Biosynthesis of Lophocerine in *Lophocereus schottii*. Part II[1]

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The biosynthesis of lophocerine (1,2,3,4-tetrahydro-1-isobutyl-6-methoxy-2-methylisoquinolin-7-ol) in intact *Lophocereus schottii* plants has been studied. The C_5 unit of the alkaloid probably arises independently from both leucine and mevalonic acid. 3-Methylbut-3-enyl pyrophosphate, 3-methylbutan-1-ol, and 3-methylbutanal have been shown to be intermediates in the biosynthesis.

THE structures of the alkaloids of the Cactaceae are based on phenethylamine, tetrahydroisoquinoline, or 1-methyltetrahydroisoquinoline units.[2] However, the alkaloids of the cactus *Lophocereus schottii*, *e.g.* lophocerine (VI),[3] are unique among the isoquinoline alkaloids in having a 1-isobutyl group.

We have shown previously that lophocerine arises from tyrosine, mevalonic acid, and leucine.[1] Our work suggested that mevalonic acid (I) was a more efficient precursor of lophocerine than was leucine (V). However too great a reliance could not be placed on this result as the cacti used in the two experiments were not in a comparable state of vigour. We have now repeated these experiments using identical cacti under identical feeding conditions and confirm that mevalonate is incorporated approximately twice as efficiently as leucine into lophocerine.

Although this result suggests a dual origin for the C_5 fragment of lophocerine, the possibility exists that leucine is metabolised to mevalonic acid, by an established pathway,[4] and so incorporated into the alkaloid. To test this hypothesis it was decided to feed 3-methylbutanoic acid, which, in the form of its coenzyme A ester, is reported to be an intermediate on the pathway from leucine to mevalonic acid,[5] to the cactus. It may be argued that 3-methylbutanoic acid could be reduced to the aldehyde and so incorporated into the isoquinoline ring system. It could conceivably also be incorporated directly *via* *N*-(dihydroxyphenethyl)-3-methylbutanamide. A negative result in this experiment however would be meaningful. 3-Methyl[1-^{14}C]butanoic acid (total activity 0·1 mCi) was injected into two *L. schottii* plants. The plants were grown on for 14 days and pure lophocerine was isolated as described previously.[1] The lophocerine was inactive. This suggests that leucine is not incorporated *via* mevalonic acid. However the non-incorporation of 3-methylbutanoic acid may also be attributed to the absence from the plant of the appropriate thiokinase enzyme.

Incorporation of tracers into lophocerine and activity of degradation product

Tracer	Amount fed	Specific activity (mCi mmol^{-1})	Activity of lophocerine (disint. min^{-1} mmol^{-1} × 10^{-6})	Incorporation (%)
DL-[2-^{14}C]Leucine	0·1 mCi	40	6·33	0·023
DL-[2-^{14}C]Mevalonolactone	0·1 mCi	5	11·28	0·040
3-Methyl[1-^{14}C]butanoic acid	0·1 mCi	5	0	0
3-Methyl[1-^{14}C]but-3-enyl pyrophosphate	0·01 mCi	50	1·40	0·052
3-Methyl[1-^{14}C]butan-1-ol	8·8 μCi	0·6	3·90	0·160
3-Methyl[1-^{14}C]butanol	9·8 μCi	0·5	0·49	0·027

Activity of 3,4-dimethoxyphthalic anhydride (from 3-methylbutanol feed) 0·48 × 10^5 disint. min^{-1} mmol^{-1}.

In our earlier work we had suggested 3-methylbutanal (IV) as being the most acceptable intermediate in the formation of the isoquinoline ring. Subsequently the involvement of an aldehyde in isoquinoline ring formation was demonstrated by the incorporation of secologanin into ipecoside.[6] We now report tracer studies on possible intermediates in the biosynthesis of lophocerine.

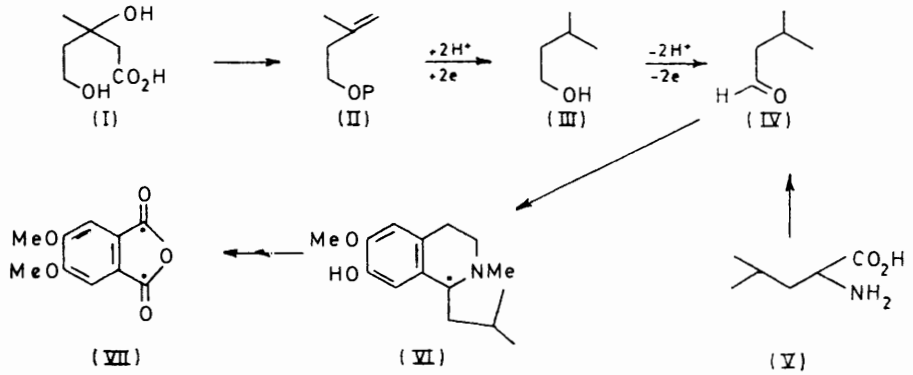
In separate experiments 3-methyl[1-^{14}C]but-3-enyl pyrophosphate (II) (total activity 0·01 mCi), 3-methyl[1-^{14}C]butan-1-ol, (III) (8·8 μCi), and 3-methyl[1-^{14}C]-

[1] D. G. O'Donovan and H. Horan, *J. Chem. Soc.* (*C*), 1968, 2791, is considered as Part I.
[2] D. G. O'Donovan and H. Horan, *J. Chem. Soc.* (*C*), 1969, 1737.
[3] C. Djerassi, S. K. Fodor, J. M. Bobbitt, and F. X. Markley, *J. Amer. Chem. Soc.*, 1957, **79**, 2203.
[4] F. Lynen, Proceedings of International Symposium on Enzyme Chemistry, Tokyo–Kyoto, 1957, p. 57.
[5] K. Bloch, *J. Biol. Chem.*, 1944, **155**, 255; M. J. Coon, *ibid.*, 1950, **187**, 71; M. J. Coon and D. Robinson, *Ann. Rev. Biochem.*, 1958, **27**, 561.
[6] A. R. Battersby and R. J. Parry, *Chem. Comm.*, 1971, 901.

butanal (IV) (9·8 μCi) were each injected into two *L. schottii* cacti. The cacti were grown on for 14 days and pure active lophocerine was isolated as described previously. Specific activities and percentage incorporations are reported in the Table. The lophocerine from the 3-methylbutanal feed was degraded to 3,4-dimethoxyphthalic anhydride (VII) which had the same specific activity as the lophocerine, showing that the aldehyde was incorporated specifically into the alkaloid.

The comparatively low but definite specific incorporation of 3-methylbutanal suggests that this compound is the intermediate involved in the ring closure reaction. The low incorporation of the aldehyde may be attributable to the obvious damage caused to the plant by the injection solution (an emulsion of the aldehyde in 95 : 5 water–ethanol).

The incorporations into lophocereine of the pyrophosphate (II) and the alcohol (III) support the biosynthetic pathway shown in the Scheme.



SCHEME

EXPERIMENTAL

M.p.s are corrected. Radioactive assays were carried out with a Nuclear Chicago Unilux II liquid scintillation counter by use of the usual scintillants; the results were processed by an off-line Olivetti P101 computer, corrections being made for background and quenching.

Administration of Tracers to L. schottii *and Isolation of Lophocerine.*—DL-[2-^{14}C]Leucine (total activity 0·1 mCi), DL-[2-^{14}C]mevalonolactone (0·1 mCi), 3-methyl[1-^{14}C]butanoic acid (0·1 mCi), 3-methyl[1-^{14}C]but-3-enyl pyrophosphate (0·1 mCi), 3-methyl[1-^{14}C]butan-ol (8·8 μCi), and 3-methyl-[1-^{14}C]butanal (9·8 μCi) were each injected into two *L. schottii* plants in separate experiments. The plants were harvested 14 days later. Lophocerine (m.p. 176—177°) was isolated in each case as described previously.[1] Specific activities and percentage incorporations are reported in the Table.

3-*Methyl*[1-^{14}C]*butan*-1-*ol.*—Sodium 3-methyl[1-^{14}C]-butanoate (124 mg; specific activity 600 μCi mmol) was dissolved in water (1 ml) and acidified to pH 2. The solution was extracted with ether and the dried (Na_2SO_4) extract evaporated to yield 3-methyl[1-^{14}C]butanoic acid (95 mg). To lithium aluminium hydride [800 mg dissolved in dry ether (5 ml)], the 3-methylbutanoic acid (95 mg) in dry ether (5 ml) was added dropwise over 30 min. After a further 30 min water (2 ml) was added and the solution was acidified and extracted with ether. The dried (Na_2SO_4) extract was evaporated to yield a colourless liquid (70 mg), shown to be pure by g.l.c.; $\nu_{max.}$ 3430 cm^{-1} (Found: C, 68·05; H, 13·65. Calc. for $C_5H_{12}O$: C, 68·2; H, 13·65%).

3-*Methyl*[1-^{14}C]*butanal.*—Sodium 3-methyl[1-^{14}C]butanoate (248 mg; specific activity 500 Ci mmol^{-1}) was converted into 3-methyl[1-^{14}C]butanoic acid (180 mg) as before. Thionyl chloride (1 ml) was added to the acid (180 mg) and the solution was refluxed for 1 h. The excess of thionyl chloride was distilled off with benzene yielding 3-methyl[1-^{14}C]butanoyl chloride (195 mg). The acid chloride was dissolved in dry ether (10 ml) in a flask under nitrogen and cooled to −70°. 0·5M-Lithium triethoxyhydridoaluminate (3·5 ml) was added during 30 min with stirring. The mixture was allowed to come to room temperature (1 h), hydrolysed with 5N-H_2SO_4, and extracted with ether. The extract was washed with 5N-NaOH, dried (Na_2SO_4), and evaporated to leave 3-methyl[1-^{14}C]-butanal, purified by conversion into its hydrogen sulphite adduct and regeneration with acid; $\nu_{max.}$ 1710 cm^{-1} (Found: C, 81·3; H, 11·6. Calc. for $C_5H_{10}O$: C, 81·4; H, 11·65%).

3,4-*Dimethoxyphthalic Anhydride.*—*O*-Methyl-lophocerine methiodide (42 mg) was dissolved in water (2 ml) and 2M-KOH (0·19 ml) and potassium permanganate (100 mg) were added. The solution was refluxed for 4 h, then acidified, and the manganese dioxide was dissolved by the addition of concentrated H_2SO_4 and aqueous sodium sulphite. The solution was extracted with ether and the dried (Na_2SO_4) extract was evaporated to yield 3,4-dimethoxyphthalic anhydride (10 mg), m.p. 174—175° (from benzene–light petroleum) (lit.,[7] 175°).

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[7] K. O. Olsen and S. O. Almquist, *Acta Chem. Scand.*, 1970, **24**, 3777.