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Xanthones in *Hypericum*: Synthesis and Biological Activities

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Abstract There has been an increasing interest in the genus *Hypericum*, because it is a source of a variety of compounds with different biological activities. Xanthones are

one of these compounds within *Hypericum* species. Recently, growing attention has been given to these heterocyclic compounds containing oxygen because of their many interesting pharmacological and biological properties, such as monoamine oxidase inhibition and antioxidant, antifungal, cytotoxic, and hepatoprotective activities.

Keywords Xanthones · *Hypericum* · Pharmacological properties · Biosynthesis · Synthesis

Abbreviations

AA	Arachidonic acid
ABTS	2,2′-Azinobis(3-ethylbenzothiozoline-6-sulfonate)
AcOH	Acetic acid
APTT	Active partial thromboplastin time reagent
CC	Column chromatography
CL	Chemiluminescence
CoMFA	Comparative molecular field analysis
COX	Cyclooxygenase
CPC	Centrifugal partition chromatography
DMSO	Dimethyl sulfoxide
ED_{50}	Effective dose
h	Hour
HPLC	High-pressure liquid chromatography
IC_{50}	Inhibitor concentration
MAO	Monoamine oxidase
MCPBA	*meta*-Chloroperoxybenzoic acid
MIC	Minimum inhibition concentration
PG	Prostaglandin
PT	Prothrombin time reagent
QSAR	Quantitative structure–activity relationship
RP	Reverse phase
TEAC	Trolox equivalent antioxidant capacity

1 Introduction

Xanthones are secondary metabolites commonly occurring in a few higher plant families. Their high taxonomic value and their pharmacological properties, such as monoamine oxidase inhibition, in vitro toxicity, and in vivo antitumor activity, have provoked great interest [1].

Their structures are related to those of flavonoids and their chromatographic behavior is also similar. While flavonoids are frequently encountered in nature, xanthones have been found in a small number of families. They occur in eight families, namely in the Gentianaceae, Guttiferae, Polygalaceae, Leguminosae, Lythraceae, Moraceae, Loganiaceae, and Rhamnaceae. Extensive studies on xanthones have been made in the Gentianaceae and Guttiferae families [2]. A number of species of *Hypericum* genus (Guttiferae) contain

xanthones which have been reported to possess several biological activities [3].

The aim of this chapter is not only to provide a review of the distribution of xanthones in *Hypericum* genus, but also to cover some more specific aspects such as biosynthesis, chemotaxonomic significance, biological activities, and synthesis.

2 Chemistry and Classification of Xanthones

2.1 Chemistry

Chemically xanthones (9*H*-xanthen-9-ones) are heterocyclic compounds with the dibenzo-γ-pyrone framework (**1**, Fig. 1). The xanthone nucleus is numbered according to a biosynthetic convention with carbons 1–4 being assigned to acetate-derived ring A and carbons 5–8 to the shikimate-derived ring B. The other carbons are indicated as 4a, 4b, 8a, 8b, 9, and 9a for structure elucidation purposes [4].

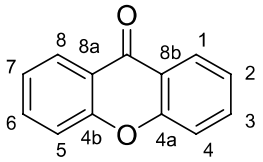


Fig. 1 Dibenzo-γ-pyrone (**1**)

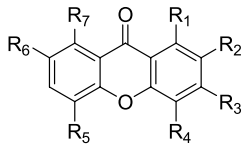
2.2 Classification of Xanthones

Xanthones isolated to date can be classified into five major groups [5]:

1. Simple oxygenated xanthones
2. Xanthone glycosides
3. Prenylated and related xanthones
4. Xanthonolignoids
5. Miscellaneous

2.2.1 Simple Oxygenated Xanthones

They can be further subdivided into six groups depending on the degree of oxygenation pattern of the basic skeleton. Genus *Hypericum* contains all



R_1=R_3=R_4=R_5=R_6=R_7= H, R_2=OH; 2-hydroxyxanthone (2)
R_1=R_3=R_4=R_6=R_7=H, R_2=OMe, R_5=OH; 5-hydroxy-2-methoxyxanthone (3)
R_1=R_2=OMe, R_3=R_5=R_6=R_7=H, R_4=OH; 4-Hydroxy-1,2-dimethoxyxanthone(4)
R_1=R_3=OMe, R_2=R_4=R_5=H, R_6=R_7=OH; 6,7-dihydroxy-1,3-dimethoxyxanthone(5)
R_1=R_6=R_7=OMe, R_4=R_5=H, R_2=R_3=OH; 2,3-Dihydroxy-1,6,7-trimethoxyxanthone(6)
R_1=OH, R_2=R_3=R_4=R_5=R_6=OMe;1-hydroxy-2,3,4,5,7-pentamethoxyxanthone(7)

Fig. 2 Examples of oxygenated xanthones

classes of oxygenated xanthones except for the hexa-oxygenated (examples of each class are given in Fig. 2):

(a) Mono-oxygenated xanthones: They are unusual and only a small number of mono-oxygenated xanthones have been isolated from natural sources up to now. 2-Hydroxyxanthone (**2**) can be given as an example of this class [6, 7].
(b) Di-oxygenated xanthones: They are more common derivatives of xanthones. 5-Hydroxy-2-methoxyxanthone (**3**) was isolated from *Hypericum roeperanum* [8].
(c) Tri-oxygenated xanthones: They can be more frequently encountered in nature. 4-Hydroxy-1,2-dimethoxyxanthone (**4**) was isolated from *H. geminiflorum* [9].
(d) Tetra-oxygenated xanthones: They are more numerous than tri-oxygenated xanthones. To date many tetra-oxygenated xanthones have been isolated. 6,7-Dihydroxy-1,3-dimethoxyxanthone (**5**) isolated from *H. geminiflorum* [9] can be shown as an example of tetra-oxygenated xanthones.
(e) Penta-oxygenated xanthones: Only a small number of this class was found in nature. 2,3-Dihydroxy-1,6,7-trimethoxyxanthone (**6**) was isolated from *H. geminiflorum* [10].
(f) Hexa-oxygenated xanthones (**7**) [11]: They have the highest degree of oxygenation observed so far and only a few compounds were identified. No hexa-oxygenated xanthones have been isolated from *Hypericum* species.

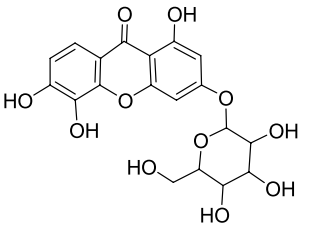
2.2.2 Xanthone Glycosides

They might be divided into *O*-glycosides and *C*-glycosides according to the nature of the glycosidic linkage:

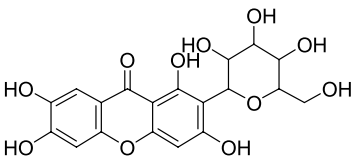
(a) *O*-Glycoside xanthones: Although most *O*-glycoside xanthones have the sugar moiety attached to position 1 of the xanthone nucleus, which is difficult to explain considering the vicinity of the carbonyl function, since this might create a strain, it is also possible to observe the glycosyl moi-

ety at any position of the xanthone nucleus. They are easily hydrolyzed in enzymatic or acid environment [13]. Only four *O*-glycoside xanthones have been isolated from *Hypericum* species. One of them is patuloside A (**8**) isolated from *H. patulum* [12].

(b) *C*-Glycoside xanthones: They are more resistant to hydrolysis compared with *O*-glycoside xanthones, but their occurrence is very much limited. Mangiferin (**9**) [5] was the first glycoside xanthone isolated in 1908 from *Mangifera indica* (Anacardiaceae) and has also been isolated from *H. montbretii* Spach. for the first time from genus *Hypericum* [14].



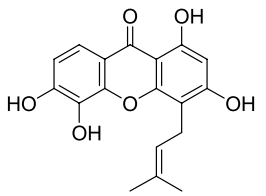
Scheme 1 3-*O*-β-D-Glucopyranosyl-1,5,6-trihydroxyxanthone; patuloside A (**8**)



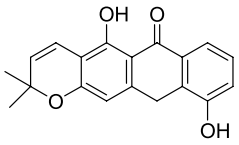
Scheme 2 2-β-D-Glucopyranosyl-1,3,6,7-tetrahydroxyxanthone; mangiferin (**9**)

2.2.3 Prenylated Xanthones

The family Guttiferae appears to produce a large number of xanthones with isopentenyl and geranyl substituents. Prenylated xanthones **10** [15] and the corresponding pyranoxanthones **11** [16] have been reported to occur in *H. japonicum* and *H. brasilianse*, respectively.



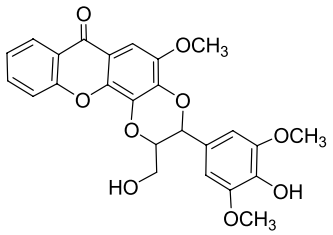
Scheme 3 1,3,5,6-Tetrahydroxy-4-(3-methyl-2-butenyl)-xanthone; ugaxanthone (**10**)



Scheme 4 6-Deoxyjacareubin (**11**)

2.2.4 Xanthonolignoids

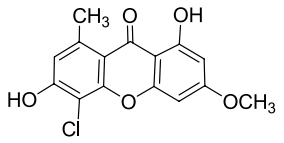
They are a relatively rare group of natural products and principally occur in some genera of the Guttiferae family: *Kielmeyera*, *Caraipa* [17], *Psorospermum* [18], and *Hypericum* [19]. These compounds are very close in the skeletal patterns formed from the association of the xanthone nucleus and the lignoid pattern (coniferyl alcohol or syringenin). The most representative ones are cadensin D (**12**) [15, 19] and kielcorin [15, 19].



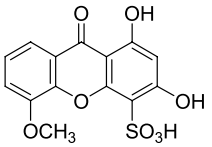
Scheme 5 Cadensin D (**12**)

2.2.5 Miscellaneous

Besides these groups, some xanthones with unusual substitutions have been isolated from different plant sources including lichens, which could not be classified in the usual manner. These compounds have been grouped as miscellaneous. We can show a chloride compound, 4-chloro-3,8-dihydroxy-



Scheme 6 4-Chloro-3,8-dihydroxy-6-methoxy-1-methylxanthone (**13**)



Scheme 7 1,3-Dihydroxy-5-methoxyxanthone-4-sulfonic acid (**14**)

6-methoxy-1-methylxanthone (**13**) from *H. ascyron* [20] and a sulfonated xanthone (**14**) from *H. sampsonii* [21] as examples of this group.

3
Biosynthesis and Synthesis of Xanthones

3.1
Biosynthesis

The biosynthetic pathways to xanthones have been discussed for 40 years. Biosynthetically, the xanthones of higher plants are formed from shikimate and acetate origins [22]. Thus phenylalanine, which is formed from shikimate by losing two carbon atoms from the side chain, is oxidized to form *m*-hydroxybenzoic acid. This combines with three units of acetate (probably via malonate) to produce the intermediate. Suitable folding and ring closure gives a substituted benzophenone, which generates the central ring of the xanthone moiety by an oxidative phenol coupling reaction [23]. Atkinson et al. [24] and Gupta and Lewis [25] performed some experiments on *Gentiana lutea* and obtained an important proof for this pathway. When plants were fed ^{14}C-labeled phenylalanine, the label was observed only in the ring B (Fig. 3). Conversely, feeding of ^{14}C-labeled acetate incorporated the label into ring A.

The other mechanisms for the intramolecular reaction of benzophenone involve quinone addition [26], dehydration between hydroxyl groups on acetate- and shikimate-derived rings (2,2′-dihydroxybenzophenone) [27], or spirodienone formation and subsequent rearrangement to form the xanthone [22, 28].

With the pioneering isolation of maclurin, 2,4,6,3′,4′-pentahydroxybenzophenone, together with 1,3,5,6- and 1,3,6,7-tetrahydroxyxanthone from the heartwood of *Symphonia globulifera* (Guttiferae), the biogenetic role of maclurin as precursor in xanthone biosynthesis was discussed by Locksey et al. [29]. Then, the following studies on the biosynthesis of xanthones in Guttiferae were reported by Bennett and Lee [30]. Cinnamic acid, benzoic acid, *m*-hydroxybenzoic acid, malonic acid, and 4′-deoxymaclurin as the intermediate benzophenone were found to be efficient precursors. This appeared to be also true for xanthone formation in cultured cells of *H. patulum*.

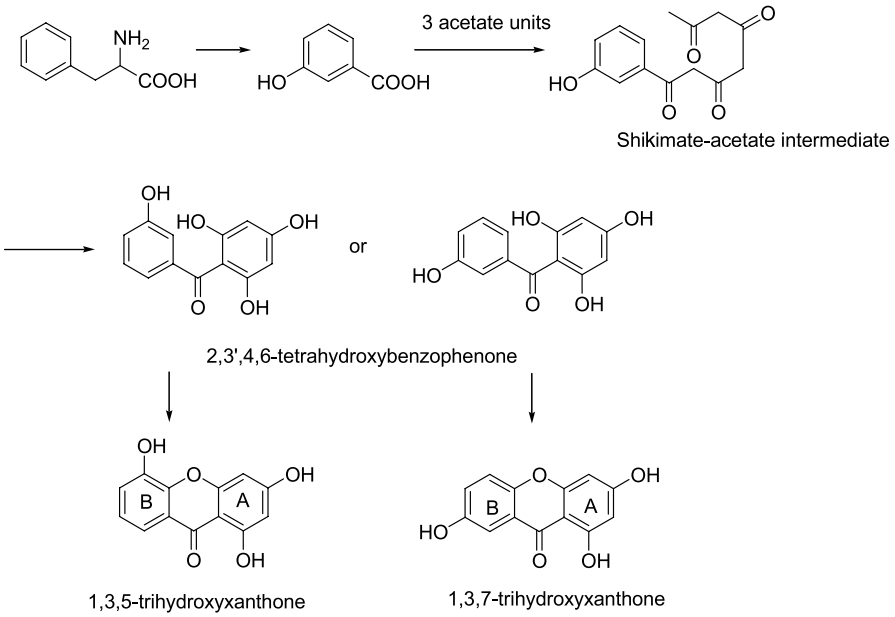


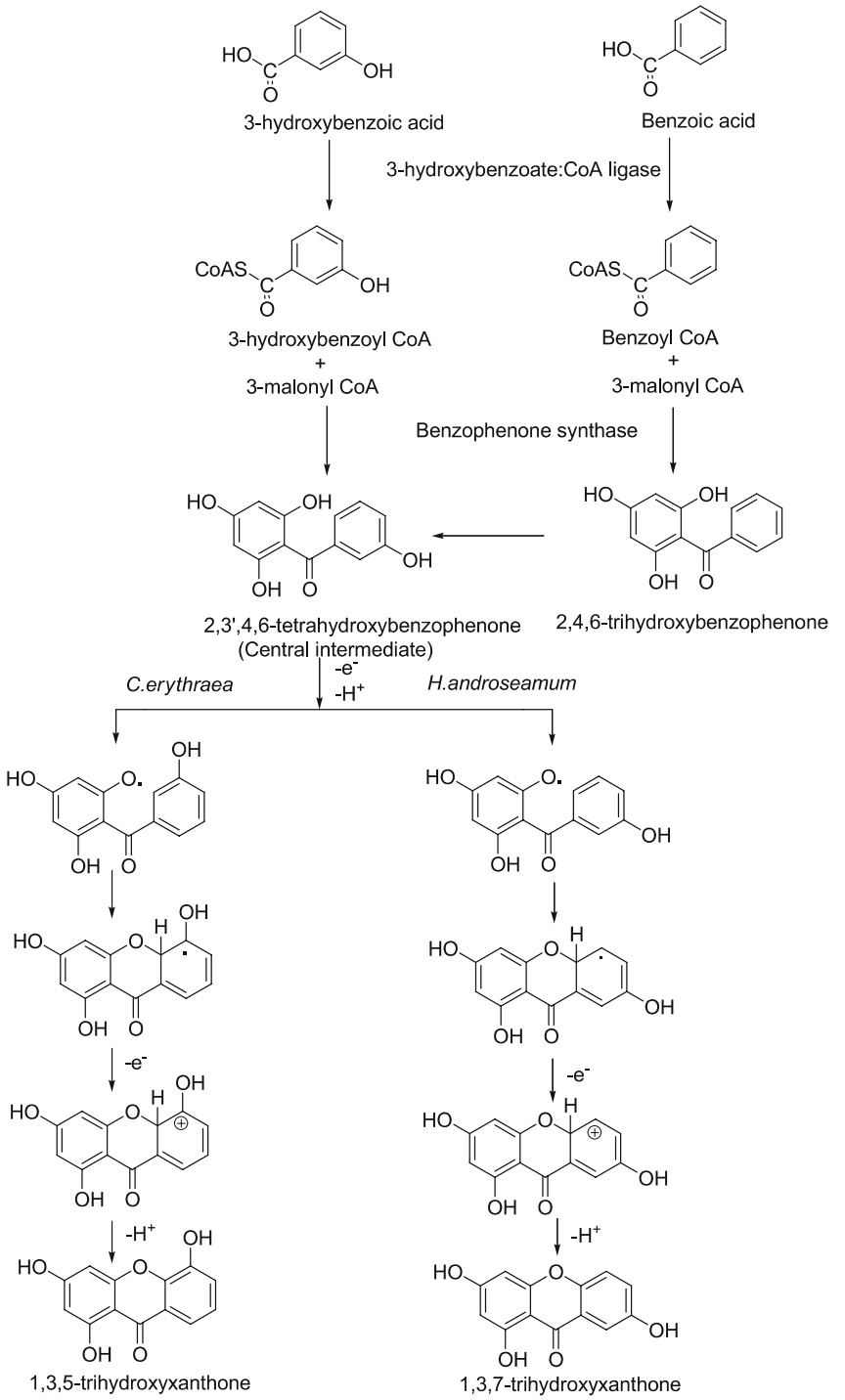
Fig. 3 Biosynthetic pathways leading to the parent xanthones 1,3,5- and 1,3,7-trihydroxyxanthone

It has been suggested that the xanthones isolated from *H. patulum* cultures could be biosynthesized from 4′-deoxymaclurin or maclurin [31–33].

Further investigation of xanthone biosynthesis was carried out by Beerhues with the detection of an enzyme named benzophenone synthase from cultured cells of *Centaurium erythraea* [34]. The formation of 2,3′,4,6-tetrahydroxybenzophenone, which is a central step in xanthone biosynthesis, was shown in cell-free extracts from cultured cells of *C. erythraea* (Fig. 4).

As part of their continuing study the same research group have reported the detection and partial characterization of another enzyme named benzophenone 3′-hydroxylase, leading to the formation of 2,3′,4,6-tetrahydroxybenzophenone in cultured *H. androsaemum* cells as well as benzophenone synthase. In contrast to the enzyme from *C. erythraea*, benzophenone synthase from *H. androseamum* acts more efficiently on benzoyl CoA than 3′-hydroxybenzoyl CoA which is supplied by 3-hydroxybenzoate:CoA ligase [35]. In *C. erythraea*, 2,3′,4,6-tetrahydoxybenzophenone is converted to 1,3,5-trihydroxyxanthone by xanthone synthase; however, in *H. androsaemum*, it is cyclized to 1,3,7-trihydoxyxanthone. Since these two isomers are precursors of the majority of higher plant xanthones [36], 2,3′,4,6-

Fig. 4 Postulated reaction mechanism of xanthones in cell cultures of *C. erythraea* and *H. androseamum* ►


3-hydroxybenzoic acid
Benzoic acid
3-hydroxybenzoate:CoA ligase
3-hydroxybenzoyl CoA
+
3-malonyl CoA
Benzoyl CoA
+
3-malonyl CoA
Benzophenone synthase
2,3',4,6-tetrahydroxybenzophenone
(Central intermediate)
2,4,6-trihydroxybenzophenone
-e⁻
-H⁺
C.erythraea
H.androseamum
-e⁻
-H⁺
-e⁻
-H⁺
1,3,5-trihydroxyxanthone
1,3,7-trihydroxyxanthone

tetrahydoxybenzophenone (THBP) represents a central intermediate in xanthone biosynthesis [37]. The formation of these isomers was explained by the regioselectivity of xanthone synthases, which are cytochrome P_{450} enzymes catalyzing the coupling of benzophenone in both cell cultures. The reaction mechanism underlying the regioselective intramolecular benzophenone cyclizations has been previously proposed by Lewis [23] to be an oxidative phenol coupling. This mechanism involves two one-electron oxidation steps (Fig. 4). The first one-electron transfer and deprotonation generate a phenoxy radical whose electrophilic attack at C-2′ or C-6′ leads to the cyclization of benzophenone. These intermediate hydroxy-cyclohexadienyl radicals are transformed by the loss of a further electron and proton to 1,3,5- and 1,3,7-trihydroxyxanthones. The results indicate that THBP is oxidatively coupled via the *ortho* position to the 3′-hydroxy group in *C. erithraea*, but via the *para* position to the 3′-hydroxy group in *H. androsaemum*. These observations indicated that THBP was the preferred substrate of xanthone synthases, and it was suggested that an oxidative phenol coupling mechanism was strongly favored by the presence of the *ortho–para* directing 3′-hydroxy group of THBP which originated from 3-hydroxybenzoic acid [38].

One of the other pathways of xanthone biosynthesis is dehydration between the hydroxyl groups of the acetate and shikimate derived rings (2,2′-dihydroxybenzophenones) via intermediates such as *O*-pyrophosphates [22, 39]. Up to 2001, however, there was no evidence for in vivo formation of polyhydroxyxanthones from benzophenones by a dehydration mechanism. In the herb *H. annulatum*, Kitanov et al. found the co-occurrence of large amounts of hypericophenoside (**15**) together with 1,3,7-trihydroxyxanthone, gentisein (**16**). The benzophenone *O*-glycoside **15** was easily transformed into gentisein (**16**) by acid or enzymatic hydrolysis (Fig. 5). This fact supports the evidence that 2,4,5′,6-tetrahydroxybenzophenone-2′-*O*-glycoside

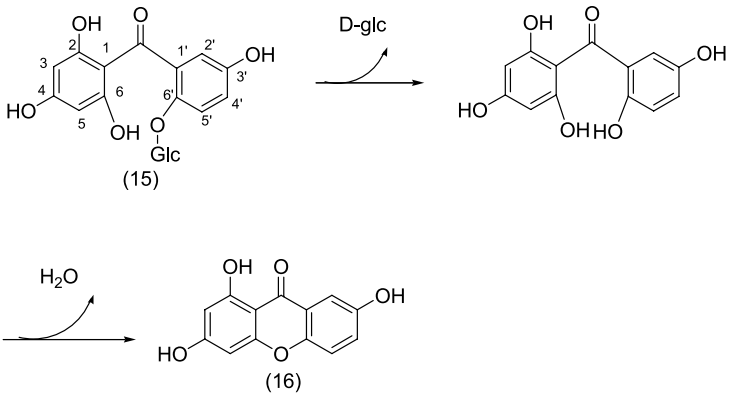


Fig. 5 Transformation of hypericophenoside (**15**) to gentisein (**16**) by a dehydration mechanism

is a precursor of 1,3,7-trihydroxyxanthone. With these results it can be concluded that some xanthones are formed in vivo by dehydration of 2,2′-dihydroxybenzophenones. This is a spontaneous reaction which appears to be regulated by deglucosylation of the precursor, which is a benzophenone with O glycosylation *ortho* to the carbonyl function [40]. The deglucosylation occurs at an earlier stage of the benzophenone biosynthesis before cyclization of two rings [40].

In the case of *C*-glycosylxanthones, it has been suggested that mangiferin is biogenetically related to flavonoids. Indeed, often mangiferin co-occurs with related *C*-glycosyl flavonoids [13]. Fujita and Inoue have reported the biosynthesis of mangiferin in *Anemarrhena asphodeloides* Bunge (Liliaceae) [41]. The results showed that the xanthone nucleus is really formed from a flavonoid-type C_6–C_3 precursor coupled with two malonate units. All the carbon atoms of phenylalanine as well as cinnamic acid and *p*-coumaric acid are incorporated into the xanthone nucleus, but benzoic acid is clearly not on the pathway which is distinct from that of normal xanthones (Fig. 6). Glycosylation seems to occur at the benzophenone stage and is followed by oxidative cyclization. For many years, the glycosylated benzophenone was considered as a hypothetical intermediate. Tanaka et al. found evidence confirming this postulated benzophenone intermediate [42]. 3-*C*-*β*-D-Glucosylmaclurin, which is a benzophenone *C*-glycoside, was isolated together with mangiferin in *Mangifera indica*. Moreover, 2,3,4′,5,6-pentahydroxybenzophenone-4-*C*-glycoside, isolated for the first time from nature, was also recently found together with mangiferin in *Gnidia involucrata* [43]. These results are important evidence for the hypothesis of Fujita and Inoue.

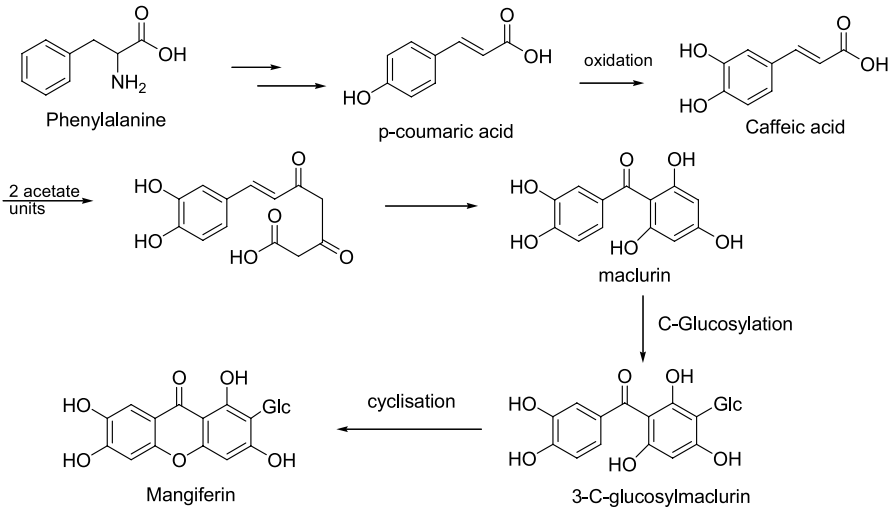


Fig. 6 Biosynthetic pathways leading to mangiferin

3.2 Synthesis of Xanthones

Since xanthones are from natural origins, they have limited type and position of substituents imposed by the biosynthetic pathways. Synthesis of new compounds enables enlargement of the possibilities of having different natures and positions of substituents on the xanthone nucleus. This will allow us to have different structures with a variety of biological activities. Based on these considerations, many xanthone compounds have been synthesized in recent years. In this section, the general methods for synthesizing xanthones and the synthesis of some pharmacologically important xanthones from genus *Hypericum* will be presented.

3.2.1 Standard Methods for the Synthesis of Xanthones

The standard methods for the synthesis of xanthones are via the benzophenone **17** and diaryl ether intermediates **18** (Fig. 7). The intermediate benzophenone derivatives **17** can be obtained by condensation between an *ortho*-oxygenated benzoic acid and an activated phenol, in the presence of phosphorus oxychloride and zinc chloride (a) [44]. This intermediate is also accessible through condensation by the Friedel–Crafts acylation of appropriately substituted benzoyl chlorides with phenolic derivatives (b) [45]. Then the oxidative or dehydrative processes cause the cyclization of 2,2′-di-oxygenated benzophenone to xanthone (c) [46].

The other method can be carried out via a suitable diaryl ether intermediate **18**, which can be obtained from the condensation of a phenol and an

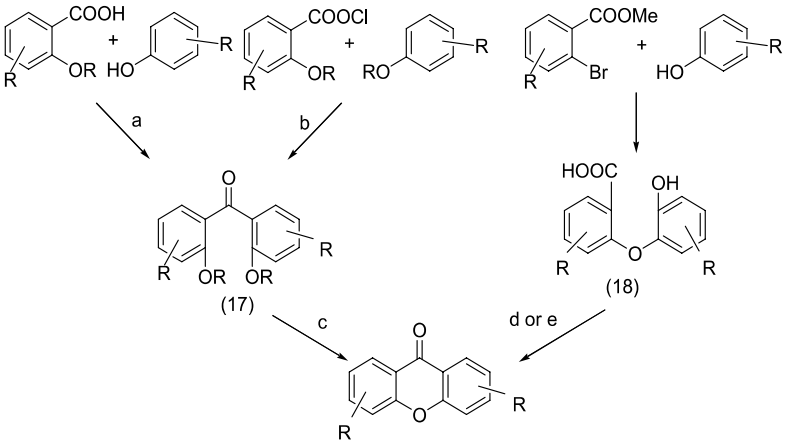


Fig. 7 General methods for the synthesis of xanthones (taken from [50] by permission from Elsevier)

o-chloro or -bromobenzoic acid. Then this biphenyl intermediate converts to the xanthone with ring formation by a one-step reaction with lithium diisopropylamide [47] or by acetyl chloride (e) [48]. Since this method was successfully applied for the first time to the synthesis of euxanthone by Ullmann and Pauchaud [49], it is called Ullmann synthesis.

The general methods for the synthesis of xanthones were shown as Fig. 7 by Pedro et al. [50]. The other well-known methods for synthesizing xanthone derivatives have also been mentioned below.

Asahina–Tanase Method

This is a useful method for the synthesis of some methoxylated xanthones or xanthones with acid-sensitive substituents [51]. Recently Vitale et al. [52] modified the procedure as shown in Fig. 8.

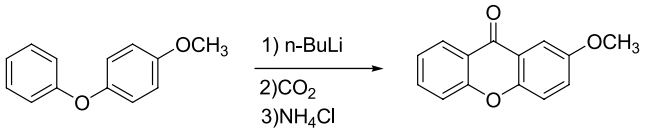


Fig. 8 Asahina–Tanase method [52]

Tanase Method

The Tanase method enables the synthesis of polyhydroxyxanthones. It has been used for the preparation of partially methylated polyhydroxyxanthones with pre-established orientation of some substituents, for example, the synthesis of 1,3-dihydroxyxanthone (Fig. 9) [53].

3.2.2 Synthesis of Prenylated Xanthones

1. O-prenylated xanthones: They have been obtained by O alkylation of a hydroxyxanthone with prenyl bromide in the presence of potassium carbonate [54–56], but very little work has been done in this area.
2. Three methods of C prenylation are known. They are as follows:
   (i) C prenylation with 2-methylbut-3-en-2-ol in the presence of boron trifluoride in ether. Only one case has been reported in the literature with 1,3-dihydroxyxanthone leading to prenylated xanthones **19–21** (Fig. 10) [57].
   (ii) The reaction of 1,3-dihydroxy-5,8-methoxyxanthone with prenyl bromide in the presence of sodium methoxide yields a mixture of O- and C-prenylated xanthones **22–24** (Fig. 11) [58].
   (iii) Another method of prenylation of the aromatic ring starts with an O prenylation followed by a C prenylation by Claisen rearrangement (Fig. 12) [55].

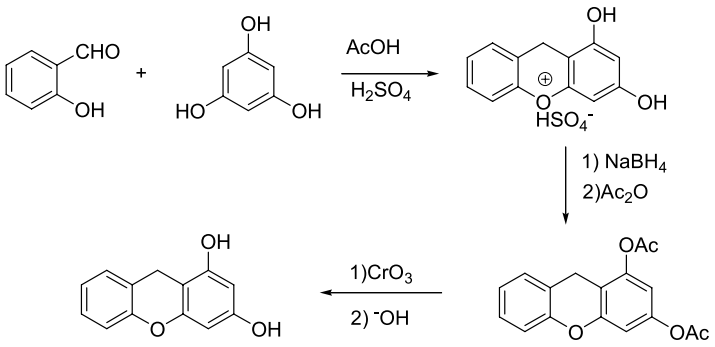


Fig. 9 Synthesis of 1,3-dihydroxyxanthone with the Tanase method [53]

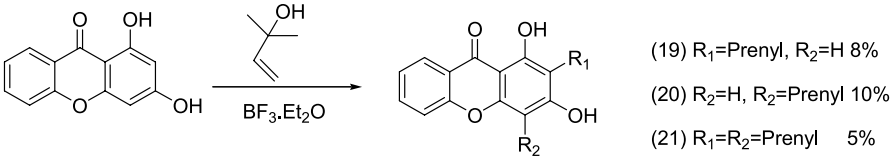


Fig. 10 C prenylation with 2-methylbut-3-en-2-ol [57]

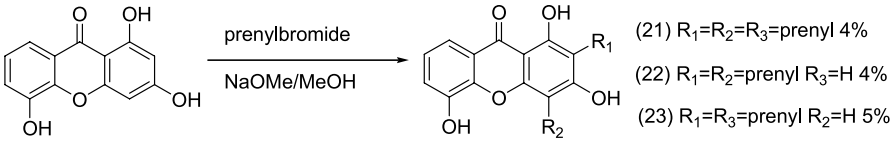


Fig. 11 C prenylation with prenyl bromide in the presence of strong base [58]

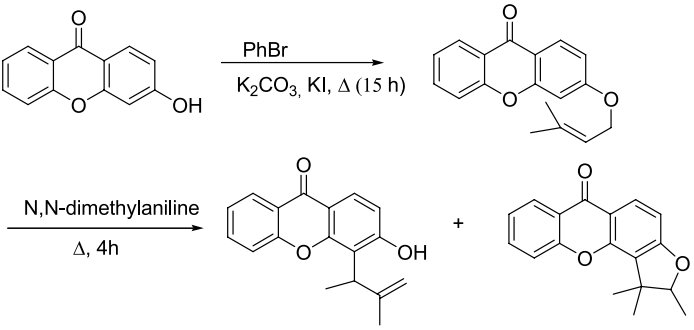


Fig. 12 C prenylation through Claisen rearrangement [55]

3.2.3
Synthesis of Xanthones Isolated from Genus *Hypericum*

2-Hydroxy-5,6,7-trimethoxyxanthone, which was isolated for the first time from *H. ericoides*, was successfully synthesized by Gil et al. [59]. The synthesis was performed from the new benzophenone precursor **25** (Fig. 13). Compound **25** was prepared by Friedel–Crafts acylation of 1,2,3,4-tetramethoxybenzene with 2,5-dibenzyloxybenzoic acid and oxalyl chloride. When benzophenone **25** was heated with Me_4NOH, 5-benzoyloxy-2-hydroxy-2′,3′,4′,5′-tetramethoxybenzophenone (**25**) underwent cyclization to 2-benzyloxy-5,6,7-trimethoxyxanthone (**26**) [60]. Hydrogenolysis of this compound with H_2/Pd-C [61] finally afforded 2-hydroxy-5,6,7-trimethoxyxanthone (**27**) which is identical to natural xanthone [60].

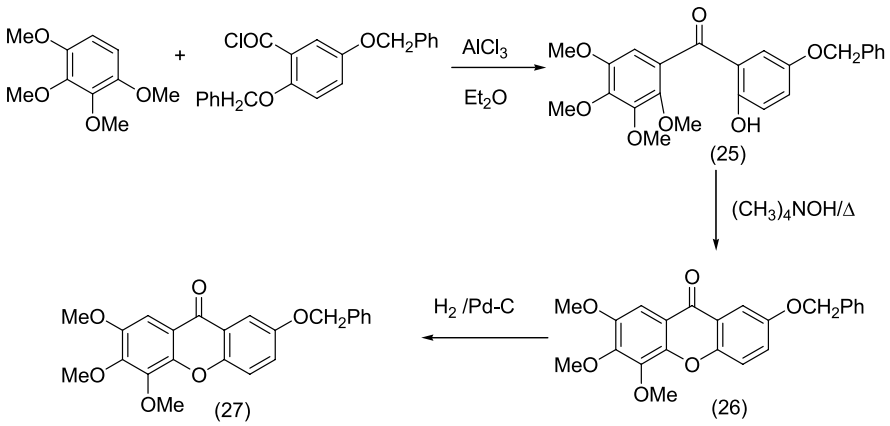


Fig. 13 Synthesis of 2-hydroxy-5,6,7′-trimethoxyxanthone [59]

The xanthonolignoid kielcorin (**35**) has been isolated from several *Hypericum* species [15, 19]. Gottlieb et al. accomplished the synthesis of kielcorin in low yield by oxidative coupling of 3,4-dihydroxy-2-methoxyxanthone and coniferyl alcohol with silver oxide [62]. Then, a facile synthesis of kielcorin (**35**) from readily available materials 3-benzyloxy-4-hydroxy-2-methoxyxanthone (**30**) and ethyl 2-bromo-3-(4-benzyloxy-3-methoxyphenyl)-3-oxopropionate (**31**) was carried out by Tanaka et al. [63]. The starting material **30** was synthesized by benzylation of 4-formyl-3-hydroxy-2-methoxyxanthone (**28**) followed by treatment with *m*-chloroperbenzoic acid in CH_2Cl_2 (the Baeyer–Villager reaction). Compound **30** was then condensed with **31** in acetonitrile in the presence of potassium *tert*-butoxide to give **32** in 74% yield; this product was subjected to catalytic hydrogenation, affording a debenzylation product **33**. Reduction of **33** with lithium borohydride in THF at 0 °C provided an inseparable mixture of alcohols (**34a,b**). The mix-

ture **34a,b** cyclized upon heating in acetic acid in the presence of concentrated hydrochloric acid to furnish kielcorin (30% yield) and *cis*-kielcorin with 3% yield (Fig. 14).

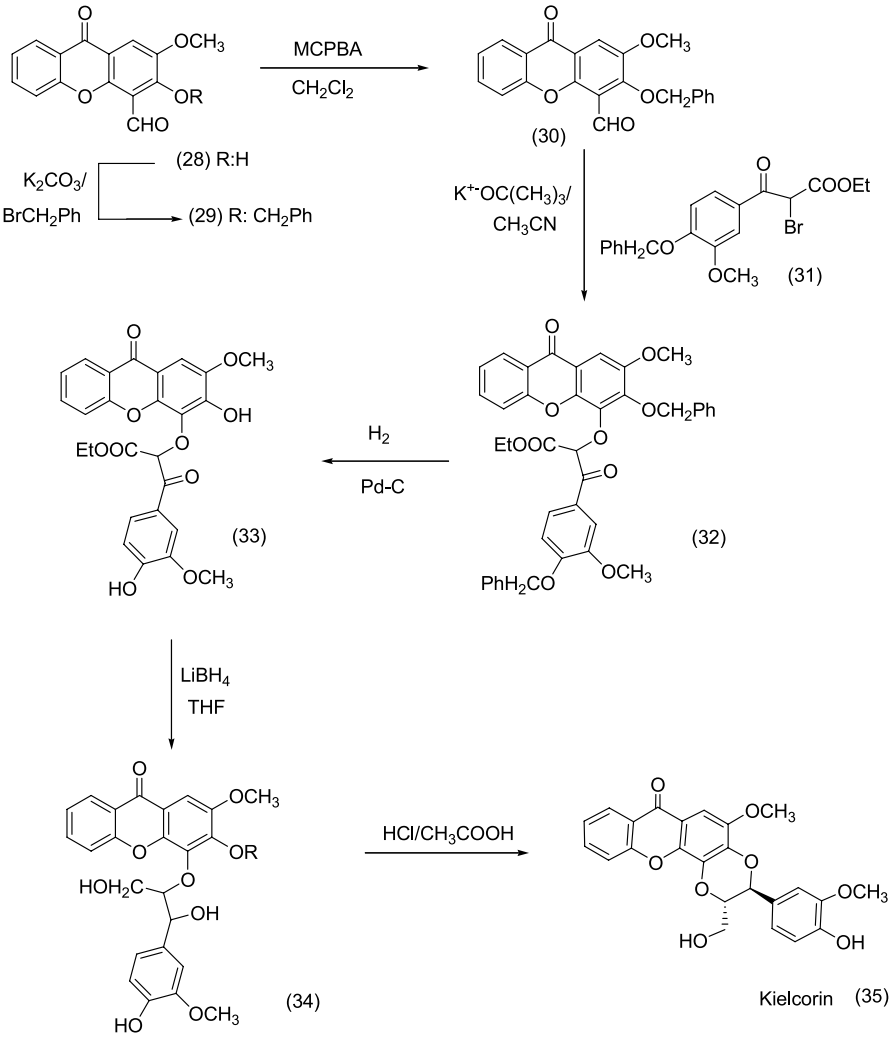


Fig. 14 Synthesis of kielcorin [63]

Recently, a study on the synthesis of novel natural secondary allylic alcohol derivatives was carried out by Helesbeux et al. [64]. The photooxygenation–reduction sequence was applied in the prenylated xanthone series; also 6-deoxyisojacareubin, which was already isolated from *H. japonicum*, was synthesized in this work. 1,3,5-Trihydroxyxanthone (**39**) was synthesized

via a polymethoxybenzophenone intermediate, easily obtained by Friedel–Crafts acylation. Thus, 2,3-dimethoxybenzoyl chloride (**36**), prepared in situ from the corresponding acid in the presence of oxalyl chloride, reacted with 1,3,5-trimethoxybenzene (**37**) to give 2-hydroxy-2′,3′,4′,6′-tetramethoxybenzophenone (**38**) with 86% yield (Fig. 15). As already described by Quillian [45], a mono-demethylation occurred on the ring provided by the acid moiety in the position *ortho* to the carbonyl function. Then subsequent base-catalyzed cyclization [65] of **38** led to 1,3,5-trimethoxyxanthone (**39**) with 93% yield along with methanol elimination. Demethylation of **39** was completed in the presence of iodhydric acid and phenol [66, 67] leading to 1,3,5-trihydoxyxanthone (**40**) with 95% yield. In the presence of an aqueous potassium hydroxide solution, **40** reacted with 4-bromo-2-methyl-2-butene to give prenylated derivatives **41**–**43**, already known as natural prod-

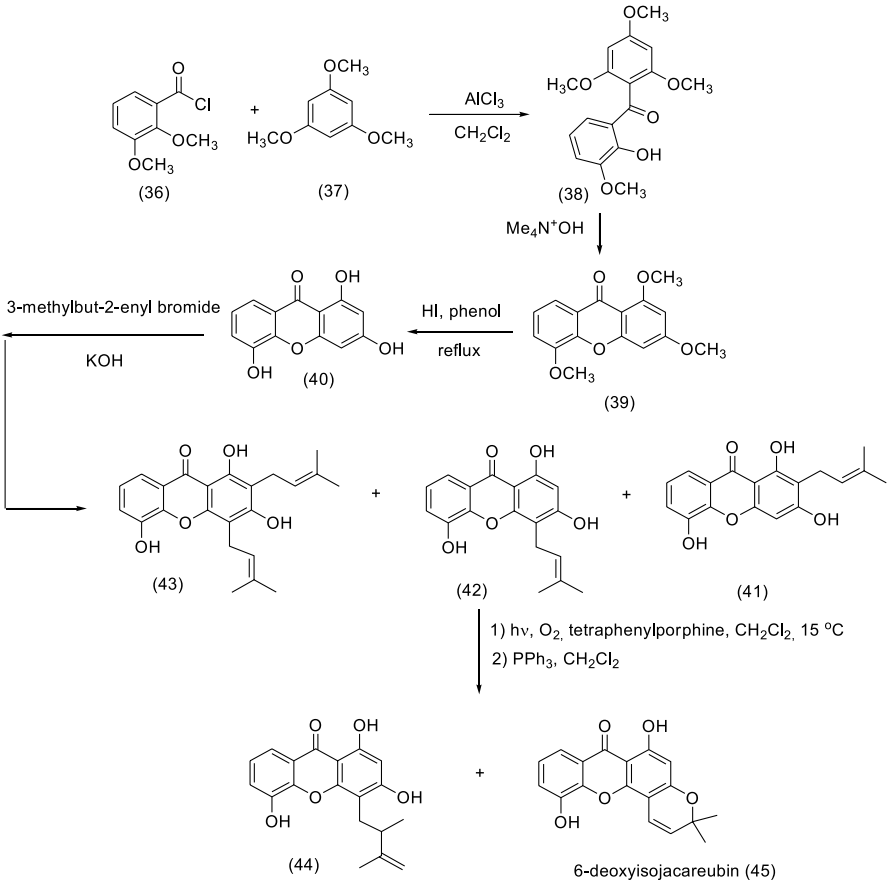


Fig. 15 Synthesis of 6-deoxyisojacareubin

ucts (Fig. 15). When the photooxygenation–reduction sequence conditions were applied to C-4-monoprenylated xanthone **42**, two products were obtained (Fig. 15): secondary allylic alcohol **44**, and as major product with 17% yield the pyranoxanthone **45** already known as a natural compound named 6-deoxyisojacareubin [15].

The C-2-monoprenylated xanthone **41** gave caledol (**46**), which is a secondary allylic alcohol, as oxidation product (Fig. 16) and similar experimental conditions led to the xanthone **47** and dicaledol (**48**) from (C-2, C-4)-diprenylated xanthone **43** (Fig. 17).

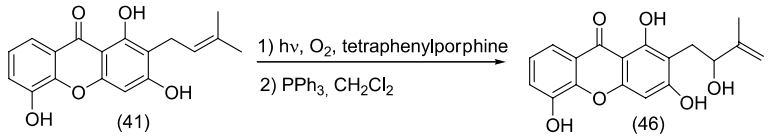


Fig. 16 Synthesis of caledol (**46**) by photooxygenation reaction

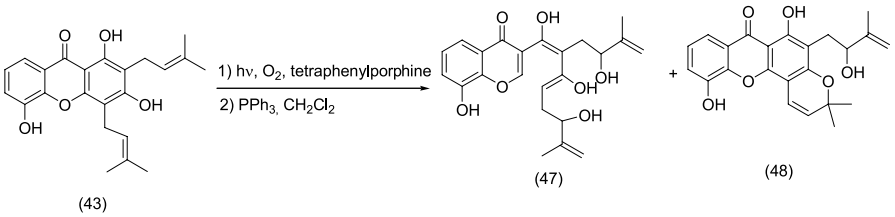


Fig. 17 Photooxygenation reaction of **43**

4
Isolation and Structures of Xanthones from *Hypericum* Species

4.1
Isolation Methods

Xanthones can be found in all parts of the plant. Extraction of xanthones from *Hypericum* species is usually carried out on dried plant material. The classical method using increasingly polar solvents has been used effectively. Lipophilic xanthones were generally separated by silica gel column chromatography (CC). For the polar xanthones, such as glycosidic and those containing hydroxyl groups, polyamide column chromatography, gel filtration chromatography on Sephadex LH-20, reversed-phase chromatography by preparative HPLC on an RP-18 column, and centrifugal partition chromatography (CPC) have been successfully employed.

Table 1 shows the part of the plant, the extracts containing xanthonoids in *Hypericum* species which have been worked, and the analytical methods used.

Table 1 Analytical methods for the isolation of xanthones from *Hypericum* species

Plant source	Plant part used	Extraction	Analysis	Refs.
H. androseamum	Roots	$CHCl_3$: MeOH	Si gel CC	[68]
H. annulatum	Aerial parts	EtOAc	Polyamide, Sephadex LH-20	[40]
H. ascyron	Whole plant	$CHCl_3$	Si gel, sephadex LH-20, ODS	[20]
H. balearicum	Twigs and leaves	Acetone	Si gel	[69]
H. beanii	Aerial parts	CH_2Cl_2	Sephadex LH-20 CC	[70]
H. brasiliense	Root and stem	CH_2Cl_2	Sephadex LH-20 CC	[16]
H. canariensis	Aerial parts	$CHCl_3$	Si gel CC	[19, 71]
H. chinense	Leaves	Hexane	Si gel CC, Toyopearl HW-20, HPLC	[72]
H. chinense	Stems	EtOAc	Si gel CC, Toyopearl HW-20, HPLC	[72]
H. erectum	Aerial parts	$CHCl_3$	Si gel CC	[73]
H. ericoides	Aerial parts	Ether	Si gel CC	[60]
H. geminiflorum	Aerial parts	MeOH	Si gel CC	[9]
H. henryi	Aerial parts	$CHCl_3$	Polyamide, Sephadex LH-20 CC	[15]
H. hookerianum	Woody stems	$CHCl_3$	Si gel column	[74]
H. inodorum	Not defined	$CHCl_3$	Si gel column	[75]
H. japonicum	Whole plant	$CHCl_3$	Si gel CC, Sephadex LH-20 CC	[76]
H. japonicum	Aerial parts	EtOAc	Si gel, polyamide, Sephadex LH-20 CC	[15]
H. japonicum	Aerial parts	CH_2Cl_2	Si gel CC, polyamide CC	[15]
H. maculatum	Not defined	CH_2Cl_2	Si gel CC	[77]
H. mysorence	Leaves and twigs	Ether	Si gel CC	[78]
H. mysorence	Timber	$CHCl_3$	Si gel CC	[79]
H. patulum	Cell cultures	EtOAc	Flash CC on Si gel	[80]
H. patulum	Cell cultures	CH_2Cl_2	Flash CC on Si gel	[80]
H. perforatum	Callus	Hexane	Si gel CC	[81]
H. reflexum	Aerial parts	$CHCl_3$	Si gel CC	[82]
H. roeperanum	Roots	CH_2Cl_2	Sephadex LH-20, HPLC on RP-18	[8]
H. sampsonii	Whole plant	EtOH	Sephadex LH-20	[21]
H. sampsonii	Whole plant	EtOAc	Si gel CC	[83]
H. scabrum	Aerial parts	EtOAc	Si gel CC, Sephadex LH-20, Toyopearl HW-20, Si gel HPLC	[84]
H. styhelioides	Leaves	BuOH	Sephadex LH-20, RP-HPLC on C18	[85]
H. subalatum	Whole plant	EtOH	Charcoal CC, Si gel CC	[86]
H. umbellatum	Aerial parts	EtOAc	Sephadex LH-20	[87]

4.2 Isolated Xanthones from *Hypericum* Species

Table 2 presents the structures of xanthones isolated and the *Hypericum* species from which they were obtained.

4.3 Structural Elucidation of Xanthones

^{1}H and ^{13}C NMR spectroscopies are the most useful methods in the structure elucidation of xanthones. ^{1}H NMR spectroscopy has been used for the determination of the substituents on each ring and it also gives information about the oxidation pattern. The observation of the signal at δ12–13 in the spectra shows chelated OH with hydroxyl substitution at position 1 or 8. When these positions are unsubstituted, aromatic protons appear between δ7.70 and 8.05 [101].

Application of the nuclear Overhauser effect (NOE) to the aromatic system may be used to determine the positions of substituent groups. The recently developed two-dimensional techniques, which can be found in numerous applications in the xanthone field, are very useful for the determination of these compounds. ^{13}C NMR analysis has played a major role in the rapid structure elucidation of xanthones. It gives information about the substitution pattern of aglycon and also about the positions of the glycosidic chains on aglycon, as well as the configuration and conformation of interglycosidic linkages [2].

The ^{13}C NMR spectra of a great number of naturally occurring xanthones have been reported and all chemical shifts assigned [102–104]. The carbonyl carbon shift is very important for identifying the oxygenation pattern of xanthone derivatives. The general observation is a decrease in electron density at the carbonyl carbon due to chelation:

1. Double chelation (1,8-di-OH): carbonyl carbon δ184
2. Monochelation (1- or 8-OH): carbonyl carbon δ178–181
3. No chelation: carbonyl carbon δ174–175

^{13}C NMR spectroscopy has now become a routine method for the structure elucidation of new xanthones. Hambloch and Frahm introduced a computer program called SEOX 1, which rapidly identifies unknown xanthones with the help of additivity rules that represents a remarkable facility in structure elucidation [105].

The UV spectrum varies in a characteristic manner depending on the oxygenation pattern. It is basically useful for locating free hydroxyl groups on the xanthone nucleus. Particularly, a free hydroxyl group at position 3 or position 6 can be easily detected by addition of NaOAc, which results in a bathochromic shift of the 300–345-nm band. A strong base such as NaOMe is capable of deprotonating all phenolic hydroxyl groups except those at-

Table 2 Structures of xanthones isolated from *Hypericum* species

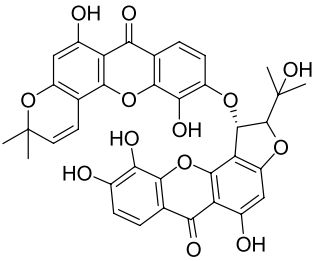
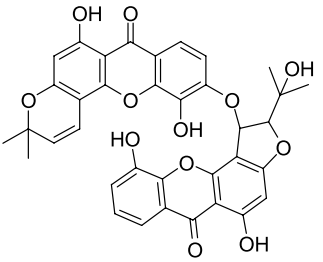
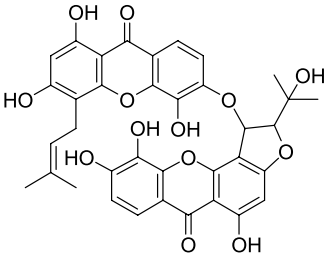
	Compound	Plant source	Refs.
1	Bijaponicaxanthone 	*H. japonicum*	[15]
2	Jacarelhypherol A: diasteromer of bijaponicaxanthone	*H. japonicum*	[88]
3	Jacarelhyperol B: 6-deoxy 	*H. japonicum*	[88]
4	Bijaponicaxanthone C 	*H. japonicum*	[89]
5	Cadensin D	*H. canariensis* and *H. mysorense*	[19, 90]

Table 2 (continued)

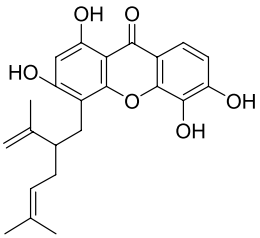
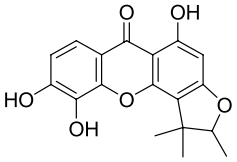
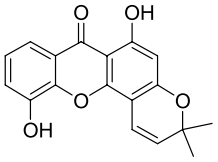
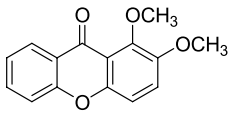
	Compound	Plant source	Refs.
6	Calycinoxanthone D; 1,3,5,6-tetrahydroxy-4-lavandulylxanthone 	*H. roeperanum*	[8]
7	4-Chloro-3,8-dihydroxy-6-methoxy-1-methylxanthone	*H. ascyron*	[20]
8	Deprenylrheediaxanthone B: 1,2-dihydro-5,9,10-trihydroxy-1,1,2-trimethyl-6*H*-furo[2,3-*c*]xanthen-6-one 	*H. japonicum* and *H. roeperanum*	[8, 15]
9	5-*O*-Methyldeprenylrheediaxanthone B	*H. roeperanum*	[8]
10	6-Deoxyisojacareubin: 6,11-dihydroxy-3,3-dimethyl-3*H*,7*H*-pyrano[2,3-*c*]xanthen-7-one 	*H. japonicum*	[15]
11	1,2-Dimethoxyxanthone 	*H. mysorense*	[79]
12	1-Hydroxy-7-methoxyxanthone	*H. mysorense*	[79]

Table 2 (continued)

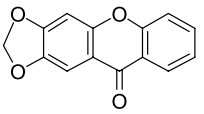
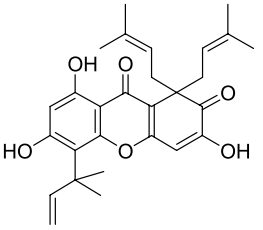
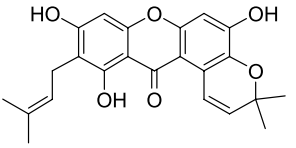
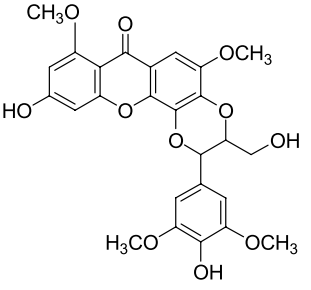
	Compound	Plant source	Refs.
13	2-Hydroxy-3-methoxyxanthone	*H. mysorense*	[79]
14	2,3-Methylenedioxyxanthone 	*H. mysorense*	[91]
15	2,5-Dihydroxyxanthone	*H. canariensis*	[71]
16	5-Hydroxy-2-methoxyxanthone	*H. inodorum*, *H. roeperanum*	[8, 75]
17	2-Hydroxy-5-methoxyxanthone	*H. canariensis*, *H. androsaemum*	[68, 71]
18	5-(1,1-Dimethyl-2-propenyl)-3,6,8-trihydroxy-1,1-bis(3-methyl-2-butenyl)-1*H*-xanthene-2,9-dione 	*H. erectum*	[73]
19	Garcinone B 	*H. patulum*	[32]
20	Gemixanthone A 	*H. geminiflorum*	[9]

Table 2 (continued)

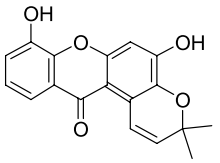
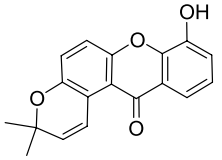
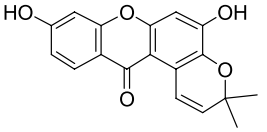
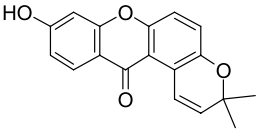
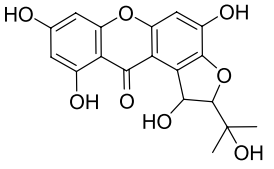
	Compound	Plant source	Refs.
21	2-Hydroxyxanthone	*Hypericum* spp.	[71, 79]
22	2-Methoxyxanthone	*Hypericum* spp.	[20]
23	Hypericanarin 	*H. canariensis*	[19]
24	Hypericanarin B 	*H. canariensis*	[92]
25	Hyperxanthone: 5,9-dihydroxy-3,3-dimethylpyrano-[3,2-*a*]xanthen-12(3*H*)-one 	*H. sampsonii*	[93]
26	Hyperireflexin 	*H. reflexum*	[82]
27	Hyperxanthone B 	*H. scabrum*	[84]

Table 2 (continued)

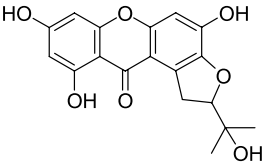
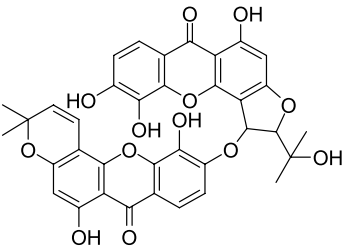
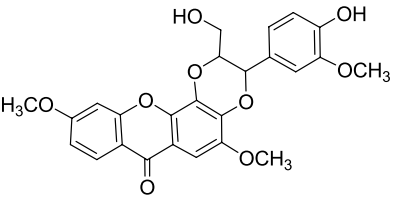
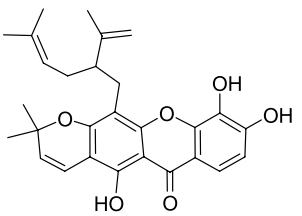
	Compound	Plant source	Refs.
28	Hyperxanthone A (1′-deoxyhyperxanthone)	*H. scabrum*	[84]
			
29	Jacarelhyperol A	*H. japonicum*	[88]
			
30	Jacarelhyperol B: 6-deoxyjacarelhyperol	*H. japonicum*	[88]
31	Kielcorin (±) form	*Hypericum* spp.	[3, 15, 19]
32	Methoxykielcorin	*H. reflexum*	[82]
			
33	Maculatoxanthone	*H. maculatum*	[77]
			

Table 2 (continued)

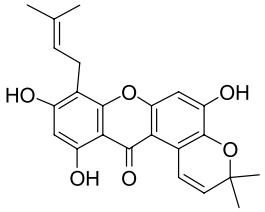
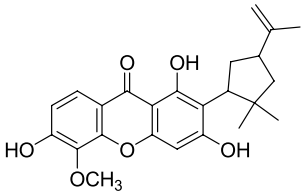
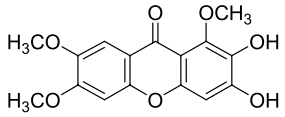
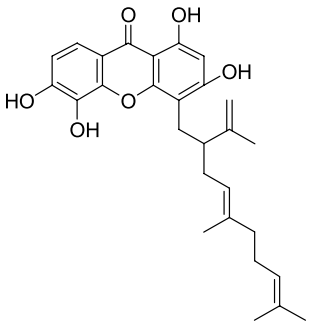
	Compound	Plant source	Refs.
34	Paxanthone B 	*H. patulum*	[32]
35	Paxanthonin 	*H. patulum*	[33, 94]
36	5-*O*-Demethylpaxanthonin	*H. patulum*, *H. roeperanum*, and *H. styphelioides*	[8, 85, 94]
37	5-*O*-Demethyl-6-deoxypaxanthonin	*H. styphelioides*	[85]
38	2,3-Dihydroxy-1,6,7-trimethoxyxanthone 	*H. geminiflorum*	[10]
39	3,6-Dihydroxy-1,5,7-trimethoxyxanthone	*H. geminiflorum*	[10]
40	Roeperanone (+),(*E*) form 	*H. roeperanum*	[8]

Table 2 (continued)

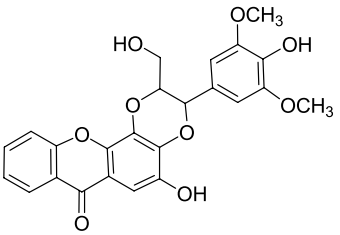
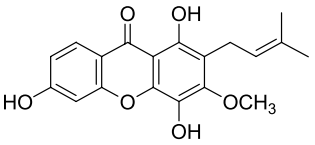
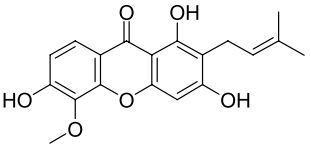
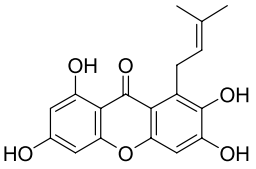
	Compound	Plant source	Refs.
41	Subalatin 	*H. subalatum*	[86]
42	1,4,6-Trihydroxy-3-methoxy-2-prenylxanthone 	*H. sampsonii*	[83]
43	Morusignin D; 1,3,6-trihydroxy-5-methoxy-2-prenylxanthone 	*H. patulum*	[33]
44	1,3,5,6-Tetrahydroxy-4-prenylxanthone; ugaxanthone	*H. japonicum*	[15]
45	2,3,6,8-Tetrahydroxy-1-prenylxanthone 	*H. androsaemum* and *H. patulum*	[32, 68]
46	Hyperxanthone C; 2,3,6,8-tetrahydroxy-1-(2-hydroxy-3-methyl-3-butenyl)xanthone	*H. scabrum*	[84]
47	Patuloside A; 1,5,6-trihydroxyxanthone 3-*O*-β-D-glucopyranoside	*H. patulum*	[12]

Table 2 (continued)

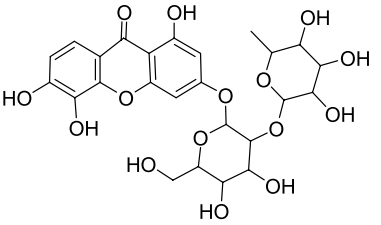
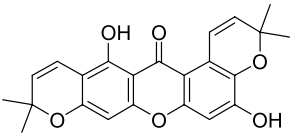
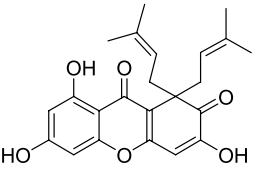
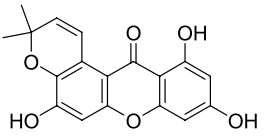
	Compound	Plant source	Refs.
48	Patuloside B; 1,5,6-trihydroxyxanthone 3-*O*-[α-L-rhamnopyranosyl-(1 → 2)-α-D-glucopyranoside] 	*H. patulum*	[12]
49	6,7-Dihydroxy-1,3-dimethoxyxanthone	*H. geminiflorum*	[9]
50	3,6-Dihydroxy-1,7-dimethoxyxanthone	*H. ascyron*	[20]
51	1,6-Dihydroxy-5,7-dimethoxyxanthone	*H. subalatum*	[95]
52	1-Hydroxy-5,6,7-trimethoxyxanthone	*H. perforatum* ssp. *Perforatum*	[81]
53	2,4-Dihydroxy-3,6-dimethoxyxanthone	*H. reflexum*	[82]
54	4-Hydroxy-2,3,6-trimethoxyxanthone	*H. reflexum*	[82]
55	2,7-Dihydroxy-3,4-dimethoxyxanthone	*H. subalatum*	[95]
56	7-Hydroxy-2,3,4-trimethoxyxanthone	*H. ericoides*	[60]
57	Padiaxanthone 	*H. patulum*	[94]
58	Patulone; 3,6,8-trihydroxy-1,1-bis(3-methyl-2-butenyl)-1*H*-xanthene-2,9-dione 	*H. patulum*	[96]
59	Toxyloxanthone B; 5,9,11-trihydroxy-3,3-dimethyl-pyrano [3,2-*a*]xanthen-12(3*H*)-one 	*H. androsaemum*, *H. patulum*	[68]

Table 2 (continued)

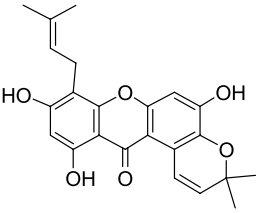
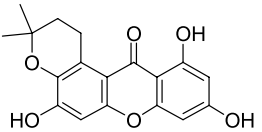
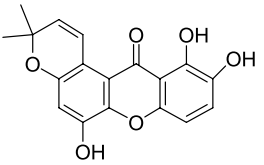
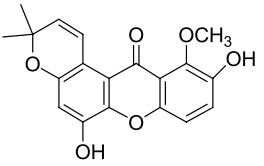
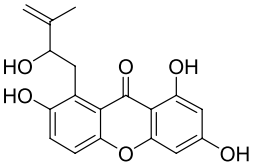
	Compound	Plant source	Refs.
60	Paxanthone 	*H. patulum*	[31]
61	3-*O*-Methylpaxanthone	*H. perforatum* ssp. *Perforatum*	[81]
62	Hyperxanthone E 	*H. scabrum*	[84]
63	Isojacareubin 	*H. japonicum* and *H. roeperanum*	[8, 15]
64	5-*O*-Methylisojacareubin 	*H. roeperanum*	[8]
65	2,6,8-Trihydroxy-1-(2-hydroxy-3-methyl-3-butenyl)xanthone: hyperxanthone D 	*H. scabrum*	[84]
66	4-Hydroxy-1,2-dimethoxyxanthone	*H. geminiflorum*	[9]

Table 2 (continued)

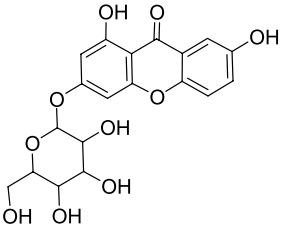
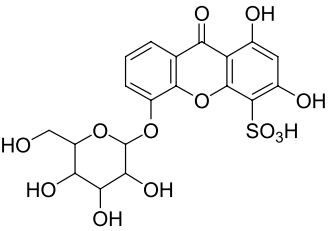
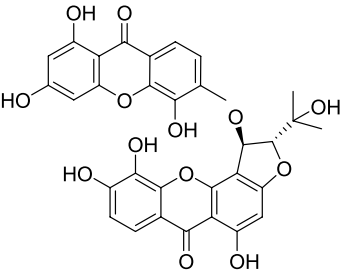
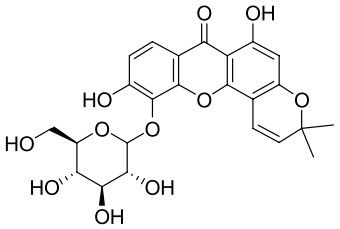
	Compound	Plant source	Refs.
67	1,2,5-Trihydroxyxanthone	*H. balearicum*	[69]
68	1,5-Dihydroxy-2-methoxyxanthone	*H. roeperanum*	[8]
69	1,3,7-Trihydroxyxanthone: gentisein	*Hypericum* spp.	[3]
70	Xanthohypericoside: 1,7-dihydroxyxanthone 3-*O*-β-D-glucopyranoside	*H. annulatum*	[97]
			
71	1,5-Dihydroxyxanthone 6-*O*-β-D-glucopyranoside	*H. japonicum*	[15]
72	1-Hydroxy-6,7-dimethoxyxanthone	*H. mysorense*	[79]
73	2-Hydroxy-3,4-dimethoxyxanthone	*H. sampsonii*	[93]
74	3-Hydroxy-2,5-dimethoxyxanthone	*H. androsaemum*	[68]
75	3,6-Dihydroxy-2-methyoxyxanthone	*H. reflexum*	[83]
76	1,3,5-Trihydroxyxanthone-4-sulfonic acid; 5-*O*-β-D-glucopyranoside	*H. sampsonii*	[21]
			
77	1,3-Dihydroxy-5-methoxyxanthone-4-sulfonic acid	*H. sampsonii*	[21]
78	Jacarelhyperol D	*H. japonicum*	[98]
			
79	4,6-Dihydroxy-2,3-dimethoxyxanthone	*H. chinense*	[99]

Table 2 (continued)

	Compound	Plant source	Refs.
80	2,6-Dihydroxy-3,4-dimethoxyxanthone	*H. chinense*	[99]
81	6-Hydroxy-2,3,4-trimethoxyxanthone	*H. chinense*	[99]
82	3,6-Dihydroxy-1,2-dimethoxyxanthone	*H. chinense*	[99]
83	4,7-Dihydroxy-2,3-dimethoxyxanthone	*H. chinense*	[99]
84	3,7-Dihydroxy-2,4-dimethoxyxanthone	*H. chinense*	[99]
85	1,6-Dihydroxyisojacareubin-5-*O*-β-D-glucoside	*H. japonicum*	[100]
			
86	3,6,7-Trihydroxy-1-methoxyxanthone	*H. japonicum*	[100]

tached at positions 1 and 8. These hydroxyls can be detected by the complex formed on addition of $AlCl_3$ which is stable to HCl. *Ortho*-dihydroxyl groups similarly may give this complex, but can be distinguished from the former by the instability of the complexes in HCl [2].

Mass spectrometry (MS) has not been applied extensively to the study of naturally occurring xanthones, but the mass spectral data provide valuable information about the structure elucidation of xanthones. As well as electron impact MS, which is a routine technique for the structure elucidation of xanthones, recently developed soft ionization techniques, such as desorption–chemical ionization MS (D/CI-MS) and fast atom bombardment MS (FAB-MS), are of great interest for the analysis of glycosides. Molecular ion peaks can be observed without derivatization. Tandem MS/MS can be extensively employed in directly characterizing constituents of complex mixtures. Recently, xanthone profiles of *H. perforatum* cell cultures were identified by HPLC-MS/MS analysis [106].

5 Biological Activities of Xanthones Isolated from *Hypericum* Species

The study of xanthones in *Hypericum* species is interesting not only for the chemotaxonomic investigation but also from the pharmacological point of

view. Several biological properties, such as strong and selective inhibition of MAO-A, in vitro toxicity, in vivo antitumor activity, as well as antibacterial and antifungal activities, have been attributed to xanthones [2]. Also, the xanthones isolated from genus *Hypericum* have been found to possess various biological activities. These pharmacological properties are explained below.

5.1 MAO Inhibitor Activity

Monoamine oxidase (MAO), which exists as two isoenzymes, MAO-A and MAO-B, plays a key role in the regulation of some physiological amines [107] and acts by desamination of some neurotransmitters, such as catecholamine, serotonin, or tyramine, as shown in Fig. 18 [108]. Inhibitors of MAO are used as antidepressive drugs [107].

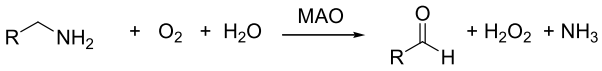


Fig. 18 Mechanism of desamination of monoamine by MAO

The antidepressive activity of *H. perforatum* has been demonstrated in vivo [109]. This activity was first explained by the presence of hypericin, which has been shown to inhibit MAO-A and B in vitro [110]. But recent investigations indicate that certain xanthones and flavonoid aglycones which have potent MAO inhibitory properties may have a more important contribution to play in the antidepressive activity of *H. perforatum* [111]. As evidence confirming these investigations, the xanthones of *H. brasiliense* showed differing degrees of inhibition of MAO-A and B. Moreover, they seemed to act in a reversible and time-independent manner. The tetracyclic xanthone, 6-deoxyjacareubin, was more potent versus MAO-A and MAO-B than the tricyclic xanthone 5-hydroxy-2-methoxyxanthone. 1,5-Dihydroxyxanthone emerged as the most potent and selective inhibitor, with an IC_{50} value of 0.73 μM for MAO-A and a selectivity index of 0.01. Thus, the internally H-bonded hydroxyl group might be operative in the mechanism of inhibition. These activities may have relevance in the search for new drugs suitable for the treatment of depression [16].

Recently, 59 xanthones (= 9*H*-xanthen-9-ones) of natural or synthetic origin were investigated for their inhibitory activity toward MAO-A and MAO-B. The majority of the compounds demonstrated reversible, time-independent activities, with selectivity toward MAO-A. The most active inhibitor (1,5-dihydroxy-3-methoxyxanthone) had an IC_{50} of 40 nM. 3D-QSAR studies revealed the importance of an OH substituent in position 1 or 5 instead of a MeO substituent and the contrary is true for position 3, where MeO

substituents lead to more active compounds than OH substituents. The CoMFA/GOLPE procedure provided information about the importance of an electron-rich zone between positions 4 and 5, and the unfavorable effect of an electron-rich zone around position 7. The ALMOND procedure showed the importance of the distance between two H-bond acceptor groups in modulating activity. These promising MAO inhibitory activities should be confirmed by in vivo experiments for the design of new antidepressant drugs [112].

5.2 Antifungal Activity

All the isolated compounds, including xanthones from *H. brasiliense*, are antifungal against *Cladosporium cucumerinum*. The minimum quantities of γ-pyrone (hyperbrasilone), 5-hydroxy-1-methoxyxanthone, 6-deoxyjacareubin, and 1,5-dihydroxyxanthone required to inhibit growth of the fungus in the bioautographic assay on TLC plates were 3, 3, 3, and 0.25 μg, respectively. Propiconazole, a triazole antifungal agrochemical, was active at 0.1 μg in the same bioassay [16].

The isolated compounds from *H. roeperanum* were tested for their antifungal activity against *Candida albicans* and *Cladosporium cucumerinum* in TLC bioautographic assays [113, 114]. The minimum amount of xanthones 2-deprenylreediaxanthone B, 5-*O*-methyl-2-deprenylrheediaxanthone B, calcinoxanthone D, roeperanone, and 5-*O*-demethylpaxanthonin required to inhibit the growth of *C. albicans* on TLC plates was 1 μg, whereas xanthone 5-*O*-methylisojacareubin showed antifungal activity at 5 μg. The reference compounds amphotericin B and miconazole were active at 1 and 0.001 μg, respectively. It must be noted that the crude dichloromethane extract did not show activity against *C. albicans* at the usual test level of 100 μg owing to the low concentration of the xanthones in *H. roeperanum*. None of the xanthones from *H. roeperanum* inhibited growth of *C. cucumerinum* at 10 μg [8].

5.3 Cytotoxic Activity

According to the first report of sulfonated xanthonoids which were isolated from *H. sampsonii*, xanthones 1,3-dihydroxy-5-methoxyxanthone-4-sulfonate and 1,3-dihydroxy-5-*O*-β-D-glycopyranosylxanthone-4-sulfonate exhibited significant cytotoxicity against the P388 cancer cell line. They were evaluated for cytotoxicity proportion against the P388 cancer cell line and were found to be moderately active (ED_{50} of 3.46 and 15.69 $\mu mol\,L^{-1}$, respectively). By contrast, VP-16 (positive control) had an ED_{50} of 0.064 $\mu mol\,L^{-1}$ [21].

5.4 Coagulant Activity

The compounds from *H. japonicum* were tested for their coagulant activity in in vitro systems. They were found to exert an interesting coagulant activity. Compound 1,5-dihydroxyxanthone-6-*O*-β-D-glucoside showed activity by promoting coagulation of PT (prothrombin time reagent) and isojacareubin showed anticoagulation of APTT (active partial thromboplastin time reagent) [15].

5.5 Antioxidant Activity

The xanthone compounds 1,3,5-trihydroxy-2-(2′,2′-dimethyl-4′-isopropenyl) cyclopentanylxanthone, 5-*O*-demethyl-6-deoxypaxanthonin, and 5-*O*-demethylpaxanthonin, as well as 3,5-dihydroxybenzophenone-4-β-D-glucoside and 3-geranyl-1-(3-methylbutanoyl)phloroglucinol isolated from the leaves of *Hypericum styphelioides*, were evaluated for their antioxidative properties in Trolox equivalent antioxidant capacity (TEAC) and chemiluminescence (CL) assays.

The free-radical-scavenging activity of compounds was evaluated in the TEAC and CL assays. The first measures the relative ability of antioxidant substances to scavenge the radical cation 2,2′-azinobis(3-ethylbenzothiozoline-6-sulfonate) ($ABTS^{\bullet+}$) as compared to a standard amount of the synthetic antioxidant Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid). The CL assay measures the inhibition of iodophenol-enhanced chemiluminescence by a horseradish peroxidase/perborate/luminol system. Trolox was used as the reference antioxidant. The results showed that xanthones exhibited free-radical-scavenging activity at potency levels comparable to those of reference antioxidant compounds quercetin and rutin, while the benzophenone and phloroglucinol type compounds had more moderate activities [87].

5.6 Antimicrobial Activity

Isojacareubin, which was found for the first time in *H. japonicum*, exhibited antimicrobial activity together with a new isopentenylated flavonol, salothranol. The antimicrobial test was carried out by the agar-well method using *Staphylococus aureus*. The compounds were examined as a 50% DMSO solution. The activity was expressed by the inhibitory diameter, which was measured after incubation for 18 h at 37 °C. The minimum inhibitory concentrations (MICs) of isojacareubin and salothranol were 125 μg/mL [77].

5.7 Anti-inflammatory Activity

The five xanthone compounds, demethylpaxantonin, patulone, garcinone B, tripteroside, and 1,3,5,6-tetrahydroxyxanthone, purified from a callus tissue culture of *H. patulum* were evaluated for their anti-inflammatory activity.

It is very important in anti-inflammatory drug development to search for natural leading compounds which exert the following pharmacological actions: (1) prevention of release of prostaglandins (PGs), major chemical mediators in the regulation of inflammation, by direct inhibition of the enzymes responsible for arachidonic acid (AA) and PG biosynthesis, including phospholipase A_2 and cyclooxygenase-2 (COX-2); and (2) suppression of transcription control of genes encoding enzymes responsible for PG biosynthesis or inflammatory cytokines. Two compounds were found with such pharmacological actions: garcinone B, which inhibits both A23187-induced PGE_2 release and LPS-induced NF-κB-dependent transcription, and patulone, which prevents COX-1 activity and A23187-induced PGE_2 release, raising the possibility of its anti-inflammatory action. These results suggested that garcinone B could become a neuropharmacological tool to elucidate intracellular signaling pathways involved in inflammation [115].

5.8 Use of Some *Hypericum* Species Containing Xanthones

It has been observed that a growing number of *Hypericum* species containing xanthones exhibit various biological properties and are used as chemotherapeutic agents in indigenous medicine for the treatment of many diseases. Typical examples are given below:

- *Hypericum* was widely used in the folk medicine of a number of European countries as a shooting agent, an antiphlogistic in the treatment of inflammation of the bronchi and the urogenital tract, a hemorrhoid treatment, and a healing agent in the treatment of traumas, burns and scalds, ulcers of various kinds, and other local and general illnesses [116].
- In the Canary Islands, various species of *Hypericum* genus have been used in folkloric medicine as a vermifuge, diuretic, and wound healer, as well as a sedative, antihysteric, and antidepressant agent [117].
- *H. perforatum* Linn. is used as an antiseptic or anthelmintic in Sri Lanka and India [118].
- *H. japonicum* Thunb. has been used in Chinese herbal medicine for the treatment of some bacterial diseases, infectious hepatitis, and tumors [119].
- Stems, leaves, and flowers of *H. ericoides* are used in Valentian folk medicine [74].

- *H. ascyron* is used in the treatment of numerous disorders, such as abscesses, boils, headache, nausea, and stomach ache in Chinese herbal medicine [119].
- *H. patulum* has been used in Chinese herbal medicine for the treatment of hepatitis, bacterial diseases, and nasal hemorrhage [119].
- *H. roeperanum* is employed, alone or in association with various plants, to cure female sterility [120].
- *H. sampsonii* is a herbal medicine used in the treatment of blood statis, to relieve swelling, and as an antitumor herb in Taiwan [121].
- *H. scabrum* is one of the most popular medicinal herbs in Uzbekistan and is used in the treatment of bladder, intestinal, and heart diseases, rheumatism, and cystitis [122, 123].
- *H. styphelioides* has been employed in traditional Cuban herbal medicine as a depurative, diaphoretic, diuretic, and tonic against blennorrhea, cold, cough, and dysmenorrhea and for the treatment of arthritis, rheumatism, hepatitis, herpes, and syphilis [124].
- Today this medicinal plant is used for these traditional purposes, but it is also largely used for the treatment of depression [118].

6 Conclusion

The recent widespread interest in the antidepressant activity of *H. perforatum* has encouraged the investigation of secondary metabolites from other *Hypericum* species. Since species of this genus occur widely in the temperate regions of the world, they have been used as traditional plants in various parts of the world. They produce several types of secondary metabolites, including flavonoids, biflavonoids, xanthones, anthraquinones, prenylated phloroglucinols, and benzophenones. Among these compounds xanthones show outstanding biological activities, such as monoamine oxidase inhibition and cytotoxic and antitumor properties, although they are relatively rare in nature in comparison with other phenolic compounds. For this reason these compounds have provoked great interest.

In this chapter, as well as the methods currently used for the isolation, separation, and structure elucidation of xanthones, their biosynthesis, synthesis, and importance as therapeutic agents was also discussed. The use of recently developed chromatographic techniques will provide characterization of these compounds and lead to the discovery of new xanthones. There are still many xanthones waiting to be discovered and evaluated by researchers for their many more biological activities.

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