Studies on the Constituents of Artemisia annua
Part II

Tu You-you, Ni Mu-yun, Zhong Yu-rong, Li Lan-na, Cui Shu-lian, Zhang Mu-qun, Wang Xiu-zhen, Ji Zheng** and Liang Xiaotian**

* Institute of Chinese Materia Medica, Academy of Traditional Chinese Medicine, Beijing, China

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.

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_3. Hydroxy and lactonic carbonyl groups can be inferred from its IR spectrum (3450, 1728 cm\(^{-1}\)). The \(^1\)HNMR 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), 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 → 0.96 → 1.16 → 1.16; for 11-CH\(_3\), 1.20 → 1.42 → 2.20 → 2.40; for 4-CH\(_3\), 1.54 → 1.55 → 1.74 → 1.75; and for H-C-5, 5.55 → 5.75 → 6.20 → 6.40. Qinghaosu IV (V) gave the corresponding shifts: 10-CH\(_3\), δ 1.04 → 1.46 → 1.77 → 2.15; 11-CH\(_3\), 1.27 → 1.99 → 2.60 → 3.42; 4-CH\(_3\), 1.65 → 3.93 → 5.74 → 7.25; H-C-5, 5.70 → 6.91 → 8.06 → 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\(\frac{1}{2} \approx 6\) Hz of the H-C-3 multiplet (hence equatorial) indicates an \(\alpha\)-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 \(^1\)HNMR 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 its preparation from qinghaosu (I) by catalytic hydrogenation [5].

\[ \text{Qinghao acid (VII) has m.p. 125–126°, and a formula of } \text{C}_{21}\text{H}_{33}\text{O}_{3} \text{from MS data. IR data (3420, 1700, 1630 cm}^{-1} \text{)} \text{showed the presence of OH and } \alpha, \beta\text{-unsaturated lactone functionalities. The } \text{OH} \text{ group should be placed at C}_{4}5 \text{and not } \text{C}_{3}4. \text{Biogenetic considerations led to the proposal of VIII as the structure of qinghao acid.} \]

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Chrysosplenol (6a) has very similar UV and \(^1\)HNMR 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\(_2\)N\(_2\) in CH\(_3\)OH 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°, [α]_D\(^{19}\) +36° (0.01, CHCl\(_3\)) and MS m/e 234 (M^+) compatible with C_{15}H_{22}O_3. 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 \(^1\)HNMR 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 methylene 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\(\frac{1}{2} \approx 5-6\) Hz) is much sharper (for 2 and 2a, we have δ 5.60 and 5.50, respectively, with W\(\frac{1}{2} = 11\) Hz, due to the coupling with two neighboring protons). Hence the endocyclic double bond of qinghao acid should be placed at C_45 and not C_34. Biogenetic considerations led to the proposal of VIII as the structure of qinghao acid.
Qinghaosu-IV

Plant material from Sichuan Province was extracted with petroleum ether and the solvent removed. The crude extract was chromatographed. Qinghaosu-IV displayed only a single spot on TLC (1:1 mixed solvent, 2% phosphomolybdate 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 (s), 1260 (m), 1220 (w), 1185 (m), 1170 (w), 1140 (m), 1080 (m), 1050 (m), 1015 (m), 970 (m), 940 (w), 920 (m), 865 (9), 765 (w), 715 (w).

Comounds Vla, VIII and the fatty alcohol were isolated from the plant material of the Beijing area. The ethereal extracts were shaken with 2% aqeous 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 (Vla) respectively.

The non-acidic fraction from ether as mentioned above was concentrated, mixed with polyamide powder and percolated with 2% vanillin. It was purified by recrystallization from petroleum ether as transparent prisms, soluble in so-

Qinghaosu-IV came down from the 6:4 portion, 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).

Acknowledgements

We thank Prof. T. J. MABRY of the University of Texas at Austin for a sample of eupatin. Liu Hongming of the Sichuan Institute of Chinese Materia Medica for the sample of 3,4-diethoxybenzoic acid obtained on alkali fusion of chrysosplenol (identical 1R with our sample) and our analytical colleagues for the recorded spectra.

References


Address: Prof. Liang Xiaotian, Institute of Materia Medica, Nan-wei Road, Beijing 100050, China.