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 compounds, 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.
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,* 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.

Treatment of 1 with an acid [p-toluenesulfonic acid monohydrate (p-TsOH·H2O) or 14% hydrogen chloride (HCl)-MeOH] in anhydrous methyl alcohol (MeOH) gave three products: MT-I (5, 5.5%), MT-II (6, 20.4%), and MT-III (7, 3.9%). 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
The assignment of the conformation of MT-I was based on a comparison of its 1H-nmr spectrum with that of 1. Compound 1 displayed proton signals at δ 2.43(ddd, Ha-3), 2.06(ddd, Hβ-3), 1.50(ddd, Hβ-2), 2.01(m, Ha-2), and 1.43(m, Hβ-10) with J1,10=J1,2β=11.5 Hz, J1,2α=6.7 Hz, J2α,3α=3.9 Hz, J2β,3α=13.0 Hz, and J2β,3β=3.2 Hz, whereas MT-I showed proton peaks at δ 1.20(ddd, Ha-1), 1.72(m, Ha-2), 1.79(m, Hβ-2), 2.31(ddd, Hβ-3), 2.02(ddd, Hβ-3), 5.30(d, Ha-5), 1.93(m, Hβ-10), and 2.86(dq, H-11) with nearly identical coupling constants of J1,10=J1,2β=11.5 Hz, J1,2α=6.7 Hz, J1,5α=1.6 Hz (W-type long-range coupling between H-1 and Hα-5), J2α,3α=4.1 Hz, J2α,3β=4.5 Hz, J2β,3α=12.9 Hz, and J2β,3β=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 Ha-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 1H- and 13C-nmr spectral data (CDCl3) of MT-II {oil, C17H28O6, [α]D +152.6° (c 0.9, MeOH)} indicated the presence of one methoxy group (1H-nmr: δ 3.47(3H, s); 13C-nmr: δ 51.42(q)) and one carbomethoxy group (1H-nmr: δ 3.65(3H, s); 13C-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-II was deduced as a pair, according to their 1H-nmr coupling patterns (J1,10=12.0 Hz, J1,2β=11.5 Hz, J1,5α=1.6 Hz, J2β,3β=3.2 Hz, J2β,3α=12.9 Hz, and J2α,3α=4.1 Hz) and their 13C-nmr 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-O2]⁺, 265 [236-Me0]+, and 207 [238-MeO]+ in its ms spectrum, together with the 13C-nmr (CDCl3) peaks at δ 55.32(d, C-1), 24.45(t, C-2), 31.67(s, C-3), 31.67(s, C-4), 105.07(s, C-5), and 86.84(s, C-6).

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 1H-nmr spectral characteristics.9

Treatment of 1 with an acid [p-TsOH·H2O 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 1H- and 13C-nmr (utilization of 1H-1H-COSY, 1H-13C-COSY, and NOESY techniques) and ms spectral data11 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.

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.

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REFERENCES AND NOTES

4. Compound 3b showed \( ^1H\text{-nmr}(400 \text{ MHz}, \text{CDCl}_3) \) signals at \( \delta \) 0.88(3H, d, J=6.6 Hz, Me-5), 0.95(1H, dt, J=1.5, 13.0 Hz, H\( \alpha \)-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, H\( \beta \)-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 stereostructure assigned for 3c previously must be revised to 3b, as irradiation of H-8 at \( \delta \) 5.04 increased the intensity (a positive nOe effect) of H-7 at \( \delta \) 5.40. Furthermore, the presence of a W-type long range coupling (J=1.5 Hz) between H-8 and H\( \alpha \)-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.
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\(_2\)O in MeOH.
9. Compound (7) was obtained as colorless needles; mp 50-51\(^\circ\)C, [\( \alpha \)]\( \text{D} \) -75.5\(^\circ\) (c 1.0, MeOH); ms m/z: 268[M]+, 225[M-COMe]+, 237[M-OMe]+, and 209[M-COOMe]+; \( ^1H\text{-nmr}(200 \text{ MHz}, \text{CDCl}_3, \delta) \): 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\(_2\)O in EtOH.
11. Selected spectral data for:

ET-I (8): oil; [\( \alpha \)]\( \text{D} \) +97.5\(^\circ\) (c 0.7, MeOH); C\(_19\)H\(_{32}\)O\(_6\); FAB ms(m/z): 357[M +1], and 379[M + Na]; \( ^1H\text{-nmr}(400 \text{ MHz}, \text{CDCl}_3, \delta) \) 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, H\( \alpha \)-2), 1.78(1H, m, H\( \beta \)-
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, CDCl₃, 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; [α]D +117.5° (c 0.9, MeOH); C₁₉H₃₂O₆; FAB ms(m/z): 357[M + 1], and 379[M + Na]; $^1$H-nmr(400 MHz, CDCl₃, 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, dddd, 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; [α]D -43.8° (c 1.0, MeOH); C₁₆H₂₆O₄; Ms(m/z): 282[M]+, 253[M-Et]+, 249[M-0Me]+, 237[M-OEt]+, 209[M-COEt]+, and 43[MeCO]+; $^1$H-nmr(200 MHz, CDCl₃, 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, MeCH₂OCO-7), 2.12(3H, s, MeCO-12), and 4.13(2H, q, J=7.1 Hz, MeCH₂OCO-7).

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