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Study on the Mechanism of Action of Artemether against Schistosomes: The Identification of Cysteine Adducts of Both Carbon-Centred Free Radicals Derived from Artemether

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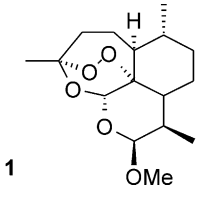
Abstract—Reaction of the antimalarial and anti-schistosome drug artemether (**1**) and catalytic amount of ferrous ion in the presence of excess cysteine gave two adducts of cysteine and previous postulated primary and secondary carbon-centred free radicals besides their other rearrangement products. This piece of further evidence for the presence of carbon-centred radicals, especially the secondary carbon-centred free radical for the first time by the isolation of its coupling adduct, will be help to understand the mechanism of action of artemether and other qinghaosu derivatives against parasites.

Artemether (**1**), a derivative of qinghaosu (artemisinin, **2**), was firstly reported as a more active antimalarial compound than its parent one by Li et al. in 1979.[1] Now it has been used as a very effective drug to fight the most serious parasitic disease worldwide, malaria, especially for the multi-drug resistant malarial parasite. During the last decade it was found that artemether also possessed potent effect against the juvenile stages of another parasite schistosome and could be used as a prophylactic drug for prevention of schistosomiasis.[2] Recently we performed a comparative study on adult *Schistosoma japonicium* survival after incubation in two media, Hank's balanced salt solution (HBSS) and RPMI 1640, supplemented with calf serum, antibiotics, artemether and haemin. It was interesting to observe that worm mortalities were consistently higher and occurred faster in HBSS in comparison with those in RPMI 1640. A control experiment (without the incubation of *S. japonicium*) showed that decomposition of artemether to free radical reaction products in RPMI 1640 was much faster than in HBSS. In this respect, it was indicated that these free radical reaction products of artemether exhibited no damage to the schistosomes. It was suggested that the worms needed to ingest artemether and haemin to the gut. Then the free radical reaction took place in the gut. The radical was toxic to the worms and killed the worms eventually.[3] For the detection of these products of minor quantity in the incubation media, we prepared their authentic samples from the free radical reaction of artemether and ferrous ion. Herewith, we would like to report the chemistry of isolation and identification of these products.



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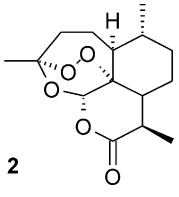

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The reaction of qinghaosu and its derivatives with ferrous ion produced mainly two products, that is compounds **3** and **4**, which were proposed to be formed through a primary carbon-centred free radical and a secondary carbon-centred free radical, respectively.[4–7] Lately the reaction of qinghaosu and its 12-deoxy-12-aryl derivatives with catalytic amount of Fe(II/III) (ferrous or ferric ion) in the presence of cysteine or reduced glutathione also could take place and produced a hydrogen attracted product and a cysteine adduct or a glutathione adduct of the primary carbon-centred free

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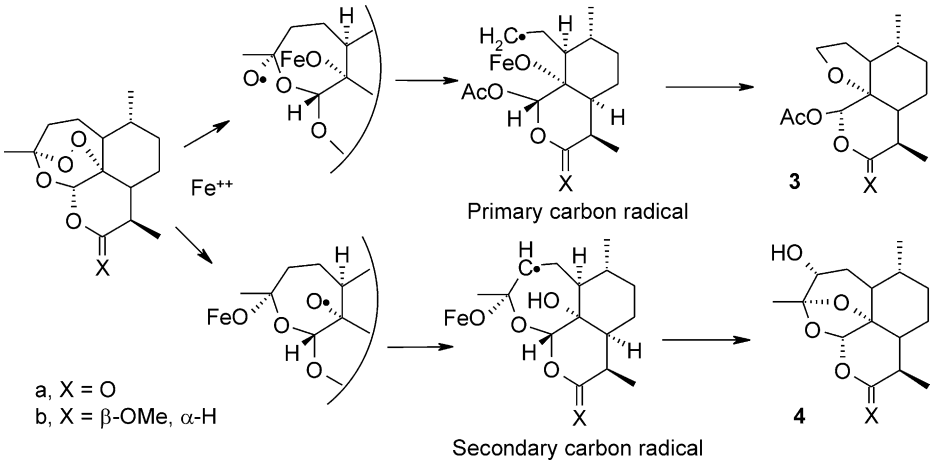
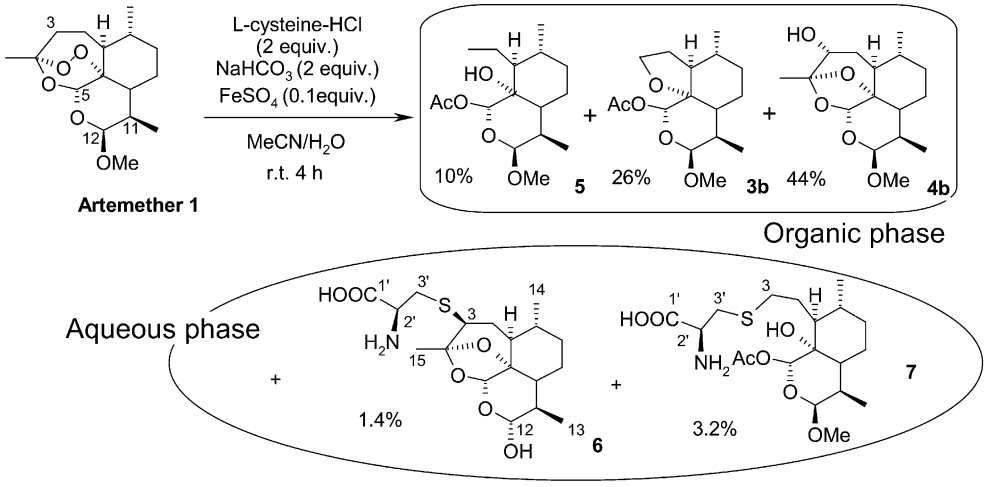


radical.[8–10] At almost the same time the heme–qinghaosu adduct and heme–artemether derived from the primary carbon-centred free radical was also reported from French laboratory.[11]

Considering the presence of cysteine in the incubation medium RPMI 1640, the reaction of artemether was carried out with catalytic amount of ferrous sulfate and excess cysteine. Thus the reaction was run in aqueous acetonitrile (1:1) at room temperature for 4 h, when all starting artemether was consumed. Working-up gave an organic phase and an aqueous one. From the organic phase two known tetrahydrofuranyl product (**3b**) and 3-hydroxyl product (**4b**) was isolated in 26 and 44% yield, respectively. Besides, a hydrogen-attracted product **5**[12] of the proposed primary carbon-centred free radical was also isolated in 10% yield. From the aqueous phase both adducts **6**[13] and **7**[14] of cysteine with the proposed secondary and primary carbon-centred free radical could be detected by TLC developed by using ^{n}BuOH/AcOH/H_2O as eluent and visualized by spraying with ninhydrin as a pink spot, and were isolated by reversed phase column chromatography. The structure of major adduct **7** (in 3.2% yield) was easily determined by its high resolution ESI mass spectrum and NMR spectra, which had similar pattern with corresponding adduct of other qinghaosu derivative.[10] The structure of the foregoing fraction **6** (in 1.4% yield) was quite different with those adducts derived from the primary carbon-centred free radical of qinghaosu and its derivatives. However, it contained the cysteine segment and the artemether skeleton too as shown by ^{1}H NMR and ^{13}C NMR spectra. The NMR pattern of cysteine part was similar to that of adduct **7** and the pattern of the artemether part was similar to that of compound **4b** except the OMe signal. These NMR data and the molecular weight 388 measured by ESI-MS showed this compound was the adduct from the proposed secondary carbon-centred free radical. The configuration of C-12-hydroxyl was determined to be α because of the axial–axial coupling constant (8.6 Hz) between H-11 and H-12. The configuration of cysteine residue at C-3 was proposed to be β according to the coupling pattern of H-3. The proton at C-3 had chemical sift 2.97 as a doublet-doublet peak with *J* value of 5.6 and 11.3 Hz, which meant that H-3 was an axial proton and in α orientation calculated by the molecular modelling (AM1).

The hydrogen-attracted product and the cysteine adduct of primary carbon-centred free radical from qinghaosu or its derivatives have been identified in our laboratory.[8,10] However, the cysteine adduct of secondary carbon-centred free radical from qinghaosu series was isolated for the first time. Therefore, up to now, the



postulated primary and secondary carbon radicals were confirmed not only by ESR spectra,[4,5] but also by the identification of both free radical adducts. On the other side, with these standard samples in our hand we have detected the products after incubation of artemether with or without *S. japonicium* in different media. It was found that RPMI 1640 contained a number of amino acids including cysteine, therefore, artemether already converted to its free radical reaction products and cysteine adducts before it reached to the gut of parasite. The observation and deduction of biological respect were reported in detail elsewhere.[3]

Acknowledgements

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12. **Compound 5**: Mp: 66–68 °C. $[\alpha]_D^{20} = +86$ (*c* 0.59, $CHCl_3$), IR (KBr) ν: 3552, 2989, 1758, 1466, 1240, 1094 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ: 0.89 (3H, d, $J = 7.3$ Hz), 0.94 (3H, d, $J = 6.5$ Hz), 0.97 (3H, t, $J = 5.9$ Hz), 2.12 (3H, s), 2.48 (1H, m), 3.43 (3H, s), 4.59 (1H, d, $J = 4.0$ Hz), 6.29 (1H, s). EI–MS (*m*/*z*): 281, 267, 209, 163. Anal. calcd for $C_{16}H_{28}O_5$: C, 63.97; H, 9.40. Found: C, 63.94; H, 9.42.
13. **Compound 6**: Syrup, $[\alpha]_D^{25} = -24.2$ (*c* 0.5, CH_3CN/H_2O = 1:1). IR (KBr) ν: 3420, 2930, 1634, 1386. ^1H NMR (400 MHz, D_2O) δ: 0.96 (3H, d, $J = 6.2$ Hz, 13-H_3), 1.00 (3H, d, $J = 7.2$ Hz, 14-H_3), 1.77 (3H, s, 15-H_3), 1.95 (2H, m), 2.20 (1H, m), 2.35 (1H, m), 2.97 (1H, dd, $J = 5.6$ Hz, 11.3 Hz, 3-H), 3.19 (1H, dd, $J = 7.0$ Hz, 14.5 Hz, 3′-H), 3.25 (1H, dd, $J = 4.6$ Hz, 14.6 Hz, 3′-H), 3.99 (1H, dd, $J = 4.4$ Hz, 7.0 Hz, 2′-H), 4.93 (1H, d, $J = 8.6$ Hz, 12-H), 5.59 (1H, s, 5-H). ^{13}C NMR (75 MHz, D_2O–CD_3COCD_3) δ: 13.73 (CH_3), 18.72 (CH_3), 22.83 (CH_3), 22.90 (CH_2), 30.93 (CH_2), 32.68 (CH_2), 33.35 (CH), 34.22 (CH_2), 35.12 (CH), 41.89 (CH), 47.72 (CH), 49.68 (CH), 54.86 CH), 83.93 (C), 95.92 (CH), 97.19 (CH), 111.18 (C), 172.81 (CO). ESI-MS (*m*/*z*): 388 (M + 1), 775 (2M + 1). ESI-HRMS calcd for $C_{18}H_{30}NO_6S$: 388.1788. Found: 388.1771.
14. **Compound 7**: Syrup, $[\alpha]_D^{25} = +27.6$ (*c* 1.0, CH_3CN/H_2O = 1:1), $[\alpha]_D^{25} = +33.9$ (*c* 0.8, H_2O). IR (KBr) ν: 3408, 2878, 1762, 1633, 1224 cm^{-1}. ^1H NMR (400 MHz, D_2O) δ: 0.96 (3H, d, $J = 7.3$ Hz, 14-H_3), 0.99 (3H, d, $J = 6.4$ Hz, 13-H_3), 2.26 (3H, s, CH_3CO), 2.38 (1H, m), 2.59 (2H, m), 2.84 (1H, m), 3.13 (1H, dd, $J = 7.1$ Hz, 14.8 Hz, 3′-H), 3.20 (1H, dd, $J = 4.4$ Hz, 14.8 Hz, 3′-H), 3.46 (3H, s, OCH_3), 3.96 (1H, dd, $J = 4.4$ Hz, 7.2 Hz, 2′-H), 4.78 (1H, d, $J = 4.0$ Hz, 12-H), 6.33 (1H, s, 5-H). ^{13}C NMR (100 MHz, D_2O–CD_3COCD_3) δ: 11.96 (CH_3), 20.28 (CH_3), 20.77 (CH_3), 24.34 (CH_2), 27.92 (CH_2), 31.28, 32.01 (CH_2), 33.24 (CH_2), 35.14 (CH_2), 35.14, 47.57, 53.51, 53.62, 55.46, 73.20 (C), 87.02, 103.76, 171.77 (CO), 172.35 (CO). ESI-MS (*m*/*z*): 420 (M + 1), 839 (2M + 1). ESI-HRMS calcd for $C_{19}H_{34}NO_7S$: 420.2050. Found: 420.2039.