Természetes eredetű, potenciálisan biológiailag aktív *O*- és *C*-prenilezett flavanonok szintézise

Doktori (PhD) értekezés tézisei

Kenéz Ágnes

Témavezető: Prof. Dr. Antus Sándor

Synthesis of naturally occurring *O*- and C-prenylated flavanones with potential biological activity

PhD theses

Ágnes Kenéz

Supervisor: Prof. Dr. Sándor Antus

Debreceni Egyetem

Természettudományi Kar

Debrecen, 2007

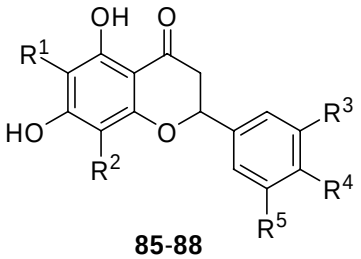
1. Introduction and objectives

Flavonoids are one of the most important groups of naturally occurring *O*-heterocycles and besides their important role in the colours of plants, they have a wide range of biological activity. Regarding their classification there are several categories depending on their versatile structures, from which flavanones, flavones, flavanoles and antocyanides can be considered the most important ones.

Most of naturally occurring flavanones usually have some alkyl side-chains, mainly methyl- prenyl- and geranyl units attached either to the ring (*C*-alkyl) or to a hydroxyl group (*O*-alkyl). The different substitution of these groups makes a large number of flavanone derivatives. Besides the characterization of isolated compounds there are many cases in which the total syntheses were achieved to prove the exact structure. Flavanones of biological activity, several pharmacological studies were made to explore the structure-activity relationship.

Most natural flavanones containing prenyl and 2,2-dimethyl-2*H*-pyran (it forms in the ring-closure of prenyl group) moiety are *C*-prenylated derivatives, which also show remarkable biological activity such as antioxidant activity arising from the flavonoid moiety as well as antibacterial, antiviral, anti-tumor, antifungal , anti-HIV and enzyme inhibition activity.

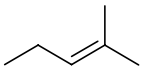
The aim of my dissertation was to develop efficient synthetic methods for the preparation of naturally occurring flavanones (**85-88**), which have potential biological activity against *Candida albicans* and were isolated from *Monotes engleri* by Hostettmann and coworkers. Moreover, the syntheses of their structural analogues were also aimed in order to study their structure-activity relationship.



85-88

Table 1. Flavanones isolated from *Monotes engleri*

	Name	R^1	R^2	R^3	R^4	R^5
85	Selinone	H	H	H	OPre	H
86	Monotesone A	H	H	OH	OPre	H
87	Lonchocarpol A	Pre	Pre	H	OH	H
88	Monotesone B	Pre	Pre	OH	H	OH

Pre = 

2. Applied methods

Macro, semi macro and micro methods of modern preparative organic chemistry were applied in the synthetic work. Reactions were monitored by thin layer chromatography; isolation, purification and separation of the crude products were carried out by preparative and column chromatography or crystallisation. Elemental analysis, melting points, optical rotation, ^{1}H and ^{13}C NMR spectroscopy and mass spectrometry measurements were applied for the identification and characterization of the prepared compounds.

The study of the antifungal activity of our compounds was carried out on different *Candida* species by agar diffusion method. The yeast cells incubated in Sabouraud-dextróz (SDB) solution for 18 hours at 37 ºC were suspended in SDA (Sabouraud-dextrose-agar) containing 1% agar (final cell concentration: 3 x 10^6 cell/mL) and then poured onto solid agar plate. The samples dissolved in 70% ethanol containing 1.5 volume% DMSO (c = 10, 25, 50 és 100 μg/mL) were incubated for 24-48 hours at 37 ºC together with the blank (solvent) and control materials (nystatine and fluconazole).

3. New results

The syntheses of naturally occurring *O*- and *C*-prenylated flavanones of potential biological activity and their structural analogues as well as the results of our pharmacological study are summarized below.

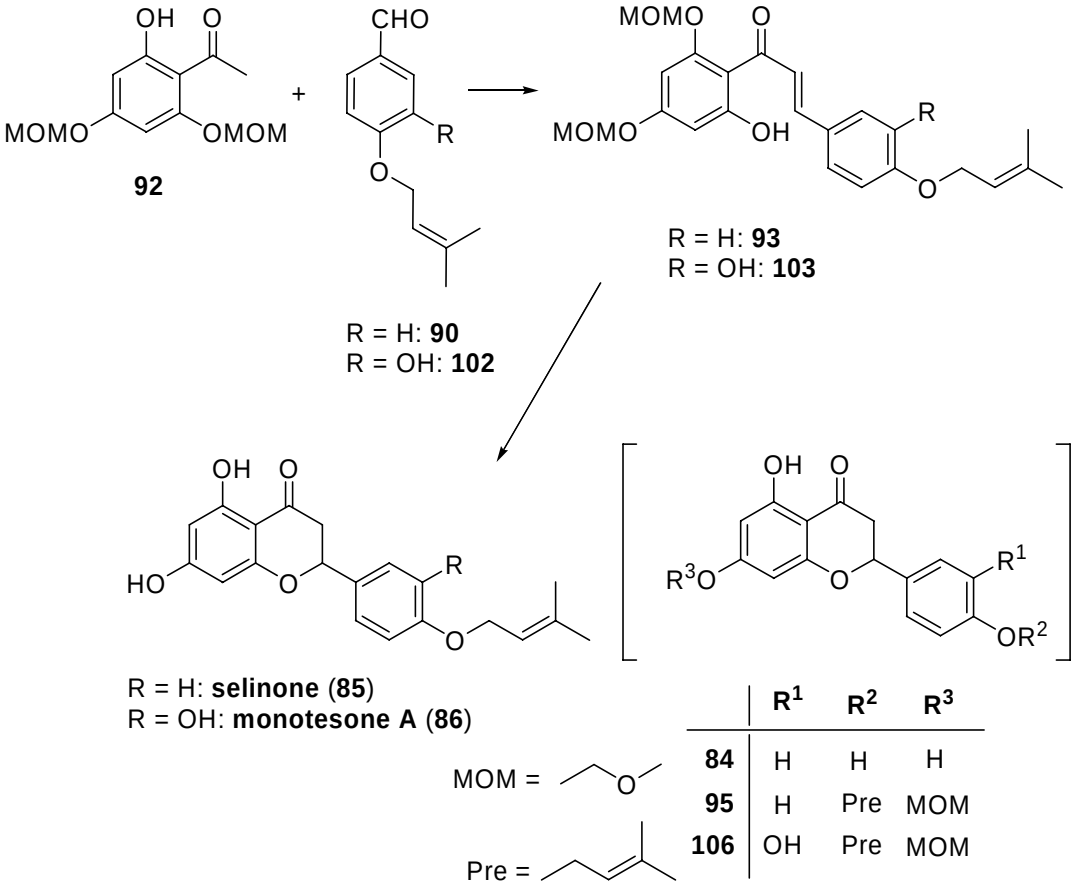
3.1. Syntheses of *O*-prenylated flavanones

3.1.1. Synthesis of (±)-selinone, monotesone A and their analogues

The first synthesis of racemic selinone [(±)-**85**] isolated also from *Selinum vaginatum clarke* was accomplished by Wagner and coworkers starting from phloracetophenone 4-*O*-*β*-neohesperidoside and 4'-(*γ*,*γ*-dimethylallyloxy)benzaldehyde. Due to the difficulties arising from the preparation of the starting material phloracetophenone 4-*O*-*β*-neohesperidoside and the cleavage of the sugar residue, we decided to develop a more efficient synthetic method allowing a detailed pharmacological study.

First, the chalcone derivative **93** was synthesized from the partially protected phloracetofenone derivative **92** and 4-prenyloxybenzaldehyde **90** under alkaline conditions. After the cleavage of methoxymethyl protecting groups of **93** under acidic conditions, a ring-closure was carried out with NaOAc in a "one-pot" reaction. Surprisingly, besides (±)-selinone [(±)-**85**], its 7-methoxymethyl ether (**95**) and naringenin (**84**) could be also isolated. The TLC monitoring of the reaction clearly showed that not only its precursor hydroxychalcone derivative was formed but also those of 7-methoxymethyl ether and naringenin due to the different reactivity of methoxymethyl groups and the cleavage of the prenyl group (Scheme 1). This method was applied for the synthesis of monotesone A [(±)-**86**] via the chalcone derivative (**103**) as well, but only its 7-methoxymethyl ether (**106**) could be isolated.

Method 1



	R1	R2	R3
84	H	H	H
95	H	Pre	MOM
106	OH	Pre	MOM

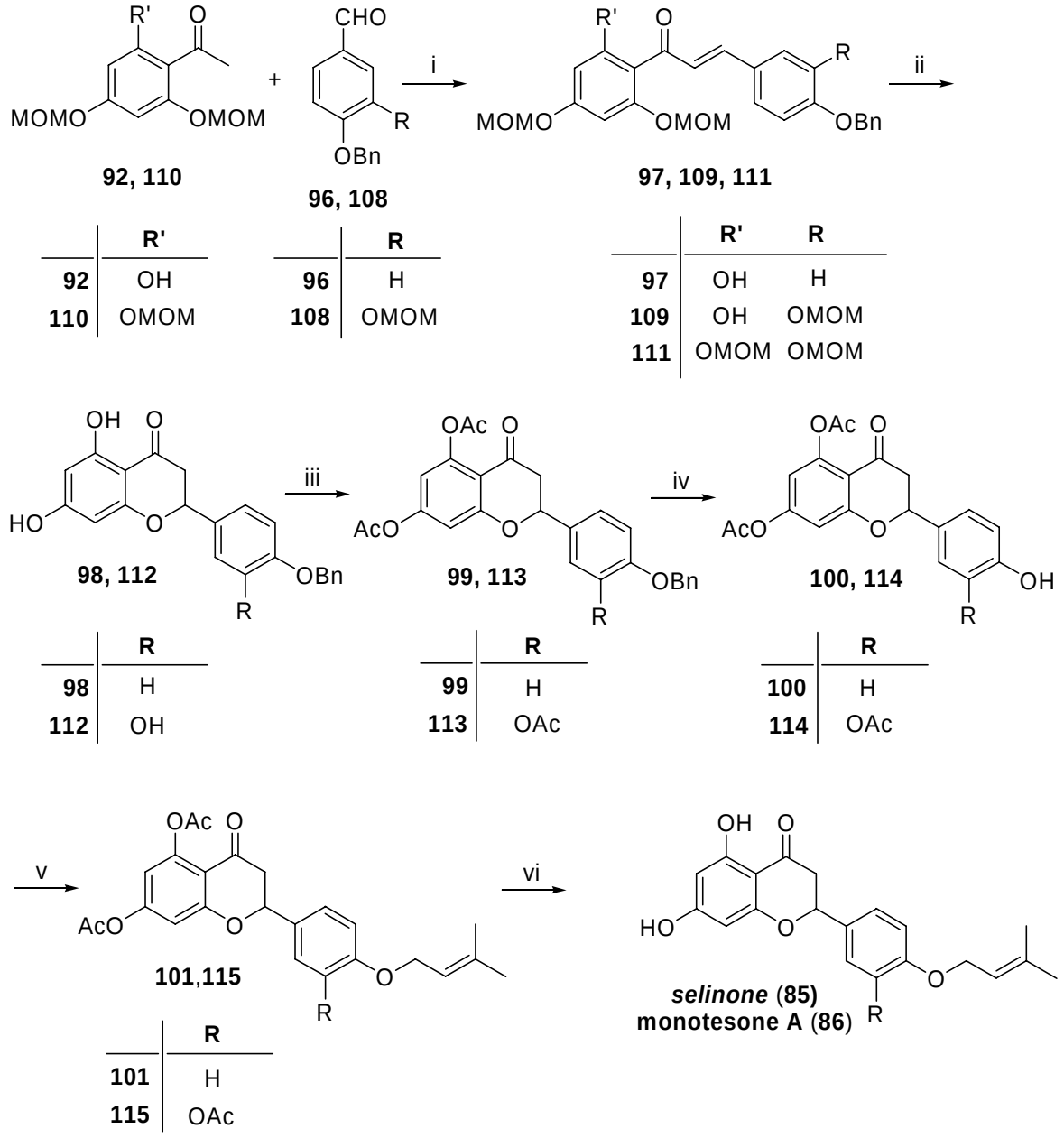
Scheme 1

In order to change our strategy on protecting groups, we decided to introduce prenyl group at the end of the synthesis under Mitsunobu conditions (**Scheme 2**). For this purpose, the C-4 hydroxyl group of the corresponding benzaldehyde derivatives was protected by a benzyl group instead of a methoxymethyl group. Claisen-Schmidt condensation of these compounds (**96**, **108**) with the partially and totally blocked phloracetophenone derivatives (**92**, **110**) afforded chalcones **97**, **109**, **111**. Since the benzyl group was stabile under both acidic and basic conditions, after the acetylation of the flavanones **98**, **112** (**98→99**, **112→113**) it was selectively cleaved by catalytic hydrogenation to yield the corresponding derivatives **100**, **114**. Prenylation of the free hydroxyl groups was achieved under Mitsunobu condition to give the peracetylated derivatives **101**, **115** whose saponofication by Zemplén's method afforded the

desired compounds selinone [(±)-**85**] and monotesone A [(±)-**86**] in pure form. Their spectroscopical data were in good accordance with those of natural ones.

The synthesis of *levo* and *dextro*rotatory selinone [(-)-**85**, (+)-**85**] was also tried.

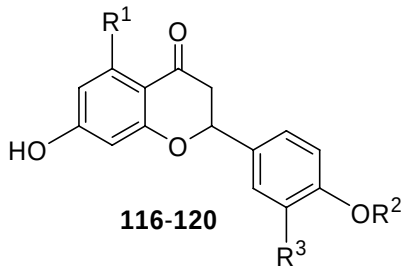
Method 2



i: KOH, ethanol/25°C, ii: 1. 10% HCl, methanol/Δ 2. NaOAc, methanol/Δ; iii: Ac_2O, dry pyridine/25°C; iv: H_2-Pd/C, methanol/25°C; v: 3-methylbut-2-ene-1-ol, Ph_3P, DIAD, dry THF/25°C; vi: NaOMe, dry methanol/25°C

Scheme 2

For the pharmacological studies several analogues (**116-120**) of selinone [(±)-**85**] and monotesone A [(±)-**86**] were prepared by the above sequence (**Scheme 3**).



	R^1	R^2	R^3
116	H	Pre	H
117	OH	Pre	OMe
118	OH	Pre	Cl
119	OH	Allil	H
120	OH	DHPre	H

Scheme 3: Analogues of selinone and monotesone A

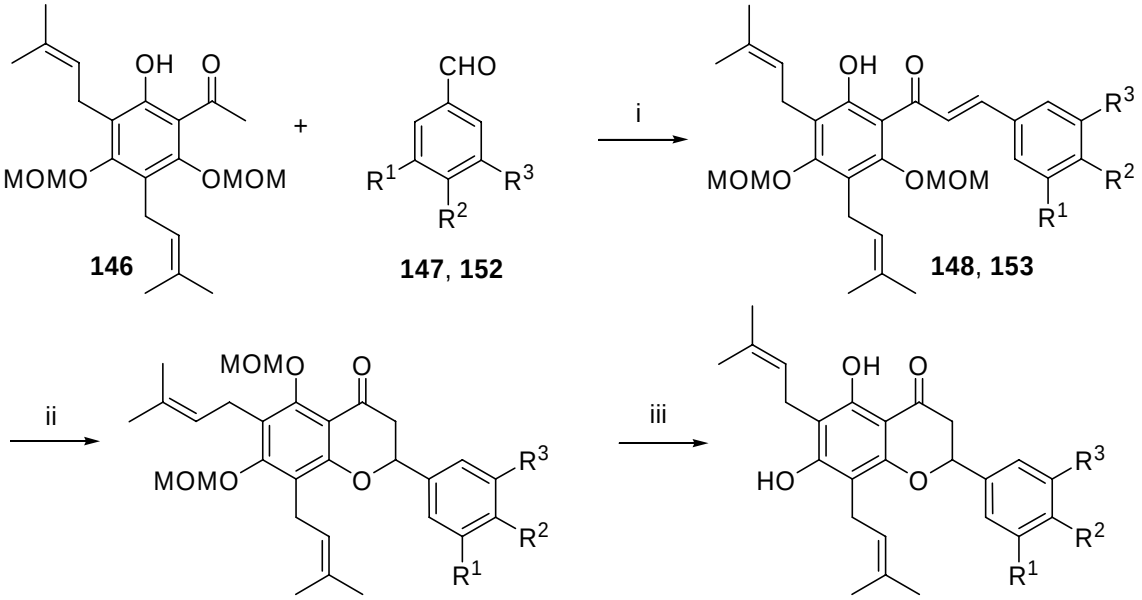
3.2. Syntheses of *C*-prenylated flavanones

3.2.1. Synthesis of (±)-lonchocarpol A, monotesone B and their analogues

Besides the *O*-prenylated flavanones such as (±)-selinone [(±)-**85**] and (-)-monotesone A [(-)-**86**], two *C*-prenylated derivatives, namely (±)-lonchocarpol A [(±)-**87**] and (±)-monotesone B [(±)-**88**], have been also isolated from *Monotes engleri* by Hostettmann and coworkers [84]. Since antifungal activity of lonchocarpol A was also published [92] it seemed reasonable to synthetize it and its analogues for the study of their structure-activity relationship.

Phloracetophenone and the corresponding MOM-protected benzaldehyde derivatives **147**, **152** were the starting materials. Phloracetophenone was *C*-prenylated under conditions developed by Xiao [103] and then partially protected by methoxymethylation (**146**). The Claisen-Schmidt condensation of **146** with benzaldehyde derivatives **147**, **152** gave the corresponding chalcones **148**, **153** whose ring-closure took place smoothly. Since the protecting groups of the resulted flavanones **149**, **154** could not be cleaved in acidic condition without destroying the molecules, BF_3•Et_2O and Me_2S system was used. After deprotection, (±)-lonchocarpol A [(±)-**87**] and (±)-monotesone B [(±)-**88**] could

be isolated in pure form whose spectroscopic data were in good agreement with those of the natural products.



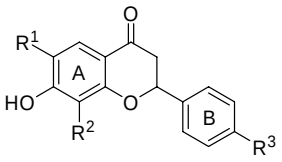
	R1	R2	R3
147	H	OMOM	H
148	H	OMOM	H
149	H	OMOM	H
152	OMOM	H	OMOM
153	OMOM	H	OMOM
154	OMOM	H	OMOM

	R1	R2	R3
87	H	OH	H
88	OH	H	OH

i: ethanol, 50% KOH-sol, 0 °C ii: NaOAc, ethanol-water/Δ; iii: BF_3•OEt_2, Me_2S, dry CH_2Cl_2

Scheme 4

Most of the *C*-prenylated analogues (**155-160**) used for pharmacological study are also naturally occurring flavanone derivatives, whose syntheses were carried out in a very similar manner discussed above starting from resacetophenone and the corresponding benzaldehyde derivatives.



155-160	Common name	R^1	R^2	R^3
155	bavachin	Pre	H	OH
156	isobavachin	H	Pre	OH
157	-	Pre	Pre	OH
158	-	Pre	H	H
159	Ovaliflavanone B	H	Pre	H
160	Ovaliflavanone A	Pre	Pre	H

Scheme 5: Analogues of lonchocarpol A and monotesone B

3.3. Results of pharmacological study

Hostettmann et al. found that the most active component of *Monotes engleri* against *Candida albicans* was (±)-selinone [(±)-**85**] (MIC: 10 μg/ml), and (-)-monotesone A [(-)-**86**] as well as monotesone B [(±)-**88**] showed much lower activity (MIC: 20 μg/ml, 50 μg/ml, respectively).

Synthetic *O*- and *C*-prenylated flavanone derivatives (**85-88**) and their analogues (**116-120**, **155-160**) were tested against different *Candida* species. The results of these examinations were summarized in Table 2 which clearly shows only (±)-selinone [(±)-**85**] and (±)-monotesone A [(±)-**86**] possess significant antifungal activity against *Candida albicans* (ATCC 10231). The *C*-prenylated flavanone derivatives, (±)-lonchocarpol A [(±)-**87**], monotesone B [(±)-**88**] and their analogues (**155-160**) did not show any antifungal activity. Tests of *O*-prenylated flavanone analogues clearly showed that the structure of selinone is responsible for the fungistatic activity because any modification of this structure led to the total loss of the fungistatic activity.

Table 2: Study of the biological activity of nystatine, fluconazole, and analogues (**116**-**120**) of (±)-selinone [(±)-**85**], (±)-monotesone A [(±)-**86**] by agardiffusion method. (Inhibition zone: mm, concentration: 100 μg/mL)
Ca1 = *Candida albicans* (14053), Ca2 = *Candida albicans* (ATCC 10231), Ci = *Candida inconspicua*, Cd = *Candida dubliniensis* and Ck = *Candida krusei*.

Code	Ca1	Ca2	Ci	Cd	Ck
nystatine	27	**28**	26	28	27
fluconazole	22	**23**	24	23	22
85	0	**12**	0	0	0
86	0	**10**	0	0	0
116	0	0	0	0	0
117	0	0	0	0	0
118	0	0	0	0	0
119	0	0	0	0	0
120	0	0	0	0	0