

Studies on the Constituents of *Artemisia annua* Part II¹

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Key Word Index:

Artemisia annua L.; Compositae; Qinghaosu; Qinghaosu I–V; Qinghao acid; Flavones; Alkanol; Scopoletin; Essential oil.

Abstract

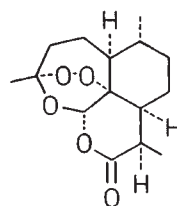
The present paper is a continuation of our study on the Chinese traditional herb *Artemisia annua* L. [1–5], describing several additional constituents: quinghaosu IV and V (V, VII), quinghao acid (VIII) [6], chrysosplenol (VIa) [7] and a paraffinic alcohol; V, VII and VIII are compounds with unreported structures.

Introduction

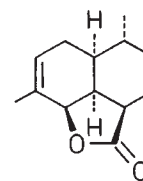
A number of our earlier papers have been devoted to studies of chemical constituents isolated from *Artemisia annua* L. (Compositae), the most notable constituent being the antimalarial quinghaosu (I) [1–4], a peroxidic lactone with unique structure. Other constituents include quinghaosu-I-III (II–IV), a flavonol (VI), scopoletin and a few terpenes from the essential oil [5].

This paper deals with the isolation and characterization of V, VIa, VII, VIII and a paraffinic alcohol.

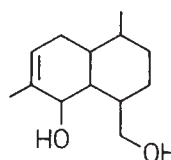
It is pertinent here to point out the close stereochemical kinship among the previously established structures I–IV. They all belong to the amorphane series (IX) [9], which has a *cis* decalin skeleton with the isopropyl group *trans* to the hydrogen on the ring juncture. Compounds I and IV are further distinguished by the presence of a modified seven-membered A-ring as the result of insertion of an extra ether oxygen.



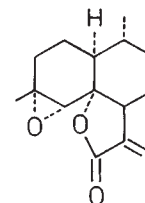
I quinghaosu



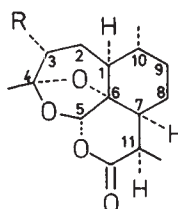
II quinghaosu-I



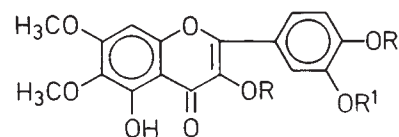
IIa LAH product of II



III quinghaosu-II
(arteannuin B) [8]

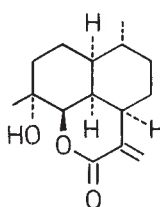


IV quinghaosu-III, R=H

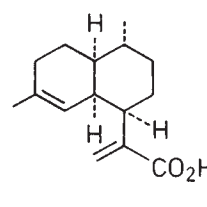


VI R=H, R¹=CH₃

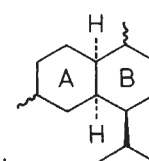
V quinghaosu-IV, R=OH VIa R=CH₃, R¹=H (chrysosplenol)



VII quinghaosu-V



VIII quinghao acid



IX amorphane [9]

¹ For Part I, see ref. 5.

Results and Discussion

Qinghaosu-IV (V) is a colourless crystalline compound with m.p. 172–173°. MS molecular weight 282 (M^+) agreed with $C_{15}H_{22}O_5$. Hydroxy and lactonic carbonyl groups can be inferred from its IR spectrum (3450, 1728 cm^{-1}). The 1H NMR spectrum displayed the following features: δ 0.95 (d, $J = 6$ Hz, 10- CH_3), 1.20 (d, $J = 6$, 11- CH_3), 1.65 (s, 4- CH_3), 3.20 (m, H-C-11), 3.60 (br, H-C-3), 5.60 (s, H-C-5) and an exchangeable OH group at 1.88. Except for the two signals at δ 1.88 and 3.60, the NMR spectra of IV and V are almost superposable, thus leading to the conclusion that the extra oxygen of V is in the form of a hydroxyl group with only four possible places (C-2, 3, 8 and 9) for its accommodation. Eu(fod)₃ was used for its allocation. Addition of successive aliquots of the shift reagent gave rise to linear changes of chemical shifts. In the case of qinghaosu III (IV), we have for 10- CH_3 , δ 0.95 \rightarrow 0.96 \rightarrow 1.16 \rightarrow 1.16; for 11- CH_3 , 1.20 \rightarrow 1.42 \rightarrow 2.20 \rightarrow 2.40; for 4- CH_3 , 1.54 \rightarrow 1.55 \rightarrow 1.74 \rightarrow 1.75; and for H-C-5, 5.55 \rightarrow 5.75 \rightarrow 6.20 \rightarrow 6.40. Qinghaosu IV (V) gave the corresponding shifts: 10- CH_3 , δ 1.04 \rightarrow 1.46 \rightarrow 1.77 \rightarrow 2.15; 11- CH_3 , 1.27 \rightarrow 1.99 \rightarrow 2.60 \rightarrow 3.42; 4- CH_3 , 1.65 \rightarrow 3.93 \rightarrow 5.74 \rightarrow 7.25; H-C-5, 5.70 \rightarrow 6.91 \rightarrow 8.06 \rightarrow 9.18. The marked shifts for 4- CH_3 and H-C-5 in compound V is compatible only with an OH group at position-3, and the $W_{1/2}$ 6 Hz) of the H-C-3 multiplet (hence equatorial) indicates an α -orientation for the OH group (axial). The structure of IV has been firmly established by its preparation from qinghaosu (I) by catalytic hydrogenation [5].

Chrysosplenol (6a) has very similar UV and 1H NMR spectra to eupatin (3, 5, 3'-triOH, 6, 7, 4'-triOMe) [7]. However, large discrepancies in m.p. of the acetates (155–157°; 219–221° for eupatin acetate) led us to a direct comparison of their IR spectra which also displayed conspicuous differences. The structure of chrysosplenol (VIa) was confirmed by the following facts. Methylation gave the hexamethyl ether, identical with an authentic specimen. The 5-OH showed a characteristic chelated NMR shift at δ 12.60. Methylation with CD_2N_2 in CH_3OH gave three partially deuterated methyl groups onto the original phenolic hydroxyls, and solvent shifts (benzene vs. chloroform [10]) revealed 5, 3' and 4' as the labelled positions. Further confirmation came from alkali fusion of the ethylated derivative, whereby 3,4-diethoxybenzoic acid (m.p. and MS) was obtained.

Qinghao acid (VIII) forms colourless cubes with m.p. 131°, $[\alpha]_D^{20} + 36^\circ$ (0.01, $CHCl_3$) and MS m/e 234 (M^+), compatible with $C_{15}H_{22}O_2$. IR peaks at 3480–2590 (s, br), 1690 (s) and 1625 (m) are indicative of an α , β -unsaturated acid. These are borne out by 1H NMR data, δ 0.83 (d, $J = 6$, 10- CH_3), 1.60 (s, 4- CH_3), 4.94 (br, H-C-5), 5.54, 6.46 (br, 11 = CH_2)

and 11.56 (br, CO_2H). Irradiation of 4- CH_3 caused an NOE increase of H-C-5 by 40 %. No NOE was found between H-C-5 and the endocyclic methylenic hydrogens, presumably due to free rotation of the $C_{7,11}$ bond.

Comparison with II or its LAH reduction product (IIa) showed that the olefinic signal of qinghao acid at 4.94 ($W_{1/2} = 5$ –6 Hz) is much sharper (for 2 and 2a, we have δ 5.60 and 5.50, respectively, with $W_{1/2} = 11$ Hz, due to the coupling with two neighboring protons). Hence the endocyclic double bond of qinghao acid should be placed at $C_{4,5}$ and not $C_{3,4}$. Biogenetic considerations led to the proposal of VIII as the structure of qinghao acid.

Qinghaosu V (VII) has m.p. 125–126°, and a formula of $C_{15}H_{22}O_3$ from MS data. IR data (3420, 1700, 1630 cm^{-1}) showed the presence of OH and α , β -unsaturated lactone functionalities. The 1H NMR spectrum showed two methyl groups at δ 0.85 (d) and 1.36 (s), and terminal methylene protons at 5.56 (s) and 6.16 (s). The carbinyl hydrogen at C-5 (δ 3.82) is a doublet with $J = 3$, hence should be in *cis* relationship with the hydrogen on the ring juncture (C-6). Further studies have been thwarted by scanty supply of material, and the structure as shown by VII is thus tentatively proposed, leaning heavily on biogenetic considerations.

We also isolated a straight chain fatty alcohol, m.p. 74–76°, characterized by its IR and 1H NMR spectra. The MS peak at m/e 392 (M-18) [11] showed it to be octacosanol ($C_{28}H_{57}OH$), probably contaminated by some C_{30} alcohol (*ca.* 5 %) as evidenced by a tiny peak at m/e 420. Further fragmentations of interest involved successive losses of 28 units from m/e 392, giving peaks at m/e 364 and 336. The last mentioned peak however was stronger than usual [11], indicating the possible contamination by a C_{26} alcohol. ULUBELEN et al. [12] reported the isolation of a C_{26} alcohol from the same species, using elemental analysis as the main evidence. Since C_{26} and C_{28} alcohols cannot be adequately differentiated by elemental analysis, there is room for the possibility of their sample being also octacosanol.

Experimental

Melting points were not corrected. IR spectra were taken with KBr discs on an IR-S spectrometer. 1H NMR spectra were taken with $CDCl_3$ solutions on WH-90, with TMS as the internal standard. MS were recorded with MM70–70H spectrometer.

Plant Material

Artemisia annua L. is a regular commodity, available in practically all warehouses for Chinese herbs. However, there might well be variations in chemical constituents with different localities, which were therefore specified below.

Silica gel columns and plates were used and eluted with the mixed solvent of petroleum ether and ethyl acetate in individually specified proportions.

Qinghaosu-IV

Plant material from Sichuan Province was extracted with petroleum ether and the solvent removed. The crude extract was chromatographed. Qinghaosu IV came down when the mixed solvent was of 9:1 proportion.

The crude qinghaosu-IV displayed only a single spot on TLC (1:1 mixed solvent, 2 % phosphomolybdic acid spray). It was purified by recrystallization from ethanol. MS, m/e (%): 282 (M^+ , 5), 238 (1), 222 (75), 207 (3), 204 (11), 194 (9), 178 (14), 166 (18), 150 (23), 137 (14), 122 (5), 107 (11), 93 (11), 81 (9), 74 (4), 69 (7), 55 (15), 43 (100). IR (cm^{-1}): 3450 (s), 2950 (m), 1728 (s), 1465 (m), 1420 (m), 1390 (m), 1345 (w), 1260 (m), 1220 (w), 1185 (m), 1170 (w), 1140 (m), 1080 (m), 1050 (m), 1015 (m), 970 (m), 940 (w), 920 (m), 865 (m), 820 (w), 785 (w), 765 (w), 715 (w).

Compounds VIa, VIII and the fatty alcohol were isolated from the plant material of the Beijing area. The ethereal extracts were shaken with 2 % aqueous sodium hydroxide, which upon acidification gave the crude acid fraction. From the chromatographic fractions with 95:5, 85:15 and 65:35 solvent compositions, were obtained qinghao acid (VIII), a fatty alcohol and chrysosplenol (VIa) respectively.

The non-acidic fraction from ether as mentioned above was concentrated, mixed with polyamide powder and percolated with 47 % ethyl alcohol. After stripping of solvent, the residue was extracted again with ether and the crude extract was chromatographed on a column. Qinghaosu V came down from the 6:4 portion, showing one spot on TLC (1:1 mixed solvent, violet spot when sprayed with 2 % vanillin).

Qinghao acid

The crude acid displayed a single spot on TLC (9:1 mixed solvent, 2 % vanillin- H_2SO_4 spray). It was purified by recrystallization from petroleum ether as transparent prisms, soluble in sodium bicarbonate. MS, m/e (%): 234 (M^+ , 63), 219 (5), 216 (6), 206 (6), 201 (3), 189 (13), 178 (6), 173 (6), 161 (8), 147 (6), 136 (28), 121 (100), 105 (17), 93 (38), 87 (6), 71 (25), 67 (9), 55 (16), 41 (22). IR (cm^{-1}): 3480–2590 (s, br), 2550 (w), 1900 (w), 1690 (s), 1625 (m), 1445 (m), 1400 (w), 1380 (w), 1320 (w), 1274 (m), 1210 (w), 1180 (w), 1155 (m), 1110 (w), 1080 (w), 1030 (w), 990 (w), 940 (m), 920 (w), 880 (w), 820 (w), 795 (w), 715 (w).

Diol from reduction of qinghaosu-I by LAH. The diol (IIa) so obtained was an oil. 1H NMR: 0.85 (d, J = 6, 10- CH_3), 1.25 (s, 4- CH_3), 5.50 (br, H-C-3), 3.80 (d, J = 3, 7- CH_2OH), 4.19 (br, H-C-5).

The fatty alcohol Crude product as one spot on TLC (9:1 mixed solvent, 5 % ethanolic phosphomolybdic acid spray). White powder from petroleum ether-chloroform. MS, m/e (%): 392 (9), 378 (2), 364 (22), 350 (2), 336 (14), 322 (2), 308 (4), 294 (3), 280 (3), 266 (3), 252 (4), 238 (4), 224 (4), 210 (5), 196 (6), 182 (6), 167 (11), 153 (14), 137 (21), 125 (37), 111 (58), 97 (97), 83 (100), 69 (75), 57 (92), 43 (75).

Chrysosplenol (VIa) Crude product as one spot on paper chromatography (Xinhua filter paper, 30 % acetic acid as eluant, 1 %

$AlCl_3$ spray). Light yellow crystals from ethanol (twice), m.p. 221–223° (lit. 235–6° [7]). MS, m/e (%): 360 (M^+ , 100), 359 (44), 346 (11), 345 (50), 343 (5), 342 (8), 341 (21), 332 (5), 317 (8). The triacetate had m.p. 155–157° (lit. 159–160° [7]). When fully methylated, m.p. 141–142°.

Qinghaosu V

The crude material was twice recrystallized from ethyl alcohol. MS, m/e (%): 250 (M^+ , 24), 235 (7), 233 (6), 232 (15), 217 (7), 208 (18), 192 (18), 180 (15), 177 (10), 174 (8), 161 (11), 147 (15), 135 (16), 134 (12), 133 (13), 121 (15), 119 (15), 107 (26), 105 (19), 95 (27), 93 (27), 91 (31), 84 (13), 82 (23), 81 (28), 79 (28), 77 (20), 71 (35), 67 (25), 65 (10), 55 (32), 53 (30), 43 (100).

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