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In 1958 (unpublished work) we investigated the alkaloid content of germinating seeds of this poppy.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.
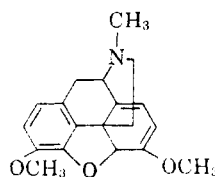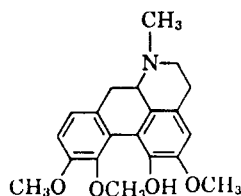

Thebaine (I)
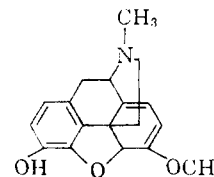
Oripavine (II)
Konovalova and Kiselev
(1948)

Isothebaine (III)
Bentley and Dyke (1957)

This unexpected result indicated the possibility of conversion of the relatively more stable ring system of isothebaine to the less stable system of thebaine during plant development. Since this interpretation runs counter to general expectation, we have reinvestigated the phenomenon using isotopic tracer.

RESULTS AND DISCUSSION

When the germinating seeds were supplied with tyrosine-2-C^{14} in aqueous solution for a period of 2 weeks, all three alkaloids became radioactive (Tables I, II). Battersby and Harper (1958, 1960) and Leete (1958, 1959) have shown that labeled tyrosine is incorporated into morphine, codeine, and thebaine in *Papaver somniferum*. Our data demonstrate that tyrosine is a building block for alkaloids with the morphothebaine ring structure as well as for those with the morphine nucleus.

If the plants were washed free of unabsorbed tyrosine after two weeks of growth (experiment II, Table II) and were left to develop for an extra week, there was observed a distinct shift from isothebaine in the direction of the other two. This is shown by the changes in ratios of isothebaine to thebaine and to oripavine, respectively, in Table II.

When the seedlings were harvested two days after germination (experiment III) the sprayed chromatogram showed no colored spot for thebaine. When the area of the paper where thebaine was usually found was eluted and the radioactivity counted a small amount of activity could be detected.

If the seedlings were washed free of tyrosine 2 days

after germination and then left to grow for another week, the radioactivity was distributed among all three alkaloids in virtually the same proportions as was the case when labeled tyrosine was supplied for a full week after germination. The data are given under experiment IV in Table II.

According to our estimates based upon ultraviolet extinctions, 10 g of seed contained 5.43 × 10^{-3} mg isothebaine. The amounts of thebaine, if present, were too small to measure. After 2 weeks of germination, the seedlings obtained from 10 g of seed contained 0.11 mg of isothebaine and 0.35 mg of thebaine.

The indication is that tyrosine is used to form isothebaine first. The latter is then converted to thebaine and to oripavine by pathways which are presently unknown. The work of Stermitz and Rapoport (1961) affords an interesting additional point. They supplied radioactive thebaine to plants (rosette stage) of this species of poppy and noted a transfer of label to oripavine but not to isothebaine. It is reasonable to assume that thebaine was demethylated *in vivo* to yield oripavine. The failure of isothebaine to acquire a radioactive label, however, indicates that the conversion reported by us is not likely to be reversible. In the simplest case, therefore, the biosynthetic sequence appears to be isothebaine → thebaine → oripavine. The pathway and mechanisms involved in this transformation of ring systems will afford a most interesting and enlightening subject for future investigations.

EXPERIMENTAL

The Experimental System.—Twenty-four grams of seeds were surface sterilized by immersion in a 50% solution of a commercial sodium hypochlorite preparation (Clorox) for 15 minutes. They were then washed several times with sterile distilled water and distributed into 12 sterile Petri dishes. Each Petri dish contained a filter paper liner and 8 ml of sterile distilled water. After 5 days at room temperature, the seeds began to germinate. They were then supplied with a few ml of labeled tyrosine in distilled water.

In an illustrative situation, after 2 weeks, all 12 plates were washed many times with distilled water to remove any adhering tyrosine. Six of the dishes were then emptied of seedlings and the latter extracted for alkaloids. The remaining six dishes were left so that the seedlings might continue their growth for another 5 days (e.g., experiments I and II). The same procedure was used in experiments III and IV, but the time sequence differed.

Extraction and Separation of the Alkaloids.—The washed plants were ground in a mortar with a few ml of 20% acetic acid and left in the acid overnight. The solution was pressed through cheesecloth and made basic with ammonia. The alkaloids were extracted with ether in a separatory funnel. The ethereal solution was, in turn, extracted with 1% aqueous citric acid solution. The citric acid solution was made

TABLE I

INCORPORATION OF TYROSINE-2-C^{14} INTO TOTAL ALKALOID FRACTION

Experiment Number	Total Activity in Tyrosine (cpm)[a]	Total Period of Growth (days)	Tyrosine Washed Away After (days)	Activity in Total Alkaloids (cpm)[a]
I	3 × 10^5	14		1.35 × 10^4
II	3 × 10^6	20	14	1.04 × 10^4
III	3.8 × 10^5	8		7.01 × 10^3
IV	3.8 × 10^5	13	8	1.15 × 10^3

[a] All counts corrected for background.

TABLE II

SEPARATION OF ALKALOIDS OF EXPERIMENTS I–IV (TABLE I)

Experiment Number	Total Activity Chromatographed (cpm)[a]	Radioactivity in Individual Alkaloids			Ratios of Activities		
		Isothebaine (cpm)[a]	Thebaine (cpm)[a]	Oripavine (cpm)[a]	I/T	I/O	T/O
I	9203	1660	1998	2257	0.84	0.74	0.89
II	7702	849	1523	1687	0.56	0.50	0.90
III	3611	543	127	44	4.30	12.34	2.90
IV	783	74	117	121	0.63	0.61	0.97

[a] All counts are corrected for background.

alkaline with ammonia, and the alkaloids were extracted with ether. The dried ether solution was evaporated. The alkaloids were dissolved in a few drops of methanol and spotted on sheets of Whatman no. 1 filter paper buffered in 2% ammonium sulfate solution. The solvent system employed was a mixture of isobutanol (100), acetic acid (20), and water (ca. 40 parts to saturation) in terms of volume (Nadeau et al., 1958). The R_F value for isothebaine in this system is 0.69, that for thebaine 0.79, and for oripavine 0.94. Dragendorff's reagent was used to detect the spots. The spots were eluted onto planchets with formic acid solution to prepare the alkaloids for radioactivity assays.

Identification of Oripavine.—Twenty grams of seeds were extracted, and the alkaloids were separated as above. Only the spots with R_F 0.69 and 0.94 were obtained on chromatograms. The faster-running spot was eluted with acetic acid solution, the acid solution was made alkaline with ammonia, and the alkaloid was extracted with ether. The dried ethereal solution was treated with a small amount of diazomethane. After evaporation of the solvent, the residue was chromatographed on paper as described above. A spot at R_F 0.79 was obtained which was presumably thebaine.

Amounts of Alkaloids Involved.—Although the extraction cycles were not devised for quantitative recovery,

it is possible to ascertain the approximate amounts of the different alkaloids involved. Ultraviolet absorption spectra of chromatogram eluates were used as a basis for this assay, and the amounts of individual alkaloids were estimated with the help of extinction coefficients established for the purpose.

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Biological Activity of an l-Epimer of d-α-Tocopheryl Acetate*

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2l,4′D,8′D-α-Tocopheryl acetate, an l-epimer of natural d-α-tocopherol, was 21% as active as d-α-tocopheryl acetate in the resorption-gestation bioassay using vitamin E-deficient female rats. 2d,4′D,8′D-α-Tocopheryl acetate had essentially the same biological activity as d-α-tocopheryl acetate derived from natural sources. 2dl,4′D,8′D-α-Tocopheryl acetate synthesized from natural phytol and 2dl,4′DL,8′DL-α-tocopheryl acetate synthesized from racemic isophytol both had about 60% the activity of d-α-tocopheryl acetate. The epimeric configuration at the 2-position of α-tocopheryl acetate is apparently dominant in determining biological activity.

α-Tocopherol, the most active form of vitamin E, has optically active centers at the 2, 4′, and 8′ carbon atoms (see Fig. 1). Robeson and Nelan (1962) recently reported the isolation of a d-epimer (2d,4′D,8′D) and an l-epimer (2l,4′D,8′D) by fractionation of 2dl,-4′D,8′D-α-tocopherol via a piperazine complex. The d-epimer had practically the same physicochemical properties as natural d-α-tocopherol.

Bioassays of these two preparations are of special

interest, since knowledge of the biological activity of α-tocopherol has heretofore been limited to the naturally occurring d-α-tocopherol and to synthetic preparations, racemic at the 2-position and, in some cases, racemic also at the 4′- and 8′-positions. To add to the pertinent biological data, we are also reporting the bioassays of the partially separated mixtures that were examined during the progress of the fractionation studies.

EXPERIMENTAL

Synthetic Tocopherols.—Preparations of 2dl,4′D,8′D-α-tocopherol[1] were synthesized by condensing trimethylhydroquinone with natural phytol[2] by the procedure of Smith and Ungnade.[3] The reaction products
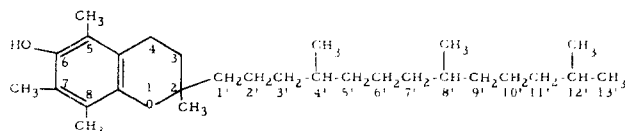

FIGURE 1

[1] Natural phytol contains two centers of optical activity with D-configurations (Burrell et al., 1959; Crabbe et al., 1959). Thus, the configurations at the 4′ and 8′ carbon atoms of α-tocopherol synthesized from natural phytol can be assigned a D-configuration.

[2] Isophytol and natural phytol were obtained from Charles Bowman and Company, N. Y.

[3] U. S. 2,411,942, Dec. 3, 1946.