Flavonoids, isoflavonoids and other constituents from the fresh mature seeds of Sophora tomentosa L.

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Abstract

The chloroform-soluble fraction in a methanol extract from mature seeds of *Sophora tomentosa* L.was subjected to chromatographic separation and purification to give two flavonoids (**4** and **5**), two isoflavonoids (**3** and **6**), one lupin alkaloid (**1**) and one polyphenol (**2**), respectively. These compounds were identified as 7,4'-dihydroxy- 3'-methoxyflavone (**4**, geraldone), 7,3'-dihydroxy-4'-methoxyflavone (**5**, farnisin), 7,3'-dihydroxy-5'-methoxyisoflavone (**3**), 7,4'-dihydroxy-3'-methoxyisoflavone (**6**), *N*-methylcytisine (**1**) and methyl 3,4-dihydroxybenzoate (**2**, methyl protocatechuate), respectively, by spectroscopic methods.

Introduction

Sophora tomentosa L. (Leguminosae) is a small tree with yellow flower commonly found at the coastal area in Okinawa Islands. Flavonoids, isoflavonoids, alkaloids and benzofurane derivatives have been isolated from the aerial parts, leaves, roots and stems of *S. tomentosa*.[1-8] It has been reported that there is a potent microbial activity against methicillin resistant *Staphylococcus aureus* in some flavonoids and that isoflavonoids revealed estrogenic activity.[9-10] The review of flavonoids in *Sophora* species described that these flavonoids have antitumor, antimicrobial, anti-HIV, radical scavenging and enzyme inhibitory activities.[11]

In connection with studies on the useful constituents from the fresh legumes of plants grown subtropical and tropical regions, we investigated the constituents from the fresh mature legumes of *S. tomentosa* L. and isolated two flavonoids, one lupin alkaloid, two isoflavonoids and one polyphenol from the mature seeds.

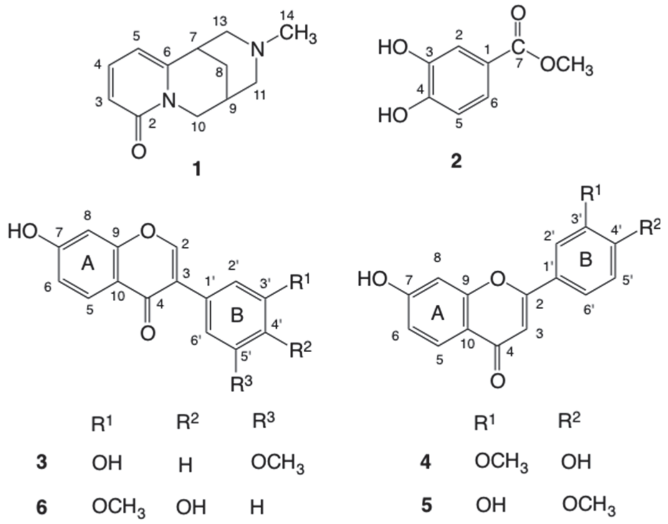
Herein, we describe the separation and structural elucidation of these compounds.

[1]

Results and Discussion

The chloroform-soluble fraction from a methanol extract of the fresh mature seeds of *Sophora tomentosa* L. was subjected to several chromatographic separation and purification to give two flavonoids (**4** and **5**), two isoflavonoids (**3** and **6**), one lupin alkaloid (**1**) and one polyphenol (**2**)

Compound **5** was obtained as a pale yellow amorphous and had a molecular formula $C_{16}H_{12}O_5$ by observation of a quasi-molecular ion peak at *m*/z 285.0741 $[M+H]^+$ (calcd for $C_{16}H_{13}O_5$: 285.0763) in the high resolution electron spray ionization mass spectrum (HR-ESI-MS). Its UV spectrum showed absorption maxima at 240 (log ε 3.99) , 290 (3.99,sh) and 341 nm (4.08) characteristic of flavone, indicating that **5** was a flavone derivative. Its IR spectrum showed a band due to OH at 3364 cm^{-1}, indicating that **5** was a flavone possessing hydroxy group(s).



Structures

1 Received: January 22, 2016

Table 1. [1]H NMR spectral data of **1–6**

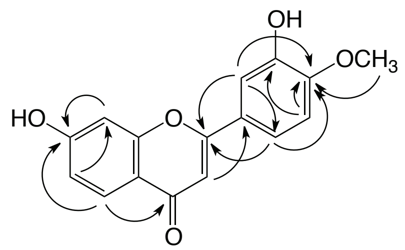
H	**1** in $CDCl_3$	**2** in $CDCl_3$	**3** in CD_3OD	**4** in CD_3OD	**5** in CD_3OD	**6** in CD_3OD
2		7.64 d (2.0)	8.14 s			8.18 s
3	6.43 dd (9.0, 1.0)*			6.60 s	6.62 s	
4	7.27 dd (9.0, 6.6)					
5	5.98 d (6.6)	6.91 d (8.3)	8.07 d (8.8)	7.87 d (8.7)	7.93 d (8.7)	8.08 d (8.7)
6		7.56 dd (8.3, 2.0)	6.95 dd (8.8, 2.2)	6.83 dd (8.7, 2.0)	6.89 dd (8.7, 2.0)	6.96 dd (8.7, 2.2)
7	2.93 t (2.4)					
8α	1.73 dt (12.7, 2.4)		6.86 d (2.2)	6.88 d (2.0)	6.92 d (2.0)	6.87 d (2.2)
8β	1.85 dd (12.7, 1.4)					
9	2.42 m					
10α	4.04 d (15.4)					
10β	3.89 dd (15.4, 6.8)					
11α	2.88 d (10.4)					
11β	2.21 d (10.4)					
13α	2.83 dt (10.8, 1.4)					
13β	2.25 dd (10.8, 2.4)					
14	2.12 s					
2'			6.98 s	7.41 d (1.8)	7.37 d (1.7)	7.18 d (1.9)
3'						
4'			7.06 s			
5'				6.85 d (8.4)	7.04 d (8.5)	6.87 d (8.1)
6'			6.98 s	7.43 dd (8.4, 1.8)	7.47 dd (8.5, 1.7)	6.98 dd (8.1, 1.9)
OCH_3-4'					3.90 s	
OCH_3-3'				3.87 s		3.91 s
OCH_3-5'			3.90 s			
OCH_3-7		3.89 s				

* Coupling constants (*J*) are expressed in Hz.

Table 2. [13]C NMR spectral data of **1–6**

C	**1** in $CDCl_3$	**2** in $CDCl_3$	**3** in CD_3OD	**4** in CD_3OD	**5** in CD_3OD	**6** in CD_3OD
1		122.5				
2	163.7	116.7	153.4	164.5	164.3	153.4
3	116.7	143.1	124.8	104.1	104.3	124.6
4	138.6	148.8	176.6	178.9	178.8	176.7
5	104.7	114.9	127.1	126.3	126.4	127.1
6	151.5	123.9	115.1	114.9	115.0	115.2
7	35.5	167.4	163.3	163.5	163.7	163.4
8	25.4		101.8	102.1	102.1	101.9
9	28.0		158.3	158.3	158.3	158.4
10	50.0		116.8	115.9	115.8	116.7
11	62.2					
13	62.5					
14	46.2					
1'			124.4	122.6	124.0	123.4
2'			120.2	109.2	112.5	112.7
3'			146.0	148.1	146.8	147.4
4'			116.0	150.5	150.1	146.5
5'			147.8	115.4	111.3	114.8
6'			111.2	120.2	118.5	121.5
OCH_3-4'					55.1	
OCH_3-3'				55.3		55.0
OCH_3-5'			55.0			
OCH_3-7		52.2				

The ^{1}H and ^{13}C NMR spectra of **5** showed a carbonyl carbon signal at δ_C 178.8 due to a chromone skeleton, ABX pattern signals at δ_H 7.93 (1H, d, *J*=8.7 Hz), 6.89 (1H, dd, *J*=8.7, 2.0 Hz) and 6.92 (1H, d, *J*=2.0 Hz) due to aromatic protons, AMX pattern signals at δ_H 7.47 (1H, dd, *J*=8.5, 1.7 Hz), 7.37 (1H, d, *J*=1.7 Hz) and 7.04 (1H, d, *J*=8.5 Hz) due to aromatic protons, and signals at δ_C 163.4, 158.4, 147.4 and 146.5 due to oxygenated aromatic carbons and a singlet due to a methoxy group at δ_H 3.91. These data indicated that **5** was a flavone derivative possessing one methoxy and two hydroxy groups and that both ring A and B were 1, 2, 4-trisubstituted aromatic rings. Complete assignments of ^{1}H and ^{13}C NMR signals were performed by COSY, HSQC and HMBC NMR analyses and analyses of sprit patterns of signals (Tables 1 and 2). The ABX pattern signals including doublet (1H, *J*=8.7 Hz) at δ_H 7.93 due to H-5 were identified as aromatic protons at A ring and suggested no substitution at C-6 position, because correlation between the doublet at δ_H 7.93 and a signal due to carbonyl carbon at δ_C 178.8 was observed in the HMBC spectrum (Fig. 1). The AMX pattern signals including the doublet of doublets (1H, *J*=8.5 1.7 Hz) due to H-6' at δ_H 7.47 were assigned as aromatic protons at B ring and suggested no substitution at C-5' position, because both doublet (1H, *J*=1.7 Hz) at δ_H 7.37 due to H-2' and the doublet of doublets at δ_H 7.47 due to H-6' showed



correlation to the signal at δ_C 164.3 due to C-2 oxygenated olefinic carbon in the HMBC spectrum (Fig. 1). Furthermore, observation of the correlation between a singlet at δ_H 3.90 due to the methoxy proton and signal at δ_C 150.1 due to C-4' aromatic carbon revealed that the methoxy group was bonded to C-4'. Therefore, the bond positions of two hydroxy groups were assigned to be C-7 and C-3'.

Thus, **5** was identified as 7, 3'-dihydroxy-4'-methoxyflavone (**5**, farnisin). The physical and spectral data of **5** were agreement with those in reference. [12)]

Compound **4** was obtained as a pale yellow amorphous and had a molecular formula of $C_{16}H_{12}O_5$ by observation of quasi-molecular ion peak at *m*/*z* 285.0759 $[M+H]^+$ (calcd for $C_{16}H_{13}O_5$: 285.0763) in its HR-ESI-MS. The UV spectrum showed absorption maxima at 241 (log ε 4.47) 290 (4.42) and 359 nm (4.44) characteristic of flavone, indicating that **4** was a flavone derivative as the same as **5**.

The IR spectrum showed a band due to OH at 3297 cm^{-1}, indicating that **4** was a flavone possessing hydroxy group(s).

The ^{1}H and ^{13}C NMR spectra showed AMX pattern signals [δ_H 7.87 (1H, d, *J*=8.8 Hz), 6.83 (1H, dd, *J*=8.8 2.0 Hz) and 6.88 (1H, d, *J*=2.0 Hz)] and ABX pattern signals [δ_H 6.85 (1H, d,*J*=8.4 Hz), 7.43 (1H , dd, *J*=8.4,1.8 Hz) and 7.41 (1H, d, *J*=1.8 Hz)], indicating that both of A and B aromatic rings were 1, 2, 4-trisubstituted. Moreover, these spectra revealed the presence of a methoxy group [δ_H 3.87 (3H, s)] and a carbonyl group (δ_C 178.9).

Complete assignments of ^{1}H and ^{13}C NMR signals were archived by COSY, HMQC and HMBC NMR analyses and analyses of sprit patterns of the signals (Tables 1 and 2). In the HMBC spectrum (Fig. 2), doublet (1H, *J*=8.7 Hz) at δ_H 7.87 due to H-5 showed a cross peak to a signal due to a carbonyl carbon (C-4) at δ_C 178.9, indicating that the AMX pattern signals including the doublet due to H-5 at δ_H 7.87 were assigned to be aromatic protons of A ring and suggested no substitution at C-6 position. The ABX pattern signals including doublet of doublets (1H, *J*=8.4, 1.8 Hz) at δ_H 7.43 due to H-6' were assigned to be those of B ring and suggested no substitution at C-5' position. The facts were supported by observation of correlations between both doublet (1H, *J*= 1.8 Hz) at δ_H 7.41 due to H-2' and the doublet of doublets at δ_H 7.43 due to H-6' and the signal at

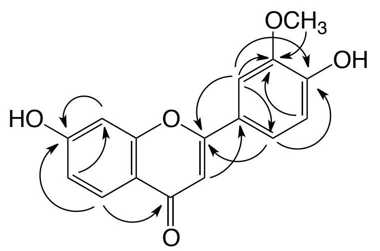


Fig. 2. Selected HMBC correlations of **4**.

δ_C 164.5 due to C-2 in the HMBC spectrum (Fig. 2). Moreover, a singlet at δ_H 3.87 due to the methoxy protons showed a cross peak to a signal at δ_C 148.1 due to C-3', which indicated that the methoxy group was bonded to C-3' (Fig 2). Therefore, each of carbons at C-7 and C-4' was bonded to a hydroxy group.

Thus, **4** was identified as 7, 4'-dihydroxy-3'-methoxyflavone (**4**, geraldone). The physical and spectral data of **4** were coincided with those in reference. [13)]

Compound **6** was obtained as a pale yellow oils and has a molecular formula of $C_{16}H_{12}O_5$ by observation of a quasi-molecular ion peak at *m*/z 285.0765 $[M+H]^+$ (calcd for $C_{16}H_{13}O_5$: 285.0763) in its HR-ESI-MS. Its UV spectrum showed absorption maxima at 249 (log ε 4.55) , 292 (4.45) and 339 nm (4.23, sh) characteristic of isoflavone, indicating that **6** was an isoflavone derivative. Its IR spectrum showed a band due to OH at 3238 cm^{-1}, indicating that **6** was an isoflavone possessing hydroxy group(s). Its ^{1}H and ^{13}C NMR spectra showed a singlet at δ_H 8.18 due to an oxygenated olefinic proton (H-2) characteristic of isoflavone and a signal at δ_C 153.4 due to oxygenated olefinic carbon (C-2) of isoflavone, supporting that **6** was the isoflavone derivative.

The ^{1}H and ^{13}C NMR spectra of **6** showed a carbonyl carbon signal at δ_C 176.7 due to a chromone skeleton, AMX pattern signals at δ_H 8.08 (1H, d, *J*=8.7 Hz), 6.96 (1H, dd, *J*=8.7, 2.2 Hz), and 6.87 (1H, d, *J*=2.2 Hz) due to aromatic protons, ABX pattern signals at δ_H 7.18 (1H, d, *J*=1.9 Hz), 6.98 (1H, dd, *J*=8.1, 1.9 Hz), and 6.87 (1H, d, *J*=8.1 Hz) due to aromatic protons, and signals at δ_C 163.4, 158.4, 147.4 and 146.5 due to oxygenated aromatic carbons and a singlet at δ_H 3.91 due to a methoxy group. These data indicated that **6** was an isoflavone derivative possessing one methoxy and two hydroxy groups and that both ring A and B were 1, 2, 4-trisubstitution aromatic rings. Full assignments of ^{1}H and ^{13}C NMR signals were performed by COSY, HSQC, and HMBC NMR analyses and analyses of coupling patterns (Tables 1 and 2). The AMX pattern signals including the doublet (1H, *J*=8.7 Hz) at δ_H 8.08 due to H-5 were identified as aromatic protons on A of ring, in suggesting no substitution at C-6 position, because a correlation between the doublet at δ_H 8.08 due to H-5 and a signal at δ_C 176.7 due to carbonyl carbon was observed in the HMBC

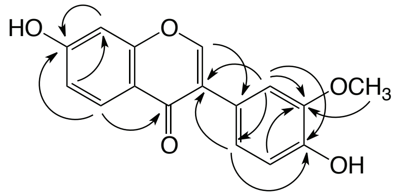


Fig. 3. Selected HMBC correlations of **6**.

spectrum (Fig. 3).The ABX pattern signals including doublet of doublet (1H, *J*=8.1, 1.9 Hz) at δ_H 6.98 were assigned as aromatic protons on B of ring, in indicating no substitution at C-5'position, because correlation the doublet of doublets at δH 6.98 due to H-6' and the signal due to C-3 aromatic carbon at δ_C 124.6 was observed in the HMBC spectrum (Fig. 3). Moreover, observation of the correlation between a singlet due to the methoxy protons and signal due to C-3' aromatic carbon at δ_C 147.4 revealed that the methoxy group was bonded to C-3'. The bond positions of two hydroxy groups were assigned to be C-7 and C-4'.

Thus, **6** was identified with 7, 4'-dihydroxy-3'-methoxyisoflavone (**6**). The physical and spectral data of **6** were agreement with those in reference. [14)]

Compound **3** was obtained as a pale yellow amorphous and has a molecular formula of $C_{16}H_{12}O_5$ by observation of quasi-molecular ion peak at *m*/z 285.0748 $[M+H]^+$ (calcd for $C_{16}H_{13}O_5$: 285.0763) in its HR-ESI-MS. Its UV spectrum showed absorption maxima at 250 (log ε 4.30) and 293 (4.23) characteristic of isoflavone, indicating that **3** was a isoflavone derivative. Its IR spectrum showed a band due to OH at 3175cm^{-1}, indicating that **3** was an isoflavone possessing hydroxy group(s). Its ^{1}H NMR spectrum showed a singlet at δ_H 8.14 due to H-2 characteristic of chromone skeleton of the isoflavone as the same as that of **6**. The ^{1}H and ^{13}C NMR spectra indicated the presence of 1, 2, 4-trisubstitution aromatic ring [AMX pattern signals: δ_H 8.07 (1H, d, *J*=8.8 Hz), 6.95 (1H, dd, *J*=8.8 2.2 Hz) and 6.86 (1H, d, *J*=2.2 Hz)], 1, 3, 5-trisubstituted aromatic ring [δ_H 7.06 (1H, s) and 6.98 (2H, each s)], a methoxy group [δ_H 3.90 (3H, s)] and a carbonyl group (δ_C 176.6).

Complete assignments of ^{1}H and ^{13}C NMR signals were archived by COSY, HMQC and HMBC NMR analyses and analyses of sprit patterns of the signals (Tables 1 and 2). In the HMBC spectrum (Fig. 4), doublet (1H, *J*=8.8 Hz) at δ_H 8.07 due to H-5 showed a cross peak to a signal at δ_C 176.6 due to a carbonyl carbon (C-4), indicating that the AMX pattern signals including the doublet at δ_H 8.07 due to H-5 were assigned to be aromatic protons of A ring and that suggested no substitution at C-6 position. Other aromatic proton signals were assigned to be those of B ring. The facts were supported by an observation of a correlation between singlet at δ_H 6.98 due to aromatic protons (H-2' and -6') and the signal at δ_C 124.8 due to C-3 in the HMBC spectrum (Fig. 4). Therefore, A ring was assigned to be

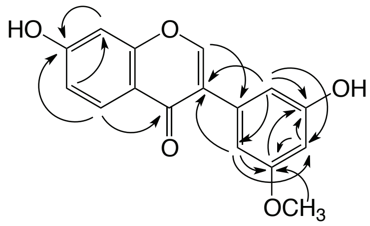


Fig. 4. Selected HMBC correlations of **3**.

1, 2, 4-trisubstitution aromatic ring and B ring to be 1, 3, 5-trisubstitution aromatic one. Moreover, a singlet at δ_H 3.90 due to the methoxy protons showed a cross peak to a signal at δ_C 147.8 due to C-5', indicating that the methoxy group was bonded to C-5'. Therefore, each of carbons at C-7 and C-3' was bonded to a hydroxy group.

Thus, **3** was elucidated to be 7, 3'-dihydroxy-5'-methoxyisoflavone (**3**). Isolation of **3** was reported, but no physical and spectral data was given.[15)]

Compound **1** was obtained as white powders and has a molecular formula of $C_{12}H_{16}N_2O$ by observation a quasi-molecular ion peak at *m/z* 205.1075 $[M+H]^+$ (calcd for $C_{12}H_{17}N_2O$: 205.1341) in its HR-ESI-MS. Compound **1** was positive to Dragendorff reagent, indicating that **1** was an alkaloid. Compound **1** was identified as *N*-methylcyticine.

The ^{1}H and ^{13}C NMR spectra indicated presences of a 1, 2, 3-trisubstituted aromatic ring [δ_H 7.27 (1H, dd, *J*=9.0, 6.6 Hz), 6.43 (1H, dd, *J*=9.0, 1.0 Hz) and 5.98 (1H, d, *J*=6.6 Hz), assigning to each *ortho*-coupled aromatic protons, and δ_C 138.6, 116.7 and 104.7], a methyl group [δ_H 2.12 (3H,s) and δ_C 46.2], four methylene groups [δ_H 4.04 (1H, d, *J*=15.4 Hz, Hα-10), 3.89 (1H, dd, *J*=15.4, 6.8, Hβ-10), 2.88 (1H, d, J=10.4, Hα-11), 2.83 (1H, dt, *J*=10.8, 1.4 Hz, Hα-13), 2.25 (1H, dd, *J*=10.8, 2.4 Hz, Hβ-13), 2.21 (1H, d, *J*=10.4 Hz, Hβ-11), 1.85 (1H, dd, *J*=12.7, 1.4, Hβ-8) and 1.73 (1H, dt, *J*=12.7, 2.4 Hz, Hα-8) and δ_C 62.5, 62.2, 50.0 and 25.4], two methyne groups [δ_H 2.93 (1H, t, *J*=2.4 Hz, H-7) and 2.42 (1H, m, H-9) and δ_C 35.5,and 28.0] and a carbonyl group (δ_C 163.7). Complete assignments of ^{1}H and ^{13}C NMR signals were performed by COSY, HSQC, and HMBC NMR analyses and analyses of coupling patterns (Tables 1 and 2). In the HMBC spectrum (Fig. 5), correlations were observed between a singlet at δ_H 2.12 due to the methyl group and

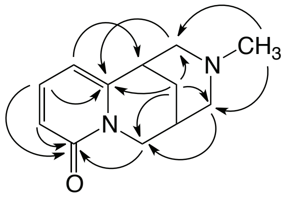


Fig. 5. Selected HMBC correlations of **1**.

both signals due to C-11 and C-13 at δ_C 62.2 and 62.5, indicating that the methyl group was bonded to the N-12 atom.

Thus, **1** was identified as *N*-methylcyticine (**1**). The spectral data of **1** were coincided with those in reference.[16)]

Compound **2** was obtained as a pale brown amorphous and had a molecular formula of $C_8H_8O_4$ by observation a quasi-molecular ion peak at *m/z* 167.0319 $[M+H]^+$ (calcd for $C_8H_9O_4$: 167.0344) in its HR-ESI-MS. The ^1H and ^{13}C NMR spectra of showed a singlet at δ_H 3.89 due to methyl protons of a methoxyl group, a carbonyl carbon signal at δ_C 167.4 due to a carboxyl group, ABX pattern signals at δ_H 6.91 (1H, d, *J*=8.3 Hz), 7.56 (1H, dd, *J*=8.3, 2.0 Hz), and 7.64 (1H, d, *J*=2.0Hz) due to aromatic protons, and signals at δ_C 143.1 and 148.8 due to aromatic carbons oxygenated. From these spectral data, **2** was identified as methyl 3, 4-dihydroxybenzoate (methyl protocatechuate, **2**). The spectral data of **2** were agreement with those in reference.[17)]

A number of flavonoids and isoflavonoids were isolated from aerial parts, roots, stems and stem barks of *S. tomentosa* and all of them were 5-hydroxy derivatives. Those isolated from mature seeds in this investigation were no substitution skeletons at C-5 position. Equol derived from isoflavone such as daidzein has estrogenic effect to human and animals.[18)] Daidzein possesses the structure similar to that (no substitution at C-5) isolated from mature seeds of *S. tomentosa*. The estrogenic effect was expected in the isoflavones from mature seeds of *S. tomentosa*.

Experimental

Analytical and preparative TLCs were carried out on Merck 60 F_{254} silica gel plate (thickness: 0.25 mm) and on the plates (thickness: 0.5 and 2.0 mm), respectively. Column chromatography (C.C.) and flash-column chromatography (F.C.C.) were carried out with Kieselgel 60 F_{254} (Merck). ^1H-, ^{13}C-, and two-dimensional NMR spectra were acquired on a Bruker Avance III 400 (^1H: 400 MHz, ^{13}C: 100 MHz) spectrometer in CD_3OD for **3**, **4**, **5** and **6** and in $CDCl_3$ for **1** and **2**. The symbols s, d, m, t, q and dd denote singlet, doublet, multiplet, triplet, quartet and doublet of doublets, respectively. HR-ESI-MS was obtained on a JEOL JMS-T100LP mass spectrometer.

Extraction and isolation. Fresh mature seeds (weight: 2.8 kg) of *Sophora tomentosa* collected at the campus of University of the Ryukyus, Okinawa-prefecture in April were ground in a blender and immersed in methanol (MeOH) for ca. 2 weeks. After filtration, the residue was re-extracted with MeOH at room temperature 3 times and at 60 ˚C for 8 hours 8 times. The MeOH soln combined were concentrated *in vacuo* into dryness to give tarry matters. The tarry matters obtained was successively extracted with hexane, chloroform ($CHCl_3$), ethyl acetate (EtOAc), 1-butanol (*n*-BuOH) and MeOH to give hexane- (50.6 g), $CHCl_3$- (23.8 g), EtOAc- (1.6 g), *n*-BuOH- (46.8g) and MeOH- soluble fractions (160.6 g), respectively. The $CHCl_3$- soluble fraction (23.8 g) was subjected to F.C.C. on silica gel (Si-gel) with solvent system of $CHCl_3$ increasing MeOH and H_2O as solvent ratio of 10:0:0, 9:1:0.1, 7:3:0.5, EtOAc-MeOH-H_2O (7:3:0.5) and EtOAc to give fractions A−E. As fraction B showed several absorption spots on analytical TLC under UV light at 254 nm and luminescence spots on analytical TLC under UV light at 365 nm, this fraction was subjected to F.C.C. on Si-gel with solvent system of $CHCl_3$-MeOH-H_2O (9:1:0.1) to give fractions B1-B5. The fraction B4 was re-chromatographed on Si-gel with $CHCl_3$-MeOH (12:1) to give fractions B451-B456. The fraction B453 was subject to preparative TLC on Si-gel with $CHCl_3$-acetone-MeOH (13:1:1) to give **1** (16 mg). Fraction B454 was subjected to C.C. on Si-gel to give fractions B4541-B4543. The fraction B4541 was subjected to preparative TLC on Si-gel with solvent system of $CHCl_3$-acetone (8:1) to give compounds **2** (4 mg) and **3** (7 mg). The fraction B4542 was subject to preparative TLC on si-gel with $CHCl_3$-acetone (8:1) to give **4** (6 mg), **5** (7 mg) and **6** (3 mg).

***N-methylcyticine* (1)**. White powders. Dragendorff reagent: positive. HR-ESI-MS: *m/z* 205.1075 $[M+H]^+$ (Calcd for $C_{12}H_{17}N_2O$: 205.1341). ^1H ($CDCl_3$, 400 MHz): Table 1; ^{13}C NMR ($CDCl_3$, 100 MHz): Table 2. These spectral data coincided with those in reference.[16)]

***Methyl 3, 4-dihydroxybenzoate* (2, *methyl protocatechuate*)**. Pale brown amorphous. HR-ESI-MS : *m/z* 167.0319 $[M+H]^+$ (Calcd for $C_8H_9O_4$: 167.0344). ^1H ($CDCl_3$, 400 MHz): Table 1; ^{13}C NMR ($CDCl_3$, 100 MHz): Table 2. These spectral data coincided with those in reference.[17)]

***7, 3'-dihydroxy-5'-methoxyisoflavone* (3).** Pale yellow amorphous. HR-ESI-MS: *m*/z 285.0748 $[M+H]^+$ (Calcd for $C_{16}H_{13}O_5$: 285.0763. UV: λmax (log ε) nm: 250 (4.30) and 293 (4.23). IR ν cm^{-1}: 3175 (OH); ^1H (CD_3OD, 400 MHz): Table 1; ^{13}C NMR (CD_3OD, 100 MHz): Table 2. Isolation of **3** was reported, but no physical and spectral data was given.[15)]

***7, 4'-dihydroxy-3'-methoxyflavone* (4, *geraldone*).** Pale yellow amorphous. HR-ESI-MS: *m/z* 285.0759 $[M+H]^+$ (Calcd for $C_{16}H_{13}O_5$: 285.0763). UV: λmax (log ε) nm: 241 (4.47) 290 (4.42) and 359 (4.44). IR ν cm^{-1}: 3297 (OH) ; ^1H (CD_3OD, 400 MHz): Table 1; ^{13}C NMR (CD_3OD, 100 MHz): Table 2. These physical and spectral data coincided with those in references. [13)]

***7, 3'-dihydroxy-4'-methoxyflavone* (5, *farnisin*).** Pale yellow amorphous. HR-ESI-MS: *m*/z 285.0741 $[M+H]^+$ (Calcd for $C_{16}H_{13}O_5$: 285.0763). UV: λmax (log ε) nm: 240 (3.99), 290 (3.99, sh) and 341 (4.08). IR ν cm^{-1}: 3364 (OH); ^1H (CD_3OD, 400 MHz): Table 1; ^{13}C NMR (CD_3OD, 100 MHz): Table 2. These physical and spectral data coincided with those in reference. [12)]

***7, 4'-dihydroxy-3'-methoxyisoflavone* (6).** Pale yellow oils. HR-ESI-MS: *m*/z 285.0765 $[M+H]^+$ (Calcd for $C_{16}H_{13}O_5$: 285.0763. UV: λmax (log ε) nm: 249 (4.55), 292 (4.45) and 339 (4.23, sh). IR ν cm^{-1}: 3238 (OH); ^1H (CD_3OD, 400 MHz): Table 1; ^{13}C NMR (CD_3OD, 100 MHz): Table 2. These spectral data coincided with those in references. [14)]

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