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RARE 6- AND 8-*O*-METHYLATED EPICUTICULAR FLAVONOLS FROM TWO *CISTUS* SPECIES

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Key Word Index—*Cistus albanicus*; *C. parviflorus*; Cistaceae; 6- and 8-*O*-methylated flavonols; other methylated flavonols; leaf resin; colour reactions; systematic implications.

Abstract—Leaves of *Cistus albanicus* and *C. parviflorus* secrete a resin which contains several rare 6- and 8-*O*-methylated flavonols. In addition, some common kaempferol, quercetin methyl ethers and one myricetin methyl ether were identified. Some typical colour reactions of the 6- and 8-methoxy derivatives on polyamide TLC plates are reported. The systematic implications concerning the flavonoids found are discussed briefly.

INTRODUCTION

The genus *Cistus* L. (Cistaceae) comprises 18 species which are perennial shrubs. *Cistus* species are characteristic elements of the macchias and garigues of the Mediterranean region [1]. Members of this genus secrete a resin on the surfaces of leaves and stems which mainly consists of terpenoids [2, 3] but phenolic compounds, mostly flavonoid aglycones, have also been identified in the resinous exudate [4, 5]. In the course of our continued study of the genus, we have now analysed the resinous material of *C. albanicus* E. F. Warburg ex Heywood and *C. parviflorus* Lam. for flavonoid constituents. Besides common kaempferol and quercetin methyl ethers and a myricetin methyl ether in *C. parviflorus*, which have been detected in other *Cistus* species [4, 6], we have found that these two taxa have a rather similar resin pattern of 6- and 8-*O*-methylated derivatives of kaempferol and quercetin. In addition to the known UV and mass spectral data for the differentiation of 6- and 8-methoxy flavonoids [7–10], we now propose a method of easy differentiation based on colour reactions with Naturstoffreagenz A (NA) on Polyamide DC-11.

RESULTS AND DISCUSSION

Washing the leaves and twigs of the two *Cistus* species with chloroform, precipitation of the waxes by methanol and removal of the terpenoids by column chromatography on Sephadex LH-20 yielded a mixture of flavonoid aglycones. From these mixtures we isolated and identified 20 different flavonoids, mainly 3-methoxyflavonols.

C. albanicus resin contained 11 different methylated flavonols including nine 6- and 8-*O*-methylated derivatives of kaempferol and quercetin, which are listed in Table 1. In addition to these rare methyl ethers, we isolated kaempferol 3-methyl ether and kaempferol 3,4′-dimethyl ether, which have been previously identified in other *Cistus* species [4, 5].

C. parviflorus secretes a complex mixture of 17 flavonol methyl ethers: seven 6- and 8-methoxyflavonols (Table 1) were found together with kaempferol 3-methyl ether, kaempferol 3,7-dimethyl ether, kaempferol 3,4′-dimethyl ether, kaempferol 3,7,4′-trimethyl ether, quercetin 3,3′-dimethyl ether, quercetin 3,7,3′-trimethyl ether, quercetin 3,7,3′,4′-tetramethyl ether and myricetin 3,7,3′,4′-tetramethyl ether. In addition, kaempferol 7- and 4′-monomethyl ethers were identified in trace amounts. Identification was achieved by co-chromatography with samples previously isolated from other *Cistus* species [4, 5], by colour reactions with and without NA, and by UV spectral data. Among the 6- and 8-methoxyflavonoids identified, four are of rare natural occurrence.

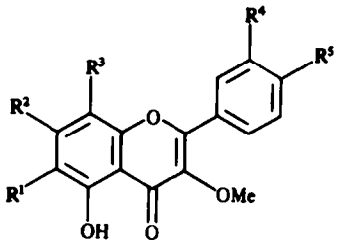
Thus, 5-hydroxy-3,7,8,3′,4′-penthamethoxyflavone (gossypetin 3,7,8,3′,4′-pentamethyl ether) (**1**) isolated from *C. albanicus* was identified by co-chromatography with an authentic sample [11] and comparison with literature data [12, 13].

5,7-Dihydroxy-3,8,3′,4′-tetramethoxyflavone (gossypetin 3,8,3′,4′-tetramethyl ether) (**2**) was obtained only in very small amount from *C. parviflorus*. UV, mass spectral data and R_f values as well as colour reactions with and without NA strongly suggested the proposed structure. Direct comparison with an authentic marker [14] confirmed our identification. The first isolation of **2** as a natural compound was published recently [14], so this is the second report of this flavonol in nature.

5-Hydroxy-3,7,8,4′-tetramethoxyflavone (herbacetin 3,7,8,4′-tetramethyl ether) (**4**) was separated from gossypetin pentamethyl ether by preparative TLC on cellulose. To the best of our knowledge, this herbacetin derivative (flindulatin) has been identified only once as a natural compound [15]. The UV data in methanol, with aluminium chloride and aluminium chloride–hydrochloric acid are identical with those published by Voirin [8]. The mass spectral data $[M]^+$ 358, $[M-15]^+$ 343 and $[M-43]^+$ 313 indicate an 8-methoxyflavonol with one hydroxyl and four methoxyl groups [16]. Colour reactions with and without NA and the R_f values support the identity of the isolated compound.

Table 1. 6- and 8-Methoxyflavonols in the leaf resins of *Cistus albanicus* and *C. parviflorus*

Flavonol	*C. albanicus*	*C. parviflorus*	R^1	R^2	R^3	R^4	R^5	
Gossypetin 3,7,8,3',4'-pentamethyl ether	+		H	OMe	OMe	OMe	OMe	(1)
Gossypetin 3,8,3',4'-tetramethyl ether		+	H	OH	OMe	OMe	OMe	(2)
Gossypetin 3,8,3'-trimethyl ether	+	+	H	OH	OMe	OMe	OH	(3)
Herbacetin 3,7,8,4'-tetramethyl ether	+		H	OMe	OMe	H	OMe	(4)
Herbacetin 3,8,4'-trimethyl ether	+	+	H	OH	OMe	H	OMe	(5)
Herbacetin 3,8-dimethyl ether	+	+	H	OH	OMe	H	OH	(6)
Quercetagetin 3,6,3'-trimethyl ether	+	+	OMe	OH	H	OMe	OH	(7)
Quercetagetin 3,6-dimethyl ether	+		OMe	OH	H	OH	OH	(8)
6-OH-Kaempferol 3,6,4'-trimethyl ether	+	+	OMe	OH	H	H	OMe	(9)
6-OH-Kaempferol 3,6-dimethyl ether	+	+	OMe	OH	H	H	OH	(10)



The identity of the fourth compound, 5,7-dihydroxy-3,8,4'-trimethoxyflavone (herbacetin 3,8,4'-methyl ether) (5), was confirmed by co-chromatography and UV and mass spectral data, which are in good agreement with literature values [17]. It was further corroborated by direct comparison with a synthetic sample [18].

The occurrence of quercetagetin 3,6,3'-trimethyl ether (jaceidin) and quercetagetin 3,6-dimethyl ether (axillarin) in the genus *Cistus* is rather unexpected, but like all the other 6- and 8-*O*-methylated flavonoids of *Cistus* they were identified by UV data, colour reactions with NA, and by direct comparison with authentic samples (see Experimental).

6- and 8-*O*-Methylated kaempferol and quercetin derivatives are easily detected by their dark colour reaction before and after spraying with NA. In addition to published data [19], we noticed remarkable colour differences between the 6-methoxy derivatives and the 8-methoxy derivatives. Without spraying, both were deep purple on Polyamide DC-11. When sprayed with NA, the 8-methoxyflavonols remained deep purple, but the 6-methoxy derivatives turned brown. When exposed to daylight for some hours all the 7-hydroxy-8-methoxyflavonols analysed so far turned brown, whereas the 7,8-dimethoxy and the 6-methoxyflavonols gave a bright yellow colour. Further studies of 6- and 8-substituted flavonols will show how far the colour reactions observed can be generalized. In addition to UV and mass spectral data this would be a useful quick method for distinguishing some 6- and 8-substituted compounds.

We have already noted the rather similar pattern of 6- and 8-substituted flavonols in resins of *C. albanicus* and *C. parviflorus*. It is of interest that all these 8- and especially the 6-methoxyflavonols are quite uncommon in the Dilleniidae and related orders of the plant kingdom. 6-Methoxyflavonoids, for example, are characteristic of the Tubiflorae and have been identified mainly in the Asteraceae and Betulaceae [20]. Considering these facts and previous reports of flavonoid aglycones from other *Cistus* species where 6- and 8-methoxyflavonoids were not found, we conclude a rather close systematic correlation between *C. albanicus* and *C. parviflorus* and an isolated position with respect to other *Cistus* species analysed so far. The endemic occurrence of *C. albanicus* in Albania and the north-west of Greece and the occurrence of *C. parviflorus* in the eastern Mediterranean region in the neighbourhood of *C. albanicus* support the close relationship between the two species.

EXPERIMENTAL

Plant material was cultivated under natural conditions at the Botany Institute of the University of Cologne. Plants were harvested in August 1984, buds and faded leaves removed and twigs washed with $CHCl_3$ to remove the resinous material and waxes. These extracts yielded 2.0% (*C. parviflorus*) and 6.8% (*C. albanicus*) of the dry wts.

The extracts, 2.8 g in both cases, were dissolved in 250 ml warm MeOH (50–60°), and the solns cooled to − 20° to precipitate the MeOH-insoluble material, mainly hydrocarbons and wax esters. The resinous MeOH-soluble material (*C. albanicus* 2.2 g; *C. parviflorus* 1.3 g) containing the flavonoids was, after evaporation, chromatographed directly over Sephadex LH-20 (100 g Sephadex LH-20; column 1 m; 2.5 cm diameter) with MeOH as eluant. This procedure separated the flavonoids from the terpenoids, which were eluted to more than 90% prior to the flavonoid material. The remaining fractions were analysed by prep. CC on polyamide SC-6 (eluted with Toluol and increasing amounts of MeCOEt and MeOH), prep. and analytical TLC on polyamide DC-6 (Tol–MeCOEt–MeOH, 10:2:1) and analytical TLC on polyamide DC-11 (Tol–MeCOEt–MeOH, 10:2:1 and 13:5:3). TLC on cellulose (H_2O–HOAc, 3:2) was used for the separation of gossypetin 3,7,8,3',4'-pentamethyl ether (1) from herbacetin 3,7,8,4'-tetramethyl ether (4) (R_f values 0.83:0.90) and gossypetin 3,8,3',4'-tetramethyl ether (2) from herbacetin 3,8,4'-trimethyl ether (5) (R_f values 0.75:0.85). TLC on silica gel 60 precoated plates (E. Merck, Darmstadt) was used to separate herbacetin 3,8-dimethyl ether from 6-hydroxykaempferol 3,6-

dimethyl ether (R_f values 0.32:0.35). All compounds were purified over Sephadex LH-20 before UV analysis. Identification was achieved by co-chromatography with authentic samples, R_f values, colour reactions with and without spraying with NA, and UV and MS data (Varian, MAT 311, 70 eV). Authentic markers of most of the flavonoids were available. Sources of gossypetin 3,7,8,3',4'-pentamethyl ether, gossypetin 3,8,3',4'-tetramethyl ether and herbacetin 3,8,4'-trimethyl ether are given in the Results. Quercetagetin 3,6,3'-trimethyl ether was isolated from *Alnus glutinosa* [21], quercetagetin 3,6-dimethyl ether from *Neurolaena oaxacana* [22] and *Hemizonia* sp. [23], gossypetin 3,8,3'-trimethyl ether from *Gutierrezia microcephala* [24], 6-hydroxykaempferol 3,6-dimethyl ether and 3,6,4'-trimethyl ether from *Betula ermanii* [25], and herbacetin 3,8-dimethyl ether from *Pityrogramma triangularis* [26].

Characterization of flavonoids. Gossypetin 3,7,8,3',4'-pentamethyl ether (**1**). Yellow needles, mp 162°; deep purple (unchanged with NA); on Polyamide DC-11 (Tol–MeCOEt–MeOH, 13:5:3) R_f 0.90; UV λ_{max}^{MeOH} nm: 257 (sh), 275, 337 (sh), 359; + NaOMe 285, 309 (sh), 384; + $AlCl_3$ 243 (sh), 283, 305 (sh), 357, 418; + $AlCl_3$ + HCl 243 (sh), 283, 305 (sh), 352, 418; + NaOAc 257 (sh), 274, 337 (sh), 357 (sh); + NaOAc + H_3BO_3 257 (sh), 274, 337 (sh), 357 (sh); MS *m/z* (rel. int.): 388 $[M]^+$ (85), 373 $[M-15]^+$ (100).

Gossypetin 3,8,3',4'-tetramethyl ether (**2**). Deep purple (unchanged with NA); on Polyamide DC-11 (Tol–MeCOEt–MeOH, 13:5:3) R_f 0.63; UV λ_{max}^{MeOH} nm: 257, 274, 340; + NaOMe 283, 312 (sh), 391; + $AlCl_3$ 266 (sh), 283, 302 (sh), 361, 412; + $AlCl_3$ + HCl 266 (sh), 283, 302 (sh), 355, 410; + NaOAc 283, 316 (sh), 384; + NaOAc + H_3BO_3 255, 275, 334; MS *m/z* (rel. int.): 374 $[M]^+$ (80), 359 $[M-15]^+$ (100), 331 $[M-43]^+$ (6).

Gossypetin 3,8,3'-trimethyl ether (**3**). Deep purple (unchanged with NA); on Polyamide DC-11 (Tol–MeCOEt–MeOH, 13:5:3) R_f 0.31; UV λ_{max}^{MeOH} nm: 260 (sh), 275, 332 (sh), 360; + NaOMe 280, 336 (sh), 416; + $AlCl_3$ 282 (sh), 304, 365, 413; + $AlCl_3$ + HCl 283, 304 (sh), 359, 414; + NaOAc 283, 324, 398; + NaOAc + H_3BO_3 259 (sh), 275, 332 (sh), 360.

Herbacetin 3,7,8,4'-tetramethyl ether (**4**). Yellow needles, mp 169°; deep purple (unchanged with NA); on polyamide DC-11 (Tol–MeCOEt–MeOH, 13:5:3) R_f 0.90; UV λ_{max}^{MeOH} nm: 273, 321, 357 (sh); + NaOMe 284, 304 (sh), 385; + $AlCl_3$ 282, 311, 350, 417; + $AlCl_3$ + HCl 282, 311, 350, 417; + NaOAc 273, 301 (sh), 321, 357; + NaOAc + H_3BO_3 273, 301 (sh), 321, 357; MS *m/z* (rel. int.): 358 $[M]^+$ (85), 343 $[M-15]^+$ (100), 315 $[M-43]^+$ (5).

Herbacetin 3,8,4'-trimethyl ether (**5**). Yellow crystals, mp 169°; deep purple (unchanged with NA); on Polyamide DC-11 (Tol–MeCoEt–MeOH, 13:5:3) R_f 0.63; UV λ_{max}^{MeOH} nm: 274, 321, 357 (sh); + NaOMe 283, 303 (sh), 387; + $AlCl_3$ 230, 283, 310, 349, 412; + $AlCl_3$ + HCl 230, 283, 310, 347, 411; + NaOAc 283, 307 (sh), 383; + NaOAc + H_3BO_3 275, 322 (sh), 358 (sh); MS *m/z* (rel. int.): 344 $[M]^+$ (88), 329 $[M-15]^+$ (100), 301 $[M-43]$ (8), 135 $[B_2]^+$ (15).

Herbacetin 3,8-dimethyl ether (**6**). Deep purple (unchanged with NA); on Polyamide DC-11 (Tol–MeCOEt–MeOH, 13:5:3) R_f 0.32; UV λ_{max}^{MeOH} nm: 273, 321, 354 (sh); + NaOMe 281, 331, 404; + $AlCl_3$ 230, 281, 311, 353, 410; + $AlCl_3$ + HCl 230, 282, 310, 349, 410; + NaOAc 281, 305 (sh), 384; + NaOAc + H_3BO_3 274, 326, 355 (sh).

Quercetagetin 3,6,3'-trimethyl ether (**7**). Deep purple (brown with NA); on Polyamide DC-11 (Tol–MeCOEt–MeOH, 13:5:3) R_f 0.35; UV λ_{max}^{MeOH} nm: 256, 270 (sh), 350; + NaOMe 271, 337, 409; + $AlCl_3$ 265, 279 (sh), 301, 369; + $AlCl_3$ + HCl 267, 369, 409 (sh); + NaOAc 273, 318, 378; + NaOAc + H_3BO_3 256, 271, 354; MS *m/z* (rel. int.): 360 $[M]^+$ (100), 345 $[M-15]^+$ (40), 317 $[M-43]^+$ (14), 135 $[A_1]^+$ (8), 151 $[B_2]^+$ (8).

Quercetagetin 3,6-dimethyl ether (**8**). Purple (reddish orange with NA) on Polyamide DC-11 (Tol–MeCOEt–MeOH, 13:5:3) R_f 0.13; UV λ_{max}^{MeOH} nm: 264, 372; + NaOMe 278, 333, 417; + $AlCl_3$ 279, 305 (sh), 441; + $AlCl_3$ + HCl 270, 295 (sh), 365, 410; + NaOAc 275, 409; + NaOAc + H_3BO_3 266, 382.

6-OH-Kaempferol 3,6,4'-trimethyl ether (**9**). Deep purple (brown with NA); on Polyamide DC-11 (Tol–MeCOEt–MeOH, 13:5:3) R_f 0.65; UV λ_{max}^{MeOH} nm: 272, 338; + NaOMe 274, 373; + $AlCl_3$ 280, 305 (sh), 361; 407 (sh); + $AlCl_3$ + HCl 280, 298 (sh), 305 (sh), 361, 407 (sh); + NaOAc 274, 296 (sh), 371; + NaOAc + H_3BO_3 272, 338.

6-OH-Kaempferol 3,6-dimethyl ether (**10**). Deep purple (brown with NA); on Polyamide DC-11 (Tol–MeCOEt–MeOH, 13:5:3) R_f 0.23; UV λ_{max}^{MeOH} nm: 270, 340; + NaOMe 275, 328, 398; + $AlCl_3$ 276, 305, 358; + $AlCl_3$ + HCl 281, 298 (sh), 305 (sh), 357; + NaOAc 273, 301, 377; + NaOAc + H_3BO_3 272, 344.

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REFERENCES

1. Warburg, E. F. (1968) in *Flora Europeae* (Tutin, T. G., Heywood, V. H., Burges, N. A., Moore, D. M., Valentine, D. H., Walter, S. M. and Webb, D. A., eds.) Vol. 2. Cambridge University Press, Cambridge.
2. Pasqual Teresa, J. de, Jara, A., Urones, J. G. and San Feliciano, A. (1972) *Quimica* **68**, 727.
3. Gülz, P. G., Kobold, U., Michaelis, K., and Vostrowsky, O. (1984) *Z. Naturforsch. Teil C* **39**, 699.
4. Wollenweber, E. and Mann, K. (1984) *Z. Naturforsch. Teil C* **39** 303.
5. Proksch, P. and Gülz, P.-G. (1984) *Phytochemistry* **23**, 470.
6. Berti, G., Livi, O., Segnini, G. and Cavero, I. (1967) *Tetrahedron* **23**, 2295.
7. Mears, T. J. and Mabry, T. J. (1972) *Phytochemistry* **11**, 411.
8. Voirin, A. (1983) *Phytochemistry* **22**, 2107.
9. Goudard, M., Favre-Bonvin, J., Lebreton, P. and Chopin, J. (1978) *Phytochemistry* **17**, 145.
10. Goudard, J., Favre-Bonvin, J., Nogradi, M. and Chopin, J. (1979) *Phytochemistry* **18**, 186.
11. Boven, I. H. and Perera, K. P. W. C. (1982) *Phytochemistry* **21**, 433.
12. Henrick, C. A. and Jefferies, P. R. (1964) *Aust. J. Chem.* **17**, 934.
13. Tatum, J. H. and Berry, R. E. (1972) *Phytochemistry* **11**, 2283.
14. Loyola, L. A., Naranjo, S. J. and Morales, B. G. (1985) *Phytochemistry* **24**, 1871.
15. Brown, R. F. C., Gilham, P. T., Hughes, G. K. and Ritchie, E. (1954) *Aust. J. Chem.* **7**, 181.
16. Wollenweber, E. and Dietz, V. H. (1979) *Phytochem. Bull.* **12**, 48.
17. Tandon, S. and Rastogi, R. G. (1977) *Phytochemistry* **16**, 1455.
18. Horie, T., Konrai, H., Osaka, H. and Nakayama, M. (1982) *Bull. Chem. Soc. Jpn.* **55**, 2933.
19. Wollenweber, E. (1982) Suppl. Chromatographie 1982, p. 50. GIT, Darmstadt.

20. Wollenweber, E. and Dietz, V. H. (1981) *Phytochemistry* **20**, 869.
21. Wollenweber, E., Bouillant, M.-L., Lebreton, P. and Egger, K. (1971) *Z. Naturforsch. Teil B* **26**, 1188.
22. Ulubelen, A., Kerr, K. M. and Mabry, T. J. (1980) *Phytochemistry* **19**, 1761.
23. Proksch, P., Budzikiewicz, H., Tanowitz, B. D. and Smith, D. M. (1984) *Phytochemistry* **23**, 679.
24. Roitman, J. N. and James, L. F. (1985) *Phytochemistry* **24**, 835.
25. Wollenweber, E. and Lebreton, P. (1971) *Biochimie* **53**, 935.
26. Wollenweber, E., Dietz, V. H., Schilling, G., Favre-Bonvin, J. and Smith, D. M. (1985) *Phytochemistry* **24**, 965.