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A Pterocarpan from *Erythrina variegata*

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

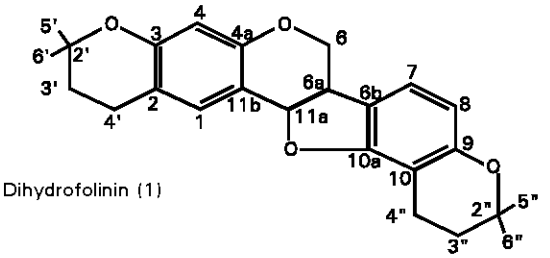
Key words: *Erythrina variegata*, Leguminosae, roots, pterocarpans, natural dihydrofolinin

Introduction

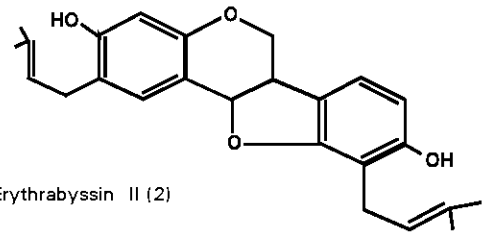
Erythrina variegata (Leguminosae) is known to occur in regions south of Himalaya and China. It represents one out of more than 100 *Erythrina* species that are widely distributed in tropical and subtropical regions of the world. Different parts of *E. variegata* have been used as folk medicine by the Malays in peninsula Malaysia for sores, tooth-ache, febrifuge, dysentery, blood in urine, antidote to snake bites, stimulating a child's appetite and increasing milk flow (Burkill, 1966). The tree is also used for support of pepperines and as shades in coffee plantations. Previous phytochemical studies on different parts of this plant have led to the isolation of pterocarpans (Telikepalli *et al.*, 1990; Tanaka *et al.*, 2000), isoflavonoids (Deshpande *et al.*, 1977; Telikepalli *et al.*, 1990; Huang and Yen, 1996, 1997; Tanaka *et al.*, 2000), erythrinan alkaloids (Ghosal *et al.*, 1970; El-Olemy *et al.*,1978; Chawla *et al.*, 1988; Sharma and Chawla, 1992, 1998; Chawla and Sharma, 1993) and others (Ghosal *et al.*, 1970, 1972; Deshpande *et al.*, 1977; El-Olemy *et al.*, 1978; Telikepalli *et al.*, 1990; Chawla and Sharma, 1993; Huang and Yen, 1997). Pterocarpans from *E. variegata* like crycristagallin, erythrabyssin II and phaseollin were shown to have antimicrobial activities against *Staphylococcus aureus* and *Mycobacterium smegmatis* (Telikepalli *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 dihydrofolinin (1), along with two known compounds from the roots of the plant. Synthetic dihydrofolinin was prepared previously from folinin by catalytic hydrogenation (Brink *et al.*, 1970). Erythrabyssin 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 dihydrofolinin (Baker and Mitscher, 1995). Octacosyl ferulate (3), on the other hand, which is also known as erythrinasinate is new in this plant.

Materials and Methods

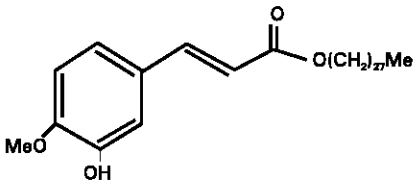
General: TLC and prep. TLC were performed using manual-coated glass plates with silica gels 60 GF_{254} and PF_{254}, whereas CC was carried out on silica gel (230-400 mesh). Spots and bands for compounds were detected using UV light at 254 and 360 nm. UV spectra were recorded on a JASCO spectrophotometer. CD spectra were recorded on a JASCO J-729WI spectropolarimeter. ^{1}H NMR (600 MHZ) and ^{13}C NMR (151 MHZ) spectra were recorded on JEOL ECP-600 and chemical shifts in ppm δ were referenced to int. TMS and to $CDCl_3$, respectively. ^{1}H -^{1}H COSY, HMQC and HMBC spectra were acquired using the standard JEOL software.



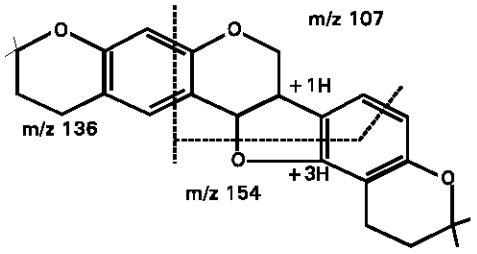
Dihydrofolinin (1)



Erythrabyssin II (2)



Octacosyl ferulate (3)



Three mass spectrum fragments of dihydrofolinin (4)

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

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Plant material: The roots of *Erythrina 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 *Erythrina variegata* were twice extracted with Me_2CO 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 fr. was 20 ml. Frs 30-37 contained dihydrofolinin (1) (5.2 mg). Frs 57-66 (102 mg) were purified by prep. TLC (hexane-EtOAc, 7:3) to give octacosyl ferulate (0.8 mg), R_f 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 $CHCl_3$-MeOH (9:1) to afford erythrabyssin II (2 mg), R_f 0.65 (hexane-EtOAc, 6:4), R_f 0.60 ($CHCl_3$-MeOH, 9:1). Identification of octacosyl ferulate and erythrabyssin II were made by comparison with the data from previous NMR and mass spectra (Kamat *et al.*, 1981; Fomum *et al.*, 1986; Nkengfack *et al.*, 1989).

Dihydrofolinin (1): White needles, UV (MeOH) λ_{max} nm (log ϵ): 210.5 (4.53), 290 (3.62); CD (MeOH, *c* 0.000255): $[\theta]_{310}$ 0, $[\theta]_{293}$ +3.2, $[\theta]_{260}$ 0, $[\theta]_{239}$ -7.9, $[\theta]_{225}$ -3.8, $[\theta]_{211}$ -18.6. FABMS m/z (rel. int.): 392 (100), 377 (3), 337 (8), 307 (13), 289 (7), 281 (8), 215 (9), 191 (7), 154 (62), 136 (45), 107 (15), 89 (13), 77 (14), 41 (6), 31 (2). HRFABMS m/z: 392.1953 ($[M]^+$, calcd for $C_{25}H_{28}O_4$: 392.1987). ^1H NMR and ^{13}C NMR (Table 1).

Erythtrabyssin II (2): FABMS m/z (rel. int.): 392 (45), 337 (6), 307 (24), 289 (13), 281 (4), 215 (5), 189 (6), 154 $[M]^+$ (100), 136 (66), 107 (20), 89 (17), 77 (16), 41 (6), 31 (2).

Table 1: ^1H NMR (600 MHZ, $CDCl_3$) and ^{13}C NMR (151 MHZ, $CDCl_3$) spectral data for compound 1[a]

Position	δ ^{13}C	δ ^1H
1	131.6	7.25 (s)
2	115.2	-
3	155.5	-
4	104.9	6.38 (s)
4a	154.9	-
6	66.8	eq 3.57 (t-like, 11.1) ax 4.20 (dd, 10.9, 5.1)
6a	40.0	3.48 (m)
6b	117.3	-
7	122.4	6.95 (d, 8.0)
8	109.3	6.35 (d, 8.0)
9	155.1	-
10	105.4	-
10a	158.1	-
11a	78.8	5.46 (d, 6.9)
11b	112.1	-
2'	74.7	-
3'	33.0	1.80 (t, 6.7)
4'	21.9	2.78 (t, 7.0)
5'	27.3[b]	1.35 (s)[b]
6'	26.63[b]	1.31 (s)[b]
2"	74.3	-
3"	32.0	1.76 (t, 6.9)
4"	17.1	2.66 (m) 2.69 (m)
5"	27.0[c]	1.34 (s)[c]
6"	26.60[c]	1.29 (s)[c]

[a]Assignments were based on COSY, HMQC and HMBC spectra.
[b,c]Assignments in the same vertical column may be interchanged.

Results and Discussion

Silica gel chromatography from the Me_2CO extracts of the roots of *Erythrina variegata* gave the pterocarpan 1 as a natural product and two known compounds 2 and 3. Erythrabyssin II has been previously isolated from several plant sources including from the root extracts of *E. variegata* (Telikepalli *et al.*, 1990) while octacosyl ferulate from the stem bark extracts of *E. burttii* (Yenesew *et al.*, 1998).

Compound 1 was obtained as white needles. Signals and absorptions characteristic of pterocarpans having a 6a, 11a-dihydro-6H-benzofurobenzopyran backbone was found in the ^1H NMR (Table 1) and the UV spectrum. In particular, a typical ABMX aliphatic spin system is clearly shown for the axial H-6, equatorial H-6, H-6a and H-11a at δ 4.20, 3.57, 3.48 and 5.46, respectively (Pachler and Underwood, 1967). Also obvious were signals for four aromatic protons, representing a *para*-situated pair at δ 6.38 (H-4) and 7.25 (H-1); and an *orto*-coupled pair at δ 6.35 (H-8) and 6.95 (H-7). The two ethyleneisopropylether moieties which form parts of the two dimethyldihydropyran rings in 1 displayed four singlets for methyl protons at δ 1.29 (3H-6"), 1.31 (3H-6'), 1.34 (3H-5") and 1.35 (3H-5') and five methylene proton signals at δ1.76 (2H-3"), 1.80 (2H-3'), 2.66 (1H-4"), 2.69 (1H-4") and 2.78 (2H-4'). ^1H-^1H COSY spectrum confirmed the coupling connectivity within the molecule 1. The ^{13}C-decoupled NMR spectrum of 1 (Table 1) was confirmed by HMQC and HMBC correlation spectra. The placement for the two ethyleneisopropylether moieties in 1 were decided by the HMBC experiment, showing interactions first from H-4' (2.78) to C-2 (δ 115.2) and C-3 (δ 155.5) and from H-3' (δ 1.80) to C-2 and second from H-4" (δ 2.66 and 2.69) to C-10 (δ 105.4) and C-9 (δ 155.1) and from H-3" (δ 1.76) to C-10. Molecular formula of $C_{25}H_{28}O_4$ for dihydrofolinin (1) was assigned by the HRFAB mass spectrum ($[M]^+$ m/z 392.1953). The FAB mass spectrum of 1 revealed a base peak at m/z 392, a peak for $[M\text{-}Me]^+$ at m/z 337, typical of compounds with isopropylether groups and a peak at m/z 377 representing loss of a C_4H_7 unit from either one of the two ethyleneisopropylether groups. The latter fragment further looses CH_2O, C_4H_8 and $C_7H_5O_2$ to give respective peaks at m/z 307, 281 and 215. Fragment at m/z 307 then loss H_2O to yield a peak at m/z 289 whereas fragment at m/z 215 loss C_2 to produce a peak at m/z 191. Three prominent peaks at m/z 154 (62%), 136 (45%) and 107 (15%) could arise from three fragments (Structure 4). The FAB mass spectrum for erythrabyssin II, on the other hand, showed almost identical fragments as of its isomer dihydrofolinin, but with a base peak at m/z 154 and other prominent peaks at m/z 136 (66%), 107 (20%), 392 (45%) and 307 (24%). Both peaks at m/z 189 for erythrabyssin II and at m/z 191 for dihydrofolinin were comparable in which both derived from the fragment at m/z 215 by losing C_2H_2 and C_2, respectively.

From all of the above UV, NMR and mass spectra observations, the structure for dihydrofolinin 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-dimethyldihydropyran rings. Synthetic compound 1 could be prepared from folinin where a 2,2-dimethylpyran ring on the benzofurano side was converted to 2,2-dimethyldihydropyran ring by catalytic hydrogenation (Brink *et al.*, 1970).

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References

Baker, W.R. and L.A. Mitscher, 1995. Isoflavonoid antibacterial compounds, compositions and use. Patent No. US 5399558 A, 17 pp.

Brink, C.V.D.M., J.P. Engelbrecht and D.E. Graham, 1970. Neorautanenia isoflavanoids. Part IV. Ficifolinol, folitenol and folinin, three new pterocarpans from the root bark of *Neorautanenia ficifolia* (Benth. Ex Harv.) C.A. Sm. J. South African Chemical Institute, 23: 24-33.

Burkill, I.H., 1966. A Dictionary of the Economic Products of the Malay Peninsula. Ministry of Agriculture and Cooperative, Malaysia.

Chawla, A.S., T.R. Krishnan, A.H. Jackson and D.A. Scalabrin, 1988. Alkaloidal constituents of *Erythrina variegata* bark. Planta Medica, 54: 526-528.

Chawla, H.M. and S.K. Sharma, 1993. Erythritol, a new isoquinoline alkaloid from *Erythrina variegata* flowers. Fitoterapia, 64: 15-17.

Deshpande, V.H., A.D. Pendse and R. Pendse, 1977. Erythrinins A, B and C, three new isoflavones from the bark of *Erythrina variegata*. Indian J. Chem. Section B, 15B: 205-207.

El-Olemy, M.M., A.A. Ali and M.A. El-Mottaleb, 1978. *Erythrina* alkaloids. I. The alkaloids of the flowers and seeds of *Erythrina variegata*. Lloydia, 41: 342-347.

Fomum, Z.T., J.F. Ayafor, J. Wandji, W.G. Fomban and A.E. Nkengfack, 1986. Erythrinasinate, an ester from three *Erythrina* species. Phytochem., 25: 757-759.

Ghosal, S., D.K. Ghosh and S.K. Dutta, 1970. Occurrence of erysotrine and other alkaloids in *Erythrina variegata*. Phytochem., 9: 2397-2398.

Ghosal, S., S.K. Dutta and S.K. Bhattacharya, 1972. Erythrinachemical and pharmacological evaluation II: Alkaloids of *Erythrina variegata* L. J. Pharmaceutical Sci., 61: 1274-1277.

Huang, K.F. and Y.F. Yen, 1996. Three prenylated isoflavones from *Erythrina variegata*. J. Chinese Chem. Soc. (Taipei), 43: 515-518.

Huang, K.F. and Y.F. Yen, 1997. Constituents of *Erythrina variegata*. (II). Chinese Pharmaceutical J. (Taipei), 49: 21-29.

Kamat, V.S., F.Y. Chuo, I. Kubo and K. Nakanishi, 1981. Antimicrobial agents from an East African medicinal plant *Erythrina abyssinica*. Heterocycles, 15: 1163-1170.

Nkengfack, A.E., D.R. Sanson, M.S. Tempesta and Z.T. Fomum, 1989. Two new flavonoids from *Erythrina eriotriocha*. J. Natural Products, 52: 320-324.

Pachler, K.G.R. and W.G.E. Underwood, 1967. A proton magnetic resonance study of some pterocarpan derivatives: The conformation of the 6a, 11a-dihydro-6H-benzofuro [3,2-c][1]benzopyran ring system. Tetrahedron, 23: 1817-1826.

Sharma, S.K. and H.M. Chawla, 1992. Isococcolinine: A new isoquinoline alkaloid from *Erythrina variegata* flowers. Indian J. Heterocyclic Chem., 2: 71-74.

Sharma, S.K. and H.M. Chawla, 1998. Structure elucidation of erythrosotidienone and erythromotidienone- two new isoquinoline alkaloids from *Erythrina variegata* flowers. J. Indian Chem. Soc., 75: 833-837.

Tanaka, H., H. Etoh, H. Shimizu, T. Makita and Y. Tateishi, 2000. Two new isoflavonoids from *Erythrina variegata*. Planta Medica, 66: 578-579.

Telikepalli, H., S.R. Gollapudi, A. Keshavarz-Shokri, L. Velazquez, R.A. Sandmann, E.A. Veliz, K.V.J. Rao, A.S. Madhavi and L.A. Mitscher, 1990. Isoflavonoids and a cinnamyl phenol from root extracts of *Erythrina variegata*. Phytochem., 29: 2005-2007.

Yenesew, A., J.O. Midiwo, M. Miessner, M. Heydenreich and M.G. Peter, 1998. Two prenylated flavanones from stem bark of *Erythrina burttii*. Phytochem., 48: 1439-1443.