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CHEMICAL TRANSFORMATIONS OF QINGHAOSU, A PEROXIDIC ANTIMALARIAL

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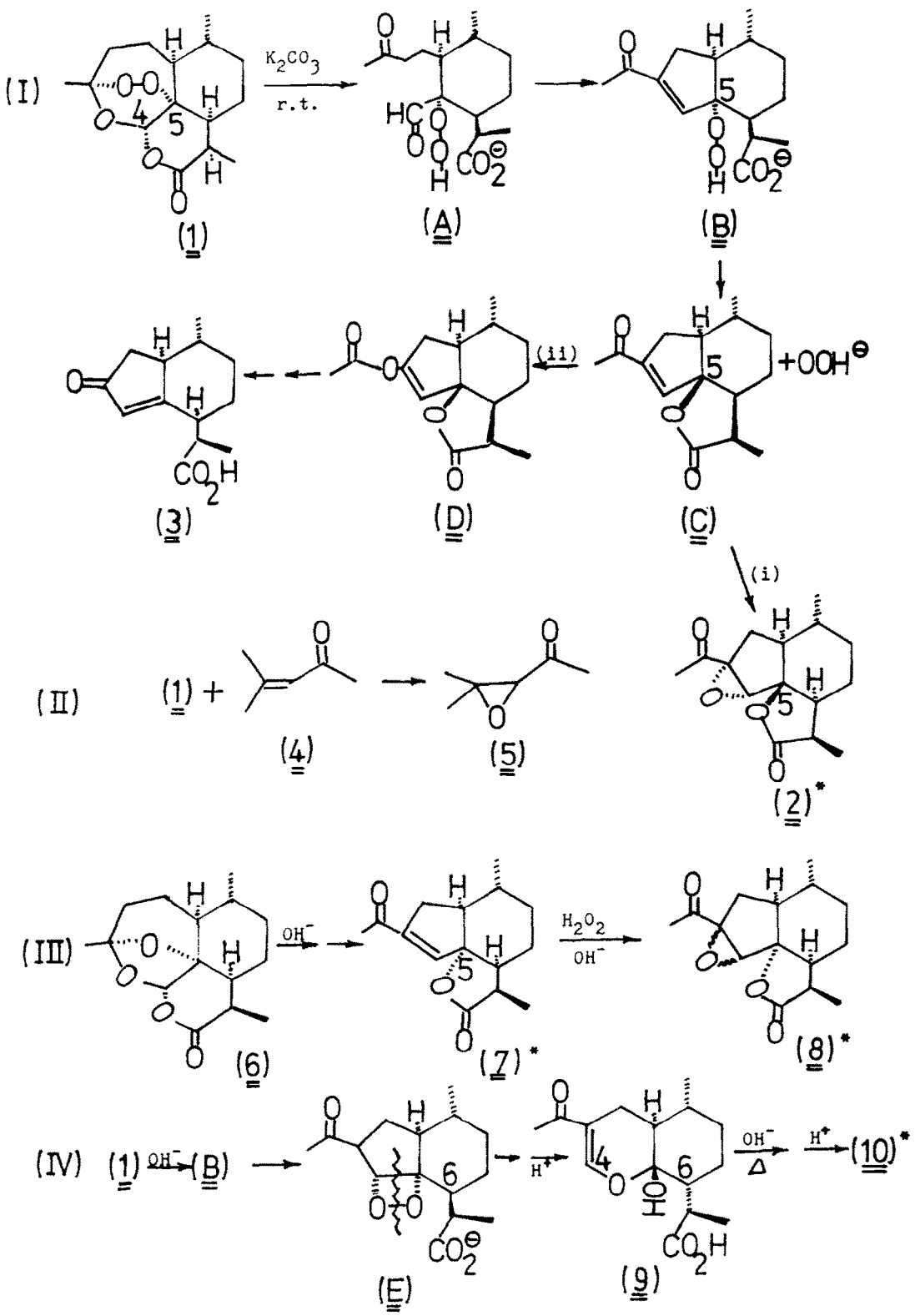
Abstract - This paper discusses the stereochemistry of 2 and 10, two previously reported transformation products of qinghaosu (1). The structures of two other products, 3 and 9, are also presented.

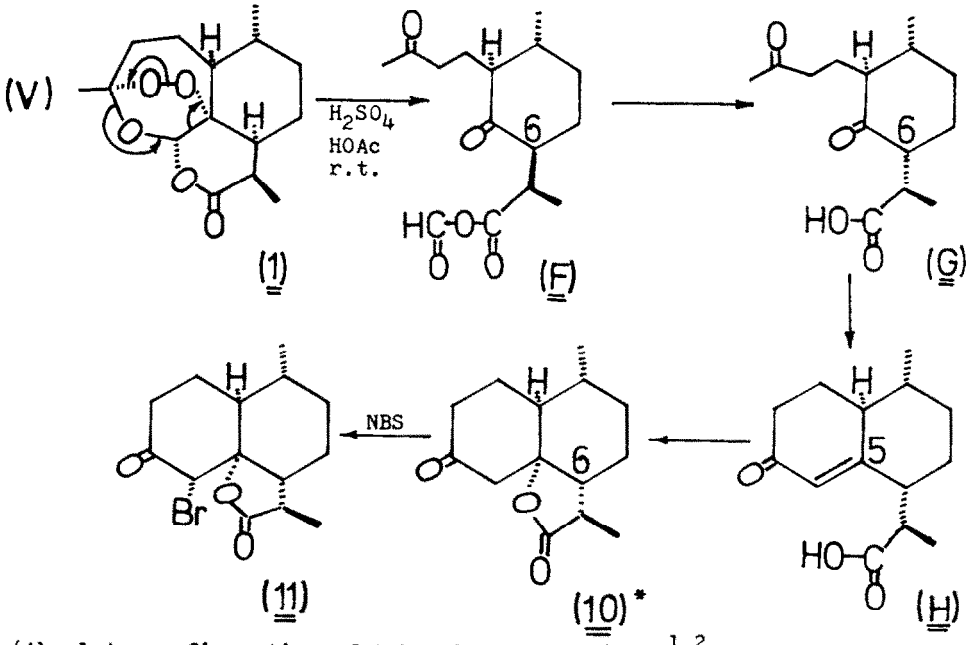
Qinghaosu (1)[1,2] is an antimalarial isolated from the aerial parts of Artemisia annua L., a wild growing plant used in Chinese traditional herbal medicine. In this paper we wish to report the stereochemistry of 2 and 10, and structural characterization of 3 and 9, all transformation products of qinghaosu. The structures of 2 and 10, apart from their stereochemistry, have been described previously[2].

Mechanistically speaking, treatment of 1 with potassium carbonate in aqueous methanol at room temperature first of all opens up the lactonic ring to give A, where all the interlocked functionalities are set free zipper-like. Subsequent condensation of the aldehyde group onto the active methylene gives the unsaturated ketone B. The hydroperoxy group at C-5 then suffers displacement by the carboxylate anion to give compound C along with a molecule of hydrogen peroxide, which can readily epoxidize the α,β-unsaturated ketone to give 2 in ca. 10% isolated yield. According to this mechanism, the oxygen function at C-5 of compound 2 is β-oriented as contrasted with the original α-orientation in qinghaosu (1). The α-configuration for the epoxide in 2 is assigned on steric grounds.

Scheme 1 Transformations of qinghaosu (1).
Structures labeled with capital letters are proposed intermediates.



Scheme 1 (continued)



(Absolute configuration of 1 has been established[1,2] as shown, from which follow all the other configurational representations).
*Spectral data[2] appended at the end of the Experimental section.

Deoxyqinghaosu (6), obtained from 1 by catalytic hydrogenation, gave compound 7 upon treatment with ethanolic potassium hydroxide[2]. Here retention of configuration at C-5 is considered to be reasonable since the hydroxyl is not a good leaving group and should be immune to displacement by the carboxylate anion (not like the case of B). Further treatment of 7 with alkaline hydrogen peroxide gave 8 which is distinctly different from 2 in physicochemical properties[2]. Unfortunately we have thus far not been able to isolate C from the complex reaction mixture (reaction I), whereby a direct comparison of C and 7 would have been much more revealing as regards the stereochemistry at C-5. Two deoxygenation reactions[3] were also tried without success in our efforts to get C from 2.

The generation of free hydrogen peroxide in reaction I was further supported by two pieces of evidence. First, when three equivalents of mesityl oxide (4) were added at the beginning, the yield of 2 was markedly diminished as monitored by comparative examination of tlc spots. The presence of 5 was detected by glc (reaction II). This result suggests that mesityl oxide has indeed served as a hydrogen peroxide scavenger. Here again because of the complexity of the reaction mixture, attempts to isolate C were unsuccessful.

Second, an oily product (3) was also isolated from reaction I in 1.6% yield. The structure of 3 follows mainly from its spectral characteristics. Thus the IR spectrum has absorptions at 1610 (C=C), 1670 (α,β-unsaturated ketone) and 1700 cm^{-1} (CO_2H), and the conjugated keto-system also finds expression in its UV spectrum with a peak at 238 nm (ε12500). The ^1H NMR spectrum displays two methyl doublets at δ 1.02 and 1.25 and an olefinic proton at 5.93 (s). The molecular formula agrees with the MS molecular ion at m/z 222.

The intermediate C has been invoked (vide supra) to account for the formation of 2 through epoxidating attack by the hydrogen peroxide on the olefinic end of the conjugated system

(path i). Alternative attack on the carbonyl carbon will lead to a Baeyer-Villiger type reaction[4] (path ii) forming the acetate (D), saponification of which then gives 3.

The scavenging action of mesityl oxide (4) and the formation of 3, to reiterate, strongly support the generation of free hydrogen peroxide in the reaction medium, thus implicating inversion of configuration at C-5 in compound 2 via the intermediate C.

In dilute sodium hydroxide, qinghaosu (1) was transformed to the enolate (and carboxylate) of 9 with a UV peak at 292 nm. Under optimized conditions (0.2% NaOH, 50°, 30 min; the reaction takes longer time at room temperature to reach approximately the same UV absorbance), the ϵ-value with respect to the starting material (1) was calculated to be 16500. The ϵ-value for pure 9 in alkaline medium was 19400, hence the conversion of 1 to 9 was better than 88% (taking into account the slight decomposition of 9 at 50°).

Pertinent spectral evidence for the structure of 9 includes MS molecular ion at m/z 282; IR at 1690 (CO_2H), 1630 and 1603 cm^{-1} (-CO-$\dot{C}$=$\dot{C}$-O-); UV peak at 292 (in OH^-), reversibly changing to 260 nm (in H^+, ϵ 14200); ^1H NMR peaks at δ0.85 (3H, d), 1.18 (3H, d), 2.12 (3H, s), 7.55 (1H, s, C_4-H).

The hemiacetal hydroxyl in 9 is tentatively assigned a β-configuration as shown in Scheme 1, since a trans A/B fusion leads to less crowding in the whole system. Inversion of configuration at C-6 parallels the case of 10 to be described below.

Conversion of 9 to a known compound was desired as a structural proof. To this end, pure 9 was refluxed in 5% NaOH; from a plethora of products the one with the matched Rf was singled out by preparative tlc and identified as 10. The formation of 10 involves expulsion[5] of a formyl group (C-4) from 9, followed by annelation as outlined in reaction V.

The mechanism of formation of 9 can be visualized as follows (see reaction IV). In the strongly alkaline medium the hydroperoxy group of B is sufficiently ionized for the occurrence of a virtually exclusive intramolecular Michael addition, forming the dioxetane ring of E. This ring, being fused as it is into a tricyclic system, is strained and decomposes spontaneously[6] to form 9. The unique fate of the intramolecular involvement of the hydroperoxy group outlined here constitutes, by exclusion, yet another piece of convincing evidence for the intermolecular counterpart in the formation of 2 through the intermediacy of free hydrogen peroxide.

There is reason to believe that 9 should also be formed in potassium carbonate solution (reaction I). The UV spectrum of the crude reaction mixture indicates that 9 was present in *ca.* 15% yield.

If we take this yield as a reasonable estimate and accept B → C and B → E as competitive intramolecular key steps, the change of K_2CO_3 to NaOH will not affect the rate of B → C because of the invariant concentration of the fully ionized carboxylate as the attacking group; if anything the tiny portion of the ROOH group that is ionized actually discourages attack by the carboxylate ion since the dianion $O_2^=$ is no longer a good leaving group. In the meantime, as the result of the increase in the degree of ionization of the ROOH group, the rate of B → E will be proportionately raised to the right order of magnitude (10^2; two pH units) for a predominant production of 9, as found experimentally.

The crude mixture of reaction I exhibited also a strong UV peak at 233 nm attributable to the presence of 3 and C (C should have comparable absorption as 7^2), among other possible products. To be optimistic, the so far elusive compound C could have been present as a final product with a maximal yield of

20-30%.

Finally let us discuss the acid treatment of qinghaosu (1). The stereochemistry of the product (10) as determined by X-ray single crystal analysis[7] is unexpected in the inversion of configuration at C-6. The rationale is offered as follows. The peroxidic linkage of qinghaosu is weakened by strong acid which triggers a concerted fragmentation (arrows in 1 of reaction V) at room temperature[8](in contrast, qinghaosu is stable in neutral aprotic solvents up to at least 150°). The intermediate F thus formed has all three substituents equatorially disposed, with the branched group at C-6 severely brushing against the carbonyl oxygen. Enolization-induced inversion at C-6 gives the less congested G which cyclizes to afford H or its ketol equivalent with a 5-α hydroxyl[9].

NBS bromination of 10 gives 11. The IR ketonic carbonyl frequencies of 10 and 11 are 1710 and 1730 cm^{-1} respectively, in line with an equatorial bromine[10]. However, the CD ketonic absorption of 11 (320 nm) is 4-fold as large as that of 10 (295 nm), with a bathochromic shift of 25 nm to be expected of an axial bromine[11]. Probably ring A of 11 is no longer a normal chair as is the case for 10, the α-bromine being twisted out of the plane containing the ketonic carbonyl.

EXPERIMENTAL

M.ps are uncorrected. IR spectra were recorded on a Perkin-Elmer 399B instrument. ^{1}H NMR spectra were run with internal TMS on Varian XL-200 and Cameca 250 MHz spectrometers. MS were taken with a JMS-02-SB spectrometer. CD measurements were made on Jobin-Yvon Autodichrograph Mark V. Tlc silica was purchased from Qingdao Haiyang Chemical Works.

Isolation of 3 To a soln of 1 (2g) in MeOH (40 ml) was added with stirring 10% K_2CO_3 soln (20 ml). After 1 hr, water (50 ml) was added and the whole extracted with ether. The aqueous layer was acidified with dil HCl to pH 2 and extracted lavishly with ether. The second ethereal extracts were washed with saturated NaCl soln and dried (Na_2SO_4). Evaporation gave an oily residue which was taken up with some MeOH and refrigerated for several days when white crystals came down and were removed. Tlc of the mother liquor with silica G ($CHCl_3$-MeOH, 4:0.3, I_2 vapor detection) showed three major spots at Rf 0.8, 0.6 and 0.3, along with a number of minor ones. Preparative separation was performed on Chromatotron with silica GF, where $CHCl_3$-MeOH (100:5) removed first the component of Rf 0.8. This was followed by gradient elution and then a mixed solvent of 100:20 proportion, out of which a few fractions contained the pure component of Rf 0.3. This was passed once more through the Chromatotron to afford 26 mg of oil (3). MS, 222 (M^+), 177, 149. ^{1}H NMR (200 MHz, $CDCl_3$), δ 1.02 (3H, d), 1.25 (3H, d), 2.80 (1H, qnt), 5.93 (1H, s).

Isolation of 9 Qinghaosu (0.3 g) in 95% EtOH (100 ml) was mixed with 0.2% aqueous NaOH (400 ml) and allowed to react at 50° for 30 min. After cooling and extraction with $CHCl_3$, the aqueous layer was acidified to pH 2 and extracted again with $CHCl_3$. The second $CHCl_3$ extracts were washed with water, dried (Na_2SO_4) and evaporated to ca. 2 ml. Acetone (5 ml) was added to the residue and the whole refrigerated, depositing crystalline granules. Recrystallization from acetone-petroleum ether (1:1) gave 45 mg of 9 (15%), m.p. 161-162°. IR (KBr), 1690(s), 1630 (s), 1603 (vs), 1468 (m), 1225 (s), 1210 (s). MS, 282 (M^+), 264, 246, 236, 221, 207, 203, 191, 180, 171, 165, 151. ^{1}H NMR (250 MHz, acetone-d_6), δ 0.85 (3H, d), 1.18 (3H, d), 2.12 (3H, s), 3.35 (1H, m), 3.55 (2-3H, br, D_2O exchangeable), 7.55 (1H, s).

NBS bromination of 10 Compound 10^2 (472 mg, 2 mmol), NBS (177 mg, 1 mmol) and benzoyl peroxide (5 mg) in CCl_4 (50 ml) were refluxed under irradiation with a 200W lamp. The gradually developed orange coloration faded out abruptly at about 1.5 hr. Upon cooling, some starting material (10) was removed by filtration. The filtrate was passed through a silica column and eluted with CH_2Cl_2 in six 50 ml fractions. The fourth fraction afforded 10 mg of Br-free material. Further elution with MeOH gave 15 mg of 11, m.p. 155-158°. MS m/z 316 & 314 (M^+). IR (KBr), 1760, 1730 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$), δ1.12 (3H, d), 1.26 (3H, d), 4.96 (1H, s). CD ($\Delta\varepsilon$ in MeOH), +1.15 (320 nm), -3.45 (270 nm). This is to be compared with +0.26 (295 nm), -2.01 (270 nm) for 10.

Compound 2^2 M.p. 222-223°. MS m/z 264 (M^+). IR (KBr), 1710, 1770 cm^{-1}. ^1H NMR (100 MHz, $CDCl_3$), δ1.08 (3H, d), 1.35 (3H, d), 2.04 (3H, s), 3.98 (1H, s).

Compound 7^2 M.p. 126-127°. UV (EtOH), 231 nm (ε16500). MS m/z 249 (M^++1). IR (KBr), 1610, 1680, 1770 cm^{-1}. ^1H NMR (100 MHz, $CDCl_3$), δ 0.96(3H, d), 1.06 (3H, d), 2.20(3H, s), 6.68 (1H, s).

Compound 8^2 M.p. 160-161°. IR (KBr), 1710, 1770 cm^{-1}. ^1H NMR (100 MHz, $CDCl_3$), δ0.88 (3H, d), 1.14 (3H, d), 2.04 (3H, s), 3.66 (1H, s).

Compound 10^2 M.p. 144-146°. MS m/z 237 (M^++1). IR (KBr), 1710, 1770 cm^{-1}. ^1H NMR (100 MHz, $CDCl_3$), δ0.94 (3H, d), 1.11 (3H, d).

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