ACID DEGRADATION PRODUCTS OF QINGHAOSU AND THEIR STRUCTURE-ACTIVITY RELATIONSHIPS

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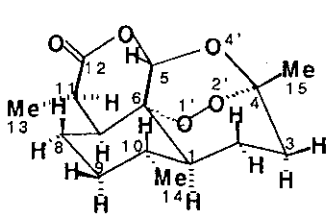
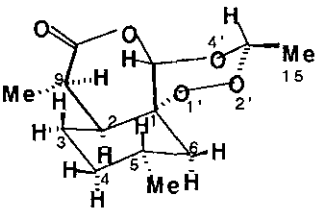
Abstract – Treatment of qinghaosu (**1**) with acid yielded 1',2',4'-trioxanes (**5** and **8**), endoperoxides (**6** and **9**), and diketones (**7** and **10**). Structures of **5**, **6**, **7**, **8**, **9**, and **10** were assigned based on their physical and spectral data. Structure-activity correlation among these compounds indicated the steric requirement of the 1',2',4',-trioxane ring system as found in **1** for potent antimalarial activity.

Qinghaosu (artemisinin or arteannuin, **1**), a sesquiterpene lactone endoperoxide isolated from the Chinese drug "Qing Hao" (*Artemisia annua* L.),[1,2] has recently been used clinically as a new type of antimalarial agent with rapid action and low toxicity against chloroquine-resistant *Plasmodium falciparum*. In order to investigate the structure-activity relationships, we have previously reported on the synthesis of **1**-related componds, including the 1',2',4'-trioxane lactone (-C-O-O-C-O-C-O-CO-, **2**),[3] 1',2',4'-trioxane (-C-O-O-C-O-, **3a** and **3b**),[3,4] and endoperoxide (-C-O-O-C-, **4**)[5] *ring systems.*



1



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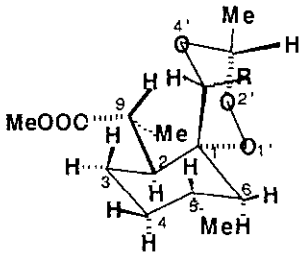
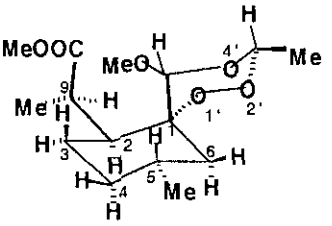
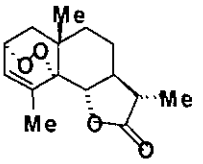


3a: R=β-OMe
3c: R=α-OMe

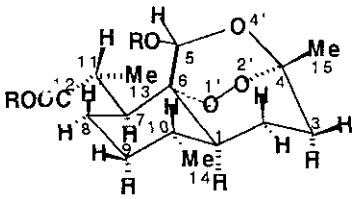


3b

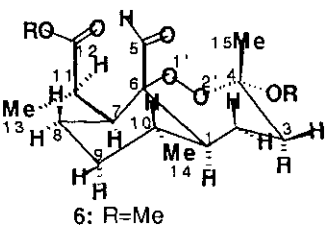


4

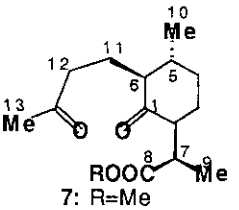
Since these compounds (2, 3a, 3b and 4) were found to be 30~100 times less active than 1 in the *in vitro* antimalarial assay against the chloroquine-resistant *P. falciparum*,[6] it indicates that the antimalarial activity of 1-related analogs may be affected significantly by the steric environment of the 1',2',4'-trioxane ring among these molecules. The ethane bridge between C-1 and C-4 of 1 might play an important role with this respect. We report herein on the synthesis of C-1/C-4 ethane bridge-bearing 1',2',4'-trioxanes (5 and 8) , endoperoxides (6 and 9), and diketones (7 and 10) by acidic degradation of 1.



5: R=Me
8: R=Et



6: R=Me
9: R=Et



7: R=Me
10: R=Et

Treatment of 1 with an acid [p-toluenesulfonic acid monohydrate(p-TsOH·H_2O) or 14% hydrogen chloride (HCl)-MeOH] in anhydrous methyl alcohol (MeOH) gave three products: MT-I (5, 5.5 %),[7] MT-II (6, 20.4 %),[7] and MT-III (7, 3.9 %).[7] These products were purified by silica gel column chromatography.

The ^{1}H- and ^{13}C-nmr spectra of MT-I and MT-II were assigned on the basis of ^{1}H-^{1}H COSY and ^{1}H-^{13}C-COSY spectra as well as decoupling experiments between each proton signal. The ^{1}H- and

^{13}C-nmr ($CDCl_3$) and ir (film) spectral data of MT-I {Syrup, $C_{17}H_{28}O_6$, [α]D +60° (c 0.7, MeOH)} indicated the presence of one methoxy group [^{1}H-nmr: δ 3.47 (3H, s); ^{13}C-nmr: δ 51.42 (q)] and one carbomethoxy group [^{1}H-nmr: δ 3.65 (3H, s); ^{13}C-nmr: δ 56.00 (q); ir: 1740 cm^{-1}], which were absent in **1**. The presence of a 1',2',4'-trioxane ring (devoid of the stereochemistry) in MT-I was evidenced by the appearance of the characteristic ion peaks at m/z 328 $[M]^+$, 296 $[M-O_2]^+$, 238 $[296-Me_2CO]^+$, and 207 $[238-MeO]^+$ in its ms spectrum, together with the ^{13}C-nmr ($CDCl_3$) peaks at δ 55.32(d, C-1), 24.45(t, C-2), 37.44(t, C-3), 105.07(s, C-4), 99.11(d, C-5), and 86.84(s, C-6)]. The assignment of the conformation of 1',2',4'-trioxane ring skeleton of MT-I as depicted in **5** was based on a comparison of its ^{1}H-nmr spectrum with that of **1**. Compound **1** displayed proton signals at δ 2.43(ddd, Hα-3), 2.06(ddd, Hβ-3), 1.50(dddd, Hβ-2), 2.01(m, Hα-2), and 1.43(m, Hβ-10) with $J_{1,10}=J_{1,2\beta}=11.5$ Hz, $J_{1,2\alpha}=6.7$ Hz, $J_{2\alpha,3\alpha}=3.9$ Hz, $J_{2\alpha,3\beta}=4.4$ Hz, $J_{2\beta,3\alpha}=13.0$ Hz, and $J_{2\beta,3\beta}=3.2$ Hz, whereas MT-I showed proton peaks at δ 1.20(ddt, Hα-1), 1.72(m, Hα-2), 1.79(m, Hβ-2), 2.31(ddd, Hα-3), 2.02(ddd, Hβ-3), 5.30(d, Hα-5), 1.93(m, Hβ-10), and 2.86(dq, H-11) with nearly identical coupling constants of $J_{1,10}=J_{1,2\beta}=11.5$ Hz, $J_{1,2\alpha}=6.7$ Hz, $J_{1,5\alpha}=1.6$ Hz(W-type long-range coupling between H-1 and Hα-5),[8] $J_{2\alpha,3\alpha}=4.1$ Hz, $J_{2\alpha,3\beta}=4.5$ Hz, $J_{2\beta,3\alpha}=12.9$ Hz, and $J_{2\beta,3\beta}=3.3$ Hz. A significant deshielding effect was also observed. This included a downfield shift of 0.29, 0.37, 0.29, and 0.50 ppm when compared the chemical shifts of Hα-3 and Hβ-3 of MT-I, Hα-3 and Hβ-3 of **1**, Hβ-2 of **1** and Hβ-2 of MT-I, and Hβ-10 of **1** and Hβ-10 of MT-I, respectively. The above evidence led to the assignment of MT-I as **5**, in which it possesses a 1',2',4'-trioxane ring nearly identical sterically to that of **1**.

The ^{1}H- and ^{13}C-nmr spectral data ($CDCl_3$) of MT-II {oil, $C_{17}H_{28}O_6$, [α]D +152.6° (c 0.9, MeOH)} indicated the presence of one methoxy [^{1}H-nmr: δ 3.36(3H,s); ^{13}C-nmr: δ 49.13(q)], one carbomethoxy [^{1}H-nmr: δ 3.65(3H, s); ^{13}C-nmr: δ 51.42(q)], and one formyl [^{1}H-nmr: δ 9.93(1H, d, J=2.8 Hz); ^{13}C-nmr: δ 200.75(d)] groups as new signals, which were not seen in **1**. The presence of an endoperoxide group (devoid of the stereochemistry) in MT-II was substantiated by the the characteristic ms spectral ion peaks at m/z 328 $[M]^+$, 296 $[M-O_2]^+$, 265 $[296-MeOH]^+$, 236 $[265-CHO]^+$, and 177 $[236-COOMe]^+$ as well as by the ^{13}C-nmr signals at δ 59.49(d, C-1), 22.11(t, C-2), 40.60(t, C-3), 94.12(s, C-4), 200.75(d, C-5), and 108.51(s, C-6). The relative configuration at H-1[δ 1.16(m)], Me-4[δ 1.19(s)], CHO-6[δ 9.93(d)], and H-10[δ 2.23(m)] in MT-II was deduced as α, β, β, and β, respectively, by their ^{1}H-nmr coupling patterns [$J_{1,10}=12.0$ Hz, $J_{1,2\beta}=11.5$ Hz, $J_{1,2\alpha}<1.0$ Hz, $J_{1,5}=2.7$ Hz(W rule signals between H-1 and H-5), $J_{2\alpha,2\beta}=14.9$ Hz, $J_{2\alpha,3\alpha}<1.0$ Hz, $J_{2\alpha,3\beta}=7.1$ Hz, $J_{2\beta,3\alpha}=11.5$ Hz, $J_{2\beta,3\beta}<1.0$ Hz, and $J_{3\alpha,3\beta}=14.5$ Hz], and by use of NOESY technique, which confirmed the interactions between Hβ-3[δ 2.05(ddd)] and Me-4[δ 1.19(s)]; H-1[δ 1.16(m)] and Hα-3[δ 1.94(ddd)]; H-1, Hα-9(δ 0.94(m), and Hα-7[(δ 1.52(ddd)]; Hα-2[δ 1.79(ddt)] and Me-10[δ 0.89(d)]; and Hβ-2[δ 1.38(ddt)] and Me-4[d 1.19(s)]. The strong shielding effect by an upfield shift of 0.41 ppm when compared the chemical shifts of Hβ-2 in MT-II and Hβ-2 in MT-I, plus the deshielding

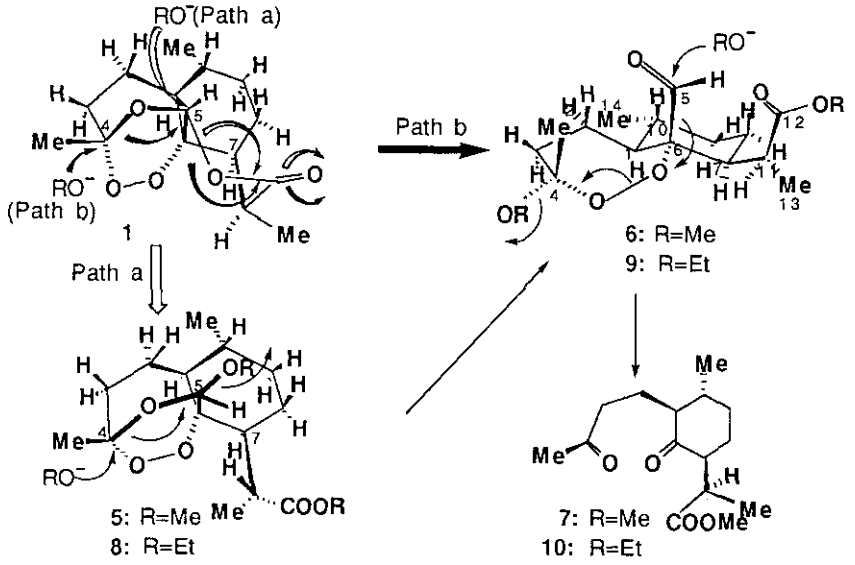
downfield shifts by 0.30 and 0.33 ppm of Hβ-10 and H-11[δ 3.19(dq)] when compared to their corresponding protons in MT-II and MT-I, respectively, led to the assignment of the stereochemistries of C-4, C-6, and C-11 of MT-II as shown in **6**. The stereostructure of MT-II was thus established as **6**.

The stereostructure of MT-III was determined to be **7** on the basis of its ms and ^{1}H-nmr spectral characteristics.[9]

Treatment of **1** with an acid [p-TsOH·H_2O or 14 % HCl-ethyl alcohol(EtOH)] in anhydrous EtOH gave rise to ET-I (**8**, 9.9 %),[10] ET-II (**9**, 38.0 %),[10] and MT-III (**10**, 3.5 %)[10] after purification by silica gel column chromatography.

The stereostructures of ET-I, -II and -III were established as 1',2',4'-trioxane (**8**), endoperoxide (**9**), and diketone (**10**), respectively, on the basis of ^{1}H- and ^{13}C-nmr (utilization of ^{1}H-^{1}H-COSY, ^{1}H-^{13}C-COSY, and NOESY techniques) and ms spectral data[11] as described in the structural elucidation of MT-I, -II, and -III.

The formational mechanism of compounds (**5**~**10**) from **1** by acid-ROH(R=Me, Et) can be considered as shown in Scheme.



Scheme

The 1',2',4'-trioxanes (**5** and **8**) were found to be almost equipotent active with **1** in the *in vitro* antimalarial assay against the chloroquine-resistant *P. falciparum*.[6] On the other hand, the endoperoxides (**6** and **9**) did not show noteworthy activity (about 100 times less active than **1**).[6] These results clearly indicate that the steric environment of the 1',2',4'-trioxane ring system as

found in **1**, **5**, and **8** is vital for expressing antimalarial activity. Further investigation along this line is currently in progress.

ACKNOWLEDGEMENT

The authors are grateful to Dr. Ai Jeng Lin of Walter Army Institute of Research for his interest and assistance in this research.

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2. D. L. Klayman, *Science*, 1985, 1049, and literature contained therein.
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4. Compound **3b** showed ^{1}H-nmr(400 MHz, $CDCl_3$) signals at δ 0.88(3H, d, J=6.6 Hz, Me-5), 0.95(1H, dt, J=1.5, 13.0 Hz, Hα-6), 1.21(3H, d, J=7.3 Hz, Me-7), 1.35(3H, d, J=5.4 Hz, Me-9), 1.93(1H, m, H-5), 2.82(1H, dq, J=3.0, 7.3 Hz, H-7), 2.76(1H, ddd, J=1.5, 4.0, 13.0 Hz, Hβ-6), 3.48(3H, s, MeO-8), 3.68(3H, s, MeOOC-9), 5.04(1H, d, J=1.5 Hz, H-8), and 5.40(1H, q, J=5.4 Hz, H-7). We have previously[3] reported the synthesis of **3a** and **3c**. However, the stereo-structure assigned for **3c** previously must be revised to **3b**, as irradiation of H-8 at δ 5.04 increased the intensity (a positive nOe effect) of H-7 at δ 5.40. Furthermore, the presence of a W-type long range coupling (J=1.5 Hz) between H-8 and Hα-6 was also observed. This work was presented at the 27th Chugoku Shikoku Branch Annual Meeting of Pharmaceutical Society of Japan, Shimane, 1988, Abstracts of paper, p. 40.
5. S. Tani, N. Fukamiya, H. Kiyokawa, H. A. Musallam, R. O. Pick, and K. H. Lee, *J. Med. Chem.*, 1985, **28**, 1743.
6. *The details of the antimalarial activity of these compounds will be reported elsewhere.*
7. Based on the reaction of **1** with p-TsOH·H_2O in MeOH.
8. M. Barfield and B. Chakrabarti, *Chem. Rev.*, 1969, **69**, 757.
9. Compound (**7**) was obtained as colorless needles; mp 50~51°C, [α]D -75.5° (c 1.0, MeOH); ms m/z: 268[M]$^+$, 225[M-COMe]$^+$, 237[M-OMe]$^+$,and 209[M-COOMe]$^+$; ^{1}H-nmr(200 MHz, $CDCl_3$, δ): 1.09(3H, d, J=6.0 Hz, Me-10), 1.19(3H, d, J=7.0 Hz, Me-11), 2.16(3H, s, COMe), and 3.21(3H, s, OMe)
10. Based on the reaction of **1** with p-TsOH·H_2O in EtOH.
11. Selected spectral data for:
ET-I (**8**): oil; [α]D +97.5° (c 0.7, MeOH); $C_{19}H_{32}O_6$; FAB ms(m/z): 357[M +1], and 379[M + Na]; ^{1}H-nmr(400 MHz, $CDCl_3$, δ) for 1',2',4'-trioxane ring: 1.20(1H, dddd, J=1.0, 1.6, 11.4, 11.8 Hz, H-1), 1.37(3H, s, Me-4), 1.71(1H, dddd, J=1.0, 3.8, 4.5, 13.3 Hz, Hα-2), 1.78(1H, m, Hβ-

2), 2.01(1H, ddd, J=3.3, 4.4, 14.6 Hz, Hβ-3), 2.31(1H, ddd, J=4.5, 12.6, 14.6 Hz, Hα-3), and 5.37(1H, d, J=1.6 Hz, H-5); ^{13}C-nmr(100 MHz, $CDCl_3$, δ): 24.11(t, C-2), 25.65(q, C-15), 34.47(t, C-3), 55.34(d, C-1), 86.22(s, C-6), 97.93(d, C-5), and 104.95(s, C-4).

Et-II (9): oil; $[\alpha]_D$ +117.5° (c 0.9, MeOH); $C_{19}H_{32}O_6$; FAB ms(m/z): 357[M + 1], and 379[M + Na]; ^{1}H-nmr(400 MHz, $CDCl_3$, δ) for endperoxide ring: 1.18(1H, m, H-1), 1.19(3H, s, Me-4), 1.34(1H, dddd, J=2.0, 2.5, 7.1, 14.6 Hz, Hβ-2), 1.79(1H, dddd, J=1.0, 11.5, 13.5, 14.6 Hz, Hα-1), 1.94(1H, ddd, J=2.0, 13.5, 14.0 Hz, Hα-3), 2.05(1H, ddd, J=1.0, 7.1, 14.0 Hz, Hβ-3), and 9.93(1H, d, J=2.7 Hz, CHO-6); ^{13}C-nmr(100MHz, δ): 20.47(q, C-15), 22.09(t, C-2), 40.68(t, C-3), 59.16(d, C-1), 94.03(s, C-4), 100.36(s, C-6), and 200.82(d, C-5).

ET-III (10): oil; $[\alpha]_D$ -43.8° (c 1.0, MeOH); $C_{16}H_{26}O_4$; Ms(m/z): 282[M]$^+$, 253[M-Et]$^+$, 249[M-0Me]$^+$, 237[M-OEt]$^+$, 209[M-COOEt]$^+$, and 43{MeCO]$^+$; ^{1}H-nmr(200 MHz, $CDCl_3$, δ): 1.08(3H, d, J=5.9 Hz, Me-5), 1.17(3H, d, J=6.6 Hz, Me-7), 1.25(3H, t, J=7.1 Hz. MeCH$_2$OCO-7), 2.12(3H, s, MeCO-12), and 4.13(2H, q, J=7.1 Hz, MeCH$_2$OCO-7).

Received, 30th March, 1990