ChemComm

A possible antimalarial action mode of qinghaosu (artemisinin) series compounds. Alkylation of reduced glutathione by *C*-centered primary radicals produced from antimalarial compound qinghaosu and 12-(2,4-dimethoxyphenyl)-12-deoxoqinghaosu

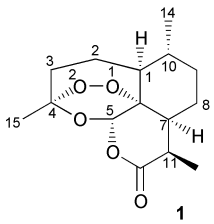
Dong-Ye Wang and Yu-Lin Wu

State Key Laboratory of Bio-organic & Natural Products Chemistry, Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences, 354 Fenglin Road, Shanghai 200032, China. E-mail: ylwu@pub.sioc.ac.cn

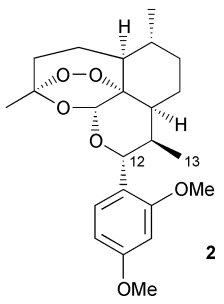
Received (in Cambridge, UK) 23rd August 2000, Accepted 3rd October 2000
First published as an Advance Article on the web 31st October 2000

Antimalarial compound qinghaosu (1) and its phenyl derivative 2 were reacted with reduced glutathione (GSH) and Fe(II/III) to give, besides other known degradation products, an interesting adduct from a primary *C*-centered free radical and GSH. Because GSH plays a very important role in the cell cycle, this finding may eventually lead to a new understanding of its mode of action.

Qinghaosu (artemisinin, **1**) originally isolated from a traditional Chinese herb,[1] and its derivatives are the most promising antimalarial medicines. Their unique 1,2,4-trioxane peroxy



1



2

structure has been proved to be essential for antimalarial activity[2] and is entirely different from that of previous generations of antimalarial drugs such as quinine and chloroquine. Cell-biological studies showed that qinghaosu's toxicity appeared to be the consequence of the membrane damage that might result from protein alkylation.[3] On the other hand, alkylation of a simplified heme *in vitro* has also been reported,[4] but this does not provide a full description of the antimalarial mechanism of qinghaosu and its derivatives. We have reported the reaction mechanism of the Fe(II)-induced cleavage of the peroxide bond in qinghaosu and its derivatives and certified the presence of *C*-centered radicals derived from this cleavage and the following radical rearrangement.[5,6] At the same time the DNA damage associated with this process has also been observed in our laboratory.[5] A parallel correlation[7] between the chemical reactivity of Fe(II)-induced cleavage of the peroxide in qinghaosu's derivatives and their antimalarial activity *in vivo* and also the intermolecular interaction[8]—abstraction of a hydrogen atom from cysteine and attachment to the cysteine sulfur atom—of the primary *C*-centered radicals involved in the degradation of qinghaosu have been found in our previous work. Continuing these explorations we have synthesized a pair of C-11 epimers of 12-(2,4-dimethoxyphenyl)-12-deoxoqinghaosu (**2**) and found once again the parallel relationship of reactivity of their Fe(II)-induced cleavage and antimalarial activity. In the presence of cysteine and catalytical ferrous or ferric ion a stable product from cysteine and the primary free radical from phenyl qinghaosu **2** could be isolated and identified. These experimental results[9] provided further evidence for the participation of a primary carbon centered free radical in the antimalarial action of qinghaosu compounds.

With the adduct of cysteine and the primary free radical derived from qinghaosu and its derivatives in hand, it would be interesting to observe if an adduct could form from glutathione and this free radical. Malaria parasite infected red cells have a high concentration of the reduced glutathione (GSH, the main reducing agent in physiological systems).[10] It was also reported that an excess GSH in the parasite may be responsible for protecting the parasite from the toxicity of heme.[11] In general, GSH takes part in many biological functions, including the detoxification of cytosolic hydrogen peroxide and organic peroxides and then protects cells from oxidative stress. Therefore, depletion of GSH or inhibiting glutathione reductase in tumor or parasite cell will induce oxidative stress and then kill these cells,[12] which may explain the mechanism of some antitumor or antiparasite agents. In addition, an alternative promising approach to finding new antitumor or antiparasite agents is provided.

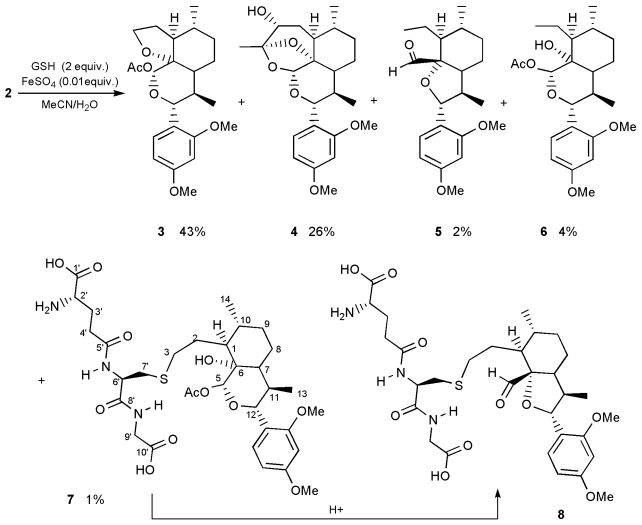
Here we report that the *C*-centered radicals of compound **2** and qinghaosu (**1**) can abstract a hydrogen atom from GSH, and, the novel adducts between **2**, **1** and GSH are characterized.

The 12-(2,4-dimethoxyphenyl)-12-deoxoqinghaosu (**2**) prepared from dihydroqinghaosu is a UV detectable, stable, lipophilic and hence easily handled qinghaosu derivative. It is also a more active antimalarial agent than qinghaosu and artermether. Therefore compound **2** was firstly used as the substrate to react with GSH in the presence of catalytic amounts of ferrous sulfate. Thus, a mixture of GSH, AcONa, a catalytic amount of $FeSO_4 \cdot 7H_2O$ and 0.5 equivalents of compound **2** in aq. MeCN was stirred at rt for 3 days. From the organic extract, starting material **2** (20%) and known compounds[9] **3**, **4**, **5**, and **6** (43%, 26%, 2%, and 4%, respectively) were obtained. On the other hand adduct **7**[13] (1%) was isolated from the aqueous layer by column chromatography (RP-C-18) (Scheme 1).

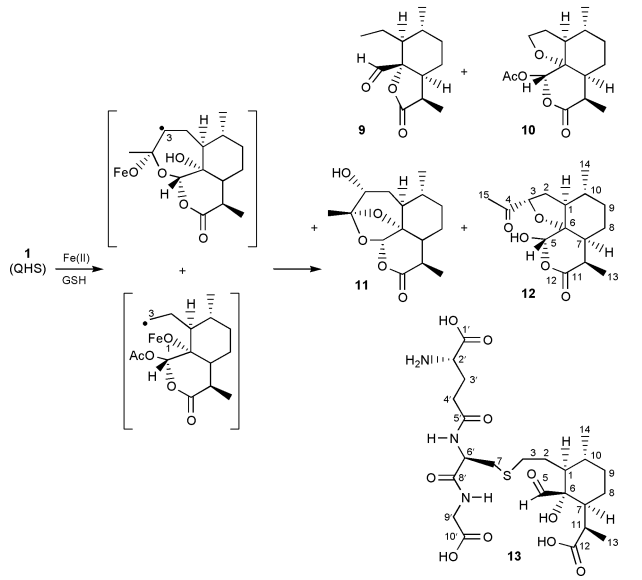
It is reasonable to propose that adduct **7** is formed by coupling the primary *C*-centered free radical and GSH. Adduct **7** gives a typical ESI mass spectrum peak at $m/z = 735$ ($M + Na - 1$) and all the corresponding proton resonances in the NMR spectra. Adduct **7** tends to rearrange to give compound **8** in acidic medium, partial rearrangement could be observed even in deuteriated water in the NMR tube.

The successful isolation of adduct **7** encouraged us to explore the reaction of GSH with qinghaosu–Fe(II/III) and search for the products from both the organic and water phases. The reaction of qinghaosu (**1**) with 2 equivalents of GHS and 0.04 equivalent Fe(II) was performed at 37 °C under argon. After 12 hours all qinghaosu was consumed to yield known compounds **9**[8] (5%), **10**[6] (41%), **11**[6] (26.4%), and **12** (1%, a C-3 epimer of known compound), all collected from the organic phase, and **13**[14] (4%) collected from the aqueous phase of the reaction mixture after work-up (Scheme 2). The reaction also proceeds wider acid conditions in the range pH 1–7, giving slight variations in the yields of **9** and **13**.

The most interesting adduct **13** is a highly polar, water soluble compound, which was recovered from the reaction



Scheme 1



Scheme 2

mixture by chromatography on reverse phase silica gel (RP-C18, H_2O). On thin-layer chromatography (TLC, with 3 : 1 : 1 n-BuOH–AcOH–H_2O) **13** could be visualized by 0.5% ninhydrin in EtOH as a pink spot. The mass spectrum (ESI) exhibited typical peaks at *m/z* 548 $(M + H)^+$, 570 $(M + Na)^+$, and 586 $(M + K)^+$. Its high resolution mass spectrum (SI) exhibited the peaks at *m/z* 570.2090 $(M + Na)^+$, and 592.1916 $(M - H + 2Na)^+$. NMR analyses were performed in D_2O, including ^1H and ^{13}C NMR, DEPT, DQ-COSY, NOESY, HMQC (^1J) and long-range (^3J) proton-carbon correlation (HMBC). Compound **13** showed several cross-peaks (H-7′/H-3, H-6′/H-3) in NOESY and (H-7′/C-3, H-3/C-7′) in HMBC spectrum. The data undoubtedly showed that a σ-bond was present between the sulfur of the glutathione residue and C-3 in the qinghaosu part. Compound **13** was therefore the result of an adduct between primary carbon-radical derived from qinghaosu and GS$^{\cdot}$.

In conclusion, qinghaosu (**1**) and its phenyl derivative **2** could be degraded in the presence of reduced glutathione (GSH) and a catalytical amount of Fe(II/III). Besides known products previously isolated from the reaction of qinghaosu and its derivative with ferrous ion, an interesting adduct from the primary *C*-centered free radical derived from qinghaosu and *S*-centered free radical from GSH was isolated and structurally elucidated by NMR and other spectroscopy. In *Plasmodium* parasite the GSH concentration is maintained by means of the glutathione reductase catalyzed reduction of glutathione disulfide (GSSG) at the expense of NADPH. GSH plays an important role in protecting the parasite from oxidative stress. The formation of a GSH–qinghaosu adduct reduces the amount of GSH and the adduct itself might also cause inhibition of the glutathione reductase in the parasite. Therefore the present finding may lead to a new understanding of the mode of action of these antimalarial drugs.

This work was supported by the Chinese Academy of Sciences (no. KJ951-A1-504-04), the National Natural Science Foundation (no. 29572075, 09561423, 29832020, 39870899), and the Ministry of Science and Technology of China (no. 970211006-6). We also thank Dr Yikang Wu for the helpful discussion.

Notes and references

1 (*a*) J.-M. Liu, M.-Y. Ni, Y.-F. Fan, Y.-Y.Tu, Z.-H. Wu, Y.-L. Wu and W.-S. Chou, *Huaxue Xuebao*, 1979, **37**, 129; (*b*) D. L. Klayman, *Science*, 1985, **228**, 1049; (*c*) A. R. Butler and Y.-L. Wu, *Chem. Soc. Rev.*, 1992, 85.

2 (*a*) Y. Li, P.-L. Yu, Y.-X. Chen, L.-Q. Li, Y.-Z. Gai, D.-S. Wang and Y.-P. Zheng, *Yaoxue Xuebao*, 1981, **16**, 429; (*b*) Y.-L. Wu and Y. Li, *Med. Chem. Res.*, 1995, **5**, 569.

3 S. R. Meshnick, T. E. Taylor and S. Kamchonwongpaisan, *Microbiol. Rev.*, 1996, **60**, 301, and references cited therein.

4 (*a*) A. Robert and B. Meunier, *Chem. Soc. Rev.*, 1998, **27**, 273; (*b*) A. Robert and B. Meunier, *J. Am. Chem. Soc.*, 1997, **119**, 5968.

5 W.-M. Wu, Z.-J. Yao, Y.-L. Wu, K. Jiang, Y.-F. Wang, H.-B. Chen, F. Shan and Y. Li, *J. Chem. Soc., Chem. Commun.*, 1996, 2213.

6 W.-M. Wu, Y.-K. Wu, Y.-L. Wu, Z.-J. Yao, C.-M. Zhou, Y. Li and F. Shan, *J. Am. Chem. Soc.*, 1998, **120**, 3316, and references cited therein.

7 D.-Y. Wang, Y.-K. Wu, Y.-L. Wu, Y. Li and F. Shan, *J. Chem. Soc., Perkin Trans. 1*, 1999, 1827.

8 Y. Wu, Z.-Y. Yue and Y.-L. Wu, *Angew. Chem., Int. Ed.*, 1999, **38**, 2580.

9 D.-Y. Wang, Y.-L. Wu, Y. Wu, J. Liang and Y. Li, *J. Chem. Soc., Perkin Trans. 1*, submitted for publication.

10 H. Atamna and H. Ginsburg, *Eur. J. Biochem.*, 1997, **250**, 670.

11 (*a*) H. Ginsburg, O. Famin, J. Zhang and M. Krugliak, *Biochem. Pharmacol.*, 1998, **56**, 1305; (*b*) O. Famin, M. Krugliak and H. Ginsburg, *Biochem. Pharmacol.*, 1999, **58**, 59.

12 R. H. Schirmer, J. G. Muller and R. L. Krauth-Siegel, *Angew. Chem., Int. Ed. Engl.*, 1995, **34**, 141.

13 Compound **7**: mp: > 210 °C. ^1H NMR (600 MHz, D_2O) δ: 7.37 (d, 1H, J = 7.8 Hz, Ar-H), 6.60 (d, 1H, J = 8.4 Hz, Ar-H), 6.56 (d, 1H, J = 2.4 Hz, Ar-H), 6.15 (s, 1H, H-5), 4.70 (m, 1H, H-12), 4.50 (m, 1H, H-6′), 3.76 (6H, $2OCH_3$), 3.74 (2H, 2H-9′), 3.08 (m, 1H, H-2′), 3.02 (m, 1H, H-7′), 2.80 (m, 1H, H-7′), 2.64 (m, 1H, H-3), 2.57 (m, 1H, H-11), 2.47 (m, 1H, H-3), 2.40 (m, 2H, 2H-4′), 2.22 (m, 1H, H-2), 2.04 (s, 3H, Ac), 1.98 (m, 2H, 2H-3′), 1.78–1.61 (m, 4H, H-9β, H-8α, H-7, H-8β), 1.39 (m, 1H, H-10), 1.26 (m, 1H, H-2), 1.12 (m, 1H, H-1), 1.05 (m, 1H, H-9α), 0.85 (d, 3H, J = 6.6 Hz, CH_3 at C-10), 0.50 (d, 3H, J = 6.6 Hz, CH_3 at C-11); MS (ESI) *m/z*: 779 ($M - 1 + 3Na$), 757 ($M + 2Na$), 735 ($M + 1 + Na$).

14 Compound **13** ^1H NMR (600 MHz, D_2O) δ: 9.98 (s, 1H, CHO), 4.63 (m, 1H, H-6′), 3.85 (m, 3H, 2H-9′ and H-2′), 3.14 (dd, 1H, J = 14.1, 5.1 Hz, H-7′), 2.93 (dd, 1H, J = 14.1, 8.7 Hz, H-7′), 2.62 (m, 4H, 2H-4′, 2H-3), 2.47 (m, 1H, H-11), 2.24 (m, 2H, 2H-3′), 1.98 (m, 1H, H-9ß), 1.82-1.91 (m, 3H, H-10, H-7, H-8α), 1.79 (m, 1H, H-8β), 1.63 (m, 1H, H-2), 1.56 (m, 1H, H-2), 1.39 (m, 1H, H-1), 1.31 (m, 1H, H-9α), 1.16 (d, 3H, J = 7.2 Hz, CH_3 at C-11), 1.04 (d, 3H, J = 6.6 Hz, CH_3 at C-10); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 209.99 (HC=O), 187.32 (C-12), 178.97 (C-10′), 177.59 (C-5′), 176.72 (C-1′), 174.67 (C-8′), 86.01(q, C-6), 56.95 (C-1 or C-2′), 56.86 (C-2′ or C-1), 55.75 (C-6′), 53.45 (C-7), 46.50 (C-11), 46.13 (C-9′), 37.89 (C-10), 37.52 (C-9), 35.44 (C-7′), 34.67 (C-4′ or C-3), 34.11 (C-4′ or C-3), 30.43 (C-2), 28.94 (C-3’ or C-8), 28.88 (C-3′ or C-8), 23.00 (CH_3 at C-1), 20.26(CH_3 at C-11); MS (ESI) *m/z*: 548 ($M^+ + 1$), 570 (M + Na); HRMS (SI) *m/z:* Calcd for $C_{23}H_{37}O_{10}N_3S$ + Na: 570.2092; Found: 570.2090; Calcd for $C_{23}H_{37}O_{10}N_3S - H + 2Na$: 592.1911; Found: 592.1916.