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Pharmacological evaluation of a novel oxime derived from isosorbide-5-mononitrate on isolated rat superior mesenteric artery

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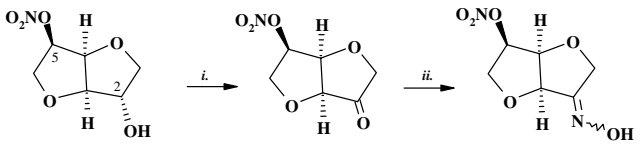
Organic nitrates are chiefly used for the the prevention and treatment of stable, unstable and Prinzmetal variant types of angina, and sometimes in patients with heart failure, especially those unable to take angiotensin-converting enzyme inhibitors [1, 2]. These drugs act by relaxing vascular smooth muscle, and cause marked venorelaxation and arteriolardilatation (with larger doses) as well as dilatation of collateral vessels of ischaemic areas. The basis of their cellular effects is the increase of cGMP formation. The release of nitric oxide (NO) from organic nitrates at concentrations achieved during therapeutic use involves an enzymic step and a reaction with tissue -SH groups. NO which is the main physiological vasodilator normally produced by the endothelial cell, activates a soluble cytosolic form of guanylate cyclase in vascular smooth muscle, probably by interacting with porphyrin binding site in the enzyme. cGMP formation is thereby increased, leading to changes in the degree of phosphorylation of various smooth muscle proteins and ultimately to de-phosphorilation of the myosin light chain, and hence relaxation [3, 4].

Classical compounds such as glyceryl trinitrate (GTN) and isosorbide dinitrate (ISDN) are still in general clinical use. However, these drugs have a number of disadvantages such as low oral bioavailability, first-pass effect, and development of tolerance during chronic administration. Isosorbide-5-mononitrate (IS-5-MN), which is an active metabolite of ISDN, is now marketed as a nitrate having only a weak first-pass effect, thus showing a bioavailability of 93% [5]. In this paper, we describe the synthesis and vasoactive effect of new nitrate derived from IS-5-MN. Our approach to modify IS-5-MN (**1**) was directed to change the tetrahydrofuran ring, having a free hydroxyl group, with retention of stereochemistry and functionality of the tetrahydrofuran ring bearing a nitrate group, responsible for pharmacological activity. For this purpose we achieved oxidative transformation of the free hydroxyl group of IS-5-MN (**1**) to the corresponding keto-nitrate of isosorbide (**2**), which was then converted to the isosorbide-5-nitrate-2-ketoxime (**3**, Scheme). It was of interest to investigate how the synthesized nitro compound and IS-5-MN affect the rat superior mesenteric artery with endothelium, aswell as which one of them has the more intensive relaxant effect on the artery.

Scheme



1 2 3

Reagents and conditions: i. PDC, Ac_2O, CH_2Cl_2, 50 °C, 4 h
ii. $NH_2OH \cdot HCl$, NaOAc, MeOH, 65 °C (80)

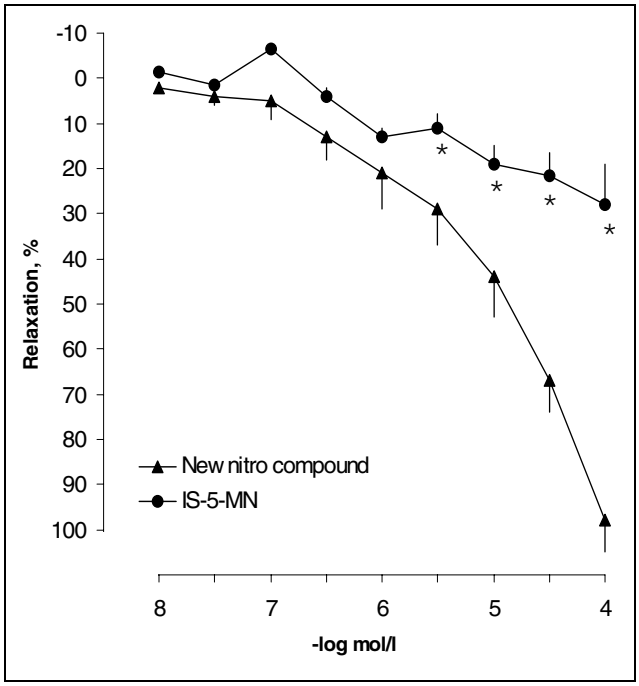


Fig.: Cumulative concentration-response curves to IS-5-MN and new nitro compound in rings with endothelium of the rat superior mesenteric artery. The relaxation was obtained during contractions induced by phenylephrine (3×10^{-7}–10^{-6} mol/l). The data are shown as mean ±SEM (n = 6), and expressed as the percentage of maximal relaxation induced by papaverine (10^{-4} mol/l).
* $P < 0.05$ compared to the new nitro compound relaxation

IS-5-MN (10^{-8}–10^{-4} mol/l) caused a concentration-dependent relaxation in rings of the superior mesenteric artery made to contract by phenylephrine (3×10^{-7}–10^{-6} mol/l) which gave 50% of the maximal tone. The maximal tone of the artery was obtained by the maximum concentration of phenylephrine (10^{-5} mol/l). The synthesized oxime-nitrate (10^{-8}–10^{-4} mol/l) induced a concentration-dependent relaxation in the precontracted preparation, too, but this inhibitory effect was statistically more intensive than that caused by IS-5-MN (Fig.). In lower concentrations (10^{-8}–10^{-6} mol/l) there were no statistical differences between the two vasoactive agents, but they were of statistical importance in large concentrations (3×10^{-6}–10^{-4} mol/l) of both compounds (Fig.). Thus, it appears that the new synthesized oxime nitrate is more effective as vasodilator agent on the rat superior mesenteric artery with endothelium than classical IS-5-MN. It has been suggested that the new nitro compound could be of interest in the treatment of angina caused by coronary artery spasm (Prinzmetal variant angina).

Experimental

1. Chemicals

The following drugs were used: acetylcholine iodide (Serva), phenylephrine (Sigma), papaverine hydrochloride (Merck), IS-5-MN (synthesized according to the procedure of Stoss [6]), pyridinium dichromate (freshly prepared according to Corey and Schmidt [7]). The solvents and commercial reagents were dried and purified by conventional methods before use.

2. Synthesis

Oxidation of IS-5-MN (**1**) to isosorbide-2-keto-5-nitrate (**2**) was carried out by pyridinium dichromate (PDC) in the presence of acetic anhydride and in methylene chloride solution [8, 9]. The corresponding ketoxime **3** was prepared by the reaction of ketone **2** with hydroxylamine hydrochloride in the presence of sodium acetate in methanol solution [10]. After crystallization from ethyl acetate – petroleum ether, ketoxime **3** was obtained as

colorless crystalline compound. M.p. 110 °C. $[\alpha]_D^{18}$ +143.3° (c 1, EtOAc). Anal. Calcd for $C_6H_8N_2O_6$: C, 35.29; H, 3.92; N, 13.72. Found: C, 35.00; H, 4.04; N, 13.51. IR and ^{1}H NMR spectral data are in accordance with those in literature [10]. The melting point was determined in open capillaries on an Electrothermal apparatus and was uncorrected. Optical rotation was measured using a Perkin–Elmer 141 MC polarimeter.

3. Pharmacology

Rats of either sex weighing 280–360 g, were killed by a blow on the head and the superior mesenteric artery was isolated. The artery was immersed in Krebs–Ringer bicarbonate solution (mmol/l: NaCl, 118.3; KCl, 4,7; $CaCl_2$, 2.5; $MgSO_4$, 1.2; KH_2PO_4, 1.2; $NaHCO_3$, 25; CaEDTA, 0.026; glucose, 11,1). The rings (4 mm long) were mounted on pairs of stainless steel wire hooks, and connected to a force transducer (Hugo Sachs Elektronik), and suspended in an organ chamber filled with 60 ml of Krebs-Ringer bicarbonate solution (37 °C; pH 7.4), which was bubbled with a gas mixture of 95% O_2/5% CO_2. Isometric tension was continuously recorded.

Each ring was gradually stretched to the optimal point (2 g) on its length-tension curve and allowed to equilibrate for 30 min [11]. The functional integrity of the endothelium was confirmed by the presence of an immediate relaxation induced by acetylcholine (10^{-6} mol/l) in rings contracted with phenylephrine (10^{-6} mol/l). All experiments were done on the pre-contracted preparations with phenylephrine [12].

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Received November 21, 2001
Accepted February 6, 2002

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Detection of allergenic urushiols in *Ginkgo biloba* leaves

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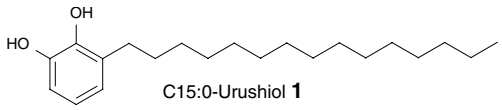
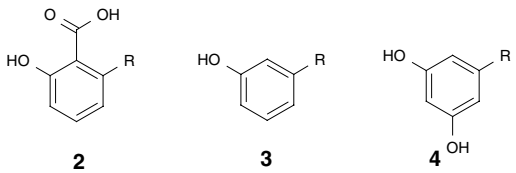
Leaves of *Ginkgo biloba* are widely used to prepare extracts for the treatment of peripheral and cerebral circulatory disorders as well as dementia of different aetiology [1–3]. *Ginkgo*, like the members of the Anacardiaceae family [4], which include mango tree (*Mangifera indica*), cashew nut tree (*Anacardium occidentale* [5]), lacquer tree (*Rhus vernicifera* [6], *Melanorrhoea usitate* [7]), Indian marking nut tree (*Semecarpus anacardium* [8]), and the poison ivy, oak and sumac genera (*Toxicodendron*) [9, 10], is known to accumulate long chain alkylphenols. All of these plants are well known to induce allergic contact dermatitis [11]. Allergies towards Anacardiaceae are extremely frequent in the USA, where about 50–85% of the population is sensitive to members of this plant family [12]. A mixture of 3-n-pentadec(en)yl or heptadec(en)ylcatechols, commonly referred to as urushiols (e. g. derivative **1** with C15 : 0 side chain) has been found to be responsible for these reactions [13, 14].



C15:0-Urushiol **1**

In *Ginkgo* several long chain alkyl phenols such as ginkgolic acids **2,** cardanols **3** and cardols **4** have been observed to posses contact allergenic properties [15–18]. Since these alkylphenols have been show to cross react with poison ivy allergens, it has been suggested that **2** and **3** can be biotransformed to yield urushiols **1** [15]. These would provide a plausible explanation for the strong allergenic effects of *Ginkgo biloba*. However, it cannot be excluded that the biochemical machinery of *Ginkgo biloba* itself is able to process these long chain alkyl phenols to form urushiols **1**. Indeed, to our knowledge we here report for the first time that urushiols are natural constituents of *Ginkgo* leaves.



2 **3** **4**

Since long chain alkylphenols represent a substantial risk factor for adverse drug reactions, suitable techniques for elimination of these compounds from therapeutically used *Ginkgo* leaf extracts should be applied. As extremely low doses (5–50 ng) of urushiols are sufficient to elicit patch test reactions in humans [19], for the determination of such small quantities, especially in extracts, it is necessary to use high resolution separation techniques in combination with suitable detection sensitivity and selectivity. Method of choice for this application is derivatization gas chromatography mass spectroscopy (GC/MS [20], see ref. [21] for LC-ES-MS of acid derivatives). In addition, known and supposed constituents are required as reference substances. Compounds **2** and **3** (R = C13 : 0, C15 : 0,