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Two New Antimicrobial Diterpenoids from the Roots of *Mimosa pudica*

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From the root of *Mimosa pudica* L. (Fabaceae), two new diterpenoids, named 19-*O*-trans-feruloyl-labd-8(17)-en-15,19-diol (1) and 19-*O*-[(*E*)-3',4'-dimethoxy cinnamoyl]-labd-8(17)-en-15,19-diol (2) had been isolated. Their structures were determined by analysis of spectroscopic data, including 1D and 2D NMR. Compounds 1-2 showed antimicrobial activity against two yeast and two bacteria tested. The strongest activity was observed against *Malassezia pachydermatis*, followed by *Candida albicans* and *Staphylococcus aureus* in the range of 12-16 mm.

Key words: *Mimosa pudica*, diterpenoids, antimicrobial activity

Introduction

Mimosa pudica L. (Fabaceae) is called a sensitive plant. The root of this plant has been used traditionlly in the treatment of diarrhoea, gastritis and enteritis.[1-2] *M. pudica* contains the toxic alkaloid mimosine, which has been found to have antiproliferative and apoptotic effects.[3] It has also antibaterial,[4-6] antioxidant,[7-8] wound healing[9], and anti-ophidian properties.[10] The organic solvent extract from *M. pudica* exhibited good activities against phytopathogen.[4] The ethanolic extracts of *M. pudica* were found to be active with MIC of 0.25-0.5 mg/ml against *Mycobacterium smegmatic*.[5] *M. pudica* were proven for antibacterial properties against 15 Gram-posttive and Gram-negative human pathogenic bacterial.[6] In this paper, isolation and antimicrobial activity of two new diterpenoids were reported.

Material and Methods

General methods

Optical rotations were measured on a JASCO DIP-360 digital polarimeter. EI-MS were recorded with a JMS-HX-100 instrument and HR EIMS with a JEOL LMS-SX 102 system. IR spectra were recorded on a JASCO FT-IR-110 infrared spectrophotometer. ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker AM-500 NMR. MPLC was carried out on a Buchi 688 MPLC system.

Plant material

The dried root of *M. pudica* was collected in

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Hsinchu county, Taiwan, in September 2009. A voucher specimen was deposited at the Department of Applied Technology of Living, Ta-Hwa University of Science and Technology, Hsinchu of Taiwan, R.O.C.

Extraction and separation

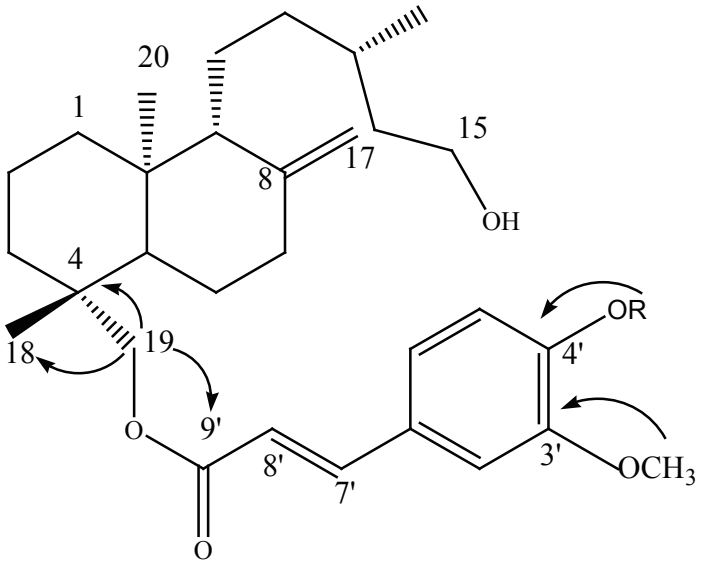
The dried root powders of *M. pudica* (2 Kg) were successively extracted under reflux with hot MeOH (50-60 °C) (5L × 3) and concentrated to give a deep brown syrup (180g). This syrup was partitioned between 1:1 EtOAc/H_2O (2L). The EtOAc layer was concentrated to give brown residue (56.0g) and then subjected to a silica gel CC eluting with *n*-hexane and increasing amounts of EtOAc (0-100%) to give seven fractions (A-G). Based on the TLC and antimicrobial assay, fractions C-E were combined and then separated by MPLC (silica gel, *n*-hexane-EtOAc gradient) followed by prep. TLC (silica gel, 5% MeOH/CH_2Cl_2) to afford 1 (178mg) and 2 (125mg).

19-*O*-trans-feruloyl-labd-8(17)-en-15,19-diol (1)

a pale brown oil. $[\alpha]_D^{25}$ – 3.17 (c, 0.16 , $CHCl_3$). HR-MS *m/z*: 484.3183 (M^+, Cald for $C_{30}H_{44}O_5$). EI-MS m/e (rel. int.): 484 (M^+, 7), 290 (10), 194 (12), 177 (100), 105(27). IR(neat) ν_{max} cm^{-1}: 3350, 2920, 1720, 1640, 1595, 1515, 1300, 1150. ^{1}H-NMR ($CDCl_3$, 500MHz) : δ Table 1. ^{13}C-NMR ($CDCl_3$, 125MHz) : δ Table 1.

19-*O*-[(*E*)-3',4'-dimethoxy cinnamoyl]-labd-8(17)-en-15,19-diol (2)

a pale brown oil. $[\alpha]_D^{25}$ – 48.6 (c, 0.05, $CHCl_3$). HR-MS *m/z*: 498.3345 (M^+, Cald for $C_{31}H_{46}O_5$). EI-MS m/e (rel. int.): 498 (M^+, 15), 468 (2), 290 (6), 208 (14), 191 (100), 163 (5). IR (neat) ν_{max} cm^{-1}: 3350, 2920, 1725, 1640, 1590, 1510, 1290, 1100. ^{1}H-NMR ($CDCl_3$, 500MHz) : δ Table 1. ^{13}C-NMR ($CDCl_3$, 125MHz) : δ Table 2.



1 R=H

2 R=CH_3

Fig. 1 HMBC (H→C) correlations of 1 and 2.

Table 1 Antimicrobial activity of the fractions A-G and compounds 1-2 of *M. pudica* using agar disk diffusion method[a]

microorganisms	A	B	C	D	E	F	G	1	2	CF[c]
M. pachydermatis	12	na[d]	11	15	15	na	na	16	14	na
C. albicans	9	na	12	14	13	na	11	15	13	na
S. aureus	na	10	15	13	14	11	na	13	15	19
E. coli	na	na	na	na	na	na	na	na	na	19

[a]Diameter of zone of inhibition (mm) including disk diameter of 8 mm. [b]Inhibitory effects at an equivalent concentration of 1 mg/disk. [c]CF=Ciprofloxacin, 5 μg/disc. [d]na=not active.

Table 2 Selected ^{1}H-NMR spectral data of compounds 1 and 2 (δ, ppm, in $CDCl_3$)

H	1	2
7	2.40 (*dd*,12.3, 1.0), 1.91 (*ddd*, 12.3, 12.1, 2.2)	2.39 (*dd*, 12.4, 1.8), 1.92 (*ddd*, 12.4, 12.1, 2.5)
15	3.64 (*m*)	3.64 (*m*)
16	0.87 (*d*, 6.6)	0.86 (*d*, 6.8)
17	4.48 (*brs*), 4.80 (brs)	4.48 (*brs*), 4.80 (*brs*)
18	1.01 (*s*)	1.06 (*s*)
19	3.92 (*d*, 11.0), 4.35 (*d*, 11.0)	3.93 (*d*, 11.2), 4.39 (*d*, 11.2)
20	0.67 (*s*)	0.66 (*s*)
2'	7.00 (*d*, 1.1)	7.03 (*d*, 1.2)
5'	6.89 (*d*, 8.2)	6.84 (*d*, 8.5)
6'	7.08 (*dd*, 8.2, 1.1)	7.09 (*dd*, 8.5, 1.2)
7'	7.68 (*d*, 16.2)	7.57 (*d*, 16.3)
8'	6.25(*d*, 16.2)	6.26 (*d*, 16.3)
OMe	3.91 (*s*)	3.92×2 (*s*)

Antimicrobial assay

The fractions C-E and compounds 1-2 of *M. pudica* were individually tested against a panel of microorganisms, including Gram-positive cocci, *Staphylococcus aureus* ATCC 25923, Gram-negative bacilli, *Escherichia coli* ATCC 25922 and the yeasts, *Candida albicans* ATCC 10239 and *Malassezia pachydermatis* ATCC 14522. Bacterial strains were cultured overnight at 37°C in Mueller Hinton agar (MHA). Yeasts were cultured overnight at 30°C in Sabouraud dextrose agar. The fractions C-E and compounds 1-2 against the test organisms were determined by the disk diffusion method.[11] All tests

Table 3 ^{13}C-NMR spectral data of compounds 1 and 2 (δ, ppm, in $CDCl_3$)

C	1	2
1	38.5	38.2
2	19.2	18.9
3	39.6	39.6
4	38.9	38.7
5	56.3	55.9
6	24.5	24.5
7	36.3	36.6
8	147.9	151
9	57.4	56.3
10	40.1	39.6
11	21.0	21.0
12	36.2	36.3
13	30.3	30.3
14	38.8	38.6
15	61.3	61.2
16	19.8	19.8
17	106.7	106.7
18	27.7	27.7
19	66.7	66.7
20	15.3	15.3
1'	127.1	127.4
2'	114.7	111.0
3'	148.2	148.2
4'	146.7	149.2
5'	115.7	116
6'	123.2	122.6
7'	144.5	144.4
8'	109.3	109.5
9'	167.5	167.4
OMe	56.1	55.9×2

were performed in duplicate.

Results and Discussion

The antimicrobial activities of the fractions C-E and compounds 1-2 against the microorganisms in the present study were assessed by evaluating the inhibition zone diameter. The results showed that fractions C-E and compounds 1-2 had antimicrobial activity against two yeast and two bacteria tested (Table 1). The strongest activity was observed against *M. pachydermatis*, followed by *C. albicans* and *S. aureus* in the range of 12-16 mm. The fractions A-B and F-G showed weak or no activity. Thereby the fractions C-E and compounds 1-2 provided potential leads in the control of vaginitis and dermatitis.[12,13]

Compound 1 was obtained as a pale brown oil. $[\alpha]_D^{25}$ – 3.17 (c = 0.16, $CHCl_3$). The molecular formula was determined as $C_{30}H_{44}O_5$ on the basis of HR-EI-MS (484.3183). The IR spectrum implied the presence of hydroxy groups (3350cm^{-1}), conjugated ester group (1720cm^{-1}), olefinic group (1640cm^{-1}) and aromatic group (1595, 1515cm^{-1}). The structure of 1 was deduced from detailed analysis of ^{1}H- and ^{13}C-NMR data and 2D NMR experiments (^{1}H-^{1}H COSY, HMQC and HMBC). The ^{1}H-, ^{13}C-NMR, ^{1}H-^{1}H COSY and HMQC spectral data of 1 indicated the presence of two tertiary methyls, one secondary methyl, one exomethylene, three methines, nine methylenes and one oxymethylene (Table 2, 3). The NMR spectra and the optical rotation of 1 was suggested the diterpene moiety as labd-8(17)-en-15,19-diol.[14-16] Moreover, feruloyl moiety was found at δ 7.01 (1H, *d*, J=1.1, H-2'), 6.89 (1H, *d*, J=8.2, H-5'), 7.08 (1H, *dd*, J=8.2, 1.1, H-6'), 7.68 (1H, *d*, J=16.2, H-7'), 6.25 (1H, *d*, J=16.2, H-8') and 3.91 (3H, *s*, OMe). The fragments at *m/z* 177 (100%), 194 (12%) in ms correspond to feruloyl moiety.[17]

HMBC correlation between H_2-19 and C-9' (feruloyl carbon) indicated that feruloyl group was attached to C-19. Thus, compound 1 was elucidated to be 19-*O*-trans-feruloyl-labd-8(17)-en-15,19-diol.

Compound 2 was obtained as a pale brown oil. $[\alpha]_D^{25}$ – 48.6 (c = 0.05, $CHCl_3$). The molecular formula of 2 was determined as $C_{31}H_{46}O_5$ on the basis of HR-EI-MS (HR-MS *m/z*: 498.3345). The IR spectrum revealed hydroxy (3350cm^{-1}), conjugated ester (1725 cm^{-1}), olefinic (1640cm^{-1}) and aromatic (1590, 1510 cm^{-1}) absorptions. The ^{1}H- and ^{13}C-NMR spectra of 2 was similar to that of 1 (Table 2, 3), except of one more methoxyl groups [δ 3.92 (s, 6H)]. The fragments at *m/z* 191 (100%), 208 (14%) in ms and NMR data correspond to (*E*)-3,4-dimethoxy cinnamic moiety.

HMBC correlation between two methoxyl groups [δ 3.92 (s, 6H)] and C-3',4' indicated that two methoxyl groups were attached to C-3',4' (cinnamic carbon). Finally, through HMBC results, the attachment of (*E*)-3',4'-dimethoxy cinnamic was determined. The correlation between H_2-19 and C-9' indicated that (*E*)-3',4'-dimethoxy cinnamic group was attached to C-19. Thus, compound 2 was elucidated to be 19-*O*-[(*E*)-3',4'-dimethoxy cinnamoyl]-labd-8(17)-en-15,19-diol.

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References

1. Pal M, Roychaudhury A, Pal A, Biswas S. A novel tubulin from *Mimosa pudica* purification and characterization. *Eur. J. Biochem*., 11:329-335, 1993.
2. Chaudhuri AR, Biswas S. Cold stability of microtubules of *Mimosa pudica. Biochem. Mol. Biol. Int*., 29:421-428, 1993.
3. Makkar HP, Siddhuraju P, Becker K. Plant secondary metabolites. *Methods Mol. Biol*., 393:1-122, 2007.
4. Kangk JG, Shin SY, Kim MJ, Bajpai V, Maheshwari DK, Kang S. C. Isolation and anti-fungal activities of 2-hydroxymethyl-chroman-4-one produced by *Burkholderia* sp. MSSP *J. Antibiot*., 57:726-731, 2004.

5.Gupta VK, Shukla C, Bisht GR, Saikia D, Kumar, S, Thakur RL. Detection of anti-tuberculosis activity in some folklore plants by radiometric BACTEC assay. *Lett. Appl. Microbiol*., 52:33-40, 2011.

6.Akter A, Neela FA, Khan MS, Islam MS, Alam MF. Detection of anti-tuberculosis activity in some folklore plants by radiometric BACTEC assay. *Indian J. Pharm. Sci*., 72:388-392, 2010.

7.Freire KR, Lins AC, Dórea MC, Santo, FA, Camara CA, Silva TM. Palynological origin, phenolic content, and antioxidant properties of honeybee-collected pollen from Bahia, Brazil. *Molecules*, 7:1652-1664, 2012.

8.Zhang J, Yuan K, Zhou WL, Zhou J, Yang P. Studies on the active components and antioxidant activities of the extracts of *Mimosa pudica* Linn. from southern China. *Pharmacogn. Mag*., 7:35-39, 2011.

9.Kokane DD, More RY, Kale MB, Nehete MN, Mehendale PC, Gadgoli CH. Evaluation of wound healing activity of root of *Mimosa pudica*. *J. Ethnopharmacol*., 15:311-315, 2009.

10.Ambikabothy J, Ibrahim H, Ambu S, Chakravarthi S, Awang K, Vejayan J. Efficacy evaluations of *Mimosa pudica* tannin isolate (MPT) for its anti-ophidian properties. *J. Ethnopharmacol*., 137:257-262, 2011.

11.Ines T, Amina B, Khaled S, Kamel G. Screening of antimicrobial activity of marine sponge extracts collected from Tunisian coast. *Proc. West. Pharmacol. Sc*., 50:152-155, 2007.

12.Wenjin Q, Yifu S. Epidemiological study on vaginal *Candida glabrata* isolated from pregnant women *Scand. J. Infect Dis*., 38:49-54, 2006.

13.Ahman S, Perrins N, Bond R. Carriage of *Malassezia* spp. yeasts in healthy and seborrhoeic *Devon Rex* cats. *Med. Mycol*., 45:449-455, 2007.

14.Ohsaki A, Yokoyama R, Miyatake H, Fukuyama Y. Two diterpene rhamnosides, mimosasides B and C, from *Mimosa hostilis. Chem. Pharm. Bull*., 54:1728 -1729, 2006.

15.Fukuyama Y, Yokoyama OA, Takahashi H, Minami, H. An example of the co-occurrence of enantiomeric labdane-type diterpenes in the leaves of *Mimosa hostilis. Chem. Pharm. Bull*., 47:454-455, 1999.

16. Popova MP, Chinou IB, Marekov IN, Bankova VS. Terpenes with antimicrobial activity from *Cretan propolis. Phytochemistry*,70:1262-1271, 2009.

17.Shirota O, Sekita S, Satake M. Two phenylpropanoid glycosides from *Sparganium stoloniferum. Phytochemistry*, 44:695-698, 1997.

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由含羞草根部得到兩個新的二萜類抗菌成分

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（102 年 3 月 28 日受理，102 年 10 月 24 日接受刊登）

從含羞草根部分離得到兩個新的二萜化合物，命名為 19-*O*-trans-feruloyl-labd-8(17)-en-15,19-diol (1) and 19-*O*-[(E)-3',4'-dimethoxy cinnamoyl]-labd-8(17)-en-15,19-diol (2)。化合物 1-2 對酵母菌和細菌具有抗菌活性，對厚皮馬拉色菌的抗菌活性最強，其次為白色念珠菌和金黃色葡萄球菌，抑菌圈在 12-16 毫米的範圍內。

關鍵字：含羞草、二萜類、抗菌活性

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