



**SYNTHESIS OF POTENTIALLY PHARMACOLOGICAL ACTIVE *O*-
HETEROCYCLES**

Theses of Doctoral (Ph.D.) Dissertation

KATALIN GULÁCSI

Debreceni Egyetem

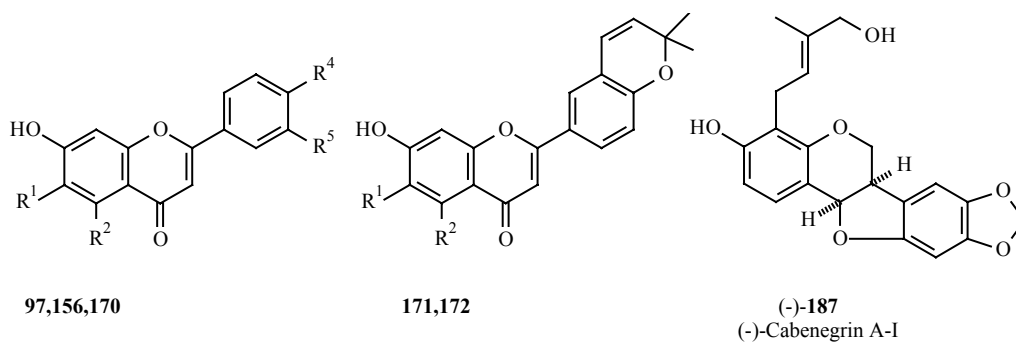
Debrecen, 2004

1. Introduction

Flavonoids possessing a wide range of biological activities represent an important class of naturally occurring *O*-heterocyclic compounds. They are important due to not only determining the beautiful plant colours but also demonstrating remarkable biological activity.

Besides their isolation, identification and synthesis, there had also been a growing interest for their structure-activity relationship study in the last decade .

The aim of my dissertation was to develop an efficient synthetic method for the preparation of naturally occurring flavones of potentially biological activity such as luteolin (**97**), chrysin (**156**), kanzonol-D (**170**), kanzonol-E (**171**) and yinyanghuo-C (**172**). Moreover, the total synthesis of the potent antidote (-)-cabenegrin A-I [(-)-**187**] and the preparation of its analogues for structure-activity study was also aimed.



	R ¹	R ²	R ³	R ⁴
luteolin (97)	H	OH	OH	OH
chrysin (156)	H	OH	H	H
kanzonol-D (170)	H	H	3,3-dimethylallyl	OH
kanzonol-E (171)	3,3-dimethylallyl	H		
yinyanghuo-C (172)	H	OH		

2. Applied Methods

The macro-, semimicro- and micro preparative methods of organic synthesis have been used. The reactions were monitored by thin-layer chromatography. The isolation and purification of the crude products and separation of isomers were carried out by crystallisation, preparative and column chromatography.

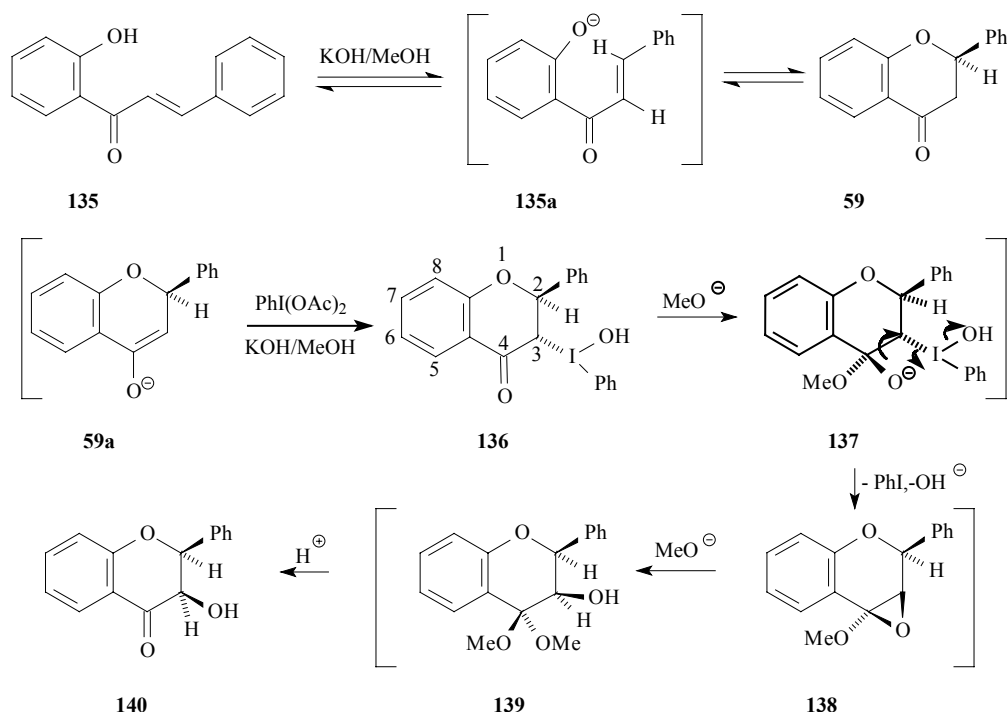
The obtained products were identified and characterised by elemental analysis, melting point determination, H-NMR, MS, CD and polarimetric technique.

3. New results of the Dissertation

In the course of our research, we developed an efficient method for the cyclodehydrogenation of 2'-hydroxychalcones and their 3,3-dimethylallyl derivatives with iodosobenzene diacetate (PIDA) and the total synthesis of (-)-cabenegrin A-I [(**187**)] and its analogues was also achieved.

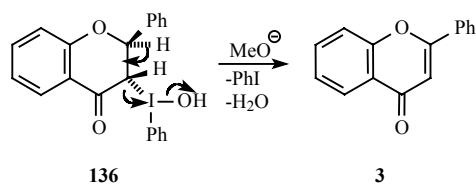
3.1. Cyclodehydrogenation of 2'-Hydroxychalcones and their 3,3-Dimethylallyl Derivatives with Iodosobenzene Diacetate

In the last two decades, the use of hypervalent iodine reagents became very popular, replacing successfully the mercury(II), thallium(III) or lead(IV) compounds, due to their similar behaviour in oxidation reactions and their more environmental friendly nature. Particularly, the reagent PIDA (with methanol as solvent and in the presence of potassium hydroxide) has been found useful in the transformation of 2'-hydroxychalcone (**135**) to *cis*-3-hydroxyflavanone (**140**), reported by Moriarty and co-workers. The proposed mechanism of this transformation is the following:



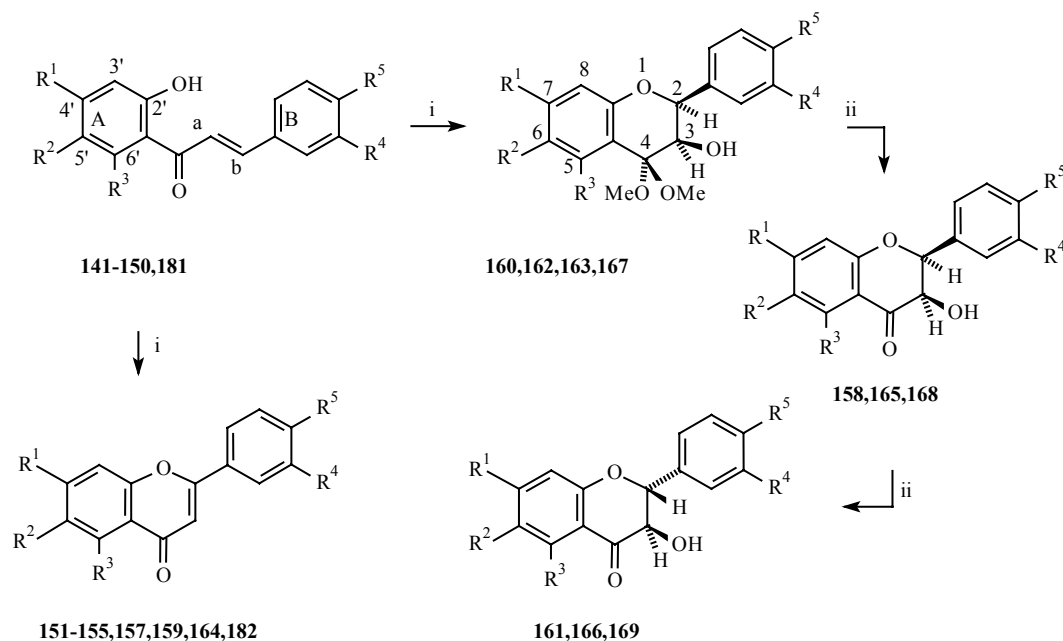
The key step of the proposed mechanism of this stereospecific transformation is the addition of the methoxide ion to the a carbonyl group of intermediate **136** to form

intermediate **137**. It seemed reasonable to assume that the prevention of this step might result in the loss of hydrogen at C-2 of **136** to give flavone **3**.



Therefore a bulky, electron-donating substituent in *peri* position (C-5) to the carbonyl group was introduced, which not only sterically hindered the nucleophilic attack of the methoxide ion but also decreased the electrophilicity of the carbonyl-function. Furthermore, the presence of an electron-donating substituent at C-7 of the intermediate **136** also decreased the electrophilicity of the carbonyl group.

According to our assumption, cyclodehydrogenation of 2'-hydroxychalcones **141-145**, resulted under standard conditions (PIDA/methanol/KOH) in the corresponding flavone derivatives **151-155** respectively, in good yields (60-75%).



	R ¹	R ²	R ³	R ⁴	R ⁵
141,151	OMe	H	OMe	H	H
142,152	OMe	H	OMe	H	OMe
143,153	OMe	H	OMe	-OCH ₂ CH ₂ O-	
144,154	MeOCH ₂ O	H	MeOCH ₂ O	H	H
145,155	MeOCH ₂ O	H	MeOCH ₂ O	OBn	OBn
146,157,158	MeOCH ₂ O	H	H	-OCH ₂ CH ₂ O-	
147,159,161	H	Me	H	OMe	OMe
148,162	H	H	H	H	OMe
149,163-166	H	H	H	-OCH ₂ CH ₂ O-	
150,167-169	Cl	H	Cl	H	H
181,182	MeOCH ₂ O	H	H	3,3-dimethylallyl	MeOCH ₂ O

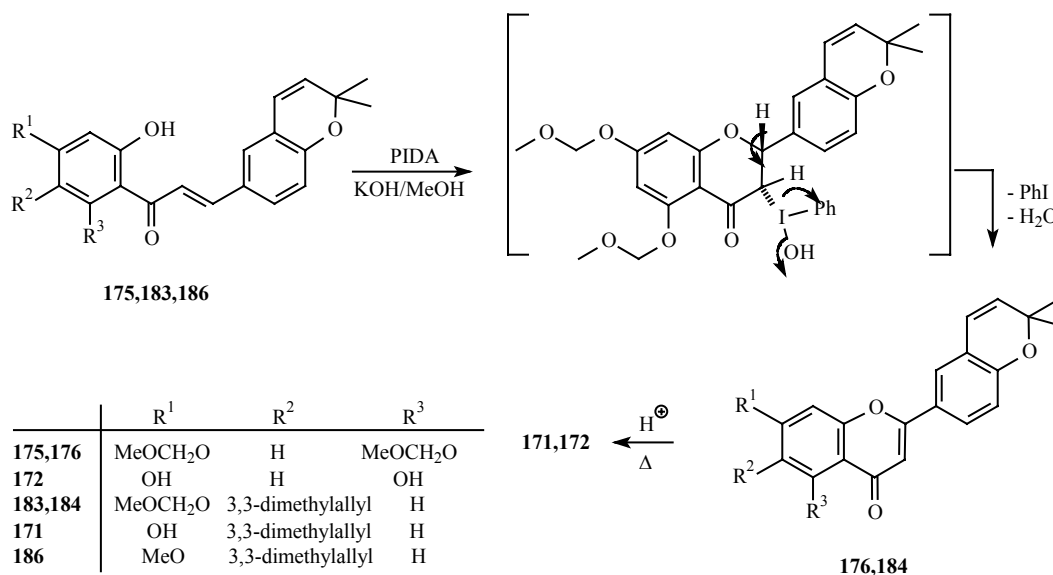
i = PIDA/KOH/MeOH
ii = HCl/H₂O

A new synthesis of naturally occurring flavones, chrysin (**156**) and luteolin (**97**), possessing significant HIV-inhibitory effect, could be performed starting from the readily available chalcones **144** and **145**, respectively.

When there is electron-donating substituent only in position 4' or 5' of the chalcone (**146,147**), the cyclodehydrogenation leads to *cis*- and *trans*-3-hydroxyflavanone derivatives **158** and **161**, beside flavones **157** and **159**, respectively.

In good agreement with our assumption, in the lack of electron-donating substituents in the ring A of the chalcones (**148,149**), the cyclodehydrogenation resulted in *cis*-3-hydroxyflavanone dimethyl acetal derivatives **162** and **163**, respectively and only traces of flavone **164**. Finally, in the presence of electron-withdrawing chloro-substituent, the chalcone **150** promoted exclusively the acetal **167**, which was transformed *via cis*-3-hydroxy-5,7-dichloroflavanone (**168**) to the more stable *trans*-isomer (**169**).

Our method could be successfully applied to the cyclodehydrogenation of 3,3-dimethylallyl and 2,2-dimethyl-2*H*-pyran moiety containing chalcones (**175,181,183**), which allowed to accomplish the first synthesis of naturally occurring yinyanghuo-C (**172**), kanzonol-D (**170**) and kanzonol-E (**171**), isolated from *Vancouveria hexandra* or *Epimedium sagittatum* and *Glycyrrhiza eurycarpa*, respectively.

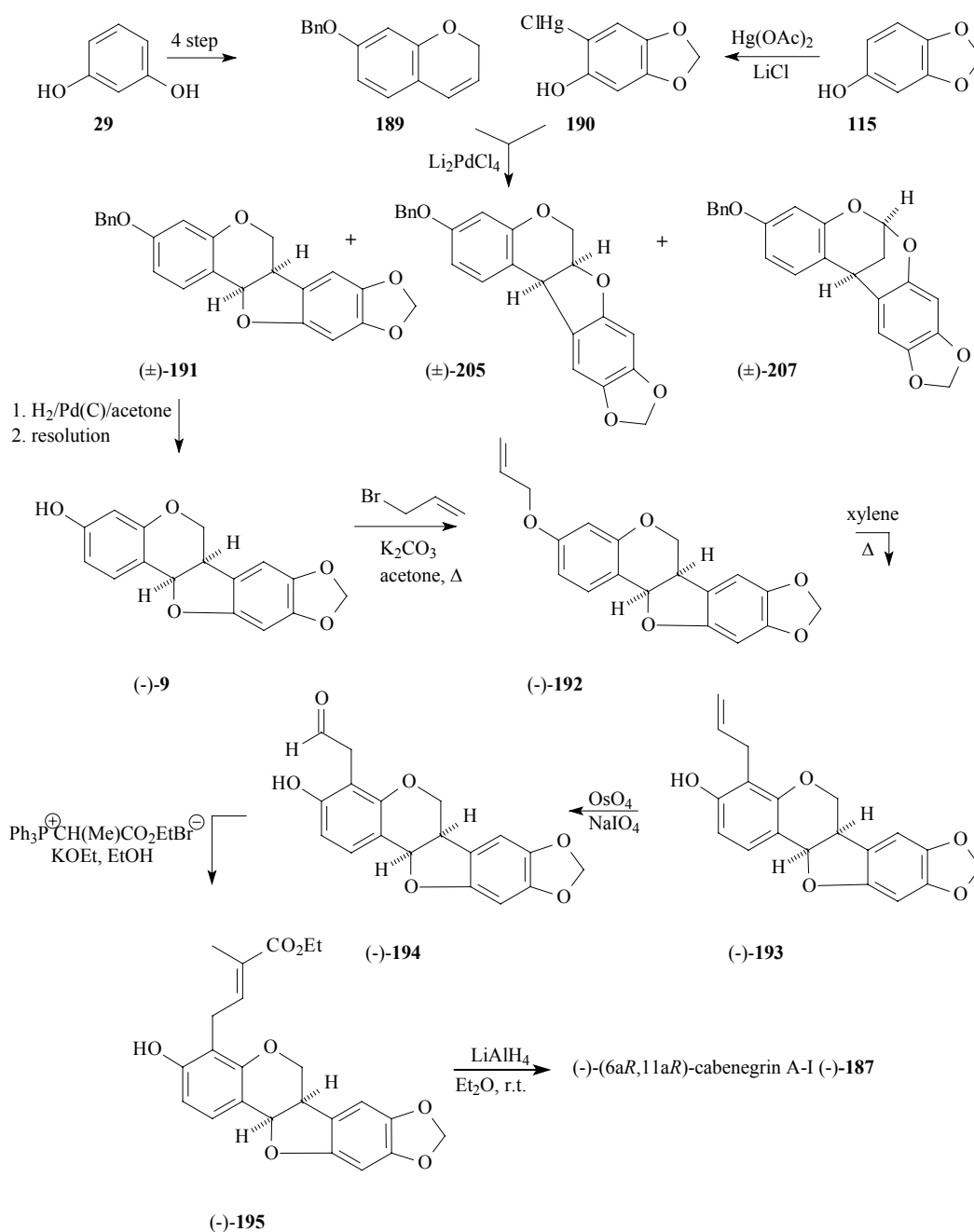


These results clearly showed that the attack of the electrophilic reagent PIDA took place regioselectively at the olefinic carbon neighbouring the carbonyl group which possessed the highest electron density among the olefinic carbons of the molecule. This high regioselectivity could also be explained by PM 3 semiempirical quantum chemical calculations.

3.2. Total Synthesis of (-)-Cabeneigrin A-I and its Analogues

The aqueous alcoholic extract of the root of a South-America plant called *Cabeça de Negra* is used against snake and spider venom. From this source, Nakanishi and co-workers isolated the (-)-cabeneigrin A-I [(-)-**187**] which has been found to be responsible for the biological activity of the extract.

Starting from the commercially available resorcinol (**29**) and sesamol (**115**), the total synthesis of (-)-cabeneigrin A-I [(-)-**187**] was accomplished in twelve steps:

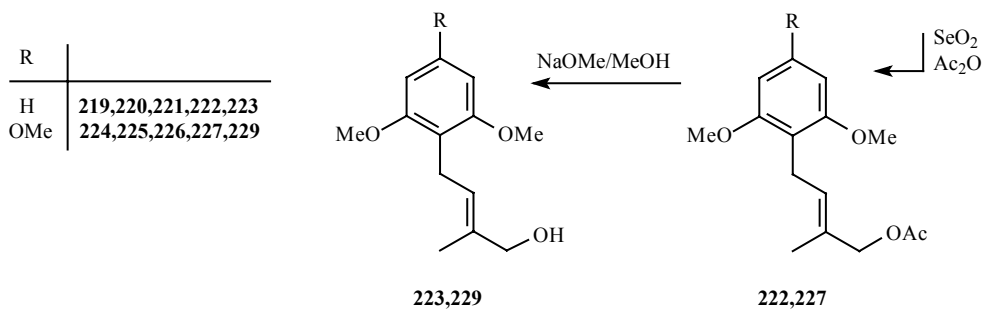
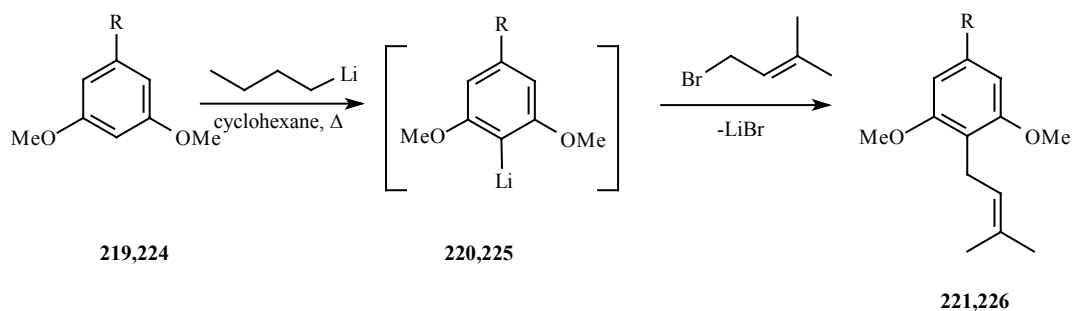


The strategy of our synthesis was based on the well-documented synthetic availability of racemic maackiain [(±)-**9**] which was obtained by Heck type oxyarylation of **189** with **190** using lithium tetrachloropalladate as catalyst. In contrast to the report of Breytenbach and Horino, we have shown that this oxyarylation process did not take place with complete regioselectivity and beside the main product *rac*-*O*-benzylmaackiain [(±)-**191**], its regioisomers **205** and **207** could also be obtained. Removal of the benzyl protecting groups by catalytic hydrogenation of (±)-**191** resulted in the *rac* maackiain [(±)-**9**], which could be resolved using (*S*)- α -methylbenzyl isocyanate. Chiroptical measurements clearly showed that the levorotatory enantiomer of maackiain [(-)-**9**] possessed (6*aR*,11*aR*) absolute configuration and was homochiral with cabenegrin A-I [(-)-**187**] and hence the synthesis of the latter could be performed from (-)-maackiain [(-)-**9**].

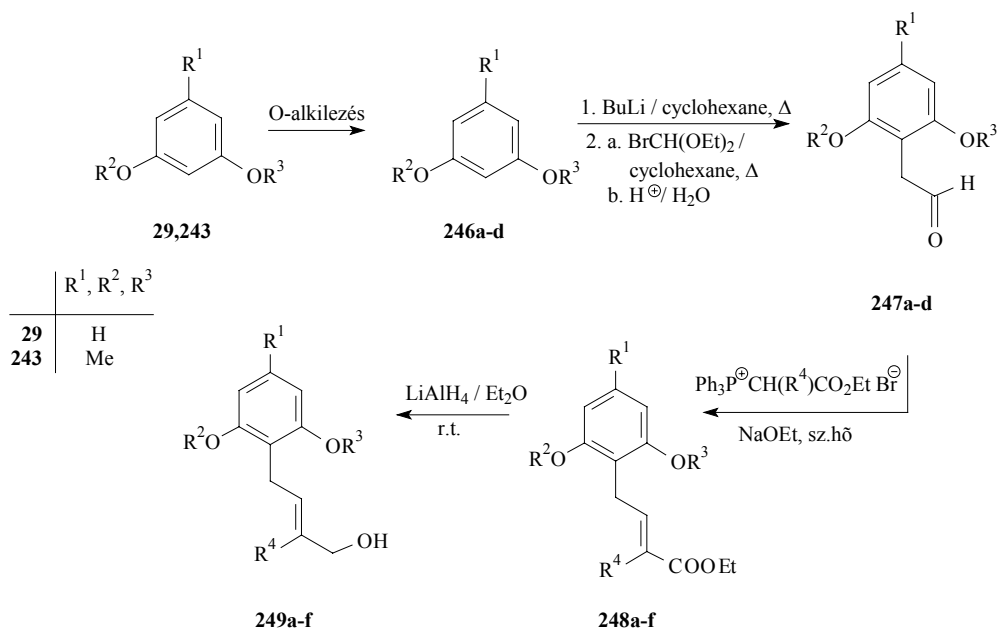
In the first step, (-)-maackiain [(-)-**9**] was alkylated with allyl bromide to give the allyl ether (-)-**192** whose thermal Claisen rearrangement in xylene at 192°C resulted in (-)-4-allylmaackiain [(-)-**193**]. Then it was treated with sodium metaperiodate in dioxane, in the presence of catalytic amount of osmium tetroxide to give the corresponding aldehyde (-)-**194** in moderate yield (34%). The (*E*)-olefinic side chain of (-)-**187** was stereoselectively introduced by Wittig reaction of (-)-**194** with α -ethoxycarbonylethyltriphenylphosphonium bromide in the presence of sodium methoxide to furnish the ester (-)-**195**. Reduction of this compound with lithium aluminum hydride at room temperature resulted in (-)-cabenegrin A-I [(-)-**187**] whose UV, CD and NMR data were identical with those reported in the literature. Thus its structure and (6*aR*,11*aR*) absolute configuration were unambiguously established.

In order to study the structure-activity relationship of (-)-cabenegrin A-I [(-)-**187**], a series of 4-arylbutene and butanol derivatives (**223**, **229**, **249a-f**, **252-254**, **268b,c**, **264**, **270**, **271**), were synthesized starting from resorcinol and 7-hydroxychromane. The 4-hydroxy-3-methylbut-2-ene-1-yl side chain was accomplished in two routes.

The 3,3-dimethylallyl derivatives **221** and **226** were prepared starting from phenol derivatives **219** and **224** *via* their lithium salts **220** and **225** and trapped with 3,3-dimethylallyl bromide respectively. The side chain was oxidized with stoichiometric amount of selenium dioxide yielding the (*E*)-acetoxysteroids **222**, **227** whose saponification with sodium methoxide afforded the (*E*)-hydroxyderivatives **223** and **229**, respectively.

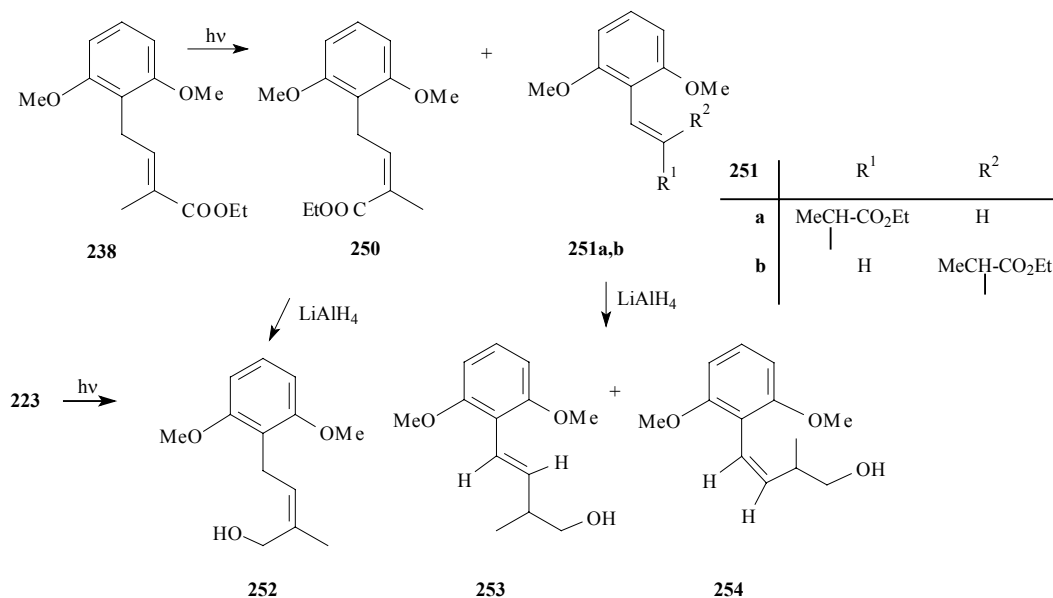


In the other route, lithium salts of **246a-d** were alkylated with bromoacetaldehyde diethyl acetal. After the acidic hydrolysis of the acetal function, the resulted aldehydes **247a-d** were treated with different phosphonium salts to obtain the (*E*)- α,β -unsaturated esters **248a-f**, whose reduction with lithium aluminum hydride afforded the allyl alcohols **249a-f**.



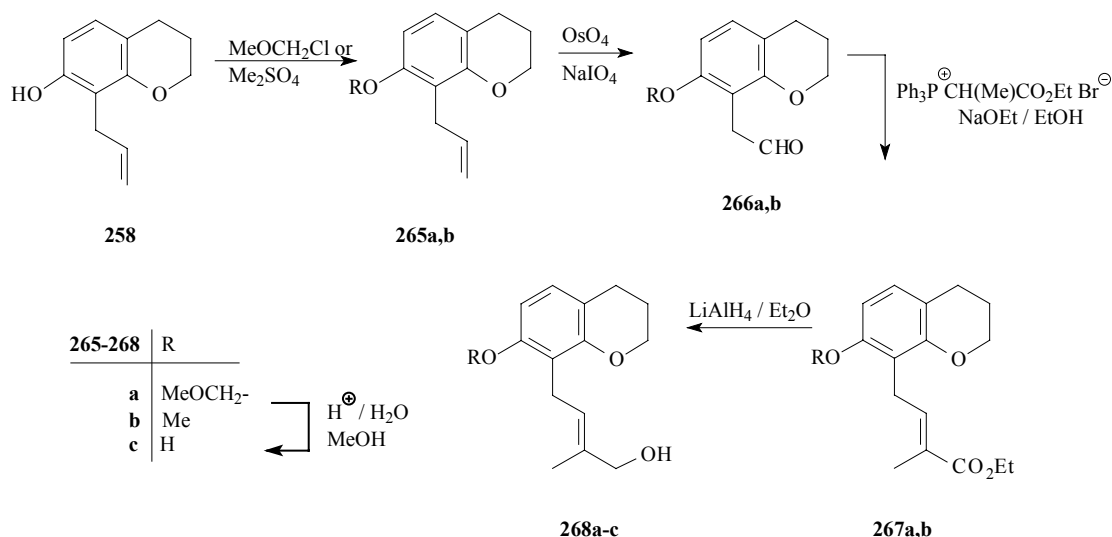
246,247	R^1	R^2	R^3	248,249	R^1	R^2	R^3	R^4
a	H	Et	Et	a	H	Et	Et	Me
b	H	Me	Et	b	H	Me	Et	Me
c	Me	Me	Me	c	Me	Me	Me	Me
d	Pr	Me	Me	d	Pr	Me	Me	Me
				e	H	Me	Me	Et
				f	H	Me	Me	Pr

It also seemed reasonable to find out whether the position of the double bond and the stereochemistry of the side chain would influence the biological activity of compound **223**. Thus, its isomers (**252-254**) were prepared by photoisomerization of ester **238** in benzene, followed by reduction with LiAlH_4 .

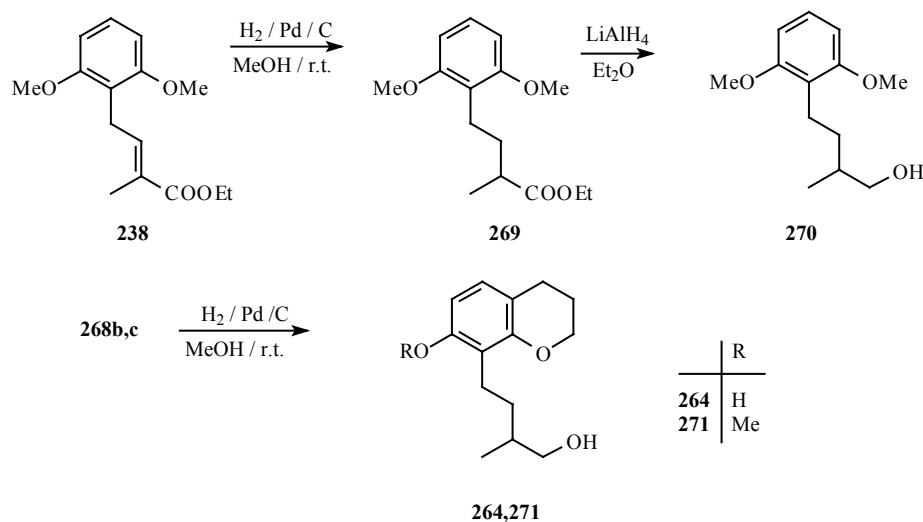


Interestingly, irradiation of alcohol **223** gave only the (*Z*)-isomer **252** and the migration of the double bond was not observed.

The chromane moiety containing analogues **268b,c** were prepared from 8-allylchromane-7-ol (**258**) in four steps as described at the synthesis of (-)-cabenegrin A-I [(**187**)].



For further pharmacological studies, some analogues containing saturated side-chain (**264,270,271**) were also prepared starting from the esters **238** and **268b,c**, respectively.



4. Summary

1. We have developed a convenient method for cyclodehydrogenation of 2'-hydroxychalcones and their *O*- and *C*-dimethylallyl derivatives using the hypervalent iodine reagent, iodosobenzene diacetate. An efficient synthesis of naturally occurring, pharmacologically active flavones such as luteolin (**97**) and chrysin (**156**) and the first synthesis of kanzonol-D (**170**), kanzonol-E (**171**) and yinyanghuo-C (**172**) were accomplished by this method.
2. Starting from commercially available resorcinol (**29**) and sesamol (**115**), the total synthesis of (-)-(6*a**R*,11*a**R*)-cabenegrin A-I [(-)-**187**] possessing significant activity against snake venom was accomplished *via rac*-maackiain [(±)-**9**] in twelve steps. We demonstrated that *rac-O*-benzylmaackiain [(±)-**191**] could indeed be obtained in the Heck arylation reaction of **189** and **190**, prepared from resorcinol (**29**) and sesamol (**115**) in four and one steps, respectively but this transformation did not take place with complete regioselectivity. Thus, besides *rac-O*-benzylmaackiain [(±)-**191**], its regioisomers (**205,207**) were also formed.
3. *Rac*-maackiain [(±)-**9**] could be resolved with (*S*)-(-)- α -methylbenzyl isocyanate and the levorotatory enantiomer [(-)-**9**] has been found to possess 6*a**R*,11*a**R* absolute configuration by chiroptical measurements namely, it is homochiral with (-)-cabenegrin A-I [(-)-**187**].
4. For the structure-activity relationship study a series of phenylbutene and butanol derivatives were prepared whose pharmacological examinations showed that the

chromane part of (-)-cabenegrin A-I [(-)-**187**] is mainly responsible for its biological activity.

List of Publications

1. Gy. Litkei, **K. Gulácsi**, S. Antus, G. Blaskó, Cyclodehydrogenation of 2'-Hydroxychalcones with Hypervalent Iodine Reagent: A New Synthesis of Flavones, *Liebigs Ann.*, 1711-1715, (1995)
2. Gy. Litkei, **K. Gulácsi**, S. Antus, G. Blaskó, An Efficient Conversion of 2'-Hydroxychalcones to Flavones by a Hypervalent Iodine Reagent, in *Flavonoids and Bioflavonoids 1995* (Ed. S. Antus, M. Gábor, K. Vetschera). Akadémia Kiadó, Budapest, **1996**, p. 335-337.
3. **K. Gulácsi**, Gy. Litkei, S. Antus, T. E. Gunda, A Short and Facile Synthetic Route to Prenylated Flavones. Cyclodehydrogenation of Prenylated 2'-Hydroxychalcones by a Hypervalent Iodine Reagent, *Tetrahedron*, **54**, 13867-13876, (1998)
4. A. L. Tőkés, Gy. Litkei, **K. Gulácsi**, S. Antus, E. Baitz-Gács, Cs. Szántay, L. L. Darkó, Absolute Configuration and Total Synthesis of (-)-Cabnegrin A-I, *Tetrahedron*, **55**, 9283-9296, (1999)
5. **K. Gulácsi**, Gy. Litkei, S. Antus, Cs. Szántay, L. L. Darkó, J. Szelényi, Gy. Haskó and Sz. E. Vízi, Synthesis and Biological Activity of the Structural Analogues of (-)-Cabnegrin A-I, *Arch. Pharm. Pharm. Med. Chem.*, **334**, 53-61 (2001)
6. S. Antus, **K. Gulácsi**, L. Juhász, L. Kiss, T. Kurtán, Synthesis of Naturally Occuring *O*-Heterocyclic Compounds of Biological Activity, *Pure and Applied Chem.*, in press
7. Gy. Litkei, **K. Gulácsi**, S. Antus, Z. Dinya, Reaction of Chalcones with NBS, a Simple One-pot Synthesis of 2-Aroylbenzo[b]furanes, *Synth. Commun.*, **26** (16), 3061-3074 (1996)

Lectures and posters:

1. Gy. Litkei, **K. Gulácsi**, S. Antus, G. Blaskó, An Efficient Conversion of 2'-Hydroxychalcones to Flavones by a Hypervalent Iodine Reagent, International Bioflavonoid Symposium, Vienna, July 16-19, **1995**.
2. Litkei Gy., **Gulácsi K.**, Antus S., Blaskó G., 5,7-Diszubsztituált flavonok új szintézise, MKE Vegyészkonferencia, Debrecen, **1995**. Augusztus 29-31.

3. **Gulácsi K.**, L. Tőkés A., Litkei Gy., Antus S., Problémák a Cabenegrin A-I és analogonjainak szintézisének, MTA Flavonoidkémiai Munkabizottsága 1996. évi ülése, **1996**, nov. 21.
4. **Gulácsi K.**, Litkei Gy., Gunda T., Antus S., Természetben előforduló polihidroxi-flavon-származékok szintézise, MTA Flavonoidkémiai és DAB Gyógyszerkémiai és Vegyipari Munkabizottság tudományos előadóülése, Debrecen, **1998**. Nov. 2-3.
5. **Gulácsi K.**, Antus S., Szántay Cs., Szelényi J., Vízi E. Sz., Dioxocin vázas cabenegrin A-I analogon szintézise és farmakológiai vizsgálata, MTA Flavonoidkémiai Munkabizottság tudományos előadóülése, Budakalász, **2000**. Dec. 11.