Pergamon

0031-9422(94)00591-5

Phytochemistry, Vol. 38, No. 1, pp. 255–257, 1995
Elsevier Science Ltd
Printed in Great Britain
0031-9422/95 $9.50 + 0.00

FLAVONOIDS AND CHROMENES FROM *ARTEMISIA ANNUA*

SHI-LIN YANG,* MARGARET F. ROBERTS, MELANIE J. O'NEILL,† FRANZ BUCAR‡ and J. DAVID PHILLIPSON

Department of Pharmacognosy, The School of Pharmacy, 29–39 Brunswick Square, London WC1N 1AX, U.K.; †Department of Biomolecular Screening, Glaxo Group Research and Development, Greenford, Middlesex UB6 OHE, U.K.; ‡Institut für Pharmakognosie der Universität Graz, Universitätsplatz 4/I, A-8010 Graz, Austria

(*Received in revised form* 27 *June* 1994)

Key Word Index—*Artemisia annua*; flavonoids; chromenes; artemisinin; antimalarial activity.

Abstract—The ethyl acetate fraction obtained from the extraction of *Artemisia annua* yielded 11 flavones, four flavone glycosides and two chromene derivatives. Three new compounds were isolated: quercetagetin 4'-methyl ether, 2,2-dihydroxy-6-methoxychromene and 2,2,6-trihydroxychromene.

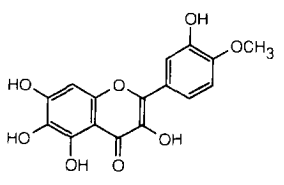
INTRODUCTION

Artemisia annua L. has been used in traditional Chinese medicine for centuries, for the treatment of malaria and dysentery. In the search for effective novel antimalarial drugs, artemesinin (qinghaosu, QHS) was isolated from the plant [1] and was found to be active against chloroquine-resistant *Plasmodium falciparum* in the treatment of cerebral malaria [2]. Previously we have reported the isolation of 17 methoxylated flavones and four coumarins from the *n*-hexane and chloroform fractions of *A. annua* L. [3]. Some flavones, e.g. casticin, chrysoplenetin, chrysosplenol-D and cirsilineol markedly enhanced the antimalarial activity of artemisinin [4]. Casticin was the most effective inhibitor of parasite-mediated transport systems controlling the influx of L-glutamine and myo-inositol across the host cell membrane in erythrocytes infected with human and murine malaria [5]. Hence, it was of interest to isolate such flavones from *A. annua* L. in order to ascertain the extent to which other flavones may enhance the activity of artemisinin or have antimalarial activity in their own right.

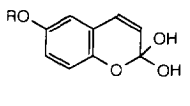
RESULTS AND DISCUSSION

The leaves and stems of Chinese grown *A. annua* were extracted with methanol. After partition of the methanolic residue between water and a series of organic solvents, the ethyl acetate fraction was investigated. This fraction yielded 11 flavones, four flavone glycosides and two chromene derivatives. The known compounds were characterized by their UV, MS and ^{1}H NMR spectra by comparison with the literature, reference compounds and identified as follows: apigenin [6], luteolin [6], kaempferol [6], quercetin [6], isorhamnetin [6], luteolin-7-methyl ether [7], isokaempferide [8], quercetagetin 3-methyl ether [9], tomentin [10], astragalin [11], luteolin 7-*O*-glucoside [6], quercetin 3'-*O*-glucoside [12], isoquercitrin [6] and quercimeritrin [13].

Three new compounds (**1**–**3**) were isolated and identified. MS data of **1** suggested a flavone with $[M]^+$ 332, which is therefore substituted with five hydroxyl groups and one methoxyl group. The absence of an ion peak at *m/z* 317 $[M-15]^+$ indicated that there was no C-6 methoxyl and the absence of *m/z* 289 $[M-43]^+$ indicated there was no C-3 methoxyl. The ion peak *m/z* 151 $[B_2]$, as well as the absence of *m/z* 137 [B_2, if *O*-di-OH ring **B**] were in accordance with a B-ring with one hydroxyl and one methoxyl group. The UV data showed a band II shift of 17 nm in NaOAc indicating a free C-7 hydroxyl. The bathochromic shift of band I with NaOAc–H_3BO_3 of 10 nm compared to MeOH showed an *O*-di-OH group in ring A (6, 7 or 7, 8). These results indicated that the methoxyl substitution was in the B-ring. The MeOH band I at λ 358 nm was consistent with a hypsochromic shift due to a C-4' methoxyl substitution, whereas for quercetagetin 3'-*O*-methyl ether the ethanol band I is at λ



1



2 R = CH_3

3 R = H

*Permanent address: Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences, Beijing, China.

366 nm [14]. The bathochromic shift in sodium methoxide with loss of intensity and slow decomposition supported a C-4′ methoxyl substitution and three hydroxyls in the A-ring. The ^{1}H NMR exhibited proton signals at δ7.80, 7.65 and 6.90 consistent with a B-ring with a C-3′ hydroxyl and C-4′ methoxyl. A singlet at δ6.72 was commensurate with a proton at C-8. The ^{1}H NMR further confirmed one methoxyl with a signal at δ4.01 (4′-OMe). NOE supported methoxyl substitution at C-4′ when irradiation at δ4.01 caused enhancement of the signal at δ6.90 (H-5′). Compound **1** was therefore identified as quercetagetin 4′-methyl ether.

The ^{1}H NMR of **2** showed a chromene pattern with proton signals at δ7.60 (1H, *d*, J = 10 Hz), 7.18 (1H, *d*, J = 2 Hz), 7.04 (1H, *dd*, J = 2, 6 Hz), 6.82 (1H, *d*, J = 6 Hz), and 6.31 (1H, *d*, J = 10 Hz). A methoxyl was indicated with a signal at δ3.86. The MS data with a $[M]^+$ 194 suggested substitution with one methoxyl and two hydroxyl groups. The ^{1}H NMR data from NOE experiments suggested two hydroxyl substituents with signals at C-2 and a methoxyl group at C-6 (see Experimental). Compound **2** was therefore identified as 2,2-dihydroxy-6-methoxychromene. The ^{1}H NMR of **3** exhibited similar data to **2**, except only for the absence of a signal at δ3.86. The MS data with a *m/z* at 180.0429 (calcd for $C_9H_8O_4$ 180.0423) suggested three hydroxyl substituents. Hence, **3** is 2,2,6-trihydroxychromene.

To date, it has been shown that *A. annua* contains seven sesquiterpene lactones, five coumarins, two chromenes and 34 flavonoids.

EXPERIMENTAL

Plant material. Fr. plant material of *A. annua* L. was collected in August 1987, 20 km west of Beijing. Samples were authenticated by Prof. W. Lian (IMPLAD, Beijing) and a voucher specimen deposited in the herbarium, IMPLAD, Beijing.

General. Plant material (19 kg fr. leaf and stem) was extracted with MeOH. The concd extract (970 g) was further partitioned between H_2O and a sequence of solvents [3]. The residue from the EtOAc fr. (21.2 g) was fractionated using chromatography on Polyclar AT (Graf Ltd, U.K.) using $CHCl_3$ as solvent, followed by the gradual introduction of MeOH to 100%. Compounds were further purified where necessary using CC on silica gel using the same solvent system as was used for the Polyclar AT column. Sephadex LH-20 (Pharmacia) was used for the prepn of compounds for spectral analysis. This procedure was carried out according to ref. [13].

Compounds were isolated from EtOAc frs eluted from a column of Polyclar AT with $CHCl_3$–MeOH in the following order: luteolin-7-methylether (2 mg, 0.01%), isorhamnetin (5.8 mg, 0.028%), isokaempferide (2 mg, 0.01%), apigenin (3 mg, 0.015%), luteolin (3 mg, 0.015%), kaempferol (6 mg, 0.03%), quercetin (6 mg, 0.03%), quercetagetin-4′-methyl ether (5 mg, 0.024%), 2,2-dihydroxy-6-methoxychromene (3 mg, 0.015%), 2,2,6-trihydroxychromene (2 mg, 0.01%), tormentin (2 mg, 0.01%), quercetagetin 3-methyl ether (4 mg, 0.02%), luteolin 7-*O*-glucoside (2 mg, 0.01%), astragalin (3 mg, 0.015%), quercetin 3′-glucoside (25 mg, 0.125%), isoquercitrin (6 mg, 0.03%), quercimeritrin (7 mg, 0.035%).

Quercetagetin 4′-methyl ether (**1**). UV: λ_{max}^{MeOH} (nm): 257, 267sh, 358; +MeONa: 269sh, 290sh, 321sh, 383 (slow decomp.); +$AlCl_3$: 277, 339sh, 441; +$AlCl_3$–HCl: 269, 295sh, 399; +NaOAc: 274, 300sh, 341sh, 378sh (slow decomp.); +H_3BO_3: 266, 368. MS: *m/z* (%) 332 (100) $[M]^+$, 151 (7), 149 (37), 136 (51), 121 (23), 109 (31). ^{1}H NMR (CD_3OD): δ7.80 (1H, *d*, J = 2 Hz, C-2′), 7.65 (1H, *dd*, J = 9 and 2 Hz, C-6′), 6.90 (1H, *d*, J = 9 Hz, C-5′), 6.72 (1H, *s*, C-8), 4.01 (3H, *s*, C-4′-OMe). NOE irradiation at δ4.01 caused enhancement of the signal at δ6.90 (C-5′).

2,2-Dihydroxy-6-methoxychromene (**2**). UV: λ_{max}^{MeOH} (nm): 285, 315. MS: *m/z* (%) 194 (100) $[M]^+$, 179 (21), 161 (48), 144 (41), 135 (35), 116 (32), 107 (22). ^{1}H NMR (CD_3OD): δ7.60 (1 H, *d*, J = 10 Hz, C-3), 7.18 (1H, *d*, J = 2 Hz, C-5), 7.04 (1H, *dd*, J = 6 and 2 Hz, C-7), 6.82 (1H, *d*, J = 6 Hz, C-8), 6.31 (1H, *d*, J = 10 Hz, C-4), 3.86 (3H, *s*, C-6-OMe). NOE: irradiated δ3.86, 7.18 and 7.04 enhanced; 6.81, 7.60 and 7.18 enhanced; 7.04, 3.86 and 6.82 enhanced; 6.82, 7.04 enhanced; 7.18, 3.86 and 6.31 enhanced; 7.60, 6.31 enhanced.

2,2,6-Trihydroxychromene (**3**). UV: λ_{max}^{MeOH} (nm): 282, 314. MS: *m/z* (%) 180.0429 (64) $[M]^+$ (calcd for $C_9H_8O_4$ 180.0423), 163 (27), 136 (100), 110 (19), 89 (58). ^{1}H NMR (CD_3OD): δ7.48 (1H, *d*, J = 10 Hz, C-3), 7.05 (1H, *d*, J = 2 Hz, C-5), 6.90 (1H, *dd*, J = 6 and 2 Hz, C-7), 6.72 (1H, *d*, J = 6 Hz, C-8), 6.20 (1H, *d*, J = 10 Hz, C-4).

Acknowledgements—S.Y. would like to acknowledge financial support from Glaxo Group Research during part of this project. F.B. acknowledges financial support from the Austrian Government. ^{1}H NMR were provided by the University Service Unit at King's College, University of London and MS by David Carter of the MS unit at The School of Pharmacy, University of London.

REFERENCES

1. China Cooperative Research Group on Qinghaosu (1982) *J. Trad. Chin. Med.* **2**, 3.
2. Jiang, J. B. (1982) *Lancet* **ii**, 285.
3. Yang, S.-L., Roberts, M. F. and Phillipson, J. D. (1989) *Phytochemistry* **28**, 1509.
4. Liu, K. C.-S., Yang, S.-L., Roberts, M. F., Elford, B. C. and Phillipson, J. D. (1989) *Planta Med.* **55**, 654.
5. Elford, B. C., Roberts, M. F., Phillipson, J. D. and Wilson, R. J. M. (1987) *Trans. R. Soc. Trop. Med. Hygiene* **81**, 434.
6. Mabry, T. J., Markham, K. R. and Thomas, M. B. (1970) *The Systematic Identification of Flavonoids.* Springer, New York.
7. Bohlmann, F., Banerjee, S. and Jakupovic, J. (1985) *Phytochemistry* **24**, 511.
8. Liu, Y.-L. and Mabry, T. J. (1982) *Phytochemistry* **21**, 209.

9. Roitmann, J. N. and James, L. F. (1985) *Phytochemistry* **24**, 835.
10. Ulubelen, A. and Mabry, T. J. (1981) *J. Nat. Prod.* **44**, 457.
11. Nakabayashi, T. (1952) *J. Agr. Chem. Soc. Jpn* **26**, 539.
12. Wagner, J. (1964) *Z. Physiol. Chem.* **335**, 232.
13. Harborne, J. B., Mabry, T. J. and Mabry, H. (1975) *The Flavonoids.* Chapman and Hall, London.
14. Harborne, J. B., Heywood, V. H. and Saleh, N. A. M. (1970) *Phytochemistry* **9**, 2011.