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Isoflavonoids from the roots of *Erythrina poeppigiana*

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Abstract

Five isoflavonoids, 7,4′-dihydroxy-2′-methoxy-8-(γ,γ-dimethylallyl)isoflav-3-ene, 4′-hydroxy-2′-methoxy-6″,6″-dimethylpyran[2″,3″:7,8]isoflav-3-ene, 5,7,4′-trihydroxy-2′-methoxy-5′-(γ,γ-dimethylallyl)isoflavanone, 5,4′-dihydroxy-7,2′-dimethoxy-5′-(γ,γ-dimethylallyl)isoflavanone and 3,9-dihydroxy-4-(γ,γ-dimethylallyl)pterocarpene as well as six known compounds were isolated from the roots of *Erythrina poeppigiana*. Their structures were established on the basis of spectroscopic evidence. © 2002 Elsevier Science Ltd. All rights reserved.

Keywords: *Erythrina poeppigiana*; Leguminosae; Isoflavonoids; Isoflav-3-enes; Isoflavanones; Erypoegins A–E

1. Introduction

Erythrina poeppigiana (Leguminosae) is widely distributed in Central and South America and has been used as a shade tree and an ornamental plant. Phytochemical studies on this plant showed the presence of erythrinan alkaloids possessing curare-like action (Barton et al., 1973; Jackson and Singh, 1982), and a recent study on the wood of this plant reported the isolation of a new erythrinan alkaloid (8-oxo-α-erythroidine epoxide) and some isoflavonoids (Tanaka et al., 2001). In continuation of our investigation on the secondary metabolites of the genus *Erythrina*, we now describe the isolation and structural elucidation of five isoflavonoids, erypoegins A–E (**1**–**5**) along with six known compounds (**6**–**11**) from the roots of this source.

2. Results and discussion

Repeated chromatography of the dichloromethane extract of the fresh roots of *E. poeppigiana* gave five new isoflavonoids (**1**–**5**) (Fig. 1) together with six known compounds (**6**–**11**) (Fig. 2). The known compounds were identified as sandwicensin (**6**) (Mitscher et al., 1988), erythrabyssin II (**7**) (Kamat et al., 1981), angolensin (**8**) (Jain and Paliwal, 1988), phaseollidin (**9**) (Perrin et al., 1972), sophorapterocarpan A (**10**) (Komatsu et al., 1981) and erystagallin A (**11**) (Tanaka et al., 1997) by comparison of spectroscopic and physical data with those of authentic samples or the reported values.

The molecular formula of erypoegin A (**1**) was determined as $C_{21}H_{22}O_4$ ($[M]^+$ m/z 338.1527) from the HREI mass spectrum. The UV spectral data and characteristic A_2X system signals [singlet aliphatic protons at C-2 (δ 4.97) and an olefinic proton at C-4 (δ 6.50)] in the ^{1}H NMR spectrum showed that **1** is an isoflav-3-ene derivative (Miyase et al., 1981). The ^{1}H NMR spectrum displayed a set of *ortho*-coupled aromatic protons (δ 6.40 and 6.82), three aromatic protons in an ABX system (δ 6.41, 6.42 and 7.15) and a methoxyl group (δ 3.79) as well as a prenyl group (δ 1.74, 1.82, 3.41 and 5.27). The methoxyl group at the C-2′ position was assigned by the NOESY experiment which exhibited NOE interactions between the methoxyl group and the aromatic proton at C-3′ (δ 6.42) and between the methoxyl group and the aliphatic protons at C-2 (δ 4.97). The placement of the prenyl group at the C-8 position was confirmed from the HMBC spectrum (Fig. 3), indicating

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correlations from the aliphatic protons at C-1″ (δ 3.41) to carbons at C-7 (δ 155.2), C-8 (δ 114.4) and C-9 (δ 151.9). Therefore, the structure of erypoegin A is represented by **1**.

The molecular formula of erypoegin B (**2**) was determined as $C_{21}H_{20}O_4$ ($[M]^+$ m/z 336.1358) by the HREI mass spectrum. Comparison of the ^1H NMR spectral data of **2** with those of **1** showed the same substituent pattern signals of a peculiar A_2X type (δ 5.00 and 6.47), a set of *ortho*-coupled aromatic protons (δ 6.34 and 6.82), three aromatic protons in an ABX system (δ 6.41, 6.42 and 7.15) and a methoxyl group (δ 3.79); these same partial structures were also supported by comparison of the ^{13}C NMR spectrum of **2** with that of **1**. The remaining 2,2-dimethylchromene ring (δ 1.42, 5.59 and 6.65) was observed in the ^1H NMR spectrum. The presence of the dimethylchromene moiety was also evident from the EI mass spectrum which displayed the typical fragment at m/z $[M-15]^+$ (Takayama et al., 1992). The location of the dimethylchromene ring fused to the C-7 and C-8 positions was assigned by the HMBC experiment (Fig. 4), revealing correlations from the olefinic proton at C-1″ (δ 6.65) to a carbon at C-7 (δ 153.4) and from the other olefinic proton at C-2″ (δ 5.59) to a carbon at C-8 (δ 109.6). Therefore, the structure of erypoegin B is represented by **2**. Isoflav-3-enes have been rarely found in natural sources because of their low stability (Dewick, 1988).

Erypoegin C (**3**) was obtained in a racemic form and its molecular formula was determined as $C_{21}H_{22}O_6$ ($[M]^+$ m/z 370.1425) from the HREI mass spectrum. The IR spectrum showed the presence of conjugated carbonyl (1640 cm^{-1}) and hydroxyl (3400 cm^{-1}) groups. The UV spectrum and a set of aliphatic proton signals (δ 4.25, 4.40 and 4.54) in the ^1H NMR spectrum revealed that **3** is an isoflavanone derivative. The ^1H NMR spectrum exhibited a hydrogen bonded hydroxyl group (δ 12.27), a pair of *meta*-coupled aromatic protons (δ 5.92 and 5.97), two singlet aromatic protons (δ 6.42 and 6.79) and a methoxyl group (δ 3.73) in addition to a prenyl group (δ 1.75, 3.25 and 5.27). The prenyl group at the C-5′ position was assigned from the NOESY spectrum which revealed NOE interaction between the aliphatic protons at C-1″ (δ 3.25) and the aromatic proton at C-6′ (δ 6.79). The methoxyl group at the C-2′ position was assigned by the NOESY experiment, indicating NOE interaction between the methoxyl group and the aromatic proton at C-3′ (δ 6.42). Further assignment of the methoxyl group was obtained by the HMBC spectrum (Fig. 5), revealing correlation to the C-2′ carbon (δ 156.9). Therefore, the structure of erypoegin C is represented by **3**.

Erypoegin D (**4**) was also obtained in racemic form and its molecular formula was determined as $C_{22}H_{24}O_6$ ($[M]^+$ m/z 384.1579) from the HREI mass spectrum. The IR spectrum showed also the presence of conjugated carbonyl (1640 cm^{-1}) and hydroxyl (3410 cm^{-1}) groups. Comparisons of ^1H and ^{13}C NMR spectral data with those of **3** revealed that one OH group was replaced by a methoxyl group (δ 3.82 in the ^1H NMR spectrum and δ 55.6 or 55.7 in the ^{13}C NMR spectrum). The methoxyl group at the C-7 position was assigned by

Table 1
^1H NMR spectral data for compounds **1–5** in $CDCl_3$

H	1	2	3	4	5
1					7.26 *d* (8.8)
2	4.97 *s*	5.00 *d* (1.5)	4.40 *dd* (11.0, 5.9) 4.54 *dd* (11.7, 11.0)	4.41 *dd* (11.2, 5.4) 4.54 *t* (11.2)	6.47 *d* (8.8)
3			4.25 *dd* (11.7, 5.9)	4.25 *dd* (11.2, 5.4)	
4	6.50 *s*	6.47 *d* (1.5)			
5	6.82 *d* (8.1)	6.82 *d* (8.8)			
6	6.40 *d* (8.1)	6.34 *d* (8.8)	5.97 *d* (2.2)	6.08 *d* (2.0)	5.56 *s*
7					7.19 *d* (8.1)
8			5.92 *d* (2.2)	6.00 *d* (2.0)	6.77 *dd* (8.1, 1.5)
10					7.01 *d* (1.5)
1′					3.43 *d* (7.3)
2′					5.27 *t* (7.3)
3′	6.42 *d* (2.2)	6.42 *d* (2.2)	6.42 *s*	6.42 *s*	
4′					1.83 *s*
5′	6.41 *dd* (8.1, 2.2)	6.41 *dd* (8.1, 2.2)			1.75 *s*
6′	7.15 *d* (8.1)	7.15 *d* (8.1)	6.79 *s*	6.79 *s*	
1″	3.41 *d* (7.3)	6.65 *d* (10.3)	3.25 *d* (7.3)	3.25 *d* (7.3)	
2″	5.27 *t* (7.3)	5.59 *d* (10.3)	5.27 *t* (7.3)	5.28 *t* (7.3)	
4″	1.82 *s*	1.42 *s*	1.75 *s*	1.75 *s*	
5″	1.74 *s*	1.42 *s*	1.75 *s*	1.75 *s*	
7-OMe				3.82 *s*	
2′-OMe	3.79 *s*	3.79 *s*	3.73 *s*	3.73 *s*	
5-OH			12.27 *s*	12.27 *s*	

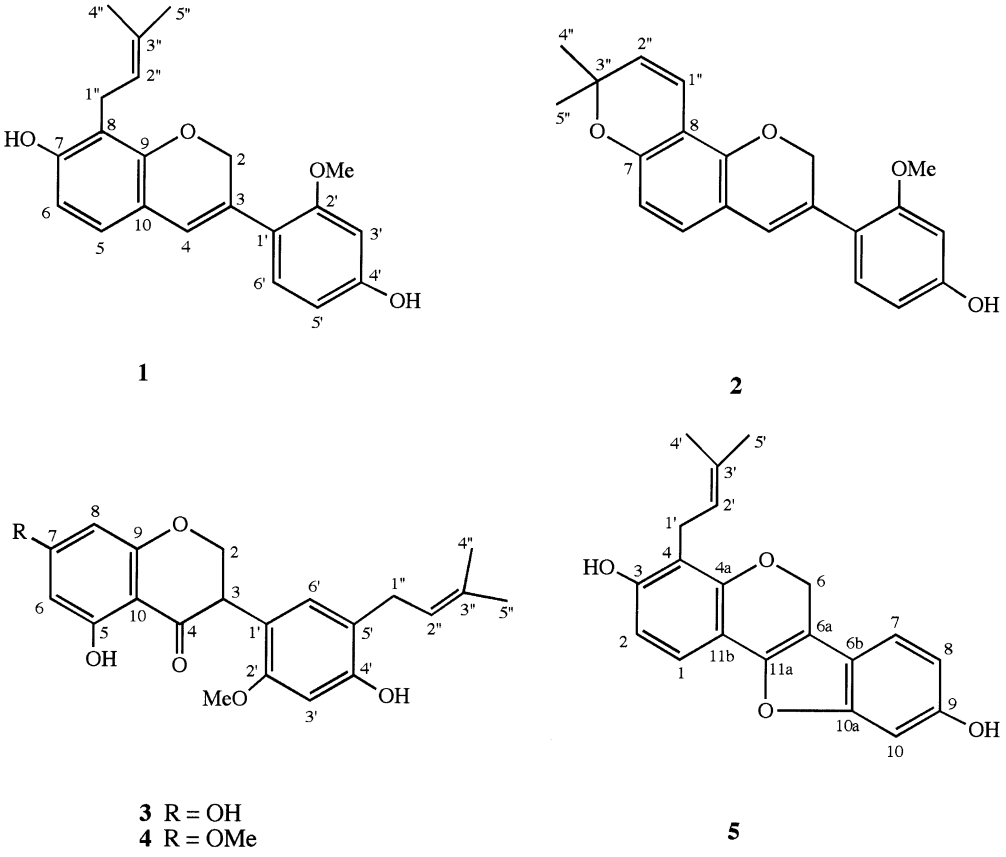

Fig. 1. Isolated compounds from *Erythrina poeppigiana*.

the NOESY experiment which exhibited NOE interactions between the methoxyl group and the aromatic proton at C-6 (δ 6.08), the methoxyl group and the aromatic proton at C-8 (δ 6.00). The assignment of the methoxyl group at C-7 was further confirmed from the following HMBC correlations (Fig. 6): the methoxyl group with a carbon at C-7 (δ 167.6); the carbon at C-7 with the aromatic proton at C-6; the aromatic carbon at C-6 (δ 94.9) with a hydrogen bonded hydroxyl group at C-5 (δ 12.27). Therefore, the structure of erypoegin D is represented by **4**.

The molecular formula of erypoegin E (**5**) was determined as $C_{20}H_{18}O_4$ ($[M]^+$ *m*/*z* 322.1211) from the HREI mass spectrum. The UV spectrum and the characteristic singlet signal at C-6 (δ 5.56) in the ^1H NMR spectrum showed that **5** has a pterocarpene skeleton (Prasad et al., 1985). The ^1H NMR spectrum displayed AMX type aromatic protons (δ 6.77, 7.01 and 7.19), a pair of *ortho*-coupled aromatic protons (δ 6.47 and 7.26) and a prenyl group (δ 1.75, 1.83, 3.43 and 5.27). The aromatic proton at the C-7 position (δ 7.19) in the AMX system was indicated by the DIFNOE experiment which exhibited NOE interaction between the aromatic proton at C-7 and the aliphatic protons at C-6. The assignment of the prenyl group at the C-4 position was confirmed from the HMBC experiment (Fig. 7), revealing that the aliphatic protons at C-1′ (δ 3.43) correlated with carbons at C-3 (δ 155.9), C-4 (δ 115.4) and C-4a (δ 152.1). Therefore, the structure of erypoegin E is represented by **5**.

3. Experimental

3.1. General

UV spectra were taken on a Beckman DU-530 spectrophotometer and IR spectra were recorded on a JASCO IR-810 spectrophotometer. The ^1H NMR spectra were measured on JEOL JNM-A 600 and 400 MHz spectrometers, while ^{13}C NMR spectra were recorded at 150.8 and 100.4 MHz on the same instruments. Mass spectra were obtained on a JEOL JMS-D 300 spectrometer. CC was performed using silica gel (230–400 mesh). TLC was carried out using Merck precoated silica gel (60 F_{254}). UV light and iodine vapor were used for the detection of compounds.

3.2. Plant material

The roots of *E. poeppigiana* were collected in Okinawa Prefecture, Japan, in April 2001. A voucher specimen was deposited at the Department of Natural Product Chemistry in the Faculty of Pharmacy, Meijo University.

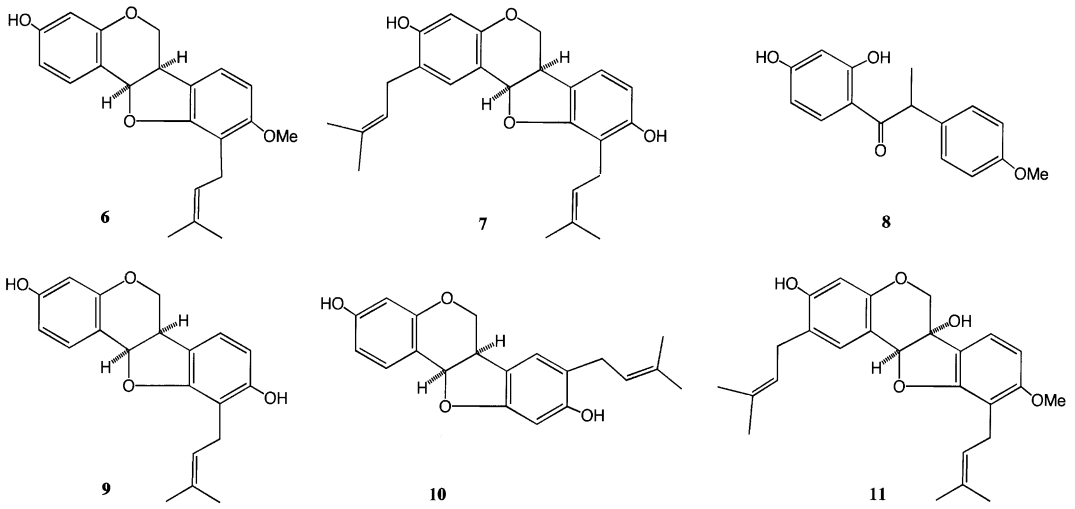

Fig. 2. Isolated compounds from *Erythrina poeppigiana* (continued).

Table 2
^{13}C NMR spectral data for compounds **1**–**5**[a] in $CDCl_3$

C	1	2	3	4	5
1					118.8
2	68.4	68.4	70.4	70.5	108.7
3	128.9	128.8	46.9	46.9	155.9
4	121.9[b]	121.6	197.6	197.6	115.4
4a					152.1
5	125.0	126.5	164.5[b]	164.5	
6	108.7	109.3	96.6	94.9	65.4
6a					105.4
6b					119.7
7	155.2	153.4	164.6[b]	167.6	118.5
8	114.4	109.6	95.1	93.9	111.9
9	151.9	149.1	163.6	163.3	153.2
10	117.1	116.9	103.4	103.5	98.9
10a					156.2
11a					148.1
11b					109.8
1′	120.8	120.7	114.4	114.4	22.6
2′	158.4	158.5	156.9	156.9	121.5
3′	99.1	99.1	100.2	100.2	134.7
4′	156.6	156.6	155.3	155.3	17.9
5′	107.3	107.3	118.5	118.5	25.8
6′	129.4	129.5[b]	131.4	131.4	
1″	22.4	116.7	29.3	29.2	
2″	121.8[b]	129.3[b]	121.8	121.8	
3″	134.5	76.1	135.2	135.0	
4″	17.9	27.8	17.8	17.8	
5″	25.8	27.8	25.7	25.7	
7-OMe				55.6[b]	
2′-OMe	55.4	55.4	55.7	55.7[b]	

[a] Assignments are based on HSQC and HMBC spectra.
[b] Assignments may be interchanged.

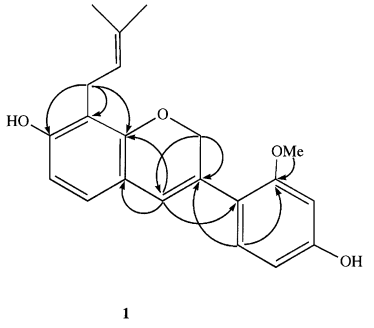

Fig. 3. Selected HMBC correlations observed in compound **1**.

3.3. Extraction and isolation

The finely powdered roots (3.2 kg) were macerated two times with acetone (18 l) and the solvent was removed to give a residue which was divided into *n*-hexane, CH_2Cl_2- and EtOAc-soluble fractions. The CH_2Cl_2-soluble fraction (20 g) was applied to silica gel column eluted with solvents of varying polarity of $CHCl_3$–acetone (10:1→1:1) and $CHCl_3$–MeOH (10:1) (each volume, 40 ml) to afford 18 fractions (fractions 1–18). Fractions 2 and 3 (1.1 g) were chromatographed on silica gel using $CHCl_3$–acetone (40:1) (each volume, 10 ml) to yield 20 fractions (fractions 19–38). Fractions 22 and 23 (188 mg) were purified by CC on silica gel using *n*-hexane–acetone (3:1) to furnish **4** (49 mg). Fractions 24–26 (549 mg) were separated by CC on silica gel using *n*-hexane–acetone (3:1) to provide **2** (5 mg), **6** (31 mg) and **7** (153 mg). Fractions 27 and 28 (165 mg) were repeatedly chromatographed on silica gel using *n*-hexane–acetone (3:1) to give **8** (21 mg). Fraction 3 (1.5 g) was separated by CC on silica gel successively using *n*-hexane–acetone (2:1) and benzene–EtOAc (10:1) to afford **1** (5 mg) and **5** (5 mg). Fraction 4 (0.8 g) was submitted to CC on silica gel using benzene–EtOAc (10:1→5:1) (each volume, 10 ml) to yield 30 fractions (fractions 39–68). Fractions 41–43 (228 mg) were rechromatographed on silica gel using $CHCl_3$–acetone (20:1→10:1.5) to furnish **9** (49 mg) and **10** (5 mg). Fractions 45–47 (250 mg) were subjected to CC on silica gel using $CHCl_3$–acetone (20:1) to provide **11** (192 mg). Fractions 48–51 (115 mg) were purified by

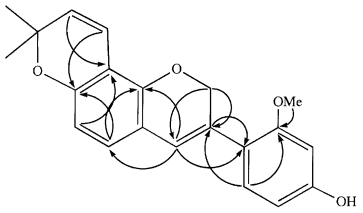

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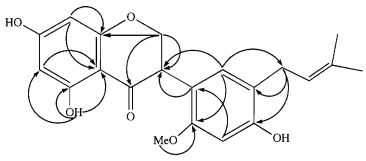
Fig. 4. Selected HMBC correlations observed in compound **2**.



3

Fig. 5. Selected HMBC correlations observed in compound **3**.

repeated CC on silica gel using *n*-hexane–acetone (2:1) to give **3** (21 mg).

3.4. Erypoegin A (*1*)

Colorless oil; UV (MeOH) λ_{max} nm (log ε): 207 (4.39), 215 (4.39), 252 *sh* (4.03), 289 *sh* (3.99), 321 (4.21); IR (KBr) ν_{max} cm^{-1}: 3400, 1610; ^1H and ^{13}C NMR in $CDCl_3$ (see Tables 1 and 2, respectively); EIMS *m/z* (rel. int.): 338 ($[M]^+$, 100), 324 (11), 295 (7), 293 (7), 282 (76), 268 (15), 251 (6); HREIMS *m/z*: 338.1527 ($[M]^+$, calc. for $C_{21}H_{22}O_4$: 338.1517).

3.5. Erypoegin B (*2*)

Colorless oil; UV (MeOH) λ_{max} nm (log ε): 205 (4.30), 236 (4.15), 255 *sh* (4.11), 288 (4.08), 318 (4.08); IR (KBr) ν_{max} cm^{-1}: 3390, 1610; ^1H and ^{13}C NMR in $CDCl_3$ (see Tables 1 and 2, respectively); EIMS *m/z* (rel. int.): 366 ($[M]^+$, 65), 321 (100), 307 (39), 279 (8), 197 (6), 173 (7), 161 (7), 153 (10), 105 (6), 77 (8); HREIMS *m/z*: 336.1358 ($[M]^+$, calc. for $C_{21}H_{20}O_4$: 336.1360).

3.6. Erypoegin C (*3*)

Colorless oil; UV (MeOH) λ_{max} nm (log ε): 206 (4.63), 230 *sh* (4.28), 288 (4.23); IR (KBr) ν_{max} cm^{-1}: 3400, 1640; ^1H and ^{13}C NMR in $CDCl_3$ (see Tables 1 and 2, respectively); EIMS *m/z* (rel. int.): 370 ($[M]^+$, 62), 315 (10), 218 (88), 203 (12), 163 (100), 153 (20); HREIMS *m/z*: 370.1425 ($[M]^+$, calc. for $C_{21}H_{22}O_6$: 370.1415).

3.7. Erypoegin D (*4*)

Colorless oil; UV (MeOH) λ_{max} nm (log ε): 206 (4.66), 230 *sh* (4.35), 287 (4.32); IR (KBr) ν_{max} cm^{-1}: 3410, 1640; ^1H and ^{13}C NMR in $CDCl_3$ (see Tables 1 and 2, respectively); EIMS *m/z* (rel. int.): 384 ($[M]^+$, 88), 367 (14), 329 (8), 218 (100), 203 (10), 201 (11), 167 (76), 163 (80); HREIMS *m/z*: 384.1579 ($[M]^+$, calc. for $C_{22}H_{24}O_6$: 384.1571).

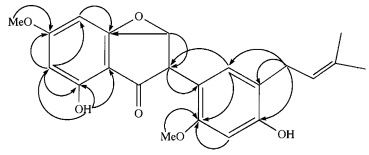


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Fig. 6. Selected HMBC correlations observed in compound **4**.

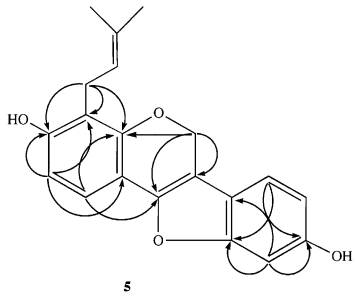

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Fig. 7. Selected HMBC correlations observed in compound **5**.

3.8. Erypoegin E (*5*)

Yellowish oil; UV (MeOH) λ_{max} nm (log ε): 207 (4.40), 254 *sh* (3.84), 290 (3.78), 333 (4.08), 350 (3.98); IR (KBr) ν_{max} cm^{-1}: 3400, 1620; ^1H and ^{13}C NMR in $CDCl_3$ (see Tables 1 and 2, respectively); EIMS *m/z* (rel. int.): 322 ($[M]^+$, 75), 307 (20), 279 (10), 277 (7), 266 (100), 237 (5); HREIMS *m/z*: 322.1211 ($[M]^+$, calc. for $C_{20}H_{18}O_4$: 322.1204).

References

Barton, D.H.R., Gunatilaka, A.A.L., Letcher, R.M., Lobo, A.M.F.T., Widdowson, D.A., 1973. Phenol oxidation and biosynthesis. Part XXII. The alkaloids of *Erythrina lysistemon, E. abyssinica, E. poeppigiana, E. fusca,* and *E. lithosperma*: the structure of erythratidine. Journal of the Chemical Society, Perkin Transactions I, 874–880.

Dewick, P.M., 1988. Isoflav-3-enes. In: Harborne, J.B. (Ed.), The Flavonoids: Advances in Research since 1980. Chapman and Hall, London, pp. 172–173.

Jackson, A.H., Singh, C.A., 1982. Studies of *Erythrina* alkaloids, part IV. GC/MS investigations of alkaloids in the leaves of *E. poeppigiana, E. macrophylla, E. berteroana*, and *E. salviiflora*. Allertonia, 39–45.

Jain, A.C., Paliwal, P., 1988. A facile synthesis of α-methyldesoxybenzoins including racemates of natural angolensin, 2-*O*-methylangolensin & 4-*O*-methylangolensin. Indian Journal of Chemistry 27B, 985–988.

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

Komatsu, M., Yokoe, I., Shirataki, Y., 1981. Studies on the constituents of *Sophora* species. XIV. Constituents of the root of

Sophora franchetiana DUNN (1). Chemical and Pharmaceutical Bulletin 29, 532–538.

Mitscher, L.A., Gollapudi, S.R., Gerlach, D.C., Drake, S.D., Veliz, E.A., Ward, J.A., 1988. Erycristin, a new antimicrobial pterocarpan from *Erythrina crista-galli*. Phytochemistry 27, 381–385.

Miyase, T., Ueno, A., Noro, T., Fukushima, S., 1981. Studies on the constituents of *Lespedeza cyrtobotrya* MIQ. II. The structures of haginin C, haginin D and lespedeol C. Chemical and Pharmaceutical Bulletin 29, 2205–2209.

Perrin, D.R., Whittle, C.P., Batterham, T.J., 1972. The structure of phaseollidin. Tetrahedron Letters, 1673–1676.

Prasad, A.V.K., Kapil, R.S., Popli, S.P., 1985. Structures of pterocarponoids: Anhydrotuberosin, 3-*O*-methylanhydrotuberosin & tuberostan from *Pueraria tuberosa*. Indian Journal of Chemistry 24B, 236–239.

Takayama, M., Fukai, T., Hano, Y., Nomura, T., 1992. Mass spectrometry of prenylated flavonoids. Heterocycles 33, 405–434.

Tanaka, H., Tanaka, T., Etoh, H., 1997. Three pterocarpans from *Erythrina crista-galli*. Phytochemistry 45, 835–838.

Tanaka, H., Etoh, H., Shimizu, H., Oh-Uchi, T., Terada, Y., Tateishi, Y., 2001. Erythrinan alkaloids and isoflavonoids from *Erythrina poeppigiana*. Planta Medica 67, 871–873.