Studies on the Constituents of Artemisia annua
Part II

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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 peroxicid 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.
Results and Discussion

Qinghaosu-IV (V) is a colourless crystalline compound with m.p. 172—173°C. MS molecular weight 282 (M+) agreed with C15H22O3. 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-CH3), 1.20 (d, J = 6, 11-CH3), 1.65 (s, 4-CH3), 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-CH₃, δ 0.95 → 0.96 → 1.16 → 1.16; for 11-CH₃, 1.20 → 1.42 → 2.20 → 2.40; for 4-CH₃, 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-CH₃, δ 1.04 → 1.46 → 1.77 → 2.15; 11-CH₃, 1.27 → 1.99 → 2.60 → 3.42; 4-CH₃, 1.65 → 3.93 → 5.74 → 7.25; H-C-5, 5.70 → 6.91 → 8.06 → 9.18. The marked shifts for 4-CH₃ and H-C-5 in compound V is compatible only with an OH group at position-3, and the W½ 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 (6α) has very similar UV and 1H NMR spectra to eupatip (3, 5, 3'-%riOH, 6, 7, 4'-trioMe) [7]. However, large discrepancies in m.p. of the acetates (155—157°C; 219—221°C for eupatip 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 hexamethy ether, identical with an authentic specimen. The 5-OH showed a characteristic chelated NMR shift at δ 12.60. Methylation with CD₂N₂ in CH₃OH gave three partially deuterated methyl groups onto the original phenolic hydroxyls, and solvent shifts (benzene vs. chloroform [10]) revealed 5, 3', 4' as the labelled positions. Further confirmation came from alkali fusion of the ethylated derivative, whereby 3,4-diethoxybenzoic acid was formed by acid hydrolysis of the ethylated derivative, and the 6-CH₃ and 11-CH₃ signals at δ 1.20 and 1.27 were replaced by broad bands at δ 1.60 and 1.74. The marked shifts for 4-CH₃ and H-C-5 in compound V is compatible only with an OH group at position-3, and the W½ 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].

Experimental

Melting points were not corrected. IR spectra were taken with KBr discs on an IR-S spectrometer. 1H NMR spectra were taken with CDCl₃ 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 displayed only a single spot on TLC (1:1 mixed solvent, 2% phosphomolybdic acid spray). It was purified by recrystallization from ethanolic. 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<sup>-1</sup>): 3450 (s), 2950 (w), 1625 (m), 1445 (m), 1400 (w), 1380 (w), 1320 (w), 1274 (m), 1265 (m), 1185 (m), 1170 (w), 1140 (m), 1080 (m), 1050 (m), 1015 (m), 970 (m), 940 (w), 920 (m), 865 (01), 820 (w), 785 (w), 765 (w), 715 (w), 705 (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 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).

Qinghaosu acid

The crude acid displayed a single spot on TLC (9:1 mixed solvent, 2% vanillin-H<sub>2</sub>SO<sub>4</sub> 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<sup>-1</sup>): 3480—2590 (s), 2550 (w), 1900 (w), 1690 (s), 1625 (m), 1445 (m), 1400 (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 (Ia) so obtained was an oil. <sup>1</sup>HNMR: 0.85 (d, J = 6, 10-CH<sub>2</sub>), 1.25 (s, 4-CH<sub>3</sub>), 5.50 (br, H-C-3), 3.80 (d, J = 3.7-CH<sub>2</sub>OH), 4.19 (br, H-C-5).

The fatty alcohol Crude product as one spot on TLC (9:1 mixed solvent, 2% vanillin-H<sub>2</sub>SO<sub>4</sub> 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% AICI<sub>3</sub> 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 (6), 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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References


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