Pergamon

Tetrahedron 56 (2000) 865–872

TETRAHEDRON

Fissistigmatins A–D: Novel Type Natural Products with Flavonoid–Sesquiterpene Hybrid Structure from *Fissistigma bracteolatum*

Andrea Porzel,[a] Trinh Phuong Lien,[b] Jürgen Schmidt,[a] Susanne Drosihn,[a] Christoph Wagner,[c] Kurt Merzweiler,[c] Tran Van Sung[b] and Günter Adam[a,*]

[a]*Institute of Plant Biochemistry, Weinberg 3, D-06120 Halle/Saale, Germany*
[b]*Institute of Chemistry, National Centre for Natural Science and Technology of Viet Nam, Nghia Do, Tu Liem, Hanoi, Viet Nam*
[c]*Department of Chemistry, Martin-Luther University Halle-Wittenberg, Kurt-Mothes-Str. 2, D-06120 Halle/Saale, Germany*

Received 16 September 1999; revised 25 November 1999; accepted 30 November 1999

Abstract—From *Fissistigma bracteolatum* Chatt. (Annonaceae), a Vietnamese folk medicinal plant, the novel type natural products fissistigmatin A–D (**1**–**4**), being composed of a flavonoid and a sesquiterpene moiety, were isolated. Their structures were elucidated by extensive NMR techniques, MS, CD, molecular modelling calculations and X-ray analysis.

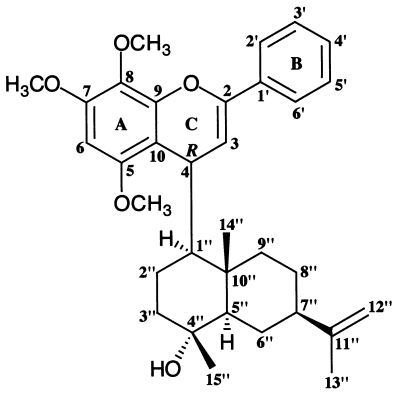
Fissistigma bracteolatum Chatt. (Annonaceae) is a creeper growing in the north of Viet Nam.[1] In South East Asia this plant, like other species of the genus *Fissistigma*, is used in traditional medicine, especially to stop wound bleeding, for treatment of infections and for enhancement of blood circulation.[2,3] Within a program concerning phytochemical studies on Vietnamese medicinal plants, we recently reported the isolation and structure elucidation of five new chalconoids from *F. bracteolatum*.[4] In continuation of our investigations on the constituents of this plant we now report on the isolation and structure determination of fissistigmatins A–D (**1**–**4**), the first examples of a new group of natural products consisting of a flavonoid and a sesquiterpene moiety.

A methanolic extract of air dried leaves and branches of *F. bracteolatum* was processed as described in the materials and methods section to afford compounds **1**–**4**. Their structures were elucidated by extensive NMR techniques, MS data, CD spectra and for **1** completed by molecular modelling calculations and X-ray crystallographic analysis.

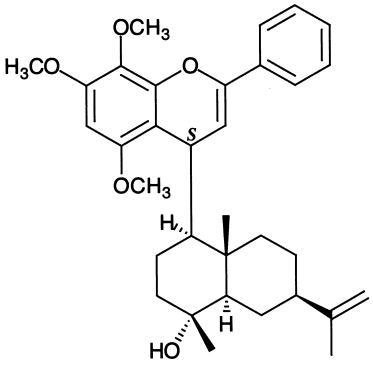
Fissistigmatin A (**1**) was isolated as white needles in a yield of 0.0080% on the dry material. The molecular formula of compound **1**, $C_{33}H_{42}O_5$, was deduced from combined analysis of HR-ESI-MS at *m/z* 541.2922 $[M+Na]^+$ (calc. 541.2930) and ^1H and ^{13}C-APT NMR spectra (Tables 1 and 2). The electron impact MS of **1** displayed a highly conjugated flavylium type ion (**a**)[5] at *m/z* 297 as base peak. The low-field ^1H NMR signals at δ 7.78 (2H, d, J=7.4 Hz, H-2′/6′), 7.43 (2H, t, J=7.4 Hz, H-3′/5′) and 7.36 (1H, t, J=7.4 Hz, H-4′) are characteristic of a monosubstituted phenyl ring. The corresponding ^{13}C resonances were assigned due to their ^{13}C–^1H correlations via $^1J_{CH}$ (HSQC). The ^1H singlets at δ 3.88 (6H) and 3.82 (3H) together with their HSQC correlations with ^{13}C signals at δ 56.6/56.0 and 61.2, respectively, revealed the presence of three methoxy groups. The ^1H chemical shifts as well as the ^1H–^{13}C correlations via $^3J_{CH}$ (HMBC) (Table 3) with ^{13}C resonances at δ 152.9/152.8 and 132.1, respectively, strongly indicated that all methoxy groups are attached to a phenyl ring. Interpretation of the NOESY correlations of the ^1H singlet at δ 6.49 (H-6) with the methoxy groups at δ 3.88, its strong HMBC correlations with ^{13}C signals at δ 152.9/152.8 (C-5/C-7), 132.1 (C-8) and 108.9 (C-10) and a weak HMBC correlation (via $^4J_{CH}$) with the ^{13}C resonance at δ 148.1 (C-9) permitted the positional assignment of the methoxy substituents. The assignment was supported by the NOE between H-2′/6′ and the methoxy signal at δ 3.82 (OMe-8) as well as the high field shift of C-6 (δ 93.3) and C-8 (δ 132.1), each caused by two oxygen substituents in *ortho* position. The ^{13}C signal at δ 135.3 was assigned to C-1′ because of its HMBC correlations with H-3′/5′. H-2′/6′ displayed an HMBC correlation with the ^{13}C resonance at δ 150.3, which was therefore assigned to C-2. The ^1H doublet at δ 5.68 showed $^3J_{CH}$ couplings with C-10 as well as C-1′ and was accordingly assigned to H-3. Furthermore, the vicinal coupling of H-3 (δ 5.68, d, J=6.0 Hz) with the ^1H double

Keywords: *Fissistigma bracteolatum*; Annonaceae; flavonoids; sesquiterpenes; NMR; X-ray analysis.
* Corresponding author. Tel.: +49-345-5582-216; fax: +49-345-5582-102; e-mail: gadam@ipb.uni-halle.de

0040–4020/00/$ - see front matter
PII: S0040-4020(99)01049-2



1



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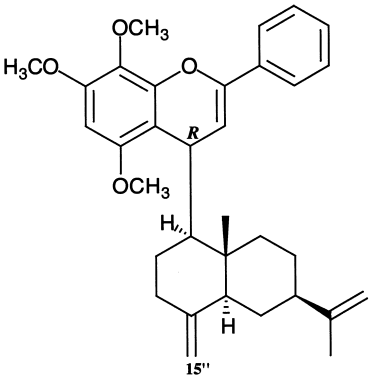

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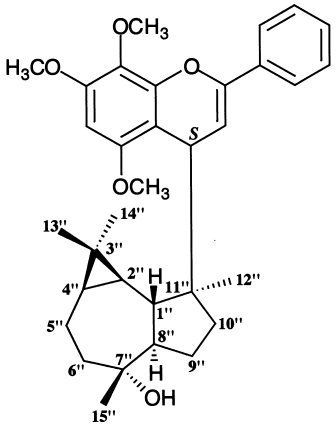
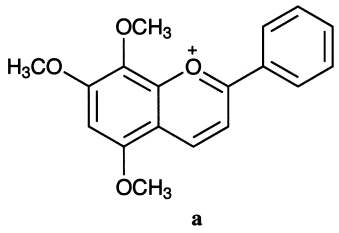
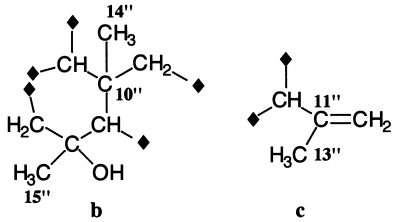


4

doublet at δ 4.09 (J=6.0/1.2 Hz) permitted the assignment of H-4. The chemical shift of C-4 of δ 30.1 (known from the HSQC spectrum) clearly indicated C-4 as sp^3 hybridized carbon.



a



b c

The remaining 15 carbon signals (3×CH_3, 5×>CH_2, 3×CH, 2×C_q, 1×=CH_2, 1×=C< belong to the second moiety of **1**. The mass spectrum showed no corresponding fragment ion, however, from the difference of the molecular formula and the fragment ion **a** the fragment formula could be derived as $C_{15}H_{25}O$. By combination of homo- and heteronuclear two-dimensional NMR experiments this moiety was recognized as a sesquiterpene unit. Namely, the HMBC correlations of the three methyl group singlets at δ 1.76 (Me-13″), 1.14 (Me-14″) and 1.02 (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, identified the two partial structures **b** and **c**. Interpretation of the ^{1}H–^{1}H-COSY spectrum and further HMBC correlations (H-9β″→C-8″; H-6α″→C-8″/C-10″; H-2β″→C-1″/C-2″/C-10″) completed by the HSQC correlations allowed an unambiguous assignment of the ^{1}H and ^{13}C resonances of the remaining positions 2″, 6″ and 8″. These data clarified that the sesquiterpene moiety of **1** possesses an eudesmane-type structure. The relative stereochemistry was deduced from the NOESY and NOE difference experiments (Fig. 1) and multiplet patterns determined from the ^{1}H NMR spectrum (or NOE difference spectra in the case of submerged signals). Unfortunately, the signal of H-1″ was overlapped by H-9α″ in acetone-d_6 solution and by H-2α″ in chloroform-d solution. However, based on the multiplet pattern of the HSQC correlation peak C-1″/H-1″ the axial orientation of H-1″ could be deduced and hence its α position.

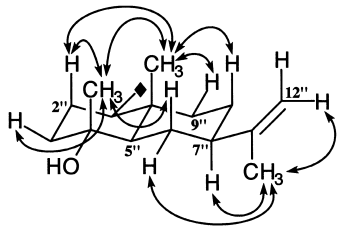


Figure 1. Key NOE correlations of the sesquiterpene moiety of **1**.

The flavonoid and the sesquiterpene moiety of **1** could be merged by the vicinal coupling of H-4 and H-1″ (J=1.2 Hz) on the one hand and by HMBC correlations of H-4 with C-1″, C-2″ and C-10″ on the other hand. The relative orientation of the two parts of **1** as well as the absolute stereochemistry at C-4 was assigned with reasonable confidence on the basis of NOE contacts found between the two moieties of the molecule accompanied by molecular modelling calculations. A strong NOE was detected between H-3/H_3-14″ and H-4/H-9β″ indicating a close spatial relationship of these protons. Ring C of the flavonoid moiety can adopt two boat-like conformations with the substituent at C-4 in *quasi*-axial and *quasi*-equatorial position, respectively. For the molecular modelling calculations (SYBYL 6.5 software package, TRIPOS, St. Louis, MO, USA) four starting structures were built with either (4*R*) or (4*S*) configuration and the two possible conformations of ring C for each case. The absolute stereochemistry of the sesquiterpene moiety was assumed to be that of eudesmane (5α, 10β-form), because in higher plants generally eudesmanes were found, whereas *ent*-eudesmanes were observed only in some species of liverworts.[6] The relative position of the two parts of the molecule were chosen according to the results of the NOE experiments. The four structures were energy minimized using the TRIPOS force field[7,8] and the Powell method[8] until a gradient of 1×10^{-3} kcal mol^{-1} Å^{-1} was achieved. Partial charge contributions were calculated according to the Gasteiger–Marsili[9] method and electrostatic interactions were taken into account by using a constant dielectric function with ϵ=5 (according to chloroform). The energy minimization clearly showed that only those conformations with the sesquiterpene substituent in *quasi*-axial position are stable ones. With these conformations, in a second step a systematic conformational search around the C-4/C-1″ bond in 10° steps was performed for both configurations (4*R*) and (4*S*), respectively. The obtained conformations were again energy minimized as described above resulting in each case in three low energy conformations. For energy comparison, these conformations were subsequently energy minimized using the semiempirical method PM3 (MOPAC 6.0 software package).[10] For the (4*R*) configurated molecule the energy of the obtained conformations was −142.5, −136.1 and −135.7 kcal mol^{-1}, respectively, and for the (4*S*) configurated molecule the energy of the obtained conformations were −140.8, −140.1 and −139.5 kcal mol^{-1}, respectively. The best correspondence of calculated structure and experimental determined NOE correlations and the observed vicinal coupling constant $^3J_{\text{H-4/H-1}''}$=1.2 Hz was found for the energetically favored structure with (4*R*) configuration (d H-3/H_3-14″ 1.8 Å; d H-4/H-9β″ 1.8 Å; dihedral angle H-4/C-4/C-1″/H-1″ −80°). To obtain final confirmation of the absolute configuration an X-ray crystallographic analysis of the *p*-bromo-benzoate derivative of **1** was carried out. The X-ray crystal structure (Fig. 2) (d H-3/H_3-14″ 2.0 Å; d H-4/H-9β″ 2.0 Å; dihedral angle H-4/C-4/C-1″/H-1″(−81°) showed the proposed *R*-configuration at C-4 as well as the proposed relative orientation of the flavonoid and the sesquiterpene moiety (dihedral angle C-3/C-4/C-1″/C-2″:X-ray: 45.0°, molecular modelling: 44.7°) (Fig. 3).

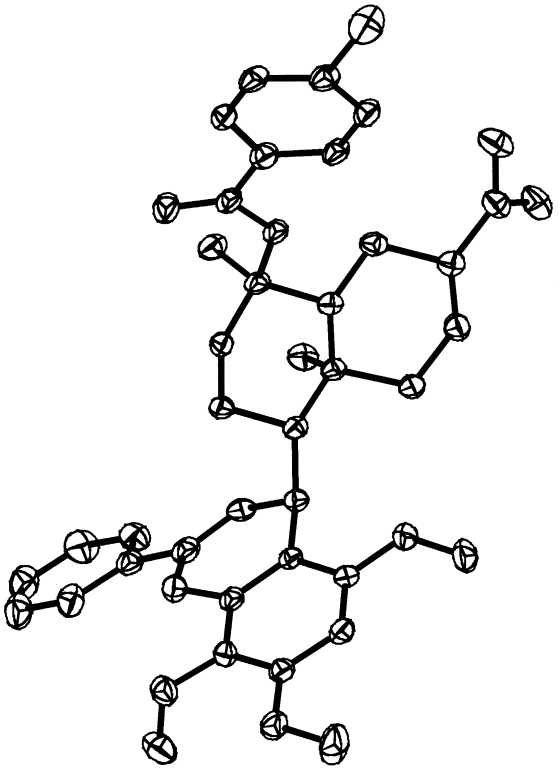


Figure 2. Molecular structure of the p-bromobenzoate of **1** with thermal ellipsoids (30% probability).

Fissistigmatin B (**2**) was obtained as colorless oil in a yield of 0.0012%, exhibiting the same EI and ESI-MS spectra as fissistigmatin A (**1**) and consequently the same molecular formula, $C_{33}H_{42}O_5$. Moreover, ^{1}H and ^{13}C NMR data of **2** (Tables 1 and 2) were quite similar to those of **1**, and the analysis of one- and two-dimensional NMR experiments led to the same constitution as **1**. Noticeable differences in chemical shifts in comparison with **1** were observed for positions, 2, 3, 4, 1″, 2″, 9″ and 14″ only, suggesting that **2** is the stereoisomer of **1** with *S*-configuration at C-4. This assumption was supported by the vicinal coupling constant $^3J_{\text{H-4/H-1}''}$ of 5.4 Hz (**1**:1.2 Hz) and strong NOEs between H-3/H-9β″ and H-4/H_3-14″. But, in contrast to **1**, only a weak NOE between H-3 and H_3-14″ was observed. Furthermore, no HMBC correlation (Table 3) was found between H-4 and C-2″, whereas in **1** the dihedral angle H-4/C-4/C-1″/C-2″ was about 160° and accordingly a strong HMBC correlation between these signals was found. In **2**, H-1″ and H-2 β″ showed a vicinal coupling with $^3J_{\text{HH}}$=12.4 Hz and hence H-1″ is *axial* oriented at the α-face. The NMR data for **2** are in good agreement with the energetically most favored structure with *S*-configuration at C-4 (d H-3/H-9β″ 1.8 Å; d H-3/H_3-14″ 3.5 Å; d H-4/H_3-14″ 1.8 Å; dihedral angle H-4/C-4/C-1″/H-1″-142°; dihedral angle H-4/C-4/C-1″/C-2″96°) obtained by molecular modelling calculations as described above. The (4*S*) configuration of **2** was confirmed by the CD spectrum, which showed a mirrored curve compared with that of **1** (Fig. 4).

Fissistigmatin C (**3**) was isolated as white needles in a yield of 0.0011%. The molecular formula of **3** as $C_{33}H_{40}O_4$, established by ESI-MS (*m*/*z* 523, $[M+Na]^+$) as well as analysis of ^{13}C{^{1}H}, ^{13}C-APT and HSQC NMR spectra,

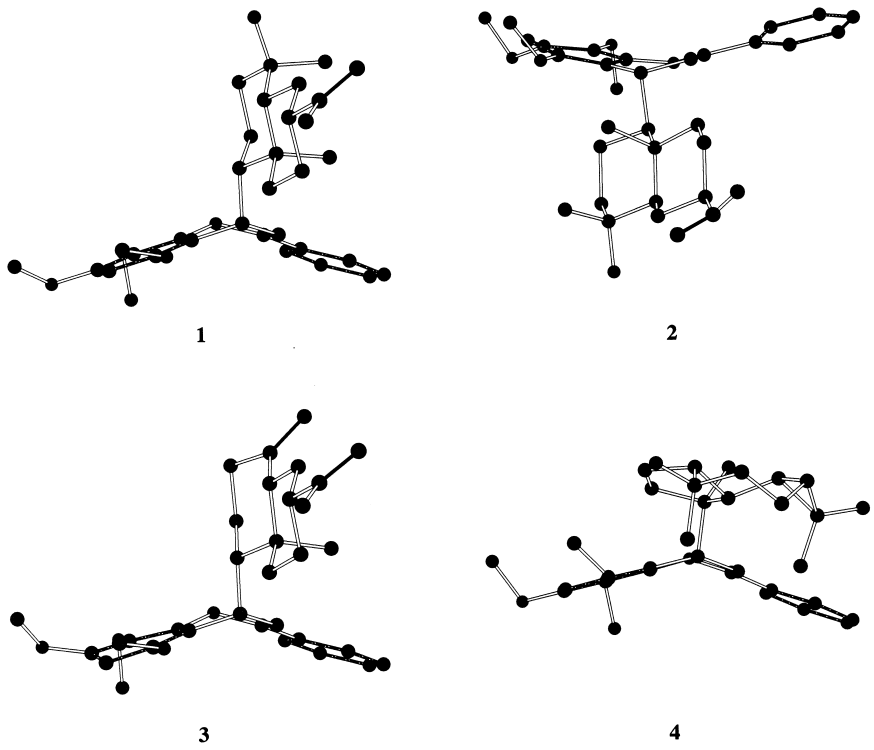


Figure 3. Perspective view of fissistigmatins A–D (**1**–**4**) (for clarity protons are not shown).

indicated a loss of one molecule of H_2O compared with compounds **1** and **2**. The ^1H and ^{13}C NMR spectral data (Tables 1 and 2) of **3** were quite similar to those of **1**, except for one methyl group replaced by a terminal methylene group (δ ^{13}C 105.9; δ ^1H 4.64 and 4.43, br s each). Based on the HMBC correlations (Table 3) of both of the methylene proton signals with C-3″ (δ 37.3) and C-5″ (δ 51.7), this double bond was assigned to C-4″-C-15″. The ^1H resonance at δ 4.64 was assigned to H-15″E due to its NOE correlation with H-3β″ (δ 2.24), whereas H-15″Z (δ 4.43) showed an NOE with H-6α″ (δ 1.52). The *R*-configuration at C-4 was proposed by

Table 1. ^1H NMR data of **1**–**4** (500 MHz, acetone-d_6)

Position	**1** δ[a] mult. *J* (Hz)	**2** δ[a] mult. *J* (Hz)	**3** δ[a] mult. *J* (Hz)	**4** δ[a] mult. *J* (Hz)
3	5.68 d 6.0	6.05 d 6.4	5.68 d 6.1	6.00 d 6.8
4	4.09 dd 6.0/1.2	3.77 dd 6.4/5.4	4.16 dd 6.1/1.3	3.69 d 6.8
6	6.49 s	6.49 s	6.51 s	6.52 s
5-OMe	3.88 s	3.86 s	3.90 s	3.84 s
7-OMe	3.88 s	3.89 s	3.89 s	3.89 s
8-OMe	3.82 s	3.83 s	3.82 s	3.82 s
2′/6′	7.78 d 7.4	7.81 d 7.5	7.76 d 7.4	7.83 d 7.4
3′/5′	7.43 t 7.4	7.43 t 7.5	7.43 t 7.4	7.44 t 7.4
4′	7.36 t 7.4	7.35 t 7.5	7.35 t 7.4	7.34 t 7.4
1″	*1.44*	1.60 ddd 12.4/5.4/2.9	*1.68*	*1.45*
2″	*1.36/1.56*[b]	*1.13/1.46*[b]	*1.47/1.64*[b]	*0.59*
3″	*1.28/1.66*[b]	*1.37/1.66*[b]	*1.90/2.24*[b]	–
4″	–	–	–	*0.58*
5″	*1.31*	*1.27*	1.86 br d 11.5	*1.81/1.10*[b]
6″	*2.01/1.26*[b]	*1.96/1.20*[b]	*1.52/1.49*[b]	*1.53/1.73*[b]
7″	*1.94*	*1.86*	*2.02*	–
8″	*1.67/1.51*[b]	*1.53/1.39*[b]	*1.71/1.49*[b]	*2.02*
9″	*1.43/2.22*[b]	*1.20/1.94*[b]	*1.61/2.29*[b]	*1.62/1.78*[b]
10″	–	–	–	*0.97/1.79*[b]
12″	4.75 br s/4.69 br s[c]	4.70 br s/4.66 br s[c]	4.77 br s/4.72 br s[c]	0.86 s
13″	1.76 dd 1.3/0.7	1.72 t 1.1	1.78 br s	1.16 s
14″	1.14 s	1.08 s	0.94 s	1.01 s
15″	1.02 s	1.02 s	4.43 br s/4.64 br s[c]	1.20 s

[a] Values in *italics* are chemical shifts of HSQC correlation peaks.
[b] H-α/H-β.
[c] H-*Z*/H-*E*.

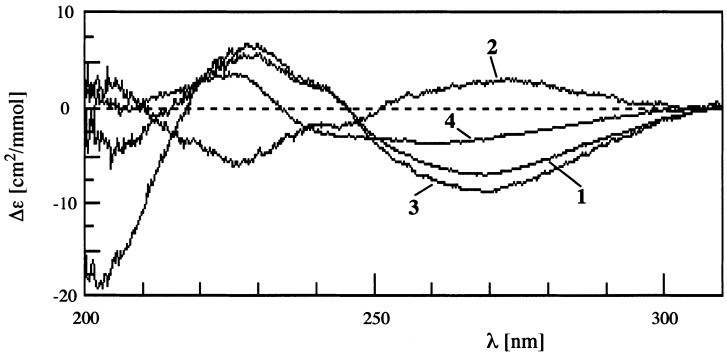


Figure 4. CD spectra (MeOH) of **1**–**4**.

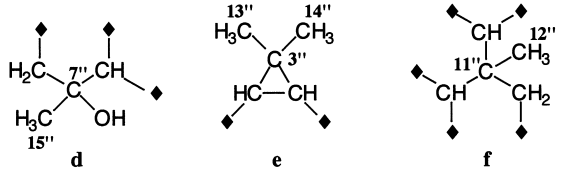
Table 2. ^{13}C chemical shifts of **1**–**4** (76.5 MHz, acetone-d_6)

Position	1	2	3	4
2	150.3	149.1	150.4	151.9
3	101.9	106.7	101.7	103.9
4	30.1[a]	32.2	30.5	39.2
5	152.9[b]	153.6	152.9[b]	153.6
5-OMe	56.0[c]	55.8	56.1	55.7
6	93.3	92.8	93.4	93.0
7	152.8[b]	152.8	152.8[b]	152.6
7-OMe	56.6[c]	56.6	56.6	56.7
8	132.1	132.7	132.2	132.7
8-OMe	61.2	61.3	61.2	61.3
9	148.1	148.2	148.2	149.5
10	108.9	108.6	108.7	107.9
1′	135.3	135.4	135.2	135.0
2′/6′	125.2	125.2	125.2	125.3
3′/5′	129.3	129.3	129.3	129.2
4′	129.1	129.0	129.2	129.1
1″	57.5	58.8	57.1	45.8
2″	22.4	25.2	25.8	31.4
3″	44.1	44.8	37.3	20.4
4″	71.2	71.2	151.4[c]	27.2
5″	56.9	57.2	51.7	21.0
6″	26.4	26.8	30.1[a]	45.6
7″	47.0	46.8	46.3	74.4
8″	27.8	28.1	27.8	59.7
9″	42.6	43.6	39.4	25.6
10″	39.2	39.8	40.5	37.9
11″	151.6	151.6	151.5[c]	53.6
12″	108.5	108.4	108.8	17.6
13″	21.2	21.1	21.1	17.3
14″	17.7	16.0	15.0	29.3[a]
15″	22.9	23.1	105.7	20.7

[a] Chemical shift of HSQC correlation peak.
[b] The assignments may be interchanged in the same column.
[c] The assignments may be interchanged in the same column.

comparison with compounds **1** and **2** based on the vicinal coupling constant $^3J_{\text{H-4/H-1}''}$ of 1.3 Hz, strong NOEs between H-3/H_3-14″ and H-4/H-9β″ and a strong HMBC correlation peak between H-4 and C-2″. This configuration was verified by the conspicuous resemblance of the CD spectra of **3** and **1** (Fig. 4).

Fissistigmatin D (**4**) was obtained as colorless amorphous solid in yield of 0.0012%. Its molecular formula was deduced from HR-ESI-MS with $[M+Na]^+$ at *m/z* 541.2926 (calc. 541.2930) and ^{13}C NMR as well as HSQC spectra to be $C_{33}H_{42}O_5$. The EI mass spectrum showed the base peak at *m/z* 297, characteristic for the fragment ion **a**. Analysis of the NMR spectra confirmed the presence of a flavonoid moiety with the same substitution pattern as for **1**–**3**. However, the signal H-4 (δ 3.69, d, *J*=6.8 Hz) showed only a vicinal coupling with H-3, suggesting that the flavonoid moiety was bound to a quarternary carbon of the sesquiterpene unit. The ^{1}H and ^{13}C NMR data (Tables 1 and 2) of the sesquiterpene moiety of **4** were quite different from those of **1**, **2** and **3**, indicating that **4** possesses a different sesquiterpene type. The sesquiterpene part of the ^{13}C NMR spectrum displayed signals due to 4 tertiary methyl, 4 methylene, 4 methine groups and 3 quarternary carbons, one of which oxygen-substituted. Two methine protons at δ 0.59 and 0.58 suggested the presence of a cyclopropane ring. HMBC correlations (Table 3) of the four methyl singlets at δ 1.20 (Me-15″), 1.16 (Me-13″), 1.01 (Me-14″) and 0.86 (Me-12″) with C-6″/C-7″/C-8″, C-2″/C-3″/C-4″/C-14″, C-2″/C-3″/C-4″/C-13″ and C-4/C-1″/C-10″/C-11″, respectively, led to three partial structures **d**, **e** and **f** and revealed that the flavonoid and the sesquiterpene moiety are connected via a C-4–C-11″ bond. The corresponding proton signals were assigned by the HSQC spectrum.



H-1″ (δ 1.45) displayed a ^{1}H–^{1}H COSY cross-peak with H-8″ (δ 2.02), which also shared cross-peaks with the proton resonances at δ 1.78 and 1.62, both belonging to the methylene carbon at δ 25.6. The two protons attached to C-10″ (δ 1.79 and 0.97) displayed ^{1}H–^{1}H COSY cross-peaks with the signals at δ 1.78 and 1.62, too. Thus, the carbons C-1″ and C-9″ (δ 25.6) were connected with C-8″ on the one hand and C-9″ with C-10″ on the other hand to build a five-membered ring system. Both C-4″ and C-6″ were connected to the methylene at δ 21.0 based on the COSY cross-peaks displayed by the corresponding protons. Namely, H-4″ (δ 0.58) showed ^{1}H–^{1}H COSY correlations with both protons attached to C-5″ (δ 1.81 and 1.10) and the *axial*-oriented proton at C-6″ (δ 1.53) displayed strong ^{1}H–^{1}H COSY crosspeaks with the *axial*-oriented proton at C-5″ (δ 1.10). The proposed constitution of the sesquiterpene moiety of **4** was verified using the computer program for automated structure elucidation COCON,[11] Internet on-line version WEBCOCON.[12] For simplification, the

Table 3. HMBC correlations of **1**–**4**

H	**1** C#	**2** C#	**3** C#	H	**4** C#
3	2, 4, 10, 1′	2, 4, 10, 1′	2, 4, 10, 1′	3	2, 4, 10, 1′
4	2, 3, 5, 9, 10, 1″, 2″, 10″	2, 3, 5, 9, 10, 1″, 10″	2, 3, 5, 9, 10, 1″, 2″, 10″	4	2, 3, 5, 9, 10, 1″, 10″, 11″, 12″
5-OMe	5	5	5	5-OMe	5
6	4, 5, 7, 8, 9, 10	4, 5, 7, 8, 9, 10	5, 7, 8, 10	6	4, 5, 7, 8, 9, 10
7-OMe	**7**	**7**	7	7-OMe	7
8-OMe	8	8	8	8-OMe8	
2′/6′	2, 6′/2′, 4′	2, 6′/2′, 4′	2, 6′/2′, 4′	2′/6′	2, 6′/2′, 4′
3′/5′	1′, 2′/6′, 5′/3′	1′, 2′/6′, 5′/3′	1′, 2′/6′, 5′/3′	3′/5′	1′, 5′/3′
4′	2′/6′	2′/6′	2′/6′	4′	2′/6′
1″α		4, 2″, 3″, 10″, 14″		1″β	4, 2″, 7″, 8″, 11″
2″α		4, 1″, 10″		6″α	4″, 7″, 15″
2″β	1″, 3″, 10″	1″, 3″, 10″		8″α	1″, 2″, 7″, 9″, 15″
3″β		1″, 2″, 4″, 5″, 15″		9″α	1″
5″α		4″, 6″, 10″, 14″, 15″		10″α	4, 1″, 8″, 9″, 11″, 12″
6″α	7″, 8″, 13″	4″, 5″, 7″		Me-12″	4, 1″, 10″, 11″
7″α	6″, 8″, 13″	6″, 8″, 13″		Me-13″	2″, 3″, 4″, 14″
9″β	5″, 7″, 8″, 10″, 14″	7″, 8″, 10″, 14″		Me-14″	2″, 3″, 4″, 13″
12″-*E*; 12″-*Z*	7″, 13″	7″, 13″	7″, 13″	Me-15″	6″, 7″, 8″
Me-13″	7″, 11″, 12″	7″, 11″, 12″	7″, 11″, 12″		
Me-14″	1″, 5″, 9″, 10″	1″, 5″, 9″, 10″	1″, 5″, 9″, 10″		
Me-15″	3″, 4″, 5″	3″, 4″, 5″	3″, 5″[a]		

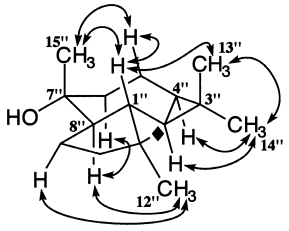


Figure 5. Key NOE correlations of the sesquiterpene moiety of **4**.

flavonoid moiety was replaced by a methyl group for the calculation. Using 9 unequivocal $^{1}H-^{1}H$ COSY correlations and 37 unequivocal HMBC correlations as connectivity information input, the only structural proposal calculated by the COCON program was identical with the aromadendran-type sesquiterpene proposed by us. Based on NOE correlations (Fig. 5), proton multiplet pattern and

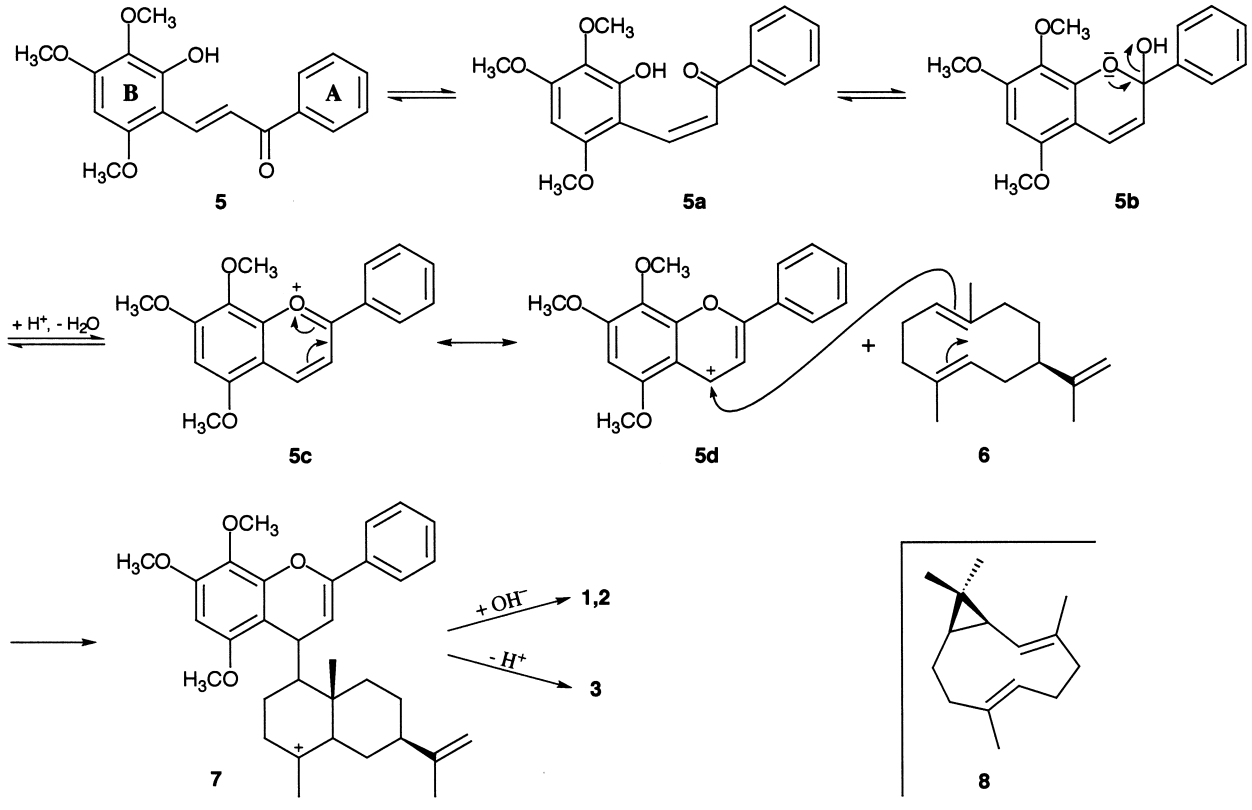


Figure 6. Proposed biosynthesis of fissistigmatins from chalcone **5**.

comparison of the NMR data with those of diastereoisomers globulol,[13-15] 7-*epi*-globulol,[14] (+)-ledol,[15,16] (−)-ledol[17] and viridiflorol[18] the stereochemistry of the sesquiterpene moiety was established as globulol. As indicated by the HMBC correlations of H-4 with C-1″/C-11″/C-12″ and H_3-12″ with C-4, the flavonoid and the sesquiterpene moiety of **4** were connected via a C-4–C-11″ bond. Unfortunately, no crystals of **4** suitable for X-ray analysis could be obtained. However, strong NOESY cross-peaks between H-3 and H-1β″/H_3-13″ and between H-4 and H-1β″/H-10β″ as well as weaker NOEs between H-3 and H_3-14″ between OMe-5 and H-10β″ and between H-2′/6′ and H_3-13″ strongly indicated a (4*S*) configuration (**4** possessed the same stereochemistry at C-4 as **1** and **3**, even though formally the descriptor had changed to (4*S*) because of the higher substitution at the sesquiterpene carbon attached to C-4) and a relative orientation of the two parts of the molecule as shown in Fig. 3. The (4*S*) configuration is supported by the CD spectrum which showed similar shape to those of **1** and **3** (Fig. 4).

The fissistigmatins A–D represent an unprecedented novel type of natural product bearing a flavonoid and a sesquiterpene unit connected via a C–C bond. Such hybrid structures require an original mixed biosynthetic pathway. Concerning the flavonoid part, chalcones play an important role as biosynthetic key intermediates.[19] Recently, we reported on a series of chalconoids from *F. bracteolatum*, among them the scarce retro-type compound 2-hydroxy-3,4,6-trimethoxychalcone **5**.[4] In analogous manner as described for other chalcones[20] this constituent can be biogenetically created from cinnamoyl-CoA and malonyl-CoA. Compound **5** exhibits the same substitution pattern at both aromatic rings as fissistigmatins A–D (**1**–**4**), suggesting its nature as putative biosynthetic precursor. In such a sequence (Fig. 6), isomerization of **5** to the *s-cis*, *cis* compound **5a** and cyclization to the hemiacetal **5b** followed by proton-catalyzed transformation could lead via the flavylium ion[19] **5c** to carbon cation **5d** as suitable species for the biosynthetic connection with a germacrene-type sesquiterpene **6** to afford the fissistigmatyl cation **7**. Nucleophilic attack of OH^- or deprotonation leads to the fissistigmatins A (**1**), B (**2**) and C (**3**), respectively. In a similar biosynthetic sequence, bicyclogermacrene (**8**) could act as a suitable sesquiterpene precursor leading to fissistigmatin D (**4**).

Experimental

General

Melting points are uncorrected. The specific rotations were measured by a JASCO-DIP-1000 polarimeter with MeOH as solvent. CD spectra were recorded on a JASCO J710 spectrometer in MeOH solution. The IR spectra were obtained on a BRUKER IFS28 instrument in $CHCl_3$ as the solvent. UV spectra were recorded on a KONTRON UNIKON 940 instrument using MeOH as solvent. EI-MS spectra (70 eV, DIS) and HR-EI-MS spectra (resolution ca. 5000) were measured on a AMD Intectra AMD-402 instrument, electrospray MS on a Finnigan MAT TSQ 7000 instrument and HR-electrospray MS on a Finnigan MAT 95 XL (resolution ca. 8000) instrument. X-Ray reflection data were collected with a STOE IPDS diffractometer using MoKα radiation ($\lambda = 0.71073$ Å). Molecular modelling calculations were carried out on Silicon graphics workstation R10000 using the SYBYL 6.5 software package (TRIPOS, St. Louis, MO, USA). $^{13}C\{^1H\}$ and ^{13}C-APT spectra were obtained with a VARIAN GEMINI 2000/300BB spectrometer at 75.5 MHz in acetone-d_6 and in $CDCl_3$ as the solvent with the solvent signal as internal reference (acetone-d_6: δ 29.8; $CDCl_3$: δ 77.0). ^1H, 1D NOE difference, 1D TOCSY and 2D spectra were recorded on a VARIAN UNITY 500 spectrometer at 499.83 MHz in acetone-d_6 and partly in $CDCl_3$ as the solvent with TMS as internal reference.

Collection, extraction and isolation

Leaves and branches of *F. bracteolatum* Chatt. were collected in Hoa Binh, Viet Nam in August 1997. The species was identified by Mr Ngo Van Trai, Hanoi. A voucher specimen is deposited in the Herbarium of the Institute of Materia Medica, Hanoi. The plant material was dried at room temperature to give 850 g, ground and extracted with 95% MeOH at room temperature. MeOH was evaporated in vacuo, and the aq. solution was extracted with *n*-hexane, followed by EtOAc and *n*-BuOH. The solvents were evaporated in vacuo. The *n*-hexane (11 g) and EtOAc (16 g) extracts were separated on silica gel with gradient *n*-hexane/EtOAc (8:2→6:4) in case of *n*-hexane extract and *n*-hexane/acetone (7:3→0:10) in case of EtOAc extract. The crude compounds **1**–**3** were isolated from *n*-hexane extract and the compound **4** from EtOAc extract. These crude compounds were purified by chromatography on silica gel and reverse-phase RP-8, which finally afforded fissistigmatin A (**1** 68 mg, 0.0080% dry wt.), fissistigmatin B (**2**, 10 mg, 0.0012% dry wt.), fissistigmatin C (**3**, 9 mg, 0.0011% dry wt.), and fissistigmatin D (**4**, 10 mg, 0.0012% dry wt.).

Fissistigmatin A (1). The compound was purified by CC [silica gel, *n*-hexane/acetone (9:1) and RP-8, MeOH/H_2O (9:1)]; white needles (cyclohexane/acetone); mp 80–82°C; R_f=0.35 [silica gel, *n*-hexane/acetone (7:3)]; $[\alpha]_D^{28.0}$ −108° (MeOH, *c* 1.00); IR ($CHCl_3$) ν_{max}: 3593, 2934, 2855, 1669, 1641, 1611, 1589, 1502, 1425, 1345, 1282, 1135, 1115, 1048, 983, 953 cm^{-1}; UV (MeOH) λ_{max} (log ϵ): 272 nm (3.73), 228 (4.25); CD (MeOH): $\Delta\epsilon_{204}$ −4.28, $\Delta\epsilon_{227}$ +5.96, $\Delta\epsilon_{240}$ +2.48, $\Delta\epsilon_{269}$ −7.01 $cm^2\,mmol^{-1}$; HR-ESI-MS *m/z*: 541.2922 $[M+Na]^+$ ($C_{33}H_{42}NaO_5$, calc. 541.2930); ESI/MS *m/z* (rel. int): 541 $[M+Na]^+$ (38), 519 $[M+H]^+$ (32), 299 (100), 267 (18); EI-MS (70 eV) *m/z* (rel. int.): 297 **a** (100), 282 (3), 267 (12), 253 (2); ^1H and ^{13}C NMR data see Tables 1 and 2.

Benzoylation of 1. 20 mg of **1** was dissolved in 0.5 ml pyridine and 50 mg *p*-bromobenzoyl chloride were added under ice cooling. The reaction mixture was stirred for 6 days at room temperature and then concentrated in vacuo. The residue was subjected to silica-gel column chromatography using *n*-hexane/EtOAc (7:3) as eluent to yield pure *p*-bromo-benzoate of **1** (10 mg, 37% yield).

***p*-Bromo-benzoate of 1.** White needles (acetone/methanol); mp 194–196°C; ^1H NMR (300 MHz, acetone-d_6): δ

7.86–7.76 (m, 2H, H-2′/6′), 7.83 (d, J=8.5 Hz, 2H), 7.65 (d, J=8.5 Hz, 2H), 7.47–7.37 (m, 3H, H-3′/5′+H-4′), 6.50 (s, 1H, H-6), 5.71 (d, J=6.0 Hz, 1H, H-3), 4.79 (s, 1H, H-12″Z), 4.72 (s, 1H, H-12″E), 4.14 (br d, J=6.0 Hz, 1H, H-4), 3.91 (s, 3H, OMe), 3.82 (s, 3H, OMe), 3.82 (s, 3H, OMe), 1.79 (s, 3H, Me-13″), 1.53 (s, 3H, Me-15″), 1.26 (s, 3H, Me-14″). *Crystal data*: Recrystallized from acetone/methanol; orthorhombic, space group $P2_12_12_1$; a=7.1978 (12) Å, b=22.4567 (40) Å, c=24.2431 (45) Å; V=3918.63 (12) Å^3; Z=4; D=1.227 g cm^{-3}; μ=1.094 mm^{-1}. 28203 reflections were collected at 220 K; 7428 independent reflections (R_{int}=0.1021). The structure was solved by direct methods using SHELXS-86[21] and refined by full matrix least-squares (SHELXL-93[22]), all non H-atoms refined anisotropically, for ($I > 2\sigma$(I)) R_1=0.0516, wR_2=0.1161, Flack parameter −0.019 (10).[23]

Fissistigmatin B (2). The compound was purified by CC [silica gel, *n*-hexane/ether (2:8) and RP-8, MeOH/H_2O (9:1)]; oil; R_f=0.32 [silica gel, *n*-hexane/acetone (7:3)]; $[\alpha]_D^{26.3}$=8.1° (MeOH, *c* 0.76); IR ($CHCl_3$) ν_{max}: 3593, 3511, 2933, 2855, 1737, 1641, 1610, 1504, 1465, 1425, 1342, 1237, 1136, 1114, 908, 891 cm^{-1}; UV (MeOH) λ_{max} (log ϵ): 268 nm (3.67), 210 (4.26); CD (MeOH): $\Delta\epsilon_{202}$ +2.02, $\Delta\epsilon_{227}$ −5.97, $\Delta\epsilon_{243}$ −1.99, $\Delta\epsilon_{273}$ 3.22 cm^2 mmol^{-1}; ESI-MS *m/z* (rel. int): 519 $[M+H]^+$ (16), 297 (100), 267 (32); EI-MS (70 eV) *m/z* (rel. int.): 297 **a** (100), 282 (2), 267 (11), 253 (2); ^{1}H and ^{13}C NMR data see Tables 1 and 2.

Fissistigmatin C (3). The compound was purified by CC [silica gel, *n*-hexane/ether (2:8) and RP-8, MeOH/H_2O (8.5:1.5)]; white needles (*n*-hexane/acetone); mp 75–77°C; R_f=0.34 [silica gel, *n*-hexane/acetone (7:3)]; $[\alpha]_D^{22.7}$=−162.2° (MeOH, *c* 0.05); IR ($CHCl_3$) ν_{max}: 3631, 3451, 2938, 2839, 1669, 1644, 1611, 1503, 1465, 1437, 1237, 1135, 1115, 1016, 890 cm^{-1}; UV (MeOH) λ_{max} (log ϵ): 273 nm (4.03), 219 (4.69); CD (MeOH): $\Delta\epsilon_{202}$ −18.99, $\Delta\epsilon_{228}$ +6.83, $\Delta\epsilon_{242}$ +2.32, $\Delta\epsilon_{269}$ −8.86 cm^2 mmol^{-1}; ESI-MS *m/z* (rel. int): 523 $[M+Na]^+$ (28), 501 $[M+H]^+$ (10), 299 (100); EI-MS (70 eV) *m/z* (rel. int.): 297 **a** (100), 282 (3), 267 (11), 253 (2); ^{1}H and ^{13}C NMR data see Tables 1 and 2.

Fissistigmatin D (4). The compound was purified by CC [silica gel, *n*-hexane/ether (1:1) and RP-8, acetonnitrile/H_2O (6:4)]; amorph.; R_f=0.29 [silica gel, *n*-hexane/acetone (7:3)]; $[\alpha]_D^{23.3}$=−72.5° (MeOH, *c* 1.00); IR ($CHCl_3$) ν_{max}: 3594, 2929, 2857, 1723, 1661, 1609, 1588, 1503, 1464, 1437, 1424, 1378, 1342, 1236, 1135, 1110, 1042, 982 cm^{-1}; UV (MeOH) λ_{max} (log ϵ): 269 nm (3.95), 241 (4.26); CD (MeOH): $\Delta\epsilon_{207}$ −0.07, $\Delta\epsilon_{226}$ +3.70, $\Delta\epsilon_{244}$ −2.98, $\Delta\epsilon_{262}$ −3.72 cm^2 mmol^{-1}; HR-ESI-MS *m/z*: 541.2926 $[M+Na]^+$ ($C_{33}H_{42}NaO_5$, calc. 541.2930); ESI-MS *m/z* (rel. int): 541 $[M+Na]^+$ (49), 519 $[M+H]^+$ (9), 297 (100), 267 (60); EI-MS (70 eV) *m/z* (rel. int.): 297 **a** (100), 282 (3), 267 (11), 253 (3); ^{1}H and ^{13}C NMR data see Tables 1 and 2.

Acknowledgements

We thank the Volkswagenstiftung and the Bundesministerium für Bildung, Wissenschaft, Forschung und Technologie, Germany, for financial support; Dr H. Münster, Finnigan MAT, Bremen, for the high resolution electrospray MS data; Mr Ngo Van Trai, Hanoi, for the identification of the plant material. One of us (T. P. L.) is indebted to the Deutscher Akademischer Austauschdienst, Bonn, for a grant.

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