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AMENTOFLAVONES FROM *CALLITRIS* SPECIES

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Key Word Index—*Callitris columellaris*; *C. endlicheri*; *C. preissii* subsp. *murrayensis*; *C. preisii* subsp. *verrucosa*; *C. canescens*; *C. macleayana*; Cupressaceae; biflavonoid; amentoflavone.

Abstract—The biflavonoid patterns of the leaves of five species of *Callitris* are characterized as amentoflavone based derivatives only. This contrasts markedly with the diversity of biflavones reported for northern genera of the Cupressaceae.

The family Cupressaceae is divided into two sub-families, the Northern Hemisphere Cupressoideae K. Koch and the Southern Hemisphere Callitroideae Saxton [1]. Although the occurrence of four parent biflavones among the Cupressoideae has been well documented [2], the only record for the subfamily Callitroideae is amentoflavone in *C. rhomboidea* [4], and an early report of hinokiflavone in *C. glauca* (= *C. columellaris* F. Muell.?) by Sawada [3]. This paper reports on the biflavonoids of a further five species of *Callitris* including Australian material of *C. columellaris*.

The EtOH extracts of the dried leaves and twigs contained amentoflavone as the major biflavonoid compound in all the species examined (Table 1). Amentoflavone 4‴ monomethyl ether was detected in trace amounts in all the species, but 7″4‴ dimethyl amentoflavone could only be detected and identified in *C. canescens*. Duplicate extraction from different collections of *C. columellaris* and *C. endlicheri* confirmed their biflavonoid patterns. Extracts of two different subspecies of *C. pressii* gave identical results as did the extracts from the characteristic 'reversion' juvenile foliage and adult foliage from *C. macleayana*.

Identification of amentoflavone, 4‴ monomethyl amentoflavone and 7″4‴ dimethyl amentoflavone was determined by co-chromatography with authentic standards in two solvent systems, viz. C_6H_6–pyridine–HCO_2H (100:20:7) on Si gel, *n*-BuOH–NH_4OH on cellulose. Several bands were partially and per-methylated, and the partial methyl ethers and hexamethyl ethers were co-chromatographed and characterized by TLC. Further, the initial eluants for *C. columellaris*, *C. preissii* subsp. *murrayensis*, *C. canescens* and *C. macleayana* were permethylated and found to contain amentoflavone hexamethyl ether as the only biflavonoid compound. This confirmed the absence of biflavonoids based on skeletons other than the 3′,8-linked amentoflavone.

The presence of hinokiflavone in *C. columellaris*, as reported by Sawada [3], could not be confirmed.

Although only six species of *Callitris* have now been examined for the presence of biflavonoids, it is interesting to note that all species are characterized by the presence of only amentoflavone-based biflavones. This contrasts markedly with the array of biflavonoids present amongst the Cupressoideae, viz. amentoflavone, hinokiflavone and cupressuflavone and/or their partial methyl ethers, as well as robustaflavone which has recently been reported in *Juniperus phoenica* [2, 5]. This apparent biochemical distinction between the two subfamilies emphasizes the geographic discontinuity of the northern and southern species in the family. Clearly, it will be necessary to investigate other genera in the Callitroideae, including representatives of the tribes Libocedreae and Tetraclineae [1]. The existing data are sufficient to indicate that the distribution of biflavonoids is likely to contribute significantly to an understanding of affinities between genera in the Cupressaceae.

Table 1. Distribution of amentoflavone, 4‴ monomethyl amentoflavone and 7″4‴ dimethyl amentoflavone in *Callitris*

	A	B	C
C. columellaris	+++	+	-
C. endlicheri	+++^{p}	+^{p}	-
C. preissii subsp. *murrayensis*	+++	+	-
C. preissii subsp. *verrucosa*	+++	+	
C. canescens	+++	+^{p}	+^{p}
C. macleayana	+++	+^{p}	-

A = amentoflavone, B = 4‴ monomethyl amentoflavone, C = 7″4‴ dimethyl amentoflavone, +++ = major presence, + = minor trace detected, *p* = compound permethylated.

EXPERIMENTAL

Collections from which samples for biflavonoid analysis were taken are lodged at the herbarium of UNSW. *C. columellaris* F. Muell. UNSW 9341, UNSW 9287. *C. endlicheri* (Parl.) F. M. Bailey UNSW 9339, UNSW 9291. *C. preissii* Miq. subsp. *murrayensis* J. Garden UNSW 9288. *C. preissi* subsp. *verrucosa* (A. Cunn. ex Endl.) F. Muell. UNSW 9290. *C. canescens* (Parl.) S. T. Blake UNSW 9289. *C. macleayana* (F. Muell.) F. Muell. UNSW 7485 (juvenile), UNSW 9239.

The dried and crushed leaves and branchlets were extrd in 70% EtOH for 48 hr, washed with petrol (60–80°) to remove excess oils,

and then concd under red. press. The residue was re-extrd in EtOH, and examined by PC in *n*-BuOH–HOAc–H_2O (4:1:5). The biflavonyls appeared under UV as a dark absorbing band immediately behind the solvent front, and fluoresced dark yellow on addition of $AlCl_3$ indicating the presence of flavonoid type compounds. The band was eluted with 1% HOAc in 70% EtOH concd and re-chromatographed on pre-coated Si gel plates developed in C_6H_6–pyridine–HCO_2H (BPF) (100:20:7), revealing the mixture of biflavonoid bands present in the extract as a number of dark, UV-absorbing bands. Each band was extrd individually and a final sepn of each was carried out on pre-coated cellulose plates developed in fresh prepared *n*-BuOH–2 N NH_4OH, 1:1 (upper layer) (BN). Initial identification was made by co-chromatography with authentic samples of amentoflavone, 4‴ monomethyl amentoflavone and 7″4‴ dimethyl amentoflavone in BPF and BN. Confirmation of the identification of these compounds as amentoflavone-based ethers was provided by partial methylation and permethylation (CH_2N_2) of some individual bands and some eluants. The methylated eluants provided only the characteristic hexamethyl ether of amentoflavone, which co-chromatographed with an authentic sample in BPF.

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SYNTHESIS AND STRUCTURAL PROOF OF WAIROL, A NEW COUMESTAN FROM *MEDICAGO SATIVA*

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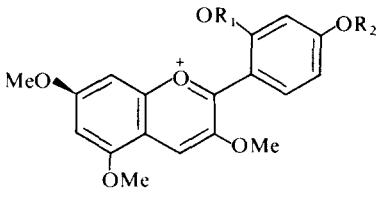
Key Word Index—*Medicago sativa*; Leguminosae; synthesis; 3-hydroxy-7,9-dimethoxycoumestan; wairol.

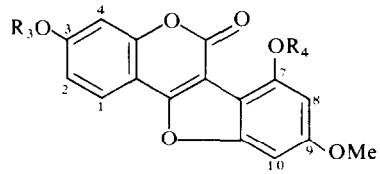
Abstract—Wairol, a coumestan from *Medicago sativa*, has been synthesized and its structure thereby has been confirmed.

In a previous investigation [1], a new coumestan was isolated from fungal-infected lucerne foliage, and tentatively identified as 3-hydroxy-7,9-dimethoxycoumestan (wairol, **3**). In this paper we report the chemical synthesis of wairol, confirming the structure previously assigned, and report some physical characteristics of this compound.

The suitably protected flavylium salt, 3,5,7-trimethoxy-2′,4′-dibenzyloxyflavylium chloride (**1**) was prepared by acid-catalysed condensation of 4,6-dimethoxysalicylaldehyde with 2,4-dibenzyloxy-ω-methoxyacetophenone [2] (76%), red needles, λ_{max} (EtOH–0.5% HCl) nm (log ε) 512 (3.91), 276 (4.12) (Found: C, 70.46; H, 5.37; $C_{32}H_{29}O_6Cl$ requires: C, 70.58; H, 5.33%.)



1 $R_1 = R_2 = Bz$
2 $R_1 = R_2 = H$



3 $R_3 = H$, $R_4 = Me$
4 $R_3 = R_4 = H$
5 $R_3 = R_4 = CD_3$
6 $R_3 = CD_3$, $R_4 = Me$
7 $R_3 = Ac$, $R_4 = Me$