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EPICUTICULAR 5-*O*-METHYL FLAVONOLS FROM *CISTUS LAURIFOLIUS*

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Abstract—Extraction of the epicuticular leaf resin of *Cistus laurifolius* revealed two new flavonol aglycones, quercetin 5,3′-dimethyl ether and quercetin 3,5,3′-trimethyl ether Both substances were only present in young immature leaves They were identified by UV, MS and ^{1}H NMR spectroscopy

INTRODUCTION

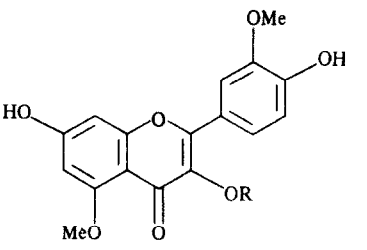
Cistus laurifolius L. [1] is a woody shrub growing in the southwest of the Iberian peninsula. Like other *Cistus* species it secretes a sticky resin from glandular trichomes Recently several methylated flavonoid aglycones have been reported from this resinous material [2–5] *Cistus laurifolius* was shown to secrete mainly quercetin, 3-methyl ethers, together with some kaempferol, apigenin and luteolin derivatives [2,5]. In no case, however, were seasonal variations of the epicuticular flavonoid pattern of *Cistus* resins taken into account The present detailed investigation of the resin of young leaves of *C laurifolius* revealed two new flavonol methyl ethers not detected previously

RESULTS AND DISCUSSION

Investigations of the qualitative and quantitative seasonal variations of the epicuticular flavonoid pattern of *C. laurifolius* revealed two spots on TLC plates, which could only be detected in immature young leaves. One fluoresced bright yellow and the other appeared bright blue under UV-light. A bright yellow fluorescence is characteristic of 3-hydroxy-5-*O*-methyl flavonols whereas the blue fluorescence might indicate a flavonol 3,5-dimethyl ether [6] The UV data suggested that both compounds were quercetin derivatives and from the characteristic fluorescence and the MS data the first was suggested to be quercetin 5,3′-dimethyl ether (**1**) and the second quercetin 3,5,3′-trimethyl ether (**2**) The ^{1}H NMR data confirmed this identification The dimethyl derivative has been isolated as its 3-glucoside [7], whereas the trimethyl ether is known only as a synthetic product [8]. In addition hydrolysis of an authentic sample of quercetin 5,3′-dimethyl ether 3-glucoside [7] gave (**1**), while partial demethylation of the 5-position of both with boron trichloride [9] gave quercetin 3′-methyl ether in the case of **1** and quercetin 3,3′-dimethyl ether in the case of **2**

The total amount of these flavonols was highest in the resin of young immature unfurled leaves. During leaf development in the summer the amount of both substances decreased. Preliminary HPLC analysis of young leaves showed that **1** made up 4% and **2** 6% of the resin flavonoids, whereas resin samples of leaves harvested in late summer showed no trace of these substances on TLC or by HPLC Total and selective disappearance of epicuticular resin flavonoids is quite unexpected Preliminary results showed that UV light of 350 nm was able to 'destroy' both substances in the resin mixture, when leaves were placed on small glass bowls Surprisingly UV light of 254 nm was less effective Isolated substances exposed to UV light were not sensitive in either case The amounts of the other known flavonoids in the resin mixture were also found to be reduced upon exposure to 350 nm UV light induced chemical reactions between the flavonoids and other resin constituents could be a possible explanation Nevertheless the selective disappearance of quercetin 5,3 -dimethyl ether and quercetin 3,5,3′-trimethyl ether in the resin would imply an enzymic block in synthesis of 5-*O*-methyl flavonols during leaf development Nothing is known about the mechanism of 5-*O*-methylation of flavonoids in plants but this suggests that there is one distinct enzyme [10] operating which is independent of the remaining methylation system in *C laurifolius*

5-*O*-Methylation is rare in nature and from the chemotaxonomic point of view it is regarded as a rather primitive evolutionary character [11] Most dicots in which these flavonoids have been identified are woody



1 R^1 = H
2 R^2 = Me

and are considered as primitive members of their groups [11]. It is interesting that *C. laurifolius* is also woody and that it probably represents a primitive form in *Cistus*, is corroborated by the epicuticular flavonoid pattern in mature leaf resin [5].

EXPERIMENTAL

Plants of *C laurifolius* L. were cultivated for 10 years at the Botany Institute of the University of Cologne under natural garden conditions. Seeds were collected in the Sierra de Guadarama, Spain by Prof. Reznik Very young unfurled leaves, harvested in June (260 g of fr. wt; 105 g of dry wt) were washed × 2 with $CHCl_3$. The combined fractions (8.6 g of extract) were dissolved in 300 ml hot MeOH. After pptn of the waxes at −20° (0 7 g) the resin (7.7 g) was separated on Sephadex LH-20 (100 g; column length 1 m; diameter 2.5 cm) with MeOH as eluent The purification of individual flavonoids follnwed the usual procedures [4, 7]. Separation of quercetin 5,3′-dimethyl ether from quercetin 3-methyl ether which was the main flavonol in the resin was achieved by low pressure liquid chromatography on a reversed-phase silica gel column (LiChroPrep RP-18, particle size 63 μm, length 30 cm, 3.5 cm diameter) [12] with H_2O (0 2% TFA)–MeOH–acetonitrile–THF (34 3:3 10) as eluents and purification of **1** was performed on Sephadex LH-20 with MeOH as eluent.

HPLC-analysis was performed on a Hypersil RP-8 column (particle size 5 μm) with H_2O (1% H_3PO_4)–MeOH–Acetonitrile–THF (34 3.3.10) Resin samples were exposed to UV light for 12 hr at a distance of 5 cm from a normal UV lamp.

NMR and MS ^{1}H NMR spectra were recorded at ambient temp. at 300.1 MHz in DMSO-d_6 Chemical shifts are given in ppm relative to TMS and coupling constants in Hz

Characterization of flavonoids. Quercetin 5,3′-dimethyl ether (**1**) (1.3 mg) bright yellow fluorescence (greenish with NA); on polyamide DC-11 (toluene–MeCOEt–MeOH, 13 5 3) R_f 0.25, UVλ_{max}^{MeOH} nm 251, 271 (sh), 362; +NaOMe 269, 317, 408, +Alcl$_3$ 261, 281 (sh), 303 (sh), 340 (sh), 423; +$AlCl_3$ +HCl 260, 281 (sh), 303 (sh), 342 (sh), 422; +NaOAc 275, 293 (sh), 320, 402, +NaOac +H_3BO_3 255, 272 (sh), 362, 431; EIMS *m/z* (rel int), 330 $[M]^+$ (100), 284 $[M-46]^+$, 167 $[A_1+H]^+$ (10), 151 $[B_2]^+$ (10), ^{1}H NMR (DMSO-d_6): δ=3.827 (*s*, 5−OMe), 3 838 (*s*, 3″ −OMe), 6 353 (*d*, *J*=2 Hz, H6), 6.521 (*d*, *J*=2 Hz, H8), 6 924 (*d*, *J*=8.5 Hz, H5′), 7.627 (*dd*, *J*=2, 8 5 Hz, H6′), 7 714 (*d*, *J*=2 Hz,, H2′) Irradiation at 5−OMe gave a NOE for H-6 and at 3′-OMe gave H-2′

Quercetin 3,5,3′-trimethyl ether (**2**). (2 0 mg) Blue fluorescence (unchanged with NA), on polyamide DC-11 (toluene–MeCOEt–MeOH, 13 . 5 : 3) R_f 0 32; UVλ_{max}^{MeOH} nm 248, 276 (sh), 344, +NaOMe 267, 311, 394, +$AlCl_3$ 252 (sh), 278 (sh), 347; +$AlCl_3$ +HCl 252 (sh), 278 (sh), 347, +NaOAc 272, 302, 363; +NaOAc +H_3BO_3 272 (sh), 300, 345, EIMS *m/z* (rel int.). 344 $[M]^+$ (100), 329 $[M-15]^+$ (75), 301 $[M-43]^+$ (18), 167 $[A_1+H]^+$ (30), 151 $[B_2]^+$ (40), ^{1}H NMR (DMSO-d_6): δ=3 719 (*s*, 3 −OMe), 3 803 (*s*, 5−OMe), 3 844 (*s*, 3′−OMe), 6.368 (*d*, *J* =2 1 Hz, H6), 6.502 (*d*, *J*=2 1 Hz, H8), 6 937 (*d*, *J*=8.4 Hz, H5′), 7 508 (*dd*, *J*=2, 8 5 Hz, H6′), 7.599 (*d*, *J*=2 Hz, H2′). Irradiation of 5−OMe gave a NOE for H-6, at 3′–OMe gave H−2′ and at 3 −OMe gave a small NOE for H-2′

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