ALKALOID BIOSYNTHESIS

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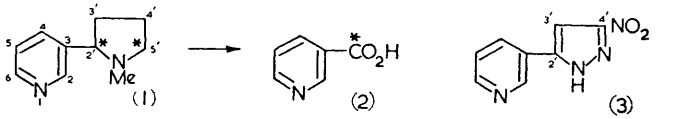
SINCE the early days of biogenetic theory, there has been much thought and discussion about the way in which plants build up the rich variety of alkaloid structures. The resultant speculations have largely been based upon structural similarities within the alkaloid series and also upon the relations of alkaloids to simpler natural products. Obviously such an approach cannot prove, and is not claimed to prove, that plants follow the suggested biosynthetic schemes. The great value of the proposals lies in the help they can give to structural studies on new alkaloids and in prompting experiments on living plants. Further progress depends upon researches *in vivo* and the present survey will cover the results gained so far from tracer experiments on alkaloid biosynthesis. The studies which predate the use of radioactive tracers were often beset with difficulties and the results obtained are fully reviewed elsewhere;[1,2] they will therefore not be discussed here. Nor is it intended to give an account of the present state of biogenetic theory, but rather to bring theory and experimental result together in those cases where the living plant has been studied.

The usual technique for biosynthetic studies of alkaloids has been to administer labelled precursors to the plants and, after a suitable period of growth, to isolate the alkaloids. These are then degraded in a controlled way to determine the positions of the labelled atoms. It must be emphasised that negative results should be treated with great caution. Thus, they may simply mean that the plant is not synthesising the alkaloid of interest at the time of the experiment, or again that the precursor used has not reached the site of synthesis. Even when incorporation has been achieved, the results must be carefully interpreted. It must always be borne in mind that incorporation of a substance which is not normally involved in the biosynthesis of an alkaloid could occur by transformation into one of the intermediates on the actual biosynthetic route. However, with proper care in execution and interpretation, the tracer technique is extremely powerful in this field.

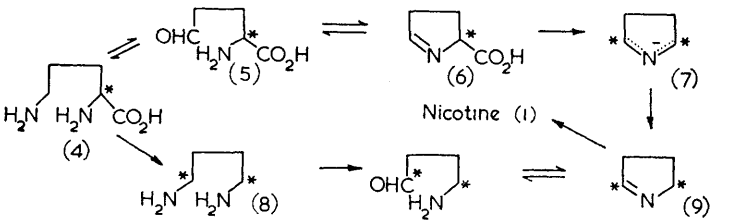
Pyridine and Piperidine Alkaloids.—The biosynthesis of nicotine (1), the major alkaloid of many *Nicotiana* species, has been extensively studied by tracer methods. This alkaloid also occurs in club mosses[3] (*Lycopodium*), in "horse-tails"[3] (*Equisetum*), and in many other genera,[4] so that it is

[1] R. F. Dawson, *Adv. Enzymol.*, 1948, **8**, 203; W. O. James, "The Alkaloids," ed. R. H. F. Manske and H. L. Holmes, Academic Press, New York, 1950, Vol. I, p. 16.
[2] K. Mothes, *Handbuch Pflanz. physiol.*, 1958, **8**, 989.
[3] Reviewed by R. H. F. Manske, "The Alkaloids," ed. R. H. F. Manske and H. L. Holmes, Academic Press, New York, 1955, Vol. V, p. 295; R. H. F. Manske and L. Marion, *Canad. J. Res.*, 1942, **20**, *B*, 88.
[4] K. Mothes, *J. Pharm. Pharmacol.*, 1959, **11**, 193.

remarkably widespread in Nature. When [2-^{14}C]ornithine (4) was fed to *N. tabacum* and *N. rustica* plants it was found[5,6] that this amino-acid is a good precursor of nicotine. Degradation of the radioactive alkaloid by oxidation with nitric acid gave nicotinic acid (2) and the nitropyrazole (3). The carboxyl group[5,6] of the acid (2) and the nitropyrazole[7] (3) each contained, within the limits of experimental error,† half the activity of the original nicotine; the *N*-methyl group of the alkaloid was inactive. Thus the activity is equally divided between positions 2′ and 5′ of nicotine and this has been confirmed by a further degradation[8] which allowed the isolation of the carbon atom from position 5′. Clearly these



results mean that a symmetrical intermediate is being formed and Leete[9] suggests putrescine (8) or the mesomeric anion (7) as possibilities. It may be significant that a plant oxidase is known which will convert putrescine into 1-pyrroline (9); this enzyme has been used in very significant *in vitro* experiments.[10]

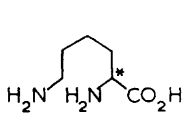


Support for the above scheme comes from the incorporation of labelled putrescine (8), proline, and glutamic acid into the pyrrolidine ring of nicotine though, compared with ornithine, these substances are less efficient precursors.[9] In the case of glutamic acid, however, the percentage incorporation will be markedly affected by the large pool[11] of the free glutamic acid present in tobacco plants.

† To avoid tedious repetition in the sequel, the words "within the limits of experimental error" should be understood in all cases where tracer results are reported.

[5] E. Leete, *Chem. and Ind.*, 1955, 537; L. J. Dewey, R. U. Byerrum, and C. D. Ball, *Biochim. Biophys. Acta*, 1955, **18**, 141.
[6] E. Leete, *J. Amer. Chem. Soc.*, 1956, **78**, 3520; 1958, **80**, 4393.
[7] E. Leete and K. Siegfried, *J. Amer. Chem. Soc.*, 1957, **79**, 4529.
[8] B. L. Lamberts, L. J. Dewey, and R. U. Byerrum, *Biochim. Biophys. Acta*, 1959, **33**, 22.
[9] E. Leete, *J. Amer. Chem. Soc.*, 1958, **80**, 2162; B. L. Lamberts and R. U. Byerrum, *J. Biol. Chem.*, 1958, **233**, 939. A. J. Clark and P. J. G. Mann, *Biochem. J.*, 1959, **71**, 596; cf. H. Tuppy and M. S. Faltaous, *Monatsh.*, 1960, **91**, 167.
[10] P. J. G. Mann and W. R. Smithies, *Biochem. J.*, 1955, **61**, 89.
[11] B. Commoner and N. Varda, *J. Gen. Physiol.*, 1953, **36**, 791; E. A. H. Roberts and D. N. Wood, *Arch. Biochem. Biophys.*, 1951, **33**, 299.

Similar feeding experiments[6] with *Nicotiana glauca* have established that lysine (10) and cadaverine (1,5-diaminopentane) can serve as precursors of the piperidine ring in anabasine (11). In contrast to the case of ornithine above, the biosynthesis from lysine does not involve a symmetrical intermediate since over 90% of the radioactivity in the alkaloid, biosynthesised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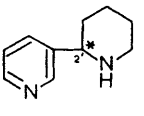
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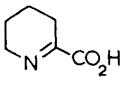
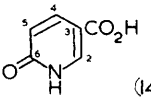

(12)


(13)

from [2-^{14}C]lysine (10), was located at position 2′ of the piperidine ring. Probably both lysine and cadaverine are converted into Δ^1-piperideine (13), with lysine undergoing oxidation first at the α-position to give the acid (12); in this way, the specific labelling of one position would be preserved. This certainly seems to be the main route from lysine to pipecolic acid (piperidine-2-carboxylic acid) in the rat[12] and in *Neurospora crassa*.[13]

The fact that the pyridine ring of anabasine was not labelled in the above experiments with lysine and cadaverine shows that the aromatic system is not derived directly from either of these precursors. These results are in agreement with the isolation[6] of inactive nicotine from *N. tabacum* plants fed with [2-^{14}C]lysine; also nicotine of very low activity was obtained after the roots of the plants had taken up generally labelled lysine.[14] Fortunately, positive information concerning the origin and mode of incorporation of the pyridine ring is rapidly accumulating. Nicotine labelled in the pyridine ring was isolated from plants fed with ring-labelled nicotinic acid,[15] but when carboxyl-labelled nicotinic acid was used[16] inactive nicotine resulted. Thus the plant can decarboxylate nicotinic acid at some stage in the incorporation of the preformed pyridine ring into nicotine. These experiments have been extended by feeding 2-, 4-, 5-, and 6-tritium-labelled nicotinic acid, and nicotinic acid generally labelled with tritium, to sterile tobacco root cultures.[17] The results show that the hydrogen atoms at positions 2, 4, and 5 are unaffected in the conversion of nicotinic acid into nicotine. The hydrogen at position 6 is lost and a reasonable explanation of this would be the intermediate formation of the pyridone (14). However, when this labelled precursor was fed to such cultures,[17] there was virtually no incorporation into nicotine. A similar result[17] was obtained by feeding the pyridone (15) labelled with tritium at position 2.

[12] M. Rothstein and L. L. Miller, *J. Amer. Chem. Soc.*, 1954, **76**, 1459.
[13] R. S. Schweet, J. T. Holden, and P. H. Lowy, *J. Biol. Chem.*, 1954, **211**, 517.
[14] A. A. Bothner-By, R. F. Dawson, and D. R. Christman, *Experientia*, 1956, **12**, 151.
[15] R. F. Dawson, D. R. Christman, R. C. Anderson, M. L. Solt, A. F. D'Adamo, and U. Weiss, *J. Amer. Chem. Soc.*, 1956, **78**, 2645; T. C. Tso and R. N. Jeffrey, *Arch. Biochem. Biophys.*, 1959, **80**, 46.
[16] R. F. Dawson, D. R. Christman, and R. C. Anderson, *J. Amer. Chem. Soc.*, 1953, **75**, 5114.
[17] R. F. Dawson, D. R. Christman, A. F. D'Adamo, M. L. Solt, and A. P. Wolf, *Chem. and Ind.*, 1958, 100; *J. Amer. Chem. Soc.*, 1960, **82**, 2628.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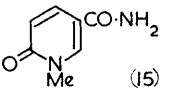
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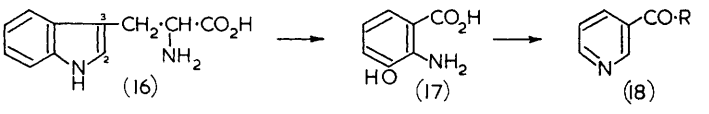
The above researches deal with a preformed pyridine ring, so that the way in which this ring is constructed still remains to be discussed. Two possible precursors, ring-labelled tryptophan[18,19] (16) and ring-labelled anthranilic acid,[20] yielded inactive nicotine; both amino-acids were fed to intact *N. tabacum* plants. Thus it seems that the route used in animals and Neurospora[21] from tryptophan (16) → kynurenine → 3-hydroxyanthranilic acid (17) → nicotinic acid (18; R = OH) is not followed in the higher plants. There is perhaps further evidence for this suggestion in the failure of ring-labelled tryptophan and tritium-labelled 3-hydroxyanthranilic acid (17) to act as precursors of nicotinamide (18; R = NH_2) in maize.[19] Also,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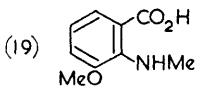
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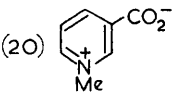
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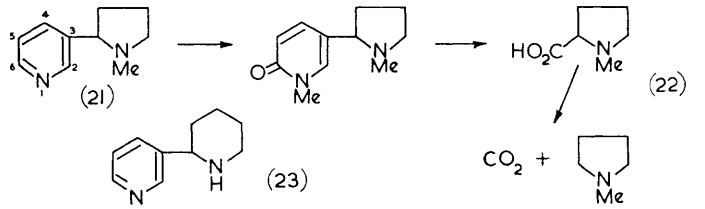
[3-^{14}C]tryptophan was not incorporated into damascenine (19) in *Nigella damascena* or trigonelline (20) in pea seedlings,[22] nor would soya-bean leaves convert ring-labelled 3-hydroxyanthranilic acid (17) into trigonelline.[23] Damascenine (19) and trigonelline are obviously closely related structurally to the amino-acids (17) and (18) respectively.

These negative results are now being followed by positive ones and at the time of writing the field is in a state of rapid development. Whereas [1-^{14}C]acetate was incorporated by tobacco plants almost entirely into the pyrrolidine ring of nicotine[24] (21), [2-^{14}C]acetate yielded nicotine with about 40% of the activity in the pyridine nucleus.[24; cf.25,26] [1-^{14}C]Propionate gave almost inactive nicotine, but [2-^{14}C]propionate led to active alkaloid with 39% of the activity in the pyridine ring.[27] The highest incorporation into nicotine was achieved with [1,3-^{14}C]glycerol as the precursor and here the isolated alkaloid had 57% of its activity in the

[18] E. Leete, *Chem. and Ind.*, 1957, 1270.
[19] L. M. Henderson, J. F. Someroski, D. R. Rao, P.-H. L. Wu, T. Griffith, and R. U. Byerrum, *J. Biol. Chem.*, 1959, **234**, 94.
[20] J. Grimshaw and L. Marion, *Nature*, 1958, **181**, 112.
[21] C. E. Dalgliesh, *Quart. Rev.*, 1951, **5**, 227; A. H. Mehler in "Amino Acid Metabolism," ed. W. D. McElroy and B. Glass, Johns Hopkins Press, Baltimore, 1955.
[22] E. Leete, L. Marion, and I. D. Spenser, *Canad. J. Chem.*, 1955, **33**, 405.
[23] S. Aronoff, *Plant Physiol.*, 1956, **31**, 355.
[24] T. Griffith and R. U. Byerrum, *Science*, 1959, **129**, 1485.
[25] G. S. Il'in, *Doklady Akad. Nauk S.S.S.R.*, 1958, **119**, 544.
[26] E. Leete, *Chem. and Ind.*, 1958, 1477.
[27] T. Griffith, K. P. Hellman, and R. U. Byerrum, *J. Biol. Chem.*, 1960, **235**, 800.

aromatic nucleus.[27] Degradation of the nicotine from the [2-^{14}C]acetate and [2-^{14}C]propionate experiments was also carried out to yield hygric acid (22) as shown; this allowed the level of activity at position 3 of the aromatic ring to be examined.[27] The present interpretation of all the results obtained is that glycerol is involved in the construction of the C-4, C-5, C-6 system of nicotine (21) and that C-2 and C-3 arise respectively from



C-3 and C-2 of propionate. Clearly, the carboxyl group of propionate is eliminated in the process. These suggestions are to some extent tentative[27] but have an analogy in the recent demonstration that glycerol is capable of supplying all the carbon atoms of nicotinic acid in *Escherichia coli*.[28] Further developments will be watched with interest.

The labelling patterns in the pyrrolidine ring of the various nicotine samples derived from the simple precursors just discussed strongly suggest that the precursors enter the tricarboxylic acid cycle and are converted into glutamic acid before incorporation.

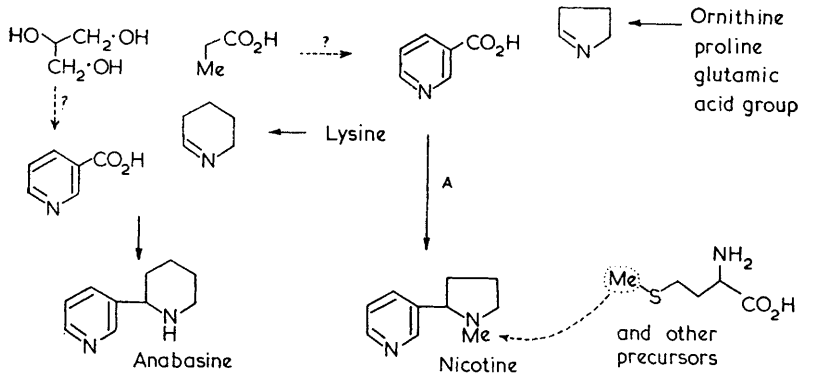
A related experiment in which [2-^{14}C]acetate was fed to *Nicotiana glauca* plants[26] afforded radioactive anabasine (23) with over 90% of the activity concentrated in the aromatic ring. It is not surprising that, in contrast to the result with nicotine (21), the incorporation into the reduced ring is low. It has been mentioned that acetate can readily pass into the ornithine–proline–glutamic acid group by way of the tricarboxylic acid cycle, whereas acetate is metabolically much further removed from lysine,[29] the precursor of the piperidine ring of anabasine. When the feeding experiment was repeated[26] with a large quantity of inactive nicotinic acid together with the [2-^{14}C]acetate, almost inactive anabasine was isolated and this suggests that acetate is converted into nicotinic acid before incorporation.

There has been considerable interest in the source of the *N*-methyl group of nicotine and here tracer experiments show that it can arise from choline[30] or methionine.[31] In the latter case, it is established that the methyl group is transferred intact, that is, a true transmethylation occurs.[32] Separate feeding experiments with [2-^{14}C]glycine,[33] [^{14}C]formate,[31] [^{14}C]formalde-

[26] M. V. Ortega and G. M. Brown, *J. Amer. Chem. Soc.*, 1959, **81**, 4437.
[29] E. Bilinski and W. B. McConnell, *Canad. J. Biochem. Physiol.*, 1957, **35**, 357.
[30] R. U. Byerrum and R. E. Wing, *J. Biol. Chem.*, 1953, **205**, 637.
[31] S. A. Brown and R. U. Byerrum, *J. Amer. Chem. Soc.*, 1952, **74**, 1523.
[32] L. J. Dewey, R. U. Byerrum, and C. D. Ball, *J. Amer. Chem. Soc.*, 1954, **76**, 3997.
[33] R. U. Byerrum, R. L. Hamill, and C. D. Ball, *J. Biol. Chem.*, 1954, **210**, 645.

hyde,[34] [3-^{14}C]serine, and [2-^{14}C]glycollate[35] all gave radioactive nicotine with most or all of the activity in the *N*-methyl group; [1-^{14}C]glycine, however, yielded inactive nicotine. This array of precursors is not surprising when it is recalled that some of them, for example, glycine, serine, and glycollate, are interconvertible in living systems. The fact that the *N*-methyl group of nicotine is labelled after feeding methyl-labelled methionine could be interpreted as a net synthesis from nornicotine or some other unmethylated precursor, or simply as a transfer of methyl groups between nicotine and methionine. It is known[36] that administration of [*Me*-^{14}C]-nicotine to intact tobacco plants resulted in the formation of [*Me*-^{14}C]-choline, so that methyl transfer from the alkaloid to acceptors can occur.

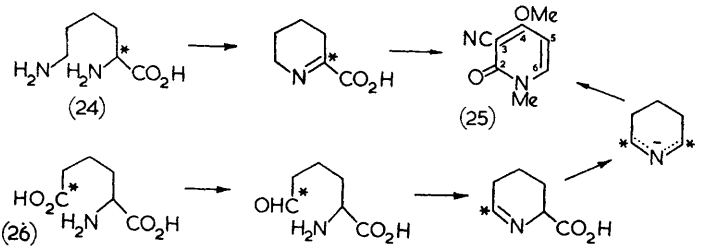
The results of the many tracer experiments outlined above can now be summarised in the biosynthetic scheme below. It seems probable that the conversion A does not involve the 6-pyridone of nicotinic acid. In this scheme, and in those which appear later in the Review, a broken circle with attached arrow has been used to indicate that the arrowed group is derived from the ringed group. Thus here the *N*-methyl group of nicotine arises by transfer of the *S*-methyl group from methionine.



Only ricinine (25) among the other pyridine alkaloids has so far been studied by tracer methods. It was isolated with its cyano-group labelled from castor-bean plants (*Ricinus communis*) which had been fed with carboxyl-labelled nicotinic acid;[37] the incorporation was 0·76%. Moreover, [1-^{14}C]acetate and [2-^{14}C]acetate are both incorporated into ricinine.[26] The two methyl groups can be derived from methionine but not from formate, choline, or hydrogen carbonate under the conditions used in the feeding experiments.[38] In contrast to the results with nicotine, it is

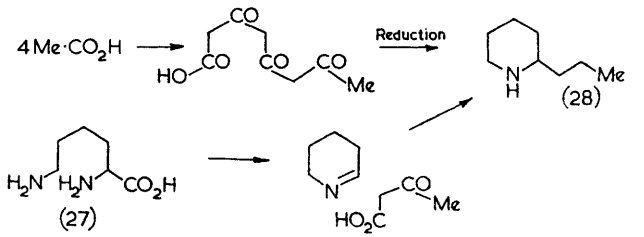
[34] R. U. Byerrum, R. L. Ringler, and R. L. Hamill, *Fed. Proc.*, 1955, **14**, 188.
[35] R. U. Byerrum, L. J. Dewey, R. L. Hamill, and C. D. Ball, *J. Biol. Chem.*, 1956, **219**, 345.
[36] E. Leete and V. M. Bell, *J. Amer. Chem. Soc.*, 1959, **81**, 4358.
[37] E. Leete and F. H. B. Leitz, *Chem. and Ind.*, 1957, 1572.
[38] M. Dubeck and S. Kirkwood, *J. Biol. Chem.*, 1952, **199**, 307.

reported that [2-^{14}C]lysine (24) can serve[39] as a precursor of the pyridine nucleus to give ricinine labelled at position 6 though the incorporation was very low (0·01%). Indeed, other workers[40] state that lysine is not used for ricinine formation. When α-amino[ε-^{14}C]adipic acid (26) was fed to the plants,[39] ricinine equally labelled at positions 2 and 6 was isolated with incorporation of 0·13%. The fact that different labelling patterns result from the unsymmetrical precursors (24) and (26) demands two routes to



ricinine and the ones suggested in the chart will rationalise the results.[39] All one can say at present is that the various findings are hard to reconcile and, moreover, are rather disturbing as they stand since they indicate a lack of specificity in the biosynthetic activities of castor-bean seedlings.

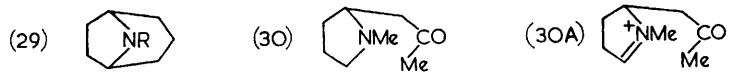
The notorious hemlock (*Conium maculatum*) has recently been the subject of tracer experiments. These plants used generally labelled lysine (27) to yield radioactive coniine (28), but the alkaloid was not degraded.[41] Further work will, therefore, be necessary to distinguish beyond all doubt between the two possible biosynthetic schemes below based on acetate and ammonia,[42] and lysine and acetate,[43] respectively. Analogy with anabasine suggests that the route from lysine will turn out to be the correct one.



Pyrrolidine and Tropane Alkaloids.—The tropane alkaloids are based upon the skeleton (29) which is obviously closely related to the simple

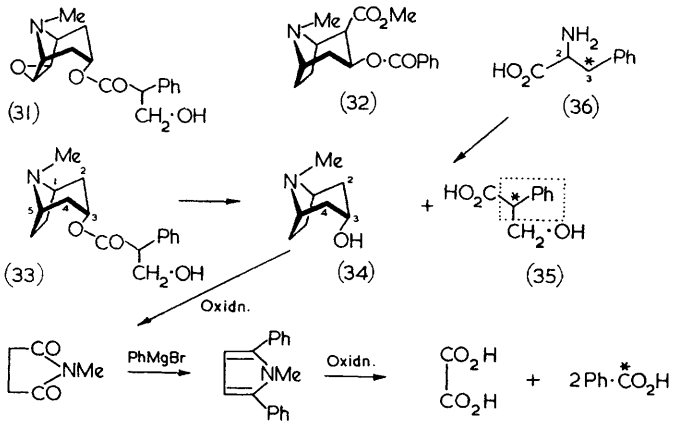
[39] H. Tamir and D. Ginsburg, *J.*, 1959, 2921.
[40] E. J. Reist and L. Marion, quoted in ref. 20.
[41] U. Schiedt and H. G. Hoss, *Z. Naturforsch.*, 1958, **13b**, 691.
[42] Cf. K. Biemann, G. Buchi, and B. H. Walker, *J. Amer. Chem. Soc.*, 1957, **79**, 5558.
[43] Sir R. Robinson, "The Structural Relations of Natural Products", Clarendon Press, Oxford, 1955.

pyrrolidine bases such as hygrine (30). Indeed, it is possible[43] that one of the stages in tropane formation is cyclisation of the pyrroline (30A). Such a linking of the pyrrolidine and tropane types is attractive because of



the occurrence of hygrine (30) with cocaine (32) in *Erythroxylon truxillense* and of a related pyrrolidine base with hyoscyamine (33) in *Atropa belladona*.[44]

The work of Marion and his colleagues[45] has shown that when [2-^{14}C]-ornithine is fed to five-months old *Datura stramonium* plants, it is incorporated into hyoscyamine (33). Hydrolysis of the alkaloid gave

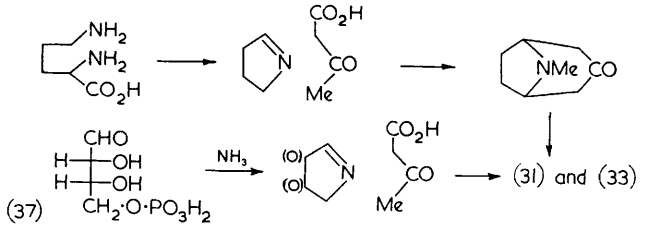


tropine (34) which was degraded as illustrated. The imide and the diphenylpyrrole both had the same specific activity as the original tropine, so that carbon atoms 2, 3, and 4 must have been inactive. Moreover, since the benzoic acid had half the specific activity of the tropine, it follows that the original activity of the hyoscyamine is located at position 1 or 5 or is distributed between these two positions. From this experiment one cannot say which of these possibilities is correct because of the symmetry of the degradation products. Nevertheless, it is clear that ornithine is incorporated in a specific manner, so that the biosynthetic scheme on p. 267 is a probable one.

It should be mentioned that there is as yet no evidence that the nitrogen atom of the alkaloid is one of those originally present in ornithine and Wenkert[46] has suggested that the true precursor of the pyrrolidine ring in

[44] P. R. van Haga, *Nature*, 1954, **174**, 833.
[45] E. Leete, L. Marion, and I. D. Spenser, *Nature*, 1954, **174**, 650; *Canad. J. Chem.*, 1954, **32**, 1116.
[46] E. Wenkert, *Experientia*, 1959, **15**, 165.

the tropane series is D-erythrose 4-phosphate (37). One attraction of his theory is that it can give a plausible account of the oxygenated alkaloids such as hyoscine (31). However, there is now evidence[47] that young *D.*



stramonium plants can bring about the oxidation of hyoscyamine (33) to hyoscine (31).

Three carbon atoms of the tropane system remain to be accounted for and it is found that *Datura metel* roots will incorporate labelled acetate into these alkaloids.[48] The active hyoscyamine (33) produced was hydrolysed to give tropine (34) which retained most of the activity of the original alkaloid. Oxidation of the tropine to *N*-methylsuccinimide then allowed the amount of activity concentrated in carbon atoms 2, 3, and 4 to be estimated. When [1-^{14}C]-acetate was fed, 85% of the activity of the tropine was located somewhere in this three-carbon chain and when [2-^{14}C]acetate was used, the figure was 75%. These results are consistent with the biosynthetic schemes given above.

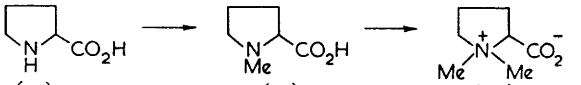
The hyoscine (31) which was also isolated from the *D. stramonium* plants fed with radioactive ornithine was shown[45] to be completely inactive. At first this result was a surprising one, but it was later found that incorporation occurred only into the *N*-methyl group of hyoscyamine (33) when [*Me*-^{14}C]methionine was fed to *D. stramonium* plants of the same age as used in the foregoing experiments.[49] This is good evidence that hyoscine (31) is not being synthesised at this stage in the plants' development and there are other indications[50] that the hyoscine present in *D. stramonium* is produced by the young plants whereas adult plants synthesise hyoscyamine exclusively. It is also established by tracer experiments that plants two months old are synthesising both alkaloids.[51] This case illustrates how little trust can be placed in completely negative results.

The biosynthesis of the tropic acid residue (35) in the alkaloids (31) and (33) has been studied by feeding [3-^{14}C]phenylalanine (36) to *D. stramonium* plants;[51] the acid (35) was then obtained by hydrolysis of the isolated alkaloids. Suitable degradation showed that the radioactive tropic acid had the label located almost entirely at the starred position.

[47] A. Romeike and G. Fodor, *Tetrahedron Letters*, 1960, **22**, 1.
[48] J. Kaczkowski, H. R. Schütte, and K. Mothes, *Naturwiss.*, 1960, **13**, 304.
[49] L. Marion and A. F. Thomas, *Canad. J. Chem.*, 1955, **33**, 1853.
[50] E. M. Trautner, *Austral. Chem. Inst. J. Proc.*, 1947, **14**, 411.
[51] E. Leete, *J. Amer. Chem. Soc.*, 1960, **82**, 612.

Thus the C_6–C_1 fragment of phenylalanine can provide the boxed part of the molecule in formula (35); the carboxyl group may or may not have been derived from C-2 of the original phenylalanine. Surprisingly, neither formate nor formaldehyde was incorporated into the hydroxymethyl group of tropic acid (35) and the origin of this feature remains to be elucidated. As in the case of nicotine, formate was used in the synthesis of hyoscyamine almost entirely as a precursor of the *N*-methyl group.[51]

Stachydrine (40) from alfalfa is the only simple pyrrolidine alkaloid to have been studied by modern methods. It now appears that the synthesis is straightforward in that both [*carboxy*-^{14}C]proline[52] (38) and [*carboxy*-^{14}C]hygric acid[53] (39), when fed separately to alfalfa plants, gave rise to



(38) (39) (40)

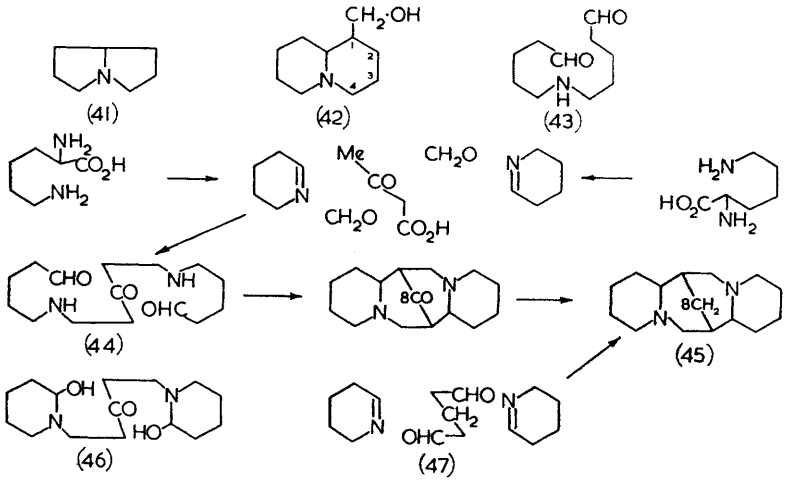
radioactive stachydrine (40), though the former amino-acid is only incorporated when mature plants are used. It thus seems that the enzyme system necessary for the conversion of proline into hygric acid is absent in young plants. The methyl groups of stachydrine were labelled after [*Me*-^{14}C]methionine had been used.[54] Incorporation of proline leads one to expect that ornithine and glutamic acid would also be used by mature plants to form stachydrine and it would be interesting to re-examine the earlier work[54] which was carried out on very young plants.

Hexahydropyrrolizine and Octahydroquinolizine Alkaloids.—Tracer studies have yet to be reported for simple alkaloids based on the hexahydropyrrolizine (pyrrolizidine) system (41). For the octahydroquinolizines (quinolizidines) the published knowledge is that [2-^{14}C]lysine and [1,5-^{14}C]-cadaverine (cadaverine = 1,5-diaminopentane) are good precursors of lupinine (42) and sparteine (45) in *Lupinus luteus*;[55] the alkaloids were not degraded. These results are in keeping with, but do not as yet establish, the proposal[43] that lupinine is derived from a precursor such as the dialdehyde (43), formed in turn from two molecules of lysine. A laboratory synthesis of (±)-epilupinine (epimeric with lupinine at C-1) by this approach has recently been achieved.[56]

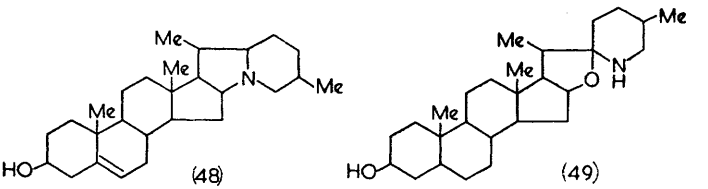
It has been proposed[43] that sparteine biosynthesis involves the intermediate (44) which could be formed from two lysine residues, one acetoacetate residue (or its equivalent), and two formaldehyde equivalents,

[52] L. Marion and D. J. McCaldin, personal communication.
[53] A. V. Robertson and L. Marion, *Canad. J. Chem.*, 1960, **38**, 396.
[54] E. Leete, L. Marion, and I. D. Spenser, *J. Biol. Chem.*, 1955, **214**, 71; A. Morgan and L. Marion, *Canad. J. Chem.*, 1956, **34**, 1704; G. Wiehler and L. Marion, *J. Biol. Chem.*, 1958, **231**, 799; A. V. Robertson and L. Marion, *Canad. J. Chem.*, 1959, **37**, 1197.
[55] H. R. Schütte and E. Nowacki, *Naturwiss.*, 1959, **46**, 493.
[56] E. E. van Tamelen and R. L. Foltz, *J. Amer. Chem. Soc.*, 1960, **82**, 502; cf. N. J. Leonard and S. W. Blum, *ibid.*, p. 503.

though there are other possibilities;[43] clearly the amino-aldehyde (44) could be in the carbinolamine form (46) rather than the open form. This scheme forms the basis of a recent neat synthesis of sparteine (45) *in vitro*.[57] In addition, tetracyclic lupin alkaloids are appearing[58] which are oxygenated at position 8, thus giving some support to the idea that an intermediate is used which is oxidised at this position. An alternative biosynthetic scheme[46] which at present is equally attractive involves the dialdehyde (47); this could be derived from lysine or possibly from shikimic acid.



Steroidal Alkaloids.—Various *Solanum* species produce steroidal bases in the form of glycosides which can be hydrolysed to the corresponding aglycones, a typical example being solanidine (48). This base was

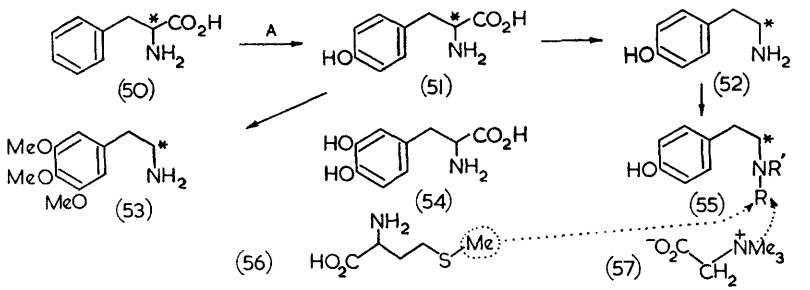


found to be radioactive after [1-^{14}C]acetate had been fed[59] to sprouting potatoes (*Solanum tuberosum*). The same precursor was used by tomato plants (*Lycopersicon pimpinellifolium*) in the construction of tomatine,[60] a glycoside based upon the aglycone tomatidine (49). These results suggest that the acetate–mevalonate pathway is being used for the biosynthesis of these bases.

[57] E. E. van Tamelen and R. L. Foltz, *J. Amer. Chem. Soc.*, 1960, **82**, 2400.
[58] M. Carmack, quoted by E. E. van Tamelen in ref. 57.
[59] A. R. Guseva and V. A. Paseshnichenko, *Biokhimiya*, 1958, **23**, 412.
[60] H. Sander and H. Grisebach, *Z. Naturforsch.*, 1958, **13b**, 755.

Phenethylamine and Isoquinoline Alkaloids.—One of the most valuable contributions of biogenetic theory has been the proposal of reasonable routes to the vast number of alkaloids in this group, starting with the same relatively simple precursors. It was suggested[61,43] before the advent of tracers that the main building stones are the aromatic amino-acids phenylalanine (50), tyrosine (51), and 3,4-dihydroxyphenylalanine (54). Decarboxylation, oxidation, and methylation, which are well known in living systems, are then required in order to convert these precursors into such simple alkaloids as hordenine (55; R = R′ = Me) and the hallucinogen, mescaline (53).

There is now sound evidence that plants do in fact use this route.[62] Thus, phenylalanine (50), tyrosine (51), and tyramine (52), all labelled with ^{14}C at the starred position, were found in separate experiments to be incorporated by sprouting barley into *N*-methyltyramine (55; R = H; R′ = Me) and hordenine (55; R = R′ = Me). Suitable degradation of the alkaloids showed that the label was located entirely at the starred position and the quantitative results suggested that hordenine (55; R = R′ = Me) is formed from tyramine (55; R = H; R′ = Me) in a stepwise methylation process. This was supported by the results from a study of the methylation by ^{14}C-tracer methods, in which it was shown that L-methionine[63] (56) and betaine[64] (57) can serve as sources of the methyl groups in these alkaloids; formate was a poor methyl precursor and choline was ineffective.[63] The biosynthesis of the barley alkaloids can thus be summarised in



the annexed scheme. However, the first step A is not necessarily on the normal pathway; for just as it is probable that in wheat and buckwheat phenylalanine and tyrosine arise from prephenic acid by separate routes,[65] as they do in *Escherichia coli*,[66] so it may well be that the major route in

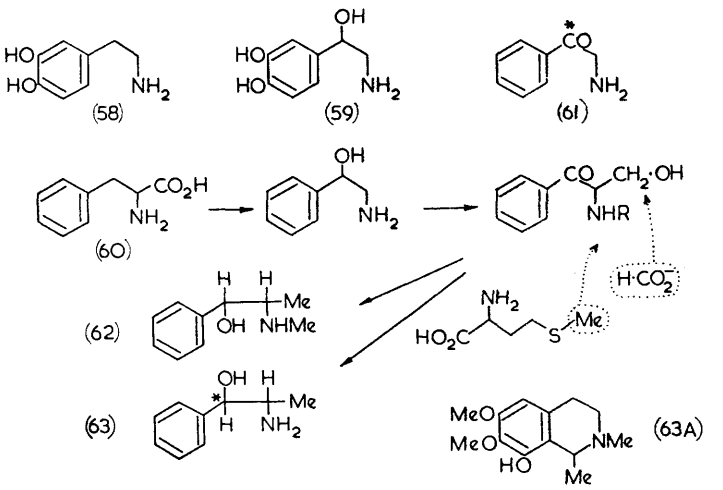
[61] E. Winterstein and G. Trier, "Die Alkaloide", Borntraeger, Berlin, 1910.
[62] E. Leete, S. Kirkwood, and L. Marion, *Canad. J. Chem.*, 1952, **30**, 749; E. Leete and L. Marion, *ibid.*, 1953, **31**, 126; J. Massicot and L. Marion, *ibid.*, 1957, **35**, 1.
[63] S. Kirkwood and L. Marion, *Canad. J. Chem.*, 1951, **29**, 30; T. J. Matchett, L. Marion, and S. Kirkwood, *ibid.*, 1953, **31**, 488; E. Leete and L. Marion, *ibid.*, 1954, **32**, 646.
[64] M. Sribney and S. Kirkwood, *Canad. J. Chem.*, 1954, **32**, 918.
[65] O. L. Gamborg and A. C. Neish, *Canad. J. Biochem. Physiol.*, 1959, **37**, 1277.
[66] I. Schwink and E. Adams, *Biochim. Biophys. Acta.*, 1959, **36**, 102 and refs. therein. B. D. Davies, *Arch. Biochem Biophys.*, 1958, **78**, 497, and refs. therein.

barley is shikimic acid → prephenic acid → tyrosine and then on through the further stages as shown in the scheme.

A similar study carried out with the cactus *Lophophora williamsii* has demonstrated[67] the conversion of [2-^{14}C]tyrosine into mescaline (53) with at least 99% of the radioactivity at the starred position.

Turning now to (−)ephedrine (62) and (+)-norpseudoephedrine (63), we find two new features, the benzylic hydroxyl group and the *C*-methyl group. Clearly these bases are similar to noradrenaline (59), the biosynthesis of which in animal systems is generally believed[68] to involve hydroxylation of dopamine (58), derived in turn from tyrosine.

When ^{15}N-labelled phenylalanine and ^{15}N-labelled alanine were fed separately to *Ephedra distachya*, the important result was obtained that nitrogen-15 was incorporated into ephedrine from the former, but hardly at all from the latter.[69] This double experiment overcomes an objection to the use of ^{15}N-labelled amino-acids that nitrogen may be transferred into and out of the general nitrogen pool of the plant by transamination. Thus, in the ephedrine case, it seems that the actual amino-acid is being



used rather than phenylpyruvic acid, for example. The *N*-methyl group of ephedrine can be derived from methionine or from formate, and formate also serves as a precursor of the *C*-methyl group.[69]

There have been complementary researches[70] on (+)-norpseudo-

[67] E. Leete, *Chem. and Ind.*, 1959, 604.

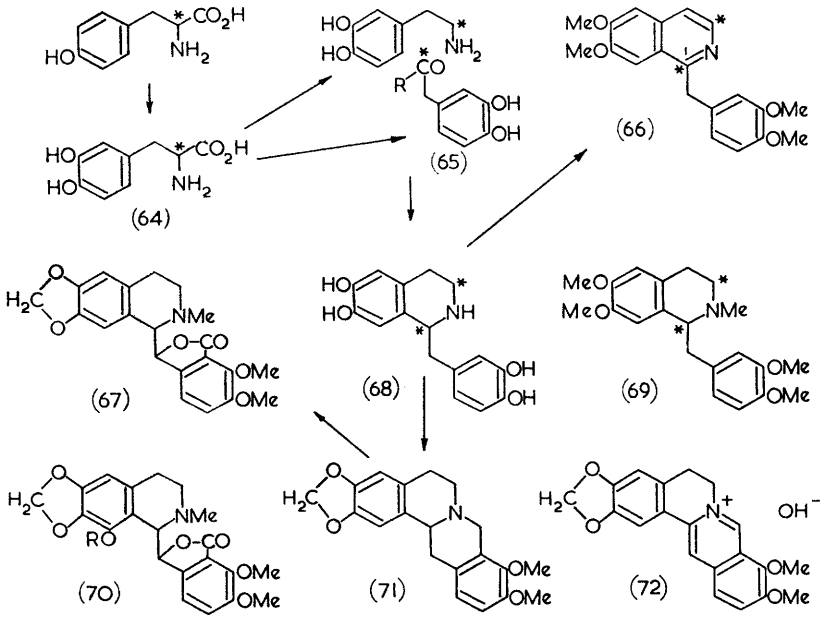
[68] S. Udenfriend and J. B. Wyngaarden, *Biochim. Biophys. Acta*, 1956, **20**, 48; M. Goodall and N. Kirshner, *J. Biol. Chem.*, 1957, **226**, 213, 821; G. Rosenfeld, L. C. Leeper, and S. Udenfriend, *Arch. Biochem. Biophys.*, 1958, **74**, 252; H. Blaschko, *Brit. Med. Bull.*, 1957, **13**, 162; S. Senoh, C. R. Creveling, S. Udenfriend, and B. Witkop, *J. Amer. Chem. Soc.*, 1959, **81**, 6236.

[69] S. Shibata and I. Imaseki, *Pharm. Bull. (Japan)*, 1956, **4**, 277; S. Shibata, I. Imaseki, and M. Yamazaki, *ibid.*, 1957, **5**, 71, 594.

[70] E. Leete, *Chem. and Ind.*, 1958, 1088.

ephedrine (63) in which this alkaloid was obtained labelled at the starred carbon atom after [3-^{14}C]phenylalanine had been taken up by a shoot of *Catha edulis*. Since in addition, ^{14}C-labelled ω-aminoacetophenone (61) has been shown to act as a precursor of ephedrine (62) in *E. distachya* without randomisation of the label,[71] the annexed tentative scheme can be written for the biosynthesis of these alkaloids. At present, other schemes are equally acceptable in which, for example, decarboxylation or *N*-methylation occurs later in the sequence of events.

The next stage in complexity in this group of alkaloids brings one to the simple isoquinolines such as pellotine (63A). This can be derived in theory[43] from tyrosine and acetic acid or some equivalents of these substances, and tracer experiments[72] on *Lophophora williamsii* suggest that at least one part of the speculation is correct. When [2-^{14}C]tyrosine was fed to the cactus, radioactive pellotine (63A) could be isolated; degradation of this material will yield information about the biosynthesis of one of the simplest isoquinoline alkaloids.



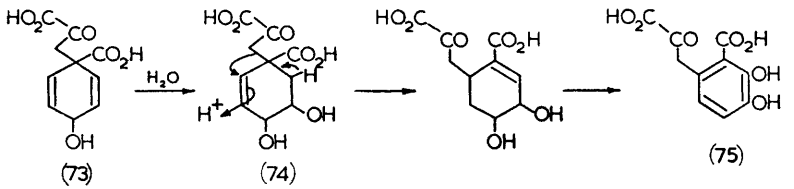
As early as 1910, it was suggested[61] that the benzylisoquinoline system known as norlaudanosoline (68) is formed from two molecules of dihydroxyphenylalanine (64) by the sort of scheme illustrated. Methylation at some stage could give laudanosine (69) and dehydrogenation of the heterocyclic system, which might possibly occur by way of the *N*-oxide,[73]

[71] I. Imaseki, S. Shibata, and M. Yamazaki, *Chem. and Ind.*, 1958, 1625.
[72] A. R. Battersby and S. Garratt, unpublished work.
[73] E. Wenkert, *Experientia*, 1954, **10**, 346.

could lead to papaverine (66). Laudanosine and papaverine occur together in the opium poppy (*Papaver somniferum*).

In addition to these two alkaloids, many other isoquinoline bases have been regarded in biogenetic theory[43,74,75] as being derived from norlaudanosoline (68) as the key intermediate. Condensation with formaldehyde or some equivalent one-carbon unit could account for the biosynthesis of canadine (71) which is found in many *Corydalis* species. Dehydrogenation of canadine could then afford berberine (72), whereas oxidative modification of the canadine skeleton (71) would give one possible route to the phthalide isoquinoline alkaloids such as hydrastine (67) which is found alongside berberine (72) in *Hydrastis canadensis*. Narcotine (70; R = Me) and narcotoline (70; R = H) would on this theory require an oxygenated canadine skeleton as the precursor. It is possible[43,74,75] to draw a large number of other alkaloids into the sort of scheme illustrated by formulæ (64) to (72).

A second hypothetical route[46] to the phthalide isoquinoline bases involves the conversion of prephenic acid (73) into the hydrated derivative (74). This may undergo an acid-catalysed rearrangement and further



transformations as illustrated. Reaction of the hydroxylated *o*-carboxyphenylpyruvic acid (75) with a phenethylamine could then give the phthalide-isoquinoline skeleton such as structure (70).

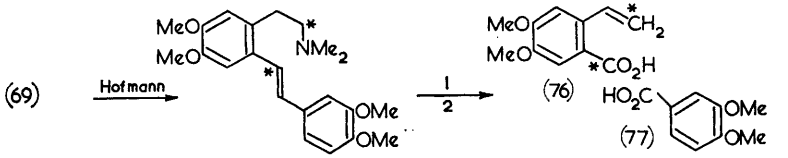
Before going further with biogenetic theory in the isoquinoline series, the experimental evidence for the cases considered so far should be examined. If the scheme tyrosine → (64) → (68) → (66) is in fact followed by the plant, it can be seen that specifically labelled papaverine (66) should be obtained from the uptake of specifically labelled tyrosine. In fact it was found[76] that when [2-^{14}C]tyrosine is fed to *Papaver somniferum* plants, it is incorporated into papaverine. The radioactive alkaloid was methylated and reduced to laudanosine (69) which was then degraded, as shown, to give the key fragment (76). The starred atoms were isolated separately from this by ozonolysis and by decarboxylation and each carried half of the activity of the original papaverine. The veratric acid (77) was inactive. The absence of labelling at positions other than the

[74] R. B. Turner and R. B. Woodward, "The Alkaloids," ed. R. H. F. Manske and H. L. Holmes, Academic Press, New York, 1953, Vol. III, p. 54.

[75] R. H. F. Manske, *J.*, 1954, 2987; R. H. F. Manske, "The Alkaloids," ed. R. H. F. Manske and H. L. Holmes, Academic Press, New York, 1954, Vol. IV, p. 1.

[76] A. R. Battersby and B. J. T. Harper, *Proc. Chem. Soc.*, 1959, 152.

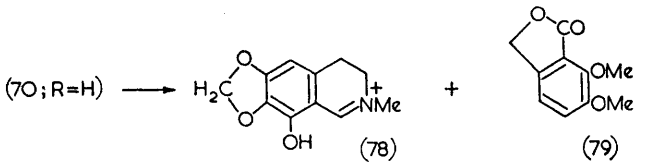
starred ones is convincing evidence against the possibility that the administered tyrosine is degraded to small fragments in the plant and that these are then used in the biosynthesis of the alkaloid. This result thus establishes the biosynthesis of papaverine (66) from two tyrosine molecules.



1, Oxidation. 2, Hofmann.

It should be stressed that "biosynthesis from tyrosine" must at present be understood to include all the close biological equivalents of tyrosine such as tyramine (52), dihydroxyphenylalanine (64), and 3,4-dihydroxyphenylpyruvic acid.

It is known[77] that radioactive berberine (72) can be isolated from plants fed with [2-^{14}C]phenylalanine, but so far the necessary controlled degradation of the alkaloid has not been reported. Tracer experiments have also been carried out on narcotoline (70; R = H); here ^{14}C-generally labelled tyrosine was fed[78] to *Papaver somniferum* plants. The radioactive narcotoline so produced was then cleaved to cotarnoline (78) and meconine (79)



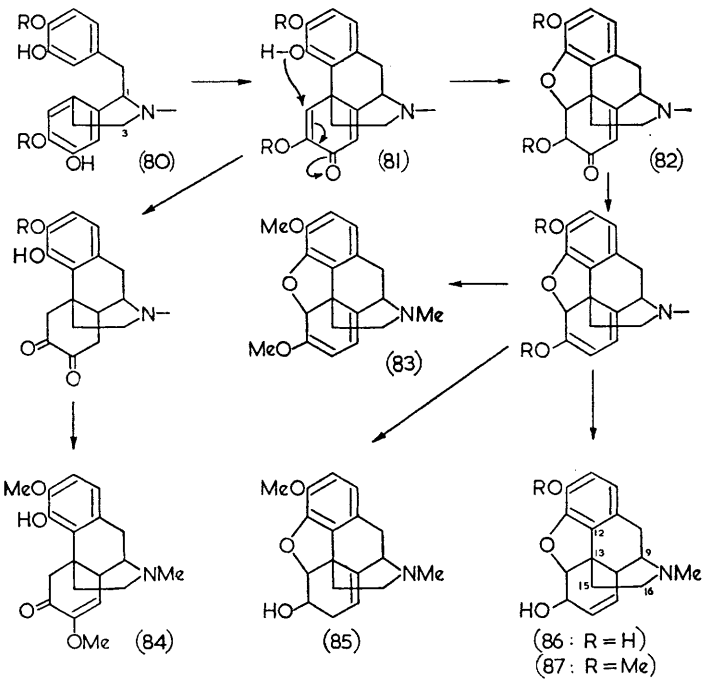
which had activities consistent with the use of the carbon skeletons of two tyrosine molecules for the synthesis of narcotoline (70; R = H). However, because the original tyrosine was generally labelled, other interpretations of this interesting result are still possible.

The suggestions involved in the scheme (64) → (72) and extensions of this scheme represent one line of thought in biogenetic theory for the isoquinoline alkaloids; a second line involves the proposal that the versatile norlaudanosoline (80; R = H) can undergo oxidative coupling. This could lead to a wide variety of alkaloids and the biosynthesis of one group of these has been extensively studied in the living plant. Accordingly, this group, containing morphine (86), codeine (87), and thebaine (83), will be discussed in the present survey.

It was first recognised by Gulland and Robinson[79] that, if norlaudano-

[77] J. L. Beal and E. Ramstad, *Naturwiss.*, 1960, **47**, 206.
[78] G. Kleinschmidt and K. Mothes, *Z. Naturforsch.*, 1959, **14b**, 52.
[79] J. M. Gulland and R. Robinson, *Mem. Proc. Manchester Lit. Phil. Soc.*, 1925, **69**, 79.

soline (80; R = H) [which is structure (68) re-written] undergoes oxidative coupling of the two aromatic rings, then the skeleton of morphine (86) can in theory be obtained. There have been many suggestions about the mechanism of the coupling process.[43,80,81] The most satisfying scheme, proposed by Barton and Cohen,[81] is illustrated here and is based upon knowledge gained in the elucidation of the structure of Pummerer's ketone.[82] The R groups may be methyl or they may represent part of an enzyme surface which is controlling the direction of the coupling process. The exact state of the nitrogen is left unspecified. Oxidation by some one-electron transfer system could generate radicals which if coupled would yield the dienone (81). The oxide bridge could then be formed by addition, as shown, to the enone system, so leading to the base (82) which by obvious

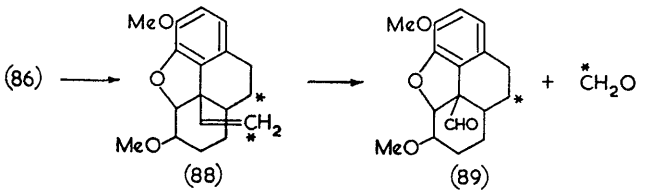


changes could afford thebaine (83), codeine (87), morphine (86), and neopine (85). If an oxide bridge is not formed from the intermediate (81), then only simple changes need be postulated to account for sinomenine (84).

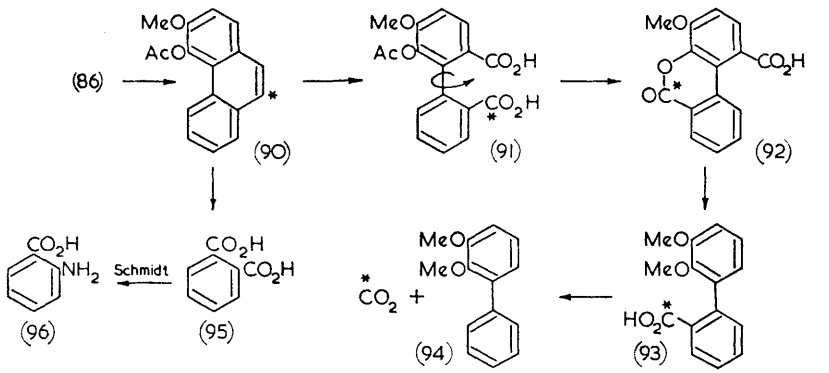
It is fortunate that tracer experiments with the opium poppy have been

[80] C. Schöpf, *Naturwiss.*, 1952, **39**, 241; K. W. Bentley, *Experientia*, 1956, **12**, 251.
[81] D. H. R. Barton and T. Cohen, "Festschrift A. Stoll", Birkhauser, Basle, 1957, p. 117.
[82] D. H. R. Barton, A. M. Deflorin, and O. E. Edwards, *J.*, 1956, 530.

very fruitful and a fairly complete picture of the biosynthesis of the alkaloids is emerging. When [2-^{14}C]tyrosine is fed to *Papaver somniferum* plants, it is incorporated[83,84] into morphine (86), codeine[83] (87), and thebaine[83] (83); the formation of labelled papaverine (66) in the same feeding experiment has already been discussed. Degradation[83] of the labelled morphine by several stages[85] gave the perhydrophenanthrene (88) from which the terminal methylene group was isolated by suitable oxidation. The formaldehyde and the major fragment (89) each contained half of the activity of the original morphine (86), so that half the alkaloid's activity is located at position 16. A second degradation[86] converted



the radioactive morphine into the phenanthrene (90) which was oxidised to the diphenic acid (91). The required carboxyl group was then selected by decarboxylation of the derived coumarin (92) followed by ring opening



to give the biphenyl-acid (93). Decarboxylation of this product gave carbon dioxide which carried half the activity of the original morphine, and the biphenyl residue (94) was almost inactive. Thus the radioactive morphine (86) is labelled equally at positions 9 and 16. Independent degradative work[84] has demonstrated that the active morphine derived from [2-^{14}C]-tyrosine yields the phenanthrene (90) which was oxidised to phthalic

[83] A. R. Battersby and B. J. T. Harper, *Chem. and Ind.*, 1958, 364; A. R. Battersby and B. J. T. Harper, *Tetrahedron Letters*, 1960, No. 27, 21.
[84] E. Leete, *Chem. and Ind.*, 1958, 977; *J. Amer. Chem. Soc.*, 1959, **81**, 3948.
[85] R. Pschorr and F. Dickäuser, *Ber.*, 1911, **44**, 2633; H. Rapoport and G. B. Payne, *J. Amer. Chem. Soc.*, 1952, **74**, 2630 and refs. therein.
[86] A. R. Battersby, R. Binks, and D. J. Le Count, *Proc. Chem. Soc.*, 1960, 287.

acid. The phenanthrene (90) and the phthalic acid (95) each contained half the activity of the original morphine, whereas anthranilic acid (96) prepared as shown contained half the activity of the phthalic acid. Because of the equivalence of the carboxyl groups of phthalic acid, these results mean that half the activity of the original morphine is located at position 9 or position 12 (or is spread between them) and it is argued[84] that the first possibility is the correct one; the other half of the activity is in the eliminated ethanamine chain. The two degradative studies are thus in full agreement.

The above results establish that two molecules of tyrosine, or a close biological equivalent, are built into morphine in the biosynthesis. Moreover, since two molecules of [2-^{14}C]tyrosine would give a norlaudanosoline system (80) labelled equally at positions 1 and 3 (p. 275), the labelling pattern of morphine is exactly in agreement with the scheme on p. 275. It seems probable that tyrosine is converted first into 3,4-dihydroxyphenylalanine.

Both groups of workers[84,87] found that [2-^{14}C]phenylalanine can serve as a precursor of morphine, but is much less efficiently incorporated than in tyrosine. Here again, it may well be that the main route used is shikimic acid → prephenic acid → tyrosine → morphine and that phenylalanine enters only by conversion into tyrosine.

The *N*-methyl group of morphine (86), and the *N*- and the *O*-methyl groups of codeine (87) and thebaine (83), can be derived from methionine;[88] formate is a poor precursor of these groups,[88] and choline is ineffective.[87]

One of the stages in the proposed biosynthesis of morphine which has attracted considerable interest is the ring-closure of norlaudanosoline (80; R = H) or some protected derivative (80). Many unsuccessful attempts have been made to carry out this conversion in the laboratory but it has now been demonstrated in the plant.[89] For this, norlaudanosoline (80; R = H), labelled with ^{14}C at position 1, was synthesised and fed to mature *Papaver somniferum* plants. The isolated morphine was highly radioactive. Indeed, norlaudanosoline is the most efficient precursor of morphine so far used. Degradation of the morphine showed[90] that all the activity is at position 9. Thus it is almost certain that the bond between positions 12 and 13 of morphine (86) is formed as was suggested in the scheme (p. 275) by a coupling reaction between two *aromatic* rings. It was further shown[90] that the plants fed with labelled norlaudanosoline (80; R = H) yielded radioactive papaverine (66) which was proved by degradation to be specifically labelled at position 1. Aromatisation of the reduced intermediate (80; R = H) is thus demonstrated.

The rate at which radioactivity is incorporated into morphine, codeine,

[87] A. R. Battersby and B. J. T. Harper, unpublished work.
[88] A. R. Battersby and B. J. T. Harper, *Chem. and Ind.*, 1958, 365.
[89] A. R. Battersby and R. Binks, *Proc. Chem. Soc.*, 1960, 360.
[90] A. R. Battersby, R. Binks, and G. V. Parry, unpublished work.

and thebaine from carbon dioxide[91] and [2-^{14}C]tyrosine[92] has been studied, and it is found that in both cases the activity moves rapidly into the thebaine fraction and then on through codeine to morphine. The simplest interpretation[91,92] of these results is that the biosynthesis runs first to thebaine (83) and then on through codeine (87) to morphine (86). This has been neatly confirmed[93] by feeding generally labelled thebaine to poppy plants, codeine and morphine both labelled in their skeletons being isolated. Similarly, generally labelled codeine was used by the plants to give labelled morphine but not labelled thebaine, whereas no activity from generally labelled morphine passed into the skeletons of codeine and thebaine. These results show the importance of demethylation as a biosynthetic process.

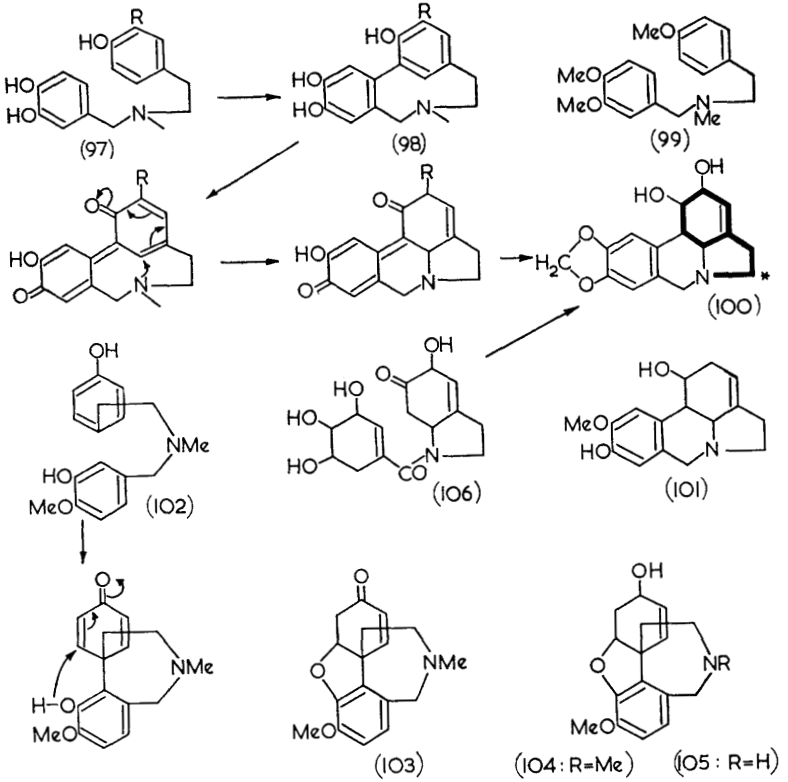
All the findings are thus falling nicely into place in the opium alkaloids. The use of two C_6–C_2 units to provide the skeletons of morphine and papaverine, and the conversion of norlaudanosoline into morphine and papaverine, have been demonstrated in the plant. The order of formation of the hydrophenanthrene alkaloids is known and the use of thebaine as a precursor of morphine and codeine suggests strongly that phenolic coupling of the intermediate (80) is partly controlled by having R = Me. In this way, many ring-closures which might occur when R = H are then blocked. It can be seen from the above biosynthetic scheme that phenolic coupling of the molecule (80; R = Me) leads readily to thebaine (83).

The Amaryllidaceae *Alkaloids.*—The intensive search among the *Amaryllidaceae* over the last few years has yielded a large number of alkaloids. Barton and Cohen[81] suggest that their biosynthesis involves a precursor of the type (97; R = H or OH), possibly with some of the hydroxyl groups protected. This could give rise to the various types of *Amaryllidaceae* alkaloid by suitable phenolic coupling. For example, the product (98; R = OH) of *ortho–para*-coupling could be further modified as shown, to give lycorine (100). Norpluviine (101) could be formed in a similar way from the precursor (97; R = H). Significantly, a simple derivative of the proposed precursor has emerged as belladine (99) in *Amaryllis belladonna.*[94] If a simple derivative of the same precursor (97; R = H) is re-written as (102) to be coupled as shown, then the structures of narwedine (103), galanthamine (104), and narcissamine (105) can be built up. Equally satisfying routes to the other alkaloids in this group can be given.[81]

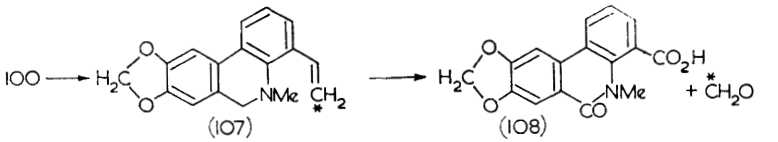
Wenkert,[46] on the other hand, suggests the use of reduced precursors based upon shikimic and prephenic acid, and the intermediate (106) derived from them could by reasonable steps be converted into lycorine (100). Here again the theory can embrace the other types of *Amaryllidaceae* alkaloids.

[91] H. Rapoport, F. R. Stermitz, and D. R. Baker, *J. Amer. Chem. Soc.*, 1960, **82**, 2765.
[92] A. R. Battersby and B. J. T. Harper, *Tetrahedron Letters*, 1960, No. 27, 21.
[93] F. R. Stermitz and H. Rapoport, *Nature*, 1961, **189**, 310.
[94] E. Warnhoff, *Chem. and Ind.*, 1957, 1385.

[2-^{14}C]Tyrosine and the phenol (102), labelled with ^{14}C on the *N*-methyl group, have been shown[95] to be incorporated by King Alfred daffodils into galanthamine (104). Good incorporations of [2-^{14}C]tyrosine were also achieved into lycorine (100) when Twink[96] and King Alfred[95,97] daffodils



were used. In addition, radioactive norpluviine (101) was isolated from the Twink plants.[96]

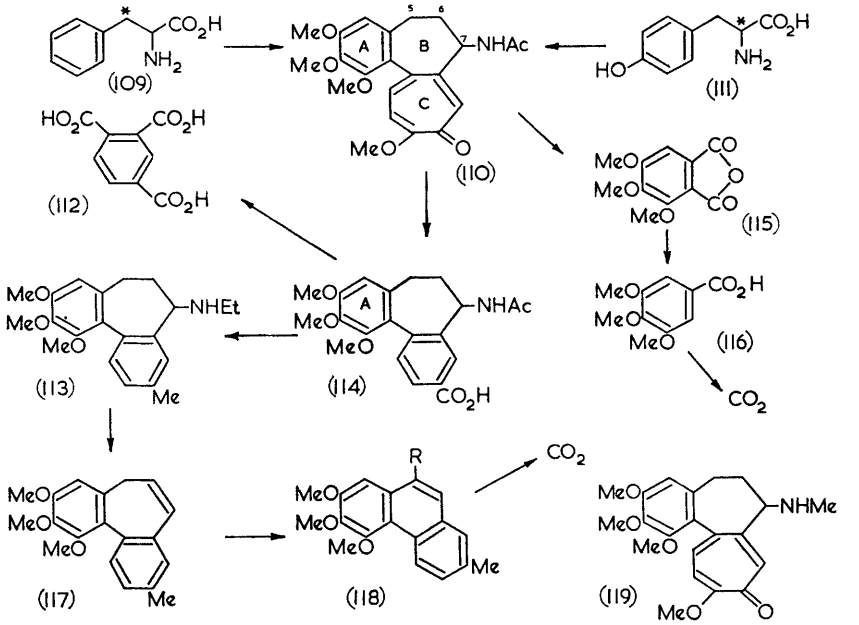


The radioactive lycorine was subjected[96] to Hofmann degradation and the terminal methylene was isolated from the methine (107) by glycol formation and periodate fission. The formaldehyde carried all the activity

[95] D. H. R. Barton and G. W. Kirby, *Proc. Chem. Soc.*, 1960, 392.
[96] A. R. Battersby, R. Binks, and W. C. Wildman, *Proc. Chem. Soc.*, 1960, 410.
[97] Personal communication from Professor D. H. R. Barton.

of the original lycorine and further oxidation of the major fragment gave the lactam acid (108) which was inactive. This interlocking evidence establishes that the activity in the lycorine is located at the starred position. Tyrosine will thus provide that part of the lycorine molecule (100) drawn with heavy bonds and, if one accepts that the conversion of prephenic acid into tyrosine is irreversible, as seems probable,[98] then these results support Barton and Cohen's theory.

Colchicine.—Colchicine (110), which occurs in the autumn crocus, presents particularly attractive biosynthetic problems because of its tropolone ring system and also because of the unusual position of the



nitrogen atom, placed three carbons away from the aromatic ring A.

[3-^{14}C]Phenylalanine (109) was incorporated[99] into colchicine (110) synthesised by *Colchicum byzantinum*, and oxidation of the active alkaloid to the anhydride (115) followed by specific decarboxylation gave the benzoic acid (116). This still retained in its carboxyl group 93% of the activity of the original colchicine. Thus the colchicine (110) derived from the precursor (109) was specifically labelled at position 5.

An independent study[100] showed that [2-^{14}C]tyrosine is incorporated into colchicine by *Colchicum autumnale*. If the C_6–C_3 system of tyrosine

[98] I. Schwinck and E. Adams, *Biochim. Biophys. Acta*, 1959, **36**, 102; O. L. Gamborg and A. C. Neish, *Canad. J. Biochem. Physiol.*, 1959, **37**, 1277.
[99] E. Leete and P. E. Nemeth, *J. Amer. Chem. Soc.*, 1960, **82**, 6055.
[100] A. R. Battersby and J. J. Reynolds, *Proc. Chem. Soc.*, 1960, 346.

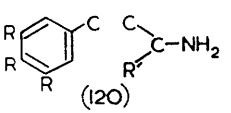
provides ring A and atoms 5, 6, and 7 of the alkaloid (110), then in this experiment position 6 should be specifically labelled. The active colchicine was converted by way of allocolchiceine (114) and the base (113) into the neutral product (117). Fission of the double bond and cyclisation of the resultant dialdehyde then gave the phenanthrene (118; R = CHO); this was oxidised and the acid (118; R = CO_2H) was decarboxylated to give the carbon atom from position 6 of colchicine (110) as carbon dioxide. The results showed that the *N*-acetyl group of the active colchicine carried half of the original activity and that the carbon atom at position 6 was virtually inactive.

The most reasonable interpretation of the above two sets of results is that the side chains of the aromatic amino-acids are degraded to the C_6–C_1 state before incorporation. The appearance of radioactivity in the *N*-acetyl group is consistent with this proposal since presumably a labelled two-carbon fragment is available; an analogy will be met later in the biosynthesis of gramine from tryptophan.

L-[*Me*-^{14}C]Methionine[100,101] and formate[100] were also incorporated into colchicine, the former being used to provide the methyl groups of the alkaloid.[101] Formate was a poor precursor.[100]

Both groups of workers[100,101] fed [1-^{14}C]acetate to the autumn crocus and obtained active colchicine. It was shown that all[101] or almost all[100] of the activity was present in the acetyl group, and Substance F (119) isolated from the same plants was inactive.[99] A small amount of activity was associated in one case with ring A and its attached atoms,[100] but ring C was isolated as trimellitic acid (112) and found to be inactive.[100] These results lead to the conclusion that the tropolone ring of colchicine (110) is not formed from acetate, in contrast to the mould tropolones.[102]

All the present theories[103,104] for the biosynthesis of colchicine now require some modification. It seems[100] that the non-tropolone part of the



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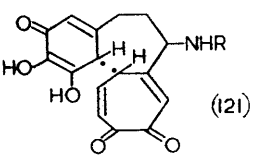


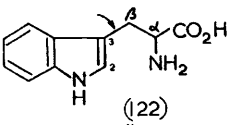
(121)

molecule is built up from the fragments shown in (120; R = H or OH) where R′ may be H, to give a glycine or an alanine equivalent, or perhaps a carbon chain or ring to provide part or all of the tropolone system. The origin of the tropolone part of the molecule is unknown and more feeding experiments are in progress to solve this fascinating problem.

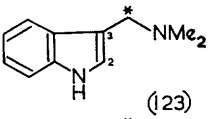
[101] Personal communication from Professor E. Leete.
[102] R. Bentley, *Biochim. Biophys. Acta.* 1958, **29**, 666; J. H. Richards and L. D. Ferretti, *Biochem. Biophys. Res. Comm.*, 1960, **2**, 107.
[103] F. A. L. Anet and R. Robinson, *Nature*, 1950, **166**, 924; B. Belleau, *Experientia*, 1953, **9**, 178; E. Wenkert, *Ibid*, 1959, **15**, 165.
[104] A. I. Scott, *Nature*, 1960, **186**, 556.

On the theoretical side, there has been the attractive proposal[104] that the bond between rings A and C of colchicine (110) is formed by a phenolic coupling process as illustrated in formula (121). Such a step would be analogous to the ring closures which give the morphine skeleton (p. 275) and the lycorine and galanthamine systems (p. 279).

Indole Alkaloids.—Gramine (123) is the simplest indole alkaloid to be examined by tracer methods. When barley seedlings were fed with [β-^{14}C]-tryptophan (122), gramine (123) was formed with labelling only at the starred position.[105] Moreover, tryptophan doubly labelled at the 2- and the β-position, *i.e.*, [β,2-^{14}C]tryptophan, gave radioactive gramine labelled at the starred position and position 2, and the ratio of the activities at the



(122)



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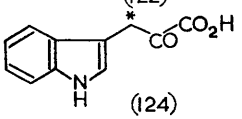

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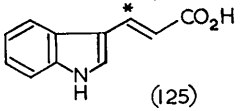


(125)

two positions was the same as in the original tryptophan.[105] This proves that the arrowed bond in tryptophan (122) remains intact during the formation of the alkaloid. Here then is another example of cleavage of the side-chain of an aromatic amino-acid between the α- and the β-position by a process which apparently operates on a three-carbon side-chain. Thus, 3-indolyl[β-^{14}C]pyruvic acid (124) and 3-indolyl[β-^{14}C]acrylic acid (125) also gave rise in sprouting barley to gramine (123) labelled only at the side-chain methylene group.[106] However, when 3-indolylacetic acid and its amide, 3-indolylglyoxylic acid, and 3-formylindole, all radioactive, were fed separately, all gave inactive gramine;[106] these four precursors were labelled in the side-chain at the carbon atom adjacent to the indole ring. The pathway used for the removal of the two carbon atoms from tryptophan does not, therefore, pass through these simple indoles and further tracer experiments here will be followed with interest.

Seven biogenetic schemes have been published which might in theory account for the structure of lysergic acid (131), the indolic component of a number of the ergot alkaloids. Some theories which made use of 5-hydroxytryptophan (cf. 130) have been eliminated by the isolation of inactive ergot alkaloids from *Claviceps purpurea* cultures fed with 5-hydroxy[β-^{14}C]trytophan.[107] As will be seen below, other precursors were satisfactorily incorporated under the same conditions. However, on the positive side, several independent researches are now giving strong

[105] K. Bowden and L. Marion, *Canad. J. Chem.*, 1951, **29**, 1037, 1045; E. Leete and L. Marion, *ibid.*, 1953, **31**, 1195.
[106] A. Breccia and L. Marion, *Canad. J. Chem.*, 1959, **37**, 1066.
[107] R. M. Baxter, S. I. Kandel, and A. Okany, *Chem. and Ind.*, 1960, 266.

support to one biogenetic scheme.[108] This, like most of the others, calls upon tryptophan (130) to provide the main part of the molecule, but it differs from the rest in suggesting the use of an isopentenyl residue to build up the piperidine ring as indicated in (130); the structure of chanoclavine[109] (127) accords well with this proposal.

Experimental support has come from feeding *C. purpurea*, growing both parasitically and saprophytically, with [β-^{14}C]tryptophan (130). The former technique[108] gave a significant incorporation into the ergot alkaloids and the latter a good one;[110–112] indeed, in the German work with saprophytic cultures, incorporations as high as 47% were achieved.[112] All the many ergot alkaloids isolated in one experiment[111] had approximately the same specific activity, including those based upon relatives of lysergic acid, such as agroclavine (128) and elymoclavine (129), a result which indicates a common biosynthetic pathway to the various bases, [*carboxy*-^{14}C]Tryptophan, fed under the conditions which gave the high incorporation already mentioned, did not yield radioactive ergot alkaloids, so that the carboxyl group must be eliminated during the biosynthesis.[112]

So far, no degradation of the active lysergic acid (131), agroclavine (128), or elymoclavine (129) from these tracer experiments has been reported and it is to be hoped that the expected location of the label entirely at the starred position will be rigorously established. This would satisfy critics who may argue that the tryptophan side-chain could have been degraded to small fragments before incorporation. However, it has been shown that the indolic part of tryptophan is used in the biosynthesis since tryptophan labelled with deuterium in the heterocycle was used by *Pennisetum typhoideum* to form deuterated agroclavine (128) and elymoclavine[113] (129).

High incorporations into agroclavine (128) and elymoclavine (129) have been achieved from mevalonolactone (126) labelled at position 2 with carbon-14 or tritium, and also from [4-^{3}H]mevalonolactone;[112] saprophytic cultures were used. Other workers[114] observed lower incorporations, but here the active alkaloids were degraded. Both agroclavine (128) and elymoclavine (129) were examined and the results obtained[114] were very similar for the two alkaloids. The bases derived from [2-^{14}C]mevalonolactone carried approximately half their activity at position 17 and no

[108] K. Mothes, F. Weygand, D. Gröger, and H. Grisebach, *Z. Naturforsch.*, 1958, **13b**, 41; A. J. Birch and H. Smith, "CIBA Foundation Symposium on Amino Acids and Peptides with Antimetabolic Activity", ed. Wolstenholme and O'Connor, Churchill, London, 1958, p. 247.

[109] A. Hofmann, R. Brunner, H. Koble, and A. Brack, *Helv. Chim. Acta*, 1957, **40**, 1358.

[110] D. Gröger, H. J. Wendt, K. Mothes, and F. Weygand, *Z. Naturforsch.*, 1959, **14b**, 355.

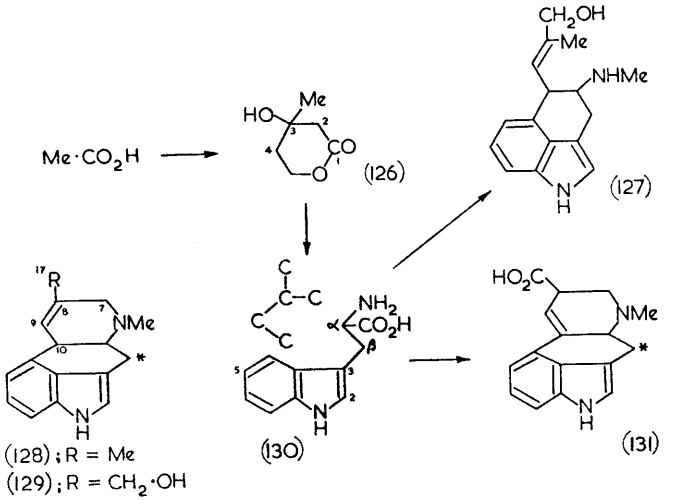
[111] W. A. Taber and L. C. Vining, *Chem. and Ind.*, 1959, 1218.

[112] D. Gröger, K. Mothes, H. Simon, H.-G. Floss, and F. Weygand, *Z. Naturforsch.*, 1960, **15b**, 141; E. H. Taylor and E. Ramstad, *Nature*, 1960, **188**, 494.

[113] H. Plieninger, R. Fischer, W. Lwowski, A. Brack, H. Kobel, and A. Hofmann, *Angew. Chem.*, 1959, **71**, 383.

[114] A. J. Birch, B. J. McLoughlin, and H. Smith, *Tetrahedron Letters*, 1960, No. 7, 1.

activity at position 8, those from [1-^{14}C]acetate had about half their activity at position 8 and none at position 17, and those from [2-^{14}C]-acetate were appreciably labelled (*ca.* 10—25% of the total) at position 17 and only weakly labelled at position 8. If these precursors are built into the ergot alkaloids as they are into terpenes and steroids, one would

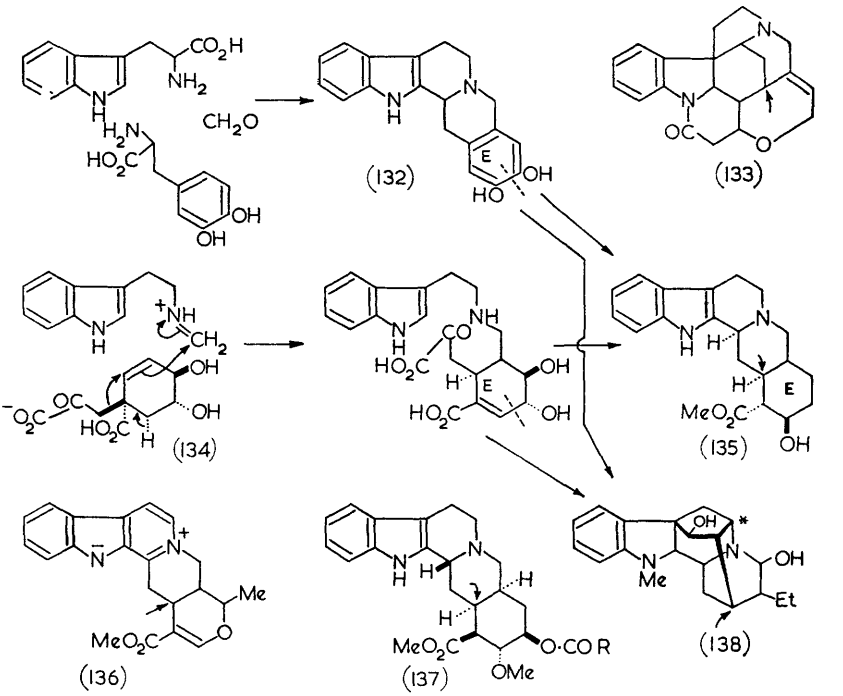


expect incorporation from [2-^{14}C]mevalonolactone into position 7 or 17, or both. Similarly, [1-^{14}C]acetate ought to pass its label into positions 8 and 10, and [2-^{14}C]acetate into positions 7, 9, and 17. It will be seen that the results are consistent with these expectations and the combined evidence from all the researches just reviewed suggests strongly that the ergot alkaloids are formed from an isopentenyl residue and tryptophan.

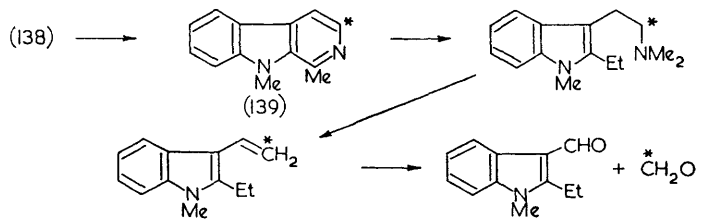
There is as yet little experimental information about the biosynthesis of the vast complex of inter-related indole alkaloids which includes such bases as yohimbine (135), strychnine (133), and the calabash curare alkaloids.[115] Current interest is centred on the origin of ring E of yohimbine (135) and the analogous carbon atoms in the many other alkaloids. Thus, one theory[43,116,117] makes use of tryptophan, a dihydroxyphenylalanine residue, and a formaldehyde equivalent to build up yohimbine (135) by way of (132) as illustrated. A valuable and ingenious alternative scheme has been elaborated by Wenkert and Bringi,[118] who propose that hydrated prephenic acid (134) may be one precursor as shown. Cleavage of ring E, first suggested[119] for the biosynthesis of strychnine (133) and known as "Woodward fission", is then invoked in both theories to accommodate

[115] K. Bernauer, *Fortschr. Chem. org. Naturstoffe*, 1959, **17**, 183; A. R. Battersby and H. F. Hodson, *Quart. Rev.*, 1960, 77.
[116] G. Barger and C. Scholz, *Helv. Chim. Acta*, 1933, **16**, 1343.
[117] R. B. Woodward, *Angew. Chem.*, 1956, **68**, 13.
[118] E. Wenkert and N. V. Bringi, *J. Amer. Chem. Soc.*, 1959, **81**, 1474.
[119] R. B. Woodward, *Nature*, 1948, **162**, 155.

such alkaloids as serpentine (136). Further ring closures after the fission step are necessary to account[117] for ajmaline (138). The difference between the two theories is that, in one, ring E of yohimbine (135), for example, is thought to be formed from an *aromatic* precursor and in the other the intermediates never become aromatic. The important point must be made that Wenkert and Bringi's theory leads to the correct absolute stereo-



chemistry at the arrowed position (see 133—138) of the various indole alkaloids, and this configuration is constant for all the other bases in this



group with the sole exception of ψ-akuammicine.[120] Though it is not possible here to give the details, these theories can embrace a large number of indole alkaloids.

[120] P. N. Edwards and G. F. Smith, *Proc. Chem. Soc.*, 1960, 215.

Recently it has been proved[121] that *Rauwolfia serpentina* plants will build [2-^{14}C]tryptophan into ajmaline (138), reserpine (137), and serpentine (136), and the first was degraded by alkali fusion to yield *ind*-*N*-methylharman (139). The further degradations shown then allowed the isolation of the starred carbon atom as formaldehyde and it carried all the activity of the original ajmaline. Thus, the alkaloid (138) is labelled solely at the starred position and there can be little doubt that tryptophan is a direct precursor of ajmaline.

In conclusion, it can be said that the present state of research on alkaloid biosynthesis is that the breakthrough has been made. Not only has it been demonstrated that plants will incorporate amino-acids, acetate, mevalonolactone, and some still simpler materials into alkaloids, but also it is known that the large intermediates can be successfully introduced into the plant's biosynthetic system. The direction in which future researches should be aimed is clear and a rapid increase in knowledge of the way alkaloids are made can be predicted with confidence.

[121] E. Leete, *Chem. and Ind.*, 1960, 692.