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ANTIOXIDATIVE ACTIVITIES OF CONSTITUENTS ISOLATED FROM *PANDANUS ODORATISSIMUS*

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Key Word Index—*Pandanus odoratissimus*; Pandanaceae; roots; lignans; benzofurans; antioxidative activity.

Abstract—Chemical component analysis of the root parts of *P. odoratissimus* led to the isolation of two phenolic compounds, four lignan type compounds plus a new benzofuran derivative. Among them, pinoresinol and 3,4-*bis*(4-hydroxy-3-methoxybenzyl) tetrahydrofuran showed strong antioxidative activities when BHA was used as a standard in the thiocyanate method. The new compounds were identified as 4-hydroxy-3-(2′,3′-dihydroxy-3′-methylbutyl)-benzoic acid methyl ester and 3-hydroxy-2-isopropenyl-dihydrobenzofuran-5-carboxylic acid methyl ester, by spectroscopic analysis.

INTRODUCTION

Plants of the genus *Pandanus* are prevalent in tropical areas, especially on the pacific islands, Malaysian islands and Australia. The leaf extract is used as a food flavour additive in India and the roots are used in folk medicine to cure thyroid problems and treat fever in Taiwan. Many fragrant essential oils, including monoterpenes and sesquiterpenes, were isolated from *P. latifolius* [1] and several alkaloids were found in *P. amaryllifolius* [2]. Fatty acid contents of the fruit of *P. conoideus* were analyzed by Southwell [3]. Also, it has recently been reported that the root extract of *P. odorus* shows a strong hypoglycaemic effect [4].

Pandanus odoratissimus flourishes in southern Taiwan and its MeOH extract shows great antioxidative activity in our continuing study of the crude drugs used in Taiwan. We herein report its chemical components and their activities.

RESULTS AND DISCUSSION

The methanol extract of *P. odoratissimus* was subjected to column chromatography to isolate a total of 15 compounds. Steroids, including phytosteroid mixtures; α-spinasterol and stigmast-7-en-3β-ol mixture; α-spinasterol caproate; stigmast-4-en-6β-ol-3-one and three phenolic compounds; vanillin (**1**); 2(*E*)-3-(3′-methoxy-4-hydroxyphenyl)-prop-2-enal (**2**); 4-hydroxy-3-(2′,3′-dihydroxy-3′-methylbutyl)-benzoic acid methyl ester (**3**) and a new benzofuran derivative, 3-hydroxy-2-isopropenyl-dihydrobenzofuran-5-carboxylic acid methyl ester (**4**); plus six lignans; eudesmin (**5**) [5]; kobusin (**6**) [5]; pinoresinol (**7**) [6]; epipinoresinol (**8**) [7]; de-4′-*O*-methyleudesmin (**9**) [8] and 3,4-*bis*(4-hydroxy-3-methoxy-benzyl)-tetrahydrofuran (**10**) [9], were isolated and identified by comparing their data with authentic materials on the basis of their mass, UV, IR and ^{1}H and ^{13}C NMR spectra.

Among them, compound **3** is a rare phenolic compound, which has only been isolated from *Eriodictyon sessilifolium* (Hydrophyllaceae) [10], and a closely related structure, compound **4**, has for the first time been isolated from nature. Its structure was identified by spectroscopic analysis and comparisons of data with the known compounds **4a** and **4b**.

Compound **3**, m.p. 143–144°, molecular formula $C_{13}H_{18}O_5$ ($[M]^+$ *m/z* 254), the UV λ_{max} at 220 (*sh*), 259, 290 (*sh*) nm and IR ν_{max} at 3440 (–OH), 1716, 1690 cm^{-1} (C=O), indicated a *para*-hydroxy-carbonyl-disubstituted aromatic compound. In the downfield region of the ^{1}H NMR spectrum, an ABX-type three proton signal was exhibited at δ 7.86 (1H, *dd*, *J* = 8.1 and 2.1 Hz), 7.77 (1H, *d*, *J* = 2.1 Hz), 6.94 (1H, *d*, *J* = 8.1 Hz) and a phenolic hydroxyl at δ 8.84 (1H, *br s*). In the high-field area, there appeared another ABX-type three

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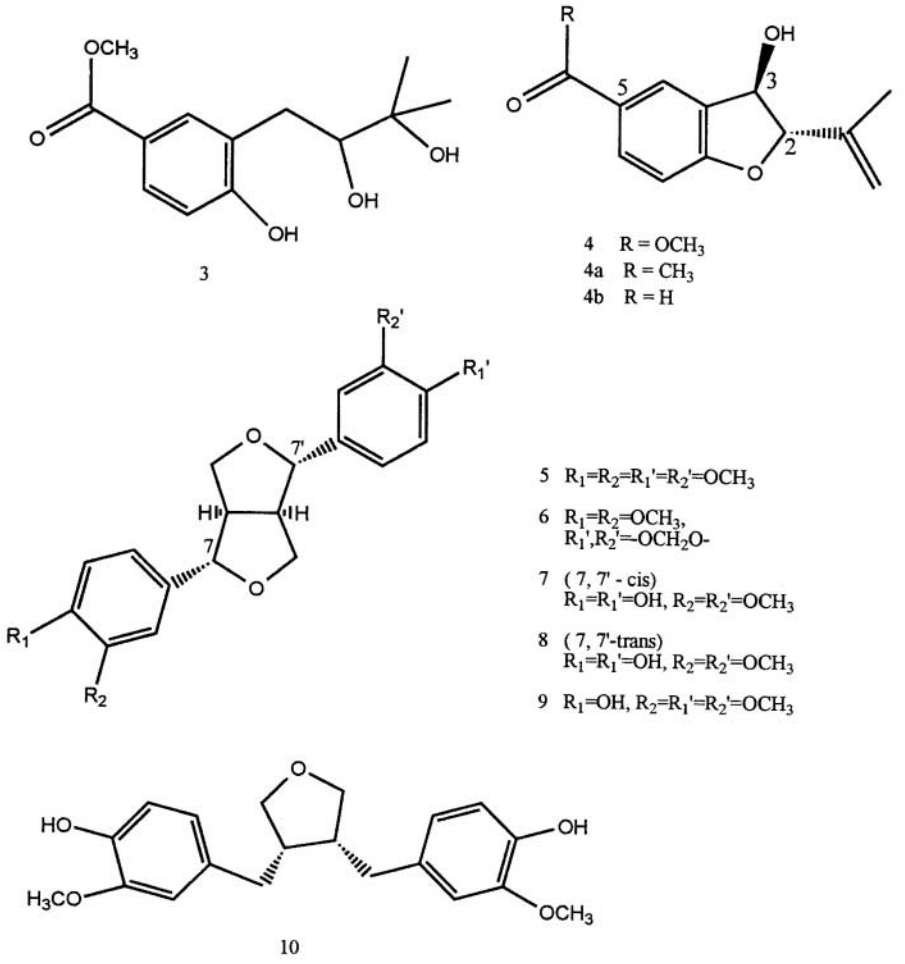

proton signal at δ 2.64 (*dd*, J = 14, 1.5 Hz), 2.85 (*dd*, J = 14, 9 Hz) and 3.70 (*dd*, J = 9, 1.5 Hz), plus two methyls at δ 1.29 (3H, *s*) and 1.36 (3H, *s*), which represented a 2,3-dihydroxyisopentane moiety attached to the benzene ring. A methoxyl signal at δ 3.88 indicated a carbonyl ester functionality. Based upon the above information, the structure of **3** is depicted as 4-hydroxy-3-(2′,3′-dihydroxy-3′-methylbutyl)-benzoic acid methyl ester.

Compound **4**, oily, molecular formula $C_{13}H_{14}O_4$ ($[M]^+$ m/z 234), the UV λ_{max} at 220 (*sh*), 258, 290 (*sh*) nm and IR ν_{max} at 3436 (-OH), 1718 cm^{-1} (C=O) resembled those in **3**. Also, in the ^1H NMR spectrum an ABX-type three proton signal at δ 8.07 (*d*, J = 2 Hz), 8.0 (*dd*, J = 8.7, 2 Hz) and 6.90 (*d*, J = 8.7 Hz) suggested a 1,2,4 trisubstituted benzene structure, as in **3**. A terminal olefinic signal appearing at δ 4.92 and 5.07 (each 1H, *br s*), plus a methyl signal at δ 1.72 indicate a isopropylene group. Since the phenolic –OH absorption is missing in the NMR spectrum and because a seven double-bond equivalence was calculated from its molecular formula, dihydrobenzofuran skeleton is assumed. An isopropylene and a hydroxyl group were substituted at C-2 and C-3, respectively, due to the signals at δ 5.15 (1H, *d*, J = 3.9 Hz) and 4.95 (1H, *d*, J = 3.9 Hz) in the NMR spectrum.

Base upon the above information, compound **4** is very similar to the known toxol **4a** [11] and compound **4b** [12], except for the 5-carboxylic acid methyl ester substituent on **4** instead of an acetyl group on the toxol and a formyl group on **4b**. ^{13}C NMR ($CDCl_3$) and the EI mass spectrum supported the proposed structure of **4**. The relative configuration of **4** is tentatively assigned *trans* (J = 3.9 Hz) by comparison with the C-2, C-3 proton coupling constant, provided by the studies of Zalkow *et al*. [11]. However, this compound decomposed soon after taking its NMR spectra, and some of its spectra were unobservable. Still, this is a new dihydrofbenzofuran compound which has been isolated from nature for the, first time.

Anti-oxidative activity was examined on the crude methanol extract and also on the pure components isolated from *P. odoratissimus*, including phenolic compounds **1**, **3** and lignans **5**, **6**, **7** and **10**, using linoleic acid as substrate in the thiocyanate method. Among them, phenolic compound,

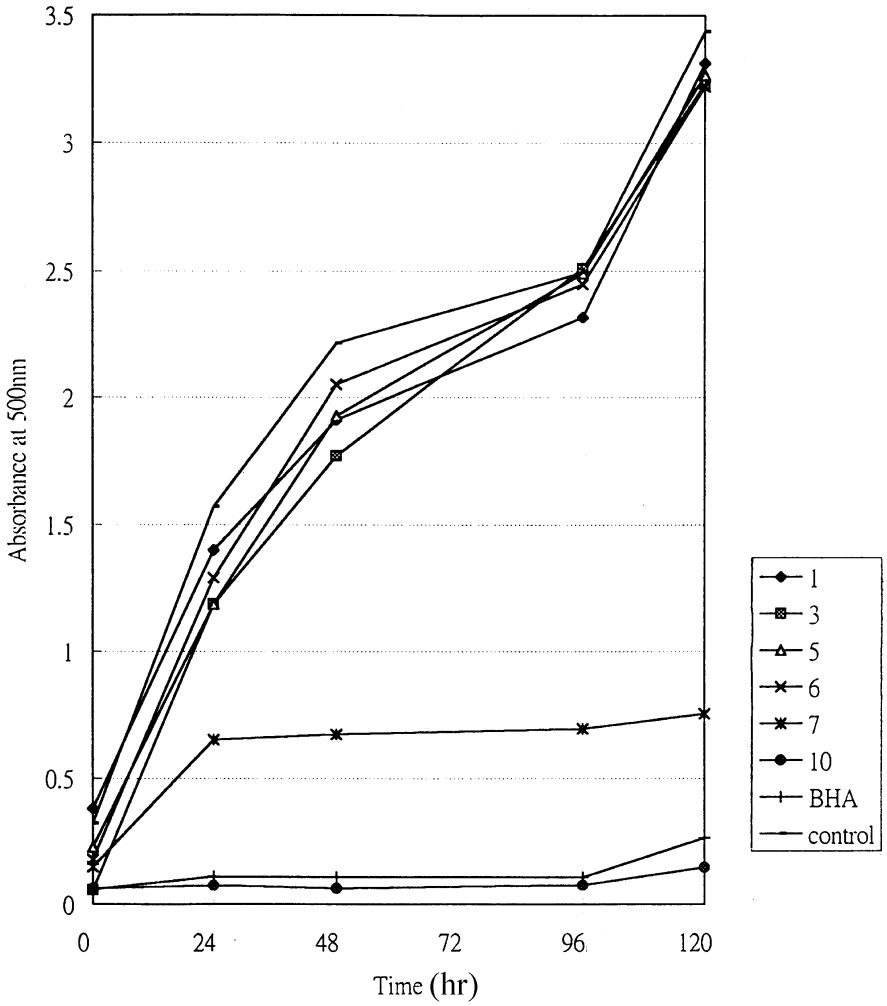

Fig. 1. Effect of compounds **1**, **3**, **5**, **6**, **7** and **10** and BHA on autooxidation of linoleic acid.

such as vanillin (**1**) and compound **3** did not show any activity, while pinoresinol (**7**) showed moderate activity and 3,4-*bis*(4-hydroxy-3-methoxybenzyl) tetrahydrofuran (**10**) exhibited activities as strong as BHA.

EXPERIMENTAL

General

NMR spectra (δ, J in Hz) were recorded on a Varian VXR-300 NMR spectrometer. TMS was used as an int. ref. for ^{1}H and ^{13}C NMR spectra measured in $CDCl_3$ and MeOD-d_4.

Isolation

Root of *P. odoratissimus* were purchased from the folk drug store in Taichung, Taiwan. A voucher specimen (NCHU 040) is deposited in our laboratory at the National Chung-hsing University. Crushed roots (5 kg) were extracted (×3) with cold MeOH. After removal of solvent, the residues were subjected to CC on silica gel using hexane and EtoAc mixts increasing polarity as eluants give 5 main frs. The first two frs contained mainly steroids. After successive chromatography on silica gel, fr. 3 (hexane–EtoAc, 4:1) gave compounds **1**, **2**, **4**, **5** and **6**. From fr. 4 (hexane–EtoAc, 1:1) the obtained lignans were **7**, **9** and **10**. Fr. 5 (EtoAc) gave compounds **3** and **8** respectively.

Compound **4**

EIMS m/z: 234 ([M]$^+$, 65%), 219 (32), 203 (41), 178 (100), 147 (21). ^{1}H NMR; δ 1.72 (3H, *s*, Me), 3.87 (3H, *s*, OMe), 4.92, 5.07 (2H, *br s*, $=CH_2$); 4.95, 5.15 (2H, *d*, J = 3.9 Hz, H-2, H-3), 6.90 (1H, *d*, J = 8.7 Hz, H-7), 8.0 (1H, *dd*, 8.7 and 2.0 Hz, H-6), 8.07 (1H, *d*, J = 8.7 Hz, H-4). ^{13}C NMR: δ 17.4 (*q*, Me), 52.0 (*q*, OMe), 76.1 (*d*, C-3), 94.8 (*d*, C-2), 110.1 (*d*, C-7), 113.0 (*t*, C-2′) 123.4 (*s*, C-5), 127.2

(*d*, C-4), 128.3 (*s*, C-9), 133.4 (*d*, C-6), 141.0 (*s*, C-1′) 163.9 (*s*, C-8), 166.6 (*s*, –C=O).

Anti-oxidative assay

The methods of Refs. [13] and [14] were slightly modified. Briefly, 1 mg of each pure compound was dissolved in 1 ml of MeOH as sample soln. To 5 ml of 1/30 M Pi buffer (pH 7), was added 0.1 ml of sample soln and 5 ml linoleic acid (0.13% in EtOH) plus 2.4 ml H_2O. The mixt. was incubated at 40° in the dark. To 0.1 ml of this sample soln, was added 4.7 ml of 75% EtOH and 0.1 ml of 30% ammonium thiocyanate. Precisely 3 min after the addition of 0.1 ml of 0.02 M ferrous chloride in 3.5% HCl to the reaction mixt., the absorbance of the red colour developed was measured at 500 nm.

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