CHAPTER II

HISTORICAL

1. Chemical constituents of genus *Nepenthes*

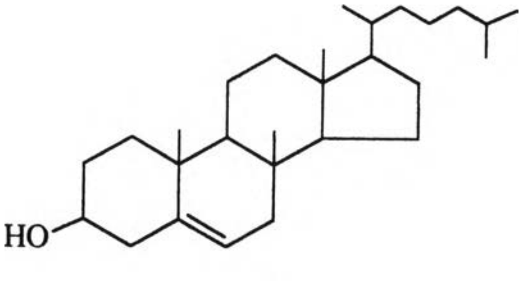
Several types of compounds were found in the genus *Nepenthes*. They are steroids, triterpenoids, non-aromatic hydrocarbons, non-aromatic carboxylic acids, aromatic esters and naphthoquinones, as shown in Table 1 and Figure 1.

Table 1 Chemical constituents of genus *Nepenthes*

Plant	Plant part	Chemical compound	Reference
Nepenthes albomarginata	Whole plant	Cholesterol (**1**) Campesterol (**2**) Stigmasterol (**3**) Sitosterol (**4**) Isofucosterol (**5**) α-Amyrin (**6**) β-Amyrin (**7**) Obtusifoliol (**8**) Cycloeucalenol (**9**) Citrostadienol (**10**) Cycloartenol (**11**) 24-Methylenecycloartanol (**12**)	Wan, *et al* 1972

Table 1 (continued)

Plant	Plant part	Chemical compound	Reference
N. albomarginata	Whole plant	Hexadecane (**13**)	Adirukmi, Noor Asimah AB and Noor Saleh, 1994
N. ampullaria	Whole plant	Pentadecane (**14**)	
N. sanguinea	Whole plant	1,2-benzene-dicarboxylic acid (**15**) 1,2-benzene-dicarboxylic 3-nitro (**16**) 9-octadecanoic acid (**17**)	
N. rafflesiana	root	Plumbagin (**18**) Droserone (**19**) Hydroxy droserone (**20**) Nepenthone A (**21**) Nepenthone B (structure not determined) Nepenthone C (**22**) Nepenthone D (**23**) Nepenthone E (**24**)	Cannon *et al*, 1980



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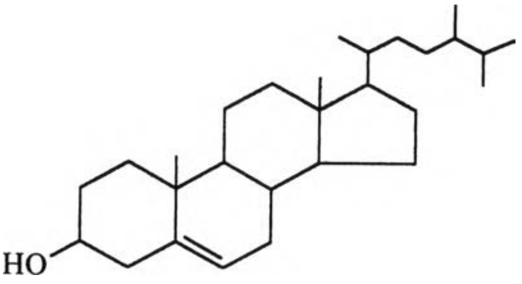

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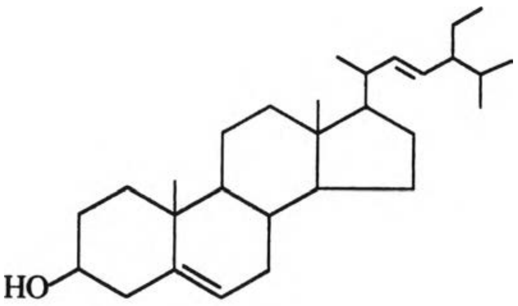

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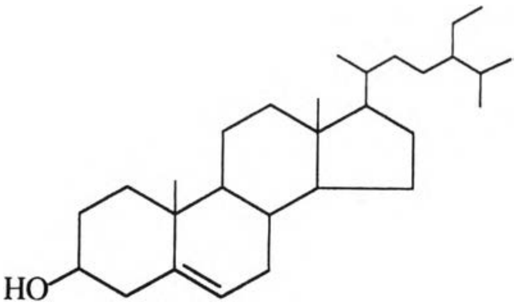
Figure 1 Chemical constituents of genus *Nepenthes*



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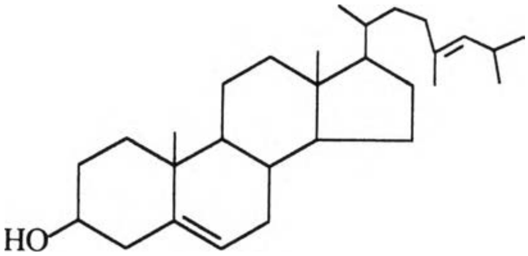

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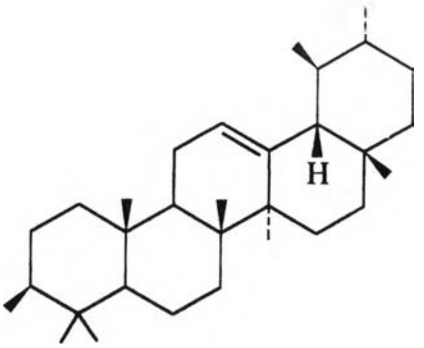

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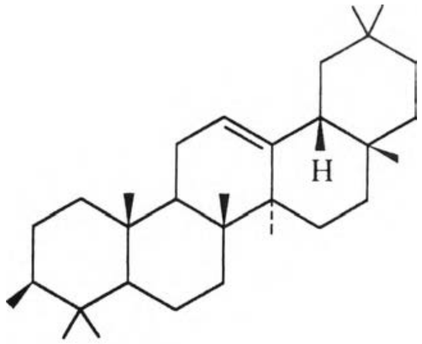
Chemical constituents of genus *Nepenthes* (continued)



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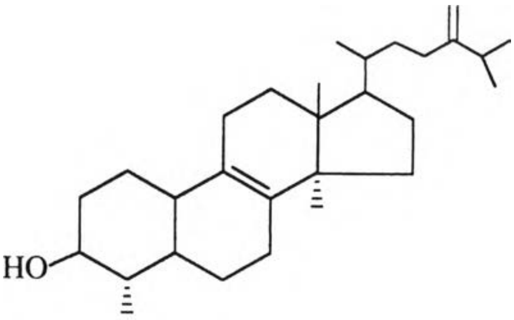

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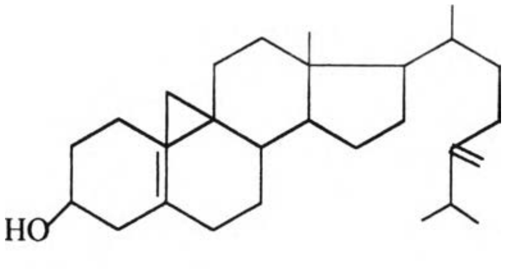

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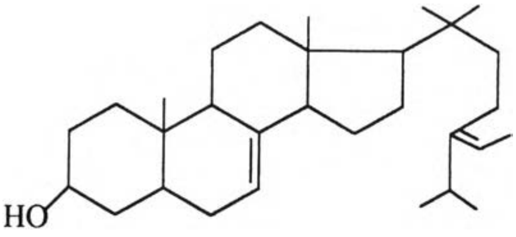

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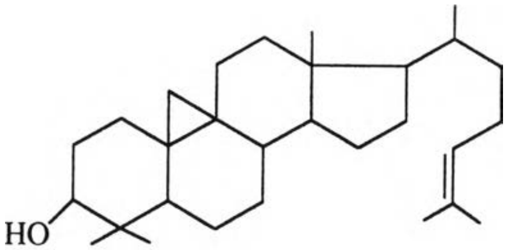

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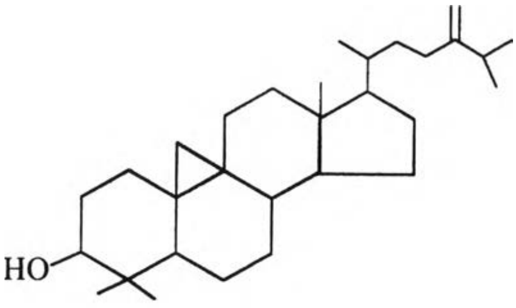

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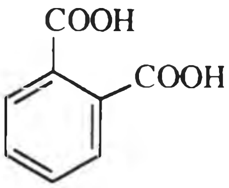
Chemical constituents of genus *Nepenthes* (continued)

$$H_3C-(CH_2)_{14}-CH_3$$

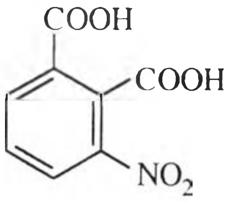
(**13**)

$$H_3C-(CH_2)_{13}-CH_3$$

(**14**)



(**15**)

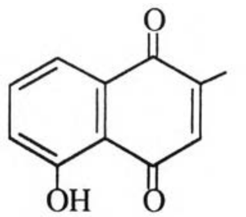


(**16**)

$$H_3C-(CH_2)_7-HC=CH-(CH_2)_7-COOH$$

(**17**)

Chemical constituents of genus *Nepenthes* (continued)



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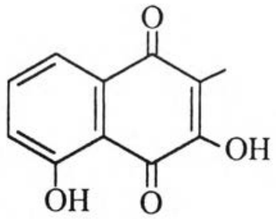

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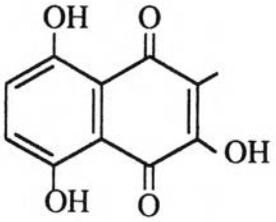

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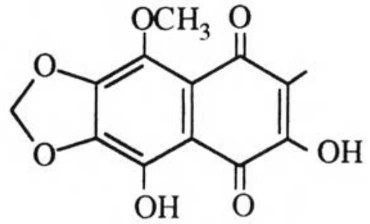

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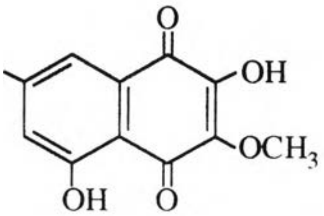

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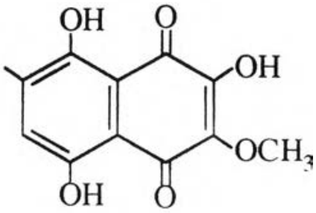

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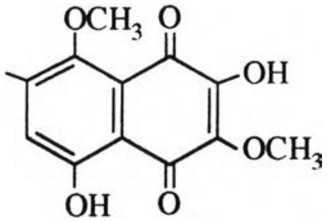

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Chemical constituents of genus *Nepenthes* (continued)

2. Naturally occuring naphthoquinones

Naphthoquinones are one group of quinones. The basic structure is related to the quinone structure mentioned by Isaksen, 1993. The quinone pigments are based on an unsaturated system of cyclic diketone, and the main structure can be derived from benzene, naphthalene and anthracene.

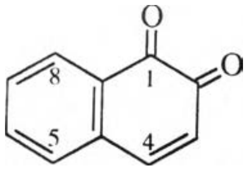
Naphthoquinones can be divided into three groups, as shown in Figure 2 : 1,2-naphthoquinones (**25**), 1,4-naphthoquinones (**26**) and 1,5-naphthoquinones (**27**). Examples of 1,2-naphthoquinones are (Figure 3) azanza A (**28**) (Thomson, 1971), hibicoquinone A (**29**) (Ferreira, *et al* 1980), mansonone H (**30**) (Letcher and Shirley, 1992), hibicoquinone B (**31**) (Ferreira *et al*, 1980) and rhinacanthone (**32**) (Kuwahara, Awai and Kodama, 1995). The 1,5-naphthoquinones are found very rarely. Cordeauxiaquinone (**33**) is an example (Figure 3). The 1,4-naphthoquinones are more common than 1,2-, and 1,5-naphthoquinones (Gibb, 1979).

3. 1,4-naphthoquinones

3.1 Distribution of 1,4-naphthoquinones

1,4-naphthoquinones are sporadically distributed. More than half of them occur in higher plants. Some are scatteredly found in animals and microorganisms (Thomson, 1971).

Microorganism sources are fungi and bacteria. The Aspergilli, especially the *Fusarium*, are important fungal sources to produce naphthoquinones. Examples are 2,5,8-trihydroxy-6-methoxy-3-(2-oxopropyl)-1,4-naphthoquinone (**34**), 8-hydroxy-2,5,6-trimethoxy-3-(2-oxopropyl)-1,4-naphthoquinone (**35**), 2,5-dihydroxy



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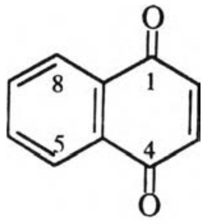

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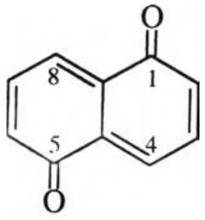

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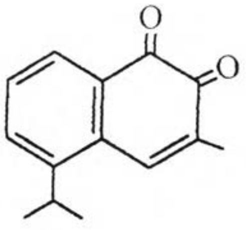
Figure 2 Structures of naphthoquinones



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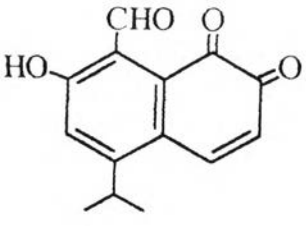

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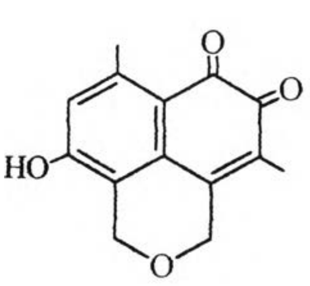

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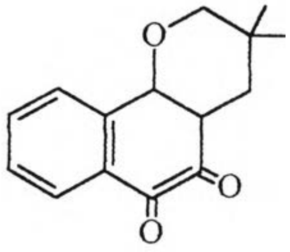

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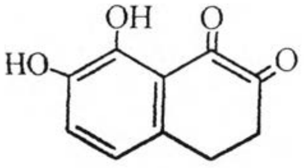

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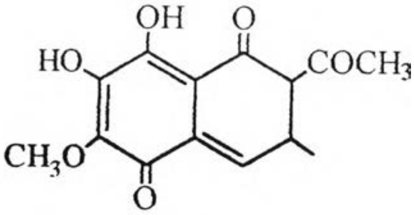
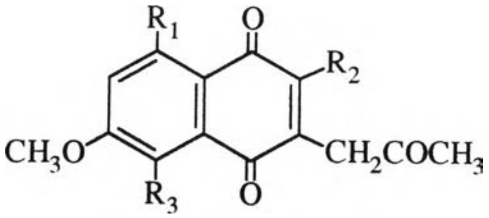
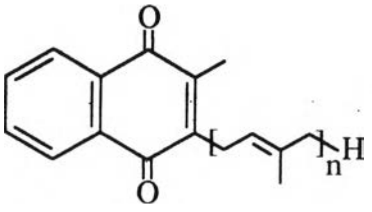


(33)

Figure 3 Examples of 1,2- and 1,5-naphthoquinones



	R_1	R_2	R_3
(34)	OH	OH	OH
(35)	OH	OCH_3	OCH_3
(36)	OCH_3	OH	OH

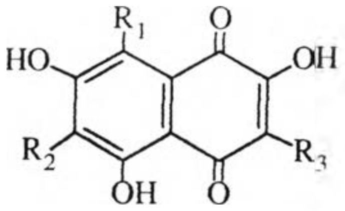


(37) n = 6

(38) n = 7

(39) n = 8

Figure 4 Examples of 1,4-naphthoquinone from microorganism sources



	R_1	R_2	R_3
(40)	OH	H	$COCH_3$
(41)	H	H	OH
(42)	OH	OH	$COCH_3$
(43)	OH	H	OH
(44)	OH	OH	OH
(45)	OH	CH_2CH_3	OH

Figure 5 Some 1,4-naphthoquinones from animals

-6,8-dimethoxy-3-(2-oxopropyl)-1,4-naphthoquinone (**36**) (Tatum, Baker and Berry, 1987) (Figure 4). In the bacteria, menaquinones e. g. MK6 (**37**), MK7 (**38**), and MK8 (**39**) (Figure 4) occur widely (Thomson, 1971).

In the animal kingdom, strongly coloured, red, purple, or blue naphthoquinone pigments have been found in echinoderms, mostly in sea urchins, but also in brittle stars and star fish. These compounds known as spinochromes or echinochromes were first isolated form the calcareous parts (spines, shell of sea urchins) but are also present in perivisceral fluid, eggs and internal organs. Most species yield a mixture of six or more pigments, which are present in the spines and shell chiefly as calcium and magnesium salts, and in other tissues probably as protein complexes. Structurally these echinoderm pigments are highly substituted and highly oxygenated derivatives. Examples of the animal naphthoquinones (Figure 5) are spinochrome A (**40**), spinochrome B (**41**), spinochrome C (**42**), spinochrome D (**43**), spinochrome E (**44**) and echinochrome A (**45**), (Britton, 1983).

In plants, naphthoquinones occur in leaves, flowers, wood, bark, roots and fruit. They are found in dicotyledons more than in monocotyledons. Some of them are found in brown algae, as shown in Table 2.

Table 2 Distribution of 1,4-naphthoquinones from plants.

Family	Genus	Reference
Acanthaceae	*Rhinacanthus*	Wu *et al*, 1988
Bignoniaceae	*Bignonia*	Thomson, 1971

Table 2 (continued)

Family	Genus	Reference
Bignoniaceae	*Kigelia*	Akunyili and Houghton, 1993
	Marhamia	Thomson, 1971
	Paratecoma	Thomson, 1971
	Tabebuia	De Oliveira *et al*, 1993
	Tecomella	Thomson, 1971
	Zeyhera	De L. Duarte Weinberg and Gottlieb, 1976
Boraginaceae	*Alkanna*	Papageorgiou, 1980
	Arnebia	Papageorgiou, 1980
	Catalpa	Inouye, Okuda and Hayashi, 1975
	Cordia	Bieber *et al*, 1990
	Crescentia	Heltzel *et al*,1993
	Cynoglossum	Papageorgiou, 1980
	Echium	Papageorgiou, 1980
	Lithospermum	Papageorgiou, 1980
	Macrostomia	Papageorgiou, 1979
	Mansoa	Itokawa *et al*, 1992
	Onosma	Papageorgiou, 1980
Compositae	*Cacaria*	Thomson, 1971

Table 2 (continued)

Family	Genus	Reference
Droseraceae	*Aldrovanda*	Thomson, 1971
	Dionaea	Kreher, Neszmelyi and Wagner, 1990
	Drosophyllum	Thomson, 1971
Ebenaceae	*Diospyros*	Van der Vijver and Gerritsma, 1974
		Alves, Costa and Paul, 1983
		Zhong, Waterman and Jeffreys, 1984
		Zakaria *et al*. 1984
	Euclea	Costa *et al*, 1976
Euphobiaceae	*Jatropha*	Papageorgiou, 1980
Gesneriaceae	*Streptocarpus*	Inoue *et al*, 1983
Juglandaceae	*Juglans*	Hirakawa *et al*, 1986
		Binder, Benson and Flath, 1989
	Pterocarya	Hirakawa *et al*, 1986
Labiatae	*Coleus*	Thomson, 1971
Lythraceae	*Lawsonia*	Gupta, Ali and Alam, 1993
Malvaceae	*Hibicus*	Ferreira *et al*, 1980
Nepenthaceae	*Nepenthes*	Cannon *et al*, 1980

Table 2 (continued)

Family	Genus	Reference
Plumbaginaceae	*Ceratostigma*	Yue *et al*, 1994
	Plumbago	Bhattacharrya and De Calvaho, 1986
Proteaceae	*Conospermum*	Dai *et al*, 1994
	Lomatia	Moir and Thomson, 1973
	Stenocarpus	Mock *et al*, 1973
Polygonaceae	*Polygonum*	Kimura *et al*, 1983
Pyrolaceae	*Pyrola*	Kosuge *et al*,1985
Rhamnaceae	*Ventilago*	Ali, Read, and Sotheeswaran, 1994
Rubiaceae	*Rubia*	Koyama *et al*, 1992
Scrophuliaceae	*Capraria*	Thomson, 1971
Verbenaceae	*Caryopteris*	Thomson, 1971
	Gmelina	Joshi, Singh and Pardasani, 1977
	Lantana	Abeygurnawardena *et al*, 1991
	Tectona	Thomson, 1971
Zingibenaceae	*Zingiber*	Amatayakul *et al*, 1979
Zygophyllaceae	*Larrea*	Comber and Sargent, 1991
Liliaceae	*Dianella*	Colegate, Dorling and Huxtable, 1987
	Lomandra	Thomson, 1971

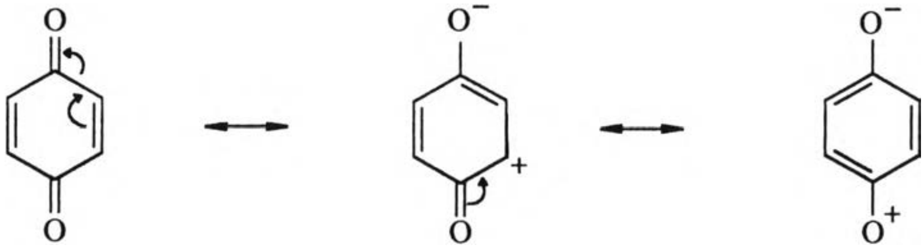
Table 2 (continued)

Family	Genus	Reference
Liliaceae	*Stypandra*	Colegate, Dorling and Huxtable, 1987
Iridaceae	*Eleutherine*	Thomson, 1971
	Tritonia	Masuda *et al*, 1987

3.2 Chemistry of 1,4-naphthoquinones

Almost all of 1,4-naphthoquinones are crystallines or solids. The colors range from yellow to red. They are readily soluble in organic solvents such as chloroform and benzene.

The naphthoquinones, like the other quinones (benzoquinones, anthraquinones) are conjugated cyclic diketones rather than aromatic systems. They are, however stabilised considerably by resonance involving charged aromatic contribution structure.



Many of them have additional phenolic hydroxy substituents and are thus slightly acidic, consequently ionising and forming salts in alkaline solution. This property is more pronounced in 2-hydroxy-1,4-naphthoquinones which are vinylogous carboxylic acids (Britton, 1983).

3.3 Derivatives of 1,4-naphthoquinone

3.3.1 Hydroxy derivatives

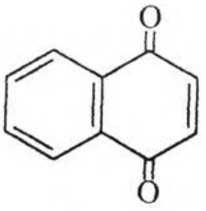
Hydroxy is the most important substituting group of naphthoquinones. It can be located at the 2, 3, 5, 6, 7 and 8 positions. They can be found as mono-, di-, tri-, tetra- or polyhydroxy substituents. Some 1,4-naphthoquinones have only one hydroxy substitutent appearing with other chemical groups (e. g. methyl, ethyl).

Examples (Figure 6) are lawsone (**46**), juglone (**47**), 6-hydroxyplumbagin (**48**), 2-methoxy-7-methyljuglone (**49**), diosmuscione (**50**), diomelquinone A (**51**), shikonin (**52**), flaviolin (**53**), mompain (**54**) and lapachol (**55**) (Thomson, 1971).

3.3.2 Alkyl, allyl and alkoxy derivatives

These derivatives are found in methyl, ethyl, methoxy, ethoxy forms. But some derivatives occur with isoprene units. Menaquinones are all bacterial metabolites (except phyllquinone found in the green part of plants). Menaquinones involve in bacterial electron transport systems. Phylloquinone is believed to play a major role in the photosynthesis pathway.

Examples (Figure 7) are 6-methyl-1,4-naphthoquinone (**56**), chimaphilin (**57**), deoxylapachol (**58**) and phylloquinone (**59**) (Thomson, 1971).



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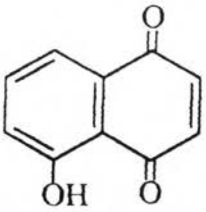

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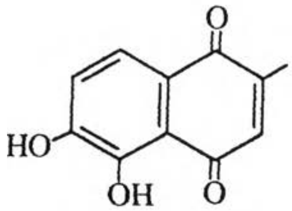

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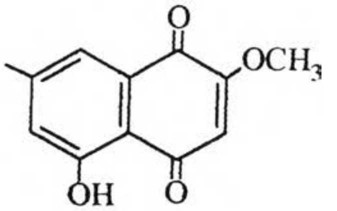

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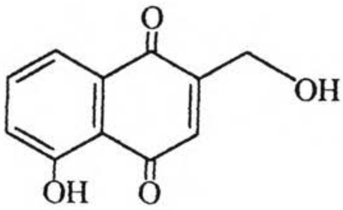

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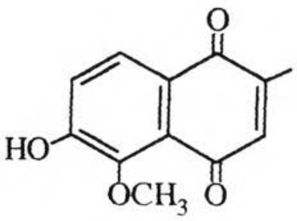

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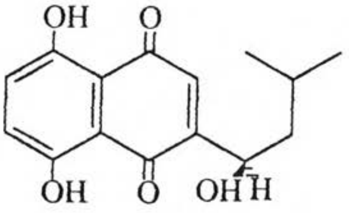

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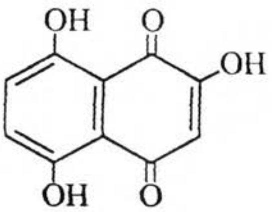

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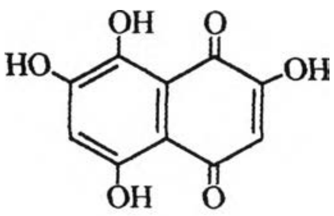

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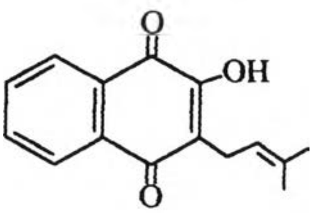

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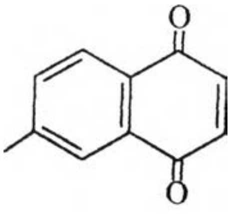
Figure 6 Examples of hydroxy derivatives of 1,4-naphthoquinone



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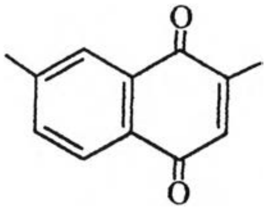

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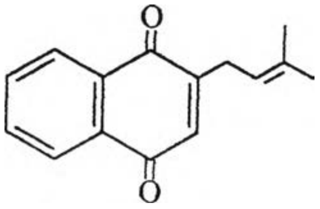

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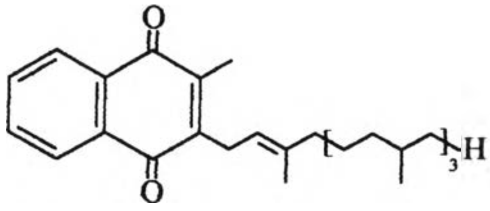


(59)

Figure 7 Examples of alkyl, allyl, and alkoxy derivatives of 1,4-naphthoquinone

3.3.3 Furano and pyrano derivatives

They are found in many plant families such as Verbenaceae (Abeygunawardena *et al*, 1991), Rhamnaceae (Thomson, 1971) and Acanthaceae (Wu *et al*,1988).

Examples (Figure 8) are rhinacanthone (**60**), lambertellin (**61**), ventilaglone (**62**) and marturinone (**63**).

3.3.4 Polymer derivatives

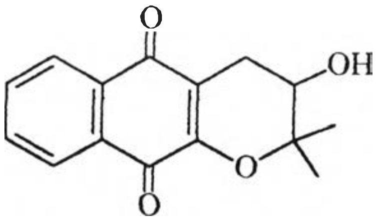
They are usually found as dimers. Most of them are found in Ebenaceae, some in Plumbaginaceae (Thomson, 1971) and Juglandaceae (Hirakawa *et al*, 1986). Trimer and tetramers are rarely found in nature.

Examples are 3′-methoxy-diospyrin (**64**), batacanone (**65**), 6,6′-diplumbagin (**66**), 2,3′-bijuglone (**67**) and cyclotrijuglone (**68**) (Figure 9).

3.3.5 Miscellaneous groups

3.3.5.1 Halogen substituted 1,4-naphthoquinones

Halogens (bromine and chlorine) are found in 1,4-naphthoquinone, for examples : 3-chloroplumbagin (**69**) and 3-bromoplumbagin (**70**). They have been isolated from the genus *Diospyros* of the family Ebenaceae (Higa, *et al* 1987) and the genus *Plumbago* of the family Plumbaginaceae (Thomson, 1971).



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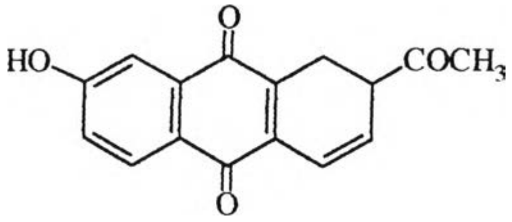

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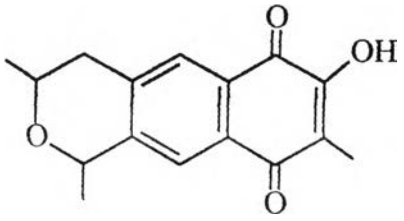

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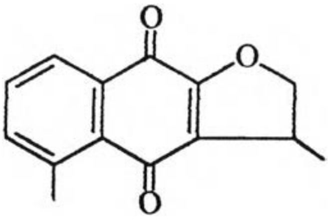

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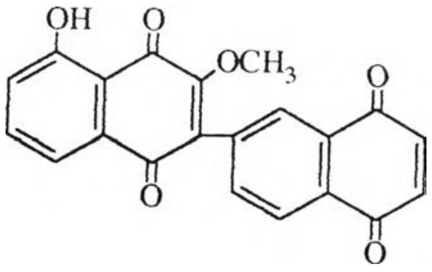
Figure 8 Examples of furano- and pyrano-1,4-naphthoquinones



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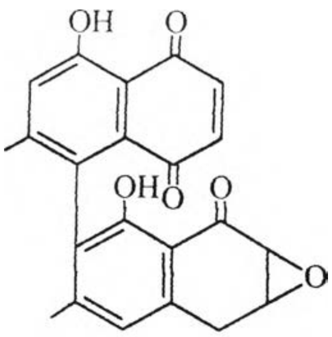

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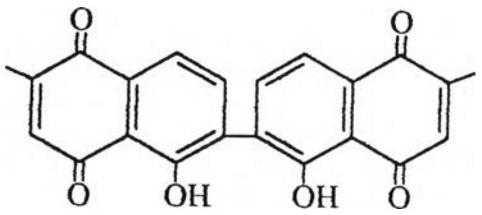

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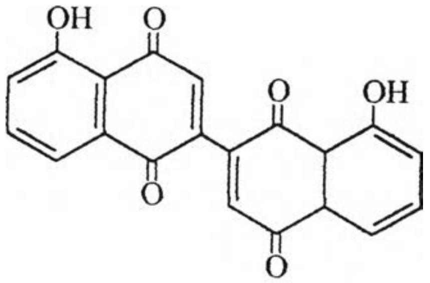

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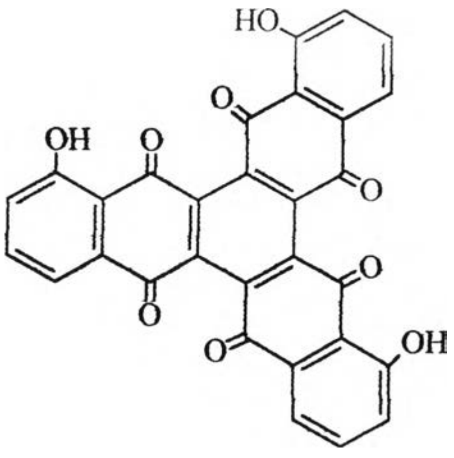


(68)

Figure 9 Examples of 1,4-naphthoquinone polymers

3.3.5.2 Carbamoyl substituted 1,4-naphthoquinones

1,4-naphthoquinones containing a carbamoyl group are found in Rubiaceae (Koyama *et al*, 1992).

Examples (Figure 10) are 2-carbamoyl-3-methoxy-1,4-naphthoquinone (**71**) and 2-carbamoyl-3-hydroxy-1,4-naphthoquinone (**72**).

3.3.5.3 Phenyl derivatives

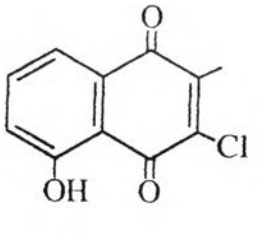
The derivatives have been isolated from the genus *Zingiber* of the family Zingiberaceae (Amatayakul *et al*, 1979) and the genus *Larrea* of Family Zygophyllaceae (Comber and Sargent, 1991). Lassumunaquinone (**73**) is an example (Figure 10).

3.4 Biosynthesis of 1,4-naphthoquinones

There are 2 main pathways proposed for the biosynthesis of 1,4-naphthoquinones. One pathway involves the acetate-mevalonate route, whereas the other involves the shikimate pathway. Condensation products, e.g. binaphthoquinones can be described by the phenolic coupling pathway (Britton, 1983).

3.4.1 Acetate-malonate pathway (Polyketide pathway)

In the first stage, condensation of acetyl-Co A (**74**) with malonyl-Co A (**75**) gives acetoacetyl-Co A (**76**). The keto group formed is not reduced at this stage, the chain lengthening process continues by addition of further C_2 units from malonyl-Co A until a chain of the required lenght has been built up. This pattern of alternating CO and CH_2 groups is the polyketide system.



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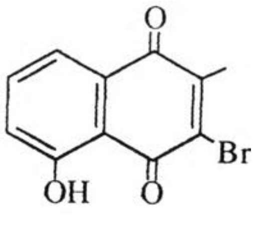

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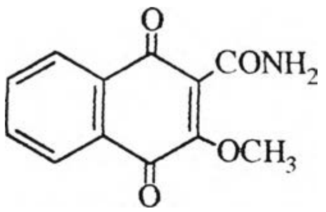

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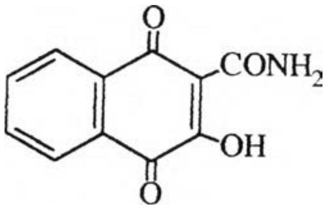

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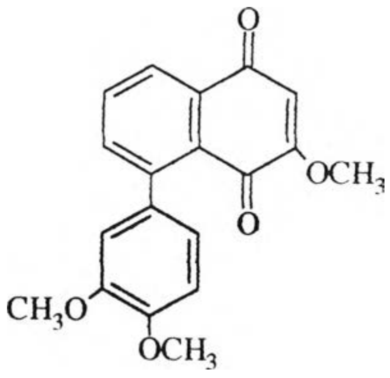


(73)

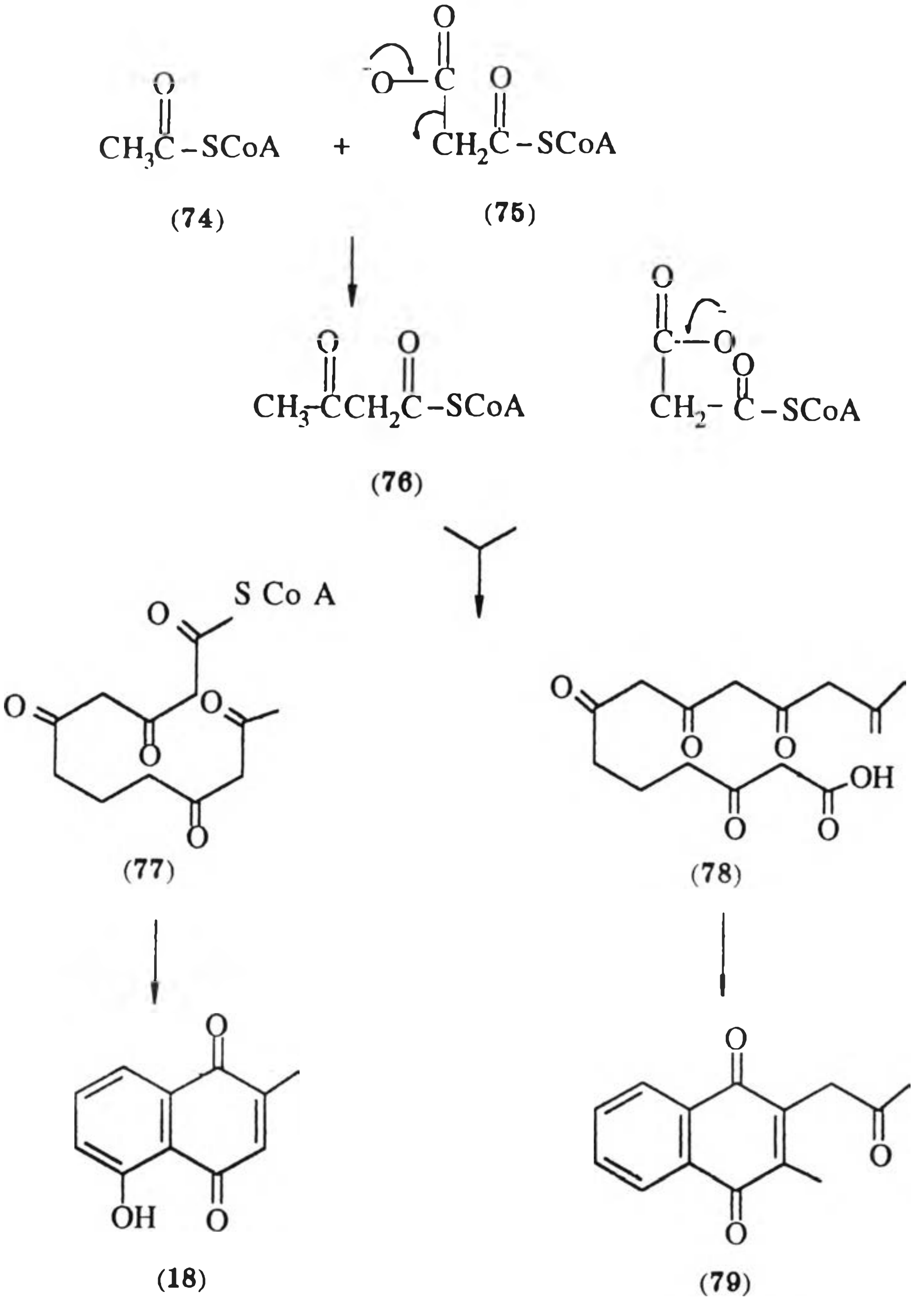
Figure 10 Examples of miscellaneous 1,4-naphthoquinones

When the polyketide is folded in a suitable way, cyclization can take place by elimination of water from appropriately positioned CO and CH_2 groups, leading to an aromatic or a quinone molecule.

The pattern of oxygen functions on alternate carbon atoms can frequently be discerned in molecules biosynthesised by this route, although addition or removal of substituent groups often disguises the polyketide pattern. It may be possible to demonstrate that a quinone is formed by incorporation experiments with acetate or malonate labelled with radioactive or stable isotopes. The manner of polyketide folding may be revealed by degradation of the labelled molecule (Scheme 1).

This transformation process is the pathway operated in the synthesis of 1,4-naphthoquinones in microorganisms. Feeding experiments with labelled acetate and malonate in cultures of the mold *Fusarium japonicum* have established the origin of javanicin (**79**) from a heptaacetyl polyketide (**78**). Reduction of the terminal carboxyl group to methyl is unusual. A less simple example is provided by mollisin, another fungal metabolite produced by cultures of *Mollisia caesia.* Biosynthesis again occurs from acetate and malonate, but in this instance two polyketide chains of three and four C_2 units, respectively, are used rather than one longer chain (Britton, 1983).

In plants, this pathway occured in the plumbagin (**18**), 7-methyljuglone biosynthesis, from a hexaketide (**77**) progenitor, but juglone derived at least part of its skeleton from shikimate (80). These are metabolites of certain carnivorous plants which live on nitrogen poor soils and depend upon insects as a source of nitrogen (Mann, 1978).



Scheme 1 Biosynthesis of 1,4-naphthoquinones by the acetate-mevalonate pathway

3.4.2 The shikimate related pathway

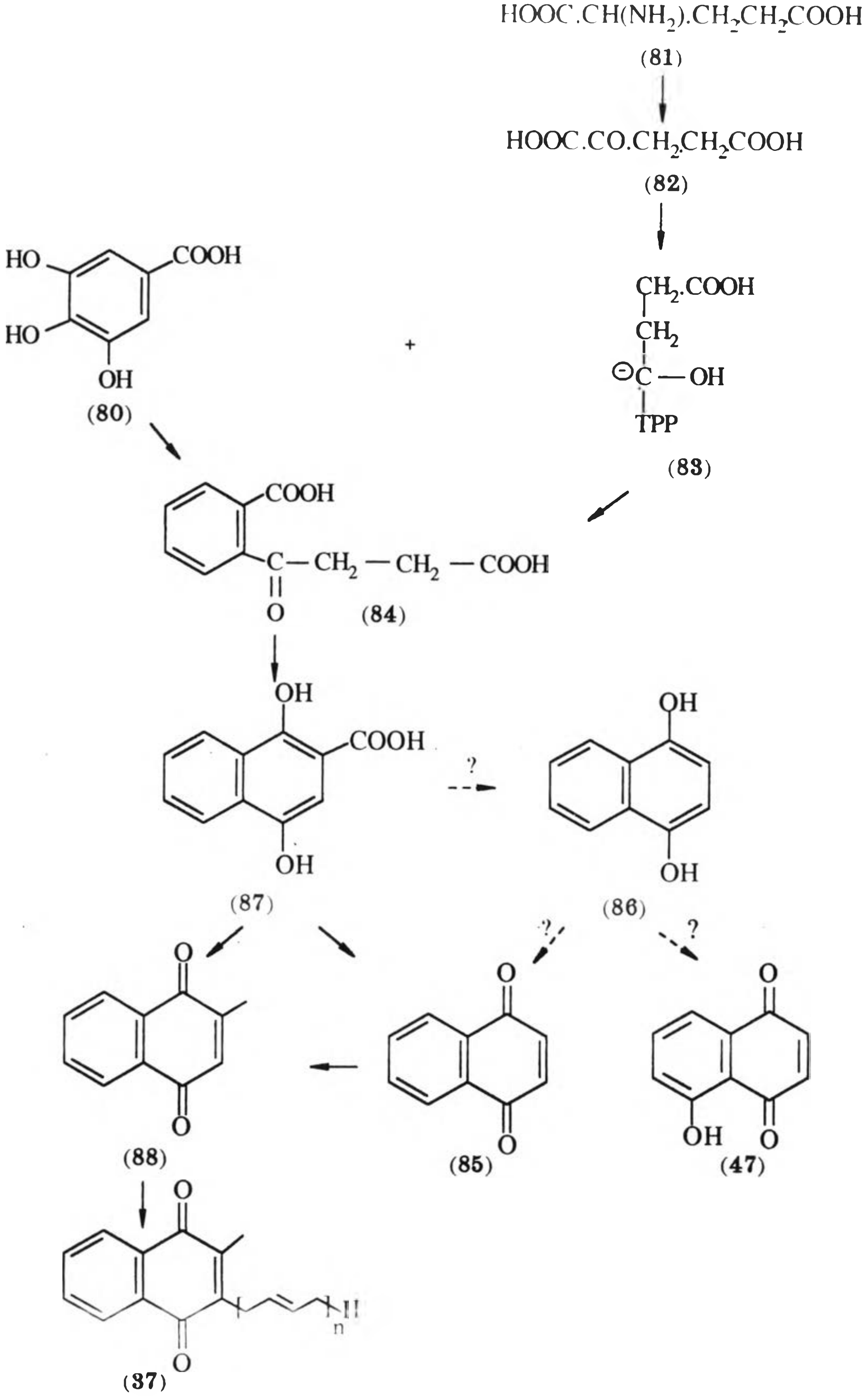
Many naphthoquinones are synthesized by the shikimate pathway. Precursors from the general carbohydrate metabolic pool give the C_7 sugar 3-deoxy-D-arabinohepturosonic acid-7-phosphate (DAHP) which under goes cyclization to give 5-hydroquinic acid. This is converted into shikimic acid, then associated with other pathways to produced various naphthoquinones (Britton, 1983).

3.4.2.1 The shikimate pathway

The main details of the pathway for formation of menaquinones and phylloquinone, lawsone and juglone have been elucidated. Feeding experiments show that the shikimate (**80**) is incorporated as an intact C_7 unit to form the benzenoid (A) ring and one of the quinone carbonyl groups. The three remaining carbon atoms are derived form the non carboxyl carbons of glutamate (**81**) or α-oxyglutarate (**82**). The key intermediate seems to be o-succinylbenzoate (**84**) formed by addition to shikimate of the thiamine pyrophosphate derivative of succinyl semialdehyde (**83**) (obtained form glutamate or α-oxyglutarate). The final steps by which o-succinylbenzoate is converted into lawsone (**46**), juglone (**47**) and related quinones are still not clear. It is thought that 1,4-naphthoquinone (**85**) itself and perhaps also 1,4-naphthoquinal (**86**) are intermediates, in the pathway leading to juglone. In the menaquinone (**37**) biosynthesis a naphthalene carboxylic acid (**87**) and 2-methyl-naphthoquinone (e. g. menadione (**88**)) are involved (Britton, 1983).

3.4.2.2 The shikimate-mevalonate pathway

The mevalonic acid pathway is obviously used to provide the isoprenoid side-chians. Examples are also known in which a short



Scheme 2 Biosynthesis of 1,4-naphthoquinones by the shikimate pathway

isoprenoid substituent (C_5 and C_{10}) in an intermediate is used to supply some of carbon atoms of a naphthoquinone ring system. The naphthoquinone so formed are therefore biosynthically substituted naphthoquinones (Britton, 1983).

3.4.2.2.1 The homogentisate intermediate pathway

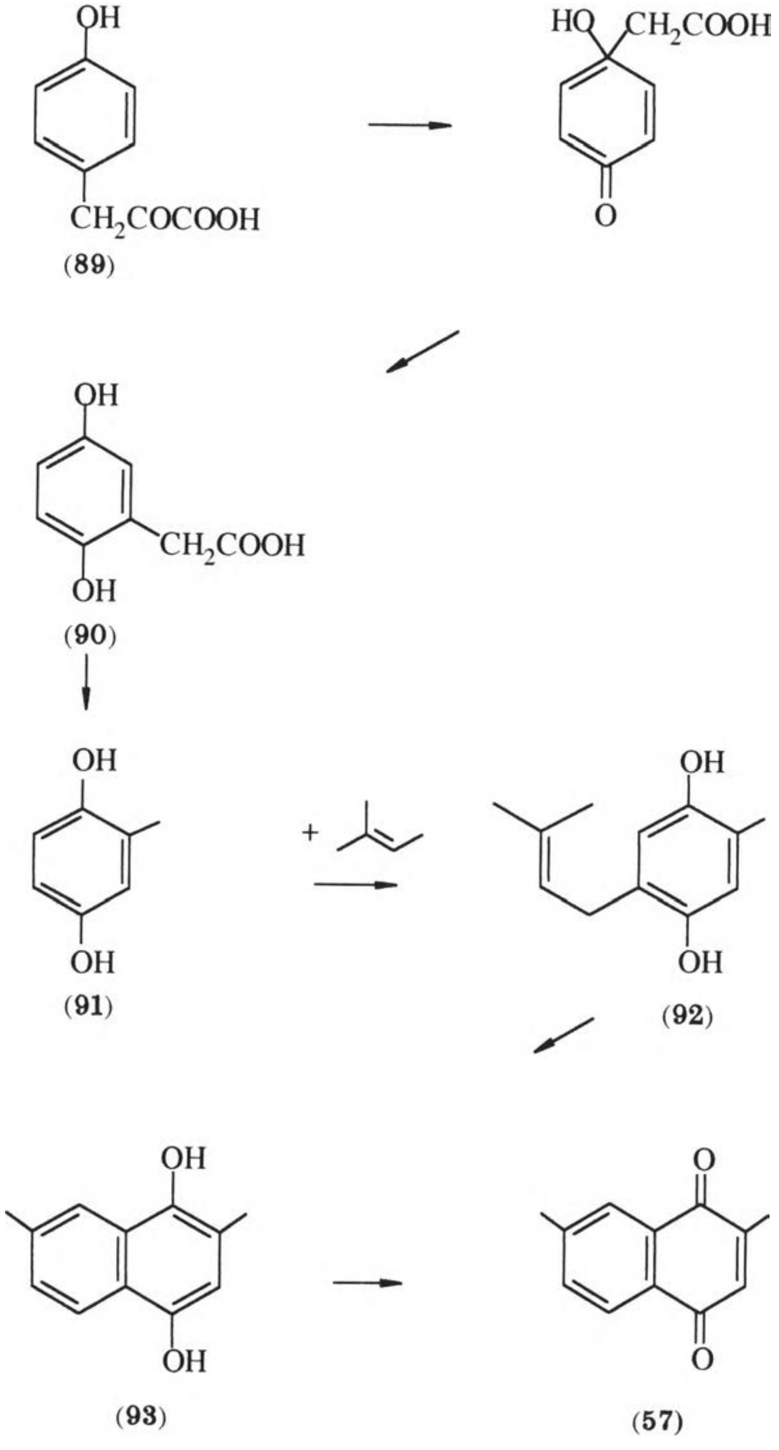
This route for naphthoquinone biosynthesis is used by plants of the Pyrolaceae. An example is chimaphilin (**57**). The quinone (ring B) is conventionally formed from shikimate. The remainder of the benzenoid (ring A) and the methyl substituent are derived from mevalonate. The probable biosynthetic pathway is outlined in scheme 3. It involves rearrangement of *p*-hydroxyphenylpyruvate (**89**) into homogentisate (**90**) and conversion into homoarbutin (**91**). Prenylation of the latter gives the substituted quinol (**92**) which cyclizes to the quinol of chimaphilin (**93**) which is oxidized to chimaphilin itself (Britton, 1983).

3.4.2.2.2 The *p*-hydroxybenzoate intermediate pathway

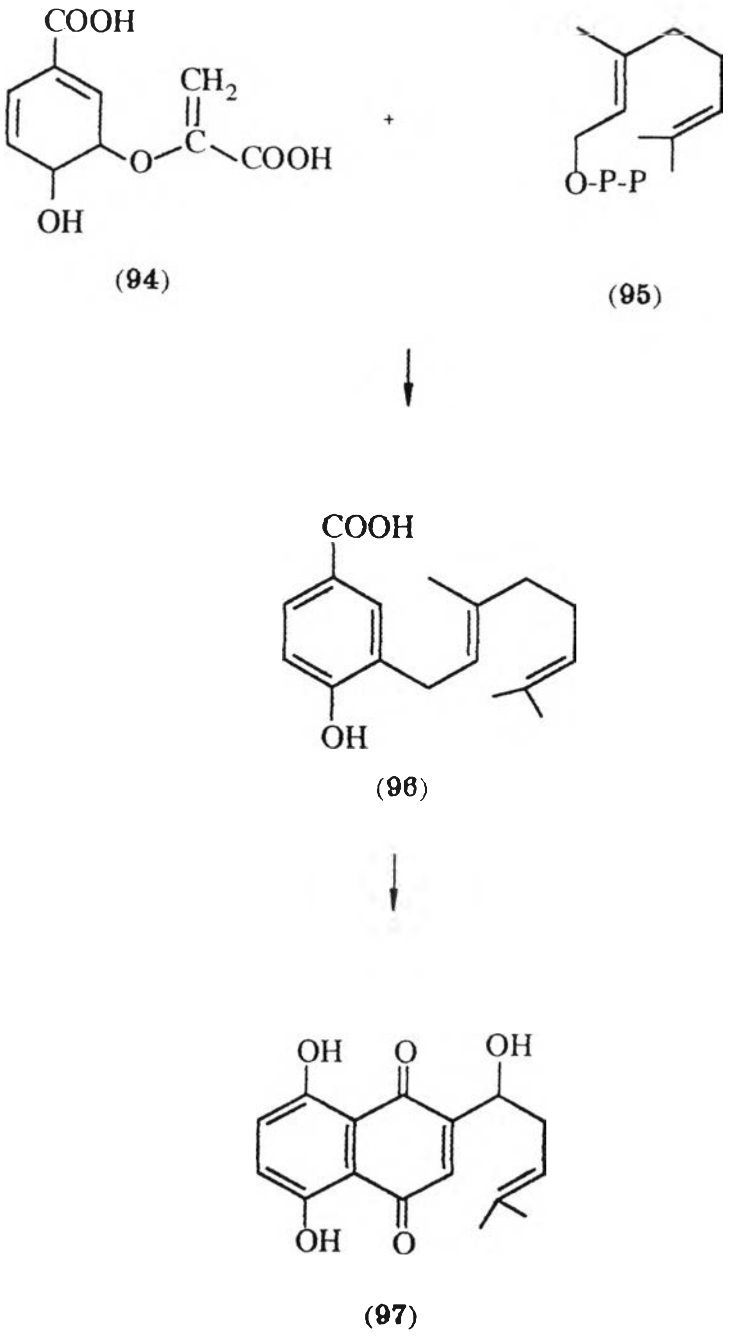
This route is found in Boraginaceae. In alkannin (**97**), it is ring A that is derived from shikimate *via* *p*-hydroxybenzoate (**94**) and two molecules of mevalonate then provide the remaining ten carbon atoms, including those of the quinone ring (ring B). The biosynthesis involves essentially prenylation of *p*-hydroxybenzoate with geranyl phosphate (**95**) to give **96**, followed by oxidative cyclization and introduction of oxygen function (Scheme 4). (Britton, 1983).

3.4.3 Phenolic coupling

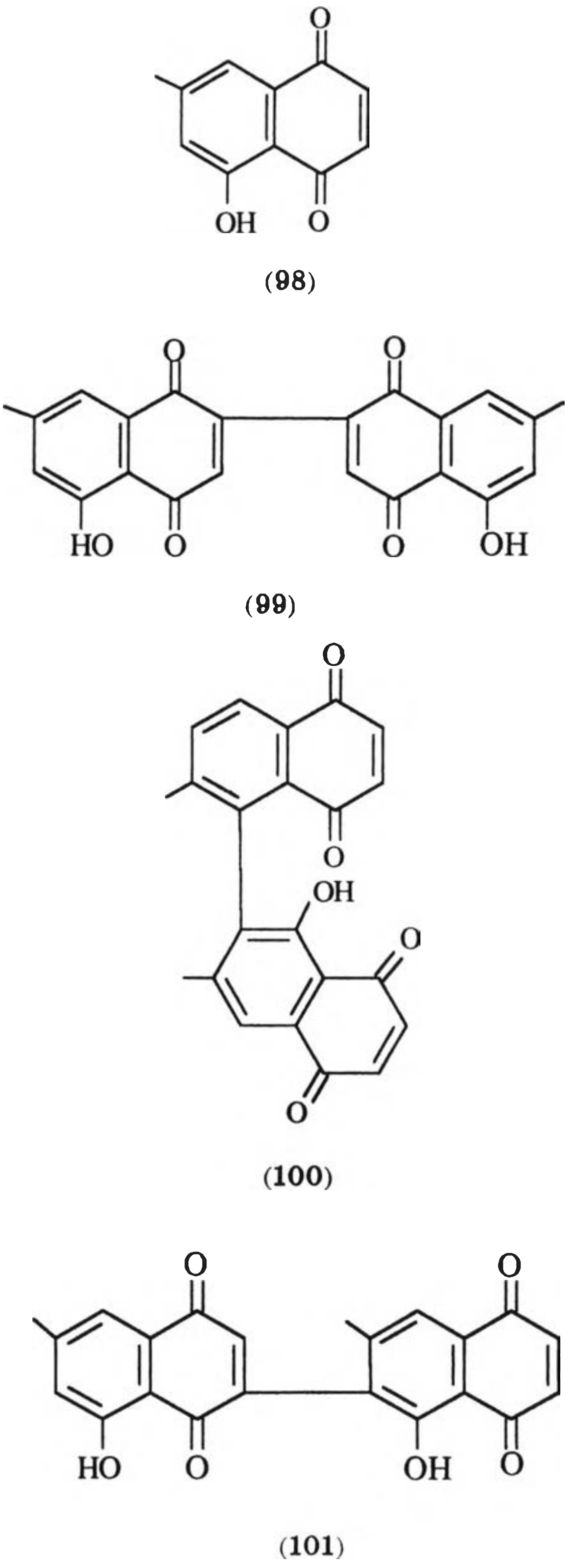
Phenolic coupling is a biosynthetesis process frequently encountered in organisms which elaborate phenols and quinones. In this process dimerization occurred by a free radical reaction in which a bond is between reactive positions on the monomers. Many of the larger, naturally occuring quinones have structures which indicate them to be dimers or other condensation products of naphthoquinones. Sometimes the monomers may also be present in the same tissue, as illustrated by the co-occurrence of 7-methyljuglone (**98**) and several dimers (**99-101**) in *Diospyros* species (Scheme 5) (Britton, 1983).



Scheme 3 Biosynthesis of 1,4-naphthoquinones by the homogentisate pathway



Scheme 4 Biosynthesis of 1,4-naphthoquinones by the *p*-hydroxybenzoate pathway



Scheme 5 The proposed coupling pattern of binaphthoquinones