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THE NATURE OF TANNIN IN RAPESEED (*BRASSICA CAMPESTRIS*)*

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Abstract-Polyphenols were present in the seed coats or testas of rapeseed. Although some oligomeric phenol was found in aqueous extracts of defatted seeds, most of the polymeric material was apparently strongly bound to other structures or constituents in the seed coat. Digestion of the seed coats with hot acidic butanol liberated alcohol-soluble pigments which were identified as anthocyanidins. It may be concluded from these results that a condensed tannin occurs in rapeseed.

INTRODUCTION

CONDENSED tannins are for the most part flavonoid polymers which, upon treatment with hot acid, yield phlobaphenes or tannin reds together with some anthocyanidin. The formation of both phlobaphene and anthocyanidin suggest that the flavonoid units are flavan-3-ols or flavan-3,4 diols[1-3] similar to catechins and leucoanthocyanidins respectively.

Very little is known regarding the polyphenols in rapeseed. In 1958, Bate-Smith and Ribéreau-Gayon[4] observed a red solution when the seeds of *Brassica napus* and *B. nigra* were treated with hot dilute acid. Other phenols such as sinapine[5] and flavonol glycosides[6,7] have been reported as constituents of the seeds.

Since tannins may have an adverse effect on the nutritive value of rapeseed meal or isolated protein, it is important to study the nature of the tannin in this material. This communication reports evidence for a condensed tannin in rapeseed.

RESULTS AND DISCUSSION

Extracts with pronounced red colorations were obtained when the seed coats of rapeseed (*Brassica campestris cv.* Echo) were digested with the butanol-hydrochloric acid mixture of Swain and Hillis.[8] The red pigment after chromatography on paper usually separated into three red bands (unknowns I-III-see Table 1). Unknowns I and III gave color reactions

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[1] E. HASLAM in *Chemistry of Vegetable Tannins*, p. 67, Academic Press, London (1966).

[2] E. C. BATE-SMITH and T. SWAIN, *Chem. & Ind.* 377 (1953).

[3] JUDITH L. GOLDSTEIN and T. SWAIN, *Phytochem.* 2, 371 (1963).

[4] E. C. BATE-SMITH and P. RIBÉREAU-GAYON. *Qual. Plant. Mater. Vegetables* 5, 189 (1958).

[5] D. R. CLANDININ, *Poultry Sci.* 40, 484 (1961).

[6] L. HORHAMMER, H. WAGNER, H. G. ARNDT, H. KRAEMAR and L. FARKAS, *Tetrahedron Letters* 567 (1966).

[7] M. T. FRANCOIS and L. CHAIZ. *Chem. Abs.* 55, 3744 (1961).

[8] T. SWAIN and W. E. HILLIS, *J. Sci. Food Agri.* 10, 63 (1959).

TABLE 1. PIGMENTS DERIVED FROM RAPESEED CONDENSED TANNINS

	R_f*				Color†		
	BAW	Forestal	Vis Max. nm	Co-Chrom with unknowns	AM	LA	Vis
Unknown I	0·45	0.49	543		Violet	Blue	Pink
Unknown II	0·80	0.65	535		no change	no change	Pink
unknown III	0·90	0.80	—		Violet	Blue	Pink
Cyanidin	0·45	0.50	544	Unk. I	Violet	Blue	Pink
Pelargonidin	0·80	0·63	532	Unk. II	no change	no change	Red
Malvidin	0.45	0.56	550		—	—	Purple

* BAW = butanol-acetic acid-water (4: 1: 1).
Forestal = acetic acid–HCl–water (150 : 15 : 50).
† AM-ammonium molybdate spray, LA-lead acetate spray.

with the molybdate and lead acetate sprays, whereas unknown II did not. This result indicated that unknowns I and III contained vicinal hydroxy groups in the 'B' ring. By comparison of spectra, R_f values in BAW and Forestal and finally by co-chromatography with authentic cyanidin, unknown I was tentatively identified as this anthocyanidin. Unknown II was identified as pelargonidin using the same tests. Unknown III, a band of high R_f value in both solvent systems, increased in intensity when the butanol-HCI solutions stood for some time in the refrigerator. This substance was likely the butyl derivative of cyanidin, the same artifact produced from arecanut by Mathew *et al.*[9] These workers also identified cyanidin and pelargonidin as the anthocyanidins produced from the proanthocyanidins of arecanut.

Rapeseed kernels produced pigmented solutions after treatment with the acidic alcohol but no evidence of anthocyanidin could be obtained after paper chromatography of the pigments. The proanthocyanidin was in the seed coat only, where it was strongly bound to other constituents. Some of the material could be solubilized if the coats were ground up in distilled water containing polyvinylpyrrolidone (PVP), which is a good hydrogen bonding agent. When the defatted seeds or seed coats were extracted with methanol to remove low molecular weight phenols, and then extracted with distilled water in a soxhlet extractor, this extract contained some oligomeric proanthocyanidin which was shown by paper chromatography to be leucocyanidin only. However, the bulk of proanthocyanidin, as such, could not be isolated from the seed coats, using ordinary solvents. The anthocyanidins formed by acid treatment of the proanthocyanidins were soluble and therefore separable on paper chromatograms.

It would appear then that the rapeseed coats contain a condensed tannin similar to the complex proanthocyanidins of arecanut[9] and of apple,[10] but different from the proanthocyanidin found in grain sorghum.[11] Paper chromatography of alcoholic extracts of seeds both after acid and after alkaline hydrolysis revealed the presence of several unknown phenolic acids and therefore the possibility exists that hydrolyzable tannins also may occur in rapeseed.

[9] A. G. MATHEW, H. A. B. PARPIA and V. S. GOVINDARAJAN, *Phytochem.* **8**, 1543 *(1969)*.
[10] S. ITO and M. A. JOSLYN, *J. Food Sci.* **30**, 44 *(1965)*.
[11] E. C. BATE-SMITH and V. RASPER, *J. Food Sci.* *34*, 203 (1969).

EXPERIMENTAL

Material. Seed coats and kernels of ***Brassica campestris cv.*** Echo were separated from each other without the use of solvents of any kind, by an entirely dry mechanical process developed by Khanzada.[12]

Method. The coats and/or kernels, after soxhlet extraction with hexane to remove the oil, were digested by heating with a 20: 1 *n*-BuOH–conc. HCl mixture for 10 min at 100”. The pigmented solutions obtained, together, and with standard anthocyanidins, were either streaked or spotted on 3 MM Whatman filter papers and developed in Forestal and in BAW solvents. For spectrophotometry (Bausch & Lomb 502 recording spcctrophotometer), the pigmented substances were elutcd with methanolic-HCl[13] and their spectra compared with those of the standard pigments. Sprays used to identify O-dihydroxy compounds were 4% ammonium molybdate in water and 1% lead acetate in 75% ethanol.[14,15]

Aqueous Extracts of Seed Coats

After removal of low mol. wt. phenols by extraction with methanol, the residues were extracted with water in a soxhlet extractor.

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[12] G. Khanzada, personal communication (unpublished results).

[13] J. B. Harborne, in *Comparative Biochemistry of the Flavonoids,* p. 9, Academic Press, London (1967).

[14] D. B. Mullick, *Phytochem.* **8,** 2003 *(1969).*

[15] T. Fuleki and F. J. Francis, *Phytochem.* *6,* 1161 (1967).