Biosynthetic Production of Aberrant Alkaloids in *Dolichothele sphaerica* (Cactaceae)

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Abstract □ Preliminary studies of the production of aberrant alkaloids were carried out by introducing unnatural precursors of dolichotheline into *Dolichothele sphaerica* in the hope that the corresponding dolichotheline analogs would be formed. Authentic samples of the required dolichotheline analogs were synthesized and served both as standards and as carriers during the extraction procedure. Radioactive analogs were separated from dolichotheline by preparative TLC, recrystallized to constant specific activity, and degraded to localize the label. Using appropriate precursors, the aberrant alkaloids *N*-isocaproylhistamine and 4(5)-[*N*-isovalerylaminomethyl]imidazole were produced by *D. sphaerica*, while the plant appears to be unable to produce *N*-benzoylhistamine, *N*-isobutyrylhistamine, and *N*-isovaleryl-*N*-isopropylhistamine.

Keyphrases □ *Dolichothele sphaerica* (Cactaceae)—biosynthesis of aberrant alkaloids □ Alkaloids, aberrant—biosynthesis of dolichotheline analogs from unnatural precursors in *Dolichothele sphaerica* □ Unnatural alkaloids—biosynthesis of dolichotheline analogs from unnatural precursors in *Dolichothele sphaerica* □ Aberrant alkaloid biosynthesis—*Dolichothele sphaerica* (Cactaceae) □ Biosynthesis of aberrant alkaloids—dolichotheline analogs in *Dolichothele sphaerica*

Studies on the biosynthesis of alkaloids in higher plants have been carried out almost entirely by means of labeled precursor feeding experiments. Such investigations assume that only the natural precursor will be incorporated efficiently into the alkaloid of interest. Yet, there are published accounts that document the incorporation of unnatural precursors into naturally occurring alkaloids (1, 2). In addition, the possibility of the incorporation of unnatural precursors into unnatural or aberrant alkaloids exists. Recently, Leete *et al.* (3) succeeded in inducing the formation of 5-fluoronicotine by feeding 5-fluoronicotinic acid to *Nicotiana tabacum*. Rueppel and Rapoport (4, 5) tested 2- and 3-methyl-substituted pyrrolinium precursors in *Nicotiana glutinosa* and reported their incorporation into the aberrant alkaloids 3′-methylnicotine, 2′-methylnicotine, and 3′,3′-dimethylnicotine.

Dolichotheline (I), a monosubstituted amide alkaloid isolated from the cactus *Dolichothele sphaerica* (Dietrich) Britton and Rose, appears to arise biosynthetically by means of a mechanism involving the condensation of histamine and isovaleryl-CoA (6). Thus, *D. sphaerica* was considered to be suitable for carrying out experiments to determine whether the enzyme(s) responsible for the linkage of histamine and isovaleric acid could also yield aberrant alkaloids following administration of selected precursors to the plant.

The following compounds were tested in the expectation that their corresponding dolichotheline analogs would be formed: isobutyric acid, isocaproic acid, benzoic acid, 4(5)-aminomethylimidazole, and *N*-isopropylhistamine. Authentic samples of the required dolichotheline analogs were synthesized and served both as standards and as carriers during the extraction procedure.

Such investigation into the biosynthesis of unnatural or aberrant alkaloids is of interest for several reasons:

1. There are few reports that deal with the incorporation of an unnatural precursor into an unnatural alkaloid.
2. Such experiments with a series of related unnatural precursors may shed light on the specificity of the enzyme(s) involved.
3. The formation of unnatural alkaloids *in vivo* has broad potential application for the preparation of analogs of biologically active natural products.

EXPERIMENTAL[1]

***N*-Isobutyrylhistamine (II)**—Isobutyric anhydride (2.2 g.) and histamine (1.1 g.) were gently refluxed for 1 hr. Several drops of water were added and the reaction mixture was condensed on a steam bath to a thick, oily residue. The addition of a few drops of acetone caused the crystallization of *N*-isobutyrylhistamine. It was recrystallized from methanol–ether, yielding 1.39 g., m.p. 139–140° [lit. (7) m.p. 123°[2]]; IR (amide) 1640 cm.$^{-1}$. NMR spectral data for all dolichotheline analogs are presented in Table I.

Anal.—Calc. for $C_9H_{15}N_3O$: C, 59.64; H, 8.34; N, 23.19. Found: C, 59.76; H, 8.34; N, 23.25.

***N*-Isocaproylhistamine (III)**—Histamine (0.9 g.) and 4-methylvaleryl chloride (1.7 g.) were refluxed gently for 20 min. The reaction mixture was then cooled, dissolved in 10 ml. of 1 *N* HCl, and extracted three times with equal portions of ether. The aqueous solution was made alkaline with concentrated ammonium hydroxide to pH 9.5 and submitted to extraction with hot chloroform in a liquid–liquid extractor for 24 hr. The chloroform extract was dried over anhydrous sodium sulfate and evaporated to yield the crude *N*-isocaproylhistamine. It was recrystallized from methanol–ether, yielding 0.95 g., m.p. 137–138°; IR (amide) 1635 cm.$^{-1}$.

Anal.—Calc. for $C_{11}H_{19}N_3O$: C, 63.13; H, 9.15; N, 20.08. Found: C, 62.97; H, 9.24; N, 20.14.

***N*-Benzoylhistamine (IV)**—Histamine (1.1 g.) and benzoyl chloride (1.4 g.) were gently refluxed for 15 min. The reaction mixture was then cooled, treated with 10 ml. of 1 *N* HCl, and extracted three times with equal portions of ether. The aqueous solution was made alkaline with concentrated ammonium hydroxide to pH 9.5 and extracted with hot chloroform for 24 hr. The chloroform extract was dried over anhydrous sodium sulfate and evaporated to yield crude *N*-benzoylhistamine. It was recrystallized from methanol–ether, yielding 0.93 g., m.p. 147–149°; IR (amide) 1640 cm.$^{-1}$.

Anal.—Calc. for $C_{12}H_{13}N_3O$: C, 66.96; H, 6.09; N, 19.52. Found: C, 67.13; H, 6.22; N, 19.47.

***N*-Isopropylhistamine Dihydrochloride**—Histamine (1.11 g.), in 25 ml. absolute ethanol and 1.5 ml. of acetone, was hydrogenated over 300 mg. of platinum oxide at atmospheric pressure for 4 hr. After the catalyst was removed, the free base was converted to the

[1] Melting points were determined on a Fisher-Johns melting-point apparatus and are corrected. IR absorption spectra were recorded using KBr pellets and a Perkin-Elmer 337 grating spectrophotometer. NMR spectra were determined in deuterated dimethyl sulfoxide using tetramethylsilane as the reference standard on a Varian A-60 spectrometer. Microanalyses were carried out by Spang Microanalytical Laboratory, Ann Arbor, Mich.

[2] The reported melting point appears to have been interchanged with that of *N*-acetylhistamine, which was also synthesized by the author.

Table I—NMR Spectra of Dolichotheline Analogs (60 Hz. in Dimethyl Sulfoxide using Tetramethylsilane Reference)[a]

Compound		Aromatic 2-CH	Aromatic 4(5)-CH	Imino N-H	—CH_2— Next to Ring	Gem Dimethyl	Miscellaneous
							C-2 and C-3 of isovaleryl group
VI: 4(5)-[*N*-Isovaleryl-aminomethyl]imidazole	p.p.m.	7.52	6.80	8.05	4.37	1.11	2.22
	J, c.p.s.	s	s	s	d 4.0	d 5.0	m
							C-2 isocaproyl group
III: *N*-Isocaproylhistamine	p.p.m.	7.39	6.67	7.75	2.61	0.88	2.02
	J, c.p.s.	s	s	s	t 7.0	d 5.0	t 7.0
							C-2 isobutyryl group
II: *N*-Isobutyrylhistamine	p.p.m.	7.26	6.54	7.53	2.54	0.99	2.20
	J, c.p.s.	s	s	s	t 7.0	d 5.0	m
							Aromatic (benzene)
IV: *N*-Benzoylhistamine	p.p.m.	7.61	6.89	9.33	2.80	—	7.88 and 7.55
	J, c.p.s.	s	s	s	t 7.0	—	m
							N-Isopropyl
V: *N*-Isopropyl-*N*-isovalerylhistamine	p.p.m.	8.96	7.36	4.40	3.04	0.94	1.24
	J, c.p.s.	s	s	s	t 7.0	d 5.0	d 5.0

[a] s = singlet, d = doublet, m = multiplet, and t = triplet.

dihydrochloride by dissolving it in 25 ml. of methanol and adding 5 ml. of 6 *N* HCl. The resulting solution was evaporated to dryness and the residue was recrystallized from absolute ethanol–ethyl acetate, yielding 1.23 g., m.p. 195–197° [lit. m.p. 197–199° (14) and 195–196° (15)].

Anal.—Calc. for $C_8H_{17}Cl_2N_3$: C, 42.49; H, 7.58; N, 18.58. Found: C, 42.71; H, 7.74; N, 18.61.

N-Isopropyl-N-isovalerylhistamine (V)—*N*-Isopropylhistamine (0.82 g.) and isovaleric anhydride (2 g.) were gently refluxed for 4 hr. When cool, several drops of water were added to the reaction mixture, which was then condensed on a steam bath to a syrupy residue. This residue could not be induced to crystallize. As a consequence, the picrate derivative was formed by dissolving the residue in 2 ml. of ethanol and adding 10 ml. of a saturated solution of picric acid in ethanol. The crystalline picrate derivative was filtered and recrystallized from water, yielding 817 mg., m.p. 145°; IR (amide) 1640 $cm.^{-1}$.

Anal.—Calc. for $C_{19}H_{26}N_6O_8$: C, 48.92; H, 5.62; N, 18.02. Found: C, 48.89; H, 5.63; N, 18.25.

Imidazole-4(5)-formaldehyde—Hydroxymethylimidazole (13 g.) (8, 9) and nitric acid (19 ml.) were digested in a covered beaker on a steam bath until the evolution of brown fumes was almost complete. The cover was then removed and the liquid was evaporated to dryness.

This residue was dissolved in a warm concentrated solution of sodium carbonate and kept at room temperature for 24 hr., at which time the insoluble imidazole-4(5)-formaldehyde was filtered and dried. It was recrystallized from water, yielding 5.1 g., m.p. 173–174° [lit. (10) m.p. 170°].

Imidazole-4(5)-formaldoxime—The above aldehyde (2.0 g.), hydroxylamine hydrochloride (1.4 g.), and sodium carbonate (1.0 g.) were dissolved in 10 ml. of water and the resulting solution was allowed to stand at room temperature for 24 hr., at which time the insoluble imidazole-4(5)-formaldoxime was filtered and dried. It was recrystallized from alcohol, yielding 1.8 g., m.p. 183° [lit. (11) m.p. 183–184°].

4(5)-Aminomethylimidazole Dihydrochloride—A solution of 1.11 g. of the above oxime in 80 ml. of methanol containing 0.05 mole of dry hydrogen chloride was hydrogenated at 15 lb. pressure over 5% palladium-on-charcoal. After 90 min., the mixture was filtered and the filtrate was evaporated to dryness. The residue was recrystallized from methanol–water to give 1.20 g. of 4(5)-aminomethylimidazole dihydrochloride, m.p. 238–241° [lit. m.p. 246–247° (12) and 244° (13)].

4(5)-[*N*-Isovalerylaminomethyl]imidazole (VI)—The free base of the above amine (0.63 g.) and isovaleryl chloride (1.1 g.) were gently refluxed for 20 min. The reaction mixture was then cooled, dissolved in 10 ml. of 1 *N* HCl, and extracted three times with equal portions of ether. The aqueous solution was basified with concentrated ammonium hydroxide to pH 9.0 and extracted with hot chloroform in a liquid–liquid extractor for 24 hr. The chloroform extract was dried over anhydrous sodium sulfate and evaporated to dryness. Crude VI was recrystallized from methanol–ether, yielding 0.61 g., m.p. 117°; IR (amide) 1630 $cm.^{-1}$.

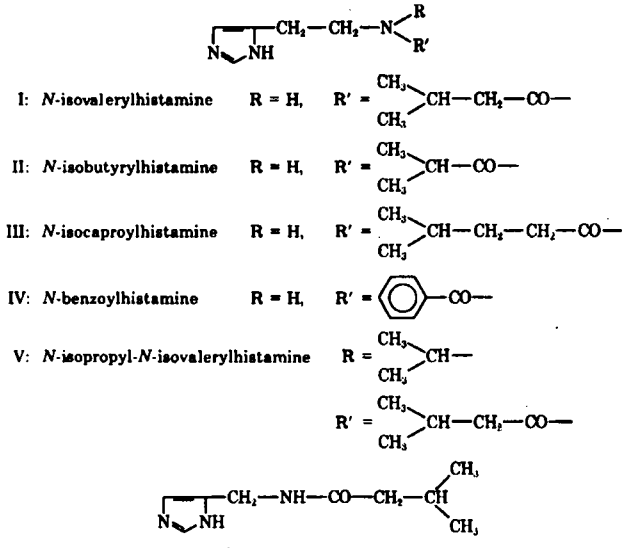
Anal.—Calc. for $C_9H_{15}N_3O$: C, 59.64; H, 8.34; N, 23.19. Found: C, 59.45; H, 8.37; N, 23.23.

Preparation of Labeled Precursors—Isovaleric acid (carboxyl-^{14}C), isocaproic acid (carboxyl-^{14}C), isobutyric acid (carboxyl-^{14}C), and benzoic acid (carboxyl-^{14}C) were purchased from commercial sources.

4(5)-Aminomethylimidazole (ring-2-^{14}C) was prepared by the method of Pyman (13, 16). This synthesis was initially accomplished using nonradioactive material. The product displayed an IR spectrum identical with that of 4(5)-aminomethylimidazole dihydrochloride and showed no melting-point depression when mixed with authentic 4(5)-aminomethylimidazole dihydrochloride. Diaminoacetone dihydrochloride (100 mg.) was added to a hot solution of 66 mg. of potassium thiocyanate-^{14}C in 0.2 ml. of water, and the mixture was heated on a steam bath for 30 min. The resulting crystalline product was filtered, added to 5 ml. of hot water, and filtered again.

The filtrate was added to 900 mg. of ferric chloride in 10 ml. of water, and the mixture was digested for 30 min. on the steam bath. Three milliliters of 10% aqueous sodium carbonate was added, followed by a hot solution of 400 mg. of picric acid in 10 ml. of boiling water. The mixture was boiled with a little charcoal and filtered. On cooling, 96 mg. of 4(5)-aminomethylimidazole-2-^{14}C (ring) dipicrate separated, m.p. 210–212° [lit. (16) m.p. 212°].

The dihydrochloride was prepared by treating the picrate with 5 ml. of 6 *N* HCl, removing the picric acid with four successive 5-ml.



portions of benzene, evaporating the acidic aqueous solution to dryness, and recrystallizing the residue from methanol–water, yielding 22 mg., m.p. 241–243°.

Plants—Living plants of *D. sphaerica*[3] were maintained in a controlled environment lab to provide optimal light, temperature, and humidity (17, 18).

Administration of Test Compounds—All precursors, with the exception of benzoic acid, were dissolved in 1 ml. of sterile water and slowly injected, by means of a 1.0-ml. sterile disposable syringe and a sterile 26-gauge, 1.3-cm. (0.5-in.), hypodermic needle, into the fleshy roots of 10 plants. The benzoic acid was dissolved in 1 ml. of 0.05 *N* NaOH and similarly injected into 10 plants. Nonradioactive *N*-isopropylhistamine was introduced simultaneously with isovaleric acid-1-^{14}C (sodium salt). After each injection, the needle was left in place for a minute to allow the solution to disperse. Following this procedure, the plants were carefully repotted, watered, and returned to the environment lab.

Isolation of Aberrant Alkaloids—Three weeks after the injection of the labeled precursors, the plants were removed from the controlled environment lab and sliced, dried in an oven at 45° for 96 hr., and ground. In each case, 500 mg. of the appropriate, nonradioactive dolichotheline analog was added to the ground material. The method used for the extraction and isolation of dolichotheline and its unnatural analogs was identical to that previously described for the isolation of dolichotheline (19). Any radioactivity residing in the unnatural analog was monitored using TLC and radiochromatogram scanning. When the analog was devoid of activity, no further work was attempted. On the other hand, when the analog did contain radioactivity, it was separated from dolichotheline by preparative TLC.

Separation of III from Dolichotheline—A mixture (600 mg.) of III and dolichotheline was dissolved in a small volume of methanol and applied (100 mg./plate) to a 2-mm. layer of silica gel GF_{254}, 20 × 20-cm. plates. The plates were then subjected to multiple development (three times) using benzene–methanol–chloroform–concentrated ammonium hydroxide (3:1:6:0.1) as the solvent system. The solvent was allowed to migrate to 2 cm. from the top edge of the plate, and the plate was carefully dried each time upon removal from the developing chamber. The R_f value for dolichotheline was 0.20; for III, it was 0.28. The area corresponding to III on each plate was carefully scraped off, and the silica gel was eluted with three 100-ml. portions of methanol. The eluant was filtered and evaporated to dryness. The residue was dissolved in 100 ml. of hot chloroform, which was then dried over anhydrous sodium sulfate, filtered, and reduced to dryness to yield *N*-isocaproylhistamine, 273 mg., m.p. 136–137°.

Separation of VI from Dolichotheline—A mixture (300 mg.) of VI and dolichotheline was separated by a method similar to that employed for the separation of III and dolichotheline. The preparative plates in this case were developed three times using chloroform–ethanol–1 *N* HCl (8:2:0.1) as the solvent system. The R_f for dolichotheline was 0.39; for VI, it was 0.55. The silica gel scraped off the plates was extracted with three 100-ml. portions of methanol. The methanol extract was reduced to dryness and dissolved in 15 ml. of water; the pH was adjusted to 9.5 with concentrated ammonium hydroxide, and the solution was extracted with hot chloroform in a liquid–liquid extractor for 24 hr. The chloroform extract was dried over anhydrous sodium sulfate and evaporated to yield 113 mg. of VI, m.p. 115–116°.

***N*-Isocaproylhistamine Picrate**—III (100 mg.) was dissolved in 1 ml. of ethanol, 1 ml. of a saturated solution of picric acid in ethanol was added, and the mixture was stirred with a glass rod. The resulting yellow crystals were filtered and washed with a few drops of water. The picrate was recrystallized from water, yielding 82 mg., m.p. 138°.

Anal.—Calc. for $C_{17}H_{23}N_6O_8$: C, 46.57; H, 5.06; N, 19.17. Found: C, 46.50; H, 5.11; N, 19.29.

Using an identical procedure, 20 mg. of radioactive III was reacted with picric acid to yield 13 mg. of *N*-isocaproylhistamine picrate, m.p. 137–138°.

4(5)-[*N*-Isovalerylaminomethyl]imidazole Picrate—The amide (100 mg.) was dissolved in 1 ml. of ethanol, and 1 ml. of a saturated solution of picric acid in ethanol was added. The resulting yellow crystals were filtered and rinsed with a few drops of water. This picrate derivative was recrystallized from water, yielding 79 mg., m.p. 171°.

Anal.—Calc. for $C_{15}H_{18}N_6O_8$: C, 43.90; H, 4.42; N, 20.48. Found: C, 43.69; H, 4.38; N, 20.41.

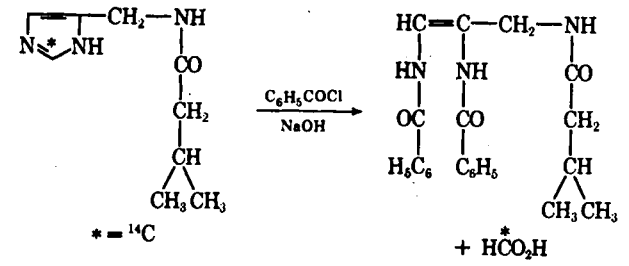
Using the above procedure, 20 mg. of the separated radioactive amide was reacted with picric acid to obtain the picrate derivative, 15 mg., m.p. 170–171°.

Degradation of VI (Scheme I)—The amide (190 mg.) was added to a cold solution of 1 g. of sodium hydroxide in 5 ml. of water. This was followed by the addition of 0.5 ml. of benzoyl chloride and 2 drops of benzene. The mixture was stirred vigorously for 2 hr. with cooling, after which the solid product was filtered, washed with a little water, and dried. The 1,2-dibenzoylamino-3-isovalerylaminopropene-1 was recrystallized from ethanol–water, yielding 170 mg., m.p. 205°.

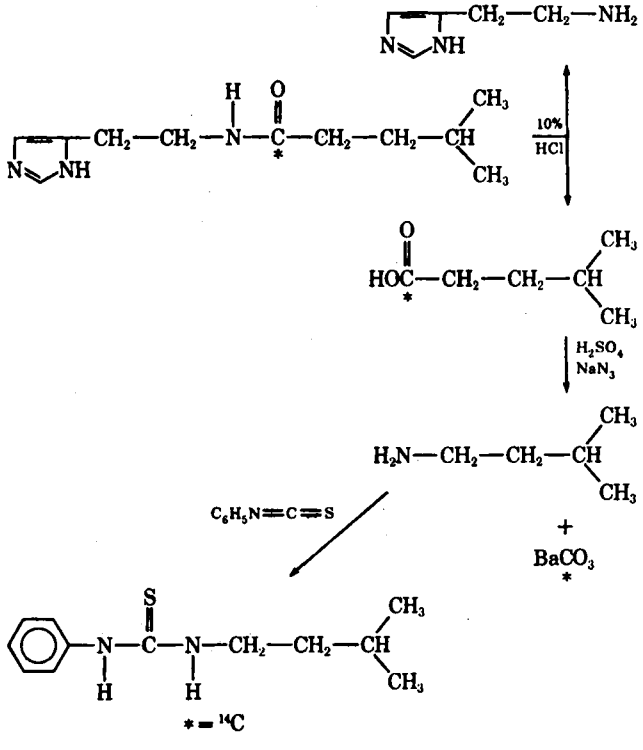
Anal.—Calc. for $C_{22}H_{25}N_3O_3$: C, 69.64; H, 6.64; N, 11.07. Found: C, 69.46; H, 6.58; N, 11.19.

Using an identical procedure, 40 mg. of the separated radioactive amide was reacted with benzoyl chloride to yield 21 mg. of 1,2-dibenzoylamino-3-isovalerylaminopropene-1, m.p. 204–205°.

Degradation of III (Scheme II)—III was hydrolyzed with 10% HCl, and the resulting histamine and isocaproate were recovered as the dihydrochloride and sodium salts, respectively, by identical procedures described for the hydrolysis of dolichotheline (6). The sodium isocaproate was further degraded by means of a Schmidt decarboxylation essentially as described by Phares (20). The sodium isocaproate and sodium chloride mixture (300 mg.) was dissolved



Scheme I—Degradation of VI



Scheme II—Degradation of III

[3] Purchased from El Paso Cactus Gardens, Anthony, N. M.-Tex. Living voucher specimens are on deposit at the University of Michigan Botanical Gardens, Ann Arbor, Mich.

Table II—^{14}C-Labeled Precursors Tested

Precursor	Position of Label	Specific Activity, mc./mM	Amount Injected, μ	Percent Injected Activity Recovered as Dolichotheline Analog
Isocaproic acid (sodium salt)	1-^{14}C	2.1	300	0.81
Isobutyric acid (sodium salt)	1-^{14}C	30.0	212	—
Benzoic acid	Carboxyl-^{14}C	2.65	179	—
4(5)-Aminomethylimidazole dihydrochloride	2-^{14}C (ring)	0.70	75.5	1.27
N-Isopropylhistamine and isovaleric acid (sodium salt)	1-^{14}C	2.0	70.5	—

Table III—Compound III Obtained from Isocaproic Acid-1-^{14}C-Injected Plants

Compound	Yield, mg.	Melting Point	Reference Melting Point	Mixed Melting Point	Specific Activity, d.p.m./mM
III (crude)	273	136–137°	137–138°	—	2.44×10^6
III recrystallized one time	246	137–138°	137–138°	—	2.44×10^6
III recrystallized two times	208	137–138°	137–138°	137–138°	2.45×10^6
III picrate	13	137–138°	138°	137–138°	2.27×10^6
Histamine dihydrochloride	53	239–243°	243–246°	242–244°	0.002×10^6
Isoamylphenylthiourea	19	100–101°	102°	101–102°	0.019×10^6

in cold 100% sulfuric acid (3 ml.) in a 25-ml. pear-shaped flask, and powdered sodium azide (250 mg.) was added in small amounts, with shaking, to the chilled solution. The flask was immediately connected to two bead towers in series, the first containing 10 ml. of 5% potassium permanganate in 1 N H_2SO_4 and the second containing 10 ml. of 1 N carbon dioxide-free sodium hydroxide. A slow stream of carbon dioxide-free air was drawn through the solution while the flask was heated to 60–65° for 7 hr. The bead tower containing the sodium hydroxide was then washed down with carbon dioxide-free water, and 2 ml. of a saturated solution of barium chloride was added. The resulting precipitate was centrifuged and the supernate was decanted. The barium carbonate was resuspended in water and again centrifuged. This process was repeated with ethanol and the carbonate was finally dried in a desiccator.

To recover the isoamylamine, the contents of the reaction flask were basified to pH 12 with 20% sodium hydroxide and the isoamylamine was extracted three times with equal portions of ether. The isoamylamine was then reacted with phenylisothiocyanate to form isoamylphenylthiourea (21).

Radioactive Assay—The method employed for the preparation of samples for scintillation counting was described previously (17, 18). The aberrant products were recrystallized to constant specific activity, and the single derivative of each product was also counted as a check of the radiopurity. The two degradation products were assayed to localize the label. The barium carbonate (0.5 mg.) was assayed as a suspension in scintillation fluid containing a thixotropic gel[4] (5 g./1000 ml. of scintillation cocktail). All samples were counted in a liquid scintillation system[5] at a preset 2σ statistical counting error of 1%. The external standard ratio method was used to determine losses due to quenching. All counts were corrected for counter efficiency.

RESULTS AND DISCUSSION

The data obtained from the *in vivo* studies on the production of aberrant alkaloids are summarized in Tables II–IV. They indicate that isocaproic acid and 4(5)-aminomethylimidazole can serve as precursors of aberrant alkaloids in *D. sphaerica*. The data also show that isobutyric acid, benzoic acid, and N-isopropylhistamine do not serve as precursors of their corresponding dolichotheline analogs.

When III obtained from plants injected with isocaproic acid-1-^{14}C was hydrolyzed, the activity resided in the isocaproate portion of the molecule. Decarboxylation by the Schmidt reaction provided carbonate which contained greater than 98% of the activity present in the sodium isocaproate. The remaining isoamylamine, isolated as isoamylphenylthiourea, was essentially inactive.

The plants injected with 4(5)-aminomethylimidazole (ring-2-^{14}C) yielded VI which, when benzoylated, yielded nearly inactive 1,2-dibenzoylamino-3-isovalerylaminopropene-1. This degradation demonstrated that greater than 98% of the activity resided in the expected position.

Calculations of percent injected activity recovered as dolichotheline analog are based on the 500 mg. of the analog added as carrier. Since the actual amount of analog formed is less than 1 mg., the error made in calculating percent incorporation based on a total yield of 500 mg. is very small.

Of the five unnatural precursors tested, only two, isocaproic acid and 4(5)-aminomethylimidazole, were incorporated into unnatural or aberrant alkaloids. With respect to the specificity of the enzymes involved, it appears that the activating enzyme(s) is capable of activating isocaproic acid, the higher homolog of isovaleric acid, to isocaproyl-CoA. In addition, the condensing enzyme can utilize the isocaproyl-CoA as a substrate and bind it to histamine in amide linkage. Furthermore, this condensing enzyme can form an amide bond between isovaleryl-CoA and a compound with an aminomethyl side chain at the 4(5)-position of the imidazole nucleus in addition to an aminoethyl side chain.

The differences in the incorporation (0.81% in III and 1.27% in VI) are reasonable because 4(5)-aminomethylimidazole occupies a more proximal position to the final biosynthetic step than isocaproic acid, which must first be activated to isocaproyl-CoA. However, the differences may also be due to differences in *in vivo* metabolism of the two precursors, although such metabolic differences are probably of little significance in this case.

The positive result obtained from the isocaproic acid experiment makes it seem surprising that isobutyric acid was not utilized as a precursor of its corresponding dolichotheline analog. This failure may be attributed to the specificity of the enzymes involved. However, a more plausible explanation is that isobutyric acid was activated to isobutyryl-CoA, a naturally occurring intermediate in the catabolism of valine (22), and then was further metabolized along the normal pathway for valine degradation,

In the animal body, benzoic acid is activated to benzoyl-CoA, which is then condensed with glycine to form hippuric acid (23–25). These reactions may be analogous to the activation of isovaleric acid to isovaleryl-CoA and the subsequent condensation of the latter with histamine. It was reasonable to assume that the benzoic acid injected into *D. sphaerica* would result in the benzoylation of

[4] Cab-O-Sil.
[5] Beckman LS-150.

Table IV—Compound VI Obtained from 4(5)-Aminomethylimidazole-2-^{14}C(ring)-Injected Plants

Compound	Yield, mg.	Melting Point	Reference Melting Point	Mixed Melting Point	Specific Activity, d.p.m./mM
VI (crude)	113	115–116°	117°	—	7.72×10^5
VI recrystallized one time	91	116–117°	117°	—	7.70×10^5
VI recrystallized two times	60	116–117°	117°	116–117°	7.79×10^5
VI picrate	15	170–171°	170°	170–171°	7.73×10^5
1,2-Dibenzoylamino-3-isovalerylamino-propene-1	21	204–205°	205°	204–205°	0.10×10^5

histamine. This expected formation of IV did not take place, possibly because of enzyme specificity and/or *in vivo* metabolism of the benzoic acid.

The remaining unnatural precursor, *N*-isopropylhistamine, also did not yield the expected dolichotheline analog. Perhaps the specificity of the condensing enzyme is such that it requires a primary amine rather than a secondary amine to be present in the histamine side chain in order to form the amide linkage.

These investigations with unnatural precursors are attempts to prepare aberrant dolichotheline analogs in the hope that these model techniques can be applied in the preparation of analogs of biologically active natural products that are difficult to synthesize. Furthermore, this work is an *in vivo* approach to the study of the specificity of the enzyme system that catalyzes the condensation of isovaleric acid and histamine. In this respect, the present experiments not only show that this enzyme system is not completely specific but also partially defines the specificity. Finally, the experiments provide additional support for the hypothesized final step in the biosynthesis of dolichotheline.

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