Cyanidin 3-monoglucoside, acylated. Acid hydrolysis gave cyanidin and glucose. Spectral analysis of the glycoside indicated that glucose was at the 3-position, and sequential acid hydrolysis indicated that it was a 3-monoglucoside. This pigment had a chromatographic mobility identical to authentic cyanidin 3-glucoside (four solvents) only after mild alkaline hydrolysis, indicating that it was acylated. The acylated and non-acylated glucoside had identical absorption spectra, indicating that the acyl group was not a cinnamic acid derivative. The nature of the acyl group was not determined.

Kaempferol and quercetin 7-monoglucosides. Acid hydrolysis gave the corresponding aglycones and glucose. The color reactions of these glycosides on paper chromatograms[10] and UV analysis indicated they were 7-glucosides, and R_f data indicated they were 7-monoglucosides.

Kaempferol and quercetin 3-glucosides. These were identified in a manner similar to the 7-monoglucosides, except that satisfactory R_fs were not obtained. Therefore, the number of glucose units at the 3-position cannot be stated.

Kaempferol and quercetin 7-diglycosides. Color reactions of these glycosides on paper chromatograms, and UV spectral data indicated these to be 7-diglycosides of kaempferol and quercetin. Their positions on two-dimensional paper chromatograms relative to the 7-monoglucoside derivatives mentioned above indicated that these are probably 7-diglycosides.

Apigenin and luteolin 7-glycosides. UV spectral data indicated these to be 7-glycosides of apigenin and luteolin.

Herbacetin 7-monoglucoside (herbacitrin). The color reactions on paper chromatograms, and UV spectral data confirmed the existence of this pigment in *M. luteus.*

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Key Word Index—Mimulus luteus; Scrophulariaceae; flower pigments; anthocyanins; flavonol glycosides; flavones.

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SOLANACEAE

CAFFEOYLPUTRESCINE IN *NICOTIANA TABACUM*

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Extensive studies have been made of the polyphenols of the leaves of tobacco resulting in the identification of many hydroxycinnamic acid derivatives.[1,2] However, there has been little work done on this class of compounds in the floral parts of the plant. Besides chloro-

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[2] A. ZANE, W. STECK and S. H. WENDER, *Tob. Sci.* 7, 21 (1965).

genic acid, Watanabe and Wender found a caffeoyl derivative in the pistil of *Nicotiana* flowers which was not characterized further.[3]

Several polyphenols were separated from chlorogenic acid by paper chromatography of methanolic extracts of the apical portions of four different varieties of *Nicotiana*. One compound was isolated in sufficient quantity by polyvinylpyrrolidone chromatography to allow further characterization. Acid hydrolysis of the compound yielded caffeic acid and putrescine (1,4-butanediamine) and the NMR spectrum indicated a 1:1 combination of the two moieties. The UV spectrum indicated a caffeoyl moiety with a significant portion of the molecules in the *cis* configuration.[4] Caffeoylputrescine 1-(3,4-dihydroxycinnamoylamino)-4-aminobutane was synthesized and its identity to the isolated compound was substantiated by IR, NMR and co-chromatography.

We know of no report of the occurrence of caffeoylputrescine in specific organs of the intact *Nicotiana* plant. The amide is found only in the apex of the vegetative plant and later the flowers, and is absent from fully expanded leaves of the plant under normal growing conditions. Recently the compound has been reported in callus tissue cultures of *Nicotiana tabacum* where putrescine or ornithine had been added to the culture medium.[5] An examination of a callus tissue culture of *Nicotiana tabacum* cv. Catterton grown without the addition of these compounds gave no indication of the presence of caffeoylputrescine in detectable quantities.

EXPERIMENTAL

Extraction and purification. Fresh apical tissue (100 g) was extracted in boiling 95% EtOH. After concentration under vacuum and extraction with pentane, the residue was applied to a column of polyvinylpyrrolidone–celite (1:1). Water was used as the solvent for the elution of caffeoylputrescine and a second chromatography of the combined fractions containing the amide was sufficient to give adequately pure compound (13 mg) for further analysis.

Identification. The R_fs × 100 for caffeoylputrescine on Whatman No. 1 paper (ascending) were: 51 (*n*-BuOH–HOAc–H_2O, 6:1:2); 44 (*n*-BuOH–EtOH–H_2O, 4:1:2·2); 74, 61 (HOAc–H_2O, 1:49); 41 (*n*-BuOH-Py-H_2O, 14:3:3); 05 (H_2O pH 5·6). The corresponding R_fs for chlorogenic acid were: 63; 33; 65, 50; 22; 80.

The UV max were at 290, 315 nm (MeOH). The IR spectrum (KBr) indicated amide C=O, ν 1645 cm^{-1} and NH_3^+, 1613 cm^{-1} (amine salt). The NMR spectrum had peaks (DMSO-d_6): δ 1·6 (—CH_2—); 2·8, 3·2 (N—CH_2—C); 6·4, 7·2 (vinyl CH, doublets); 6·8, 7·0 (aromatic CH); 8·0 (NH). The compound was hydrolyzed in 3 N H_2SO_4 at 90° for 3 hr. After removal of the excess acid, the ether-soluble fraction contained caffeic acid (R_f and UV) and the aqueous fraction contained putrescine sulfate (R_f). Caffeoylputrescine was synthesized by the addition of excess putrescine to 3,4-diacetylcaffeoyl chloride in $CHCl_3$.

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Key Word Index—Nicotiana tabacum; Solanaceae; caffeoylputrescine.