An Isoflovonoid, Warangalone from the Stem Bark of Dadap Ayam (*Erythrina variegata*)

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ABSTRACT

In the course of our continuing search for novel paralytic compound from Indonesian plants, the methanol extract of the stem bark of *Erythrina variegata* (Leguminosae) showed significant paralytic activity against the third instar larvae of silkworm (*Bombyx mori*). The purposes of this research were isolation and structural elucidation of paralytic compound from the stem bark of *E. variegata*. Using the paralytic activity following the separation, the methanol extract was separated by combination of column chromatography to yield prenylisoflavone, warangalone. The chemical structure of warangalone was identified based on spectroscopic evidence and comparison with the previous reported. The paralytic activity of warangalone showed weak activity against the third instar larvae of silkworm (*B. mori*).

Keywords: *Erythrina variegata*, Leguminosae, isoflavonoid, warangalone, paralytic activity

INTRODUCTION

The genus *Erythrina* (Leguminosae) comprises 107 spesies distributed in tropical and subtropical regions of the world (Corner & Watanabe, 1969). Extract of the leaves, stem bark, seed, and roots have a significant history of use in indigenous medical practice for the treatment of diseases such as skin tumors due to insect bites and pathological inflammations (Hanum & Maesen 1997). Previous studies have shown that the leaves, stem bark and seed of many *Erythrina* species principally contains alkaloids, which are known to display interesting biological activities (Chawla & Jackson 1986, Supratman *et al*. 2000, Herlina *et al*. 2003, 2004, 2005,2006). In our on going research on the Indonesian *Erythrina* plants, we have studied the stem bark of *E. variegata,* which is widely used in traditional medicine to treat various diseases, including dysentry, asthma, venereal diseases, and leprosy. *E. variegata* known as "dadap ayam" in Indonesia (Heyne 1987). As part of our continuing search for novel paralytic compound from Indonesian *Erythrina* plants, we report the isolation and structural identification of an isoflovonoid, warangalone (Figure 1).

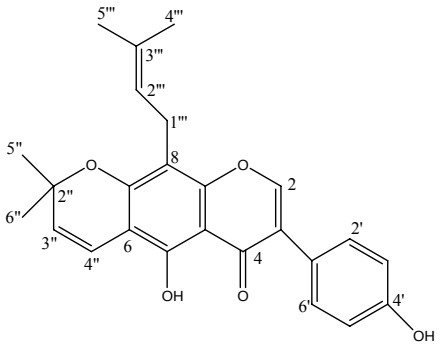

Figure 1. Chemical Structure of Warangalone.

METHODS

General experimental procedure

Melting points (mp) were uncorrected. The IR spectra were recorded with a Perkin-Elmer 1760 X FT-IR spectrophotometer, and the UV spectra were recorded with a Hitachi model U-3210. Mass spectra were recorded with JEOL JMS-DX300 instrument. The ^{1}H- and ^{13}C-NMR spectra were obtained with JEOL JNM GX 270 and JNM A-500 spectrometer. Chemical shifts are given on a δ (ppm) scale with TMS as an internal standard. Column chromatography was carried out using Merck Kieselgel 60 (70-200 mesh), and TLC analysis on precoated plates (Merck Kieselgel GF_{254}, 0.2 mm).

Paralytic assay

Larvaes of *B. mori* were used for the bioassay, and were cultured on an artificial diet purchased from Nippon Nosan Kogyo Co., Ltd. To one gram of the diet, 100 μl of the methanol extract or a certain amount of the sample to be tested was added in a petri dish. After removing the solvent, five larvaes of the third instar stage were introduced into the petri dish, and the paralytic rate of the silkworm was observed within 1-6 hours at 25°C after initiating the administration (Hayashi *et al*. 2000).

Plant material

Samples of the stem bark of *E. variegata* were collected in June, 2004, in Bandung District, West Java, Indonesia. The plant was identified by a staff at the Laboratory of Plant Taxonomy, Department of Biology, Bandung Insitute of Technology, Bandung, Indonesia, and a voucher specimen has been deposited at the herbarium.

Extraction and isolation

The dried stem bark (2.2 kg) of *E. variegata* were soaked in MeOH. Evaporation of the MeOH extract gave concentrated aqueous extract, which was extracted with CH_2Cl_2. The resulting CH_2Cl_2 extract was partitioned between *n*-hexane and MeOH containing 10% water, and then lower layer was concentrated and extracted with EtOAc. The EtOAc layer was subsequently dried over anhydrous sodium sulphate, filtred, evaporated to dryness. The EtOAc fraction (15.8 g) was chromatographed on Kieselgel 60 (70-230 mesh) by eluting with *n*-hexane and an increasing ratio of EtOAc, and by EtOAc and an increasing ratio of MeOH to afford the 20% MeOH eluate (1.2 g). The fraction (683 mg) eluted with 10% and 20% methanol were further flash-chromatographed on Kieselgel 60 with 5% MeOH in $CHCl_3$ to yield a crude active compound (42.3 mg), which was crystallized from MeOH to yield an active compound (25.5 mg).

RESULTS AND DISCUSSION

The methanolic extract of the dried stem bark of *E. variegata* was concentrated and extracted with CH_2Cl_2. The CH_2Cl_2 extract exhibited a paralytic activity toward silkworms. The CH_2Cl_2 extract was partitioned between *n*-hexane and MeOH containing 10% water. The active lower layer was extracted with EtOAc. By using the paralytic activity to follow the separations, the EtOAc fraction was separated by combination of column chromatography on Kieselgel 60 to afford an isoflavonoid (**1**).

Compound **1** was obtained as pale yellowish needles (*n*-hexane-acetone); mp 163-164°C. Its molecular formula, $C_{25}H_{24}O_5$ was deduced by FAB-MS data which showed the $[M]^+$ at *m/z* 404 together with ^{1}H- and ^{13}C-NMR spectral data, indicating that **1** has fourteen bond equivalents. The UV absorption maximum of **1** in MeOH were absorved at 343, 261, 241 nm, suggested the presence of an isoflavonoid skeleton. The bathochromic shift observed by adding NaOH ($\Delta\lambda$ = 41, 17 and 13 nm) respectively, suggested the presence of a free hydroxyl group at C-5 which chelated with the carbonyl function at C-4 (Marby *et al*., 1970). Its IR spectrum showed vibration bands at 3453 (free OH), 1710 (C=O chelated), 1070 (C-O-C asymmetric) and 3077, 1513, and 825 cm^{-1} (*para* substituted aromatic ring), respectively.

The ^{1}H-NMR and ^{13}C-NMR in combination with DEPT spectra of **1** showed signals assignable to two methyl groups [δ_H 1.68 (3H, s), 1.81 (3H, s); δc 18, 26], and one gem-dimethyl at [δ_H 1.47 (6H, s)]; δc 28], an olefinic proton at [7.89 (1H, s); δc 152.7], a chelated hydroxyl [δ_H 13.1 (1H, s); δc 181.4], one non-chelated hydroxyl [δ_H 9.88, (1H, s); δc 156.9], and two set of ortho couple doublets (J = 8.5 Hz) resonating at δ_H 6.86 and 7.37, one carbonyl at δc 171.4, indicating **1** to be tetracyclyc structure. The presence of AB type aromatic proton signals were observed in the ^{1}H- and ^{13}C-NMR spectra [δ_H 5.63 (1H, d, J = 10 Hz); δc 128 and 6.74 (1H, d, J = 10 Hz); δc 116] were typical of 4" and 5", respectively, indicated the presence of a pyran ring. In the extended spectrum of ^{1}H-NMR indicated that the other prenyl group was observed from the presence of A_2X at 3.40 (2H, d, J = 7 Hz) and 5.17 (1H, dd, J = 7 and 8 Hz), suggesting that the prenyl group was located at C-8. Besides these, the signals arising from a methine carbon [δc 152.7 (C-2), 130.2 (C-2'), 115.0 (C-3'), 115.6 (C-5'), 130.2 (C-6'), 116 (C-4"), 128 (C-3"), 122 (C-2'''), and 152 (C-2), one oxygenated methane [δc 78 (d, C-2") were confirmed by the ^{13}C-NMR and DEPT spectra. These observations together with a detailed comparison of spectral data with those previously reported (Talla *et al*. 2003) led us to identify **1** as warangalone, 83,3-dimethyl-allyl)-4'-hydroxy-2''',2'''-dimethylpyran[6,7,b]isoflavon (Figure 1).

Biological activities of warangalone were examined against the third instar larvae silkworm (*B. mori*). Upon oral administration, warangalone exhibited paralysis with ED_{50} value more than 100 ppm, indicated that warangalone showed weak activity.

Table 1. The NMR data of isolate **1**[*]

Position	$\delta_{C(ppm)}$	$\delta_{H(ppm)}$ [integral, mult., and $J_{(Hz)}$]
2	152.7	7.89 (1H, s)
3	121.0	-
4	181.4	-
5	154.0	-
6	106.0	-
7	156.0	-
8	107.0	-
9	154.0	-
10	105.0	-
1’	123.0	-
2’	130.0	7.37 (1H, d, 8.5)
3’	115.0	6.86 (1H, d, 8.5)
4’	157.0	-
5’	115.6	6.86 (1H, d, 8.5)
6’	130.2	7.37 (1H, d, 8.5)
2”	78.0	-
3”	128.0	5.63 (1H, d, 10.0)
4”	116.0	6.74 (1H, d,10.0)
5”	28.0	1.47 (3H, s)
6”	28.0	1.47 (3H, s)
1’’’	21.0	3.40 (2H, d, 7.6)
2’’’	120.0	5.17 (1H, dd, 7 and 8)
3’’’	131.0	-
4’’’	26.0	1.68 (3H, s)
5’’’	18.0	1.81 (3H, s)

Note: *taken in $CDCl_3$ at 500 MHz for ^1H and 125 MHz for ^{13}C

CONCLUSION

The prenylisoflavone, warangalone has been isolated from the bark of *E. variegate.* The chemical structure of warangalone was identified on the basis of spectroscopic evidences and comparison with previously related compounds.

Warangalone showed paralytic against third instar larvae of silkworm (*B. mori*) with their ED_{50} values more than 100 ppm, indicated that warangalone showed weak activity.

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