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Trypanocidal Flavonoids from *Sophora flavescens*

Kenji Matsuo,[a] Michiho Ito,[a] Gisho Honda,*[,a] Tran Kim Qui,[b] Fumiyuki Kiuchi[a,†]

[a]*Graduate School of Pharmaceutical Sciences, Kyoto University, 46-29 Yoshida, Sakyo-ku, Kyoto, 606-8501, Japan*
[b]*Research Center for Applied Chemistry, Vietnam National University, Ho Chi Min City, Vietnam*

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The acetone extract of *Sophora flavescens* Aiton (Leguminosae) exhibited lethal activity against *Trypanosoma cruzi*. Column chromatographic separation of the extract guided by trypanocidal activity afforded a new prenylated flavanone (**4**), together with nine known flavonoids: sophoraflavanone G (**1**), (–)-kurarinone (**2**), kushenol L (**3**), 2'-methoxykurarinone (**5**), 7,4'-dihydroxy-5-methoxy-8-(γ,γ-dimethylallyl)-flavanone (**6**), leachianone A (**7**), 8-prenylnaringenin (**8**), noranhydroicaritin (**9**) and alopecurone G (**10**). The structure of the new flavanone **4** was determined on the basis of spectroscopic analyses. The minimum lethal concentrations of these compounds against epimastigotes of *T. cruzi* were 3.7 μM (**1**), 14 μM (**2**), 7.1 μM (**3**), 7.2 μM (**4**), 6.9 μM (**5**), 71 μM (**6**), 5.5 μM (**7**), 18 μM (**8**), 4.4 μM (**9**) and 3.6 μM (**10**).

Keywords: *Sophora flavescens, Trypanosoma cruzi,* flavonoid, Leguminosae

The dried roots of *Sophora flavescens* Aiton (Leguminosae) are used in Asian countries for treatment of gastrointestinal disorders, diarrhea, fever, pain, and skin parasites.[1] In our screening of natural medicines used in Vietnam for trypanocidal activity against *T. cruzi*, the ethiologic agent of Chagas' disease,[2] we found that the acetone extract of the dried roots of this plant, which is called "Khô sâm Bắc" in Vietnam,[3] showed lethality against epimastigotes of this parasite. In this paper, we report the identification of the trypanocidal constituents of *S. flavescens*.

EXPERIMENTAL

General procedures Melting points were determined on a Yanagimoto micro melting point apparatus. Optical rotation ($[\alpha]_D$) and CD spectrum were measured on JASCO DIP-370 polarimeter and JASCO J-720 spectropolarimeter, respectively. ^{1}H- and ^{13}C-NMR spectra were measured on a JEOL JNM-LA500 spectrometer with TMS as an internal standard and chemical shifts were recorded in δ ppm. Fuji Silysia BW-127ZH silica gel and Cosmosil 75C$_{18}$-Prep (Nacalai Tesque) were used for column chromatography.

Plant materials Khô sâm Bắc (dried roots of *S. flavescens*) was purchased from a market in Ho Chi Min city in June 2000.

Extraction and isolation Dried roots of *S. flavescens* (2 kg) was extracted with acetone under reflux (3 h × 3 times) and the extract was concentrated under reduced pressure to give 68.6 g of dark brown residue (MLC= 6.25 μg/ml). A part of the residue (5.2 g) was applied to a silica gel column and eluted with $CHCl_3$-MeOH=19:1 to give 7 fractions. Fraction 4 (716 mg, MLC=6.25 μg/ml) was separated by silica gel column chromatography (hexane-AcOEt-MeOH=3:2:0.1) and crystallized from benzene to give sophoraflavanone G (**1**)[4] (60 mg). Fraction 5 (1.1 g, MLC=12.5 μg/ml) was subjected to silica gel column chromatography with hexane-acetone=1:1 and purified by Lobar RP-18 column (MeOH-H_2O=7:3) to give (–)-kurarinone (**2**)[5] (389 mg). Another part of the acetone extract (58.2 g) was separated by silica gel column chromatography ($CHCl_3$-MeOH=19:1, 9:1 and MeOH) to give 8 fractions. Fraction 5 (7.6 g, MLC=6.25 μg/ml) was separated by silica gel column chromatography (hexane-AcOEt-MeOH=100:100:2.5, 10:20:1 and

† Present address: Tsukuba Medicinal Plant Research Station, National Institute of Health Sciences, Ministry of Health, Labour and Welfare, 1 Hachimandai, Tsukuba 305-0843, Japan

MeOH) to give 6 fractions. The second fraction (fraction 5-2, 2.6 g) was separated by silica gel (benzene-AcOEt=2:1, $CHCl_3$-AcOEt=3:2), Lobar RP-18 (MeOH-H_2O=7:3) and Sephadex LH-20 (MeOH) to give kushenol L (**3**)[6] (10 mg). Fractionation of the fourth (fr. 5-4, 317 mg) and fifth (fr. 5-5, 1.1 g) fractions by silica gel (ether-acetone=15:1, hexane-acetone=5:3, $CHCl_3$-acetone=15:1, hexane-AcOEt=1:1) and Lobar RP-18 (MeOH-H_2O=8:2) gave a new flavanone (**4**) (11 mg), and (2*S*)-2'-methoxykurarinone (**5**)[5] (111 mg) and (2*S*)-7,4'-dihydroxy-5-methoxy-8-(γ,γ-dimethylallyl)-flavanone (**6**),[7] respectively. Further separation of fraction 4 (2.9 g) by combinations of silica gel and ODS as described above gave leachianone A (**7**)[8] (42 mg), 8-prenylnaringenin (sophoraflavanone B, **8**)[9] (11 mg), noranhydroicaritin (**9**)[10] (8 mg), and alopecurone G (**10**)[11] (15 mg).

Compound 4: Pale yellow amorphous, mp 126-127°C. $[\alpha]_D$ −105.2° (*c*=0.54, MeOH). CD (MeOH) λ_{ext} 333.7 nm (Δε +4.11), 312.1 (0.0), 290.7 (−13.3). IR (KBr) cm^{-1}: 3306, 2974, 2928, 1655, 1605, 1582, 1462, 1300, 1107. UV (MeOH) λ_{max} nm (log ε): 224 (sh, 4.31), 288 (4.19). FAB-MS *m/z*: 439.2116 ($M+H^+$, Calcd for $C_{26}H_{31}O_6$: 439.2120). ^{1}H- and ^{13}C-NMR in acetone-d_6: see Table 1.

Trypanocidal assay Trypanocidal assays were performed as described previously.[12] Each assay was performed in duplicate. The minimum lethal concentration (MLC) of a positive control, gentian violet, was 6.3 μM under this condition.

RESULTS AND DISCUSSION

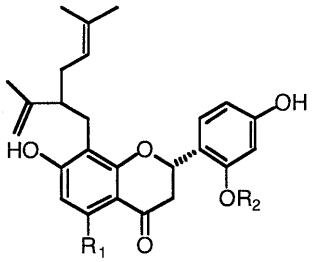
Fractionation of the acetone extract of the roots of *S. flavescens* under the guidance of trypanocidal activity resulted in the isolation of ten trypanocidal compounds. Of the ten constituents isolated, nine were known flavonoids; sohporaflavanone G (**1**),[4] (−)-kurarinone (**2**),[5] kushenol L (**3**),[6] (2*S*)-2'-methoxy-kurarinone (**5**),[5] (2*S*)-7,4'-dihydroxy-5-methoxy-8-(γ,γ-dimethyl-allyl)-flavanone (**6**),[7] leachianone A (**7**),[8] 8-prenylnaringenin (sophoraflavanone B, **8**),[9] noranhydroicaritin (**9**),[10] and alopecurone G (**10**).[11] Their structures were confirmed by comparison of their spectral data with those reported.

Compound **4** was obtained as a pale yellow amorphous. Its molecular formula was established as $C_{26}H_{30}O_6$ by high resolution FAB-MS. The ^{1}H- and ^{13}C-NMR spectra (Table 1) were similar to those of leachianone B (**11**),[13] except for the chemical shift of the carbonyl carbon: the carbonyl carbon of **4** appeared higher field at δ 189.0 than that of leachianone B (**11**) at δ 198.3. Analyses of HMQC and HMBC spectra (Table 1) revealed that the methoxy group in **4** was at C-5 instead of C-2' in **11**. Thus, the structure was concluded as indicated. The stereochemistry at C-2 was concluded to be *S* from the positive Cotton effect in its CD spectrum.[14] However, the stereochemistry at C-2" could not be determined. The MLC of the isolated compounds against epimastigotes of *T. cruzi* were 3.7 μM (**1**), 14 μM (**2**), 7.1 μM (**3**), 7.2 μM (**4**), 6.9 μM (**5**), 71 μM (**6**), 5.5 μM (**7**), 18 μM (**8**), 4.4 μM (**9**), 3.6 μM (**10**). Alopecurone G (**10**) showed the most potent activity, however, the content of this compound was small. Taking the contents of the trypanocidal compounds into account, the major part of the trypanocidal activity of the extract can be ascribed to sophoraflavanone G (**1**) and (−)-kurarinone (**2**). Although some flavonoids have been reported to have trypanocidal activity,[15-17] this is the first report of

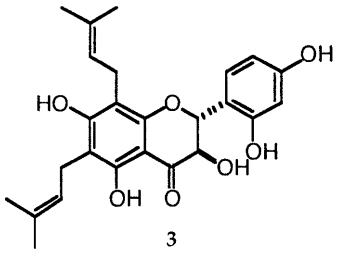
Table 1. NMR Data of Compound **4** in acetone-d_6

No.	**11**	**4**		
	$^{13}C^a$	^{13}C	$^1H^b$	HMBC^c
2	75.7	75.2	5.64 (dd, *J*=13.1, 2.8)	1', 2', 6'
3	43.0	45.4	2.65 (dd, *J*=16.2, 2.8)	4, 10
			2.85 (dd, *J*=16.2, 13.1)	2, 4, 1'
4	198.3	189.0	-	
5	163.0	161.2	-	
6	97.7	94.2	6.00 (s)	5, 7, 8, 10
7	163.6	160.5	-	
8	103.8	102.7	-	
9	161.9	162.8	-	
10	103.4	106.0	-	
1'	119.5	118.2	-	
2'	159.3	156.1	-	
3'	100.5	103.5	6.47 (d, *J*=2.5)	1', 2', 4', 5'
4'	160.5	159.3	-	
5'	108.5	107.8	6.42 (dd, *J*=8.2, 2.5)	1', 3'
6'	128.8	128.2	7.32 (d, *J*=8.3)	2, 2', 4'
1"	23.0	22.8	2.21 (dd, *J*=16.8, 9.8)	7, 8, 9, 2",3", 8"
			2.72 (dd, *J*=16.8, 5.5)	7, 8, 9, 2", 8"
2"	42.3	41.8	1.69 (m)	8"
3"	30.5	30.1	1.85 (m)	2", 4", 5"
			2.27 (m)	
4"	124.0	123.4	5.18 (t-like)	6", 7"
5"	134.0	133.5	-	
6"	26.4	25.9	1.66 (3H, s)	4", 5", 7"
7"	18.3	17.9	1.57 (3H, s)	4", 5", 6"
8"	80.6	79.6	-	
9"	21.6	21.3	1.22 (3H, s)	2", 8", 10"
10"	28.3	27.8	1.41 (3H, s)	2", 8", 9"
OMe	56.4	55.9	3.76 (3H, s)	5
2'-OH			8.59 (s)	2', 1'
4'-OH			8.34 (s)	3', 5'

[a] In DMSO-d_6 (from ref. 13); [b] *J* values in Hz; [c] carbons correlated to the proton.



1 : R_1=OH; R_2=H
2 : R_1=OMe; R_2=H
5 : R_1=OMe; R_2=Me
7 : R_1=OH; R_2=Me
10 : R_1=H; R_2=Me



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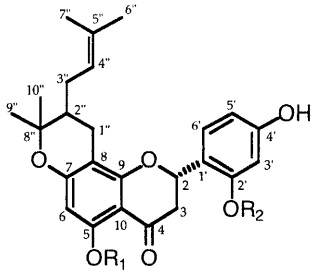
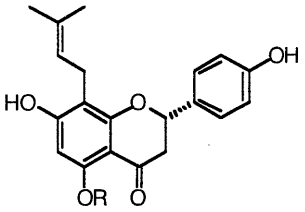
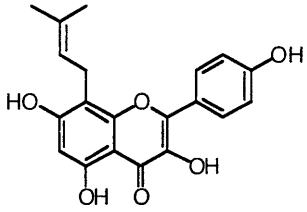


4 : R_1=Me; R_2=H
11 : R_1=H; R_2=Me



6 : R=Me
8 : R=H



9

trypanocidal activity for prenylated flavonoids. Among the isolated flavanones, those with a lavandulyl group (**1**, **2**, **4**, **5**, **7**, **10**) showed stronger activity compared to those with a prenyl group (**6**, **8**), suggesting that prenyl groups may play an important role in the activity. In fact, naringenin, a flavanone without any prenyl group, showed only a very weak trypanocidal activity (MLC=370 μM).

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