Total synthesis of arteannuin (Quinghaosu) and related compounds

ZHOU Wei-Shan
Shanghai Institute of Organic Chemistry, Academia Sinica
345 Lingling Lu, Shanghai, China

Abstract — A new sesquiterpene peroxide was isolated from the Chinese herbal medicine Qinghao (Artemisia annua L.). Its structure and absolute stereochemistry have been firmly established by combined spectral, chemical and X-ray crystallographic methods. Based on pharmacological and chemical studies it was shown to be the antimalarial principle and named arteannuin (qinghaosu). Citronellal was used as a starting material for the total synthesis of arteannuin. The peroxide group of the arteannuin molecule was introduced by photooxidation. Deoxyarteannuin, arteannuin A, arteannuin B, arteannuin E, and arteannuin F which occur together with arteannuin in the same plant were also synthesized.

The Chinese herbal medicine Qinghao (Artemisia annua L. Compositae) is abundant and grows indigenously all over China. It has been used in practice for treatment of malaria in China for more than one thousand years. However, the therapeutic effect in traditional form was not so definite and consistent.

Further studies on antimalarial effect of Qinghao are done recently. An active antimalarial fraction of Qinghao was identified in 1971, from which a new sesquiterpene peroxide was isolated in 1972. Based on pharmacological and chemical studies, it was found to be the antimalarial principle and named arteannuin (qinghaosu). This natural constituent is fast acting and is effective against malaria resistant to chloroquine. However, clinical trials revealed that in treatment with arteannuin the disease recurred sooner than with chloroquine, despite complete disappearance of parasites from patient’s blood.

From indigenous Artemisia annua L., nine sesquiterpenes (Fig. 1 1—9) have been isolated (ref. la—e), of which all but arteannuin B (3) (ref. 2) are new compounds. From biogenetic viewpoint, they are all closely related to the amorphene series which is characterized by the presence of a cis-decalin skeleton with the isopropyl group trans to the hydrogen on the ring juncture.

Arteannuin is a novel type of sesquiterpene lactone with a peroxy linkage (ref. 1a). X-ray structure analysis (ref. 3) showed that in arteannuin all the five oxygen atoms crowded on the same side of the molecule, and starting from O5 an alternate carbon-oxygen chain of $O_5-C_{12}-O_6-C_{11}-O_7-C_8$ is formed. In this chain the carbon-oxygen bond distances, starting from $C_{12}-O_5$, are in a sequence of short, long, short, long, short..., but all lying well within the ranges of that of a normal single bond or of a partial double bond. Probably, the lone pair of electrons on oxygen is no longer confined only on the oxygen atom and a variation of bond type has occurred. This may tend to make the entire molecule more stable. The result was in agreement with the properties of arteannuin being stable towards both heat and light.
Probably, alternation in bond-length is also responsible for the chemotherapeutic activity of arteannuin molecule.

Hydrogenation of arteannuin 1 gave compound 2, which gave an α, β-unsaturated ketone 10 upon treatment with 10% alcoholic KOH solution (ref. 1a) (Scheme 1). This could be easily accounted for by a ring-opening of 2, followed by intramolecular aldol condensation. 10 on treating with 30% H2O2/KOH afforded the α-epoxide 11. On the other hand, treatment of 1 with K2CO3 in CH3OH gave an α-epoxide 12 (ref. 4). Further investigation of its reaction mechanism is in progress. Under the action of strong acid (H2SO4/HOAc), arteannuin 1 gave 14 through the intermediary of 13, which was formed by rupture of the 6-membered ring containing C4, C5, and C6 probably triggered by cleavage of the peroxidic linkage. The isopropyl group of arteannuin 1 on treatment with strong acid underwent isomerization to give α-cis lactone (ref. la and 5). The lactonic carbonyl of arteannuin 1 can be reduced by sodium borohydride to give the hemiacetal 15a. A number of derivatives have been prepared therefrom such as acetal acetate 15b which was shown to be more active than dihydroarteannuin 15a and arteannuin 1 (ref. 6).

The alternate C=O chain of arteannuin molecule can be visualized as a ketal-acetal-lactone system formed from the attack on the hydroperoxy group in the molecule 16 (Scheme 2), the enol methyl ether compound 17 might be used as a key intermediate for the total synthesis of 1. In order to achieve the transformation of 17 to 16, which could be cyclized to 1, the key intermediate 17 was hydroperoxydized on the C6-position by photooxidation. 17 was obtained either from 10R(+)-citronellal 18 or from 11R-methyldihydroarteannuic acid 25, which could be obtained from the arteannuic acid 9 (Scheme 3).

Kinetic deprotonation of 20 and reaction of the resulting enolate with 3-trimethylsilyl-3-buten-2-one provided 1,5-diketone 21 in 55% yield, which gave α, β-unsaturated ketone 22 on cyclization and dehydration in 62% overall yield in two steps. Reduction of 22 followed by oxidation gave 23 in 47% yield. Both 22 and 23 showed a positive CE in their CD, therefore the α-orientation of 1-H in 22, and of 6-H in 23 could be assigned, respectively. When 23 was reacted with methyl Grignard reagent followed by dehydration, the mixture of 24 and its Δ3-isomer was obtained in 1:1 ratio in 93% yield. The pure 24 was separated by repeated flash chromatography. In order to obtain the methyl dihydroarteannuic acid 25, 24 was first treated with Na-AH, then oxidized with Jones reagent and finally esterified with CH2N2 to give the desired product 25 in 77% yield in 3 steps. Compound 25 can also be obtained from the arteannuic acid 9 first through esterification with diazomethane, then controlled hydrogenation with NaBH4 in the presence of NiCl2. Ozonization of 25 afforded aldehyde-ketone 26. Selective protection of the ketonic carbonyl of 26 with 1,3-propanediol and the transformation of aldehydic carbonyl into the enol methyl ether gave 17. The overall yield of 17 from 25 was 33% in 4 steps. Irradiation of the methanolic solution of 17 in the presence of oxygen and a sensitizer (Rose Bengal) with a 200-W high pressure mercury lamp at −78°C followed by acid treatment gave arteannuin 1 (ref. 7 and 8) in 28% yield (2 steps).

Hydroxylation of 17 with osmium-tetroxide in ether at room temperature followed by treatment with hydrogen sulfide yielded deoxyarteannuin 2 in 45% yield (ref. 7). At about the same
time, Schmid and Hofheinz published the total synthesis of arteannuin B. They also used photoreaction to introduce the hydroperoxy group (—OOH) to the C6-position in the same key intermediate 17 obtained from the ring aldehydeic carbonyl compound which came from ring ketonic carbonyl (ref. 9).

We also planned to prepare this enol methyl ether compound 17 from 33 which could also be obtained from citronellal 18 by converting its ring ketonic carbonyl into the ring aldehydeic carbonyl as Schmid and Hofheinz (ref. 10). In order to establish the stereochemistry of 32, it was transformed into the degradation product 14 of arteannuin. 32, after treatment with alkali to remove the formyl group, was submitted to cyclization to afford the α,β-unsaturated ketone carboxylate 34 which was treated with dilute HCl to give a mixture of the norsesquiterpenoid lactone 14 and its stereoisomer 35. When NaOH was used, 14 was the major product. On the other hand, when Ba(OH)2 was used, 35 became the major product (ref. 5).

14 and 35 exhibit different Cotton effect in their CD. Since 14 showed a positive Cotton effect and 35 showed a negative one, thus a cis A/B ring fusion and a trans A/B ring fusion might be assigned, respectively (Fig. 2). The prediction from the octant rule is in consistency with the experimental results. Because an axial substituent is present in β-position of the keto group, in consideration of anti-octant rule occurring in ORD (ref. 11), the NOE measurement was used to confirm these assignments. Irradiation of C9–H and C15–H of compound 35 produced 16.4% and 13.9% enhancement of the signal of the equatorial hydrogen at C5, respectively. Therefore the configuration of A,B ring was further proved and the β-cis-lactone ring was thus assigned for 35, as shown in Fig. 3.
However, irradiation of the C7—H of compound 14 had no effect on the intensity of the C5 equatorial hydrogen (Fig. 3). Thus the configuration of lactone ring of 14 might be in trans fusion. But this postulation from the NOE experiments was shown to be incorrect, since a lot of related compounds were synthesized from 14, revealing the cis configuration of this lactone ring. Thus, the isodeoxyarteannuin 46 (Scheme 5) (ref. 12), and isoarteannuin B 53 (Scheme 7) (ref. 13) from this norsesquiterpenoid lactone 14 as starting material were synthesized by the sequence of reactions shown below.

Scheme 5
Because lactone 39 could not be transformed into its hydroxy acid, 39 was first reduced with LiAlH₄ and then selectively acetylated with Ac₂O-Py to the monoacetylated compound 40 in 77% yield which on ozonolysis furnished epoxy compound 41 in 90% yield. Its formation might be considered as a result of an intramolecular ketal and acetal formation. 41 was oxidized with Jones reagent to give C₅-carbonyl compound 42 in 86% yield. In order to obtain methyl ester 44 (R=CH₃), 42 was first hydrolyzed with K₂CO₃ in CH₃OH then oxidized with Jones reagent and finally esterified with diazomethane to give the desired product 44 (R=H). Since the circular dichroism of 44 (R=CH₃) coincides with that of 50 obtained from arteannuin 1, the epoxy group of 44 is also in a-orientation.

Scheme 6

Reduction of epoxy lactone 44 with DIBAL afforded a 91% yield of the hydroxy compound 45. The configuration of the hydroxy group was assigned as a by using NOE measurement. Irradiation of the C₅-H of 45 and 49 produced 11.3% and 13% enhancement of the signal of C₅-H, respectively. Hence the configuration of C₅-H could be assigned as a and C₅-CH₃ as a', which is the same with that of 49 obtained from arteannuin 1. On irradiation of the C₅-H of both 45 and 49, only 45 produced 9.7% enhancement of C₅-H, so the configuration of the isopropyl carboxyl group could be assigned as a. 45 after treatment with DCC-Py in the presence of p-TsOH at room temperature was submitted to lactonization to give the target compound 46 in 61% yield.

The cis lactone ring of compound 46 was further proved by the NOE experiment. Irradiation of the C₅-H of the isodeoxyarteannuin 46 led to 11.4% enhancement of C₅-H, while on irradiation of the C₅-H of the natural deoxyarteannuin 2 no enhancement was found. Irradiation of the C₇-H of both 46 and 2 produced 26.9% and 9.6% enhancement of C₅-H, respectively. On the basis of coupling constant of C₅-H and C₇-H of 46 and 2 obtained from decoupling technique, the configuration of the methyl group at C₁₁ was assigned as a'. An alternative approach to 46 was achieved from 41 through the following sequence of reactions p–q–r. But the yield involving three steps of p–q–r was very low (17%), especially, in the step of oxidation with Ag₂O. Reaction of 41 with CH₃OH·BF₃·Et₂O afforded a cyclic ether instead of C₅-methyl ether. Oxidation of this cyclic ether to lactone with Jones reagent or t-butyl chromate failed.

39 on exposure to lithium diisopropylamide (LDA) with subsequent treatment of the enolate with diphenyl diselenide gave the phenylselenide 51 in 32% yield (Scheme 7), which was oxidized with H₂O₂ to yield C₅-methylene Y-lactone 52 in 61% yield. 52 was oxidized with m-chloroperbenzoic acid to give a mixture of two stereoisomers in 1:1 ratio in 75% total yield, which was then separated into isoaarteannuin B 53 and its stereoisomer 54 by TLC. The epoxy configuration were postulated based on their 1H-nmr spectra in combination with the result of diaxial opening of epoxide on treatment with formic acid. The configuration of C₅-methylene Y-lactone was determined by circular dichroism (ref. 14). Since 53 and 54 showed a positive Cotton effect, a cis-lactone ring fusion might be assigned. The a-cis-lactone ring configuration of 54 was further proved by X-ray diffraction (ref. 16).
The best solution to this problem was the X-ray single crystal diffraction (ref. 15). The result finally confirmed the presence of both a cis A,B ring fusion and a cis lactone ring of 14. The absolute configuration of cis-lactone ring was determined by ORD. Treatment of 14 and 35 with triethylxonium fluoroborate afforded the stereoisomeric α, β-unsaturated keto ester 36 and 37, respectively. Compounds 36 and 37 all exhibited positive Cotton effect but with different amplitude (Fig. 5). Therefore the α-orientation of C1-H could be postulated. Because C1-H of 14 and 35 is in α-configuration and A,B ring of 14 and that of 35 are in cis and trans fusions, respectively, therefore the absolute configuration of cis-lactone ring of 14 must be 6 α and 7 α and that in 35, 6 β and 7 β.

In conclusion, the isopropyl group of arteannuin 1 on treatment with strong acid was indeed isomerized to form the α-cis lactone ring. Although it is the most unusual reaction, probably it tends to form the most stable α-cis-lactone ring. The further evidence is that the lactone compound 39 obtained from 14 could not be transformed into its hydroxy acid (Scheme 5).

Arteannuin B 3 occurs together with arteannuin 1 in the plant of Artemisia annua L. Its structure has been determined by Stefanovic et al (ref. 2) and its several stereoisomers have been synthesized by Dreiding (ref. 17). We have synthesized this natural sesquiterpene lactone by photoreaction of arteannuinic acid 9 which also exists in the same plant. A solution of arteannuinic acid 9 and hematoporphyrin in pyridine-water was irradiated with a 200-W high pressure mercury lamp to give the arteannuin B 3 and the isodeoxyarteannuin B 60 (ref. 18). The cis-lactone ring fusion for 60 was established on the basis of allylic coupling constant of C7-H and C13-H in 1H-NMR spectrum and the Cotton effect in circular dichroism in comparison with arteannuin B. 60 on reaction with m-chloroperbenzoic acid gave isoarteannuin B 61. The α-epoxy configuration was proved by diaxial opening of epoxide on treatment with acetic acid. 60 upon reduction with NaBH4 in presence of a small amount of NiCl2 gave the S2' reaction product 64. 50 upon reduction with NaBH4 without NiCl2 only gave 1,4-addition product 63, which was reduced with NaBH4-NiCl2 or H2-Pd/CaCO3 to give the S2' reaction product 64 (Scheme 8).
Dihydroarteannuinic acid 65 obtained from arteannuinic acid 9 on irradiation in the same way as 9 gave the \( \alpha \)-hydroperoxide 66. Ozonolysis of 66 in dichloromethane-pyridine afforded the acetal 67. Activation of the carboxyl group in 67 with TsCl-Et\textsubscript{3}N resulted in the cyclization of the lactone ring to afford the final product, arteannuin analog 68 (ref. 19). Antimalarial tests showed that 68 possessed the same activity as arteannuin. (Scheme 8).

Arteannuines E, F, 5 and A 7 also exist together with arteannuin 1 in the same plant. 6 and 5 have been prepared from \( \alpha \)-oxide 69 and \( \beta \)-oxide 70 obtained from arteannuinic acid 9 by epoxidation with MCPBA, respectively (ref. 20). When 69 reacted with HCOOH, the mixture of 71 and its 4 \( \alpha \)-CH\textsubscript{3} isomer was obtained in 2:1 ratio. It is clear that the trans fusion lactone 5 is not a trans-diaxial product, while cis-fusion lactone 6 is. The steric hindrance of 5-axial OH group, probably rendered the lactonization of 73 rather difficult.
Arteannuin A has also been prepared from α-oxide. Ozonolysis of α-oxide followed by oxidative cleavage with alkaline hydrogen peroxide furnished the γ-lactone in 81% overall yield in 2 steps. Dehydration of the γ-lactone with POCl₃-Py gave arteannuin A in 43% yield (ref. 21).

**Scheme 10**

**REFERENCES**


   c. Zhou Wei-shan, Zhang Lian, Xu Xing-xiang, Fan Zhao-chang, Submitted for publication.


   i. Zhou Wei-shan, Zhang Lian, Xu Xing-xiang, Submitted for publication.