Isolation of Octopamine From Annual Rye Grass

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In considering the biogenesis of the oxazole alkaloid, annuloline, which occurs in annual rye grass (Lolium multflorum), and whose structure was determined by Karimoto et al. (1), the participation of β-hydroxyphenylethylamine seemed likely. This idea was given support by the discovery of synephrine in tangerine leaves (2) and octopamine in lemon leaves (3), showing that such compounds do exist in plants. It is conceivable that a precursor of annuloline is derived from such a β-hydroxyphenylethylamine and a substituted cinnamic acid:

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\begin{align*}
\text{Octopamine} & \quad \text{Annuloline} \\
\end{align*}
\]

The demonstration of octopamine in rye grass, as reported below, supports the possibility that it may be a precursor of annuloline.

In the isolation of octopamine from rye grass, approximately 2 kg of fresh seedlings, 10 to 12 days old, were extracted with methanol in a Soxhlet apparatus. The extract was passed through a 5.5 × 40 cm Amberlite IR 120 cation exchange column in the hydrogen form. The column was washed with water prior to elution with 2 N ammonium hydroxide. The eluate was taken to dryness in vacuo, and the residue was dissolved in a small volume of methanol. Two-dimensional paper chromatograms of the concentrate revealed a phenolic amine whose behavior indicated that it was octopamine. It chromatographed satisfactorily in n-butanol-acetic acid-water (4:1:1), \( R_F \) 0.62, and in i-butanol-methyllethylketone-ammonium hydroxide-water (4:3:2:1), \( R_F \) 0.80. Its color reactions with various reagents matched those of the authentic compound: ninhydin, brown; diazotized sulfanilic acid, yellow; diazotized p-nitroaniline, red, and 2,6-dichloroquinone-chlorimide (Gibb's reagent), blue. Periodate oxidation and mild acetylation of portions of the extract, performed according to the procedures of Kakimoto and Armstrong (4), produced \( p \)-hydroxybenzaldehyde and \( N \)-acetyl octopamine respectively, as detected on paper chromatograms.

The concentrated basic fractions from different batches of seedlings were each streaked on several sheets of Whatman No. 3 MM paper. The developing solvent was the \( n \)-butanol-acetic acid-water mixture. The edges of the papers were sprayed with diazotized sulfanilic acid to locate the bands containing octopamine, which were cut out and eluted. After the eluate was evaporated to dryness, the residue was dissolved in methanol and streaked on several sheets of Whatman No. 3 MM paper. The developing solvent was the \( t \)-butanol-methyllethylketone-ammonium hydroxide-water mixture. Again the bands containing octopamine were located with diazotized sulfanilic acid and eluted. The eluate was put on a 1 × 8 cm Dowex 50 W × 4 column which was in the hydrogen form. A gradient elution of 0.5 M ammonium hydroxide dripping onto 1 liter of water was used to remove the octopamine. Fractions of 10 ml were collected and the absorbance of each measured at 290 nm. Those fractions with significant absorbance were also spot tested on paper with periodate-benzidine (4). The contents of these tubes, usually numbers 20 to 25, were pooled and evaporated to dryness. The dried residue was pulverized and sublimed. This compound and authentic octopamine both melted between 164 to 168° and no depression was observed in a mixed melting point. The infrared spectrum run in Nujol resembled that of the authentic DL compound (Winthrop Laboratories). Ultraviolet spectra were observed at acid and alkaline pH and fluorescence spectra were observed at acid pH according to Kakimoto and Armstrong (4). The spectra were identical for the isolated material and the synthetic compound (Fig.

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Literature Cited


