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ANTIMICROBIAL FLAVONOIDS FROM *PSIADIA TRINERVIA* AND THEIR METHYLATED AND ACETYLATED DERIVATIVES

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Key Word Index—*Psiadia trinervia*; Compositae; flavonoids; antifungal; antibacterial; *Cladosporium cucumerinum*; *Bacillus cereus*; bioautography.

Abstract—From a dichloromethane extract and a hydrolysed methanolic extract from the leaves of *Psiadia trinervia*, 13 3-methylated flavonols have been isolated. Their structures were established by the usual spectroscopic methods (UV, EIMS, ^{1}H and ^{13}C NMR). Ayanin, casticin, chrysosplenol-D and 5,7,4′-trihydroxy-3,8-dimethoxyflavone were responsible for the antifungal activity found in the preliminary screening. Chrysosplenol-D, isokaempferide, 5,7,4′-trihydroxy-3,3′-dimethoxyflavone and 5,7,4′-trihydroxy-3,8-dimethoxyflavone displayed antibacterial activity. Twenty-nine derivatives were prepared by permethylation and selective methylation of the free hydroxyl group at C-5. The antimicrobial activities of the isolates and derivatives were determined by bioautographic assays using *C. cucumerinum* and *B. cereus* as test organisms.

INTRODUCTION

Species of the genus *Psiadia* (Compositae) are used in African traditional medicine as an expectorant for the treatment of bronchitis and asthma [1], a poultice for rheumatoid arthritis and as an analgesic for brain and nerves [2]. Previous phytochemical investigations of the genus *Psiadia* led to the isolation of the diterpenoids psiadiol [3, 4], isopsiadiol and 6-deoxypsiadiol [5] from *P. altissima*. In addition, some terpenes in the essential oil of *P. arabica* [6] and *P. salviifolia* [7] have been identified. The shrub *Psiadia trinervia* Willd. is an endemic species of the Mascarene Islands [8]. There has been no report on previous phytochemical investigations of this species. In a preliminary screening for biological activity, the dichloromethane extract and a hydrolysed methanolic extract from the leaves of *P. trinervia* were active against the plant pathogenic fungus *Cladosporium cucumerinum* and the gram-positive bacterium *Bacillus cereus*. We now report on a series of 13 flavonol 3-*O*-methyl ethers from *P. trinervia* and on 29 other flavonoid derivatives and their antimicrobial activities.

RESULTS AND DISCUSSION

The leaves of *Psiadia trinervia* were extracted successively with petrol, dichloromethane and methanol. The dichloromethane extract showed antimicrobial activity against *Cladosporium cucumerinum* and *Bacillus cereus* in bioassays on TLC [9, 10]. Activity-guided isolation yielded antimicrobial compounds **2**, **5**, **6** and **10**, together with seven additional methylated flavonols (**1**, **3**, **4** and **7–9**). The methanolic extract, inactive in the preliminary screening, also displayed antimicrobial activity after acidic hydrolysis. The four flavonol 3-methyl ethers **10–13** were subsequently isolated by column chromatography on silica gel and Diol from the hydrolysed methanolic extract. Compounds **10**, previously isolated from the dichloromethane extract, **11** and **13** were responsible for the antimicrobial activity.

A dark purple absorption of the spots on TLC under long wavelength UV light (366 nm) and the UV spectra recorded with the classical shift reagents [11] clearly indicated a free hydroxyl group at C-5 for compounds **1–13**. This was confirmed by the ^{1}H NMR spectra exhibiting a low field singlet (δ12.31–12.70, 1H) of a chelated hydroxyl group. Oxygenation patterns of the compounds were determined with the aid of ^{1}H and ^{13}C NMR spectra. A pair of *meta* coupled aromatic protons was indicative of a 5,7-substituted ring A in compounds **1–3**, **5**, **11** and **12**. 7-Methyl ethers and 7-hydroxy compounds could be distinguished with the aid of the UV spectra recorded with sodium acetate and the fragment $[A]^+$ in the EIMS [12]. A 5,6,7-substitution pattern as encountered in flavonols **6**, **7**, **9** and **10** was distinguished from the isomeric 5,7,8-substituted compounds **4**, **8** and **13** by the chemical shift of the aromatic singlet, appearing at δ6.85–6.94 for H-8 and δ6.27–6.30 for H-6. The relative abundance of the ions $[M]^+$, $[M-1]^+$ and $[M-15]^+$ in the EIMS were in support of the proposed structures [13, 14]. The substitution pattern of ring B was also deduced by ^{1}H NMR spectroscopy. An AA′BB′ system in the spectra of compounds **1**, **9**, **11** and **14** was indicative of a 4′-substitution, whereas the other compounds exhibited a three proton system with a coupling pattern typical of a 3′,4′-substituted B ring. The substituents, whether free hydroxyl or methoxyl groups, could be ascertained by UV spectroscopy with shift reagents (NaOAc and NaOAc+H_3BO_3), with the aid of the characteristic $[B_2]^+$ and $[B_2-28]^+$ fragments in the EIMS [12], and by chemical shift differences in the ^{1}H NMR spectra.

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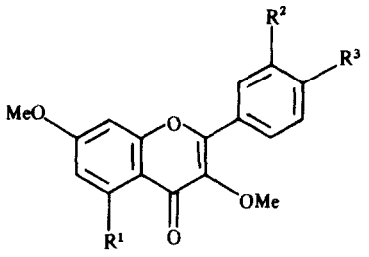
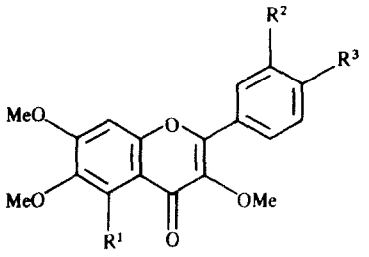
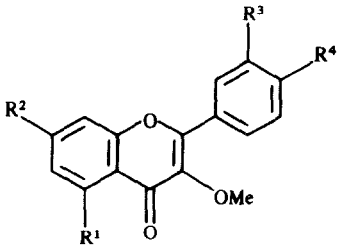


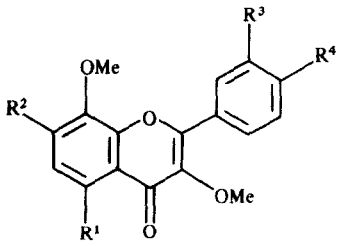
	R^1	R^2	R^3
1	OH	H	OH
1a	OMe	H	OMe
1b	OH	H	OAc
1c	OMe	H	OAc
1d	OMe	H	OH
3	OH	OH	OH
3b	OH	OAc	OAc
3c	OMe	OAc	OAc
3d	OMe	OH	OH
5	OH	OH	OMe
5b	OH	OAc	OMe
5c	OMe	OAc	OMe
5d	OMe	OH	OMe



	R^1	R^2	R^3
6	OH	OH	OMe
7	OH	OMe	OMe
9	OH	H	OH
9a	OMe	H	OMe
9b	OH	H	OAc
9c	OMe	H	OAc
9d	OMe	H	OH
10	OH	OH	OH
10a	OMe	OMe	OMe
10b	OH	OAc	OAc
10c	OMe	OAc	OAc
10d	OMe	OH	OH



	R^1	R^2	R^3	R^4
2	OH	OH	OMe	OH
2a	OMe	OMe	OMe	OMe
2b	OH	OAc	OMe	OAc
2c	OMe	OAc	OMe	OAc
2d	OMe	OH	OMe	OH
11	OH	OH	H	OH
12	OH	OH	OH	OH



	R^1	R^2	R^3	R^4
4	OH	OH	OMe	OH
4a	OMe	OMe	OMe	OMe
4b	OH	OAc	OMe	OAc
4c	OMe	OAc	OMe	OAc
4d	OMe	OH	OMe	OH
8	OH	OH	OMe	OMe
8b	OH	OAc	OMe	OMe
8c	OMe	OAc	OMe	OMe
8d	OMe	OH	OMe	OMe
13	OH	OH	H	OH

Thus, the substitution pattern was 3',4'-OH for flavonols **3**, **10** and **12**, 3',4'-OMe in the case of compounds **7** and **8**, while 3'-OH, 4'-OMe and 3'-OMe, 4'-OH substitution was found for compounds **5**, **6**, and **2**, **4**, respectively. The ^{13}C NMR spectra of compounds **1**–**13** confirmed the proposed structures in all cases [15, 16]. The compounds **1**–**13** have all been isolated previously from other plant sources [17–26], but flavonoids have not been reported before from species belonging to the genus *Psiadia*.

Previous investigations have shown that lipophilic flavonoids display antimicrobial activity, and it was argued that this property was due to their ability to penetrate biological membranes [27]. Methylation of 5-OH has been considered to be a structural feature essential to good antifungal activity [28], whereas 3-methyl flavonols have been reported to exhibit antiviral activity [29]. In order to investigate the antimicrobial activity of a large number of related compounds and in view of further biological tests, a series of partially and fully methylated derivatives of the isolates were prepared. In extrapolation of the above cited investigations [27, 28], an improved antimicrobial activity was anticipated for both the 5-methyl ethers and the permethylated compounds, compared to the original flavonoids.

Treatment of flavonoids **1, 2, 4, 9** and **10** with dimethyl sulphate in the presence of potassium carbonate gave permethyl ethers **1a, 2a, 4a, 9a** and **10a** in high yield. Selective methylation of the hydroxyl at C-5 was achieved by the method described by Wagner *et al.* [30]. Partial acetylation of **1–5** and **8–10** yielded derivatives **1b–5b** and **8b–10b**. The corresponding 5-methyl ethers **1c–5c** and **8c–10c** were prepared through treatment with dimethyl sulphate, and compounds **1d–5d** and **8d–10d** were obtained after deprotection in acidic solution. While the 5-methyl ethers and most of the corresponding acetates have been synthesized earlier, the eight partially acetylated derivatives **1b–4b, 8b–10b** and **10c** have not been reported.

In order to determine the antimicrobial activity for the 13 isolates and 29 derivatives, geometric dilution series were prepared for each compound and tested in the bioautographic assays [9, 10]. The amounts spotted ranged from 20 to 1 μg, and from 4 to 0.25 μg, when testing for antifungal and antibacterial activity, respectively. This procedure does not yield an absolute measure for antimicrobial activity, comparable to MIC values, but provides a rapid and reproducible determination of the relative activity of the test compounds. Thus, 20 μg of compounds **5** and **6**, and 5 μg of flavonols **10** and **13** were sufficient to inhibit growth of *C. cucumerinum* (Table 1). The permethylated flavonols, the 5-methyl ethers and the partially acetylated compounds were all inactive when tested at 20 μg. However, reaction intermediates **1c** (1 μg), **3c**(5 μg) and **4c**(5 μg) exhibited good activity. Four of the isolates, namely compounds **2, 10, 11** and **13** were active against *B. cereus* (Table 1), but all the derivatives prepared failed to inhibit bacterial growth when spotted at 4 μg.

As can be seen from Table 1, there is no clear correlation between antifungal and antibacterial activity for the compounds tested. Although it is not possible to establish a general structure–activity relationship, some trends can be observed. For a good antibacterial activity, a 5,7-dihydroxy substitution, as encountered with compounds **2, 11** and **13**, seems to be important. The presence of a 7-OMe group significantly reduces the activity. To a lesser degree, 3′,4′-dihydroxyflavonols possess antibacterial activity. However, these structural features cannot be solely responsible for bioactivity, as a comparison of flavonoids **2, 11, 12** and quercetin illustrates. While the phenolic groups may interact with biological structures through hydrogen bonding [31], a certain degree of lipophilicity is apparently required for the flavonoids to be active.

Compared to the antibacterial flavonols, the antifungal compounds tend to be more lipophilic. Contrary to the expectations, the 5-methyl ethers **2d–5d**, and **8d–10d**, and all the permethylated compounds were inactive at the amounts tested. It seems that free phenolic groups are required for flavonol 3-methyl ethers with a substituted B ring to be active. However, the acetylated reaction intermediates **1c, 3c** and **4c** were the best antifungals among the 42 compounds tested. An explanation may be that these lipophilic compounds are deacylated in the fungal cell to yield an active phenol. Further investigations are however required to substantiate these observations.

Table 1. Results of bioassays

Compound	*C. cucumerinum*	*B. cereus*
1c	1*	—
2	—	0.25*
3c	5	—
4c	5	—
5	20	—
6	20	—
10	5	4
11	—	0.25
13	5	0.5
quercetin		2.5

*Minimal amount (μg) of compound required to inhibit growth on the TLC plate.

EXPERIMENTAL

General. Mp: uncorr. UV-shift reagents were prepared according to ref. [11]. ^1H and ^{13}C NMR spectra were measured in DMSO-d_6, $CDCl_3$ or $CDCl_3$–CD_3OD (1:1) at 200.06 MHz for proton and 50.30 MHz for carbon, respectively. TMS was used as an int. standard.

TLC was carried out on pre-coated silica gel 60 F_{254} aluminium sheets (Merck), and RP-8 or Diol HPTLC plates (Merck). The following solvent systems were employed: $CHCl_3$–MeOH (9:1) and toluene–EtOAc (3:1) (silica gel), MeOH–H_2O(4:1) (RP-8), and $CHCl_3$–MeOH (9:1) (Diol). The compounds were revealed in UV light (254 and 366 nm), and after spraying with 2-aminoethyl diphenylborate/polyethyleneglycol reagent [32]. Column chromatography was achieved on silica gel (40–63 μm, Merck). Low pressure liquid chromatography was carried out on pre-packed Lobar column type B (LiChroprep Diol, 40–63 μm, Merck) and the mobile phase was delivered by a Duramatic 80 pump at flow rate of 1 ml/min. The purity of the compounds was checked by HPLC (Spectra-Physics 8700 pump, photodiode array detector HP-1040 A, coupled with an HP-85 personal computer and an HP-7470A plotter). The HPLC columns (Knauer) were packed with LiChrosorb RP-8(7 μm), LiChrosorb CN (7 μm) or LiChrosorb Diol (5 μm).

Plant material. Leaves of *Psiadia trinervia* were collected in November 1987 in Mauritius and a voucher specimen is deposited at the herbarium of Mauritius Sugar Industry Research Institute, Réduit, Mauritius.

Extraction and isolation. The air-dried leaves (156 g) were ground and extracted at room temp. successively with petrol, CH_2Cl_2 and MeOH. 16.9, 24.8 and 34.4 g of extracts were obtained respectively.

A portion (20 g) of CH_2Cl_2 extract was subjected to CC on silica gel using $CHCl_3$–MeOH (49:1→4:1) as eluent. A total of 14 fractions (1–14) was collected. Compounds **7** (11 mg), **8**(30 mg), **1**(109 mg) and **9**(69 mg) were isolated from fraction 3, 5 and 6, respectively, by CC on silica gel with toluene–EtOAc (3:1) and subsequent recrystallization from $CHCl_3$–MeOH (1:1). Fractions 8, 10, 12 and 13 recrystallized from $CHCl_3$–MeOH (1:1) giving pure compounds **4** (105 mg), **2** (125 mg), **3** (61 mg) and **10** (110 mg). Chromatography of fraction 4 on silica gel with toluene–MeOH (4:1) and further recrystallization from $CHCl_3$–MeOH (1:1) afforded pure compounds **5** (72 mg) and **6** (53 mg).

A portion (10 g) of methanolic extract was dissolved in 100 ml of ethanol. After addition of 50 ml of 1 M HCl, the mixture was refluxed for 2 hr. EtOH was removed under red. pressure, remaining aq. reaction mixture was extracted with $CHCl_3$ (4 × 100 ml) and EtOAc (3 × 100 ml). The organic layers were washed with water, combined and evapd to dryness under red. pres. The residue (5.7 g) was submitted to CC on silica gel using

$CHCl_3$–MeOH (19:1 → 1:1) as mobile phase. 10 fractions (1′–10′) were collected. Compound **12** (34 mg) was obtained from fraction 6′ by low pressure liquid chromatography on Diol with $CHCl_3$–MeOH–HOAc (950:50:1). Fraction 2′ was rechromatographed on silica gel with petrol–EtOAc (1:1) as eluent. 4 fractions (1″–4″) were collected. Fraction 3″ and 2″ were subjected to low pressure chromatography on Diol with $CHCl_3$–MeOH–HOAc (995:5:1) and $CHCl_3$–MeOH (99:1), respectively, yielding compounds **10** (8 mg), **11** (40 mg) and **13** (13 mg).

Preparation of derivatives. Permethylation: the flavonol (10 mg) was refluxed for 1 hr in 10 ml of dried Me_2CO in the presence of K_2CO_3 (0.7 g) and $(Me)_2SO_4$ (0.3 ml). The mixture was filtered and the residue washed with Me_2CO several times. The permethylated flavonol was purified by CC on silica gel with $CHCl_3$–EtOAc (40:1).

Selective methylation of 5-OH: (i) partial acetylation: the flavonol (*ca* 30 mg) in 0.5 ml of dry pyridine was treated at room temp. for 12 hr with accurate molar equivalent of Ac_2O required to achieve acetylation of all hydroxyls with the exception of 5-OH. The mixture was poured into ice-water. The ppt. was filtered off and the acetylated flavonol was purified by CC on silica gel with $CHCl_3$–EtOAc (40:1). (ii) Methylation: methylation of 5-OH was achieved under the conditions used for permethylation. The product was purified by CC on silica gel with $CHCl_3$–EtOAc (40:1) or $CHCl_3$–EtOAc (9:1). (iii) Deprotection: the compound was dissolved in EtOH (5 ml) with 1 M HCl (5 ml) and refluxed for 1.5 hr. EtOH was removed under red. pres., and the aq. solution extracted with EtOAc (2 × 5 ml). The organic layer was washed with H_2O (3 × 5 ml) and evapd to dryness. The 5-methyl ether was recrystallized from $CHCl_3$–MeOH (1:1).

Bioautography. Testing for antifungal activity was carried out according to [9]. Direct bioautography for antibacterial compounds was performed according to [10], with some modifications. Glass backed silica gel GF_{254} TLC plates (Merck) were used. *B. cereus* strain 8601 served as test organism. After a preincubation of 2 hr at 37°, *ca* 8–9 ml of the bacterial suspension was sprayed onto a 10 × 20 cm plate. Enzymatic activity was detected with MTT (Fluka) as substrate.

Compounds **1**–**13** have all been isolated from other sources [17–26], but incomplete spectral data have been published for these flavonoids. Mp and ^{13}C NMR data are therefore listed below. For the derivatives prepared, physico-chemical data are given only for new compounds.

5,4′-Dihydroxy-3,7-dimethoxyflavone (**1**). Mp 224–226°; ^{13}C NMR (DMSO-d_6): δ155.77 (C-2)[a], 137.71 (C-3), 177.93 (C-4), 160.90 (C-5)[b], 97.57 (C-6), 164.95 (C-7), 92.09 (C-8), 156.16 (C-9)[a], 105.10 (C-10), 120.41 (C-1′), 130.04 (C-2′), 115.52 (C-3′), 160.19 (C-4)[b], 115.52 (C-5′), 130.04 (C-6′), 59.51 (MeO-3), 55.86 (MeO-7).

5,7,4′-Trihydroxy-3,3′-dimethoxyflavone (**2**). Mp 230–232°; ^{13}C NMR (DMSO-d_6): δ155.43 (C-2), 137.70 (C-3), 177.88 (C-4), 161.22 (C-5), 98.55 (C-6), 164.12 (C-7), 93.80 (C-8), 156.33 (C-9), 104.18 (C-10), 120.79 (C-1′), 111.95 (C-2′), 147.42 (C-3′), 149.72 (C-4′), 115.60 (C-5′), 122.16 (C-6′), 59.67 (MeO-3), 55.67 (MeO-3′).

5,3′,4′-Trihydroxy-3,7-dimethoxyflavone (**3**). Mp 222–225°; ^{13}C NMR (DMSO-d_6): δ156.18 (C-2), 137.81 (C-3), 177.94 (C-4), 160.86 (C-5), 97.61 (C-6), 165.00 (C-7), 92.11 (C-8), 155.89 (C-9), 105.09 (C-10), 120.64 (C-1′)[a], 115.47 (C-2′)[b], 145.17 (C-3′), 148.75 (C-4′), 115.63 (C-5′)[b], 120.57 (C-6′)[a], 59.57 (MeO-3), 55.95 (MeO-7).

5,7,4′-Trihydroxy-3,8,3′-trimethoxyflavone (**4**). Mp 216–218°; ^{13}C NMR (DMSO-d_6): δ155.86 (C-2)[a], 137.68 (C-3), 177.99 (C-4), 155.09 (C-5)[a], 98.74 (C-6), 156.87 (C-7)[a], 127.45 (C-8), 148.49 (C-9), 104.40 (C-10), 120.90 (C-1′), 111.60 (C-2′), 147.43 (C-3′), 149.75 (C-4′), 115.73 (C-5′), 122.04 (C-6′), 59.60 (MeO-3), 60.87 (MeO-8), 55.48 (MeO-3′).

5,3′-Dihydroxy-3,7,4′-trimethoxyflavone (*ayanin*, **5**). Mp 172–174°; ^{13}C NMR (DMSO-d_6): δ155.59 (C-2)[a], 138.17 (C-3), 178.05 (C-4), 160.89 (C-5), 97.73 (C-6), 165.12 (C-7), 92.24 (C-8), 156.27 (C-9)[a], 105.18 (C-10), 122.14 (C-1′), 115.03 (C-2′), 146.32 (C-3′), 150.28 (C-4′), 111.84 (C-5′), 120.36 (C-6′), 59.69 (MeO-3), 56.03, 55.61 (MeO-7 and MeO-4′).

5,3′-Dihydroxy-3,6,7,4′-tetramethoxyflavone (*casticin*, **6**). Mp 185–187°; ^{13}C NMR (DMSO-d_6): δ151.72 (C-2)[a], 137.95 (C-3), 178.23 (C-4), 151.62 (C-5)[a], 131.57 (C-6), 158.64 (C-7), 91.25 (C-8), 155.57 (C-9), 105.57 (C-10), 122.20 (C-1′), 115.05 (C-2′), 146.34 (C-3′), 150.27 (C-4′), 111.82 (C-5′), 120.34 (C-6′), 59.98, 59.65 (MeO-3 and MeO-6), 56.41, 55.62 (MeO-7 and MeO-4′).

5-Hydroxy-3,6,7,3′,4′-pentamethoxyflavone (*artemetin*, **7**). Mp 160–161°; ^{13}C NMR (DMSO-d_6): δ151.81 (C-2)[a], 138.07 (C-3), 178.28 (C-4), 151.63 (C-5)[a], 131.60 (C-6), 158.73 (C-7), 91.53 (C-8), 155.49 (C-9), 105.64 (C-10), 122.23 (C-1′), 111.58 (C-2′)[b], 148.49 (C-3′), 151.34 (C-4′), 111.28 (C-5′)[b], 122.08 (C-6′), 59.78, 60.06 (MeO-3 and MeO-6), 56.53, 55.68, 55.68 (MeO-7, MeO-3′ and MeO-4′).

5,7-Dihydroxy-3,8,3′,4′-tetramethoxyflavone (**8**). Mp 211–213°; ^{13}C NMR (DMSO-d_6): δ155.90 (C-2), 138.04 (C-3), 178.08 (C-4), 154.80 (C-5), 98.85 (C-6), 157.07 (C-7), 127.51 (C-8), 148.46 (C-9)[a], 104.07 (C-10), 122.32 (C-1′), 111.76 (C-2′)[b], 148.58 (C-3′)[a], 151.23 (C-4′), 110.93 (C-5′)[b], 121.76 (C-6′), 59.73, 60.98 (MeO-3 and MeO-8), 55.66, 55.42 (MeO-3′ and MeO-4′).

5,4′-Dihydroxy-3,6,7-trimethoxyflavone (*penduletin*, **9**). Mp 216–218°; ^{13}C NMR (DMSO-d_6): δ151.73 (C-2)[a], 137.69 (C-3), 178.20 (C-4), 151.65 (C-5)[a], 131.58 (C-6), 158.61 (C-7), 91.35 (C-8), 156.92 (C-9), 105.65 (C-10), 120.44 (C-1′), 130.16 (C-2′), 115.65 (C-3′), 160.33 (C-4′), 115.65 (C-5′), 130.16 (C-6′), 59.64, 60.02 (MeO-3 and MeO-6), 56.45 (MeO-7).

5,3′,4′-Trihydroxy-3,6,7-trimethoxyflavone (*chrysosplenol-D*, **10**). Mp 238–240°; ^{13}C NMR (DMSO-d_6): δ151.68 (C-2), 137.64 (C-3), 178.18 (C-4), 151.68 (C-5), 131.54 (C-6), 158.58 (C-7), 91.20 (C-8), 155.95 (C-9), 105.51 (C-10), 120.73 (C-1′)[a], 115.68 (C-2′)[b], 145.26 (C-3′), 148.82 (C-4′), 115.53 (C-5′)[b], 120.61 (C-6′)[a], 59.61, 60.01 (MeO-3 and MeO-6), 56.41 (MeO-7).

5,7,3′-Trihydroxy-3-methoxyflavone (*isokaemferide*, **11**). Mp >300°; ^{13}C NMR (DMSO-d_6): δ155.06 (C-2)[a], 137.08 (C-3), 177.36 (C-4), 159.63 (C-5), 98.00 (C-6), 163.57 (C-7), 93.20 (C-8), 155.83 (C-9)[a], 103.68 (C-10), 120.04 (C-1′), 129.60 (C-2′), 115.10 (C-3′), 160.71 (C-4′), 115.10 (C-5′). 129.60 (C-6′), 59.15 (MeO-3).

5,7,3′,4′-Tetrahydroxy-3-methoxyflavone (**12**). Mp 252–255°; ^{13}C NMR (DMSO-d_6): δ156.26 (C-2)[a], 137.61 (C-3), 177.82 (C-4), 161.21 (C-5), 98.46 (C-6), 164.02 (C-7), 93.50 (C-8), 155.53 (C-9)[a], 104.13 (C-10), 120.76 (C-1′)[b], 115.36 (C-2′)[c], 145.15 (C-3′), 148.61 (C-4′), 115.68 (C-5′)[c], 120.51 (C-6′)[b], 59.57 (MeO-3).

5,7,4′-Trihydroxy-3,8-dimethoxyflavone (**13**). Mp 235–238°; ^{13}C NMR (DMSO-d_6): δ155.84 (C-2)[a], 137.53 (C-3), 177.94 (C-4), 155.38 (C-5)[a], 98.77 (C-6), 156.96 (C-7)[a], 127.53 (C-8), 148.50 (C-9), 103.93 (C-10), 120.68 (C-1′), 129.88 (C-2′), 115.67 (C-3′), 160.12 (C-4′), 115.67 (C-5′), 129.88 (C-6′), 59.59, 60.87 (MeO-3 and MeO-8).

[a–c]The assignments may be reversed.

4′-Acetyloxy-5-hydroxy-3,7-dimethoxyflavone (**1b**). Mp 163–165°; UV λ_{max}^{MeOH} nm (log ε): 340 sh (4.05), 300 (4.12), 266 (4.40), $\lambda_{max}^{AlCl_3}$ nm: 392, 332, 279, 253. $\lambda_{max}^{AlCl_3-HCl}$ nm: 392, 329, 279, 252. ^1H NMR ($CDCl_3$): δ12.55 (1H, *s*, 5-OH), 8.13 (2H, *d*, *J* = 8.8 Hz, H-2′ and H-6′), 7.26 (2H, *d*, *J* = 8.8 Hz, H-3′ and H-5′), 6.45 (1H, *d*, *J* = 2.2 Hz, H-8), 6.37 (1H, *d*, *J* = 2.2 Hz, H-6), 3.88 (6H, *s*, 3- and 7-MeO), 2.35 (3H, *s*, 4′-OAc).

7,4′-Diacetyloxy-5-hydroxy-3,3′-dimethoxyflavone (**2b**). Mp 168–170°; UV λ_{max}^{MeOH} nm (log ε): 340 (4.09), 308sh (4.03), 267 (4.26), 246 (4.22). $\lambda_{max}^{AlCl_3}$ nm: 393, 332, 278. $\lambda_{max}^{AlCl_3-HCl}$ nm: 399, 329, 278. ^1H NMR ($CDCl_3$): δ12.55 (1H, *s*, 5-OH), 7.73 (1H, *dd*, *J* = 8.3, 2.0 Hz, H-6′), 7.68 (1H, *d*, *J* = 2.0 Hz, H-2′), 7.18 (1H, *d*, *J*

= 8.3 Hz, H-5'), 6.82 (1H, *d*, *J* = 2.0 Hz, H-8), 6.55 (1H, *d*, *J* = 2.0 Hz, H-6), 3.91, 3.88 (3H each, *s*, 3- and 3'-MeO), 2.36, 2.33 (3H each, *s*, 7- and 4'-OAc).

3',4'-*Diacetyloxy*-5-*hydroxy*-3,7-*dimethoxyflavone* (**3b**). Mp 160–162°; UV λ_{max}^{MeOH} nm (log ε): 340 sh (3.97), 300 sh (4.05), 267 (4.34). $\lambda_{max}^{AlCl_3}$ nm: 392, 333, 280, 251. $\lambda_{max}^{AlCl_3-HCl}$ nm: 393, 330, 280. ^{1}H NMR ($CDCl_3$): δ 12.50 (1H, *s*, 5-OH), 8.04 (1H, *dd*, *J* = 8.6, 2.0 Hz, H-6'), 7.97 (1H, *d*, *J* = 2.0 Hz, H-2'), 7.35 (1H, *d*, *J* = 8.6 Hz, H-5'), 6.44 (1H, *d*; *J* = 2.2 Hz, H-8), 6.36 (1H, *d*, *J* = 2.2 Hz, H-6), 3.90, 3.87 (3H each, *s*, 3- and 7-OMe), 2.34, 2.35 (3H each *s*, 3'- and 4'-OAc).

7,4'-*Diacetyloxy*-5-*hydroxy*-3,8,3'-*trimethoxyflavone* (**4b**). Mp 141–143°; UV, λ_{max}^{MeOH} nm (log ε): 350 sh (3.99), 320 (4.04), 271 (4.30), 249 (4.18). $\lambda_{max}^{AlCl_3}$ nm: 412, 338, 281. $\lambda_{max}^{AlCl_3-HCl}$ nm: 417, 333, 282. ^{1}H NMR ($CDCl_3$): δ 12.21 (1H, *s*, 5-OH), 7.82 (1H, *dd*, *J* = 8.4, 2.0 Hz, H-6'), 7.77 (1H, *d*, *J* = 2.0 Hz, H-2'), 7.20 (1H, *d*, *J* = 8.4 Hz, H-5'), 6.54 (1H, *s*, H-6), 3.92, 3.91 (3H and 6H, *s*, 3-, 8- and 3'-OMe), 2.38, 2.36 (3H each, *s*, 7- and 4'-OAc).

7-*Acetyloxy*-5-*hydroxy*-3,8,3',4'-*tetramethoxyflavone* (**8b**). Mp 173–177°; UV λ_{max}^{MeOH} nm (log ε): 356 (4.15), 275 sh (4.09), 252 (4.20). $\lambda_{max}^{AlCl_3}$ nm: 410, 366, 267. $\lambda_{max}^{AlCl_3-HCl}$ nm: 412, 358, 264. ^{1}H NMR ($CDCl_3$): δ 12.32 (1H, *s*, 5-OH), 7.85 (1H, *dd*, *J* = 8.6, 2.1 Hz, H-6'), 7.77 (1H, *d*, *J* = 2.1 Hz, H-2'), 7.01 (1H, *d*, *J* = 8.6 Hz, H-5'), 6.51 (1H, *s*, H-6), 3.97, 3.95, 3.93, 3.88 (3H each, *s*, 3-, 8-, 3'- and 4'-OMe), 2.37 (3H, *s*, 7-OAc).

4'-*Acetyloxy*-5-*hydroxy*-3,6,7-*trimethoxyflavone* (**9b**). Mp 157–159°; UV λ_{max}^{MeOH} nm (log ε): 319 (4.24), 270 (4.40), 247 (4.17). $\lambda_{max}^{AlCl_3}$ nm: 339, 280, 256. $\lambda_{max}^{AlCl_3-HCl}$ nm: 340, 284, 254. ^{1}H NMR ($CDCl_3$): δ 12.50 (1H, *s*, 5-OH), 8.11 (2H, *d*, *J* = 9.0 Hz, H-2' and H-6'), 7.25 (2H, *d*, *J* = 9.0 Hz, H-3' and H-5'), 6.50 (1H, *s*, H-8), 3.95, 3.92, 3.87 (3H each, *s*, 3-, 6- and 7-OMe), 2.34 (3H, *s*, 4'-OAc).

3',4'-*Diacetyloxy*-5-*hydroxy*-3,6,7-*trimethoxyflavone* (**10b**). Mp 144–147°; UV λ_{max}^{MeOH} nm (log ε): 320 (4.22), 271 (4.40), 246 (4.16). $\lambda_{max}^{AlCl_3}$ nm: 342, 282, 255. $\lambda_{max}^{AlCl_3-HCl}$ nm: 340, 285, 254. ^{1}H NMR ($CDCl_3$): δ 12.45 (1H, *s*, 5-OH), 8.04 (1H, *dd*, *J* = 8.6, 2.1 Hz, H-6'), 7.96 (1H, *d*, *J* = 2.1 Hz, H-2'), 7.35 (1H, *d*, *J* = 8.6 Hz, H-5'), 6.50 (1H, *s*, H-8), 3.95, 3.92, 3.89 (3H each, *s*, 3-, 6- and 7-OMe), 2.34, 2.35 (3H each, *s*, 3'- and 4'-OAc).

3',4'-*Diacetyloxy*-3,5,6,7-*tetramethoxyflavone* (**10c**). Mp 137–140°. UV λ_{max}^{MeOH} nm (log ε): 316 (4.18), 261 (4.17). $\lambda_{max}^{AlCl_3}$ nm: 314, 261. ^{1}H NMR ($CDCl_3$): δ 8.03 (1H, *dd*, *J* = 8.8, 1.9 Hz, H-6'), 7.94 (1H, *d*, *J* = 1.9 Hz, H-2'), 7.27 (1H, *d*, *J* = 8.8 Hz, H-5'), 6.73 (1H, *s*, H-8), 3.99, 3.95, 3.89, 3.87 (3H each, *s*, 3-, 5-, 6- and 7-OMe), 2.33, 2.32 (3H each, *s*, 3'- and 4'-OAc).

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