FLAVONOIDS OF *ASCARINA LUCIDA*

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ABSTRACT.—Flavonoids of the primitive angiosperm *Ascarina lucida* (Chloranthaceae) were investigated. The species contains quercetin and kaempferol 3-O-mono- and diglycosides, orientin, and isoorientin, vitexin, isovitexin, as well as orientin and vitexin derivatives that could not be positively identified. The fundamental flavonoid structures of *A. lucida* are similar to those reported for members of the primitive angiosperm families Idiospermaceae, Lauraceae and Piperaceae where C-glycosylflavones and flavonols also occur.

Ascarina lucida Hook. f. (Chloranthaceae) is a shrub or small tree occurring in lowland to lower montane forests in New Zealand (1). The genus *Ascarina* comprises a total of perhaps ten species distributed throughout Malaysia, Polynesia, and New Zealand (1). Despite the status of the Chloranthaceae as a primitive angiosperm family and the taxonomic difficulties surrounding its affinities (*vide infra*), virtually no chemical data appear to be available for the family. We therefore undertook the present study of *A. lucida* in order to improve the meager flavonoid data base for the Chloranthaceae, as well as for primitive angiosperms in general.

EXPERIMENTAL

PLANT MATERIAL.—Leaves of *A. lucida* were obtained from the following sources: broadleaf forest near track into Anatoki Hut, 1500 ft., New Zealand *N.W. Nelson s. n.* 1980; Lake Kaniere, Westland, New Zealand (plants maintained in greenhouse culture at the Botany Division Garden, Department of Scientific and Industrial Research, Private Bag, Christchurch, New Zealand). Vouchers are deposited at UBC.

GENERAL TECHNIQUES.—Plant material was extracted continuously with methanol. The extracts subsequently were taken to dryness, and phenols were extracted with boiling water (filtration with Celite filter aid). Leaf tissue (90 g, dry weight) from the first of the above noted collections yielded 3.3 g of *n*-butanol soluble phenolics; 33 g of the latter collection yielded 1.0 g *n*-butanol soluble phenolics. Flavonoids were isolated and purified following the methods of Wilkins and Bohm (2). Purified flavonoids were analyzed by use of a com-

TABLE 1. Flavonoids isolated from *Ascarina lucida* (Chloranthaceae).

Kaempferol	Quercetin 3-O-xyloside
Vitexin	Quercetin 3-O-rhamnoside
Isovitexin	Kaempferol 3-O-rhamnosylglucoside (rutinoside)
Orientin	Quercetin 3-O-rhamnosylglucoside (rutinoside)
Isoorientin	Quercetin 3-O-rhamnosylglucoside
Kaempferol 3-O-glucoside	Quercetin-like derivative (7-substituted)[a]
Kaempferol 3-O-galactoside	Orientin derivative (7-substituted)[a]
Kaempferol 3-O-xyloside	Vitexin derivative[a]
Kaempferol 3-O-rhamnoside	
Quercetin 3-O-glucoside	
Quercetin 3-O-galactoside	

[a]Compound that could not be characterized completely.

bination of uv spectral and H nmr data, hydrolysis with trifluoroacetic acid (TFA), and tlc against known compounds (when available) with the solvent systems of Wilkins and Bohm (2). Uv spectrophotometry and H nmr methods[1] followed those described by Mabry *et al.* (3). Sugar determinations were conducted according to the methods of Wilkins and Bohm (2). Results are given in table 1.

[1]Uv was done on Unicam SP8–100; nmr was done on Varian EM–390.

DISCUSSION

The two population samples of *A. lucida* examined possess identical flavonoid profiles. Nineteen compounds were isolated and either wholly or partially identified (table 1). Three of these compounds could not be characterized completely and were minor components of the samples we analyzed. Among the flavonols are quercetin 3-0-glycosides and trace amounts of kaempferol 3-0-glycosides. The monosides are kaempferol and quercetin 3-0-glucosides, galactosides, xylosides, and rhamnosides. Three diglycosides were isolated and identified. Quercetin and kaempferol 3-0-rhamnosylglucosides (rutinosides) are present, as well as very large quantities of a second quercetin 3-0-diglycoside that also yielded rhamnose and glucose on complete acid hydrolysis. Partial hydrolysis of this compound yielded quercetin 3-0-glucoside and rhamnose, establishing the structure as a quercetin 3-0-rhamnosylglucoside. The position of attachment of the rhamnose was not determined. The corresponding kaempferol diglycoside was not observed.

Two flavonol aglycones were isolated: kaempferol and a quercetin-like derivative that could not be positively identified. Analysis of uv spectral data for the quercetin-like derivative indicates that the 7 position is substituted, but hydrolysis with TFA, as well as concentrated HCl, did not remove the substituted moiety. Proton nmr data confirm that this compound is a quercetin derivative and also indicate that the substituted moiety is not organic. The identity of this substituent could not be ascertained, however.

Ascarina lucida also contains several C-glycosylflavones. Large quantities of orientin and isoorientin were observed, as well as trace amounts of vitexin and isovitexin. Trace amounts of two additional C-glycosylflavones were detected, but these could not be positively identified. One of these compounds did not migrate well in either of our two standard solvent systems (2). The migration of this compound on polyamide could be increased, however, by acidification of the solvents. This behavior is typical of flavonoids bearing a glucuronic acid or other charged moiety. Acid hydrolysis failed, however, to yield any detectable sugars but did give orientin as the aglycone. Uv spectral data for the parental compound indicate a substitution at the 7 position. The compound was not present in sufficient quantity to permit H nmr analysis. These data tentatively establish the compound as a 7-substituted orientin derivative.

The second of the unknown C-glycosylflavones isolated from *Ascarina* gave H nmr signals identical to those observed for vitexin. However, the compound differed from vitexin, as well as isovitexin, in color reaction to our standard spray reagent (0.1% β-aminoethyl diphenylborinate). The unknown compound initially turns a dull brown and slowly changes to a dull green-brown in response to this spray, whereas vitexin and isovitexin yield a green color. This color reaction suggests a possible B ring substitution. Furthermore, uv spectral data clearly indicate that the 7 position is unsubstituted. The compound is unaltered following treatment with TFA or HCl. Proton nmr data indicate that the substituted moiety is not organic. The available data indicate a vitexin derivative with an inorganic substitution, perhaps at the 4' position.

This is the first report of flavonoid data for *Ascarina*. Furthermore, there have been very few chemical investigations of the Chloranthaceae in general. Bate-Smith (4) indicated that *Chloranthus officinalis* had been investigated chemically, but no flavonoid constitutents were reported. Gornall *et al.* (5) did not report flavonoid data for the Chloranthaceae in their recent review of the distribution of flavonoids in the angiosperms.

The majority of families of the Magnoliiflorae for which flavonoid data are available have profiles based predominantly on the flavonols quercetin and kaempferol (5, 6). *Ascarina lucida* possesses kaempferol and quercetin glycosides, as well as C-glycosylflavones. The fundamental flavonoid structures of this taxon,

therefore, seem most similar to those reported for taxa of the Idiospermaceae, Lauraceae, and Piperaceae (5, 6) where C-glycosylflavones and flavonols also occur.

The Chloranthaceae has attracted interest because of its primitive wood anatomy (7–10). In addition, as noted above, the taxonomic placement of this primitive angiosperm family is problematic. Cronquist (11) and Hutchinson (12) referred the Chloranthaceae to the Piperales, along with the Piperaceae and Saururaceae. Vijayaraghavan (13), Swamy (9), Dahlgren (14), and Takhtajan (15), in contrast, maintained that the summation of morphological and anatomical data fails to support any close relationship between the Chloranthaceae and Saururaceae and Piperaceae. Although the flavonoid information presented here for *A. lucida* hardly provides a representative flavonoid data base for the Chloranthaceae, it is interesting to note that both the Chloranthaceae and Piperaceae contain C-glycosylflavones (the Saururaceae contains quercetin and kaempferol glycosides). This may not necessarily indicate, however, a close affinity between the Chloranthaceae and Piperaceae. Young (pers. comm.) cautions that C-glycosylflavones may have evolved independently in several different families of primitive angiosperms, and their pattern of distribution in the Magnoliiflorae and Nymphaeiflorae (*sensu* Dahlgren, 14) may, therefore, be indicative of parallel evolution.

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