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A New Aromatic Amide, Caffeoylputrescine from Callus Tissue Culture of *Nicotiana tabacum*

Sir:

During tracer studies of the nicotine bio-synthesis in callus tissue cultures of *Nicotiana tabacum* we observed that considerable amounts of the radioactivity from administered ^{14}C -labelled putrescine or ornithine were incorporated into some ninhydrin-positive basic compounds. These compounds gave highly fluorescent spots under ultra-violet light on paper chromatograms.

In connection with our studies on the biosynthesis of nicotine, it seems to be of some importance and interest to identify such compounds. One of these was isolated and identified as 1-(3,4-dihydroxycinnamoylamino)-4-aminobutane (caffeoylputrescine) from callus tissue cultures of *N. tabacum*.

Five hundred g of the well washed tissue was repeatedly extracted with 75% aqueous ethanol. The extracts were passed through a column of Amberlite CG-50 resin in the hydrogen form. The column was washed with water and eluted with 4N acetic acid. The eluate was concentrated to dryness under reduced pressure at 40°C. The residue was dissolved in a small volume of water and subjected to cellulose column chromatography with *n*-butanol-acetic acid-water (65:10:25) as a solvent. The fractions containing the compound were dried and the residue was dissolved in a small volume of water. Repeated recrystallization from water afforded 110 mg of fine needles of the compound. The

hydrochloride of the compound decomposed at 245~250°C with changing a form at about 96°C. This change seems to be resulted from a loss of water of crystallization. Elementary analysis of the compound gave, C, 48.27; H, 7.00; N, 8.78 per cent. The calculated values for $\text{C}_{13}\text{H}_{18}\text{N}_2\text{O}_3 \cdot \text{HCl} \cdot 2\text{H}_2\text{O}$ were C, 48.28; H, 7.18; N, 8.68 per cent.

The compound gave violet color with ninhydrin and high fluorescence. The Le Rosen test for aromatic compounds,¹⁾ Pauly's reaction²⁾ and FeCl_3 reaction were all positive. These results indicate that the presence of the amino group and phenolic moiety.

The UV absorption peaks of the compound in water are at 219, 235, 295 and 320 m μ . The spectrum is closely similar to that of caffeic acid.

Hydrolysis of the compound by refluxing in 2N NaOH for 4 hr under a nitrogen atmosphere gave caffeic acid and putrescine, which were identified by paper chromatography and infrared absorption spectroscopy.

The IR spectrum of the compound in KBr disk shows the usual feature for mono-substituted amides. The amide band A (carbonyl) is present at 1598 cm^{-1} and the amide band B at 1536 cm^{-1} . The absorption at 1655 cm^{-1} seems to be ascribed to the C=C stretching vibration in conjugation with the carbonyl. The absorption at 980 cm^{-1} is considered to be due to the unsaturated C-H deformation vibration at the *trans*-double bond.³⁾

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These results indicate that a possible structure of the compound to be 1-(3,4-dihydroxycinnamoylamino)-4-aminobutane. This is further confirmed by its NMR spectrum. The NMR spectrum of the compound in deuterium oxide shows the presence of three aromatic protons (δ 6.88, singlet and δ 6.76, triplet formed by partial overlapping of two doublets) and two protons (δ 7.10, doublet and δ 6.11, doublet, J value 15.0 c/s) corresponding to vinyl protons. The signal of the vinyl proton which is adjacent to the carbonyl group shows a chemical shift at the lower field than the other proton because of the effects of the conjugated carbonyl group and the aromatic ring current. The coupling constant between the vinyl protons of 15.0 c/s confirmed the *trans* arrangement about the double bond.⁴⁾ The spectrum also reveals four protons (δ 3.05) attributable to two methylene groups adjacent to amino groups and four additional protons (δ 1.61) corresponding to the other methylene groups of 1,4-diaminobutane.

Final confirmation of the structure was obtained by comparison with synthetic 1-(3,4-dihydroxycinnamoylamino)-4-aminobutane. To a solution of excess putrescine in chloroform was added diacetylcaffeic acid chloride. Acetylcaffeoylputrescine thus obtained was separated by cellulose column chromatography with a solvent of *n*-butanol-acetic acid-water (65:10:25). The acetyl derivative was treated with 2N NaOH and acidified with conc. HCl giving caffeoylputrescine hydrochloride (decomp. 245~250°C).

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The IR, UV and NMR spectra of both compounds were perfectly identical, respectively. These properties clearly show that the compound is 1-(3,4-dihydroxycinnamoylamino)-4-aminobutane.

Feruloylputrescine has been isolated and identified from citrus leaves and fruits by Wheaton and Stewart,⁵⁾ and some related aromatic amides such as aegelin,⁶⁾ heraclavin, cinnamoylhistamine,⁸⁾ and *p*-coumaroylagmatine⁹⁾ have been found in plants. The occurrence of caffeoylputrescine in nature has not previously been reported. Predominance of cinnamic acid derivatives in tissue cultures of tobacco plants are notable. Bergmann *et al.* observed the occurrence of the glucosamine ester of hydroxycinnamic acid derivatives in tissue cultures of *N. tabacum*.

Further work is now under way to study its physiological role in the cells and investigate its possible role in the biosynthesis of nicotine.

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