Arch. Pharm. Res. **14**(2), 105-108 (1991)

Insecticidal Isoflavon Glycoside from *Maackia amurensis*

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(Received January 3, 1991)

Abstract □ An insecticidal isoflavon glycoside was isolated from the roots of *Maackia ammurensis*. Its structure was shown to be formononetin-7-*O*-β glucosyl[1-6]glucoside[1] by chemical and spectroscopic methods and to have insecticidal activities against Brown planthopper female adults by spray and topical applications.

Keywords □ *Maackia amurensis*, formononetin, brown planthopper, spray method, topical application.

In our search for natural insecticides, the extract of *Maackia amurensis* roots was shown to possess insecticidal properties. The crude methanol extract of *M. amurensis* exhibited 88% mortality against brown planthopper (BPH) at 1% concentration (Table II). The insecticidal components were transferred to the *n*-butanol fraction and their insecticidal activities were evaluated (Table III). Each fraction was very toxic against BPH and the mode of action was speculated as contact and stomach poison. Especially the fractions 4 & 5 showed distinguished activities and the insecticidal principal (Compound **1**) of the fractions was purified and isolated. Compound **1** afforded aglycon and sugars on acid hydrolysis and monoglycoside[**2**] on partial hydrolysis. The ^{13}C-NMR spectrum of aglycon[**3**] was exactly same as that of formononetin[1-3] and was confirmed by co-IR and co-mp with authentic sample. The sugar moiety was proved to be D-glucose by paper chromatography and the β-linkage was revealed by coupling constant (6.8 Hz)[4]. In the ^{13}C-NMR spectrum of **1**, all carbon multiplicities were determined by DEPT, and the down field shift of 6′ carbon of inner glucose revealed that the inner glucosidic linkage was [1-6] pattern (Table I). The effect of glycosylation of 7-OH on the C-7 signal was same as reported value (1.4 ppm)[5]. Because glycosylation of sugar hydroxyls produces a sizable down field shift in the resonance of the hydroxylated carbon, we could determine the interglycosidic linkage by the chemical shift changes[6]. In the mass spectrum of peracetate of **1**, *m/z* 619 ion accorded with peracetate of two glucose moieties and terminal glucose ion (*m/z* 331) was also detected[7,8]. The ^{1}H-^{1}H cosy and ^{1}H-^{13}C cosy spectral data were consistent with those of the proposed structure. Although the interglycosidic linkage was not established at all, formononetin-7-*O*-β-glucosylglucoside was already reported as a new compound from *Cladrastis platycarpa*[9] and isolated first time from the title plant.

EXPERIMENTAL METHODS

Plant materials

The dried roots (6 kg) of *M. amurensis* were collected in Kyungki-Do province of Korea in spring, 1989.

General experimental procedure

All melting points were determined on a Buchi 510 and uncorrected. The following instruments were used: NMR; JNM-GSX 270 and JNM-GSX 500 FT NMR spectrometer with TMS as an internal reference. HPLC; Waters Model 510 with gradient controller and programmable multiple wavelength detector, prep-HPLC; Waters Delta Prep 3000, Mass; JMS-DX 300 mass spectrometer, IR; Digilab fts-80. For column chromatography, Kieselgel (230-400 mesh, Merck) was used. TLC was performed on Kieselgel G (Merck) using the lower phase of

Table I. CMR data of compounds 1, 2 and 3 (ppm, DMSO)

Carbon No.	Compound 1	Compound 2	Compound 3
C-1′	123.74	123.39	123.35
C-2′	130.42	130.03	130.17
C-3′	113.99	113.62	113.64
C-4′	159.38	159.03	159.2
C-5′	113.99	113.62	113.64
C-6′	130.42	130.03	130.17
C-2	153.83	153.5	152.92
C-3	124.37	124.01	124.44
C-4	175.19	174.66	174.2
C-5	127.24	126.92	127.35
C-6	115.13	115.5	115.25
C-7	162.71	161.46	162.71
C-8	101.47	103.47	102.3
C-8a	157.79	157.01	157.7
C-4a	118.83	118.5	117.01
OMe	55.5	55.13	55.19
Glc-1′	100.35	100.2	
Glc-2′	73.56	73.17	
Glc-3′	76.83	76.51	
Glc-4′	70.3	69.71	
Glc-5′	76.83	77.22	
Glc-6′	69.33	60.7	
Glc-1″	104.47		
Glc-2″	74.05		
Glc-3″	76.07		
Glc-4″	70.62		
Glc-5″	77.1		
Glc-6″	61.54		

$CHCl_3$-MeOH-H_2O (65:35:10) as a solvent system and detection was performed with following agents: for aglycon and glycosides; spraying with 10% H_2SO_4 followed by heating, for sugars; aniline hydrogen phthalate reagent.

Extraction and isolation

The roots were extracted with MeOH (5 *l*, twice, 2 hr) at 80℃. The extracts were combined and concentrated *in vacuo* to give brown residue (170g), which was suspended with H_2O (1.5 *l*) and subsequently extracted with *n*-hexane (fraction 1), ether (fraction 2), EtOAc (fraction 3), *n*-BuOH saturated with H_2O (fraction 4). The *n*-BuOH solution was evaporated to give *n*-BuOH soluble fraction (27g). The fraction was developed on silica gel column chromatography with $CHCl_3$-MeOH-H_2O (65:35:10, lower phase) to give fraction 5 (Rf 0.7-1), fraction 6 (Rf 0.5-0.7), fraction 7 (Rf 0-0.5). The main component of fraction 6 (Compound **1**, 875 mg) was isolated on reverse phase prep-HPLC with H_2O-MeOH-AcOH-EtOAc (80:20:5:2) as a mobile phase. mp. 178-180℃; $[\alpha]_D$: −0.724 (MeOH); ^{1}H-NMR (DMSO-d_6, ppm): 8.45 (1H, s, H-2), 8.11 (1H, d, J= 8.3 Hz, H-5), 7.57 (2H, d, J=8.2 Hz, H-2′, 6′), 7.34 (1H, d, d, H-6), 7.20 (1H, d, J=3 Hz, H-8), 7.09 (2H, d, J=8 Hz, H-3′, 5′), 3.81 (3H, s, OCH_3); IR (ν_{max}^{KBr} cm^{-1}): 3380, 2930, 1620, 1250, 1080; EI mass, *m/z* (% Rel. Int.): 268 (100), 132 (65); ^{13}C-NMR: see Table I.

Partial hydrolysis compound 1

Compound **1** was hydrolyzed with 0.5 N H_2SO_4 (2 hrs) at 60℃. The reaction mixture was extracted with *n*-BuOH and chromatographed on silica gel to give monoglucoside [2]. mp. 208-210℃; $[\alpha]_D$: −20.3 (Pyridine); ^{1}H-NMR (DMSO-d_6, ppm): 8.41 (1H, s), 8.07 (1H, d, J=9.2 Hz), 7.54 (1H, d, J=8.8 Hz), 7.24 (1H, d, J=2.4 Hz), 7.16 (1H, d, d, J=2.3, 8.1), 6.99 (1H, d, J=8.3 Hz), 5.12 (1H, d, J=6.83), 3.80 (3H, s); ^{13}C-NMR: see Table I.

Acid hydrolysis of compound 1

Compound **1** was refluxed with aq. 2 N H_2SO_4 for 3 hrs to afford aglycon [3] as precipitates. The agly-

Table II. Insecticidal activity of crude MeOH extract of *Maackia amurensis*

Key pest	Method	Solvent	Conc. (%)	% Mortality (blank)
HF(A)	TA	MeOH	1	7(2)
BPH(A)	TA	MeOH	1	80(3)
BPH(A)	SP	Water(S)	1	88(4)
DBM(5L)	TA	MeOH	1	10(4)
DBM(3L)	SP	Water(S)	1	25(5)
GPA(A)	SP	Water(S)	1	5(1)
TSM(A)	LD	Water(S)	1	5(2)

HF : House Fly
BPH(A) : Brown planthopper adult
DBM(5L) : Dimond back moth instar larva
GPA(A) : Green peach aphid adult
TSM : Two spotted spider mite
TA : Topical Application
SP : Spay Method
(S) : Solution Emulsified with Surfactant

Table III. Insecticidal activity of each fraction against brown planthopper

Sample	Key pest	Method	Solvent	Conc. (%)	% Mortality (blank)
MeOH Extract	BPH	TA	MeOH	1	80 (6)
	BPH	SP	Water	1	88 (3)
Fraction 1	BPH	TA	MeOH	1	23 (5)
	BPH	SP	Water	1	63 (4)
Fraction 2	BPH	TA	MeOH	1	10 (3)
	BPH	SP	Water	1	20 (3)
Fraction 3	BPH	TA	MeOH	1	28 (5)
	BPH	SP	Water	1	33 (8)
Fraction 4	BPH	TA	MeOH	1	93 (7)
	BPH	SP	Water	1	100 (6)
Fraction 5	BPH	SP	MeOH	1	87 (0)
Fraction 6	BPH	SP	MeOH	1	95 (0)
Fraction 7	BPH	SP	MeOH	1	45 (3)
Compound **1**	BPH	SP	MeOH	1	100 (4)
	BPH	SP	MeOH	0.1	92 (7)

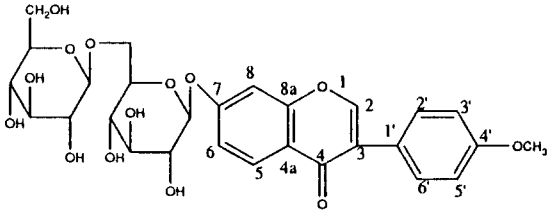

Fig. 1. The structure of compound 1.

con was crystallized in MeOH and was identified as formononetin by co-IR and co-mp with authentic sample. mp. 252-253℃; ^{1}H-NMR (DMSO-d_6, ppm): 8.26 (1H, s), 7.97 (1H, d, J=9 Hz), 7.50 (2H, d, J=9 Hz), 6.96 (2H, d, 9 Hz), 6.91 (1H, d, d, J=2.9 Hz), 6.85 (1H, d, J=2 Hz), 3.79 (3H, s); ^{13}C-NMR: see Table I; EI mass, *m/z* (%, Rel. Int.): 268 (100), 132 (75).

Peracetylation of compound 1

Compound **1** (10 mg) was dissolved in Ac_2O (1 ml) and pyridine. After 24 hr at room temperature, the reaction mixture was worked up as an usual manner to yield hepta acetate [**4**] as white needles. mp. 123-126℃; ^{1}H-NMR ($CDCl_3$, ppm): 8.37 (2H, d, J=8 HZ), 8.12 (1H, s), 7.57 (2H, d, J=9 Hz), 7.21 (1H, d, J=2 Hz), 7.19 (1H, d, J=1.6 Hz), 6.95 (1H, d, J=8.7 Hz), 3.93 (3H, s), 1.94-2.4 (21H, s); EI mass, *m/z* (%, Rel. Int): 886 (2), 619 (0.4), 331 (23), 268 (34), 169 (52), 109 (30), 43 (100); IR (ν_{max}^{KBr} cm^{-1}): 1750, 1240, 1060.

Insecticidal activity test

Each fraction was tested primarily with house fly, dimond back moth, green peach aphid, brown plant hopper, and two spotted spider mite by topical application, spray and leaf dipping methods[10]. The test methods were described briefly as follows; Formulation 1 was 1% methanol solution of the test compounds. Formulation 2 was the emulsified solution which was made by shaking up the compounds in 10% methanol and surfactant (Triton X-100, 0.01 %). Brown planthopper was chosen because of its selective insecticidal responses (Table II, III). Newly emerged BPH female adults from the laboratory colonies were picked up individually with a vacuum pencil, anesthetized briefly with CO_2 gas, and treated with formulation 1 by topical applicator (0.2 μl scale) to the dorsum of the thorax of each insect for the detection of contact-poison effects and treated with formulation 2 by spray method for the detection of broad insecticidal activities. After treatments, all insects were held in 30×120 test tube (5 rice seedlings were placed in each test tube with 10 insects, 5 replications) in a room maintained at 25℃ and 60 RH with fluorescent daylight under alternating 16 day/ 8 night cycle for 24 hr. After 24 hr, the mortality was evaluated as follows; when the insect did not move with gentle disturbance, we regarded the insect as dead one.

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