# Biosynthesis of the Morphine Alkaloids

The major biosynthetic steps leading from tyrosine to morphine in the opium poppy have been elucidated.

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Complex organic molecules abound in nature, and the way in which they are synthesized in living organisms has been of interest to biochemists and organic chemists alike. Recently, organic chemists have studied the biosynthesis of many natural products with the use of radiotracer techniques (*1*). Special attention has been paid to the complex "secondary metabolites" (*2*) produced by plants and, to a lesser extent, by animals. In this article I shall illustrate the chemist's approach to biosynthetic problems by reviewing recent work on the morphine alkaloids.

## Structural and Chemical Arguments

Morphine, the major alkaloid of the opium poppy, *Papaver somniferum*, has the structure I. Our task is to discover how this complicated ring system is constructed in the poppy from the primary building units present in all organisms. A necessary preliminary to any biosynthetic study is the development of a working hypothesis based on structural and chemical arguments. Only then can fruitful tracer experiments be planned. In 1925, Gulland and Robinson (*3*) made the first important structural proposal. If bonds *a* and *b* in morphine are broken and the molecule is rotated (see structure I) then we obtain the structure II, which has a carbon skeleton resembling that of unsubstituted benzylisoquinoline III. Derivatives of the base III are well known and several have been isolated from opium. Winterstein and Trier (*4*) had earlier suggested that the benzylisoquinolines themselves could be derived from two eight-carbon units. Dihydroxyphenylacetaldehyde IV and dihydroxyphenethylamine (dopamine) V are especially suited to this purpose since condensations such as the reaction of IV with V to yield III can be carried out in the laboratory without the aid of an organism or enzyme. Both $C_8$ units, IV and V, might be derived ultimately from the essential amino acid tyrosine VI. Thus, our working hypothesis is that two molecules of tyrosine, a known (*5*) constituent of the poppy, are used to construct a benzylisoquinoline (as III) which in turn is elaborated to give the morphine ring system.

Battersby and Harper (*6*) and, independently, Leete (*7*) were the first to test this hypothesis experimentally. Tyrosine, containing $^{14}C$ at position 2, designated by the asterisk in VI, was fed to mature *Papaver somniferum* plants. After a suitable period for metabolism, morphine was isolated and found to be radioactive. The activity was retained even after rigorous purification of the isolated compound, and it must therefore have been contained somewhere in the morphine molecule. According to theory, radiocarbon from tyrosine-2-$^{14}C$ should reside only at two positions, C-9 and C-16, as indicated by the asterisks in I, in morphine. "Scrambling" of radioactivity throughout the alkaloid would be a trivial result indicating that the tyrosine had been broken into smaller fragments before incorporation. In fact, chemical degradation showed that all the activity in the radiolabeled morphine was at C-9 and C-16, each position containing approximately half the total. Moreover, a parallel experiment with dopamine-1-$^{14}C$ V gave (*8*) morphine labeled only at C-16, again supporting the biosynthetic theory. In these and later experiments only a small proportion (about 1 percent) of the labeled precursor was incorporated into the final alkaloid. This is not surprising and certainly does not invalidate the results. The precursor may be inefficiently absorbed by the plant, and, on its way to the actual site of alkaloid biosynthesis, may be converted into substances unrelated to the desired end product. For example, the plant may use tyrosine more efficiently for the synthesis of protein than for the synthesis of morphine.

It is convenient at this point to introduce two other opium alkaloids. Codeine VII and thebaine VIII contain, respectively, one and two methyl groups more than morphine does. However morphine is the end product rather than the progenitor of the series. This was shown in two ways. Battersby and Harper (*9*) fed tyrosine-2-$^{14}C$ to a large number of plants. Small batches were extracted from time to time, and the activities of the morphine alkaloids were determined. The activity from the tyrosine was passed first through thebaine VIII, then through codeine VII, and finally into morphine I. Stermitz and Rapoport (*10*) used a different and more direct method. They fed plants separately with labeled specimens of thebaine, codeine, and morphine. The radioactivity from thebaine passed into both codeine and morphine, while that from codeine passed into morphine but not into thebaine. Morphine itself was not converted into either of the other two alkaloids. With tyrosine and its derivatives firmly established as precursors, we can turn to the later stages of morphine biosynthesis.

## Benzylisoquinoline Precursors

Norlaudanosoline III provides the starting point for the elaboration of the complete morphine skeleton. Material labeled with $^{14}C$ at either C-1 or C-3, indicated by asterisks in III, was incorporated (*11*) into morphine with an efficiency greater than that observed for tyrosine. Again chemical degradation was used to show that the morphine was labeled only in the predicted (C-9 or C-16) positions. We have seen that thebaine VIII is the first morphine alkaloid to be formed in the plant. Let us assume that both the methoxyl (MeO) groups and the *N*-methyl (NMe) group in thebaine are already present in the benzylisoquinoline precursor. That is, norlaudanosoline must be methylated three times before further biosynthe-

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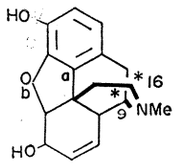

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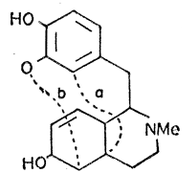

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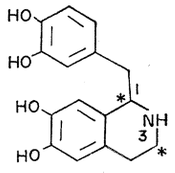

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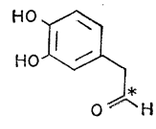

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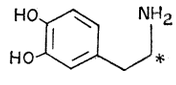

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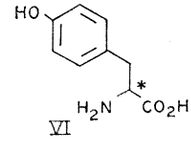


VI

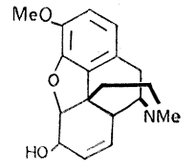
tic modification occurs. This assumption leads us to consider four compounds as possible thebaine precursors. One of these, reticuline, has the structure IX; the other three isomers differ only in the relative positions of the methoxyl and hydroxyl (OH) groups. A comparison of structures VIII and IX suggests at once that reticuline has the orientation of methoxyl groups required for conversion into thebaine, and the following consideration of reaction mechanisms supports this view. Accordingly, labeled reticuline was fed, and good incorporation into all three morphine alkaloids was observed (*12*). It could, however, still be argued that demethylation of reticuline, with regeneration of the known precursor norlaudanosoline had occurred. To test this point, reticuline, labeled with $^{14}C$ in all three methyl groups and at C-3 in the nucleus, was fed to poppies in the usual way. The activity ratios of the various labels in reticuline were known from the method of preparation and could be compared with those of the derived thebaine by appropriate degradation. With the use of the nuclear (C-3) activity as an internal standard, it was shown that no demethylation had taken place during biosynthesis. The precise methylation sequence leading from norlaudanosoline to reticuline is as yet ill-defined, but *N*-norreticuline (that is, reticuline without the *N*-methyl group) is also an efficient precursor of the morphine alkaloids.

The skeptical reader may at this point remark that almost any benzylisoquinoline will be incorporated into morphine if one tries hard enough to bring about such a result. Let me disillusion him. At various times all four of the isomers of reticuline mentioned above have been fed to *P. somniferum* (*13*). Only one of these, reticuline itself, was incorporated.

## Phenol Oxidation

In 1957, before any tracer experiments had been reported, Barton and Cohen (*14*) put forward a theory for the late stages of morphine biosynthesis. They based their arguments on the known chemical reactions of phenols with one-electron oxidizing agents, such as ferricyanide. Reticuline contains two phenolic hydroxyl groups and might be converted, by oxidation, into a diradical X which may be rewritten in the form XI. Coupling of the unpaired electrons, which are represented by a heavy dot in X and XI, would ultimately lead to the new structure XII. Only reticuline, and not its structural isomers, has the phenolic groups correctly disposed for this transformation. The hypothetical intermediate XII was not at that time a known compound but eventually a synthesis was devised from thebaine as the starting material (*15*). Samples were labeled, either with tritium ($^3H$) at C-1 or with $^{14}C$ at C-16 (XII), and fed separately to opium poppies (*12*). High incorporation into the morphine alkaloids was observed, and again no "scrambling" of the labels took place. Soon after the synthesis of the intermediate XII had been completed, the isolation of a new alkaloid, salutaridine, from *Croton salutaris* was reported (*16*). This natural substance was identical with the synthetic material.

Since salutaridine XII had never been detected in opium one might object that its conversion into the morphine alkaloids, though efficient, was not a normal biosynthetic process and had, in fact, been induced by feeding an unnatural precursor. This objection was easily overcome (*12*). Plants were injected with tyrosine-2-$^{14}C$, and after a few days the total plant alkaloids were isolated. Inactive salutaridine was added to this radioactive mixture and was then recovered and carefully purified. In this way even traces of radioactive salutaridine, formed in the plant from tyrosine-2-$^{14}C$, would be "diluted



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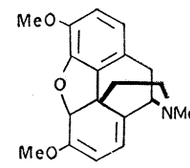

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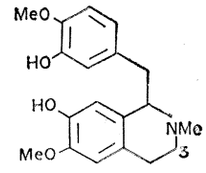

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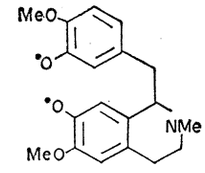

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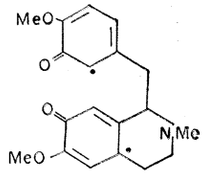

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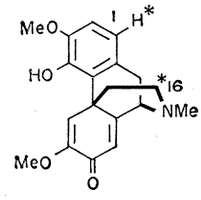
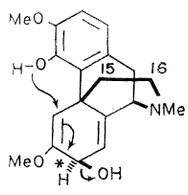


XII

out" of the mixture and retained in the recovered salutaridine. In fact, the recovered salutaridine was radioactive. Thus the conversions, tyrosine into salutaridine and salutaridine into morphine, have been separately established. The overall transformation, tyrosine into morphine, must therefore pass, at least some extent, through salutaridine. A similar dilution experiment, with radioactive norlaudanosoline as the primer, gave essentially the same result. Since then, salutaridine has been isolated (*17*) as a minor constituent of *Papaver orientale*, which also produces thebaine, and its importance in morphine biosynthesis seems established beyond doubt.

The transformation of salutaridine into thebaine requires the closing of one more ring. This can readily be done by chemical means (*15*). Reduction of salutaridine with sodium borohydride gives two alcohols, called salutaridinol-I and salutaridinol-II. They differ only in the spacial orientation (stereochemistry) of the new, alcoholic, hydroxyl group. In salutaridinol-I (XIII) the hydroxyl group is on the same side of the molecule as the bridge from C-15 to C-16; in salutaridinol-II the hydroxyl is on the opposite side (*18*), that is, H and OH are interchanged in XIII. Under mildly acidic conditions both alcohols lose the elements of water to give thebaine. The arrows in XIII

illustrate how bonds are formed and broken in this process. We cannot assume, of course, that biological processes must follow chemical equivalents. In this example the analogy is close but not complete. Both salutaridinols were labeled with tritium on the carbon bearing the new hydroxyl group, H*-C-OH in XIII. An internal reference label was introduced elsewhere in the molecule. It was found (*12*) that salutaridinol-I, but not salutaridinol-II, was converted very efficiently into thebaine in *P. somniferum*. Moreover, tritium was fully retained during biosynthesis, an indication that reconversion into salutaridine had not taken place (the transformation H*-C-OH into C=O demands complete loss of H*). It is not surprising to find that only one of the salutaridinols will serve as a thebaine precursor in vivo since enzymic processes are normally highly selective. Why the particular configuration XIII should be favored is not clear and merits further study.

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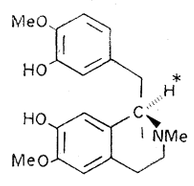


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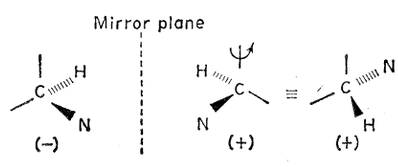


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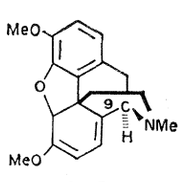


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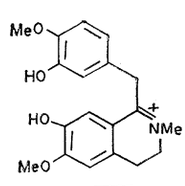


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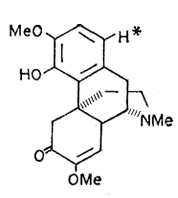


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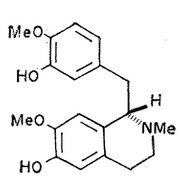


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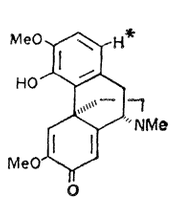


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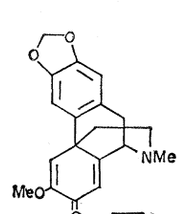
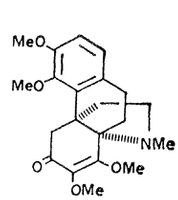



XXI




XXII

## Optically Active Precursors

An important feature of the structure of the reticuline molecule has so far been ignored. Reticuline contains one carbon atom, C-1 in XIV, which is attached to four different atoms or groups. Two different orientations of the atoms about C-1 are possible, each corresponding to a different optical isomer of reticuline. The two possible orientations are related to each other as an object to its mirror image (XV). The particular form of reticuline shown in formula XIV is designated (–)-reticuline and has the configuration about C-1 corresponding to that about C-9 in thebaine (compare XIV and XVI). It follows, therefore, that (–)-reticuline and not (+)-reticuline should be the precursor of thebaine.

In the feeding experiments described, synthetic (±)-reticuline was used, that is, an equal mixture of both optical isomers. However, Battersby and his colleagues (*13*) succeeded in resolving the mixture into its components and were then able to feed each isomer separately. Surprisingly, both isomers were incorporated into the morphine alkaloids with similar efficiency. This result showed that another, previously unsuspected, step must be involved in the metabolism of reticuline. The simplest explanation invokes a rapid and reversible conversion of (+)- and (–)-reticuline into the dehydro derivative XVII. This can be reduced back to each optical isomer of reticuline by the introduction of hydrogen either from above or below the plane of the molecule. In fact, dehydroreticuline (XVII) was found to be an excellent precursor for thebaine. Also, both (+)- and (–)-reticuline, labeled with tritium at C-1, asterisk in XIV, lost tritium during conversion to thebaine. The loss from (+)-reticuline was almost complete, but (–)-reticuline did retain appreciable and variable amounts of the isotope. This is understandable if (+)-reticuline is first converted into dehydroreticuline XVII with, of course, complete loss of tritium. Reduction then gives (–)-reticuline which, in turn, is oxidized to salutaridine XII. (–)-Reticuline can be converted into salutaridine directly, but the reversible process, the conversion of XIV to XVII and vice versa, causes some loss of tritium. The recent isolation of (±)-reticuline from opium, by Brochmann-Hanssen and Furuya (*19*), provides valuable confirmation of these ideas and also, of course, shows that reticuline is indeed a naturally occurring alkaloid in poppies. Further isolation studies, and experiments with plants grown in an atmosphere containing carbon dioxide–$^{14}C$, have shown (*20*) that both optical isomers of reticuline are present in *P. somniferum* in amounts that vary with the plant's age.

I now turn briefly to some alkaloids which do not occur in opium but are nonetheless structurally related to morphine. Sinomenine XVIII occurs in the climbing plant *Sinomenium acutum*. The structure of sinomenine resembles that of morphine but lacks one ring, contains one extra oxygen atom, and has the bridge from C-15 to C-16 on the opposite side of the molecule. Two benzylisoquinolines have been considered as likely precursors for sinomenine. (+)-Protosinomenine XIX is a structural isomer of (+)-reticuline and has the hydroxyl and methoxyl groups in the positions at which they appear in sinomenine [one hydroxyl group would become a carbonyl (C=O) group after oxidative ring closure]. (+)-Reticuline itself would, at least superficially, appear a less probable precursor. However, when tritium-labeled (±)-protosinomenine and (±)-reticuline were fed, side by side, to *S. acutum* only (±)-reticuline was incorporated into sinomenine (*21*). As customary, the labeled sinomenine was then degraded in the laboratory to show that no "scrambling" of the tritium had occurred. By analogy with the biosynthesis of thebaine, the optical isomer XX of salutaridine should be the next intermediate on the biosynthetic pathway from reticuline to sinomenine. A most timely reinvestigation of the alkaloids of *S. acutum* led to the isolation of this substance,

now called sinoacutine (*22*). Fortunately, it was possible to label the natural sinoacutine directly with tritium (*23*) (marked by an asterisk in XX) and to carry out the critical feeding experiment. Good incorporation of sinoacutine into sinomenine was observed (*21*), the tritium being located in the expected position (see XVIII) in the derived alkaloid. How this conversion is effected in the plant is still not known.

Amurine XXI from *Papaver nudicaule* (*24*) and hasubanonine XXII from *Stephania japonica* (*25*) represent other interesting variations of the morphine skeleton. Indeed, the rearranged ring system of hasubanonine provides a special challenge to theoreticians and experimenters concerned with biosynthesis of alkaloids. However, there is little doubt that these compounds also will eventually be related to one or other of the simple benzylisoquinolines.

## Summary

Tracer experiments, supported throughout by the analogous chemical transformations, have firmly established the biosynthetic sequence tyrosine →norlaudanosoline →reticuline →salutaridine →salutaridinol-I →thebaine →codeine → morphine in *Papaver somniferum*. In general, the farther a precursor lies along this sequence, the more efficient its conversion to morphine in the intact plant. Several intermediates remain to be discovered, such as those lying between tyrosine and norlaudanosoline and between thebaine and codeine. Proof that morphine is made only by the reticuline-salutaridine route is still lacking and would require a careful comparison of the rate of morphine synthesis with the turnover rates for the various intermediates. More importantly, detailed knowledge of the mechanism of each biochemical step can come only with isolation of the enzyme system involved. The chemical oxidation of (−)-reticuline, to give salutaridine, can only be accomplished in very low (0.02 percent) yield (*15, 26*), whereas, even with whole plants, the biological incorporation of reticuline into the morphine alkaloids can reach 8 percent (*13*). One would like to know just how an enzyme system directs the oxidative cyclization of reticuline in the desired sense.

Kleinschmidt and Mothes and Fairbairn and Wassel (*27*) have shown that the latex isolated from opium poppies is capable of transforming tyrosine into morphine. Perhaps further work with opium latex will provide the key to the remaining problems of morphine biosynthesis.

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