Phytochemistry, Vol. 27, No. 8, pp. 2651–2653, 1988.
Printed in Great Britain.

0031-9422/88 $3.00+0.00

FLAVONOIDS AND THE RELATIONSHIP OF *ITEA* TO THE SAXIFRAGACEAE

BRUCE A. BOHM, GAVIN CHALMERS and U. G. BHAT

Department of Botany, University of British Columbia, Vancouver, British Columbia, V6T 2B1, Canada

IN MEMORY OF TONY SWAIN, 1922 1987

Key Word Index—*Itea*; Iteaceae; Iteoideae; Saxifragaceae; Hydrangeaceae; *C*-glycoflavones; orientin; isoorientin; vitexin; isovitexin; absence of flavonols; chemotaxonomy.

(*Received* 4 *February* 1988)

Abstract—The flavonoids of *Itea virginica*, *I. parviflora* and *I. japonica* were isolated and shown to be based on orientin, isoorientin, vitexin and isovitexin. These compounds occur free in small amounts, and as X″-glucosides and X″-xylosides. The xylosides are the prominent diglycosides in all cases. A very small amount of isoorientin 7-*O*-glucoside was detected in *I. parviflora*. Two dimensional TLC of 12 additional species showed patterns similar to each other and to those of the three species that we studied in greater detail. No flavonols were detected in any of these taxa. These results allow a clear distinction to be drawn between *Itea* and the Saxifragaceae s.l. whose flavonoid profiles are based upon mono-, di- and triglycosylated flavonols. *C*-Glycoflavones have not been detected in any of the *ca* 30 genera examined to date.

INTRODUCTION

Itea is a genus of shrubs and small trees native to eastern Asia with a single species known from eastern North America. *Itea virginica*, known locally (and in cross-word puzzles) as 'Virginia willow', occurs on the Atlantic coastal plain from southern New Jersey to Florida and westward to southern Illinois, eastern Missouri, Oklahoma and Texas. The remaining species of *Itea* are found distributed from Japan southward to the Philippines, tropical India and Java and into the northwestern Himalayas [1]. Both Cronquist [2] and Takhtajan [3] give the number of species in the genus as *ca* 20. Although *Itea* has most often been put in, or at least near, the Saxifragaceae, several lines of evidence suggest that its affinities may lie elsewhere. We undertoook an investigation of the flavonoids of *Itea* with the hope that such information might offer further insights into the relationships of the genus.

RESULTS

Examination of the flavonoids isolated from *Itea japonica*, *I. parviflora* and *I. virginica* showed them to be *C*-glycoflavones based on orientin, isoorientin, vitexin and isovitexin. *Itea japonica* exhibited the four parent compounds, X″-xylosides of each as major components of the diglycoside fraction and minor amounts of vitexin and isovitexin X″-glucosides. *Itea parviflora* exhibited the four parent compounds, major amounts of the X″-xylosides of each, lesser amounts of the X″-glucosides of each and a trace of eriodictyol 7-*O*-glucoside (TLC only). Trace amounts of apigenin and luteolin may also have been present. *Itea virginica* yielded vitexin, the X″-xylosides and X″-glucosides of vitexin and isovitexin, a X″-glucoside of isoorientin and a trace of isoorientin 7-*O*-glucoside.

Two-dimensional TLC analysis of 12 additional species of *Itea* showed patterns similar to those observed for the other three species. Small spots corresponding to the chromatographic position and exhibiting colour properties of eriodictyol 7-*O*-glucoside were seen in a few of the these as well. There was considerable variation in amounts of individual compounds (as judged by spot size) among the species studied. Owing to the availability of only small leaf samples of these additional species further work on the identities of the pigments was not undertaken.

DISCUSSION

The taxonomic placement of *Itea* has been the subject of some discussion. In his very broadly based view of the Saxifragaceae, Engler [4] first placed *Itea* in the Escallonioideae but later revised his view [5] and placed it in its own subfamily, the Iteoideae. Hutchinson [6, 7] preferred to recognize the Iteaceae J. G. Agardh, but maintained its apparent relationship with the escalloniaceous group. Cronquist [2] sees *Itea* as a member of his 'broadly defined' Grossulariaceae, which he maintains within the Rosales. Takhtajan [3] places the Iteaceae (*Itea* and *Coristylis*) within Hydrangeales superorder Cornanae. In this system Takhtajan [3] places Saxifragaceae and Grossulariaceae in his Saxifragales within superorder Rosanae.

Several detailed experimental studies of the Saxifraga-

*No. 28 in the series, 'Chemotaxonomic studies in the Saxifragaceae s.l.'.

ceae s.l. have been published which include data that have bearing on the relationships of *Itea*. An excellent review of the earlier literature was presented by Spongberg [1]. Spongberg [1] pointed out differences in features of *Itea* (chambered pith, stipulate leaves, confluent styles and stigmas) that set it apart from members of the Escallonioideae. Features of the ovules and development and structure of the embryo sac are sufficiently similar to those of the Saxifragoideae that Mauritzon [8, 9] suggested that *Itea* should either be within or near that subfamily. On the other hand, a highly distinctive pollen morphology [10], places *Itea*, along with *Choristylis* (which has been included in the Escallonioideae), clearly apart from the Saxifragoideae. Studies of floral morphogenesis led Klopfer [11] to consider *Itea* as a genus of uncertain relationships. The problem of placement of *Itea* was not solved by Bensel and Palser's [12, 13] very thorough study of floral anatomy in the Saxifragaceae s.l. despite their observation that, "Among all the subfamilies studied, the morphology and vascular system of the *Itea* flower (Iteoideae) are most similar to those of the Saxifragoideae. Indeed, one essentially cannot differentiate between flowers of *Itea* and those of the Saxifragoideae, either externally or internally." After remarking on the pollen differences and the difference in chromosome number between *Itea* ($n = 11$) [14, 15] and members of the Saxifragoideae, Bensel and Palser [13] concluded that the lack of correlation between floral anatomy and other characters may indicate that *Itea* is not as closely related to the Saxifragoideae as the floral characters would suggest.

Comparatively few chemical examinations of *Itea* have been published. Plouvier [16] reported the presence of allitol in *Itea* and suggested that this might be taken as indicating a relationship with *Brexia* which accumulates dulcitol, an isomer of allitol. Floral anatomy, however, argues against this alliance [13]. Plouvier [16] wondered if *Itea* and *Brexia* might be related to some element of the Celastraceae based upon the presence in that family of dulcitol. To the best of our knowledge members of the Celastraceae accumulate flavonols, not *C*-glycoflavones [17]. Two reports of flavonoids in *Itea* have appeared. Bate-Smith [18] reported the presence of leucodelphinidin in *I. illicifolia*, while Jay [19] recorded leucodelphinidin and leucocyanidin as major and minor components, respectively, of *I. virginica*. Jay also reported [19] that kaempferol, quercetin and myricetin were absent.

Our studies of the flavonoids of Saxifragaceae s.l. [20, 21 and refs therein] and Hydrangeaceae [22] provide us with an excellent base for comparison. The principal flavonoids present in all of the genera included in these studies (*ca* 30) are mono- di- and triglycosides of kaempferol, quercetin and myricetin in most cases. A few taxa lack myricetin glycosides. In a few species lesser amounts of flavones and/or dihydroflavonols were also observed [23, 24], and the flavonols of *Chrysosplenium* are extensively methylated [25]. In no instance, however, were C-glycoflavones observed. The flavonoid data clearly serve to distinguish *Itea* from both the Saxifragaceae s.s. and the Hydrangeaceae. Too few chemical data are available to allow meaningful comparisons with the Escalloniaceae. Unfortunately, the flavonoid data do not tell us where *Itea* belongs. Serological studies of the sort reported by Grund and Jensen for the Saxifragales [26], or other macromolecular approaches, might assist in the solution of this problem.

EXPERIMENTAL

Plant material. Itea parviflora Hemsley (310 g) was obtained from Taiwan. *Itea virginica* L. (200 g) was collected in North Carolina. Vouchers for these specimens are in UBC. Material of *Itea japonica* Oliver (2.0 g) came from a specimen collected in Honshu, *M. Togasi TSM No.* 1189 (GH). The remaining material was obtained from herbarium specimens (100–500 mg): *I. amoena* Chun, Kwangtung, H. Y. Liang 69500 (A); *I. arisanensis* Hayata, Formosa, E. H. Wilson 10772 (A); *I. chinensis* Hooker & Arn., Kwangtung, T. & T. Lingnan 12615 (A); *I. formosana* Li, Taiwan, E. H. Wilson 11123 (A); *I. ilicifolia* Oliver, Sichuan, W. P. Fang 10190 (A); *I. indochinensis* Merrill, Yunnan, H. T. Tsai 60933 (A); *I. macrophylla Wall.*, Herb. late East India Co. No. 2501 (GH); *I. maesifolia* Elmer, Philippine Isl. Plts., 18023 Elmer (A); *I. mutans Royle, Ramsu, Jumu-Kashmir* Rd., R. R. Stewart 13563 (A); *I. oblonga* Hand.-Mazz., Anhwei, R. C. Ching 2894 (A); *I. oldhamii* Schneider, Ryukyu, T. Yamazaki 2279 (A); and *I. yunnanensis* Franchet, Yunnan, J. F. Rock 6448 (A). Specimens are housed at the Gray Herbarium (GH) or Arnold Arboretum herbarium (A).

Isolation, purification and identification of flavonoids. The extraction and purification procedures used were described in detail in Gornall and Bohm [24]. Structural identification was based upon standard methods of UV [27] and mass spectroscopy [28]. Hydrolyses were done using trifluoroacetic acid; sugar analysis was described in [24]. Standard samples of orientin, isoorientin, vitexin and isovitexin were available from earlier studies. TLC was performed on home-made Polyamide 6.6 plates using solvent systems described in [24].

Acknowledgements—This study was supported by operating and equipment grants from the Natural Sciences and Engineering Research Council of Canada. We should like to thank Drs Douglas Soltis for material of *I. virginica*, Kuo-Shih Hsu for *I. parviflora* and S. S. Spongberg for material of the other taxa. We also wish to acknowledge the directors of the herbaria mentioned for permission to sample their specimens.

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