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Investigations on the Darzens Condensation of 2-Bromo-4,6-dimethoxybenzofuran-3(2*H*)-one

Hradec Králové, 2006 Eva Lepičová

I would like to thank to Professor Michael Gütschow, Department of Pharmaceutical Chemistry, University of Bonn, for leadership and kind assistance.

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Content:

1. Index of Abbreviations

BA	benzaldehyde
CHD	coronary heart disease
DHBF	4,6-dihydroxybenzofuran-3(2*H*)-one
DHF	3',4'-dihydroxyflavonol
DMF	dimethylformamide
DMD	dimethyldioxirane
DMSO	dimethoxysulfoxide
HMBC	heteronuclear multiple bond correlation
HSQC	heteronuclear single quantum correlation
LDL	low-density protein
M.p.	melting point
MS	mass spectrum
M_w	molecular weigh
NADPH	nicotinamide adenine dinucleotide phosphate
NBA	2-nitrobenzaldehyde
NMR	nuclear magnetic resonance
PTT	phenyltrimethylammonium tribromide
THF	tetrahydrofuran
Triton B	benzyltrimethylammonium hydroxide

2. Introduction

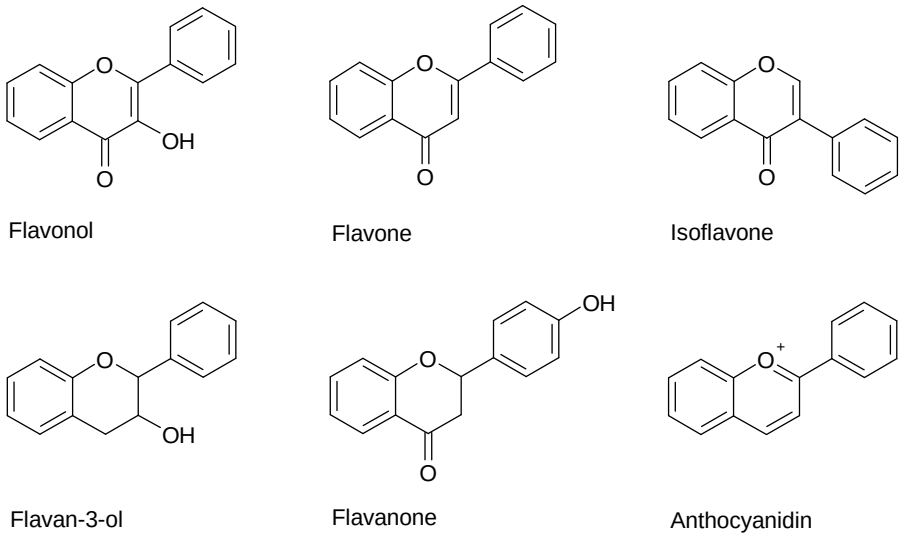
My work continues the previous research works of Prof. M. Gütschow and his co-workers. This team is interested in preparative methods of synthesis of prototypes of naturally occurring flavonoids and testing their biological activities. My diploma thesis is the indirect follow up of the diploma thesis of David Bolek[22].

The aim of my work was to investigate the reactions of 2-bromo-4,6-dimethoxybenzofuran-3(2*H*)-one with various aldehydes by the means of Darzens condensation. This reaction as an synthetic entry to flavonoid derivatives is not sufficiently described in the literature.

3. Theoretical Part

3.1. General

Flavonoids are polyphenolic secondary metabolites widely dispersed throughout the plant kingdom and found in substantial levels in commonly consumed fruits, vegetables and beverages (especially red wine, green and black tea). The flavonoid family is divided into a number of sub-groups. The six main classes are flavonols, flavones, flavan-3-ols, isoflavones, flavanones and anthocyanidins (Scheme 1).



Scheme 1: Main classes of flavonoids

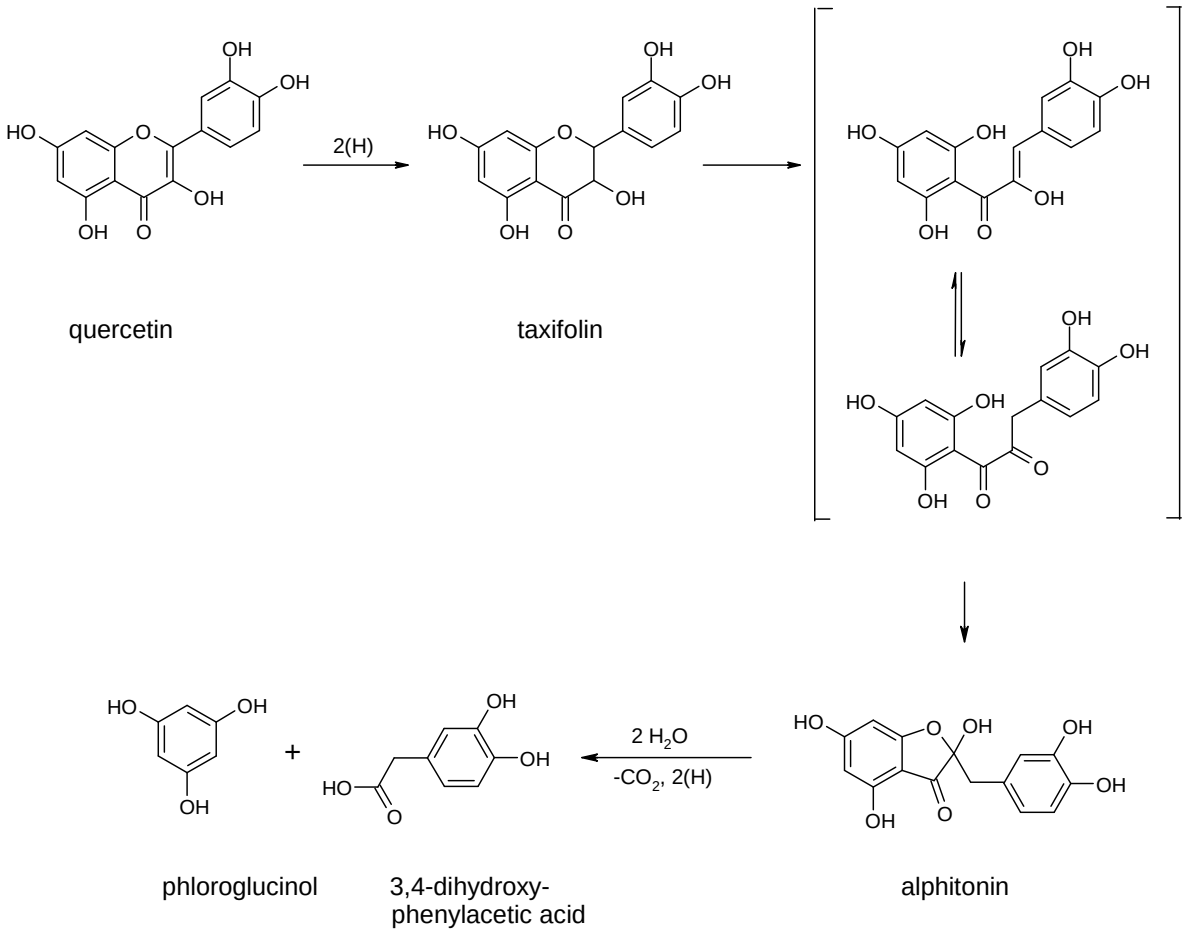
Nutritionists became interested in flavonoids in the 1930´s when it was shown that flavonoids from citrus fruits decreased capillary permeability and had vitamin C sparing properties[1].

Flavonoids are synthesised in plants tissues from shikimate as secondary metabolites, where can be found conjugated to sugars, especially glucose, rhamnose and rutinose[21].

The daily intake of flavonoids[2] calculated on the basis of the aglycones was estimated to range from approximately 3 to 70 mg in different countries. A major part of ingested flavonoids are not absorbed and are largely degraded by the intestinal microflora. Intestinal bacteria play important roles not only in deconjugation of flavonoids but also in their further degradation.

Eubacterium ramulus play the main role. It is a strict anaerobe resident in the human intestinal tract and grows with quercetin-3-glucoside (isoquercetin) as the sole carbon and energy source.

It has been shown that the degradation of the quercetin to phloroglucinol and (3,4-dihydroxyphenyl)pyruvic acid proceeds via the hydroxyflavanone taxifolin and the auronol derivative alphitonin[2] (Scheme 2).



Scheme 2: Degradation of the quercetin

To study the bioactivity of flavonoids, it is necessary to develop suitable preparative methods to provide sufficient amounts for biological investigations.

3.2. Bioactivity of Flavonoids

Flavonoids possess wide spectrum of biological activity.

3.2.1. Antioxidant Activity

Flavonoids have been shown to act as scavengers of various oxidising species i.e. superoxide anion, hydroxyl radical or peroxy radicals[10]. They are also able to stabilise membranes by decreasing membrane fluidity.

3.2.2. Anti Coronary Heart Disease Activity

Naturally presented flavonoids have been found in vitro to inhibit oxidation of low-density protein (LDL). In most countries a high intake of saturated fats is strongly correlated with high mortality from coronary heart disease (CHD). This is not the case in some southern French regions. This so called “French Paradox” shows low incidence of CHD attributed to the regular intake of red wine despite the high saturated fat intake[13]. The concentration of free and conjugated grape skin-derived flavonols is higher in red than in white wines, in which are grape skins removed during the production[1].

3.2.3. Vascular Activity

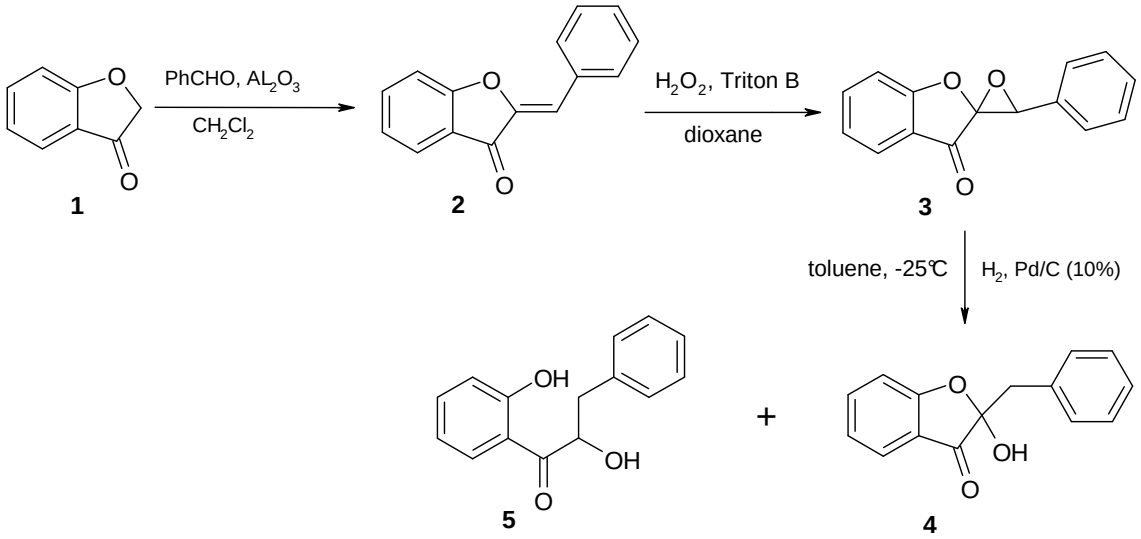
Flavonoids may act in a number of different ways on the various components of blood such as platelets, monocytes, low-density protein (LDL) and smooth muscles. Platelets are key participants in atherogenesis and pro-inflammatory mediators are produced from them. Flavonoids may inhibit C, aggregation and secretion[10]. Flavonoids can also physiologically antagonise vascular NADPH oxidase activity, which is primarily responsible for the enhanced vascular oxidative stress in pathological states. Natural flavonol quercetin and the synthetic flavonol DHF effectively decreased the oxidative stress in aortic tissues from apoliprotein(E)-deficient mice as a model of atherosclerosis[20].

3.2.4. Other Flavonoids Activities

Flavonoids have anti-inflammatory activity[23,32] (they may inhibit the cyclo-oxygenase and the 5-lipoxygenase pathways of arachidonate metabolism), cytotoxic antitumor activity[19,27] (actions on tumor cells with a variety of anticancer effects such as cell growth and kinase activity inhibition, apoptosis induction, suppression of the secretion of matrix metalloproteinases and of tumor invasive behaviour), antibacterial[24,28], antifungal[25,26], antiprotozoal[29], antiviral[26,30], analgesic[32] and oestrogenic[31] activity.

3.3. Synthesis of Flavonoid Derivatives

The ways of synthesis auronols and alphitonins are described in literature. The successful synthetic route of preparation 2-benzyl-2-hydroxybenzofuran-3(2*H*)-one (**4**), as a prototype of naturally occurring auronols, was described in work of Löser et al.[3]. The auronol derivative was prepared by reductive opening of aurone-derived epoxide (**3**) under Pd-catalyzed hydrogenolysis (Scheme 3).



Scheme 3: Sythesis of auronol derivative

Epoxidation and hydrogenolysis of aurone-derived epoxides promises to be an efficient method to prepare auronols and other flavonoids[3].

3.3.1. Epoxidation

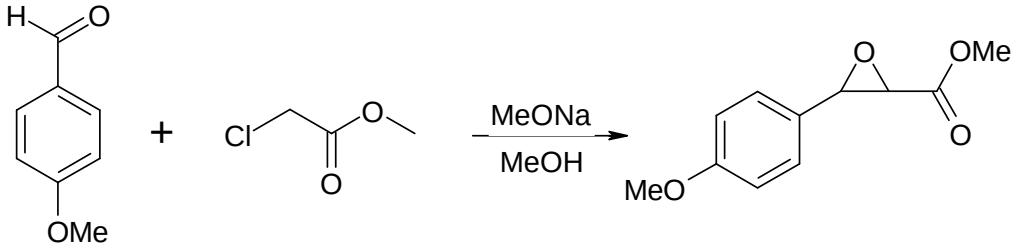
The epoxidation of flavonoid compounds is described in the literature:

1. By means of hydrogen peroxide in the presence of base[4]
2. By means of dimethyldioxirane[5, 6]
3. By means of m-chloroperoxobenzoic acid in benzene[7]
4. By Darzens condensation[4]

Oxidative epoxidation was not the main goal of my work. The first three epoxidation reactions are described in the diploma thesis of David Bolek[22] (2004).

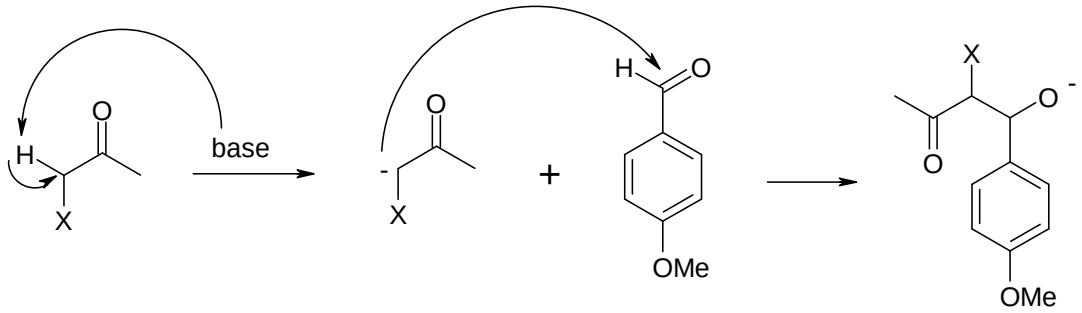
3.3.1.4. Darzens Condensation

Darzens condensation is used to synthesize an epoxide[8]. It was discovered by the organic chemist Georges Darzens (1867-1954), and was first published in 1904 as a glycidic ester condensation[9] (Scheme 4).



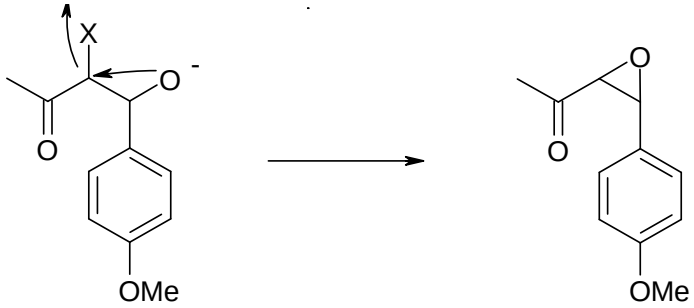
Scheme 4: Darzens condensation

The reaction occurs in two steps. The first step involves the aldol reaction of a halocarbon and an aldehyde (Scheme 5).



Scheme 5: Aldol reaction

The second is an intramolecular nucleophilic substitution via a S_N2 mechanism. The negatively charged oxygen attacks the carbon with the halogen (a leaving group), forming the epoxide (Scheme 6).



Scheme 6: Intramolecular nucleophilic substitution

The Darzens condensation with benzofuranone is further described in the methodical part.

4. Methodical Part

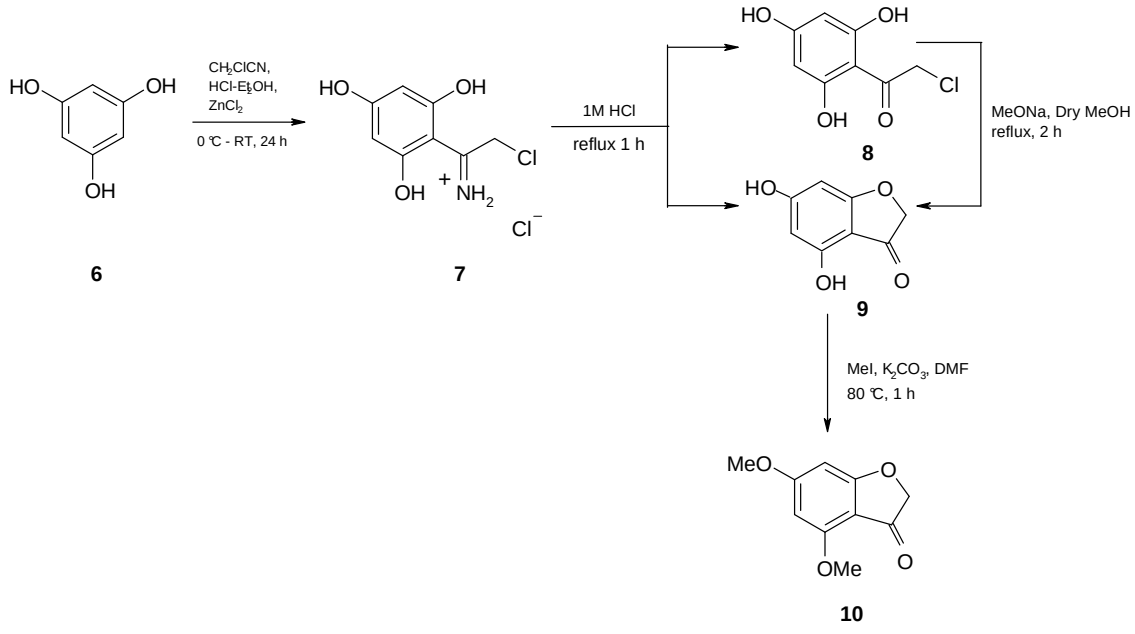
4.1. General Strategies and the Main Objectives

The whole synthesis of 4,6-dimethoxybenzofuranone from phloroglucinol is described in the work of Beney et al.[11] and in the work of Bolek and Gütschow[12]. For bromination, combined methods from the literature[14, 15] were used.

The reactions of 2-bromo-4,6-dihydroxybenzofuran-3(2*H*)-one with aromatic aldehydes by Darzens condensation were not sufficiently described. The only mention is in the work of Brady et al.[4] in the synthesis of flavonoid epoxides. As it was already mentioned there was described only one reaction with 2-nitrobenzaldehyde to give an epoxide as a product. However, as a nitro derivate, this product does not contain the typical substitution pattern of the natural occurring flavonoids. The Darzens reaction with another aldehydes was mentioned, but neither structures of the products nor the NMR data were reported.

4.2. Synthesis of 4,6-Dimethoxybenzofuran-3(2H)-one

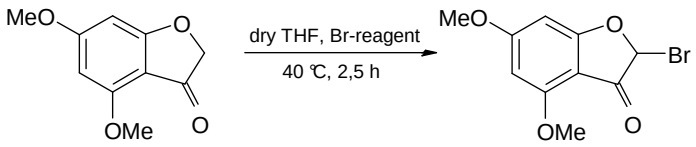
Phloroglucinol (**6**) is condensed to 2´,4´,6´-trihydroxy-2-chloroacethophenon iminium chloride (**7**) by the reaction with 2-chloracetonitrile using dry zinc chloride as catalyst. 4,6-Dihydroxybenzofuranone (**8**) was prepared by the following acid hydrolysis and cyclisation in sodium methoxide from 2–chloro-(2´,4´,6´-trihydroxy)acetophenone (**9**). Methyl iodide under basis conditions was used as an alkylation reagent for alkylation (**10**) (Scheme 7).



Scheme 7

4.3. Synthesis of 2-Bromo-4,6-dimethoxybenzofuran-3(2H)-one

Instead of using a solution of bromine in diethyl ether/dioxan[14] phenyltrimethyammoniumbromide dibromide in dry tetrahydrofuran[15] was used as a better bromination reagent (Scheme 8). PPT has the advantage of high stability and ease of preparation. We used a commercially available reagent. When dissolved in tetrahydrofuran, PTT (like pyridine hydrobromide perbromide) is a source of Br_3^- ions, the properties of which are different from those of molecular bromine. In particular, it is much less electrophilic and less reactive toward aromatic rings and double bonds, and is thus is a better selective brominating reagent when the molecule has double bonds or activated aromatic nuclei which would be attacked by bromine. Reaction of bromine gives a mixture of products what results from ring bromination[15]. 5,7-Dimethoxyflavanone can be brominated in good yield at the position alpha to the keto group, although the aromatic ring is activated by two methoxy groups[16].

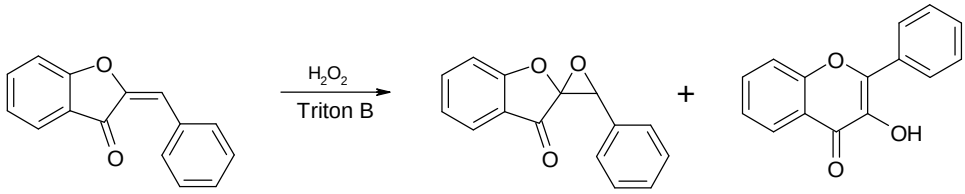


10 **12**

Scheme 8

4.4. Darzens Condensation of 2-Bromo-4,6-dimethoxybenzofuran-3(2H)-one

Brady et al.[4] described oxidation of aurones (**2**) by means of hydrogen peroxide in alkaline solution of sodium or potassium hydroxide, which gives a mixture of aurone epoxide (**3**) and flavonol (**11**) (Scheme 9). These reactions give relatively low yields. By using benzyltrimethylammonium hydroxide instead of alkaline hydroxide, the yield of aurone epoxide was higher. When the duration of the reaction is extended, flavonol is the main product.

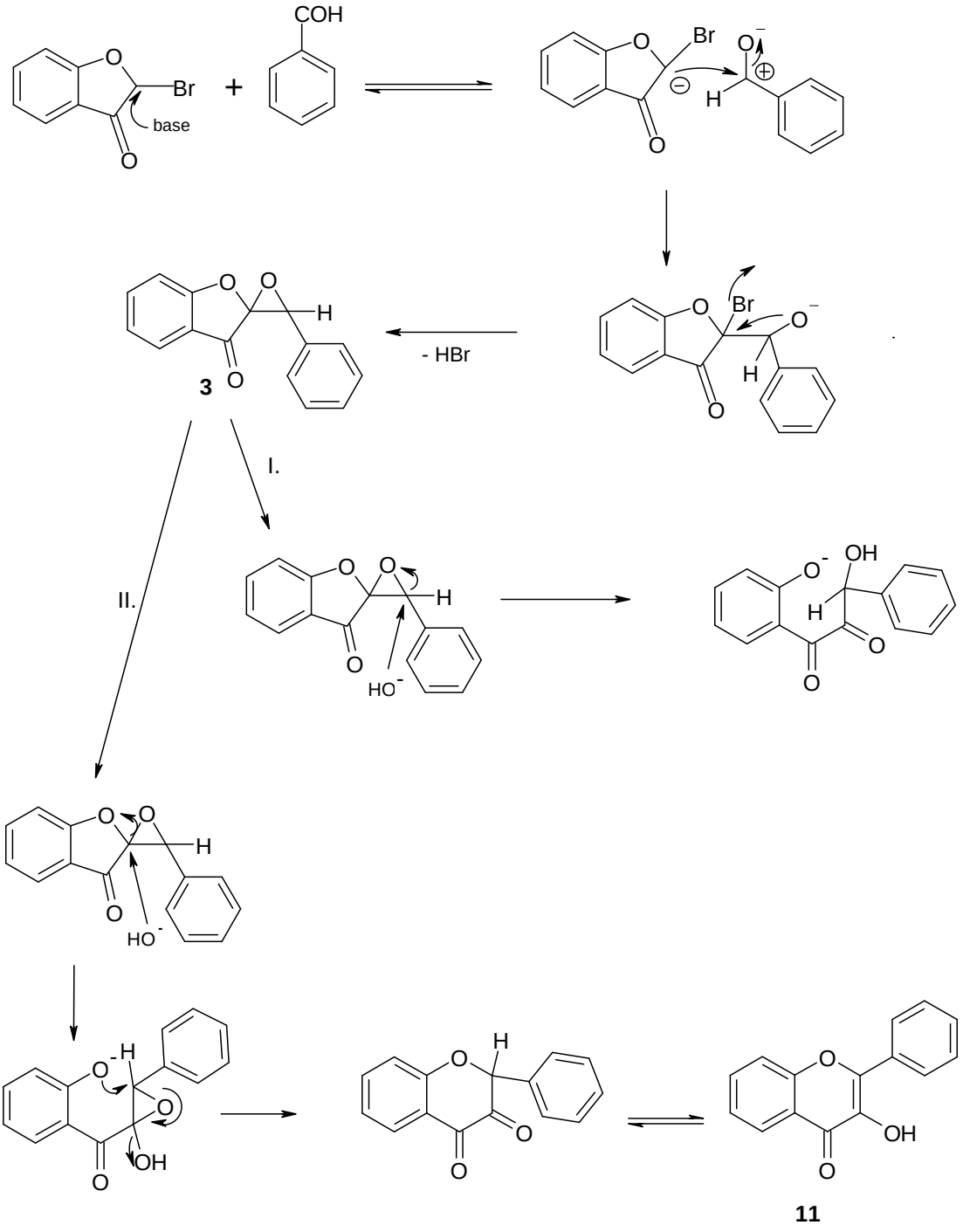


2 **3** **11**

Scheme 9

The mechanism for flavonol formation from aurone epoxides has not been studied yet, though a number of possibilities have been considered. Nucleophiles displace the epoxide oxygen of αβ-epoxyketones by attack either at the α-position or, more frequently, at the β-position (Scheme 10). Possible initial steps in a mechanism for flavonol (**11**) formation from aurone epoxides (**3**) (pathway I.) might involve base attack at the β-position (or at the α-position to give an anion followed by proton migration) to form a ring-open phenolat anion. This mechanism did probably not result in the formation of the flavonol.

The pathway II. is more probable. It involves initial attack at the α-carbon atom, but instead of decyclisation of the oxiran ring, with concomitant release of steric strain, to produce the unstable anion, the furanone ring is decyclised to produce resonance-stabilised acylphenoxide ion. This can then undergo a ring closure to form the six-membered product[4].



Scheme 10

Another study has shown that aurone epoxides may also be synthesized by Darzens condensation. Brady et al.[4] used 2-bromo-6-methoxybenzofuranon-3-one. It was condensed with a number or aryl aldehydes. The best results were obtained with 2-nitrobenzaldehyde. The reactions with other aldehydes are not described.

5. Experimental Part

5.1. General Data

5.1.1. Materials and Apparatus

A Boetius apparatus (VEB Rapido) was used for the determination of melting points, the values were not corrected.

The NMR spectra were measured in the „Department of Pharmaceutical Chemistry, Poppelsdorf, University of Bonn“ on the BRUKER AVANCE 500 MHz instrument. Whereas the ^{1}H-spectra were recorded at 500 MHz, the ^{13}C-spectra were recorded at 125 MHz. The chemical shifts are not introduced directly from tetramethylsilan as inner standard but with the help of residual signal of the solvent.

The elemental analysis was carried out in the „Department of Pharmaceutical Chemistry, Endenich, University of Bonn“ on the Vario EL from ,,Elemental Analysensysteme GmbH”.

The X-ray structure analysis was measured in the „Department of Inorganic Chemistry, Endenich, University of Bonn“ on the KappaCCD-Diffractometer and MACH3-Diffractometer.

Mass spectra were obtained on a API 2000 spectrometer from Applied Biosystems at the “Department of Inorganic Chemistry, Endenich, University of Bonn“

The thin-layer chromatography was carried out using aluminium sheets coated with silica gel 60 F_{254} (Merck). The chromatograms were detected by the help of UV-Lamp Desaga HP-UV/VIS with the wavelength of 254 and 366 nm.

5.1.2. Chemicals

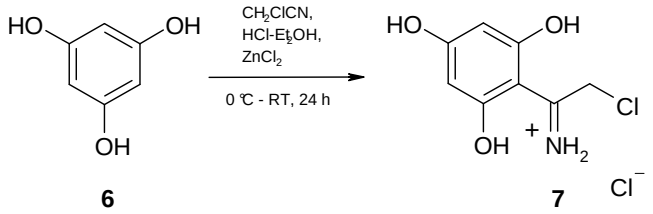
The starting chemicals were bought from Aldrich, Fluka, and Merck.

For the detection of DC the following spray solutions were used:

- $FeCl_3$ – 2 % solution in 0,5 N hydrochloric acid
- I_2/KI – Solution from 13 g of iodine and 20 g potassium iodine in 100 ml of ethanol and water added to one litre.
- HCL – 2 N solution

5.2. Prescriptions

5.2.1. Preparation of 2-Chloro-(2´,4´,6´-trihydroxy)acetophenone Iminium Chloride



6 **7**

Procedure:

A solution of 25.2 g (200 mmoles) phloroglucinol (**6**) in 125 ml of anhydrous diethyl ether was prepared at 0°C. Chloroacetonitrile (15.1 g = 12.7 ml, 200 mmoles), freshly glow zinc chloride (0.9 g, 6.6 mmoles) and a solution of HCl in anhydrous diethyl ether (375 ml, 1M, 375 mmoles) was added. In the course of reaction, a yellow precipitate was formed. It was stirred at 0 °C for 2 hours and then kept stirring for other 20 hours while the ice melted. The precipitate was removed by filtration, washed with anhydrous diethyl ether (2 x 40 ml) and dried to give a yellow solid (25.39 g).

Yield: 54 %

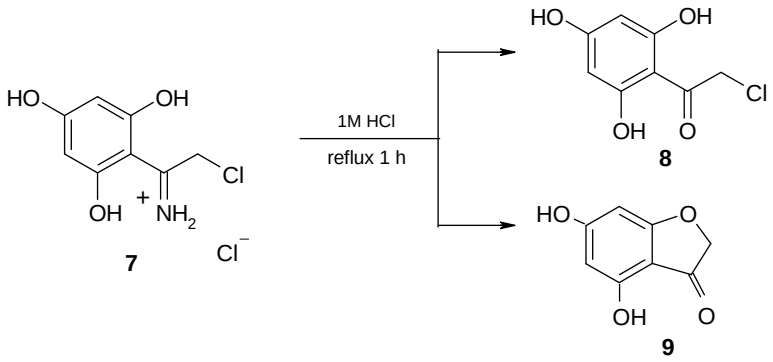
M.p.: 230-234 °C, ref.[12] 230-233 °C

Molecular formula: $C_8H_9Cl_2NO_3$ (M_w = 238,07)

NMR: ^{1}H-NMR (500 MHz, DMSO-d_6): δ (ppm) 5.45 (s, 2H, CH_2), 6.08, 6.24 (each d, J = 1.6 Hz, 1H, 3´-H, 5´-H), 7.52 (s, 2H, NH_2^+), 9.87, 10.80, 12.50 (each s, total 3H, OH)

^{13}C-NMR (125 MHz, DMSO-d_6): δ (ppm) 75.39 (C-2), 90.20, 97.03 (C-3´, C-5´), 99.49 (C-1´), 160.55, 172.99, 173.84, 176.16 (C-1, C-2´, C-4´, C-6´)

5.2.2. Preparation of 2–Chloro-(2´,4´,6´-trihydroxy)acetophenone and 4,6-Dihydroxy-benzofuran-3(2H)-one



Procedure:

A mixture of the salt 2-chloro-(2´,4´,6´-trihydroxy)acetophenone iminium chloride (**7**) (23.8 g, 100 mmoles) and 1M HCl (500 ml) was refluxed for 1 hour. The red solution was kept at 0 °C for 4 hours in the ice-bath and then kept in the fridge overnight. The precipitate was filtered off, wash with ice-cold water (20 ml), kept drying in an open vessel for 24 hours and then in vacuum over phosphorus pentoxide to obtain a pink solid (17.013 g).

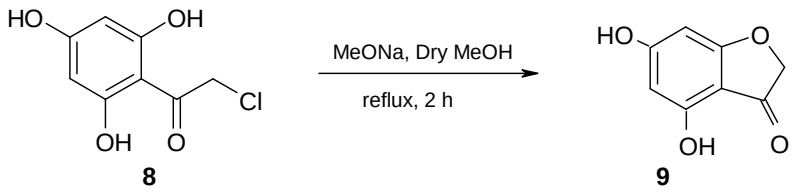
Yield (of the mixture)**:** 92 % (calculated from the approximately ratio of 1:1)

NMR: ^{1}H-NMR (500 MHz, DMSO-d_6): δ (ppm) 4.53 (s, 2H, CH_2), 4.96 (s, 2H, CH_2)*, 5.84 (s, 2H, 3´-H, 5´-H), 5.90, 5.91 (each d, J = 1,7 Hz, 2H, 5-H, 7-H), 10.52 (s, total 3H, OH), 12.04 (s, 2H, OH)*

^{13}C-NMR (125 MHz, DMSO-d_6): δ (ppm) 51.06 (C-2)*, 75.03 (C-2), 90.34, 96.42 (C-5, C-7), 94.94 (C-3´, C-5´)*, 102.70 (C-1´)*, 102.86 (C-3a), 164.12, 164.23, (C-2´, C-6´)*, 165,67 (C-4´)*, 157.66, 167.78, 175.82 (C-4, C-6, C-7a), 194.14 (CO), 194,85 (CO)*

Note *: signals for 2–chloro-(2´,4´,6´-trihydroxy)acetophenone

5.2.3. Preparation of 4,6–Dihydroxybenzofuran–3(2H)-one



8 **9**

Procedure:

Five g (approximately 27 mmoles) of a mixture of 2–chloro-(2´,4´,6´-trihydroxy)–acetophenone and 4,6-dihydroxybenzofuran-3(2*H*)-one (in molar relation of approximately 1:1) were added to a solution of sodium methoxide prepared from sodium (2.1 g, 91.3 mmoles) and dry methanol (50 ml). The red solution was refluxed for 2 hours and became violet. After evaporation under reduced pressure almost to dryness, the residue was partitioned between 1M HCl (180 ml) and ethyl acetate (60 ml). Insoluble material was separated by filtration. The yield was a pure product (1.62 g of a light-pink solid).

Some precipitate went trough the funnel No. 4 and was mainly in the aqueous phase. The organic layer was collected and the aqueous phase was extracted with ethyl acetate (3 x 60 ml), the organic layers were combined and washed with ethyl acetate (90 ml) and brine (90 ml), dried over sodium sulphate. The clear red solution was evaporated to dryness to obtain additional pure product as a light-brown solid (1.917 g). Total yield: 3.52 g.

Yield: 78 %

Mp.: filtrated product: 246 - 248 °C

evaporated product: 247 - 250 °C

ref.[12] 253- 256 °C

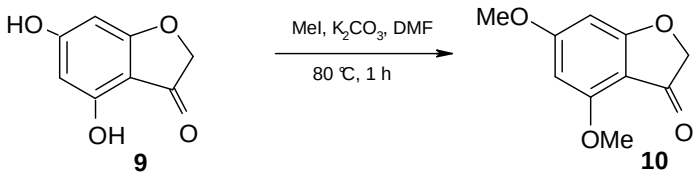
Molecular formula: $C_8H_6O_4$ (M_w = 166,13)

NMR: The identity of both products was checked by NMR.

^{1}H-NMR (500 MHz, DMSO-d_6): δ (pip) 4.53 (s, 2H, CH_2), 5.90, 5.91 (each d, J = 1,75 Hz, 2H, 5-H, 7-H), 10.53 (s, 2H, OH)

^{13}C-NMR (125 MHz, DMSO-d_6): δ (pip) 74.98 (C-2), 90.27, 96.37 (C-5, C-7), 102.82 (C-3a), 157.62, 167.73 (C-4,C-6), 175.76 (C-7a), 194.06 (C-3, CO)

5.2.4. Preparation of 4,6-Dimethoxybenzofuran-3(2H)-one



Procedure:

Methyl iodide (4.9 g, 2.2 ml, 34.5 mmoles), potassium carbonate (3.2 g, 23 mmoles) and dimethylformamide (75 ml) were added to 4,6-dihydroxybenzofuranone (**9**) (1.91 g, 11.5 mmoles). The mixture was stirred at 80 °C for 1 hour. It was evaporated under reduced pressure, and the residue was partitioned between water (150 ml) and ethyl acetate (150 ml). Insoluble material was separated by filtration as a first crude product.

The aqueous phase was extracted with ethyl acetate (1 x 150 ml, 2 x 60 ml), the organic layers were combined, washed with water (100 ml) and brine (100 ml), dried over sodium sulphate and evaporated to dryness to obtain additional crude product.

The combined crude materials (90 %) were recrystallized from ethyl acetate (100 ml) to obtain dark yellow crystals (1.3157 g).

Yield: 59 %

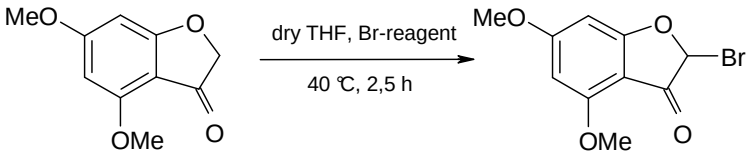
M.p.: 137 – 140 °C, ref.[12] 132- 136 °C

Molecular formula: $C_{10}H_{10}O_4$ (M_w = 194,19)

NMR: ^{1}H-NMR (500 MHz, DMSO-d_6): δ (ppm) 3.82, 3.84 (each s, total 6H, CH_3), 4.64 (s, 2H, CH_2), 6.16, 6.35 (each d, J = 1,9 Hz, 1H, 5-H, 7-H)

^{13}C-NMR (125 MHz, DMSO-d_6): δ (ppm) 56.01, 56.37 (CH_3), 75.39 (C-2), 89.57, 93.03 (C-5, C-7), 104.25 (C-3a), 158.43, 169.36 (C-4, C-6), 176.39 (C-7a), 194.07 (C-3, CO)

5.2.5. Preparation of 2-Bromo-4,6-Dimethoxybenzofuran-3(2H)-one



10 **12**

Procedure:

Dimethoxybenzofuranone (0.7 g, 3.6 mmoles) was dissolved in dry tetrahydrofuran (20 ml). The mixture was stirred at 40 °C in oil bath. Within 20 minutes a solution of phenyltrimethylammoniumbromide dibromide (1.36 g, 3.6 mmoles) in dry THF (10 ml) was added. The mixture was stirred at 40 °C for additional 2 hours. The solvent was evaporated and the residue was dissolved in 120 ml of a 1:1 mixture of dichloromethane/water. The organic layer was washed with water (2 x 40 ml), dried with sodium sulphate and charcoal, filtered and evaporated. The crude material (95 %) was recrystallized from toluene (10 ml) to obtain yellow crystals.

Yield: 40 %

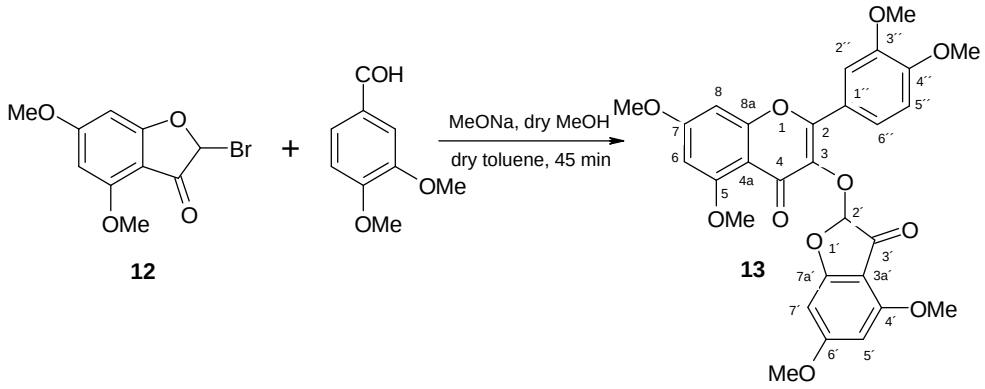
M.p.: 144 – 147 °C, ref.[14] 143 – 145 °C

Molecular formula: $C_{10}H_9O_4Br$ (M_w = 273,085)

NMR: ^{1}H-NMR (500 MHz, DMSO-d_6): δ (ppm) 3.84, 3.82 (s, 3H, CH_3), 5.43 (s, 1H, 2-H), 6.14, 6.25 (each d, J = 1,7, 1H, 5-H, 7-H)

^{13}C-NMR (125 MHz, DMSO-d_6): δ (ppm) 56.06, 56.41 (CH_3), 89.48 (C-2), 92.76, 98.48, (C-5, C-7), 102. 51 (C-3a), 159.04, 170.13 (C-4, C-6), 173.34 (C-7a), 192.49 (C-3, CO)

5.2.6. Darzens Condensation with 3,4-Dimethoxybenzaldehyde



2-Bromo-4,6-dimethoxybenzofuran-3(2*H*)-one (**12**) (1 g, 3.66 mmoles) was dissolved in hot dry toluene (40 ml) and cooled to room temperature. Then 3,4-dimethoxybenzaldehyde (0,61 g) was added. After dissolving, a sodium methoxide solution was added. This solution was prepared from dry methanol (5 ml) and sodium (84 mg). The mixture was stirred for 45 minutes and the precipitate was filtered off and dried.. The precipitate was washed with water (20 ml) and dried. The material (yield: 0.0924 g) was recrystallized from toluene/DMF (3:1, 40 ml) to obtain a pure product (0.0437 g). The material was dissolved in hot ethyl acetate, and the filtrate was kept at room temperature in an open vessel. Slow evaporation of ethyl acetate furnished the crystals.

Yield: 4.6% (2.2% after recrystallization)

M.p.: 213 - 217 °C, conversion 198 - 204 °C, no references

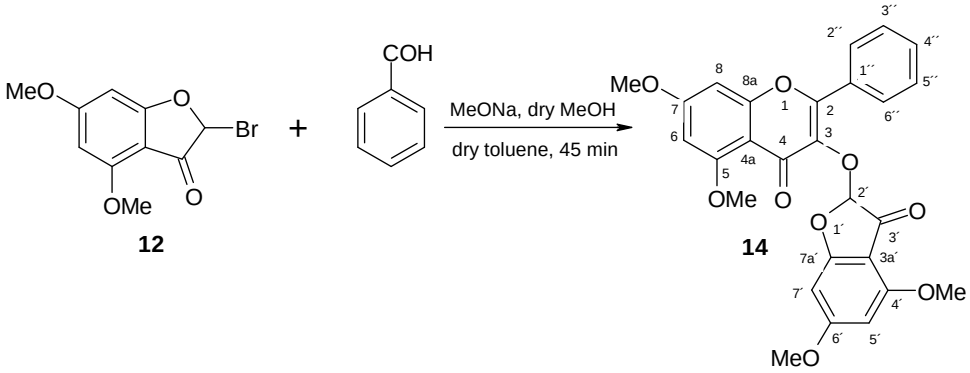
Molecular formula: $C_{29}H_{26}O_{11}$ (M_w = 550,52)

MS (70eV): 550 (M^+, 49%), 358 (16%), 239 (100%)

NMR: ^{1}H-NMR (500 MHz, DMSO-d_6): δ (ppm) 3.50 (3H, s, C-3´´, CH_3), 3.77, 3.78 (3H, s, C-4´´or C-6´, CH_3), 3.85 (3H, s, C-4´, CH_3), 3.86 (3H, s, C-5, CH_3), 3.91 (3H, s, C-7, CH_3), 6.10 (1H, d, J = 1,7 Hz, C-7´), 6.19 (1H, d, J = 1,7 Hz, 5´-H), 6.28 (1H, s, 2´-H), 6.54 (1H, d, J = 2,2 Hz, 6-H), 6.85 (1H, d, J = 2,2 Hz, 8-H), 6.99 (1H, d, J = 8,8 Hz, 5´´-H), 7.44 (1H, d, J = 2,0 Hz, 2´´-H), 7.53 (1H, dd, J_1 = 8,8 Hz, J_2 = 2,0 Hz, 6´´-H)

^{13}C-NMR (125 MHz, DMSO-d_6): δ (ppm) 55.01 (C3´´- CH_3), 55.70 (C4´´or C6´- CH_3), 56.23 (C7 - CH_3), 56.29 (C4´ - CH_3), 56.35 (C5 - CH_3), 56.50 (C-4´´or C-6´- CH_3), 89.88 (C-7´), 93.31 (C-8), 93.65 (C-5´), 96.38 (C-6), 98.45 (C-2´), 101.91 (C-3a), 108.38 (C-4a), 111.37 (C-5´´), 111.69 (C-2´´), 121.64 (C-6´´), 122.19 (C-1´´), 135.39 (C-3), 148.13 (C-3´´), 150.91 (C-4´´), 153.26 (C-2), 158.35 (C-8a), 159.30 (C-4´), 160.54 (C-5), 164.18 (C-7), 170.31 (C-6´), 171.28 (CO, C-4), 173.60 (C-7´a), 188.41 (CO, C-3´)

5.2.7. Darzens Condensation with Benzaldehyde



2-Bromo-4,6-dimethoxybenzofuran-3(2*H*)-one (**12**) (0.5 g, 1.83 mmoles) was dissolved in a high temperature in dry toluene (20 ml) and cooled to room temperature. Then benzaldehyde (0.19 g, 1.83 mmoles, 0.19 ml) was added. After dissolving, a sodium methoxide solution was added. This solution was prepared from dry methanol (2.5 ml) in which metallic sodium (42 mg) was dissolved. The mixture was stirred for 45 minutes and the precipitate was filtered off and dried. The precipitate was washed with water (10 ml) and filtered. The material (0.0363 g from the yield of 0.0604 g) was recrystallized from toluene/DMF (3:1, 30 ml) and after slow evaporation, pure product was obtained (0.0178 g).

Yield: 7% (2% after recrystallization)

M.p.: 213 - 217 °C

Molecular formula: $C_{27}H_{22}O_9$ (M_w = 490,47)

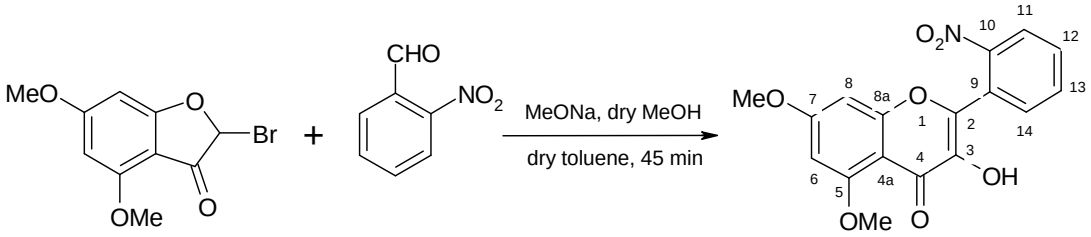
MS (70 eV): 490 (M^+ 95%), 282 (100%)

NMR: ^{1}H-NMR (500 MHz, DMSO-d_6): δ (ppm) 3.79, 3.84, 3.87, 3.90 (3H, s, CH_3), 6.09 (1H, d, J = 1,9 Hz, 7´-H), 6.18 (1H, d, J = 1,9, Hz, 5´-H), 6.55 (1H, d, J = 2,2 Hz, 6-H), 6.84 (1H, d, J = 2,2 Hz, 8-H), 7.36-7.41 (3H, m, aromatic ring), 7.85-7.87 (2H, m, aromatic ring)

^{13}C-NMR (125 MHz, DMSO-d_6): δ (ppm) 56.25, 56.40, 56.53 (CH_3), 89.77 (C-5´), 93.30 (C-6), 93.60 (C-7´), 96.49 (C-8), 98.65 (C-2´), ?102.12 (C-3a), ?108.73 (C-4a), 128.34, 128.42, 130.05, 130.70 (C-aromatic), ?136.18 (C-3), ?145.91, ?149.14 (C-aromatic), 153.63 (C-2), 158.53 (C-8a), 159.24 (C-4´), 160.63 (C-5), 164.32 (C-7), 170.27 (C-6´), 171.45 (C-4), 173.60 (C-7´a), 188.20 (CO, C-3´)

Some signals are badly visible in the NMR spectrum.

5.2.8. Darzens Condensation with 2-Nitrobenzaldehyde



10 **15**

2-Bromo-4,6-dimethoxybenzofuran-3(2*H*)-one (**10**) (0.5 g, 1.83 mmoles) was dissolved in a high temperature in dry toluene (20 ml) and cooled to room temperature. Then 2-nitrobenzaldehyde (0.277 g, 1.83 mmoles) was added. After dissolving, a sodium methoxide solution was added. This solution was prepared from dry methanol (2.5 ml) in which metallic sodium (42 mg) was dissolved. The mixture was stirred for 45 minutes and the precipitate was filtered off and dried. The precipitate was washed with water (10 ml) and filtered. The material (0.2330 g from the yield of 0.2399) was recrystallized from toluene/DMF (3:1, 20 ml) to receive yellow crystals (0,1070 g).

Yield: 38% (17% after recrystallization)

M.p.: 209 – 213 °C

Molecular formula: $C_{17}H_{13}NO_7$ (M_w = 343,29)

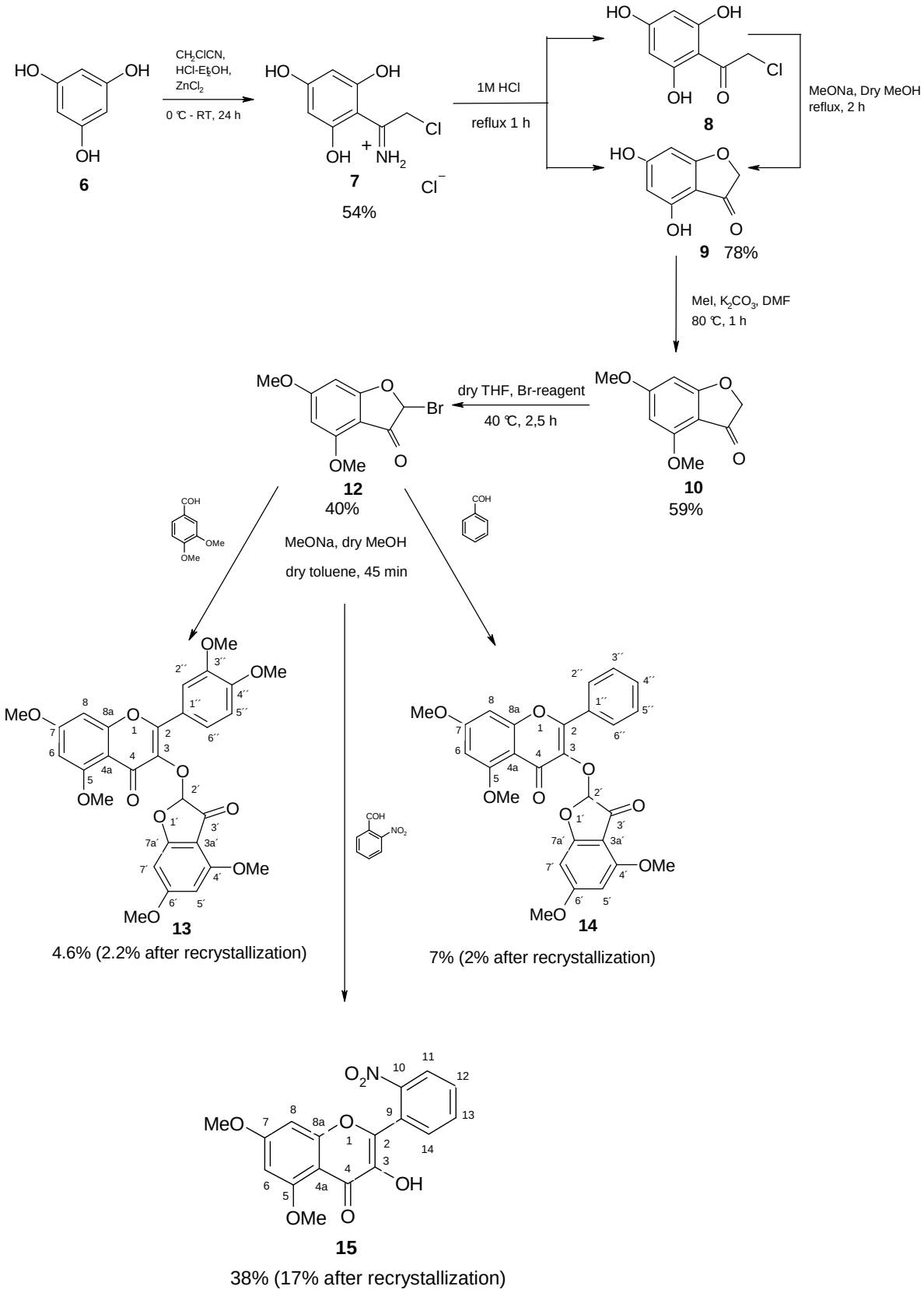
Elemental analysis: calculated: C 59.48%, H 3.82%, N 4.08%

found: C 59.34%, H 4.33%, N 4.36%

NMR: ^{1}H-NMR (500 MHz, DMSO-d_6): δ (ppm) 3.86 (3H, s, CH_3), 3.87 (3H, s, CH_3), 6.51, 6.59 (1H, d, J = 2,2 Hz, 6,8-H), 7.74-7.78 (1H, m, aromatic), 7.86-7.92 (2H, m, aromatic), 8.10 (1H, dd, J_1 = 8,2 Hz, J_2 = 1,3 Hz, 11-H), 9.06 (1H, s, OH)

^{13}C-NMR (125 MHz, DMSO-d_6): δ (ppm) 56.15, 56.45 (CH_3), 92.79, 96.06 (C-6,8), 107.25 (C-4a), 124.69 (C-11), 124.93 (C-9), 131.31, 131.52, 133.51 (C-12, 13, 14), 139.10, 140.44 (C-2, 3), 148.05 (C-10), 158.69 (C-8a), 160.63, 164.03 (C-5, 7), 170.88 (C-4)

5.3. Genaral Scheme of Synthesis



Scheme 11: General Scheme of Synthesis

6. Discussion

6.1. Synthesis of 4,6-Dimethoxybenzofuran-3(2H)-one

This synthesis has been already described and works well with sufficient yields. There was no reason to change the procedure.

6.2. Synthesis of 2-Bromo-4,6-Dimethoxybenzofuran-3(2H)-one

Bromination of benzofuranone was described by using bromine in diethyl ether/dioxan in the work of Benett et al.[14]. But this reaction gives a mixture of two bromo compounds 2-Bromo-4,6-dimethoxybenzofuran-3(2*H*)-one (**12**) and 2,5-Dibromo-4,6-dimethoxybenzofuran-3(2*H*)-one in 62 and 24% yield. We needed to receive only the 2-bromo compound (**12**) as a pure product. We used mild conditions (30-40 °C), time of 2 hours and as a bromination reagent phenyltrimethyammoniumbromide dibromide (Phenyltrimethylammonium tribromide, PTT) in dry tetrahydrofuran (Methodical Part).

The reaction was made under various temperatures and the same time of duration. The temperatures had not big influence on the yield but on the purity of the product (Table 1). The best conditions seem to be temperature of 35-40 °C and time of 2 hours.

Table 1: Dependence of purity on reaction temperature

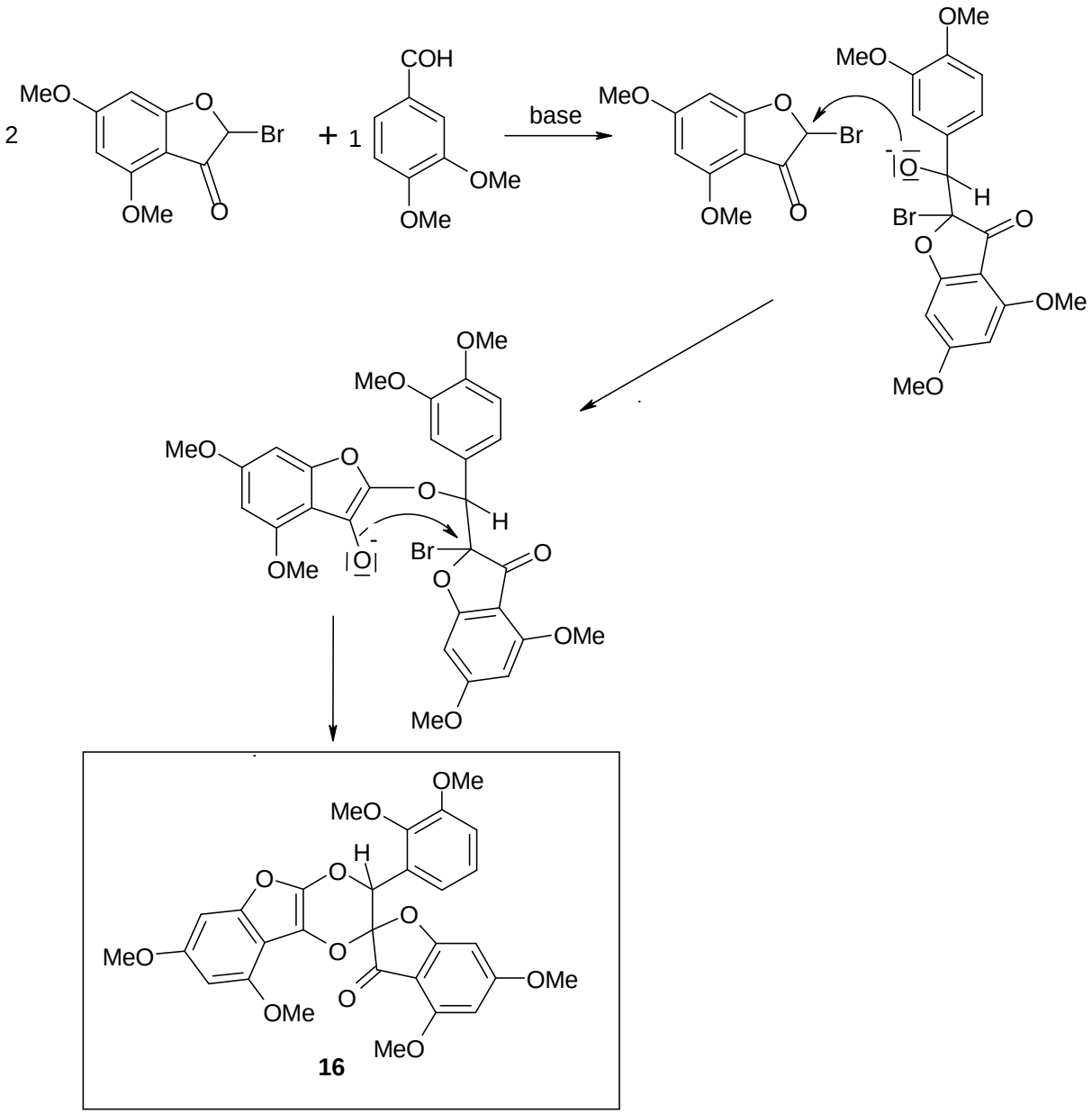
Temperature (°C)	Time (hours)	Yield of recrystallized product (%)	Purity (checked by TLC and NMR)
40	2	30	pure
40	2	35	pure
35	2	44	pure
35	2	40	pure
30	2	37	impure
35	4	52	impure

6.3. Darzens Condensation

6.3.1. Darzens Condensation with 3,4-Dimethoxybenzaldehyde

The main goal of this synthesis was to determine the structure of the product (**13**). It was not easy to assign the structure only from the NMR data, because neither the reaction process nor the product was known.

From the first received NMR data was proposed a structure outgoing from the theoretical course of reaction (Scheme 12) and known facts from NMR data.



Scheme 12

From ^{1}H-NMR it was sure that there must be one singlet hydrogen in the structure because of the only singlet signal (6.28 ppm). Six methoxy groups' signals (55.01 – 56.50 ppm) and number of 29 carbons showed that the ratio of the reacting compounds is 2:1 (bromo compound to benzaldehyde). Because of the only signal in the chemical shift of ketones in the range of 182-215 ppm[17] was the signal of 188,41 ppm, it seemed the structure has only one keto group. All signals were not possible to determine with sure without knowledge of the real structure of the product. It was necessary to prepare crystals good enough for the X-ray structure analysis.

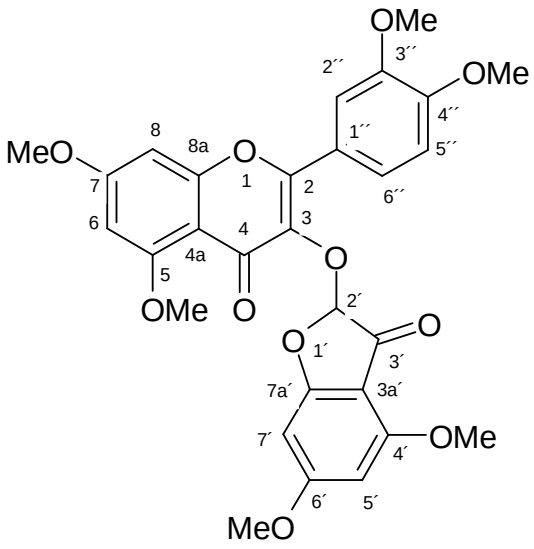
The product is very badly soluble in most of the solvents. Good solubility was in DMF and DMSO. But these solvents are unusable for recrystallization. It is soluble in acetone, toluene, ethyl acetate, ethanol, 1-propanol in high temperatures, but in relatively big amount of solvent. It was used hot toluene and DMF in the ratio of 3:1 as the first solvent for recrystallization. After 3 weeks in the fridge (-20 °C) a precipitate was formed. The precipitate was a very pure product, but the crystals were not good enough.

This pure product was dissolved in hot 1-propanol and recrystallized. After several weeks in the fridge, a precipitate was formed (colourless gel), but no crystals. The solvent was evaporated and the residue was recrystallized this time from ethyl acetate. The small amount of precipitate (floating in the solvent) was filtered off and the filtrate was let to stand in an open vessel to allow the solvent to slowly evaporate. This time crystals were formed and given to Dr. Martin Nieger from the Department of Inorganic Chemistry, University of Bonn, Endenich. Finally we receive the structure (Fig. 15, 16, 17).

The results testified, that a six membered ring is formed, but instead of a 1,4-dioxane derivative (**16**), a flavonol derivative (**13**) was obtained.

It was checked by NMR that the final recrystallized product is the same compound as the pure product, because of the number of hot recrystallizations from several solvents and a possible instability, that might lead to a further reaction.

The spectral assignment (Table 2) was done with the help of the NMR spectrum of quercetin, and those of other flavonol derivatives[18, 33,34], as well as the HSQC spectrum and HMBC spectrum of our product.



13

Table 2: NMR data of 3-[(2,3-Dihydro-4´6´-dimethoxy-3-oxo-2-benzofuranyl)oxy]-5,7-dimethoxy-2-(3´´,4´´-dimethoxyphenyl)-4H-1-benzopyran-4-one

	^{1}H-NMR δ (ppm)	^{13}C-NMR δ (ppm)
C-2	-	153.26
C-3	-	135.39
C-4 (CO)	-	171.28
C-4a	-	108.38
C-5	-	160.54
C-5 (CH_3)	3,86 (s)	56.35
C-6 (H)	6,54 (d, J = 2,2)	96.38
C-7	-	164.18
C-7 (CH_3)	3,91 (s)	56.23
C-8 (H)	6,85 (d, J = 2,2)	93.31
C-8a	-	158.35
C-2´	6,28 (s)	98.45
C-3´ (CO)	-	188.41
C-3a	-	101.91
C-4´	-	159.30
C-4´ (CH_3)	3,85 (s)	56.29
C-5´ (H)	6,19 (d, J = 1,7)	93.65

C-6´	-	170.31
C-6´ (CH_3)	3.77 or 3.78 (s)	55.70 or 56.50
C-7´ (H)	6,10 (d, J = 1,7)	89.88
C-7´a	-	173.60
C-1´´	-	122.19
C-2´´ (H)	7,44 (d, J = 2,0)	111.69
C-3´´	-	148.13
C-3´´ (CH_3)	3,50 (s)	55.01
C-4´´	-	150.91
C-4´´ (CH_3)	3.77 or 3.78 (s)	55.70 or 56.50
C-5´´ (H)	6,99 (d, J = 8,8)	111.37
C-6´´ (H)	7,53 (dd, J_1 = 8,8, J_2 = 2,0)	121.64

Recorded in DMSO-d_6: ^{1}H-NMR at 500 MHz, ^{13}C-NMR at 125 MHz
J in Hz

The only signals we were not able to distinguished with sure were signals of 3.77 or 3.78 ppm.

6.3.2. Darzens Condensation with Benzaldehyde

On the basis of the results with 3,4-Dimethoxybenzaldehyde it was then possible to assign the NMR data of the product obtained with benzaldehyde (**14**).

6.3.3. Darzens Condensation with 2-Nitrobenzaldehyde

From the further works[4] it was expected that the epoxide would be formed. But the NMR data showed no epoxide even in the crude product. It is probably not very stable and reacts in the base immediately to form a flavonole derivate (**15**). But there still exists a question, why NBA does not react into a 2:1 compound as BA or dimethoxybenzaldehyde.

7. Conclusion

The main goal of the work was to determine the reaction product of a Darzens condensation useful for flavonoid syntheses. We have synthesized a new type of flavonoid compounds, which are not described in the literature. The structure was confirmed by X-ray structure analysis and assigned with NMR signals.

We have found that Darzens condensation can be one of the ways to prepare flavonol-derivatives, but in very low yields. The unexpected formation of flavonol derivatives prevented the isolation of epoxides in the case of the reactions with dimethoxybenzaldehyde and benzaldehyd. Therefore, such epoxides are not available to be used in the next step to be transformed through a hydrogenolytic cleavages to auronol derivatives, such as tetramethylalphitonin.

The next step will be testing of biological activity and possible biological utilization of the non-nitro compounds.

8. Summary in Czech Language

8.1. Úvod

Moje práce navazuje na předchozí výzkum Prof. M. Gütschowa a jeho spolupracovníků. Tento tým se zabývá preparativními metodami syntézy prototypů v přírodě se vyskytujících flavonoidů a testováním jejich biologické aktivity. Moje diplomová práce nepřímo navazuje i na výzkum Davida Bolka[22].

Úkolem mé práce bylo objasnit reakci 2-brom-4,6-dimethoxybenzofuran-3(2*H*)-onu s různými aromatickými aldehydy prostřednictvím Darzensovi kondenzace. Tato reakce, jako možná syntetická cesta k derivátům flavonoidů, není v literatuře dosud dostatečně popsána.

8.2. Teoretická část

8.2.1. Obecně

Flavonoidy jsou polyfenolické sekundární metabolity, široce zastoupené v rostlinné říši. Vyskytují se v běžně konzumovaném ovoci, zelenině a nápojích (především v červeném víně, zeleném a černém čaji).

Dietologové se začali více o flavonoidy zajímat v roce 1930, kdy se ukázalo, že flavonoidy z citrusového ovoce snižují kapilární permeabilitu a mají ochranné vlastnosti pro vitamín C[1] a v souvislosti s „Francouzským paradoxem“[13].

Denní příjem flavonoidů[2] se pohybuje v rozmezí od 3 do 70 mg. Většina potravou přijatých flavonoidů se nevstřebává a je ve velkém množství rozložena střevní mikroflórou. Střevní mikroorganizmy hrají důležitou roli nejen v dekonjugaci, ale také v následném rozkladu flavonoidů.

Nejdůležitějším mikroorganismem v tomto ohledu je *Eubacterium ramulus*, striktní anaerob žijící ve střevě člověka. Jako zdroj uhlíku a energie využívá kvercetin-3-glucosid (isokvercetin). Bylo dokázáno, že degradace kvercetinu na fluoroglucinol (**6**) a kyselinu (3,4-dihydroxyfenyl)pyrohroznovou probíhá přes hydroxyflavanon taxifolin a derivát auronolu – alphitonin (viz str. 8, Schéma 2).

Pro studium bioaktivity flavonoidů a jejich další biologické studie je nezbytné vyvinout takové preparativní metody, díky kterým je bude možné získat v dostatečném množství.

8.2.2. Bioaktivita flavonoidů

Flavonoidy mají široké spektrum biologické aktivity:

- Antioxidační aktivita
- Pozitivní působení při ischemické chorobě srdeční
- Cévní aktivita (protiagregační, protizánětlivá)
- Cytotoxická protinádorová
- Antibakteriální, antimykotická, antivirotická, antiprotozoická aj.

8.2.3. Syntéza derivátů flavonoidů

Löser et al.[3] připravili derivát auronolu reduktivním otevřením auron-epoxidu (**3**) hydrolýzou katalyzovanou paladiem (viz str. 10, Schéma 3). Na základě tohoto výzkumu se epoxidace a hydrogenolýza epoxidů auronových derivátů staly slibnými metodami pro přípravu auronolů a jiných flavonoidů[3].

Epoxidace flavonoidů jsou v literatuře popsány několika způsoby. Mým úkolem však nebyly epoxidační reakce všeobecně, ale pouze Darzensova kondenzace, jako jeden z možných způsobů přípravy derivátů flavonoidů, dosud dostatečně neprozkoumaný. Darzensova kondenzace probíhá ve dvou krocích. První krok zahrnuje aldolovou reakci halogenovaného uhlíku a aldehydu. (viz str. 11, Schéma 5). Druhým krokem je intramolekulární substituce, kdy negativně nabitý kyslík napadne uhlík nesoucí halogen jako odstupující skupinu za tvorby epoxidu (viz str. 11, Schéma 6).

8.3. Metodická část

8.3.1. Syntéza

Syntéza vychází z fluoroglucinolu (**6**), který je kondenzován na 2´,4´,6´-trihydroxy-2-chloracetofenon (**7**) použitím aktivovaného chloridu zinečnatého jako katalyzátoru. 4,6-Dihydroxybenzofuranon (**8**) byl připraven kyselou hydrolýzou a cyklizací v methoxidu sodném z 2-chlor-(2´,4´,6´-trihydroxy)acetofenonu. Pro alkylaci byl použit methyljodid v bazickém prostředí. Pro bromaci byl použit fenyltrimethylamonium-tribromid v bezvodém THF jako selektivní bromační činidlo pro bromaci do polohy 2 (viz str. Schéma 7 a 8).

V literatuře[4] je popsána oxidace auronu (**2**) prostřednictvím peroxidu vodíku v alkalickém roztoku hydroxidu sodného nebo draselného. Tato reakce dává vznik směsi epoxidu auronu a flavonolu (Schéma 9). Tato reakce probíhá v nízkých výtěžcích, použitím benzyltrimethylamonium-hydroxidu se výtěžek epoxidu zvýší. Při prodloužení reakčního času, je hlavním produktem flavonol (**11**).

Mechanismus formace flavonolu z epoxidu auronu (**3**) nebyl dosud prostudován. Nukleofil nahradí epoxidický atom kyslíku atakem na α-pozici nebo častěji na β-pozici (viz str. Schéma 10).

Jiná studie ukázala, že epoxid auronu může být také syntetizován Darzensovou kondenzací. Brady a kol.[4] použili 2-brom-6-methoxybenzofuranon-3-on, který podrobili kondenzaci s arylaldehydy. Nejlepšího výsledku dosáhli s 2-nitrobenzaldehydem. Reakce s jinými aldehydy nebyly popsány.

8.4. Diskuse a závěr

Hlavním cílem mé práce bylo určit reakční produkt Darzensovi kondenzace, který by bylo možné použít k syntéze flavonoidů. Určit strukturu ze samotného výsledku NMR analýzy nebylo možné, protože jak proces reakce, tak i produkt samotný byl neznámý. Z prvních NMR dat byla navržena teoretická struktura (viz str. Schéma 12). Rozhodující byl jediný singletový signál (6.28 ppm), poukazující na přítomnost vodíkového atomu a šest signálů pro methoxyskupinu. Protože jediný signál v rozmezí chemických posunů ketonů (182-215 ppm)[17] byl signál 188,41 ppm, zdálo se, že struktura má jen jednu ketoskupinu. Nebylo možné určit s jistotou všechny signály bez znalosti skutečné struktury. Proto bylo nezbytně nutné připravit krystaly vhodné pro krystalografickou analýzu. Vhodné krystaly jsem připravila pomalým odpařením etylacetátu. Dr. M. Niegerem byla následně určena struktura produktu (viz str., Obr. 15, 16, 17).

Výsledky potvrdily vznik šestičlenného kruhu, ale místo očekávaného derivátu 1,4-dioxanu (**16**) vznikl derivát flavonolu (**13**). Signál pro druhou ketoskupinu se objevil vlivem chemického posunu ve vyšším poli (171.28 ppm).

Pro následné určení signálů (viz str. Tabulka 2) bylo použito naměřené NMR spektrum kvercetinu, spektra derivátů flavonoidů uvedené v literatuře[18, 33,34] a HSQC a HMBC spektrum našeho produktu.

V případě 2-nitrobenzaldehydu se nepodařilo prokázat vytvoření epoxidu, popisované v literatuře[4].

Podařilo se nám získat nový typ flavonoidní sloučeniny, který není doposud popsán v literatuře. Jeho struktura byla potvrzena krystalografickou analýzou a popsána pomocí NMR spektra.

Na základě mé práce bylo zjištěno, že Darzensova kondenzace může být s úspěchem použita jako jedna z cest pro přípravu flavonolového derivátu, ale ve velice nízkých výtěžcích. Neočekávaná formace flavonolového derivátu znemožňuje izolaci epoxidu v případě reakce s dimethoxybenzaldehydem a benzaldehydem. Proto tyto epoxidy není možné použít pro hydrogenolytické štěpení na auronolové deriváty (tetramethylalphitonin).

Dalším krokem bude testování biologické aktivity pro možné biologické využití sloučenin neobsahující nitroskupinu.

8. NMR Spectra

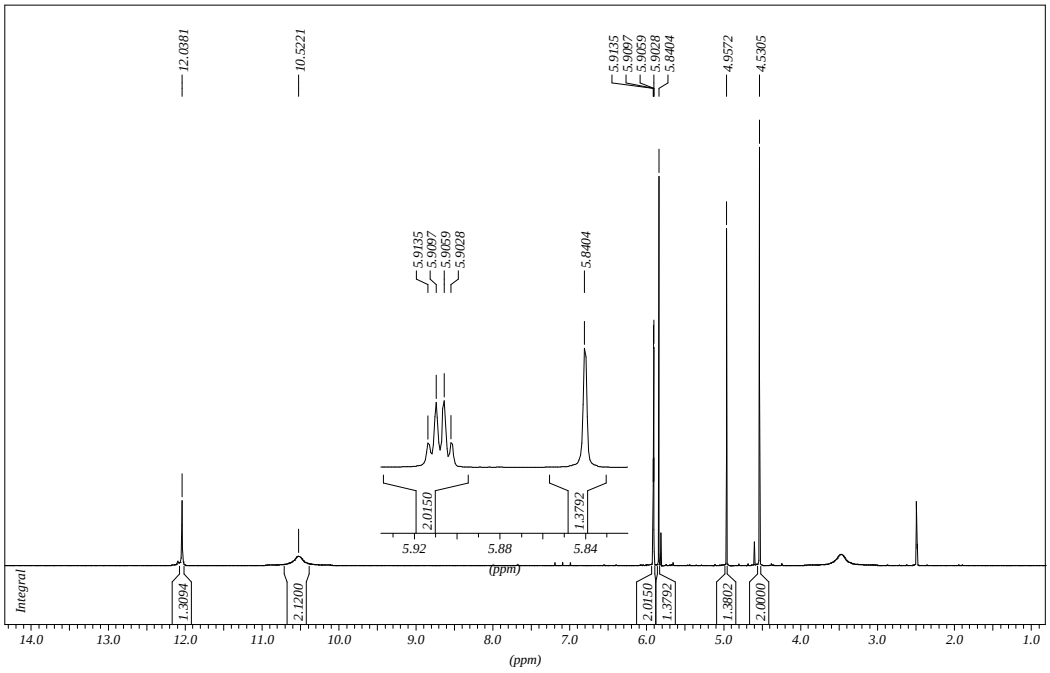


Fig.1: ^{1}H-NMR of 2´, 4´,6´-Trihydroxy–2–chloroacetophenone and 4,6-Dihydroxybenzofuran-3(2*H*)-one

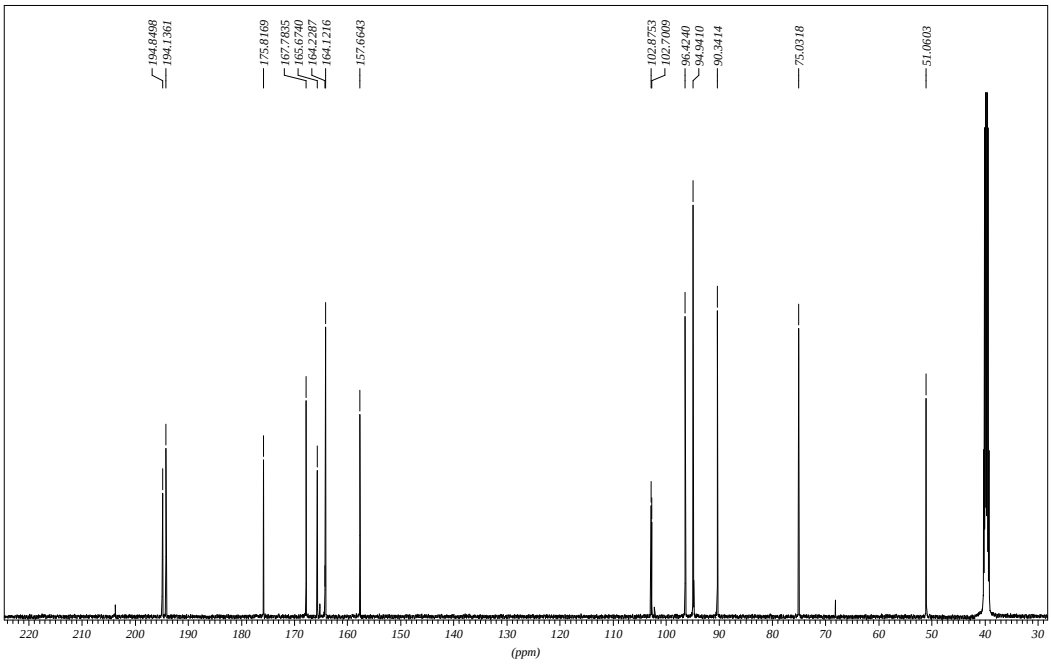


Fig.2: ^{13}C-NMR of 2´,4´,6´-Trihydroxy–2–chloroacetophenone and 4,6-Dihydroxybenzofuran-3(2*H*)-one

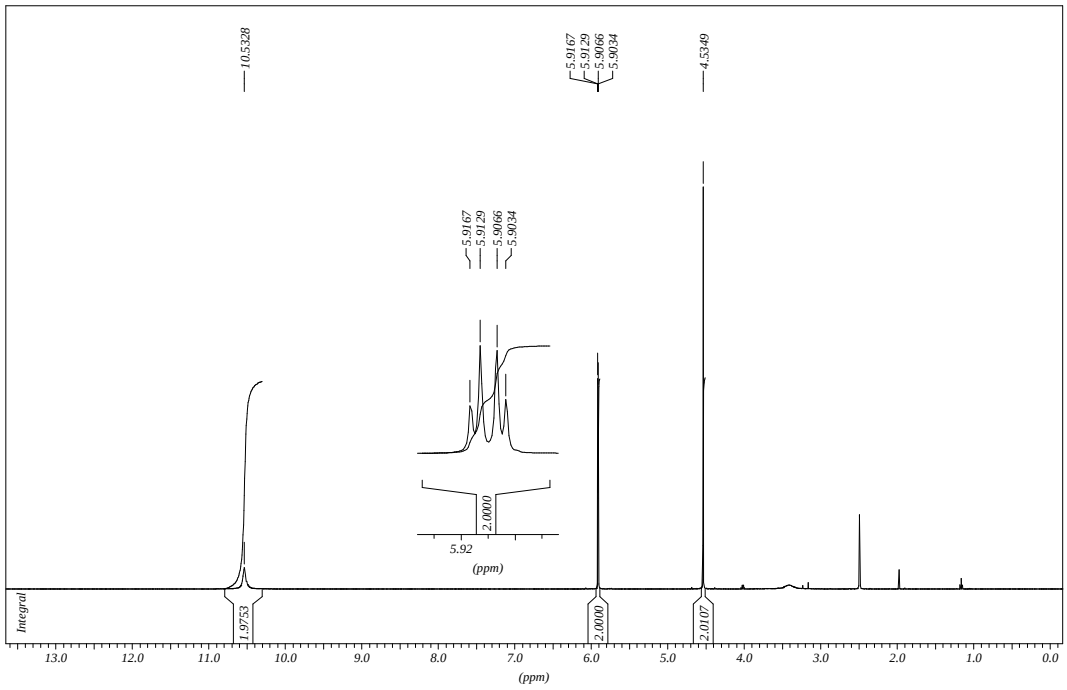


Fig.3: ^{1}H-NMR of 4,6–Dihydroxybenzofuran–3(2*H*)-one

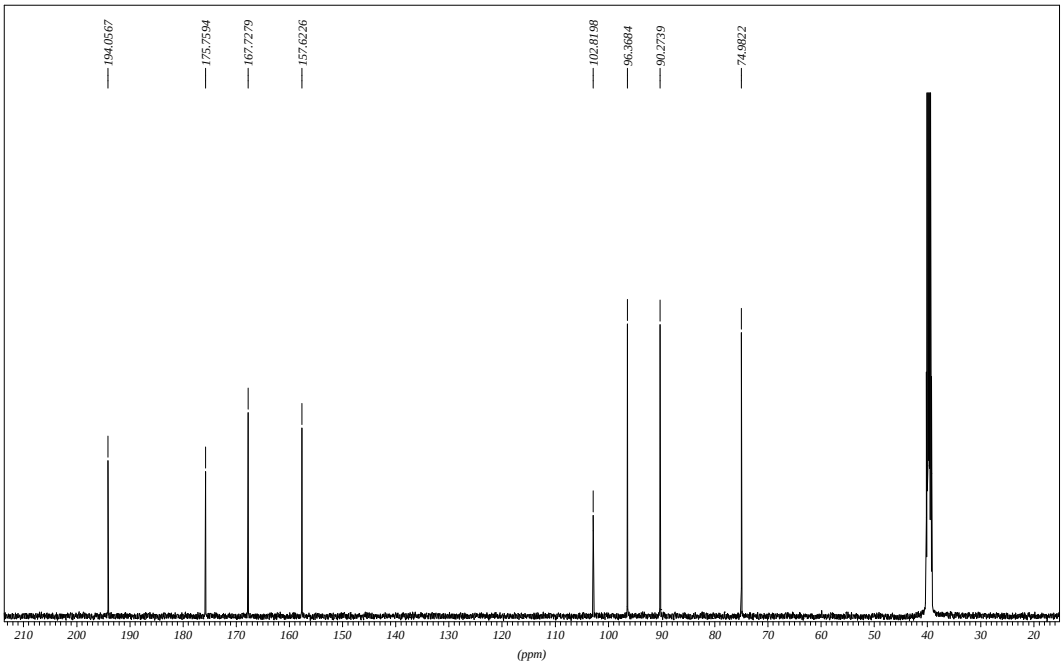


Fig.4: ^{13}C-NMR of 4,6–Dihydroxybenzofuran–3(2*H*)-one

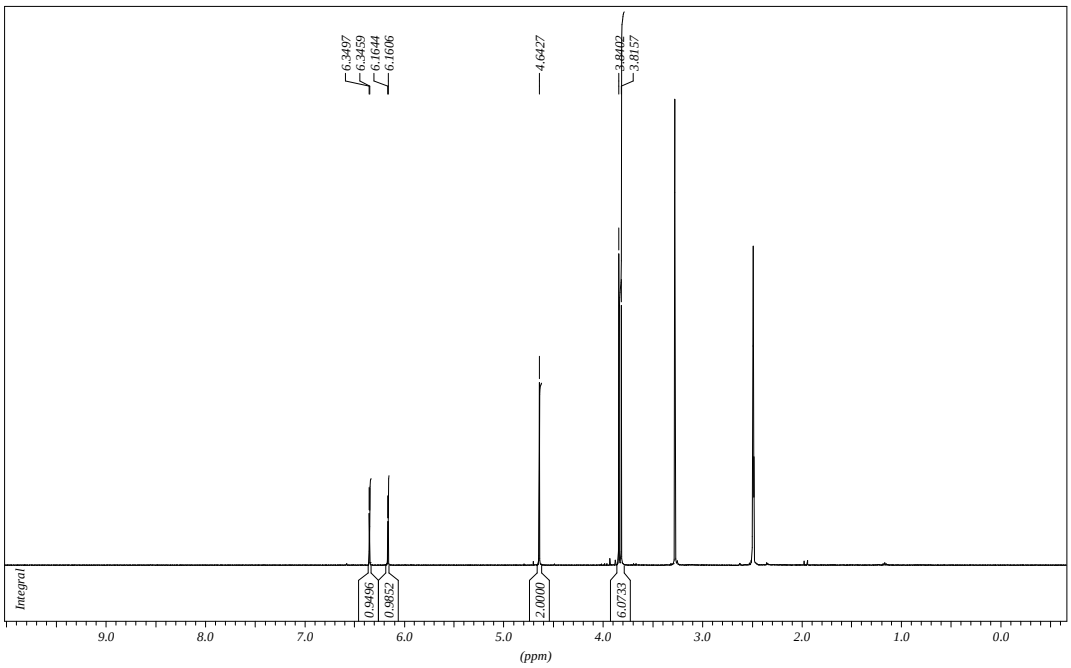


Fig.5: ^{1}H-NMR of 4,6-Dimethoxybenzofuran-3(2*H*)-one

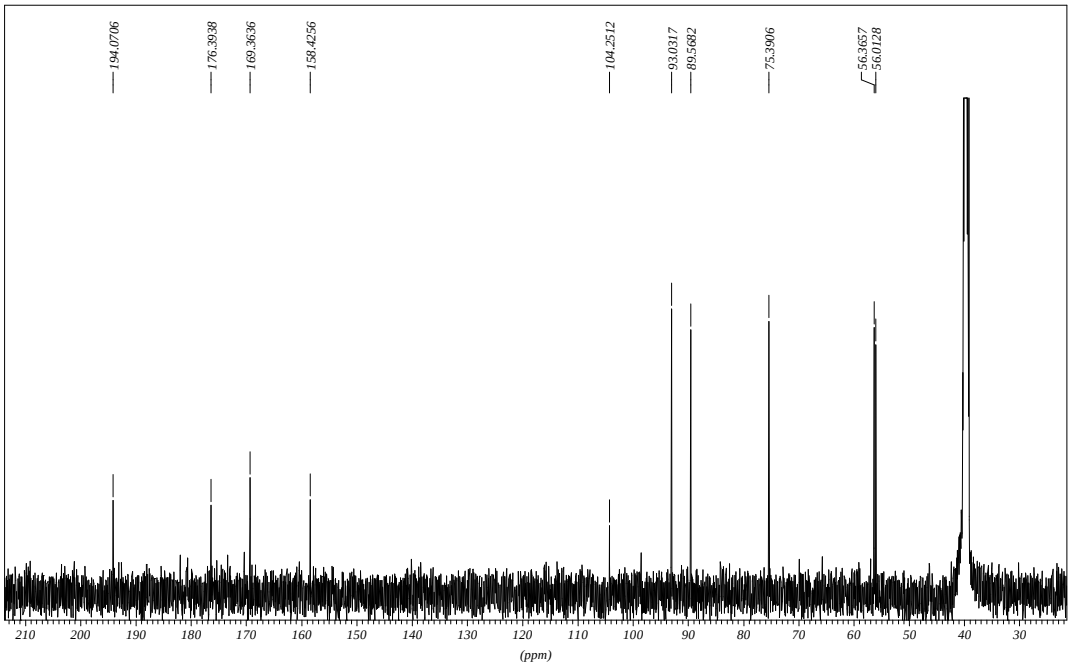


Fig.6: ^{13}C-NMR of 4,6-Dimethoxybenzofuran-3(2*H*)-one

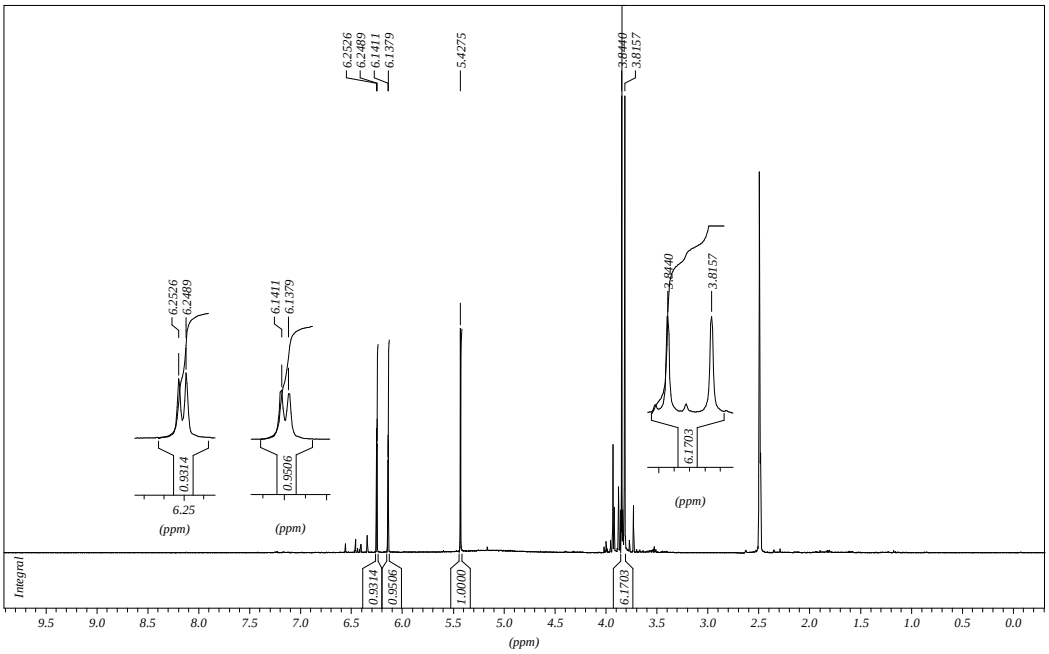


Fig.7: ^{1}H-NMR of 2-Bromo-4,6-Dimethoxybenzofuran-3(2*H*)-one

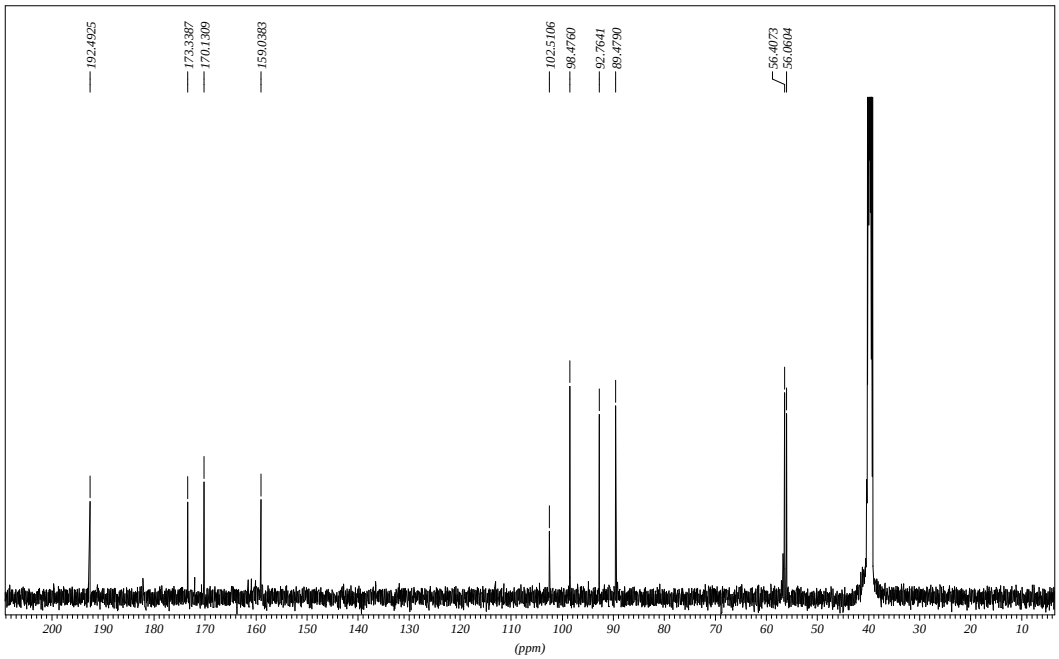


Fig.8: ^{13}C-NMR of 2-Bromo-4,6-Dimethoxybenzofuran-3(2*H*)-one

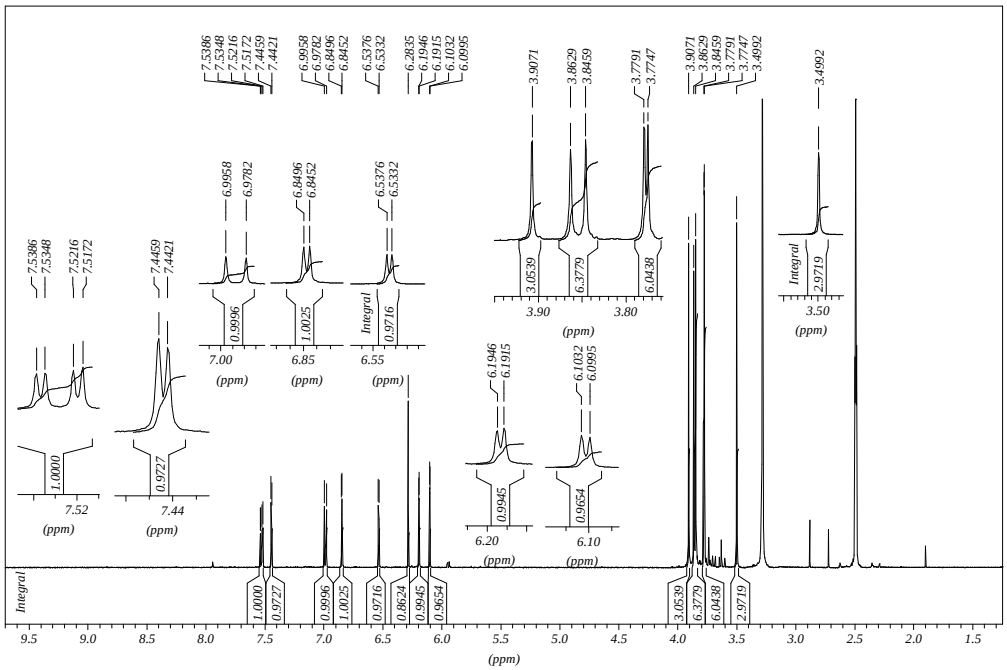


Fig.9: [1]H-NMR of Darzens Condensation Product with 3,4-Dimethoxybenzaldehyde

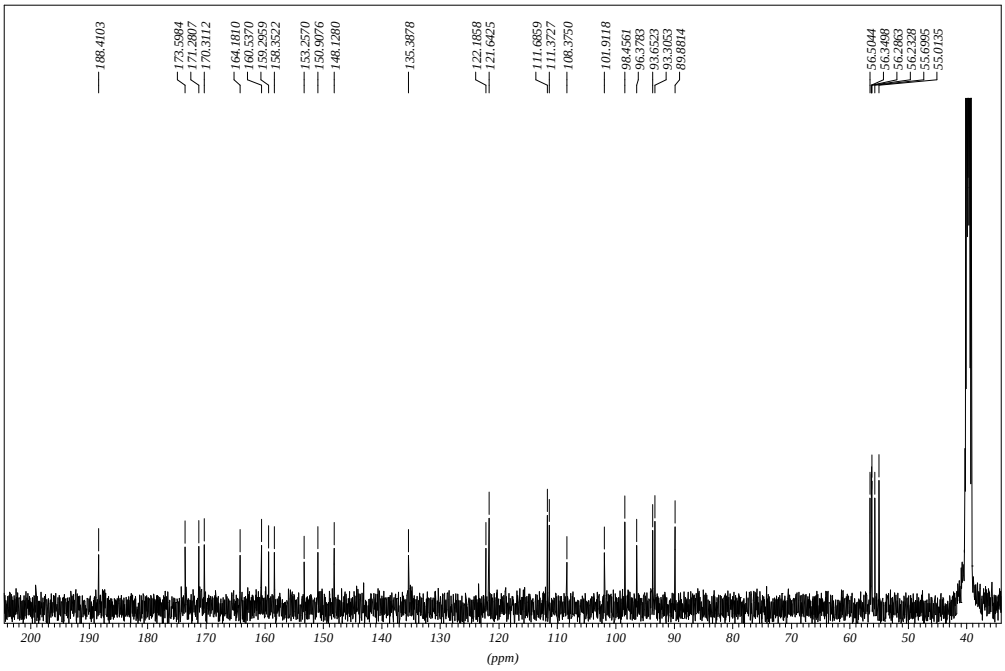


Fig.10: [13]C-NMR of Darzens Condensation Product with 3,4-Dimethoxybenzaldehyde

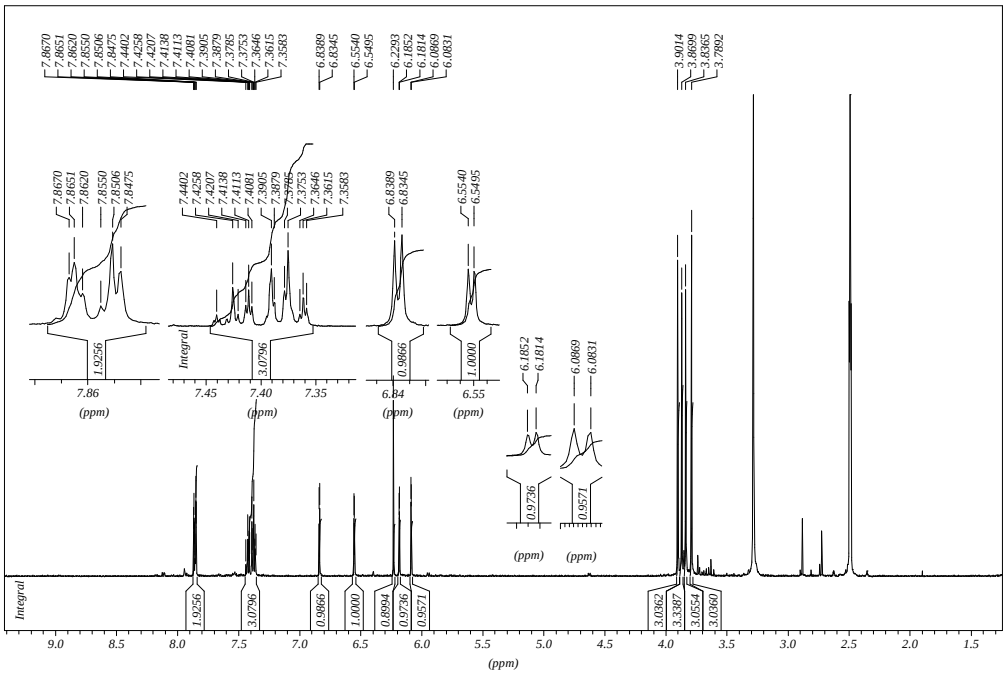


Fig.11: ^{1}H-NMR of Darzens Condensation Product with Benzaldehyde

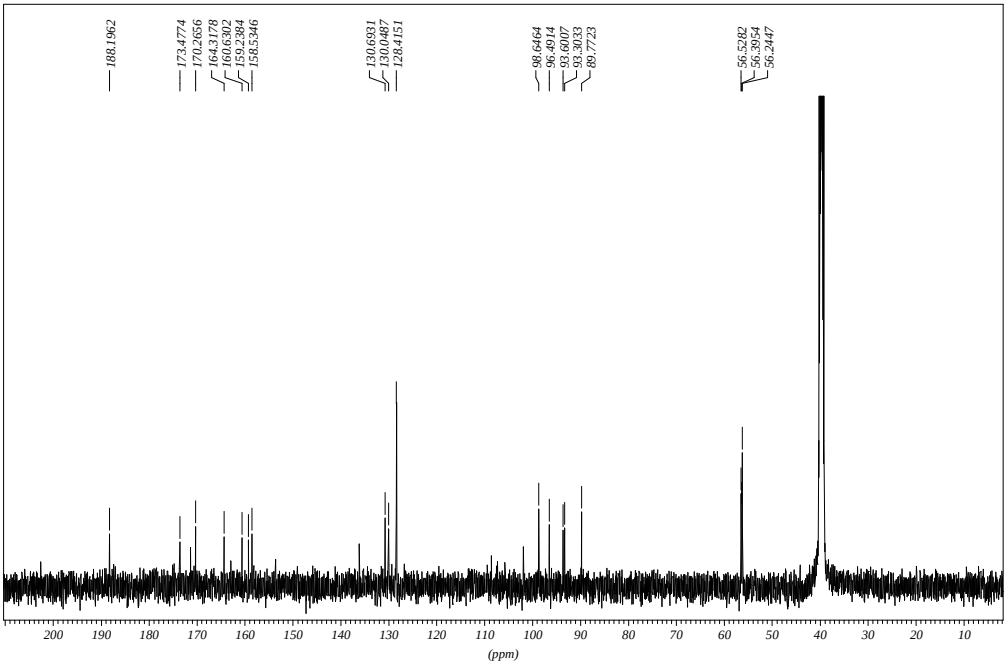


Fig. 12: ^{13}C-NMR of Darzens Condensation Product with Benzaldehyde

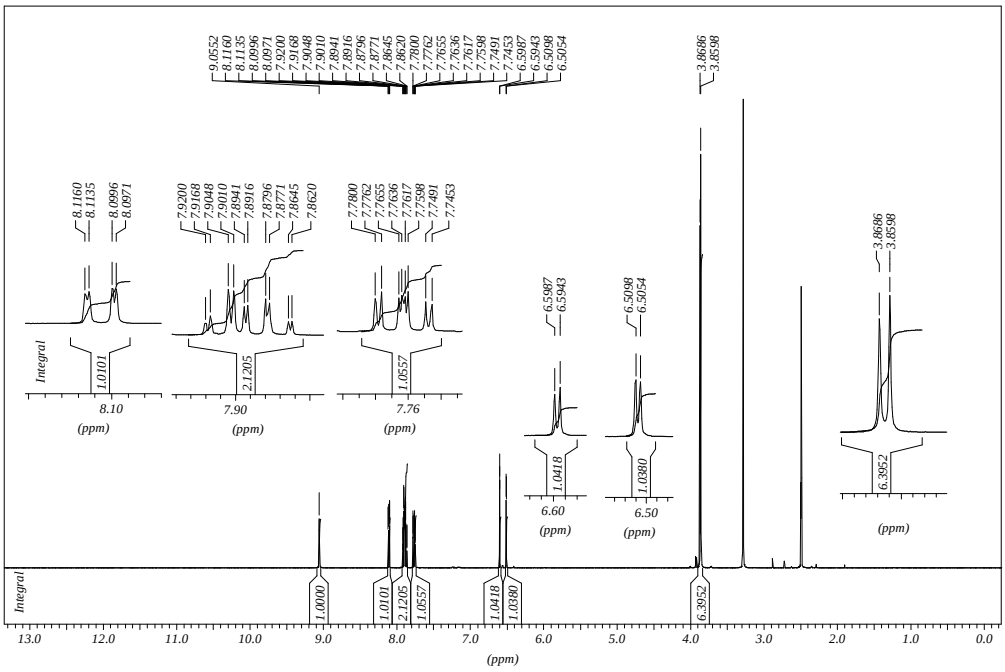


Fig.13: ^{1}H-NMR of Darzens Condensation Product with 2-Nitrobenzaldehyde

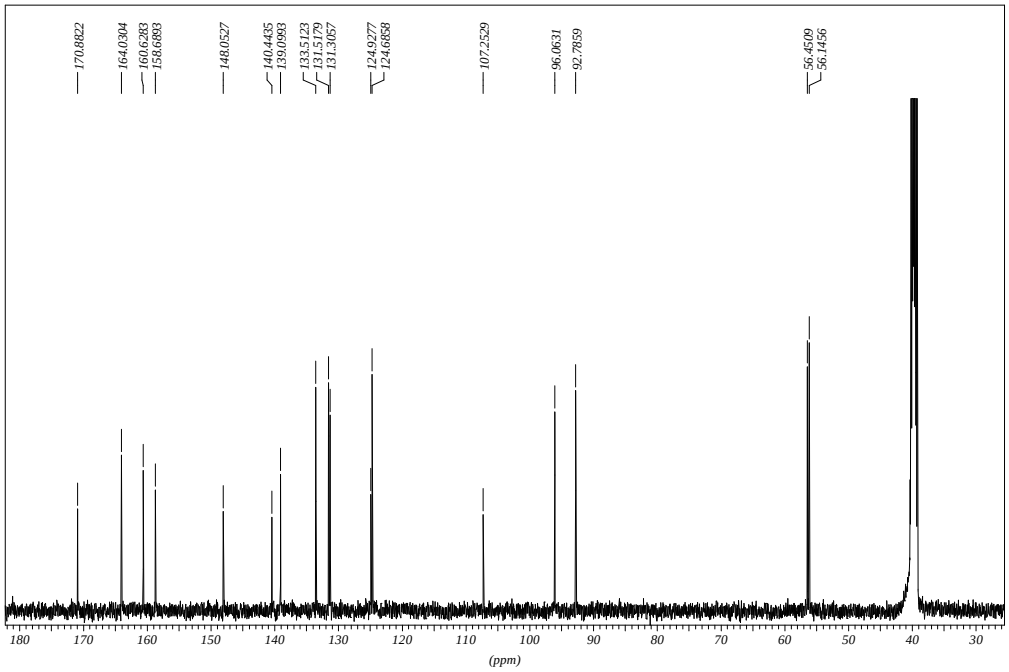


Fig.14: ^{13}C-NMR of Darzens Condensation Product with 2-Nitrobenzaldehyde

9. X-ray Crystal Structure

3-[(2,3-Dihydro-4´6´-dimethoxy-3-oxo-2-benzofuranyl)oxy]-5,7-dimethoxy-2-(3´´,4´´-dimethoxyphenyl)-4*H*-1-benzopyran-4-one

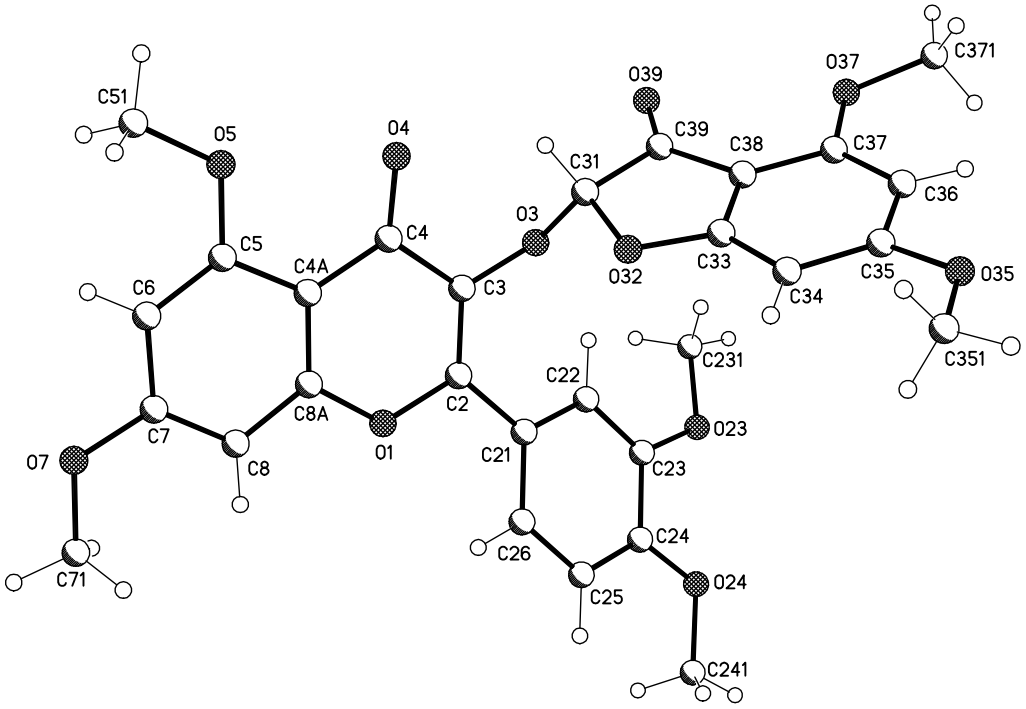


Fig. 15

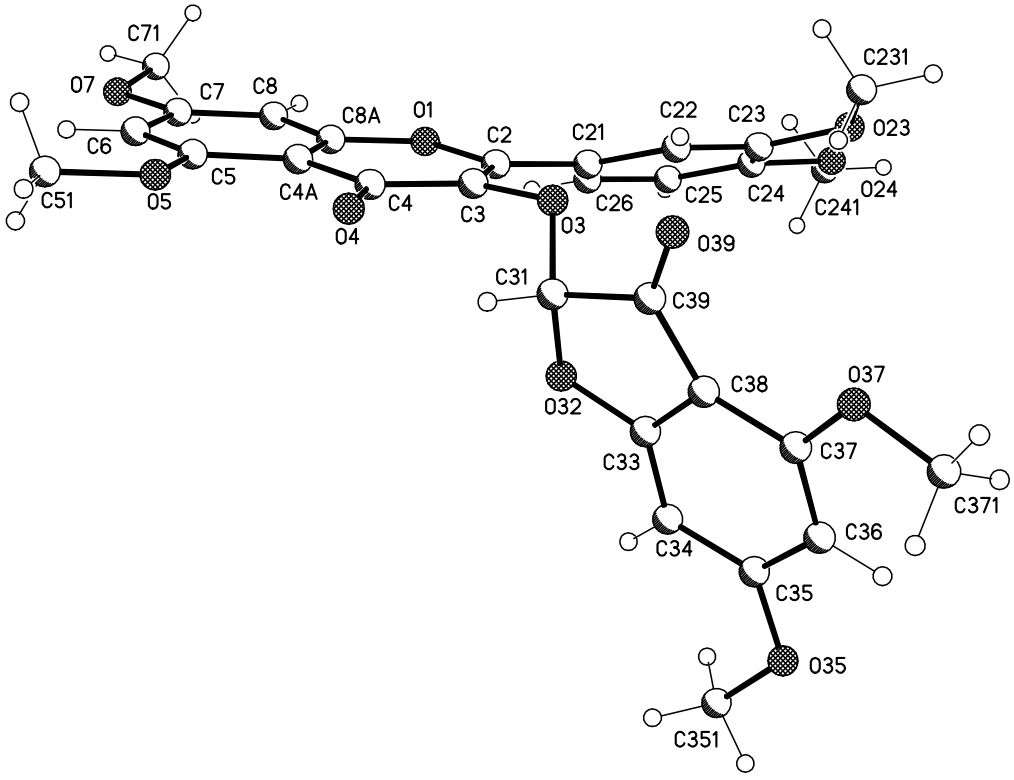

C71
O7
C7
C8
C8A
O1
C2
C21
C22
C23
C231
O23
C6
C5
C4A
C4
C3
O3
C26
C25
C24
C241
O24
C51
O5
O4
O39
C31
C39
O32
C38
O37
C33
C37
C371
C34
C36
C35
O35
C351

Fig. 16

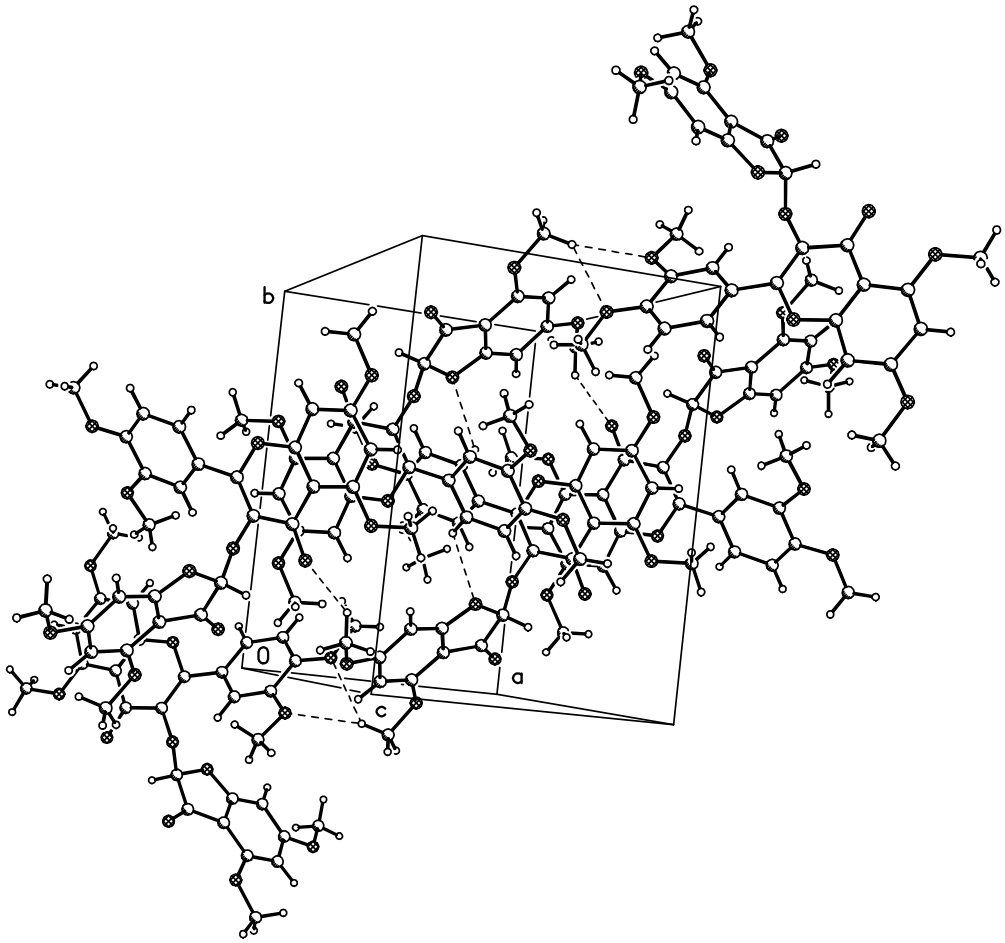


Fig. 17

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