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## 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*.<sup>1-8)</sup> 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.<sup>9-10)</sup> The review of flavonoids in *Sophora* species described that these flavonoids have antitumor, antimicrobial, anti-HIV, radical scavenging and enzyme inhibitory activities.<sup>11)</sup>

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.

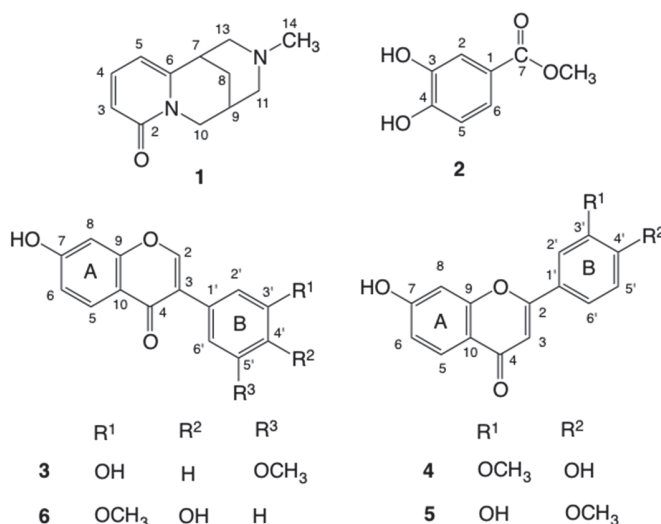
<sup>1</sup>

### 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<sub>16</sub>H<sub>12</sub>O<sub>5</sub> by observation of a quasi-molecular ion peak at *m/z* 285.0741 [M+H]<sup>+</sup> (calcd for C<sub>16</sub>H<sub>13</sub>O<sub>5</sub>: 285.0763) in the high resolution electron spray ionization mass spectrum (HR-ESI-MS). Its UV spectrum showed absorption maxima at 240 (log  $\epsilon$  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<sup>-1</sup>, indicating that **5** was a flavone possessing hydroxy group(s).



Structures

<sup>1</sup> Received: January 22, 2016

Table 1. <sup>1</sup>H NMR spectral data of 1–6

H	1	2	3	4	5	6
	in CDCl <sub>3</sub>	in CDCl <sub>3</sub>	in CD <sub>3</sub> OD	in CD <sub>3</sub> OD	in CD <sub>3</sub> OD	in CD <sub>3</sub> OD
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 <sub>3</sub> -4'					3.90 s	
OCH <sub>3</sub> -3'				3.87 s		3.91 s
OCH <sub>3</sub> -5'			3.90 s			
OCH <sub>3</sub> -7		3.89 s				

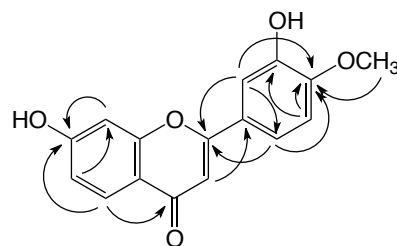
\* Coupling constants (*J*) are expressed in Hz.

The <sup>1</sup>H and <sup>13</sup>C NMR spectra of **5** showed a carbonyl carbon signal at δ<sub>C</sub> 178.8 due to a chromone skeleton, ABX pattern signals at δ<sub>H</sub> 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<sub>H</sub> 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<sub>C</sub> 163.4, 158.4, 147.4 and 146.5 due to oxygenated aromatic carbons and a singlet due to a methoxy group at δ<sub>H</sub> 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 <sup>1</sup>H and <sup>13</sup>C NMR signals were performed by COSY, HSQC and HMBC NMR analyses and analyses of split patterns of signals (Tables 1 and 2). The ABX pattern signals including doublet (1H, *J*=8.7 Hz) at δ<sub>H</sub> 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<sub>H</sub> 7.93 and a signal due to carbonyl carbon at δ<sub>C</sub> 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<sub>H</sub>

Table 2. <sup>13</sup>C NMR spectral data of 1–6

C	1	2	3	4	5	6
	in CDCl <sub>3</sub>	in CDCl <sub>3</sub>	in CD <sub>3</sub> OD	in CD <sub>3</sub> OD	in CD <sub>3</sub> OD	in CD <sub>3</sub> OD
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 <sub>3</sub> -4'					55.1	
OCH <sub>3</sub> -3'				55.3		55.0
OCH <sub>3</sub> -5'			55.0			
OCH <sub>3</sub> -7		52.2				

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<sub>H</sub> 7.37 due to H-2' and the doublet of doublets at δ<sub>H</sub> 7.47 due to H-6' showed



correlation to the signal at δ<sub>C</sub> 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<sub>H</sub> 3.90 due to the methoxy proton and signal at δ<sub>C</sub> 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.<sup>12)</sup>

Compound **4** was obtained as a pale yellow amorphous and had a molecular formula of C<sub>16</sub>H<sub>12</sub>O<sub>5</sub> by observation of quasi-molecular ion peak at *m/z* 285.0759 [M+H]<sup>+</sup> (calcd for C<sub>16</sub>H<sub>13</sub>O<sub>5</sub>: 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  $\text{cm}^{-1}$ , indicating that **4** was a flavone possessing hydroxy group(s).

The  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra showed AMX pattern signals [ $\delta_{\text{H}}$  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 [ $\delta_{\text{H}}$  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 [ $\delta_{\text{H}}$  3.87 (3H, s)] and a carbonyl group ( $\delta_{\text{C}}$  178.9).

Complete assignments of  $^1\text{H}$  and  $^{13}\text{C}$  NMR signals were archived by COSY, HMQC and HMBC NMR analyses and analyses of split patterns of the signals (Tables 1 and 2). In the HMBC spectrum (Fig. 2), doublet (1H,  $J=8.7$  Hz) at  $\delta_{\text{H}}$  7.87 due to H-5 showed a cross peak to a signal due to a carbonyl carbon (C-4) at  $\delta_{\text{C}}$  178.9, indicating that the AMX pattern signals including the doublet due to H-5 at  $\delta_{\text{H}}$  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  $\delta_{\text{H}}$  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  $\delta_{\text{H}}$  7.41 due to H-2' and the doublet of doublets at  $\delta_{\text{H}}$  7.43 due to H-6' and the signal at

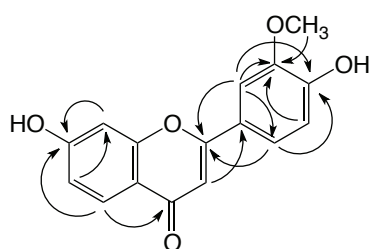


Fig. 2. Selected HMBC correlations of **4**.

$\delta_{\text{C}}$  164.5 due to C-2 in the HMBC spectrum (Fig. 2).

Moreover, a singlet at  $\delta_{\text{H}}$  3.87 due to the methoxy protons showed a cross peak to a signal at  $\delta_{\text{C}}$  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.<sup>13)</sup>

Compound **6** was obtained as a pale yellow oils and has a molecular formula of  $\text{C}_{16}\text{H}_{12}\text{O}_5$  by observation of a quasi-molecular ion peak at  $m/z$  285.0765  $[\text{M}+\text{H}]^+$  (calcd for  $\text{C}_{16}\text{H}_{13}\text{O}_5$  : 285.0763) in its HR-ESI-MS. Its UV spectrum showed absorption maxima at 249 (log  $\epsilon$  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  $\text{cm}^{-1}$ , indicating that **6** was an isoflavone possessing hydroxy group(s). Its  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra showed a singlet at  $\delta_{\text{H}}$  8.18 due to an oxygenated olefinic proton (H-2) characteristic of isoflavone and a signal at  $\delta_{\text{C}}$  153.4 due to oxygenated olefinic carbon (C-2) of isoflavone, supporting that **6** was the isoflavone derivative.

The  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra of **6** showed a carbonyl carbon signal at  $\delta_{\text{C}}$  176.7 due to a chromone skeleton, AMX pattern signals at  $\delta_{\text{H}}$  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  $\delta_{\text{H}}$  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  $\delta_{\text{C}}$  163.4, 158.4, 147.4 and 146.5 due to oxygenated aromatic carbons and a singlet at  $\delta_{\text{H}}$  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  $^1\text{H}$  and  $^{13}\text{C}$  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  $\delta_{\text{H}}$  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  $\delta_{\text{H}}$  8.08 due to H-5 and a signal at  $\delta_{\text{C}}$  176.7 due to carbonyl carbon was observed in the HMBC

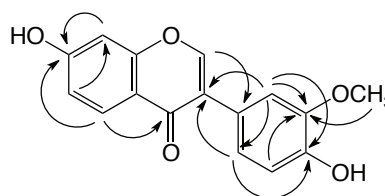


Fig. 3. Selected HMBC correlations of **6**.

spectrum (Fig. 3). The ABX pattern signals including doublet of doublet (1H,  $J=8.1, 1.9$  Hz) at  $\delta_{\text{H}}$  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  $\delta_{\text{H}}$  6.98 due to H-6' and the signal due to C-3 aromatic carbon at  $\delta_{\text{C}}$  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  $\delta_{\text{C}}$  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.<sup>14)</sup>

Compound **3** was obtained as a pale yellow amorphous and has a molecular formula of  $\text{C}_{16}\text{H}_{12}\text{O}_5$  by observation of quasi-molecular ion peak at  $m/z$  285.0748  $[\text{M}+\text{H}]^+$  (calcd for  $\text{C}_{16}\text{H}_{13}\text{O}_5$ : 285.0763) in its HR-ESI-MS. Its UV spectrum showed absorption maxima at 250 (log  $\epsilon$  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  $3175\text{cm}^{-1}$ , indicating that **3** was an isoflavone possessing hydroxy group(s). Its  $^1\text{H}$  NMR spectrum showed a singlet at  $\delta_{\text{H}}$  8.14 due to H-2 characteristic of chromone skeleton of the isoflavone as the same as that of **6**. The  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra indicated the presence of 1, 2, 4-trisubstitution aromatic ring [AMX pattern signals:  $\delta_{\text{H}}$  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 [ $\delta_{\text{H}}$  7.06 (1H, s) and 6.98 (2H, each s)], a methoxy group [ $\delta_{\text{H}}$  3.90 (3H, s)] and a carbonyl group ( $\delta_{\text{C}}$  176.6).

Complete assignments of  $^1\text{H}$  and  $^{13}\text{C}$  NMR signals were archived by COSY, HMQC and HMBC NMR analyses and analyses of split patterns of the signals (Tables 1 and 2). In the HMBC spectrum (Fig. 4), doublet (1H,  $J=8.8$  Hz) at  $\delta_{\text{H}}$  8.07 due to H-5 showed a cross peak to a signal at  $\delta_{\text{C}}$  176.6 due to a carbonyl carbon (C-4), indicating that the AMX pattern signals including the doublet at  $\delta_{\text{H}}$  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  $\delta_{\text{H}}$  6.98 due to aromatic protons (H-2' and -6') and the signal at  $\delta_{\text{C}}$  124.8 due to C-3 in the HMBC spectrum (Fig. 4). Therefore, A ring was assigned to be

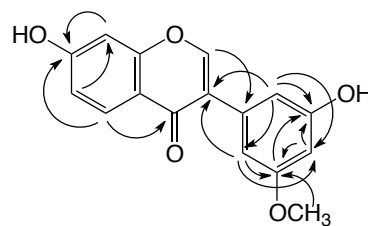


Fig. 4. Selected HMBC correlations of **3**.

1, 2, 4-trisubstitution aromatic ring and B ring to be 1, 3, 5-trisubstitution aromatic one. Moreover, a singlet at  $\delta_{\text{H}}$  3.90 due to the methoxy protons showed a cross peak to a signal at  $\delta_{\text{C}}$  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.<sup>15)</sup>

Compound **1** was obtained as white powders and has a molecular formula of  $\text{C}_{12}\text{H}_{16}\text{N}_2\text{O}$  by observation a quasi-molecular ion peak at  $m/z$  205.1075  $[\text{M}+\text{H}]^+$  (calcd for  $\text{C}_{12}\text{H}_{17}\text{N}_2\text{O}$ : 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*-methylcycitine.

The  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra indicated presences of a 1, 2, 3-trisubstituted aromatic ring [ $\delta_{\text{H}}$  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  $\delta_{\text{C}}$  138.6, 116.7 and 104.7], a methyl group [ $\delta_{\text{H}}$  2.12 (3H, s) and  $\delta_{\text{C}}$  46.2], four methylene groups [ $\delta_{\text{H}}$  4.04 (1H, d,  $J=15.4$  Hz, H $\alpha$ -10), 3.89 (1H, dd,  $J=15.4, 6.8$ , H $\beta$ -10), 2.88 (1H, d,  $J=10.4$ , H $\alpha$ -11), 2.83 (1H, dt,  $J=10.8, 1.4$  Hz, H $\alpha$ -13), 2.25 (1H, dd,  $J=10.8, 2.4$  Hz, H $\beta$ -13), 2.21 (1H, d,  $J=10.4$  Hz, H $\beta$ -11), 1.85 (1H, dd,  $J=12.7, 1.4$ , H $\beta$ -8) and 1.73 (1H, dt,  $J=12.7, 2.4$  Hz, H $\alpha$ -8) and  $\delta_{\text{C}}$  62.5, 62.2, 50.0 and 25.4], two methyne groups [ $\delta_{\text{H}}$  2.93 (1H, t,  $J=2.4$  Hz, H-7) and 2.42 (1H, m, H-9) and  $\delta_{\text{C}}$  35.5, and 28.0] and a carbonyl group ( $\delta_{\text{C}}$  163.7). Complete assignments of  $^1\text{H}$  and  $^{13}\text{C}$  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  $\delta_{\text{H}}$  2.12 due to the methyl group and



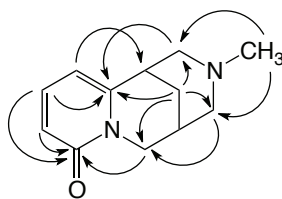


Fig. 5. Selected HMBC correlations of **1**.

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

Thus, **1** was identified as *N*-methylcytisine (**1**). The spectral data of **1** were coincided with those in reference.<sup>16)</sup>

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  $\delta_H$  3.89 due to methyl protons of a methoxyl group, a carbonyl carbon signal at  $\delta_C$  167.4 due to a carboxyl group, ABX pattern signals at  $\delta_H$  6.91 (1H, d,  $J=8.3$  Hz), 7.56 (1H, dd,  $J=8.3$ , 2.0 Hz), and 7.64 (1H, d,  $J=2.0$ Hz) due to aromatic protons, and signals at  $\delta_C$  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.<sup>17)</sup>

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.<sup>18)</sup> 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<sub>254</sub> 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<sub>254</sub> (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-methylcysticine (1)***. White powders. Dragendorff reagent: positive. HR-ESI-MS:  $m/z$  205.1075 [M+H]<sup>+</sup> (Calcd for C<sub>12</sub>H<sub>17</sub>N<sub>2</sub>O: 205.1341). <sup>1</sup>H (CDCl<sub>3</sub>, 400 MHz): Table 1; <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz): Table 2. These spectral data coincided with those in reference.<sup>16)</sup>

***Methyl 3, 4-dihydroxybenzoate (2, methyl protocatechuate)***. Pale brown amorphous. HR-ESI-MS:  $m/z$  167.0319 [M+H]<sup>+</sup> (Calcd for C<sub>8</sub>H<sub>9</sub>O<sub>4</sub>: 167.0344). <sup>1</sup>H (CDCl<sub>3</sub>, 400 MHz): Table 1; <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz): Table 2. These spectral data coincided with those in reference.<sup>17)</sup>

***7, 3'-dihydroxy-5'-methoxyisoflavone (3)***. Pale yellow amorphous. HR-ESI-MS:  $m/z$  285.0748 [M+H]<sup>+</sup> (Calcd for C<sub>16</sub>H<sub>13</sub>O<sub>5</sub>: 285.0763). UV:  $\lambda_{\max}$  (log  $\epsilon$ ) nm: 250 (4.30) and 293 (4.23). IR  $\nu_{\text{cm}^{-1}}$ : 3175 (OH); <sup>1</sup>H (CD<sub>3</sub>OD, 400 MHz): Table 1; <sup>13</sup>C NMR (CD<sub>3</sub>OD, 100 MHz): Table 2. Isolation of **3** was reported, but no physical and spectral data was given.<sup>15)</sup>

***7, 4'-dihydroxy-3'-methoxyflavone (4, geraldone)***. Pale yellow amorphous. HR-ESI-MS:  $m/z$  285.0759 [M+H]<sup>+</sup> (Calcd for C<sub>16</sub>H<sub>13</sub>O<sub>5</sub>: 285.0763). UV:  $\lambda_{\max}$  (log  $\epsilon$ ) nm: 241 (4.47) 290 (4.42) and 359 (4.44). IR  $\nu_{\text{cm}^{-1}}$ : 3297 (OH); <sup>1</sup>H (CD<sub>3</sub>OD, 400 MHz): Table 1; <sup>13</sup>C NMR (CD<sub>3</sub>OD, 100 MHz): Table 2. These physical and spectral data coincided with those in references.<sup>13)</sup>

***7, 3'-dihydroxy-4'-methoxyflavone (5, farnisin)***. Pale yellow amorphous. HR-ESI-MS:  $m/z$  285.0741 [M+H]<sup>+</sup> (Calcd for C<sub>16</sub>H<sub>13</sub>O<sub>5</sub>: 285.0763). UV:  $\lambda_{\max}$  (log  $\epsilon$ ) nm: 240 (3.99), 290 (3.99, sh) and 341 (4.08). IR  $\nu_{\text{cm}^{-1}}$ : 3364 (OH); <sup>1</sup>H (CD<sub>3</sub>OD, 400 MHz): Table 1; <sup>13</sup>C NMR (CD<sub>3</sub>OD, 100 MHz): Table 2. These physical and spectral data coincided with those in reference.<sup>12)</sup>

***7, 4'-dihydroxy-3'-methoxyisoflavone (6)***. Pale yellow oils. HR-ESI-MS:  $m/z$  285.0765 [M+H]<sup>+</sup> (Calcd for C<sub>16</sub>H<sub>13</sub>O<sub>5</sub>: 285.0763). UV:  $\lambda_{\max}$  (log  $\epsilon$ ) nm: 249 (4.55), 292 (4.45) and 339 (4.23, sh). IR  $\nu_{\text{cm}^{-1}}$ : 3238 (OH); <sup>1</sup>H (CD<sub>3</sub>OD, 400 MHz): Table 1; <sup>13</sup>C NMR (CD<sub>3</sub>OD, 100 MHz): Table 2. These spectral data coincided with those in references.<sup>14)</sup>

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