detail (Tomić et al. 2004). Briefly, the rat was placed with its hind paws on two transducer platforms of the apparatus (Hugo Sachs Elektronik, March-Hugstetten, Germany) and pushed slowly and smoothly downwards, until one of the paws exceeds the trigger level set at 100 g. The difference (d) in pressures applied to non-inflamed (vehicle-injected) and inflamed (Con A-injected) rat hind paw is determined after each measurement.

2. Experimental protocol

In order to examine the peripheral effects of OXC, the drug and the Con A were coadministered intraplantarly (i.pl.), into the right hind paw. Control animals received the same volume of Con A. To exclude the possible systemic effect of the i.pl. injected drug, the highest dose of OXC used was given contralaterally. The influences of caffeine and DPCPX on the peripheral anti-hyperalgesic actions of OXC were examined after i.pl. coadministration of each antagonist with OXC and Con A. The comparative group of animals received Con A with OXC. To exclude the possible systemic effect of intraplantarly injected antagonists, the highest doses tested were given contralaterally. Finally, the effects of the highest doses of caffeine and DPCPX coadministered with Con A have been evaluated and compared with the effect of Con A alone.

3. Chemicals

OXC (Novartis Pharma AD), caffeine (Galenika) and DPCPX (Sigma) were dissolved or suspended in a vehicle containing 50% polyethylene glycol 400 and 50% saline, and sonicated for 15 min. Con A was used in a fixed dose of 0.8 mg/paw. All substances were injected i.pl. in a final volume of 0.1 ml/paw.

4. Statistics

Results are expressed as means ± S.E.M. Calculations for percent anti-hyperalgesic activity (%AA), percent inhibition (%I) of that activity, and ED_{50} values were done according to Tallarida and Murray (1986) and Tomić et al. (2004). Statistical difference was determined by Student's t-test or one-way analysis of variance (ANOVA), followed by Tukey's HSD test. A value of $P < 0.05$ was considered significant.

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NO-Synthase inhibitors provide influence on protective effect of modified endotoxine diphosphoryl lipid A in a rat heart model of ischemic-reperfusion injury

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The present study was designed to assess whether a protective effect of the modified diphosphoryl lipid A (modLA) against myocardial ischemia-reperfusion injury (IRI) in rats can be related to the mechanism involving inducible nitric oxide synthase (iNOS). Pre-treatment with modLA significantly reduced the duration of both ventricular tachycardia ($p < 0.01$) and ventricular fibrillation ($p < 0.001$) compared to controls. Under these conditions the incidence of animal death was reduced ($p < 0.05$). The beneficial effect of modLA was markedly attenuated by the prior administration of selective iNOS inhibitor S-methylisothiourea (SMT). In this animal group, mortality was significantly increased ($p < 0.01$) partially in consequence of sustained ventricular arrhythmias. These results indicate that induction of iNOS can be responsible for cardioprotection of modLA.

Reperfusion of previously obstructed coronary arteries is known to produce paradoxically both morphological and functional damage of the myocardial tissue to a considerable greater extent than ischemia alone (Bolli 1990). Although numerous drugs have been shown to exert a protective effect against ischemic-reperfusion injury (IRI), new pharmacological strategies are being developed (Maxwell and Lip 1997).

Recently the modified lipids A obtained from bacterial lipopolysaccharides have been demonstrated to be a suitable means to decrease deleterious effects of IRI. Modified lipids A possess the immunomodulatory activity of parent lipids A (Salkowski 1997), but enjoy reduced toxicity. Monophosphoryl lipid A (MLA) has been the most widely studied substance, which represents a novel agent capable of enhancing myocardial tolerance to IRI, when pre-treated 24 h prior to ischemia. As with delayed ischemic preconditioning this cardioprotective activity of MLA manifests itself as a reduction in infarct size, polymorphonuclear leucocyte infiltration into the ischemic myocardium, myocardial stunning and arrhythmias in multiple animal species and various animal models of IRI, as

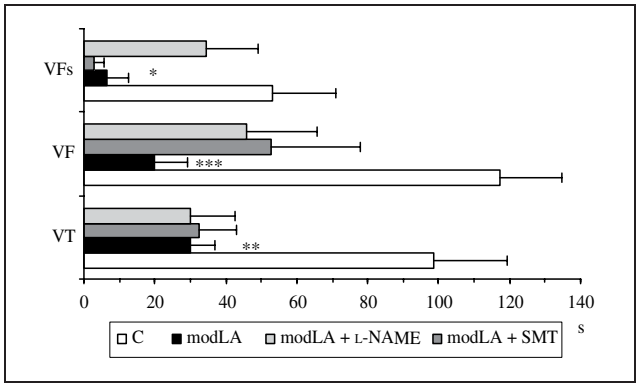

Fig. 1: Duration of ventricular tachycardia (VT), ventricular fibrillation (VF) and sustained ventricular fibrillation (VFs) in control (C), modLA-treated (modLA), modLA and L-NAME-treated (modLA + L-NAME) and modLA and SMT-treated (modLA + SMT) rats
The data are expressed as means ± SEM, n = 6–8 for each group
* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ significant difference from control group

well (Elliott et al. 1998). Although multifactorial mechanisms of cardioprotection may be induced by MLA, current evidence suggests that cardioprotective effects of MLA involve myocardial iNOS enzyme activation (Zhao et al. 1997; Maulik et al. 1998; Gyorgy et al. 1999; Wang et al. 2002). The maximal NO or iNOS expression was found between 4 and 6 h of MLA pre-treatment and NO may be responsible for the delayed cardioprotection observed 24 h after MLA pre-treatment.

Modified diphosphoryl lipid A (modLA) used in this study was obtained from an *E. coli* strain adapted to amine oxide. Fatty acid and hydroxy fatty acid profile of modLA differs from the lipid A isolated from the natural sensitive strain (Bukovský et al. 1991). In experiments where reperfusion of the isolated ischemic heart was performed an administration of modLA to rats was demonstrated to improve the contractile properties of the myocardium as well as to reduce the severity of ventricular arrhythmias (Šperglová et al. 2002).

Based on the cardioprotective effect of MLA mediated by signalling through production of inducible nitric oxide synthase, this study was designed to test whether modLA may mediate any cardioprotection by a similar mechanism.

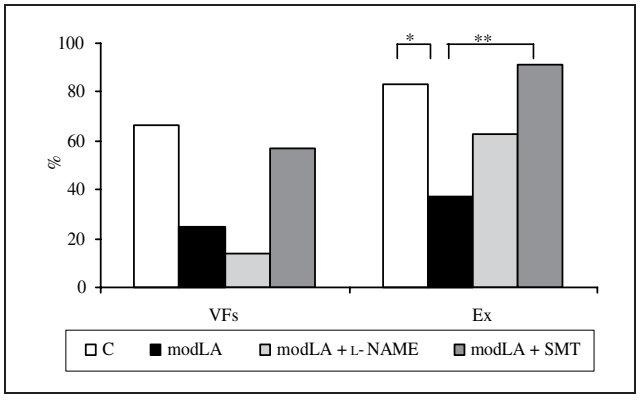

Fig. 2: Incidence of sustained ventricular finrillation (VFs) and death (Ex) in control (C), modLA-treated (modLA), modLA and L-NAME-treated (modLA + L-NAME) and modLA and SMT-treated (modLA + SMT) rats
* $p < 0.05$ significant difference from control group, ** $p < 0.01$ significant difference from modLA-treated group

The experiments were performed in male Wistar rats (Anlab, Prague, Czech Republic). The experimental protocol of study was performed in accordance with the 'Guide for the Care and Use of Laboratory Animals' and with the approval of the Ethics Committee of the Faculty. After an adaptation period, the rats were randomly divided into the four experimental groups. Rats from control group (C) received saline i.v. 24 h before ischemia, while rats from the other groups were treated i.v. with modLA (500 $\mu g \cdot kg^{-1}$) 24 h before ischemia. Rats from one treated group received the nonselective NOS inhibitor L-nitroarginine methyl ester (L-NAME, 10 $mg \cdot kg^{-1}$, Sigma) i.v. 24 h before ischemia. Rats from the other treated group received the selective iNOS inhibitor *S*-methylisothiourea (SMT, 3 $mg \cdot kg^{-1}$, Sigma) i.p. 30 min before ischemia. ModLA was obtained from the *Escherichia coli* strain ATCC 11229 which was adapted to 1-(methyldodecyl)dimethylamine oxide (ATDNO) (820 mM, 200 µg/ml). Lipid A was isolated and purified according to Schromm et al. (1998). 24 h after administration of modLA, hearts were subjected to 6 min left coronary-descending artery occlusion followed by 10 min reperfusion. The heart action was recorded on an electrocardiograph (Seiva, Czech Republic) and the arrhythmias were analyzed according to guidelines known as The Lambeth Conventions (Walker et al 1988). Differences among the groups in duration of ventricular arrhythmias were compared using the Student's t-test. For comparison of incidence of both arrhythmias and animal death the Fisher test for very small samples using the table 2×2 was used. A value of $p < 0.05$ was considered statistically significant.

The results of this study suggest that administration of modLA protects the myocardium subjected to IRI. The significant shortening of duration of both ventricular tachycardia (VT, $p < 0.01$) and fibrillation (VF, $p < 0.001$) together with shortened duration of sustained ventricular fibrillations (VFs, $p < 0.05$) induced by reperfusion was observed after pre-treatment of rats with modLA. A decreased tendency of incidence of ventricular arrhythmias (VF 62.5% vs. 100%) and arrhythmic score (4.8 vs. 5.8) was in a correlation with the duration of arrhythmias. Moreover, the incidence of death in the group of animals pre-treated with modLAs was significantly decreased ($p < 0.05$). Results from previous studies have established a similar cardioprotective activity of MLA (Elliott 1998). Many investigators made an attempt to chemically modify the parent lipid A in order to reduce toxicity while retaining its immunomodulatory and cardioprotective properties. Dubničková et al. (2003) have shown that the imunomodulatory effect of modLA is similar to that of MLA regarding *in vitro* modulation of an immune response of human mononuclear cells.

Current evidence suggests that the protective effect of MLA may be due to myocardial iNOS enzyme activation (Zhao et al. 1997; Maulik et al. 1998; Gyorgy et al. 1999; Wang et al. 2002). In order to confirm an involvement of iNOS in modLA mediated cardioprotection, animals were pretreated with either L-NAME or SMT, NOS inhibitors, which differ in their specificities to inhibit the activity of iNOS. NOS inhibitors were administered in doses suggested by other authors, who studied a mechanism of MLA-mediated cardioprotection in animal models of myocardial IRI (Tosaki et al. 1998; Lei et al. 1999).

The pretreatment with nonselective NOS inhibitors did not significantly affect the protective effect of modLA against myocardial IRI. The beneficial effect of modLA was markedly attenuated by pre-treatment with selective iNOS inhi-

bitor SMT. In this animal group, mortality was significantly increased ($p < 0.01$) in consequence of increased incidence and duration of VT, VF and mainly VFs.
In conclusion, it could be suggested that modLA may exert its protective effect against myocardial IRI at least in part by inducing NO synthesis.

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An eudesmane glycoside from *Fissistigma pallens*

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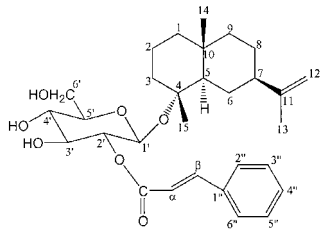
From *Fissistigma pallens* (Fin. & Gagn.) Merr. (Annonaceae), a Vietnamese folk medicinal plant, a novel eudesmane glycoside named fissispallin (**1**) has been isolated, besides afzelin. Their structures were elucidated by spectroscopic methods (^{1}H, ^{13}C and 2D NMR).

Fissistigma pallens (Fin. & Gagn.) Merr. (Annonaceae) is growing in the North of Vietnam (Ban 2000), its chemical constituents have not yet been studied. In continuation of phytochemical studies on Vietnamese *Fissistigma* plants (Porzel et al. 2000), we have carried out a phytochemical investigation on the leaves of *F. pallens*, which resulted in a novel sesquiterpene glycoside, named fissispallin, besides the known flavonol glycoside, afzelin (Thuy et al. 1998). This paper deals with the isolation and structural elucidation of the new fissispallin (**1**) on the base of studies of its MS, 1D and 2D NMR.



1 : Fissispalin

Compound **1** was obtained as powder from EtOAc extract by chromatography on silica gel. The HR ESI MS of compound **1** gave the $[M + Na]^+$ peak at m/z 537.28345 (calc. 537.28227) leading to the molecular formula $C_{30}H_{42}O_7$. The sugar moiety was identified from its characteristic signals in the ^{1}H and ^{13}C NMR spectra (Table) as β-D-glucopyranose. The low-field ^{1}H NMR signals at δ 7.54 (2 H, d, J = 7.4 Hz, H-2″/6″), 7.42 (2 H, dd, J = 7.4; 2.5 Hz, H-3″/5″) and 7.43 (1 H, m, H-4″) are characteristic of a mono-substituted phenyl ring. The corresponding ^{13}C resonances were assigned due to their ^{13}C-^{1}H correlation (HMQC). The ^{13}C NMR spectrum showed the presence of the cinnamate moiety by a singlet at δ 166.39 (C=O), two doublets at δ 117.81 (β-CH=), 145.44 (α-CH=), a singlet at δ 134.37 (C-1″), two doublets at δ 128.16 (C-2″, C-6″), 128.86 (C-3″, C-5″) and a doublet at δ 130.32 (C-4″). The presence of the *trans*-cinnamate moiety is also confirmed by the appearance of two doublets at δ 7.73 and 6.44 (each 1 H, d, J = 16.0 Hz, H-α and H-β) in the ^{1}H NMR spectrum. This was supported by the