Phytochemistry, Vol. 28, No. 5, pp. 1509–1511, 1989.
Printed in Great Britain.

0031-9422/89 $3.00+0.00

METHOXYLATED FLAVONES AND COUMARINS FROM *ARTEMISIA ANNUA*

YANG SHILIN,* MARGARET F. ROBERTS and J. DAVID PHILLIPSON

Department of Pharmacognosy, The School of Pharmacy, University of London, 29-39 Brunswick Square, London WC1N 1AX, U.K.

(*Received* 11 *August* 1988)

Key Word Index—*Artemisia annua*; Asteraceae; methoxylated flavones; coumarins.

Abstract—Chinese grown *Artemisia annua* contained 14 known methoxylated flavonoids and three new naturally occurring compounds: quercetagetin-3-methyl ether, 5,2′4′-trihydroxy-6,7,5′trimethoxyflavone and 5,7,8,3′-tetrahydroxy-3,4′-dimethoxyflavone. Four known commonly occurring coumarins were also isolated.

INTRODUCTION

In the search for effective novel antimalarial drugs, the sesquiterpene lactone qinghaosu (QHS, artemisinine) was isolated from *Artemisia annua* L. used in traditional Chinese medicine [1]. This drug is active against chloroquine-resistant *Plasmodium falciparum* in the treatment of cerebral malaria [2]. Our studies with QHS and some of its derivatives have indicated that in an *in vitro* growth assay, the antimalarial activity of this sesquiterpene lactone is markedly enhanced by the presence of such methoxylated flavones as artemetin (**1**) and casticin (**2**). At the concentrations used they have no antimalarial activity. By contrast the antimalarial activity of chloroquine was unaffected by the presence of these flavones [3]. Although casticin has been reported as occurring in *A. annua* L. [4], the only other flavones reported for this species are quercetagetin 6,7,3′,4′-tetra-methyl ether [5] and 3,6,7,3′-tetra-*O*-methyl-5,4′-dihydroxyflavone [4]. Hence it was of interest to isolate other methoxylated flavones from *A. annua* to ascertain the extent to which these or other methoxylated flavones may enhance the activity of QHS, particularly when given in crude extract form.

RESULTS AND DISCUSSION

The leaves and stems of Chinese grown *A. annua* were extracted with 70% methanol. After partition of the methanolic residue between water and a series of organic solvents, the flavonoid aglycones and coumarins of the chloroform and *n*-hexane fractions were isolated. These fractions yielded 24 aglycones, many of which had hydroxyl methoxy substitution patterns which made them potential candidates for further investigation of the flavonoid potentiated antimalarial activity of QHS.

The following methoxylated flavones were isolated and spectroscopic data obtained. The known compounds were characterised by their spectroscopic data, by comparison with the literature and reference compounds. They were as follows: casticin **1** [6]; chrysoplenetin **2** [7]; chrysosplenol-D **3** [8]; circilineol **4** [9]; penduletin **5** [10]; eupatorin **6** [11]; axillarin **8** [8]; cirsiliol **9** [12]; tamarixetin **10** [13]; rhamnetin **11** [13]; quercetin 3-methyl ether **12** [10]; cirsimaritin **13** [9]; rhamnocitrin **14** [14] and chrysoeriol **15** [15].

Three new compounds (**7**, **16**, **17**) were also isolated and identified. MS data of compound **7** suggested a flavone with $[M]^+$ 346, which is therefore substituted with four hydroxyls and two methoxyls. An ion at *m/z* 303 (90%) $[M-43]^+$, indicated methoxyl substitution at C-3. The UV data was consistent with a C-5 hydroxyflavone with C-6, C-7, C-3′ hydroxyls and a C-4′ methoxyl. The bathochromic shift in sodium methoxide with loss of intensity supported C-4′ methoxyl substitution. The Band I 370 nm (MeOH) and 424 nm ($AlCl_3$–HCl) suggested substitution with a hydroxyl at C-6 [6]. The ^{1}H NMR exhibited protons at 7.88, 7.75 consistent with a B-ring with a C-3′ hydroxyl and a C-4′ methoxyl. A singlet at 6.82 was commensurate with a proton at C-8 (compared to 6.46 for C-6 in **17** [10]. The ^{1}H NMR further confirmed two methoxyls with signals at 4.04 (4′-OMe) and 3.89 (3-OMe). Compound **7** was therefore considered to be quercetagetin 3,4′-dimethyl ether.

MS data with a M^+ 360 (100) for compound **16** suggested a flavone with three hydroxyl and three methoxyl groups. An ion at *m/z* 345 (82) was consistent with a C-6 methoxyl, an ion at *m/z* 167 (12) suggested a B_1 fragment with two hydroxyls and one methoxyl and an ion at *m/z* 181 (34) was consistent with an A_1-Me fragment suggesting an A-ring with C-5 hydroxyl and C-6,7, methoxyl substitution. The UV data was consistent with hydroxyl substitution at C-5, and C-4′ and a C-7 methoxyl. The λ_{max} at 410 nm (MeONa) compared with 360 nm (MeOH) suggested a flavone with hydroxyl substitution at C-4′. No complexing with boric acid was observed and the λ_{max} at 398 nm ($AlCl_3$) compared with 398 nm ($AlCl_3$/HCl) and 360 nm (MeOH) was consistent with C-5 hydroxyl and C-6 methoxyl substitution. No shift in the Band I in NaOAc compared with NaOAc was observed indicating substitution at C-7 with a methoxyl group. The ^{1}H NMR of **16** has three signals at δ3.73, 3.81 and 3.93 consistent with methoxyl substitution at C-6, C-5′ and C-7. The spectrum also contained the somewhat unusual pattern of four further singlets at δ6.57, 6.96, 7.11

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

and 7.45. These were assigned to protons at C-3, C-8, C-3′ and C-6′, respectively by comparison with literature data for compounds with similar A- or B-ring substitution patterns [9, 16]. These assignments were also supported by the NOE data where irradiation at 3.93 (7-OMe) caused enhancement of the signal at 6.96 (H-8) and irradiation at 3.81 (C-5′) gave enhancement at 7.45 (H-6′), whilst irradiation at 7.11 (H-3′) and 6.57 (H-3) produced no enhancement of other signals. Compound **16** was therefore identified as 5,2′4′-trihydroxy-6,7,5′-trimethoxyflavone.

MS data of compound **17** suggested a flavone $[M]^+$ 346 substituted with four hydroxyl and two methoxyl groups similar to **7** in that an ion at *m/z* 303 (87) suggested substitution at C-3 with a methoxyl. The UV data was similar to that for **7** and similarly suggested a C-5 hydroxy flavone with C-7, C-3′ hydroxyl and C-4′ methoxyl groups. However the ^{1}H NMR differed from that of **7** particularly in the signal at 6.48 (**17**) as opposed to 6.82 (**7**) suggesting that the third hydroxyl in **17** was at C-8 rather than C-6 as in **7**. Compounds **7** and **17** separated on TLC with R_fs 0.71 and 0.58, respectively on silica gel with chloroform–methanol (15:2) as solvent. Compound **17** was therefore identified as 5,7,8,3′-tetrahydroxy-3,4′-dimethoxyflavone.

The chloroform fraction also contained a number of coumarins namely scopoletin, scoparon, 6,8-dimethoxy-7-hydroxy coumarin and 5,6-dimethoxy-7-hydroxy coumarin, which were identified from their spectral data and comparison with literature values [17–19].

Artemisia annua yielded an interesting series of methoxylated flavones, many of which have previously been recorded for other species of *Artemisia*. For example casticin previously isolated from *A. annua* [4] has been isolated also from *A. judaica* [9]. Circilineol has been isolated previously from *A. ludoviciana*, *A. herba-alba* [9], *A. mesatlantica* [21], *A. monosperma* [9] and *A. capillaris* [22], whilst axillarin has been characterized from *A. taurica* [23], *A. incanescans* [24] and *A. ludoviciana* [20] and cirsimaritin from *A. scoparia* [25], *A. mesatlantica* [20] and *A. capillaris* [22]. Rhamnocitrin has been identified from *A. scoparia* [25] and eupatorin from *A. ludoviciana* [20]. The remaining methoxylated flavones other than 17 though not previously isolated from *Artemesia* spp. are commonly found within members of the tribe Anthemideae of the Asteraceae [26].

However only *A. annua* has been found to produce the sesquiterpene lactone artemisinine. Although flavonoids have a wide range of biological activities no well defined structure–activity relationship has emerged to account for the cytotoxic properties of some of these compounds. Our earlier work has shown that of a wide range of flavonoids artemetin and casticin were the most effective inhibitors 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, respectively [3], but whether this property is directly relevant to the synergistic effects on growth inhibition is not known at present. The present range of compounds allows for studies at both levels of structure-related activity.

EXPERIMENTAL

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

General. Plant material (19 kg leaf and stem) was extracted with MeOH. The concd extract (970 g) was further partitioned between H_2O and a sequence of solvents. The residues obtained were: *n*-hexane (172 g); $CHCl_3$ (224 g), EtOAc (21.2 g) and *n*-BuOH (288 g). Flavonoids from the *n*-hexane and $CHCl_3$ fractions were chromatographed on Polyclar AT (Graf Ltd UK) using $CHCl_3$, followed by the gradual introduction of MeOH to 100%. Compounds were further purified where necessary using prep. TLC on silica gel in $CHCl_3$–MeOH, (9:1). Sephadex LH-20 (Pharmacia) was used for the preparation of compounds for spectral analysis, these were carried out according to ref. [10].

Compounds **1** and **2** were isolated from the *n*-hexane fraction and all other compounds (**3**–**18**) were isolated from the $CHCl_3$ fraction eluted from a column of Polyclar AT with $CHCl_3$–MeOH as follows. Fraction F12 chrysoplenetin (12 mg, 0.035%), eupatorin (3 mg 0.01%); F22-8, penduletin (1.5 mg 0.004%); F22-10 cirsimaritin (1.45 mg, 0.004%); chrysosplenol D (35 mg, 0.1%), and cirsilineol (2 mg, 0.006%); F35 rhamnocitrin (1.34 mg 0.003%). F36 cirsiliol (1.42 mg, 0.0017%) F43 5,7,8,3-tetrahydroxy-3,4′-dimethoxyflavone (1.2 mg 0.0036%) F48 5,2′4′-trihydroxy-6,7,5′-trimethoxyflavone (2.3 mg 0.062%); F50 chrysoeriol (2.6 mg, 0.0067%); F56 quercetagetin 3,4′-dimethyl ether (1 mg 0.0003%); F78 tamarixetin (1 mg 0.0037%); F83 rhamnetin (1.1 mg 0.0033%); F109 axillarin (1 mg 0.003%), and quercetin 3-methyl ether (1 mg, 0.003%).

Quercetagetin 3,4′-dimethyl ether (**7**). UV λ_{max}^{MeOH} 216, 258, 370; +MeONa 216, 274, 428; $AlCl_3$ 216, 276, 452; +$AlCl_3$/HCl 216, 270, 424; +NaOAc 216, 270, 390; +H_3BO_3 216, 265, 390. MS: M^+(%) 346 (100), 303 (86), 164 (19), 153 (10), 137 (27), 121 (11), 109 (9), ^{1}H NMR: (CD_3OD), δ7.81 (1H, *d*, *J* = 2, C-2′), 7.75 (1H, *dd*, *J* = 9 and 2, C-6′), 7.01 (1H, *d*, *J* = 9, C-5′), 6.82 (1H, *s*, C-8), 4.04 (3H, *s*, C-4′-OMe), 3.89 (3H, *s*, C-3 -OMe).

5,2′4′-Trihydroxy-6,7-dimethoxyflavone (**16**). UV λ_{max}^{MeOH} 207, 270, 360; + MeONa 214, 270, 410↑; +$AlCl_3$ 210, 280, 398; +$AlCl_3$/HCl 210, 280, 398; +NaOAc 215, 270, 370; +H_3BO_3 215, 270, 368. MS: M^+ (%) 360 (100), 345 (82), 331 (22), 300 (32), 181 (34), 167 (12), 165 (24), 153 (43), 151 (17), 137 (12), 69 (51). ^{1}H NMR: (DMSO) δ, 7.45 (1H, *s*, C-6′), 7.11 (1H, *s*, C-3′), 6.96 (1H, *s*, C-8), 6.57 (1H, *s*, C-3), 3.93 (3H, *s*, C-7, OMe), 3.81 (3H, *s*, C-5′-OCH_3), 3.73 (3H, *s*, C-6-OCH_3). NOE irradiated 3.93, 6.96 enhance, irradiated 3.81, 7.45 enhance irradiated 7.11 no change.

5,7,8,3′-Tetrahydroxy-3,4′-dimethoxyflavone (**17**). UV λ^{MeOH} 208, 260, 370; +MeONa 209, 278, 420↓; +$AlCl_3$ 208, 270, 430; +$AlCl_3$/HCl 208, 270, 430; +NaOAc 216, 278, 390; +H_3BO_3 218, 260, 370. MS: M^+ (%) 346 (100), 328 (43), 303 (87), 173 (12), 151 (17), 135 (6), 120 (8). ^{1}H NMR (CD_3OD) δ7.82, (1H, *d*, *J* = 2, C-2′), 7.72 (1H, *dd*, *J* = 9 and *J* = 2, C-6′) 6.91 (1H, *d*, *J* = 9, C-5′), 6.46 (1H, *s*, C-6), 3.93 (3H, *s*, C-4′ OMe), 3.86 (3H, *s*, C-3 OMe).

Acknowledgements—S. Y. would like to acknowledge financial support from the Royal Society. ^{1}H NMR were provided by the University Service Unit at King's College, London University and MS by David Carter of the MS unit at The School of Pharmacy, London University.

REFERENCES

1. China Cooperative Research Group on Qinghaosu (1982) *J. Trad. Chin. Med.* **2**, 3.
2. Jiang, J. B. (1982) *Lancet ii*, 285.
3. Elford, B. C., Roberts, M. F., Phillipson, J. D. and Wilson,

R. J. M. (1987) *Trans. R. Soc. Trop. Med. Hygiene* **81**, 434.
4. Stefanovic, M., Jokic, A. and Behbul, A. (1972) *Bull. Soc. Chim Beograd* **37**, 463.
5. Djermanovic, M., Jokic, A., Mladenovic, S. and Stefanovic, M. (1975) *Phytochemistry* **14**, 1873.
6. Timmermann, B. N., Miles, R., Mabry, T. J. and Powell, A. M. (1979) *Phytochemistry* **18**, 1855.
7. Lee, K. H. (1969) *Phytochemistry* **8**, 1515.
8. Rodriguez, E. Carman, N. G., Van der Velde, G., McReynolds, J. H., Mabry, T. J., Irwin, M. A. and Geissman, T. A. (1972) *Phytochemistry* **11**, 3509.
9. Saleh, N. A. M., El-Negoumy, S. I. and Abou-Zaid, M. M. (1987) *Phytochemistry* **26**, 3059.
10. Mabry, T. J., Markham, K. R. and Thomas, M. B. (1970) *The Systematic Identification of Flavonoids*. Springer, New York.
11. Kupchan, M. (1969) *Tetrahedron* **25**, 1603.
12. Morita, N., Shimizu, M. and Arisawa, M. (1973) *Phytochemistry* **12**, 421.
13. Stuck, R. F. and Kirk, M. C. (1970) *J. Agric. Food Chem.* **18**, 548.
14. Gonnet, J. F. and Jay, M. (1972) *Phytochemistry* **11**, 2313.
15. Briescorn, C. H. and Riedel, W. (1979) *Planta Med.* **31**, 308.
16. Malan, E. and Roux, D. G. (1979) *J. Chem. Soc. Perkin I.* (1979), 2696.
17. Brown, D. R., Asplund, A. and McMahon, V. A. (1975) *Phytochemistry* **14**, 1083.
18. Von Schmersahl, P. (1966) *Planta Med.* **14**, 179.
19. Yusupov, M. I. and Sidyakin, G. P. (1973) *Khim. Prir. Soedin.*, 430.
20. Liu, Y.-L. and Mabry, T. J. (1982) *Phytochemistry* **21**, 209.
21. Bonzid, N., Moulis, C. and Fouraste, I. (1982) *Planta Med.* **44**, 157.
22. Namba, T., Hattari, M., Takehana, Y., Tsunezuka, M., Tomimori, T., Kuzu, H. and Miyaichi, Y. (1983) *Phytochemistry* **22**, 1057.
23. Oganesyan, E. T., Smirnova, L. P., Dzhumyrko, S. F. and Kechatova, N. A. (1976) *Khim. Prir. Soedin*, 599.
24. Barberá, O., Marco, J. A., Sanz, J. F., Sanchez, Paranreda, J. (1986) *Phytochemistry* **25**, 2357.
25. Chandrasekharan, I., Khan, H. A. and Ghanim, A. (1981) *Planta Med.* **43**, 310.
26. Wollenweber, E. (1983) in *Recent Advances in Flavonoid Research* (Harborne, J. B. and Mabry, T. J., eds). Chapman & Hall, London.