An Isotopic Study of Nicotine Biosynthesis in Relation to the Krebs Tricarboxylic Acid Cycle*

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Certain intermediates in general metabolism are incorporated into the pyrrolidine ring of nicotine with minimal randomization of isotopic label. With the experimental system in steady state with respect to nicotine production, additions of intermediates which normally occur in very small pools (*e.g.* acetic acid) lead to relatively greater randomization of label than do additions of metabolites which normally occur in larger pools within the plant. Label from the carboxyl group of acetic, malonic, succinic, and citric acids and from positions 2 of alanine and 3 of β-alanine was equally divided between pyrrolidine ring positions 2 and 5. Label from position 2 of acetic and malonic acids and from positions 3 of alanine and 2 of β-alanine was found in pyrrolidine ring positions 3 and 4 up to 80% of total. Label from position 2 of succinic and fumaric acids and from position 3 of aspartic acid was divided equally between positions 2, 3, 4, and 5. These results are interpreted in a manner consistent with known operational aspects of the Krebs tricarboxylic acid cycle. Acetic acid and other Krebs cycle intermediates were also incorporated into the pyridine ring in a manner consistent with cycle involvement. Glycerol was utilized in pyridine ring biosynthesis by at least two pathways, one of which probably involved oxidation to acetate and entry of the latter *via* the Krebs cycle.

The experiments of Byerrum and his group (Wu *et al.*, 1962) have shown that glutamic acid is very likely the initial intermediate of a series of well-established biochemical reactions which leads to the pyrrolidine ring of nicotine (Leete, 1958). Glutamic acid is also a take-off product of the Krebs tricarboxylic acid cycle. Wu *et al.* (1962) have adduced strong evidence for the participation of this cycle in pyrrolidine ring biosynthesis by the tobacco plant. They have shown that incorporation of C^{14} from certain metabolites such as acetic, propionic, and aspartic acids may be interpreted readily in terms of both constitutive and operational aspects of the cycle.

Granting the validity of the above interpretations, a notable feature of these experiments and of those relating to the biosynthesis of the pyrrolidine ring of hyoscyamine reported by Bothner-by *et al.* (1962) is the clear indication of minimal recycling of certain of the added intermediates prior to their incorporation into the alkaloid molecules. This indication of a predominance of the synthetic over the degradative functions of the cycle at the loci of alkaloid biosynthesis is of interest owing to the fact that these loci are known to occur in the root tips of tobacco and of Datura, where general biosynthetic activity is at a high level (*cf.* Dawson, 1948; Mothes, 1959; and Dawson *et al.*, 1960c, for review). Of still greater interest, however, is the opportunity afforded through explorations of the extent of recycling of added intermediates to define still more clearly the detailed relations between pyrrolidine ring biosynthesis and the tricarboxylic acid cycle.

In the tobacco leaf the acetic acid pool is known to be extremely small (Hoskin *et al.*, 1953), whereas the pool, *e.g.*, of succinic acid may approximate 0.06% of the dry weight of the leaf (Palmer, 1955). Under steady state conditions, addition of succinate to a biosynthetically active system might be expected to result in less disturbance of cycle kinetics than addition of acetate. Additions of intermediates which convert slowly to acetate might be expected to avoid such disturbance. One consequence of kinetic perturbation, when it occurs, would be a tendency toward extensive recycling of the added intermediate leading to relatively diffuse patterns of isotope distribution within the pyrrolidine ring. Conversely, if disturbance of the steady state is avoided, much more specific distribution patterns should be observed.

We present data herein which confirm the above expectations with respect to the entry of carbon-14 from specifically labeled intermediates into the pyrrolidine ring of nicotine. The requirement for steady state kinetics was met by suitable manipulations (Dawson, 1960) of excised tobacco root cultures. Estimation of radioisotope concentrations at various positions in the pyrrolidine ring was facilitated as a consequence of the fact (Dewey *et al.*, 1955; Leete, 1955; Leete and Siegfried, 1957) that a symmetrical intermediate stands between glutamic acid and the nicotine pyrrolidine ring. Furthermore, Wu *et al.* (1962) have shown that label from intermediates of the glycolytic and tricarboxylic acid cycle pathways is incorporated to a negligible extent by the pyrrolidine-*N*-methyl group of nicotine. Thus, the distribution of isotopic carbon within the ring is established simply by ascertaining the amount present in any one of the four ring positions. The activity of each carbon of the pyrrolidine ring can be obtained by difference, since the activity of the methyl group is negligible and that of C-2 = C-5 and C-3 = C-4 in all known cases of these compounds in nicotine biosynthesis. The activity of C-2 is easily obtained by decarboxylation of nicotinic acid obtained by oxidation of the appropriate nicotine.

Additional data are presented which bear upon the question of the pathway of pyridine ring biosynthesis. A tentative hypothesis is presented which involves Krebs tricarboxylic acid cycle intermediates.

EXPERIMENTAL

The biosynthesis of nicotine by excised tobacco (*Nicotiana tabacum* L, var. Turkish) roots in sterile culture, the isolation from these roots of nicotine as the picrate, the oxidation of nicotine to nicotinic acid, and the dry combustion and mass spectrometric analysis

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TABLE I
"ACETATE GROUP" COMPOUNDS AND GLYCEROL

Compound	Concn. in Medium (μg/ml)	Total Radiochemical Yield (%)	% of Total in Pyridine Ring	% of Total in Pyrrolidine Ring	% of Pyrrolidine Activity in C-2	% of Total in Pyrrolidine C-2
Acetate-1-C^{14}	0.35	0.29	1	92	51	47
Acetate-2-C^{14}	0.28	0.76	38	61	11	7
Malonate-1-C^{14}	3.80	0.08	1.2	86	50	43+
Malonate-2-C^{14}	3.17	0.20	32	65	15	9.5
Alanine-1-C^{14}	5.0	0.0004	—	—	—	—
Alanine-2-C^{14}	1.5	1.19	1.7	95	40	38
Alanine-3-C^{14}	6.6	5.0	28	72	10	7
β-Alanine-1-C^{14}	5.2	0.0002	—	—	—	—
β-Alanine-2-C^{14}	2.5	6.33	40	60	13	8
β-Alanine-2-C^{14}	2.93	4.0	30	69	13	9
β-Alanine-3-C^{14}	4.53	1.40	0.3	96	41	39
α-Alanine-$C_3{}^{14}$	0.06	1.8	30	67	28	19
Glycerol-1-C^{14}	1.23	0.94	50	49	12	6
Glycerol-2-C^{14}	3.1	2.13	43	53	42	22
Pyruvate-2-C^{14}	1.92	0.48	—	98	54	53.5

of N^{15}-containing compounds have been described elsewhere (Dawson *et al.*, 1960a). Radiochemical analyses were performed by means of dry combustion and proportional gas counting of CO_2 (Christman *et al.*, 1955).

Decarboxylation of Nicotinic Acid.—Between 30 and 50 mg of nicotinic acid is dissolved in water with less than one equivalent of carbonate-free aqueous NaOH and a slight excess of $CaCl_2$, and the solution is taken to dryness by means of a stream of dry N_2 gas in a quartz tube provided with a standard taper joint. This tube is connected to a radiator trap provided with a stopcock at each side, then to a second radiator trap which is part of a vacuum gas handling system (Christman *et al.*, 1955). After the system is pumped until the residue is thoroughly dry, the first trap is cooled with a dry-ice slush and the second with liquid N_2. Then the solid material is heated carefully with a burner for several minutes until the residue has turned black. After the tube has cooled, the CO_2 in the second trap is purified by gas chromatography, retrapped with liquid N_2, and introduced into a gas counting tube (Christman, 1961). The yield of CO_2 is 30–40%. Pyridine present in the first trap is distilled by means of liquid N_2 on a small vacuum manifold into several cc of a solution of picric acid in methanol. Then the container is brought to 1 atm. with N_2 gas, the tube removed from the vacuum line and warmed, and the pyridine picrate obtained by refrigeration and subsequent removal of the supernatant methanol. After one recrystallization from methanol, the picrate is sufficiently pure for combustion and for radioactivity analysis to obtain the activity of the pyridine ring alone.

Degradation to Pyridine Ring Carbon-2.—Nicotinamide methiodide is oxidized to the corresponding 2-pyridone as previously described (Dawson *et al.*, 1960a). This material is reduced to the *N*-methyl-2-piperidone-3-carboxamide by reduction with H_2 at 1 atm. in acetic acid solution, half the sample weight of 5% Rh on alumina being used as catalyst. The reduction takes place within about 30 minutes, and *N*-methyl-2-piperidone-3-carboxamide is isolated by filtration, removal of the acetic acid *in vacuo*, and crystallization of the residue from ethanol and a little ether. The compound melts at 140–1° (uncorrected). The yield is about 80%.

Anal. Calcd. for $C_7H_{12}O_2N_2$: C, 53.83%; H, 7.74%; N, 17.94%; O, 20.49%. Found: C, 54.08%; H, 7.88%; N, 17.78%; O, 20.53%.

This material is hydrolyzed by refluxing for 12 hours with excess 6 N HCl, then taking the solution to dryness *in vacuo*. Chloride ion is removed by taking the residue up in water and treating it with small portions of thoroughly washed IRA-401 (OH^- form) until the solution has a *p*H of 3. Removal of the water and crystallization of the residue twice from methanol-ether gives colorless crystals of 2-(γ-methylaminopropyl)-malonic acid, m.p. 185–90° (decomposition) (yield about 45%).

Anal. Calcd. for $C_7H_{13}O_4N$: C, 47.99%; H, 7.48%; N, 8.00%. Found, C, 47.91%; H, 7.38%; N, 8.10%.

The compound is introduced into a tube similar to that used for the decarboxylation of nicotinic acid and is placed on a vacuum line with two traps, as before. Heating of the reaction vessel to about 200° produces CO_2, which is captured in the second radiator trap by liquid N_2 and treated as previously described. The remaining δ-methylaminovaleric acid ring closes under these conditions to produce *N*-methyl-2-piperidone (Ruzicka, 1921), which is contained in the first trap. Its hydrolysis with HCl produces δ-methylaminovaleric acid again, and the deamination and subsequent carbon-by-carbon degradation of this compound will yield a specific degradation of the pyridine ring. The activity of the 2-position is obtained by difference of the malonate decarboxylation result from that obtained by decarboxylation of the nicotinic acid itself (Dawson *et al.*, 1960a).

In the present case, the specific activity of the carboxyl carbon of nicotinic acid obtained from nicotine produced during feedings of β-alanine-2-C^{14} was 762 mμc/mmole. The activity of the CO_2 produced by decarboxylation of 2-(γ-methylaminopropyl)-malonic acid from this system was 738 mμc/mmole. That of the original nicotinic acid itself was 3639 mμc/mmole, so that about 25% of the pyridine ring activity is apparently in the 2-position of the ring. The activity of C-2 of the pyridine ring, by difference, is 714 mμc/mmole.

RESULTS AND DISCUSSION

Radiochemical Yields.—The data (Tables I and II) show that a wide variety of smaller molecules in the category of general metabolic intermediates may be incorporated into nicotine in appreciable amounts. However, comparisons between different compounds with respect to over-all radiochemical yields (ratio of total activity recovered as nicotine to total activity

TABLE II
"SUCCINATE GROUP" COMPOUNDS

Compound	Concn. in Medium (μg/ml)	Total Radiochemical Yield (%)	% of Total in Pyridine Ring	% of Total in Pyrrolidine Ring	% of Pyrrolidine Activity in C-2	% of Total in Pyrrolidine C-2
Succinate-1-C^{14}	3.6	0.04	14.9	85.7	15.1	12.9
Succinate-2-C^{14}	4.72	0.42	49	48	25	12
Fumarate-2-C^{14}	1.57	0.44	55	45	25	11
l-Aspartate-2,3-C^{14}	2.23	0.40	51	47	32	15
d,l-Aspartate-3-C^{14}	5.03	1.10	50	45	27	12
d,l-Aspartate-4-C^{14}	4.37	0.0025	—	—	—	—
Aspartate-C_4^{14}	0.25	0.57	43	53	26	14
		Miscellaneous Compounds[a]				
Aspartate-N^{15}[b]	5.0	4	50	50	—	—
Glycine-2-C^{14}	7.8	4.33	0.07	~100	—	—
Citrate-1-C^{14}	6.1	0.06	0.15	100	3	3
Glucose-1-C^{14}	0.17	0.05	40	63	4	2.5
Glucose-6-C^{14}	0.37	0.12	52.5	48	19	9

[a] Other compounds which were tested but which supported radiochemical yields in nicotine of less than 0.0% included mevalonic-2-C^{14} acid, β-hydroxy-β-methyl glutaric-β-C^{14} acid, glycine-1-C^{14}, and sodium carbonate-C^{14}. [b] The approximate mean excess of N^{15} present in the tobacco roots and culture fluids was 2%, and this is also the concentration in each ring of the nicotine produced.

fed) are not especially useful owing to differences in rates of utilization of these compounds in the available pathways of metabolism and to some variations in the experimental system.

In an earlier paper (Dawson *et al.*, 1960b), we demonstrated that in our experimental system substrate concentration effects upon radiochemical yield were small. Glycerol-1-C^{14} was supplied at concentrations of 1.23 and 3.10 μg per ml of culture medium. The radiochemical yields (Table I) were identical. The concentration variable was not regulated otherwise in the present experiments.

Malonic acid was obtained commercially under the listing malonic-1,3-C^{14} acid. However, none of the molecules was actually doubly labeled, and a more appropriate designation would be malonic-1-C^{14} acid. The distinction is important because malonic-1-C^{14} acid supported a radiochemical yield approximately one-half as great as that supported by malonic-2-C^{14} acid. One may conclude that malonic acid is converted in a symmetrical fashion to a C_2 intermediate with some of the biosynthetic properties of acetic acid (*cf.* Table I). If the malonic-1,3-C^{14} acid actually had been doubly labeled the interpretation would differ substantially. Similar considerations affect the interpretation of results obtained with glycerol-1,3-C^{14}. Only one position in the molecule was labeled. We have used the designation glycerol-1-C^{14} accordingly. The ratios of radiochemical yields in Table III have been corrected to reflect these facts, but actual radiochemical yields are given in Tables I and II.

The carboxyl carbon of alanine and of β-alanine did not reappear in nicotine. Selective conservation of carbon atoms in positions 2 of β-alanine and 3 of alanine suggests that both compounds were oxidatively deaminated so that the C atoms to which the amino group was originally attached became the carboxyl carbon of the probable C_2 intermediate. Comparisons of the relative quantities of isotopic carbon contributed to nicotine from different positions in the precursor molecules yield the results contained in Table III.

Distribution of Label Between Rings.—The data of Tables I and II show a tendency for label from C_4 precursors and glycerol to enter the pyridine and pyrrolidine rings almost equally, and for C_2 and C_3 precursors other than glycerol to enter the pyrrolidine ring preferentially. The data also show a somewhat greater relative incorporation of label into the pyridine ring from the methylene groups of the C_4 acids than from the methyl group of acetic acid, carbon-2 of malonate, carbon-3 of alanine, and carbon-2 of β-alanine. Assuming that the C_3 compounds are converted first to C_2 compounds, the results suggest that 2-carbon and 4-carbon intermediates are involved in the biosynthesis of both rings but that circumstances may alter the relative contributions made by each.

When aspartic acid-N^{15} was added to the cultures, the results indicated that the nitrogen label was equilibrated during the culture period with the general nitrogen pool. The N^{15} content of nicotine as isolated and of the nicotinic acid produced from it by degradation were the same and were approximately equal to the average N^{15} content of all nitrogen present in the system. It appears therefore that the nitrogen atoms in both rings arose from the general nitrogen pool and not from some specific metabolite of aspartic acid such as β-alanine, for example.

Distribution of Label Within the Pyrrolidine Ring.—Approximately 90% of label acquired from the carboxyl positions of acetic, malonic, succinic, and citric acids and from positions 2 of alanine and 3 of β-alanine entered the pyrrolidine ring. Between 40 and 50% of this label was recovered from pyrrolidine ring position 2 except in the cases of succinic and citric acids, where the amount recovered was much less.

TABLE III
A COMPARISON OF THE RELATIVE AMOUNTS OF CARBON-14 INCORPORATED INTO NICOTINE FROM PRECURSOR MOLECULES LABELED IN DIFFERENT POSITIONS

	Positions Compared	Ratios of Radiochemical Yields
Acetic acid	2 and 1	3:1
Malonic acid	2 and 1 or 3	1:1[a]
α-Alanine	2 and 1	3000:1
	3 and 2	4:1
β-Alanine	2 and 1	25,000:1
	2 and 3	4:1
Glycerol	2 and 1 or 3	1:1[a]
Succinic acid	2 and 1	10:1
Aspartic acid	3 and 4	440:1

[a] Radiochemical yield of C-1-labeled form multiplied by 2 to weight figures for absence of label in C-3.

On the other hand, label acquired from position 2 of acetic and malonic acids and from positions 3 of alanine and 2 of β-alanine entered to the extent of 10 to 15% in ring position 2. Label entering from position 2 of succinic and fumaric and from position 3 of aspartic acids occurred in the pyrrolidine ring to the extent of about 50% of the total, but of this only about 25% occurred in ring position 2.

Our figures for distribution of label between pyridine and pyrrolidine rings and for incorporation of label into pyrrolidine ring position 2 from acetic acid and from aspartic-3-C^{14} acid agree very closely with figures reported by Wu *et al.* (1962). Likewise, our figures for incorporation of label from position 2 of β-alanine agree very closely with those for the incorporation of label from position 2 of propionic acid as reported by the same workers.

Although the general trend is clearly one of conversion of C_3 compounds to C_2 compounds and the incorporation of C_2 and C_4 compounds into the pyrrolidine ring of nicotine, there are some interesting variations. These variations can be viewed in terms of certain operational parameters of the Krebs tricarboxylic acid cycle.

For example, two carbon atoms of malonic acid are incorporated into nicotine with roughly equal radiochemical yields (assuming loss of half of the activity from malonic-1-C^{14} acid). On the other hand, the carbon atom in position 2 of acetic acid is incorporated into nicotine slightly more than twice as readily as the carbon atom in position 1. This does not make it less likely that malonic acid is converted to acetate prior to its incorporation into the pyrrolidine ring. Rather it may mean that malonic acid is converted to acetic acid at a sufficiently slow rate to avoid appreciable change in steady-state concentrations of acetate. Under such conditions, one may find that added precursor is recycled to a minimum extent before being taken off in the form of α - ketoglutarate (Wu *et al.*, 1962; Bothner-by *et al.*, 1962). Conversely, if acetate is added to the cultures in sufficient quantity, or if another additive is converted rapidly to acetate, then the steady-state concentration of the latter would be seriously disturbed and more or less extensive recycling could occur as a result. In this instance, there would be an accumulation of label in reservoirs of, *e.g.*, succinate or malate. Label from the latter could then be fed into the precursors of the pyrrolidine ring in a somewhat more complicated fashion. Thus, one might expect an attrition of carboxyl-C^{14} due to decarboxylation steps at the oxalosuccinate and the α-ketoglutarate stages. By this means, unequal incorporation of the two carbon atoms from acetate and of those from compounds rapidly convertible to acetate may be given what appears to be a simple explanation.

It is possible also to explain the distribution of label within the pyrrolidine ring by recourse to the citric acid cycle. For example, the occurrence of 40 to 50% of pyrrolidine label in ring position 2 would follow if carboxyl-labeled acetate were to unite with nonlabeled oxaloacetate to produce citric-1-C^{14} acid. Assuming that the Ogston effect prevails in the tobacco root, citric acid so labeled should yield α-ketoglutaric-5-C^{14} acid. The latter would yield in turn a symmetrical pyrrolidine precursor containing 50% of its label in position 2 and the other 50% in position 5. This result should be essentially unaffected by a substantial amount of recycling of acetate for the reason that decarboxylation reactions would result in loss of label rather than intramolecular distribution. Wu *et al.* (1962) have arrived at similar conclusions.

Likewise, if succinic-2-C^{14} acid is supplied, if this acid enters the normal endogenous pool, and if it then enters the citric acid cycle by reaction with nonlabeled acetate without seriously affecting steady state conditions (*i.e.*, without recycling), the pyrrolidine ring of nicotine should contain 25% of its total label in each of positions 2, 3, 4, and 5. This outcome was also realized in the present investigation.

When acetic-2-C^{14} acid was supplied to the cultures, distribution of label within the pyrrolidine ring was such as to suggest either substantial recycling with consequent spreading out of label and/or a substantial amount of label in the *N*-methyl group. Wu *et al.* (1962) have shown that only 3% of label incorporated into nicotine from acetic-2-C^{14} acid enters the *N*-methyl group under their conditions. Therefore, the data are consistent with the concept of recycling to form uniformly labeled succinate. The latter, upon conversion to oxaloacetate, could accept acetic-2-C^{14} acid and thus lead in the simplest case to α-ketoglutarate with *ca.* 12.5% of label in positions 2 and 5, respectively, and 37.5% in each of positions 3 and 4. The figures with respect to relative amount of label recovered from position 2 are consistent with this interpretation.

Only 12% of the total activity in the pyrrolidine ring was recovered from position 2 when label was in position 1 of glycerol. Wu *et al.* (1962) reported very similar figures for distribution of label within the pyrrolidine ring and between the two rings. However, they did not observe the higher radiochemical yield for incorporation of label from glycerol-2-C^{14}. As noted by these workers, glycerol is incorporated in a manner such that the carbon atom at position 2 becomes the carboxyl carbon of, for example, acetate. However, another even more significant pathway appears to lie between glycerol and the pyridine ring (cf. Table I).

Distribution of Label Within the Pyridine Ring.—Excepting glycerol, the compounds used in these experiments also entered the pyridine ring in two discernible groups, the C_2 ("acetate") group and the C_4 ("succinate") group (*cf.* Tables I and II). We have suggested (Dawson and Christman, 1961) that the carbon atoms of positions 1 through 4 of the pyridine ring may arise from an intermediate related to citric acid in a manner such that the carbon of position 3 of citrate becomes the carbon of pyridine ring position 3 of nicotinic acid and subsequently of nicotine. This view is supported by the findings of Griffith *et al.* (1960) and of Byerrum and Griffith (1961) that about 50% of the pyridine ring activity acquired from acetic-2-C^{14} acid and from β-alanine-2-C^{14} is located in ring position 3, and by our observation that about 25% of the pyridine ring activity acquired from β-alanine-2-C^{14} is located in ring position 2. If citric acid were converted symmetrically into nicotinic acid (a proximal precursor of the nicotine pyridine ring) by a pathway which avoids the Ogston effect, the remaining 25% of label should occur in ring position 4. We are developing a degradation scheme capable of affording an answer to this question. It may be noted that the citric acid hypothesis has the advantage of accounting for the branched carbon skeleton of nicotinic acid. If aromatization should occur prior to loss of citric acid carboxyl groups, it would appear that quinolinic acid is not a likely intermediate on the path to nicotinic acid. Tritium-labeled quinolinic acid (Wilzbach labeling) is not incorporated into nicotine in our experimental system. We have not yet tested labeled cinchomeronic acid.

Label from glycerol is incorporated into the pyridine ring in such a manner (Table I) as to lead to the conclusion that there must be two pathways involved. One is probably through acetic acid, in which case the

carbon atom in position 2 of glycerol becomes a carboxyl carbon. However, label from position 2 of glycerol is incorporated much more extensively than would be the case if this were the only pathway. It seems reasonable to suppose, in the light of the previous discussion, that some of the glycerol may be incorporated into positions 5 and 6 of the pyridine ring via a C_2 unit such as glycolaldehyde. If glycolaldehyde and citric acid, or related compounds, are indeed the precursors of the pyridine ring of nicotinic acid and hence also of nicotine, it is evident that glycerol may provide, at least indirectly, all of the carbons of this ring. Ortega and Brown (1959) have shown that glycerol is a sufficient precursor for nicotinic acid biosynthesis in *Escherichia coli*, but our data appear to exclude the possibility that the route of incorporation of glycerol involves simply a combination of two C_3 units, at least in the tobacco plant.

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The Biochemical Transformation of the Morphothebaine to the Morphine Ring System*

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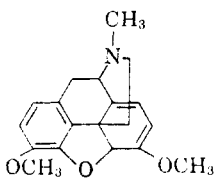
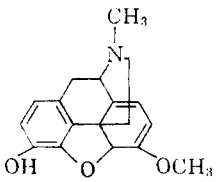
Carbon-14-labeled tyrosine supplied to seedlings of oriental poppy (*Papaver orientale* L.) was incorporated into thebaine, oripavine, and isothebaine. By removing labeled precursor at a critical stage in seedling development, it was possible to show that label from isothebaine can be transferred to thebaine and to oripavine. Spectrophotometric evidence is presented which indicates a shift from isothebaine to thebaine and oripavine production by the seedlings. It is concluded that the morphothebaine ring system can be converted *in vivo* to that of the morphine type.

The oriental poppy (*Papaver orientale* L.) produces three main alkaloids. Two of these, thebaine and oripavine, have the hydrophenanthrene nucleus of the morphine alkaloids, and the third, isothebaine, has a morphothebaine ring system.

In 1958 (unpublished work) we investigated the alkaloid content of germinating seeds of this poppy. If suitable extracts were made of the seeds themselves and of the seedlings after only a few days of growth, paper chromatography of these extracts showed only two spots which on elution and measurement of the ultraviolet spectra could be identified as isothebaine and another phenolic alkaloid which we assume to be oripavine. No indication of thebaine appeared at this time. If the germinated seedlings were grown for one or two weeks a new spot appeared on the chromatogram of the extract which could be identified by its spectrum to be thebaine.



Thebaine (I)



Oripavine (II)
Konovalova and Kiselev (1948)

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