CHEMICAL TRANSFORMATIONS OF SOME KNIPHOFIA QUINONES

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ABSTRACT

Chemical Transformations
of Some Kniphofia Quinones.
By
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Chemical investigation of the rhizome of *Kniphofia fotiosa* (Hochst), Asphodelaceae, led to the isolation of a minor product code named KKA-1 **(40)**. The spectral data suggested that it was a dimer of knipholone **(32)** and its anthrone **(33)**. This was further confirmed by reductive cleavage to 4,6-dihydroxy-2-methoxyacetophenone **(37)**, acetylphlorogtucinol **(36)**, and chrysophanol **(5)**. It was synthesized from knipholone anthrone **(33)** through oxidative coupling reactions.

The synthesis of 4,6-dihydroxy-2-methoxyacetophenone **(37)**, a consitiuent of AT. *fotiosa*, is reported. Knipholone **(32)** was synthesized from knipholone anthrone **(33)** through oxidation. Reductive cleavage of knipholone **(32)** gave chrysophanol **(5)**, 4,6-dihydroxy-2-methoxyacetophenone **(37)** and acetylphloroglucinol **(36)**. Bromination of knipholone anthrone **(33)** resulted in four bromides: **41**, **42**, **43** and **45**. These bromides were not stable in solution.

LIST OF FIGURES

LIST OF ABBREVIATIONS

NOE	Nuclear overhauser effect
COSY	Correlation spectroscopy
dqCOSY	Double quantum correlation spectroscopy
NOESY	Nuclear overhauser and exchange spectroscopy
INEPT	Insensitive enhancement by polarization transfer
DEPT	Distortionless enhancement by polarization transfer
TLC	Thin layer chromatography
pTLC	Preparative thin layer chromatography
Ca.	Approximately
NMR	Nuclear magnetic resonance
IR	Infra red spectroscopy
UV	Ultra-violet spectroscopy
NMR multiplicities	s: singlet
	d: doublet
	t: triplet
	m: multiplet
IR peak strengths	s: strong
	m: medium
	w: weak
DMF	Dimethylformamide
DMSO	Dimethylsulfoxide
SAM	S-adenosyl methionine

anthraquinone dyes have now been replaced by better and cheaper synthetic dyes though they still retain their place as models for the synthesis and development of such dyes [12].

It is therefore not surprising that organic chemists continue to work on plants in the hope of coming up with compounds beneficial to man and his subjects. This is in addition to the chemical knowledge gathered in the process.

1.1 Occurrence and structural diversity of anthranoids

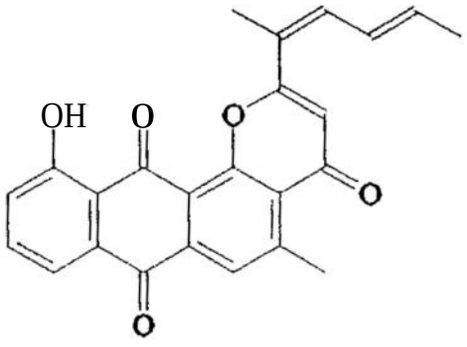
Over 1200 naturally occurring quinones had been known by the year 1986. They are widely distributed in both animals and plants, as pigments and as intermediates in cellular respiration and photosynthesis. Thomson presented a compact and authoritative review on the recent advances in their structure and chemistry, backed with comprehensive references [13].

Anthraquinones, and anthranoids in general, represent a large number of naturally occurring quinones. They are widely distributed in nature and have been isolated from both higher and lower organisms. In plants, they have been isolated from many families including Rhainnaceae, Rubiaceae, Polygonaceae, Bignoniaceae, Verbanaceae, Scrophlariaceae, Leguminaceae and several genera of the family Liliaceae.

Anthranoids, as the name suggests, are anthracene derivatives. In this work, common names and numbering (1, Table 1) have been employed. Structure 1 represents the basic skeleton of anthraquinones. It is possible to have the carbonyl functions on other positions. They can be 1,2-, 1,4- or 9,10-anthraquinones, the last class being the most abundant in nature.

Anthraquinones found in nature are mostly hydroxylated. Except with the kidamycin group of antibiotic antitumour agents, there are relatively few departures from the usual C_{14} and C_{15} basic skeletons [14]. A few examples are listed in Table 1.

Dimeric anthraquinones are also common, examples being the variously coupled bi-chrysophanol such as 2-2' (cassiamin C), 2-7' (microcarpin), 4-7' (asphodelin), 5-5' (dihydrorugulosin) and 7-7' (chrysotalunin) [13]. Miscellaneous anthranoids are also known, an example being a-indomycinone (13), which was isolated from the mycelium of *Streptomyces* spp. [6].



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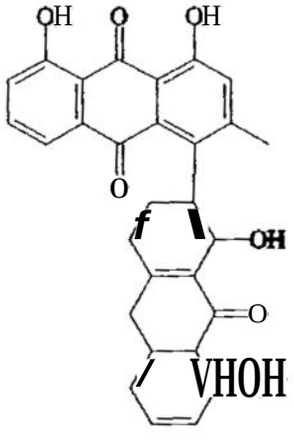

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1.2 Structure elucidation of anthraquinones.

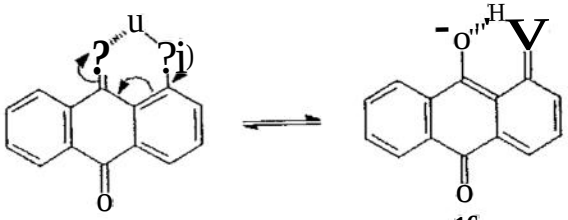
Complex natural products structure elucidation was a formidable task to the organic chemists of the 19th and early 20th century. Spectroscopic methods provide extremely powerful tools in the hands of chemists for the elucidation of complex natural products. Analysis of spectra has now replaced, to a large extent, an extremely laborious and time consuming chemical degradation employed by classical organic chemists.

One of the greatest advantages of the new approach is the small amount of sample (0.01-20 mg) that can be analyzed and later recovered (non-destructive analysis), a notable exception being mass spectroscopy. Spectrum acquisition takes short time, usually up to 15 minutes for simple spectroscopic analysis. A review on modern techniques in one- and

two-dimensional nuclear magnetic resonance (NMR) spectroscopy has been edited by Nakanishi [16].

In infrared (IR) spectroscopy, anthranoids show characteristic carbonyl stretching. Since most are hydroxylated, the effects of the hydroxyl groups dominate the spectrum. Chelation (Scheme 1), possible when a-hydroxyl groups are present, shifts the carbonyl stretching to the lower frequencies. In addition, a second carbonyl stretching at a lower frequency results when such a-hydroxyl groups are present. The hydroxyl stretching frequency disappears or is lowered [17], as a result of chelation and resonance (Scheme 1). The spectrum usually shows a strong peak at 1280 cnr^{1} as is common with most a-6 unsaturated and cross conjugated ketones. Extensive studies on carbonyl stretching of anthraquinones were conducted by Bloom *et al.* [17].

Scheme 1. Conjugate addition in hydroxylated anthraquinones.



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In UV-VIS spectroscopy, anthraquinones show intense benzenoid absorptions at 240-260 nm, a medium band at 320-330 nm and a strong quinonoid electron transfer (ET) band at 270-290 nm. There is also a weak quinonoid (n-π^{*}) band at 405 nm. The UV region of the spectrum is not seriously affected by substitution. However, the visible region of the spectrum is influenced by the number of a-hydroxyl groups. 6-Hydroxyl groups do not seriously affect the positions of the absorption maxima. Substituent effects have been studied in the past and several works appear in literature [18, 19].

Nuclear magnetic resonance (NMR) spectroscopy is a powerful tool in structure determination of natural products. a-Hydroxylated anthraquinones show characteristic signals far downfield (above 5 11) due to the chelated hydroxyl protons. The splitting pattern as well as the coupling constants give information on the protons involved. Though coupling constants are highly dependent on dihedral angles, they are fairly constant for aromatic protons. *Ortho* coupling constants range between 7 and 9 Hz while those of *meta*

coupling range between 2 and 3 Hz. Para coupling constants are too small (approximately 1 Hz) and are not usually considered.

In 'H NMR spectroscopy, it is not possible, at present, to calculate theoretical substituent effects. This is because they are thought to be a combination of a number of factors including inductive, steric and anisotropic effects. However, attempts have been made to arrive at statistical correlations after studying a large number of differently substituted anthraquinones. These are done by assuming that the effects are additive. Several papers have appeared in the past, a good example being that of Bailantine *et al.* [20] where they computed substituent effects from data obtained from 450 aromatic compounds. The values obtained are close enough to help in the assignment of signals of unknown aromatic compounds.

^{13}C NMR spectroscopy has now become a routine procedure in structure determination of anthraquinones. It still requires a sizeable amount of sample (at least 10 mg for operating at 25 MHz) in comparison with proton NMR spectroscopy. However, the duration required to obtain a spectrum has been greatly reduced ranging from minutes to hours depending on the number of scans needed.

Anthraquinones show a general close agreement in their ^{13}C NMR spectra. The carbonyl carbons characteristically resonate way downfield (ca. 5 200) as a result of deshielding by the oxygen substituent. The chemical shift profiles closely resemble those of 'H NMR spectra though the range is broad, down up to 5 210 or even more, while that of 'H NMR spectra is rarely beyond 5 16.

Improvement on signal separation has been attained by the use of Lanthanide shift reagents. $Yb(fod)_3$ and $Pr(fod)_3$ have been found [21] to be superior in ^{13}C NMR studies. In 1978, Berger *et al.* [22] published a paper on the hydroxyl substituent effects on the ^{13}C NMR chemical shifts of variously hydroxylated anthraquinones. However, the data is just a starting point since a large number of anthraquinones contain not only hydroxyl substituents but also other functional groups as well. Hofle *et al.* [23] published chemical shifts of various anthraquinones with variously oriented acetate substituents.

The value of mass spectroscopy (MS) is that it requires microgram amounts of sample, that it can provide accurate measurement of molecular weight and that it may yield a complex fragmentation pattern which is often characteristic of (and may identify) that compound. The major disadvantage is that MS spectrophotometers are much more

complicated and expensive than those of UV and IR which are operated by the chemist himself. Therefore, they are normally operated by trained personnel.

Mass spectroscopy has been used in the structural elucidation of anthraquinones. Rao *et al*. [24] used mass spectroscopy for the determination of the structure of ventinone B. Hydoxylated anthraquinones give peaks due to progressive loss of -OH, and H20, as well as CO. The other substituents confer upon the molecule definite fragmentation patterns. X-ray is a useful tool in determination and confirmation of structural arrangement but it is limited to crystalline compounds. Optical rotation still remains a powerful tool in stereochemical studies and determination of optical purity of natural products. Circular dichroism has been used to determine absolute configurations of 1,1'-bianthryl derivatives through the exciton chirality method [25]. In addition to the largely spectroscopic structural determination methods so far described, there are also the classical functional group identification through chemical means, which are useful in preliminary examination.

1.3 Synthesis

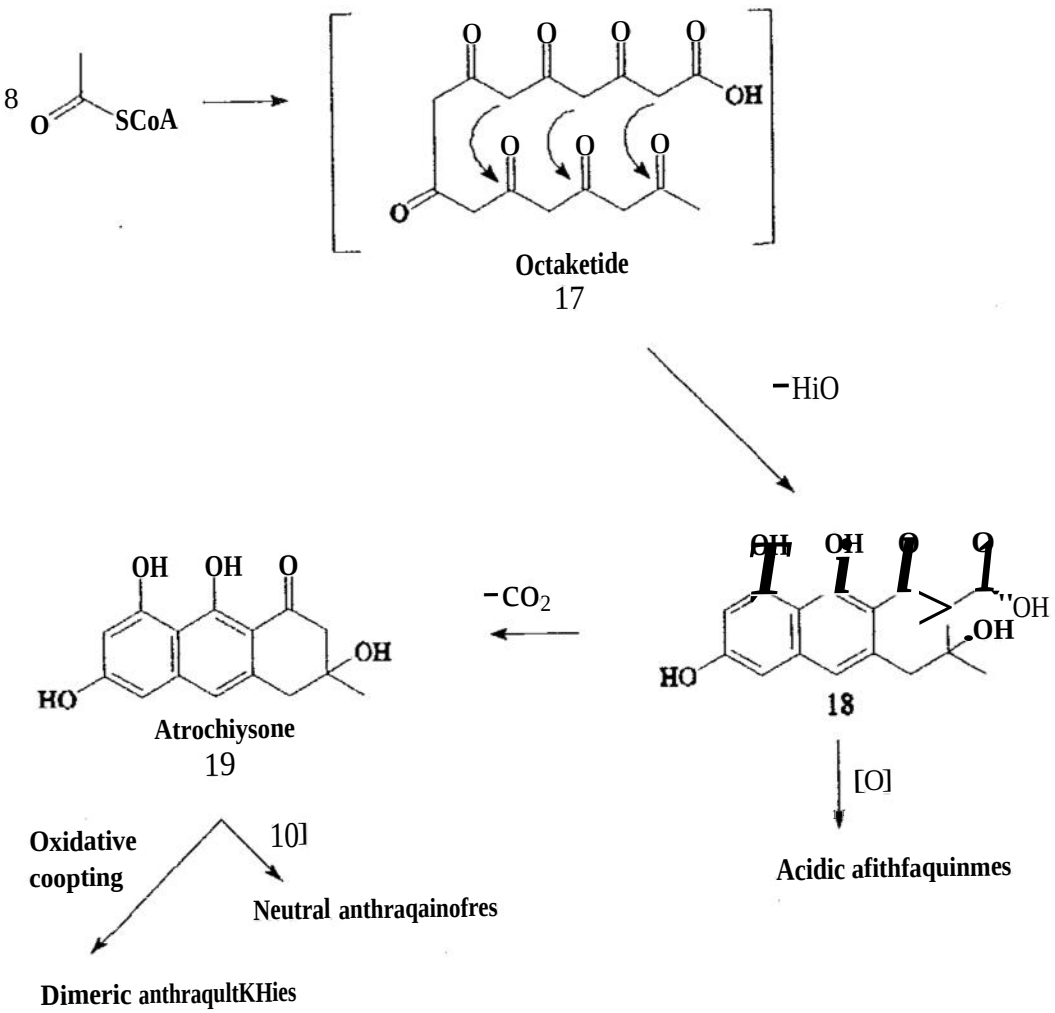
A wide array of approaches have been made towards the synthesis of anthraquinones. These range from simple functional group transformations of naturally occurring compounds to elegant total syntheses from readily available starting materials. It is out of scope of this work to go into details on various syntheses but it will suffice to say that a wide range of such synthetic approaches, including the relevant references, have been covered by Thomson [9, 13].

1.3.5 Biosynthesis.

Biosynthetically, anthraquinones arise by at least two pathways: The acetate-malonate and the shikimate-mevalonate pathways. These two are schematically represented in Schemes 2 [26] and 3 [27].

Acetate-malonate pathway leads to quinones substituted in both rings A and C. On the other hand, shikimate-mevalonate pathway gives rise to quinones substituted in only ring C. We will see later, section 1.5, that all kniphofia quinones are substituted in both rings and hence are most likely derived through the acetate-malonate pathway.

Scheme 2. Acetate-malonate pathway [26].



Dimeric anthraquinones undergo reductive cleavage [10] to yield the corresponding monomers. The reduction is commonly done using alkaline sodium dithionite. Other methods have also been used including the use of formic acid [31].

In addition, anthraquinones undergo aromatic ring reactions like halogenation, an old example [32] being in the bromination of some anthraquinone acetates using N-bromosuccinimide.

1.4.1 Phenol coupling reactions

Phenolic anthraquinones undergo phenol coupling reactions under the appropriate conditions. Pioneer work on coupling was carried out by Pummerer [33] early this century. He managed to synthesize what is referred as the Pummerer's ketone through intramolecular coupling. Extensive reviews have been published including those of Scott [34] and Musso [35].

These are radical reactions and hence side products are common. The more stable the radicals leading to a given product, the more the yields of that product. The coupling can either be C-O or C-C. Both types have been observed [34, 35] to occur depending on the conditions and the nature of the phenolic compounds.

The reactions are best carried out using one electron carrier systems such as ferric chloride and potassium ferricyanide. Various other reagents have been used [34, 35] to effect such reactions. These include ammonium molybdate, organic peroxides, peroxidase + hydrogen peroxide, barium hydroxide, MnO_2/silica gel, AgiCCh on celite, Elbs persulphate, Fenton's reagent (H_2O_2) and Fremy's salt (potassium nitrosodisulphonate). Unfortunately, there are no standard reagents and conditions.

1.5 Kniphofia quinones

Liliaceae is a large and heterogenous family. As a result, it has recently been divided into smaller and more homogenous families [36]. These are: Asparagaceae, Alliaceae, Asphodelaceae, Dracaenaceae, Eriospermaceae and Hyacinthaceae. Asphodelaceae comprise of two sub-families; the Asphodeloideae and Alooideae. The Alooideae comprise of the genera *Aloe*, *Gasteria*, *Haworthia*, *Lomatophyllum* and *Poellinitzia*. The Asphodeloideae comprises of the genera *Asphodeline*, *Asphodelus*, *Bulbine*, *Bulbininella*,

of this study to come up with the best means of transforming the anthrone into the anthraquinone. In addition, it was the objective of this study to try and synthesize derivatives of the anthrone (33) in preparation for x-ray structural studies of this compound in order to correctly assign the spatial arrangement. This would greatly help structural assignments of similar compounds. This would also be interesting since very few bromides of anthraquinones have been investigated.

In the course of studies on the pigments of *K. foliosa*, it has been observed that there are some minor pigments which could be dimers of the anthraquinones so far isolated from the plant. One of these occurs in tangible amounts. The purpose of this study is to isolate and identify this pigment which has been given the code, KKA-1 (knipholone-knipholone anthrone). It is an objective in this study to do synthetic studies on this dimer from the corresponding monomers through coupling reactions. A second pigment, of less polarity than KKA-1, code named KKA-2, was also to be isolated and characterised. However, its low amount in the plant as well as problems in getting it in pure form thwarted our efforts.

2.1.2 Phenol coupling reactions

Coupling of chrysophanol (5) and 37 would constitute a biogenetic type synthesis of knipholone. Due to the little amount available, it was thought necessary to first try coupling of closely related model compounds. All the compounds tried including emodin anthrone, torosachrysone and aloesaponol I were natural products and hence only limited amounts were available. Therefore, not enough tests were carried out in order to arrive at a definite conclusion.

Action of potassium ferricyanide on aloesaponol I gave only the oxidized product, aloesaponarin I, in excellent yields but there was no evidence of the coupled products. Emodin anthrone gave the corresponding anthraquinone upon treatment with basic 7 potassium ferricyanide with minute quantities of side products, which could be the coupled products, and which were not investigated further due to the low amounts. Treatment with iron chloride did not give any reliable data.

Limited attempts on the coupling of 5 and 37 using both $FeCl_3$ and K_3FeCN_6, both of which had earlier been used successfully to effect coupling (see section 1.4.1) did not give any coupled products. This could in itself mean that coupling occurred earlier in the biosynthetic pathway, possibly during the pre-anthraquinone stage [38]. This possibility was the main reason which led us to choose aloesaponol I and torosachrysone as model compounds since they are pre-anthraquinones.

2.1.3 Oxidation of knipholone anthrone (33) to knipholone (32).

Anthrones show two modes of oxidation: addition of an oxygen atom or, and often concomitantly, oxidative coupling [29]. Synthesis of 32 from 33 would therefore involve the first type. We set to maximize the yields of 32 at the expense of the coupling products. The coupling mode of oxidation is discussed in the synthesis of 40 (see section 2.2.3)

Several oxidation systems were tried including conventional oxidizing reagents, like Jones reagent and others based on Cr^{6+}, but all gave mixtures which were difficult to separate. Aerial oxidation in several solvents including acetone, methanol, DMSO and acetonitrile gave 32. In addition there were several side products which are most likely to be the coupled products. Indeed, two of them corresponded to KKA-1 and KKA-2 (see

section 2.2.3). However, it was difficult to reproduce the results in terms of rate and product distribution.

Solid supported reagents have been used in the past to effect oxidation [55]. An adaptation of the procedure employed by Keinan *et al.* [55] using silica gel supported iron chloride was employed resulting in efficient oxidation of **33** to **32**. It was observed that the reaction proceeds cleanly only if the reagent is kept dry (it is sensitive to both light and moisture (see section 3.5)). The process is highly reproducible.

2.2 KKA-1 (40)

The rhizomes of *K. foliosa* have been known to contain some small quantities of un-identified pigments. These are most likely to be the coupled products of the anthraquinones so far isolated from the plant. Some of these compounds are probably artifacts formed during the isolation process.

2.2.1 Isolation of KKA-1 (40)

There is an anthraquinone (hereby code-named KKA-1) which occurs in tangible amount. This was isolated according to the procedure outlined in section 3.8. To eliminate the possibility of KKA-1 being an artifact, careful experiments were done several times by extraction of fresh material within a short period of time. This was done to minimize any transformations which may occur during the extraction process. The profiles of the components of the crude extracts were then compared with a standard sample on both normal and oxalic acid impregnated TLC plates. The results showed that the compound is really a natural compound and not an artifact. The data available showed that KKA-1 has structure **40**.

2.2.2 Structure determination of KKA 1 (40)

The proton NMR spectrum of KKA-1 (**40**) (Figs. 2 and 3 and Table 3) shows a general proton duplicity. The assignment was greatly aided by comparison with the spectra of closely related anthranoids (Table 6). There are six singlets downfield at δ 11.91, 12.25, 12.46, 12.64 (1-, 1'-, 8-, 8'-OH) and 14.09, 14.41 (2''-, 2'''-OH). These shifts are

Fig. 2. 400 MHz ^{1}H NMR spectrum of KKA-1 (**40**).

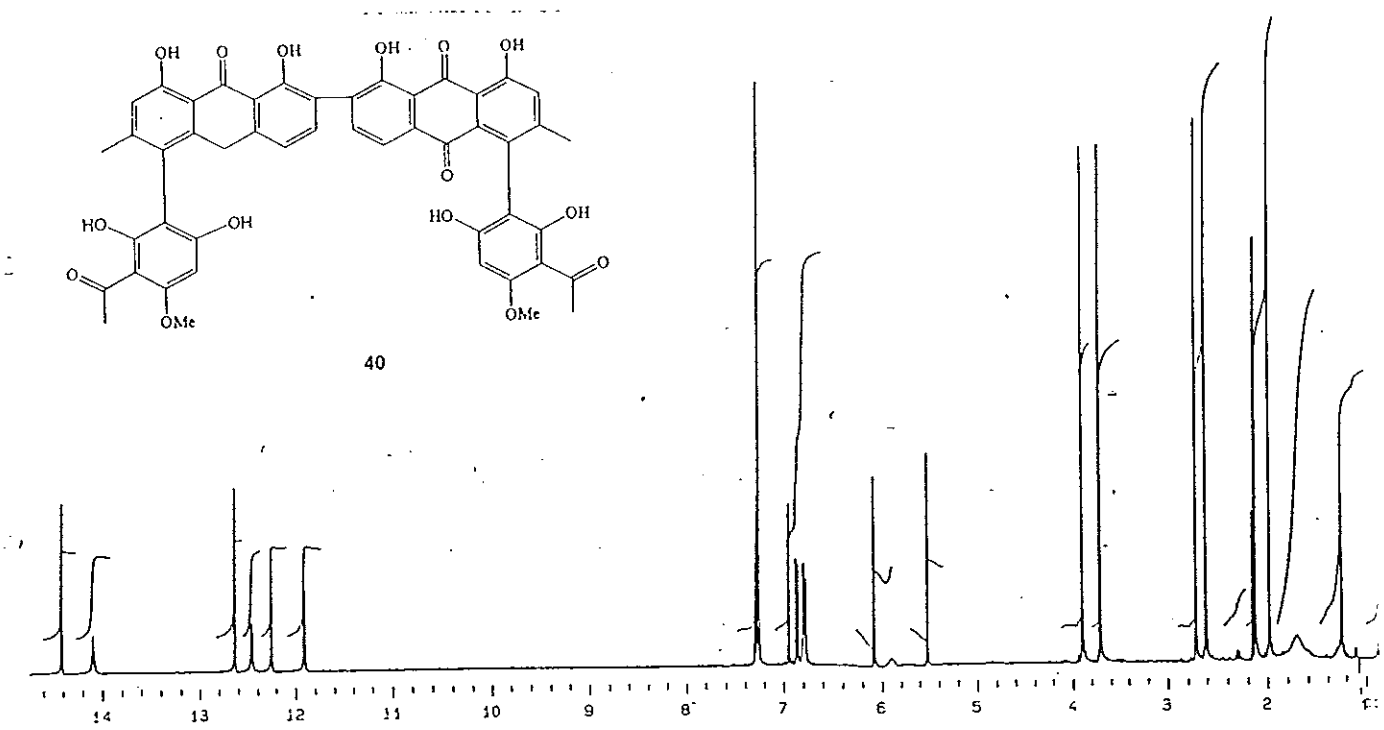
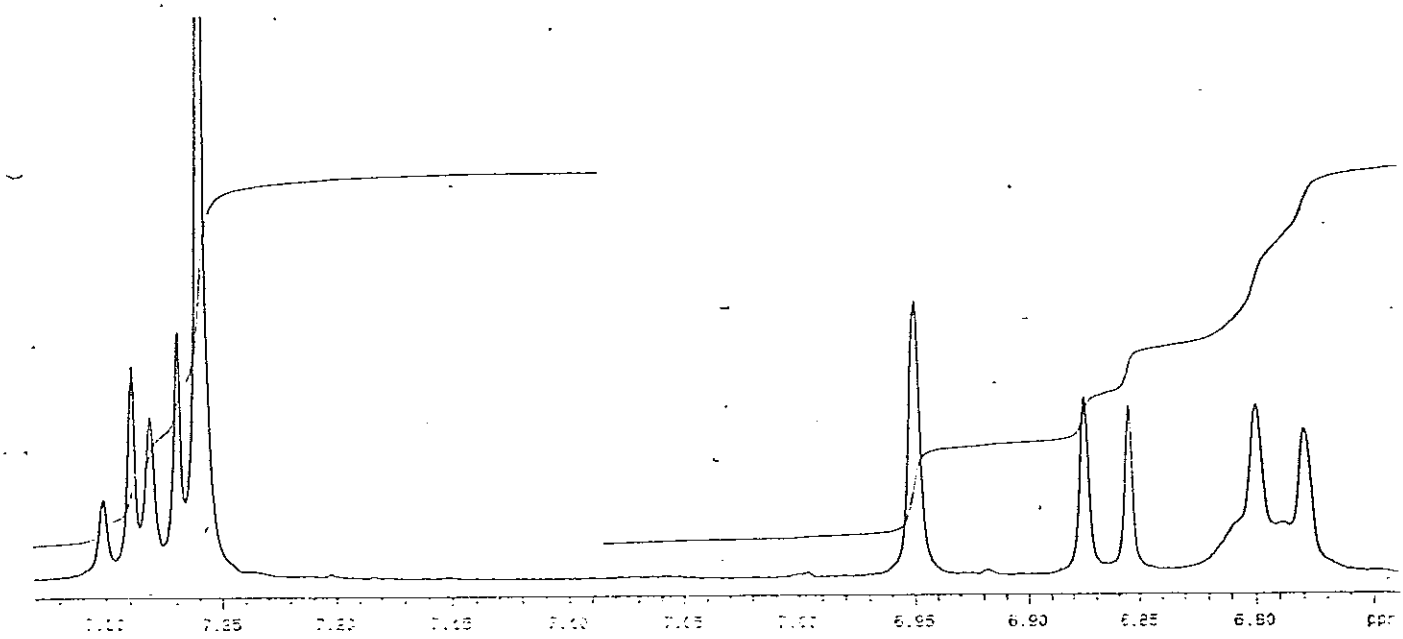


Fig. 3. 400 MHz ^{1}H NMR spectrum of KKA-1 (**40**) (blow-up).



carbons can be estimated from the $[M + 1]^+$ peak at 853.2. The % abundance relative to the $[M^+]$ peak is (0.45/0.87) x 100 = 51.724. The number of carbons are therefore approximated at 51.724/1.1 = 47. Synthesis (see section 2.2.3) and NMR data indicate that the compound contains 48 carbons which is quite close. This corresponds to a knipholone-knipholone anthrone dimer (**32-33**). A knipholone-knipholone dimer (**32-32**, $C_{48}H_{34}O_{16}$) would give a molecular ion peak at m/z 867.2 while the corresponding bi-anthrone (**33-33**, $C_{48}H_{38}O_{14}$) would give a molecular ion peak at m/z 838.2. This was further supported by the general mass spectrum. There are peaks at m/z 835 and 834 corresponding to loss of -OH and H_2O, respectively. Peaks at m/z 421 and 434 correspond to knipholone and knipholone anthrone moieties resulting from symmetrical cleavage.

The ion at m/z 434 corresponding to the knipholone moiety loses a methyl radical, as is common with methyl ketones, to yield a peak at m/z 419. A peak at m/z 239 can be attributed to the loss of an acyl phloroglucinol unit from the knipholone anthrone ion (a diminished peak at m/z 252 can be attributed to a similar loss from the knipholone unit ion). It was expected that there will be a peak at m/z 182 for the acyl phloroglucinol unit. This was absent but instead, there was a prominent peak at m/z 167 attributed to the loss of a methyl radical (compare with the spectrum of **37** in section 2.1.1) from the ion at m/z 182.

The UV spectrum of **40** shows absorption maxima at λ_{max} (MeOH) 202.1, 292.5, 363.4 and 437.4 nm, the last three peaks suggesting that the compound is a polyhydroxylated anthraquinone [56]. A mixture of **32** and **33** gives peaks at λ_{max} 292.6, 259.3, 354.5, 223.1, 288.4 and 448.9 which closely resemble those of compound **40**. This compares well with the UV spectrum of **32** and **33**. This indicates that the compound is polyhydroxylated.

The assignment of the signals in the ^{13}C NMR spectrum (Table 5, Fig. 5) was aided by comparison with the shifts of closely related anthranoids (Table 4). The spectrum shows the presence of 5 carbonyl carbons at δ 193.9, 192.6 (C9, C-9'), 182.5 (C-10) and 203.4, 203. 5 (3''- $\underline{C}OCH_3$, 3'''- $\underline{C}OCH_3$). There is a triplet of multiplets at δ 29.7 (C-10') (Fig.7). This strongly supports the suggestion that **40** is composed of the moieties of **32** and **33**. The question that now remains is the position of the coupling.

Fig. 4. Mass spectrum of KKA-1 (40).

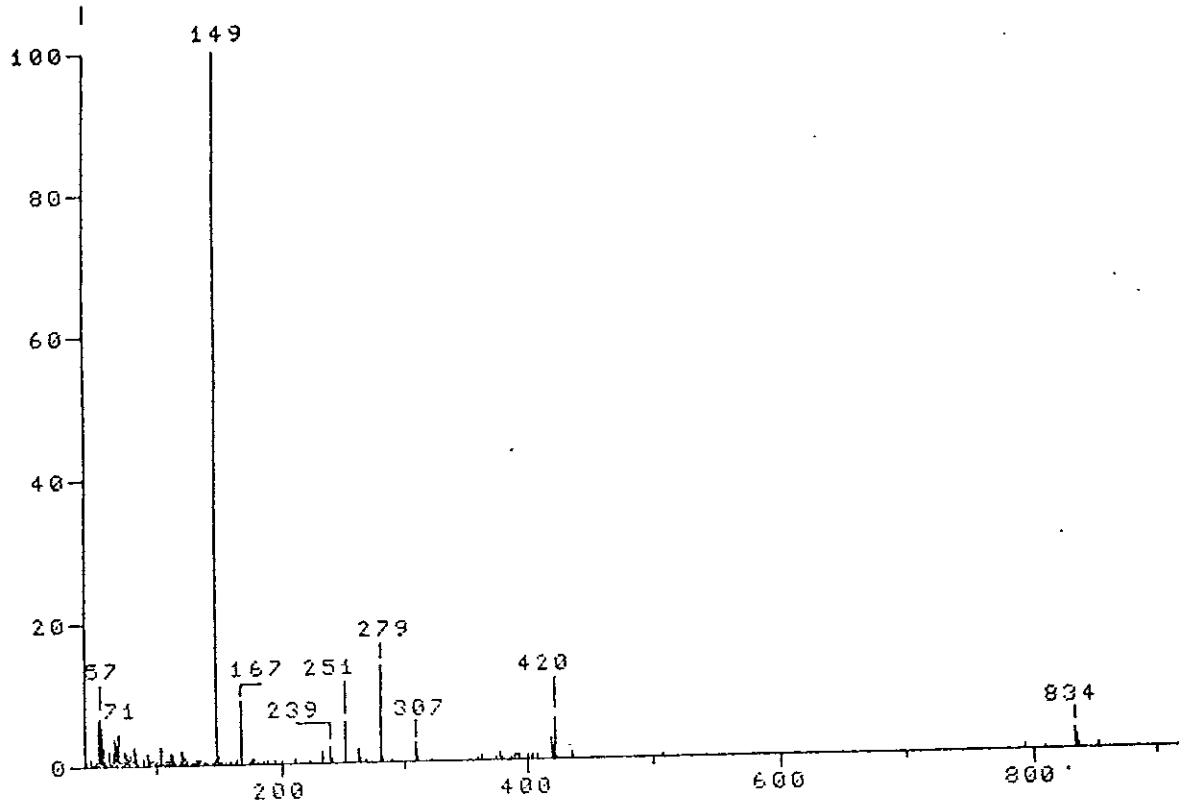


Fig. 5. 100 MHz ^{13}C NMR spectrum of KKA-1 (40).

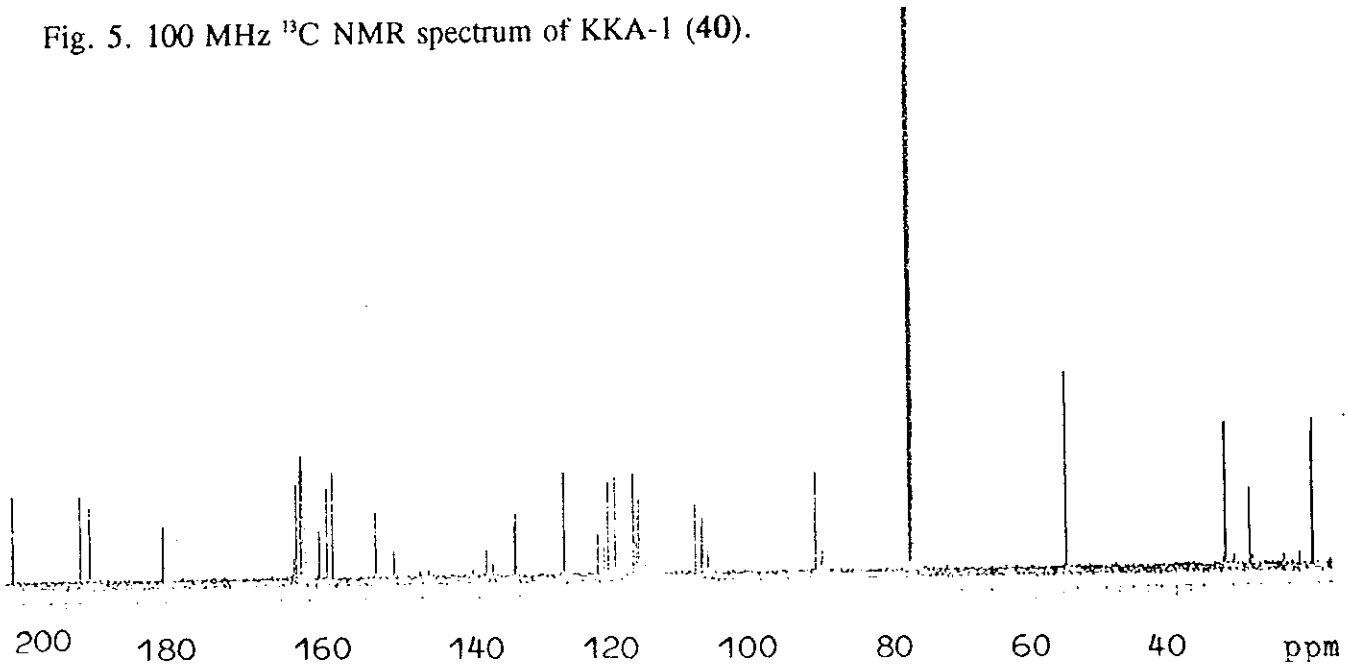


Table 4. ^{13}C NMR data for compounds **5**, **32** and **33**

C	5	32	33	C	5	32	33
1	162.4	161.7	163-165.8	9	192.5	192.5	195.6
1a	113.7	114.7	116.5	10	181.9	181.9	32.2
2	124.5	124.6	123	1'		104.7	107.5
3	149.3	151.6	150.8	2'		163.3	163-165.8
4	121.8	128.5	123	3'		107.3	107.0
4a	133.2	131.6	144.2	4'		162.4	163-165.8
5	119.9	119.3	116.6	5'		91.2	92.5
5a	133.6	134.4	143.6	6'		161.9	163-165.8
6	136.9	137.4	137.4	Ar$\underline{C}H_3$	22.3	20.4	21.3
7	124.3	123.3	117.6	O$\underline{C}H_3$		55.6	56.6
8	162.7	161.1	163-165.8	CO$\underline{C}H_3$		32.6	33.2
8a	115.8	115.5	115.5	$\underline{C}OCH_3$		202.3	204.2

There are three possible positions of coupling assuming that **40** is a dimer of **32** and **33**: positions 2, 5 and 7 (*ortho, para* to the -OH groups) of either monomer. It is also possible to have coupling on the 5'' and 5''' positions of the acyl phloroglucinol units. The various combinations should result in a wide range of products, of which **40** is one of them. The ratios would be dependent on several factors like steric hinderance and reactivities of the various positions during the coupling process. The aromatic proton signals at δ 5.52, 6.09 (H5'', H5''') in the ^{1}H-NMR spectrum confirs that the phloroglucinol units are intact, and hence coupling could not have occurred at the 5'' and 5''' positions.

The ^{1}H NMR spectrum of **40** (Fig. 3) shows two singlets, at δ 6.95 and 7.26 (H2, H2') which indicate that coupling could not have occurred at these positions. The two singlets give cross peaks with the aromatic methyl signals at δ 1.99 and 2.13 in the NOE (NOESY) spectrum (Fig. 8). Further confirmation of the presence of the two protons (H2, H2') is obtained from the ^{13}C NMR spectrum (Fig. 7) which gives two doublets of quartets at δ 21.0 and 20.8 (3-$\underline{C}H_3$, 3'-$\underline{C}H_3$) due to coupling with the three directly attached protons as well as long range coupling with H2 and H2'. The signal would have been a quartet if

Fig. 6. DEPT spectrum of KKA-1 (40).

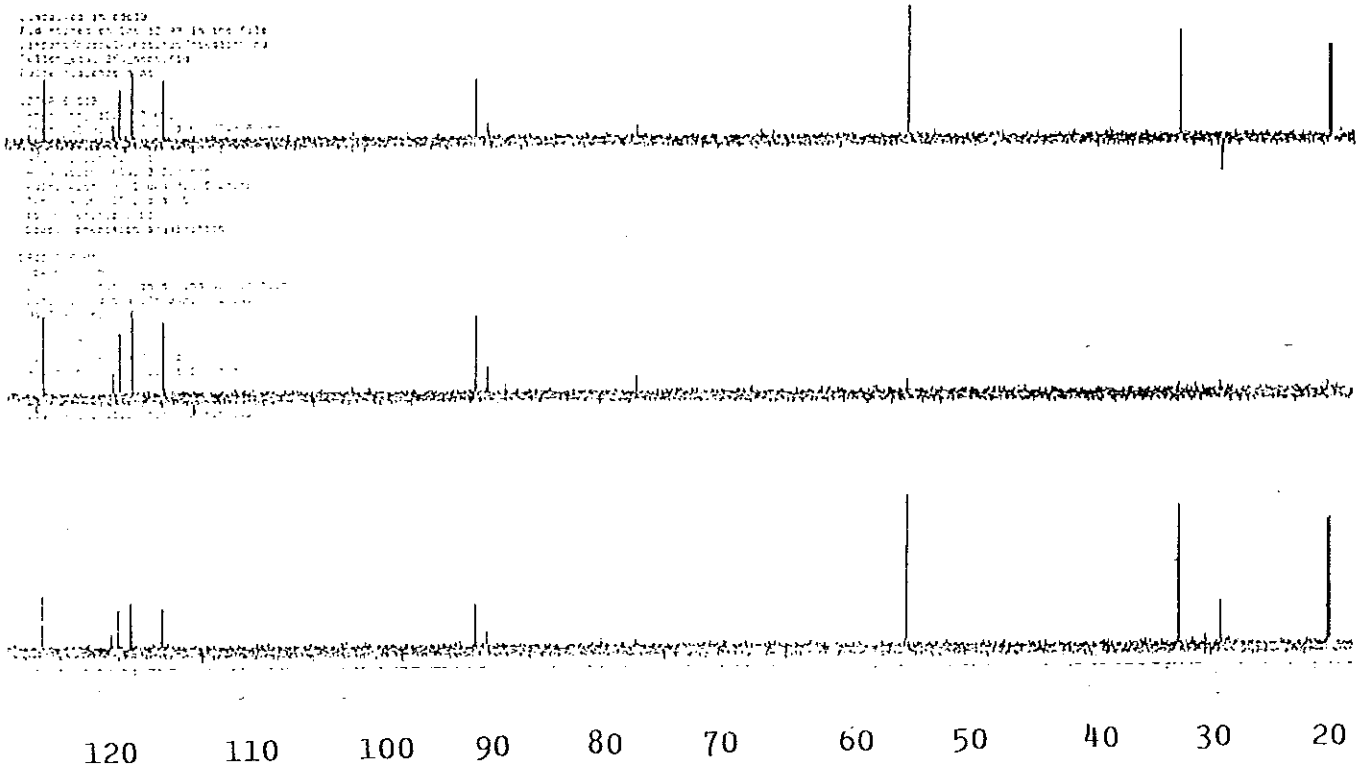


Fig. 7. Proton coupled ^{13}C NMR spectrum of KKA-1 (40).

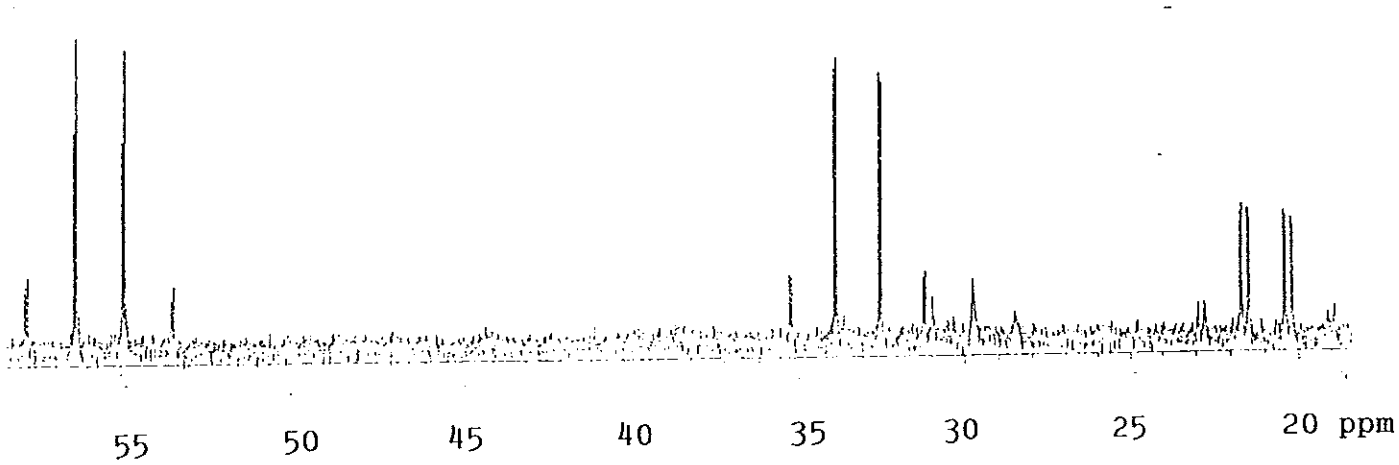


Fig. 8. NOESY spectrum of KKA-1 (40).

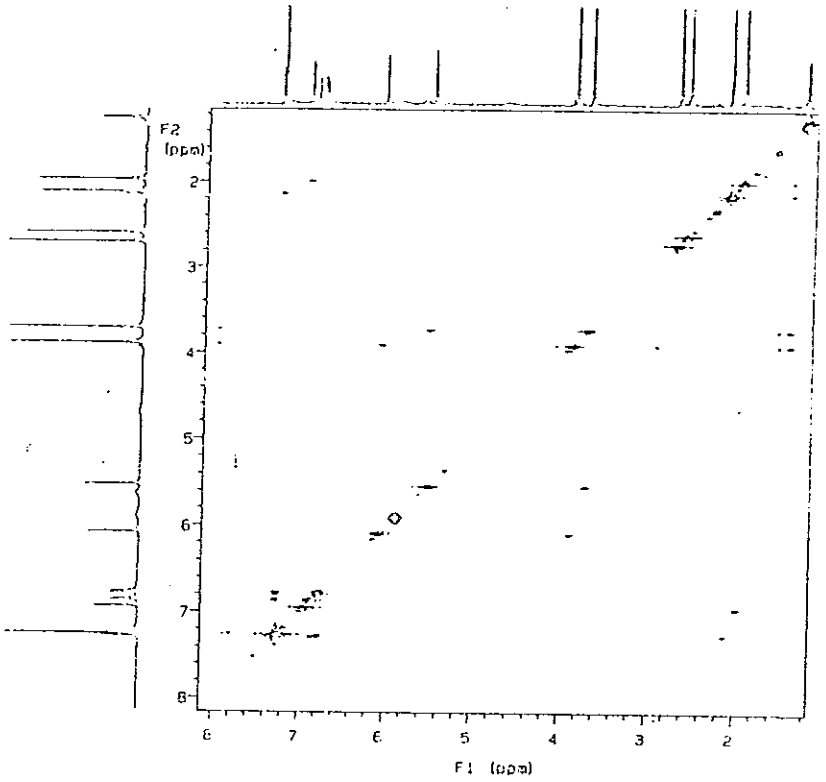
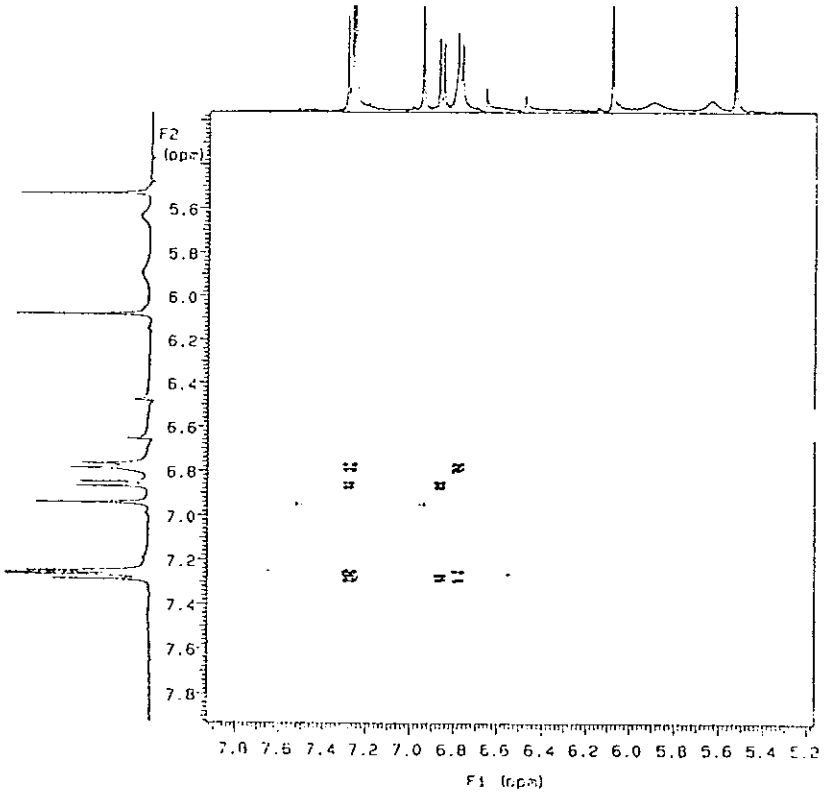


Fig. 9. dqCOSY spectrum of KKA-1 (40).



triplet due to coupling with the two directly attached protons, but a triplet of multiplets due to long range coupling with the peri-proton on C-5'. These strongly suggests that the anthrone moiety couples through C-7'. Indeed, the week multiplets at δ 145.0 and 146.5 can be attributed to C-7 and C-7'.

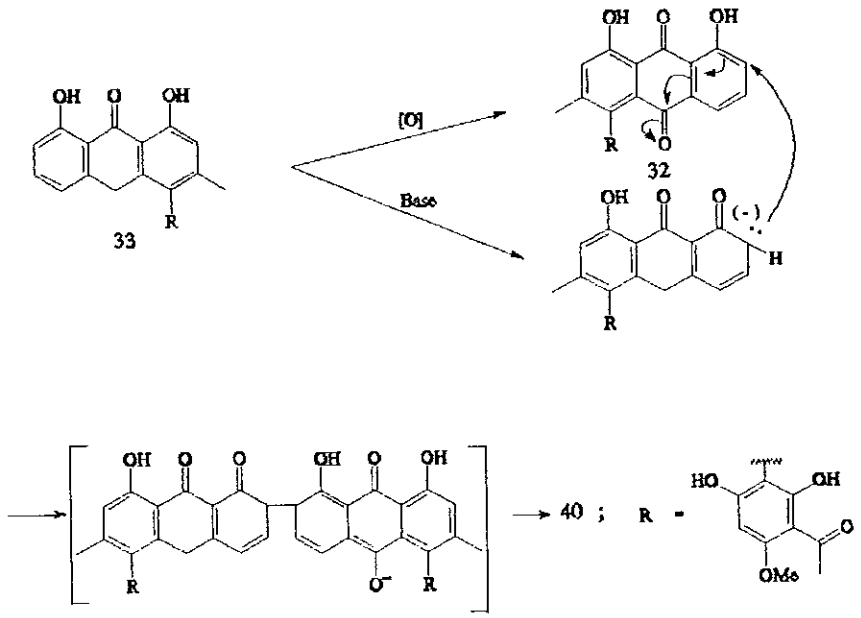
In the ^{1}H NMR spectrum (Fig. 3 and Table 6), there are four doublets centred at δ 7.28, 7.27, 6.865, 6.79 (H5, H5', H6, H6'). The coupling constant value (^3J = 8) is typical of vicinal coupling. The homonuclear COSY (dq COSY) spectrum (Fig. 9) shows cross peaks between the doublet at δ 7.28 and 6.87 while the doublet at δ 7.27 gives a cross peak with the doublet at δ 6.79. Each of the pairs showing cross peaks represents a pair of *ortho* coupled protons (H5, H6 and H5', H6'). To assign which is which, we have to first locate the position of the methylene (10'-H_2) protons signal(s), which is not clear from the proton NMR spectrum, for there is bound to be long range coupling between them and H5'. There is therefore need for heteronuclear correlation experiments.

2.2.3 Synthesis of KKA-1 (40)

The coupling mode of anthrone oxidation (see section 2.1.3) leads to coupled products. The experiments in this section were geared towards favouring this mode. Aerial oxidation has the advantage of affording products which are easier to purify. Aerial oxidation of knipholone anthrone (**33**) was attempted in various solvents including acetonitrile, acetone, methanol, dimethylformamide (DMF) and dimethylsulfoxide (DMSO). In all cases, three main products were formed: knipholone (**32**), KKA-1 (**40**), and a minor product code named KKA-2. In addition, there were other products in various amounts which could be the extensively coupled products. This was especially so in DMSO. Acetonitrile with catalytic amount of silica gel affords the cleanest products and these were separated through the usual chromatographic methods. However, such a method was found to be unsatisfactorily slow upon scale up. A quick method was found through the use of acetone and a catalytic amount of base (NaOH). In such a case, the mechanism would involve proton abstraction, nucleophilic attack on an aromatic carbon and re-aromatization [57] (Scheme 6). However, the process was not very reproducible in terms of products distribution and there were difficulties in purification of the products.

In an effort to come up with reproducible results, aerial oxidation of knipholone anthrone (33) was re-investigated, and found out that coupling accompanied by oxidation went rapidly and in a reproducible manner at room temperature in acetone containing silica gel. This gave 32, 40 and small amounts of KKA-2. These were separated on a chromatotron (care being taken to avoid any silica gel residue in the solutions containing KKA-1 as it decomposed rapidly).

In the synthesis of KKA-1, there consistently occurred a minor second compound which was code-named KKA-2. The NMR data on this compound is very close to that of KKA-1. The major difference is in that the proton NMR spectrum of KKA-2 lacks a signal for the acyl protons of the acyl phloroglucinol unit. The structure elucidation of this compound is still in progress.



Scheme 6. Michael addition-type mechanism for the synthesis of KKA-1 (40).

2.2.4 Reductive cleavage of KKA-1 (40)

KKA-1 (40) was efficiently cleaved by use of basic sodium dithionite to yield chrysophanol (5), 4,6- dihydroxy-2-methoxyacetophenone (37) as well as the demethylated product, acetylphloroglucinol (36), just as is the case with the reductive cleavage of knipholone (32, see section 2.1). This is in further support of the close relationship of KKA-1 to knipholone as well as its dimeric nature.

2.3 Bromination of knipholone anthrone (33)

Bromination of **33** was done using bromine in acetic acid as well as with N-bromosuccinimide to yield various bromides.

2.3.1 Bromination using bromine in acetic acid

Bromination of **33** using the procedure of Kitagawa *et al.* [58] gave two principal products, **41** and **42**. The fact that the compounds were indeed bromides was confirmed by the classical flame test experiment of burning a small sample on the tip of a previously red-hot heated copper wire over a flame of a bunsen burner. A greenish flame indicates a bromide (assuming there were no chlorides, which also give positive tests).

The structure of the compounds were elucidated using 90 MHz ^{1}H NMR spectra and by comparing these with the spectra of **32**, **33** and other closely related compounds. A search in the chemical literature revealed that similar compounds were not reported before.

The spectrum of **41** (Table 6, Fig. 10) shows presence of two *ortho* coupled protons. There are two doublets at δ 6.75 and 7.6 (^3J = 9 Hz) which can be assigned to either protons H5 and H6 or H6 and H7. The coupling constant is correct for *ortho* coupled protons. The other alternative of *meta* coupled protons would give a coupling constant of 2-3 Hz. This together with the lack of a triplet in the aromatic region strongly suggests that ring A is mono-substituted.

Comparison of the spectra of **41** and **33** (Table 6) indicates that the compound is di-brominated. This is so because of the lack of a singlet for H2 as expected in the aromatic region and the presence of a singlet at δ 6.3 attributed to the H5'. This indicates that the acyl phloroglucinol unit is not brominated (this is peculiar in that we expected bromination to be faster on the acyl phloroglucinol unit). In addition, there are signals at δ 2.30 (3H, *s*, ArC$\underline{H}_3$) and 2.70 (3H, *s*, ArCOC$\underline{H}_3$) indicating there was no side chain bromination. The singlet at δ 4.0 is for the methoxy protons.

Downfield, there are three singlets for chelated hydroxyl proton signals at δ 13.7 (1H, *s*, 2'-OH), 13.2 (1H, *s*, 1-OH), and 12.20 (1H, *s*, 8-OH). The 6'-OH proton signal does not appear in the spectrum possibly due to its acidic nature and hence experiencing rapid proton exchange. 2'-OH proton resonates farther downfield than the others due to a

Fig. 10. 90 MHz ^{1}H NMR spectrum of **41**.

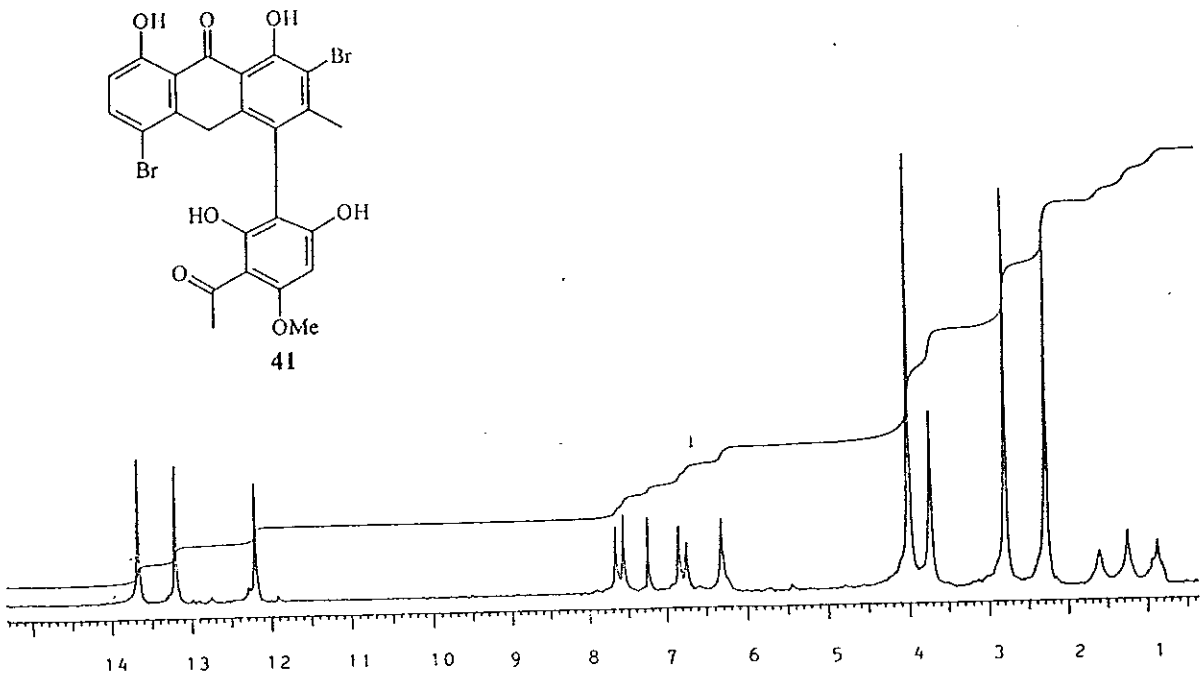
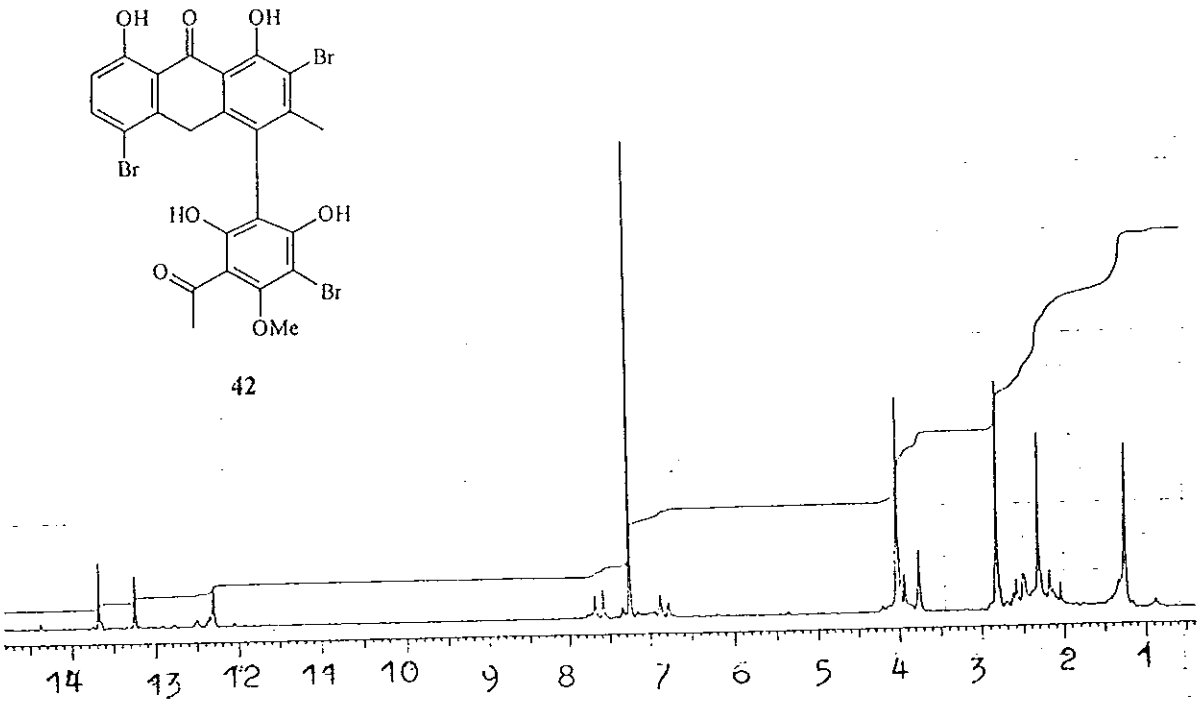
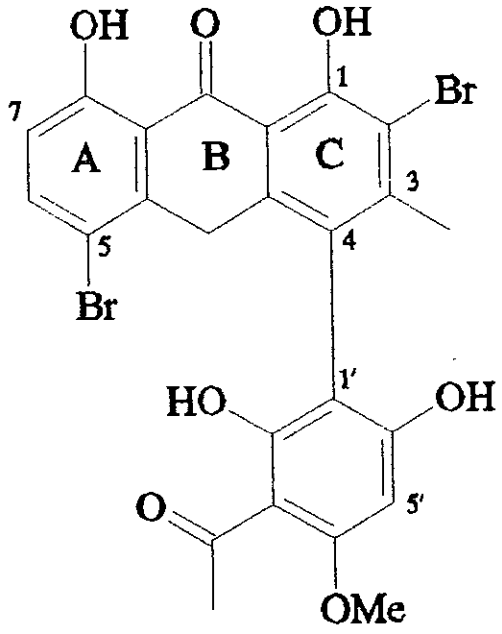


Fig. 11. 90 MHz ^{1}H NMR spectrum of **42**.





41

stronger chelation and hence high deshielding. In the spectrum of 33 (Table 6), the 1-OH and 8-OH protons resonate close to each other at δ 12.25 and 12.35. Hence there is a considerable deshielding of one of this signals in the bromide. This can only mean that the two hydroxyl (1-OH and 8-OH) protons have been affected differently by bromination. Since we have already established that there is bromination on ring C (due to lack of signal for the H2 proton), it is reasonable to expect the same substituent effects on 1-OH and 8-OH proton resonances if the bromide on ring A was on C-7.

Since this is not the case, we conclude that the bromide must be on C-5 (it can not be on C-6 since then we should expect two *ortho* coupled protons. In any case it is difficult to brominate *meta* to the hydroxyl groups).

Ballantine *et al.* [20] concluded that bromination has little effect on the ring protons except a 0.1 ppm deshielding of the *ortho* protons. Therefore, we can assign the doublet at δ 7.6 to H6 and that at δ 6.75 to H7 simply by comparing with the corresponding shifts in the spectrum of 33. We can therefore conclude that the bromide substituent has a marked de-shielding effect on *ortho* hydroxyl protons in 1,8-hyroxylated anthraquinones. This is possibly due to chelation during that short period when the hydroxyl is not chelated to the keto oxygen. There is little effect of a *para* bromide on the hydroxyl substituent.

Table 6. 90 MHz ^{1}H NMR chemical shift data for compounds **32, 33, 41, 42, 43** and **45.**

	Shifts						
Proton	**5**	**32**	**33**	**41**	**42**	**43**	**45**
1-OH	12.10	12.53	12.35	13.2 *s*	13.25 *s*	12.25 *s*	12.15 *s*
2	7.1	7.32	6.88	-	-	6.9 *s*	7.00 *s*
5	7.75	7.75	6.40	-	-	7.5 *m*	-
6	7.65	7.56	7.49	7.6 *d*	7.65 *d*	7.5 *m*	7.65 *s*
7	7.30	7.30	6.67	6.75 *d*	6.8 *d*	7.0 *m*	-
8-OH	12.00	12.00	12.25	12.2 *s*	12.3 *s*	11.65 *s*	11.65 *s*
2'-OH	-	14.22	14.32	13.7 *s*	13.7 *s*	15.0 *s*	14.35 *s*
5'	-	6.24	6.30	6.3 *s*	-	6.35 *s*	-
6'-OH	-	8.95	-	-	-	-	-
Ar$\underline{CH_3}$	2.45	2.13	2.12	2.3 *s*	2.25 s	2.45 *s*	2.45 *s*
O$\underline{CH_3}$	-	3.98	4.00	4.0 *s*	4.0 *s*	3.9 *s*	4.05 *s*
CO$\underline{CH_3}$	-	2.62	2.63	2.7 *s*	2.7 *s*	2.7 *s*	2.85 *s*
10'	-	-	4.07	3.6 *brs*	3.75 *s*	5.5 *s*	5.65 *s*

In contrast, shielding field effects of the *peri*-bromide are experienced by $H_2$10, resulting in a slight upfield shift from that in **33**. Interestingly, there is a considerable shielding of the hydroxyl protons of the acyl phloroglucinol unit up from δ 14.32 (in **33**) to 13.7. It therefore seems reasonable to conclude that the phloroglucinol unit is arranged in such a way that the chelated hydroxyl is close to the 5-Br and experiences shielding field effects.

The IR spectrum of **41** shows the general profile as expected for a polyhydroxylated anthraquinone. It shows a medium rather broad peak at 3461 cm^{-1} for the hydroxyl groups. The methyl peaks occur at 2925 and 2860 cm^{-1}. There is a strong peak for the carbonyl absorptions at 1594 cm^{-1} franked by shoulders on both sides. However, it is not possible to differentiate the quinonoid carbonyl from that of the acyl phloroglucinol unit as they vibrate at almost the same frequency. The region between 1600 and 1450 cm^{-1}, though

2.3.2 Bromination using N-bromosuccinimide: The Wohl-Zeigler reaction

Bromination using N-bromosuccinimide [59], mostly used to effect allylic bromination, was also done leading to two compounds in approximately equal amounts. Their 90 MHz ^{1}H NMR spectra suggest structures **43** and **45**. The spectrum of **43** (Table 6, Fig. 13) shows a singlet at δ 5.5 which was attributed to H10. It is considerably deshield by the *geminal*- bromide. To eliminate any doubt that this was really H10 and not a hydroxyl proton signal, the spectrum was regenerated from a sample containing D_2O. The signal did not disappear as expected for rapidly exchanging acidic protons.

The appearance of a signal at δ 6.35 (1H, *s*, H5') indicated there was no bromination on the phloroglucinol unit. Again there was little shifting of the chelated hydroxyl of the anthrone unit. The data generated was not enough to distinguish which of the two hydroxyl, 1-OH and 8-OH, corresponds to which of the two signals at δ 11.65 and 12.25 which indicates that one of these hydroxyl group protons has been shielded by allylic bromination at C-10 as compared to that in **33**. In the aromatic region there are two multiplets centred at δ 7.0 and 7.5 attributed to protons 5, 6 and 7. A singlet is clearly visible at δ 6.9 which is attributed to H2.

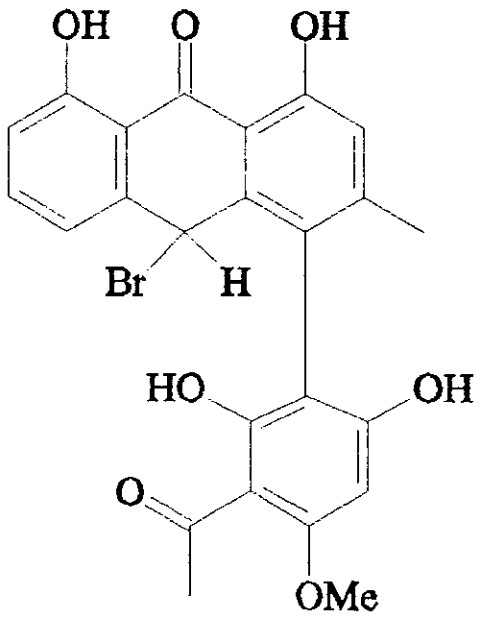


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Fig. 12. 90 MHz ^{1}H NMR spectrum of **43**.

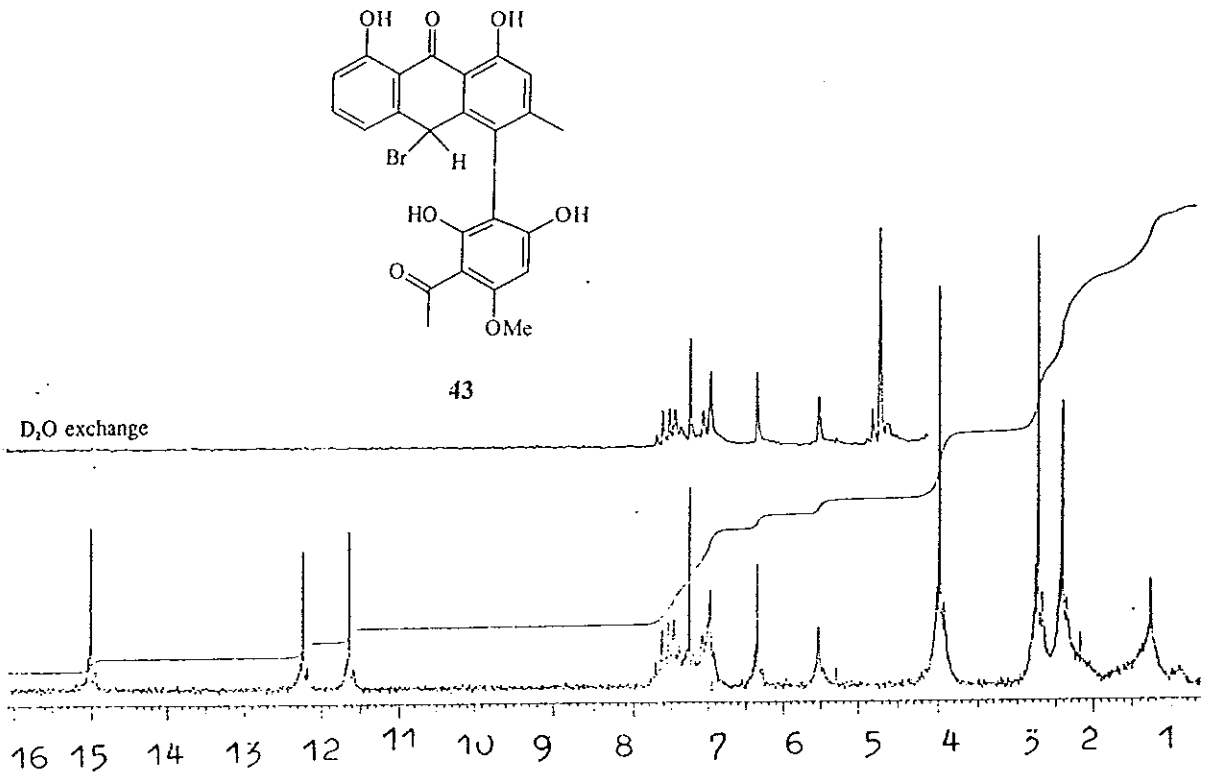
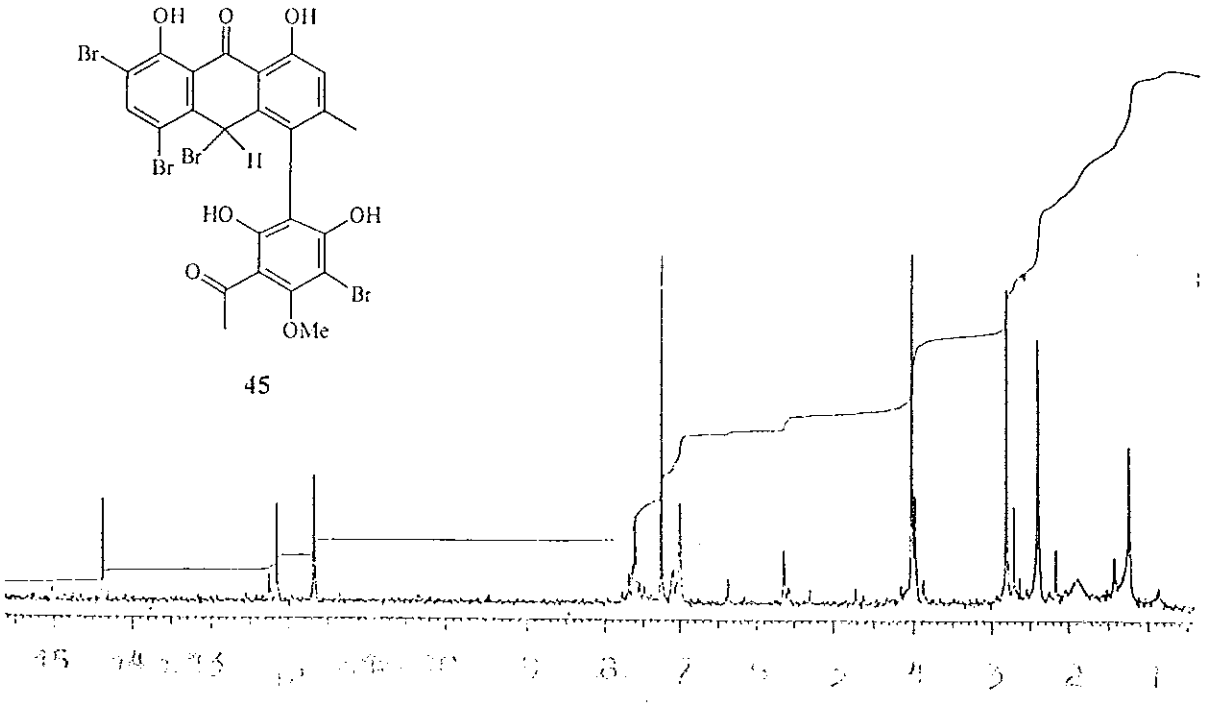
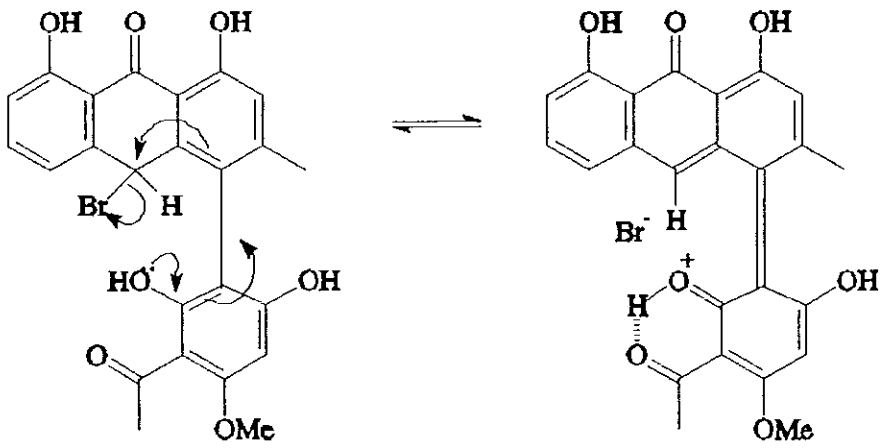


Fig. 13. 90 MHz ^{1}H NMR spectrum of **45**.



Scheme 7. Conjugation and resonance in bromide **43**.

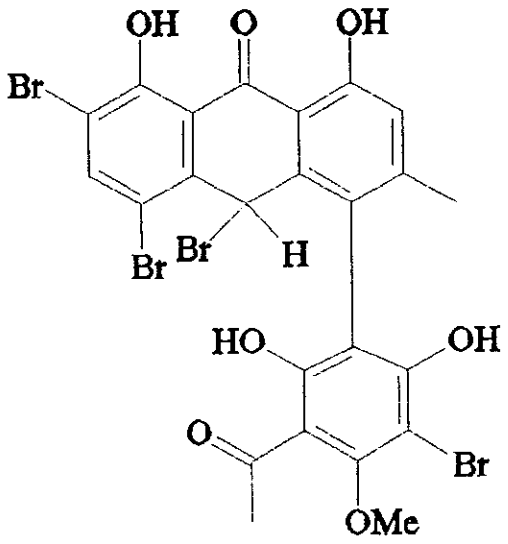


43 **44**

Interestingly, the phloroglucinol chelated -OH proton resonates far downfield at δ 15.0, a deshielding possibly explained by resonance and the fact that bromine is a good leaving group (Scheme 7). This creates a positive charge on the oxygen holding the hydrogen and hence the strong deshielding. Note is taken that such a system is not possible if the bromide was attached to an aromatic carbon. The rest of the spectrum resembles that of **33** (Table 6).

The spectrum of **45** (Table 6, Fig. 13) shows two singlets in the aromatic region which can be attributed to H2 (δ 7.0) and H6 (δ 7.65). This means there is dibromination on ring A which does not appreciably shift the *ortho* proton (H6) just as explained earlier. Placement of a bromide on C-5 closely agrees with our conclusion that bromide field effects cause shielding and hence the upfield shift of the chelated hydroxyl of the phloroglucinol unit to δ 14.35 as compared to that of **43** at δ 15.0.

However, the effect of the bromide on an *ortho* hydroxyl group, as discussed here above (see section 2.3.1), is not clear when we consider the spectrum of tetrabromide **45** (Table 6, Fig. 13). The *ortho* bromide on C-7 has little effect on the 8-OH contrary to our earlier conclusion and it is hard to explain this unless the other bromides on the molecule have unexplained counter balancing effects. There is therefore need for more study on these and other closely related systems before a definite conclusion can be drawn.



45

2.4 Conclusion and suggestions

This work mainly revolved around the chemistry of kniphofia quinones. Special attention has been given to the spectroscopic methods in the determination of structure owing to their relatively easy applicability and in conformity with the current development in these fields. The work started with the synthesis of the relatively simple acetyl phloroglucinol monomethyl ether unit of knipholone and its anthrone. No attempt was made to the synthesis of the chrysophanol unit since several papers have appeared in recent publications on various methods of its synthesis. As mentioned earlier, coupling of the two units would constitute a biogenetic type synthesis of knipholone. This is yet to be accomplished . It is possible that in nature, coupling occurs during the pre-anthraquinone stage and indeed several pre-anthraquinones have been isolated in nature. Model experiments with pre-anthraquinones, though not exhaustive due to availability of only small amounts of starting materials, failed to yield coupled products, instead giving only the corresponding anthraquinones.

Biotransformations are not new reactions since indeed they are used in the preparation of alcoholic drinks. Trial experiments on biotransformation of knipholone anthrone in several biological systems, though not discussed in this thesis, failed to yield any products other than the starting material, an outcome attributed to the poor solubility of the anthrone

in the biological media, which was aqueous. One way to overcome this problem would be to make more soluble derivatives like glycosides.

The bromides made were quite unstable and much more work is needed. There are very few anthraquinone bromides which have been isolated from natural sources and hence very little literature occurs on such systems. However, we hasten to say that the data so far obtained and discussed in section 2.3 as well as the reaction procedures employed strongly suggests the structures presented in that section. ^{13}C NMR and MS spectroscopic analysis would greatly help in confirming their structures.

It is apparent that *Kniphofia foliosa* is quite rich in anthraquinonoid pigments and there is a lot to be done before its phytochemistry is exhausted. There still remains unidentified minor pigments which most probably are dimeric and isomeric to the pigments so far isolated.

Despite the problems in the location of the H_2'10 in the ^1H NMR spectrum, all the other evidences point at **40** as the structure for KKA-1.

In contrast, more work needs to be done to establish the structure of the closely related KKA-2. KKA-2 shows close relationship with KKA-1 in regard to the ^1H NMR spectroscopy, the major difference being that it lacks the acyl proton signal expected in the neighbourhood of δ 4.0. This was difficult to explain owing to the expected difficulties in the loss of such a group.

3.2 *Methylation of 38*

The acetate (**38**) (149 mg, 0.753 mmol) was dissolved in $CHCl_3$ (3 ml). Methyl iodide (0.05 ml, 0.8 mmol) and Ag_2O (1.3 g, 5.6 mmol) were added and the mixture stirred at room temperature for 2 h. The resulting mixture was diluted with $CHCl_3$ (15 ml) and run through a short column packed with silica gel to remove the Ag_2O. The column was washed with $CHCl_3$ to give a clear solution which was concentrated *in vacuo* to give:

39, R_f = 0.9 (solvent system A). ^1H NMR ($CDCl_3$, 90 MHz): δ 2.3 (3H, *s*, OAc), 2.65 (3H, *s*, $COCH_3$), 3.85 (3H, *s*, OMe), 6.15, 6.35 (2H, *m*, arom.H), 13.55 (1H, *s*, OH).

3.3 *Acetylation of 37*

The reaction was done as in part (3.1) above, using 6.7 mg (0.0037 mmol) of **37**, 10 ml acetone, 1 drop of pyridine, and 4 drops of Ac_2O to yield the same product (**39**) as (3.2) above (4.7 mg, 61%).

3.4 *4,6-dihydroxy-2-methoxyacetophenone (37)*

39 (50 mg, 0.238 mmol) was dissolved in acetone (10 ml) and 2 ml 2N HCl added. The mixture was refluxed for 30 minutes. The resulting mixture was cooled and extracted with $CHCl_3$. The organic layer was washed with water (3 x 15 ml), dried over anh. Na_2SO_4 and concentrated *in vacuo* to give:

37, R_f = 0.44 (solvent system A), 0.04 g (93%). Mp 198-200° (lit. [50] 201-202°). **MS** $[M]^+$ found 182.0799, $C_9H_{10}O_4$ requires 182.05791. MS (see Fig. 1), ^1H NMR ($CDCl_3$, 90 MHz): δ 2.6 (3H, s, COMe), 3.85 (3H, s, OMe), 5.95 (2H, m, arom.), 13.95 (1H, s, OH).

3.5 *Preparation of silica gel supported iron chloride*

Silica gel (Woelm 30-63 μm, 10.65 g) was dried in an oven at 150° for 1.5 h. After cooling, it was mixed with 2.13 g of $FeCl_3.6H_2O$ (i.e 20% w/w) and the mixture suspended in 5% methanol in absolute ether. The solvent was removed under vacuum and the mixture dried in an oven at 80° for 4 h. The resulting orange powder, which was light

and moisture sensitive, was stored in a tightly closed bottle, lapped with an aluminium foil, in the desiccator.

3.6 *Oxidation of knipholone anthrone (33): formation of 32*

Knipholone anthrone (**33**) (50 mg, 0.12 mmol) was dissolved in 10 ml acetone. Silica gel supported $FeCl_3$ prepared above (section 3.5), (0.6 g), was added and the solvent removed under vacuum. The mixture was left to rotate for one hour on the rotary evaporator. The resulting mixture was extracted in ethyl acetate and concentrated to yield a crude material which was purified by column chromatography to yield:

Knipholone (**32**), R_f = 0.61 (solvent system D), 44.1 mg (85.6%). ($C_{24}H_{18}O_8$). Mp 224-224.5°. UV λ_{max} (MeOH) nm: 223.1, 254.5, 288.4, 428.9. ^{1}H NMR ($CDCl_3$, 90 MHz): δ 2.15 (3H, *s*, 3-CH_3), 2.75 (3H, *s*, 3'-$COCH_3$), 3.95 (3H, *s*, 4'-OMe), 6.15 (1H, *s*, 5'H), 7.2 (1H, *m*, H2), 7.6 (3H, ABC splitting, H5, H6, H7), 11.95 (1H, *s*, OH), 12.55 (1H, *s*, OH), 14.15 (1H, *s*, 2'-OH).

3.7 *Reductive cleavage of knipholone (32)*

Knipholone (**32**) (24 mg, 0.00553 mmol) was dissolved in 5 ml 5% aq.NaOH and $Na_2S_2O_4$ (40 mg, 0.23 mmol) added. The solution was stirred in an oil bath at 80° for 2.5 h. The deep red solution was cooled to room temperature and acidified with 10 ml of 2N HCl to give a precipitate which was taken up in EtOAc (3 x 10 ml). This organic layer was concentrated *in vacuo* and run through a silica gel column using neat $CHCl_3$ as the mobile solvent. This gave:

Chrysophanol (**5**), 10.8 mg (83%), R_f = 0.95 (solvent system A), ^{1}H NMR ($CDCl_3$, 90 MHz): δ 2.45 (3H, *s*, 3-CH_3), 12.00, 12.10 (2H, 2*s*, -OH), 7.10 (1H, *s*, H2), 7.65 (1H, *d*, H4), 7.75 (H5, *dd*, H5), 7.85 (1H, *t*, H6), 7.30 (1H, *dd*, H7) which was identical with an authentic sample. The other products were 4,6-dihydroxy-2-methoxyacetophenone (**37**) and acetylphloroglucinol (**36**), both identified by comparison of their R_f values with those of authentic samples.

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