Pergamon

PII: S0031-9422(97)00071-X

Phytochemistry, Vol. 45, No. 4, pp. 835–838, 1997

0031-9422/97 $17.00+0.00

THREE PTEROCARPANS FROM *ERYTHRINA CRISTA-GALLI*

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(*Received* 28 *October* 1996)

Key Word Index—*Erythrina crista-galli*; Leguminosae; wood; phytoalexin; pterocarpan; 6a-hydroxypterocarpan; erystagallins A–C.

Abstract—Three new pterocarpans, erystagallins A–C, were isolated from the wood of *Erythrina crista-galli*, together with the three known pterocarpans, cristacarpin, phaseollidin and 2-(γ,γ-dimethylallyl)-6a-hydroxyphaseollidin. Their structures were elucidated on the basis of the spectroscopic evidence. © 1997 Elsevier Science Ltd. All rights reserved

INTRODUCTION

During our investigation of the genus *Erythrina*, we reported [1] the isolation and structural determination of the pterocarpan, hydroxycristacarpone, from *Erythrina orientalis*. We have now carried out a study of the non-alkaloidal secondary metabolites of *E. crista-galli*, which is widely distributed in subtropical and tropical regions and has antimicrobial activity against Gram-positive bacteria (*Staphylococcus aureus* and *Mycobacterium smegmatis*) [2, 3]. There are some reports of chemical studies on its non-alkaloidal constituents, in which flavonoids [4], cinnamylphenols [5] and pterocarpans (cristacarpin **1**, phaseollidin **2**, demethylmedicarpin, erycristin, sandwicensin, erycristagallin and erythrabyssin-II) functioning as phytoalexins and possessing antimicrobial activity [2, 3, 6] have so far been isolated. We now describe the isolation and structural elucidation of three novel pterocarpans (**4–6**), named erystagallins A–C, along with three known pterocarpans, **1**, **2** and 2-(γ, γ-dimethylallyl)-6a-hydroxyphaseollidin **3** from the wood of this species.

RESULTS AND DISCUSSION

Silica gel chromatography from the methylene chloride extract of the wood of *E. crista-galli* afforded three novel pterocarpans (**4–6**) and three known pterocarpans (**1–3**).

Compound **3**, colourless oil, was the known 6a-hydroxypterocarpan with an equivocal stereochemistry [7]. The absolute stereochemistry at C-6a and C-11a was established as *S* from the negative optical rotation value [6].

Erystagallin A (**4**) was obtained as a colourless oil and the molecular formula was confirmed to be $C_{26}H_{30}O_5$ by the HR mass spectrum (*m/z* 422.2080). The UV spectral data and the characteristic three protons (δ 3.99, 4.11 and 5.24) in the ^{1}H NMR spectrum (Table 1) suggested that **4** was a 6a-hydroxypterocarpan derivative. The ^{1}H NMR spectrum revealed signals of four aromatic protons (δ 6.33, 6.53, 7.16 and 7.19) and two prenyl groups (δ 1.72, 1.731, 1.735, 1.738, 3.21, 3.28, 5.16 and 5.35), which were similar to those of **3**. The ^{13}C NMR spectrum (Table 2) of the partial structures was also in good agreement with that of **3**, indicating the same substitution patterns for aromatic rings A and D. The remaining methoxyl group was located at the C-9 position as deduced from the HMBC spectrum, indicating correlation between C-9 (δ 160.2) and the methoxyl proton (δ 3.80); further confirmation of this assignment was obtained from a DIFNOE experiment, which displayed NOE interaction between the methoxyl group and an aromatic proton (δ 6.53) at the C-8 position. Assignment of all the ^{1}H NMR and ^{13}C NMR signals of **3–6** was accomplished by analyses of the ^{1}H-^{1}H COSY, HMQC and HMBC spectra. The absolute stereochemistry at C-6a and C-11a was *S* from the negative optical rotation value. Therefore, the structure of erystagallin A was represented as formula **4** (6a-*S* and 11a-*S*).

Erystagallin B (**5**) was obtained as a colourless oil whose molecular formula was confirmed to be $C_{26}H_{30}O_6$ by the HR mass spectrum (*m/z* 438.2056). The UV and ^{1}H NMR spectra (δ 3.88, 4.03 and 5.39) indicated that **5** also had a 6a-hydroxypterocarpan skeleton. In the ^{1}H NMR spectrum of **5**, three aromatic protons (δ 6.23, 6.46 and 7.01) on rings A and

Table 1. ^{1}H NMR spectral data of compounds **2**–**6** in acetone-d_6

H	2	3	4	5	6
1	7.34 *d* (8.3)	7.19 *s*	7.19 *s*		7.34 *d* (8.3)
2	6.56 *dd* (8.3, 2.2)				6.56 *dd* (8.3, 2.4)
4	6.35 *d* (2.2)	6.32 *s*	6.33 *s*	6.23 *s*	6.37 *d* (2.4)
6	3.58 *t* (10.3)	3.97 *d* (11.5)	3.99 *d* (11.2)	3.88 *d* (11.2)	3.57 *dd* (10.3, 9.3)
	4.23 *dd* (10.3, 4.4)	4.09 *d* (11.5)	4.11 *d* (11.2)	4.03 *d* (11.2)	4.25 *dd* (10.3, 5.4)
6a	3.53 *m*				3.56 *m*
7	6.94 *d* (8.0)	7.00 *d* (8.2)	7.16 *d* (8.2)	7.01 *d* (8.3)	7.05 *d* (8.2)
8	6.38 *d* (8.0)	6.43 *d* (8.2)	6.53 *d* (8.2)	6.46 *d* (8.3)	6.25 *d* (8.2)
11a	5.45 *d* (6.6)	5.23 *s*	5.24 *s*	5.39 *s*	5.52 *d* (5.5)
1′	3.26 *d* (7.0)	3.28 *d* (7.3)	3.28 *d* (7.3)	3.25–3.30 *m*	3.00 *dd* (15.6, 9.8)
				3.38 *dd* (14.2, 7.0)	3.17 *dd* (15.6, 8.3)
2′	5.27 *t* (7.0)	5.36 *t* (7.3)	5.35 *t* (7.3)	5.27–5.29 *m*	4.60 *dd* (9.8, 8.3)
4′	1.61 *s*	1.73 *s*	1.731 *s**	1.78 *s*	1.21 *s*
5′	1.73 *s*	1.74 *s*	1.72 *s*†	1.66 *s*	1.25 *s*
1″		3.23 *d* (7.3)	3.21 *d* (7.3)	3.25–3.30 *m*	
2″		5.23 *t* (7.3)	5.16 *t* (7.3)	5.27–5.29 *m*	
4″		1.73 *s*	1.735 *s**	1.69 *s*	
5″		1.60 *s*	1.738 *s*†	1.61 *s*	
1-OMe				3.99 *s*	
9-OMe			3.80 *s*		
3-OH	8.15 *br s**	8.47 *s*	2.85 *s*‡	8.59 *s*	8.55 *s*
9-OH	8.53 *br s**	8.29 *s*	8.45 *s*‡	8.32 *s*	
6a-OH				4.83 *s*	
3′-OH					8.01 *s*

*, †, ‡ Assignments in same vertical column may be interchanged.

D and two prenyl groups (δ 1.61, 1.66, 1.69, 1.78, 3.25–3.30, 3.38 and 5.27–5.29) were assigned by comparison of the ^{1}H NMR and ^{13}C NMR spectra with those of **3**. The signal at δ 23.4 due to C-1′ in the ^{13}C NMR spectrum of **5** showed that the pterocarpan had oxygenated substituents at di*ortho*-positions to the prenyl group [8]. The methoxyl group was ascribed to the C-1 position from the HMBC spectrum, which indicated correlation between C-1 (δ 161.3) and the methoxyl protons (δ 3.99); this was confirmed by a DIFNOE experiment (Fig. 1). The absolute stereochemistry at C-6a and C-11a was also *S* (negative optical rotation). Therefore, the structure of erystagallin B was represented as formula **5** (6a-*S* and 11a-*S*).

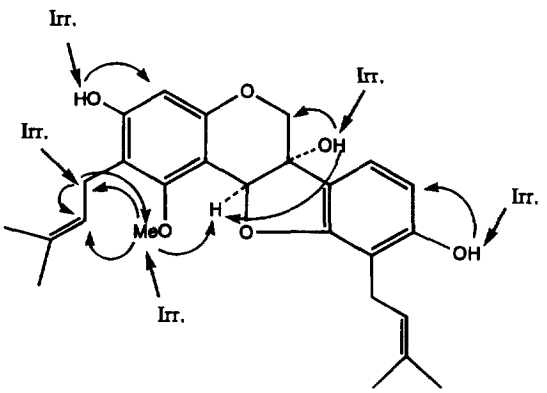
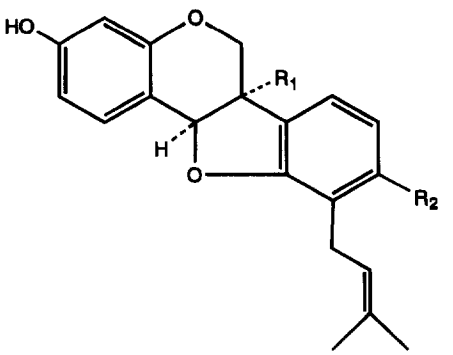


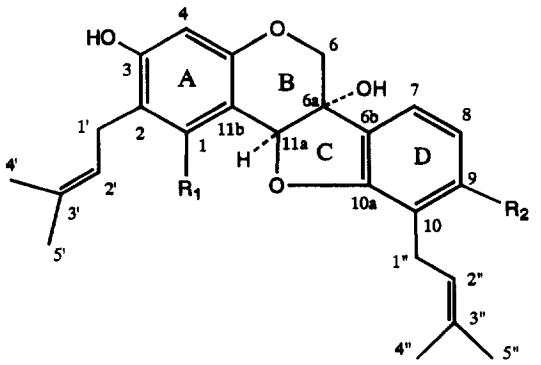
Fig. 1. Difference NOEs in compound **5**.

Erystagallin C (**6**) was obtained as a colourless oil and the molecular formula was confirmed to be $C_{20}H_{20}O_5$ by the HR mass spectrum (*m/z* 340.1299). The UV and ^{1}H NMR spectra (δ 3.56, 3.57, 4.25 and 5.52) indicated it to be a pterocarpan derivative. Comparison of the ^{1}H NMR spectrum of **6** with that of **2** displayed identical positions of five aromatic protons (δ 6.25, 6.37, 6.56, 7.05 and 7.34), an oxymethylene group (δ 3.57 and 4.25) and two methine protons (δ 3.56 and 5.52); these partial structures were also indicated by comparison of the ^{13}C NMR spectrum of **6** with that of **2**. The remaining signals [two methyl groups on a carbinol carbon (δ 1.21 and 1.25) and ABX-type aliphatic protons (δ 3.00, 3.17 and 4.60)] were attributable to a hydroxyisopropyldihydrofuran substituent which, in the mass spectrum, was verified by the characteristic fragments at *m/z* 281 $[M-59]^+$ and *m/z* 59 [9]. The C-1′ position on the hydroxyisopropyldihydrofuran moiety was linked to the C-10 position as deduced by HMBC, showing correlation between C-10 (δ 109.6) and H-1′ (δ 3.0 and 3.17 each), C-10 and H-8 (δ 6.25). Furthermore, the aliphatic proton at the C-1′ position (δ 3.0) revealed NOE interaction with a hydroxyl proton at the C-3′ position (δ 8.01), whose signal was ascribed from the HMBC spectrum. The absolute stereochemistry at C-6a and C-11a was *R* (negative optical



1 $R_1 = OH$, $R_2 = OMe$

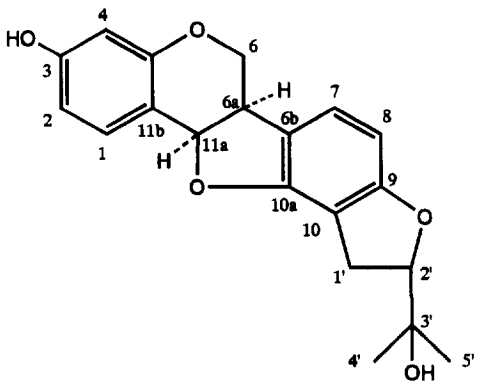
2 $R_1 = H$, $R_2 = OH$



3 $R_1 = H$, $R_2 = OH$

4 $R_1 = H$, $R_2 = OMe$

5 $R_1 = OMe$, $R_2 = OH$



6

rotation), which was confirmed by the CD spectrum which displayed a negative Cotton effect at 236 nm [6]. The configuration of C-2′, however, was obscure. Therefore, the structure of erystagallin C was represented as formula **6** (6a-*R* and 11a-*R*).

EXPERIMENTAL

General. Mps: uncorr. CC: Merck silica gel 60 (230–400 mesh). TLC: Kieselgel 60 F_{254} (Merck); spots were detected by spraying with 50% H_2SO_4 and by UV light. ^1H NMR 400 and 600 MHz) and ^{13}C NMR (67.5 MHz) spectra were measured in acetone-d_6 (TMS int. standard). UV spectra were recorded in MeOH.

Plant material. Wood of *E. crista-galli* was collected at Kagoshima prefecture, Japan, in July 1995.

Extraction and isolation. Wood (10.3 kg) was extracted with MeOH and evapd to give a dark green residue. The residue was divided into *n*-hexane-, CH_2Cl_2-, and EtOAc-sol. frs. The CH_2Cl_2-sol. fr. (8 g) was chromatographed on silica gel and eluted with $CHCl_3$, $CHCl_3$–Me_2CO (10:1), $CHCl_3$–Me_2CO (1:1) and $CHCl_3$–MeOH (10:1); 20 ml frs were collected. Frs 59–65 were sepd by CC [bezene–EtOAc (10:1)] to afford **2** (7 mg) and **4** (42 mg), which was further purified by CC [*n*-hexane–Et_2O (1:2)]. Frs 66–72 were sepd by repeated CC [benzene–EtOAc (10:1) and benzene–EtOAc (1:1)] to give **1** (103 mg) and **6** (15 mg), which was further purified by CC [*n*-hexane–Me_2CO (2:1)]. Frs 138–200 were sepd by CC [benzene–EtOAc (5:1) and subsequently by $CHCl_3$–MeOH (20:1)] to provide **5** (9 mg) and **3** (5 mg). Identification of **1** was made by direct comparison with an authentic sample [1].

Phaseollidin (**2**). Oil. $[\alpha]_D - 165°$ (MeOH, *c* 0.1). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3560, 1620, 1600. UV λ_{max} nm: 208, 282, 287. MS *m/z*: 324 ([M]$^+$, 100%), 309, 307, 281, 268, 251, 239. HRMS *m/z*: 324.1357 ([M]$^+$ calcd for $C_{20}H_{20}O_4$: 324.1360). Spectral data identical to those

Table 2. ^{13}C NMR spectral data of compounds **2–6** in acetone-d_6

C	2	3	4	5	6
1	133.0	132.5	132.5	161.3	133.2
2	110.4	123.1	123.0*	116.5	110.5
3	159.6*	156.9	156.9	158.4	159.8
4	103.9	103.4	103.4	100.1	104.0
4a	157.6	154.9	154.9	155.5	157.8
6	67.1	70.5	70.4	70.8	67.3
6a	40.9	77.2	77.0	76.8	40.4
6b	118.9	121.4	123.1*	121.6	120.4
7	122.6	121.9	122.0	121.8	124.4
8	108.1	108.6	104.3	108.7	101.7
9	156.8	157.8	160.2	157.7	163.3
10	112.0	112.0	113.3	112.0	109.6
10a	159.7*	160.0	159.5	159.7	156.8
11a	78.9	85.8	85.8	82.6	79.8
11b	113.2	113.3	113.1	107.7	112.8
1′	23.4	28.5	28.5	23.4	28.1
2′	123.6	124.0	123.9	124.7	90.8
3′	131.2	132.3	132.3	131.0	71.5
4′	17.8	17.9	17.8	18.0	25.5
5′	25.8	25.9	25.9	25.8	26.1
1″		23.2	23.1	23.2	
2″		123.4	123.2	123.6	
3″		131.3	131.5	131.4	
4″		17.9	17.8	17.9	
5″		25.9	25.9	25.8	
1-OMe				63.2	
9-OMe			56.3		

*Assignments in same vertical column may be interchanged.

reported for phaseollidin [10, 11]. ^1H NMR: Table 1; ^{13}C NMR: Table 2.

2-(γ,γ-Dimethylallyl)-6a-hydroxyphaseollidin (**3**). Oil. $[\alpha]_D -218°$ (MeOH, *c* 0.1). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3560, 1620, 1600. UV λ_{max} nm: 209, 285. MS *m/z*: 408 ($[M]^+$, 100%), 390, 380, 352, 347, 335, 325, 297, 291, 279, 269, 255, 251, 231, 221, 205, 203. ^1H NMR ($CDCl_3$): δ 1.79 (9H, *s*, 3 × Me), 1.74 (3H, *s*, Me), 2.35 (1H, *s*, OH), 3.34 (4H, *d*, *J* = 7.3 Hz, 1′-H and 1″-H), 3.95 (1H, *d*, *J* = 11.7 Hz, 6-H), 4.18 (1H, *d*, *J* = 11.7 Hz, 6-H), 5.24 (1H, *s*, 11a-H), 5.25 (1H, *s*, OH), 5.25 (1H, *t*, *J* = 7.3 Hz, 2′-H or 2″-H), 5.32 (1H, *t*, *J* = 7.3 Hz, 2′-H or 2″-H), 5.38 (1H, *s*, OH), 6.40 (1H, *s*, 4-H), 6.45 (1H, *d*, *J* = 8.2 Hz, 8-H), 7.08 (1H, *d*, *J* = 8.2 Hz, 7-H), 7.23 (1H, *s*, 1-H). HRMS *m/z*: 408.1927 ($[M]^+$, calcd for $C_{25}H_{28}O_5$: 408.1935). ^1H NMR: Table 1; ^{13}C NMR: Table 2. Spectral data identical to those reported for 2-(γ,γ-dimethylallyl)-6a-hydroxyphaseollidin [7].

Erystagallin A (**4**). Oil. $[\alpha]_D -182°$ (MeOH, *c* 0.1). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600, 1620, 1600. UV λ_{max} nm: 209, 283. MS *m/z*: (422 $[M]^+$, 100%), 404, 394, 366, 361, 351, 349, 339, 323, 297, 295, 283, 269, 231, 217, 203, 201. HRMS *m/z*: 422.2080 ($[M]^+$ calcd for $C_{26}H_{30}O_5$: 422.2092). ^1H NMR: Table 1; ^{13}C NMR: Table 2.

Erystagallin B (**5**). Oil. $[\alpha]_D -278°$ (MeOH, *c* 0.1). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600, 1620, 1610. UV λ_{max} nm: 210, 284. MS *m/z*: 438 ($[M]^+$, 100%), 420, 410, 395, 379, 377, 365, 355, 349, 342, 339, 323, 309, 299, 293, 283, 261, 245, 231, 217, 205, 203. HRMS *m/z*: 438.2056 ($[M]^+$ calcd for $C_{26}H_{30}O_6$: 438.2041). ^1H NMR: Table 1; ^{13}C NMR: Table 2.

Erystagallin C (**6**). Oil. $[\alpha]_D -58°$ (MeOH, *c* 0.1). CD (MeOH; $c\ 2.85 \times 10^{-5}$): Δ ε +4.01 (287), −7.44 (236), −3.71 (225), −13.15 (212). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600, 1620, 1600. UV λ_{max} nm: 209, 281, 286. MS *m/z*: 340 ($[M]^+$, 100%), 325, 307, 282, 281, 268, 253, 239, 223, 213, 211, 147, 59. HRMS *m/z*: 340.1299 ($[M]^+$ calcd for $C_{20}H_{20}O_5$: 340.1310). ^1H NMR: Table 1; ^{13}C NMR: Table 2.

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