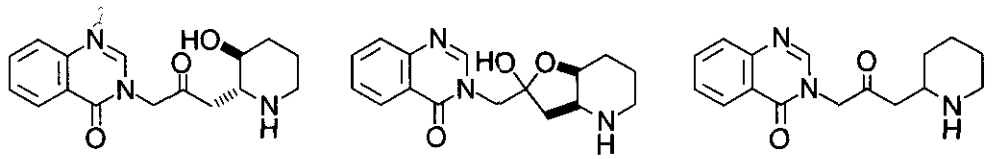
SYNTHESIS AND ANTIMALARIAL ACTIVITY OF *DL*-DEOXYFEBRIFUGINE

Yasuo Takeuchi,* Shin-ichiro Tokuda, Tomoko Takagi, Midori Koike, Hitoshi Abe, Takashi Harayama, Yasuharu Shibata, Hye-sook Kim, and Yusuke Wataya

Faculty of Pharmaceutical Sciences, Okayama University, Okayama 700-8530, Japan

Abstract- *dl*-Deoxyfebrifugine (**3**) was synthesized from 2-piperidone (**4**) by two methods *via* the Wittig reaction of 2-hydroxypiperidine (**6**). The antimalarial activity of **3** was discussed.

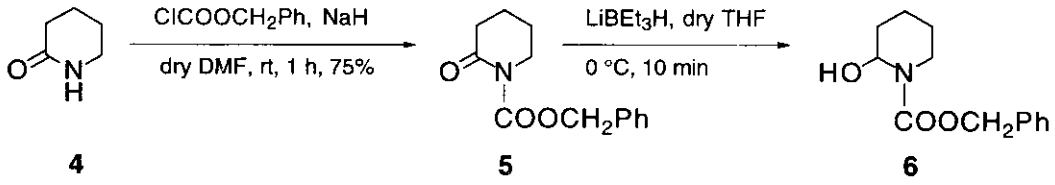
Febrifugine (**1**) is the antimalarial agent that was isolated from *Dichroa febrifuga* or *Hydrangea umbellata* with isofebrifugine (**2**).[1a, b] Recently, Kobayashi *et al.* corrected the error in the absolute structures of **1** and **2** as shown in Figure 1 by achieving the asymmetric syntheses of all the stereoisomers.[2] The synthesis[3] and antimalarial activity[4] of *dl*-deoxyfebrifugine (**3**) have already been reported and it was concluded that **3** is 1/100 as active as **1** against *Plasmodium lophurae* in ducks. We were interested in the antimalarial activity of **3** against *Plasmodium falciparum* to clarify the structure-activity relationship of febrifugine derivatives. However, the yields of the intermediates in the reported synthetic method[5] were low. In this paper, we describe a new method of synthesizing *dl*-deoxyfebrifugine (**3**), and its antimalarial activity.



Febrifugine (**1**) Isofebrifugine (**2**) *dl*-Deoxyfebrifugine (**3**)

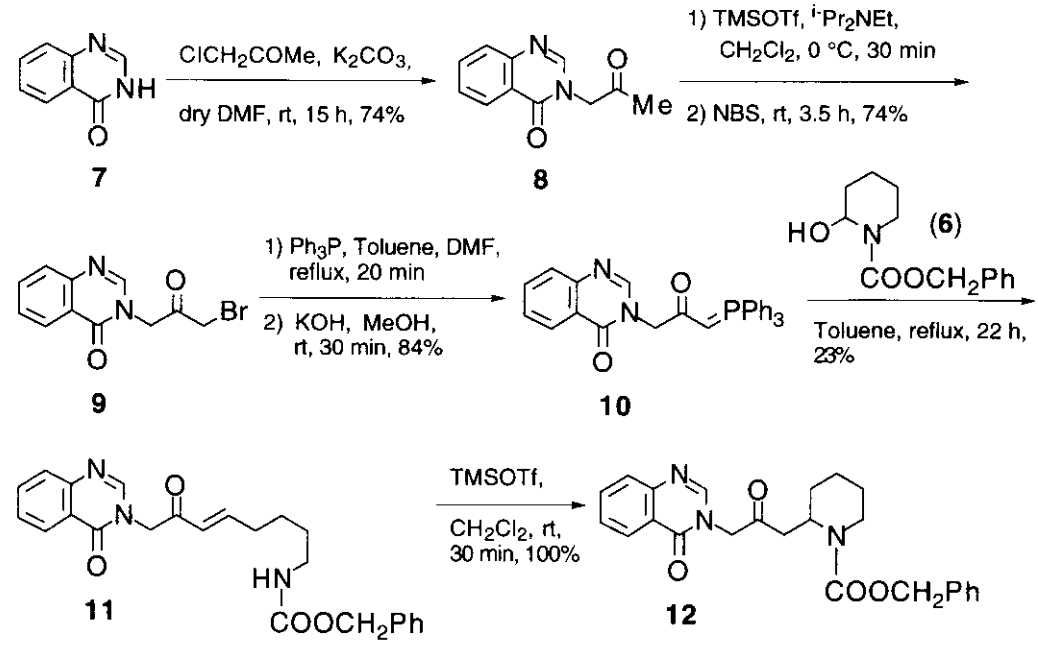
Figure 1. Structures of Febrifugine Derivatives.

We synthesized **3** by two methods *via* the Wittig reaction of the key intermediate, an *N*-protected cyclic aminal (**6**) (Scheme 1). Compound (**6**) was synthesized by reduction[6] of *N*-benzyloxycarbonyl-2-piperidone (**5**),[7] which was obtained by benzyloxycarbonylation of 2-piperidone (**4**), with lithium triethylborohydride ($LiBHEt_3$) and used for the next reaction without purification.



Scheme 1

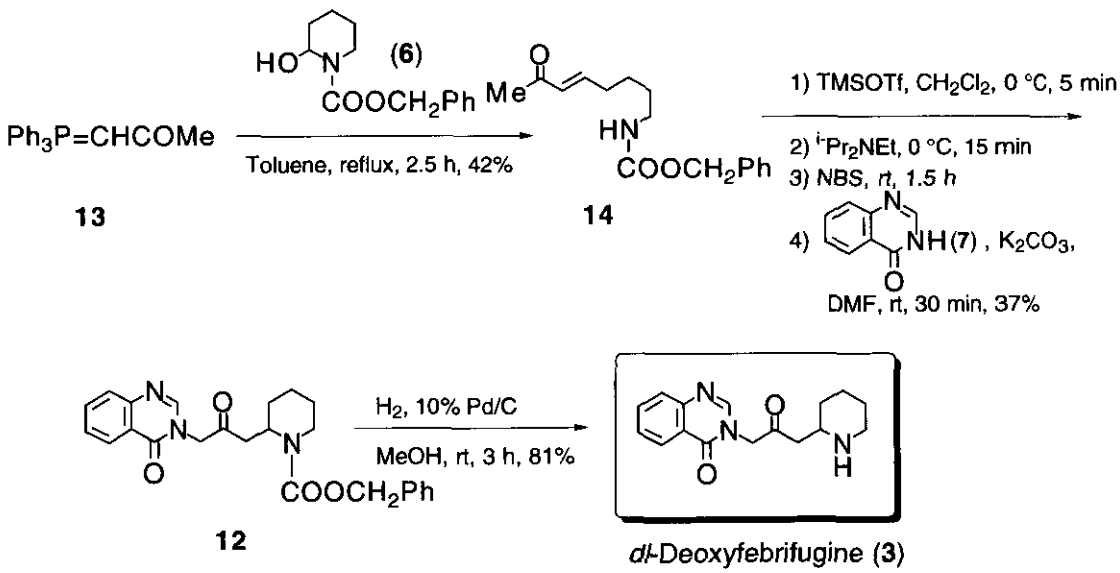
In the first synthetic method (Scheme 2), the Wittig reagent (**10**) was successfully prepared from 4(3*H*)-quinazolinone (**7**) in three steps with high yield. The bromination[8] of 3-(2-oxopropyl)-4(3*H*)-quinazolinone (**8**)[9] *via* silyl enol ether proceeded highly regioselectively to give bromomethyl ketone (**9**).[10] The Wittig reaction of **9** with **6** afforded the compound (**11**) and involved opening the piperidine ring. The yield of this reaction was low, but we found that the Wittig reaction of *N*-protected cyclic aminal proceeded with the cleavage of the C-N bond. We determined that the double bond in **11** had an *E* configuration based on the coupling constant (J = 16 Hz) of the olefinic protons seen in the ^{1}H-NMR. The Michael reaction of **11** with trimethylsilyl trifluoromethanesulfonate (TMSOTf) easily afforded the closed-ring product (**12**).



Scheme 2

In the second synthetic method (Scheme 3), the Wittig reaction of **6** was tried using acetylmethylene-triphenylphosphorane (**13**) instead of **10** as the Wittig reagent to afford the open-ring product (**13**) in 42%

yield. Compound (**14**) was unstable and was used immediately for the next reaction after purification by column chromatography. A series of four steps, the Michael reaction, silylation, bromination, and coupling with **7**, successfully afforded **11**, which had the same physicochemical properties and spectrum data as **11** prepared by the first method. Deprotection of **11** was performed by hydrogenolysis to afford the desired product, *dl*-deoxyfebrifugine (**3**). This method was superior to the first our method in the overall yield.



Scheme 3

Table 1. Antimalarial Activity and Toxic Selectivity of Febrifugine Derivatives.

Compound	*FM3A* EC_{50}, μM	*P. Falciparum* EC_{50}, μM	Toxic Selectivity
Febrifugine (**1**)	0.17	0.0007	243
Isofebrifugine (**2**)	0.19	0.0034	56
dl-deoxyfebrifugine (**3**)	2.1	0.1	21
Quinine	100	0.11	909
Chloroquine	32	0.018	1778
Pyrimethamine	0.12	0.001	120
Artemisinin	10	0.0079	1266

Although **3** has antimalarial activity against *Plasmodium falciparum in vitro* similar to quinine, the activity of **3** was remarkably lower than that of febrifugine (**1**) or isofebrifugine (**2**). The antimalarial activity we observed was the same as the reported result, which shows that there were no differences in the *in vitro* and

in vivo activities against *Plasmodium falciparum* and *Plasmodium lophurae*, and that the hydroxy group of **1** and **2** plays an important role in the antimalarial activity.

ACKNOWLEDGMENT

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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. MS spectra were recorded on a VG-70SE spectrometer. ^{1}H-NMR spectra were run on a Hitachi R-1500 (60 MHz) spectrometer.

1-Benzyloxycarbonyl-2-pipeidone (5)[7]

To a solution of 2-piperidone (**4**, 7.93 g, 80.0 mmol) in dry DMF (70 mL), sodium hydride (4.61 g, 62.5% in mineral oil, 120 mmol) was added at 0 ℃ and benzyl chloroformate (17.0 mL, 120 mmol) was added dropwise at 0 ℃ during 30 min. The mixture was stirred at rt for 1 h. The mixture was poured into cold water (500 mL) and extracted with AcOEt (150 mL x 2). AcOEt layer was washed with brine (100 mL), dried over anhydrous $MgSO_4$, and the solvent was removed by evaporation. The residue was chromatographed (SiO_2; hexane:AcOEt=1:1) to give **5** (14.0 g, 75%) as colorless oil. IR (neat) cm^{-1}: 1770, 1710. ^{1}H-NMR ($CDCl_3$) δ: 1.40—2.20 (m, 4H), 2.30—2.70 (m, 2H), 3.60—3.80 (m, 2H), 5.72 (s, 2H), 7.20—7.50 (m, 5H). HRMS (FAB) calcd for $C_{13}H_{16}NO_3$: 234.1130. Found: 234.1088.

3-(2-Oxopropyl)-4(3*H*)-quinazolinone (8)[9]

To a mixture of 4(3*H*)-quinazolinone (7.31 g, 50.0 mmol) and anhydrous potassium carbonate (8.34 g, 60.0 mmol) in dry DMF (60 mL), chloroacetone (4.78 mL, 60.0 mmol) was added and the mixture was stirred at rt for 15 h. The mixture was poured into cold water (600 mL) and extracted with AcOEt (200 mL x 3). AcOEt layer was washed with brine (200 mL x 3), dried over anhydrous $MgSO_4$, and the solvent was removed by evaporation. The residue was recrystallized from AcOEt to give **8** (7.49 g, 74%) as colorless needles, mp 166—168 °C (AcOEt) (lit.,[9] 166—167 °C). IR (neat) cm^{-1}: 1720, 1670. ^{1}H-NMR ($CDCl_3$) δ: 2.31 (s, 3H), 4.79 (s, 2H), 7.27—7.83 (m, 3H), 7.81 (s, 1H), 8.27 (dd, 1H, J = 7.6, 1.1 Hz).

3-(3-Bromo-2-oxopropyl)-4(3*H*)-quinazolinone (9)[10]

To a solution of **8** (4.04 g, 20.0 mmol) and diisopropylethylamine (4.40 mL, 25.3 mmol) in dry CH_2Cl_2 (40 mL), trimethylsilyl trifluoromethanesulfonate (4.40 mL, 24.3 mmol) was added at 0 ℃. The mixture was stirred at 0 ℃ for 30 min under an argon atmosphere. To the mixture, NBS (4.28 g, 24.0 mmol) was added. The mixture was stirred at rt for 3.5 h. The precipitates were filtered off, washed with ether

(50 mL) and water (50 mL), and recrystallized from AcOEt to give **8** (4.18 g, 74%) as colorless needles, mp 218—222 °C (AcOEt) (decomp) (lit.,[10] 211—213 °C). IR (KBr) cm^{-1}: 1740, 1670. ^{1}H-NMR (DMSO-d_6) δ: 4.55 (s, 2H), 5.14 (s, 2H), 7.40—7.93 (m, 3H), 8.10—8.27 (m, 1H), 8.27 (s, 1H).

3-(2-Oxo-3-triphenylphosphoranediylpropyl)-4(3*H*)-quinazolinone (10)

A solution of **9** (5.00 g, 17.8 mmol) and triphenylphosphine (4.67 g, 17.8 mmol) in toluene (30 mL) and DMF (30 mL) was stirred at reflux for 20 min. The precipitates were filtered off and washed with AcOEt. A solution of the precipitates and KOH (20 g) in MeOH (200 mL) was stirred at rt for 30 min. The mixture was poured into ice water (200 mL) and the precipitates were filtered off. The precipitates were washed with water and Et_2O and recrystallized from AcOEt to give **10** (6.90 g, 84%) as colorless needles, mp 186—189 °C (AcOEt). IR (KBr) cm^{-1}: 1670. ^{1}H-NMR ($CDCl_3$) δ: 3.80 (br d, 1H, J = 24.0 Hz), 4.74 (s, 2H), 7.27—7.83 (m, 18H), 8.17 (s, 1H), 8.32 (dd, 1H, J = 7.3, 1.1 Hz). *Anal.* Calcd for $C_{29}H_{23}N_2O_2P$: C, 75.31; H, 5.01; N, 6.06. Found: C, 75.59; H, 5.12; N, 6.04.

3-(8-Benzyloxycarboxamido-2-oxo-3-octenyl)-4(3*H*)-quinazolinone (11)

To a solution of **5** (0.70 g, 3.0 mmol) in dry THF (5 mL), lithium triethylborohydride (3.3 mL of 1.0 M solution in THF, 3.3 mmol) was added to dropwise at 0 ℃ and the mixture was stirred at 0 ℃ for 10 min. The mixture was poured into a saturated aqueous NH_4Cl solution (50 mL) and extracted with AcOEt (50 mL x 2). The AcOEt layer was washed with brine (50 mL), dried over anhydrous $MgSO_4$, and the solvent was removed. A solution of the residue and **10** (1.16 g , 2.50 mmol) in toluene (5 mL) was stirred at reflux for 12 h. After cooling, the precipitates were filtered off and recrystallized from AcOEt to give **11** (0.23 g, 22%) as colorless plates, mp 145—147 ℃ (AcOEt). ^{1}H-NMR ($CDCl_3$ and DMSO-d_6 (1:1)) δ: 1.18—1.75 (m, 4H), 1.94—2.64 (m, 2H), 2.83—3.42 (m, 2H), 5.06 (s, 4H), 6.23 (br d, 1H, J = 16.4 Hz), 6.58—6.82 (m, 1H), 7.20—7.82 (m, 3H), 7.32 (s, 5H), 8.08 (s, 1H), 8.22 (br d, 1H, J = 8.5 Hz). FABMS (positive ion mode) *m/z*: 420 $(M+1)^+$. *Anal.* Calcd for $C_{24}H_{25}N_3O_4$: C, 68.72; H, 6.01; N, 10.02. Found: C, 68.56; H, 6.06; N, 9.88.

3-[2-Oxo-3-(1-benzyloxycarbonyl-2-piperidyl)propyl]-4(3*H*)-quinazolinone (12)

From **11**: To a solution of **11** (0.18 g, 0.43 mmol) in dry CH_2Cl_2 (5 mL), trimethylsilyl trifluoromethanesulfonate (0.08 mL, 0.44 mmol) was added to dropwise at 0 ℃. The mixture was stirred at rt for 30 min. The mixture was poured into a saturated aqueous $KHCO_3$ solution (30 mL) and extracted with AcOEt (30 mL x 2). The AcOEt layer was washed with brine (30 mL), dried over anhydrous $MgSO_4$, and the solvent was removed to give **12** (0.18 g, 100%) as colorless needles, mp 127—128 ℃ (AcOEt:hexane=1:1). IR (KBr) cm^{-1}: 1730, 1690. ^{1}H-NMR ($CDCl_3$) δ: 1.42—1.83 (m, 6H), 2.75—2.98 (m, 2H), 3.00—3.98 (m, 2H), 4.06—4.18 (m, 1H), 4.90 (br s, 2H), 5.13 (s, 2H), 7.32 (s, 5H), 7.39—7.84 (m, 3H), 7.91 (s, 1H), 8.27 (br d, 1H, J = 7.3 Hz). FABMS (positive ion mode) *m/z*: 420 $(M+1)^+$. *Anal.* Calcd for $C_{24}H_{25}N_3O_4$: C, 68.72; H, 6.01; N, 10.02. Found: C, 68.67; H, 5.92; N, 9.74.

From **5**: To a solution of **5** (1.17 g, 5.0 mmol) in dry THF (10 mL), lithium triethylborohydride (5.0 mL of 1.0 M THF solution, 5.0 mmol) was added with stirring at 0 ℃ for 10 min. The mixture was poured into a saturated aqueous NH_4Cl solution (50 mL) and extracted with AcOEt (30 mL x 2). AcOEt layer was washed with brine (50 mL), dried over anhydrous $MgSO_4$, and the solvent was removed by evaporation. A solution of the residue and **13** (1.91 g, 6.0 mL) in toluene (10 mL), was stirred at reflux for 2.5 h. After removal of the solvent, the residue was chromatographed (SiO_2; hexane:AcOEt=3:1) to give **14** (0.58 g, 42%) as a light yellow oil, which was immidately used in the next reaction. To a solution of **14** (0.75 g, 2.7 mmol) in dry CH_2Cl_2 (5 mL), trimethylsilyl trifluoromethanesulfonate (0.60 mL, 3.3 mmol) was added with stirring at 0 ℃. The mixture was stirred at 0 ℃ for 5 min. To the mixture, diisopropylethylamine (0.57 mL, 3.3 mmol) was added. The mixture was stirred at 0 ℃ for 15 min. To the mixture, NBS (0.58 g, 3.3 mmol) was added and the mixture was stirred at rt for 1.5 h. The mixture was poured into water (50 mL) and extracted with AcOEt (50 mL). The AcOEt layer was washed with water (50 mL) and brine (50 mL), dried over anhydrous $MgSO_4$, and the solvent was removed. A mixture of the residue, **7** (0.40 g, 2.7 mmol), and anhydrous potassium carbonate (0.38 g, 2.7 mmol) in dry DMF (5 mL) was stirred at rt for 30 min. The mixture was poured into water (100 mL) and extracted with AcOEt (50 mL x 2). The AcOEt layer was washed with brine (50 mL), dried over anhydrous $MgSO_4$, and the solvent was removed. The residue was chromatographed (Al_2O_3; hexane:AcOEt=1:2) to give **12** (0.42 g, 37%).

dl-Deoxyebrifugine (3)[3]

A mixture of **12** (0.20 g, 0.48 mmol) and 10%-Pd/C (0.10 g) in MeOH (10 mL) was stirred at rt for 3 h under a balloon of H_2 gas. The mixture was filtered off and the filterate was concentrated to dryness to give **3** (0.11 g, 81%) as a colorless needles, mp 139—141 ℃ (AcOEt:hexane=1:1) (lit.,[3] dihydrochloride: 228—230 ℃ (dec)). IR (KBr) cm^{-1}: 3300, 1730, 1670. ^{1}H-NMR ($CDCl_3$) δ: 1.06—1.96 (m, 6H), 2.49—3.12 (m, 6H), 4.79 (s, 2H), 7.32—7.81 (m, 3H), 7.92 (s, 1H), 8.27 (dd, 1H, J = 7.3, 1.4 Hz). FAB–MS (positive ion mode) *m/z*: 286 $(M+1)^+$. *Anal.* Calcd for $C_{16}H_{19}N_3O_2$: C, 67.35; H, 6.71; N, 14.73. Found: C, 67.37; H, 6.52; N, 14.65.

Bilological assays **Parasites of *P. falciparum*** *Plasmodium falciparum* strain FCR-3 (ATCC 30932) was used in our study. *P. falciparum* was maintained *in vitro* at 37 ℃ in RPMI 1640 medium plus 10% human serum (Gibco, NK) containing human red blood cells (RBCs, type A) at 5% hematocrit in 24-well microplates. The microplates were placed in a CO_2 incubator (5% CO_2, 5% O_2, 90% N_2) at 37 ℃ and the medium was changed daily.

Mammalian cells A wild-type mouse mammary tumor FM3A cell line (subclone F28-7) was supplied by the Japanese Cancer Research Resources Bank (JCRB). FM3A cells were maintained in suspension culture at 37 ℃ in a 5% CO_2 atmosphere in plastic bottles containing ES medium (Nissui Pharmaceuticals, Tokyo, Japan)supplemented with 2% heat-inactivated fetal bovine serum (Gibco, NY).

***In vitro* antimalarial activity of anthraquinones.** The following procedures were used for routine assay of antimalarial activity. Various concentrations of compounds in DMSO were prepared. Ten μL of each solution was added to individual wells of a 24-well plate. Erythrocytes with 0.3% parasitemia were added to each well containing 990 μL of culture medium to give a final hematocrit level of 3%. The plates were incubated at 37 ℃ for 72 h in a CO_2 incubator (5% CO_2, 5% O_2, 90% N_2). To evaluate the antimalarial activity of anthraquinones, we prepared thin smears from each culture and stained them with Giemsa (E. Merck, Germany). More than 10,000 erythrocytes were examined under microscopy. All of the test compounds were assayed in duplicate at each concentration. Drug-free control cultures were run simultaneously. All data points represent the mean of at least two experiments. The fifty percent inhibitory concentration. EC_{50} was determined by comparison to drug-free controls incubated under the same conditions.

Toxicity against mammalian cell line Cells grew with a doubling time of about 12 h. Prior to exposure to drugs, cell density was adjusted to 5 x 10^4 cells/mL. A cell suspension of 990 μL was dispensed to the test plate, and compounds at various concentrations suspended in either 10 μL of DMSO, were added to individual wells of a 24-well plate. The plates were incubated at 37 ℃ in a 5% CO_2 atmosphere for 48 h. Cell numbers were measured using a blood cell counter CC-108 (Toa Medical Electric Co., Japan). All data points represent the mean of at least two experiments. EC_{50} was determined compared with those in drug-free controls incubated under the same conditions.

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