A STUDY OF THE BIOSYNTHESIS OF COLCHICINE AND MERENDERINE IN Merendera raddeana

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In an investigation of the biosynthesis of colchicine in some species of the genus Colchicum L. (family Liliaceae) it was established that the hydrocarbon skeleton of this alkaloid is formed from amino acids – phenylalanine and tyrosine [1-4]. The methoxy groups in colchicine arise as the result of the methylation of the hydroxy groups of methylmethionine [1]. The source of the N-acetyl group is sodium acetate [1, 5] and, in part, tyrosine [5]. In addition, it was shown that the biosynthesis of colchicine takes place through phenethyltetrahydroisoquinoline bases [6].

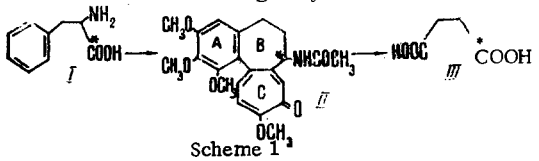
Thus, the simultaneous occurrence of tropolone and isoquinoline bases in some genera of the family Liliaceae is explained by their common biogenetic pattern.

In recent years, homoaporphine and homoproaporphine bases have been isolated from many species of the Liliaceae family, in addition to tropolone and phenethyltetrahydroquinoline alkaloids [7]. These compounds differ completely in structure, although they have common precursors [6, 8]. However, the question of their simultaneous biosynthesis from the precursors mentioned has been little studied. Consequently, it appeared of interest to investigate with the aid of labeled amino acids the biosynthesis of alkaloids in plants containing both tropolone and nontropolone compounds.

Such plants include Merendera raddeana Rgl., in which the main components of the alkaloids are colchicine and the homoaporphine base merenderine [9]. The biosynthesis of colchicine has been considered previously only in species of the genus Colchicum L.; this process has not been investigated in other plant genera, in particular, in Merendera Ram.

The present paper gives the results of a study of the biosynthesis of colchicine and merenderine in M. raddeana. The plants were supplied separately with the suggested precursors of the colchicine alkaloids: [1-^{14}C]phenylalanine, generally labeled tyrosine, and sodium [1-^{14}C]acetate. We found that phenylalanine and tyrosine are included in the molecules of alkaloids of both neutral and basic nature. The inclusion of sodium acetate takes place only in alkaloids of neutral character. We observed a greater radioactivity and a greater degree of inclusion of the labeled amino acids in the subsurface parts of the plant (Table 1).

For the accumulation of the labeled colchicine, the phenolic substances of the neutral-phenolic fraction of alkaloids [10] were methylated with diazomethane. In order to determine the position of the radioactive isotopes included in the colchicine molecule, the latter was subjected to oxidative degradation. The chromium-trioxide oxidation of the colchicine (II, Scheme 1) isolated when the plant was supplied with [1-^{14}C]phenylalanine (I) yielded radioactive succinic acid. According to this result and literature information on the biosynthesis of colchicine, the inclusion of phenylalanine in the colchicine molecule and its oxidative composition can be represented in the following way:



Scheme 1

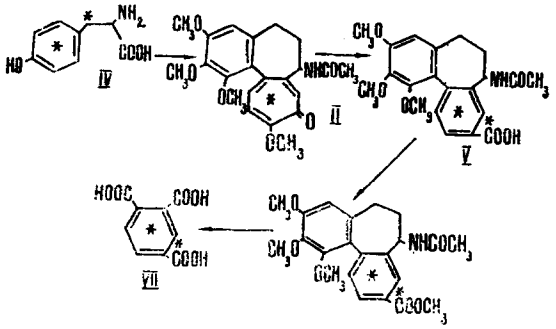
V. I. Lenin Tashkent State University. Translated from Khimiya Prirodnykh Soedinenii, No. 6, pp. 764-770, November-December, 1972. Original article submitted April 3, 1972.

TABLE 1. Amounts of Alkaloids in M. raddeana and Their Specific Activities (counts/min · mmole)

Precursors added	Parts of the plant	Weights of the plant samples, g	Alkaloids isolated: neutral fraction mg	%	sp. activity	% inclusion	basic fraction mg	%	sp. activity	% inclusion
[1-^{14}C]Phenylalanine	Leaves and flowers	17,9	59,5	0,33	$1,01 \cdot 10^5$	0,06	50,1	0,28	$2,02 \cdot 10^5$	0,11
$1,75 \cdot 10^8$	Bulbs	27,0	48,6	0,18	$1,39 \cdot 10^6$	0,79	8,2	0,03	$7,01 \cdot 10^5$	0,40
	Bulb sheaths	6,5	11	0,17	$7,44 \cdot 10^5$	0,42	2,6	0,04	$1,02 \cdot 10^6$	0,59
The same	Leaves and flowers	2,1	6,3	0,30	$1,73 \cdot 10^5$	0,10	5,6	0,27	$5,23 \cdot 10^4$	0,03
Ordinary tyrosine $5,22 \cdot 10^7$	Leaves and flowers	2,5	6,9	0,27	$2,2 \cdot 10^5$	0,42	5,7	0,23	$3,7 \cdot 10^5$	0,7
	Bulbs	5,3	8,4	0,16	$1,2 \cdot 10^6$	2,30	1,6	0,03	$4,48 \cdot 10^5$	0,85
	Bulb sheaths	2,0	3,2	0,16	$2,28 \cdot 10^6$	4,30	1,0	0,05	$5,90 \cdot 10^5$	1,1
Sodium [1-^{14}C]acetate, $1,17 \cdot 10^8$	Leaves and flowers	11,7	31,1	0,26	$4,8 \cdot 10^4$	0,04	24,5	0,21	—	—
	Bulbs	29,0	58,0	0,20	$2,5 \cdot 10^5$	0,21	11,6	0,04	—	—
	Bulb sheaths	3,6	6,9	0,19	$1,31 \cdot 10^6$	1,12	1,4	0,04	—	—
Control	Leaves and flowers	3,2	9,6	0,30	—	—	7,0	0,22	—	—
	Bulbs	4,1	6,4	0,15	—	—	1,2	0,03	—	—
	Bulb sheaths	2,5	4,6	0,18	—	—	0,7	0,03	—	—

Thus, in M. raddeana, as well, ring A and carbon atoms 5, 6, and 7 of ring B of the colchicine molecule are formed from phenylalanine.

In order to determine the site of inclusion of the generally labeled tyrosine (IV, Scheme 2), in the colchicine molecule, the latter was converted by a known method [11] into colchicinic acid (V). The methyl ester of this acid was oxidized with potassium permanganate. Radioactive trimellitic acid (VII) was isolated.



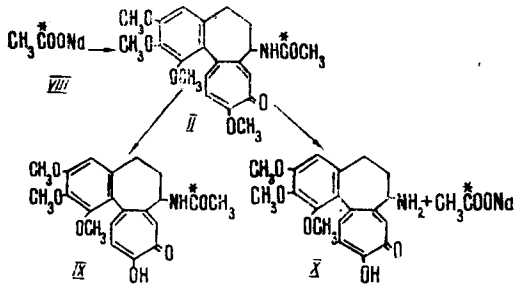
Scheme 2

The isolation of this acid shows the inclusion of the generally labeled tyrosine into the colchicine molecule with the formation of its tropolone ring, which is in harmony with literature information. The colchicine was hydrolyzed in an alkaline medium [12] to determine the position of inclusion of the sodium acetate in its molecule. This gave colchiceine (IX, Scheme 3) which had retained the initial radioactivity of the colchicine. Another part of the radioactive colchicine was subjected to more far-reaching hydrolysis in acid solution [13]. The deacetylcolchiceine (X) isolated proved to be nonradioactive. Radioactivity was

TABLE 2. Specific Radioactive (counts/min · mmole) of the Decomposition Products of Colchicine

Precursors added	Colchicine	Decomposition products	Activity found, %
[1-^{14}C]Phenylalanine $1.75 \cdot 10^8$	$1.62 \cdot 10^6$	Succinic acid $2.99 \cdot 10^5$	19.04
Generally labeled tyrosine $5.22 \cdot 10^7$	$1.26 \cdot 10^5$	Trimellitic acid $2.96 \cdot 10^4$	23.5
Sodium [1-^{14}C]acetate $1.17 \cdot 10^8$	$5.06 \cdot 10^5$	Sodium acetate $1.64 \cdot 10^4$	3.0

detected in the acid mother solution containing acetic acid:

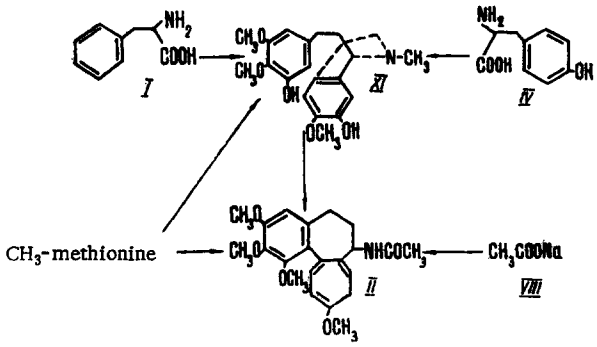


Scheme 3

Thus, we have shown that the radioactivity is concentrated in the N-acetyl group of the colchicine molecule.

The specific radioactivities of the products of the degradation of colchicine can be judged from the figures of Table 2. What has been said above shows the common nature of the biosynthesis of colchicine in species of autumn crocus and Merendera.

The biosynthesis of colchicine in M. raddeana can be represented in the form of a generalized scheme through a phenethylisoquinoline base of type (XI) (Scheme 4), which corresponds to the results reported in the literature for other species of plants.



Scheme 4

In view of the inadequate amount of labeled merenderine, the decomposition of this alkaloid was not performed. At the same time, the inclusion of phenylalanine and tyrosine in the merenderine molecule shows that these amino acids are also precursors for this alkaloid. The noninclusion of sodium acetate in the merenderine molecule can be explained by absence of an N-acetyl group in it.

On studying the question of the site of formation of the alkaloids in Merendera raddeana, we supplied part of the plants with [1-^{14}C]phenylalanine through the stem, after having first removed the corms. Radio-

active colchicine, 2-demethylcolchicine, and merenderine were obtained. Consequently, it may be assumed that the biosynthesis of the alkaloids in this plant takes place in the epigeal parts as well as in the corms.

EXPERIMENTAL

Supply of Precursors to the Plants. Flowering plants were supplied in crystallizing dishes with solutions containing the various compounds as follows: 240 whole plants and 20 plants without corms and root systems were supplied with [1-^{14}C]phenylalanine (26.4 mg) with a specific activity of $1.75 \cdot 10^8$ counts/min · mmole; 20 plants were supplied with generally labeled tyrosine (0.081 mg) with a specific activity of $5.22 \cdot 10^7$ counts/min · mmole; and 220 plants were supplied with sodium [1-^{14}C]acetate (19 mg) with a specific activity of $1.17 \cdot 10^8$ counts/min · mmole. The plants were supplied additionally with Knopp's solution.

After seven days' exposure, the plants were carefully washed with water and separated into three separate parts – corms, the sheaths of the corms, and the epigeal organs (flowers, leaves, and stems). The parts of the plants were fixed at 110°C for 15 min and were dried to constant weight at 80°C.

Isolation of the Alkaloids. The dried and comminuted parts of the plant were extracted with methanol. The combined alkaloids were isolated and separated into individual fractions by a method described previously [10]. The qualitative composition of the alkaloid fractions were studied by thin-layer chromatography on alumina in the chloroform–methanol (24 : 1) system, the spots being revealed with iodine vapor. The fractions of neutral-phenolic alkaloids contained colchicine (the main component, R_f 0.57), 2-demethylcolchicine (R_f 0.40), and, in very small amount, β-lumicolchicine (R_f 0.83), N-formyldeacetylcolchicine (R_f 0.49), and 2-demethyl-β-lumicolchicine (R_f 0.43), and also substances with a nonmethylated tropolone hydroxyl (R_f 0.00). After the methylation of this fraction with diazomethane and its purification by chromatography on alumina, colchicine insignificantly contaminated with other substances was obtained.

The fractions of basic-phenolic alkaloids contained mainly merenderine (R_f 0.57) and a small amount of unknown bases.

The colchicine, 2-demethylcolchicine, and merenderine isolated by thin-layer chromatography in the individual state proved to be radioactive. The radioactivity of the alkaloids was measured in an end-window counter.

Oxidation of [7-^{14}C]Colchicine to Succinic Acid. With constant stirring, 1.24 g of chromium trioxide was added to a solution of 132 mg of colchicine (specific activity $1.62 \cdot 10^6$ counts/min · mmole) isolated from the plants supplied with [1-^{14}C]phenylalanine in 8 ml of 25% sulfuric acid. The mixture was heated in the water bath for 1 h and was left at room temperature for 3 h. The excess of oxidizing agent was destroyed with methanol. Then the acid solution was extracted with ether. After the drying of the ethereal extract and the distillation of the solvent, the residue was crystallized from acetone, giving 7 mg (18.3%) of succinic acid with mp 189°C and a specific activity of $2.99 \cdot 10^5$ counts/min · mmole). This acid was additionally identified by paper chromatography [butan-1-ol–formic acid–water (40 : 10 : 50) system; R_f 0.76] together with an authentic sample.

Formation of Trimellitic Acid from Colchicine Labeled Generally in the Tropolone Ring. Colchicinic Acid. Nonradioactive colchicine was added to 13 mg of colchicine having a specific activity of $4.86 \cdot 10^5$ counts/min · mmole isolated from the plants supplied with generally labeled tyrosine to give a total sample of 50 mg. The product obtained, with a specific activity of $1.26 \cdot 10^5$ counts/min · mmole, was dissolved in 3 ml of absolute methanol, and a solution of sodium methoxide (100 mg of metallic sodium in 3 ml of methanol) was added. The mixture was boiled for 45 min, and the solvent was distilled off in vacuum. An aqueous solution of the residue was acidified with hydrochloric acid to pH 1 and extracted with chloroform. Crystals with mp 260-262°C deposited.

The methylation of colchicinic acid with diazomethane in methanol formed its methyl ester.

Trimellitic Acid. A solution of 46 mg of methyl colchicinate in a mixture of 0.5 ml of 13% nitric acid and 0.5 ml of acetic acid was heated in the water bath for 20 min. After cooling, a mixture of 160 ml of water, 2 ml of 2 N caustic soda, and 6.0 g of potassium permanganate was added to the solution until the purple coloration ceased to disappear. The excess of oxidizing agent was destroyed with methanol.

After the separation of the manganese dioxide, the aqueous filtrate was concentrated in vacuum. The residue was acidified with sulfuric acid to pH 1 and was extracted with ether, giving 26 mg of a mixture of

carboxylic acids with R_f 0.61 (oxalic acid) and R_f 0.82 (trimellitic acid). By their preparative separation using paper chromatography in the system given above, the trimellitic acid was isolated in the individual state with R_f 0.82. The substance was extracted from the corresponding chromatogram strips with ether in a Soxhlet apparatus. The yield of trimellitic acid was 14 mg (58.3%), mp 212-240°C, specific activity $3.6 \cdot 10^4$ counts/min · mmole. The acid was identified by its melting point and R_f value in comparison with an authentic sample. The latter was prepared from p-cymene by a known method [14].

Alkaline Hydrolysis of Colchicine Labeled in the N-Acetyl Group. A mixture of 3 ml of caustic soda solution and 24 mg of colchicine (specific activity $5.06 \cdot 10^5$ counts/min · mmole) isolated from plants supplied with sodium [1-^{14}C]acetate was heated in the water bath for 1 h. After extraction with chloroform, colchiceine retaining the original radioactivity of the colchicine was isolated.

Acid Hydrolysis of Colchicine Labeled in the N-Acetyl Group. A solution of 50 mg of radioactive colchicine with an activity of $5.06 \cdot 10^5$ counts/min · mmole in 2 ml of 10% hydrochloric acid was heated under reflux in the water bath for 12 h. Then the acid solution was brought to pH 9 with caustic soda and was extracted with chloroform. This gave 42 mg of a nonradioactive base identified in a thin-layer chromatogram as deacetylcolchiceine. The alkaline aqueous mother solution was evaporated to constant weight, and the radioactivity of the dry residue was determined.

SUMMARY

The biosynthesis of colchicine and merenderine in Merendera raddeana Rgl. has been studied.

The results of supplying the plants with [1-^{14}C]phenylalanine, generally labeled tyrosine, and sodium [1-^{14}C]acetate, and also of the degradation of the molecule of the colchicine isolated, have shown the general nature of the biosynthesis of this alkaloid in species of plants of the genera Colchicum and Merendera Ram.

The inclusion of labeled tyrosine and phenylalanine in the merenderine molecule shows that the tropolone and nontropolone alkaloids of Merendera have the same precursors but different biosynthetic routes.

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