

Calcium Dobesilate in Prevention and Treatment of Diabetic Retinopathy

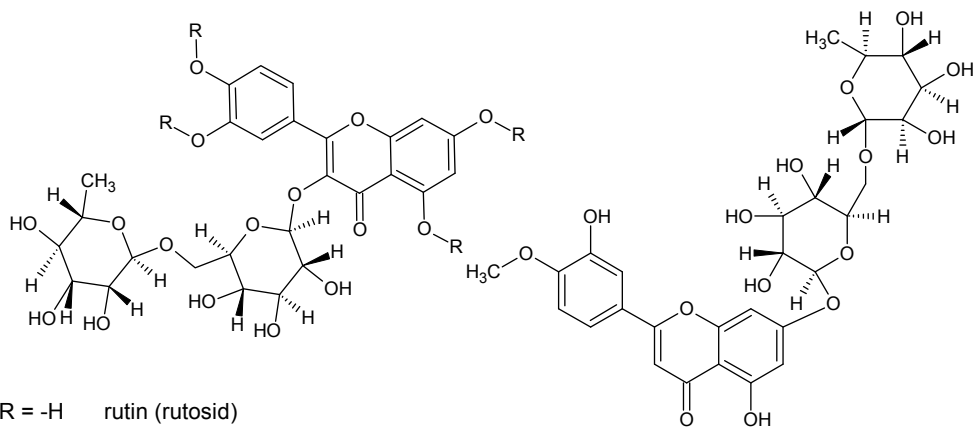
Oldřich Farsa

*Faculty of Pharmacy, University of Veterinary and Pharmaceutical Sciences Brno
Czech Republic*

1. Introduction

Diabetic retinopathy is a leading cause of adult vision loss and blindness. Angioprotective drugs are one of possibilities, which can be used in treatment of diabetic retinopathy. Nearly all of them are very efficient antioxidants or reactive oxygen and nitrogen species (RONS) scavengers. They often contain phenolic groups. Most of them are glycosides of plant origin with aglycones of flavonoid structure, i.e. aglycones are polyhydroxylated derivatives of 2-phenyl-4H-chromen-2-one or 2-phenyl-4H-1-benzopyran-4-one. Quercetin, which is the aglycone of many glycosides including rutoside, can serve as a typical example. Other glycosides, such as a saponoside escine (Reparil®) isolated from seeds of horse chestnut *Aesculus hippocastaneum*, have triterpenic aglycones. (In fact, escin is a mixture of several related compounds. The particular structure on the Figure I express escin I that is a mixture of two geometric isomers. They differ in *E* or *Z* configuration of 2-methylbut-2-enoyl, which is attached to originally hydroxyl oxygen in position 21 β of the triterpenoid scaffold.) (Kim et al., 2004, Carrasco & Vidrio, 2007). Flavonoid glycosides have poor bioavailability that is due to their low lipophilicity. This problem has been solved by etherification of phenolic groups of flavonoid aglycones with hydroxyalkyl such as hydroxyethyl groups (troxerutin) in past (Agolini & Cavallini, 1987, Wadworth & Faulds, 1992) Troxerutin was demonstrated to attenuate neovascularization in retinopathy in streptozocin-induced diabetic rats (Chung et al., 2005). More recently, increased bioavailability of flavonoid glycosides is reached by micronisation without changing their chemical structures. Preparations such as Detralex® or Daflon® contain standardized and micronized flavonoid fraction characterized by its content of diosmin, which is their main active constituent. Their activity in the treatment of diabetic retinopathy was clearly demonstrated (Lacombe et al., 1989). There is nearly only one exception among these quite complex compounds: calcium dobesilate (Danium®, Doxium®, Dexium®), which is a very simple synthetic molecule: calcium 2,5-dihydroxybenzenesulfonate (see Figure 1)

Calcium dobesilate is also almost the only one angioprotective drug that recently remains the object of interest in treatment of diabetic retinopathy including clinical studies (Leal et al. 2010, Einarsdottir & Stefansson, 2009, Ribeiro et al., 2006), and even it was used as a standard for determination of the particular activity of a traditional Chinese herbal medicine preparation (Luo et al. 2009).

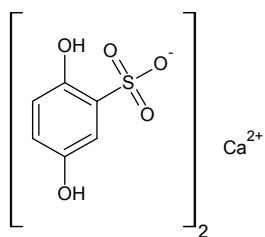


R = -H rutin (rutosid)

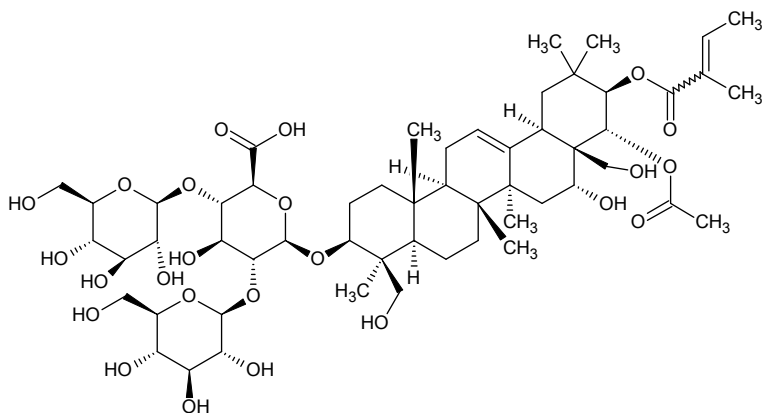
R = -OCH₂CH₂OH troxerutin

free aglycone with R = -H and free
-OH in pos. 3 = quercetin

diosmin



calcium dobesilate



escin I

Fig. 1. Calcium dobesilate as the simplest structure among angioprotective drugs.

2. Calcium dobesilate: Chemical, historical and bibliographical data

2.1 Early syntheses of 2,5-dihydroxybenzenesulfonic acid and its salts

The first synthesis of 2,5-dihydroxybenzenesulfonic acid that can be found is that in the article of Seyda (Seyda, 1883). On principle it was sulfonation of hydroquinone with diluted sulfuric acid in mild conditions, the reaction temperature did not exceed 50°C, for 3 hours. During this period, starting hydroquinone gradually dissolved in diluted sulfuric acid. After cooling of the solution for 24 hours, formed crystals of 2,5-dihydroxybenzenesulfonic acid were filtered off. The filtrate was diluted with water and under boiling saturated with barium carbonate. The excessive barium carbonate was then together with formed barium sulfate filtered off. The filtrate was being concentrated *in vacuo* until a white crystalline syrupy mass was formed. This viscous liquid was then poured on a porous ceramic plate to be dried. Thus barium 2,5-dihydroxybenzenesulfonate was yielded. Potassium 2,5-dihydroxybenzenesulfonate was prepared by adding of potassium carbonate into an aqueous solution of barium 2,5-dihydroxybenzenesulfonate followed by filtering-off of formed barium carbonate and concentration of the filtrate *in vacuo* into a viscous liquid that was then diluted with double volume of absolute ethanol. A brown precipitate, which had been formed was then filtered off and all the ethanol was distilled off from the filtrate with exclusion of air. Crystalline mass formed from the filtrate was then recrystallized from water to give transparent octaedric crystals. Sodium 2,5-dihydroxybenzenesulfonate was prepared similarly by adding of sodium carbonate into a solution of the barium salt. It was formed in the concentrated aqueous solution as a mass consisted from microscopic octaedric crystals. Lead 2,5-dihydroxybenzenesulfonate was prepared from the crude 2,5-dihydroxybenzenesulfonic acid and lead carbonate. It precipitated from a concentrated aqueous solution as an amorphous solid that was no more soluble in water. However, it was soluble in concentrated acetic acid. The lead salt gave the free 2,5-dihydroxybenzenesulfonic acid by reaction with sulfane (see Figure 2).

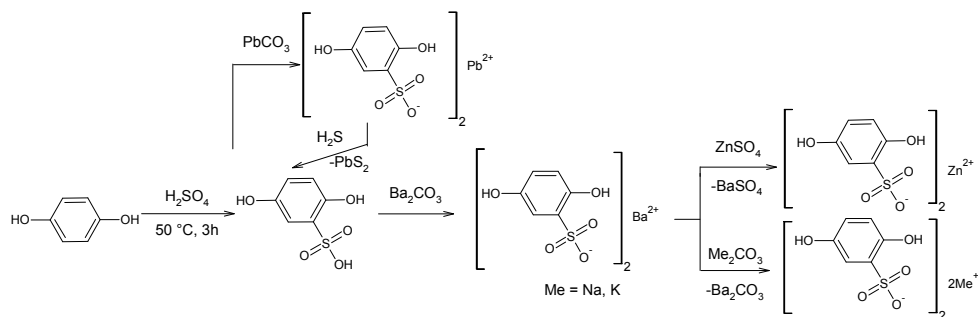


Fig 2. Early syntheses of 2,5-dihydroxybenzenesulfonic acid and some its metallic salts.

2.2 Industrial „redox“ one-pot synthesis of calcium dobesilate vs. sulfonation followed by neutralization

Estève-Subirana (Estève-Subirana, 1970) has developed a different synthesis of calcium dobesilate, which is also simple and suitable for the industrial production of it. It consists of only one reaction of calcium hydrogensulfite with 1,4-benzoquinone. Such a procedure could be classified as a „redox substitution“ due to changes of the oxidation states of both sulfur atom and benzene ring. However, such a synthesis is not suitable for low scale

preparations of calcium dobesilate including those that are performed by students in practical classes in subjects such as medicinal or organic chemistry. These practical educations can be met by students of fields such as pharmacy or chemistry at appropriate schools or faculties. The main problem of the Estéve-Subirana's procedure consists in use of calcium hydrogensulfite, which is not commercially available in solid state. Its concentrated aqueous solution is frequently prepared by reaction of gaseous sulfur dioxide with an aqueous suspension of calcium carbonate. This reaction can proceed in a special bubble column reactor typical for industrial processes such as the procedure developed by Rückauf and colleagues (Rückauf et al., 1990).

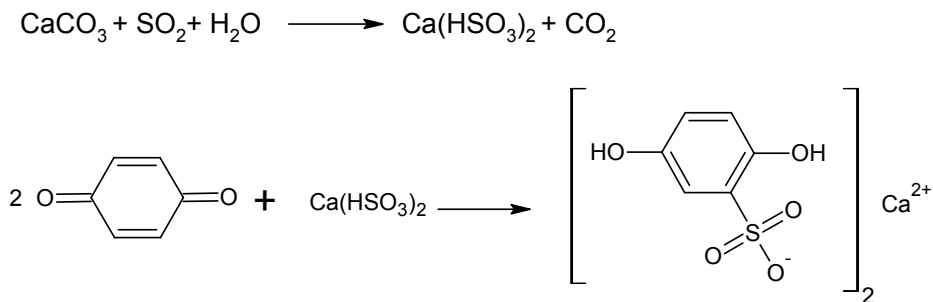


Fig. 3. Preparation of calcium dobesilate according to Estéve-Subirana (Estéve-Subirana, 1970) preceded with preparation of calcium hydrogensulfite according to Rückauf et al. (Rückauf et al., 1990).

To develop a simple and effective synthesis of calcium dobesilate that does not require calcium hydrogensulfite, we returned to sulfonation of hydroquinone. After we had tried several procedures using diluted sulfuric acid without significant successes (Šablatura, 2005), we came back to the original preparation of Seyda (Seyda, 1883). We modified his synthesis of barium 2,5-dihydroxybenzenesulfonate for preparation calcium dobesilate in conditions of today. Hydroquinone was sulfonated with concentrated sulfuric acid, a resulted mixture was then neutralized short boiling with calcium carbonate. Poorly soluble calcium sulfate precipitate was then filtered off. The filtrate was evaporated nearly to dryness. The residuum was dispersed in concentrated ethanol and filtered. The ethanolic filtrate was evaporated to dryness. Recrystallization of the residuum from the mixture butan-1-ol / chloroform gave calcium dobesilate in the form of its monohydrate (see Fig. 4).

Crystallization trials from several with various ratios alcohol / water mixtures led in most to solid solvates, e.g. calcium dobesilate : propan-2-ol 1 : 1.3 (dobesilate : alcohol ratio was estimated from $^1\text{H-NMR}$ spectrum). Simple confirmation of identity of the product by determination of its melting point is impossible because of high value of its melting temperature, but it can be identified by simple spectrophotometry in UV region according to Czech Pharmacopoeia 2005 Edition (Czech Pharmacopoeia 2009, 2009) (see also bellow). This procedure was published in a journal devoted to chemical education (Farsa & Šablatura, 2008) and in more detailed and modified form also in an instruction manual for practical courses in medicinal chemistry (Beneš & Farsa, 2007). It is notable that, short time after its publishing, very similar procedures of preparation of calcium dobesilate appeared in Chinese patents (Yao 2010, Yang et al. 2010).

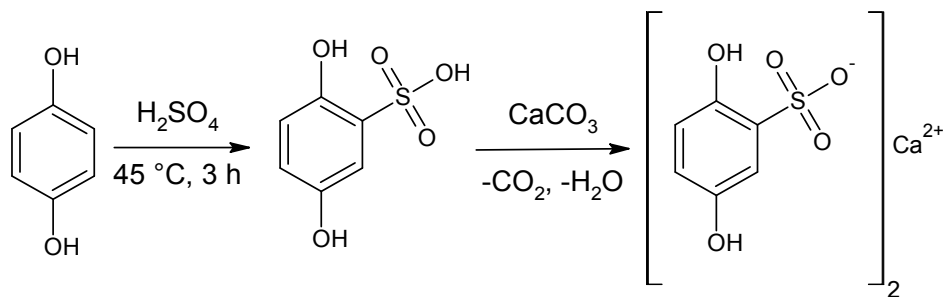


Fig. 4. Preparation of calcium dobesilate proposed for practical courses in chemical disciplines

2.3 Spectral data and other important physico-chemical properties of calcium dobesilate

Infrared spectra of calcium dobesilate measured in a potassium bromide tablet contained significant absorption bands at wavenumbers 3410, 1500, 1470, 1220, 1080, 880, 815 and 640 cm^{-1} (Estéve-Soler, 1977). A ^1H nuclear magnetic resonance spectrum (^1H -NMR) of calcium dobesilate recorded in deuterium oxide contained following signals (values of chemical shifts δ are in ppm): 7.13 (bs, 3H, aromatic hydrogens), 6.70 (s, 2H, 2 OH) and 4.75 (s, 2H, H_2O). The least signal served also as the reference one. There was evident from the ratio of integral areas that the investigated sample was calcium dobesilate monohydrate (Estéve-Soler, 1977). ^1H -NMR measured by the author in deuterated dimethyl sulfoxide (DMSO-D_6) was a little different. It contained the following signals (chemical shifts δ are in ppm, coupling constants J in Hz): 9.78 (s, 1H, OH), 8.84 (s, 1H, OH), 6.80 (d, 1H, $J=2.4$, arom. H), 6.57 (m, 2H, arom. H), 3.37 (s, 4H, 2 H_2O). The integral area of the signal assigned to water hydrogens corresponded to 4 hydrogen atoms per one dobesilate anion so that this sample was calcium dobesilate tetrahydrate (see Fig. 5). A ^{13}C nuclear magnetic resonance spectrum (^{13}C -NMR) of the same sample of calcium dobesilate was recorded by the author also in DMSO-D_6 in attached proton test (APT) mode. This type of carbon NMR spectrum enables to differ between carbon atoms with odd and even number of hydrogen atoms attached to every particular carbon atom so that in our case to distinguish between aromatic carbons with one hydrogen and those with no hydrogen. It contained the following signals (the values of chemical shift in ppm): 148.8 (CH), 145.8 (CH), 130.6 (CH), 117.9 (CSO_3^-), 116, 5 (COH), 112.8 (COH) (see Fig. 5).

Also ultraviolet spectra, which are advantageous namely for purity determination, have been known for decades. They have been recorded from various solvents such as water, methanol or aqueous hydrochloric acid. (Negritescu et al., 1979, Kračmár et al., 1988, Chen & Xiao, 2008). These electronic spectra are relatively easy to obtain because of low prices of needed UV spectrophotometers in comparison with NMR or IR spectrometers. This is probably one of the reasons for which some pharmacopoeias use this method for confirmation of identity of calcium dobesilate monohydrate, which is official as a drug substance (Czech Pharmacopoeia 2009, 2009, European Pharmacopoeia Edition 7.2, 2011). In accordance with both pharmacopoeias, the UV spectrum of calcium dobesilate measured in water can have absorption maxima at 221 and 301 nm and the specific absorbance at 301 nm in the range 174 to 181 (see Fig. 6).

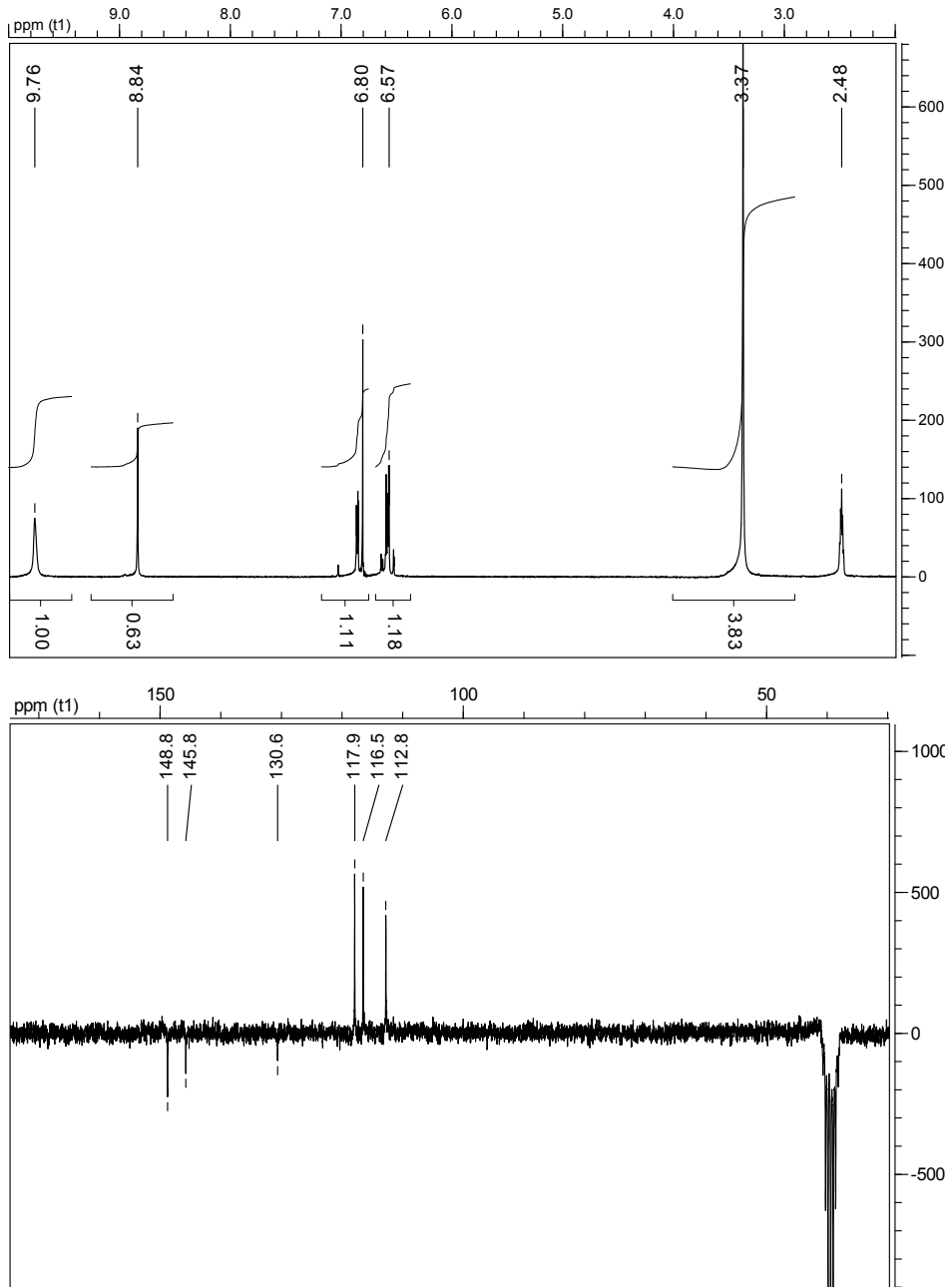


Fig. 5. $^1\text{H-NMR}$ spectrum of calcium dobesilate dihydrate recorded at 200 MHz Varian Gemini FT-NMR spectrometer in DMSO-D_6 solution (top) and its $^{13}\text{C-NMR}$ spectrum measured in DMSO-D_6 solution at 50 MHz at the same instrument (bottom).

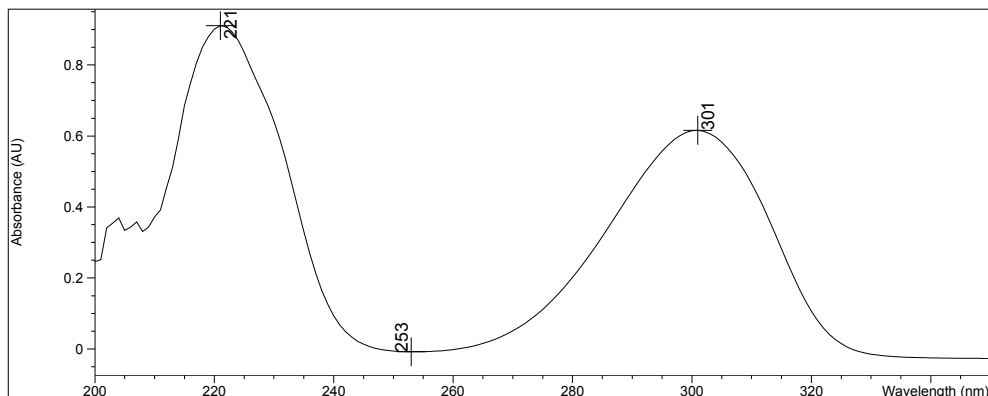


Fig. 6. Ultraviolet spectrum of calcium dobesilate measured in an aqueous solution at Agilent 8453 diode array UV-VIS spectrophotometer in accordance with requirements of the European Pharmacopoeia (European Pharmacopoeia Edition 7.2, 2011).

As it was mentioned above, melting point of calcium dobesilate monohydrate is out of range of measurement of common melting point apparatuses and thus it is not suitable for identification of calcium dobesilate monohydrate as a substance. Milne states that anhydrous calcium dobesilate melts above 300°C under decomposition (Milne, 2002).

The pharmacopoeias state that calcium dobesilate monohydrate is very soluble in water, freely soluble in anhydrous ethanol, very slightly soluble in propan-2-ol and practically insoluble in dichloromethane (Czech Pharmacopoeia 2009, 2009, European Pharmacopoeia Edition 7.2, 2011). In fact, it is extremely soluble in water: three weight parts of calcium dobesilate monohydrate dissolve in one part of boiling water. This ratio of the compound and the solvent can be used for purifying of the drug by recrystallization (Nováček et al., 1987).

Since calcium dobesilate is a hydroquinone derivative it can be easily oxidized into calcium 3,6-dioxocyclohexa-1,4-diene-1-sulfonate (see Fig. 7).

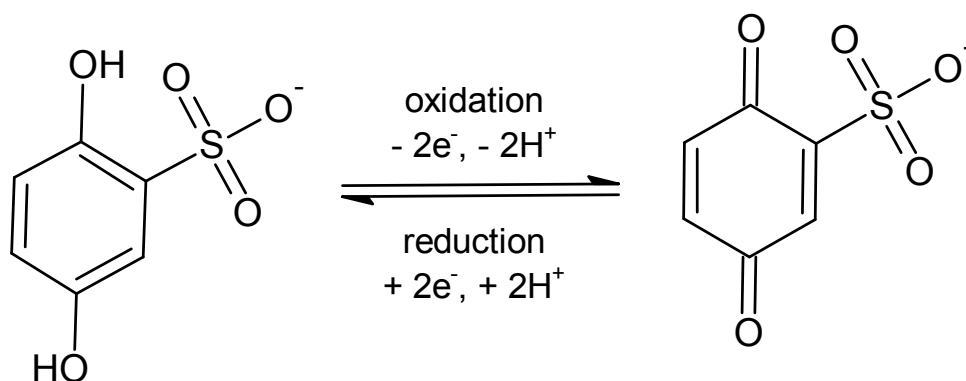


Fig. 7. Reversible oxidation of dobesilate anion into 3,6-dioxocyclohexa-1,4-diene-1-sulfonate.

Reversibility of this reaction has been repeatedly demonstrated by cyclic voltammetry with various types of electrodes. (Zheng et al., 2007, Zhang & Zhang, 2009, Zhang et al., 2011). Such methods have reached very low detection limit for calcium dobesilate up to 4.0×10^{-8} mol L⁻¹ and have been resistant to interference with other reducing compounds occurring in body liquids such as uric acid, serotonin, and ascorbic acid (Hu et al., 2009). That is why they have been successfully used not only for identification and assay of calcium dobesilate in substance samples and drug formulations (Guangzhi et al., 2009) but also in biological samples (Zheng et al., 2007). The influence of bovine serum albumin (BSA) on the electrochemical behavior of calcium dobesilate was also investigated. The stoichiometric coefficient and the association constant of calcium dobesilate with BSA were obtained by this technique in addition to those obtained by UV-spectrophotometry. It was found that the peak currents in cyclic voltammetry diagrams decreased and the peak potentials stayed almost unchanged in the presence of BSA, indicating that there might exist an interaction between calcium dobesilate and BSA (Xu et al., 2009). Oxidation of calcium dobesilate is also used for its titrimetric assay by means of cerimetry with potentiometric end-point determination required by some pharmacopoeias (European Pharmacopoeia 7.2, 2011, Czech Pharmacopoeia 2009, 2009). This method gives more accurate results than chelatometric titrimetry using N,N'-ethylenediaminetetraacetic acid (EDTA) for complexation of calcium ion of calcium dobesilate (Zhang & Xu, 1999). The activity of calcium dobesilate as a reduction agent is also reason of its radical-scavenging activity, which plays an important role in its mechanism of action as a drug of diabetic retinopathy (see further).

The chromatographic behaviour of calcium dobesilate is determined by its extremely low lipophilicity as it is an ionic salt. Thin-layer chromatography (TLC) on a silica gel normal phase was being used for identification of hydroquinone as an impurity in calcium dobesilate substance. While hydroquinone migrates in the required mobile phase dichloromethane : methyl acetate : ethyl acetate 20 : 30 : 50, calcium dobesilate remains at the start (Czech Pharmacopoeia 2002, 2002, Šablatura, 2006). Reversed phase high performance liquid chromatography (reversed phase-high performance liquid chromatography) methods for identification and content assay of calcium dobesilate namely in various samples have been reported. Assays of calcium dobesilate in dog blood plasma for pharmacokinetic purposes after its single (Plessas et al., 1986a) and repeated (Plessas et al., 1986b) administration were performed using a C18 column. A similar method using a Hypersil® octadecylsilyl silica gel (ODS) column and UV detection in absorption maximum of calcium dobesilate at 301 nm with mobile phase methanol : water 7 : 3 and flow rate 1.0 mL min⁻¹ has been developed for determination of calcium dobesilate in pharmaceuticals (Zhu & Chen, 2005). Slightly different conditions, mobile phase acetonitrile : water buffer pH 2.5 1 : 1, flow rate 1.0 mL min⁻¹ and detection wavelength 290 nm, were used in a procedure suitable for simultaneous determination of calcium dobesilate and lidocaine hydrochloride, dexamethasone acetate, butylhydroxyanisol and hydroquinone as a degradation product of calcium dobesilate in suppositories and an ointment. The retention times ranged between 2.4 min for the most hydrophilic calcium dobesilate to 17.1 min for the most hydrophilic BHA (Zivanovic et al., 2005). Also recent pharmacopoeias utilize reversed phase-high performance liquid chromatography using a spherical end-capped ODS column, mobile phase acetonitrile : aqueous buffer solution 1 : 9, flow rate 0.8 mL min⁻¹ and detection wavelength 220 nm for the test for presence of impurities, namely

hydroquinone. calcium dobesilate could have the retention time about 6 min in this system (European Pharmacopoeia 7.2, 2011, Czech Pharmacopoeia 2009, 2009). Finally, Rona and Ary reported a reversed phase-high performance liquid chromatography procedure suitable for determination of calcium dobesilate in human plasma for purposes of bioequivalence studies. They used a C16 reverse phase modified with inserted amide moiety. Such phases have been recommended as suitable for separation of polar compounds (Sigma-Aldrich, 2011). The mixture phosphate buffer pH 2.5 : acetonitrile 3 : 1 was used as a mobile phase, the detection wavelength was 305 nm (Rona & Ary, 2001). It is interesting that no high performance liquid chromatography procedure using electrochemical detection has been reported although redox properties of calcium dobesilate offer this possibility of sensitive detection.

2.4 Polymorphism, solvate complexes and co-crystals of calcium dobesilate

Calcium dobesilate monohydrate is the most common crystalline form of calcium dobesilate and is also official in many pharmacopoeias. It is also a subject of a mild misunderstanding between some pharmacopoeias and Chemical Abstracts or their electronic version, the data base SciFinder, because the Chemical Abstracts Service reference number (CAS RN) 20123-80-2 is in the pharmacopoeias (European Pharmacopoeia 7.2, 2011, Czech Pharmacopoeia 2009, 2009) assigned to this monohydrate while in SciFinder to anhydrous calcium dobesilate. Calcium dobesilate monohydrate is then referred in SciFinder under CAS RN 117552-79-1 (SciFinder, 2011). Calcium dobesilate monohydrate has been characterized by powder X-ray diffraction spectrum (Lu, 2005) and by two methods of differential thermal analysis (DTA); thermogravimetric analysis (TGA) a differential scanning calorimetry (DSC). Caproiu and colleagues reported based on DTA that calcium dobesilate monohydrate showed dehydration starting at 115°C and ending at 180°C (Caproiu et al., 1988) while Lu stated the dehydration interval between 134°C and 180°C (Lu, 2005). Calcium dobesilate dihydrate, sesquihydrate, i.e. hydrate containing 1.5 mol water per 1 mol calcium dobesilate, and non-stoichiometric hydrates containing 5/7, 5/4, 1.6 and 2.6 mol water per 1 mol calcium dobesilate respectively have been patented (Huang, 2007).

Calcium dobesilate has been patented as an active ingredient of its mixed-phase co-crystalline form. Such co-crystals are based on a theory that the additive(s), which are also known as co-crystal formers, are co-crystallized as minor non-stoichiometric components in the active agent's crystalline matrix. This co-crystalline phase has then semi-crystalline nature and contains a high incidence of crystal defects. Any excess additive that is not co-crystallized with the active agent forms a separate phase (Additive Phase) apart from the active agent, which results in the mixed phase co-crystal composition. The Additive Phase and the active agent crystals containing co-crystallized additive can be tightly agglomerated forming a mixed phase co-crystal particle. The unique physical properties obtained by this process of preparation can include changes in apparent solubility, crystallinity, water wetability, dissolution rates, physical powder properties (e.g., bulk density, absolute density, refractive index, x-ray diffraction, spectral, flowability, hygroscopicity, adsorption, and compaction), bioavailability, apparent permeability, apparent taste, and/or stability (Goldman, 2005). Calcium dobesilate is believed to form co-crystals with co-crystal former, which has at least one functional group selected from amine, amide, pyridine, imidazole, indole, pyrrolidine, carbonyl, carboxyl, hydroxyl, phenol, sulfone, sulfonyl, mercapto and

methylsulfanyl, such that calcium dobesilate and co-crystal former are capable of co-crystallizing from a solution phase under crystallization conditions (Almarsson et al., 2011). Calcium dobesilate has also been patented as a component of an ionic liquid (Rogers et al., 2010) that can be used for overcoming of influence polymorphism in drug substances (Rogers et al., 2007). calcium dobesilate can also form solvates with propylene glycol, which could be more stable than hydrates (Tawa et al, 2010). However, none of these patents contains a particular example of processing of calcium dobesilate.

3. Activities found in *In Vitro* and animal models

3.1 Antioxidant and antiradical activity

Antioxidant or reduction activity of calcium dobesilate is a result of its hydroquinone structure. This was mentioned above in section 2.3 in relationship to its analytical behaviour (compare Fig. 8) but this type of reactivity has also an important impact to its biological activity. The ability of 2,5-dihydroxybenzenesulfonic acid salts to reduce mercurous and silver nitrates to metallic mercury and silver respectively was first observed by Seyda (Seyda, 1883). Calcium dobesilate was shown to actively interact with toxic superoxide anion radical $O_2^{\cdot-}$. This reactive oxygen species (ROS) was generated either by UV irradiation of an aqueous solution of glycytryptophan at 280 nm, or by irradiation of a mixture of glycytryptophan with riboflavine with visible light. Thus calcium dobesilate showed the activity similar to that of superoxide dismutase (Lozovskaia et al., 1990). This result was confirmed in the experiment in which superoxide radicals were generated in the system xanthine/xanthine oxidase/ferrous chloride, but potency of calcium dobesilate to scavenge superoxide radicals was 23 times less than that of rutin that was used for comparison. However, calcium dobesilate was as potent as rutin in scavenging hydroxyl radicals generated *in vitro* via the iron-catalyzed Haber-Weiss reaction from superoxide anion ($IC_{50} = 1.1$ vs $0.7 \mu M$, respectively). In human erythrocytes, calcium dobesilate reduced phenazine methosulfate dependent lipid peroxydation, although the effect was observed at high concentration (Brunet et al., 1998a). Oral treatment with calcium dobesilate significantly protected diabetic rat retina against a free radicals mediated injury induced by ischemia/reperfusion. This was observed in an *in vivo* experiment with streptozotocin-induced diabetic rats (Szabo et al., 2001). The results of these experiments support the hypothesis that the antioxidant properties of calcium dobesilate could play a role in its angioprotective properties *in vivo*. The antioxidant effects of calcium dobesilate were further investigated in relation to the oxidative status, apoptosis and *in vitro* proliferation of human peripheral blood mononuclear cells (PBMC) isolated from healthy donors. Calcium dobesilate alone did not modify cell growth *in vitro* until $10 \mu M$. This molecule counteracted oxidative damages generated by the high reducing aldose 2-deoxy-D-ribose (dR). PBMC incubated with the reducing sugars D(-)-ribose and 2-deoxy-D-ribose (dR) exhibited characteristic patterns of apoptosis, such as DNA fragmentation and morphological changes, which can be prevented by exogenous antioxidants such as N-acetyl-L-cysteine (Barbieri et al., 1994) and/or other agents (Anderson et al., 1994). Calcium dobesilate was shown to reduce apoptosis by delaying both membrane permeability changes and DNA fragmentation. Calcium dobesilate $10 \mu M$ affected in a time-dependent dynamics several parameters representative of the cellular oxidative status. In particular, calcium dobesilate

significantly increased the activity of glutathione S-transferase (GST) after three days of treatment and also, but to a lower extent, the activity of γ -glutamyltransferase (γ -GT). Both enzymes are known to be involved in the glutathione (GSH) metabolic cycle. This enzymatic behaviour was reversed at seven days of treatment, with a significant GST decrease and a γ -GT activation. After seven days of calcium dobesilate exposure, the intracellular GSH content was enhanced and this resulted in a dramatic decrease in lipid peroxidation, underlining the powerful antioxidant properties of calcium dobesilate in human PBMC. (Graber et al., 1998). These results indicate that *in vitro* antioxidant effects of calcium dobesilate manifest *in vivo* by reduced lipid peroxidation and contribute to the prevention of apoptosis.

3.2 Angioprotective action of calcium dobesilate against reactive oxygen species-induced capillary permeability

Microvascular permeability seems to be strongly increased by ROS generated *in situ*. In an *in vivo* experiment in rats, calcium dobesilate administered intraperitoneally (i.p.), intravenously (i.v.) and orally (p.o.) significantly reduced microvascular permeabilization induced by ROS in the rat peritoneal cavity. ROS were generated again either with the system xanthine / xanthine oxidase or with PMS/NADH. Microvascular permeabilization was quantified by Evans blue extravasation. The activity of calcium dobesilate was comparable to that of rutin (Brunet et al., 1998b).

3.3 Enhancement of nitric oxide synthase activity in endothelial cells

The term endothelium-derived relaxing factor was originally proposed by Robert Furchgott for a then unknown factor leading to relaxation of the smooth muscle of large arteries in response to acetylcholine. Nitric oxide NO, which is in fact a free radical, was later found to be the mediator of this response. Most of NO in the body is synthesized by the endothelial isoform of NO synthase (NOS) from its precursor L-arginine (Palmer et al., 1988a), which is inhibited by false substrates of NOS, e.g., L-N^G-monomethyl arginine (L-NMMA) (Palmer et al., 1988b). NOS is a highly regulated protein and the endothelial form (eNOS) is predominantly found in endothelial cells. In them, two different isoenzymes can be expressed, depending on the activation state of these cells. In resting, non-activated endothelial cells a constitutive enzyme (ecNOS) is expressed (Moncada & Higgs, 1993) and after challenge with proinflammatory cytokines and/or bacterial endotoxin a cytokine-inducible enzyme (iNOS) is expressed in addition (Suschek et al., 1993). The constitutive, calcium-dependent isoenzyme produces low amounts of NO for short periods of time (Palacios et al., 1989). This endothelium-derived NO plays a crucial role in blood pressure regulation (Rees et al., 1989), in inhibition of platelet aggregation and platelet adhesion (Radomski et al., 1987a, b), and in modulating leukocyte adhesion, an essential step early in tissue inflammation (Kubes et al., 1991, Zimmerman et al., 1992). The inducible, cytosolic, calcium-independent NO synthase is expressed only after cell activation and releases large amounts of NO for longer periods of time that functions as cytotoxic and immune regulatory effector molecule (Kröncke et al., 1995). Diabetes-induced vascular function abnormalities are besides other biochemical and morphological changes also reflected by decreased synthesis of NO (Durante et al., 1988, McVeigh et al., 1992). Magnesium 2,5-dihydroxybenzenesulfonate or magnesium dobesilate was used instead of calcium

dobesilate in an experiment concerned with investigation of influence of dobesilate on NOS activities. The reason of usage of this compound was to eliminate a direct activation of the constitutive calcium-dependent NOS by calcium ions. In search for an effect on endothelial NO production, macrovascular endothelial cells from rat aorta, microvascular endothelial cells from rat exocrine pancreatic tissue, and capillary endothelial cells from rat islets, were cultured in the presence or absence of magnesium dobesilate. The activity of eNOS in resident cells as well as of iNOS in cytokine-activated cells was measured indirectly by recording the citrulline concentrations in culture supernatants. In each of the different endothelial cells magnesium dobesilate incubation (0.25 ± 1 mM) for 24 h led to a significant and concentration-dependent increase in eNOS-activities. With cytokine-activated endothelial cell cultures only moderate effects were observed with little or no concentration-dependency. Addition of the NOS-inhibitor L-NMMA led to a significant suppression of citrulline formation in all cultures as an evidence for the enzyme specificity of these effects. Both iNOS- and eNOS-specific reverse transcription and semi-quantitative polymerase chain reaction (RT-PCR) with RNA from resident or cytokine-activated endothelial cells gave no evidence for an increase in NOS-specific mRNA after MgD-treatment. Furthermore, dobesilate-mediated enhancement of NO synthesis in resting endothelial cells was not due to iNOS induction in these cells, as no iNOS-specific signal was found by RT-PCR (Suschek et al., 1997). Results of these experiments were supported by an additional study in which calcium dobesilate was found to enhance the endothelium-dependent relaxation induced by acetylcholine in rabbit isolated aorta artery. This effect was clearly endothelium dependent because, after the endothelium had been removed, the effect disappeared. The experiments were carried out on approximately 2 – 3 wide rings prepared by cutting of the arteries. The effect of calcium dobesilate was inhibited when the rings were incubated with increasing concentration of another known NOS inhibitor N^G -nitro-L-arginine methyl ester (L-NAME), and this effect was reversed with L-arginine, the substrate in NO synthesis (Ruiz et al., 1997).

3.4 Influence of calcium dobesilate on apoptosis in vessel and other tissues

Since apoptosis is closely linked with oxidation stress some aspects of anti-apoptotic effects of calcium dobesilate were mentioned above in section 3.1. In a clinical study, the influence of calcium dobesilate to apoptosis of varicose veins in comparison with diosmin-hesperidin combination was investigated. Patients were treated either with calcium dobesilate or with diosmin-hesperidin six weeks prior to the surgical removal of the particular varicose vein. Tissue samples obtained from such veins of 56 patients were immunohistochemically stained with antibodies of anti-bcl-2 and anti-bax thus aimed at anti-apoptotic (bcl-2) and pro-apoptotic (bax) proteins. Significant differences in the presence of bcl-2 protein expression between the untreated patient group and the group treated with calcium dobesilate suggest that calcium dobesilate could be of benefit in treatment of vascular disorders by down-regulating apoptosis (Iriz et al., 2008). On the other hand, calcium dobesilate is capable to elicit growth arrest of some types of cancer, in particular glioma (Cuevas et al., 2005) and basal cell carcinoma (Cuevas & Arrazola, 2005), and induce apoptosis of their cells.

3.5 Effects of calcium dobesilate on expression of adhesion molecules ICAM-1 and VCAM-1

Diabetes causes metabolic and physiologic abnormalities in the retina, and inflammation seems to play a critical role in the development of diabetic retinopathy. Those changes

include the upregulation of inducible NOS, cyclooxygenase-2, intercellular adhesion molecule-1 (ICAM-1), caspase-1, vascular endothelial growth factor (VEGF), and nuclear factor kappa B (NF- κ B), which leads to increased production of NO, prostaglandin E2, and cytokines (Adamis & Berman, 2008, Kern, 2007). It has also been demonstrated that the adhesion of leukocytes to retinal vessels is increased in the retinas of diabetic animals, and this increase is correlated with changes in tight junction proteins and increased blood-retinal barrier (BRB) permeability (Barber et al., 2000, Klaassen et al., 2009). The increase in leukostasis is also associated with an increase in the expression of ICAM-1 by retinal endothelial cells (Miyamoto et al., 1999). NF- κ B regulates the expression of adhesion molecules, such as ICAM-1, and NF- κ B activation has been correlated with the increase in leukostasis and BRB breakdown in diabetic rat retinas (Joussen et al., 2002). In a study on streptozotocin-induced diabetic rats, diabetes increased the BRB permeability and retinal thickness. These changes were inhibited by Calcium dobesilate treatment. Calcium dobesilate also inhibited the increase in leukocyte adhesion to retinal vessels or endothelial cells and in ICAM-1 levels, induced by diabetes or elevated glucose. Moreover, CaD decreased oxidative stress and p38 mitogen-activated protein kinase (p38 MAPK) and NF- κ B activation caused by diabetes. Thus, calcium dobesilate can prevent the BRB breakdown induced by diabetes, by restoring tight junction protein levels and organization and decreasing leukocyte adhesion to retinal vessels. The protective effects of calcium dobesilate probably involves the inhibition of p38 MAPK and NF- κ B activation, possibly through the inhibition of oxidative/nitrosative stress (Leal et al., 2011).

Vascular cell adhesion molecule-1 (VCAM-1) is also an important marker of the endothelial function. Similarly to ICAM-1, the concentration of its soluble form (sVCAM-1) is elevated in diabetes and diabetic retinopathy (Nowak et al., 2008, Clausen et al., 2000). The influence of calcium dobesilate on the levels of sVCAM-1 and sICAM-1 was investigated in a clinical trial in mildly obese male smokers. Endothelial dysfunction in these subjects was confirmed by means of increased levels of sVCAM-1, sICAM-1 and related parameters, but no effect of calcium dobesilate on levels of cell adhesion molecules was observed after 3 months of treatment with with 1000 mg calcium dobesilate dobesilate daily (Schram et al., 2003).

3.6 Angiogenesis inhibition

Calcium dobesilate was investigated for its ability to interfere with the process of angiogenesis in a mouse gelatine sponge assay using acidic fibroblast growth factor (aFGF) as an inducer of neovascularization. According to the reported results, calcium dobesilate remarkably reduced vessel ingrowth in aFGF-containing subcutaneous sponges in mice. These findings suggest that calcium dobesilate could be an effective agent in the treatment of angiogenesis-dependent diseases involving FGFs (Cuevas et al., 2005). This knowledge was successfully used in the treatment of erythematotelangiectatic rosacea, which is characterized by uncontrolled angiogenesis (Cuevas & Arrazola, 2005). Angiogenesis is, however, recently considered to be a main contributor to the pathogenesis of diabetic retinopathy (Aiello, 2003, Campochiaro, 2000; Campochiaro and Hackett, 2003). The anti-angiogenic effect of calcium dobesilate can also be mediated by aminopeptidases inhibition, namely by inhibition of aminopeptidase N (Yang et al., 2007).

3.7 Reduction of retinal albumin leakage by calcium dobesilate

The action of calcium dobesilate on retinal albumin leakage in streptozotocin-diabetic rats was investigated together with relevant *in vivo* retinal antioxidant and permeability markers, i.e., carboxymethyl-lysine-advanced glycation end product (CML-AGE) formation and vascular endothelial cell growth factor (VEGF) overexpression. Twenty days after streptozotocin administration, diabetic rats were treated for 10 days with calcium dobesilate (100 mg/kg/day per os) or vehicle. Retinal albumin leakage, CML-AGE formation, and VEGF overexpression were evaluated by immunohistochemistry of frozen eye sections. Diabetic rats exhibited dramatic increases in retinal albumin leakage (31% of positive vessels vs. 0.2% in nondiabetic rats, CML-AGE retinal occurrence (40±3% vs. undetectable positive vessels), and retinal VEGF protein expression (14.6±1.1 vs. 3.5±0.5 VEGF-positive spots/field). Calcium dobesilate significantly reduced retinal albumin leakage (by 70%), retinal CML-AGEs contents (by 62%), and retinal VEGF expression (by 69.4%). In conclusion, calcium dobesilate orally given to diabetic rats markedly reduced retinal hyperpermeability, CML-AGE contents, and VEGF overexpression. These results strongly suggest that calcium dobesilate stabilizes blood-retinal barrier in diabetic retinopathy (Rota et al., 2004).

3.8 Influence of calcium dobesilate on platelets and blood viscosity

Platelet-active drugs are in general of potential benefit in the prevention of diabetic microangiopathy. Calcium dobesilate was shown to reduce aggregation and the release reaction induced by thrombin and collagen in rabbit platelets (Michal & Gotti, 1988). Calcium dobesilate also increased platelet cAMP concentrations *in vitro* and *ex vivo* probably through activation of adenylate cyclase (Michal and Gotti, 1988). In addition, this drug reduced platelet electrophoretic mobility (Heidrich et al., 1983), and it inhibited, in a time- and concentration-dependent manner, platelet-activating factor (PAF) production by the EA926 endothelial cell line stimulated by thrombin (Bussolino et al., 1986). The effects of PAF on the microvasculature bed of glomerular or pulmonary microcirculation suggest that PAF might play a role in microcirculation disease. The rheological properties of blood in diabetes mellitus have received increasing attention since Skovborg and colleagues demonstrated elevation of whole-blood viscosity in diabetics (Skovborg et al., 1966). In particular, evidence has accumulated that abnormal blood viscosity plays a role in the pathogenesis of diabetic retinopathy. Increased blood viscosity in diabetes mellitus has been attributed to changes in plasma protein composition (Barnes et al., 1977, Hoare et al., 1976, Hudomel et al., 1977, Skovborg et al., 1966); and to increased aggregation of red blood cells (McMillan, 1976). The mechanism by which hyperviscosity contributes to the deterioration of the microcirculation in the retina and other organs in diabetics is that, by increasing the resistance to blood flow, it causes blood stasis, especially in the capillaries and postcapillary venules, which are the sites where the very early lesions of diabetes microangiopathy characteristically appear. In some studies, calcium dobesilate has been demonstrated to decrease hyperviscosity in blood (Barras & Graf, 1980, Vojnikovic, 1991), perhaps through a fibrinogen-lowering effect (Barras & Graf, 1980, Vinnazzer & Hachen, 1987).

3.9 Influence of calcium dobesilate on plasma levels of endothelin

Endothelin-1 (ET-1) was discovered by Yanagisawa and colleagues in 1988 (Yanagisawa et al., 1988) in the supernatant of aortic endothelial cells. ET-1 is a potent and prolonged

vasoconstrictor and mitogenic endothelium derived peptide, and has been considered as a marker for endothelial damage and potential contributor to the development of the atherogenic process. Increased circulating ET-1 levels were found in patients with atherosclerosis, as well as in patients with Type 2 diabetes mellitus suggesting a role in the pathogenesis of these disorders (Ak et al., 2001). Plasma ET-1 plays also an important role in the whole patho-physiological process of diabetic retinopathy. Its plasma concentrations correlate to the severity of the disease, measures of their plasma concentrations can help to predict the severity of the disease. They also enable to distinguish between proliferative and non-proliferative diabetic retinopathy (Cao et al., 2009). In a clinical study, the plasma ET-1 concentrations were determined in 45 diabetes mellitus patients. The changes of ET-1 in 20 diabetic retinopathy patients before and after treatment with calcium dobesilate and 30 normal controls were observed. The level of plasma endothelin was higher in diabetes mellitus patients than that of the controls, which was even higher in diabetic retinopathy. After treatment with calcium dobesilate, the plasma ET-1 level decreased significantly (Zhong & Guo, 1997).

4. Pharmacokinetics and metabolism of calcium dobesilate

Studies on the metabolism and pharmacokinetics of calcium dobesilate were carried out by Benakis and colleagues (Benakis et al., 1974). They reported the results of blood levels of calcium dobesilate labeled with ³⁵S isotope, protein binding and urinary excretion in humans. The studies in humans were performed by the administration of the drug *p.o.* or *i.v.* After *i.v.* medication, 500 mg, the maximum value is obtained 5 min after administration and is about 65 mg/ml. This value decreases rapidly, the plasma half-life being 1 hr. After administration by the oral route, 500 mg in a capsule, the maximum value was obtained at the 6th hour after medication and is about 8mg/ml, and a plateau was obtained between the 3rd and the 10th hour. Levels decreased slowly and were undetectable 24 hours after administration. Intestinal absorption was 15% per hour during the first 7 hours and then decreased to 5% per hour. More than 80% of the drug is absorbed in the first 8 hours. The drug is 20–25% protein bound. The specific affinity of the drug to aggregated and nonaggregated platelets has been demonstrated. Calcium dobesilate does not cross the blood-brain barrier. Urinary elimination in the first 24 hours reaches 75% after *i.v.* medication and 50% after oral medication.

5. Clinical findings and usage of calcium dobesilate in prevention and treatment of diabetic retinopathy

5.1 Results of clinical studies

While the results of various *in vitro* experiments suggest that calcium dobesilate would have to be effective in the treatment of diabetic retinopathy, the results of clinical trials are ambiguous. One of the first clinical studies was a double-blind, randomized trial, which lasted for 2 years. The authors investigated the efficacy of calcium dobesilate on diabetic retinopathy in 51 patients, 17 were on placebo. The statistical analysis of the results indicated that calcium dobesilate acts as a potent angioprotector, capable of preventing both intra- and extraretinal hemorrhages. The drug also lowered the incidence of exudate

formation and improved visual acuity (Salama Benarroch et al., 1977). A double-blind crossover clinical trial performed on 18 diabetics, the results of which were published in the same year, investigated the effect of calcium dobesilate on capillary resistance and background retinopathy in comparison with placebo. Each treatment lasted 8 months. The study gave no evidence of a significant beneficial effect of calcium dobesilate on the capillary resistance in diabetics or on the course of the diabetic retinopathy (Larsen et al., 1977). The additional two independent, double-blind, controlled studies were performed to evaluate the efficacy of calcium dobesilate for the treatment of nonproliferative diabetic retinopathy. In the first study, forty-two patients underwent a six-month crossover evaluation while receiving calcium dobesilate (750 mg per day) and placebo in random order. In the second one, thirty-six patients received calcium dobesilate (1,000 mg per day) or placebo for one year. Evaluation by clinical examination, fluorescein angiography, angiography, and fundus photography failed to demonstrate any beneficial effect of calcium dobesilate (Stamper et al., 1978). On the other hand, the study with 50 patients who had diabetic retinopathy open-angle glaucoma, raised intraocular pressure, and hyperviscosity of whole blood, plasma, and aqueous humor and received 1,500 mg of calcium dobesilate daily for 3 months or placebo exhibited the significant improvement of the state of the retina, the visual acuity, the intraocular pressure and the 3 viscosity values in the calcium dobesilate group (Vojnikovic, 1984). In a retrospective controlled study, 54 patients with diabetic retinopathy received calcium dobesilate (mean 650 mg/day) for 6-30 months (mean 18 months) and were compared to a correspondingly selected control group. The patients were divided into three subgroups (mild, moderate, and severe diabetic retinopathy). Microaneurysms, blot hemorrhages, striate hemorrhages, and hard exudates were assessed semiquantitatively from panorama fundus photographs, using a scoring system. The effect of calcium dobesilate was statistically significant for cases with moderate diabetic retinopathy on summing up the scores of the various retinal lesions. No effect on diabetic maculopathy or visual acuity was observed (Adank & Koerner, 1985). A more recent study, however, carried out in 197 patients, showed that 2 g of calcium dobesilate daily for 2 years had exhibited a significantly better activity than placebo on prevention of BRB disruption, independently of diabetes control. Tolerance was very good (Ribeiro et al., 2006). One of the most recent studies was concerned with the influence of calcium dobesilate on development of clinically significant macular oedema (CSME) within a follow-up period of 5 years. It was performed on 635 patients from 40 centers in 11 countries. They were treated with 1500 mg of calcium dobesilate per day in three divided doses of 500 mg or with placebo for period up to 5 years. Unfortunately, this trial showed that calcium dobesilate did not reduce the risk of development of CSME (Haritoglou et al., 2009).

6. Safety and important adverse effects of calcium dobesilate

Adverse events with calcium dobesilate do not occur very frequently and have the following distribution in terms of frequency: fever (26%), gastrointestinal disorders (12.5%), skin reactions (8.2%), arthralgia (4.3%), and agranulocytosis (4.3%). No deaths have been attributed to calcium dobesilate. Most adverse events are rare and unrelated to the pharmacological properties of calcium dobesilate. (Allain et al., 2004). Agranulocytosis, which was first reported in 1992 (Kulesa et al., 1992), is probably the most serious adverse

effect of calcium dobesilate. The estimated prevalence of agranulocytosis is 0.32 cases/million treated patients, i.e. ten times less than the calculated prevalence of agranulocytosis in the general population (Allain et al., 2004). Moreover, there is no case report that would attribute agranulocytosis clearly to calcium dobesilate during a simple treatment of diabetic retinopathy.

7. Dosage and application forms of calcium dobesilate

Calcium dobesilate is predominantly administered *p.o.* in uncoated tablets. Initial dosage in early stages of microangiopathies, namely diabetic retinopathy, is 250 mg calcium dobesilate three times a day (three tablets per day). After 2-3 weeks it is possible to decrease dosage to the keeping level 250-500 mg calcium dobesilate per day. Diminished performance of liver or kidneys has no impact to dosage of calcium dobesilate (Medicinal products database of the State institute for Drug Control, 2011).

7.1 Intraocular preparations of calcium dobesilate

Except of usually administered oral tablets, some topic preparations of calcium dobesilate have also been reported. Ocular preparations for a local treatment of diabetic retinopathy and related disorders like glaucoma are the matter of the patent application of Velpandian (Velpandian, 2006). The simplest eye solution, which is the Composition I of the Example 1 of this patent, contain 1 % weight-volume (w/v) calcium dobesilate, 0.76 % w/v sodium chloride for isotonicity and 0.1 % w/v sodium metabisulfite as an antioxidant. The Composition II is similar but contains only 0.70 % w/v sodium chloride and furtherly 0.4 % w/v hydroxypropylmethylcellulose as a viscosity-increasing agent. The Compositions III and IV are water insoluble and water soluble eye ointments respectively. The micronized form of calcium dobesilate in concentration 1 % w/v is used for preparation of both. The vehicle for water insoluble ointment is the white petrolatum mixed with a mineral oil, for the water soluble one it is a mixture of polyethylene glycols. Both ointments contain suitable antimicrobial preservatives in concentration 0.01 % w/v and 0.001 % w/v respectively. The composition V is a controlled-release gel based on a suitable gel-forming polymer and contain also 1 % w/v calcium dobesilate.

8. Conclusion

Calcium dobesilate is one of the oldest drugs used in the treatment of diabetic retinopathy. The first reports concerning its usage appeared in the late 1960th (Sevin & Cuendet, 1969a, b). Its mechanisms of action have been gradually elucidated. Many of them are related to its reactivity as a reducing agent. Now it is known that they include lowering of reactive oxygen species-induced capillary permeability, enhancement of nitric oxide synthase activity in endothelial cells, influence of calcium dobesilate on apoptosis in vessel and other tissues, effects of calcium dobesilate on expression of cellular adhesion molecules, angiogenesis inhibition, reduction of retinal albumin leakage, influence of calcium dobesilate on platelets and blood viscosity, influence of calcium dobesilate on plasma levels of endothelin and others. Despite certain doubts of its efficiency originating from results of some clinical trials calcium dobesilate still remains the only angioprotective agent that reduces the progression of diabetic retinopathy (Garay et al., 2005).

9. Acknowledgements

The author is grateful to the Internal grant agency of the University of Veterinary and Pharmaceutical Sciences Brno for the financial support by the grant No. 26/2010/FaF.

10. References

- Adamis, A. P. & Berman, A. J. (2008). Immunological mechanisms in the pathogenesis of diabetic retinopathy. *Semin Immunopathol*, Vol. 30, No. 2, (April 2008), pp.(65-84), ISSN 1863-2297
- Adank, C. & Koerner, F. (1985). Calcium dobesilate in diabetic retinopathy. A retrospective controlled study. *Ophthalmologica*, Vol. 190, No. 2, (February 1985), pp. (102-111), ISSN 0030-3755
- Aiello, L.M.. (2003). Perspectives on diabetic retinopathy. *American journal of ophthalmology*, Vol. 136, No. 1, (July 2003), pp. (122-135), ISSN 0002-9394
- Agolini, G. & Cavallini, G. M. (1987). Long-term therapy of retinal vasculopathies with oral administration of high doses of O-(beta-hydroxyethyl)-rutoside. *La Clinica terapeutica*, Vol. 120, No. 2, (January 1987), pp. (101-110), ISSN 0009-9074
- Ak, G., Buyukberber, S., Sevinc., A, Turk, H. M., Ates, M., Sari, R., Savli, H. & Cigli, A. (2001). The relation between plasma endothelin-1 levels and metabolic control, risk factors, treatment modalities, and diabetic microangiopathy in patients with Type 2 diabetes mellitus. *Journal of diabetes and its complications*, Vol. 15, No. 3, (May-June 2001), pp. (150-157), ISSN 1056-8727
- Allain, H., Ramelet, A. A., Polard, E. & Bentué-Ferrer, D. (2004). Safety of calcium dobesilate in chronic venous disease, diabetic retinopathy and haemorrhoids. *Drug Safety*, Vol. 27, No. 9, (August 2004), pp. (649-660), ISSN 0114-5916
- Almarsson, O., Bourghol Hickey, M., Peterson, M. L., Zaworotko, M. J., Moulton, B. & Rodriguez-Hornedo, N. (2011). *US 7927613* (19.04.2011)
- Anderson, D., Yu, T. W., Phillips, B. J. & Schmezer, P. (1994). The effect of various antioxidants and other modifying agents on oxygen-radical-generated DNA damage in human lymphocytes in the COMET assay. *Mutation Research - Fundamental and Molecular Mechanisms of Mutagenesis*, Vol. 307, No. 1, (May 1994), pp. (261-270), ISSN 1386-1964
- Barbieri, D., Grassilli, E., Monti, D., Salvioli, S., Franceschini, M. G., Franchini, A., Bellesia, E., Salomoni, P., Negro, P., Capri, M., Troiano, L., Cossarizza, A. & Franceschi, C. (1994). D-ribose and 2-deoxy-D-ribose induce apoptosis in human quiescent peripheral blood mononuclear cells. *Biochemical and Biophysical Research Communications*, Vol. 201, No. 3, (June 1994), pp. (1109-1116), ISSN 1090-2104
- Barras, J. & Graf, C. (1980) Hyperviscosity in diabetic retinopathy treated with Doxium (calcium dobesilate). *Vasa*, Vol. 9, No. 2, (April-June 1980), pp. (161-164), ISSN0301-1526
- Barber, A. J., Antonetti, D.A. & Gardner, T. W. (2000). Altered expression of retinal occludin and glial fibrillary acidic protein in experimental diabetes. *Investigative Ophthalmology and Visual Science*, Vol. 41, No. 11, (October 2000), pp. (3561-3568), ISSN 1552-5783

- Barnes, A.J., Locke, P., Scudder, P.R., Dormandy, T. L., Dormandy, J. A. & Slack, J. (1977). Is hyperviscosity a treatable component of diabetic microcirculatory disease? *Lancet*, Vol. 2, No. 8042, (October 1977), pp. (789-791), ISSN: 0140-6736
- Benakis, A., Glasson, B., Bouvier, C. A., Ritschard, J., Krahenbuhl, B., Jung, A. & Hachen, H. J. (1974). Metabolism and pharmacokinetics of calcium dobesilate in humans. *Therapie*, Vol. 29, No. 2, (March-April 1974), pp. (211-219), ISSN 0040-5957
- Beneš, L. & Farsa, O. (2007). Medicinal Chemistry. An instruction manual for practical courses. University of Veterinary and Pharmaceutical Sciences, ISBN 978-80-7305-003-0, Brno, Czech Republic
- Brunet, J., Farine, J. C., Garay, R. P. & Hannaert, P. (1998a). In vitro antioxidant properties of calcium dobesilate. *Fundamental & clinical pharmacology*, Vol. 12, No. 2, (February 1998), pp. (205-212), ISSN 0767-3981
- Brunet, J., Farine, J. C., Garay, R. P. & Hannaert, P. (1998b). Angioprotective action of calcium dobesilate against reactive oxygen species-induced capillary permeability in the rat. *European Journal of Pharmacology*, Vol. 358, No. 3, (October 1998), pp. (213-220), ISSN 0014-2999
- Bussolino, F., Biffignandi, P. & Arese, P. (1986). Platelet-activating factor: a powerful lipid autacoid possibly involved in microangiopathy. *Acta Haematologica*, Vol. 75, No. 3, (March 1986), pp. (129-140), ISSN 0001-5792
- Campochiaro, P. A. (2000). Retinal and choroidal neovascularization. *Journal of Cellular Physiology*, Vol. 184, No. 3, (September 2000), pp. (301-310), ISSN 0021-9541
- Campochiaro, P. A. & Hackett, S. F. (2003). Ocular neovascularization: a valuable model system. *Oncogene*. Vol. 22, No.429, (September 2003), pp. (6537-6548). ISSN . 0950-9232
- Cao, W., Jiang, Y. & Chen, Y. (2009). Clinical significance of changes of ET-1/CGRP contents in patients with diabetic retinopathy. *Guoji Yanke Zazhi*, Vol. 9, No. 2, (February 2009), pp. (309-310), ISSN 1672-5123
- Caproiu, R., Praisler-Stoica, M., Tanase, A. & Georgescu, D. (1988). Characterization of calcium 2,5-dihydroxybenzenesulfonate (calcium dobesilate) by thermal analysis. *Revistade Chimie*, Vol. 39, No. 6, (June 1988), pp. (528-530), ISSN 0034-7752
- Carrasco, O. F. & Vidrio, H. (2007). Endothelium protectant and contractile effects of the antivaricose principle escin in rat aorta. *Vascular pharmacology*, Vol. 47, No. 1, (July 2007), pp. (68-73), ISSN 1537-1891
- Chen, Y. & Xiao, Y. (2008). Detection of calcium dobesilate in calcium dobesilate capsules: a comparison of HPLC and UV spectrophotometry. *Guangdong Yaoxueyuan Xuebao*, Vol. 24, No. 5, (May 2008), pp. (480-483), ISSN 1006-8783
- Chung, H. K., Choi, S. M., Ahn, B. O., Kwak, H. H., Kim, J. H. & Kim, W. B. (2005). *Arzneimittelforschung*, Vol. 55, No. 10, (November 2005), pp.(573-80), ISSN 0004-4172
- Clausen, P., Jacobsen, P., Rossing, K., Jensen, J. S., Parving, H. H. & Feldt-Rasmussen, B. (2000). Plasma concentrations of VCAM-1 and ICAM-1 are elevated in patients with Type 1 diabetes mellitus with microalbuminuria and overt nephropathy. *Diabetic medicine : a journal of the British Diabetic Association*, Vol. 17, No. 9, (September 2000), pp.(644-649), ISSN 0742-3071

- Cuevas, P. & Arrazola, J. M. (2005). Treatment of basal cell carcinoma with dobesilate. *Journal of the American Academy of Dermatology*, Vol. 53, No. 3, (September 2005), pp. (525-526), ISSN 1368-5031
- Cuevas, P., Díaz-González, D., Sánchez, I., Lozano, R. M., Giménez-Gallego, G. & Dujovny, M. (2006). Dobesilate inhibits the activation of signal transducer and activator of transcription 3, and the expression of cyclin D1 and bcl-XL in glioma cells. *Neurological Research*, Vol.28, No. 2, (March 2006), pp. (127-130, ISSN 0161-6412
- Cuevas, P., Sanchez, I., Lozano, R. M. & Gimenez-Gallego, G.. (2005). Dobesilate is an angiogenesis inhibitor. *European Journal of Medical Research*, Vol. 10, No. 9, (September 2005), pp. (369-372), ISSN 0949-2321
- Czech Pharmacopoeia 2002*. (2002). Grada, ISBN 80-247-0464-1, Prague, Czech Republic
- Czech Pharmacopoeia 2009*. (2009). Grada, ISBN 80-247-2994-7, Prague, Czech Republic
- Durante, W., Sen, A. K., Sunahara & F. A. (1988). Impairment of endothelium-dependent relaxation in aortae from spontaneously diabetic rats. *British Journal of Pharmacology*, Vol. 94, No. 2, (June 1988), pp. (463-468), ISSN 0007-1188
- Einarsdottir, A. B. & Stefansson, E. (2009). Prevention of diabetic retinopathy. *Lancet*, Vol. 373, No. 9672, (April 2009), pp. (1316-1318), ISSN 0140-6736
- Estève-Soler, J. (1977). Verfahren zur Herstellung des Kalziumsalzes der 2,5-dihydroxybenzolsulfonsäure. *DD 125668* (11.5.1977)
- Estève-Subirana, A. (1970): Therapeutically active derivatives of p-dihydroxybenzene. *US 3509207* (28.4.1970)
- European Pharmacopoeia 7.2*. (2011). European Directorate for the Quality of Medicines & Health Care, ISBN 978-92-871-6704-0, Strasbourg Cedex, France
- Farsa O., Šablatura M. (2008). An alternative synthesis of calcium dobesilate, a simple venous insufficiency drug, suitable for use in medicinal or organic Chemistry practical courses. *Khimija/Chemistry*, Vol. 17, No. 4 (July-August 2008), pp. (281-285), ISSN 0861-9255
- García Benayas, E., García Díaz, B. & Pérez, G. (1997). Calcium dobesilate-induced agranulocytosis. *Pharmacy world & science*, Vol. 19, No. 5, (October 1997), pp. (251-252), ISSN 0928-1231
- Garay, R.P., Hannaert, P. & Chiavaroli, C. (2005). Calcium dobesilate in the treatment of diabetic retinopathy. *Treatments in Endocrinology*, Vol. 4, No. 4, (July-August 2005), pp. (221-232), ISSN 1175-6349
- Goldman, D. (2005). Method of preparation of mixed phase co-crystals with active agents. *CA 2548281* (23.06.2005)
- Graber, R., Farine, J. C., Fumagalli, I., Tatti, V. & Losa, G.A. (1998). Calcium Dobesilate protects human peripheral blood mononuclear cells from oxidation and apoptosis. *Apoptosis*, Vol 3, No. 1, (1998), pp. (41-49), ISSN 1360-8185
- Guangzhi, H., Long, C., Yong, G., Shijun, S. & Xiaolai, W. (2009). Selective electrochemical sensing of calcium dobesilate based on the nano-Pd/CNTs modified pyrolytic graphite electrode. *Talanta*, Vol. 78, No.3, (March 2009), pp. (1211-1214), ISSN . 0039-9140
- Haritoglou, C, Gerss, J., Sauerland, C., Kampik, A. & Ulbig, M. W. (2009). Effect of calcium dobesilate on occurrence of diabetic macular oedema (CALDIRET study): randomised, double-blind, placebo-controlled, multicentre trial. *The Lancet*, Vol. 373, No. 9672, (April 2009), pp. (1364 - 1371), ISSN 0140-6736

- Heidrich, H., Gerke, E. & Nekarda, H. (1983). Thrombozytenaggregationshemmung unter Calciumdobesilat. *Arzneimittel-Forschung/Drug Research*, Vol. 33, No. 4, (April 1983), pp. (580-582), ISSN 0004-4172
- Hoare, E., Barnes, A. & Normandy, J. (1976) Abnormal blood viscosity in diabetes mellitus and retinopathy. *Biorheology*, Vol. 13, No. 1, (January - February 1976), pp. (21-25), ISSN0006-355X
- Hu, G. Ma, Y., Guo, Y. & Shao, S. (2009). Selective electrochemical sensing of calcium dobesilate based on an ordered mesoporous carbon-modified pyrolytic graphite electrode. *Journal of Electroanalytical Chemistry*, Vol. 633, No. 1, (August 2009), pp. (264-267), ISSN 1572-6657
- Huang, Z. (2007). New formulations of 2,5-dihydroxybenzenesulfonic acid for improving microcirculation and protecting blood vessel. *CN 101081823* (05.12.2007)
- Hudomel, J., Nemeth, B., Palfalvy, M. & Farkas A. (1977). Effect of calcium dobesilate (Doxium) on blood hyperviscosity in cases of diabetic retinopathy. *Ophthalmic Research*, Vol. 9, No. 1, (January - February 1977), pp. (25-30), ISSN 0030-3747
- Iriz, E., Vural C., Ereren, E., Poyraz, A., Erer, D., Oktar, L., Golgot, L., Halit, V., Soncul, H. (2008). Effects of calcium dobesilate and diosmin-hesperidin on apoptosis of venous wall in primary varicose veins. *Vasa*, Vol. 37, No. 3, (August 2008), pp. (233-240), ISSN 0301-1526
- Joussen, A. M., Poulaki, V., Mitsiades, N., Kirchhof, B., Koizumi, K., Döhmen, S. & Adamis, A. P. (2002). Nonsteroidal anti-inflammatory drugs prevent early diabetic retinopathy via TNF-alpha suppression. *The FASEB Journal*, Vol. 16, No. 3, (March 2002), pp. (438-440), ISSN 0892-6638
- Kern, T. S. (2007). Contributions of inflammatory processes to the development of the early stages of diabetic retinopathy. *Experimental Diabetes Research*, Vol. 2007, Article ID 95103, pp. (95-103), ISSN 1687-5214
- Kim, M., Park, B.-Y., Moon, C.-H., Park, E.-K. & Kim, K. (2004). Composition containing horse chestnut extract for anti-angiogenic and matrix metalloproteinase inhibitory activity. *EP 1438059* (July 21, 2004)
- Klaassen, I., Hughes, J. M., Vogels, I. M., Schalkwijk, C. G., Van Noorden, C. J. & Schlingemann, R. O. (2009). Altered expression of genes related to blood-retina barrier disruption in streptozotocin-induced diabetes. *Experimental eye research*, Vol. 89, No. 1, (June 2009), pp. (4-15), ISSN 0014-4835
- Kračmár, J., Kračmárová, J., Kovářová, A. & Stejskal, Z. (1988). UV spectrophotometry in drug control. 37: New active substances containing benzene, pyridine and quinoline chromophores. Part 8: Impact of substitution and solvent. *Pharmazie*, Vol. 43, No. 3, (March 1988), pp.(173-176), ISSN 0031-7144
- Kröncke, K.D., Fehsel, K. & Kolb-Bachofen, V. (1995). Inducible nitric oxide synthase and its product nitric oxide, a small molecule with complex biological activities. *Biological Chemistry Hoppe-Seyler*, Vol. 376, No. 6, (June 1995), pp. (327-343), ISSN 0177-3593
- Kubes, P., Suzuki, M. & Granger, D.N. (1991). Nitric oxide: An endogenous modulator of leukocyte adhesion. *Proceedings of the National Academy of Sciences of the United States of America*, Vol. 88, No. 11, (June 1991), pp. (4651-4655), ISSN 0027-8424
- Kulesa, W., Becker, E. W., Berg, P. A. (1992). Recurrence of agranulocytosis after taking calcium dobesilate. *Deutsche medizinische Wochenschrift*, Vol. 117, No. 10, (March 1992), pp. (372-374), ISSN 0012-0472

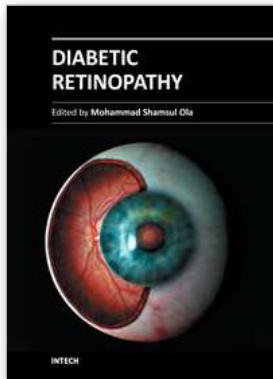
- Lacombe, C., Lelievre, J. C., Bucherer, C. & Grimaldi, A. (1989). Activity of Daflon 500 mg on the hemorheological disorders in diabetes. *International angiology : a journal of the International Union of Angiology*, Vol. 8, Supl. 4, (October-December 1989), pp. (48-48), ISSN 0392-9590
- Larsen, H. W., Sander, E., Hoppe, R. (1977). The value of calcium dobesilate in the treatment of diabetic retinopathy. A controlled clinical trial. *Diabetologia*, Vol. 13, No. 2, (April 1977), pp. (105-109), ISSN 0012-186X
- Leal, E. C., Martins, J., Voabil, P., Liberal, J., Chiavaroli, C., Bauer, J., Cunha-Vaz, J. & Ambrosio, A. F. (2010). Calcium dobesilate inhibits the alterations in tight junction proteins and leukocyte adhesion to retinal endothelial cells induced by diabetes. *Diabetes*, Vol. 59, No. 10, (October 2010), pp. (2637-2645), ISSN 0012-1797
- Lozovskaia, E. L., Kaplunskii, G. D., Sapezhinskii, I. I. (1990). Superoxide dismutase activity and photosensitizing properties of 2,5-dihydroxybenzolsulfonate. *Biofizika*, Vol. 35, No. 6, (June 1990), pp. (912-916), ISSN 0006-3029
- Lu, J. R. (2005). Synthesis and structural analysis of calcium dobesilate monohydrate. *Zhongguo Xiandai Yingyong Yaoxue*, Vol. 22, No. 2, (February 2005), pp. (124-126), ISSN 1007-7693
- Luo, X. X., Duan, J. G., Liao, P. Z., Wu, L., Yu, Y. G., Qiu, B., Wang, Y. L., Li, Y. M., Yin, Z. Q., Liu, X. L. & Yao, K. (2009). Effect of qiming granule on retinal blood circulation of diabetic retinopathy: a multicenter clinical trial. *Chinese journal of integrative medicine*, Vol. 15, No. 5, (October 2009), pp. (384-388), ISSN 1672-0415
- McMillan, D. (1976). Plasma protein changes, blood viscosity and diabetic microangiopathy. *Diabetes*, Vol. 25, No. Suppl. 2, (January 1976), pp. (25-33), ISSN 0012-1797
- McVeigh, G. E., Brennan, G. M., Johnston, G. D., McDermott, B. J., McGrath, L. T., Henry, W. R., Andrews, J. W. & Hayes, J. R. (1992). Impaired endothelium-dependent and independent vasodilation in patients with type 2 (non-insulin-dependent) diabetes mellitus. *Diabetologia*, Vol. 35, No. 8, (August 1992), pp. (771-776), ISSN 0012-186X
- Michal, M. & Gotti, C. (1988). Effect of calcium dobesilate on platelet function. *Thrombosis Research*, Vol. 51, No. 6, (September 1988), pp. (593-605), ISSN 0049-3848
- Milne, G. W. A. (Ed.) (2002). *Drugs: Synonyms & Properties*. Ashgate Publ. Co., ISBN: 0-566-08228-4, Brookfield, Vt., USA
- Miyamoto, K., Khosrof, S., Bursell, S. E., Rohan, R., Murata, T., Clermont, A. C., Aiello, L. P., Ogura, Y. & Adamis, A. P. (1999). Prevention of leukostasis and vascular leakage in streptozotocin-induced diabetic retinopathy via intercellular adhesion molecule-1 inhibition. *Proceedings of the National Academy of Sciences of the United States of America*, Vol. 96, No. 19, (September 1999), pp. (10836-10841), ISSN 0027-8424
- Moncada, S. & Higgs, A. (1993). The L-arginine-nitric oxide pathway. *The New England Journal of Medicine*, Vol. 329, No. 27, (December 1993), pp. (2002-2012), ISSN 0028-4793
- Negritescu, S., Belu, D., Dragoi, R., Sisman, E., Cosmin, A., Candidatu, A. & Georgescu, D. (1979). Analytical studies on 2,5-dihydroxybenzenesulfonic acid salts as antihemorrhagic drugs. *Revista de Chimie*, Vol. 30, No. 9, (September 1979), pp. (912-915), ISSN: 0034-7752
- Nováček, A., Sedláčková, V., Horáček, L. & Šimek, P. (1987). A method of purification of 2,5-dihydroxybenzenesulfonic acid calcium salt. CS 8402981, (15.09.1987)

- Nowak, M., Wielkoszyński, T., Marek, B., Kos-Kudła, B., Świętochowska, E., Siemińska, L., Kajdaniuk, D., Glogowska-Szelag, J. & Nowak, K. (2008). Blood serum levels of vascular cell adhesion molecule (sVCAM-1), intercellular adhesion molecule (sICAM-1) and endothelial leucocyte adhesion molecule-1 (ELAM-1) in diabetic retinopathy. *Clinical and experimental medicine*, Vol. 8, No. 3, (September 2008), pp. (159-164), ISSN 1591-8890
- Palacios, M., Knowles, R. G. & Palmer, R. M. J. (1989). Nitric oxides from L-arginine stimulates the soluble guanylate cyclase in adrenal glands. *Biochemical and Biophysical Research Communications*, Vol. 165, No. 2, (December 1989), pp. (802-810), ISSN 1090-2104
- Palmer, R. M. J., Ashton D. S. & Moncada, S. (1988). Vascular endothelial cells synthesize nitric oxide from L-arginine. *Nature*, Vol. 333, No. 6174, (June 1988), pp. (664-666), ISSN 0028-0836
- Palmer, R. M. J., Rees, D. D., Ashton, D. S. & Moncada, S. (1988). L-Arginine is the physiological precursor for the formation of nitric oxide in endothelium-dependent relaxation. *Biochemical and Biophysical Research Communications*, Vol. 153, No. 3, (June 1988), pp. (1251-1256), ISSN 1090-2104
- Plessas, C. T., Karayannakos, P., Plessas, S. T., Costakis, A., Donta, I. & Skalkeas, G. (1986a). Pharmacokinetics of calcium dobesilate in beagle dogs after a single administration. *European journal of drug metabolism and pharmacokinetics*, Vol. 11, No. 4, (April 1986), pp. (303-308), ISSN 0378-7966
- Plessas, C. T., Karayannakos, P., Plessas, S. T., Costakis, A., Donta, I. & Skalkeas, G. (1986b). Pharmacokinetics of calcium dobesilate in beagle dogs after repeated administration. *European journal of drug metabolism and pharmacokinetics*, Vol. 11, No. 4, (April 1986), pp. (309-312), ISSN 0378-7966
- Radomski, M. W., Palmer, R. M. J. & Moncada, S. (1987a). Comparative pharmacology of endothelium-derived relaxing factor, nitric oxide and prostacyclin in platelets. *British Journal of Pharmacology*, Vol. 92, No. 3, (November 1987), pp. (181-185), ISSN 0007-1188
- Radomski, M. W., Palmer, R. M. J. & Moncada, S. (1987b). Endogenous nitric oxide inhibits human platelet adhesion to vascular endothelium. *Lancet*, Vol. 2, No. 8567, (November 1987), pp. (1057-1059), ISSN 0140-6736
- Rees, D. D., Palmer, R. M. J. & Moncada, S. (1989). Role of endothelium-derived nitric oxide in the regulation of blood pressure. *Proceedings of the National Academy of Sciences of the United States of America*, Vol. 86, No. 9, (May 1989), pp. (3375-3381), ISSN 0027-8424
- Ribeiro, M. L., Seres, A. I., Carneiro, A. M., Stur, M., Zourhani, A., Caillon, P. & Cunha-Vaz, J. G. (2006). Effect of calcium dobesilate on progression of early diabetic retinopathy: a randomised double-blind study. *Graefe's archive for clinical and experimental ophthalmology*, Vol. 244, No. 12, (December 2006), pp. (1591-1600), ISSN 0721-832X
- Rogers, R. D., Daly, D. T., Gurau, G., MacFarlane, D., Turanjanin, J., Dean, P. M., Scott, J., Bica, K. & Seddon, K. R. (2010). Dual functioning ionic liquids and salts thereof. *World Patent Application WO 2010/078300* (08.07.2010)
- Rogers, R. D., Daly, D. T., Swatloski, R. P., Hough, W. L., Davis, J. H. Jr., Smiglak, M., Pernak, J. & Spear, S. K. (2007). Multi-functional ionic liquid compositions for

- overcoming polymorphism and imparting improved properties for active pharmaceutical, biological, nutritional, and energetic ingredients. *US Patent Application 20070093462* (26.04.2007)
- Róna, K. & Ary, K. (2001). Determination of calcium dobesilate in human plasma using ion-pairing extraction and high-performance liquid chromatography. *Journal of Chromatography B: Biomedical Sciences and Applications*, Vol. 755, No. 1-2, (May 2001), pp. (245-251), ISSN 0378-4347
- Rota, R., Chiavaroli, C., Garayc, R. P. & Hannaert, P. (2004). Reduction of retinal albumin leakage by the antioxidant calcium dobesilate in streptozotocin-diabetic rats. *European Journal of Pharmacology*, Vol. 495, No. 2-3, (July 2004), pp.(217- 224), ISSN 0014-2999
- Rückauf H., Turek, F. & Dietrich, W. (1990). Verfahren zur Herstellung von Hydrogensulfidlösungen. *DD 282899* (26.9.1990)
- Ruiz, E., Lorente, R. & Tejerina, T. Effects of calcium dobesilate on the synthesis of endothelium-dependent relaxing factors in rabbit isolated aorta. *British Journal of Pharmacology*, Vol. 121, No. 4, (June 1997), p. (711-716), ISSN 0007-1188
- Šablatura, M. (2006). *Development and optimization of a novel exercise for practical courses in Medicinal Chemistry I. Master thesis*. University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic
- Salama Benarroch, I., Nano, H., Pérez, H., Elizalde, F., Bisceglia, H. & Salama. A. (1977). Assessment of calcium dobesilate in diabetic retinopathy. A double-blind clinical investigation. *Ophthalmologica*, Vol. 174, No. 1, (January 1977), pp. (47-51), ISSN 0030-3755
- Schram, M. T., Stam, F., de Jongh, R. T., de Vries, G., van Dijk, R. A., Serné, E. H., Lampe, D., Nanayakkara, P. W., Tushuizen, M. E., Scheffer, P. G., Schalkwijk, C. G., Kamper A. M., Stehouwer, C. D . (2003). The effect of calcium dobesilate on vascular endothelial function, blood pressure, and markers of oxidation in obese male smokers: a placebo-controlled randomised clinical trial. *Atherosclerosis*, Vol. 170, No. 1, (September 2003), pp. (59-72), ISSN 0021-9150
- Sci Finder (July 2011). Substance Detail 20123-80-2. In: *Sci Finder, a Data Base of the American Chemical Society*, 12. 07. 2011, Available from <https://scifinder.cas.org/scifinder/view/scifinder/scifinderExplore.jsf>
- Sevin, R., Cuendet, J. F. (1969a). The action of calcium dobesilate in diabetic retinopathy. *Bulletins et mémoires de la Société française d'ophtalmologie*, Vol. 82, pp. (170-180), ISSN 0081-1092
- Sevin, R., Cuendet, J. F. (1969b). Calcium dobesilate in diabetic retinopathy. *Ophthalmologica*, Vol. 159, No. 1, (January-February 1969), pp. (126-135), ISSN 0030-3755
- Seyda, A. (1883). Über Sulfonsäuren des Hydrochinons. *Berichte der Deutschen Chemischen Gesellschaft*, Vol.16, No.1, (January-June 1883), pp. (687-694), ISSN 0009-2940
- Sigma-Aldrich. (July 2011). SUPELCOSIL™ ABZ+Plus HPLC Column, In: *Sigma-Aldrich online catalog*, 12. 07. 2011, Available from http://www.sigmaaldrich.com/catalog/ProductDetail.do?lang=en&N4=59194C30|SUPELCO&N5=SEARCH_CONCAT_PNO|BRAND_KEY&F=SPEC
- Skovborg, F., Nielsen, A.V., Schlichtkrull, J. & Ditzel, J. (1966). Blood-viscosity in diabetic patients. *Lancet*, Vol. 1, No. 7429, (January 1966), pp. (129-131), ISSN 0140-6736

- Stamper, R. L., Smith, M. E., Aronson, S. B., Cavender, J. C., Cleasby, G. W., Fung, W. E. & Becker, B. (1978). The effect of calcium dobesilate on nonproliferative diabetic retinopathy: a controlled study. *Ophthalmology*, Vol. 85, No. 6, (June 1978), pp. (594-606), ISSN 0161-6420
- State Institute for Drug Control. (August 2011). SPC 98886. Summary of data concerning the preparation Danium ®. In: *Medicinal products database*, 02. 08. 2011, Available from [http://www.sukl.eu/modules/medication/search.php?data\[search_for\]=Danium&data\[code\]=&data\[atc_group\]=&data\[material\]=&data\[path\]=&data\[reg\]=&data\[radiio\]=none&data\[rc\]=&data\[with_adv\]=0&search=Search&data\[listing\]=20](http://www.sukl.eu/modules/medication/search.php?data[search_for]=Danium&data[code]=&data[atc_group]=&data[material]=&data[path]=&data[reg]=&data[radiio]=none&data[rc]=&data[with_adv]=0&search=Search&data[listing]=20)
- Suschek, C., Kolb, H. & Kolb-Bachofen, V. (1997). Dobesilate enhances endothelial nitric oxide synthase-activity in macro- and microvascular endothelial cells. *British Journal of Pharmacology*. Vol. 122, No. 7, (December 1997), pp. (1502-1508), ISSN 0007-1188
- Suschek, C., Rothe, H., Fehsel, K., Enczmann, J. & Kolb-Bachofen, V. (1993). Induction of a macrophage-like nitric oxide synthase in cultured rat aortic endothelial cells. *Journal of Immunology*, Vol. 151, (September 1993), pp. (3283-3291), ISSN 0022-1767
- Szabo, M. E., Haines, D., Garay, E., Chiavaroli, C., Farine, J. C., Hannaert, P., Berta, A. & Garay, R. P. (2001). Antioxidant properties of calcium dobesilate in ischemic /reperfused diabetic rat retina. *European Journal of Pharmacology*, Vol. 428, No. 2, (October 2001), pp. (277-286), ISSN 0014-2999
- Tawa, M., Almarsson, O. & Remenar, J. (2010) Pharmaceutical propylene glycol solvate compositions. *US 7790905* (07.09.2010)
- Velpandian, T. (2006). Pharmacetical compositions of calcium dobesilate. *IN 1327/DEL/2006* (01.06.2006)
- Vinazzer, H. & Hachen, H. J. (1987). Influence of calcium dobesilate (Doxium) on blood viscosity and coagulation parameters in diabetic retinopathy. *Vasa*, Vol. 16, No. 2, (March - April 1987), pp. (190-192), ISSN 0301-1526
- Vojnikovic, B. (1984). Hyperviscosity in whole blood, plasma, and aqueous humor decreased by doxium (calcium dobesilate) in diabetics with retinopathy and glaucoma: a double-blind controlled study. *Ophthalmic Research*, Vol. 16, No. 3, (May-June 1984), pp. (150-162), ISSN 0030-3747
- Vojnikovic, B. (1991). Doxium (calcium dobesilate) reduces blood hyperviscosity and lowers elevated intraocular pressure in patients with diabetic retinopathy and glaucoma. *Ophthalmic Research*, Vol. 23, No. 1, (January-February 1991), pp. (12-20), ISSN 0030-3747
- Wadworth, A. N. & Faulds, D. (1992). Hydroxyethylrutosides. A review of its pharmacology, and therapeutic efficacy in venous insufficiency and related disorders. *Drugs*, Vol. 44, No. 6, (June 1992), pp. (1013-1032), ISSN 0012-6667
- Xu, H., Li, X., Zhang, J., Zhang, Z. & Liu, K. (2009). *Analytical Letters*, Vol. 42, No. 8, (May 2009), pp. (1094-1110), ISSN 0003-2719
- Yanagisawa, M., Kurihara, H., Kimura, S., Tomobe, Y. & Kobayashi, M. (1988). A novel potent vasoconstrictor peptide produced by vascular endothelial cells. *Nature*, Vol. 332, No. 6163, (March 1988), pp. (411-415), ISSN 0028-0836
- Yang, E., Shim, J.S., Woo, H.-J., Kim, K.-W. & Kwon, H.J. (2007). Aminopeptidase N/CD13 induces angiogenesis through interaction with a pro-angiogenic protein, galectin-3.

- Biochemical and Biophysical Research Communications*, Vol. 363, No. 2, (November 2007), pp. (336-341), ISSN 1090-2104
- Yang, Y., Ma G. & Long, H. (2010). Process for preparation of calcium dobesilate hydrate. CN 101880248 (10.11.2010)
- Yao, C. (2010). Process for preparation of calcium dobesilate nanopowder. CN 101898980 (1.12.2010)
- Zhang, Y., Du, F. Y. & Wei, L. L. (2011). Electrochemical behavior of calcium dobesilate at a gold nanoparticles modified glassy carbon electrode and its determination. *Fenxi Shiyanshi*, Vol. 30, No. 3, (March 2011), pp. (50-53), ISSN 1000-0720
- Zhang, Y. & Zhang, Z. F. (2009). Lihua Jianyan, *Huaxue Fence*, Vol. 45, No. 11, (June 2009), pp. (1296-1301), ISSN 1001-4020
- Zhang, Z. & Xu, Z. (1999). Comparison of two titration methods of calcium 2,5-dihydroxybenzenesulfonate. *Huagong Shikan*, Vol. 13, No. 11, (November 1999), pp. (36-38), ISSN 1002-154X
- Zheng, J., Zhang, Y. & Yang, P. (2007). An ionic liquid-type carbon paste electrode for electrochemical investigation and determination of calcium dobesilate. *Talanta*. Vol. 73, No. 5, (April 2007), pp.(920-925), ISSN 0039-9140
- Zhong, H. & Guo, L. (1997). The plasma levels of endothelin in diabetic retinopathy and their changes after treatment with doxium. *Hunan Yike Daxue Xuebao - Bulletin of Hunan Medical University*, Vol. 22, No. 1, (January 1997), pp. (56-58), ISSN 1000-5625
- Zhu, F. & Chen, J. (2005). Determination of calcium dobesilate in drugs by HPLC. *Baoji Wenli Xueyuan Xuebao, Ziran Kexueban*, Vol. 25, No. 2, (February 2005), pp. (119-121), ISSN 1007-1261
- Zimmerman, G.A., Prescott, S.M. & McIntyre, T.M. (1992). Endothelial cell interactions with granulocytes: tethering and signalling molecules. *Immunology Today*, Vol. 13, No. 3, (March 1992), pp. (93-100), ISSN 0167-5699
- Zivanovic, L., Zecevic, M., Markovic, S., Petrovic, S. & Ivanovic, I. (2005). Validation of liquid chromatographic method for analysis of lidocaine hydrochloride, dexamethasone acetate, calcium dobesilate, butylhydroxyanisol and degradation product hydroquinone in suppositories and ointment. *Journal of Chromatography A*, Vol. 1088, No. 1-2, (September 2005), pp. (182-186), ISSN 0021-9673



Diabetic Retinopathy

Edited by Dr. Mohammad Shamsul Ola

ISBN 978-953-51-0044-7

Hard cover, 356 pages

Publisher InTech

Published online 24, February, 2012

Published in print edition February, 2012

The aim of this book is to provide a comprehensive overview of current concepts in pathogenesis, diagnosis and treatments of diabetic retinopathy. It provides a collection of topics written by excellent authors, covering discussions on advances in understanding of pathophysiology, immunological factors and emerging concepts, relating to clinical aspects and treatment strategies. The contents of the book will not only provide a resource for our knowledge but also improve diagnosis and treatment options for those patients who suffer vision loss due to diabetic retinopathy.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Oldřich Farsa (2012). Calcium Dobesilate in Prevention and Treatment of Diabetic Retinopathy, Diabetic Retinopathy, Dr. Mohammad Shamsul Ola (Ed.), ISBN: 978-953-51-0044-7, InTech, Available from: <http://www.intechopen.com/books/diabetic-retinopathy/calcium-dobesilate-in-prevention-and-treatment-of-diabetic-retinopathy>

INTECH

open science | open minds

InTech Europe

University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China

Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

© 2012 The Author(s). Licensee IntechOpen. This is an open access article distributed under the terms of the [Creative Commons Attribution 3.0 License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.