AN EFFICIENT SYNTHESIS OF NOVEL SPIRODEOXO-ARTEMISININ WITH 2-PYRAZOLINE RING

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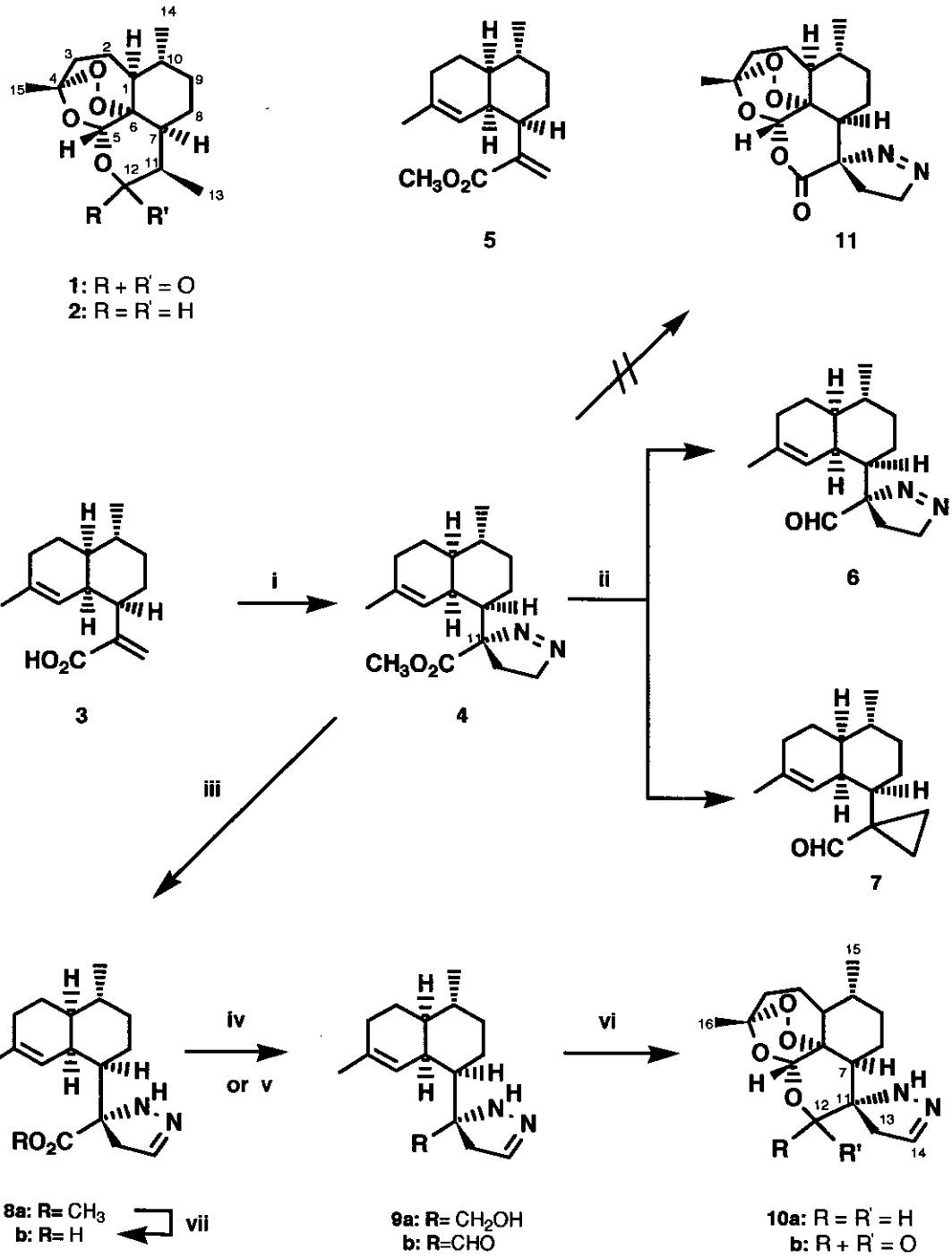
Abstract -Water-soluble, novel spirodeoxoartemisinin (**10a**) with 2-pyrazoline ring at C-11 was prepared from artemisinic acid (**3**) in four steps.

Artemisinin (Qinghaosu) (**1**), a sesquiterpene lactone endoperoxide is a first natural trioxane isolated from *Artemisia annua*, L. This compound is of special biological interest because of its outstanding anti-malarial activity,[1] *in vitro* activity against *Pneumocystis carinii*,[2] and *T. gondii*.[3] The first anti-HIV activity of artemisinin-related trioxanes was also reported by our group.[4] Deoxoartemisinin (**2**) was prepared from artemisinic acid (**3**)[5] *via* photooxygenative cyclization of dihydroartemisinyl alcohol. A variation of this photooxygenative cyclization was later appeared for the synthesis of artemisinin and artemisitene.[6] Deoxoartemisinin (**2**) shows more antimalarial activity than artemisinin *in vitro* and *in vivo*.[7a] Since then, a series of novel derivatives at C-12 have been prepared to increase stability[7b-c] and solubility.[7d] Water-soluble and stable deoxoartemisinins recently draw some attention for better bioavailability.[7d] Although C-11 spiro compound (**11**) has been prepared directly from scarce artemisitene,[8] no synthesis of water-soluble spiroartemisinin from naturally abundant artemisinic acid (**3**) is available. In this paper, we would like to report the first synthesis of novel spirodeoxoartemisinin (**10a**) as a water-soluble and more stable analog. The synthetic strategy for **10a** is outlined in Scheme 1. Treatment of artemisinc acid (**3**) with excess (5 eq.) diazomethane afforded the 1, 3-dipolar addition adduct (**4**)[9,10] in 98 % yield *via* methyl artemisinate (**5**). The stereochemistry at C-11 of **4** was confirmed as *S*-configuration by a single crystal X-Ray analysis.[9] Direct reduction of the adduct (**4**) with DIBALH provided the spiro 1-pyrazolinealdehyde (**6**) (38 %) and spirocyclopropyl aldehyde (**7**) (28 %), respectively. No alcohols of **6** and **7** were produced by this reduction. We believe that the unsuccess of photooxygenative cyclization of **4** into **11** was due to the denitrogenation of the relatively unstable azo group of spiro 1-pyrazoline of **4** into a short-lived biradical prior to allylic hydroperoxidation at C-4 and C-6 of **4**. To maintain somewhat more inert spiro moiety for the survival under the strong acid-catalyzed phootoxygenative cyclization, treatment of the adduct (**4**) with hydrochloric acid tautomerized the azo group within the spiro 1-pyrazoline of **4** into the more stable 2-pyrazoline (**8a**). Second reduction of **8a** with LAH cleanly afforded spiro 2-pyrazoline alcohol (**9a**). Partial reduction of **8a** with DIBALH (1.2 eq.) provided the aldehyde **9b** (42 %). The aldehydes (**6**, **7** and **9b**) are versatile chiral intermediates for the synthesis of C-12 derivatives of **10a**. Chiral dye-sensitized photooxidation and subsequent strong acid-catalyzed cyclization of **9a** afforded the target spirodeoxoartemisinin (**10a**) in 23 % yield. MME (molecular mechanics minimum energy)



Scheme 1. *Reagents and Conditions*: (i) CH_2N_2(5 equiv.), anhydrous ether, O°C,10 h, 98 %. (ii) DIBALH (2.5 equiv.),CH_2Cl_2, -78 °C, 5 h, 38 % for **6** and 28 % for **7**. (iii) conc-HCl (cat.), $CHCl_3$, rt, 30 min, 90 %. (iv) LAH (1.5 equiv.), anhydrous ether, O°C to r t, 5 h, 59 %. (v) DIBALH (1.2 equiv.), CH_2Cl_2, -78 °C, 5 h, 42 %. (vi) oxygen, irradiation, methylene blue, CH_2Cl_2, -78 °C, 2 h, then Dowex resin (strongly acidic) or triflic acid (cat.), CH_2Cl_2, -23 °C to r t, 5 h, 23 %. (vii) 2.5 % KOH/ CH_3OH, rt, 48 h, 93 %.

calculation[11] predicted 11-*S*-isomer (**10a**) (57.5 kcal/mol) as the slightly more stable stereoisomer than 11-*R*-isomer (58.6 kcal/mol). While the NOESY spectrum of **10a** showed interaction between 13-Hα (δ 3.02) and the 12-Hβ (δ 3.62) proton, no NOE enhancement was observed between 13-Hα (δ 3.02) and 12-Hα (δ 3.74) and between 13-Hβ (δ 2.76) and 7-Hα (δ 1.45) of **10a**, establishing the stereochemistry at C-11 of **10a** as *S*-configuration. This study shows *S*-configuration at C-11 of the compound (**4**) was retained during the conversion into **10a**. Neither the ester (**8a**) nor the spiro acid (**8b**), prepared by hydrolysis of **8a** with methanolic potassium hydroxide, was converted to **10b** under the above conditions. In the absence of relatively activity-reducing and stability-decreasing carbonyl group at C-12, spiro 2-pyrazoline ring effect on antimalarial activity will be reported in due course.

In conclusion, this synthesis provides water-soluble, novel spirodeoxoartemisinin analog (**10a**) (as a HCl salt) from naturally abundant artemisinic acid by short, highly regio-, and stereo-specific route. It also demonstrates that the bulkyness of C-11 as the spiro 2-pyrazoline of **9a** does not prevent the photooxygenative cyclization into the spiroartemisinin derivative (**10a**).[9]

EXPERIMENTAL

General

Melting points were determined in open capillary tubes with a Device MEL-TEMP II. IR spectra were obtained as KBr pellet and neat on NaCl plate by a Nicolet Impact 400 FT-IR Spectrophotometer. The NMR spectra were measured on a Bruker AC 250 NMR Spectrometer operating at 250 MHz for ^{1}H NMR and 63 MHz for ^{13}C NMR. All spectra were recorded in $CDCl_3$ as solvent and chemical shifts were reported in ppm relative to TMS. The NOE experiment was carried out with a Bruker AMX-500 NMR. GC/MS spectra were obtained from a Shimadzu GC/MS-QP 2000A. Specific rotation were determined in $CHCl_3$ as solvent by a RUDOLPH Research AUTOPOL III. A medium pressure, mercury-vapor lamp of 300W as a light source was used in photooxidation reaction. Analytical thin-layer chromatography was performed on Merck pre-coated silica gel glass plate(silica gel 60, F-254, 0.25 mm) and flash column chromatography was performed on Merck 230~400 mesh silica gel. Visualization was achived with UV(254 nm), solution of molibdic acid and cerium sulfate in H_2SO_4, anisaldehyde solution or $KMnO_4$.

Reduction of (4) to aldehyde (6) and spirocyclopropanealdehyde (7)

A solution of **4** (200 mg, 0.6 mmol) in 50 mL of methylene chloride was cooled in a dry ice-acetone bath. To a solution of DIBALH(1.76 mmol, 1.1 mL of 1.6 M in toluene), the solution of **4** was slowly added during 30 min under a N_2 atmosphere and then stirred for 5 h. In order to remove an excess DIBALH, a few drops of EtOAc and continuously small amount of distilled water were added in the reaction mixture, which was allowed to rt. After excess EtOAc was added, the reaction mixture was washed with 0.1 M H_2SO_4 solution (3 x 30 mL), saturated-$NaHCO_3$ solution and brine and dried over $MgSO_4$ and concentrated *in vacuo*. The resulting oil was chromatographed on silica gel (hexane /ethyl acetate=5:2) to give the pyrazolinealdehyde (**6**) (68.1 mg, 38 %), and the spirocyclopropanealdehyde (**7**) (44.8 mg, 28 %), which both were colorless oils. Compound (**6**): NMR (250 MHz, $CDCl_3$,): δ 8.80 (s, 1H, CHO), 4.90 (s, 1H, olefinic H-5), 3.85 (dt, J=9.7, 0.7 Hz, 2H, H-13), 3.0 -2.70 (m, 2H, 14-CH_2), 1.62

(s, 3H, 15-CH_3), 0.90 (d, J= 6.15 Hz, 3H, 14-CH_3), C^{13}-NMR ($CDCl_3$, 75 MHz): 165.2, 160.5, 136.9, 120, 43.8, 42.1, 41.5, 39, 35.1, 34, 27.9, 26.7, 25.9, 24.9, 24, 20.1. IR ($CHCl_3$): νmax 2929, 2874, 2733, 1750, 1675, 1442, 1363, 1253, 1130, 1057, 794 cm^{-1}; MS m/z: 260 (M^+), 231, 162, 125, 112, 99, 91, 79, 55, 41, 29. Anal. Calcd for $C_{16}H_{24}N_2O$: C 73.85, H 9.23, N 10.77. Found C 73.63, H 9.31, N 10.69. Compound (**7**): NMR (250 MHz, $CDCl_3$,): δ 9.21 (s, 1H, CHO), 5.27 (s, 1H, olefinic H-5), 1.62 (s, 3H, 15-CH_3), 0.88 (d, J= 6.3 Hz, 3H, 14-CH_3), C^{13}-MNR ($CDCl_3$, 75 MHz): 202.6, 135.2, 120.6, 41.7, 41.3, 39.7, 35.6, 33.7, 27.8, 26.2, 25.5, 24.1, 23.9, 19.7, 14.8, 10.8. IR ($CHCl_3$): νmax 2926, 2872, 2721 (CHO), 1714, 1454, 1375, 1261 cm^{-1}; MS m/z: 233 (M^++1), 218, 201, 188, 162, 147, 133, 119, 105, 91, 79, 55, 41, 28.

Isomerization of 4 to 8a

To a solution of compound (**4**) (500 mg, 1.72 mmol) in 10 mL of chloroform, a drop of conc-HCl (catalytic amount) was added and stirred at rt for 30 min. After saturated-$NaHCO_3$ solution was added, the reaction mixture was extracted with $CHCl_3$ (3 x 100 mL). The extract was washed with brine, dried over $MgSO_4$ and concentrated *in vacuo* to afford an oil. The oil was purified by flash column chromatography (silica gel, hexane/ethyl acetate=5:1) to give the product (**8a**) (449 mg, 90 %) as a colorless oil. Compound (**8a**): $[\alpha]_D^{25}$ -87.7° (c 0.49, $CHCl_3$); NMR (250 MHz, $CDCl_3$,): δ 6.68 (s, 1H, imine), 5.23 (s, 1H, olefinic H-5), 3.72 (s, 3H, methyl ester), 3.20 (dd, J=17 Hz, 1H, H-13), 2.83 (dd, J=17 Hz, 1H, H-13), 1.63 (s, 3H, 16-CH_3), 0.90 (d, J= 6.5 Hz, 3H, 15-CH_3), C^{13}-NMR ($CDCl_3$, 75 MHz): 176, 143, 136, 120, 74, 53, 47, 42, 39, 38, 35, 28, 26, 25, 24, 19. IR ($CHCl_3$): νmax 3344 (N-H), 1733 (C=O), 1650 (C=C) cm^{-1}; MS m/z: 291 (M^++1), 232 (M^+-N_2-O_2). Anal. Calcd for $C_{17}H_{26}N_2O_2$: C 70.34, H 8.97, N 9.66. Found C 70.23, H 8.74, N 9.29.

Hydrolysis of 8a to 8b

The pyrazoline ester (**8a**) (50 mg, 0.17 mmol) in 3 mL of 2.5 % KOH/MeOH solution was allowed to stand at rt for 2 days. To the reaction mixture was added 10 mL of H_2O and then the mixture was concentrated to remove MeOH. To the aqueous solution was acidified with conc-HCl to pH 2 and extracted with Et_2O (3 x 50 mL). The extracts were dried over $MgSO_4$ and evaporated to dryness. Flash column chromatography (silica gel, hexane/ethyl acetate=1:1) afforded the light yellow oil product (**8b**) (43.7 mg, 93 %). Compound (**8b**): $[\alpha]_D^{25}$ +8.0 ° (c 0.05, $CHCl_3$); NMR (250 MHz, $CDCl_3$,): δ 6.78 (s, 1H, imine), 5.26 (s, 1H, olefinic H-5), 3.20 (d, J=17.6 Hz, 1H, H-13), 2.85 (d, J=17.6 Hz, 1H, H-13), 2.45 (m, 1H, H-7), 1.61 (s, 3H, 16-CH_3), 0.87 (d, J= 6.4 Hz, 3H, 15-CH_3), IR ($CHCl_3$): νmax 3332, 2924, 2870, 1719 (C=O), 1611 (C=C), 1451, 1388, 1256 cm.$^{-1}$ Anal. Calcd for $C_{16}H_{24}N_2O_2$: C 69.57, H 8.70, N 10.14. Found C 70.03, H 8.74, N 10.47.

Reduction of 8a to 9a

To a solution of LAH (35.3 mg, 0.93 mmol) in 10 mL of freshly dried ether, a solution of **8a** (100 mg, 0.34 mmol) in 10 mL of dried ether was slowly dropped at 0 °C during 10 min. The solution was stirred at rt for 5 h. After the reaction mixture was filtered through a celite pad, to the filtrate was added 10 mL of water, then extracted with ether (3 x 30 mL). The extract was washed with saturated-$NaHCO_3$, brine,

and dried over $MgSO_4$, and concentrated to dryness. The crude product was purified by flash column chromatography on silica gel (hexane/ethyl acetate=5:1) to give **9a** as a colorless oil (95.8 mg, 59 %). Compound (**9a**): $[\alpha]_D^{25}$ -56.00° (c 0.025, $CHCl_3$); NMR (250 MHz, $CDCl_3$,): δ 6.70 (s, 1H, imine), 5.07 (s, 1H, olefinic H-5), 3.50 (d, J=2 Hz, 2H, 12-CH_2), 2.81 (dd, J=19.2, 1.5 Hz, 1H, H-13), 2.58 (dd, J=18, 1.5 Hz, 1H, H-13), 1.62 (s, 3H, 16-CH_3), 0.88 (d, J= 6.4 Hz, 3H, 15-CH_3), C^{13}-NMR ($CDCl_3$, 75 MHz): 145, 136, 120, 68, 66, 46, 42, 39, 37, 35, 28, 26, 25.5, 24.23, 20. IR ($CHCl_3$): ν max 3333 (OH), 2936, 2863, 1610, 1449 cm^{-1}. Anal. Calcd for $C_{16}H_{26}N_2O$: C 73.28, H 9.92, N 10.69. Found C 73.03, H 9.46, N 10.98.

Reduction of 8a to 9b

A solution of **8a** (50 mg, 0.17 mmol) in dried methylene chloride(10 mL) was added to DIBALH(0.21 mmol, 0.13 mL of 1.6 M in toluene) at -78 °C and stirred under a N_2 atmosphere for 5 h. After work-up as it was described for the compound (**6**), flash column chromatography on silica gel (hexane/ethyl acetate=5:2) afforded a colorless oil (**9b**) (18.5 mg, 42 %). Compound (**9b**): NMR (250 MHz, $CDCl_3$,): δ 9.50 (s, 1H, CHO), 6.65 (s, 1H, imine), 5.18 (s, 1H, olefinic H-5), 2.98 (dd, J=17.7, 1.6 Hz, 1H, H-13), 2.74 (dd, J=6.5 Hz, 1H, H-13), 1.59 (s, 3H, 16-CH_3), 0.84 (d, J= 6.4 Hz, 3H, 15-CH_3). IR ($CHCl_3$): νmax 3338 (OH), 1743 (C=O), 1650, 1448 cm^{-1}. MS m/z: 260 (M^+), 231 (M^+-CHO), 207, 175, 163, 135, 121, 95, 81, 69, 28, 18. Anal. Calcd for $C_{16}H_{24}N_2O$: C 73.85, H 9.23, N 10.77. Found C 73.03, H 9.87, N 10.66.

Photooxidation of 9a

While dried oxygen was passed through a solution of **9a** (90 mg, 0.34 mmol) and methylene blue (catalytic amount) in 10 mL of freshly dried methylene chloride at -78 °C, the solution was irradiated with a mercury-vapor lamp (300 W) for 2 h. To the reaction mixture, Dowex resin or triflic acid (cat. amount) was added at -23 °C and the whole mixture was stirred at rt for 5 h. To the solution was added saturated-$NaHCO_3$ solution, and the mixture was extracted with CH_2Cl_2 (3 x 30 mL) and concentrated under reduced pressure to provide the crude product. Flash column chromatography on silica gel (hexane/ethyl acetate=5:2) of the crude product gave **10a** (24 mg, 23 %) as a light yellow oil. Compound (**10a**): $[\alpha]_D^{25}$ -223.33° (c 0.06, $CHCl_3$); NMR (250 MHz, $CDCl_3$,): δ 6.83 (s, 1H, imine), 5.09 (s, 1H, H-5), 4.58 (m, 1H, N-H), 3.74 (1H, d, J= 8.8 Hz, Hα-12), 3.62 (d, J= 8.8 Hz, Hβ-12), 3.02 (d, J=17.8 Hz, 1H, Hα-13), 2.76 (dd, J=17.8, 1.3 Hz, 1H, Hβ-13), 1.60 (s, 3H, 16-CH_3), 1.45 (m, 1H, H-7), 0.89 (d, J= 6.4 Hz, 3H, 15-CH_3), C^{13}-NMR ($CDCl_3$, 75 MHz): 146.6, 136.2, 120.1, 89.7, 75.1, 47.5, 44, 42, 37.5, 35.4, 28.2, 25.7, 25, 24.1, 19.7, 15.9. IR ($CHCl_3$): νmax 3400 (NH), 2924, 2870, 1683, 1448 cm^{-1}; MS m/z: 308 (M^+), 280 (M^+-N_2), 264 (280-O). Anal. Calcd for $C_{16}H_{24}N_2O_4$: C 62.34, H 7.79, N 9.09. Found C 62.71, H 7.30, N 9.42.

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10. Compound (**4**) is identical by comparison of mmp, specific rotation and spectral properties with those of the known compound.[9]
11. Hyper Chem (version 4.0) obtained from Hypercube, Inc., Ontario, Canada was used for molecular mechanics calculations.

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