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Dihydroflavonols from *Lannea coromandelica*

Md. Tofazzal Islam[a], Satoshi Tahara[a,b,*]

[a]*Division of Applied Bioscience, Graduate School of Agriculture, Hokkaido University, Kita-ku, Sapporo, 060-8589, Japan*
[b]*CREST, Japan Science and Technology Corporation, Japan*

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Abstract

The dihydroflavonols, (2*R*,3*S*)-(+)-3′,5-dihydroxy-4′,7-dimethoxydihydroflavonol and (2*R*,3*R*)-(+)-4′,5,7-trimethoxydihydroflavonol were isolated from the stem bark of *Lannea coromandelica*, along with the known (2*R*,3*R*)-(+)-4′,7-di-*O*-methyldihydroquercetin, (2*R*,3*R*)-(+)-4′,7-di-*O*-methyldihydrokaempferol and (2*R*,3*R*)-(+)-4′-*O*-methyldihydroquercetin. All five compounds were isolated for the first time from the genus *Lannea*; furthermore, (2*R*,3*S*)-(+)-3′,5-dihydroxy-4′,7-dimethoxydihydroflavonol, was a rare *cis*-type isomer. The structures of all compounds were elucidated by spectroscopic methods including 2D NMR and CD analysis. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: *Lannea coromandelica* L. (Anacardiaceae); *cis*-type Dihydroflavonol, (2*R*,3*S*)-(+)-3′,5-dihydroxy-4′,7-dimethoxydihydroflavonol; (2*R*,3*R*)-(+)-4′,5,7-trimethoxydihydroflavonol; Isolation; Structure elucidation

1. Introduction

Lannea coromandelica L. (Anacardiaceae) is a deciduous tropical tree widely distributed in Bangladesh, India and some other tropical countries. Plants belonging to this genus are used in folk medicine for treatment of elephantiasis, impotence, ulcers, vaginal troubles, halitosis, heart disease, dysentry, gout and rheumatism (Yusuf et al., 1994; Oliver, 1968). Various phytochemicals have been isolated from *Lannea* spp., including quercetin-3-*O*-arabinoside and ellagic acid (Subramanian and Nair, 1971), 6,6-dimethyl-[2,3:7,6]-pyrano-8-(γ,γ-dimethylallyl)flavanone (Sultana and Ilyas, 1986a), rutin and quercetin (Sulochana and Sastry, 1968), and lanceolatin-B and 7,2′-dimethoxy-4′,5′-methylenedioxyflavone (Sultana and Ilyas, 1986b) from the leaves and flowers; phlobatannin and leucocyanidin (Nair et al., 1963), β-sitosterol, physcion and physcion anthranol B from the bark (Subramanian and Nair, 1971); and a ferulic acid ester (Govindachari et al., 1971) from the roots. Two cytotoxic hydroquinones, lanneaquinol and 2′-(*R*)-hydroxylanneaquinol, were recently reported from *L. welwitchii* (Groweiss et al., 1997). We investigated the chemical constituents of *L. coromandelica* bark and isolated five dihydroflavonols (compound **1**–**5**), two of which (**1** and **2**) are novel plant compounds. All the compounds are reported here for the first time from the genus *Lannea*; furthermore, compounds **1** is a rare *cis*-type isomer. Stereostructure determination of naturally occurring *cis*-type dihydroflavonols is described briefly from a historical perspective.

2. Results and discussion

Compound **1** immediately gave an intense purple–pink colour when treated in MeOH solution with granular magnesium and conc. HCl (Shinoda test), indicative of a flavonoid ring system (Dean, 1963). Ultraviolet absorption maxima at 291 and 317 (*sh*) nm suggested that it was a flavanone analogue (Bohm, 1975). An intense molecular ion peak was observed at

* Corresponding author. Tel.: +81-11-706-3840; fax: +81-11-706-4182.

E-mail address: tahara@abs.agr.hokudai.ac.jp (S. Tahara).

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m/z 332 ($[M]^+$, 100%) in the FD MS spectra along with m/z 314 ($[M–H_2O]^+$, 67%). The HR-FD MS spectrum of the compound exhibited exact molecular mass (found, 332.0924 and calcd., 332.0896) corresponding to the molecular formula $C_{17}H_{16}O_7$. Two methoxy singlet peaks were observed at δ 3.82 and 3.84 in the ^{1}H-NMR spectrum (CD_3OD). The UV spectrum was unchanged upon addition of NaOAc, indicating that one of the methoxy groups was at the C-7 position, and a bathochromic shift on the addition of $AlCl_3$ suggested the presence of a chelated hydroxyl group *peri* to the C-4 carbonyl (Mabry et al., 1970). In the ^{1}H-NMR spectrum of **1**, signals at δ 5.75 and 5.03 (each 1H, *d*, J = 5.0 Hz) could be assigned to H-2 and H-3, respectively, the coupling constant (J = 5.0 Hz) indicated a *cis*-configuration (Ingham et al., 1986). The ^{13}C-NMR spectral data (CD_3OD) [δ 82.2 (C-2) and 77.8 (C-3)] also clearly indicated that **1** was a dihydroflavonol (C-3-OH) (Kasai et al., 1988). The two remaining substitutents, one hydroxyl and one methoxy group, were located at C-3′ and C-4′, respectively, based on the spectral features of the H-2′ (doublet at δ 6.87 coupled to H-6′ with J = 2.3 Hz), H-5′ (doublet at δ 6.87 coupled to H-2′ with J = 8.3 Hz), H-6′ (double doublets at δ 6.79 coupled to H-2′ and H-5′ with J = 8.3 and 2.3 Hz) and, H-6 and H-8 (AB system centered at δ 6.03 and 6.13, $J_{6,8}$ = 2.3 Hz) resonances. In the EI MS spectrum, a peak at m/z 330 $[M-2]^+$ may be explained by the thermal loss of H_2. The base peak at m/z 314 was due to the loss of water from the molecule. The retro-Diels-Alder (RDA) fragmentation, which gave rise to ions m/z 167 (16%) from the A-ring and 166 (4%) from the B-ring, was in accordance with the above deduction. The locations of the substituents were unambiguously deduced by NOE measurements (Fig. 1). HMBC and HMQC correlations (Table 1) supported the suggestion that compound **1** is a *cis*-isomer of 3′,5-dihydroxy-4′,7-dimethoxydihydroflavonol. The absolute configuration of this compound was confirmed as (2*R*,3*S*) by comparison of its CD curve with those of reported *cis*-type dihydroflavonols (Nonaka et al., 1987) (Table 2). Thus, the structure of the first isolate is confirmed as

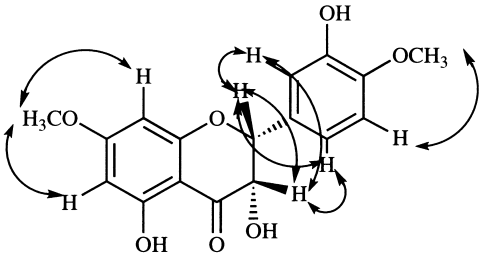


Fig. 1. NOE correlations observed in the NOESY spectrum of **1**.

(2*R*,3*S*)-(+)-3′,5-dihydroxy-4′,7-dimethoxydihydroflavonol (**1**), a new natural product.

In the FD MS spectrum, compound **2** showed an intense molecular ion peak at m/z 330 (M^+ 100%). The HR-EI MS spectrum of the compound exhibited the exact molecular mass (found, 330.1073 and calcd., 330.1103) corresponding to the formula $C_{18}H_{18}O_6$. The ^{1}H-NMR data were also consistent with the deduced formula. Compound **2** also immediately responded to Shinoda test to afford an intense purple–pink colour indicative of a flavonoid ring system. The UV λ_{max} at 283 and 310 (*sh*) nm were closely related to dihydroflavonol (Bohm, 1975). Compound **2** showed a white–blue florescence under UV light characteristic of 5-methoxy flavonoids. The EI MS spectrum showed a fragment indicating the loss of HCO, m/z 301 (88%) and the base peak was obtained by RDA fragmentation, giving rise to ions at m/z 181 (100%) from dimethoxylated A-ring and 150 (24%) from monomethoxylated B-ring. The ^{1}H-NMR spectrum exhibited the typical AX system of H-2 and H-3 at δ 5.01 (H-2) and 4.43 (H-3, $J_{2,3}$ = 11.9 Hz). The magnitude of the coupling constant ($J_{2,3}$ = 11.9 Hz) indicated a *trans*-type dihydroflavonol (Clark-Lewis, 1968). Among the three substituents ($3 \times OCH_3$), one methoxy group was allocated to C-4′ position because of the signal features H-3′ and H-5′ at δ 7.00 (2H, *d*-like, J = 8.8), and H-2′ and H-6′ at δ 7.52 (2H, *d*-like, J = 8.8). The remaining two aromatic protons, H-6 and H-8, were resonating at δ 6.15 and 6.25, (each 1H, *d*, J = 2.3 Hz). Since the UV spectrum in methanol was unaltered on addition of NaOAc and $AlCl_3$, two of the three methoxy groups were allocated to C-7 and C-5 positions (Mabry et al., 1970). The B-ring mass fragment from **2** and the ^{1}H-NMR spin patterns for B-ring protons were in agreement with those of known compound **4**, where only a methoxy group was located on C-4′. Compound **2** was therefore deduced to be 4′,5,7-trimethoxydihydroflavonol. The absolute configuration of **2** was determined to be (2*R*,3*R*) by comparison of its CD curve with those of reported compounds (Nonaka et al., 1987) (Table 2). Thus, the structure of **2** was elucidated as (2*R*,3*R*)-(+)-4′,5,7-trimethoxydihydroflavonol, which has not been reported as a natural product. The 4′,5,7-trimethoxydihydroflavonol (*trans*-type, racemate) was synthesized by Guider et al. (1955) and van der Westhuizen et al. (1980). Later, Takahashi and co-workers stereoselectively synthesized (2*R*,3*R*)-(−)-4′,5,7-trimethoxydihydroflavonol in 1988 (Takahashi et al., 1988).

Compound **3**, $C_{17}H_{16}O_7$ from the HR-EI MS spectrum, differed from **1** only in the H-2 to H-3 coupling constant ($J_{2,3}$ = 11.6 Hz) in ^{1}H-NMR and in the chemical shift values of respective carbon in ^{13}C-NMR, indicating that it was the *trans*-isomer of **1**. All the spectral data of **3** were reasonably matched with

Table 1
^{1}H- and ^{13}C-NMR assignments and HMBC correlations of compound **1**[a]

Carbon number	Chemical shift of carbon δ (ppm) (CD_3OD)	Chemical shift of proton, δ (ppm) & J (Hz) (DMSO)	HMBC correlation H → C
2	82.2	6.03, *d* (2.2)	C-4, C-1′, C-9
3	77.8	4.80, *d* (2.2)	C-4
4	191.6	–	–
5	163.3	11.47, *s* (OH)	–
6	95.8	6.16, *d* (2.3)	C-8
7	170.3	3.80, *s* (OCH_3)	C-7
8	95.0	6.29, *d* (2.3)	C-6, C-9
9	165.4	–	–
10	103.6	–	–
1′	129.3	–	–
2′	119.4	6.77, *d* (2.0)	C-4′
3′	147.8	9.28, *s* (OH)	-
4′	149.3	3.91, *s* (OCH_3)	C-4′
5′	112.7	6.95, *d* (8.3)	C-1′, C-3′
6′	114.8	6.67, *dd* (8.3 & 2.0)	C-4′
2 × OCH_3	57.0, 56.9	–	–

[a] Carbon assignments are based on HMQC and HMBC data.

these reported for (2*R*,3*R*)-dihydroquercetin-4′,7-di-*O*-methyl ether (Ruangrungsi et al., 1981). The structure of **3** was confirmed by analysis of its 1D and 2D NMR spectra (^{1}H–^{1}H COSY, HMBC, HMQC and NOESY), and the absolute configuration was confirmed by analysis of its CD spectra (Table 2). Thus, the structure of **3** was elucidated as (2*R*,3*R*)-(+)-4′,7-di-*O*-methyldihydroquercetin, which has previously been found in the leaves of *Blumea balsamifera* (Compositae) (Ruangrungsi et al., 1981).

The molecular formula of compound **4** was deduced from the HR-EI MS spectrum to be $C_{17}H_{16}O_6$. The characterization of **4** by ^{1}H-NMR, EI MS, ^{13}C-NMR, and 2D NMR (^{1}H–^{1}H COSY, HMBC, HMQC and NOESY), and CD measurement (Table 2) elucidated its structure as (2*R*,3*R*)-(+)-4′,7-di-*O*-methyldihydrokaempferol, which was previously isolated from *Cephalanthus spathelliferus* (Rubiaceae) (Lima and Polonsky, 1973) and the epicuticle of *Haplopappus baylahuen* (Compositae) (Nuñez-Alarcon et al., 1993) as well as some other plant sources (Rossi et al., 1997).

Compound **5**, with a molecular formula of $C_{16}H_{14}O_7$ as deduced from the HR-EI MS spectrum, exhibited the typical AX system of H-2 and H-3 in its ^{1}H-NMR spectrum, with signals at δ 5.07 (H-2, $J_{2,3}$ = 11.5 Hz) and 4.64 (H-3, *dd*, $J_{2,3}$ = 11.5 Hz and $J_{3,OH}$ = 4.3 Hz), where H-3 signal was split further by coupling with the C-3-OH proton resonating at δ 4.70 ($J_{3,OH}$ = 4.3 Hz). The large coupling constant ($J_{2,3}$ = 11.5 Hz) showed a *trans*-diaxial relationship and indicated a dihydroflavonol structure for **5**. Its B-ring proton and carbon signals were similar to the respective signals of compound **3**. The substitution of two remaining OH-groups at C-5 and C-7 was confirmed by UV spectrum (bathochromic shifts with NaOAc and $AlCl_3$). The absolute configuration of **5** was confirmed as (2*R*,3*R*) by CD measurement (Table 2). Thus, the structure of compound **5** is represented by (2*R*,3*R*)-(+)-4′-*O*-methyldihydroquercetin. This compound was previously found in the leaves of *Blumea balsamifera* along with compound **3** (Ruangrungsi et al., 1981)

Table 2
CD data and absolute configurations of *Lannea coromandelica* dihydroflavonols

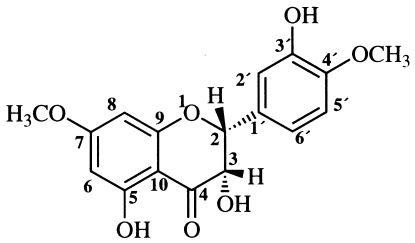
Compound (g/100 ml MeOH)	Reading	Absolute configuration
1 (0.0190)	$[\theta]_{289}$ 0, $[\theta]_{300}$ − 10100, $[\theta]_{310}$ 0, $[\theta]_{334}$ + 12400, $[\theta]_{386}$ 0	2*R*,3*S*
2 (0.0015)	$[\theta]_{282}$ 0, $[\theta]_{288}$ − 18000, $[\theta]_{316}$ 0, $[\theta]_{332}$ + 9000, $[\theta]_{381}$ 0	2*R*,3*R*
3 (0.0397)	$[\theta]_{270}$ 0, $[\theta]_{295}$ − 38100, $[\theta]_{317}$ 0, $[\theta]_{333}$ + 10500, $[\theta]_{386}$ 0	2*R*,3*R*
4 (0.0371)	$[\theta]_{274}$ 0, $[\theta]_{300}$ − 17400, $[\theta]_{313}$ 0, $[\theta]_{332}$ + 8460, $[\theta]_{380}$ 0	2*R*,3*R*
5 (0.0250)	$[\theta]_{277}$ 0, $[\theta]_{294}$ − 31600, $[\theta]_{313}$ 0, $[\theta]_{330}$ + 9090, $[\theta]_{379}$ 0	2*R*,3*R*
(+)-Taxifolin[a] (0.0397)	$[\theta]_{274}$ 0, $[\theta]_{297}$ − 4280, $[\theta]_{318}$ 0, $[\theta]_{332}$ + 901, $[\theta]_{375}$ 0	2*R*,3*R*
(−)-Taxifolin[a] (0.0920)	$[\theta]_{274}$ 0, $[\theta]_{297}$ + 4020, $[\theta]_{318}$ 0, $[\theta]_{332}$ − 977, $[\theta]_{375}$ 0	2*S*,3*S*
(+)-Epitaxifolin[a] (0.0658)	$[\theta]_{268}$ 0, $[\theta]_{295}$ + 3190, $[\theta]_{322}$ 0, $[\theta]_{341}$ − 1520, $[\theta]_{378}$ 0	2*S*,3*R*
(−)-Epitaxifolin[a] (0.0789)	$[\theta]_{268}$ 0, $[\theta]_{295}$ − 4180, $[\theta]_{322}$ 0, $[\theta]_{341}$ + 2030, $[\theta]_{378}$ 0	2*R*,3*S*

[a] Nonaka et al. (1987).

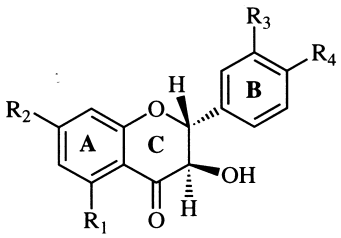
The ^{1}H- and ^{13}C-NMR data of known compounds **3**, **4** and **5** were previously measured using low resolution instruments. Moreover, ^{13}C-NMR data of **3** and **5** were not reported earlier. Hence, we have presented the spectroscopic data for compounds **3**, **4** and **5**.

The substitution pattern of 4′-OCH_3 and 3′-OH in B-ring in *L. coromandelica* dihydroflavonols (**1**, **3** and **5**) is an isoferulic acid-type. Owing to the asymmetric carbons C-2 and C-3, dihydroflavonols can occur in four stereoisomeric forms. However, in nature, they occur predominantly as the 2,3-*trans* isomers with the C-2 phenyl and C-3 hydroxyl group in equatorial positions (Bohm, 1975); 2,3-*cis*-type isomers are rare in nature. Clark-Lewis et al. (1968) first synthesized 2,3-*cis*-analogues (*cis*-3-methoxyflavanone) by the oxidation of a flavan-3,4-diol. They discussed the relative thermodynamic stability of *cis*- and *trans*-dihydroflavonols and suggested that, owing to hydrogen bonding between the carbonyl group and the equatorial 3-hydroxyl group, the 2,3-*trans* isomer should be favored. The CD, optical rotatory dispersion and absolute configuration of some *cis*- and *trans*-dihydroflavonols were studied by Gaffield (1970). The isolation of a racemic 2,3-*cis*-dihydroflavonol (3-*O*-methyl-2,3-*cis*-fustin) from the heartwood of *Trachylobium verrucosum* (Gaertn.) Oliv. was claimed as early as 1972 (Merwe et al., 1972). A 7-*O*-glycoside of (−)-*cis*-5,7-dihydroxy-3′-methoxy-2,3-*cis*-dihydroflavonol was first isolated in pure form from the peelings of *Citrus junos* (Kumamoto et al., 1985). The racemic *cis*-dihydroflavonol was also reported (Moriarty and Prakash, 1985) as a synthetic product from the oxidation of flavanone by hypervalent iodine. In 1986, Ingham and co-workers isolated a 2,3-*cis*-dihydroflavonol, shuterone B, from the fungus-inoculated leaflets of *Shuteria vestita* (Ingham et al., 1986), and Foo (1986) isolated 3′,4′,7,8-tetrahydroxydihydroflavonol from *Acacia melanoxylon*. The latter compound was suggested to be the precursor of proanthocyanidins with 2,3-*cis* flavanol units previously found in the same plant (Foo, 1987). The absolute configuration of shuterone B was established as 2*S*,3*R*; a year later, the absolute stereochemistry of 3′,4′,7,8-tetrahydroxydihydroflavonol was confirmed as 2*R*,3*S* (Foo, 1987). In 1987, Nonaka et al. reported a group of dihydroquercetin 3-*O*-β-xylopyranosides, namely (2*R*,3*R*)-(+)-and (2*S*,3*S*)-(−)-taxifolin 3-*O*-xylosides, and (2*S*,3*R*)-(+)- and (2*R*,3*S*)-(−)-epitaxifolin 3-*O*-xylosides, from *Thujopsis dolobrata* (Cupressaceae). They rigorously determined the absolute configurations of all isolates together with those of the corresponding aglycones, and their CD patterns were correlated with the stereostructures. One year later, Kasai et al. (1988) isolated four diastereomers of dihydroquercetin 3-*O*-α-rhamnosides from the leaves of *Engelhardtia chrysolepis* (Juglandaceae) and deduced their absolute configurations. The co-occurence of *cis* and *trans* isomers of a dihydroflavonol in *Pinus sylvestris* has been reported by Lundgren and Theander (1988). Clearly, few *cis*-type dihydroflavonols of natural origin are yet known.

The biosynthetic significance of the occurrence of 2,3-*cis*-dihydroflavonol **1** in *Lannea coromandelica* is not clear at present due to lack of phytochemical data on this plant. Therefore, detailed phytochemical investigations on plants of the *Lannea* genus are necessary in order to formulate the possible biosynthetic pathway of a *cis*-type isomer.



1: (2*R*,3*S*)-(+)-3′,5-dihydroxy-4′,7-dimethoxydihydroflavonol



(2*R*, 3*R*)-dihydroflavonols)

2: R_1=OCH_3, R_2=OCH_3, R_3=H R_4=OCH_3
3: R_1=OH, R_2=OCH_3, R_3=OH, R_4=OCH_3
4: R_1=OH, R_2=OCH_3, R_3=H R_4=OCH_3
5: R_1=OH, R_2=OH, R_3=OH, R_4=OCH_3

3. Experimental

3.1. General procedures

Mp.s were uncorrected. Instrumental analyses were conducted using a JEOL JMS-AX 500 (EI) and JEOL JMS-SX102A (FD) for mass spectrometry, a HITACHI U-3210 spectrophotometer for UV spectrometry, a JEOL JNM-EX 270 for 1D (^{1}H- and ^{13}C-) NMR spectrometry, a JASCO Model J-20 spectrometer for CD, a digital polarimeter JAS CO DIP-370 for specific rotation and a Bruker AMX500 for 2D (^{1}H–^{1}H COSY, NOESY, HMQC and HMBC) NMR spectrometry. TMS was used as the internal standard in NMR spectrometry. Gibbs and vanillin reagents were used to detect dihydroflavonols on TLC plates. The

Shinoda test was used to confirm the dihydroflavonol skeleton. Analytical and prep. TLC separations were carried out on thin layer plates (Merck Kieselgel 60 F_{254}, 0.25 mm thickness) using $CHCl_3$/MeOH (25:1, solvent A; 25:2, solvent B & 25:4, solvent C). Silica gel 60 (spherical, 100–210 μm) (SiO_2, Kanto Chemical), Sephadex® LH-20 (Pharmacia) and RP-18 (Cosmosil 75C_{18}-OPN, Nacalai, Tesque) were used for open column chromatography.

3.2. Plant material

The stem bark of *L. coromandelica* was collected in March 1998 in Comilla, Bangladesh and identified by M. Iqbal Hossain, BADC, Bangladesh. Voucher specimens are on deposit at the National Herbarium, Dhaka, Bangladesh. The material was then air dried and coarsely powdered.

3.3. Extraction and isolation

The dried powdered stem bark (1.9 kg) of *L. coromandelica* was extracted with acetone (280 g) and successively fractionated according to the solubility in *n*-hexane (1.5 g), diethyl ether (3.5 g), ethyl acetate (19.0 g) and methanol (255.0 g). The methanol solubles (70 g) were subjected to column chromatography (CC) on silica gel (1.45 kg) eluting with *n*-hexane–$CHCl_3$–MeOH with increasing amounts of $CHCl_3$ and MeOH to give 11 fractions. Fraction 8 (eluting solvent: $CHCl_3$–MeOH = 70: 30, 500 ml; solute, 800 mg) was further chromatographed on silica gel (16 g) eluting with $CHCl_3$–MeOH (50:1) followed by prep. TLC to give (2*R*,3*S*)-(+)-3′,5-dihydroxy-4′,7-dimethoxydihydroflavonol (**1**) (85 mg) (eluate from 650 to 850 ml), (2*R*,3*R*)-(+)-4′,7-di-*O*-methyldihydroquercetin (**3**) (15 mg) (eluate from 350 to 550 ml), and (2*R*,3*R*)-(+)-4′,7-di-*O*-methyldihydrokaempferol (**4**) (20 mg) (eluate from 150 to 350 ml).

One gram of methanol solubles was subjected to Sephadex LH-20 CC eluting with MeOH to give four fractions. Fraction 3 (eluate from 500 to 800 ml, 366 mg) was further rechromatographed by reverse phase (RP-18) open CC eluating with 50% aq. MeOH followed by prep. TLC to give (2*R*,3*R*)-(+)-4′,5,7-trimethoxydihydroflavonol (**2**) (2.0 mg) and (2*R*,3*R*)-(+)-4′-*O*-methyldihydroquercetin (**5**) (2.5 mg), and additional **3** (8.0 mg) and **4** (2.5 mg).

3.4. (2R,3S)-(+)-3′,5-dihydroxy-4′,7-dimethoxydihydroflavonol (1)

Colourless powder from MeOH; mp 183.5–184°; under UV light, quenched (UV_{254}, short wave) and dark (UV_{365}, long wave); R_f 0.02 (Solvent B) and 0.13 (Solvent C); $[\alpha]_D^{24}$ + 156° (MeOH: *c* 0.2); CD data (Table 2), UV λ_{max} MeOH: 231 (*sh*), 291, 317 (*sh*); +NaOMe: 238 (*sh*), 292, 364; +$AlCl_3$: 224, 293 (*sh*), 316, 381; +$AlCl_3$/HCl: 225, 292 (*sh*), 317, 388; +NaOAc: 291, 328 (*sh*), FDMS: *m*/*z* 332 ($[M]^+$ 100), 314 ($[M-H_2O]^+$, 67); HR-FD MS: $[M]^+$ 332.0924, calcd. for $C_{17}H_{16}O_7$ 332.0896. EIMS (*m*/*z*): 332 ($[M]^+$, 2), 330 ($[M-2H]^+$, 34), 314 ($[M-H_2O]^+$, 100), 285 (19), 271 (22), 243 (16), 167 (16), 166 (4), 151 (4), 143 (6), 138 (5), 137 (3), 133 (7), 105 (5), 44 (8), 40 (8), ^{1}H-NMR spectral data (270 MHz, CD_3OD): δ 6.87 (1H, *d*, *J* = 8.3 Hz, H-5′), 6.83 (1H, *d*, *J* = 2.3 Hz, H-2′), 6.79 (1H, *dd*, *J* = 8.3 & 2.3 Hz, H-6′), 6.13 (1H, *d*, *J* = 2.3 Hz, H-8), 6.03 (1H, *d*, *J* = 2.3 Hz, H-6), 5.75 (1H, *d*, *J* = 5.0 Hz, H-2), 5.03 (1H, *d*, *J* = 5.0 Hz, H-3), 3.82 and 3.84 (each 3H, *s*, 2 × OCH_3), ^{1}H-NMR spectral data (270 MHz, acetone-d_6): δ 11.65 (1H, *s*, 5-OH), 7.71 (1H, *s*, 3′-OH), 6.89 (1H, *d*, *J* = 8.3 Hz, H-5′), 6.84 (1H, *d*, *J* = 2.1 Hz, H-2′), 6.77 (1H, *dd*, *J* = 8.3 and 2.1 Hz, H-6′), 6.14 (1H, *d*, *J* = 2.1 Hz, H-8), 6.10 (1H, *d*, *J* = 3.2 Hz, H-2), 6.00 (1H, *d*, *J* = 2.1 Hz, H-6), 5.02 (1H, *d*, *J* = 3.2 Hz, H-3), 3.80 and 3.88 (each 3H, *s*, 2 × OCH_3); ^{1}H-NMR (DMSO) and ^{13}C-NMR spectral data: see Table 1.

3.5. (2R,3R)-(+)-4′,5,7-trimethoxydihydroflavonol (2)

Colourless powder from EtOAc; under UV light, quenched (short wave) and white blue florescence (long wave); R_f 0.66 (Solvent A); $[\alpha]_D^{24}$ + 30° (MeOH: *c* 0.2) (Takahashi et al., 1988, $[\alpha]_D$ − 12.9°, $CHCl_3$, *c* 1.25); CD data (Table 2), UV λ_{max} MeOH: 228, 283, 310 (*sh*); +NaOMe: 227 (*sh*), 282, 310 (*sh*); +$AlCl_3$: 221, 283, 315, 348 (*sh*); +NaOAc: 237 (*sh*), 285, 310 (*sh*), FD MS: *m*/*z* 330 ($[M]^+$, 100), HR-EIMS: $[M]^+$ 330.1073, calcd. for $C_{18}H_{18}O_6$ 330.1103, EIMS (*m*/*z*): 330 ($[M]^+$, 15), 301 (88), 193 (20), 181 (100), 167 (10), 150 (24), 148 (8), 121 (28), 77 (5), 40 (5), ^{1}H-NMR spectral data (acetone-d_6, 270 MHz): δ 7.52 (2H, *d*-like, *J* = 8.8 Hz, H-2′, H-6′), 7.00 (2H, *d*-like, *J* = 8.8 Hz, H-3′, H-5′), 6.25 (1H, *d*, *J* = 2.3 Hz, H-8), 6.15 (1H, *d*, *J* = 2.3 Hz, H-6), 5.01 (1H, *d*, *J* = 11.9 Hz, H-2), 4.43 (1H, *dd*, *J* = 11.9 & 3.6 Hz, H-3), 4.29 (1H, *d*, *J* = 3.6 Hz, 3-OH), 3.87, 3.84 and 3.82 (each 3H, *s*, 3 × OCH_3).

3.6. (2R,3R)-(+)-4′,7-di-O-methyldihydroquercetin (3)

Colourless needles from EtOAc; mp 184–186° (MeOH) (Ruangrungsi et al., 1981, 164–167°); under UV light, quenched (short wave) and yellow (long wave); R_f 0.56 (Solvent B), $[\alpha]_D^{29}$ + 32.8° (MeOH: *c* 0.0148) (Ruangrungsi et al., 1981, $[\alpha]_D$ + 14.8°); CD data (Table 2), UV λ_{max} MeOH: 228 (*sh*), 289, 324 (*sh*); +NaOMe: 240 (*sh*), 290, 359; +$AlCl_3$: 224, 293 (*sh*), 315, 373; +$AlCl_3$/HCl: 223, 290 (*sh*), 314, 382;

+NaOAc: 289, 323 (*sh*), FD MS (*m/z*): 332 ($[M]^+$, 100); HR-EI MS: $[M]^+$ 332.0883, calcd. for $C_{17}H_{16}O_7$ 332.0896, EI MS data were identical with literature values (Ruangrungsi et al., 1981). ^{1}H-NMR spectral data (acetone-d_6, 270 MHz): δ 11.69 (1H, *s*, 5-OH), 7.72 (1H, *s*, 3′-OH), 7.09 (1H, *d*, J = 1.9 Hz, H-2′), 7.03 (1H, *dd*, J = 8.3 and 1.9 Hz, H-6′), 6.99 (1H, *d*, J = 8.3 Hz, H-5′), 6.09 (1H, *d*, J = 2.3 Hz, H-8), 6.06 (1H, *d*, J = 2.3 Hz, H-6), 5.10 (1H, *d*, J = 11.6 Hz, H-2), 4.77 (1H, *d*, J = 4.3 Hz, 3-OH), 4.69 (1H, *dd*, J = 11.6 and 4.3 Hz, H-3), 3.88 (3H, *s*, 4′-OCH_3), 3.87 (3H, *s*, 7-OCH_3); ^{13}C-NMR spectral data (acetone-d_6, 270 MHz): δ 198.6 (C-4), 169.3 (C-7), 164.7 (C-9), 164.0 (C-5), 148.9 (C-4′), 147.3 (C-3′), 131.0 (C-6′), 120.5 (C-2′), 115.5 (C-5′), 112.0 (C-1′), 102.1 (C-10), 95.8 (C-6), 94.7 (C-8), 84.5 (C-2), 73.2 (C-3), 56.4 and 56.3 ($2 \times OCH_3$).

3.7. (2*R*,3*R*)-(+)-4′,7-di-*O*-methyldihydrokaempferol (**4**)

Colourless needles from EtOAc; mp 185–186° (Nuñez-Alarcon et al., 1993, 84–186°); under UV light, quenched (short wave) and yellow (long wave); R_f, 0.89 (Solvent B), $[\alpha]_D^{24}$ + 34.4° (MeOH, *c* 0.4575) (Lima and Polonsky, 1973, $[\alpha]_D$ + 89°, pyridine, *c* 1), CD data (Table 2), UV λ_{max} MeOH: 217, 290, 324 (*sh*); +NaOMe: 244 (*sh*), 288, 359; +$AlCl_3$: 225, 316, 384; +$AlCl_3$/HCl: 225, 314, 386; +NaOAc: 290, 326 (*sh*), FDMS *m/z* (%): 316 ($[M]^+$, 100); HR-EIMS: $[M]^+$ 316.0916, calcd. for $C_{17}H_{16}O_6$ 316.0947, ^{1}H-NMR spectral data (270 MHz, acetone-d_6): δ 11.67 (1H, *s*, 5-OH), 7.52 (2H, *d*-like, J = 8.7 Hz, H-2′, H-6′), 7.00 (2H, *d*-like, J = 8.7 Hz, H-3′, H-5′), 6.09 (1H, *d*, J = 2.3 Hz, H-8), 6.06 (1H, *d*, J = 2.3 Hz, H-6), 5.16 (1H, *d*, J = 11.6 Hz, H-2), 4.75 (1H, *d*, J = 3.6 Hz, 3-OH), 4.70 (1H, *dd*, J = 11.6 Hz and 3.6 Hz, H-3), 3.86 (3H, *s*, 4′-OMe), 3.84 (3H, *s*, 7-OMe), EI MS and ^{13}C-NMR data were identical with literature values (Rossi et al., 1997).

3.8. (2*R*,3*R*)-(+)-4′-*O*-methyldihydroquercetin (**5**)

Colourless amorphous powder from EtOAc; mp 181.5–182.5° (Ruangrungsi et al., 1981, 173–174°); under UV light, quenched (short wave) and dark (long wave); R_f, 0.32 (Solvent A). $[\alpha]_D$ + 20.4° (MeOH, *c* 0.75) (Ruangrungsi et al., 1981, $[\alpha]_D$ + 14.9°); CD data (Table 2), UV λ_{max} MeOH: 289, 332 (*sh*); +NaOMe: 246, 326; +$AlCl_3$: 271 (*sh*), 315, 385; +$AlCl_3$/HCl: 292 (sh), 314, 375; +NaOAc: 286 (*sh*), 327; +NaOAc/H_3BO_3: 291, 325 (*sh*), FD MS *m/z* (%): 318 ($[M]^+$, 100); HR-EIMS calcd. for $C_{16}H_{14}O_7$ $[M]^+$ 318.0739, found, 318.0704, EI MS data were identical with literature values (Ruangrungsi et al., 1981), ^{1}H-NMR spectral data (270 MHz, acetone-d_6): δ 11.71 (1H, *s*, 5-OH), 7.70 (1H, *s*, 3′-OH), 7.09 (1H, *d*, J = 2.0 Hz, H-2′), 7.03 (1H, *dd*, J = 8.3 and 2.0 Hz, H-6′), 6.98 (1H, *d*, J = 8.3 Hz, H-5′), 6.00 (1H, *d*, J = 2.3 Hz, H-8), 5.96 (1H, *d*, J = 2.3 Hz, H-6), 5.07 (1H, *d*, J = 11.5 Hz, H-2), 4.70 (1H, *br s*, 3-OH), 4.64 (1H, *br d*, J = 11.5 Hz, H-3), 3.88 (3H, *s*, 4′-OCH_3), ^{13}C-NMR spectral data (270 MHz, acetone-d_6): δ 198.2 (C-4), 167.8 (C-7), 165.7 (C-9), 164.1 (C-5), 148.9 (C-4′), 147.3 (C-3′), 131.1 (C-6′), 120.5 (C-2′), 115.5 (C-5′), 112.0 (C-1′), 101.6 (C-10), 97.1 (C-6), 96.1 (C-8), 84.2 (C-2), 73.2 (C-3), 56.3 (OCH_3).

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