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METHYLATED FLAVONOIDS FROM *CISTUS LADANIFER* AND *CISTUS PALHINHAE* AND THEIR TAXONOMIC IMPLICATIONS

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Key Word Index—*Cistus ladanifer*; *C. palhinhae*; Cistaceae; leaf resin; methylated flavonoids; chemotaxonomy.

Abstract—Three methylated kaempferol and two methylated apigenin derivatives were identified in the leaf resins of *Cistus ladanifer* and *C. palhinhae*. The two species produced identical secreted flavonoids which supports their close affinity based on morphological similarities.

INTRODUCTION

The genus *Cistus* L. is one of the characteristic genera of the Mediterranean region [1, 2]. It consists of 16 species which are some of the dominating shrubs of the maquis and garigues ecosystems [2]. Several species of *Cistus*, including *C. ladanifer* L. and *C. palhinhae* Ingram, secrete large amounts of resin on the surfaces of leaves and stems. Epicuticular alkanes and wax esters [3–6] and terpenoids [7–10] have been identified previously as resin constituents of several *Cistus* species.

In continuation of our chemotaxonomic studies on *Cistus*, we now report the flavonoid composition of the leaf resins of *C. ladanifer* and *C. palhinhae*.

RESULTS AND DISCUSSION

The leaf resins of *Cistus ladanifer* and *C. palhinhae* were found to have identical flavonoid patterns. Preparative TLC on Polyamid DC 6 yielded 3,7-dimethylkaempferol, 3-methylkaempferol, 4′-methylapigenin and apigenin. Further chromatography of the 3,7-dimethylkaempferol and 4′-methylapigenin bands on Polyamid DC 11 allowed the separation of small quantities of 3,4′-dimethylkaempferol and 7-methylapigenin. Preliminary studies have shown that the secreted flavonoid patterns of other *Cistus* species are significantly different and more complex than those of *C. ladanifer* and *C. palhinhae* and appear to be species-specific (Proksch, P. and Gülz, P.-G., unpublished results). *C. palhinhae* is also morphologically very similar to *C. ladanifer* and has been recognized as a separate species only quite recently [2]. The present flavonoid evidence thus supports their close affinity. Both species were also previously shown to be very similar in regard to other epicuticular components such as wax esters [4].

A previous study on flavonoid glycosides from *Cistus* showed that the flavonoid patterns were rather uniform throughout the whole genus and did not allow a clear distinction between species or groups of related species based on qualitative differences [11]. However, the secreted flavonoids of *Cistus* show very distinct patterns that might be more useful in delineating species than the flavonoid glycosides.

Poetsch and Reznik [11, 12] showed that quercetin, myricetin and kaempferol glycosides were the major flavonoid constituents in the cell vacuoles of *Cistus ladanifer* and *C. palhinhae*. The total lack of quercetin and myricetin and the appearance of apigenin-type flavonoids in the leaf resins of these two species reflect differences in the regulation of the biosynthesis of flavonoid glycosides and secreted flavonoid aglycones.

EXPERIMENTAL

Cistus ladanifer and *C. palhinhae* were grown from seeds and cultivated at the Botanisches Institut over several years. Leafy branches were harvested in April 1983 and immediately washed with $CHCl_3$. The yields of the crude resins were between 10 and 13% of the dry wts. Analytical TLC on Polyamid DC 6 revealed the presence of several flavonoid aglycones. The flavonoids were isolated by prep. TLC on Polyamid DC 6, solvent system C_6H_6–MEK–MEOH (14:2:1) and on Polyamid DC 11, solvent system C_6H_6–*n*-pentane–MEK–MeOH (6:12:1:1) [13]. All compounds were purified by CC on Sephadex LH-20 with MeOH as eluant prior to UV analysis. Identification was achieved by co-chromatography with authentic standards and by UV analysis according to standard procedures [14]. Flavonoid standards were purchased from Carl Roth KG (West Germany) or supplied by Professor E. Rodriguez (University of California, Irvine, U.S.A.).

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MUKONAL, A PROBABLE BIOGENETIC INTERMEDIATE OF PYRANOCARBAZOLE ALKALOIDS FROM *MURRAYA KOENIGII*

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Key Word Index—*Murraya koenigii*; Rutaceae; mukonal; carbazole alkaloid.

Abstract—Mukonal, a carbazole alkaloid has been isolated from *Murraya koenigii*. The structure of the compound has been established as 2-hydroxy-3-formyl carbazole based on physical (UV, IR, ^{1}H NMR, ^{13}C NMR and mass spectrometry) and chemical transformations.

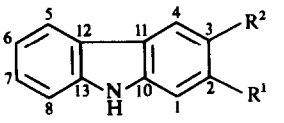
INTRODUCTION

Murraya koenigii is known to be the richest source of carbazole alkaloids so far reported [1]. The present investigation reveals the presence of a new carbazole alkaloid, mukonal, from the petrol extract of the stem bark of *M. koenigii*.

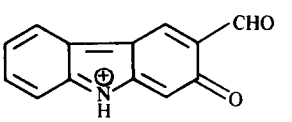
RESULTS AND DISCUSSIONS

Mukonal **1**, $C_{13}H_9NO_2$ $[M]^+$ *m/z* 211, mp 238° was homogeneous by TLC and mass spectrometry. It gave a 2,4-dinitrophenylhydrazone and reduced ammoniacal silver nitrate solution showing the presence of an aldehyde function. A deep blue colouration with ferric chloride indicated a chelated phenolic hydroxyl group. The UV spectrum of **1** (λ_{max}^{EtOH} nm: 234, 247, 278, 297 and 342 with log ε 4.42, 4.21, 4.54, 4.58 and 4.06) is characteristic of a 3-formyl carbazole [2]. The IR spectrum (KBr) showed absorption peaks at 3380 (OH or NH), 1640 (chelated aldehyde), 1610 and 1590 cm^{-1} (aromatic system).

The 100 MHz ^{1}H NMR spectrum (DMSO-d_6) displayed characteristic signals at δ 11.76 (1H, *s*, OH proton, exchangeable with D_2O), δ 11.0 (1H, *br s*, NH proton, exchangeable with D_2O), δ 10.16 (1H, *s*, CHO proton), δ 8.4 (1H, *s*, aromatic proton), δ 8.0–7.3 (4H, complex multiplet, aromatic proton) and δ 7.0 (1H, *s*, aromatic proton). The appearance of four protons in the region δ 8.0–7.3 as a complex multiplet suggests lack of substitution of one of the benzene rings of the carbazole moiety. The relatively deshielded singlet at δ 8.4 could be assigned to the C-4-H *ortho* to the aldehyde group. Since the C-4 proton is not *meta* coupled and the hydroxyl is chelated, the hydroxyl group could be located at C-2.



1 R^1 = OH, R^2 = CHO
2 R^1 = H, R^2 = H
4 R^1 = OH, R^2 = H
5 R^1 = OAc, R^2 = CHO
6 R^1 = OH, R^2 = COOH
7 R^1 = OH, R^2 = COOMe



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