From Qinghao, Marvelous Herb of Antiquity, to the Antimalarial Trioxane Qinghaosu—and Some Remarkable New Chemistry

RICHARD K. HAYNES*,† AND
SIMONE C. VONWILLER*,‡

Department of Chemistry, Hong Kong University of Science and Technology, Kowloon, Hong Kong, and School of Chemistry, University of Sydney, New South Wales 2006, Australia

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The famous Qing Dynasty novel *Honglou Meng* or *The Dream of Red Mansions* by Xao Xueqin provides a wonderful insight into the extraordinarily sophisticated political and social life led by upper class Chinese in the late 16th and early 17th centuries. In one memorable sequence, as recorded in Chapter 51 of David Hawkes's distinguished translation of the novel,[1] a physician is called in to diagnose the malady of a maid-servant of Master Bao-Yu, who because of the cold weather, is ensconced in Master Bao-Yu's bedroom. The physician, young and inexperienced, conducts the consultation with the patient concealed behind the bed curtain, and prescribes a decoction containing herbal constituents—perilla, kikio root, wind-shield, nepeta seed, thorny lime, ephedra, and others. However, Master Bao-Yu is not happy with the prescription, and in contrast to a prevailing acceptance of diagnosis and prescription today, is able to sum up sufficient courage to query the wisdom of the young physician in prescribing such harsh decongestants as thorny lime (*Citrus* spp.) and ephedra (*Ephedra* spp.) to a young lady. He calls for a re-examination by a physician of more established repute. The latter, of considerably greater age than the first, actually presents quite a similar prescription, although the thorny lime and ephedra are now replaced by the more gentle angelica (probably *Angelica dahurica*), bitter peel (*Citrus* spp.), and white peony root (probably *Paeonia lactiflora*). Bao-Yu thereupon orders the decoction to be prepared immediately within his household, "for the scent of boiling herbs is the finest in the world, far superior to the perfume of any flower...".

The knowledge which led Bao-Yu to question the prescription of the first physician is indicative of the sophistication of Chinese medicine at the time, and contrasts markedly with contemporaneous European practice. Knowledge was accessible to the wealthy household through detailed, carefully, and elegantly scripted pharmacopoeia. One herb which also was featured prominently in these pharmacopoeia, especially in relation to decoctions used to treat fever, was *qinghao*, the "blue-green" herb (*Artemisia annua*). Recorded use of qinghao spans over 2000 years, with written descriptions first appearing in 168 B.C. in the Mawangdui Han Dynasty *Wu Shi Er Bing Fang Lun* (*Treatments for 52 Sicknesses*), and as late as 1798 in the *Wen Bing Tiao Bian* (*Book of Fevers*). The most detailed description appears in the mammoth *Ben Cao Gang Mu* (*Compendium of Materia Medica*) compiled in 1596 by the great Ming Dynasty physician Li Shi-Zen, and which is still printed in China today.[2] With this background of use, qinghao was a prominent target for investigation in a Chinese program, involving Chinese chemists, pharmacologists, and botanists, designed to isolate and identify possible new antimalarial drugs.[3] In 1972, after activity-guided bioassay involving ether extracts, there was isolated a remarkable new compound which the Chinese called *qinghaosu* (compound **1**), the "active principle of qinghao". The compound was demonstrated to have substantial antimalarial activity. Chinese chemists then embarked on a major program which entailed both derivatization of qinghaosu to provide compounds with better formulation characteristics and clinical trials on qinghaosu and selected derivatives.[3] Within this program, the Chinese prepared the oil-soluble artemether (**2**) and arteether (**3**) and the water-soluble artesunate (**4**). The program was noteworthy for its success in demonstrating to the world the advent of a new antimalarial drug and its derivatives, which structurally are entirely unrelated to the classical antimalarials based on quinine and synthetic analogues. Qinghaosu, artemether, and artesunate are now used for treatment of severe malaria, and with sanction and support from the World Health Organization, Geneva, it may be said that

Richard K. Haynes, after obtaining a Ph.D. degree from the University of Western Australia in 1970, carried out postdoctoral research as a Gillette International Fellow for two years in Professor Musso's group at the University of Karlsruhe, Germany, and then worked as a Visiting Lecturer in Professor Sir Derek Barton's group at Imperial College, London, until 1975. He thereafter took academic positions in Australia, first at Monash University, Melbourne, and then at the University of Sydney until the end of 1992. In 1988 and 1990, Dr. Haynes participated in the Australian Academy of Science-Chinese Academy of Science exchange program which enabled him to visit institutes in China associated with qinghaosu research. Since 1993, Dr. Haynes has been a Reader in Chemistry at the Hong Kong University of Science and Technology. Research interests are in methods-based synthetic organic chemistry, biological oxygenation processes, and drug design and mechanism of action.

Simone C. Vonwiller graduated with First Class Honours in 1983 and obtained a Ph.D. degree from the University of Sydney in 1988. She worked for one year as a DAAD Ferdinand von Müller Fellow in Professor Winterfeldt's group at the University of Hannover, Germany, before returning to Sydney as an Australian Research Council Research Fellow. In 1992, Dr. Vonwiller was awarded the Queen Elizabeth II Fellowship at the University of Sydney by the Australian Research Council. Her main chemical interests are in the chemistry of organic peroxides and organic synthesis. She also has a strong extracurricular interest in the fine arts.

† Hong Kong University of Science and Technology.
‡ University of Sydney.

(1) Xueqin, Xao *Honglou Meng*, translated as *The Story of the Stone* by David Hawkes: Penguin Classics: Harmondsworth, London, 1977; Vol. 2 (The Crab-Flower Club), Chapter 51.
(2) A description of uses of qinghao appears on pp 944−946 of the 1991 edition of *Ben Cao Gang Mu*; People's Health Publishing, Lanzhou Printing Press: Lanzhou, China, 1991.
(3) For reviews with references to development and clinical use, see: Qinghaosu Antimalaria Coordinating Research Group. Antimalaria Studies on Qinghaosu. *Chin. Med. J.* **1979**, *92*, 811. Klayman, D. L. *Science* **1985**, *228*, 1049. Luo, X.-D.; Shen, C.-C. *Med. Res. Rev.* **1987**, *7*, 29. Trigg, P. I. *Econ. Med. Plant Res.* **1989**, *3*, 19. Woerdenbag, H. J.; Lugt, C. B.; Pras, N. *Pharm. Weekbl., Sci. Ed.* **1990**, *12*, 169.

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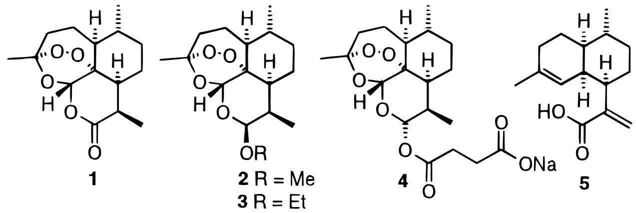
these drugs have truly "come of age".[4,5] Qinghao has thus yielded a compound whose usage together with its derivatives indeed promises to become as prominent as that of quinine,[5] a drug whose development from the cinchona tree of Peru also has a fascinating history.[6]



1 **2** R = Me **3** R = Et **4** **5**

With the unique juxtaposition of peracetal, acetal, and lactone substructures within which the trioxane nucleus confers the potent antimalarial activity, qinghaosu has much to interest the organic chemist. Relatively efficient totally synthetic routes have been developed both the parent compound itself[7] and to derivatives which bear structural modifications about the periphery of the molecule.[7−9] Such derivatives display enhanced activity against the malaria parasite. Because of the need both to prepare optimum, specialized derivatives and to map structure−activity relationships, current activity in the area is intense. However, because of the structural complexity of the compound, it is most unlikely that any totally synthetic approach to the parent compound will supplant the natural source of the compound. Extraction of qinghaosu by means of hexane from the dried leaves of *A. annua*, within which concentrations to 0.5% have been recorded, is relatively facile.[10] However, with increasing world demand, coupled with the need to prepare the specialized, optimum derivatives, semisynthetic routes from readily available, structurally-related natural products will assume greater importance in the future. One such natural product is qinghao (artemisinic) acid (**5**),[10] the biogenetic precursor of qinghaosu in *A. annua*, and whose concentrations therein, at least in samples grown outside China, are 2−8-fold greater than those of qinghaosu.[11] Samples of *A. annua* assayed at the Tasmanian Department of Primary Industry's Agricultural Research Station uniformly returned high qinghao acid assays relative to those of qinghaosu.[12] The one as yet uncontrolled variable is the age of the leaf sample used in the assay; it may well be that concentrations of qinghao acid decrease with aging of the samples, probably via an autoxidative pathway.[13]

Our early interests in the area of oxygenation chemistry, and the chemistry of peroxides,[14] led us in 1988 to consider possible biomimetic pathways from qinghao acid in *A. annua*.[15] Formal requirements for the conversion are reduction of the α,β-unsaturation in qinghao acid to provide dihydroqinghao acid (**6**), oxidative cleavage of the 3−4 double bond to the ketoaldehyde intermediate **10**, and autoxidation of the latter to the α-hydroperoxy aldehyde **11**.[16] With all oxygen atoms now in place, closure of the latter to generate qinghaosu may then follow (Scheme 1). However, in a biosynthetic sense, the oxidative cleavage of the internal alkene presents the major problem in the sequence. While a number of routes might be envisaged, that proceeding via the allylically transposed hydroperoxide **7** (Scheme 1) attracts on the basis that formation of the hydroperoxide has ample analogy in lipoxygenase-mediated autoxidation processes involving unsaturated acids. The hydroperoxide **7** may undergo Hock cleavage[17,18] via C to O migration[18] to form hemiacetal **9** which collapses to the ketoaldehyde **10** (Scheme 1).

In order to probe the cleavage process, we examined the behavior of allylic hydroperoxides in the presence of outer sphere oxidants and Lewis and protic acids. For pinene hydroperoxide (**12**), treatment with catalytic $FeCl_3{\cdot}Et_2O$ in CH_2Cl_2, $Fe(phen)_3(PF_6)_3$, or $Cu(OTf)_2$ in CH_2Cl_2−MeCN gave the ketoaldehyde **15**, corresponding to the classical "Hock" product.[19] Triflic acid in CH_2Cl_2 also gave the same product.[20] In view of the oxidizing nature

(4) For usage in treatment of severe malaria, see *inter alia*: Li, G-Q.; Guo, X.-B.; Jin, R,; Wang, Z.-C.; Jian, H.-X.; Li, Z.-Y. *J. Tradit. Chin. Med.* **1982**, *2*, 125. Hien, T. T.; White, N. J. *Lancet* **1993**, *341*, 603. Arnold, K. *J. Hong Kong Med. Assoc.* **1993**, *45*, 189. White, N. J. *Trans. R. Soc. Trop. Med. Hyg.* **1994**, *88*, Suppl. 1, S5. Looareesuwan, S. *Trans. R. Soc. Trop. Med. Hyg.* **1994**, *88*, Suppl. 1, S9.

(5) For details of comparative trials with artemether (**2**) and quinine in the treatment of severe falciparum malaria, see: van Hensbroek, M. B.; Onyiorah, E.; Jaffar, S.; Schneider, G.; Palmer, A.; Frenkel, J.; Enwere, G.; Forck, S.; Nusmeijer, A.; Bennet, S.; Greenwood, B.; Kwiatowski, N. *N. Engl. J. Med.* **1996**, *335*, 69. D. Hien, T. T.; Day, P. J. N.; Phu, N. H.; Hoang, N. T.; Chau, T. T. H.; Loc, P. P.; Sinh, D. X.; Cuong, L. V.; Vinh, H.; Waller, D.; Peto, T. E. A.; White, N. J. *N. Engl. J. Med.* **1996**, *335*, 76.

(6) For an excellent overview see: Bruce-Chwatt, L. J. In *Malaria: Principles and Practice of Malariology*; Wernsdorfer, W. H., McGregor, I., Eds.; Churchill Livingstone: Edinburgh, 1988, Vol. 1, Chapter 1.

(7) Xu, X.-X.; Zhu, J.; Huang, D.-Z.; Zhou, W.-S. *Huaxue Xuebao* **1983**, *41*, 574. Schmid, G.; Hofheinz, W. *J. Am. Chem. Soc.* **1983**, *105*, 624. Xu, X.-X.; Zhu, J.; Huang, D.-Z.; Zhou, W.-S. *Tetrahedron* **1986**, *42*, 819. Avery, M. A.; Jennings-White, C.; Chong, W. K. M. *Tetrahedron Lett.* **1987**, *28*, 4629. Ravindranathan, T.; Kumar, M. A.; Menon, R. B.; Hiremath, S. V. *Tetrahedron Lett.* **1990**, *31*, 755. Avery, M. A.; Chong, W. K. M.; Jennings-White, C. *J. Am. Chem. Soc.* **1992**, *114*, 974. Lansbury, P. T.; Nowak, D. M. *Tetrahedron Lett.* **1992**, *34*, 4435. Liu, H.-J.; Yeh, W.-L.; Chew, S. Y. *Tetrahedron Lett.* **1993**, *34*, 4435.

(8) For reviews, see: Zaman, S. S.; Sharma, R. P. *Heterocycles* **1991**, *32*, 1593. Butler, A. R.; Wu, Y.-L. *Chem. Soc. Rev.* **1992**, 85. Zhou, W.-S.; Xu, X.-X. *Acc. Chem. Res.* **1994**, *27*, 211.

(9) Avery, M. A.; Jennings-White, C.; Chong, W. K. M. *J. Org. Chem.* **1989**, *54*, 1789. Avery, M. A.; Jennings-White, C.; Chong, W. K. M. *J. Org. Chem.* **1989**, *54*, 1792. Avery, M. A.; Chong, W. K. M.; Bupp, J. E. *J. Chem. Soc., Chem. Commun.* **1990**, 1487. Avery, M. A.; Chong, W. K. M.; Detre, G. *Tetrahedron Lett.* **1990**, *31*, 1799. Avery, M. A.; Bupp, J. Internat. Pat. Applic. WO 91/14689, Oct 1991.

(10) Deng, D.; Zhu, D.; Gao, Y.; Dai, J.; Xu, R. *Kexue Tongbao* **1982**, *27*, 1359. Klayman, D. L.; Lin, A. J.; Acton, N.; Scovill, J. P.; Hoch, J. M.; Milhous, W. K.; Theoharides, A. D.; Dobek, A. S. *J. Nat. Prod.* **1984**, *47*, 715−717.

(11) Analysis of dried leaf samples provided by the Kunming Pharmaceutical Factory, Kunming, China, indicate concentrations of qinghao acid 3-fold less than those of qinghaosu. In contrast, the concentration of qinghao acid in *A. annua* samples from Shandong Province is reported at 3.8% dry wt (top leaves of the main stem): Huang, J.-J.; Zhou, F.-Y.; Wu, L. F.; Zhen, G.-H. *Acta Chim. Sin.* **1988**, 383.

(12) Haynes, R. K.; Vonwiller, S. C. *Trans. R. Soc. Trop. Med. Hyg.* **1994**, *88*, Suppl. 1, S23.

(13) Kim, N.-C.; Kim, S.-U. *J. Korean Agric. Chem. Soc.* **1992**, *35*, 106.

(14) See *inter alia*: Arain, M. F.; Haynes, R. K.; Vonwiller, S. C.; Hambley, T. W. *J. Am. Chem. Soc.* **1985**, *107*, 4582. Haynes, R. K.; Hilliker, A. E. *Tetrahedron. Lett.* **1986**, *27*, 509. Arain, M. F.; Haynes, R. K.; Vonwiller, S. C. *Aust. J. Chem.* **1988**, *41*, 505 and references therein.

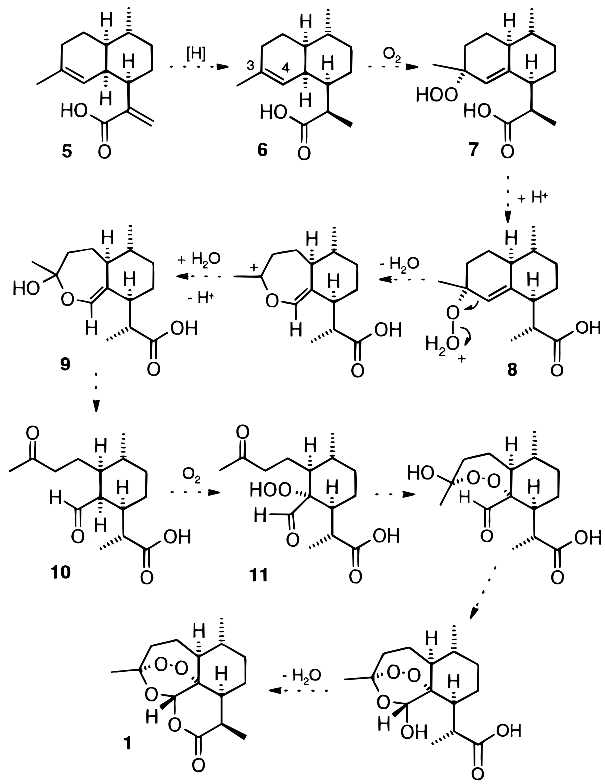
(15) During a visit to the Chinese Academy of Science Shanghai Institute of Organic Chemistry in June 1988, historical development of qinghaosu was related to R.K.H. in discussions with Professor Zhou Wei-Shan's group.

(16) The significance of the stereochemistry of oxygen insertion into aldehyde **10** (Scheme 1) is discussed in ref 40.

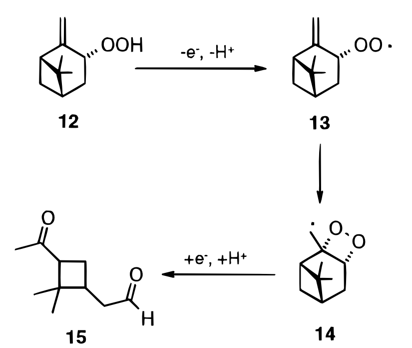
(17) For reviews, see: Frimer, A. A. *Chem. Rev.* **1979**, *79*, 363. Kropf, H. *Methoden der Organischen Chemie (Houben-Weyl)*; Band E13, *Organische Peroxoverbindungen*; George Thieme Verlag: Stuttgart, 1988; Teilband II, Chapter B, pp 1084−1095 and references therein.

(18) Porter, N. A. In *Organic Peroxides*; Ando, W., Ed.; Wiley, New York, 1992; pp 143−146 and references therein.

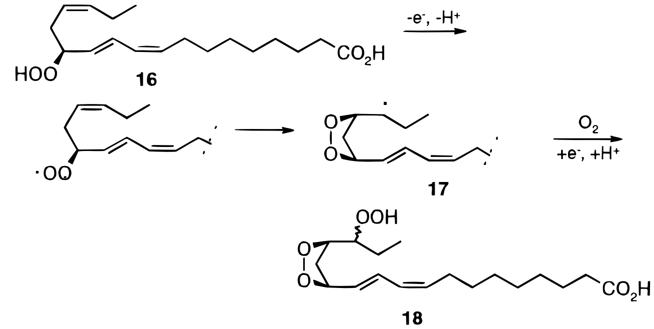
Scheme 1



Scheme 2



Scheme 3



of the most effective catalysts, it was not clear whether Hock rearrangement was necessarily being followed in these reactions.[21] As a second possibility, we therefore proposed oxidation of the hydroperoxide **12** by the catalyst to the peroxy radical **13**. The peroxy radical cyclizes to the dioxetanyl radical **14**. Back-electron transfer from the catalyst, or hydrogen atom abstraction from the starting hydroperoxide in a radical chain process and cleavage of the dioxetane, provides the ketoaldehyde (Scheme 2).[19] In support of the radical proposal, we discovered that fatty acid hydroperoxides such as **16** are converted into hydroperoxydioxolanes, for example, **18**, with oxygen and $Cu(OTf)_2$ in MeCN.[22] The intermediacy of a peroxy radical generated by oxidation of the hydroperoxide, which cyclizes to the dioxolanyl radical **17** concisely accounts for formation of **18** (Scheme 3).

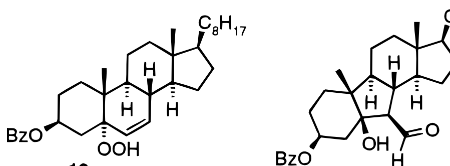
The most important point to emerge thus far was that, irrespective of the actual mechanism, the facile conversion of the allylic hydroperoxide **12**, obtained from the parent alkene by the action of singlet oxygen, into the ketoaldehyde **15**, provides an acceptable formal biomimetic means of oxidative cleavage of the alkene.[23]

However, as we continued the work, it soon became apparent that ketoaldehydes were not necessarily the primary products of the cleavage reactions. Thus, treatment of the hydroperoxide **19** from cholesteryl benzoate with all catalysts gave exclusively the aldol product **20** in high yield.[19] The ketoaldehyde **21** was not observed. We also established, as was earlier intimated by literature data on closely related compounds,[24] that the ketoaldehyde **21** *does not undergo conversion into the aldol **20** under the conditions of formation of the latter from the hydroperoxide **19***. Thus, the ketoaldehyde **21** is not the precursor to the aldol **20**. However, the *actual* precursor must *possess enolic character*, in order for it to transform into the aldol. Clearly, in the case of pinene hydroperoxide (**12**), aldolization of the enolic precursor is unable to take place, and instead, tautomerism into the ketoaldehyde occurs. A noteworthy and related case indicative of an enolic intermediate involves the hydroperoxide **22** from β-pinene. This with $Cu(OTf)_2$ of $Fe(phen)_3(PF_6)_3$ in MeCN provided the aldol **23** as the major product; nopinone (**24**) was a relatively minor product.[25]

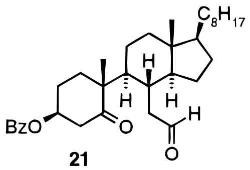
In turning to the special case of the methyl ester **25** of qinghao acid, we found that this reacted rapidly with singlet oxygen to provide the tertiary hydroperoxide **26**. This upon treatment with catalytic $Fe(phen)_3(PF_6)_3$ in MeCN under nitrogen gave the ketoaldehydes **27** and **28**.[19,26] Also isolated was a small amount of the aldol **29**, a remarkable result in view of the congested nature of the compound.[26] The formation of aldol **29** indicates that, here also, an enolic intermediate must be formed during cleavage of the hydroperoxide.

By far the most exciting event was to observe that when the cleavage reaction involving the tertiary hydroperoxide **26** was carried out in the presence of $Cu(OTf)_2$ in MeCN *under oxygen*, a new mixture of products was formed (53–70% overall) *which did not contain the ketoaldehydes **27***

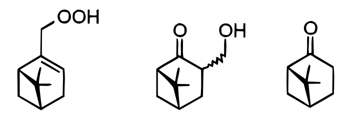
(19) Haynes, R. K.; Vonwiller, S. C. *J. Chem. Soc., Chem. Commun.* **1990**, 449.
(20) Courtneidge, J. L. *J. Chem. Soc., Chem. Commun.* **1992**, 381.
(21) We did *not* discount Hock cleavage as a possible route to the dicarbonyl compounds, as is assumed in ref 20 in a criticism of our work in ref 19.
(22) Haynes, R. K.; Vonwiller, S. C. *J. Chem. Soc, Chem. Commun.* **1990**, 1102.
(23) For biosynthetic conversions invoking Hock cleavage in fatty acid metabolism, see ref 18.
(24) Morand, P.; Kaufman, M. *J. Org. Chem.* **1969**, *34*, 2175.
(25) Haynes, R. K.; Vonwiller, S. C.; Warner, J. A. Unpublished results.
(26) Haynes, R. K.; Vonwiller, S. C. *J. Chem. Soc., Chem. Commun.* **1990**, 452.



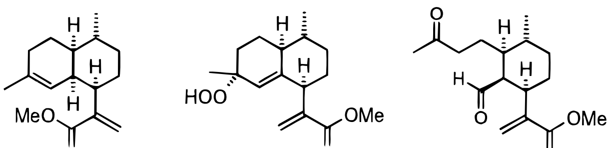
19 20



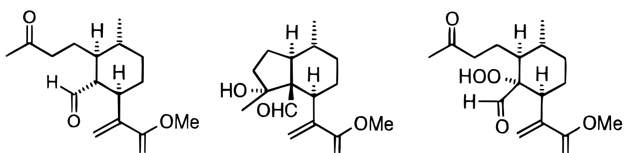
21



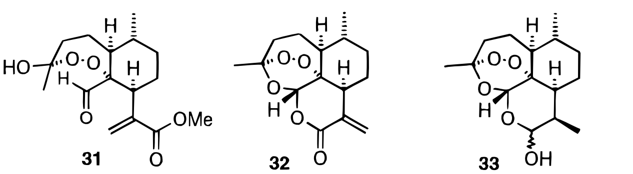
22 23 24



25 26 27



28 29 30

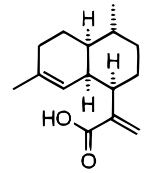
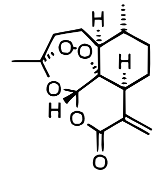
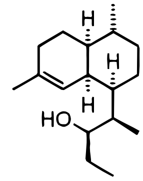
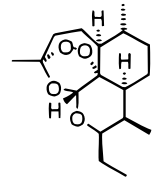
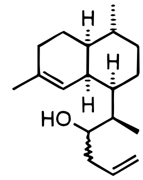
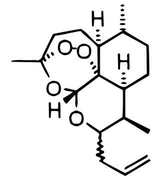
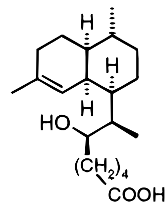
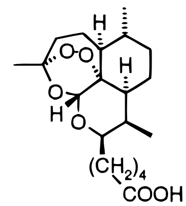
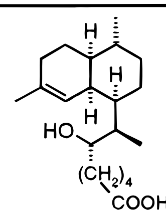
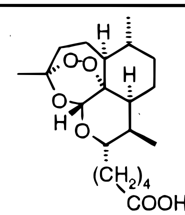
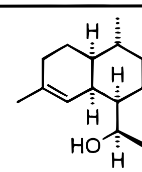
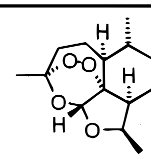
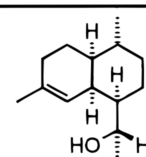
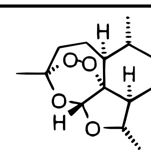


31 32 33

and ***28*** and at most only trace amounts of the aldol **29**.[26–28] The new mixture consisted of the formyl hydroperoxide **30** in equilibrium with the peroxyhemiacetal **31**; each compound was clearly identified by ^{13}C NMR spectra. This mixture was then cleanly converted into dehydroqinghaosu (artemisitene) (**32**) by treatment with *p*-TsOH in CH_2Cl_2 (*cf.* Scheme 1). It is to be noted that dehydroqinghaosu is a minor constituent of *A. annua*,[29] although it has approximately 20% of the activity of qinghaosu against *P. falciparum in vitro*.[30] The compound serves as a convenient substrate for preparation of new qinghaosu derivatives, and as a result of the new methodology, it is now readily available.[31]

The sequence was refined so that dehydroqinghaosu, qinghaosu, or structurally related compounds could be prepared in one-pot processes directly from qinghao or dihydroqinghao acid or the corresponding derivatives. Thus, through use of simple chemical transformations, qinghao acid was converted into new derivatives (Table 1). The acids and their derivatives were each converted by singlet oxygen into the corresponding tertiary hydroperoxides. Without removal of small amounts of the secondary hydroperoxide regioisomers also formed in the oxygenation, the tertiary hydroperoxides were treated *in situ* with $Cu(OTf)_2$ (0.1 equiv) in CH_2Cl_2/MeCN under oxygen at −20 °C until cleavage−oxygenation was observed by TLC to be complete. Gradual warming of the

Table 1. Products Obtained from Qinghao Acid and Derivatives[32]

Qinghao acid derivative	Qinghaosu derivative	Isolated Yields
		46%
		34%
		36%
		34%
		35%
		39%
		25%

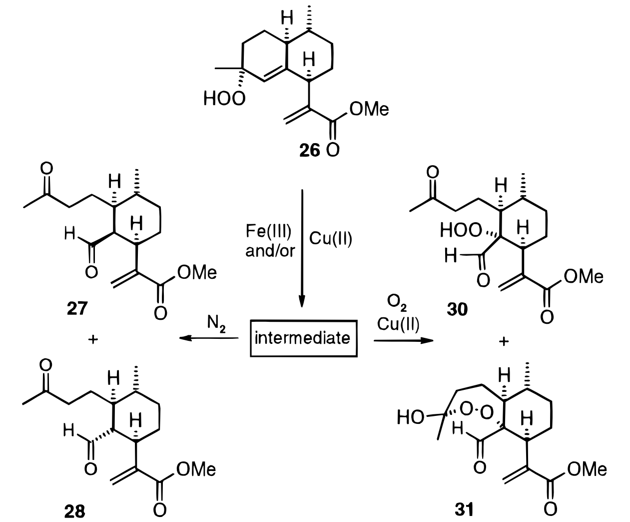
(27) $Fe(phen)_3(PF_6)_3$ induces allylic hydroperoxide cleavage under both nitrogen and oxygen. It is ineffective as an oxygenation catalyst; see: Haynes, R. K.; King, G. R.; Vonwiller, S. C. *J. Org. Chem.* **1994**, *59*, 4743.
(28) Vonwiller, S. C.; Warner, J. A.; Mann, S. T.; Haynes, R. K. *J. Am. Chem. Soc.* **1995**, *117*, 11098.
(29) Acton, N.; Klayman, D. L. *Planta Med.* **1985**, 441.
(30) Acton, N.; Klayman, D. L. *Planta Med.* **1987**, 266.
(31) For an alternative approach to dehydroqinghaosu from qinghaosu, see: El-Feraly, F. S.; Ayalp, A.; A.;-Yahya, M. A.; McPhail, D. R.; McPhail, A. T. *J. Nat. Prod.* **1990**, *53*, 66.

reaction mixture to room temperature effected the final ring closure. In this way, dehydroqinghaosu and the compounds listed in Table 1 were prepared in yields generally greater than 30% overall from qinghao acid and derivatives.[32] While some of these compounds have been obtained from qinghaosu via dihydroqinghaosu (dihydroartemisinin) (**33**),[33] the semisynthesis of qinghaosu derivatives from qinghao acid derivatives represents an attractive alternative means of obtaining these potentially very useful antimalarial drugs. As indicated above, qinghao acid is an abundant constituent of *A. annua*, and extraction procedures have been developed which recover *both* the qinghaosu and qinghao acids.[34]

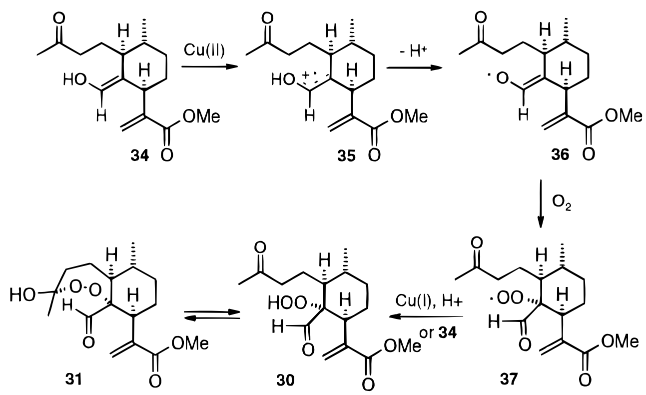
The success of the cleavage–oxygenation sequence is remarkable, but the process itself is not unique. It is important to point out that, at about the same time as our discovery, Roth and Acton independently discovered a similar transformation involving reaction of dihydroqinghao acid hydroperoxide (**7**) with oxygen in the presence of catalytic trifluoroacetic acid in hexane at room temperature to provide qinghaosu in 30% overall yield from dihydroqinghao acid (**6**).[35]

In both the Roth–Acton and our cases, we needed to pinpoint the precursor which is capable of reacting with oxygen. As a first step, it was necessary to probe the relationship between the anaerobic cleavage reactions leading to the aldols and/or ketoaldehydes and the aerobic cleavage–oxygenation reaction leading to the formyl hydroperoxide–peroxyhemiacetal mixtures. The methyl ester hydroperoxide **26** of qinghao acid was submitted to ^{1}H NMR examination from 180 to 300 K in CD_2Cl_2 containing triflic acid in the absence of oxygen, conditions under which it was observed to undergo clean transition through a first intermediate, whose identity later provided the key to an understanding of this chemistry, and then a second intermediate, the aldol **29**. Upon warming to 300 K, the aldol **29** was completely transformed into the ketoaldehydes **27** and **28**, the final products.[28] On the other hand, TLC analysis of mixtures of the hydroperoxide **26** and $Fe(phen)_3(PF_6)_3$ or $Cu(OTf)_2$ in MeCN revealed initial generation of an intermediate more polar than the starting hydroperoxide *regardless of whether the rection was conducted under oxygen or nitrogen*. Under nitrogen, this polar intermediate eventually transformed into the ketoaldehydes **27** and **28**, and under oxygen, into the oxygenation products **30** and **31** (Scheme 4).[28] The polar intermediate was able to be isolated at low temperature,

Scheme 4



Scheme 5



and this was demonstrated by NMR spectroscopy to be identical with the first intermediate in the triflic acid reaction. Further low-temperature ^{1}H, ^{13}C, and NOE NMR experiments clearly revealed that this was the simple enol **34** (Scheme 5). NMR data indicate a stereostructure with diaxial substituents on the cyclohexane ring consistent with the effects of $A^{1,3}$ strain.[28]

The isolation of this simple aldehyde enol is noteworthy. Simple ketone enols as chemically distinct species are well known, but these are characterized by features such as steric encumbrance that confer stability in the absence of a proton source and which in certain cases render isolation possible.[36] Aldehyde enols are rare; in addition to the current case, two other examples of isolable aldehyde enols have been reported.[37] The stability of the enol **34** may be due to the large flanking diaxial substituents, which will hinder protonation required for tautomerism to the aldehyde.

Although simple ketone enols are relatively inaccessible, it has been possible to measure their redox properties with some precision; reduction potentials generally lie within a range which enables electron transfer to one-

(32) Haynes, R. K.; Vonwiller, S. C. Provisional Patent P6989, September 1989. Haynes, R. K.; Vonwiller, S. C. Int. Applic. PCT/Au90/00456 (27/9/90) WO; *Chem. Abstr.* **1992**, *116*, 59094a. Haynes, R. K.; Vonwiller, S. C. *Synlett* **1992**, 481. Haynes, R. K.; Vonwiller, S. C. PCT Int. Applic. WO 9308195 (29/4/93); *Chem. Abstr.* **1993**, *119*, 181047q. Haynes, R. K.; Vonwiller, S. C.; Wang, H.-J. *Tetrahedron Lett.* **1995**, *36*, 4641.

(33) Pu, Y. M.; Ziffer, H. *J. Med. Chem.* **1995**, *38*, 613.

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(35) Roth, R. J.; Acton, N. *J. Nat. Prod.* **1989**, *52*, 1183. Roth, R. J.; Acton, N. *J. Chem. Educ.* **1991**, *68*, 612. For related transformations see: Bustos, D. A.; Jung, M.; ElSohly, H. N.; McChesney, J. D. *Heterocycles* **1989**, *29*, 2773. Jung, M.; Li, X.; Bustos, D. A.; ElSohly, H. N.; McChesney, J. D. *Tetrahedron Lett.* **1989**, *30*, 5973. Jung, M.; Bustos, D. A.; ElSohly, H. N.; McChesney, J. D. *Synlett* **1990**, 743. Jung, M.; Yu, D.; Bustos, D. A.; ElSohly, H. N.; McChesney, J. D. *Bioorg. Med. Chem. Lett.* **1991**, *1*, 741. Jung, M.; Bustos, D. A.; ElSohly, H. N.; McChesney, J. D. *Synlett* **1993**, 43.

(36) For reviews, see: Hart, H. *Chem. Rev.* **1979**, *79*, 515. Rappoport, Z.; Biali, S. E. *Acc. Chem. Res.* **1988**, *21*, 442.

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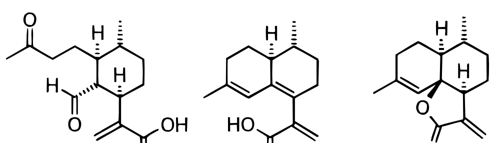
electron oxidants to take place.[38] The properties of the resulting cation radical have been examined with respect to dimerization or electron transfer reactions with nucleophiles, but we are unaware of reports of their reaction with oxygen as distinct from simple autoxidation of the enols themselves.[39] In our case, the enol **34** undergoes electron transfer with $Cu(OTf)_2$ to generate a cation radical **35**, deprotonation of which and reaction of the resulting enol (or formyl) radical **36** with oxygen will provide the peroxy radical **37** (Scheme 5).[40] A radical chain reaction is set up as the peroxy radical **37** converts the enol into an enol radical via hydrogen atom abstraction. Alternatively, electron transfer from Cu(I) to, and protonation of, the peroxy radical generates the formyl hydroperoxide **30** and Cu(II). Under the Roth−Acton conditions, where no oxidant is present, the oxygenation is initiated by peroxy radical-mediated autoxidation of the enol to the radical and propagated through the radical chain process. The enol is produced through the action of low concentrations of trifluoroacetic acid on the hydroperoxide **7**.

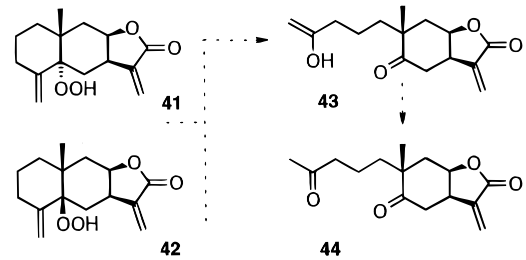
Thus, the mode of formation of aldols under anaerobic conditions is now clear; the reaction involves enols. The aldolization may be promoted by the $Cu(OTf)_2$ or protic acid complexing to the remote ketonic carbonyl. The important point though is that we may make a new generalization about the venerable Hock cleavage of allylic hydroperoxides: *the cleavage reaction proceeds via enols*. We thereby have an entirely unanticipated, yet highly effective, means of producing simple enols.[41]

It is of interest to note that the use of enolic intermediates underpins total synthesis of qinghaosu and derivatives. Oxygenation, however, has usually been effected through use of singlet oxygen.[7] In the biosynthesis of qinghaosu from qinghao acid, the involvement of a hydroperoxide and the enol arising via decomposition of the hydroperoxide must now be considered as very likely. In addition, we note that dihydroqinghao acid (**6**), as well as the seco-derivative **38** of qinghao acid, dehydroqinghao acid (**39**), and desoxyepiarteannuin B (**40**), are metabolites

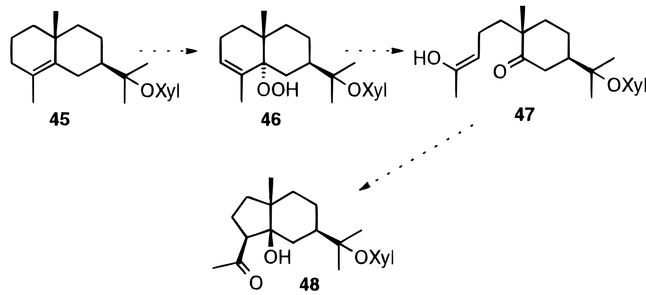


38 **39** **40**

Scheme 6



Scheme 7



isolated from *A. annua*.[13,42] We have observed that these and related products are also formed in small amounts during the catalyzed decomposition of qinghao acid and ester-derived hydroperoxides such as **7** and **26**. Biosynthetic formation of the hydroperoxide **7** and the free acid hydroperoxide of **26** may be mediated by lipoxygenase-like processes in the plant.

The occurrence of dicarbonyl compounds and the corresponding aldols as natural products suggests that the allylic hydroperoxide−enol conversion is of general importance in secondary metabolism. 5-Desoxy-5-hydroperoxytelekin (**41**) and 5-dexosy-5-hydroperoxy-5-*epi*-telekin (**42**) co-occur with umbellifolide (**44**) in *Artemisia umbelliformis*.[43] Decomposition of either of the former compounds will produce the enol **43**. While the enol may aldolize, this is not expected to be facile, and thus tautomerism to umbellifolide (**44**) will supervene (Scheme 6). Eudesmol (**45**) and iphionane (**48**) co-occur in *Iphiona scabra*.[44] It is rational to consider **45** as the precursor to **48** and that the conversion *in vivo* proceeds via the hydroperoxide **46** and the enol **47** (Scheme 7). This enol, in contrat to enol **43**, will now undergo facile aldolization to provide iphionane (**48**). Investigation in this area was initiated, and is currently being undertaken by S.C.V.

Concluding Remarks. The advent of the qinghaosu class of antimalarial has ushered in a new era for treatment of malaria, and work in all areas, from that involving preparation of new derivatives, to uncovering the mode of action, to pharmacokinetic studies, drug formulation, clinical studies, and field trials, continues apace. The herb of antiquity, qinghao, has provided a fascinating compound whose trioxane nucleus is the focus of development of new methodology to provide structures containing this nucleus. In our case, we have happened upon a reaction which is eminently suited for this purpose, but which also

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(40) The conversion of enol **34** into dehydroqinghaosu (**32**) requires installation of three new chiral centers (*cf.* Scheme 1). Addition of oxygen to the *Re*-face of the enol radical **36** leading to hydroperoxide **30** is preferred because of the adjacent β-configured *diaxial* side chains. In **30**, the hydroperoxy group is equatorial and the distal carbonyl group is accessible for both *Re*-face attack, leading to peroxyhemiacetal **31**, and *Si*-face attack, leading to "unnatural" peroxyhemiacetal. As unnatural qinghaosu-like products are never observed, it is possible that equilibration of any unnatural hemiacetal precursor may occur via ring-opening and reclosure to the "natural" qinghaosu or precursor.
(41) Rearrangement of hydroperoxide **26** to enol **34** is presumed to proceed via a cyclic enol ether (*cf.* compound **9**, Scheme 1), an intermediate which we have so far not been able to detect.
(42) Brown, G. D. *Phytochemistry* **1994**, *36*, 637.
(43) Appendino, G.; Gariboldi, P.; Calleri, M.; Chiari, G.; Viterbo, D. *J. Chem. Soc., Perkin Trans. 1* **1983**, 2705.
(44) El-Ghazouly, M. G.; El-Sebakhy, N. A.; Seif El-Din, A. A.; Zdero, C.; Bohlmann, F. *Phytochemistry* **1987**, *26*, 2603.

provides an unexpected route to otherwise relatively inaccessible enols in a general way.

Finally, it should be noted that the trioxane nucleus of qinghaosu itself displays a chemistry which is really too varied and rich to allow for an uncluttered view of the mode of action of the drug against the malarial parasite.[45] Thus, work in this area is continuing. Irrespective of a decisive acquisition of understanding the mode of action, there is reasonable expectation for development of drugs encapsulating the trioxane nucleus against other targets.[46]

We express our deep gratitude to Professor Zhou Wei-Shan for providing generous samples of qinghao acid in the early phase of this work and to Professor Lin Guo-Qiang, director of the Chinese Academy of Science Shanghai Institute of Organic Chemistry Institute, for support for initiation of the research in Sydney. We thank the Australian Research Council for generous financial support (Grant A29131196) and for award of a Queen Elizabeth II Fellowship to S.C.V. The Hong Kong Research Grants Council and HKUST are thanked for generous financial support to R.K.H. (Grants HKUST 591/95P and HKUST RIG.SC03 95/96) for ongoing research associated with the mode of action and drug development at the Hong Kong University of Science and Technology.

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