ABSTRACT

A benzoate, ethyl 4-hydroxybenzoate (1) was isolated from the stems of Synsepalum dulcificum Daniell (Sapotaceae). The structure of the benzoate was elucidated by chemical and physical evidence.

Keywords
Synsepalum dulcificum, Sapotaceae, Stems, Benzoate.

Introduction
Synsepalum dulcificum Daniell (Sapotaceae) is an evergreen shrub native of tropical West Africa; and its fruit, red berries, have the property of remarkably altering the sour taste into sweet taste [1]. This explains why the berry has been called “miracle fruit or mysterious fruit”. In the course of screening for chemically novel agents from plants, S. dulcificum was chosen for further phytochemical investigation [2-5]. In the course of screening for biologically and chemically novel agents from Formosan Sapotaceous plants [6,7], S. dulcificum was chosen for further phytochemical investigation. In continuation of some studies of chemotaxonomy and biologically active metabolites from this plant, a methanol extraction of the stems of S. dulcificum afforded 7 compounds, including ethyl 4-hydroxybenzoate (1), propane-1,2,3-triol (2), 2,5-dimethoxyphenol (3), 3,4,5-trimethoxybenzoic acid (4), nicotinic acid (5), β-sitosterol (6) and stigmasterol (7) [8]. In this paper, we report the isolation and structural elucidation of this benzoate (1).

Ethyl 4-hydroxybenzoate (1), obtained as a white powder, established by the molecular formula C_9H_10O_3 by HR-EIMS at m/z [M + Na]^+ 189.0522 (calcd for C_9H_10O_3Na, 189.0528). Two IR bands at ν_{max} 3300 and 1670 cm^{-1} and a signal appearing at δ 168.1 in the 13C NMR spectrum suggested that hydroxyl groups and an ester group might be present. The 1H NMR spectrum revealed an AA’BB’ pattern at δ 7.36 (2H, d, J = 9.0), and 6.84 (2H, d, J = 9.0) for H-2,6′ and H-3,5′, one methylene protons at δ 4.01 (2H, q, J = 7.2) for H-1′, one methyl protons at δ 1.39 (3H, t, J = 7.2) for H-2′, respectively. 13C NMR and DEPT experiments on 1 showed 9 resonance lines consisting of one methyl, one methylene, four methines and three quaternary carbons (including one carbonyl signal at δ 168.1).

The mass, UV, IR and 1H NMR data suggested that 1 is a type of benzoate and that the position of hydroxyl group should be located on the skeleton. The sequential correlations of the NOESY spectrum were successfully established as shown in Figure 1. Thus, the structure of this compound was named to be ethyl 4-hydroxybenzoate (1), which was further confirmed by HMBC experiments (Table 1).
Table 1: NMR data of 1 in CDCl₃ (δ in ppm, J in Hz, 400 MHz for ¹H NMR, and 100 MHz for ¹³C NMR).

<table>
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<tr>
<th>Position</th>
<th>δ_ C</th>
<th>δ_H</th>
<th>mult.</th>
<th>J (Hz)</th>
<th>HMBC (δH → δC)</th>
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<td>1</td>
<td>130.8</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>2</td>
<td>121.9</td>
<td>7.36</td>
<td>d, 9.0</td>
<td>C-1, C-3(5), C-4</td>
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<td>C-1, C-2(6), C-4</td>
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<td>7</td>
<td>168.1</td>
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<tr>
<td>1'</td>
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<tr>
<td>2'</td>
<td>14.8</td>
<td>1.39</td>
<td>t, 7.2</td>
<td>C-1'</td>
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</tr>
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</table>

Figure 1: NOESY correlations of 1.

Experimental
General
UV spectra were obtained in CH₂CN, IR spectra were measured on a Hitachi 260-30 spectrophotometer. ¹H NMR (400 MHz), ¹³C NMR (100 MHz), HETCOR, HMBC, COSY, NOESY, and DEPT spectra were obtained on a Varian (Unity Plus) NMR spectrometer. Low-resolution ESI-MS spectra were obtained on an API 3000 (Applied Biosystems) and high-resolution ESI-MS spectra on a Bruker Daltonics APEX II 30e spectrometer. Silica gel 60 (Merck, Kieselgel 60 70~230 mesh, 230~400 mesh) was used for column chromatography. Precoated Silica gel plates (Merck, Kieselgel 60 F-254), 0.20 mm and 0.50 mm, were used for analytical TLC and preparative TLC, respectively, visualized with 50% H₂SO₄.

Plant Material
The specimen of the fruits of S. dulcificum was collected from Kaohsiung County, Taiwan, in October 2007. A voucher specimen was identified by Dr Fu-Yuan Lu (Department of Forestry and Natural Resources College of Agriculture, National Chiayi University) and was deposited in the School of Medical and Health Science, Fooyin University, Kaohsiung County, Taiwan.

Extraction and Isolation
The stems (6.14 kg) of S. dulcificum were extracted repeatedly with MeOH at room temperature for 24–48 h. The MeOH extract was dried and evaporated to leave a viscous residue (425.8 g). The residue was placed on a silica gel column and eluted with CH₂Cl₂ gradually enriched with MeOH to produce 4 fractions. Fraction 1 (6.38 g) was subjected to silica gel CC by eluted with n-hexane–EtOAc (5:1) to obtain β-sitosterol (6) (12 mg) and stigmastanol (7) (8 mg), respectively. Fraction 2 (4.15 g) eluted with CH₂Cl₂–MeOH (80:1) was further separated using silica gel CC (n-hexane–acetone, 2:1) to yield 3,4,5-trimethoxybenzoic acid (4) (3 mg). Fraction 3 (8.61 g) eluted with CH₂Cl₂–MeOH (50:1) was further purified by silica gel CC using the same solvent system and preparative TLC (CH₂Cl₂–MeOH, 10:1) to give propane-1,2,3-triol (2) (8 mg), 2,5-dimethoxyphenol (3) (5 mg) and nicotinic acid (5) (6 mg). Fraction 4 (7.33 g) eluted with CH₂Cl₂–MeOH (40:1) was further purified by silica gel CC using the same solvent system and preparative TLC (CH₂Cl₂–MeOH, 15:1) to give ethyl 4-hydroxybenzoate (1) (4 mg).

Ethyl 4-hydroxybenzoate (1). White powder. UV λmax (MeCN, log ε) 213 (2.75), 253 (3.53), 273 (2.42) nm. IR (νmax, cm⁻¹): 3300 (OH), 1670 (C=O). ESI-MS m/z 189 [M+Na]+; HR-ESI-MS m/z 189.0522 [M+Na]+ (calcd for C9H10O3Na, 189.0528). 1H NMR (400 MHz, δ in ppm, J in Hz, 400 MHz for ¹H NMR, and 100 MHz for ¹³C NMR): 6.84 (2H, d, 9.0 Hz), 7.24 (2H, d, 9.0 Hz).

References