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FLAVONOIDS OF *WYETHIA ANGUSTIFOLIA* AND *W. HELENIOIDES*

SUSAN MCCORMICK*, KATHLEEN ROBSON and BRUCE BOHM

Department of Botany, University of British Columbia, Vancouver, British Columbia, V6T 2B1, Canada

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Key Word Index—*Wyethia angustifolia*; *Wyethia helenioides*; Compositae; Heliantheae; prenylated flavanones; prenylated dihydroflavanol; isoflavones; prenylated isoflavones; flavanones; dihydrooxepin ring.

Abstract—Seventeen flavonoids, including seven new natural products, were isolated from a dichloromethane extract of *Wyethia angustifolia*. Known compounds are: 8-*C*-prenyleriodictyol, 6-*C*-prenyleriodictyol, 8-*C*-prenylnaringenin, 6-*C*-prenylnaringenin, orobol 7-methyl ether, orobol 3'-methyl ether, naringenin 4'-methyl ether, orobol, eriodictyol and naringenin. The new compounds are 6-*C*-prenylorobol, 6-*C*-prenylorobol 3'-methyl ether, orobol 7,3'-dimethyl ether, 8-*C*-prenyldihydroisorhamnetin, 7,8-dihydrooxepinoeriodictyol, 7,8-dihydrooxepinodihydroquercetin and 3',4'-dihydrooxepino-6'-hydroxybutein. A dichloromethane extract of *Wyethia helenioides* yielded eleven compounds only five of which were previously reported from the species. All these compounds appear to occur on the leaf surface.

INTRODUCTION

As part of a chemical and morphological study of the related genera *Wyethia* and *Balsamorhiza* [1, 2], the leaf surface flavonoids of *Wyethia angustifolia* (DC.) Nutt. were examined. This is our first report of the chemistry from a member of *Wyethia* section *Wyethia*. An investigation of the epicuticular components of *Wyethia mollis* Gray [3], also in section *Wyethia*, yielded only two isoflavones, orobol 7-methyl ether and orobol 3'-methyl ether. Since *Wyethia angustifolia* exhibits a great deal of morphological variation among populations and individuals, it was of interest to see if this variation was reflected in the flavonoid chemistry. Extracts of 15 plants from three different populations were compared by cochromatography. Although there was some variation at both inter- and intra-populational levels this was mainly in the amounts of individual compounds. The plant material used in this study was pooled from two populations.

As part of this study, *Wyethia helenioides* (DC.) Nutt. (section *Alarconia*) was examined also. This species was reported to contain 8-*C*-prenylnaringenin, 8-*C*-prenyleriodictyol, 6-*C*-prenyleriodictyol, orobol 7-methyl ether and orobol 3-methyl ether [4]. Our collection of *W. helenioides* yielded six additional compounds.

RESULTS AND DISCUSSION

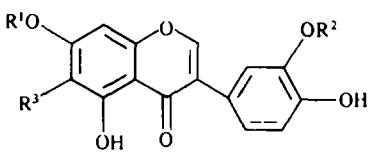
A dichloromethane wash of the leaves of *Wyethia angustifolia* afforded 6-*C*-prenylorobol (4 mg, **1**), 6-*C*-prenylorobol 3'-methyl ether (10 mg, **2**), 8-*C*-prenyldihydroisorhamnetin (8 mg, **3**), orobol 7,3'-dimethyl ether (10 mg, **4**), 7,8-dihydrooxepinoeriodictyol (12 mg, **5**), 7,8-dihydrooxepinodihydroquercetin (7 mg, **6**), 3',4'-dihydrooxepino-6'-hydroxybutein (10 mg, **7**), 8-*C*-prenyleriodictyol (20 mg, **8**) [4], naringenin 4'-methyl ether (15 mg, **9**) [5], eriodictyol (21 mg, **10**) [6], orobol (**11**) [7], 6-*C*-prenyleriodictyol (20 mg, **12**) [4], orobol 7-methyl ether (15 mg, **13**) [8], 8-*C*-prenylnaringenin (10 mg, **14**) [9], orobol 3'-methyl ether (10 mg, **15**) [10], 6-*C*-prenylnaringenin (20 mg, **16**) [1] and naringenin (10 mg, **17**) [11].

A dichloromethane wash of *W. helenioides* yielded **4**, **8**, **9**, **12**, **13**, **14**, **15**, **16**, jaceidin (**18**), axillarin (**19**) and 6-methoxyluteolin (**20**).

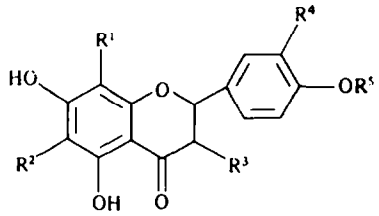
Compounds **1**–**6** appeared dark when viewed under UV light indicating that in each case there was a 5-hydroxyl group present. The UV spectrum of **1** in methanol exhibited a major absorbance at 267 nm typical of an isoflavone. The mass spectrum exhibited a molecular ion at *m/z* 345 with losses of 15, 43 and 55 mass units characteristic of a *C*-prenylated compound [12]. The appearance of an A fragment at *m/z* 165 and a B_1 fragment at *m/z* 134 indicated that the prenyl and two hydroxyls were present on the A ring and two hydroxyls were present on the B-ring. The absence of a bathochromic shift in the UV (methanol) spectrum after the addition of aluminum chloride suggested that the prenyl group was *ortho* to the 5-hydroxyl group [13]. Insufficient material was available for an NMR spectrum. On the basis of the available spectral data, **1** was identified as 6-*C*-prenylorobol. The 6,8-di-*C*-prenyl analog has been isolated from *Milletia pachycarpa* [14].

The UV spectrum of **2** recorded in methanol exhibited a major absorbance at 265 nm which shifted only 1 nm after the addition of aluminum chloride. This suggested that **2** was an isoflavone with a substituent *ortho* to a 5-hydroxyl group. The mass spectrum of **2** exhibited a molecular ion at *m/z* 368 with losses of 15, 43 and 55 mass units characteristic of a compound with a *C*-linked prenyl group. The molecular weight of 368 required in addition to a prenyl, one methoxyl and three hydroxyl groups. B_1 and B_1 – Me fragments at *m/z* 148 and 133 supported a B-ring with one hydroxyl group and one methoxyl group. The ^{1}H NMR spectrum was consistent with a prenylated isoflavone. A one proton singlet at δ6.28, consistent with H-8, and the absence of a shift in the UV spectrum after

*Present address: USDA-SRRC, 1100 R.E. Lee Blvd, New Orleans, LA 70179, U.S.A.

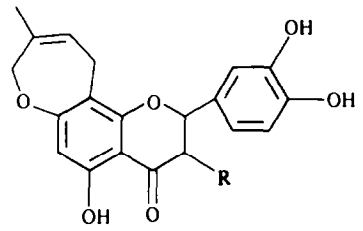


	R^1	R^2	R^3
1	H	H	pr
2	H	Me	pr
4	Me	Me	H
11	H	H	H
13	Me	H	H
15	H	Me	H

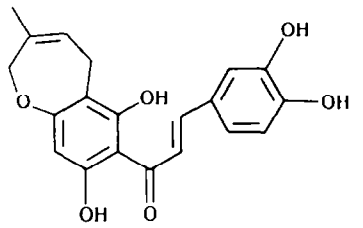


	R^1	R^2	R^3	R^4	R^5
3	pr	H	OH	Me	H
8	pr	H	H	OH	H
9	H	H	H	H	Me
10	H	H	H	OH	H
12	H	pr	H	OH	H
14	pr	H	H	H	H
16	H	pr	H	H	H
17	H	H	H	H	H

(pr = $CH_2CH{=}CMe_2$)



	R
5	H
6	OH



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the addition of aluminum chloride, supported the placement of the prenyl group at the C-6 position. The pattern of B-ring protons supported a 3'-methoxyl 4'-hydroxyl B-ring. On the basis of the spectral data, **2** was identified as 6-*C*-prenylorobol 3'-methyl ether.

A major UV absorbance at 289 nm and the appearance of AB doublets at δ4.9 and 4.1 in the ^{1}H NMR spectrum indicated that **3** was a dihydroflavanol. A bathochromic shift of 10 nm after the addition of aluminum chloride and HCl and the appearance of a one proton singlet at δ5.87 were consistent with a proton at the 6-position. A prenyl, assigned to the 8-position, was indicated by the presence in the NMR of a broad multiplet at δ5.1, a doublet at δ3.1 and two methyl singlets at δ1.63 and 1.53. A methyl singlet was present at δ3.81. The mass spectrum of **3** exhibited a molecular ion at *m/z* 386 consistent with a dihydroflavanol with a prenyl, three hydroxyls and one methoxyl. B_4 and B_3 fragments, at *m/z* 137 and 166 respectively, were consistent with the presence of one hydroxyl and one methoxyl in the B-ring. The B-ring protons which appeared in the 90 MHz carbon tetrachloride spectrum as a three-proton multiplet at δ6.7–7.0 were resolved in the 400 MHz acetone-d_6 spectrum. The appearance of the H-2' doublet downfield from the H-6' double doublet supported a B-ring with a 3'-methoxyl, 4'-hydroxyl substitution pattern [15]. On the basis of the spectral data **3** was assigned the structure of 8-*C*-prenyldihydroisorhamnetin.

The UV spectrum of **4** in methanol exhibited a major absorbance at 263 nm which shifted 10 nm after the addition of aluminum chloride was consistent with an isoflavone with a proton *ortho* to the 5-hydroxyl group. The mass spectrum of **4** exhibited a molecular ion at *m/z* 314 which required two hydroxyls and two methoxyls. An A_1 at 167 and a B_1 at 148 indicated that the A-ring and B-ring each had one methoxyl and one hydroxyl group. The ^{1}H NMR spectrum of **4** recorded in CCl_4 exhibited a six proton singlet at δ3.87. When the spectrum was recorded in benzene-d_6, two singlets appeared at δ3.53 and 3.31. Shifts of δ0.34 and 0.56 are consistent with 3'- and 7-methoxyls, respectively [16]. On the basis of the spectral data, **4** was identified as orobol 7,3'-dimethyl ether.

The UV spectrum of **5** had a major absorbance at 290 nm of a flavonone. A bathochromic shift of 20 nm after the addition of aluminum chloride suggested that there was a proton *ortho* to a 5-hydroxyl. The ^{1}H NMR spectrum of **5** was nearly superimposable with that of 8-*C*-prenyleriodictyol (**8**). The major difference was the absence of one of the γ-methyl singlets in the NMR of **5**. Compound **8** had two methyl singlets at δ1.65 and 1.55.

Instead, **5** had a methyl singlet at δ1.6 and a two-proton singlet at δ3.95, the latter consistent with a methylene adjacent to an oxygen. This suggested that one of the γ-methyls had cyclized with the 7-hydroxyl group to form a seven membered dihydrooxepin ring. This type of ring system has been found in a series of bibenzyl derivatives and one dihydrochalcone isolated from species of the liverwort genus, *Radula* [17–20]. The mass spectrum of **5** exhibited a molecular ion at *m/z* 354 ($C_{20}H_{18}O_6$) requiring twelve rings/double bonds. This was consistent with a flavanone with a dihydrooxepin ring. A fragment at *m/z* 136 was consistent with the B_4 of eriodictyol. An $A_1 + 1$ fragment at *m/z* 219 with losses of 15 and 53 was consistent with a A-ring with one hydroxyl group and a dihydrooxepin ring. On the basis of these data, **5** was identified as 7,8-dihydrooxepinoeriodictyol.

A major absorbance of 292 nm in the UV spectrum of **6** indicated a flavanone or dihydroflavanol skeleton. A bathochromic shift of 20 nm after the addition of aluminum chloride showed that there was a proton *ortho* to the 5-hydroxyl group. The ^{1}H NMR had a methyl singlet at δ1.57, a methylene singlet at 3.93, a multiplet at 5.3 and a doublet at 3.1, all consistent with a dihydrooxepin ring. The appearance of AB doublets at δ4.1 and 4.8 indicated that **6** was a dihydroflavonol. A singlet at δ5.9, consistent with an H-6, supported the placement of the seven-membered ring at the 7 and 8 positions. The mass spectrum of **6** exhibited a molecular ion at *m/z* 370 consistent with a dihydroflavonol with three hydroxyls and a dihydrooxepin ring. The appearance of B_3 and B_4 fragments, at *m/z* 152 and 123 respectively, supported a dihydroquercetin skeleton. On the basis of the spectral data, **6** was identified as 7,8-dihydrooxepinodihydroquercetin.

Compound **7** appeared bright yellow in both visible and UV light and had a major absorbance at 376 nm which suggested that it was a chalcone. A hypsochromic shift of 40 nm in the aluminum chloride spectrum after the addition of HCl indicated that there was an *ortho*-dihydroxyl group in the B-ring. The appearance of AB doublets at δ8.1 and 7.65 confirmed that **7** was a chalcone. A singlet at δ5.97 was consistent with H-5′. The pattern of B-ring signals confirmed the presence of a 3,4-disubstituted B-ring. The remaining signals indicated that there was a dihydrooxepin ring. The mass spectrum of **7** exhibited a molecular ion at *m/z* 354 with losses of 15, 43 and 53 mass units, consistent with a dihydrooxepin ring. The presence of B-fragments at 136 and 123 confirmed the presence of an *ortho*-dihydroxyl B-ring. $A_1 + 1$, $A_1 - 15$ and $A_1 - 53$ fragments, at *m/z* 219, 203 and 165 respectively, confirmed an A-ring with two hydroxyl groups, a dihydrooxepin ring and one proton. On the basis of the spectral data **7** was identified as 3′-4′-dihydrooxepino-6′-hydroxybutein.

EXPERIMENTAL

Plant material. Leaves of *Wyethia angustifolia* were collected May, 1984 in Jasper Ridge Biological Preserve (Santa Clara Co.; B. A. Bohm # 1772) and in Napa State Park (Napa Co.; B. A. Bohm # 1774, 1775). Leaves of *W. helenioides* were collected April 26, 1984, in Mendocino County, mile 74 on Rt. 101 (B. A. Bohm, #1768). Vouchers are deposited in UBC.

Extraction and separation. Leaves of *W. angustifolia* (39 g) and *W. helenioides* (20 g) were treated in the same way. The air-dried leaves were extracted several times with CH_2Cl_2. The combined extracts were dried under red. pres. and then chromatographed over Polyclar AT columns using CH_2Cl_2–MeOH (3:1) and increasing amounts of MeOH. Fractions from this column were separated further on polyamide TLC using toluene–petrol (80–100)–MeCOEt–MeOH (12:6:2:1) and/or toluene–MeCOEt–MeOH (12:5:3). Isolated compounds were cleaned over Sephadex LH-20 columns prior to spectral analysis. Individual compounds were identified on the basis of UV, ^{1}H NMR, MS and co-chromatography with standard compounds.

6-*C*-*Prenylorobol* (**1**). UV λ_{max}^{MeOH} nm: 292 sh, 267; + NaOMe: 340, 269; + $AlCl_3$: 300 sh, 268; + $AlCl_3$–HCl: 267; + NaOAc 269; + NaOAc–H_3BO_3: 268. EIMS (probe) 70 eV, *m/z* (rel. int.): 354 $[M]^+$ (32), 339 $[M - Me]^+$ (12), 311 $[M - C_3H_7]^+$ (56), 299 $[M - C_4H_7]^+$ (55), 221 $[A_1 + 1]^+$ (6), 185 $[A_1' - Me]^+$ (8), 177 $[A_1 - C_3H_7]^+$ (14), 165 $[A_1 - C_4H_7]^+$ (22), 134 $[B_1]^+$ (20).

6-*C*-*Prenylorobol 3′-methyl ether* (**2**). UV λ_{max}^{MeOH} nm: 265; + NaOMe: 275; + $AlCl_3$: 266; + $AlCl_3$–HCl: 266. ^{1}H NMR (90 MHz, CCl_4, TMS ether): δ7.8 (1H, *s*, H-2), 7.2/6.8 (3H, *m*, H-2′, 5′, 6′), 6.25 (1H, *s*, H-8), 5.1 (1H, *m*, H-β), 3.85 (3H, *s*, OMe), 3.28 (2H, *d*, J = 0 Hz, H-αs), 1.75 (3H, *s*, γ-Me), 1.67 (3H, *s*, γ-Me). EIMS (probe) 70 eV, *m/z* (rel. int.): 368 $[M]^+$ (59), 353 $[M - Me]^+$ (31), 325 $[M - C_3H_7]^+$ (72), 313 $[M - C_4H_7]^+$ (70), 148 $[B_1]^+$ (28), 221 $[A_1 + 1]^+$ (5), 165 $[A_1 - C_4H_7]^+$ (40).

8-*C*-*Prenyldihydroisohamnetin* (**3**). UV λ_{max}^{MeOH} nm: 289; + $AlCl_3$: 314; + $AlCl_3$–HCl: 309. EIMS (probe) 70 eV, *m/z* (rel. int.): 386 $[M]^+$ (6), 221 $[A_1 + 1]^+$ (23), 165 $[A_1 - C_4H_7]^+$ (100), 166 $[B_3]^+$ (34), 137 $[B_4]^+$ (45). ^{1}H NMR (90 MHz, CCl_4, TMS ether): δ6.9 (3H, *m*, H-2′, 5′, 6′), 5.9 (1H, *s*, H-6), 4.95 (1H, *d*, J = 12 Hz, H-2), 4.13 (1H, *d*, J = 12 Hz, H-3), 3.8 (3H, *s*, OMe), 3.1 (2H, *d*, J = 7.5 Hz, H-αs), 5.1 (1H, *m*, H-β), 1.5 (3H, *s*, γ-Me), 1.6 (3H, *s*, γ-Me); (400 MHz, Me_2CO-d_6): 7.23 (1H, *d*, H-2′), 7.06 (1H, *dd*, H-6′), 6.88 (1H, *d*, H-5′).

Orobol 7,3′-dimethyl ether (**4**). UV λ_{max}^{MeOH} nm: 263; + NaOMe: 272; + $AlCl_3$: 273; + $AlCl_3$–HCl 273. EIMS (probe) 70 eV, *m/z* (rel. int.): 314 $[M]^+$ (79), 299 $[M - Me]^+$ (22), 167 $[A_1 + 1]^+$ (72), 148 $[B_1]^+$ (36), 133 $[B_1 - Me]^+$ (45). ^{1}H NMR (90 MHz, CCl_4, TMS ether): δ7.65 (1H, *s*, H-2), 7.15, 6.8 (3H, *m*, H-2′, 5′, 6′), 6.4 (1H, *d*, J = 2 Hz, H-8), 6.2 (1H, *d*, J = 2 Hz, H-6), 3.9 (6H, *s*, 2-OMe); (C_6D_6): 3.53 (3H, *s*, OMe), 3.33 (3H, *s*, OMe).

7,8-*dihydrooxepinoeriodictyol* (**5**). UV λ_{max}^{MeOH} nm: 290; + NaOMe: 330; + $AlCl_3$: 310; + $AlCl_3$–HCl 310. ^{1}H NMR (90 MHz, CCl_4, TMS ether): δ6.83 (3H, *m*, H-2′, 5′, 6′), 5.9 (1H, *s*, H-6), 5.32 (1H, *br t*, J = 8 Hz, H-β), 5.2 (1H, *dd*, J = 3, 13 Hz, H-2), 3.9 (2H, *br s*, γ-CH_2–), 3.2 (2H, *d*, J = 8 Hz, H-αs), 1.53 (3H, *s*, γ-Me). EIMS (probe) 70 eV, *m/z* (rel. int.): 354 $[M]^+$ (79), 339 $[M - Me]^+$ (52), 311 $[M - C_3H_7]^+$ (14), 301 $[M - C_4H_5]^+$ (23), 219 $[A_1 + 1]^+$ (96), 203 $[A_1 - Me]^+$ (93), 165 $[A_1 - 53]^+$ (87), 136 $[B]^+$ (84).

7,8-*Dihydrooxepinodihydroquercetin* (**6**). UV λ_{max}^{MeOH} nm: 293; + NaOMe: 325; + $AlCl_3$: 316; + $AlCl_3$–HCl 305. EIMS (probe) 70 eV, *m/z* (rel. int.): 370 $[M]^+$ (4), 355 $[M - Me]^+$ (2), 219 $[A_1 + 1]^+$ (32), 165 $[A_1 - 53]^+$ (14), 203 $[A_1 - Me]^+$ (8), 152 $[B_3]^+$ (17), 123 $[B_4]^+$ (27). ^{1}H NMR (90 MHz, CCl_4, TMS ether): δ6.9 (3H, *m*, H-2′, 5′, 6′), 6.0 (1H, *s*, H-6), 5.3 (1H, *m*, H-β), 4.9 (1H, *d*, J = 10 Hz, H-2), 4.15 (1H, *d*, J = 10 Hz, H-3), 3.2 (2H, *d*, J = 8 Hz, H-α), 3.95 (2H, *s*, γ-CH_2–), 1.58 (3H, *s*, γ-Me).

3′,4′-*Dihydrooxepino-6′-hydroxybutein* (**7**). UV λ_{max}^{MeOH} nm: 375, 316 sh; + NaOMe: 436, 360 sh; + $AlCl_3$: 428; + $AlCl_3$–HCl 384. EIMS (probe) 70 eV, *m/z* (rel. int.): 354 $[M]^+$ (58), 339 $[M - Me]^+$ (28), 311 $[M - C_4H_5]^+$ (9), 219 $[A_1 + 1]^+$ (53), 203 $[A_1 - Me]^+$ (100), 177 $[A_1 - C_3H_7]^+$ (25), 165 $[A_1 - 53]^+$ (24), 136 $[B_4]^+$ (42). ^{1}H NMR (90 MHz, CD_3OD, TMS ether): δ8.1 (1H, *d*, J = 15 Hz, H-β), 7.63 (1H, *d*, J = 15 Hz, H-α), 7.13 (1H, *d*, J = 1.5 Hz, H-2), 7.0 (1H, *dd*, J = 1.5, 8 Hz, H-6), 6.82 (1H, *d*, J

= 8 Hz, H-5), 5.96 (1H, s, H-5'), 5.5 (1H, *br t*, H-2"), 3.9 (2H, s, H-5"), 3.3 (2H, *d*, *J* = 7 Hz, H-1"), 1.7 (3H, s, γ-Me).

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REFERENCES

1. McCormick, S., Robson, K. and Bohm, B. (1985) *Phytochemistry* **24**, 1614.
2. McCormick, S., Robson, K. and Bohm, B. (1985) *Phytochemistry* **24**, 2133.
3. Waddell, T. G., Thomasson, M. H., Moore, M. W., White, H. G., Swanson-Bean, P., Green, M. E., Van Horn, G. S. and Fales, H. M. (1982) *Phytochemistry* **21**, 1631.
4. Bohlmann, F., Zdero, C., Robinson, H. and King, R. M. (1981) *Phytochemistry* **20**, 2245.
5. Ghosal, S., Jaiswal, D. K. and Biswas, K. (1978) *Phytochemistry* **17**, 2119.
6. Waterman, P. G. and Crichton, E. G. (1980) *Phytochemistry* **19**, 1187.
7. Mabry, T. J., Markham, K. R. and Thomas, M. B. (1970) *The Systematic Identification of Flavonoids*, pp. 204, 322, 2359. Springer, New York.
8. Akisanya, A., Bevan, C. E. L. and Hirst, J. (1959) *J. Chem. Soc.* 2679.
9. Mabry, T. J., Markham, K. R. and Thomas, M. B. (1970) *The Systematic Identification of Flavonoids*, pp. 215, 329. Springer, New York.
10. de AlMeida, M. E. and Gottlieb, O. R. (1974) *Phytochemistry* **13**, 751.
11. Cook, I. F. and Knox, J. R. (1975) *Phytochemistry* **14**, 2510.
12. Rama Rao, A. V., Rathi, S. S. and Venkataram, K. (1972) *Indian J. Chem.* **10**, 989.
13. Sherif, E. A., Gupta, R. K. and Krishnamurti, M. (1980) *Tetrahedron Letters* **21**, 641.
14. Singhal, A. K., Sharma, R. P., Thyagarajan, G., Herz, W. and Govindan, S. V. (1980) *Phytochemistry* **19**, 929.
15. Mabry, T. J., Markham, K. R. and Thomas, M. B. (1970) *The Systematic Identification of Flavonoids*, pp. 265–266. Springer, New York.
16. Rodriguez, E., Carmen, N. J. and Mabry, T. J. (1972) *Phytochemistry* **11**, 409.
17. Asakawa, Y., Toyota, M. and Takemoto, T. (1978) *Phytochemistry* **17**, 2005.
18. Asakawa, Y., Takeda, R., Toyota, M. and Takemoto, T. (1981) *Phytochemistry* **20**, 858.
19. Asakawa, Y., Kasube, E., Takemoto, T. and Suire, C. (1978) *Phytochemistry* **17**, 2117.
20. Asakawa, Y., Toyota, M. and Takemoto, T. (1978) *Experientia* **34**, 971.