A Pterocarpan from *Erythrina variegata*

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Abstract: A pterocarpan, natural dihydrofolin, was isolated from the roots of *Erythrina variegata* and its structure was established on the basis of spectroscopic evidence. Two known compounds, the pterocarpan erythabysinin II and the alkyl ester of ferulic acid, octacosyl ferulate, were also isolated.

Key words: *Erythrina variegata*, Leguminosae, roots, pterocarpans, natural dihydrofolin

Introduction

*Erythrina variegata* (Leguminosae) is known to occur in regions south of Himalaya and China. It represents one out of more than 100 *Erythrina* species that are widely distributed in tropical and semitropical regions of the world. Different parts of *E. variegata* have been used as folk medicine by the Malas in peninsula Malaysia for sores, tooth-ache, febrifuge, dysentery, blood in urine, antite to snake bites, stimulating child's appetite and increasing milk flow (Burkill, 1966). The tree is also used for support of peppers in and as shades in coffee plantations. Previous phytochemical studies on different parts of this plant have led to the isolation of pterocarpans (Telikepalii et al., 1990; Tanaka et al., 2000), isoflavonoids (Deshpande et al., 1977; Telikepalii et al., 1990; Huang and Yen, 1996, 1997; Tanaka et al., 2000), erythinan alkaloids (Ghosal et al., 1970; El-Olemy et al., 1978; Chawla et al., 1988; Sharma and Chawla, 1992, 1996; Chawla and Sharma, 1993) and others (Ghosal et al., 1970, 1972; Deshpande et al., 1977; El-Olemy et al., 1978; Telikepalii et al., 1990; Chawla and Sharma, 1993; Huang and Yen, 1997). Pterocarpans from *E. variegata* like cryoritigallin, erythabysinin II and phaselinin were shown to have antimicrobial activities against Staphylococcus aureus and Mycobacterium smegmatis (Telikepalii et al., 1990). In an investigation on other secondary metabolites from *E. variegata*, we now describe the isolation and a comprehensive structural elucidation of a natural product, the pterocarpan dihydrofolin (1), along with two known compounds from the roots of the plant. Synthetic dihydrofolin was prepared previously from folin by catalytic hydrogenation (Brink et al., 1970). Erythabysinin II (2) as an isomer of 1 has two uncyclized sets of an isoprenyl side chain that is adjacent to a phenolic hydroxyl group. Compound 2 was cyclized using acid catalyst to afford dihydrofolin (Baker and Mitscher, 1995). Octacosyl ferulate (3), on the other hand, which is also known as erythrinamine is new in this plant.

Materials and Methods

General: TLC and prep. TLC were performed using manual-coated glass plates with silica gels 60 GF254 and PF254, whereas CC was carried out on silica gel (230-400 mesh). Spots and bands for compounds were detected using UV light at 254 and 360 nm. UV spectra were recorded on a JASCO spectrophotometer. CD spectra were recorded on a JASCO J-720W spectropolarimeter. 1H NMR (600 MHz) and 13C NMR (151 MHz) spectra were recorded on JEOL ECP-600 and chemical shifts in ppm δ were referenced to int. TMS and to CDCl3, respectively. 1H-1H COSY, HMOC and HMBC spectra were acquired using the standard JEOL software.

**Dihydrofolin (1)**

**Erythabysinin II (2)**

**Octacosyl ferulate (3)**

**Three mass spectrum fragments of dihydrofolin (4)**

**Mass spectra were recorded on a JEOL JMS HX-110 spectrometer.**

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542
Plant material: The roots of *ERYTHRAIA VARIEGATA* were collected from Dengkil, Selangor, Malaysia, in April, 2001. A voucher specimen was deposited at the Herbarium of Universiti Kebangsaan Malaysia.

Extraction and isolation: The air-dried powdered roots (150 g) of *ERYTHRAIA VARIEGATA* were twice extracted with MeCO and the combined extract evaporated to give a dark-brown residue (5 g). The extract was subjected to CC on silica gel with hexane containing increasing percentages of EtOAc as eluent and each collected fraction was 20 ml. Frs 30-37 contained dihydrofolin (1) (6.2 mg), Frs 57-66 (102 mg) were purified by prep. TLC (hexane-EtOAc, 7:3) to give octacysyl furate (0.8 mg), Rf 0.25 (hexane-EtOAc, 7:3). Frs 115-147 (100 mg) were purified twice by prep. TLC, first with hexane-EtOAc (6:4) and second with CHCl<sub>3</sub>-MeOH (9:1) to afford erthyribassilin (2) (2 mg), Rf 0.85 (hexane-CHCl<sub>3</sub>, 6:4), Rf 0.60 (CHCl<sub>3</sub>-MeOH, 9:1). Identification of octacysyl furate and erthyribassilin were made by comparison with the data from previous NMR and mass spectra (Kamat et al., 1981; Forum et al., 1986; Nkenfack et al., 1989).

**Dihydrofolin (1):** White needles, UV (MeOH) l<sub>max</sub> nm (log e) 210.5 (4.53), 290 (3.82): CD (MeOH, c 0.000250) [b] 98.50, [b] 192.1 +3.2, [b] 190.0, [b] 193.6. 6.9, [b] 191.4 -3.8, [b] 191.4 -196.6. FABMS m/z (rel. int.): 307 (2), 289 (100), 271 (77), 257 (19), 239 (13), 233 (8), 221 (6), 215 (1), 191 (1), 174 (82), 146 (45), 107 (15), 89 (13), 77 (14), 41 (8), 31 (2).

**HRFAB MS m/z: 392.1953 (M+), calcd for C₂₂H₂₆O₂.** 392.1987. H<sup>1</sup>NMR and <sup>13</sup>C NMR (Table 1).

**Erythribassilin (2):** FABMS m/z (rel. int.): 392 (45), 373 (6), 307 (24), 289 (131), 261 (4), 215 (6), 189 (8), 154 (MI<sup>+</sup>) (100), 138 (68), 107 20), 89 (17), 77 (16), 41 (8), 31 (2).

**Results and Discussion**

Silica gel chromatography from the MeCO extracts of the roots of *ERYTHRAIA VARIEGATA* gave the pterocarpan 1 as a natural product and two known compounds 2 and 3. Erythribassilin II has been previously isolated from several plant sources including the root extracts of *E. variegata* (Telliprolu et al., 1990) while octacysyl furate is isolated from the stem bark extracts of *E. bella* (Yenesew et al., 1998). Compound 1 was obtained as white needles. Signals and absorptions characteristic of pterocarpanos having a 6a, 11a-dihydro-6β-benzofuranbenzypanyl backbone was found in the <sup>1</sup>H NMR (Table 1) and the UV spectrum. In particular, a typical ABM xenon shown in the axial H-6, equatorial H-6, H-6a and H-11a at 6 0.20, 3.57, 3.48 and 6.45, respectively (Pachler and Underwood, 1967). Also obvious were signals for four aromatic protons, representing a para-situated pair at 6 6.58 (H-4) and 7.25 (H-1); and an ortho-coupled pair at 6 6.35 (H-8) and 6.95 (H-7). The two ethylidenepropiophenol moieties which form parts of the two dimethylhydroydpyran rings in 1 displayed four singlets for methyl protons at 6 1.29 (3H-8<sup>α</sup>), 1.31 (3H-8<sup>β</sup>), 1.34 (3H-6<sup>α</sup>) and 1.35 (3H-6<sup>α</sup>) and five methane proton signals at 6 0.76 (2H-3<sup>α</sup>), 0.80 (2H-3<sup>β</sup>), 2.65 (4H-4<sup>α</sup>), 2.69 (4H-4<sup>β</sup>) and 2.78 (2H-4<sup>α</sup>). <sup>1</sup>H COSY spectrum confirmed the coupling connectivity within the molecule 1. The <sup>1</sup>C-decoupled NMR spectrum of 1 (Table 1) was confirmed by HMOC and HMB correlation spectra.

The placement for the two ethylidenepropiophenol moieties in 1 were deduced by the HMB correlation experiment, showing interconversions first from H-4<sup>α</sup> (2.78) to C-2 (6 115.2) and C-3 (6 155.6) and from H-3<sup>α</sup> (6 0.80) to C-2 and second from H-4<sup>α</sup> (6 0.56 and 6.69) to C-10 (6 106.4) and C-9 (6 155.4) and from H-3<sup>β</sup> (6 1.78) to C-10. Molecular formula of C<sub>32</sub>H<sub>32</sub>O<sub>2</sub> for dihydrofolin (1) was assigned by the HRFAB mass spectrum (MI<sup>+</sup>) m/z 392.1983). The FAB mass spectrum of 1 revealed a base peak at m/z 392, a peak for [M+Me<sup>+</sup>] at m/z 377 representing loss of a C<sub>2</sub>H<sub>5</sub>ON from either one of the two ethylidnepropiophenol groups. The latter fragment further loss ChO, C<sub>2</sub>H<sub>3</sub> and C<sub>2</sub>H<sub>2</sub>O to give respective peaks at m/z 307, 281 and 215. Fragment at m/z 307 then loses H<sub>2</sub>O to yield a peak at m/z 289 whereas fragment at m/z 215 loss C<sub>2</sub>H to produce a peak at m/z 191. Three prominent peaks at m/z 154 (162%), 136 (45%) and 107 (15%) could arise from three fragments (Structure 4). The FAB mass spectrum for erythribassilin II, on the other hand, showed almost identical fragments as of its isomer dihydrofolin, but with a base peak at m/z 154 and other prominent peaks at m/z 135 (66%), 107 (20%), 392 (45%) and 307 (24%). Both peaks at m/z 189 for erythribassilin II and at m/z 191 for dihydrofolin were comparable which both derived from the fragment at m/z 215 by losing CH<sub>2</sub>H<sub>2</sub> and C<sub>2</sub>H<sub>5</sub>, respectively.

From all of the above UV, NMR and mass spectra observations, the structure for dihydrofolin is represented by 1. This report appears to be the first on the occurrence of pterocarpan as a natural compound with two terminal 2,2-dimethylhydroydpyran rings. Synthetic compound 1 could be prepared from folin in which 2,2-dimethylpyn ring on the benzofurancide was converted to 2',2'-dimethylhydroydpyran ring by catalytic hydrogenation (Brink et al., 1970).

**Acknowledgment**

We wish to express our thanks to Mr. A. Zainuddin Ibrahim of Universiti Kebangsaan Malaysia for collecting and identifying the plant sample.

**References**


Ahmad et al.: Erythrina variegata, Leguminosae, roots, pterocarps, natural dihydrofolinin


