Total synthesis of arteannuin (Quinghaosu) and related compounds

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Abstract – A new sesquiterpene peroxide was isolated from the Chinese her-
bal medicine Qinghao (Artemisia annua L.). Its structure and absolute
stereochemistry have been firmly established by combined spectral, chemi-
cal and X-ray crystallographic methods. Based on pharmacological and chemi-
cal studies it was shown to be the antimalarial principle and named artean-
nuin (qinghaosu). Citronellal was used as a starting material for the
total synthesis of arteannuin. The peroxide group of the arteannuin mole-
cule 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 in-
digenously 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 iso-
lated in 1972. Based on pharmacological and chemical studies, it was found to be the anti-
malarial 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 disappearence of parasites from patient's blood.

From indigenous Artemisia annua L. nine sesquiterpenes (Fig. 1 1-9) have been isolated (ref.
1a-e), of which all but arteannuin B (3) (ref. 2) are new compounds. From biogenetic view-
point, 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
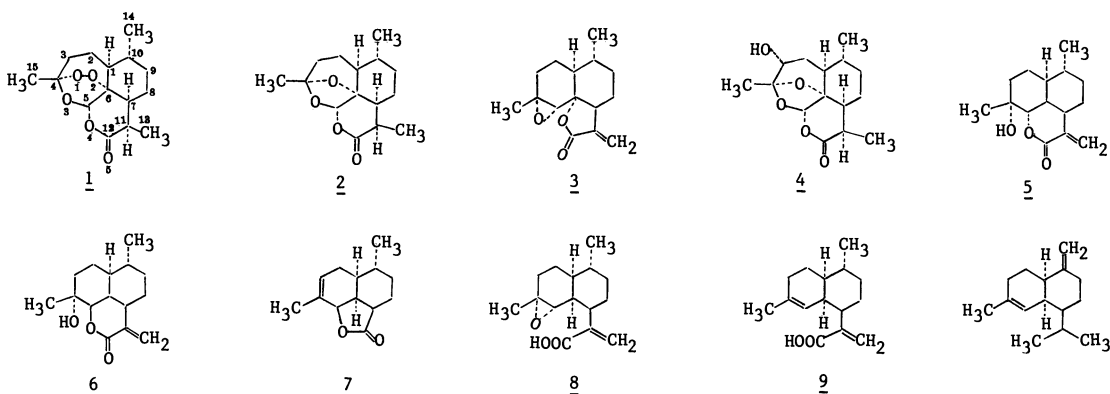juncture.

Fig. 1

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 O_5 an alternate carbon-oxygen chain of $O_5-C_{12}-O_4-C_5-O_3-C_4-O_1-O_2-C_6$ is formed. In this chain the carbon-oxygen bond distances, starting from $C_{12}-O_4$ 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.

Scheme 1   *Scheme 2*
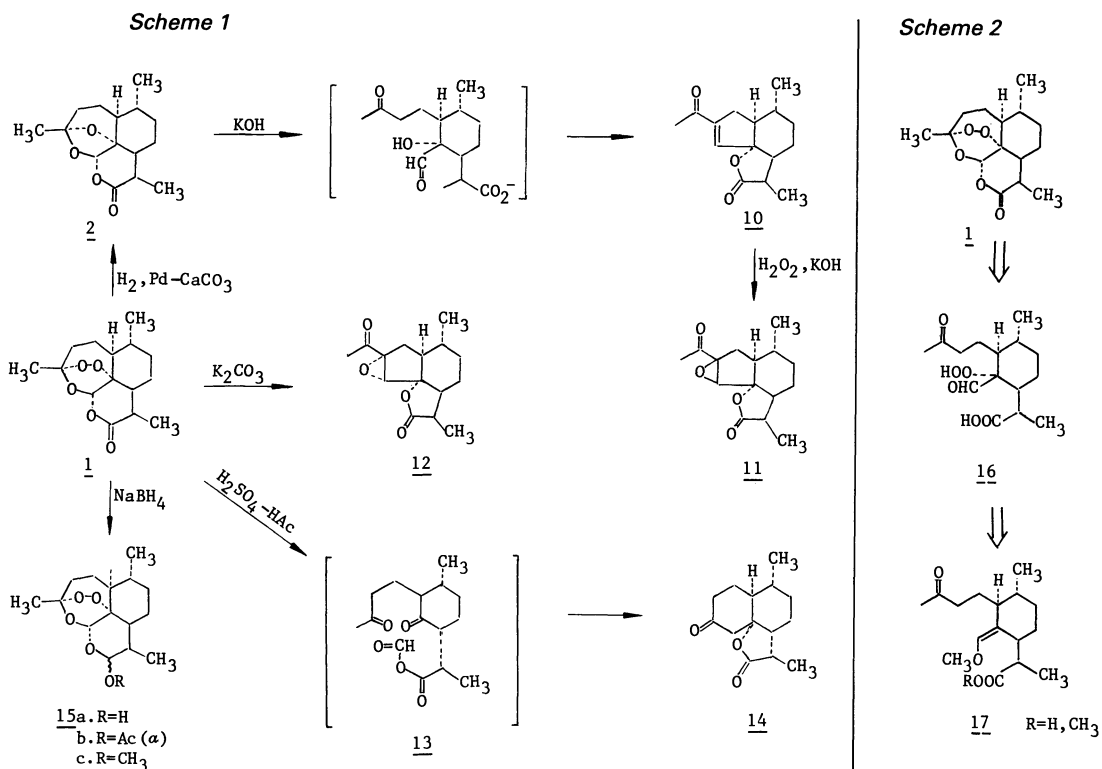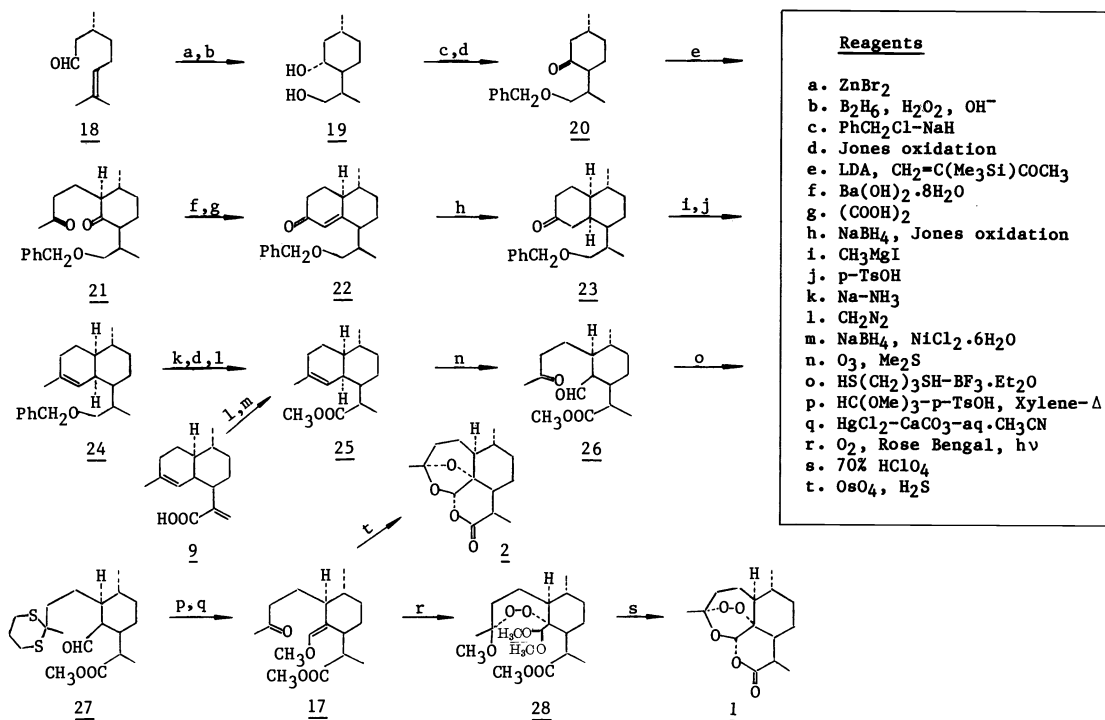

Probably, alternation in bond-length is also responsible for the chemotherapeutic activity of arteannuin molecule.

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 account-ed for a ring-opening of 2, followed by intramolecular aldol condensation. 10 on treating with 30% H_2O_2/KOH afforded the β-epoxide 11. On the other hand, treatment of 1 with K_2CO_3 in CH_3OH gave α-epoxide 12 (ref. 4). Further investigation of its reaction mechanism is in progress. Under the action of strong acid (H_2SO_4/HOAc), arteannuin 1 gave 14 through the intermediary of 13, which was formed by rupture of the 6-membered ring containing C_4, C_5, and C_6 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. 1a 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 ace-tate 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 C_6-position by photooxidation. 17 was obtain-ed either from 10R(+)-citronellal 18 or from 11-(R)-methyldihydroarteannuate 25, which could be obtained from the arteannuinic 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 Δ^3-isomer was obtained in 1:1 ratio in 93% yield. The pure 24 was separated by repeated flash chromotography. In order to obtain the methyl dihydroarteannuate 25, 24 was first treated with Na-NH$_3$ then oxidized with Jones reagent and finally esterified with CH_2N_2 to give the desired product 25 in 77% yield in 3 steps. Compound 25 can also be obtained from the arteannuinic acid 9 first through esterification with diazomethane, then controlled hydrogenation with NaBH$_4$ in the presence of NiCl$_2$. Ozonization of 25 afforded aldehyde-ketone 26. Selective protection of the ketonic carbonyl of 26 with 1,3-propanedithiol 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 17 in the methanolic solution of 17 in the pre-sence 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

Scheme 3

Reagents

a. ZnBr$_2$
b. B$_2$H$_6$, H$_2$O$_2$, OH$^-$
c. PhCH$_2$Cl-NaH
d. Jones oxidation
e. LDA, CH$_2$=C(Me$_3$Si)COCH$_3$
f. Ba(OH)$_2$·8H$_2$O
g. (COOH)$_2$
h. NaBH$_4$, Jones oxidation
i. CH$_3$MgI
j. p-TsOH
k. Na-NH$_3$
l. CH$_2$N$_2$
m. NaBH$_4$, NiCl$_2$·6H$_2$O
n. O$_3$, Me$_2$S
o. HS(CH$_2$)$_3$SH-BF$_3$·Et$_2$O
p. HC(OMe)$_3$-p-TsOH, Xylene-Δ
q. HgCl$_2$-CaCO$_3$-aq.CH$_3$CN
r. O$_2$, Rose Bengal, hν
s. 70% HClO$_4$
t. OsO$_4$, H$_2$S

time, Schmid and Hofheinz published the total synthesis of arteannuin. They also used photo-reation to introduce the hydroperoxy group (-OOH) to the C$_6$-position in the same key inter-mediate 17 obtained from the ring aldehydic 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 aldehydic 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-unsa-turated 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).
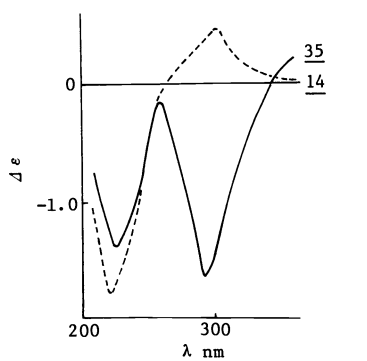
Fig.2 CD spectra of 14 and 35
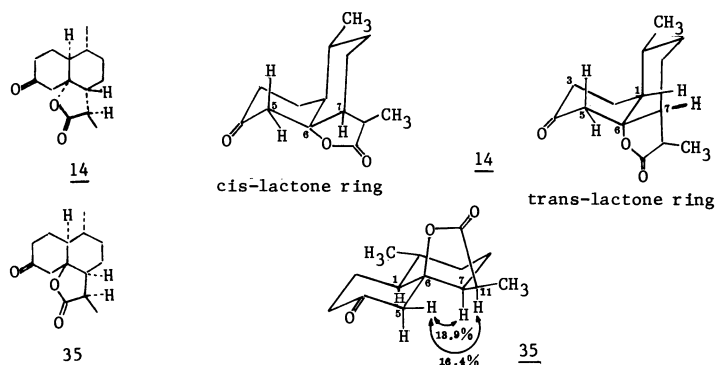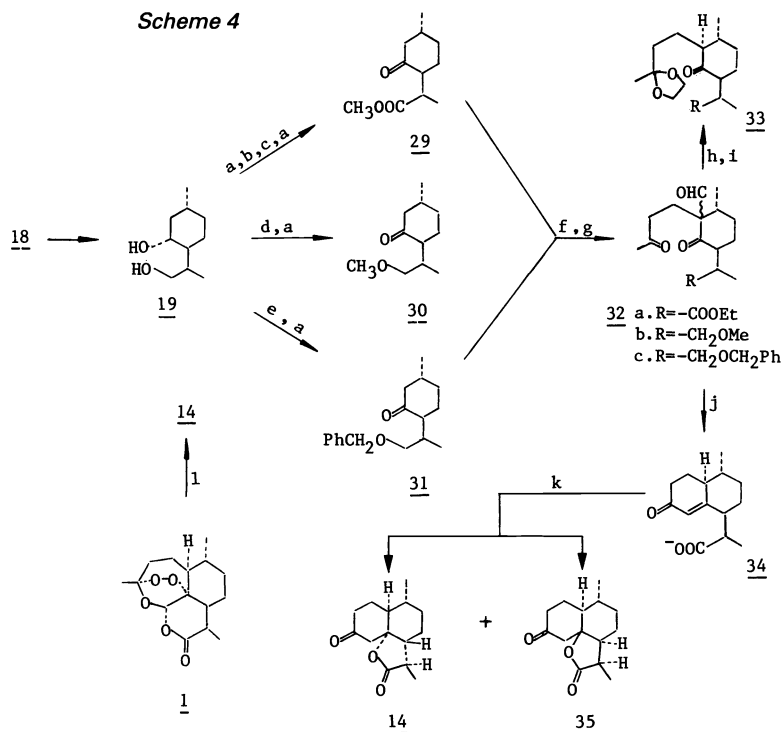
cis-lactone ring

trans-lactone ring

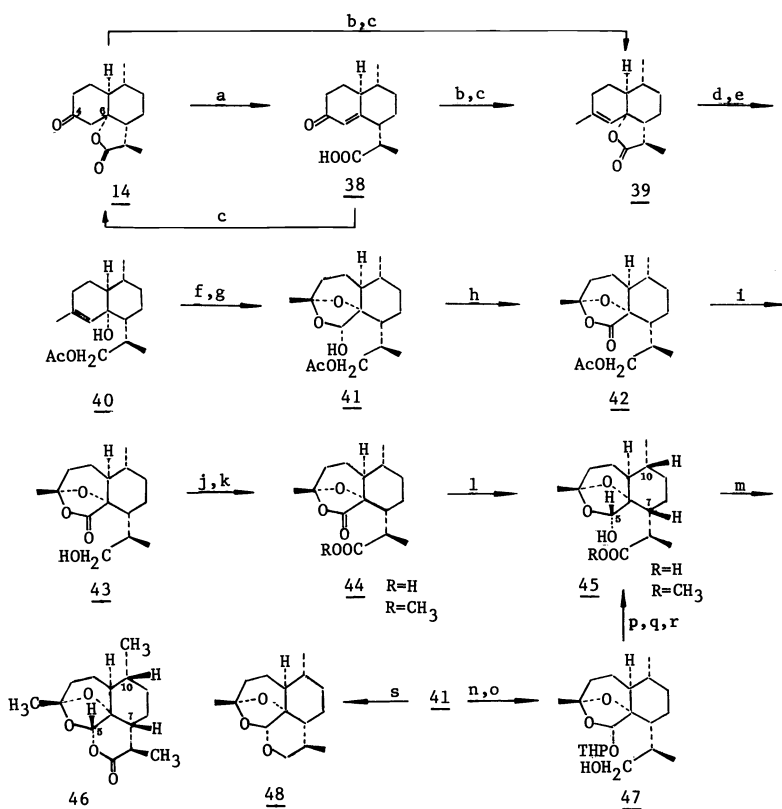**Fig.3** Conformations of 14 and NOE of 35

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 consist-ency 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 C$_{11}$-H and C$_7$-H of compound 35 produced 16.4% and 13.9% enhancement of the signal of the equatorial hydrogen at C$_5$, res-pectively. 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.

Scheme 4

Reagents

a. CrO_3
b. K_2CO_3
c. CH_2N_2
d. CH_3I, NaH
e. BzCl, NaH
f. HCO_2Et, NaH
g. ⟨O⟩, Et_3N
h. $Ba(OH)_2 \cdot 8H_2O$
i. ⟨OH, OH⟩, p-TsOH
j. $NaOH-C_2H_5OH-H_2O$ or $Ba(OH)_2-C_2H_5OH-H_2O$
k. H_3O^+
l. $HAc-H_2SO_4$ (10:1)

32 a. R=-COOEt
    b. R=-CH$_2$OMe
    c. R=-CH$_2$OCH$_2$Ph

However, irradiation of the C_7-H of compound 14 had no effect on the intensity of the C_5 equatorial hydrogen (Fig. 3).[7] 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

Reagents

a. KOH, H_2O
b. CH_3MgI, Et_2O
c. H_2SO_4
d. $LiAlH_4$, THF
e. Ac_2O, Py
f. O_3, CH_2Cl_2
g. Zn-HAc
h. Jones oxidation
i. K_2CO_3, CH_3OH
j. Jones oxidation
k. CH_2N_2
l. DIBAL
m. DCC, Py, p-TsOH
n. ⟨O⟩, THF, $POCl_3$
o. K_2CO_3, CH_3OH
p. PCC, CH_2Cl_2
q. Ag_2O
r. H_3O^+
s. CH_3OH, $BF_3 \cdot Et_2O$

Because lactone 39 could not be transformed into its hydroxy acid, 39 was first reduced with LiAlH$_4$ and then selectively acetylated with Ac$_2$O-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 C$_5$-carbonyl compound 42 in 86% yield. In order to obtain methyl ester 44 (R=CH$_3$), 42 was first hydrolyzed with K$_2$CO$_3$ in CH$_3$OH 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=CH$_3$) coincides with that of 50 obtained from arteannuin 1 (Scheme 6), the epoxy group of 44 is also in α -orientation.

Scheme 6
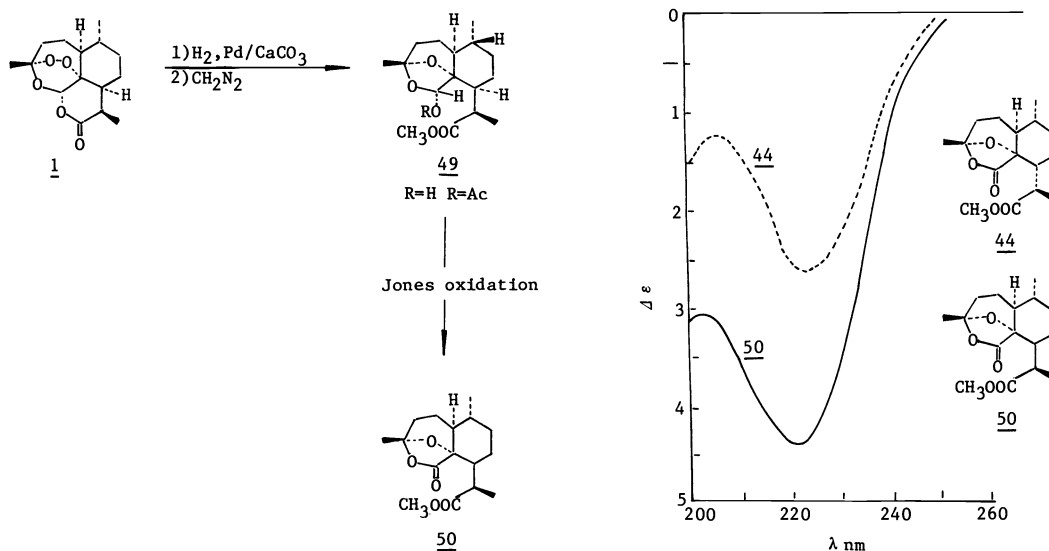
Fig.4   CD spectra of compounds 44 and 50

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 α by using NOE measurement. Irradiation of the C$_{10}$-H of 45 and 49 produced 11.3% and 13% enhancement of the signal of C$_5$-H , respectively, hence the configuration of C$_5$-H could be assigned as β and C$_5$-OH as α which is the same with that of 49 obtained from arteannuin 1. On irradiation of the C$_7$-H of both 45 and 49, only 45 produced 9.7% enhancement of C$_5$-H, so the configuration of the isopropyl carboxyl group could be assigned as α . 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 C$_7$-H of the isodeoxyarteannuin 46 led to 11.4% enhancement of C$_5$-H, while on irradiation of the C$_7$-H of the natural deoxyarteaninuin 2 no enhancement was found. Irradiation of the C$_{10}$-H of both 46 and 2 produced 26.9% and 9.6% enhancement of C$_5$-H, respectively. On the basis of coupling constant of C$_7$-H and C$_{11}$-H of 46 and 2 obtained from decoupling technique, the configuration of the methyl group at C$_{11}$ was assigned as β . An alternative approach to 46 was achieved from 41 through the following sequence of reactions n→ o→ p→ q→ r. But the yield involving three steps of p→ q→ r was very low (17%), especially, in the step of oxidation with Ag$_2$O. Reaction of 41 with CH$_3$OH-BF$_3$·Et$_2$O afforded a cyclic ether instead of C$_5$-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 diphenyldiselenide gave the phenylselenide 51 in 32% yield (Scheme 7), which was oxidized with H$_2$O$_2$ to yield α -methylene γ-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 isoarteannuin B 53 and its stereoisomer 54 by TLC. The epoxy configuration were postulated based on their ^{1}H-nmr spectra in combination with the result of diaxial opening of epoxide on treatment with formic acid. The configuration of α -methylene-γ -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 α -cis-lactone ring configuration of 54 was further proved by X-ray diffraction (ref. 16).
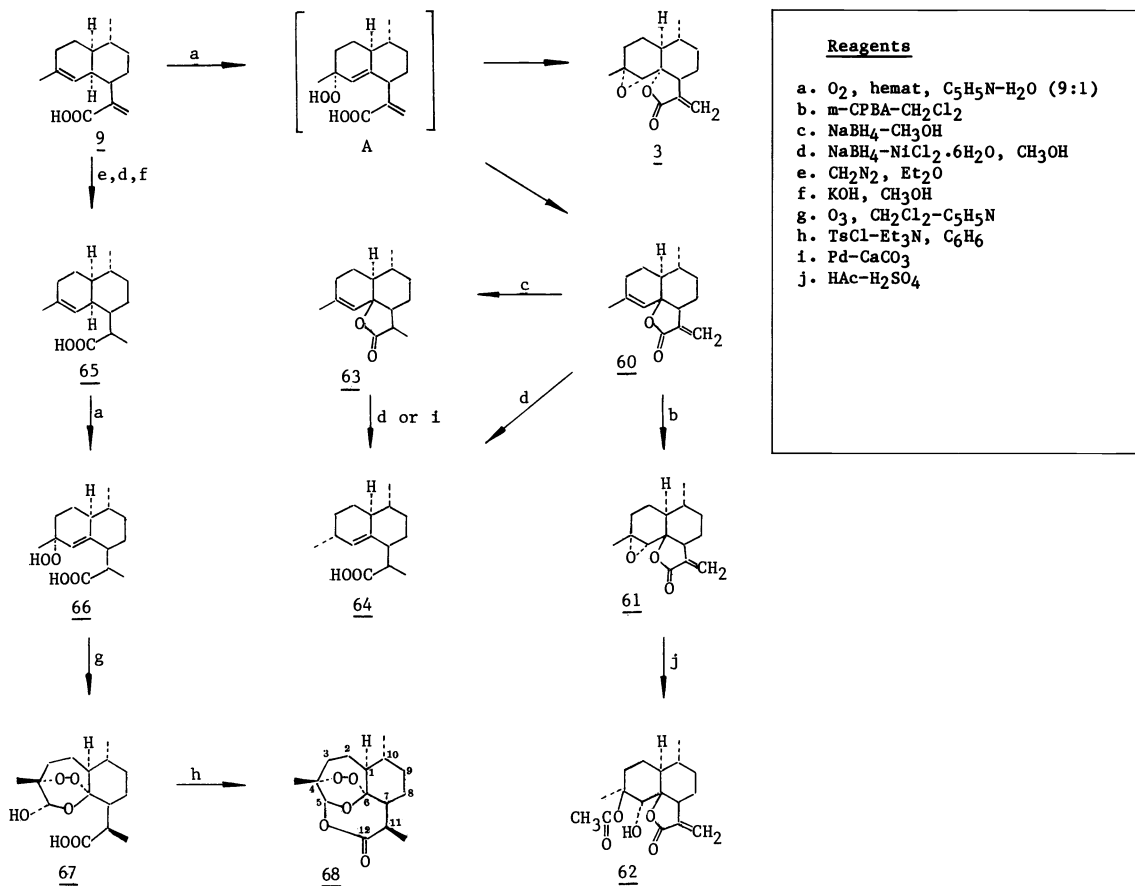
Scheme 7
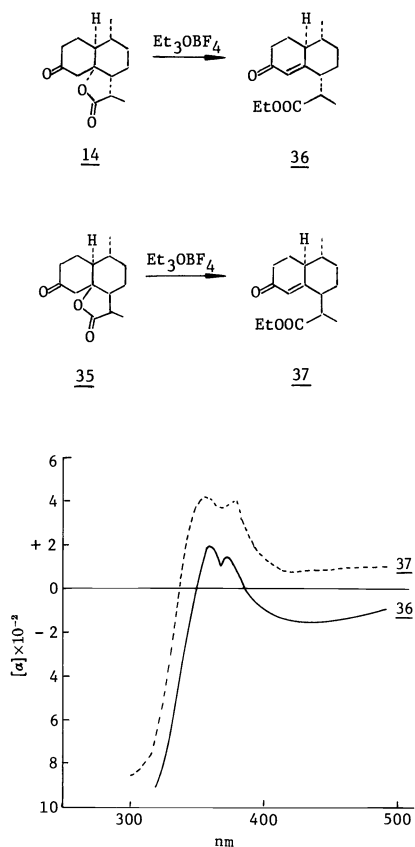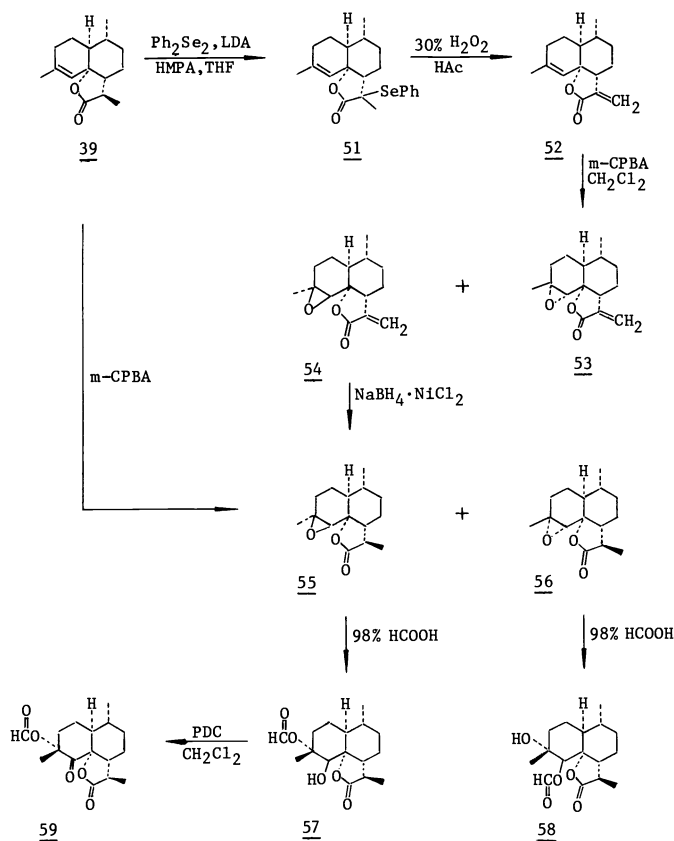
Fig.5 ORD spectrum of <u>36</u> and <u>37</u>

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 <u>14</u>. The absolute configuration of cis-lactone ring was determined by ORD. Treatment of <u>14</u> and <u>35</u> with triethyloxonium fluoroborate afforded the stereoisomeric α, β -unsaturated keto ester <u>36</u> and <u>37</u>,respectively. Compounds <u>36</u> and <u>37</u> all exhibited positive Cotton effect but with different amplitude (Fig. 5). Therefore the α -orientation of C_1-H could be postulated. Because C_1-H of <u>14</u> and <u>35</u> is in α -configuration and A,B ring of <u>14</u> and that of <u>35</u> are in cis and trans fusion, respectively,therefore the absolute configuration of cis lactone ring of <u>14</u> must be 6 α and 7 α and that in <u>35</u>, 6 β and 7 β .

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

Arteannuin B <u>3</u> occurs together with arteannuin <u>1</u> in the plant of <u>Artemisia annua L.</u> 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 <u>9</u> which also exists in the same plant. A solution of arteannuinic acid <u>9</u> and hematoporphyrin in pyridine-water was irradiated with a 200-W high pressure mercury lamp to give the arteannuin B <u>3</u> and the isodeoxyarteannuin B <u>60</u> (ref. 18). The cis-lactone ring fusion for <u>60</u> was established on the basis of allylic coupling constant of C_7-H and C_{13}-H in ^{1}H-NMR spectrum and the Cotton effect in circular dichroism in comparison with arteannuin B. <u>60</u> on reaction with m-chloroperbenzoic acid gave isoarteannuin B <u>61</u>. The α -epoxy configuration was proved by diaxial opening of epoxide on treatment with acetic acid. <u>60</u> upon reduction with $NaBH_4$ in presence of a small amount of $NiCl_2$ gave the S_N2' reaction product <u>64</u>. <u>60</u> upon reduction with $NaBH_4$ without $NiCl_2$ only gave 1,4-addition product <u>63</u>, which was reduced with $NaBH_4$-$NiCl_2$ or H_2-Pd/$CaCO_3$ to give the S_N2' reaction product <u>64</u> (Scheme 8).

Scheme 8

Reagents

a. O_2, hemat, $C_5H_5N-H_2O$ (9:1)
b. m-CPBA-CH_2Cl_2
c. NaBH$_4$-CH$_3$OH
d. NaBH$_4$-NiCl$_2$·6H$_2$O, CH$_3$OH
e. CH$_2$N$_2$, Et$_2$O
f. KOH, CH$_3$OH
g. O$_3$, CH$_2$Cl$_2$-C$_5$H$_5$N
h. TsCl-Et$_3$N, C$_6$H$_6$
i. Pd-CaCO$_3$
j. HAc-H$_2$SO$_4$

Dihydroateannuinic acid **65** obtained from arteannuinic acid **9** on irradiation in the same way as **9** gave the α -hydroperoxide **66**. Ozonolysis of **66** in dichloromethane-pyridine afforded the acetal **67**. Activation of the carboxyl group in **67** with TsCl-Et$_3$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).

Arteannuins E **6**, F **5** and A **7** also exist together with arteannuin **1** in the same plant. **6** and **5** have been prepared from α -oxide **69** and β -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 α-CH$_3$ 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.

Scheme 9
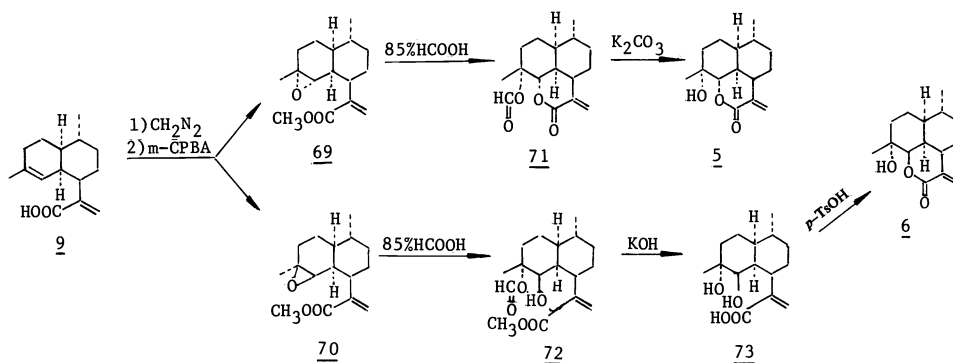
Arteannuin A $\underline{7}$ has also been prepared from α-oxide $\underline{69}$. Ozonolysis of $\underline{69}$ followed by oxidative cleavage with alkaline hydrogen peroxide furnished the γ-lactone $\underline{75}$ in 81% overall yield in 2 steps. Dehydration of $\underline{75}$ with $POCl_3$-Py gave arteannuin A $\underline{7}$ in 43% yield (ref. 21).

Scheme 10
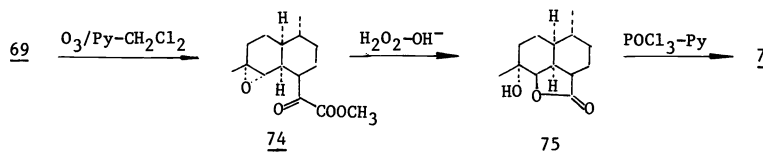
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