Supporting Information to:

Antibacterial Constituents from the Roots of *Erythrina herbacea*
Against Methicillin-Resistant *Staphylococcus aureus*

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**General experimental procedures**

Optical rotations were determined using a JASCO DIP-370 digital polarimeter. CD spectra were measured on a JASCO J-725 spectropolarimeter. UV spectra were obtained on a Beckman DU-530 spectrophotometer, and IR spectra were obtained using a JASCO FT/IR-4200 spectrophotometer. ¹H-NMR (500 MHz) and ¹³C-NMR (125 MHz) spectra were
recorded on a JEOL ECA-500 spectrometer, and the chemical shifts were expressed on the \( \delta \) (ppm) scale with TMS as an internal standard. EI-MS and HR-EI-MS were recorded on a JEOL JMS-SX 102A spectrometer. Column chromatography (CC) was carried out on Merck silica gel (230–400 mesh). Spots on TLC plates (Merck silica gel 60 F\(_{254}\)) were detected with a UV lamp or by I\(_2\) vapor.

**Extraction and isolation**

The finely powdered roots (2.5 kg) were macerated with acetone (2 \( \times \) 14 L) at room temperature and the solvent was removed to give a residue, which was extracted successively into \( n \)-hexane, CHCl\(_3\), and EtOAc. The CHCl\(_3\) extract (183.1 g) was applied to silica gel (3500 g, 10 \( \times \) 90 cm) CC eluting with CHCl\(_3\)–Me\(_2\)CO (20:1, 20:3, 3:1, 1:1, 10 L each) and Me\(_2\)CO (10 L) to give 7 fractions (A1–A7). Fr. A3 (33.0 g) was subjected to silica gel (1200 g, 10 \( \times \) 30 cm) CC eluting with CHCl\(_3\)–Me\(_2\)CO (40:1, 20:3, 1:1, 2 L each) to give 8 fractions (B1–B8). Fr. B3 (5.08 g) was purified by silica gel (200 g, 4.5 \( \times \) 24 cm) CC eluting with \( n \)-hexane–Me\(_2\)CO (5:1, 1:1, 125 mL each) to give 8 fractions (C1–C8). Fr. C4 (303 mg) was purified by silica gel (10 g, 1.0 \( \times \) 27 cm) CC eluting with CHCl\(_3\)–Me\(_2\)CO (40:1, 20:3) to give eryvariestyrene (4) (44.4 mg). Fr. C7 (235 mg) was purified by repeated silica gel CC using benzene–EtOAc (40:1) to give bidwillol A (7) (7.1 mg). Frs. B4 and B5 (14.8 g) were subjected to silica gel (450 g, 5.5 \( \times \) 38 cm) CC eluting with \( n \)-hexane–Me\(_2\)CO (5:1, 3:1, 600 mL) to give 6 fractions (D1–D6). Fr. D4 (10 g) was subjected to silica gel CC using CHCl\(_3\)–Me\(_2\)CO (40:1, 20:3) and subsequent silica gel CC using benzene–EtOAc (20:1, 10:1, 3:1) to give a crude mixture (136 mg), which was separated by preparative TLC using \( n \)-hexane–Me\(_2\)CO (3:1) to give phaseollinisoflavan (8) (8.6 mg, \( R_f = 0.34 \)) and erythbidin A (9) (25.9 mg, \( R_f = 0.28 \)). Fr. D5 (1.22 g) was purified twice by preparative TLC using benzene–EtOAc (3:1) to give eryvarin L (11) (16.3 mg, \( R_f = 0.34 \)). Fr. A4 (40.0 g) was applied to silica gel (1200 g, 10 \( \times \) 31 cm) CC eluting with CHCl\(_3\)–Me\(_2\)CO (20:1, 10:1, 1:1, 500 mL each) to give 25 fractions (F1–F25). Frs. F7 and F8 (7.93 g) were separated by silica gel (240 g, 4.5 \( \times \) 29 cm) CC eluting with \( n \)-hexane–Me\(_2\)CO (3:1, 2:1, 200 mL each) to give 8 fractions (G1–G8). Fr. G5 (192 mg) was purified by repeated silica gel CC using benzene–EtOAc (10:1) to give phaseollidin isoflavan (10) (27 mg). Fr. G7 (363 mg) was purified by silica gel CC using \( n \)-hexane–Me\(_2\)CO (3:1, 1:1) to give eryvarin H (6) (56.4 mg). Frs. F12 and F13 (5.13 g) were subjected to silica gel (140 g, 4.5 \( \times \) 17 cm) CC eluting with
benzene–EtOAc (20:1, 10:1, 1:1, 80 mL each) to give 10 fractions (H1–H10). Fr. H4 (4 g) was purified by silica gel CC eluting with CHCl3–Me2CO (40:1, 20:3) and subsequent silica gel CC using benzene–EtOAc (20:1, 10:1) to give glabrocoumarone A (12) (15.5 mg). Fr. H8 (393 mg) was separated by silica gel CC using CHCl3–Me2CO (40:1) to give 6-formyl-2,2-dimethyl-2H-chromene (3) (6.4 mg) and erybacin A (1) (10.1 mg). Frs. F14 and F15 (2.49 g) were applied to silica gel CC using n-hexane–Me2CO (5:1, 3:1, 2:1, 300 mL each) to give 9 fractions (I1–I9). Fr. I3 (345 mg) was separated by silica gel CC using CHCl3–Me2CO (40:1, 20:3) and subsequently benzene–EtOAc (3:1) to give erybacin B (2) (13.8 mg) and (±)-glyasperin F (5) (60.8 mg).

6-Formyl-2,2-dimethyl-2H-chromene (3): Colorless oil; UV (MeOH): \( \lambda_{\text{max}} \) (log \( \varepsilon \)) = 296 (3.84), 255 (4.47), 229 (4.24) nm; IR (KBr): \( \nu_{\text{max}} \) = 3430, 1640, 1540, 1510, 1460 cm\(^{-1}\); \(^1\)H-NMR (500 MHz, CDCl3): \( \delta = 1.47 \) (6H, s, CH\(_3\) \times 2), 5.69 (1H, d, \( J = 9.8 \) Hz, H-3), 6.37 (1H, d, \( J = 9.8 \) Hz, H-4), 6.86 (1H, d, \( J = 8.3 \) Hz, H-8), 7.52 (1H, d, \( J = 2.3 \) Hz, H-5), 7.64 (1H, dd, \( J = 8.3, 2.3 \) Hz, H-7), 9.83 (1H, s, CHO); \(^{13}\)C-NMR (125 MHz, CDCl3): \( \delta = 190.7 \) (CHO), 158.7 (C-9), 132.0 (C-7), 131.5 (C-3), 130.0 (C-6), 127.7 (C-5), 121.4 (C-4), 121.1 (C-10), 116.8 (C-8), 77.9 (C-2), 28.5 (CH\(_3\)); EI-MS: \( m/z \) (rel. int.) = 188 [M]\(^+\) (11), 173 (100), 159 (2), 149 (15), 144 (7), 115 (11); HR-EI-MS: \( m/z = 188.0830 \) [M]\(^+\) (calcd. for C\(_{12}\)H\(_{12}\)O\(_2\): 188.0837).

Phaseollidin isoflavan (10): Colorless oil; \([\alpha]_D^{23} = +9 \) (c 0.10, MeOH); CD (MeOH) (c 3.07 × 10\(^{-5}\)) \( \Delta \varepsilon \): 233 (−4.42), 274 (0), 288 (−0.33) nm; EI-MS: \( m/z \) (rel. int.) = 326 [M]\(^+\) (18), 252 (100), 235 (41), 219 (23), 204 (16), 191 (12), 148 (12); HR-EI-MS: \( m/z = 326.1517 \) [M]\(^+\) (calcd. for C\(_{20}\)H\(_{22}\)O\(_4\): 326.1518).

**Antibacterial assay**

Bacterial cell suspensions (10\(^7\) colony-forming units/mL) of 13 MRSA strains were prepared using Mueller–Hinton broth (Difco). MIC was determined by the broth dilution technique. Fresh Mueller–Hinton broth (890 \( \mu \)L) and twofold serial dilutions of the compounds in DMSO (10 \( \mu \)L) were added to the wells of a multi-well plate, followed by inoculation of bacterial cell suspensions (100 \( \mu \)L). The final concentration range of the tested compounds was 1.56–50 \( \mu \)g/mL. The plates were aerobically incubated at 37 °C for 24 h. MIC was
defined as the lowest concentration at which no visible bacterial growth was observed. Aliquots (20 µL) were removed from the wells that inhibited visible bacterial growth and streaked onto Muller–Hinton agar plates. The agar plates were incubated in the same conditions. MBC was defined as the lowest concentration at which colonies failed to grow.

6-Formyl-2,2-dimethyl-2H-chromene

![Structure of 6-formyl-2,2-dimethyl-2H-chromene (3).](image)

**Fig. 1S** Structure of 6-formyl-2,2-dimethyl-2H-chromene (3).
MS fragments of erybacin A (1)

![Structures and molecular formulas for erybacin A](image1.png)

MS fragments of erybacin B (2)

![Structures and molecular formulas for erybacin B](image2.png)

**Fig. 2S** MS fragments of erybacin A (1) and erybacin B (2).
Fig. 3S Plausible biosynthesis route of erybacin B (2) to glyinflanin H.