THE FORMATION OF ALKALOIDS IN MAMMALIAN TISSUES[1]

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Condensation of the amino acids L-dopa and L-tryptophan with acetaldehyde, a major metabolite of ethanol, may take place in mammalian systems. The following review summarizes the present knowledge on this interesting subject and includes the synthesis of a variety of substances which might be formed during such interactions.

Plants have long been considered to be the sole source of alkaloids. However, evidence obtained in recent years suggests that mammalian systems may be capable of synthesizing substances recognized as intermediates in the biosynthesis of certain plant alkaloids[2]. These findings are of considerable importance since some of these compounds exhibit significant pharmacological effects or may be converted into real active alkaloids by further biological transformations[2].

It is not easy to assess whether the so-called "biosynthesis" of (±)-salsolinol (II = SAL)[2] by direct condensation of dopamine (I) with acetaldehyde by Schöpf et al.[3,4] in vitro at pH 5 has biosynthetic significance, since SAL has never been detected in plants as yet. In contrast its 7-O-methyl ether, salsoline (III), has been isolated in racemic[5] and optically active form[6] (Scheme I).

Studies regarding the biosynthesis of isoquinoline Cactus alkaloids, such as (-)-anhalonidine (IV) of established absolute configuration[7], suggest that the biosynthesis of 1-methyl substituted tetrahydroisoquinoline alkaloids may be more complicated than originally assumed[3,4]. Kapadia et al.[8] have recently shown that the 1-methyl group of IV and related alkaloids might originate from pyruvic acid rather than from acetaldehyde.

Scheme I

Scheme I

I

II : R = H

III : R = Me

IV

V

Whereas organic chemists exploring the field of alkaloid biosynthesis became early aware of the importance of stereochemical and configurational features of starting materials, intermediates and final products, biochemists working with mammalian systems have only recently recognized the importance of such refined knowledge which may well be essential for the interpretation of biological data of alkaloids[9].

The first communication that precursors of alkaloids might be formed in mammalian tissues was presented by Holtz et al.[10] in 1964. This group reported that in experimental animals the hypertensive drug dopamine (I) was converted in the presence of a monoamine oxidase concentrate into an antihypertensive substance recognized as tetrahydropapaveroline (THP), (V=norlaudanosoline), easily convertible via O,N-methylation into well-known 1-benżylisoquinoline alkaloids[11].

Neither Holtz et al., nor Sandler et al., who discovered SAL and THP as urinary excretion products from patients treated with L-dopa[12], nor Collins et al., who reported the formation of SAL in the brain of rats after oral administration of L-dopa and ethanol[13], have reported whether or not these products were optically active. Such data could help to explain whether these substances are formed by enzymatic processes in mammalian tissues, intestinal

bacteria or by nonenzymatic condensations in the gastrointestinal tract. No substantial evidence has ever been presented so far that man could synthesize morphine or aporphine alkaloids per se or after treatment with *L*-dopa[14]. It is, however, interesting to note that Davis et al. have recently presented physicochemical evidence that small quantities of two isomeric tetrahydroxy berbines[2] were detected in the urine of patients treated with large quantities of L-dopa[15].

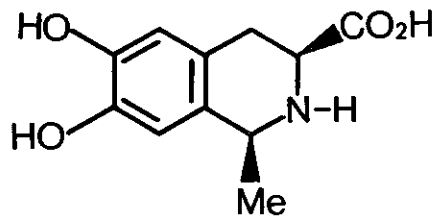
Whether the formation of these berbines originates from laudanosoline (N-methyl-THP), through biosynthetic sequences well established in plants[16] or from methionine, is open to speculation.

In connection with the discovery of the beneficial effect of *L*-dopa in Parkinson's syndrome[17] a program was initiated to prepare and characterize a series of optically active condensation products formed by reaction of L-dopa with acetaldehyde, a well-known metabolite of ethanol.

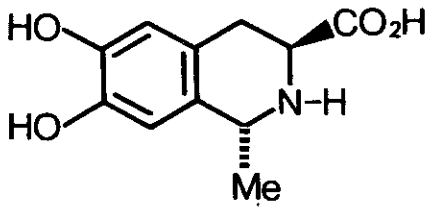
Condensation reactions of L-dopa with acetaldehyde[18]: Reaction of L-dopa with acetaldehyde under acidic conditions afforded a mixture of two isomeric tetrahydroisoquinoline-3-carboxylic acids (Scheme II).

Scheme II

Scheme II



VI



VII

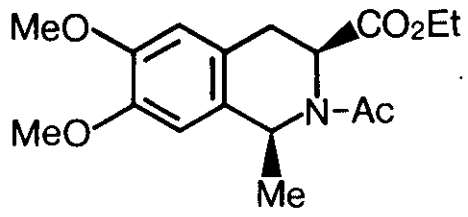
Whereas under acidic conditions the formation of the cis acid VI is strongly favored, at neutral pH considerably more of the trans acid VII, with yields up to 25%, was obtained. The absolute configuration of VI was established by X-ray analysis of its ethyl ester hydrochloride. The absolute stereochemistry of VII was elucidated by chemical correlation.

Base-catalyzed epimerisation of the derivatives VIII and XI, prepared from VI and VII, afforded mixtures containing 60-70% of the trans isomers IX and XI in addition to minor amounts of the cis isomers VIII and XII[19].

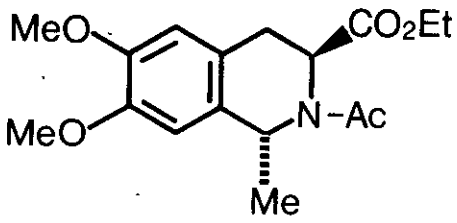
The two optically active derivatives IX and XII could easily be converted by acid hydrolysis into the two tetrahydroisoquinoline-3-carboxylic acids X and XIII, obtained alternatively from D-dopa by condensation with acetaldehyde, O-methylation with diazomethane, followed by acid hydrolysis with 3N hydrochloric acid. This clearly demonstrates that the structures assigned to the products shown in Scheme III are correct and that all four possible isomeric *1-methyl-tetrahydroisoquinoline-3-carboxylic acids* can be prepared from L-dopa.

Scheme III

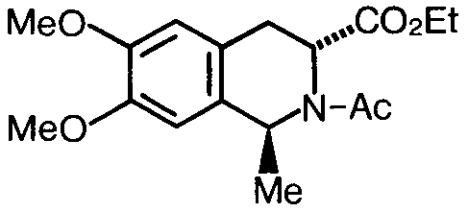
Scheme III



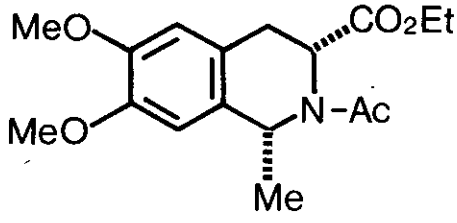
VIII



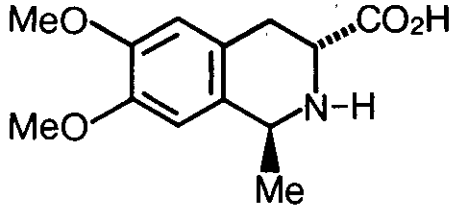
XI



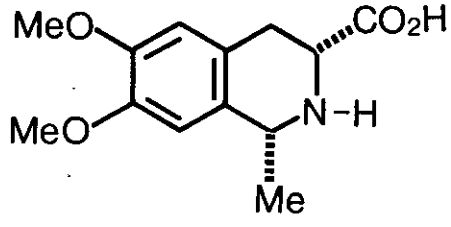
IX



XII



X



XIII

These conversions are of chemical interest and have no bearing on the biological significance of transformations taking place in vivo under physiological conditions.

Condensation reactions of L-tryptophan and L-5-hydroxytryptophan with acetaldehyde[20]:

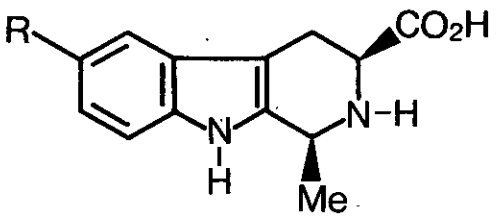
As an extension, the acid-catalyzed condensation of the important amino acids L-tryptophan and L-5-hydroxytryptophan, both found in mammalian tissues[21], was studied. The results paralleled earlier findings observed in the L-dopa series and resulted in the formation of the two cis acids XIV and XVI as major, and the trans acids XV and XVII as minor products. The known absolute configuration of XIV[22] together with ORD and CD spectra obtained in this series of compounds and compared with the spectra of the condensation products obtained in the L-dopa series made it possible to assign their structures as shown in Scheme IV.

Scheme IV

Preparation of optically active salsolinols (SAL) and tetrahydropapaverolines (THP)[23]:

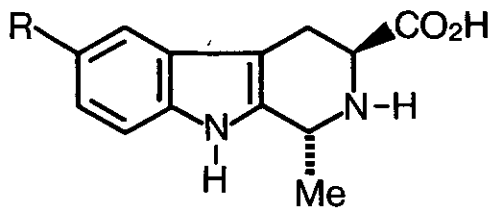
Since SAL and THP were detected as urinary excretion products in man treated with L-dopa[12], it seemed logical to prepare their optical isomers and to make them available

Scheme IV



XIV : R = H

XVI : R = OH



XV : R = H

XVII : R = OH

for biological investigation. The preparation of S(-)-SAL (XXa) and its R(+)-enantiomer (XXb), isolated and characterized in form of their hydrobromides, was accomphlished by O-demethylation of known S-(-)-salsolidine (XVIIIa) and its enantiomer XVIIIb with 48% hydrobromic acid. The absolute configurations of these compounds, shown in Scheme V, could easily be established by conversion of XXa into S(-)-carnegine (N-methyl derivative of XVIIIa). In a similar manner, O-demethylation of S(-)-N-norlaudanosine (XIXa) and its enantiomer XIXb with boiling hydrogen iodide afforded, after treatment with ammonia and hydrochloric acid, the hydrochlorides of S(-)-THP (XXIa) and its enantiomer XXIb.

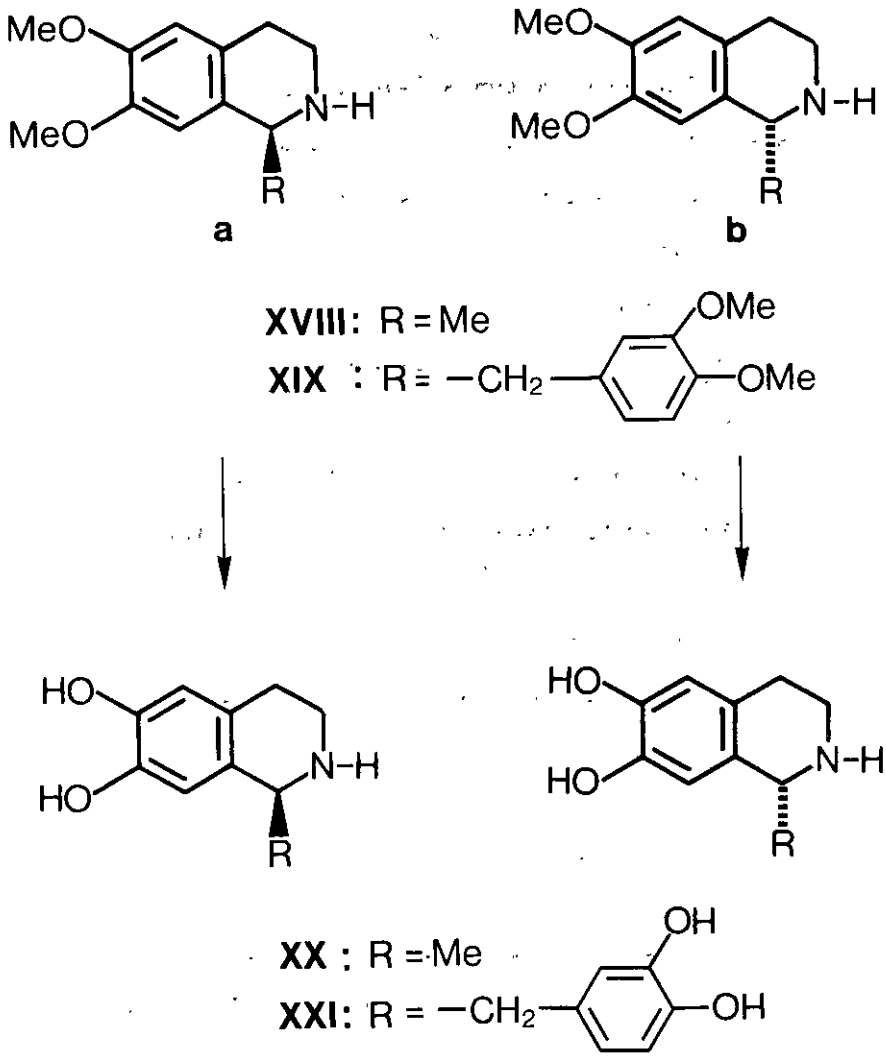
Thus, for the first time the important reaction products of dopamine with acetaldehyde and 3,4-dihydroxyphenylacetaldehyde became available in optically active forms.

Scheme V

Optically active salsolines and isosalsolines[24] (Scheme VI)

A well established metabolic pathway of compounds containing vicinal aromatic hydroxy groups is the conversion of the catechol group into a monomethyl ether. Such reactions can take place with the aid of enzymes, such as catechol-O-methyltransferase. It is, therefore, not astonishing that

Scheme V


MeO
MeO
N-H
R
a
MeO
MeO
N-H
R
b
XVIII: R = Me
XIX : R = —CH2
OMe
OMe
HO
HO
N-H
R
HO
HO
N-H
R
XX : R = Me
XXI: R = —CH2
OH
OH

a metabolite of L-dopa in man, which accumulates in mammalian tissues, is its 3-O-methyl ether[25]. Since it can be expected that SAL could undergo a similar transformation, it seemed logical to prepare all four optically active monomethyl ethers of SAL.

The synthesis of the two enantiomeric salsolines (XXII a and b) and their isomeric 6-monomethyl ethers (XXIII a and b) could easily be accomplished by optical resolution of their known benzyl ethers. Cleavage of the benzyloxy group was achieved by acid hydrolysis.

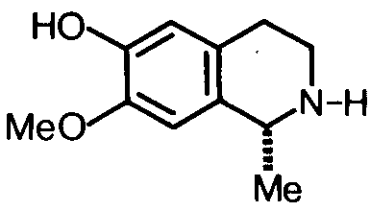
The absolute stereochemistry of the various isomers was proven in the R-series (XXIIa and XIIIa) by N-methylation, followed by O-methylation which afforded R-(+)-carnegine (XXIV) of established absolute configuration[26].

Scheme VI

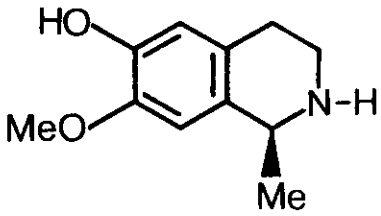
Biological activities of optically active SAL's and THP's[27]:

Sheppard et al.[28] have examined the optical isomers of SAL and THP for agonist and antagonist activity with adenylate cyclases of the caudate nucleus (dopamine-type) and the erythrocyte (β-type) of the rat. With the former enzyme all showed pronounced antagonist activities. With the β-type adenylate cyclase, only the optically active

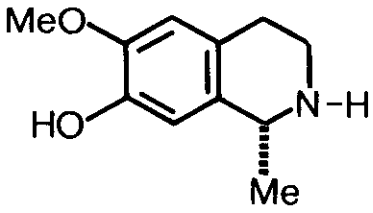
Scheme VI



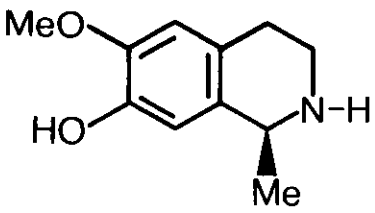
XXII a



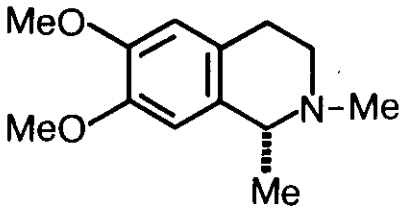
XXII b



XXIII a



XXIII b



XXIV

THP's showed pronounced agonist activity, the S-(-)-THP being the more active isomer. Cohen et al. who studied the inhibitory effects on the accumulation of ^{3}H-dopamine by rat brain slices found that S(-)-SAL was the most active inhibitor. S-(-)-THP was found to be a very effective lipolytic agent with isolated fat cells, whereas the R-(+)-form was inactive[29]. Studies performed in the laboratory of Wade et al. showed that both enantiomers of SAL are dopamine agonists, whereas S-(-)-THP was found to be a potent antagonist[30].

CONCLUSIONS The detection of SAL and THP as urinary excretion products in patients with Parkinson's syndrome treated with L-dopa may be related to drug therapy, but to what extent will only be known if more data on treated and untreated patients become available. The proper identification, hopefully combined with the isolation of chemically pure products, seems, however, necessary to substantiate the many hypotheses presented thus far.

From the first biological data reported for the optically pure isomers of SAL and THP, it may be concluded that distinct differences exist between the various enantiomers in vitro and in experimental animals. Whether the observations with these and other products observed in mammalian systems in vitro are of practical value in man, remains to be seen.

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