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.

1 For Part I, see ref. 5.
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 1HNMR 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 are compatible only with an OH group at position-3, and the W1/2 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 1HNMR spectra to eupatin (3, 5, 3′-triOH, 6, 7, 4′-triOME)[7]. However, large discrepancies in m.p. of the acetates (155—157°C; 219—221°C 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 hexamethylene 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′ 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.

Qinghaosu acid (VIII) forms colourless cubes with m.p. 131°C, [α]D3 +36°C (0.01, CHCl3) and MS m/e 234 (M+) compatible with C15H22O2. IR peaks at 3170-2930 (s, br), 1690 (s) and 1625 (m) are indicative of an α, β-unsaturated acid. These are borne out by 1HNMR data, δ 0.83 (d, J = 6, 10-CH3), 1.60 (s, 4-CH3), 4.94 (br, H-C-5), 5.54, 6.46 (br, 11 = CH2) and 11.56 (br, CO2H). Irradiation of 4-CH3 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 C7, 11 bond.

Comparison with II or its LAH reduction product (IIa) showed that the olefinic signal of qinghao acid at 4.94 (W1/2 = 5-6 Hz) is much sharper (for 2 and 2a, we have δ 5.60 and 5.50, respectively, with W1/2 = 11 Hz, due to the coupling with two neighboring protons). Hence the endocyclic double bond of qinghao acid should be placed at C4, 5 and not C3, 4. Biogenetic considerations led to the proposal of VIII as the structure of qinghao acid.

Qinghaosu V (VII) has m.p. 125—126°C, and a formula of C15H22O3 from MS data. IR data (3420, 1700, 1630 cm−1) showed the presence of OH and α, β-unsaturated lactone functionalities. The 1HNMR spectrum showed two methyl groups at δ 0.85 (d) and 1.36 (s), and terminal methylene protons at 5.56 (s) and 16.16 (s). The carbonylic 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 scantly 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°C, characterized by its IR and 1HNMR spectra. The MS peak at m/e 392 (M-18) [11] showed it to be octacosanol (C28H56OH), probably contaminated by some C30 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 C26 alcohol. ULUBELEN et al. [12] reported the isolation of a C26 alcohol from the same species, using elemental analysis as the main evidence. Since C26 and C28 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. 1HNMR spectra were taken with CDC13 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 only 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^+), 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), 2880 (m), 2350 (w), 1785 (m), 1625 (m), 1445 (m), 1400 (w), 1380 (w), 1320 (w), 1274 (m), 1185 (w), 1110 (w), 1080 (m), 1050 (m), 1015 (m), 970 (m), 940 (w), 920 (w), 865 (w), 765 (w), 715 (w).

Compounds Vla, 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 (Vla) respectively.

The non-acidic fraction from ether as mentioned above was concentrated, mixed with polyamide powder and percolated with 47 % ethereal 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).

Qinghaos acid

The crude acid displayed a single spot on TLC (9:1 mixed solvent, 2 % vanillin-H$_2$SO$_4$ spray). It was purified by recrystallization from petroleum ether as transparent prisms, soluble in sodium bicarbonate. MS, m/e (%): 282 (M^+), 238 (1), 222 (75), 207 (3), 204 (11), 194 (9), 178 (14), 166 (18), 150 (23), 137 (14). IR (cm^-1): 3450 (s), 2950 (m), 2880 (m), 2350 (w), 1785 (m), 1625 (m), 1445 (m), 1400 (w), 1380 (w), 1320 (w), 1274 (m), 1185 (w), 1110 (w), 1080 (m), 1050 (m), 1015 (m), 970 (m), 940 (w), 920 (w), 865 (w), 765 (w), 715 (w).

Diol from reduction of qinghaosu-I by LAH. The diol (Vla) so obtained was an oil. ^1HNMR: 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$_3$OH), 4.19 (br, H-C-5).

The fatty alcohol Crude product as one spot on TLC (9:1 mixed solvent, 2 % 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 (Vla) 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 (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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