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SHORT COMMUNICATION

Identification of Antioxidant Compound from *Asparagus racemosus*

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Roots of *Asparagus racemosus* were found to possess antioxidant property. DPPH autography-directed separation resulted in the identification of a new antioxidant compound named racemofuran (3) along with two known compounds asparagamine A (1) and racemosol (2). The structure of 3 was fully characterized by spectroscopic data (UV, MS, ^{1}H NMR, ^{13}C NMR, and 2D NMR). Racemofuran revealed antioxidant property against DPPH with IC_{50} value of 130 μM. Copyright © 2004 John Wiley & Sons, Ltd.

Keywords: Asparagus racemosus; antioxidant; benzofuran; racemofuran.

INTRODUCTION

Reactive free radicals have been known to mediate causative diseases such as cancer, atherosclerosis, and aging. Consequently, compounds with free radicals scavenging, complexing with pro-oxidant metals or quenching of singlet-oxygen formation are currently considered as protective or therapeutic agents against these diseases. In our ongoing search for antioxidants from Thai medicinal plants (Aree, 2003a,b), we found antioxidant activity in the crude extract of *Asparagus racemosus* Willd (Liliaceae) roots. In Thailand, decorticated roots have been used as a remedy for diseases of the spleen, liver and other internal organs, including for preventing miscarriage (Pongboonrod, 1976). Recently, *A. racemosus* extract has been reported for antioxidant properties against damage induced by γ-radiation in rat liver mitochondria (Kamat *et al.*, 2000), but the active principles have not been identified. We employed DPPH autography in our screening for rapid locating of active components in *A. racemosus* extract. Although asparagamine A was detected as a major principle in the active CH_2Cl_2 extract, it failed to scavenge DPPH free radical on TLC autography.

Thus, an attempt to identify the components actually responsible for this activity was accomplished, resulting in the isolation of a new antioxidant compound named racemofuran (**3**) (Figure 1). This paper describes the isolation, structure elucidation, and antioxidant property of the new compound.

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MATERIALS AND METHODS

General. Spectroscopic data were recorded with the following instruments: FISON MS 8000 (EIMS); PERKIN-ELMER 1760X (FTIR); JEOL JNM-α 500 (500 MHz, ^{1}H; 125 MHz, ^{13}C, NMR). Chemical shifts are reported in ppm (δ) referenced to TMS.

Extraction and isolation. Roots of *A. racemosus* were collected in Kanchanaburi in February 1994. The specimen was authenticated (by Associate Professor Kosum Peeraman, Department of Botany, Faculty of Science, Chulalongkorn University) through comparison with a voucher specimen (BKF 81621) deposited at the herbarium of the Royal Forest Department, Ministry of Agriculture and Cooperatives, Thailand.

Air-dried roots (13 kg) of *A. racemosus* were minced into tiny pieces and extracted as described by Tip-pyang *et al.* (2000). The CH_2Cl_2 extarct (154 g), which was active against DPPH on TLC autography, was chromatographed on silica gel using gradients of CH_2Cl_2 in hexane and MeOH in CH_2Cl_2, yielding 122 fractions. Fractions 73–96 eluted with CH_2Cl_2 were recrystallized to afford asparagamine A (**1**, 3.56 g). The combined fractions 39–61 were purified on chromatotron® using EtOAc-hexane 1:1–7:3 to yield racemosol (**2**, 152 mg) and EtOAc-hexane 1:4 to furnish racemofuran (**3**, 146 mg).

DPPH free radical scavenging activity. TLC autography was employed for screening crude extracts and rapid detection of active components (Table 2), which appeared as pale yellow spots on purple background after spraying with DPPH reagent (Hostettmann *et al.*, 1997). Pure active compounds were further validated by the UV absorbing method (Yen and Hsieh, 1997). Briefly, test sample solution (0.25 mM, 0.5 mL) was added to a 1 mL methanolic solution of DPPH (final concentration of DPPH was 0.2 nM). The mixture was vigorously shaken and kept in the dark for 30 min. The

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absorbance of the resulting solution was measured at 518 nm with a UV spectrometer. Test sample was run in triplicate and averaged. The activity was expressed as IC_{50} value, and BHT was used as a positive control (IC_{50} 87 μM).

RESULTS AND DISCUSSION

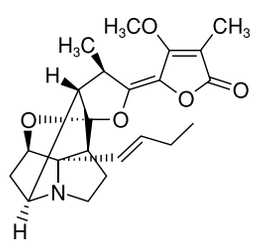
By using DPPH autography method, we succeeded in identification of a new antioxidant compound named racemofuran (**3**) together with known compounds, asparagamine A (**1**) (Sekine *et al.*, 1994) and racemosol (**2**) (Sekine *et al.*, 1997), of which spectroscopic data reminiscent with those previously reported.

A new benzofuran **3** was isolated as a light brown powder. Its molecular formula $C_{17}H_{16}O_4$ was established by HREIMS. IR spectrum revealed the presence of hydroxy group (3500–3100 cm^{-1}) and aromatic ring (1600 cm^{-1}). ^1H NMR spectrum showed characteristic signals of 4,2″-substituted benzofuran at 6.88 (H-1″), 7.14 (H-2), 6.64 (H-3), and 7.15 (H-5). The hydroxy group of 4.84 ppm were placed at C-4 according to HMBC correlations to C-3, C-4, and C-5, including *ortho* coupling of H-2/H-3 ($^3J_{2,3}$ = 6.1 Hz) and *meta* coupling of H-3/H-5 ($^3J_{3,5}$ = 2.2 Hz). The presence of aromatic singlet at 6.91 (H-6′) and four functionalities [2.18 and 2.39 (2 × CH_3), 3.87 (OCH_3), and 5.18 (OH)] indicated pentasubstitution on another benzene ring. To confirm the substitution pattern, 1D NOE was employed. Irradiation of H-1″ (6.88) caused enhancement of the singlet peak at 2.39, which was ascribable to 2′-CH_3. By the same way, NOE difference suggested that the methoxy group (5′-OCH_3) was adjacent to H-6′.

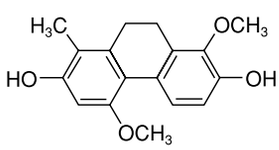
The exact positions of remaining hydroxyl and methyl groups could not be pointed out by NOE data alone since they demonstrated no enhancement on irradiation of 2′-CH_3 and 5′-OCH_3. Based on HMBC cross peaks (OH → C-2′, 3′, 4′ and CH_3 → C-3′, 4′, 5′), the hydroxyl and methyl groups were accommodated at C-3′ and C-4′, respectively. Therefore, the structure of racemofuran was depicted for **3**. Racemofuran is nearly identical to stemofurans E and G from *Stemona collinsae* (Pacher *et al.*, 2002) except for 4-OH in racemofuran and 2-OH in stemofurans. Racemofuran showed antioxidant activity towards DPPH with IC_{50} value of 130 μM, while asparagamine A (**1**) was not active (IC_{50} > 500 μM: Table 3). Due mainly to rapid detection by DPPH autography, racemofuran (**3**) was identified as the actual principle responsible for antioxidant property instead of asparagamine A (**1**), a very major component presented in CH_2Cl_2 extract. In addition, other antioxidant compounds reported from genus *Asparagus* have been exemplified by the presence of lipoic and dihydrolipoic acids (Navari-Izzo *et al.*, 2002), including aminoethylcystein decarboxylated dimer (Macone *et al.*, 2002). Unlike polyphenols and flavanoids, the antioxidant property of benzofurans has been less documented, and little is known about their roles in free radical scavenging. Thus, it is of great interest to investigate how benzofurans exert their antioxidant property towards free radicals such as DPPH.

EXPERIMENTAL DATA

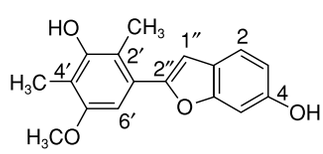
Compound **3**. Light brown powder. EIMS *m/z* 284 $[M]^+$ (100), 267 (20), 241 (25), 197 (28), 115 (45); HREIMS *m/z* 284.1029 (calcd for $C_{17}H_{16}O_4$, 284.1049) UV (MeOH) λmax (log ε) 240 (3.45), 272 (3.45), 324 (3.46) nm; IR (KBr) ν_{max} 3500–3100, 1600, 1480, 1120, 1050, 790 cm^{-1}. ^1H NMR and ^{13}C NMR see Table 1.



asparagamine A (1)



racemosol (2)



racemofuran (3)

Figure 1. Structures of isolated compounds.

Table 1. NMR data for 3 ($CDCl_3$)

Position	$^1H^a$	$^{13}C^b$
1		118.4
2	7.14 d (6.1)	104.3
3	6.64 dd (6.1, 2.2)	107.9
4		149.0
4-OH	4.84 s	
5	7.15 d (2.2)	124.8
6		156.1
1′		128.4
2′		112.4
2′-CH_3	2.39 s	13.1
3′		153.0
3′-OH	5.18 s	
4′		114.5
4′-CH_3	2.18 s	8.5
5′		156.0
5′-OCH_3	3.87 s	55.8
6′	6.91 s	103.3
1″	6.88 s	101.7
2″		154.9

[a] Coupling constants in Hz were given in parenthesis.
[b] Multiplicities were determined by DEPT 90 and DEPT 135.

Table 2. DPPH free radical scavenging activity of extract and fractions from *A. racemosus*[a]

Extract/fr. no.	Free radical scavenging activity[b]
dichloromethane extract	+ +
1–10	–
11–18	–
19–20	–
21–33	–
34–38	+
39–61	+ + +
62–72	+
73–96	–
97–110	–
111–122	–

[a] 10 μL of test sample was used.
[b] Estimated as the lowest concentration at which pale yellow spots appeared after spraying with DPPH reagent; + + + (0.5 mg/mL), + + (2.0 mg/mL), + (10.0 mg/mL), – (not active at 10 mg/mL).

Table 3. DPPH free radical scavenging activity of isolated compounds

Compound	IC_{50} (μM)
asparagamine A (**1**)	>500
racemosol (**2**)	>300
racemofuran (**3**)	130
BHT	87

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