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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References

Bolli R (1990) Mechanism of myocardial "stunning". Circulation 82: 723–738.
Bukovský M, Mlynarčík D, Nagy A, Bella J (1991) Outer membrane alternations in *Escherichia coli* cells adapted to amine oxides. Acta Facultatis Pharm XLXI: 153–168.
Dubničková M, Bukovský M, Mlynarčík D (2003) Activation of human leucocytes by lipid A from *E. coli* strains adapted to quaternary ammonium salts and amine oxide. Folia Microbiol 48: 543–547.
Elliott GT (1998) Monophosphoryl lipid A induces delayed preconditioning against ischemia reperfusion injury. J Moll Cell Cardiol 30: 3–17.
Gyorgy K, Muller B, Végh A, Kleschyov AL, Stoclet JC (1999) Triggering role of nitric oxide in the delayed protective effect of monophosphoryl lipid A in a rat heart. Br J Pharmacol 127: 1892–1898.
Maulik N, Tosaki A, Elliott GT, Maulik G, Das DK (1998) Induction of iNOS gene expression by monophosphoryl lipid A: a pharmacological approach for myocardial adaptation to ischemia. Drugs Exp Clin Res 24: 117–124.
Maxwell SRJ, Lip GYH (1997) Reperfusion injury: a review of the pathophysiology, clinical manifestations and therapeutic options. Int J Cardiol 58: 95–117.
Salkowski CA, Detore GR, Vogel SN (1997) Lipopolysaccharide and monophosphoryl lipid A differentially regulate interleukin-12, gamma interferon and interleukin-10 mRNA production in murine macrophages. Infect Immun 65: 3239–3247.
Schromm AB, Brandenburg K, Loppnow H, Zähringer U, Rietschel ET, Carroll SF, Koch MH, Kusumoto S, Seydel U (1998) The charge of endotoxin molecules influences their conformation and IL-6-inducing capacity. J Immunol 161: 5464–5471.
Šperglová L, Stankovič M, Jusko M, Švec P, Stankovičová T (2002) Modified lipid A affects the function of isolated perfused rat heart. Brat Lek Listy 103: 338–339.
Tosaki A, Maulik N, Elliot GT, Blasig IE, Engelman RM, Das DK (1998) Preconditioning of rat heart with monophosphoryl lipid A: role for nitric oxide. J Pharmacol Exp Ther 285: 1274–1279.
Walker MJA, Curtis MJ, Hearse DJ, Campbell RWF, Janse MJ, Yellon DM, Cobbe SM, Coker SJ, Harness JB, Harron DWG, Higgins AJ, Julian DG, Lab MJ, Manning AS, Northover BJ, Parratt JR, Riemersma RA, Riva E, Russell DC, Sheridan DJ, Winslow E, Woodward B (1988) The Lambeth Conventions: guidelines for the study of arrhythmias in ischemia, infarction, and reperfusion. Cardiovasc Res 22: 447–455.
Wang YP, Sato C, Mizoguchi K, Yamashita Y, Oe M, Maeta H (2002) Lipopolysaccharide triggers late preconditioning against myocardial infarction via inducible nitric oxide synthase. Cardiovasc Res 56: 33–42.
Xi L, Jarrett NC, Hess ML, Kukreja RC (1999) Essential role of inducible nitric oxide synthase in monophosphoryl lipid A – induced late cardioprotection. Evidence from pharmacological inhibition and gene knockout mice. Circulation 99: 2157–2163.
Zhao L, Weber PA, Smith JR, Comeford ML, Elliott GT (1997) Role of inducible nitric oxide synthase in pharmacological "preconditioning" with monophosphoryl lipid A. J Mol Cell Cardiol 29: 1567–1576.

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An eudesmane glycoside from *Fissistigma pallens*

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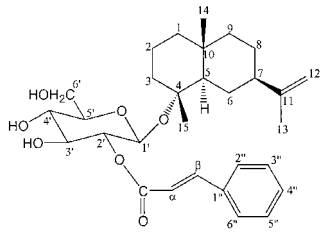
From *Fissistigma pallens* (Fin. & Gagn.) Merr. (Annonaceae), a Vietnamese folk medicinal plant, a novel eudesmane glycoside named fissispallin (**1**) has been isolated, besides afzelin. Their structures were elucidated by spectroscopic methods (^{1}H, ^{13}C and 2D NMR).

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

peak at m/z 131 due to fragment $[C_9H_7O]^+$ from the EI MS and absorption at 1638 cm^{-1} in FT-IR spectrum. The remaining 15 carbon signals ($3 \times CH_3$, $6 \times CH_2$, $2 \times CH$, $2 \times Cq$, $1 \times =CH_2$, $1 \times =C<$) belong to the first moiety of **1**. The mass spectrum showed the corresponding fragment ions at m/z 221 $[C_{15}H_{25}O]^+$ and 293 $[M\text{-}C_{15}H_{25}O]^+$. From the results of 2D NMR experiments this moiety was determined as a sesquiterpene unit. The HMBC correlations of three methyl singlets at δ 1.69 (Me-13), 0.91 (Me-14) and 1.10 (Me-15) with C-7/C-11/C-12; C-1/C-5/C-9/C-10 and C-3/C-4/C-5, respectively, confirmed the partial structures of the sesquiterpene. The remaining positions C-2, C-6 and C-8 were determined by HMBC (H-9β → C-8; H-6α → C-8/C-11; H-2β → C-4) as well as by HMQC correlations. A literature search revealed that the sesquiterpene is eudesm-11-en-4-α-ol (Consolacion et al. 1997; Waßmuth-Wagner et al. 1995). This was supported by the COSY spectrum with two olefinic protons at δ 4.87 and 4.85 (each 1 H, br s), the allylic protons at δ 1.69 (3 H, s), 2.27 (1 H, br s, H-7). The relative configuration of **1** was deduced from NOESY and HMBC experiments, particularly the attachment of the glucose to the sesquiterpene and the position of linkage between glucose and cinnamic acid. It indicated that the methyl groups (C-14 and C-15) of the sesquiterpene moiety are close together in the molecule. Since the C-14 of eudesm-11-en-4-α-ol is in an axial position, then C-15 should also be in axial position and therefore the glucoside is in equatorial position. The NOESY spectral data supported the results of the coupling constants analyses. The anomeric carbon atom has the β-configuration based on the axial H-1′ at δ 4.75 (1 H, d, J = 7.8 Hz). The linkage between cinnamic acid and glucose could be revealed by a broad triplet in the lower field at δ 4.79 (J = 7.9 Hz, H-2′) in the ^{1}H NMR and confirmed by the HMBC correlations C=O/H-2′, H-α, H-β. Peracetylation of **1** supported its proposed structure. From the above data, the structure of **1** is elucidated as eudesm-11-en-4-α-*o*-β-D-2-cinnamoyloxy glucopyranoside, named as fissispallin. Fissispallin is the first natural representative of ester of cinnamic acid and sesquiterpene glycoside.

Table: ^{1}H and ^{13}C-NMR data of 1 [$CDCl_3$, δ (J in Hz)]

	δC, 125 MHz	δH (J in Hz), 500 MHz	HMBC
1	40.85	1.25 m; 1.05 1 H, m	H_3-14
2	19.61	2.0 m; 1.5 m	H_2-3
3	39.77	1.62 dt (13.8, 3.9); 1.68[a] m	H_3-15
4	79.69	–	H-1′, H_2-2, H_2-3, H_3-15
5	47.49	1.43 br dt (13.7, 3.3)	H_3-14, H_3-15
6	22.54	1.97 dd (13.3; 2,0); 1.27 m	H-5
7	39.34	2.27 br s	H_3-13
8	23.27	1.60 m	H_2-6, H_2-9
9	40.56	1.35 m; 1.26 m	H_3-14
10	35.05	–	H_3-14
11	146.80	–	H-6b, H-7, H_2-12, H_3-13
12	110.81	4.87 br s; 4.85 br s	H_3-13
13	22.82	1.69 s	H_2-12
14	18.87	0.91 s	H-5
15	18.21	1.10 s	H_2-1, H-5
1′	94.34	4.75 d (7.8)	H-2′
2′	74.37	4.79 t[b] (7.9)	H-1′
3′	75.20	3.39 m	H-4′
4′	75.60	3.61 br t (9.1)	H-1′, H-2′
5′	70.77	3.69 t (9.1)	H_2-6′
6′	62.09	3.91 dd (11.8, 3.5) 3.79 dd (11.8, 5.5)	H-5′
C=O	166.39	–	H-2′, H-α, H-β
α	145.44	7.73 d (16.0)	H-2″/H-6″
β	117.81	6.44 d (16.0)	H-α
1″	134.37	–	H-α, H-β H-2″/H-6″
2″, 6″	128.16	7.54 d (7.4)	H-β, H-3″/H-5″
3″, 5″	128.86	7.42 dd (7.4, 2.5)	H-2″/ H-6″, H-4″
4″	130.32	7.43 m	H-2″/ H-6″, H-3″/ H-5″

[a] hidden under δ 1.69; [b] t like dd

Experimental

1. Equipment

Optical rotation $[\alpha]_D$: JASCO-DIP-1000 Digital Polarimeter with $CHCl_3$ as solvent. FT-IR: Nicolet IMPACT 410. CD spectra: JASCO J710 (MeOH). EI MS (70 eV, DIS): ADM 402, Finnigan TSQ 700 and HR-ESI-MS (resolution ca. 5000): QStar Pulsar (Applied Biosystems). NMR: Bruker Avance 500, 499.84 MHz (^{1}H) and 125 MHz (^{13}C, ^{13}C DEPT). TMS (δ = 0.0, ^{1}H) and $CDCl_3$ (δ = 77.0, ^{13}C) were references. CC: Silica gel 60, 0.06–0.2 mm (Merck) for the first column, silica gel 60, 40–63 μm (Merck) for the following columns. TLC: silica gel 60 F_{254} (Merck).

2. Plant material

Leaves of *F. pallens* were collected from Nghe An province, Vietnam in November 2004. The species was identified by Dr. Ngo Van Trai, Institute of Materia Medica, Hanoi. A voucher specimen Poilane 16533 (VT4256, HN) is deposited in the Herbarium at the Institute of Ecology and Natural Resources, Vietnamese Academy of Science and Technology, Hanoi.

3. Extraction and isolation

The plant material (1.3 kg) was dried, ground and extracted with 95% MeOH at room temperature. MeOH was evaporated in *vacuo*, and the solution was extracted with *n*-hexan, followed by EtOAc and *n*-BuOH. The EtOAc extract (30 g) was separated on silica gel with gradient $CHCl_3$/MeOH (95 : 5 → 8 : 2) and then $CHCl_3$/MeOH/H_2O (65 : 35 : 5) to afford 45 fractions. The crude compound **1** was separated from fraction 25 and further purified by chromatography on silica gel with MeOH/EtOAc/H_2O (2 : 12 : 1).

Fissispallin (eudesm-11-en-4α-o-β-D-2-cinnamoyloxy glucopyranoside, **1***):*

Powder from EtOAc/MeOH; yield 0.028%; $[\alpha]_D^{24}$ − 44° ($CHCl_3$, c1.00); FT-IR (KBr) ν_{max}: 3406, 3084, 2934, 1638, 882 cm^{-1}; CD (MeOH): $\Delta\varepsilon_{197} - 11.37$, $\Delta\varepsilon_{216} + 5.63$, $\Delta\varepsilon_{222} + 5.61$, $\Delta\varepsilon_{276} - 6.28$ cm^2 $mmol^{-1}$; HR ESI MS: 537.28346 $[M + Na]^+$ ($C_{30}H_{42}NaO_7$, calc. 537.28227); ESI MS m/z (rel. int): 537 $[M + Na]^+$ (100), 293 $[M + H\text{-}221]^+$ (20), 104 $[C_8H_7 + H]^+$ (10). ESI MS negative ions m/z: 549 $[M + Cl]^-$ (16), 513 $[M - H]^-$ (30), 383 $[M\text{-}C_8H_7CO]^-$ (100); EI MS (70 eV) m/z (rel. int.): 514 $[M]^+$ (2), 496 (4), 352 (8), 311 (8), 293 $[M\text{-}C_{15}H_{25}O]^+$ (73), 275 (8), 205 (75), 204 (100), 189 (13), 161 (17), 149 (24), 147 (20), 131 (93), 123 (32), 103 (19), 95 (20); ^{1}H and ^{13}C NMR data see Table.

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References

Ban NT (2000) Thuc vat chi Vietnam (Flora of Vietnam), Hanoi Publishing House for Science and Technics, p. 209–210.

Consolacion YR, John AR, Diana ST, John C (1997) Sesquiterpene glycosides from *Pittosporum pentandrum*. Phytochemistry 45: 545–547.

Porzel A, Lien TP, Schmidt J, Susane D, Sung TV, Adam G (2000) Fissistigmatins A-D: Novel type natural products with flavonoid-sesquiterpene hybrid structure from *F. brateolatum*. Tetrahedron 56: 865–872.

Thuy TT, Sung TV, Ripperger H, Adam G (1998) Flavonol glycosides from *Premna flavescens*. Vietnam J Chem 36 (3): 49–52.

Waßmuth-Wagner HO, Kalinowski HJ (1995) Isolation and identification of 11-selinen-4α,7β-ol and 10-aromadendranol in the essential oil of *Murraya koennigii*. Planta Med 61: 196–197.