxylation pattern the C-6 and C-8 protons occur as doublets (J= 2.5 Hz) and are therefore readily distiguished from the C-3 proton singlet. Some naturally occurring flavonoids have oxygenation only at position 7 in the A-ring. In this case the C-5 proton is deshielded by the C-4 keto

group and therefore absorbs near 8.0 ppm, and thus at lower field than most aromatic protons. The C-5 proton in these 7-oxygenated flavonoids appears as a doublet (J= ca.9 Hz) as a result of ortho coupling between the C-5 and C-6 protons. The signals for protons on C-6 and C-8 both occur at lower field than in the 5,7-dihydroxyflavonoids. In 5-deoxyflavonoids the C-8 proton may absorb at either higher or lower field than the C-6 proton.

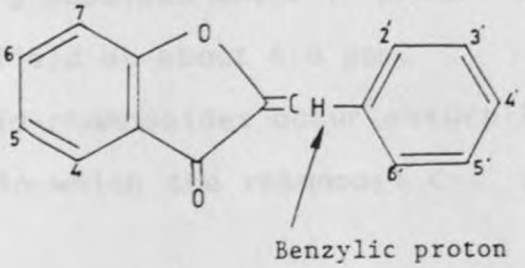
The B-ring ($\underset{\sim}{X}$) protons usually appear in the range 6.7 - 7.9 ppm, which is downfield from the region where the A-ring protons absorb. The signal pattern observed for B-ring protons is characteristic for the substitution pattern of that ring and, in addition suggests the oxidation level of the ring C. When ring B is oxygenated at C-4', a four-peak pattern of two doublets (each J=8.5 Hz) is observed. The doublet for the C-3' and -5' protons always appears upfield from the C-2' and - 6' protons and generally falls in the range 6.65 - 7.1 ppm for all types of flavonoids. The C-2', -6' doublet appears at 7.1 - 8.1 ppm . The C-5' proton of 3',4'-oxygenated flavones and flavonols appears as a doublet centered between 6.7 and 7.1 ppm (J = 8.5 Hz), and the C-2' and -6' proton signals usually occur between 7.2 and 7.9 ppm. 3',4'-

oxygenated isoflavanones, flavanones and dihydroflavonols give a complex multiplet,usually two peaks for the C-2', -5' and -6' protons in the region 6.7 -7.1 ppm. The C-3 proton of flavones gives a sharp singlet near 6.3 ppm which usually overlaps the signals produced by the A - ring protons. The C-2 proton in isoflavones, which is in a beta position to the C-4 keto function, occurs in the range 7.6 - 7.8 ppm (in CCl_4).

The H-α and H-β protons of chalcones (XI) occur as doublets (J = ca. 17 Hz) in the ranges 6.7 - 7.4 ppm (H-α) and 7.3 -7.7 ppm (H-β) while the aurone (XII) benzylic proton appears as a singlet at 6.5 -6.7 ppm.



XI



XII

The signal for the C-2 proton of flavanones appears as a quartet (two doublets J_{cis} = ca. 5Hz) (J_{trans} = ca. 11Hz) near 5.2 ppm as a result of the coupling of the C-2 proton with the two C-3 protons. The C-3 protons couple with each other (J = 17Hz) in addition to their spin - spin interaction with C-2 proton, thus giving rise to two overlapping quartets near 2.8 ppm. In naturally occurring dihydroflavonols, the C-2 proton signal occurs as a doublet (J = ca. 11Hz) near 5.2 ppm, while the C-3 proton doublet appears further upfield at about 4.3 ppm.

In flavonoids, methoxyl proton signals appear in the region 3.5 - 4.1 ppm, while most aromatic acetoxyl proton signals occur in the rage 2.25 - 2.50 ppm. Most of the trimethylsilyl proton signals of flavonoid TMS ethers occur between 0.1 and 0.5 ppm. With glucosides (and presumably some other glycosides) a sugar on the C-3 hydroxyl group can be readily distinguished from one at C-4', C-5 or C-7. In the latter three types of flavonoid O-glucosides, the C-1" proton signal occurs near 5.0 ppm, while in flavonol 3-O-glucosides the C-1" proton signal appears further downfield at about 5.8 ppm.

Flavonoid rhamnosides occur naturally as α- L - rhamnosides in which the rhamnosyl C-1" proton has an

equatorial-equatorial coupling with the C-2" proton (J = ca. 2Hz). In both the 3- and 7-O rhamnosides the C-1" proton signal occurs in the range 5.0 - 5.3 ppm. In the NMR spectra of flavonoid rhamnosides, the signal for the rhamnose methyl group which occurs at 0.8 -1.2 ppm, is also a distinguishing feature[54].

Infra-red (I.R.) spectra of flavonoids form useful adjuncts to structural elucidation and are usually quoted in support of identities of newly isolated compounds. However little development has taken place over the last years with regard to the use of I.R. spectra for diagnostic purposes in the flavonoid field. This is probably because U.V. spectra are more informative, require much less material and are generally easier to carry out using polar solvents in which the flavonoids are fully soluble[53].

1.40 ANTHRAQUINONES

Anthraquinones are found in both higher plants and fungi and frequently they exist as glycosides in flowering plants and occasionally in higher fungi. Of the two sub-families of the family Polygonaceae; Eriogonoideae and Polygonoideae, only the latter produce anthraquinones. Many species of the genera

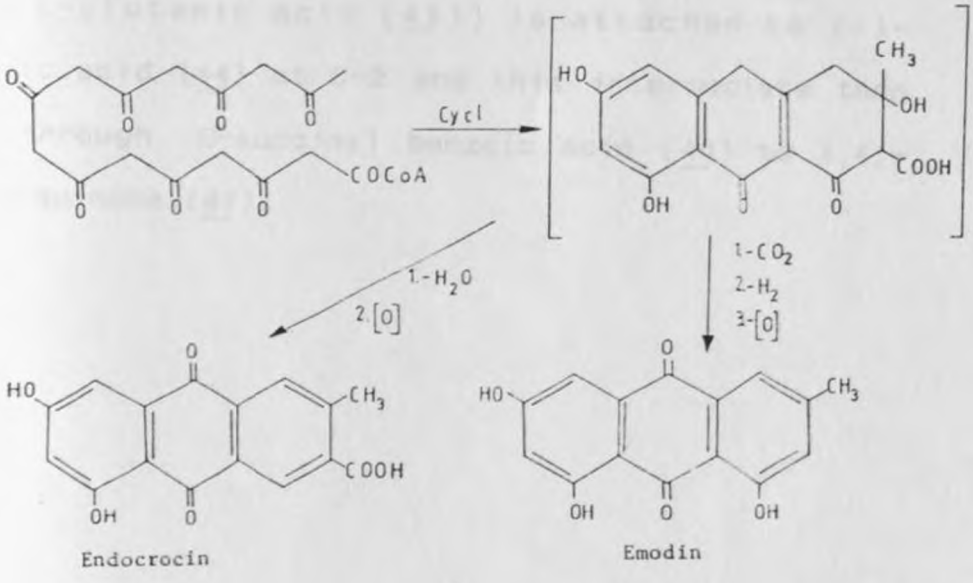
Ernex, Rumex , Rheum, as well as species of Atraphaxis, Oxygonum, polygonum, and Muhlenbeckia produce anthraquinones[55]. Matasi (1987) working with the Kenyan *Polygonum pulchrum* and *P. setosulum* isolated the anthraquinones emodin (21), physcion (20) and chrysophanol (28) from the leaves, stems and roots. Most anthraquinones are simply polyhdroxy / methoxy derivatives with or without a one-carbon side chain (CH_3, CH_2OH, CHO,CO_2H) which invariably occupies a ß position[56].

1.41 BIOSYNTHESIS OF ANTHRAQUINONES

It is difficult to systematize the biosynthesis of quinones because they show such a diversified picture[57]. Benzoquinones originate from shikimic acid, polyketides, or mevalonate. Naphthoquinones may either be completely synthesized from acetate or in the polyhydroxylated spirochrones. Natural phenolic anthraquinones are supposedly formed by head-to-tail condensation of acetate units[58]. Many anthraquinones found in higher plants are of polyketide origin[59] and they are often structurally distinquishable by the existence of substituents in the two benzenoid rings of the anthraquinone nucleus. This acetate hypothesis is in accord with the "Emodin type" of structures, and

does not agree with the "alizarin type" compounds.

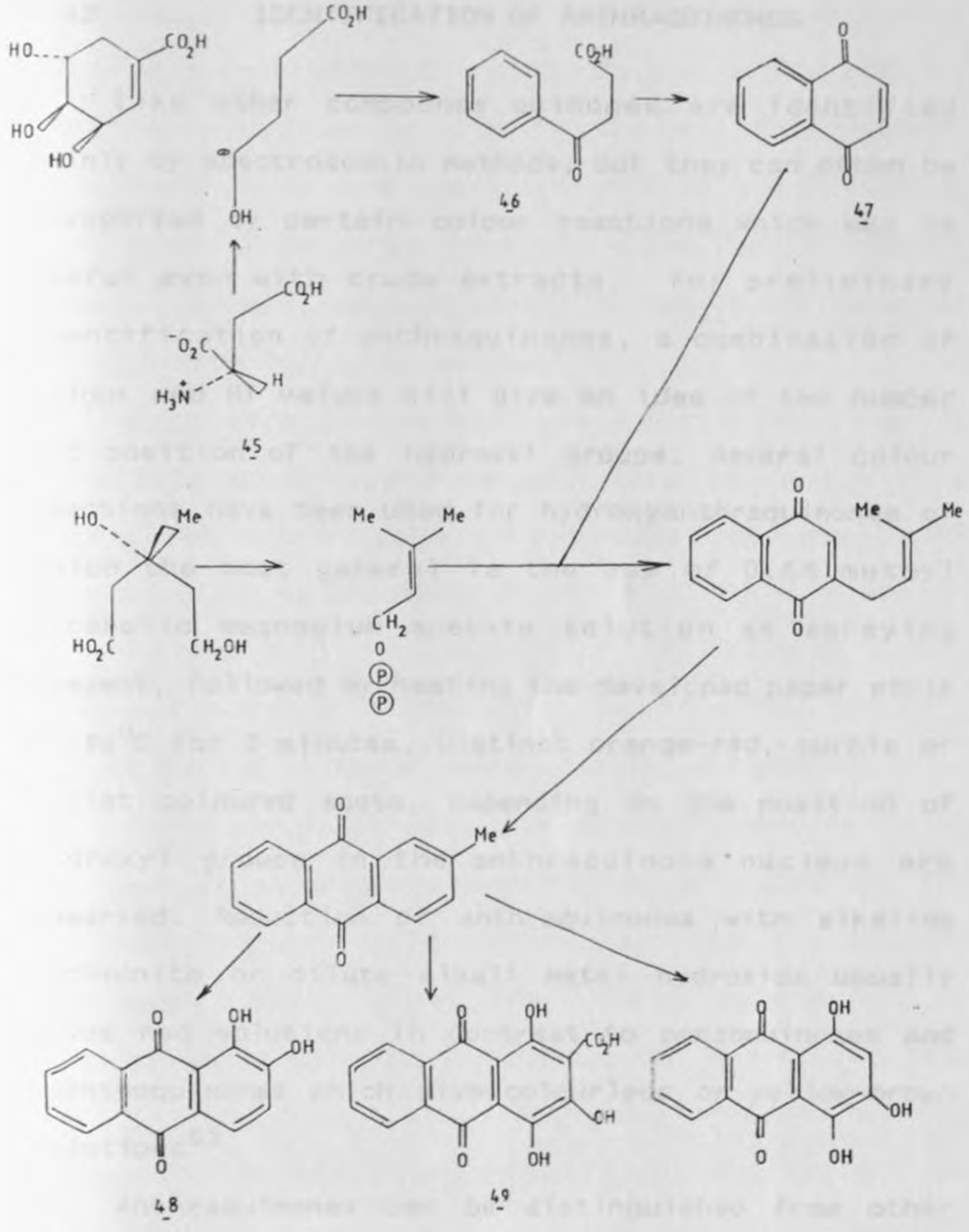
Emodin is constructed from one acetate and seven malonate units[60] scheme 5. Numerous anthraquinones are produced by the octaketide pathway conforming to the basic emodin structure. They arise via different folding, O-methylation, side chain oxidation, nuclear hydroxylation or elimination of hydroxyl groups, chlorination, dimerization via phenol oxidation etc.



Scheme 5

Polyketide origin of anthraquinones.

It has been proposed that "alizarin type" anthraquinones originate from a naphthalenic precursor[61] to which a branched C_5 chain is attached and which then undergoes cyclization and oxidation. The details of the biosynthetic origin of rings A and B in the anthraquinone nucleus is shown in scheme 6. 1,6 -($^{14}C_2$)-(I)-shikimic acid is incorporated directly into ring A of alizarin (48) and pseudopurpurin (49) by degradation of the labelled metabolites to phthalic acid which retained all the activity of the quinonoid pigment. A C_3 fragment [from L-glutamic acid (45)] is attached to (-)-shikimic acid (44) at C-2 and this intermediate then leads through O-succinyl benzoic acid (46) to 1,4,-naphthoquinone (47).



Scheme 6

Shikimic acid pathway for the biosynthesis of anthraquinones.

1.42 IDENTIFICATION OF ANTHRAQUINONES

Like other compounds quinones are identified mainly by spectroscopic methods, but they can often be recognized by certain colour reactions which may be useful even with crude extracts. For preliminary identification of anthraquinones, a combination of colour and Rf values will give an idea of the number and position of the hydroxyl groups. Several colour reactions have been used for hydroxyanthraquinones of which the most general is the use of 0.5% methyl alcoholic magnesium acetate solution as spraying reagent, followed by heating the developed paper strip at 90^{o}C for 5 minutes. Distinct orange-red, purple or violet coloured spots, depending on the position of hydroxyl groups in the anthraquinone nucleus are observed. Reduction of anthraquinones with alkaline dithionite or dilute alkali metal hydroxide usually gives red solutions in contrast to benzoquinones and naphthoquinones which give colourless or yellow-brown solutions[63].

Anthraquinones can be distinguished from other classes of quinones by the fact that they have four or five absorption bands in the Ultra Violet (UV) and

Visible (Vis) regions. The spectra of most anthraquinones show absorption bands in the region 240-260,260-290 nm and 320 - 330 nm. The shorter wavelength bands are characteristic of the quinonoid nucleus of the anthraquinone while the long wavelength absorption band, usually broad, is due to the carbonyl group of the quinonoid nucleus[64].

With very few exceptions hydroxy and alkoxy groups intensify the long-wave band and there is a bathochromic shift,absorption above 360 nm being dominated by the number of α- hydroxy groups. β-Hydroxy groups have relatively little influence unless adjacent to an α-hydroxyl. As in other quinone series alkali shifts provide additional information.

Infra-red spectroscopy is another technique which is used in structure determination of quinones. The carbonyl frequencies of quinones are the most popular although these values are rarely sufficient in classifying a quinone. p-Benzoquinone absorbs at 1669 cm^{-1} (in solution). The frequency is raised as the number of linear fused rings increases (e.g 1,4-naphthoquinone 1675 cm^{-1} , 9,10- anthraquinone 1678 cm^{-1}, naphthacene - 5,12-quinone 1682 cm^{-1}),and is lowered by hydrogen bonding, by the introduction of

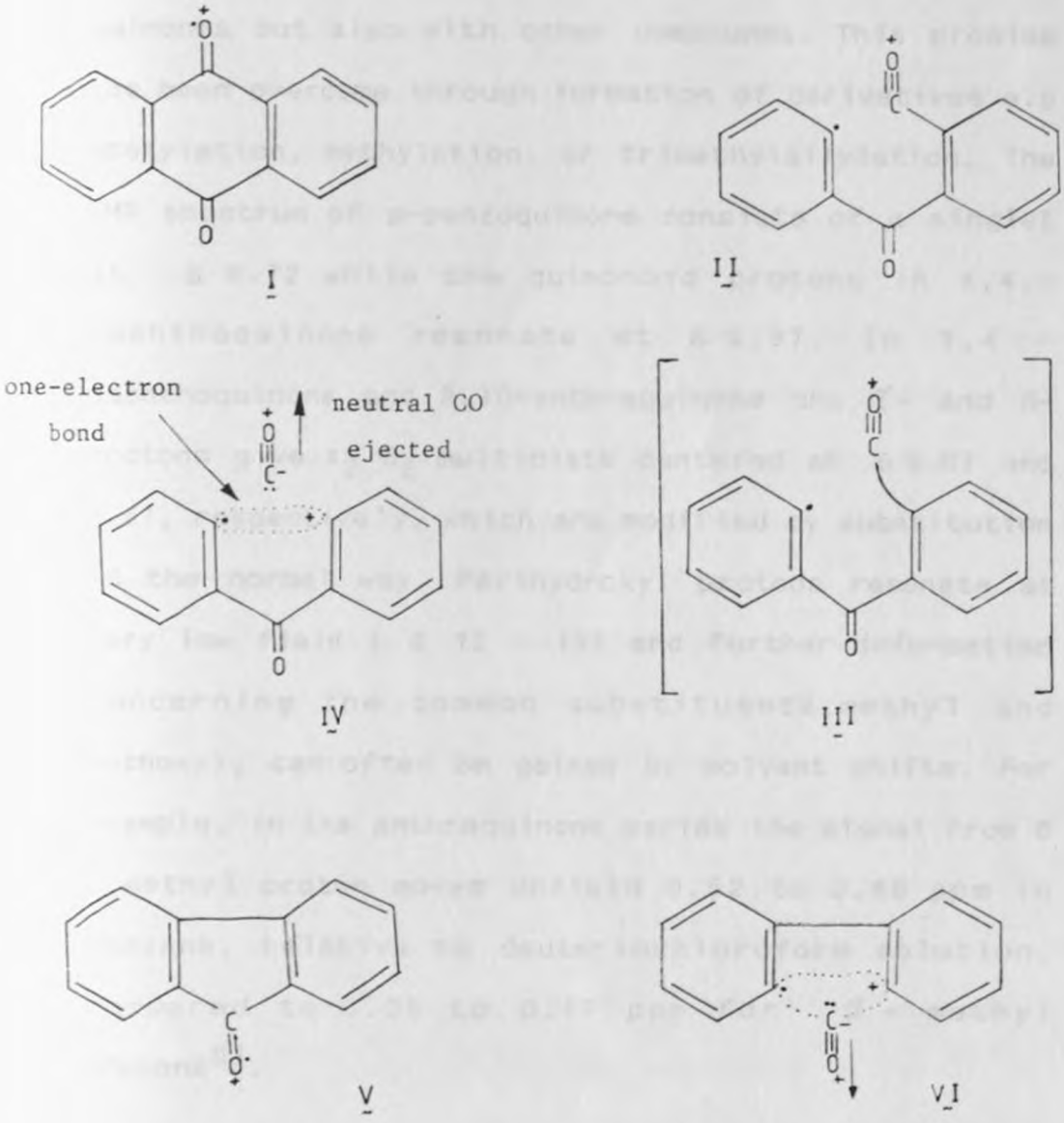
electron donating substituents and by extending quinonoid conjugation through more than one ring. Many symmetric p-quinones show multiple absorption in the carbonyl region.

Chelated quinones are recognised by the lowered frequency of both carbonyl and hydroxyl absorptions. Anthraquinones without α-hydroxyl groups usually show only one carbonyl peak and its position is little affected by substitution apart from β-hydroxyl groups which may lower the frequency by < 20cm^{-1}. Most anthraquinones show intense absorptions in the region 1600-1575 cm^{-1}, in some cases overlapping with chelated carbonyl peak[63].

Mass spectroscopy is yet another important technique used in structure elucidation of quinones. Fragmentation of quinones is characterized by loss of one or two molecules of carbon monoxide. Benzoquinones and naphthoquinones also eliminate an acetylenic fragment from the quinone ring.

Anthraquinones are very stable, and the spectrum of the parent compound consist of the molecular ion (the base peak), strong M-CO and M-2CO peaks, doubly charged ions for each of these and very little else.

In anthraquinones the most loosely - bound electron, which is therefore removed preferentially during formation of the molecular ion (from electrons of low energy) is one of the lone-pair electrons on the oxygen atom. This is followed by uncoupling of the electron pair in one of the C-C bonds adjacent to the carbonyl group (scheme 7). The positive charge on the oxygen (II) will induce a polarity in the adjacent bonds, and this will be a maximum in the remaining C-C bond, hence weakening it (III). The dissociation of a neutral CO molecule leaves a highly excited flourenone ion (IV) which attains ground state by the donation of one of the oxygen lone-pair electrons to the one-electron bond. The fluorenone ion then breaks in analogous fashion eliminate a further neutral CO molecule to give a highly excited O-diphenylene ion[65].



Scheme 7

Fragmentation of Anthraquinones.

Proton magnetic resonance forms one of the most important technique in structure determination. Solubility of sample is a common snag not only with quinones but also with other compounds. This problem has been overcome through formation of derivatives e.g acetylation, methylation, or trimethylsilylation. The NMR spectrum of p-benzoquinone consists of a singlet at δ 6.72 while the quinonoid protons in 1,4,-naphthoquinone resonate at δ 6.97. In 1,4 - naphthoquinone and 9,10-anthraquinone the α- and β-protons give A_2 B_2 multiplets centered at δ 8.07 and 7.67, respectively, which are modified by substitution in the normal way. Perihydroxyl protons resonate at very low field (δ 12 - 13) and further information concerning the common substituents,methyl and methoxyl, can often be gained by solvent shifts. For example, in the anthraquinone series the signal from β - methyl proton moves upfield 0.52 to 0.60 ppm in benzene, relative to deuteriochloroform solution, compared to 0.06 to 0.17 ppm for α - methyl protons[63].

CHAPTER 2

RESULTS AND DISCUSSION

A preliminary spot-test survey was carried out on the inner tissue aglycones of both leaves and flower heads of *P. senegalense*, before embarking on large scale extraction, isolation and purification.

The accumulated flavonoid aglycones in the leaf resin of *P. senegalense* were first washed[27] off using fresh portions of acetone (fraction 1) until the last portion was clear. The leaves were dried under shade and then macerated. A flow chart showing the procedure used for extraction and purification of compounds is shown in Figure 1.

Extraction of the macerated leaves was done using 80% methanol in water followed by a further extraction using 70% and 50% methanol[66]. Methanol was then removed from this extract in a rotary evaporator. The remaining aqueous extract was then fractionated with dichloromethane to remove any remnant aglycones (fraction 2). The aqueous extract was then hydrolysed[67] using 2M HCl for two hours to obtain the inner tissue aglycones. The hydrolysate was then extracted first using dichloromethane (fraction 3) and then a more polar solvent, ethyl acetate (fraction 4).

Analysis of fraction 1 to 4 using T.L.C. revealed that fraction 2 comprised of a few compounds all of which were present in fraction 1 plus chlorophyll; when the T.L.C. was developed using solvent 1 (Table 3).

Table 3

Location of surface compounds on analytical T.L.C.plates.

Compound	Appearance under light		Appearance after fuming with NH3	RF.
	Vis	UV		
1.	Yellow	Yellow-purple	Bright Yellow	0.93
2.	Yellow	Yellow-purple	Orange-Brown	0.79
3.	Yellow	Purple	Bright Yellow	0.67
4.	Faint yellow	Purple	Orange	0.58
5.	Yellow	Yellow-Purple	Orange pink	0.50
6.	Deep yellow	Yellow-purple	Greenish-orange	0.39
7.	Clear	Deep purple	Greenish-blue	0.10
8.	Yellow	Purple	Light pink	0.014

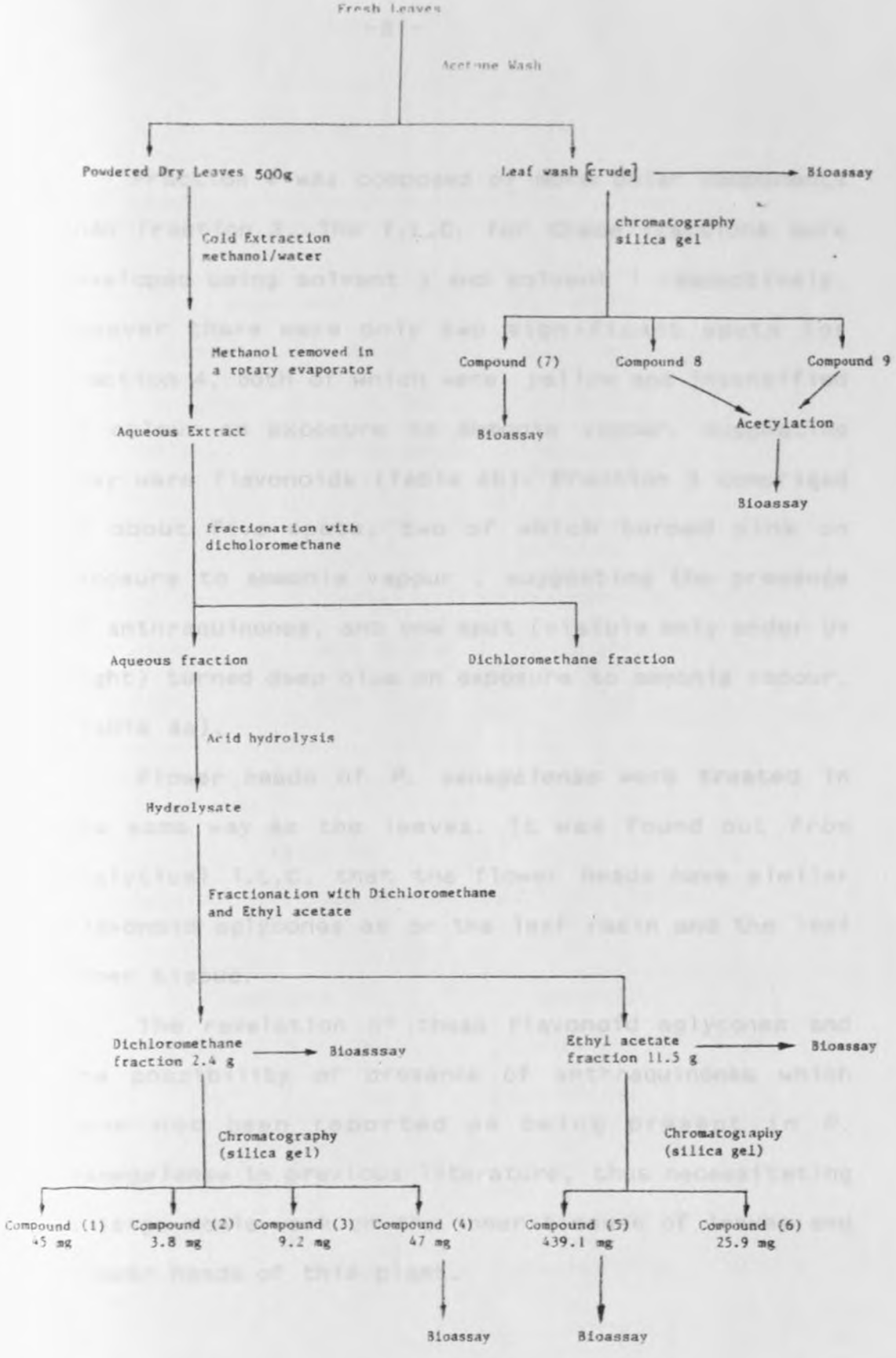


Fig. 1:

Fractionation of Polygonum senegalense leaves.

Fraction 4 was composed of more polar components than fraction 3. The T.L.C. for these fractions were developed using solvent 3 and solvent 1 respectively. However there were only two significant spots for fraction 4, both of which were yellow and intensified in colour on exposure to ammonia vapour, suggesting they were flavonoids (Table 4b). Fraction 3 comprised of about five spots, two of which turned pink on exposure to ammonia vapour , suggesting the presence of anthraquinones, and one spot (visible only under UV light) turned deep blue on exposure to ammonia vapour, (Table 4a).

Flower heads of *P. senegalense* were treated in the same way as the leaves. It was found out from analytical T.L.C. that the flower heads have similar flavonoid aglycones as on the leaf resin and the leaf inner tissue.

The revelation of these flavonoid aglycones and the possibility of presence of anthraquinones which have not been reported as being present in *P. senegalense* in previous literature, thus necessitating a large scale work on the inner tissues of leaves and flower heads of this plant.

Table 4a

Location of internal aglycones on analytical T.L.C.plates:
Dichloromethane soluble fraction.

Compound	Appearance under light		Appearance after fuming	RF.
	Vis	UV	with NH_3	
1.	Yellow	Yellow-purple	Light Pink which fades	0.91
2.	Yellow	Yellow-purple	Pink which persists	0.40
3.	Yellow	Purple	Greyish blue	0.35
4.	Clear	Deep purple	Faint grey	0.27
5.	Clear	Deep purple	Deep blue	0.07

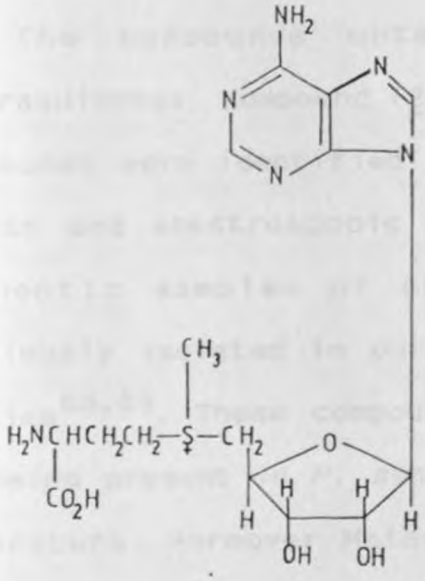
Table 4b

Location of internal aglycones on analytical T.L.C.plates:
Ethyl acetate soluble fraction.

Compound	Appearance under light		Appearance after fuming	RF.
	Vis	UV	with NH3	
6.	Yellow	Yellow-purple	Orange	0.84
7.	Yellow	Yellow-purple	Intense yellow	0.50
8.	Yellow	Yellow-purple	Greyish-brown	0.27

Large scale washing of leaves with acetone was carried out on freshly collected plants of *P. senegalense*. The concentrate of this extract was stored for the determination of its biological activities.

Five hundred grams of dry powdered previously acetone washed leaves of *P. senegalense* were successively extracted with 80, 70, and 50% methanol. During the extraction, heat and an obnoxious gas were liberated. The gas smelled of mercaptans and although it was not immediately analysed, it was suspected to contain low molecular weight thiols probably from the biosynthetic -O methylating agents such as S-adenosylmethionine[45,48] (50) or methionine (51).



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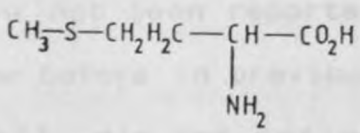


51

All extracts were combined and methanol removed in a rotary evaporator before partitioning with dichloromethane. The aqueous layer, which was dark brown in colour was then hydrolysed with 2M HCl for two hours to give a dark red hydrolysate, which was partitioned with dichloromethane and then ethyl acetate. The concentrate of these two fractions gave 2.4 g of a dark brown oily substance and 11.5 g of a brownish thick substance respectively.

Isolation of the components of the dichloromethane soluble fraction was carried out using column chromatography. The eluting solvents were n-hexane, dichloromethane and methanol. Purification of the compounds was done using preparative T.L.C; flash chromatography and fractional crystallization.

The compounds obtained included the two anthraquinones, compound (2) and compound (3). The two compounds were identified by comparing their melting points and spectroscopic properties with those of authentic samples of chrysophanol and emodin, previously isolated in our laboratory from the Rumex species[68,69]. These compounds have not been reported as being present in *P. senegalense* before in previous literature. Moreover Matasi (1987), did not notice presence of anthraquinones in *P. senegalense* during

his work. Anthraquinones especially emodin, physcion, and chrysophanol have been reported in many other Polygonum species.[18,25,70,71,72,73]

The flavonoid aglycones obtained from this fraction in appreciable amounts are compound (1) and compound (4).

Their identification was achieved through spectral analysis and literature correlation. The detailed spectroscopic data for these compounds, and others to be discussed are summarised in tables 14 to 19 in appendix B and spectra in appendix C.

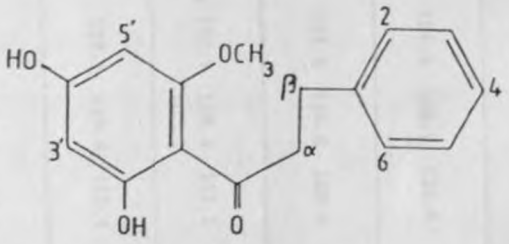
Column chromatographic separation of the ethyl acetate fraction, using dichloromethane and methanol followed by preparative thin layer chromatography gave two flavonoids, compound (5), and compound (6). Identification of compounds was carried out using spectroscopic data and literature correlation.

The major absorption band in the UV spectrum of compound (1) was at λ max (MeOH) 286 nm suggested a flavonoid lacking conjugation of A- and B- rings. The proton NMR signals at 2.87 ppm (triplet, 2H) and 3.22 ppm (triplet, 2H) were ascribed to C-α and C-β protons respectively. This was confirmed through ^{1}H NMR literature correlation with dihydrochalcones isolated from *Viburnum davidii* and *V. lantanoids*[74], thereby

requiring compound (1) to have a dihydrochalcone skeleton. ^{1}H NMR signals at 7.24 ppm (a doublet of multiplets integrating for 2H and 3H) and the ion peak at m/e 91 in the mass spectrum required an unsubstituted B-ring. The observed molecular ion peak at m/e 272 in the mass spectrum required dihydroxylation and monomethylation. The ion peak at m/e 167 and 140 required all substuituents to be on A-ring.

The bathochromic shift of 36 nm for band I in the UV spectrum on addition of NaOMe to a methanolic solution of compound (1) led to positioning of a hydroxyl group at the C-4' position. Lack of any shift on addition of HCl to a MeOH / $AlCl_3$ solution of the compound in the UV spectrum led to the positioning of a hydroxyl group at the C-2' position and to the conclusion that the two hydroxyl groups are not *ortho* to one another. Moreover,from ^{1}H NMR spectrum,the signals at 5.98 ppm (d, J = 2.2 Hz integrating for 1 H) and 5.88 ppm (d, J = 2.2 Hz also integrating for 1 H) showing *meta* coupling, implied trisubstitution and that the two protons in the A-ring are in totally different environments. The signal at 3.80 ppm (s, 3H) in the ^{1}H NMR spectrum was attributed to a methoxyl group.

These observations together with IR results led to the assignment of compound (1) as 2',4'-dihydroxy-6'-methoxydihydrochalcone. The structure of compound (1) was further confirmed through literature correlation with ^{13}C NMR spectra for some dihydrochalcones,[75] (Table 5).



1

Compound (1) was first isolated from *Uvaria angolensis*[76] and its structure confirmed by synthesis from 2-hydroxy-4-benzyloxy-6-methoxyacetophenone.

Table 5

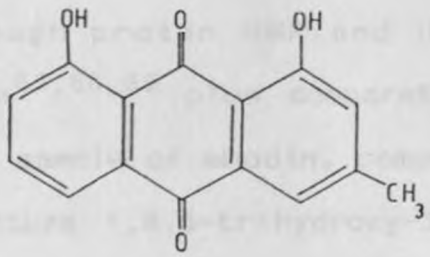
A selection of chalcones and dihydrochalcones carbon-13 spectra:

COMPOUND	C-1	C-2	C-3	C-4	C-5	C-6	C-β	C-α	C=O	C-1'	C-2'	C-3'	C-4'	C-5'	C-6'
Chalcone	134.7	128.3	128.3	130.3	128.3	128.3	144.4	121.7	189.8	138.0	128.3	128.7	132.6	128.7	128.3
2'-hydroxychalcone	134.5	128.9	128.6	130.8	128.6	128.9	145.3	118.5	193.6	119.9	163.6	118.8	136.3	119.9	129.6
2'-hydroxy-4'-methoxychalcone	134.6	128.8	128.4	130.5	128.4	128.8	144.2	120.2	191.6	113.9	166.5	101.0	166.0	107.6	131.1
2',4-dihydroxy-4'-methoxydihydrochalcone	131.7	128.3	115.1	155.6	-	-	29.2	39.5	200.1	114.6	165.3	100.7	166.1	107.1	132.2
2',4,4'-trihydroxydihydrochalcone	132.1	129.6	115.4	155.8	-	-	29.5	39.7	200.7	113.3	165.6	103.0	164.7	108.1	133.0
Compound (1)	141.5	128.3	125.8	125.8	125.8	128.3	30.1	45.0	203.7	104.4	164.8	95.7	163.0	91.4	166.0

The UV spectrum of compound (2) in methanol showed absorption bands at λ max, 426 nm, 284 nm, 274 nm sh. and 234 nm. When 5% KOH solution was added to a methanolic solution of compound (2), the absorption bands were observed at λ max, 504 nm, 281 nm, 274 nm sh; which displays absorption bands characteristic of anthraquinones. The UV absorption bands in dichloromethane were at λ max, 430, 288, 278, and 256 nm. The IR spectrum had a strong absorption peak at 1625 cm^{-1} due to C=O stretch. The proton NMR spectrum displayed signals at 12.04 ppm (s) and 12.14 ppm (s) which were attributed to two hydroxyl groups. The low field position of these signals was ascribed to the acidity of the protons certainly due to hydrogen bonding with the carbonyl oxygen. The ^1H NMR signal at 2.48 ppm (s, 3H), was ascribed to the methyl protons. The mass spectrum portrayed a molecular ion peak at m/e 254. The other ion peaks at m/e 226 and 197 were attributed to the loss of C=O groups during fragmentation. Other ion peaks were observed at m/e 169, 152, and 127. From the proton NMR spectrum the signal at 7.28 ppm (d, J = 1.2 Hz) was ascribed to C-2 proton and the signal at 7.32 ppm (d, J = 1.2 Hz) attributed to C-4 proton. The multiplet of signals at ppm, 7.67 - 7.85 are due to C-5, C-6, C-7 protons.

Through literature[63,68,69] correlation of the UV and proton NMR spectroscopic values, compound (2) was assigned the structure 1,8-dihydroxy-3-methylanthraquinone.

Compound (2) is being reported present in *P. senegalense* for the first time.

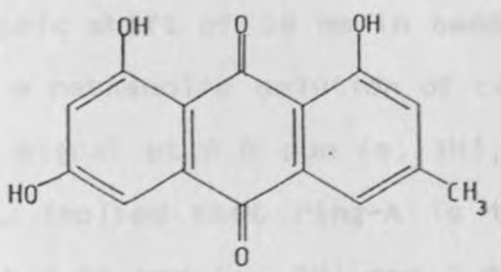


2

The UV spectrum of compound (3) in dichloromethane showed absorption bands at λ max, 430, 298 sh; 284, and 262 nm, characteristic of anthraquinones. The IR spectrum displayed a strong absorption peak at 1620 cm^{-1} due to C=O stretch, which is diaryl conjugated and hydrogen bonded. The mass spectrum displayed a molecular ion peak at m/e 270. This required a trihydroxylated and monomethylated anthraquinone. The characteristic fragmentation of anthraquinones was shown by the mass spectrum at m/e 242 and 213. Other ion peaks were at m/e 185, 139,

135, and 121. The proton NMr spectrum displayed a signal at 2.37 ppm (s, 3H) which was ascribed to the methyl protons. The ^{1}H NMR signal at 6.53 ppm (d, J = 2.5 Hz) and 7.04 ppm (d, J = 2.5 Hz) showing *meta* coupling suggested that one of the rings is disubstituted at meta positions. Similarly the proton NMR signals at 7.08 ppm and 7.39 ppm J = 0.83 Hz implied that the other ring is also disubstituted.

Through proton NMR and UV correlation with literature[63,68,69] plus comparative analysis with an authentic sample of emodin, compound (3) was assigned the structure 1,6,8-trihydroxy-3-methylanthraquinone. This structure was further confirmed using ^{13}C NMR signals (Table 16, appendix B). Compound (3) is being reported present in *P. senegalense* for the first time.



3

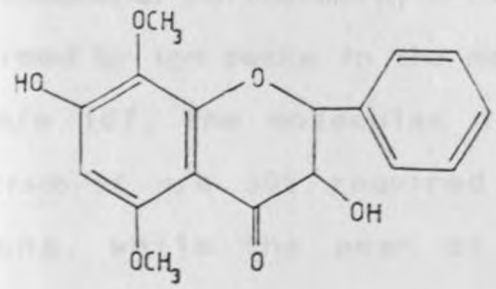
An intriguing compound was the UV active compound (4), which turned blue on T.L.C. after exposure to ammonia fumes. Its UV spectrum in methanol displayed one major absorption peak (band II) at λ max 288 nm, the other one (band I) at λ max 330 nm was weak. This absorption phenomenon is exhibited by flavanones and dihydroflavonols. Its IR spectrum displayed a strong absorption band at 1630 cm^{-1} due to C=O stretch.

The, mass spectrum showed a molecular ion peak at m/e 316 which corresponds to $C_{17}H_{16}O_6$. From the proton NMR spectrum, the multiplet of signals at 7.4 ppm required one of the rings of compound (4) to be unsubstituted.

The presence of a hydroxyl group at C-7 and oxygenation at C-5 was inferred from the bathochromic shift of 38 nm in band II, when NaOMe was added to a methanolic solution of compound (4). Presence of the hydroxyl group at C-7 was further confirmed by the bathochromic shift of 38 nm in band II when NaOAc was added to a methanolic solution of compound (4).

The signal at 6.0 ppm (s, 1H), in the proton NMR spectrum, implied that ring-A is trisubstituted. The signal at 3.92 ppm (s, 3H) and 3.93 ppm (s, 3H) were ascribed to the methoxyl protons. Presence of the methoxyl groups at C-5 and C-8 was deduced from lack

of shift in band II when $AlCl_3$ was added to a methanolic solution of compound (4) and when HCl was added to the MeOH / $AlCl_3$ solution of the compound. This was further confirmed from the ion peak at m/e 196 in the mass spectrum. From the proton NMR spectrum, the signal at 5.0 ppm (d, J = 8.80 Hz) was ascribed to the proton at C-2 while the signal at 4.0 ppm (d, J = 3.84 Hz) was ascribed to the proton at C-3. The structure was further confirmed through ^{13}C NMR spectroscopic data, (Table 17, appendix B). From literature correlation of the proton NMR values[77] and ^{13}C NMR values[78] of dihydroflavonols, compound (4) was assigned the structure 3,7,-dihydroxy-5,8-dimethoxyflavanone.



4

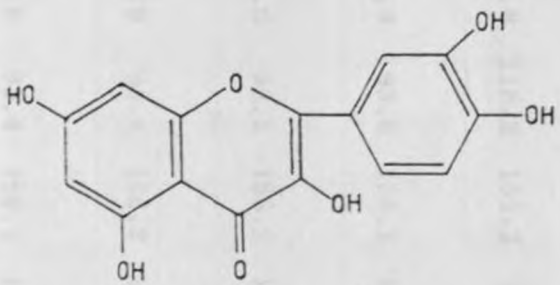
Compound (5) was the major compound of the hydrolysate of *P. senegalense*, and formed about 0.088% of dry acetone-washed leaves by weight.

A methanolic solution of compound (5) exhibited two major absorption peaks in the UV spectrum at λ max 370 nm and λ max 254 nm, which are in the absorption region for flavones and flavonols. The presence of 3,3',4'-trihydroxyl system was inferred from the observed bathochromic shift of 50 nm in band I and the rapid degeneration of this band[79] when NaOMe was added to a methanolic solution of compound (5). Presence of a B-ring *ortho*-dihydroxyl group was further confirmed by a bathochromic shift of 15 nm in band I when H_3BO_3 was added to a MeOH / NaOAc solution of compound (5) and a hypsochromic shift of 25 nm in band I when HCl was added to a MeOH / $AlCl_3$ solution this compound. Furthermore, B-ring dihydroxylation was confirmed by ion peaks in the mass spectrum at m/e 137 and m/e 167. The molecular ion peak in the mass spectrum at m/e 302 required a pentahydroxylated flavone, while the peak at m/e 153 required a dihydroxylated A-ring system, thereby further confirming C-3 hydroxyl group. A bathochromic shift of 55 nm in band I even after addition of HCl to a MeOH / $AlCl_3$ solution of compound (5), was attributed

to the presence of C-5 hydroxyl group.

From the proton NMR spectrum the signals at 6.26 ppm (d, J = 2.1 Hz) and 6.43 ppm (d, J = 2.1 Hz) showing *meta* coupling implied that the two protons in A-ring are in different environments. This revelation and the observed 24 nm bathochromic shift in band II when NaOAc was added to a methanolic solution of compound (5) confirmed C-7 hydroxyl group.

Through literature correlation of the UV spectrum and that of quercetin[79] and the ^{13}C NMR spectrum[75] (Table 6), compound (5) was assigned the structure 3,5,7,3',4'-pentahydroxyflavone (quercetin).



5

Quercetin was first obtained as the aglycone of the glycoside quercetrin and is the most commonly occurring of all the flavones[80]. It occurs both free and in the form of several glycosides. The glycoside of quercetin [quercetin-3-(2"-galloylglucoside)] (18) was first isolated from *P. senegalense* by Dossaji[38].

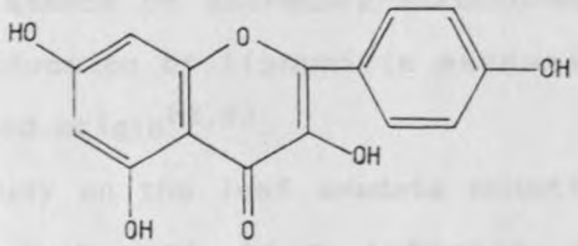
Table 6

A selection of Flavone and Flavonol ^{13}C-NMR spectra.

COMPOUND	C-2	C-3	C-4	C-5	C-6	C-7	C-8	C-9	C-10	C-1'	C-2'	C-3'	C-4'	C-5'	C-6'
Flavone	162.1	106.7	176.7	125.1	124.5	133.8	118.2	155.3	123.1	130.8	126.0	128.7	131.4	128.7	126.0
Kaempferol	146.8	135.6	175.9	160.7	98.2	163.9	93.5	156.2	103.1	121.7	129.5	115.4	159.2	115.4	129.5
Quercetin	146.9	135.8	175.9	160.8	98.3	164.0	93.5	156.2	103.1	122.1	115.2	145.1	147.7	115.7	120.1
Compound (5)	146.8	135.8	175.9	160.8	98.2	163.9	93.4	156.2	103.0	122.0	115.1	145.1	147.7	115.6	120.0
Compound (6)	146.7	135.6	175.9	160.7	98.1	163.8	93.4	156.1	103.0	121.6	129.4	115.4	159.1	115.4	129.4

Compound (6) gave a UV spectrum which displayed two major absorption bands at λmax (MeOH), 363 nm and 268 nm, which is characteristic of flavones and flavonols. From the proton NMR spectrum, the signals at ppm; 9.35 (s), 12.44 (s), 10.75 (s) and 10.07 (s) plus the molecular ion peak at m/e 286 in the mass spectrum, suggested the presence of four hydroxyl groups in compound (6). The presence of an ion peak at m/e 153 in the mass spectrum suggested that two hydroxyl groups are resident in ring-A while an ion peak at m/e 121 suggested only one hydroxyl group in ring-B. the fact that compound (6) has no *ortho*-dihydroxyl groups was elucidated by lack of shifts in all absorption bands when HCl was added to a MeOH / $AlCl_3$ solution of the compound, and no shift at all when H_3BO_3 was added to a MeOH / NaOAc solution of compound (6). The presence of a 3,4'-dihydroxyl system was confirmed by a bathochromic shift in band I of 49 nm and subsequent degeneration of the band. the assigning of the hydroxyl group at C-4' was further substantiated by the signals in the proton NMR spectrum at 6.90 ppm (d, J = 8.9 Hz, 2H) and 8.00 ppm (d, J = 8.9 Hz, 2H) confirming that there are two pairs of equivalent protons. The hydroxyl group at C-7 was confirmed from the bathochromic shift of 25 nm in

band I and 6 nm bathochromic shift in band II, when NaOAc was added to a methanolic solution of compound (6). The proton NMR spectrum displayed two signals at 6.15 ppm (d, J = 1.9 Hz) and 6.40 ppm (d, J = 1.9 Hz) showing *meta* coupling and implying that the two protons in the A-ring are in different environments. This together with the observed bathochromic shift of 59 nm when $AlCl_3$ was added to a methanolic solution of this compound was considered sufficient evidence for assigning compound (6) as 3,5,7,4'-tetrahydroxyflavone (kaempferol). Moreover, the UV spectrum of this compound compared well with a UV spectrum of Kaempferol from literature[79]. The structure was further confirmed through literature correlation of its ^{13}C NMR values with those of Kaempferol from literature[75] (Table 6).



6

Kaempferol is one of the flavonoids which are widely distributed and exist mainly in glycosidic combination. Though being reported present in *P. senegalense* for the first time, between 1975 and 1980, Kaempferol was reported in the leaf of *Larrea tridentata* (Zyg), *Larrea nitida* (Zyg), *Aesculus* spp (Hipp), *Betula* spp (Bet), *Populus* spp (Salic) bud excretion, *Cheilanches* spp, *Notholaena* spp and *Pityrogramma* spp[81].

Flavonoids are widely distributed as glycosides dissolved in the cell sap, that is, located in the cell vacoule of the inner tissues. Their existence as externally deposited free aglycones has long been known for some conspicous cases such as the bud excretion of of poplars, or the whitish farina on the leaves and inflorescences of primroses[34]. Usually the occurrence of flavonoid aglycones is correlated with the existence of secretory structures and also with the production of lipophilic excretions, mostly of terpenoid origin[82,83].

Study on the leaf exudate constituents has been approached with keen interest over the last years[30,31,32,84,85].

A comparison of the results discussed in this work, that is, hydrophilic components of P. senegalense, and the externally deposited free flavonoid aglycones of this plant, [27] (compounds 20,21,22,23,24,25,26,27) show that, although basic hydroxyflavonoids are also found in the leaf resin, it is evident that the flavonoid aglycones encountered are mostly methylated chalcones. Except for compound (1) the inner tissue flavonoids are flavonols and a dihydroflavonol which are more hydrophilic than the chalcones from the leaf resin. The presence of anthraquinones in the leaf inner tissues and their absence in the leaf resin should also be noted.

It is for these differences that it is felt that researchers should always screen for resinous material from plant parts before embarking on the conventional extraction methods so as to establish a clear cut picture on the distribution of compounds. The obtained information would be quite useful especially to chemotaxonomists.

2.10 Mosquito and locust bioassays of fractions and pure compounds from P. senegalense

The other part of this work was to carry out bioassays on the crude leaf wash, some compounds from the leaf wash, and from the inner tissues. The concentrate of the crude leaf wash was tested for its larvicidal activity against 2nd instar mosquito larvae of Aedes aegypti.

The tests in the experiments required a quantal type of response where the animals die or not die. The tolerance concetration was taken as the LC_{50}; the concentration above which fifty per cent (50%) of the population is killed within a given time.

Probit analysis methods[86,87] were used, where logarithmic concentrations were used to show the distribution of tolerance to avoid the extended tail (Tables 9, 11, and 13). In plotting the graphs, the probits of percentage mortality was plotted against the logarithm of concentration, and the LC_{50} was determined by taking the antilogarithm at probit 5 (Figs. 2, 3, and 4).

Table 8, appendix A, shows that the surface extract has larvicidal activity and that the activity is dependent on concentration and time of exposure.

It can also be derived from the table that high concentrations deter metamorphosis (growth retardation). Table 9 shows the probit transformations of mortality. A plot of the probits of percentage mortality against logarithm of concentration, (Fig.2), shows that, the LC_{50} of this extract is 11.21 ug/ml in 24 hours, 5.75 ug/ml in 96 hours and 3.98 ug/ml in 168 hours. Larvae treated with 7 ug/ml or above were found to remain small in size and sluggish in movement as compared to the controls.

Tests for antifeedant activity were carried out with mid-fifth instar nymphs of the desert locust *Schistocerca gregaria* using the Butterworth and Morgan method[11]. When 24-hour starved nymphs were given access to filter papers soaked in 0.25 M sucrose solution and sprayed with extracts at different concentrations, total protection for 8 hours was obtained up to an extract concentration of 10 ug/ml (Table 7). Compound (7) was isolated from the leaf resin[27] and its spectral data is in Table 20 and the spectra in appendix C. This compound was tested for its larvicidal activity against 2nd instar larvae of *Aedes aegypti*. Tables 10 and 11 (appendix A) show that this compound is very toxic. At concentrations

of 8 ug/ml and above, the larvae which survived remained small in size compared to the controls, and did not pupate. Fig.3 shows that the LC_{50} of this compound after 24 hours was 11.85 ug/ml, 4.38 ug/ml in 96 hours and 2.26 ug/ml in 168 hours.

Antifeedant tests on 5th instar nymphs of *Schistocerca gregaria* showed the dichloromethane fraction, of the hydrolysate, to have a relative antifeedant activity (RAP) of -59.1 at a concentration of 100 ug/ml (Table 7). This implies that the fraction is phagostimulatory.

The RAP values were calculated from the formula;

$$RAP = \frac{\text{Aver. \% consumed CP} - \text{Aver. \% consumed TP}}{\text{Aver. \% consumed CP} + \text{Aver. \% consumed TP}} \times 100\%$$

Where CP = Control paper

TP = Treated paper

Aver. = Average

Table 7

Relative Antifeedant Percentages (RAP) of *P. senegalense* leaf at various concentrations against 5th instar nymphs of *Schistocerca gregaria*.

Concentration ug/ml	Crude leaf wash	Crude dichloro-methane fraction	Compound (4)	Crude ethyl-acetate fraction	Compound (5)	Acetate of compound (8)	Acetate of compound (9)
100	100	-59.1	-45.1	-79.6	-89.4	52.2	65.0
10	100	-8.0	-12.8	-19.2	-23.0	8.1	11.5
1	68.7	-2.6	-3.3	-7.1	2.9	-3.5	4.7

Fig.2: Plot of the probit of P against log dose for the crude leaf wash.

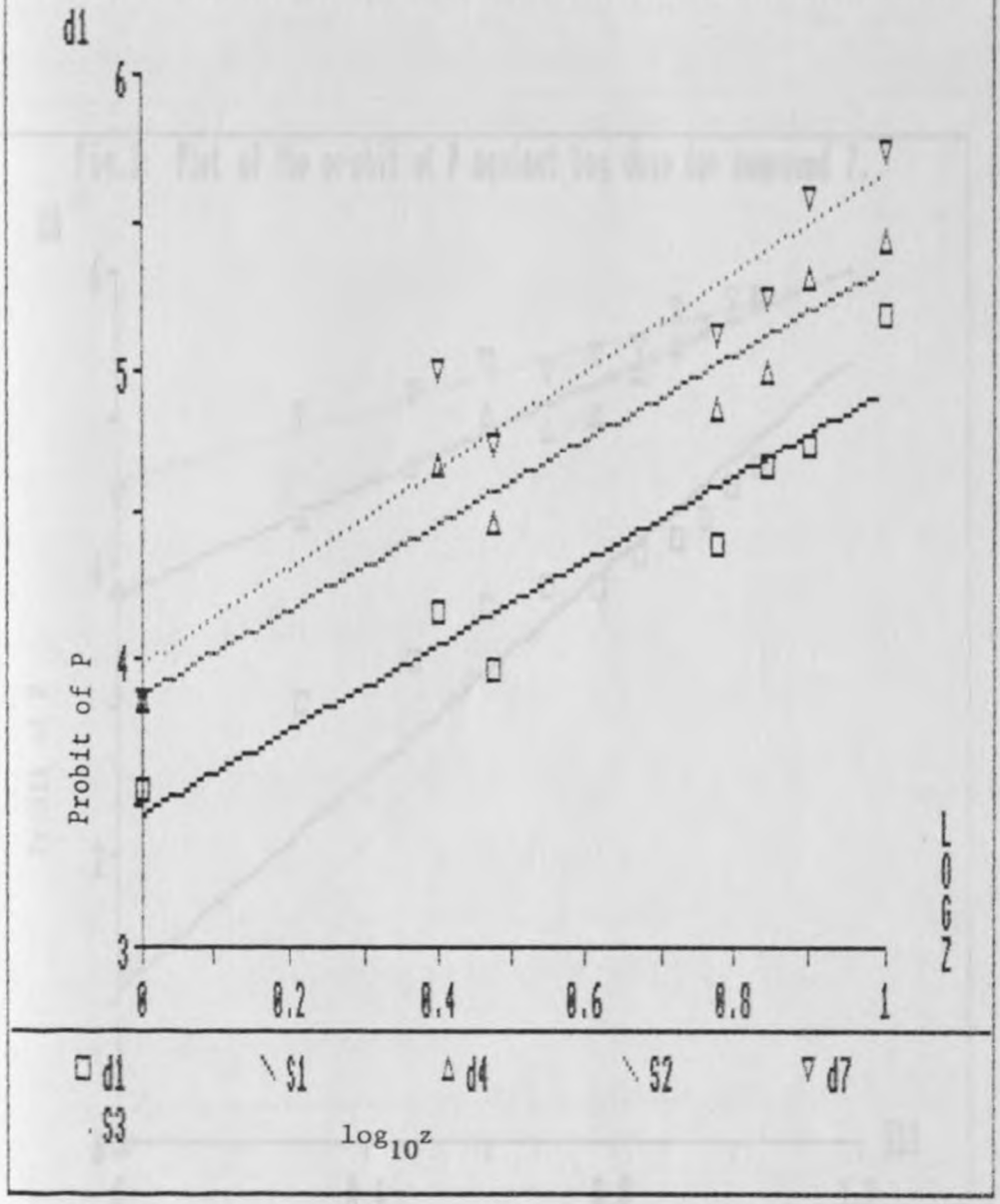


Fig.3: Plot of the probit of P against log dose for compound 7.

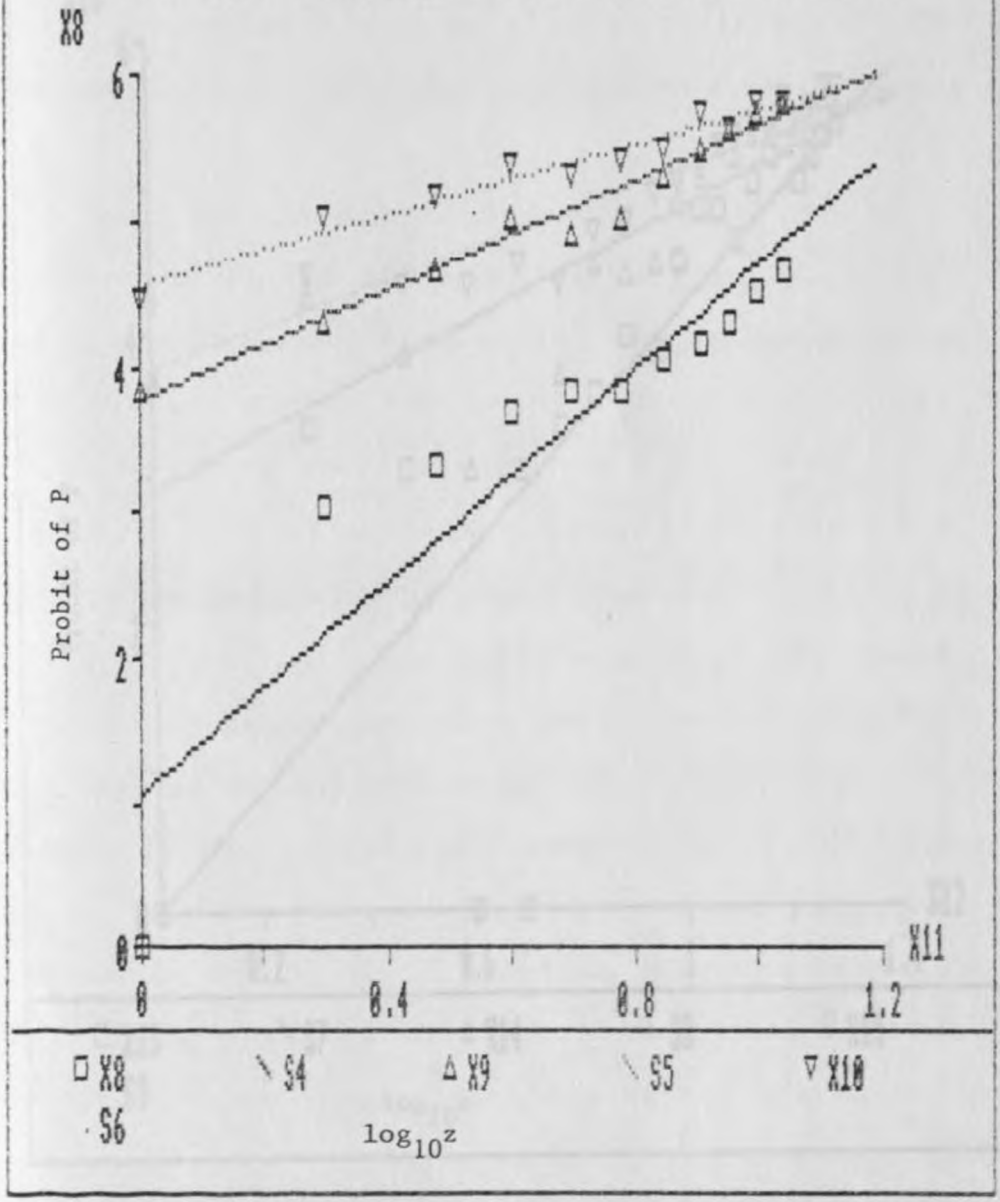
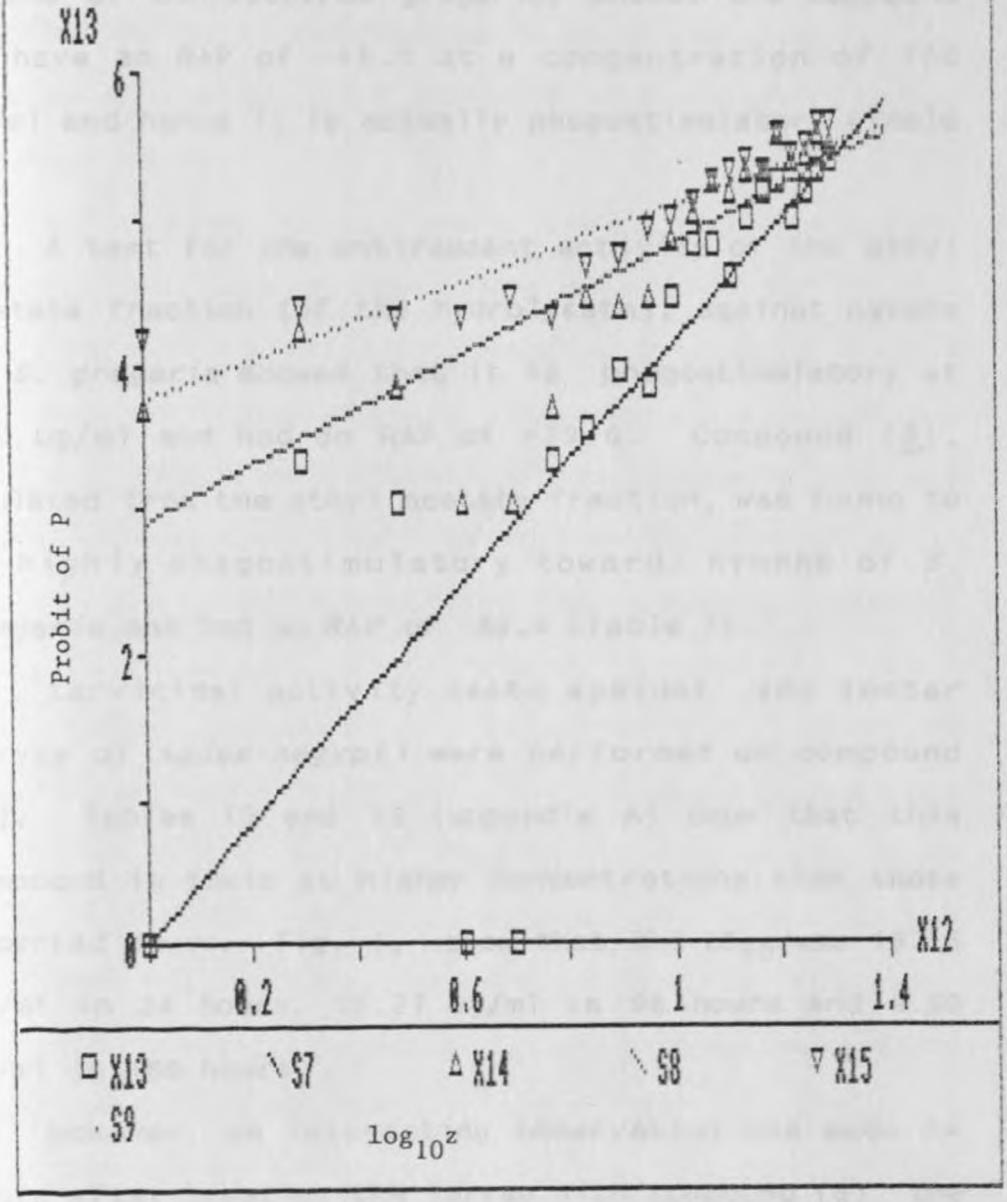


Fig.4: Plot of the probit of P against log dose for compound 5.



Compound (4), obtained from the dichloromethane fraction, showed no larvicidal activity even upto a concentration of 15 ug/ml against 2nd instar larvae of *Aedes aegypti*, but antifeedant tests on 5th instar nymphs of *Schistocerca gregaria*, showed the compound to have an RAP of -45.1 at a concentration of 100 ug/ml and hence it is actually phagostimulatory (Table 7).

A test for the antifeedant activity of the ethyl acetate fraction (of the hydrolysate), against nymphs of *S. gregaria* showed that it is phagostimulatory at 100 ug/ml and had an RAP of -79.6. Compound (5), isolated from the ethyl acetate fraction, was found to be highly phagostimulatory towards nymphs of *S. gregaria* and had an RAP of -89.4 (Table 7).

Larvicidal activity tests against 2nd instar larvae of *Aedes aegypti* were performed on compound (5). Tables 12 and 13 (appendix A) show that this compound is toxic at higher concentrations than those reported above. Fig. 4, show that the LC_{50} was 16.15 ug/ml in 24 hours, 12.27 ug/ml in 96 hours and 8.50 ug/ml in 168 hours.

However, an interesting observation was made 24 hours after treating the larvae with compound (5); the thorax of all larvae in the treated jars were reddish

in colour and brownish in the abdomen. The intensity of the pigmentation increased with the concentration of the compound. Observation of the larvae under the microscope revealed that the red pigment was localised in pockets on either side of the thorax and that this pigment was absent in the tracheal system.

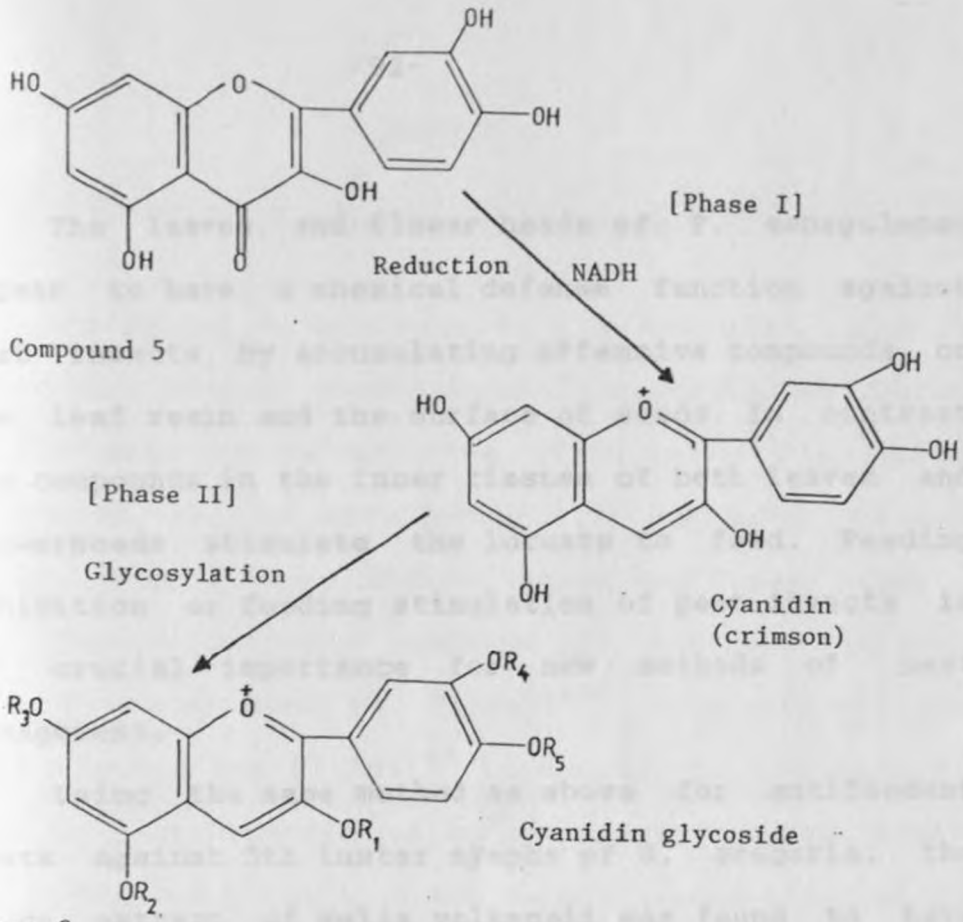
When some larvae were dissected under the microscope, the pigment was found to be in the thoracic muscles and faint brown pigmentation in the alimentary canal. However, the nervous system and the salivary glands were devoid of the pigment. It was later noticed that this pigmentation disappeared after 3 to 5 days from the surviving larvae and the water in the jars attained a slight reddish colouration.

Although no chemical analysis of this pigment in the larvae was carried out, it can be suggested that there was a biochemical reaction taking place, most probably detoxification of the compound.

Detoxification enzymes or enzyme systems, increase the water solubility of foreign compounds (xenobiotics) and thus render them more easily eliminated by the excretory mechanisms of the organism. These substances are adapted for the elimination of the water-soluble end products of

metabolic processes. The process by which such compounds are eliminated is divided into two phases; the first involves oxidations, reductions and hydrolyses to make the compound more hydrophilic and the second phase involves combination of the functional groups with highly water-soluble endogenous metabolites such as glucose, glutathione, and various amino acids, to give rise to conjugation products which are readily excreted[88,89,90].

Using an appropriate reducing agent such as NADH, it is possible that compound (5) (quercetin), gets converted to cyanidin which is crimson in colour. This may be followed by glycosylation to give an intensely coloured cyanidin glycoside which may be easily excreted. The most plausible explanation for the above observation is summarised in scheme 8.



Scheme 8

Detoxification mechanism of compound (5) (quercetin) by larvae of A. aegypti.

Compounds (8) and (9), whose spectral data is in appendix B and C were isolated from the leaf resin[27], but were found to have no antifeedant activity. However on acetylation, they were found to have some appreciable antifeedant activities. The acetate of compound (8) had an RAP of 52.2 while that of compound (9) had an RAP of 65.0 at a concentration of 100 ug/ml (Table 7).

The leaves and flower heads of P. senegalense appear to have a chemical defense function against pest insects, by accumulating offensive compounds on the leaf resin and the surface of seeds. In contrast the compounds in the inner tissues of both leaves and flowerheads stimulate the locusts to feed. Feeding inhibition or feeding stimulation of pest insects is of crucial importance for new methods of pest management.

Using the same method as above for antifeedant tests against 5th instar nymphs of S. gregaria, the crude extract of Melia volkensii was found to have total protection upto a concentration of 1 ug/ml,[13] while azadirachtin from the seeds of Azadirachta indica was found to completely inhibit feeding at a concentration of 0.04 ug/ml.[11]

The highest larvicidal activity against larvae of A. aegypti was from compound (7) from the leaf resin and then from the crude leaf wash while the internal compounds have little or no activity at all. Such larvicidal activities have been determined on fruit kernel extract of Melia volkensii against Anopheles arabiensis mosquito larvae and it was found to have an LC_{50} of 5.4 ug/ml in 48 hours[91].

CHAPTER 3

COMMENT AND CONCLUSION

Polygonum senegalense is a plant with several compounds, most of them externally deposited on leaves and flower heads as free lipophilic flavonoid aglycones, and the more hydrophilic compounds located in the inner tissues of both leaves and flower heads.

Two glycosides of compound (5) have been isolated from *P. senegalense* by Dossaji[40] (compound 18) and Abdel-Gaward and El-Zait[41] (compound 10). It would, however, be interesting to find out how the other compounds are present in the inner tissues and also to find out whether there is another glycoside of compound (5).

The resinous material covering the leaves and flower heads of *P. senegalense* is a potential source of several biologically active ingredients. The observed larvicidal activity against *A. aegypti* and its high antifeedant activity against *S. gregaria* together with the molluscicidal activity[31] against *Lymnaea natalensis* and *Biomphalaria pfeifferi* makes this plant commercially important especially since the plant grows quite easily in the highlands and the ease

of extraction of the resinous material.

The inner tissue compounds of *P. senegalense* exhibit a feeding response towards *S. gregaria*, which is a very important activity especially in the development of new chemical products for the protection of cultivated plants.

In general the inner tissue compounds do not appear to exhibit high larvicidal activities against *A. aegypti*. This could in part be due to resistance of larvae or lack of toxicity of the compounds. From the phenomenon observed for compound (5) while carrying out larvicidal tests, the former appears to be true.

In the last decades, many species of insects have acquired resistance to insecticides. This resistance is inherited and has proved to be the biggest single barrier to successful chemical control of insects. Although most of the scientific investigations of detoxication mechanisms in insects have involved either insecticides or compounds related to them[88,89,90], it should be recalled that almost all of these compounds are synthetic organic chemicals of recent origins and the mechanisms themselves must have evolved in response to selection pressure by other, naturally occurring, toxic substances. This would

include primarily the secondary plant metabolites such as terpenes, alkaloids, flavonoids, etc., many of which are lipophilic and of low but appreciable toxicity. Since the number of potential toxicants is large, the enzymes which have evolved are nonspecific. Their not-infrequent role as detoxifying enzymes would appear to be the result of this lack of specificity, the many variations of chemical structure metabolized, including some in which the metabolites are more toxic than the parent compound.

It would therefore be interesting to know whether intoxication reactions are more common among naturally occurring toxicants, in response to which the enzymes were evolved, or among the synthetic organic toxicants, to which they have been exposed more recently.

The array of compounds in the leaf resin of P. senegalense which appear to be natural toxicants would therefore form a basis for this kind of research. The proposed detoxication mechanism of compound (5) by larvae of A. aegypti is quite attractive in studying the detoxicant intermediates and the final excretory product.

The other area that needs further research is the analysis of the gas liberated during methanol/water

extraction of dry powdered leaves of *P. senegalense*. This might elucidate the biosynthetic agents in the leaf and should therefore explain the presence of the array of O-methylated flavonoids in the leaf resin.

CHAPTER 4

4.10 GENERAL

4.11 Instruments

The UV/Vis spectra were determined using a Pye-Unicam SP8-150 UV/Vis spectrophotometer. Melting points were determined using a Gallenkamp melting point apparatus and are uncorrected. The infrared spectra were run using KBr pellet technique using a Perkin-Elmer 598 infrared spectrophotometer. The ^{1}H and ^{13}C NMR spectra were obtained from a JEOL GSX-400 spectrometer and mass spectrometry was done on a JEOL JMS-D 300 Machine at Meiji college of Pharmacy, Tokyo, Japan.

4.12 Chromatography

The diameters of the columns used were 4.5 cm, 2.0 cm and 1 cm. Column chromatography was carried out using Merck silica gel (70-230) mesh A.S.T.M. Merck silica gel suitable for thin layer chromatography (T.L.C.) was used for preparative T.L.C.

A slurry of silica gel in deionized water was spread uniformly to a depth of 1-2 mm on 20x20 cm plates using an electric spreader. They were air dried for 24 hours and then activated in an oven at 100 ^{o}C for one hour.

Flash chromatography was used which consisted of a column with fritted glass bed supports and a flow controller valve. The flow controller valve is made from a glass/teflon needle valve and a standard 24/40 joint. The pump used was a Second nature Whisper 400 instrument. The silica gel used was 40-63 um (400-230 mesh) silica gel 60. Commercial analytical Merck silica gel G plates were used for analytical T.L.C. and for monitoring the eluents. Solvent removal from fractions was carried out in vacuo using a rotary evaporator.

The solvents used for developing the plates were:

dichloromethane 100% (Solvent 1),
40% ethyl acetate in n-hexane (Solvent 2),
50% ethyl acetate in n-hexane (Solvent 3),
50% n-hexane in dichloromethane (Solvent 4),
2% methanol in dichloromethane (Solvent 5).

4.13 Plant Material

Polygonum senegalense is a locally available plant. It was collected from the southern shores of Lake Naivasha in the Kenyan rift valley. Identification of the plant was done at the University of Nairobi, Botany department herbarium. Leaves and flower heads were separated out and placed separately for purposes of study.

4.21 Qualitative Composition of Surface Components on Leaves and Flower Heads.

The wet (fresh) leaves of Polygonum senegalense were extracted with acetone by dipping the leaves into beakers containing acetone. This was done until the last beaker was almost clear. The process of washing a single leaf took not more than two minutes. The flower heads were similarly extracted.

Qualitative analysis for these extracts was performed using analytical Merck silica gel G plates and developed using solvent 1 (dichloromethane).

The relative positions of the constituent compounds of the extracts on the analytical T.L.C. plates were determined by observing the plates under UV light and then exposing them to ammonium vapour.

The components observed and their relative front values are shown in Table 1. Plant material collected from Nairobi were also analysed alongside those collected in Naivasha, and all gave the same values as shown in table 3. Leaves with either glabrous or tomentose surface showed identical surface compounds. Table 3 therefore summarises the surface phenomena of leaves and flower heads of P. senegalense.

4.22 Qualitative Composition of internal aglycones in macerated *P. senegalense* leaves and flower heads

The previously acetone-washed *P. senegalense* leaves and flower heads were allowed to dry under shade. These were then ground into a powder using an electric grinder. The powder was put into an erlnmeyer flask and added 70% methanol in water and cold extracted overnight. The extract was filtered off and methanol removed using a rotary evaporator. The aqueous extract was allowed to cool and then partitioned using dichloromethane to remove all residual aglycones. The dichloromethane soluble fraction was concentrated using a rotary evaporator and analysed alongside the acetone wash (surface material) using analytical TLC plates. These showed that apart from chlorophyll the other components were similar to those on the leaf surface.

The aqueous layer was added 2N HCl solution to make a solution of 1N HCl. This was then refluxed for 2 hours. On cooling the hydrolysate was partitioned using dichloromethane and then using ethyl acetate.

The two fractions were then analysed using analytical Merck silica gel G plates developed using solvent 1 and solvent 3 respectively. The results are shown in Table 4a and Table 4b.

4.30 LARGE SCALE EXTRACTION OF P. SENEGALENSE LEAVES

4.31 Extraction of surface compounds using acetone.

The leaves of *P.senegalense* collected from the southern shores of lake Naivasha were washed with acetone by dipping into a series of beakers each containing 400 mls of acetone; the acetone in the last beaker remaining clear. The extracts were combined and filtered. The solvent was removed in a rotary evaporator leaving a dark brown oily substance. This was stored away since the constituents are known (Wanjau 1990).

The extracted leaves were spread on a clean dry bench and allowed to dry under shade for one week.

The dry leaves were then ground into a powder using an electrically powered grinder to give 500g of powder. This was stored for further extraction.

4.32 Re-extraction of macerated *P. senegalense* leaves with methanol in water.

The powder of previously acetone-washed leaves of *P. senegalense* were placed in a five litre erlnmeyer flask. This was extracted with 80% methanol in water for twenty four hours after which it was

filtered. The residue was re-extracted with 70% methanol in water and then 50% methanol in water. Each extraction was carried out overnight.

All extracts were combined and then concentrated over a rotary evaporator until most of the methanol had been removed.

On cooling, the aqueous extract was exhaustively re-extracted with dichloromethane in a separatory funnel. The dichloromethane fraction contained chlorophyll and some flavonoid aglycones previously established as present on the leaf surface.

The aqueous extract was stored ready for hydrolysis. During extraction of the powder with 80% methanol in water a gas was being evolved from the mixture which on smelling was reminiscent of mercaptans, probably low molecular weight mercaptans. This gas or mixture of gases was not immediately analysed.

4.33 Acid hydrolysis of methanol extract of *P. senegalense* leaves

The aqueous fraction (500 mls) of the methanol extract after partitioning with dichloromethane was mixed with 2N HCl solution to make a solution of IN HCl. This was placed in a 2-litre round bottomed flask

and refluxed for two hours. The solution changed colour during the hydrolysis from dark brown to dark red.

On cooling, the hydrolysate was exhaustively extracted with dichloromethane by shaking in a separatory funnel. The dichloromethane soluble fraction was concentrated in a rotary evaporator to give 2.4g of a dark brown oily substance which is about 0.5% per gram weight of dry leaves. This was analysed using analytical Merck silica gel G plates using solvent 1.

The remaining aqueous fraction was exhaustively extracted using ethyl acetate and concentrated to give 11.5g (about 2.3% per gram weight of dry leaves) of a brownish thick substance.

The concentrate was qualitatively analysed by T.L.C. using solvent 3 together with acetone leaf wash. The dichloromethane fraction was found to be constituted of five aglycones while the ethyl acetate fraction comprised of two major aglycones. The methanol extract was found to have aglycones of relatively lower Rf-values than those of the acetone leaf wash.

4.34 Test for presence of flavonoids in eluents during column chromatography

(a) To about 3 mls of the solution, 3 mls of 1N NaOH solution was added. Colour intensification in the aqueous layer was suggestive of flavonoid presence.

(b) To the same volume of eluent was added 2 mls of 1% $AlCl_3$ solution. Intensification of colour was taken to suggest presence of flavonoids.

(c) To 2 mls of the solution was added 0.5 mls of conc. HCl and a few magnesium turnings. Colour change to brick red confirmed presence of flavonoids.

4.35 Test for presence of anthraquinones in eluents during column chromatography

To the same volume of organic solution was added 1N KOH solution and shaken . A violet colour developing in the aqueous layer was suggestive of presence of anthraquinones.

4.40 LARGE SCALE COLUMN CHROMATOGRAPHY FOR THE HYDROLYSATE EXTRACTS

4.41 Column Chromatography for the Dichloromethane Extract of the Hydrolysate

About 2.3 g of the sample was adsorbed onto 5g of silica gel and then loaded on a glass column (2.0 cm in diameter) previously packed with 60g of silica gel in dichloromethane slurry. The column was eluted with dichloromethane and then polarity increased using methanol.

The first yellow band eluted with dichloromethane turned a solution of 1M KOH violet, suggesting presence of an anthraquinone. Analysis of the fraction using TLC and solvent 4, revealed only one significant spot at R.F. 0.4, which turned pink on exposure to ammonia vapour. Further purification of this fraction using flash chromatography produced 3.8 mg of compound (2) which was recrystalised from n-hexane / dichloromethane mixture. The identity of this compound was confirmed by performing mixed melting points and co-spotting with an authentic sample of chrysophanol and showed to be identical. The column was further eluted with dichloromethane and produced a second fraction which tested positive for

anthraquinones. A TLC analysis using solvent 1 showed two major spots. One spot at R.F. Ø.45 was UV active and turned faint brown several hours after exposure to ammonia vapour. The other spot at R.F. Ø.17 turned pink on exposure to ammonia vapour. The separation of these two compounds was carried out using flash chromatography. The UV active compound (1) crystallised out to give 45 mg of white needle like crystals mpt. 177-179 °C. The other compound (3) crystallised from n-hexane / ethyl acetate mixture to give 9.2mg of reddish crystals mpt. 242-244 °C. This compound was counterchecked with an authentic sample of emodin and mixed melting points carried out. The column was then eluted with 1% methanol in dichloromethane, to give a fraction which tested positive for flavonoids. The T.L.C. showed three U.V. active spots. All turned deep blue on exposure to ammonia vapour. They had R.F. values of Ø.63, Ø.53 and Ø.39 when the T.L.C. plate was developed using solvent 2. This fraction was further purified through a small column of silica gel to give 47mg of compound (4), R.F. Ø.39 with solvent 2. The other two compounds were only in trace amounts and therefore could not be identified.

Compound (1), mpt. 177-179 °C, showed a strong

and sharp absorption band in its infrared spectrum at 3270 cm^{-1}. Other bands were observed at cm^{-1}, 3070-3010 which were weak, 1615 strong and sharp, 1560 sharp, 1490, 1470, 1440, 1400, 1370, 1295, 1158 strong and sharp. Its UV absorption band in methanol appeared at λ max 286 nm and 222 nm. When shift reagents were used, the UV λ max were observed as follows; NaOMe (322 nm with an increase in intensity, 246 nm and 222 nm), NaOAc (322 nm,268 nm sh; 244 nm sh; and 226 nm), NaOAc / H_3BO_3 (328 nm sh; 290 nm, 226 nm), $AlCl_3$ (358 nm med; 308 nm, 222 nm), $AlCl_3$ / HCl (358 nm med; 308 nm, 222 nm). The mass spectrum showed ion peaks at m/e 272, 255, 167, 141, 125, and 91. The proton NMR spectrum showed signals at ppm; 5.88 (d, J = 2.2 Hz), 5.98 (d, J = 2.2 Hz), 2.87 (t), 3.22 (t), 7.24 (m, 3H) and 3.80 (s, 3H). Its ^{13}C NMR spectrum showed signals at ppm; 30.1, 45.0, 55.9, 91.4, 95.7, 104.4, 125.8, 128.3, 141.5, 163.0, 164.8, 166.0, and at 203.9.

The IR spectrum for compound (2) (mpt. 197 - 198^{o}C) showed an absorption band at 3410 cm^{-1}broad and weak. Others were at cm^{-1}, 2920, 2850, 1620 strong, 1555, 1475, 1450, 1365, 1265 strong, 1215, 1155, and 750. Its UV absorption bands in methanol appeared at 426 nm, 284 nm, 234 nm and 274 nm sh. In presence of

5% KOH in the methanolic solution, the bands appeared at 504 nm, 281 nm, 246 nm and 274 nm sh. When dichloromethane was used as solvent the absorption bands were observed at 430 nm, 288 nm, 256 nm and a shoulder at 278 nm. Its mass spectrum showed peaks at m/e, 254, 226, 197, 169, 152, and 127. The proton NMR spectrum showed signals at ppm; 2.48 (s,3H), 7.28 (d, J = 1.2 Hz), 7.32 (d, J = 1.2 Hz) and 7.67-7.85 (m, 3H).

The IR spectrum for compound (3), showed absorption bands at cm^{-1}, 3450, 3015 weak, 2900, 1610 strong, 1570, 1470, 1370, 1330, 1215, 1165, 1260, and 1090. Its UV spectrum in dichloromethane showed peaks at 4330 nm, 284 nm, 262 nm and a shoulder at 298 nm. Its proton NMR spectrum showed signals at ppm; 7.08 (d, J = 0.80 Hz), 7.39 (d, J = 0.83 Hz), 7.04 (d, J = 2.47 Hz), 6.53 (d, J = 2.48 Hz) and 2.37 (s, 3H). The ^{13}C NMR spectrum showed signals at ppm; 21.5, 107.8, 108.8, 108.7, 113.2, 120.4, 124.0, 132.7, 134.9, 148.2, 161.4, 164.4, 165.5, 181.2, and 189.6.

Compound (4), which turned to deep blue on T.L.C. when exposed to ammonia vapour showed absorption bands on the IR spectrum at cm^{-1}, 3420 sharp, 3340 broad, 3060, 3010, 2925, 2840, 1620, 1580, 1510, 1485, 1400, 1315, 1245 and 1155. The UV spectrum in

methanol had absorption peaks at λmax, 288 nm strong and 330 nm very weak,almost a shoulder of the major peak. When shift reagents were used, the absorption bands appeared at λmax, NaOMe, 326 nm, NaOAc, 326 nm, NaOAc / H_3BO_3, 328 nm sh, 292 nm, $AlCl_3$, 356 nm weak, 281 nm, 294 nm sh; $AlCl_3$ / HCl, 356 nm weak, 280 nm, 292 nm sh. Its mass spectrum showed peaks at m/e, 316, 195, 167, 149, 117, and 79. The spectrum from proton NMR showed signals at ppm; 3.93 (s, 6H), 6.0 (s, 1H), 4.0 (1H), 5.0 (d, J = 8.80 Hz), 7.38 (m, 5H). Its ^{13}C NMR spectrum showed peaks at ppm, 94.4, 102.7, 126.7, 128.7, 128.9, 139.8, 125.6, 154.7, 158.1, 159.0, 199.1, 127.9, 72.7, 68.1, 61.0, and 51.2.

4.42 Column Chromatography for the Ethyl Acetate Extract of the Hydrolysate

A glass column of diameter 4.5 cm was packed with 140g of silica gel in dichloromethane slurry. About 5.5 g of the ethyl acetate extract was adsorbed onto about 10g of silica gel and then introduced into the column. Eluation was first carried out using dichloromethane and later polarity increased using methanol. Dichloromethane eluted only one yellow band which tested positive for both anthraquinones and flavonoids. Analytical T.L.C. carried out for this fraction using solvent 4 revealed three spots at R.F.

0.50, a yellow spot which intensified on exposure to ammonia vapour, a UV active spot at R.F. 0.48 and a yellow spot which turned pink on exposure to ammonia vapour at R.F. 0.4. When this fraction was co-spotted with compound (2), the spot at R.F. 0.4 tarried with this compound . However this fraction could not be further purified because of its little amount. The polarity of the elution solvent was increased to 1% methanol in dichloromethane then 2%, 3% and 5% when another dark yellow band started moving down the column. This fraction tested positive for flavonoids and a T.L.C. analysis using solvent 3 showed two yellow spots at R.F. 0.50 which intensified after exposure to ammonia fumes and R.F. 0.30 which intensified through dark yellow to almost grey. Further elution with increased amounts of methanol to pure methanol did elute any other band. The above yellow compounds were further isolated and purified using a small column and preparative T.L.C. using solvent 3. This yielded 12.4 mg of yellow crystals mpt. 242-244^{o}C, compound (6), having R.F. of 0.50 using solvent 3. Compound (6) was recrystalized from n-hexane / ethyl acetate mixture. The compound at R.F. 0.30 using solvent 3 was recrystalized from dichloromethane / methanol mixture to give 210 mg of

yellow needle like soft crystals; compound (5), which had a melting point of 315-317°C. Compound (6) showed absorption bands in its IR spectrum at 3400 cm^{-1} sharp and 3320 cm^{-1} broad. Other bands included, cm^{-1}, 3000, 1658, 1610, 1570, 1500, 1450, 1380, 1175, 1250, and 1225. Its UV spectrum showed absorption bands in methanol at λ max 363 nm, 322 nm sh, 292 nm sh, and 268 nm. Using shift reagents the absorption peaks were observed at λ max; NaOMe (412 nm, which degenerated, 320 nm, 278 nm), NaOAc (388 nm, 310 nm weak, 274 nm), NaOAc / H_3BO_3 (368 nm, 322 nm sh, 292 nm sh, 268 nm), $AlCl_3$ (422 nm, 346 nm, 303 nm sh, 266 nm), $AlCl_3$ / HCl (422 nm, 345 nm, 303 nm sh, 267 nm). The mass spectrum of compound (6) showed peaks at m/e 286, 258, 229, 153, 121, and 93. Its proton NMR spectrum displayed signals at ppm, 6.15 (d, J = 1.93 Hz), 6.40 (d, J = 1.93 Hz), 8.0 (d, J = 8.9 Hz, 2H), 6.90 (d, J = 8.9, 2H), and 9.35 (s, 1H), 12.44 (s, 1H), 10.75 (s, 1H), 10.07 (s, 1H). Its ^{13}C NMR spectrum showed signals at ppm, 93.4, 98.1, 115.4, 129.4, 159.1, 121.6, 103.0, 156.1, 163.8, 160.7, 175.9, 135.6, and 146.7.

Compound (5) had an IR spectrum which portrayed absorption bands at, cm^{-1}, 3390, 3300 broad, 3010 weak, 1660, 1610, 1555, 1515, 1450, 1370, 1195, 1165, 1315, and 1260. Its UV spectrum in methanol appeared

at λ max, 370 nm, 254 nm sh, 266 nm. In the presence of shift reagents, the absorption bands were observed at λ max; NaOMe, (420 nm, degenerates, 330), NaOAc, (442 nm, degenerates, 328 nm weak, 278 nm), NaOAc / H_3BO_3, (440 nm sh, 385 nm, 254 nm), $AlCl_3$, (450 nm, 272 nm), $AlCl_3$ / HCl, (425 nm, 358 nm sh, 300 nm sh, 266 nm). The mass spectrum gave peaks at m/e, 302, 167, 153, 137, and 272. The proton NMR displayed signals at, ppm, 6.26 (d, J = 2.09 Hz), 6.43 (d, J = 2.12 Hz), 7.69 (d, J = 2.16 Hz), 7.66 (d, J = 2.16 Hz), and 7.77 (d, J = 2.17 Hz). The ^{13}C NMR spectrum gave signals at ppm, 147.7, 135.8, 175.9, 160.8, 98.2, 163.9, 93.4, 156.2, 103.0, 122.0, 115.1, 145.1, 146.8, 115.6, and 120.0.

4.51 Acetylation of Compound (5)

A Sample of mass 30 mg was placed in a 100 ml flat bottomed flask containing 1ml of freshly distilled acetic anhydride and 1 ml of dry pyridine. This was then stirred for 24 hours using a magnetic stirrer at room temperature. Ice cold water was then poured into this solution with stirring. The resulting precipitate was filtered and dissolved in ethyl acetate, dried using anhydrous magnesium sulphate and concentrated. The solid was crystallised from ethyl acetate / n-hexane mixture slowly. This

produced 24 mg of soft white crystals. The compound (5) acetate was found to melt at 188-190°C. Its RF using solvent 3 was 0.38 while using the same solvent the Rf of compound (5) was 0.30. The infra-red spectrum of this acetate displayed a strong absorption band at 1775 cm^{-1} and a shoulder at 1715 cm^{-1}. Other bands appeared at cm^{-1}, 1645 sharp, 1625 sharp, 1615,1500, 1480, 1430, 1370, 1195 strong and sharp, 1015 and 900. Its UV spectrum in methanol showed absorption bands at λ max, 296 nm, and 249 nm. The mass spectrum gave peaks at m/e, 470, 429, 387, 345, 303, 273, 245, 217, 153, 137 and 109. Its proton NMR spectrum displayed signals at ppm, 1.59 (s), 2.44 (s), 6.88 (d, J = 2.2 Hz), 7.34 (d, J = 2.0 Hz), 7.74 (d, J = 2.14 Hz), 7.71 (d, J = 2.14 Hz), and 7.69 (d, J = 2.0 Hz). while the ^{13}C NMR spectrum displayed signals at ppm, 21.2, 21.0, 20.7, 20.5, 169.3, 167.9, 167.8, 134.1, 123.9, 144.4, 142.2, 124.0, 127.8, 126.5, 170.1, 150.4, 113.9, 154.3, 109.0, 156.9, and 114.8.

4.52 Acetylation of Compound (8) (from the leaf resin)

30mg of this compound were mixed with 4 mls of acetic anhydride and 1ml of dry pyridine in a 100 ml quick-fit flask. The reaction mixture was stirred for

24 hours using a magnetic stirrer at room temperature. Ice cold water was added into the reaction flask and the resultant precipitate filtered off. This precipitate was dissolved in diethyl ether and dried using anhydrous magnesium sulphate. After concentration the solid crystallised from diethyl ether / n-hexane mixture to give 25mg light yellow crystals. The acetate of compound (8) displayed signals in the proton NMR spectrum at ppm; 2.17 (s, 3H), 3.80 (s, 3H), 3.84 (s, 3H), 6.30 (d, J = 2.2 Hz), 6.41 (d, J = 2.2 Hz), 6.98 (s), 7.02 (s), 7.36-7.55 (m). The ^{13}C NMR spectrum showed signals at ppm, 191.7, 169.2, 162.1, 159.2, 150.1, 144.4, 134.8, 130.4, 128.9, 128.4, 128.0, 115.9, 100.3, 96.8, 56.1, 55.7, and 20.9.

4.53 Acetylation of Compound (9) (from the leaf resin)

100mg of this compound was placed in a 100 mls flat bottomed flask containing 2 mls of dry pyridine and 6 mls of acetic anhydride. It was then stirred for 24 hours at room temperature after which ice cold water was added to the reaction flask. The resulting precipitate was filtered off, dissolved in diethyl ether and then dried. The solid crystallised from diethyl ether / n-hexane mixture to give white

crystals of mass 65mg.

This acetate gave signals in the proton NMR spectrum at ppm, 2.19 (s, 3H), 2.36 (s, 3H), 3.76 (s, 3H), 3.77 (s, 3H), 6.64 (s, 1H), 6.93, 6.97, 7.38-7.56 (m), while the ^{13}C NMR spectrum displayed signals at ppm 20.4, 20.8, 56.3, 61.2, 104.6, 121.7, 127.3, 128.6, 128.9, 130.7, 134.5, 138.4, 142.6, 145.4, 146.2, 152.8, 168.2, 168.5, and 191.5.

4.60 BIOASSAYS

4.61 Larvicidal Tests

Tests for activities of crude acetone leaf wash and methanol extract hydrolysates (dichloromethane soluble extract and ethyl acetate soluble extract) and some of the isolated compounds both internal and external aglycones against *Aedes aegypti* mosquito larvae were performed. Eggs of *Aedes aegypti* were submerged in a 0.08% sodium chloride solution of deionized water and left undisturbed to hatch.

At their second instar, the larvae were distributed into glass jars containing 40 mls of 0.08% sodium chloride solution plus the test sample. The stock solutions were prepared by dissolving the crude extracts or compound in a small volume of

ethanol and then diluting using deionized water [91]. The sample solution were then transferred into the test glass jars using micro-pipettes so as to make various concentrations. The larvae were then fed with Ca dog biscuits. The controls contained 40 mls of 0.08% sodium chloride solution into which ethanol whose amount was equivalent to the amount added in the highest concentration of test jars was added. Each jar contained 40 larvae. All tests were carried out in duplicate. The mortality was recorded after every 24 hours until most of the controls reached adulthood; this took about one week. The mortality at day 1, 4 and 7 was analysed, (Tables 8, 10, and 12).

4.62 Antifeedant tests

Antifeedant activities against the desert locust Schistocerca gregaria was carried out using the Butterworth and Morgan procedure. The activity was tested for crude acetone leaf wash, methanol extract hydrolysates (both dichloromethane soluble extract and ethyl acetate soluble extract), and some of the isolated pure compounds and the acetates of two compounds from the leaf resin. Filter papers (Whatman No.1) were impregnated with test solution, allowed to dry and then sprayed with 0.25M sucrose

solution and allowed to dry again. For each test the insects were presented with filter papers impregnated with sucrose only, to act as a control. These test papers were presented to separate groups of ten mid 5th instar nymphs of S. gregaria which had been starved for 24 hours. After 8 hours, the papers were removed and the area of paper consumed calculated. The Relative Antifeedant Percentage (RAP) (Table 7) was calculated as follows:

$$RAP = \frac{\text{Aver. \% Consumed CP} - \text{Aver. \% Consumed TP}}{\text{Aver. \% Consumed CP} + \text{Aver. \% Consumed TP}} \times 100\%$$

Where CP = Control paper

TP = Treated paper

Aver.= Average

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APPENDIX A

Table 8

Number of 2nd instar larvae of *Aedes aegypti* surviving after treatment with various concentrations of the crude leaf wash of *P. senegalense*.
N = 40 in each test group.

Concentration ug/ml (z)	POST-TREATMENT DAYS						
				7			
	0	1	4	L	P	A	Total surviving
1	40	37	35	11	20	4	35
3	40	34	28	7	15	2	24
5	40	32	25	6	13	1	20
6	40	29	22	14	4	0	18
7	40	25	20	11	5	0	16
8	40	24	15	7	4	0	11
10	40	17	13	8	1	0	9
(Control) = 0	40	40	40	5	15	20	40

Where L = Larvae

P = Pupae

A = Adults

Table 9

Probit transformations of mortality on larvae of *A. aegypti* when treated with various concentrations of the crude acetone wash of *P. senegalense* leaf.

concentration ug/ml (z)	$\log_{10}z$	f^{d1}	P_1	Probit of P_1	f^{d4}	P_4	Probit of P_4	f^{d7}	P_7	Probit of P_7
1	0.000	3	7.5	3.56	5	12.5	3.85	5	12.5	3.85
3	0.477	6	15.0	3.96	12	30.0	4.48	16	40.0	4.75
5	0.699	8	20.0	4.16	15	37.5	4.68	20	50.0	5.00
6	0.778	11	27.5	4.40	18	45.0	4.87	22	55.0	5.13
7	0.845	15	37.5	4.68	20	50.0	5.00	24	60.0	5.25
8	0.903	16	40.0	4.75	25	62.5	5.32	29	72.5	5.60
10	1.000	23	57.5	5.19	27	67.5	5.45	31	77.5	5.76
(Control) = 0	-	0	0.0	0.00	0	0.0	0.00	0	0.0	0.00

Where f = frequency of deaths
P = percentage frequency
d = day

Table 10

Number of 2nd instar larvae of *Aedes aegypti* surviving after treatment with various concentrations of compound (7) from *P. senegalense*

N = 40

Concentration ug/ml (z)	POST-TREATMENT DAYS						
				7			
	0	1	4	L	P	A	Total surviving
1	40	40	35	11	11	6	28
2	40	39	30	7	9	3	19
3	40	38	25	10	4	3	17
4	40	36	19	10	3	1	14
5	40	35	21	9	6	0	15
6	40	35	19	10	3	0	13
7	40	33	15	10	2	0	12
8	40	32	12	9	0	0	9
9	40	30	10	10	0	0	10
10	40	27	9	8	0	0	8
11	40	25	8	8	0	0	8
Control) = 0	40	40	40	3	11	26	40

Table 11

Probit transformations of mortality when larvae of *A. aegypti* were treated with various concentrations of compound 7.

concentration ug/ml (z)	$\log_{10}z$	f^{d1}	P_1	Probit of P_1	f^{d4}	P_4	Probit of P_4	f^{d7}	P_7	Probit of P_7
1	0.000	0	0.0	0.00	5	12.5	3.85	12	30.0	4.48
2	0.301	1	2.5	3.04	10	25.0	4.33	21	52.5	5.06
3	0.477	2	5.0	3.36	15	37.5	4.68	23	57.5	5.19
4	0.602	4	10.0	3.72	21	52.5	5.06	26	65.0	5.39
5	0.699	5	12.5	3.85	19	47.5	4.94	25	62.5	5.32
6	0.778	5	12.5	3.85	21	52.5	5.06	27	67.5	5.45
7	0.845	7	17.5	4.07	25	62.5	5.32	28	70.0	5.52
8	0.903	8	20.0	4.16	28	70.0	5.52	31	77.5	5.76
9	0.954	10	25.0	4.33	30	75.0	5.67	30	75.0	5.67
10	1.000	13	32.5	4.55	31	77.5	5.76	32	80.0	5.84
11	1.041	15	37.5	4.68	32	80.0	5.84	32	80.0	5.84
(Control) = 0	-	0	0.0	0.00	0	0.0	0.00	0	0.0	0.00

Table 12

Number of survivals of 2nd instar larvae of *Aedes aegypti* surviving after treatment with various concentrations of compound 5 from *P. senegalense*

N = 40

Concentration ug/ml (z)	POST-TREATMENT DAYS						
				7			
	0	1	4	L	P	A	Total surviving
1	40	40	36	6	7	19	32
2	40	38	31	4	12	13	29
3	40	39	35	8	7	15	30
4	40	40	39	8	10	12	30
5	40	40	39	8	10	10	28
6	40	38	36	12	9	9	30
7	40	37	28	7	8	10	25
8	40	34	29	10	3	11	24
9	40	35	28	2	10	9	21
10	40	28	22	1	9	10	20
11	40	23	19	3	7	8	18
12	40	23	16	2	5	9	16
13	40	26	17	1	5	9	15
14	40	20	15	2	9	3	14
15	40	17	15	4	6	5	15
16	40	16	11	2	3	6	11
17	40	20	14	5	3	5	13
18	40	17	13	3	3	6	12
19	40	15	11	4	4	2	10
20	40	14	12	5	2	3	10
(Control) = 0	40	40	40	6	11	23	40

Table 13

Probit transformations of mortality when larvae of *A. aegypti* were treated with various concentrations of compound 5.

concentration ug/ml (x)	$\log_{10}x$	I^{d1}	P_1	Probit of P_1	I^{d4}	P_4	Probit of P_4	I^{d7}	P_7	Probit of P_7
1	0.000	0	0.0	0.00	4	10.0	3.72	8	20.0	4.16
2	0.301	2	5.0	3.36	9	22.5	4.24	11	27.5	4.40
3	0.477	1	2.5	3.04	5	12.5	3.85	10	25.0	4.33
4	0.602	0	0.0	0.00	1	2.5	3.04	10	25.0	4.33
5	0.699	0	0.0	0.00	1	2.5	3.04	12	30.0	4.48
6	0.778	2	5.0	3.36	4	10.0	3.72	10	25.0	4.33
7	0.845	3	7.5	3.56	12	30.0	4.48	15	37.5	4.68
8	0.903	6	15.0	3.96	11	27.5	4.40	16	40.0	4.75
9	0.954	5	12.5	3.85	12	30.0	4.48	19	47.5	4.94
10	1.000	12	30.0	4.48	18	45.0	4.87	20	50.0	5.00

Table 13 continued...

11		1.041	17	42.5	4.81	21	52.5	5.06	22	55.0	5.13
12		1.079	17	42.5	4.81	24	60.0	5.25	24	60.0	5.25
13		1.114	14	35.0	4.61	23	57.5	5.19	25	62.5	5.32
14		1.146	20	50.0	5.0	25	62.5	5.32	26	65.0	5.39
15		1.176	23	57.5	5.19	25	62.5	5.32	25	62.5	5.32
16		1.204	24	60.0	5.25	29	72.5	5.60	29	72.5	5.60
17		1.230	20	50.0	5.00	26	65.0	5.39	27	67.5	5.45
18		1.255	23	57.5	5.19	27	67.5	5.45	28	70.0	5.52
19		1.279	25	62.5	5.32	29	72.5	6.50	30	75.0	5.67
20		1.301	26	65.0	5.39	28	70.0	5.52	30	75.0	5.67
(Control) = 0		-	0	0.0	0.00	0	0.0	0.00	0	0.0	0.00

APPENDIX B

Table 14

NMR Chemical shift positions for 2',4'-dihydroxy-6'-methoxydihydrochalcone (1)

Position of C	$\delta^{13}C$ NMR	$\delta^{1}H$ NMR
1'	104.4	-
2'	164.8	-
3'	95.7	5.88 d, J = 2.2 Hz
4'	163.0	-
5'	91.4	5.98 d, J = 2.2 Hz
Keto	203.7	-
	45.0	3.22 t.
	30.1	2.87 t.
1	141.5	-
3	125.8	
4	125.8	7.24 m.
5	125.8	
6	128.3	
6'-OCH_3	55.9	3.80 s.

Table 15

NMR Chemical shift positions for compound (2)
1,8-dihydroxy-3-methylanthraquinone

Position of C	δ^1H NMR
1	-
2	7.28 d, J = 1.17 Hz
3	-
4	7.32 d, J = 1.15 Hz
5	7.82 d, J = 1.16 Hz
6	7.67 t
7	7.85 d, J = 1.12 Hz
8	-
9	-
10	-
11	-
12	-
13	-
14	-
C_1-OH	12.04 s
C_8-OH	12.14 s
C_3-CH_3	2.48 s

Table 16

NMR Chemical shift positions for
1,6,8-trihydroxy-3-methylanthraquinone (3)

Position of C	$\delta^{13}C$ NMR	$\delta^{1}H$ NMR
1	161.4	-
2	120.4	7.08 d, J = 0.80 Hz
3	148.2	-
4	124.0	7.39 d, J = 0.83 Hz
5	108.7	7.04 d, J = 2.47 Hz
6	165.5	-
7	107.8	6.53 d, J = 2.48 Hz
8	164.4	-
C-9 Keto	189.6[a]	-
C-10 Keto	181.2[a]	-
11	134.9[b]	-
12	108.8[c]	-
13	113.2[c]	-
14	132.7[b]	-
3-CH_3	21.5	2.37 s.

Table 17

NMR Chemical shift positions for compound (4) 3,7-dihydroxy-5,8-dimethoxyflavanone

Position of C	δ^{13}C NMR	δ^{1}H NMR
2	72.7	5.0 d, J = 8.80 Hz
3	68.1	4.0 d, J = 3.84 Hz
4-Keto	199.1	-
5	154.7	-
6	94.4	6.0 s
7	158.9	-
8	158.1	-
9	139.8	-
10	102.7	-
1'	128.8	-
2'	126.7	
3'	128.7	
4'	128.6	7.4 m
5'	128.7	
6'	126.7	
5-OCH_3	61.0	3.93 s
8-OCH_3	51.2	3.92 s

Table 18

NMR Chemical shift positions for compound (5) 3,5,7,3',4'-pentahydroxyflavone

Position of C	δ^{13}C NMR	δ^{1}H NMR
2	146.8	-
3	135.8	-
4-Keto	175.9	-
5	160.8	-
6	98.2	6.26 d, J = 2.09 Hz
7	163.9	-
8	93.4	6.43 d, J = 2.12 Hz
9	156.2	-
10	103.0	-
1'	122.0	-
2'	115.1	7.69 d, J = 2.16 Hz
3'	145.1	-
4'	147.7	-
5'	115.6	7.66 d, J = 2.16 Hz
6'	120.0	7.77 d, J = 2.17 Hz

Table 19

NMR Chemical shift positions for compound (6) 3,5,7,4'-tetrahydroxyflavone

Position of C	$\delta^{13}C$ NMR	$\delta^{1}H$ NMR
2	146.7	-
3	135.6	-
4-Keto	175.9	-
5	160.7	-
6	98.1	6.15 d, J = 1.93 Hz
7	163.8	-
8	93.4	6.40 d, J = 1.93 Hz
9	156.1	-
10	103.0	-
1'	121.6	-
2'	129.4	8.00 d, J = 8.89 Hz
3'	115.4	6.90 d, J = 8.89 Hz
4'	159.1	-
5'	115.4	6.90 d, J = 8.89 Hz
6'	129.4	8.00 d, J = 8.89 Hz
3-OH	-	9.35 s
5-OH	-	12.44 s
7-OH	-	10.75 s
4'-OH	-	10.07 s

Table 20

NMR Chemical shift positions for compound (7)
2',6'-dihydroxy-4'-methoxydihydrochalcone

Position of C	δ^{13}C NMR	δ^{1}H NMR
1'	104.4	-
2'	156.5	-
3'	94.9	5.92 s
4'	165.6	-
5'	94.9	5.92 s
6'	156.5	-
Keto	204.4	-
	45.3	3.4 t
	30.8	3.0 t
1	141.7	-
2	128.7	7.30 m
3	126.1	7.30 m
4	128.8	7.30 m
5	126.1	7.30 m
6	128.7	7.30 m
4'-OCH_3	55.8	3.70 s

Table 21

NMR Chemical shift positions for compound (8)
2'-hydroxy-4',6'-dimethoxychalcone

Position of C	δ^{13}C NMR	δ^{1}H NMR
1'	106.3	-
2'	168.4	-
3'	93.8	6.09 d, 2.0 Hz
4'	162.5	-
5'	91.3	5.95 d, 2.0 Hz
6'	166.2	-
Keto	192.6	-
	127.5	7.77 d, 15.8 Hz
	142.3	7.89 d, 15.8 Hz
1	135.5	-
2	128.8	7.58 m
3	128.3	7.38 m
4	130.0	7.38 m
5	128.8	7.38 m
6	128.8	7.58 m
4'-OCH_3	55.8	3.90 s
6'-OCH_3	55.8	3.82 s

Table 22

NMR Chemical shift positions for compound (9)
2',4'-dihydroxy-3',6'-dimethoxychalcone

Position of C	δ^{13}C NMR	δ^{1}H NMR
3'	94.9	5.92 s
1'	106.4	-
2'	158.7	14.34 s
3'	135.3	-
4'	155.2	6.38 s
5'	89.7	6.06 s
6'	158.7	-
Keto	193.0	-
	127.4	7.79 d, 15.5 Hz
	142.5	7.87 d, 15.6 Hz
1	128.4	-
2	128.8	7.60 m
3	128.4	7.39 m
4	130.0	7.39 m
5	128.4	7.39 m
6	128.8	7.60 m
3'-OCH_3	60.8	3.92 s
6'-OCH_3	55.9	3.90 s

APPENDIX C

Key to UV spectrum, solvent and shift reagents

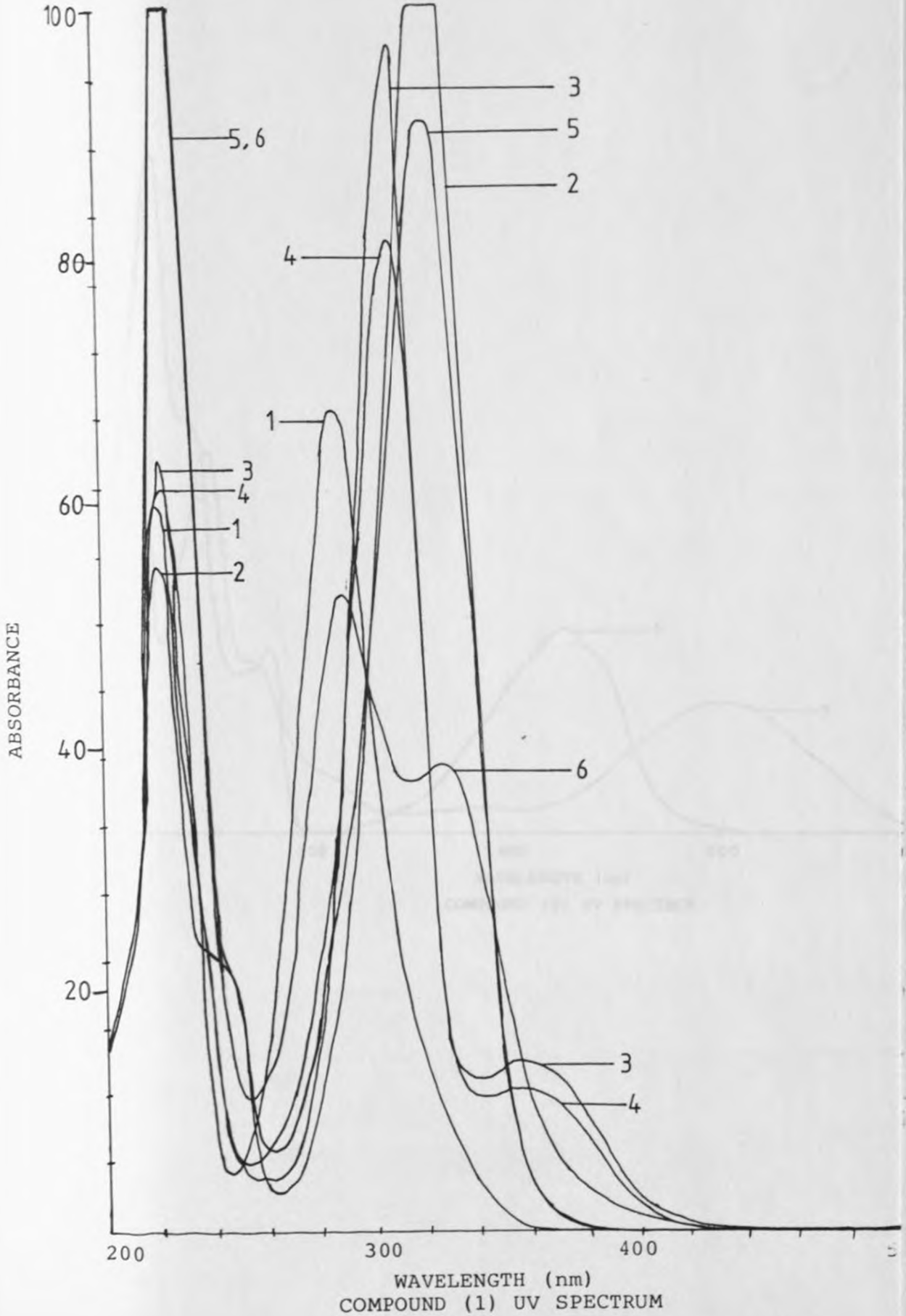
1. MeOH
2. MeOH + NaOMe
2b. MeOH + NaOMe after 5 minutes
3. MeOH + $AlCl_3$
4. MeOH + $AlCl_3$ + HCl
5. MeOH + NaOAc
5b. MeOH + NaOAc after 8 minutes
6. MeOH + NaOAc + H_3BO_3
7. MeOH + 5%KOH
8. CH_2Cl_2

Key to IR spectra

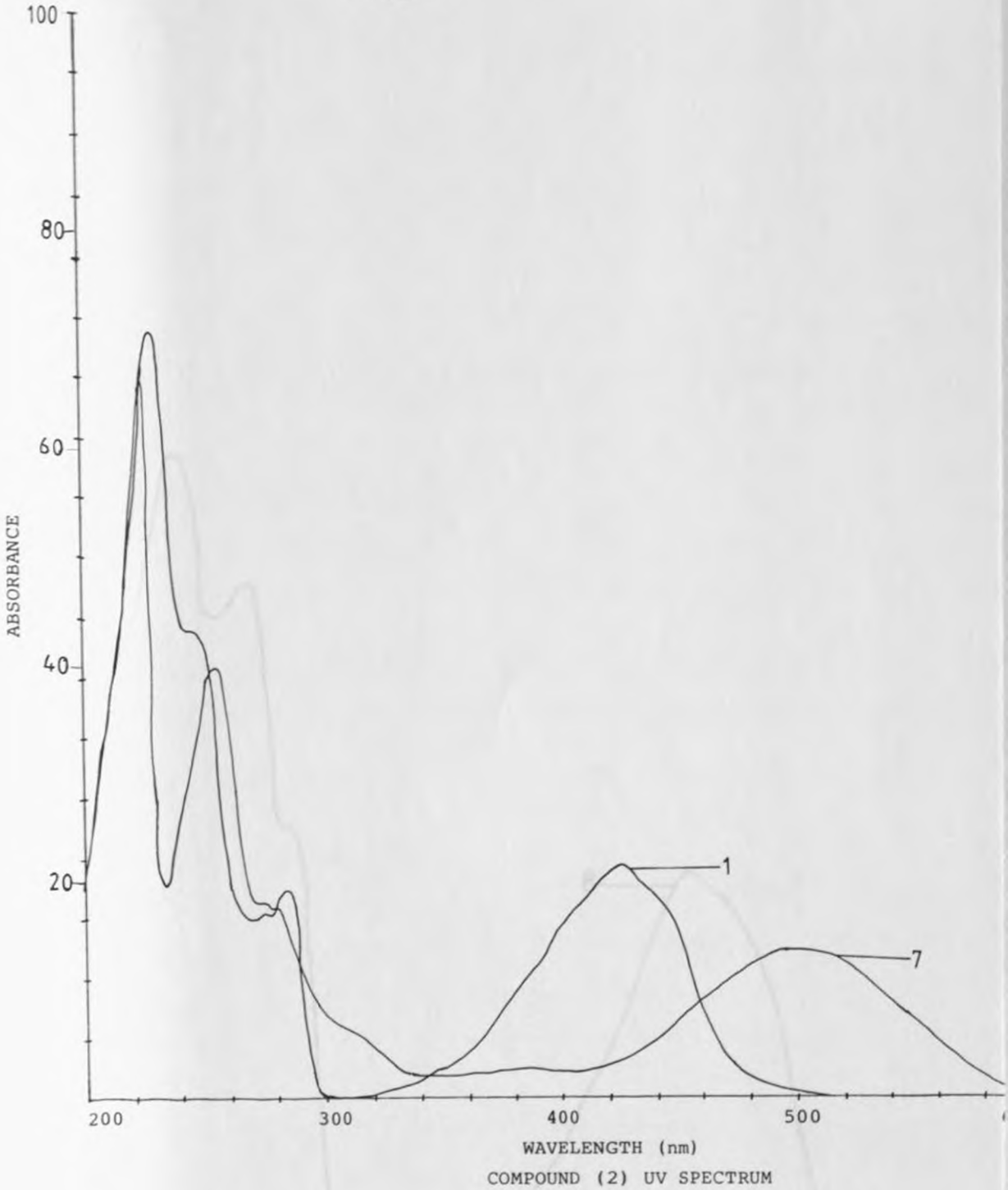
W.(cm^{-1}) = Wavelength in wavenumbers

% T = Percent transmittance

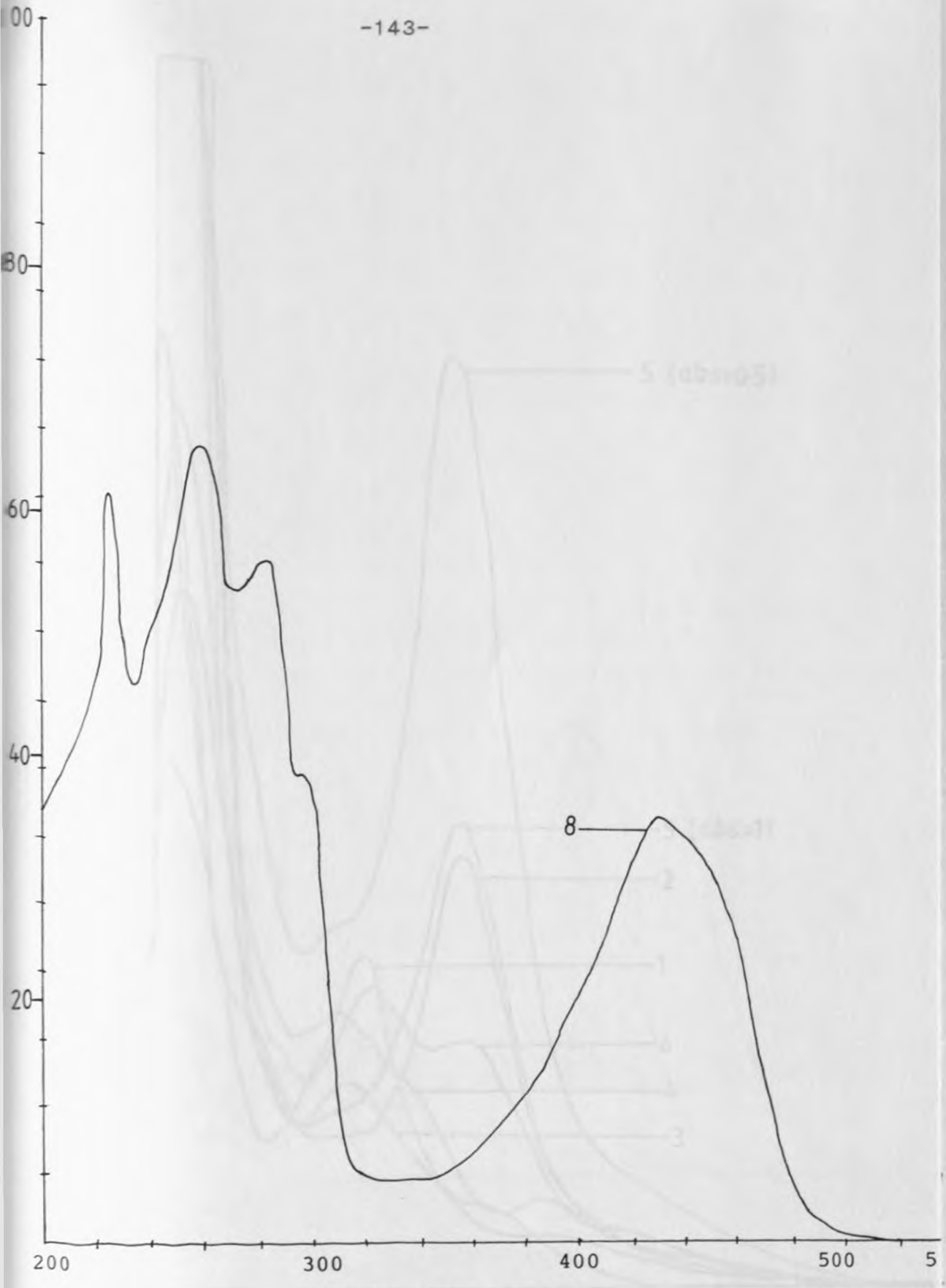
W. (μm) = Wavelength in micrometers



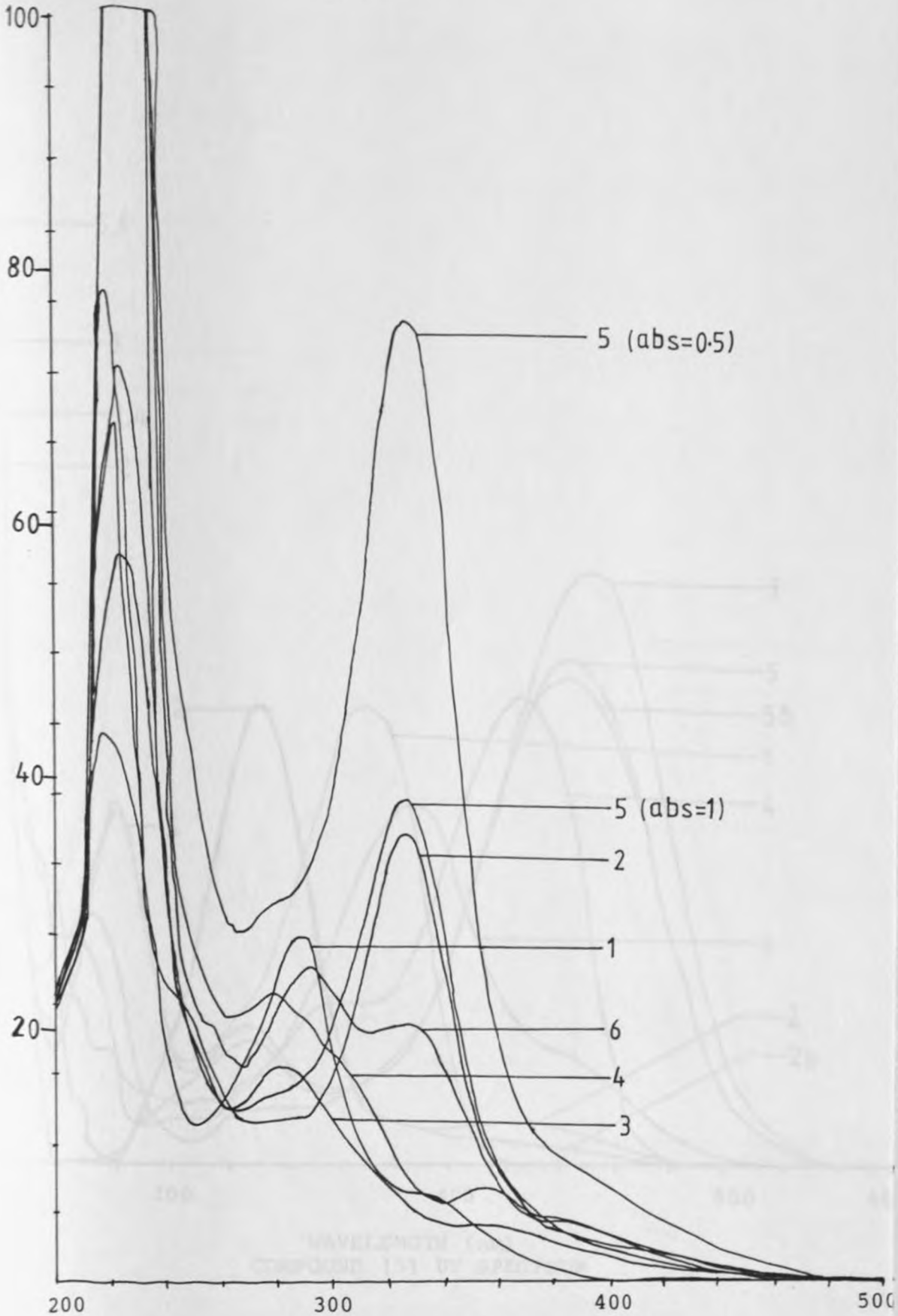
COMPOUND (1) UV SPECTRUM



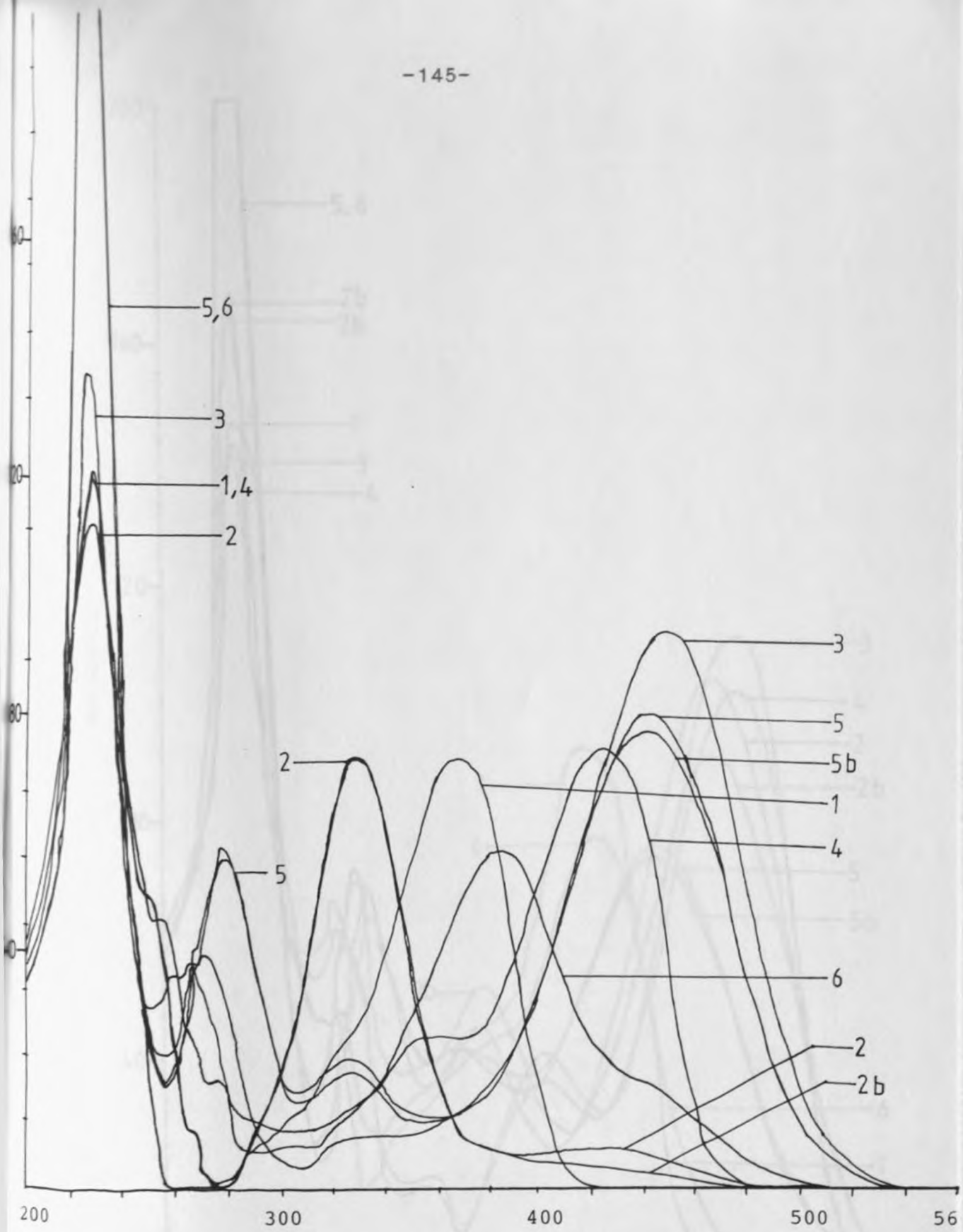
COMPOUND (2) UV SPECTRUM



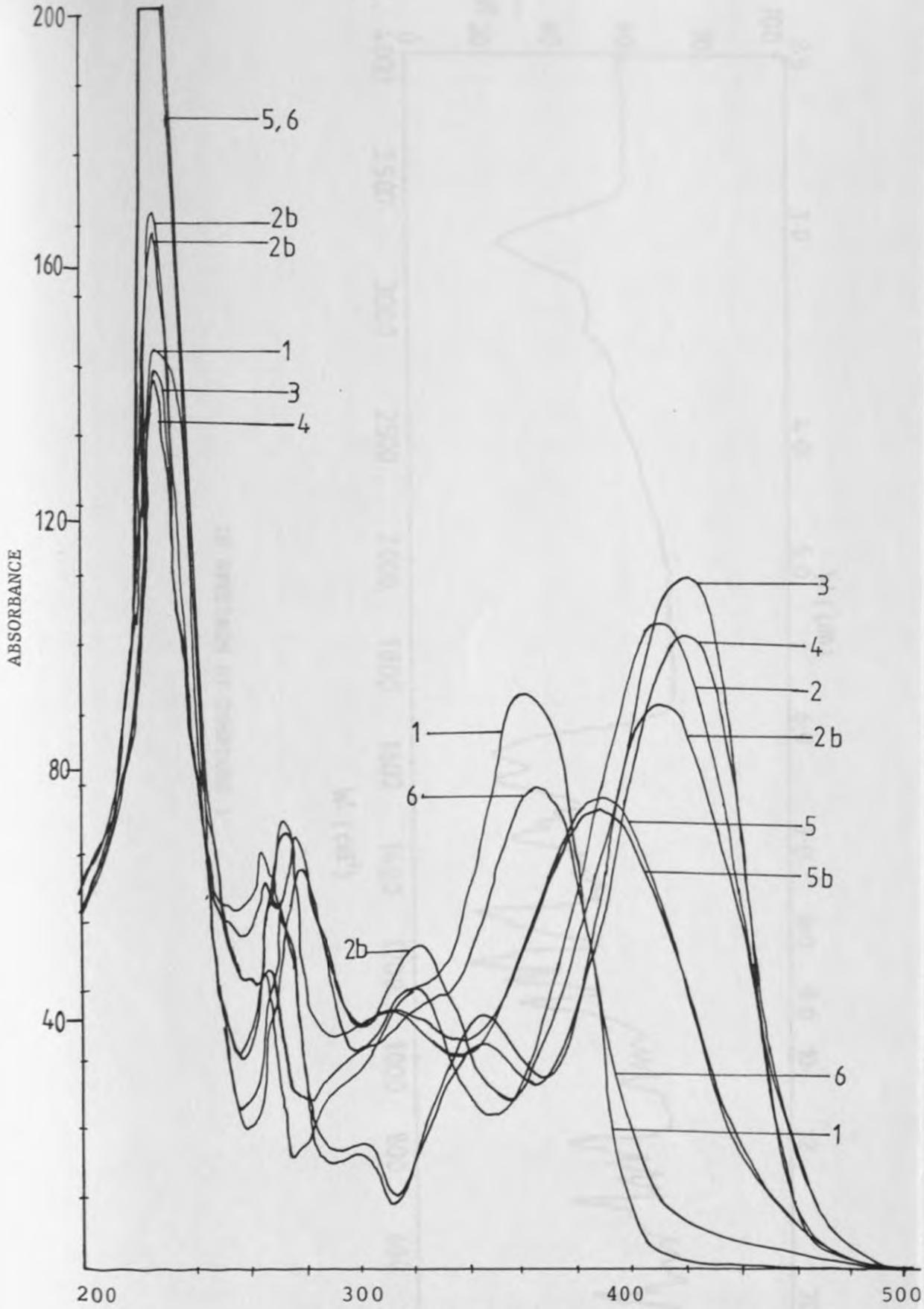
COMPOUND (3) UV SPECTRUM



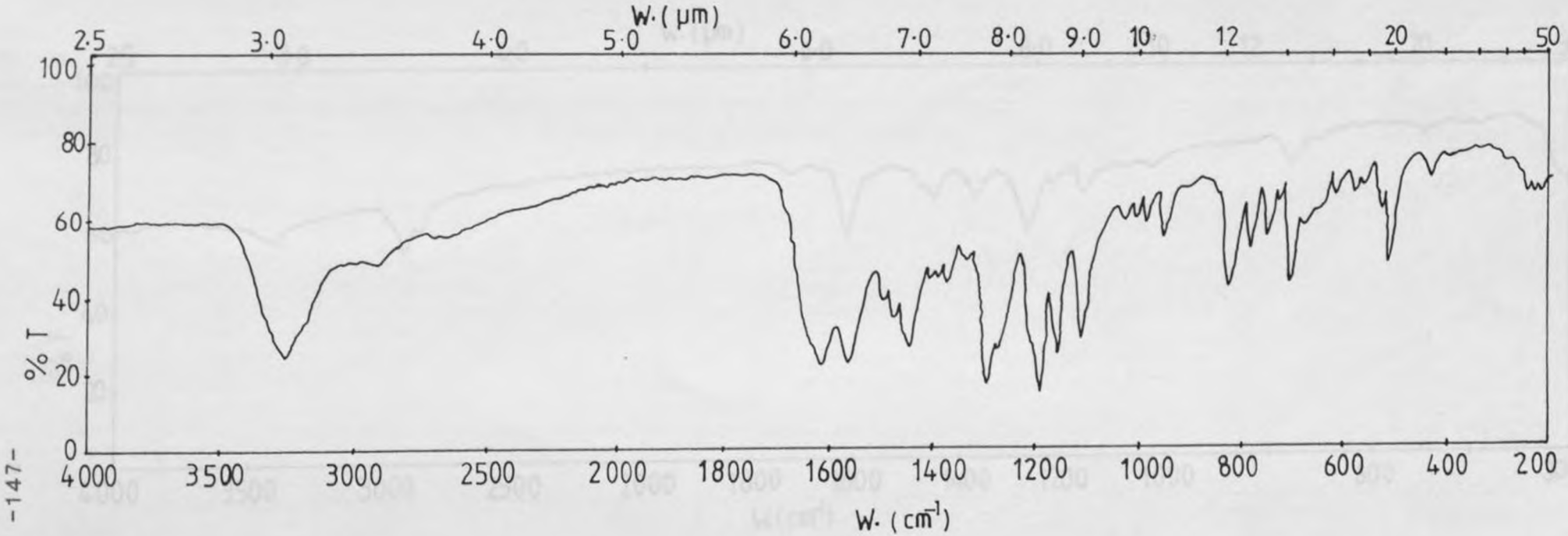
COMPOUND (4) UV SPECTRUM



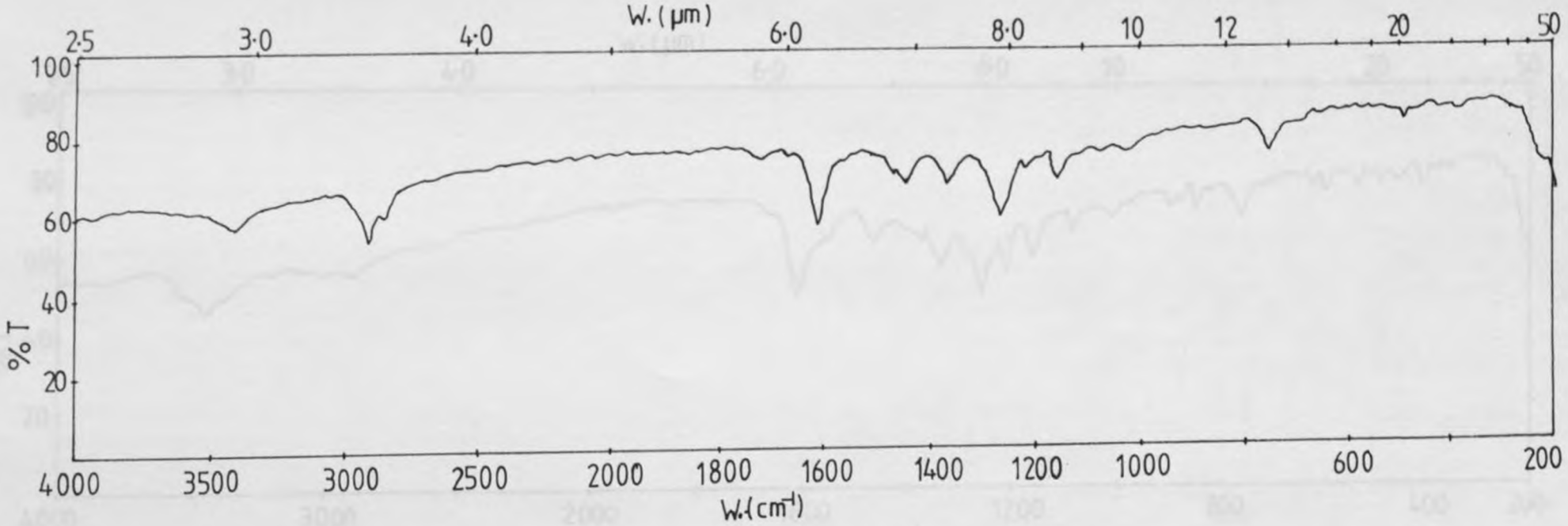
WAVELENGTH (nm)
COMPOUND (5) UV SPECTRUM



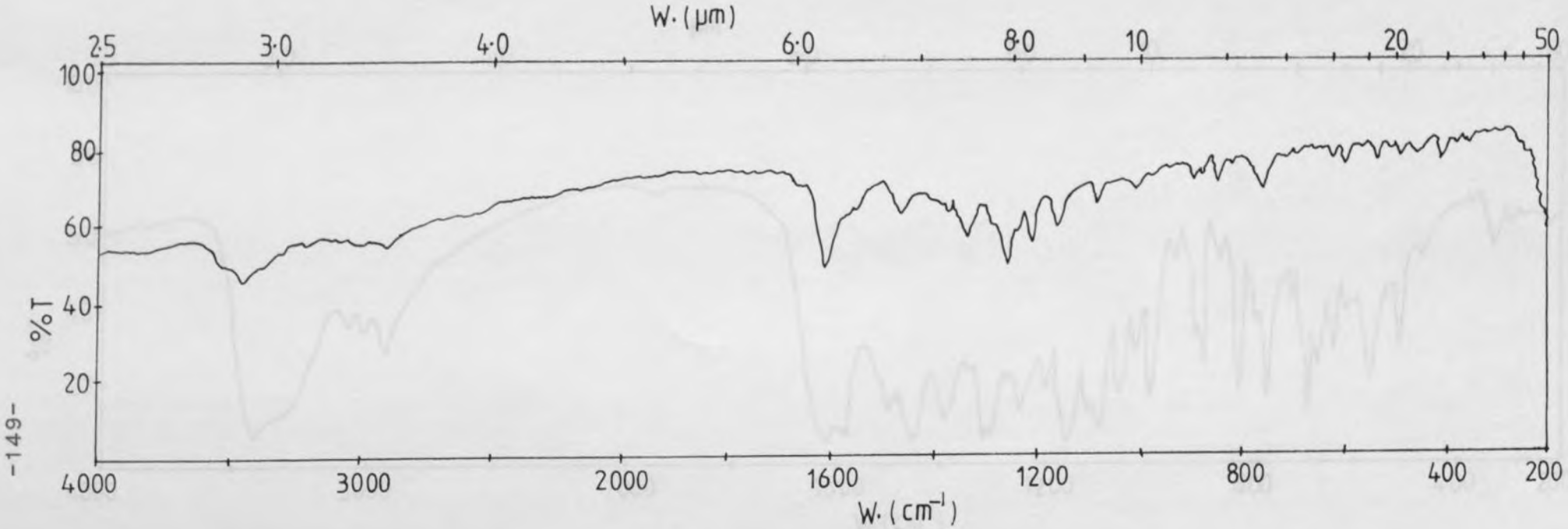
COMPOUND (6) UV SPECTRUM



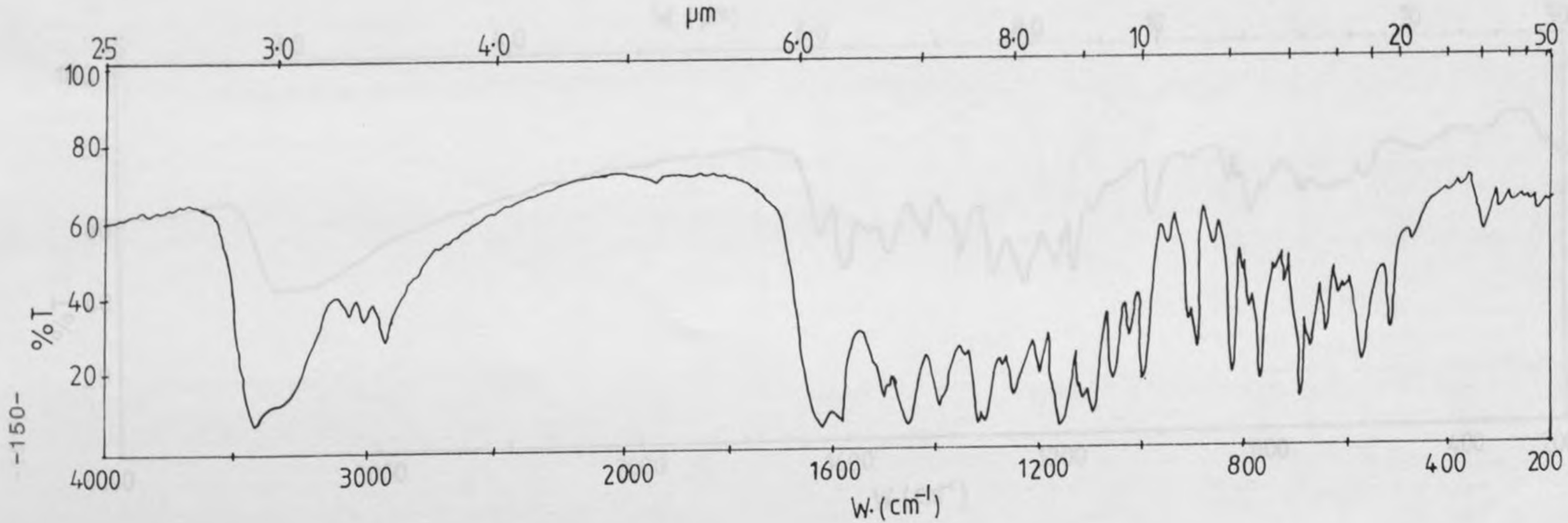
IR SPECTRUM OF COMPOUND 1



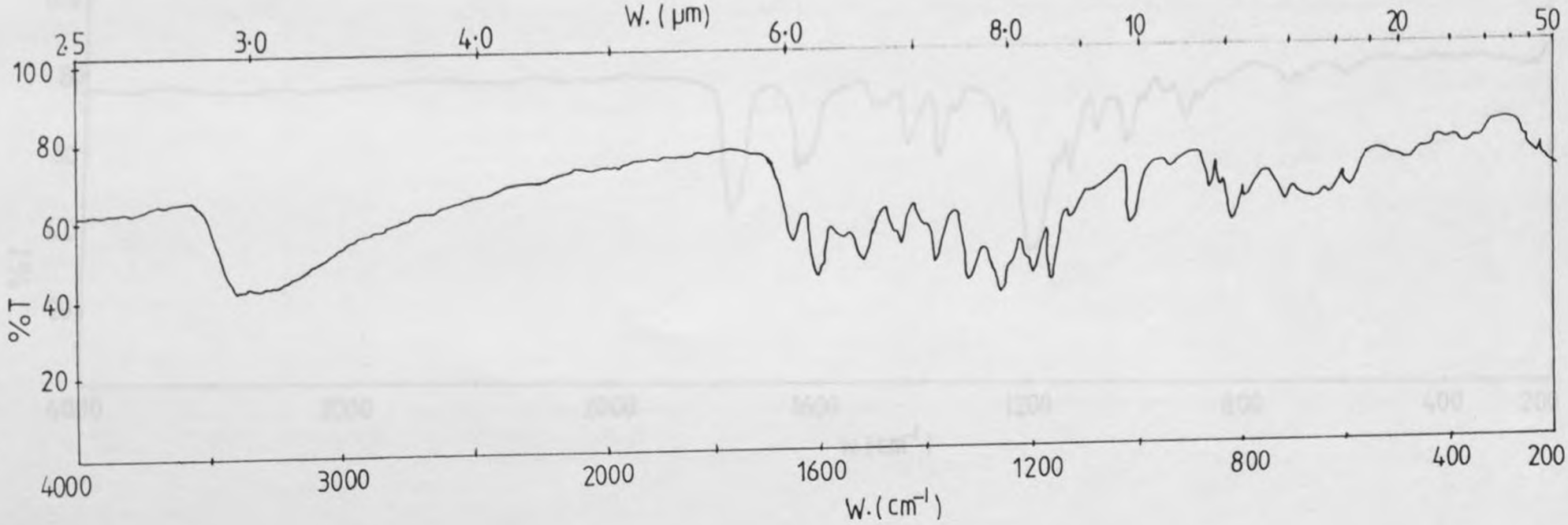
IR SPECTRUM OF COMPOUND 2



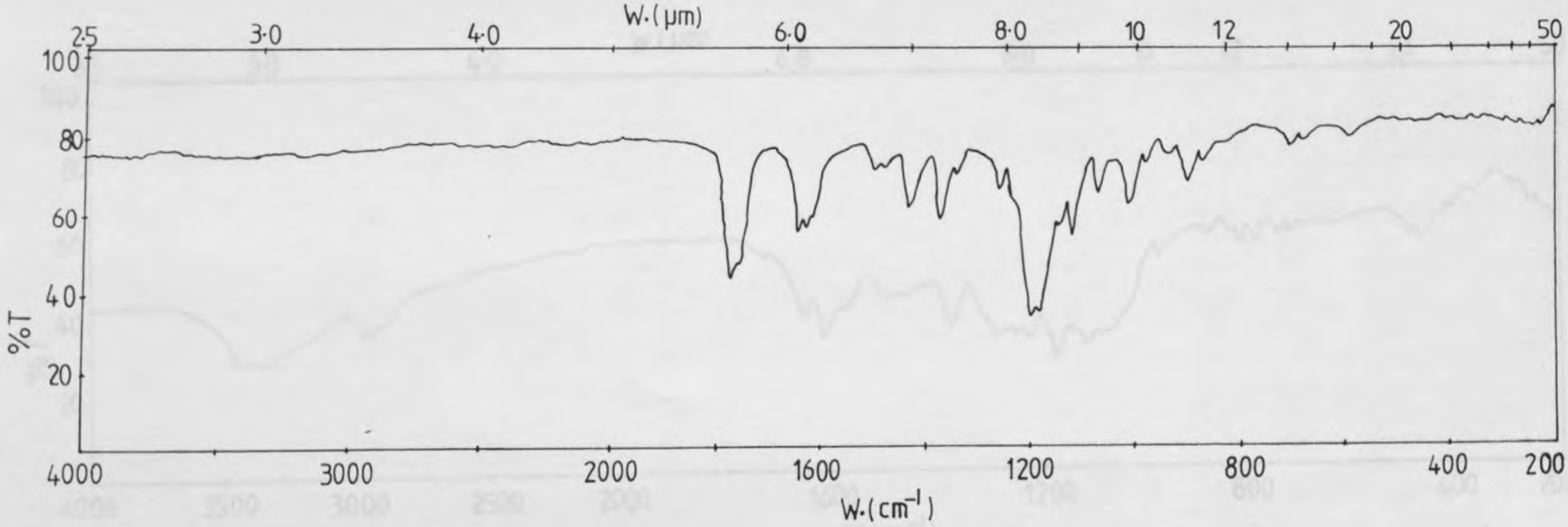
IR SPECTRUM OF COMPOUND 3



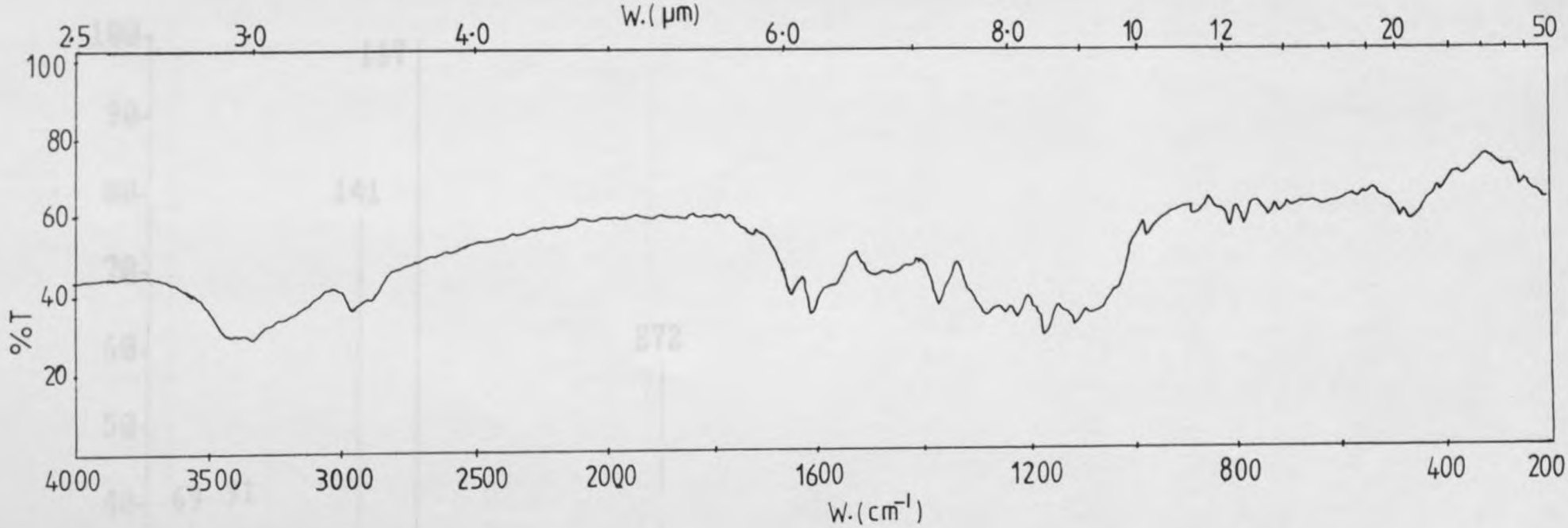
IR SPECTRUM OF COMPOUND 4



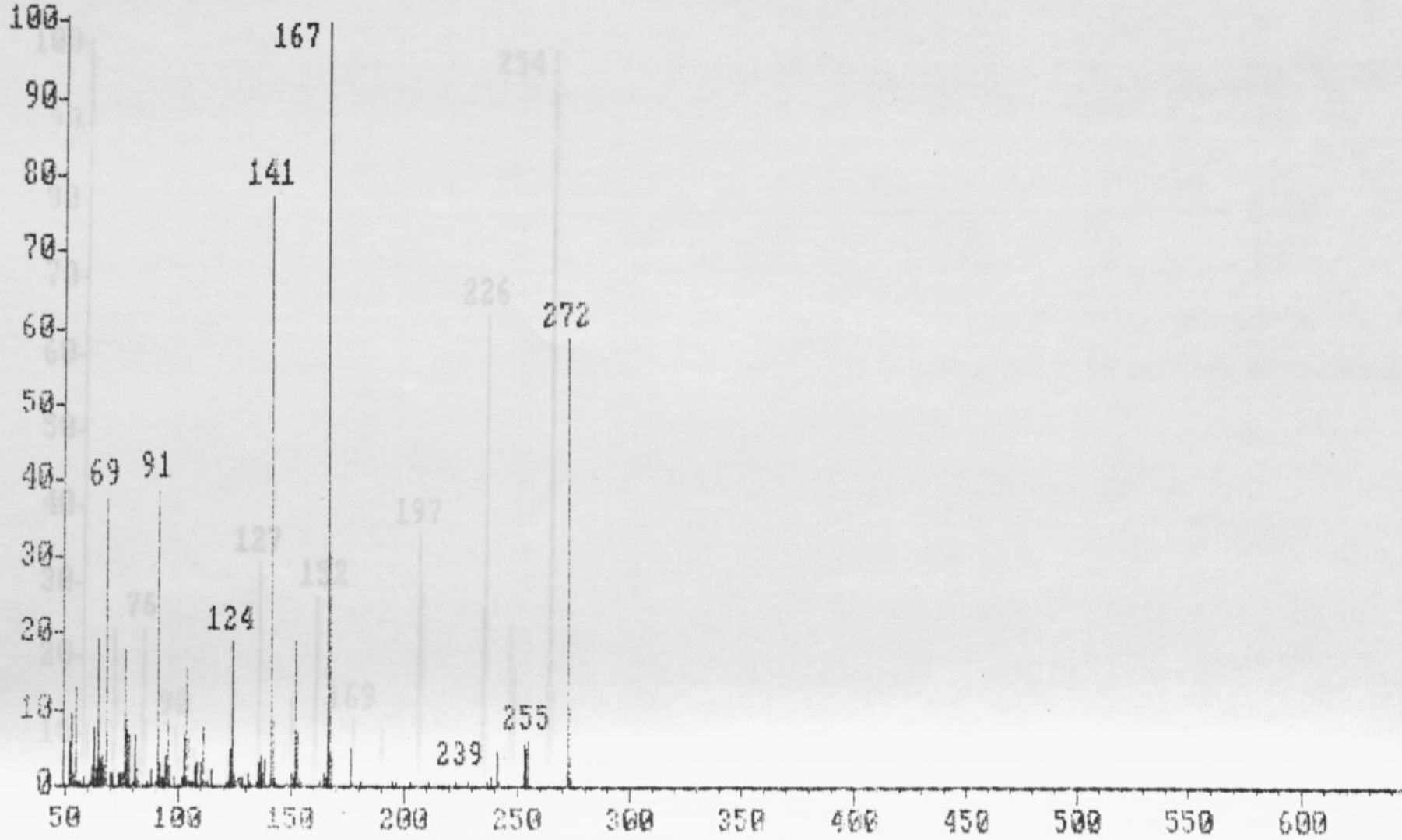
IR SPECTRUM OF COMPOUND 5



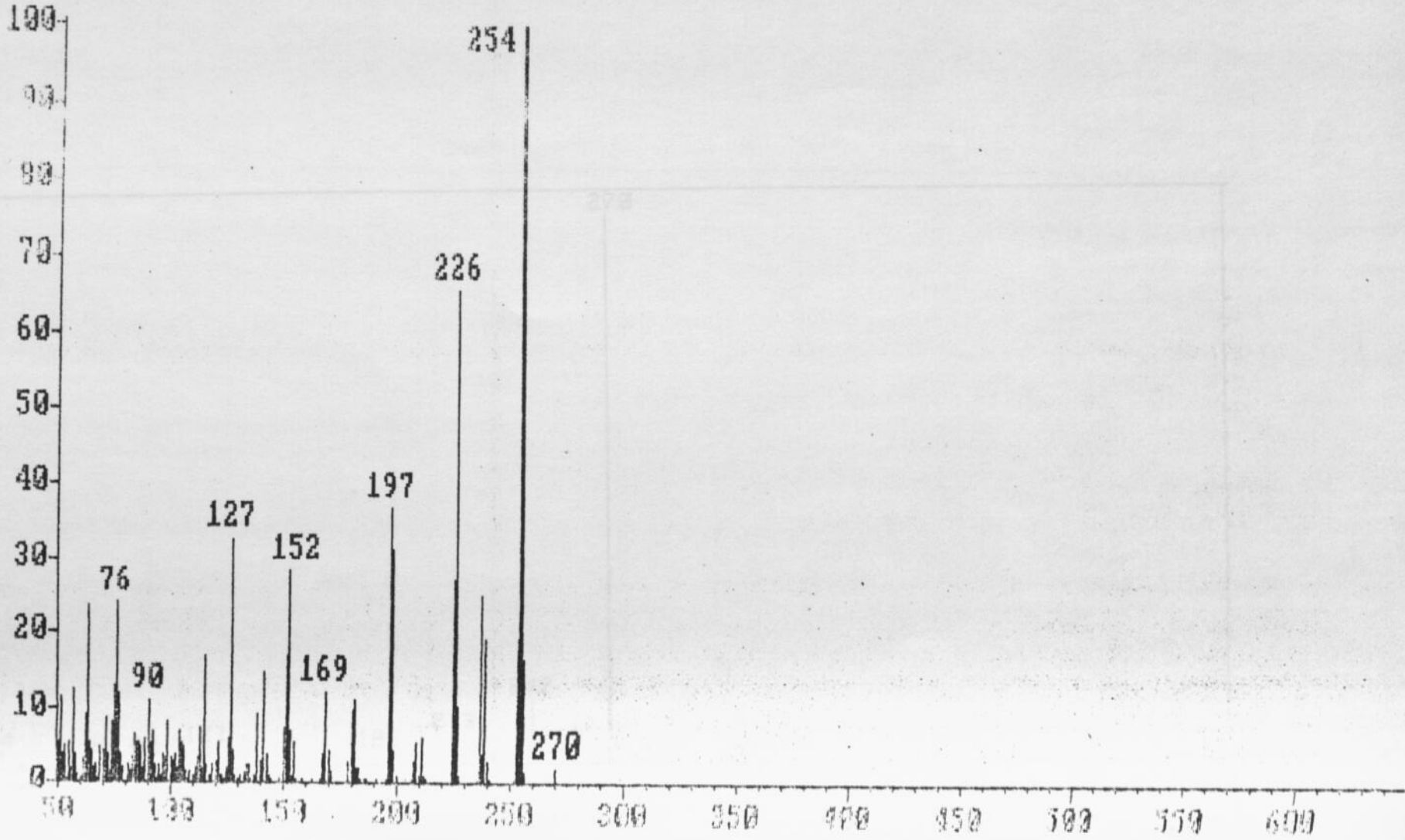
IR SPECTRUM OF COMPOUND 5 ACETATE



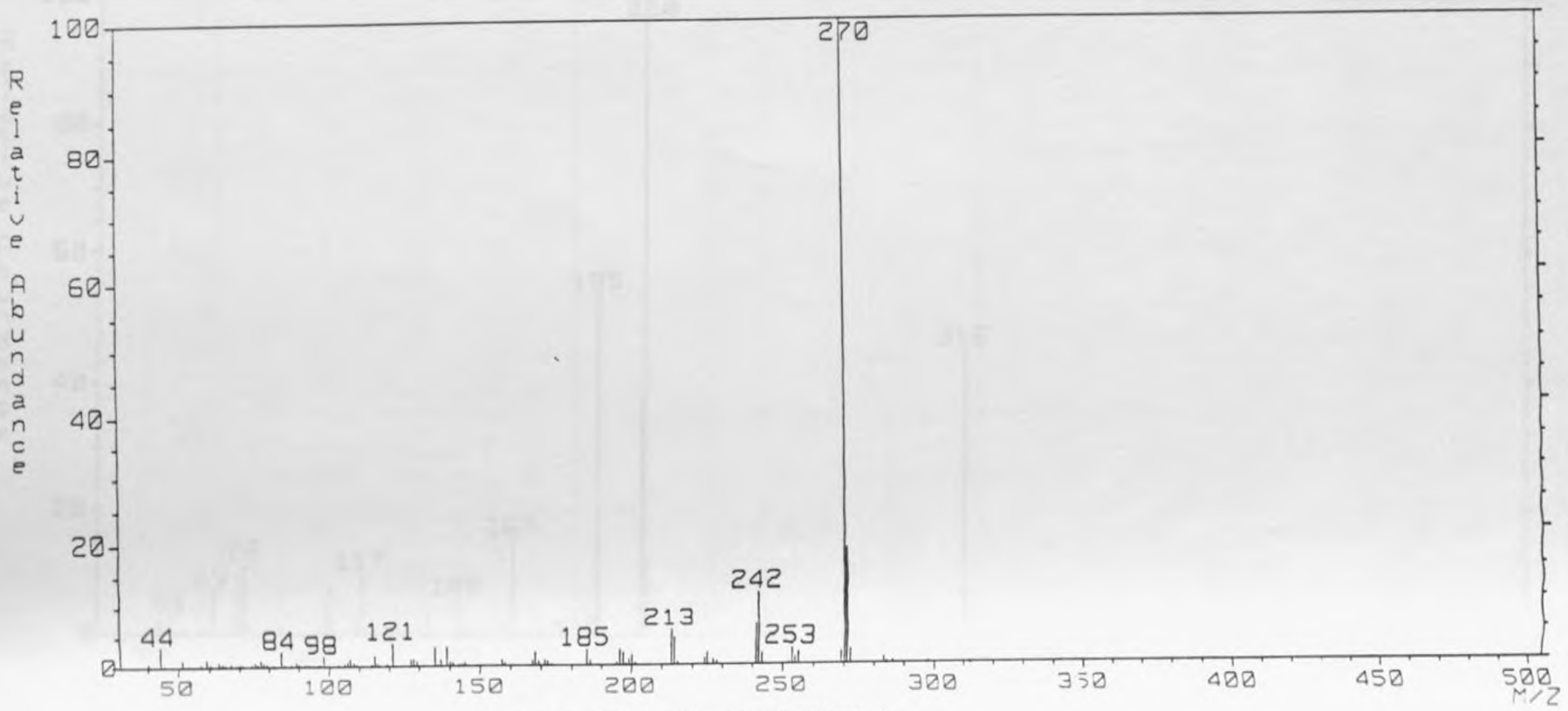
IR SPECTRUM OF COMPOUND 6



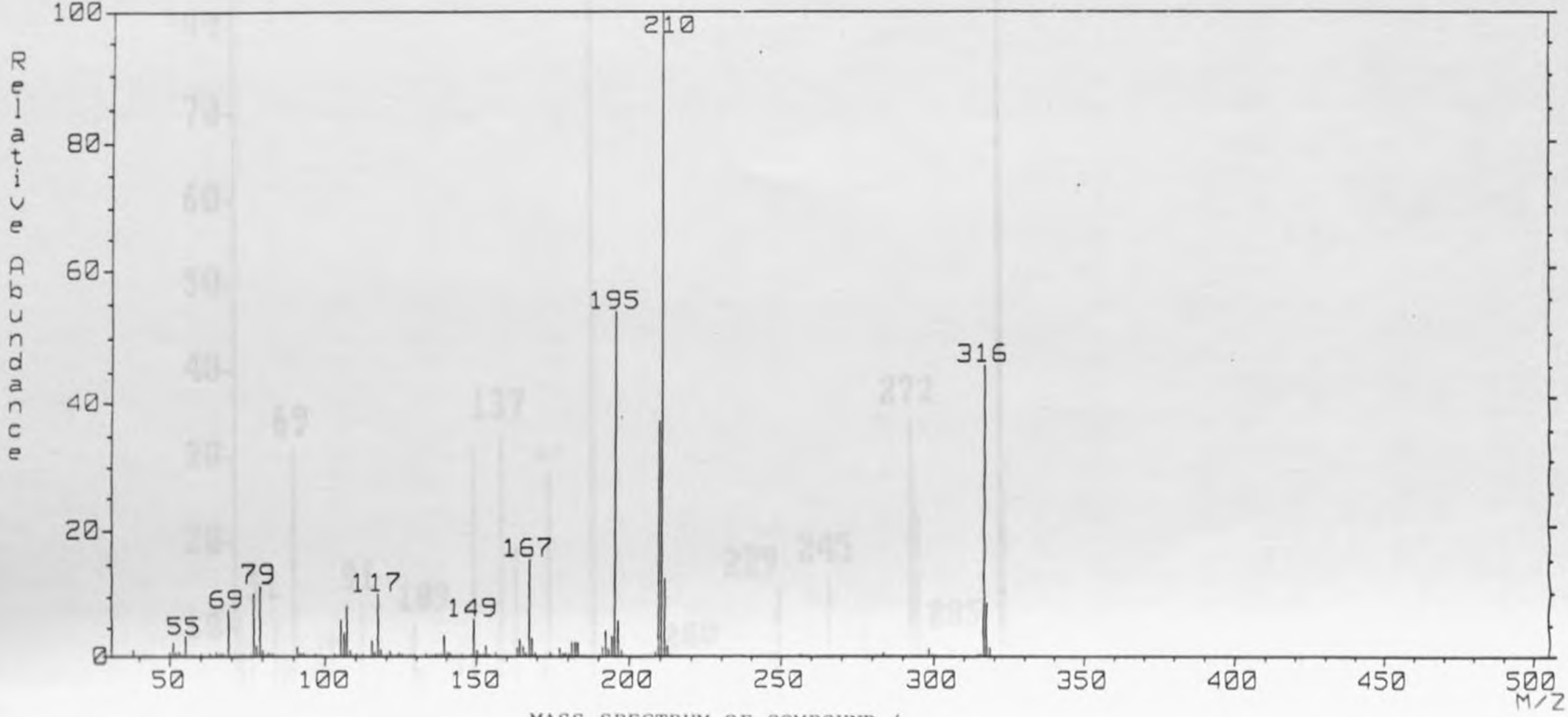
MASS SPECTRUM OF COMPOUND 1



MASS SPECTRUM OF COMPOUND 2

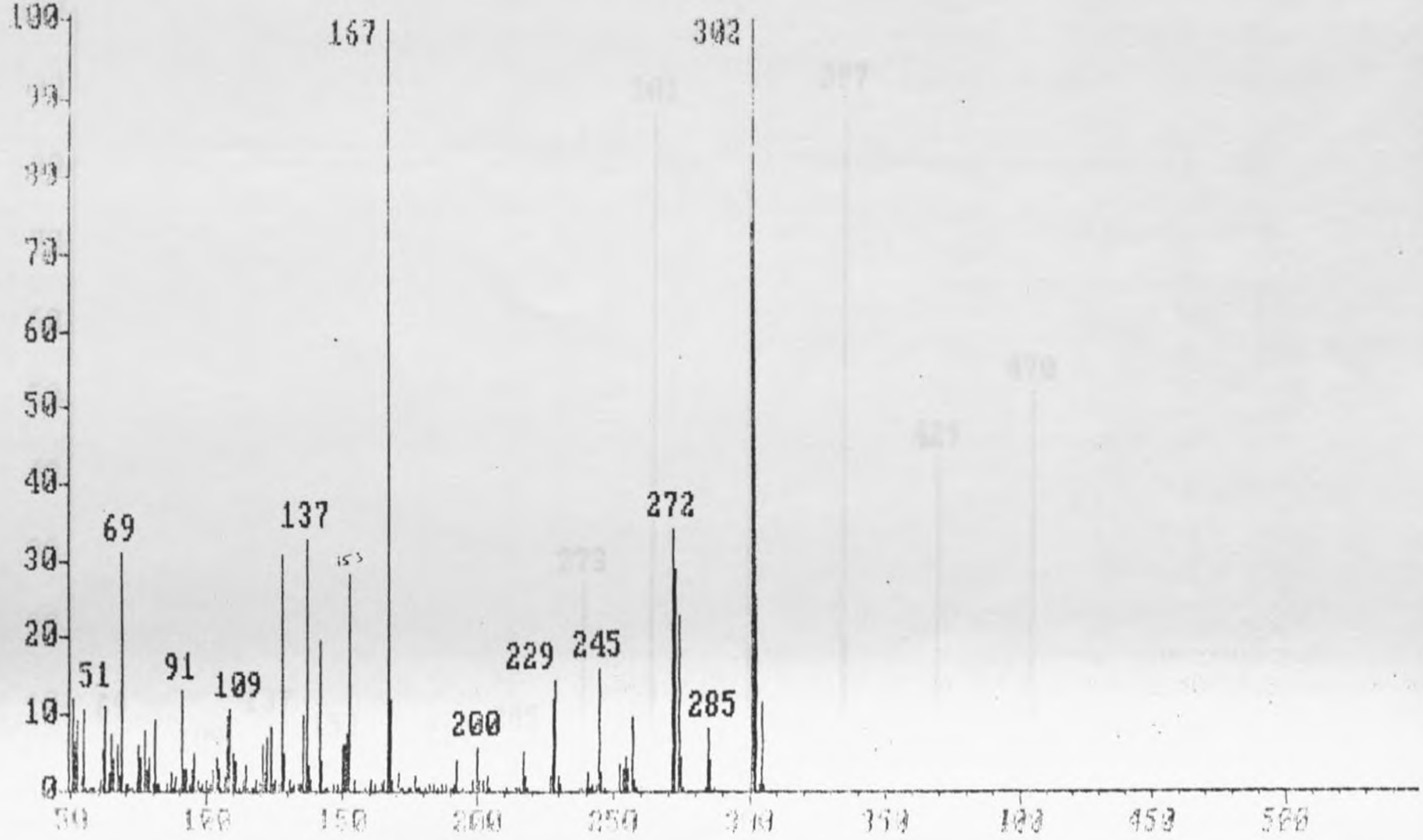


MASS SPECTRUM OF COMPOUND 3

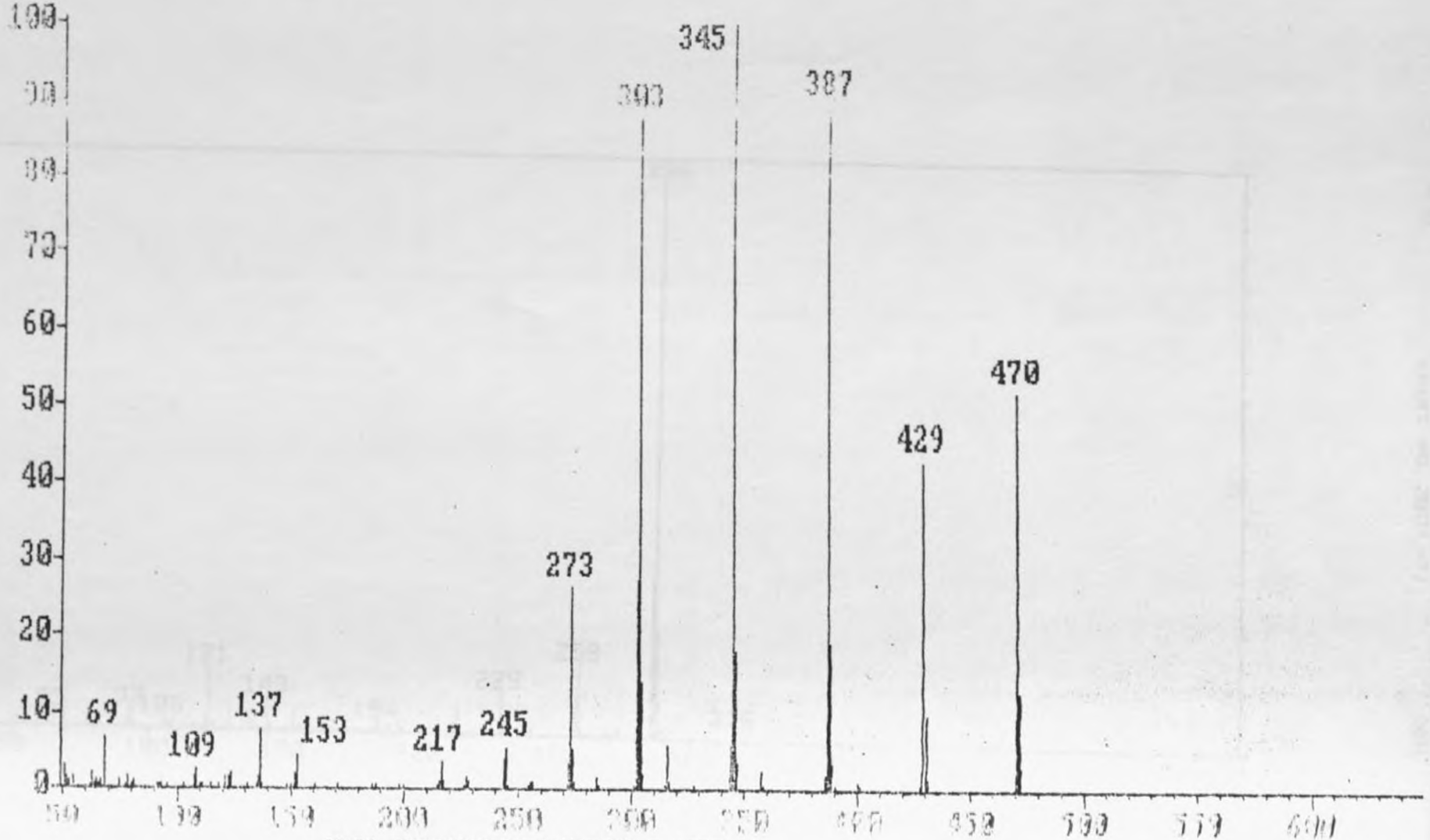


MASS SPECTRUM OF COMPOUND 4

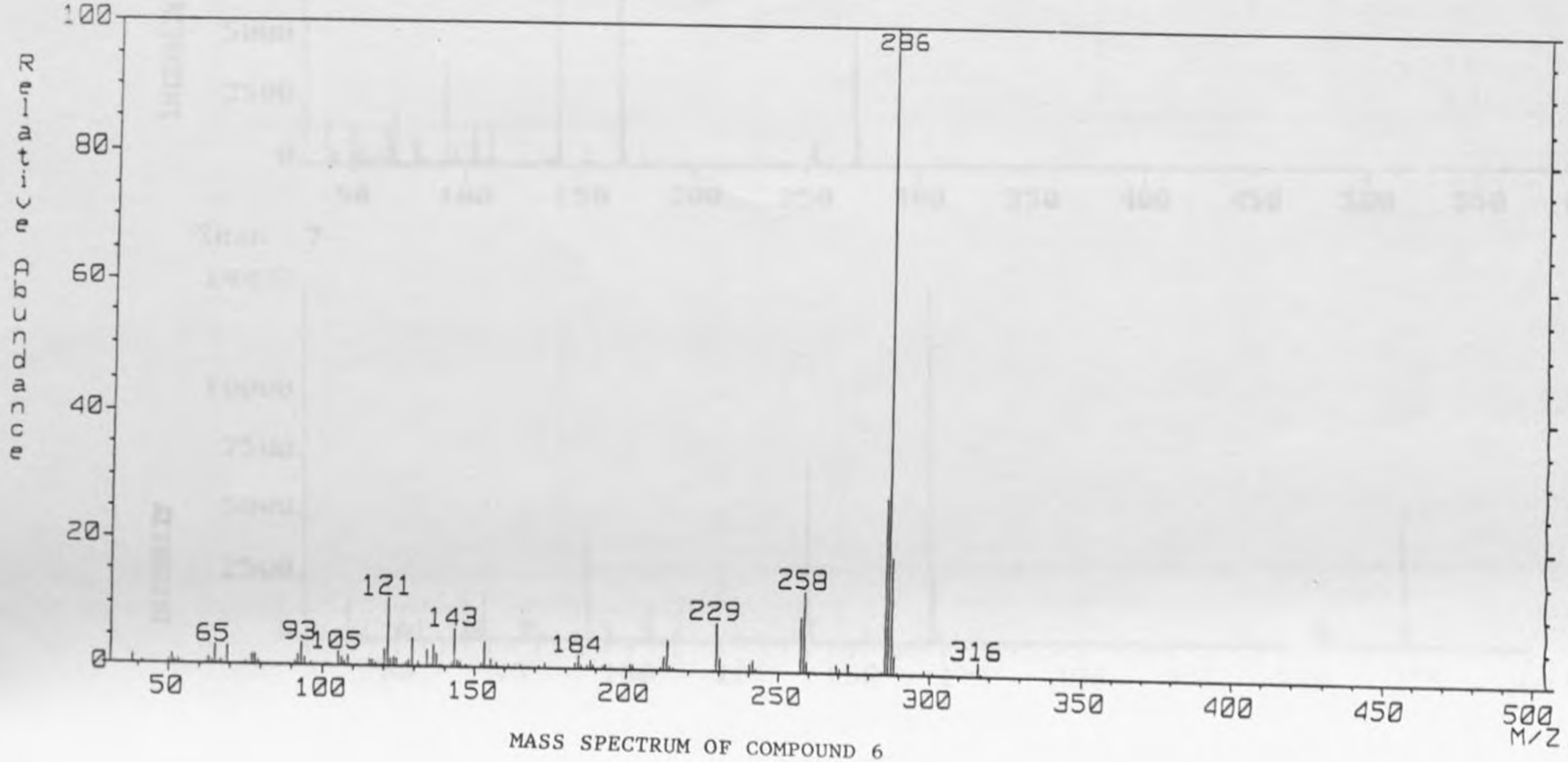
JEOL DATUM



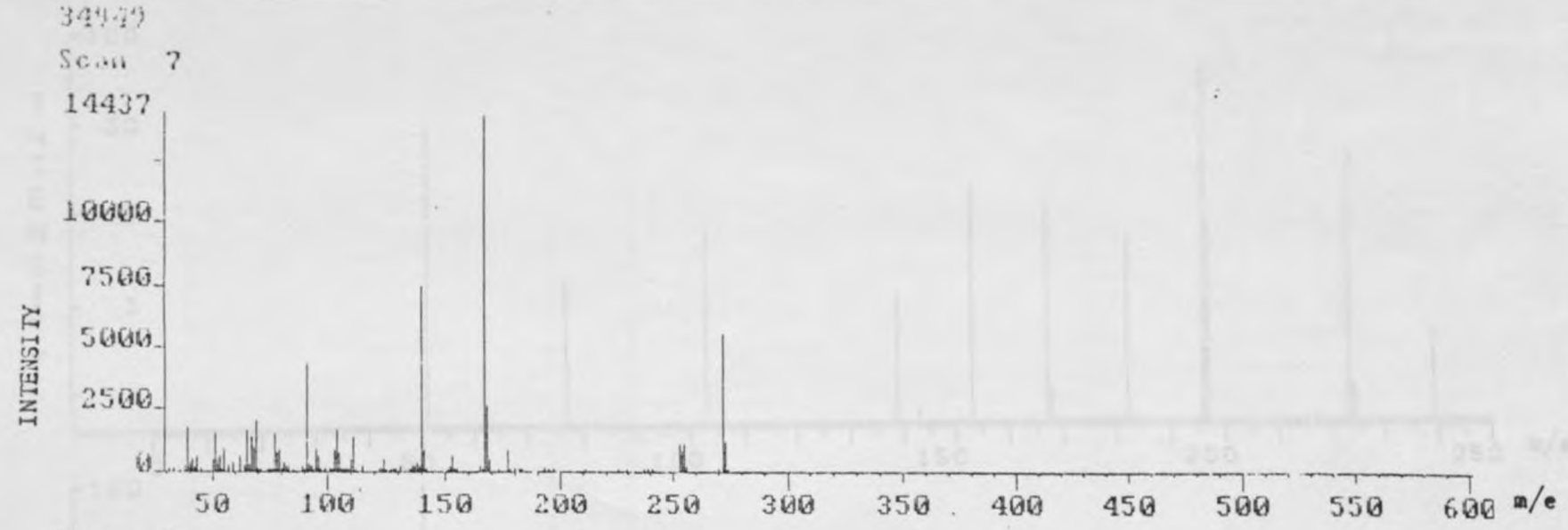
MASS SPECTRUM OF COMPOUND 5

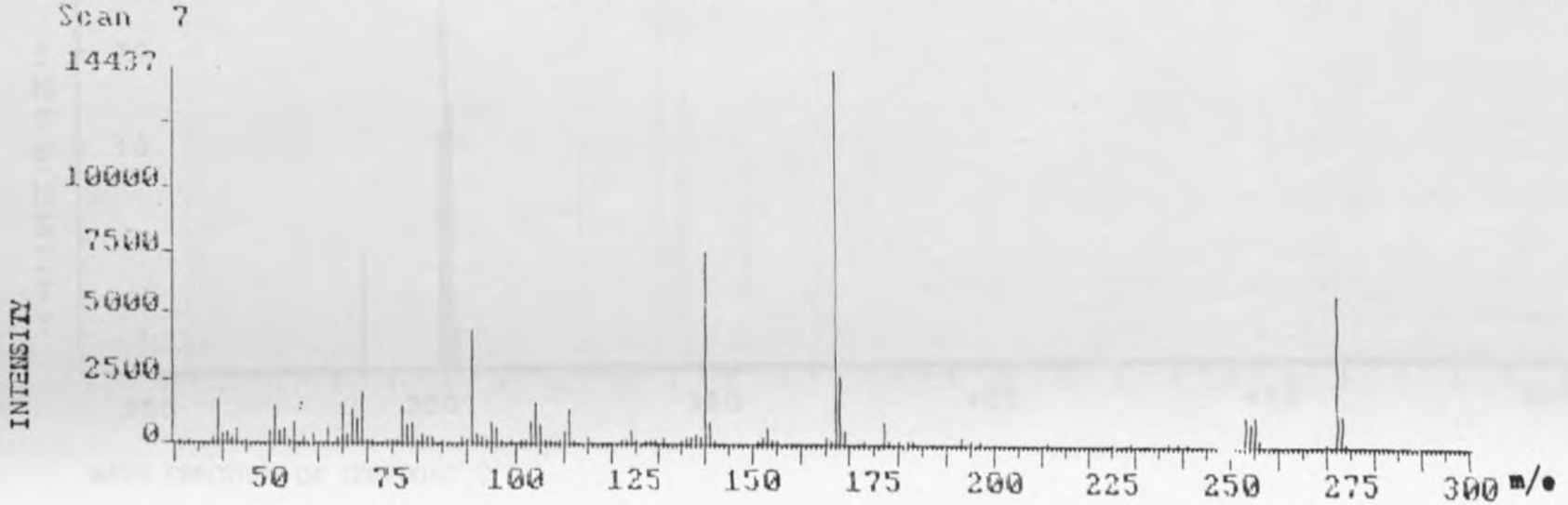


MASS SPECTRUM OF COMPOUND 5 ACETATE

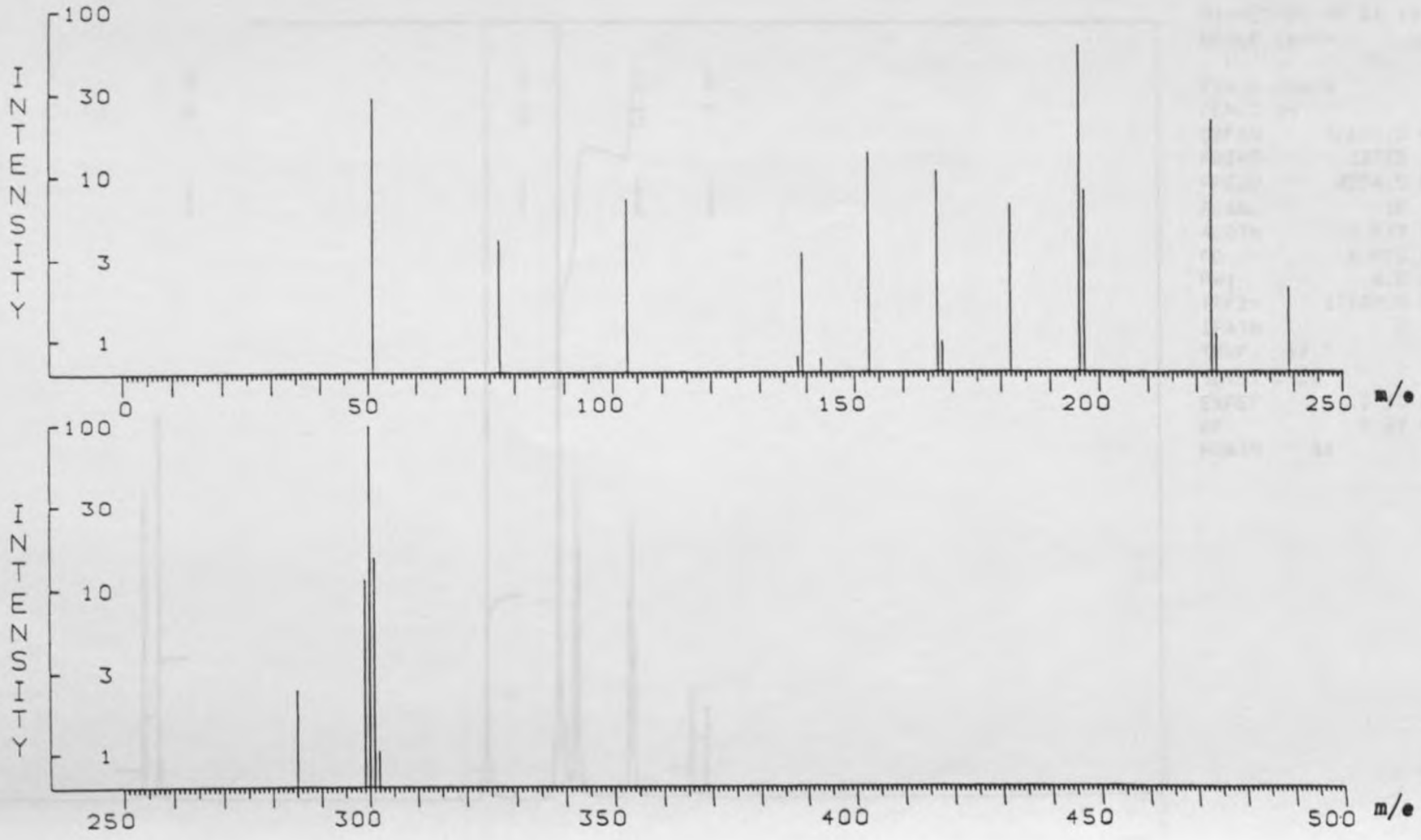


MASS SPECTRUM OF COMPOUND 6

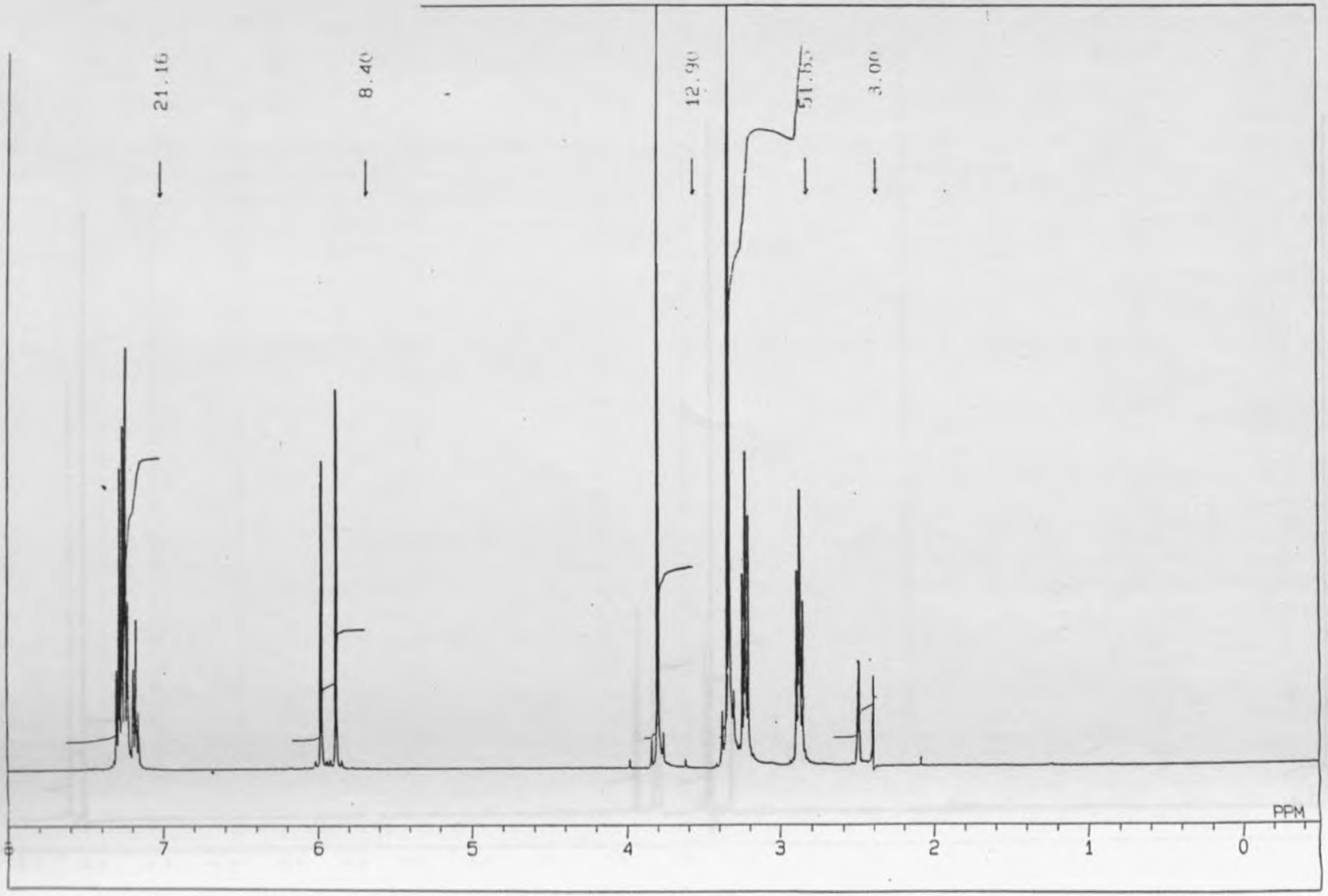




MASS SPECTRUM OF COMPOUND (7)



MASS SPECTRUM OF COMPOUND 9



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SCANS 16
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PD 1.000 sec
PW1 4.0 us
IRFIN 10300.0 Hz
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TEMP. 27.0 C
SLVNT DMSO
EXREF 2.50 ppm
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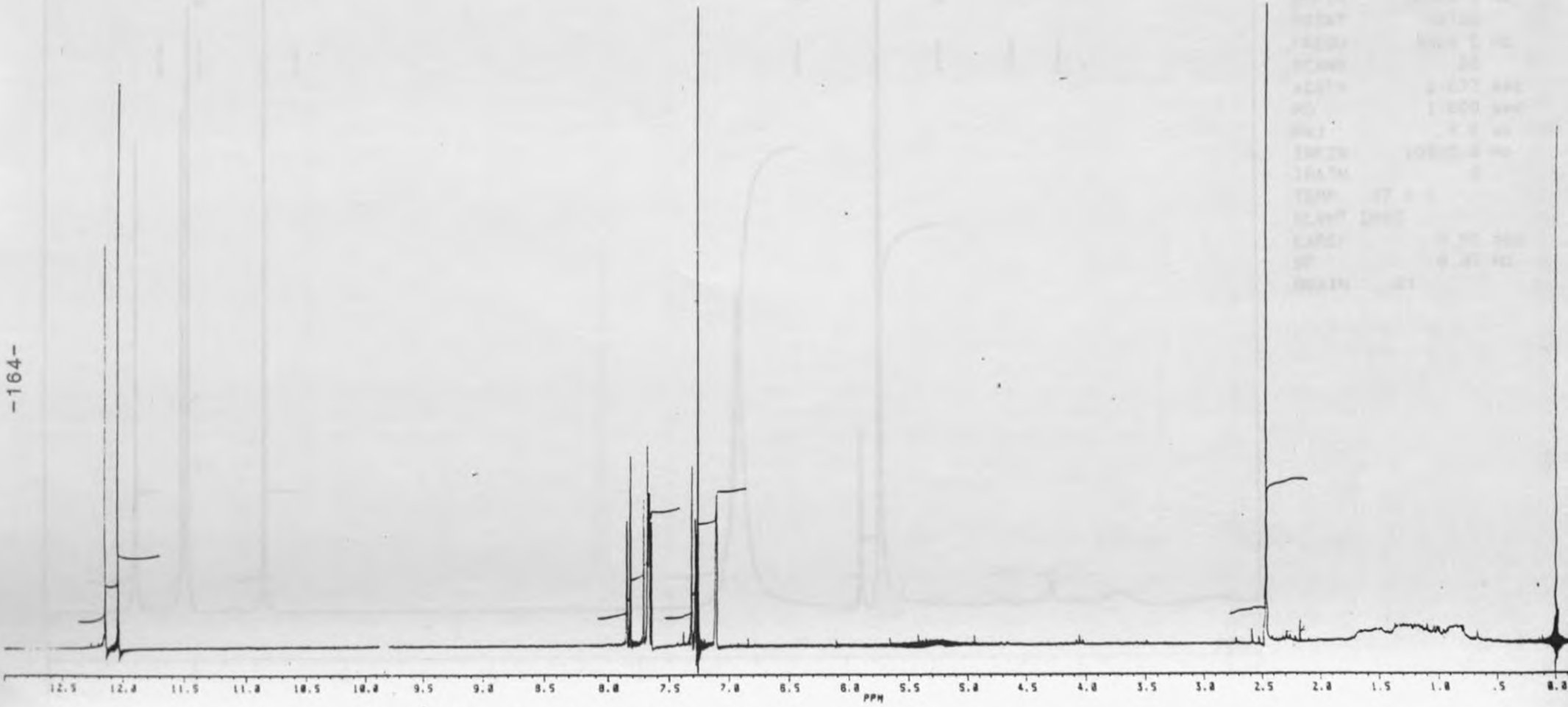
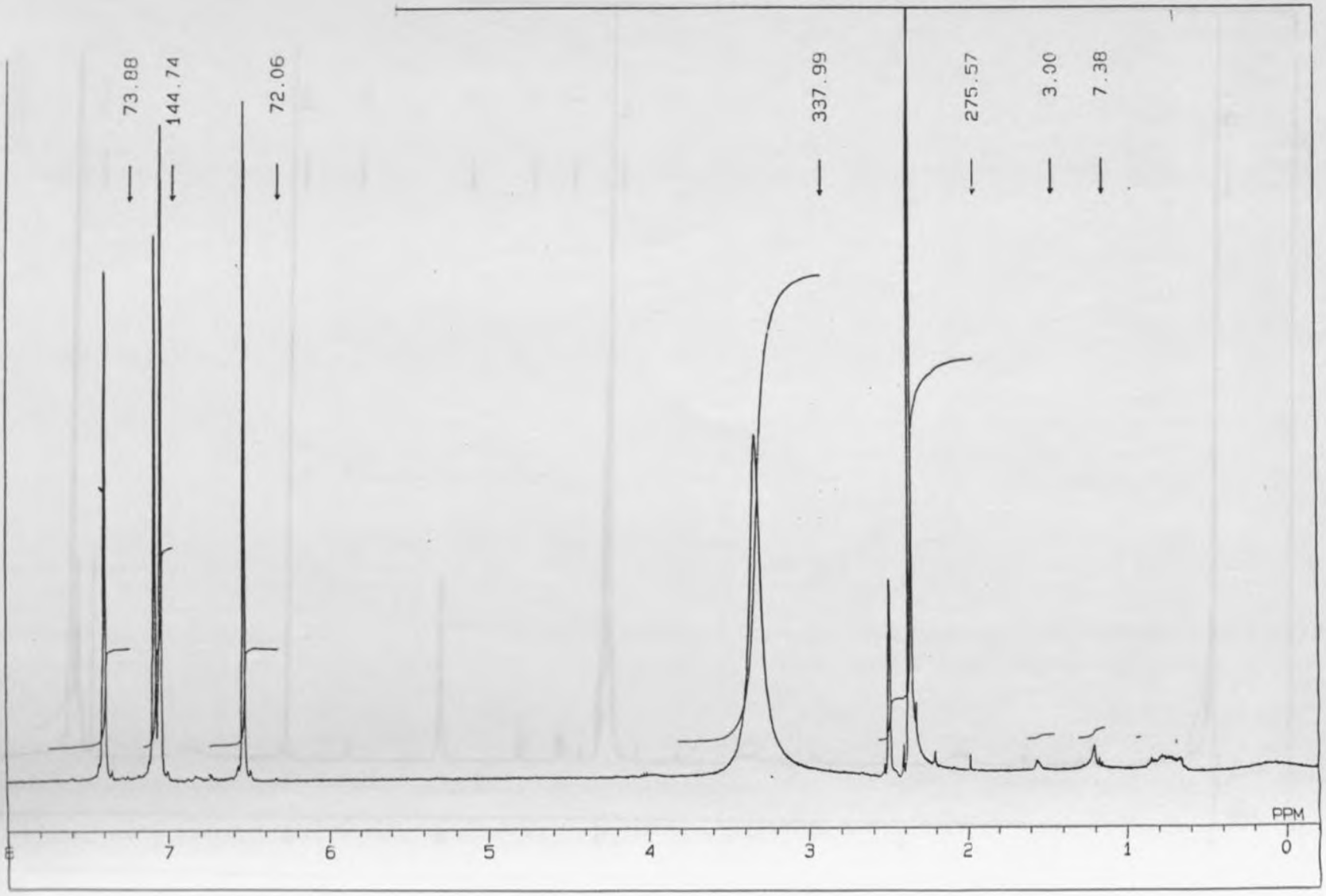
RGAIN 18

^{1}H NMR SPECTRUM OF COMPOUND 1



^{1}H NMR SPECTRUM OF COMPOUND 2



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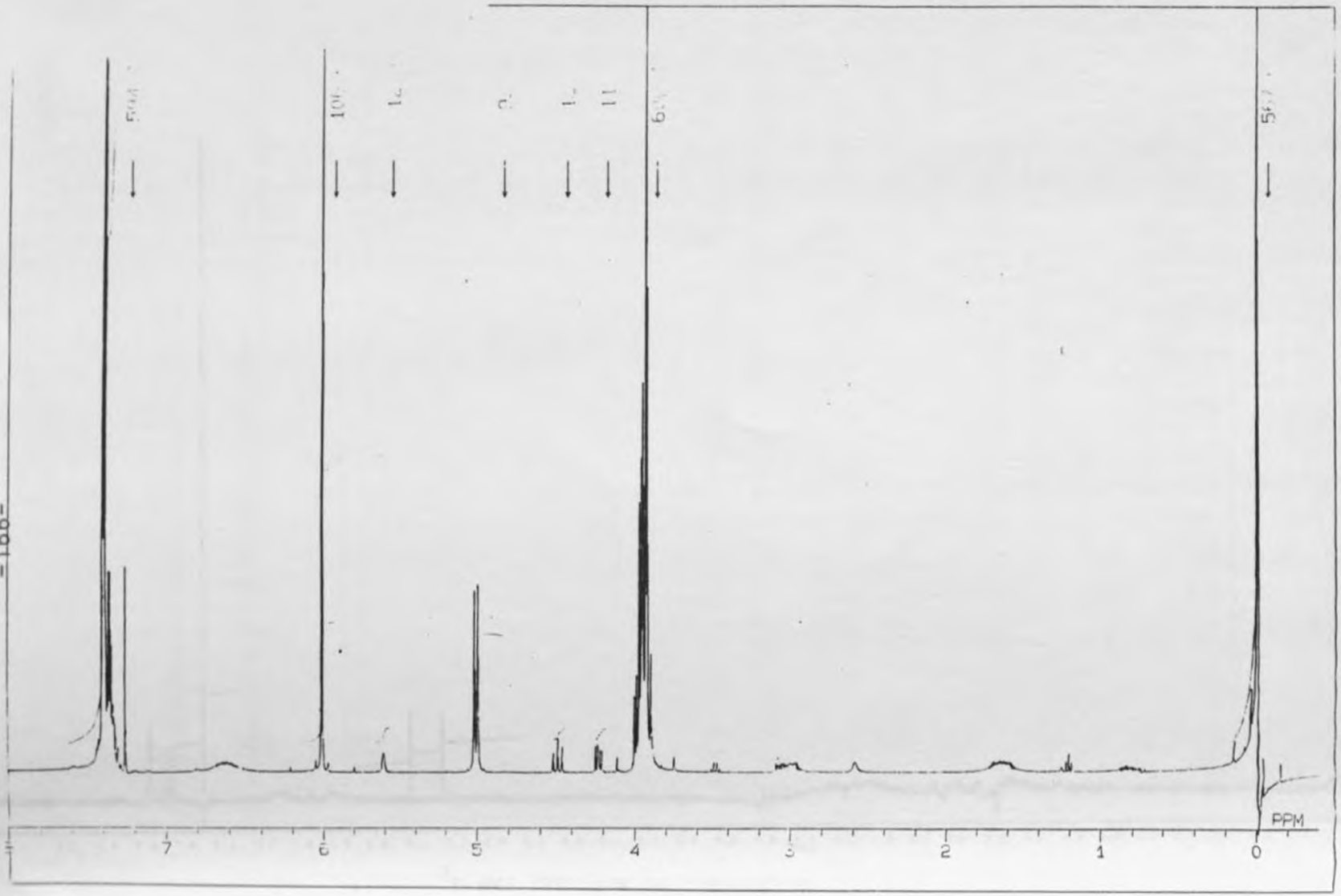
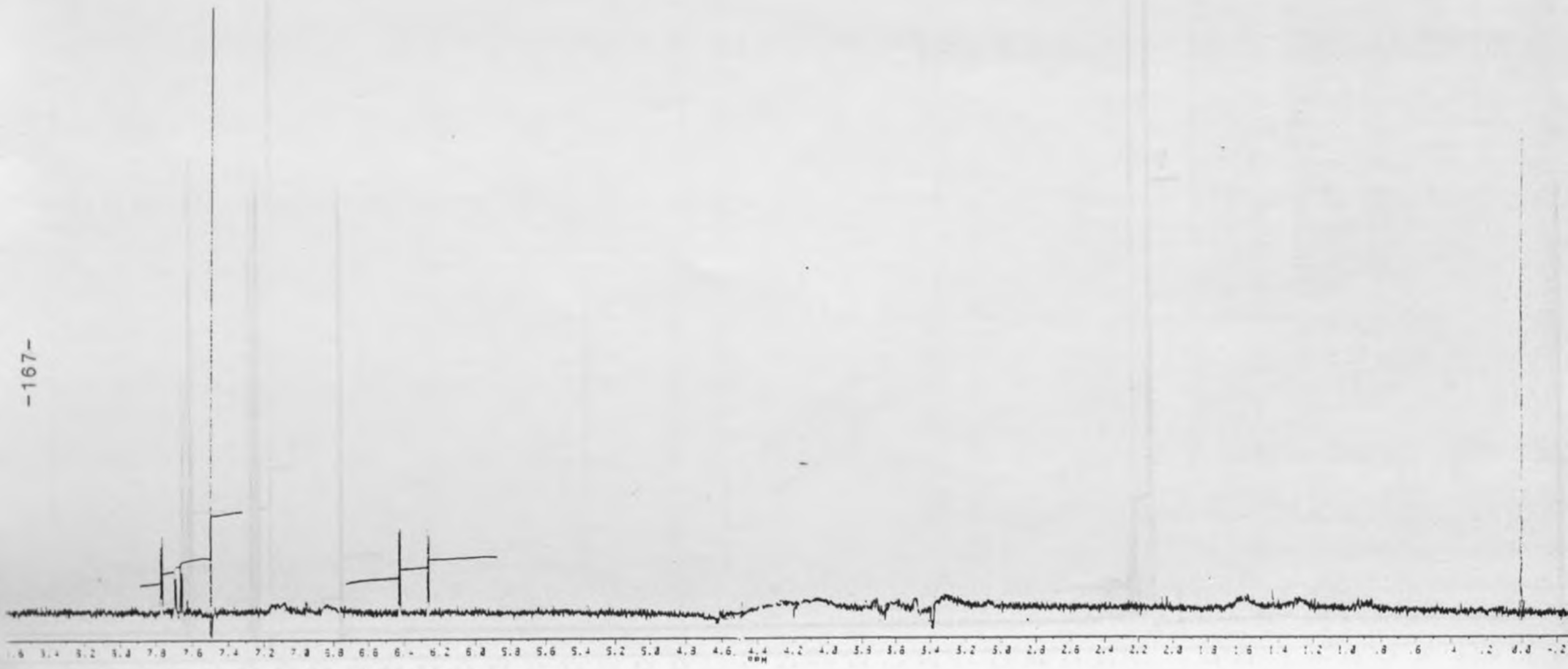
RGAIN 21

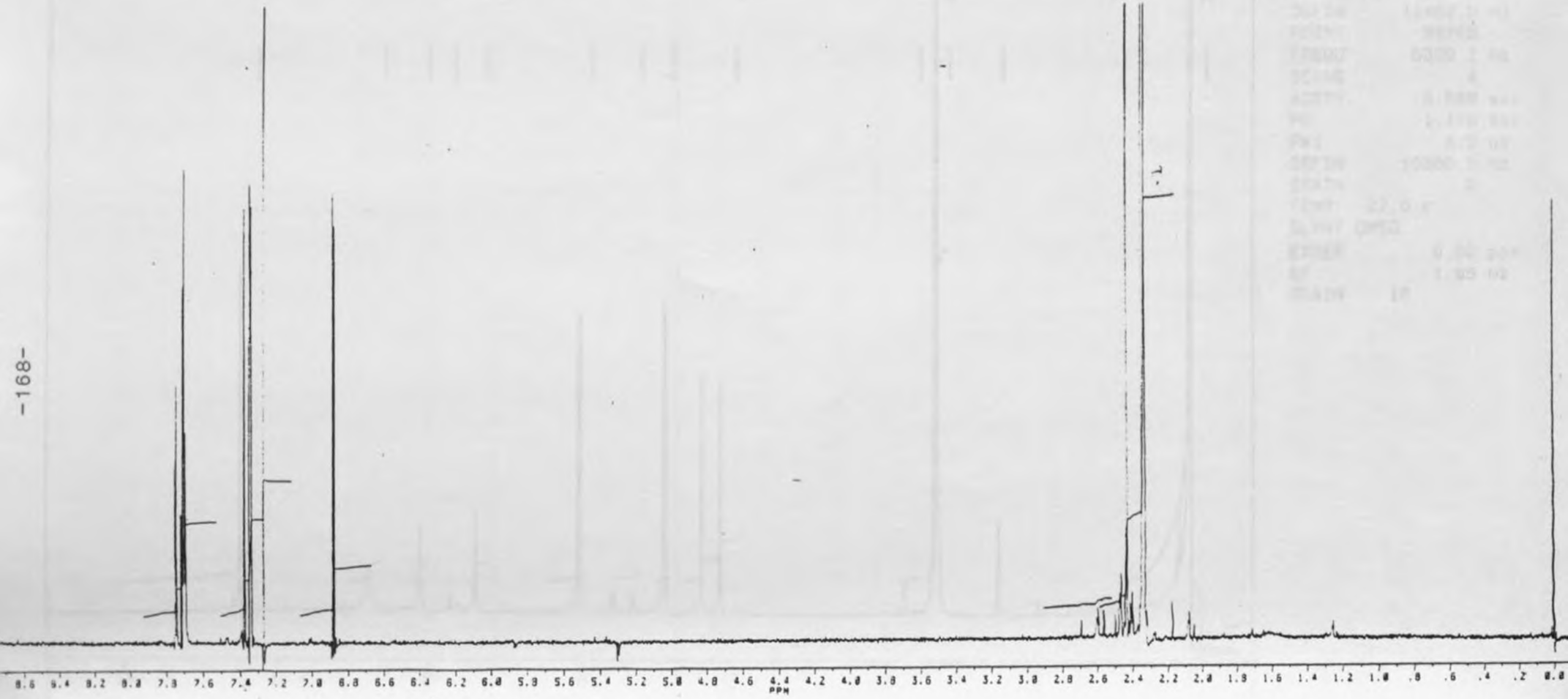
^{1}H NMR SPECTRUM OF COMPOUND 3



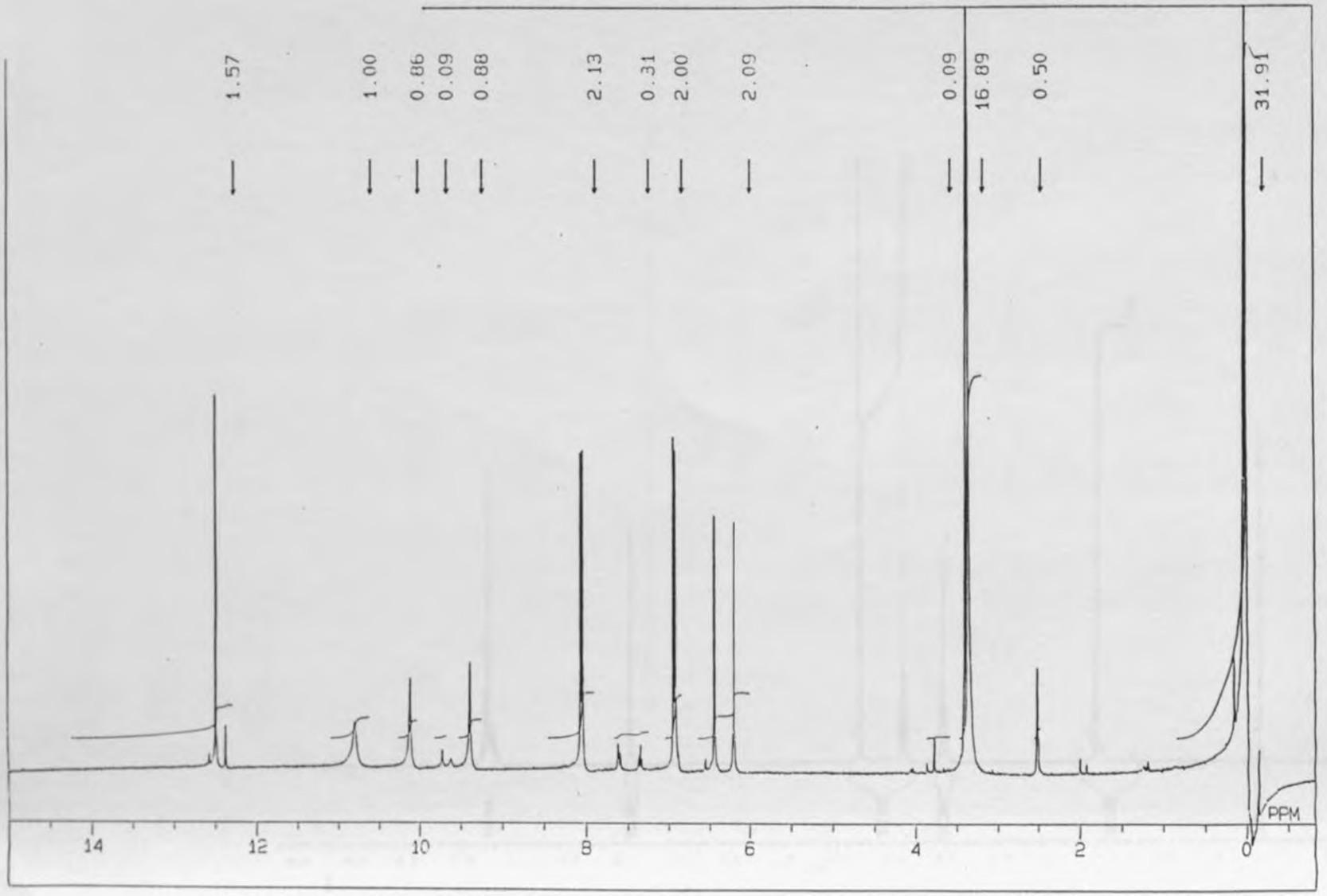
^{1}H NMR SPECTRUM OF COMPOUND 4



^{1}H NMR SPECTRUM OF COMPOUND 5



^{1}H NMR SPECTRUM OF COMPOUND 5 ACETATE



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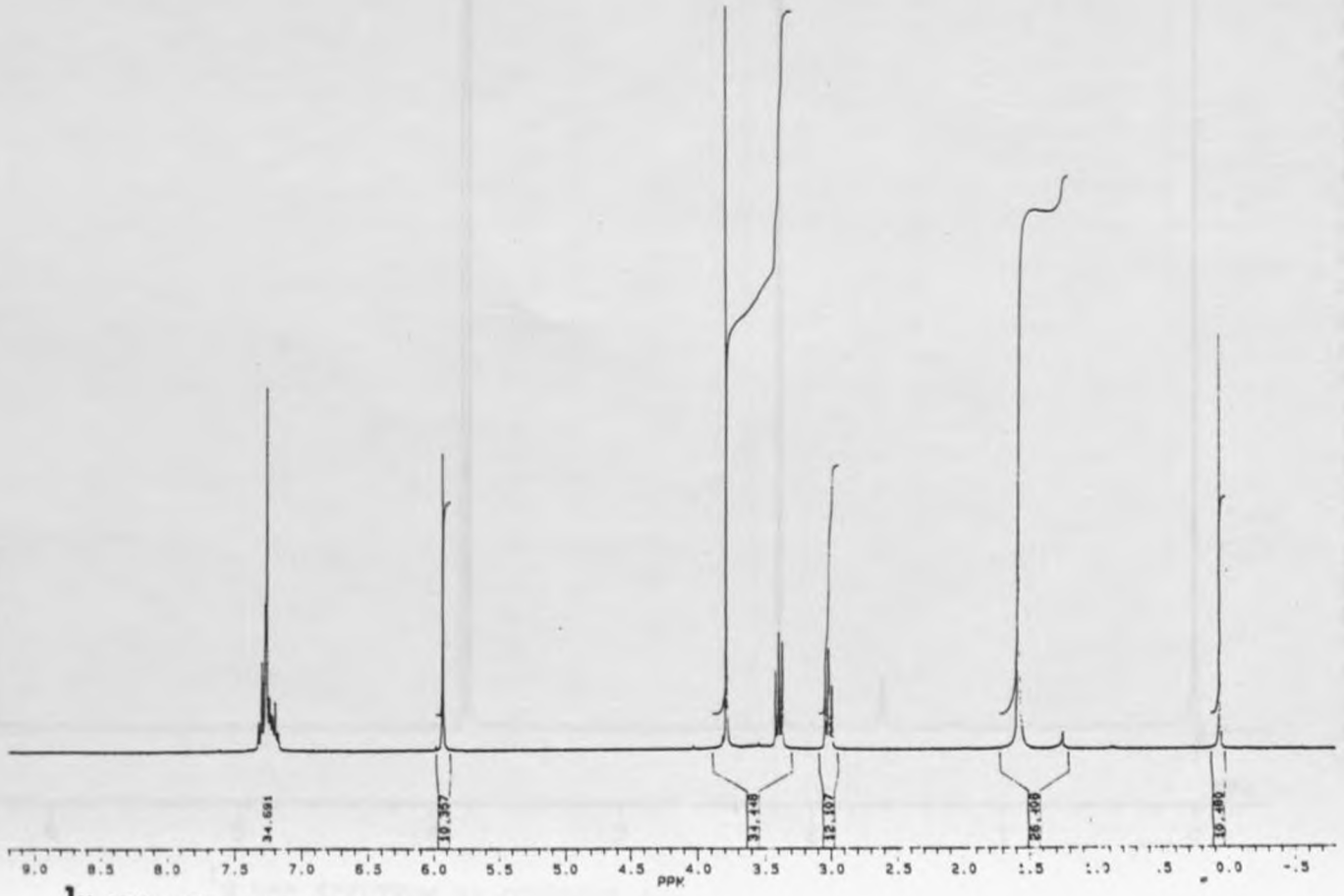
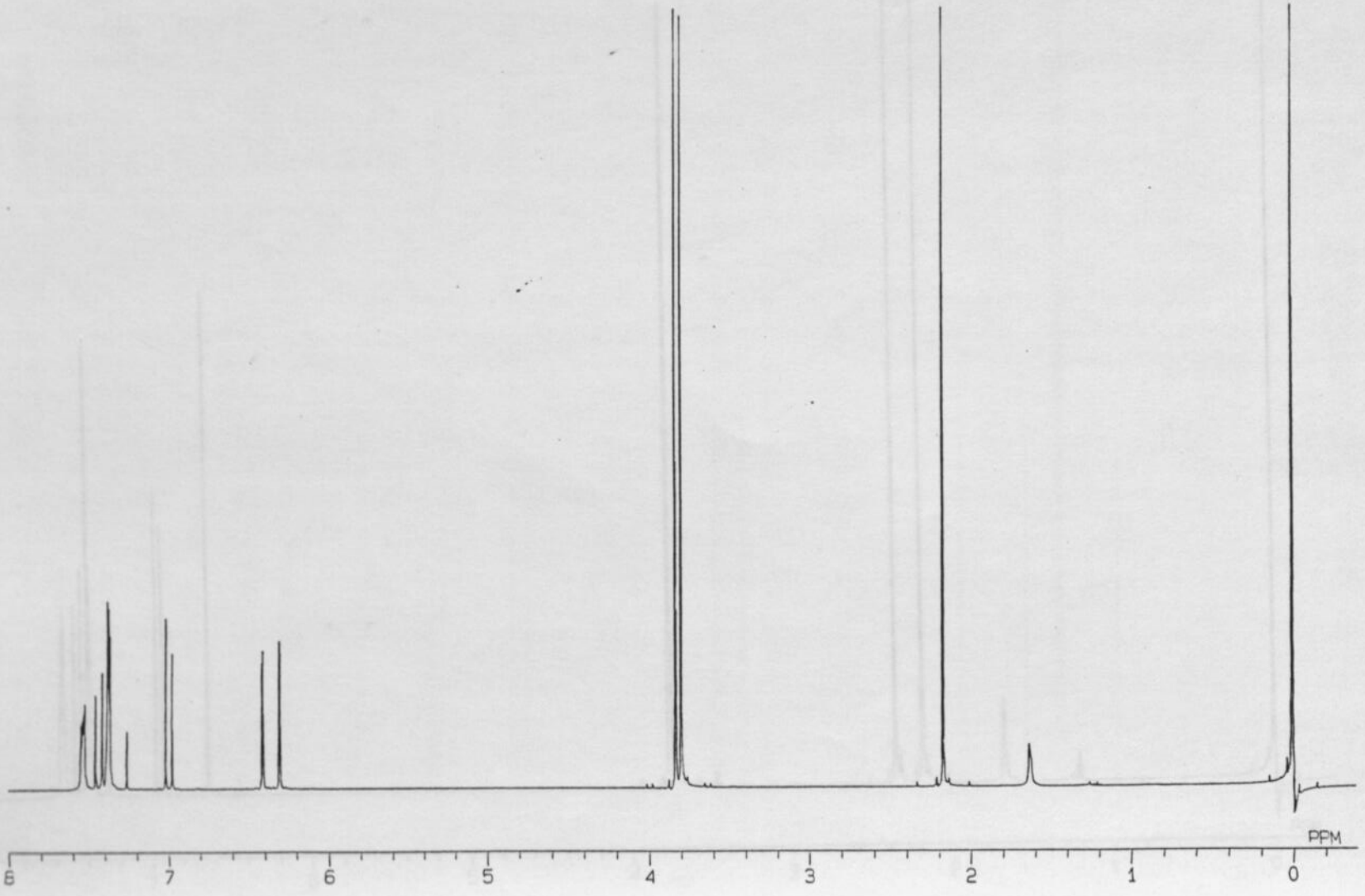
RGAIN 16

^{1}H NMR SPECTRUM OF COMPOUND 6



^{1}H NMR SPECTRUM OF COMPOUND 7



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OBSET 124.00 kHz
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CLPNT 256
CLFRQ 100.0 Hz
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PD 1.000 sec
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PW3 13.0 us
PI1 1.000 ms
PI2 1.000 ms
PI3 1.000 ms
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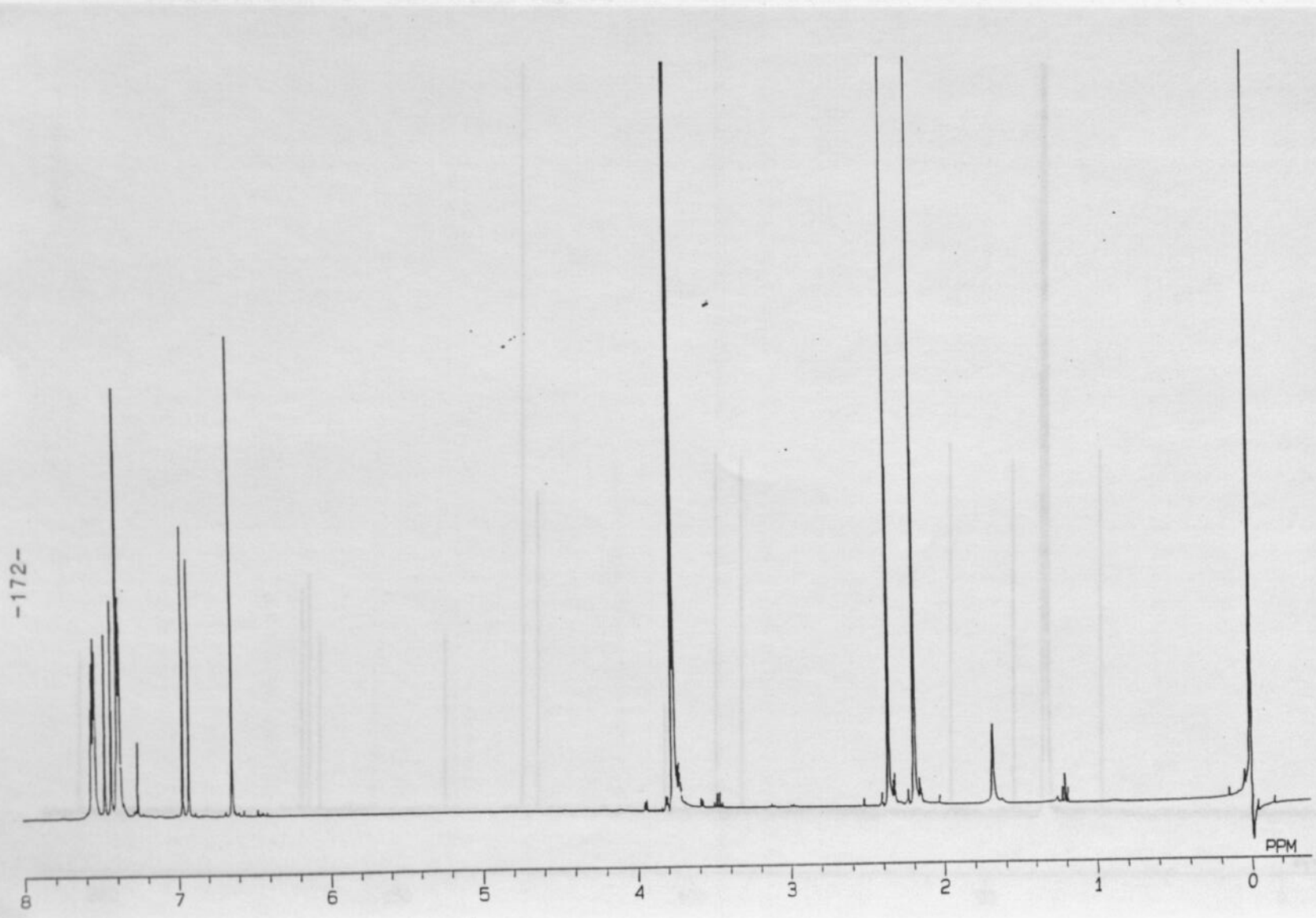
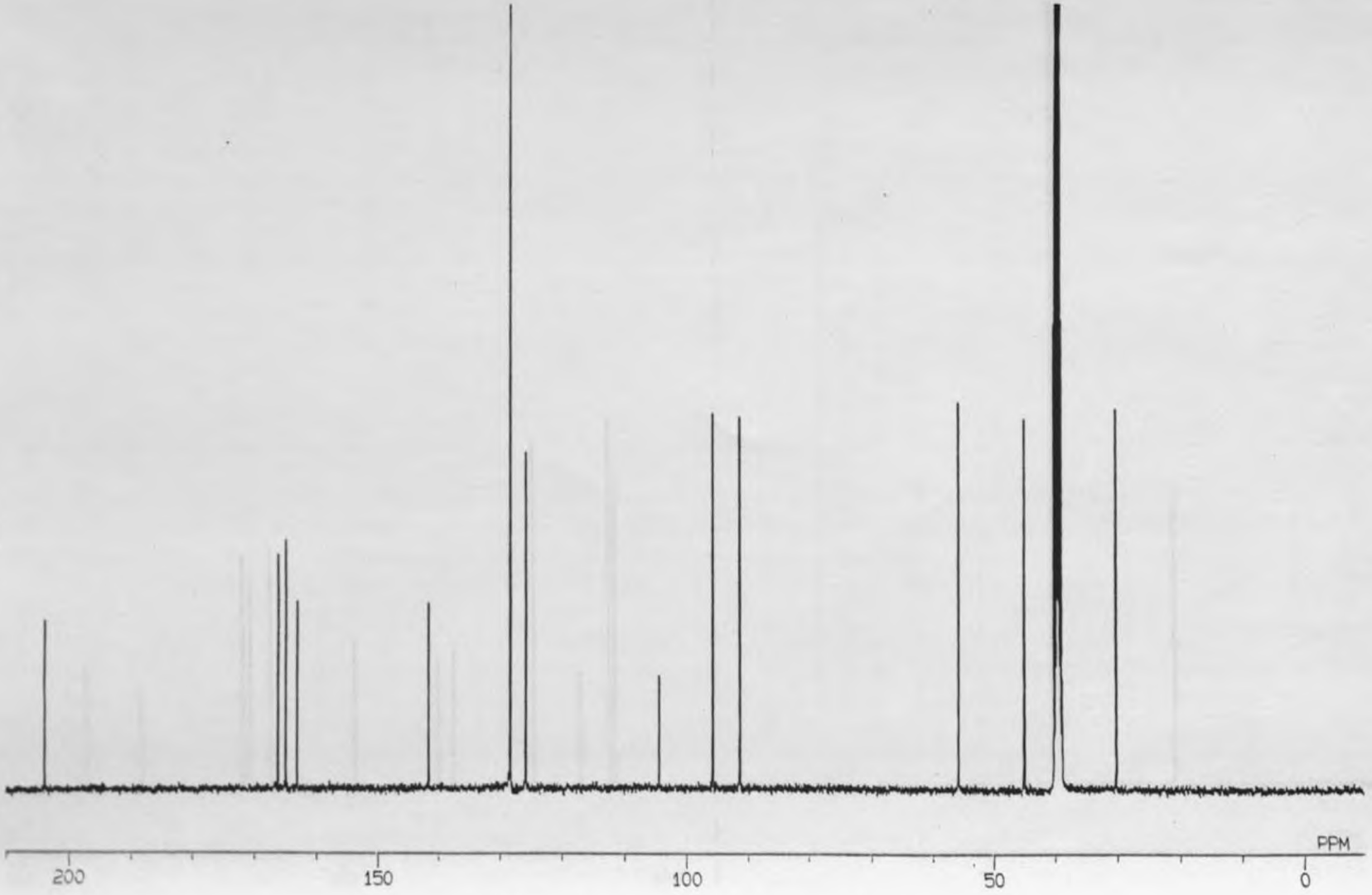
CLREF 0.00 ppm

^{1}H NMR SPECTRUM OF COMPOUND 8



^{1}H NMR SPECTRUM OF COMPOUND 9

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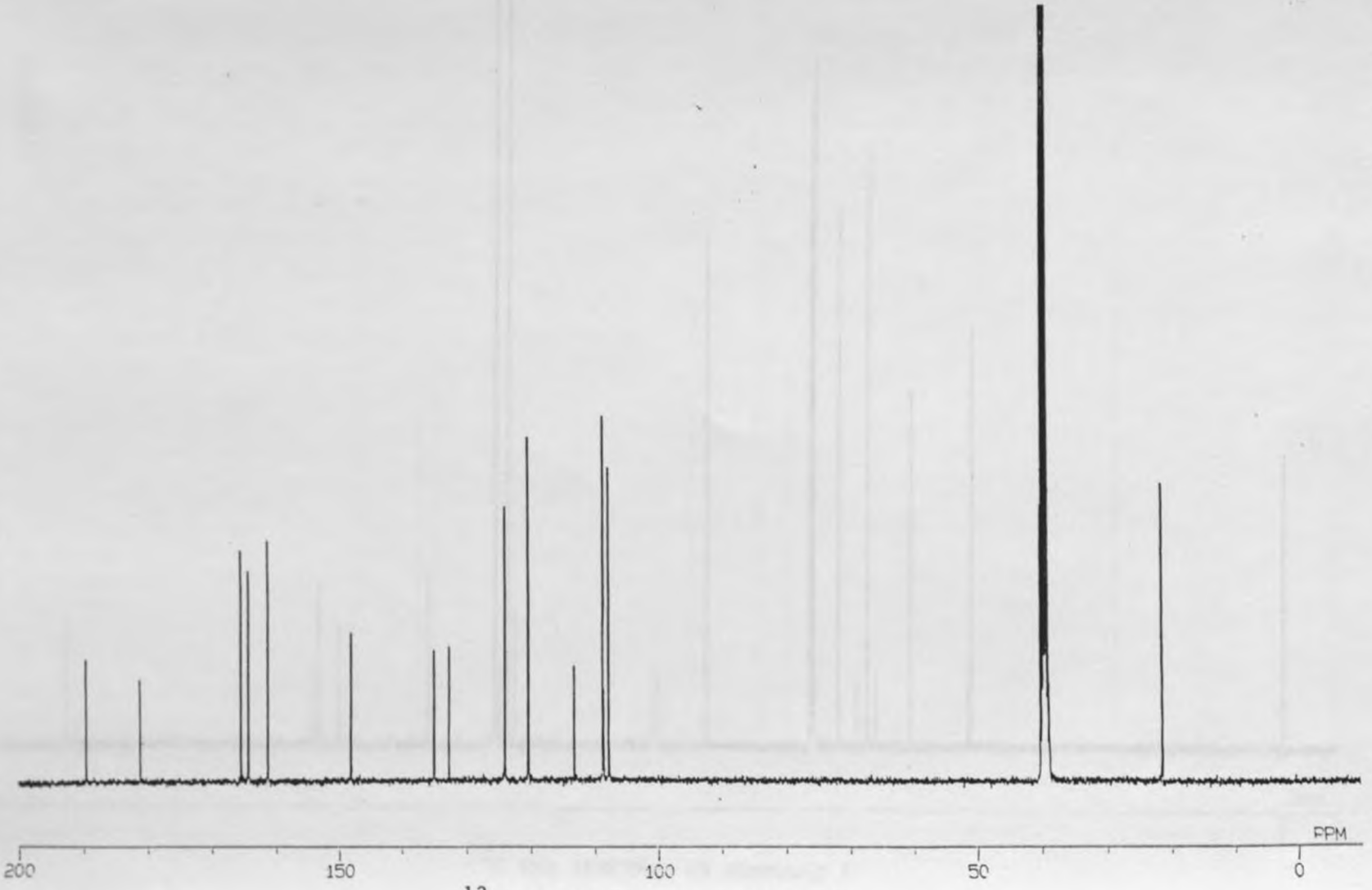
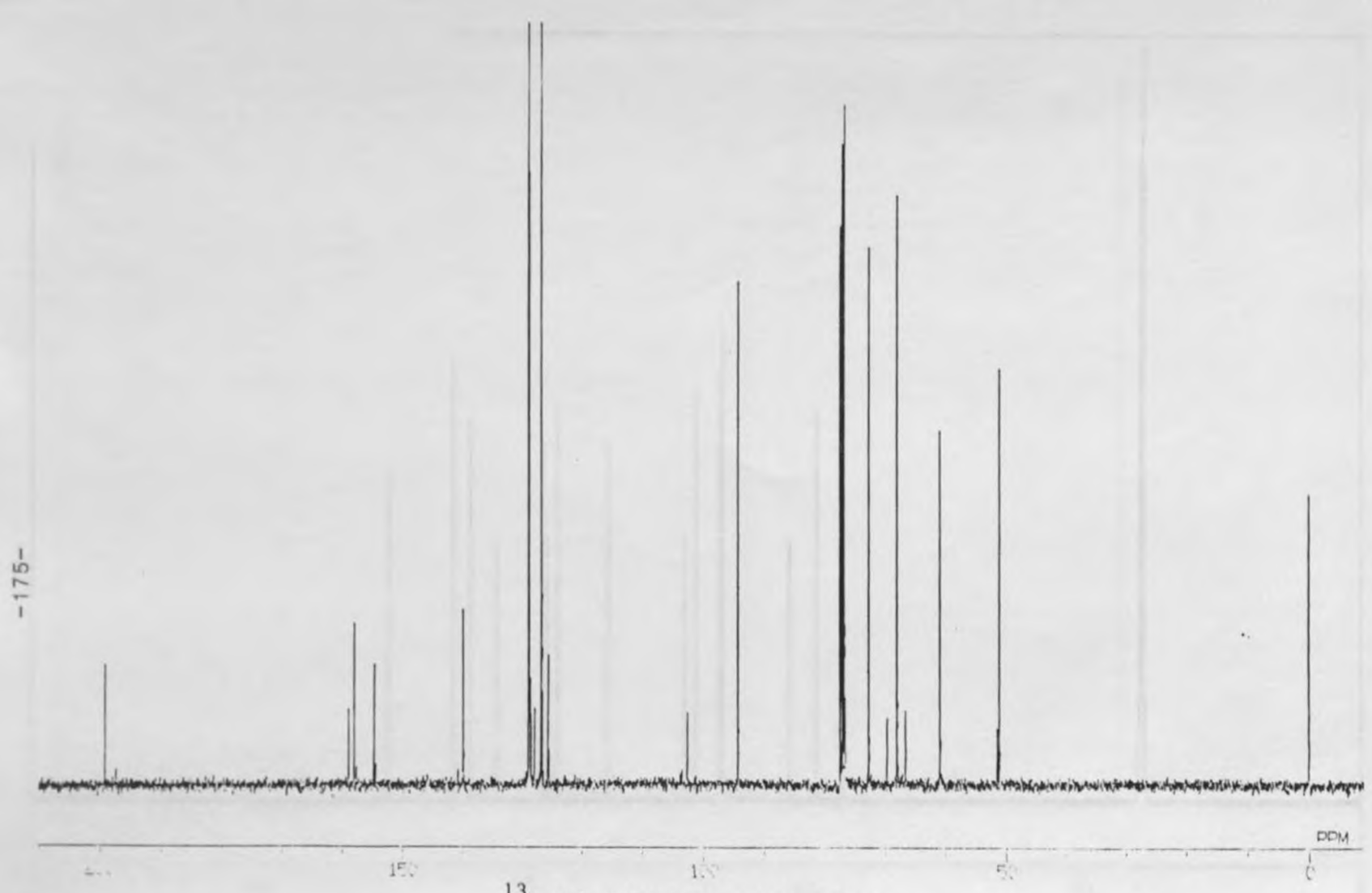
COMNT NKG-01 (DMSO)
EXMOD SGCMLP
OBNUC 13C
OBFIN      11500.0 Hz
POINT        32768
FREQU      24038.5 Hz
SCANS        20000
ACQTM        0.682 sec
PD           0.800 sec
PW1            5.0 us
IRFIN      10300.0 Hz
IRATN            15
TEMP.  27.0 c
SLVNT DMSO
EXREF        39.50 ppm
BF            1.47 Hz
RGAIN    29
```


^{13}C NMR SPECTRUM OF COMPOUND 1

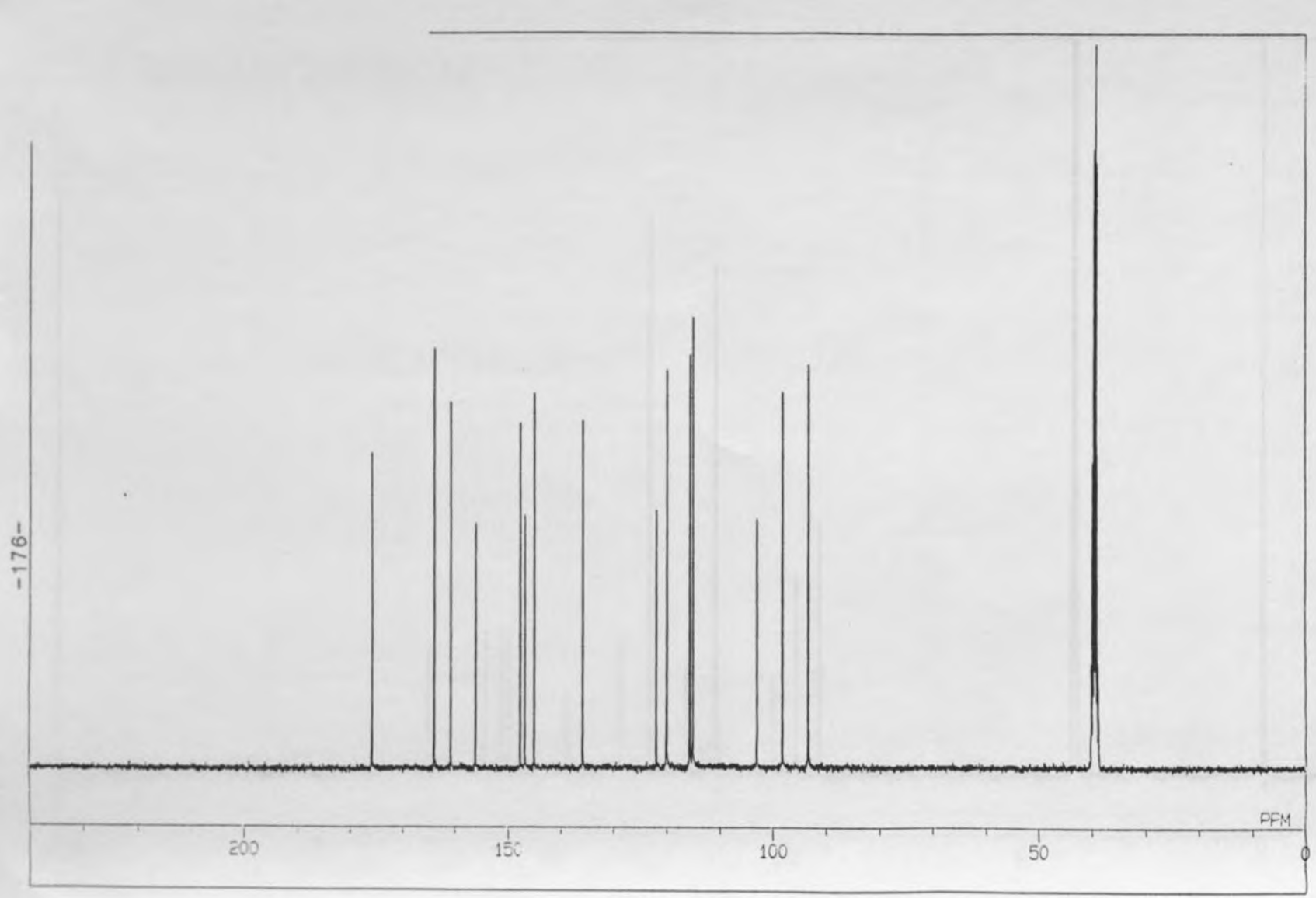


02-OCT-90 09.21.56
DFILE SAVING
COMNT NKB-1E (DMSO
EXMOD SGCMLP
OBNUC 13C
OBFIN 11500.0 Hz
POINT 32768
FREQU 24038.5 Hz
SCANS 20000
ACQTM 0.682 sec
PD 0.800 sec
PW1 5.0 us
IRFIN 10300.0 Hz
IRATN 15
TEMP. 27.0 c
SLVNT DMSO
EXREF 39.50 ppm
BF 1.47 Hz
RGAIN 28

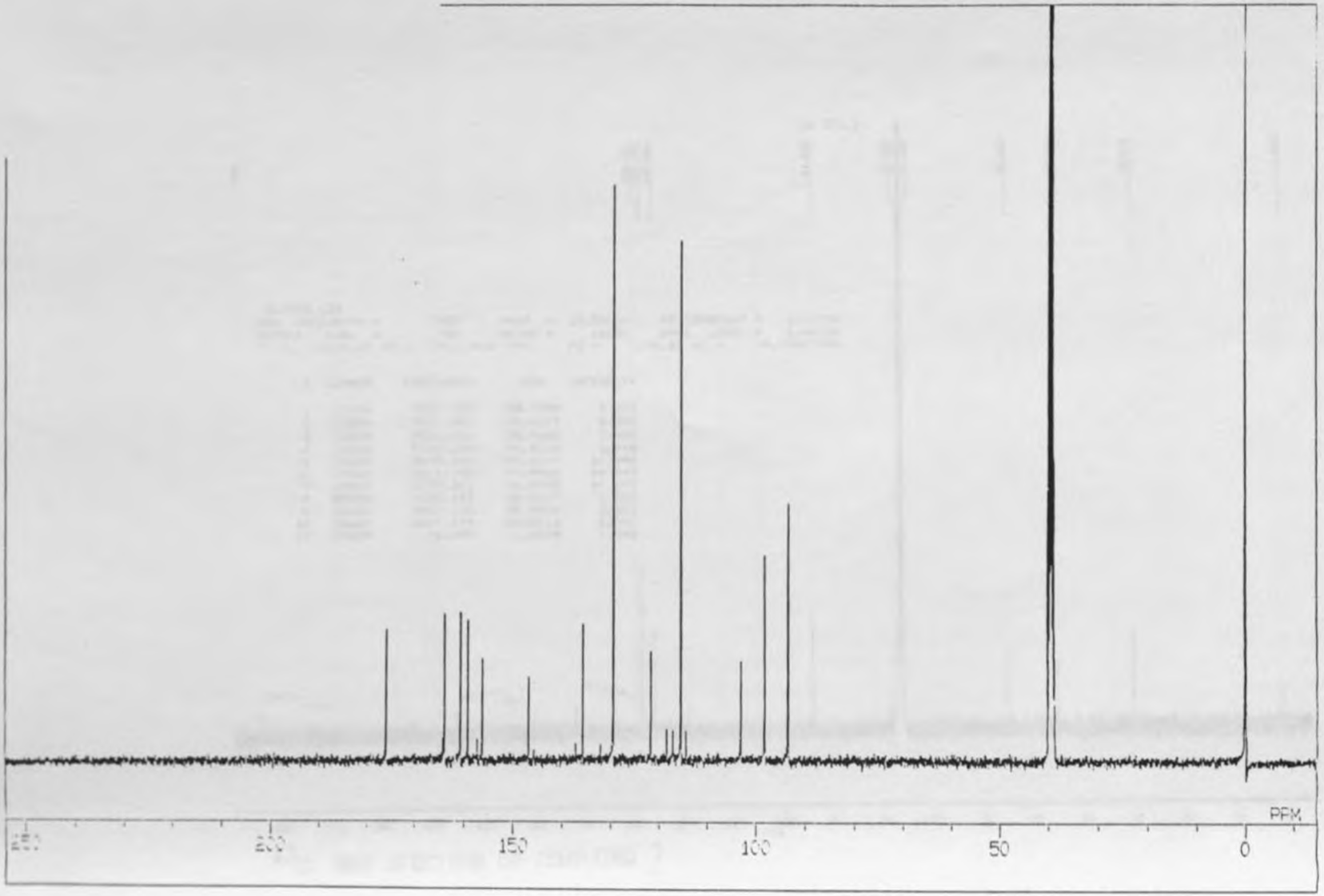
^{13}C NMR SPECTRUM OF COMPOUND 3



^{13}C NMR SPECTRUM OF COMPOUND 4

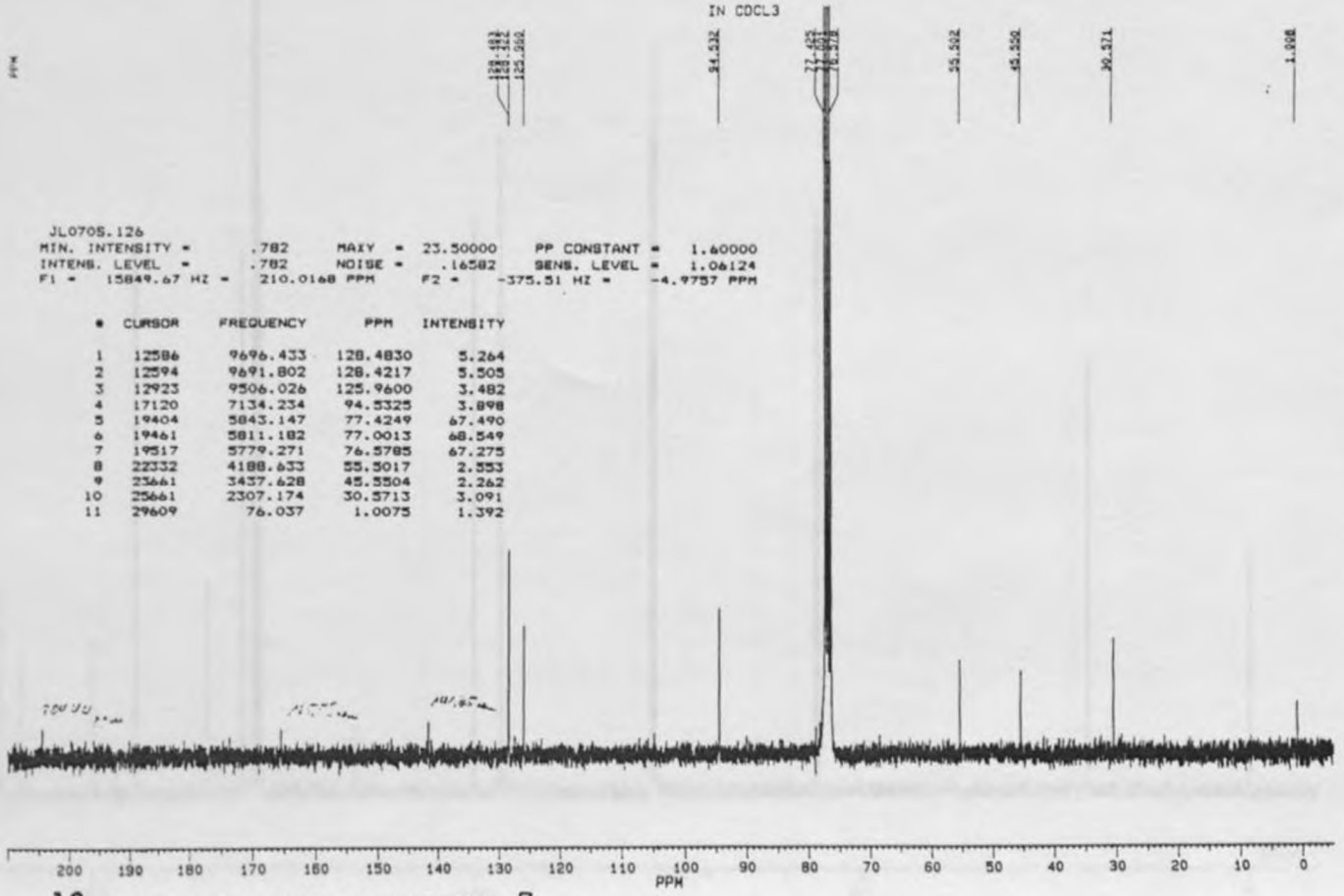


^{13}C NMR SPECTRUM OF COMPOUND 5

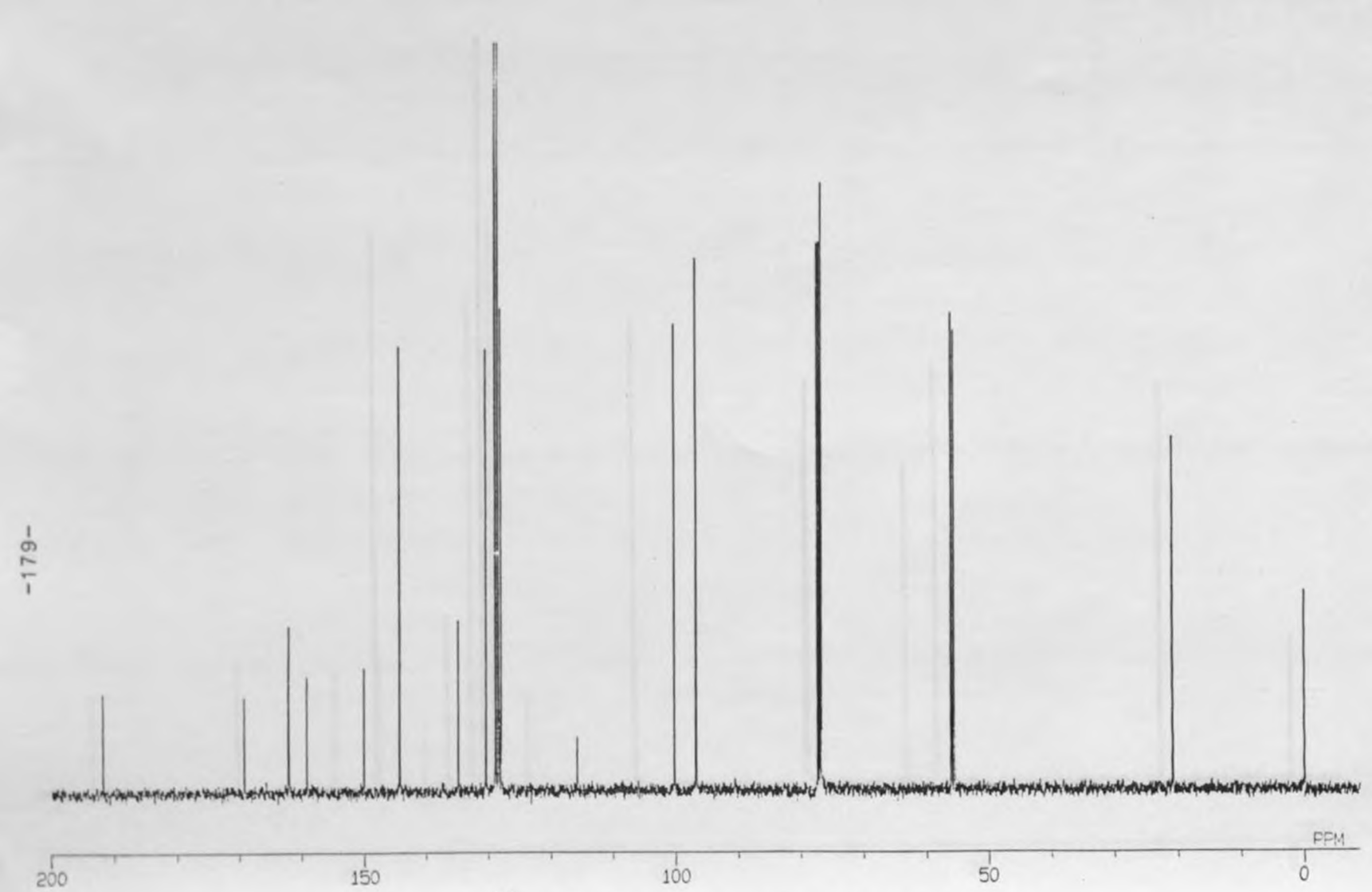


09-OCT-90 16.11.32
DFILE SAVING
COMNT NKG-07 DMSO
EXMOD SGCMLP
OBNUC 13C
OBFIN 12500.0 Hz
POINT 32768
FREQU 27027.0 Hz
SCANS 9581
ACQTM 0.606 sec
PD 0.800 sec
PW1 5.0 us
IRFIN 10300.0 Hz
IRATN 15
TEMP. 27.0 c
SLVNT DMSO
EXREF 0.00 ppm
BF 1.65 Hz
RGAIN 28

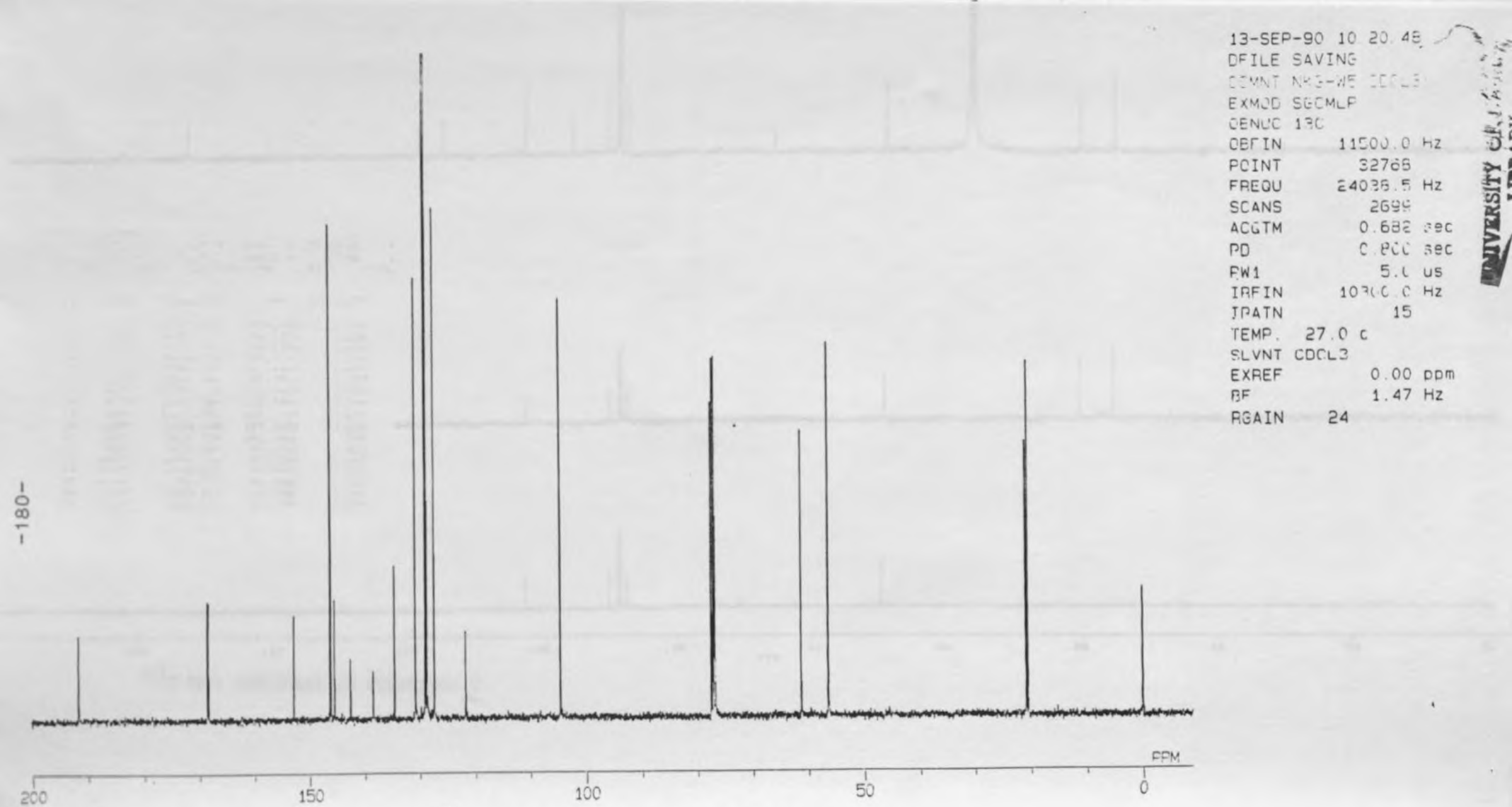
^{13}C NMR SPECTRUM OF COMPOUND 6



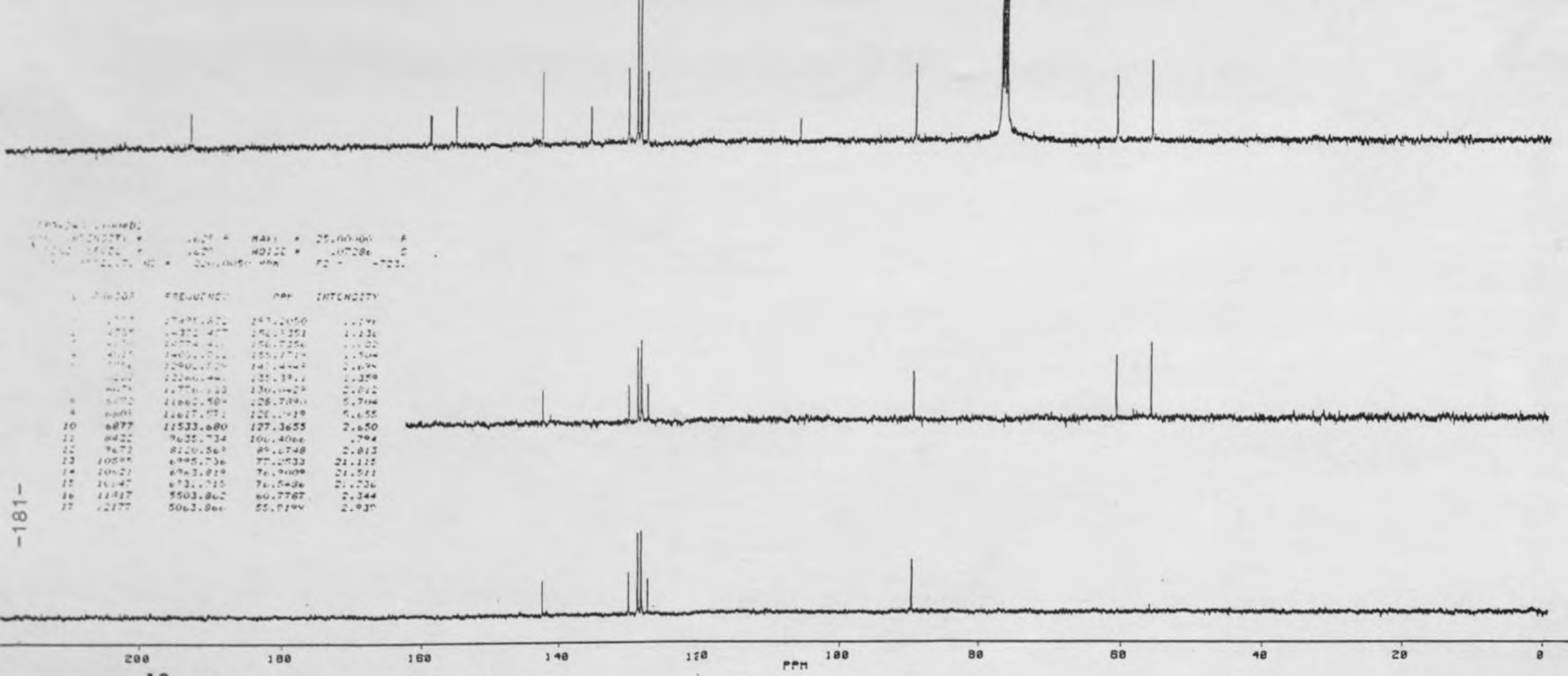
^{13}C NMR SPECTRUM OF COMPOUND 7



^{13}C NMR SPECTRUM OF COMPOUND 8 ACETATE



^{13}C NMR SPECTRUM OF COMPOUND 9 ACETATE



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		[illegible]	[illegible]	[illegible]
		[illegible]	[illegible]	[illegible]
		[illegible]	[illegible]	[illegible]
		[illegible]	[illegible]	[illegible]
		[illegible]	[illegible]	[illegible]
9		[illegible]	[illegible]	[illegible]
10	6877	11533.680	127.3655	2.650
11	8422	9635.734	106.4066	.794
12	9673	8120.563	89.6748	2.813
13	10585	6995.736	77.2633	21.115
14	10621	6951.819	76.7909	21.511
15	10647	6921.715	76.5486	21.736
16	11417	5503.862	60.7767	2.344
17	12177	5063.866	55.8194	2.930

^{13}C NMR SPECTRUM OF COMPOUND 9