ANTHRANOIDS OF SOME KNIPHOFIA SPECIES FROM ETHIOPIA

A thesis

Presented to the

School of Graduate Studies

Addis Ababa University

In partial Fulfillment

of the requirements of the Degree

Master of Science in Chemistry

by

Esaiyas Berhanu

June 1984

ACKNOWLEDGEMENTS

I would like to express my appreciation to my advisor, Dr. Ermias Dagne for constant guidance and help in the course of this work. I would also like to thank Dr. Berhanu Abegaz for his constructive suggestions an encouragements. I am grateful to Prof. Dr W. Steglich, Bonn University, FRG, for the high resolution NMR and MS spectra and for helpful discussions. I wish to express my appreciation to W/t Menbere Habtemariam and Ato Dawit Dado for their constant encouragment. Thanks are also due to members of the Chemistry Department, AAU and Co-ordinating Office for Traditional Medicine, Ministry of Health, for their interest in the work and to Ato Yilma Tamiru who typed the manuscript.

Financial supports from the Swedish Agency for Research Cooperation with the Developing Countries (SAREC) and from UNDP/WHO for the Development of Traditional Medicine under the project ETH/78/023 are gratefully acknowledged.

Abstract

Anthranoids of Some *Kniphofia* Species from Ethiopia

by

Esaiyas Berhanu

Research Advisor Dr. Ermias Dagne

There are at least six reported *Kniphofia* species in Ethiopia of a total of 65 species of *Kniphofia* in the world all of which are restricted to tropical Africa and Yemen. *Kniphofia foliosa* (family Liliaceae) known in Amharic as *Ashenda* or *Shemetmete* is used in Ethiopian traditional medicine for the treatment of abdominal cramps. Recent examination of the rhizomes of this species by our research group resulted in the isolation and characterization of chrysophanol and the new anthranoid knipholone.

Chloroform soluble fractions of the leaves of *K. foliosa* afforded a new anthracene derivative, aloe-emodin monoacetate, in addition to knipholone and chrysophanol. The structure of this new compound was determined by spectral analysis of the natural product and its derivatives. The leaves, flowers and rhizomes of *K. foliosa* all contain knipholone as the major anthranoid with trace amounts of chrysophanol. Aloe-

../..

emodin acetate is localized only in the leaves while the pigments designated as K_{f_7} and K_{f_8} are found only in the rhizomes.

From the acetone extract of the rhizomes of K. isoetifolia Cf two pigments K_{f_7} and K_{f_8} were isolated. The sodium dithionite reductive cleavage of K_{f_8} as well as a mixture of K_{f_7} and K_{f_8} gave chrysophanol and islandicin as fragments, a contribution towards the final structural elucidation of these as yet unknown pigments.

On further investigation islandicin was isolated for the first time from the rhizomes of K. foliosa, K. isoetifolia Cf and from the leaves of K. foliosa and this finding probably constitutes the first detection of islandicin in higher plants as it was earlier reported only in fungi particularly in Penicillium islandicum.

TABLE OF CONTENTENTS

../..

../..

../..

1. Introduction

Kniphofia foliosa Hochst (family Liliaceae), synonyms K. quartiniana, K. densiflora and K. arussi, which is known in Amharic as Ashenda or Shemetmete, is a robust clump-forming perennial herb with a short root stalk, widely distributed and restricted to the Ethiopian plateau at altitudes from 2050 - 4000 meters. Marais[1] registered the occurrence of K. foliosa in seven administrative regions namely, Tigrai, Gondar, Shoa, Arssi, Bale and Hararghe. This plant is particularly popular because of its colorful flowers and is known to bloom from June to September and also during December and January. K. foliosa is the most often encountered Kniphofia species out of five other Kniphofia species known in Ethiopia namely, K. isoetifolia, K. hidebrandtti, K. schimperi, K. pumila and K. insignis.[1,2] The regional distribution of Kniphofia in Ethiopia is given in Table 1. In Ethiopian traditional medicine the rhizomes of K. foliosa are used in the treatment of abdominal cramps.

As to the chemical composition of Kniphofia species an earlier study established the presence of rhein(7) in seeds of K. uvaria[3] a plant used in Zulu folk medicine in South Africa[4]. Besides a report on the isolation of putrescine amides from leaves of K. foliosa,

K. flavovirens and K. tuckii[5], no further studies on Kniphofia could be found in the literature.

Preliminary screening tests on the leaves, flowers and rhizomes of K. foliosa as well as on the rhizomes of K. isoetifolia Cf showed all of them to be highly endowed with pigments which show positive reactions for anthranoids. Aside from the above cited reference on the presence of rhein (7) in K. uvaria no other anthranoids have been reported from the genus Kniphofia. This deficiency in the literature on this genus as well as its local medicinal use prompted us to first of all investigate different parts of the readily available species K. foliosa. The study was also extended to other Kniphofia species of Ethiopia in the hope of establishing a chemotaxonomic correlation in this genus.

Table 1. Distribution of Kniphofia species in Ethiopia[1]

K. foliosa Hochst	K. isoetifolia Hochst
28 Km. W. of Feche (S)*	Enchetcab (Go), Semien Mt (Go)
Between Debre-markos and Feche (S)	Ankober (S), Entoto (S) Mt. Zuquala (S)
22 Km. N.E. of Gondar	Seru (A), Mt. Chilalo (B),
Adwa (T), Choke Mt. (G).	Asela (A)
Galama Mt. (A), Boruluccu Mt (A)	Goba (B), 25 Km. W. of Dinshu (B)
Goba (B), Garamulata Mt. (H)	Gore (I), upper Gojeb valley (K)
Hara Wobalo Kebele on the road to Woreilu from Dessie (W)	Denkano (K)

K. Schimperi Barker	K. pumila (Ait.) Kunth
Chilga (Go), Choke Mt. (G)	Senafe (E), Gafate (Go), Degen Meda (Go),
2 Km. E. of Bahir Dar (G)	Welkeit pass (Go), Choke Mts. (G)
Mullusayu (S), Blue Nile Gorge (S)	Near Giga 120 Km. N. of Debre-markos (G)
Mt. Entoto 8 Km. S.E of A.A (S)	Timeccia valley W. of Arat Mekannaker (G)
Garamuleta Mts. (H)	Gara Akun (H)
Mt. Buruluccu 20 Km. E. of Asela (A)	17 Km. S. of A.A (S), 30 Km. N.E. of A.A (S)
8 Km. E.S. of Hagereselam (Si.)	97 Km. N. of Robe (Si.)
E. Slopes of Mt. Delo (Si.)	30 Km. W.S.W of Jima (K)

K. hilelebrandtii Cufod	K. insignis Renelle
102 Km. from A.A on Jimma road (S) 10 Km. W. of Ghedo (S)	130 Km. N. of A.A on the way to Debre-Markos (S) 145 Km. N. of A.A on the way to Debre-Markos (S), Seru Aba (A)

Key*:

S	= Shoa	H	= Hararghe
GO	= Gondar	W	= Wollo
G	= Gojam	I	= Illubabor
T	= Tigrai	Si	= Sidamo
A	= Arssi	K	= Keffa
B	= Bale	E	= Eritrea

2. Literature background

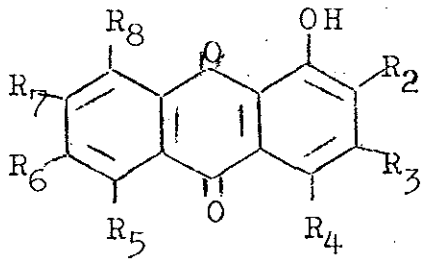
2.1 General aspects of the chemistry of anthranoids

The largest group of natural quinones is made up of anthranoids. Some of them have been important as dyestuffs and others as purgatives. The plant families richest in these types of compounds are Rubiaceae, Rhamnaceae and Polygonaceae.[6] Several genera of the family Liliaceae, Viz. Aloe, Asphodeline, Bulbine etc. are also known to contain anthranoids and bisanthranoids.[5,7]

The anthraquinone derivatives constitute an important class of compounds with important biological properties. These compounds, elaborated both by higher and lower plants, are also one of the most well known naturally occurring pigments. As they are all derivatives of anthracene Marini et al[8] have proposed the general name of "anthranoids" by analogy with "flavonoids". In this thesis the name "anthranoids" and "anthracene derivatives" will be used interchangeably.

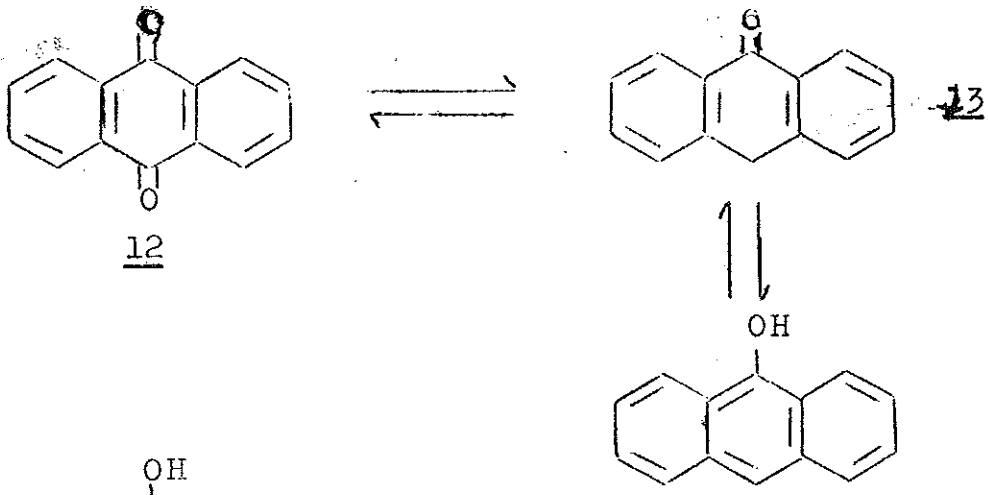
Most anthranoids from higher plants and fungi are hydroxylated and commonly exist as glycosides, although anthraquinone itself has been reported to occur free in plants. Most

anthranoid s are high melting crystalline compounds, and are usually yellow or red in color, although colors ranging from yellow to brown are also encountered. It is interesting to note that the color of anthranoids is closely associated with their chemical structure. Thus the presence of hydroxyl groups at both the 1 and 4 positions impart red color to an anthranoid as exemplified by islandicin (1) quinizarin (2), purpurin (3), dermocybin (8) [isolated from Cortinarius sanguineus Fr.][9], erthroglaucin (9) [Dermocybe sanguina][10], dermorubin (10) [Dermocybe sanguina][10] and xanthorin (11) [Xanthoria elegans (Link)][11]. On the other hand chrysophanol (4), emodin (5) and phscion (6) lack the 4-OH group and are all yellow in color.

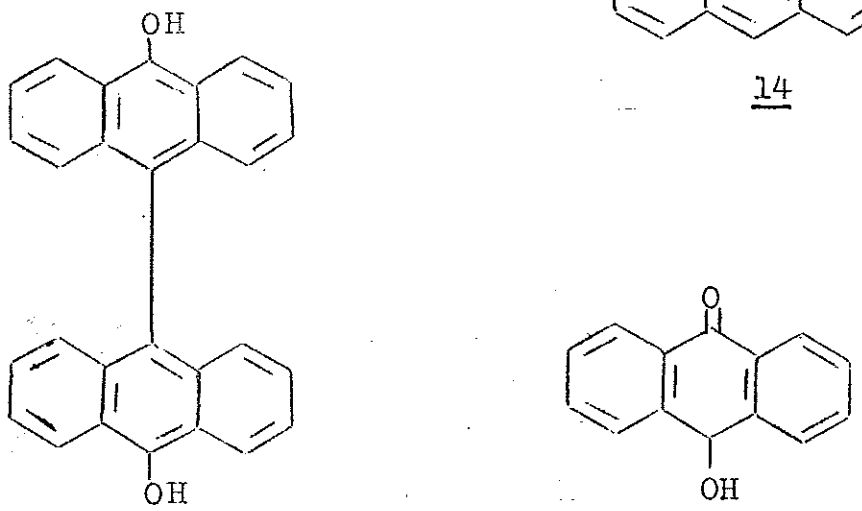


	R_2	R_3	R_4	R_5	R_6	R_7	R_8
Islandicin 1	H	CH_3	OH	H	H	H	OH
Quinizarin 2	H	H	OH	H	H	H	H
Purpurin 3	H	OH	OH	H	H	H	H
Chrysophanol 4	H	CH_3	H	H	H	H	OH
Emodin 5	H	CH_3	H	H	OH	H	OH
Physcion 6	H	CH_3	H	H	OCH_3	H	OH
Rhein 7	H	COOH	H	H	H	H	OH
Dermocybin 8	H	CH_3	H	OH	OCH_3	OH	OH
Erythroglaucin 9	H	CH_3	OH	H	OCH_3	H	OH
Dermorubin 10	COOH	CH_3	OH	H	OH	H	OCH_3
Xanthorin 11	H	CH_3	H	OH	OCH_3	H	OH

Anthranoids having positions 9 and 10 in the keto form (12) were the first to be recognized both in the free state and as glycosides.[12] Further work showed the existence of natural products which also contained reduced anthracene derivatives namely anthrones (13), anthranol (14) and Oxanthrones (15) and compounds formed by the union of two monomer molecules, the dianthranoids (16).



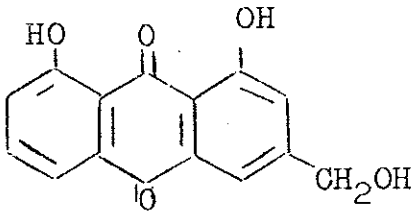
12 13 14



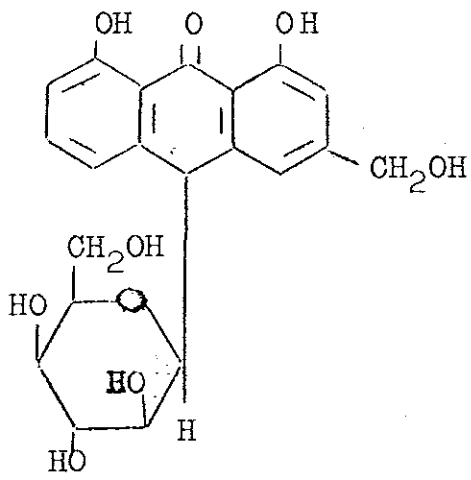
16 15

Many anthracene derivatives occur as glycosides with the sugar residue linked through one of the phenolic hydroxyl groups. The sugar in the reduced glycosides may be linked as usual through phenolic oxygens of the outside ring or they may be attached at C-9 to the enol form of an anthrone or anthranol.

Although aloe-emodin (17) occurs in Rheum species as an ordinary glycoside[6,13], in Aloe it is found as barbaloin (18) an unsual compound in which a glucose like group is linked by a carbon-carbon bond to a partially reduced anthrone.



17



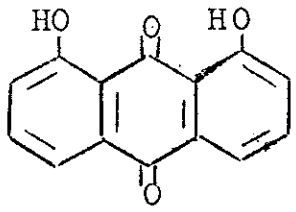
18

Other compounds apparently similar to barbaloin (18) occur in the species of Aloe. Unlike O-glycosides they are stable towards acid hydrolysis but may be split with ferric chloride[13] to form aloe-emodin 17.

The physiological activity of several of these anthracene derivatives has made them important laxatives and cathartics. Although the laxative properties

of anthranoid containing plants such as Cascara and Senna species have been known for centuries it was only recently that it has been shown that both the free anthranoids and the glycosides are pharmacologically inactive, activity being induced through their bio-conversion into anthranols.[6]

The principal anthranoid cathartics are available as senna, cascara sagrada and danthrone drugs. Senna is obtained from dried leaflets or pods of Cassia spp. while cascara is obtained from barks of Cascara spp. The commercial drug "Danthron" (Dorbame) is 1,8-dihydroxyanthraquinone (19) and is useful as a cathartic. The fact that some pharmacopeia[6] recommend storage of purgative plants for periods up to one year before use is explained by the necessity for slow reduction and hydrolysis of the glycosides to free anthranols. Furthermore anthracene derivatives are active as cathartics because they are reduced to anthranols by intestinal microflora.



19

Very little is known regarding the function of these various anthracene derivatives in plants. The existence of the equilibrum anthranoid $\rightleftharpoons$ anthrone in plant cells has led to the speculation that these compounds may some how participate in hydrogen transfer or oxidation-reduction reactions.[6]

2.2 Chemical and physical properties of anthranoids

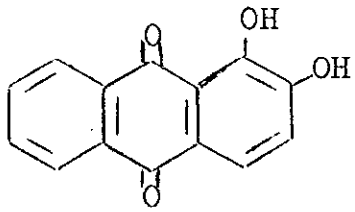
2.2.1 Chemical detection

Borntrager's test[12] is often used for the detection of anthranoids.

Borntrager's test:- If maceration of powdered plant material with organic solvent such as ether followed by filtration and addition of aqueous ammonia or sodium hydroxide leads to formation of pink, red or violet color, it signifies the presence of free anthranoids. If only glycosides are present the test should be modified by first hydrolysing with alcoholic potassium hydroxide. Borntrager's test is negative with either very stable anthranoid glycosides or the reduced derivatives of the anthranol type.

Anthracene derivatives containing

free carboxylic acid group such as rhein (7) can be separated from the other anthranoids by extraction from an organic solution with sodium bicarbonate solution.



20

The Borntrager's test is not specific for anthracene derivatives as naphthoquinones also give a positive reaction.

2.2.2 Color reactions

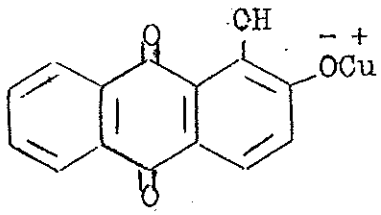
The color given by hydroxyanthracene derivatives when treated with 0.5% methanoic magnesium acetate can be used to predict the substitution patterns.[14] Thus compounds containing two hydroxyl groups in 1,3 or 1,8-positions such as in emodin (5), chrysophanol (4) or aloe-emodin (17), give an orange-red or pink color when treated with the above reagent

../..

while those with two hydroxyls in the 1,4-positions such as islandicin (1), quinizarin (2) produce a purple color, and those with two hydroxyls in 1,2-positions, i.e. alizarin (20) exhibit a violet color.

2.2.3 Salt formation

Certain hydroxyanthranoids such as alizarin (20) form Cu, Cd, Fe and Ni salts when boiled in an organic solvent with metallic chlorides and sodium acetate.[15] They appear to be normal salts in which the beta hydrogen of the OH group is replaced by the metal. These complexes are practically insoluble in cold water but dissolve readily in ethanol, methanol and acetone. Thus copper alizarate, $C_{14}H_7O_4Cu$ (21) was prepared by boiling a mixture of alizarin, $CuCl_2$ and sodium acetate in nitrobenzene.



21

2.2.4 Thin layer chromatography of anthranoids

Most thin layer chromatographic work on anthranoids which appears in the literature is that performed to separate the active constituents in commercial laxative drugs. The vast majority of publications in this connection are concerned with the separation of Aloe, Rhamnus and senna natural drug constituents.[16]

In most cases the adsorbent preferred is silica gel although separation of some anthracene derivatives using polyamide layers has also been reported.[16] Some common solvent systems widely used in the separation of anthracene derivatives are given in Table 2.

Visualization:- Anthranoids may be detected on silica gel GF_{254} layer through their quenching of short wave UV light. They fluoresce brown-yellow to red in long wave UV light. After spraying with alkali solution, a more intense yellow, orange or red fluorescence is observed in long wave UV light. If this is followed by further spraying with fast Blue B salt[16] the fluorescent zones of many compounds become visible in day light (orange, yellow or green).

Table 2. Solvent systems for separating anthranoids on silica gel G layer[16]

A. For anthranoid glycosides

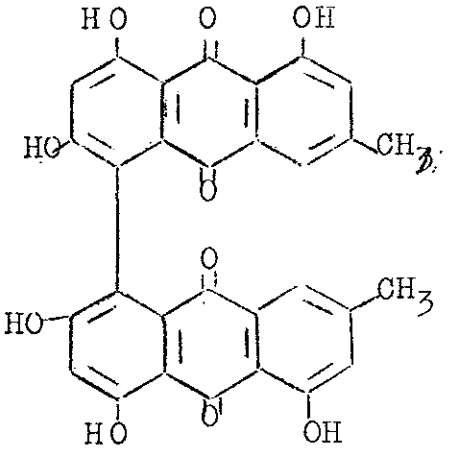
No.	Solvents	Ratio
1	Ethylacetate-methanol-water	100:16.5:13.5
2	Benzene-ethylformate-formic acid	75:24:1
3	Benzene - acetic acid	2:1
4	Chloroform - 95% ethanol	3:1
5	Chloroform - 95% ethanol - water	30:15:1
6	Methylene chloride - methanol	83.5:16.5
7	n-propanol - ethylacetate - water	4:4:3

B. For aglycones[16]

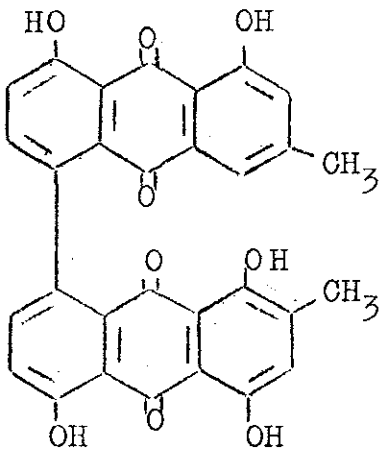
No.	Solvents	Ratio
1	Benzene	-
2	Benzene - methanol	9:1
3	Isopropyl ether	-
4	Heptane -benzene - chloroform	1:1:1

2.2.5 Reductive cleavage of dimeric anthranoids

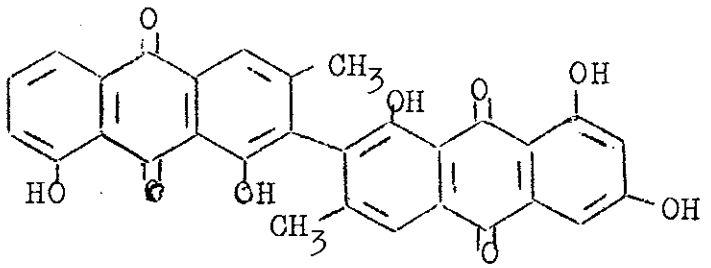
Dimeric anthranoids contain almost all possible combinations of monomeric anthranoids, most being linked through the $5,5^1$ positions. Thus skyrin (22) is a dimer of emodin (5) while roseoskyrin (23) is a dimer of chrysophanol (4) and islandicin (1).[17] Bisanthranoids such as cassiamin (24) isolated from the root bark of Cassia siamea Lam, linked through $2,2^1$ positions are also known.[18]



22

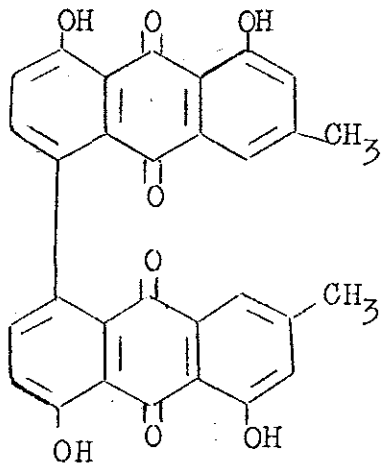


23



24

Most dimeric anthranoids show optical activity arising from restricted rotation about C-C bond connecting the two monomeric groups and they can be reductively cleaved to the corresponding monomers by means of alkaline sodium dithionite ($Na_2S_2O_4$). Reductive cleavage of compounds such as skyrin (22) in which the units are linked ortho to the hydroxyl groups occurs readily while compounds such as bischrysophanol (25) where the linkage is para to the hydroxyl groups show little or no tendency to split.[19,20]



25

2.3 Spectral properties of anthranoids

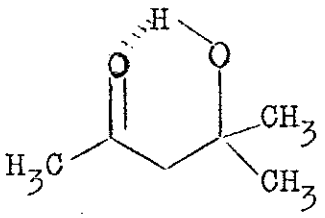
2.3.1 IR spectra

The carbonyl stretching vibrational

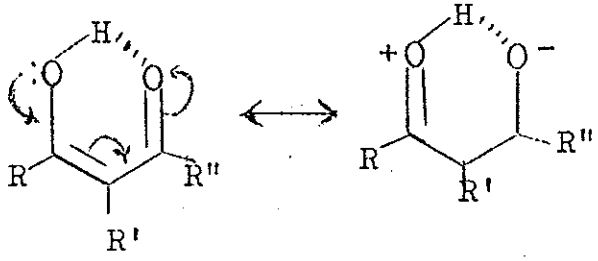
absorption region gives important indications about presence of the basic anthraquinone unit as well as the hydroxylation pattern on the anthraquinone nucleus.

To understand the carbonyl absorption in anthraanoids, first it would be helpful to discuss the absorption pattern in simple molecules such as nonconjugated, β-hydroxy, α,β-unsaturated ketones and β-diketones.

Nonconjugated ketones exhibit carbonyl absorption very near to 1715 cm^{-1} while β-hydroxyketones show very small shifts to lower frequency due to simple intramolecular hydrogen bonding as in diacetone alcohol (26). Conjugation of the carbonyl group with a carbon-carbon double bond in acetophenone and isophorone effects a shift in the order of 30 cm^{-1} to the lower frequency (see Table 3).



26



27 28

β-diketones such as acetyl acetone are enolizable to usually formulated structures 27 and 28. The large shift of the bond from the usual ketone carbonyl absorption position as well as its high intensity may be explained reasonably in terms of the resonance structures 27 and 28. The enhanced participation of the ionic structure leads to greater decrease in the double bond character of the carbonyl bond.

Table 3. Carbonyl frequencies of some simple ketones in cm^{-1} [21]

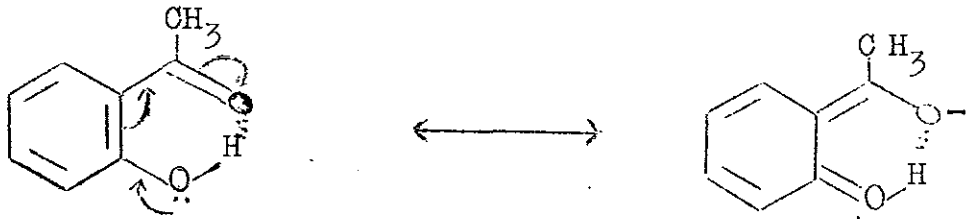
Compound	Type	Frequency
Acetone	Nonconjugated	1715
Diacetone alcohol	β-hydroxyketone	1706
Acetophenone	Conjugated ketone	1685
Isophorone	Conjugated ketone	1672
Acetyl acetone	α,β-diketone	1620

The resonance of type 27 and 28 can be written for compounds in which the carbon-carbon double bond is part of aromatic ring. Thus the carbonyl bond of ortho-hydroxyacetophenone (29) is found to be shifted to 1639 - 1613 cm^{-1}.

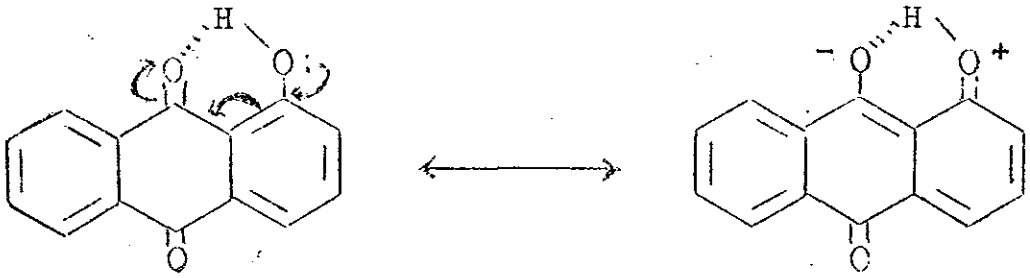
../..

Hydroxyanthranoids can be treated as "conjugated chelate systems" when the hydroxyl group is in the alpha position and the resonance structures 30 and 31 can be formulated as in enolized β-diketones.

The stretching vibration of the carbonyl group which has a hydroxyl group in the alpha position shows profound changes in the frequency and intensity, so that a second more intense band at a lower frequency results due to conjugation and chelation. Table 4 lists the carbonyl frequencies of anthracene derivatives grouped according to the arrangement of hydroxyl groups on the anthraquinone nucleus. Depending on the number of carbonyl peaks and their frequency range, it is possible to place an anthranoid in one of the five groups of Table 4.



29



30 31

In the hydroxyl region of the IR spectra anthranoids having a hydroxyl group at the beta position on the anthraquinone nucleus exhibit sharp hydroxyl stretching band between 3600 and 3150 cm^{-1}. The spectra of alpha hydroxyanthranoids show no noticeable absorption in the hydroxyl region but exhibit a broad and weak absorption band centered at approximately 2700 cm^{-1} corresponding to the stretching frequency of a chelated hydroxyl bond.[22]

Table 4. Carbonyl frequencies of hydroxyanthraquinones in cm^{-1}[21]

Group type	Type of anthranoid	Carbonyl frequencies		Δ
1	no α-OH	1678-1653	-	
2	1-OH	1675-1647	1637-1621	24-38
3	1,4 and 1,5-$(OH)_2$	1645-1608	-	-
4	1,8-$(OH)_2$	1678-1661	1626-1616	40-57
5	1,4,5-$(OH)_3$	1616-1592	-	-

2.3.2 UV/Visible spectra

In enones conjugation of the double bond

with a carbonyl group leads to intense absorption corresponding to transfer of a π electron from the ethylene orbital to the carbonyl orbital. This absorption is designated as electron transfer (E.T) band. Electron transfer bands are found between 220-250 nm in simple enones. The second characterstic band in enones appear at 310-330 nm and corresponds to the displaced n --> π^* band of carbonyl group.

Benzene absorbs at λ_{max} (log ε), 184 (4.8), 203.5 (3.9) and 254 (2.3) in hexane solution while acetophenone exhibits a four band spectrum with bands at 199 (4.3), 246 (4.1), 278 (3) and 320 (1.7).

Quinones:- Benzoquinones have spectra characterized by intense absorption at 240-90 (E.T band) together with a weaker band in the 400 nm region. Naphthoquinones and higher quinones possess multi-banded spectra which may be roughly divided into

A. E.T. bands 250-300 nm

B. Overlapping E.T. and benzenoid bands 300-400 nm

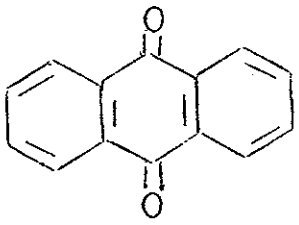
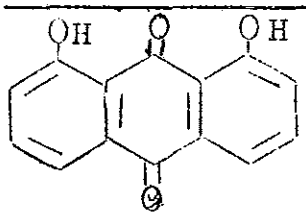
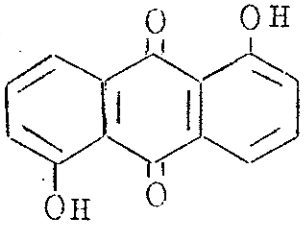
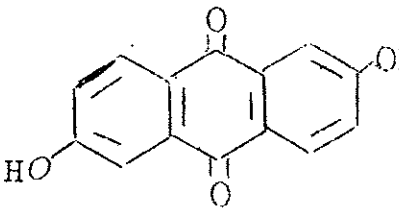
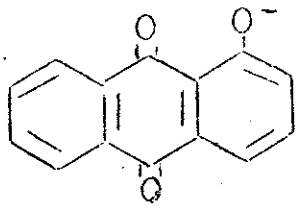
C. Weak (n --> π^*) band at 400-500 nm.

Substitution by a hydroxyl group masks the last of these bands by generation of intense absorption in the 400-500 nm region. The spectra of anthranoids may be regarded as cross conjugated acetophenone - benzoquinone type spectra. We designate the transition due

to acetophenone and benzoquinone chromophores as A and B respectively. Thus for anthraquinone in ethanol we have λ_{max} 243, 252 (A-band), 263 (B-band), 325 (A-band) and 405 (B-band). The 405 nm quinonoid absorption is susceptible to intensity increase and shift to longer wavelength by the introduction of nuclear hydroxyl groups.

In Table 5 the absorption bands of some hydroxy-anthranoids are given.[23]

Table 5. UV/Visible absorption bands of hydroxyanthraquinones[23]

Anthranoid	E.T. band λ_{max}(log. ε) (nm)	Overlapped E.T. benzenoid band λ_{max}.(log.ε)	n--->π^* λ_{max}(log.ε)	Solvent
	243.5 (4.5) 252.5 (4.7) 263 (4.3) 276 (4.1)	325 (3.7)	405 (1.95)	Hexane
	254 (4.4) 274 (4.2) 284 (2.2)	-	430 (3.2)	Hexane
	225 (4.6) 253 (4.2) 275 (3.9) 287 (3.9)	330 (7.7)	418 (3.9) 437 (3.9)	Ethanol
	274 (4.5) 301 (4.3)	249 (3.9)	385 (3.3)	Ethanol
	248 (4.5) 278 (4.0)	315 (3.7)	484 (2.8)	NaOH

The 1-OH derivatives in alkaline solution show one large absorption band with a maximum around 500 nm, the 2-hydroxy compounds one large band with a maximum at 492 nm and two smaller ones with maximum at 300 and 238 nm.

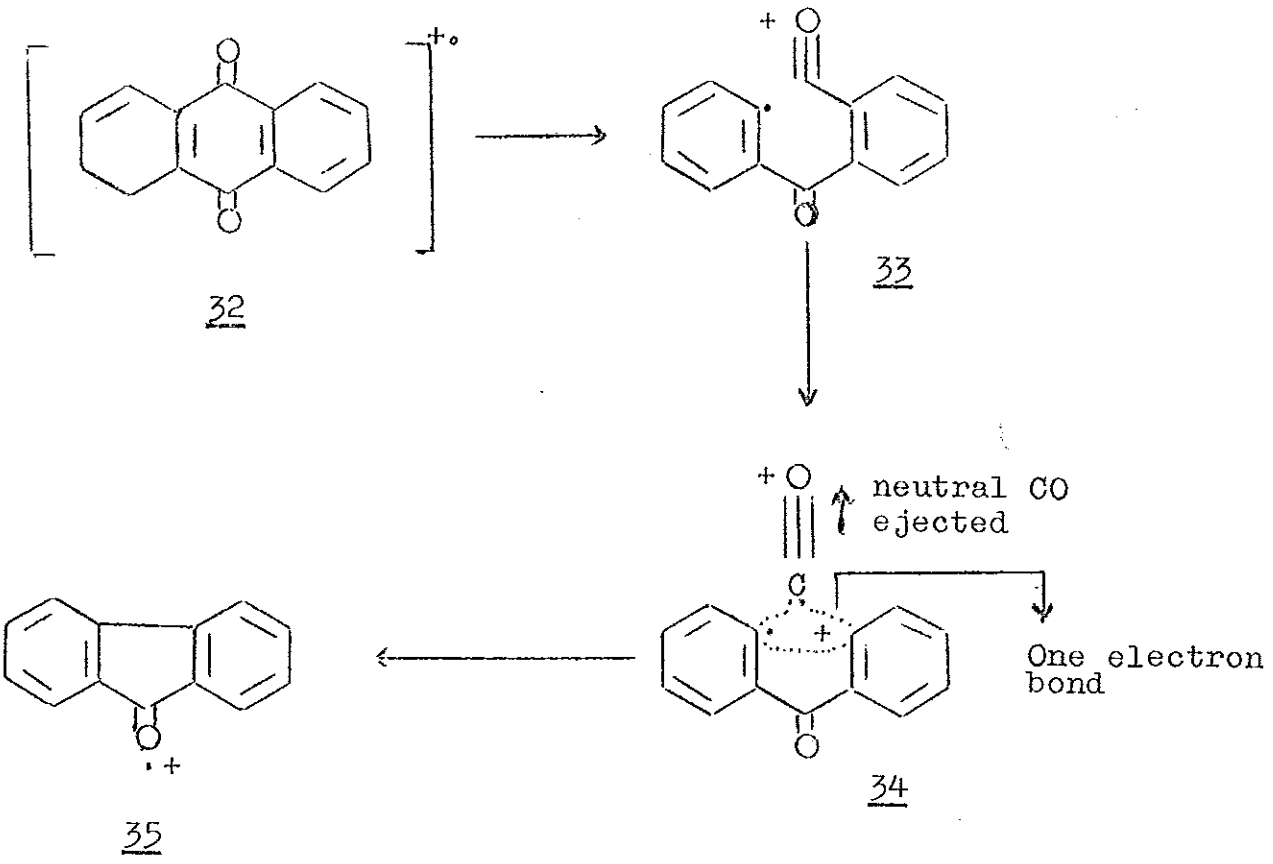
Hydroxyl groups in positions 2,6 and 7 give rise to more strongly auxochromic bands than those with hydroxyls in 1,4 or 5 positions probably because in the former a hydroxyl group is para to one of the carbonyl group[24].

2.3.3 MS fragmentation pattern in anthranoids

The tendency to lose neutral carbon monoxide from the parent or fragment ions of many oxygenated compounds was first noticed by the study of the high resolution mass spectra of anthraquinones. In the mass spectrum of anthraquinone (32) the most abundant ions are of mass 208 (M^+), 180 (M^+-CO) and 152 (M^+-2CO). Accurate mass measurements at high resolution have established that these two latter ions were not produced by the elimination of C_2H_4 fragment but by successive loss of two neutral CO fragments.

In anthraquinones, the most loosely bound

electron, which is therefore, removed preferentially during the formation of the molecular ion by electron impact of low energy is one of the lone pair electrons on the oxygen atom. Since O^+ is trivalent one of the C-C bonds adjacent to the carbonyl group may become uncoupled to provide an orbital which can give a π bonding effect with the lone pair oxygen orbital as in 33. The positive charge on the oxygen atom will induce a polarity in the adjacent bonds and this will be a maximum in the remaining C-C bond. In this state the carbonyl group is so weakly attached to the remainder of the molecule and loses CO readily (34) to give the fluorenone ion (35).[25]



32 33 34 35

Anthranoids generally show the same tendency to eliminate neutral carbon monoxide. This point is exemplified by monohydroxyanthraquinone whose molecular ions tend to fragment with loss of a neutral CO molecule, which is followed by a second CO and then either a third CO or a CHO fragment.

2.3.4 NMR spectroscopy of anthranoids

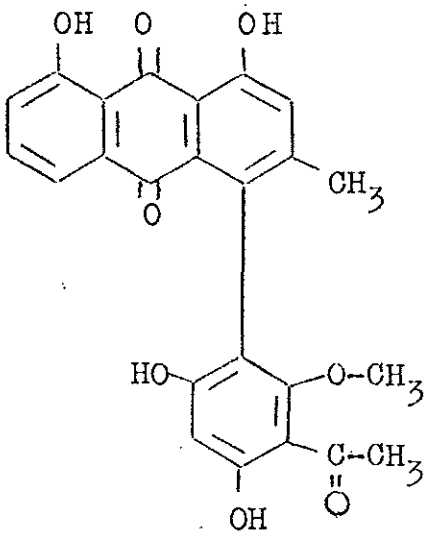
^{1}H and ^{13}C NMR spectroscopy serve as powerful tools in the structural elucidation of anthranoids. Analysis of chemical shifts as well as of coupling constant data greatly aid in structural assignment.

In ^{1}H NMR spectra of anthranoids the hydroxyl protons on 1,4,5 and 8 positions are easily distinguished from other types of hydroxyl protons by their appearance at unsually low field resonance between 11 and 14 ppm, a shift accounted for by the chelation of hydroxyl groups with 9, 10 keto groups. Compounds of the chrysophanol type are easily recognized by the characteristic pattern of the protons at the 5, 6 and 7 positions which give rise to two double doublets and a triplet while compounds of the emodin type give highly simplified spectra.

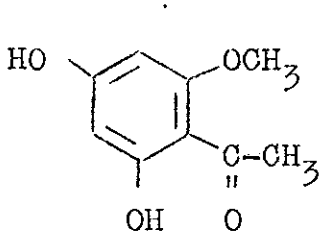
Considerable amount of information is also obtained by examining spectra of acetyl, methyl and trimethylsilyl derivatives of anthranoids. Thus Steglich and Lösel[26] studied the chemical shift differences for the corresponding aromatic protons in the ^{1}H NMR spectra of per-acetylated and per-trimethylsilylated anthranoids and found the acylation shift data as useful parameters in determining the positions of O-alkyl or O-glycosyl substituents in 1,8-dihydroxy-anthraquinone derivatives. These findings are based on the well-known acetylation shifts in phenols where o-protons are shifted by -0.25 ppm and p-protons by -0.20 ppm where as m-protons remain unaffected. The presence of many hydroxyl groups leads to an additive effect.

In ^{13}C NMR spectra of anthranoids carbons 9 and 10 are easily recognized by their resonance occurring between 180 and 185 ppm while carbons bearing hydroxyl groups appear at about 160 ppm.[27] The introduction of proton coupled ^{13}C NMR measurements has increased the potential of ^{13}C in structural assignment work. Thus in the structural elucidation of Knipholone (36) by E. Dagne and W. Steglich[5] the attachment of the acetyl phloroglucinol unit (37) to the chryscophanol system of 36 where C-10 displays a doublet (J = 4Hz)

arising from ^{3}J-coupling with the peri-proton at C-5. This confirmed the presence of the substituent at C-4. A double of doublet would have been expected for C-10 if both peri-protons at C-4 and C-5 were available. Consistent with this argument the signal for C-9 in compound 36 was a sharp singlet.



36



37

2.4 Biosynthesis of anthranoids

The structural and substitution pattern of anthranoids generally reflect two modes of biosynthetic path ways.[28,29] Many common anthranoids found in microorganisms, lower animals and plants

are mainly of polyketide origin.

Anthranoids occurring in Rubiaceae, however, are not derived from acetate but rather by the second pathway namely the shikimic acid-o-succinoylbenzoic acid route.

2.4.1 Polyketide derived anthranoids

This route was found to be valid in the biosynthesis of anthracene derivatives because it was found that labelled acetate was specifically incorporated into some anthronoids such as islandicin (1) and emodin (5). Most of the acetate derived anthranoids examined so far are formed by cyclization of an octaketide chain. Other steps in the biosynthesis of these anthranoids involves decarboxylation, reduction and aromatization of the cyclized polyketide.

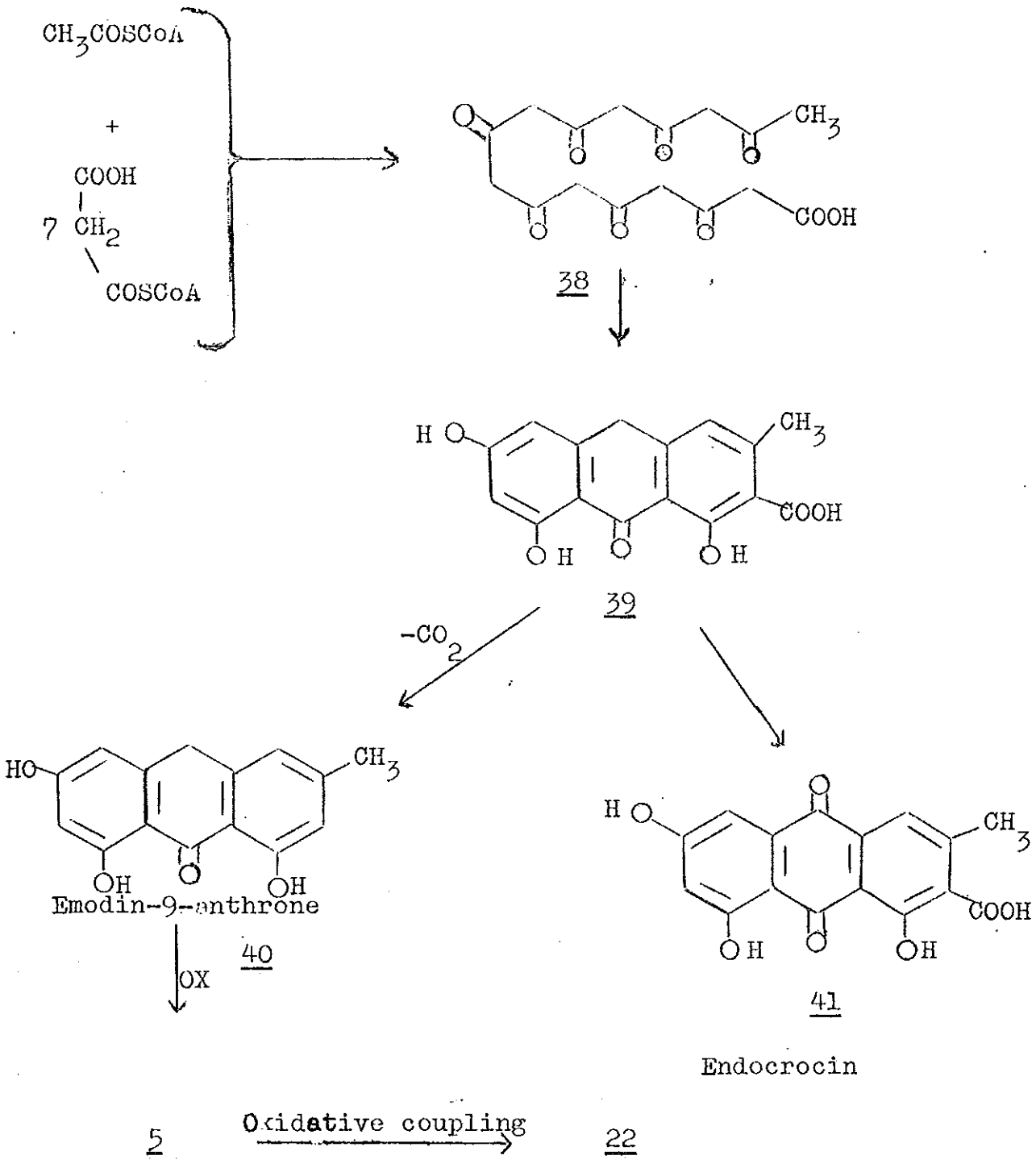
Decarboxylation of the tricyclic skeleton produces anthracene derivatives with 15-carbon atoms. Such derivatives are common and are usually found as an anthrone or an anthranoid dimer. Anthrones are biosynthetic intermediates which lead to anthranoid structures and the oxidative coupling of anthranoids leads to dimeric anthranoids. The biosynthetic pathway which

leads to emodin (5) and endocrocin (41) is given in Scheme 1.

Anthranoids can arise from either a single polyketide chain or from more than one polyketide chain. The active form of the first C_2 unit is acetyl CoA, while malonyl CoA, formed by carboxylation of acetyl CoA, is used for subsquent C_2 units. This utilization of two different units by the enzyme accounts for the different distribution of radioactivity in polyketide molecules obtained after incorporation of labelled malonic acid. The positions corresponding to the first C_2 unit (derived from acetyl CoA) and the ones corresponding to the successive C_2 units (derived from malonyl CoA) are lebelled to different extent. This behaviour of the organism is general and illustrates how anthranoids such as islandicin are formed by one chain process.

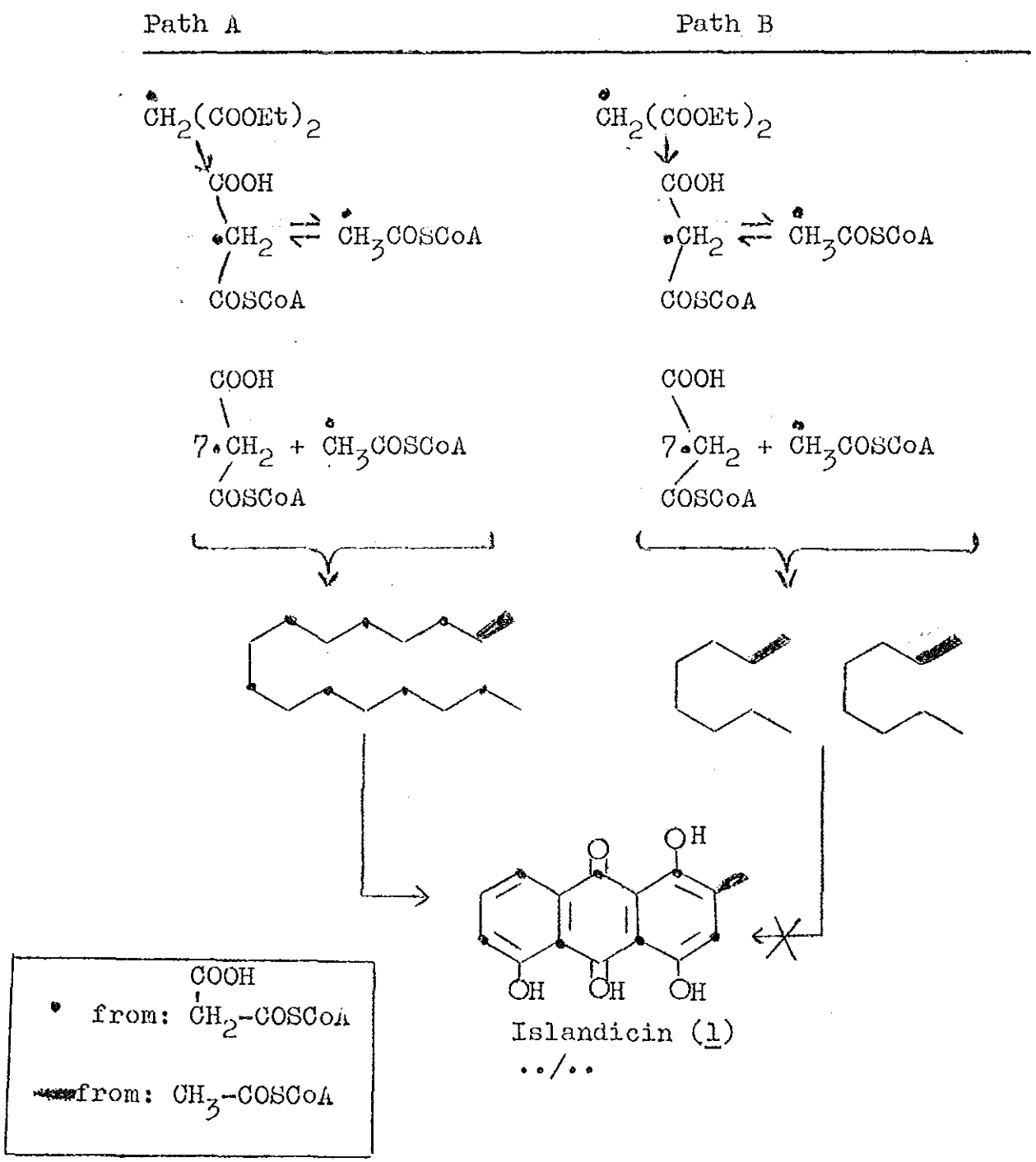
Radioactive islandicin obtained after feeding Penicillium islandicum with diethyl [2-^{14}C] malonate demonstrated the utilization of a single starter acetyl CoA unit and the subsquent incorporation of seven malonyl CoA for building the anthranoid molecule.[28,30] This is consistent with path A of Scheme 2 in which a long continuous chain is first formed which then ring closes to give the anthracene skeleton. A two chain

Scheme 1. Biosynthesis of anthrones and anthranoids by the polyketide pathway[28]



synthesis as in path B is ruled out by labelling studies which after feeding of diethyl [2-^{14}C] malonate gave different specific activities as opposed to the expected identical activities for the methyl carbon and C-10 of the isolated islandicin.

Scheme 2. Biosynthesis of islandicin[28]



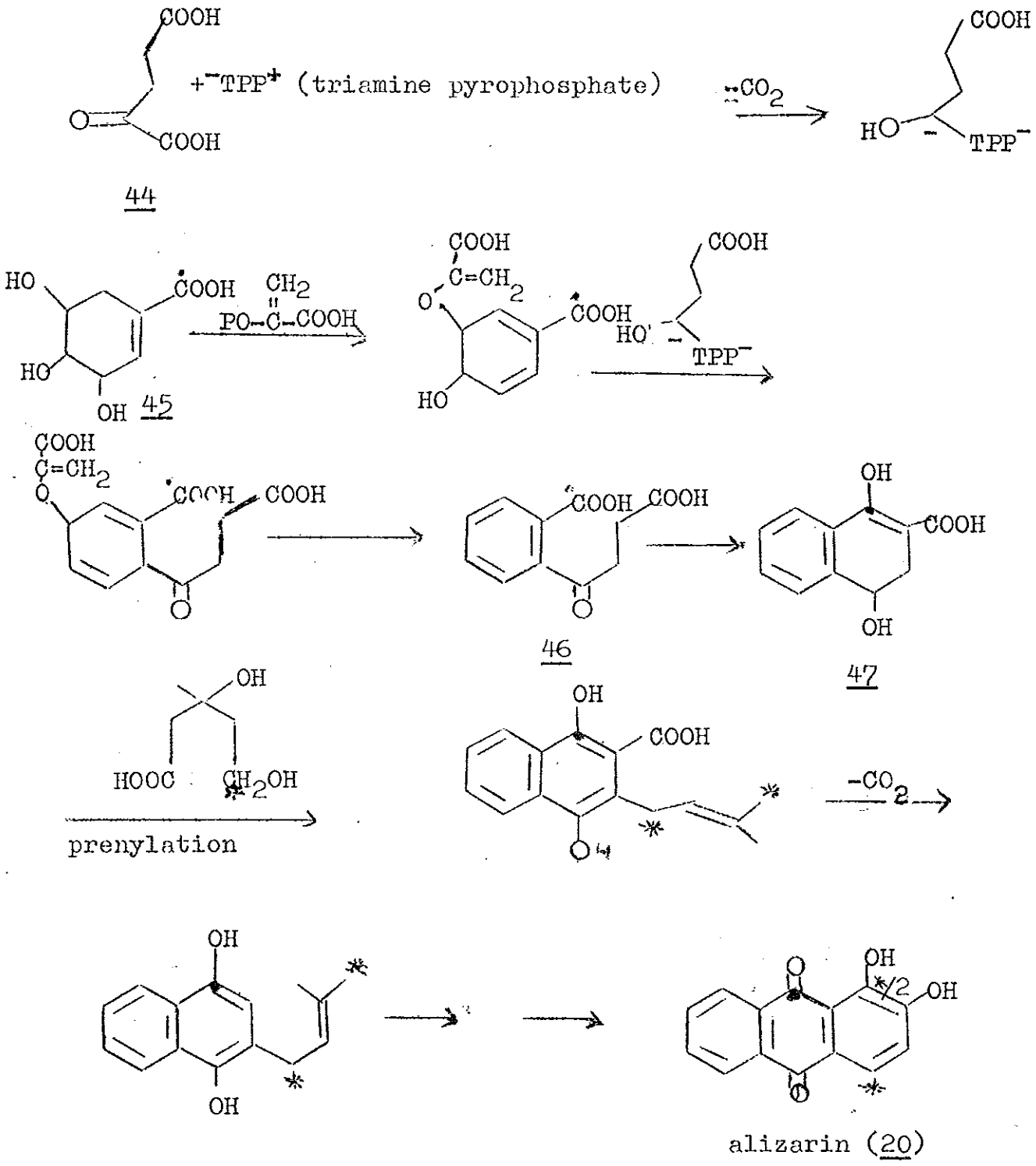
Islandicin (1)

../..

2.4.2 Shikimic acid-o-succinoylbenzoic acid derived anthranoids

Anthranoids produced by plants can be classified into two categories, depending on how only one, or both A and C rings bear particular substituents such as OH, OCH_3 CH_3, etc. The compounds substituted in both rings A and C usually with a C-15 skeleton, have polyketide origin as exemplified by emodin (5). Other compounds such as alizarin (20) and purpurin (3) are formed by the shikimic acid-o-succinoylbenzoic acid route in some plant families, especially in Rubiaceae. By separatey feeding shikimic acid (^{14}C at the carbonyl), and (RS)-[-^{14}C] mevalonic acid to Rubia tinctorum (Rubiaceae) radioactive alizarin (20) has been isolated. Chemical degradation indicated that ring C is derived from MVA. This biosynthetic pathway is shown in Scheme 3.

Scheme 3. Biosynthesis of alizarin



Shikimic acid (45) derived from phosphoenolpyruvic acid and erythro-4-phosphate is incorporated into alizarin together with the three central carbon of α-ketoglutaric acid (44), an intermediate of tricarboxylic acid cycle. O-Succinoylbenzoic acid (46) is a necessary intermediate in this class of anthranoids. Although this has been proved by incorporation experiments o-Succinoylbenzoic acid itself has not so far been isolated from nature. Prenylation of the naphthalene intermediate (47) and subsquent cyclization and oxidation of ring C leads to alizarin.

2.4.3 Natural distribution of anthranoids

About half of the natural anthracene derivatives occur in the bark, wood and flowers of flowering parts specially in the family Rubiaceae. Some anthranoids are also found in the Rhamnaceae, polygonaceae, Leguminosae,[29] Serophulariaceae, Gesneriaceae etc. In monocotyledons this class of compounds is found only in Liliaceae.[12]

Other rich sources of anthranoid pigments are the higher fungi and lichens which are known to elaborate a number of interesting anthracene derived pigments besides the common

anthranoids emodin, chrysophanol and their respective glycosides.

Thus Xanthoria elegans a bright red fungal component of lichen is rich in the bright red pigment xanthorin (11).[11] Penicillum islandicum produces the red compound islandicin (1) Dermocybe sanguinea yields erthroglaucin (9), dermocybin (8) and dermorubin (10).[10]

More than 100 anthranoids[8] have been isolated from higher plants and it has been shown that dimeric anthracene derivatives which are commonly encountered in lower plants are rare in higher plants except for few examples such as Cassiamin (24), isolated from the root bark of Cassia siamea Lam.[8]

3. Results and discussion

3.1 Extraction and fractionation of K. foliosa leaves

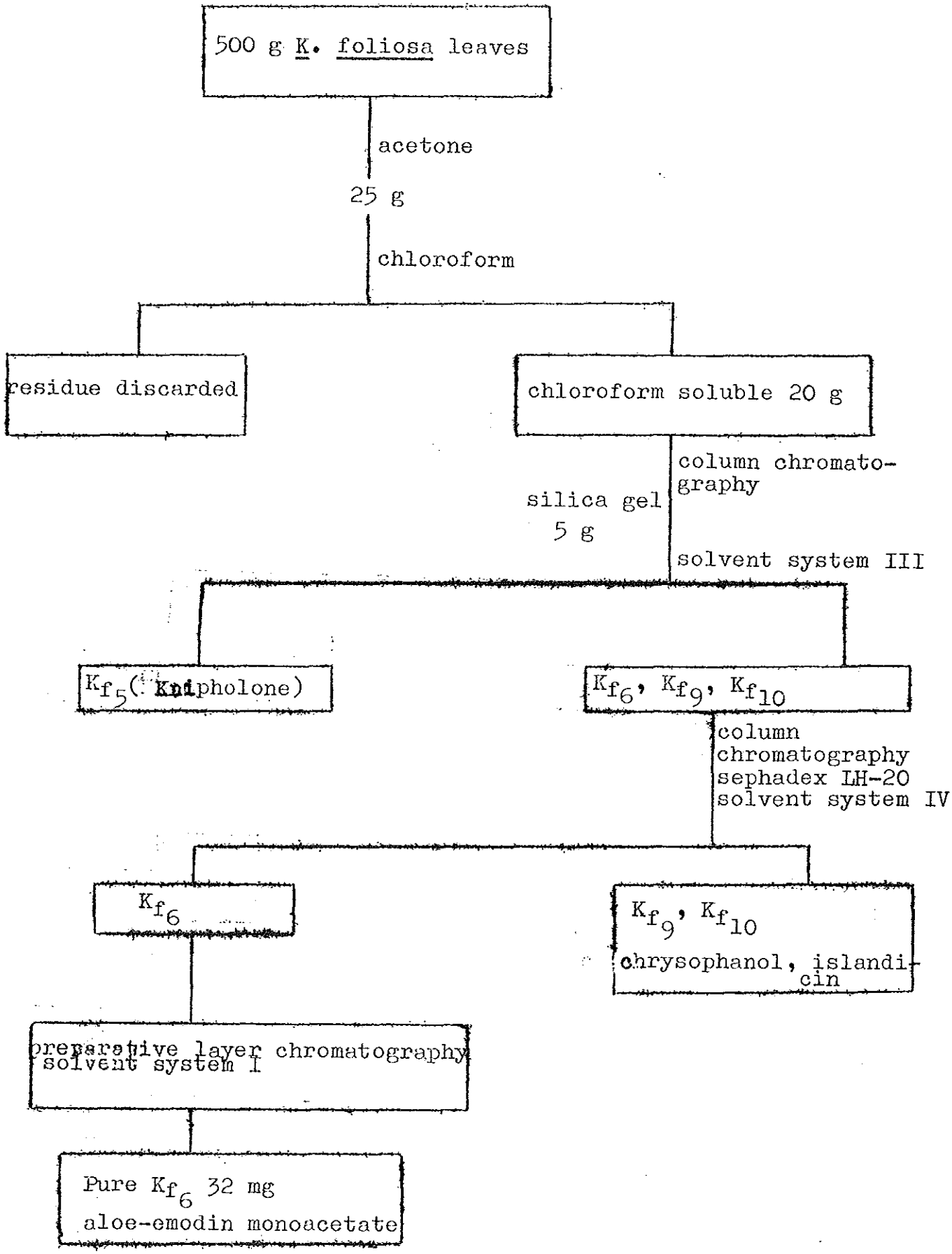
The leaves of K. foliosa collected from Wollo province in Ethiopia in September 1983 were examined for their chemical composition since it has been established earlier that the rhizomes of this plant were rich in anthranoids. Preliminary screening tests on the leaves also revealed that the leaves were rich in anthranoids.

Extraction of K. foliosa leaves with acetone yielded a greenish solid. The crude acetone extract was subsequently taken up in chloroform and the chloroform insoluble part was discarded. The chloroform soluble fraction on TLC, using solvents system I (see experimental) showed three major spots K_{f_5}, K_{f_6} and a third spot of higher R_f value. The third spot upon rechromatography with solvent system II was found to be composed of two compounds designated as K_{f_9} and $K_{f_{10}}$ The spots below K_{f_5} designated as K_{f_1} --> K_{f_4} are found only in trace amounts. A schematic flow chart for the extraction and fractionation of K. foliosa leaves is given is Scheme 4.

Silica gel column chromatography of the chloro-

form extract using solvent system III as eluting solvent enabled the separation of K_{f_5} from the other components. The mixture containing K_{f_6}, K_{f_9} and $K_{f_{10}}$ was applied on a column packed with sephadex LH-20 and eluted with solvent system IV thus separating K_{f_6} from the other two compounds. The final purification of K_{f_6} was brought about by preparative layer chromatography using solvent system I.

Scheme 4. Flow chart for extraction and fractionation of *K. foliosa* leaves



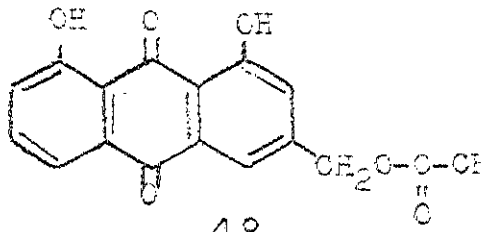
3.1.1 Characterization of a new anthranoid $k_{f_{16}}$ as aloe-emodin monoacetate.

Thin layer chromatographic comparison of crude acetone extract of rhizomes, flowers and leaves of K. foliosa revealed that K_{f_6} is localized only in the leaves.

K_{f_6}, orange needles from acetone, mp 203-206 (dec.) was found to give a positive reaction for the characteristic alkaline anthranoid test and further produced an orange-red color upon treatment with alcoholic magnesium acetate. This characterstic color is an important indication of anthranoids with two hydroxyl groups in 1,3 or 1,8-positions on the anthraquinone nucleus.

The high resolution MS (fig. 1) revealed an empirical formula of $C_{17}H_{12}O_6$ which readily lost a fragment with mass 42 to yield a base peak $C_{15}H_{10}O_5$, $(M-CH_2CO)$, a peak characterstic of aryl and benzylic acetates.[31] The appearance in the IR spectrum of a strong carbonyl absorption at 1745 cm^{-1} was also suggestive of the presence of an acetyl group further confirmed by a methyl absorption signal at δ 2.15 in the ^1H NMR spectrum (see Table 6).

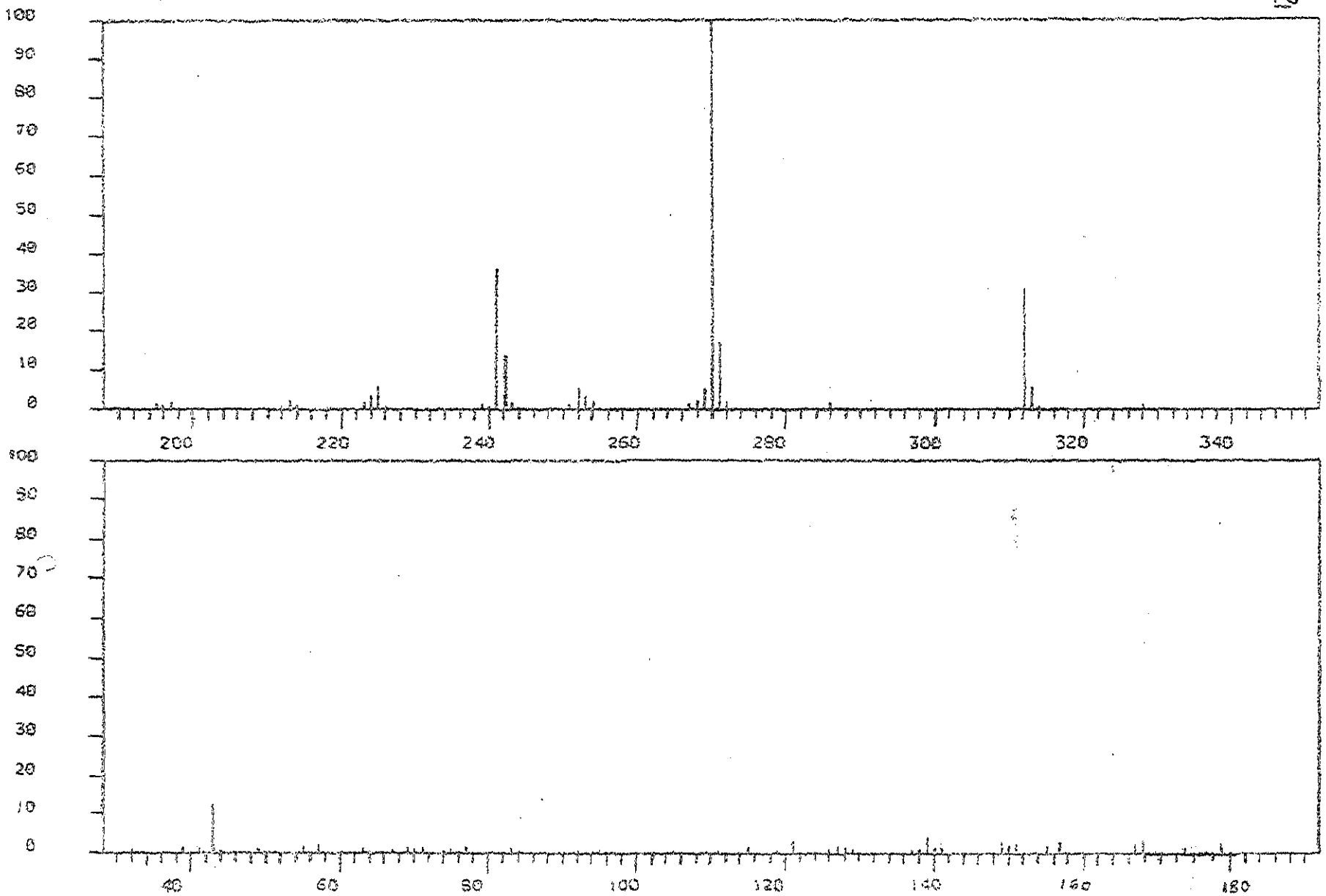
Fig. 1. Mass spectrum of aloe-emodin monoacetate



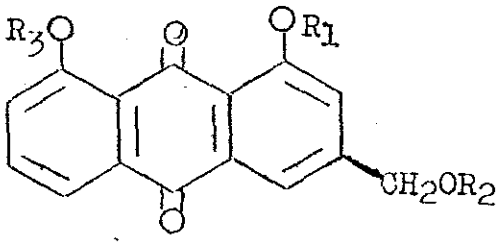
48

MASS INTENSITY REPORT:

290AV.12 [TIC=148767. 100%=27565]



Further analysis of the ^{1}H NMR spectrum revealed the presence of a methylene group at δ 5.24 broadend by long range coupling with aromatic protons. The chemical shifts as well as splitting patterns of the aromatic protons were indicative of the presence of an anthraquinone moiety of the chrysophanol (4) type. This was further supported by the existence of two sharp signals in the IR spectrum at 1680 for the non-chelated carbonyl and 1635 cm^{-1} for the chelated carbonyl. The above data is consistent with structure 48. To our knowledge this constitutes the first report of the existence of 48 as a natural product.



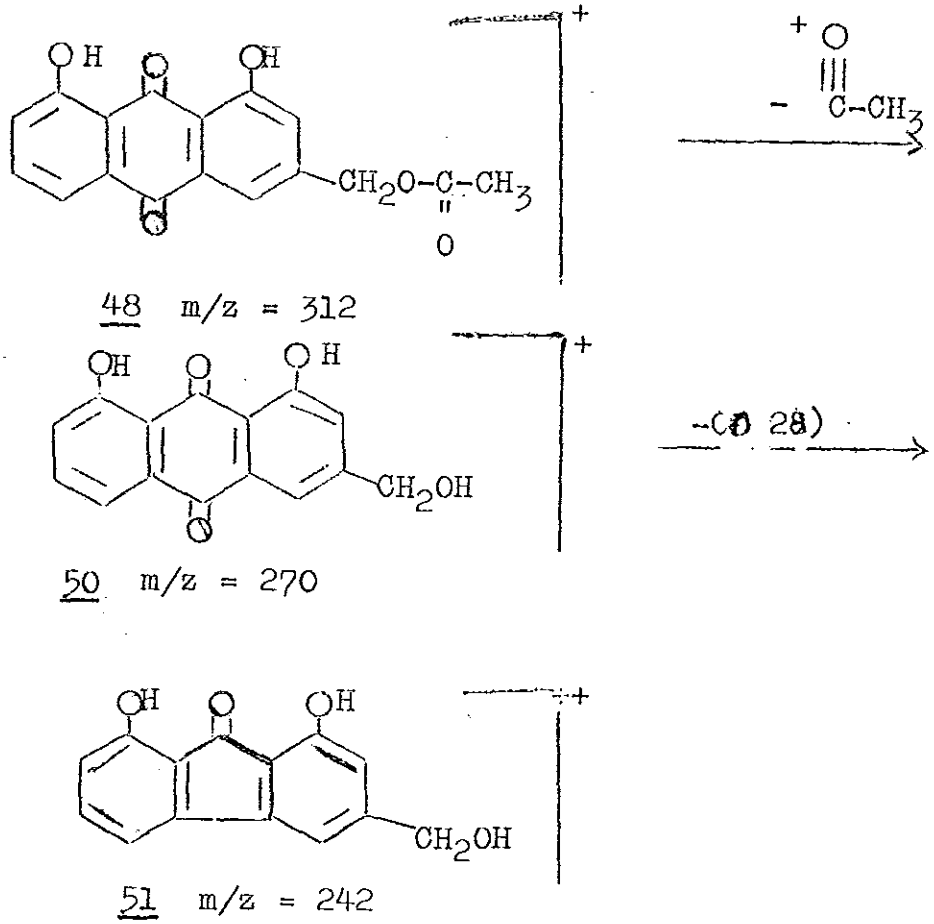
48 $R_1 = R_3 = H$, $R_2 = AC$

49 $R_1 = R_2 = R_3 = AC$

50 $R_1 = R_2 = R_3 = H$

../..

The MS spectrum of compound 48 reproduced in figure 1 exhibits a typical fragmentation pattern for anthranoids, as the parent ion readily loses an acetyl moiety followed by subsquent ejection of a carbon monoxide to yield the fluorenone fragment (51) as shown in the scheme below.



The UV and visible absorption spectrum of compound 48 (λ_{max} 252, 284, 312, 422) resembles those of 1,8-dihydroxyanthranoids such as chrysophanol (4) (λ_{max} 277, 255, 278, 287, 427) and emodin (5) (λ_{max} 222, 252, 265, 289, 437).[18]

Acetylation of compound 48 with acetic anhydride/pyridine yielded a yellow acetate (49), mp 169-171 (lit. 170-175)[32]. The mass spectrum of compound 49 (fig. 2) showed the most prominent peaks at 396 ($C_{21}H_{16}O_8$), 354 ($C_{19}H_{14}O_7$), 312 ($C_{17}H_{12}O_6$) and 270 ($C_{15}H_{10}O_5$). The successive loss of two acetyl groups gave the peak of mass 312 (i.e. M^+ for compound 48) and further loss of the remaining acetyl group resulted in the base peak at mass 270. The mass spectral data is consistent with diacetylation of compound 48, clearly indicating the presence of two hydroxyl groups in compound 48.

^{1}H NMR of 49 (fig. 3) showed three acetyl signals at δ 2.15 (s, $COOCH_3$) and 2.44 (s, 6H, 2x $COOCH_3$). The fact that the acetyl group at δ 2.15 in 49 exhibit the same chemical shift as in 48 and the identity of the chemical shifts of the two introduced acetyl groups constitute further proof for the location of the acetyl group in compound 48 on the hydroxymethyl group. The two proton singlet at δ 5.22 broadened due to long range coupling with aromatic protons was assigned to methylene

../..

DS-50 MASS INTENSITY REPORT: 312AU1.31 [TIC-97473, 100%-23205]

Fig. 2. Mass spectrum of aloe-emodin triacetate

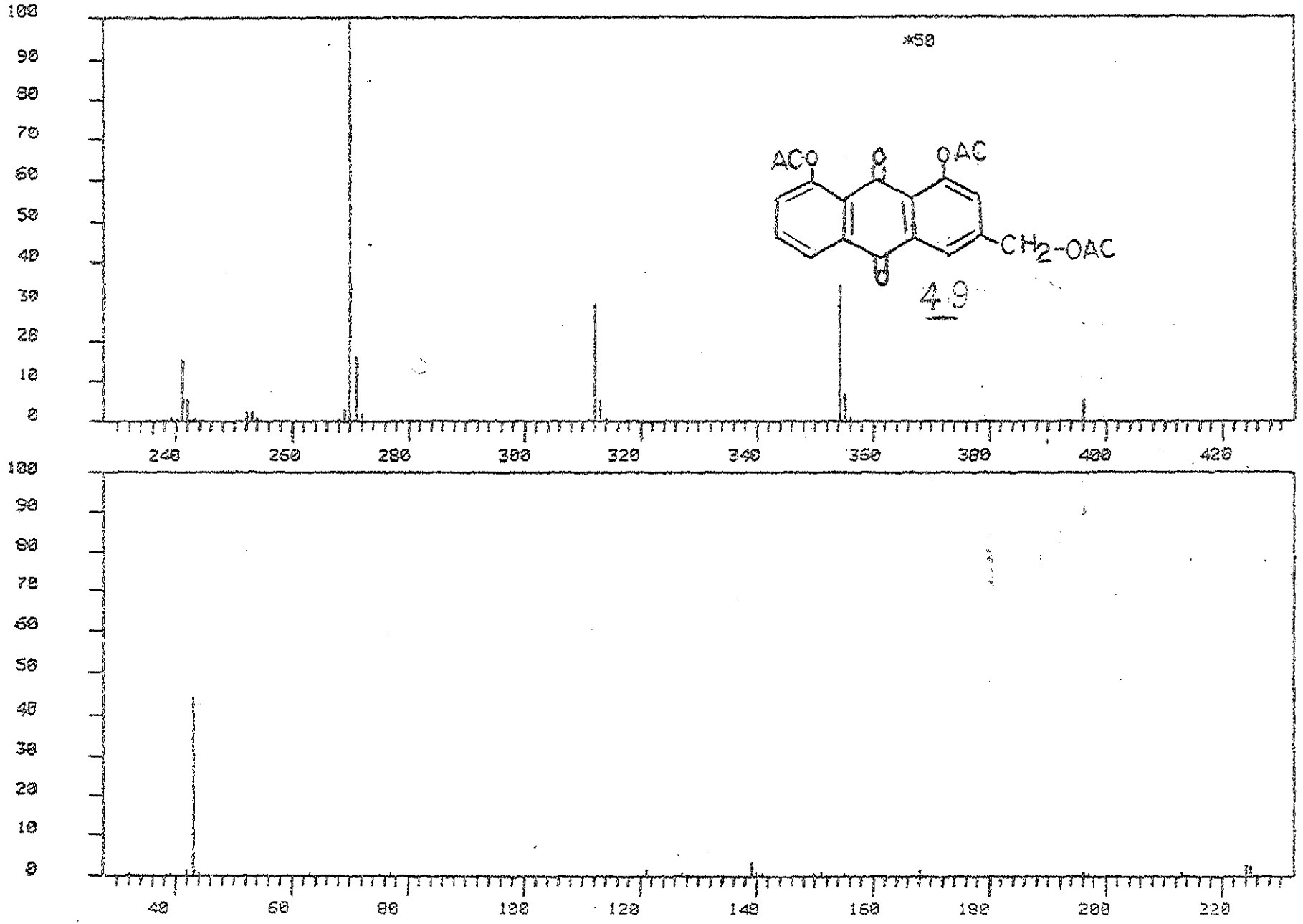
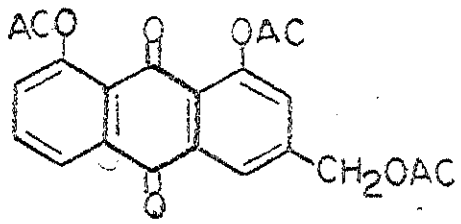


Fig. 3. 90 MHz NMR spectrum of aloe-emodin triacetate ($CDCl_3$)



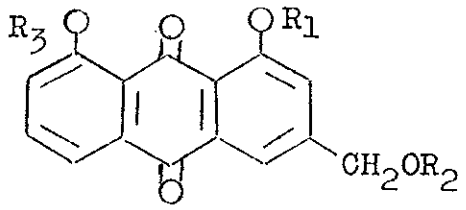
49

protons.

In the aromatic region two doublets at δ 7.40 (J = 2Hz) and 8.20 (J = 2Hz) were assigned to protons at positions 2 and 4 respectively, a double doublet at δ 7.40 (J = 8, 2Hz) to H-7, a double doublet at δ 8.24 (J = 8, 2Hz) to H-5 and a triplet at δ 7.7 (J = 8Hz) to H-6 (see Table 6).

On alkaline hydrolysis 48 afforded aloe-emodin (50), mp 216-220 (lit. 222)[33] whose molecular formula, $C_{15}H_{10}O_5$, was confirmed from its MS which gave the molecular ion peak at 270.0527 with 100% relative intensity.

Table 6. 90 MHz proton NMR data of aloe-emodin monoacetate (48) and aloe-emodin triacetate (49)



	δ (multiplicity), J(Hz)	
Protons	Compounds	
	48	49
2	7.39 (d), 1.6	7.40 (d), 2
4	7.88 (m)	8.20 (d), 2
5	7.88 (m)	8.24 (dd), 8, 2
6	7.88 (m)	7.7 (t), 8
7	7.44 (dd), 7, 2.2	7.40 (dd), 8, 2
$-CH_2-$	5.24 (br s)	5.22 (br s)
R_1	12.00 (br)	2.44 (s)
R_2	2.15 (s)	2.15 (s)
R_3	12.00 (br)	2.44 (s)

3.1.2 Identification of K_{f_5} as knipholone and K_{f_9} as chrysophanol

Earlier investigation of the rhizomes of K. foliosa had shown the presence of an interesting anthracene derivative, knipholone (36) and trace amounts of chrysophanol (4)[5]. Compound 36 whose structure was determined by spectroscopic methods as well as by degradation to known compounds represented a new type of anthranoid pigment in which the chromophore is connected to an acylphloroglucinol unit.

Knipholone designated earlier as K_{f_5}, an orange-brown pigment, which on treatment with alkaline sodium dithionite afforded chrysophanol (4) and 2-methoxy-4, 6-dihydroxyacetophenone (37) was found to be a major constituent of the rhizomes of K. foliosa. We have also established here that K_{f_5} isolated from the leaves of K. foliosa was the major anthranoid, making knipholone the major secondary metabolite of K. foliosa. Examination of K_{f_9} isolated from the leaves showed that it was identical with chrysophanol (4) (TLC, IR). Furthermore we have examined the acetone extract of the flowers of K. foliosa and established 36 to be the major anthranoid accompanied by chrysophanol as a minor constituent.

../..

3.2 Extraction and fractionation of K. isoetifolia Cf

As indicated earlier there are at least 6 reported Kniphofia species in Ethiopia of a total of 65 species[34] of Kniphofia in the world. Most of these species are restricted to tropical Africa except for one species recorded from Yemen. Although it was not possible in the course of this project to investigate all the Ethiopia species we have attempted to look into other Kniphofia species collected in October 1983 from the vicinity of Addis Ababa namely K. isoetifolia Cf.*

Extraction of powdered rhizomes of K. isoetifolia Cf with acetone yield a pink sticky substance. Thin layer chromatogrphy of crude extract on silica gel using solvent system I as a developing solvent showed four major spots, K_{f_5} (R_f = 0.57), K_{f_7} (R = 0.86), K_{f_8} (R_f = 0.89), K_{f_9} (R_f = 0.93).

---

*The Cf signifies that the species identification is not yet fully ascertained. The plant is now being grown in our garden for submission for a taxonomical study and proper botanical identification.

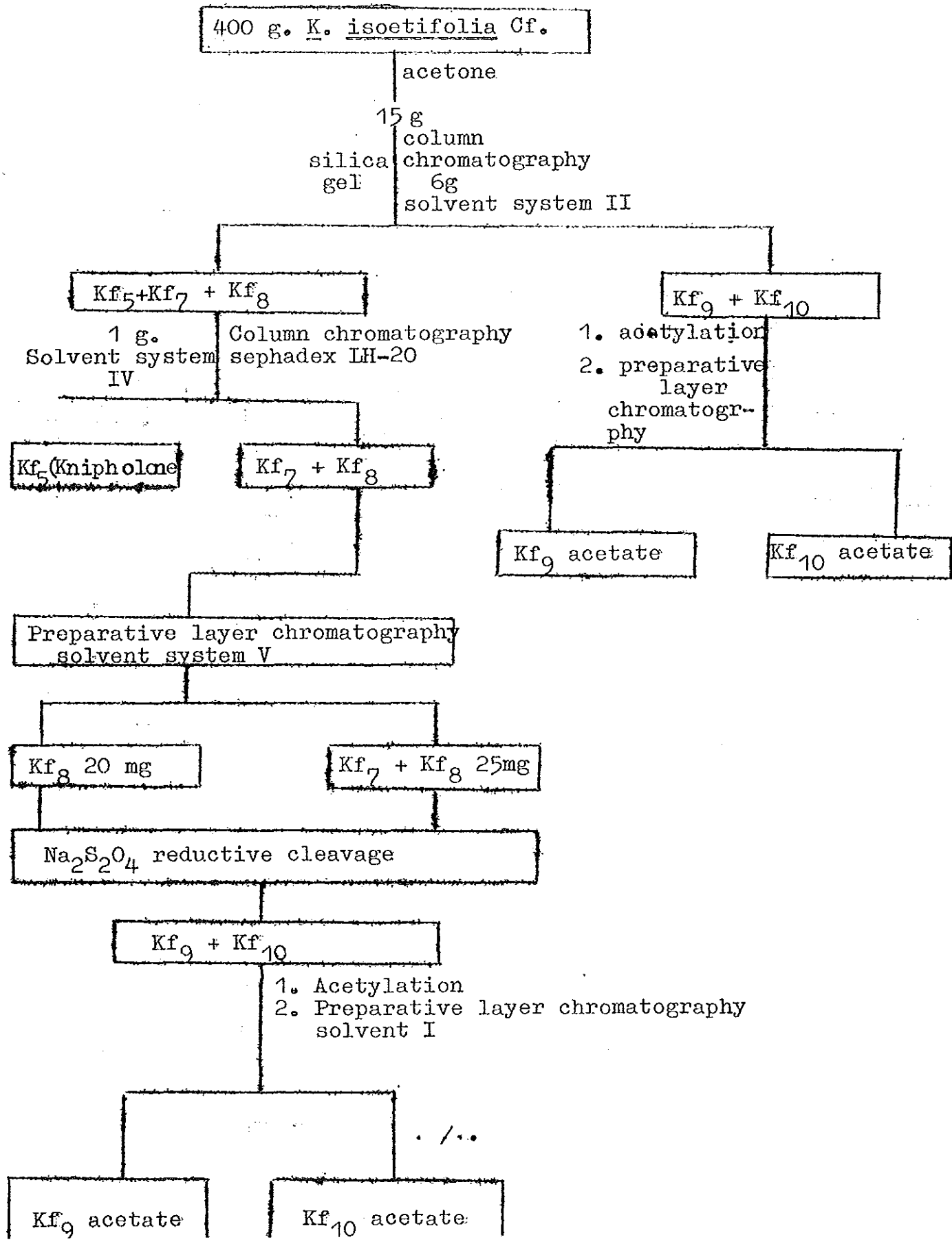
Comparison of the crude acetone extract on TLC with the crude extract of the rhizomes of K. foliosa revealed that there is no significant difference in chemical composition as far as presence of anthranoids are concerned but there is considerable difference in the relative abundance of the constituents. Thus in K. isotifolia K_{f_7} is present in relatively greater amount than K_{f_8} as can be gauged from the intensity of these spots on TLC.

A schematic flow chart for extraction and fractionation of the rhizomes of K. isoetifolia Cf is given in Scheme 5.

The crude acetone extract was applied on column packed with silica gel and eluted with solvent system II thus dividing the components into two groups of mixtures, the first group containing K_{f_9} and $K_{f_{10}}$ while the latter fractions were composed of K_{f_5}, K_{f_7} and K_{f_8}.

The mixture containing components K_{f_5}, K_{f_7} and K_{f_8} was subjected to column chromatography on sephadex LH -20 using solvent system IV. This readily separated K_{f_5} from the other two components.

Scheme 5. Extraction and fractionation of K. isoetifolia Cf



3.2.1 Degradation of K_{f_7} and K_{f_8}

K_{f_7} and K_{f_8} are yellow and red pigments respectively which were isolated from K. foliosa, K. isoetifolia Cf and detected in another as yet unidentified species of Kniphofia. These observations confirm significance of both K_{f_7} and K_{f_8} in the genus Kniphofia. Examination of the leaves, flowers, rhizomes and roots of K. foliosa has established that K_{f_7} and K_{f_8} are to be found only in the rhizomes and r' o s of this plant.

$Na_2S_2O_4$ reductive cleavage of dimeric compounds to the corresponding monomeric fragments makes the structural determination work in these compounds more simpler because the fragments obtained are usually known anthranoids or simple molecules. Structural determination work of skyrin (22) was greatly aided by $Na_2S_2O_4$ degradation of the molecule because it gave emodin a well known anthracene derivative as the only product[19] and as mentioned earlier degradation of knipholone (36) afforded chrysophanol and a structurally simple molecule 2-methoxy-4,6-dihydroxyacetophenone (37). It has been shown that alkaline $Na_2S_2O_4$ reductive cleavage of

.../..

dimeric anthranoids takes place readily[19] when two units are linked ortho to the hydroxyl group, as in 22 and 36.

K_{f_7} and K_{f_8} have nearly identical R_f values in many solvent systems and their separation by chromatographic methods was quite difficult although small amount of each was obtained by preparative layer chromatography using solvent system V. Crystals containing the two pigments could be obtained by concentration of the fractions collected from silica gel column of acetone extract using solvent II. Attempts to separate them by fractional crystalization were also not successful.

The MS of these two compounds show molecular ion at mass 492, $C_{30}H_{20}O_7$ for K_{f_7} and at mass 508 $C_{30}H_{20}O_8$ for K_{f_8}. Since C-30 anthracene derivatives are usually bisanthranoids, it was assumed that K_{f_7} and K_{f_8} could also be bisanthranoids. Both compounds were subjected to reductive cleavage using alkaline $Na_2S_2O_4$.

The cleavage experiment was performed first on pure K_{f_8} and since there was not enough pure K_{f_7} at our disposal the cleavage was done on a mixture containing both K_{f_7} and K_{f_8}. Interestingly, the same products were collected in both cases. The two fragments obtained were chrysophanol (4) and a fragment which on TLC was identical to the earlier mentioned compound $K_{f_{10}}$. 4 was

identified in a similar manner as discussed in section 3.1.2 and characterization of the other pigment as islandicin (1) will be discussed is section 3.2.2.

The close similarity in the TLC behaviour of K_{f_7} and K_{f_8} and their nearly identical empirical formulae lead one to believe that the two compounds should have nearly similar structures. Interestingly K_{f_7} is bright yellow while K_{f_8} is strikingly red and the former gives an orange-red color with alcoholic magnesium acetate while K_{f_8} gives a purple color. These observations coupled with the degradation work on K_{f_8} and mixtures of K_{f_7} and K_{f_8} which yielded chrysophanol (4) and islandicin (1) may eventually lead to a full structural assignment of K_{f_7} and K_{f_8}. In the following section evidence will be presented to show the identity of $K_{f_{10}}$ with islandicin thus clearly establishing the fragment of K_{f_8} to be present in the rhizomes and leaves of K. foliosa and rhizomes of K. isoetifolia Cf.

3.2.2 Characterization of fragments of K_{f_8} as islandicin and chrysophanol

As indicated earlier sodium dithionite reductive cleavage of either K_{f_8} or a mixture of

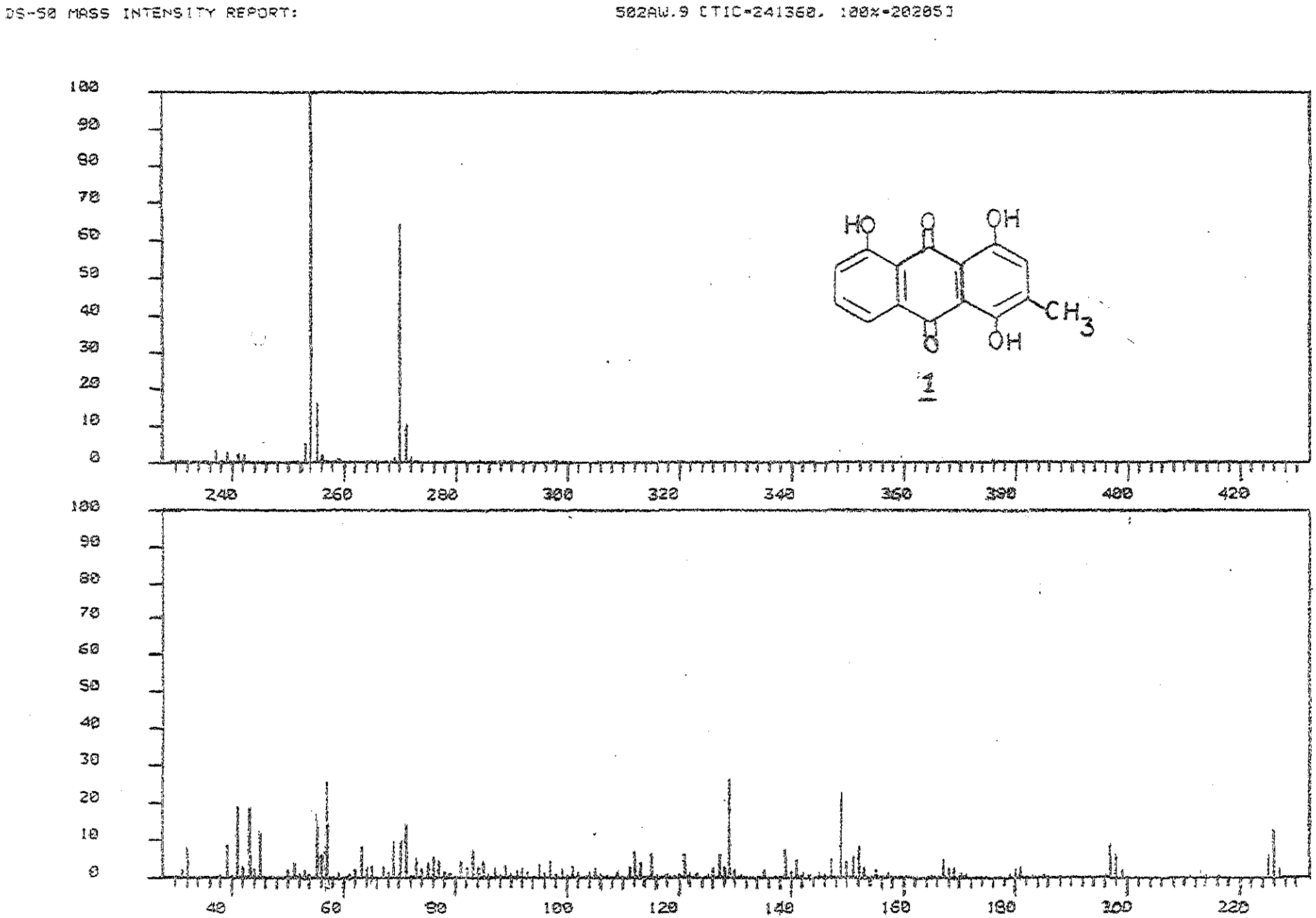
K_{f_8} and K_{f_7} afforded compound 4 and a red pigment. The separation of these two compounds by means of chromatographic methods was difficult and only small amounts of each were obtained by means of preparative layer chromatography using solvent systems II and VI.

The red pigmented fragment (mp 212-215°) is readily soluble in chloroform and only sparingly soluble in acetone and methanol. In 5% NaOH solution it gave a violet color fading gradually to pale yellow on standing in air. In cold conc. H_2SO_4 it gave rise to bright purple-red and in acetic acid a yellow orange color. Treatment with alcoholic magnesium acetate gave a light purple color, an indication of an anthraquinone moiety which contains two hydroxyls at the 1,4-positions.

The high resolution MS (fig. 4) displayed a molecular formula of $C_{15}H_{10}O_5$, suggestive of a compound containing one more oxygen than chrysophanol. Examination of the ^1H NMR spectrum showed sharp singlet at δ 2.33 which can be ascribed to a methyl absorption and three singlets at δ 12.24, 12.30 and 13.45 for absorption of the three chelated hydroxyl groups. The rather low field absorption for the third hydroxyl strongly indicates that this hydroxyl is singly chelated to a carbonyl thus restricting the third hydroxyl to position 4 or 5.

../..

Fig. 4. Mass spectrum of islandicin



Analysis of the chemical shifts and coupling constants of the aromatic protons was not possible as the sample was contaminated by presence of trace amounts of chrysophanol.

Because of the problem confronted with in separation of compound 4 and the red pigment, a mixture containing the two was directly acetylated using acetic anhydride and pyridine. The two acetate derivatives were readily separated by preparative layer chromatography using solvent system I. After separation 4-diacetate was found to be identical with the 4-diacetate prepared from an authentic sample of 4 (TLC, mp, IR), thus confirming one fragment of K_{f_8} to be chrysophanol.

Acetate of the red pigment, pale yellow needles from methanol, had mp of 203-206. Investigation of its ^{1}H NMR spectrum (fig. 5) showed two singlets at δ 2.47 (6H, 2xCOOC<u>H</u>$_3$) and at 2.53 (3H, COOC<u>H</u>$_3$) clearly indicating the presence of three hydroxyl groups in the red compound. A sharp singlet at δ 2.36 was assigned to the absorption of methyl protons. Examination of the aromatic region (see fig. 6) revealed a typical absorption pattern for the aromatic protons of the anthraquinone nucleus having protons at 5, 6 and 7 positions.

The presence of a strong absorption band at 1675 cm^{-1}

Fig. 5. 90 MHz NMR spectrum of islandicin triacetate ($CDCl_3$)

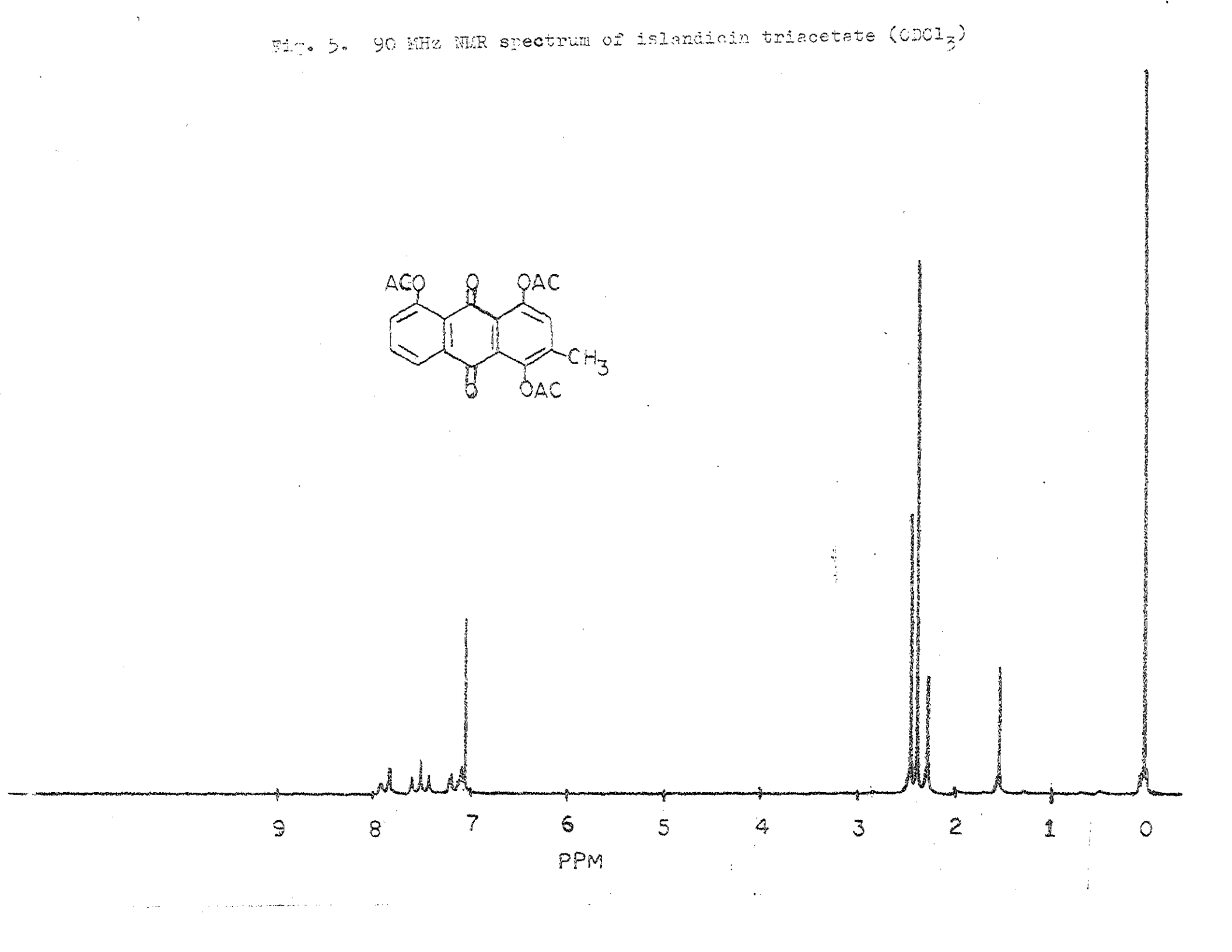
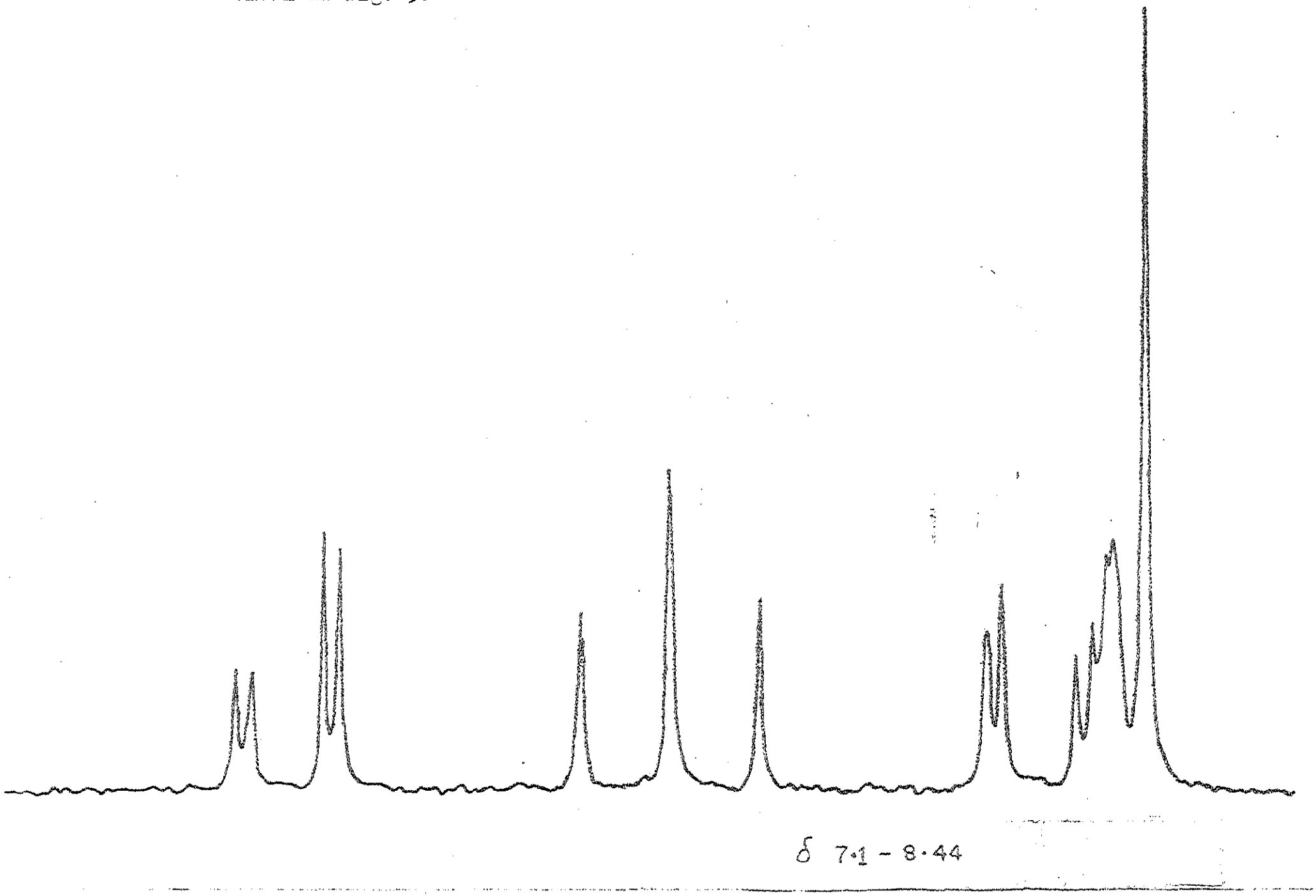


Fig. 6. Expansion of the aromatic region of the NMR spectrum of islandicin triacetate shown in Fig. 5.



in the IR spectrum for non-chelated carbonyl groups was an additional proof for the presence of an anthraquinone moiety.

The above data for the red pigment was consistent with the structure of islandicin (1), which has been isolated from the dried mycelium of penicillium islandicum[35]. The literature reporte mp for islandicin and its triacetate is 216 and 208 respectively which are in good agreement with our findings. Thus degradation of both K_{f_8} or a mixture of K_{f_8} and K_{f_7} resulted in chrysophanol and islandicin. 90 MHz proton NMR data for islandicin, chrysophanol, islandicin triacetate and chrysophanol diacete are given Table 7.

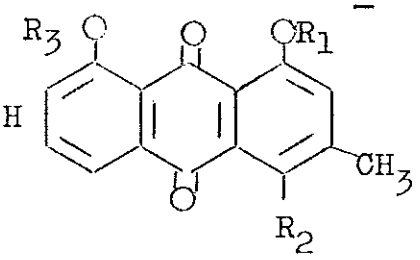
Table 7. 90 MHz proton NMR data of islandicin (1), chrysophanol (4), islandicin triacetate and chrysophanol triacetate

1 $R_1 = R_3 = H$, $R_2 = OH$

1-triacetate $R_1 = R_3 = AC$, $R_2 = OAC$

4-diacetate $R_1 = R_3 = AC$, $R = H$

4 $R_1 = R_2 = R_3 = H$



	δ(multiplicity), J(Hz)			
	Compounds			
Protons	1	4	1-triacetate	4-diacetate
2	7.3(d),0.8	7.02(br s)	7.36(br s)	7.14(br s)
5		7.79(d d)	8.13(d d), 7.8, 1.4	8.13(d d), 7.8, 1.4
6			7.78(t),8.0	7.70(t),8.0
7		7.24(d d)	7.40(d d),8, 1.3	7.34 (d d), 8, 1.4
CH_3	2.33(s)	2.40(s)	2.36(s)	2.44(s)
R_1	12.24(s)	11.98(s)	2.47(s)	2.40(s)
R_2	13.45(s)		2.53(s)	7.98(br m)
R_3	12.30(s)	12.06(s)	2.47(s)	2.40(s)

../..

3.2.3 Isolation of islandicin from the rhizomes of K. isoetifolia Cf

Crude acetone extract of the rhizomes of K. isoetifolia Cf was applied on a column packed with silica gel and eluted with solvent system II. TLC analysis (solvent II) after combination and concentration of the first three fractions, showed two pigments earlier designated as K_{f_9} and $K_{f_{10}}$ (see section 3.2.1 Scheme 5). Further TLC comparison of the mixture of K_{f_9} and $K_{f_{10}}$ with degradation products of K_{f_7} and K_{f_8} i.e. chrysophanol and islandicin, showed the identity of K_{f_9} with chrysophanol and $K_{f_{10}}$ with islandicin.

Examination of the leaves and rhizomes of K. foliosa in a similar manner extablished the presence of islandicin both in the leaves and rhizomes.

To further confirm the identity of the two groups of compounds i.e. K_{f_9} $K_{f_{10}}$ and (obtained from the plants) with the degradation products of K_{f_7} and K_{f_8}, the mixture containing K_{f_9} and $K_{f_{10}}$ was acetylated as in section 3.2.2. The acetates separated were found to be identical with 4-diacetate and 1-triacetate (TLC mp) thus confirming the presence of the two fragments of

K_{f_8} in the plants. It is interesting to note that to our knowledge there is no earlier report in the literature of the occurrence of islandicin in higher plants.

3.2.4 Preliminary data on K_{f_7} and K_{f_8}

As mentioned earlier K_{f_7} and K_{f_8}, isolated from the rhizomes and roots of K. foliosa and K. isoetifolia Cf are significant components in these species next to kniphlone (36). For a full structural elucidation of these pigments, besides the reductive cleavage experiment which clearly established that K_{f_7} and K_{f_8} are dimeric anthranoids, some spectral data of these compounds were recorded and these will be presented in this section.

The MS of K_{f_8} showed molecular ion at mass 508 ($C_{30}H_{20}O_8$) and two fragment ion at mass 270, $C_{15}H_{10}O_5$ and at mass 254, $C_{15}H_{10}O_4$ which were reasonably assigned for compounds 1 and 4 respectively. In MS of per-methylether of K_{f_8} the molecular ion appeared at mass $C_{35}H_{30}O_8$. Methylation introduced five methyl groups indicating the presence of five hydroxyl groups

in K_{f_8}. MS data for K_{f_8} and its per-methylether for some prominent peaks ar given in Table 8.

In ^1H NMR spectrum of K_{f_8} two sharp signals at δ 2.21 and 2.24 were assigned for two groups of methyl protons and these methyl protons were also clearly seen in the spectrum of K_{f_8}-permethylether. Five O-CH_3 protons in the spectrum of K_{f_8}-permethylether appeared at δ 3.08, 3.83, 3.86, 3.94 and 3.95. 400 MFz proton NMR data for K_{f_8} and its per-methylether are given in Table 9.

The IR spectrum of K_{f_8} showed a broad absorption band at 3470cm^{-1} which can be due to chelated hydroxyl absorption and a strong band at 1610 due to chelated carbonyl absorption. The absence absorption band at about 1680 cm^{-1} was indicative of the chelation of all carbonyl groups in K_{f_8}.

The molecular ion for K_{f_7} appeared at mass 492, $C_{30}H_{20}O_7$ which exactly showed a mass difference of one oxygen atom than the molecular ion mass of K_{f_8} and only the fragment at mass 254, $C_{15}M_{10}O_4$ for chrysophanol was observed. 90 MHz ^1H NMR spectrum of K_{f_7} showed 2 methyl groups at δ 2.24 and 2.47. Aromatic region protons and hydroxyl protons were not resolved.

Final structural assignment for both K_{f_7} and K_{f_8} awaits better ^1H NMR data to assign aromatic region protons and ^{13}C studies on these natural products and their derivatives.

../..

Table 8. MS, UV and IR spectra data of K_{f_8} and MS spectra dat of K_{f_8}-permethylether and K_{f_7}

Anthranoid	IR		UV* (λ_{max}, nm)
	Cm^{-1}	Nature	233, 255, 268, 297
K_{f_8}	3470	broad	
	1610	strong	
	1440	strong	

MS

Anthranoid	Formula for a fragment	Mean mass		Rel. intensity
		Found	Calculated	
K_{f_8}	$C_{30}H_{20}O_8$	508.1171		36.94
	$C_{30}H_{18}O_8$	506.1009	506.1002	12.60
	$C_{15}H_{10}O_5$	270.0536	270.0528	50.00
	$C_{15}H_{10}O_4$	254.0573	254.0573	60.00
K_{f_7}	$C_{30}H_{20}O_7$	492.1197		60.15
	$C_{30}H_{18}O_7$	490.1062	490.1053	79.54
	$C_{15}H_{10}O_4$	254		30
	$C_{15}H_{11}O_4$	255		40
K_{f_8}-permethylether	$C_{35}H_{30}O_8$	578		100
	$C_{34}H_{28}O_8$	564		60

*Visible region absorption was not included.

Table 9. 400 MHz proton NMR data of K_{f_8} and K_{f_8} permethylether

K_{f_8}				K_{f_8}-permethylether			
δ(multiplicity)	J(Hz)	No. of Protons	Assignmet	δ(multiplicity)	J(Hz)	No. of Protons	Assignment
2.21(s)	-	3	CH_3	2.27(s)	-	3	CH_3
2.24(s)	-	3	CH_3	2.37(s)	-	3	CH_3
6.64(br s)	-	1	-	3.08(s)	-	3	C-CH_3
6.76(d)	7.5	1	-	3.83(s)	-	3	C-CH_3
6.78(br s)	-	1	-	3.86(s)	-	3	O-CH_3
6.92(d d)	8.3,1	1	-	3.94(s)	-	3	O-CH_3
7.15(br s)	-	1	-	3.95(s)	-	3	O-CH_3
7.25(br s)	-	1	-	6.71(br s)	-	1	-
7.50(t)	7.8	1	-	6.75(br s)	-	1	-
7.97(d br)	7.5	1	-	6.90(d d)	7.5,1	1	-
8.70(br s)	-	1	-	6.94(d)	8.0	1	-
12.00(br s)	-	-	OH*	7.08(s)	-	1	-
12.20(br s)	-	-	"	7.35(t)	8.0	1	-
12.27(br s)	-	-	"	8.00(d)	8.0	1	-
				8.39(d)	8.0	1	-

*From permethylation the presence of five hydroxyls in K_{f_8} is unequivocally established thus confirming the MS data for the presence of a total of 20 protons in K_{f_8}.

3.3 Conclusion

The original aim of this project was to investigate all the Kniphofia species of Ethiopia for their anthranoid content in order to establish a chemotaxonomic correlation. However, time-limitations and the fact that not all of the Kniphofia species have been botanically identified and properly collected have compelled us to restrict this study to essentially two species namely K. foliosa and K. isoetifolia Cf.

The first part of our work was the extention of the earlier investigation on the rhizomes of K. foliosa by E. Dagne and W. Steglich to the leaves and flowers of the same plant. Knipholone, earlier isolated from the rhizomes was found to be a major constituent in the leaves as well as in the flowers. It has been also detected in the very fine roots emanating from the rhizomes of this plant. Thus knipholone is the major anthranoid of K. foliosa existing in all parts of the plant and rendering itself to easy separation from the other chemical components of the plant.

Another secondary metabolite which has been detected in all parts of the plant was chrysophanol. It has been shown that in rhizomes and leaves of

K. foliosa chrysophanol was found with another structurally very closely related pigment islandicin. In the early stage of our work we were not aware of the existence of islandicin because in many solvent systems the two pigments had exactly the same R_f values and they overlap. But latter on islandicin was also found to be one of the constituents of the leaves and rhizomes of K. foliosa. Aloe-emodin monoacetate was found to be localized only in the leaves of K. foliosa. Extention of the chemical investigation to the leaves of other Kniphofia species would be interesting because it will show the interrelationship or the difference between these plants interms of the anthranoid compositions.

Not much work was done on the flowers of K. foliosa except a TLC comparison of the acetone extract with the extracts of other parts of the plant, which showed the presence of knipholone and chrysophanol. Therefore further chemical examination of the flowers of this plant and others, in the genus Kniphofia, will contribute much to the chemotaxonomic correlation.

The second part of this work was the chemical investigation done on the rhizomes of K. isoetifolia Cf. We were able to isolate from the rhizomes of this plant knipholone, chrysophanol and islandicin, knipholone being the major constituent.

Beside the above three pigments other anthranoids

designated as K_{f_7} and K_{f_8} (yellow and red respectively) whose structures were not yet determined, were isolated. On TLC and IR spectral comparison they were found to be identical with the yellow and red pigments isolated from the rhizomes of K. foliosa. Thus it was established that the chemical compositions of the rhizomes of the two plants are the same as far as anthracene derivatives are concerned. Table 10 summarizes anthranoids isolated from different parts of K. foliosa and from the rhizomes of K. isoetifolia Cf.

Furthermore, in an attempt to shade light on the structure elucidation of K_{f_7} and K_{f_8}, we found that the degradation of these compounds resulted in chrystophanol and islandicin a finding considered as significant because it revealed that K_{f_7} and K_{f_8} were dimeric anthranoids composed of the two monomeric anthracene derivatives isolated from the same plant. Although it has been established that K_{f_7} and K_{f_8} are dimeric anthranoids, it was not possible in the course of this work to assign the final structures for these pigments.

Table 10. Summary of anthranoids isolated from K. foliosa and K. isoetifolia Cf.

	K. foliosa				K. isoetifolia Cf.			
Compound	Rh	R	L	F	Rh	R	L	F
Knipholone	✓	✓	✓	✓	✓	*	*	*
Chrysophanol	✓	✓	✓	✓	✓	*	*	*
Islandicin	✓	*	✓	*	✓	*	*	*
K_{f_7}	✓	✓	X	X	✓	*	*	*
K_{f_8}	✓	✓	X	X	✓	*	*	*

Rh = rhizomes

R = roots

L = leaves

F = flowers

✓ = indicates the presence

* = indicates that no work was done

X = indicates the absence

../..

4. Experimental

4.1 General

Plant material:- The leaves of Kniphofia foliosa were collected in 1983 from Wollo, Ethiopia, on the road from Dessie to Woreilu, Hara Wobalo Kebele at an altitude of 2600 m. and identified by Mrs. Sue Edwards, Department of Biology, Addis Ababa University. The rhizomes of Kniphofia isoetifolia Cf were collectedin 1983 from Bole (Addis Ababa) and identified by Ato Zerhun Woldu of the Biology Department, Addis Ababa University. For both plant materials collected a voucher sample was kept in the National Herbarium, Addis Ababa.

Apparatus:- Melting points were determined on a Kofler Block apparatus. UV spectral (in methanol) were recorded on a Perkin Elmer 555 spectrophotometer. IR spectra (KBr) were run on an SP 2000 Pye Unicam spectrophotometer. Mass spectra were recorded at 70 eV on AEI MS 30 and MS 50 instruments. ^{1}H NMR spectra were measured at 90 or 400 MHz using Bruker WH 90 or WH 400 instruments and chemical shifts are given in δ-values with TMS as internal standard.

../..

Analytical thin layer chromatograms were run on a 0.25 mm thick layer of silica gel UV_{254}(Merck). Preparative layer chromatograms were run on 2.00 mm thick layer of silica gel 60 PF_{254} + 360 (Merck). Column chromatography was performed on silica gel 70-230 mesh (Merck) and on sphadex LH-20.

Solvent systems:- The following solven systems were used for thin layer, preparative layer and column chromatography.

Solvent system	I	benzene-Ethyl acetate (4:1)
Solvent system	II	hexane-acetone (9:1)
Solvent system	III	chloroform-Ethyl acetate (9:1)
Solvent system	IV	methanol-chloroform (1:1)
Solvent system	V	dichloromethane
Solvent system	VI	petroleum ether 60-80°-acetone (9:1)

4.2 Extraction and fractionation

4.2.1 Extraction and fractionation of K. foliosa leaves

The powdered leaves of K. foliosa (500 g), when soaked in acetone at room temperature, yield after filtration and evaporation of the solvent a greenish substance 25 g (5%)

which on TLC (solvent I) showed at least 5 spots of which three R_f 0.57 (K_{f_5}), 0.86 (K_{f_6}) and 0.89 (K_{f_9} + $K_{f_{10}}$) were the most prominent. The crude acetone extract was soaked in $CHCl_3$ (100 ml), after filtration the residue was discended. The chloroform soluble part which contained all the major spots, upon filtration and removal of the solvent yielded 20.16 g (4%) of a greenish solid. 5 g of this was applied on column packed with silica gel (100 g) and eluted with solvent III. A total of 10 fractions each 250 ml were collected. The residue obtained after combination and evaporation of fractions 2-5 contained mainly K_{f_6}. This was applied on a column packed with sephadex LH-20 (100 g) and eluted with solvent IV. A total of 19 fractions each 10 ml were collected. K_{f_6} was principally in fractions 10-19. These fractions were combined and evaporated. Final purification of K_{f_6} was done by preparative layer chromatography (solvent I) and 32 mg of pure K_{f_6} was collected. Fractions 1-9 from sephadex column contained mainly K_{f_9} and $K_{f_{10}}$. Fractions 6-10 from silica gel column contained mainly K_{f_5} which was purified by preparative layer chromatography (solvent I).

4.2.2 Extraction and fractionation of K. isoetifolia Cf rhizomes

400 g of powdered rhizomes of K. isoetifolia

../..

Cf were packed in a column and eluted with 2 liters of acetone. Removal of the solvent under reduced pressure gave 15 g (4%) of a pink sticky material which on TLC (solvent I) showed at least 8 spots of which four with R_f 0.57, 0.86, 0.89 and 0.93, were the most prominent. The crude acetone extract (6 g) was applied on a column packed with silica gel (110 g) and eluted with solvent II. A total of 42 fractions were collected. Fractions 1-30 each 40 ml, fractions 31-35, each 60 ml, and fractions 36-42, each 250 ml, collected. Fractions 1-4 were mainly composed of K_{f_9} and $K_{f_{10}}$. Fractions 5-32 containing K_{f_5}, K_{f_7} and K_{f_8} were combine, and upon evaporation of the solvent 2.5 g was collected. 1 g of this was applied on a column packed with sephadex LH-20 (150 g) and eluted with solvent IV. A total of 27 fractions each 10 ml were collecte. Fractions 1-15 principally contained K_{f_7} and K_{f_8}. Fractions 16-27 which were mainly composed of K_{f_5} were combined and the solvent was removed. Pure K_{f_5} was obtained by means of preparative layer chromatography (solvent I). Upon combination and evaporation of fractions 1-15 200 mg K_{f_7} and K_{f_8} mixture was collected. Application of this on preparative layer (solvent V) afforded 20 mg K_{f_8} and 25 mg of a mixture of the two.

../..

4.3 Characterization of anthranoids

4.3.1 Characterization of kniopholone and chrysophanol

Kniopholone (36):- The details of isolation and charactreization of this compound have been reported earlier.[5] K_{f_5} isolated from the leaves of *K. foliosa* and from the rhizomes of *K. isoetifolia* Cf was identical with an authentic sample of 36 (TLC IR).

Chrysophanol (4):- The chrysophanol isolated from the leaves of *K. foliosa* and the rhizomes of *K. isoetifolia* Cf was identical with an authentic sample (TLC IR) and the diacetate prepared was characterized by comparing its mp and TLC with chrysophanol diacetate obtained by acetylation of degradation product of K_{f_8} (see section 4.4.6).

4.3.2 Characterization of K_{f_6} as aloe-emodin monoacetate (48).

Thin layer chromatograph:- It gave a single yellow spot (R_f = 0.86) solvent I) on silica gel layer.

../..

Melting point:- Orange needles from acetone, mp 203-206 (dec.)

Color test:- Turned violet with 5% aqueous NaOH and upon treatment with 0.5% methanolic magnesium acetate produced an orange color.

IR ν_{max}(KBr) 3500, 1745, 1680, 1635, 1575, 1460, 1390, 1280 and 1170 cm^{-1}; UV λ_{max}^{MeOH} (log ε), 252 (4.06), 284 (4.3), 312 (3.73), 422 (3.9); ^1H NMR (90 MHz, DMSO-d_6): δ 2.15 (s, $COCH_3$), 5.24 (br s, $-CH_2$), 7.39 (d, J= 1.6 Hz), H-2), 7.44 (d d, J = 7.0 and 2.2 Hz; H-7), 7.88(M, H-4 , H-5, H-6), 12.00 (br, 2OH); MS m/z/ (rel. int.) $C_{17}H_{12}O_6$ 312.0642 (M^+, 30), $C_{15}H_{10}O_5$ 270.0527 (100), 241 (35).

K_{f_6}-triacetate(aloe-emodin triacetate)(49):- Yellow needles from benzene mp 169-171 (lit. 170-175)[32] ^1H NMR (90 MHz, $CDCl_3$); δ 2.15 (s, $COOCH_3$), 2.44 (s,6H, 2x $COOCH_3$) 5.22 (br s,$-CH_2-OAC$), 7.40 (d, J = 2Hz, H-2), 7.40 (d d, J = 8 and 2Hz, H-7), 7.7 (t, J = 8Hz, H-6), 8.20 (d, J = 2Hz, H-4), 8.24 (d d, J = 8 and 2Hz, H-5); MS m/z/ (rel. int.) $C_{21}H_{16}O_8$ 396.0833 (M^+, 7), 354 (33), 312 (29) $C_{15}H_{10}O_5$ 270.0528 (100).

Hydrolysis of K_{f_6} (aloe-emodin (50):- Yellow needles from toluene mp 216-220 (lit. 222)[33] MS $C_{15}H_{10}O_5$ 270.0527 (M^+, 100/) Calcd. 270.0528.

../..

4.4 Reactions

4.4.1 Anthranoid color test:-

To an anthranoid dissolved in acetone, chloroform or methanol 2 drops of 0.5% methanolic magnesium acetate was added. Formation of a purple color indicated the presence of two hydroxyl groups in 1,4-positions on the anthraquinone nucleus, frormation of an orange-red color two hydroxyl groups in 1,3 or 1,8 positions and two hydroxyl in the 1,2-positions produce a blue color.[14]

4.4.2 Acetylation of aloe-emodin monoacetate

To aloe-emodin monoacetate (10 mg) dissolved in acetic anhydride (5 ml) pyridine (3 drops) was added and the mixture was heated with stirring at 60-70° for 4 hrs and then stirred without heating for 24 hrs. 5 ml of ice water was added and the mixture stirred for 30 mins. After neutralization with saturated sodium bicarbonate solution extracted with chloroform and chloroform solution was washed with 2N H_2SO_4, dried with anhydrous sodium sulphate. After removal of the solvent

crude reaction product collected was recrystalized from benzene. Yellow needles mp 169-171, were obtained.

4.4.3 Hydrolysis of aloe-emodin monoacetate:

5.2 mg aloe-emodin monoacetate in 3 ml 0.5% methanoic KOH (violet solution) was heated on water bath for 10 min. After removal of methanol the residue was acidified with 2N H_2SO_4 and extracted with 3 x 10 ml portions of ethyl acetate and dried with anhydrous Na_2SO_4. After removal of the solvent 4.5 mg of a brown-red product was obtained and recrystalized from toluene, mp 216-220°.

4.4.4 $Na_2S_2O_4$ reductive cleavage of K_{f_8}

To a solution of K_{f_8} (10 mg) in 10 ml 5% NaOH solution, $Na_2S_2O_4$ (15 mg) was added and the mixture was heated at 80° for 1½ hrs. On cooling the mixture was acidified with 2N H_2SO_4 and extracted with ethyl acetate and dried with Na_2SO_4. After removal of the solvent 8.7 mg of a mixture of chrsyophanol and islandicin was collected.

../..

4.4.5 $Na_2S_2O_4$ reductive cleavage of K_{f_7} and K_{f_8} mixture

To a solution of K_{f_7} and K_{f_8} mixture (56.7 mg) in 15 ml 5% NaOH solution was added $Na_2S_2O_4$ (85 mg) and the mixture was heated at 80° for 1½ hrs. On cooling the mixture was acidified with 2N H_2SO_4 and extracted with ethyl acetate. Then dried with Na_2SO_4. After removal of the solvent a 45.5 mg mixture of chrysophanol and islandicin was obtained.

Islandicin separated from chrysophanol by applying the degradation mixture on a preparative layer using solvent II has the following properties.

Thin layer chromatography:- Single spot (R_f = 0.6 solvent II) on silica gel layer.

Melting point;- Brownish-red compound mp 212-215°.

Color test:- It turned violet in 5% aqueous NaOH solution fading gradually to pale yellow color on standing in air and upon treatment with 0.5% methanolic magnesium acetate gave light purple color. In conc. H_2SO_4 bright purple-red color and in acetic acid a yellow orange color.

^{1}H NMR (90 MHz, $CDCl_3$) δ 7.31 (d, J = 0.8, H-2) 2.33 (s, $\underline{CH_3}$), 12.30 (s, OH), 12.24 (s, OH), 13.45 (s, OH); MS m/z/ (rel. int.) $C_{15}H_{10}O_5$ 270.0523 (M^+, 64), $C_{15}H_{10}O_4$ 254.057 (100), $C_{14}H_9O_3$ 225.0553 (6), $C_{13}H_{10}O_2$ 198.0673 (6).

4.4.6 Acetylation of amixture of islandicin and chrysophanol

To 28.9 mg of the mixture in acetic anhydride was added 3 drops of pyridine, the mixture was heated at 60-70° for 4 hrs with stirring and stirred without heating for 24 hrs. After work up as in section 4.4.2 the two acetate were separated by preparative layer chromatography using solvent I. Recrystalization from methanol afforded 15 mg of chrysophanol diacetate and 8 mg of islandicin triacetae.

Islandicin triacetate:- Pale yellow needles from methanol mp. 203-206. ^{1}H NMR (90 MHz $CDCl_3$) δ 2.36 (s, CH_3) 2.40 (s, 6H, 2x $COO\underline{CH_3}$), 2.53 (s, $COO\underline{CH_3}$), 7.36 (br s, H-2) 8.30 (d d, J = 7.8 and 1.4 Hz, H-5), 7.78 (t, J = 8Hz, H-6) 7.40 (d d, J = 8 and 1.3 Hz, H-7; IR ν_{max}(KBr) 1780, 1760, 1675, 1590cm^{-1}.

Chrysophanol diacetate:- Yellow needles from metha ol mp 201-203 (lit. 204.5)[36]. IR,

../..

ν_{max}(KBr) 1760, 1680, 1665; ^{1}H NMR (90 MHz, $CDCl_3$) δ 2.40 (s, 6H, 2xCOO$\underline{CH_3}$) 2.44 (s, $\underline{CH_3}$), 7.14 (br s, H-2), 8.13 (d d, J = 7.8 and 1.4 Hz, H-5), 7.70 (t, J = 8Hz, H-6), 7.34 (d d, J = 8 and 1.4 Hz, H-7), 7.98 (br m, H-4).

4.4.7 Characterization of $K_{f_{10}}$ as islandicin

The first few fractions obtained from silica gel column chromatography of acetone extracts of the rhizomes of K. foliosa, K. isoetifolia Cf and the leaves of K. foliosa mainly contain $K_{f_{10}}$ and chrysophanol (see section 4.2.2). On TLC (solvent II) $K_{f_{10}}$ was found to be identical with islandicin obtained by degradation of K_{f_8} or a mixture of K_{f_7} and $K_{f_{10}}$.

Acetylation of a mixture of $K_{f_{10}}$ and chrysophanol

To 20 mg of a mixture of $K_{f_{10}}$ and crysophanol (ofcourse containing other nonanthranoid compounds as impurities) in acetic anhydride was added 3 drops of pyridine. The mixture was heated at 60-70° for 4 hrs with stirring and stirred without heating for 24 hrs. After work up as in section 4.4.2 very small amounts of the two acetates, enough only for TLC and mp determination were collected by preparative layer

chromatography (solvent I). $K_{f_{10}}$ - acetate was found to be identical with islandicin triacetate obtained by acetylation of degradation product of K_{f_8} or a mixture of K_{f_7} and K_{f_8} (TLC. mp).

References

1. Marias, W., Kew Bull., 28 (3) (1973) 465.

2. Edward, S., "Some Wild Flowering Plants of Ethiopia", Artistic Press, Addis Ababa, 1976, p. 8.

3. Boross, L., Acta Chim. Acad. Sci. Hung., 35 (1963)195 (Chem. Abst., 58 (1963) 10505 h.).

4. Watt, J.M. and M.G. Breyer - Brandwijk, "The Medicinal and Poisonous Plants of Southern and Estern Africa", E and S. Living Stone Ltd., London, 1962, p 707.

5. Dagne, E. and W. Steglich, Phytochemistry, in press.

6. Robinson, T., "The Organic constituents of Higher Plants" Cordus Press, New York, 1975, p. 103.

7. Rizk, H.M, F.M. Hammounda and M.M. Gawad, Phytochemistry, 11 (1972) 2122,

8. Lemi, J., "Review of the Chemistry and Physiological properties of Quinones in Higher Plants" in the Proceedings of the 2nd Inter. meeting on Medicinal and Aromatic Plants Citta di Castello, Sept. 1981 p. 99.

9. Steglich, W., and V. Austel, Tetrahedron Letters, 26 (1966) 3077.

10. Steglich, W., L. Walter and A. Volkhard, Chem. Ber., 102 (1960) 4104.

../..

11. Steglich, W., L. Walter and R. Wolfgang, Tetrahedron Letters, 47 (1967) 4719.

12. Trease, G.E and W. C. Evan, "Pharmacogonsy", BaiDicre, London, 1978, p. 373.

13. Evelyn Hay, J. and L.J. Haynes, J. Chem. Soc., (1956) 3141-45.

14. Shibata, S., M. Takito and O. Tanka, J. Am. Chem. Soc., 72 (1950) 2789-90.

15. Crossley, M.L., J. Am. Chem. Soc., 41 (1919) 2018 (Chem. Abst. 14 (1920) 185^{8}.).

16. Stahl, E., "Thin Layer Chromatography, 2nd ed., Springer-Verlag, New York, 1969, p. 707.

17. Nakanishi, K., T. Goto, S. Ito, S. Natori and S. Nozoe, "Natural Product Chemistry" Vol. 2, Academic Press, New York, 1975 p. 172.

18. Dutta, N.L., A.C. Ghosh, P.M. Nair and K. Venkataraman, Tetrahedron Letters, 40 (1964) 3023,

19. Shibata, S., O. Tanaka and I. Kitagawa, Pharm. Bull. (Tokyo), 3 (1955) 274.

20. Bentley, K.W, "The Natural Pigments", Vol. 4, Int., New York, 1960, p. 206.

21. Rasmusser, R.S., D.D. Tunnicliff and R. Robert BraHain, J. Am. Chem. Soc., 71 (1949) 1068.

../..

22. Bloom, H., L.H. Briggs and B. Cleverley, J. Chem. Soc., (1959) 178.

23. Scott, A.I., "Interpretation of the UV Spectra of Natural Products" Vol.7, Macmillan Comp., New York, 1964, p. 286.

24. Meyer and O. Fischer, Braunchweing Bor, 46, 85. (Chem. Abst. 7 (1913) 1198[1]).

25. Beynon, J.H., R.A. Saunders and A.E. Williams, "The Mass Spectra of Organic Molecules", Elsevier, London, 1968, p. 204.

26. Steglich, W. and W. Losel Tetrahedron, 25 (1960) 4391-4399.

27. Berger, Y. and A. Castonguay, Org. Magn. Reson. 11 (1978) 375.

28. Manitto, p. and P.G. Sammes, "Biosynthesis of Natural Products", Ellis Horwood, New York 1981, p. 196.

29. Goodwin, T.W., "Chemistry and Biochemistry of Plant pigments", Academic Press, New York, 1965, p. 310.

30. Gatenbeck, Acta. Chem. Scand. 16 (1962) 1053. (Chem. Abst. 57 (1962) 12864 d.)

31. Melafferty, F. W., "Interpretation of Mass Spectra 2nd ed. W.A. Benjamin Inc., London, p. 137.

../..

32. Paul, G. Stecher "An Encyclopidia of Chemicals and drugs" 8th ed. N. J. Merk and CO Inc., USA. p. 40.

33. Rizk, A.M., F.M. Hammouda and M.M. Abdel-Gawad, Phytochemistry, 11 (1972) 2122.

34. Plowes, D.L.H and R.B. Drumond, "Wild Flowers of Rhodesia", Longman Rhodesia, Salsbury, 1976 p. 19.

35. Howard, B.H. and H. Raistrick, Bichem. J 44(1949). 227-33 (Chem. Abst. 44 (1950) 591 f).

36. "Dictionary of Organic Compounds", Vol. 2, 4th ed., Oxford Press, New York, 1965, p. 707.