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The chemistry and biology of bicoumarins

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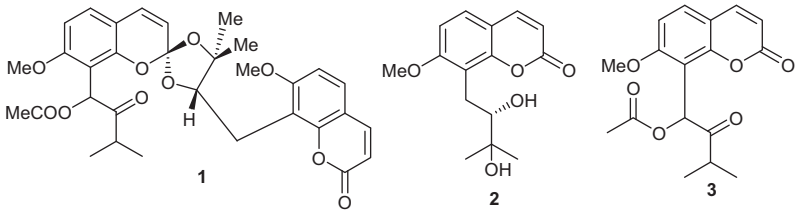
1. Introduction

Coumarins have been found to be distributed extensively in various types of flora and in all parts of the plant. They have also been reported from microorganisms and animals.[1] The simple hydroxylated and methoxylated coumarins in the free state or as glycosides occur widely in different plant families, but as the structural complexity of the compound increases, they seem to be restricted more and more to familial occurrence. The coumarin content of the plants may vary in different stages of their growth. Some plants contain the maximum amount at the time of sprouting and the younger leaves are richer in coumarin content than the older ones. The role of these compounds in the plant economy has not yet been fully elucidated.[1] Apart from the identification of numerous new bicoumarins, their biological activities and targets, in recent decades much has been learned about their biosynthesis and biological functions, and novel synthetic routes have been established. This review aims to give an overview on the structural diversity, biological activities, biosynthesis, and synthesis of bicoumarins.

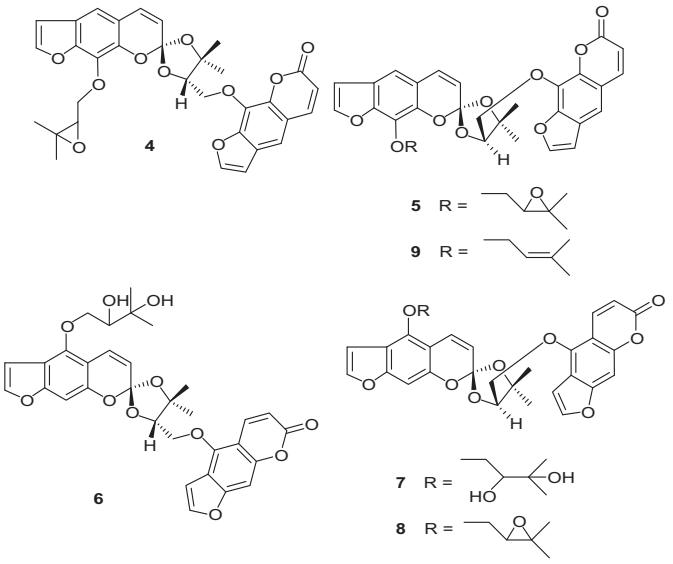


2. Bicoumarins

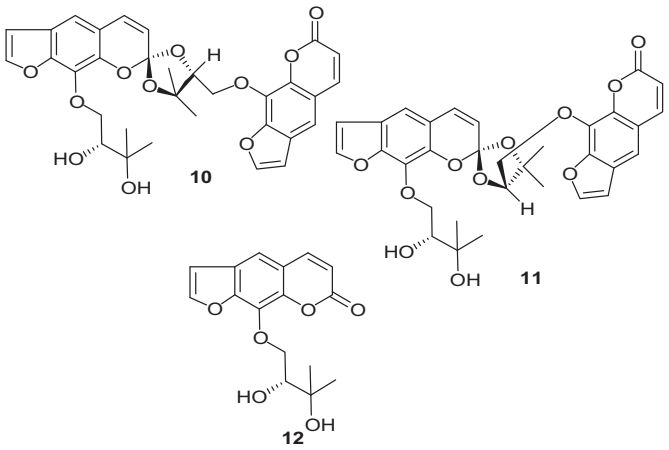
2.1. Spirobicoumarins

Murraya spp. have been used in different folk medicines in India, Australia, and South Africa. A decoction of the leaves is a remedy for bruises and has been used to treat certain fungoid skin troubles; the leaves and fruits are astringent, antidysenteric, and act as a febrifuge; the leaves have been used as a tonic, a toothache remedy, an emmenagogue, stimulant, and astringent; an infusion of the leaves or bark is regarded as antidiarrheic and antidysenteric. Moreover, *Murraya exotica* extracts have been tested successfully as an anticancer drug.[2] Bioguided investigation of *M. exotica* resulted in the isolation of Murramarin A (**1**).[2,3] Murramarin A (**1**) is a rare type of bicoumarin that connects two coumarin moieties by an *ortho*ester structure. The entire structure was established by NMR spectroscopy, and it was clarified to be composed of meranzin hydrate (**2**)[4] and hainanmurpanin (**3**).[5] Murramarin A (**1**) does not contain a furanocoumarin moiety.

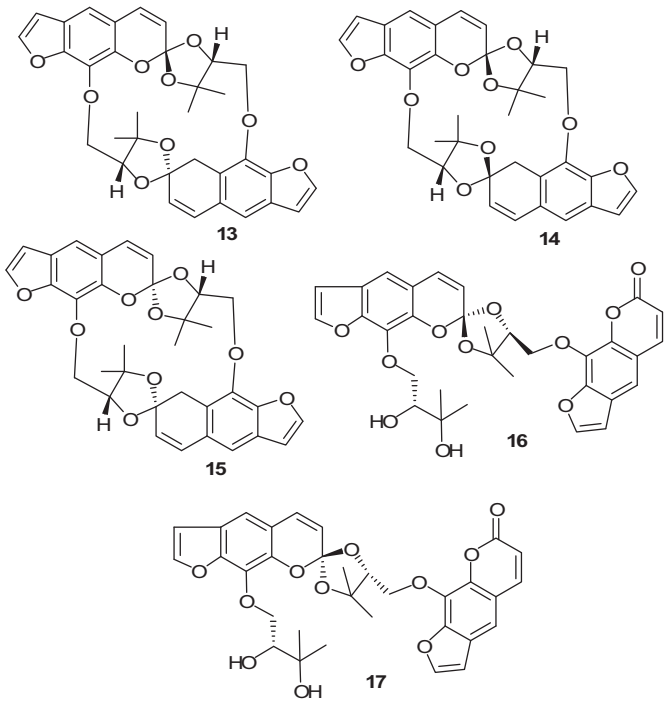
The genus *Ferula* comprises 130 species distributed from the Mediterranean region to Central Asia. This genus is well documented as a good source of biologically active compounds, such as coumarins, terpene alcohols, and sesquiterpene derivatives. Several species have been used in folk medicine. The methanol extract of the dried roots of a *Ferula sumbul* afforded six spirobicoumarins, fesumtuorins D–H (**4**–**8**), and **9**.[6]



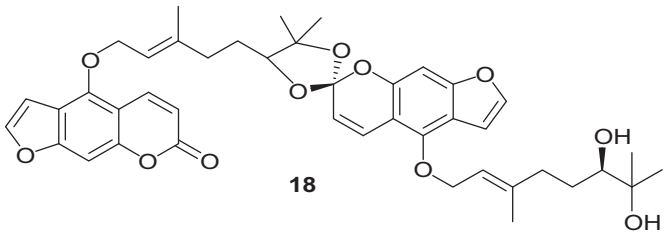
Two new spirobicoumarins, rivulobirins C (**10**), and D (**11**), were isolated from the underground parts of *Pleurospermum rivulorum*.[7] They were characterized as two stereoisomers having a different configuration at the C-2 position resulting from the condensation of the two heraclenol (**12**)[8] units, respectively.



P. rivulorum is a rich source of spirobicoumarins. Three spirobicoumarins, cyclorivulobirins A–C (**13**–**15**), have been isolated from *P. rivulorum*.[1] The absolute configurations of these compounds were established by chemical and spectral means, including NOESY experiments.[9] Taniguchi reported two bicoumarins named rivulorubin C (**16**) and D (**17**)[10] from *P. rivulorum*. Further, spirobicoumarins isolated from this plant were examined for their inhibitory effects on CYP3A activity. As a result, rivulorubins C (**16**) and D (**17**) showed strong inhibitory effects, with IC_{50} values similar to that of the typical CYP3A inhibitor, ketoconazole.

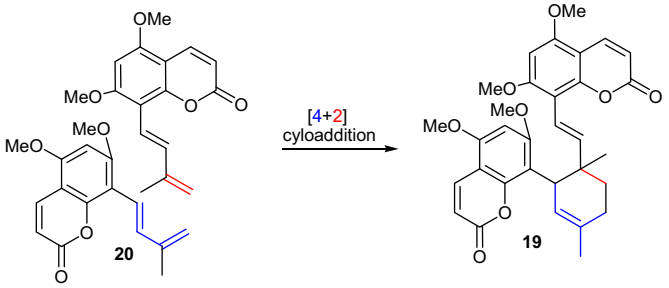


Concomitant oral administration of grapefruit juice affected the bioavailability of dihydropyridine-type calcium channel blockers, such as felodipine and nifedipine, and similar phenomena in pharmacokinetics have been reported for various clinically important drugs. In the course of their study on CYP3A4 inhibitors in the diet, Ohta et al. reported the isolation of a spirobicoumarin, paradisin C (**18**). Paradisin C (**18**) is a good cytochrome P450 (CYP) inhibitor with an IC_{50} value of 1.0 μM.[11]

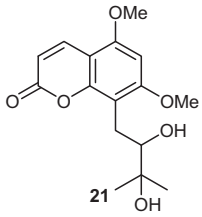


2.2. Monoterpene-unit linked bicoumarins

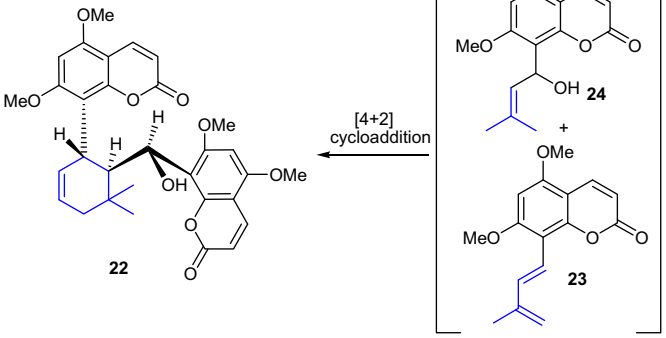
Celebrated at one time in European medicine under the name, Lopez root *Toddalia asiatica* Lamk. (Rutaceae), a climbing shrub, found in Northern, Western, and Southern parts of India, has been claimed in the indigenous system of medicine to have cardiotonic, stimulant, and antipyretic properties.[12] Ethanolic extract of *T. asiatica* showed significant diuretic activity.[12] A new dimeric coumarin, taddasin (**19**), possessing a cyclohexene ring with a vinylic side chain interposed between the two coumarin moieties has been isolated from *T. asiatica*. Biogenetically, taddasin (**19**) was considered to be formed from (*E*)-5,7-dimethoxy-8-(3-methylbuta-1,3-dienyl)-2*H*-chromen-2-one (**20**) through a [4+2] cycloaddition reaction.[12] In a preliminary evaluation, toddasin (**19**) possessed diuretic activity (71%) at a dose of 21 mg/kg in rats, when compared with chlorothiazide (125 mg/kg, 100%).



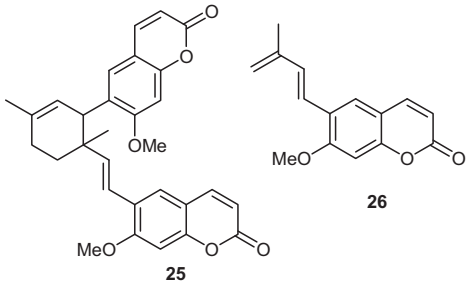
A phytochemical investigation of *M. exotica* Linn. (Syn. *Murraya paniculata*) produced the dimeric coumarin mexolide.[13] It would seem likely that despite the 23 °C difference in the reported melting points, taddasin (**19**) from *T. asiatica*, and mexolide from *M. exotica*, are the same compound. Both are optically inactive and both compounds a published with different names, but same structure. Since mexoticin (**21**), a coumarin isolated from the same plant, has a potential diene system required for the aforementioned cycloaddition, it was subjected to dehydration with P_2O_5 in boiling xylene, when cycloaddition of the diene system (**20**) generated in situ took place, yielding mexolide as one of the reaction products.



A reinvestigation of *T. asiatica* produced another dimeric coumarin, taddalosin (**22**), possessing a cyclohexene ring with a vinylic side chain interposed between the two coumarins.[14,15] Taddalosin (**22**) contains a new type of 1,3,4,5,5-pentasubstituted cyclohexene ring in its molecule in place of the common 1,3,4,4-tetrasubstituted cyclohexene in the known biscoumarins described above, and could be biosynthetically produced by a Diels–Alder-type reaction, in which 5,7-dimethoxy-8-(3-methyl-1,3-butadienyl)coumarin (**23**) and 5,7-dimethoxy-8-(1-hydroxy-3-methyl-2-butenyl)coumarin (**24**) act as diene and dienophile, respectively, in the plants. The structure and relative stereochemistry of toddalosin (**22**) were established by X-ray crystallography.

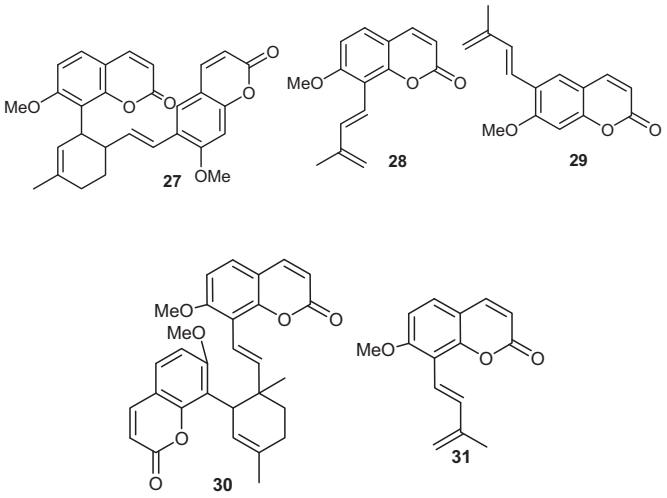


Another monoterpene-linked biscoumarin, thamnosin (**25**), was first isolated from *Thamnosma montana* Torr and Frem.[16] Biogenetically, thamnosin (**25**) was considered to be formed from two molecules of (*E*)-7-methoxy-6-(3-methylbuta-1,3-dienyl)-2*H*-chromen-2-one (**26**) through a [4+2] cycloaddition reaction.

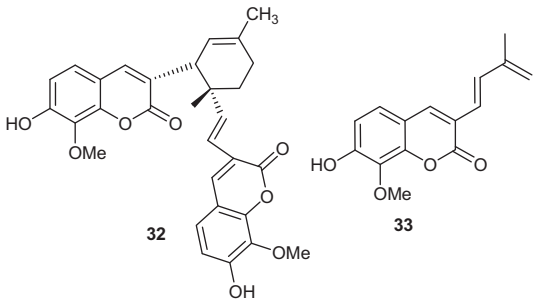


Similarly isothamnosin A (**27**) was isolated from *Ruta* sp.[17] Biogenetically, isothamnosin A (**27**) was considered to be formed from *trans*-dehydroosthol (**28**)[18] and citrubuntin (**29**)[19] through a [4+2] cycloaddition reaction.[20]

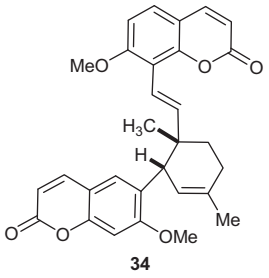
A phytochemical investigation of *Phebalium nudum* yielded phebalin (**30**) and the structure was confirmed by X-ray crystallography. Biosynthetically it may be formed from **31** via a [4+2] cycloaddition reaction.[21]



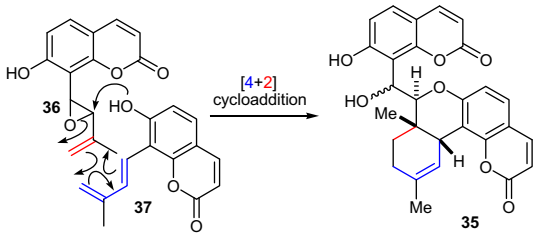
A novel dimeric 3-prenylated coumarin named microcybin (**32**) has been isolated from the aerial parts of *Microcybe multiflorus* (Rutaceae). Microcybin is a novel structure, but is closely related to a number of other dimeric coumarins that have been isolated from the Rutaceae. As with other such dimers, it lacks optical activity and is, therefore, a racemic mixture, possibly formed through a Diels–Alder addition of (*E*)-7-hydroxy-8-methoxy-3-(3-methylbuta-1,3-dienyl)-2*H*-chromen-2-one (**33**).[22]



From the roots of several hybrid seedlings resulting from crosses of *Citrus paradise* MACF. (Duncan), a new biscoumarin, named bisparasin (**34**), was isolated.[23] Bisparasin (**34**) is regioisomer of isothamnosin A (**27**). The biogenetic route of bisparasin (**34**) was considered to be same as that of isothamnosin A (**27**).

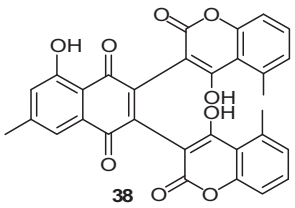


A phytochemical investigation of *Citrus hassaku* HORT. Ex Tanaka (Rutaceae) resulted in the isolation of a new biscoumarin, named hassmarin (**35**).[24] Hassmarin (**35**) is considered to be formed biogenetically by a Diels–Alder-type condensation between 7-hydroxy-8-(3-(prop-1-en-2-yl)oxiran-2-yl)-2*H*-chromen-2-one (**36**) and (*E*)-7-hydroxy-8-(3-methylbuta-1,3-dienyl)-2*H*-chromen-2-one (**37**), followed by cleavage of the epoxide ring by the phenolic hydroxyl group of (*E*)-7-hydroxy-8-(3-methylbuta-1,3-dienyl)-2*H*-chromen-2-one (**37**). Hassmarin (**35**) is the first example of a biscoumarin having an ether bond as well as a monoterpene linkage.



2.3. 7-Methyljuglone-linked bicoumarin

Diospyros ismailii Ng (Ebenaceae), a tree endemic to West Malaysia, produced a novel compound, named ismailin (**38**). 7-Methyljuglone and its oligomers are well-known metabolites of *Diospyros* sp. Ismailin (**38**) has one 7-methyljuglone and two 4-hydroxy-5-methylcoumarin-3-yl units.[25]

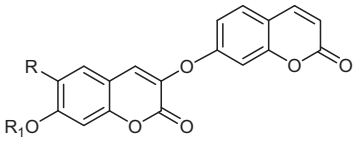


2.4. C3–C7-linked bicoumarins

2.4.1. Coumarins linked by ether bridge. Edgeworthia gardneri Meissn (*syn*. E. tornentosa Nakai) is known locally as Aryili. The plant grows in the Middle–Hill and Birch–Hill areas of Darjeeling, where the fruits of this plant are used as a fish-poison. In China, the roots and stems are used as a remedy for buboes. Besides daphnoretin (**40**) and edgeworthin (**41**), a new biscoumarin was isolated from the stem bark of *E. gardneri*.[26] The spectral and chemical evidence led to a structure in which two coumarin units are linked by an oxygen bridge between carbons 3 and 7′. Daphnoretin (**40**) is reported in more than thirty plant species.[27–30] Daphnoretin (**40**) was bioassayed for classical pathway complement inhibitory activity in vitro and had a significant effect on the classical pathway of the complement system with an IC_{50} value of 11.40 μM, when compared with tiliroside (IC_{50} 78.56 μM), which was used as a positive control.[29] Biological studies of daphnoretin (**40**) have reported that it inhibits DNA polymerase β lyase, and protein kinase C activation[31] and exhibits antifungal activity.[32] A reinvestigation of *E. gardneri* yielded biscoumarins named edgeworin (**39**), daphnoretin (**40**), edgeworthin (**41**)[31] along with demethyldaphnoretin,[33] and 7-*O*-acetyl daphnoretin (**42**).[34]

Compounds **39**–**41** inhibited the lyase activity of DNA polymerase with IC_{50} values of 7.3 μg/mL (22.5 μM), 43.0 μg/ml (122.3 μM), and 32.1 μg/ml (94.8 μM), respectively. The three biscoumarins from *E. gardneri* possess very similar structures, but exhibit rather different inhibitory potencies toward DNA polymerase β-lyase. The dearth of functional groups available for interaction with the enzyme and DNA substrates suggest that some or all of the existing groups must be important for specific interaction. This is reinforced by the negative effect of a substituent or bulky functionality at the 6-position, which must interfere with this interaction, thus resulting in a lesser inhibitory effect. Additionally studied was the ability of edgeworin (**39**) to potentiate the cytotoxicity of bleomycin toward cultured A549 cells by blocking the repair of bleomycin-mediated DNA damage. Compound **39** clearly increased the cytotoxicity of bleomycin when the two were employed jointly.

Compound **40** (daphnoretin) is the best-known biscoumarin derivative and was reported to show in vivo antineoplastic activity against the Ehrlich ascites carcinoma in mice and to inhibit a number of enzymes involved in DNA synthesis in Ehrlich ascites cells.[35] It has also been reported that daphnoretin (**40**) is a protein kinase C activator and suppresses hepatitis B virus gene expression in human hepatoma cells.[36] Presently, it is demonstrated that daphnoretin (**40**) inhibits the lyase activity of polymerase β. It is unclear at present whether its inhibition of polymerase β lyase activity is related to its earlier-reported in vivo antineoplastic activity.[35] In this context, it is worth mentioning that polymerase β has been found to be overexpressed in some human tumor tissues, and more recently it has been shown that overexpression of polymerase β results in a mutator and genome instability phenotype.[37,38]



39 R = H, R_1 = H
40 R = Me, R_1 = H
41 R = OH, R_1 = H
42 R = Me, R_1 = Ac

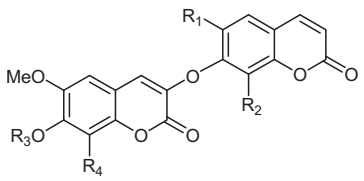
The genus *Artemisia* (Compositae family) comprises approximately 300 species widely distributed in Europe, North America, Asia, and South Africa, with about 200 species occurring in China.[39] Many *Artemisia* spp. are popular Chinese traditional medicinal plants, and they are frequently used for the treatment of dysmenorrhea, amenorrhea, hepatitis, inflammation, bruising, jaundice, hemostasia, malaria, and cancer.[40] Arteminorin A (**43**), a bicoumarin, was isolated from the aerial parts of *Artemisia minor*. The in vitro cytotoxicity of arteminorins A (**43**) isolated from *A. minor* was examined with HepG2 cells, a cancer cell line. The inhibitory effects on the activity of XOD and PTP1B enzymes of these compounds were also tested but arteminorins A (**43**) was inactive in the tests.[41]

The bark and fruits of *Daphne mezereum* L. (Rutaceae), indigenous to Europe, North America, and Western Asia, have been used in traditional medicine as a remedy for the treatment of ulcers and rheumatism and as a purgative and abortifacient. In homeopathy, dilutions of the tincture 'Mezereum' (ethanolic extract of the bark) are used against various skin diseases (herpes, eczema, and allergy) and neuralgia. *D. mezereum* is a rich source of coumarins and phytochemical studies have reported the isolation of the bicoumarins daphnorin (**44**) and daphnorin 6′-hydroxymethylglutarate (**45**).[42]

Hymenaea courbaril L. (Fabaceae) is a large tropical hardwood tree. This species is widespread in Central and South America; there are related species in Madagascar and Africa. Extracts of *H. courbaril* (Jatoba) have found utility for candida and yeast infections, for fungal infections (athlete's foot, nail fungus, etc.), prostatitis, cystitis, and urinary tract infections. It has also been used as a natural stimulant and energy tonic (tones, balances, and strengthens overall body functions).

Hymenain (**46**), a biscoumarin, was isolated from the germinated seeds of *H. courbaril* var. *stilbocarpa*.[43] Bicoumarin **46** did not show antifungal activity against the filamentous fungi *Fusarium solani*, *Cladosporium cladosporioides*, and *Aspergillus niger* and against yeast *Candida albicans*. A bioautographic assay against *Cladosporium sphaerospermum* with **46** also showed a lack of antifungal activity. A TLC autographic test using a DPPH revealing solution was performed with compound **46**. Hymenain

(**46**) showed a powerful DPPH radical scavenging-activity and reduced 50% of DPPH free radicals at a concentration of 100 μM.[44]

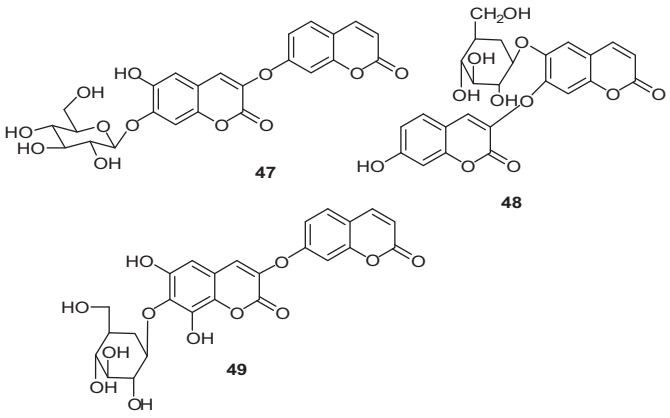


43 $R_1 = R_2 = R_4 = Me$, $R_3 = H$
44 $R_1 = R_2 = R_4 = H$, $R_3 = Glc$
45 $R_1 = R_2 = R_4 = H$, R_3 = (3-hydroxy-3-methylglutarate)-Glc
46 $R_1 = Me$, $R_2 = R_3 = R_4 = H$

Daphne oleoides Linn., belonging to Thymelaeaceae family, is a xerophytic shrub, found at high altitudes in the Himalaya mountains of Pakistan. It is locally used as folk medicine against many diseases and infections.[45] Thymelaeaceae is regarded as a principal source of coumarins and their dimers. A dicoumarinyl ether glycoside, 7-*O*-[β-D-glucopyranosyl]-6-hydroxy-3-[(2-oxo-2*H*-1-benzopyran-7-yl)-2*H*-1-benzopyran-2-one (**47**), belonging to a very rare class of dimeric coumarins', was isolated from an ethyl acetate fraction of the roots of *D. oleoides*.[46]

Similarly another dimeric coumarin glycoside **48** and was isolated from *D. oleoides*. The structure was established by means of 1D and 2D NMR spectroscopy as 3-({6-[(β-D-glucopyranosyl)oxy]-2-oxo-2*H*-1-benzopyran-7-yl}oxy)-7-methoxy-2*H*-1-benzopyran-2-one (**48**).[47]

Daphsaifnin (**49**), a new dimeric coumarin glucoside, was isolated from the roots of *D. oleoides*.[48]



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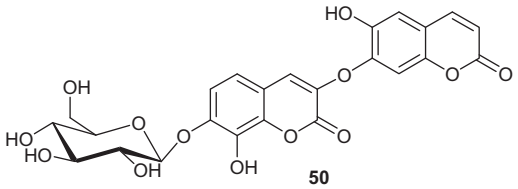
49

Two dicoumarins, 3-[[6-(α-D-glucopyranosyloxy)-2-oxo-2*H*-1-benzopyran-7-yl]oxy]-7-methoxy-2*H*-1-benzopyran-2-one (**50**), and 7-(β-D-glucopyranosyloxy)-8-hydroxy-3-[(6-hydroxy-2-oxo-2*H*-1-benzopyran-7-yl)oxy]-2*H*-1-benzopyran-2-one (**51**), isolated from *D. oleoides* were evaluated for urease inhibition assays, but these compounds did not show significant activity.[49]

Ruta chalepensis L., a member of the family Rutaceae, is a shrub originating in Southern Europe, but has now spread over North America and some other places. Little is known about its use in folk medicine. It is reported to be useful against intestinal colic, atonic amenorrhea, and rheumatic diseases.[50] Like other Rutaceae, *R. chalepensis* contains coumarins, alkaloids, and terpenes.

An investigation of a plant cell culture of *R. chalepensis* L. yielded a biscoumarin named rutarensin (**52**). Rutarensin (**52**) is one of only a few glucosides esterified at C-6 with 3-hydroxy-3-methylglutaric acid.[50]



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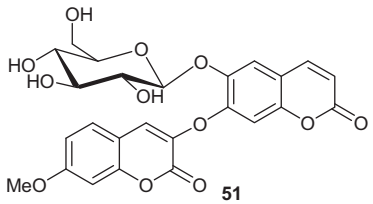

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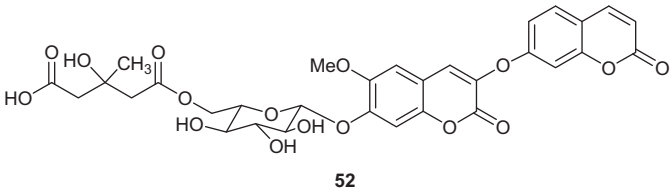
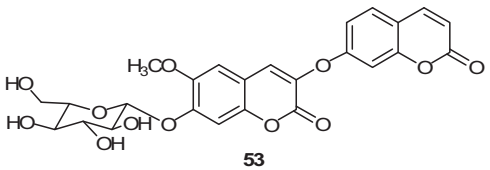


52

Narantuyaa et al. recovered a new bicoumarin glycoside chamaejasmoside (**53**) from the aerial parts of *Stellera chamaejasme*.[51] Yang et al. reinvestigated *S. chamaejasme* and reported isodaphnoretin B (**54**).[52]



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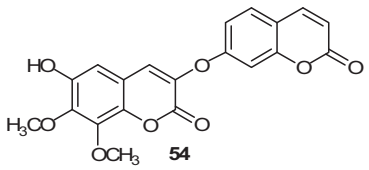
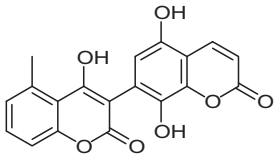


54

2.4.2. Coumarins linked through carbon–carbon bonds. The triphenol (**55**), isolated from *Gerbera anandia*, is the only C3–C7 bicoumarin in which the two coumarin units are linked through a carbon–carbon bond.

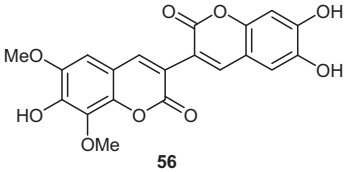


55

2.5. C3–C3′-linked bicoumarins

2.5.1. Coumarins linked through carbon–carbon bonds. A. minor plants grow uniquely on the Qinghai-Tibet Plateau of China. They have long been used as a substitute for the traditional Tibetan, medicine *Artemisia sieversiana*, for the treatment of fever, rheumatism, dysentery, scabies, and bruising.[39] A bicoumarin, arteminorin C (**56**), was isolated from the aerial parts of *A. minor*. The in vitro cytotoxicity of compound **56** was examined with HepG2 cells, a cancer cell line. The inhibitory effects on the activity of XOD and PTP1B enzymes of compound **56** were also tested. Arteminorin C (**56**) showed inhibition of the activity of XOD with GI_{50} 7.71 μM.[41]

Fungi of the genus *Aspergillus* have repeatedly been isolated from marine sources, such as sponges or algae. Even though terrestrial strains of *Aspergillus* sp. have been extensively studied in the past with regard to their natural product chemistry, marine-derived strains of the same genus nevertheless proved to be prolific sources of new secondary metabolites up to now unknown from terrestrial fungi. A phytochemical investigation of a strain of *A. niger* that was obtained from the Mediterranean sponge *Axinella damicornis* resulted in a 3,3′-bicoumarin named bicoumanigrin (**57**).[53] Prior to the isolation of compound **57** from *A. niger*, toddasiatin (3,3′-linkage) from the plant *T. asiatica* (family Rutaceae)[54] and 4,4′-biisofraxidin (4,4′-linkage) from root cultures of *Impatiens balsamina* (Balsaminaceae)[55] had been the only examples of naturally occurring bicoumarins that are linked via the lactone rings of their monomeric subunits. Bicoumanigrin (**57**) was subjected to a panel of four bioassays including a test for insecticidal properties, a brine shrimp assay, an agar plate diffusion assay, and a cytotoxicity test employing human cancer cells. Compound **57** was not toxic to brine shrimps and did not inhibit the growth of the assayed microorganisms in the agar plate diffusion assay. With regard to antiproliferative activity toward various leukemia and carcinoma cell lines, bicoumanigrin A (**57**) showed a moderate inhibitory effect on cell growth when measured with the MTT assay or using the incorporation of 3*H*-thymidine as a marker. Counting of the cells 48 h after the addition of 1–20 μg/mL of bicoumanigrin A (**57**) resulted in a mean growth inhibition of up to 50% relative to the controls, depending on the cell line chosen.[55]



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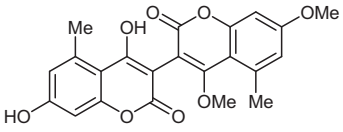
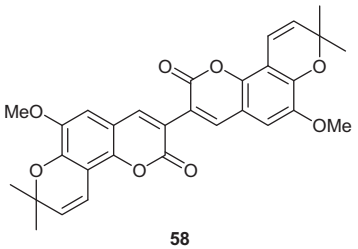


57

Examination of the root wood of Formosan *T. asiatica* led to the isolation of one dimeric coumarin named toddasiatin (**58**). The structure of toddasiatin (**58**) was elucidated to be a dimer of braylin with a C-3–C-3 linkage.[54] However, the isolation of braylin and norbraylin from *T. asiatica*[56] provides good evidence for the existence of the new dimeric coumarin, toddasiatin (**58**). This is the first example of pyranocoumarin dimer.

Phytochemical studies on extracts from the wood of the decay-resistant tropical tree *Dyphysa robinioides* Benth., known as 'guachipelin', resulted in the isolation and characterization of diphysin (**59**), a 3–3′-dimeric 4-phenyldihydrocoumarin.[57] Diphysin (**59**) showed weak activity against several gram-negative and gram-positive microorganisms. Diphysin (**59**) showed optical activity and its structure was determined by an X-ray analysis of the hexaacetate derivative of **59**. Compound **59** is the first reported dimer of a 4-arylcoumarin or 4-aryldihydrocoumarin. A few monomeric 3,4-dihydro-4-arylcoumarins have been encountered in the Guttiferae and Rubiaceae families.[57]

Erycibe obtusifolia Benth (Convolvulaceae) is distributed in Southeast Asia and Australia. The roots, stems and twigs of this plant are also found in the south of China and are used in Chinese folk medicine to relieve the symptoms of rheumatoid arthritis.[58] A new bicoumarin, named 7,7′-dihydroxy-6,6′-dimethoxy-3,3′-biscoumarin (**60**), was isolated from the roots of *E. obtusifolia*.[59] Bicoumarin **60** was a symmetrical dimer of scopoletin at C-3.



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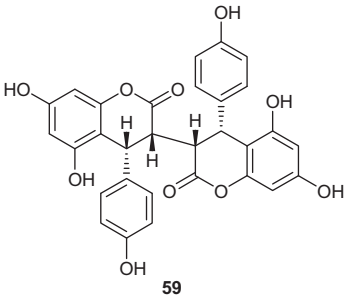

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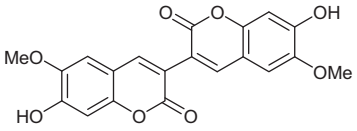
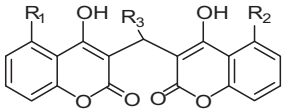


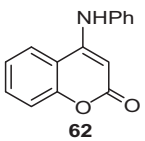
60

2.5.2. Methylene and methine group-linked bicoumarins. Young leaves of *Melilotus alba* have been used for tea, cooked greens, salads, and flavoring. White sweet clover is considered a good plant for soil restoration. *M. alba* is regarded as an important plant for honey production. A phytochemical investigation of *M. alba* (sweet clover hay) produced dicoumarol (**61**), where the two coumarin units are linked at C3–C3′ through a methylene group.[60] Since the presence of the anticoagulant dicoumarol (**61**) in mouldy clover can cause sickness and death in cattle,[61] a variety of techniques have been developed for its quantitative measurement in clover. Thus, the reaction of dicoumarol with aniline at 180 °C was reported to give 4-anilidocoumarin (**62**) (2 mol) in an almost quantitative amount. A reinvestigation of this reaction to discover the fate of the methylene group has revealed that the product is, in fact, an equimolar mixture of 4-anilidocoumarin and (**63**). The authors trivially named this product mean coumarin since it contained the methylene group yet was a derivative of the anil. They also commented that, considering the difficulty of isolation and separation, the name seemed appropriate.

Sengupta et al. isolated another C3–C3′ methylene group-linked bicoumarin, named gerberinol (**64**), from *Gerbera lanuginose*.[62] 1-Methylgerberinol (**65**) has been isolated from the roots of *Diospyros kakis*.[63]



61 $R_1 = R_2 = R_3 = H$
64 $R_1 = R_2 = Me$, $R_3 = H$
65 $R_1 = R_2 = R_3 = Me$



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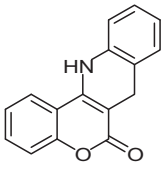
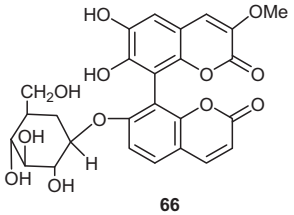


63

2.6. C8–C8′-linked bicoumarins

2.6.1. Coumarins linked through carbon–carbon bonds. *Daphne* spp. (Thymelaeaceae) have shown a variety of pharmacological actions. Some compounds isolated from *Daphne mezereum*, *Daphne odora*, *Daphne acuminate*, *Daphne genkwa*, and *Daphne papyracea*, have shown antileukemic, nematocidal, and cardiotoxic activity, and antischistosomial, sedative and hypotensive effects.[33] *D. oleoides* also yielded another C8′–C8-linked dicoumarin named, gulsamanin (**66**).[64]

Daphne feddei Levl (Thymelaeaceae) is an evergreen shrub. Its used as a folk medicine under the name of 'aluobaluoji' for healing wounds, rheumatoid arthritis, and stomach ache. Liang et al.[65] investigated *D. feddei* and isolated a new C8–C8′ coupled bicoumarin, giraldoid A (**67**).



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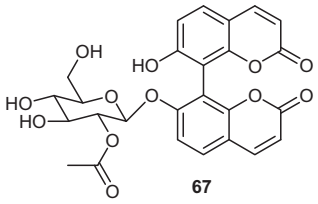
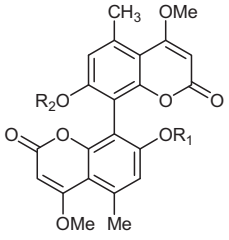


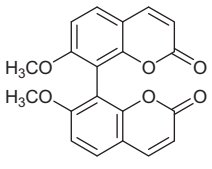
67

Chemical studies of the sclerotia of *Aspergillus alliaceus* Thom and Church (Aspergillaceae) have yielded two bicoumarin metabolites, named kotanin (**68**), desmethyl kotanin (**69**).[66] Orlandin (**70**) is a new bicoumarin that has been isolated from *A. niger* found growing on orange leaves. It was nontoxic to day-old cockerels, but significantly inhibited wheat coleoptile growth at and 10^{-3}, 10^{-4}, 10^{-5} M.[67]



68 $R_1 = R_2 = Me$
69 $R_1 = H$, $R_2 = Me$
70 $R_1 = R_2 = H$

The plant *Boennrnghausenra albijiora* Relchb and Melssner is a slender, erect, perennial herb found mostly at a temperate climate in the Himalayan ranges between 1000 and 2800 m above sea level.[68] A phytochemical investigation of *B. albijiora* yielded one dimeric coumarin designated as jayantmin (**71**). Another C8–C8′ linked bicoumrain, edgeworoside (**72**), was isolated from *Edgeworthia chrysantha* and the axial 8,8′-chirality of **72** was concluded to be *R* from the CD spectrum of the aglycone.[69]



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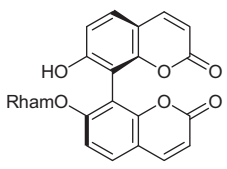
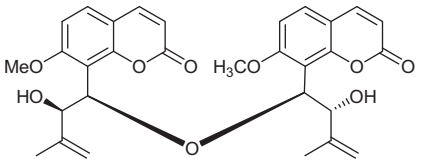


72

2.6.2. Miscellaneous C8–C8′-linked bicoumarins. Negi et al., isolated bismurrangatin (**73**), a new bicoumarin, from the vegetative branches of *M. exotica*.[2] Dondon et al., isolated a new bicoumarin (**74**) from the leaves and stems of *Triphasia trifolia*. Its structure was determined by spectral data and it appeared that the two coumarinic moieties of **74** are derivatives of mexoticin and meranzin hydrate, which are known constituents of the plant.[70]



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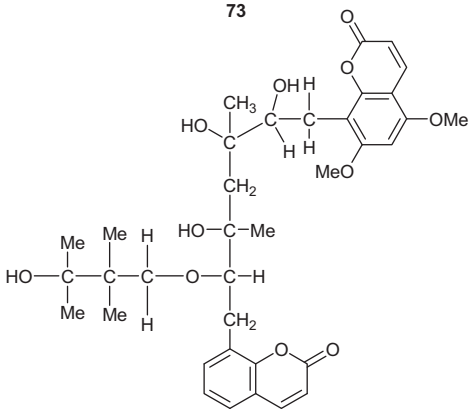
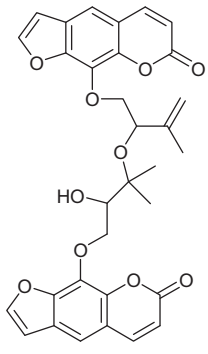


74

P. rivulorum 'Yunnan Qiang Huo' is a Chinese folk medicine used as an antipyretic, analgesic and diaphoretic agent in local areas of Yunnan province, China. It has been proved by pharmacological experiments that the water extract possesses an antiarrhythmic effect.[71] A new bicoumarin, rivulobirin A (**75**), was isolated from the underground parts of *P. rivulorum*. Teshima et al. isolated a bicoumarin named murradimerin A (**76**), from the leaves of *M. exotica* (Rutaceae) collected in Ishigaki island, Japan.[3] Taniguchi reported one bicoumarin named rivulobirin E (**77**), from *Pleurospermum rivulorum*.[9]



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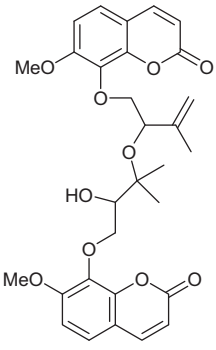

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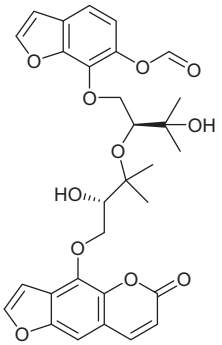
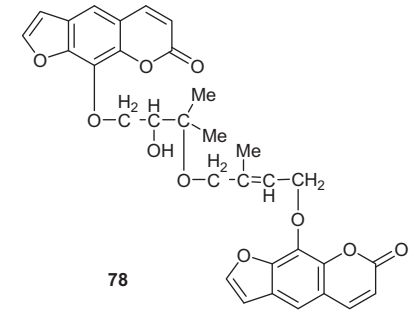


77

A phytochemical investigation of *Heracleum candicans* produced a dimeric coumarin, named candicanin (**78**).[72] *Clausena excavata* Burm., a wild shrub of the Rutaceae family, is extensively distributed in Southeast Asia. It has been used as a folk medicine in Eastern Thailand for the treatment of cancer and several other disorders. In some countries, its leaves and stem have been used for colic, cough, headache, rhinitis, sores, wounds, yaws, and detoxification.[73] Two new bicoumarins, cladimarins A (**79**) and B (**80**), were isolated from the branches of *C. excavata* (Rutaceae) collected in Indonesia.[74]

Angelica gigas grows in Korea on moist soils in altitudes 200 m above sea level. The plant, especially the roots, is used under the Korean names, 'Zam Dang Gui' and 'Sung Gem Zo', in folk medicines for headaches, wounds, and arrhythmia. Gigasol (**81**), the first dimethyleneoxybiscoumarin, has been isolated from *A. gigas*.[75]



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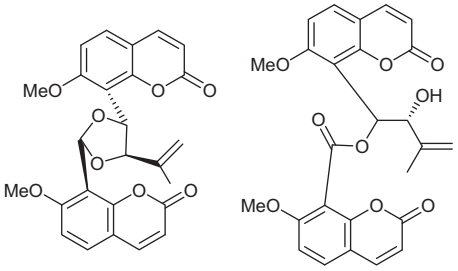
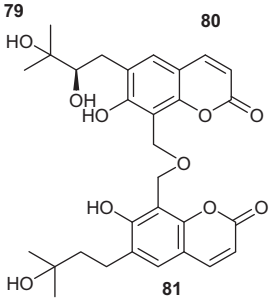


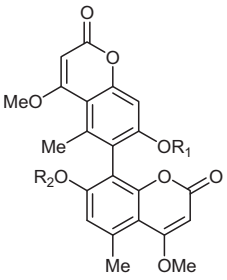
79 **80**



81

2.7. C6–C8′-linked bicoumarins

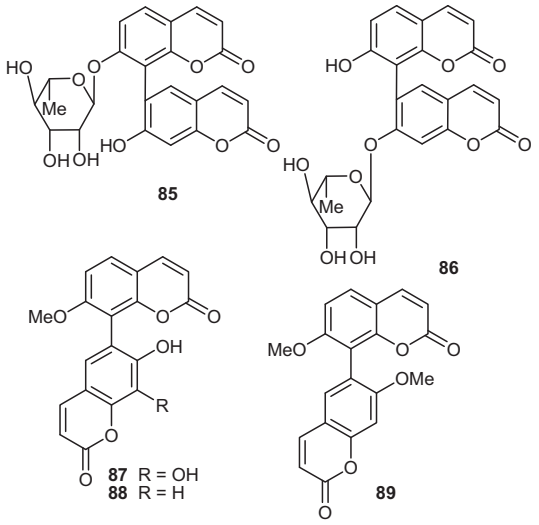
2.7.1. Coumarins linked through carbon–carbon bonds. In the course of searching for emestrin and related compounds in members of the *Aspergillus nidufans* group (teleomorph genus: *Emericefla*), three new bicoumarins, desertorins A (**82**), B (**83**), and C (**84**), were isolated from the mycelium of *E. desertorurn* Samson and Mouchacca, strain CBS 653.73, which originates from desert soils in Egypt.[76] Desertorin C (**84**) was optically active because of restricted rotation about the carbon–carbon single bond joining the two monomer units.[76]



82 $R_1 = R_2 = H$
83 $R_1 = H, R_2 = Me$
84 $R_1 = R_2 = Me$

Riaz et al. reported daphjamilin (**85**), a dimeric coumarin glycoside with a rare C–C linkage between C6′–C8 of two coumarin units, isolated from *D. oleoide*.[77] Daphjamilin (**85**) was evaluated for urease enzyme inhibitory activity. Interestingly compound **85** showed significant activity with IC_{50} values of 22.05 and 26.30 μM, respectively, against *Bacillus pasteurii* and jack bean urease enzymes in a concentration-dependent fashion.[49] Daphwazirin (**86**), a new biscoumarin glycoside with a rare C–C linkage between C6′–C8 of two coumarin units, has been isolated from the roots of *D. oleoides*.[78]

Trifolium repens L. (Leguminosae), which is distributed worldwide, is used as an important health food for humans. It has estrogenic, antispasmodic, and expectorant properties.[79] A bicoumarin, named repensin A (**87**), was isolated from *T. repens* L. The two coumarin units in repensin A (**87**) are linked by C8 with C6′. Interestingly, bicoumarin **87** was optically active.[80] Similarly, Xu et al., isolated a bicoumarin, named bicoumastechamin (**88**) from *S. chamaejasme* L. (Thymelaeaceae).[81] A bicoumarin named matsukaze-lactone (**89**), was isolated previously from *Ipomopsis aggregate*,[82] and from *B. albijiora*.[83,84]

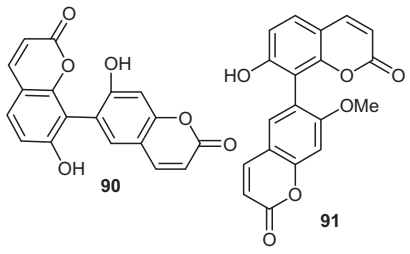


85 **86**

87 R = OH
88 R = H

89

A bicoumarin named bicoumol (**90**), have been isolated from *Euphorbia quinquecostata*[85] as well as from ladino clover.[86] *Boenninghausenia albiflora*, belonging to the family Rutaceae and is well known for its medicinal properties in traditional systems of medicine. In the ethnobotanical literature, the aerial as well as the root parts have been described as antiseptics. The leaf parts have been applied to cuts and wounds, whereas the root powder has been used as an antiseptic. Its juice has also been given as a therapy for vomiting and dysentery. *B. albiflora* has flea-repellent and calcium-blocking activity.[87] Bhubaneswin (**91**), a new bicoumarin, was isolated from *B. albiflora*.[88]



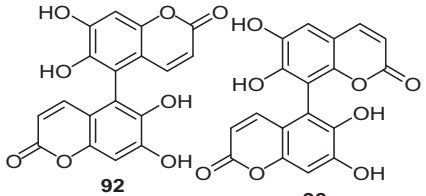
90 **91**

2.8. C5–C8′-linked bicoumarins

2.8.1. Coumarins linked through carbon–carbon bonds. The dried whole plant (including the roots) of *Viola yedoensis* Makino (Violaceae) is an important Chinese traditional medicine named 'Zi Hua Di Ding' (Herba Violae), which is used as an antifebrile and

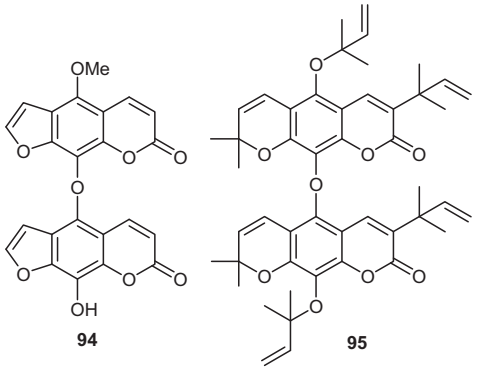
detoxicant agent for the treatment of acute pyogenic infections, such as boils, furuncles, and carbuncles.[89] A bioguided phytochemical investigation of *V. yedoensis* resulted in the isolation of one dicoumarin, named euphorbetin (**92**).[90] Previously, euphorbetin (**92**) was isolated from *Euphorbia lathyris* L.[91]

The anticoagulant activities of compound **92** were measured by activated partial thrombosis time (APTT), thrombin time (TT), and prothrombin time (PT), and then compared with those of heparin. Euphorbetin (**92**) could increase anticoagulant activity, which was characterized by APTT, PT, and TI. However, the enhancement by euphorbetin (**92**) in anticoagulant activity might be due to its stabilization of and structural similarity to, vitamin K. As found for the mechanism of other dicoumarins, euphorbetin (**92**) may competitively inhibit epoxide reductase of vitamin K, preventing the reduction of vitamin K into a hydroquinone, and then produce the anticoagulant activity by blocking the recycling of vitamin K.[90] Another C5–C8-coupled bicoumarin isoeuphorbetin (**93**), has been obtained from *V. yedoensis*[92] and *E. lathyris*.[93]



92 **93**

2.8.2. Coumarins linked by ether bridge. Among bicoumarins linked that are C5–C8′ ether linked, rivulobirin B (**94**), a new bicoumarins, was isolated from the underground parts of *P. rivulorum*.[10,71] *Citrus paradisi* Macfad (Rutaceae) is popularly called grapefruit. In humans, grapefruit seed extract has been documented to have broad-spectrum antibacterial (through its bactericidal mechanism), antifungal, wound-healing, and antioxidant properties. In addition, there is clinical evidence of its effectiveness in the treatment of urinary tract infections caused by *Pseudomonas aeruginosa*, *Klebsiella* sp., *Staphylococcus aureus*, and *Escherichia coli*.[9] It has also been widely used in veterinary medicine as a panacea.[94] One dimeric coumarin, named marshdimerin (**95**), has been isolated from the roots of Marsh grapefruit (*C. paradisi* Macf.).[95] Daphgilin (**96**), a bicoumarin, has been isolated from the stem bark of *Daphne giraldii*.[96]



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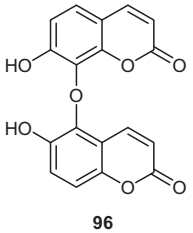
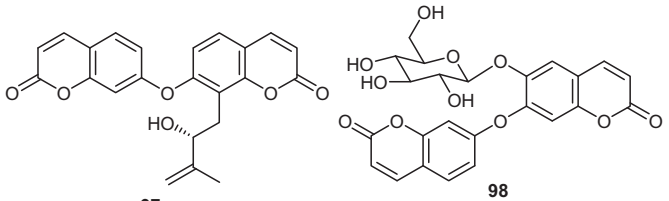


96

2.9. C7–C7′-linked bicoumarins

2.9.1. Coumarins linked by ether bridge. Limonia acidissima (Rutaceae) is distributed throughout India. In Sanskrit, it is known as 'Kapitthah' and, in Hindi, it is well known as 'Katbel'. A decoction of the leaves is used in the treatment of constipation and vomiting and as a cardiotonic, and diuretic agent. The leaves contain coumarins, triterpinoids, and steroids.[97] The steam distillates were found to act as antimicrobial, insecticidal, and antifungal agents. As the plant is reported to have a number of medicinal uses, various authors have attempted a pharmacognostic study of the leaves. A dimeric coumarin, limodissimin A (**97**), was isolated from *L. acidissima*. Limodissimin A (**97**) was isolated as a yellow gum and showed a positive optical rotation value of **97** ($[\alpha]_D^{25}$ +3.8) that was in good agreement with that of the related (2′*R*)-hydroxy-coumarin derivatives. Limodissimin A (**97**) was evaluated for inhibitory effects on NO production in LPS-activated BV-2 cells, a microglial cell line, but it did not show a significant inhibitory effect on NO production in the range from 5 to 20 μM.[97]

Liang et al.[65] investigated *D. feddei* and isolated another new C7–C7′ dicoumarin glucoside, 6-hydroxy-7-[(2-oxo-2*H*-1-benzopyran-7-yl)oxy]-2*H*-1-benzopyran-2-one (**98**). A bicoumarin, arteminorin B (**99**), was isolated from the aerial parts of *A. minor*. The in vitro cytotoxicity of compound **99** was examined with HepG2 cells, a cancer cell line. The inhibitory effects on the activity of XOD and PTP1B enzymes of these compounds were also tested. Compound **99** did not show significant activity in the above tests.[41]



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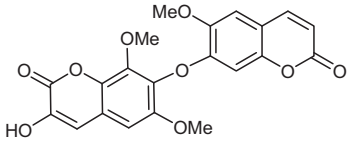

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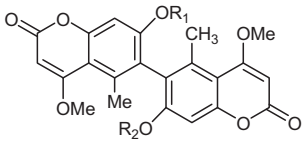
2.10. C6–C6′-linked bicoumarins

2.10.1. Coumarins linked through carbon–carbon bonds. Chemical studies of the sclerotia of *A. alliaceus* Thom and Church (Aspergillaceae) have yielded three new bicoumarin metabolites, named isokotanins A (**100**), B (**101**), and C (**102**).[66] Isokotanins A–C (**100**–**102**) are chiral, due to restricted rotation about the single bond connecting the two coumaryl units. Each compound showed optical activity, but no stereochemical assignments were made.[66] Interestingly, isokotanins A (**100**) and B (**101**) are dextrorotatory, whereas isokotanin C (**102**) is levorotatory, and this had led to the suggestion that racemic isokotanin C is the biogenetic precursor of isokotanin A and B. Significantly, chemical conversion of isokotanin C (**102**) into isokotanin A (**100**) yielded a product with an optical rotation opposite in sign to that of naturally occurring isokotanin A, whereas the analogous reaction of isokotanin B (**101**) gave isokotanin A (**100**) identical in all respects, including optical rotation, with naturally occurring isokotanin A (**100**). Isokotanins B (**101**) and C (**102**) show activity against the corn earworm *Helicoverpa zea* and the dried fruit beetle *Carpophilus hemipterus*.[66]

Petromyces alliaceus Malloch and Cain, the teleomorph of *A. alliaceus* Thorn and Church in the *Aspergillus ochraceus* group

(section Circumdati, subgenus Circumdati), is characterized by producing gray-black sclerotial bodies, which may mature slowly into ascostroma.[98] This species is widely distributed in the world, including U.S.A., Mexico, South Africa, Egypt, U.K., India, and Australia. A bicoumarin, 7-*O*-demethyl-6,6′-bisiderin (**103**), was isolated from the ascostromata of *Petromyces alliaceus*.[99]



100 $R_1 = R_2 = Me$
101 $R_1 = H$, $R_2 = Me$
102 $R_1 = R_2 = H$
103 $R_1 = Me$, $R_2 = H$

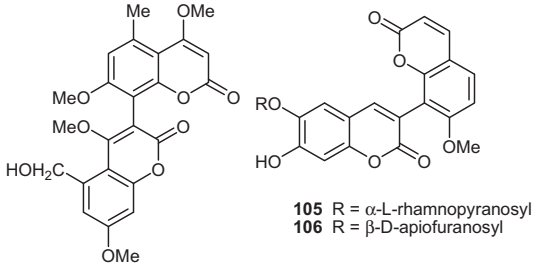
2.11. C3–C8′-linked bicoumarins

2.11.1. Coumarins linked through carbon–carbon bonds. Extracts of the sclerotia of *Aspergillus fivus* (Eurotiaceae) exhibit anti-insect activity against the fungivorous beetle *C. hemipterus* (Nitidulidae). A phytochemical investigation of *A. fivus* produced a new bicoumarin, aflavarin (**104**), along with two known bicoumarins, kotanin (**68**), and desmethyl kotanin (**69**).[100]

It was originally postulated that desertorins and kotanins might be shikimate derived. However, it has been shown in isotope labeling studies with *A. niger* and *Aspergillus variecolor* that both kotanin (**68**) and the presumed siderin precursor[101] are formed via the polyketide pathway.[102,103] Thus, it seems likely that aflavarin (**104**) is also a polyketide metabolite of analogous origin, arising via dimerization of a siderin precursor with connectivity at a different site. Unlike the desertorins and kotanins, the linkage between the two coumaryl subunits of aflavarin (**104**) involves one of the pyrone rings, and one of the sideryl subunit Me groups (C-12′) is oxygenated.

Aflavarin exhibits anti-insect activity against both adults and larvae of *C. hemipterus*, causing respective feeding reductions of 66 and 53% relative to the controls when incorporated into a standard test diet at a concentration of 100 ppm. Interestingly, desmethyl kotanin (**69**) exhibits no activity against *C. hemipterus* at the same concentration in this assay. Although studies of *Aspergillus sclerotia* have afforded a variety of compounds with activity against the corn earworm, *H. zea*, aflavarin is the first non-aflavinine derivative that showed significant activity against the more ecologically relevant fungivorous insect *C. hemipterus*. However, it does not exert significant effects on *H. zea*. In other tests, aflavarin exhibits no antimicrobial activity against *Bacillus subtilis*, *S. aureus*, or *C. albicans* in standard disc assays at 100 μg/disc. Aflavarin (**104**) showed some cytotoxicity toward human solid tumor cell lines, affording ED_{50} values of 7.5, 55.0, and 5.8 μg/ml against non-small-cell lung carcinoma A-549, breast adenocarcinoma MCF-7, and colon adenocarcinoma HT-29 cells, respectively [100].Aflavarin (**104**) is a major metabolite of the sclerotia of this strain of *A. flavus*. Considering its sclerotial concentration and the potency of its effects on *C. hemipterus*, aflavarin (**104**) could be significant in helping to defend sclerotia from feeding by fungivorous insects. Analytical HPLC analysis of sclerotial extracts from eleven other strains of *A. flavus* and *Aspergillus parasiticus* showed that at least seven contained aflavarin (**104**).[100]

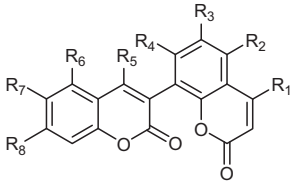
Two new biscoumarin glycosides 6-*O*-α-L-rhamnnopyranosyl daphnogirin (**105**) and 6-*O*-β-D-apiofuranosyl daphnogirin (**106**) were isolated from the stem barks of *D. giraldii* NITSCHE (Thymelaeaceae).[104] The stems and roots of *D. giraldii* have been used in Chinese folk medicine to treat rheumatism, and coumarins of this plant are commonly considered as the major bioactive constituents. Compounds **105** and **106** were tested for inhibitory effects against the production of nitric oxide (NO) in RAW264.7 cells, and cytotoxicity toward human tumor cell lines A549, LOVO, QGY-7703, 6T-CEM, but neither reduced the production of NO, nor inhibited the human tumor cell lines.[104]



104

105 R = α-L-rhamnopyranosyl
106 R = β-D-apiofuranosyl

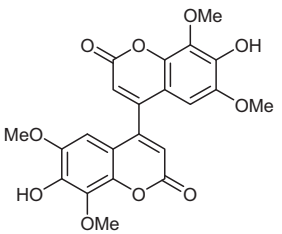
A biscoumarin, named ipomopsin (**107**), was isolated from the germinated seeds of *H. courbaril* var. *stilbocarpa*.[43] Surprisingly, **107** did not show antifungal activity against the filamentous fungi *F. solani*, *C. cladosporioides*, and *A. niger* and the yeast *C. albicans*. A bioautographic assay against *C. sphaerospermum* with **107** also showed a lack of antifungal activity. A TLC autographic test using a DPPH revealing solution was performed with compound **107**. Ipomopsin (**107**) showed powerful DPPH radical-scavenging activity. Ipomopsin (**107**) reduced 50% of DPPH free radicals at concentrations of 300 and 500 μM, respectively.[44] Ipomopsin (**107**) was isolated previously from *I. aggregate*.[82] A similar C3 and C8′-linked biscoumarin like **107**, named 7,7′-dihydroxy-3,8′-biscoumarin (**108**), was isolated from the leaves and twigs of *Gnidia socotrana* (Balf. f.) Gilg (Thymelaeaceae), a plant occurring endemically on Socotra island (Yemen).[105] A bicoumarin, named 7-*O*-demethyl-3,8′-bisiderin (**109**), was isolated from the ascostromata of *Petromyces alliuceus*.[99]



107 $R_1 = R_2 = R_5 = R_6 = H$, $R_3 = R_7 = Me$, $R_4 = R_8 = OH$
108 $R_1 = R_2 = R_3 = R_5 = R_6 = R_7 = H$, $R_4 = R_8 = OH$
109 $R_3 = R_7 = H$, $R_1 = R_4 = R_5 =$ OMe, $R_2 = R_6 = Me$, $R_8 = OH$

2.12. C4–C4′-linked bicoumarins

2.12.1. Coumarins linked through carbon–carbon bonds. I. balsamina L. (Balsaminaceae) has long been used in Thailand as a traditional folk medicine. Its leaves are usually used for the treatment of abscesses, nail ingrowth, and dermatophytosis. A new biscoumarin, 4,4′-biisofraxidin (**110**), has been isolated from the root cultures of *I. balsamina*.[55]



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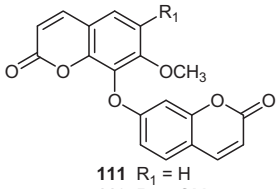
2.13. C7–C8′-linked bicoumarins

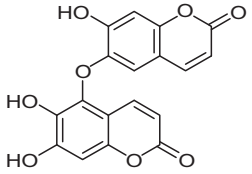
2.13.1. Coumarins linked by ether bridge. Some plants from the genus *Ruta* are used as folk medicines. The plants showed anti-inflammatory activity. They are used as contraceptives, to relieve the symptoms of hangovers, and are applied externally as a poultice against rheumatic pain. *Ruta* plant constituents showed antifungal properties, which could be beneficial for agriculture and medicine.[106] A phytochemical investigation of *Ruta oreojasma* produced two C7–C8′ ether-coupled bicoumarins, named fatafarin (**111**) and oreojasmin (**112**).[107]



111 R_1 = H
112 R_1 = OMe

2.14. C5–C6′-linked bicoumarins

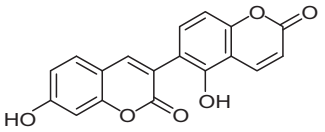
2.14.1. Coumarins linked by ether bridge. A bioguided phytochemical investigation of *V. yedoensis* resulted in the isolation of a bicoumarin, named as dimeresculetin (**113**)[90] The anticoagulant activities of compound **113** where measured by APTT, TT, and PT, and then compared with those of heparin. On PT and TT, dimeresculetin (**113**) increased the anticoagulant activity with increasing concentration and prolonged the TT to 74.97 s, a little more significant than heparin at the test concentration. However, APTT of dimeresculetin (**113**) was seldom prolonged, even at 100 μg/ml, suggesting that dimeresculetin (**113**) may usually express anticoagulant activity correlating with the extrinsic coagulation process. Meanwhile, euphorbetin (**92**) could also increase anticoagulant activity, which was characterized by APTT, PT, and TT. Although dimeresculetin (**113**) showed a structure similar to that of euphorbetin (**92**), there were still some different effects in their anticoagulant activities, which might be attributed to the linkage difference between the two coumarin mother nuclei,[90] i.e., an ether linkage in dimeresculetin (**113**) and a linkage attaching two phenyl rings directly in euphorbetin (**92**). It is presumed that the ether linkage may be easy to cleave into esculetin (monomer nuclei of dimeresculetin (**113**)) and euphorbetin (**92**) to produce anticoagulant activity, which can explain the similar APTT, PT, and TT between dimeresculetin (**113**) and esculetin. However, the enhancement (**92**) in anticoagulant activity of euphorbetin might be due to its stabilization and the structural similarity to that of vitamin K. As in the mechanism of other dicoumarins, euphorbetin (**92**) may competitively inhibit epoxide reductase of vitamin K, preventing the reduction of vitamin K into hydroquinone, and then produce the anticoagulant activity by blocking the recycling of vitamin K.[90]



113

2.15. C3–C6′-linked bicoumarins

2.15.1. Coumarins linked through carbon–carbon bonds. There is only one example of C-3 with C-6′-coupled bicoumarins named repensin B (**114**), isolated from *T. repens* L. Interestingly, repensin B (**114**) was optically active.[80]



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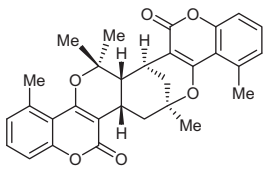
2.16. Miscellaneous bicoumarins

A phytochemical investigation of the roots and rhizomes of *Gerbera piloselloides* yielded two new types of dicoumarins (dicoumaro-*p*-menthanes), named dibothrioclinins I (**115**) and II (**116**).[108] Comparison of the NMR data of **115** with those of bothrioclinin, a known coumarin, showed that these were identical for rings A, A′, B, B′, C, C′. Therefore, **115** was considered a dimer of bothrioclinin. Obviously, the D ring of **115** was in fact a *p*-menthane, i.e., **115** was dicoumaro-*p*-menthane. So far, many dicoumarins have been isolated from plants, but the structure of **115** is different from other known dicoumarins in its dimerization manner. However, dibothrioclinin II (**116**) did not have optical activity, unlike dibothrioclinin I (**115**). The single-crystal X-ray diffraction data indicated that it was a racemic mixture (space group *P*2/*a*), including a pair of enantiomers.[108]

D. feddei Levl is a common evergreen shrub cultivated in Yunnan, Sichuan, and Guizhou provinces in China. Its stem barks are used as a folk medicine for the treatment of injuries from falls and bruises.[109] Liang et al.[65] investigated *D. feddei* and isolated a novel dicoumarinolignoid, feddeiticin (**117**), the first example with a dicoumarinolignoid skeleton. Furthermore, compound **117** was optically inactive and showed no ellipticity in the CD spectrum, which suggested that it occurs as a racemate.



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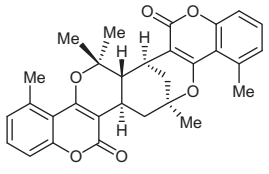

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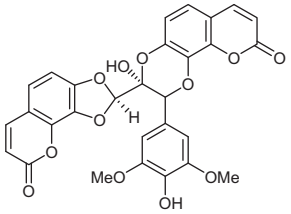
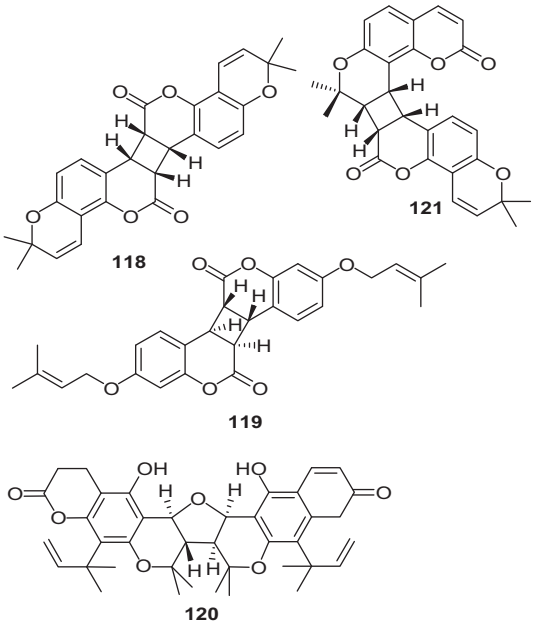
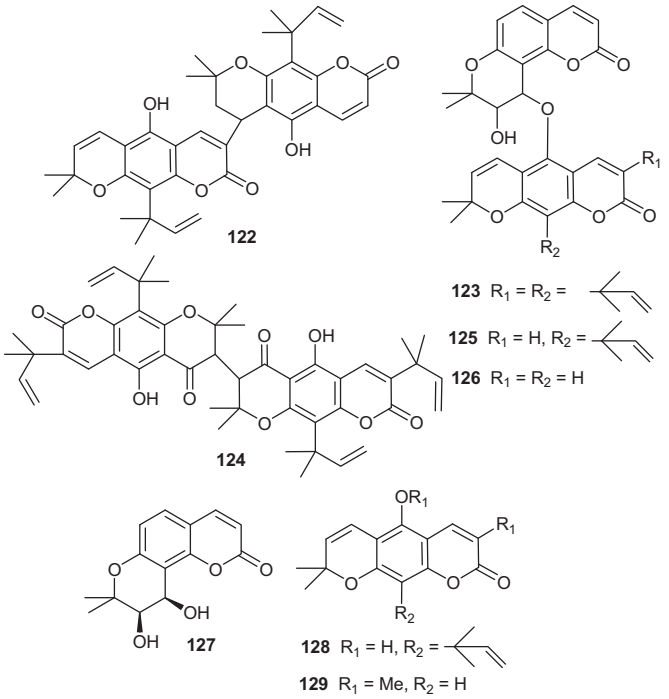


117

Leaves and bark of *Clausena lenis* have been used for the treatment of dysentery and arthritis and a new dimeric coumarin, diseselin B (**118**), was isolated from the aerial parts of this plant. In the ^{1}H and ^{13}C NMR spectra of **118**, the number of H- and C-atoms observed was only half of that corresponding to the molecular formula, suggesting that compound **118** was a completely symmetrical structure.[110] A phytochemical investigation of *Haplopappus deserticola* produced a dimeric coumarin **119**.[111]

Many new coumarins and acridone alkaloids have been isolated from *Citrus* plants.[112] In particular; novel types of acridone-coumarin dimers and bicoumarins were characteristic constituents of *Citrus* plants. A phytochemical investigation of *Citrus yuko* Hort. Ex Tanaka (Rutaceae) produced a bicoumarin, named furobinodentin (**120**).[113] The structure of **120** was confirmed by X-ray crystallographic analysis. Furobinodentin (**120**) is a novel type of bicoumarin composed of two nordentin[114] units linked with the formation of a dihydrofuran ring.

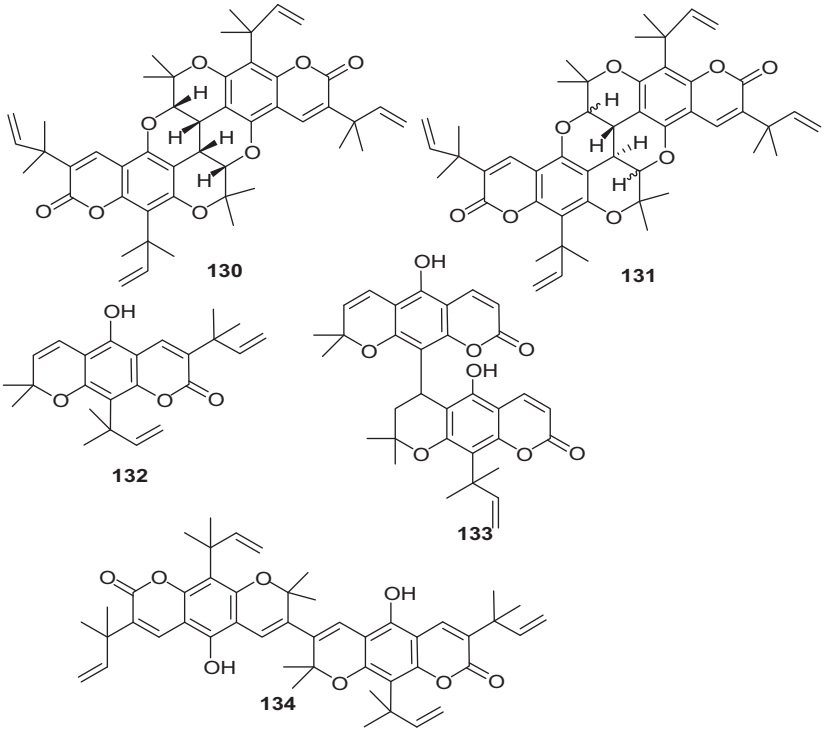




C. lenis Drake (Rutaceae) is a shrub growing in Yunnan province. Leaves and bark of this plant have been used for the treatment of dysentery, and arthritis. A phytochemical investigation of the aerial parts of *C. lenis* collected in Xishuangbanna, Yunnan province, resulted in a new dimeric coumarin, named diseselin A (**121**), a dimer of seselin.[115–117]

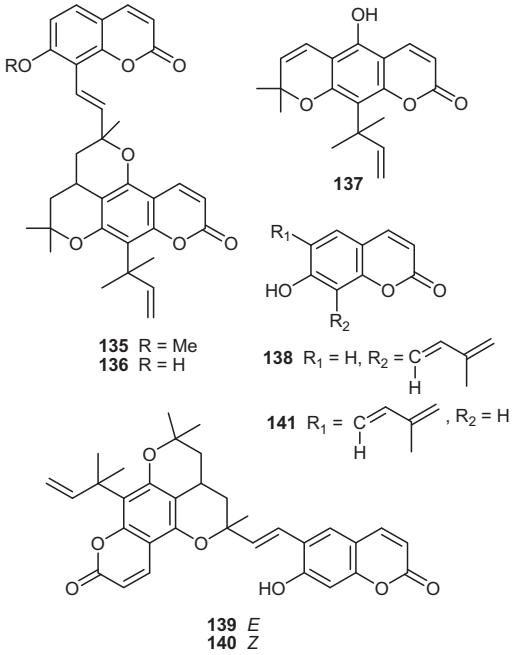
Citrus plants have been reported to contain coumarins. A phytochemical investigation of *C. hassaku* HORT. Ex Tanaka (Rutaceae) resulted in the isolation of three new bicoumarins named bisnorponcitrin (**122**), khelmarin C (**123**), and bishassanidin (**124**).[118] Two new bicoumarins named khelmarins A (**125**) and B (**126**), were isolated from the root bark of *Poncirus trifoliata*, which had been used as rootstock of *C. hassaku*. The structures of khelmarins A (**125**) and B (**126**) contain cis-khelactone (**127**) as a common structural component, which is linked with a nordentin (**128**) or xanthoxyletin (**129**) unit, respectively.[119]

A reinvestigation of *C. hassaku* HORT. Ex Tanaka (Rutaceae) resulted in the isolation of novel dimeric coumarins named claudimerins A (**130**)[112,120] and B (**131**).[2] Claudimerins A (**130**) and B (**131**) were optically inactive and the complete structure and relative stereochemistry of **130** were obtained from a single-crystal X-ray crystallographic analysis. The relative configurations of the four protons on the pyranopyran ring of **131** could not be determined. The authors point out that the lack of optical activity of **130** and **131** suggests that either they are artifacts or they are formed in the plant cells without the participation of enzymes. The structure of **130** was

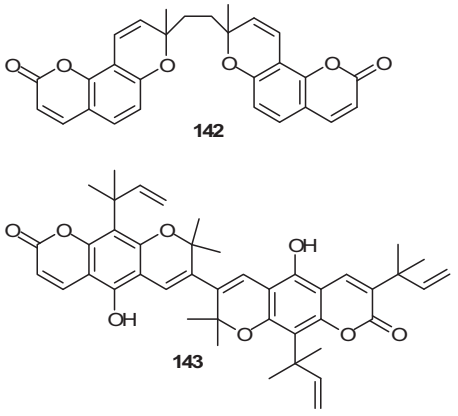


confirmed by single-crystal X-ray crystallographic analysis. The structure of claudimerin A (**130**) consisted of two clausarin (**132**)[121] units linked symmetrically with the formation of the pyranopyran ring. Nordenletin (**133**) has been extracted from *C. hassaku* roots.[122] Similarly, bisclausarin (**134**) from *C. hassaku* roots, comprising two clausarin (**132**) units, is the first example of a bicoumarin having a direct carbon–carbon linkage at the pyran ring.[123]

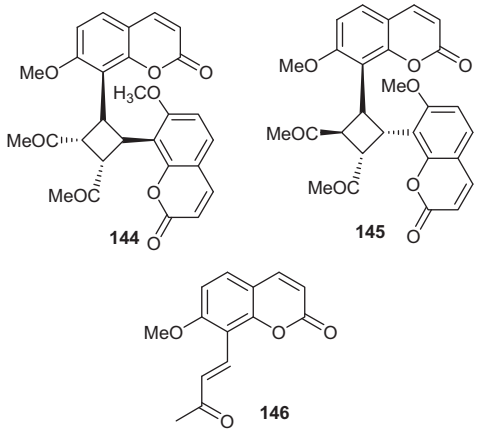
Citrumarin A (**135**) and the corresponding phenol, citrumarin B (**136**), have been isolated from the roots of the hybrid *P. trifoliata*×*C. paradise*. Citrumarin A (**135**) is considered biogenetically to be the dimer of nordentatin (**137**) and *trans* dehydroosthol (**138**). Citrumarins C (**139**) and D (**140**), both from the roots of *C. hassaku*, correspond to the dimer of nordentatin (**137**) with de-*O*-methyl citrubuntin (**141**) and its cis analogue, respectively.[124]



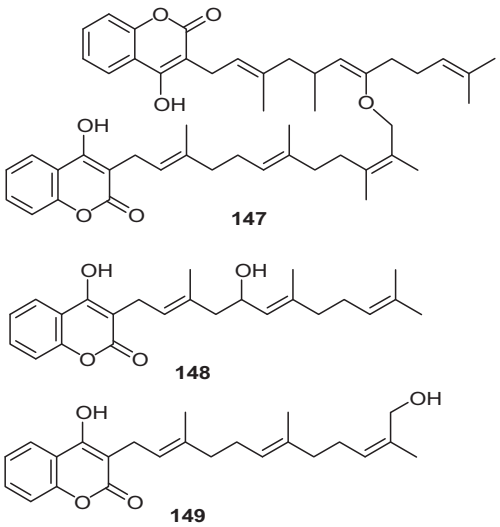
Two new bicoumarins, named biseselin (**142**) and yukomarin (**143**), were isolated from the roots of Yuko (*C. yuko* Hort. ex Tanaka) and their structures were elucidated by spectroscopic analyses.[125]



Bisosthenon (**144**),[126,127] a new dimeric coumarin, was isolated from the roots of *Citrus funadoko* HORT. Because the structure having a *cis*–*cis*, *cis*-cyclobutane ring system may be excluded due to the severe steric strain among the substituents, an alternative structure (**144**) having a *trans*–*cis trans*-cyclobutane system was proposed. The structure of bisosthenon (**144**) was confirmed by an X-ray crystallographic analysis. A stereoisomer of bisosthenon (**144**), named bisosthenon B (**145**), was isolated from the roots of Marsh grapefruit (*C. paradisi* Macf.).[95] Bisosthenon (**144**) and bisosthenon B (**145**) are the head-to-head dimer of osthenon (**146**),[128] which was previously isolated from *Citrus* plants.



Ferulenoloxyferulenol (**147**), from *Ferula communis* var. *genuina*, is a dimer arising from the etherification of a molecule of ε-hydroxyferulenol (**148**) by a molecule of its ω isomer (**149**).[129]



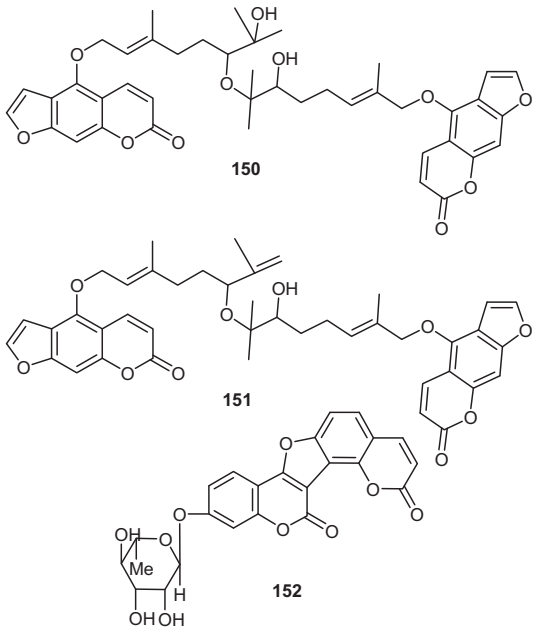
Two new bicoumarins, named GF-I-1 (**150**) and GF-I-4 (**151**), from grapefruit (*C. paradisi* Macf.) juice are tail–tail dimers.[130]

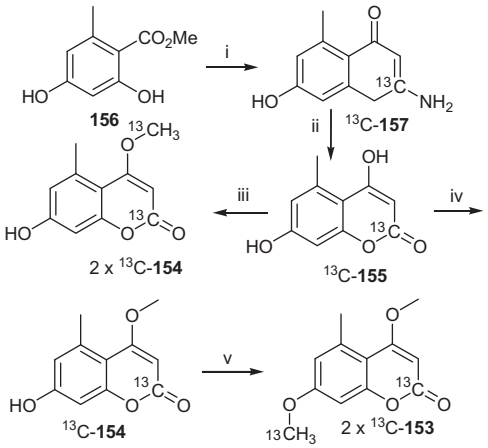
Lasiosiphon eriocephalus Decne (Syn. *Gnidia glauca*), belonging to family Thymaleaceae, is a medicinally important tropical plant. It has medicinal uses as a rubifacient, has a teeth-loosening effect and demonstrates in vitro anticoagulant activity, due to the biscoumarin found in this plant.[131] A new furanobiscoumarin rhamnoside, named eriocephaloside (**152**), has been isolated from the whole plants of *L. eriocephalus*.[132] Eriocephaloside (**152**), represents a new group of furanobiscoumarins in which the aryl ring of one unit is linked through both oxygen and carbon to the lactone ring of the second unit.

3. Biosynthetic studies

3.1. Biosynthesis of kotanin

Kotanin (**68**) belongs to a larger group of regioisomeric bisiderins synthesized by diverse *Aspergillus* sp.. It has been reported





Scheme 1. Synthesis of ^{13}C-labeled monomeric substrates: Reaction conditions: (i). 1 equiv, $H_3C^{13}CN$, 5 equiv, LDA, THF, −78 °C to rt, 2.5 h, 80%; (ii). 12% aq HCl, 3 h, 90%; (iii). $^{13}CH_3OH$, BCl_3, rt, 3 days, 81%; (iv). HCl (1.25 M)/MeOH, rt, 3 days, 71%; (v). 1 equiv, $^{13}CH_3I$, K_2CO_3, HMPT, rt, 20 h, 76%.

that kotanin (**68**), as well as its regioisomers, isokotanin A (**100**) and desertorin C (**84**), are readily obtained by chemical synthesis;[133] this encourages incorporation experiments and the validation of analytical assays. Stothers and Stoessl postulated a polyketide origin for the biosynthesis of kotanin (**68**) following incorporation experiments with [1,2-^{13}C]-acetate in *A. niger* strain ATCC 36626, although the incorporation rates were low (0.18%).[102]

Huettel et al.[134] reported the regio- and stereoselective intermolecular oxidative phenol coupling in the *A. niger* biosynthesis of kotanin (**68**), by using the incorporation of ^{13}C-labeled monomeric precursors. Singly and doubly labeled substrates were used to identify the monomeric precursor for the phenol coupling. The stereoselectivity of this reaction was confirmed by CD spectroscopy and chiral HPLC. Additionally, the regio- and chemoselectivity of the biosynthetic step was confirmed by HPLC assays optimized for the detection of siderin-type coumarins in crude fungal extracts.

3.1.1. Chemical synthesis of ^{13}C-labeled monomeric substrates. Since O-methylation steps might occur before, as well as after, phenol coupling in kotanin biosynthesis, it was important that ^{13}C-labeled siderin (**153**) as well as its hydroxyl derivatives, demethylsiderin (**154**), and 3,7-dihydroxy-4-methylcoumarin (**155**), putative substrates for the oxidative phenol-coupling step were available. For the same reason, ^{13}C-labeling was introduced into the carbon skeleton and not at the methoxy groups. In the course of the feeding experiments, however, it was necessary to introduce additional labeling at the C-4 and C-7 methoxy groups.[134]

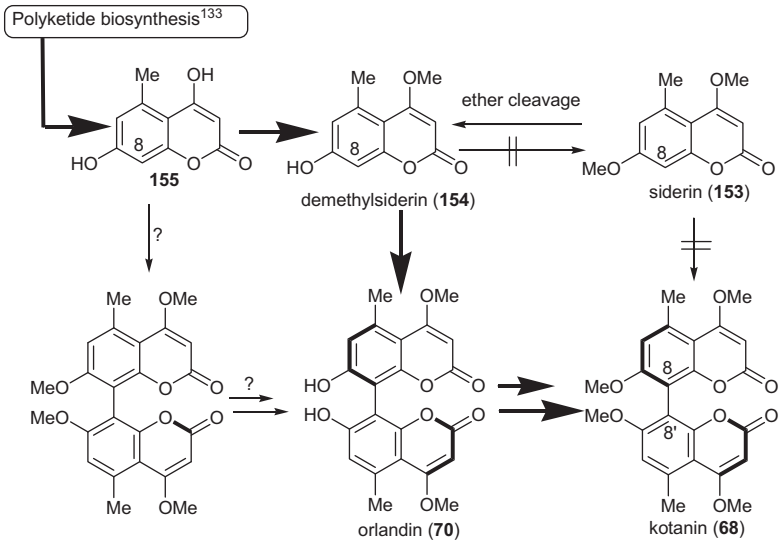
The lithium salt of [1-^{13}C]-acetonitrile was added (Scheme 1) to methyl orsellinate (**156**) to obtain aminochromenone (^{13}C-**157**). This was hydrolyzed in hydrochloric acid to the dihydroxycumarin (^{13}C-**155**). Acid-catalyzed etherification of ^{13}C-**155** in methanol–HCl (1.25 M) yielded regioselectively the 7-hydroxy-4-methoxycumarin, [2-^{13}C]-demethylsiderin (^{13}C-**155**). The same type of reaction with ^{13}C-methanol delivered a ^{13}C-labeled methoxy group at C-4 so that doubly labeled demethylsiderin (2×^{13}C-**154**) was obtained in 80% yield. Siderin (**153**), ^{13}C-labeled at C-2 and at the methoxy group at C-7 (2×^{13}C-**153**), was generated by O-methylation of ^{13}C-**154** with [^{13}C]-methyl iodide under standard conditions.[134]

*3.1.2. First feeding experiment: [2-^{13}C]-dihydroxycoumarin (^{13}C-**155**).* If a polyketide origin is assumed for the biosynthesis of **68**, **69**, and **70**, then **155** is expected to be a precursor of all three metabolites, since it is the direct product of pentaketide condensation (Scheme 2). Additionally, it can be assumed that it has the best solubility in water of the monomeric substrates (**153**–**155**), so ^{13}C-**155** was selected for the first incorporation experiment.

Huettel et al.[134] reported that a $CHCl_3$ extract from an *A. niger* culture was treated with dimethyl sulfate to increase the yield of **68** by methylation of the hydroxy derivatives, **70** and **69**, and 1.8 mg of **68** was isolated from the crude extract and analyzed by NMR spectroscopy. In the ^{13}C NMR spectrum, the signal of the carboxylic C atoms (C-2 and C-2′) was ten-fold more intense than that of **68** from the reference experiment. This corresponds to an incorporation rate of ∼9% for monomer **155**.

*3.1.3. Second feeding experiment: [2-^{13}C]-demethylsiderin (^{13}C-**154**).* In order to ascertain whether *A. niger* is able to incorporate other monomeric coumarins, ^{13}C-**154** was fed in a second experiment (no methylation of the crude extract was carried out). Only **68** was isolated (4.5 mg) and analyzed by NMR spectroscopy. Similarly to the previous experiment, a considerable increase in the intensity of the corresponding ^{13}C NMR signal was observed eightfold, ∼7% incorporation rate.[134]

*3.1.4. Third feeding experiment: [2-^{13}C,7-O$^{13}CH_3$]-siderin (2×^{13}C-**153**).* Feeding experiments 1 and 2 showed that *A. niger* is able to incorporate at least two of the putative monomeric precursors into **68**, but it remained unclear, which of these substrates is the direct precursor for the phenol-coupling step. To investigate this, the next incorporation experiment was performed with a substrate that was labeled both at C-2 and at the C-7 methoxy group (2×^{13}C-**153**). Coumarins were isolated from the crude extract as follows: 2×^{13}C-**154** (38 mg, 63% recovered), ^{13}C-**154** (17 mg, 30%), and the ^{13}C-enriched bicoumarins, **70** (0.3 mg) and **68** (0.5 mg). No demethylkotanin (**69**) was detected. The extraordinarily low yields of the bicoumarins might be due to purification difficulties, although an inhibitory effect of **153** on the production of the dimers cannot be excluded. The ^{13}C enrichment at the carboxylic C atom was clearly resolved (10-fold increase in intensity; ∼9% incorporation rate). Surprisingly, no intensity increase was observed for the C-7 methoxy group. This proves that the substrate for the phenol-coupling step is not **153**, but a hydroxyl derivative, probably **154**, generated by cleavage of the aromatic methoxy group.[134]



Scheme 2. Biosynthetic pathways of kotanin (**68**) according to ^{13}C-labeling experiments.

*3.1.5. Fourth feeding experiment: [2-^{13}C,4-O^{13}CH$_3$]-demethylsiderin (2×^{13}C-**154**).* To determine whether **154** is incorporated into the dimeric coumarins without prior cleavage of the C-4 methoxy group, [^{13}C]-demethylsiderin with an additional labeling at the corresponding C atom (2×^{13}C-**154**) was fed to *A. niger*. Bicoumarins were isolated from the crude extract as follows: **70** (1.9 mg), **69** (0.4 mg), and **68** (1.4 mg), and ^{13}C incorporation was observed at the carboxylic C atom as well as at the methoxy group at C-4. This result proved that **154** was incorporated into the bicoumarins without cleavage of the methyl ether. Integration of the ^{13}C–H-coupled signal of the methoxy groups in the ^{1}H NMR spectra allowed precise determination of the ^{13}C incorporation rates at this position. These were significantly higher than those in the previous experiments: 40% for **70**, 30% for **69**, and 16% for **68**. This might be due to feeding the substrates at an earlier stage of growth. It is striking that the incorporation rate was significantly higher for **70** than for the *O*-methylated derivatives, **69** and **68**; this supports a biosynthetic pathway from **70** via **69** to **68**. A fast dynamic equilibrium between these compounds during biosynthesis can be excluded. The results give no hint of cleavage of the 2×^{13}C-**154** methoxy group. No ^{13}C-**155** was detected in the HPLC crude extract. A partial incorporation of this compound into the bicoumarins should result in a reduced intensity of the methoxy-^{13}C NMR signal relative to that of the carboxylic C atom. However, the relative intensities of these signals remained constant. Nevertheless, this pathway cannot be entirely excluded.[134]

In conclusion, all three monomeric substrates **153**–**155** were incorporated into **68** by *A. niger*. An oxidative dimerization of **153** can be excluded, while **154** was proved to be dimerized to **69**. There is no evidence that **155** is a substrate for the oxidative phenol coupling. Besides the phenol coupling, no other conversion of the monomeric substrates was observed, except a demethylation of **153** to **154**. However, the cleavage of aromatic ethers by *A. niger* is well known and not substrate specific.[134]

From this study and the results from Stothers and Stoessl,[102] who have postulated a polyketide origin for kotanin (**68**), the biosynthetic pathway of **68** can be derived as shown in Scheme 3. The initial product of polyketide coumarin biosynthesis is expected to be **155**; this is *O*-methylated to **154**, which undergoes a regio- and stereoselective oxidative phenol-coupling step to **70**. Finally, **70** is *O*-methylated stepwise (via **69**) to kotanin (**68**).

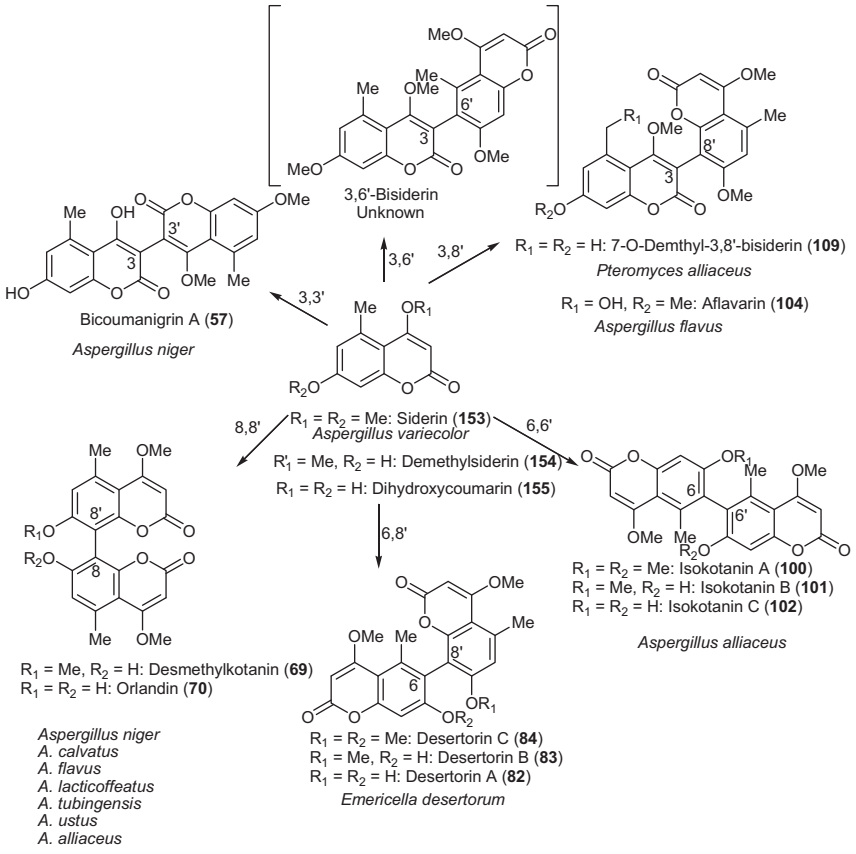
3.2. Biosynthesis of bicoumarins

The biaryl axis is a common feature of many herbal, fungal, and bacterial natural products.[135] For many of these biaryl compounds, the biosynthesis contains an oxidative phenolic-coupling step with a regio- and stereochemistry characteristic of the biological origin.[136] For this reason, families of closely related dimeric phenolic secondary metabolites are found in nature differing only in the linkage and spatial arrangement of their monomeric precursors. This kind of biodiversity is, for example, common to the dimeric coumarins kotanin (**68**), isokotanins A (**100**), B (**101**), and C (**102**), and desertorin C (**84**) etc. (Scheme 3). The different regio- and stereoselectivities at the dimerization of some sets of aromatic compounds give rise to a remarkable diversity of optically active, regioisomeric biaryls in diverse species, notably fungi (Scheme 3). The biosynthesis of these compounds is chemically very interesting, since sterically hindered biaryls are generated with high regio- and stereocontrol. Although an oxidative phenol coupling is generally assumed, this reaction has been demonstrated only twice in a fungal system.[134,137,138]

4. Chemical synthesis

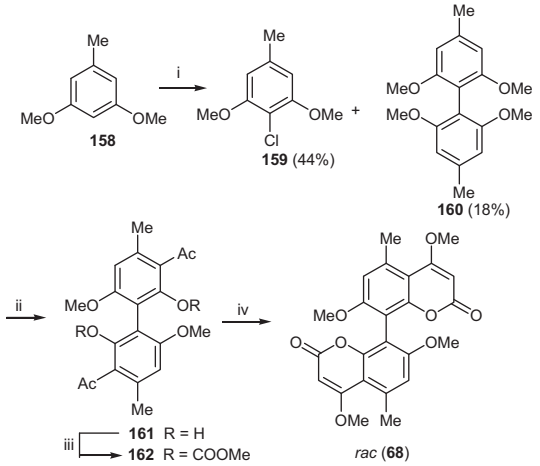
4.1. Total synthesis of racemic kotanin

Büchi et al. reported the first racemic synthesis of kotanin (**68**).[139] For the synthesis of kotanin **68**, the tetramethoxybiphenyl **160** was required. Metalation of orcinol dimethyl ether (**158**) with butyllithium followed by oxidation of the organolithium derivative with cupric chloride afforded only 18% of the desired biphenyl **160** accompanied by 44% of the chloride **159**. Acetylation of **160** with acetic anhydride catalyzed by titanium tetrachloride furnished the diketone **161**. Efforts to add the remaining two rings in one operation using malonyl chloride failed. When the carbonate **162** available from the phenol **161** and methyl chloroformate in pyridine was subjected to the



Scheme 3. Putative biosynthetic pathways for bicoumarins.

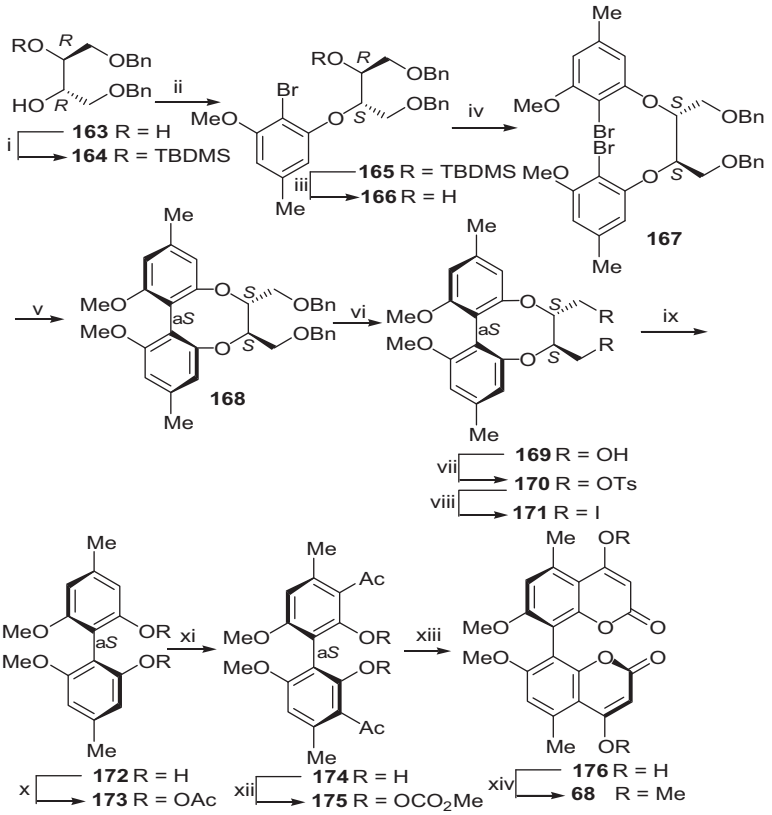
action of potassium *tert*-butoxide in *tert*-butyl alcohol, the desired cyclized product was formed (Scheme 4). Due to its highly polar nature and extreme insolubility, this intermediate could not be fully characterized and was methylated in its crude form with dimethyl sulfate in the presence of potassium carbonate. The resulting tetramethoxy compound was identical except for optical rotation with kotanin (**68**).[139]



Scheme 4. Total synthesis of racemic kotanin (**68**); Reagents and conditions: (i) *n*-BuLi, −78 °C, $CuBr_2$; (ii) $(MeCO)_2O$, $TiCl_4$, 4 h, 95%; (iii) py, Methyl chloroformate, 75 °C, 4 h, 94%; (iv) a. K, CH_2Cl_2, reflux, 2 h; b. $(Me)_2SO_4$, K_2CO_3, reflux, 4 h, 29%.

4.2. Lin asymmetric synthesis of the naturally occurring (+)-kotanin

In 1971, the fungal metabolites kotanin (**68**) was isolated as the first enantiomerically pure 8,8′-bicoumarins from *Aspergillus clavatus*.[139,140] The optical activity of kotanin (**68**) was caused by the restricted rotation around the carbon–carbon single bond connecting the two aryl monomers. Although racemic kotanin (**68**) was synthesized by Büchi et al.[139] the stereochemistry still remained unsolved for over twenty years. Lin and Zhong[140] reported the first asymmetric synthesis of the naturally occurring (+)-kotanin (**68**), in which the asymmetric intramolecular oxidative coupling of the cyanocuprate intermediate of **167** developed by Lipschutz group[141] and the Fries rearrangement of **173** were employed as the key steps. The absolute configuration of (+)-kotanin (**68**) was assigned as a*S*, based on a comparison of the CD Cotton effects of the intermediate **174** with that of the known compound. As shown in Scheme 5, 1, 4-di-*O*-benzyl-L-threitol **163** was converted into its monosilyl ether **164**. Mitsunobu reaction of **164** with 2-bromo-3-methoxy-5-methylphenol[142] gave **165** in 85% yield. Cleavage of the silyl ether of **165** with *n*-Bu_4NF in THF gave **166** in 91% yield, which was followed by treatment with 2-bromo-3-methoxy-5-methylphenol again to afford **167** in 54% yield. Treatment of **167** with *n*-BuLi followed by the addition of CuCN/TMEDA (1:3) led to the formation in situ of a higher-order cyanocuprate intermediate, which transformed in to **168** upon exposure to dry oxygen at −78 °C in 60% yield. Catalytic hydrogenation of **168** gave the threitol **169** in 90% yield. The threitol **169** was converted into the ditosylate **170** in 92% yield, which upon treatment with NaI gave the diiodide **171** in 96% yield. Reduction of **171** by activated zinc powder in ethanol



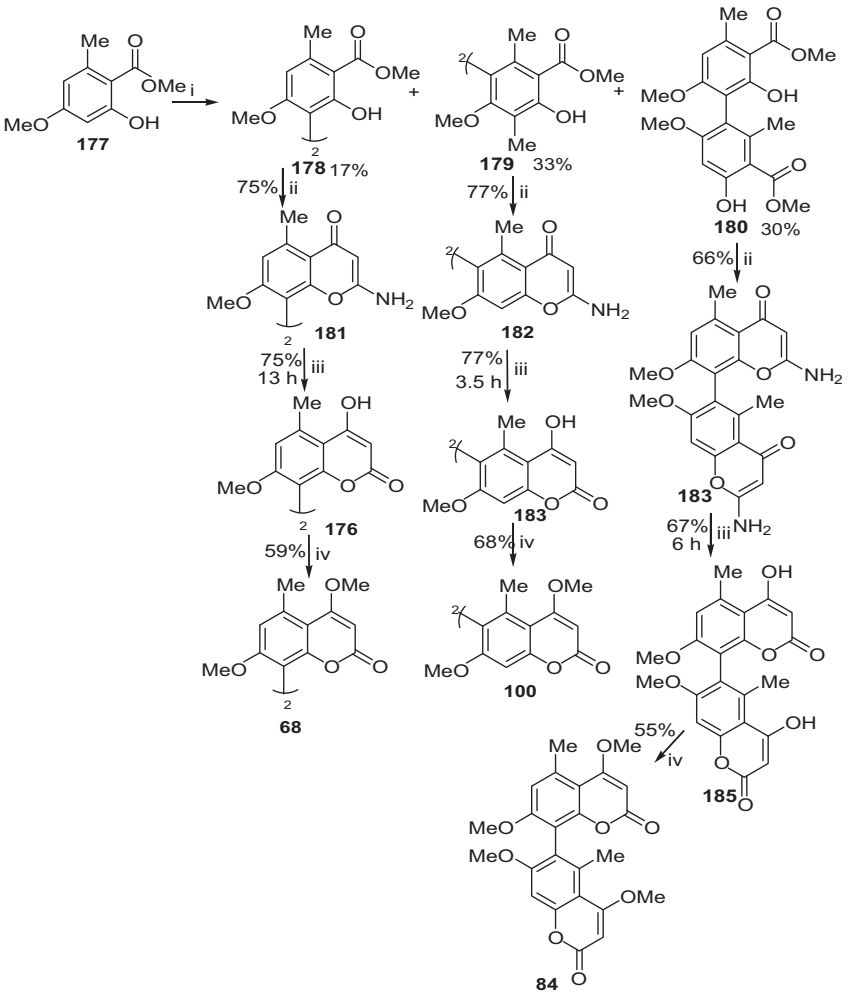
Scheme 5. Total synthesis of natural (+) kotanin (**68**); Reagents and conditions: (i) TBDMSCl, imidazole, DMF, rt, 24 h, 84%; (ii) 2-bromo-3-methoxy-5-methylphenol, DEAD, *n*-Bu_3P, THF, rt, 24 h, 85%; (iii) *n*-Bu_4NF, THF, 2 h, 91%; (iv) 2-bromo-3-methoxy-5-methylphenol, DEAD, *n*-Bu_3P, THF, rt, 42 h, 54%; (v) *n*-BuLi, THF, −78 °C, 1 h; CuCN/TMEDA (1:3), −78 °C to −40 °C, 1 h; dry O_2, −78 °C, 4 h, 60%; (vi) 10% Pd/C, H_2, EtOAc, 12 h, 90%; (vii) TsCl, py, 0 °C, 8 h, 92%; (viii) NaI, acetone, reflux, 3 h, 96%; (ix) activated Zn powder, EtOH, reflux, 1 h, 80%; (x) $(CH_3CO)_2O$, py, 2 h, 93%; (xi) $TiCl_4$, benzene, reflux, 4 h, 68%; (xii) $ClCO_2Me$, py, 4-DMAP, 50 °C, 8 h, 85%; (xiii) *t*-BuOK, *t*-BuOH, 60 °C, 2 h; (xiv) NaI, HMPA, rt; $(Me)_2SO_4$, HMPA, rt, 20 min, 42% (**175**→**68**).

provided the biphenol **172** in 80% yield. The enantiomeric excess of **172** was determined to be 82% by examination of the ^1H NMR spectra of its corresponding (*S*)-Mosher's ester. The enantiomerically pure **172** was obtained by recrystallization from ethyl acetate and hexane. The diacetate **173**, generated from **172** on treatment with acetic anhydride in pyridine, underwent a Fries rearrangement promoted by $TiCl_4$ as Lewis acid to afford **174** in 68% yield. The dicarbonate **175**, prepared from **174** and methyl chloroformate in pyridine, was subjected to treatment with *t*-BuOK in *t*-BuOH to afford the desired cyclized product **176**. Then, the crude **176** was directly methylated with NaH/HMPA/$(Me)_2SO_4$ to give the naturally occurring (+)-kotanin **68** [$[\alpha]_D^{20}$ +38.4 (*c* 0.44, $CHCl_3$); lit.[143] [$[\alpha]_D^{20}$ +40.0 (*c* 1.65, $CHCl_3$)] in 42% yield. Accordingly, the absolute configuration of the biaryls **168**–**176** and the naturally occurring (+)-kotanin **68** were all assigned as a*S*. This assignment was also in agreement with Lipschutz group's conclusion[141] that the (2*S*,3*S*)-tetraether generally induced the formation of (a*S*)-biaryl in the coupling process (**167**→**168**).

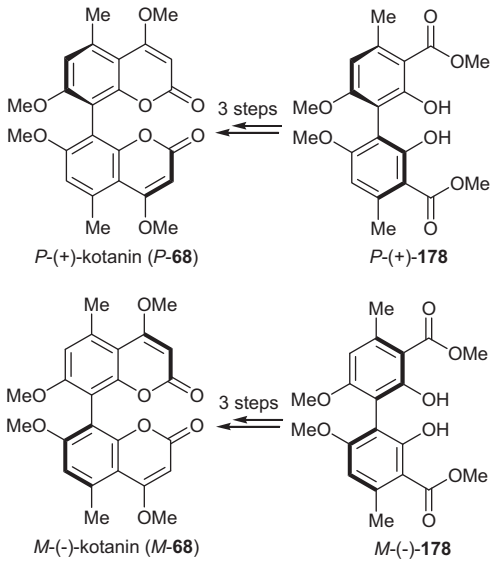
4.3. Hüttel total synthesis of coumarins kotanin, isokotanin A, and desertorin C

The biaryl unit is a widely distributed structural element in many natural product classes.[133] However, its chemical synthesis often requires multistep procedures, especially if the synthesis has to be atroposelective. Hüttel et al.[133] reported the synthesis of the dimeric coumarins, kotanin (**68**) isokotanin A (**100**), and desertorin C (**84**) (Scheme 6). Since the biaryl precursors **178**–**180** are available in a single step by an unselective oxidative phenol coupling[144] of readily available methyl 2-hydroxy-4-methoxy-6-methylbenzoate (**177**), the syntheses of the three regioisomeric bicoumarins, kotanin (**68**), isokotanin A (**100**), and desertorin C (**84**), could be realized efficiently. For the transformation of benzoate **177** into 4-hydroxycoumarin, acetic ester enolate was added to the ester moiety followed by acidic hydrolysis and cyclization. However, neither the conversion of **177** with the lithium enolate of *tert*-butyl acetate nor treatment with LDA gave the desired product. In a further attempt, acetonitrile was used as an acetic ester equivalent to obtain compounds **181**, **182**, and **184** in reasonable yield. The acidic hydrolysis of compounds **181**, **182**, and **184** was carried out by refluxing in a methanol–hydrochloric acid mixture to give the pure 4-hydroxycoumarins **176**, **183**, and **185**. For the acidic hydrolysis of the resulting aminobichromenones **181**, **182**, and **184**, the reaction time had to be adjusted, since the reactivity of these compounds decreases from the 6,6′-dimer to the 8,8′-dimer. On the other hand, decarboxylation products were observed if the reaction time was too long. Because of their low solubility even in polar solvents, the 4,4′-dihydroxybicoumarins **176**, **183**, and **185** were used for the next step without further purification. Due to the low reactivity of the hydroxy group in **176**, **183**, and **185**, an optimized method described in the literature was adopted for the final methylation step using dimethyl sulfate, sodium hydride, and HMPT.[145] Alternatively, these highly toxic reagents can be avoided by using acid-catalyzed etherification for O-methylation.

Moreover, both atropisomers of kotanin (**68**) were prepared from the pure atropisomers of the dimeric ester **178**[144,146] in 24 and 10% yield, respectively (Scheme 7). P-(+)-kotanin (*P*-**68**) was obtained from *P*-(+)-**178** and M-(−)-kotanin (*M*-6**8**) from the *M*-(−)-isomer of **178**.



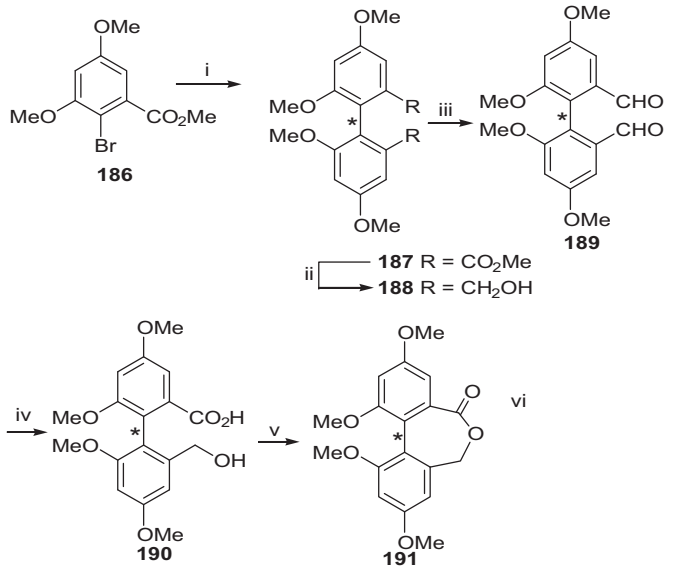
Scheme 6. Reagents and conditions: (i) $FeCl_3/SiO_2$, 60 °C, 20 h; (ii) $LiCH_2CN$, THF, −78 °C to rt; (iii) HCl (32%), MeOH, Δ, 5 h; (iv) Me_2SO_4, NaH, HMPT, rt, 2 h.



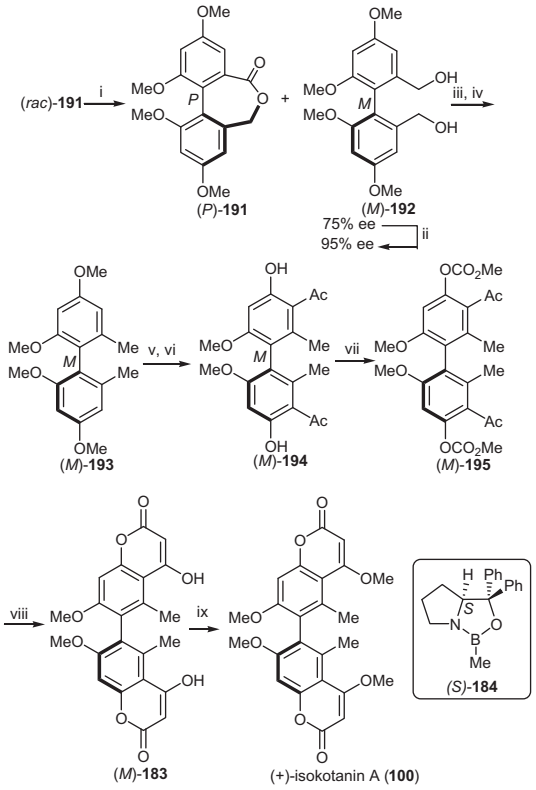
Scheme 7.

4.4. Bringmann atropo-enantioselective synthesis of (+)-isokotanin A

Bringmann et al.[147] described the atropo-enantioselective total synthesis of the axially chiral bicoumarin, (+)-isokotanin A (**100**) (Schemes 8 and 9). Key steps were the formation of a configurationally stable seven-membered biaryl lactone and its kinetic resolution by atroposelective ring cleavage. For the synthesis of the key lactone **191** required here, bromo ester **186** was prepared from 3,5-dimethoxybenzoic acid by esterification[148] and bromination according to a procedure reported by Danishefsky[149] Ullmann coupling of **186** gave the corresponding racemic diester **187**[150] (Scheme 8), reduction of which with $LiAlH_4$ provided diol **188**, which was then oxidized to the dialdehyde **189**. Under Cannizzaro conditions, **189** underwent an intramolecular disproportionation to the hydroxy acid **190**. The final ring closure was achieved with DCC and DMAP under modified Steglich conditions[151] to give the seven-membered lactone **191** in an overall yield of 39% (seven steps). On a preparative scale, the oxazaborolidine-mediated kinetic resolution of **191** (k_{rel}=27) was stopped at 56% conversion (see Scheme 9). After chromatographic separation, the optical purity of the product alcohol (*M*)-**192** was increased from 75 to 95% ee by a single crystallization step. Both (*M*)-**192** were transformed into the respective tetramethyl ethers **193** (Scheme 9) by hydroxy/bromo exchange and subsequent $LiAlH_4$ reduction, as previously used for other biaryl systems.[152,153]



Scheme 8. Reagents and conditions: (i) Cu, DMF, 89%; (ii) $LiAlH_4$, THF, 97%; (iii) MnO_2, CH_2Cl_2, 92%; (iv) KOH, EtOH, 94%; (v) DCC, DMAP, CH_2Cl_2, 72%.



Scheme 9. Reagents and conditions: (i) (*S*)-**184**, BH_3·THF, THF, 46% of (*M*)-**192** and 43% of (*P*)-**191**; (ii) recrystallized from EtOAc/cyclohexane; (iii) PPh_3, $(CBrCl_2)_2$, CH_2Cl_2; (iv) $LiAlH_4$, Et_2O, 83% (two steps); (v) $(CH_3CO)_2O$, $TiCl_4$, CH_2Cl_2, 94%; (vi) $TiCl_4$, benzene, 82%; (vii) $ClCO_2Me$, py, 55%; (viii) KO*t*-Bu, *t*-BuOH; (ix) NaH, Me_2SO_4, HMPA, 26%.

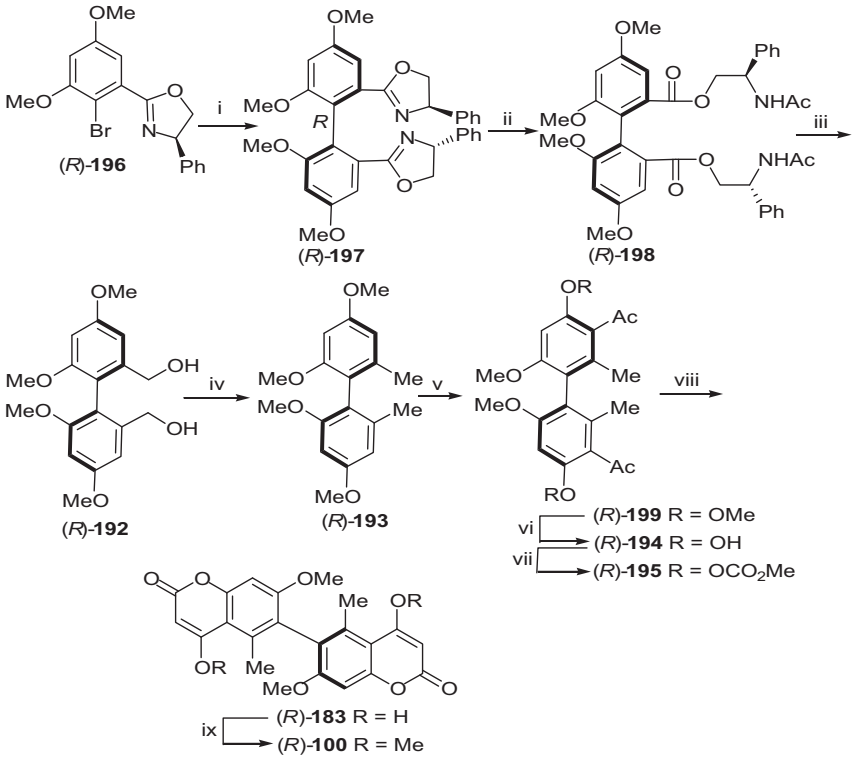
The final steps toward the target molecule, (+)-isokotanin A (**100**), were accomplished by the use of Lin's procedure,[154] according to a sequence already well known for the preparation of dimeric coumarins.[139,140,155] The tetramethyl ether (*M*)-**193** was acylated in the presence of $TiCl_4$[139] in almost quantitative yield (Scheme 9). In the next step, the methyl ethers *para* to the biaryl axis were cleaved selectively to give the phenol (*M*)-**194** in 82% yield. Treatment of the phenol (*M*)-**194** with methyl chloroformate gave the carbonate (*M*)-**195**. In the presence of KO*t*-Bu as a base, (*M*)-**195** cyclized to give the bicoumarin (*M*)-**183** (Scheme 9). This very polar compound contained some impurities, but could not be purified by column chromatography, due to its very low solubility in common organic solvents. Therefore, the crude (*M*)-**183** obtained was directly transformed into (+)-isokotanin A (**100**) by the use of the reagent combination NaH, Me_2SO_4, and HMPA[145,154] The spectroscopic data and the optical rotation of (+)-isokotanin A (**100**) were in full accordance both with those of the natural product[66] and with those of material synthesized earlier.[154]

4.5. Lin synthesis of natural (+)-isokotanin A

Lin et al[154] reported the first asymmetric synthesis of optically pure natural (+)-isokotanin A (**100**) (Scheme 10). As shown in Scheme 10, asymmetric Ulmann coupling of **196** in the presence of activated Cu powder and DMF for 72 h produced the bis(oxazoline) **197**. Because the bis(oxazoline) **197** was unstable in acidic media, it was converted directly into **198** by treatment with TFA/H_2O followed by acetylation. $LiAlH_4$ reduction of **198** in THF at room temperature gave the dicarbinol **192**. The diastereomeric excess of **192** was determined to be 83% by the examination of the ^1H NMR spectrum of the corresponding (*S*)-Mosher's ester. The optically pure dicarbinol **192** was obtained by recrystallization from acetyl acetate. The biaryl **193** was prepared from **192** by catalytic hydrogenation in the presence of 10% Pd/C and a catalytic amount of TFA in ethanol. Compound **193** was acetylated with $(MeCO)_2O/TiCl_4$ in CH_2C1_2 to afford **199**, which was selectively demethylated with $TiCl_4$/benzene to give **194**. The carbonate **195**, generated from the phenol **194** and methyl chloroformate in pyridine, was subjected to treatment with *t*-BuOK in *t*-BuOH to afford the desired cyclized product **183**. Finally, **183** was methylated with NaH/HMPA/$(Me)_2SO_4$ to give the optically pure (+)-isokotanin A (**100**).

4.6. Total synthesis of racemic desertorin C

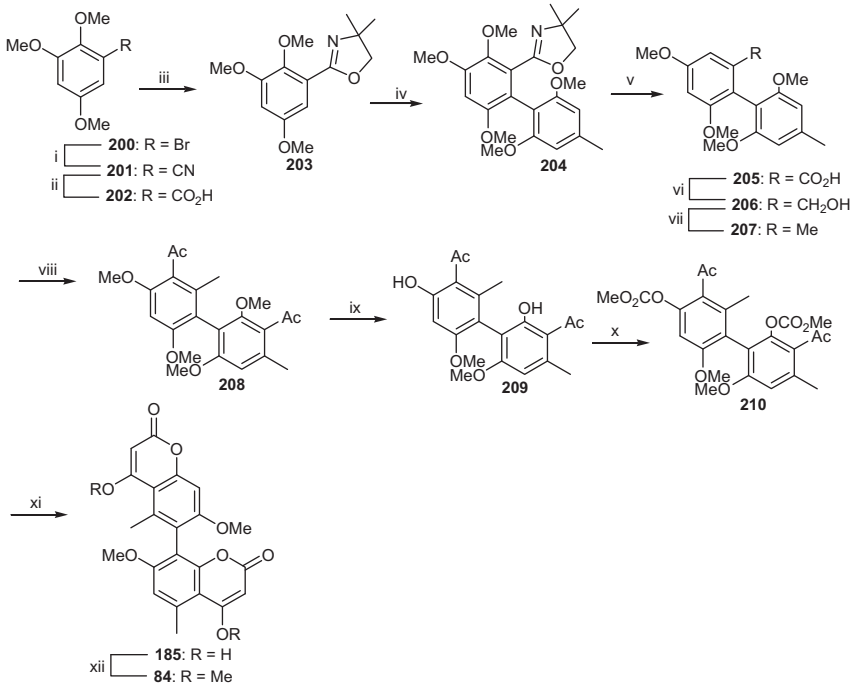
Sargent et al.[155] reported the racemic synthesis of desertorin C (**84**) (Scheme 11). Addition of CuCN into the known bromo compound (**200**)[156] gave the nitrile (**201**). Hydrolysis of the nitrile (**201**) gave the acid (**202**), which was converted into the dihydro-oxazole (**203**) by the usual method.[157] On reaction with the Grignard reagent derived from the 2-bromo-1,3-dimethoxy-5-methylbenzene, the dihydro-oxazole (**203**) gave 92% of the substitution product (**204**). The masked carboxy group in **204** was deprotected and the resultant carboxylic acid was converted into its methyl ester **205**. On reduction with $LiAlH_4$, the ester **205** yielded the compound **206**, which on further reduction with hydrogen over Pd/C gave the biphenyl **207** with 97% yield. Acetylation of **207** with 2 mol equiv of the mixed anhydride derived from acetic acid and trifluoroacetic anhydride gave the monoacetyl compound and this was accompanied by the diacetyl compound **208**. Further acetylation of the biphenyl **207** with an excess of the acetylating agent gave the diacetyl compound **208** in 85% yield. On demethylation with boron trichloride, the diacetyl compound **208** gave the crucial product **209**. Compound **209** was treated with methyl chloroformate and pyridine and the resultant biscarbonate **210**, on cyclization with potassium *tert*-butoxide in *tert*-butyl alcohol, gave a high yield of the dimeric 4-hydroxycoumarin **185**. Finally methylation of **185** gave the racemic form of the natural product, desertorin C (**84**). The structure of desertorin C, a metabolite of the mould *Emericella desertorum*, was confirmed as 4,4′,7,7′-tetramethoxy-5,5′-dimethyl-6,8′-bicoumarin (**84**) by its synthesis in racemic form. The key step involved the construction of the unsymmetrical biphenyl, 2,2′,4,6′-tetramethoxy-4′,6-dimethylbiphenyl (**207**), using dihydro-oxazole chemistry.



Scheme 10. Reagents and conditions: (i) activated Cu, DMF, reflux, 72 h; (ii) TFA, H_2O, THF, rt; Ac_2O, py, 51%; (iii) $LiAlH_4$, THF, rt 80%; (iv) 10% Pd/C, cat. TFA, EtOH; (v) $TiCl_4$, CH_2Cl_2, rt; (vi) $TiCl_4$, C_6H_6, reflux; (vii) $ClCO_2Me$, py, 55 °C, 90%; (viii) *t*-BuOK, *t*-BuOH, 60 °C, 89%; (ix) a. NaH, HMPA, rt; b. $(CH_3)_2SO_4$, HMPA, rt, 64%.

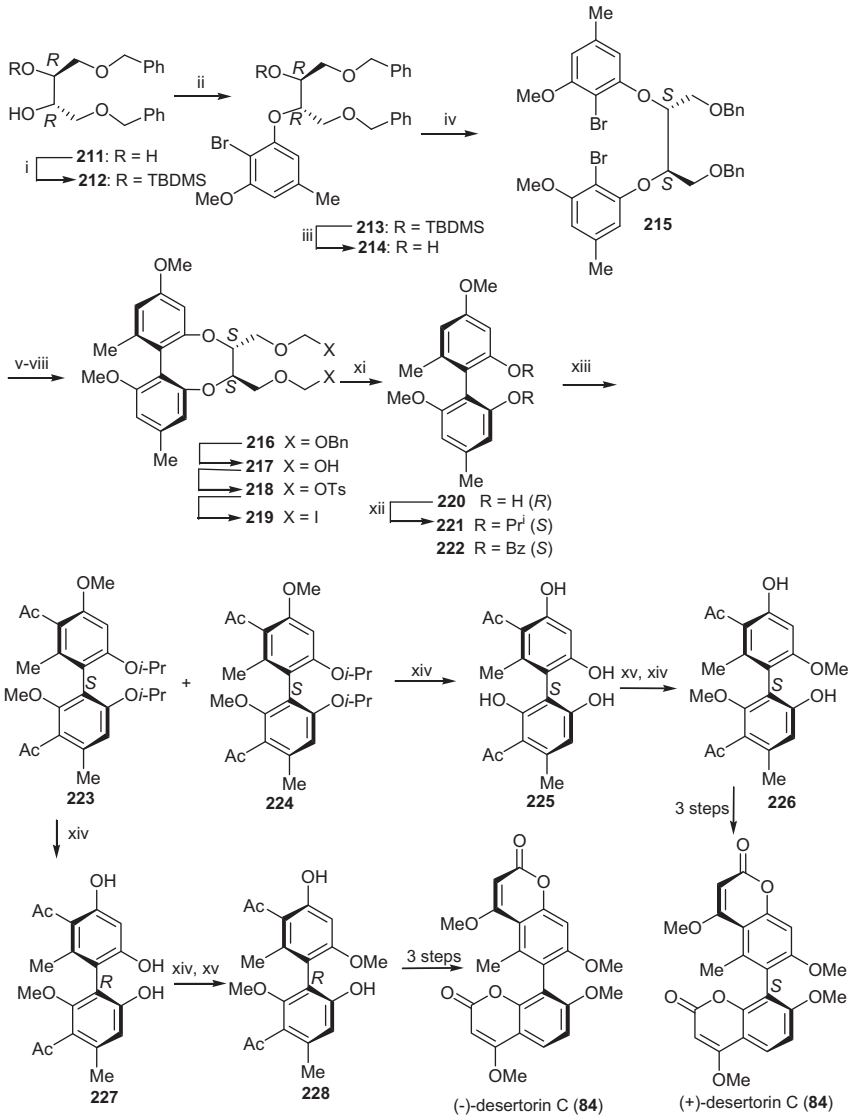
4.7. Total synthesis of both atropenantiomers of desertorin C

Desertorin C (**84**) belongs to a family of unsymmetrical coumarin dimers of fungal origin, which are optically active on account of restricted rotation about their stereogenic axes.[76] Sargent et al.[155] have previously synthesized desertorin C (**84**) in racemic form (Scheme 11) using the (±)-diketone **220** as the key intermediate. Subsequently, the absolute configuration of the

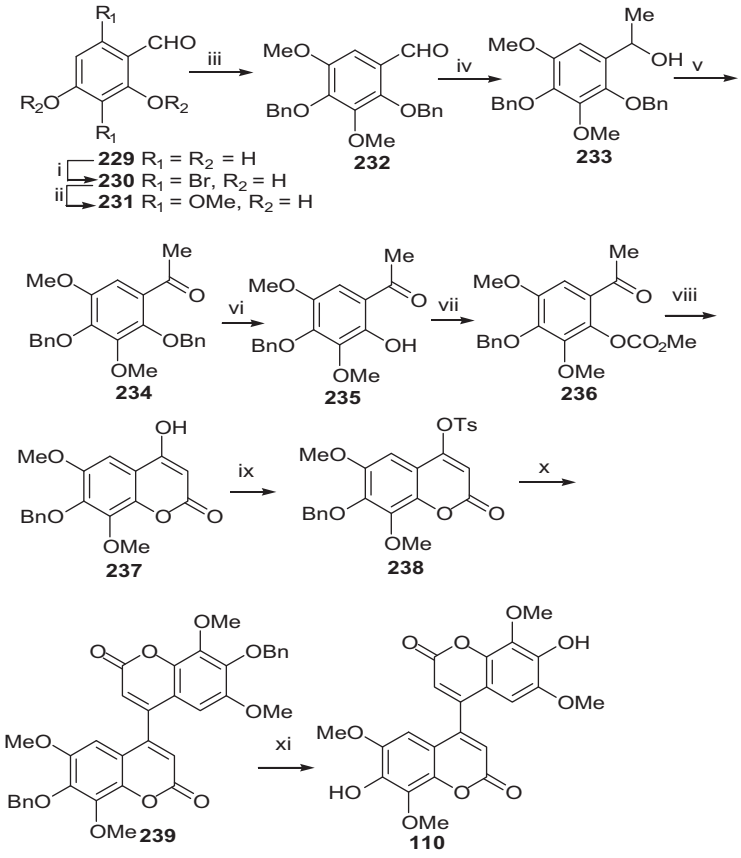


Scheme 11. Reagents and conditions: (i) CuCN, 16 h, 98%; (ii) NaOH, MeOH, 3 days, 84%; (iii) (a): $SOCl_2$, rt, 20 h; (b): 2-amino-2-methylpropan-1-ol, 0 °C→rt, 2 h, 94%; (iv) 2-bromo-1,3-dimethoxy-5-methylbenzene, Mg, THF, 92%; (v) (a): MeI, rt, 70 °C, 25 h; (b): MeI, K_2CO_3, DMF, 95%; (vi) $LiAlH_4$, THF, rt, 0.5 h, 97%; (vii) Pd/C, HCl, 94%; (viii) TFAA, AcOH, CH_2Cl_2, 85%; (ix) BCl_3, CH_2Cl_2, 96%; (x) $ClCO_2Me$, py, 70 °C, 2 h, 94%; (xi) Potassium *tert*-butoxide, *tert*-butyl alcohol, 2 h, 98%; (xii) $(Me)_2SO_4$, K_2CO_3, reflux, 18 h, 22%.

desertorins was established as *R* by an X-ray crystal structure determination of the bis-bromobenzoate.[158] Sargent et al.[159] then reported the asymmetric synthesis of both enantiomers of 1,1′-(2′,4-dihydroxy-6,6′-dimethoxy-2,4′-dimethylbiphenyl-3,3′-diyl)-bisethanone, which allow the formal synthesis of both enantiomers of desertorin C (**84**) (Scheme 12). Mitsunobu reaction (Scheme 12) between 2-bromo-3-methoxy-5-methylphenol[160] and the mono(*tert*-butyldimethylsilyl) ether **212** of 1,4-di-*O*-benzyl-L-threitol **211**[161] gave the ether **213** (68%), which on deprotection afforded the alcohol **214** (90%). This alcohol was subjected to another Mitsunobu reaction with the 2-bromo-5-methoxy-3-methylphenol.[162] The resultant D-threitol derivative **215** (45%) was subjected sequentially to the Lipschutz et al.[141] procedure, which gave the cyclized product **216** (40%). Deprotection was achieved by hydrogenolytic debenzylation and tosylation of the resultant diol **217**. The tosylate **218** was converted into the iodide **219**, which on reductive elimination with activated zinc supplied the diol **220**. In order for the intramolecular coupling **214**→**215** to occur, the aryloxy substituents in the intermediate higher-order cyanocuprate are predicted to adopt, on account of the anomeric effect, the gauche conformation. Hence, the axial configuration of the intermediate cyclic compound **216** is *S* and that of the diol **220** is *R*. The diol appeared to be enantiomerically pure since it was not resolved on HPLC on two chiral columns. The CD spectrum (MeCN) of the derived dibenzoate **222** showed an exciton splitting centered at λ 226 nm with a positive first Cotton effect (λ 237 nm, $\Delta\varepsilon$ 24.3) and a negative second effect (λ 215 nm, $\Delta\varepsilon$ −9.0), in keeping with the *R* configuration of the diol **220**. The diol **220** was isopropylated and the resultant ether **221** was acetylated with AcOH and TFAA, which supplied an inseparable mixture of the compounds **223** and **224**. Selective dealkylation of this mixture with BCl_3 yielded the tetrol **225** (30%), and the triol **227** (35%). Methylation and selective demethylation of the tetrol **225** gave the (*S*)-**226** (69%), which had previously been obtained by basic hydrolysis of desertorin C (**84**).[76] The (*R*)-diketone **228** (82%), was obtained in a similar fashion from the triol **227**. Since the racemic compound has been converted into desertorin C (**84**) (Scheme 12), this constitutes a formal synthesis of both of the enantiomers [(+)-desertorin C and (−)-desertorin C] of this metabolite.



Scheme 12. Reagents and conditions: (i) TBDMSCl, imidazole, DMF, 25 °C, 15 h, 76%; (ii) 2-bromo-3-methoxy-5-methylphenol, Bu_3P, DEAD, THF, 25 °C, 24 h; (iii) Bu_4NF, THF, 25 °C, 1 h; (iv) 2-bromo-5-methoxy-3-methylphenol, Bu_3P, DEAD, THF, 25 °C, 48 h; (v) BuLi, Ar, THF, −78 °C, 1 h; (vi) CuCN, TMEDA, −78 to −40 °C, 15 min; (vii) O_2, −78 °C, 3 h; (viii) H_2, Pd/C, EtOAc, 94%; (ix) TsCl, py, 0 °C, 7 h, 78%) NaI, Me_2CO, reflux, 5 h, 91%; (xi) Zn, EtOH, reflux, 1 h, 80%; (xii) Pri-Br, K_2CO_3, DMF, 45 °C, 48 h, 68%; (xiii) TFAA, AcOH, CH_2Cl_2, 25 °C, 7 h, 69%; (xiv) BCl_3, CH_2Cl_2, 0 °C, 2 h; (xv) MeI, K_2CO_3, DMF, 40 °C, 15 h.



Scheme 13. Reagents and conditions: (i) Br_2/AcOH, 25 °C, rt or Bu_4NBr_3/EtOH, rt; (ii) NaOMe, CuCl/DMF, reflux; (iii) BnBr/py, rt; (iv) MeMgCl, THF, −78 °C; (v) PCC/CH_2Cl_2, rt; (vi) HCl/AcOH, rt; (vii) $ClCO_2Me$/pyridine, rt; (viii) *t*-BuOK/*t*-BuOH, reflux; (ix) TsCl/py, rt; (x) $NiCl_2(PPh_3)_2$, PPh_3, NaH, toluene, 90 °C; (xi) HCl/AcOH, rt.

4.8. Total synthesis of 4,4′-biisofraxidin

Lei et al.,[163] synthesized the bicoumarin, 4,4′-biisofraxidin (**110**), through 11 steps in 7% overall yield (Scheme 13). Bromination of 2,4-dihydroxy-benzaldehyde (**229**) with bromine or Bu_4NBr_3 afforded compound **230** in a yield of 74 or 78%, respectively. Methoxylation of **230** with NaOMe gave compound **231** and benzylation of compound **231** gave compound **232** in 73% yield. The ketone **233** was obtained after an addition reaction of compound **232** with MeMgCl and oxidation of compound **233** by PCC, and debenzylation of **234** selectively gave compound **235**. Cyclization of **235** with sodium and $CO(OEt)_2$ under reflux failed, the free hydroxyl in compound **235** was protected with $ClCO_2Me$ to give compound **236** in 80% yield and then cyclization under the basic conditions provided compound **237** in 79% yield. According to coupling the coupling-reaction conditions, attempts to prepare the brominated or iodinated precursor failed. Therefore, the free hydroxyl group was transformed into tosylate **238** and subjected to a coupling reaction. The expected compound **239** was isolated in 60% yield. Under acidic conditions, debenzylation of the coupling product gave directly the target molecule, 4,4′-biisofraxidin (**110**). In summary, natural 4,4′-biisofraxidin (**110**) was successfully synthesized through 11 steps in 7% overall yield.

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Javid Hussain was born in Kirman Kurram Agency Pakistan. He received his B.Sc. degree from F.G Degree College Peshawar and M.Sc. from Gomal University, D.I. Khan. J. Hussain obtained his Ph.D. degree in 2004 under the supervision of Prof. Viqar Uddin Ahmad from International Center for Chemical and Biological Sciences H.E.J.Research Institute of Chemistry, Karachi Pakistan in Natural Product Chemistry and Synthetic Organic Chemistry. J. Hussain worked as HEC postdoctoral fellow at University of Glasgow, UK with Prof. Pavel Kocovsky 'Sir William Ramsay Professor of Chemistry' and worked on asymmetric synthesis. He started his career as Lecturer, at Department of Chemistry, Kohat University of Science and Technology Kohat, Pakistan in 2004 and was promoted to Assistant Professor in the same Department in 2005. In September, 2010 he joined the Department of Biological Sciences and Chemistry University of Nizwa, Oman as Associate Professor.

Ahmed Al-Harrasi received his B.Sc. in Chemistry from Sultan Qaboos University (Oman) in 1997. Then he moved to the Free University of Berlin from which he obtained his M.Sc. in Chemistry in 2002 and then his Ph.D. in Organic Chemistry in 2005 as a DAAD-fellow under the supervision of Prof. Hans-Ulrich Reissig. His Ph.D. work was on New Transformations of Enantiopure 3,6-Dihydro-2*H*-1,2-oxazines. Then he received the Fulbright award in 2008 for postdoctoral research in chemistry for which he joined Prof. Tadhg Begely group at Cornell University where he worked on synthesis of isotopically-labeled thiamin pyrophosphate. His current research focuses on the drugs discovery from Omani Medicinal plants as well as on the synthesis of biologically-active compounds. He is currently Associate Professor of Organic Chemistry and Assistant Dean for Graduate Studies and Research at the University of Nizwa, Oman.

Karsten Krohn was born in 1944 and graduated from the University of Kiel in 1968. He obtained his Ph.D. in 1971 under the supervision of Prof. A. Mondon with a thesis on the isolation and synthesis of alkaloids (narciclasin and lycoricidin) from daffodils. He spent his postdoctoral work first with Prof. Mondon and then with Prof. Winterfeldt as a DFG stipendiary, working on the synthesis of camptothecin. In 1975 he moved to the University of Hamburg, where he started his work on quinone antibiotics (in particular the anthracyclines) and achieved his Habilitation in 1979. He became Associate Professor at the Technical University of Braunschweig in 1981 and Full Professor in 1991 at the University of Paderborn. In 1984 he was Visiting Professor in Madison, Wisconsin, 1996 in Nancy (France), 2001 in Manila (Philippines), and 2006 in Le Mans (France). He is member of several editorial boards of chemical journals (J. Antibiotics, J. Carbohyd. Chem., ARKIVOK, Chin. J. Nat. Med., Nat. Prod. Commun.). He is Honorary Member of the Hungarian Humbold Society, Fellow of the Japan Society for the Promotion of Science, Doctor Honoris Causa of the University of Debrecen, Hungary and Doctor Honoris Causa of the University of Le Mans, France. His research interests are in the isolation and synthesis of natural products from fungi and medicinal plants, synthesis of quinone antibiotics (anthracyclines, angucyclines, anthrapyranes), and the chemistry of sugars (*C*-glycosides, *O*-glycosides, conversion of sugars into useful chiral building blocks). He is in retirement since September 2009.