

# Flavones and New Isoflavone Derivatives from Microorganisms: Isolation and Structure Elucidation

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In the course of our chemical screening of actinomycetes and other bacteria from terrestrial and marine sources, several extracts showed colourless middle polar bands with strong UV absorption at 254 nm and brown to grey colouration with anisaldehyde/sulphuric acid. Working-up of such strains led to the isolation of a number of isoflavonoids. Daidzein (**1a**) and genistein (**1b**) are very wide-spread, however, compounds like kakkatin (**2b**, *Streptomyces* sp. GW39/1530) were known only from plant sources. Additionally, three new isoflavonoids were obtained, namely 4',7-bis-( $\beta$ -cymaropyranosyl)-genistein (**1e**) and 4'-hydroxy-6,7-methoxyisoflavone (**2c**) from the actinomycete isolate HKI 129-L, and genistein-4'-(6''-methyl)-salicylate (**1d**) from *Streptomyces* sp. isolate GW27/2506. **1d** is the first natural 4'-ester of an isoflavonoid and an aromatic acid. For the first time, also two flavonoids were isolated from bacteria, apigenin (**5a**) and luteolin-3'-methyl ether (**5b**).

**Key words:** *Streptomyces* sp., Flavones, Isoflavones, Genistein

## Introduction

Isoflavonoids like daidzein (**1a**), genistein (**1b**) and many derivatives are widely distributed plant metabolites. Some of them show antioxidative properties, are inhibitors of various lytic enzymes, or have antifungal or estrogenic activities. For a recent review see [1]. The genistein/piperazine complex was found to possess anti-cancer activity [2]. Public interest has focussed especially on their xenoestrogenic activity which is believed to influence the human health positively across the food chain.

Although more than 40 isoflavonoids have been isolated from microorganisms, a biosynthetic origin in bacteria and fungi has not been proven. More likely is a microbial biotransformation of glycosidic plant isoflavonoids which are present in widely used nutrient constituents like soybean flour or malt extract. The isolation of microbial isoflavonoids from bacteria does therefore most probably not reflect *de novo* biosyntheses rather than the presence of glycosidases and further metabolic capabilities.

In this paper we describe the structure elucidation of genistein-4'-(6''-methyl)-salicylate (**1d**) and

4',7-bis-( $\beta$ -cymaropyranosyl)-genistein (**1e**), two new isoflavonoids isolated from terrestrial actinomycetes. Additionally, several known methylated genisteins were isolated and the NMR patterns of 4'-O-methylgenistein (**1f**), 7-O-methylgenistein (**1c**), 4',5-di-O-methylgenistein (**1g**), 4',6-dihydroxy-7-methoxyisoflavone (kakkatin, **2b**), 4'-hydroxy-6,7-dimethoxyisoflavone (**2c**), and 4',6,7-tri-O-methylisoflavone (**2d**) are discussed.

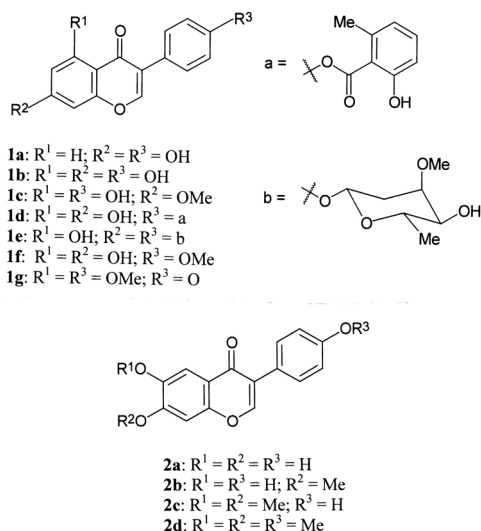
After screening more than 1000 terrestrial and marine streptomycetes, we have now obtained two flavones, apigenin (**5a**) and luteolin-3'-methyl ether (**5b**), for the first time from bacteria. While there is a general consensus about the plant origin of bacterial isoflavonoids, the isomeric flavones have never been isolated from microorganisms to the best of our knowledge, although they are again very common plant metabolites.

## Results and Discussion

Genistein-4'-(6''-methyl)-salicylate (**1d**) was obtained as a colourless solid from *Streptomyces* sp. GW27/2506, a strain which was found also to produce a variety of phenazine antibiotics [3]. The com-

pound was readily soluble in organic solvents like dichloromethane or acetone and showed the characteristic  $^1\text{H}$  NMR splitting of a *p*-disubstituted electron-rich benzene derivative, *m*-coupled  $^1\text{H}$  signals and singlets at  $\delta$  8.30, 10.40, and 12.90, the latter two being exchangeable with  $\text{D}_2\text{O}$ . This pattern was very similar to that of genistein (**1b**), a very common by-product of fermentations using soybean flour or malt extract as carbon source. Additional signals of aromatic protons in 1,2,3-position, however, and a methyl singlet at  $\delta$  2.80 (3 H) were noticed.

The  $^{13}\text{C}$  NMR spectrum contained two carbonyl signals at  $\delta$  181.2 and 170.0 of a conjugated ketone and an ester or acid group, respectively. Six signals between  $\delta$  166 and 150 represented aromatic carbon atoms directly connected with oxygen atoms. The spectrum showed twelve additional signals between  $\delta$  142 and 94, where those at  $\delta$  131.2 and 122.6 represented two carbon atoms each. In the aliphatic region only one methyl signal at  $\delta$  22.9 was present. The ESI and EI mass spectra led to the molecular weight  $m/z$  404, and EI HRMS measurements resulted in the molecular formula  $\text{C}_{23}\text{H}_{16}\text{O}_7$ . A search in AntiBase [4] and the Dictionary of Natural Products (DNP) [5] with the  $^1\text{H}$  and  $^{13}\text{C}$  NMR data, the molecular weight and formula did not lead to a known structure.



Careful interpretation of the cross signals in the  $^1\text{H}$ ,  $^1\text{H}$  COSY, HMQC and HMBC spectra combined with the coupling constants of the signals in the  $^1\text{H}$  NMR spectrum resulted in the fragments I and II for the compound A, of which the former represents the genistein chromophore.

Table 1. Comparison  $^1\text{H}$  NMR data of fragment I of **1d** with genistein (**1b**) in DMSO.

Atom	$^1\text{H}$ NMR shift		
	<b>1d</b>	<b>1b</b>	$\Delta\delta$
2	8.30	8.28	0.02
2', 6'	7.73	7.32	0.41
3', 5'	7.40	6.95	0.45
6	6.30	6.28	0.02
8	6.42	6.42	0.00

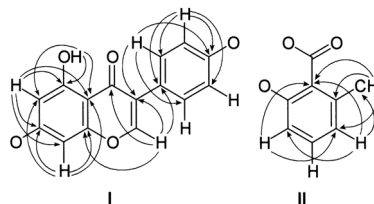
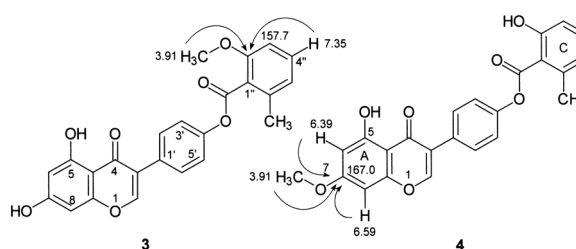


Fig. 1. Fragments I and II of compound **1d** constructed with the aid of HMBC ( $\text{H} \rightarrow \text{C}$ ),  $^1\text{H}$ ,  $^1\text{H}$  COSY, and HMQC couplings.

There were no cross signals visible between fragments I and II to connect them. Comparison of the proton NMR data in fragment I with those of genistein (**1b**), however, showed negligible differences for H-6 and H-8 of ring A, whereas the signals for H-2', 6' and H-3', 5' of ring C were clearly shifted to low field ( $\Delta\delta \approx 0.5$ ) (Table 1). It follows that the substitution must have occurred at ring C *via* an ether or ester bond.

Methylation of compound **1d** with diazomethane resulted in a 1:1 mixture of two monomethyl derivatives **3** and **4** with methoxy signals at  $\delta$  3.91. Further methylation or separation was not necessary, because the required information was already available from the mixture: In the HMBC spectrum, the methoxy signal and the H-4'' triplet at  $\delta$  7.35 were coupling with the same carbon atom C-2'' of the salicylate residue at  $\delta$  157.7, which clearly shows that the methoxy group in **3** exists as an ether but not as ester.



This was further confirmed by the coupling of the methoxy signal of the second component **4** with C-7 at  $\delta$  167.0 to which the proton signals of ring A at  $\delta$  6.59 and 6.39 also showed couplings: OH-7 must have

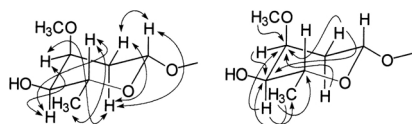


Fig. 2. NMR correlation of H,H COSY ( $\leftrightarrow$ ) and HMBC ( $\text{H} \rightarrow \text{C}$ ) experiments in the cymaropyranosyl fragments of isoflavone **1e**.

been free before methylation: The parent compound was therefore genistein-4'-(6''-methylsalicylate) (**1d**), a new natural product.

Among the more than 800 naturally occurring isoflavones [5], only about 30 exist as esters and all these are acetates. The metabolite **1d** is the first isoflavone 4'-ester of an aromatic acid.

A second isoflavone was obtained from *Actinomyces* sp. isolate HKI 129-L as a colourless solid with strong UV absorption on TLC as well. The DCI mass spectrum showed *quasi* molecule ion signals at  $m/z$  576 ( $[\text{M}+\text{NH}_4]^+$ ) and 559 ( $[\text{M}+\text{H}]^+$ ), which indicated the molecular weight to be 558.

The aromatic region of the  $^1\text{H}$  NMR spectrum showed again typical proton signals of the genistein chromophore: an H/D exchangeable singlet of intensity 1 at  $\delta$  12.83, a singlet at  $\delta$  7.89 (1 H), two doublets each of intensity 2 at  $\delta$  7.46 and 7.12 and two signals each of intensity 1 at  $\delta$  6.53 and 6.46. At  $\delta$  5.23 (1 H) and 5.16 (1 H) two dd signals were visible which could be assigned to two anomeric protons of sugar residues leading to the assumption that this metabolite must be a glycoside of genistein. The  $^{13}\text{C}$  NMR spectrum supported this by signals at  $\delta$  97.4 and 96.8 of the anomeric carbon atoms of two sugar residues.

The aliphatic region of the  $^1\text{H}$  NMR spectrum showed signals in pairs indicating that both sugar residues were either very similar or even identical. The constitution of the sugars was derived with the aid of the H,H COSY, HMQC and HMBC couplings shown in Fig. 2.

The relative stereochemistry of the sugars was derived from coupling constants of the sugar protons in the  $^1\text{H}$  NMR spectrum (Table 1), which led to the constitution and configuration of  $\beta$ -cymarose. Comparison of the NMR data with literature values for the  $\beta$ -D-cymaropyranosyl substructure in (20S)-18,20-epoxystrophanthidin- $\beta$ -D-cymaroside [6] showed good agreement of the  $^{13}\text{C}$  shifts; the absolute configuration remained open.

The connection of the two  $\beta$ -cymaropyranosyl units at both C-4' and C-7 was finally derived by the

Table 2. Comparison of NMR data of the  $\beta$ -cymaropyranosyl substructure of compound **1e** with literature values for the  $\beta$ -D-cymaropyranosyl part of (20S)-18,20-epoxystrophanthidin- $\beta$ -D-cymaroside [6]; np = not published.

Atom	$^1\text{H}$		$^{13}\text{C}$	
	<b>1e</b>	cymaroside [6]	<b>1e</b>	[6]
1	5.23 (dd, 11, 2 Hz)	5.12 (dd, 10, 2 Hz)	97.4	97.6
2	2.58 (m)/1.79 (ddd, 21, 11, 2 Hz)	np	34.7	36.3
3	3.32 (m)	3.70 (q, 3 Hz)	80.2	78.7
4	3.26 (dd, 9, 1 Hz)	3.52 (dd, 9, 3 Hz)	75.1	73.8
5	3.52 (dq, 9, 6 Hz)	np	72.2	71.1
6	1.40 (d, 6 Hz)	1.51 (d, 6 Hz)	17.9	18.9
3-OMe	3.46	3.41	56.6	58.0

HMBC couplings of the anomeric protons with the respective carbon signals of the genistein chromophore giving 4',7-bis-( $\beta$ -cymaropyranosyl)-genistein (**1e**), a new natural product. Monoglycosides of genistein (**1b**) were often isolated from plants. A database search [5] resulted, however, only in four 4',7-diglycosides: Sarothamnose, neobacin [8], genistein-4',7-diglucoside [9], which were reported from plants, and the genistein 4',7-di- $\alpha$ -L-rhamnoside was obtained from an *Actinomyces* sp. [10].

Another new isoflavone ether obtained from *Streptomyces* sp. isolate HKI 129-L gave a molecular ion peak at  $m/z$  298 (EI MS), and its high resolution led to the molecular formula  $\text{C}_{17}\text{H}_{14}\text{O}_5$ . The proton NMR spectrum showed two doublets of doublets each of intensity 2 at  $\delta$  7.46 and 6.85, which agreed well with the values found for the 4-hydroxyphenyl substituent (ring C) in daidzein (**1a**) and genistein (**1b**). In addition, there were three singlets each of one proton in the aromatic region at  $\delta$  7.95, 7.68 and 6.90 and a singlet of two methoxy groups at  $\delta$  3.99. This indicates clearly a dimethyl ether of 4',6,7-trihydroxyisoflavone. As the NMR data were different from those of afrormosin (4',6-dimethoxy) [11,12] and alfarone (4',7-dimethoxy) [13], our compound must be the new 4'-hydroxy-6,7-dimethoxyisoflavon (**2c**). The constitution was finally confirmed by the HMBC couplings as shown in Fig. 3.

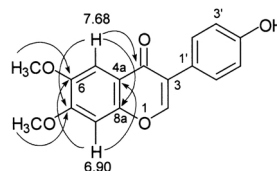
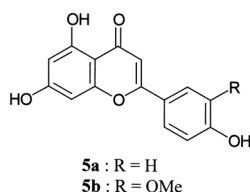


Fig. 3. HMBC couplings ( $\text{H} \rightarrow \text{C}$ ) in 4'-hydroxy-6,7-dimethoxy isoflavone (**2c**).

Additionally to the previous isoflavones, we have often isolated genistein (**1b**) and daidzein (**1a**) from *Streptomyces* of marine and terrestrial origin when grown on malt extract or soybean flour media. A further plant isoflavone kakkatin (**2b**) was isolated from *Streptomyces* sp. isolate GW39/1530. This compound was readily identified by the typical proton NMR signal pattern in the aromatic region. For a further confirmation and for comparison, genistein (**1b**) and 4',6,7-trihydroxy-isoflavone (**2a**) were partially methylated by brief treatment with diazomethane. Only one (**1c**) of the expected monoethers was formed. A second (**1f**) was, however, commercially available. While chelated hydroxyl groups in anthraquinones do not react easily with diazomethane, **1b** yielded in addition the dimethyl ether **1g**.

From the culture of the terrestrial *Streptomyces* sp. isolate GW10/1811 grown on soybean flour medium, in addition to daidzein (**1a**), genistein (**1b**) and genistein-7-methyl ether (**1c**), two more polar UV absorbing slightly yellow compounds were isolated which showed characteristic differences in their NMR spectra with respect to the isoflavonoids. While the latter gave deepfield singlets usually beyond  $\delta$  8 for 2-H, the yellow compounds from GW10/1811 showed a singlet at  $\delta$  6.8 which is characteristic for 3-H of flavonoids. Using the ESI MS and NMR data, both compounds were easily identified as apigenin [14] (**5a**) and luteolin-3'-methyl ether (**5b**). The identification was finally confirmed by comparison with reference data [14].



As both flavonoids are known plant metabolites, it is plausible that **5a** and **5b** are originally constituents of the nutrient composition and were isolated as artefacts. It should be mentioned, however, that we are using this soybean flour medium as a standard composition since many years and never isolated **5a** or **5b** before.

## Experimental Section

Material and methods were used as described earlier [15].

### Description of the producers

The terrestrial strain *Actinomyces* sp. HKI 129-L was isolated from a soil sample in Thuringia, Germany, and is kept

in the strain collection of the Hans-Knöll-Institute, Jena, Germany, and was not further characterized. The terrestrial isolates GW27/2506, and GW39/1530 are according to their morphological characteristics *Streptomyces* spp. They were obtained from the culture collection of bioLeads GmbH, Heidelberg, Germany.

### Fermentation

All strains were fermented on a 20 l scale. The terrestrial *Streptomyces* sp. GW27/2506 and *Streptomyces* sp. GW48/1530 were cultured on SM medium [16]. Stock cultures of HKI 129-L were suspended in glycerol (10%)/lactose (5%) solution and preserved by storage in liquid nitrogen. This suspension served as inoculum for a medium containing (g/l): glucose 15, soybean flour 15, CaCO<sub>3</sub> 1, NaCl 5, KH<sub>2</sub>PO<sub>4</sub> 1. A pH 6.2 was adjusted prior to sterilization. Inoculated 250 ml Erlenmeyer flasks containing 50 ml medium were incubated for 48 h at 28 °C on a rotary shaker orbiting in a 10 cm circle at 150 rpm. The resulting culture was transferred into 400 ml volumes of the same medium in 2-liter shake flasks. After further incubation period of 48 h, the mycelium was used as 5% inoculum for a 10 liters fermentor. The fermentor medium contained (g/l): glucose 20, soy bean flour 20, NaCl 5, CaCO<sub>3</sub> 3 and was adjusted to pH 7.2 prior to sterilization. The well aerated culture was harvested after 96 h of cultivation at 28 °C and lyophilized. This material was finally extracted with ethyl acetate.

*S. sp.* GW 10/1811 was cultivated on the above soybean medium with mannitol instead of glucose in the same way. For working-up and defatting of the crude extracts, our standard procedures were used [15].

### Work-up procedures

#### *Streptomyces sp. isolate HKI 129-L*

The defatted extract from 10 l of the fermentation broth of the *Actinomyces* sp. isolate HKI 129-L was pre-separated on silica gel (25 × 500 mm) into six fractions, using a stepwise gradient of 1.5 l CHCl<sub>3</sub>/2 % CH<sub>3</sub>OH, 1 l CHCl<sub>3</sub>/4% CH<sub>3</sub>OH, 1.5 l CHCl<sub>3</sub>/6 % CH<sub>3</sub>OH).

Fraction I (810 mg,  $R_f$  = 0.65 – 0.40, CHCl<sub>3</sub>/5% CH<sub>3</sub>OH) was further separated on Sephadex LH-20 (30 × 600 mm, CHCl<sub>3</sub>/40 % CH<sub>3</sub>OH). The first fraction showed a strong UV absorption on TLC and gave a yellowish brown colouration on spraying with anisaldehyde/sulphuric acid. Further separation by preparative HPLC (RP<sub>18</sub> silica gel, CH<sub>3</sub>CN-H<sub>2</sub>O azeotrope/H<sub>2</sub>O: 50/50; flow rate 18.5 ml/min,  $R_t$  = 15.4–17 min) yielded 7.5 mg of 4',7-bis-( $\beta$ -cymaropyranosyl)-genistein (**1e**) as a white solid. Similarly fraction III (550 mg,  $R_f$  = 0.35 – 0.22, CHCl<sub>3</sub>/5% CH<sub>3</sub>OH) yielded 5.3 mg of 4'-hydroxy-6,7-dimethoxyisoflavone (**2c**) after final purification by HPLC (linear CH<sub>3</sub>CN/H<sub>2</sub>O gradient). From fraction VI, 14 mg of genistein (**1b**) were obtained.

**4',7-Bis-( $\beta$ -cymaropyranosyl)-genistein (1e)**

Colourless solid,  $R_f = 0.41$  ( $\text{CHCl}_3/5\% \text{ CH}_3\text{OH}$ ). – IR (KBr):  $\nu = 3446 \text{ cm}^{-1}$  (OH), 2928, 1732, 1654, 1510, 1458, 1376, 1240, 1181, 1119, 1064, 993, 837, 668. –  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 500.0 MHz):  $\delta = 12.83$  (s; 1 H, 5-OH), 7.89 (s; 1 H, 2-H), 7.46 (d,  $^3J = 8.4 \text{ Hz}$ ; 2 H, 2'-H, 6'-H), 7.12 (d,  $^3J = 8.4 \text{ Hz}$ ; 2 H, 3'-H, 5'-H), 6.53 (d,  $^4J = 2.4 \text{ Hz}$ ; 1 H, 8-H), 6.46 (d,  $^4J = 2.4 \text{ Hz}$ ; 1 H, 6-H), 5.23 (dd,  $^3J_{aa} = 11.4 \text{ Hz}$ ,  $^3J_{ae} = 1.5 \text{ Hz}$ ; 1 H, 1''-H), 5.16 (dd,  $^3J_{aa} = 11.4 \text{ Hz}$ ,  $^3J_{ae} = 1.5 \text{ Hz}$ ; 1 H, 1'''-H), 3.53/3.51 (2 dq,  $^3J_{aa} = 9.3 \text{ Hz}$ ,  $^3J = 5.9 \text{ Hz}$ ; 2 H, 5''-H, 5'''-H), 3.47/3.45 (2 s; 6 H, 3''-OCH<sub>3</sub>, 3'''-OCH<sub>3</sub>), 3.32 (m; 2 H, 3''-H, 3'''-H), 3.27/3.25 (2 dd,  $^3J_{aa} = 9.3 \text{ Hz}$ ,  $^3J_{ae} = 1.0 \text{ Hz}$ ; 2 H, 4''-H, 4'''-H), 2.58 (m; 2 H, 2''-H<sub>e</sub>, 2'''-H<sub>e</sub>), 1.80/1.78 (2 ddd,  $^2J = 21.0 \text{ Hz}$ ,  $^3J_{aa} = 11.4 \text{ Hz}$ ,  $^3J_{ae} = 1.5 \text{ Hz}$ ; 2 H, 2''-H<sub>a</sub>, 2'''-H<sub>a</sub>), 1.40/1.39 (2 d,  $^3J = 5.9 \text{ Hz}$ ; 6 H, 6''-H<sub>3</sub>, 6'''-H<sub>3</sub>). –  $^{13}\text{C}$  NMR and APT ( $\text{CDCl}_3$ , 125.7 MHz):  $\delta = 180.8$  (C<sub>q</sub>-4), 162.6 (C<sub>q</sub>-5), 162.5 (C<sub>q</sub>-7), 157.6 (C<sub>q</sub>-8a), 153.0 (CH, C-2), 130.1 (2 CH, C-2', C-6'), 124.5 (C<sub>q</sub>-1'), 123.6 (C<sub>q</sub>-3), 116.6 (2 CH, C-3', C-5'), 107.1 (C<sub>q</sub>-4a), 99.8 (CH, C-6), 97.4 (CH, C-1''), 96.8 (CH, C-1'''), 94.7 (CH, C-8), 80.4 (CH, C-3''), 80.2 (CH, C-3'''), 75.3 (CH, C-4''), 75.1 (CH, C-4'''), 72.2 (CH, C-5''), 71.9 (CH, C-5'''), 56.6 (CH<sub>3</sub>, 3''-OCH<sub>3</sub>), 56.5 (CH<sub>3</sub>, 3'''-OCH<sub>3</sub>), 34.9 (CH<sub>2</sub>, C-2''), 34.7 (CH<sub>2</sub>, C-2'''), 17.9 (CH<sub>3</sub>, C-6''), 17.9 (CH<sub>3</sub>, C-6'''). – EI-MS (70 eV):  $m/z(\%) = 270$  (100), 153 (10), 87 (61). – DCI-MS ( $\text{NH}_3$ ):  $m/z(\%) = 576$  ( $[\text{M} + \text{NH}_4]^+$ , 90), 559 ( $[\text{M} + \text{H}]^+$ , 48), 162 (58), 130 (100).

**4'-Hydroxy-6,7-dimethoxyisoflavon (2c)**

Colourless solid,  $R_f = 0.21$  ( $\text{CHCl}_3/5\% \text{ CH}_3\text{OH}$ ). –  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 200.1 MHz):  $\delta = 7.95$  (s; 1 H, 2-H), 7.68 (s; 1 H, 5-H), 7.46 (d,  $^3J = 7.6 \text{ Hz}$ ; 2 H, 2'-H, 6'-H), 6.90 (s; 1 H, 8-H), 6.85 (d,  $^3J = 7.6 \text{ Hz}$ ; 2 H, 3'-H, 5'-H), 4.00/3.98 (2 s; 6 H, 6-OCH<sub>3</sub>, 7-OCH<sub>3</sub>). –  $^{13}\text{C}$  NMR and APT ( $\text{CDCl}_3$ , 75.5 MHz):  $\delta = 176.2$  (C<sub>q</sub>-4), 156.1 (C<sub>q</sub>-4'), 154.6 (C<sub>q</sub>-7), 152.5 (C<sub>q</sub>-8a), 152.2 (CH, C-2), 147.8 (C<sub>q</sub>-6), 130.4 (2 CH, C-6'), 124.7 (C<sub>q</sub>-3), 123.8 (C<sub>q</sub>-1'), 117.8 (C<sub>q</sub>-4a), 115.8 (2 CH, C-3', C-5'), 104.8 (CH, C-5), 99.5 (CH, C-8), 56.5 (CH<sub>3</sub>, OCH<sub>3</sub>), 56.4 (CH<sub>3</sub>, OCH<sub>3</sub>). – EI-MS (70 eV):  $m/z(\%) = 298.0841$  ( $\text{M}^+$ , 100; calcd. 298.0841 for  $\text{C}_{17}\text{H}_{14}\text{O}_5$ ), 256 (23), 198 (73), 169 (23). – DCI-MS ( $\text{NH}_3$ ):  $m/z(\%) = 316$  ( $[\text{M} + \text{NH}_4]^+$ , 10), 299 ( $[\text{M} + \text{H}]^+$ , 100).

***Streptomyces* sp. isolate GW27/2506**

Fraction 5 obtained through the column chromatography of the ethyl acetate extract of the strain *Streptomyces* sp. isolate GW27/2506 on silica gel was separated into two subfractions 5A and 5B. The purification of the first subfraction on HPLC RP 18 ( $\text{MeCN}/\text{H}_2\text{O}$ ) delivered saphenic acid and Genistein-4'-(6''-methyl-salicylat) (**1d**, 2.0 mg). From the other fractions many known and new phenazine antio-

otics are isolated whose purification and structures will be discussed later somewhere else.

Kakkatin (**2b**, 10 mg) was isolated from *Streptomyces* sp. GW39/1530 in a similar way. After chromatographic separation, the isoflavone precipitated from the chloroform/methanol solution on slow evaporation.

**Genistein-4'-(6''-methyl)-salicylate (1d)**

Colourless solid,  $R_f = 0.59$  ( $\text{CHCl}_3/10\% \text{ MeOH}$ ), grey-brown with anisaldehyde/sulphuric acid. –  $^1\text{H}$  NMR (acetone- $d_6$ , 300 MHz):  $\delta = 12.90$  (s, H/D exchangeable, 1 H, 5-OH), 10.20 (br s, H/D exchangeable, 2 H, 6-OH, 2''-OH), 8.30 (s, 1 H, 2-H), 7.73 (d,  $^3J = 8.8 \text{ Hz}$ , 2 H, 2'-H, 6'-H), 7.40 (d,  $^3J = 8.8 \text{ Hz}$ , 2 H, 3'-H, 5'-H), 7.34 (t,  $^3J = 8.5 \text{ Hz}$ , 1 H, 4''-H), 6.83 (d,  $^3J = 8.6 \text{ Hz}$ , 2 H, 3''-H, 5''-H), 6.42 (d,  $^4J = 2.1 \text{ Hz}$ , 1 H, 8-H), 6.30 (d,  $^4J = 2.1 \text{ Hz}$ , 1 H, 6-H), 2.60 (s, 3 H, CH<sub>3</sub>). –  $^{13}\text{C}$  NMR and APT (acetone- $d_6$ , 75.5 MHz):  $\delta = 181.2$  (C<sub>q</sub>-4), 170.0 (COO), 165.3 (C<sub>q</sub>-7), 163.5 (C<sub>q</sub>-5), 161.8 (C<sub>q</sub>-2''), 159.0 (C<sub>q</sub>-8a), 155.3 (CH-2), 151.2 (C<sub>q</sub>-4'), 141.2 (C<sub>q</sub>-6''), 134.8 (CH-4''), 131.2 (CH-2', -6'), 130.2 (C<sub>q</sub>-1'), 123.5 (CH-5''), 123.3 (C<sub>q</sub>-3), 122.6 (CH-3', -5'), 115.8 (C<sub>q</sub>-3''), 115.4 (C<sub>q</sub>-1''), 106.1 (C<sub>q</sub>-4a), 100.5 (CH-6), 94.5 (CH-8), 22.9 (Me). – HMBC (acetone- $d_6$ , IN4PLRND, F1 75.5 MHz, F2 300 MHz) see Fig. 1 – (+)-ESI-MS:  $m/z = 427$  ( $\text{M} + \text{Na}$ ), 831 ( $2\text{M} + \text{Na}$ ). – (–)-ESI-MS:  $m/z = 403$  ( $\text{M} - 1$ ). – IR (KBr):  $\nu$  ( $\text{cm}^{-1}$ ) = 3425, 3011, 2986, 1651, 1249. – EI-MS (70 eV):  $m/z(\%) = 404.0892$  ( $\text{M}$ , 22; calcd. 404.08960 for  $\text{C}_{23}\text{H}_{16}\text{O}_7$ ), 270 (100), 241 (4), 153 (12), 135 (53).

**4',6-Dihydroxy-7-methoxyisoflavone (Kakkatin, 2b)**

Colourless solid,  $R_f = 0.33$  ( $\text{CHCl}_3/10\% \text{ MeOH}$ ). –  $^1\text{H}$  NMR ( $[\text{D}_6]\text{DMSO}$ , 300 MHz):  $\delta = 10.50$  (s br, H/D exchangeable; 1 H, OH), 9.46 (s, H/D exchangeable; 1 H, OH), 8.26 (s, 1 H, 2-H), 7.43 (s, 1 H, 5-H), 7.39 (d, 2 H, 2'-H, 6'-H), 6.93 (s, 1 H, 8-H), 6.80 (d, 2 H, 3'-H, 5'-H), 3.88 (s, 3 H, OMe). – EI-MS (70 eV):  $m/z = 284$  ( $\text{M}^+$ , 100), 166, 69.

***Streptomyces* sp. isolate GW10/1811**

The crude extract (4.50 g) from *S. sp.* GW10/1811 was separated on silica gel (column 40  $\times$  6 cm,  $\text{CH}_2\text{Cl}_2/\text{MeOH}$  gradient). The fraction 5 containing the polar UV absorbing isoflavones genistein (**1b**), genistein-7-methyl ether (**1c**) and daidzein (**1a**) was re-chromatographed on Sephadex LH 20 ( $\text{CH}_2\text{Cl}_2/40\% \text{ CH}_3\text{OH}$ ). Genistein-7-methyl ether (**1c**, 4.2 mg,  $R_f = 0.60$ ,  $\text{CH}_2\text{Cl}_2/8\% \text{ CH}_3\text{OH}$ ), genistein (**1b**, 60 mg,  $R_f = 0.50$ ,  $\text{CH}_2\text{Cl}_2/8\% \text{ CH}_3\text{OH}$ ) and daidzein (**1a**, 20 mg,  $R_f = 0.46$ ,  $\text{CH}_2\text{Cl}_2/8\% \text{ CH}_3\text{OH}$ ) were obtained as white solids.

Fraction 10 of the silica gel column was re-chromatographed on Sephadex LH 20. The first subfraction yielded 7.2 mg apigenin (**5a**,  $R_f = 0.47$  ( $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}$ : 90/10) as a slightly yellow solid. From the second sub-

fraction, 2.2 mg luteolin-3'-methyl ether (chrysoeriol, **5b**) were obtained.

#### Luteolin-3'-methyl ether (**5b**)

Light yellow solid,  $R_f = 0.65$  ( $\text{CH}_2\text{Cl}_2/10\%\text{CH}_3\text{OH}$ ).  $^1\text{H}$  NMR (DMSO, 300 MHz):  $\delta = 6.19$  (d,  $J = 1.4$  Hz, 1 H, 6-H), 6.48 (d,  $J = 1.4$  Hz, 1 H, 8-H), 6.80 (s, 1 H, 3-H), 6.95 (dd,  $J = 8.0$  Hz, 1 H, 5'-H), 7.5 (d,  $J = 1.4$  Hz, 1 H, 2'-H), 7.52 (dd,  $J = 8.0$  Hz, 1.4 Hz, 1 H, 6'-H), 3.88 (s, 3 H, 3'-OCH<sub>3</sub>). – (–)-ESIMS:  $m/z = 299$  [ $\text{M} - \text{H}$ ]<sup>–</sup>.

#### Isoflavone methyl ethers by methylation with diazomethane

To a solution containing 14 mg of a mixture of genistein (**1b**) and 4',6,7-trihydroxy-isoflavone (**2a**) in 2 ml  $\text{CH}_2\text{Cl}_2/\text{MeOH}$  (4:1), 0.5 ml of a 0.2 M diazomethane solution in ether was added and kept for ca. 30 sec at room temp. The reaction mixture was then evaporated to dryness and finally separated by PTLC ( $\text{CH}_2\text{Cl}_2/15\%$  MeOH) to afford **1c**, **1g**, **2c**, and **2d**.

#### 7-O-Methyl-genistein (4',5-Dihydroxy-7-methoxyisoflavone), (**1c**)

Colourless solid,  $R_f = 0.37$  ( $\text{CHCl}_3/10\%\text{CH}_3\text{OH}$ ). –  $^1\text{H}$  NMR (DMSO- $d_6$ , 300 MHz):  $\delta = 12.92$  (s br, 1 H, 5-OH), 9.60 (s br, 1 H, 4'-OH), 8.37 (s, 1 H, 2-H), 7.37 (d,  $^3J = 8.7$  Hz, 2 H, 2'-H, 6'-H), 6.82 (d,  $^3J = 8.7$  Hz, 2 H, 3'-H, 5'-H), 6.63 (d,  $^4J = 2.2$  Hz; 1 H, 8-H), 6.39 (d,  $^4J = 2.2$  Hz; 1 H, 6-H), 3.86 (s, 3 H, OMe).

#### 7-Hydroxy-4',5-dimethoxyisoflavone (**1g**)

Colourless solid,  $R_f = 0.51$  ( $\text{CHCl}_3/10\%\text{CH}_3\text{OH}$ ). –  $^1\text{H}$  NMR (DMSO- $d_6$ , 300 MHz):  $\delta = 8.43$  (s, 1 H, 2-H),

7.51 (d,  $^3J = 8.7$  Hz, 2 H, 2'-H, 6'-H), 7.01 (d,  $^3J = 8.7$  Hz, 2 H, 3'-H, 5'-H), 6.65 (d,  $^4J = 2.2$  Hz; 1 H, 8-H), 6.41 (d,  $^4J = 2.2$  Hz; 1 H, 6-H), 3.86, 3.79 (2 s, each 3 H, 2 OMe).

#### 4'-Hydroxy-6,7-dimethoxyisoflavone (**2c**)

Colourless solid (see above),  $R_f = 0.55$  ( $\text{CHCl}_3/10\%\text{CH}_3\text{OH}$ ). –  $^1\text{H}$  NMR (DMSO- $d_6$ , 300 MHz):  $\delta = 8.34$  (s, 1 H, 2-H), 7.44 (s, 1 H, 5-H), 7.40 (d,  $^3J = 8.7$  Hz, 2 H, 2'-H, 6'-H), 7.19 (s, 1 H, 8-H), 6.81 (d,  $^3J = 8.7$  Hz, 2 H, 3'-H, 5'-H), 3.91, 3.86 (2 s, each 3 H, 2 OMe).

#### 4',6,7-Trimethoxy-isoflavone (**2d**)

Colourless solid,  $R_f = 0.71$  ( $\text{CHCl}_3/10\%\text{CH}_3\text{OH}$ ). –  $^1\text{H}$  NMR (DMSO- $d_6$ , 300 MHz):  $\delta = 8.40$  (s, 1 H, 2-H), 7.53 (d,  $^3J = 8.7$  Hz, 2 H, 2'-H, 6'-H), 7.45 (s, 1 H, 5-H), 7.21 (s, 1 H, 8-H), 6.98 (d,  $^3J = 8.7$  Hz, 2 H, 3'-H, 5'-H), 3.92, 3.86, 3.79 (3 s, each 3 H, 3 OMe).

#### 5,7-Dihydroxy-4'-methoxyisoflavone (**1f**)

The ether **1f** was purchased from Fluka Chemie GmbH, Switzerland, as a colourless solid,  $R_f = 0.38$  ( $\text{CHCl}_3/10\%\text{CH}_3\text{OH}$ ). –  $^1\text{H}$  NMR (DMSO- $d_6$ , 300 MHz):  $\delta = 12.93$  (s br, 1 H, 5-OH), 10.92 (s br, 1 H, 4'-OH), 8.36 (s, 1 H, 2-H), 7.50 (d,  $^3J = 8.7$  Hz, 2 H, 2'-H, 6'-H), 6.88 (d,  $^3J = 8.7$  Hz, 2 H, 3'-H, 5'-H), 6.40 (d,  $^4J = 2.1$  Hz; 1 H, 8-H), 6.24 (d,  $^4J = 2.1$  Hz; 1 H, 6-H), 3.79 (s, 3 H, 4'-OMe).

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