Phytochemistry, 1970, Vol. 9, pp. 1855 to 1858. Pergamon Press. Printed in England.

PHENOLIC CONSTITUENTS OF THE OIL FLAX (*LINUM USITATISSIMUM*)

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(*Received* 25 *November* 1969)

Abstract—The major phenolic constituents in the cotyledons and young shoots of *Linum usitatissimum* L. consist of (a) nine glycosides and esters of *p*-coumaric, caffeic, ferulic and sinapic acids and (b) six *C*-glycosides of *O*-glycoflavones. Substituted benzoic acid derivatives and flavonol glycosides were absent in both parts of the plant.

INTRODUCTION

APART FROM the reports on the isolation of linocinnamarin[1,2] (glucosyl-*p*-coumaric methylester) and linocaffein[3] (glucosyl caffeic methylester) from flaxseed hulls and the identification of chlorogenic acid[4] in young shoots, our knowledge of the phenolic constituents of *Linum* or its family, Linaceae, is deficient.[5-7] Only recently, a 5,7-dihydroxy-3′,4′-dimethoxy flavone-7-rhamnoside[8] has been isolated and identified from *L. maritimum*. This prompted us to investigate the cinnamyl and flavonoid constituents of flax cotyledons and young shoots in view of understanding the role of plant phenolics in disease resistance or susceptibility, as was previously reported with chlorogenic acid in flax rust.[4]

RESULTS AND DISCUSSION

Cinnamic Acid Derivatives

Nine purified fractions were isolated, from cotyledons or young shoots, by a combination of chromatographic (column, paper & TLC) and spectrophotometric techniques. Identification of these fractions was based on spectral shifts with NaOH and with NaOAc,[9] alkaline and acid hydrolysis, fluorescence in u.v. light, and comparison with authentic samples. They were identified as *p*-coumaryl quinic acid, *p*-coumaryl glucose, 3-*O*-caffeoyl quinic (chlorogenic), glucosyl caffeic acid, caffeoyl glucose, glucosyl ferulic acid, feruloyl glucose and a glycoside and an ester of sinapic acid whose non-phenolic moieties were not identified. None of the substituted cinnamic acids was found free in either tissue. Apart from slight quantitative differences between cotyledons and young shoots, caffeic acid derivatives, especially chlorogenic acid, were found to be the major cinnamyl compounds in both tissues.

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The absence of substituted benzoic acids, especially *p*-hydroxybenzoic, protocatechuic, vanillic and syringic acids, in both cotyledons and young shoots, is remarkable. Although the biosynthesis of C_6–C_1 acids has been shown to proceed through their C_6–C_3 isomers, by a removal of two-carbon fragment,[10] it seems that this mechanism is absent in flax tissues.

C-Glycoflavones

Table 1 lists the u.v. absorption spectra and chromatographic characteristics of the six flavone glycosides (*A–F*) isolated from flax tissues. The u.v. spectral data for compounds *B* and *C* are in agreement with those known for apigenin, whereas the corresponding maxima for compounds *A*, *D*, *E* and *F* coincide with those of luteolin.[11] This was confirmed by the bathochromic shifts produced with NaOAc and boric acid for the u.v. maxima of the latter compounds and their colour with Benedict's reagent in u.v. light. It appears that all six flavones have their 7-hydroxyl glycosylated, since none of their short wavelength peaks gave a shift with NaOAc. The fact that the spectrum of compound *C* did not give a shift with $AlCl_3$ suggests that its 5-hydroxyl is substituted, being free in the other compounds (Table 1).

Acid hydrolysis of each individual flavone glycoside released sugar residues within 1–2 hr. The sugars were identified as rhamnose in compounds *A* and *D*, both rhamnose and glucose in *B* and *C*, and two unidentified sugar residues in compounds *E* and *F*. The chromatographic behaviour of the aglycones were suggestive of C-glycoflavone nature.[12] Their u.v. absorption data showed that no *O*-glycosidic links were present.

Prolonged acid hydrolysis of each compound gave no extra sugar residues, but several spots of interconvertible intermediates which may be considered as acid-equilibrium isomers characteristic of 6-*C*- and 8-*C*-glycoflavones.[13] This was more evident for the aglycones of compounds *D*, *E* and *F* and less so for those of compounds *A*, *B* and *C*.

Comparison of the hydrolysis products with authentic samples of *C*-glycoflavones, showed close similarity of their u.v. absorption values and chromatographic characteristics. The aglycone from compound *A* was similar to lucenin-1; those from *B* and *C* to vicenin; that from *D* to orientin and those from both *E* and *F* to *iso*-orientin (Fig. 1). There were slight differences in R_f values between the hydrolysis products of the flax flavones and the authentic samples which may be due to the nature of C-linked sugar(s) attached to 6-, 8- or both positions. It appears that the flax compounds are *O*-glycosides of *C*-glycoflavones. Their tentative identification, based on the above evidence, is given in Table 1.

These previously unreported results indicate a rich glycoflavone chemistry in flax which may now be added to the few angiosperm families[14] containing flavone-*C*-glycosides, some of which are known to contain *O*-glycosides of *C*-glycoflavones.[15,16] It appears also that the phenolic pattern in *Linum* is very characteristic in view of the variety of bound forms of substituted cinnamic acids, absence of hydroxybenzoic acids, predominance of *C*-glycoflavones and absence of flavones and flavonols; both substituted benzoic acids and flavonols are almost ubiquitous. This characteristic pattern may contribute significantly to chemotaxonomy of the family if more genera or species are examined.

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TABLE 1. U.V. ABSORPTION SPECTRA AND CHROMATOGRAPHIC CHARACTERISTICS OF FLAX FLAVONES

Reaction mixture	Compounds	λ_{max} (mμ) *A*	*B*	*C*	*D*	*E*	*F*
50% MeOH		252, 265, 348	268, 340	269, 335	256, 268, 345	253, 268, 348	257, 268, 348
+ NaOH		269, 410	278, 328, 402	282 330, 400	273, 328, 410	272, 410	272, 403
+ NaOAc		261, 326	268, 344	271, 341	266, 348	262, 360	262, 368
+ NaOAc + H_3BO_3		257, 368	268, 348	270, 341	263, 368	259, 368	260, 370
+ $AlCl_3$		269, 300,* 405	273, 296,* 337, 382	270, 300,* 335	269, 386	270, 390	273, 385
R_f values (×100)†,‡							
t-BAW		12	15	25	30	40	45
n-BAW		18	30	33	38	46	50
15% HOAc		70	50	60	40	55	60
3% KCL		18	20	27	20	15	18
Sugar residues§		Rhamnose	Rhamnose, glucose	Rhamnose, glucose	Rhamnose	Unidentified sugar	Unidentified sugar
Tentative identification		Lucenin-7-rhamnoside	Vicenin-7-rhamnoglucoside	Vicenin-5-glucoside-7-rhamnoside	Orientin-7-rhamnoside	*Iso*-orientin-7-glycoside	*Iso*-orientin-7-glycoside

* Inflection.
† All were brownish in u.v. light changing to yellow or orange-yellow with ammonia.
‡ See text for composition of solvent systems.
§ After hydrolysis with 1 N HCl in 50% MeOH.

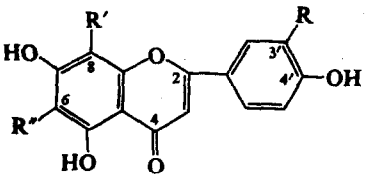

FIG. 1. *C*-GLYCOFLAVONES ISOLATED FROM FLAX TISSUES.

R	R′	R″	
H	Glucose	Glucose	Vicenin
OH	Glucose	H	Orientin
OH	H	Glucose	*Iso*-orientin
OH	Glucose	Glucose	Lucenin

EXPERIMENTAL

Plant Material

Seeds of *Linum usitatissimum* L. (var. Bison, Bombay or Koto) were grown in large flats filled with soil under greenhouse conditions. Preliminary investigations indicated slight quantitative but no qualitative difference in the phenolic pattern of 8-day-old cotyledons or 4-week-old shoots of the three varieties.

Isolation of Cinnamyl Compounds and Flavone Glycosides

Fresh plant material was homogenized with boiling 95% then 60% EtOH in a Waring blender. The filtered extracts were evaporated *in vacuo* to an aqueous residue which was filtered and defatted with light petroleum. Due to the complexity of phenolic pattern and presence of large amounts of extraneous matter, the aqueous extract was passed through a moderately acidic resin column (IRC-50, H^+) and the phenolic compounds fractionally eluted with 20% aqueous *iso*-PrOH followed by increasing alcohol concentrations. Successive chromatographic separation and purification were carried out on paper and cellulose TLC plates using *n*-BuOH–HOAc–H_2O (6:1:2), *n*-BuOH–pyridine–H_2O (14:3:2), methyl-*iso*-butyl ketone–HCOOH–H_2O (14:3:2), benzene–HOAc–H_2O (125:72:3) and 2% aq. HOAc for glycosides and esters of cinnamic acid derivatives; *n*-BuOH–HOAc–H_2O (4:1:2·2), *tert*-BuOH–HOAc–H_2O (3:1:1), 15% aq. HOAc and 3% KCl for flavone glycosides.

Hydrolytic Conditions

Cinnamyl compounds were hydrolyzed with alkali (1 *N* NaOH for 2 hr, under N_2 at room temp.) or acid (1 *N* HCl for 30 min at 100°). Flavone glycosides were hydrolyzed with 1 *N* HCl in 50% MeOH under reflux for various time periods and the course of reaction was followed by chromatographing ethylacetate extracts of the acid hydrolysates.

Acknowledgements—We wish to thank the National Research Council of Canada for financial support of this project. We are also grateful to Dr. B. A. Bohm for authentic samples of cinnamyl esters and glucosides, Drs. Jerry McClure and the late M. Seikel for reference compounds of *C*-glycoflavones. The excellent assistance of Miss Barbara Naegele, Mr. Leroy Schrubb and Mr. Shiu-kuen Ho is greatly appreciated.