BIOGENESIS OF THE AMARYLLIDACEAE ALKALOIDS. II. STUDIES WITH WHOLE PLANTS, FLORAL PRIMORDIA AND CELL FREE EXTRACTS[1]

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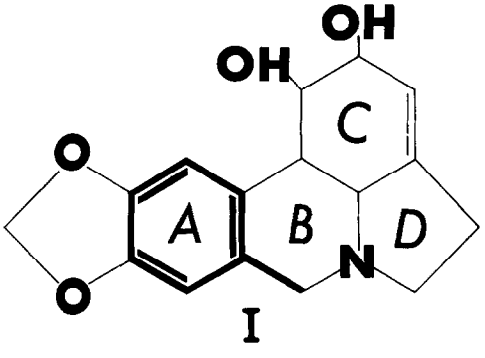
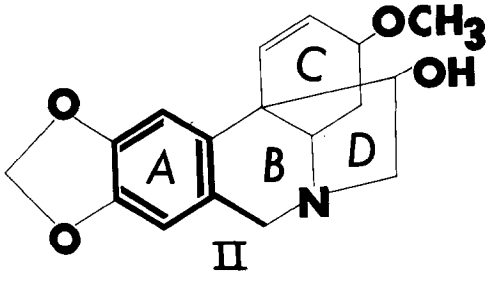
Tyrosine, but not phenylalanine, provides the eight carbon atoms of rings C and D of the Amaryllidaceae alkaloids (Barton, et al., 1960, Battersby, et al., 1960). Phenylalanine and 3,4-dihydroxybenzaldehyde are incorporated into rings A and B of the alkaloids, lycorine (I), haemanthamine (II) and norbelladine (Suhadolnik, et al., 1962, 1963, Wildman, et al., 1962). Tyrosine is not incorporated into rings A and B of these alkaloids.



I



II

This communication concerns the manner in which phenylalanine can serve as the precursor for rings A and B (I and II, heavy

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bonds) of the Amaryllidaceae alkaloids. Two pathways were considered: 1) the conversion of phenylalanine⟶phenylserine ⟶benzaldehyde⟶p-hydroxybenzaldehyde⟶3,4-dihydroxy-benzaldehyde; 2) the deamination of phenylalanine⟶trans-cinnamic acid⟶p-hydroxycinnamic acid⟶3,4-dihydroxycinnamic acid or p-hydroxybenzaldehyde⟶3,4-dihydroxybenzaldehyde.

Two kinds of experiments have been performed: 1) administration of labeled compounds to growing intact plants, followed by the isolation of the alkaloid, haemanthamine; 2) culturing floral primordia from N. pseudonarcissus L. on a supplemented agar medium in the presence of radioactive compounds. The floral primordia were obtained by dissecting away the tunicates, ensiform leaves and part of the rhizomes of plants that had been maintained at 10°C in the dark for 3 mo. The floral primordia (2-3 cm in length and weighing 0.3-0.8 g) were placed into 100 ml of an antibiotic solution containing penicillin (100,000 units), streptomycin (50,000 units) and fungizone (3,000 units) for 3 min. The tissue was then removed and rinsed with sterile water. The culture flasks contained 30 ml of supplemented agar medium of the following composition: 2% White's basic medium (Difco Company, Detroit, Michigan), 15% fresh coconut water, 0.001% naphthaleneacetic acid and 1.5% agar. To each flask was added 1.48 mg (9,980 mμc) of trans-cinnamic acid-3-C^{14}. The flasks and medium were sterilized at 121°C for 15 min and cooled to about 50°C. Penicillin (3,000 units), streptomycin (1,500 units) and fungizone (600 units) were added to the liquified agar medium. The medium was then allowed to solidify. Two primordia were added to each flask. They were grown for 6 days at 30°C and illuminated 14 hrs a day by two 40-watt fluorescent lamps placed 30 cm above the flasks. The primordia were removed, rinsed with distilled water and ground in a mortar and pestle. Twenty milligrams of carrier haemanthamine

was added and the haemanthamine was isolated and crystallized to constant specific activity.

As can be seen from Table I, in the whole plant experiments, trans-cinnamic acid-3-C^{14} and p-hydroxycinnamic acid-3-C^{14} were incorporated into haemanthamine while benzaldehyde-7-C^{14} and p-hydroxybenzaldehyde-7-C^{14} were not incorporated. trans-Cinnamic acid-3-C^{14} was also incorporated into haemanthamine by floral primordia (Table I). The haemanthamine isolated from the primordia had a specific activity of 3,500 mμc/mmole. This compares with 216 mμc/mmole for the haemanthamine isolated from the whole plant. The haemanthamine from the cinnamic acid and p-hydroxycinnamic acid experiments is being degraded to determine the exact location of the radioactivity.

TABLE I. ORIGIN OF RING A AND THE BENZYLIC CARBON ATOM OF HAEMANTHAMINE IN N. PSEUDONARCISSUS

Compound Added	Administered			Found in Haemanthamine	
	Amount		Sp. Act.	Sp. Act.	Dilution
	mg	mμc	mμc/mmole	mμc/mmole	
trans-cinnamic acid-3-C^{14}	14.8	99,800	998,000	216	4,620
trans-cinnamic* acid-3-C^{14}	1.48	9,980	998,000	3,500	285
p-hydroxycinnamic acid-3-C^{14}	75.0	3,330	7,280	11.8	617
benzaldehyde-7-C^{14}	5.0	10,000	210,000	0	———
p-hydroxy-benzaldehyde-7-C^{14}	3.07	4,600	183,000	0.79	232,000

* Floral primordia were used for this experiment.

When 3,4-dihydroxycinnamic acid and threo-DL-phenylserine (both compounds were randomly labeled with tritium) were admin-

istered to N. incomparabilis, only 3,4-dihydroxycinnamic acid was incorporated into lycorine (Table II).

TABLE II. ORIGIN OF RING A AND THE BENZYLIC CARBON ATOM OF LYCORINE IN N. INCOMPARABILIS

Compound Added	Administered			Found in Lycorine	
	Amount		Sp. Act.	Sp. Act.	Dilution
	mg	mμc	mμc/mmole	mμc/mmole	
3,4-dihydroxy-cinnamic acid*	4.1	16,000	702,000	41	17,100
threo-DL-phenyl-serine*	12.6	6,700	96,300	0	———

* Randomly labeled with tritium.

Although tritium-labeled phenylserine was not incorporated into the alkaloid, this amino acid is metabolized by N. pseudo-narcissus. This was shown by the administration of threo-DL-phenylserine-2-C^{14} followed by the isolation of radioactive glycine from the hydrolyzed plant protein. The radioactive glycine could arise by threonine aldolase cleaving the phenyl-serine. If benzaldehyde arises from the enzymic cleavage of phenylserine, it is not incorporated into haemanthamine as evi-denced by the results of the benzaldehyde-7-C^{14} experiment (Table I). The data shown in Tables I and II support the phenyl-alanine-cinnamic acid pathway.

To show that the incorporation of the trans-cinnamic acid into haemanthamine does not proceed by amination to form phenyl-alanine, the plant protein from the trans-cinnamic acid-3-C^{14} experiment was hydrolyzed. The phenylalanine was not radioactive.

Since Neish (1961) and Koukol and Conn (1961) reported the isolation of phenylalanine deaminase from plant tissue, this

enzyme was studied in the Amaryllidaceae. An acetone powder was extracted with 0.05 M borate buffer, pH 8.9. Phenylalanine deaminase was assayed according to the procedure of Koukol and Conn (1961). The ultraviolet absorption spectrum of the product of the phenylalanine deaminase reaction was identical with that of authentic trans-cinnamic acid. Both spectra had a single maximum at 268 mμ. The specific activity of the cinnamic acid isolated from the enzyme reaction mixture was 4.0 μc/mmole. This compares with a specific activity of 5.6 μc/mmole of the L-phenylalanine-3-C^{14} added to the incubation mixture.

The use of floral primordia and cell free extracts should greatly facilitate studies on the interconversion of alkaloids, regulation of their synthesis and their function in plants.

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