PHYTOCHEMICAL AND SOME BIOACTIVITY STUDIES OF *TECLEA TRICHOCARPA*, *TOVOMITA MANGLE*, *ASPILIA PLURISETA*, *TEPHROSIA HILDEBRANDTII*, AND *MILLETIA THONNINGII*

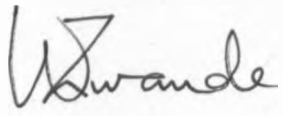
Wilber Lwande

M.Sc., B.Sc., Dip. Ed.

A thesis submitted in fulfilment for the Degree of Doctor of Philosophy in the University of Nairobi.

1984

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



W. Lwande

This thesis is submitted for examination with my approval as University supervisor.

Dr. R. M. Munavu

C O N T E N T S

ACKNOWLEDGEMENTS

I would like to express my gratitude to the International Centre of Insect Physiology and Ecology (ICIPE) for the generous financial and material support that has enabled me to carry out the work for this thesis.

My sincere thanks are due to Dr. A. Hassanali, Dr. R.M. Munavu and Dr. A.N. Mengech for their continuous help and encouragement during the course of this work.

I am very grateful to the Istituto Italo Africano for a fellowship to Italy in 1981. The constant help and guidance given to me by Prof. G.B. Marini Bettolo and Prof. F. Delle Monache at Istituto Chimica, Universita Cattolica del S. Cuore, Rome, Italy, is deeply appreciated.

The interest and encouragement afforded to me by the Director of ICIPE, Prof. T.R. Odhiambo during the course of this work is deeply appreciated. Thanks are also due to my colleagues at the Chemistry and Bioassay research unit, ICIPE, Nairobi, for their kindness and co-operation and to Dr. P. MacDowell for running some of the mass spectra.

Finally, I would like to acknowledge the cheerful help and encouragement of my wife, Winnifred Ajambo.

ABSTRACT

This thesis describes a chemical and bioactivity study of five tropical plant species, namely Teclea trichocarpa Eng., Tovomita mangle G. Mariz., Aspilia pluriseta Schweinf., Tephrosia hildebrandtii Vatke. and Milletia thonningii (Schumach & Thonn.) Bak. From the bark of T. trichocarpa (Rutaceae) has been isolated three 9-acridone alkaloids, melicopicine, tecleanthine and 6-methoxy tecleanthine. Both melicopicine and tecleanthine exhibited mild antifeedant activity against the African Armyworm, Spodoptera exempta while all the three alkaloids showed antimicrobial activity against the fungus, Cladosporium cucumerinum and the bacterium Bacillus subtilis. A chemical study of the root extract of T. mangle (Guttiferae) led to the isolation of two new benzophenones, benzophenone A and B, both containing the unusual lavundalyl chain. Three kauranoid diterpenoids were isolated from the leaves of A. pluriseta (Compositae), a Kenyan folk-medicinal plant that is widely used for the cure of wounds. The antibacterial activity of these compounds against six gram-positive and gram-negative bacteria is reported. From the roots of the shrub T. hildebrandtii (Leguminosae) was isolated a new flavanonol acetate together with the flavone trans-anhydro-techrostachin. The flavanonol acetate exhibited mild antifeedant activity against the African armyworm. Chemical studies on the seeds of

M. thonningii (Leguminosae) led to the isolation of a new pyrano-isoflavone together with three known isoflavones and robustic acid.

Structural elucidation of these compounds was performed on the basis of their spectroscopic data and in some cases by chemical transformations. Antifeedant activity tests against S. exempta were done using the leaf disc bioassay with Zea mays. The antifungal activity was determined by the Cladosporium spray bioassay while antibacterial activity was established by the paper-disc method.

The range of compounds isolated indicate that there may be a vast reservoir of yet untapped bioactive natural products in tropical flora which may be useful as medicines and pesticides or which may serve as useful models for medicinal and pesticidal compounds.

CHAPTER 1

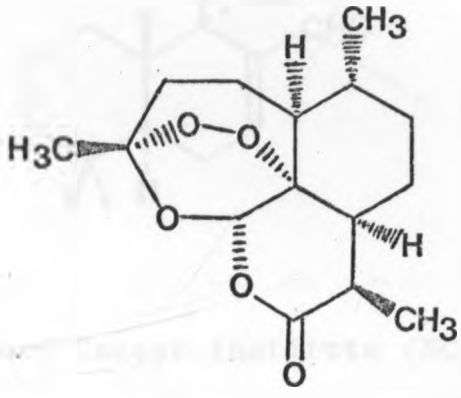
1. I N T R O D U C T I O N

1.0 GENERAL CONSIDERATIONS

Plants have been subjected to predation by a variety of organisms throughout evolutionary time. The degree of predation is particularly intense in the favourable tropical climates where plants, in contrast to their temperate zone counterparts have evolved more efficient and varied chemical defense mechanisms for survival. Tropical flora thus provide a rich source of natural chemical compounds which exhibit useful activity against insect pests and disease organisms. Indeed, crude infusions from a wide variety of plant species have been used for centuries to kill and repel insect pests and vectors and to combart a variety of ailments. Todate, despite the dominance of synthetics, the plant kingdom still serves to provide mankind with more than 25 percent of its useful drugs [1,2]. However, a suprisingly large number of plants remain uninvestigated except perhaps in the antitumor area where support funds have been relatively more forthcoming.

It is for this reason that the World Health Organisation (WHO) has set up more than 6 research centres all over the world to find new and effective

drugs from folk medicinal plants for treating diseases [3]. In this connection, over 22,000 medicinal plants have been documented by WHO [4]. Already, WHO has come up with a number of promising drugs. For instance, from a Chinese folk medicinal herb, Qinghao (Artemisia annua L.), has been isolated and characterized an active antimalarial principle named Qinghaosu (or artemisinine), a sesquiterpene peroxide 1 [5]. The herb

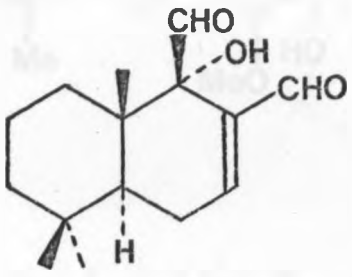


1

has been used in China for Malaria therapy for over 1,000 years. Qinghaosu and its analogues are currently undergoing clinical trials and may prove especially useful against chloroquine-resistant malarial parasites [5].

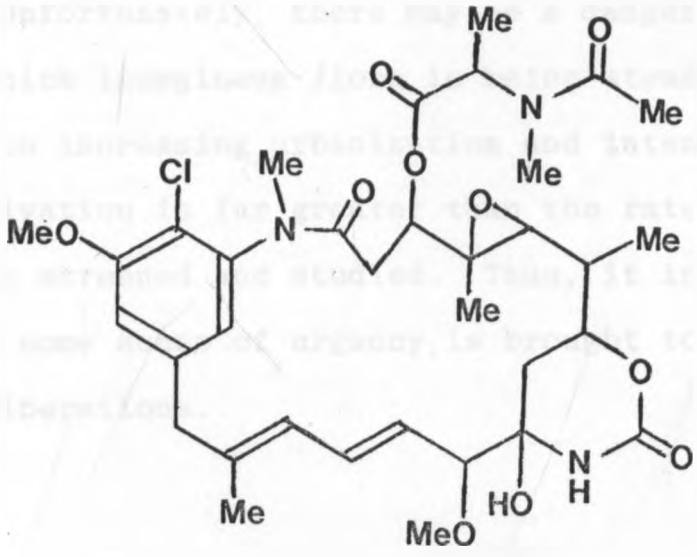
Meanwhile a number of Universities and Research Centres like the International Centre of Insect Physiology and Ecology (ICIPE) in Nairobi, Kenya, have set up research programmes to screen plants for substances that are active against insect pests.

At the ICIPE, many African plants have been screened for insect antifeedant, larvicidal, antijuvenile hormonal, moulting and antimoulting hormonal, repellant and insecticidal activities. This programme has led to the isolation of several bioactive natural products including warbuganal 2, the most potent antifeedant compound against the African armyworm, Spodoptera exempta, a very serious crop pest in East Africa [6].



2

The National Cancer Institute (NCI) in the United States has been screening thousands of plants from all over the world for possible presence of tumor inhibitors. Presently, over 750,000 plant species are candidates for consideration as sources of tumor inhibitors [7]. One of the most interesting results was the isolation of a potent antileukemic ansa macrolide, maytansine 3 from a number of Maytenus plant species including Maytenus buchananii, 15,000kg of which were collected in Kenya in 1976 for the isolation of maytansine [8]. Maytansine has recently been undergoing clinical trials under the auspices of the National Cancer Institute [9,10].



3

Of interest is the fact that M. buchananii had for centuries been used earlier in folk medicine in Kenya for the cure of leukemia [11].

Although Africa is extremely rich in diverse types of plant species many of which have been widely used in folk medicine, very little research work has been done in Africa in this field as compared to countries like China where of the 5,000 chinese folk medicinal plant species, 1,000 have already been commercialized, giving rise to 1.2 million US dollars in earnings per year [12]. It is for this reason that the Scientific Technical and Research Commission (STRC) of the Organisation of African Unity (OAU) is trying to promote research on medicinal plants. Already, STRC is

publishing the Journal of African Medicinal Plants and a newsletter on Research into African Medicinal plants.

Unfortunately, there may be a danger that the rate at which indeginous flora is being steadily wiped out due to increasing urbanization and intensification of cultivation is far greater than the rate at which it is being screened and studied. Thus, it is important that some sense of urgency is brought to these considerations.

1.1. AIM OF THE PROJECT

The aim of this work was fwo-fold:-

1. To isolate and characterize chemical compounds from five tropical plant species, namely:

a) *Teclea trichocarpa* Eng.

b) *Tovomita mangle* G. Mariz.

c) *Aspilia pluriseta* Schweinf.

d) *Tephrosia hildebrandtii* Vatke.

e) *Milletia thonningii* (Schumach & Thonn.) Bak.

Isolation of the compounds was to be carried out by solvent extraction of parts of the plants followed by separation of the extracts using chromatographic techniques, while structure elucidation was to be performed by spectroscopy and chemical transformations.

2. To carry out some bioactivity tests on compounds isolated from the above plant species. The bioactivity tests were to include insect antifeedant activity tests against the African armyworm, *Spodoptera exempta* by the leaf-disc bioassay with *Zea mays* [41], antibacterial activity tests against the bacteria *Escherichia coli*, *Bacillus mycoides*, *Macrococcus luteus*, *Bacillus subtilis*, *Staphylococcus aureus* and *Xanthomonas pelargonii* by the paper disc method [100], and anti-fungal tests against the fungus *Cladosporium cucumerinum* by the *Cladosporium* spray bioassay [76].

1.2. PREVIOUS WORK ON THE GENERA TECLEA DELILE., TOVOMITA AUBL., ASPILIA THOU., TEPHROSIA PERS. AND MILLETIA WRIGHT & ARN.

1.2.1 Teclea Delile.

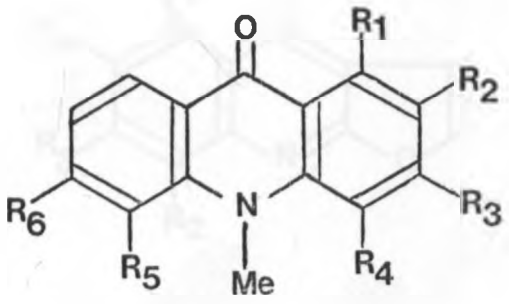
The genus Teclea Delile. belongs to the family Rutaceae and subfamily Toddalioideae [13, 14]. It comprises of trees and shrubs, with over 30 known species that are distributed in Tropical Africa, Comoro Islands and Madagascar [15].

Several species of Teclea have been used in folk medicine in Africa for the treatment of a variety of ailments, while one species has been employed in exorcising and the fruits of another are known to be edible [11, 16, 17]. Table 1 lists the uses of some Teclea species.

Teclea is known to be rich in alkaloids [14]. In phytochemical studies of seven Teclea species, seven acridone alkaloids (Table 2) [18-26], ten furoquinoline alkaloids (Table 3) [18, 21, 27-32] and one O-amino-benzophenone alkaloid 23 [21, 33, 34] have so far been isolated. Both the acridone and furoquinoline alkaloids are considered to be biogenetically derived from anthranilic acid 24 [14]. The isolation of tecleanone 23 supports the proposal that acridone alkaloids are formed via condensation of anthranilic acid 24 and acetate [35]. Acridone alkaloids may be of value as

Table 1. Uses of some Teclea species

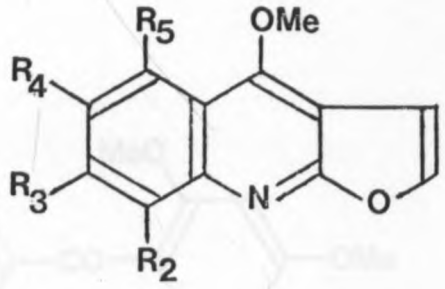
Plant species	Uses	Region	References
T. nobilis	For treatment of fever, pneumonia, rheumatism, gonorrhea, syphilis and as an anthelmintic	E. Africa	11, 16
T. pilosa	For treatment of heart pain	E. Africa	11, 16
T. emaniensis	For treatment of headache	E. Africa	16
T. simplicifolia	For treatment of pneumonia and leaf-lung diseases	E. Africa	16
T. unifoliata	For treatment of gonorrhea and wounds	E. Africa	16
T. utilis	For driving away spirits	E. and S. Africa	16
Trafzelii	Fruit edible	W. Africa	17
T. grandifolia	Stems used as chewsticks For treatment of cough	Ghana	17 17



4

Table 2. Acridone alkaloids isolated from Teclea [18-26]

Acridone alkaloids	Substitution in 4					
	R_1	R_2	R_3	R_4	R_5	R_6
1,3, 4-Trimethoxy-10-methyl acridone 5	OMe		OMe	OMe		
1,3, 5-Trimethoxy-10-methyl acridone 6	OMe		OMe		OMe	
Arborinine 7	OH	OMe	OMe			
Melicopicine 8	OMe	OMe	OMe	OMe		
Tecleanthine 9	OMe	O-CH_2-O			OMe	
6-Methoxy tecleanthine 10	OMe	O-CH_2-O			OMe	OMe
Evoxanthine 11	OMe	O-CH_2-O				

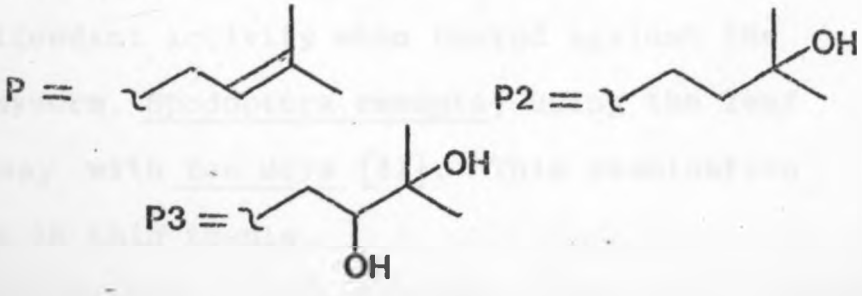


12

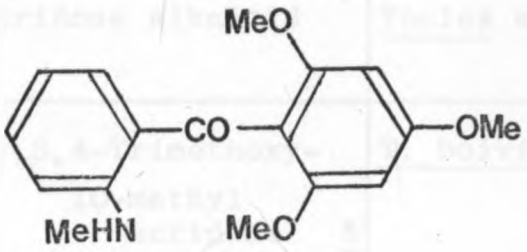
Table 3 Furoquinoline alkaloids isolated from Teclea Delile. [18, 21, 27, 32]

Furoquinoline alkaloid		Substitution in 12			
		R_2	R_3	R_4	R_5
Halfordinine	13	OMe	OMe	OMe	
Kokusaginine	14		OMe	OMe	
Skimmianine	15	OMe	OMe		
Evoxine	16	OMe	P3		
Nkolbisine	17		OMe	P2	
Flindersiamine	18	OMe	$O-CH_2-O$		
Tecleaverdoornine	19	OH	$O-CH_2-O$		P
Tecleaverdine	20	OH	$O-CH_2-O$		P2
Tecleine	21	OH	$O-CH_2-O$		
Maculine	22		$O-CH_2-O$		

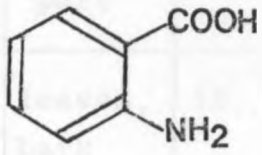
Formulae to table 3.



chemotaxonomic indicators for this genus [14]. Table 4 gives the distribution of the acridone alkaloids from Teclea.



23



24

Two phenylethylamines [30, 36], three tetranor-triterpenes [32, 37], six flavanosides [38, 39] and lupeol [21, 29, 36, 38, 40] have also been reported to occur in this genus.

1.2.1.1 Teclea trichocarpa Eng.

Teclea trichocarpa Eng. is a small evergreen tree which in Kenya grows mainly in forest areas of the Coast and Central Provinces [13]. No previous phytochemical or biological activity work has so far been reported on it. The search for insect anti-feedant compounds from natural sources led to the examination of extracts from the bark of T.trichocarpa which, in a random screening programme, revealed potent antifeedant activity when tested against the African armyworm, Spodoptera exempta, using the leaf disc bioassay with Zea mays [41]. This examination is recorded in this thesis.

Table 4 The distribution of acridone alkaloids in *Teclea* Delile [18-26]

Acridone alkaloid	*Teclea* species	Plant part	References
1,3,4-Trimethoxy-10-methyl acridone 5	*T. boiviniana*	leaves, bark	18, 20
1,3,5-Trimethoxy-10-methyl acridone 6	*T. boiviniana*	bark	20
Arborinine 7	*T. boiviniana*	leaves	18
	T. natalensis		19
Melicopicine 8	*T. boiviniana*	bark	20
Tecleanthine 9	*T. boiviniana*	leaves, bark.	18, 20
	T. verdoorniana	root — bark	21
	T. natalensis	bark	19, 26
6-Methoxytecleanthine 10	*T. boiviniana*	leaves	
		bark	18, 20
Evoxanthine 11	*T. boiviniana*	leaves, bark	18, 20
	T. verdoorniana	root bark	21,22,23
	T. grandifolia	bark	24,25
	T. natalensis.		19

1.2.2 Tovomita Aubl.

The genus Tovomita Aubl. belongs to the family Guttiferae and subfamily Clusioideae [42]. It is known to contain over 60 species which are centred mainly in Central and Tropical South America [15].

Chemical studies on three Tovomita species have led to the isolation of four new Xanthones: tovoxanthone (25) from T. choisyana Pl. et Tr. [43], tovophyllin A (26) and B (27) from T. macrophylla (Pl. et Tr.) Walp. [44] and manglexanthone (28) from T. mangle G. Mariz [42]. Also isolated were betulinic acid from T. choisyana [43], T. macrophylla [44] and T. mangle [42], sitosterol from T. choisyana and T. macrophylla [43, 44], stigmasterol from T. choisyana [43] and β-amyrin from T. macrophylla [44].

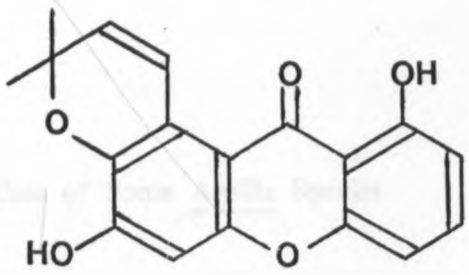
1.2.2.1 Tovomita mangle G. Mariz

Later in this thesis, a chemical study of the roots of Tovomita mangle G. Mariz will be described.

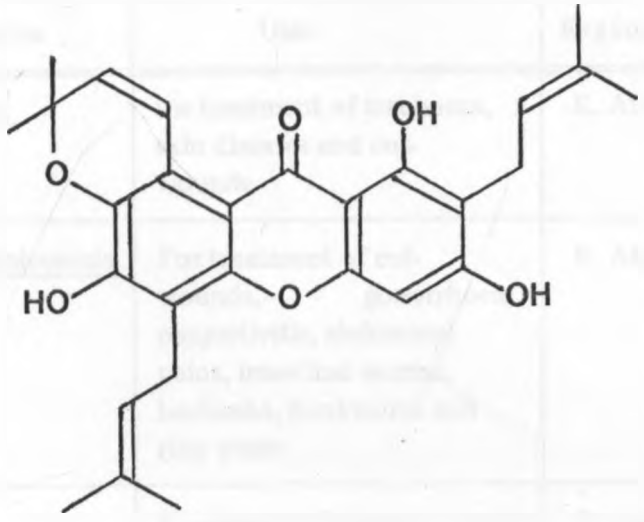
1.2.3 Aspilia Thou.

Aspilia Thou. is a large genus belonging to the family Compositae, subfamily Heliantheae and the subtribe Ecliptinae [13, 45]. Over 125 species of Aspilia are known to occur in tropical Africa, Central and South America, and in Madagascar [15].

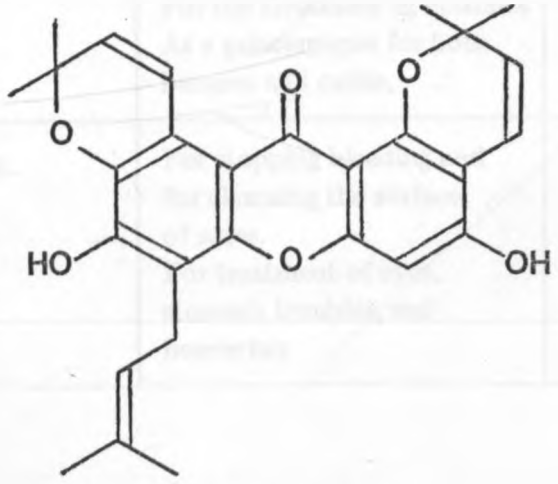
Table 5 lists the uses of some Aspilia species. Most noteworthy is the use of a number of them for the cure of cut wounds. In East Africa, the pounded



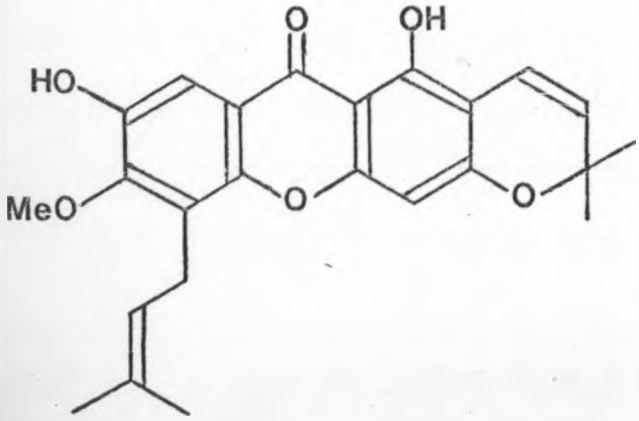
25



26



27



28

Table 5 Uses of Some Aspilia Species

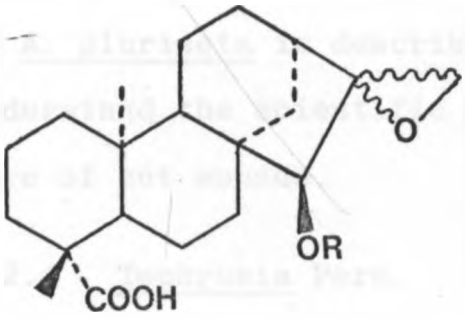
Aspilia species	Uses	Region	References
A. pluriseta	for treatment of trachoma, skin diseases and cut-wounds	E. Africa	11
A. mossambiccensis	For treatment of cut-wounds, gonorrhoea, conjuctivitis, abdominal pains, intestinal worms, backache, hookworm and ring worm.	E. Africa	11
A. hostii	For relief of lumbago, sciatica and neuralgia.	S. Africa	16
	For the treatment of eclamsia	S. Africa	16
	As a galactagogue for both humans and cattle,		
A. latifolia	For stopping bleeding and for cleansing the surface of sores.	W. Africa	17
	For treatment of eyes, stomach troubles, and headaches	W. Africa	17

leaves of Aspilia pluriseta and Aspilia mossambiccensis have been widely used for the cure of cut wounds by their application onto the wounds [11]. Similarly, the bruised leaves and flowers of Aspilia latifolia are widely used in parts of West Africa for the same purpose [16]. In W. Africa A. latifolia is known to be quite effective in stopping bleeding, hence its common name the "haemorrhage plant." [16].

Chemical studies on one Aspilia species, A. parvifolia led to the isolation of a range of terpenoids. From the roots and aerial parts of A. parvifolia was isolated as its methyl ester the tiglate epoxide 29, the angelate epoxide 30, ent-kaurenic acid 31, the angelate ester 32, the tiglate esters 33 and 34, ent-kaurane-16-ol 35, ent-kauren-19-al 36, ent-kauren-19-ol 37, the thiopheneacetylene 38, α and β pinene, γ-humulene, sitosterol, ent-manool, 6-acetyl-2,2-dimethyl chromene, spathulenol, phellandrene, p-cymene, β-farnesene, germacrene and α-gurjunene [46].

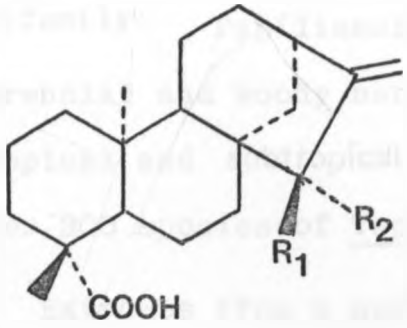
1.2.3.1 Aspilia pluriseta Schweinf.

Aspilia pluriseta Schweinf. is a woody shrub that grows abundantly in most parts of Kenya, especially in the black cotton and dry bushed grasslands [47]. No phytochemical or biological activity studies have been reported on this plant.



29. R = Tiglate

30. R = Angelate

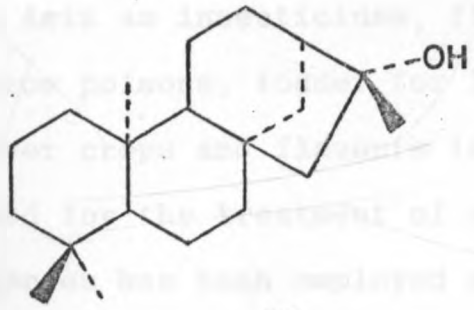


31. $R_1 = R_2 = H$

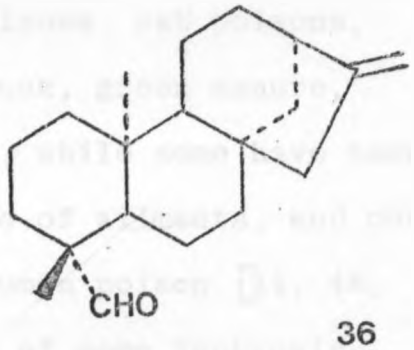
32. R_1 = OAng, R_2 = H

33. R_1 = OTigl., R_2 = H

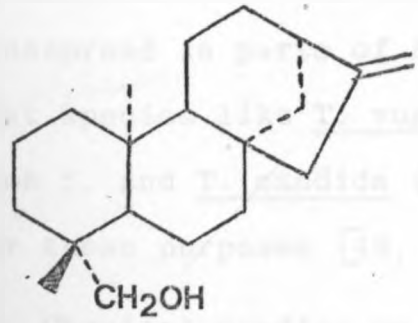
34. R_1 = H, R_2 = OTigl.



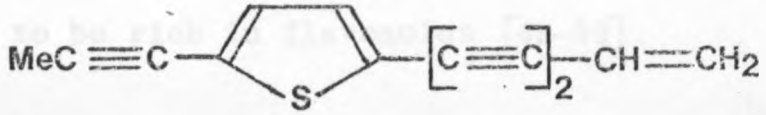
35



36



37



38

In this thesis, a chemical and bioactivity study of A. pluriseta is described in an attempt to understand the scientific basis for its use in the cure of cut wounds.

1.2.4 Tephrosia Pers.

The genus Tephrosia Pers. (Family: Leguminosae, Subfamily: Papilionoideae) is a large genus of perennial and woody herbs that are distributed in the tropical and subtropical regions of the world [13, 48]. Over 300 species of Tephrosia are known [15].

Extracts from a number of Tephrosia species have been used in Africa, Central and South America and in Asia as insecticides, fish poisons, rat poisons, arrow poisons, fodder for livestock, green manure, cover crops and flavours in milk, while some have been used for the treatment of a range of ailments, and one species has been employed as a human poison [11, 48, 16, 17]. Table 6 lists the uses of some Tephrosia species. The use of extracts from Tephrosia species as insecticides and fish poisons has been quite widespread in parts of Africa and Asia to the extent that species like T. vogelli Hook f., T. densiflora Hook f. and T. candida (Roxb.) DC. have been cultivated for these purposes [48, 16].

Chemical studies on twenty one species of Tephrosia have shown it to be rich in flavonoids [49-59].

Table 6 Uses of some Tephrosia species

Plant Species	Uses	Region	References
T. aequilata Bak.	For treatment of abdominal pains and venereal diseases.	E. Africa	16
T. anselli	As fodder for horses, cattle, sheep and goats.	W. Africa	17
T. atroviolacea E. G. Bak.	Root administered to women after birth.	Tanzania	16
T. bracteolata	As fodder for horses	W. Africa	17
T. candida D.C	As a fish poison and cover crop.	S. Himalaya, E. Bengal, Burma, and Congo	16
		E. Africa	48
T. capensis Pers.	As an arrow poison and emetic for biliousness.	S. Africa	16
	For treatment of weak heart and nervousness	S. Africa	16
T. dasyphylla Welw. ex Bak.	As a fish poison.	S. Zimbabwe	16
T. densiflora Hook. f.	As a fish and arrow poison.	Tropical Africa and S. Africa	16, 17
T. diffusa Harv.	As a parasiticide	S. Africa	16

Table 6 Contd.....

Plant Species	Uses	Region	References
T. elegans Schum. & Thonn.	As an arrow poison.	S. Africa	16
T. kraussiana Meisn.	For treatment of cough.	S. Africa	16
T. linearis	As fodder for horses, sheep, cattle and goats.	W. Africa	18
	Twigs used as brooms	W. Africa	18
	As a flavour in milk.	W. Africa	18
T. lupinifolia D.C.	For abortion and committing suicide in women.	S. Africa	16
T. macropoda Harv.	As a fish poison, insecticide and anthelmintic in cattle.	S. Africa	16
T. noctiflora	For treatment of cough.	E. Africa	11
	Administered to women after birth.	E. Africa	11
T. paucijuga	For treatment of wounds	E. Africa	11
T. pentaphylla	For treatment of sore throat and cold.	E. Africa	11
T. pumila	For treatment of cold and venereal diseases.	E. Africa	11

Table 6 Contd.....

Plant Species	Uses	Region	References
T. purpurea	For treatment of stomach pains, snake bites and headache.	E. Africa	11
	As a fish poison	W. E. and S.E. Africa, E. Indies, Queensland, S. Asia, E. Indies	16, 17
	As a flavour in milk, green manure and cover crop.	W. Africa	16, 17
	As a purgative.	E. Africa and Vietnam.	11, 16
	As an anthelmintic	E. Indies	16
T. purpurea	As a deobstruent and diuretic.	India	16
	For treatment of fevers, coughs, tightness of chest, biliary and splenic troubles.	India	16
T. toxicaria	As an insecticide, fish and arrow poison.	S. Africa, E. Indies	16
	As a source of a blue dye	S. Mexico, C. America, W. Indies and S. Pacific Islands.	16

Table 6 Contd.....

Plant Species	Uses	Region	References
T. vogelli Hook f.	As a fish poison, insecticide and arrow poison.	Tropical Africa	16, 17
	For treatment of sores, skin diseases in dogs and goats, toothache, scabies, yaws and constipation.	S., C. and E. Africa	11, 16, 17
	As an abortifacient	S. Africa	16
	As an ornamental plant	E. Africa	11
T. villosa	For treatment of pain in the liver and spleen.	E. Africa	11

Figure 1. illustrates the proposed biogenetic relationship of the major structural types of flavonoids isolated from Tephrosia [49]. A range of flavanones 40' and 40'.1 [49-53], chalcones 40.1 [51, 54], one flavanonol 40.2 [49], flavonols 40.2.1 [49, 55], β- hydroxy chalcones 40.3 and 40.3.1 [49], flavones 40.4 and 40.4.1 [49, 56, 57], isoflavones 40.5, 40.5.1, 40.5.2 and 40.5.3 [49], and rotenoids 40.6 and 40.6.1 [49, 58, 59] have so far been isolated from Tephrosia. All these flavonoids are considered to be biogenetically derived from a common cinnamate-triacetate precursor, initially of chalcones 40 [49]. In addition, a number of them are O-methylated to varying degrees and the majority of them sustain mevalonate-derived C-5 chains in the form of unmodified or oxidatively modified prenyl side chains [49, 59]. Prominent among the flavones is a group of 5, 7-oxygenated and 7-oxygenated compounds that are characterized by the occurrence of a C-8 prenyl unit which has, in many cases, undergone substitution and cyclization of varying degree of complexity. Figure 2 shows the various types of prenyl derived substituents in flavonoids from Tephrosia. It would appear that the complex substituents at C-8 arise from the ability of Tephrosia species to initially oxidise a 7-OMe group to a methyleneoxy cation, $-\overset{+}{O}=CH_2$, in the same way that closely related species of Leguminosae, as well as a number of Tephrosia species, oxidise the 2'-OMe group of isoflavonoids to yield rotenoids [60].

Figure 1. Biogenetic relationships of major structural types of flavonoids in Tephrosia.

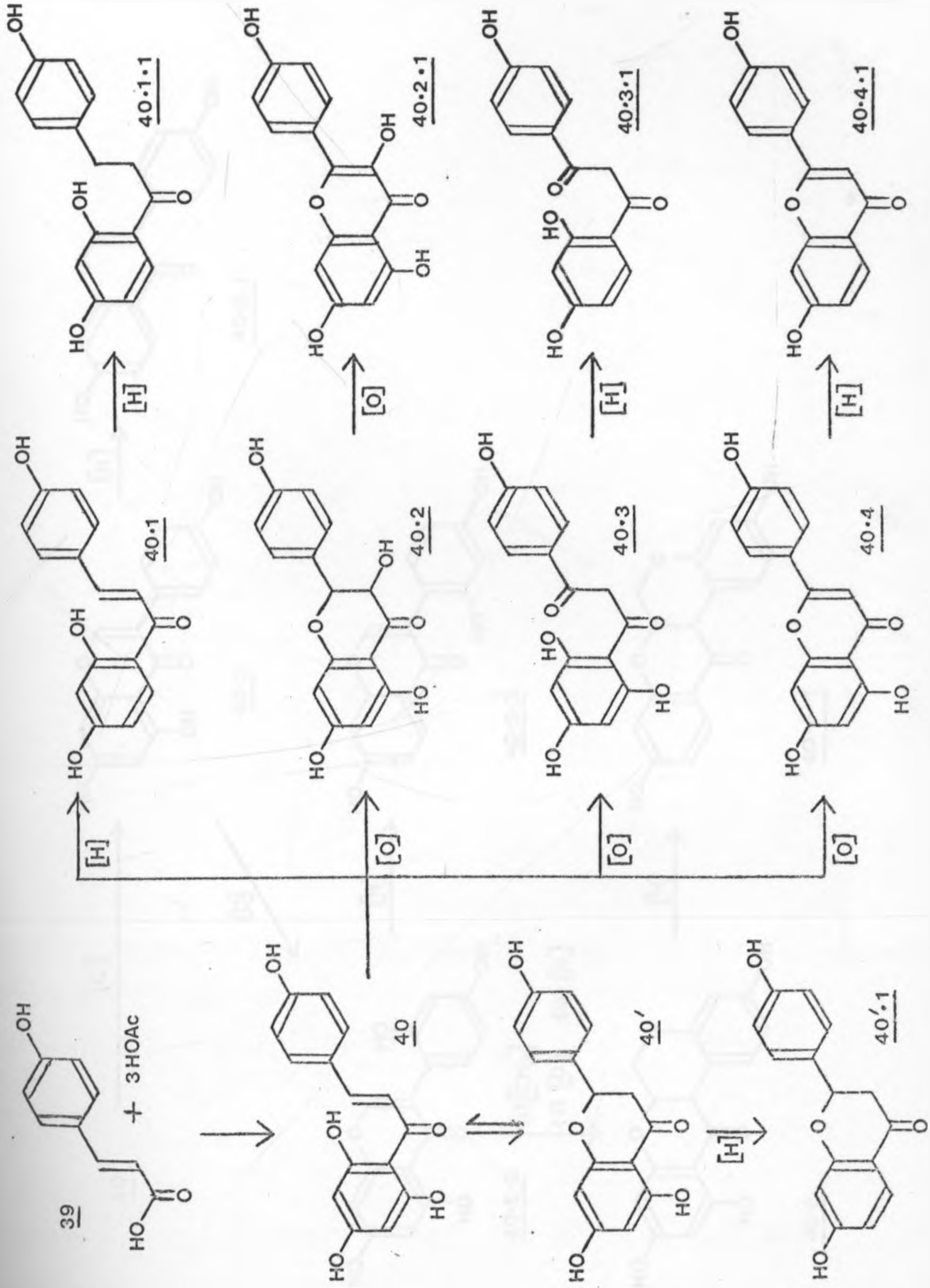
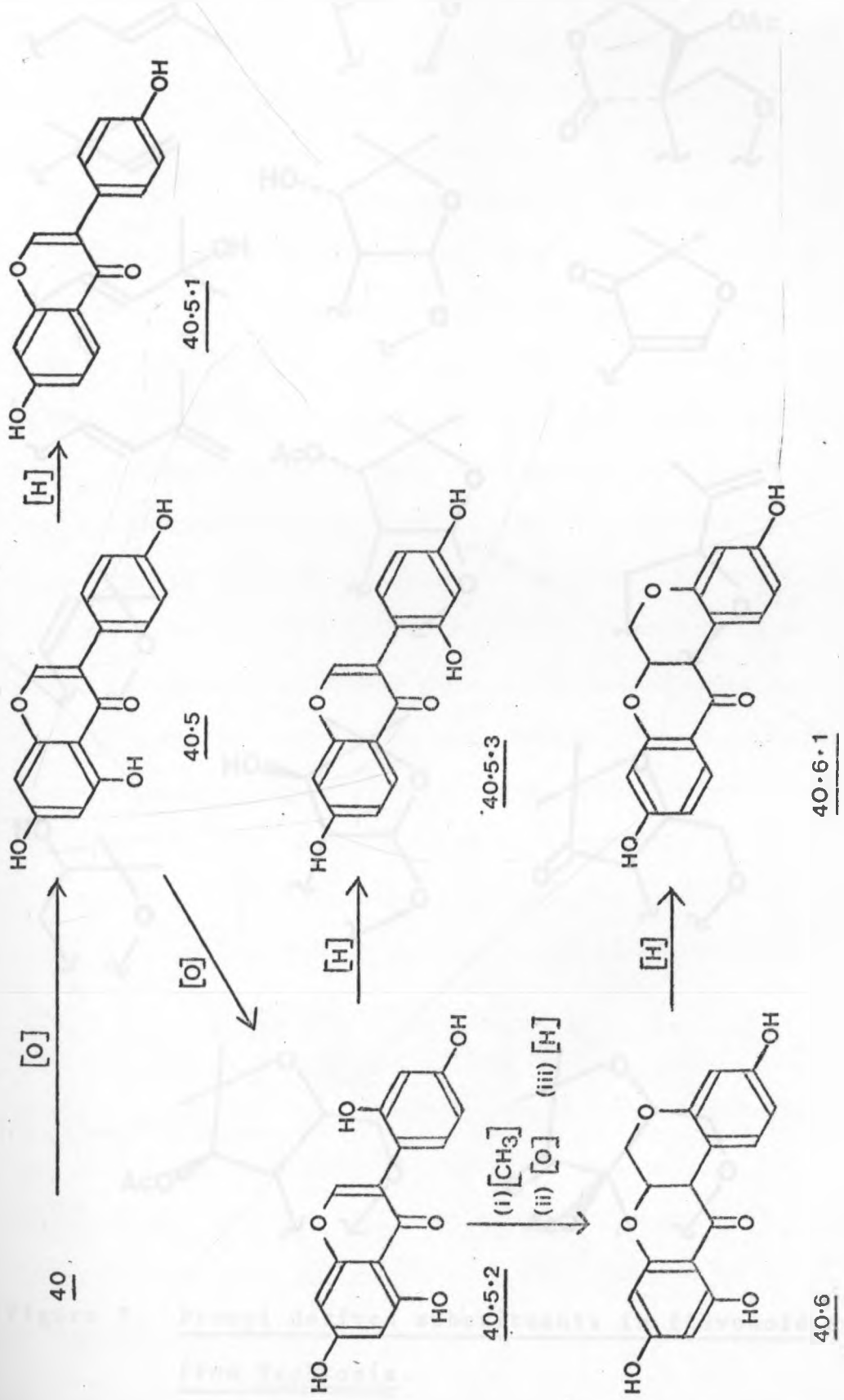


Figure 1. Contd.....



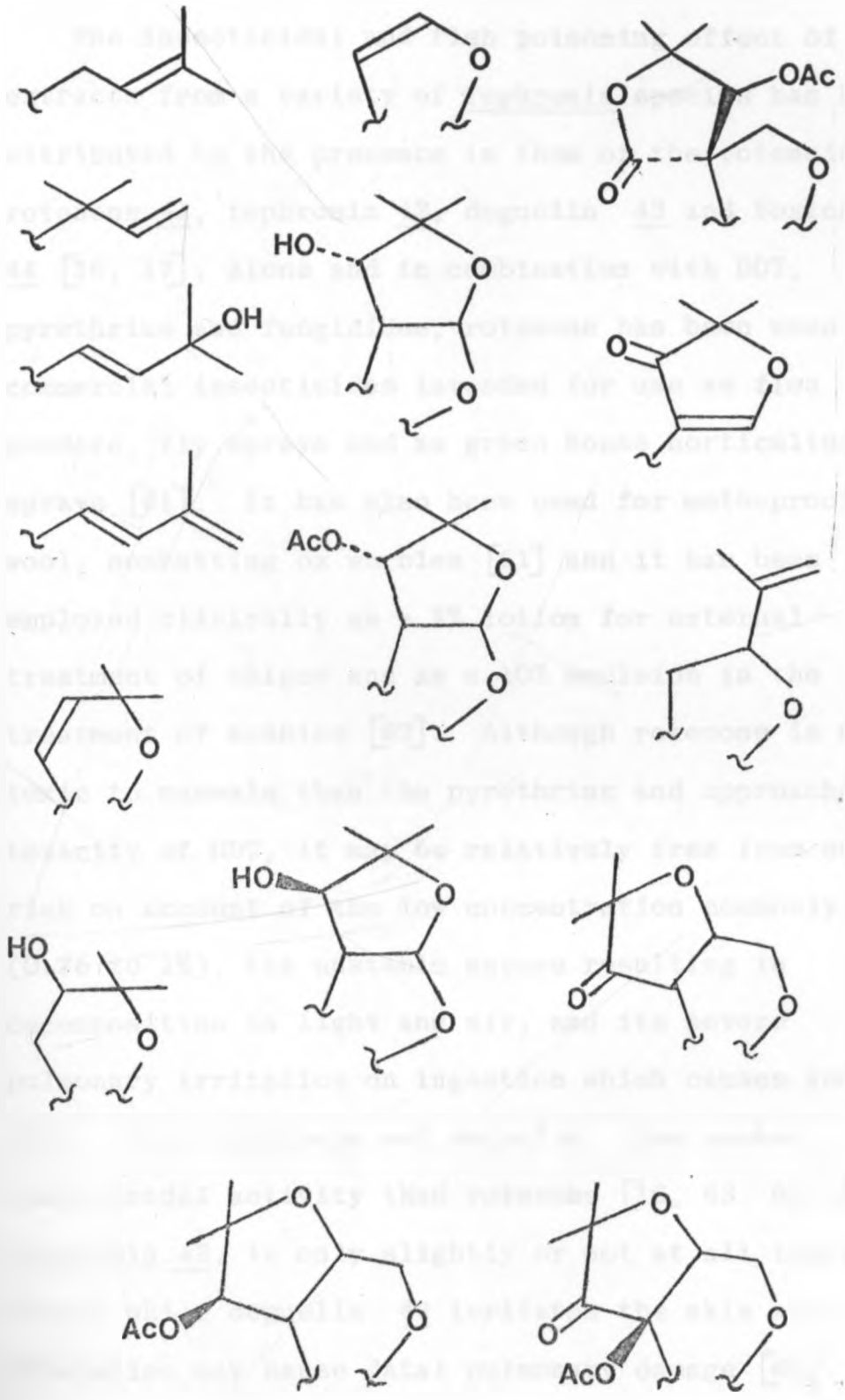
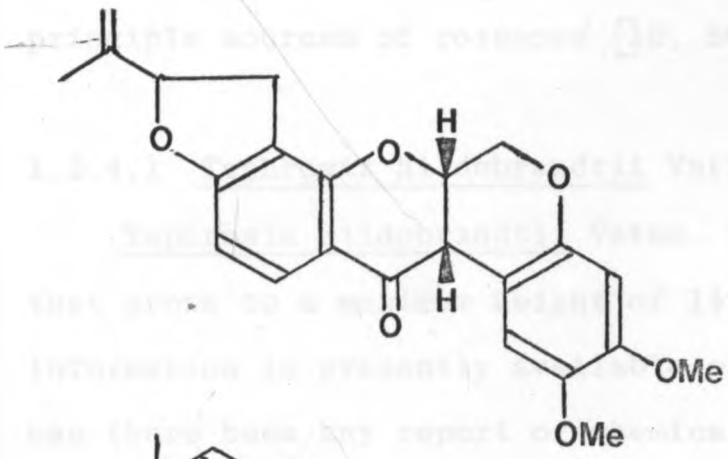
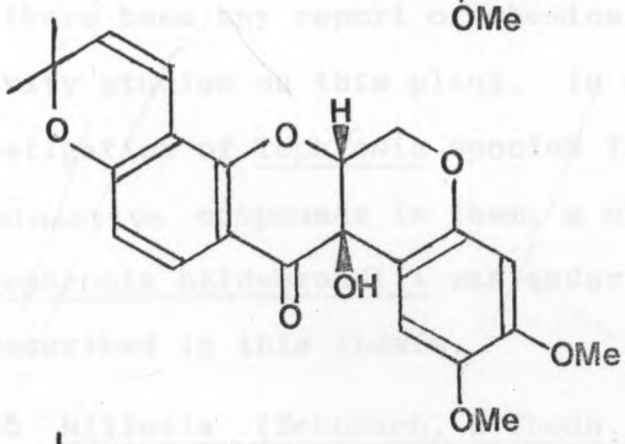


Figure 2. Prenyl derived substituents in flavonoids from Tephrosia.

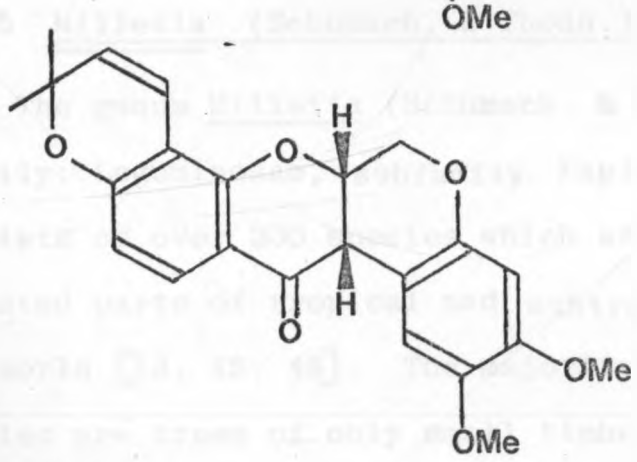
The insecticidal and fish poisoning effect of extracts from a variety of Tephrosia species has been attributed to the presence in them of the rotenoids rotenone 41, tephrosin 42, deguelin 43 and toxicarol 44 [16, 17]. Alone and in combination with DDT, pyrethrins and fungicides, rotenone has been used in commercial insecticides intended for use as flea powders, fly sprays and as green house horticultural sprays [61]. It has also been used for moth-proofing wool, combatting ox warbles [61] and it has been employed clinically as a 2% lotion for external treatment of chigoe and as a 10% emulsion in the treatment of scabies [62]. Although rotenone is more toxic to mammals than the pyrethrins and approaches the toxicity of DDT, it may be relatively free from serious risk on account of the low concentration commonly used (0.75 to 1%), its unstable nature resulting in decomposition in light and air, and its severe pulmonary irritation on ingestion which causes vomiting [62]. Both tephrosin and deguelin show weaker insecticidal activity than rotenone [16, 63, 64, 65]. Tephrosin 42, is only slightly or not at all toxic to humans while deguelin 43 irritates the skin and inhalation may cause fatal pulmonary damage [65]. Toxicarol 44 is highly toxic to fish but almost non-toxic to insects [66, 67]. None of the tephrosia species that have been examined contain as much rotenone, deguelin and other insecticidal constituents as are


H
O
O
O
O
H
O
OMe
OMe

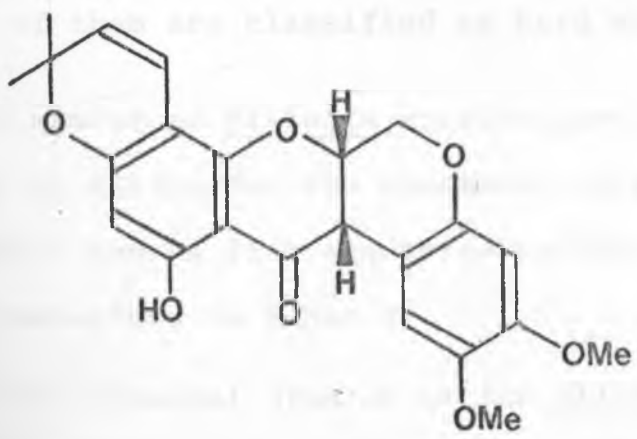
41


H
O
O
O
OH
O
OMe
OMe

42


H
O
O
O
H
O
OMe
OMe

43


H
O
O
O
O
H
HO
O
OMe
OMe

44

found in derris or cubē roots, which have been the principle sources of rotenone [16, 50, 59, 60, 65].

1.2.4.1 Tephrosia hildebrandtii Vatke.

Tephrosia hildebrandtii Vatke. is a small shrub that grows to a maximum height of 1½ feet. No information is presently available on its uses, nor has there been any report of chemical or biological activity studies on this plant. In a systematic investigation of Tephrosia species for possible presence of bioactive compounds in them, a study of the roots of Tephrosia hildebrandtii was undertaken. This study is described in this thesis.

1.2.5 Milletia (Schumach. & Thonn.) Bak.

The genus Milletia (Schumach. & Thonn.) Bak. (Family: Leguminosae, subfamily Papilionoideae) consists of over 200 species which are distributed in forested parts of tropical and subtropical regions of the world [13, 15, 48]. The majority of Milletia species are trees of only small timber size although some of them are classified as hard woods [17].

A number of Milletia species have been used in parts of Africa for the treatment of a variety of ailments and as fish and arrow poisons. These uses are summarized in Table 7.

Phytochemical studies on ten Milletia species have shown them to be rich in flavonoids of structural

Table 7 Uses of some *Milletia* species

Milletia species	Uses	Region	References
M. lasiantha	As an aphrodisiac	E. Africa	11
M. makondensis	For treatment of toothache	E. Africa	11
M. usaramensis	For treatment of snake bite	E. Africa	11
M. oblata	For treatment of stomach-ache, coughs, swollen parts of the body, and bladder troubles.	E. Africa	11
	For treatment of bladder	S. Africa	
M. eriocalyx	For treatment of skin eruptions	E. Africa	16
M. ferruginea	As a fish poison	S. Africa	16
M. grandis	As a vermifuge particularly for round worms.	S. Africa	16
	As a fish and arrow poison	S. Africa	16

types similar to the ones from Tephrosia (Fig. 1). A range of flavanones 40'.1 [49, 68], chalcones 40 and 40.1 [49, 68], dihydrochalcones 40.1.1 [49], flavanonols 40.2 [69], flavonols 40.2.1 [49], β-hydroxychalcones 40.3 and 40.3.1 [49], flavones 40.4 and 40.4.1 [49], isoflavones 40.5, 40.5.1, 40.5.2 and 40.5.3 [49, 69-71] and rotenoids 40.6 and 40.6.1 [49, 72] have so far been isolated from Milletia. The similarity of the flavonoids from Tephrosia and Milletia provides a taxonomic support for their placement in the same subfamily.

1.2.5.1 Milletia thonningii (Schumach & Thorn.) Bak.

Milletia thonningii is a decidous tree growing to a height of 30 to 40 ft. In this thesis, a phytochemical study of the seeds of M.thonningii which was carried out on the plant, is described.

CHAPTER 2

2. RESULTS AND DISCUSSION

2.1 9-ACRIDONE INSECT ANTIFEEDANT ALKALOIDS FROM THE BARK OF TECLEA TRICHOCARPA Eng.

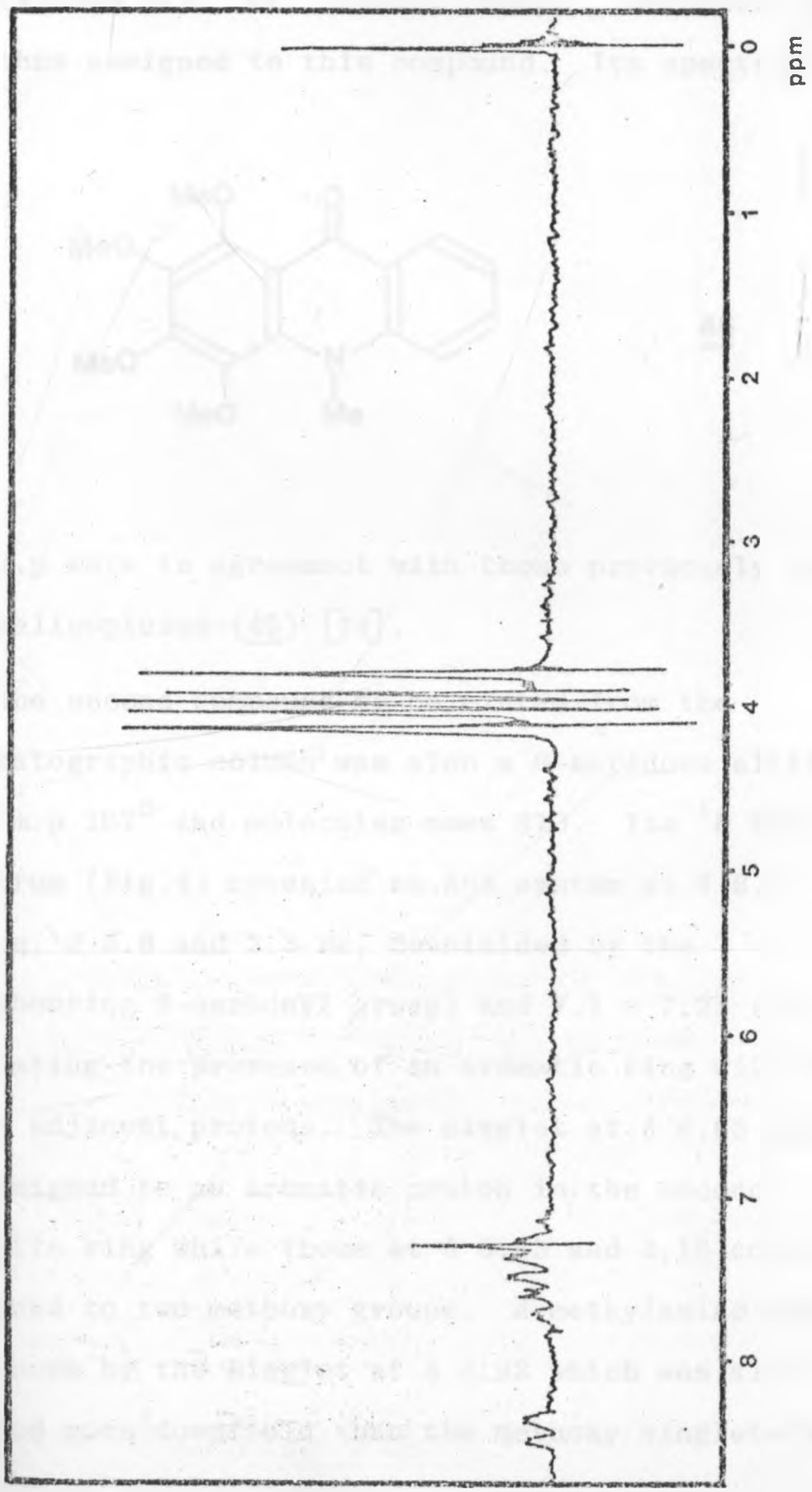
The dried ground bark of Teclea trichocarpa Eng. was extracted with methanol. Separation of the methanol extract by column chromatography using silica gel and chloroform-ethyl acetate (4:1 v/v) as eluant afforded three chromatographically pure compounds.

The first compound to be eluted from the chromatographic column, m.p 133-134°, had Molecular mass 329, an IR band at 1740 cm^{-1}, UV bands at 203sh, 218, 268, 310sh and 397 nm and a ^{1}H NMR spectrum (Fig.3) which, in addition to chemotaxonomic considerations, suggested it was a 9-acridone alkaloid.

The ^{1}H NMR spectrum of the compound showed the presence of four methoxy groups at δ 3.80, 3.97, 4.02 and 4.11; and one methylamino group at δ 3.91. The signal of the methylamino group was shifted more downfield than the methoxy singlets in trifluoroacetic acid [73].

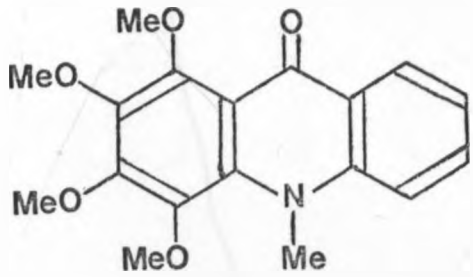
The doublet of a doublet centred at δ 8.41 (1H, deshielded by the neighbouring 9-carbonyl group) and the multiplet at δ 7.78 - 7.10 (3H) suggested that

Figure 3. ^{1}H NMR spectrum of melicopicine (45) in deuterochloroform.



there was present an aromatic ring with four adjacent protons.

The second aromatic ring could then be fully substituted with four methoxy groups. Structure 45 was thus assigned to this compound. Its spectral data

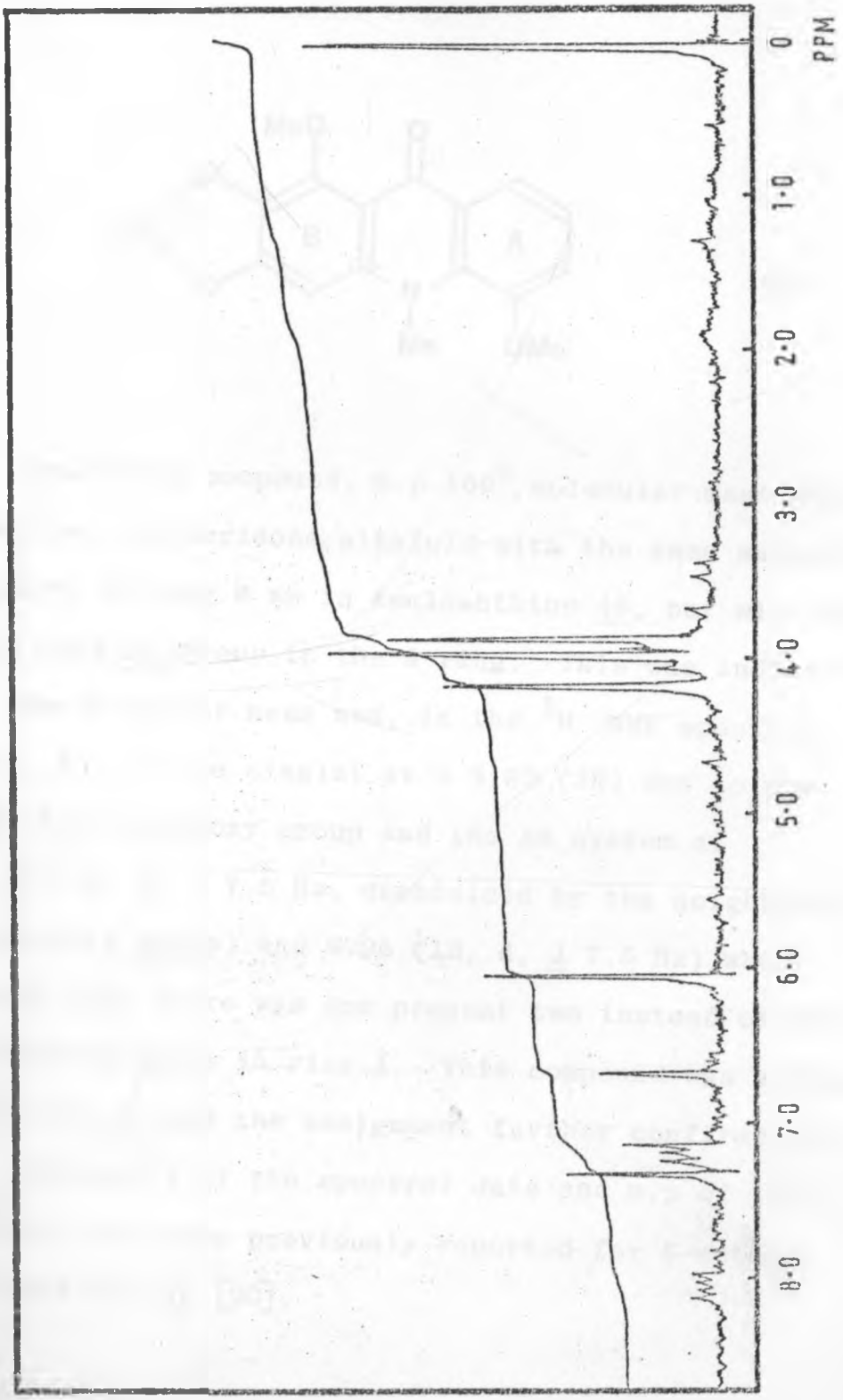


45

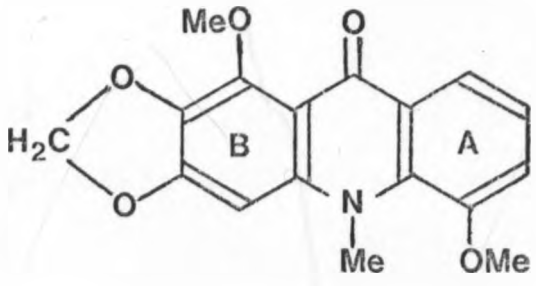
and m.p were in agreement with those previously reported for melicopicine (45) [74].

The second compound to be eluted from the chromatographic column was also a 9-acridone alkaloid with m.p 157^{o} and molecular mass 313. Its ^{1}H NMR spectrum (Fig.4) revealed an ABX system at δ 8.2 (1H, q, J 6.6 and 3.3 Hz, deshielded by the neighbouring 9-carbonyl group) and 7.1 - 7.21 (2H, m) indicating the presence of an aromatic ring with only three adjacent protons. The singlet at δ 6.65 could be assigned to an aromatic proton in the second aromatic ring while those at δ 3.83 and 4.15 could be assigned to two methoxy groups. A methylamino group was shown by the singlet at δ 3.92 which was also shifted more downfield than the methoxy singlets in

Figure 4. ^{1}H NMR spectrum of tecleanthine (46) in deuterochloroform.



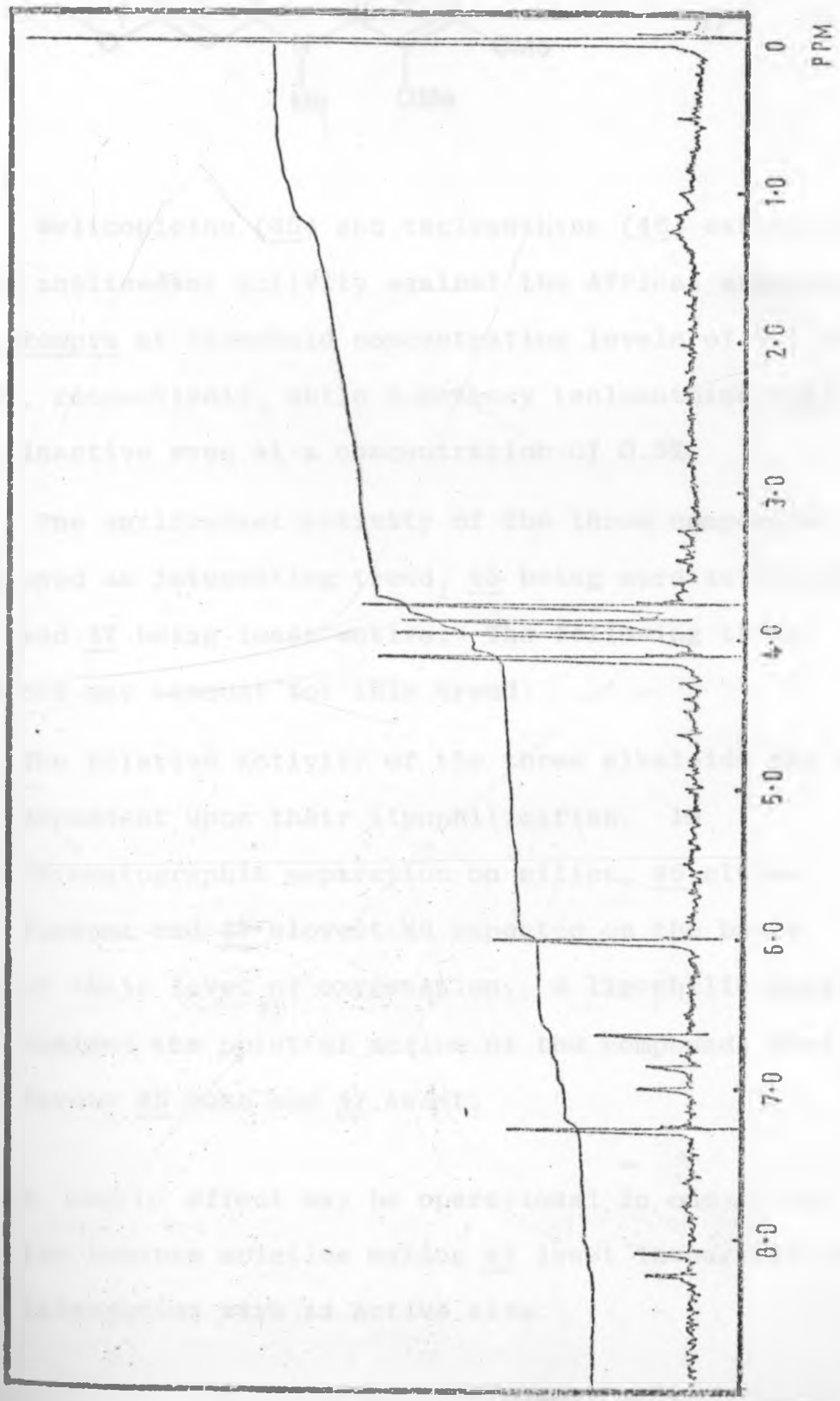
trifluoroacetic acid. The singlet at δ 6.02 (2H) was typical of a methylene dioxy group. The spectral data and m.p of this compound were found to be in agreement with those previously reported for tecleanthine (46) [19].

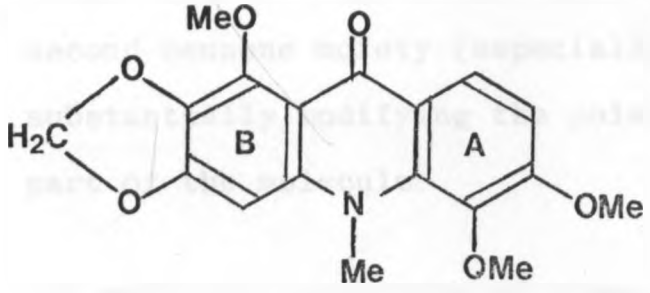


46

The third compound, m.p 169°, molecular mass 343, was also a 9-acridone alkaloid with the same substitution pattern in ring B as in tecleanthine 46, but with one more methoxy group in the A ring. This was indicated by the molecular mass and, in the ^1H NMR spectrum (Fig. 5), by the singlet at δ 3.80 (3H) due to the additional methoxy group and the AB system at δ 8.2 (1H, d, J 7.5 Hz, deshielded by the neighbouring 9-carbonyl group) and 6.95 (1H, d, J 7.5 Hz) which showed that there was now present two instead of three adjacent protons in ring A. This compound was assigned structure 47 and the assignment further confirmed by the similarity of the spectral data and m.p of the compound to those previously reported for 6-methoxy tecleanthine 47 [20].

Figure 5. ^{1}H NMR spectrum of 6-methoxy tecleanthine 47 in deuterochloroform.





47

Melicopicine (45) and tecleanthine (46) exhibited mild antifeedant activity against the African armyworm, S. exempta at threshold concentration levels of 0.1 and 0.5%, respectively, while 6-methoxy tecleanthine (47) was inactive even at a concentration of 0.5%.

The antifeedant activity of the three compounds followed an interesting trend, 45 being more active than 46, and 47 being least active. The following three factors may account for this trend:

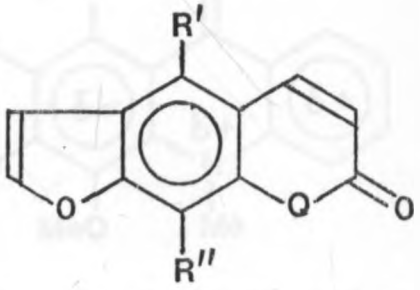
a) The relative activity of the three alkaloids may be dependent upon their lipophilicities. In chromatographic separation on silica, 45 elutes fastest and 47 slowest as expected on the basis of their level of oxygenation. A lipophilic barrier against the point of action of the compounds would favour 45 most and 47 least.

b) A steric effect may be operational in one of the two benzene moieties making 47 least favourable in interaction with an active site.

c) The overall charge distribution in the acridone nucleus may be important, with methoxylation of the second benzene moiety (especially in the 6 position) substantially modifying the polarisation of this part of the molecule.

The relative importance of these factors will become evident when other substituted acridones are tested for antifeedant activity. However, it is interesting to make a tentative comparison with a structurally similar furocoumarin group of antifeedants which have been tested against Spodoptera litura [75]. In this class, structural differences and, in particular, the pattern of oxygen substitution were found to be much more important than relative lipophilicity of the compounds. Thus bergapten (48) and isopimpinelline (49) have the same level of activity (despite obvious differences in polarity), while xanthotoxin (50) and psoralin (51) which lack a methoxy function at the 4-position have a relatively low activity.

There is an interesting hint of similarity in charge distribution between acridones and furocoumarins (Fig. 6) suggesting that one of the methoxy oxygens in bergapten and isopimpinelline may play the same role as the carbonyl oxygen of acridones and that a triply or quadruply oxygenated benzene group of acridones may fulfil the function



	R'	R''
48.	OMe	H
49.	OMe	OMe
50.	H	OMe
51.	H	H

of the fused furan moiety which has been shown to be important for antifeedant activity of the furocoumarins [75].

In view of the speculation outlined above, it may be informative to undertake a closer comparative study of structure-activity relationships in the two classes of compounds using the same species of experimental insects. In particular it would be helpful to determine if in fact there is a common physiological basis of action in the two sets of compounds. It may be noted that one of the main difficulties in structure-activity studies in insect antifeedants, is that they represent physiologically diverse groups and that the mode of action of most classes of compounds are yet to be investigated.

All the three alkaloids showed antifungal activity when tested against the fungus, Cladosporium cucumerinum,

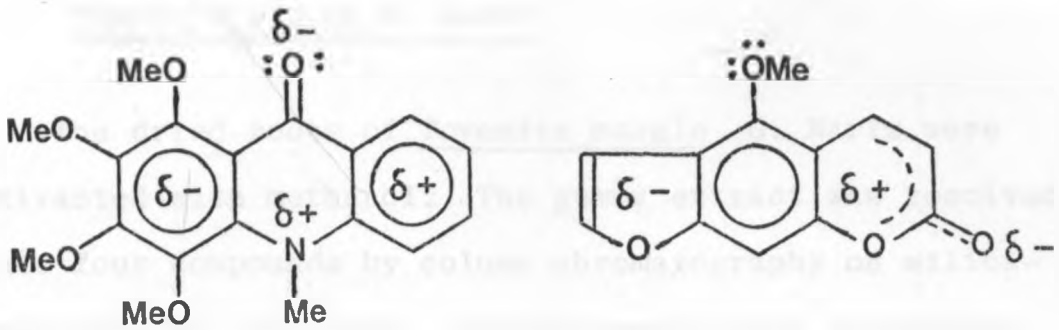


FiG 6. An acridone and a furocoumarin antifeedant

by the Cladosporium spray bioassay [76] (see experimental section). Using the paper disc method (see experimental section), the compounds also showed activity against the bacterium Bacillus subtilis, above a concentration level of 200 µg/disc.

Melicopicine (45), tecleanthine (46) and 6-methoxy tecleanthine (47) were first isolated from Melicope fareana [74], Teclea natalensis [19] and Teclea boiviniana [20] respectively. Subsequently, the distribution of tecleanthine and 6-methoxy tecleanthine was found to be restricted largely to the genus Teclea, while melicopicine appears to have a wider taxonomic distribution, having also been isolated from the genera Melicope and Acronychia, both belonging to the family Rutaceae [77].

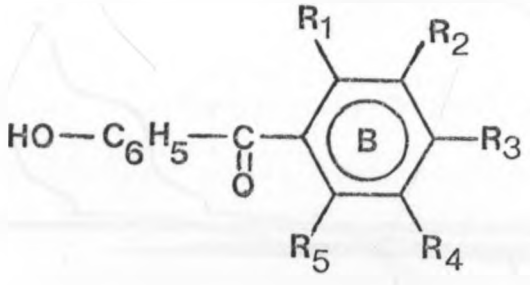
2.2 TWO NEW BENZOPHENONES WITH LAVUNDALYL CHAIN FROM TOVOMITA MANGLE G. MARIZ.

The dried roots of Tovomita mangle G. Mariz were extracted with methanol. The gummy extract was resolved into four compounds by column chromatography on silica-gel using as the eluant, benzene containing increasing quantities of ethyl acetate.

The first compound to be eluted from the column was an unknown oil, named tovophenone A. Tovophenone A, molecular mass 464, showed negative rotatory power. Its ^1H NMR spectrum showed two peaks at δ 8.53 (1H) and 8.30 (1H) which were exchangeable with D_2O, a multiplet at δ 7.30 - 6.60 corresponding to four aromatic protons, a broad singlet at δ 4.53 (1H) suggestive of two terminal methylene groups, $=CH_2$, and a singlet at δ 3.68 indicative of an aromatic methoxy group. The signals at δ 5.10 (broad t, 1H), δ 3.20 (broad d, 2H) and δ 1.70 (broad s, 6H) were indicative of a γ,γ-dimethylallyl chain, $-CH_2-CH=C(CH_3)_2$ [78].

The IR spectrum of tovophenone A showed a broad band at ν_{max} 3500 to 3200 cm^{-1} depicting the presence of an H-bonded hydroxyl function, intense bands at ν_{max} 1610 and 1580 cm^{-1} due to a carbonyl group and a band at ν_{max} 880 cm^{-1} which confirmed the presence of terminal methylene groups.

The UV-visible spectrum [λ_{max} 235, 266 and 304 nm (logε 4.07, 3.89, 4.08)], the peaks at ν_{max} 1610 and 1580 cm^{-1} in the IR spectrum as well as the fragment ions at m/e 121 ($HO\text{-}C_6H_4\text{-}C{\equiv}O^+$) and 93 ($HO\text{-}C_6H_4^+$) in the mass spectrum were consistent with a polyhydroxylated benzophenone with one mono-hydroxylated ring(52).

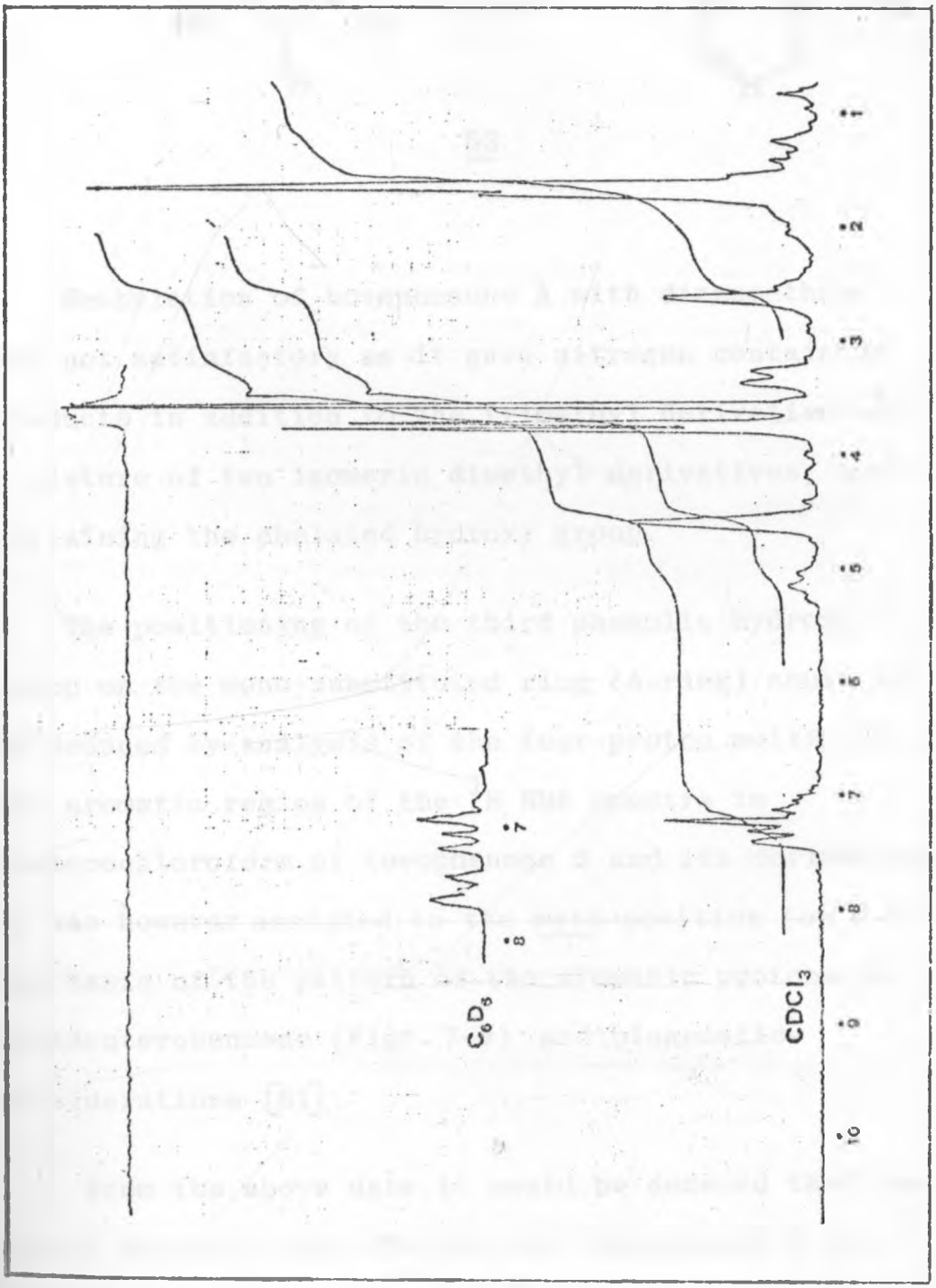


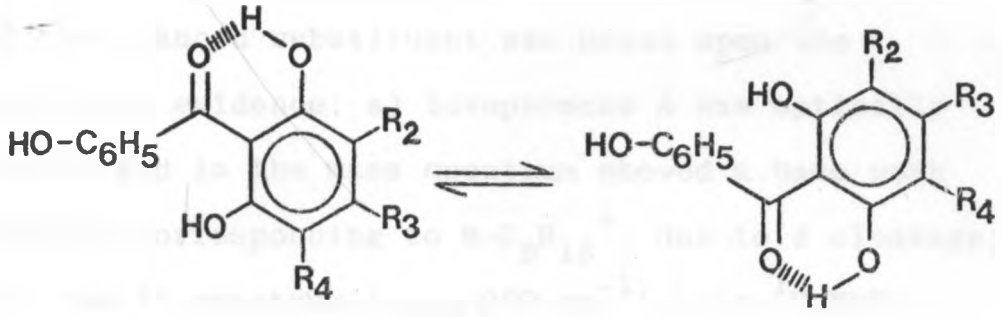
52

On acetylation with acetic anhydride in pyridine, tovophenone A gave the triacetyl derivative while on methylation with dimethyl sulfate-potassium acetate, it yielded the trimethyl derivative , showing that there was present in tovophenone A, three aromatic hydroxyl groups. Figure 7 shows the ^1H NMR spectrum of the trimethyl derivative.

Thus, the two signals at δ 8.53 and 8.30 in the ^1H NMR spectrum of tovophenone A were due to two H-bonded hydroxyl groups. At first sight, the chemical shifts of these signals may appear too low for H-bonded hydroxyl groups but they are normal values for 2,6-dihydroxy benzophenones where chelation with the carbonyl group is shared between the two hydroxyl groups (53)[79, 80].

Figure 7. ^{1}H NMR spectrum of the trimethyl derivative of tovophenone A in deuterochloroform and deuterobenzene.





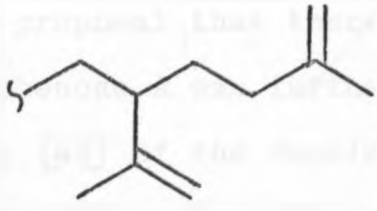
53

Methylation of tovophenone A with diazomethane was not satisfactory as it gave nitrogen containing products in addition to the trimethyl derivative and a mixture of two isomeric dimethyl derivatives, both containing the chelated hydroxy group.

The positioning of the third phenolic hydroxy group on the mono-substituted ring (A-ring) could not be deduced by analysis of the four-proton multiplet in the aromatic region of the ^{1}H NMR spectra in deuterochloroform of tovophenone A and its derivatives. It was however assigned to the meta-position (on C-3') on the basis of the pattern of the aromatic protons in hexadeuterobenzene (Figs. 7-9) and biogenetic considerations [81].

From the above data it could be deduced that the second aromatic ring (B-ring) of tovophenone A was totally substituted by one methoxy group, two hydroxyl groups (on C-2 and C-6), one γ,γ-dimethyl allyl chain

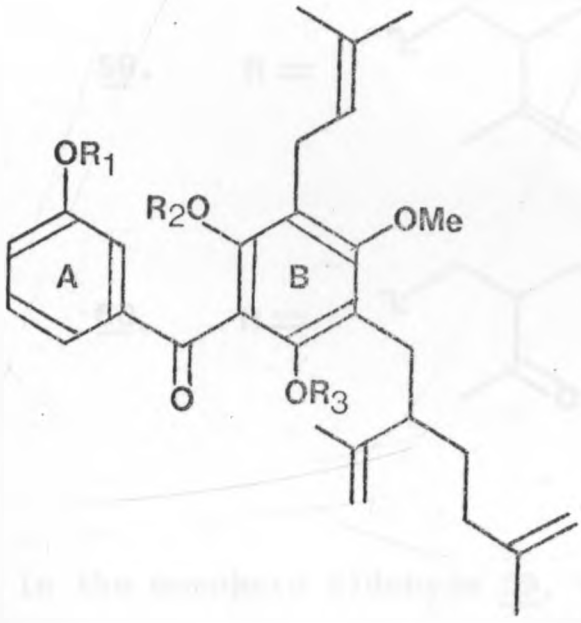
and one yet undetermined substituent. Formulation of the unknown substituent was based upon the following evidence: a) tovophenone A was optically active and in the mass spectrum showed a base peak (M-123) corresponding to $M-C_9H_{15}^+$, due to β cleavage; b) the IR spectrum (ν_{max} 880 cm^{-1}), the ^1H NMR (δ 4.53, broad singlet, 4H) and the ^{13}C NMR (two triplets at δ 111.5 and 109.4 in the trimethyl derivative) indicated the presence of two terminal methylene groups, which, together with two further vinyl methyl groups (δ 1.70) accounted for two isopropenyl groups; c) the broad multiplet (3H) around δ 2.6 (Fig. 7) could be attributed to the benzylic methylene and the allylic methine, while the other two methylene groups gave signals between δ 2.10 and 1.30, partially hidden by the isopropenyl methyl signals. The above data suggested that the unknown substituent was a lavundalyl chain (54).



54

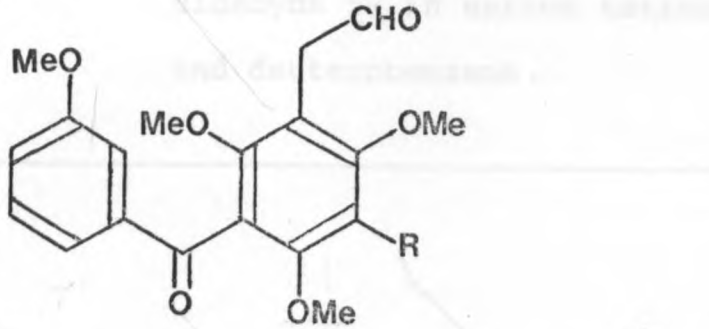
The delay in the occurrence of the bathochromic shift in the UV spectrum (λ_{max} 266 to 335 nm) on addition of aluminium chloride indicated the presence

of bulky substituents ortho to the 2,6-dihydroxyl system [82]. This suggested that the bulky γ,γ-dimethyl allyl chain and the proposed lavundalyl chain were ortho to the 2,6-dihydroxyl groups. Thus, the structure of tovophenone A was proposed as being 55 and those of the triacetyl, dimethyl and trimethyl derivatives as 56, 57 and 58 respectively.

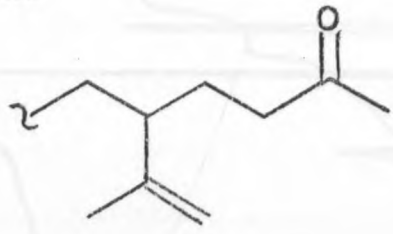


	R_1	R_2	R_3
55.	H	H	H
56.	Ac	Ac	Ac
57.	Me	Me	H
58.	Me	Me	Me

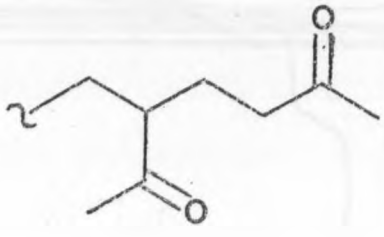
The proposal that there was a lavundalyl chain in tovophenone A was further verified by a one-step cleavage [83] of the double bonds in tovophenone A with OsO_4-HIO_4. Depending on the time of the reaction, this process on the trimethyl derivative 53 resulted in the formation of two compounds which were formulated as 59 and 60.



59. R =

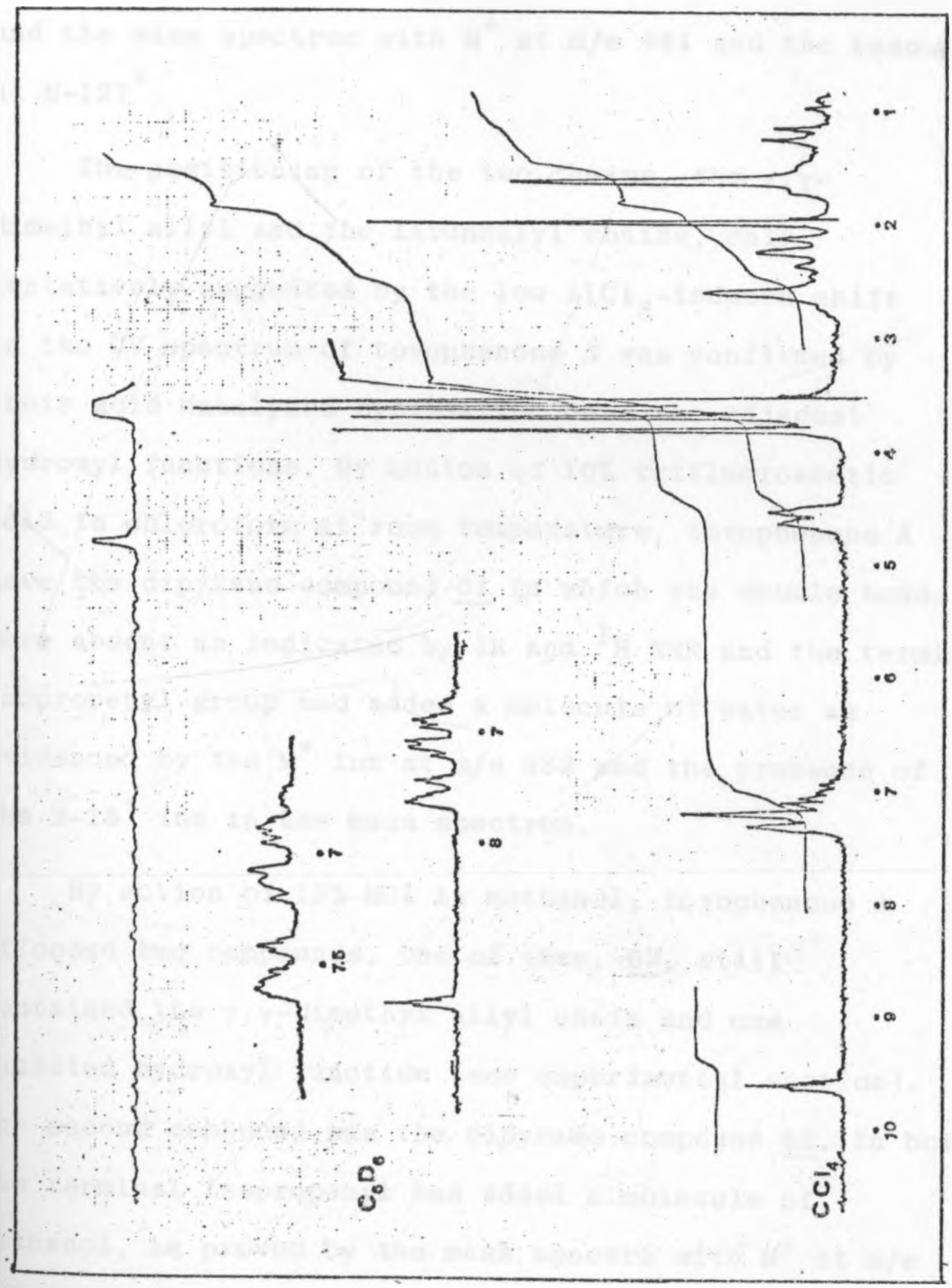


60. R =



In the monoketo aldehyde 59, the γ,γ-dimethyl allyl chain was cleaved to $-CH_2CHO$ while only one terminal methylene of the lavundalyl chain was affected. This was evident in the IR spectrum which showed peaks at ν_{max} 1730-1710 and 896 cm^{-1} due to carbonyl and terminal methylene functions. The ^1H NMR spectrum (Fig. 8) showed a singlet at δ 9.60 due to -CHO, signals at δ 4.60 (1H), 4.50 (1H), and 1.63 (3H) due to one isopropenyl group, and a singlet at δ 1.98 (3H) due to the $-CO-CH_3$ group. The mass spectrum of 59, with M^+ at m/e 482 and the base peak at $M-125^+$ instead

Figure 8. ^{1}H NMR spectrum of the monoketo aldehyde 59 in carbon tetrachloride and deuterobenzene.

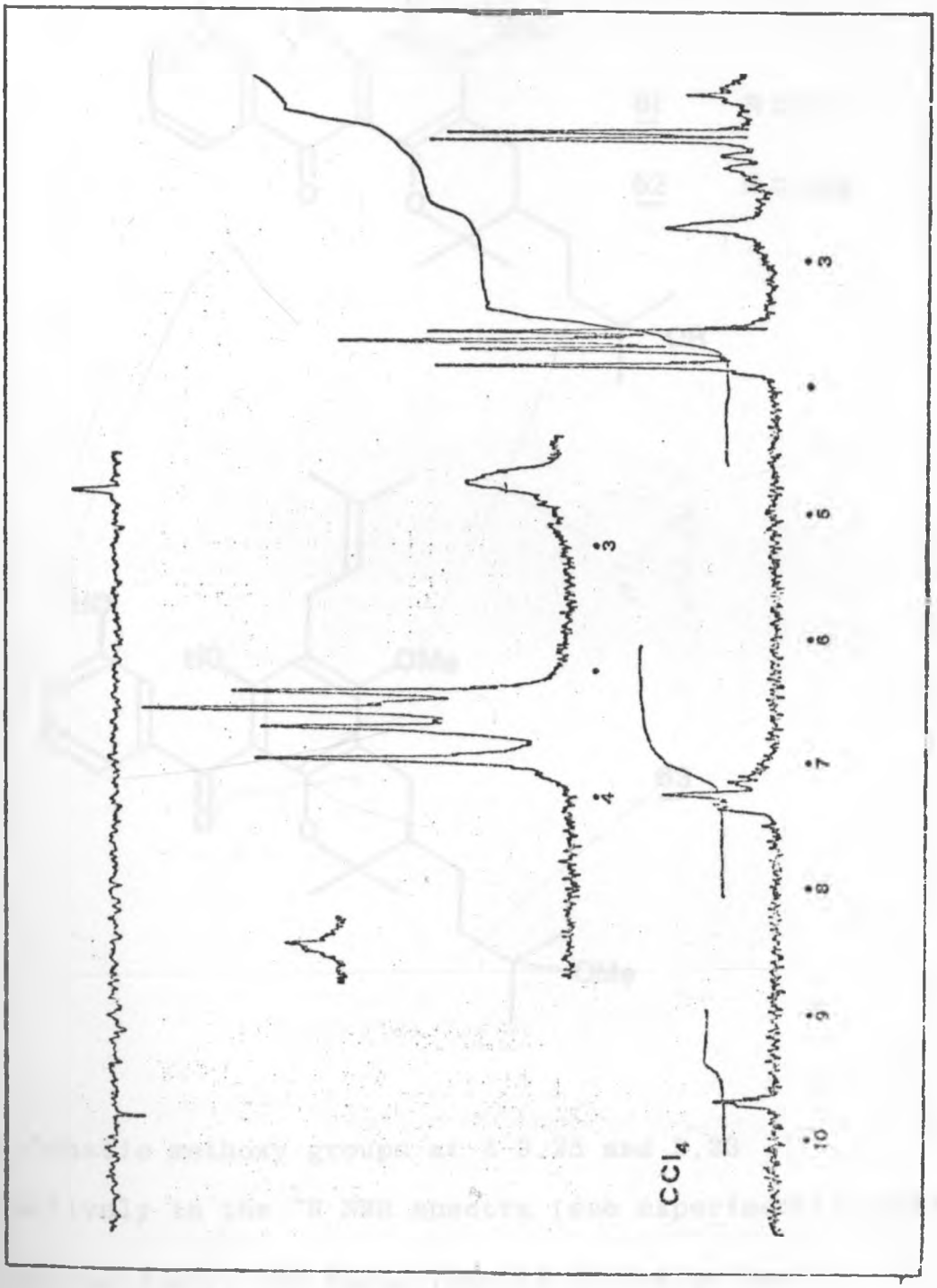


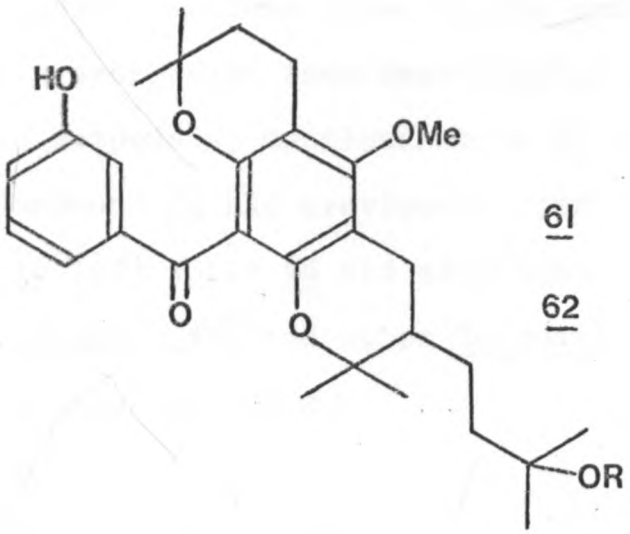
of $M-123^+$ as in tovophenone A, also reflected the structural variations. The diketoaldehyde 60, arose from the cleavage of all the double bonds of 55. This was indicated by the IR and NMR (Fig. 9) spectra in which the peaks due to the terminal methylene groups were absent and the mass spectrum with M^+ at m/e 484 and the base peak at $M-127^+$.

The positioning of the two chains, the γ,γ-dimethyl allyl and the lavundalyl chains, only tentatively suggested by the low $AlCl_3$-induced shift in the UV spectrum of tovophenone A was confirmed by their acid catalyzed cyclisation onto the adjacent hydroxyl functions. By action of 10% trifluoroacetic acid in chloroform at room temperature, tovophenone A gave the dipyrano compound 61 in which the double bonds were absent as indicated by IR and ^1H NMR and the terminal isopropenyl group had added a molecule of water as evidenced by the M^+ ion at m/e 482 and the presence of the $M-18^+$ ion in the mass spectrum.

By action of 15% HCl in methanol, tovophenone A afforded two compounds. One of them, 63, still contained the γ,γ-dimethyl allyl chain and one chelated hydroxyl function (see experimental section). The second compound was the dipyrano compound 62. In both, the terminal isopropenyl had added a molecule of methanol, as proved by the mass spectra with M^+ at m/e 496 and an ion corresponding to $M-32^+$, and by the presence

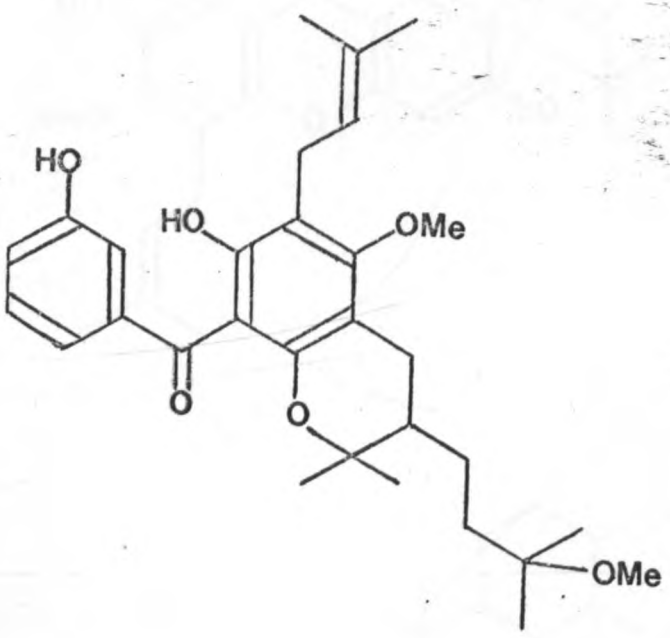
Figure 9. ^{1}H NMR spectrum of the diketo aldehyde 60 in carbon tetrachloride.





61 R = H

62 R = Me

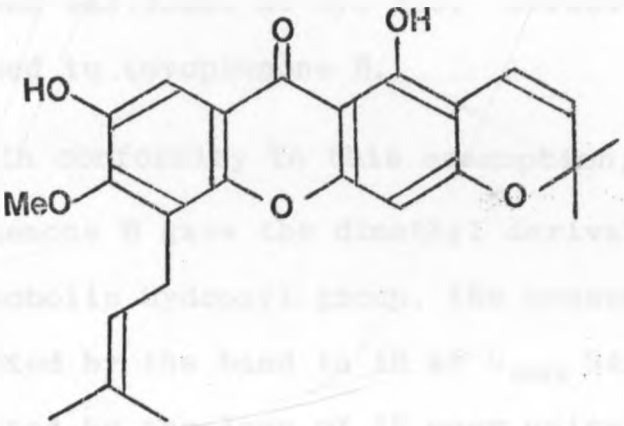


63

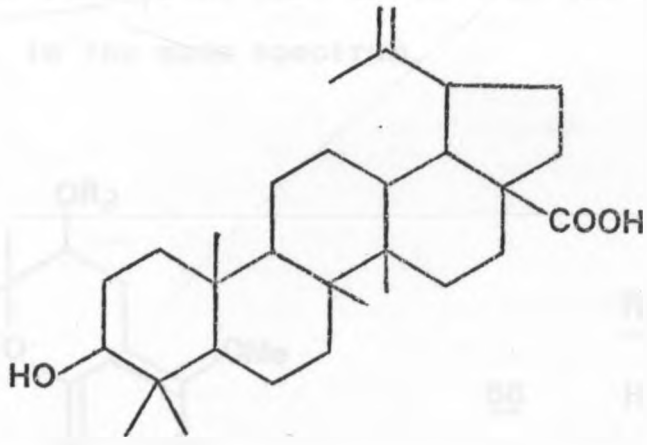
of aliphatic methoxy groups at δ 3.25 and 3.23 respectively in the ^{1}H NMR spectra (see experimental section).

Collectively, the above results permitted the structure of tovophenone A to be conclusively formulated as 55.

The second and third compounds to be eluted from the chromatographic column were, on the basis of their spectroscopic properties (see experimental section) found to be the known compounds, manglexanthone 64 and betulinic acid 65. Compound 64 had previously been isolated from T.mangle [42] while 65 had also been reported to occur in T. mangle [42] and other Tovomita species [43, 44] (see section 1.2.2).



64

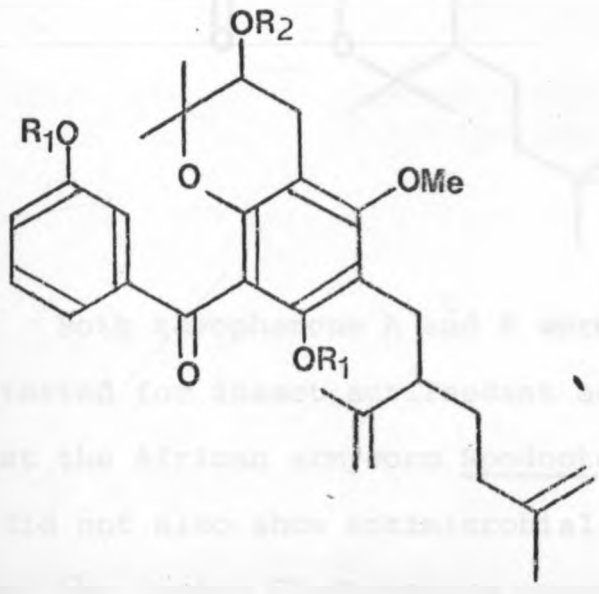


65

The fourth compound to be eluted from the chromatographic column was an unknown oil, named tovophenone B. Tovophenone B displayed some spectral

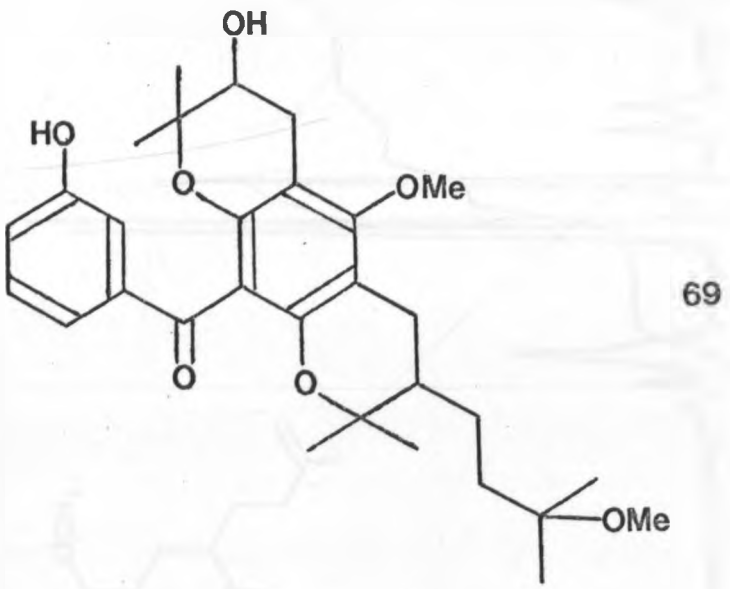
features that were similar to those of tovophenone A. Its NMR, IR and Mass spectra revealed the presence of a mono-hydroxylated A ring, one methoxy group and a lavundalyl chain (see experimental section). It however did not contain the γ,γ-dimethyl allyl chain, and only one chelated hydroxyl group was evident in the ^1H NMR spectrum at δ 12.0. This meant that the C_5-chain must be in a cyclised form and it must contain the elements of a molecule of water, since the molecular ion peak was found at m/e 480. Structure 66 was thus assigned to tovophenone B.

In conformity to this assumption, on methylation, tovophenone B gave the dimethyl derivative 67 containing an alcoholic hydroxyl group, the presence of which was indicated by the band in IR at ν_{max} 3490 cm^{-1} and supported by the loss of 18 mass units from the base peak, $M-C_9H_{15}^+$, in the mass spectrum.



	R_1	R_2
66	H	H
67	Me	H
68	Me	COMe

Acetylation of compound 67 gave the monoacetyl derivative 68. The most significant feature in the ^1H NMR spectrum of 68 (Fig. 10) was the appearance of signals of an AX_2 system at δ 4.80 (1H, t) and δ 3.20 (2H, d), which in 66 and 67 were partially hidden by other signals. These signals, in addition to three-proton singlets at δ 1.38 and 1.23 may be attributed to one 2,2-dimethyl-3-hydroxychroman system, thus permitting the structure of Tovophenone B to be formulated as 66. As with 55, under acidic conditions (15% HCl in methanol) tovophenone B gave cyclotovophenone B (69), which exhibited the expected spectral data (see experimental section).

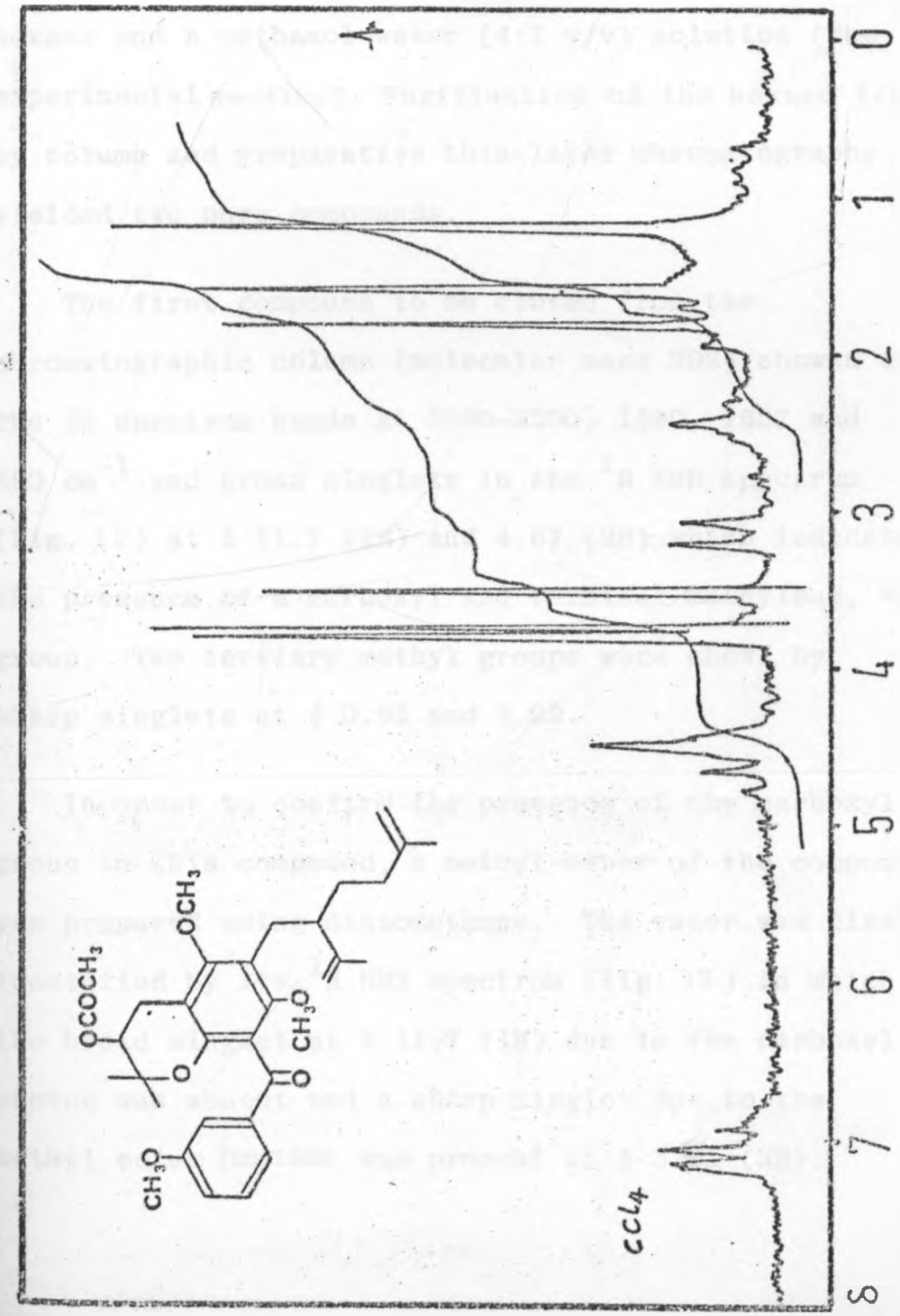


69

Both tovophenone A and B were inactive when tested for insect antifeedant activity against the African armyworm Spodoptera exempta. They did not also show antimicrobial activity against the fungus Cladosporium cucumerinum and

the bacteria Escherichia coli, Bacillus mycoides, Macrococcus luteus, Bacillus subtilis, Staphylococcus aureus and Xanthomonas pelargonii.

Figure 10. ^1H NMR spectrum of compound 68 in carbon tetrachloride.



2.3 ANTIBACTERIAL KAURANOID DITERPENOIDS FROM THE LEAVES OF ASPILIA PLURISETA SCHWEINF.

The dried ground leaves of Aspilia pluriseta Schweinf. were extracted with methanol, the methanol extract partitioned between water and chloroform, and the chloroform fraction partitioned further between hexane and a methanol-water (4:1 v/v) solution (see experimental section). Purification of the hexane fraction by column and preparative thin-layer chromatography yielded two pure compounds.

The first compound to be eluted from the chromatographic column (molecular mass 302) showed in the IR spectrum bands at 3500-2500, 1690, 1657 and 880 cm^{-1} and broad singlets in the ^1H NMR spectrum (Fig. 11) at δ 11.7 (1H) and 4.67 (2H) which indicated the presence of a carboxyl and terminal methylene, $=CH_2$, group. Two tertiary methyl groups were shown by sharp singlets at δ 0.93 and 1.22.

In order to confirm the presence of the carboxyl group in this compound, a methyl ester of the compound was prepared using diazomethane. The ester was clearly identified by its ^1H NMR spectrum (Fig. 12) in which the broad singlet at δ 11.7 (1H) due to the carboxyl proton was absent and a sharp singlet due to the methyl ester function was present at δ 3.61 (3H).

Figure 11. ^{1}H NMR spectrum of ent-kaurenic acid 70 in carbon tetrachloride.

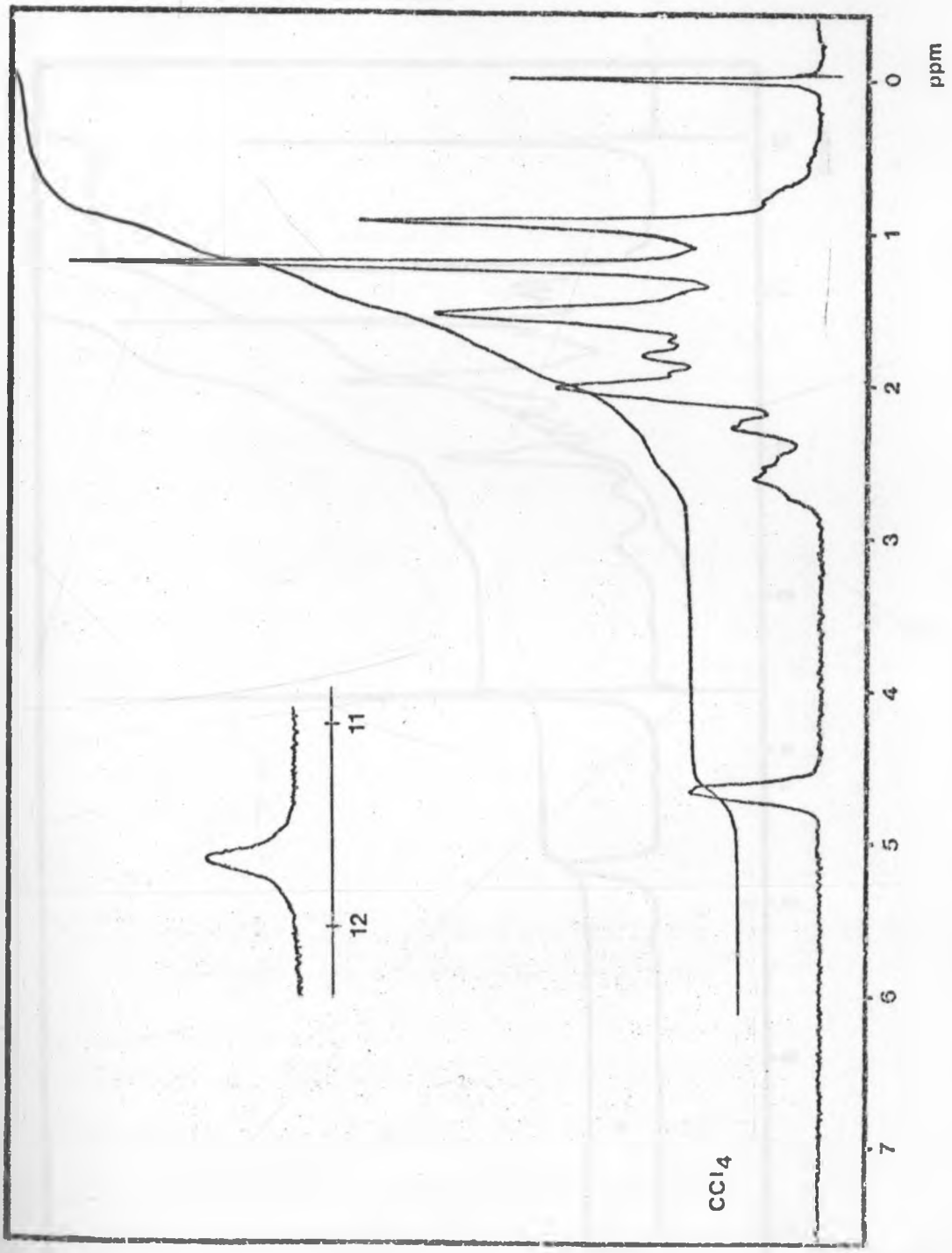
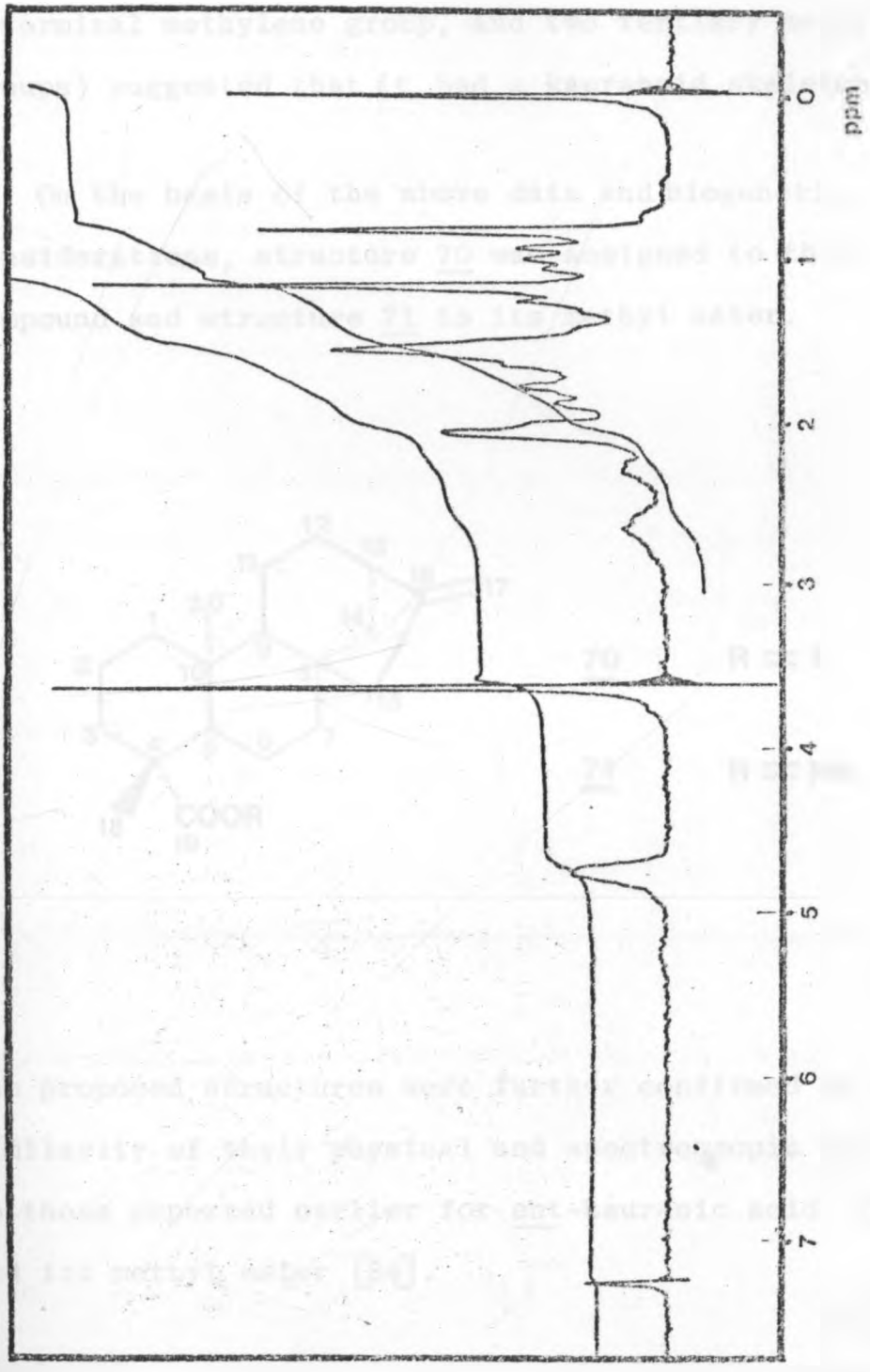
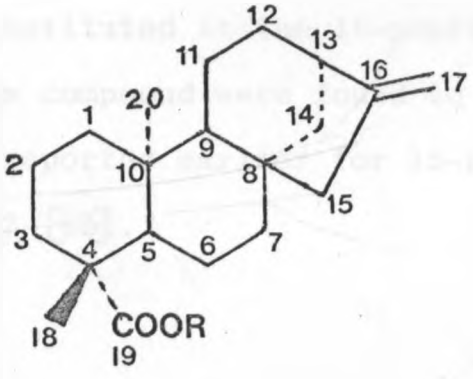


Figure 12. ^{1}H NMR spectrum of the methyl ester of ent-kaurenic acid 71 in deutero-chloroform.



The ^{1}H NMR spectral pattern of this compound as well as its molecular mass suggested a diterpene. Assuming that the diterpene obeyed the isoprene rule and that it had a common diterpene skeleton, the fact that it possessed four pendant groups (a carboxyl group, a terminal methylene group, and two tertiary methyl groups) suggested that it had a kauranoid skeleton.

On the basis of the above data and biogenetic considerations, structure 70 was assigned to this compound and structure 71 to its methyl ester.

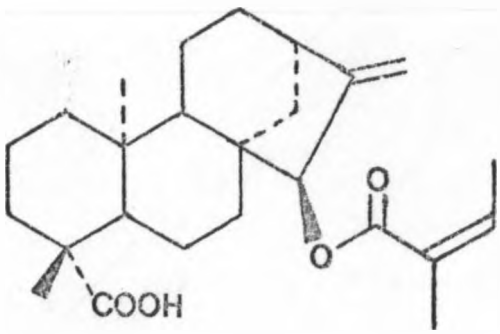


70 R = H

71 R = Me

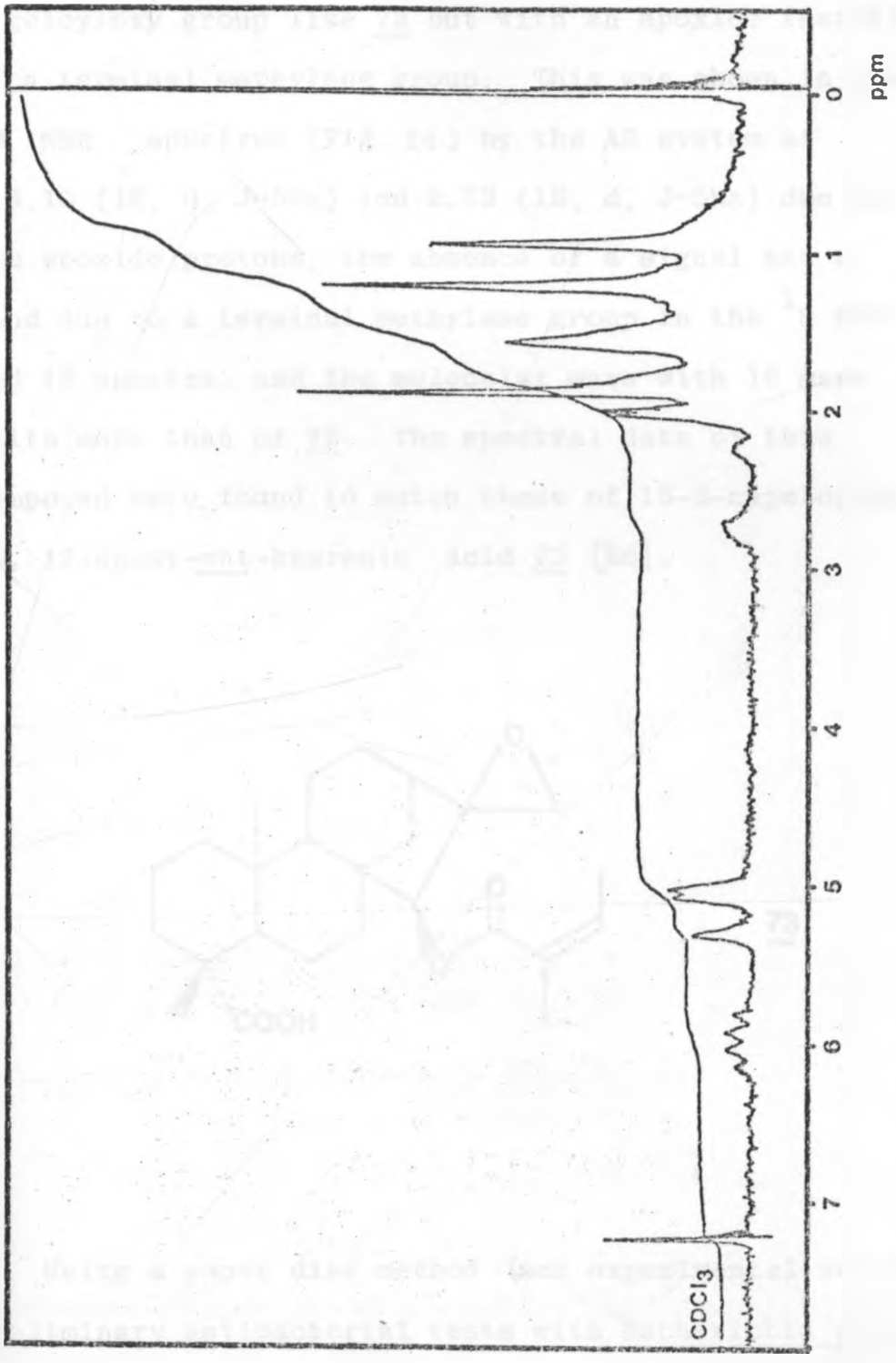
The proposed structures were further confirmed by the similarity of their physical and spectroscopic data to those reported earlier for ent-kaurenic acid (70) and its methyl ester [84].

The spectral features of the second compound (molecular mass 400) to be eluted from the chromatographic column indicated that it was an ent-kaurenic acid derivative with an angeloyloxy group. This was suggested in the ^{1}H NMR spectrum (Fig. 13) by the signals at δ 6.00 (1H, qq), 2.03, and 1.90 which are typical of an angeloyloxy grouping, and the M-100 peak in the mass spectrum corresponding to loss of the angeloyloxy from the molecular ion. The fact that the signal due to the terminal methylene group was now split into two broad singlets [δ 5.10 (1H) and 5.33 (1H)] and was shifted downfield in relation to that of ent-kaurenic acid (70) suggested that the angeloyloxy group was substituted at the 15-position. The spectral data of this compound were found to be in agreement with those reported earlier for 15-β-angeloyloxy-ent-kaurenic acid 72 [85].

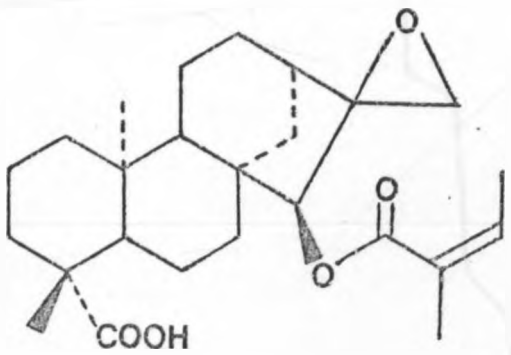


72

Figure 13. ^{1}H NMR spectrum of compound 72 in deuterochloroform.



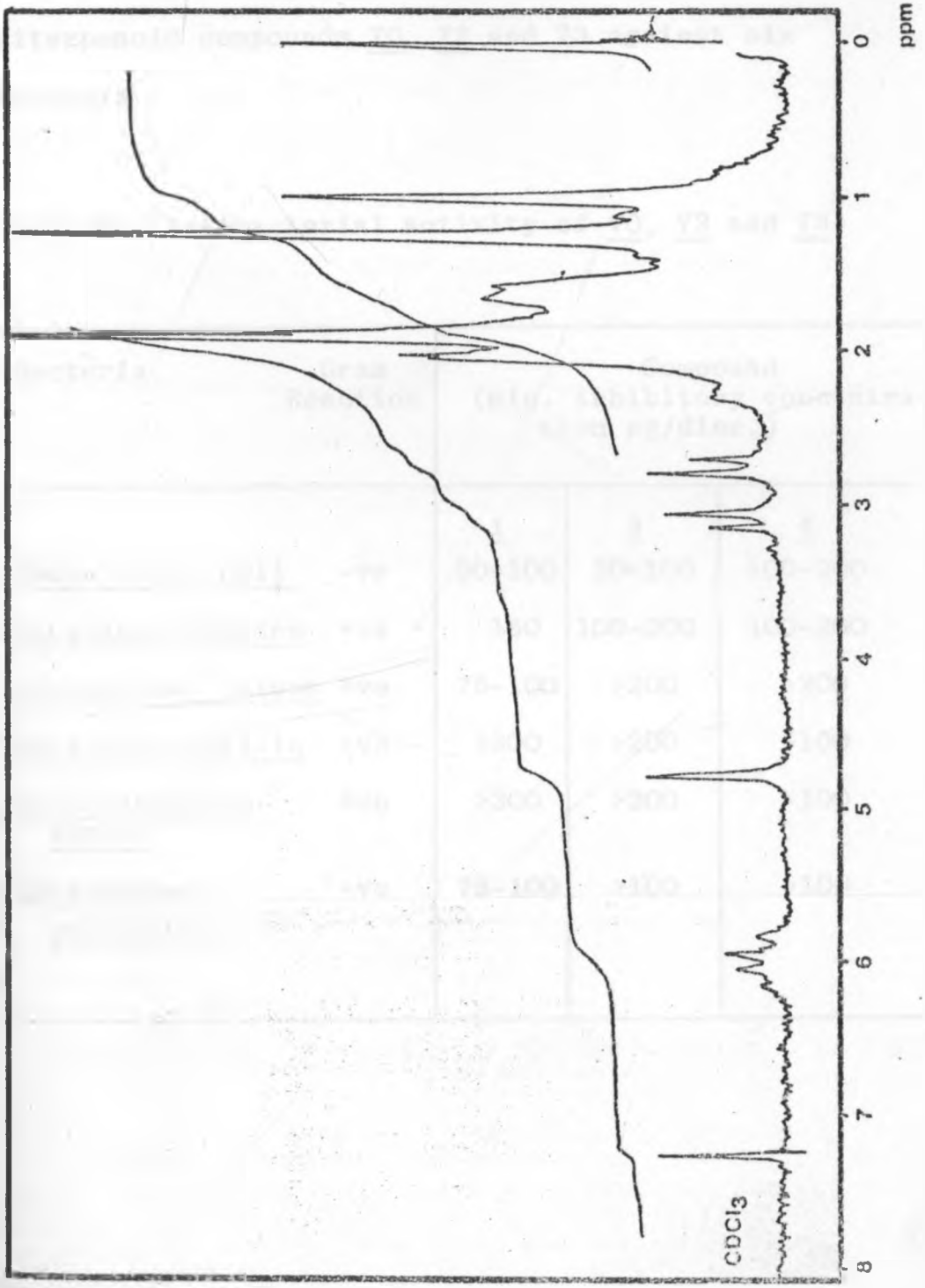
Purification of the water-methanol fraction by column and preparative thin-layer chromatography afforded a third compound, molecular mass 416. This compound was also a kaurenic acid derivative, with an angeloyloxy group like 72 but with an epoxide instead of a terminal methylene group. This was shown in the ^1H NMR spectrum (Fig. 14) by the AB system at δ 3.10 (1H, d, J=5Hz) and 2.73 (1H, d, J=5Hz) due to the epoxide protons, the absence of a signal and a band due to a terminal methylene group in the ^1H NMR and IR spectra, and the molecular mass with 16 mass units more than of 72. The spectral data of this compound were found to match those of 15-β-angeloyloxy-16, 17-epoxy-ent-kaurenic acid 73 [86].



73

Using a paper disc method (see experimental section), preliminary antibacterial tests with Escherichia coli, Micrococcus luteus and Xanthomonas pelargonii

Figure 14. ^{1}H NMR Spectrum of compound 73 in deuterochloroform.



indicated that the crude methanol extract of the leaves of A. pluriseta had antibacterial activity against all three bacteria. Table 8 shows the results for the minimum inhibitory concentration of the three purified diterpenoid compounds 70, 72 and 73 against six bacteria.

Table 8: Antibacterial activity of 70, 72 and 73

Bacteria	Gram Reaction	Compound (min. inhibitory concentration µg/disc.)		
		1	3	4
Escherichia coli	-ve	50-100	50-100	100-200
Bacillus mycoides	+ve	150	100-200	100-200
Macrococcus luteus	+ve	75-100	>200	>200
Bacillus subtilis	+ve	>300	>200	>100
Staphylococcus aureus	+ve	>300	>200	>100
Xanthomonas pelargonii	-ve	75-100	>100	>100

The antimicrobial activity of ent-kaurenic acid 70 against several sensitive cocci such as Micrococcus citrus, Sarcina lutea and staphylococcus aureus W. has been reported earlier in independent communications [84, 87]. The antibacterial activity of the leaves of A. pluriseta lends some justification to its use in wound healing.

Although no clear structure-activity relationship can be inferred from the above table, it is evident that for the two bacteria B. subtilis and S. aureus, compound 4 which contains an epoxide group is more active than 3, while 1 which does not bear an angeloyloxy group is least active. In the case of B. mycoides, M. luteus and X. pelargonii, 1 is more active than 3 and 4 which contain angeloyloxy groups while for E. coli, 4 which bears an epoxide group is less active than 1 and 3.

2.4 A NEW FLAVANONOL ACETATE FROM THE ROOTS OF TEPHROSIA HILDEBRANDTII VATKE.

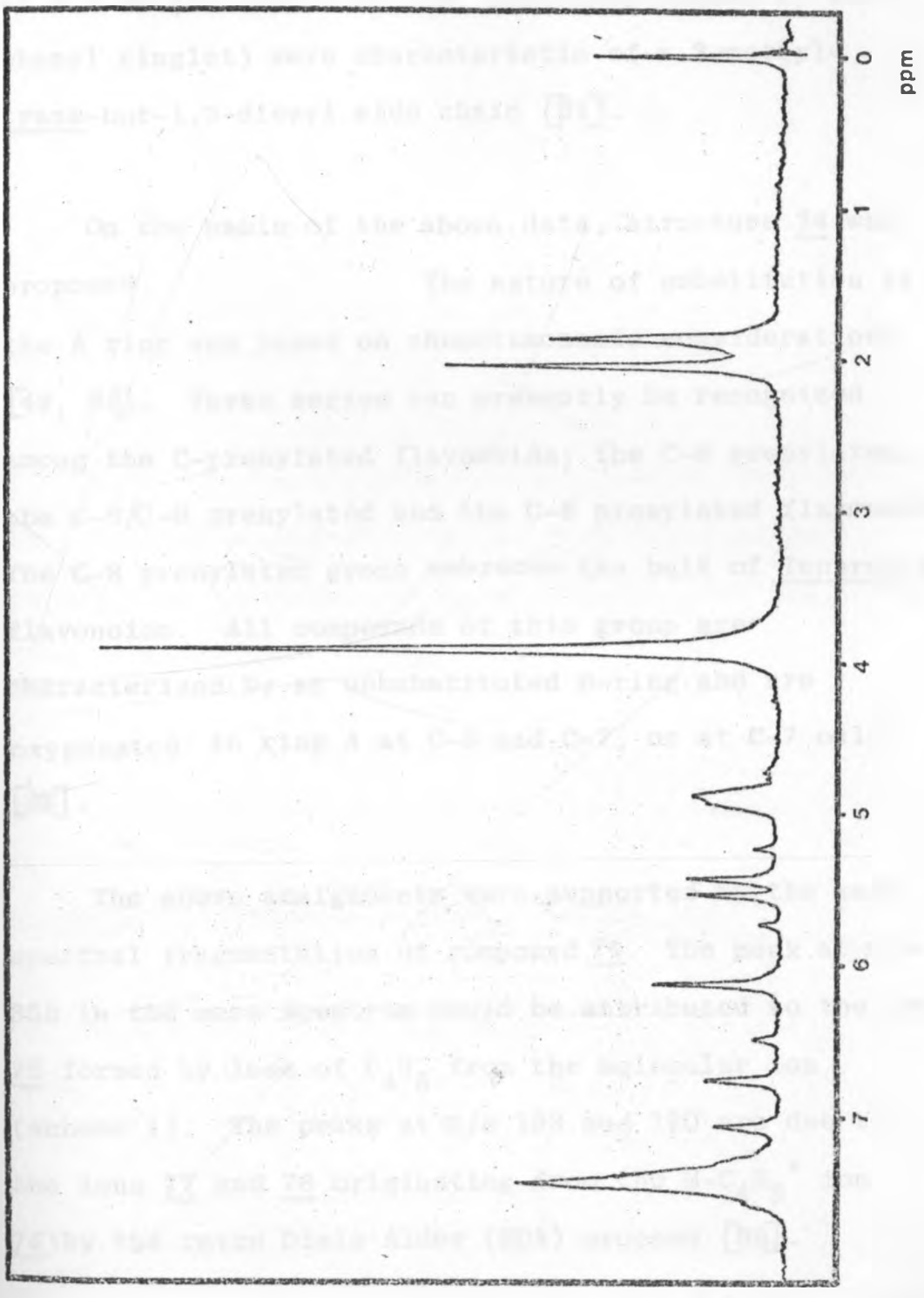
The dried ground roots of Tephrosia hildebrandtii Vatke were extracted with methanol. The methanol extract was partitioned between water and chloroform and the chloroform fraction further partitioned between hexane and a methanol-water (4:1 v/v) mixture. Evaporation of methanol from the methanol-water fraction and subsequent extraction of the residue with chloroform yielded a gummy extract. Column chromatography of this extract followed by repeated preparative thin-layer chromatography yielded two chromatographically pure compounds 74 and 80.

Compound 74 was an oil with molecular mass of 408. Its absorption at λ_{max} 280 and 315 n.m in the UV spectrum and at ν_{max} 1690 cm^{-1} in the IR spectrum suggested that it had a flavanonol or flavanone structure [88, 89]. The two doublets at δ 5.32 (1H) and 5.63 (1H) with J=12 Hz, and the sharp singlet at δ 2.05 (3H) in the ^1H NMR spectrum (Fig. 15) were characteristic of the C (2), C (3) and acetyl protons of a flavanonol acetate [90].

The presence of an unsubstituted B ring was shown by the singlet at δ 7.4 (5H) in the ^1H NMR spectrum [88].

The substituents on the A ring were indicated by the ^1H NMR spectrum. The singlet at δ 3.90 (6H)

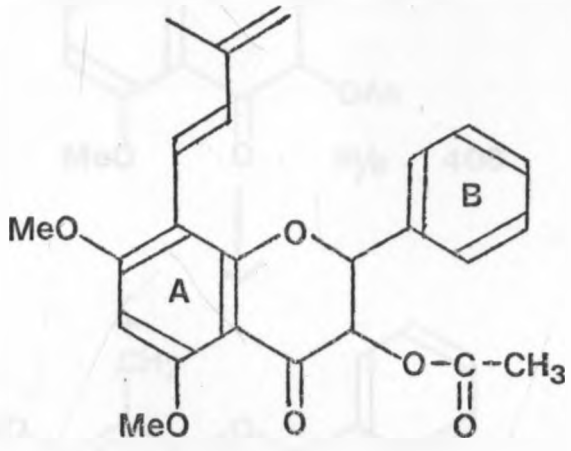
Figure 15. ^{1}H NMR spectrum of compound 74 in deutero-chloroform.



corresponded to two methoxy groups while the sharp singlet at δ 6.12 (1H) was due to one aromatic proton. The singlet at δ 1.86 (3H),a broad singlet at δ 4.9 (2H) and an AB system with doublets (J=16 Hz) centered at δ 6.60 (1H) and 7.20 (1H, partially hidden by the phenyl singlet) were characteristic of a 3-methyl-trans-but-1,3-dienyl side chain [91].

On the basis of the above data, structure 74 was proposed. The nature of substitution in the A ring was based on chemotaxonomic considerations [49, 92]. Three series can presently be recognized among the C-prenylated flavonoids; the C-6 prenylated, the C-6/C-8 prenylated and the C-8 prenylated flavonoids. The C-8 prenylated group embraces the bulk of Tephrosia flavonoids. All compounds of this group are characterized by an unsubstituted B-ring and are oxygenated in ring A at C-5 and C-7, or at C-7 only [92].

The above assignments were supported by the mass spectral fragmentation of compound 74. The peak at m/e 355 in the mass spectrum could be attributed to the ion 75 formed by loss of C_4H_5 from the molecular ion (scheme 1). The peaks at m/e 193 and 120 are due to the ions 77 and 78 originating from the $M-C_4H_5^+$ ion (76)by the retro Diels Alder (RDA) process [89].

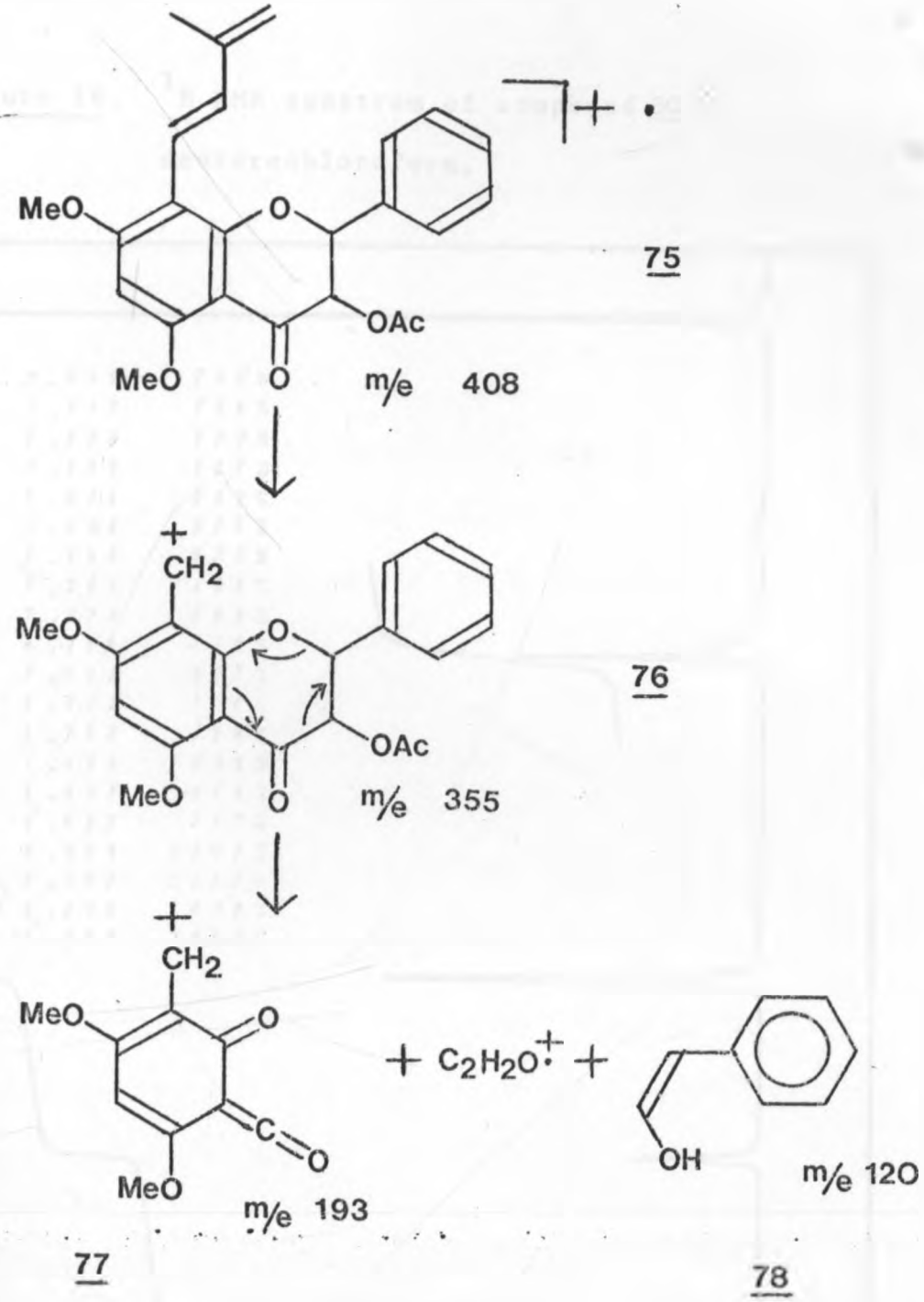


74

74 exhibited mild antifeedant activity at a threshold concentration of 0.2% when tested against the African armyworm, Spodoptera exempta, by the leaf disc bioassay with Zea Mays [41].

Flavanonols and flavonols acetylated at the 3-hydroxyl position as in 74 are rare in nature. Only one such compound, alpinone-3-O-acetate 79 has been isolated earlier from Alpinia japonica [93].

Compound 80 was a yellow solid. It was purified further by crystallization in ethyl acetate to yield yellow crystals with m.p 216-220° and of molecular mass 348. It showed absorption at λ_{max} 260, 276 and 346sh nm, ν_{max} 1640 cm^{-1} and a singlet in the ^1H NMR spectrum (Fig. 16) at δ 6.42 (H-3) which were characteristic of a flavone [88]. The multiplets centred at δ 7.48 (3H)


MeO
O
OAc
MeO
O
m/e 408
75
CH2
MeO
O
OAc
MeO
O
m/e 355
76
CH2
MeO
O
C
O
MeO
m/e 193
77
+ C2H2O
OH
m/e 120
78

Scheme 1

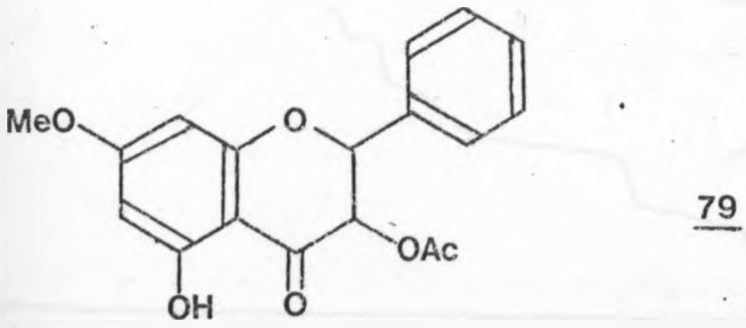
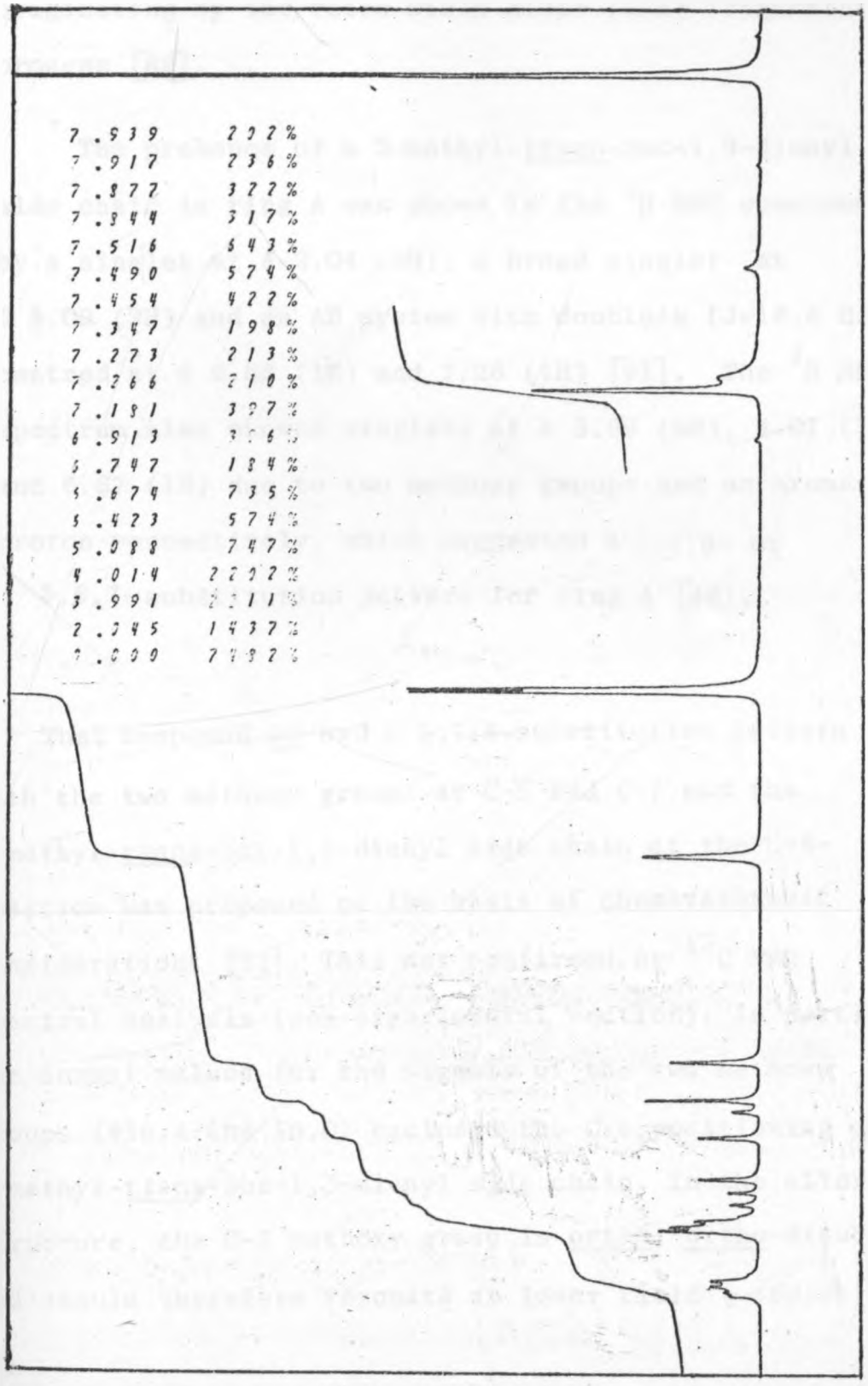

MeO
O
OAc
OH
O
79

Figure 16. ^{1}H NMR spectrum of compound 80 in deuterochloroform.

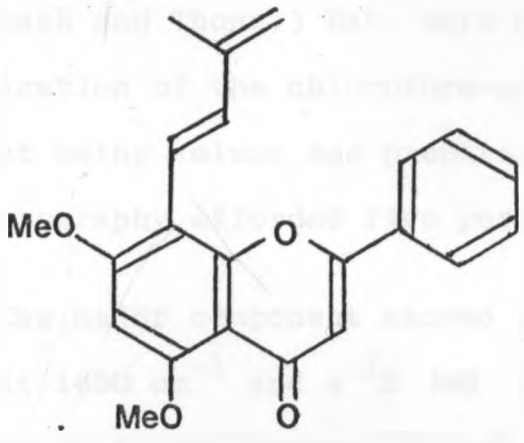


and 7.81 (2H) indicated the B ring to be unsubstituted. This was confirmed by the fragment ions in the mass spectrum at m/e 105 (C_6H_5-CO^+) and 102 (C_6H_5-$C{\equiv}CH^+$.) originating by the retro-Diels Alder (RDA) fragmentation process [89].

The presence of a 3-methyl-*trans*-but-1,3-dienyl side chain in ring A was shown in the ^{1}H NMR spectrum by a singlet at δ 2.04 (3H), a broad singlet at δ 5.09 (2H) and an AB system with doublets (J=16.6 Hz) centred at δ 6.83 (1H) and 7.26 (1H) [91]. The ^{1}H NMR spectrum also showed singlets at δ 3.99 (3H), 4.01 (3H) and 6.67 (1H) due to two methoxy groups and an aromatic proton respectively, which suggested a 5,7,8- or 5,6,7-substitution pattern for ring A [49].

That compound 80 had a 5,7,8-substitution pattern with the two methoxy groups at C-5 and C-7 and the 3-methyl-*trans*-but-1,3-dienyl side chain at the C-8-position was proposed on the basis of chemotaxonomic considerations [92]. This was confirmed by ^{13}C NMR spectral analysis (see experimental section). In particular, the normal values for the signals of the two methoxy groups (δ56.4 and 56.0) excluded the C-6 positioning of the 3-methyl-*trans*-but-1,3-dienyl side chain. In the alternative structure, the C-5 methoxy group is *ortho*, *ortho*-disubstituted and should therefore resonate at lower field (~δ60.0) [94]

On the basis of the above data, structure 80 was assigned to Compound 80. The spectral data and m.p



80

of compound 80 were found to be in close agreement with that previously reported for trans-anhydro-tephrostachin 80, a constituent of Tephrosia bracteolata [52].

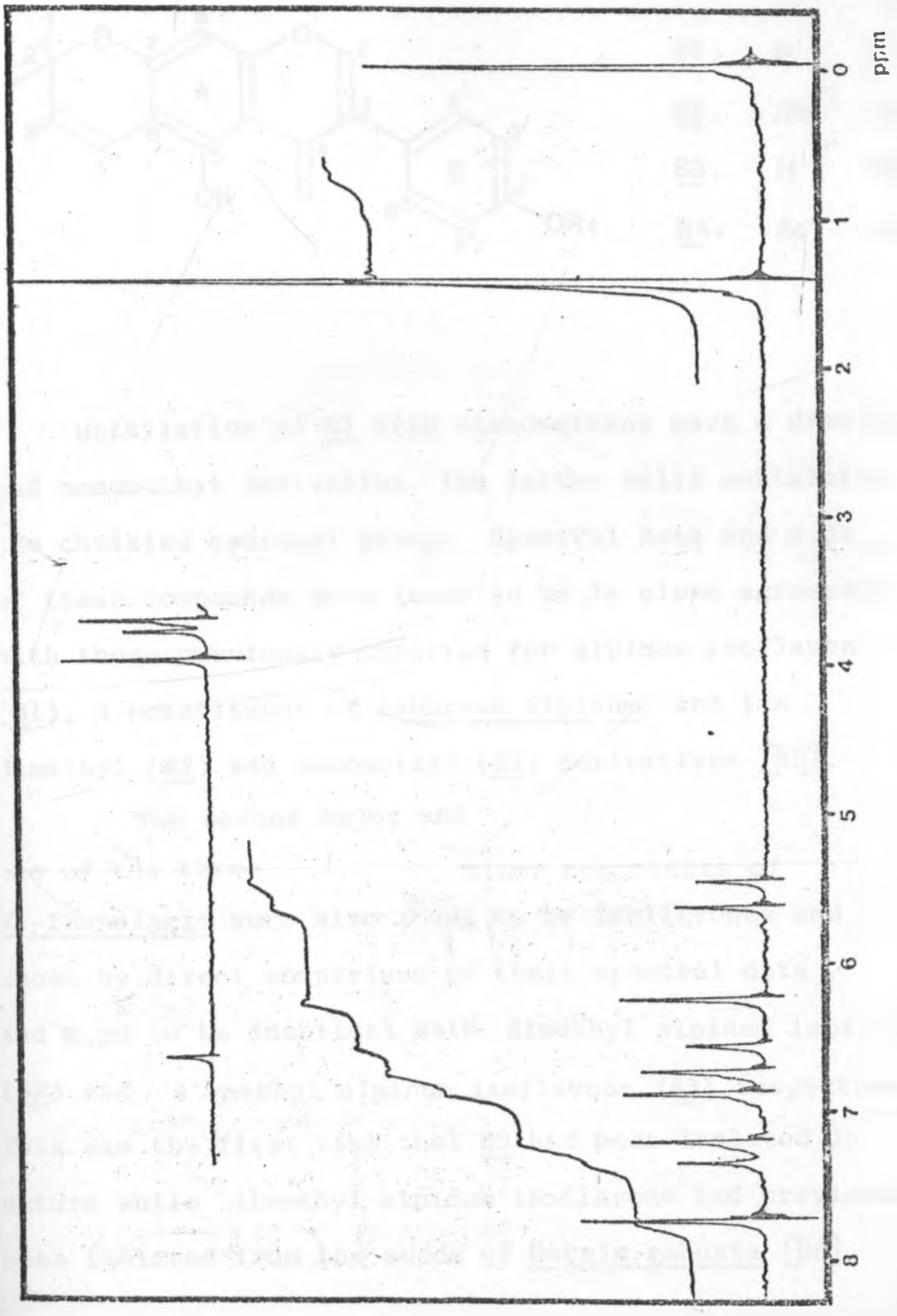
Unlike compound 74, trans-anhydro tephrostachin 80 did not show antifeedant activity against the African armyworm Spodoptera exempta even at a high concentration of 0.5%, using the leaf disc bioassay with Zea Mays [41].

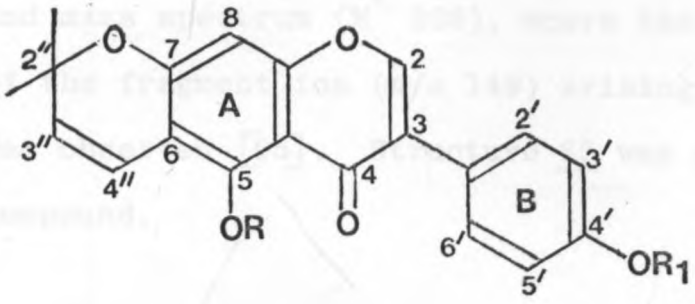
2.5 A NEW PYRANO-ISOFLAVONE FROM THE SEEDS OF MILLETIA THONNINGII (SCHUMACH AND THONN.) BAK.

The dried ground seeds of Milletia thonningii (Schumach and Thonn.) Bak. were extracted with methanol. Purification of the chloroform-soluble portion of this extract using column and preparative thin-layer chromatography afforded five pure compounds.

The major component showed a UV spectrum, an IR band at 1650 cm^{-1} and a ^1H NMR spectrum (Fig. 17) typical of an isoflavone [88,89]. The ^1H NMR had a sharp singlet at δ 13.20 (≈0.5H) which was indicative of a chelated hydroxyl group and a sharp singlet at δ 7.72 (1H) typical of H-2 of an isoflavone. The doublets at δ 7.25 (2H, J=8.5 Hz) and 6.80 (2H, J=8.5 Hz) suggested that there was present a para-disubstituted B ring while those at δ 6.60 (1H, J=10 Hz) and 5.53 (1H, J=10 Hz) and the sharp singlet at δ 1.40 (6H) indicated that a dimethylpyran group was substituted in the A ring. The sharp singlet at δ 6.23 corresponded to the single proton in the A ring. The slow change of the maximum in the UV spectrum on addition of aluminium chloride and biogenetic considerations suggested that the dimethylpyran group was substituted at the 6- and 7- positions. On the basis of the above data, the compound was assigned the structure 81.

Figure 17. ^{1}H NMR spectrum of alpinum isoflavone (81) in carbon tetrachloride.



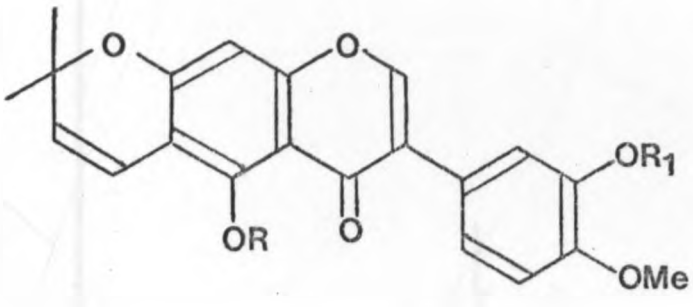


	R	R_1
81.	H	H
82.	Me	Me
83.	H	Me
84.	Ac	Me

Methylation of 81 with diazomethane gave a dimethyl and monomethyl derivative, the latter still containing the chelated hydroxyl group. Spectral data and m.ps of these compounds were found to be in close agreement with those previously reported for alpinum isoflavone (81), a constituent of Laburnum alpinum, and its dimethyl (82) and monomethyl (83) derivatives [95].

The second major and one of the three minor components of M. thonningii were also found to be isoflavones and shown by direct comparison of their spectral data and m.ps to be identical with dimethyl alpinum isoflavone (82) and 4′-methyl alpinum isoflavone (83) respectively. This was the first time that 83 had been isolated in nature while dimethyl alpinum isoflavone had previously been isolated from the seeds of Derris robusta [96].

The fourth compound was a new isoflavone with the same substitution pattern in the A ring as alpinum isoflavone, but with a methoxy group in the B ring. This was indicated by the ^1H NMR spectrum (Fig. 18) and mass spectrum (M^+ 366), where the appropriate shift of the fragment ion (m/e 149) arising from the B ring was observed [95]. Structure 85 was assigned to this compound.



	R	R_1
85.	H	H
86.	Ac	Ac

The linear fusion of the dimethylpyran ring in 85, suggested by a slow change of the maximum in the UV spectrum on addition of aluminium chloride, was corroborated by the downfield shift ($\Delta\delta$ + 0.17) of the H-4" signal in the ^1H NMR of 85 in deuteropyridine [82]. Confirmation of this was obtained by preparation of the diacetyl derivative 86, in which the acetylation of the 5-hydroxyl caused the expected [97] diamagnetic shift ($\Delta\delta$ - 0.20) of the H-4" signal in the ^1H NMR spectrum (Fig. 19).

Figure 18. ^{1}H NMR spectrum of compound 85 in carbon tetrachloride.

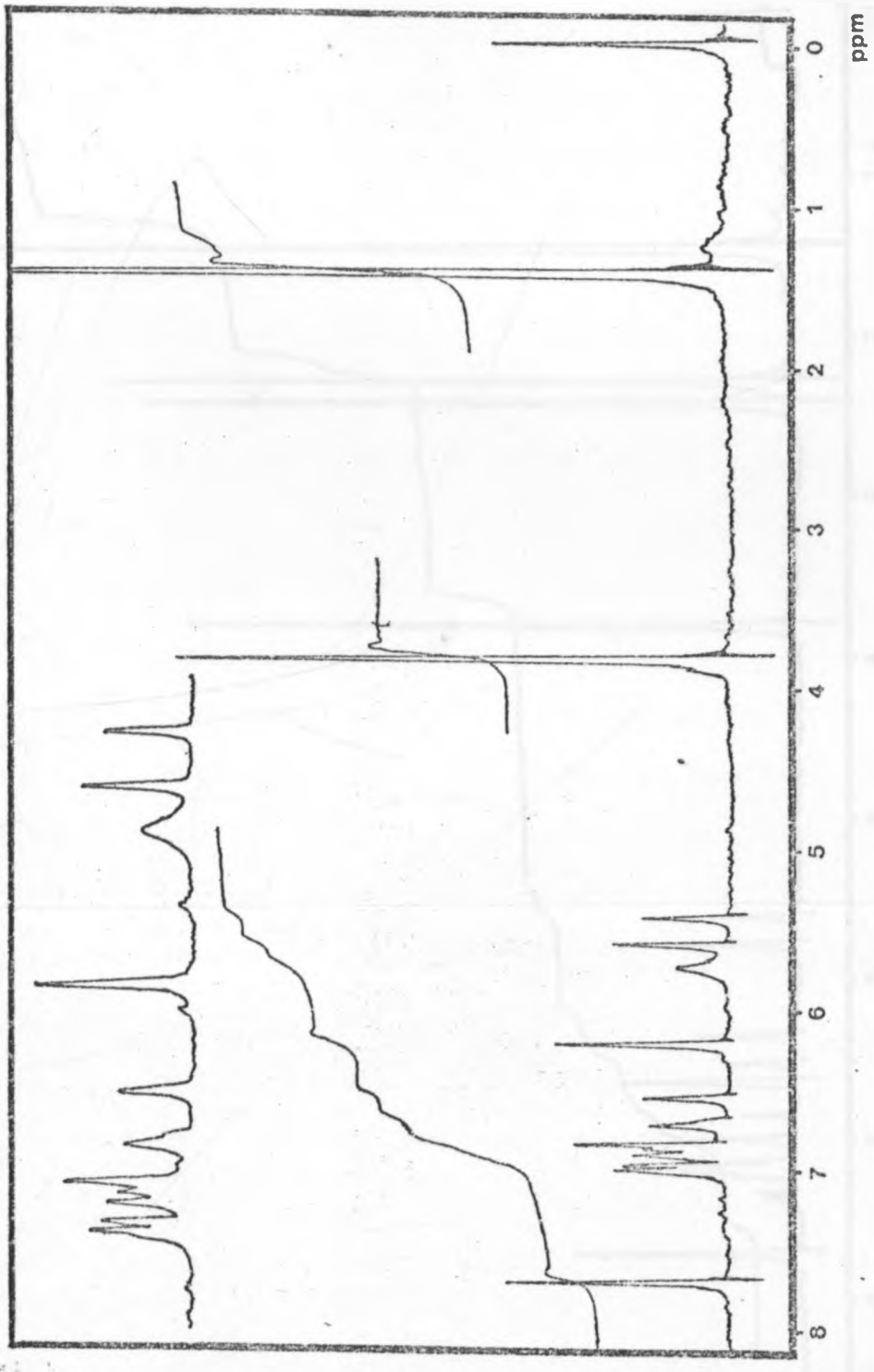
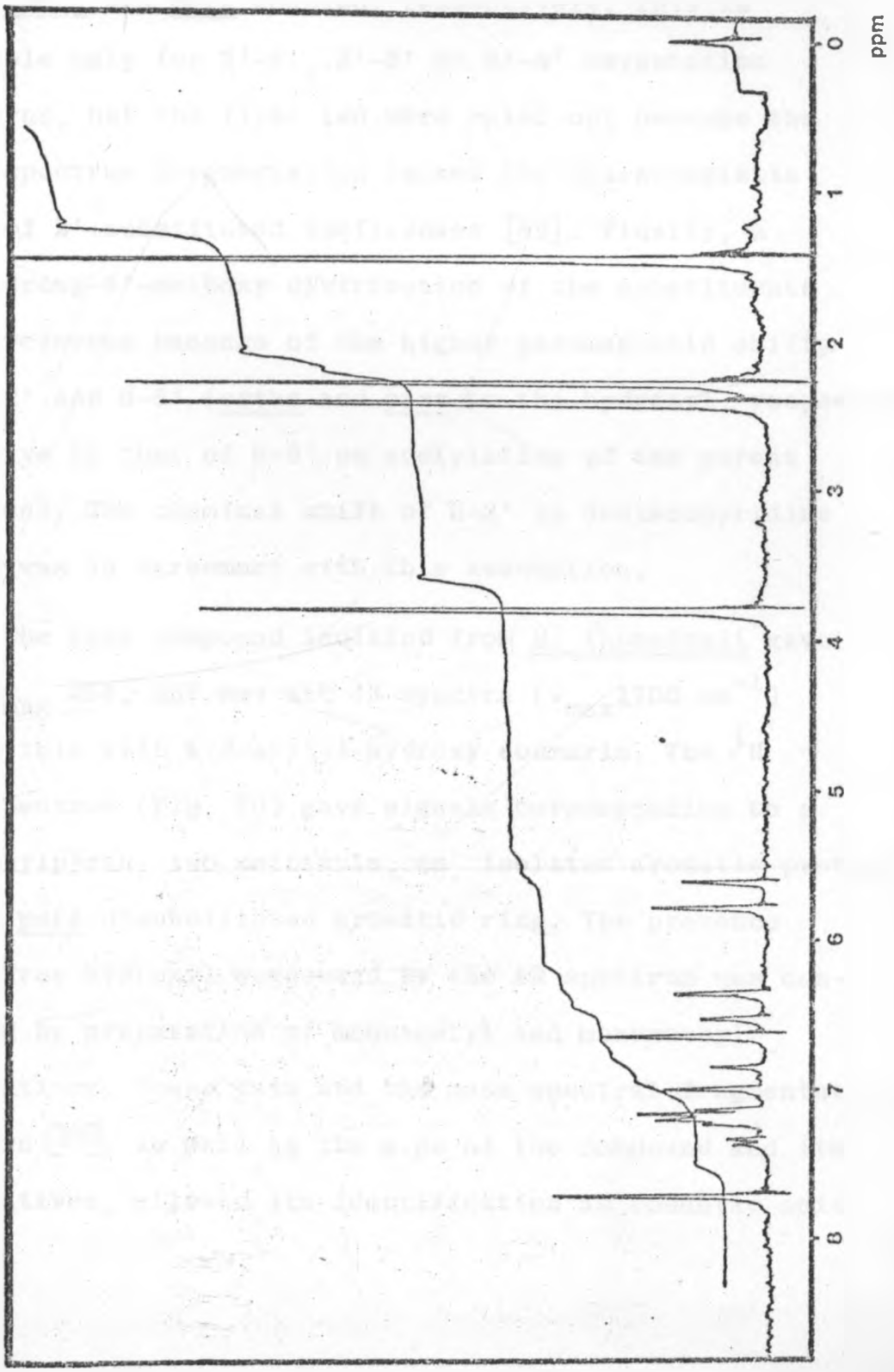


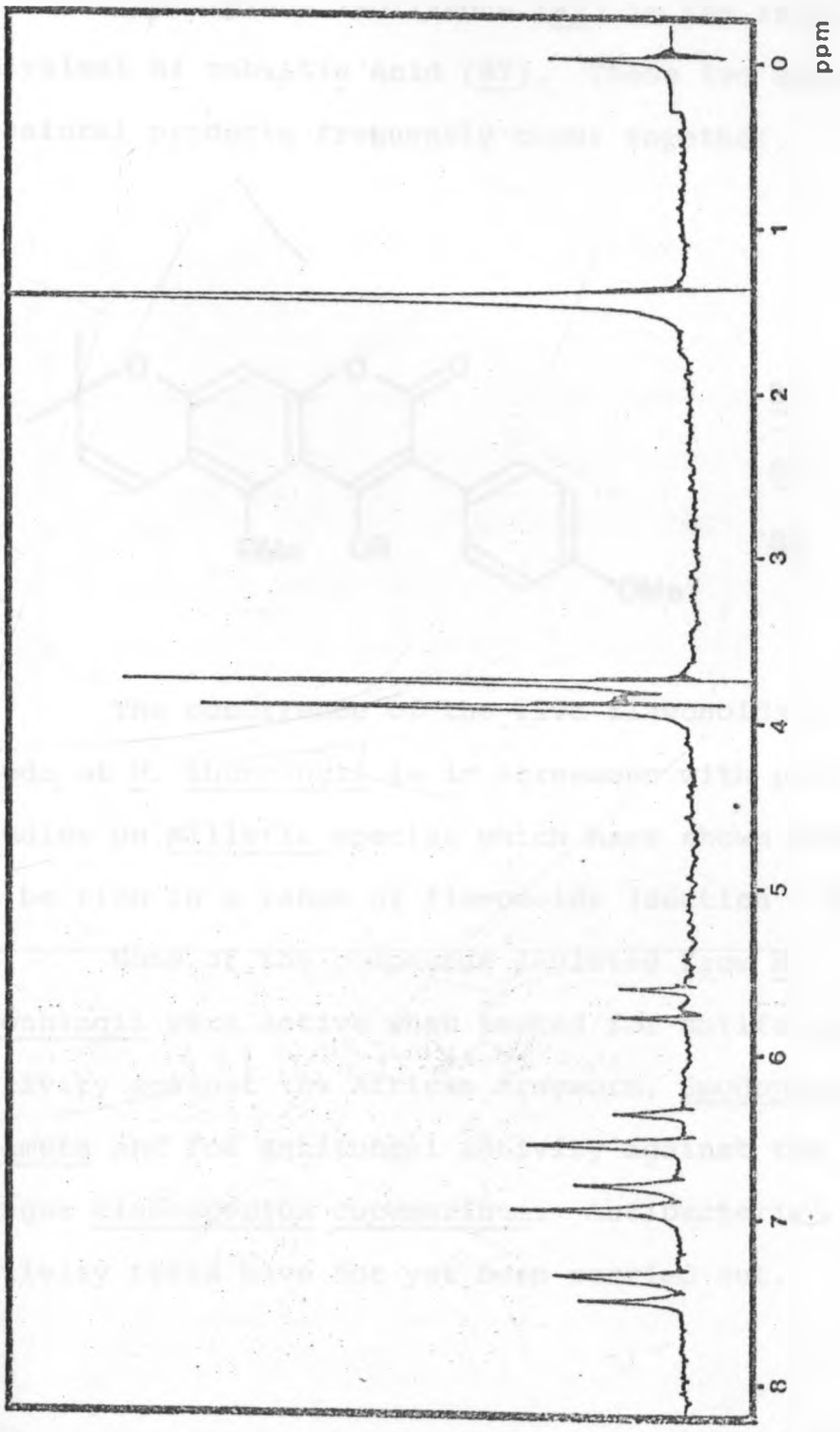
Figure 19. ^{1}H NMR spectrum of compound 86 in carbon tetrachloride.



The substitution in the B ring of 85 could not be inferred from the complicated pattern in the aromatic region of its ^{1}H NMR spectrum (Fig. 18), but in that of 86 (Fig. 19) the three aromatic protons appeared as ortho, ortho-meta and meta coupled, respectively. This is possible only for 2'-4', 2'-5' or 3'-4' oxygenation patterns, but the first two were ruled out because the mass spectrum fragmentation lacked the characteristic ions of 2'-substituted isoflavones [89]. Finally, a 3'-hydroxy-4'-methoxy distribution of the substituents was preferred because of the higher paramagnetic shifts on H-2' and H-6' (ortho and para to the hydroxyl, respectively) relative to that of H-5' on acetylation of the parent compound. The chemical shift of H-2' in deuteropyridine in 85 was in agreement with this assumption.

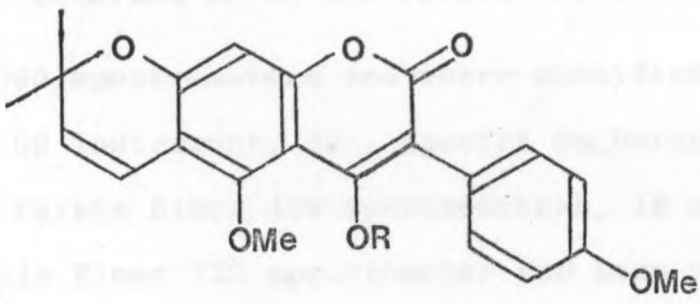
The last compound isolated from M. thonningii gave UV (λ_{max} 254, 337 nm) and IR spectra (ν_{max} 1705 cm^{-1}) compatible with a 3-aryl-4-hydroxy coumarin. The ^{1}H NMR spectrum (Fig. 20) gave signals corresponding to a dimethylpyran, two methoxyls, an isolated aromatic proton and a para-disubstituted aromatic ring. The presence of a free hydroxyl suggested by the IR spectrum was confirmed by preparation of monoacetyl and monomethyl derivatives. These data and the mass spectral fragmentation pattern [98], as well as the m.ps of the compound and its derivatives, allowed its identification as robustic acid

Figure 20. ^{1}H NMR spectrum of robustic acid (87) in carbon tetrachloride.



87, previously reported [98, 99] from Derris robusta, and that of the acetyl and methyl derivatives as 88 and 89 respectively.

Dimethylalpinum isoflavone (82) is the isoflavone equivalent of robustic acid (87). These two classes of natural products frequently occur together.



	R
87.	H
88.	Ac
89.	Me

The occurrence of the five flavonoids in the seeds of M. thonningii is in agreement with previous studies on Milletia species which have shown them to be rich in a range of flavonoids (section 1.2.5).

None of the compounds isolated from M. thonningii were active when tested for antifeedant activity against the African armyworm, Spodoptera exempta and for antifungal activity against the fungus Cladosporium cucumerinum. Antibacterial activity tests have not yet been carried out.

CHAPTER 3

3. E X P E R I M E N T A L

TLC was performed on "Merck" precoated silica-gel 60 F_{254} plates (0.25 mm thickness) and column chromatography on silica-gel 60(0.063-0.200 mm, 70-230 mesh, ASTM) and on silica-gel 60(0.040-0.063 mm, 230-400 mesh ASTM) using an HPLC Quickfit column with application of nitrogen pressure. ^1H NMR spectra were recorded on 60 MHz Perkin Elmer R-24A and Varian EM 360 spectrometers and where specified, on 100 MHz Jeol FX-100 instrument, UV spectra on Beckmann ACTA C III and Perkin Elmer 402 spectrometers, IR spectra on a Perkin Elmer 720 spectrometer and Mass spectra on a GC/MS Finnigan 1015D and an AEI 12 spectrometer.

3.1 BIOLOGICAL ACTIVITY TESTS

Antifeedant activity

The antifeedant activity was determined against the African armyworm,, Spodoptera exempta using the leaf disc bioassay with Zea mays [41]. Zea mays leaf discs (2 cm diameter) were cut out with a cork borer, 5 of the leaf discs were dipped for 2 sec in an acetone solution of the sample and another 5 control leaf discs were immersed for 2 sec in acetone. The treated and control discs were alternately arranged concentrically in a Petri dish and ten S. exempta larvae placed in the centre. The minimum antifeedant activity level was

taken as the concentration of the acetone solution of the sample which after 2 h of each of three trials, completely inhibited the larvae from feeding on the treated discs.

Antifungal activity

The Cladosporium spray bioassay was used [76]. The fungus Cladosporium cucumerinum (Ell. & Arth.) was cultured on a mixture of dextrose agar and tomato juice. Commercial tomato juice (40 ml) was centrifuged and the clear juice poured into 3 Petri dishes containing cultures of C. cucumerinum. The mixture of the fungus and tomato juice was gently scraped off the culture medium and filtered through 3 layers of bandage cloth to remove the larger mycelial fragments. The conidial suspension so formed was evenly sprayed onto a silica gel plate, initially spotted with the compounds under investigation and eluted using a $CHCl_3$-EtOAc (4-1) solvent system. The plate was incubated under humid conditions for 48 h after which the fungus had grown evenly on the plate and the antifungal compounds could be detected as clear zones, with no fungal growth.

Antibacterial activity

A paper-disc method was used. [100]. The antibiotic assay discs (Whatman No. 1, 0.6 cm diameter) were loaded with a prescribed amount of compound (10-300 µg/disc) in chloroform. In each case there was a control disc treated with chloroform (100 µl). Sufficient time was allowed for the chloroform to evaporate

before transferring the discs to the agar surface.

The discs were arranged in Petri dishes containing Tryptone-soya agar which had been streaked with 0.1 ml of the bacterium (3×10^8 cells/ml by McFarland nephlometer barium sulphate standard). Plates were incubated at 37°C and diameters of inhibition zones measured after 24 h. The experiment was repeated three times, and the minimum inhibitory concentration recorded.

3.1 TECLEA TRICHOCARPA ENG.

Plant material

The bark of T. trichocarpa was collected from Karura Forest, in Nairobi, Kenya.

Extraction and purification

The air dried plant material (1.5 kg) was extracted with methanol in the cold and the extract evaporated in vacuo giving rise to an oily residue (39 g). The crude oily residue (10 g) was eluted through a short silica-gel column (hexane-acetone, 1-1, 1ℓ). The eluant was evaporated in vacuo to yield an oil (0.54 g) which was rechromatographed on silica-gel ($CHCl_3$-EtOAc, 4-1) affording melicopicine 45 (32 mg), tecleanthine 46 (29 mg) and 6-methoxy tecleanthine 47 (34 mg) eluted in that order.

Melicopicine 45. M.p. 133-134° (MeOH) (Lit. [74] m.p. 133-134°). UV: λ_{max} 203sh, 218, 268, 310sh and 397 nm. IR: ν_{max} 1640, 1610, 1580, 1220 and 765 cm^{-1}. ^1H NMR ($CDCl_3$): δ 3.80 (3H, s, OCH_3), 3.91 (3H, s, NCH_3), 3.97 (3H, s, OCH_3), 4.02 (3H, s, OCH_3), 4.11 (3H, s, OCH_3), 7.42 (3H, m, H-5, H-6 and H-7), 8.41 (1H, dd, H-8). M.S: m/e 329 (M^+), 314(M-15, 100%), 270, 256, 228, 157, 149, 143 and 77.

Tecleanthine 46.M.p. 157° (EtOAc) (Lit. [19] m.p. 158°). UV: λ_{max} 220sh, 235, 268, 281sh, 306sh and 401 nm (logε 4.28,4.23, 4.74, 4.67, 3.83 and 4.04). IR: ν_{max} 2810, 1742, 1620 and 1590 cm^{-1}. ^1H NMR ($CDCl_3$): δ 3.83 (3H, s, OCH_3), 3.92 (3H, s, NCH_3), 4.15 (3H, s, OCH_3), 6.02 (2H, s, O-CH_2-O), 6.65 (1H, s, H-4), 7.1 - 7.21 (2H, m, H-6 and H-7) and 8.2 (1H, q, H-8, $J_{6,8}$ 3.3; $J_{7,8}$ 6.6 Hz). M.S: m/e 313 (M^+), 298 (M-15), 285, 270, 267, 255, 240, 239, 224, 212 and 78 (100%).

6-Methoxy tecleanthine 47 M.p. 169° (EtOAc) (Lit. [20] m.p. 168°). UV: λ_{max} 221, 323 and 392 nm (logε 4.15 4.74, 3.74 and 3.88). IR: ν_{max} 2790, 1730, 1620 and 1590 cm^{-1}. ^1H NMR ($CDCl_3$): δ 3.8 (3H, s, OCH_3). 3.9 (3H, s, NCH_3), 3.99 (3H, s, OCH_3), 4.12 (3H, s, OCH_3), 6.03 (2H, s, O-CH_2-O), 6.67 (1H, s, H-4), 6.95 (1H, d, H-7, J 7.5 Hz) and 8.2 (1H, d, H-8, $J_{7.8}$ 7.5 Hz). M.S: m/e 343 (M^+, 100%), 328, 315, 300, 297, 285, 281, 268, 253, 238, 266, 211, and 77.

3.2 TOVOMITA MANGLE G. MARIZ

Plant Material.

The roots of Tovomita mangle G. Mariz were collected in north-eatern Brazil (Pernambuco). A voucher sample is kept in the Herbarium of Instituto de Antibioticos (Recife) under the cipher 2846.

Extraction and purification.

The dried ground root was extracted in the cold with acetone. The gummy residue of the extraction (20 g) was stirred with $CHCl_3$ - hexane (2-1) and the insoluble material discarded by filtration. The soluble portion was evaporated and the residue (12 g) purified on a silica-gel column. Impure tovophenone A (4.5 g), manglexanthone [42] (500 mg), betulinic acid [42] (350 mg), and tovophenone B (620 mg) were successively eluted with benzene containing increasing quantities of ethyl acetate (20% for the last two compounds). The two benzophenones were again purified by further chromatography.

Tovophenone A (55), yellow oil, $[\alpha]_D$ -20 (c 2.2, $CHCl_3$). UV: λ_{max} 235, 266 and 304 nm ($\log\varepsilon$ 4.07, 3.89, 4.08); (+ NaOMe) 235, 304, 428 nm; (+ AcONa) unaltered; (+ $AlCl_3$ after 30 min) 335 nm. IR: ν_{max} 3500, 3300, 1610, 1580, 880 cm^{-1}. ^1H NMR(CCl_4): δ8.53 (H1, broad s, exchangeable with D_2O), 8.30 (1H, broad s, exchangeable with D_2O), 7.30-6.60 (5H, m), 5.10 (1H, broad t, J=7Hz), 4.53 (4H, broad

3.68 (3H, s), 3.20 (2H, broad d, J=7 Hz), 2.75-2.20 (3H, m), 2.10-1.30 (4H, m), 1.70 (12H, broad) s). ^{1}H NMR (C_5D_5N) : δ3.57 (2H, broad d, J=7 Hz), 2.93 (2H, broad s); m/e 464 (M^+, 18), 447 (6), 420 (12), 396 (4), 379 (6), 357 (12), 341(M-C_9H_{15}, 100), 325 (6), 297 (85), 285 (341 - C_4H_7, 20), 203 (297 - HOC_6H_5, II), 191 (285 - HOC_6H_5, 11), 145 (6), 123 (C_9H_{15}, 20), 121 (Ho-C_6H_{14}-$C{\equiv}O^+$, 62), 93 (18), 69 (10); m^* 250.6 (464→341), m^* 238.2 (341→285), m^* 138.7 (297→203), m^* 128.0 (285→191).

Acetylation with pyridine-acetic anhydride overnight at room temperature afforded triacetyltovophenone A (56), oil. IR: ν_{max} 1780, 1685, 1600, 1380, 1205, 900 cm^{-1}. ^{1}H NMR (CCl_4): δ 7.70-7.25 (4H, m), 5.03 (1H, broad t, J=7Hz), 4.58 (4H, broad s), 3.77 (3H, s), 3.32 (2H, broad d, J=7 Hz), 2.77-2.30 (3H, m), 2.24 (3H, s), 2.10-1.40 (4H, m), 1.87 (3H, s), 1.82 (3H, s), 1.67 (12H, broad s).

Methylation of tovophenone A (1 g) with diazomethane and chromatographic purification gave a 4-1 mixture (200 mg) of 2,3'-dimethyl tovophenone A (57) and 6,3'-dimethyl tovophenone A, 80 mg of 2,6,3'-trimethyl tovophenone A (58), and many CH_2N_2 addition products (not studied).

Methylation of tovophenone A (100 mg) by refluxing it for 5h with dimethyl sulphate and potassium acetate in anhydrous acetone afforded, after standard work up, 58 (75 mg).

Dimethyl derivative mixture, oils, IR : ν_{max} 3500, 1600, 1580 cm^{-1}. ^1H NMR ($CDCl_3$): δ10.87 (0.8 H, s), 10.70 (0.2 H, s), 3.82 (3H, s), 3.78 (3H, s), 3.27 (3H, s); CH_2 (C_5-chain), $\delta(C_5D_5N)-\delta(CDCl_3)$= + 0.07. Trimethyl tovophenone A (58), oil, $[\alpha]_D$-14 (c 1.8, $CHCl_3$). IR: ν_{max} 1660, 1580, 880 cm^{-1}. ^1H NMR spectrum, fig. 7; ^{13}C NMR ($CDCl_3$): δ 181.4 (s, CO), 159.5-153.1 (s x 4; C-2, C-4, C-6, C-3'), 147.4 (s, >C=), 146.3 (s, >C=), 139.0 (s, >C=), 132.7 (s), 130.8 (d), 124.8 (s), 123.4 (d), 123.0 (s), 122.3(s), 120.0 (d, =CH-), 112.9 (d x 2), 111.5 (t, $=CH_2$), 109.4 (t, $=CH_2$), 62.8 (q, OCH_3), 62.6 (q, OCH_3), 61.2 (q, OCH_3), 55.4 (q, OCH_3 on C-3'), 46.8 (d), 35.6 (t), 30.5 (t), 29.9 (t), 25.8 (q), 23.6 (t), 22.6 (q), 18.1-18.0 (qx2). MS: m/e 506 (M^+, 11), 399 (5), 397 (6), 384 ($M-C_9H_{14}$, 100), 315 (3) 149 (2), 135 ($CH_3O-C_6H_4-C\equiv O^+$, 20), 121 (25), 107 (7), 69 (8); m^* 291.4 (506→384), m^* 258.4 (384→315).

Action of Acids on Tovophenone A. - a) Tovophenone A (100 mg) in $CHCl_3$ containing 10% trifluoroacetic acid was left at room temperature for 18 h. ^1H NMR and IR spectra indicated the disappearance of the double bonds and formation of a trifluoroacetyl derivative; IR: ν_{max} 1780 cm^{-1}, δ1.53 [6H, s, $(CH_3)_2$ C-OTFA]. The crude product was stirred (4 h) in methanolic 10% NaOH.

Acidification with 2N HCl, standard work-up and chromatographic purification on silica gel column (benzene-ethylacetate, 9-1) gave 60 mg of pure dipyrano derivative,oil, (61), IR: ν_{max} 3500, 1660, 1600 cm^{-1}, ^1H NMR ($CDCl_3$): δ7.50-7.10 (4H, m), 5.90 (broad s, OH), 3.80 (3H, s), 2.95-2.50 (4H, m, benzylic CH_2). MS: m/e 482 (M^+, 20), 464 (M-18, 58), 449 (464-15, 50), 409 (464-55, 25), 395 (16), 393 (15), 379 (15), 341 (464-C_9H_{15}, 100), 325 (15), 297 (10), 285 (341-56, 33), 244 (25), 211 (20), 191 (285-HOC_6H_5, 20), 123 (200), 121 (600); m^* 446.7 (482→464), m^* 434.5 (464→449), m^* 360.5 (464→409), m^* 250.6 (464→341), m^* 238.2 (341→285). b) Tovophenone A (300 mg) in MeOH containing 15% HCl was stirred overnight at room temperature. Standard work-up and preparative TLC ($CHCl_3$-ethylacetate, 9-1) afforded the monopyrano derivative, 63, (95 mg) and the dipyrano derivative, 62 (115 mg). Monopyrano derivative (63), oil, UV: λ_{max} 326 nm (logε3.70); (+$AlCl_3$ after 30') 340 nm. IR: ν_{max} 3600, 3300, 1600 cm^{-1}. ^1H NMR ($CDCl_3$): δ11.60 (1H, s), 7.30-6.70 (4H, m), 5.20 (1H, broad t, J=7 Hz), 3.76 (3H, s), 3.25 (2H, broad d, J=7 Hz), 3.13 (3H, s), 2.75-2.45 (2H, m), 1.78 (3H, broad s), 1.70 (3H, broad s), 1.30 (6H, s), 1.13 (6H, s). MS: m/e 496 (M^+, 35), 464 (M-MeOH, 27), 449 (464-15, 27), 441 (11), 421 (8), 409 (464-55, 25),

396 (12), 393 (8), 379 (11), 341 (464-C_9H_{15}, 100), 325 (28), 297 (17), 285 (341-56, 31), 191 (285-HOC_6H_5, 14), 167 (8), 149 (19), 123 (18), 121 (60). m* 434.5 (464→449), m* 434.1 (496→464), m* 250.6 (464→341), m* 238.2 (341→285). Dipyrano derivative (62), oil, UV: λ_{max} 324 nm (logε 3.58). IR: ν_{max} 3600, 3300, 1665, 1600 cm^{-1}. ^1H NMR ($CDCl_3$): δ 7.30-6.80 (4H, m), 3.77 (3H, s), 3.23 (2H, t, J=7 Hz), 3.13 (3H, s), 2.80-2.40 (2H, m), 1.80-1.0 (7H, m), 1.23 (3H, s), 1.13 (15H, s). MS: m/e 496 (M^+, 34), 479 (M-CH_3, 12), 464 (M-MeOH, 36), 449 (464-15, 55), 447 (8), 409 (464-55, 18), 396 (11), 395 (13), 341 (464-C_9H_{15}, 100), 325 (18), 297 (13), 285 (25), 191 (285-HOC_6H_{15}, 18), 167 (12), 149 (21), 123 (18), 121 (67).

Osmium Tetroxide-Catalized Periodate Oxidation of Tovophenone A. - Trimethyl tovophenone A (300 mg, 0.6 x 10^{-3}mol.) in dioxane-H_2O, 3-1 (10 ml) was stirred for 3 h with OsO_4 (75 mg, 0.3 x 10^{-3} mol.) and $NaIO_4$ (250 mg, 1.16 x 10^{-3} mol.). After dilution with H_2O and ethyl ether extraction, the raw product was purified on silica gel column (benzene-ethylacetate, 9-1) giving 102 mg of pure monoketo aldehyde, 59, oil, $[\alpha]_D$-10 (c 1, $CHCl_3$); IR: ν_{max} 2840, 2730, 1730-1710, 1668, 1585, 896 cm^{-1}. ^1H NMR spectrum, fig. 8. MS: m/e 482 (M^+,5), 357 (M-$C_8H_{13}O$, 100), 343 (9), 329 (6), 313 (4), 311 (5), 221 (6), 207 (5), 191 (4), 149 (9), 135 (MeO-C_6H_4-$C{\equiv}O^+$, 52), 125 (6), 121 (55), 107 (23). Monoketo

aldehyde 59 in dioxane-H_2O, 3-1 (3 ml) was treated again with OsO_4 (50 mg) and $NaIO_4$ (100 mg) and stirred for 18 h. Standard work-up and purification as above gave 32 mg of pure diketo aldehyde, 60, oil, IR: ν_{max} 2850, 2730, 1730-1710, 1670, 1590 cm^{-1}. ^1H NMR spectrum, fig. 9. MS: m/e 484 (M^+, 24), 456 (15), 455 (24), 357 ($M-C_7H_{11}O_2$, 100), 329 (44).

Manglexanthone 64, yellow needles, m.p. 209-10° (MeOH) (Lit. [42] m.p. 208-10°). UV: λ_{max}^{MeOH} 230sh, 255sh, 284, 335, 376 nm (log ε 4.30, 4.35, 4.84, 4.25, 4.00). IR: ν_{max} 3240, 1660, 1640, 1620, 1590, 1470, 1380, 1330, 1300, 1280, 1250, 1200, 1180, 1165, 1130. 890, 790, 710 cm^{-1}. ^1H NMR ($CDCl_3$): δ 7.60 (1H, s), 6.72 (1H, d, J = 10Hz), 6.30 (1H, s), 5.56 (1H, d, J = 10Hz), 5.22 (1H, t), 3.92 (3H, s), 3.56 (2H, d), 1.78 (3H, s), 1.72 (3H, s), 1.42 (6H, s). MS: m/e 408 (M^+, 60), 393 (100), 377 (7), 361 (8), 349 (5), 335 (20), 323 (10), 204 (10), 203 (10).

Betulinic acid 65 m.p. 309-314° (MeOH) (Lit. [42] m.p. 310-4°). MS: m/e 456 (M^+, 75), 438 (18), 423 (15), 248 (40), 234 (25), 220 (30), 207 (46), 203 (40), 201 (18), 191 (30), 190 (38), 189 (100), 187 (35), 177 (18), 175 (35). IR (KBr): ν_{max} 3450, 1690, 1635, 890 cm^{-1}. Methyl betulinate: m.p. 221-3° (MeOH) (Lit. [42] m.p. 222-4°). M.S.: m/e 470 (M^+, 60), 455 (13), 452 (10), 411 (30), 262 (60), 220 (35), 207 (55),

203 (35), 189 (100). Acetyl derivative: m.p. 287-9° (EtOH) (Lit. [42] m.p. 288-90°). IR: ν_{max} 1720, 1690, 890 cm^{-1}. ^1H NMR ($CDCl_3$): δ 4.75 (IH, s), 4.55 (1H, s), 4.49 (1H, t), 3.03 (1H, broad t), 2.00 (3H, s), 1.7 (3H, broad s), 1.0 (3H, s), 0.95 (3H, s), 0.85 (9H, s).

Tovophenone B (66), yellow oil. UV: λ_{max} 242, 262 sh and 305 nm; ($+AlCl_3$ after 30 min) 333 nm. IR: ν_{max} (CCl_4) 3350, 1620, 1590, 887 cm^{-1}. ^1H NMR (CCl_4): δ 12.0 (1H, s), 7.30-6.60 (5H, m), 4.55 (4H, broad s), 4.20 (1H, t, J=7 Hz), 3.83 (3H, s), 3.10 (2H), 2.70-2.30 (3H, m), 2.0 - 1.30 (4H, m) 1.66 (6H, broad s), 1.0 (3H, s), 0.91 (3H, s). MS: m/e 480 (M^+, 6), 424 (10), 412 (3), 396 (8), 392 (4), 357 ($M-C_9H_{15}$, 75), 349 (9), 339 ($357-H_2O$, 12), 297 (6), 285 (9), 279 (6), 271 (7), 259 (8), 255 (11), 251 (6), 248 (9), 205 (9), 203 (7), 191 (9), 167 (14), 149 (42), 123 (C_9H_{15}, 15), 121 ($HO-C_6H_4-C\equiv O^+$, 100), 93 (121-28, 36), 69 (32); m* 265.5 (480→357), m* 321.9 (357→339), m* 71.4 (121→93). Refluxing of Tevophenone B (75 mg) for 5h with dimethyl sulphate and potassium acetate in anhydrous acetone gave dimethyl tovophenone B (67), oil, 45 mg, IR ν_{max} 3400, 1660, 1595,885 cm^{-1}. ^1H NMR (CCl_4): δ 7.35-6.70 (4H, m), 4.53 (4H, broad s), 4.28 (1H, t), 3.84 (3H, s), 3.77 (3H, s), 3.48 (3H, s), 3.30 (2H, d), 2.70-2.35 (3H, m), 2.10-1.30 (4H, m), 1.61 (6H, broad s), 1.04 (6H, s). MS: m/e

508 (M^+, 2), 440 (1), 385 (M-C_9H_{15}, 100), 367 (385-H_2O, 11), 343 (7), 325 (5), 313 (9), 277 (3), 149 (4), 135 (CH_3O-C_6H_4-C≡O^+, 35), 121 (15), 107 (12); m* 349.6 (385→367). Acetylation (pyridine-acetic anhydride) of the methylderivative (67) gave monoacetyl dimethyl tovophenone B (68), oil, IR: λ_{max} 1730, 1660, 1595, 890 cm^{-1}, δ (CCl_4) 7.30-6.80 (4H, m), 4.80 (1H, t, J = 7.5 Hz), 4.55 (4H, broad s), 3.85 (3H, s), 3.80 (3H, s), 3.57 (3H, s), 3.20 (2H, d, J = 7.5 Hz), 2.70 - 2.20 (3H, m), 2.0 - 1.30 (4H, m), 1.87 (3H, s), 1.63 (6H, broad s), 1.38 (3H, s), 1.23 (3H, s).

Action of HCl on Tovophenone B. Tovophenone B (200 mg) in MeOH containing 15% HCl waS stirred overnight at room temperature. Standard work-up and preparative TLC (C_6H_6-ethyl acetate, 6-4) afforded compound 69, (105 mg), Oil, IR: ν_{max} 3350, 1670, 1600 cm^{-1}. ^1H NMR ($CDCl_3$): δ 7.40 - 6.90 (4H, m), 4.50 (1H, t), 3.93 (3H, s), 3.15 (3H, s). MS: m/e 512 (M^+, 13), 497 (M-15, 1.5), 480 (M-MeOH,9), 465 (480-15, 11), 357 (480-C_9H_{15}, 32), 339 (357-H_2O, 7), 307 (7), 298 (15), 284 (8), 279 (33), 256 (11), 167 (45), 149 (100), 123 (23), 121 (40), 93 (15); m* 450 (512→480), m* 71.5 (121→93).

3.3 ASPILIA PLURISETA SCHWEINF.

Plant material.

The aerial parts of Aspilia pluriseta were collected in Chiromo, Nairobi, Kenya.

Extraction and purification.

The air dried plant material (1.22 Kg) was extracted with methanol in the cold and the extract evaporated. The crude oily residue (41.6 g) was partitioned between chloroform and water. The chloroform extract was evaporated to yield an oil (9.3 g) which was further partitioned between hexane and methanol-water, 5-1. The hexane fraction was evaporated to yield an oil (4.2 g). This oil (2.8 g) was separated by column chromatography (hexane-EtOAc, 9-1) yielding impure 70 (1.2 g) and 72 (130 mg). Preparative TLC ($CHCl_3$) of impure 70 (270 mg) and impure 72 (130 mg) yielded pure 70 (128 mg) and pure 72 (42 mg). The water-methanol fraction was evaporated and the resulting aqueous mixture was extracted with chloroform. Evaporation of the chloroform extract yielded an oil (810 mg) column chromatography ($CHCl_3$-EtOAc, 8-2) of which afforded impure 73 (120 mg) which was purified further by preparative TLC (Hexane-EtOAc, 6-4) yielding pure 73 (73 mg).

(-) Kaur-(16)en-(19)oic acid (ent-kaurenic acid). 70 IR: $\nu_{max}^{CCl_4}$ cm^{-1} 3500-2500, 1690, 1657, 880. ^1H NMR (CCl_4): δ 11.7 (br s, 19-H), 4.67 (br s, 17H), 2.58 (m, 13-H), 1.22 (s, 18-H), 0.93 (s, 20-H). MS; m/e (rel. int.) 302 (M^+, 51), 287 (M-15, 30), 259 (47), 257 (M-45, 25), 243 (25), 241 (30), 239 (17), 213 (34), 201 (12.7), 199 (27), 131 (64), 121 (51), 105 (68), 91 (100).

Esterification of 70 (106 mg) in Et_2O with CH_2N_2 afforded the methyl ester 71. ^1H NMR ($CDCl_3$): δ 4.74 (s, 17-H), 3.61 (s, 19-H), 1.15 (s, 18-H), 0.82 (s, 20-H.). MS; m/e (rel. int) 316 (M^+, 97), 301 (M-15, 38) 273 (M-43, 83), 257 (M-59, 95), 91 (100).

15-β-Angeloyloxy-ent-kaurenic acid 72 IR: $\nu_{max}^{CCl_4}$ cm^{-1} 3500-2500, 1710, 1690, 1260, 1230, 1160, 845. ^1H NMR ($CDCl_3$): δ 6.00 (q, J=7, 1Hz, 3'-H), 5.33 (br s, 15-H), 5.10 (br s, 17-H), 5.03 (br s, 17-H), 2.76 (m, 13-H), 2.03 (dq, J=7, 1Hz, 5'-H), 1.90 (dq, J=1, 1Hz, 4'-H), 1.23 (s, 18-H), 0.95 (s, 20-H). MS; m/e (rel. int) 400 (M^+, 6.5), 300 (M-100 , 28.6), 285 (300-15, 18.8), 272 (300-28, 11.9), 257 (6.5), 255 (300-45, 7), 239 (285-46, 5.3), 147 (9), 123 (10.7), 121 (12.9), 91 (22.6), 83 ($C_4H_7CO^+$, 100); m^* 270.75 (300→285), m^* 200.42 (285→239).

15-β-Angeloyloxy-16, 17-epoxy-ent-kauranic acid 73. IR: $\nu_{max}^{CCl_4}$ cm^{-1} 3500-2500, 1710, 1290, 1260 br, 1190. ^1H NMR ($CDCl_3$): δ 6.00 (q, J=7 Hz, 3'-H), 4.78 (s, 15-H),

3.10 (d, J=5 Hz, 17-H), 2.73 (d, J=5 Hz, 17-H), 2.03 (dq, J=7 Hz, 5'-H), 1.90 (dq, J=1, 1Hz, 4'-H), 1.23 (s, 18-H) 1.00 (s, 20-H). MS; m/e (rel. int) 416 (M^+, 5.3), 316 (M-100, 22), 301 (316-15, 12), 298 (316-18, 10.3), 283 (298-15, 6.3), 271 (316-45, 9), 149 (23.3), 123 (14.6), 121 (16.3), 91 (21), 83 ($C_4H_7CO^+$, 100); m^* 286.7 (316→301), m^* 281.02 (316→298), m^* 268.75 (298→285), m^* 231.40 (316→271).

3.4 TEPHROSIA HILDEBRANDTII VATKE

Plant material.

The roots of Tephrosia hildebrandtii Vatke. (Leguminosae) were collected from Kilimambogo, Kenya.

Extraction and separation

The dried roots (621 g) were powdered and extracted with methanol in the cold. Evaporation of the extract yielded a gummy residue (44.7 g). The residue was partitioned between water and chloroform and the chloroform fraction (19.1 g) partitioned further between hexane and methanol-water (4:1 v/v) mixture. Evaporation of methanol from the methanol-water fraction and subsequent extraction of the residue with chloroform yielded a gummy extract (11.6 g). Column chromatography of this extract (10 g) using chloroform-ethyl acetate (4:1 v/v) mixutre as eluant afforded two chromatographically pure compounds which were purified further by preparative thin layer chromatography to yield

compound 74 (186 mg) and 80 (155 mg).

3-Acetyl-5, 7-dimethoxy -8-methyl-trans-but-1, 3-dienyl flavanonol 74, oil. UV: λ_{max}^{MeOH} 280, 315 nm. IR: ν_{max} 1770, 1690, 1585 and 885 cm^{-1}. ^1H NMR ($CDCl_3$): δ 1.86 (3H, s, Me), 2.05 (3H, s, Me), 3.90 (6H, s, OMe), 4.90 (2H, br s, $=CH_2$), 5.32 (1H, d, J=12 Hz, H-1"), 5.63 (1H, d, J=12 Hz, H-2"), 6.12 (1H, s, H-6), 6.60 (1H, d, J=16 Hz, H-2), 7.20 (1H, d, J=16 Hz, H-3) and 7.4 (5H, br s, H-2', 3', 4', 5' and 6'). MS: m/e (rel. int.) 408 (M^+, 28), 376 (M-32, 8), 355 (M-53, 8), 348 (6), 325 (9), 260 (22) 229 (100), 214 (61), 209 (59), 205 (80), 194 (30), 193 (53), 120 (28), 91 (61).

Trans-anhydro tephrostachin

Compound 80. M.p 127-133° (EtOAc) (Lit. [52] m.p 128-134°). UV: λ_{max} 260, 276 and 346 sh nm. IR: ν_{max} 3100, 1640, 1590 and 880 cm^{-1}. ^1H NMR (100 MHz, $CDCl_3$): δ2.04 (3H s, Me), 3.99 (3H, s, OMe), 4.01 (3H, s, OMe), 5.09 br s, $=CH_2$), 6.42 (1H, s, H-3), 6.67 (1H, s, H-6), 6.83 (1H, d, J=16.6 Hz, H-1"), 7.26 (1H, d, J=16.6 Hz, H-2"), 7.48 (3H, m, H-3' 4' and 5'), 7.81 (2H, m, H-2' and 6'). ^{13}C NMR δ/ppm 178.1 (CO); 161.5, 160.8, 159.8 (C-2, C-5, C-7); 156.1 (C-8a); 143 (C-1"); 136 (C-2"); 131.8 (C-1'); 131.2 (C-4'); 129 (C-3', C-5'); 126.2 (C-2', C-6'); 117.8, 117.0 (C-8, C-4"); 109.1 (C-4a); 108.5 (C-2); 107.7 (C-3"); 91.8 (C-6); 56.4, 56.0 (OCH_3); 18.2 (Me). MS m/e (rel.int.):

348 (M^+, 100), 319 (73.3), 317 (20.1), 387 (26.6), 225 (10.5), 129 (11.2), 115 (12.8), 105 (C_6H_5-CO^+, 38.3), 102 ($C_6H_5C{\equiv}CH^{+\cdot}$, 11.1), 91 (13.2), 77 (23.9).

3.5 MILLETIA THONNINGII (SCHUMACH & THONN.) BAK.

Plant material.

The seeds of Milletia thonningii (Schumach & Thonn.) Bak. from West Africa were supplied by the Royal Batanic Gardens, Kew, and authenticated by the Professor A. Polhill herbarium.

Extraction and fractionation.

29 g of seeds were powdered and continously extracted with hot MeOH and the extract evaporated. The residue was dissolved in $CHCl_3$-H_2O and the organic layer separated and evaporated (4 g). The crude oily residue was roughly separated on silica-gel in five fractions: F_1 (C_6H_6; 1.9 g of triglycerides), F_2, F_3, F_4 (C_6H_6-EtOAc, 97-3; 90 mg, 250 mg and 1.2 g respectively) and F_5 (C_6H_5-EtOAc, 9-1; 300 mg, not examined further). Extended column chromatography of F_2, F_3, F_4 (C_6H_6-EtOAc, 97-3; 90 mg, 250 mg and 1.2 g respectively) and F_5 (C_6H_5-EtOAc, 9-1; 300 mg, not examined further). Extended column chromatography of F_2 gave 4'-methyl alpinum isoflavone (40 mg). Crystallization (C_6H_6) of F_3 afforded 3',5 dihydroxy-4'-methoxy-2",2"-dimethyl-pyrano-(5",6";6,7)-isoflavone (125 mg). Extended column chromatography of F_4 gave alpinum isoflavone

(745 mg) and a mixture of two compounds which were separated by preparative TLC ($CHCl_3$, three developments) yielding robustic acid (96 mg) and dimethylalpinumisoflavone (143 mg).

Alpinum isoflavone, 81, M.p 210-2 °C (acetone-hexane) (lit [95] m.p 213-4 °C). UV: λ_{max}^{MeOH} 284 (4.77) nm; λ_{max}^{AcONa} 284 nm; $\lambda_{max}^{AlCl_3}$ 299 nm after 30 minutes, IR: $\nu_{max}^{CHCl_3}$ 3570, 3300, 1650 cm^{-1}. ^1H NMR (60 MHz; $CDCl_3$-CD_3OD, 9-1): δ 13.20 (0.5 H, s), 7.72 (1H, s, H-2), 7.25 (2H, d, J=8.5 Hz; H-2', H-6'), 6.80 (2H, d, J=8.5 Hz; H-3', H-5'), 6.60 (1H, d, J=10 Hz, H-4"), 6.23 (1H, s, H-8), 5.53 (1H, d, J=10 Hz, H-3"), 1.40 (6H, s). Methylation (CH_2N_2) gave monomethylderivative, 83 and dimethylderivative 82 identical (mixed m.p., TLC and ^1H NMR) to the natural products.

Dimethyl alpinum isoflavone, 82. M.p. 119-21 °C (C_6H_6) (lit. [95, 96] m.p 119-20 °C). ^1H NMR (60 MHz, $CDCl_3$): δ 7.60 (1H, s, H-2), 7.30 (2H, d, J=8.5 Hz; H-2', H-6'), 6.78 (2H, d, J=8.5 Hz; H-3', H-5') 6.63 (1H, d, J=10 Hz, H-4"), 6.40 (1H, s, H-8), 5.53 (1H, d, J=10 Hz, H-3"), 3.80 (3H, s), 3.73 (3H, s), 1.40 (6H, s).

4'-Methyl alpinum isoflavone, 83. M.p. and mixed m.p. 137-8 °C (C_6H_6) (lit. [95] m.p 136-7 °C). ^1H NMR (60 MHz, $CDCl_3$): δ 13.20 (1H, s), 7.77 (1H, s, H-2), 7.40 (2H, d, J=8.5 Hz; H-2'; H-6'), 6.92 (2H, d, J=8.5 Hz; H-3' H-5'), 6.67 (1H, d, J=10 Hz, H-4"),

6.27 (1H, s, H-8), 5.56 (1H, d, J=10 Hz, H-3"), 3.80 (3H, s), 1.43 (6H, s), OCH_3, $\Delta\delta$ ($CDCl_3$-C_6D_6), +0.5 ppm. H-4", $\Delta\delta$ (C_5D_5N-$CDCl_3$), + 0.16 ppm. Acetylation (Ac_2O-AcONa) gave 5 acetyl-4' methylalpinumisoflavone (84), m.p 180-1 °C (heptane) (lit. [95] m.p 179-81 °). ^1H NMR (60 MHz, $CDCl_3$): δ 7.70 (1H, s), 7.33 (2H, d, J=8.5 Hz), 6.87 (2H, d, J=8.5 Hz), 6.63 (1H, s, H-8), 6.43 (1H, d, J=10, H-4"), 5.7 (1H, d, J=10 Hz, H-3"), 3.78 (3H, s), 2.40 (3H, s), 1.46 (6H, s). $\Delta\delta$ (84-83) H-4", -0.24 ppm; H-3", + 0.14 ppm; H-8, + 0.38 ppm,

3', 5-dihydroxy-4'-methoxy-2", 2"-dimethylpyrano (5", 6"; 6,7)-isoflavone, 85 M.p 155-6 ° (C_6H_6), yellow plates. UV: λ_{max}^{MeOH} 285 (4.62) nm; λ_{max}^{AcONa} 284 nm. $\lambda_{max}^{AlCl_3}$ 303 nm after 20 minutes. IR: $\nu_{max}^{CHCl_3}$ 3550, 1660, 1630 cm^{-1}. ^1H NMR (60 MHz, $CDCl_3$): δ 13.0 (1H, s), 7.70 (1H, s, H-2), 7.03-6.85 (3H, m; H-2', H-5', H-6'), 6.63 (1H, d, J=10 Hz, H-4"), 6.22 (1H, s, H-8), 5.75 (1H, broad s, OH, exchanged with D_2O), 5.53 (1H, d, J=10 Hz, H-3"), 3.80 (3H, s), 1.42 (6H, s): (C_5D_5N) 7.55 (1H, d, J=2 Hz, H-2'), 6.80 (1H, d, J=10 Hz, H-4"). H-4", $\Delta\delta$ (C_5D_5N-$CDCl_3$) + 0.17 ppm. EIMS (probe) 70 eV; m/e (rel. int.) 366 (M^+, 40), 365 (9), 351 ($M-15^+$, 100), 336 ($351-15^+$, 26), 247 (18), 233 (4), 204 (3), 203 (RDA of ($M-15^+$, 5), 175.5 ($M-15^+$, 27), 168 (7), 154 (18), 149 (B ring , 4), 140 (3), 135 (6), 105 (4). Acetylation (Ac_2O-AcONa) gave 3', 5 diacetoxy-4'-methoxy-2", 2" dimethyl-pyrano-(5", 6"; 6, 7)

isoflavone (86), m.p 206-8 ^{o} (CH_2Cl_2-heptane), white needles. ^{1}H NMR (60 MHz, $CDCl_3$): δ 7.70 (1H, s, H-2), 7.27 (1H, dd, J=2 and 8.5 Hz, H-6'), 7.13 (1H, d, J=2 Hz, H-2'), 6.91 (1H, d, J=8.5 Hz, H-5'), 6.63 (1H, s, H-8), 6.43 (1H, d, J=10 Hz, H-4"), 5.70 (1H, d, J=10 Hz, H-3"), 3.77 (3H, s), 2.40 (3H, s), 2.27 (3H, s), 1.43 (6H, s). Δδ(86-85) H_{α}, -0.20 ppm; H_{β}, + 0.17 ppm; H-8, + 0.41 ppm.

Robustic acid, 87. M.p 202-5 ^{o}C (C_6H_6) (lit. [98], m.p 208-10 ^{o} (lit. [99], m.p 208-9 ^{o}). UV: λ_{max}^{MeOH} 233, 256, 338 nm; λ_{max}^{AcONa} and λ_{max}^{NaOMe} 248, 277, 330 nm. IR: $\nu_{max}^{CHCl_3}$ 3520, 3280, 1705, 1630, 1610 cm^{-1}. ^{1}H NMR (60 MHz, $CDCl_3$): δ 7.42 (2H, d, J=8.5 Hz; H-2', H-6'), 6.90 (2H, d, J=8.5 Hz; H-3', H-5'), 6.57 (1H, s, H-8), 6.45 (1H, d, J=10 Hz, H-4"), 5.70 (1H, d, J=10 Hz, H-3"), 3.96 (3H, s), 3.77 (3H, s), 1.44 (6H, s). EIMS (probe) 70 eV; m/e (rel. int.) 380 (M^{+},100), 365 ($M-15^{+}$, 92), 337 (18), 321 (8), 307 (6), 233 (30), 232 (15), 217 (67), 203 (14), 189 (4), 175 (11), 161 (15), 148 (21), 135 (8), 133 (4). Acetylation (Ac_2O-pyridine) gave 4'-acetylrobustic acid (88), m.p 194-6 ^{o} (CH_2Cl_2-heptane) (lit. [99] 198-9 ^{o}). ^{1}H NMR (60 MHz, $CDCl_3$): δ 7.35 (2H, d, J=8 Hz; H-2', H-6'), 6.90 (2H, d, J=8 Hz; H-3', H-5'), 6.60 (1H, s, H-8), 6.53 (1H, d, J 10 Hz, H-4"), 5.70 (1H, d, J=10 Hz, H-3"), 3.80 (3H, s), 3.76 (3H, s), 2.13 (3H, s), 1.44 (6H, s). Methylation of robustic acid (87) with CH_2N_2 gave the corresponding

methylether (robustic acid methyl ether), 89 , m.p 194-5 °C (EtOH) (lit [98], m.p 198-200 °C; lit. [99], m.p 195-6 °C). ^{1}H NMR (60 MHz, $CDCl_3$)δ 7.36 (2H, d, J=8.5 Hz; H-2', H-6') 6.93 (2H, d, J=8.5 Hz; H-3', H-5'), 6.57 (1H, d, J=10 Hz, H-4"), 6.53 (1H, s, H-8), 5.67 (1H, d, J=10 Hz, H-3"), 3.83 (3H, s), 3.80 (3H, s), 3.53 (3H, s), 1.46 (6H, s).

REFERENCES

1. Farnswoth N.R. and Morris R.W. Am. J. Pharm. 148 (1976) 46-52.

2. Farnsworth N.R. and Bingel A.S. New Natural Products and Plant drugs with Pharmacological, Biological or Therapeutical Activity. (1977) (In Wagner H. and Wolff eds.) Springer-Verlag, New York, p. 61-73.

3. The Primary Health Care, WHO, UNICEF Meeting Report, Alma Acta (1978), Geneva.

4. Penso G. J. Ethnopharmacol. 2 (1980) 183.

5. The Chemotherapy of Malaria, WHO, SWG, 4th Meeting Report, (1981) Beijing, China.

6. Meinwald J., Prestwich G., Nakanishi K. and Kubo I. Science, 199 (1978) 1167.

7. Farnsworth N.R. and Kaas C.J. J. Ethnopharmacol. 3 (1981) 85-99.

8. Sneden A. T. and Beemsterboer G.L. J. Nat. Prod. 43 (1980) 637-640.

9. Kupchan S.M., Komoda Y., Branfman A.R., Sneden A.T., Court W.A., Thomas G.J., Hintz H.P.J., Smith A.K., Howie G.A., Verma A.K., Nagao Y., Dailey R.G., Zimmerly V.A. and Summer W.C. J. Org. Chem., 42 (1977) 2439.

10. Douros J. and Suffness M. Cancer Chemother. Pharmacol., 1 (1978) 91.

11. Kokwaro J.O. Medicinal Plants of East Africa (1976) 1st Edition, East African Literature Bureau, Nairobi, 384 pp.

12. Xiao Peigen. Proceedings of the 2nd International Seminar on Medicinal and Aromatic Plants, 1981, Citta di Castello, Italy. In press.

13. Dale I.R. and Greenway P.J. Kenya trees and shrubs, (1961) Buchanan's Kenya Estates Limited, Nairobi and Hatchards, London. 654 pp.

14. Waterman P.G. Biochem. System. Ecol. 3 (1975) 149-180.

15. Willis J.C. Dictionary of Flowering plants and Ferns. (1973) The University Press, Cambridge, 1245 pp.

16. Mitchell Watt, J. and Brandwijk M.G. The Medicinal and Poisonous Plants of Southern and Eastern Africa. (1962) 1st Edition, E & S Livingstone Limited, London. 1457 pp.

17. Dalziel J.M. The Useful Plants of West Tropical Africa. (1955) 1st edition, Crown Agents for Overseas Governments and Administrations, London. 612 pp.

18. Vaquette J., Hocquemiller R., Pousset J.L. and Cave A., Planta Med. 33 (1978) 78-84.

19. Pegel K.H. and Wright W.G. J. Chem. Soc. C (1969) 2327-9.

20. Vaquette J., Cleriot M.O., Paris M.R., Pousset J.L., Cave A. and Paris R.R. Plant. Med. Phytother. 8 (1974) 57-62.

21. Okugun J.I. and Ayafor J.F. J. Chem. Soc., Chem. Commun. (1977) 652-3.

22. Waterman P.G. Biochem. Syst. 1 (1973) 153-61.

23. Tackie A.N., Kofi-Tsekpo W.M. and Hadzija B.W. W. Afr. Pharm. 9 (1967) 24-5, 43.

24. Popp F.D. and Chakraborty D.P. J. Pharm . Sci. 53 (1964) 968.

25. Paris R.R. and Stambouli A. Compt. rend. 247 (1958) 2421-3.

26. Laing M. and Sommerville P. J.S. Afr.Chem. Inst. 27 (1974) 162-3

27. Ayafor J.F. and Okogun J.I. J. Chem. Soc., Perkin Trans. I (1982) 909-15.

28. Paris R.R. and Stambouli A. Compt. rend. 248 (1959) 3736-8.

29. Fish F., Meshal I.A. and Waterman P.G. J. Pharm. Pharmacol. 28 (1976), Suppl. 72p

30. Fish F., Meshal I.A. and Waterman P.G. Fitoterapia. 48 (1977) 170-2.

31. Vaquette J., Pousset J.L. and Cave A. Plant. Med. Phytother. 8 (1974) 72-5.

32. Ayafor J.F. and Okogun J.I. J. Nat. Prod. 45 (1982) 182-5.

33. Waterman P.G. Phytochem. 14 (1975) 2092-3.

34. Casey A.C. and Malhotra A. Tetrahedron lett. (1975) 401-4.

35. Waterman P.G. Phytochem. 14 (1975) 2092-2093.

36. Badger G.M., Christie B.J. and Rodda H.J. Australian J.Chem. 16 (1963) 734-5.

37. Ayafor J.F., Sondengam B.L., Connolly J.D., Rycro ft D.S. and Okogun J.I. J.Chem. Soc., Perkin Trans.1. (1981) 1750-3.

38. Candy H.A., Pegel K.H. and Wright W.G. J.S. Afr. Chem. Inst. 26 (1973) 11-14.

39. Paris R., and Etchepare S. Ann. Pharm. Fr. 26 (1968) 51-3.

40. Gellert E. Australian J. Chem. 10 (1957) 209-10.

41. Kubo I. and Nakanishi K. In Advances in Pesticide Science (1979) (Geissbühler H., Brooks G.T. and Kearney P.C., eds.) IUPAC Pesticide Chemistry,

Pergamon Press, Oxford, p. 285. -

42. Marta M., Delle Monache F., Marini-Bettolo G.B., Lyra D.A., De Mello F., and De Lima O.G. Gazz. Chim. Ital. 106 (1976) 651. -

43. Gabriel S.J. and Gottlieb O.R. Phytochem. 11 (1972) 3035-6.

44. Goncalves De Oliveira W., Gottlieb O.R. and Lins Mesquita A.A. Phytochem. 11 (1972) 3323-3325.

45. Stuessy T.F. The Biology and Chemistry of the Compositae. 1977 (Heywood V.H., Harborne, J.B. and Turner B.L., eds.) Academic Press, London, p. 621.

46. Bohlmann F., Ziesche J., King R.M. and Robinson H. Phytochem. 20 (1981) 751-756.

47. Agnew A.D.Q. Upland Kenya Wild Flowers. 1974, Oxford University Press. London. 827 pp.

48. Gillett J.B., Polhill R.M. and Verdcourt B. In Flora of Tropical East Africa. 1971 (Milne-Redhead E. and Polhill R.M., eds.) The Government Printer, Nairobi, 501 pp.

49. Gomes C.M.R., Gottlieb O.R., Marini Bettolo G.B., Delle Monache F. and Polhill R.M. Biochem. System. Ecol. 9 (1981) 129-147.

50. Rao P.P., and Srimanarayana G. Curr. Sci. 50 (1981) 319-20.

51. Pelter A., Ward R.S., Rao E.V. and Raju N.R. J.Chem. Soc., Perkin Trans. I. (1981) 2491-8.

52. Khalid S.A. and Waterman P.G. Phytochem. 20 (1981) 1719-20.

53. Chibber S.S., Dutt S.K., Sharma R.P. and Sharma A. Indian J. Chem., Sect. B. 20B (1981) 626-7

54. Sinha B., Natu A.A. and Nanavati D.D. Phytochem. 21 (1982) 1468-1470.

55. Dutt S.K. and Chibber S.S. Phytochem. 22 (1983) 325-6.

56. Chibber S.S. and Dutt S.K. Phytochem. 20 (1981) 1460.

57. Mahmoud Z.F., Saleh M.R.I. and Khafagy S.M. Sci.Pharm. 49 (1981) 184-6.

58. Kamal R., and Jain S.C. Agric. Biol. Chem. 44 (1980) 2985.

59. Singhal A.K., Sharma R.P., Baruah J.N., Govindan S.V. and Herz W. Phytochem. 21 (1982) 949-951.

60. Crombie L., Dewick P.M. and Whiting D.A. J.Chem. Soc., Perkin Trans. I. (1973) 1285.

61. Roark R.C. Industr. Engng Chem. 25 (1933) 639.

62. Gleason M.N., Gosselin R.E. and Hodge H.C. Clinical Toxicology of Commercial Products. 1957, Williams and Wilkins, Baltimore.

63. Clark E.P. Science. 71 (1930) 396; J. Amer. Chem. Soc. 53 (1931) 313, 729, 2369, 3431; 54 (1932) 3000.

64. Clark E.P. J. Amer. Chem. Soc. 52 (1930) 2461.

65. The Merck Index. (1976) 9th Edition, (Windholz M., Budavari S., Stroumtsos L.Y. and Ferting M.N.eds.) Merck & Co., Inc., U.S.A. 1313 pp.

66. Roark R.C. J. Econ. Ent. 26 (1933) 587.

67. Roark R.C. U.S. Dept. Agric. Div. Insectic. Invest. Feb. 1937, E402.

68. Islam A., Gupta R.K. and Krishnamurti M. Phytochem. 19 (1980) 1558-1559.

69. Singhal A.K., Sharma R.P., Thyagarajan G., Herz W. and Govindan S.V. Phytochem. 19 (1980) 929-34

70. Raju K.V., Srimannarayana G., Ternai B., Stanley R. and Markham K.R. Tetrahedron 37 (1981) 957-62.

71. Singhal A.K., Sharma R.P., Madhusudanan K.P., Thyagarajan G., Herz W. and Govindan S.V. Phytochem. 20 (1981) 803-6.

72. Singhal A.K., Sharma R.P., Baruah J.N., Govindan S.V. and Herz W. Phytochem. 21 (1982) 949-951.

73. Ma J.C.N. and Warnhoff E.W. Canad. J. Chem. 43 1965, 1849.

74. Price J.R. Australian J. Sci. Res. A2 (1949) 249-254.

75. Yajina T. and Munakata K. Agric. Biol. Chem. 43 (1979) 8 .

76. Macfoy C.A. and Smith I.M. Physiol. Plant Pathology 14 (1979) 99-111.

77. Price J.R. (1963) Chemical Plant Taxonomy, 1963 (Swain T., edition), Academic Press, London, p. 429.

78. Smalberger T.M., Vleggaar R. and Weber J.C. Tetrahedron, 30, (1974) 3927-3931.

79. Locksley H.D. and Murray I.G. J. Chem. Soc. (C) (1970) 392.

80. Atkinson J.C., Gupta P. and Lewis J.R. Tetrahedron 25 (1969) 1057.

81. Carpenter I., Locksley H.D. and Scheinmann F. Phytochem. 8 (1969) 2013.

82. Alves de Lima R. and Delle Monache G. Rend. Acad. Naz. dei XL, (Rome) 3 (1977-8) (V) 181.

83. Rappo R., Allen Jr. D.S., Lemieux R.U. and Johnson W.S. J. Org. Chem. 21 (1956) 478.

84. D'Alburquerque I.L., Corio E., Delle Monache F., Tucci A.P. and Marini Bettolo G.B. Mart. Ann. Inst. Sup. sanita. 5 (1969) 557-8.

85. Bohlmann F., Fritz U., King R.M. and Robinson H. Phytochem. 19 (1980) 2655.

86. Bohlmann F. and Zdero C. Phytochem. 16 (1977) 786.

87. D'Albuquerque M.M.F., De Andrade Lyra F.D., De Mello J.F., De Lima O.G., Delle Monache F., De Santana M.B. and Moreira L.C. Rev. Inst. Antibiot. Recife, 14 (1974) 83.

88. Mabry T.J., Markham K.R. and Thomas M.B. The Systematic Identification of Flavonoids, 1970, Springer-Verlag, Berlin. 354 pp.

89. Harborne J.B., Mabry T.J. and Mabry H. The Flavonoids., 1975, Chapman & Hall, London. 1106 pp

90. Smalberger T.M., Vlegaar R. and Weber J.C. Tetrahedron. 30 (1974) 3927.

91. Gray A.I., Waigh R.D. and Waterman P.G. J. Chem. Soc. Perkin Trans. I (1975) 488.

92. Waterman P.G. and Khalid S.A. Phytochem. 19 (1980) 909-915.

93. Gripenberg J., Honkanen E. and Silander K. Acta Chem. Scand. (1956) 393-6.

94. Nakato T., Alonso J., Gillet R. and Martin A. J. Chem. Soc. Perkin Trans. I. (1979) 2107.

95. Jackson B., Owen P.J. and Scheinmann F. J.Chem. Soc. C (1971) 3389.

96. Chibber S.S. and Sharma R.P. Indian J. Chem. 18B (1979) 471.

97. Arnone A., Cardillo G., Merline L. and Mondelli R. Tetrahedron Letters (1967) 4201.

98. Johnson A.P. and Pelter A. J. Chem. Soc. C (1966) 606.

99. East A.J., Ollis W.B. and Wheeler R.E. J. Chem. Soc. C (1969) 365.

100. Lwande W., Gebreyesus T., Chapya A., MacFoy C., Hassanali A. and Okech M. Insect Sci. Application. (In press).