Synthesis and Antimalarial Activity of Febrifugine Derivatives

Yasuo Takeuchi,* Midori Koike, Kumiko Azuma, Hiromi Nishioka, Hitoshi Abe, Hye-Sook Kim, Yusuke Wataya, and Takashi Harayama*

Faculty of Pharmaceutical Sciences, Okayama University, Okayama 700–8530, Japan.
Received January 12, 2001; accepted February 28, 2001

The regioisomers (2a,b) of the piperidine ring of febrifugine (1a) and isofebrifugine (1b) were synthesized from 4-allyl-3-piperidone (5). Reduction of 5 afforded a mixture of the trans and cis alcohols (6a,b) without diastereoselectivity; this result differentiated it from the reduction of 2-allyl-3-piperidone (14). The antimalarial activity of 2a,b and related compounds was tested.

Key words antimalarial activity; febrifugine; synthesis; structure–activity relationship

Febrifugine (1a) is an antimalarial agent which was isolated from Dichroa febrifuga or Hydrangea umbellata with isofebrifugine (1b).1) Recently Kobayashi et al. corrected the error in the absolute structures of 1a,b, as shown in Fig. 1, by achieving the asymmetric syntheses of all the stereoisomers.2) We have developed a new synthetic method for 1a,b,3) and our interest next focused on the structure–activity relationship (SAR) of 1a,b. The difficulty in the purification or antimalarial screening of 1a,b is isomerization4) between 1a and 1b, which occurs via a reversible Michael reaction. We thought that a derivative in which isomerization did not occur might be a more potent compound. Although much is reported on the SAR of substituents5) on the 4(3H)-quinazolinone ring, the only known modification of the piperidine ring involves regioisomers of the hydroxy group.6) In this report, we describe the synthesis and antimalarial activity of derivatives (2a,b) that are regioisomers of the nitrogen atom on the piperidine ring of 1a,b.

We prepared 2a,b from 1-benzyl-3-hydroxypyridinium chloride (3) in seven isolated steps by modifying our method for synthesizing 1a,b (Chart 1). The successive O-allylation, reduction,7) and replacement8) of the benzyloxycarbonyl (Cbz) group from 3 afforded benzyl 1-(3-allyloxy-1,2,5,6-tetrahydropyridine)carboxylate (4) in 47% yield. The Claisen rearrangement of 4 by heating at 140°C in xylene proceeded smoothly to give benzyl 4-allyl-3-oxo-1-piperidinecarboxylate (5) in 99% yield. Reduction of 5 with sodium borohydride (NaBH4) afforded trans (6a) and cis (6b) benzyl 4-allyl-3-hydroxy-1-piperidinecarboxylate as an inseparable mixture.

Purification and structural determination of the inseparable mixture of 6a and 6b were achieved as shown in Chart 2. Although trans (12a) and cis (12b) benzoate produced from 6a,b were separated by column chromatography, the existence of rotomers9) in the 1H-NMR spectrum made the structural analysis of 12a,b difficult. Hydrogenolysis of 12a,b produced trans (13a) or cis (13b) 4-propyl-3-piperidinyl benzoate, respectively. In the 1H-NMR spectrum, the proton at the 3 position on the piperidine ring of 13a was observed at 4.73 ppm with a coupling constant of 4.0 and 9.5 Hz. The proton on 13b, on the other hand, was observed at 5.10 ppm as a single broad peak. Pure 6a and 6b were prepared by hydrolysis of 12a and 12b and led to 7a and 7b, respectively.

We previously found that reduction of benzyl 2-allyl-3-oxo-1-piperidinecarboxylate (14) with NaBH4 at room tem-

Fig. 1. Febrifugine Derivatives

Chart 1

* To whom correspondence should be addressed.  e-mail: take@pharm.okayama-u.ac.jp © 2001 Pharmaceutical Society of Japan
perature gave cis-benzyl 2-allyl-3-hydroxy-1-piperidinecarboxylate (15b) in high yield as the sole product, without involving the diastereomeric isomer.3a,b As an additional experiment, we attempted reduction of 14 with super hydride® (LiBEt3H) or L-selectride® (LiBi2Bu3H) (Table 1). The high cis selectivity in the reduction was maintained, although it decreased in the order NaBH4, LiBEt3H, LiBi2Bu3H. In contrast, reduction of the 4-allyl derivative (5) with NaBH4 afforded trans selectivity to generate a mixture of 6a and 6b.

We predicted cis selectivity in the reduction of 14 with hydride from a conformational analysis of 14 using molecular calculations.3a,b In order to confirm our prediction, we calculated10) the stable conformers of 16 and 17, which were selected as convenient models of 5 and 14 (Fig. 2). The difference in the heat of formation (H.F.) between the minimized conformer (16b) having the allyl group at the axial position and the optimized conformer (16a) having the allyl group at the equatorial position was about 3.0 kcal/mol; between 17b and 17a it was −0.6 kcal/mol. Considering the generation of cis alcohol from 16b or 17b, our prediction is consistent with experimental determinations of selectivity.

The reaction of a mixture of 6a,b with N-bromosuccimide (NBS) gave a mixture of separable intramolecular bromoetherified products (7a,b). The HPLC data for 7a indicated that this was a 3.6 : 1 mixture of the diastereomeric isomers. The methoxy compound (8a) could be prepared in high yield (83%) as a 1 : 2 mixture of the diastereomeric isomers by dehydrobromination using potassium tert-butoxide and bromoetherification using NBS and methanol. Deacetalization of 8a followed by a coupling reaction with 4(3H)-quinazolinone (9) afforded 10a in 81% yield. The hydrogenolysis of 10a gave 2a in 37% yield as a crystalline solid. To increase the yield of hydrogenolysis, we treated 10a with acid in an unsuccessful attempt to give the N-benzylated compound (11a) of 2a along with 2a. Similarly, the diastereomer (2b) of 2a was synthesized from 7b. Contrary to our expectations, the 13C-NMR spectrum made it clear that 2b was present in the keto form, not the hemi-acetal form (2b' in Chart 1).

The in vitro antimalarial activities of compounds 1a,b, 2a,b, 10a,b, and 11a against Plasmodium falciparum were...
tested (Table 2). Both 2a,b regioisomers of the nitrogen atom in the piperidinone ring of 1a,b were inactive, while the Cbz derivatives of 2a,b exhibited very weak activity compared to 1a,b.

Experimental

Melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. IR spectra were recorded on a JASCO A-102 spectrometer. Mass spectra (MS) were recorded on a VG-7070E spectrometer. 1H- and 13C-NMR spectra were run on a JASCO MY 60FT or a Varian VXR-500 spectrometer. Analytical HPLC was performed with a Shimadzu SPD-6A instrument on a silica gel column, Chemosorb 5Si-U (Chemco). Merck Silica gel 60 (230—400 mesh) and Wako activated alumina (300 atom in the piperidine ring of 1a,b) were employed for column chromatography.

Reduction of 5 with LiBEt3H

Reduction of 14 with NaBH4

Reduction of 14 with LiBi3H

Reduction of 14 with NaBH4

Reduction of 14 with LiBEt3H

Reduction of 14 with LiBi3H

Reduction of 14 with NaBH4

Reduction of 14 with LiBEt3H

Reduction of 14 with NaBH4

Reduction of 14 with LiBi3H
a mixture was stirred at room temperature for 1 h. Methanol (15 ml) and NBS (1.39 g, 7.80 mmol) were added and the mixture was stirred at room temperature for 2 h. It was then poured into aqueous 10% Na₂S₂O₃ solution and extracted with AcOEt. The AcOEt layer was washed with aqueous saturated KHC₂O₃ solution and brine, and solvent was removed. The residue was purified by column chromatography (SiO₂, AcOEt:hexane=1:1; flow rate 1.8 ml/min; wavelength, 254 nm; tₚ=8.9 and 10.1 min (17.66). IR (neat) cm⁻¹: 1700, 1220. H-NMR (60 MHz, CDCl₃, rotomers) δ: 1.24—2.59 (7H, m), 3.22 (3H, s), 3.15—3.40 (2H, m), 3.52 (2H, s), 3.99—4.26 (1H, m), 5.13 (2H, s), 7.35 (5H, s). FAB-MS m/z: 384 (M⁺)1, 386 (M⁺)3). FAB-HR-MS m/z: 384085 (M⁺)1 (Calcd for C₁₇H₂BrNO₃: 3840810). The mixture was stirred at room temperature for 1 h, then poured into water and extracted with AcOEt. The AcOEt layer was washed with aqueous saturated KHC₂O₃ solution and brine, and solvent was removed. The residue was recrystallized from AcOEt to give 10a (2.67 g, 81%) as colorless needles, mp 191—192 °C (AcOEt). IR (KBr) cm⁻¹: 3440, 1720, 1600, 1280. H-NMR (500 MHz, CDCl₃, rotomers) δ: 0.98—1.32 (1H, m), 1.81 (2H, m), 2.10—2.31 (2H, m), 2.30—3.40 (2H, m), 4.20—4.80 (1H, m), 5.12—5.21 (1H, m), 6.35 (2H, m), 7.03—7.30 (1H, m). FAB-MS m/z: 386 (M⁺)1, 388 (M⁺)2. FAB-HR-MS m/z: 388070 (M⁺)1 (Calcd for C₁₇H₂BrNO₃: 388075). The mixture was stirred at room temperature for 1 h, then poured into water and extracted with AcOEt. The AcOEt layer was washed with aqueous saturated KHC₂O₃ solution and brine, and solvent was removed. The residue was recrystallized from CH₂Cl₂ to give 10b (1.48 g, 61%) as colorless needles, mp 205—207 °C (CH₂Cl₂). IR (KBr) cm⁻¹: 3440, 1720, 1600, 1280. H-NMR (500 MHz, CDCl₃, rotomers) δ: 0.24—1.20 (1H, m), 1.55—1.75 (2H, m), 1.77 (2H, br), 1.81—2.30 (2H, m), 2.30—3.40 (2H, m), 3.80—4.50 (3H, m), 5.15 (2H, s), 7.35 (5H, s). FAB-MS m/z: 354 (M⁺)1, 356 (M⁺+1). FAB-HR-MS m/z: 354069 (M⁺)1 (Calcd for C₁₇H₁₄BrNO₃: 3540705).
the mixture of chromatography (MeOH) to give MeOH (3 ml) and CH2Cl2 (5 ml) was stirred at room temperature for 32 h. The layer, which was continuously extracted for 4 d, was dried over anhydrous MgSO4, and the solvent was removed. The residue was purified by column chromatography (MeOH) to give 11a (0.215 g, 28%) as colorless needles, mp 153.5—156 °C (EtOH). IR (KBr) cm−1: 3440, 1720, 1660. 1H-NMR (500 MHz, CDCl3) δ: 1.24—1.35 (3/2H, m), 1.81 (1/2H, dd, J = 13.5, 3.3 Hz), 1.90 (2H, br s), 1.98—2.04 (1H, m), 2.43 (1H, t, J = 11.5 Hz), 2.50 (1H, dd, J = 15.8, 5.3 Hz), 2.57 (1H, td, J = 12.0, 2.5 Hz), 2.82 (1H, dd, J = 15.8, 8.0 Hz), 2.98 (1H, br d, J = 12.0 Hz), 3.20 (1H, dd, J = 15.5, 4.5 Hz), 3.31 (1H, td, J = 10.0, 4.5 Hz), 4.89 (2H, dd, J = 49.5, 17.3 Hz), 7.51 (1H, td, J = 8.0, 1.5 Hz), 7.73 (1H, d, J = 8.0 Hz), 7.77 (1H, td, J = 8.0, 1.5 Hz), 7.90 (1H, s), 8.28 (1H, dd, J = 8.0, 1.5 Hz). 13C-NMR (125 MHz, CDCl3) δ: 31.99, 41.02, 43.54, 45.71, 54.12, 54.77, 71.18, 121.60, 126.22, 127.32, 127.42, 134.65, 148.18, 148.22, 160.16, 203.98. FAB-MS m/z: 302 (M+1)+, 603 (2M+1)+. FAB-MS m/z: 302.1539 (M+1)+. (Calcd for C16H20N3O3: 302.1505). Anal. Calcd for C16H19N3O3·1/2 H2O: C, 61.92; H, 6.50; N, 13.54. Found: C, 62.01; H, 6.22; N, 13.56.

cis-3-[3-(3-Hydroxy-4-piperidinyl)-2-oxopyrrol]-4(3H)-quinazolinone (2b) A mixture of 10b (0.103 g, 0.24 mmol) and 10% Pd/C (2.87 mg) in MeOH (3 ml) and CH2Cl2 (5 ml) was stirred at room temperature for 32 h under a balloon of H2 gas. The mixture was filtered and the solvent was removed. The residue was recrystallized from EtOH to give 2b (0.528 g, 82%) colorless needles, mp 233—235 °C (dec.). IR (KBr) cm−1: 3300, 1720, 1690. 1H-NMR (500 MHz, CD3OD) δ: 1.73 (1H, dd, J = 14.0, 3.5 Hz), 1.90 (1H, ddd, J = 22.5, 13.0, 4.0 Hz), 2.27—2.34 (1H, m), 2.66 (1H, dd, J = 17.5, 7.0 Hz), 2.87 (1H, dd, J = 17.5, 7.0 Hz), 3.02 (1H, td, J = 13.5, 3.5 Hz), 3.13 (1H, dd, J = 12.8, 1.3 Hz), 3.23—3.28 (1H, m), 3.28—3.32 (3H, m), 4.02 (1H, br s), 4.83—4.98 (2H, m), 7.57 (1H, s, J = 8.0 Hz), 7.71 (1H, d, J = 8.0 Hz), 7.85 (1H, td, J = 8.0, 0.8 Hz), 8.17 (1H, s), 8.21 (1H, d, J = 8.0, 0.8 Hz). 13C-NMR (125 MHz, CDCl3) δ: 24.01, 35.04, 42.85, 45.04, 51.18, 56.00, 64.46, 122.80, 127.40, 128.08, 128.72, 135.99, 149.23, 149.24, 162.49, 203.73. FAB-MS m/z: 302 (M+1)+, 604 (2M+2)+. FAB-HR-MS m/z: 302.1527 (M+1)+. (Calcd for C16H19N3O3: 302.1505).

**Antimalarial Activity** Assays and evaluation of siderophore activities were carried out according to the methods described previously.11

**Acknowledgements** We are grateful to the SC-NMR Laboratory of Okayama University for use of the facilities.

**References**


