Studies on the Constituents of Artemisia annua Part II\(^1\)

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 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.
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-3), 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-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 a 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 (VIIa) 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 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]D²⁰ +36° (0.01, CHCl₃) and MS m/e 234 (M+), compatible with C15H22O3. IR peaks at 3480–3290 (s, br), 1690 (s) and 1625 (m) are indicative of an 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 carboxylic hydrogen at C-5 (δ 3.82) is a doublet eith 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°C, characterized by its IR and ¹HNMR spectra. The MS peak at m/e 392 (M-18) [11] showed it to be octacosanol (C₂₈H₅₇OH), probably contaminated by some C₂₀ 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 C₂₀ alcohol. ULUBELEN et al. [12] reported the isolation of a C₂₈ alcohol from the same species, using elemental analysis as the main evidence. Since C₂₀ 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 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.

This document was downloaded for personal use only. Unauthorized distribution is strictly prohibited.
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 one 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 (w), 2870 (w), 1660 (m), 1500 (w), 1420 (m), 1390 (w), 1350 (w), 1260 (m), 1220 (w), 1185 (m), 1170 (w), 1140 (m), 1080 (m), 1050 (m), 1015 (m), 970 (m), 940 (w), 920 (m), 865 (1), 820 (m), 765 (w), 715 (w).

Compounds VIa, VIII and the fatty alcohol were isolated from the plant material of the Beijing area. The etereal 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.

Qinghaosu 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 (%): 564 (M+, 1), 392 (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 (Ia) 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-5). 3.80 (d, J = 3.7-CH_2OH), 4.19 (br, H-C-3).

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 eupatlin. Liu Hongming of the Sichuan Institute of Chinese Materia Medica for the sample of 3,4-dioxybenzoic acid obtained on alkalif fusion of chrysosplenol (identical IR with our sample) and our analytical colleagues for the recorded spectra.

References

1 Cooperative research group on the structure of qinghaosu: Kexue Tongbao 14, 142 (1977).
6 Liu Hongming of the Sichuan Institute of Chinese Materia Medica for the sample of 3,4-dioxybenzoic acid obtained on alkalif fusion of chrysosplenol (identical IR with our sample) and our analytical colleagues for the recorded spectra.

Address: Prof. Liang Xiaotian, Institute of Materia Medica, Nan-wei Road, Beijing 100020, China.