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SHORT COMMUNICATION

CHEMOTAXONOMICAL STUDIES OF SOME *CASUARINA* SPECIES

S. NATARAJAN, V. V. S. MURTI and T. R. SESHADRI

Department of Chemistry, University of Delhi, Delhi-7, India

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Abstract—A chemical examination of the leaves of seven *Casuarina* species from India, Australia and Thailand has been carried out to find chemical clues of possible taxonomic value. Kaempferol, quercetin and (+)catechin were identified in all the plants, cupressuflavone in one plant, hinokiflavone in two plants and ellagic acid in five plants. These findings suggest that *Casuarina* is a woody dicotyledon which shows characters putting it on the primitive side of the evolutionary scale.

INTRODUCTION

CASUARINACEAE comprising about 40 species is the only family included under Casuarinales. Members of this family are found chiefly in Australia, the South Western Pacific and in the Indo-Malayan regions. Only *Casuarina equisetifolia* shows an extended distribution. The taxonomic status of this genus has been the subject of much discussion. In gross morphology it resembles some of the gymnosperms and this superficial similarity is considered to be due to convergence. *Casuarina* appears to be a highly isolated genus. Earlier it was considered to be the most primitive genus of the dicots but the idea has now been abandoned; it is now thought that *Casuarina* is a reduced plant rather than a primitive type. There is agreement that *Casuarina* is eventually related to some of the families of Hamamelidae.[1-3]

In the context of these differences of opinion regarding the taxonomic position of Casuarinaceae, their chemistry has special significance. After the recognition of the wide occurrence of biflavones in the leaves of gymnosperms attempts were made to utilize their distribution for taxonomical purposes.[2] Hinokiflavone was found to be present in the leaves of *Casuarina stricta*;[4] the leaf wax of this species yielded (on hydrolysis) juniperic acid (16-hydroxypalmitic acid) which is considered to be a typical gymnosperm constituent.[2] No biflavone was recorded in *C. cunninghamiana*.[5] In the present work a detailed chemical examination of *Casuarina* species for polyphenolics of possible taxonomic value has been carried out.

RESULTS

A study of the leaves of the following Casuarinas has now been carried out.

Casuarina littoralis Salisb. (Australia); *C. suberosa* Otto and Dietr. (Australia); *C. cunninghamiana* Miq (Australia); *C. cunninghamiana* Miq (India); *C. rigida* Miq (Australia); *C. junghuhniana* Miq (Thailand); *C. equisetifolia* L. (Thailand); *C. equisetifolia* L. (India); *C. montana* Leschen (India).

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[2] V. V. S. MURTI, *Bull. Nat. Inst. Sci. India* **34**, 161 (1967).
[3] A. CRONQUIST, *The Evolution and Classification of Flowering, Plants* p. 172, Nelson, London (1968).
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[5] E. C. BATE-SMITH, *J. Linn. Soc., Lond.* **58**, 95 (1962).

Only *Casuarina equisetifolia* of Indian origin could be procured in sufficient quantity for detailed studies; in other cases most of the examination was carried out by TLC in a number of solvent systems.

The leaves were extracted exhaustively with hot ethanol. From the alcoholic concentrate light petroleum solubles were removed and the residue was separated into ether-soluble and ether-insoluble fractions. These were examined by TLC employing authentic substances as markers. Multiple development was frequently used for better resolution of compounds with close R_f values. The hydroxy compounds were located by alcoholic ferric chloride spray and also by characteristic u.v. fluorescence. Ethanolic lead acetate spray and zinc-hydrochloric acid test were also carried out to locate other flavonoids on TLC plates. The crude alcohol and ether-soluble portions were examined by TLC before and after methylation using authentic flavonoids and biflavonoids and their methyl ethers as markers.[6] The main findings of the present study are given in Table 1.

TABLE 1

Name of species	*K*	*Q*	(+)*C*	*E*	*H*	*C*
C. littoralis (Australia)	+	+	+	−	−	−
C. suberosa (Australia)	+	+	+	−	+	−
C. cunninghamiana (Australia)	+	+	+	+	−	−
C. cunninghamiana (India)	+	+	+	−	−	+
C. rigida (Australia)	+	+	+	+	−	−
C. junghuhniana (Thailand)	+	+	+	+	+	−
C. equisetifolia (Thailand)	+	+	+	+	−	−
C. equisetifolia (India)	+	+	+	+	−	−
C. montana (India)	+	+	+	−	−	−

K, Kaempferol; *Q*, Quercetin; (+)*C*, (+)Catechin; *E*, Ellagic acid; *H*, Hinokiflavone; *C*, Cupressuflavone.

DISCUSSION

The biflavones are widely distributed in gymnosperms but only occasionally are found in angiosperms.[2,7,8] They have also been reported from Pteriodophytes.[2,9] Though the significance of the occurrence of biflavones in the plant kingdom is not clearly understood it appears that the capacity to synthesize these dimeric compounds is a primitive metabolic character which is lost in the succeeding generations.[2]

The distribution of ellagic acid in plants has been considered to be significant.[10] It is not found in ferns, gymnosperms and monocotyledons and is confined to certain families in dicotyledons. Kaempferol and quercetin occur commonly in woody plants.[11] The occurrence of ellagic acid, kaempferol and quercetin in the Casuarinas examined now (see also Ref. 12) lends support to the view that *Casuarina* is a woody dicotyledon and that the Casuarinales could be grouped together with several other orders of dicots in the sub-class Hamamelidae.[3] In this context, it may be relevant to mention that Hamamelidaceae contain ellagic acid and

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[11] E. C. BATE-SMITH in *Chemical Plant Taxonomy* (edited by T. SWAIN), p. 138, Academic Press, London (1963).
[12] J. N. USMANI, N. U. KHAN and W. RAHMAN, *J. Indian Chem. Soc.* **47**, 179 (1970).

glycosides of kaempferol, quercetin and myricetin.[13] On the other hand, the identification of biflavones (hinokiflavone in *C. junghuhniana* and *C. suberosa* and cupressuflavone in *C. cunninghamiana* in the present work, and hinokiflavone in *C. stricta* by Sawada)[4] cannot be overlooked. But these compounds have not been found in all the Casuarinas examined; further even in those species now examined these compounds are present only in very small quantities. The present investigation lends support to the view that Casuarinaceae is a woody dicotyledon which shows characters towards the primitive side of the evoluationary scale. However, in order to get a complete picture of the taxonomic position of *Casuarina* species it is essential that various other chemical components should also be examined.

EXPERIMENTAL

TLC and dry column chromatography were carried out on silica gel supplied by the National Chemical Laboratory, Poona, India. The following solvent systems were used for TLC.

(A) ϕCH_3–Py–AcOH (10:1:1); (B) ϕCH_3–DMF–AcOH (10:1:1); (C) ϕCH_3–AcOEt–AcOH (20:4:1); (D) ϕCH_3–HCOOEt–HCOOH (5:4:1); (E) ϕH–acetone (4:1); (F) AcOH–conc. HCl–H_2O (30:1:30).

General Method of Extraction and Examination of the Extracts

The air-dried leaves were exhaustively extracted with hot ethanol in a Soxhlet. The concentrate was repeatedly boiled with light petroleum to remove chlorophyll and waxes. The residue was extracted repeatedly with warm Et_2O and the residue dissolved in EtOH.

The ether solubles were examined by TLC using the solvent systems (A), (B), (D), (E). Ethanolic $FeCl_3$ (2%), and 25% H_2SO_4 were used to locate the spots. Ethanolic neutral $Pb(Ac)_2$ spray followed by NH_4OH revealed the flavonols as pink or red spots. The hydroxy compounds appeared as dull red fluorescent spots in u.v. light.

The alcoholic extract was examined in solvent systems (A), (C), (E) and (F) and ethanolic. $FeCl_3$ and 25% H_2SO_4 were used as sprays.

Methylation Procedure

The dried Et_2O and EtOH solubles were methylated by refluxing with purified Me_2SO_4 (2 ml) and K_2CO_3 (5 g) in dry acetone solution till the Fe^{3+} reaction became negative. The sticky dark solid product was purified by chromatography over silica gel and elution with $CHCl_3$.

The methyl ethers were examined by TLC in solvent systems (A) to (E) using u.v. fluorescence and 25% H_2SO_4 spray. The methyl ethers of kaempferol and quercetin could be detected by spraying a suspension of Zn dust in acetone followed by conc. HCl when they appeared as red spots.

Dry Column Chromatography of the Ether Extract of C. equisetifolia *(India)*

The crude concentrate (200 mg) was dissolved in acetone (5 ml), silica gel (2 g) added and mixed well, dried at 80° for 2 hr and over P_2O_5. This silica gel was placed on a column (12 × 1·5 cm) of dry silica gel and solvent system (E) was allowed to percolate slowly into the column; the individual components of the extract separated as distinct yellow bands. These were washed down with the same solvent (E) and 10 ml fractions were collected. After the removal of the solvent the fractions were monitored by TLC and the individual components isolated. Kaempferol, quercetin and (+)catechin were identified by m.p., mixed m.p. and comparison with authentic samples.

Isolation of Ellagic Acid

When the alcoholic concentrate of *Casuarina equisetifolia* (Thailand), *C. junghuhniana* (Thailand), *C. cunninghamiana* (Australia) and *C. rigida* (Australia) was allowed to stand in the refrigerator for 2 weeks a yellow solid was deposited from extracts. This was filtered and boiled with more EtOH; m.p. > 360° (pyridine–MeOH). It gave a green Fe^{3+} reaction and a deep red Griessmayer test characteristic of ellagic acid.

Its identification as ellagic acid was confirmed by co-TLC with an authentic specimen and by preparation of its methyl ether (m.p., mixed m.p. and superimposable i.r.).

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