PERGAMON

Phytochemistry 54 (2000) 891–896

PHYTOCHEMISTRY

www.elsevier.com/locate/phytochem

Minor phenolics from *Crinum bulbispermum* bulbs

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Received 17 November 1999; received in revised form 11 May 2000

Abstract

From the bulbs of *Crinum bulbispermum* Milne, four new minor compounds were isolated viz. 4-hydroxy-2′,4′-dimethoxy-dihydrochalcone (**1**), 4,5-methylenedioxy-4′-hydroxy-2-aldehyde[1,1′-biphenyl] (**4**), hippacine (**6**), and 4′-hydroxy-7-methoxy-flavan-3-ol (**7**). In addition, four known compounds were isolated and identified as 2(*S*),3′,4′-dihydroxy-7-methoxy flavan (**2**), isolarrien (**3**), isoliquiritigenin (**5**) and liquiritigenin (**8**).

The structures of the isolated compounds were established by spectral evidence.

Keywords: Crinum bulbispermum; Amaryllidaceae; Flavans; Flavanones; Chalcones; Dihydrochalcones; Pyrrolophenanthridone alkaloid

1. Introduction

The genus *Crinum* (family Amaryllidaceae, subfamily Amaryllidoidae) has attracted considerable attention due to its alkaloidal content (Ghosal et al., 1985). The alkaloidal content of *Crinum bulbispermum* Milne has been established (Ramadan, 1986; El-Moghazi and Ali, 1976; Abd El-Baky, 1976; Ali et al., 1984). The non-nitrogenous constituents of *Crinum bulbispermum* has not attracted much attention from phytochemists (Ramadan, 1986). Therefore, we are interested in identifying the phenolic constituents of the plant. Extensive column chromatography and HPLC of the defatted acidic organic layer of the ethanolic extract of the bulbs resulted in the isolation and characterisation of a new alkaloid named hippacine and 4′-hydroxy-7-methoxy-flavan-3-ol, 4-hydroxy-2′,4′-dimethoxydihydrochalcone and 4,5-methylenedioxy-4′-hydroxy-2-aldehyde[1,1′-biphenyl]. In addition, four known compounds were isolated and identified as liquiritigenin, isoliquiritigenin, isolarrien and 2(*S*),3′,4′-dihydroxy-7-methoxy flavan.

Isolarrien and 2(*S*),3′,4′-dihydroxy-7-methoxy flavan are reported here for the first time in the family Amaryllidaceae, while liquiritigenin is reported here for the first time in the genus.

2. Results and discussion

Compound **1** exhibited UV absorption maxima at 227, 268 and 270 nm and its ^{1}H NMR in DMSO-d_6 revealed signals at δ 2.77 and 3.07 (2 H each, triplet, $J = 8$ Hz, phenyl–$\underline{CH_2}$–$\underline{CH_2}$–C═O). Inspection of the ^{13}C NMR spectrum indicated that compound **1** must be a dihydrochalcone derivative (Tanaka et al., 1982). HR-FAB mass spectral analysis indicated its molecular formula as $C_{17}H_{18}O_4$ with nine double bond equivalents, eight of them corresponding to the two benzene rings and the last one to a carbonyl group. The ^{1}H NMR spectrum showed an ABX and AA′BB′ pattern of substitution on two aromatic rings and two sets of triplets for the aliphatic protons. The spectrum did not exhibit a hydrogen-bonded phenolic hydroxyl which indicated that H-2′ was free or substituted by OCH_3. The spectrum also showed two signals for two aromatic methoxyls at δ 3.70 and 3.75 which appeared as

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0031-9422/00/$ - see front matter
PII: S0031-9422(00)00184-9

quartets in the ^{13}C NMR at δ 55.07 and 55.27. The two methoxyls are positioned on one ring (ring A) at C-2′ and C-4′. This could be confirmed from the non-bathochromic shift with NaOAc. Furthermore, the ^{13}C NMR downfield chemical shift of C-1′ and the upfield shift of C-2′, C-3′ and C-4′ in comparison with C-2′ hydroxylated derivatives (Wollenweber and Siegler, 1982; Drewes and Hudson, 1983; Agrawal, 1989). The above-mentioned data are consistent with the structure 4-hydroxy,2′,4′-dimethoxydihydrochalcone and this is a new dihydrochalcone.

The identification of compound **2** was based on comparison of its spectral data with those reported in Ref. (Achenbach et al., 1988). The ^{13}C and ^{1}H NMR spectral data of compound **2** were coincident with the published data (Achenbach et al., 1988) of 2(*S*)-3′,4′-dihydroxy-7-methoxy flavan isolated from *Bauhinia manca* and reported here for the first time in the family Amaryllidaceae.

The ^{1}H NMR spectrum of compound **3** exhibits signals due to one methoxyl at δ 3.71, an ortho coupled aromatic protons at δ 6.58 (1H, *d*, $J = 8.5$ Hz, H-6) and 7.55 (1H, *d*, $J = 8.5$, H-5), five other aromatic protons at δ 7.37–7.45 (5H, *m*, protons of ring B) and an ABX system at δ 5.62 (1H, *dd*, $J = 12.3$, 3 Hz, H-2), 2.75 and 3.1 (each one-proton, H-3_{eq} and H-3_{ax}). These data strongly suggest that compound **3** is a flavanone. The chemical shift of the three proton ABX system being particularly characteristic of this type of flavonoid (Markham and Mabry, 1975).

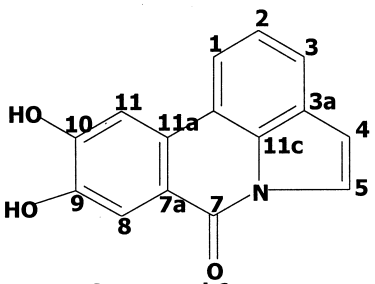
The ^{13}C NMR exhibits signals typical for flavanones at δ 43.20 (*t*, C-3), 79.12 (*d*, C-2), and 189.89 (s, C-4). The shifts of C-2 and C-4 are in accordance with a flavanone unsubstituted in C-2′, C-6′ and C-5, respectively (Agrawal, 1989). The position of methoxyl and hydroxyl groups on ring A are substituted at positions which provide protons exhibit ortho coupling in the ^{1}H NMR spectrum. Of the three available possibilities, the 8,7-substitution pattern is the most probable. Since in this structure one proton is deshielded by the adjacent carbonyl this would agree with the observed resonances at δ 7.55 (H-5), and the shift of carbonyl group (189.89). Also, the ^{13}C NMR revealed signal for C-8 at δ 135.20 indicating the *O*-methylation of this carbon (Agrawal, 1989). From the above-mentioned data, it could be possible to identify compound **3** as 7-hydroxy-8-methoxyflavanone (isolarrien) (Kemp et al., 1979). This is the second report of this compound in nature and its ^{13}C NMR is reported here for the first time.

The molecular formula of compound **4** was determined as $C_{14}H_{10}O_4$ from HR-FAB mass spectral analysis. The UV spectrum showed typical maxima for methylenedioxy substituted benzene ring at λ_{max} 212, 237 and 260 nm (Ali et al., 1981). The methylenedioxy group could be confirmed by a singlet integrating for two protons at δ 6.16 in ^{1}H NMR and a triplet at δ 102.28 in ^{13}C NMR spectra. The ^{1}H NMR spectrum also revealed six aromatic protons, two of which were para oriented and occurred as singlets at δ 7.28 and 6.97 and located at the ring carrying the methylenedioxy group (H-3 and H-6, respectively). The other four protons form a typical AA′BB′ system for para substituted benzene ring at δ 7.19 (2H, *d*, $J = 8.6$ Hz, H-2′,H-6′) and δ 6.85 (2H, *d*, $J = 8.6$ Hz, H-3′, H-5′) which confirmed the presence of two signals each for two carbons at δ 131.34 and 115.23 in the ^{13}C NMR. The ^{1}H NMR spectrum also displayed a characteristic singlet signal at δ 9.63 and a doublet at δ 189.89 in the ^{13}C NMR spectrum characteristic for aldehyde proton and aldehyde carbonyl group, respectively, (Tagashira and Ohtake, 1998). The position of aldehyde group in position 2 could be explained by the downfield shift of C-3 (δ 7.28) and also on biogenitic ground. So, the hydroxyl group must be located at C-4′. The above-mentioned data are consistent with a biphenyl derivative containing methylenedioxy and an aldehyde in one ring and a hydroxy substituent on the other ring. Consequently, compound **4** was assigned as 4,5-methylenedioxy, 4′-hydroxy, 2-aldehyde [1,1′-biphenyl]. To the best of our knowledge, compound **4** has not been encountered before in nature nor has it been prepared synthetically. The relationship in structure between our compound and the alkaloid ismine (Ghosal et al., 1985) may give evidence that our compound may play a role in the biosynthesis of this compound.

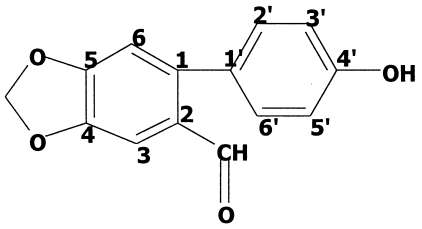
The identification of isoliquiritigenin **5** was based on comparison of its spectral data with those reported in Ref. (Saitoh et al., 1978; Agrawal, 1989). The ^{13}C and ^{1}H NMR spectral data of compound **5** were coincident with the published data (Saitoh et al., 1978; Agrawal, 1989).

The molecular formula of compound **6** was determined as $C_{15}H_9NO_3$ from HR-FAB mass spectral analysis and gave a positive response to the $FeCl_3$ test for phenols. In accordance with the formula, the ^{1}H NMR spectrum shows signals for seven protons and the other two are incorporated as hydroxyls. Two one proton singlets were found at δ 7.61 and 7.79 ppm for the aromatic protons H-11 and H-8 in para positions. In the aromatic range appear three signal groups for H-1, H-2 and H-3 protons. H-2 is twice ortho coupled with H-1 and H-3 ($J = 7.6$ Hz), respectively. H-1 and H-3 exhibit a supplementry meta coupling. Moreover, the ^{1}H NMR spectrum also showed resonances at δ 7.01 and 8.05 (each 1H, *d*, $J = 3.4$ Hz) which refer to H-4 and H-5 with a typical coupling constant 3.4 Hz for the indole system.

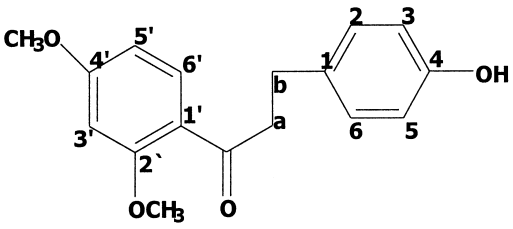
The ^{13}C NMR spectrum of **6** showed resonances for 15 carbon atoms. The APT and DEPT experiments revealed the presence of seven doublets (C-1, C-2, C-3, C-4, C-5, C-8 and C-11) and eight singlets, among



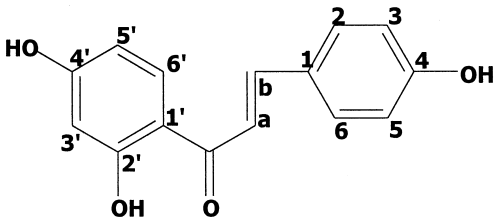
Compound 6



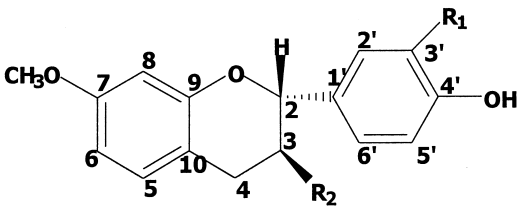
Compound 4



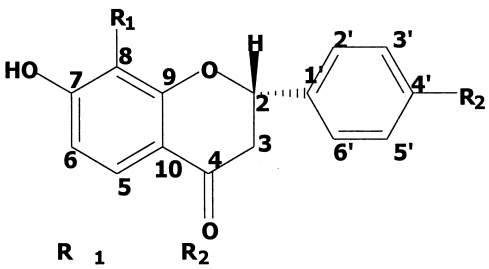
Compound 1



Compound 5



	R_1	R_2
Compound 2	OH	H
Compound 7	H	OH



	R_1	R_2
Compound 3	OCH_3	H
Compound 8	H	OH

which were three oxygenated carbons which appeared downfield (δ 157.45, 152.00 and 146.92) and corresponded to C-7, C-10 and C-9 respectively, together with resonances at δ 130.27, 127.81, 127.79, 118.66 and 116.56 for C-11c, C-11a, C-3a, C-7a and C-11b, respectively. The above-mentioned data showed close similarity to the ^{1}H and ^{13}C NMR of the known alkaloid hippadine (Ali et al., 1981a) but the methylenedioxy in hippadine was replaced by two hydroxyls. Therefore, this compound was named as 4,5-ethano-9,10-dihydroxy-7-phenanthridone and provisionally named hippacine.

Compound **7** was obtained as an amorphous powder. Its UV spectrum showed two major absorption maxima at 220 and 280 nm, characteristic for flavans (Cooke and Down, 1970). The molecular formula of compound **7** was determined as $C_{16}H_{16}O_4$ from HR-FAB mass spectrometry in accordance with a flavan containing two hydroxyls and one methoxyl group. The ^{1}H NMR spectrum exhibited signals for seven aromatic protons: two sets of two proton doublets ($J = 8.3$ Hz) at δ 6.73 and 7.14 for a typical para substituted benzene ring (H-3′, H-5′ and H-2′, H-6′). Three sets of one proton, each, from which two sets of one proton doublets at δ 6.94 (1 H, *d*, $J = 8.4$ Hz, H-5) and 6.35 (1 H, *d*, $J = 2.7$ Hz, H-8), while the other set exhibited doublet of doublets at δ 6.43 (1 H, *dd*, $J = 8.4$ and 2.7 Hz, H-6). The ^{13}C NMR exhibited a downfield chemical shift of C-2 resonance (δ 81.31) indicating that the flavan unit has the 2,3-trans configuration (Foo, 1987; Porter et al., 1982) which is also substantiated by the large H-2, H-3 proton coupling constant ($J = 7.6$ Hz) (Clark-Lewis et al., 1964) observed in the ^{1}H NMR spectrum. The location of the hydroxyl group in ring B could be ascertained by the presence of the fragment at *m*/*z* 135

$[M^+-H-C_8H_8H_2]^+$. The ^{13}C NMR spectrum exhibited one quartet for aromatic methoxyl at δ 55.03 and one triplet at δ 32.54 for C-4. The other aliphatic oxygenated carbons C-2 and C-3 appeared as doublets at δ 81.31 and 66.10 indicating the flavan-3-ol type. The carbons of the AA′ BB′ pattern appeared at δ 114.84 and 128.38 for H-3′, H-5′ and H-2′, H-6′, respectively.

From the above-mentioned data, it is possible to elucidate the structure of the new compound **7** as 4′-hydroxy-7-methoxy-flavan-3-ol.

The identification of (−)-liquiritigenin **8** was based mainly on the comparison of its spectral data with those reported in (Youssef et al., 1998). Its ^{13}C NMR spectrum showed resonances of 15 carbons. These signals, based on DEPT experiments, were classified into six doublets (C-2, C-5, C-6, C-8, C-2′/C-6′ and C-3′/C-5′), one triplet for C-3 and six singlets (C-4, C-7, C-9, C-10, C-1′ and C-4′). Among the singlets were three oxygenated ones appearing at 166.78, 165.57 and 158.98 for C-7, C-9 and C-4′, respectively.

3. Experimental

3.1. Instruments

NMR spectra were recorded in DMSO-d_6 using JEOL JNM A-400 spectrometer (400 MHz for ^1H NMR and 100 MHz for ^{13}C NMR) with TMS as internal standard. The mass spectra were recorded by JEOL JMS-SX 102 spectrometer. Optical rotations were measured by Union PM-1 digital polarimeter. Preparative HPLC was carried out on columns of ODS (150 × 20 mm i.d., YMC) with a Tosoh refraction index (RI-8) detector. The flow rate was 6 ml/min. TLC was carried out on precoated silica gel plates (kieselgel 60 F_{254}, Merck). For CC, silica gel G (Merck).

3.2. Plant materials

The plant material was collected in April 1997 from Experimental Station of Medicinal Plants, Pharmacognosy Department Assiut University. The identity of the plant was confirmed by Prof. N. EL-Hadidi, Department of Botany and Plant Taxonomy, Faculty of Science, Cairo University. A voucher sample is kept in the Herbarium of the Faculty of Pharmacy, Assiut University, Egypt.

3.3. Extraction and isolation

The dried bulbs (10 kg) of *Crinum bulbispermum* were extracted by maceration in EtOH for 96 h (4 × 20 L). The combined extracts were evaporated and the concentrated viscous extract was partitioned between $CHCl_3$ and 2% H_2SO_4. The organic layer was washed with distilled water then concentrated under reduced pressure to give viscous residue (100 g). This fraction was defatted with petrol. The defatted residue showed several $FeCl_3$-positive spots on TLC. The residue (60 g) was fractionated by flash CC eluting with petrol followed by EtOAc–petrol gradients. Fractions 50 ml each, were collected and monitored by TLC. Similar fractions were combined to give three main fractions (A–C). Fraction A (300 mg) contains compounds **1**–**3**, while fraction B (150 mg) contains Compounds **4**–**6**. Fraction C (105 mg) contains compounds **7**–**8**. Fine separation of the components of each fraction was carried out by repeated Preparative HPLC on ODS columns using CH_3CN 45% for the separation of Fraction C components. CH_3CN 50% is used for the fine separation of the components of fractions A and B. The separated compounds **1**–**8** have the following retention times (min): 23, 18, 17.5, 16, 12.5, 11, 13 and 10, respectively.

3.4. Identification of compound *1*

Compound **1**: 4-hydroxy,2′,4′-dimethoxydihydrochalcone. Amorphous powder (20 mg); negative HR-FAB mass; found: 285.1112 $[M-H]^-$ ($C_{17}H_{18}O_4$–H requires 285.1127); UV λ_{max}: (MeOH) 227, 228, 263, 270 nm; ^1H NMR (400 MHz, DMSO-d_6): δ 2.77 (2H, *t*, $J = 8$ Hz, H_2-α), 3.07 (2H, *t*, $J = 8$ Hz, H_2-β), 3.70 (3H, *s*, OCH_3), 3.75 (3H, *s*, OCH_3), 6.40 (1H, *dd*, $J_{5',6'} = 8.3$ Hz, $J_{5',3'} = 2.5$ Hz, H-5′), 6.50 (1H, *d*, H-3′), 7.04 (1H, *d*, H-6′), 6.82 (2H, *d*, $J = 8$ Hz, H-3, H,5), 7.82 (2H, *d*, $J = 8$Hz, H-2, H-6); ^{13}C NMR (100 MHz, DMSO-d_6): 24.42 (*t*, C-β), 37.88 (*t*, C-α), 55.07 (*q*, OCH_3), 55.27 (*q*, OCH_3), 98.34 (*d*, C-3′), 104.38 (*d*, C-5′), 115.23 (*d*, C-3, C-5), 121.17 (*s*, C-1′), 128.27 (*s*, C-1), 129.90 (*d*, C-6′), 130.42 (*d*, C-2, C-6), 157.94 (*s*, C-4), 159.06 (*s*, C-2′), 161.94 (*s*, C-4′), 197.72 (*s*, C═O).

3.5. Identification of compound *2*

Compound **2**: 2(*S*)-3′,4′-dihydroxy-7-methoxy flavan. Amorphous powder (12 mg); ^1H NMR (400 MHz, DMSO-d_6): δ 1.87 (1H, *m*, H_A-3), 2.03 (1H, *m*, H_B-3), 2.62 (1H, *m*, H_A-4), 2.82 (1H, *m*, H_B-4), 3.66 (3H, *s*, OCH_3), 4.86 (1H, *dd*, $J = 10$, 2.2 Hz, H-2), 6.43 (1H, *d*, $J = 2.4$ Hz, H-8), 6.41 (1H, *dd*, $J = 8.3$, 2.4 Hz, H-6), 6.64 (1H, *dd*, $J = 8$, 1.9 Hz, H-6′), 6.70 (1H, *d*, $J = 8$ Hz, H-5′), 6.77 (1H, *d*, $J = 1.9$ Hz, H-2′), 6.95 (1H, *d*, $J = 8.3$ Hz, H-5); ^{13}C NMR (100 MHz, DMSO-d_6): 23.78 (*t*, C-3), 29.22 (*t*, C-4), 54.99 (*q*, OCH_3), 77.00 (*d*, C-2), 101.24 (*d*, C-6), 106.78 (*d*, C-8), 113.66 (*d*, C-2′), 113.90 (*s*, C-10), 115.28 (*d*, C-5′), 117.16 (*d*, C-6′), 129.92 (*d*, C-5), 132.43 (*s*, C-1′),

144.86 (*s*, C-3′), 145.05 (*s*, C-4′), 155.61 (*s*, C-9), 158.55 (*s*, C-7).

3.6. Identification of compound **3**

Compound **3**: 7-hydroxy-8-methoxy-flavanone. Amorphous powder (6 mg); ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.37–7.45 (5H, *m*, protons of ring B), 6.58 (1H, *d*, $J = 8.5$ Hz, H-6), 7.55 (1H, *d*, $J = 8.5$ Hz, H-5), 5.62 (1H, *dd*, $J = 12.3$, 3 Hz, H-26), 3.71 (3H, *s*, OCH_3), 3.10 (1H, *dd*, $J = 16.6$, 12.3 Hz, H-3_{ax}), 2.75 (1H, *dd*, $J = 16.6$, 3 Hz, H-3_{eq}); ^{13}C NMR (100 MHz, DMSO-d_6): δ 43.20 (*t*, C-3), 60.20 (*q*, OCH_3), 79.12 (*d*, C-2), 110.57 (*d*, C-6), 114.43 (*s*, C-10), 122 (*d*, C-5), 126.39 (*d*, C-3′, C-5′), 128.38 (*s*, C-4′), 128.54 (*d*, C-2′, C-6′), 135.20 (*s*, C-8), 139.19 (*s*, C-1′), 155.62 (*s*, C-9).157.16 (*s*, C-7), 189.89 (*s*, C═O (C-4)).

3.7. Identification of compound **4**

Compound **4**: 4,5-methylenedioxy-4′-hydroxy-2-aldehyde[1,1′-biphenyl]. Amorphous powder (10 mg); negative HR-FAB mass; found: 241.0497 [M − H]$^-$ ($C_{14}H_{10}O_4$–H requires 241.0500); UV λ_{max}: (MeOH) 212, 237, 260 (sh), 324 nm; ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.63 (1H, *s*, aldehyde proton), 7.28 (1H, *s*, H-3), 7.19 (2H, *d*, $J = 8.6$ Hz, H-2′, H-6′), 6.97 (1H, *s*, H-6), 6.85 (2H, *d*, $J = 8.6$ Hz, H-3′, H-5′), 6.16 (2H, *s*, OCH_2O); ^{13}C NMR (100 MHz, DMSO-d_6): δ 189.88 (*d*, HC═O), 157.57 (*s*, C-4′), 151.92 (*s*, C-4), 147.11 (*s*, C-5), 143.28 (*s*, C-2), 127.85 (*s*, C-1), 127.47 (*s*, C-1′), 131.34 (*d*, C-2′, C-6′), 115.23 (*d*, C-3′, C-5′), 110.08 (*d*, C-3), 105.18 (*d*, C-6), 102.28 (*t*, OCH_2O).

3.8. Identification of compound **5**

Compound **5**: isoliquiritigenin (4,2′,4′-trihydroxychalcone). Yellow needles (MeOH) (7 mg), mp 194–196°C.

3.9. Identification of compound **6**

Compound **6**: 4,5-etheno-9,10-dihydroxy-7-phenanthridone (hippacine). Amorphous powder (10 mg); negative HR-FAB mass; found: 250.0495 [M − H]$^-$ ($C_{15}H_9O_3N$–H requires 250.0504); ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.01 (1H, *d*, $J_{4,5} = 3.4$ Hz, H-4), 8.05 (1H, *d*, $J_{4,5} = 3.4$ Hz, H-5), 7.46 (1H, *t*, $J_{2,3} = 7.6$ Hz, $J_{2,1} = 7.6$ Hz, H-2), 7.78 (1H, *dd*, $J_{3,1} = 1$ Hz, H-3), 8.02 (1H, *dd*, H-1), 7.61 (1H, *s*, H-11), 7.79 (1H, *s*, H-8); ^{13}C NMR (100 MHz, DMSO-d_6): 157.45 (*s*, C-7), 152.00 (*s*, C-10), 146.92 (*s*, C-9), 130.27 (*s*, C-11c), 127.81 (*s*, C-11a), 127.79 (*s*, C-3a), 123.97 (*d*, C-5), 123.26 (*d*, C-2), 121.78 (*d*, C-3), 118.66 (*s*, C-7a), 118.06 (*d*, C-1), 116.56 (*s*, C-11b), 113.97 (*d*, C-4), 110.55 (*d*, C-11), 108.79 (*d*, C-8).

3.10. Identification of compound **7**

Compound **7**: 4′-hydroxy-7-methoxy-flavan-3-ol. Amorphous powder (10 mg); negative HR-FAB mass; found: 271.0972 [M − H]$^-$ ($C_{16}H_{16}O_4$–H requires 271.0970); UV λ_{max}: (MeOH) 205, 212, 220, 280 nm; ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.14 (2H, *d*, $J = 8.3$ Hz, H-2′, H-6′), 6.94 (1H, *d*, $J = 8.4$ Hz, H-5), 6.73 (2H, *d*, $J = 8.3$ Hz, H-3′, H-5′), 6.43 (1H, *dd*, $J = 8.4$, 2.7 Hz, H-6), 6.35 (1H, *d*, $J = 2.7$ Hz, H-8), 4.65 (1H, *d*, $J = 7.6$ Hz, H-2), 3.92 (1H, *ddd*, $J = 7.6$, 5.1, 8.5 Hz, H-3), 3.67 (3H, *s*, OCH_3), 2.80 (1H, *dd*, $J = 15.8$, 5.1 Hz, H-4_{eq}), 2.64 (1H, *dd*, $J = 15.8$, 8.5 Hz, H-$4_{ax.}$); ^{13}C NMR (100 MHz, DMSO-d_6): δ 32.54 (*t*, C-4), 55.03 (*q*, OCH_3), 66.10 (*d*, C-3), 81.31 (*d*, C-2), 100.77 (*d*, C-8), 107.00 (*d*, C-6), 112.77 (*s*, C-10), 114.84 (*d*, C-3′, C-5′), 128.38 (*d*, C-2′, C-6′), 129.71 (*s*, C-1′), 130.15 (*d*, C-5), 154.74 (*s*, C-9), 156.96 (*s*, C-4′), 158.71 (*s*, C-7).

3.11. Identification of compound **8**

Compound **8**: (−)-liquiritigenin (7,4′-dihydroxyflavanone). White needles (MeOH) (10 mg), mp 205–206°C.

Acknowledgements

We are grateful to the Japan Society for Promotion of Science (JSPS) and Grant in Aid, Ministry of Education and Culture, Japan, for the financial support of this work. Also, we would like to thank the Research Center for Molecular Medicine, Hiroshima University for NMR spectral analysis.

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