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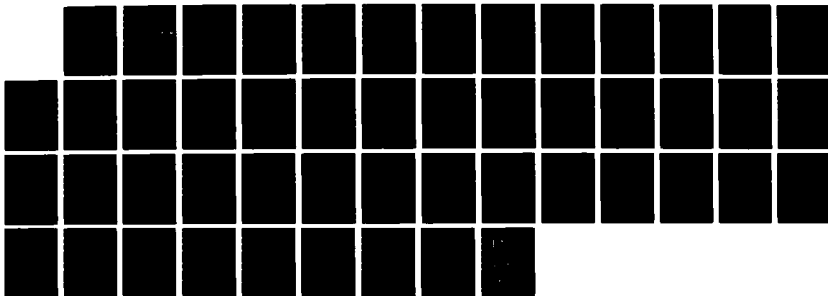
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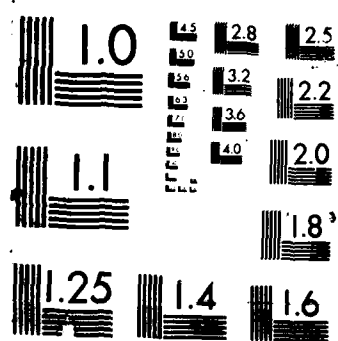
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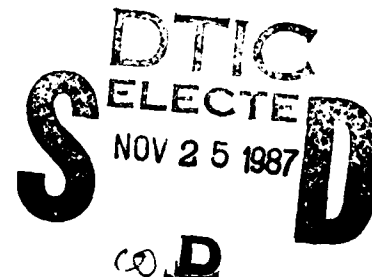
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ANTIMALARIAL CYCLIC PEROXIDE LACTONES

FINAL REPORT

KUO-HSIUNG LEE

May 31, 1987



Supported by

U. S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21701-5012

Contract No. DAMD17-83-C-3098 & DAMD17-85-C-5010

University of North Carolina
Chapel Hill, North Carolina 27514

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REPORT DOCUMENTATION PAGE				Form Approved OMB No. 0704-0188	
1a. REPORT SECURITY CLASSIFICATION UNCLASSIFIED			1b. RESTRICTIVE MARKINGS		
2a. SECURITY CLASSIFICATION AUTHORITY			3. DISTRIBUTION/AVAILABILITY OF REPORT Approved for public release; distribution unlimited		
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE					
4. PERFORMING ORGANIZATION REPORT NUMBER(S)			5. MONITORING ORGANIZATION REPORT NUMBER(S)		
6a. NAME OF PERFORMING ORGANIZATION University of North Carolina		6b. OFFICE SYMBOL (If applicable)		7a. NAME OF MONITORING ORGANIZATION	
6c. ADDRESS (City, State, and ZIP Code) School of Pharmacy Chapel Hill, North Carolina 27514				7b. ADDRESS (City, State, and ZIP Code)	
8a. NAME OF FUNDING/SPONSORING ORGANIZATION U.S. Army Medical Research & Development Command		8b. OFFICE SYMBOL (If applicable)		9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER DAMD17-83-C-3098* DAMD17-85-C-5010**	
8c. ADDRESS (City, State, and ZIP Code) Fort Detrick Frederick, MD 21701-5012		10. SOURCE OF FUNDING NUMBERS			
		PROGRAM ELEMENT NO. 62772A		PROJECT NO. 3M1- 62770A870	
		TASK NO. AJ		WORK UNIT ACCESSION NO. 033	
11. TITLE (Include Security Classification) (U) Antimalarial Cyclic Peroxide Lactones					
12. PERSONAL AUTHOR(S) Kuo-Hsiung Lee					
13a. TYPE OF REPORT Final		13b. TIME COVERED FROM 10/15/84 TO 2/28/87		14. DATE OF REPORT (Year, Month, Day) 1987 May 31	
15. PAGE COUNT					
16. SUPPLEMENTARY NOTATION * Period of time covered by contract 15 March 1983 - 14 September 1984 ** Period of time covered by contract 15 October 1984 - 28 February 1987					
17. COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)		
FIELD	GROUP	SUB-GROUP			
06	01		Antimalarial agents, synthesis of α -santonin-derived		
07	03		cyclic peroxide, brusatol-related quassinoid glycoside, ester and carbamate, qinghaosu and qinghaosu-related analogs		
19. ABSTRACT (Continue on reverse if necessary and identify by block number) An α-santonin-derived cyclic peroxide (VI of Scheme-1) related to qinghaosu (1 of Scheme-4) has been synthesized and tested for antimalarial activity. The result indicates that the requirement of the cyclic peroxide function for potent antimalarial activity among the sesquiterpene lactones might be quite specific and could involve a unique C-O-C-O-C-O-C-O linkage as found in qinghaosu. One of the mechanisms of action of qinghaosu and artesunate was found to inhibit the activity of the cytochrome oxidase which is located in the plasma, the nuclear and the food vacuole limiting membranes as well as in the mitochondria of the trophozoites of <i>Plasmodium berghei</i>. The quassinoids bruceoside-A, brusatol and bruceolide were tested for antimalarial activity <i>in vitro</i> against the chloroquine-resistant (Smith) isolates of <i>Plasmodium</i>					
20. DISTRIBUTION/AVAILABILITY OF ABSTRACT ☐ UNCLASSIFIED/UNLIMITED ☒ SAME AS RPT ☐ DTIC USERS			21. ABSTRACT SECURITY CLASSIFICATION Unclassified		
22a. NAME OF RESPONSIBLE INDIVIDUAL Mary Frances Bostian			22b. TELEPHONE (Include Area Code) 301-663-7325		22c. OFFICE SYMBOL SGRD-RM1-S

falciparum. Brusatol was quite active, bruceoside-A was not active, and bruceolide showed only a trace of activity. The fact that 15[(E)-non-2-enoyl] bruceolide synthesized from brusatol was eight times less active than brusatol would indicate that the requirement of a C-15 ester moiety among brusatol related quassinoids for enhanced antimalarial activity could be quite specific. The semi-synthetic bruceoside-A acetonide and 15-methylcarbamoyl bruceolide were also found to be less active than brusatol. The antimalarial activity of 15-phenylalaninyl bruceolide is currently under evaluation.

Attempted synthesis of qinghaosu by methods shown in Schemes-5 and -6 was carried out. Compound 16 (Scheme-6) was synthesized in good yield. However, it failed to yield 18 by alkylation with 17 and LDA. By use of Zhou's method, we have synthesized qinghaosu from (-)-isopulegol.

New synthesis of qinghaosu (Scheme 9) involves the alkylation of cyclohexanone (11) with benzothiazolium lithium followed by dehydration with carbomethoxy-sulfamoyl triethylamine salt has led to the formation of the desired product 16a. Synthesis of 33 and 20 has also been achieved (Scheme-9).

Photooxygenation of the enol methyl ether of 8 (Scheme-10) led to the simpler qinghaosu analogs, 9a, 9b and 10. The antimalarial activity of these compounds will be evaluated shortly.

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(1) Objectives of the Contract -

The objective of this contract was to develop, via synthesis, potential novel antimalarial drugs including (A) α -santonin derived cyclic peroxides, (B) the brusatol derived glycosides, esters and carbamates, (C) the total synthesis of qinghaosu based upon Zhou's method as advised by my COTR and the Division of Experimental Therapeutics, Walter Reed Army Institute of Research (see my Annual Report dated 5/29/86 and 11/14/86), and (D) the new synthesis of qinghaosu and its novel simpler analogs. This report describes the synthesis of compounds for the foregoing objectives.

(2) Synthetic procedures for each target compound listed above -

A. α -Santonin-derived cyclic peroxide, ST-ST-peroxide-1 (BK70493)-Scheme-1.

a. 1,2-Dihydro- α -Santonin (II) - This known compound was prepared from santonin (I) according to an exact literature procedure² in quantitative yield.

b. 3-Hydroxy-6, 11 β H-eudesm-4-en-6, 13-olide (III) - This compound was prepared based upon Corey's procedure³ in his synthesis of an analogous compound. A solution of II (11.7 g) and aluminum isopropoxide (26 g) in dry 2-propanol (150mL) was allowed to boil gently in a 250-mL flask fitted with a Vigreux column. The acetone vapor formed was allowed to escape from the reaction mixture. After 6 hr. the 2-propanol was distilled and the reaction mixture was reduced to ca. a 50-mL volume. Cold 1 N hydrochloric acid was added, and the resulting acidic solution was extracted with methylene chloride. The extracts were washed with saturated sodium bicarbonate and saturated sodium chloride solutions and dried over sodium sulfate to afford an oily mixture (III) in ca. 80% yield. Compound III: IR (CHCl₃) 3620(OH), 3030 (C=C), 1775 and 1765 cm⁻¹ (mixture lactone C=O) cm⁻¹; NMR (250 MHz, CDCl₃, TMS) δ 1.18 (s, Me-10), 1.21 (d, J = 7.5 Hz, Me-11), 1.93 (s, Me-4), 2.85 (br. s, OH) (disappeared upon addition of D₂ O), 3.94 (t, J = 7.5 Hz, H-3), 4.62 (d, J = 9 Hz, H-6) and 3.32 (m, H-11).

c. 6, 11 H-eudesm-2, 4-dien-6, 13-olide (V) - This compound was synthesized from III via IV. To a solution of III (3 g) in anhydrous tetra-hydrofuran (50 mL) was added 4 mL of triethylamine. The mixture was cooled to 5° and treated with mesyl chloride (2 g). Stirring was continued for 2 hr. at 5°. The resulting triethylamine hydrogen chloride salt was filtered and the filtrate was evaporated in vacuo. The residue was added with water and extracted with methylene dichloride, dried over anhydrous magnesium sulfate and distilled under reduced pressure to yield crystalline residue (IV) upon cooling. Without further purification this residue was dissolved in DMSO (10mL) and treated with DBU (1,5-diazabicyclo [5.4.0] undecene-5) (1.5g). After stirring for 15 hr., the mixture was added 50 mL of ice-water and the resulting oil was extracted with methylene dichloride, washed with HCl, water, sodium bicarbonate and water, dried over anhydrous magnesium sulfate and evaporated in vacuo to furnish a residue. Recrystallization from ether-hexane gave V as colorless crystals in 25% yield. Compound V: MP 148°; IR (KBr) 3030 (C=C) and 1770 (lactone C=C) cm⁻¹; NMR (250 MHz, CDCl₃, TMS) δ 1.05 (3H, s, Me-10), 1.25 (3H, d, J = 7.5 Hz, Me-11), 1.97 (3H, d, J = 2.0 Hz Me-4), 2.28 (1H, m, H-11), 4.51 (1H, d, J = 11.0 Hz H-6) and 5.72 (2H, m, H-2 and H-3).

Anal calcd for C₁₅H₂₀O₂: m/z 232.1462. Found: m/z 232.1467.

Considerable efforts were directed toward the improvement of yield for the conversion of IV to V. Table-1 lists various reaction conditions which were carried out for this purpose. The reason for this low-yield step is due to the formation of Va.

d. ST-ST-peroxide-1 (or 2,5- α -peroxy-6,11 H-eudesm-3-en-6,13-olide) VI) - A solution of V (300 mg) and eosin Y (20mg) in pure benzene (300 mL) was irradiated with a 260-w photoflood lamp while a finely dispersed stream of purified oxygen was bubbled through the reaction mixture. After 30 hr. of irradiation, benzene was distilled under reduced pressure and the resulting oil was column chromatographed on silica gel (30 g). The benzene-ethyl acetate (1:1) eluate yielded 200 mg (60% yield) of the desired peroxide V as colorless prisms after recrystallization from ether. Compound VI: mp 143-145 $^{\circ}$; IR (KBr) 3030 (C-H) and 1760 (lactone C=O) cm^{-1} ; NMR (250 MHz, CDCl_3 , TMS) δ 0.99 (3H, s, Me-10), 1.24 (3H, d, J=7.5 Hz, Me-11), 2.07 (3H, d, J= 2.0 Hz, Me-4), 4.35 (1H, d, J=11.0 Hz, H-6), 4.58 (1H, m, H-2), 6.31 (1H, dd, J=7.5 and 2.0 Hz, H-3) and 2.32 (1H, m, H-II).

Anal. Calcd for $\text{C}_{15}\text{H}_{20}\text{O}_4$: m/z 264.1360. Found: m/z 264.1360.

B. The brusatol-derived glycosides, esters and carbamates

1. Bruceoside-A Acetonide (BK 69703) - Scheme-2.

a. The starting material bruceoside-A (1) was obtained by an initial extraction of air-dried fruits of Brucea javanica according to an exact procedure described in the literature¹ by the P.I., followed by column and preparative scale high performance liquid chromatographic purification.

b. Treatment of bruceoside-A (100 mg) with acetaldehyde diethyl acetal (40 mg) and p-toluene sulfonic acid (10mg) in chloroform (20mL) at room temperature for two days followed by silica gel column chromatography (1 g) of the resulting product yielded bruceoside-A acetonide as amorphous powder (60% yield): mp 185-190 $^{\circ}$; IR 3450 (br, OH), 1730-1760 (br, C=O) and 1640 (C=C) cm^{-1} ; NMR (250 MHz, CDCl_3 , TMS) δ 1.17 (3H, d, J=7.0 Hz, MeCHOO), 1.60 (3H, s, Me-10), 1.39 (3H, d, J = 5.0 Hz, Me-4), 1.6-2.1 (5H, m, H-5, H-6, H-9 and H-14), 1.94 (3H, d, J=1.5 Hz, Me-23), 2.20 (3H, d, J=1.5 Hz, Me-23), 2.4.-2.6 (1H, m, H-4), 3.35-3.80 (10H, m, sugar CH-O (6H) + 4 OH), 3.81 (3H, s, COOMe), 4.15-4.28 (2H, m, H-11 and H-12), 4.44 (1H, br. s, H-15), 4.6-4.8 (4H, m, H-7, H-17 and anomeric H), 5.62 (1H, s, H-22), 6.20 (1H, br. MeCHOO) and 6.85 (1H, s, H-1).

2. 15(E)-Non-2-enoylbruceolide (7) - Scheme 2.

a. The synthesis of this target compound was also based upon the discussion and recommendation during the site visit of Drs. R. O. Pick and H. A. Musallam on July 26, 1983.

b. 3-O-dimethyl-t-butylsilyl brusatol (8). A mixture of brusatol (2, 1.04g, 2 mmol), dimethyl-t-butylsilyl chloride (360 mg, 2.4 mmol) and imidazole (340 mg, 5 mmol) in dimethyl formamide (2 mL) was heated at 40-50 $^{\circ}\text{C}$ for 1.5 hr. The reaction mixture was poured into water and the resulting crystals were filtered and recrystallized from ether-petroleum ether to yield colorless crystals (1.14 g, 90% yield) of 8: mp 242-243 $^{\circ}\text{C}$; IR (KBr) 3500 (OH), 1735 (ester and lactone C=O), 1670 (α,β -unsat. C=O) and 1610 (C=C) cm^{-1} ; NMR (CDCl_3) δ 0.15 and 0.18 (3H each, s, Me_2Si), 0.96 (9H, s, Me_3C), 1.39 (3H, s, Me-10), 1.84 (3H, s, Me-4), 1.93 and 2.19 (3H each, s, Me-23), 2.32 and 2.93 (2H, ABq, J=15.2 Hz, H_2 -1), 2.39 and 2.96 (1H each, d-like,

H₂-6) 3.78 (3H, s, COOMe), 4.20 (1H, m, H-11), 4.26 (1H, m, H-12), 4.80 (1H, m, H-7), 5.62 (1H, s, H-22) and 6.27 (1H, br. s, H-15). Anal. (C₃₂H₄₆O₁₁Si) C, H; calcd m/z 619.2572 (m⁺-Me). Found m/z 619.2571.

c. 3-O-dimethyl-t-butylsilyl bruceolide (9). To a solution of 8 (500 mg) in methanol (30 mL) was added 1 N potassium methoxide in methanol (100 mL) at 0°C. After 12 hr, the reaction mixture was neutralized with Dowex 50W-X2 cation exchange resin and evaporated in vacuo. The residue was extracted with chloroform, washed with sodium bicarbonate, water, dried over anhydrous magnesium sulfate and distilled under reduced pressure to furnish 9 in 70% yield as amorphous powder: mp 158-160°C; IR (CHCl₃) 3580 (OH), 1730 (ester and lactone C=O), 1670 (α,β-unsat. C=O) and 1610 (C=C) cm⁻¹; NMR (CDCl₃) δ 0.13 and 0.18 (3H each, s, Me₂Si), 0.96 (9H, s, Me₃C-), 1.38 (3H, s, Me-10), 1.84 (3H, s, Me-4), 2.33 and 2.92 (2H, ABq, J=15.2 Hz, H₂-1), 2.37 and 2.90 (1H, each, d-like, H₂-6), 3.84 (3H, s, COOMe), 4.21 (1H, m, H-11), 4.26 (1H, m, H-12), 4.72 (1H, m, H-7) and 5.28 (1H, d, J = 11 Hz, H-15). Anal. (C₂₇H₄₀O₁₀Si) C, H; calcd m/z 537.2153 (m⁺-15). Found m/z 537.2157.

d. 3-O-dimethyl-t-butylsilyl, 15[(E)-non-2-enoyl bruceolide (10). To a 50 mL of chloroform solution of 15(E)-non-2-enoyl chloride (900 mg, 5.1 mmol), ²¹ obtained from treatment of 2-nonenic acid with thionyl chloride, was added dropwise with 9 (720 mg, 1.3 mmol) in pyridine (2 mL). After the mixture was stirred for 1 hr., it was further refluxed for 5 hrs. The mixture was added with diluted hydrogen chloride to remove pyridine, and then extracted with chloroform. The chloroform layer was washed with aqueous sodium chloride, water, sodium bicarbonate, aqueous sodium chloride and then water, dried over anhydrous magnesium sulfate and evaporated in vacuo to yield a red oil. This oil was column chromatographed over silica gel (10 g) and eluted with benzene and then chloroform-ethyl acetate (7:3). The eluate from the latter was dissolved in ether, and then added with petroleum ether to yield white powders (765 mg, 85% yield). Further purification of this product was achieved by preparative TLC (silica gel, chloroform-ethyl acetate (9:1), R_f = 0.30) to furnish 10 as amorphous white powders (585 mg, 65% yield): mp 120-125°C; IR (CHCl₃) 3700-3600 (OH), 1730 (C=O), 1670 (α,β-unsat. C=O) and 1600 (C=C) cm⁻¹; NMR (CDCl₃) δ 0.15 and 0.16 (3H each, s, Me₂Si), 0.90 (3H, m, Me-29), 0.96 (9H, s, Me₃C-), 1.50-1.12 [8H, m, (CH₂)₄-25-28] 1.39 (3H, s, Me-10), 1.84 (3H, s, Me-4), 2.07 (1H, d, J=4.0 Hz, H-9), 2.22 (2H, q, J=7.5 Hz, H₂-24), 2.37 and 2.92 (2H, ABq, J=15.2 Hz, H₂-1), 2.41 and 2.96 (1H each, d-like, H₂-6), 3.13 (1H, d, J=15.0 Hz, H-14), 3.76 (3H, s, COOMe), 4.20 (1H, s, H-12), 4.26 (1H, br. d, J=4.0 Hz, H-11), 4.72 and 3.81 (1H each, d, J=7.5 Hz, H-17), 4.78 (1H, m, H-7), 5.77 (1H, d, J=17.0 Hz, H-22), 6.33 (1H, d, J=15.0 Hz, H-15) and 7.05 (1H, sextet, J=17.0 and 7.5 Hz, H-23). Anal. (C₃₆H₅₄O₁₁Si) C, H; calcd m/z 633.2728 (M⁺-57). Found m/z 633.2750.

e. 15[(E)-non-2-enoyl] bruceolide (7). To a solution of 10 (611 mg, 0.9 mmol) in anhydrous tetrahydrofuran (1 mL) was added dropwise a 1.0 M solution of tetrabutylammonium fluoride in tetrahydrofuran (1.8 mL, 1.8 mmol). After the mixture was stirred for 15 min., it was poured into 30 mL of iced water. The resulting oily substance was extracted with chloroform. The chloroform layer was washed with diluted hydrogen chloride, water, diluted sodium bicarbonate and water, dried over anhydrous magnesium sulfate and evaporated under reduced pressure to afford an oil (460 mg). This oil was dissolved in ethyl acetate (1 mL) and then added with petroleum ether to yield a precipitate. Further purification of this precipitate by washing with petroleum ether several times and drying gave rise to colorless amorphous powders (7, 460 mg, 90% yield): mp 110-115°C; IR (CHCl₃) 3600-3400 (OH), 1750 (lactone C=O), 1730 (ester C=O), 1640 and 1665 [CO-C(OH)-C] and 1720 (C-C-COO) cm⁻¹; NMR (CDCl₃) δ 0.90 (3H, m, Me-29), 1.20-1.82 (8H, m, (CH₂)₄-25-28), 1.39 (3H, s, Me-

10), 1.84 (3H, s, Me-4), 2.13 (1H, d, J=7.0 Hz, H-9), 2.22 (2H, q, J=7.5 Hz, H₂-24), 2.39 and 2.98 (1H, each, d-like, H₂-6), 2.43 and 2.99 (2H, ABq, J=15.2 Hz, H₂-1), 3.13 (1H, d, J=15.0 Hz, H-14), 3.36 (1H, br.s, OH-12), 3.76 (3H, s, COOMe), 4.20 (1H, s, H-12), 4.26 (1H, d, J=7.0 Hz, H-11), 3.81 and 4.72 (1H each, d, J=7.5 Hz, H-17), 4.78 (1H, m, H-7), 5.77 (1H, d, J=17.0 Hz, H-22), 6.09 (1H, br.s OH-3), 6.33 (1H, d, J=15.0 Hz, H-15) and 7.05 (1H, sextet, J=17.0 and 7.5 Hz, H-23). Anal. (C₃₀H₄₀O₁₁2H₂O) C, H; calcd m/z 576.2568 (M⁺). Found 576.2579.

3. 15-Methylcarbamoyl bruceolide XII- Scheme -3.

a. The synthesis of XII employed a procedure using brusatol as a starting material. Compound IX, prepared from brusatol was described above.

b. Synthesis of Compound XI: To a solution of IX (530 mg) in absolute benzene (50 mL) was added MeNCO (82 mg). The mixture, after stirred at room temp. for 30 hrs., was washed with saturated NaCl solution, dried over anhydrous MgSO₄ and evaporated in vacuo to yield a residue. Recrystallization of this residue from ether-petroleum ether afforded XI (480 mg, 82% yield) as white prisms: mp 145-157°; IR (CHCl₃) 3450, 3550 (OH and NH), and 1730 (C=O) cm⁻¹ NMR (250 MHz, CDCl₃) δ 5.95 (1H, d, J = 15 Hz, H-15), 5.40 (1H, br. s, NH), 4.83 (1H, br. s, H-7), 4.71, 3.75 (2H, d each, J = 7.5 Hz, H-17), 4.24 (2H, br. s, H-11 and H-12), 3.79 (3H, s, COOCH₃), 2.80 (3H, d, J = 5.6 Hz, N-CH₃), 1.86 (3H, s, CH₃-4), 1.39 (3H, s, CH₃-10), 0.96 (9H, s, Si-t-butyl group), 0.16, 0.15 (6H, s each, SiMe₂) and 2.97 (1 H, d, J = 15.0 Hz, H-14).

Anal. Calcd for C₂₉H₄₃O₁₁ N Si: m/z 609.2596. Found: m/z 609 (M⁺-C₄H₉): m/z 552.1898. Found: m/z 552.1898.

c. Synthesis of 15-Methylcarbamoyl Bruceolide (XII): A solution of XI (222 mg, 0.36 mmol) in 80% acetic acid (2 mL) was heated at 80°C for 1.5 hr. with stirring. After the acetic acid was evaporated in vacuo, the residue was added with water (10 mL). The water-insoluble substances were filtered. The filtrate was evaporated under reduced pressure to give an oil (150 mg) which showed two spots on TLC (silica gel - EtOAc). Separation and isolation of Compound XII (80 mg) was achieved by PTLC (silica gel - EtOAc). Compound XII (50% yield) was recrystallized from acetone-ether: mp 204-206°; IR (IBr) 3600-3200 (br., OH, NH), 1730 (ester and lactone CO), 1640 (OCONHMe), and 1660 (α,β-unsat. CO) cm⁻¹; NMR (250 MHz, DMSO-d₆-TMS) δ 7.77 (1H, br. s, NH), 5.44 (1H, br. s, H-15), 4.92 (1H, br. s, H-7), 4.42, 3.69 (2H, d each, J= 7.5 Hz, H-17), 4.06 (1H, s, H-12), 4.02 (1H, d, J = 7.5 Hz, H-11), 3.69 (3H, s, COOCH₃), 2.86 (3H, s, N-CH₃), 2.76 (1H, d, J= 15.0 Hz, 14-H), 2.76 (2H, q, J = 5.0 Hz, H-1), 2.11 (1H, dd, J = 15.0 Hz and 2.0 Hz, H-5), 1.81 (2H, dt, J = 15.0 Hz and 2.0 Hz, H-6), 1.69 (3H, s, CH₃-4) and 1.17 (3H, s, CH₃-10).

Anal. Calcd for C₂₃H₂₉O₁₁ N: m/z 495.1739. Found m/z 495.1722.

d. Considerable efforts were made to remove the dimethyl-t-butylsilyl protecting group from IX in order to make XII. When XI was treated with tetrabutylammonium fluoride in THF at room temperature, Compound XIII instead of Compound XII was obtained in ca. 80% yield.

4. 15-Phenylalaninyl bruceolide (XVI) - Scheme 3.

a. Synthesis of Compound XIV - To a stirred solution of IX (600 mg) in anhydrous benzene (30 mL) was added freshly distilled anhydrous pyridine (2mL) and N-tert butoxycarbonyl-L-phenylalanine anhydride⁵ (700 mg). After 20 hrs., the mixture was washed with water, diluted HCl, water, NaHCO₃, saturated NaCl and water. The benzene

layer was dried over anhydrous MgSO_4 and evaporated in vacuo to yield a residue (800 mg). Recrystallization of this residue from ether afforded XIV as colorless needles (630 mg, 72% yield): mp 233-235 $^\circ$; IR (KBr) 3600-3400 (OH and NH), 1740 (lactone CO), 1725 (ester CO), 1670 (NHCO), 1640 (C=C-CO) and 1610 (C=C-CO) cm^{-1} ; NMR (250 MHz, CDCl_3) δ 0.15, 0.16 (3H each, s, SiMe_2), 0.93 (9H, s, SiMe_3), 1.35 (9H, s, O-CMe $_3$), 1.38 (3H, s, Me-10), 1.83 (3H, s, Me-10), 1.83 (3H, s, Me-4), 2.02 (H-9), 2.34 (2H, m, H-6 and H-1), 2.92 (2H, m, H-6 and H-1), 3.19 (1H, d, $J = 7.50$ Hz, H-14), 3.79 (3H, s, COOMe), 4.24 (2H, brm, H-11 and H-12), 3.75, 4.69 (2H, d each, $J = 7.50$ Hz, H-17), 4.78 (1H, s-like, H-7), 6.30 (1H, d, H-15) and 7.27 (5H, aromatic protons).

Anal. Calcd. for $\text{C}_{41}\text{H}_{57}\text{O}_{13}\text{N}$ Si: m/z 799.3583. Found: m/z 742 [$\text{M}^+ - 57$ (t-BOC group)] and m/z 784 [$\text{M}^+ - 15$ (Me group)]

b. Synthesis of XV - To a stirred solution of XIV (1.23 g, 1.53 mmol) in THF (8 mL) was added $\text{Bu}_4\text{N}^+\text{F}^-$ (4 mL, 4 mmol) at 0 $^\circ$. After 10 min., the reaction mixture was poured into iced water (20 mL) and extracted with chloroform. The chloroform extract was washed with water, 3% aqueous Na_2CO_3 and water, dried over anhydrous MgSO_4 , and evaporated under reduced pressure to give a residue. Recrystallization from EtOAc-ether yielded XV as colorless prisms (990 mg, 94% yield): mp 213-215 $^\circ$; TLC (silica gel, EtOAc- CHCl_3 (1:1), Rf = 0.3); IR (KBr) 3550-3300 (OH and NH), 1740 and 1720 (lactone and ester CO), 1640 (NHCO) and 1610 (α,β -unsat. CO) cm^{-1} ; NMR (250 MHz CDCl_3) δ 1.35 (9H, s, CMe $_3$), 1.39 (3H, s, Me-10), 1.84 (3H, d, $J = 1.0$ Hz, Me-4), 3.20 (2H, d-like, $J = 13.0$ Hz, CH_2Ph), 3.81 (3H, s, COOMe), 4.26 (2H, m, H-11 and H-12), 4.53 (1H, m, $\text{CH-CH}_2\text{Ph}$), 3.75, 4.70 (1H each, d, $J = 7.5$ Hz, H-17), 4.77 (1H, s-like, H-7, 5.00 (1H, d, $J = 11.0$ Hz, NHCO), 6.32 (1H, d, $J = 13.0$ Hz, H-15) and 7.27 (5H, m, aromatic protons).

Anal. Calcd for $\text{C}_{35}\text{H}_{43}\text{O}_{13}\text{N}$: m/z 685.2747. Found (FAB): m/z 686 ($\text{M}^+ + \text{H}$) and m/z 586 [$(\text{M}^+ - 99)$ (t-BOC)]

c. Synthesis of XVI - A solution of XV (830 mg) in anhydrous EtOAc (20 mL) was cooled to 0 $^\circ$, and anhydrous HCl gas was introduced into this solution for about 40 min. The reaction mixture was allowed to stand at 0 $^\circ$ for 30 min in the nitrogen atmosphere. The resulting crystals were filtered, washed with cold EtOAc and dried to furnish XVI (700 mg, 93% yield) as colorless needles: mp 225-227 $^\circ$; TLC (silica gel, EtOAc, Rf = 0.40); IR (KBr) 3500-3200 (OH and NH $_2$), 1760 (lactone CO) and 1740 (ester CO) cm^{-1} ; NMR (250 MHz, CD_3OD) δ 1.38 (3H, s, Me-10), 1.84 (3H, s, Me-4), 1.90 (1H, d, $J = 5.1$ Hz, H-9), 3.00 (1H, H-5), 2.55, 2.86 (1H each, d, $J = 15.0$ Hz, H-1), 3.44 (1H, d, $J = 15.0$ Hz, H-14), 3.69 (3H, s, COOMe), 3.06, 3.34 (1H each, q, $J = 15.0$, 4.0 and 15.0, 7.0 Hz, H-23), 4.38 (1H, q, $J = 4.0$ and 7.0 Hz, H-22), 4.18 (1H, d; $J = 5.6$ Hz, H-11), 4.32 (1H, s-like, H-12), 3.72, 4.70 (1H each, d, $J = 7.0$ Hz, H-17), 4.92 (1H, m, H-7), 6.37 (1H, d, $J = 15.0$ Hz, H-15) and 7.35 (5H, m, aromatic protons).

Anal. Calcd for $\text{C}_{30}\text{H}_{35}\text{O}_{11}\text{N} \cdot \text{HCl} \cdot 3\text{H}_2\text{O}$: C 55.25%, H 6.08% and N 2.14%. Found: C 55.09%, H 6.30% and N 2.51%.

Also Anal. Calcd for $\text{C}_{30}\text{H}_{35}\text{O}_{11}\text{N}$ (FAB): m/z 585 (M^+). Found: m/z 586 ($\text{M}^+ + \text{H}$).

C. The total synthesis of qinghaosu (1) - Schemes-4-8.

1. Earlier attempted new synthesis of 1 - Schemes-4-6

As stated in the original contract proposal, pages 47 and 48, our initial efforts were directed toward a much simpler and more efficient synthesis of qinghaosu (1) compared to the first total synthesis of 1 by Schmid and Hofheinz (Scheme-4) which was published as a short communication.⁶ Based upon our proposed Scheme-5, compound 16 was synthesized from (-)-isopulegol (2) via 3, 4 and 15 according to Scheme-6 without difficulty. Compound 4 and 15 were characterized as crystalline derivatives 4b and

15b, respectively. Attempted alkylation of 15 or 16 with ketal chloride 17 in LDA/THF failed to yield the target compound 18. Benzoylation of 4 with benzyl chloride in NaH-DMSO according to Iwashige and Saeki⁷ also failed to provide 4f in good yield. Thus, 4 was benzoylated to 4c. Oxidation of 4c with $\text{CrO}_3\text{-AcOH}$ gave 4d which was planned for an alkylation reaction study. An alternative synthesis of 18 via a key intermediate 2b was also planned. Thus, isopulegone (2a) was prepared from 2 by Jones' oxidation. However, attempted alkylation of 2a with 17 or 17a (prepared from 1-chloro-3-butanone) in LDA/THF at -78° failed to yield 2b. Model studies on alkylation of R-(+)-pulegone (30) with 17a in LDA/THF or in Lithium N-isopropylcyclohexylamide (LiICA)/THF also failed to give 31 despite the fact that it gave 70% yield of a 3:1 mixture of 32 and 33 with MeI in LiICA/THF. Alkylation of 30 with trimethyl-(3-iodo-1-methyl-1-propenyl) silane (17c) in LiICA/THF to yield 34 was planned. Compound 17c was prepared according to a literature method.⁸ The compounds listed in Scheme-6 were synthesized as follows.

a. (-)-Isopulegol (2) - The commercially available (-)-isopulegol (from Pfaltz and Bauer Co.) was purified by column chromatography on silica gel in hexane to a product which showed $[\alpha]_D -10^\circ$ (c 0.5, CHCl_3).

b. Compound 3 - (-)-Isopulegol (2, 4.62 g), dimethyl-t-butyldimethylsilyl chloride (5.43 g), imidazole (4.89 g) was dissolved in 2 mL of dimethyl formamide. After standing at room temperature for 3 hrs, the mixture was poured into ice water and extracted with benzene. The benzene extract was washed with water, dried over anhydrous magnesium sulfate and evaporated in vacuo to give 3 as an oil (85%); TLC (silica gel-petroleum ether, $R_f = 0.4$, one spot); IR 3080 and 1640 ($\text{C}=\text{C}$) cm^{-1} .

c. Compound 4 - To a solution of 3 (2.68 g) in absolute THF (5 mL) was added dropwise 5 mL of a $\text{B}_2\text{H}_6\text{-THF}$ 1 solution under dry nitrogen and cooled to 0°C . After stirring the reaction mixture for 1 hr at room temperature, 1.5 mL of water was added slowly, and the mixture was further stirred until the cease of the generation of hydrogen gas. The mixture was then cooled, and added slowly with 3M aqueous NaOH (2 mL) and 30% H_2O_2 (1.1 mL). After stirring for 1 hr at room temperature, the mixture was added with water and extracted with benzene. The benzene layer was washed with saturated aqueous NaCl solution, dried over MgSO_4 and evaporated under reduced pressure to yield a residue. Column chromatography of this residue on silica gel in chloroform afforded 4 (1.9 g; 66% yield; TLC $R_f = 0.21$) as an oil: IR 3400 (OH) cm^{-1} (the IR spectrum also showed the disappearance of double bond absorption); NMR (CDCl_3 , 250 MHz) δ 3.6-3.3 (3H, m, H-3 and H-10), 2.02-0.90 (9H, m, H-1, H-2, H-4, H-5, H-6 and H-8), 0.85 (3H, d, $J = 7.0$ Hz) and 0.82 (3H, d, $J = 7.0$ Hz) (Me-1 and Me-8), 0.81 (9H, s, $\text{Me}_3\text{-Si}$) and 0.01 (6H, s, SiMe_2).

d. Compound 4a - The 3,5-dinitrobenzoate derivative of 4 was prepared by treatment of 4 with 3,5-dinitrobenzoyl chloride in dry benzene as usual. Compound 4a: mp $94\text{-}95^\circ\text{C}$; IR (KBr) 1720 (ester CO), 1640 (aromatic), 1540 and 1345 (NO_2) cm^{-1} .

e. Compound 4b - To a solution of 4 (200 mg, ca. 7 mmole) in tetrahydrofuran (5 mL) was added a solution of $n\text{-Bu}_4\text{NF}$ (1 mL, ca. 1 mmole) in tetrahydrofuran (3 mL). After the mixture was stirred for 2 hrs at room temperature, it was poured into water and extracted with chloroform. The chloroform extract was washed with saturated NaCl solution, dried over anhydrous MgSO_4 , and evaporated in vacuo to yield a crystalline residue. Recrystallization of the residue from ether-petroleum ether afforded the diol 4b (102 mg, 85% yield): mp $99\text{-}102^\circ\text{C}$; MS m/z 172 (M^+) ($\text{C}_{10}\text{H}_{20}\text{O}_2$), 154 ($\text{M}^+ - 120$) and 136 ($\text{M}^+ - 2\text{H}_2\text{O}$); IR 3300-3100 (OH) and 1040 (C-O) cm^{-1} ; NMR (CDCl_3) δ 0.921 (3H, d, $J = 6.6$ Hz), 0.960 (3H, d, $J = 7.3$ Hz) (two methyl groups at C-1 and C-8), 3.464 (1H, dt,

J = 4.2 and 10.0 Hz, H-3) and 3.588 and 3.665 (2H, AB part of an ABX, J = 3.6, 5.4 and 10.7 Hz, CH_2OH).

f. Compound 15 - The keto acid (15) was prepared by either one of the following two methods:

Method-a: To a solution of 4 (573 mg) in acetone (10 mL, purified by addition of KMnO_4 followed by distillation) was added dropwise Jones' reagent at 5°C . After 30 min. the mixture was further added dropwise with stirring at room temperature until the absence of the orangish yellow color of the reagent. Addition of small amount of water to dissolve the precipitate was needed. The unreacted reagent was destroyed by addition of isopropanol. The resulting mixture was distilled in vacuo and the residue partitioned between water and chloroform. The chloroform layer was washed with water and extracted with aqueous saturated Na_2CO_3 solution. The Na_2CO_3 layer was acidified with dilute HCl and further extracted with CHCl_3 . The CHCl_3 extract was washed with water, dried and evaporated to furnish 15 as an oil (227 mg, 61.5% yield, $R_f = 0.49$) after column chromatographic purification on silica gel in chloroform.

Method-b: This involves the isolation of an aldehyde intermediate (15a) followed by oxidation to 15. To a solution of 4 (1 g, 3.5 mmol) in acetone (30 mL) was added dropwise 2 mL of Jones' reagent at 5°C . After the reaction was complete, the excess chromic acid was decomposed by addition of isopropanol. The mixture was adjusted to pH 6-7 with aqueous NaHCO_3 , followed by distillation of acetone. The residue was added with water and extracted with chloroform. The chloroform extract was washed with water, dried (MgSO_4), and distilled to yield 979 mg of an oil (15a). This oil was dissolved in acetone (30 mL) and oxidized with Jones' reagent in the same way described above. The resulting product was extracted with ether. The ether layer was washed with water and further extracted with saturated aqueous Na_2CO_3 solution. Acidification of the Na_2CO_3 layer with diluted HCl followed by extraction with CHCl_3 gave rise to 15 [455 mg, 70.7% yield, TLC $R_f = 0.49$ (silica gel, $\text{CHCl}_3\text{-MeOH} = 10:1$)]: IR (CHCl_3) 3300-2700 (br, COOH), 1770 (six-membered CO) and 1700 (COOH) cm^{-1} .

It is of note that the NMR spectrum of 15 indicated that it might still be a mixture despite it showing only a foregoing single spot on TLC. However, this compound can be used directly without further purification for the synthesis of pure 16 in good yield.

g. Compound 15b - A mixture of 15 (85 mg), thionyl chloride (500 mg) and dry benzene (5 mL) was refluxed for 1 hr. The solvent and excess thionyl chloride were distilled off. The oily residue, without purification, was dissolved in dry benzene (5 mL) and added dropwise to a solution of 3,5-dinitrobenzyl alcohol (80 mg) containing a small amount of pyridine. After the mixture was refluxed with stirring for 2 hrs, it was washed successively with aqueous NaHCO_3 , diluted HCl , saturated NaCl and water, and dried over anhydrous MgSO_4 . Distillation of the solvent in vacuo gave a residue. Purification of this residue by preparative TLC (silica gel in CHCl_3 , $R_f = 0.32$) followed by recrystallization from ether furnished 15b: mp $118\text{-}120^\circ\text{C}$; MS m/z 364 (M^+) ($\text{C}_{17}\text{H}_{20}\text{O}_7\text{N}_2$), 336 ($\text{M}^+ - 28$) and 320 ($\text{M}^+ - 44$); NMR (CDCl_3) δ 8.961 (1H, t, J = 2.0 Hz), 8.467 (2H, d, J = 2.0 Hz) (aromatic protons), 4.894, 4.841 (2H, ABq, J + 13.0 Hz, $-\text{OCH}_2\text{-Ph}$), 1.170 (3H, d, J = 7.3 Hz) and 1.008 (3H, d, J = 6.7 Hz) (two methyl groups at C-1 and C-8).

h. Compound 16 - A solution of 15 (1 g, 5.4 mmol) in ether (10 mL) was methylated with an ether solution of diazomethane (500 mg). The solution was allowed to stand at room temperature for 10 hrs. The excess diazomethane and ether were

distilled to give 16 as an oil [1.02 g, 95% yield, TLC Rf = 0.53 (silica gel, CHCl_3)]: IR (CHCl_3) 1730 (COOMe) and 1710 (six-membered CO) cm^{-1} ; NMR (CDCl_3 , 250 MHz) δ 3.67 (3H, s, COOCH_3), 1.21 (3H, d, $J = 7.0$ Hz), 1.05 (3H, d, $J = 7.0$ Hz) (Me-1 and Me-8), 2.79 (1H, m, H-8) and 2.59 (1H, m, H-4).

i. Compound 17 - To a solution of 4-chloro-2-butanone (5 g) in dry benzene (5 g) which was placed in a Dean-Stark apparatus, was added ethylene glycol (5 g) and p-toluene-sulfonic acid (100 mg). The mixture was heated at ca. 90°C . After 80 mL of benzene was distilled off, the reaction mixture was poured into saturated aqueous NaHCO_3 solution and extracted with benzene. The benzene extract was washed successively with aqueous NaCl and water, dried over anhydrous MgSO_4 and evaporated *in vacuo*. The resulting residual oil was distilled under reduced pressure to yield pure 17 as an oil (3 g, 42.6% yield): bp $48^\circ\text{C}/1$ mm Hg; NMR (CDCl_3) δ 1.37 (3H, s, CH_3), 2.290 (2H, dd, $J = 6.0$ and 9.0 Hz, $-\text{CH}_2\text{CH}_2\text{Cl}$), 3.62 (2H, dd, $J = 6.0$ and 9.0 Hz, $-\text{CH}_2\text{CH}_2\text{Cl}$) and 3.98 (4H, s, $-\text{OCH}_2\text{CH}_2\text{O}-$).

j. Alkylation of 15 - Alkylation of the keto acid (15) with LDA/THF at -78° based upon a procedure described below for the alkylation of 16 to 16a gave rise to the recovery of the starting material only.

k. Alkylation of 16 to 16a - Absolute tetrahydrofuran (THF, 2 ml) was placed in a three-necked 50 ml flask under N_2 stream at -78° (dry ice-acetone bath). To which, diisopropylamine (0.5 ml) and 1.5M n-BuLi-hexane (1.7 ml) were added. After 30 min., a solution of keto ester (16) (500 mg) in THF (2 ml) was added dropwise. After the mixture was stirred for 30 min., a solution of ketal chloride (17, 380 mg) in THF was added dropwise. After the mixture was allowed to stand overnight, it was poured into dil. HCl and extracted with CHCl_3 . The CHCl_3 layer was washed with dil. Na_2CO_3 and H_2O , and dried over anhydrous MgSO_4 . Evaporation of the solvent *in vacuo* gave an oily residue (649 mg). Separation of this residue afforded a starting material (210 mg) and a lactonic substance [16a (180 mg): IR (CHCl_3)-1740 and 1680 cm^{-1} ; NMR (CDCl_3) δ 4.619 (1H, m, CH-O), 1.807 (3H, t, $J = 1.5$ Hz vinyl- CH_3) and 1.009 (3H, d, $J = 6.7$ Hz, sec- CH_3)]. No alkylation product, such as 18, was detected.

l. Benzylation of 4 - Benzylation of 4 (19.5 g) with NaH-DMSO under N_2 according to Iwashige and Saeki⁷ gave poor yield (0.4 g) of 4f, 12g of the starting material 4 and 0.5 g of 4e.

m. Benzoylation of 4 to 4c - A mixture of hydroxy silyl ether (4, 1.27 g), benzoic anhydride (1.13 g), and pyridine (3 ml) was heated at 60° in a water bath for 6 hrs. the reaction mixture was poured into water (30 ml) and extracted with chloroform. The chloroform layer was washed successively with dil. HCl, dil. Na_2CO_3 , and H_2O , and dried over MgSO_4 . Evaporation of the solvent under reduced pressure gave an oily residue (2.6 g). This residue was chromatographed on silica gel (48 g) and eluted with hexane and 10%- CHCl_3 -hexane to furnish an oily substance [4c (1.45 g, 84% yield): IR (CHCl_3) - 1705, 1600 and 1270 cm^{-1} ; NMR (CDCl_3 , with a CH_2Cl_2 line as an internal standard as silylmethyl groups were present in the molecule) δ 8.087-7.433 (5H, aromatic protons), 4.322 (1H, dd, $J = 4.5$ and 10.8 Hz), 4.180 (1H, dd, $J = 8.7$ and 10.8 Hz, AB part of ABX) (CH_2OCOPh), 3.582 (1H, dt, $J = 4.2$ and 10.2 Hz, i-O-CH), 1.107 (3H, d, $J = 7.0$ Hz, sec-Me), 0.939 (3H, d, $J = 6.8$ Hz, sec-Me), 0.919 (9H, s, tert-butyl) and 0.106 and 0.088 (3H, each, s, Me_2Si).

n. Oxidation of 4c to 4d - To a solution of silyl ether benzoate (4c, 1.04 g) in AcOH (2ml) was added dropwise a solution of CrO_3 (280 mg) in AcOH (4 ml) containing H_2O (1 ml) in an ice-water bath. After the mixture was stirred at room temperature

for 8 hrs. it was poured into water and extracted with ether. The ether layer was washed with water to remove as much acetic acid as possible, and then with dil. Na_2CO_3 and H_2O . The organic layer was dried over MgSO_4 and evaporated to afford an oily residue (963 mg). The residue was chromatographed on silica gel (41 g) and eluted with 10% CHCl_3 -hexane to give an oily substance [4d (501 mg,) 70% yield]: IR (CHCl_3) - 1700, 1600 and 1270 cm^{-1} ; NMR (CDCl_3) δ 8.039-7.405 (5H, aromatic protons), 4.322 (1H, dd, $J = 5.2$ and 10.9 Hz), 4.284 (1H, dd, $J = 6.3$ and 10.9 Hz , AB part of ABX) ($\text{CH}_2\text{O-COPh}$), 1.087 (3H, d, $J = 6.7\text{ Hz}$, sec-Me) and 1.014 (3H, d, $J = 6.1\text{ Hz}$, sec-Me).

o. Preparation of Isopulegone (2a) - To a stirred solution of isopulegol (2, 2 g) in acetone (10 ml) at 0° was added dropwise Jones' reagent until a pale-orange color just disappeared. The solution was stirred at below 10°C for 2 hrs. After evaporation of acetone, the residue was extracted with CHCl_3 . The CHCl_3 layer was washed with water, dried (MgSO_4), and evaporated to dryness to give an oil. The crude product was separated by column chromatography (silica gel in benzene) to give isopulegone (2a) as an oil (yield: 1.5 g): IR (CHCl_3)-1700 (CO) and $1642\text{ (CH}_2\text{=C) cm}^{-1}$; NMR (CDCl_3 , 60 MHz) δ 1.78 (3H, s, vinyl CH_3), 4.75 and 4.97 (1H each, s-like x 2, -CH_2).

p. Alkylation of 2a - To a stirred solution of diisopropylamine (400 mg) in THF (5 ml) at -78°C was added n-butyllithium (2.6 ml, 1.66 M in hexane). The reaction was stirred at -78°C for 1 hr, and then isopulegone (2a) (250 mg) in THF (2 ml) was added. After 1.5 hrs., 1-chloro-3, 3-ethylene-dioxybutane (17) (450 mg) in thf (2 ml) was added. The reaction was stirred for 3 hrs at -78°C and 12 hrs at r.t. The reaction was quenched with water, and extracted with ether. The ether layer was washed with brine, dried (MgSO_4), and evaporated to dryness to give an oil. However, this oil corresponded to the starting material 2a and 17 and not the target compound 2b.

q. Preparation of 1-Iodo-3,3-ethylenedioxybutane (17a) - Compound 17a was prepared according to Trost and Kunz [*J. Am. Chem. Soc.*, **97**, 7152 (1975)]. A solution of sodium iodide (8.7 g) in acetone (90 ml) was added to 1-chloro-3-butanone (5 g), and reaction was refluxed for 5 hrs. and further stirred for 12 hrs. at room temperature. After evaporation of acetone, the residue was extracted with CH_2Cl_2 , and the CH_2Cl_2 layer was washed with 5% aqueous sodium thiosulfate solution, aqueous sodium chloride solution, dried (MgSO_4), and evaporated to dryness to give 1-iodo-3-butanone as an oil. A mixture of crude 1-iodo-3-butanone, ethylene glycol (5 g) and p-toluene sulfonic acid (0.1 g) in benzene (100 ml) was refluxed with a Dean-Stark trap for 5 hrs.

After the reaction was allowed to cool, the solution was washed with aqueous sodium bicarbonate solution, 5% aqueous sodium thiosulfate solution, aqueous sodium chloride solution, dried (MgSO_4), and evaporated to dryness to give a brown oil (8 g). This product was purified by distillation to give the iodo ketal (17a) (3g) as a yellow liquid; bp $75^\circ\text{C}/1.5\text{ mm Hg}$; NMR (CDCl_3 , 60 MHz) δ 3.95 (4H, s, $\text{-O-CH}_2\text{CH}_2\text{-O-}$), 3.18 (2H, m), 2.25 (2H, m) ($\text{C-CH}_2\text{CH}_2\text{I}$) and 1.30 (3H, s, CH_3)

r. Alkylation of R-(+)-pulegone (30) - This was based upon a method described by Lee et al [R. A. Lee, C. McAndrews, K. M. Patel and W. Reusch, *Tetrahedron Lett.*, 965 (1973)]. To a stirred solution of N-isopropylcyclohexylamine (690 mg) in THF (3 ml) at 0°C was added n-butyllithium (3.2 ml). The reaction was stirred at 0°C for 1.5 hr., and then pulogone (30, Aldrich Chemical Company, 200 mg) in THF (2 ml) was added at 0°C . After 15 min., methyl iodide (295 mg) in THF (2 ml) was added. The

reaction was stirred for 30 min. at 25° C. The reaction was quenched with water, and extracted with ether. The ether layer was washed with brine, dried (MgSO₄), and evaporated to dryness to give an oil. The crude product was separated by preparative thin layer chromatography. Upper produce: 3:1 mixture of 32 and 33 (70% yield) (one spot on TLC); NMR (CDCl₃, 60 MHz, H for protons of 32 and H' for protons of 33) δ 0.97-1.82 (6H + 3 H', dx₃, CH-CH₃), 1.25 (3H', s, -c-CH₃), 1.83 (3H + 3H' s -CH₃), 1.91 (3H, s, -c-CH₃), 4.89 and 4.97 (1H each, s-like x2, -CH₂). Lower product: starting material (10% yield) (30).

s. Preparation of Trimethyl-3-iodo-1-methyl-1-propenyl silane (17c) - Compound 17c was prepared according to Gawley⁸ from propargyl alcohol 35 via 36, 37, 38 and 39 as shown in Scheme-6.

2. Total synthesis of 1 based upon Zhou's method - Scheme-8.

At this stage our efforts were directed toward the synthesis of qinghaosu (1) based upon a most recent synthetic route (Scheme-7) presented by Zhou⁹ as an Abstract at the 1984 Hawaii ACS meeting after consulting with Dr. A. J. Lin, our new Contracting Officer's Technical Representative. If we are successful in this synthesis, then we will work on the improvement of yield to provide an efficient total synthesis of 1. The key intermediate 13 (Scheme-8) involved in this synthesis was synthesized by us prior to Zhou's publication of his oral presentation as a full paper in April 1986.¹⁰ We have very recently also achieved the synthesis of 1 from 13 (see Annual Report dated 11/14/86). The synthesis of compounds listed in Scheme-8 are described below. It is to note that our synthesis of Zhou's compound 4 was from (-)-isopulegol instead of from (+)-citronellal according to Schmid and Hofheinz⁶ mentioned above. The synthesis of 6 from x via y, z and a was based upon Boeckman's method.¹¹

a. Compound 3 from Methoxymethylation of (-)-Isopulegol - Methoxymethyl chloride (89.0 g) was added dropwise to a mixture of (-)-isopulegol (2) (113.6 g), dimethylaniline (134.2 g), and dry dichloromethane (100 ml) with stirring in an ice-water bath. The reaction mixture was stirred at room temperature for 3 hrs. The mixture was poured into water and extracted with benzene. The benzene layer was washed with dil. HCl and H₂O, and dried over anhydrous magnesium sulfate. The solvent was evaporated in vacuo to afford an oily residue (143.5 g, almost pure, yield 98.2%). This residue was distilled under reduced pressure to give pure 3 as a colorless liquid (83-84°/1.5 mm Hg, 82.0 g): IR (CHCl₃) 1040 (C-O), 1645 and 900 (C = CH₂) cm⁻¹; and NMR (CDCl₃) δ 4.790 (2H, m, C = CH₂), 4.716, 4.548 (2H, ABq, J = 7.0 Hz, -O-CH₂-O-), 3.447 (1H, dt, J = 6.4, and 9.5 Hz, O-CH<), 3.340 (3H, s, OCH₃), 1.733 (3H, dd, J = 1.4 and 0.9 Hz, C = C-CH₃), 0.933 (3H, d, J = 6.5 Hz, sec-CH₃).

b. Compound 3a from Hydroboration and Oxidation of 3 - To a solution of 3 (97.8 g) in absolute THF (25 ml) was added dropwise borane-THF complex (1 M solution, 250 ml) with stirring under nitrogen atmosphere at 0°C. After the addition was complete, the reaction mixture was stirred at room temperature for 1 hr. Water (75 ml) was added to the mixture and stirred until evolution of H₂ gas ceased. The reaction mixture was cooled in an ice-water bath, and then added with 3 M NaOH solution (100 ml) and 30% hydrogen peroxide solution (56.3ml) dropwise, successively. After the addition, the mixture was warmed up to room temperature and further stirred for an additional hour. The reaction mixture was poured into ice-water and extracted with benzene. The benzene layer was washed with a saturated NaCl solution and dried over MgSO₄. Evaporation of the solvent gave an oil (104.7 g). This oil was distilled under reduced pressure to give 3a as a pure oil (84.6 g, 79.2%): NMR (CDCl₃) δ 4.777, 4.635 (2H, ABq, J = 6.8 Hz, -O-CH₂-O-), 3.759-3.353 (3H, m, -CH₂-OH and -CH-OH), 3.407

(3H, s, OCH₃), 0.934 (3H, d, J = 7.2 Hz, sec-CH₃), and 0.915 (3H, d, J = 6.5 Hz, sec-CH₃); IR 3600, 3450 (OH) and 1060 (C-O cm⁻¹).

c. Compound 3b from Benzylation of 3a - A solution of 3a (73.6 g) in absolute THF (180 ml) and DMF (60 ml) was added dropwise to a suspension of sodium hydride (12.7 g in THF (180 ml) and DMF (60 ml) with stirring at 0°C under nitrogen atmosphere. The reaction mixture was stirred at 0°C for 1.5 hrs., added with benzyl bromide (115.6 g), and further stirred at room temperature for 2.5 hrs. The reaction mixture was poured into ice-water and extracted with ether. The ether layer was washed with water and dried over anhydrous magnesium sulfate. Evaporation of the solvent gave an oily residue (167.2 g). This oil chromatographed on silica gel column to give 3b, a pure oil (74.8 g, 72%): IR (CHCl₃) 1040 (C-O) cm⁻¹; and NMR (CDCl₃) δ 7.349-7.200 (5H, aromatic protons), 4.739, 4.605 (each 1H, Abq, J = 6.8 Hz, -O-CH₂-O-), 4.490 (2H brs, PhCH₂-O), 3.355 (3H, s, OCH₃), 3.541-3.219 (3H, m, -CH₂-O and CH-O), 0.991 (3H, d, J = 7.0 Hz, sec-CH₃) and 0.907 (3H, d, J = 6.5 Hz, sec CH₃).

d. Compound 3c from Demethoxymethylation of 3b - A solution of 3b (73.6 g) in MeOH (400 ml) containing 1 ml of HCl was heated at 65° in a water bath for 10 hrs. The solvent was distilled, added with H₂O and extracted with CHCl₃. The CHCl₃ layer was washed with dil. Na₂CO₃ and H₂O and dried over anhydrous MgSO₄. Evaporation of the solvent gave 3c as an oil (62.5 g, 99%): IR (CHCl₃) 3400 cm⁻¹ (OH); and NMR (CDCl₃) δ 7.356-7.200 (5H, aromatic protons), 4.551, 4.476 (each 1H, ABq, J = 11.9 Hz, Ph CH₂-O-), 3.505 (1H, dd, J = 6.1 and 9.1 Hz), 3.384 (1H, dd J = 3.7 and 9.1 Hz, AB part of ABX) (-O-CH₂-CH), 0.958 (3H, d, J = 7.3 Hz, sec-CH₃) and 0.909 (3H, d, J = 6.5 Hz, sec-CH₃).

e. Compound 4 from Oxidation of 3c - A solution of 3c (18.8 g) in dry CH₂Cl₂ (25 ml) was added to a suspension of pyridinium chlorochromate (22.1 g) in dry CH₂Cl₂ (100 ml) with stirring. The reaction mixture was stirred at room temperature for 2 hrs and the supernatant was decanted from the black gum. The insoluble residue was washed with ether (3 x 100 ml). The combined solvent was passed through a short column of Florisil. The solvent was evaporated to afford an oil (18.6 g). This oil was distilled under reduced pressure to give 4 as a pure oil (170-174°/1.2-1.5 mm Hg, 11.3 g, 61%): IR (CHCl₃) 1700 (C=O) cm⁻¹; and NMR (CDCl₃) δ 7.356-7.200 (5H, aromatic protons), 4.479 (2H, s, PhCH₂-O), 3.482 (1H, d, d, J = 5.3 and 9.1 Hz, AB part of ABX) (-O-CH₂CH), 1.017 (3H, d, J = 6.8 Hz, sec-Me), and 1.002 (3H, d, J = 6.0 Hz, sec-Me).

f. Compound y from Trimethylsilylation of x - A 3 L three necked flask was charged with magnesium turnings (61 g) and absolute THF (2000 ml), and a solution of vinyl bromide (x, 268 g) in absolute THF (500 ml) was added dropwise. After the mixture was heated at reflux for 1 hr., a solution of chlorotrimethylsilane (270 g) in absolute THF (250 ml) was added dropwise. The reaction mixture was stirred for 2 hrs., under reflux, then cooled to room temperature and stirred overnight. The reaction mixture was distilled using a 30 cm Vigreux column. The distillate (bp 60-65°) was collected, and washed with water (15 x 200 ml) to afford y as a colorless liquid (190.4 g, 76.1%): NMR (CDCl₃, 250 MHz, CHCl₃ as internal reference) δ 6.150 (1H, dd, J = 14.6,

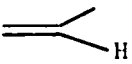
20.0 Hz, $\text{H}=\text{CH}-\text{SiMe}_3$), 5.901 (1H, dd, J = 4.1, 14.6 Hz, $\text{H}-\text{CH}=\text{SiMe}_3$),

5.646 (1H, dd, J = 4.1, 20.0 Hz, $\text{H}-\text{CH}=\text{SiMe}_3$), and 0.058 (9H, s, SiMe₃). The NMR

spectrum also suggested the presence of 7.0% of H₂O and 5.6% of THF.

g. Compound z from Bromination of y - Bromine (356 g) was added dropwise to trimethylvinylsilane (y, 190.4 g) with stirring at -78°C over 2 hrs. The resulting red mixture was warmed up to room temperature. Then diethylamine (1270 ml) was added dropwise to the mixture with stirring. After the addition was complete, the reaction mixture was heated at reflux for 14 hrs. The mixture was cooled to room temperature and the precipitate was filtered off. The precipitate was washed with ether and the ether was combined with the filtrate. The ether solution was washed with 10% HCl to remove diethylamine, and with water and saturated aqueous sodium chloride. The solvent was dried over anhydrous magnesium sulfate, concentrated and distilled under reduced pressure through a 30 cm Vigreux column, to give bromotrimethylsilylethylene z

(210 g, 62%) bp $65-68^{\circ}/120$ mm Hg): NMR (CDCl_3) δ 6.250 (1H, d, $J = 1.8$ Hz,

), 6.166 (1H, d, $J = 1.8$ Hz, , and 0.134 (9H, s, SiMe_3).

h. Compound a from Grignard Reaction of z - The 2 L three-necked flask was charged with Mg (20.8 g) and absolute THF (230 ml), and 1,2-bromoethane (4.5 g) was added with stirring. A solution of bromo-trimethylethylene (z, 113 g) in absolute THF (170 ml) was added to the stirred mixture. After the addition was complete, the reaction mixture was refluxed for 1 hr. Then acetaldehyde (57 g) was added dropwise and the temperature was maintained at reflux. The stirring was continued for 1 hr. at reflux. The reaction mixture was distilled until a distillate amounting to ca. 100 ml was collected. The condensed reaction mixture was cooled and diluted with ether and hydrolyzed by addition of saturated ammonium chloride (100 ml). The salts were filtered and washed with ether. The aqueous layer was extracted with ether. The combined ether fraction was washed with saturated NaCl solution, dried over MgSO_4 , and concentrated by distillation at atmospheric pressure to afford the crude silylbutenol (a, ca. 105 g).

i. Compound b from Oxidation of a - 250 ml of an aqueous solution (300 ml) containing CrO_3 (70 g) and H_2SO_4 (112 g) was added dropwise to a solution of silylbutenol (a) (129.1 g) in acetone (220 ml) with stirring in an ice-water bath over 3 hrs. After completion of the addition, isopropyl alcohol was added to the reaction mixture. The mixture was poured into ether (500 ml), and 300 ml of aqueous saturated sodium chloride was added. The aqueous layer was extracted with ether (4 x 200 ml). The combined ether solutions were washed with saturated aqueous sodium chloride (2 x 200 ml), dried over anhydrous magnesium sulfate, and concentrated by distillation at atmospheric pressure through a 30 cm Vigreux column. Continued distillation under reduced pressure gave silylbutenone (b) (3-trimethylsilyl-3-buten-2-one) (40.6 g; yield 31.9%; bp $81-84^{\circ}\text{C}/92-93$ mmHg; lit. bp $98-103^{\circ}\text{C}/100$ mmHg) as a pale yellow liquid: NMR (CDCl_3 , MHz) δ 6.479 (1H, d, $J = 1.9$ Hz, olefinic H), 6.149 (1H, d, $J = 1.9$ Hz, olefinic H), 2.270 (3H, s, $\text{CH}_3\text{CO-}$), and 0.123 (9H, s, $\text{Si}(\text{CH}_3)_3$).

j. Compound 5 from Alkylation of 4 - n-BuLi (1.55 M/L, 81.8 ml) was added dropwise to a solution of diisopropylamine (18.7 ml) in anhydrous tetrahydrofuran (THF) at -78°C with stirring for 30 min., and the mixture was stirred for an

additional hour. To the mixture was added dropwise a solution of keto-benzyl ether (4) (27.5 g) in anhydrous THF (40 ml) for 30 min. After stirring for 30 min., 3-trimethylsilyl-3-buten-2-one (b) (22.5 g) in anhydrous THF (25 ml) was added dropwise. The mixture was stirred at -78°C for 1 hr., then warmed to 0°C and further stirred at 0°C for 3 hrs. The reaction mixture was acidified with 10% HCl and stirred for 15 min., then neutralized with 5% NaHCO_3 , and extracted with ether. The ether layer was washed with water, dried over anhydrous MgSO_4 , and concentrated to give a crude product (47.0 g). Column chromatography of the crude product on silica gel (1 kg) with elution with 10% ether-hexane afforded the diketone (5) (19.6 g, yield 56.0%) as a colorless oil: IR (CHCl_3) $1705\text{ (C=O cm}^{-1}\text{)}$; and NMR (CDCl_3 , 200 MHz) δ 7.390-7.210 (5H, aromatic protons), 4.477 (2H, s, $\text{PhCH}_2\text{-O}$), 3.459 (1H, dd, $J = 5.0$ and 9.1 Hz), 3.373 (1H, dd, $J = 5.6$ and 9.1 Hz, AB part of ABX, $\text{O-CH}_2\text{CH<}$), 2.117 (3H, s, -C(=O)-CH_3), 1.060 (3H, d, $J = 5.9$ Hz, sec- CH_3), and 1.000 (3H, d, $J = 6.4$ Hz, sec- CH_3).

k. Compound 6 from Cyclization of 5 via 6a - To a solution of diketone (5) (19.0 g) in ethanol (750 ml) was added $\text{Ba(OH)}_2 \cdot 8\text{H}_2\text{O}$ (20.7 g). After the mixture was stirred at room temperature for 2.5 hrs, it was neutralized with 10% HCl and concentrated under reduced pressure (by vacuum pump, room temperature). The residue was extracted with dichloromethane, washed with water, dried over anhydrous magnesium sulfate, and concentrated to afford a crude product (23.1 g): IR (CHCl_3) 3400 (OH) and $1710\text{ (C=O cm}^{-1}\text{)}$.

A mixture of the above products (23.0 g) and 2% oxalic acid in ethanol (800 ml) was refluxed for 2.5 hrs. The reaction mixture was neutralized with 5% NaHCO_3 and concentrated under reduced pressure (by vacuum pump, room temperature) to furnish a residue. Extraction of the residue with dichloromethane followed by washing with water, drying over anhydrous magnesium sulfate, and evaporation afforded a crude oily product (21.9 g). This product was chromatographed on silica gel (410 g) to yield an α,β -unsaturated ketone (6) (12.0 g, 66.8% in 2 steps; recrystallized from hexane) as colorless prisms: mp $75\text{-}76^{\circ}\text{C}$; IR (CHCl_3) 1660 and $1610\text{ (}\alpha,\beta\text{-unsaturated ketone cm}^{-1}\text{)}$; and NMR (CDCl_3 , 200 MHz) δ 7.373-7.230 (5H, aromatic H), 5.861 (1H, s, olefinic H), 4.514, 4.442 (each 1H, ABq, $J = 12.1$ Hz, $\text{CH}_2\text{-O}$), 3.470 (1H, dd, $J = 3.2$ and 9.0 Hz), 3.343 (1H, dd, $J = 5.9$ and 9.0 Hz, AB part of ABX, $\text{O-CH}_2\text{-CH<}$), 1.061 (3H, d, $J = 6.3$ Hz, sec- CH_3), and 1.023 (3H, d, $J = 6.4$ Hz, sec- CH_3).

This compound was proven to be 1 - 5,10 -methyl-7 -[2' -1'-benzyloxy)-propane]-decalone-4 by an x-ray analysis as shown in Figure-1.

l. Compound 7 from Reduction and Oxidation of 6 and its Intermediate - To a solution of sodium borohydride (8.27 g) in pyridine (110 ml) was added dropwise a solution of the α,β -unsaturated ketone (6, 11.6 g) in pyridine (140 ml) in an ice-water bath. After the mixture was stirred at room temperature for 7 hrs., water (18 ml) was added, and the mixture was further stirred for 30 min. The mixture was diluted with ether (500 ml) and acidified with 10% HCl. The ether layer thus separated was added with a solution of sodium iodate (79.8 g) in water (800 ml). After the mixture was further stirred for 15 hrs., at room temperature, the organic layer was separated and the aqueous layer was extracted with ether. The combined ether extracts were washed successively with 5% $\text{Na}_2\text{S}_2\text{O}_3$, H_2O , 10% HCl, 5% NaHCO_3 , H_2O , dried over anhydrous magnesium sulfate, and concentrated to give the oily residue (12.3 g).

To a solution of the foregoing residue (12.3 g) in acetone (70 ml) was added dropwise an aqueous solution (30 ml) containing CrO_3 (7.8 g) and H_2SO_4 (9 ml) in an

ice-water bath. After the mixture was stirred for 15 min., isopropanol was added until the color of the reagent disappeared. The mixture was extracted with ether and the ether extract was washed with 5% NaHCO_3 , and water, dried over anhydrous magnesium sulfate, and evaporated to give an oily residue (11.6 g). Column chromatography of the residue on silica gel (200 g) afforded the saturated ketone (7) (4.47 g, 38.2% in 2 steps; recrystallized from ether-hexane) as colorless needles: mp 62-64°C; IR (CHCl_3) 1700 (6-membered ketone); and NMR (CDCl_3 , 250 MHz) δ 7.345-7.230 (5H, aromatic H), 4.508, 4.435 (each 1H, ABq., $J = 12.1$ Hz, $\text{O}-\text{CH}_2-$), 3.431 (1H, dd, $J = 3.2$ and 9.2 Hz), 3.326 (1H, dd, $J = 5.3$ and 9.2 Hz, AB part of ABX, $\text{O}-\text{CH}_2-\text{CH}<$), 0.974 (3H, d, $J = 6.6$ Hz, sec- CH_3), and 0.942 (3H, d, $J = 6.3$ Hz, sec- CH_3).

m. Compound 8 from Grignard Addition followed by Dehydration of 7 - A 200 ml three-necked flask was charged with magnesium turnings (560 mg) and absolute ether (35 ml), and iodomethane (3.6 g) was slowly added dropwise to the stirred mixture under nitrogen atmosphere. After the addition was complete, the mixture was cooled to room temperature. Then a solution of the saturated ketone (7, 546 mg) in absolute ether (25 ml) was added dropwise and stirred at room temperature for 1.5 hrs.

After the foregoing reaction mixture was added dropwise with saturated aqueous ammonium chloride, it was extracted with ether. The ether extract was washed successively with 10% HCl, 5% sodium bicarbonate and H_2O , dried over anhydrous magnesium sulfate, and evaporated to give an oily residue (605 mg), whose IR spectrum showed the absence of carbonyl functions.

A solution of p-toluenesulfonic acid (550 mg) in tetrahydrofuran (1 ml) was added to a solution of the above oily residue (595 mg) in benzene (60 ml). The mixture was refluxed using a Dean-Stark apparatus for 2 hrs. The reaction mixture was cooled to room temperature and washed with dil. Na_2CO_3 and H_2O , dried over anhydrous magnesium sulfate, and evaporated to provide an oily residue (560 mg). This was subjected to preparative TLC using silver nitrate-impregnated silica gel plates (detection by spraying water and elution with 5% methanol-chloroform mixture) to yield 8 (110 mg) and 8a (90 mg). Compound 8 showed NMR (CDCl_3 , 250 MHz) δ 7.367-7.220 (5H, aromatic H), 5.216 (1H, br. s, olefinic H) 4.537, 4.454 (each 1H, ABq, $J = 12.2$ Hz, $\text{Ph}-\text{CH}_2-\text{O}-$), 3.533 (1H, dd, $J = 3.4$ and 9.0 Hz), 3.301 (1H, dd, $J = 6.8$ and 9.0 Hz, AB part of ABX, $\text{O}-\text{CH}_2-\text{CH}<$), 1.620 (3H, br. s, $\text{C}=\text{C}-\text{CH}_3$), 1.015 (3H, d, $J = 6.8$ Hz, sec- CH_3), and 0.856 (3H, d, $J = 6.4$ Hz, sec- CH_3).

n. Compound 8a from Debenzylation of 8 with Na/NH_3 - To a two-necked 1 liter flask was introduced liquid ammonia (300 ml) in a dry ice-acetone bath. This was then added with a solution of 8 (3.90 g) in ether (30 ml) and several pieces of metallic sodium (ca 5 g) with stirring. After 1 hr., the reaction was quenched by addition of aqueous ammonium chloride. The mixture was warmed up to room temperature, and then evaporated to dryness. The residue was extracted with ether. The ether layer was washed with water, dried over anhydrous magnesium sulfate, and evaporated in vacuo to afford a crystalline residue (2.76 g, 99%), which was recrystallized from hexane to give 8a as colorless needles: mp 77-78°C. IR (CHCl_3) 3630 and 3450 (OH) cm^{-1} ; NMR (CDCl_3 , 250 MHz) δ 5.213 (1H, br. s, olefinic H) 3.742 (1H, d, d, $J = 3.3$ and 10.6 Hz) 3.522 (1H, dd, $J = 6.1$ and 10.6 Hz, AB part of ABX, $\text{HO}-\text{CH}_2-\text{CH}<$), 1.632 (3H, br. s, $\text{C}=\text{C}-\text{CH}_3$) 0.998 (3H, d, $J = 6.8$ Hz, sec- CH_3) and 0.865 (3H, d, $J = 6.5$ Hz, sec- CH_3).

o. Compound 9 from Oxidation of 8a with Jones' Reagent - To a solution of 8a (2.75 g) in acetone (50 ml) was added dropwise Jones' reagent at 0°C until the color of the reagent did not disappear. After stirring for 40 min., isopropyl alcohol was added to the mixture to reduce the excess reagent. The mixture was then diluted with

water and extracted with CHCl_3 . The CHCl_3 layer was washed with water, dried over anhydrous MgSO_4 , and evaporated to afford an oily residue (2.95 g). The residue was dissolved in ether and extracted with dil. K_2CO_3 . The alkaline layer was acidified with 10% HCl and extracted with CHCl_3 . The CHCl_3 layer was washed with water, dried over MgSO_4 , and evaporated to give a crystalline residue (2.60 g, 89%), which was recrystallized from acetone hexane to yield 9 as colorless prisms: mp 130-132°C; IR (CHCl_3) 3400-3000 and 1700 (OH) cm^{-1} ; NMR (CDCl_3 , 200 MHz) δ 5.116 (1H, s, C-CH), 1.635 (3H, br.s, C-C- CH_3), 1.192 (3H, d, J = 6.9 Hz, sec- CH_3) and 0.870 (3H, d, J = 6.3 Hz, sec- CH_3).

p. Compound 10 from Methylation of 9 with CH_2N_2 - To a solution of 9 (2.57 g) in ether (50 ml) was added on ethereal diazomethane at 0°C. The mixture was allowed to stand for 5 min. The excess diazomethane was decomposed with acetic acid. The ether solution was washed with dil. K_2CO_3 and water, dried over anhydrous MgSO_4 , and evaporated to furnish 10 as an oily residue (2.30 g, 94%): IR (CHCl_3) 1720 and 1170 (COO-) cm^{-1} ; NMR (CDCl_3 , 250 MHz) δ 5.121 (1H, br.s, olefinic H), 3.674 (3H, s, - COOCH_3), 1.134 (3H, d, J = 6.9 Hz, sec- CH_3), and 0.862 (3H, d, J = 6.4 Hz, sec- CH_3).

q. Ozonization of 10 to 11 - Ozone was introduced into a solution of 10 (185 mg) in MeOH (0.7 ml) and CH_2Cl_2 (4 ml) in a dry ice-acetone bath for 135 sec until the starting material disappeared on TLC. After purging the excess ozone with Argon, Me_2S (0.7 ml) was added to the reaction solution with stirring at 0°-20°C for 2 hrs., and then at room temperature for 1 hr. The reaction solution was concentrated in vacuo to give the aldehyde-ketone (11), which shows almost one spot on TLC [R_f = 0.31, silica gel, benzene-acetone (10:1)].

r. Selective protection of the ketonic carbonyl group of 11 to 12 - To a solution of crude 11, obtained from 185 mg of 10, in CH_2Cl_2 (6 ml) was added dropwise 1,3-propanedithiol (138 mg) with stirring, and then BF_3 -ether (90 mg). After the reaction mixture was stirred at room temperature for 3 hrs., CH_2Cl_2 (50 ml) was added. The solution was washed with 5% NaHCO_3 (25 ml x 2) and water (20 ml x 3), dried over anhydrous MgSO_4 , and concentrated in vacuo to give an oily residue, which was purified by P-TLC [silica gel, benzene-acetone (10:0.7)] to afford the desired product (12, 76.8 mg, R_f = 0.53) together with three other compounds [R_f = 0.61 (13 mg), R_f = 0.42 (38.2 mg), R_f = 0.36 (14.5 mg)].

s. Transformation of the aldehydic carbonyl group of 12 into the enol methyl ether (13) - A mixture of 12 (76 mg), trimethyl orthoformate (0.5 ml), MeOH (1 ml) and catalytic amount of p-TsOH. H_2O was stirred at room temperature for 12 hrs. The reaction solution was added to a boiling solution of xylene (5 ml) and refluxed with a water-separator for 1.5 hrs. After cooling, the solution was worked up in the usual way to give the enol ether [13, 47.6 mg, R_f = 0.50 (silica gel, benzene-acetone (10:0.5), 62.3%], which was purified by column chromatography [silica gel, n-hexane n-hexane-benzene (1:1) benzene benzene-acetone (10:0.5)]. Compound 13 was isolated from benzene-acetone (10:0.5): IR: 1730 (COOMe) and 1652 (enol ether) cm^{-1} , NMR δ 0.95 (3H, d, J = 6.9 Hz, 10-Me), 1.11 (3H, d, J = 6.9 Hz, 11-Me), 1.63 (3H, s, 4-Me), 2.60-2.90 [4H, m, SCH_2], 3.556, 3.562 (3H, 2s, -OMe, mixture of 1:1.6), 3.69 (3H, s, COOMe) and 5.889, 5.949 (1H, 2s, =CH- , mixture of 1:1.6).

t. Conversion of the thioketal (13) into the carbonyl group (14) - To a stirred solution of HgCl_2 (66 mg) and powdered CaCO_3 (40 mg) in 80% aq. MeCN (1.5 ml) was added a solution of 13 (20 mg) in 80% aq. MeCN (1 ml) at room temperature. The mixture was stirred and refluxed under N_2 for 1.5 hrs., cooled and filtered. The filtered cake was washed with CH_2Cl_2 (50 ml). The organic layer was washed

successively with 5M aq. NH_4OAc (20 ml) and water (20 ml x 3), and concentrated in vacuo to give 14 [14.6 mg, Rf - 0.24, silica gel, benzene-acetone (10:0.5), 94.8%]: IR 1725 (COOMe), 1710 ($\text{C}=\text{O}$) and 1652 (enol ether) cm^{-1} ; NMR δ 0.92 (3H, d, J = 6.7 Hz, 10-Me), 1.05 (3H, d, J = 6.7 Hz, 11-Me), 2.125, 2.142 (3H, 2s, $\text{CH}_3\text{CO}-$, mixture of 1:1.6), 3.530, 3.545 (3H, 2s, OMe, mixture of 1:1.6), 3.67 (3H, s, COOMe) and 5.843, 5.950 (1H, 2s, =CH- , mixture of 1:1.6).

u. Synthesis of Qinghaosu (1) from 14 via 15 - To a solution of 14 (17 mg) in MeOH (20 ml) was added Rose Bengal (4 mg). The resulting red solution through which oxygen was bubbled using 1% $\text{Na}_2\text{Cr}_2\text{O}_7\cdot\text{H}_2\text{O}$ as filter, was cooled to $-70 - 60^\circ\text{C}$, irradiated with a high pressure mercury lamp (200 W) for 6 hrs., and then passed the HCl gas until the red color disappeared. After further stirring at room temperature for 2 hrs., the solution was neutralized with 5% NaHCO_3 and concentrated in vacuo to give an aqueous solution, which was extracted with ether (20 ml x 2). The ethereal layer was washed with water, dried over MgSO_4 and evaporated to give a residue (12 mg), which showed no starting material (14) on TLC [Silica gel, benzene-acetone (10:1)]. To a solution of the residue (12 mg) in ether (1.5 ml) was added a solution of 60% HClO_4 (0.3 ml) and water (0.6 ml). The mixture was stirred at $20-25^\circ\text{C}$ for 40 hrs. The ethereal layer was separated and water (10 ml) was added to the aqueous layer. The combined aqueous solution was further extracted with ether (20 ml x 2). The ethereal solution was washed, dried over MgSO_4 and evaporated to give a residue (10 mg) which showed the presence of the target compound (1, Rf = 0.43) together with three spots on TLC [Silica gel, benzene-acetone (10:1)]. The product was purified to give the target compound (1, Ca * 1.1 mg, 7.3%) together with major compound (Ca. * 2.7 mg) by HPLC [solvent MeOH, Partisil M9 10/15 ODS-2, Col. No 4H, Whatman]. The nmr spectrum [400 MHz, CDCl_3 1.01 (3H, d, J = 5.7 Hz, Me-10), 1.22 (3H, d, J = 7.5 Hz, Me-11), and 5.87 (1H, s, H-5)] of this synthetic compound is identical with that of an authentic sample of qinghaosu (1) isolated from Artemisia annua.

All nmr spectra corresponding to each synthetic intermediate described in this report have been sent to Dr. A. J. Lin, my Contracting Officer's Technical Representative on October 20, 1986.

*The yield was calculated from the intensity curve of HPLC compared with an authentic sample of qinghaosu.

D. New synthesis of qinghaosu (1) and its simpler analogs - Scheme -9 and 10.

1. New Synthetic Methods - Scheme-9.

In addition to the foregoing total synthesis, we are currently developing new improved methods for the practical synthesis of qinghaosu. Thus,

a. We have accomplished the alkylation of 16 via enamine and methyl vinyl ketone (Scheme-9). Compound 16 was described above (Scheme-6). The alkylated products 9 and 10 were isolated as a mixture in 20% yield from 16. It is planned to convert 9 to 10 with weak base, such as $\text{Ba}(\text{OH})_2\cdot 8\text{H}_2\text{O}$. Compound 10 could serve as an intermediate for the synthesis of Zhou's compound 11.

Synthesis of 9 and 10 from 16 - In a 100 ml flask equipped with a Dean-Stark trap a mixture of the keto-ester (16, 1g) pyrrolidine (0.5 g), p-toluene sulfonic acid (0.1 g), and anhydrous benzene (50 ml) was refluxed and stirred for 72 hrs. The solvent was evaporated in vacuo to give an oil which was distilled to yield an enamine (8).

To a stirred solution of this enamine (0.3 g) in anhydrous MeOH (10 ml) was added methyl vinyl ketone (0.2 ml). After the mixture was refluxed for 20 hrs., it was cooled to room temperature and added with a solution of sodium acetate (0.5 g), acetic acid (1 ml), and water (1 ml). After further reflux for 4 hrs., MeOH was evaporated and the resulting liquid was extracted with benzene. The benzene layer was washed with brine, dried and evaporated to give an oil (20% yield), which was separated by PTLC to furnish a pure 1,5-diketone (9) and a mixture of 9 and 10. Compound 9 showed: IR (CHCl_3) at $1710 \text{ (C=O) cm}^{-1}$ and NMR (CDCl_3) δ 0.94 (3H, d, $J = 7 \text{ Hz}$, CH_3), 1.13 (3H, d, $J = 7 \text{ Hz}$, CH_3), 2.13 (3H, s, COCH_3), and 3.68 (3H, s, COOCH_3).

b. By an application of Corey's method,¹⁴ we have accomplished the synthesis of 16 (Scheme-9) which was prepared from the alkylation of cyclohexanone (11) with benzothiazonium lithium followed by dehydration with carbomethoxysulfamoyl triethylamine salt in good yield. Further transformation of 16 to qinghaosu 1 is in progress.

Preparation of 12 from 11 - Under an argon atmosphere 6.50 ml of 1.55 M nBuli in hexane was added to 25 ml of dry ether. The resulting solution was cooled to -78°C (dry ice-acetone) and 1.09 ml of benzothiazole was added dropwise. The solution was allowed to stir for 10 min and 2.61 g of 11 was added dropwise. The resulting solution was allowed to stir for 1 hour and then quenched with 50 ml of H_2O . The ether layer was removed and the remaining aqueous phase was extracted with ether (25 ml x 3). The organic layer were combined, dried (MgSO_4), and evaporated. The product was purified by flash chromatography (hexane/ether, 4:1, silica gel) to give 1.6 g (57%) of product as a slightly yellow oil: IR (CCl_4) 3340 (O-H) , 1585 (C-N) , 1072 (C-O) ; NMR (200 MHz, CDCl_3) δ 0.82 (3H, d, 5-Me), 0.91 (3H, d, 7-Me), 4.58 (2H, s, Ar- CH_2 -O), 7.15-7.47 (7H, m, Ar-H), 7.75-8.05 (2H, m, benzothiazole 4', 7'-H).

Preparation of carbomethoxysulfamoyl triethylamine inner salt, (15) To 200 ml of benzene was added 40.1 ml of chlorosulfonyl isocyanate 13. To this under a nitrogen atmosphere was added, dropwise over 1 hour, 24 ml of dry MeOH in 25 ml of benzene. The excess solvent was removed under reduced pressure and the resulting residue was recrystallized from toluene to give 72 g (83%) of 14. Carbomethoxysulfamoyl chloride (14, 10 g) was added to 125 ml of benzene. This solution was added dropwise to a solution of 100 ml of benzene containing 18.0 ml of triethylamine. When the addition was complete (1 hr) the precipitate was removed by filtration and the filtrate was evaporated to afford an oil which solidified upon standing. The product was recrystallized from toluene to give 9.0 g (65%) of 15: mp $68-70^\circ\text{C}$; lit mp $71-72^\circ\text{C}$.

Dehydration of 12 to give 16a - In 50 ml of benzene was dissolved 10.0 g of 12. To this solution was added 5.4 g of 15 in 10 ml of benzene. The resulting solution was stirred under nitrogen at room temperature for 1 hour and the temperature was then raised to 55°C for 5 hours. The reaction was quenched with 25 ml of H_2O . The aqueous layer was removed and the organic phase was washed with an additional 25 ml of H_2O . The aqueous layers were combined and extracted with benzene (15 ml x 3). The organic layers were combined, dried (MgSO_4) and the benzene removed under reduced pressure to afford an oil which was purified by flash chromatography (hexane/ether, 4:1, silica gel) to give 2.0 g (39%) of 16a as an oil: IR (CCl_4) 1550 (c-n) , 1090 (C-O) ; NMR (400 MHz, CDCl_3) δ 0.96 (3H, d, 5-Me), 1.06 (3H, d, 7-Me), 4.32 (2H, m, Ar- CH_2 -O), 6.45 (1H, d, C=C-19), 7.15-7.47 (7H, m, Ar-H), 7.75-8.05 (CH, dd, benzothiazole 4', 7'-H).

Preparation of 33 from 16a - In 1.0 ml of CH_2Cl_2 was dissolved 500 mg (1.3 mmol) of unsaturated benzothiazole 16a. To this was added 1.07 ml (10 eq.) of methyl fluorosulfonate and the resulting solution was stirred at room temperature under a

nitrogen atmosphere for 48 hours. The solvent was removed under reduced pressure and the resulting brown oil was washed twice with ether and the product dried under reduced pressure. The product was dissolved in 2.0 ml of absolute ethanol and the solution cooled to -20°C (dry ice- CCl_4). To this was added 0.49 g (10 eq.) of NaBH_4 and the resulting solution was stirred for 2 hours. The cooling bath was removed and 50 ml of ether was added. The organic phase was washed with 3×10 ml of 0.5N Na_2CO_3 , dried (MgSO_4), and evaporated to give the crude N-methyl benzothiazoline. The crude product was dissolved in 5 ml of CH_3CN and 1.0 ml of H_2O containing 1.5 eq. of AgNO_3 was added. The solution was allowed to stir at room temperature for 15 min, when an additional 1.5 eq. of AgNO_3 in 1.0 ml of H_2O was added. The solution was allowed to stir for 15 min at which time 1 equivalent of triethylamine was added. The reaction was stirred an additional 10 min and filtered through celite (ethereal wash). To the organic phase was added 10 ml of brine and the precipitate (AgCl) was removed by filtration through celite. The aldehyde was isolated by preparative TLC (hexane/ether, 4:1, silica gel) to give 100 mg (30%) of 33 as a clear oil: IR (CCl_4) 2880 (aldehyde C-H), 1680 (C=O) cm^{-1} ; $^1\text{H-NMR}$ (CDCl_3) δ 0.94 (d, 3H, $-\text{CH}_3$), 1.19 (d, 3H, $-\text{CH}_3$), 4.42 (s, 2H, Ar- CH_2 -O), 6.57 (d, 1H, C=C-H), 7.30 (s, 5H, ArH).

Synthesis of Di-3-butenyl Mercury (18) from 4-Bromo-1-butene (17) - To 3.40 g (0.14 mo.) of Mg in 100 ml of THF (distilled) was added dropwise 15 g (0.11 mol) of 4-bromo-1-butene (17) in 75 ml of THF at such a rate as to just maintain reflux. When the addition was complete the resulting solution was stirred at reflux for 2 hours. The Grignard solution was transferred via canula to a second flame dried three neck flask in order to remove any unreacted Mg. To this solution was carefully added dropwise 15 g (0.055 mol) of Hg Cl_2 suspended in 50 ml of THF. After the addition was complete the solution was stirred at reflux for 10 hours under an atmosphere of N_2 . The reaction was quenched by the careful addition of H_2O . The product was taken up in ether, dried (MgSO_4), and the solvent removed under reduced pressure. The crude product was purified by distillation to afford 6.6 g of 18: bp = $96-98^{\circ}\text{C}/6.0$ mm Hg; lit bp $103-104^{\circ}\text{C}/14$ mm Hg.

Preparation of 3-Butenyl-lithium (19) - Into a flame dried three neck flask with argon flowing through was placed 6.0 cm of Li wire which had been first cut into pieces. The vessel was quickly closed and 15 ml of cyclopentane was added. To this was added 1.0 g (3.2 mmol) of di-3-butenyl mercury (18) via syringe. The joints of the flask were wrapped with parafilm and the reaction was allowed to stir for 24 hours at room temperature under an atmosphere of argon. In the morning the lithium had become black and was no longer floating on the solvent.

Preparation of Compound 20 - Into a flame dried three neck flask under an argon atmosphere was placed 3.0 ml of 0.42 M solution of 3-butenyl-lithium in cyclopentane. The resulting yellow solution was stirred at room temperature for 45 minutes and quenched with MeOH. To this was added 25 ml of ether and the organic phase was washed with 3×5 ml of sat. NaCl. The ether was dried (MgSO_4) and evaporated to give the crude product which was purified by spinning band chromatography (hexane: silica gel) to give 35 mg of 20 as a clear oil: IR (CCl_4) 1632 cm^{-1} (C=N); $^1\text{H-NMR}$ (CDCl_3) δ 0.86 (d, 3H, $-\text{CH}_3$), 0.94 (d, 3H, $-\text{CH}_3$), 4.38 (s, 2H, Ar- CH_2 -O), 4.70-5.70 (m, 3H, CH_2 -CH-C) 7.30 (s, 5H, phenyl-H), 7.6-8.1 (m, 4H, benzothiazole H).

2. New Synthetic simpler analogs related to qinghaosu (1) - Scheme-10.

We have also very recently achieved the synthesis of first three simpler qinghaosu (1) analogs (9a, 9b and 10). Compounds 9a, 9b and 10 have almost the same structure and stereochemistry compared to 1 for the essential moieties which are

thought to be responsible for the potent antimalarial activity of 1.¹⁵ Compounds 9a, 9b and 10 were synthesized from the enol methyl ether (8) by photooxygenation as shown in Scheme-10. The antimalarial activity of 9a, 9b and 10 is currently under evaluation.

Photooxygenation of 8 to 9a and 9b - Through a solution of the enol ether 8 (260 mg), acetaldehyde (1 ml) and Rose Bengal (20 mg) in THF (6 ml) was continuously bubbled oxygen using 1% $\text{Na}_2\text{Cr}_2\text{O}_7 \cdot \text{H}_2\text{O}$ as a filter and the solution was irradiated with a 200 W high pressure Hg lamp at -70 to 78°C for h. After filtration, the red precipitate was washed with CHCl_3 (40 ml) and the CHCl_3 solution was combined with the filtrate. The resulting solution was evaporated under reduced pressure to give a residue, which was purified by column chromatography [benzene-acetone (20:1), silica gel] to give a residue (200 mg). This residue was purified by HPLC [MeOH, Partisil Mg 10/15 ODS-2, Col No 4H, Whatman] to give three compounds 7 (37 mg), 9a (24.8 mg) and 9b (37.9 mg) together with two unknown compounds A (60 mg) and B (30 mg). Compound 7: IR (film) 1703 and 1728 cm^{-1} (ketone and ester); NMR (CDCl_3) δ 1.05 (1H, d, J = 6.25 Hz, 5-Me) and 1.22 (1H, d, J = 7.01 Hz, 9-Me). This sample was identified with an authentic sample. Compound 9a: oil, IR (CHCl_3) 1722 cm^{-1} (ester); NMR 400 MHz, (CDCl_3) δ 0.88 (3H, d, J = 6.4 Hz, 5-Me) 1.21 (3H, d, J = 7.1 Hz, 9-Me), 1.36 (3H, d, J = 5.7 Hz, 7-Me), 3.40 (3H, s, J = OMe), 3.67 (3H, s, 9-COOMe), 5.04 (1H, s, 8-H) and 5.40 (1H, 1, J = 5.7 Hz, 7-H). Compound 9b: mp $88-89^\circ\text{C}$ (CHCl_3 -n-hexane); IR (CHCl_3) 1720 cm^{-1} (ester); NMR (400 MHz, CDCl_3) δ 0.89 (3H, d, J = 6.50, 5-Me), 1.26 (3H, d, J = 5.37 Hz, 7-Me), 1.27 (3H, d, J = 7.65, 9-Me), 3.40 (3H, s, 8-OMe), 3.62 (3H, s, 9-COOMe), 3.80 (1H, dq, J = 2.85, 7.65 Hz, 9-H), 4.79 (1H, s, 8-H) and 5.61 (1H, q, J = 5.5 Hz, 7-H).

Photooxygenation of 8 to 10 - Through a solution of the enol ether 8 (260 mg), acetaldehyde (1 ml) and Rose Bengal (15 mg in dry MeOH (6 ml) was continuously bubbled oxygen using 1% $\text{Na}_2\text{Cr}_2\text{O}_7 \cdot \text{H}_2\text{O}$ as a filter and the solution was irradiated with a 200 W high pressure Hg lamp at -70 to 78°C for 3 h. The reaction mixture was further bubbled with hydrogen chloride gas until the red solution was decolorized. After stirring at 20°C for 2 h, the reaction mixture was neutralized with 5% NaHCO_3 and concentrated in vacuo to give an aqueous solution, which was extracted with ether (30 ml x 3). The ethereal layer was washed with water, dried (MgSO_4), and evaporated to give a residue (258 mg). The residue was dissolved in ether (15 ml) and 60% HClO_4 (1.0 ml) was added to the solution. The mixture was stirred at $20-25^\circ\text{C}$ for 20 h. The ethereal layer was separated and the remaining aqueous layer was further extracted with ether (30 ml x 4). The ethereal layer was combined, washed with water, dried (MgSO_4) and evaporated to give an oily residue (260 mg), which was purified by preparative TLC [Benzene-acetone (20:0.5), silica gel] to afford the desired product 10 (31 mg, Rf = 0.46) together with 7 (66.5 mg, Rf = 0.36). Compound 7 was identified with an authentic sample. Compound 10: mp $95-96^\circ\text{C}$ (MeOH-n-hexane); NMR (250 MHz, CDCl_3) δ 1.01 (3H, d, J = 6.33 Hz, 5-Me), 1.18 (3H, d, J = 7.28, 9-Me), 1.34 (3-H, d, J = 5.44 Hz, 7-Me), 2.75 (1H, ddd, J = 13.49, 3.85, 1.75 Hz, 2-H), 3.04 (1H, q.d, J = 7.28, 3.85 Hz, 9-H), 5.63 (1H, s, 8-H) and 5.66 (1H, q, J = 5.44 Hz, 7-H).

(4) Literature References

1. K. H. Lee, Y. Imakura, Y. Sumida, R. Y. Wu, I.H. Hall and H. C. Huang, J. Org. Chem., **44**, 2180 (1979).
2. A. E. Greene, J. Muller and G. Ourisson, J. Org. Chem., **39**, 186 (1974).
3. E. J. Corey and A.G. Hortmann, J. Am. Chem. Soc., **87**, 5736 (1965).
4. T. Honda, K. Imao, M. Inoue, N. Nakazuka, T. Tachioka and T. Nakanishi, Abstract of 1983-Annual Meeting of Japanese Pharmaceutical Society, Tokyo, Japan.

5. D. H. Rammler and H. G. Khorana, J. Am. Chem. Soc., **85**, 1997 (1963).
6. G. Schmid and W. Hofheinz, J. Am. Chem. Soc., **105**, 624 (1983).
7. T. Iwashige and H. Saeki, Chem. Pharm. Bull., **15**, 1803 (1967).
8. R. E. Gawley, Synthesis, 792 (1976).
9. X. X. Xu, J. Zhu, D. Z. Huang and W. S. Zhou, 1984 Hawaii Am. Chem. Soc. Meeting Abstract, 10E102.
10. X. X. Xu, J. Zhu, D. Z. Huang and W. S. Zhou, Tetrahedron, **42**, 819 (1986).
11. R. K. Boeckman, D. M. Blum, B. Ganem and N. Halvey, Org. Syn., **58**, 152, (1978).
12. R. E. Desjardins, C. J. Camfield, J. D. Haynes and J. D. Chulay, Antimicrob. Agents Chemother., **16**, 710 (1979).
13. T. S. Osdene, P. B. Russell and L. Rane, J. Med. Chem., **10**, 431 (1967).
14. E. J. Corey and D. L. Boger, Tetrahedron Letters, **5**, 9 and 13 (1978) and literature cited therein.
15. S. Tani, N. Fukamiya, H. Kiyokawa, H. A. Musallam, R. O. Pick and K. H. Lee, J. Med. Chem., **28**, 1743 (1985).

(5) Biological Test Data

The in vitro and in vivo antimalarial assays were carried out by the Department of Medicinal Chemistry, Division of Experimental Therapeutics, Walter Reed Institute of Research, Washington, DC. Compounds 1 - 4 were found to be less active than qinghaosu [ED_{50} (Smith) <3.4 ng/ml] against the chloroquine-resistant isolates of Plasmodium falciparum. Their test data were shown below:

1. ST-ST-peroxide-1 (BK 70493) - ED_{50} (Smith) = 728.59 ng/ml and in vivo assay showed toxicity at 160 and 640 mg/kg.
2. Bruceoside-A Acetonide (BK 69703) - ED_{50} (Smith) > 125 ng/ml, ED_{50} (Camp) = 1017.93 ng/ml and in vivo assay showed toxicity at 320 mg/kg.
3. Brusatolyl-1-nonenoate (BK 73047) - ED_{50} (Smith) = 61.54 ng/ml and ED_{50} (Camp) = 85.26 ng/ml.
4. 15-Methylcarbamoyl bruceolide (BK 84184) - ED_{50} (W-2 clone) = 416.81 ng/ml and ED_{50} (Sierra Leone clone) = 448.19 ng/ml.
5. 15-Phenylalaninyl bruceolide - data not received yet.
6. Brusatol (BJ 58983) - ED_{50} (Smith) = 7.5799 ng/ml.
7. Bruceolide (BJ 58992) - ED_{50} (Smith) = 75.1985 ng/ml.
8. Bruceoside-A (BJ 58974) - ED_{50} (Smith) = 581.1406 ng/ml.
9. IMK-Q-20-2 - data not received yet.
10. IMK-Q-25-4 - data not received yet
11. IMK-Q-25-5 - data not received yet
12. IMK-Q-28-2 - data not received yet

(6) Publication and Patents

1. S. Tani, N. Fukamiya, H. Kiyokawa, H. A. Musallam, R. O. Pick, and K. H. Lee "Antimalarial Agents. 1. -Santonin-Derived Cyclic Peroxide as Potential Antimalarial Agent", J. Med. Chem., **28**, 1743 (1985).
2. Y. Zhao, W. K. Hanton and K. H. Lee, "Antimalarial Agents. 2. Artesunate, An Inhibitor of Cytochrome Oxidase Activity in Plasmodium berghei", J. Nat. Prod., **49**, 139 (1986).
3. K. H. Lee, S. Tani and Y. Imakura, "Antimalarial Agents. 4. Synthesis and Biological Activity of Brusatol Related Compounds", J. Nat. Prod. submitted.

(7) List of Personnel Receiving Contract Support

1. Dr. Narihiko Fukamiya (6/1/83-8/31/83), an Assistant Professor of synthetic organic chemistry on leave from Hiroshima University, Japan, was appointed as Postdoctoral Fellow for the synthesis of target compounds.

2. Dr. Shohei Tani (7/15/83-3/11/85) an Associate Professor of Pharmaceutical Chemistry on leave from Kobe Gakuin University, Japan, was appointed as Postdoctoral Fellow to replace Dr. Fukamiya's position.

3. Dr. Hiroshi Irie (11/4/83-12/14/83), (7/1/84-8/31/84 and 7/1/86-7/31/86), a Professor of Synthetic Pharmaceutical Chemistry on leave from Nagasaki University participated in synthetic work of this contract research. Dr. Irie is a distinguished Synthetic Organic Chemist well-known in Japan.

4. Dr. Toshio Yokoi (3/4/85-9/30/86), Assistant Professor of Pharmaceutical Chemistry on leave from Kobe Gakuin University (Ph.D. from Kyoto University), a synthetic organic chemist, was appointed as Postdoctoral Fellow to succeed Dr. S. Tani's position. Dr. Tani returned to Kobe Gakuin University as Professor of Pharmaceutical Chemistry in March 1985.

5. Dr. Junko Koyama (4/23/85-4/8/86), Instructor of Synthetic Organic Chemistry, Kobe Women's College of Pharmacy, was appointed as a postdoctoral fellow working on the alternative synthesis of qinghaosu. Dr. Koyama is an experienced synthetic organic chemist and has published more than 10 articles in the synthesis of natural products.

6. Dr. Venkataraman Amarnath (4/1/86-7/1/86), a Research Assistant Professor of Medicinal Chemistry, participated in the synthesis of qinghaosu. Dr. Amarnath is an experienced synthetic organic chemist.

7. Dr. Yasuhiro Imakura (7/15/86-5/31/87), Visiting Assistant Professor on leave from Faculty of Pharmaceutical Science, The University of Tokushima, Japan, replaced Dr. Toshio Yokoi's position. Dr. Imakura is author and co-author of more than 40 publications in the area of isolation and synthesis of natural products. He succeeded in the synthesis of qinghaosu (1) as well as its simpler analogs 9a, 9b and 10.

8. Dr. Forrest Smith (9/1/86-5/31/87), a synthetic medicinal chemist, replaced Dr. Amarnath's position to work on the new method for the synthesis of qinghaosu. Dr. Smith has synthesized 33 and 16a as described in this report.

9. Hong Hu (11/1/86-5/31/87), a visiting scientist who had previous training in Dr. W.S. Zhou's laboratory of Shanghai Institute of Organic Chemistry, in the synthesis of antimalarial drugs, has been working on the synthesis of simpler analogs related to 1 since 11/1/86.

10. Dr. Li-Ming Yang (1/1/87-2/28/87), a new Ph.D. from our Division, was appointed as a Postdoctoral Fellow to facilitate the progress of the synthesis of 1 and its new analogs.

Scheme 1 : Synthesis of ST-ST-Peroxide-1 (BK 70493)

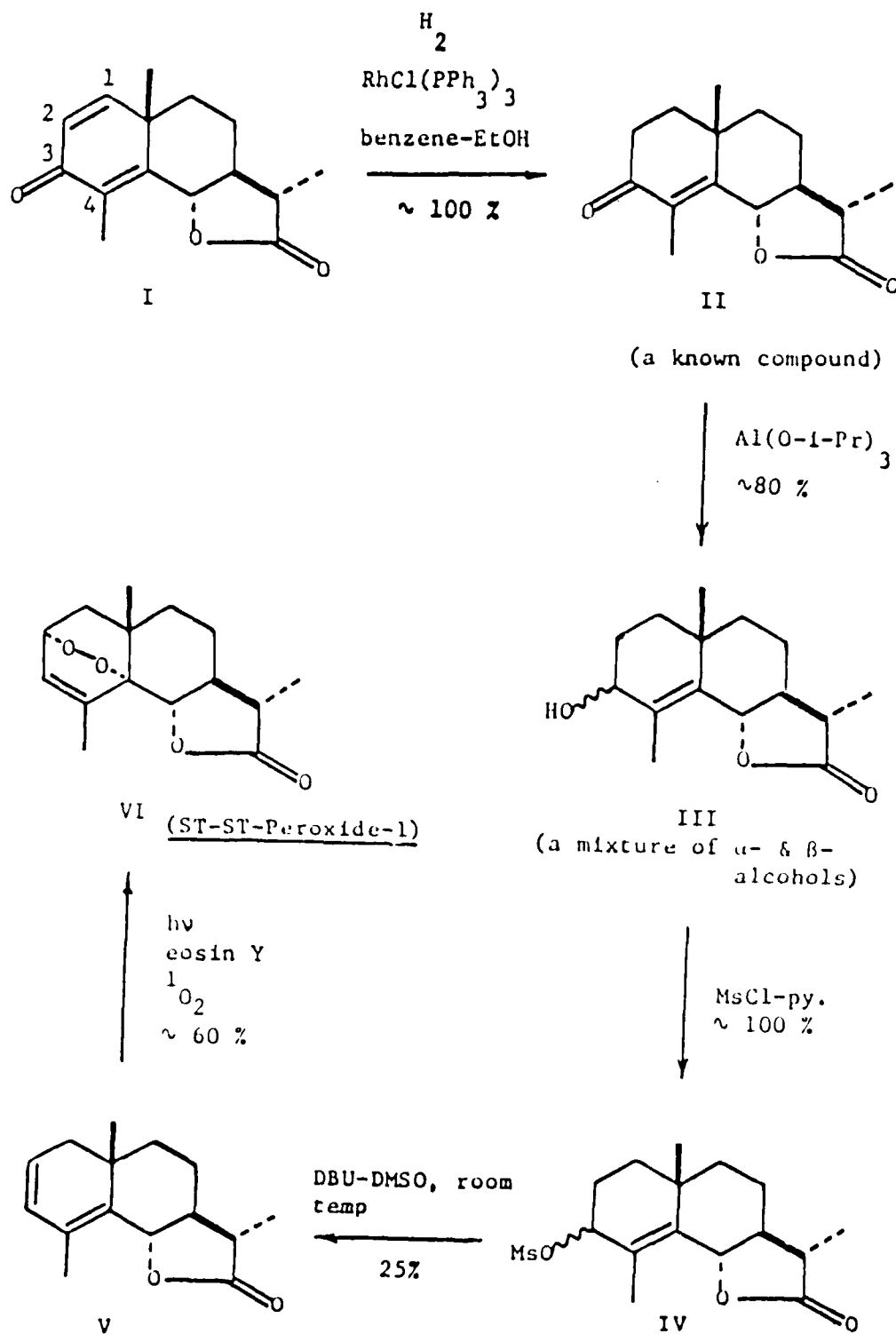
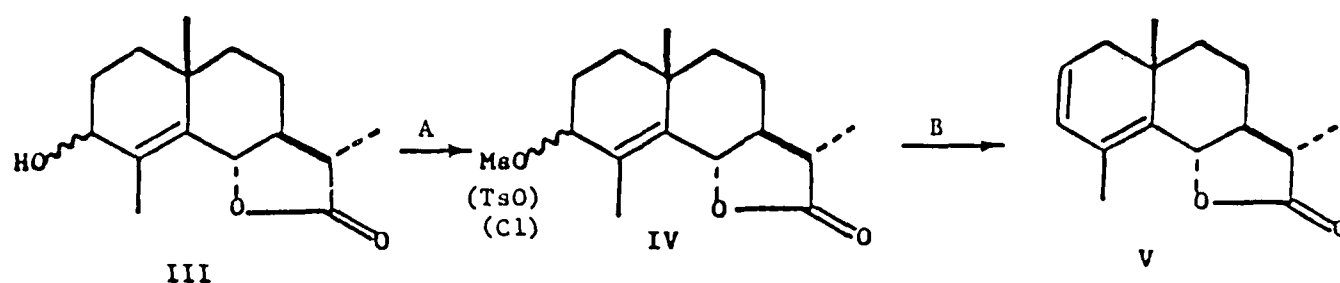


Table 1 : Conversion of Compound III to Compound V Under Various Conditions



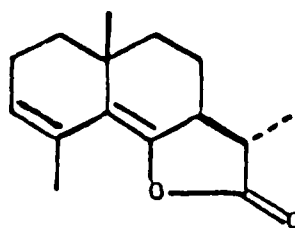
Reagents

	A	B	
1	MsCl/Et ₃ N - THF	DBU-DMSO, Δ	I
2	MsCl/Et ₃ N - CH ₂ Cl ₂	DBU-DMSO, room temp	II
3	MsCl/pyridine - THF	pyridine, Δ	III
4	MsCl/pyridine	t-BuOK-DMSO, Δ	IV
5	SOCl ₂ -pyridine	t-BuOK-DMSO, room temp	V
6	SOCl ₂ -pyridine	Et ₃ N-DMSO, room temp	VI
7	p-TsCl-pyridine	DBU-CHCl ₃ , room temp	VII
8	MsCl/pyridine/DMSO	pyridine-DMSO, Δ	VIII

Best Conditions

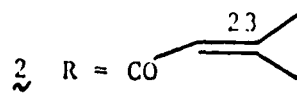
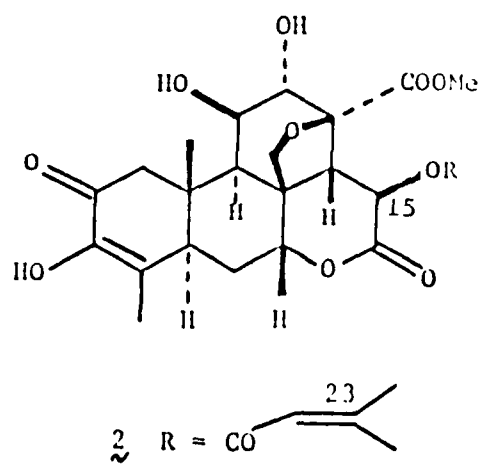
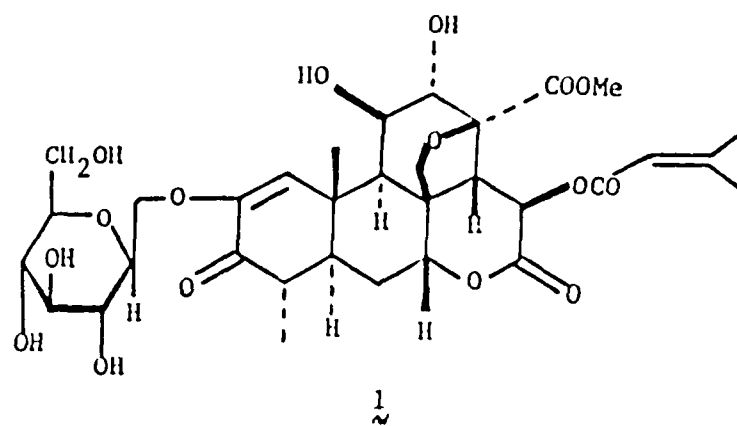
A2-BVI (25% yield) or
A2-BII (25% yield) or
A2-BVIII (25% yield)

The other combination of reaction conditions all produced less than 25% yield.

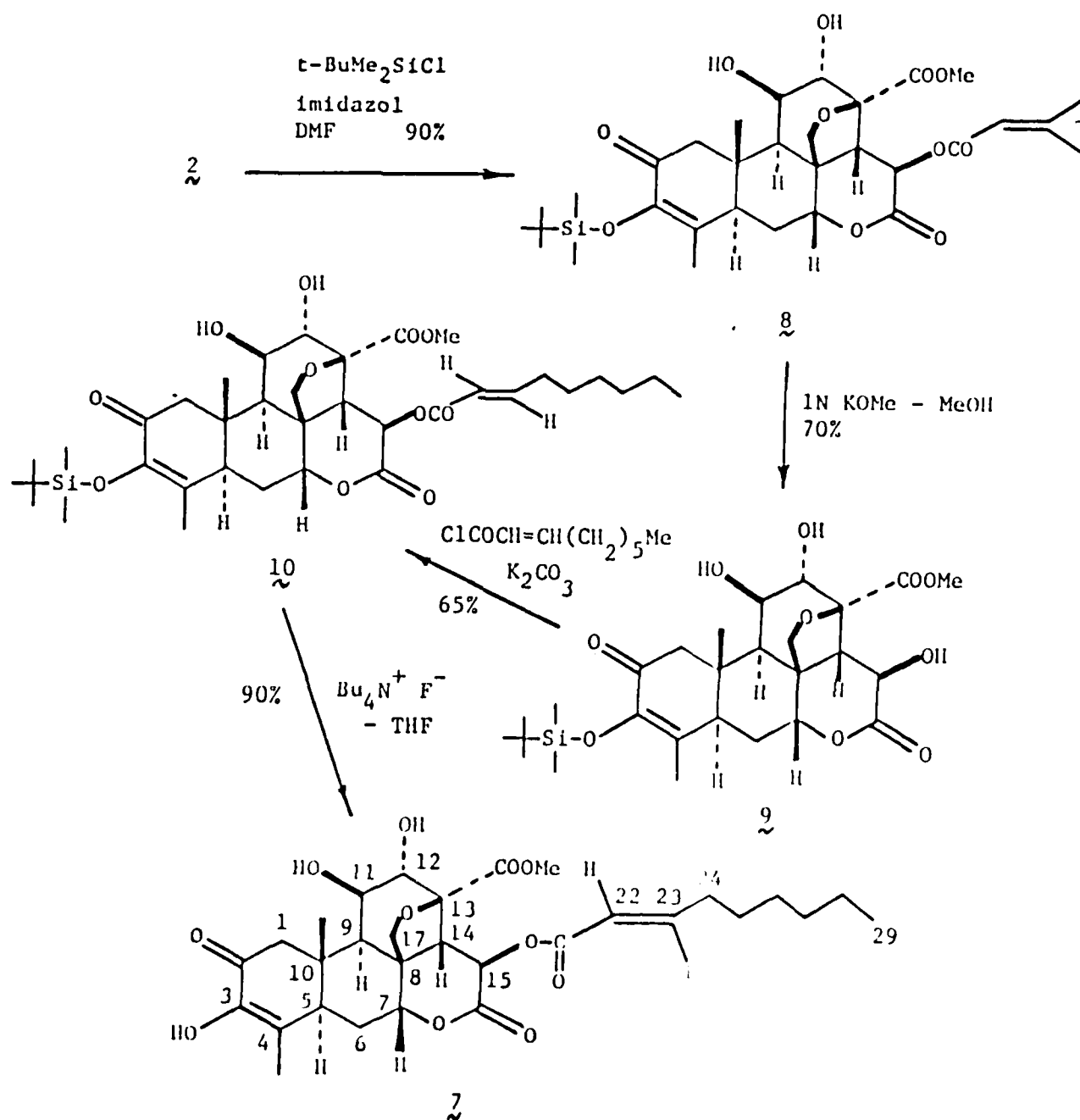


Va

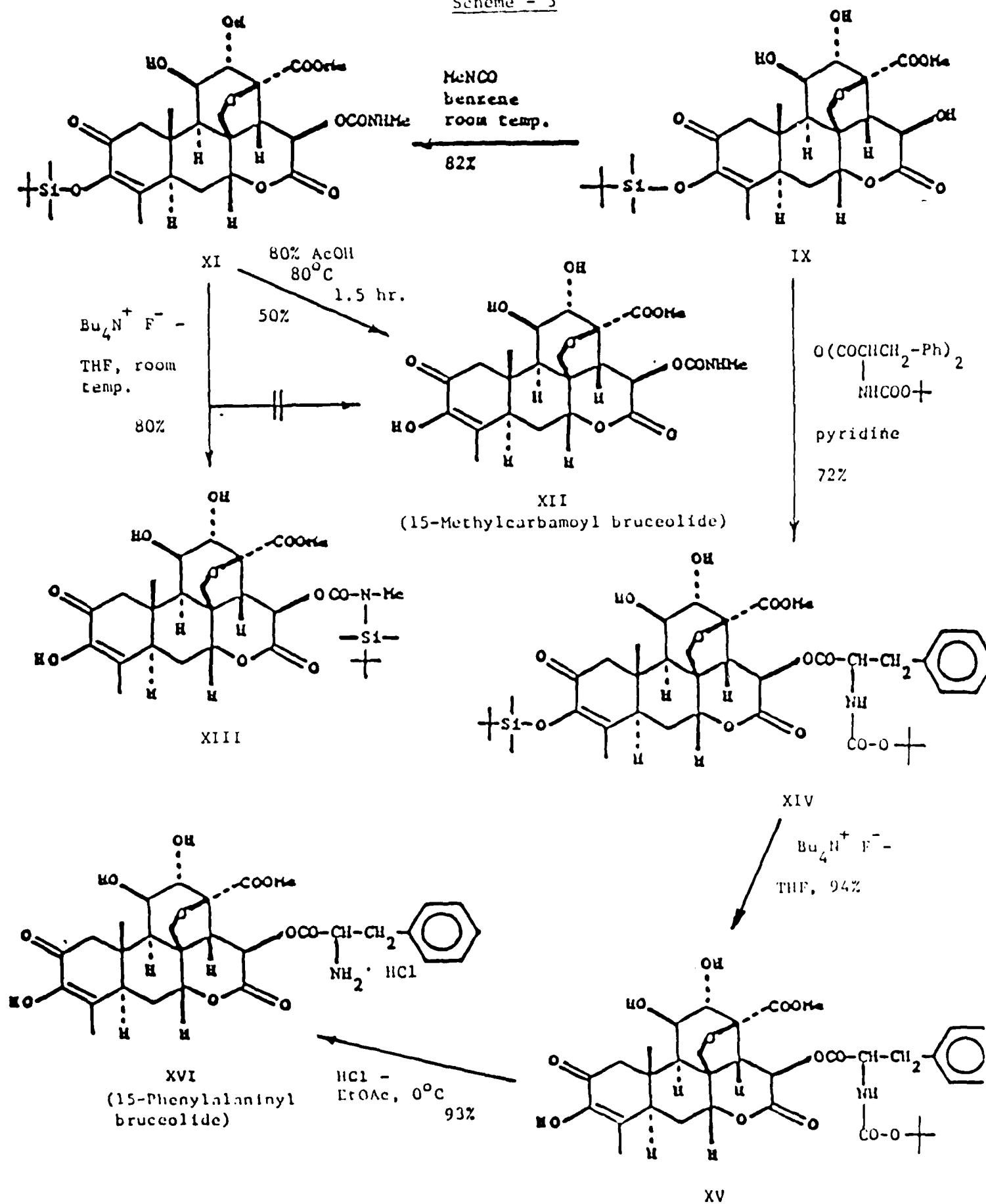
Scheme - 2



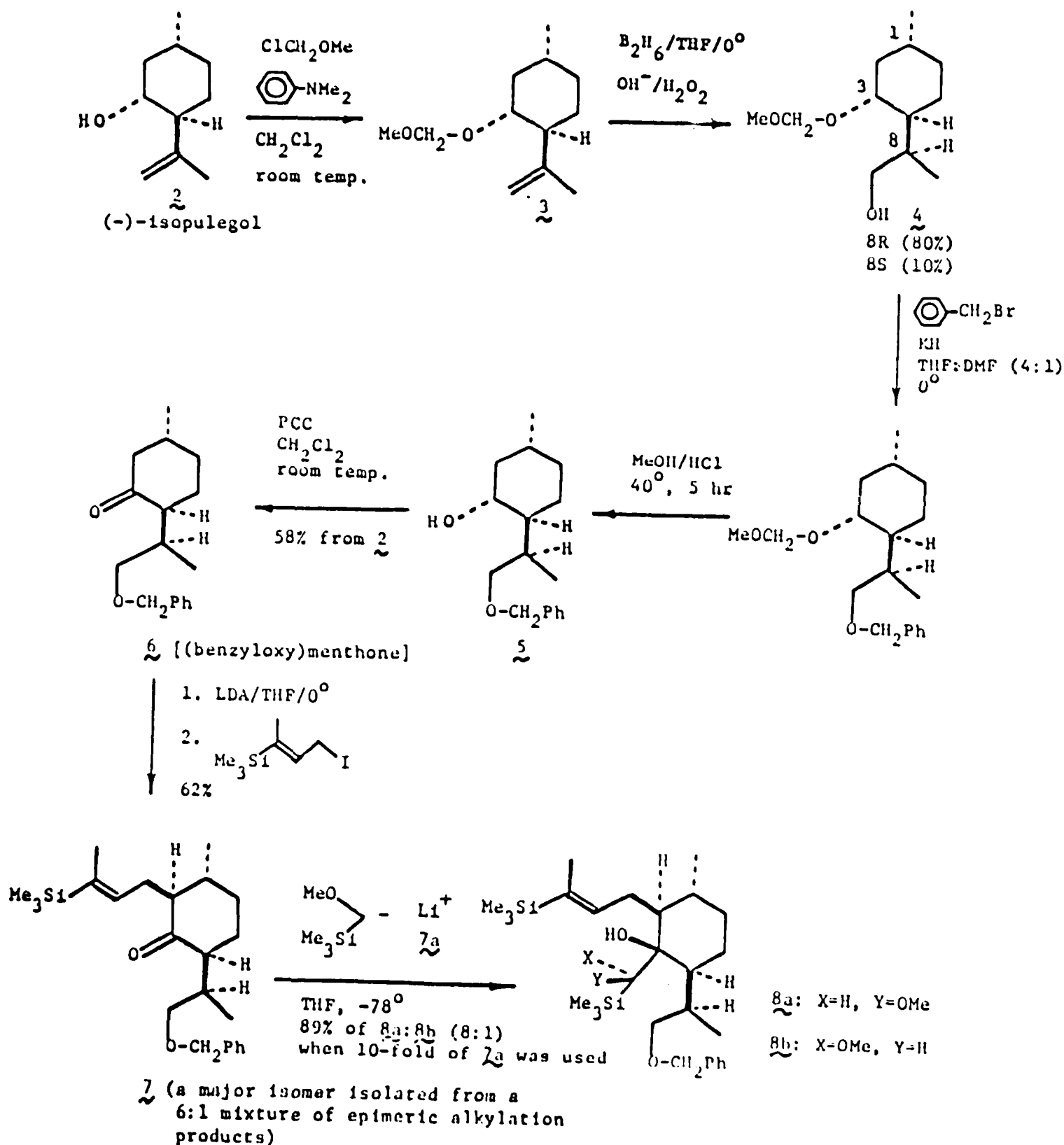
Scheme - 2 - Continued



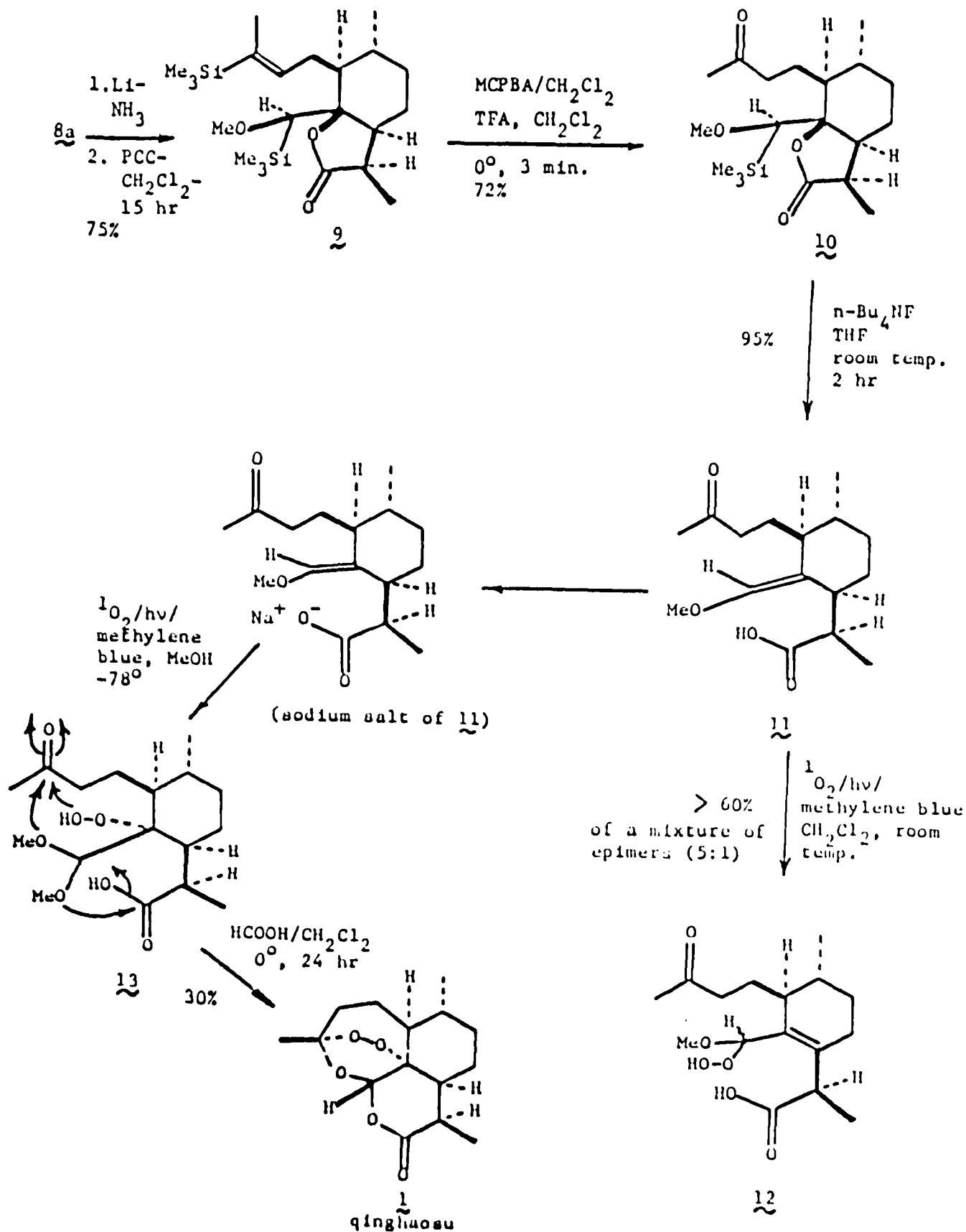
Scheme - 3



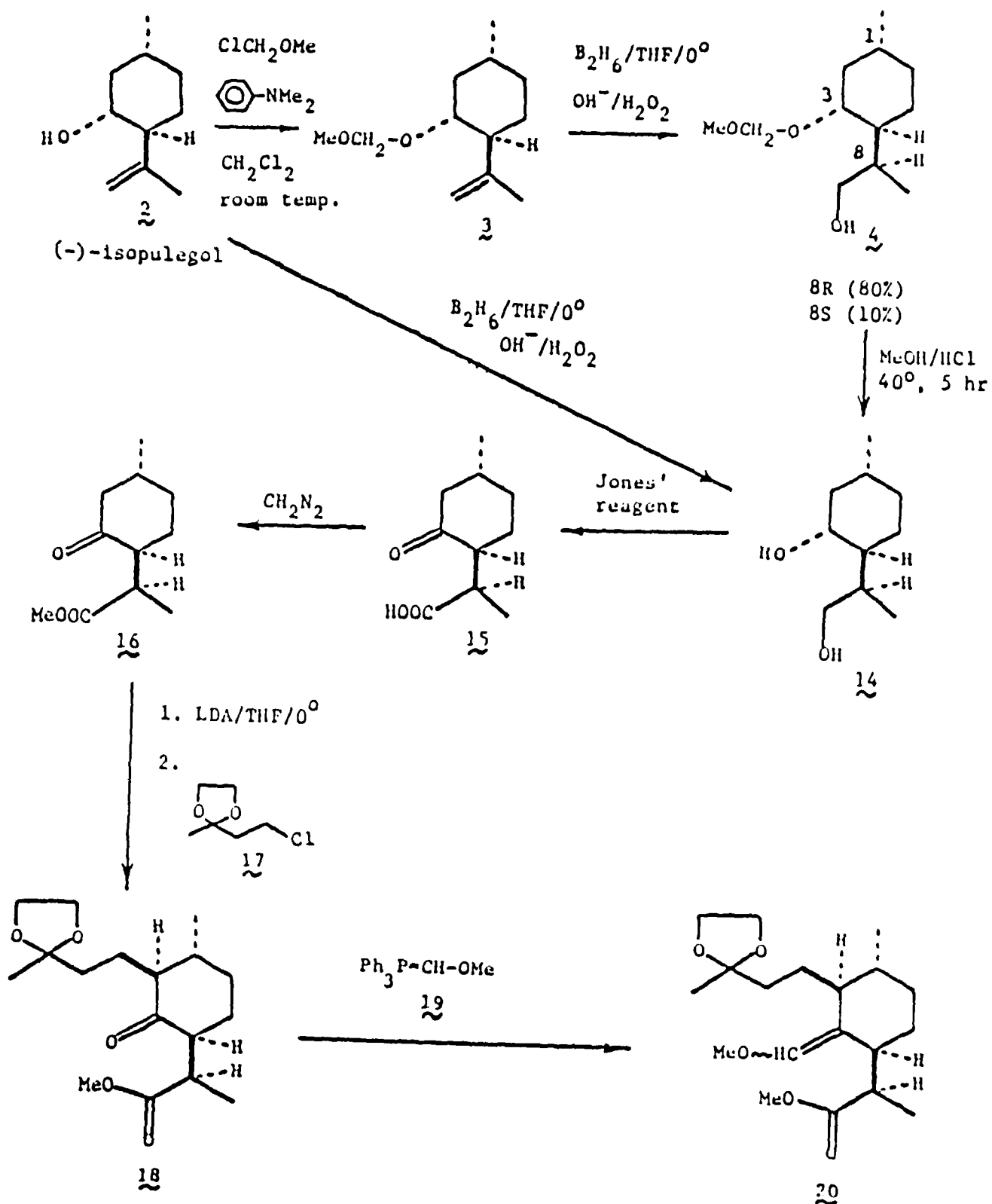
Scheme - 4 : Dr. Hofheinz's Total Synthesis of Qinghaosu
 [G. Schmid and W. Hofheinz, *J. Am. Chem. Soc.*, 105, 624 (1983)]



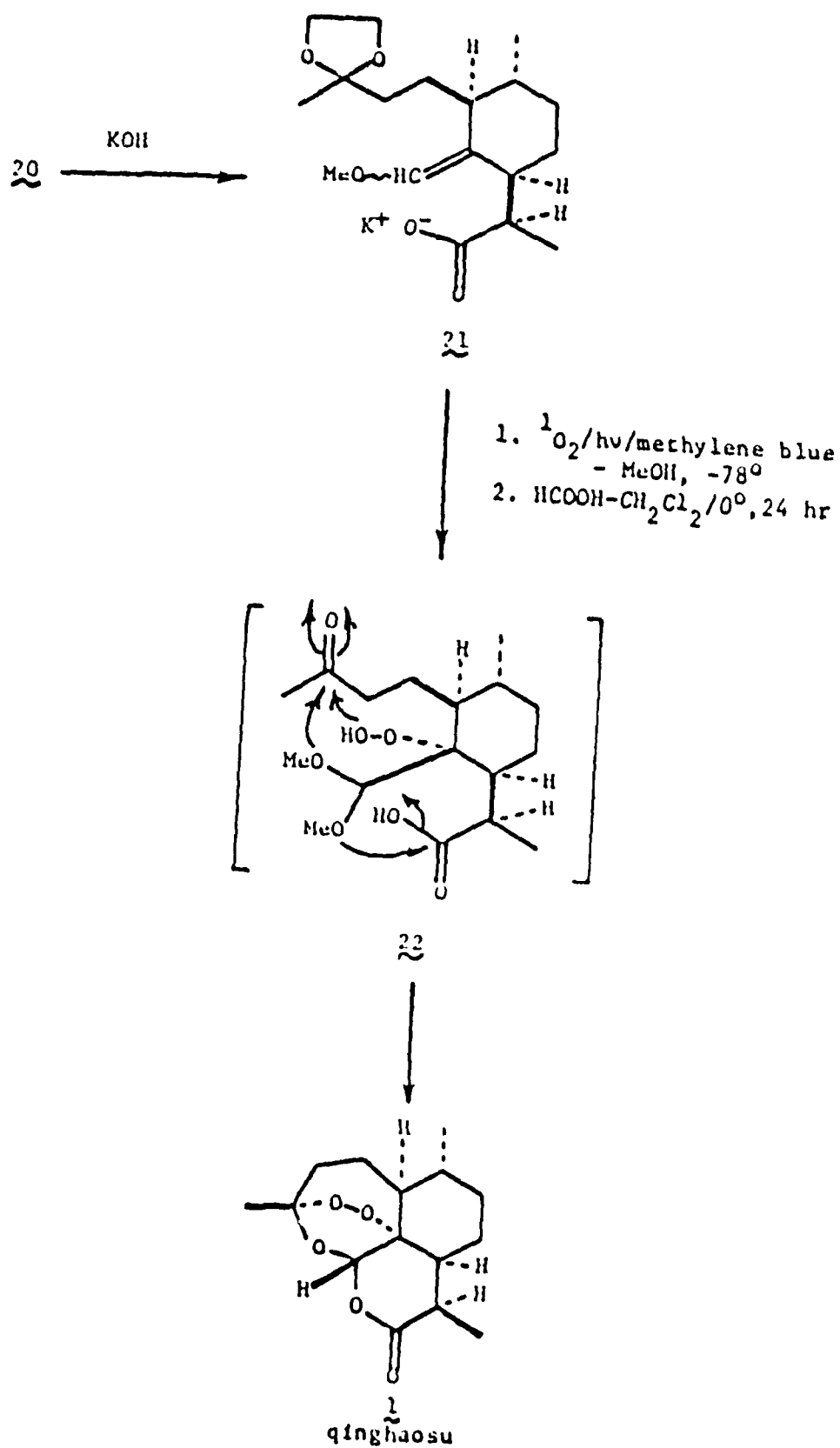
Scheme - 4 : Dr. Hofheinz's Total Synthesis of Qinghaosu - Continued



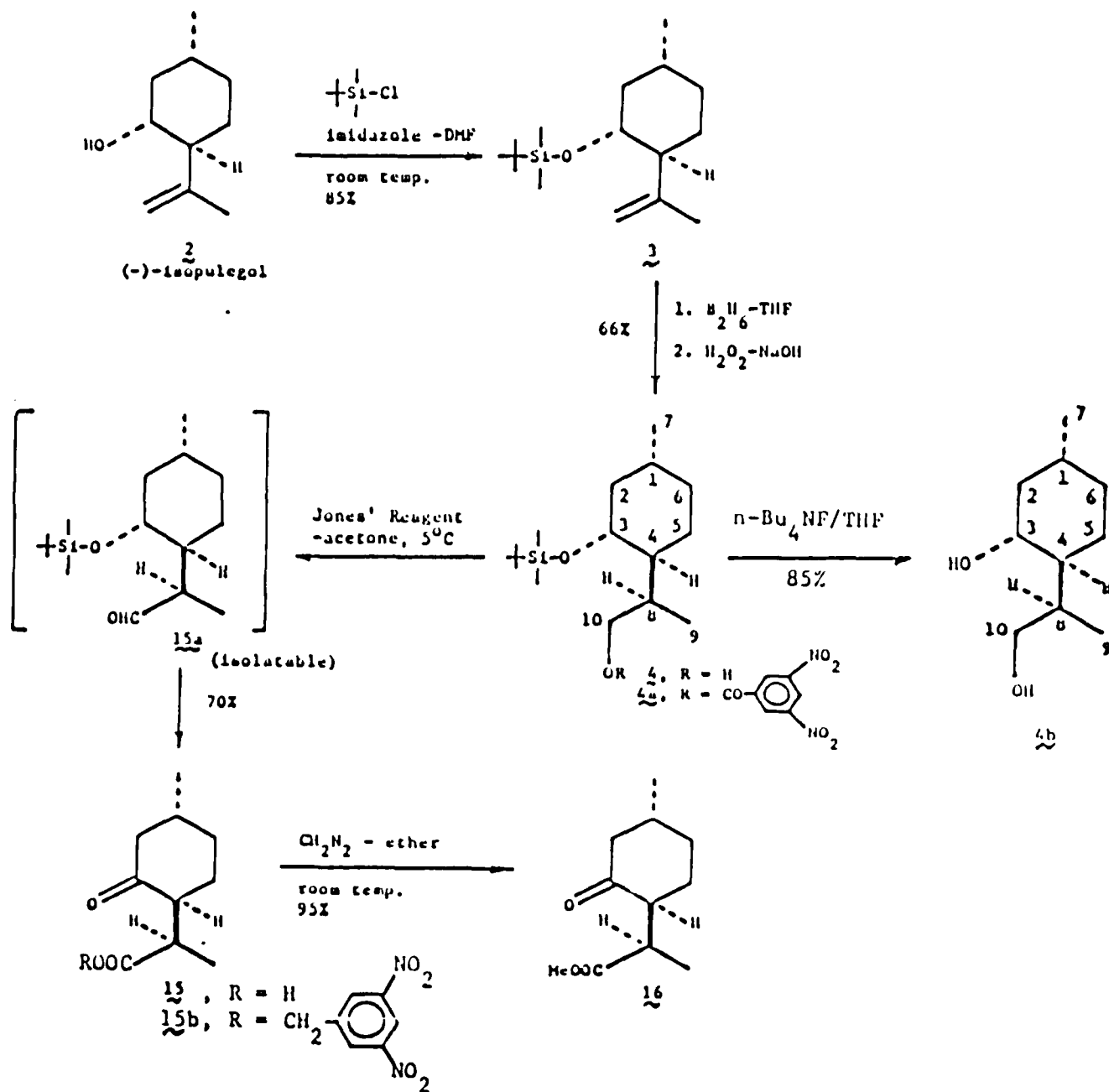
Scheme 5 : Proposed Total Synthesis of Qinghaosu



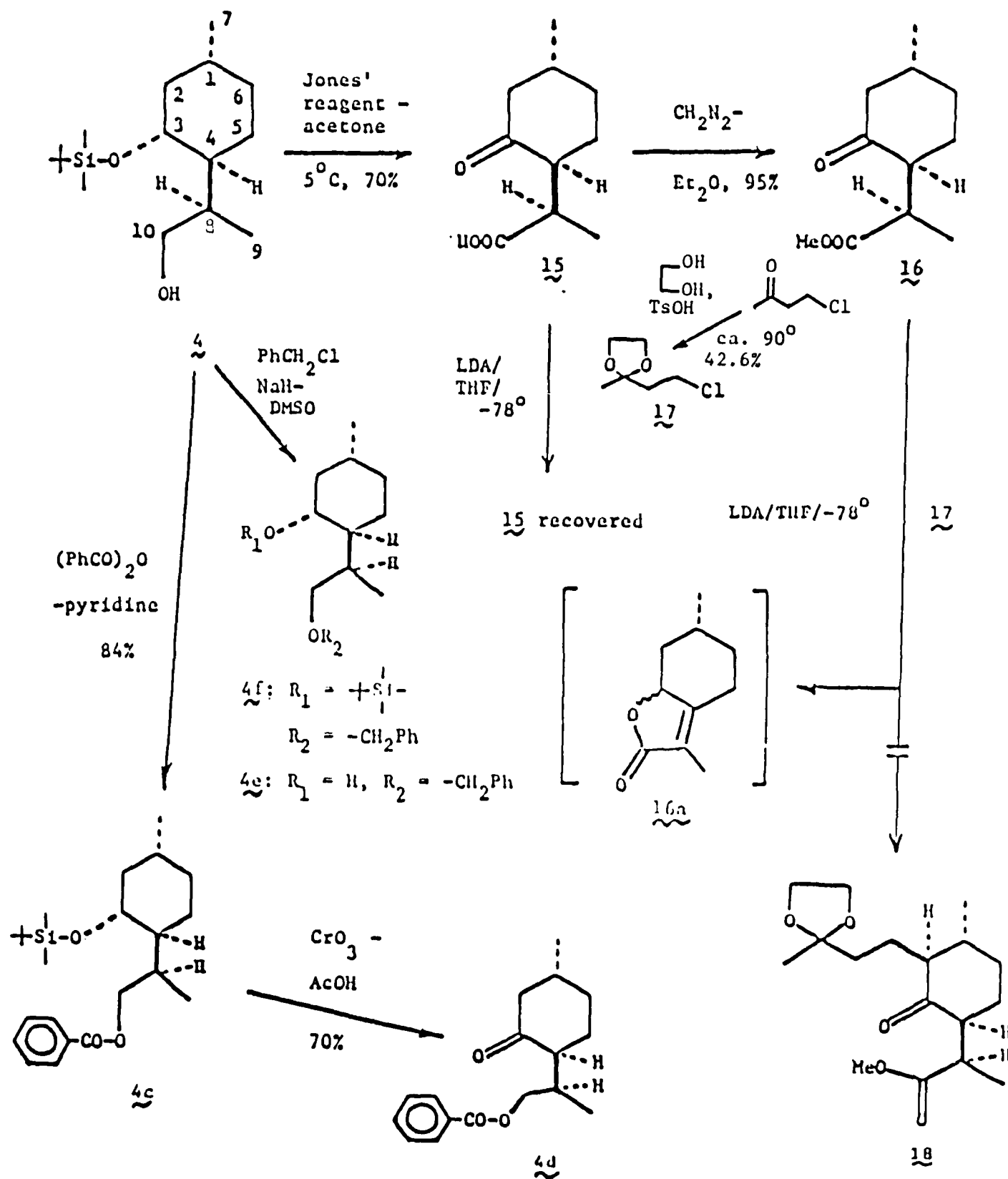
Scheme - 5 : Proposed Total Synthesis of Qinghaosu - Continued



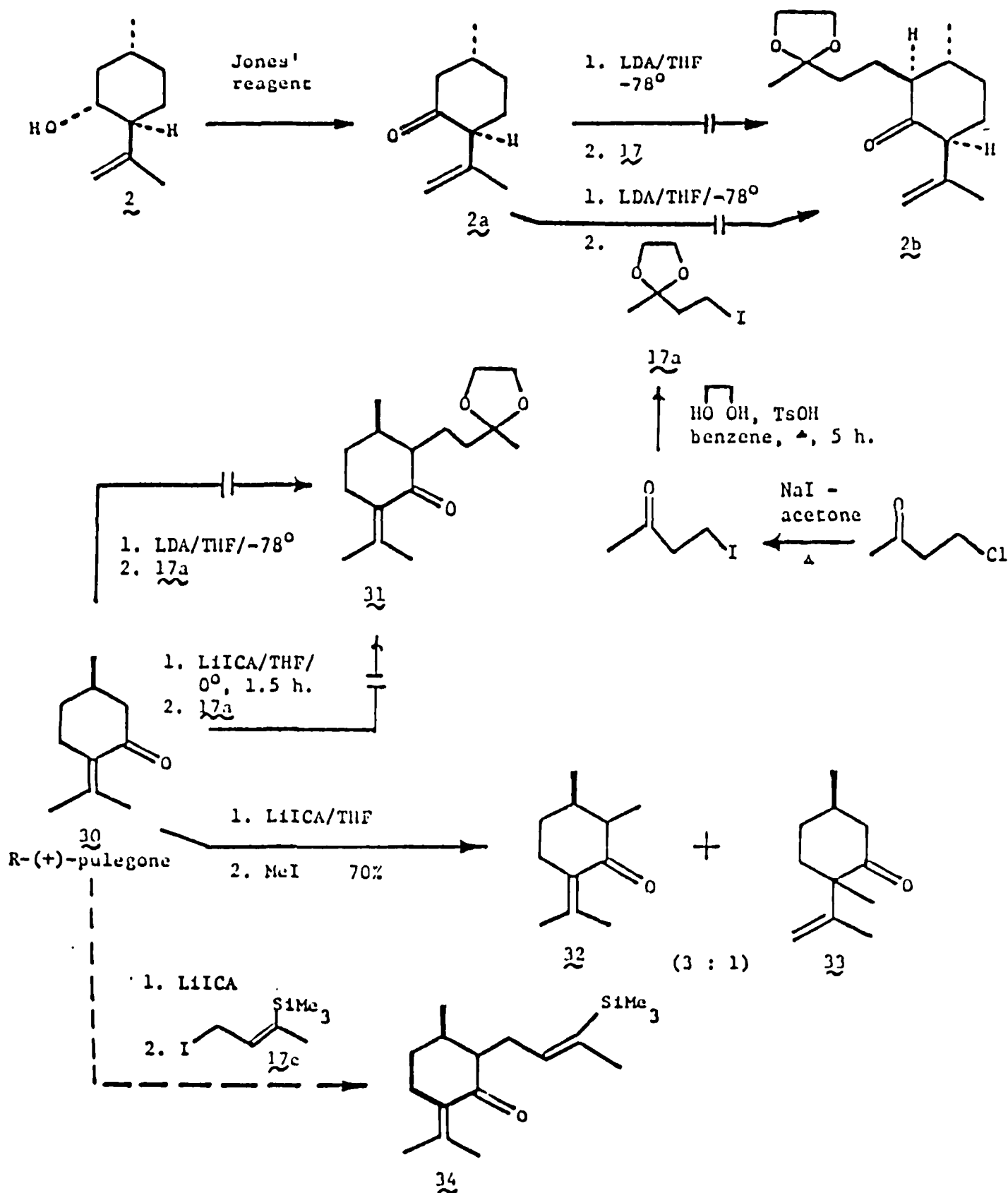
Scheme - 6



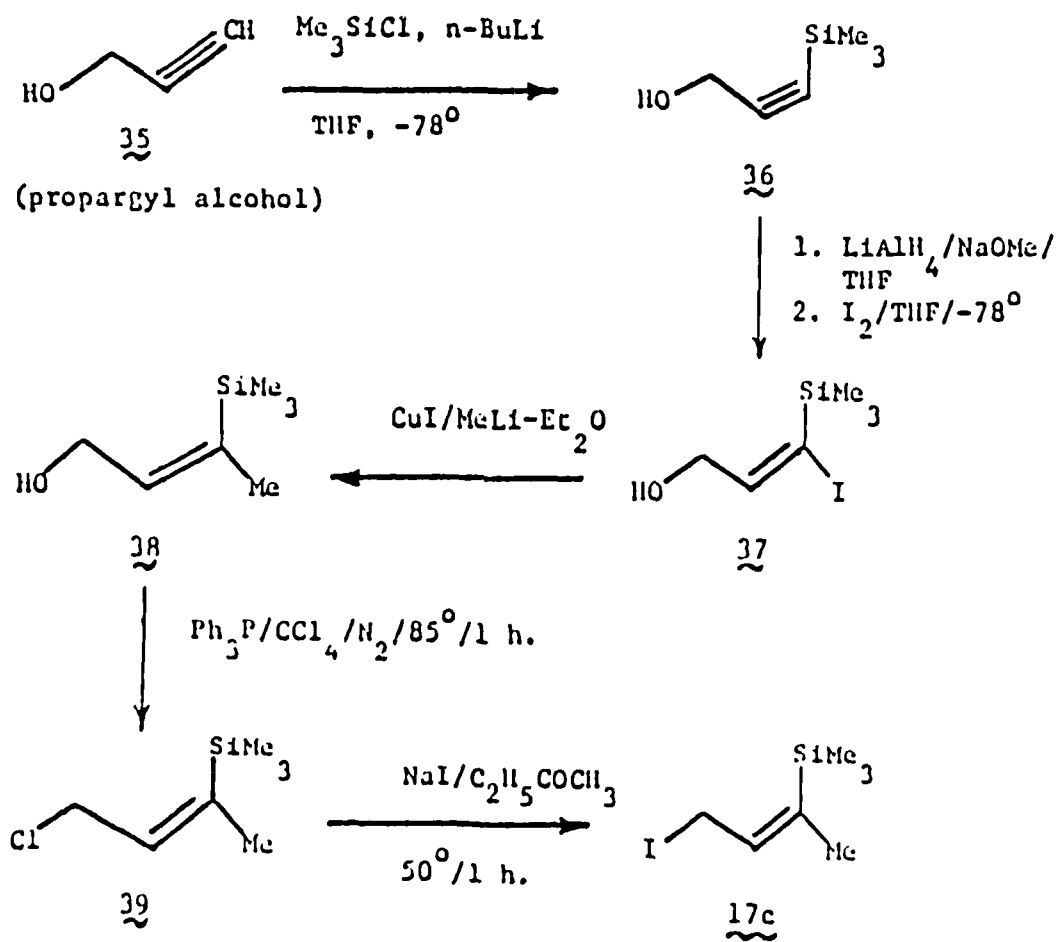
Scheme - 6 - Continued



Scheme - 6 - Continued

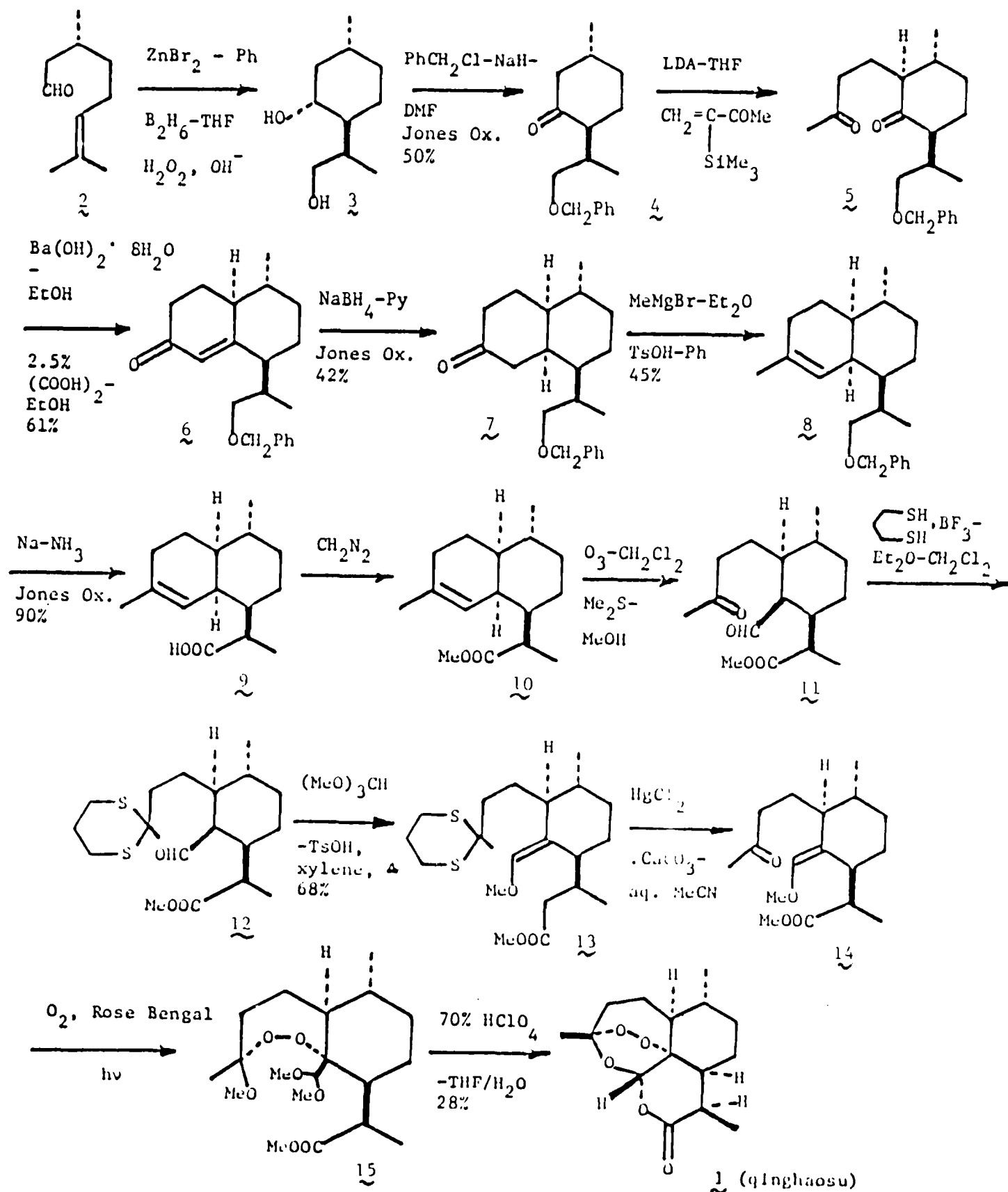


Scheme - 6 - Continued

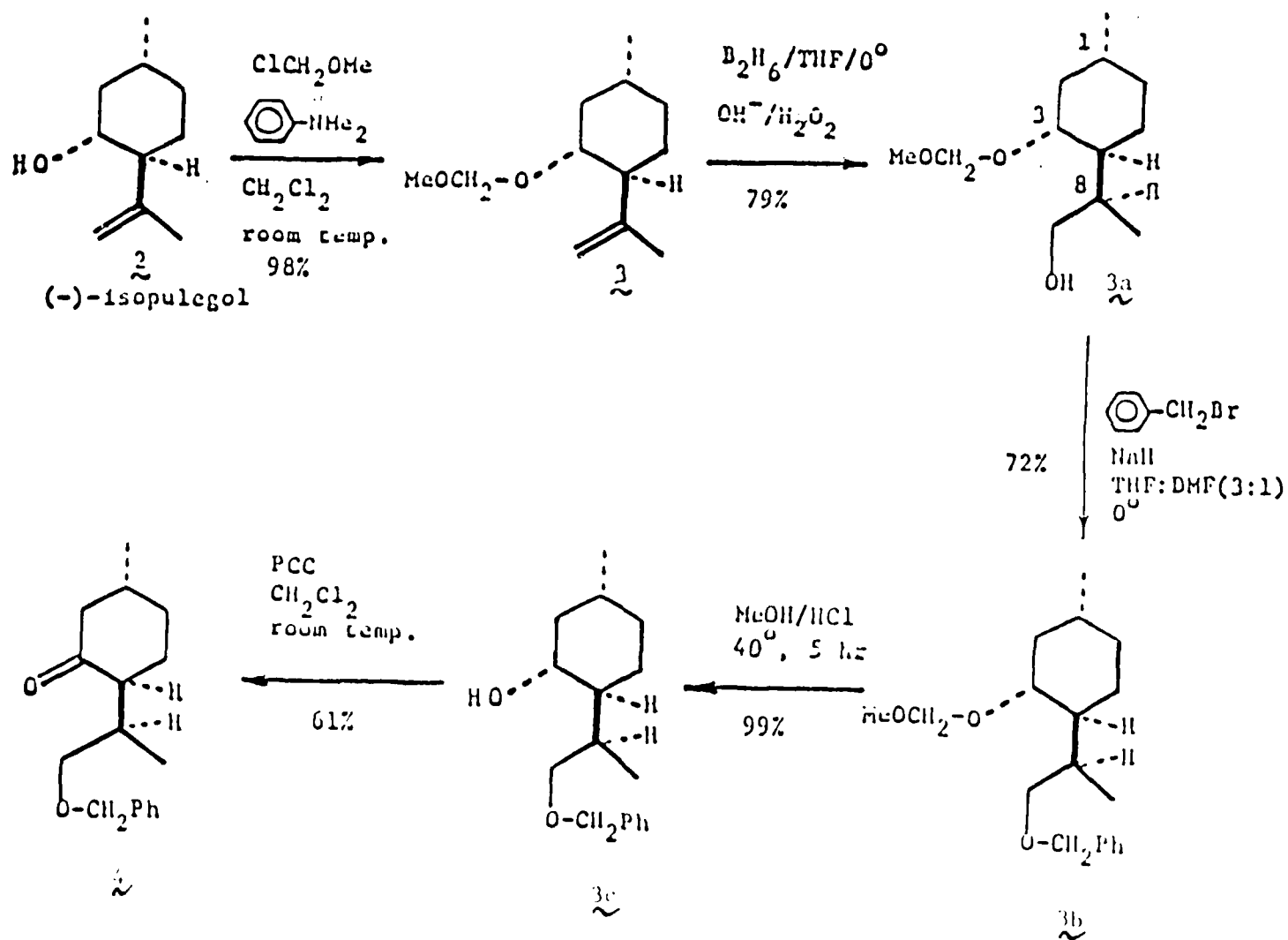


Scheme - 7 : Zhou's Total Synthesis of Qinghaosu

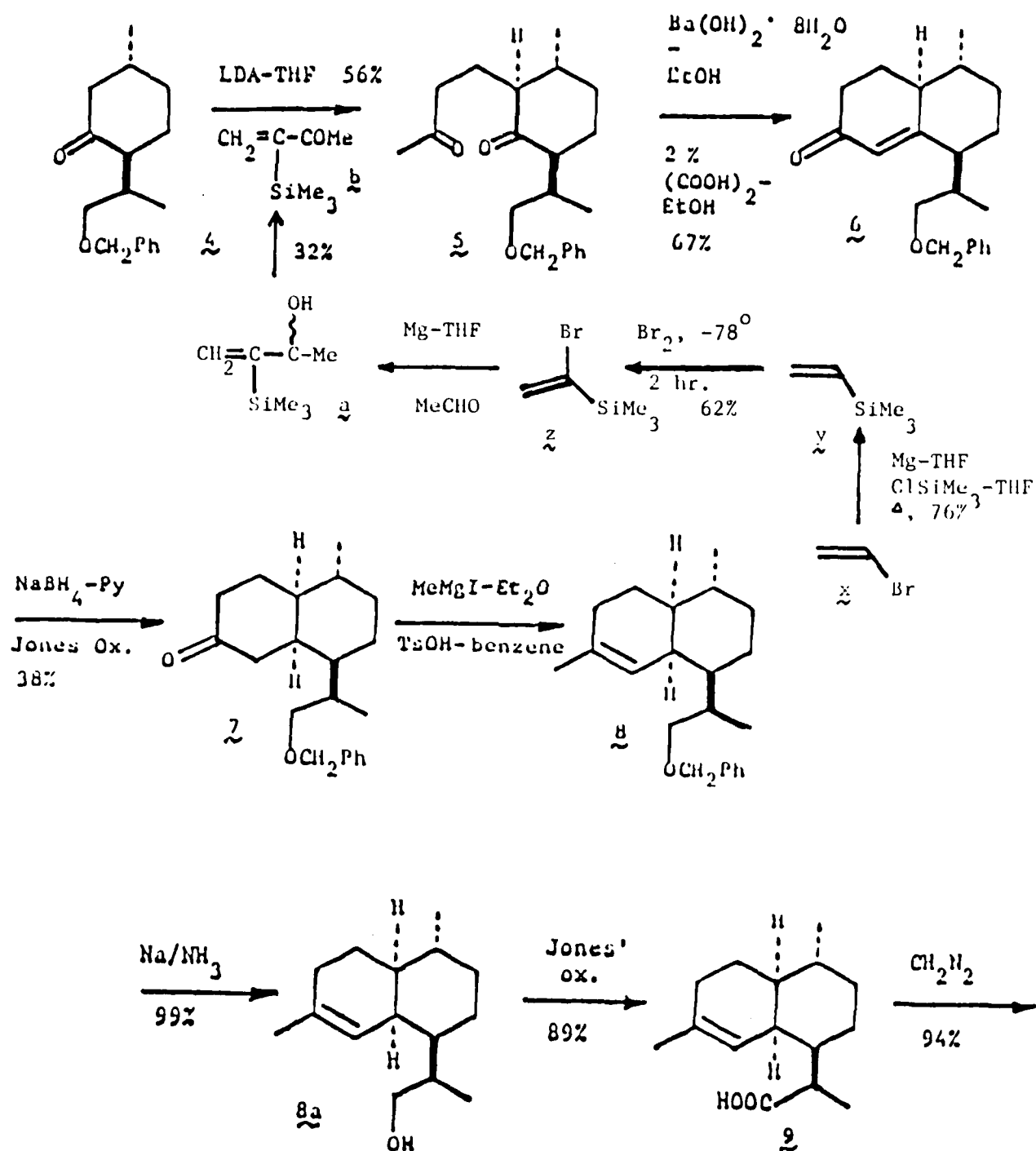
(X. X. Xu, J. Zhu, D. Z. Huang and W. S. Zhou, 1984 Hawaii ACS Meeting Abstract, and *ibid*, *Tetrahedron*, 42, 819 (1986))



Scheme - 8 - Synthesis of Qinghaosu Based Upon Zhou's Method



Scheme - 8 - Synthesis of Qinghaosu Based Upon Zhou's Method - Continued



Scheme - 8 - Synthesis of Qinghaosu Based Upon Zhou's Method - Continued

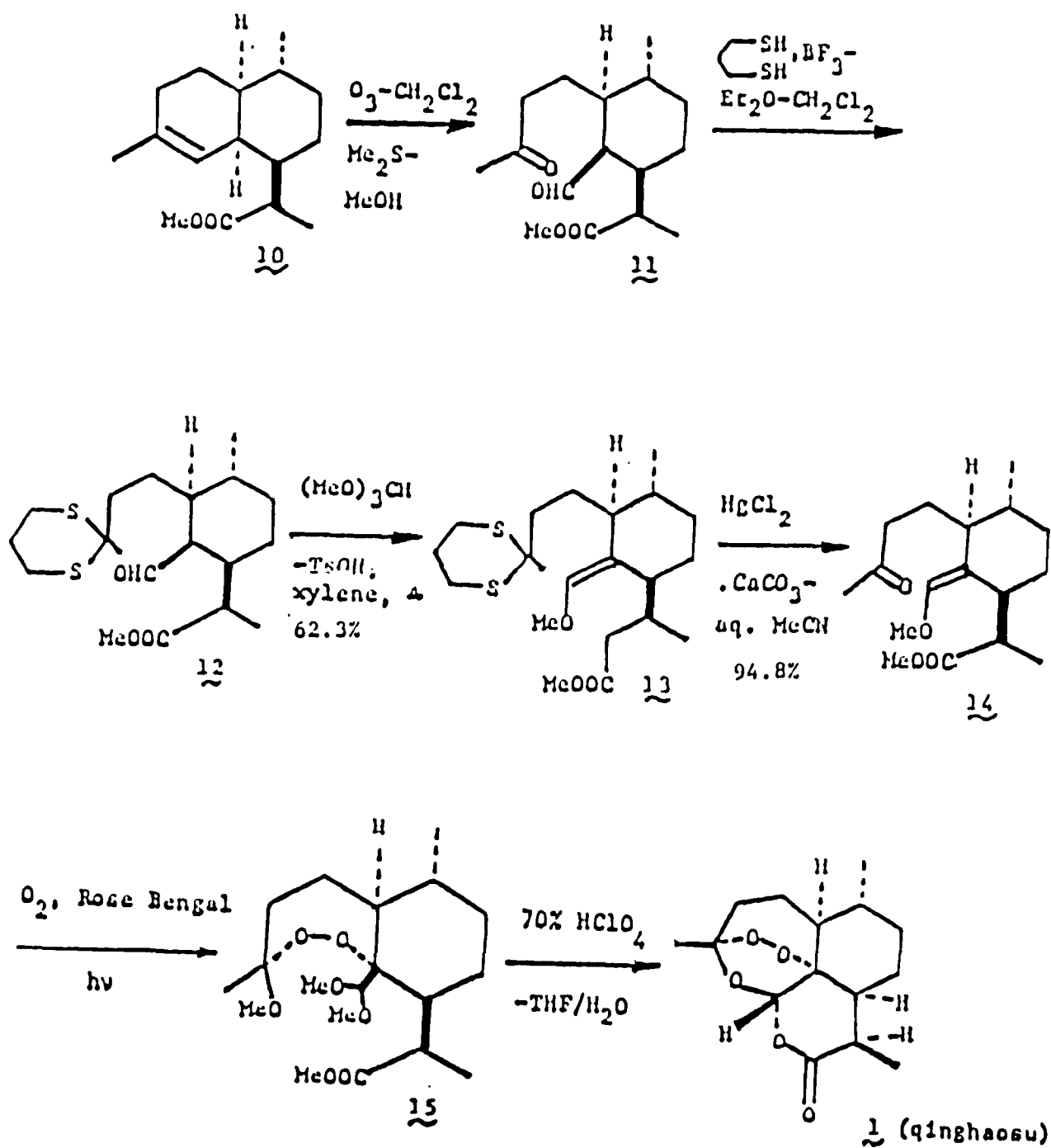
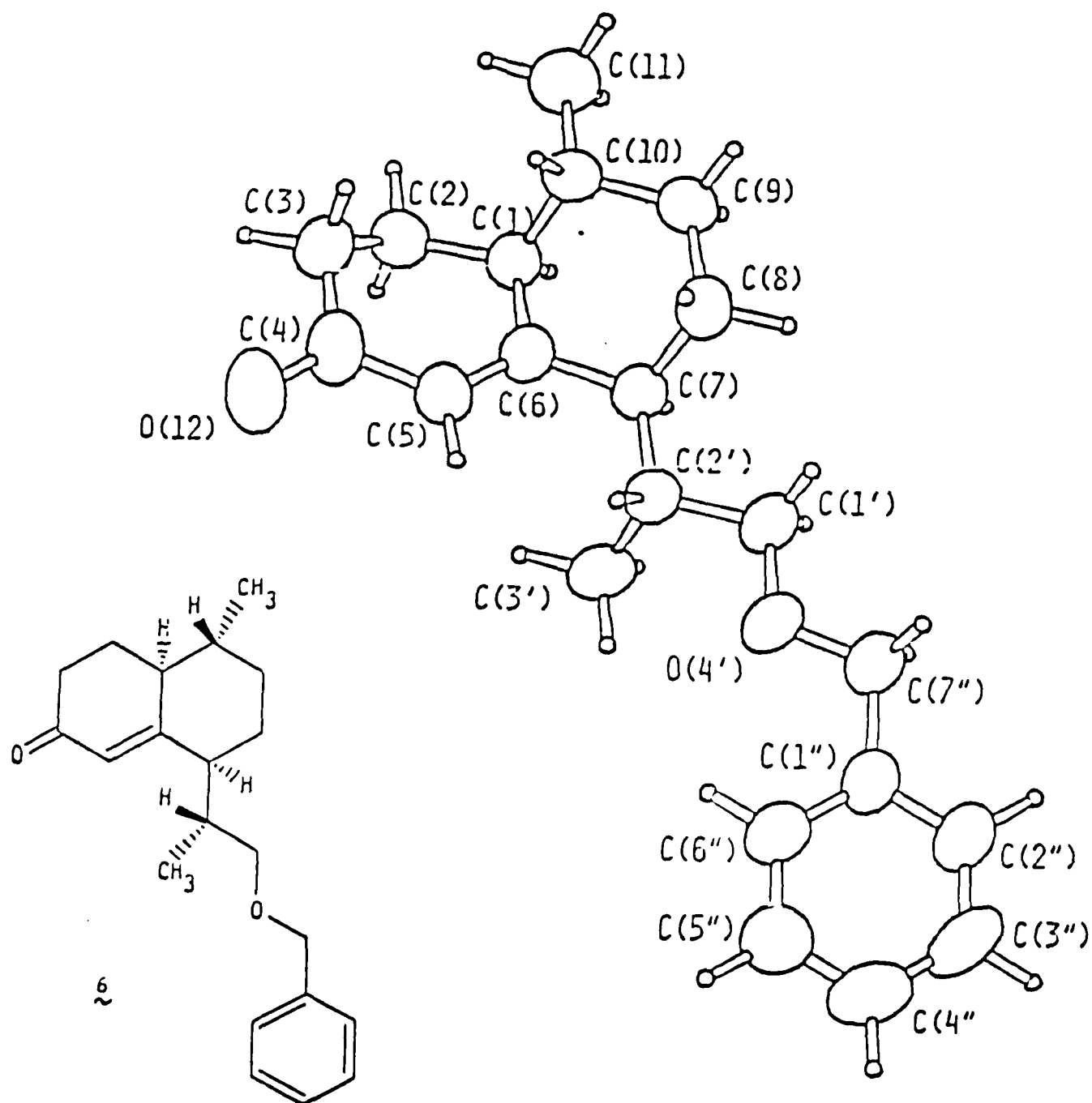
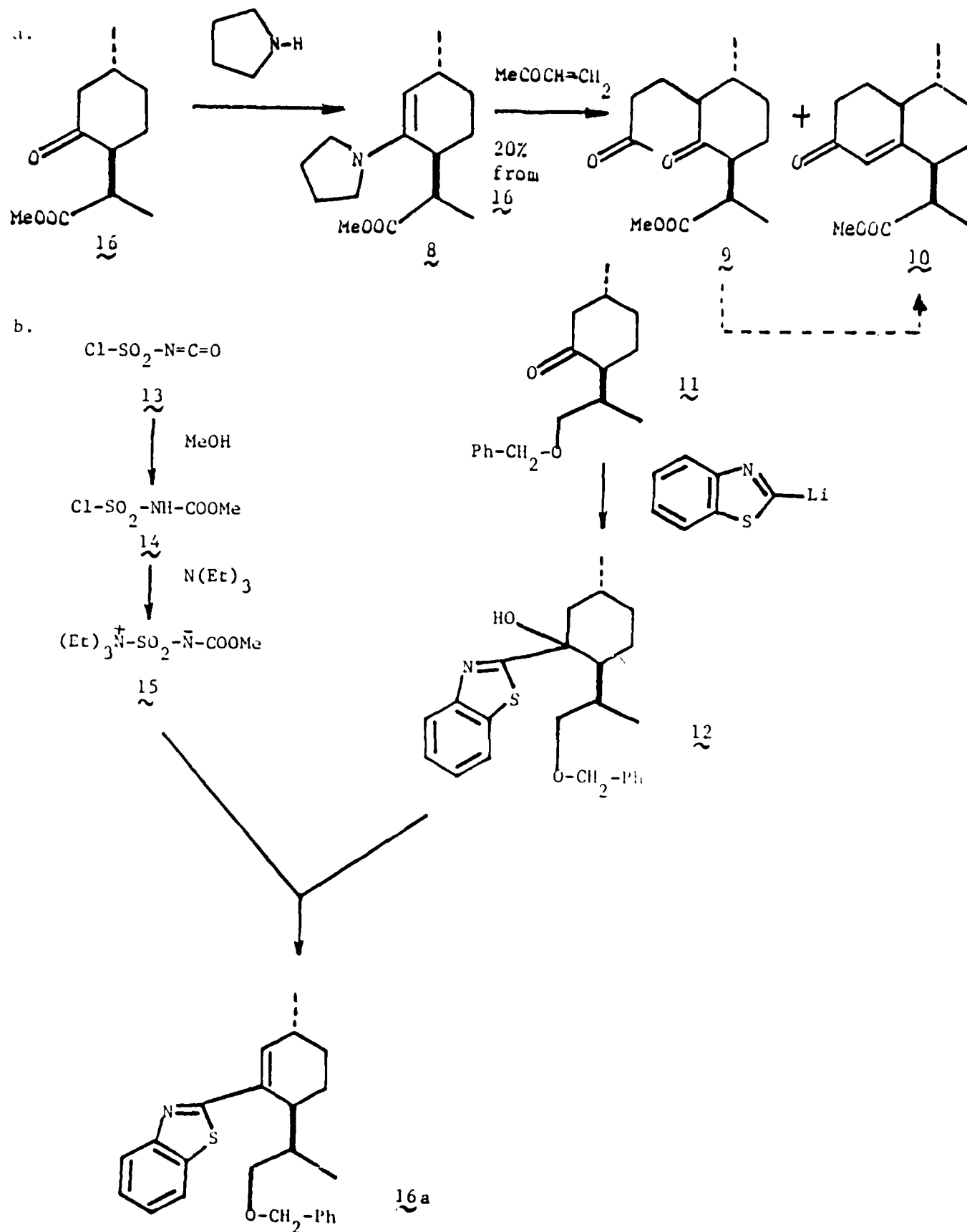


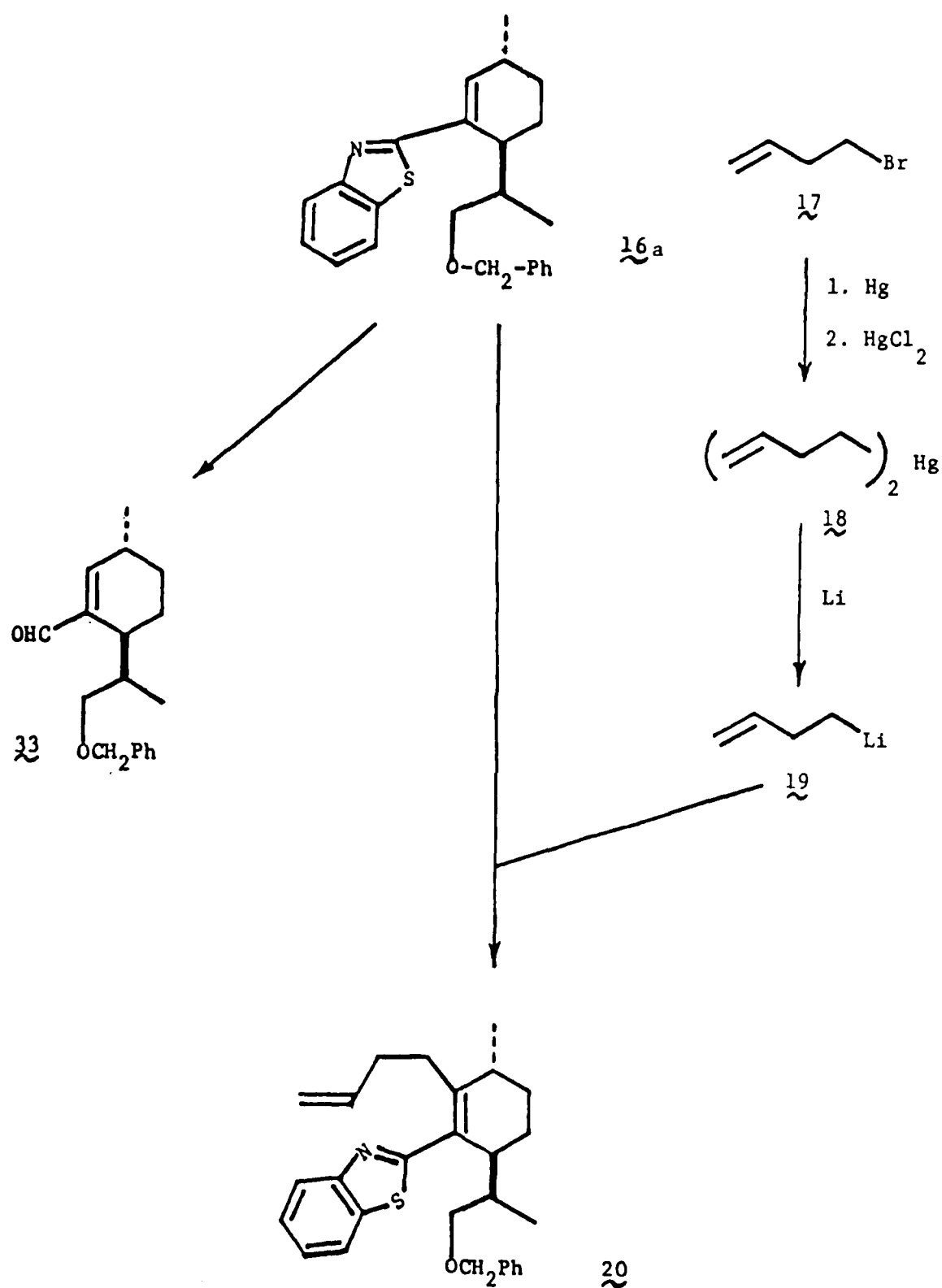
Figure 1
X-Ray Structure of Compound 6



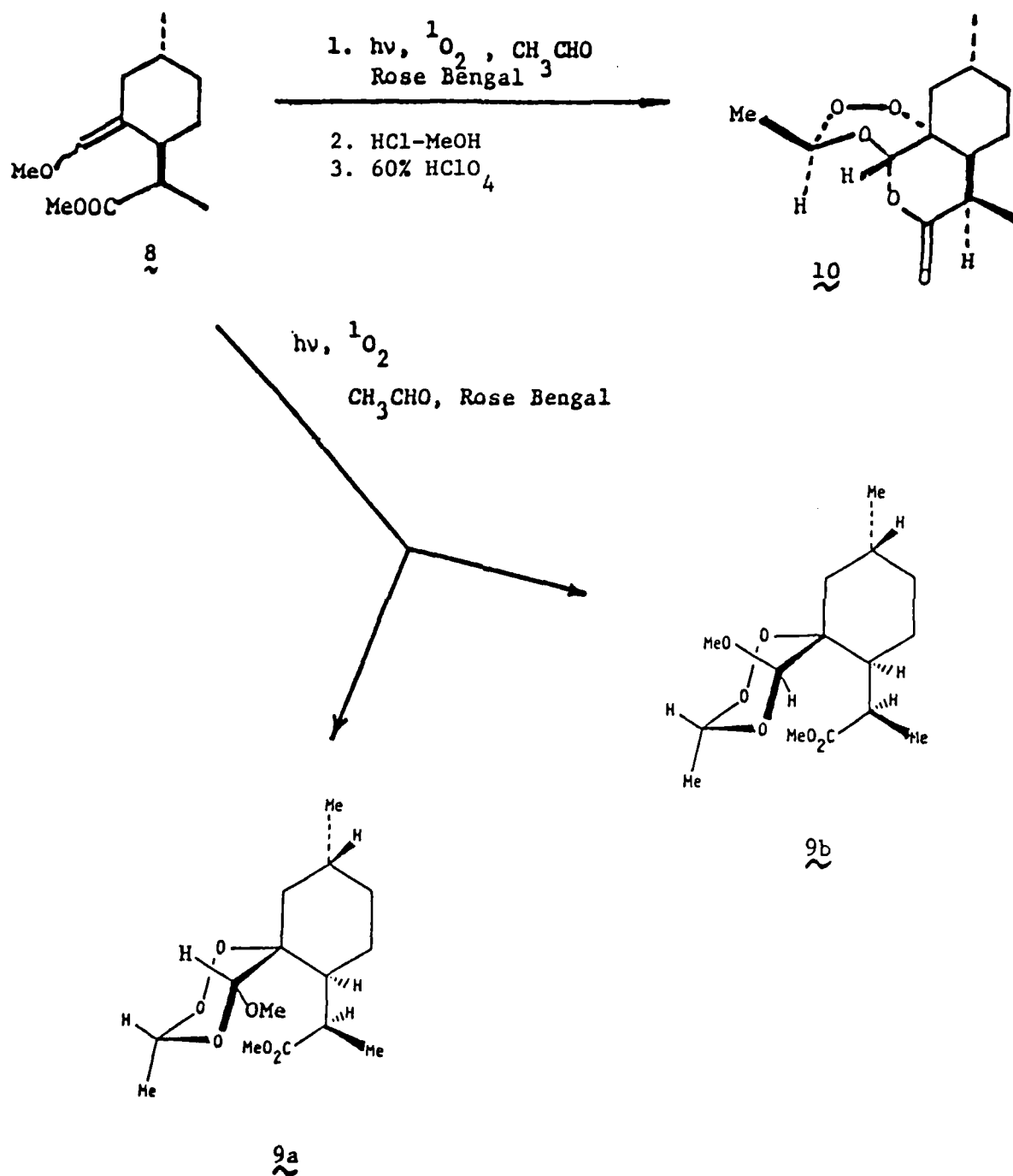
Scheme - 9 - New Synthesis of Oinghaosu



Scheme 9 - New Synthesis of Qinghaosu - Continued



Scheme 10 - Synthesis of Simpler Qinghaosu Analogs



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