Phytochemistry, Vol. 21, No. 1, pp. 209–214, 1982.
Printed in Great Britain.

0031-9422/82/010209-06 $03.00/0

FLAVONOIDS FROM *ARTEMISIA LUDOVICIANA* VAR. *LUDOVICIANA*

YONG-LONG LIU* and T. J. MABRY

The Department of Botany, The University of Texas at Austin, Austin, TX 78712, U.S.A.

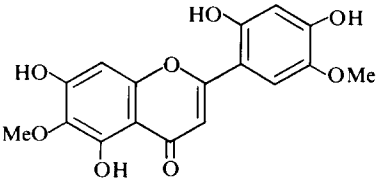
(*Received* 31 *March* 1981)

Key Word Index—*Artemisia ludoviciana* var. *ludoviciana*; Compositae; Anthemideae; *Hypochlora alba*; Acrididae; 5,7,2′,4′-tetrahydroxy-6,5′-dimethoxyflavone; 6-methoxyflavones; 6-methoxyflavonols; 5,7-dihydroxy-3,6,8,4′-tetramethoxyflavone; flavones; flavonols.

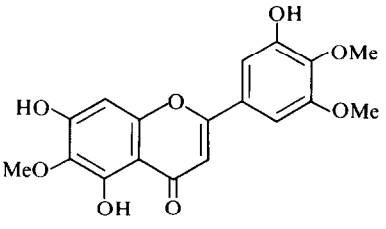
Abstract—Nineteen flavonoids were isolated from *Artemisia ludoviciana* var. *ludoviciana*, including a new 2′-hydroxy-6-methoxyflavone, 5,7,2′,4′-tetrahydroxy-6,5′-dimethoxyflavone. The known compounds include quercetagetin 3,6,3′,4′-tetramethyl ether, eupatilin, 5,7-dihydroxy-3,6,8,4′-tetramethoxyflavone, luteolin 3′,4′-dimethyl ether, jaceosidin, 5,7,4′-trihydroxy-3,6-dimethoxyflavone, tricin, hispidulin, chrysoeriol, kaempferol 3-methyl ether, apigenin, axillarin, eupafolin, selagin and luteolin together with three flavones which were previously isolated for the first time from *Artemisia frigida*: 5,7,4′-trihydroxy-6,3′,5′-trimethoxyflavone, 5,7,3′-trihydroxy-6,4′,5′-trimethoxyflavone and 5,7,3′,4′-tetrahydroxy-6,5′-dimethoxyflavone.

INTRODUCTION

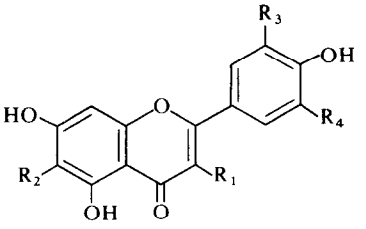
Artemisia ludoviciana var. *ludoviciana* Nutt. serves as the primary host for the grasshopper *Hypochlora alba*, while closely related grasshoppers cannot survive on this plant [1]. In our continuing investigation of the chemical bases of these feeding preferences, we report here 19 flavonoids from a Kansas collection of *A. ludoviciana* var. *ludoviciana*, including the new 5,7,2′,4′-tetrahydroxy-6,5′-dimethoxyflavone (**1**), and three highly oxygenated flavones: 5,7,4′-trihydroxy-6,3′,5′-trimethoxyflavone (**2**), 5,7,3′-trihydroxy-6,4′,5′-trimethoxyflavone (**3**) and 5,7,3′4′-tetrahydroxy-6,5′-dimethoxyflavone (**4**) which were first found in *A. frigida* [2, 3]. In our previous work



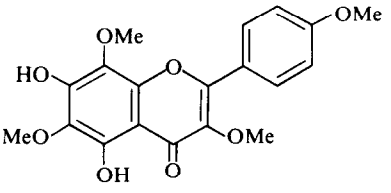
1



3



2 $R_1 = H$; $R_2 = R_3 = R_4 = OMe$
4 $R_1 = H$; $R_2 = R_4 = OMe$; $R_3 = OH$
6 $R_1 = R_2 = OMe$; $R_3 = R_4 = H$
7 $R_1 = R_2 = OMe$; $R_3 = OH$; $R_4 = H$
8 $R_1 = R_2 = H$; $R_3 = OH$; $R_4 = OMe$



5

* Permanent address: The Institute of Materia Medica, Chinese Academy of Medical Sciences, Peking, the People's Republic of China.

on the Kansas collection of *A. ludoviciana*, six sesquiterpene lactones were isolated [4]. Other workers have studied the sesquiterpene lactones from collections of *Artemisia ludoviciana* from Oklahoma, New Mexico and Mexico [5–7].

RESULTS AND DISCUSSION

The MS of the new flavonoid **1** [mp 291 294° (dec.)] exhibited a molecular ion peak at *m/z* 346 (100%) for $C_{17}H_{14}O_8$ in accord with four hydroxyl and two methoxyl groups (Table 1). **1** appeared as a purple spot on cellulose TLC under UV light and changed to yellow with ammonia, suggesting the presence of free 5- and 4′-hydroxyl groups. When sprayed with NA, the spot turned green, indicating that the compound did not contain free 3′- and 4′-hydroxyl groups. **1** exhibited UV maxima in methanol at 366, 286 and 260 nm and shifts with diagnostic reagents (Table 2) which suggested the presence of an oxygen function at C-6 (Δ + 30 nm for Band I in $AlCl_3$/HCl compared with Band I in MeOH) and hydroxyl groups at positions 5,7 and 4′ [8]. Moreover, these UV spectral studies confirmed the absence of a second hydroxyl group *ortho* to the one at the 4′ position. The ^{1}H NMR spectrum of the TMSi ether of **1** (in CCl_4) provided the most definitive data concerning its structure. In addition to two one-proton singlets at δ 6.32 and 6.47 typical for H-3 and H-8 in 6-methoxyflavones, two additional one-proton singlets were observed at δ 6.53 and 7.16. These last two signals can only be assigned to isolated protons at the 3′ and 6′ positions, thus requiring a 2′,4′,5′ substitution pattern in the B-ring. Since the UV and NMR data clearly established the oxygenation pattern of **1** as well as the presence of hydroxyl groups at the 5,7 and 4′ positions, the remaining fourth hydroxyl and the two methoxyl groups could be assigned to the other available sites, namely positions 6,2′ and 5′. The benzene-induced shifts of the two methoxyl resonances for the TMSi ester of **1** supported the presence of one methoxyl at C-6 (δ 3.72–3.80, Δ = −0.08 ppm) and a second at the 5′ position (δ 3.83 3.34, Δ = +0.49 ppm). Since the UV spectral data and color reaction with NA indicated that there was no *ortho*-dihydroxyl group in the B-ring, the fourth hydroxyl could be assigned to the 2′ position, thus confirming that the second methoxyl is at the 5′ position. The occurrence of Band I at 366 nm in the UV spectrum of **1** in methanol is unusual for flavones but has been previously observed for another 2′-oxygenated flavone, 5,7-dihydroxy-6,2′,4′,5′-tetramethoxyflavone (Band I in EtOH at 360 nm) [9].

The structure of **1** was confirmed by MS: a peak of 81% relative intensity was observed at *m/z* 331 (M^+ − Me), a result which is characteristic for 6-methoxyflavones [10, 11, 21]; moreover, other fragments for both **1** and its PDM derivative were in accord with the presence of one methoxyl and two hydroxyl groups in both the A- and B-rings of the natural product (Table 1). Together, the spectral findings establish that **1** is 5,7,2′,4′-tetrahydroxy-6,5′-dimethoxyflavone.

Compound **5** gave a mass spectrum (Table 1) showing a molecular ion peak of 58% intensity at *m/z* 374 suggesting a flavonoid aglycone bearing two hydroxyl and four methoxyl groups. The M − 15 peak appeared as the base peak supporting the presence of both 6- and 8-methoxyl groups [20]. Other fragments, notably the $[A_1]^+$, $[B_1]^+$ and $[B_2]^+$, indicated that the A-ring contained two hydroxyl and two methoxyl groups with only one methoxyl in the B-ring. Therefore, the fourth methoxyl must be at position 3. The ^{1}H NMR spectrum of the TMSi ether of **5** confirmed the oxygenation pattern. In addition to signals for four methoxyl groups, the spectrum exhibited only a pair of two-proton doublets at δ 6.85 and 7.60 for the C-3′, 5′ and C-2′, 6′ protons, respectively (Table 3). Therefore, **5** was identified as 5,7-dihydroxy-3,6,8,4′-tetramethoxyflavone, a compound previously isolated from *Ambrosia grayi* [12]. The UV data for **5** (Table 2) were identical with those previously reported [12].

The mass spectrum of **6** (Table 1) gave a molecular ion peak (100%) at *m/z* 330 for a flavonoid containing three hydroxyl and two methoxyl groups. The fragmentation pattern indicated that the A-ring contained one methoxyl and two hydroxyl groups with one hydroxyl group in the B-ring. Therefore, the second methoxyl must be at position 3. The UV spectral data (Table 2) suggested the presence of a 6-methoxyl group as well as hydroxyl groups at positions 5,7 and 4′. The ^{1}H NMR spectrum of the TMSi ether of **6** exhibited signals for two methoxyl groups at δ 3.70 and 3.80 and a pair of two-proton doublets at δ 6.83 and 7.93 for the 3′,5′ and 2′,6′ protons. In addition, a singlet appeared at δ 6.47 which could be assigned to H-8 (Table 3). These spectral findings confirmed **6** to be 5,7,4′-trihydroxy-3,6-dimethoxyflavone [13–15].

Flavonoid **7** appeared purple on a paper chromatogram under UV light and turned yellow with ammonia, indicating the presence of 5- and 4′-hydroxyl groups. When the paper was sprayed with NA, **7** changed to orange-yellow indicating a 3′,4′-dihydroxyl group. The UV spectral data suggested the presence of a methoxyl group at C-6 and four hydroxyl groups at the 5,7,3′ and 4′ positions (Table 2). The mass spectrum of **7** exhibited a molecular ion peak at *m/z* 346 (100%) for $C_{17}H_{14}O_8$ in accord with a flavonoid aglycone containing four hydroxyl and two methoxyl groups (Table 1). Since the fragmentation pattern supported the presence of one methoxyl and two hydroxyl groups in the A-ring and two hydroxyls in the B-ring, the remaining methoxyl must be at C-3. In addition to two methoxyl signals at δ 3.70 and 3.83, the ^{1}H NMR spectrum of the TMSi ether of **7** (in CCl_4) exhibited a singlet at δ 6.47 for H-8, a doublet at δ 6.81 for H-5′ and a two-proton double doublet at δ 7.49 for H-2′ and 6′ (Table 3). These spectral data established **7** to be 5,7,3′4′-tetrahydroxy-3.6-dimethoxyflavone (axillarin) [20].

The mass spectrum of **8** (Table 1) exhibited a molecular ion peak at *m/z* 316 indicating the presence of one methoxyl and four hydroxyl groups. The appearance of $[A_1 + 1]^+$, $[B_1]^+$ and $[B_2]^+$ fragments at *m/z* 153, 164 and 167, respectively, indicated the presence of two hydroxyl groups in the A-ring and two hydroxyl and one methoxyl groups in the B-ring. Moreover, the MS spectrum of permethylated **8** exhibited a molecular ion peak at *m/z* 372 with a fragmentation pattern similar to that of permethylated tricin. These MS data along with the UV data (Table 2) established **8** to be 5,7,3′4′-tetrahydroxy-5′-methoxyflavone (selagin), a substance previously reported from *Huperzia selago* [17] and from two species of *Isoetes* [19].

5,7,4′-Trihydroxy-6,3′,5′-trimethoxyflavone (**2**), 5,7,3′-trihydroxy-6,4′,5′-trimethoxyflavone (**3**), 5,7,3′,4′-tetrahydroxy-6,5′-dimethoxyflavone (**4**), quercetagetin 3.6.3′,4′-tetramethyl ether (**9**), 5,7-dihydroxy-6,3′,4′-

Table 1. MS data for flavonoids from *Artemisia ludoviciana**

Flavonoid	M^+	$[M-H]$	$[M-Me]^+$	$[M-18]^+$	$[M-HCO]^+$	$[M-COMe]^+$	$[A_1-Me]^+$	$[A_1-MeCO]^+$	$[A_1-MeCO-CO]^+$	$[B_1]^+$	$[B_2]^+$	$[A_1+H]^+$
5,7,2′,4′-Tetrahydroxy-6,5′-dimethoxyflavone (**1**)	346 (100)	345 (37)	331 (55)	328 (53)	317 (16)	303 (43)	167 (32)	139 (31)	111 (9)	164 (29)	167 (32)	183 (8)
PDM of **1**	414 (40)	413 (21)	399 (100)	396 (10)	385 (3)	371 (3)	201 (11)	173 (24)	145 (16)	198 (7)	201 (12)	217 (8)
5,7-Dihydroxy-3,6,8,4′-tetramethoxyflavone (**5**)	374 (58)	373 (35)	359 (100)	356 (16)	345 (49)	331 (28)	197 (6)	169 (5)	141 (9)		135 (22)	213 (9)
5,7,4′-Trihydroxy-3,6-dimethoxyflavone (**6**)	330 (100)	329 (72)	315 (65)	312 (33)	301 (12)	287 (54)	167 (12)	139 (5)	111 (4)		121 (36)	183 (3)
Axillarin (**7**)	346 (100)	345 (66)	331 (63)	328 (41)	317 (14)	303 (52)	167 (33)	139 (27)	111 (26)		137 (44)	183 (13)
Selagin (**8**)	316 (100)	315 (23)			287 (6)					164 (46)	167 (39)	153 (60)

* Previously recorded MS data are not included, see refs. [2, 3]. MS were recorded at 70 eV, source temp. 200° and probe temp. from 50° to 425°. Values are given in m/z and in parentheses the % abundance relative to the base peak. The A_1, B_1 and B_2 terminology for the fragments is given in ref. [12].

Table 2. UV data for flavonoids from *Artemisia ludoviciana**

Flavonoid	(λ_{max}, nm) MeOH	NaOMe	$AlCl_3$	$AlCl_3$/HCl	NaOAc	NaOAc/H_3BO_3
5,7,2′,4′-Tetrahydroxy-6,5′-dimethoxyflavone (**1**)	366, 286sh 260	433, 312 266	406, 326 293sh, 270	396, 322 292sh, 267	409, 326 268	425sh, 377 300sh, 367
5,7-Dihydroxy-3,6,8,4′-tetramethoxyflavone (**5**)	352, 294sh 270, 255	398, 344sh 304sh, 270	408sh, 370 300sh, 278sh 262	408sh, 366 300sh, 278 260	380, 317sh 270	347, 268
5,7,4′-Trihydroxy-3,6-dimethoxyflavone (**6**)	340, 270	398, 329 275	400sh, 366 305, 278 262sh	398sh, 358 302sh, 280 260sh	390, 329sh 273	344, 270
Axillarin (**7**)	349, 292sh 268, 254	404, 332 270	437, 348sh 306sh, 278	402sh, 365 300sh, 280sh 264	394, 328sh 269	373, 263
Selagin (**8**)	355, 298sh 267	415, 322 263	428, 298sh 271	390, 362sh 296sh, 274	396, 318sh 275, 263	377, 300sh 256sh

* Previously published UV data are not included, see refs. [2, 3]. All UV spectra were recorded using standard procedures [8].

Table 3. ^{1}H NMR data for TMSi ethers of flavonoids from *Artemisia ludoviciana**

TMSi ethers of flavonoids	H-3	H-8	H-2′	H-3′	H-5′	H-6′	–OMe† 3	6	4′	5′
5,7,2′,4′-Tetrahydroxy-6,5′-dimethoxyflavone (**1**)	6.32*s*	6.47*s*		6.53*s*		7.16*s*		3.72*s* Δ = −0.08‡		3.83*s* Δ 0.49‡
5,7-Dihydroxy-3,6,8,4′-tetramethoxyflavone (**5**)			7.60*d* (9.0)	6.85*d* (9.0)	6.85*d* (9.0)	7.60*d* (9.0)	3.79*s*	3.69*s*	3.84*s*	3.84§
5,7,4′-Trihydroxy-3,6-dimethoxyflavone (**6**)		6.47*s*	7.93*d*	6.83*d* (9.0)	7.83*d* (9.0)	7.93*d* (9.0)	3.80*s*	3.70*s*		
Axillarin (**7**)		6.47*s*	7.49*m*		6.81*d* (9.0)	7.49*m*	3.83*s*	3.70*s*		

* Only new ^{1}H NMR data are included, for previous reports see refs. [2, 3]. Spectra were recorded in CCl_4. Values are given in ppm (δ-scale) relative to TMS as an internal standard. Numbers in parentheses denote coupling constants in Hz. Signals are indicated as follows: *s* = singlet; *d* = doublet; *m* = multiplet.

† Some OMe signal assignments may need to be interchanged.

‡ ^{1}H NMR spectrum of TMSi ether of **1** in C_6D_6 showed a three-proton signal for a 6-methoxyl at δ 3.80 and another signal of δ 3.34 for a 5′-methoxyl group.

§ An 8-OMe signal.

trimethoxyflavone (eupatilin) (**10**), luteolin 3′,4′-dimethyl ether (**11**), jaceosidin (**12**), tricin (**13**), hispidulin (**14**), chrysoeriol (**15**), kaempferol 3-methyl ether (**16**), apigenin (**17**), eupafolin (**18**) and luteolin (**19**) were identified by UV, MS, color reactions, co-chromatography with authentic samples and, except for **2**, **3**, **4**, **10** and **16**–**19**, by ^{1}H NMR.

EXPERIMENTAL

Plant material. The aerial parts of *A. ludoviciana* var. *ludoviciana* Nutt. were collected by Dr. Craig C. Freeman, Oct. 10, 1979, just west of Manhattan, KS. Voucher specimen No. 326 is deposited at the Department of Entomology, Kansas State University.

General techniques. Mps are uncorr. CC employed Polyclar AT (GAF Corp.) and Sephadex LH-20 (Pharmacia). Precoated cellulose plates (E. Merck), polyamide, polygram, polyamide-6 (Macherey–Nagel) and Si gel 60 GF-254 (E. Merck) were used for TLC. The solvent systems were: TBA (*t*-BuOH–HOAc–H_2O, 3:1:1); *n*-BAW, upper layer (*n*-BuOH–HOAc–H_2O, 4:1:5); BMM (C_6H_6–MeCOEt–MeOH, 4:3:3); BPMM [C_6H_6–petrol (65–110°)–MeCOEt–MeOH, 60:26:7:7]; CAA ($CHCl_3$–Me_2CO–HCO_2H, 9:2:1) and BPA (C_6H_6–pyridine–HCO_2H, 36:9:5). All flavonoids were purified over Sephadex LH-20 using MeOH prior to spectral analyses by standard procedures [8, 18]. Visualization of the flavonoids on TLC plates was realized either by UV light + NH_3 or by spraying with NA (Naturstoffreagenz-A in MeOH).

Isolation of flavonoids. Ground, dried leaves (0.5 kg) were extracted with 85% aq. MeOH (3 l. × 3) and then 50% aq. MeOH (1 l. × 1). Both sets of MeOH extracts were evapd under red. pres. until only H_2O remained. The ppt. formed in the 50% MeOH concentrate after standing in the cold was filtered off (1 g). The conc. 85% MeOH extract was partitioned with *n*-hexane, $CHCl_3$ and EtOAc and the conc. $CHCl_3$ extract (39.4 g) was purified over a cellulose column, eluting with 15% HOAc and then 40% HOAc; 50 fractions were collected. Combined fractions 3–50 afforded 5.2 g of a mixture which was chromatographed over a Polyclar column. The column was first

Table 4. Chromatographic data (R_f × 100 and colors) for flavonoids from *Artemisia ludoviciana**

Flavonoid	Cellulose HOAc 15%	HOAc 40%	TBA	*n*-BAW	Polyamide BMM	Polyamide BPMM	Silica gel CAA	Silica gel BPA	Color in UV (366)†	UV/NH_3 (366)†	UV/NA (254)†
5,7,2′,4′-Tetra-hydroxy-6,5′-dimethoxyflavone (**1**)	5	34	69	86	55	3	5	21	p	y	g
5,7-Dihydroxy-3,6,8,4′-tetramethoxyflavone (**5**)	17	62	81	90	90	62	27	67	p	p	pbr
5,7,4′-Trihydroxy-3,6-dimethoxyflavone (**6**)	22	66	89	94	91	37	18	60	p	pbr	pbr
Axillarin (**7**)	14	53	78	87	76	10	6	33	p	y	or
Selagin (**8**)	3	24	62	77	55	5	3	17	p	yg	yor

* One-dimensional TLC; for solvent systems and other details see the Experimental.

† UV, long wavelength 366 nm; short wavelength 254 nm. Colors are: p = purple; y = yellow; br = brown; or = orange; g = green. NA = Naturstoffreagenz A in MeOH.

eluted with C_6H_6 and then a mixture of C_6H_6–MeOH with a gradual increase in the ratio of MeOH. Fractions were collected by following the bands with UV light, and 13 pure flavonoids were obtained. Fractions which contained two or three flavonoids were further separated by PC on Whatman 3MM paper yielding an additional six flavonoids.

All isolated flavonoids were crystallized from MeOH. The yields were as follows: **1**, 15 mg; **2**, 5 mg; **3**, 4 mg; **4**, 3 mg; **5**, 9 mg; **6**, 10 mg; **7**, 8 mg; **8**, 3 mg; **9**, 11 mg; **10**, 45 mg; **11**, 8 mg; **12**, 12 mg; **13**, 8 mg; **14**, 21 mg; **15**, 18 mg; **16**, 4 mg; **17**, 6 mg; **18**, 9 mg and **19**, 7 mg.

MS, UV, ^{1}H NMR and chromatographic data for **1** and other unusual flavonoids are given in Tables 1–4.

Acknowledgements—This work was supported by the Robert A. Welch Foundation (Grant F-130) at the University of Texas at Austin, and the National Institutes of Health (Grant HD-04488). Some support was also provided by a grant awarded to Profs. H. Knutson and T. L. Hopkins, Kansas State University (NSF Grant DEB 7905482) with whom we are collaborating on the *Artemisia*–grasshopper problem.

REFERENCES

1. Bajoi, A. H. and Knutson, H. (1977) *Acrida* **6**, 219.
2. Liu, Y.-L. and Mabry, T. J. (1981) *Phytochemistry* **20**, 309.
3. Liu, Y.-L. and Mabry, T. J. (1981) *Phytochemistry* **20**, 1389.
4. Ohno, N., Gershenzon, J., Roane, C. and Mabry, T. J. (1980) *Phytochemistry* **19**, 103.
5. Geissman, T. A. and Saitoh, T. (1972) *Phytochemistry* **11**, 1157.
6. Dominguez, X. A. and Cardenas, G. E. (1975) *Phytochemistry* **14**, 2511.
7. Sanchez-Viesca, F. and Romo, J. (1963) *Tetrahedron* **19**, 1285.
8. Mabry, T. J., Markham, K. R. and Thomas, M. B. (1970) *The Systematic Identification of Flavonoids*. Springer, New York.
9. Purushothaman, K. K., Sarada, A., Saraswathi, G. and Connolly, J. D. (1977) *Phytochemistry* **16**, 398.
10. Kingston, D. G. I. (1971) *Tetrahedron* **27**, 2691.
11. Lee, H. H. and Tan, C. H. (1965) *J. Chem. Soc.* 2743.
12. Herz, W., Anderson, G. D., Wagner, H., Maver, G., Flore, G. and Farkas, L. (1973) *Tetrahedron Letters* 2571.
13. Fukui, K., Matsumoto, T., Nakayama, M. and Horie, T. (1969) *Experientia* **25**, 349.
14. Lebreton, P., Wollenweber, E., Southwick, L. and Mabry, T. J. (1971) *C. R. Acad. Sci. Ser. C* **272**, 1529.
15. Rösler, H., Star, A. E. and Mabry, T. J. (1971) *Phytochemistry* **10**, 450.
16. Taylor, A. D. and Wong, E. (1965) *Tetrahedron Letters* 3675.
17. Voirin, B. and Jay, M. (1976) *Phytochemistry* **15**, 840.
18. Mabry, T. J. and Markham, K. R. (1975) *The Flavonoids* (Harborne, J. B., Mabry, T. J. and Mabry, H., eds.) pp. 78–126. Chapman & Hall, London.
19. Voirin, B. and Jay, M. (1978) *Biochem. Syst. Ecol.* **6**, 99.
20. Tomas, F., Ferreres, F. and Guirado, A. (1979) *Phytochemistry* **18**, 185.
21. Goudard, M., Favre-Bonvin, J., Lebreton, P. and Chopin, J. (1978) *Phytochemistry* **17**, 145.