Studies on the Constituents of Artemisia annua
Part II

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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 antimalerial 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.
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 lactic carboxyl groups can be inferred from its IR spectrum (3450, 1728 cm\(^{-1}\)). The \(^1\)HNMR spectrum displayed the following features: \( \delta \) 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 \( \delta \) 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\(_3\), \( \delta \) 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\(_3\), \( \delta \) 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 \( \alpha \)-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 eupatins (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].

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 lactic carboxyl groups can be inferred from its IR spectrum (3450, 1728 cm\(^{-1}\)). The \(^1\)HNMR spectrum displayed the following features: \( \delta \) 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 \( \delta \) 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\(_3\), \( \delta \) 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\(_3\), \( \delta \) 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 \( \alpha \)-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 eupatins (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 \( \delta \) 12.60. Methylation with CD\(_3\)\(_2\)N 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 an alcali fusion of the ethylated derivative, whereby 3,4-diethoxybenzoic acid (m.p. and MS) was obtained.

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 \( \alpha \), \( \beta \)-unsaturated lactone functionalities. The \(^1\)HNMR spectrum showed two methyl groups at \( \delta \) 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 (\( \delta \) 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 \(^1\)HNMR 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. \(^1\)HNMR 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 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 (s), 2340 (s), 1728 (s), 1465 (m), 1260 (m), 1220 (w), 1185 (m), 1170 (w), 1140 (m), 1080 (m), 1050 (m), 1015 (m), 970 (m), 940 (w), 920 (m), 865 (w), 845 (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 qinghaosu acid (VIII) and chrysosplenol (Vla) respectively.

The non-acidic fraction from ether as mentioned above was concentrated, mixed with polyamide powder and percolated with sodium bicarbonate. 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 (s), 2340 (s), 1728 (s), 1465 (m), 1260 (m), 1220 (w), 1185 (m), 1170 (w), 1140 (m), 1080 (m), 1050 (m), 1015 (m), 970 (m), 940 (w), 920 (m), 865 (w), 845 (w), 765 (w), 715 (w).

Qinghaosu acid

The crude acid displayed a single spot on TLC (9:1 mixed solvent, 2% phosphomolybdic acid 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 (11), 75 (21), 67 (9), 55 (16), 41 (7). IR (cm⁻¹): 3480–2590 (s), 2550 (s), 2350 (s), 1900 (s), 1690 (s), 1625 (m), 1445 (m), 1400 (w), 1380 (w), 1370 (m), 1350 (m), 1270 (m), 1260 (m), 1220 (w), 1185 (m), 1150 (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 (H-a) so obtained was an oil. 'HNMR: 0.85 (d, J = 6, 10-CH₃), 1.25 (s, 4-CH₃), 5.50 (br, H-C-5). 3.80 (d, J = 3.7-CH₂OH), 4.19 (br, H-C-5). The fatty alcohol Crude product as one spot on TLC (9:1 mixed solvent, 2% vanillin). After stripping of solvent, the residue was extracted again with ether and 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 petroleum ether as transparent prisms, soluble in sodium bicarbonate. 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 (s), 2340 (s), 1728 (s), 1465 (m), 1260 (m), 1220 (w), 1185 (m), 1170 (w), 1140 (m), 1080 (m), 1050 (m), 1015 (m), 970 (m), 940 (w), 920 (m), 865 (w), 845 (w), 765 (w), 715 (w).

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), 175 (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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