CHARLES UNIVERSITY

FACULTY OF PHARMACY IN HRADEC KRÁLOVÉ

DEPARTMENT OF PHARMACEUTICAL CHEMISTRY AND PHARMACEUTICAL ANALYSIS

Synthesis of isoprenoid naringenin derivatives

DIPLOMA THESIS

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Supervisor: prof. PharmDr. Petr Zimčík, Ph.D.

Consultant supervisor: Hubert Chapuis, Ph.D.

HRADEC KRÁLOVÉ 2020

"I declare that this thesis is my original work. All literature and other resources I used while processing is listed in the bibliography and properly cited. This thesis was not used for obtaining the same or different academic degree."

At first, I would like to thank to my supervisor prof. Petr Zimčík for his leadership through my studies. Many thanks to assoc. prof. Iva Boušová for getting in contact with the research team in France. My greatest thanks belong to dr. Hubert Chapuis for leading the research in France as well as for taking care of me during my stay abroad.

Abstrakt

Univerzita Karlova, Farmaceutická fakulta v Hradci Králové

Katedra	Katedra farmaceutické chemie a farmaceutické analýzy
Kandidát	**Jakub Kernal**
Školitel	**prof. PharmDr. Petr Zimčík, Ph.D.**
Konzultant	**Hubert Chapuis, Ph.D.**
Název diplomové práce	Syntéza isoprenoidních derivátů naringeninu

Naringenin, flavonoid ze skupiny flavanonů, a jeho deriváty jsou v současnosti předmětem intenzivního výzkumu pro své antibakteriální, antimykotické a cytotoxické účinky. Díky nejnovějším metodám organické chemie je možno uspokojit zvýšenou poptávku po těchto molekulách prostřednictvím syntézy těchto přírodních látek.

Cílem této práce byla syntéza a fyzikálně-chemická charakteristika derivátů naringeninu se zvýšenou lipofilitou. Tyto deriváty budou poté dále předmětem výzkumu a to především díky své potenciální cytotoxické aktivitě.

Při procesu získávání lipofilních derivátů naringeninu byly v této práci použity celkem dva přístupy. (Fig. 1) Modifikace molekuly naringeninu pomocí terpenoidní části, která by měla zlepšit prostup těchto látek do buněk, byla realizována zaprvé esterifikací hydroxylové skupiny flavonoidu v poloze 4′, nebo esterifikací hydroxylových skupin na obou kruzích flavonoidu A a B. Cílem druhého syntetického přístupu byla modifikace vazby uhlík-uhlík na kruhu A flavonoidu.

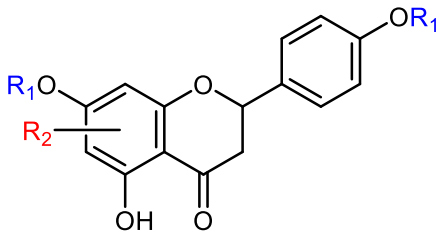

Fig. 1. Modifikace molekuly naringeninu – esterifikace (modrá) nebo tvroba vazby uhlík-uhlík (červená)

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Abstract

Charles University, Faculty of Pharmacy in Hradec Králové

Department	Department of Pharmaceutical Chemistry and Pharmaceutical Analysis
Candidate	**Jakub Kernal**
Supervisor	**prof. PharmDr. Petr Zimčík, Ph.D.**
Consultant	**Hubert Chapuis, Ph.D.**
Title of Thesis	Synthesis of Isoprenoid Naringenin Derivatives

Naringenin as a member of flavanone subclass of flavonoids and its derivatives have been subjects of intensive study for their antibacterial, antifungal, and antineoplastic properties. Recent development in current methods of synthetic chemistry allows us to satisfy the increasing demand for these molecules by providing an alternative source of these naturally occurring substances in the means of chemical synthesis.

The aim of this work was synthesis and characterization of naringenin derivatives with increased lipophilic profile which will serve in future research as potential cytotoxic agents.

Two major approaches have been developed in the process of obtaining lipophilic derivatives of naringenin (Fig. 2) where the lipophilic moiety consists of terpene or terpenoid part, which is believed to increase the uptake of the desired product by cells. In the first approach, we focused on the modification of the B phenolic ring by esterification of the hydroxyl group on the 4′ carbon or on the modification of both A and B phenolic rings. The second approach was aimed on synthesis of prenylflavonoids by creating a carbon-carbon bond on the A phenolic ring.

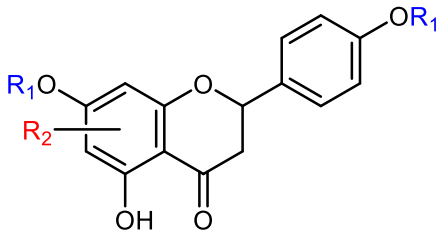

Fig. 2. Modifications to the naringenin molecule – esterification (blue) or creation of carbon-carbon bond (red).

List of abbreviations

DCC	*N*,*N*′-dicyclohexylcarbodiimide
DMAP	4-dimethylaminopyridine
MeOH	methanol
NBS	*N*-bromosuccinimide
NMR	nuclear magnetic resonance
TLC	thin-layer chromatography
UV	ultraviolet

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1 Aim of the Work

Flavonoids are plant pigments, class of secondary metabolites that have been subject to multiple studies regarding their indispensable influence on human health, mainly for their antioxidant, antibacterial and cytotoxic properties. Naringenin, flavanone type of flavonoids, commonly found in citrus fruits, and other naringenin derivatives have been researched in the past for their antineoplastic activity.

The aim of this work is synthesis of isoprenoid naringenin derivatives and their characterization by nuclear magnetic resonance (NMR) spectroscopy.

The isoprenoid modification of the flavonoid aims at increasing cell uptake and consists of two approaches (Fig. 3). The first approach aims at esterification of the hydroxyl functions present at the molecule; the purpose of the second approach is to create carbon-carbon bonds.

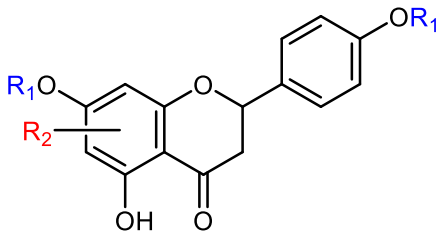


Fig. 3. Modifications to the naringenin molecule – esterification (blue) or creation of carbon-carbon bond (red).

Finally, the syntheses, if successful, will provide material for biological testing in order to confirm any cytotoxic activity of the flavonoid derivatives.

2 Theoretical Part

2.1 Terpenes and terpenoids

Terpenes and terpenoids are vastly diverse class of molecules of natural origin,[1] commonly used together with their derivatives as medicines, fragrances, or commercial flavors. They are known for their clinical usage as anticancer and antimalarial drugs, they are active against bacteria, protozoa, and fungi, and have been studied for their antiviral effects as well.[2]

2.1.1 Structure and classification

Structure of terpenes is characterized by their 5-carbon basic building block isoprene.[3] Main terpene classes (Fig. 4) include hemiterpenes (C_5), monoterpenes (C_{10}), sesquiterpenes (C_{15}), diterpenes (C_{20}), triterpenes (C_{30}) and tetraterpenes (C_{40}). Terpene derivatives that are modified by addition of functional groups, typically oxygen atoms are named terpenoids and include functions such as alcohols, aldehydes, ketones, esters, ethers, and peroxides.[4, 5] Terpenes can also be further characterized by number of cycles as acyclic, monocyclic, and bicyclic.

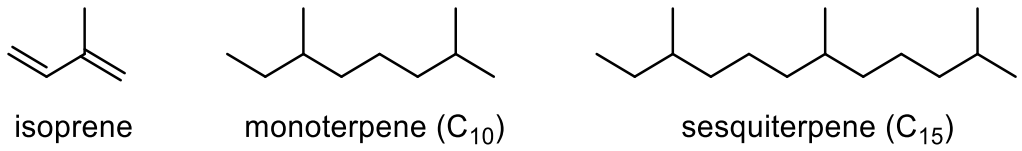


Fig. 4. Basic building block of terpenes. Isoprene with carbon backbones of the two most common terpene classes - monoterpenes and sesquiterpenes.

2.1.1.1 Monoterpenes

Monoterpenes consist of two isoprene units. Common examples of acyclic monoterpenes are citronellol, geraniol and nerol (Fig. 5). For the group of monocyclic terpenes (Fig. 6), the common examples would be limonene, cymene, pulegone. The group of bicyclic monoterpenes (Fig. 7) could be characterized by compounds such as camphor, pinene or α-thujene.[3, 6]

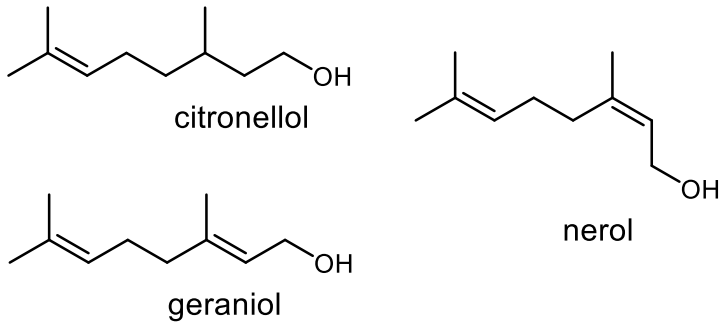


Fig. 5. Examples of acyclic monoterpenes.

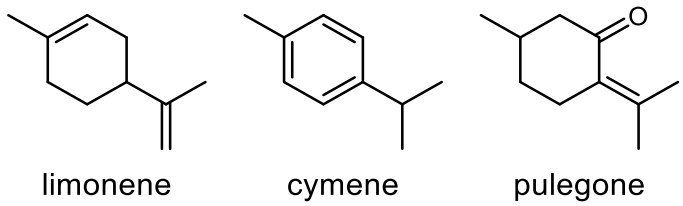


Fig. 6. Examples of monocyclic monoterpenes.

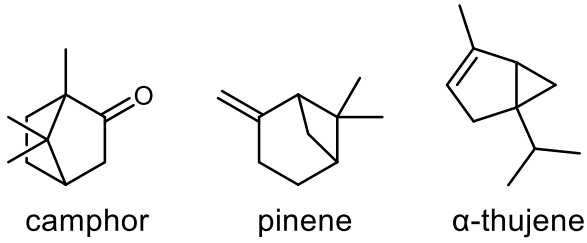
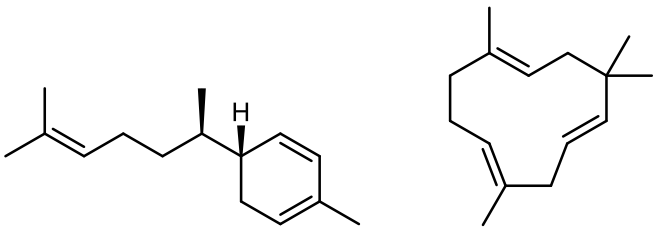


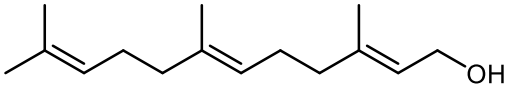
Fig. 7. Examples of bicyclic monoterpenes.

2.1.1.2 Sesquiterpenes

Sesquiterpenes are characterized by three isoprene units. The extension of the polycarbonic chain allows for greater structural diversity and increases the number of cyclic compounds.[4] Commonly known example of acyclic sesquiterpene is farnesol, cyclized examples include compounds such as zingiberene and humulene (Fig. 8).



zingiberene humulene



farnesol

Fig. 8. Examples of sesquiterpenes. Acyclic farnesol and cyclic terpenes zingiberene and humulene.

2.1.2 Biological properties of selected terpenes

Terpenes can function as pigments, hormones, component of signal transduction pathways or as defensive agents against pathogens.[7] Most terpenes possess minor antimicrobial activities. Some known terpenoids are also anticancer or antimalarial drugs.[2, 5] In terpenoids, these activities are even more abundant with modifications of functional groups – for example, the presence of hydroxyl group in phenolic terpenoids and the presence of delocalized electrons are determining factors for increased antimicrobial activity.[3]

2.1.2.1 Biological properties of squalene

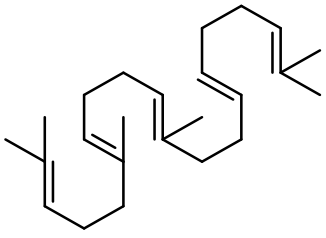


Fig. 9. Structure of squalene.

Squalene (Fig. 9) is polyunsaturated triterpene, containing six isoprene units in total. Naturally, squalene acts as precursor of cholesterol synthesis. It is frequently used to prepare emulsions, which can deliver vaccines and various drugs.[6] Squalene can be used either as a drug carrier or as a squalene-drug conjugate. This formation allows to prolong release of target drug or its

activity.[8] Squalene itself is then believed to possess antibacterial and antifungal activity as well as photoprotective[9] and radioprotective[10] properties. It has been shown that squalene functions as protective agent and decreases chemotherapy-induced side effects.[6]

2.1.2.2 Taxanes

Natural occurring diterpenoid paclitaxel and taxoid derivatives docetaxel and cabazitaxel (Fig. 10) have been in clinical use as potent chemotherapy agents. The mechanism of action lies in promotion of tubulin polymerization in cells, which affects mitotic spindle during cell division. The microtubules formed in the presence of taxanes are extraordinary stable and therefore dysfunctional, causing the death of the cell by disrupting the mitosis, and therefore the cell division. Furthermore, paclitaxel is also known to induce the expression of the gene for tumor necrosis factor α. Finally, the binding site of taxanes to tubulin differs from the binding sites of other anti-mitotic drugs. Taxanes bind to the *N*-terminal amino acids of the beta-tubulin subunit, rather than to tubulin dimers.[11]

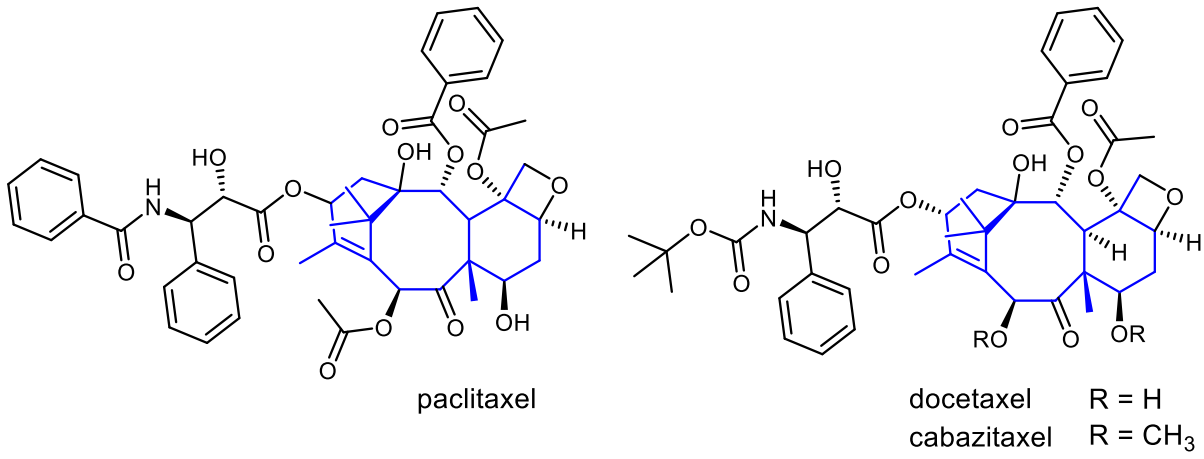


Fig. 10. Structures of paclitaxel, docetaxel and cabazitaxel with highlighted diterpenoid backbone (in blue).

2.2 Flavonoids

Flavonoids are plant pigments of terpenoid origin, that have been investigated for their *in vitro* antibacterial, antiviral and antioxidant properties.[12] Multiple studies also report their involvement in carcinogenesis, mainly by ability to block cell cycle, induce apoptosis,[13] or by ability to disrupt mitotic spindle formation.[14] Modulatory effect of flavonoids on xenobiotic-metabolizing hepatic and intestinal enzymes that are responsible for activation of environmental as well as food carcinogens have been reported as well.[15] In conclusion, all these findings suggest that flavonoids and their derivatives might perform as promising candidates in future research, especially as anticancer agents. Although antioxidant properties of flavonoids may seem at first glance contradictory to their cytotoxic properties and the indirect activation of carcinogens might theoretically antagonize the inhibitory effects on carcinogenesis, the broad structural variety of phenolics and knowledge of structure-activity relationship allows to target compounds with the desired attributes only.

2.2.1 Structure and classification

Flavonoids are members of the C_6-C_n-C_6 class of phenolic compounds. The C_6-C_n-C_6 class can be further divided by the total number of carbon atoms in the linker chain, therefore flavonoids (n = 3) share their structure similarities with xanthonoids (n = 1), stilbenoids, anthrones and anthraquinones (n = 2) and with diaryl heptanoids, also known as curcuminoids (n = 7)[16].

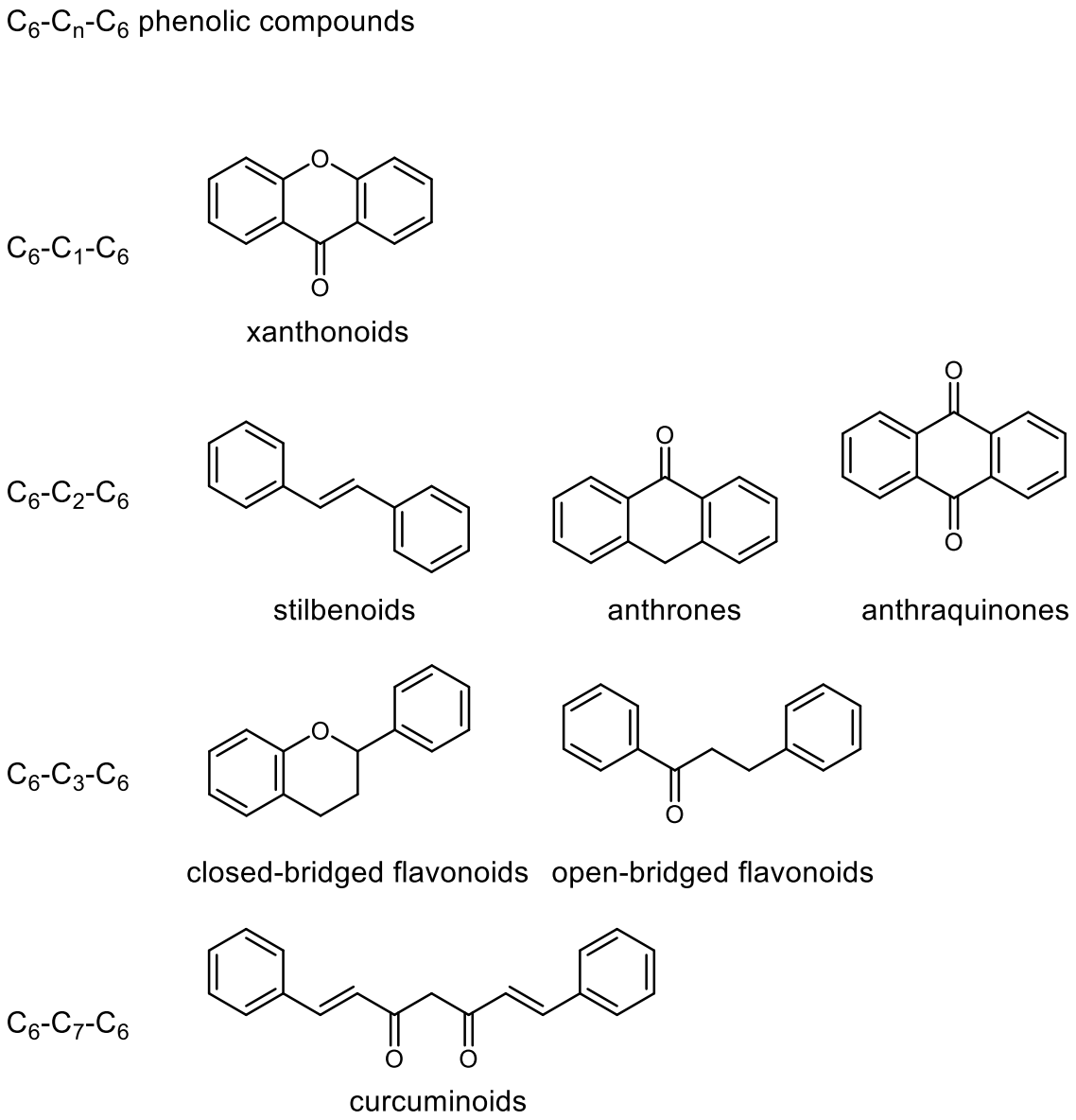


Fig. 11. Structural characteristics of C_6-C_n-C_6 phenolics.[16]

The classification of flavonoids is based on the opening of the C_3 bridge, which gives the possibility to distinguish between open bridged flavonoids, where the carbon bridge forms linker between two aromatic rings and closed bridged flavonoids, in which the carbon chain closes and forms third heterocyclic ring.[16] The naming of the three rings in flavonoids is based on their biosynthesis, in which the two aromatic rings are created first and therefore named A and B ring and the third, heterocyclic ring (if present) is known as the C ring.

2.2.1.1 Open bridged flavonoids

In most cases, open bridged flavonoids are characterized by the presence of a carbonyl group on the C_3 chain adjacent to the A ring. In chalcones as the main subgroup of open bridged flavonoids, the additional α, β-double bond is present, whereas in the case of dihydrochalcones as their reduced counterparts the molecules possess saturated C_3 chain. Minor subgroups of open bridged flavonoids (Fig. 12) consist of β-hydroxychalcones and β-ketodihydrochalcones.[16] Since open bridged flavonoids act as precursors in the biosynthesis of closed bridged flavonoids it is important to be aware of their structural difference, which might give an idea about the possible formations and structural modifications to the C ring in closed bridged flavonoids.

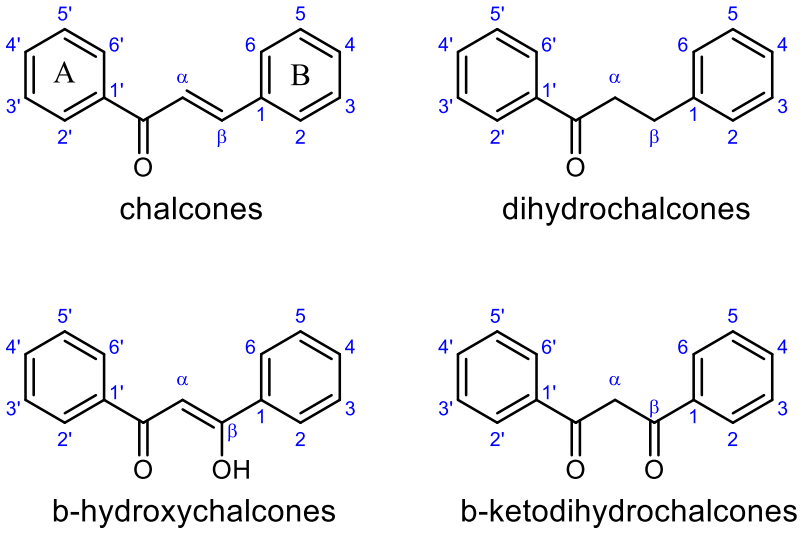


Fig. 12. Structural characteristics of open bridged flavonoids.[16]

2.2.1.2 Closed bridged flavonoids

Closed bridged flavonoids are characterized by the formation of heterocyclic C ring. The reaction mechanism of the intramolecular cyclization (Fig. 13) of chalcones involves deprotonation of 2′-hydroxyl group with subsequent intramolecular attack of the oxyanion on the α, β-unsaturated bond *via* mechanism of Michael addition.[17] Further characterization of closed bridged flavonoids is based on the number of atoms in the C heterocycle, since the nucleophilic addition can appear on α or β position, two groups of closed bridged flavonoids are formed – 5-membered and 6-membered heterocyclic ring flavonoids.

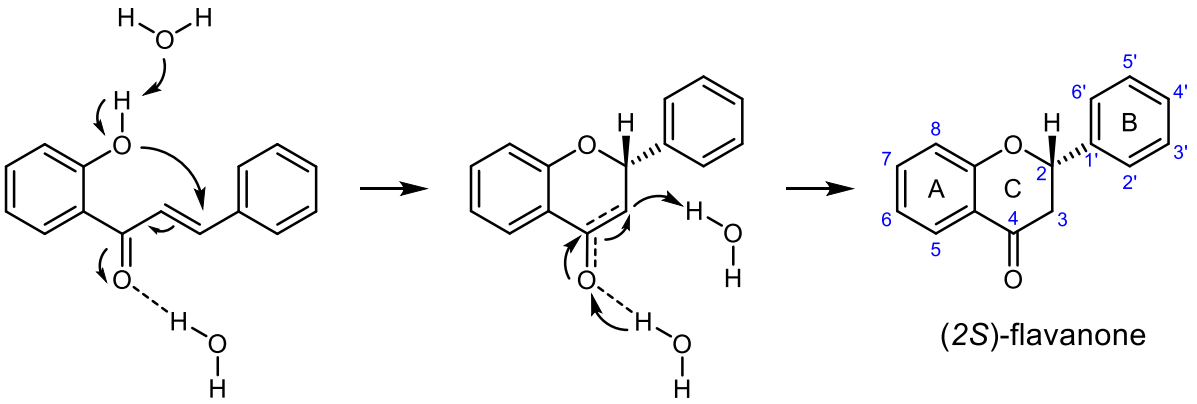


Fig. 13. Intramolecular cyclization of chalcone to flavanone. Nucleophilic attack of the 2′-oxyanion on the α, β-unsaturated bond followed by stabilization of the enolate and formation of the reaction product.[18] Although the cyclization can appear spontaneously and results in racemic mixture, the enzymatic transformation is often selective towards the (*2S*)-flavanone.[17]

The 5-membered subgroup of flavonoids consists of two major classes (Fig. 14) – aurones and auronols.[16] The name "aurone" has its origin in Latin word *aurum* (gold) and refers to the golden color of the pigments present in many plants. Aurone analogues possess various biological activities.[19]

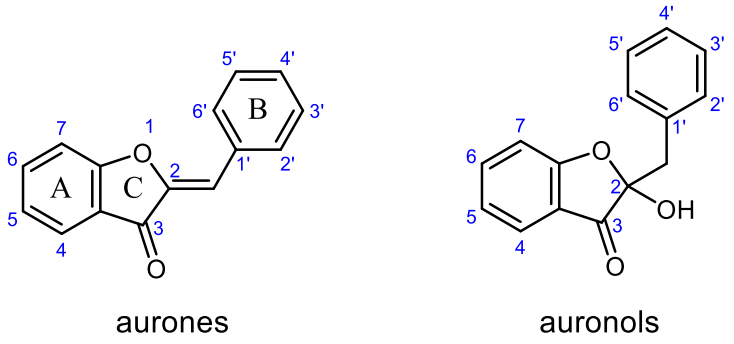


Fig. 14. Structural characteristics of 5-membered closed bridged flavonoids.[16]

The 6-membered subgroup of flavonoids consists of derivatives of phenylchromane which differ in the position in which is the phenyl group attached to the chromane backbone. Three major classes can be distinguished (Fig. 15) – 2-phenylchromane flavonoids, which can be further divided into nine major types, 3-phenylchromane flavonoids also known as isoflavonoids and 4-phenylchromane flavonoids also known as neoflavonoids.

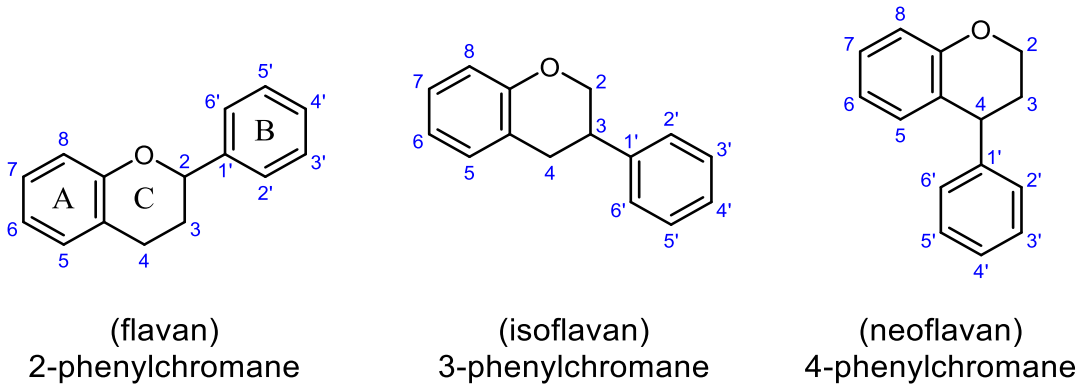


Fig. 15. Structural characteristics of 6-membered closed bridged flavonoids.[16]

2-Phenylchromane flavonoids are distinguished by modification to the C ring into nine types (Fig. 16) – flavans, flavans-3-ols, flavan-4-ols, flavanones, anthocyanidins, flavan-3,4-diols, dihydroflavonols, flavones and flavonols.[16]

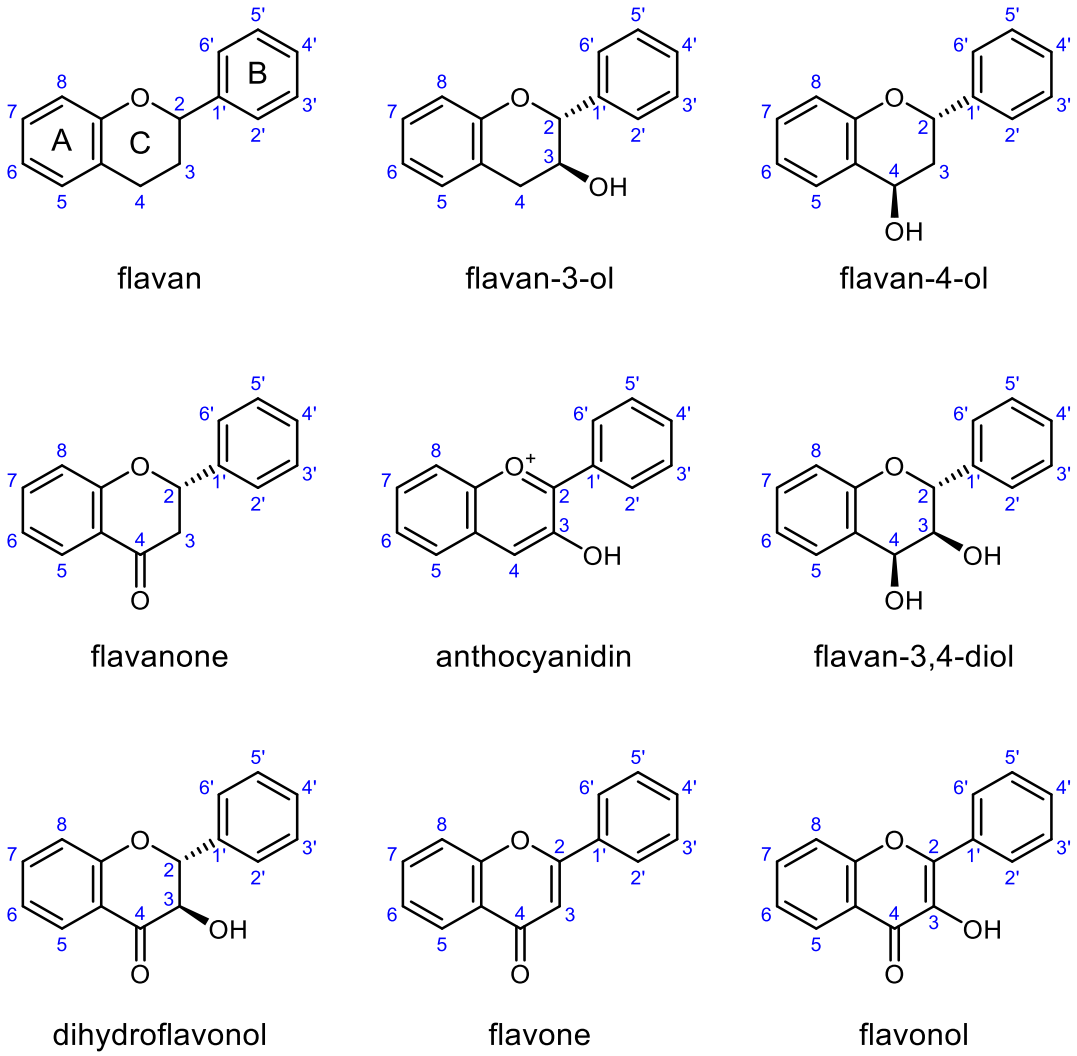


Fig. 16. Structural characteristics of 2-phenylchromane flavonoids.[16]

2.2.2 Biological properties of selected naringenin derivatives

Naringenin (Fig. 17) is trisubstituted flavanone derivative modified by hydroxyl function at positions 5, 7 and 4′. Common sources of naringenin include fruits such as citrus fruits and grapes.[20, 21]

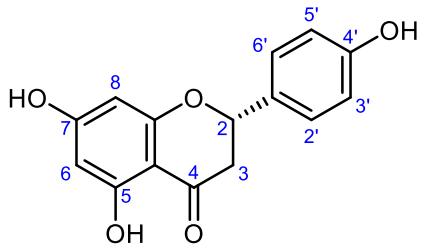


Fig. 17. Structure of naringenin.

2.2.2.1 Antiestrogenic properties

Flavonoids are generally considered to have no or very low estrogenic activity. On the other hand, there are some examples, such as apigenin, kaempferol and naringenin (Fig. 18) as well, that have been proved to have antiestrogenic effect through inhibition of estrone reduction.[22]

Certain flavonoids of the flavone type have been researched for their *in vitro* ability to inhibit the 17β-oxidation of testosterone and estradiol. Certain studies have also shown an activity in inhibition of placental aromatase. All these enzymatic pathways play an important role in regulation of estrogen hormone availability.[23, 24]

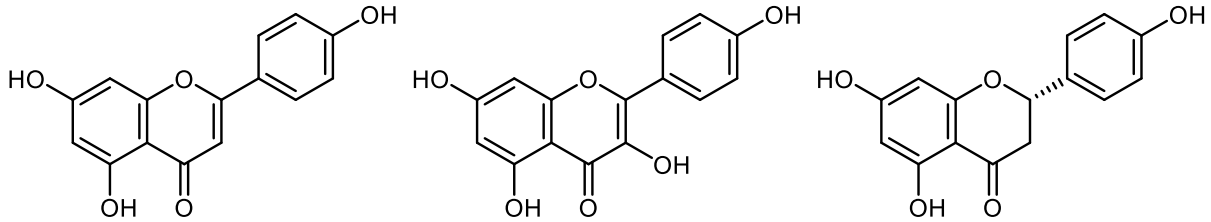


Fig. 18. Flavonoids with antiestrogenic properties.

2.2.2.2 Antiproliferative activity

Naringenin derivatives 6-prenylnaringenin, 8-prenylnaringenin, xanthohumol and isoxanthohumol have been researched in the past for their anticancer potential. These

prenylflavonoids have shown significant anticancer activity, however in case of xanthohumol and isoxanthohumol they have demonstrated antagonism against the antiproliferative effects of common anticancer drugs (5-fluorouracil, oxaliplatin, irinotecan). On the other hand, 6-prenylnaringenin has been found to potentiate antiproliferative effect of irinotecan in colorectal cancer cells. Overall, these findings suggest that 6-prenylnaringenin might be suitable candidate for anticancer combination therapy.[25]

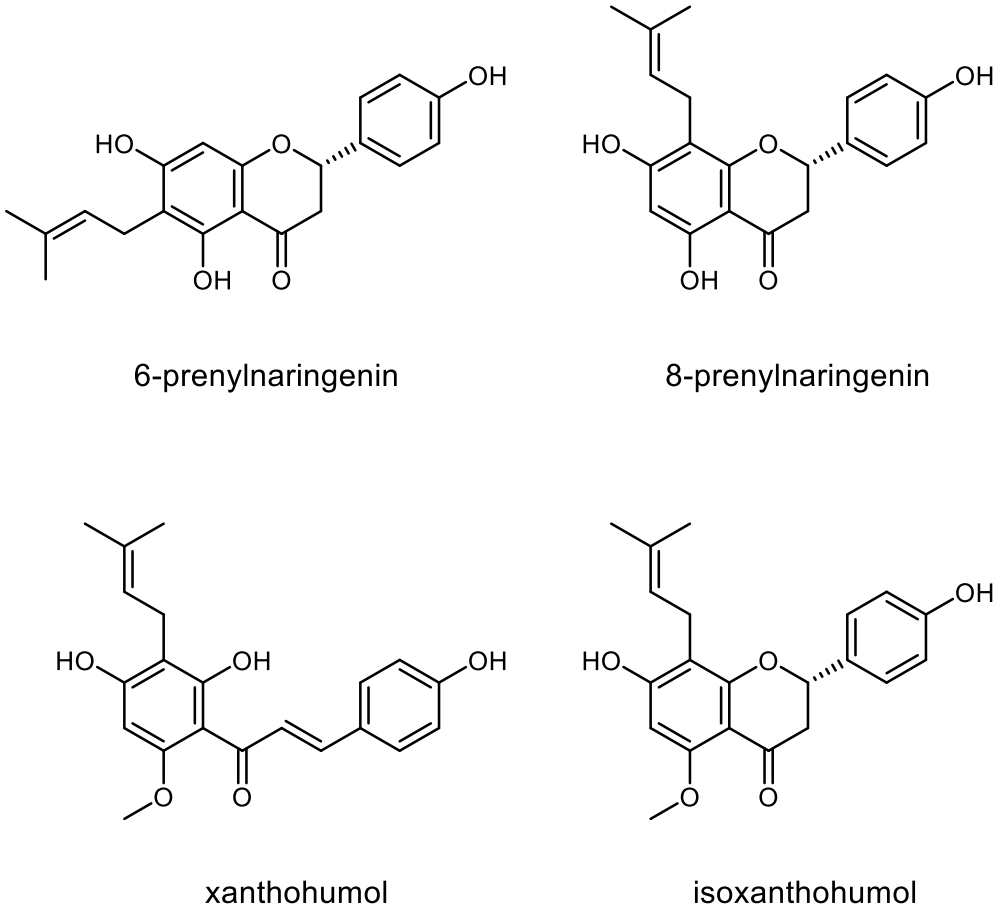


Fig. 19. Prenylated flavonoids with antiproliferative properties.

3 Experimental part

Chemicals used for the reactions and for purification were purchased from chemical vendors associated with University of Lorraine, namely Sigma-Aldrich, Fisher Scientific and VWR.

Reactions were monitored by thin-layer chromatography (TLC) on F_{254} silica gel pre-coated sheets (Merck). For UV detection, two UV lamps (emitting at 254 nm and 312 nm; model VL-6M, Vilber Lourmat, Marne la Vallée, France) were used. In cases where no aromatic bond nor any conjugated system was present in the molecule, detection with vanillin or phosphomolybdic acid was used. Stationary phase for column chromatography was silica gel Geduran Si 60 (0.063-0.200 mm). Mobile phases are stated separately for each compound. Petroleum ether refers to the fraction boiling in the 40–60 °C range. For NMR measurements spectrometer Bruker Avance III was used, with frequencies 400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR.

3.1 Preparation of Jones reagent

Chromium trioxide (13.37 g, 133.7 mmol) was dissolved in distilled water (35 ml) and under constant stirring mixed with concentrated sulfuric acid (40.70 g, 398.7 mmol).

3.2 Preparation of (E)-3,7-dimethylocta-2,6-dienoic acid (1)

Geraniol (500.1 mg, 3.24 mmol) was mixed with acetone (5 ml), previously prepared Jones reagent was added (20 drops) and the reaction was stirred for 30 hours, then quenched with few drops of isopropyl alcohol, diluted with distilled water and extracted with diethyl ether (3×20 ml). The combined organic layers were dried over sodium sulfate and solvent was removed *in vacuo*. The starting material degraded into multiple products and although the reaction was repeated under many different conditions, the oxidation of geraniol never proved to successfully result in geranic acid.

3.3 Separation of (E)-3,7-dimethylocta-2,6-dienoic acid (1) and (Z)-3,7-dimethylocta-2,6-dienoic acid (2)

Commercially available mixture of geranic and nerolic acid (1.00 g) was separated by column chromatography (200 g of silica gel, mobile phase ethyl acetate/hexane, 1:5).

The yield was 151.4 mg of yellow oil **1**.

R_f = 0.32 (ethyl acetate/hexane 1:5), ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 1.60 (s, 3H), 1.68 (s, 3H), 2.16-2.18 (m, 7H), 5.07 (br, 1H), 5.69 (d, 1H, *J*=0.9 Hz)

Commercially available mixture of geranic and nerolic acid (1.00 g) was separated by column chromatography (ethyl acetate/hexane, 1:5).

The yield was 23.8 mg of yellow oil **2**.

R_f = 0.35 (ethyl acetate/hexane 1:5), ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 1.62 (s, 3H), 1.69 (s, 3H), 1.93 (d, 3H, *J*=1.4 Hz), 2.13-2.19 (m, 2H), 2.64 (t, 2H, *J*=7.6 Hz), 5.12-5.16 (m, 1H), 5.68 (d, 1H, *J*=1.1 Hz)

3.4 Preparation of 4-(5,7-dihydroxy-4-oxochroman-2-yl)phenyl 3-methylbut-2-enoate (3)

3,3-Dimethylacrylic acid (147.2 mg, 1.47 mmol) was dissolved under constant stirring and nitrogen atmosphere in anhydrous acetonitrile (12 ml) and mixed with *N,N'*-dicyclohexylcarbodiimide (304.5 mg, 1.48 mmol). After 1 minute, precipitate formed. After 20 minutes, 4-dimethylaminopyridine (20.4 mg, 0.17 mmol) and naringenin (200.4 mg, 0.74 mmol) were added. Reaction was stirred for 25 hours at room temperature, then diluted with distilled water (30 ml) and extracted three times with dichloromethane (30 ml). Combined organic layers were dried over sodium sulfate and the solvent was removed *in vacuo*. The product was further subjected to column chromatography (ethyl acetate/hexane, 1:4) to give yield 99.5 mg (38 %) of yellow oil.

R_f = 0.23 (ethyl acetate/hexane, 1:4), ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm*)* 12.00 (s, ½H), 11.84 (s, ½H), 7.45 (d, 1H, *J*=8.5 Hz), 7.29 (d, 1H, *J*=6.1 Hz), 7.16 (d, 1H, *J*=8.6 Hz), 6.86 (d, 1H, *J*=8.6 Hz), 6.31 (dd, 1H, *J*=2.1 Hz, *J*=5.1 Hz), 5.96 (dd, 1H, *J*=2.3 Hz, *J*=7.4 Hz), 5.90 (dt, 1H, *J*=1.32 Hz, *J*=25.9 Hz), 5.34-5.41 (m, 1H), 3.00-3.15 (m, 1H), 2.77-2.85 (m, 1H), 2.23 (3H, dd, *J*=1.2 Hz, *J*=6.2 Hz), 2.00 (dd, 3H, *J*=1.2 Hz, *J*=5.4 Hz)

3.5 Preparation of 5-hydroxy-2-(4-((3-methylbut-2-enoyl)oxy)phenyl)-4-oxochroman-7-yl 3-methylbut-2-enoate (4)

3,3-Dimethylacrylic acid (147.2 mg, 1.47 mmol) was dissolved under constant stirring and nitrogen atmosphere in anhydrous acetonitrile (12 ml) and mixed with *N,N'*-dicyclohexylcarbodiimide (304.5 mg, 1.48 mmol). After 1 minute, precipitate formed. After 20 minutes, 4-dimethylaminopyridine (20.4 mg, 0.17 mmol) and naringenin (200.4 mg, 0.74 mmol) were added. Reaction was stirred for 25 hours at room temperature, then diluted with 30 ml of distilled water and extracted three times with 30 ml of dichloromethane, dried over sodium sulfate and the solvent was removed *in vacuo*. The product was further subjected to column chromatography (ethyl acetate/hexane, 1:4) to give yield 25.2 mg (7.8 %) of yellow oil.

R_f = 0.48 (ethyl acetate/hexane, 1:4), ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.00 (dd, 6H, *J*=1.1 Hz, *J*=3.8 Hz), 2.23 (dd, 6H, *J*=1.1 Hz, *J*=4.7 Hz), 2.87 (dd, 1H, *J*=3.1 Hz, *J*=17.2 Hz) 3.08 (dd, 1H, *J*=13.2 Hz, *J*=35.2 Hz) 5.46 (dd, 1H, *J*=3.0 Hz, *J*=13.2 Hz) 5.86 (t, 1H, *J*=1.3 Hz) 5.92 (t, 1H, *J*=1.3 Hz) 6.33 (s, 2H), 7.17 (d, 2H, *J*=8.6 Hz) 7.46 (d, 2H, *J*=8.5 Hz), 11.83 (s, 1H)

3.6 Preparation of 4-(5,7-dihydroxy-4-oxochroman-2-yl)phenyl (E)-3,7-dimethylocta-2,6-dienoate (5)

Previously prepared compound **1** (150.1 mg, 0.89 mmol) was dissolved under constant stirring and nitrogen atmosphere in freshly distillated acetonitrile (10.5 ml). Then *N,N'*-dicyclohexylcarbodiimide (267.1 mg, 1.29 mmol) was added and after 5 minutes precipitation occurred and the solution changed color from colorless to milk white. After 30 minutes, 4-dimethylaminopyridine (17.9 mg, 0.15 mmol) and naringenin (176.6 mg, 0.65 mmol) were added. The reaction was stirred at room temperature for 24 hours, then diluted with distilled water (30 ml) and extracted with diethyl ether (3 × 30 ml), dried over sodium sulfate, the solvent was removed in vacuo and the product was subjected to column chromatography (ethyl acetate/hexane 1:4) to give yield 31.86 mg (13.7 %) of yellow oil.

R_f = 0.47 (ethylacetate/hexane, 1:2), ^{1}H NMR ($CDCl_3$, 400 MHz): (ppm) δ 7.28 (d, 2H, *J*=8.5 Hz), 6.87 (d, 2H, *J*=8.5 Hz), 6.29-6.34 (m, 2H), 5.85 (s, 1H), 5.34 (dd, 1H, *J*=2.7 Hz, *J*=13.5 Hz), 5.06-5.17 (m, 1H), 3.10 (dd, 1H, *J*=13.3 Hz, *J*=17.3 Hz), 2.81 (dd, 1H, *J*=1.3 Hz, *J*=19.2 Hz), 2.67 (t, 2H, *J*=7.6 Hz), 2.15-2.29 (m, 5H), 1.65 (dd, 6H, *J*=31.6 Hz, *J*=10.8 Hz)

3.7 Preparation of 4-(5,7-dihydroxy-4-oxochroman-2-yl)phenyl (Z)-3,7-dimethylocta-2,6-dienoate (6)

Mixture of geranic and nerolic acid (563 mg, 3.65 mmol) was under inert atmosphere dissolved in freshly distillated acetonitrile (41.25 ml). Then *N,N'*-dicyclohexylcarbodiimide (1.212 g, 5.88 mmol) was added and the mixture was stirred for 30 minutes, then 4-dimethylaminopyridine (67.9 mg, 0.56 mmol) and naringenin (691.7 mg, 2.54 mmol) were added. The reaction was stirred at room temperature for 24 hours, diluted with distilled water (50 ml) and extracted three times with diethyl ether (50 ml), dried over sodium sulfate and the solvents were removed *in vacuo*. Product was further subjected to column chromatography (ethyl acetate/hexane, 1:4) to give yield 60.2 mg (5.6 %) of yellow oil.

R_f = 0.53 (ethylacetate/hexane, 1:2), ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.44 (d, 2H, *J*=8.5 Hz), 7.13-7.18 (m, 2H), 5.95 (d, 2H, *J*=1.1 Hz), 5.91 (dd, 1H, *J*=1.2 Hz, *J*=5.2 Hz), 5.37 (dd, 1H, *J*=3.0 Hz, *J*=10.1 Hz), 5.09-5.16 (m, 1H), 3.02 (dd, 1H, *J*=12.9 Hz, *J*=17.1), 2.76 (dd, 1H, *J*=3.0 Hz, *J*=17.1 Hz), 2.69 (t, 2H, *J*=8.4 Hz), 2.17-2.28 (m, 5H), 1.66 (dd, 6H, *J*=30.8 Hz, *J*=15.0 Hz)

3.8 Preparation of 4-(7-acetoxy-5-hydroxy-4-oxochroman-2-yl)phenyl acetate (7)

Naringenin (1.497 g, 5.50 mmol) was dissolved in pyridine (12 ml) under inert conditions and acetic anhydride (1.1 ml, 11.55 mmol) was added dropwise to the mixture. Reaction was stirred at room temperature for 24 hours, then poured over water/ice solution – orange precipitate formed. The precipitate was dried on glass frit, dissolved in chloroform and the solvent was removed *in vacuo*. Recrystallization was performed from methanol. Yield 0.792 g (36 %) of white crystals.

R_f = 0.66 (hexane/diethylether, 1:4), ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.29 (s, 3H), 2.32 (s, 3H), 2.88 (dd, 1H, *J*=17.2 Hz, *J*=3.0 Hz), 3.11 (dd, 1H, *J*=17.2, *J*=13.2), 5.46 (dd, 1H, *J*=13.2, *J*=3.0), 6.31 (d, 1H, *J*=2.1), 6.32 (d, 1H, *J*=2.1), 7.17 (d, 2H, *J*=8.6), 7.47 (d, 2H, *J*=8.4), 11.83 (s, 1H)

3.9 Preparation of 4-(7-acetoxy-5-((3-methylbut-2-en-1-yl)oxy)-4-oxochroman-2-yl)phenyl acetate (8)

Previously prepared compound **7** (200.1 mg, 0.56 mmol) and triphenylphosphine (181.9 mg, 0.69 mmol) were dissolved under constant stirring and nitrogen atmosphere in dry tetrahydrofuran (7 ml). 3-Methyl-2-buten-1-ol (0.1 ml, 0.97 mmol) was added. The mixture was cooled down to 0 °C and diethyl azodicarboxylate (178.8 mg, 1.03 mmol) dissolved in dry tetrahydrofuran (3 ml) was dropwise added. After the addition of diethyl azodicarboxylate, the mixture changed the color to bright yellow. The reaction was allowed to warp up and stirred at room temperature for 20 hours. The solvent was removed by evaporation *in vacuo* and the crude mixture was dissolved in diethyl ether (4 ml) and refrigerated overnight. The orange crystals of triphenylphosphine oxide were separated from the mixture by filtration and discarded. The dissolved mixture was then diluted with distilled water (50 ml), washed two times with diethyl ether (50 ml), dried over sodium sulfate, concentrated *in vacuo* and subjected to column chromatography (diethyl ether/hexane, 1:1) to give yield 72.4 g (30 %) of white crystals.

R_f = 0.31 (diethyl ether/hexane, 1:1), ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.74 (s, 3H), 1.79 (s, 3H), 2.30 (s, 3H), 2.31 (s, 3H), 2.82 (dd, 1H, *J*=16.5 Hz, *J*=3.0 Hz), 3.01 (dd, 1H, *J*=16.3 Hz, *J*=13.3), 4.61 (d, 2H, *J*=6.4 Hz), 5.43 (dd, 1H, *J*=13.1 Hz, *J*=2.8 Hz), 5.53 (t, 1H, *J*=6.4 Hz), 6.31 (d, 1H, *J*=2.1 Hz), 6.42 (d, 1H, *J*=2.1 Hz), 7.14 (d, 2H, *J*=8.6 Hz), 7.46 (d, 2H, *J*=8.6 Hz); ^{13}C NMR ($CDCl_3$, 101 MHz): δ 18.53, 21.27, 21.35, 25.95, 45.92, 66.56, 78.79, 100.07, 103.33, 119.06, 122.09, 127.44, 136.26, 150.95, 156.53, 161.29, 163.86, 169,48, 189.20

3.10 Preparation of (6E,10Z,14E)-3-bromo-2,6,10,15,19-pentamethylicosa-6,10,14,18-tetraen-2-ol (9)

Squalene (10.134 g, 24.67 mmol) was dissolved in tetrahydrofuran (63 ml). Water (7.5 ml) was added, the solution changed from clear to cloudy. After that, more tetrahydrofuran (18 ml) was added, the solution changed from cloudy to clear. The mixture was then cooled below 0 °C and *N*-bromosuccinimide (4.354 g, 24.46 mmol) was added portionwise. The mixture was allowed to warm up and was stirred at room temperature for 2 hours. Water (50 ml) was added to quench the reaction and the solvent was removed *in vacuo*. Purification by chromatography was performed over silica gel on glass frit with gradient elution (petrolether,

petrolether/diethylether 95:5, petrolether/diethylether 9:1), combined fractions were collected, and the solvent was removed *in vacuo*. Yield 3.147 g (29 %) of white solid.

R_f = 0.19 (petrolether/diethylether 9:1), ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 4.97-5.17 (m, 4H), 3.88 (dd, 1H, *J*=1.8 Hz, *J*=11.3 Hz), 1.85-2.09 (m, 14H), 1.60 (s, 2H), 1.52 (s, 12H), 1.25 (d, 6H, *J*=4.4 Hz), 0.78 (s, 3H)

3.11 Preparation of 2,2-dimethyl-3-((3E,7Z,11E)-3,7,12,16-tetramethylheptadeca-3,7,11,15-tetraen-1-yl)oxirane (10)

Previously prepared compound **9** (3.147 g, 7.16 mmol) was dissolved in methanol (40 ml) and stirred with anhydrous potassium carbonate (2.046 g, 14.81 mmol) at room temperature for 16 hours. Methanol was removed *in vacuo* and the crude mixture was dissolved in 0.5 N hydrochloric acid (30 ml) and extracted with ethyl acetate (4×50 ml). Organic layers were collected and then washed with saturated solution of sodium hydrogencarbonate (10 ml), then with brine (10 ml), dried over sodium sulphate and the solvent was removed *in vacuo*. The product was sufficiently pure for further reactions. Yield 1.446 g (56 %) of yellow oil.

R_f = 0.63 (diethylether/hexane 1:9), ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 4.97-5.18 (m, 4H), 2.63 (t, 1H, *J*=6.2 Hz), 1.86-2.14 (m, 14H), 1.61 (s, 3H), 1.55 (s, 3H), 1.53 (s, 9H), 1.35 (s, 2H), 1.23 (s, 3H), 1.18 (s, 3H)

3.12 Preparation of (4E,8Z,12E)-4,8,13,17-tetramethyloctadeca-4,8,12,16-tetraenal (11)

Compound **10** (1.446 g, 4.03 mmol) was dissolved in diethylether (20 ml) and was added dropwise to suspension of periodic acid (0.647 g, 2.83 mmol) in diethylether (10 ml). After the addition, the solution looked like milk. The mixture was stirred at room temperature for 2 hours. Saturated solution of sodium hydrogencarbonate was added (30 ml) and the mixture was extracted with ethyl acetate (3×50 ml), combined organic layers were washed with brine (10 ml), then dried over sodium sulphate and the solvent was removed *in vacuo*. The crude product was purified on glass frit over silica (diethylether/hexane 1:20). Yield 1,225 g (96 %) of yellow oil.

R_f = 0.38 (diethylether/hexane 1:20), ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 9.66 (t, 1H, *J*=1.9 Hz), 4.99-5.10 (br, 4H), 2.42 (t, 2H, *J*=7.8 Hz), 2.23 (t, 2H, *J*=7.7 Hz), 1.86-2.04 (br, 12H), 1.60 (s, 3H), 1.52 (s, 12H)

3.13 Preparation of (4E,8Z,12E)-4,8,13,17-tetramethyloctadeca-4,8,12,16-tetraenoic acid (12)

Previously prepared compound **11** (997.5 mg, 3.15 mmol) was dissolved in acetone (10 ml) and treated with Jones reagent (20 drops) over the course of 5 minutes. The reaction was then quenched with few drops of isopropanol. The mixture was poured into brine (50 ml) and extracted with diethyl ether (4 × 50 ml), dried over sodium sulphate, filtered, and concentrated *in vacuo*. Yield 784,7 mg (75 %) of yellow oil.

R_f below 0.1 (diethylether/hexane 1:9), ^{1}H NMR (DMSO, 400 MHz): δ (ppm) 12.03 (s, 1H), 5.00-5.13, (br, 4H), 2.12-2.29 (m, 4H), 1.87-2.05 (br, 12H), 1.61 (s, 3H), 1.54 (s, 12H)

3.14 Preparation of 4-(5,7-dihydroxy-4-oxochroman-2-yl)phenyl (4E,8Z,12E)-4,8,13,17-tetramethyloctadeca-4,8,12,16-tetraenoate (13)

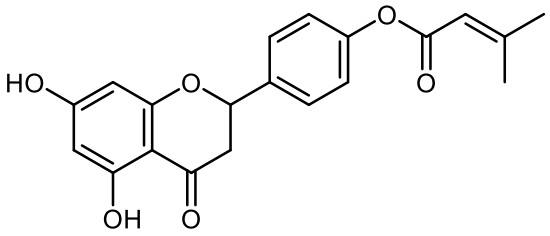
Compound **12** (296.2 mg, 0.891 mmol) was dissolved in anhydrous acetonitrile (18 ml) under nitrogen atmosphere. *N*,*N*′-Dicyclohexylcarbodiimide (161.2 mg, 781.2 mmol) was added, precipitate formed. After 20 minutes, 4-dimethylaminopyridine (13.7 mg, 112.1 mmol) and naringenin (100.0 mg, 367.3 mmol) were added. The reaction mixture was stirred at room temperature over weekend (60 hours). The reaction was quenched with distilled water (50 ml) and extracted with ethyl acetate (3×50 ml). Combined organic layers were dried over sodium sulphate and removed *in vacuo*. The product was further subjected to column chromatography (ethyl acetate/hexane 1:4) to obtain yield of 97,4 mg (45 %) of yellow oil.

R_f = 0.19 (ethylacetate/hexane 1:4), ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.43 (d, 2H, *J*=8.56 Hz), 7.11 (d, 2H, *J*=8.63 Hz), 5.95 (s, 2H), 5.36 (dd, 1H, *J*=2.9 Hz, *J*=13.0 Hz), 5.25 (t, 1H, *J*=6.7 Hz), 5.07-5.19 (br, 4H), 3.00 (dd, H1, *J*=13.2 Hz, *J*=17.1 Hz), *2*.68 (t, 2H, *J*=7.2 Hz), 2.44 (t, 2H, *J*=8.0 Hz), 1.94-2.16 (br, 12H), 1.58-1.70 (m, 15H)

4 Discussion

The aim of this work was synthesis and characterization of naringenin derivatives with increased lipophilic profile. This was achieved by synthesis and characterization of derivatives **3-6** and **13**. (Fig. 20) However, one of the desired synthetic pathways was also preparation of flavonoid with carbon-carbon modification which did not take place (Fig. 21).

3



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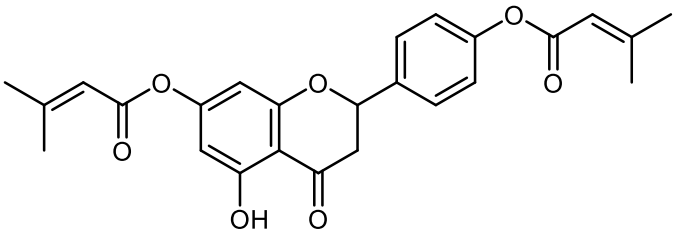

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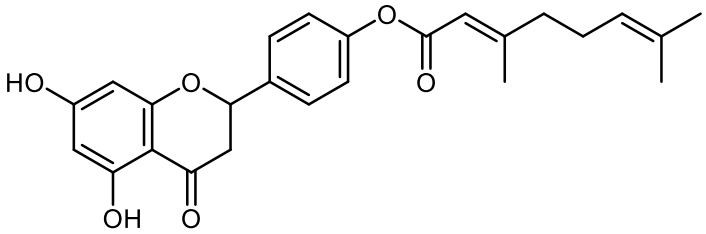

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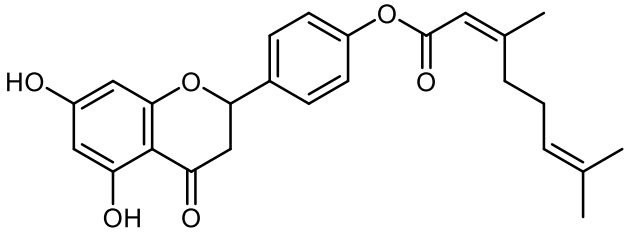

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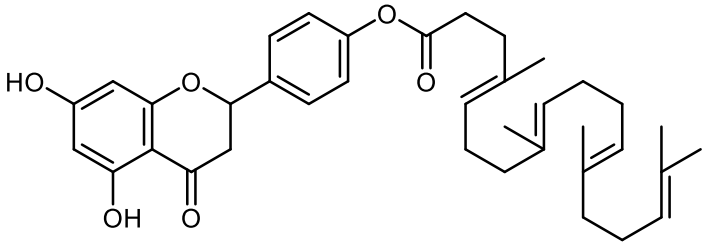


Fig. 20. Prepared naringenin derivatives.

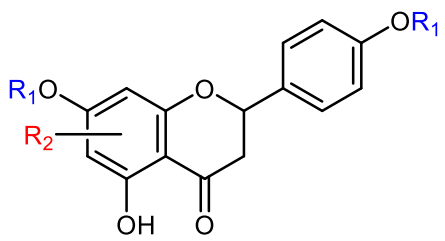


Fig. 21. Naringenin modifications – completed (in blue) and not completed (in red).

4.1 Chromic acid oxidation

Some of the acids used for esterification of naringenin were not available commercial (or not in a pure form) and therefore were prepared from their intermediates. Chromic acid is powerful oxidizing agent. It is usually prepared as required from chromic oxide in combination with aqueous solution of sulfuric acid.[26] It can be used to convert primary alcohols and aldehydes to carboxylic acids, or secondary alcohols to ketones. In the case of converting aldehyde **11** to carboxylic acid **12** (for synthesis of **11** see below) the conversion was successful, but the intended oxidation of geraniol to geranic acid **1** did not occur (Fig. 22), the allyl alcohol was always selectively converted to aldehyde. Due to the fact that whole two-step syntheses of **1** from geraniol *via* intermediate aldehyde would potentially result in mixture of two isomers, geranic and nerolic acid, the strategy to prepare these compounds was further abandoned and they were purchased as a mixture and purified by column chromatography.

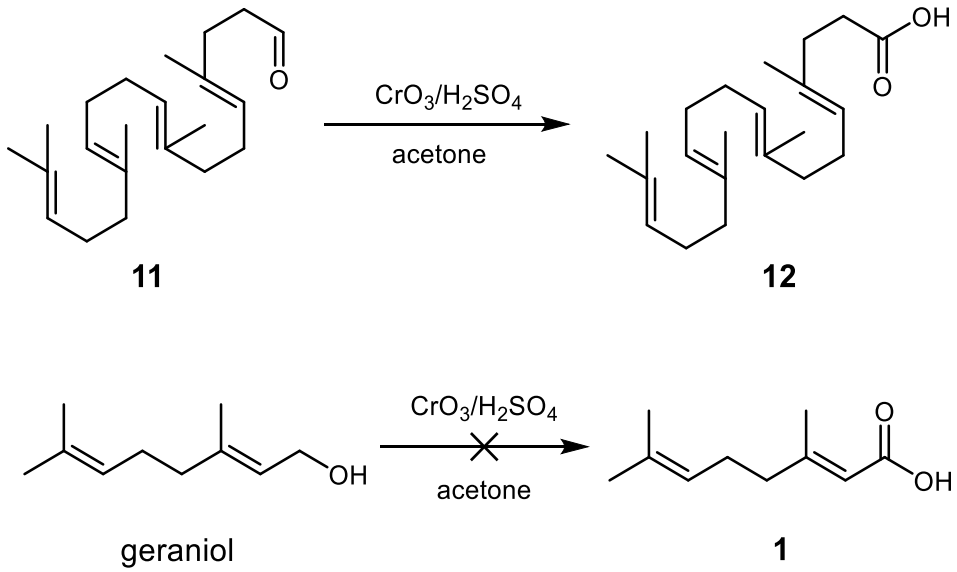
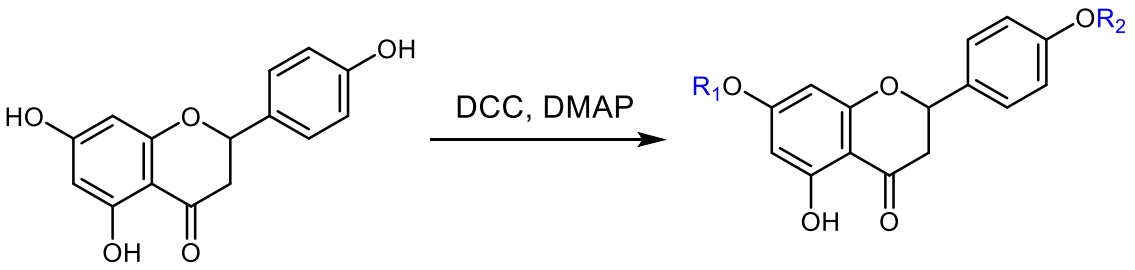


Fig. 22. Oxidation of aldehyde **11** to carboxylic acid **12** with chromic acid, unsuccessful oxidation of geraniol to geranic acid **1**.

4.2 Steglich esterification

Steglich esterification (Fig. 23) was used in preparation of the esters **3**-**6** and **13**. Esterifications were in all cases successful with variable yields (5.6 % - 45 %). The esterification always resulted in mixture of diesters on positions 7 and 4′ and monoesters on position 4′. Due to its acidity, hydroxyl group on the position 5 was not targeted and remained as it was. The products **3**, **5**, **6**, and **13** are monoesters except for **4** which is diester.

The nerolic acid derivative **6** was prepared *via* reaction of naringenin with commercial mixture of geranic and nerolic acid due to very low yield of the isolation of nerolic acid from a commercial mixture – less than 24 mg of pure nerolic acid was received from 1.00 g of the mixture.



naringenin products **3-6** and **13**

product	R_1	R_2
3	H	3,3-dimethylallylic acid
4	3,3-dimethylallylic acid	3,3-dimethylallylic acid
5	H	geranic acid **1**
6	H	nerolic acid **2**
13	H	trisnorsqualenic acid **12**

Fig. 23. Synthesis of esters **3-6** and **13**.

The reaction itself relies on the usage of *N*,*N*′-dicyclohexylcarbodiimide (DCC) and 4-dimethylaminopyridine (DMAP) and has been used in the past not only for preparations of esters, but amides and thioamides as well.[27] Standard DCC reaction takes place because diimides possess reactive cumulative double-bond system and therefore are susceptible to nucleophilic attack at the central carbon.

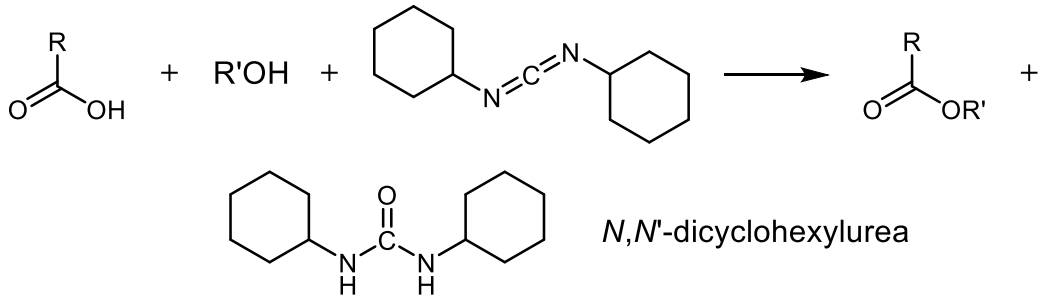


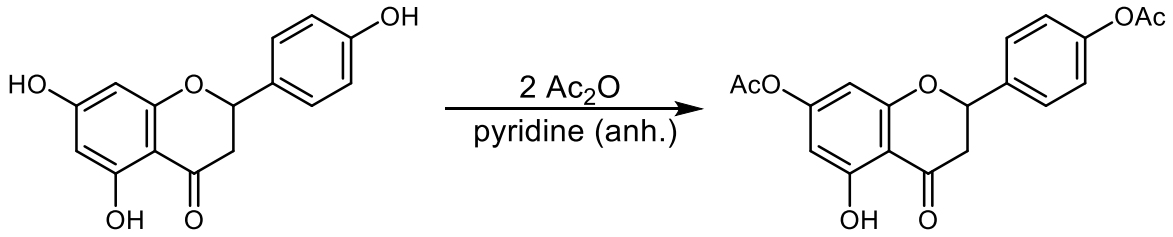
Fig. 24. Reaction mechanism of standard DCC reaction.[26]

In the first step of the coupling reaction, the carboxyl function adds to the imide to give an acyl intermediate (Fig 24). The second step is alcoholysis of the intermediate to give the coupled product and *N*,*N*′-dicyclohexylurea.[26] The DMAP acts in this reaction as an acylation catalyst and the addition of this catalyst accelerates the esterification, suppresses the formation of side products, and in some cases enables formation of sterically demanding esters.[27]

4.3 Synthesis of prenylated naringenin derivatives

The desired synthesis[28] of naringenin derivatives with carbon-carbon modifications consisted of four individual steps – protection of selected hydroxyl groups, Mitsunobu coupling, Claisen-Cope rearrangement and deprotection.

The first step towards the preparation of prenylated naringenin derivatives started with protection of the hydroxyl groups 7 and 4′ on the naringenin molecule (Fig. 25) by reaction with acetic anhydride in anhydrous pyridine and resulted in naringenin-diacetate **7**. The third available hydroxyl was the least probable to react due to its proximity to the carbonyl function.



naringenin **7**

Fig. 25. First step of the synthesis – protection of naringenin hydroxyl groups.

The second step involved Mitsunobu coupling[29] (Fig. 26) on hydroxyl on position 5 of the naringenin-diacetate **7**, which was converted to ether **8** by reaction with triphenylphosphine, diethyl azodicarboxylate and 3-methyl-2-buten-1-ol (prenol) in anhydrous tetrahydrofuran.

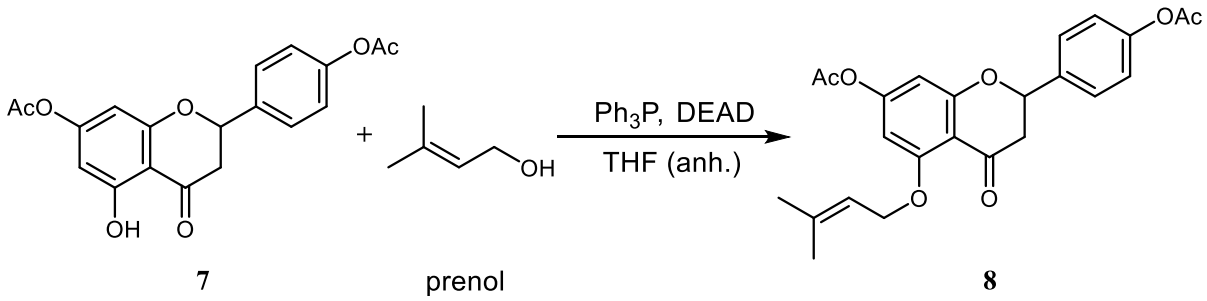


Fig. 26. Second step of the synthesis – Mitsunobu coupling.

However, the third step (Fig. 27) proved to be fatal to the whole strategy. The desired thermal rearrangement of compound **8** did not take place, probably due to bad choice of the solvent (tetralin instead of decalin) or because the high temperature needed for the reflux resulted in thermal degradation of the product, possibly to open bridged flavonoid.

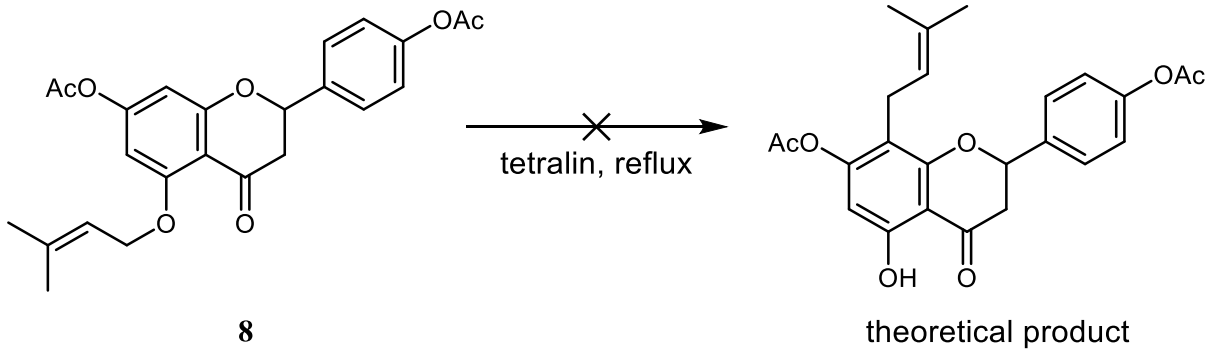


Fig. 27. Third step of the synthesis – Claisen-Cope rearrangement.

4.4 Van Tamelen synthesis

Van Tamelen synthesis[30] was used in the process of obtaining the trisnorsqualenic acid **12.** This time-tested process consists of three individual steps – preparation of bromohydrin **9**, preparation of epoxide **10** and preparation of aldehyde **11**. Finally, the target aldehyde was converted to carboxylic acid **12**.

The first step of van Tamelen sequence (Fig. 28) consists of oxidation of the terminal double bond by *N*-bromosuccinimide. The selectivity of the reaction to the terminal double bond is achieved by preparing saturated solution of squalene in aqueous tetrahydrofuran, in which the squalene exists in its coiled, compact formation. The non-terminal double bonds are then sterically shielded and less likely to react as opposed to the terminal double bond, which remains exposed.

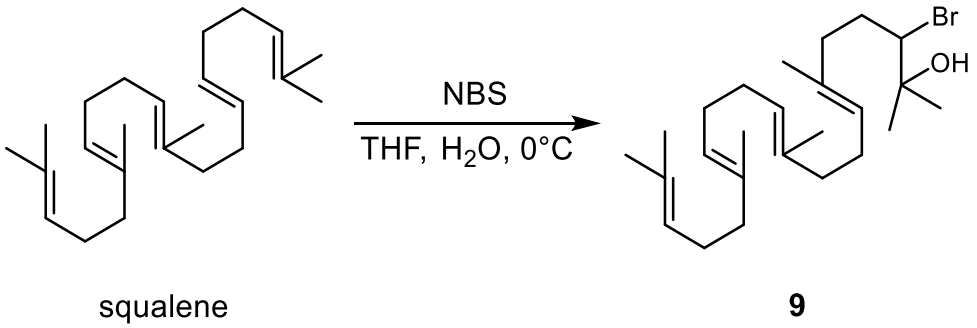


Fig. 28. The first step of van Tamelen synthesis – oxidation of terminal double bond.

The second step involves ring closure to epoxide **10**. (Fig. 29) This has been done by reaction with potassium carbonate in methanol.

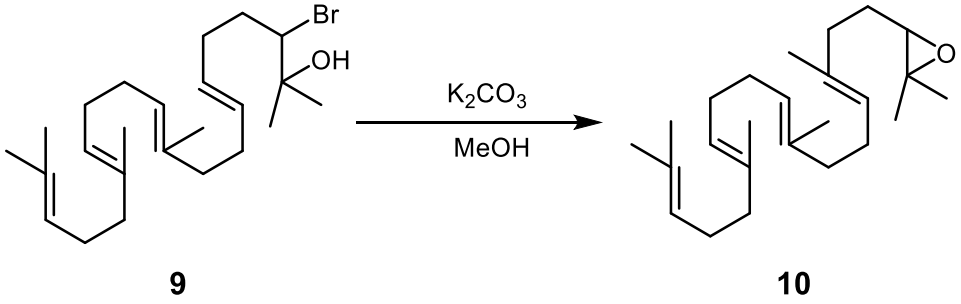


Fig. 29. The second step of van Tamelen synthesis – creation of epoxide ring.

The last step of the van Tamelen synthesis (Fig. 30) consists of periodate oxidation of the previously prepared epoxide **10** to aldehyde **11** in diethylether.

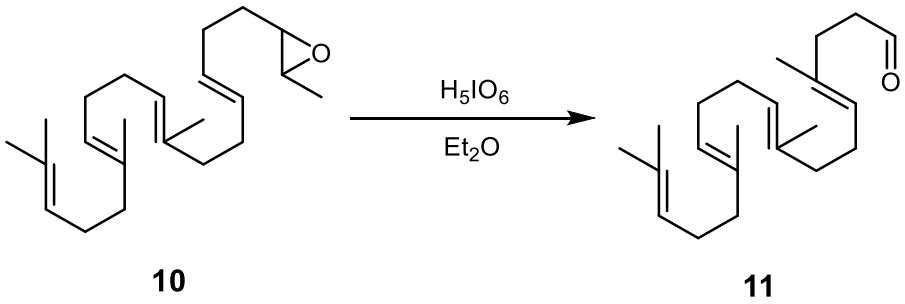


Fig. 30. The last step of van Tamelen synthesis – conversion to aldehyde.

The aldehyde **11** was then converted to carboxylic acid **12** by reaction with chromic acid (see above, Fig. 22).

In conclusion, the final products **3-6** and **13** have been prepared and are now subjects of intensive testing for their potential *in vitro* cytotoxic properties.

5 Conclusion

The following compounds have been successfully purified:

(*E*)-3,7-dimethylocta-2,6-dienoic acid (**1**)

(*Z*)-3,7-dimethylocta-2,6-dienoic acid (**2**)

The following precursors have been successfully prepared:

4-(7-acetoxy-5-hydroxy-4-oxochroman-2-yl)phenyl acetate (**7**)

4-(7-acetoxy-5-((3-methylbut-2-en-1-yl)oxy)-4-oxochroman-2-yl)phenyl acetate (**8**)

(6*E*,10*Z*,14*E*)-3-bromo-2,6,10,15,19-pentamethylicosa-6,10,14,18-tetraen-2-ol (**9**)

2,2-dimethyl-3-((3*E*,7*Z*,11*E*)-3,7,12,16-tetramethylheptadeca-3,7,11,15-tetraen-1-yl)oxirane (**10**)

(4*E*,8*Z*,12*E*)-4,8,13,17-tetramethyloctadeca-4,8,12,16-tetraenal (**11**)

(4*E*,8*Z*,12*E*)-4,8,13,17-tetramethyloctadeca-4,8,12,16-tetraenoic acid (**12**)

The following final compounds have been successfully prepared:

4-(5,7-dihydroxy-4-oxochroman-2-yl)phenyl 3-methylbut-2-enoate (**3**)

5-hydroxy-2-(4-((3-methylbut-2-enoyl)oxy)phenyl)-4-oxochroman-7-yl 3-methylbut-2-enoate (**4**)

4-(5,7-dihydroxy-4-oxochroman-2-yl)phenyl (*E*)-3,7-dimethylocta-2,6-dienoate (**5**)

4-(5,7-dihydroxy-4-oxochroman-2-yl)phenyl (*Z*)-3,7-dimethylocta-2,6-dienoate (**6**)

4-(5,7-dihydroxy-4-oxochroman-2-yl)phenyl (4*E*,8*Z*,12*E*)-4,8,13,17-tetramethyloctadeca-4,8,12,16-tetraenoate (**13**)

6 Literature

[1] GERSHENZON, J.; DUDAREVA, N. The function of terpene natural products in the natural world. *Nat. Chem. Biol.*, **2007**, *7*, 408-414.

[2] MARTIN, V. J. J. et al. Engineering a mevalonate pathway in Escherichia coli for production of terpenoids. *Nat. Biotechnol.*, **2003**, *7*, 796-802.

[3] MAHIZAN, N. A. et al. Terpene Derivatives as a Potential Agent against Antimicrobial Resistance (AMR) Pathogens. *Molecules*, **2019**, *24*, 2631.

[4] BAKKALI, F. et al. Biological effects of essential oils – A review. *Food Chem. Toxicol.*, **2008**, *46*, 446–475.

[5] NAZZARO, F. et al. Effect of Essential Oils on Pathogenic Bacteria. *Pharmaceuticals,* **2013**, *6*, 1451-1474.

[6] REDDY, L. H.; COUVREUR, P. Squalene: A natural triterpene for use in disease management and therapy. *Adv. Drug Delivery Rev.*, **2009**, *61*, 1412–1426.

[7] PENUELAS, J.; MUNNEBOSCH, S. Isoprenoids: an evolutionary pool for photoprotection. *Trends Plant Sci.*, **2005**, *4*, 166-169.

[8] WANG, J. et al. The delivery and antinociceptive effects of morphine and its ester prodrugs from lipid emulsions. *Int. J. Pharm. (Amsterdam, Neth.)*, **2007**, *1-2*, 95-104.

[9] KOHNO, Y. et al. Kinetic study of quenching reaction of singlet oxygen and scavenging reaction of free radical by squalene in n-butanol. *Biochim. Biophys. Acta, Lipids Lipid Metab.*, **1995**, *1*, 52-56.

[10] HASHIM, Y. Z. H. Y. et al. Components of Olive Oil and Chemoprevention of Colorectal Cancer. *Nutr. Rev.*, **2005**, *11*, 374-386.

[11] ROWINSKY, E. K.; DONEHOWER, R. C. Paclitaxel (Taxol). *N. Engl. J. Med.* **1995**, *15*, 1004-1014.

[12] ABOTALEB, M. et al. Flavonoids in Cancer and Apoptosis. *Cancers,* **2019**, *11*, 28.

[13] KUNTZ, S.; WENZEL, U.; DANIEL, H. Comparative analysis of the effects of flavonoids on proliferation, cytotoxicity, and apoptosis in human colon cancer cell lines. *Eur. J. Nutr*., **1999**, 38, 133–142.

[14] BUTLER, M. S. Natural products to drugs: natural product-derived compounds in clinical trials. *Nat. Prod. Rep.,* **2008**, *25*, 475–516.

[15] KŘÍŽKOVÁ, J. et al. The effects of selected flavonoids on cytochromes P450 in rat liver and small intestine. *Interdiscip. Toxicol.,* **2009**, *2(3)*, 201–204.

[16] TSIMOGIANNIS, D.; OREOPOULOU, V. Classification of Phenolic Compounds in Plants. In: Ronald Ross Watson (Ed.) *Polyphenols in Plants – Isolation, Purification and Extract Preparation*. 2nd Ed., Academic Press, London 2019, ISBN 978-0-12-813768-0

[17] JEZ, J. M.; NOEL, J. P.; Reaction Mechanism of Chalcone Isomerase. *J. Biol. Chem.,* **2002**, *2*, 1361–1369.

[18] JEZ, J. M. et al. Structure and mechanism of the evolutionarily unique plant enzyme chalcone isomerase. *Nat. Struct. Biol.,* **2000**, *9*, 786-791.

[19] HASSAN, G. S. et al. Aurones and furoaurones: Biological activities and synthesis. *Bulletin of Faculty of Pharmacy, Cairo University*. **2018**, *2*, 121-127.

[20] PARASHAR, P. et al. Augmented Therapeutic Efficacy of Naringenin Through Microemulsion-Loaded Sericin Gel Against UVB-Induced Photoaging. *AAPS PharmSciTech,* **2020**, *21*, 215.

[21] ALAM, M. A. et al. Effect of Citrus Flavonoids, Naringin and Naringenin, on Metabolic Syndrome and Their Mechanisms of Action. *Adv. Nutr*., **2014**, *5*, 404–417.

[22] ROSS, J. A.; KASUM, C. M. Dietary Flavonoids: Bioavailability, Metabolic Effects, and Safety. *Annu. Rev. Nutr.,* **2002**, *22*, 19–34.

[23] TA, N.; WALLE, T. Aromatase inhibition by bioavailable methylated flavones. *J. Steroid Biochem. Mol. Biol.*, **2007**, *107*, 127–129.

[24] KELLIS, J. T.; VICKERY, L. E. Inhibition of Human Estrogen Synthetase (Aromatase) by Flavones. *Science*. **1984**, *225*, 1032-1034.

[25] AMBROŽ, M. et al. Antiproliferative Effects of Hop-derived Prenylflavonoids and Their Influence on the Efficacy of Oxaliplatine, 5-fluorouracil and Irinotecan in Human Colorectal C Cells. *Nutrients*. **2019**, *11*, 879.

[26] ROBERTS, J. D.; CASERIO, M. C. *Basic Principles of Organic Chemistry, 2nd edition.*, W. A. Benjamin, Inc., Menlo Park 1977. ISBN 0-8053-8329-8

[27] NEISES, B.; STEGLICH, W. Simple Method for the Esterification of Carboxylic Acids. *Angew. Chem., Int. Ed. Engl.*, **1978**, *17*, 522-524.

[28] GESTER, S. et al. An efficient synthesis of the potent phytoestrogens 8-prenylnaringenin and 6-(1,1-dimethylallyl)naringenin by europium(III)-catalyzed Claisen rearrangement. *Tetrahedron*, **2001**, *6*, 1015-1018.

[29] SWAMY, K. C. K. et al. Mitsunobu and Related Reactions: Advances and Applications. *Chem. Rev. (Washington, DC, U. S.)*, **2009**, *6*, 2551-2651.

[30] VAN TAMELEN, E. E.; CURPHEY, T. J. The Selective In Vitro Oxidation of the Termial Double Bonds in Squalene. *Tetrahedron Lett.*, **1962**, *3*, 121-124.