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Feigrisolides A, B, C and D, New Lactones with Antibacterial Activities from *Streptomyces griseus*

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Four new lactone compounds, named feigrisolides A to D (**1** to **4**), have been isolated from *Streptomyces griseus*. The chemical structures were determined by detail analysis of their spectroscopic data and chemical transformations. Structurally, the feigrisolides A (**1**) and B (**2**) are hepta-lactones, feigrisolide C (**3**) and D (**4**) are 16-membered macrodiolides. Biological studies showed that feigrisolide B (**2**) exhibited strong antibacterial, as well as medium cyctotoxic, and antiviral activities. Feigrisolides A (**1**), C (**3**) and D (**4**) are medium inhibitors of 3α-hydroxysteroid-dehydrogenase (3α-HSD) inhibiting activity.

Two groups of medium-ring diolides have been isolated from fungi and *Streptomyces*: unsymmetrical 14-membered macrodiolides such as grahamimycin A_1[1] and colletodiol[2], and C_2-symmetric 16-membered lactones, like pyrenophorin[3], vermiculine[4], conglobatin[5] and elaiophylin[6]. A number of these secondary metabolites and their analogues show antibiotic activity[7]. As part of a project to discover new secondary metabolites from a chemical screening approach[8~11] as a supplement to target-orientated screening attempts, we examined the crude product from the fermentation of a new isolate of *Streptomyces griseus* (strain GT 051022). The chemical screening routine led to three highly intensive brown spots on silica gel TLC plates by staining with anisaldehyde/sulfuric acid, which prompted us to start an isolation and structure elucidation program. It was found that the strain produces two 16-membered non-symmetric macrodiolides, named feigrisolide C (**3**) and D (**4**) as well as two new hepta-lactones, feigrisolide A (**1**) and B (**2**).

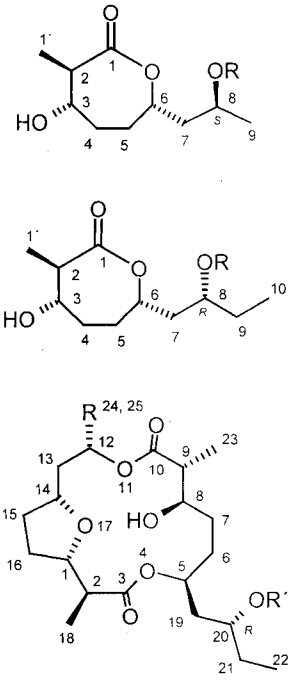
Taxonomy of Producing Strain *Streptomyces griseus*

The strain GT 051022 was isolated from a soil sample and identified as *Streptomyces griseus* on the basis of the methods described by SHIRLING and GOTTLIEB[13] and the comparison of the results with the data of HUETTER[12] and of SHIRLING and GOTTLIEB[14]. The strain is deposited in the culture collection of the Hans-Knoell-Institute for Natural Products Research, Jena, Germany.

Screening, Fermentation, and Isolation

The strain GT 051022 was cultivated in 100 ml of medium B in a 300 ml Erlenmeyer flask. The culture broth was examined with the procedures of chemical screening[11], which led to 3 characteristic brown spots detected by staining with anisaldehyde-H_2SO_4 on silica gel HPTLC plates [Rf=0.27 (**1**), Rf=0.33 (**2**), Rf=0.40 (**3** and **4**); solvent system: $CHCl_3$-MeOH, 9 : 1]. In order to isolate quantities of the secondary metabolites, fermentation of this strain was carried out in two 50-liter fermentors (medium B, 6 days at 28°C, 500 rpm, aeration 10 liter/minute). After harvesting and filtration, the culture filtrate was lyophilised to obtain about 1000 g of a crude product which was dissolved in water and extracted with *n*-butanol *via* an Extra-Flow Membrane Contactor (Liqui-Cel, Hoechst Celanese). The obtained dark oily enriched material (30 g) was sequentially separated by silica gel

Fig. 1. Structural formulae of feigrisolides A to D (**1** to **4**) and their derivatives (**5** to **14**).



1, R=H,
5, R=Ac
9, R=(2*R*)-phenylbutyryl
10, R=(2*S*)-phenylbutyryl

2, R=H
6, R=Ac
11, R=(2*R*)-phenylbutyryl
12, R=(2*S*)-phenylbutyryl

3, R=Me, R'=H
4, R=Et, R'=H
7, R=Me, R'=Ac
8, R=Et, R'=Ac
13, R=Me, R'=(2*R*)-phenylbutyryl
14, R=Me, R'=(2*S*)-phenylbutyryl

column chromatography, Sephadex LH-20 permeation chromatography and reversed-phase HPLC to yield four pure metabolites, feigrisolides A, B, C and D (**1** to **4**).

The isolated pure compounds were characterized spectroscopically. The molecular formulae were determined by mass spectrometry and the structures were elucidated by both, detailed analysis of the ^{1}H-, ^{13}C-, ^{1}H-^{1}H-, and ^{1}H-^{13}C-shift correlation NMR-spectra, and chemical transformations.

Feigrisolide A (**1**)

The molecular formula ($C_{10}H_{18}O_4$) was defined by HREI-MS (*m/z*=203.1314, $C_{10}H_{19}O_4$ $[M+H]^+$). It also gave further characteristic fragments at *m/z*=184.1109, $C_{10}H_{16}O_3$ $[M-H_2O]^+$, *m/z*=169.0868, $C_9H_{13}O_3$ $[M-H_2O-CH_3]^+$, and *m/z*=125.0622, $C_7H_9O_2$, $[M-H_2O-C_3H_7O]^+$. The ESI-MS spectrum showed three characteristic peaks at *m/z*=203.2 $[M+H]^+$, 225.2 $[M+Na]^+$ and 201.3 $[M-H]^+$. The IR absorption bands at 3435 and 1725 cm^{-1} indicated the presence of hydroxyl groups and an ester functionality.

The ^{1}H NMR spectrum of **1** shows 18 proton signals, from which the broad singlet signal at 4.35 ppm points to two hydroxyl groups, two methyl groups each attached to a methine moiety (δ 1.16, J=7.2 Hz and δ 1.21, J=6.3 Hz), three methylene groups (δ 1.68; 2.01/1.66; and 2.03/1.67), an aliphatic methine group (δ 2.50, dq, J=8.3, 7.2 Hz), as well as three additional methine groups at δ 4.19, 4.08 and 3.98, which are attached to oxygen atoms. The ^{13}C NMR spectrum exhibits the signals of ten carbon atoms. Besides four methine groups, three methylene groups and two methyl groups, there exists only one quaternary carbon atom (δ 177.5), due to the carbonyl group. These data are in accordance with the ^{1}H NMR data.

The proton-proton connections were established from both a comparison of coupling constants and ^{1}H-^{1}H COSY NMR experiments, and were confirmed by ^{1}H-^{13}C HMBC experiments. The carbonyl group is connected to C-2 according to the HMBC NMR spectrum. The molecular formula requires ring closure because of an additional degree of unsaturation. There exist two possibilities of lactone formation: (i) a 7-membered lactone formed between the carbonyl group and 6-OH, or (ii) a 9-membered lactone by linkage with 8-OH. Upon treatment with acetic anhydride in pyridine, **1** yields the mono-acetate **5**. Comparing the ^{1}H NMR spectra of **1** and **5**, 8-H showed a significant downfield shift from δ 4.08 to δ 5.02, while that of 6-H is shifted to higher field ($\Delta\delta$=0.17). This pointed to an acetylation of 8-OH.

The stereochemistry of 8-OH in **1** was determined by

Table 1. NMR data of feigrisolide A (**1**) and B (**2**) (in $CDCl_3$).

Position	1		2	
	δ ^{13}C	δ ^1H (multi. J = Hz)	δ ^{13}C	δ ^1H (multi. J = Hz)
1	177.5 (s)		177.6 (s)	
2	45.3 (d)	2.50 (dq, 8.3, 7.2)	45.3 (d)	2.50 (dq, 8.3, 7.0)
3	81.1 (d)	3.98 (q, 8.3)	81.0 (d)	3.98 (bq, 8.3)
4	29.1 (t)	2.03 (α, m)	29.1 (t)	2.03 (α, m)
		1.67 (β, m)		1.68 (β, m)
5	39.5 (t)	1.66 (α, m)	30.6 (t)	1.65 (α, m)
		2.01 (β, m)		2.01 (β, m)
6	77.1 (d)	4.19 (m)	77.3 (d)	4.21 (m)
7	42.9 (t)	1.68 (m)	40.7 (t)	1.70 (m)
8	65.2 (d)	4.08 (m)	70.4 (d)	3.78 (m)
9	23.1 (q)	1.21 (d, 6.3)	29.9 (t)	1.51 (m)
10	-	-	10.0 (q)	0.92 (t, 7.5)
1′	13.7 (q)	1.16 (d, 7.2)	13.7 (q)	1.16 (d, 7.0)
OH x 2		4.35 (2H, br. s)		4.75 (2H, br. s)

HELMCHEN's method[16] by esterification with each one of the enantiomers of 2-phenylbutyric acid resulting in the (2*R*)-2-phenylbutyrate-derivative **9** and (2*S*)-2-phenylbutyrate-derivative **10**, respectively. A comparison of the ^1H NMR data of **9** and **10** showed both, a significant high-field shift for the 9-H signal ($\Delta\delta$=0.11), and significant low-field shifts for the resonances of 3-H ($\Delta\delta$=0.15), 6-H ($\Delta\delta$=0.30), and 2-H ($\Delta\delta$=0.08) of **9** with regard to those of **10**. This indicates the *S*-configuration at C-8.

The existence of an ^1H-^1H-NOESY NMR correlation signal between δ 3.98 (H-3) and δ 4.19 (H-6) indicates that these protons and in consequence both, the hydroxyl group at C-3 and the side chain at C-6 are arranged in a *syn*-facial (equatorial) stereochemistry. The ^1H NMR coupling constant between H-2 and H-3 (J=8.3 Hz) suggests that the dihedral angle between H-3 and H-2 is close to 0° or 180°. On the basis of the *syn*-facial stereochemistry of H-3 and H-6, the dihedral angle degree between H-3 and H-2 was calculated[15] to be −165.8° for 2β-CH_3 and 3α-OH, and +81.4° for 2α-CH_3 and 3β-OH, when 2-CH_3 and 3-OH are *anti*-facial. With *syn*-facial positions, the magnitude of the angle was calculated to be +34.8° (2α-CH_3 and 3α-OH) and −81.9° (2β-CH_3 and 3β-OH). Obviously, the best fit is 2β-CH_3 and 3α-OH pointing to an *anti*-facial stereochemistry. Due to its *syn*-facial relationship with 3α-OH, the substitution at C-6 can then be proposed as α. However, the relative stereochemistry of the lactone ring substituents has no correlation to the absolute stereochemistry at C-8 obtained *via* HELMCHEN's method. Therefore, feigrisolide A is (2*R**, 3*S**, 6*S**, 2′*S*)-3-hydroxy-6-(2-hydroxy-propyl)-2-methyl-heptalacton (**1**).

Feigrisolide B (**2**)

Due to the results from the HRFAB-MS (*m/z*=217.1469 $[M+H]^+$, $C_{11}H_{21}O_4$) **2** contains an additional methylene group compared to feigrisolide A (**1**). The IR, UV, ^{13}C and ^1H NMR spectra (Table 1) of **2** showed close structural similarities to **1**. In the side chain a methyl group at C-8 in **1** is replaced by an ethyl group in **2**. Acylation of **2** with acetic anhydride in pyridine also yields a mono-acetate **6**. A comparison of the ^1H NMR spectra of **2** and **6** indicates the acetylation of the hydroxyl group at C-8 due to the downfield shift of the signals for 8-H ($\Delta\delta$=1.14 ppm). The relative stereochemistry of **2** seems to be identical to **1** based on both, identical coupling constants in the ^1H NMR spectra, and NOESY NMR experiments. However, a comparison of the ^1H NMR data of the (2*R*)-ester **11** and the (2*S*)-ester **12** exhibits a significant low-field shift for the 10-H signal ($\Delta\delta$=0.22), and significant high-field shifts for 3-H ($\Delta\delta$=0.04), 6-H ($\Delta\delta$=0.14), and 2-H ($\Delta\delta$=0.10) signals of **11** with regard to those of **12**, which indicates the *R*-configuration at C-8. Also in the case of **2**, the absolute stereochemistry at C-8 did not allow to predict the absolute stereochemistry of the ring. Therefore, feigrisolide B is (2*R**, 3*S**, 6*S**, 2′*R*)-3-hydroxy-6-(2-hydroxy-butyl)-2-methyl-hepta-lactone (**2**).

Feigrisolide C (**3**)

The molecular formula of $C_{21}H_{36}O_7$ was determined

Table 2. NMR data of feigrisolide C (**3**) and D (**4**).

Pos.	3				4	
	δ $^1H^a$ (mult, J = Hz)	δ $^{13}C^a$	δ $^1H^b$ (mult, J = Hz)	δ $^{13}C^b$	δ $^1H^a$ (mult, J = Hz)	δ $^{13}C^a$
1	3.95 (m)	80.5 (d)	3.98 (dt, 8.4, 6.5)	82.1 (d)	3.95 (m)	80.5 (d)
2	2.50 (dq, 8.3, 7.0)	44.9 (d)	2.47(dq, 8.4, 7.0)	45.7 (d)	2.47(dq, 8.3, 7.0)	44.9 (d)
3		176.9 (s)		175.8 (s)		176.4 (s)
5	4.14 (m)	77.1 (d)	4.02 (dq, 4.5, 7.5)	77.6 (d)	4.13 (m)	77.1 (d)
6	1.97 (m) / 1.60 (m)	30.5 (t)	1.95 (m) / 1.60 (m)	29.1 (t)	1.98 (m) / 1.60 (m)	28.8 (t)
7	1.96 (m) / 1.60 (m)	28.7 (t)	1.96 (m) / 1.50 (m)	31.9 (t)	1.97(m) / 1.61(m)	30.5 (t)
8	3.97 (m)	81.0 (d)	3.92 (dt, 8.4, 6.5)	81.3 (d)	3.97 (m)	81.0 (d)
9	2.46 (dq, 8.3, 7.0)	45.5 (d)	2.42 (dq, 8.4, 7.0)	46.4 (d)	2.50 (dq, 8.3, 7.0)	45.5 (d)
10		174.3 (s)		174.5 (s)		174.6 (s)
12	5.02 (ddq, 5.0, 8.2, 6.3)	68.9 (d)	4.96 (ddq, 5.8, 8.2, 6.5)	69.5 (d)	4.95 (m)	73.1 (d)
13	1.80 (m) / 1.75 (m)	42.3 (t)	1.77 (m) / 1.68 (m)	43.5 (t)	1.78 (m)	40.2 (t)
14	3.98 (m)	76.6 (d)	3.90 (m)	77.0 (d)	3.98 (m)	76.7 (d)
15	2.02 (m) / 1.58 (m)	31.0 (t)	2.01 (m) / 1.52 (m)	32.1 (t)	2.02 (m) / 1.60 (m)	31.0 (t)
16	2.02 (m) / 1.66 (m)	29.1(t)	1.95 (m) / 1.67 (m)	28.9 (t)	2.02 (m) / 1.68 (m)	29.3 (t)
18	1.15 (d, 7.0)	13.4 (q)	1.08 (d, 7.0)	13.5 (q)	1.16 (d, 7.0)	13.4 (q)
19	1.68 (m)	40.5 (t)	1.58 (m) / 1.53 (m)	43.5 (t)	1.68 (m)	40.5 (t)
20	3.74 (m)	70.4 (d)	3.62 (m)	70.3 (d)	3.74 (m)	70.4 (d)
21	1.49 (m)	29.9 (t)	1.40 (m)	31.5 (t)	1.49 (m)	29.9 (t)
22	0.92 (t, 7.4)	10.0 (q)	0.90 (t, 7.5)	10.3 (q)	0.92 (t, 7.4)	10.1 (q)
23	1.10 (d, 7.0)	13.6 (q)	1.04 (d, 7.0)	13.6 (q)	1.12 (d, 7.0)	13.8 (q)
24	1.24 (d, 6.3)	20.3 (q)	1.19 (d, 6.5)	20.9 (q)	1.60 (m)	27.3 (t)
25					0.88 (t, 7.4)	9.4 (q)
OH x 2	5.03 (brs)		2.85 (brs)		4.30 (brs)	

[a] in $CDCl_3$, [b] in Aceton-d_6

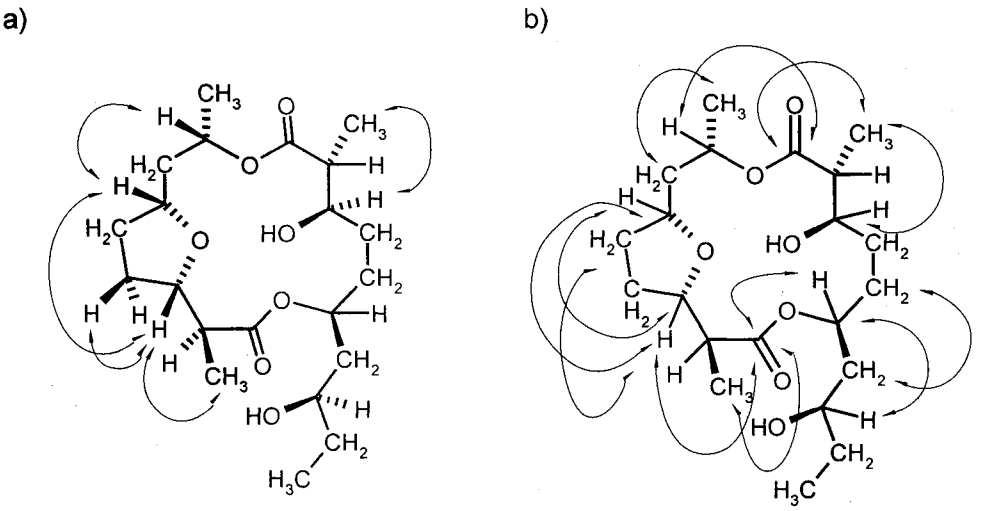
from HRFAB-MS (m/z=401.2429 $[M+H]^+$) indicates four degrees of unsaturation. The presence of an hydroxyl and two carbonyl groups was supported by the strong absorptions at 3315, 1719, and 1689 cm^{-1} in the IR spectrum, as well as by the ^{13}C NMR (125 MHz, acetone-d_6) signals at δ 174.5 and 175.8. The ^1H NMR (500 MHz, acetone-d_6) spectrum showed the signals of 36 protons, including four methyl groups. Three methylene groups were attached to methine carbons (δ 1.19, d, J=6.5 Hz; δ 1.08, d, J=7.0 Hz; δ 1.04, d, J=7.0 Hz) and one to a methylene group (δ 0.90, t, J=7.5 Hz). Two hydroxyl groups (δ 2.85, broad) are present, as well as 8 methine groups (six attached to oxygen atoms, and two to methyl groups). The ^{13}C NMR spectrum showed the signals of 21 carbon atoms: two carbonyl groups (δ 174.5 and 175.8), eight methine groups, seven methylene groups, and four methyl groups (Table 2).

A comparison of the ^1H NMR [500 MHz, $CDCl_3$] and ^{13}C NMR [125.0 MHz, $CDCl_3$] data of **3** with those of **1** and **2** suggested that metabolites **1** and **2** may act as building blocks for **3** forming a 16-membered bis-lactone from the biosynthetic point of view. This assumption resulted from detailed analysis of the 2D-NMR data (Fig. 2). The chemical shift of 12-H (δ 4.96) and its HMBC correlation signal to the carbonyl group C-10 (δ 174.5) demonstrated ester formation between C-12 and C-10. The second ester linkage was determined to be between the carbonyl group C-3 and C-5 due to the HMBC-correlation of δ 4.02 (5-H) with δ 175.8 (C-3).

According to the degrees of unsaturation, a further ring was considered. A tetrahydrofurane ring is formed *via* an ether bond and could be proved by an HMBC correlation signal between 1-H (δ 3.98) and C-14 (δ 77.0). Acylation of **3** with acetic anhydride/pyridine yielded the mono-acetate **7**, which showed a downfield shift of 20-H from δ 3.74 to δ 4.88 (^1H NMR in $CDCl_3$) in comparison to that of **3**. This indicates the acetylation of the hydroxyl group at C-20.

The coupling between 1-H and 2-H (8.4 Hz) predicts an *anti*-facial stereochemistry. This was confirmed by the strong NOE between 2-H and 16α-H in comparison to that of 2-H and 1-H, as well as to a strong NOE between 1-H and the methyl group at C-2 (δ 1.08). An NOE signal between 1-H (δ 3.98) and 14-H (δ 3.90) was observed, indicating a *syn*-facial position for both protons. A stronger NOE between 12-H (δ 4.96) and H-14 (δ 3.90) than that

Fig. 2. Selected $^3J_{H,H}$ NOE-NMR (a) and $^3J_{C,H}$ NMR (b) couplings of feigrisolide C (**3**).



between 14-H and the methyl group at C-12 (δ 1.19) suggests that both, 14-H, and 12-H are in a *syn*-facial position. Therefore, the relative stereochemistry of the fragment between C-12, (C-14) and C-2 seems to be in agreement with that of the building block **1**. This led us to the assumption that the positions C-8 and C-9 may have the same relative stereochemistry as that of C-1 and C-2. The fragment skeleton between C-10 and C-22 can be proposed to derive from **2** or a biosynthetically closely related metabolite. However, there exists no prove of this assumption. We propose an identical relative stereochemistry as in **2**, which is supported by identical coupling constants in the ^{1}H NMR spectrum and NOESY-data. The absolute stereochemistry at C-20 in **3** was determined as *R*-configuration by HELMCHEN's method. Thus, feigrisolide C is (2′*R*)-8-hydroxy-5-(2-hydroxybutyl)-2,9,12-trimethyl-4,11,17-trioxa-bicyclo[12.2.1]heptadecane-3,10-dione (**3**).

Feigrisolide D (**4**)

Due to the results from the HRFAB-MS (*m/z*=415.2563, calcd. for $C_{22}H_{39}O_7$, 415.2595, $[M+H]^+$) and ESI-MS (positive ion, *m/z*=415.5 $[M+H]^+$, 437.2 $[M+Na]^+$) **4** exhibits one more methylene group than feigrisolide C (**3**), while the UV- and IR- of **4** are almost identical to those of **3**. A comparison of the NMR data of both metabolites results in an almost identical structure except for the substituent at C-12 bearing an ethyl group in **4** instead of the methyl group in **3** (Table 2). Acylation of **4** with acetic anhydride/pyridine also only yielded a mono-acetate **9**, which independently proved the structure of **4**. The relative stereochemistry of **4** is the same as **3** based on comparative coupling constants (Table 2). This is supported by the optical rotation data (**4** $[\alpha]_D$ +15.7; **3** $[\alpha]_D$ +17.2). Therefore, feigrisolide D is (2′*R*)-8-hydroxy-5-(2-hydroxybutyl)-2,9-dimethyl-12-ethyl-4,11,17-trioxa-bicyclo [12.2.1]heptadecane-3,10-dione (**4**).

Biological Acitvities

Feigrisolide A, B, C, and D (**1** to **4**) were examined in a number of different antibacterial, antiviral, cytotoxic and enzyme assays, each performed with a number of different test organisms[17~21]. Feigrisolide B (**2**) exhibits antibacterial activity against *Sporobolomyces salmonicolor* SBUG 549 (23 mm zone of growth inhibition at 50 μg), but weak activity against the other organisms tested. However, the other three metabolites only show weak or no antibacterial activity. In the antiviral testing, **2** and **3** show moderate activity on Coxsackie virus B3. Feigrisolides A (**1**), C (**3**) and D (**4**) show medium 3α-hydroxysteroiddehydrogenase inhibiting activity, while **2** does not. In cytotoxicity and anti-proliferative testing on L-929, K562 and HeLa cell lines no general cellular toxicity was observed except **3** shows moderate activity (Table 3).

Table 3. Biological activities of feigrisolides A to D (**1** to **4**).

Compound	Antibacterial activity[a]	Virus inhibition at 25 μg/ml	Enzyme tests	Cytotoxicity IC_{50} [μg/ml][h]
1	<14[b]	<30%	<20%[f]; 40%[g]	>100
2	23[c], <14[b,d]	50%[e]	<20%[f, g]	50
3	<14[b]	50%[e]	<20%[f]; 40%[g]	>100
4	<14[b]	<30%	<20%[f]; 40%[g]	>100

a) mm zone of growth inhibition at 50 μg;
b) Inactive;
c) *Sporobolomyces salmonicolor* SBUG 549;
d) Other organisms except *Sporobolomyces salmonicolor* SBUG 549;
e) Coxsackie virus B3: 50 % inhibition at 25 μg/ml;
f) Inhibition of xanthine oxidase at 2.0 μM;
g) Inhibition of 3α-HSD at 24.97 μM;
h) HeLa cell line; L-929 cell line; K562 cell line;

Experimental

General

^1H and ^{13}C NMR spectra: Bruker Avance DPX 300 (300 MHz) and Avance DRX 500 (500 MHz) instruments. MS: High-resolution EI mass spectra: AMD-402 instrument of BE geometry equipped with direct inlet system (AMD Intectra Harpstedt, Germany). Electrospray MS spectra: triple quadrupole mass spectrometer Quattro (VG Biotech, Altrincham, England). IR spectra: Shimadzu, Model IR 470 (KBr, discs). UV/VIS spectra: Varian CARY 1/3 Bio UV/VIS spectrophotometer. Optical rotation values: Perkin Elmer 241. CD spectra: Epson EPL-4300 (Model: 40A, Seiko Epson Corp.). Preparative scale HPLC: ABiMED Gilson Instruments [306 Pump; 811C Dynamic Mixer; 806 Manometric Module; column: Licrosorb RP-18 (7 μm), 250/25, Merck]. Fermentation: 50-liter fermentor (Braun Diessel). TLC: silica gel plates (HPTLC ready-to-use plates, silica gel $60F_{254}$ on aluminium foil or glass, Merck). LC: silica gel 60 (0.040~0.063 mm, Merck). Sephadex-LH 20 (Pharmacia).

Culture Medium

Medium A: Soluble starch 10 g/liter, $(NH_4)_2SO_4$ 2 g/liter, K_2HPO_4 1 g/liter, NaCl 1 g/liter, $MgSO_4 \times 7\ H_2O$ 1 g/liter, $CaCO_3$ 2 g/liter, agar 200 g/liter, and 5 ml of trace element concentrate per liter, pH=7.0 prior to sterilisation. Medium B: Glycerol 3%, casein peptone 0.2%, K_2HPO_4 0.1%, NaCl 0.1%, $MgSO_4 \times 7\ H_2O$ 0.05%, 5 ml of trace element concentrate per liter, pH=7.0 prior to sterilization. Trace element concentrate: 3 g $CaCl_2 \times 2\ H_2O$, 1 g Fe-III-citrate, 0.2 g $MnSO_4$, 0.1 g $ZnCl_2$, 0.025 g $CuSO_4 \times 5\ H_2O$, 0.02 g $Na_2B_4O_7 \times 10\ H_2O$, 0.004 $CoCl_2$, 0.01 g $NaMoO_4 \times 2\ H_2O$ in 1 liter of de-ionized water.

Fermentation

Streptomyces griseus was cultivated on agar plates (medium A) at 28°C for 14 days. A 1 cm^2 piece was used to inoculate 100 ml of medium B in 300 ml Erlenmeyer flasks. The flasks were cultivated on a rotary shaker (180 rpm) at 28°C for 6 days and were used for metabolite pattern analysis as well as for inoculation (5% v/v) of two 50 liter fermentators containing medium B (6 days, 28°C, 500 rpm, aeration 10 liter/minute).

Isolation and Purification

After harvesting the culture broth was filtered and the culture filtrate was lyophilized to give about 1000 g of a crude product, which was dissolved in 3 liters of water and extracted with *n*-butanol *via* an Extra-Flow Membrane Contactor (Liqui-Cel, Hoechst Celanese) yielding 30 g of a dark oil after evaporation. 29 g of this oil was chromatographed on silica gel (column: 7.5×40 cm) with *n*-hexane - ethyl acetate - methanol (gradient from 1 : 1 : 0 to 1 : 1 : 0.2). 30 ml per fraction was collected, analysed by TLC (silica gel, $CHCl_3$ - MeOH, 9 : 1), and combined as following: I: fractions 74 to 93, 3.0 g; II: fractions 94 to 230, 17.0 g; and III: fractions 231 to 260, 2.4 g.

2.9 g of I was chromatographed on silica gel (column: 4×50 cm, $CHCl_3$ - MeOH, 15 : 1) to give two fractions. The first fraction (1.2 g) was further purified by silica gel chromatography [(column: 2.5×50 cm, $CHCl_3$ - MeOH,

from 50:1 to 20:1)]; Sephadex LH-20 chromatography (column: 2.5×100 cm, MeOH); and HPLC (RP-C_{18}, 2.5×25 cm, 7 μm, MeOH-H_2O, 65:35) to give **3** (240 mg, white powder) and **4** (35 mg, colorless oil). The second fraction (0.90 g) was purified on a Sephadex LH-20 column (2.5×100 cm, MeOH) and on RP-C_{18}-HPLC (2.5×25 cm, 7 μm, MeOH-H_2O, 40:60) to yield pure **2** (190 mg, colorless oil).

2.4 g of III was purified on a Sephadex LH-20 column (5×80 cm, 5 cm, MeOH, analysed by TLC on RP-C_{18}-SiO_2 in MeOH-H_2O, 1:1) and then cleaned by HPLC (RP-C_{18}, 2.5×25 cm, 7 μm, MeOH-H_2O, 35:65) to yield pure **1** (76 mg colorless oil).

Feigrisolide A (**1**)

Colorless oil. $[\alpha]_D^{20}=+3.4°$ (c=0.3 in methanol). IR (KBr) ν_{max}=3435, 2935, 2875, 1725, 1457, 1190, 1086, 1058, 937 cm^{-1}. UV (Ethanol) λ_{max} (log ε)=199 (3.47), 220 (3.27) nm. HREI-MS m/z=203.1314 (calcd. for $C_{10}H_{19}O_4$, 203.1283) $[M+H]^+$ (2); 184.1109, $C_{10}H_{16}O_3$ $[M-H_2O]^+$ (50); 169.0868, $C_9H_{13}O_3$ $[M-H_2O-CH_3]^+$ (90); 125.0622, $C_7H_9O_2$ $[M-H_2O-C_3H_7O]^+$ (100). FAB-MS m/z=203.4 $[M+H]^+$ (100); 225.1 $[M+Na]^+$ (30), ESI-MS: (positive ion) m/z=203.2 $[M+H]^+$ (15); 225.2 $[M+Na]^+$ (100). ESI-MS (negative ion) m/z=201.3 $[M-H]^-$ (100). ^1H NMR and ^{13}C NMR data in Table 1.

Feigrisolide B (**2**)

Colorless oil. $[\alpha]_D^{20}=0$ (c=0.2 in methanol), IR (KBr) ν_{max}=3385, 3375, 2935, 2875, 1727, 1457, 1198, 1065, 1034, 950 cm^{-1}. UV (Ethanol) λ_{max} (log ε)=119 (3.36), 221 (3.27) nm. CD (Ethanol) λ_{max} ([θ])=222 (+1309). HRFAB-MS (Positive ion) m/z=217.1469 (calcd. for $C_{11}H_{21}O_4$, 217.1492) $[M+H]^+$. HREI-MS m/z=198.1258 (calcd. for $C_{11}H_{18}O_3$, 198.1260) $[M-H_2O]^+$ (10); 169.0863, $C_9H_{13}O_3$ $[M-H_2O-C_2H_5]^+$ (100); 125.06218, $C_7H_9O_2$ $[M-H_2O-C_4H_9O]^+$ (70). ESI-MS (positive ion) m/z=217.3 $[M+H]^+$ (70), 239.3 $[M+Na]^+$ (100), 234.3 $[M+NH_4]^+$ (40), 455.6 $[2M+Na]^+$ (20). ESI-MS (negative ion) m/z=215.3 $[M-H]^-$ (100). ^1H NMR and ^{13}C NMR data in Table 1.

Feigrisolide C (**3**)

White powder, $[\alpha]_D^{20}=+17.2°$ (c=0.4 in methanol). IR (KBr) ν_{max}=3315, 2970, 2940, 2875, 1719, 1689, 1454, 1196, 1058, 947 cm^{-1}, UV (Ethanol) λ_{max} (log ε)=201.2 (3.98) nm. HRFAB-MS (positive ion) m/z=401.2429 (calcd. for $C_{21}H_{37}O_7$, 401.2450) $[M+H]^+$; 423.2353 (calcd. for $C_{21}H_{36}O_7Na$, 423.2359) $[M+Na]^+$. ESI-MS (positive ion) m/z=401.6 $[M+H]^+$ (45), 423.5 $[M+Na]^+$ (100), 823.8 $[2M+Na]^+$ (10). EI-MS m/z=401 $[M+H]^+$ (10), 382 $[M-H_2O]^+$ (10), 371 $[M-C_2H_5]^+$ (30), 353 $[M-C_2H_5-H_2O]^+$ (70), 338 (25), 327 (72), 258 (100). ^1H NMR and ^{13}C NMR data in Table 2.

Feigrisolide D (**4**)

Colorless oil, $[\alpha]_D^{20}=+15.7°$ (c=0.2 in methanol). IR (KBr) ν_{max}=3435, 2965, 2935, 1725, 1456, 1376, 1262, 1192, 1062 cm^{-1}. UV (Ethanol), λ_{max} (log ε)=200.7 (3.97) nm. HRFAB-MS (positive ion) m/z=415.2563 (calcd. for $C_{22}H_{39}O_7$, 415.2595) $[M+H]^+$ (100), 437 $[M+Na]^+$ (40), ESI-MS (positive ion) m/z=415.5 $[M+H]^+$ (45), 437.2 $[M+Na]^+$ (100), 852.0 $[2M+Na]^+$ (30). EI-MS m/z=415 $[M+H]^+$ (5), 385 $[M-C_2H_5]^+$ (10), 367 $[M-C_2H_5-H_2O]^+$ (20), 352 (18), 272 (20), 199 (100). ^1H NMR and ^{13}C NMR data in Table 2.

General Procedure for Acylation of Feigrisolides A, B, C and D (**1**~**4**)

0.05 mmol of the different feigrisolides were dissolved in 0.25 ml of pyridine, to which 50 μl of acetic anhydride was added. The mixtures were stirred for 24 hours, dried by a flow of nitrogen and the crude products were chromatographed on silica gel columns (1×50 cm, $CHCl_3$-MeOH, 20:1) to yield the acetylated feigrisolides **5** to **8**, respectively.

8-Acetyl-feigrisolide A (**5**)

Colorless oil, yield 96%. ^1H NMR (300 MHz, $CDCl_3$), δ=5.02 (1H, hextett, J=6.3 Hz, H-8), 4.02 (1H, m, H-6), 3.96 (1H, m, H-3), 2.50 (1H, dq, J=8.4, 7.0 Hz, H-2), 2.05 (1H, m, H-4a), 2.03 (1H, m, H-5a), 2.02 (3H, s, O*Ac*), 1.81 (2H, m, H-4b, H-5b), 1.64 (2H, m, H-7), 1.26 (3H, d, J=6.3 Hz, H-9), 1.19 (3H, d, J=7.0 Hz, H-1′), ^{13}C NMR (75 MHz, $CDCl_3$), δ=175.9 (s, C-1), 170.5 (s, *C*OCH$_3$), 80.3 (d, C-3), 77.2 (d, C-6), 68.8 (d, C-8), 44.7 (d, C-2), 42.2 (t, C-7), 31.0 (t, C-5), 29.4 (t, C-4), 21.3 (q, C-9), 20.4 (q, CO*C*H$_3$), 13.4 (q, C-1′).

8-Acetyl-feigrisolide B (**6**)

Colorless oil, yield 95%. ^1H NMR (500 MHz, $CDCl_3$), δ=6.50 (1H, br, s, OH), 4.92 (1H, m, H-8), 3.98 (1H, m, H-6), 3.95 (1H, m, H-3), 2.51 (1H, dq, J=8.4, 7.0 Hz, H-2), 2.04 (3H, s, O*Ac*), 2.00 (2H, m, H-4a, H-5a), 1.80 (1H, ddd, J=7.3, 4.5, 14.2 Hz, H-7a), 1.75 (1H, ddd, J=5.4, 8.0, 14.2 Hz, H-7b), 1.64 (1H, m, H-4b), 1.60 (3H, m, H-5b, H-9), 1.16 (3H, d, J=7.0 Hz, H-1′), 0.86 (3H, t, J=7.4 Hz, H-10). ^{13}C NMR (125 MHz, $CDCl_3$), δ=178.0 (s, C-1), 170.8 (s, *C*OCH$_3$), 80.1 (d, C-3), 77.0 (d, C-6), 73.3 (d, C-8), 44.9 (d, C-2), 39.8 (t, C-7), 31.6 (t, C-5), 28.9 (t, C-9), 27.4 (t,

C-4), 21.2 (q, $COCH_3$), 13.2 (q, C-1′), 9.4 (q, C-10).

20-Acetyl-feigrisolide C (**7**)

Colorless oil, yield 90%. HREI-MS *m/z*=443.2621 (calcd. for $C_{23}H_{39}O_8$, 443.2597) $[M+H]^+$ (5); 425 (5), 369 (30), 199 (95), 185 (100). HRFAB-MS *m/z*=465.2466 (calcd. for $C_{23}H_{38}O_8Na$, 465.2464) $[M+Na]^+$. ^1H NMR (300 MHz, $CDCl_3$), δ=5.02 (1H, ddq, *J*=5.0, 6.3, 8.2 Hz, H-12), 4.88 (1H, m, H-20), 3.97~4.02 (3H, m, H-5, H-8, H-14), 3.85 (1H, m, H-1), 2.50 (1H, dq, *J*=8.3, 7.0 Hz, H-2), 2.46 (1H, dq, *J*=8.3, 7.0 Hz, H-9), 2.04 (2H, m, H-15a, H-16a), 2.02 (3H, s, O*Ac*), 1.90 (2H, m, H-6a, H-7a), 1.82 (2H, m, H-13) 1.70 (3H, m, H-16b, H-19), 1.60 (3H, m, H-6b, H-7b, H-15b), 1.50 (2H, m, H-21), 1.25 (3H, d, *J*=6.3 Hz, H-24),1.17 (3H, d, *J*=7.0 Hz, H-18), 1.08 (3H, d, *J*=7.0 Hz, H-23), 0.87 (3H, t, *J*=7.4 Hz, H-22). ^{13}C NMR (75 MHz, $CDCl_3$), δ=176.3 (s, C-3), 174.3 (s, C-10), 170.8 (s, *C*OCH_3), 80.3 (d, C-1, C-8), 77.1 (d, C-5), 76.5 (d, C-14), 73.5 (d, C-20), 68.8 (d, C-12), 45.6 (d, C-2), 44.8 (d, C-9), 42.3 (t, C-13), 40.0 (t, C-19), 31.3 (t, C-15), 31.1 (t, C-7), 29.3 (t, C-21), 28.9 (t, C-16), 27.5 (t, C-6), 21.2 (q, $COCH_3$), 20.4 (q, C-24), 13.4 (q, C-23), 13.2 (q, C-18), 9.3 (q, C-22).

8-Acetyl-feigrisolide D (**8**)

Colorless oil, yield 91%. ^1H NMR (300 MHz, $CDCl_3$), δ=4.98 (1H, m, H-12), 4.87 (1H, m, H-20), 4.02 (2H, m, H-5, H-14), 3.85 (2H, m, H-1, H-8), 2.50 (1H, dq, *J*=8.3, 7.0 Hz, H-9), 2.47 (1H, dq, *J*=8.3, 7.0 Hz, H-2), 2.06 (2H, m, H-15a, H-16a), 2.02 (3H, s, O*Ac*), 1.92 (2H, m, H-6a, H-7a), 1.82 (2H, m, H-13) 1.70 (3H, m, H-16b, H-19), 1.60 (5H, m, H-6b, H-7b, H-15b, H-24), 1.50 (2H, m, H-21), 1.19 (3H, d, *J*=7.0 Hz, H-18), 1.10 (3H, d, *J*=7.0 Hz, H-23), 0.90 (3H, t, *J*=7.4 Hz, H-22), 0.88 (3H, t, *J*=7.4 Hz, H-25).

General Procedure of Esterification of Feigrisolides A, B and C (**1**, **2** and **3**) with both Diastereomers of 2-Phenylbutyric Acid

12 mg (0.06 mmol) of *N,N*-dicyclohexylcarbodiimide (DCC), 8 mg (0.06 mmol) of 4-(dimethylamino)-pyridine (DMAP) and 10 mg (0.05 mmol) of (2*R*)-2-phenylbutyric acid (or the 2*S*-diastereomer) were added to a 10-ml flask which contained 3 ml of dichloromethane. After cooling to −5°C, 0.05 mmol of the different feigrisolides dissolved in 2 ml of dichloromethane was added. After heating to room temperature, the mixture was stirred for 14 hours. 4 ml of water was added and the products were extracted with chloroform (4 ml, four times). The combined organic layer was dried with Na_2SO_4, filtered, the filtrate was dried in vacuum, and chromatographed on silica gel (column: 0.5× 10 cm, $CHCl_3$-MeOH, 20:1) to yield **9** (34%), **10** (30%), **11** (35%), **12** (30%), **13** (37%), and **14** (34%), respectively.

{8-[(2*R*)-2-Phenylbutyryl]}-feigrisolide A (**9**)

Colorless oil. ^1H NMR (500 MHz, $CDCl_3$), δ=7.25~7.33 (5H, m, aromatic protons), 5.00 (1H, hextett, *J*=6.3 Hz, H-8), 3.90 (2H, m, 3-H, H-6), 3.41 (1H, t, *J*=7.4 Hz, H-2″), 2.49 (1H, dq, *J*=8.1, 7.2 Hz, H-2), 2.20~1.50 (8H, m, H-4, H-5, H-7, H-3″), 1.13 (3H, d, *J*=6.3 Hz, H-9), 1.17 (3H, d, *J*=7.1 Hz, H-1′), 0.90 (3H, t, *J*=7.3 Hz, H-4″), ^{13}C NMR (125 MHz, $CDCl_3$), δ=176.7 (s, C-1), 173.4 (s, C-1″), 139.1(s), 128.4 (×2, d), 127.9 (×2, d), and 127.0 (d): six aromatic carbons, 80.1 (d, C-3), 77.2 (d, C-6), 69.0 (d, C-8), 53.7 (d, C-2″), 44.7 (d, C-2), 42.2 (t, C-7), 31.0 (t, C-5), 29.2 (t, C-4), 26.5 (t, C-3″), 20.2 (q, C-9), 13.4 (q, C-1′), 12.1 (q, C-4″).

{8-[(2*S*)-2-Phenylbutyryl]}-feigrisolide A (**10**)

Colorless oil. ^1H NMR (500 MHz, $CDCl_3$), δ 7.26~7.33 (5H, m, aromatic protons), 5.00 (1H, hextett, *J*=6.3 Hz, H-8), 3.75 (1H, q, *J*=6.3 Hz, H-3), 3.60 (1H, m, H-6), 3.45 (1H, t, *J*=7.6 Hz, H-2″), 2.41 (1H, dq, *J*=8.1, 7.2 Hz, H-2), 2.20~1.50 (8H, m, H-4, H-5, H-7, H-3″), 1.24 (3H, d, *J*=6.3 Hz, H-9), 1.13 (3H, d, *J*=7.2 Hz, H-1′), 0.89 (3H, t, *J*=7.4 Hz, H-4″), ^{13}C NMR (125 MHz, $CDCl_3$), δ=176.7 (s, C-1), 173.4 (s, C-1″), 139.4 (s), 128.4 (d, ×2), 128.0 (d, ×2), and 127.0 (d): six aromatic carbons, 79.9 (d, C-3), 77.0 (d, C-6), 69.0 (d, C-8), 53.7 (d, C-2″), 44.7 (d, C-2), 42.1 (t, C-7), 30.8 (t, C-5), 29.2 (t, C-4), 26.5 (t, C-3″), 20.6 (q, C-9), 13.2 (q, C-1′), 12.1 (q, C-4″).

{8-[(2*R*)-2-Phenylbutyryl]}-feigrisolide B (**11**)

Colorless oil. ^1H NMR (500 MHz, $CDCl_3$), δ=7.22~7.33 (5H, m, aromatic protons), 4.92 (1H, m, H-8), 3.85 (1H, m, H-3), 3.70 (1H, m, H-6), 3.47 (1H, t, *J*=7.4 Hz, H-2″), 2.39 (1H, dq, *J*=8.1, 7.2 Hz, H-2), 2.10 (3H, m, H-4a, H-5a, H-3″a), 1.80~1.50 (7H, m, H-4b, H-5b, H_2-7, H_2-9, H-3″b), 1.10 (3H, d, *J*=7.0 Hz, H-1′), 0.88 (3H, t, *J*=7.4 Hz, H-10). 0.92 (3H, t, *J*=7.3 Hz, H-4″), ^{13}C NMR (125 MHz, $CDCl_3$), δ=177.7 (s, C-1), 170.2 (s, C-1″), 139.4 (s), 128.6 (d, ×2), 128.0 (d, ×2), and 127.0 (d): six aromatic carbons, 79.9 (d, C-3), 77.2 (d, C-6), 73.2 (d, C-8), 53.0 (d, C-2″), 44.6 (d, C-2), 40.0 (t, C-7), 30.8 (t, C-5), 29.3 (t, C-4), 27.6 (t, C-9), 26.4 (t, C-3″), 13.2 (q, C-1′), 12.1 (q, C-4″), 9.4 (q, C-10).

{8-[(2*S*)-2-Phenylbutyryl]}-feigrisolide B (**12**)

Colorless oil. ^1H NMR (500 MHz, $CDCl_3$), δ=7.22~7.33 (5H, m, aromatic protons), 4.90 (1H, m, H-8), 3.89

(1H, m, H-3), 3.84 (1H, m, H-6), 3.42 (1H, t, J=7.4 Hz, H-2″), 2.49 (1H, dq, J=8.1, 7.2 Hz, H-2), 2.10 (3H, m, H-4a, H-5a, H-3″a), 1.80~1.50 (7H, m, H-4b, H-5b, H_2-7, H_2-9, H-3″b), 1.17 (3H, d, J=7.0 Hz, H-1′), 0.66 (3H, t, J=7.4 Hz, H-10). 0.91 (3H, t, J=7.3 Hz, H-4″), ^{13}C NMR (125 MHz, $CDCl_3$), δ=177.0 (s, C-1), 173.7 (s, C-1″), 139.2 (s), 128.6 (d, ×2), 128.0 (d, ×2), and 127.1 (d): six aromatic carbons, 80.1 (d, C-3), 77.2 (d, C-6), 73.2 (d, C-8), 53.1 (d, C-2″), 44.8 (d, C-2), 40.0 (t, C-7), 31.9 (t, C-5), 29.1 (t, C-4), 27.6 (t, C-9), 26.3 (t, C-3″), 13.2 (q, C-1′), 12.1 (q, C-4″), 9.0 (q, C-10).

{20-[(2*R*)-2-Phenylbutyryl]}-feigrisolide C (**13**)

Colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$), δ=7.20~7.33 (5H, m, aromatic protons), 5.03 (1H, ddq, J=5.0, 6.3, 8.2 Hz, H-12), 4.86 (1H, m, H-20), 3.90~4.00 (2H, m, H-8, H-14), 3.85 (1H, m, H-1), 3.50 (1H, m, H-5), 3. 40 (1H, t, J=7.5 Hz, H-2′), 2.50 (1H, dq, J=8.3, 7.0 Hz, H-9), 2.40 (1H, dq, J=8.3, 7.0 Hz, H-2), 2.10~1.50 (16H, m, H_2-6, H_2-7, H_2-13, H_2-15, H_2-16, H_2-19, H_2-21, H_2-3′), 1.25 (3H, d, J=6.3 Hz, H-24), 1.17 (3H, d, J=7.0 Hz, H-18), 1.08 (3H, d, J=7.0 Hz, H-23), 0.89 (3H, t, J=7.3 Hz, H-4′), 0.84 (3H, t, J=7.4 Hz, H-22). ^{13}C NMR (125 MHz, $CDCl_3$), δ=176.3 (s, C-3), 174.3 (s, C-10), 173.6 (s, C-1′), 139.4 (s), 128.6 (d, ×2), 128.0 (d, ×2), and 127.1 (d): six aromatic carbons, 80.3 (d, C-1), 80.1 (d, C-8), 77.1 (d, C-5), 76.6 (d, C-14), 73.8 (d, C-20), 68.7 (d, C-12), 53.9 (d, C-2′), 45.5 (d, C-2), 44.8 (d, C-9), 42.3 (t, C-13), 40.0 (t, C-19), 31.2 (t, C-15), 31.1 (t, C-7), 29.3 (t, C-16), 28.3 (t, C-6), 27.6 (t, C-21), 26.4 (t, C-3′), 20.4 (q, C-24), 13.4 (q, C-23), 13.2 (q, C-18), 12.1 (q, C-4′), 9.3 (q, C-22).

{20-[(2*S*)-2-Phenylbutyryl]}-feigrisolide C (**14**)

Colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$), δ=7.20~7.33 (5H, m, aromatic protons), 5.03 (1H, ddq, J=5.0, 6.3, 8.2 Hz, H-12), 4.86 (1H, m, H-20), 3.90~4.00 (2H, m, H-8, H-14), 3.85 (1H, m, H-1), 3.75 (1H, m, H-5), 3.41 (1H, t, J=7.5 Hz, H-2′), 2.50 (1H, dq, J=8.3, 7.0 Hz, H-2), 2.46 (1H, dq, J=8.3, 7.0 Hz, H-9), 2.10~1.50 (14H, m, H_2-7, H_2-8, H_2-13, H_2-15, H_2-16, H_2-19, H_2-3′), 1.45 (2H, m H-21), 1.25 (3H, d, J=6.3 Hz, H-24), 1.17 (3H, d, J=7.0 Hz, H-18), 1.08 (3H, d, J=7.0 Hz, H-23), 0.89 (3H, t, J=7.3 Hz, H-4′), 0.66 (3H, t, J=7.4 Hz, H-22). ^{13}C NMR (125 MHz, $CDCl_3$) δ=176.5 (s, C-3), 174.3 (s, C-10), 173.7 (s, C-1′), 139.3 (s), 128.6 (d), 128.4 (d), 128.1 (d), 128.0 (d), and 127.1 (d): six aromatic carbons, 80.3 (d, C-1), 80.2 (d, C-8), 77.1 (d, C-5), 76.7 (d, C-14), 73.8 (d, C-20), 68.8 (d, C-12), 53.8 (d, C-2′), 45.6 (d, C-2), 44.8 (d, C-9), 42.3 (t, C-13), 40.0 (t, C-19), 31.3 (t, C-15), 31.0 (t, C-7), 29.3 (t, C-16), 28.3 (t, C-6), 27.3 (t, C-21), 26.3 (t, C-3′), 20.4 (q, C-24), 13.4 (q, C-23), 13.2 (C-18), 12.2 (q, C-4′), 8.9 (q, C-22).

Determination of Biological Activities

Antibiotic Activity

The antibiotic activity was tested by means of agar diffusion plate assays[17,19] against the following microorganisms: *Bacillus subtilis* ATCC 6633 (IMET 10880) NA, *Staphylococcus aureus* (IMET 10760) SG 511, *Escherichia coli* SG 458, *Pseudomonas aeruginosa* SG 137 (IMET 10480), *Pseudomonas aeruginosa* K 799/61, *Sporobolomyces salmonicolor* SBUG 549, *Candida albicans* BMSY 212, *Penicillium notatum* JP 36, and *Mycobacterium phlei* SG 346.

Inhibition of Xanthin Oxidase and 3α-Hydroxysteroid-dehydrogenase (3α-HSD)

The compounds were tested in a lucigenine-coupled chemiluminescence assay for inhibitory activity on xanthine oxidase (SIGMA CHEMICAL Co.) with allopurinol used as standard[18]. Inhibitory activity on 3α-HSD was tested by measurement of NADPH-consumption[21].

Cytotoxic Properties

The testing for cytotoxic and antiproliferative activities against L-929 (mouse fibroblasts), K562 (human leukemia) and HeLa (human cervix carcinoma) was performed according to standard protocols[17].

Antiviral Activity

The antiviral activity against coxsackie virus B3 (strain Nancy), influenza virus A and herpes simplex virus type I was tested by inhibition of virus-induced cytopathic effects (zpE) on HeLa, MDCK and GMK cells, respectively[20].

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References

1) RONALD, R. C. & S. GURUSUDDAIAH: Grahamimycin A_1: a novel dilactone antibiotic from *Cytospora*. Tetrahedron Lett. 21: 680~684, 1980
2) GROVE, J. F.; R. N. SPEAKE & G. WARD: Metabolic products of *Colletotrichum capsici*: isolation and characterisation of acetylcollecttrichin and colletodiol. J. Chem. Soc. (C), 230~234, 1966
3) ISHIBASHI, K.: Studies on antibiotic from *Helminthosporium* sp. Fungi. II. Pyrenophorin, a new antibiotic produced by *Pyrenophora avenae*. J. Agr. Chem. Soc. Japan 35 (3): 257~262, 1961
4) FURKA, J.; P. MEMEC & I. KUHR: Vermiculine, a new antiprotozoal antibiotic from *Penicillium vermiculatum*. J. Antibiotics 25: 208~211, 1972
5) WESTLEY, W. J.; M. C. LIU, H. R. EVANS & J. F. BLOUNT: Conglobatin, a novel macrolide dilactone from *Streptomyces conglobatus* ATCC 31005. J. Antibiotics 32: 874~877, 1979
6) ARCAMONE, F. M.; C. BERTAZZOLI, M. CHIONE & T. SCHOTTI: Melanosporin and elaiophylin, new antibiotics from *Streptomyces melanosporus* n. sp. Giorn. Microbiol. 7: 207~216, 1959
7) SEEBACH, D.; H. F. CHOW, R. F. W. JACKSON, M. A. SUTTER, S. THAISRIVONGS & J. ZIMMEERMANN: (+)-11,11'Di-*O*-methylelaiophylidene—Preparation from elaiophylin and total synthesis from (*R*)-3-hydroxybutyrate and (*S*)-malate. Liebigs Ann. Chem. 1281~1308, 1986
8) TANG, Y. Q.; C. MAUL, R. HOEFS, I. SATTLER, S. GRABLEY, X. Z. FENG, A. ZEECK & R. THIERICKE: Gabosines L, N and O, new carba-sugars from *Streptomyces* with DNA-binding properties. European J. Org. Chem. 149~153, 2000
9) TANG, Y. Q.; I. SATTLER, R. THIERICKE, S. GRABLEY & X. Z. FENG: Xialenons, new pentalenons from *Streptomyces*. European J. Org. Chem. (submitted)
10) TANG, Y. Q.; I. SATTLER, S. GRABLEY, X. Z. FENG & R. THIERICKE: Streptoketol A and B, new secondary metabolites with DNA-binding properties. Nat. Prod. Lett. (in press)
11) GRABLEY, S.; R. THIERICKE & A. ZEECK: The chemical screening approach. *In* Drug Discovery from Nature. *Eds.*, S. GRABLEY, R. THIERICKE, pp. 124~148, Springer-Verlag, Heidelberg, 1999
12) HÜTTER, R.: Systematik der Streptomyceten. S. Karger, Basel, 1967
13) SHIRLING, E. B. & D. GOTTLIEB: Cooperative description of type cultures of *Streptomyces* III. Additional species descriptions from first and second studies. Int. J. Syst. Bacteriol. 18 (4): 279~392, 1968
14) SHIRLING, E. B. & D. GOTTLIEB: Methods for characterization of *Streptomyces* species. Int. J. Syst. Bacterio. 16 (3): 313~340, 1966
15) Alchemy32, version 2.0, Tripos Inc., St. Loius
16) HELMCHEN, G.: Über eine neue Methode zur Bestimmung der Absoluten Konfiguration von chiralen sekundären Alkoholen und Aminen: NMR-Spectroskopie von diasteromeren Estern und Amiden der α-Phenylbutter- und Hydratropasäure. Tetrahedron Lett. 16: 1527~1530, 1974
17) GRÄFE, U.; W. IHN, M. RITZAU, W. SCHADE, C. STENGEL, B. SCHLEGEL, W. F. FLECK, W. KÜNKEL, A. HÄRTL & W. GUTSCHE: Helioferins; novel antifungal lipopeptides from *Mycogene rosea*: screening, isolation, structures and biological properties. J. Antibiotics 48: 126~133, 1995 and references therein
18) HÄRTL, A.; A. STELZNER, M. RITZAU, S. HEINZE & U. GRÄFE: 5-Hydroxy-3,4,7-triphenyl-2,6-benzofurandione, a new xanthine oxidase inhibitor from *Peniophora sanguinea*. J. Antibiotics 51: 528~530, 1998 and references therein
19) DORNBERGER, K.; W. IHN, M. RITZAU, U. GRÄFE, B. SCHLEGEL & W. F. FLECK: Chrysospermins, new peptaibol antibiotics from *Apiocrea chrysosoerma* Ap101. J. Antibiotics 48: 977~989, 1995 and references therein
20) SCHMIDTKE, M.; C. KNORRE, L. BLEI, A. STELZNER & E. BIRCH-HIRSCHFELD: Penetration and antiviral activity of Coxsackievirus B3 (CVB3)-specific phosphorothioate oligodeoxynucleotides (PS-ODN). Nucleosides, Nucleotides 17: 1557~1566, 1998
21) PENNING, T. M.: Inhibition of 5β-dihydrocortisone reduction in rat liver cytosol: A rapid spectrophotometric screen for nonsteroidal anti-inflammatory drug discovery. J. Pharmceut. Sci. 651~654, 1985