CHEMICAL SCREENING OF SOME KENYAN POLYGONUM SPECIES.

BY

JULIUS JARET MATASI

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

MARCH, 1987.

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

Signature _____

JULIUS JARET MATASI
UNIVERSITY OF NAIROBI

This thesis has been submitted for examination with our approval as University Supervisors.

Signature _____

DR. J. O. MIDIWO
DEPARTMENT OF CHEMISTRY
UNIVERSITY OF NAIROBI

Signature _____

PROF. R. M. MUNAVU
DEPARTMENT OF CHEMISTRY
UNIVERSITY OF NAIROBI

This thesis is dedicated to all the members of my family.

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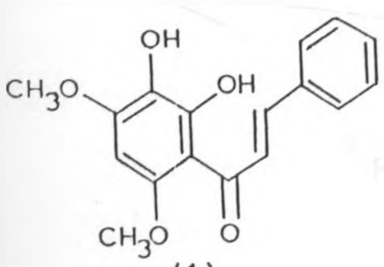
SUMMARY

Eleven species of the genus polygonum (family: Polygonaceae) are found in Kenya. They appear in the "key to species" in the following order: P.baldschuriacum Regel, P.convolvulus L, P.nepalense Meisn, P.capitatum Han, P.afromantanum Greenway, P.aviculare L, P.strigosum R.Br., P.salicifolium wild, P.senegalense Meisn, P.pulchrum Blume and P.setosulum A. Rich. Chemical screening by a process of sequential non-polar and polar solvent extraction (petroleum ether, chloroform and methanol along with acid hydrolysis), chromatographic separation followed by spectroscopic (^{1}H and ^{13}C NMR, UV, IR and MS) analysis was performed on the latter four species.

The established molluscicidal plant, P.senegalense was studied extensively. Several A-ring only tetra-and tri-oxygenated chalcones and derivative flavanones, dihydroflavonol and flavone were obtained mostly in the free but also in bonded states from leaves. These included 2',3'-dihydroxy-4', 6'-dimethoxy chalcone (1), 2',6'-dihydroxy-3',4'-dimethoxy chalcone (2), 2'-hydroxy-4',6'-dimethoxy chalcone (3), 7-hydroxy-5-methoxy flavanone (4), 7-hydroxy-5, 8-dimethoxy

flavanone (5), 7-hydroxy-5, 8-dimethoxy flavanonol(6), two polymorphic 5-hydroxy-7-methoxy flavanones (7) and (8) and 7-hydroxy-5, 8-dimethoxy flavone (9). Roots and stems of P.senegalense on the other hand contained non or only traces of these light yellow to orange solids.

The other three species do not appear to contain high concentrations of the above flavonoids and their derivatives. P.salicifolium analysis did not lead to isolation of any significant secondary metabolites. P.pulchrum roots, leaves and stems contained the anthraquinones, emodin (10), physcion (11) and chrysophanol (12). This plant also contained a triterpenoid with yet indeterminate structure. P.setosulum roots and stems again showed only the above anthraquinones and trace amounts of flavonoids.



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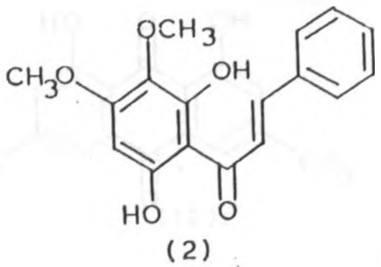

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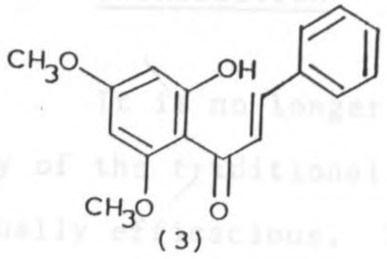

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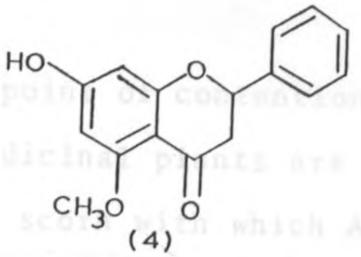

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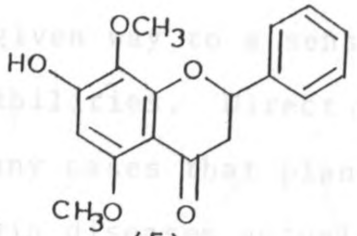

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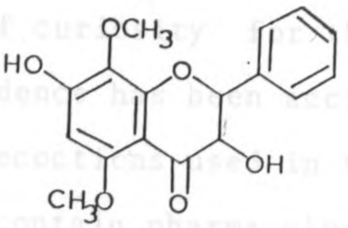

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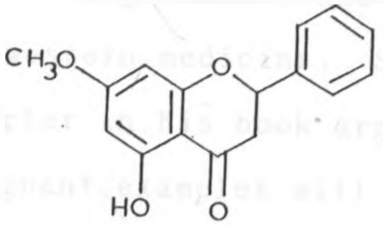

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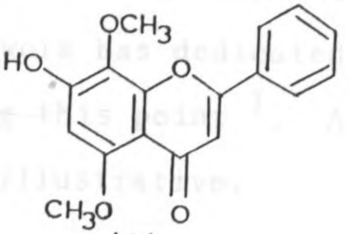

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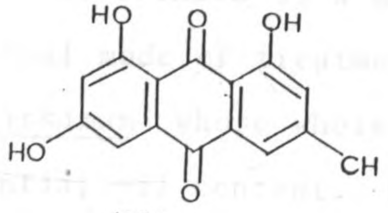

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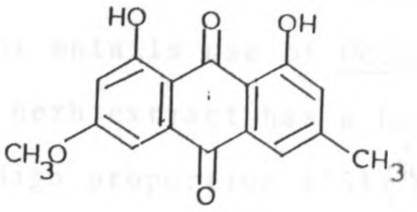

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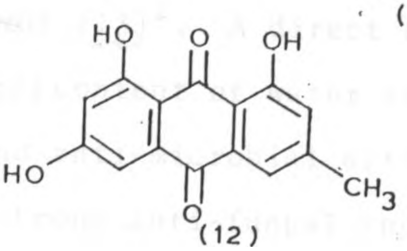

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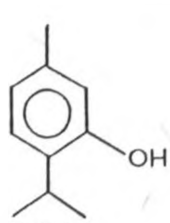
CHAPTER 1

1.0 INTRODUCTION

It is no longer a point of contention that many of the traditional medicinal plants are actually efficacious. The scorn with which African cultural mode of treatment was viewed at one time has given way to a sense of curiosity for the possibilities. Direct evidence has been accrued in many cases that plant decoctions used in treating certain diseases actually contain pharmacologically active ingredients known to cure the same illnesses in western medicine. Sofowora has dedicated a chapter in his book arguing this point [1]. A few poignant examples will be illustrative.

Diarrohoea is a microbial disease. A traditional mode of treatment entails use of Ocimum gratissimum whose whole herb extract has a high essential oil content. High proportion (75%) of this oil is thymol (13)[2]. A direct correspondence between the thymol content of water saturated essential oil and anti-microbial activity has been observed. The strong anti-fungal and anti-microbial activity of the leaves of Plumbago zeylanica used throughout Africa for parasitic skin diseases is due to the presence of plumbagin (14) [3 a,b].

A dilute concoction is active against a wide spectrum of bacteria and pathogenic fungi e.g. Coccidiodes imminites, Histoplasma capsulatum, Trichophyton spp, Candida albicans, Aspergillus niger and A. flavus [4a,b]. Decoction of Rauwolfia species is used to treat mentally disturbed patients because of its recognised sedative properties [3a]. The roots of R. vomitoria and R. serpentina contain reserpine (15) which is established to have hyportensive and sedative properties.



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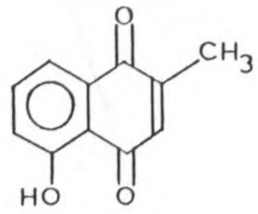

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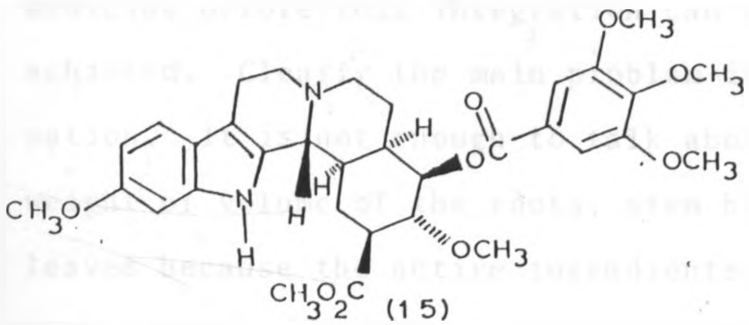

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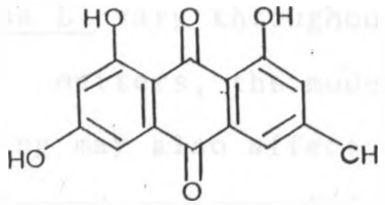
In East Africa about 120 plants have anthelmintic properties in folkloric medicine[3b]. The Rumex abyssinicus and R. usambarensis

found in Kenya and used for this purpose contain large amounts of 1,8-dihydroxy anthraquinones such as emodin (10) compounds which effect their activity by laxative action, the purpose for which they are used in formulations in British pharmacodex.



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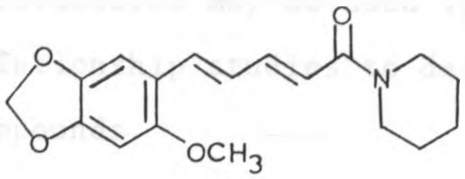
However serious considerations still remain in the overall management of African traditional medicine before full integration can be achieved. Clearly the main problem is standardisation. It is not enough to talk about a given weight or volume of the roots, stem bark or leaves because the active ingredients may vary considerably. Active ingredients in all parts of the plant are known to vary qualitatively and quantitatively by the location, season and in some cases even by the time of day. It was shown that the type of alkaloids found in Piper guinense growing in Ghana depended on the season;

wisanine (16) and α,β-dihydrowisamine (17) were found in June-October but the main alkaloids in January-February collection were piperine (18), α, β-dihydropiperine (19), and N-isobutyl-trans-2-trans-4-eicosodinomide (20) [5]. C-glycosides, O-glycosides and free anthraquinones in Rhamnus purshiana L. vary throughout the year [6]. To exacerbate matters, the mode of post-harvest processing may also affect the active ingredients. Thus the need to assay before final packaging is underlined.



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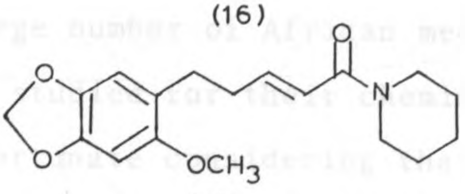

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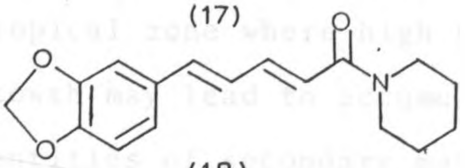

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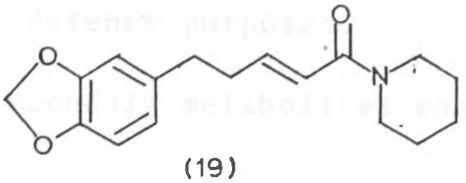


(19)

$$CH_3(CH_2)_{13} - CH_2 - (CH = CH)_2 - CONH - CH_2 - CH(CH_3)_2$$

20

To know the level of these substances in packages, their chemical identities should be established. Once this is done, by chemical analysis, assaying can easily be achieved through use of physical and chemical techniques e.g. Gas liquid chromatography, High pressure liquid chromatography, spectrophotometry, volumetry, gravimetry and electrophoresis. Furthermore identified structures may be used in structure activity relationship studies to develop more suitable compounds.

A large number of African medicinal plants are not yet studied for their chemical constituents. This is unfortunate considering that most of Africa is in the tropical zone where high propensity of microbial growth may lead to accumulation by plants of large quantities of secondary metabolites possibly for defence purposes.

The secondary metabolites which are frequently the active ingredients include alkaloids, sterols, triterpenoids, saponins, flavonoids and anthraquinones. In this project it was chosen to study four Kenyan Polygonum species.

1.1 Biological description[7]..

Four Kenyan polygonun spp. are recorded in traditional therapy mode as efficacious. These are P. pulchrum, P. salicifolium, P. senegalense, and P. setosulum. The genus polygonum is a member of the polygonaceae family (dock family) and is represented in Kenya by eleven species including the four above.. They appear as follows in the "Key to species", Polygonum baldschuriacum Regel, P. convolvulus L., P. nepalense. meisn, P. capitatum Ham, P. afromantanum Greenway, P. aviculare L., P. strigosum R. Br., P.salicifolium willd, P. senegalense, Meisn., P. pulchrum Blume and P. setosulum A. Rich. Of these, P. afromantanum, P. convolvulus, P. pulchrum, P. salicifolium and P. senegalense are considered important flowers in the highland regions of Kenya [8].

P. salicifolium is an erect annual with elliptic, almost sessile leaves and a terminal group of slender interrupted racemes of pink or white flowers. It is found by streamsides or other watersides upto an altitude of 3000 metres. Leaf decoctions is purgative and is also used for treatment of skin troubles.

P. senegalense is also an erect, variably hairy, softly shrubby perennial, and its stem is

often covered with conspicuous slightly inflated, brown stipular sheaths. Leaves are lanceolate, acute, glabrous or densely white tomentose. The racemes number 2-6 usually with white or pink flowers. It is a common plant on the riversides, streamsides and marshes upto a height of 3000 metres. Two varieties exist in Kenya: var. senegalense which is almost glabrous (except for small yellow glands) while, var. albomentosum is densely white tomentose. The former variety was used in this study. It is also noted that intermediate varieties between these two may also occur [8]. P. senegalense is reported as a medicine for cows without specification of the exact problem treated [3b].

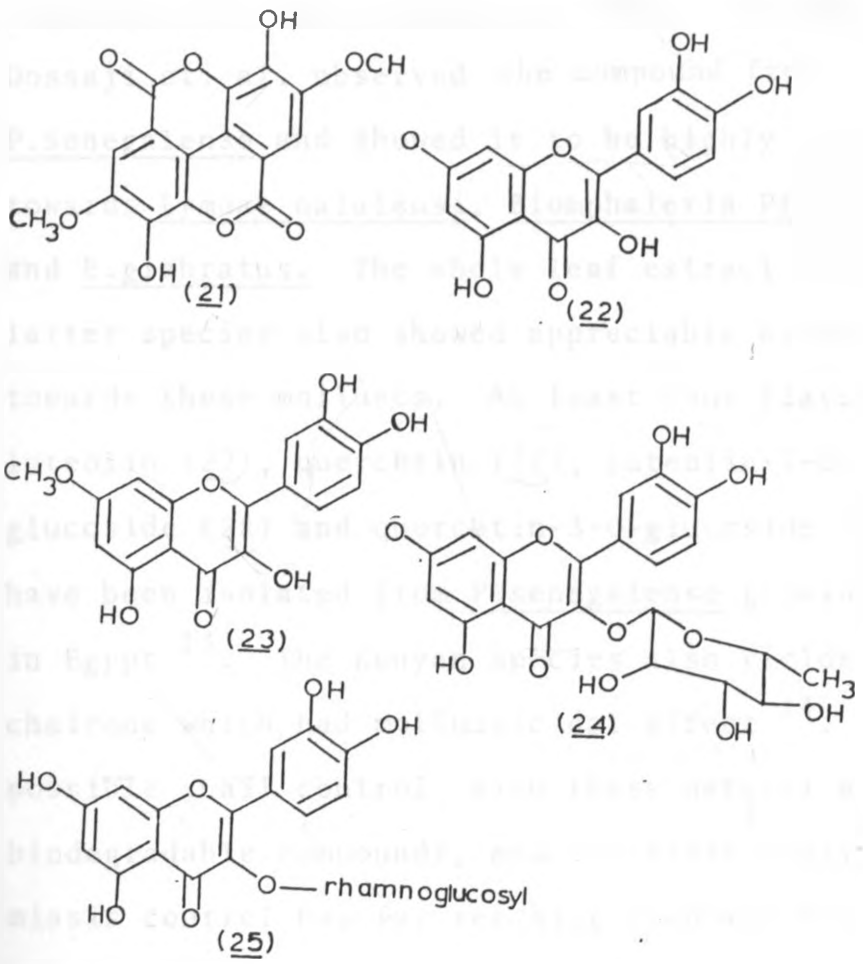
P. pulchrum which is a softly hairy perennial herb with narrow lanceolate entire leaves, often with longitudinal undulations and usually has pink flowers. It is usually found by the watersides. The leaf extracts are drunk about three times daily for the treatment of syphilis, according to one report [3b].

P. setosulum is a roughly hairy perennial herb with lanceolate-ovate or elliptic leaves and inflorescence as in P. pulchrum. It is also a common waterside plant, often growing at higher altitudes than the other species, upto 3500 metres.

Its ethno-pharmacology is not so well defined, the only record claims that Maasai doctors use it to revive comatose patients [3b].

1.2 Pharmacological background

In general most of the reports on the chemical constituents of Polygonum are due to their usage in ethno-pharmacology of various countries. The chinese P. perfoliatum (95% ethanol) extract is known to be anti-hypotensive. A total of 21 crystalline compounds were isolated from this plant amongst which was 3,3' - dimethylellagic acid (21) which when administered to renal hypertensive rats, produced a significant effect on blood pressure and heart contractile force [9]. P. tomentesum is used extensively in Indian medicine. Chemical analysis of P. tomentesum revealed the presence of flavonoids, quercetin (22) rhamnetin (23), quercitrin (24) and rutin (25). P.sachalinense has been used in the orient as a cure for various problems amongst which include use as a laxative, di-uretic, treatment of suppurative dermatitis, gonorrhoea and athletes foot. Amongst compounds which have been isolated from P.sachalinense are physcion (11), emodin (10), emodin γ-O-β-D-glucoside and β-sitosterol glucoside [10].

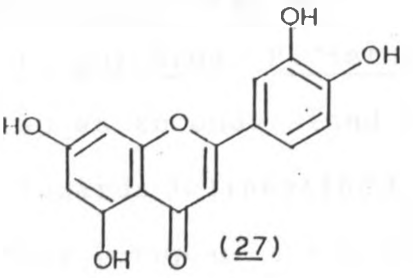


1.3 Molluscicidal activity

Perhaps the most economically important bioactivity reported for the genus is the molluscicidal activity reported for the Japanese

P. nodosum [11] and Kenyan P.senegalense [12].

Quercetin-3β-D-glucoside-2"-gallate (26) was isolated from the molluscicidal P. nodosum and reported as a new compound in 1979. In 1980, Dossaji et. al. observed the compound from P.senegalense and showed it to be highly toxic towards Lymnae nalalensi, Biomphaleria Pfeifferi and B.glabratus. The whole leaf extract of the latter species also showed appreciable bioactivity towards these molluscs. At least four flavonoids, luteolin (27), quercetin (22), luteolin-7-O-glucoside (28) and quercetin-3-O-glucoside (29), have been isolated from P.senegalense growing in Egypt [13]. The Kenyan species also yielded a chalcone which had molluscicidal effect [14]. The possible snail control, with these natural easily biodegradable compounds, and therefore schistosomiasis control has far reaching economic health implications for this region of the world where the disease is endemic. Schistosomiasis control is a priority area for WHO [15,16]



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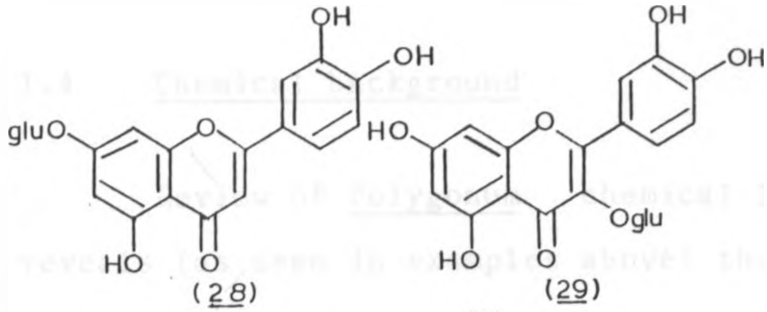
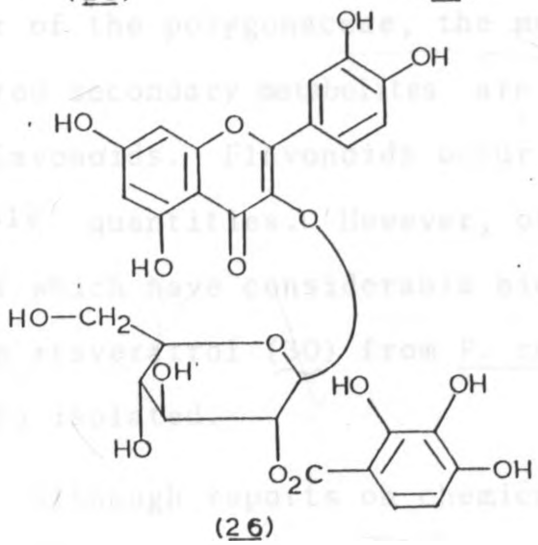




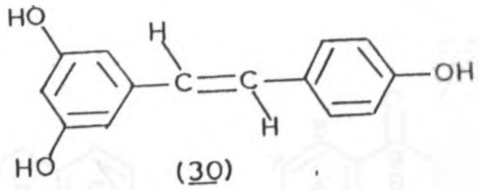
It was therefore interesting to chemically re-study the Kenyan P. senegalense along with other common species, P. pulchrum, P. setosulum and P. salicifolium so as to understand their chemistry as a first step towards delineating the health implication of this group of weed, either directly

in chemotherapy or as a molluscicide.

1.4 Chemical background

Review of Polygonum. chemical literature reveals (as seen in examples above) that as a member of the polygonaceae, the most commonly reported secondary metabolites are anthraquinones and flavonoids. Flavonoids occur most frequently in isoluble quantities. However, other metabolites, some of which have considerable bioactivity, for example resveratrol (30) from P. cuspidatum [17], are also isolated.

Although reports on chemical studies of Kenyan Polygonum species is restricted to P. senegalense, it is interesting that only from this Polygonum, has a chalcone been observed. The chalcone was detected from the hydrophobic fraction of the methanol extract of leaves of the plant [14]. Its structure proved to be 2', 4'-dihydroxy-3', 6'-dimethoxychalcone (31) - a ring A only tetra-oxygenated chalcone. The extract from which the flavone (26) was isolated was also methanol based. Thus only hydrophilic extraction had been effected. Lipophilic extraction from which large amounts of aglycones may be obtained has never been reported.



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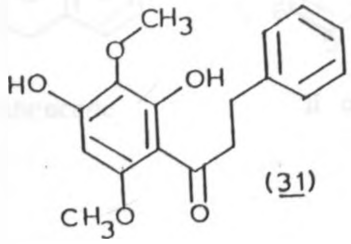
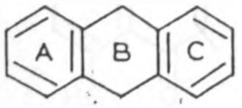


(31)

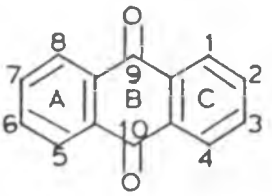
In this project lipophilic extraction for all parts of P. senegalense has been performed in the process of extractive fractionation with petroleum ether, chloroform, and methanol. This procedure was also applied to the rest of the species studied. The thesis reports on the extraction, isolation, and characterisation of aglycone secondary metabolites from the four Polygonum species. These have turned out to be anthraquinones and flavonoids.

1.5 Anthraquinones and Flavonoids background

Anthraquinones are derivatives of the hydrocarbon anthracene (I). Anthracene on oxidation is easily transformed to 9, 10-anthraquinone (II) which is shown with its numbering system.

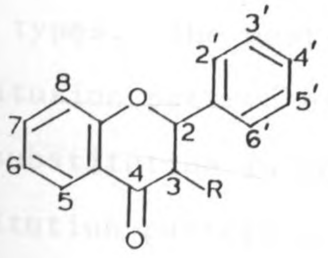


I anthracene

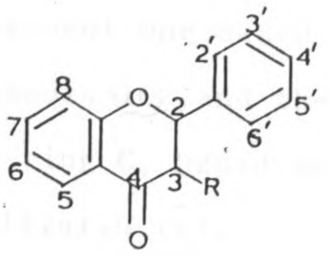


II anthraquinone

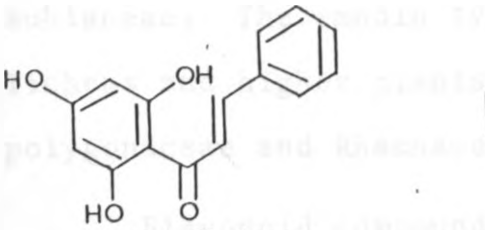
The basic structure of flavonoids is that of flavone and is shown below with its numbering system. The other classes of flavonoids are also shown.



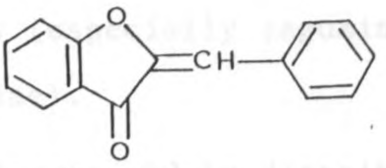
III, R = H, flavone
IV, R = OH, flavonol



V, R = H, flavanone
VI, R = OH, flavanonol



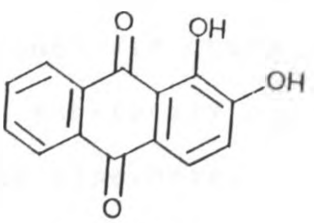
VII Chalcone



VIII Aurone

IX Pelargonidin (an anthocyanidin)

Of all the anthraquinones in the plant kingdom, about half occur in flowering plants (chiefly, Rubiaceae, Scrophulariaceae Leguminosae, Rhamnaceae, Polygonaceae and Liliaceae) and the rest in fungi, lichens and bacteria. There are two major types. The most frequent one based on the substitution pattern of emodin (10), and the other with substitution in only ring C, based on the substitution pattern of alizarin (X).



X Alizarin

The alizarin type have been found mostly in Rubiaceae. The emodin type occurs in fungi, lichens and higher plants (especially Leguminosae, polygonaceae and Rhamnaceae).

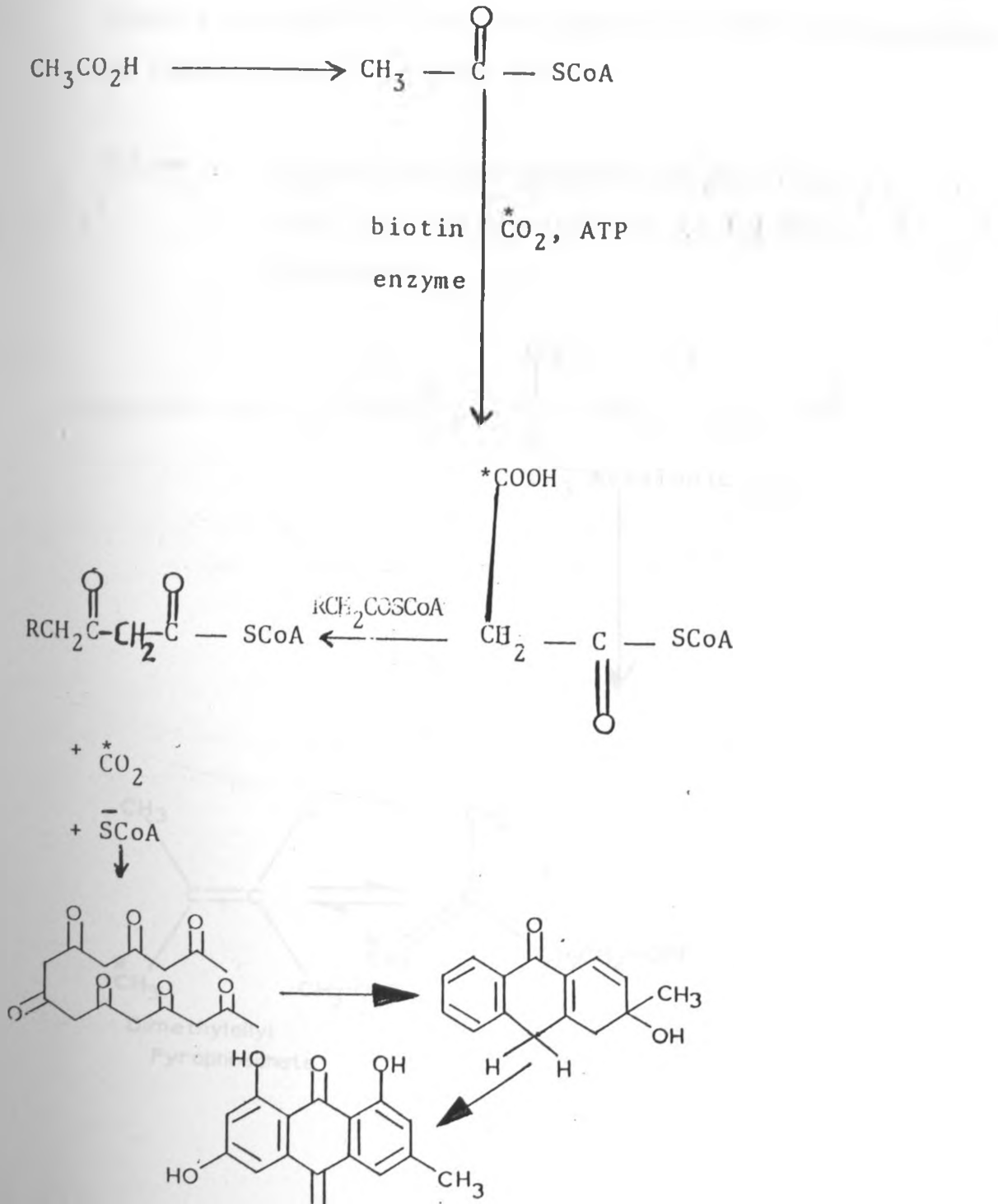
Flavonoid compounds are widely distributed in higher plants. The glycosides of quercetin, for example, occur in 62% of the leaves of dicotyledons examined by Bate-Smith (1962)[18]. They have been isolated from all the different parts of plants, although there are usually varieties in the types of compounds found in the various anatomical tissues of any one plant. In terms of plant colouring, the most striking of such plant organs are undoubtedly the flowers and fruits. But it should be remembered that flavonoids also play a part in leaf colouring. Anthocyanidins are the main colour constituents of flowers and fruits. Flavonols and flavones are widely spread in higher plants. Flavanones and dihydroflavonols are simple reductions of flavones and flavonols. They are not very frequently encountered. Chalcones and Aurones are also not highly distributed. Isoflavones are characteristic of the papilionatae, a sub-family of leguminosae and are rarely found elsewhere.

Two biosynthetic pathways have been

suggested to fit the occurrence of two major types of natural anthraquinones, the emodin and the alizarin types, in flowering plants. The fungal anthraquinones, which are of emodin type have been found to be acetate-malonate derived [19]. By performing feeding experiments, this postulate has been confirmed [20,21] (Scheme I).

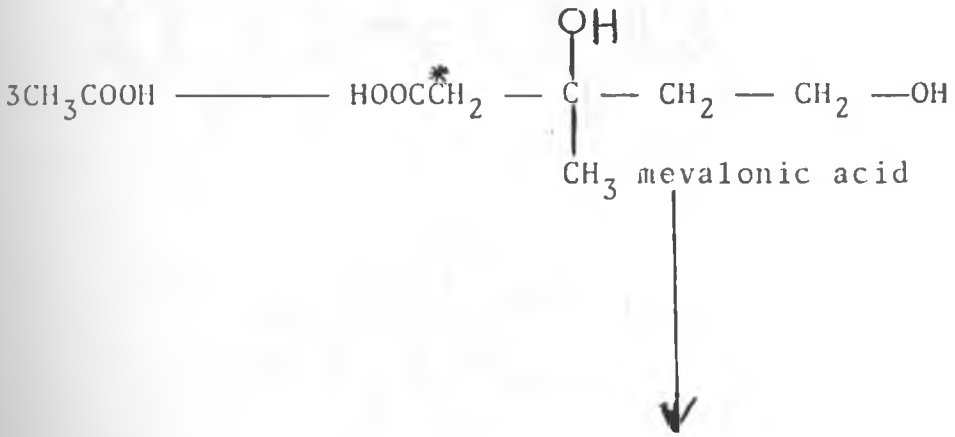
On the other hand, it has been shown that in higher plants (Rubia tinctorum), anthraquinones are derived from the shikimic acid-acetate mevalonate route. Using feeding techniques, results have been obtained showing that ring A of alizarin from Rubia tinctorum (Rubiaceae) is built up from shikimic acid and that ring C is of acetate origin [22] (Scheme II). There are therefore two biosynthetic pathways. The polyacetate-malonate pathway for the emodin type anthraquinones and the one from naphthalenic precursor to which a C_5 side chain is attached, followed by cyclization and oxidation [23,24,25] (Scheme I).

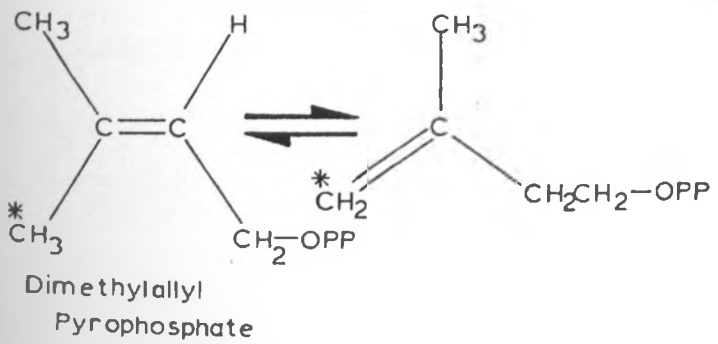
Scheme 1: Polyacetate - malonate pathway leading to anthraquinones



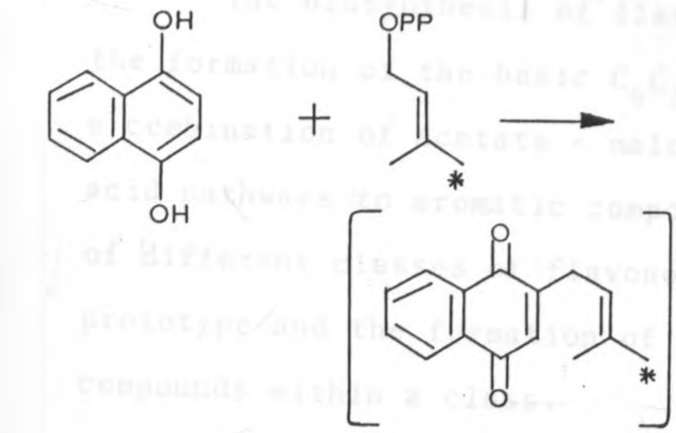
In the polymalonate pathway, AcetyCoA is carboxylated to malonylCoA in the presence of biotin. It then condenses with another molecule of malonylcoA with again, loss of carbon dioxide [26]. The poly-β-ketomethylene formed then cyclizes. Oxidation of side chain and removal of hydroxyl group are a process that can be invoked to account for the structure of a few anthraquinones of emodin type [27] (Scheme I).

Scheme 2: Pathway of incorporation of mevalonic acid into pseudopurpur in XI in Rubia tinctorum.

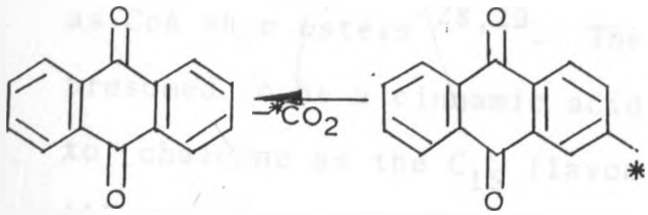


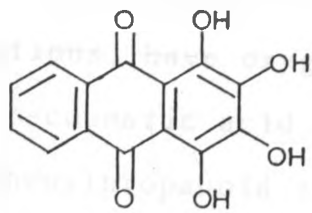


Dimethylallyl Pyrophosphate



Phenylnaphthaquinone





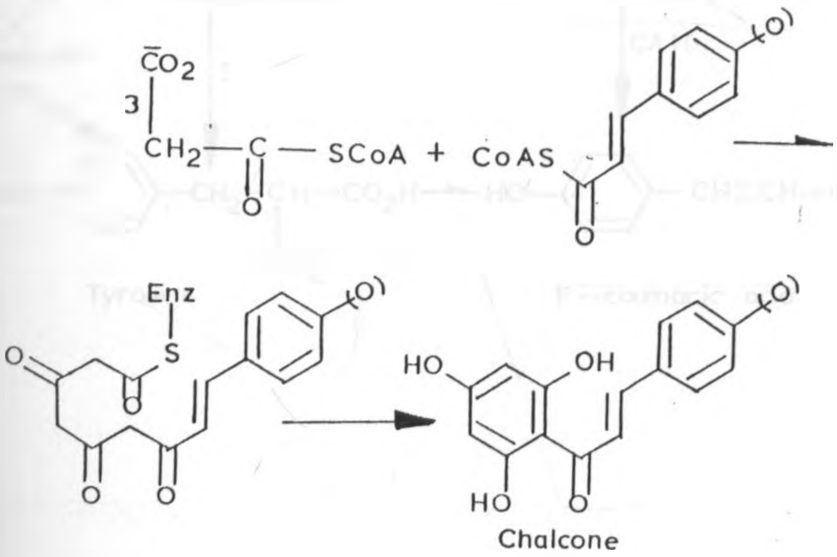
XI Pseudopurpurin

The biosynthesis of flavonoids involves, the formation of the basic $C_6C_3C_6$ skeleton from a combination of acetate - malonate and shikimic acid pathways to aromatic compounds, the formation of different classes of flavonoids from the C_{15} prototype and the formation of individual compounds within a class.

The $C_6C_3C_6$ skeleton is postulated to arise from the condensation of C_6C_3 unit with three malonate - acetate units, all being activated as CoA thio esters[28,29]. The C_6C_3 unit is presumed to be a cinnamic acid, giving rise to chalcone as the C_{15} flavonoid prototype. All available evidence support this hypothesis[30].

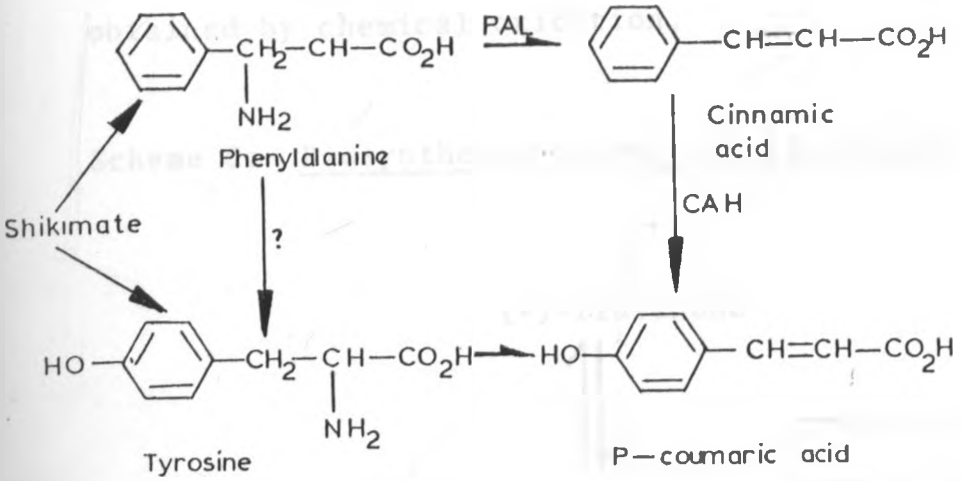
All flavonoid compounds, with a few exceptions, have oxygen at position C-4', suggesting that p-coumaric acid rather than cinnamic acid is the phenylpropanoid intermediate. The enzyme cinnamic acid 4-hydroxylase (CAH) catalyses the formation of p-coumaric acid from cinnamic acid. The biosynthesis of p-coumaric acid is also possible via tyrosin but this route is of significance in plants of Gramineae family only.

Scheme 3: Probable origin of the $C_6C_3C_6$ skeleton of flavonoid compounds.



The immediate precursor of cinnamic acid is phenylalanine. The enzyme phenylalanine ammonia lyase (PAL) effects the conversion. Studies have shown that the acetate - malonate and shikimic pathway is the same in micro-organisms and in higher plants [31].

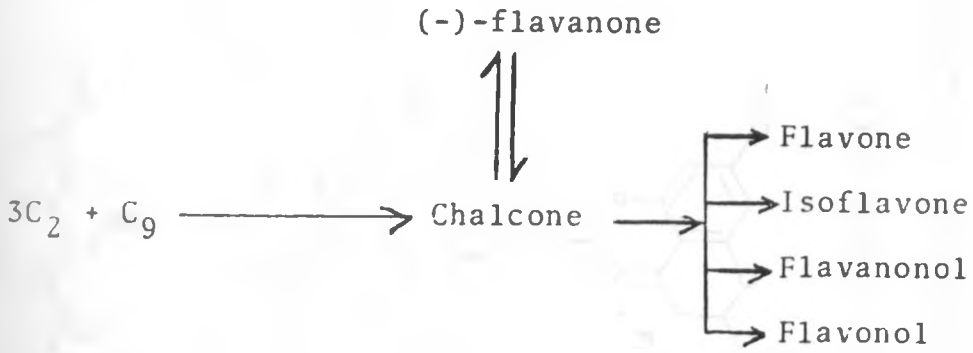
Scheme 4: Possible routes to p-coumaric acid



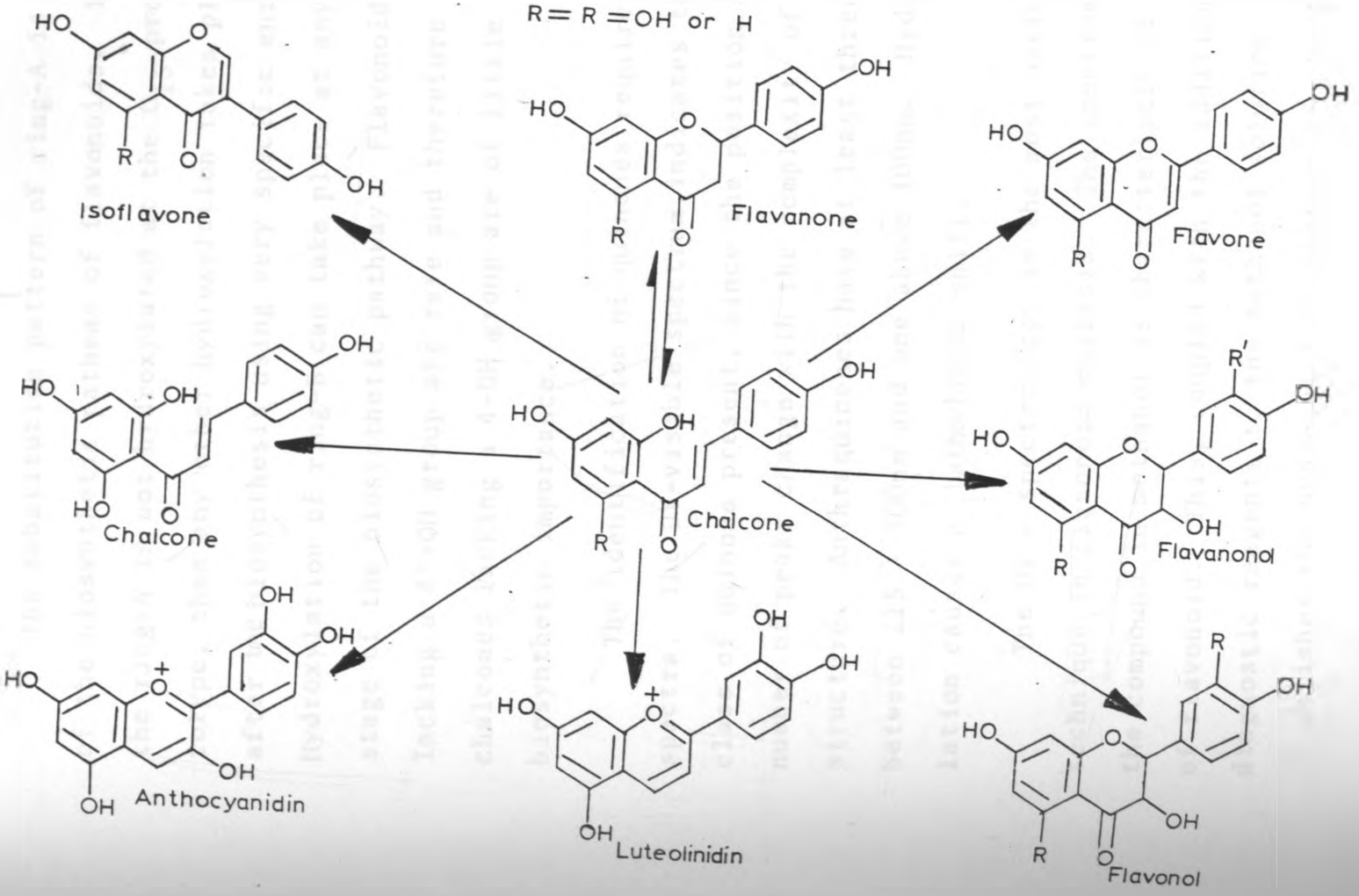
Since flavanones are thermodynamically more stable than chalcones, it was earlier presumed that flavanone must represent the primary heterocyclic intermediate in the pathway to other classes of flavonoids. But studies carried out, confirmed the contrary [32,33]. It was shown that (-)-flavanone and chalcone are biochemically interconvertible. And that reaction chalcone-flavanone catalysed by isomerase is not directly involved in the pathway from chalcones to flavanones, isoflavones, flavanonols and flavonols. Flavanone

intermediate step in the biosynthesis of the flavonoids. Aurones are always found together with chalcones from which they can easily be obtained by chemical oxidation.

Scheme 5: Biosynthetic pathway of flavonoids



Scheme 6 Incorporation of Chalcones into other flavonoid compounds-



The substitution pattern of ring-A is part of the biosynthetic pathway of flavonoids. If the ring-A is not hydroxylated at the C_{15} prototype, then any other hydroxylation takes place after the biosynthesis using very specific enzymes. Hydroxylation of ring-B can take place at any stage of the biosynthetic pathway. Flavonoids lacking a 4'-OH group are rare and therefore chalcones lacking a 4-OH group are of little biosynthetic importance.

The identification of quinones requires spectra. The UV-visible spectrum indicates the class of quinone present, since the position and number of peaks change with the complexity of the structure. Anthraquinones have at least three bands between 215 - 300nm and one above 400nm. Hydroxylation causes a bathochromic shift.

The UV - spectroscopy is the most useful technique in flavonoid analysis. The spectrum of the compound in methanol is characteristic of class of flavonoid. This, coupled with the addition of diagnostic reagents to the methanol solution establishes the hydroxylation pattern of flavonoids.

The methanol spectra of flavones and flavonols have two major peaks in the region 240-400nm. Band I (usually 300-380nm) is considered to be due to

ring-B conjugation with carbonyl group and band II (usualy 240-280nm) is due to conjugation of ring-A with carbonyl group. Isoflavones, flavanones and dihydroflavonols can be easily distinguished from flavones and flavonols because of having little or no conjugation between the ring-B and the C-4 carbonyl system. They usually have an intense band II with a low intensity peak (shoulder) for band I. The band II absorption for isoflavones (usually 245-270nm) is unaffected by increased hydroxylation of the ring-B. Both dihyroflavonols and flavanones have their band II peak in the range 270-295nm, clearly distinguished from isoflavones. Chalcones have their major peak (band I) occuring in the range 340-390nm, although those lacking ring-B hydroxylation may have band I at shorter wavelength. Band II may appear as a minor peak in region 220-270nm. Increased oxygenation of either ring-A or ring-B usually results in bathochromic shift.

The addition of sodium methoxide to sufficiently ionise all the phenolic hydroxyl groups gives bathochromic shifts to all the peaks. The extent of which depends on the class of compound and the overall substitution pattern [34]. Anthocyanidins are unstable and decompose under

these conditions. These shifts are summarised in table 1. Sodium acetate instead ionises only acidic phenolic hydroxyl groups. This is usually 7-OH group and changes involve only band I of the spectra of flavones, flavonols and to similar changes as full basification of most flananones and isoflanones. Therefore the presence of 7-OH group can be determined in these compounds. The addition of weakly acidic aluminium chloride solution to a methanolic solution of most flavonols gives an immediate yellow colour. This is due to the formation of a chelate between the carbonyl group and the adjacent hydroxyl group at C-3, or if this is substituted, that of C-5. For flavones, chalcones, flavanones, isoflavones and aurones, it is always the latter group which is involved. The shifts are also summarised in table 1.

Table 1. Variations in the U.V. and visible spectra of flavonoids by the use of shifts reagents.

Compound	λmax(nm) MeOH	$\log \varepsilon$ long wave length peak	Δλ(nm) Base (all OH)	Δλ(nm) NaOAC (7-OH)	Δλ(nm) $AlCl_3$ (3-or-5-OH)
Isoflavones	250-270	4.30-4.60	10-15	5-15	10-20
Flavanones	275-295	4.20-4.30	30-40	30-40	20-30
Flavones	255-285 315-275	4.20-4.45	10-20 40-60	10-20	40-60
Chalcones	370-385	4.42-4.49	40-60		50-65
Aurones	385-415	4.44-4.48	40-60		50-65
Anthocyanins	475-542	4.35-4.50	unstable	Unstable	15-50 (orthodihydroxy)

The flavanones having a free 7-OH group are accompanied by a two-fold increase in intensity of the peak on addition of sodium methoxide. While flavonoids with orthodihydroxy and vicinal trihydroxy groups are quite susceptible to oxidation at high pH and many compounds e.g. quercetin (22), are destroyed. This can be avoided by adding sodium borohydride [35].

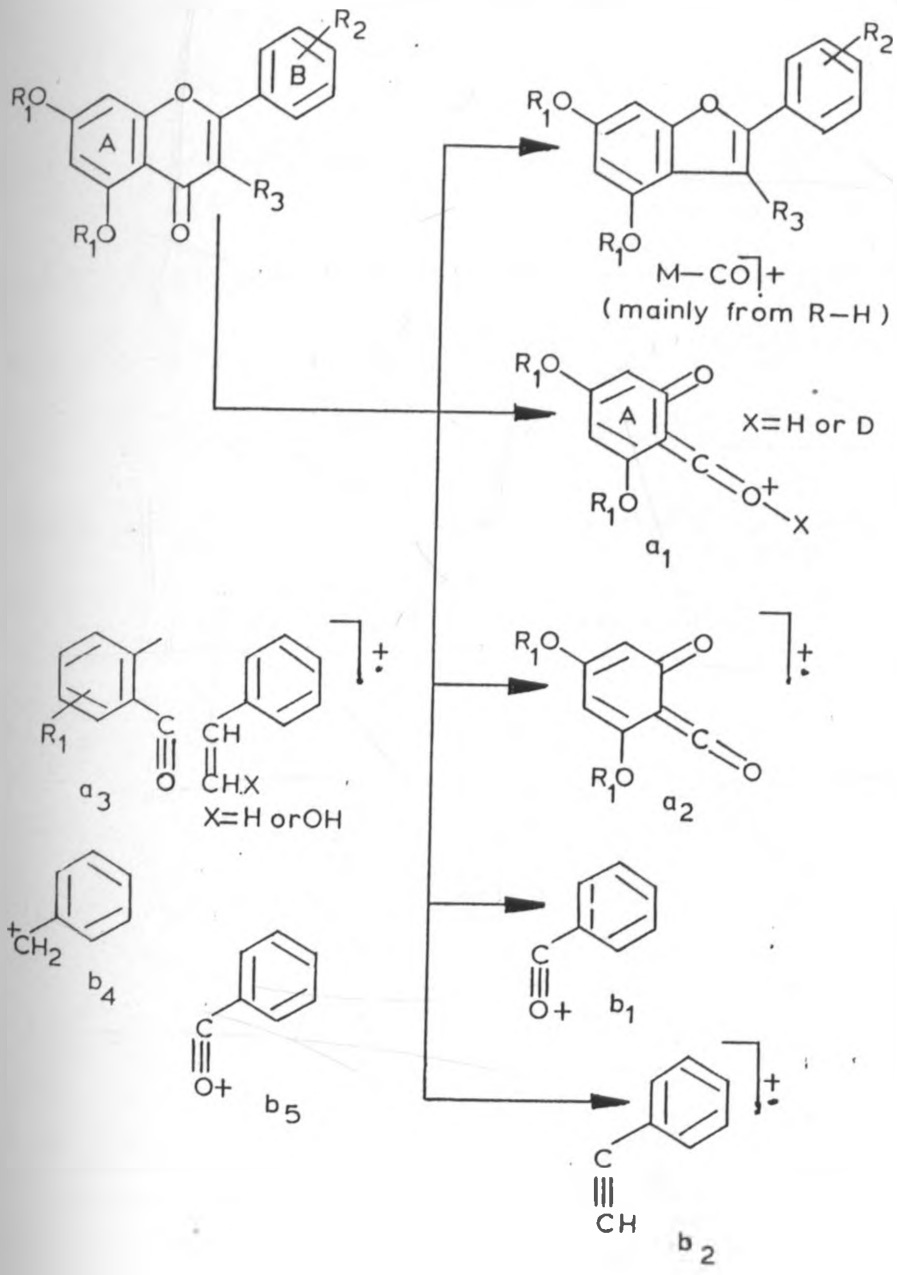
The infrared spectra is very important in identifying carbonyl frequencies of quinones.. The absorption frequencies are raised as the number of fused rings increases and is lowered by hydrogen bonding. Chelated quinones e.g. the 1,8-dihydroxyanthraquinones can be recognised by lowered frequency of carbonyl and hydroxyl absorptions. For the flavonoids, although not very documented, the infrared spectra forms useful adjuncts to structural elucidation. The carbonyl absorption with and without hydrogen bonding is again the striking factor.

The use of ^{1}H NMR spectroscopy is now well established in flavonoid analysis, due to thorough work carried out by Mabry and co-workers [34]. The most informative region from the point of view of A-or-B-ring substitution is the range

δ5.75-8.2. This is the range for flavonoid aglycones without methoxy groups. The ^{1}H NMR for quinones also give very valuable information to the anthraquinones structure identification.

Mass spectroscopy is another valuable tool in terms of structural elucidation. The anthraquinones are quite stable and the parent peak is normally the base peak, with strong M-CO and M-2CO peaks. The flavonoid mass spectra also gives the parent peak as the base peak because of their stability. The major fragmentations are due to the retro Diels-Alder reactions (i.e. split to give charged diene-like and dienophile fragments) involving the heterocyclic ring [36]. The typical fragmentation patterns are given below (Scheme 7).

Scheme 7: Principal initial ions produced in the mass spectral fragmentation of flavonoids.



Isoflavones give the same ions a_2 and b_2 as flavones. Chalcones produce ions a_3,b_3 (x = H), b_5 and flavanones give a_1, a_2 and b_3 (x = H). Flavanonols give a_2, b_3 (x = OH) and b_4.

CHAPTER 2

2.0 RESULTS AND DISCUSSION

The approach taken in studying the secondary constituents of *P.salicifolium*, *P.senegalense*. *P.pulchrum* and *P.setosulum* was to perform a preliminary spot-test survey of the inherent constituents before embarking on larger scale extraction, isolation and subsequently characterisation.

Preliminary tests were performed on 95% ethanol extracts of 10-20 g powder samples [37]. The methodology entailed soxhlet exhaustive extraction of the powder, solvent removal on rotatory evaporator to give a viscous mass. Samples used for the tests were drawn from this material. For example anthraquinones could be detected by taking a small sample and shaking with benzene. The benzene solubles were then exposed to ammonium hydroxide solution. If the aqueous layer turned violet then the presence of free anthraquinones was inferred. Flavonoids on the other hand are detected by the same procedure but with potassium hydroxide as the base using colour intensification as the criterion. Sterols, triterpenes and saponins were likewise tested as detailed in the experimental section. The results of these tests are shown in table II.

Table II: Preliminary test results

Species	Part of plant	Anthraquinones	Flavonoids	Sterols triterpenoids saponins	Alkaloids
P.Salicifolium	Roots & stems	-	+	+	-
	Leaves	-	+	+	-
P.Senegalense	Roots & stems	-	+	+	-
	Leaves	-	+ + +	+	-
P.Pulchrum	Roots	+ +	-	+ +	-
	Stems	+	-	+	-
	Leaves	+	+	+	-
P.Setosulum	Roots & stems	+ +	+	+	-
	Leaves	+	+	+	-

Key: - negative, + trace, ++ positive
+ + + very positive.

The apparent total absence of alkaloids is notable and in line with finding no Polygonum species in the literature with such compounds. The ubiquitous triterpenoids, sterols, and saponins were found, albeit in trace amounts. The presence of anthraquinones depended on the species, being observed in P.pulchrum and P.setosulum but not in P.salicifolium and P.senegalense. Flavoncids tests were positive for all the plants and in all parts except for roots and stems of P.pulchrum.

Clearly any large scale work on these plants would be geared towards extracting and isolating the component anthraquinones and flavonoids. However, in general the approach designed for extraction was one which was incidentally general for most secondary metabolites, that is sequential soxhlet extraction with petroleum ether, chloroform and methanol in that order. Since it was the inten ion to delineate only the aglycones, the methanol extract was subsequently hydrolysed with 2NHCl and extracted with an organic solvent.

2.1 P.setosulum

The petroleum ether and chloroform extracts of P.setosulum roots and stems which amounted to a

total of 4g from a 150g dry powder were usually combined because of analyatical thin layer correspondence. The methanol extract (14.6g) was treated separately as shown in figure 1. The non-polar fraction on silica gel chromatography with petroleum ether, benzene, chloroform and ethylacetate yielded a greenish portion which was positive for anthraquinone test. To resolve the phenolics from the green matter, the 300mg portion dissolved in chloroform was extracted with potassium hydroxide solution. Acidification of the extract followed by partition into chloroform gave an isolate from which emodin (10) was separated. The methanol hydrolysate from roots and stems showed compound (10) and two other anthraquinones, physcion (11) and chrysophanol (12). These anthraquinones were identified by comparing with authentic samples previously isolated in our laboratories from Rumex spps [38].

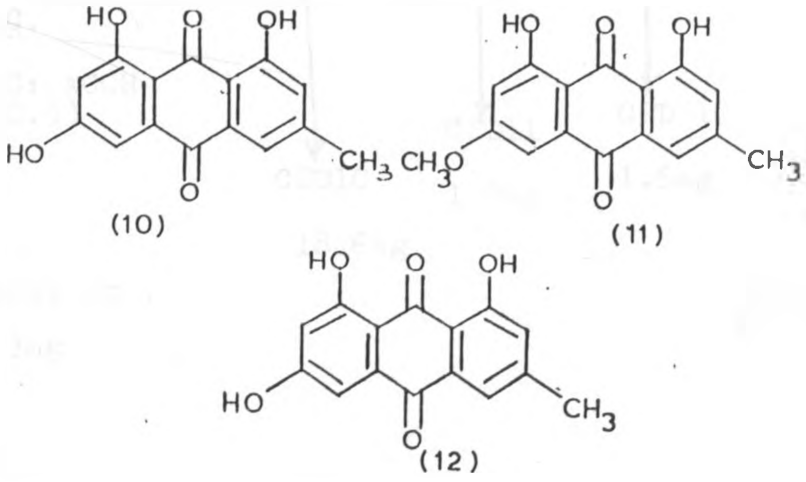


Fig. 1 Fractionation of roots and stems of P. setosulum.

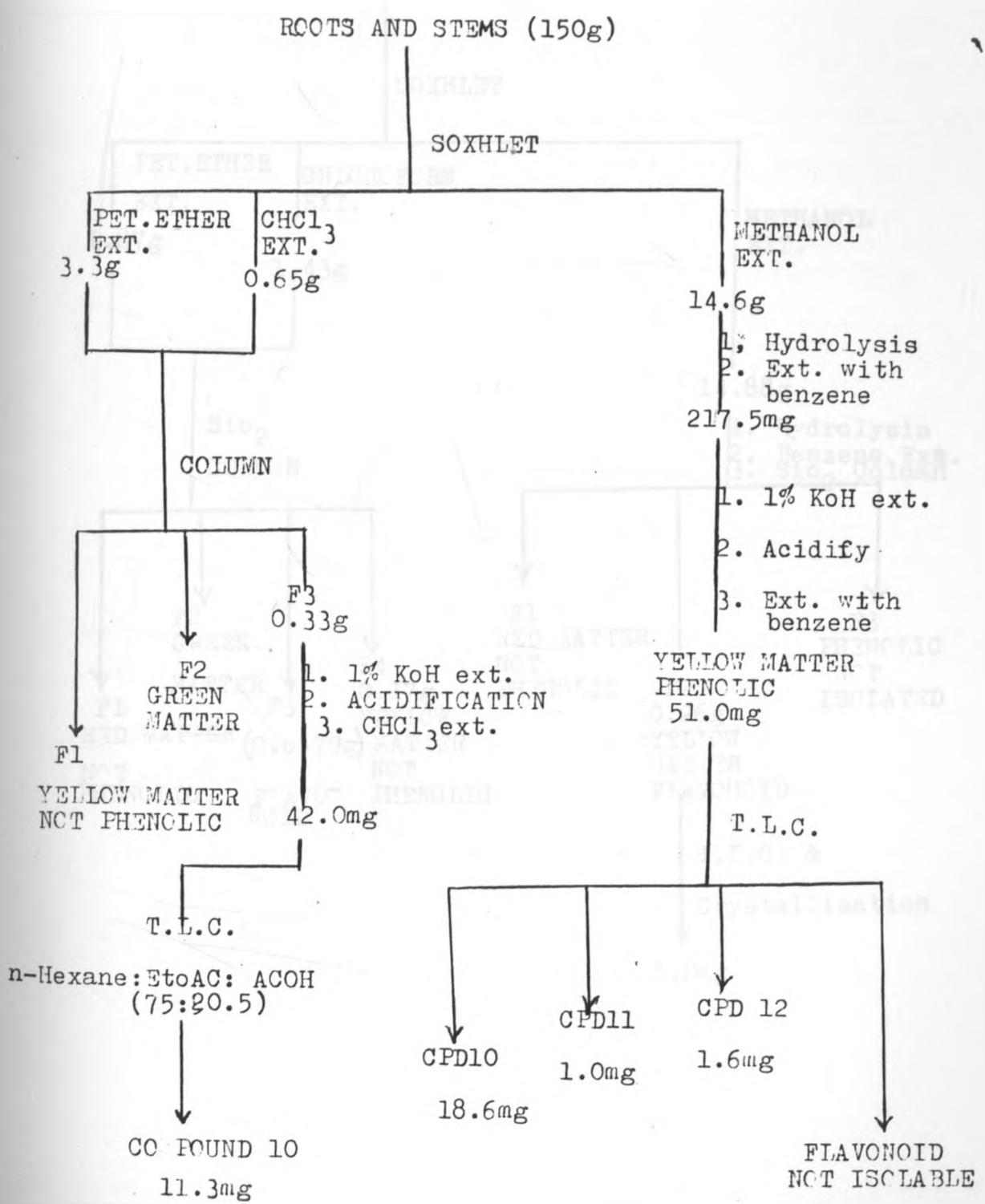
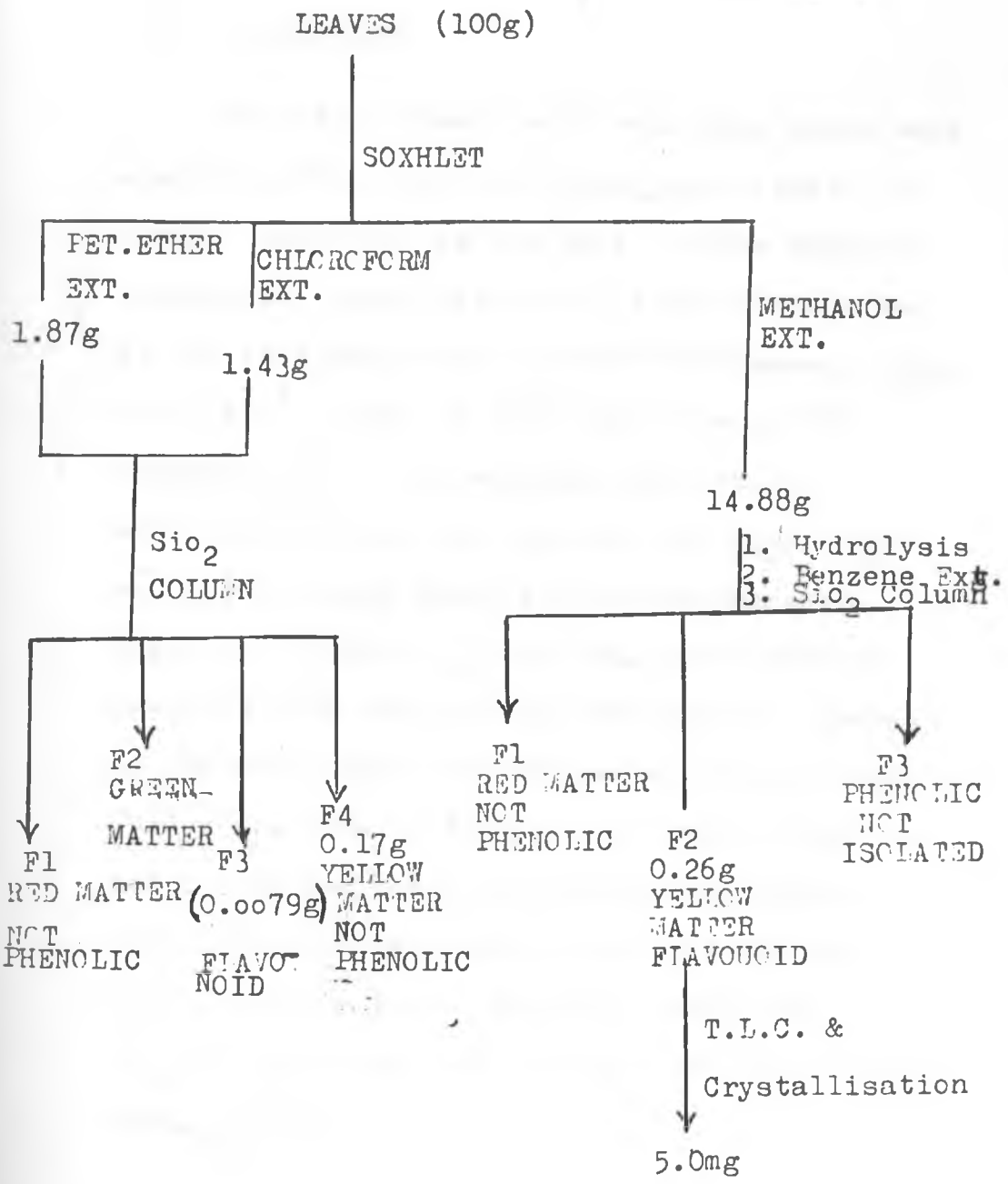


Fig.2 Fractionation of leaves of P. setosulum.



2.2. P.pulchrum

The three common anthraquinones above were again found in roots of P.pulchrum, albeit in minute quantities as the only isolabe phenolic compounds. Usual extractive fractionation led to the realisation of 1.5×10^{-3}% compound (10), 7.0×10^{-4}% compound (11) and 6.0×10^{-3}% compound (12). The methanol extract on hydrolysis showed the existence of (10), (11) and (12) through thin layer chromatographic analysis. Emodin (10) (11.2mg) was actually isolated from 800g of dry stem powder. Leaves on the other hand were devoid of anthraquinones. Cold extraction of leaves of a later collection led to the isolation of a white crystalline compound which gave a positive triterpenoid micro-chemical test. However, sufficient spectroscopic data was lacking for its positive identification.

Figure 3 Fractionation of P.pulchrum roots

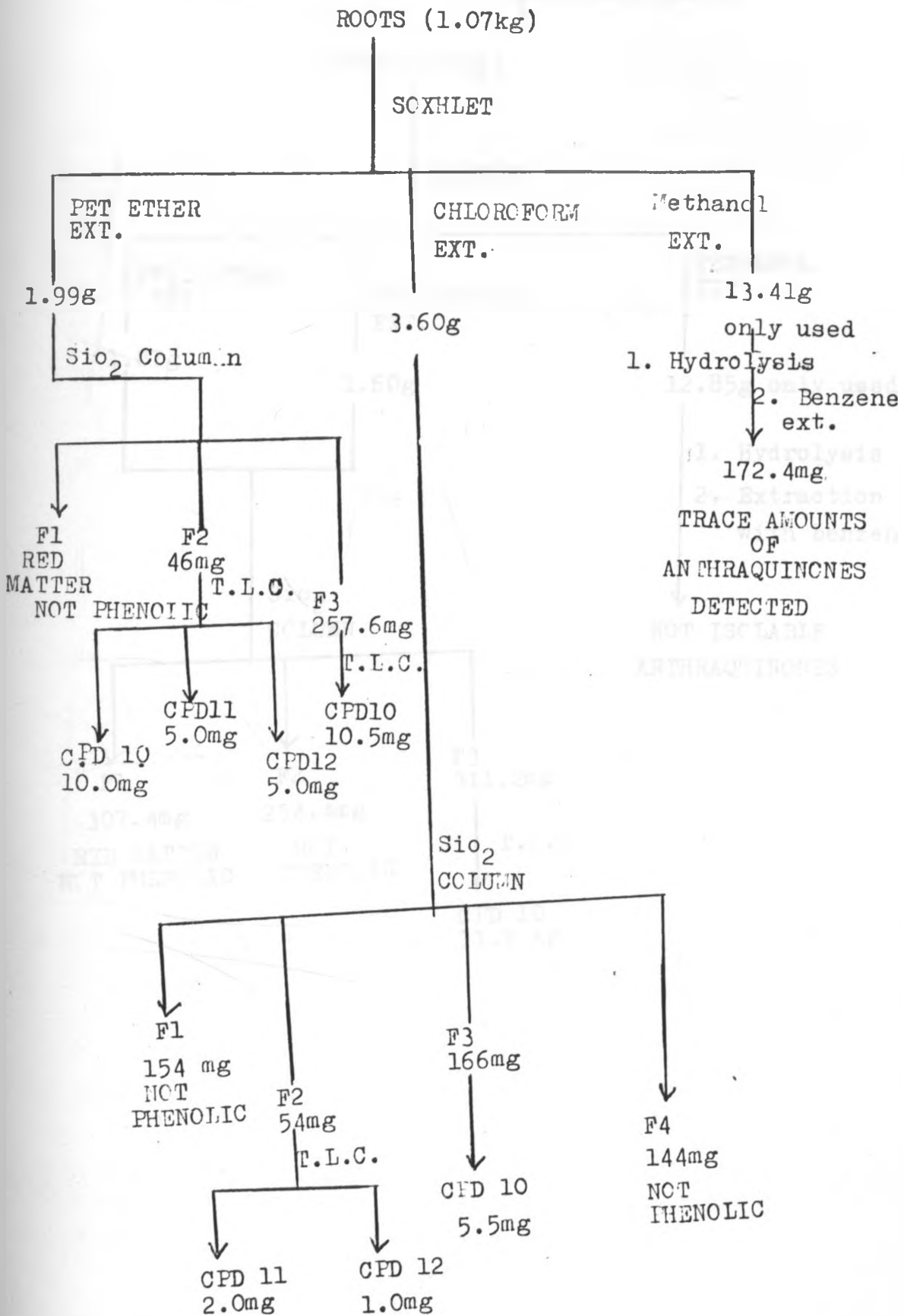
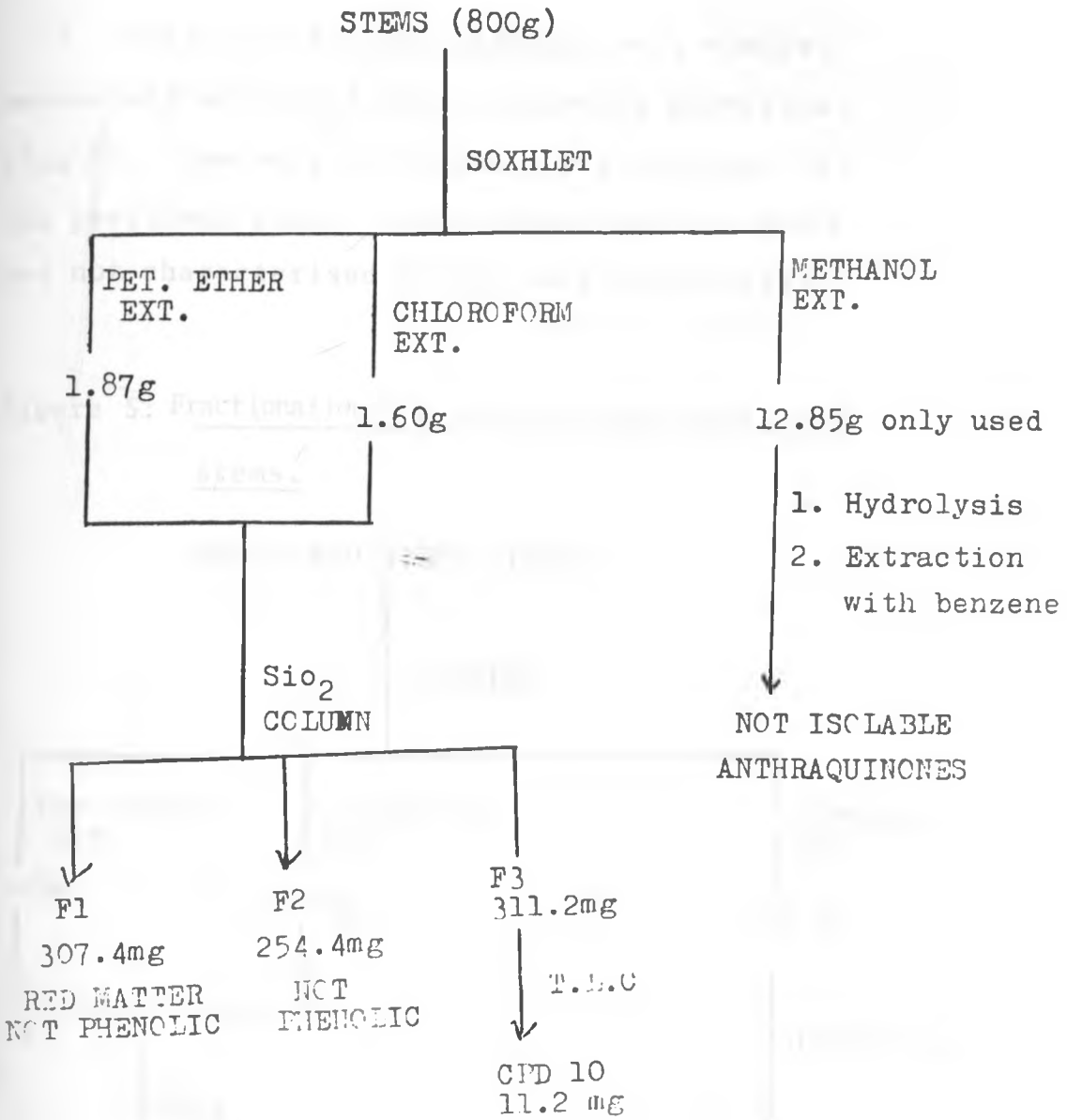


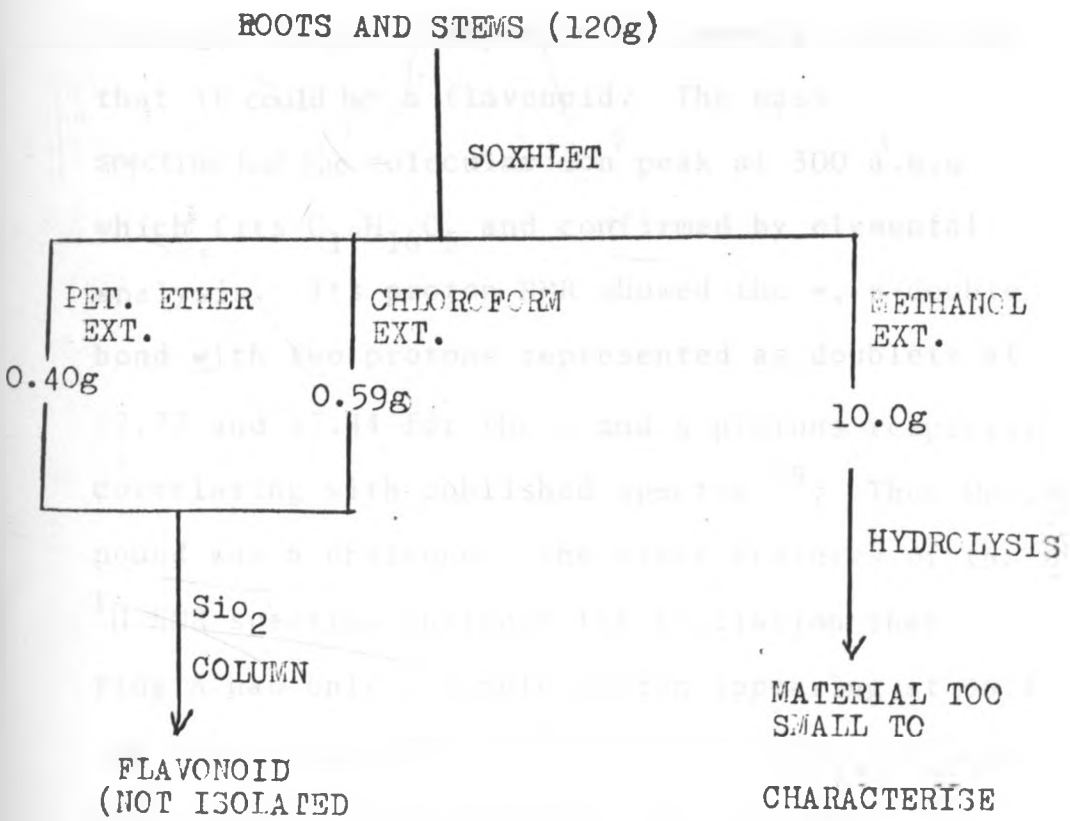
Figure 4. Fractionation of P.pulchrum stems



2.3 P.salicifolium

Analysis of P.salicifolium in a similar mannar did not yield characterisable phenolics (Fig.5). However, a flavonoid was observed in the petroleum ether - chloroform fraction which was not characterised due to lack of material.

Figure 5: Fractionation of P.salicifolium roots and stems.

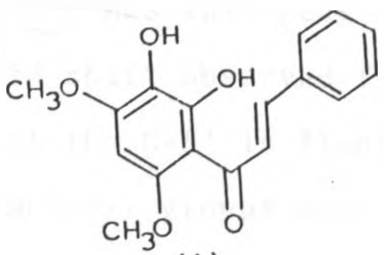


2.4 P. senegalense

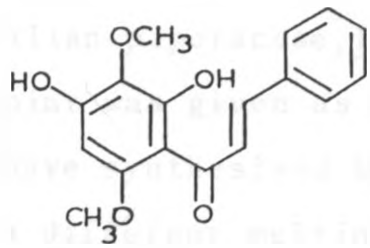
The petroleum ether and chloroform extract of P.senegalense on chromatographic fractionation revealed several flavonoids from this species in appreciable amounts (Fig.6 and 7). The first compound (1) to be characterised was eluted from two silica gel columns with benzene as 0.218% by weight of a 160g dry leaf powder. It had an R_f of 0.34 with pure chloroform as the developing solvent and its spot assumed greenish colouration through orange on exposure to ammonia indicating that it could be a flavonoid. The mass spectrum had the molecular ion peak at 300 a.m.u. which fits $C_{17}H_{16}O_5$ and confirmed by elemental analysis. Its proton NMR showed the α, β-double bond with two protons represented as doublets at δ7.72 and δ7.84 for the α and β protons respectively correlating with published spectra [39]. Thus the compound was a chalcone. The other features of the ^1H NMR spectrum included the revelation that ring A had only a single proton appearing at δ6.4 implying penta-substitution of this ring. Ring B protons were represented by two multiplets at δ7.39 and δ7.57 suggesting its mono-substitution. Compound (1) has two methoxy groups on ring A which appear at δ3.88 and δ3.90. The other oxygen

substituents of the ring were hydroxy groups as shown by the formation of a diacetate.

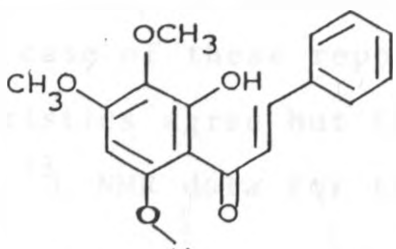
The arrangement of the four substituents on ring A was determined by ultraviolet - visible and Infra-red spectroscopy studies. A bathochromic shift on band I as obtained in methanol when aluminium trichloride was added showed that there was a 2'-hydroxy group. This was confirmed by IR analysis in which the carbonyl stretch appearing at 1618cm^{-1} moved to 1630cm^{-1} on acetylation implying removal of the chelating hydrogen bonding. That the two hydroxy groups were juxtaposed on ring A was shown by the 53nm shift to longer wavelength caused by sodium acetate-boric acid reagent on band I. The structure assigned is shown below, which compares with the other tetra-oxygenated chalcone (32) reported by Marudufu from this species and with pashanone [14,40,41]



(1)



(32)



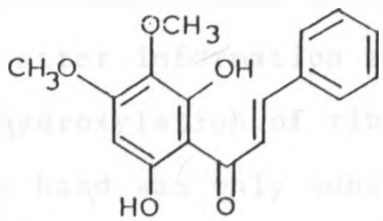
(33)

This structure was confirmed by ^{13}C NMR which showed a peak at δ89.9. The carbon NMR of compound (32) had a peak at δ92.8 assigned to C-5'. Pashanone (33) has this peak at δ91.8. The significant upfield shift observed for (1) should therefore mean that the C-5' is flanked by two methoxy groups. Methoxy groups have been assigned greater carbon shielding effect at the ortho and para - positions than the hydroxy group in flavonoids [42,43,44,45,46].

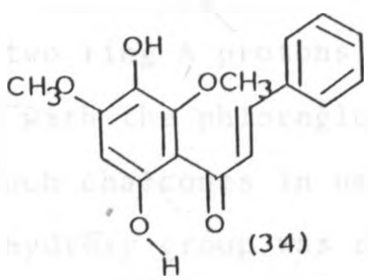
The compound 2', 3'-dihydroxy-4', 6'-dimethoxy-chalcone (1) has been reported previously before from Brazilian piperaceae, Piper hispidum when its melting point was given as 60°C [47]. Bhaskar and Seshardi have synthesised this compound but reported a different melting point (138- 40°C) from benzene-ethylacetate [41]. Compound (1) re-crystallised from benzene melted at 104-106°C. In every case of these reports, all the other characteristics agree but this is the first time that the ^{13}C NMR data for the compound are reported.

A second chalcone (2) observed was isolated as a 0.227% red powder which had a m.p. of 142-144°. The proton NMR of (2) showed the presence of an α, β-double bond. Again ring B was free of substitution. The mass spectrum showed the molecular ion at 300 a.m.u. while the base peak was at 196 a.m.u., the latter indicating a tetra-oxygenated A-ring: two hydroxy groups and two methoxy groups. The sodium acetate- boric acid test, this time did not yield a shift in band I. However, aluminium trichloride reagent caused a shift of 45 nm which implied the presence of a peri-hydroxy group. The IR spectrum proves this assumption since the carbonyl stretch occuring at 1620 cm^{-1} is moved to 1635 cm^{-1} on acetylation!

The shift with sodium acetate was not significant thus the other hydroxy group was not at C-4'. Two possible structures can be written which account for these data as below:



(2)

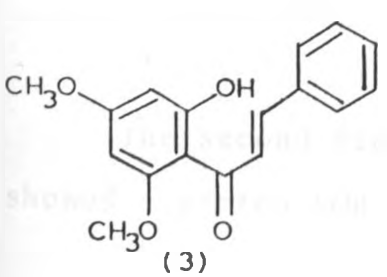


(34)

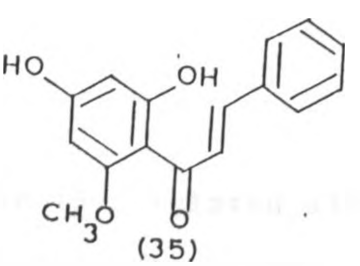
Thus (2) has identical structure to pashanone which was isolated from the Indian Gesnaraceae, Didimocarpus pedicellata [40] and later synthesized [41]. The latter report had it that its melting point was 147-49°, crystallised from benzene - light petroleum ether. The ^{13}C NMR of pashanone was reported by Patra and Mitra [46]. The chemical shift position match closely those observed for compound (2), especially in ring B. Since the

C-5' is at δ92.9, the structure of (2) is confirmed as that assigned to pashanone.

The third chalcone (3) found was ring A tri-oxygenated. The compound existed in highest concentration (0.459%). It had the molecular ion at 284 a.m.u, while the base peak was at 207 a.m.u. The latter information showed dimethoxylation, and monohydroxylation of ring A. Ring B on the other hand was only substituted with the propane skeleton. The proton NMR spectrum shows two doublets in the aromatic region δ6.09 and δ5.95 (J, 2.0Hz), thus there was a meta orientation of the two ring A protons. This finding was in line with the phloroglucinol biosynthetic origin of such chalcones in nature. The position of the hydroxy group was determined easily by UV-visible shift technique. Addition of $AlCl_3$ reagent shifted band I which placed the moeity at 2'-position, thus the structure is as shown below.



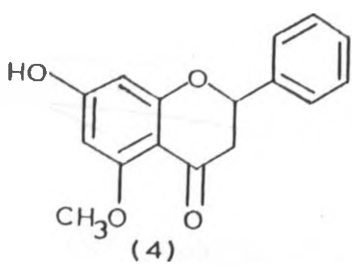
(3)



(35)

Compound (3) had a melting point of 89-90°C which agrees closely with that of monomethyl ether of (35) which was isolated from Alpinia speciosa [48] with m.p. 90-91°C.

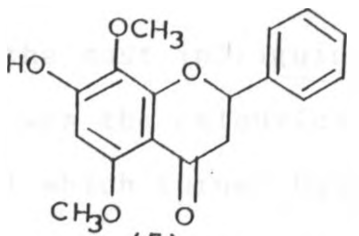
Several flavanones were expectedly observed in the isolates. The hydrolysate of the methanol extract of the leaves yielded a flavanone with the molecular ion at 270 a.m.u. reading $C_{16}H_{14}O_4$. The parent peak was from retro-Diels-Alder process at 166 a.m.u. revealing disubstitution of flavanone ring-A. The hydroxy group is at the C-7 since a significant 38 nm shift is observed with sodium acetate reagent.



(4)

The second flavanone (5) isolated also showed a proton NMR indicative of non-substituion

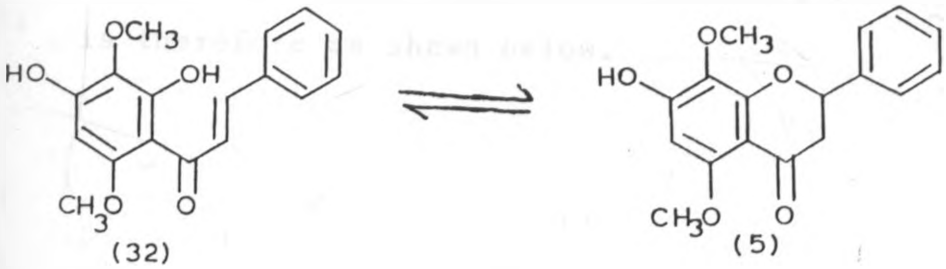
of ring B but having three oxygen substituents on ring A. The molecular ion was at 300 a.m.u. which fits $C_{17}H_{16}O_5$. The chief fragmentation process leads to an even mass daughter ion at 196 a.m.u. as expected. The UV-visible spectrum showed that the requisite single hydroxy group for ring A was at C-7 since sodium acetate caused a 35 nm shift for band II. The only arrangement of substituents possible is as shown below.



(5)

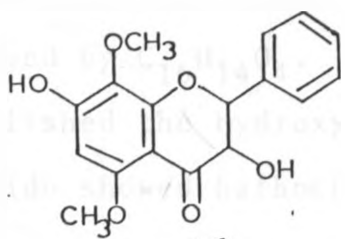
Clearly this compound is related to the chalcone reported by Marudufu, compound (32) from which it could have been formed at the intervention of flavanone isomerase as shown in figure 8.

Fig. 8. Isomerisation of chalcone (32) to flavanone (5).



Perhaps the most intriguing compound obtained from the plant was the colourless but UV-active dihydroflavonol which turned blue through green on exposure to ammonia. It was assigned the structure below because of the following spectroscopic reasons. The molecular ion was at 316 a.m.u. which corresponds to $C_{17}H_{16}O_6$. The proton NMR showed a singlet at δ6.1 for a single proton in ring A. A doublet of duoblets appeared at δ4.4. This latter set of peaks crumbled to a doublet (J, 13Hz) on deuterium exchange. The UV-spectrum has band I and band II peaks at 238 nm and 290nm respectively, typical of dihydroflavonols. Shifts in band II were caused by sodium methoxide and sodium acetate reagents only. The sodium acetate shift was by

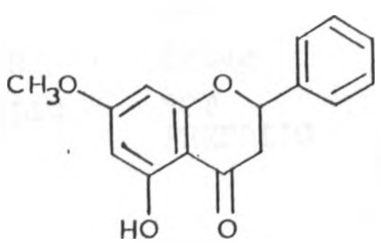
as much as 40 nm placing the ring A hydroxy group at the C-7 position. Sodium acetate-boric acid did not cause a shift. The IR showed a carbonyl absorption at 1635 cm^{-1} which implies hydrogen bonding probably to the C-3 hydroxy moeity. The most reasonable structure for (6) is therefore as shown below.



(6)

Apparently two flavanones were purified from the fraction which contained the major metabolite (3) by fractional crystallisation. The first one (designated compoud (7) crystallised out from n-Hexane-chloroform solution while the latter one crystallised after (3) from n-Hexane

alone and was numbered compound (8). The melting points of the two sets of crystals was the only different characteristics they had; all spectroscopic data match exactly prompting the conclusion that they were really polymorphic sets of the same compound. Such a phenomenon was also observed for compound (1) from different laboratories and is known to be common amongst chalcones [49,50]. The total weight of the two materials was 203.9 mg from 160g dry leaf weight (0.127%). The molecular ion occurred at 270 a.m.u. which is satisfied by $C_{16}H_{14}O_4$. The UV and IR studies established the hydroxy group at C-5. Sodium methoxide showed bathochromic shift of 40nm while aluminium chloride created a 21 nm shift which was not removed by addition of HCl.. The proton NMR showed that ring A had two phenyl protons: two close doublets around δ6.08 (J = 2Hz). A doublet of doublets with J, 3.4Hz and 12.3Hz and two doublet of doublets occurred at round δ3.0 - these being typical of flavanones. The structure assigned to the materials is shown below which makes it a phloroglucinol derivative.



(7) &(8)

Figure 6. Fractionation of P.senegalense roots and stems.

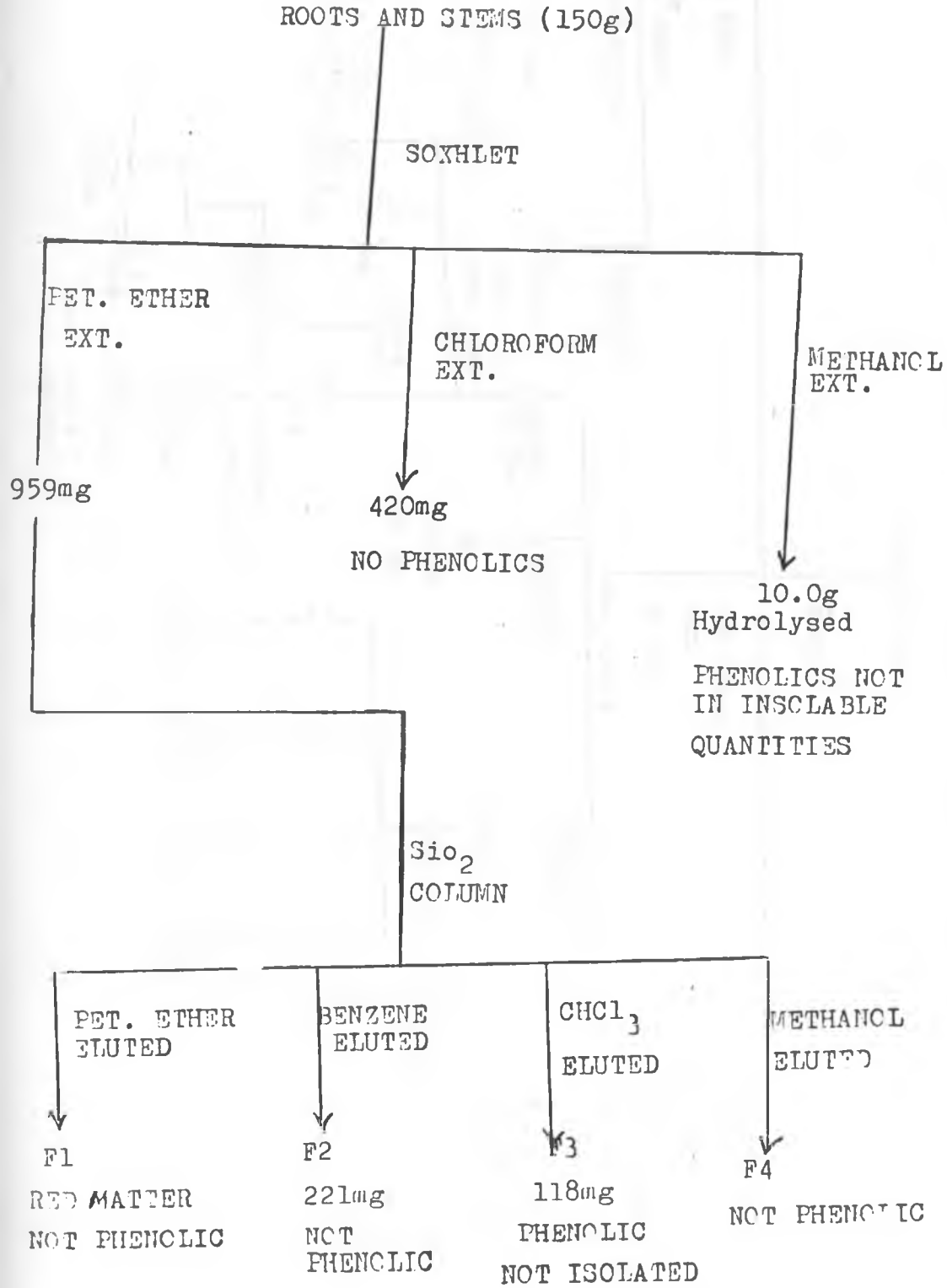
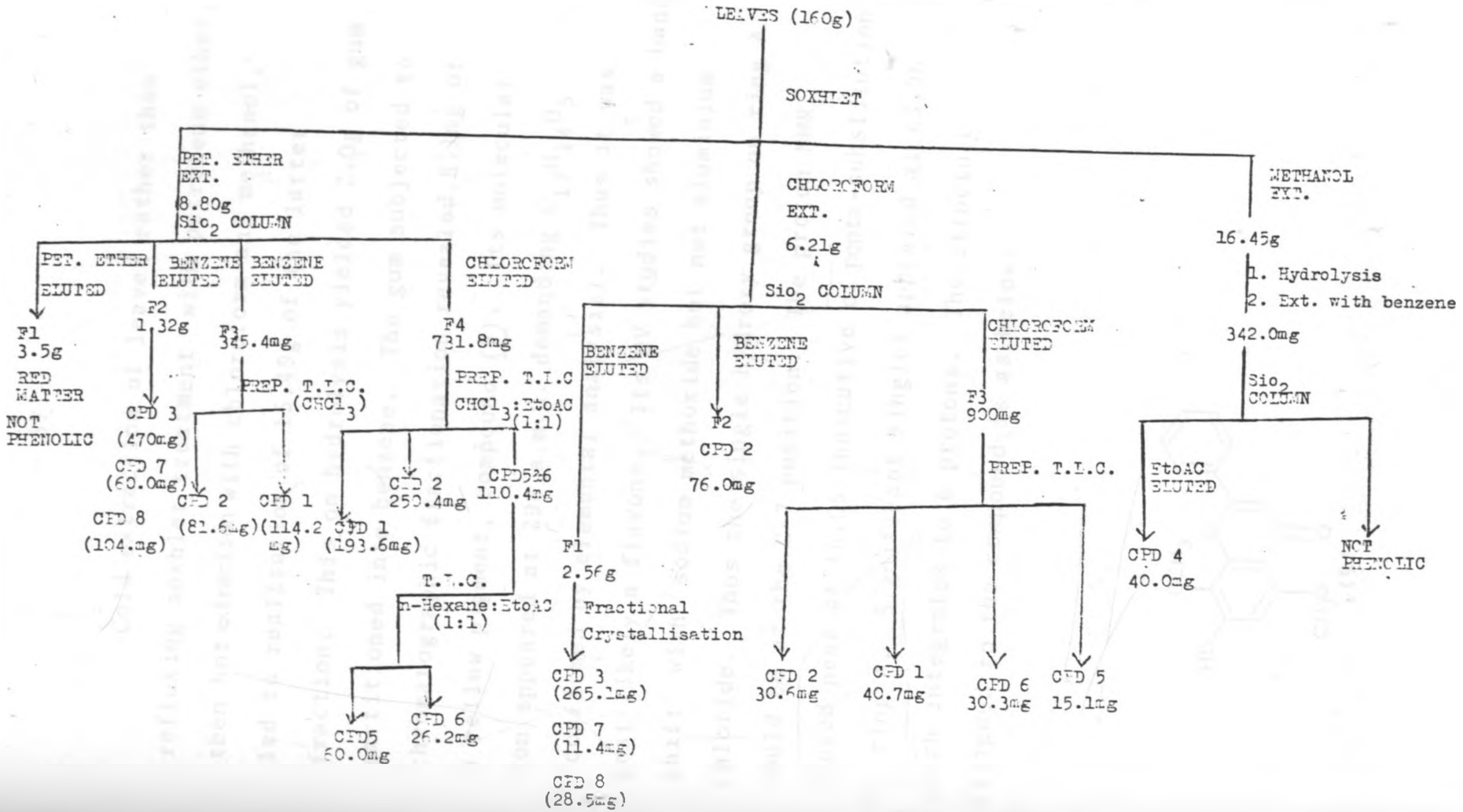
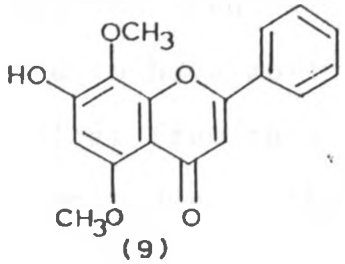


Figure 7: FRACTIONATION OF P. SENEGALENSE LEAVES FROM L. MALVASHA



Cold extraction of leaves rather than refluxing soxhlet treatment with petroleum ether, then hot extraction with chloroform and methanol, led to realisation of 15.49g of the latter fraction. This on hydrolysis yielded 2.0g of gum partitioned into benzene. The gum subjected to chromatographic fractionation revealed 8.1mg of a yellow pigment, compound (9). Its molecular ion appeared at 298 a.m.u. demanding $C_{17}H_{14}O_5$ (confirmed by elemental analysis). Thus it was most likely a flavone. Its UV studies showed a band I shift with sodium methoxide but not aluminium chloride. Thus the single hydroxy group on ring A could be at the C-7 position. The proton NMR showed peak at $\delta 6.05$ indicative of penta-substitution of ring A. Significant singlet appeared at $\delta 3.96$ which integrates to 6 protons. The structure assigned to the compound is as below:



(9)

CHAPTER 3

3.0 COMMENT AND CONCLUSION

The common phenolics, anthraquinones and flavonoids are present in Kenyan polygonum species sometimes in appreciable quantities. The anthraquinones are only observed in the two species which appear last in the key to species, that is P. pulchrum and P. setosulum in that order. Whether these are the only indigeneous members of the genus out of the eleven that contain anthraquinones was not determined but would definitely be of chemotaxonomic significance. All the four species researched contain at least trace amounts of flavonoids in all parts. P. senegalense leaf stands out as being particularly rich in chalcones and the related flavanones. Furthermore these chalcone derivatives are either tetra-oxygenated or tri-oxygenated only in the ring to which the carbonyl carbon is attached. The leaf and seed extract of this plant had been reported to have molluscicidal activity [51]. It is clear from this work that the gross activity is not only due to the two flavonoids previously reported but several such compounds. Further work on the plant, to characterise the glycoside

and molluscicidal testing of the pure compounds is necessary. Combination testing in the case of synergism is also needed.

Chalcones have wide ranging biological activities which include bacteriostatic and bacteriocidal [52], acaricidal, [53], anthelmentic [54] and fungicidal activities [55]. Therefore the economical use to which the waterside weed, P.senegalense may be put should be explored more vigorously.

CHAPTER 4

EXPERIMENTAL SECTION

4.1.0 GENERAL

Instruments

Melting points were determined using a Gallenkamp melting point apparatus and are uncorrected. The UV/vis spectra was determined using a Perkin-Elmer model Lambda 3 UV/vis spectrophotometer. Mass spectra were determined on a GC/MS Finnigan 1015D spectrometer. The infrared, proton and carbon-13 Nuclear magnetic Resonance (^{1}H NMR & ^{13}C NMR) spectra were obtained from the University of Maryland, U.S.A.

Materials

P.pulchrum was collected from Chiromo area of the University of Nairobi. The rest, P. senegalense, P.setosulum and P. salicifolium were collected from the Southern shores of L. Naivasha. The plant samples were positively identified at the Botany Department Herbarium, University of Nairobi. The plants were divided into roots, stems and leaves for purposes of study. They were immediately dried under sun-

lamp to avoid mould attack. The dried samples were then ground into fine powder before being extracted.

Chromatography

All the chromatographic solvents were purified by distillation before use. Column chromatography was performed using Merck silica gel (70-230 mesh A.S.T.M.). Solvent removal from fractions was carried out *in vacuo* with a rotatory evaporator. Merck silica gel suitable for thin layer chromatography (T.L.C.) was used for preparative T.L.C. with a glass slab holder. A slurry of silica gel in water was spread uniformly to a depth of 1-2 mm on a 20x20 cm plates. They were air dried for 24 hrs. followed by heating in an oven for 1½ hrs. at 120°C before before use. Commercial analytical Merck silica gel G plates were used for analytical T.L.C. and for monitoring the eluents. The solvent systems used were chloroform 100%, (solvent 1); n-Hexane: ethylacetate (1:1),(solvent 2); n-Hexane: ethylacetate: Acetic acid (75:20:5),(solvent 3); for analytical as well as preparative T.L.C.

4.2.0 PRELIMINARY TESTING

4.2.1 Extraction of P. Pulchrum roots for preliminary testing.

Dry powdered roots sample of mass 20g was extracted with 95% ethanol in a soxhlet extractor for 24 hrs. The extract was cooled and concentrated *in vacuo* using a rotatory evaporator leaving a thick brown syrup.

4.2.2 Test for the presence of anthraquinones in P. Pulchrum roots.

A small sample extract of the roots was shaken with 10mls of benzene and filtered. A solution of 5mls of 10% ammonium hydroxide was then added and the mixture shaken. A violet colour developed showing the presence of anthraquinones.

4.2.3 Test for the presence of alkaloids in P. Pulchrum roots.

A. A sample of mass 2g of roots extract was hydrolysed with 2M HCl solution by heating in a water bath for about 10 minutes and filtered after cooling. To 5mls of filtrate, a few drops of Mayer's reagent were added. Even a slight

turbidity which would be indicative of presence of alkaloids was not observed.

B. As a confirmatory test for absence of alkaloids, about 50mg of the root extract was heated with a mixture of chloroform: methanol (1:1) and the solution chromatographed on silica gel plates using the following solvent systems:

(a) Chloroform: methanol (9;1)

(b) Chloroform: Ethylacetate (8:2)

(c) Methanol: ammonia (100:3)

The chromatograms were then developed by spraying with freshly prepared Dragendorff's reagent. Presence of yellow spots on the plates would indicate presence of alkaloids. There were no yellow spots and hence a confirmation for absence of alkaloids.

4.2.4 Test for presence of sterols, triterpenes saponins in P. pulchrum roots.

(a) The green colouring matter was removed by washing severally about 1g of extract with petroleum spirit (40-60^{o}C). The residue was then extracted with 20mls of chloroform. To 5mls chloroform portion, 0.5mls acetic anhydride was added followed by 2 drops of conc. H_2SO_4.

A light green colour developed, confirming the presence of sterols and triterpenes in trace amounts.

(b) About 0.5g of extract was shaken with water. The appearance of froth which persists for more than half an hour showed the presence of saponins.

4.2.5 Test for presence of flavonoids in P. Pulchrum roots.

A mass of 1g of root extract was washed several times with petroleum ether (40-60^{o}C) to remove the lipid material. The residue was then taken up in 80% ethanol and the following experiments performed on the solution.

(a) To about 3mls of the solution, was added 4mls of 1% $Alcl_3$ solution. The yellow colour did not intensify showing the absence of flavonoids.

(b) To about 3mls of the solution was added 4mls of 1% KoH solution. The colour did not turn deep yellow confirming absence of flavonoids.

(c) To 2mls of the solution was added 0.5mls of conc. Hcl and a few magnesium turnings. The colour did not change to brick red, a further confirmation for absence of flavonoids.

Other parts of P. pulchrum and those of P. salicifolium, P. senegalense and P. setosulum were screened in a similar manner. The results are summarised in Table II.

4.3.0 LARGE SCALE EXTRACTIONS

4.3.1.1. Extraction of P. senegalense leaves

The dry powdered sample of mass 160g was fractionated between the solvents, petroleum ether (40-60^{o}C), chloroform and methanol respectively using a soxhlet extractor. The extraction with petroleum ether lasted for 12 hrs, when no colour could be identified by the sides of the extractor. This was then concentrated in vacuo using a rotatory evaporator leaving a sticky paste of mass, 8.8g. Chloroform was then introduced and the extraction took 27 hrs. The extract was concentrated in vacuo leaving a dark greenish mass, 6.21g. Finally, methanol was used and left extracting for a period of 2 days before complete extraction as seen by the sides of extractor. It was similarly concentrated to give a thick brown syrup of mass 16.45g. A thin layer chromatography (T.L.C.) of the petroleum ether and chloroform extracts using solvent 3,

showed two yellow spots and a greenish spot. On developing the T.L.C. plate with ammonia vapour, there was a mixture of colours developing. From yellow, the colour changed to orange and finally to three different overlapping colours: green, brown and blue. This indicated no separation of the compounds. Chloroform (100%), (solvent 2), was then tried as a solvent system. The samples were separated into four clear spots, the fourth spot, appearing only after developing the plate with ammonia vapour. There were three yellow compounds with R_f 0.34, 0.65 and 0.81. The fourth, which changed from colourles to blue on exposure of the T.L.C. plate to ammonia vapour had an R_f 0.14. The yellow compound with R_f 0.81 did not change colour on exposure to ammonia vapour. The spot with R_f 0.34, changed to green through orange, while that with R_f 0.65 changed to brown through orange. There was another UV-active compound slightly below the one which changed to blue on exposure of T.L.C. plate to ammonia vapour. Although the petroleum ether and chloroform extracts had the same compounds, they were treated to column chromatography separately because of column size restrictions.

4.3.1.2 Column chromatography of the petroleum ether extract of P.senegalense leaves.

A column was packed with 126g of silica gel in petroleum ether (40-60^{o}C). A mass of 8.8g was adsorbed on a small amount of silica gel and transferred to the column. Petroleum ether was the first solvent of elution. It eluted an orange-red coloured compound of mass 3.5g which did not respond to the preliminary tests carried before. It moved with the solvent front on application to a T.L.C. plate in all the solvents used. It was suspected to be a carotenoid and was not characterised. The yellow compound following it required another solvent to speed up the elution. Benzene was introduced and immediately eluted the yellow fraction. This fraction had three compounds, one yellow, the other two colourless and visible only after exposure to UV-light. This fraction was dissolved in chloroform and cooled in ice. Light yellow crystals separated out and were recrystallised from n-Hexane/chloroform mixture to give crystals of compound (7), 60mg, m.p. 96-8^{o}C. The compound showed ultraviolet absorption peaks in methanol at λ_{max} 284 nm and a shoulder at λ_{max} 320 nm. The infrared

spectrum showed peaks at 1570 and 1625 cm^{-1}. The mass spectrum had peaks at m/e values 270 (54.8), 193.2(100), 138.3(67.8), 95(95.5) and 77(52.4). The elemental analysis data was found to be C, 71.00; H, 5.30. $C_{16}H_{14}O_4$ requires C, 71.10; H, 5.22. The remaining yellow solution was fractionally crystallised from n-Hexane/ chloroform mixture to give monoclinic yellow crystals (3), 473.8mg, m.p. 89-90°C. The ultra-violet absorption spectrum of the compound had peaks at λ_{max} 230 sh and 337 nm. The infrared spectrum had peaks at 1575 and 1610 cm^{-1}. The mass spectrum peaks appeared at m/e values 284(30.7), 207(100.0), 181.2(39.0), 103.2(43.4), 95(26.1) and 77(64.5). Elemental analysis found: C,71.85; H, 17.60. $C_{17}H_{16}O_4$ requires: C,71.82; H,17.63. The filtrate was concentrated and crystallised from n-hexane to give colourless UV-active crystals (8), 104.0mg, m.p. 110-111°C. The ultraviolet spectrum of the compound in methanol showed peaks at λ_{max} 284 and 320 sh. nm. The in-frared spectrum had absorption peaks at 1570 cm^{-1} and 1625 cm^{-1}. The mass spectrum had peaks at m/e values 270 (63.4), 193.2(100.0), 166.1(82.1), 138.1(56.5), 95.1(79.0) and 77(43.5). Elemental analysis found: C, 71.00; H,5.30. $C_{16}H_{14}O_4$ requires: C,71.10;H,5.22.

There was another yellow band following this one, just eluted. It was removed from column with benzene. This fraction was active towards 1% KOH solution. It had two major compounds from the analytical T.L.C. Four preparative T.L.C's using solvent 1 were recommended. The process gave two compounds, one with the high R_f 0.65 and changing from yellow to brown on exposure of the T.L.C. plate to ammonia vapour. This was re-crystallised from benzene to give orange crystals (2), 81.6mg, m.p. 142-144°C. The ultraviolet spectrum of the compound in methanol had peaks at λ_{max} 232 and 335 nm. The infrared spectrum had absorption peaks at 1560 and 1620 cm^{-1}. The mass spectrum showed peaks at m/e values 300(39.0), 196(45.8), 181(100.0), 153(41.7), 77.1(38.3) and 69.0(64.0) The elemental analysis found: C,70.01; H,5.39. $C_{17}H_{16}O_5$ requires: C,69.99; H,5.37. The other yellow compound of lower R_f 0.34 and turning to green through orange on exposure to ammonia vapour was similarly re-crystallised from benzene to give yellow crystals (1), 114.2mg, m.p. 104-106°C. The ultraviolet absorption spectrum of the compound in methanol had a peak at λ_{max} 342 nm. The infrared spectrum showed the significant peaks at 1580 and 1618 cm^{-1}. The mass spectrum had peaks at m/e values 300(70.0), 181(100.0),

270(23.1), 223(33.0), 197(22.8), 195(70.9), 193(40.0), 167(65.9), 153(68.5), 139.(33.2) and 77(65.2). The elemental analysis found: C,67.92; H,5.40. $C_{17}H_{16}O_5$ requires: C,67.99: H, 5.37.

At this stage, another solvent was introduced to increase the rate of elution of the yellow band. Chloroform, however, brought out two more compounds in addition to the yellow band which was being collected. Compound changing to blue (6) on exposure to ammonia vapour and the UV-active one just below it on T.L.C. plate (5), were also eluted. A preparative T.L.C. using solvent 1, separated the compounds (1), 193.6mg and (2), 250.4mg. The other two compounds were separated using solvent 2, [n-hexane: ethylacete (1:1)] on a preparative T.L.C. to give colourless crystals, re-crystallised from benzene (5), 60mg m.p. 146-148°C. The ultraviolet spectrum of the compound in methanol had peaks at λ_{max} 290nm and a shoulder at λ_{max} 325nm. The infrared absorption spectrum showed peaks at 1580 and 1660 cm^{-1}. The mass spectrum had peaks at m/e values 300(47.3), 196(100.0), 181(63.4), 153(49.6), 139.(34.2), 103(20.5), 77(25.5), and 69.1(34.8). The elemental analysis found:

C,68.1;H,5.30. $C_{17}H_{16}O_5$ requires: C,67.99;H,5.37. The other colourless compound separated from this preparative T.L.C. was similarly re-crystallised from benzene to give (6), 20.4mg, m.p. 133-135°C.. The ultraviolet spectrum of the compound showed absorption peaks λ_{max} 290 nm and a shoulder at λ_{max} 330nm. The infrared spectrum had one significant peak at 1635 cm^{-1}. The mass spectrum had peaks at m/e values 316(100.0), 210(100.0), 167(31.5), 117.0(26.1), 107(27.8), 105(22.5), 79(76.9), 77(68.9), 69(92.7) and 55(54.7). The elemental analysis found: C,64.52;H,5.16. $C_{17}H_{16}O_6$ requires: C,64.55;H,5.10. The yellow fraction which followed, on further elution using chloroform gave (6), 5.8mg from a preparative T.L.C. using solvent 2, and other inactive impurities. Methanol did not elute any compound of interest.

4.3.1.3 Column chromatography of chloroform extract of P.senegalense leaves.

A column was packed with 120g of silica gel in petroleum ether (40-60°C). A chloroform extract of mass 6.21g was transferred to the column. This extract had been previously

adsorbed onto a small amount of silica gel. Elution was started with petroleum ether but did not remove any compound. Benzene was then introduced to speed up the movement of the compounds in the column. This solvent eluted a yellow band of mass 2.6g. This fraction was fractionally crystallised to give the compounds (3), 265.1mg; (7), 14.4mg and (8), 28.4mg. The fraction was followed by another yellow band which gave a yellow colour intensification with the 1% KoH solution. An analytical T.L.C. of this fraction using solvent 1, showed the presence of only one pure compound which changed to brown on exposure to ammonia vapour. The same compound had been isolated from the petroleum ether extract It was re-crystallised from benzene to give orange crystals (2), 76mg.

At this stage, the solvent was changed to chloroform. Part of this compound (2), was mixed up with another yellow band which was following it in the column. The fraction eluted was then treated to a prep. T.L.C. using solvent 1, to give two clearly separated compounds (1), 40.7mg and (2), 30.6mg. The lower UV-active band was treated to another preperative T.L.C. using solvent 2, to give compounds (5), 10.1mg and (6), 20.0mg. On further

elution with chloroform, more of (5) and (6) were eluted, being isolated by a prep. T.L.C. using solvent 2, to give (5), 10.3mg and (6), 5.0mg. All the other eluents that followed on changing the solvent to methanol were discarded since they contained no compounds of interest from the analytical T.L.C.

4.3.1.4 Column chromatography of methanol hydrolysate of P.senegalense leaves

The methanol extract of mass 16.45g was first hydrolysed by refluxing with 120mls of 2MHCl solution for 2 hrs. before extracting with benzene. The extract weighing 342mg was then transferred to a column packed with 30g of silica gel. The analytical T.L.C. of the sample showed one compound of interest with R_f value just the same as compound (5) in solvent 1. This compound was colourless, but visualised only after exposure to UV-light. It was eluted by ethyl acetate after running petroleum ether, benzene and chloroform respectively through the column. The compound (4), 40mg, m.p. 213-215^{o}C, was re-crystallised from benzene-ethyl acetate. The ultraviolet absorption spectrum in methanol had peaks at λ_{max} 280nm and a small shoulder at λ_{max} 310nm. The mass spectrum

had peaks at m/e values 270(26.81), 166(100.0), 138(47.2), 95(20.8), and 69(41.8).

4.3.1.5 Cold extraction of P. senegalense leaves using petroleum ether (40-60^{o}C).

A powered sample of mass 170g was extracted repeatedly using petroleum ether by leaving the leaves poweder covered with solvent for several days. This method was to ensure that any labile compounds that might have decomposed during the high temperature soxhlet extraction can be isolated. The extract weighed 5.73g after concentrating *in vacuo* using a rotatory evaporator. The most conspicuous point to note from this method was that no greenish colouring matter was extracted on first extraction and therefore the compounds of interest were clearly observed on the analytic T.L.C. Compound (4), was however, not observed.

The sample was thereafter extracted with chloroform and methanol using sochlet

extractor to ensure exhaustive extraction. This method gave a mass of 2.45g for chloroform extract and 15.49g for methanol extract. An analytical T.L.C. of the chloroform extract gave the same compounds present in the pet. ether extract, but with heavy greenish material. Qualitatively the cold extract was quite similar to soxhlet extract.

4.3.1.6 Column chromatography of methanol hydrolysate of P.senegalense leaves (From cold extraction).

The thick brown syrup of methanol extract of mass 15.49g was transferred to a 250mls distillation flask. This was hydrolysed by refluxing with 120mls of 2MHCl. for 2hrs. A heavy precipitate of dark brown material formed during the hydrolysis. After cooling, the hole material was extracted with benzene. The benzene extract was concentrated to give a mass of 2.00g. An analytical T.L.C. using solvent 1, showed the presence of only one compound, which had the same R_f as compound (1).

It also gave similar colour reaction on exposure to ammonia vapour, although the greenish colour developed was light. The compound was isolated by a preparative T.L.C. using solvent 1, giving light yellow crystals (9), 8.1mg, m.p. 194-196°C. The ultraviolet spectrum of the compound had peaks at λ_{max} 284, 290 and 340nm. The infrared spectrum had absorption peaks at 1585, 1635 and 3480 cm^{-1}. The mass spectrum had peaks at m/e values 298(12.5), 167(44.9), 115(21.98), 69.2(100.0). The elemental analysis found: C,68.40; H, 4.75. $C_{17}H_{14}O_5$ requires: C,68.45;H,4.73.

4.3.2.0 Extraction of P.senegalense roots and stems.

A powdered sample of mass 150g of combined roots and st ms was extracted with soxhlet extractor using the solvents, petroleum ether (40-60°C), chloroform and methanol respectively. The extraction with petroleum ether lasted for about 6 hrs. and after cooling, was concentrated in vacuo using a rotatory

evaporator leaving a mass of 0.96g. Chloroform was then introduced and took 12 hrs. for complete extraction. On concentration left a mass of 0.42g. Methanol extraction lasted for 1 day and left a mass of 10.00g on concentrating using a rotatory evaporator. From the analytical T.L.C. using solvent 1, the chloroform extract had practically no compound. The whole plate (T.L.C.) had green coloured spots. The petroleum ether extract had one compound showing a yellow colour intensification on exposure of the T.L.C. plate to ammonia vapour, but this compound was in trace amounts. It was colourless initially and this test showed presence of flavonoids. The methanol extract was hydrolysed, extracted with benzene and an analytical T.L.C. performed on the concentrated fraction using solvent 1. A trace of the compound appearing in the pet. ether extract was observed.

4.3.3.0 Extraction of P.pulchrum roots

A powdered sample of roots of total mass 1.07kg was extracted with a soxhlet extractor using the solvents, petroleum ether (40-60°C), chloroform and methanol respectively. Pet. ether was the first solvent of extraction and took 19 hrs. The cold extract was concentrated *in vacuo* using a rotatory evaporator to give a mass of 1.99g. Chloroform was then introduced and extracted for 16 hrs. The extract was concentrated *in vacuo* using a rotatory evaporator giving a dark green mass of weight 3.06g. Methanol was finally introduced and extraction lasted for 25 hrs. The extract was concentrated using a rotatory evaporator to give a dark brown syrup of total mass 67.1g. The petroleum ether and chloroform extracts were spotted on an analytical T.L.C. using solvent 3, [n-Hexane: ethylacetate: acetic acid (75:20:5)], along with the anthraquinones standards, emodin (10), physcion (11), and chrysophanol (12). The R_f values in the above solvent system were 0.28, 0.56 and 0.64. The T.L.C. showed presence of the above anthraquinone

standards and therefore were separated by column chromatography. The methanol extract after hydrolysis, extraction with benzene, showed the presence of the same anthraquinones using solvent 3, but in trace amounts.

4.3.3.1 Column chromatography of petroleum ether (40-60°C) extract of P.pulchrum roots.

A sample of mass 1.99g was adsorbed onto silica gel before being transferred to a column packed with 50g of silica gel under petroleum ether, petroleum ether was then introduced as the eluting agent. This eluted a red material, which was not active towards 1% KOH solution. The solvent was then changed to benzene and eluted a yellow fraction which showed a violet colouration with 1% KOH solution. An analytical T.L.C. of the fraction against the standard anthraquinones, emodin, physcion and chrysophanol, showed all the three were present. They were isolated by a preparative T.L.C. using solvent 3, giving emodin (10), 10.0mg, m.p. 257-258°C; physcion (11), 5.0mg, m.p. 204-205°C and chrysophanol (12), 10.0mg, m.p. 197-8°C. Their identities were confirmed by performing mixed melting points with authentic samples of the compounds isolated

from Rumex spp. in our laboratory. Benzene was used as the next eluting solvent for sometime but could not appreciably move the next yellow compound and so was changed to chloroform. Chloroform eluted the third fraction which contained emodin. This was isolated by a preparative T.L.C. using solvent 3, to give (10), 10.5mg. Chloroform did not elute any other compound. The solvent was changed to methanol, but could not elute any other phenolic compound.

4.3.3.2 Column chromatography of chloroform extract of P.pulchrum roots.

A mass of 3.60g of chloroform extract was transferred to a column packed with 80g of silica gel. The extract had been adsorbed on a small amount of silica gel before being transferred to the column. The analytical T.L.C. of this sample using solvent 3, indicated the presence of only one compound, emodin, the rest of the material was green. Physcion and chrysophanol were in trace amounts. After passing 1.5 litres each, of petroleum ether and benzene through the column, chloroform was introduced. This eluted the yellow fraction (166mg), which contained emodin. A prep.T.L.C. using solvent 3, led to the

isolation of emodin, 5.5 mg. The rest of the material eluted using methanol had no phenolic compounds.

4.3.3.3 Hydrolysis and chromatography of methanol extract of P.pulchrum roots

The whole extract of mass 13.41g was hydrolysed by refluxing with 140mls of 2MHCl solution for 2hrs. This hydrolysate was cooled and extracted with benzene. The benzene extract was concentrated and checked for presence of the anthraquinones appearing in the pet. ether and chloroform extracts. The mass of the material extracted was 172.4mg but had no isolable compounds.

4.3.4.0 Extraction of P.pulchrum stems

A powdered sample of mass 800g was extracced with a soxhlet extractor using petroleum ether chloroform and methanol respectively. The petroleum ether extraction lasted for 12hrs., chloroform for 8hrs. and methanol for 2 days. Each of the fractions were concentrated separately using a rotatory evaporator, dried and weighed. The petroleum ether extract weighed 1.87g, chloroform, 1.6g and the methanol extract 50.6g. An analytical T.L.C. of pet. ether and

chloroform extracts against the anthraquinone standards showed the presence of emodin alone in the extracts. The fractions were combined and handled together. The methanol extract after hydrolysis and extraction with benzene did not have any isolable amounts of phenolic compounds.

4.3.4.1 Column chromatography of the petroleum ether and chloroform extracts of P.pulchrum stems.

With the idea that we have only one compound in this fractions, the combined extracts of mass 3.47g was adsorbed on a small amount of silica gel before being transferred to a column packed with 80g of silica gel in petroleum ether (40-60^{o}C). Pet. ether was the first solvent to be introduced in the column for elution. It eluted some yellowish material, (307.4 mg) which did not intensify with 1% KOH solution. It was moving with the solvent front. No other compound came out of the column with this solvent, so benzene was introduced. This one also eluted some fraction which was not handled further, as there were no compounds of interest. Chloroform was introduced and eluted impure

emodin (311.2mg). The impurities were separated by a prep. T.LC. using solvent 3 to give pure emodin, (11.2mg). No other fraction from this column gave a yellow colour intensification with 1% KoH solution, on changing eluting solvent to methanol.

4.3.5.0 Extraction of P.pulchrum leaves

A powdered sample of mass 131.5g was extracted with a soxhlet extractor using the solvents, petroleum ether (40-60^{o}C), chloroform and methanol respectively. Petroleum ether extraction lasted for 33 hrs, chloroform for 12 hrs. and methanol for 24 hours. Each fraction was concentrated separately in vacuo using a rotatory evaporator, dried and weighed. The petroleum ether extract weighed 2.61g, chloroform extract 2.36g and methanol extract 16.74 g. After application of petroleum ether and chloroform extracts on an analytical T.L.C. using solvent 3, there was no visible compound on developing the T.L.C. plate with ammonia vapour. The extract did not respond to tests for presence of anthraquinones and flavonoids. There were trace amounts of anthraquinones in the methanol hydrolysate.

4.3.6.0 Cold extraction of P.pulchrum leaves

This was a repeat of the leaves of P.pulchrum but without the use of soxhlet extractor. The leaves (200g) were repeatedly extracted with petroleum ether (40-60^{o}C) to give a mass of 0.679g after concentrating in vacuo using a rotatory evaporator. The sample was dried and then chloroform introduced. The chloroform extract was concentrated to give a mass of 0.991g. Methanol was then finally used for the cold extraction. Apart from the anthraquinones previously isolated, another colourless compound, 60.0mg, m.p. 135-136^{o}C was isolated from the chloroform extract. The compound was eluted after passing 1.5 litres of pet.ether through the column packed with 60g of silica gel, followed by benzene at a very slow rate to effect complete separation. The spot tests showed that it was a triterpene.

4.3.7.0 Extraction of P.setosulum roots and stems.

The roots and stems were combined since in this plant species, the roots were less developed and it was hard to separate the two parts. A total mass of 150g of powdered sample was extracted

using the solvents, petroleum ether (40-60°C), chloroform and methanol respectively, in a soxhlet extractor. The extraction with pet.ether took 5 hrs. It was concentrated in vacuo using a rotatory evaporator to give a mass of 3.27g. Chloroform extraction lasted for 8 hrs. and on concentrating it gave a mass of 0.646g. Methanol was finally introduced and extraction lasted for 12 hrs. On concentrating, it gave a syrup of mass 14.6g. From the analytical T.L.C. of the petroleum ether and chloroform extracts using solvent 3, they showed similar compounds and were therefore combined. One compound behaving as emodin on T.L.C. plate was observed on developing the plate with ammonia vapour.

4.3.7.1 Column chromatography of petroleum ether and chloroform extracts of P.setosulum roots and stems.

A total mass of 3.916g was adsorbed on a small amount of silica gel and transferred to a column packed with 60g of silica gel. Pet.ether was introduced as solvent of elution. It eluted a yellow band immediately and on concentrating, left behind a red powder which could not crystallise.

The solvent was changed to benzene and the first fraction eluted with it contained only greenish material. The four consecutive fractions that followed after elution with benzene, followed by chloroform contained emodin. These were combined to give a mass of 0.326g and on application to a preperative T.L.C. using solvent 3, emodin (11.3 mg) was isolated. The other fractions were not phenolic.

4.3.7.2 Hydrolysis and chromatography of methanol extract of P.setosulum roots and stems.

A mass of 14.58g was put in a flask and hydrolysed with 140mls of 2MHCl solution, by refluxing for 2 hr. The mixture was cooled and extracted with benzene. The benzene extract was concentrated and spotted on an analytical T.L.C. using solvent 3 against the anthraquinone standards; emodin, physcion and chrysophenol. On developing the T.L.C. plate with ammonia vapour, the three standards were all present in the extract, plus a flavonoid. The flavonoid was in trace amounts. The benzene extract (217.5mg) was extracted with 1% KOH solution, acidified and re-extracted with chloroform to give yellow phenolic matter of mass 51.0mg. This was then put on a

preperative T.L.C. using solvent 3, to give emodin 18.6mg, physcion, 1.0mg and chrysophanol, 1.6mg.

4.3.8.0 Extraction of P.setosulum leaves.

A powdered sample of mass 100g was extracted with a soxhlet extractor using the solvents, petroleum ether (40-60^{o}C), chloroform and methanol respectively. Pet.ether extraction lasted for 8 hrs. and on concentrating left a solid mass of weight 1.87g. Chloroform extraction lasted for 10 hrs. leaving a green mass of weight 1.43g on concentrating. Methanol was finally used and extraction lasted for 12 hrs. On concentrating, the cold extract left a mass of 14.88g. From the analytical T.L.C. of the pet. ether and chloroform extracts, using solvent 3, showed they had similar compounds and were therefore combined. There were two visible yellow compounds from the T.L.C. plate, non of them being an anthraquinone as the were responding differently towards 1% KoH solution giving a yellow colour intensification. One of the compounds had the same R_f value as emodin, but the other had a slightly lower R_f value than physcion. They were suspected to be flavonoids.

3.8.1 Column chromatography of petroleum ether and chloroform extracts of P.setosulum leaves.

A combined mass of 3.30g of pet.ether and chloroform extracts was adsorbed on silica gel before being transferred to a column packed with 60g of silica gel. petroleum ether eluted a yellow material which did not respond towards 1% KoH solution. On introduction of benzene, came a dark green band which had no other compound from T.L.C. analysis using solvent 3. After this band, came another yellow band with further elution using benzene. This fraction gave a yellow colour intensification with 1% KOH solution (0.0079g). An analytical T.L.C. of this fraction using solvent 3, showed the presence of two yellow compounds which were showing a yellow colour intensification with ammonia vapour. The other eluents on changing the solvent to chloroform and methanol, showed no compounds from the analytical T.L.C. The two flavonoids were in trace amounts and were not isolated.

4.3.8.2 Hydrolysis and chromatography of methanol extract of P.setosulum leaves.

A mass of 14.9 was hydrolysed by refluxing with 140ml of 2MHCl for 2 hrs. It was cooled and extracted with benzene. The benzene extract was concentrated and spotted on an analytical T.L.C. using solvent 3. This gave the same compounds identified in the petroleum ether and chloroform extracts. In addition, there were trace amounts of emodin observed on exposure of the T.L.C. plate to ammonia vapour. A column chromatography of the benzene extract (0.490g) using 60g of silica gel left a red matter (not phenolic), a yellow phenolic matter (0.26g) and finally some dirty non phenolic matter, coming out of column with ethyl acetate. A prep. T.L.C. of yellow phenolic matter using solvent 3, gave a yellowish compound (5.0mg), which was not characterised.

4.3.9.0 Extraction of P.salicifolium roots and stems.

A powdered sample of mass 100g was extracted with a soxhlet extractor using the solvents, petroleum ether (40-60^{o}C), chloroform and methanol respectively. Pet.ether extraction lasted for a period of 5 hr and on concentrating in vacuo

using a rotatory evaporator, left a mass of 0.40g. Chloroform was then introduced and extraction left for 5 hr. The concentrated sample weighed 0.587g. Methanol was finally used for a period of 8 hr. and on concentrating left a mass of 10.0g. From the analytical T.L.C. of the pet. ether and chloroform extracts, they showed similar compounds and were therefore combined. Only one compound was responding positively towards the test for presence of flavonoids. It was however not in isolable amounts.

43.9.1 Hydrolysis and chromatography of methanol xtract of P.salicifolium roots and stems.

A mass of 10.0g of methanol extract was hydrolysed with 140mls of 2MHCl solution by refluxing for 2 hrs. The cold sample was extracted with benzene and concentrated to give a mass of 0.34g. A T.L.C. of this sample using solvent 1, showed the presence of the two flavonoids appearing in the methanol hydrolysate of P.setosulum. After a column chromatography using silica gel, the fraction containing the two compounds was too small to characterise - a mass of 11.0mg with five compounds from the

T.L.C. analysis.

43.10.0 Cold extraction of P.salicifolium leaves

A sample of fresh leaves of P.salicifolium was extracted using cold petroleum ether (40-60°C) for about a week; replacing the pet. ether with fresh amount after everyday. The extract was completely green and on concentrating and spotting on an analytical T.L.C. using solvent 1, there was only one compound responding positively to the flavonoid test. This compoud was colourless but showed a yellow spot on exposure of the T.L.C. plate to ammonia vapour. This was however, different from the yellow compounds showing yellow colour intensification on exposure of the T.L.C. plate to ammonia vapour in the methanol hydrolysate of roots and stems of P.salicifolium. It was however, in trace amounts and therefore not isolated.

4.4.0 Acetylation of 2'3'-dihydroxy-4',6'-dimethoxy chalcone (1)

A sample of mass 100mg was placed in 100mls RB flask containing 4 mls of freshly distilled acetic anhydride and 4mls of dry pyridine. It was then left standing at room temperature.

After 10 minutes, the reaction mixture had changed from nearly orange to colourless. This was then poured in ice cold water with stirring and filtered immediately. It had been noticed that if left for sometime at this stage, the initial colour of reactants regenerates. The residue was dissolved in diethyl ether, dried and concentrated. The resulting solid material was crystallised from n-Hexane/chloroform mixture by cooling in ice: 85.3mg, m.p. 112-113°C. The mass spectrum had significant peaks at m/e values 384(2.8), 196(19.0) and 43.0(100.0).

4.5.0 Acetylation of 2',6'-dihydroxy-3',4'-dimethoxy chalcone, 2.

A mass of 100mg of sample was put in 100ml RB flask containing 4ml of freshly distilled acetic anhydride and 4ml of dry pyridine. It was then, left standing at room temperature. The reaction was almost complete after only 10 minutes. This was then poured into ice cold water with stirring and immediately filtered. The residue was dissolved in diethyl ether, dried with magnesium sulphate and concentrated using a rotatory evaporator to give a solid mass. This mass was crystallised from n-Hexane/chloroform

to give colourless crystals: 62.4mg, m.p. 115-116^{o}C. The mass spectrum of the compound had peaks at m/e values 384(2.7), 342(11.1), 196.1 (23.7) and 43.0 (100.0).

4.6.0 A cetylation of 2'-hydroxy-4',6'-dimethoxy Chalcone, 3.

A mass of 203.4mg of the sample was put in 100mls RB flask containing 8ml of freshly distilled acetic anhydride and 4ml of pyridine. The mixture was then left standing at room temperature. The reaction for this compound was quite slow and it took upto 1hr. to observe a noticable colour change. The mixture was then poured into ice cold water with stirring and immediately filtered. The residue was dissolved in diethyl ether, dried with magnesium sulphate and concentrated to give a solid mass. This mass was crystallised from n-Hexane/chloroform mixture to give colourless crystals: 137.2mg, m.p. 132-134^{o}C. The mass spectrum had significant peaks at m/e values 326(7.0), 283(22.1), 207.1 (63.9), 181.2(22.3) and 43.1(100).

Table III. NMR chemical shift positions for 2',3'-dihydroxy-4',6'-dimethoxy chalcone, 1

Position of C	$\delta^{13}C$ NMR	$\delta^{1}H$ NMR
1'	106.5	-
2'	159.1	-
3'	130.1	-
4'	158.9	-
5'	89.9	6.04
6'	159.1	-
Keto	193.3	-
α	127.5	7.72d J,16.3
β	142.6	7.84d J,16.3
1	135.5	-
2	128.9	7.38
3	128.4	7.57
4	130.1	7.39
5	128.4	7.57
6	128.9	7.38
4'-OCH_3	56.0	3.88
6'-OCH_3	60.9	3.90

Table IV. NMR chemical shift positions for 2', 6'-dihydroxy-3',4'-dimethoxy chalcone 2.

C	$\delta^{13}C$ NMR	$\delta^{1}H$ NMR
1'	105.3	-
2'	159.2	-
3'	135.1	-
4'	160.1	-
5'	91.8	6.08s
6'	159.2	-
keto	192.7	-
α	127.6	7.84d J, 16
β	142.1	8.13d J, 16
1	135.1	-
2	129.1	7.64
3	128.4	7.62
4	130.4	7.63
5	128.4	7.62
6	129.1	7.64
3'-OCH_3	60.4	3.89
4'-OCH_3	55.9	3.84
-OH	-	10.6

Table V. NMR chemical shift positions for 2'-hydroxy-4', 6'-dimethoxy chalcone 3

Position of C	$\delta^{13}C$ NMR	$\delta^{1}H$ NMR
1'	106.3	-
2'	168.4	-
3'	93.8	6.09dJ, 2.0
4'	162.5	-
5'	91.3	5.95dJ, 2.0
6'	166.2	-
keto	192.6	-
α	127.5	7.77dJ,15.8
β	142.3	7.89dJ,15.8
1	135.5	-
2	128.8	7.58m
3	128.3	7.38m
4	130.0	7.39m
5	128.3	7.38m
6	128.8	7.58m
4'-OCH_3	55.8	3.82s
6'-OCH_3	55.8	3.90s
-OH		10.22s

Table VI. NMR chemical shift positions for 7-hydroxy-5-methoxy flavanone 4.

Postion of C	$\delta^{13}C$ NMR	$\delta^{1}H$ NMR
2	-	3.03dd
3	-	2.77m
		2.76m
keto	-	-
5	-	-
6	-	6.05d
7	-	-
8	-	6.07d
1'	-	
2'	-	
3'	-	
4'	-	7.27m
5'	-	
6'	-	
5-OCH3	-	3.88s
-OH	-	-

TABLE VII. NMR chemical shift positions for 7- hydroxy-5,8-dimethoxy flavanone 5.

Position of C	$\delta^{13}C$ NMR	$\delta^{1}H$ NMR
2	76.7	5.48dd, J3.4 & 12.5
3	75.4	2.9dd
4	189.0	-
4a	106.5	-
5	155.3	-
6	92.2	6.18s
7	157.5	-
8	155.4	-
8a	147.5	-
1'	138.7	-
2'	125.9	7.4m
3'	128.8	7.4m
4'	128.6	7.4m
5'	128.8	7.4m
6'	125.9	7.4m
5-OCH_3	61.5	3.8.
8-OCH_3	56.2	3.85s

TABLE VIII. NMR chemical shift positions for 3,7-dihydroxy-5,8-dimethoxy flavanol 6

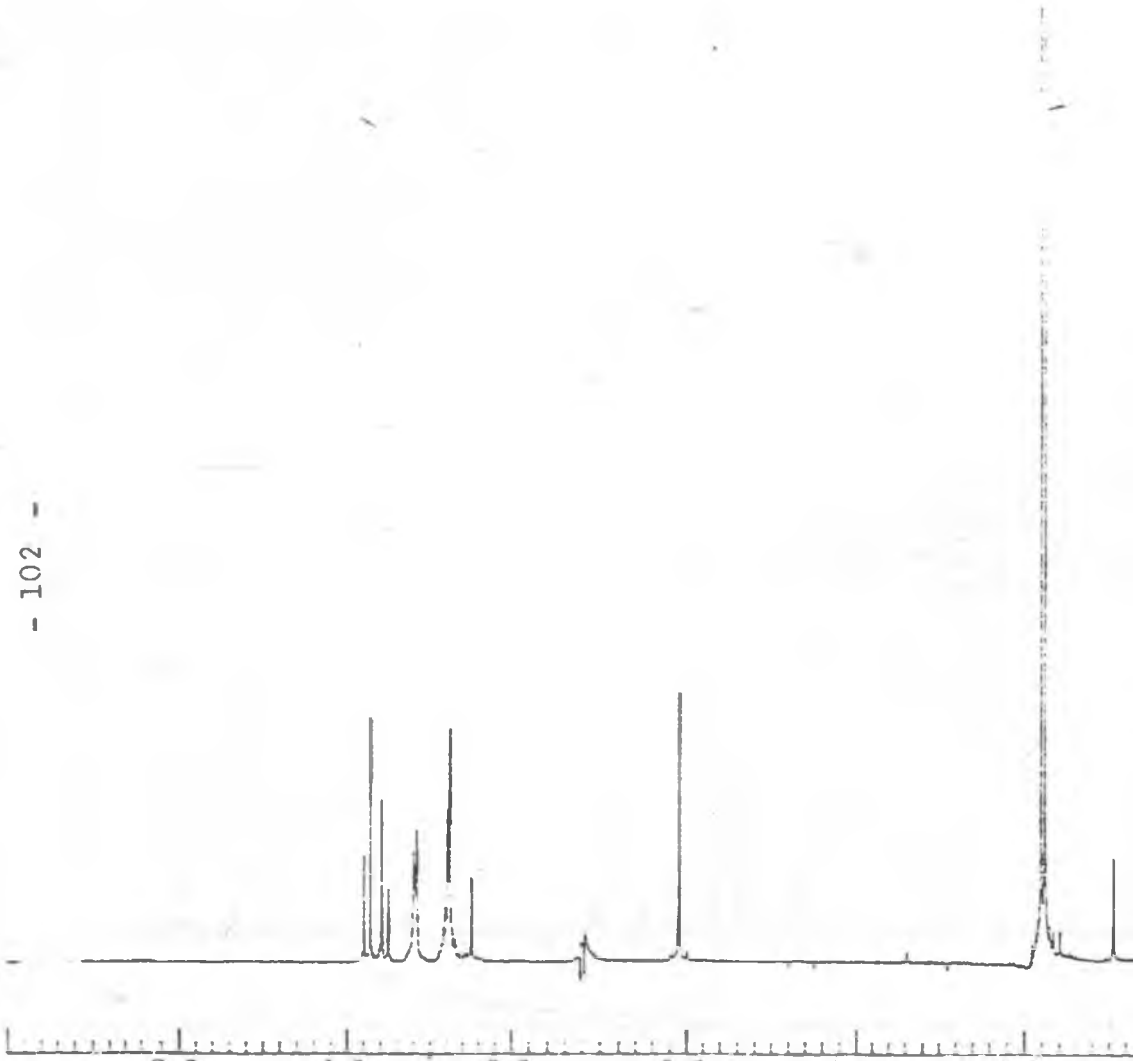
Position of C	$\delta^{13}C$ NMR	$\delta^{1}H$ NMR
2	72.5	5.2dJ,13.0
3	68.0	4.4dd,J,5.3 & 13.0
4	199.1	-
4a	158.4	-
5	156.8	-
6	94.3	6.1s
7	158.0	-
8	159.0	-
8a	158.4	-
1'	140.0	-
2'	125.0	7.4m
3'	129.8	
4'	127.5	
5'	129.8	
6'	125.0	
5-OCH_3	61.0	3.97s
8-OCH_3	51.2	3.98s

Table IX. NMR chemical shift positions for 5-hydroxy-7-methoxy flavanones, 7 & 8

Position of C	δ^{13}C NMR	δ^{1}H. NMR
2	79.2	5.47dd J,3,4 & 12.3
3	43.4	3.0m
4	195.8	-
4a	103.2	-
5	164.2	-
6	94.3	-
7	168.0	-
8	95.2	-
8a	162.8	-
7-OCH_3	55.7	3.70s
-OH		9.06s
1'	138.4	
2'	126.1	
3'	128.9	
4'	128.9	7.4m
5'	128.9	
6'	126.1	

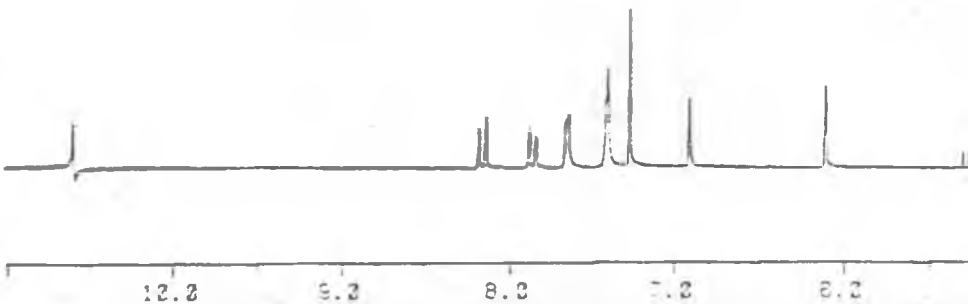
Table X. NMR chemical shift posiitions for 7-dydroxy-5,8-dimethoxy flavone 9

Position of C	$\delta^{13}C$ NMR	$\delta^{1}H$ NMR
2	158.1	-
3	137.4	
4	185.9	-
4a	103.5	-
5	157.5	-
6	94.3	6.05
7	157.3	-
8	157.5	-
8a	155.3	-
1'	129.8	-
2'	126.1	7.49m
3'	128.8	7.45m
4'	129.7	7.45m
5'	128.8	7.45m
6'	126.1	7.49m
5-OCH_3	61.0	3.96s
8-OCH_3	55.7	3.7s

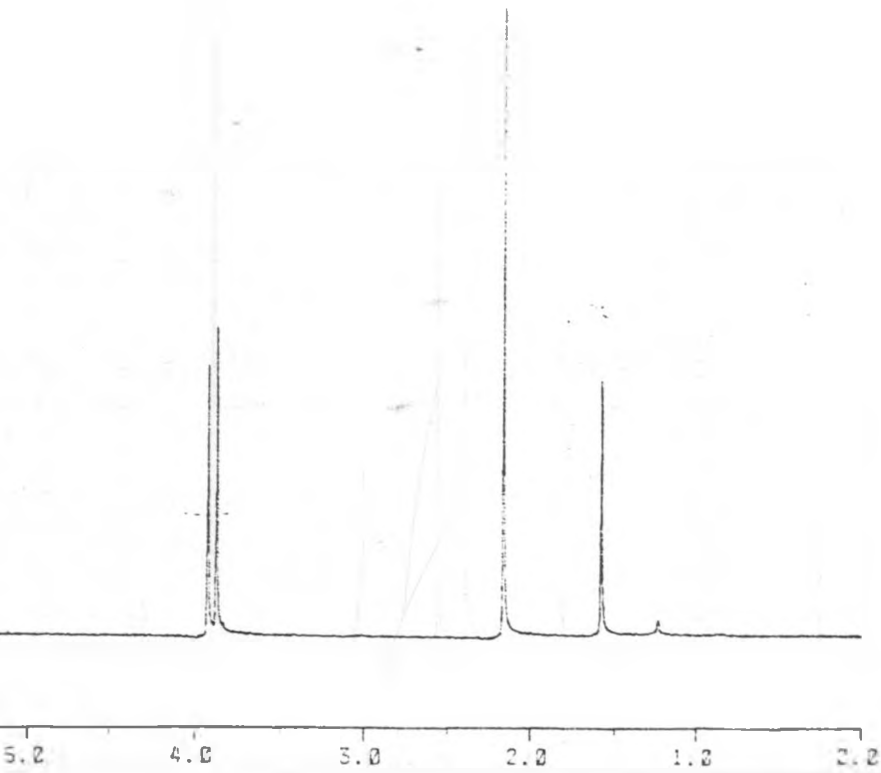


Compound 1


3.0
2.0
1.0
0.0
-1.0



Compound 2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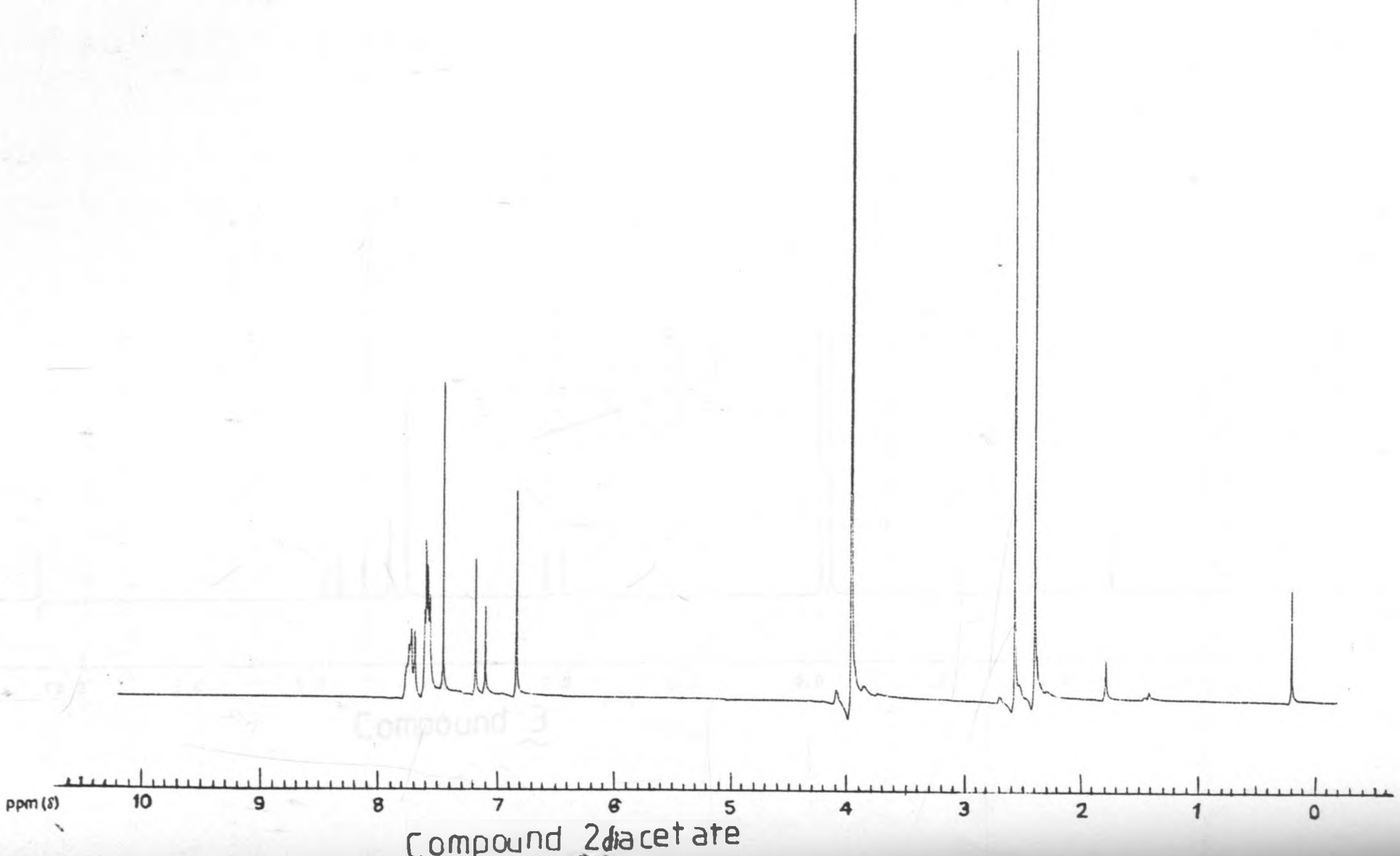
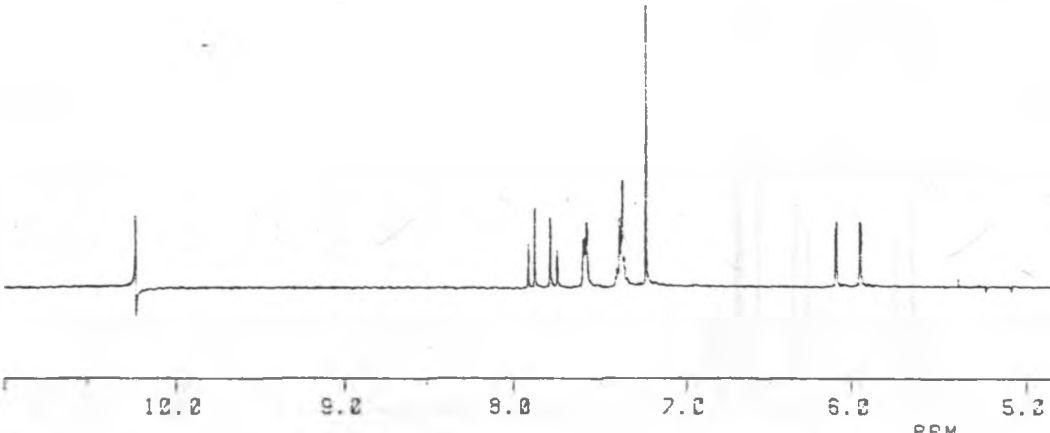
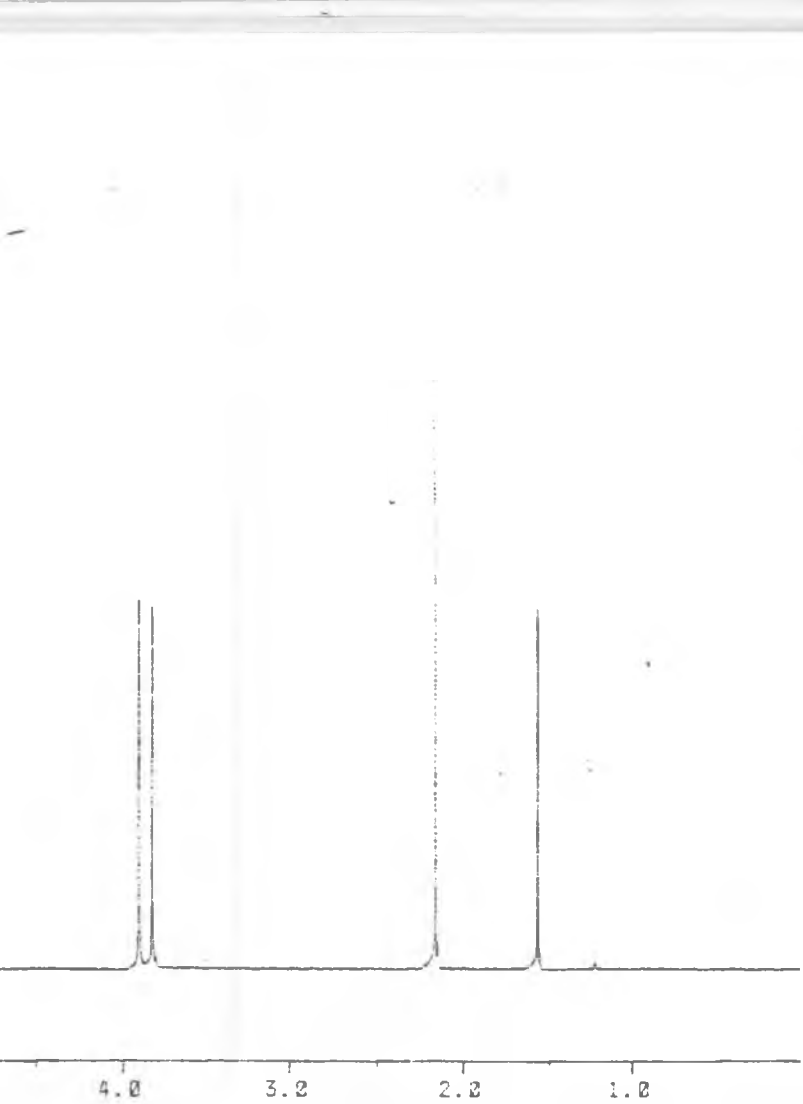
4.0
3.0
2.0
1.0
0.0

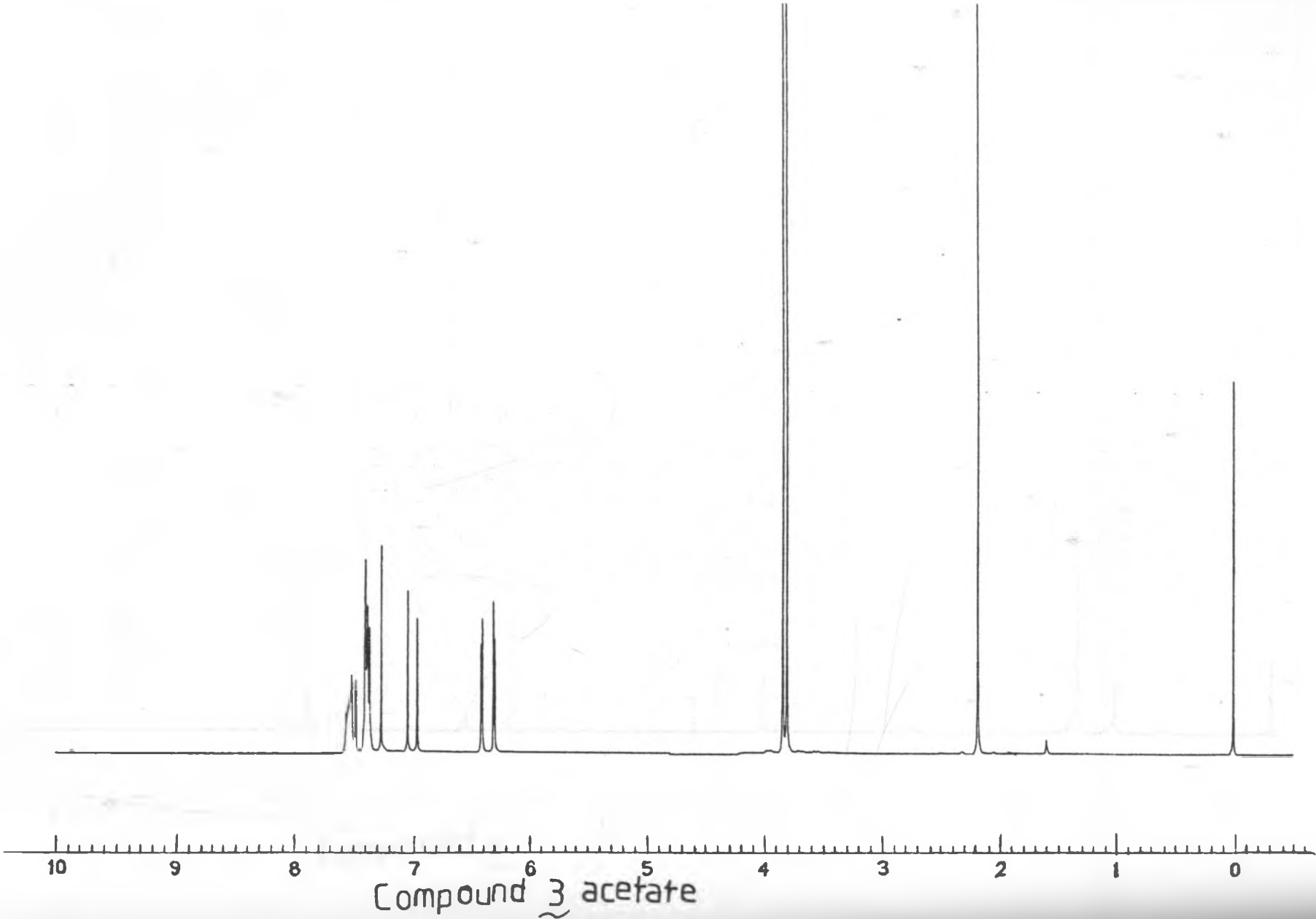


Compound 2 diacetate

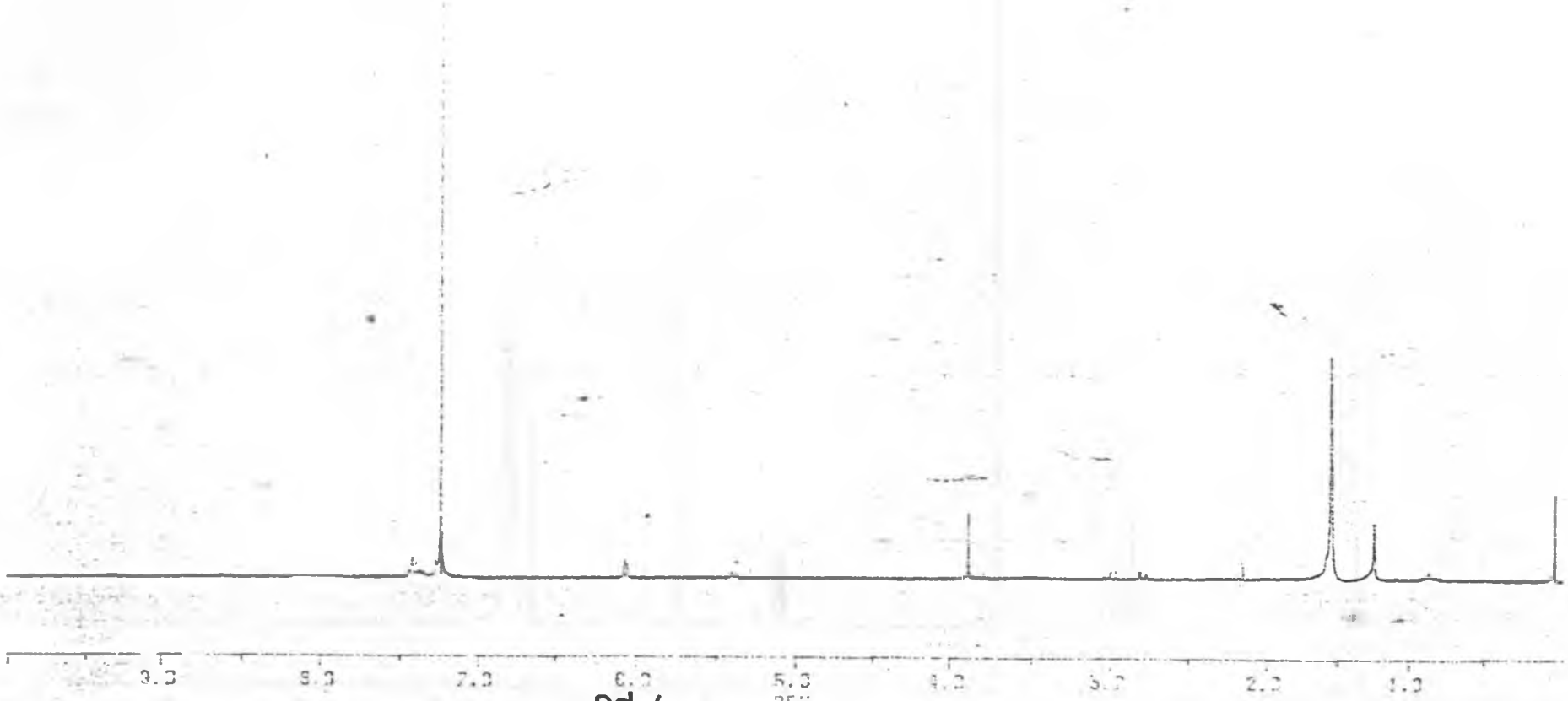


Compound 3

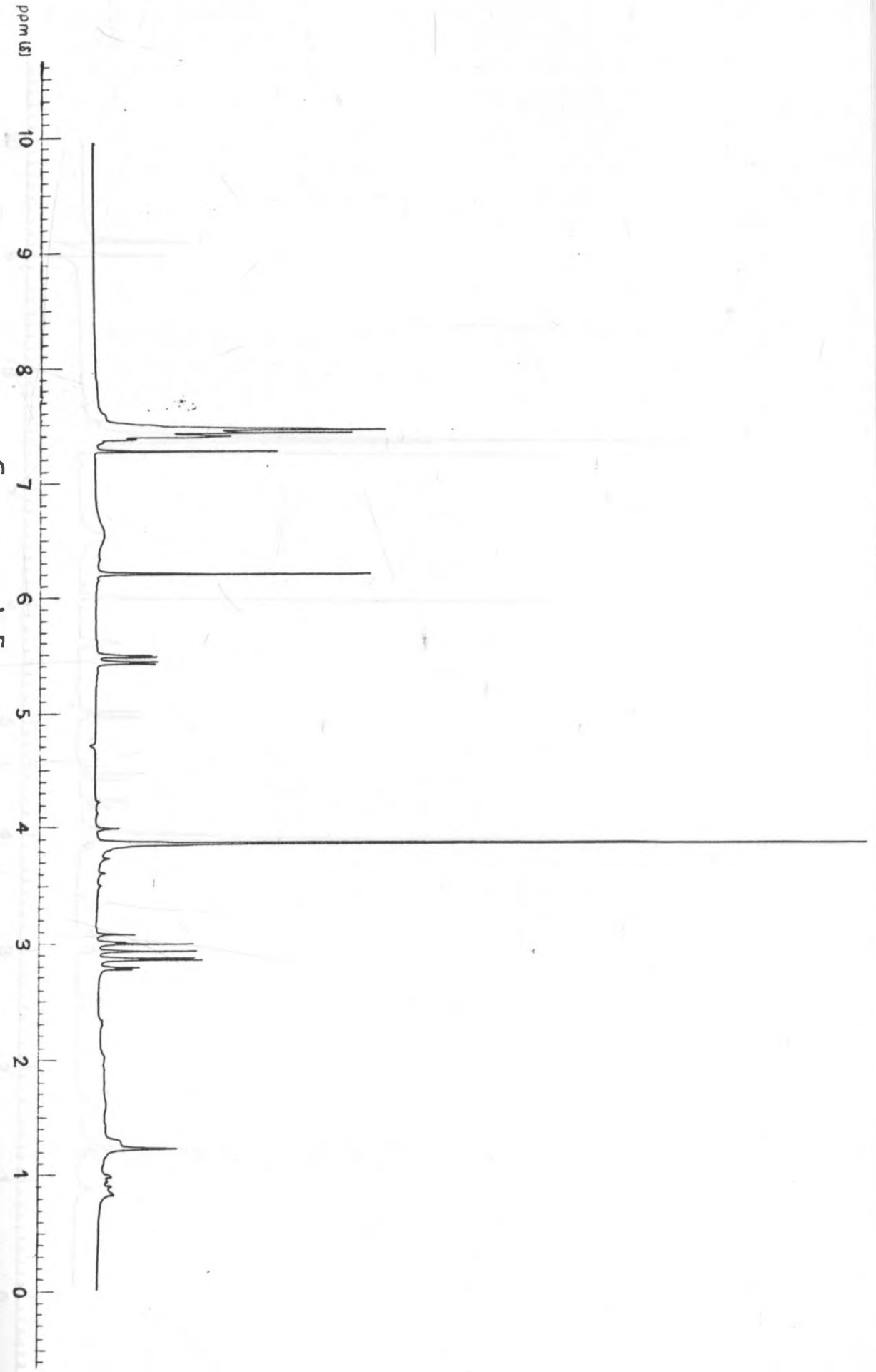

4.0
3.2
2.2
1.0



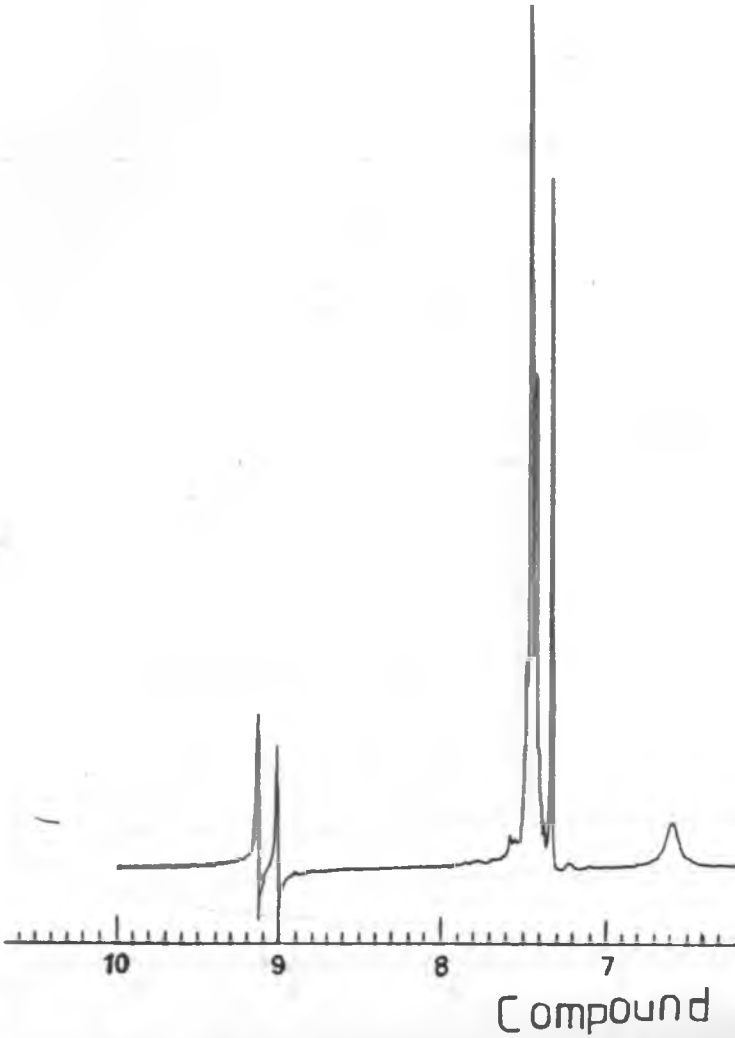
Compound 3 acetate

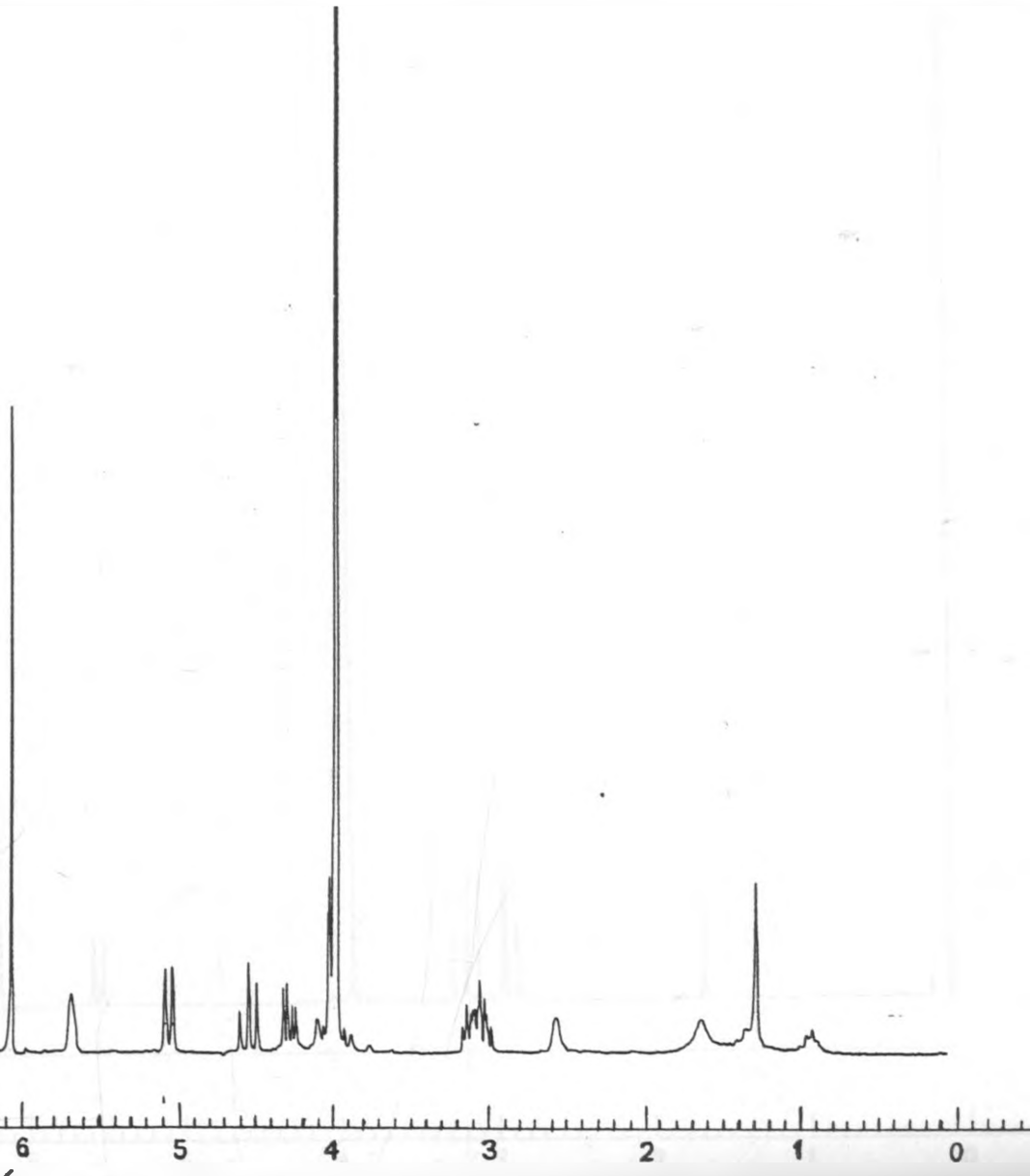


Compound 4

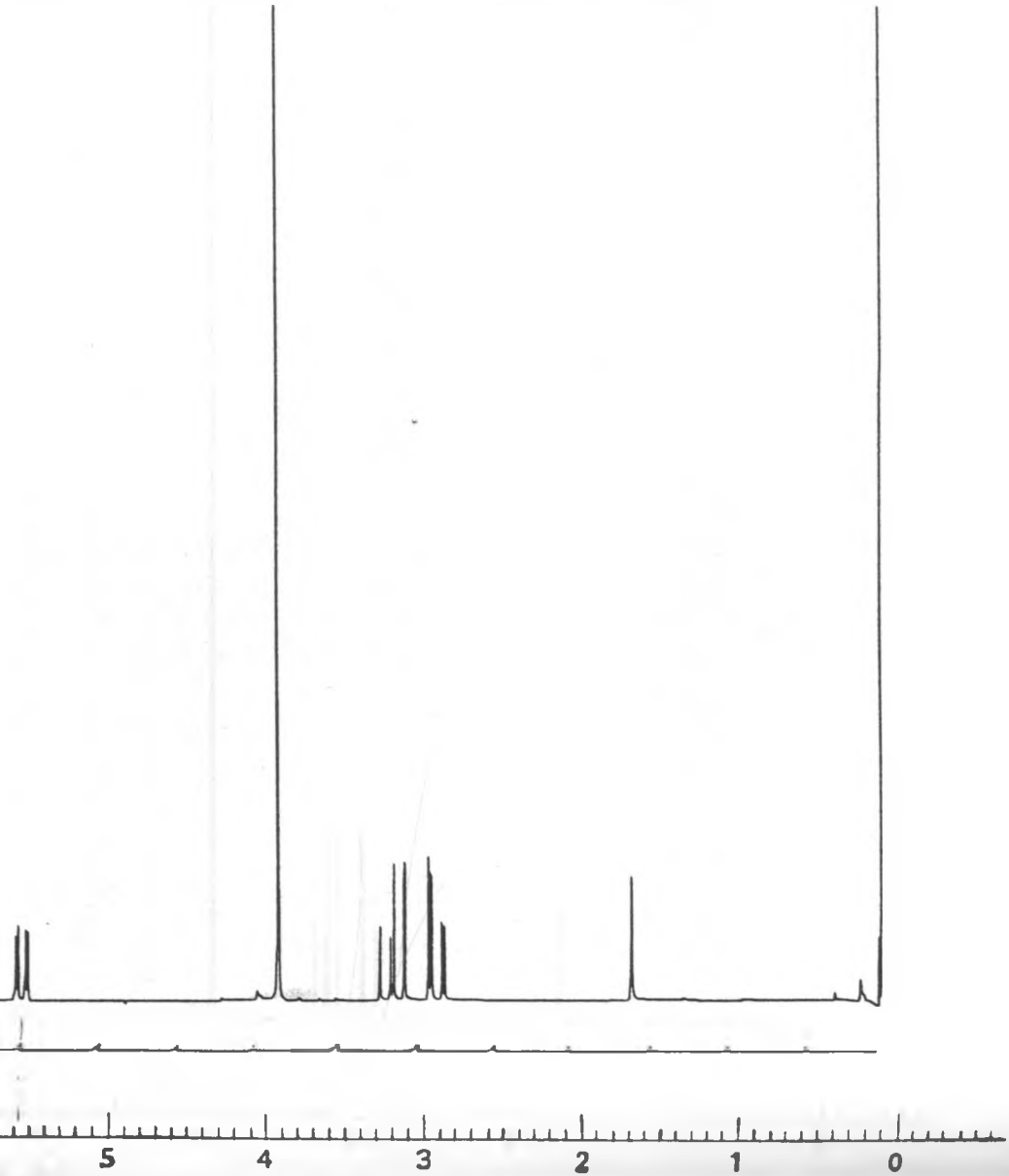


Compound 5


10
9
8
7
Compound

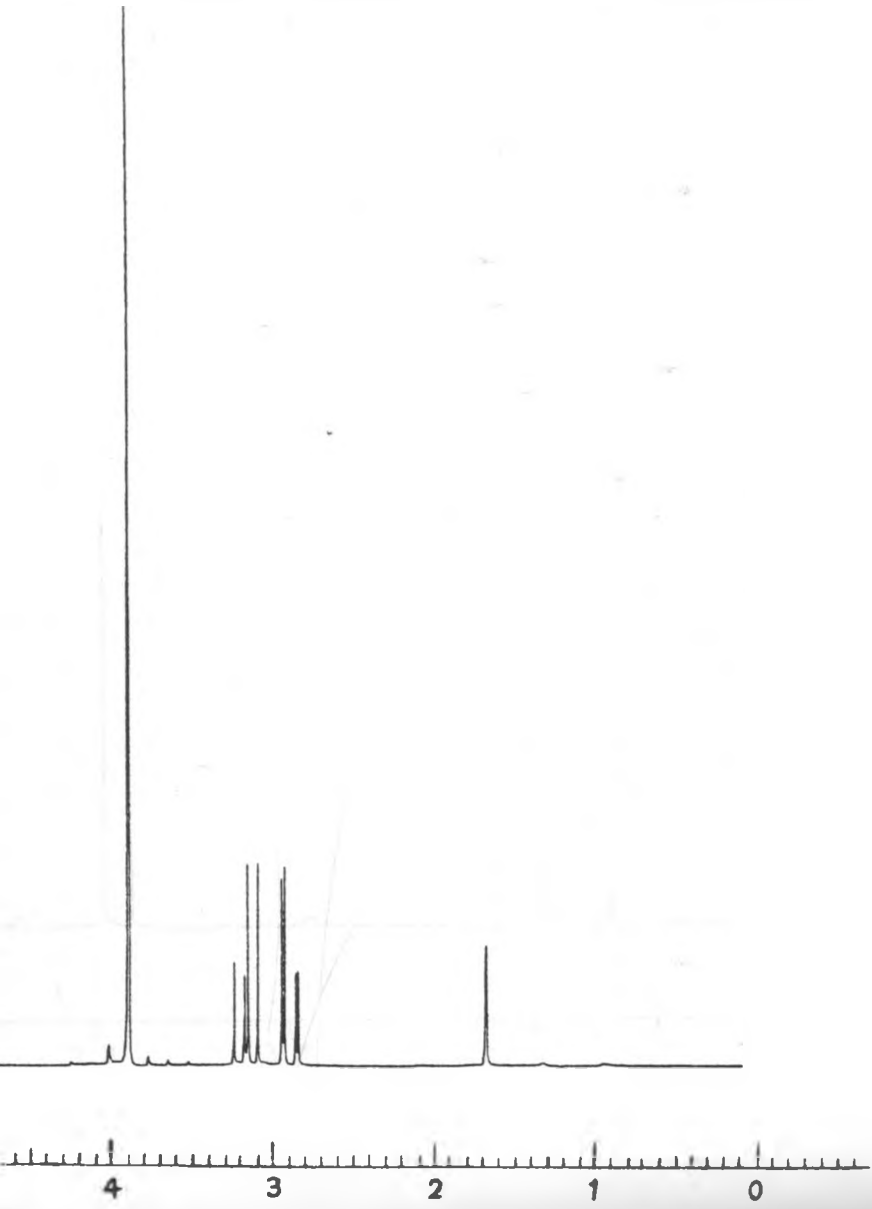


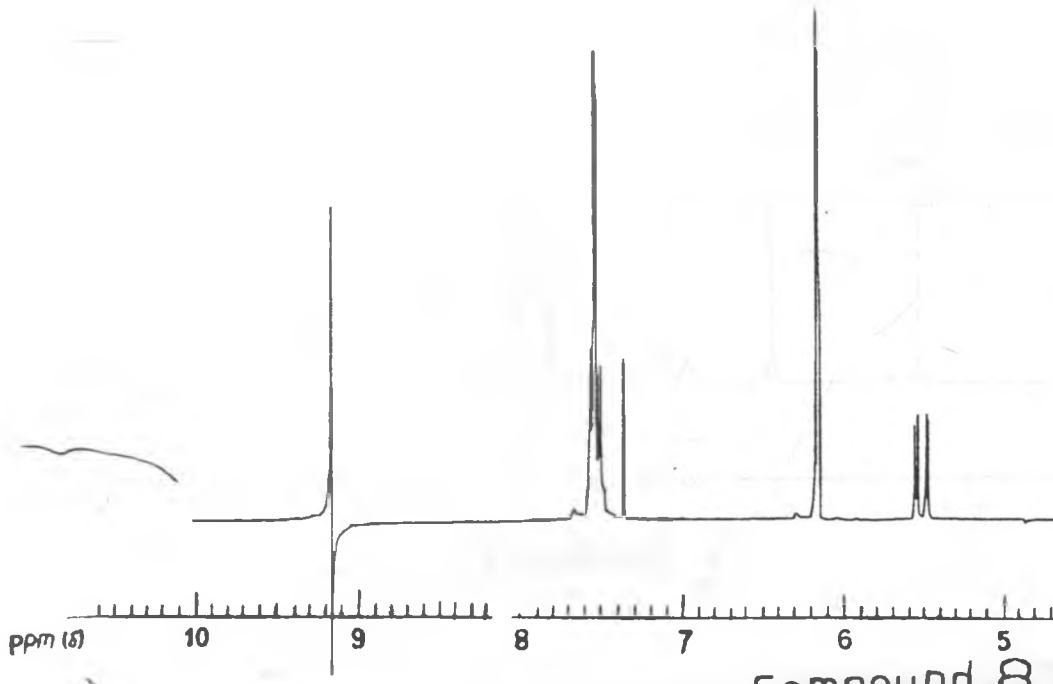
6



7

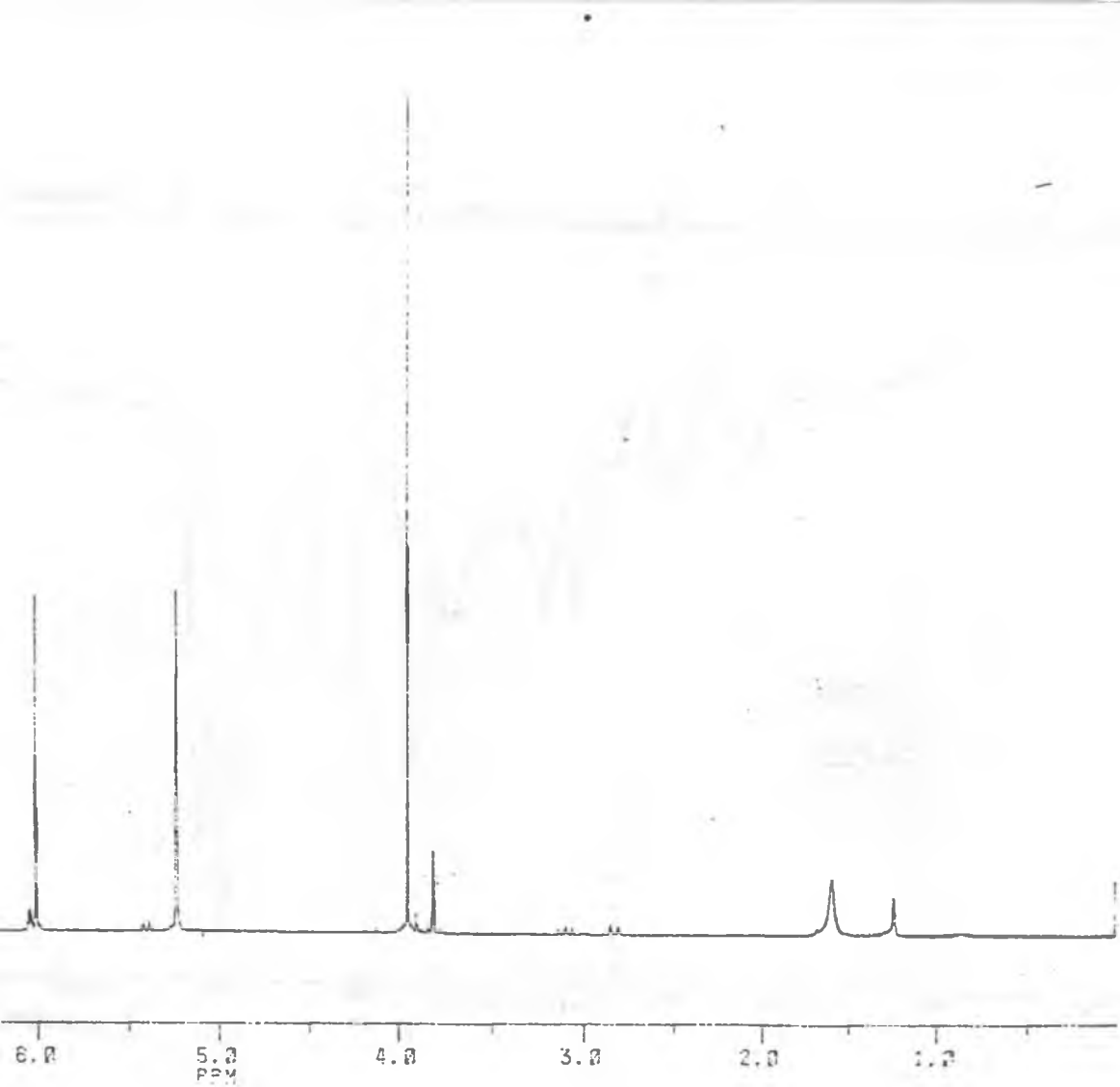
Compound C

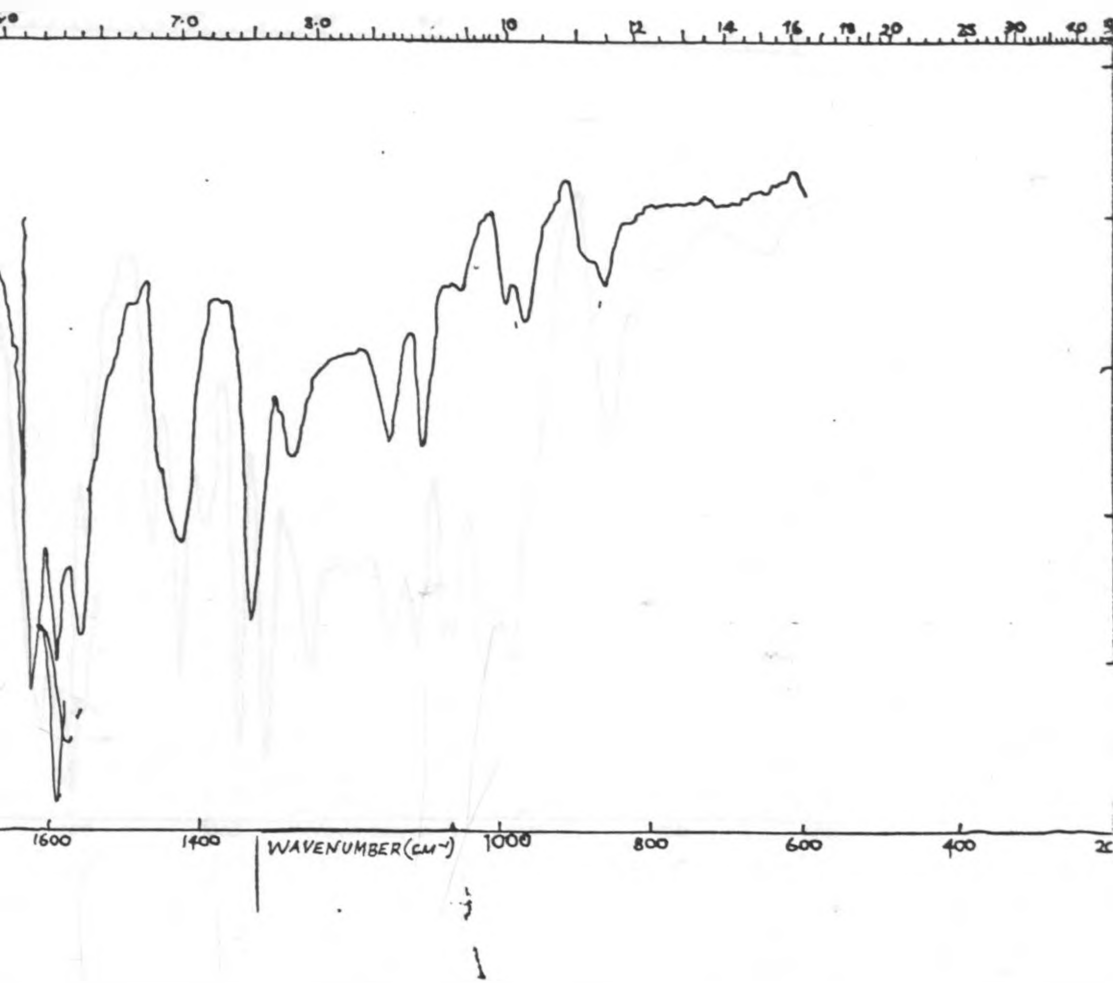

4
3
2
1
0



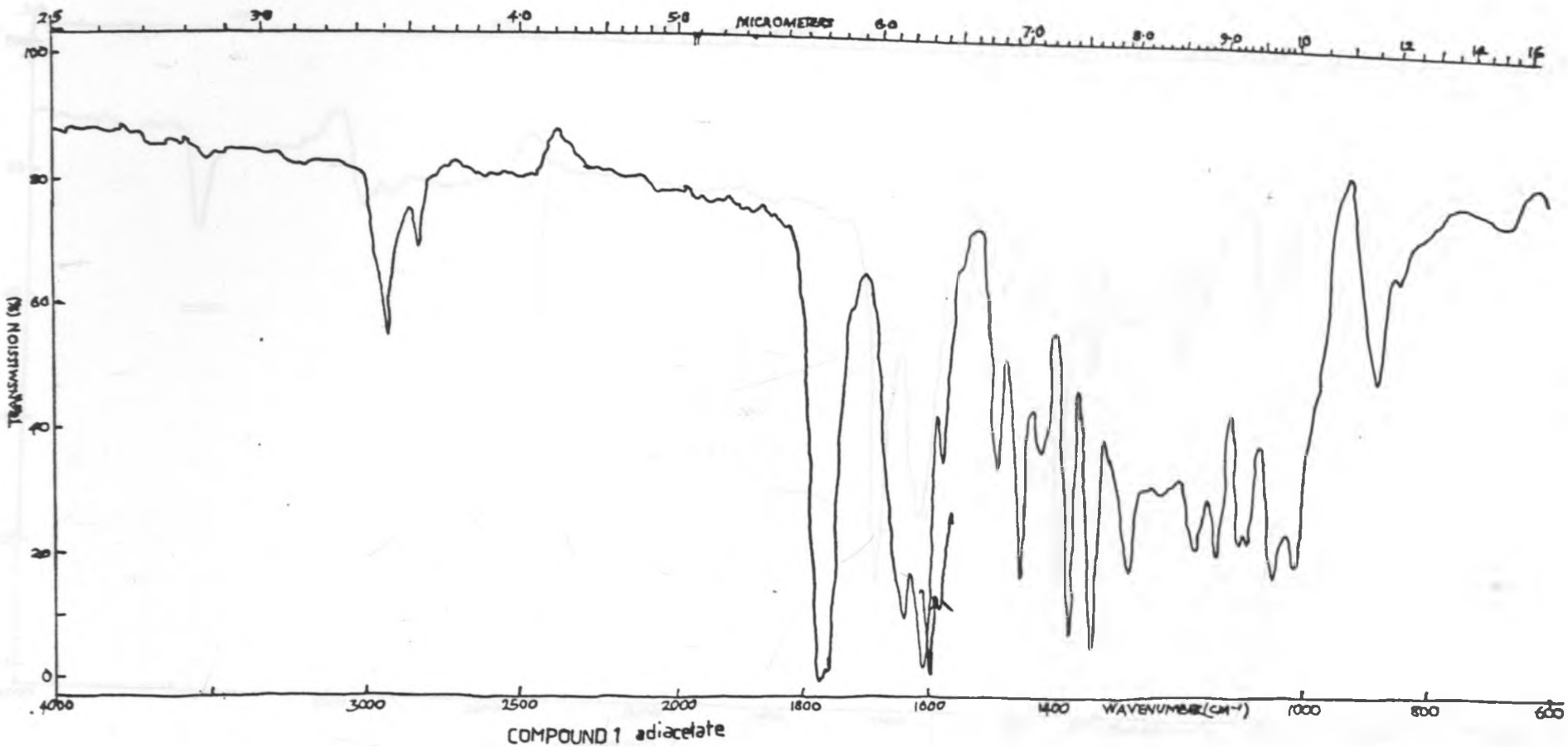
Compound 8

Compound 9

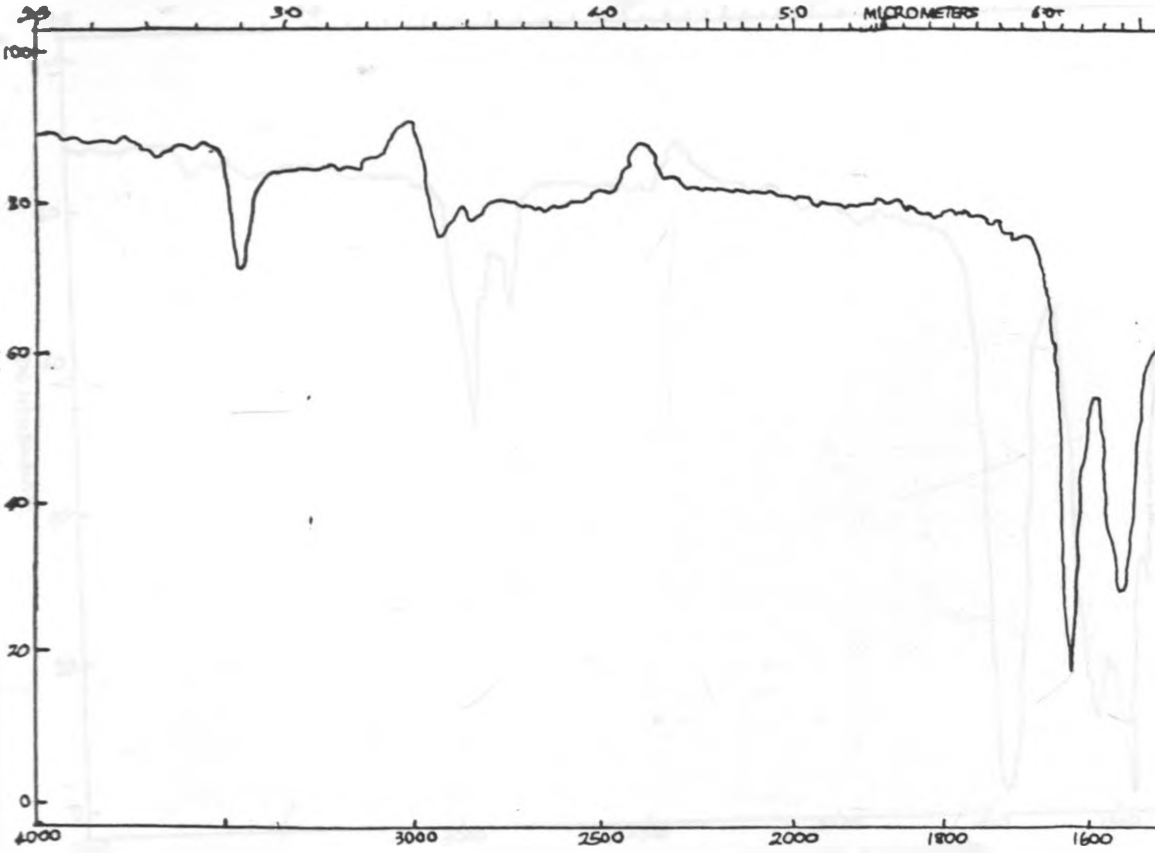

6.0
5.0
PPM
4.0
3.0
2.0
1.0


7.0
8.0
10
12
14
16
18
20
25
30
40
1600
1400
WAVENUMBER (cm⁻¹)
1000
800
600
400

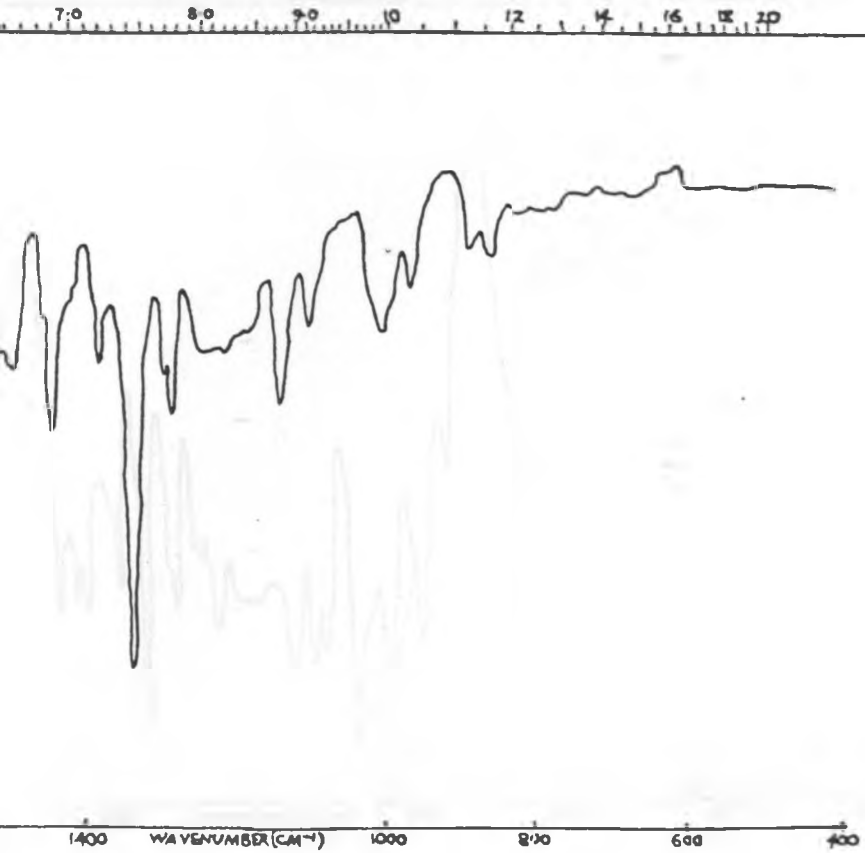
COMPOUND 1
4000
3500
3000
2500
2000
1800
0
20
40
60
80
100
TRANSMISSION (%)
2.5
3.0
4.0
5.0
MICROMETERS

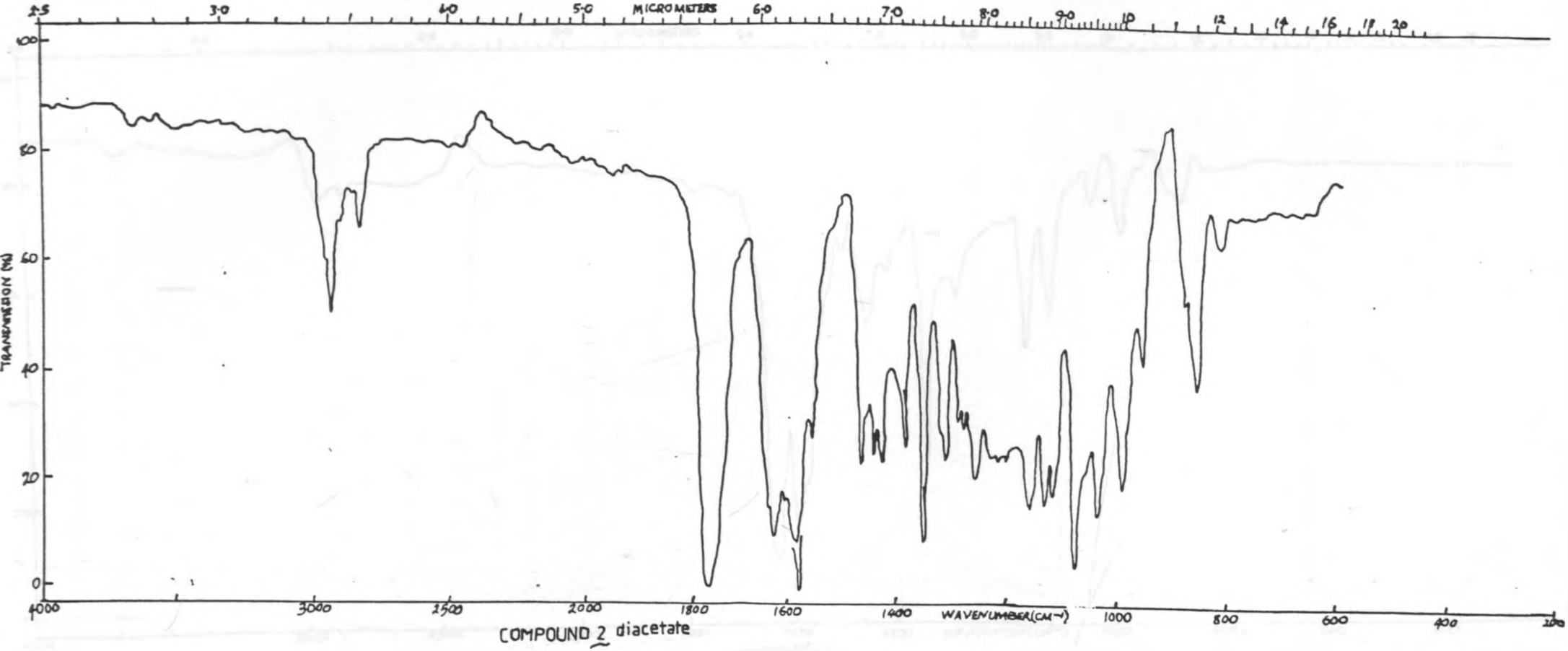


COMPOUND 1 adiacetate

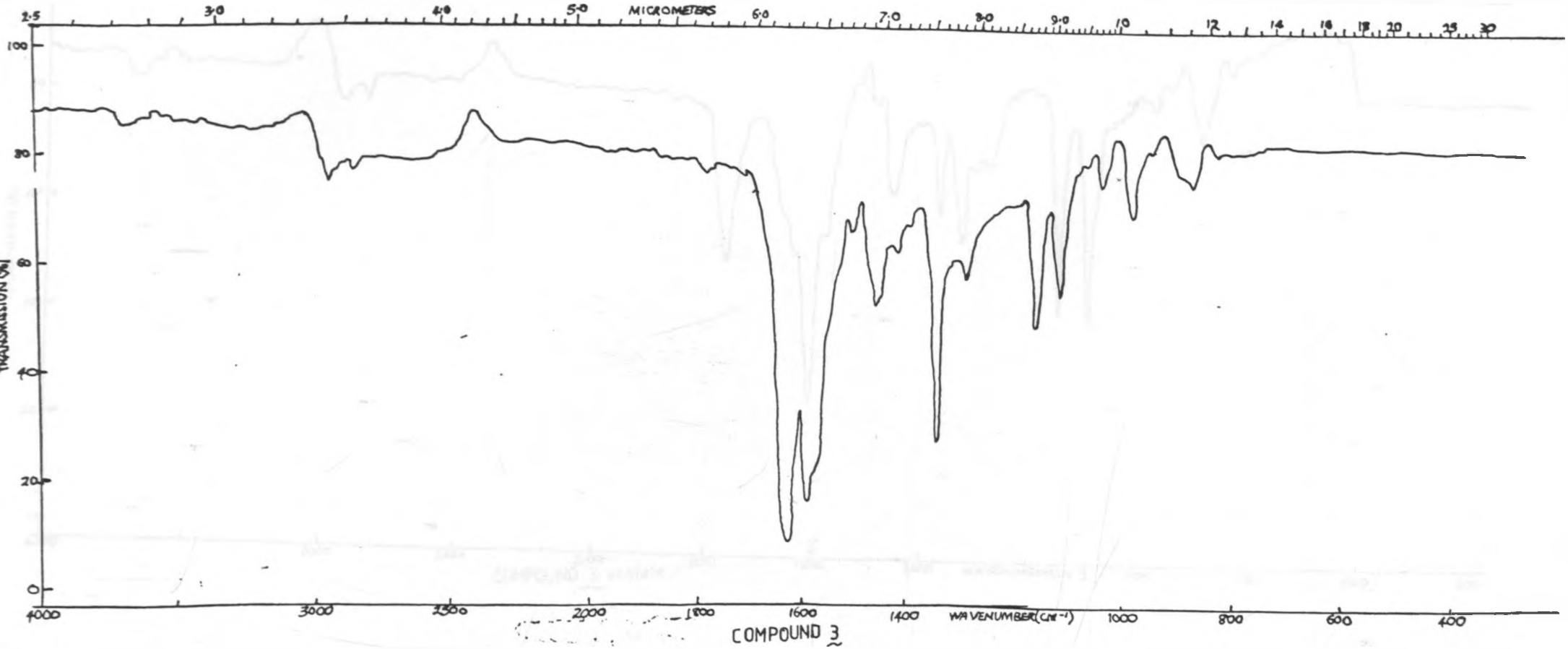

3·0
4·0
5·0
MICROMETERS
6·0
100
80
60
40
20
0
4000
3000
2500
2000
1800
1600

COMPOUND 2

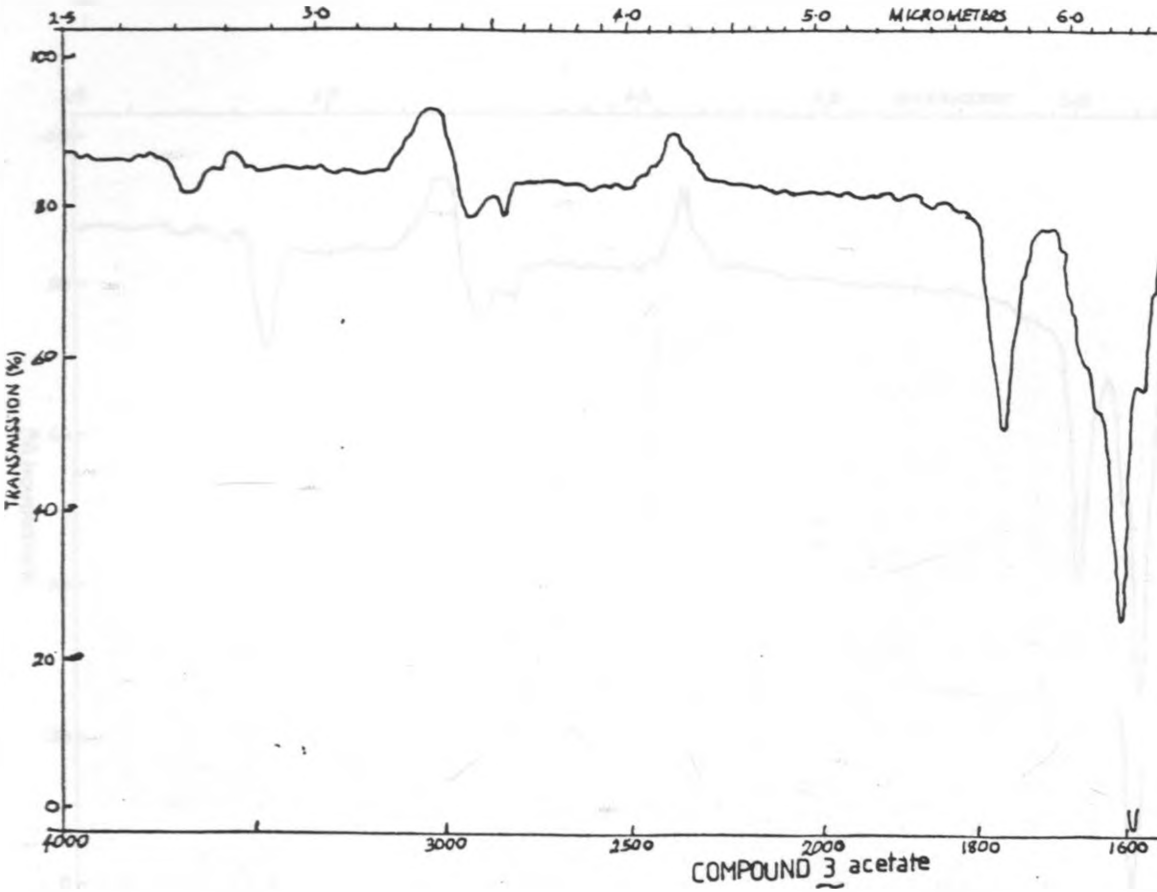

7·0
8·0
9·0
10
12
14
16
18
20
1400
WAVENUMBER (CM⁻¹)
1000
800
600
400



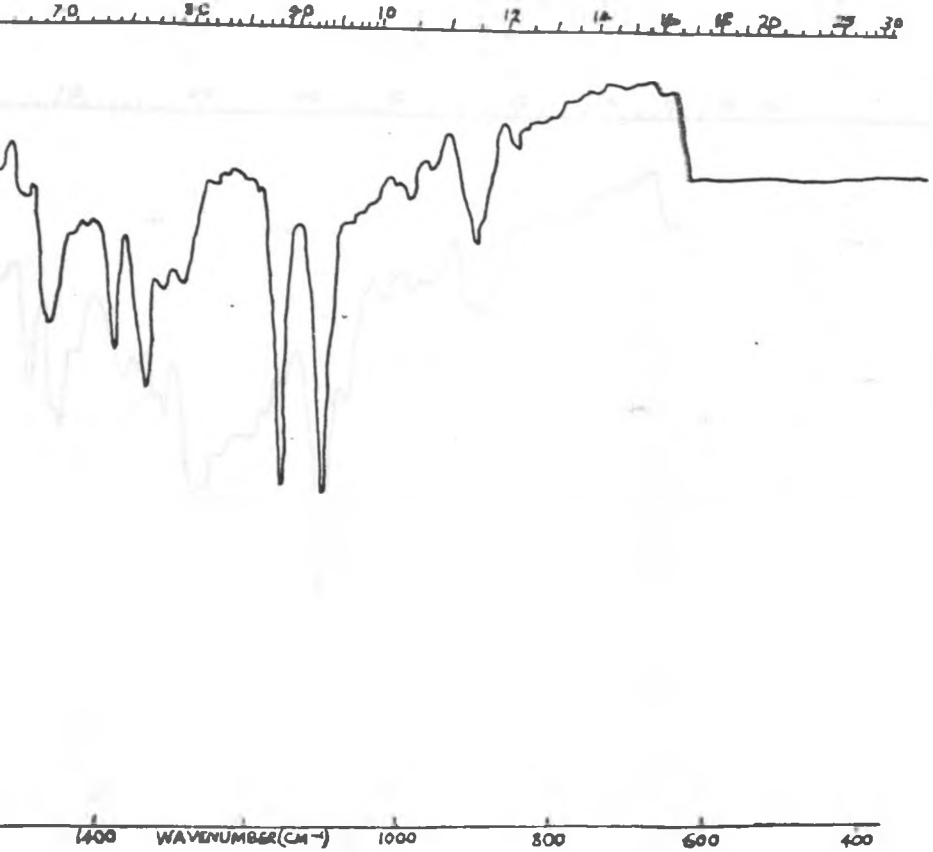
COMPOUND 2 diacetate

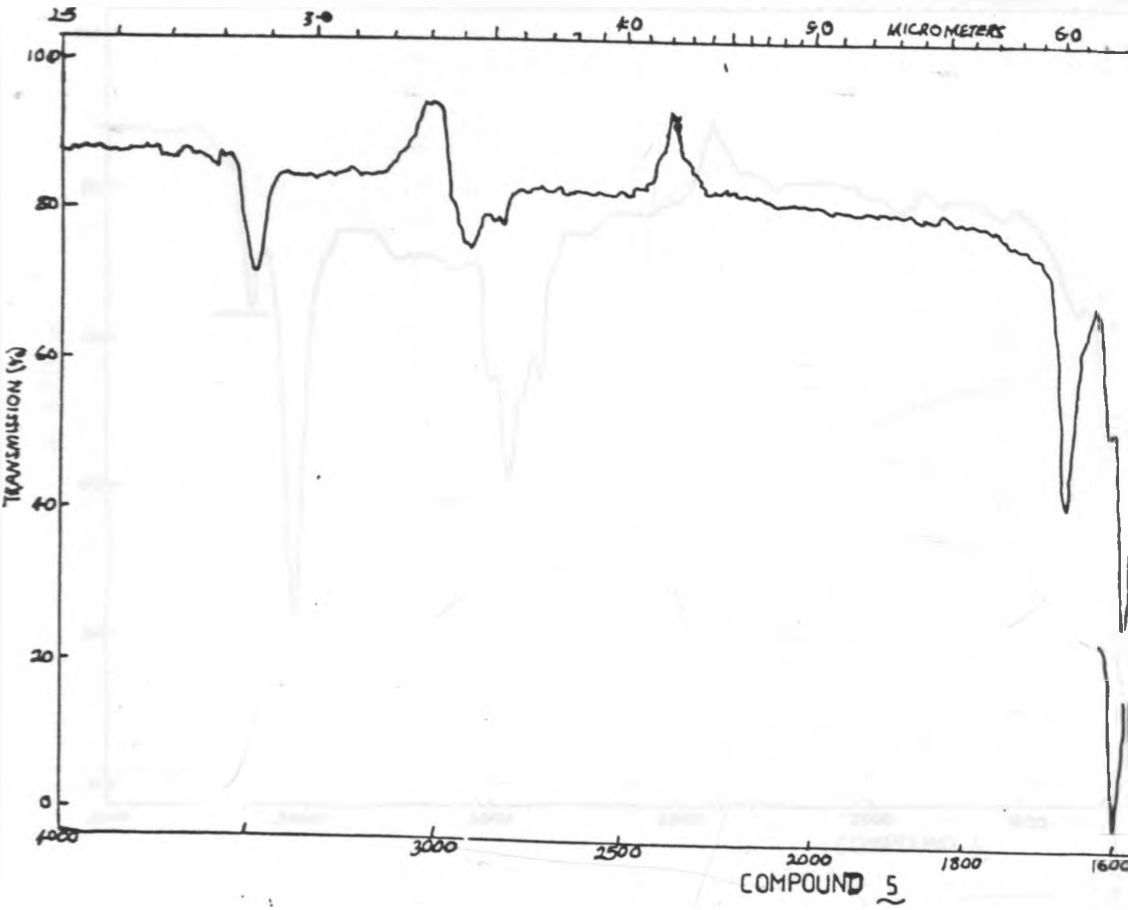

2·5
3·0
4·0
5·0
MICROMETERS
6·0
7·0
8·0
9·0
10
12
14
16
18
20
25
30
100
80
60
40
20
0
TRANSMISSION (%)
4000
3000
2500
2000
1800
1600
1400
WAVENUMBER (CM⁻¹)
1000
800
600
400

COMPOUND 3

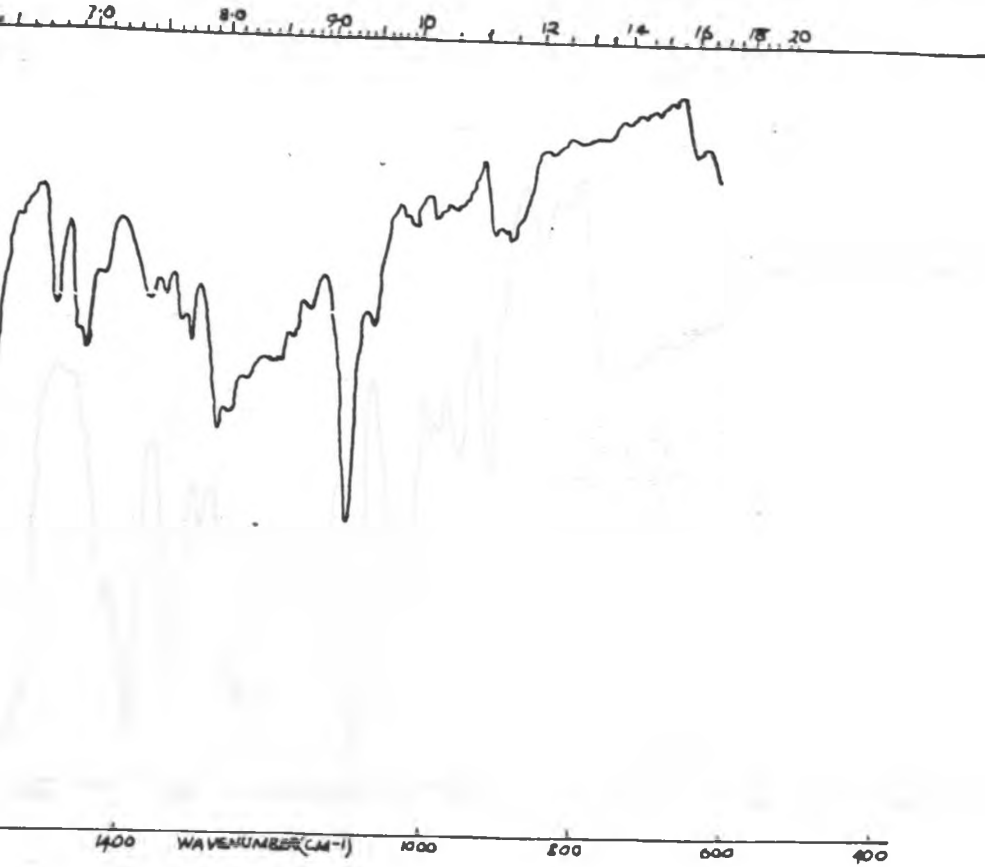


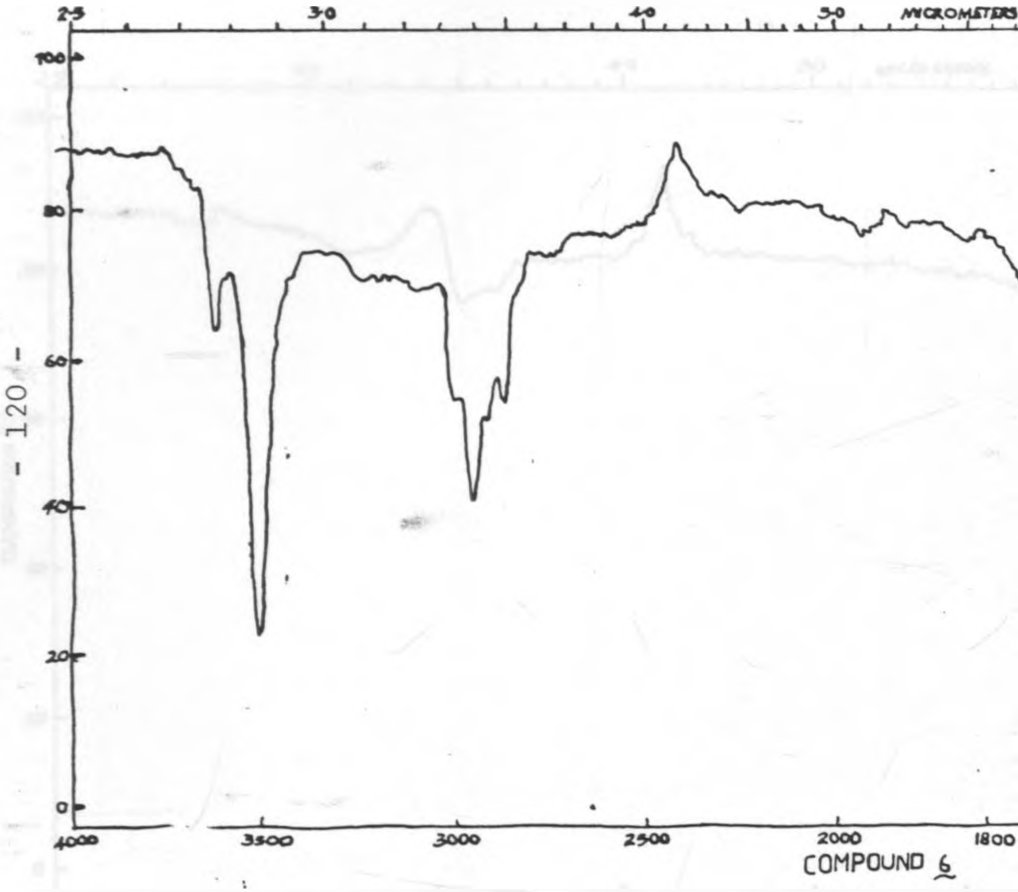
COMPOUND 3 acetate


7.0
8.0
9.0
10
12
14
16
18
20
25
30
1400
WAVENUMBER(CM⁻¹)
1000
800
600
400

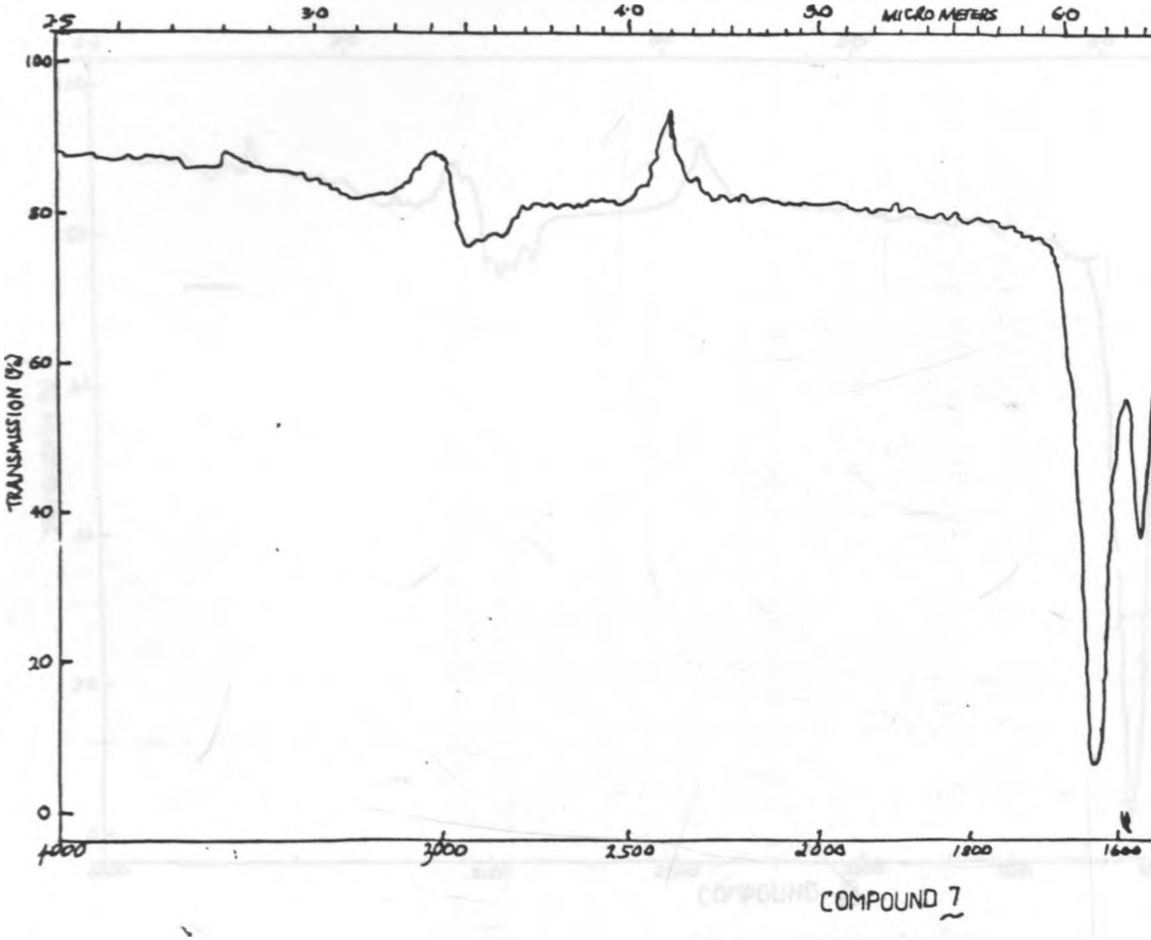

25
30
40
50
MICROMETERS
60
100
80
60
40
20
0
TRANSMISSION (%)
4000
3000
2500
2000
1800
1600

COMPOUND 5

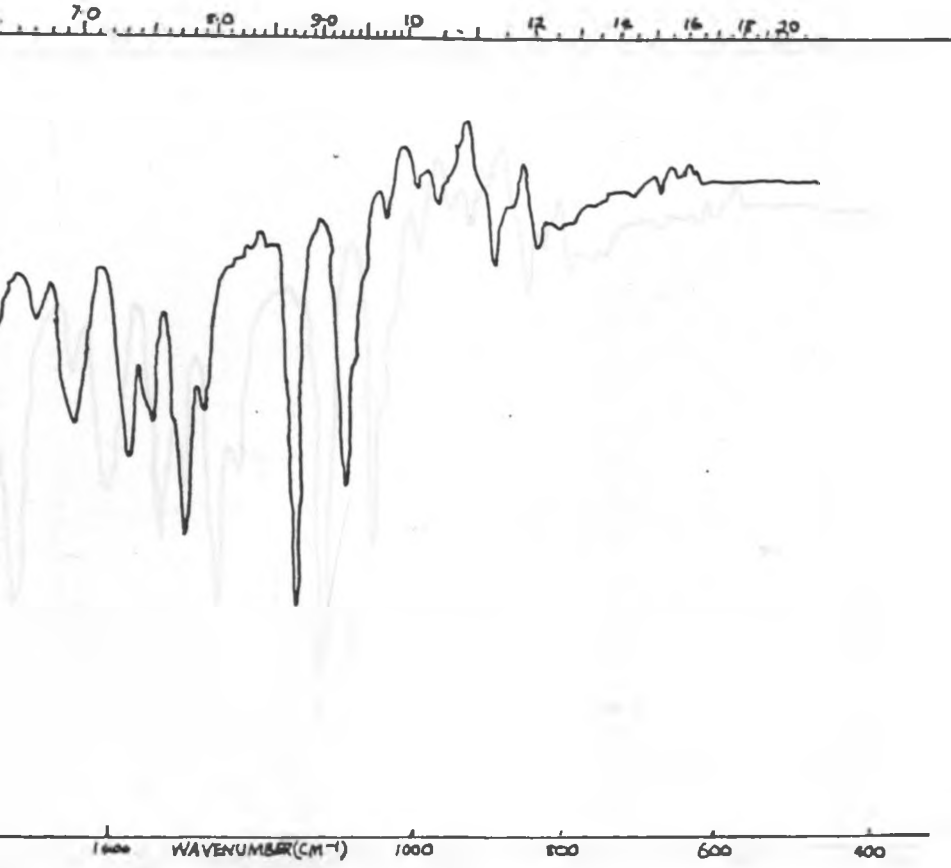

7·0
8·0
9·0
10
12
14
16
18
20
1400
WAVENUMBER (CM-1)
1000
800
600
400

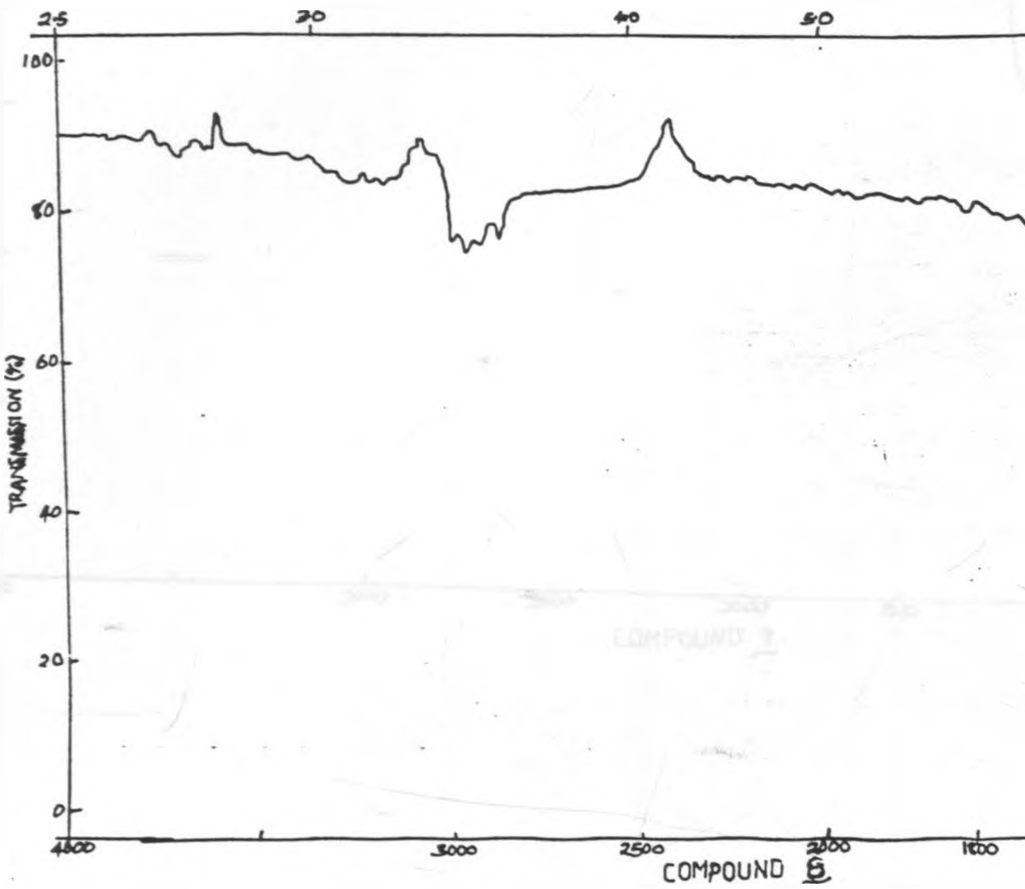

2·5
3·0
4·0
5·0
MICROMETERS
100
80
60
40
20
0
4000
3500
3000
2500
2000
1800

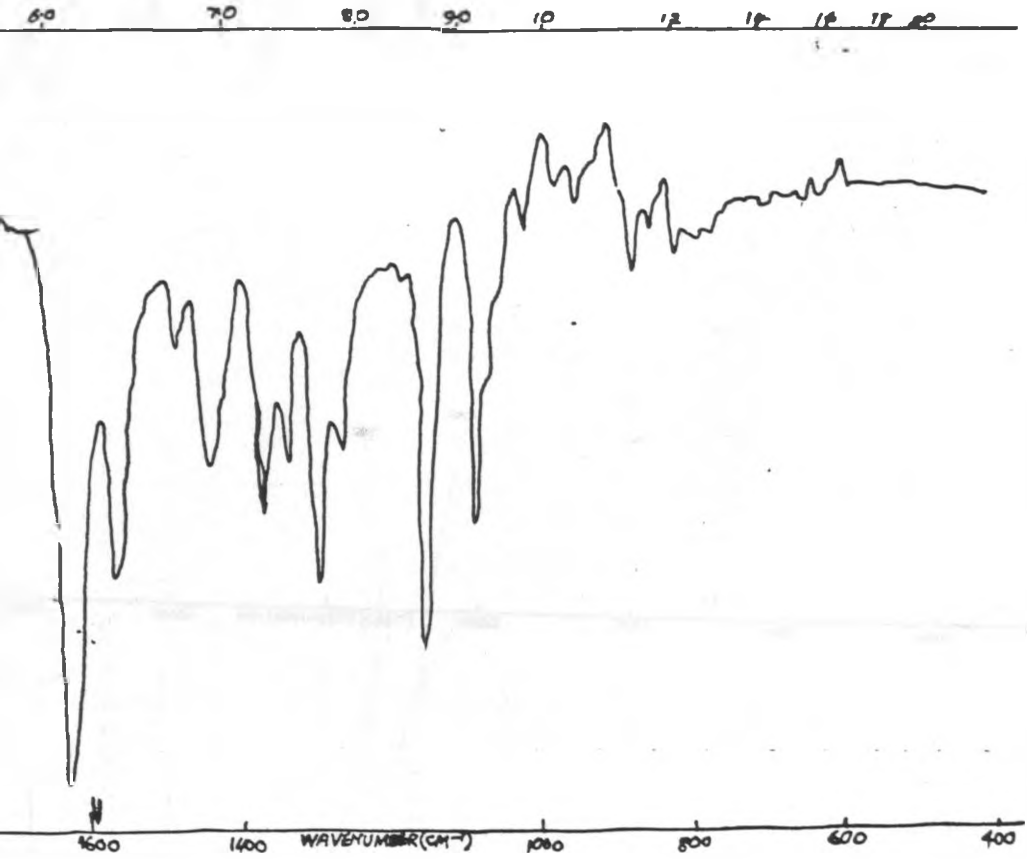
COMPOUND 6

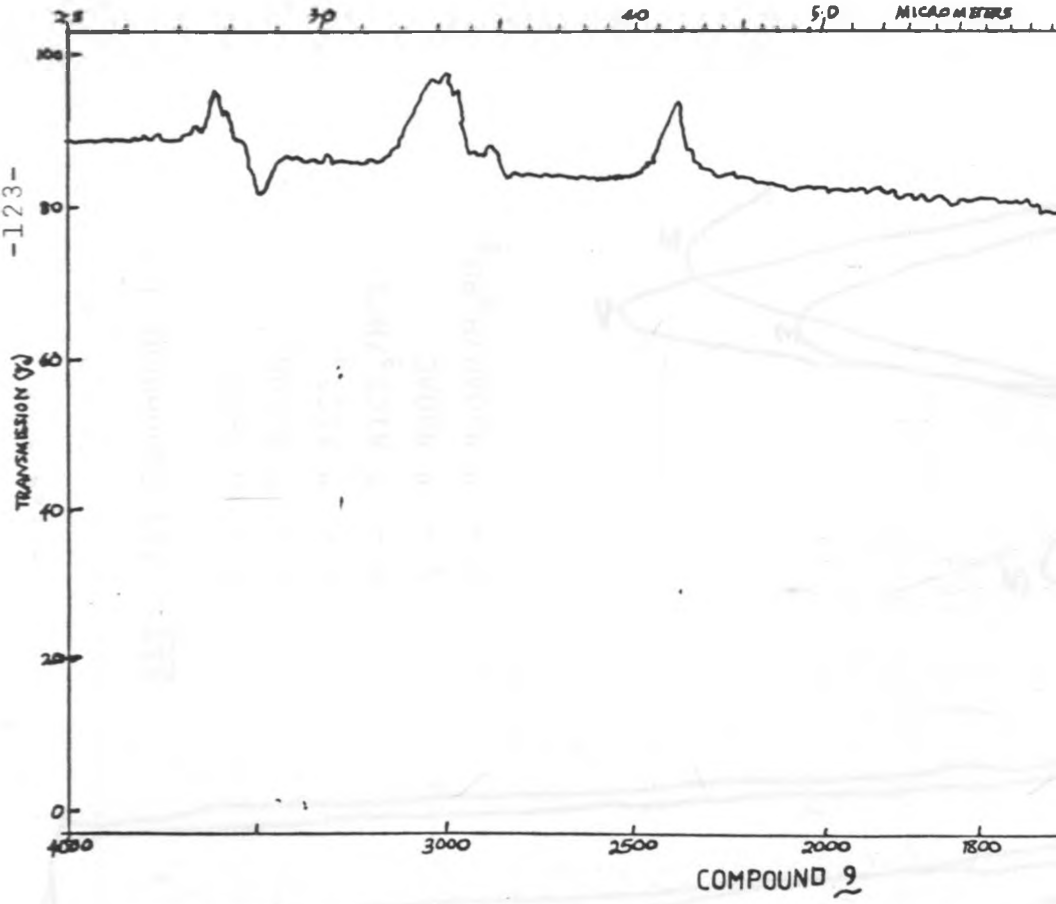

2.5
3.0
4.0
5.0
MICRO METERS
6.0
100
80
60
40
20
0
TRANSMISSION (%)
4000
3000
2500
2000
1800
1600

COMPOUND 7

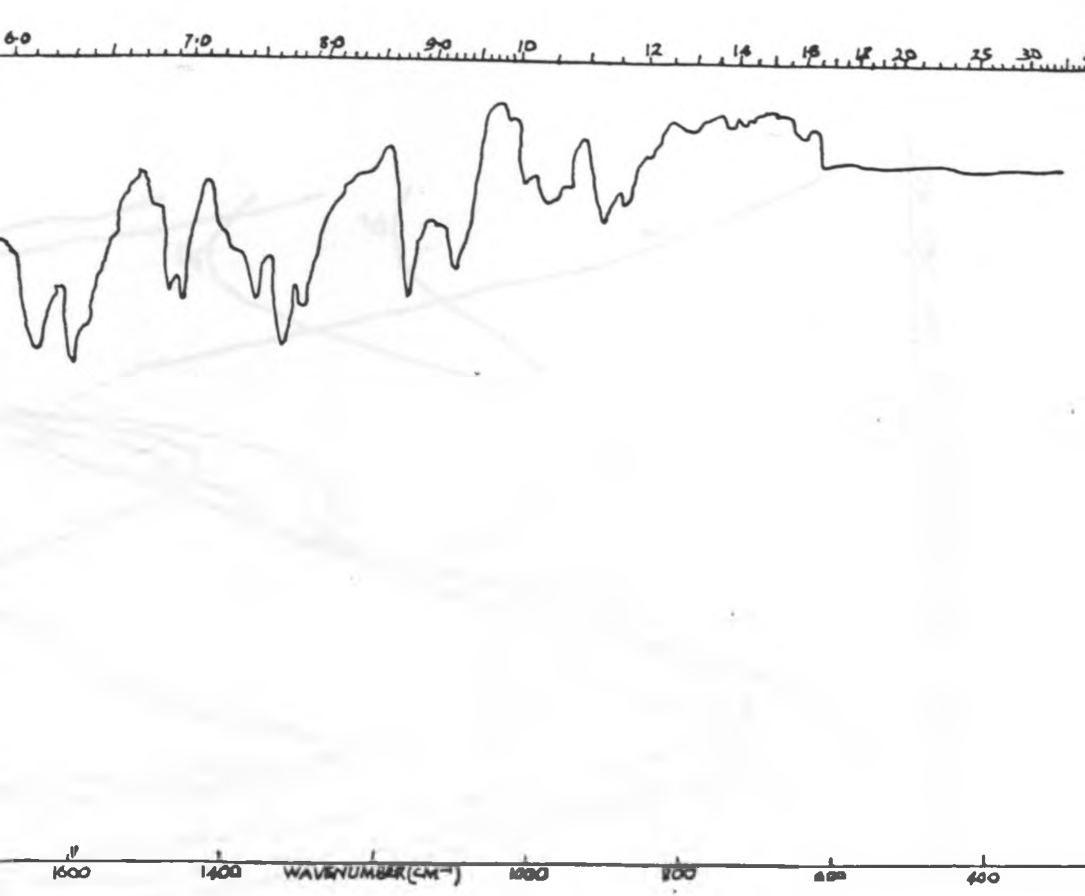

7.0
8.0
9.0
10
12
14
16
18
20
1600
WAVENUMBER(CM⁻¹)
1000
800
600
400

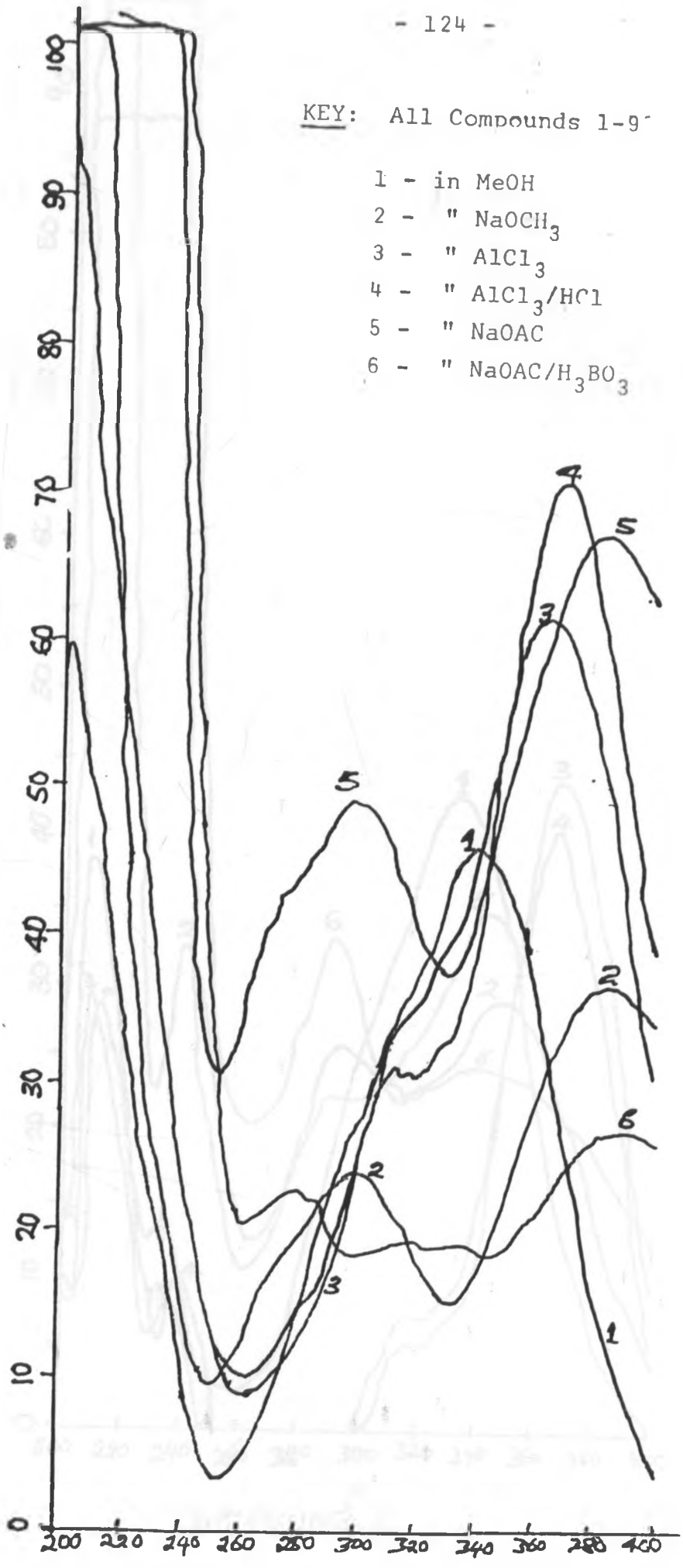

2.5
20
40
60
100
80
60
40
20
0
TRANSMISSION (%)
4000
3000
2500
2000
1600
COMPOUND 5


6.0
7.0
8.0
9.0
10
12
1600
1400
WAVENUMBER (CM⁻¹)
1000
800
600
400

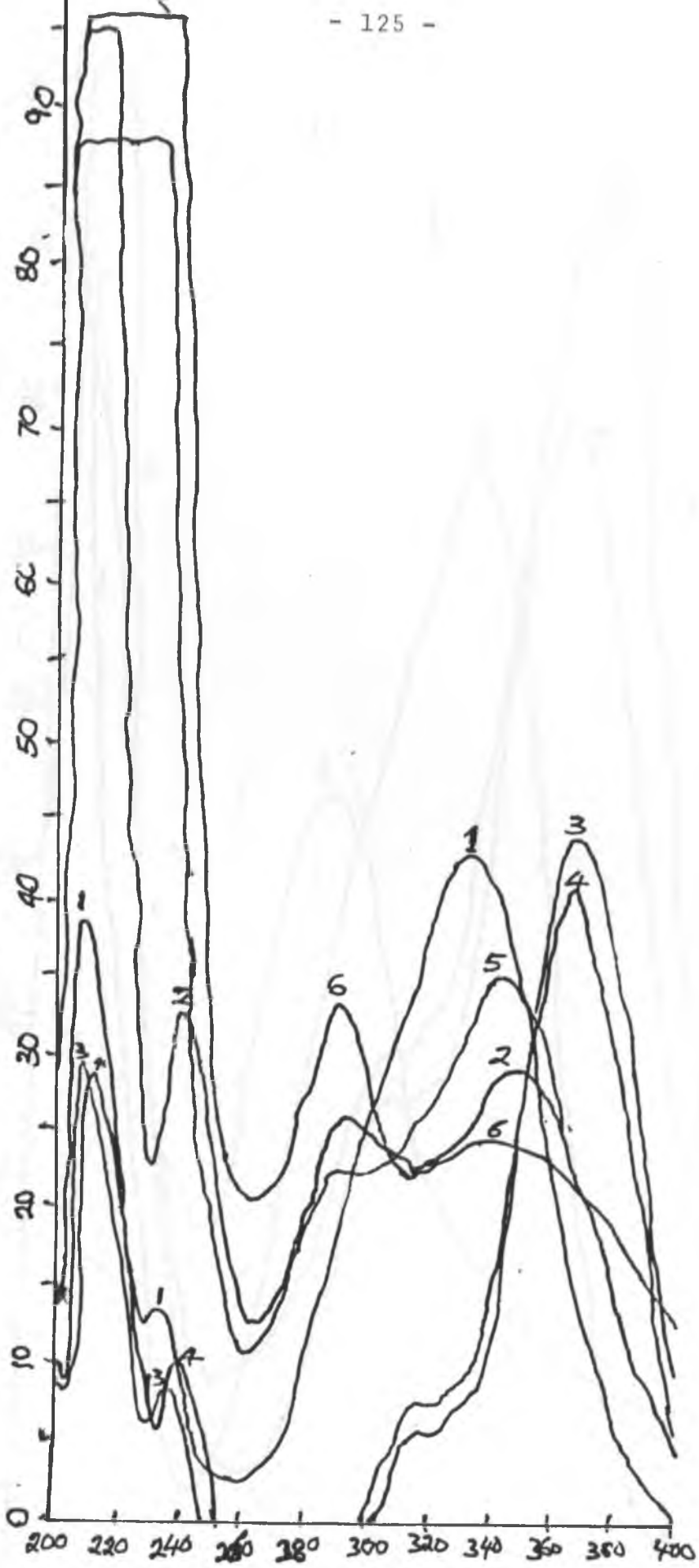

2.5
3.0
4.0
5.0
MICROMETERS
100
80
60
40
20
0
TRANSMISSION (%)
4000
3000
2500
2000
1800

COMPOUND 9

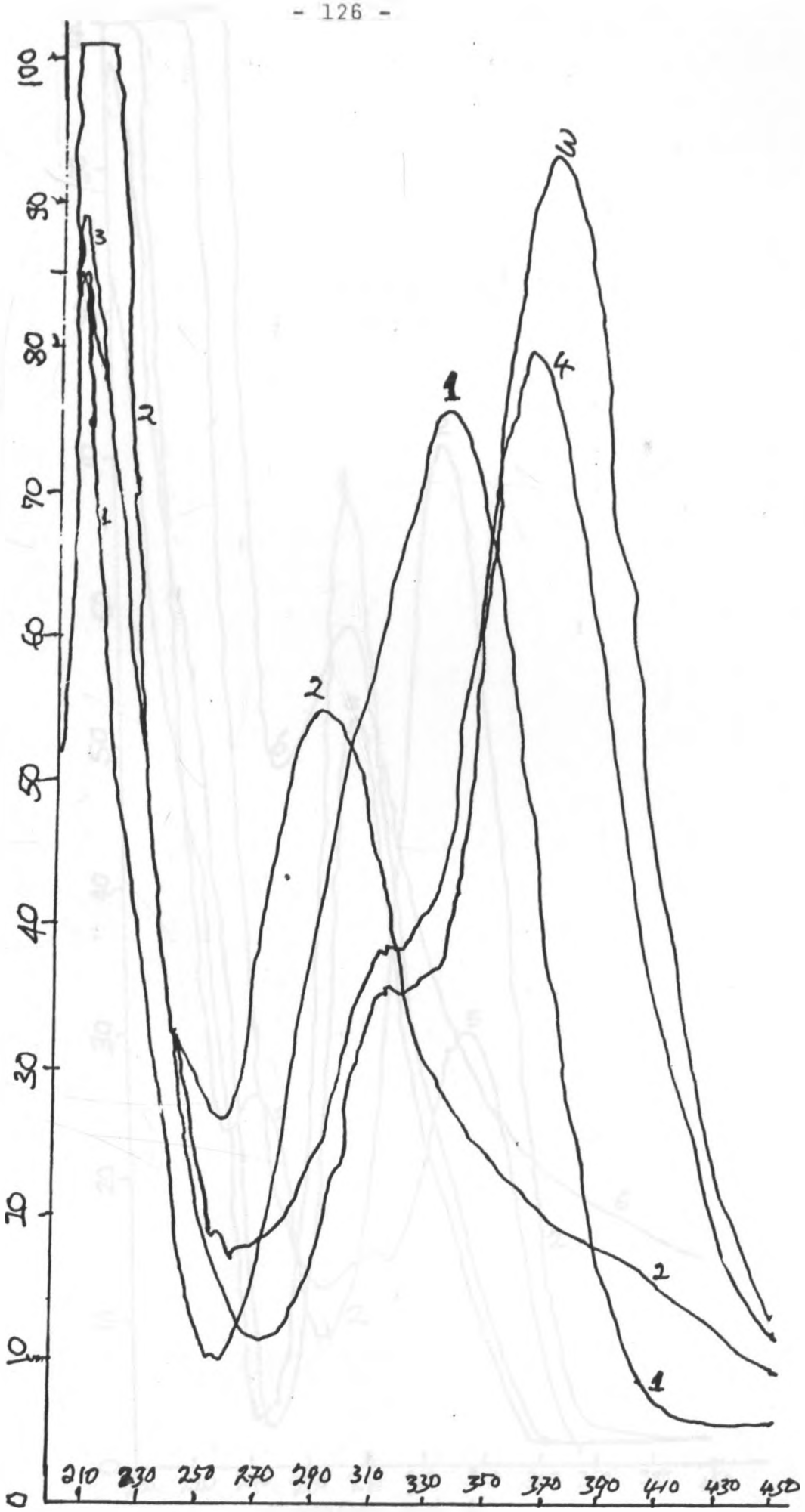

6·0
7·0
8·0
9·0
10
12
14
16
18
20
25
30
1600
1400
WAVENUMBER (CM⁻¹)
1000
800
600
400


KEY: All Compounds 1-9
1 - in MeOH
2 - " $NaOCH_3$
3 - " $AlCl_3$
4 - " $AlCl_3/HCl$
5 - " NaOAC
6 - " $NaOAC/H_3BO_3$
100
90
80
70
60
50
40
30
20
10
0
200
220
240
260
280
300
320
340
360
380
400

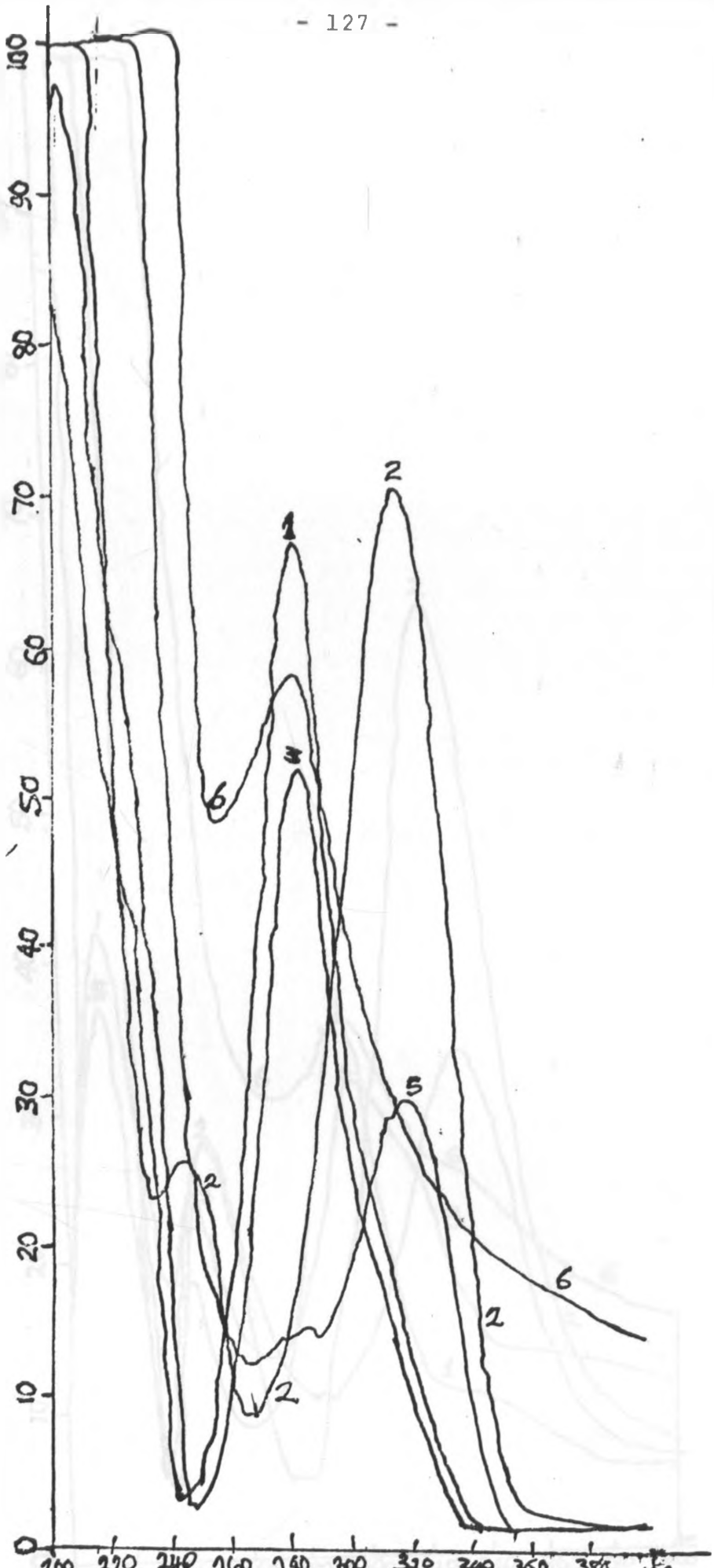
COMPOUND 1


0
10
20
30
40
50
60
70
80
90
200
220
240
260
280
300
320
340
360
380
400
1
2
3
4
5
6

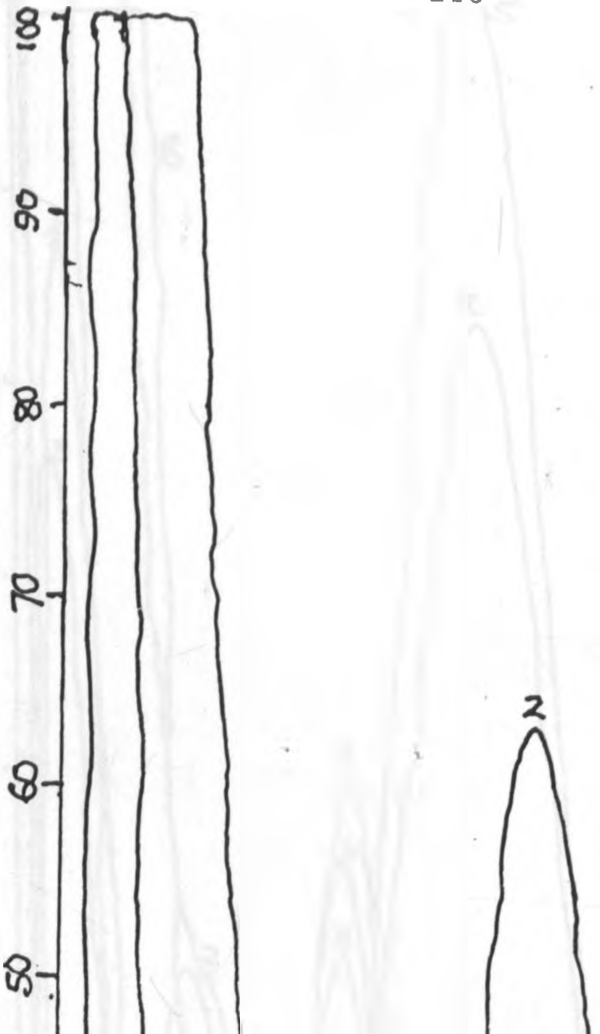
Compound 2

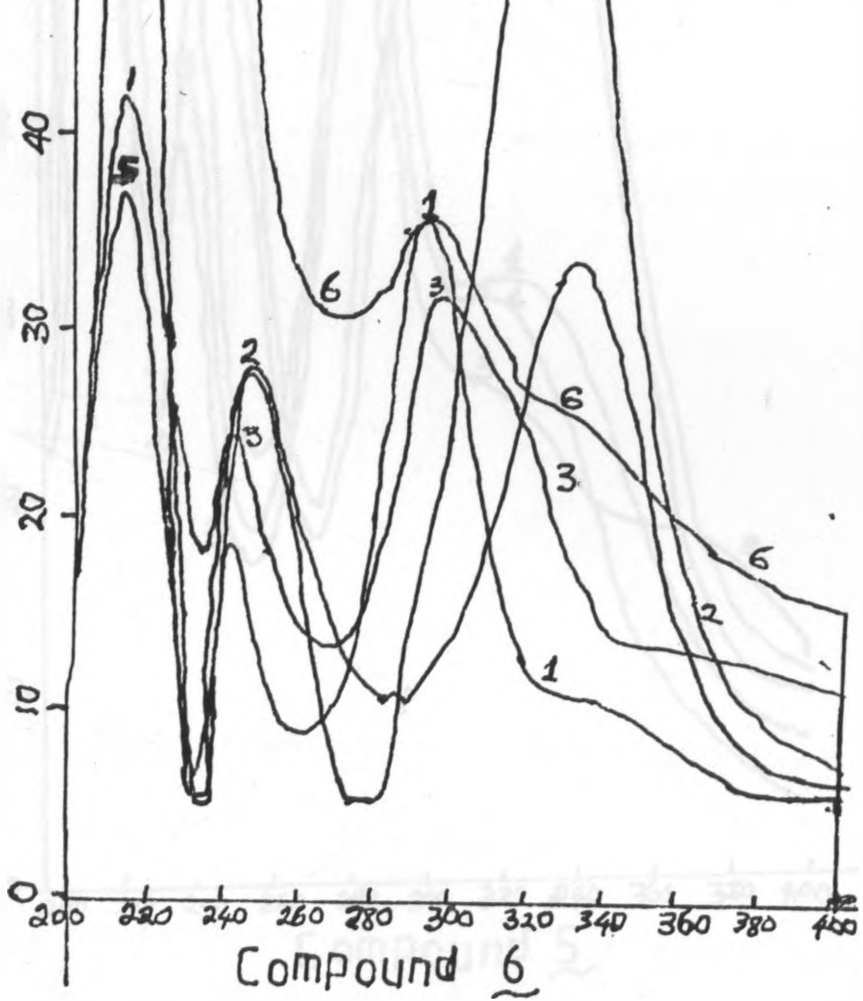

100
90
80
70
60
50
40
30
20
10
0
210
230
250
270
290
310
330
350
370
390
410
430
450
1
2
3
4

Compound 3

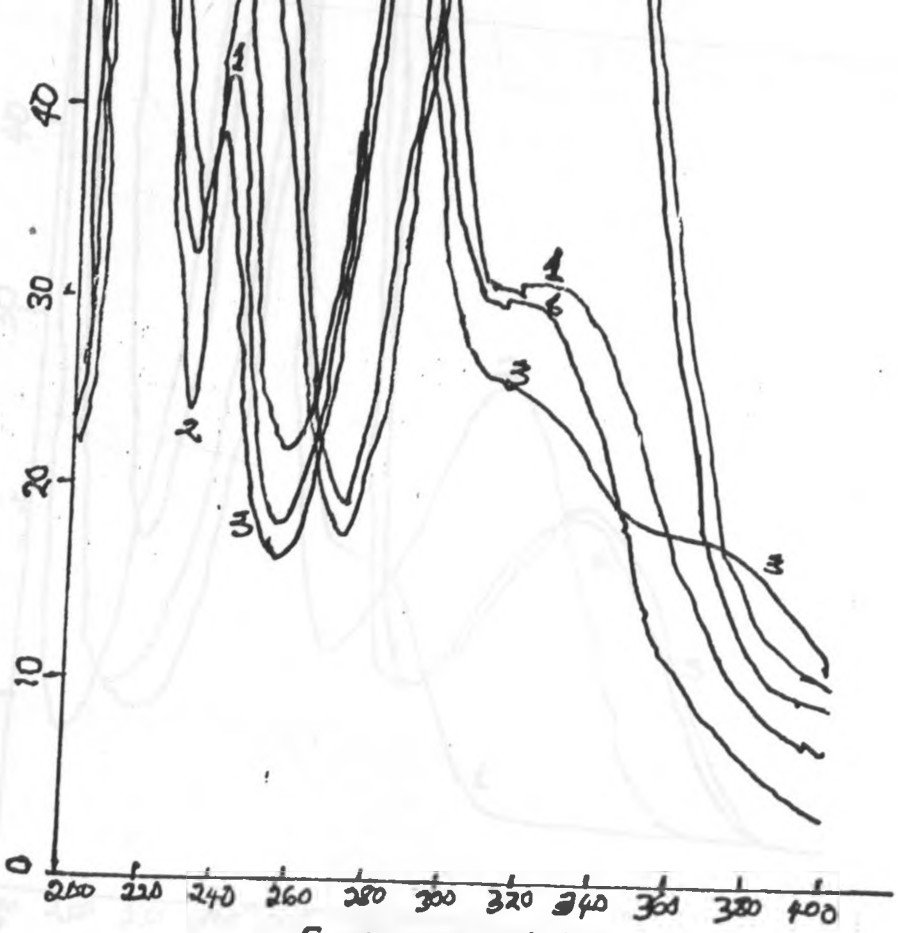

100
90
80
70
60
50
40
30
20
10
0
200
220
240
260
280
300
320
340
360
380
400
1
2
3
4
5
6

Compound 4

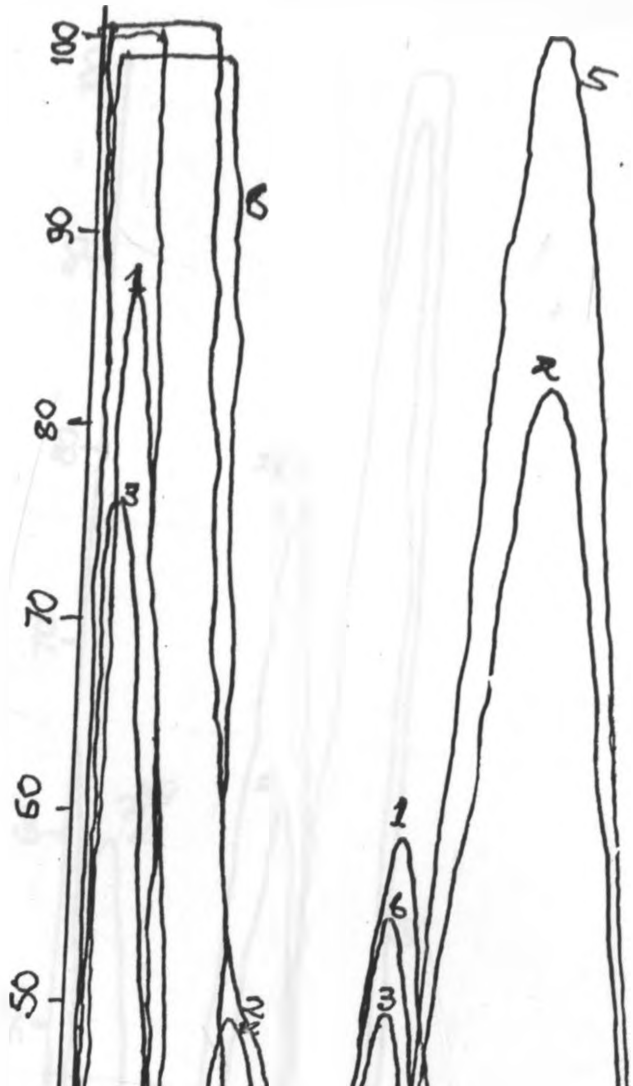

100
90
80
70
60
50
2

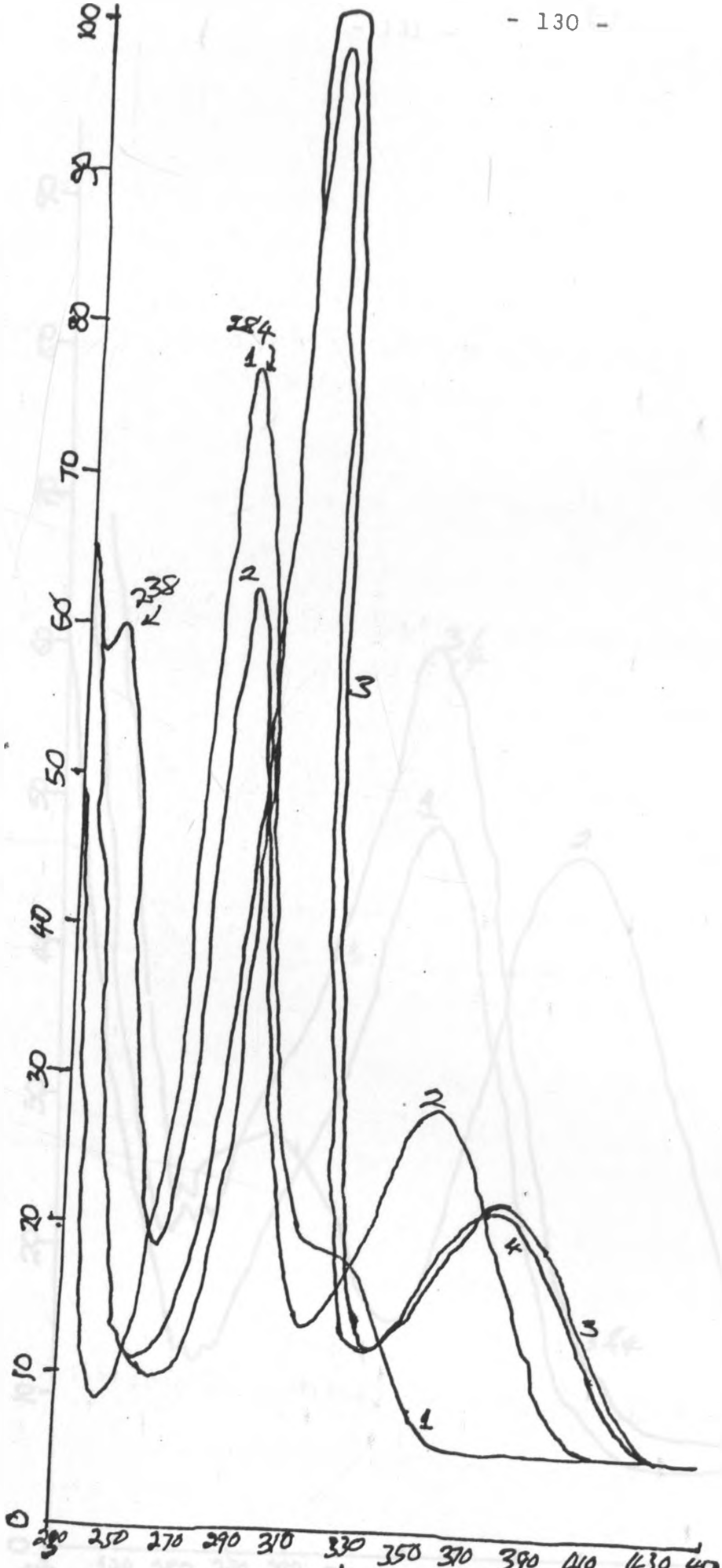

0
10
20
30
40
200
220
240
260
280
300
320
340
360
380
400
1
5
2
3
6
1
3
6
3
6
2
1

Compound 6

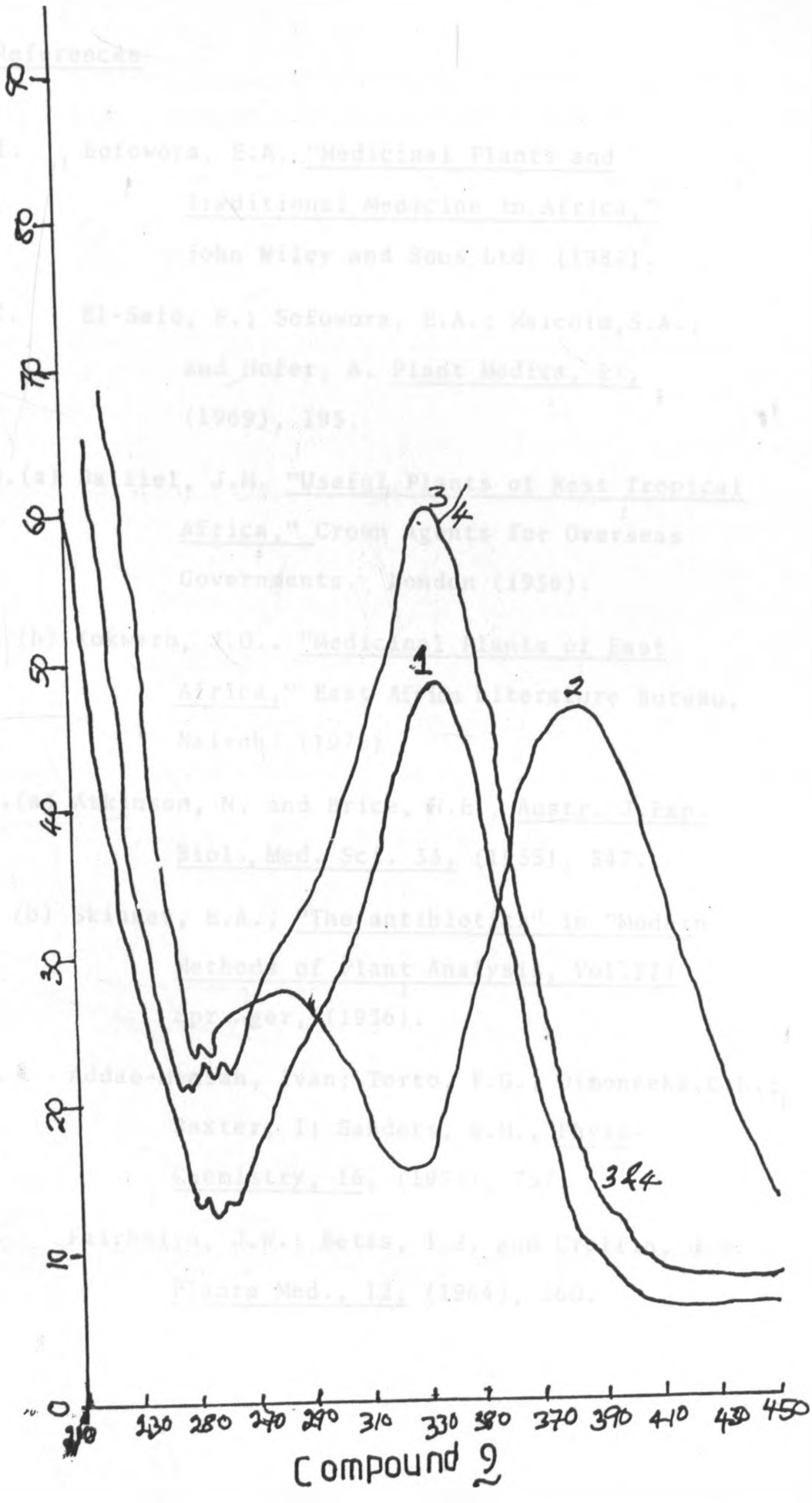


Compound 5


100
90
80
70
60
50
6
1
1a
2
5
1
6
3


100
90
80
70
60
50
40
30
20
10
0
284
238
1
2
3
2
4
3
1
230
250
270
290
310
330
350
370
390
410
430
450

Compound 7 & 8


R
80
70
60
50
40
30
20
10
0
230
250
270
290
310
330
350
370
390
410
430
450
1
2
3
4
3&4
Compound 9

References

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2. El-Said, F.; Sofowora, E.A.; Malcolm,S.A.; and Hofer, A. Plant Medica, 17, (1969), 195.

3.(a) Dalziel, J.M. "Useful Plants of West Tropical Africa," Crown Agents for Overseas Governments. London (1956).

(b) Kokwaro, J.O.. "Medicinal Plants of East Africa," East Africa Literature Bureau, Nairobi (1976)

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