Theses of doctoral (PhD) dissertation

Synthesis of potentially biologically active pterocarpan derivatives

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1. Introduction and objectives

Flavonoids are one of the largest and the most important group of naturally occurring O-heterocycles. According to the more general terminology, O-heterocyclic natural products possessing a diphenylpropane skeleton (C₆-C₃-C₆) and their related open chain derivatives are considered to belong to this family of organic compounds. In the past few decades, numerous and diverse pharmacological investigations were carried out which confirmed that flavonoids possess antibacterial, antiviral, antifungal, antiinflammatory, diuretic, anti-tumor and antiosteoporotic activity above their well-known antioxidant activity.

The aim of my dissertation was to synthetise 7-isopropyloxyisoflavone (Ipriflavone (1a))’s analogues, such as pterocarpan (1b) and its related derivatives (1c,d) of potential antiosteoporotic activity in racemic and optically pure form and to study their stereochemistry.

As a continuation of our efforts on their enantioselective synthesis, the aim of my research was also to study of oxidative transformation of enol methyl ether and enol acetate of racemic flavanone [(±)-189, 205] with thallium(III) nitrate (TTN), lead(IV) tetraacetate (LTA) and hypervalent phenyliodines (PIDA and HTIB) as well.

2. Applied methods

The macro-, semi-micro, and micro-methods of the modern preparative organic chemistry were used in the synthetic work. The purity of the
substances, the ratio of products were controlled and the reactions were monitored by thin-layer chromatography. Purification of the crude products and separation of the isomers were carried out either by crystallization, or by column chromatography. The characterisation and the structural elucidation of the compounds were obtained by determination of their melting point, one and two-dimensional (1H-1H-COSY, 13C-1H-HSQC, HMBC, NOESY) NMR spectroscopy, and MALDI/ESI-TOF mass spectrometry and by analytical RP-HPLC methods.

3. New scientific results of the dissertation

3.1. Synthesis of pterocarpan derivatives of potential antiosteoporotic activity

In order to study the antiosteoporotic activity of pterocarpans, the synthesis of a series of this type of compounds were achieved from appropriately substituted 2H-chromenes by Heck-type oxyarylation as well as from racemic 3-hydroxy-9-methoxy pterocarpan [(±)-136c] prepared from 7,2’-dihydroxy-4’-methoxyisoflavone (140) by the reduction of sodium tetrahydroborate followed, by a ring closure performed under acidic conditions at room temperature.

3.1.1. Synthesis of racemic pterocarpan [(±)-13], pterocarpin [(±)-41], 3-methoxypterocarpan [(±)-56c], 3-isopropylxysterocarpan [(±)-136a] and 3-isopropyl-oxy-8,9-methylendioxypterocarpan [(±)-136b].

The Heck-oxyarylation of 2H-chromenes (70a,b,e) with ortho-mercurphenol derivatives (79a,b) were prepared as described by Inoue et al. and the crude products have been purified by column chromatography to give the corresponding racemic pterocarpan derivatives [(±)-13, 41, 56c,
136a, 136b] in low and moderate yield (Scheme 1).

It should be noted that in good agreement with our previously results, the arylation of 2H-chromenes (70a, b, e) took place in a non-regioselective manner resulting in the bridged O-heterocycles [(±)-137a-e] due to cationic mechanism of this transformation besides racemic pterocarpan derivatives [(±)-13, 41, 56c, 136a, 136b].

\[
\text{Scheme 1: Synthesis of (±)-13, 41, 56c, 136a, b pterocarpan derivatives.}
\]


The synthesis of racemic 3-hydroxy-9-methoxypterocarpan [(±)-136c] has been carried out from 2,4-dihydroxyphenyl-2,4-dimethoxybenzylketone (138) via 7-hydroxy-2',4'-dimethoxyisoflavone (139) in four steps (Scheme 2).

The critical step of this synthesis has been found to be the selective cleavage of 2'-methoxy group of isoflavone 139 with aluminum trichloride (139→140). Its success was strongly depended on the complexation of the reagent at the carbonyl group, which could be affected by the carefully
dried acetonitrile used as solvent in this reaction. In the next step, the reduction of 140 was performed by sodium-tetrahydroborate in the mixture of THF and EtOH at room temperature to result in the sodium salt of isoflavan-4-ol [(±)-141], which after removal of EtOH, was followed by its treatment with hydrochloric acid giving rise to racemic 3-hydroxy-9-methoxypterocarpan [(±)-136c] in good yield.

![Scheme 2: Synthesis of (±)-136c pterocarpan derivative.](image)

For the study of structure-oestrogen activity relationship of pterocarpans, the isopropyl-, ethyl- and n-propyl derivatives (±)-136d-f have been also prepared.
3.1.3. Synthesis of 5-carba- [(±)-148a-d] and -azapterocarpan [(±)-159a,b].

Based on the examples published in the literature of medicinal chemistry, it is well-known that the similarity of the pterocarpan to the natural ligands of estrogen receptors can be increased by the replacement of their oxygen atom at position 5 with a methylene group. Therefore, (±)-148a-d 5-carbapterocarpan were also prepared from the appropriately substituted 1,2-dihydronaphthalene (147a-c) and ortho-mercurphenol derivatives (79a,b) using Heck-oxyarylation reaction, respectively.

![Scheme 3: Synthesis of (±)-136d-f pterocarpan derivatives.]

Interestingly, in the course of this reaction not only the ring closure leading to rac-148a-d and rac-149a,b took place, but in case of the 147b,c
1,2-dihydronaphthalene derivatives, a β elimination (147b,c+79a→149a,b) could be also observed. Moreover, the formation of the so-called O-bridged compounds (e.g 137a, where O-5= CH2) could not be detected at all. These facts have also clearly indicated, that the Heck-oxyarylation of 147b,c naphtalene derivatives took place regioselectively with a cationic mechanism.

For the synthesis of 6-aza analogues [(±)-159a,b], the corresponding protected 1,2-dihydroquinoline derivative (158) has been prepared starting from m-anisidine and methyl-acrylate in 7 steps. Surprisingly, its coupling with ortho-mercuryphenol (79a) under the conditions of Heck-oxyarylation did not lead to the rac-159a aza-pterocarpan derivative but only the formation of the O-bridged compound [(±)-160] could be observed in 26% yield.

In the case of the 79b mercuryphenol, only the formation of (±)-159b azapterocarpan derivative could be detected in low yield (20%).

3.1.4. Attemps for enantioselective synthesis of pterocarpans.
In order to have a clear correlation between the structure and antiosteoporotic activity of pterocarps discussed above, their synthesis in optically pure form has been also carried out. It seems to be quite obvious that they can be obtained from the corresponding substituted (+)-(2R)- or (2S)-2'-benzyloxyflavanone derivatives using the method recently developed by our research group. In the course of the synthesis of (+)-(6αS,11αS)-maackiain (14c) isolated from Maackia amurensis, and its 3-deoxy derivatives (14a), the oxidation of the racemic 2'-benzyloxy-6,7-methylenedioxyflavanone [(±)-163b] was performed with thallium(III) nitrate (TTN) or phenyl iodosonium diacetate (PIDA) under the conditions reported by Kapoor et al. and Prakash and Tanwar, respectively. Surprisingly, the reaction had an unexpected outcome and the predicted ring contraction [(±)-163b→(±)-164] did not occur, although it was believed that the electron-donating methylenedioxy group attached to C-6 and C-7 increased the migratory aptitude of this aryl group. Instead of the trans-2,3-dihydrobenzo[b]furan ester [(±)-164], the 2'-benzyloxy-6,7-methylenedioxy-isoflavone (165) could be isolated by repeated column chromatography in crystalline form with 47% yield as a sole product.

In order to get information about the reason of this unusual transformation the racemic 2'-benzyloxy-6,7-dimethoxyflavanone [(±)-176] and 6-methoxy-2'-benzyloxyflavanone [(±)-181] have been prepared and examined how these compounds will be reacted with TTN or PIDA.
Similarly to 2′-benzylxy-6,7-methylenedioxyflavanone [(±)-163b], the transformation of its 6,7-dimethoxy analogue [(±)-176] did not result in the ring-contracted product [(±)-177] either by TTN or by PIDA, but the corresponding isoflavone (178) and flavone (179) derivatives were formed in 21/1- and 1/32% yield, respectively.

Moreover, the oxidation of 2′-benzylxy-6-methoxy-flavanone [(±)-181], with TTN resulted in (±)-182 trans-2,3-dihydrobenzo[b]furan ester as a major product besides the 183 isoflavone and 184 flavone derivatives. In the case of PIDA, only 184 flavone derivative could be isolated in 51% yield.

Oxidation of 2′-benzylxy-6-nitroflavanone [(±)-187] with TTN or PIDA resulted in 2′-benzylxy-6-nitroisoflavone (188) in low yield (10-
These results have clearly indicated that the product’s profile of the transformation of 2’-benzyloxyflavanones by TTN or PIDA was strongly dependent on their substitution of ring A and therefore this method was not suitable for the synthesis of naturally occurring pterocarps possessing hydroxy, methoxy, or methylenedioxy group at C-8 or/and C-9.

In order to have further insight into this process and reveal the reasons of these surprising results, we examined the so-called “basic reaction” – the ring-contraction of enol ether of racemic flavanone [(±)-189] – to racemic methyl 2-phenyl-2,3-dihydrobenzo[b]furan-3-carboxylate [(±)-191a] by TTN monitored by HPLC. Thus, our research group have recently shown that this transformation took place stereoselectively resulting in the trans-[(±)-191a] and thus the carbonium ion 204 did not play a role as an intermediate of this process.

3.2. Reinvestigation of the ring-contraction of flavanone [(±)-6]

3.2.1. Ring-contraction of enol methyl ether of racemic flavanone [(±)-189]

The transformation of racemic flavanone [(±)-6] has been performed with 1.1 mol equivalent TTN in TMOF in the presence of catalytic amount of 70% perchloric acid at room temperature monitored by HPLC. It has been clearly indicated that the conversion of (±)-6 reached 98% in 30 minutes to result in a complex mixture.
Many components of this mixture could be nearly base-line separated and besides rac-flavanone [(±)-6, (2%)], rac-methyl 2,3-dihydro-2-phenylbenzo[b]furan-3-carboxylate [(±)-191a (76%)], flavone [4 (3%)] and isoflavone [8 (9%)] could be identified unequivocally by comparison with authentic samples (entry 1 in Table 1).

**Scheme 10.** Ring contraction of (±)-6 flavanone.

**Table 1.** Yields and retention times of the compounds of the transformation of flavanone [(±)-6].

<table>
<thead>
<tr>
<th>Entry</th>
<th>React.</th>
<th>Proc.</th>
<th>6 (7.20)</th>
<th>191a (7.90)</th>
<th>191b (7.20)</th>
<th>4 (6.50)</th>
<th>192a (6.90)</th>
<th>192b (7.00)</th>
<th>8 (6.70)</th>
<th>197 (6.93)</th>
<th>202 (6.14)</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>TTN</td>
<td>A</td>
<td>2</td>
<td>76</td>
<td>-</td>
<td>3</td>
<td>0.5</td>
<td>1</td>
<td>9</td>
<td>3</td>
<td>&lt;1</td>
</tr>
<tr>
<td>2</td>
<td>TTN</td>
<td>B</td>
<td>15</td>
<td>40</td>
<td>-</td>
<td>26</td>
<td>-</td>
<td>1</td>
<td>2</td>
<td>9</td>
<td>&lt;1</td>
</tr>
<tr>
<td>3</td>
<td>TTN</td>
<td>C</td>
<td>&lt;1</td>
<td>145</td>
<td>-</td>
<td>33</td>
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<td>3</td>
<td>3</td>
<td>&lt;1</td>
</tr>
<tr>
<td>4</td>
<td>LTA</td>
<td>D</td>
<td>1</td>
<td>33</td>
<td>-</td>
<td>18</td>
<td>13</td>
<td>18</td>
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<td>1</td>
<td>-</td>
</tr>
<tr>
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<td>PDDA</td>
<td>E</td>
<td>4</td>
<td>66</td>
<td>-</td>
<td>10</td>
<td>&lt;1</td>
<td>-</td>
<td>2</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>HTIB</td>
<td>E</td>
<td>3</td>
<td>49</td>
<td>-</td>
<td>6</td>
<td>2</td>
<td>-</td>
<td>1</td>
<td>5</td>
<td>-</td>
</tr>
</tbody>
</table>

**Procedure A:** TMOF/70% HClO4/r. t./5 min TTN  
**Procedure B:** TMOF/70% HClO4/-10°C/5 min TTN  
**Procedure C:** TMOF/70% HClO4/r. t./30 min TTN  
**Procedure D:** TMOF/conc. H2SO4/r. t./5 min LTA  
**Procedure E:** TMOF/conc. H2SO4/r. t./5 min HTIB
Moreover, the compound with $t_R = 8.95$ min could be also isolated by preparative TLC and it has been identified as methyl 2-phenylbenzo[b]furan-3-carboxylate (197) by its NMR and MS data. Its structure was also confirmed by chemical correlation with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) starting from (±)-191a derivative.

![Scheme 11. Synthesis of 197 derivative with DDQ.](image)

In the reaction of (±)-6 carried out at -10°C, the transformation to the 189 enol ether took place slower as expected. The conversion of (±)-6 reached only to 85% in 30 minutes, and a considerable change in the products’s profile could be observed (entry 2 in Table 1). Moreover, the by-product at $t_R = 8.14$ min could be also isolated by preparative TLC. Its structure has been established by NMR and MS evidences as the methyl 2,3-dihydro-2-methoxy-3-phenylbenzo[b]furan-3-carboxylate with (2$S^*$,3$S^*$) relative configuration [(±)-202].

![Image](image)

The HPLC monitoring of the transformation of racemic flavanone by LTA was also carried out. The conversion reached to 99% in 2 hours and the formation of (±)-191a (33%), 4 (11%), 192a (1%), 192b (18%), 8 (23%) és 197 (1%) could be detected. (entry 4 in Table 1). In contrast to the observation of Khanne, the formation of cis-(±)-191b could not be observed by at all.

The transformation of (±)-6 flavanone by PIDA or HTIB in TMOF and
catalytic sulfuric acid was also studied at room temperature. Their HPLC monitoring has clearly shown that the transformations took place significantly slower in both cases than with TTN and their product’s profiles were similar but significantly different from that obtained by TTN. The ring contraction of (+)-6 by PIDA led to trans-(±)-191a (66%) as the main product and instead of the formation of isoflavone (8), the formation of flavone (4) was favoured as side-product (entry 5 in Table 1). This fact could be explained by quantum chemical calculations, which gave important informations about the structure of the (±)-190c intermedier (190c/a és 190c/b).

As shown in the Scheme 12, the flavone (4) was formed from the thermodynamically more stable phenyliodosonium(III)-intermedier [(±)-190c/a] and at the same time 190c/b-one might be transformed via 204 carbonium or 204 epoxonium ion into trans-(±)-191a ester and isoflavone (8). Thus the HPLC monitoring of the transformation of rac-flavanone [(±)-6] has clearly shown that only the trans-(±)-191a ester was present in its crude product, the formation of the cis-one [(±)-191b] in traces could not be detected, therefore the heterolytic cleavage of C-3 and iodosonium(III) bond of (±)-190c/a or (±)-190c/b resulting in 203 carbonium ion could be
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disclosed with certainly. Instead of this carbonium ion the 204 epoxonium ion was formed by a SN2-type process, which has indicated the neighbouring group participation of C-4 methoxy group must play a determining role in this transformation.

3.2.2. Ring-contraction of enol acetate of racemic flavanone [(±)-205]

In order to examine the role of neighbouring group participation in the ring-contraction, the transformation of enol acetate of racemic flavanone [(±)-205] prepared from (±)-6 according to the literature was reacted with 1,1 mol equivalent TTN in TMOF in the presence of catalytic amount of 70% perchloric acid at room temperature. The HPLC monitoring of this reaction has clearly indicated that the conversion of (±)-205 reached 99% in 30 minutes to result in a mixture of products shown in Table 2 (entry 1).

All components of this mixture could be nearly base-line separated and besides rac-flavanone [(±)-6 (1 %)], its enol-acetate [(±)-205 (1 %)], isoflavone [8 (78 %)], flavone [4 (13 %)] could be identified. Surprisingly rac-methyl 2,3-dihydro-2-phenylbenzo[b]furan-3-carboxylate [(±)-191a], cis-3-methoxyflavanone (192), phenylbenzo[b]furan derivatives 197 and 202 did not formed at all. (entry 1 in Table 2).

<table>
<thead>
<tr>
<th>Entry</th>
<th>Reag.</th>
<th>Proced.</th>
<th>6 (7.20)</th>
<th>4 (6.10)</th>
<th>8 (6.65)</th>
<th>205 (7.76)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>TTN</td>
<td>A</td>
<td>2</td>
<td>18</td>
<td>77</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>PIDA</td>
<td>B</td>
<td>7</td>
<td>13</td>
<td>78</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>PIDA</td>
<td>C</td>
<td>4</td>
<td>65</td>
<td>1</td>
<td>15</td>
</tr>
</tbody>
</table>

Procedure A: TMOF/70%-os HClO₄/r. t./5 min TTN  
Procedure B: TMOF/conc. H₂SO₄/r. t./5 min PIDA  
Procedure C: Glacial acetic acid/5 min PIDA

Table 2.: Yields and retention times of the compounds of the transformation of enol acetate [(±)-205].

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Very similar product’s profile could be observed when PIDA was used as oxidizing agent. Besides racemic flavanone [(±)-6] and flavone (4), isoflavone (8) could be detected as a main product (entry 2 in Table 2).

These results have very clearly indicated that the substitution of the methyl group of (±)-189 with the acetyl one has prevented the ring-contraction (±)-205 to (±)-191a, but the formation of isoflavone (8) was strongly preferred in both cases. This observation could be explained by the neighboring group participation of acetoxy and methoxy groups at C-4 of (±)-206a,b intermediers formed from (±)-205 by TTN or PIDA, as depicted in Scheme 13.

Scheme 13.: The transformation of (±)-205 enol acetate in TMOF by PIDA.

The HPLC monitoring of the transformation of (±)-205 by PIDA in glacial acetic acid at room temperature gives also some interesting information about the feature of this reaction. The conversion of (±)-205 reached 85% in 2 hours and the flavone (4) as a main product was obtained in 65% yield, besides traces of isoflavone [8 (1%)], flavanone [(±)-6 (4%)] and enol acetate [(±)-205 (15%)] shown in Table 2 and in Scheme 14.
Scheme 14.: The transformation of (±)-205 enol acetate in glacial acetic acid by PIDA.

In the absence of methanol, the triacetoxy-phenyliodosonium intermediate (212) formed in the course of the stereo-controlled addition of the electrophilic reagent (PIDA) to (±)-205. In the next step, its transformation by the neighboring participation of its acetoxy group of α configuration in S_N2-type manner gave 213a 1,3-dioxolanium ion in equilibrium 213b. The deprotonation of 213a resulted in 4,4-diacetoxy-4H-flavene (214) whose hydrolysis afforded flavone (4) as a main product (65%) during the workup of reaction mixture. Although this equilibrium of 213a-213b is strongly shifted towards 213a the small amount of isoflavone (7) could be also formed by 2→3 phenyl migration followed by a deprotonation and hydrolysis (213b→210→8). This has been observed experimentally indeed (entry 3 in Table 2).

It is noteworthy that the transformation of flavanone enol acetate (205) by TTN or PIDA in TMOF in the presence of catalytic amount of 70% perchloric acid has revealed a convenient and new approach to the synthesis of isoflavone (8). While the transformation of 205 by PIDA in glacial acetic acid has discovered a new simple route to flavone (4).
4. Publikációk jegyzéke/List of Publications

4.1. Az értekezés alapjául szolgáló közlemények


4. **Németh, I.;** Kiss-Szikszai, A.; Mándi, A.; Komáromi, I.; Kurtán, T.; Antus, S.; "Oxidation of Enol-acetate of Flavonone with Thallium(III) nitrate or Phenyliodosonium Diacetate: Convenient Routes to Isoflavone or Flavone", (beküldve)

4.2. Konferencia előadások a dolgozat témájában

1. **Németh István,** Antus Sándor, Gulácsi Katalin, Kéki Sándor, Zsuga Miklós: Észrevételek a 2’-benziloxiflavanonok gyűrűszűkítési átalakításáról, Flavonoid Munkabizottsági Ülés, 2006. december 1, Budapest

2. **Németh István,** Antus Sándor, Gulácsi Katalin: 2’-Benziloxiflaanonok gyűrűszűkítési átalakításának vizsgálata, Flavonoid Munkabizottsági Ülés, 2008. okt. 20, Debrecen


4.3. Konferencia poszterek az értekezés témájában


3. **Németh István, Antus Sándor**: 2'-Benziloxiflavanonok gyűrűszűkítési reakciójának tanulmányozása, Országos Vegyészkonferencia, 2008. jún. 19-21, Hajdúszoboszló

4. **Németh István, Kiss Attila, Mándi Attila, Antus Sándor**: Flavanon gyűrűszűkülési reakciójának felülvizsgálata, Országos Vegyészkonferencia, 2010. jún. 30- júl. 2., Hajdúszoboszló
