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SHORT COMMUNICATION

A GLUCOSAMINE DERIVATIVE OF CAFFEIC ACID IN *NICOTIANA* NODAL TUMORS

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Abstract—A polyphenol from *Nicotiana suaveolens* × *langsdorffii* nodal tumors has been isolated and provisionally identified as *N*-caffeoylglucosamine.

INTRODUCTION

DURING a study of the anthocyanin, delphinidin 3-rhamnosylglucoside, in the nodal tumors of *Nicotiana suaveolens* × *langsdorffii*,[1] significant quantities of a polyphenol (compound A) were noted in addition to chlorogenic acid and scopolin, the other major polyphenols present. These compounds were separated by paper chromatography (Table 1). Compound A gave positive results with the Arnow reagent for the *O*-dihydroxyphenol moiety.[2] Although A was not cleaved readily by base as occurs with the caffeic acid depsides, acid hydrolysis yielded caffeic acid and glucosamine (2-amino-2-deoxyglucose) in a 1:1 ratio. The hydroxyl groups of the caffeoyl moiety were not substituted since methylation with diazomethane and subsequent acid hydrolysis yielded 3,4-dimethoxycinnamic acid.[3] The amino group of the glucosamine was not detected by ninhydrin until after the cleavage of the original compound, signifying that an amide linkage of the aminosugar to the caffeic acid moiety exists.

TABLE 1. R_f VALUES (×100) OF THE MAJOR *Nicotiana suaveolens* × *langsdorffii* TUMOR POLYPHENOLS

	BAW	2% HOAc	H_2O	BEW	BPW	KFW	PNW	B (7·0)
Compound A	50	61° 36′	05	60	50	20	01	34
Chlorogenic acid	65	65° 50′	80	35	20	52	02	60
Scopolin	47	70	70	55	47	14	48	70

Solvents: BAW (*n*-BuOH–HOAc–H_2O, 6:1:2); BEW (*n*-BuOH–EtOH–H_2O, 4:1:2·2); BPW (*n*-BuOH–pyridine–H_2O, 14:3:3); KFW (methyl isobutyl ketone–HCO_2H–H_2O, 14:3:2); PNW (*iso*-PrOH–NH_4OH–H_2O, 10:1:1); B (7·0) (phosphate buffer, 0·1 M, pH 7·0). Ascending chromatography on Whatman No. 1 paper.

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The u.v. spectrum of compound A indicated a *cis–trans* mixture of the 1-caffeoyl derivative[4] which was confirmed by chromatographic studies using 2% HOAc showing the *cis* isomer to be the predominant form.[5] Although the intensity of the u.v. fluorescence of the upper band demonstrated that greater than 50 per cent of the isomeric mixture was in the *cis* form, attempts at elution of the two isomers for quantitative measurements were unsuccessful due to the interconversion of the two forms as in the case of caffeic acid.[4] The lack of a strong i.r. absorption at 814 cm^{-1} would confirm the predominance of the *cis* isomer.[6] Under the experimental conditions used, chlorogenic acid was found in the *trans* form with only a trace of *cis* form occurring.

The evidence presented suggests that the structure of compound A is *N*-caffeoylglucosamine. Very small quantities of glucosamine esters of caffeic, *p*-coumaric and ferulic acids have been reported in tissue cultures of *Nicotiana tabacum* var. "Samsun".[7] The spectral and chromatographic data for the glucosamine ester of caffeic acid differ from those of the compound described here. Free glucosamine has been reported in a number of *Nicotiana* species and hybrids,[8] but no reports have been made on the presence of phenolic acid-glucosamine conjugates in the plant.

Quantitative estimations of the various polyphenols were made in newly formed tumors and the results compared to those obtained from older ones: chlorogenic acid, 1·5 per cent, 2·0 per cent; scopolin, 0·07 per cent, 0·5 per cent; caffeoyl glucosamine, 0·08 per cent, 0·24 per cent of the dry weight. An increase in polyphenol content was found as the age of the tumor increased, which is in agreement with a previous report on scopolin in similar tissues.[9]

EXPERIMENTAL

Extraction and Isolation Procedure

Lyophilized nodal tumors of *Nicotiana suaveolens* × *langsdorffii*, grown in the greenhouse, were extracted repeatedly with boiling absolute MeOH. After removal of the lipids and concentration, the extract was chromatographed on Whatman No. 1 or 3 MM paper in H_2O followed by BEW. Compound A was located by u.v. light (blue, turquoise/NH_3) and eluted with MeOH.

Cleavage

Small portions of compound A were heated at 100° in 1 N HCl for 3–4 hr. The aglycone was extracted with ether and chromatographed on paper using benzene–HOAc–H_2O (125:72:3), 2% HOAc, $CHCl_3$–HOAc–H_2O (2:1:1), *n*-BuOH–2 N NH_3 (1:1), and the compound co-chromatographed with caffeic acid. The aqueous portion of the hydrolysate was concentrated and excess acid removed. Glucosamine (2-amino-2-deoxyglucose) was identified in this portion by paper chromatography using BAW (4:1:1), phenol-H_2O (4:1), MeOH–pyridine–H_2O (20:5:1). The sugar-aglycone ratio of the purified glycoside was determined by acid cleavage of the compound, spectrophotometric measurement of the caffeic acid moiety after ether extraction and quantitative paper chromatographic determination of the glucosamine.

Spectra

Ultra violet spectra. Compound A: λ_{max} (MeOH) 285, 315 nm; λ_{max} (NaOH) 360 nm; λ_{max} (NaOAc) 285, 315 nm; chlorogenic acid: λ_{max} (MeOH) 296, 327 nm; λ_{max} (NaOH) 360 nm; λ_{max} (NaOAc) 296, 327 nm. Infra red spectra were determined as nujol mulls. Compound A: ν_{max} 1670 cm^{-1}; chlorogenic acid: ν_{max} 1695 cm^{-1}, ν_{max} 814 cm^{-1}; 3,4-dimethoxycinnamide: ν_{max} 1670 cm^{-1}, 816 cm^{-1}.

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