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Biflavonoids. Part 4[1*]. Structure and Stereochemistry of Novel Flavanone- and the First Isoflavanone-benzofuranone Biflavonoids

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Abstract: The structure and stereochemistry of (2*R*,3*S*)-naringenin-(3α→5)-(2*R*)-maesopsin, its isoflavanone analogue, (2*S*,3*R*)-dihydrogenistein-(2α→7)-(2*R*)-maesopsin, and the 2S (F-ring) diastereomers of both compounds from the heartwood of *Berchemia zeyheri* were established by ^{1}H NMR and CD data. The isoflavanone analogues represent the first isoflavanone-benzofuranone biflavonoids.

Keywords: Biflavonoids; Biogenesis; Isoflavanone; Benzofuranone; Circular Dichroism

INTRODUCTION

Our first reports[2,3] on the phenolic constituents of the red heartwood of *Berchemia zeyheri* revealed the presence of a flavanone-benzofuranoid type dimer, (2*R*,3*S*)-naringenin-(3α→7)-(2*R*)-maesopsin (zeyherin)[4] and its 2-C(F) diastereomer, (2*R*,3*S*)-naringenin-(3α→7)-(2*S*)-maesopsin. The collective utilization of chemical degradation, ^{1}H NMR, CD and computational data not only defined the structures but permitted estimation of the absolute stereochemistry of the zeyherin diastereomers as well as related structures from the same source.[2,3]

Continued investigation has now revealed the existence of a second diastereomeric pair of regio-isomers, (2*R*,3*S*)-naringenin-(3α→5)-(2*R*)-maesopsin **1** and (2*R*,3*S*)-naringenin-(3α→5)-(2*S*)-maesopsin **2** as well as a unique pair of related diastereomers, (2*S*,3*R*)-dihydrogenistein-(2α→7)-(2*R*)-maesopsin **5** and (2*S*, 3*R*)-dihydrogenistein-(2α→7)-(2*S*)-maesopsin **6**, representing the first natural isoflavanone-benzofuranoid oligomers.[1]

RESULTS AND DISCUSSION

The complex mixture of polyphenolic compounds[1-5] from the heartwood of *B. zeyheri* was extensively

* Part 3 is ref. 1, Part 2 is ref. 3 and Part 1 is ref. 2.

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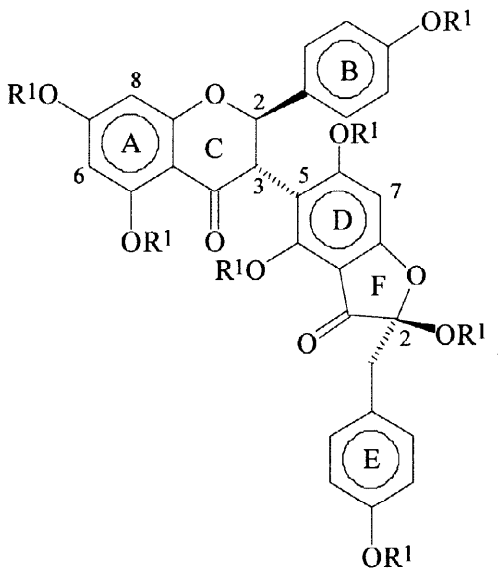
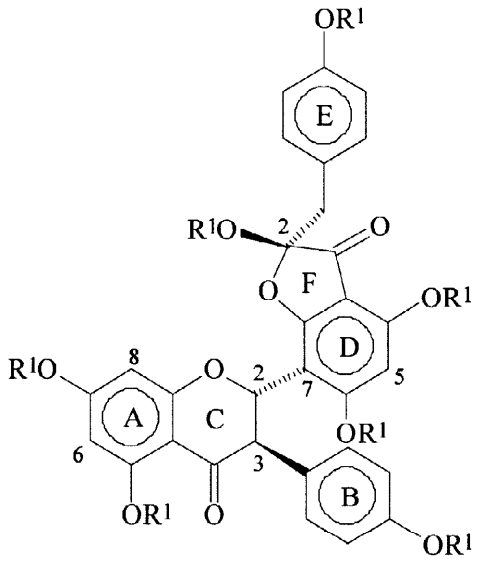
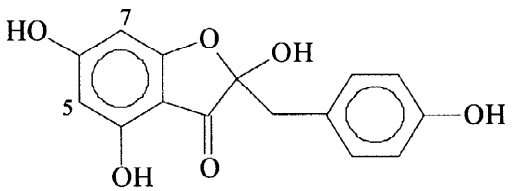
fractionated and chromatographed into various fractions.[1,5] These fractions still comprised mixtures and, following confirmation of the absence of natural methoxy groups by ^{1}H NMR, were methylated with dimethyl sulfate under rigorously anhydrous conditions. The additional chromatographic step offered by derivatization was a prerequisite for sample purity.



1 R^1=H
2 R^1=H, C-2(F) epimer
3 R^1=Me
4 R^1=Me, C-2(F) epimer



5 R^1=H
6 R^1=H, C-2(F) epimer
7 R^1=Me
8 R^1=Me, C-2(F) epimer



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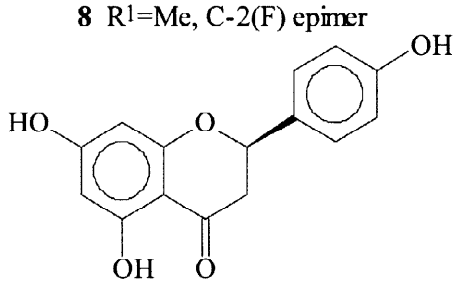

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(2*R*,3*S*)-Naringenin-(3α→5)-(2*R*)-maesopsin **1** and its 2-C(F) diastereomer **2** were purified as the yellow amorphous permethylaryl ethers **3** and **4**. Their ^{1}H NMR spectra exhibit the typical effects of slow dynamic rotational isomerism about the interflavonoid bond at ambient temperature. At elevated temperature (70°C), however, the close structural relationships with the naringenin-(3α→7)-maesopsin diastereomers[2,3] become obvious and the presence of a tetra-*O*-methylmaesopsin unit coupled with a tri-*O*-methylnaringenin moiety can clearly be discerned from the ^{1}H NMR data.

The ^{1}H NMR spectra of both diastereomers **3** and **4** exhibit the characteristic signals of the naringenin unit substituted at 3-C(C), resulting in an AM-spin system associated with 2-H(C) (δ 5.51, 5.65 for **3** and **4**, resp.) and 3-H(C) (δ 4.47, 4.56 for **3** and **4**, resp.; $^3J_{2,3}$ = 12.0 Hz for both), an aromatic AB-system (J_{AB} = 2.0

Hz for both) representing the A-ring, and an AA'BB'-spinsystem (J_{AB} = 9.0 Hz for both) originating from the *para*-substituted B-ring. NOE associations between 2-H(C) and 2/6-H(B) (δ 7.02, 7.15 for **3** and **4**, resp.)

Table : ^{1}H NMR chemical shifts (δ_H) for biflavonoid derivatives **3**, **4**, **7** and **8**. Multiplicities and coupling constants (Hz) are given in parenthesis.

Ring	Proton	**3**	**4**	**7**	**8**
A	6-H [a]	6.16 (d, 2.0)	6.15 (d, 2.0)	5.99 (d. 2.0)	6.05 (d. 2.0)
	8-H [a]	6.18 (d, 2.0)	6.18 (d, 2.0)	5.98 (d. 2.0)	6.00 (d. 2.0)
B	2,6-H	7.02 (d, 9.0)	7.15 (d, 9.0)	7.32 (d. 8.5)	7.38 (d. 8.5)
	3,5-H [b]	6.75 (d, 9.0)	6.76 (d, 9.0)	6.84 (d. 8.5)	6.82 (d. 8.5)
C	2-H	5.51 (d, 12.0)	5.65 (d, 12.0)	5.61 (d. 10.5)	5.62 (d. 10.5)
	3-H	4.47 (d, 12.0)	4.56 (d, 12.0)	4.66 (d. 10.5)	4.62 (d. 10.5)
D	5-H			5.93 (s.)	5.95 (s.)
	7-H	5.99 (s)	6.08 (s)		
E	2,6-H	7.13 (d, 9.0)	7.11 (d, 9.0)	7.01 (d. 8.5)	7.15 (d. 8.5)
	3,5-H [b]	6.74 (d, 9.0)	6.74 (d, 9.0)	6.65 (d. 8.5)	6.62 (d. 8.5)
F	2-CH_2	3.18 (d, 14.0)	3.15 (d, 14.0)	3.13 (d. 14.0)	3.19 (d. 14.0)
		3.11 (d, 14.0)	3.04 (d, 14.0)	3.05 (d. 14.0)	3.06 (d. 14.0)
	2-OMe	3.29 (s)	3.21 (s)	3.24 (s.)	3.19 (s.)
	Ar-OMe	3.93, 3.89, 3.84, 3.79, 3.76, 3.55 (each s)	3.89, 3.84, 3.80, 3.79, 3.75, 3.69 (each s)	3.89, 3.87, 3.86, 3.79, 3.78, 3.73 (each s)	3.92, 3.88 (x2), 3.82, 3.79, 3.71 (each s)

[a] May be interchanged in **3**, **4**, **7** and **8** ; [b] May be interchanged in **3** and **4**

serve to identify the latter, while 2/6-H(B) and 3/5-H(B) (δ 6.75, 6.76 for **3** and **4**, resp.) may be associated by correlations in their COSY spectra. The close proximity, however, of 3/5-H(B) and 3/5-H(E) (δ 6.74, 6.74 for **3** and **4**, resp.) precludes the unambiguous differentiation of these protons for both compounds. NOE experiments distinguish 6- and 8-H(A) (δ 6.16, 6.18 and 6.15, 6.18 for **3** and **4**, resp.) by associations with 5- and/or 7-OMe(A). The maesopsin unit, substituted on the A-ring, is characterized by the conspicuously shielded 2-OMe(F) (δ 3.29, 3.21 for **3** and **4**, resp.) and the diastereotopic α-methylene (δ 3.18, 3.11 for **3** and δ 3.11, 3.04 for **4**, J_{AB} = 14.0 Hz for both) resonances reminiscent of the benzofuranoid moiety, together with an AA'BB'-spin system and a residual proton (δ 5.99, 6.08 for **3** and **4**, resp.). An NOE association between this proton and only one methoxy group, 6-OMe(D) (δ 3.55, 3.69 for **3** and **4**, resp.), define both compounds as 5(D)-linked dimers. NOE association of 2/6-H(E) (δ 7.13, 7.11 for **3** and **4**, resp.) with the respective α-methylene protons, permit access to the E-ring protons.

The close structural relationships of the permethylaryl ethers **7** and **8** with **3** and **4** are evident from the conspicuous resemblance of their ^{1}H NMR spectra (Table). The former pair, however, are notably free of the effects of dynamic rotational isomerism about the interflavonoid bond. An AM-spin system emanating from 2-H(C) (δ 5.61, 5.62 for **7** and **8**, resp.) and 3-H(C) (δ 4.66, 4.62 for **7** and **8**, resp.) once more discloses the presence of a heterocyclic C-ring with 2,3-*trans* relative configuration ($^3J_{2,3}$ = 10.5 Hz for both) while an AB-

spin system (J_{AB} = 2.0 Hz for both) represents 6- and 8-H of the A-ring. Elements of a tetra-*O*-methylmaesopsin unit substituted at the A-ring are evident from the residual singlet (δ 5.93, 5.95 for **7** and **8**, resp.) and the presence of the shielded 2-OMe(F) (δ 3.24, 3.19 for **7** and **8**, resp.) together with the 2(F)-methylene (J_{AB} = 14.0 Hz for both). Differentiation between the two very similar AA'BB'-spin systems, reminiscent of the B- and E-ring protons, are effected *via* COSY spectra using the benzylic 2(F)-methylene and 3-H(C) resonances as reference signals.

Our initial communication[1] reported an NOE-association of the residual singlet with one methoxy group [6-OMe(D)] only, thus defining the compounds as 5(D)-linked dimers. Improved resolution of the NOESY data has since, however, revealed association of the residual singlet with two methoxy groups [4(D)- and 6-OMe(D) δ 3.89 and 3.87; 3.88 and 3.92, resp.] and we accordingly amend our initial structures to those of 7(D)-linked dimers **7** and **8**. Long-range COSY- and NOESY-experiments permit definition of compounds based on a 3-substituted flavanone by association of 2/6-H(B) with 2-H(C), as displayed by the 3α(C)→5(D)-linked dimers **3** and **4**. This contrasts notably with the analogues **7** and **8** where coupling of 2/6-H(B) occurs with 3-H(C), indicative of a 2(C)→3(C)-'shift' of the B-ring and therefore of 2α(C)→7(D)-linked dimers based on a 2-substituted isoflavanone moiety.

The above structural assignments correlate with the EI mass spectral fragmentation data of the derivatives **3**, **4**, **7** and **8**, which apart from the molecular ion M^+, m/z 656, all display prominent peaks at m/z 535, 476, 355, 313, 312, 181, and 121. The m/z 121 fragment reflects the loss of a 4-methoxybenzyl radical involving the E-ring hence affording the m/z 535 ion, whereas that at m/z 476 results from the equivalent of an RDA-fragmentation of the ABC units of the flavanone- **3** and **4** or isoflavanone moieties **7** and **8**.

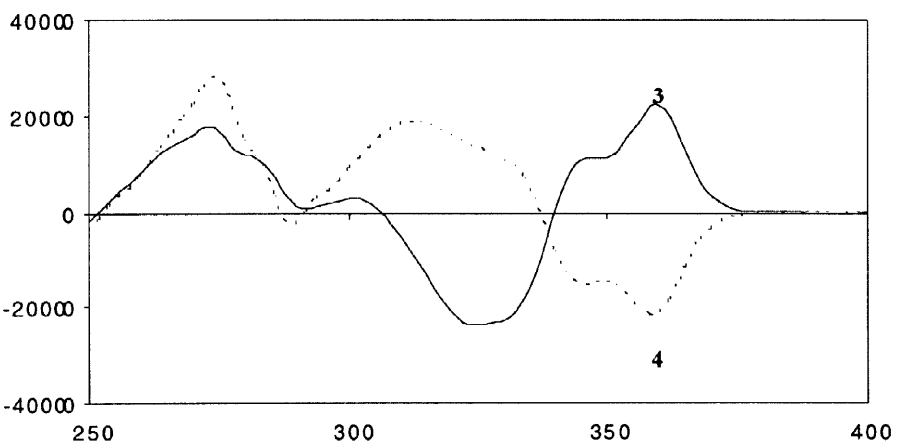

Figure 1 : CD spectra of biflavonoid derivatives **3** and **4**

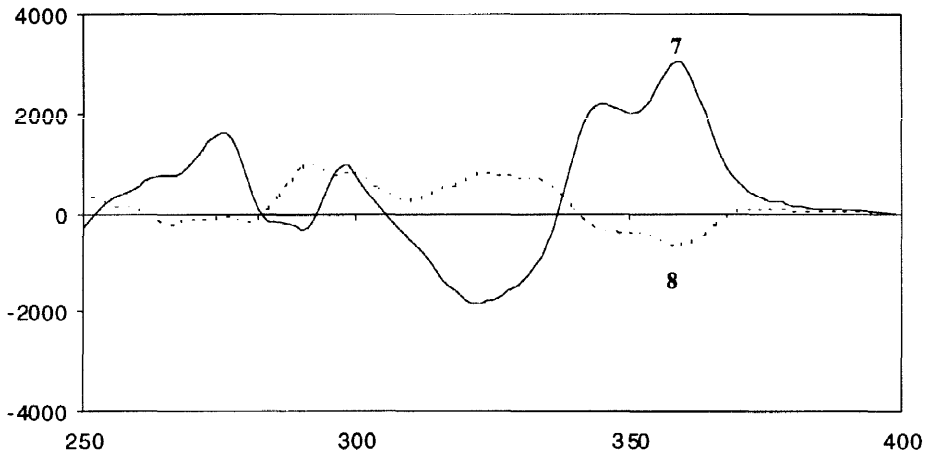

Figure 2 : CD spectra of biflavonoid derivatives **7** and **8**

The CD-curves (Figure 1) of the flavanone-(3α→5)-benzofuranone analogues **3** and **4** are nearly superimposable with those of the (3α→7)-coupled diastereomers.[2,3] Thus, from the CD data and supporting evidence generated for the latter compounds it is clear that the positive Cotton effect observed for the n → π* transition in the 340 - 380 nm region[6] for **3** corresponds with a 2*R*(F) absolute configuration for the maesopsin unit. Similarly, the negative Cotton effect in the same region observed for **4** indicates a 2*S*(F) absolute configuration for the maesopsin moiety. In agreement with the chiroptical data of the flavanone-(3α→7)-benzofuranone diastereomers,[2,3] the positive Cotton effect observed for the π → π* transition (260 - 290 nm) then indicates a 2*R*(C) absolute configuration for the flavanone unit[7] in both **3** and **4**. When taken in conjunction with the 2,3-*trans* configuration of the heterocyclic C-ring indicated by ^{1}H NMR coupling constants, this corresponds with a 2*R*,3*S*(C) absolute configuration for the flavanone unit in both compounds and thus define them as (2*R*,3*S*)-naringenin-(3α→5)-(2*R*)-maesopsin **1** and its 2*R*,3*S*(C):2*S*(F)-diastereomer **2**.

Although similar positive and negative Cotton effects in the 360 nm region[6] {$[\theta]_{359}$ $+3.1\times10^3$ and $[\theta]_{360}$ -6.9×10^2} (Figure 2) facilitate the allocation of 2*R*(F)- and 2*S*(F)- configurations, respectively, to the isoflavonoid analogues **7** and **8**, Cotton effects originating from the isoflavanone moieties are not comparable with those of flavanones. Isoflavanones display a distinctive Cotton effect in the *ca.* 350 nm region for their n → π* transitions, a positive effect reflecting a 3*R*-configuration and a negative effect a 3*S*-configuration.[8,9] The strong positive effect observed in this region for compound **7** is therefore reminiscent of a 3*R*-configuration, the high amplitude being the result of the cumulative effect of the *R*-chirality at both 3-C(C) and 2-C(F). The much weaker and negative Cotton effect displayed by the isomer **8** would then likewise be in accord with a 3*R*(C)-configuration, since the positive effect originating from the chirality at 3-C(C) and the negative effect associated with a 2S(F)-configuration would now be opposing each other, the latter being slightly dominant (Figure 2). When taken in conjunction with the 2,3-*trans* relative configuration for the C-

ring protons ($^3J_{2,3}$ = 10.5 Hz for both), a 2*S*(C)-configuration is implied for both compounds and therefore permits assignment of the absolute configuration of these novel biflavonoids as (2*S*,3*R*)-dihydrogenistein-(2α→7)-(2*R*)-maesopsin **5** and its diastereomer, (2*S*,3*R*)-dihydrogenistein-(2α→7)-(2*S*)-maesopsin **6**.

The absolute configuration of the isoflavanone ABC moieties (2*S*,3*R*) in **5** and **6**, relative to those of the flavanone moieties (2*R*,3*S*) in **3** and **4**, are in agreement with the proposed biogenetic origin of the biflavonoids **5** and **6** *via* initial 2,3-phenyl migration of the (2*R*)-4',5,7-trihydroxyflavanone (naringenin) **9**, which coexists in *B. zeyheri*.[5] Irrespective of the particular mechanism involved, *i.e.*, ionic or radical, several of which have been proposed,[10-12] such a migration always proceeds in a *suprafacial* fashion. (2*R*)-Naringenin would therefore experience phenyl migration from C-2β to C-3β, leaving C-2 electron deficient and prone to nucleophilic attack from the less hindered α-face by the phloroglucinol-type D-ring of maesopsin **10**. Since the latter coexists as the racemate in *B. zeyheri*,[5] the two diastereomers (2*S*,3*R*)-dihydrogenistein-(2α→7)-(2*R*)-maesopsin **5** and (2*S*,3*R*)-dihydrogenistein-(2α→7)-(2*S*)-maesopsin **6** originate. Proposals regarding the possible biogenetic origin of the flavanone analogues **1** and **2** are believed to parallel those of the zeyherin epimers which have been covered previously.[3]

EXPERIMENTAL

^{1}H NMR Spectra were recorded on a Bruker AM-300 spectrometer for solutions in $CDCl_3$ with Me_4Si as internal standard. Mass spectra were obtained with a Kratos MS-80 instrument, CD data in MeOH on a JASCO J-710 spectropolarimeter and IR spectra in $CHCl_3$ with a HITACHI 270-50 infrared spectro-photometer. TLC was performed on precoated Merck plastic sheets (silica gel 60 PF_{254}, 0.25 mm) and the plates were sprayed with H_2SO_4-HCHO (40:1, v/v) after development. Preparative plates (PLC), 20x20 cm, Kieselgel PF_{254} (1.0 mm) were air-dried and used without prior activation. Separations on Sephadex LH-20 were in EtOH at a flow rate of *ca.* 0.5 ml min^{-1} (30 min fractions). Flash column chromatography (FCC) was carried out in a glass column (5 cm diameter) charged with Merck Kieselgel 60 (230-400 mesh) at a flow rate of 3 ml min^{-1} (30 ml fractions) under N_2 pressure. Methylations were performed with Me_2SO_4 in dry Me_2CO containing anhydrous K_2CO_3 at reflux temperature. Water-soluble phenolics were freeze-dried with a Virtis Freeze mobile 12 SL. Evaporations were carried out under reduced pressure at *ca.* 40°C in a rotary evaporator.

The extraction of the heartwood of *B. zeyheri* with aqueous Me_2CO (8:2, v/v) and fractionation of the extract by means of countercurrent distribution and column chromatography using Sephadex LH-20 leading to fractions 7-8.9.1 to 7-8.9.25 were fully described in ref. 5. Methylation of fraction 7-8.9.15 (600 mg) and subsequent FCC in hexane-benzene-Me_2CO-MeOH (40:40:15:5, v/v) afforded three fractions 7-8.9.15.1 (tubes 1-18, 219 mg), 7-8.9.15.2 (19-28, 111 mg) and 7-8.9.15.3 (36-51, 183 mg). Fraction 7-8.9.15.1 was separated further (see ref. 5) to yield fraction 7-8.9.15.1.11 which was resolved into two components **3** (R_F 0.66) and **4** (R_F 0.62) by PLC in $CHCl_3$-Et_2O (98:2, v/v, x4).

(2R,3S)-4',5,7-tri-*O*-methylnaringenin-(3α→5)-(2R)-2,4,4',6-tetra-*O*-methylmaesopsin 3. The title compound (15 mg) was obtained as a *yellow amorphous solid.* (Found: M^+, 656.2257. $C_{37}H_{36}O_{11}$ requires M, 656.2258); δ_H (Table); CD $[\theta]_{359.3}$ 2.2x10^4, $[\theta]_{346.2}$ 1.2x10^4, $[\theta]_{325.0}$ –2.4x10^4, $[\theta]_{272.9}$ 1.8x10^4 (Figure 1); IR 1706, 1674 cm^{-1}.

(2R,3S)-4',5,7-tri-*O*-methylnaringenin-(3α→5)-(2S)-2,4,4',6-tetra-*O*-methylmaesopsin 4. Compound **4** (13 mg) was obtained as a *yellow amorphous solid.* (Found: M^+, 656.2257. $C_{37}H_{36}O_{11}$ requires M, 656,2258): δ_H (Table); CD $[\theta]_{358.6}$ –2.2x10^4, $[\theta]_{345.4}$ –1.5x10^4, $[\theta]_{312.4}$ 1.9x10^4, $[\theta]_{274.4}$ 2.19x10^4 (Figure 1); IR 1708, 1680 cm^{-1}.

Fraction 6 (14.5 g) from the countercurrent distribution (see ref. 5) was separated by column chromatography using Sephadex LH-20 in EtOH to give the following fractions : 6.1 (tubes 1-38, 7.1 g), 6.2 (39-49, 560 mg), 6.3 (50-92, 856 mg), 6.4 (93-112, 134 mg), 6.5 (113-133, 202 mg), 6.6 (134-161, 67 mg) and 6.7 (162-347, 148 mg). Fraction 6.4 (134 mg) was methylated and purified by PLC in benzene-Me_2CO (7:1, v/v) to yield a mixture (R_F 0.24) which was resolved into two components **7** (R_F 0.63) and **8** (R_F 0.53) by a further PLC separation in benzene-EtOAc (3:1, v/v, x7).

(2S,3R)-4',5,7-tri-*O*-methyldihydrogenistein-(2α→7)-(2R)-2,4,4',6-tetra-*O*-methylmaesopsin 7. The title compound (5 mg) was obtained as a *yellow amorphous solid.* (Found: M^+, 565.2257. $C_{37}H_{36}O_{11}$ requires M, 656.2258); δ_H (Table); CD $[\theta]_{359.0}$ 3.1x10^3, $[\theta]_{345.4}$ 2.2x10^3, $[\theta]_{322.1}$ –1.8x10^3, $[\theta]_{297.7}$ 9.8x10^2, $[\theta]_{290.0}$ –3.73x10^2, $[\theta]_{275.2}$ 1.6x10^3 (Figure 2); IR 1706, 1702 cm^{-1}.

(2S,3R)-4',5,7-tri-*O*-methyldihydrogenistein-(2α→7)-(2S)-2,4,4',6-tetra-*O*-methylmaesopsin 8. Compound **8** (6 mg) was obtained as a *yellow amorphous solid.* (Found: M^+, 565.2257. $C_{37}H_{36}O_{11}$ requires M, 656.2258); δ_H (Table); CD $[\theta]_{359.7}$ –6.9x10^2, $[\theta]_{349.4}$ –3.8x10^2, $[\theta]_{324.5}$ 8.0x10^2, $[\theta]_{310.0}$ 2.6x10^2, $[\theta]_{299.1}$ 8.5x10^2, $[\theta]_{291.4}$ 1.0x10^3 (Figure 2); IR 1707, 1701 cm^{-1}.

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