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CHEMICAL CONSTITUENTS OF EUCLEA schimperi

A Thesis Presented to
the School of Graduate Studies
Addis Ababa University

In Partial Fulfillment of
the Requirements for the
Degree Master of Science in Chemistry

by

Zerom Tesfai

June 1988

D E D I C A T I O N

To my father Tesfai Redda

Page

3. EXPERIMENTAL

3.1 Materials and Apparatus 61

3.2 Solvent Systems 61

3.3 Plant Material 61

3.4 Extraction and Isolation of Compounds 62

3.4.1 Extraction and Isolation of Compounds from the Root Bark of *E. schimperi* (A.Dc) Dandy 62

3.4.2 Extraction and Isolation of Compounds from the Stem Bark of *E. schimperi* (A.Dc) Dandy 64

4. REFERENCES 66

A B S T R A C T

Chemical Constituent of Euclea schimperi

by

Zerom Tesfai

Research Advisor: Dr. Ermias Dagne

There are two indegineous Euclea species in Ethiopia of a total of nearly 20 species of Euclea in the world all of which are restricted to tropical and subtropical Africa. Euclea schimperii (family Ebenaceae), known in Amharic as Dedeho, is used in Ethiopia traditional medicine for the treatment of gonorrheae.

The extract obtained from the root bark afforded seven naphthoquinones and two triterpenes. Five of the naphthoquinones are known compounds, these being 7-methyljuglone (N-1), neodiospyrin (N-2), 2-methylnaphthazarin (N-3), mamegakinone (N-4), and isodiospyrin (N-7). The other two naphthoquinones have not yet been structurally elucidated. The two triterpenes are lupeol (T-1) and unidentified triterpene (T-3).

The extract from the stem-bark of E. schimperi yielded isodiospyrin (N-7), lupeol (T-1), and another uncharacterized triterpene (T-2).

From previous phytochemical reports it appears that out of the known naphthoquinones isolated neodiospyrin (N-2) and 2-methylnaphthozarin (N-3) have not been re-

ported before from any part of the plant. Furthermore, the occurrence of neodiospyrin (N-2) from the genus Euclea is reported here for the first time.

A C K N O W L E D G E M E N T S

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L I S T O F T A B L E S

Page

1. Ebenaceae naphthoquinones and their reduced derivatives arranged in order of increasing molecular weight 3

2. Triterpenes of Euclea species 17

3. Contribution bs substituents to the chemical shifts of C-5 hydroxy-protons in juglone 33

4. Contribution of different linkage position to the chemical shift of C-5 hydroxy-protons of 7-methyljuglone is dimeric derivatives 34

5. Chemical shift of 7-methyl protons of dimeric derivatives with different linkage 35

6. R_f and colour reaction of the compounds isolated 39

L I S T O F S C H E M E S

		Page
1.	Biosynthetic route to 7-methyljuglone and plumbagin via acetate polymalonate pathway	21
2.	Formation of C-6 and C-8 radicals of 5-hydroxy naphthoquinones	22
3.	The formation of possible radical intermediates from 1,8-dihydroxy-3-methylnaphthalene (65)	24
4.	Probable biogenesis of elliptinone	25
5.	Fragmentation pattern of 1,4-naphthoquinones	29
6.	Fragmentation of diospyrin via cyclization	30
7.	EIMS fragmentation pattern of N-1	43
8.	EIMS fragmentation pattern of N-2	46
9.	EIMS fragmentation pattern of N-3	50
10.	EIMS fragmentation pattern of N-4	53

L I S T O F F I G U R E S

Page

1. Structures of naphthoquinones and their reduced derivatives of the Ebenaceae 6

2. Flow chart for the extraction and isolation procedure of the root bark of E. schimperi 40

3. Flow chart for the extraction and isolation procedure of the stem bark of E. schimperi 41

4. 90 MH_z ^1H NMR of N-1 44

5. 90 MH_z ^1H NMR of N-2 47

6. 90 MH_z ^1H NMR of N-3 51

7. 90 MH_z ^1H NMR of N-4 54

8. 90 MH_z ^1H NMR of N-7 56

9. 90 MH_z ^1H NMR of N-5 59

INTRODUCTION

The family Ebenaceae is mainly found in the tropics and subtropics, occassionally extending into temperate areas. According to Hegnauer the family comprises seven genera namely Diospyros, Euclea, Maba, Oncotheca, Rhaphidanthe, Royena, and Tetraclis.[1] However, at present the family is generally considered to be composed of three genera[2] Diospyros, Euclea, and Lassiocarpa. Thus Maba, Raphidanthe, Royena, and Tetraclis are included under Diospyros while Oncotheca is included as a monotypic family.

The Ebenaceae is usually placed in the order Ebenales, which includes some or all of the following families: Ebenaceae, Sapotaceae, Styracaceae, Lissocarpaceae, Symplocaceae, Hopleastigmataceae, and Sarocospermaceae. The Ebenaceae seems to occupy a unique position in the order because it elaborates naphthoquinones and their reduced derivatives[3].

Many Diospyros and Euclea species have been subjected to phytochemical studies. In many cases, the main reason behind these studies has been the role these plants play in traditional medicine systems of various societies. In addition the well known African ebony tree also belongs to the genus Diosypros, i.e. D. ebenum. The first report on the clinical trials of D. mollis[4] extracts showed activity against hookworm. Harper[5] et al reported the use of Diospyros species as fish poison and

as anti termites in some African traditional medicinal systems. Diospyrol[6] (67), which is important precursor in naphthoquinone biogenesis, was found to be responsible for the anthelminitic effect of the fresh fruits of D. mollie. Plumbagin (2), a naphthoquinone isolated from many Diospyros species is reported to inhibit the growth of several Sacharomyces.[7] The same compound caused skin lesion and had an irritant and necrotic action on mice during the study carried out by Debray[8] et al. The leaves and seeds of D. montana were also effective against many microorganisms in the study conducted by Goutam[9] and co-workers. The constituent from D. virginiana and D. usambarensis which was identified as 7-methyljuglone (1), an isomer of plumbagin, was found to possess termicidal, molluscidal, and fungicidal properties.[10,11] Similarly diospyrin (18), a dimer of 7-methyljuglone, was also identified as the biologically active constituent present in the bark extract of D. montana with growth inhibitory activities towards Enhrilich ascites carcinoma in mice.[12]

Hitherto nearly 50 naphthoquinones and their reduced derivatives have been isolated and structurally elucidated from the two genera Diospyros and Euclea of the Ebenaceae. These secondary metabolities are listed in Table 1, where the metabolites are arranged according to increasing molecular weights. The table contains 12 monomers (1 - 12), 32 dimers (14, 16 - 46), 3 trimers (48 - 50), a tetramer (51), and 3 metabolities (13, 15, 47) which belong to a

new class of natural product with 4-hydroxy-5-methyl-coumarin unit as a substituent in the 1,4-naphthoquinone skeleton. The structures of all these metabolities are drawn in Fig. 1.

Table 1. Ebenaceae naphthoquinones and their reduced derivatives arranged in order of increasing molecular weight.

S.No.	Name	MF	MW	Structure No	Source	Ref.
1	7-methyljuglone	$C_{11}H_8O_3$	188	1	D. melonxylon	13
2	Plumbagin	$C_{11}H_8O_3$	188	2	D. elliptifolia	14
3	Epiisoshinanolone	$C_{11}H_{12}O_3$	192	3	D. canaliculata	15
4	Isoshinanolone	$C_{11}H_{12}O_3$	192	4	D. maritima	16
5	Shinanolone	$C_{11}H_{12}O_3$	192	5	D. japonica	17
6	-	$C_{12}H_{10}O_3$	202	6	D. melanoxylon	18
7	-	$C_{12}H_{10}O_3$	202	7	D. melanoxylon	18
8	2-Methylnapthazarin	$C_{11}H_8O_4$	204	8	D. lyciodes sericea	19
9	Diomelquinone A	$C_{12}H_{10}O_4$	218	9	D. melanoxylon	20
10	-	$C_{12}H_{10}O_4$	218	10	D. melanoxylon	18
11	-	$C_{12}H_{10}O_4$	218	11	D. kaki	21
12	-	$C_{12}H_{10}O_4$	218	12	D. usambemsis	11
13	Cyclocanaliculatin	$C_{21}H_{12}O_6$	360	13	D. canaliculata	15

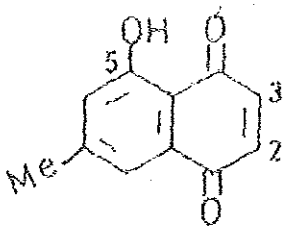
Table 1 contd.

S.No	Name	MF	MW	Structure No	Source	Ref.
14	Ebenone	$C_{22}H_{16}O_5$	360	14	D. ebenum	22
15	Canaliculatin	$C_{21}H_{14}O_6$	362	15	D. canaliculate	15
16	-	$C_{22}H_{12}O_6$	372	16	D. montana	23
17	Biramentaceone	$C_{22}H_{14}O_6$	374	17	D. melanoxylan	24
18	Diospyrin	$C_{22}H_{14}O_6$	374	18	D. mespiliformis	14
19	Elliptinone	$C_{22}H_{14}O_6$	374	19	D. elliptifolia	14
20	Isodiospyrin	$C_{22}H_{14}O_6$	374	20	D. virginiane	14
21	Mamegakinone	$C_{22}H_{14}O_6$	374	21	D. lotus	25
22	Maritinone	$C_{22}H_{14}O_6$	374	22	D. kaki	21
23	Neodiospyrin	$C_{22}H_{14}O_6$	374	23	D. kaki	21
24	Rotundiquinone	$C_{22}H_{14}O_6$	374	24	D. rotundifolia	26
25	ß-Dihydrodiospyrin	$C_{22}H_{16}O_6$	376	25	D. montana	27
26	Natelenone	$C_{22}H_{16}O_6$	376	26	E. natalensis	28
27	Tetrahydrodiospyrin	$C_{22}H_{18}O_6$	378	27	D. montana	29
28	-	$C_{23}H_{16}O_6$	388	28	D. melanoxylon	30
29	Batocanone	$C_{22}H_{14}O_7$	390	29	D. batocana	31
30	Celebaquinone	$C_{23}H_{18}O_6$	390	30	D. celebica	32
31	Diosquinone	$C_{22}H_{14}O_7$	390	31	D. montana	23
32	Isocelebaquinone	$C_{23}H_{18}O_6$	390	32	D. celebica	32
33	-	$C_{22}H_{14}O_7$	390	33	D. ferrea	16
34	-	$C_{22}H_{14}O_7$	390	34	D. montona	23

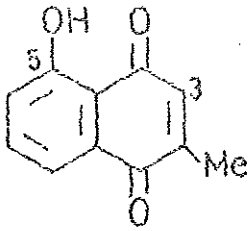
Table 1 contd.

S.No.	Name	MF	MW	Structure No	Source	Ref.
35	-	$C_{22}H_{14}O_7$	390	35	D. montana	23
36	Euclanone	$C_{22}H_{14}O_7$	390	36	E. natalensis	33
37	Diosindigo A	$C_{24}H_{20}O_6$	404	37	D. baxifolia	34
38	Diosindigo B	$C_{24}H_{20}O_6$	404	38	D. celebica	35
39	-	$C_{23}H_{16}O_7$	404	39	D. mannii	36
40	-	$C_{23}H_{17}O_7$	405	40	D. morrisiana	37
41	Eucleolatin	$C_{22}H_{14}O_8$	406	41	E. lanceolata	38
42	-	$C_{24}H_{18}O_7$	418	42	D. montana	23
43	-	$C_{22}H_{13}O_7Cl$	4245	43	D. montana	23
44	-	$C_{24}H_{16}O_8$	432	44	D. morrisiana	36
45	-	$C_{24}H_{18}O_8$	434	45	D. melanoxylon	39
46	-	$C_{22}H_{12}O_6Cl_2$	443	46	D. montana	23
47	Ismailin	$C_{31}H_{20}O_9$	536	47	D. ismaili	40
48	Isoxylospyrin	$C_{33}H_{18}O_9$	558	48	D. whyteana	41
49	Xylospyrin	$C_{33}H_{18}O_9$	558	49	D. chloroxylon	42
50	Galipinone	$C_{33}H_{20}O_9$	560	50	E. natalensis	3
51	Bisisodiospyrin	$C_{44}H_{26}O_{12}$	746	51	D. lotus	

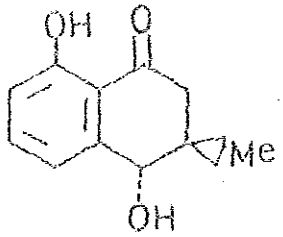
Fig. 1. Structures of naphthoquinones and their reduced derivatives of the Ebenaceae.



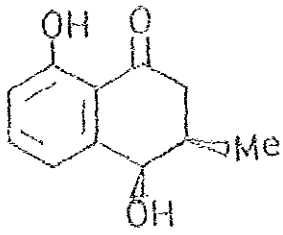
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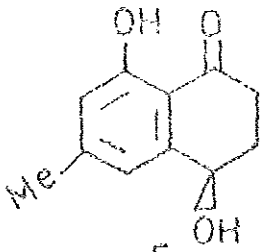
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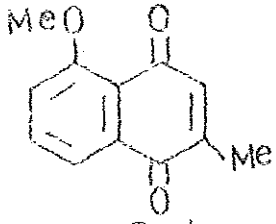
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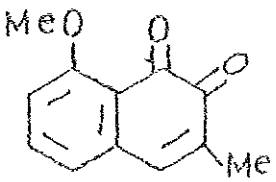
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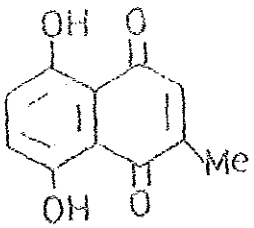
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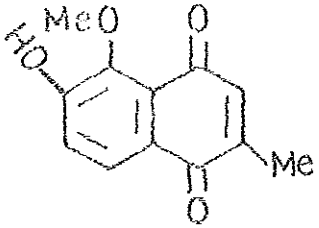
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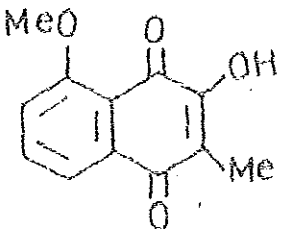
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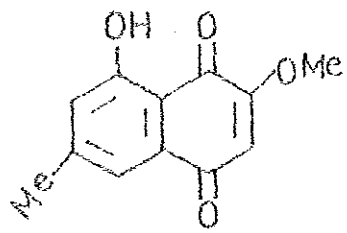
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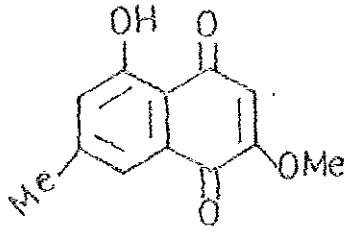
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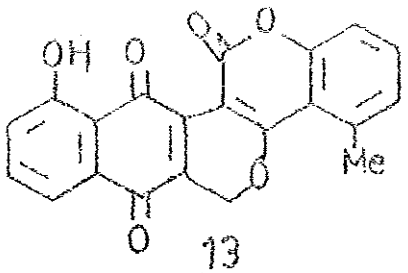
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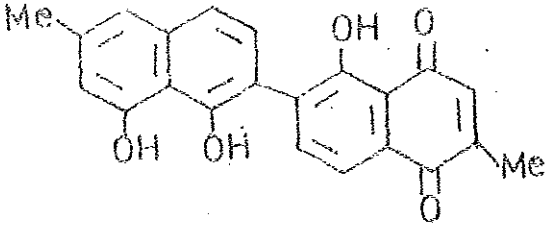
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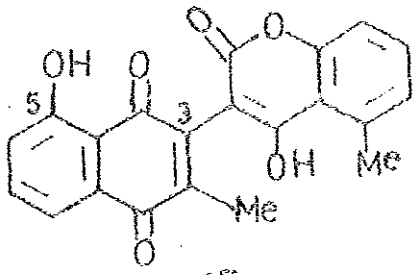
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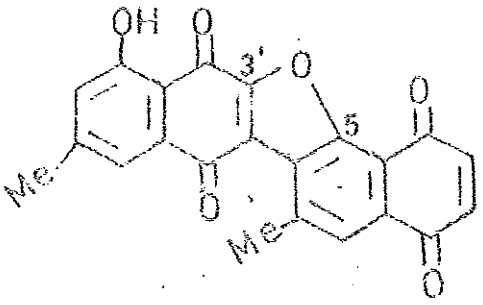
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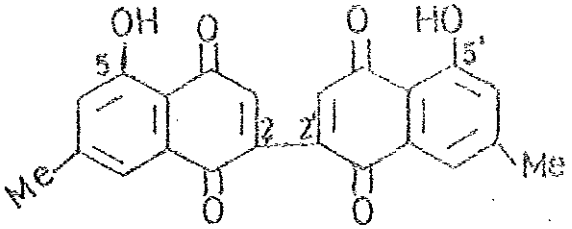
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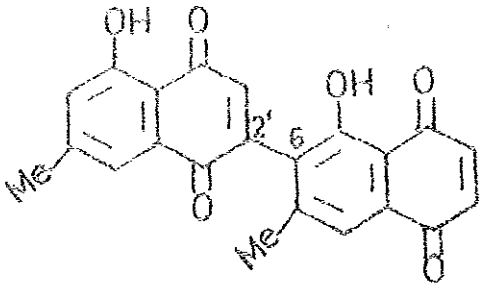
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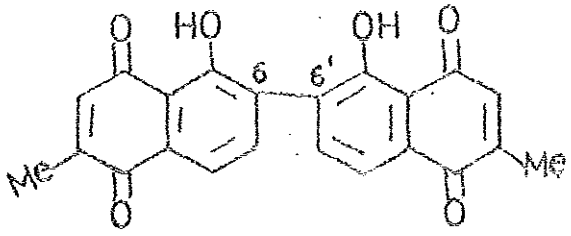
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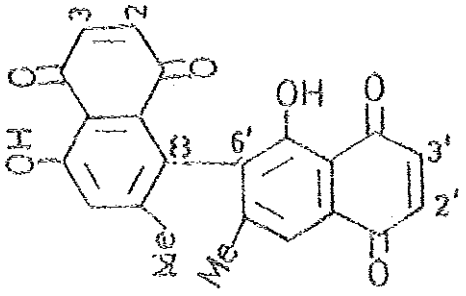
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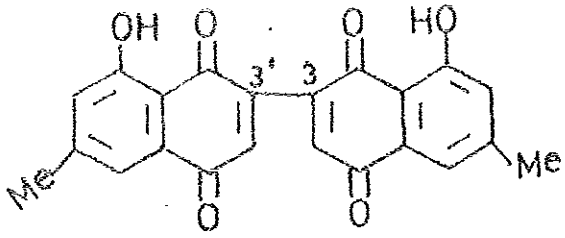
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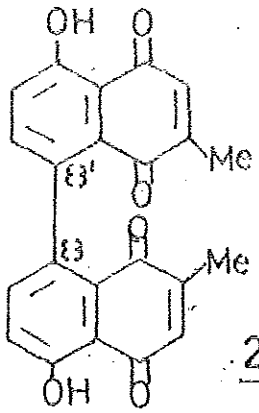
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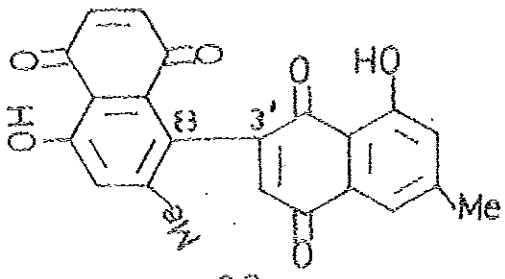
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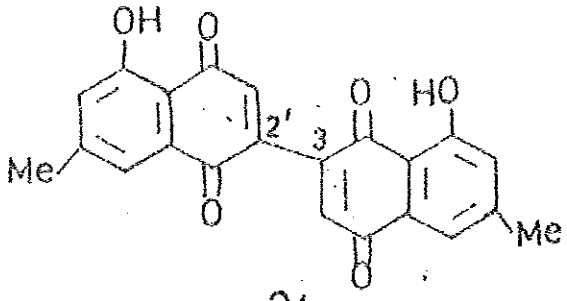
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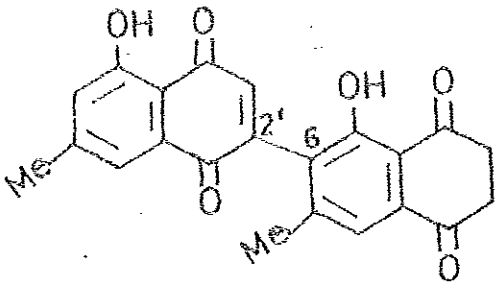
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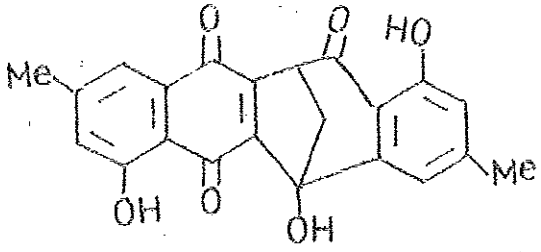
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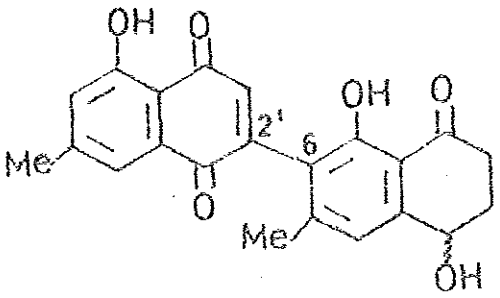
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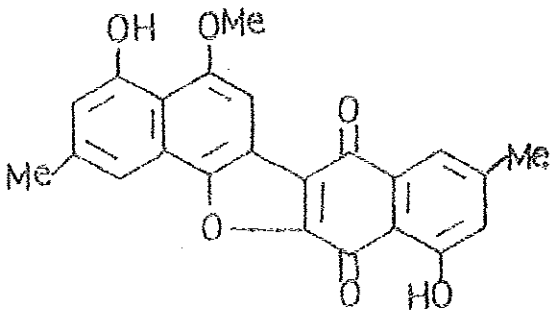
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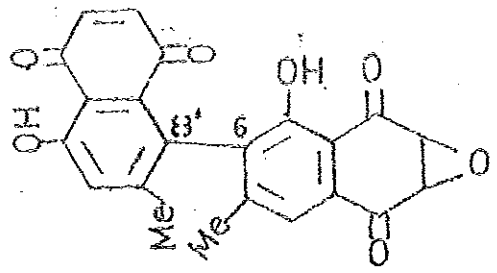
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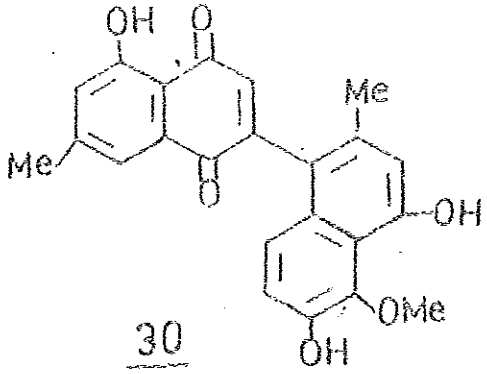
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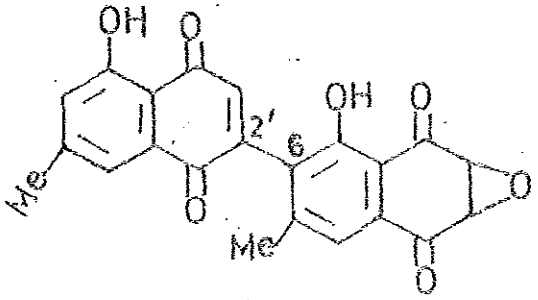
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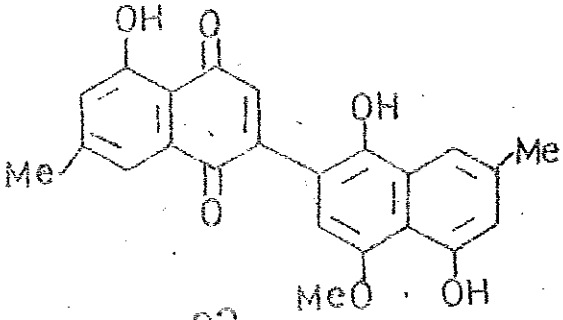
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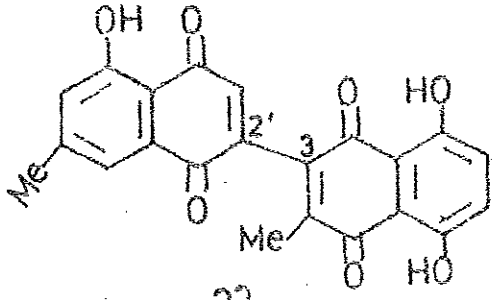
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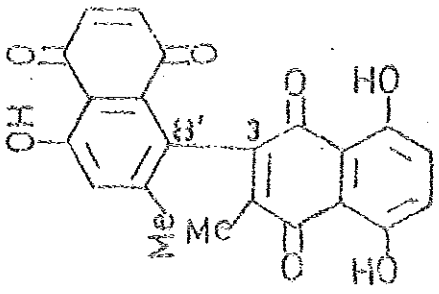
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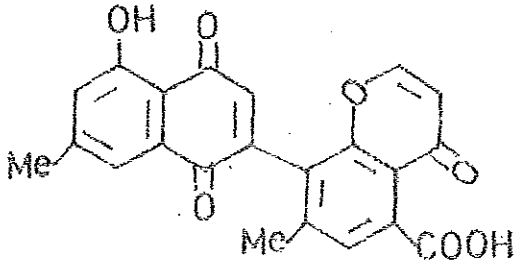
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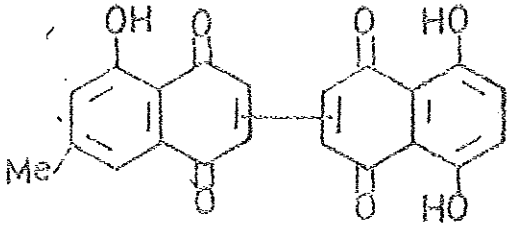
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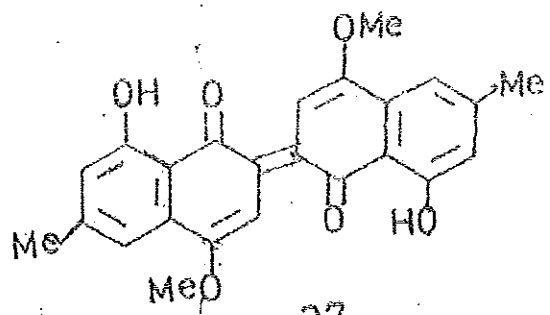
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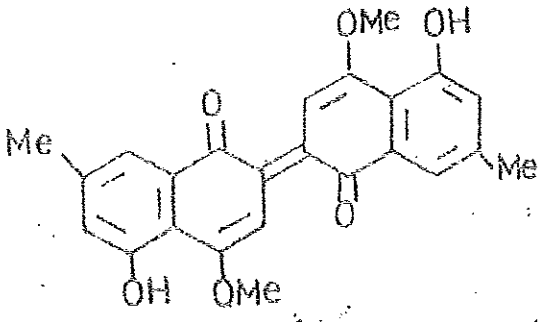
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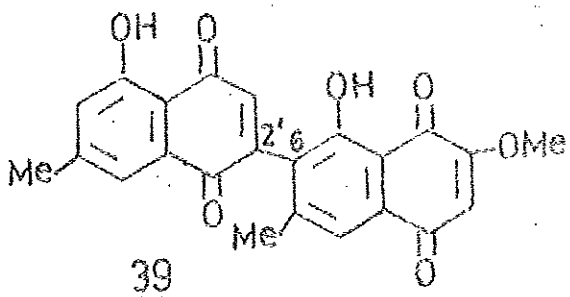
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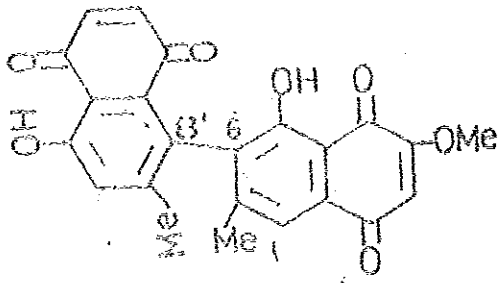
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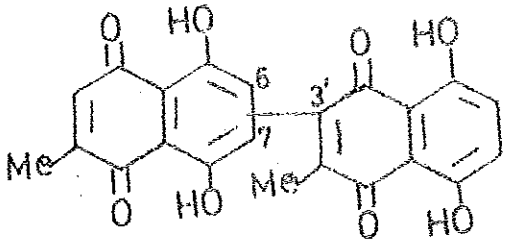
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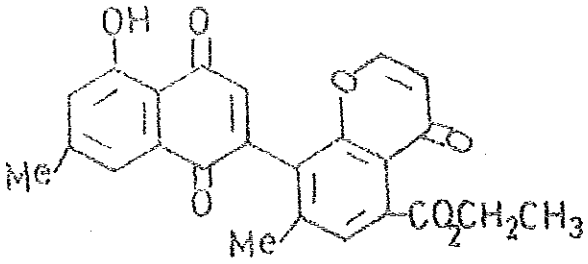
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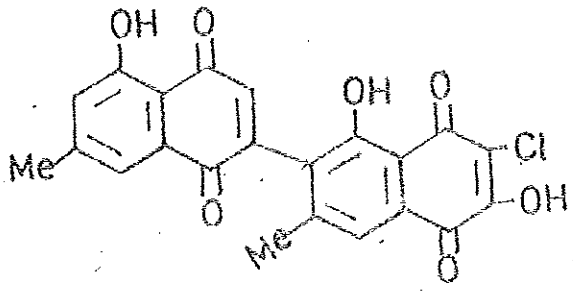
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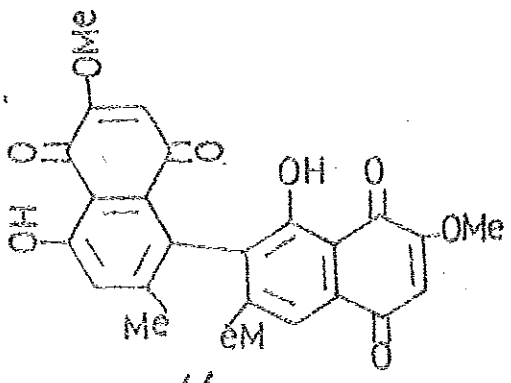
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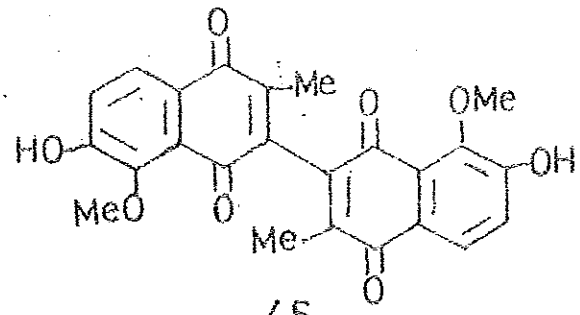
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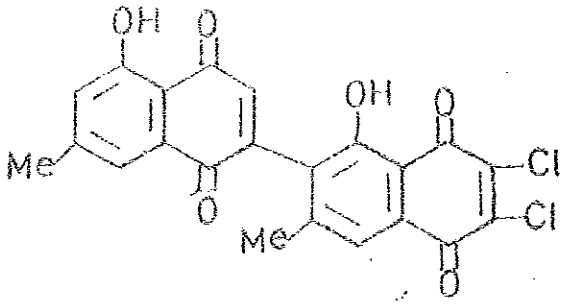
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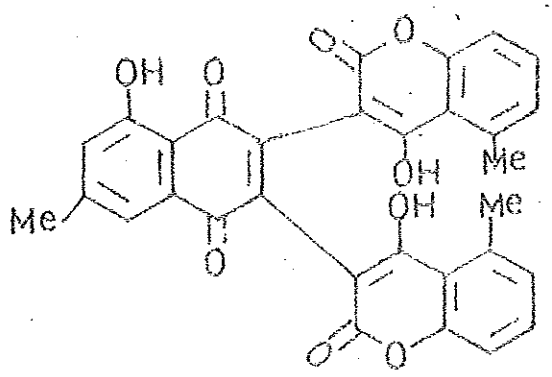
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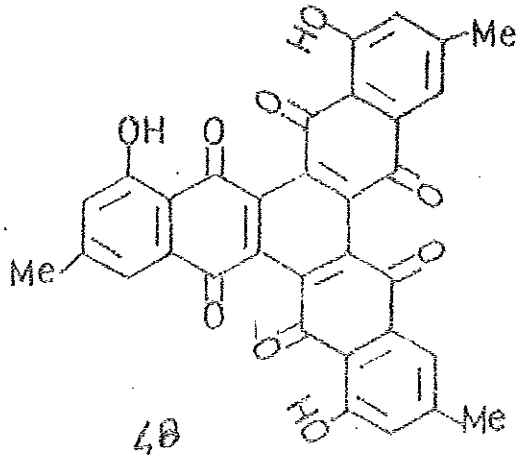
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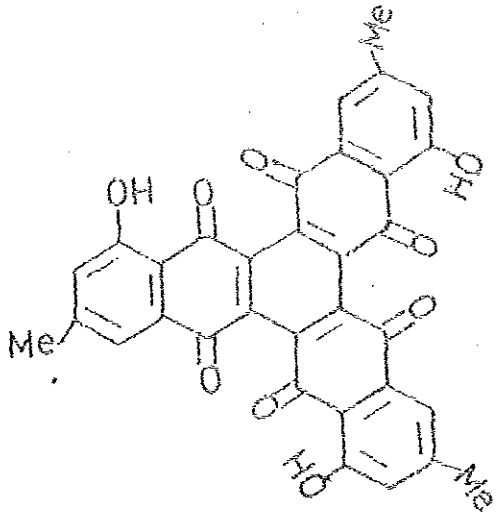
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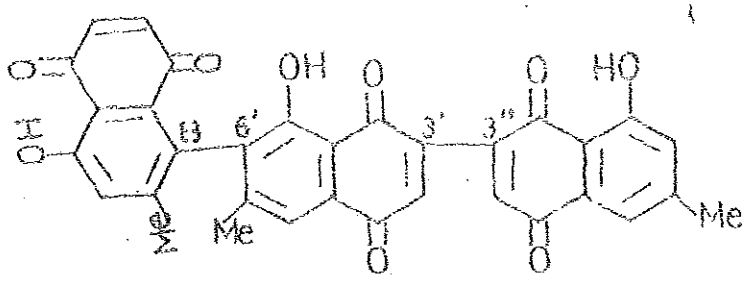
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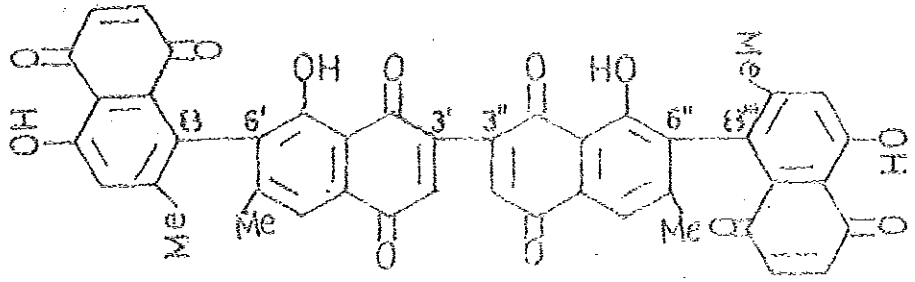
48


Me
OH
O
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O
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O
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O
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Me
OH
Me

49


O
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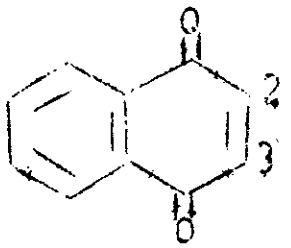
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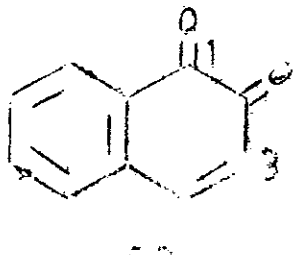
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1.1 The Chemistry of Euclea

The vast majority of naphthalene derivatives found in nature are quinones, and the others are mainly related naphthols or naphthyl ethers. Increasing number of o-naphthoquinones and binophthoquinones have been found in recent years from different sources.[43] A large number of naphthoquinones are found in nature as yellow, blue or red pigments. The fundamental naphthoquinone structures with the ring numbering system are shown below:



52



53

Naphthoquinones and triterpenoids were reported from plants belonging to the family Ebenaceae starting from the early fifties. A great number of naphthoquinones were reported from Diospyros, the largest genus of the family. The occurence of diospyrol and naphthaldehydes in Diospyros mollis and D. ebenum was noted in the early reports.[56,57]

The genus Euclea attracted the attention of phytochemists since 1969 when the first report on the occurrence of naphthoquinones in the genus appeared. The chemical investigation of Euclea

by Alves et al[44] resulted in the isolation and characterization of 2-methylnaphthazarin (8) a compound which was previously described by MacBeth et al in 1935.

Van der Vijver and co-worker[1] examined three Euclea species together with other Diospyros. The study revealed the presence of 7-methyljuglone (1), diospyrin (18), and isodiospyrin (20) from different parts of the plants. These compounds were previously reported from other Diospyros species.

In 1973 the phytochemical investigation on E. pseudebenus by Ferreira and co-workers[45] reported the isolation of five naphthoquinones namely 2-methylnaphthazarin (8), diosindigo A (37), mamegakinone (21), 7-methyljuglone (1) and euclein. Euclein was proposed as a 3,6'-dimer of 7-methyljuglone. However, a later study[23] proved it to be identical to diospyrin (18). Diosindigo A is a compound previously isolated by Musgrove and Skoyles[34] from D. baxifolia.

James[46] investigated E. natalensis, a plant used by people for toothache and headache remedy. The study ended up with already known compounds of Euclea species. But the previous claim for the confinement of naphthoquinones in the roots and fruits was found not to be general since this work showed the presence of naphthoquinones in stem extracts as well. Reinvestigation of E. lanceolata by Ferreira et al[38] led to the isolation of many naphthoquinones among which were 8'-hydroxydiospyrin (33) and a dimeric methylnaphthazarin (41). The

latter compound which was named as eucleolatin was the first example of a dimeric naphthazarin and was found to be either 3 - 6' or 3 - 7'-dimer of 2-methylnaphthazarin.

Lopes[47] reported many known naphthoquinones from the chloform extracts of the root bark of E. lanceolata. Ferreire and co-workers[48] investigated E. pseudebenus and reported the presence of 8'-hydroxyisodiospyrin (34) together with other naphthoquinones.

In 1974 Van der Vijver and Gerritsma[49] studied four Euclea species namely E. natalensis, E. divinorum, E. crispa, and E. schimperi. 7-methyljuglone (1), mamegakinone (21), diospyrin (18), and isodiospyrin (20) were the compounds isolated from each species. The first two species have been reinvestigated by different workers and resulted in the isolation of several more compounds. The last species, i.e., E. schimperi has been the subject of reinvestigation in this study and we report the isolation and characterization of additional compounds (see Results and Discussion).

Fausto et al[50] studied E. natalensis a plant known in Africa folk-medicine. Unlike previous studies which focused on naphthoquinones. this study paid attention to triterpenes, another class of secondsry metabolites common to the Ebenaceae. The workers reported the isolation of α-amyrin lupeol, uvaol (as diacetate) betulin, ursolic acid, and sitosterol (structures are given in Table 2). Identification of lupeol (57) betulin (55), biramentaceone was also reported from

E. divinorum and E. natalensis by Lopes and Paul.[51] King et al[28] characterized a new natural product named natelenone (26) from E. natalensis. Natelenon (26) is a dihydrodimer of 7-methyljuglone (1) in which the two halves are linked by two carbon-carbon bond to give a fused tetracyclic structure in which one ring carries a methylene bridge. The final structure was proposed from X-ray crystallographic analysis.

Cruz Costa et al[5] reinvestigated E. divinorum and isolated diosindigo A (37), 2-methylnaphthazarin (8), 7-methyljuglone (1), and diospyrin (18) together with two triterpenes of the lupane series, i.e., lupeol and betulin. In 1977 Ferreira[33] et al reported a new natural product euclanone (36), a trimer which was galipinone (50), and other already reported naphthoquinones of Ebenaceae. Galipinone is a trimer of 7-methyljuglone where the three units probably linked C-8 - C-8' and C-3' - C3". This compound was previously isolated by Vijver[26] from D. galpinii. Euclanone is a new dimer of 7-methyljuglone and methylnaphthazarin which is isomeric with 8'-hydroxydiospyrin. Its final elucidation was obtained by X-ray crystallographic analysis.

The latest report on Euclea species was by Khan[53] in 1985. The root bark extracts of E. natalensis gave 4,8-dihydroxy-6-methyl-1-tetralone (5). Structure of this tetralone was confirmed by oxidation of (5) to 7-methyljuglone, and subsequent reduction to the natural tetralone.

Table 2. Triterpenes of Eucleа species.

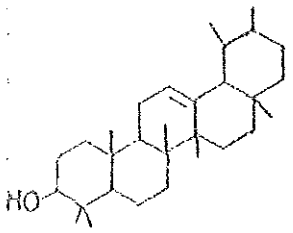
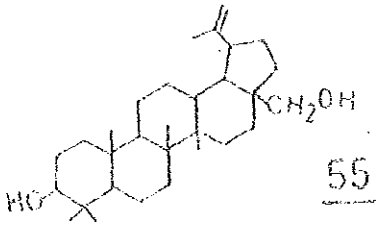
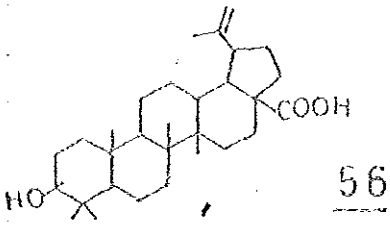
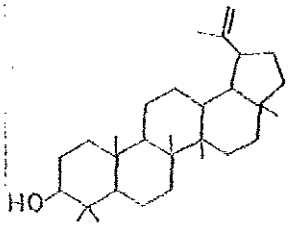
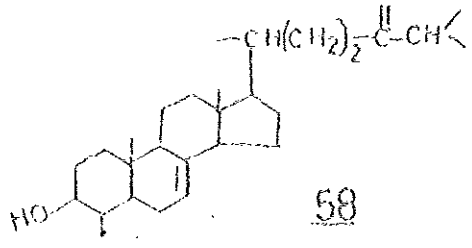
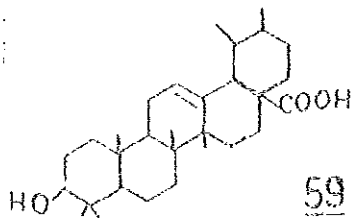
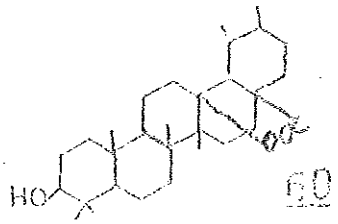
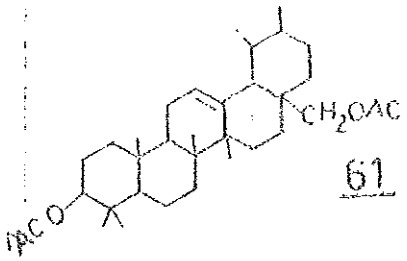
NAME	MF MW	STRUCTURE	SOURCE	REF.
α-amyrin	$C_{30}H_{50}O$ 426	 54	E. natalensis	50
Betulin	$C_{30}H_{50}O_2$ 442	 55	E. natalensis E. divinorum	50,51 52
Betulinic acid	$C_{30}H_{48}O_3$ 456	 56	E. natalensis	50
Lupeol	$C_{30}H_{50}O$ 426	 57	E. natalensis E.divinorum	50,51 52
Sitosterol	$C_{29}H_{48}O$ 412	 58	E. natalensis	50

Table 2. cont.

NAME	MF MW	STRUCTURE	SOURCE	REF.
Ursolic acid	$C_{30}H_{48}O_3$ 456	 59	E. natalensis	50
Ursolic acid lactone	$C_{30}H_{48}O_3$ 456	 60	E. natalensis	50
Uvaol diacetate	$C_{34}H_{54}O_4$ 526	 61	E. natalensis	50

1.2 Biogenesis of Naphthoquinones

The biogenesis of naphthoquinones had not been paid attention to until recently although fully structurally elucidated compounds belonging to this class[43] have been known for quite a while. Hence, the biogenetic pathways leading to the formation of naphthoquinones in the Ebenaceae are not fully understood. However, Leistner[54] proposed three principal pathways by which naphthoquinones may be derived in higher plants. These include:

i. The acetate-polymalonate pathway;
ii. The phenylalonine-thyrosine pathways;
iii. The shikimate acid-o-succinoylbenzoic acid pathway.

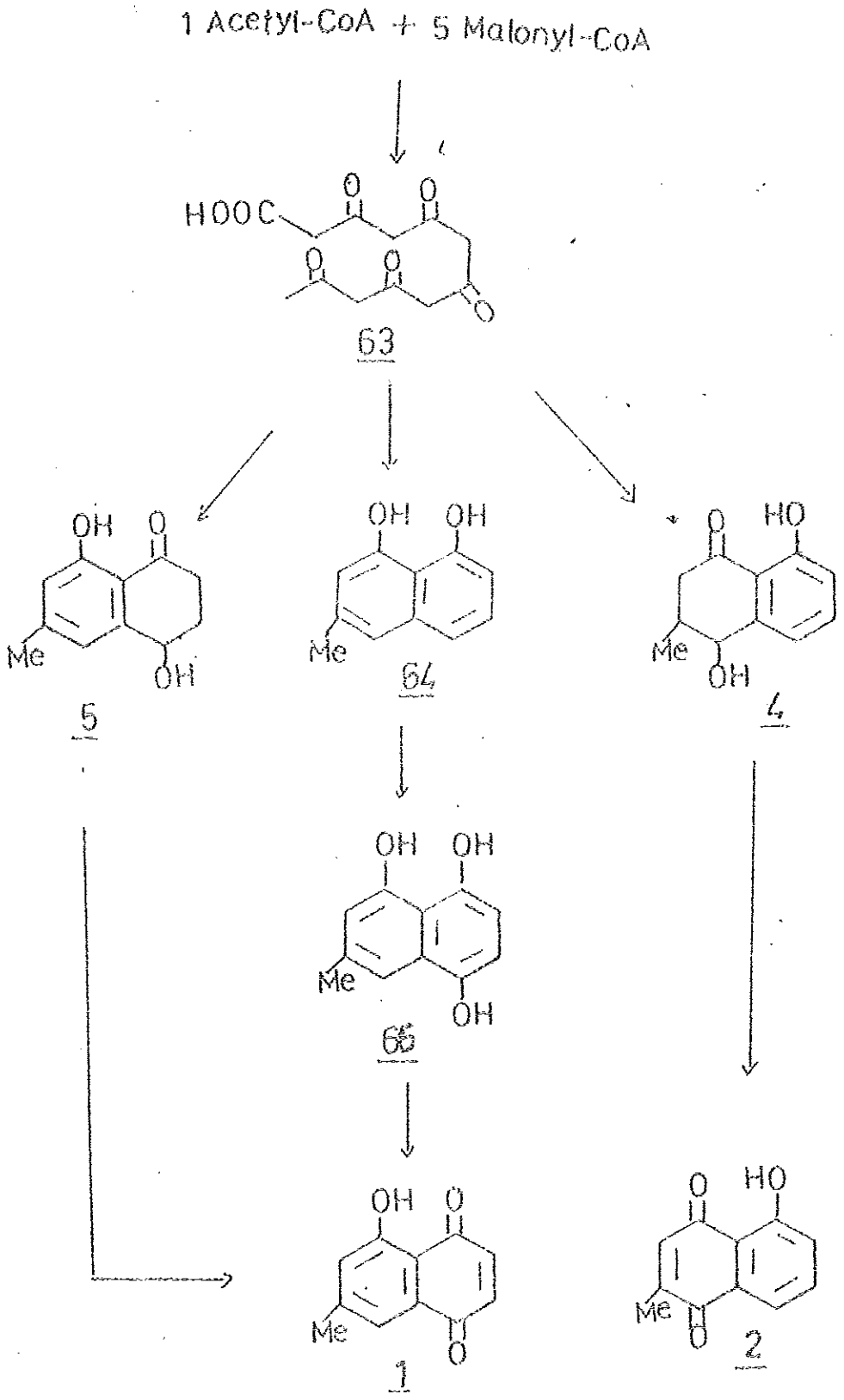
Biological and phytochemical evidences appear to favor the hypothesis that the quinones in the Ebenaceae originate from acetate. The structural features of the naphthoyl and naphthoquinone derivatives of the genera Diospyros and Euclea clearly suggest that they are formed from acetate-polymalonate units[3] through acetyl-coenzyme A(COA) and malonyl-COA.

The biosynthetic process that involves the head to tail condensation of acetyl COA with appropriate numbers of malonyl COA leading to subsequent formation of polyketomethylene (63) is suggested from

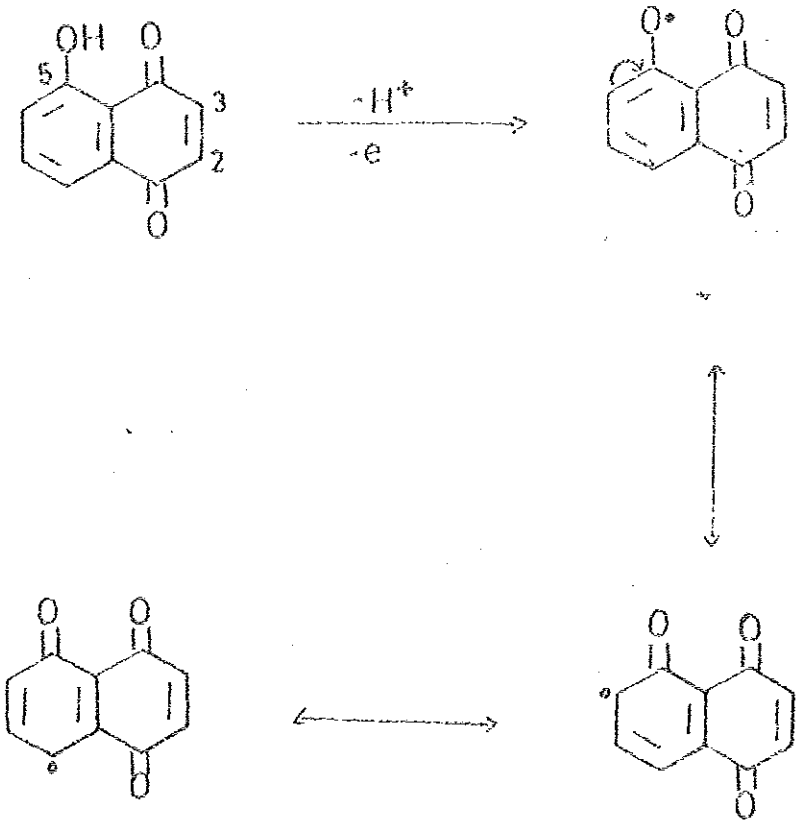
experiments with ^{14}C-labelled acetic and malonic acids. The chain is folded and cyclized to give the corresponding phenolic products (5), (64), and (4) as shown in Scheme 1.

Feeding experiments by Durand and Zenk[55] with young shoots of Plumbago europaea L. confirmed the heavy incorporation of labelled acetate and malonate into Plumbagin (2). In contrast to these observations shikimate, L-methionine and mevalonic acid incorporation was very insignificant. These experiments suggest that the polyacetate pathway may very well be the most likely route in operation in the Ebenaceae (Scheme 1) since related naphthols and reduced methyl 1,4-naphthoquinones such as shinanolone (5) and isoshinanolone (4) were isolated from Diospyrs species.[15,16]

The mechanisms proposed for the formation of dimers, trimers or tetramers found in the Ebenaceae are based on speculation rather than on experimental results. These are believed to arise as a result of phenolic oxidative coupling of two or more monomers by a radical pairing reaction. This coupling process is considered to be initiated by removal of an electron from a phenolate anion to give a radical intermediate which, by delocalization of the unpaired electron activates position C-6 or C-8 (Scheme 2). The formation of all dimers of polymers linked at C-6 and C-8 can be easily rationalized in terms of the presence of a 5-hydroxy group in both monomers.[3]



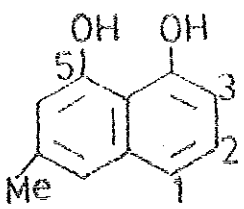
Scheme 1. Biosynthetic rout to 7-methyjuglone and plumbagin via acetat-polymalonate pathway.



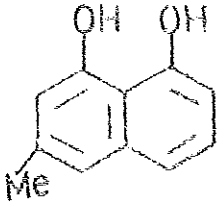
Scheme 2. Formation of reactive C-6 and C-8 radicals of 5-hydroxy naphthoquinones.

The biosynthetic intermediate 1,8-dihydroxy-3-methyl-naphthalene (65) or its biogenetic equivalent has been

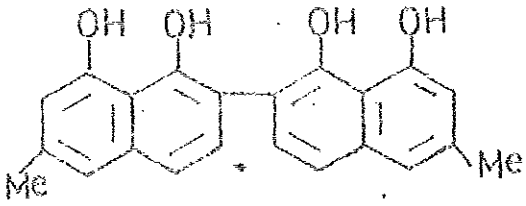
suggested by Tozuka[16] as intermediate during the formation of dimers linked at C-2 or C-3. It is likely that most 7-methyjuglone dimers are formed after C-1 of 65 had been hydroxylated to yield 66 because, if dimerization had occurred before that stage, as in the case of diospyrol (67), the chance of radical formation at C-8,



65

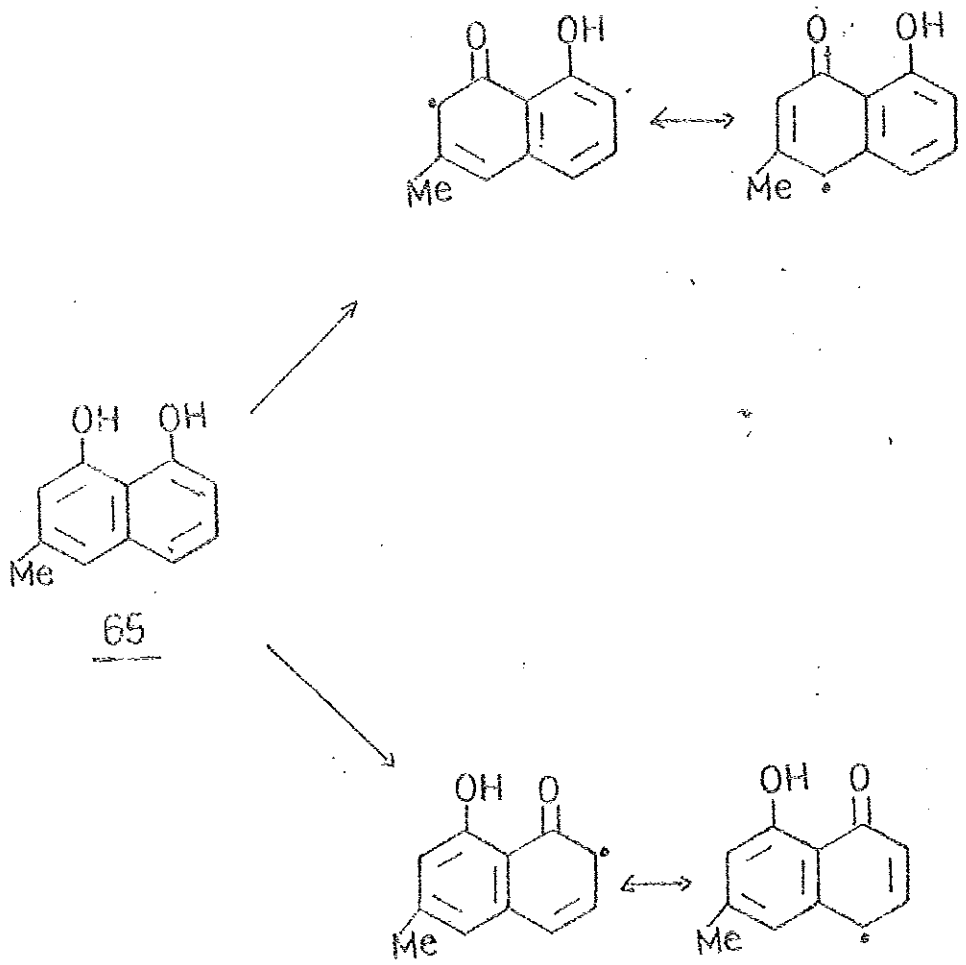


66



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C-6, C-3, and C-1 of would be equal. As a result, the naphthoquinone dimers linked at these four positions would be formed with similar probability with no dimer formation at C-2 (Scheme 3).

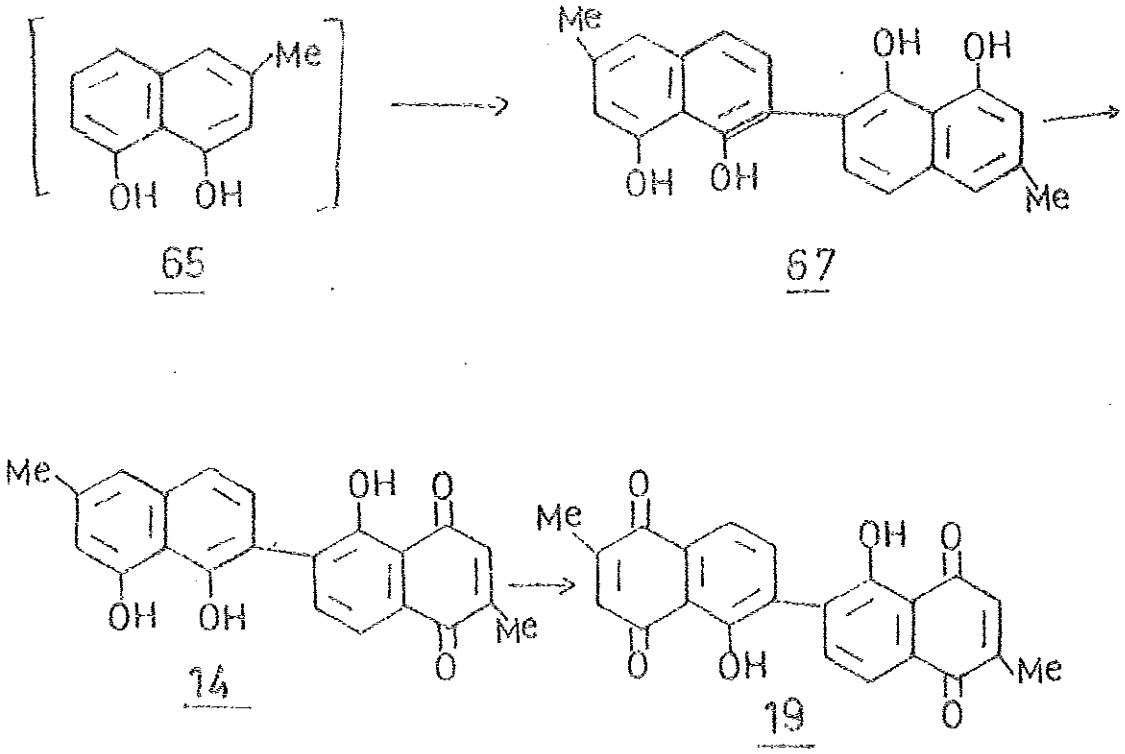


Scheme 3. The formation of possible radical intermediates from (65)

It is of interest to note that no dimers with C-8-C-8', C-6-C-6' or C-3-C-6' linkage and derived from

7-methyljuglone have been isolated to date. The former two types of linkages are unlikely because of the steric hindrance of the hydroxy and methyl groups on both benzene rings. Despite this hindrance, the occurrence of C-6-C-8' dimers, e.g. isodiospyrin (20) in plants has been ascribed to enzymatic process.

The isolation of ebenone (14) together with elliptinone (19) from D. ebenum prompted Sankaram and Reddy[22] to suggest the probable biogenesis of elliptinone via ebenone as a biogenetic precursor (Scheme 4).



Scheme 4. Probable biogenesis of Elliptinone.

1.3 Spectral Properties of Naphthoquinones

The early work on structure elucidation was mainly dependent on the preparation of derivatives, degradative experiments, and in some cases on syntheses. However, with the advent of modern spectroscopic techniques, elucidation of compounds depends very heavily on the interpretation of spectral information. The four important spectroscopic methods for naphthoquinones, like other secondary metabolites, are ultraviolet-visible, infrared, nuclear magnetic resonance, and mass spectroscopies. The important features of each of these four methods pertinent to naphthoquinone structural investigations are briefly discussed below.

1.3.1 Ultraviolet-visible Spectra[56]

The diagnostic features of the electronic spectrum of a 1,4-naphthoquinone which is unsubstituted in the benzenoid ring are:

i. intense benzenoid and quinoid electron-transfer (E.T) bands in the 240 - 290 nm region (ϵ 13,000 - 25,000),

ii. a benzenoid E.T. band at about 335 nm of medium intensity (ϵ 2600 - 3200),

iii. a quinoid E.T. band in the 330 - 450 nm region of low to medium intensity that is generally observed for 2,3-disubstituted compounds, and

iv. a broad local excitation (L.E.) band of low intensity ($\epsilon < 100$) in the 400 - 500 nm region attributable to the n-π^* transition of the quinone carbonyls.

In 1,4-naphthoquinones with hydroxyl group in position 5 (most of the naphthoquinones of the Ebenaceae are of this type), e.g. juglone, the salient features are:

i. intense benzenoid and quinoid E.T. bands in the 240 - 320 nm region (ϵ 7000 - 20,000),

ii. a benzenoid E.T. band at about 425 nm (ϵ 3000 - 5000), and

iii. a quinoid E.T. band in the 320 - 420 nm region (ϵ 1000 - 2500).

The benzenoid E.T. band in juglone is not significantly changed by further benzenoid substitution with the exception of urther o-and p-hydroxylation. Naphthazarins, with two peri-hydroxyl groups, are characterized by a combined benzenoid and quinoid E.T. band in the region 270 - 350 (ϵ 10,000) due to tautomeric phenomena.

1.3.2 Infrared Spectra[43,3]

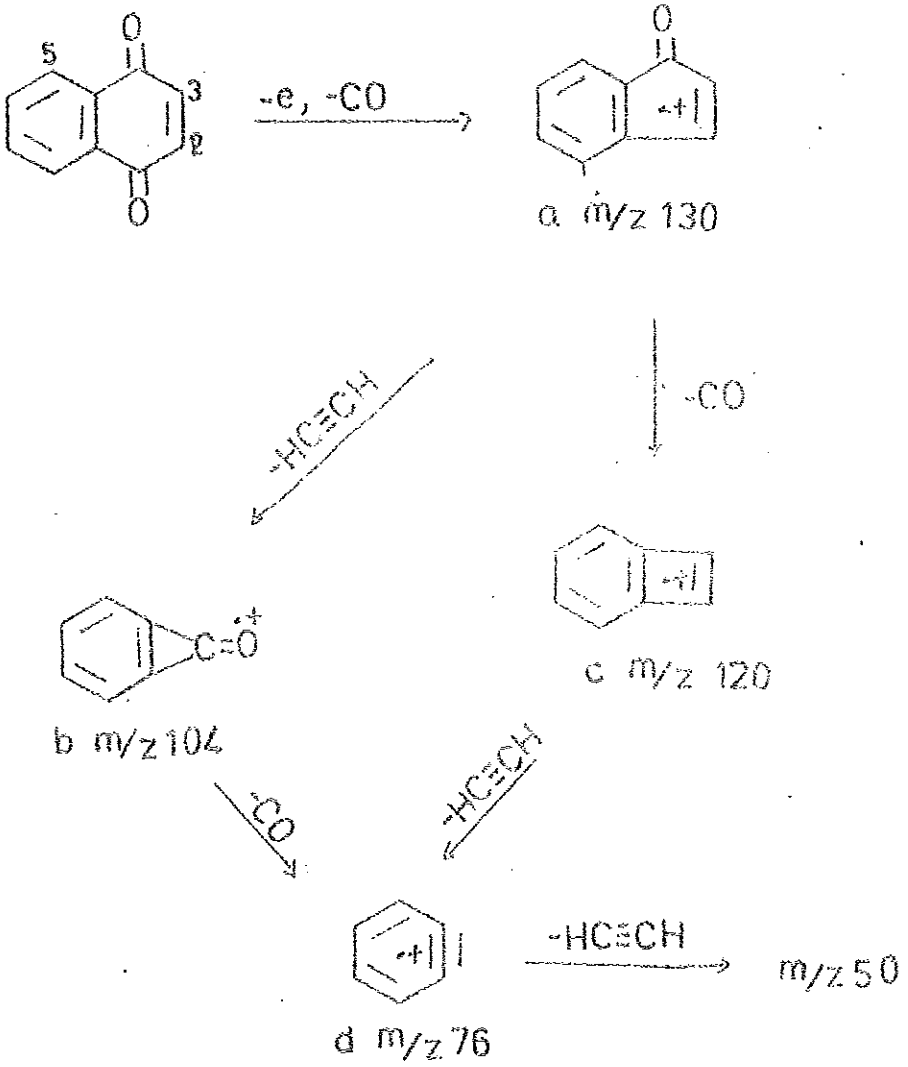
The carbonyl frequencies of quinones are useful diagnostic aids in structure determination. The carbonyl frequency is lowered by hydrogen bonding and by substitution either on the quinoid or on the adjacent benzenoid ring +I or +M groups. The carbonyl frequency is

raised by π-M substituents and by steric strain, both of which are relatively rare in natural quinones.

Chelated quinones are numerous, and can be recognized by their displaced carbonyl absorption together with the downward shift in hydroxyl frequency (o-hydroxyquinones) or the absence of hydroxyl absorption in the 3300 cm^{-1} region (peri-hydroxyquinones). In the IR spectra of 5-hydroxy-1,4-naphthoquinones, three characteristic absorptions are observed; at 3420 - 3440 cm^{-1} (sometimes obscured), due to the hydrogen bonded hydroxyl group, and two adjacent bands between 1630 - 1670 cm^{-1} which are assignable to free and hydrogen banded carbonyl groups. These bands shift bathochromically in basic medium.

1.3.3 Mass Spectra

Naphthoquinones break down in a well-defined manner upon electron impact, and plausible structures can usually be assigned to the most abundant fragment ions.[57] In most cases the molecular ion forms the base peak. The appearance of an abundant ion m/z 104 (b) and its decomposition products m/z 76 (d) and m/z 50 are characterstic naphthoquinones with no benzenoid substituents (Scheme 5). On the other hand substitution in the benzenoid ring causes these peaks to shift to the appropriate higher m/z values. Partial or almost total shift of the m/z 104 to m/z 105 corresponding to the benzoyl ion is observed for C-2 or C-3 hydroxy substituent.



Scheme 5. Fragmentation pattern for 1,4-naphthoquinones.

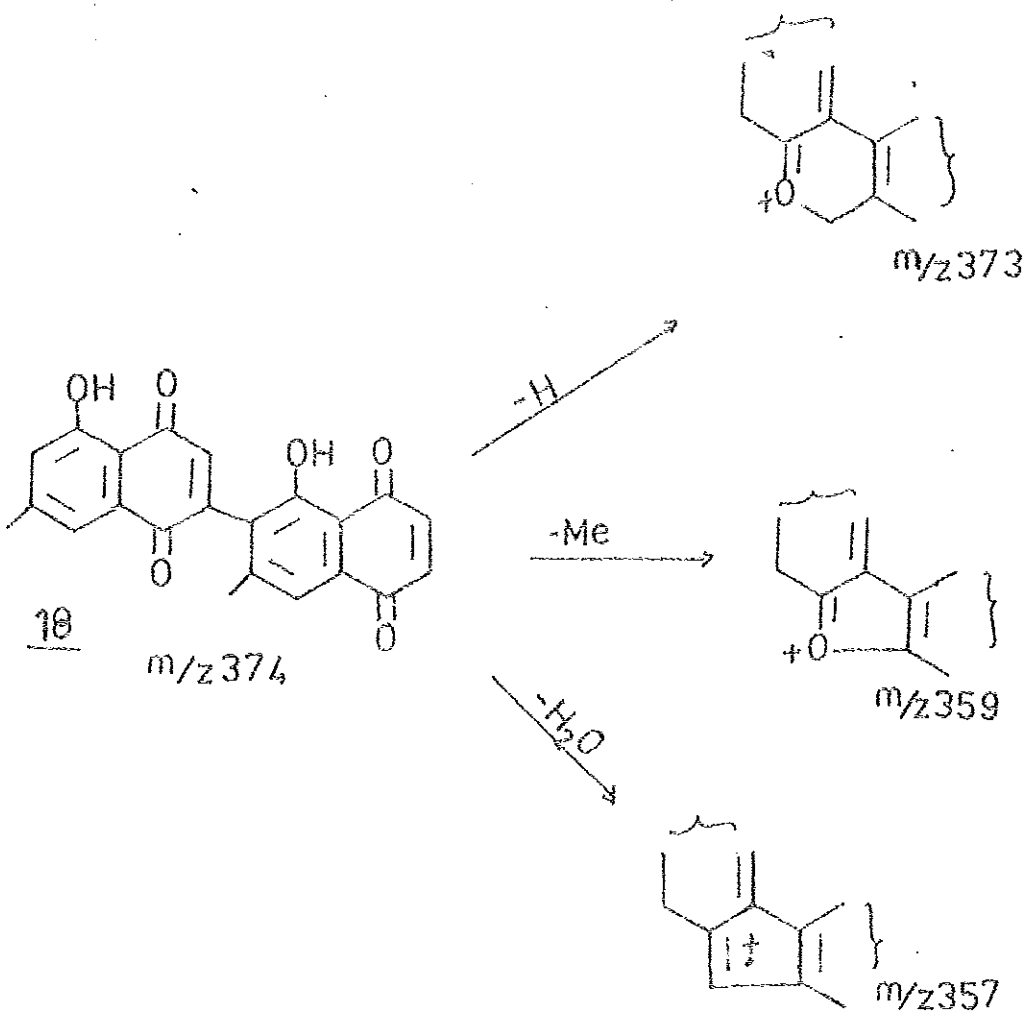
The mass spectra of the dimeric naphthoquinones can be correlated with those of simple 1,4-naphthoquinones[14] but shows a more complex pattern of mainly low-intensity peaks. The dimers molecular ion m/z as Lillie[23] suggested appear to undergo fragmentation mainly via

a. cyclization reaction,

b. repeated extrusion of carbon monoxide, and

c. scission of the quinoid rings

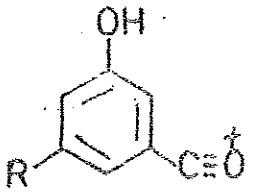
The cyclizations usually involve the substituent ortho to the internuclear link and result in the expulsion of a hydrogen atom or a methyl or hydroxyl radical, or water molecule, to give the ions at m/z 373, 359, 357, and 356 (Scheme 6).



18

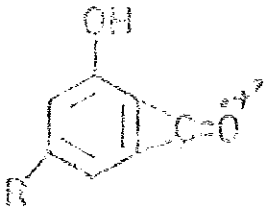
Scheme 6. Fragmentation of diospyrin via cyclization.

The ions produced by the scission of the quinone rings provide an indication of the mode of linkage of the two naphthequinone systems. Dimers from 7-methyljuglone (1) undergo cleavage of an internal quinone ring to form the expected ions at m/z 135 (68), 134 (69), and 106 (70). In addition to these there is m/z 163 (71) for those dimers possessing a methyl group ortho to the internuclear linkage. On the other hand in dimers derived from 2-methyljuglone one usually observes peaks at m/z 121 (72) 120 (73), and 92 (74). 2-methyljuglone dimers also give rise to a fragment ion at m/z 149 (75) following similar fission pathway as the m/z 163 ion of 7-methyljuglone.



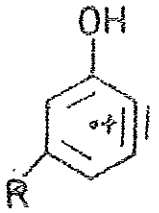
72 R=H m/z 121

68 R=Me m/z 135



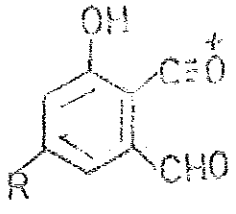
73 R=H m/z 120

69 R=Me m/z 134



74 R=H m/z 92

70 R=Me m/z 106



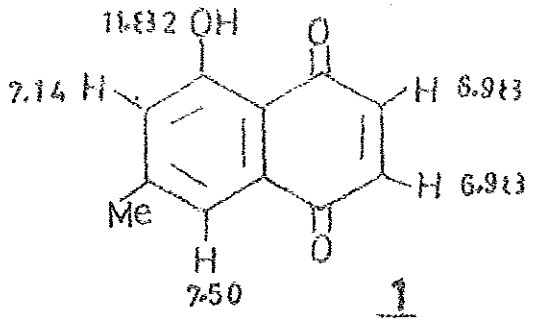
75 R=H m/z 149

71 R=Me m/z 163

1.3.4 Nuclear Magnetic Resonance Spectra

The ^{1}HNMR spectrum of 7-methyljuglone (1) is relatively simple and exhibits five signals for 8 protons. The two quinoid protons remain as unresolved singlet and the hydroxy proton resonates at lower field (11.82) because it is hydrogen-bounded. A typical meta coupling (J = 1.5 Hz) is assigned to the two aromatic protons.

In dimers of 7-methyljuglone having two free quinoid protons these appear as a singlet unless a bulky group is attached at C-8. However, when such a group is present the two protons appear as AB quartet (J = 10.0 Hz).



1

Lillie and Musgrave[58] reported the significance of the chemical shifts of the 5-OH proton of juglone derivatives in structure elucidation. They noted that the position of the signal for the 5-hydroxy-proton depends on the nature and location of the substituents present. The changes in the value of the 5-hydroxy-proton arises from the additive effect of the indi-

vidual substituents. These effects, in terms of numerical values, were calculated by substracting the juglone hydroxy-proton shift (δ 11.87) from that of the monosubstituted juglone or, in some cases, the shift of a substituted juglone from that of a juglone with one more substituent group. The substituents effects are shown in Table 3.

Table 3. Contribution by substituents to the chemical shift of C-5 hydroxy-proton in juglone

	Contribution (ppm, in $CDCl_3$)				
Substituent	C-2	C-3	C-6	C-7	C-8
Me	+0.6	+0.14	+0.35	-0.05	
Cl	-0.9	-0.21	+0.75		+0.69
Br	-0.9	-0.22			
OH	+0.41	-0.83	+0.20		+0.51

As the majority of the naphthoquinones isolated from plants belonging to the Ebenaceae are the oligomers of 7-methyljuglone (1), with an additional few based on 2-methyljuglone (plumbagin), deduction of the various linkages between the monomers is very important with respect to structural elucidation. From the published ^{1}HNMR[3] data of 7-methyljuglone dimers, attempts have been made to correlate the chemical shift of the 5-hydroxyproton with the mode of linkage of the dimer.

Thus, if the linkage occurs through the benzenoid ring (C-6 or C-8) of one of the two 7-methyljuglone units, the 5-hydroxyproton of that moiety resonates at a lower field, with a δ value $\gtrsim$ 12.0. In contrast, if the linkage is on the quinoid ring (C-2 or C-3), the value of the 5-hydroxyproton of this unit could be less than 12. Correlation of 5-hydroxy-proton with various kinds of linkage is depicted in Table 4.

Table 4. Contribution of different linkage position to the chemical shift of C-5 hydroxy-proton of 7-methyljuglone in dimeric derivatives

	Contribution (ppm, in $CDCl_3$)			
Linkage type	C-2	C-3	C-6	C-8
Q-Q	-0.02	-0.13		
B-B			+0.20	+0.58
Q-B	+0.07	-0.12	+0.29	+0.46

\+ Hydroxy proton in 7-methyljvglone as 11.82

++ Q = quinoid ring; benzenoid ring

On top of this, one can use the resonance position of 7-methyl protons for obtaining information regarding the linkage type (Table 5).

Table 5. Chemical shifts of 7-methylprotons of dimeric derivatives with different linkage

Linkage Type	Value ($CDCl_3$)
Q-Q	2.45 ± 0.03
Q(a)-B(b)	(a)2.44 (b)2.30
B-B	2.02 ± 0.03

1.4 The Objective of this Study

The genus Euclea has only attracted the attention of phytochemists since 1969 when the first study of naphthoquinones of Euclea sp. appeard. Since then many naphthoquinones have been isolated from E. lanceolata, E. natalensis, E. divinorum, E. schimperi, E. pseudebnus, E. crispa, E. undulata etc.. Some of these naphthoquinones were reported as new natural products.

The genus Euclea, unlike Diospyros which is the largest genus of the family with about 500 species, has few species under it. The botanical information reveals that the genus consists of not more than 20 species. The distribution of the species of this genus is mainly in Africa particularly in the southern part of the continent. In contrast, Diospyros species are widely distributed in Asia and Africa. In Ethiopia there are two indigenous Euclea species

namely E. schimperi (A.DC) Dandy and E. divinorum Hiern.

In this study we have reinvestigated E. schimperi (A.DC) Dandy. The plant is widely distributed in Ethiopia and is known in Amharic as "Dedeho". It is used in traditional medicine as remedy for Gonorrhea in southern part of the country. The only phytochemical report on the plant in the literature was by Van der Vijver et al[49] on the roots, stems, and fruits of E. schimperi (A.DC) Dandy var dephnoids (Hiern) De Winter collected from South Africa. The study resulted in the isolation of 4 naphthoquinones namely 7-methyl-juglone (1), mamegakinone (21), diospyrin (18), and iso-diospyrin (20). Although pentacyclic triterpenes have been reported from the family, Van der Vijver et al[49] did not report from any part of the plant investigated. The study claimed also the absence of naphthoquinones from the stem.

The main objective of the present study is to re-investigate different parts of the plant because of its importance in traditional medicine of Ethiopia.

2. RESULTS AND DISCUSSION

Preliminary screening of the EtOAc (root barks and chloroform (stem barks) extracts of Euclea schimperi (A.Dc) Dandy collected from Merha Bete (Shoa region) indicated the presence of a number of naphthoquinones and triterpenes after spraying with alc. KOH and Lieberman-Burchardt reagents, respectively.

The EtOAc extracts of three different portions of the root barks of E. schimperi under reduced pressure yielded each a residue which after chromatographic separation on a silical gel column (see Experimental part) afforded seven naphthoquinones (N-1 - N-7) and two triterpenes (T_1 and T_3). Flow chart for the extraction and separation method is depicted in Fig. 2. Similarly the chloroform extract of the stem bark gave after the usual chromatographic separation one naphthoquinone (N-7) and the triterpenes T_1 and T_2 (see flow chart in Fig. 3.).

Five of the naphthoquinones N-1, N-2, N-3, N-4, and N-7 are known compounds which correspond to 7-methyljuglone (1), neodiospyrin (23, 2-methylnaphthazarin (8), mamegakinone (21), and isodiospyrin (20), respectively. We report here the occurrence of N-2 and N-3 in E. schimperi for the first time. One of the rare dimers N-2 or neodiospyrin (23) isolated here is a new record for the genus Euclea. The remaining three known compounds mentioned above have already been reported from the plant

by Van der Vijver et al.[49] Eventhough Van der Vijver's work showed the absence of any naphthoquinone in the stem bark of the plant, we were able to isolate isodiospyrin (N-7) from the stem bark of the plant.

The remaining two naphthoquinones, N-5 and N-6, have not been elucidated due to lack of necessary data. Preliminary data on N-5 is presented in the text. Regarding N-6, no data is sofar generated due to solubility as well as volatility problems. But the methylether derivative of N-6 gave ^{1}H NMR but failed to give EIMS which hampered the structure elucidation at present.

Out of the three triterpenes T-1 is identified as lupeol which has not been reported from the parts of the plant we have investigated. The remaining two triterpenes have been sent for high resolution NMR and MS.

The characterization of these compounds have been done mainly by spectroscopic methods. The spectral and physical data of the known compounds isolated have been compared with those reported in the literature. In some cases characterization was augmented by direct comparison with authentic samples.

Table 6. R_f and colour reaction of the compounds isolated using solvent system II, P.

Spots	R_f	Colour reaction with alcoholic KOH	Compound
N-1	0.63	Pink	7-methyljuglone
N-2	0.56	Pink	Neodiospyrin
N-3	0.65	Blue	2-methylnaphthazarin
N-4	0.62	Violet	Mamegakinone
N-5	0.29	Purple	-
N-6	0.60	Pink	-
N-7	0.49	Purple	Isodiospyrin
T-1	0.40	-	Lupeol
T-2	0.23	-	-
T-3	0.22	-	

Fig. 2. Flow Chart for the Extraction and Isolation Procedure of the Root Bark of E. schimperi.

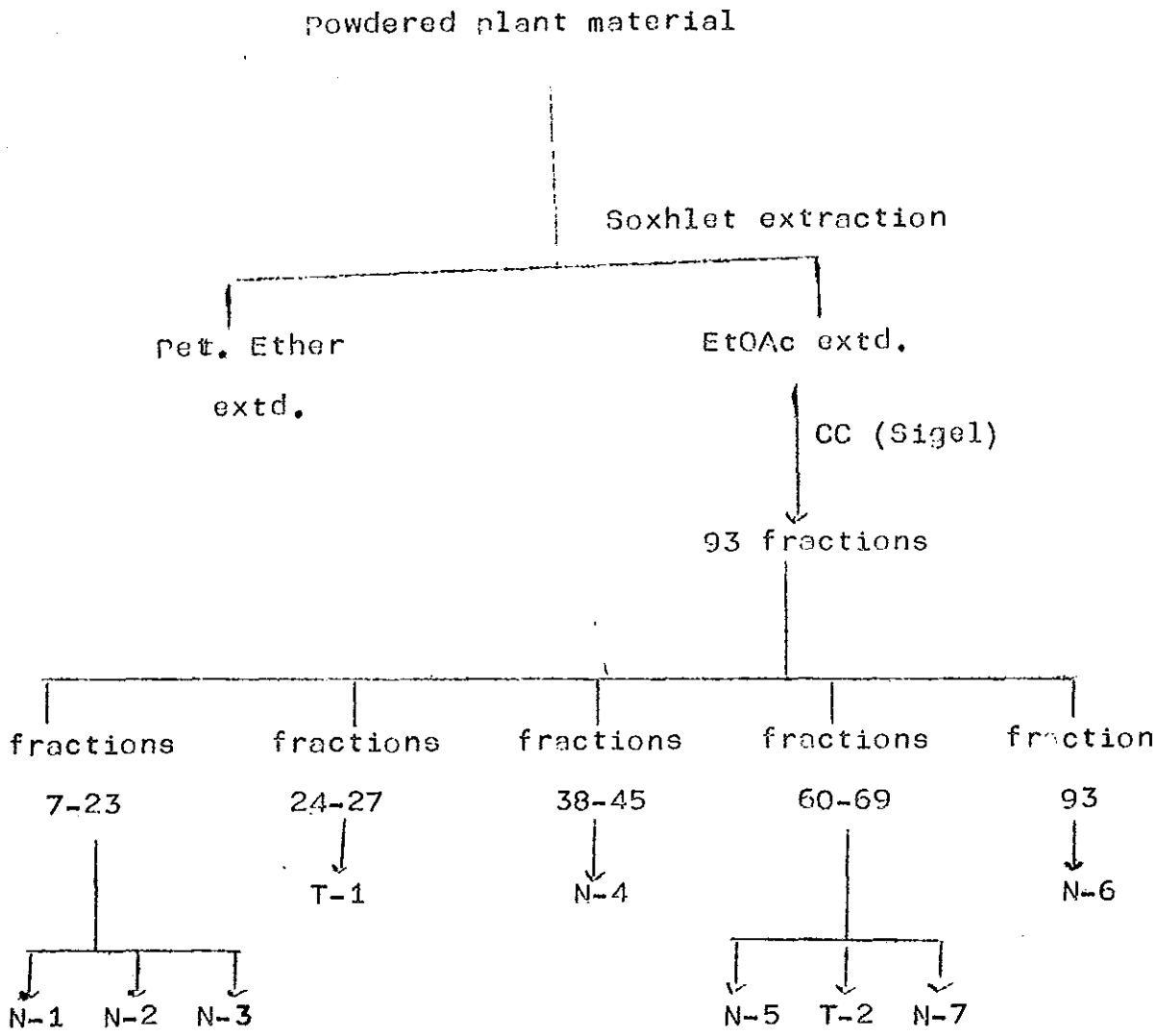
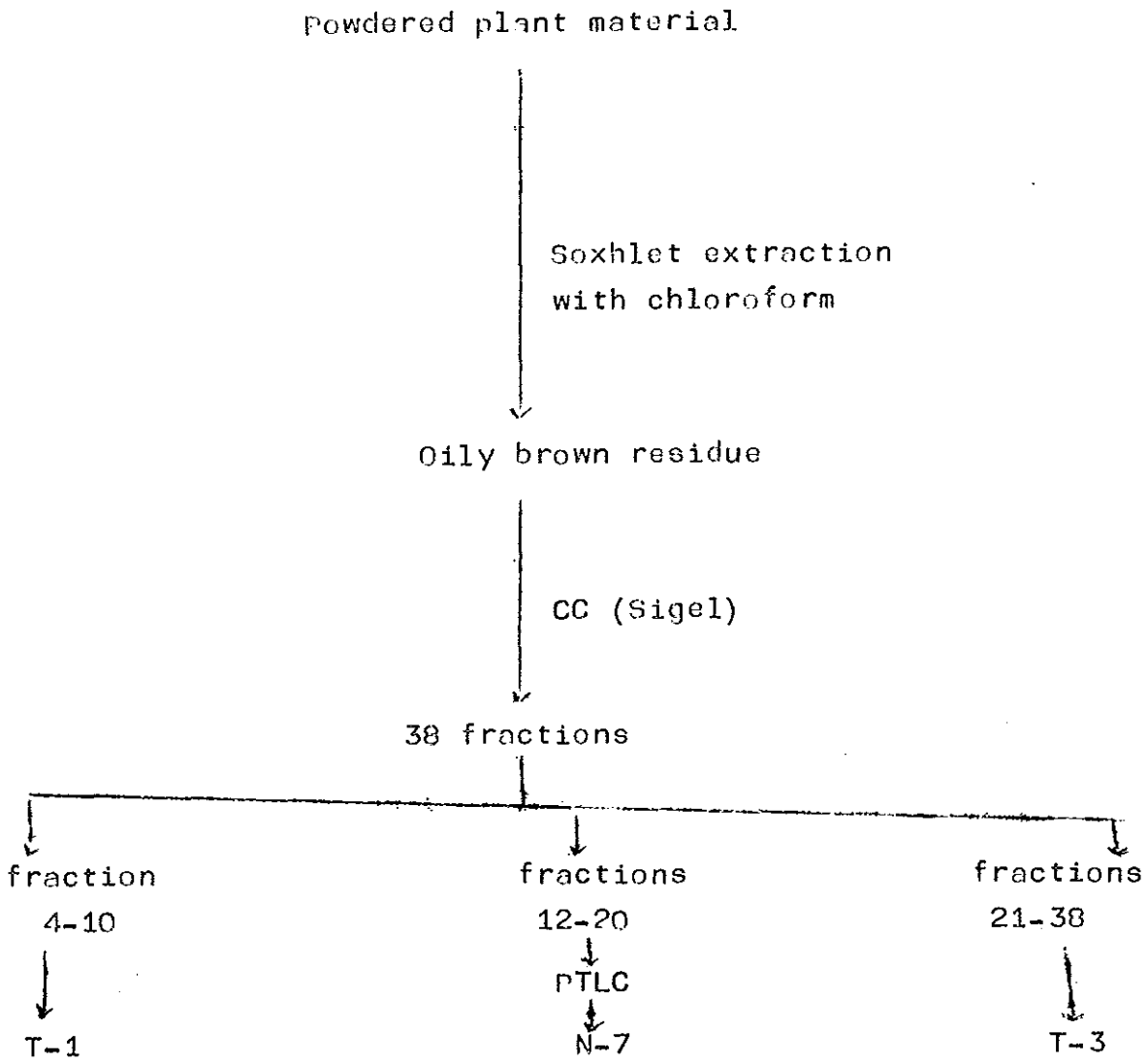


Fig. 3. Flow Chart for the Extraction and Isolation Procedure of the Stem Bark of *E. schimperi*



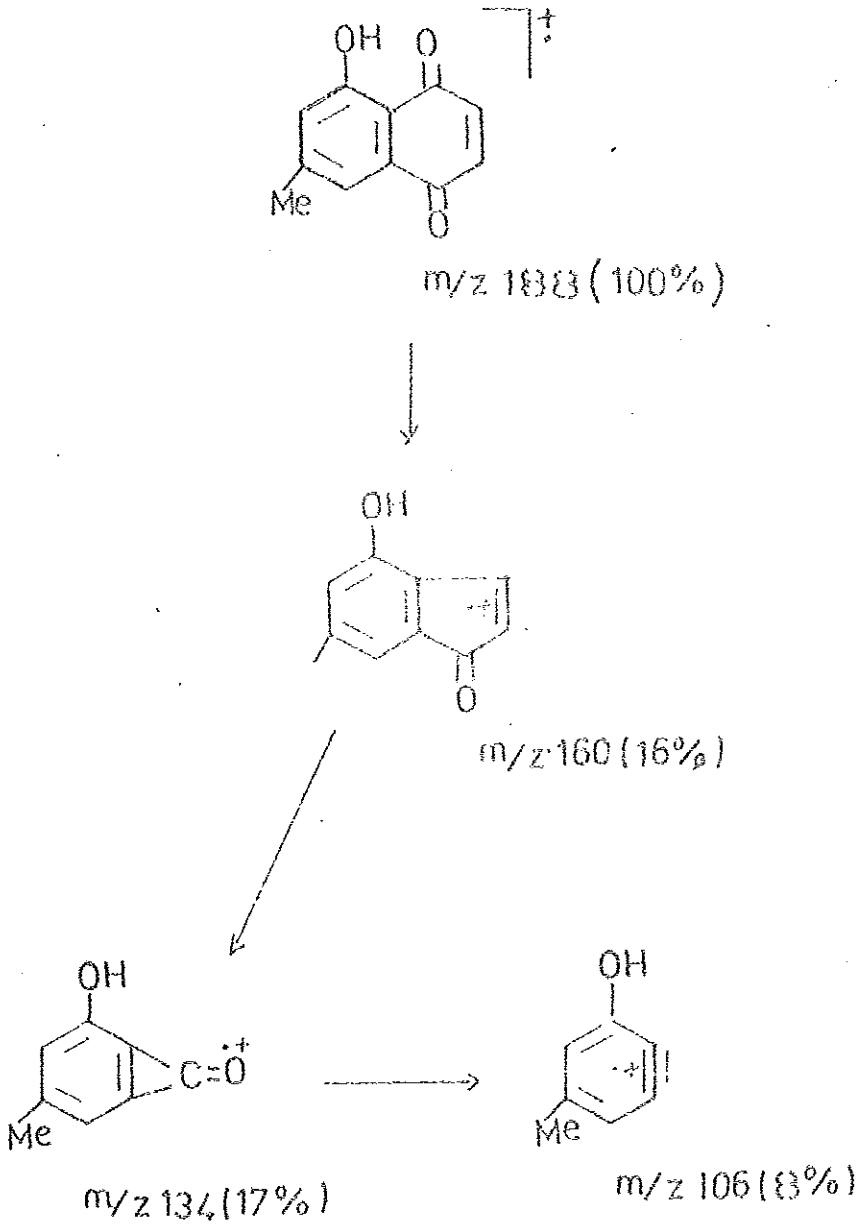
2.1 Characterization of N-1 as 7-methyljuglone (1)

It is a reddish compound, recrystallized from n-hexane, mp 122-124°. The UV spectrum showed absorption maxima at 218, 253, and 423 nm. These maxima shifted bathochromically to 263, 292 sh, and 539 nm when sodium hydroxide solution was added. These values showed a 1,4-naphthoquinone moiety. The IR (KBR) spectrum showed characterstic bonds at 1635 and and 1680 cm^{-1} which were due to hydrogen bonded and free carbonyl functions, respectively.

The ^1H NMR (90 mHz, $CDCl_3$) spectrum (Fig. 4) showed a singlet at δ 2.50 (3H) which corresponds to aromatic methyl group. A two proton singlet at δ 6.97 are assignable to quinoid protons. The two broad singlets at δ 7.15 (H) and δ 7.51 (H) can be assigned to two aromatic protons which are meta to each other. The down field chemical shift of the latter proton relative to the other one was caused by the carbonyl function which is peri to the proton. A down field singlet at δ 11.89 is attributable to the bonded hydroxy function.

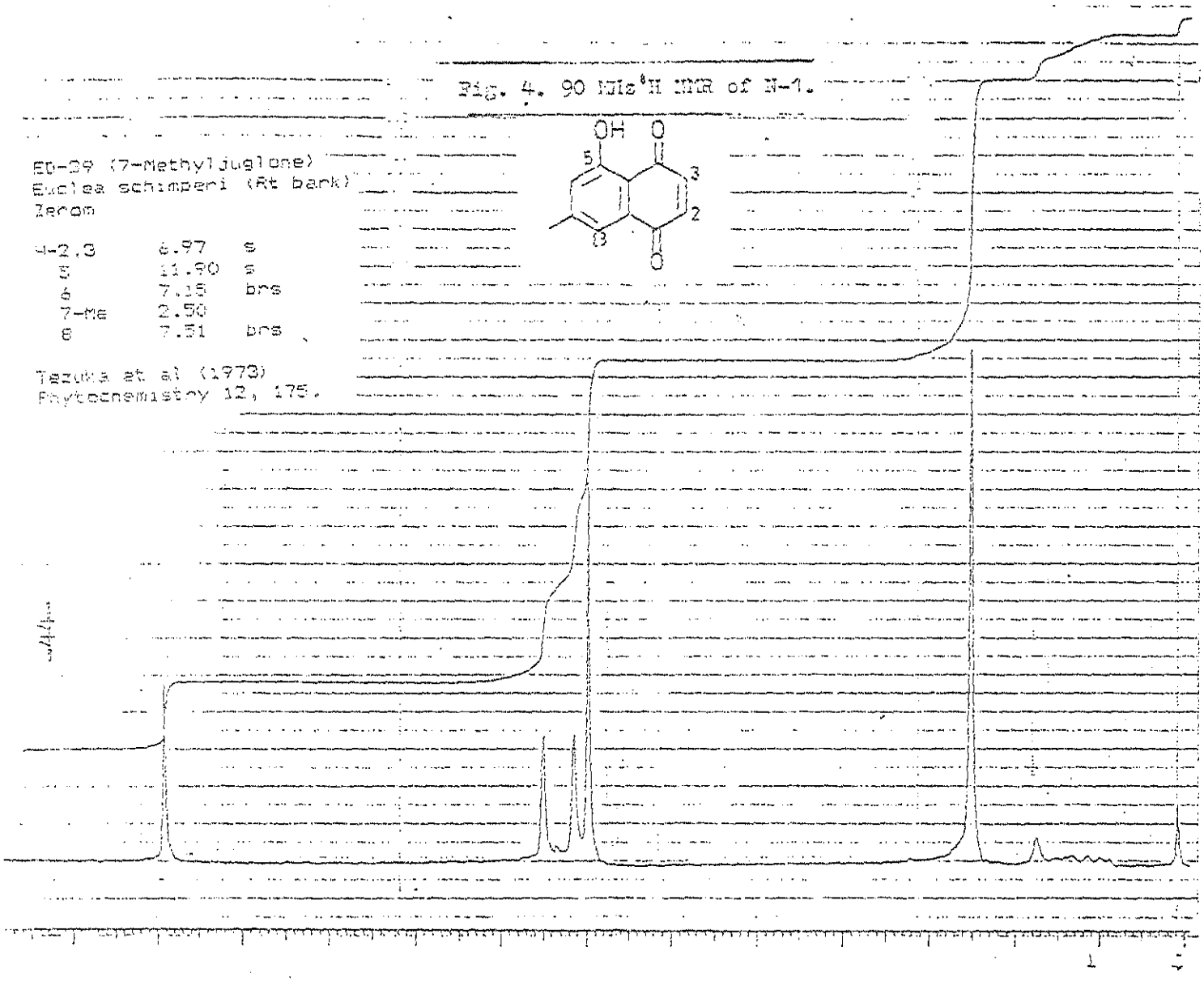
The HRMS revealed the molecular formula as $C_{11}H_8O_7$ with a molecular weight of 188. The major fragment ions appeared at 160 (M^+-CO), 134 (M^+-CO-C_2H_2), and 106 (M^+-2CO-C_2H_2). The fragmentation pattern of N-1 is shown in Scheme 7.

All the above spectroscopic data comply with



Scheme 7. EIMS fragmentation pattern of M-1.

Fig. 4. 90 MHz ^{1}H NMR of N-1.

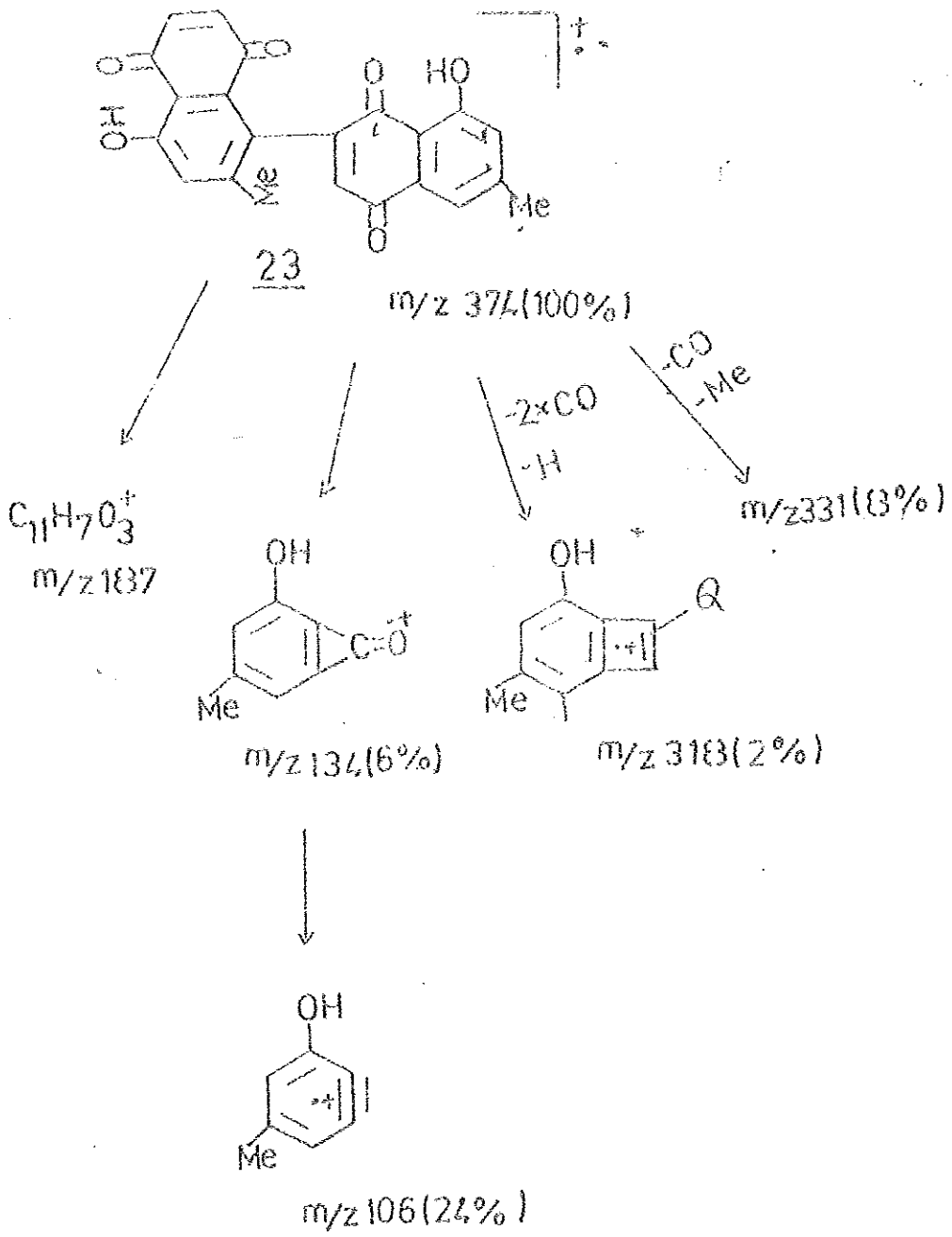


7-methyljuglone (1) which has been reported from many Diospyros and Euclea species. Its occurrence in E. schimperi was reported by Van der Vijver.[49]

2.2 Characterization of N-2 as Neodiospyrin (23)

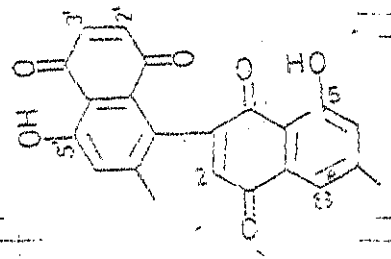
The compound is reddish-orange in colour, decomposed at 204-209°. The UV-visible spectrum had absorption maxima at 248, 292, 350 sh, and 425 nm which subsequently shifted bathochromically to 292 and 528 nm upon addition of sodium hydroxide solution which is consistent with a compound with the juglone chromophore. The IR (KBr) spectrum whowed three bands at 3450, 1658, and 1640 cm^{-1} which are indicative of the presency of hydroxy, cheleted and nonchelated carbonyl functions, respectively. The HRMS suggested the formula as $C_{22}H_{14}O_6$ (MW=374). This spectrum revealed major ions at m/z 318 (2%), 331 (8%), 134 (6(%), and 106 (24%) which are expectable fragment ions of 7-methyljuglone dimer (Scheme 8).

The ^1H NMR (90 MHz, $CDCl_3$) spectrum of N-2 (Fig. 5) exhibited singlets at δ 2.30 and δ 2.47 that are assignable to two benzenoid methyl groups. A singlet at δ 6.61 (1H) is typical of a quinoid proton and a singlet at δ 7.12 (1H) is due to aromatic proton of 7-methyljuglone and it suggested that the linkage in the dimer is from C(8') to either C(2) or C(3). Further support for the inter-



Scheme 8. EIMSfragmentation pattern of N-2.

Fig. 5. 90 MHz ^{1}H NMR of N-2



ED-40 (Neodiospyrin)
Euclea schimperi (Rt bark)
Zeroa

H-2	6.61	s	
2',3'	6.78,6.93	ABq	10.1
6	7.15	brs	
8	7.54	brs	
6'	7.26	s	
5-OH	11.4	s	
5'-OH	12.22	s	
7-Me	2.47	s	
7'-Me	2.30	s	

Zakaria, et al (1984)
Phytochemistry 23, 1481.

ED-40

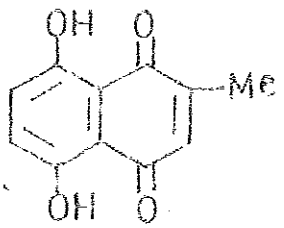
nuclear linkage involving the C(8') position is by the appearance of an AB quartet positioned at δ 6.78 (1H) and δ 6.93 (1H) with coupling constants of 10.1 Hz due to C(2') and C(3') protons, respectively. One could envisage also the arene-to-quinone linkage from the chemical shifts of the two methyl group as depicted in Table 5. The two broad signals at δ 7.25 (1H) and 7.54 (1H) are assignable to meta coupled aromatic protons. The downfield singlets at δ 11.74 and δ 12.28 are due to the hydrogen-bonded hydroxyl protons at C(5) and C(5'), respectively. Lillie[58] has shown that C(5) hydroxy group in 7-methyljuglone system has a chemical shift of 11.72 when an aryl substituent is at C-3 of this moiety. Since δ 11.74 assigned to one of the hydroxyl group in N-2 is very close to the value 11.72 obtained from the work of Lillie, it seems very likely that the linkage in N-2 is C(8') - C(3).

The above presented evidence is consistent with the structure assigned for neodiospyrin (23). To our knowledge, we isolated it here for the first time from the genus Euclea. The compound has previously been reported from different Diospyros species. This was confirmed further from comparison with authentic sample.

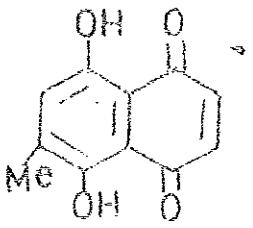
2.3 Characterization of N-3 as 2-Methylnaphthazarin (8)

The ^{1}H NMR (90 MHz, $CDCl_3$) spectrum of N-3 (Fig. 6) revealed a quartet (J 1.4 Hz) at δ 6.91 (1H) which is attributable to a proton which is allylically

coupled with a methyl group. Such allylic coupled is observed in compounds such as plumbagin (2), 14, and 36. A doublet (J 1.4 Hz) at 2.25 (3H) is assigned to a methyl group which is coupled with the proton at 6.91. The chemical shift at 7.21 (2H) is due to protons which appeared as singlet. The two downfield chemical shifts at 12.58 and 12.47 are assigned to two chelated hydroxyl groups. Based on these data, one can propose two alternative structures which are designated as A and B.



A



B

In agreement with this the HRMS suggested the molecular formula as $C_{11}H_8O_7$ (MW=204). Important fragment ions appeared at m/z 189 (M^+-CH_3), 148 (M^+-2CO), 176 (M^+-CO), 136 ($M^+-CO-C_3H_4$), and 108 ($M^+-2CO-C_3H_4$). The ions at m/z 136 and 108 are normally observed if the dihydroxy aromatic ring of naphthazarin system[57] has no substituents. These ions fragment only from structure A or 2-methylnaphthazarin. Further support comes from the absence of ions at m/z 122 and 150 which otherwise, would come from structure B. The fragmentation pattern is shown

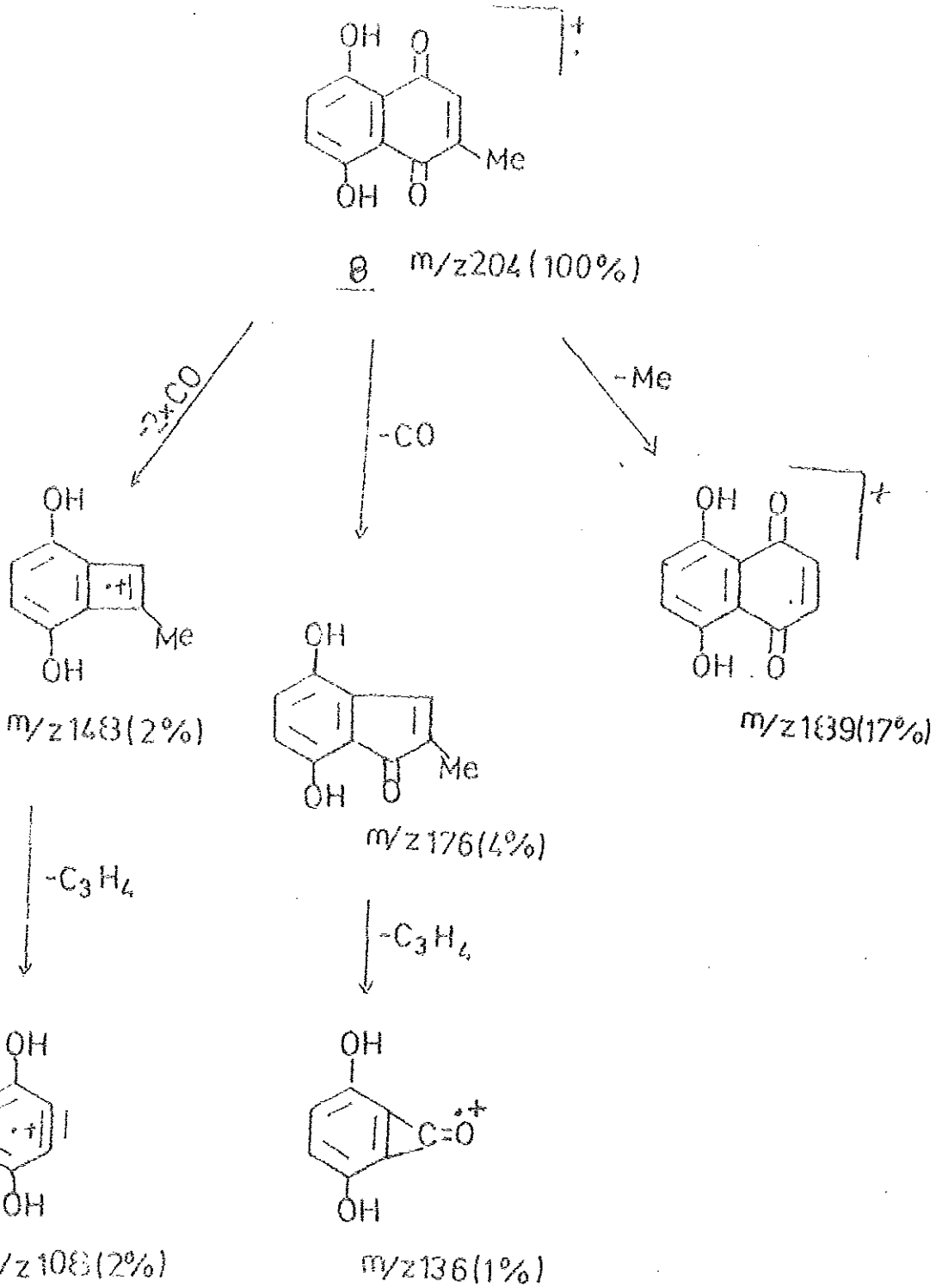
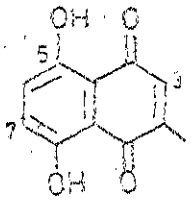

OH
O
Me
OH
O
+·
8
m/z 204 (100%)
-2×CO
-CO
-Me
OH
+·
Me
OH
m/z 148 (2%)
OH
Me
OH
O
m/z 176 (4%)
OH
O
+·
OH
O
m/z 189 (17%)
-C3H4
-C3H4
OH
+·
OH
m/z 108 (2%)
OH
C=O+·
OH
m/z 136 (1%)

Fig. 6. 90 MHz ^{1}H NMR of E-3.



ED-48 (2-methylnaphthazarin)

H-3	6.71	q	1.41 Hz
6,7	7.21	s	
2-Me	2.25	d	1.41
8-OH	12.47	s	
5-OH	12.58	s	

Ferreira, et al (1972)
Phytochemistry 11, 2352.

Zero
FT-34-218-1
(Proton (data))
NUCLEUS Z-34-220
ED-48
SOLVENT
CONCENTRATION
REFERENCE
TEMPERATURE
PULSE
DATA POINTS
WINDOW
NO OF PULSES
SPECTRAL WIDTH 1350
AMPLITUDE
DECOUPLING
DATE 27/10/87
OPERATOR
REMARKS ADRES
E. Schreier

JEC LTD

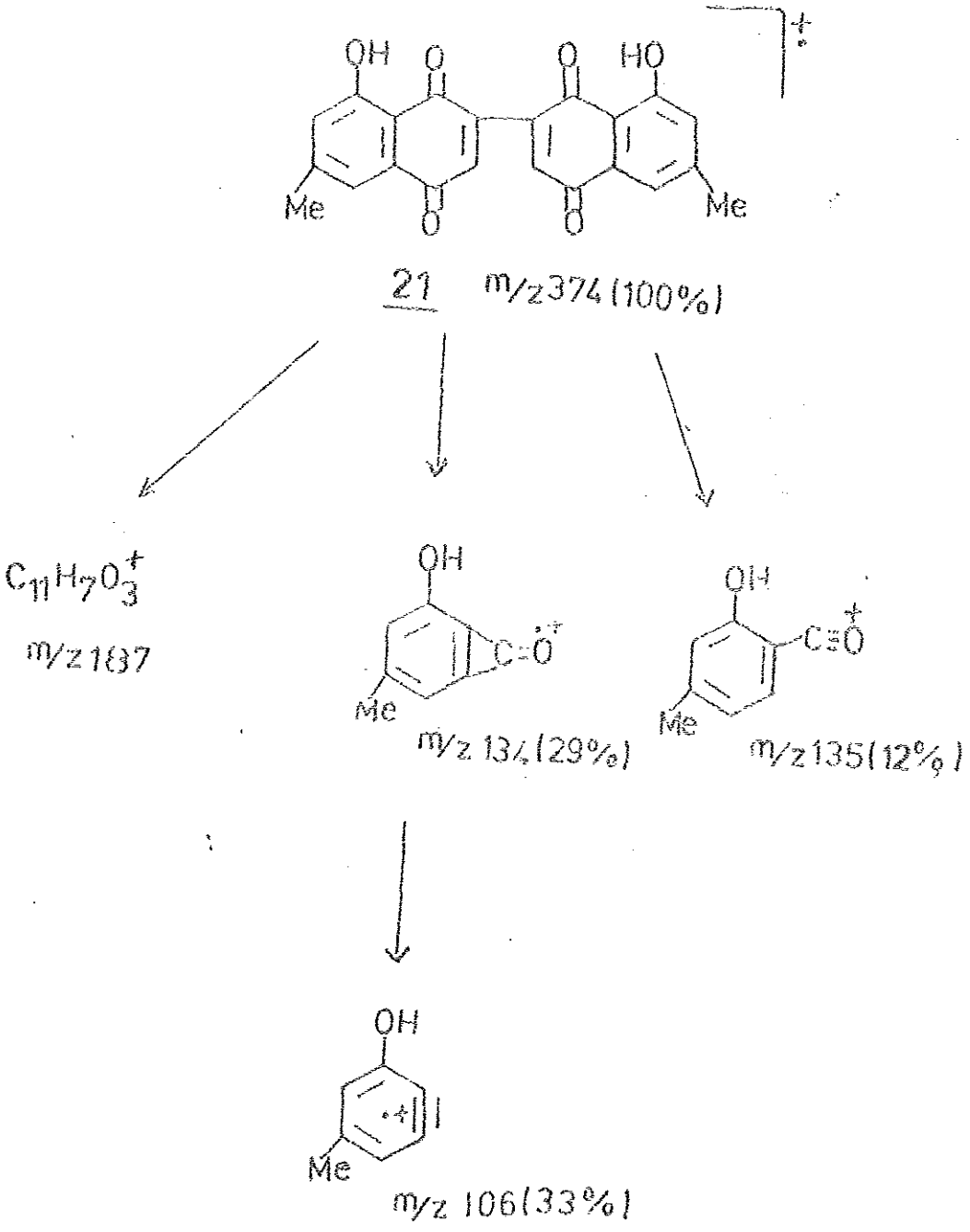
in Scheme 9.

The foregoing spectral data are compatible with 2-methylnaphthazarin (8). The compound has been previously isolated from D. lyciodes sericea,[19] D. heterotricha,[19] and E. lanceolata.[44] Both the ^{1}H NMR and HRMS information are also in close agreement to the value reported for 2-methylnaphthazarin (8) in the literature[19] except low ion intensities in the MS. There is no prior report on the occurrence of this compound in E. schimperi.

2.4 Characterization of N-4 as Mamegakinone (21)

It is orange in colour which crystallized from chloroform, mp 251-253°. The UV-visible spectrum exhibited absorption maximum at 225, 251, and 440 nm which showed bathochromic shift upon addition of sodium hydroxide. The IR (KBr) spectrum showed signals at 3465, 1645, and 1670 cm^{-1} which indicated the presence of hydroxy, cheleted and non-cheleted carbonyl functions, respectively. The HRMS suggested the molecular formula as $C_{22}H_{14}O_7$ (MW=374). The significant fragment ions at m/z 135, 134, and 106 (Scheme 10) which are produced by the cleavage of an internal quinone ring of 7-methyljuglone unit.

The ^{1}H NMR (90 MHz, $CDCl_3$) spectrum (Fig. 7) indicated a symmetrical dimer of 7-methyljuglone. A broad singlet at δ 7.13 (2H) is assignable to aromatic



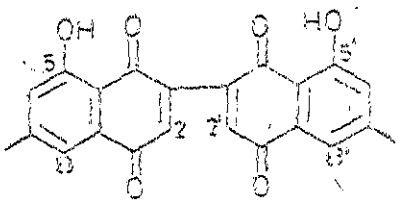
Scheme 10. EIMS fragmentation pattern of N-4.

ED-50 (Nanegakinone)
Euclea schimperi (Rt bark)
Zeron

4-2,2'	6.98	s	
6,6'	7.13	brs	
8,8'	7.50	d	1.4
7,7'-Me	2.46	s	
5,5'-OH	11.74	s	

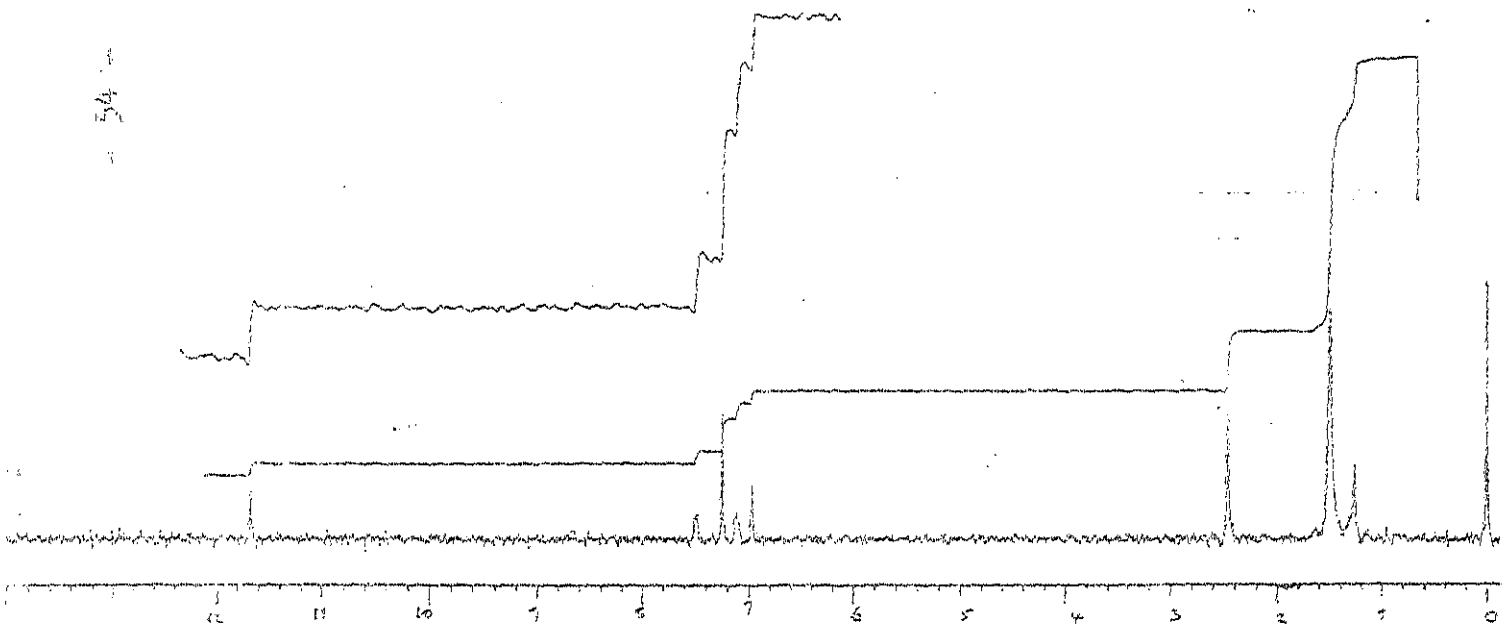
Yoshihira et al (1970)
Tetrahedron Lett. 7.

Fig. 7. 90 MHz ^{1}H NMR of K-4.



Zeron
ZT-34-238-fr.
ED-50
40 scans
45°

CDCl$_3$



E. Schimperi

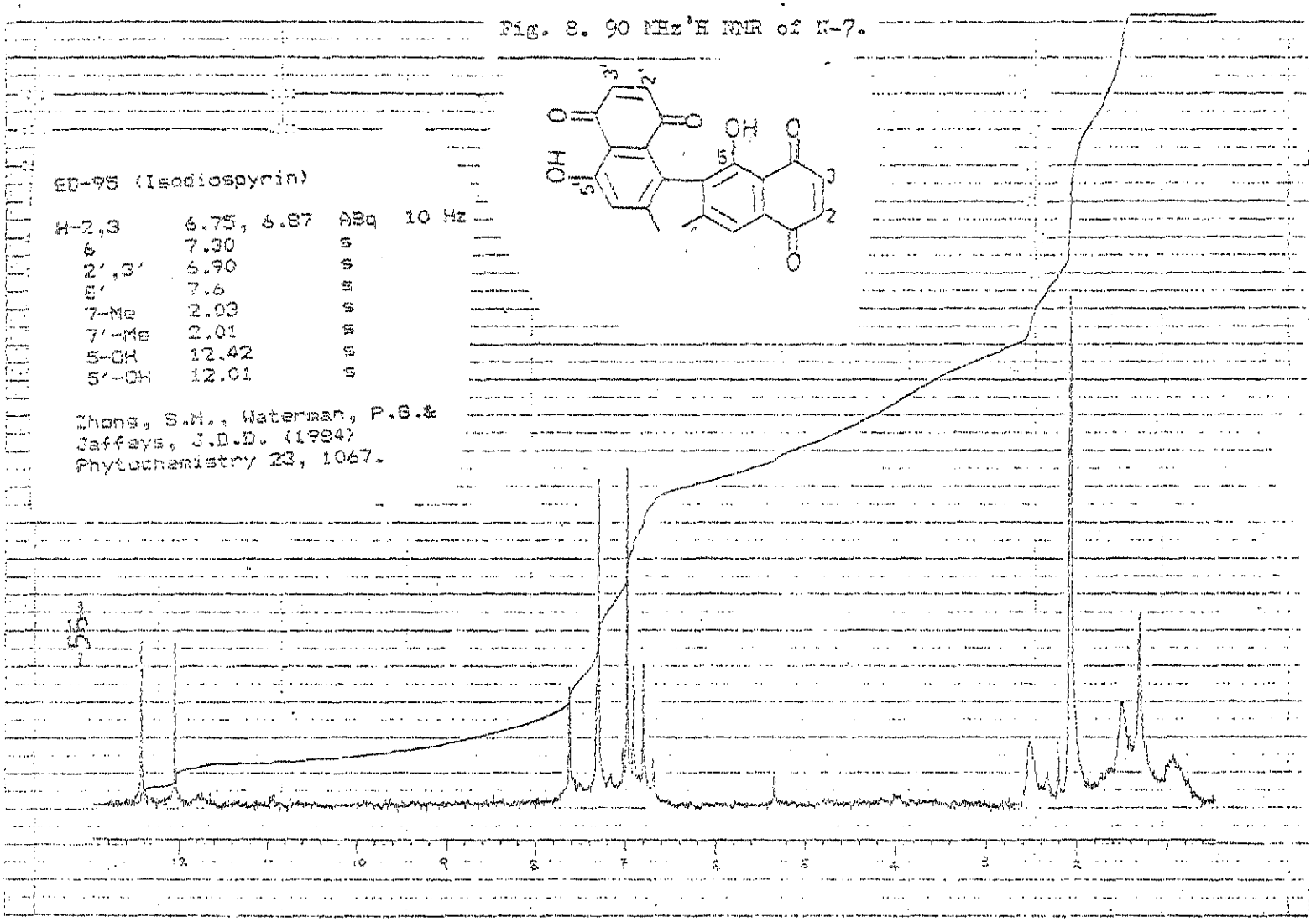
protons. A doublet (J 1.4 Hz) at δ 7.50 (2H) are due to aromatic protons which are peri to the carbonyl function. The singlet at δ 2.11 (6H) are attributed to methyl groups in the aromatic ring. The downfield chemical shift at δ 11.72 are due to two cheleted hydroxy group. These data suggest the linkage in the dimer to be quinone-to-quinone either through C(2)-C(2') or C(3)-C(3'). The two alternative mode of linkage correspond to biramentaceone (17) and mamegakinone (21), respectively. The position of linkage can be obtained from the chemical shift of the C-5 hydroxy protons of 7-methyljuglone substituted with aryl group. An aryl substituent at C-2 of the 7-methyljuglone system would[58] be expected to give a a shift at δ 11.89 while a similar substituent at C-3 would produce a hydroxy-proton shift of 11.70. The value observed for N-4 namely 11.72 is close to 11.70 which implies that the link between the two units in the dimer involves C(3)-C(3').

The spectral data presented above conforms with mamegakinone (21). Further support also comes from difference in tlc comparison with authentic biramentaceone (17). This compounds has been reported from many Diospyros species. Van der Vijver[49] also reported its occurrence in E. schimperi.

2.5 Characterization of N-7 as Isodiospyrin (20)

The compound forms orange plates from chloroform,

Fig. 8. 90 MHz ^{1}H NMR of K-7.



mp 225-228°. The UV had absorption maxima at 252 and 428 nm. These maxima shifted bathochromically to 293 and 540 nm upon addition of sodium hydroxide solution. IR (KBr) spectrum like other dimers revealed bands at 3400, 1660, and 1640 cm^{-1} which are due to hydroxyl, non-cheleted and cheleted carbonyl functions, respectively.

The ^1H NMR (90 MHz, $CDCl_3$) spectrum (Fig. 8) of N-7 exhibited the presence of four vinylic protons and two of these appear as a singlet at 6.90. The other two are well separated from each other and appeared as an AB quartet at δ 6.75 and δ 6.87 with a spin-spin coupling constant of 10 Hz. It is thus obvious that neither of the quinone rings is involved in the linking of the two naphthoquinone moieties. Also there is a signal at δ 7.6 (H) for only one downfield proton in a peri position to the carbonyl and the linkage must, therefore, be between the peri position of one naphthoquinone and the C-6 position of the other naphthoquinone moiety. The remaining one proton at δ 7.3 can be assigned to a proton flanked by a methyl and hydroxyl group. The two downfield chemical shifts at δ 12.42 and δ 12.01 are assignable to two cheleted hydroxyl group. These hydroxyl group shifts are consistent with the value given for dimers having arene-to-arene linkage as discussed in the text.

The compound showed the same tlc profile when compared with authentic isodiospyrin sample. Its occurrence in

E. schimperi was reported by Van der Vijver.[49]

2.6 Preliminary Data an N-5

The compound forms yellowish plate from chloroform, mp 127-129°. The UV spectrum showed absorption maxima at 405 sh, 378 sh, 351, and 238 nm which are indicative of naphthoquinone moiety. The IR (KBr) revealed the presence of cheleted and non-cheleted carbonyl functions from the bands at 1620 and 1650 cm^{-1} respectively.

The ^1H NMR (90 MHz, $CDCl_3$) spectrum (Fig. 9) of N-5 exhibited two cheleted hydroxyl functions at δ 11.40 and δ 11.43. Two methyl group signals appeared as singlets at δ 2.2 and δ 2.24. Two proton singlets are observed at δ 2.98 and δ 3.0. The other signals outside the aromatic region are a triplet centered at δ 4.1 (1H) and a broad singlet at δ 4.75 (1H).

Further signals in the ^1H NMR are five singlet signals between 6.5 and 7.43 which are signals found in 1,4-naphthoquinones.

The HRMS spectrum of N-5 suggested the molecular formula as $C_{22}H_{16}O_6$ (MW=376). The other major fragment ion appeared at m/z 375 (5%), 359 (40%), 358 (100%), 338 (64.8%), 382 (22%), 188 (12.2%), 135 (11.1%), 134 (8.7%), and 106 (11.2%). The latter three fragment ions suggest that N-5 is a

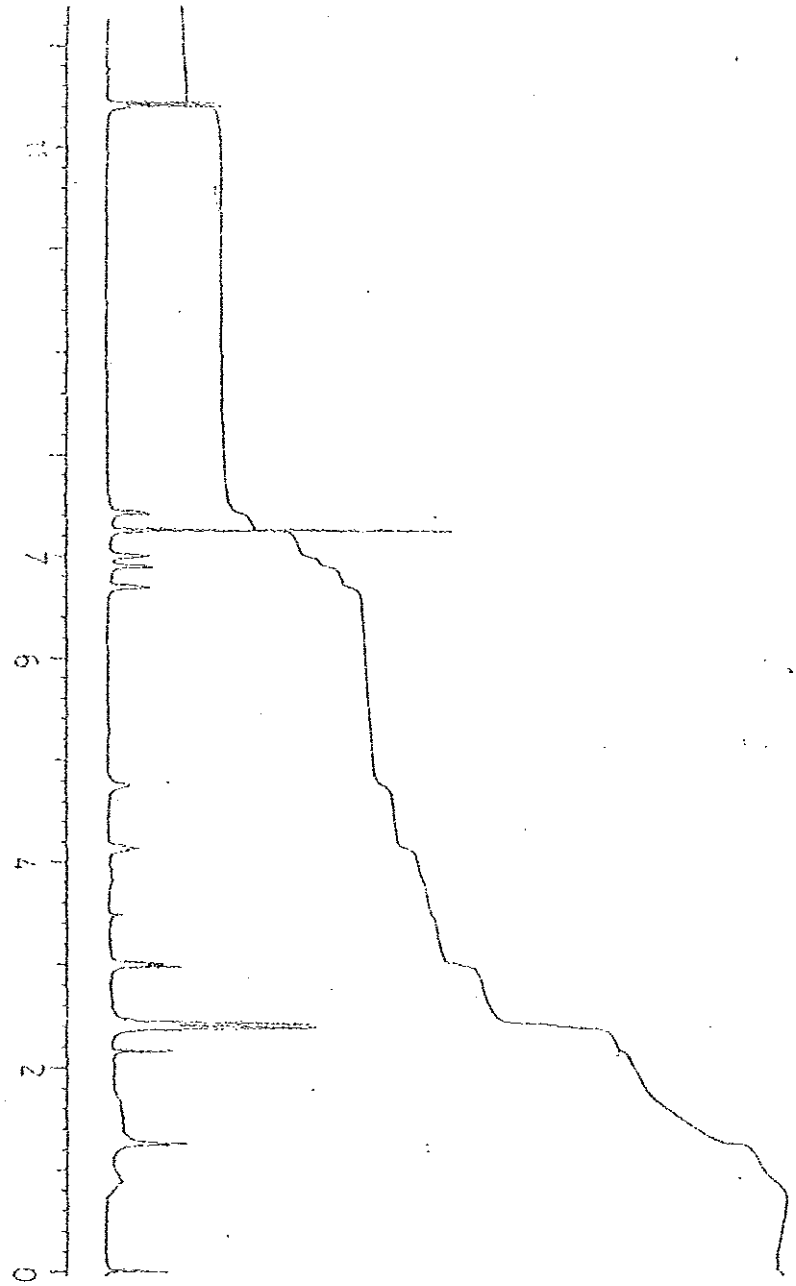


Fig. 9. 90 MHz ^{1}H NMR of E-5.

dimer of 7-methyljuglone.

2.7 Identification of T-1 as Lupeol (57)

Colourless needles from methanol, mp 270-214°. The compound was found identical (TLC, IR, mixed mp) with authentic lupeol.

3. EXPERIMENTAL

3.1 Materials and Apparatus

Melting points were determined on Kofler hot stage apparatus. IR spectra were measured for potassium bromide discs on Perkin-Elmer Infra-red Spectro-photometer model 727B, UV-visible absorption spectra for ethanolic solutions with Perkin-Elmer model 555 spectrometer, ^{1}H NMR for solutions in deuteriochloroform with Jeol 90 MH_z and 250 MH_z spectrometers (tetramethylsilane as internal standard). The mass spectra were recorded on a high resolution mass spectrometer at 70 ev using direct probe insertion.

Analytical thin layer chromatogram were run on a 0.25 mm thick layer of silica gel and the products isolated were detected by their UV fluorescence and by spraying with 5% methanolic potassium hydroxide (naphthoquinones) or Lieberman-Burchardt reagent (triterpenes). Preparative layer chromatography was run on 2 mm thick layer of silica gel. Column chromatography was performed on silica gel 60 (70 - 230 mesh) and Sephadex LH-20.

3.2 Solvent Systems

The following solvent systems were used for thin layer, preparative layer, and column chromatography. The solvent systems are:

I. Petrol^{+}- EtOAc - HOAC (48:1:1)

II. Petrol^{+}- EtOAc - HOAC (46:3:1)

III. Methanol - chloroform (1:1)

IV. Benzene: chloroform (1:9)

V. Petrol^{+}- chloroform mixtures

VI. Petrol^{+}- EtOAc mixtures

$^{+}$Fraction with a boiling point of 60 - 80^{o}C.

3.3 Plant Material

Euclea schimperi (A.DC) Dandy, roots and stems, were collected in 1987 from Shoa region near Merha Bete. A voucher specimen is kept at the National Herbarium, Biology Department, Addis Ababa University under the Cipher Mesfin T. 6946.

3.4 Extraction and Isolation of Compounds

3.4.1 Extraction and Isolation of Compounds from the Root Bark of E. schimperi (A.DC) Dandy

A. Dried sample (70 g) of the root bark was extracted in Soxhlet apparatus with EtOAc for 18 hrs. The extract was filtered and concd. under reduced pressure to give 2.5 g of oily brown residue. The residue was subjected to vacuum liquid chromatography (VLC) and eluted with petrol-chloroform mixture (solvent system V) of increasing polarities to

give 28 fractions each 100 ml.

Fractions 5 - 7 (20% chloroform) and 14 - 16 (50%) chloform) were combined and separately applied on silica gel columns (60 g each). Eluting with petrol and mixtures of petrol-chloroform of gradually polarities afforded from the former 3 mg of N-1 (10% chloroform) and N-2 (2 mg) from the latter (15% chloroform).

B. A second portion of 300 g of the root of the plant material was extracted on Soxhlet first with petrol (60 - 80^{o}) followed by EtOAc. The EtOAc extract extract was evaporated to dryness and the resulting residue (10 g) was chromatographed on silica gel column (250 g) eluting with petrol/chloroform (solvent system V) mixtures of increasing polarities. Ninety three fractions each 75 ml were collected.

Fractions 7 - 23 (30% chloroform) by PTLC (2 mm silica gel plate, solvent system I) displayed 3 bands. N-3 (2 mg) was obtained from the band with the highest R_f value. N-2 (4 mg) was obtained from the bond with the lowest R_f and N-1 (5 mg) was obtained from the middle band.

Fractions 24 - 27 (30% chloroform) afforded T-1 (20 mg) which was further recrystallized from MeOH.

Fractions 38 - 45 (30% chloroform) after applying to a silica gel column and eluting with increasing polarities of petrol/EtOAc afforded (solvent system VI)

N-4 (3 mg) in the 20% EtOAc eluting mixture.

The residue obtained from fractions 60 - 69 (40% chloroform) was further purified by PTLC (solvent system II) followed by passing through a Sephadex LH 20 column (solvent system III) to yield N-5 (3 mg and from fraction 93 (100% chloroform) N-6 (4 mg) was obtained.

C. A third portion of the root bark (300 g) was successively extracted with petrol (60 - 80^{o}) and EtOAc. The petrol extract was subjected to column chromatography on silica gel (120 g) eluting with petrol containing increasing amounts of EtOAc. A total of 38 fractions each 150 ml were collected. Fractions 14 - 20 (10% EtOAc) afforded N-1 (6 mg) and N-2 (5 mg), fractions 21 - 25 (15% EtOAc) yielded N-4 (2 mg), and fractions 26 - 28 (20% EtOAc) gave T-2 (60 mg).

The EtOAc extract was chromatographed on silica gel column (200 g) and eluted with chloroform. Fractions 6 - 20 afforded N-7 (3 mg), which was further purified by PTLC (solvent system IV). Fractions 24 - 42 gave N-5 (4 mg) after purification using PTLC (solvent system II).

3.4.2 Extraction and Isolation of Compounds from Stem Bark of E. schimperi (A.DC) Dandy

Stem bark of the plant material (200 g) was extracted on Soxhlet apparatus with chloroform for 2 days. The residue (3 g) was chromatographed on silica gel (150 g) and eluted with petrol EtOAc

mixtures (solvent system VI) of increasing polarities to give a total of 38 fractions. Fractions 4-10 (5% EtOAc) gave T-1 (10 mg), 12-20 (15% EtOAc) after purification using PTLC (solvent system II) afforded N-7 (1 mg), and 21-38 (20% EtOAc) yielded.

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