1

Biosynthesis

BY R. B. HERBERT

1 Introduction

Previous practice, of listing earlier Reports in this series as the first references,[1—9] is continued. One of the most recent reviews on alkaloid biosynthesis[10] is again cited for background information; another, more extensive, review has appeared which includes a discussion of the biosynthesis of nitrogeneous microbial metabolites, as well as that of plant alkaloids.[11]

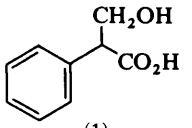
This Report is the tenth to have been published. Some perspective has been given to research surveyed in the first five Reports,[5] and it is appropriate to attempt the same for material in succeeding volumes. In the following, liberal reference is made to fuller discussion in previous Reports, and in any case, discussion is curtailed of material appearing again in this Report.

The biosynthetic route to most plant alkaloids and nitrogenous microbial metabolites is quite closely defined by the results of straightforward tracer feeding experiments. For these, work with the enzymes involved provides extra detail, with helpful confirmation for an already deduced sequence (see, *e.g.*, nicotine[12]

[1] R. B. Herbert, in 'The Alkaloids', ed. J. E. Saxton (Specialist Periodical Reports), The Chemical Society, London, 1971, Vol. 1.
[2] J. Staunton, in 'The Alkaloids', ed. J. E. Saxton (Specialist Periodical Reports), The Chemical Society, London, 1972, Vol. 2.
[3] R. B. Herbert, in 'The Alkaloids', ed. J. E. Saxton (Specialist Periodical Reports), The Chemical Society, London, 1973, Vol. 3.
[4] R. B. Herbert, in 'The Alkaloids', ed. J. E. Saxton (Specialist Periodical Reports), The Chemical Society, London, 1974, Vol. 4.
[5] R. B. Herbert, in 'The Alkaloids', ed. J. E. Saxton (Specialist Periodical Reports), The Chemical Society, London, 1975, Vol. 5.
[6] R. B. Herbert, in 'The Alkaloids', ed. M. F. Grundon (Specialist Periodical Reports), The Chemical Society, London, 1976, Vol. 6.
[7] R. B. Herbert, in 'The Alkaloids', ed. M. F. Grundon (Specialist Periodical Reports), The Chemical Society, London, 1977, Vol. 7.
[8] R. B. Herbert, in 'The Alkaloids', ed. M. F. Grundon (Specialist Periodical Reports), The Chemical Society, London, 1978, Vol. 8.
[9] R. B. Herbert, in 'The Alkaloids', ed. M. F. Grundon (Specialist Periodical Reports), The Chemical Society, London, 1979, Vol. 9.
[10] R. B. Herbert, in 'Comprehensive Organic Chemistry', ed. D. H. R. Barton and W. D. Ollis, Pergamon, Oxford, 1978, Vol. 5, p. 1045.
[11] R. B. Herbert, in "Rodd's Chemistry of Carbon Compounds", second edition, ed. S. Coffey, 1980, Vol. IV L, p. 291.
[12] S. Mizusaki, M. Tanabe, M. Noguchi, and E. Tamaki, *Phytochemistry*, 1972, **11**, 2757; *Plant Cell Physiol.*, 1971, **12**, 633; R. B. Herbert, in ref. 4, p. 7.

and coniine[13]). For others it is only with the isolation of the enzymes responsible for the various steps of biosynthesis that a clear statement about the sequence can be made. Not surprisingly, most progress has been made in isolating enzymes responsible for metabolite formation in micro-organisms. Good examples of this are to be found in studies on the biosynthesis of echinulin (this Report p. 24), indolmycin,[14] and benzodiazepine alkaloids.[15,16] It may well be that some of the problems which still defy a solution will only yield to experiments with isolated enzymes.

One such problem concerns the tropic acid moiety (1) found in the tropane alkaloids. It is derived from phenylalanine and its formation involves a 1,2-shift of the carboxy-group in the amino-acid. The mechanism and the substrate for rearrangement are uncertain, however (this Report, p. 12). There is a similar rearranged phenylalanine fragment in the microbial metabolite tenellin (2).[17] That such a rearrangement should be observed both in a plant alkaloid and a microbial metabolite argues for a simple common diversion from phenylalanine metabolism.



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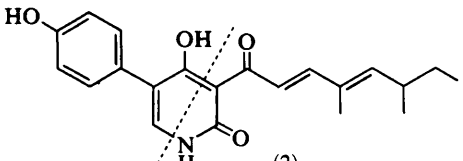

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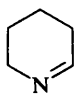
The biosynthesis of the *Lycopodium* alkaloids, *e.g.* lycopodine (5), is still a puzzle. Biosynthesis of these alkaloids from Δ^1-piperideine (3) and acetic acid, suggests that these alkaloids are formed by dimerization of a single precursor, for which isopelletierine (4; R = H) is a logical candidate. But, it is quite clear that it is the source for only one half of these molecules.[18] What the precursor for the other half is, remains unknown.



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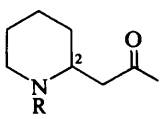

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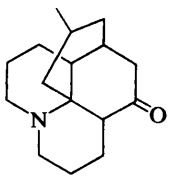


(5)

[13] M. F. Roberts, *Phytochemistry*, 1978, **17**, 107; *ibid.*, 1977, **16**, 1381; *ibid.*, 1971, **10**, 3057; *ibid.*, 1974, **13**, 1847; *ibid.*, 1975, **14**, 2393; *J. Pharm. Pharmacol.*, 1975, **27S**, 86P; R. B. Herbert, in ref. 4, p. 10; in ref. 6, p. 7; in ref. 7, p. 4; in ref. 9, p. 2.

[14] M. K. Speedie, U. Hornemann, and H. G. Floss, *J. Biol. Chem.*, 1975, **250**, 7819; R. B. Herbert, in ref. 7, p. 16.

[15] E. A. Aboutabl and M. Luckner, *Phytochemistry*, 1975, **14**, 2573; I. Richter and M. Luckner, *ibid.*, 1976, **15**, 67; L. Nover and M. Luckner, *FEBS Lett.*, 1969, **3**, 292; M. Luckner, *Eur. J. Biochem.*, 1967, **2**, 74; M. Luckner, K. Winter, and J. Reisch, *ibid.*, 1969, **7**, 380; see also this Report, p. 27.

[16] R. B. Herbert, in ref. 8, p. 26; in ref. 7, p. 24; in ref. 5, p. 39.

[17] E. Leete, N. Kowanko, R. A. Newmark, L. C. Vining, A. G. McInnes, and J. L. C. Wright, *Tetrahedron Lett.*, 1975, 4103; R. B. Herbert, in ref. 7, p. 9; in ref. 5, p. 11.

[18] W. D. Marshall, T. T. Nguyen, D. B. MacLean, and I. D. Spenser, *Can. J. Chem.*, 1975, **53**, 41; R. B. Herbert, in ref. 7, p. 3; in ref. 1, p. 5; in ref. 4, p. 1; in ref. 3, p. 28.

There are some difficulties associated with a highly attractive model for piperidine alkaloid biosynthesis (this Report, p. 9). There is still debate about the symmetrical or unsymmetrical incorporation of $^{13}CO_2$ into nicotine (this Report, p. 14). Curiously, the nature of the intermediates in quinolizidine alkaloid biosynthesis is unknown, although an attractive hypothesis has been proposed that is based on incorporation of Δ^1-piperideine (3).[19]

The terpenoid indole alkaloids are a group of plant bases derived by multiple variation on the strictosidine [(79); p. 20] skeleton. Arguably the most important work in the past five years has been done on these alkaloids with enzyme preparations from plant tissue cultures, and the research is of great potential significance for other studies in alkaloid biosynthesis. The results have allowed close definition of the early stages of biosynthesis (this Report, p. 19). Use of crude enzyme preparations in this way has been extended to the study of benzylisoquinoline biosynthesis, with enzyme-catalysed formation of norlaudanosoline-1-carboxylic acid [(57); p. 16]; this compound had earlier been identified[20] as the first of the benzylisoquinolines (this Report, p. 15). It seems that amino-acids of this general formula (6) are key intermediates in the biosynthesis of all isoquinoline alkaloids.[20,21] Lophocereine (7) is exceptional in that two routes (from leucine and mevalonate) may lead to it, only one of which potentially involves an acid like (6).[22]

Most remarkable for proving that structural relationships are not always what they seem are anatabine [(34); p. 11] and dioscorine (8). Inspection and experience with other piperidine alkaloids leads one to expect a biosynthesis for the piperidine ring [heavy bonding in (8)] from lysine *via* Δ^1-piperideine (3).[10] However, in both cases it turns out that this ring derives from nicotinic acid[23] [the exceptional derivation of the piperidine nuclei of coniine and pinidine (9) from acetate has been known for some time[24]].

The structurally unusual base securinine (10) derives in orthodox manner from lysine *via* (3), the origins of the remaining atoms being in tyrosine.[25] So far no intermediates have been identified, and the alkaloid presents an intriguing biogenetic puzzle.

[19] W. M. Golebiewski and I. D. Spenser, *J. Am. Chem. Soc.*, 1976, **98**, 6726; R. B. Herbert, in ref. 8, p. 3. For other references to work with these alkaloids see R. B. Herbert, in ref. 6, p. 6; in ref. 9, p. 2.

[20] M. L. Wilson and C. J. Coscia, *J. Am. Chem. Soc.*, 1975, **97**, 431; A. R. Battersby, R. C. F. Jones, and R. Kazlauskas, *Tetrahedron Lett.*, 1975, 1873; D. S. Bhakuni, A. N. Singh, S. Tewari, and R. S. Kapil, *J. Chem. Soc., Perkin Trans. 1*, 1977, 1662; R. B. Herbert, in ref. 6, p. 17; in ref. 9, p. 8.

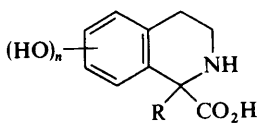
[21] G. J. Kapadia, G. S. Rao, E. Leete, M. B. E. Fayez, Y. N. Vaishnav, and H. M. Fales, *J. Am. Chem. Soc.*, 1970, **92**, 6943; J. Staunton, in ref. 2, p. 10; see also this Report, p. 15.

[22] D. G. O'Donovan and E. Barry, *J. Chem. Soc., Perkin Trans. 1*, 1974, 2528; D. G. O'Donovan and H. Horan, *J. Chem. Soc. (C)*, 1968, 2791; H. R. Schütte and G. Seelig, *Justus Liebigs Ann. Chem.*, 1970, **730**, 186; R. B. Herbert, in ref. 1, p. 17; in ref. 6, p. 16.

[23] Dioscorine: see E. Leete, *Phytochemistry*, 1977, **16**, 1705; R. B. Herbert, in ref. 8, p. 1; in ref. 9, p. 1; anatabine: see this Report, p. 11.

[24] E. Leete and J. O. Olsen, *J. Am. Chem. Soc.*, 1972, **94**, 5472; E. Leete and K. N. Juneau, *ibid.*, 1969, **91**, 5614; R. B. Herbert, in ref. 1, p. 1; in ref. 4, p. 10; J. Staunton, in ref. 2, p. 26. For recent work on pinidine, see E. Leete, J. C. Lechleiter, and R. A. Carver, *Tetrahedron Lett.*, 1975, 3779; E. Leete and R. A. Carver, *J. Org. Chem.*, 1975, **40**, 2151; R. B. Herbert, in ref. 7, p. 4.

[25] U. Sankawa, Y. Ebizuka, and K. Yamasaki, *Phytochemistry*, 1977, **16**, 561; R. J. Parry, *Tetrahedron Lett.*, 1974, 307; *J. Chem. Soc., Chem. Commun.*, 1975, 144; W. M. Golebiewski, P. Horsewood, and I. D. Spenser, *ibid.*, 1976, 217; R. B. Herbert, in ref. 5, p. 10; in ref. 6, p. 40; in ref. 7, p. 2; in ref. 8, p. 4; this Report, p. 10.



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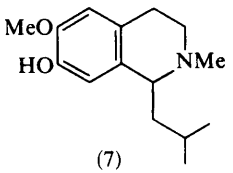

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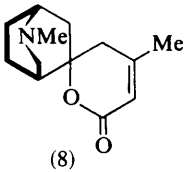

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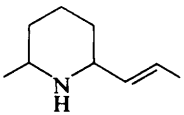

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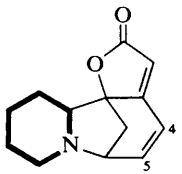


(10)

The structurally elaborate phenanthroindolizidine alkaloids of *Tylophora asthmatica*, *e.g.* tylophorinine (15), have proved to have a biogenesis from (11),[26] which is a common structural element in many pyrrolidine and piperidine alkaloids.[10] A later key intermediate is the diphenol (12), which, by oxidative phenol coupling, affords (13). This dienone then suffers modification to give the *T. asthmatica* bases.[27] Tylophorinine (15) must be formed *via* (14), rearrangement of which involves styryl (as against aryl) migration; a so far unique example among alkaloids formed by oxidative coupling of phenols.

Hasubanonine (18) and protostephanine (17) appear to be benzylisoquinoline variants, but only after extensive and painstaking research has this been established.[28,29] The key intermediate is the triphenol (16),[29] the crucial (and elusive) feature of which is two hydroxy-groups on ring C; so far, (16) is the only example of a substrate for oxidative coupling of phenols where more than one hydroxy-group must be present on an aromatic ring.

Chelidonine [(66); p. 17] was long suspected as a protoberberine variant. The truth of this has been established by the results of a series of elegant experiments, which also defined the detail of biosynthesis, including the stereochemistry of the proton losses which occur during transformation of protoberberine into chelidonine (this Report, p. 16; see also ref. 30). Corydaline (19) is a methylated protoberberine, and investigation of its biosynthesis suggests an orthodox pathway. The corydaline skeleton appears in modified form in ochotensimine (20).[31]

[26] R. B. Herbert, F. B. Jackson, and I. T. Nicolson, *J. Chem. Soc., Chem. Commun.*, 1976, 865; R. B. Herbert, in ref. 8, p. 6.

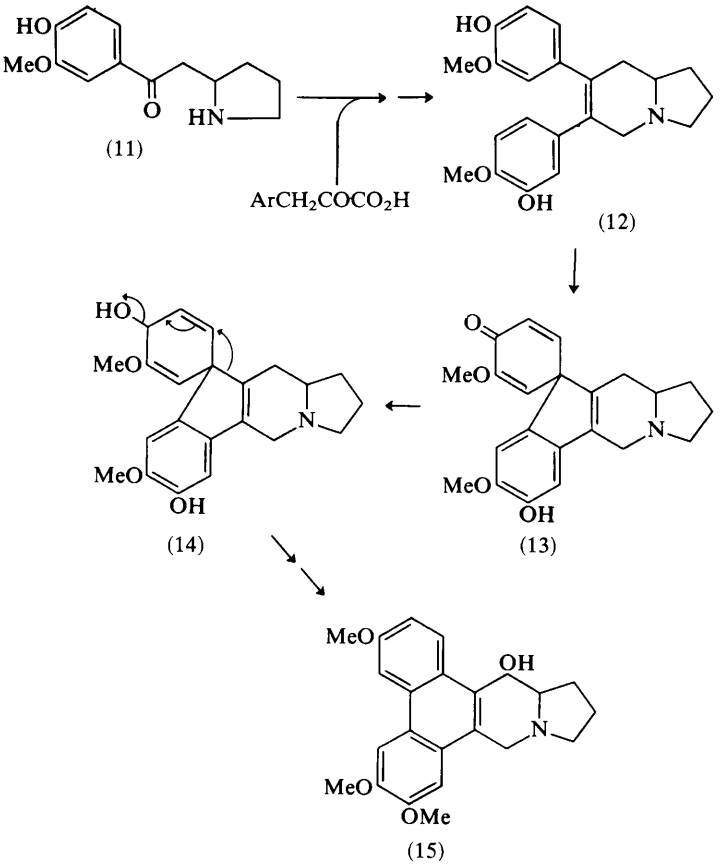
[27] R. B. Herbert and F. B. Jackson, *J. Chem. Soc., Chem. Commun.*, 1977, 955; R. B. Herbert, in ref. 9, p. 5.

[28] A. R. Battersby, R. C. F. Jones, R. Kazlauskas, C. Poupat, C. W. Thornber, S. Ruchirawat, and J. Staunton, *J. Chem. Soc., Chem. Commun.*, 1974, 773; R. B. Herbert, in ref. 6, p. 26.

[29] A. R. Battersby, A. Minta, A. P. Ottridge, and J. Staunton, *Tetrahedron Lett.*, 1977, 1321; R. B. Herbert, in ref. 8., p. 8.

[30] A. Yagi, G. Nonaka, S. Nakayama, and I. Nishioka, *Phytochemistry*, 1977, **16**, 1197; R. B. Herbert, in ref. 9, p. 14.

[31] G. Blaschke, *Arch. Pharm. (Weinheim, Ger.)*, 1968, **301**, 439; *ibid.*, 1970, **303**, 358; H. L. Holland, M. Castillo, D. B. MacLean, and I. D. Spenser, *Can. J. Chem.*, 1974, **52**, 2818; R. B. Herbert, in ref. 1, p. 21; in ref. 6, p. 23.



New investigation of bisbenzylisoquinoline biosynthesis is welcome (see ref. 32; also this Report, p. 16). Although aporphine alkaloids are the simplest developments of the benzylisoquinoline skeleton, their biosynthesis need not, as several examples show,[10,33] be simple. It has, however, been found that the biosynthesis of boldine[34] and isocorydine[35] is straightforward. Further detail has been reported[36] on the biosynthesis of *Erythrina* alkaloids, which were established to be modified benzylisoquinolines some time ago.[37] Further detail on the biosynthesis of morphine (23) and related alkaloids continues to be published.[38] Of particular

[32] R. B. Herbert, in ref. 9, p. 11.

[33] R. B. Herbert, in ref. 5, p. 15; in ref. 4, p. 17; in ref. 1, p. 19; J. Staunton, in ref. 2, p. 12.

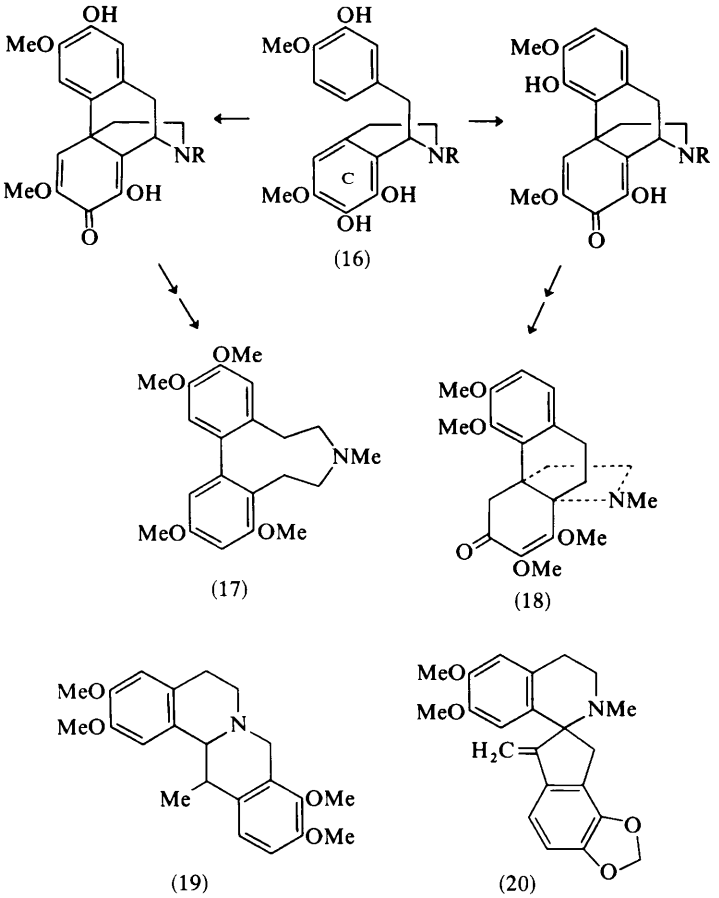
[34] S. Tewari, D. S. Bhakuni, and R. S. Kapil, *J. Chem. Soc., Chem. Commun.*, 1974, 940; R. B. Herbert, in ref. 8, p. 19.

[35] O. Prakash, D. S. Bhakuni, and R. S. Kapil, *J. Chem. Soc., Perkin Trans. 1*, 1978, 622; R. B. Herbert, in ref. 9, p. 14.

[36] D. H. R. Barton, R. D. Bracho, C. J. Potter, and D. A. Widdowson, *J. Chem. Soc., Perkin Trans. 1*, 1974, 2278; D. S. Bhakuni, A. N. Singh, and R. S. Kapil, *J. Chem. Soc., Chem. Commun.*, 1977, 211; D. S. Bhakuni and A. N. Singh, *J. Chem. Soc., Perkin Trans. 1*, 1978, 618; R. B. Herbert, in ref. 6, p. 25; in ref. 8, p. 10; in ref. 9, p. 16.

[37] R. B. Herbert, in ref. 5, p. 24; in ref. 1, p. 22.

[38] R. B. Herbert, in ref. 9, p. 8.

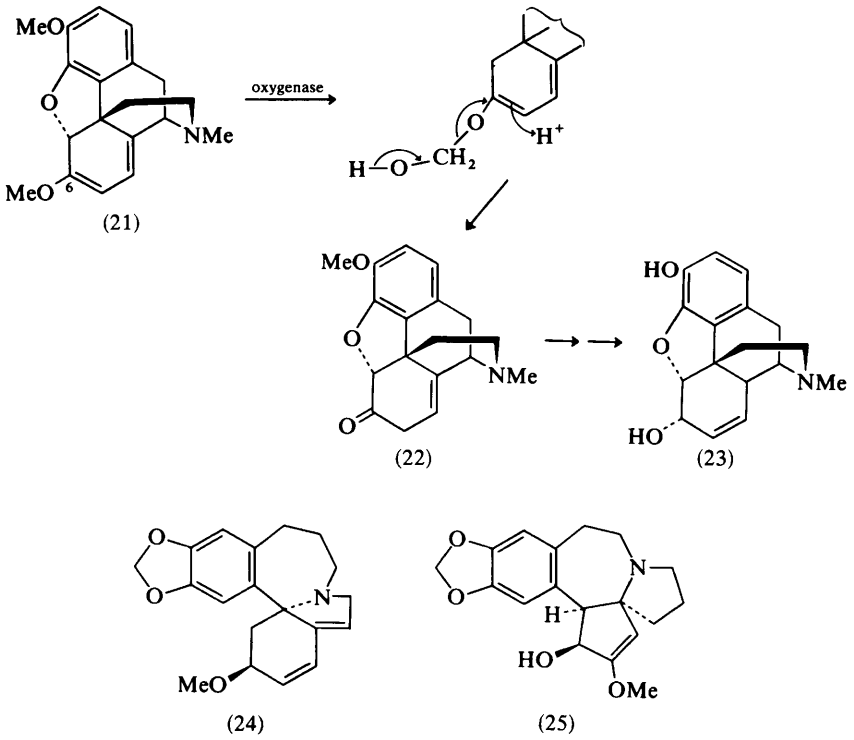


interest is the observation that the demethylation of thebaine (21) to give neopinone (22), which occurs at an intermediate state of biosynthesis, involves retention of oxygen at C-6. This is not expected of normal ether hydrolysis, and an alternative mechanism is shown.[38,39]

The 'homo-*Erythrina*' alkaloid schelhammeridine (24) appears to be derived, as expected, from a phenethylisoquinoline.[40] After some initial doubt the evidence for the biosynthesis of the related base cephalotaxine (25) has been deduced to be consistent with it too being a modified phenethylisoquinoline.[41]

Use of ^{13}C labels has transformed, especially, the study of polyketide biosynthesis, and this label has found application in the study of the biogenesis of

[39] J. S. Horn, A. G. Paul, and H. Rapoport, *J. Am. Chem. Soc.*, 1978, **100**, 1895.
[40] A. R. Battersby, E. McDonald, J. A. Milner, S. R. Johns, J. A. Lamberton, and A. A. Sioumis, *Tetrahedron Lett.*, 1975, 3419; R. B. Herbert, in ref. 7, p. 10.
[41] J. M. Schwab, M. N. T. Chang, and R. J. Parry, *J. Am. Chem. Soc.*, 1977, **99**, 2368; R. B. Herbert, in ref. 8, p. 12.



nitrogenous microbial metabolites, *e.g.* cytochalasins,[42] rifamycins,[43] geldanomycin,[44] prodiginines,[45] and nybomycin.[44] Of particular note is the greater certainty, and more detail generally obtained, with ^{13}C labelling. (For the biosynthesis of metabolites related to the rifamycins and geldanomycin, namely the mitomycins and streptovaricins, see refs. 46 and 47, respectively.)

With plants, the much lower incorporation generally obtaining has generally precluded the use of stable isotopes. In three studies so far, ^{13}C labelling has been used where incorporations were favourable: these were camptothecin,[48] colchicine,[49] dioscorine,[23] nicotine (with $^{13}CO_2$; see this Report, p. 14), and anatabine (this Report, p. 11).

Staple isotopes for biosynthetic studies continue to be ^{14}C and ^{3}H, often in combination, to indicate both intact incorporation[26,27,50] and to monitor hydrogen

[42] R. B. Herbert, in ref. 7, p. 29; in ref. 6, p. 44.
[43] R. B. Herbert, in ref. 6, p. 45; see also ref. 9, p. 34, and this Report, p. 29.
[44] R. B. Herbert, in ref. 8, p. 30.
[45] R. B. Herbert, in ref. 6, p. 50; in ref. 9, p. 32; see also ref. 8, p. 36.
[46] R. B. Herbert, in ref. 7, p. 32; in ref. 6, p. 45; in ref. 4, p. 40.
[47] R. B. Herbert, in ref. 7, p. 32; this Report, p. 29.
[48] R. B. Herbert, in ref. 6, p. 36; see also this Report, p. 22.
[49] A. R. Battersby, P. W. Sheldrake, and J. A. Milner, *Tetrahedron Lett.*, 1974, 3315; R. B. Herbert, in ref. 6, p. 28.
[50] P. W. Jeffs, H. F. Campbell, D. S. Farrier, G. Ganguli, N. H. Martin, and G. Molina, *Phytochemistry*, 1974, **13**, 933; R. B. Herbert, in ref. 5, p. 23.

loss in the course of biosynthesis, for which there are numerous examples. A combination of these labels in two different precursors has also been used to obtain accurate comparison of the relative efficiencies of incorporation of these precursors.[51,52]

A precursor with ^{14}C and ^{3}H labels is normally made by mixing two singly labelled samples. In some cases it has been observed that subsequent biotransformation leads to an increase in the relative amount of tritium-labelled species. This is explained as the result of preferential metabolism of ^{14}C-labelled material along other paths.[52,53]

Where incorporations are favourable, deuterium can provide more information than tritium, one example being the biosynthesis of microbial phenazines, where the analysis of deuterium incorporation from [2-^{2}H]shikimic acid has allowed clear definition of the way in which shikimic acid is used in the construction of the phenazine ring system.[54]

A number of microbial metabolites whose biosynthesis has been studied are (at least formally) derived from a di- (or tri-)peptide: echinulin and benzodiazepine alkaloids (both referred to already), gliotoxin,[55] mycelianamide,[56] sporidesmin,[57] and the β-lactam antibiotics.[58] In the case of the last mentioned, in spite of extensive work and the accumulation of a wealth of detail on the fates of the individual atoms in the precursor amino-acids, the mechanism of ring formation remains obscure.

A study of the biosynthesis of cyclopiazonic acid[59] is to be noted.

A feature of the biosynthesis of a number of microbial metabolites is the fracture of aromatic rings in the precursor amino-acids: pyrrolnitrin (see this Report, p. 23), anthramycin,[60,61] tomaymycin,[60] and streptonigrin (see this Report, p. 23). This is only observed rarely in the biosynthesis of plant bases, a notable example being the betalains, which are pigments in plants of the order Centrospermae.[10,62]

With much of the essential biosynthetic information uncovered, there has been little published work on furoquinoline[63] and Amaryllidaceae[63,64] alkaloids. Although a similarity in structure between mesembrine and Amaryllidaceae alkaloids indicates a similar biogenesis, the evidence is to the contrary. So far, in

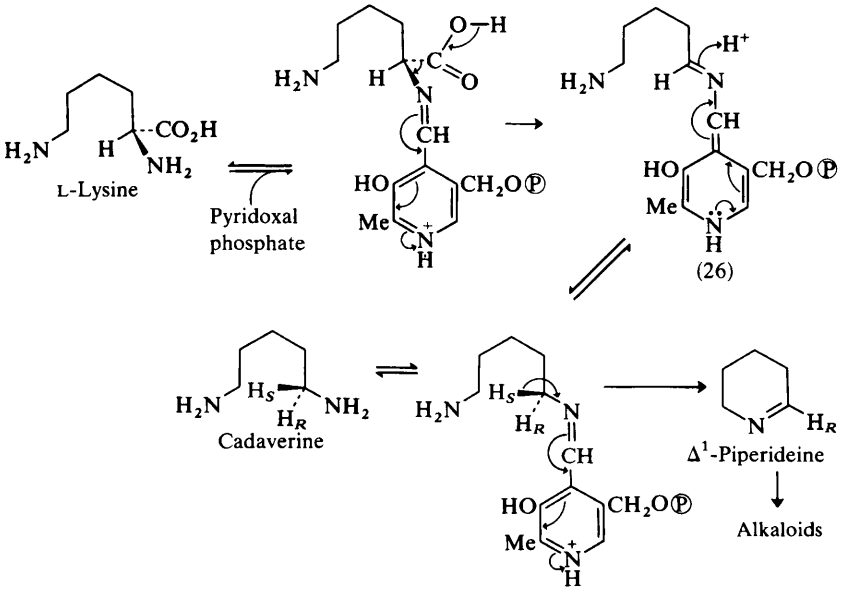
[51] E. Leistner, R. N. Gupta, and I. D. Spenser, *J. Am. Chem. Soc.*, 1973, **95**, 4040; R. B. Herbert, in ref. 5, p. 5; N. M. Bale and D. H. G. Crout, *Phytochemistry*, 1975, **14**, 2617.
[52] R. B. Herbert, in ref. 7, p. 1.
[53] A. R. Battersby, R. J. Francis, M. Hirst, E. A. Ruveda, and J. Staunton, *J. Chem. Soc., Perkin Trans. 1*, 1975, 1140; A. R. Battersby, J. Staunton, H. R. Wiltshire, R. J. Francis, and R. Southgate, *ibid.*, p. 1147.
[54] R. B. Herbert, F. G. Holliman, and J. B. Sheridan, *Tetrahedron Lett.*, 1976, 639; R. B. Herbert, in ref. 7, p. 27; see also this Report, p. 28; other references are R. B. Herbert, in ref. 8, p. 33; in ref. 9, p. 29.
[55] R. B. Herbert, in ref. 6, p. 37; in ref. 7, p. 24; this Report, p. 27.
[56] R. B. Herbert, in ref. 6, p. 37; in ref. 8, p. 35.
[57] R. B. Herbert, in ref. 6, p. 30.
[58] R. B. Herbert, in ref. 6, p. 49; in ref. 7, p. 30; in ref. 8, p. 35; in ref. 9, p. 33; this Report, p. 29.
[59] R. B. Herbert, in ref. 6, p. 30; in ref. 7, p. 18; in ref. 8, p. 27.
[60] R. B. Herbert, in ref. 7, p. 25; in ref. 5, p. 40.
[61] R. B. Herbert, in ref. 8, p. 24.
[62] R. B. Herbert, in ref. 6, p. 41; and refs. cited therein.
[63] R. B. Herbert, in ref. 6, p. 39.
[64] R. B. Herbert, in ref. 8, p. 19.

spite of extensive research, the nature of the key intermediates of mesembrine alkaloid biosynthesis remains obscure.[65]

Study of the biosynthesis of ergot[66] and steroidal[67] alkaloids continues. The biosynthesis of the necic acid components of the pyrrolizidine alkaloids has been elucidated,[68] and attention has turned to the biosynthesis of the pyrrolizidine nucleus, which is largely unknown (see this Report, p. 13).

2 Piperidine, Pyridine, and Pyrrolidine Alkaloids

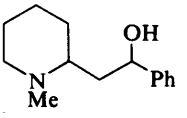
L-Lysine and cadaverine serve as precursors for the majority of piperidine alkaloids.[10] The experimental results have been interpreted in terms of an attractive series of pyridoxal-linked intermediates derivable independently from both precursors; see Scheme 1. The transformation of cadaverine into alkaloids involves stereospecific removal of one of the protons attached to C-1 (*pro-S* hydrogen) and probably involves a diamine oxidase; *cf.* refs. 5 and 6. Examination of the diamine-oxidase-catalysed oxidation of cadaverine, with enzyme from hog kidney, and analysis by an excellent ^2H n.m.r. method, has shown that this reaction also involves removal of the 1-*pro-S* proton from the diamine;[69] pea seedling diamine oxidase has been found to effect the conversion of benzylamine into benzaldehyde with similar stereochemistry.[70]



Scheme 1

[65] R. B. Herbert, in ref. 7, p. 23; in ref. 8, p. 21; in ref. 9, p. 16.
[66] R. B. Herbert, in ref. 6, p. 31; in ref. 7, p. 20; in ref. 8, p. 27; in ref. 9, p. 26; see also this Report, p. 26.
[67] R. B. Herbert, in ref. 6, p. 52; in ref. 7, p. 32; in ref. 8, p. 28; in ref. 9, p. 27.
[68] R. B. Herbert, in ref. 1, p. 8; in ref. 3, p. 40; in ref. 6, p. 11; in ref. 9, p. 4.
[69] J. C. Richards and I. D. Spenser, *J. Am. Chem. Soc.*, 1978, **100**, 7402.
[70] A. R. Battersby, J. Staunton, and M. C. Summers, *J. Chem. Soc., Perkin Trans. 1*, 1976, 1052.

The conversion of lysine into piperidine alkaloids involves retention of hydrogen isotope at C-2.[10] The sequence is suggested to be that shown in Scheme 1, and catalysis of the reaction may be attributed to L-lysine decarboxylase. This enzyme, from the micro-organism *Bacillus cadaveris*, has been found to carry out the conversion of L-lysine into cadaverine with retention of configuration. Decarboxylation of L-[2-^{3}H]lysine by this enzyme then affords [1*S*-^{3}H]-cadaverine. When this material is converted into alkaloids, *e.g.* *N*-methylpelletierine (4; R = Me), the tritium attached to what becomes C-2 is lost; *cf.* refs. 5 and 6. On the other hand, conversion of lysine into sedamine (27) in *Sedum acre* results in retention of the tritium originally present at C-2. The simplest explanation is that protonation of (26) in the micro-organism and plant proceeds with opposite stereochemistry. This is at variance, however, with current ideas on the stereochemistry of reactions that are catalysed by pyridoxal phosphate.[71]



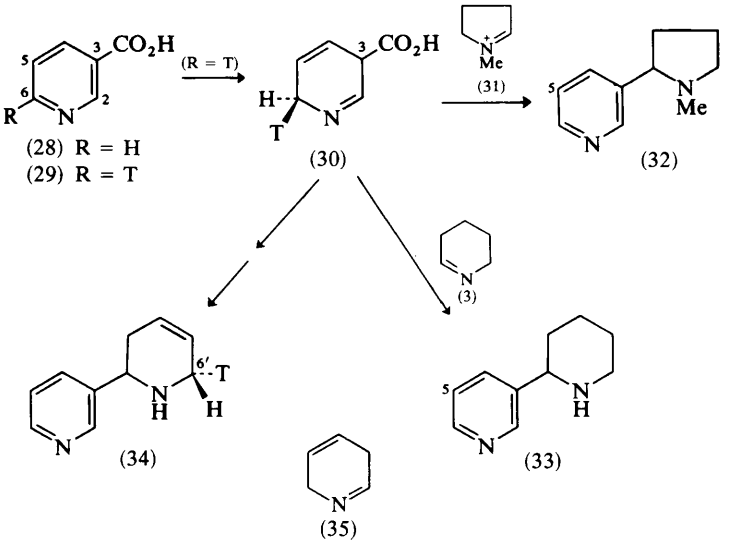
(27)

This is disturbing, and so the stereochemistry of the overall reaction L-lysine → cadaverine → Δ^1-piperideine has been checked,[72] using L-lysine decarboxylase from *Escherichia coli* and *B. cadaveris*, pea seedling diamine oxidase, and *S. acre* plants, with confirmation of the above deductions; the lysine decarboxylase from the two sources effected decarboxylation with the same stereochemical outcome. Solution of the problem must now await further experiments with the enzymes involved in alkaloid biosynthesis.

Securinine.—The C_5N unit in securinine (10), shown with thickened bonds, is derived from lysine *via* Δ^1-piperideine (3); *cf.* ref. 7. The eight-carbon unit (normal bonding) of (10) derives from tyrosine. Some of the results, published in preliminary form, are now available in full.[73] The mechanism whereby (3) combines with a tyrosine derivative remains an intriguing mystery. *p*-Hydroxyphenylpyruvic acid and *p*-hydroxyphenylacetaldehyde (30)[73] are plausibly involved. A flanking approach in feeding experiments using the more stable 4′-hydroxy-2-phenylethanol and *p*-hydroxyphenylacetic acid, potentially convertible into *p*-hydroxyphenylacetaldehyde, gave, however, negative results.[73] It has been shown that securinine (10) is the biosynthetic precursor for its 4,5-dihydro-derivative *in vivo*.[73]

Anabasine.—5-Fluoronicotinic acid [as (28)] is one of several unnatural substrates that have been metabolized by plants to alkaloid analogues; *cf.* refs. 3 and 5. The major alkaloid of *Nicotiana glauca* is anabasine (33); it is essentially racemic. 5-Fluoronicotinic acid has been found recently to be converted into racemic 5-fluoroanabasine [as (33)] in this plant.[74]

[71] H. C. Dunathan and J. G. Voet, *Proc. Natl. Acad. Sci. USA*, 1974, **71**, 3888.
[72] H. J. Gerdes and E. Leistner, *Phytochemistry*, 1979, **18**, 771.
[73] R. J. Parry, *Bio-org. Chem.*, 1978, **7**, 277.
[74] E. Leete, *J. Org. Chem.*, 1979, **44**, 165.



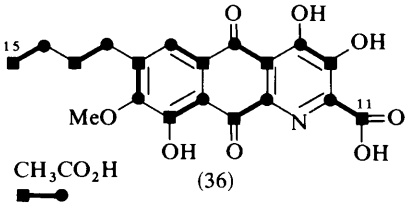
Anatabine.—It is known that anabasine (33) is derived from one molecule each of lysine and nicotinic acid (28).[10] In contrast, the co-occurring alkaloid anatabine (34) is derived exclusively from nicotinic acid, both heterocyclic rings being equally labelled by labelled precursor. Furthermore, the labelling results show that the nicotinic-acid fragments couple by the linking of C-3 of one unit to C-2 of the other. In the formation of anabasine (33) and nicotine (32), linkage is to C-3 of the nicotinic-acid fragment, which again gives the pyridine ring in these alkaloids. For these alkaloids, tritium from C-6 of nicotinic acid is lost in the course of biosynthesis; *cf.* refs. 4, 6, and 8.[75] The biosynthesis of the pyridine ring of anatabine is similar in that tritium is again lost from C-6 of nicotinic acid.[75] On the other hand, tritium from C-6 of nicotinic acid is retained in the tetrahydropyridine ring of anatabine, and is located at C-6′, as expected; the stereochemistry is *S* [see (34)].

The biosynthesis of nicotine and anabasine can reasonably proceed *via* (30), which will be electrophilic towards (31) and (3), which are intermediates for the other parts of the alkaloids,[10] subsequent aromatization leading to loss of tritium. Coupling of two molecules of (30), decarboxylation, and aromatization would give anatabine, with 50% retention of tritium observed; the stereochemistry of (30) follows from that determined in anatabine (34). [Direct coupling of two molecules of (30) makes use of the higher electrophilicity associated with this molecule (*cf.* fatty acid biosynthesis), and so is preferred to the suggested coupling with two molecules of (35).]

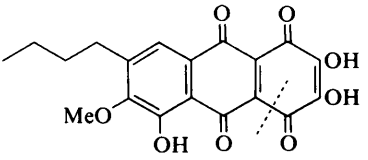
The formation of $\alpha\beta$-dipyridyl in *Nicotiana* species occurs from anatabine (34) during drying of the plants prior to isolation of alkaloids.[76]

[75] E. Leete, *J. Chem. Soc., Chem. Commun.*, 1978, 610.
[76] E. Leete, K. C. Ranbom, and R. M. Riddle, *Phytochemistry*, 1979, **18**, 75.

Phomazarin.—The structure of phomazarin, an orange pigment produced by *Pyrenochaeta terrestris*, has been defined as (36).[77] It is manifestly polyketide in structure, and the results of experiments with [1-^{14}C]- and [2-^{14}C]-acetate are in support. Precise definition of the nature of the polyketide precursor follows from experiments with [^{13}C]acetate and [^{13}C]- and [^{14}C]-malonate;[78] [1-^{13}C]- and [2-^{13}C]-acetate showed alternate and complementary labelling of the carbon atoms of phomazarin. The coupling observed in the n.m.r. spectrum of metabolite derived from [$^{13}C_2$]acetate established that assembly of phomazarin was from nine intact acetate units, as shown in (36). Observation of a lower level of radioactivity at C-15, but not C-11, relative to other labelled positions in material derived from [2-^{13}C]malonate (similar results with ^{14}C-labelled precursor) indicated that C-15 was the 'starter' acetate unit, and further therefore that only one polyketide chain was involved (C-11 was a potential site for starter acetate in a two-chain precursor). In the course of biosynthesis, this chain must be broken to allow intercalation of the nitrogen atom present in (36), and it has been suggested that this occurs on the bisquinone (37).[78]



(36)



(37)

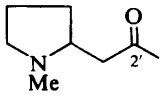
Tropane Alkaloids.—[3-^{14}C]Acetoacetate has been shown[79] to be a precursor for hygrine (38) (in *Nicandra physaloides*), the activity being localized at C-2'. Unfortunately, because of the labelling site in the precursor, it is uncertain whether the acetoacetate was incorporated intact or only after prior degradation to acetate (no comparative levels of incorporation of acetoacetate and acetate were obtained either).

It has been shown recently that (+)-hygrine was much preferred over its enantiomer, in *Datura innoxia*, for the elaboration of tropane bases, but only slightly preferred in the formation of cuscohygrine; *cf.* ref. 9. On the other hand, in *Physalis alkekengi*, *Atropa belladonna*, and *Hyoscyamus niger*, no stereoselectivity was observed in alkaloid formation.[80]

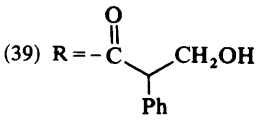
The possible role of cytochrome *P*450 as an enzyme that can hydroxylate tropane alkaloids has been examined *in vitro* by use of a rat liver microsomal preparation.[81] 3α-Tigloyloxytropane (40) was converted into meteloidine (41). The reaction was dependent on NADPH and was inhibited by carbon monoxide, thus suggesting that cytochrome *P*450 was the enzyme responsible.

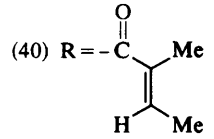
[77] A. J. Birch, D. N. Butler, R. Effenberger, R. W. Rickards, and T. J. Simpson, *J. Chem. Soc., Perkin Trans. 1*, 1979, 807.
[78] A. J. Birch and T. J. Simpson, *J. Chem. Soc., Perkin Trans. 1*, 1979, 816.
[79] B. A. McGaw and J. G. Woolley, *J. Pharm. Pharmacol.*, 1978, **30**, 83P.
[80] B. A. McGaw and J. G. Woolley, *Phytochemistry*, 1979, **18**, 189.
[81] E. W. T. Major, I. Davies, and J. G. Woolley, *J. Pharm. Pharmacol.*, 1978, **30**, 81P.

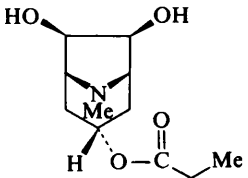
The tropic acid moiety (1) found in some tropane alkaloids, *e.g.* (39), is known to be derived from phenylalanine, and the necessary skeletal rearrangement involves intramolecular shift of the carboxy-group of the amino-acid; *cf.* ref. 7. Competitive feeding experiments with phenylpyruvic acid, phenyl-lactic acid (42), and phenylalanine in *Datura stramonium* plants has given results which indicate that phenyl-lactic acid is a later intermediate in the formation of tropic acid than phenylalanine.[82]

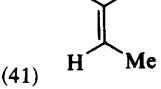


(38)

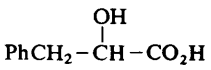








(41)



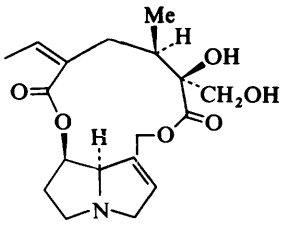
(42)

Pyrrolizidine Alkaloids.—Welcome new work has been published[83] on the origins of the pyrrolizidine ring system [as (45)], seen in alkaloids such as retrorsine (43). It was known that retronecine (45) is labelled at C-9 with one quarter of the activity from [1,4-$^{14}C_2$]putrescine, [2-^{14}C]ornithine, and [5-^{14}C]ornithine. The observation with the latter two precursors indicates that incorporation of ornithine proceeds by way of a symmetrical intermediate, at least for ring B. The new results[83] confirm this conclusion, and establish that ornithine is built into ring A also *via* a symmetrical intermediate. Putrescine was a much better precursor for retrorsine (43) than ornithine (measured in each case against arginine that bore a different isotopic label, fed at the same time), and a similar distribution of activity in the retronecine (45) was observed. This, and the symmetrization of the ornithine label, indicates strongly that retronecine (45) is formed from two molecules of ornithine *via* putrescine.

Incorporations, similar to that for putrescine, were observed[83] for spermine (46) and spermidine (47) (also measured against arginine; ^{14}C-labels on asterisked carbons), and there was a similar distribution of the label. These amines can, like putrescine, then be converted into pyrrolizidine alkaloids *via* the hypothetical (44); both polyamines are known to be degraded to pyrrolines, resembling (44), plus 1,3-diaminopropane on oxidation with plant polyamine oxidases.

It has also been found[83] that proline, 4-aminobutanoic acid, and glutamic acid were poor precursors for retrorsine (43), and label was scattered over both retronecine and the necic acid fragment.

[82] M. Ansarin and J. G. Woolley, *J. Pharm. Pharmacol.*, 1978, **30**, 82P.
[83] D. J. Robins and J. R. Sweeney, *J. Chem. Soc., Chem. Commun.*, 1979, 120.



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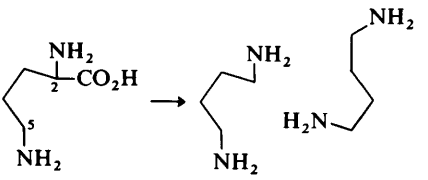
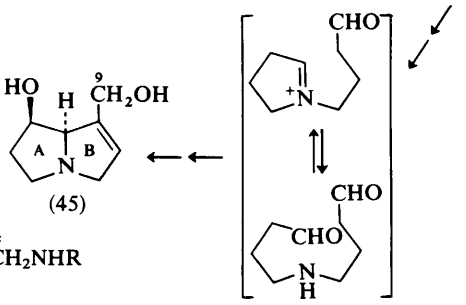


Ornithine Putrescine



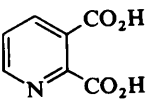
(45) (44)

$H_2N(CH_2)_3NH\overset{*}{C}H_2(CH_2)_2\overset{*}{C}H_2NHR$

(46) R = H

(47) R = $(CH_2)_3NH_2$

Ricinine.—Ricinine (49), the alkaloid of castor bean plants, is derived from nicotinic acid (28) and quinolinic acid (48), and its formation is intimately associated with the pyridine nucleotide cycle; *cf.* ref. 6. Quinolinic acid is built from a C_3 fragment that is formed from glycerol *via* glyceraldehyde and a C_4 unit that is related to succinic or aspartic acids. A recent investigation has confirmed this pathway for ricinine (49) and indicated that dihydroxyacetone phosphate lies between glycerol and glyceraldehyde (loss of tritium from C-2 of labelled glycerol).[84]



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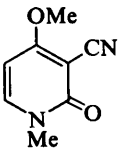


(49)

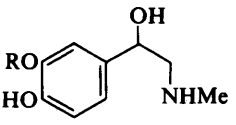
Nicotine.—Some uncertainty, associated with an n.m.r. method (*cf.* ref. 8) of analysis on nicotine (32) derived from $^{13}CO_2$, has been resolved in a recent paper.[85] It appears, in agreement with the degradative evidence, that labelling of the pyrrolidine ring of (32) by CO_2 is symmetrical overall. Not inconsistent with this, unsymmetrical labelling within individual molecules was observed. This may be taken as a reflection of the fact that assembly of the five carbon atoms of the eventual pyrrolidine ring occurs from different sources.

[84] T. Robinson, *Phytochemistry*, 1978, **17**, 1903.

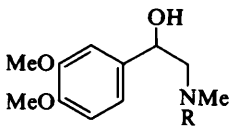
[85] M. Nakane and C. R. Hutchinson, *J. Org. Chem.*, 1978, **43**, 3922.

3 Phenethylamine and Isoquinoline Alkaloids

Normacromerine.—Normacromerine (52) is closely related to simple phenethylamines,[10] and is formed from tyrosine and tyramine *via* epinephrine (50) in the cactus *Coryphantha macromeris*; *cf.* ref. 9. Metanephrine (51), a natural constituent of the cactus, has been shown to be an efficient and specific precursor for normacromerine (52).[86] The previously observed slow conversion of (52) into macromerine (53) has again been noted.[86]



(50) R = H
(51) R = Me



(52) R = H
(53) R = Me

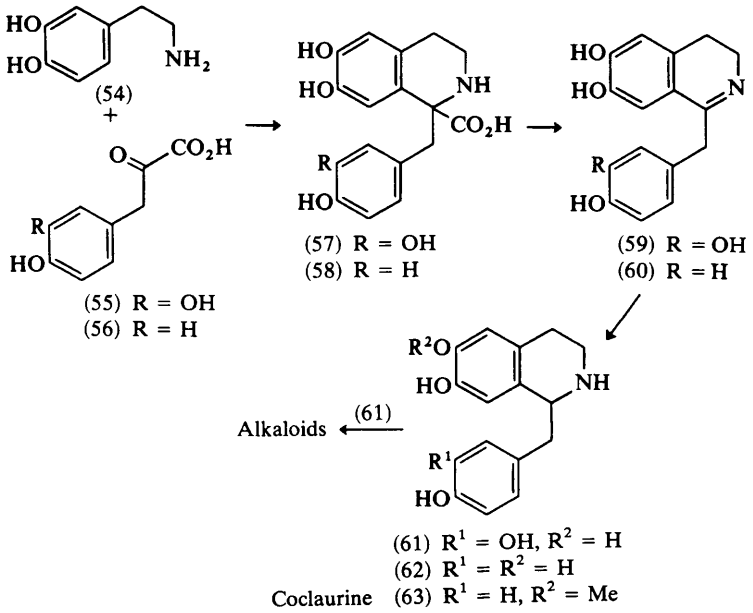
Peyote Alkaloids.—An *O*-methyltransferase has been isolated from the peyote cactus. Its ability to catalyse the methylation of various phenolic phenethylamines and isoquinolines, and the site of methylation, has indicated possible biosynthetic relationships within the peyote cactus;[87] *cf.* ref. 3.

***Papaver* Alkaloids.**—Biosynthesis of benzylisoquinoline alkaloids involves the amino-acid dopa, which is in part implicated through decarboxylation to dopamine.[10] The presence of L-dopa decarboxylase in *Papaver orientale* latex has recently been demonstrated.[88]

An early key intermediate in benzylisoquinoline biosynthesis is (57), which by decarboxylation affords (59); this in turn leads to (61) and on to alkaloids[10,20] (Scheme 2). Confirmation of this pathway has come from a study using cell-free preparations of *P. somniferum* stems and seed capsules.[89] It was found that this preparation catalysed the formation of (57), (59), and (61) from dopamine (54) plus 3,4-dihydroxyphenylpyruvic acid (55); without the addition of *S*-adenosylmethionine, NADPH, and pyridoxal phosphate, the reaction stopped at (57). The formation of the alkaloids reticuline, thebaine, codeine, and morphine, produced by whole plants, could not be detected with this cell-free system. The results confirm not only the intermediacy of (57) and (59) in benzylisoquinoline biosynthesis, but also the involvement of (54) and (55).

Coclaurine.—Coclaurine (63) is an intermediate of some significance in the biosynthesis of benzylisoquinoline alkaloids, *e.g.* bisbenzylisoquinolines (see below). Its biosynthesis, in *Annona reticulata*, has been investigated[90] and found to follow an orthodox pathway,[10] from two molecules of tyrosine. Radioactive tyramine, dopa, and dopamine (54) labelled the phenethylamine portion of (63)

[86] W. J. Keller, *J. Pharm. Sci.*, 1979, **68**, 85.
[87] G. P. Basmadjian, S. F. Hussain, and A. G. Paul, *Lloydia*, 1978, **41**, 375.
[88] M. F. Roberts and M. D. Antoun, *Phytochemistry*, 1978, **17**, 1083.
[89] A. I. Scott, S.-L. Lee, and T. Hirata, *Heterocycles*, 1978, **11**, 159.
[90] O. Prakash, D. S. Bhakuni, and R. S. Kapil, *J. Chem. Soc., Perkin Trans. 1*, 1979, 1515.



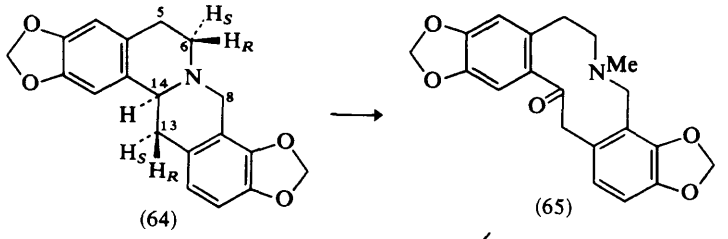
Scheme 2

whereas 4-hydroxyphenylpyruvic acid (56) was, like tyrosine, incorporated into both halves. Further orthodoxy is seen in the incorporation of (58) (shown to be specific), (60), and (62) (*cf.* refs. 10 and 20, and Scheme 2).

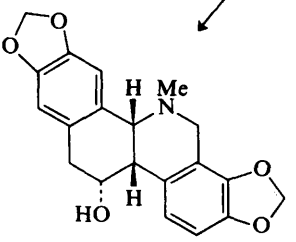
Chelidonine.—Chelidonine (66) has been proved to be a modified benzylisoquinoline arising along a pathway from (*S*)-reticuline, through (*S*)-scoulerine, (*S*)-stylopine (64), and protopine (65) (detailed pathways are given in ref. 7, p. 12, and ref. 8, p. 14). The steps which lie beyond stylopine (64) manifestly involve fracture of the C-14—N and C-6—N bonds in (64). It has been shown that the *pro-S* proton at C-13, as well as the one at C-14, is lost on formation of chelidonine (those at C-5 and C-8 are retained). The most recent results are that in the scission of the C-6—N bond it is again the *pro-S* proton which is lost.[91] It is interesting to note that all three protons are lost from the *si*-face of the molecule.

Bisbenzylisoquinoline Alkaloids.—The biosynthesis of several bisbenzylisoquinolines has now been investigated. All have been found to be based on coclaurine (63).[92,93] Investigation of the biosynthesis of oxyacanthine (69), in *Cocculus laurifolius*, has given results which show that this alkaloid too arises from coclaurine.[94] Norcoclaurine (62), coclaurine (63), and *N*-methylcoclaurine were

[91] A. R. Battersby, J. Staunton, M. C. Summers, and R. Southgate, *J. Chem. Soc., Perkin Trans. 1*, 1979, 451.
[92] D. H. R. Barton, G. W. Kirby, and A. Wiechers, *J. Chem. Soc.* (*C*), 1966, 2313.
[93] D. S. Bhakuni, A. N. Singh, S. Jain, and R. S. Kapil, *J. Chem. Soc., Chem. Commun.*, 1978, 226; D. S. Bhakuni, V. M. Labroo, A. N. Singh, and R. S. Kapil, *J. Chem. Soc., Perkin Trans. 1*, 1978, 121; D. S. Bhakuni, S. Jain, and A. N. Singh, *ibid.*, p. 380.
[94] D. S. Bhakuni, A. N. Singh, and S. Jain, *J. Chem. Soc., Perkin Trans. 1*, 1978, 1318.

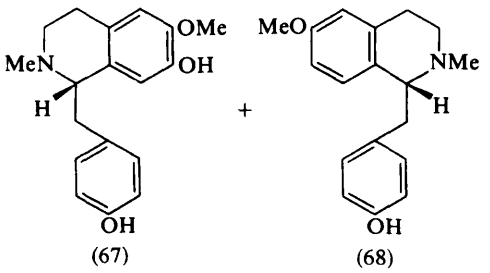


(64) (65)

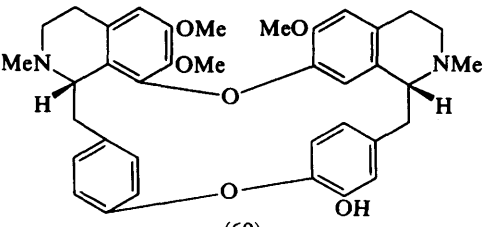


Chelidonine (66)

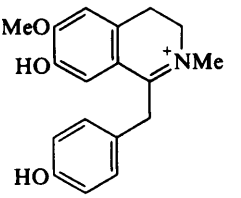
all efficiently incorporated; as expected, *NOO*-trimethylcoclaurine was not utilized for biosynthesis. It was shown further that the incorporation of (±)-*N*-methylcoclaurine was equally into both halves of (69), and without loss of hydrogen label from C-1. Results obtained with chiral samples of *N*-methylcoclaurine are that (*R*)-*N*-methylcoclaurine (68) is built into the right-hand half of (69) only; *i.e.*, the half with the same stereochemistry. Complementary results were obtained with (*S*)-*N*-methylcoclaurine (67). It afforded only the left-hand half, which has (*S*) stereochemistry. It is clear from these results that



(67) (68)



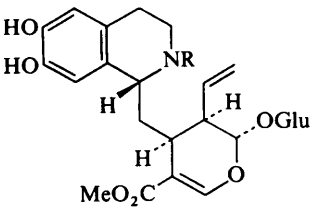
(69)



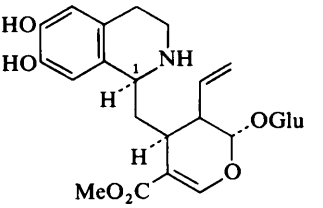
(70)

stereoisomers of *N*-methylcoclaurine are not interconvertible [*via* (70)] prior to utilization for alkaloid biosynthesis, so the efficient incorporation of (70) that is observed is not by a normal pathway, in agreement with results for other bisbenzylisoquinoline alkaloids.[93]

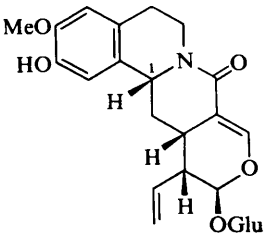
Ipecac Alkaloids.—The stereochemistry of ipecoside (71) is known by *X*-ray analysis, and this has been confirmed.[95] Feeding experiments in *Cephaelis ipecacuanha* have given results which demonstrate that it is not desacetylipecoside (72), as previously supposed (*cf.* ref. 2), but desacetylisoipecoside (73), with the same stereochemistry at C-1 (= C-11b) as cephaeline (76) and emetine (75), which is the true precursor for these alkaloids.[95,96] Similar results were obtained for cephaeline (76) in *Alangium lamarckii*.[95] On the other hand, desacetylipecoside (72), and not (73), is the precursor for ipecoside (71) (in *C. ipecacu-*



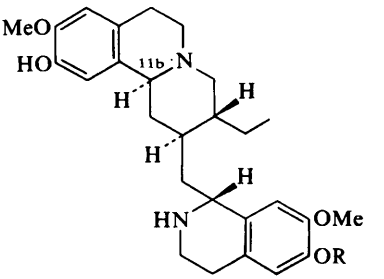
Ipecoside (71) R = Ac
(72) R = H



(73)



(74)



Emetine (75) R = Me
Cephaeline (76) R = H

[95] N. Nagakura, G. Höfle, and M. H. Zenk, *J. Chem. Soc., Chem. Commun.*, 1978, 896; N. Nagakura, G. Höfle, D. Coggiola, and M. H. Zenk, *Planta Med.*, 1978, **34**, 381.
[96] A. R. Battersby, N. G. Lewis, and J. M. Tippett, *Tetrahedron Lett.*, 1978, 4849.

anha)[95,96] and for alangiside (74) (in *A. lamarckii*),[95] now seen as biosynthetic dead ends. Conversion of either of these precursors into the corresponding alkaloids was with retention of the proton at C-1.[95] Utilization of both epimers in biosynthesis contrasts with the formation of terpenoid indole alkaloids where only one epimer [strictosidine (79), p. 20] is involved in the biosynthesis of alkaloids with both 3α- and 3β-configurations; *cf.* ref. 9 [C-3 is equivalent to C-1 in (73)]. The overall picture is consistent, however, for the formation of 3β indole alkaloids, with obvious inversion of configuration, results in the loss of hydrogen isotope from C-3.

4 Alkaloids Derived from Tryptophan

Terpenoid Indole Alkaloids.—It has been confirmed recently for ajmalicine (84), vindoline (89), and catharanthine (90), in *Catharanthus roseus* (*Vinca rosea*),[96] that strictosidine (79), and not vincoside (86), is the key intermediate in terpenoid alkaloid biosynthesis (*cf.* ref. 9).

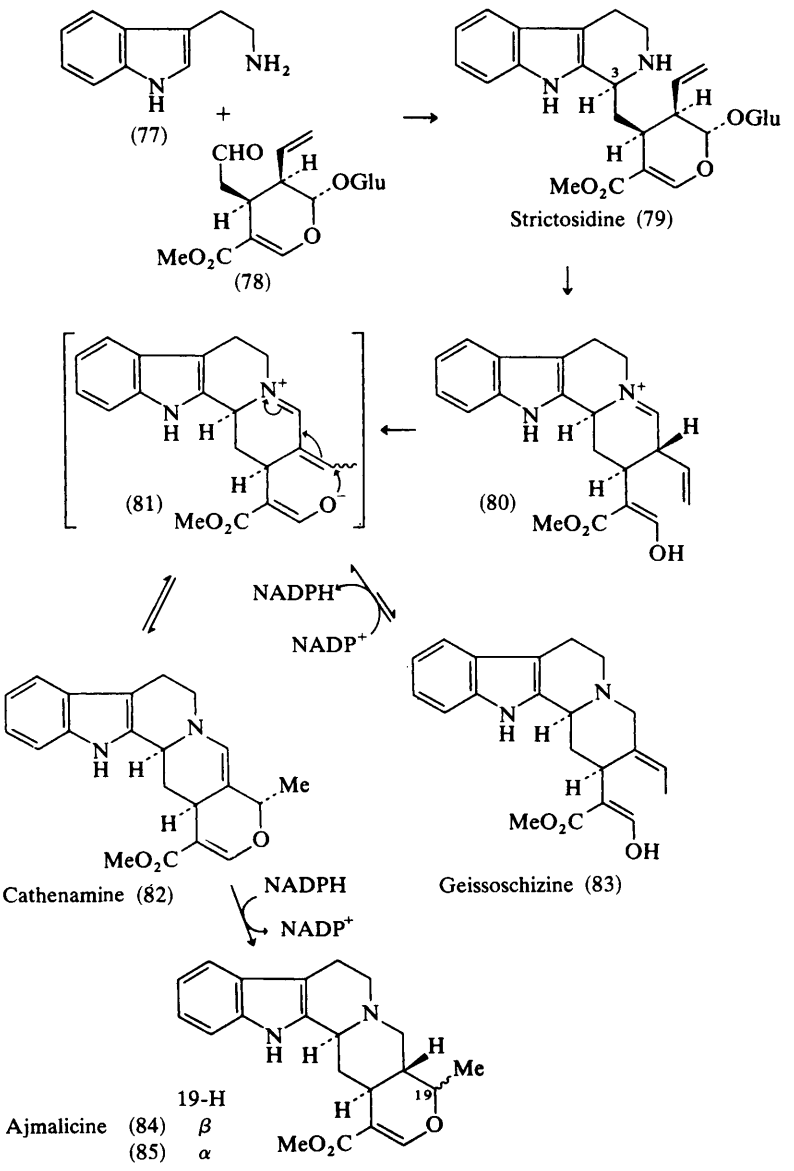
Cathenamine (82) has been identified as an intermediate after strictosidine (79). The immonium salt (80) lies logically between (79) and (82); see Scheme 3. Evidence in support has been obtained by isolating sitsikirine (91) and iso-sitsikirine (92) as new and exclusive products from a *C. roseus* enzyme preparation incubated with strictosidine (79) and potassium borohydride.[97] (It would be interesting to repeat the experiment with borodeuteride and to determine the sites of labelling.)

The cell-free synthesis of strictosidine (79) and cathenamine (82) has been further explored, and the conditions under which these key compounds are formed have been optimized.[98] Strains from *C. roseus* suspension cultures that were resistant to inhibition of their growth by various tryptophan analogues have been selected.[99] The free tryptophan level in cells of these strains could be 30—40 times higher than in normal cells. Tryptophan at this level did not induce tryptophan decarboxylase, nor the production of alkaloids. It is to be noted, however, that stimulation of alkaloid production by tryptophan and tryptamine[100] in cultures of normal cells has been reported. In the case of tryptamine the two most prominent metabolites were *N*-acetyltryptamine and *NN*-dimethyltryptamine.

5α-Carboxystrictosidine (88) is a naturally occurring compound. Neither it, nor its C-3 epimer (87), has been found to be a precursor for akuammidine (93), sarpagine (94), ajmaline (95), tetraphyllicine (96), quebrachidine (97), or gelsemine (98),[101] thus reinforcing the already deduced key role of strictosidine (79) in terpenoid indole biosynthesis. 5α-Carboxystrictosidine (88), on the other hand, is reasonably an intermediate in the formation of alkaloids such as adirubine (99).

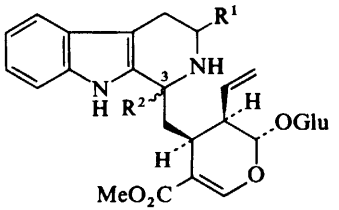
It has been found that enzyme systems from *C. roseus* deal inefficiently with geissoschizine (83)[102,103] (*cf.* ref. 8, p. 27), hitherto believed to be a key

[97] J. Stöckigt, M. Rueffer, M. H. Zenk, and G.-A. Hoyer, *Planta Med.*, 1978, **33**, 188.
[98] J. Stöckigt, *Phytochemistry*, 1979, **18**, 965.
[99] A. I. Scott, H. Mizukami, and S.-L. Lee, *Phytochemistry*, 1979, **18**, 795.
[100] R. J. Krueger and D. P. Carew, *Lloydia*, 1978, **41**, 327.
[101] J. Stöckigt, *Tetrahedron Lett.*, 1979, 2615.
[102] S. L. Lee, T. Hirata, and A. I. Scott, *Tetrahedron Lett.*, 1979, 691.
[103] J. Stöckigt, *J. Chem. Soc., Chem. Commun.*, 1978, 1097.

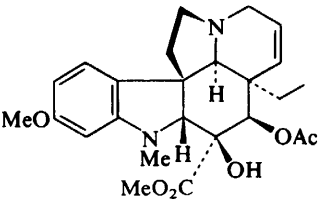


Scheme 3

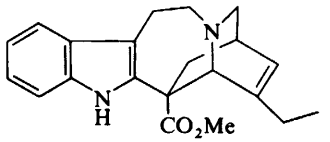
biosynthetic intermediate. Much more efficient conversions of secologanin plus tryptamine into ajmalicine (84) were recorded. The enzymic conversion of geissoschizine (83) into ajmalicine (84) and 19-*epi*-ajmalicine (85) was found to be at least partly independent of the synthesis of these two alkaloids from (77) and (78).[103] The former conversion depended on the presence of $NADP^+$ as well as



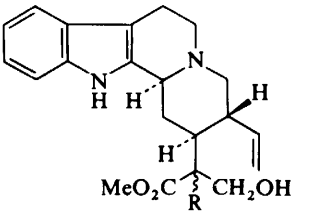
(86) R^1 = H, R^2 = β-H
(87) R^1 = CO_2H, R^2 = β-H
(88) R^1 = CO_2H, R^2 = α-H



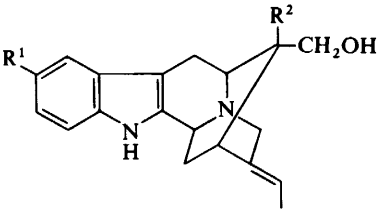
(89)



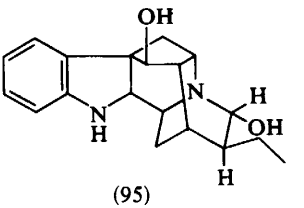
(90)



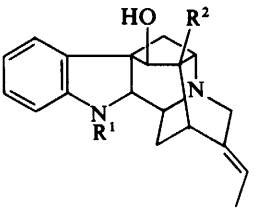
(91) R = β-H
(92) R = α-H



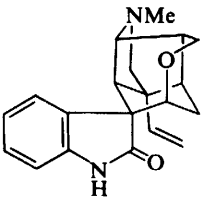
(93) R^1 = H, R^2 = CO_2Me
(94) R^1 = OH, R^2 = H



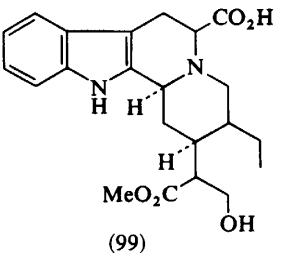
(95)



(96) R^1 = Me, R^2 = H
(97) R^1 = H, R^2 = CO_2Me



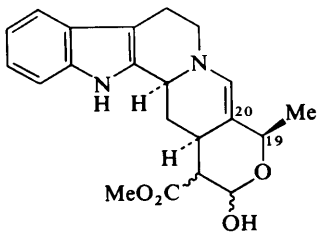
(98)



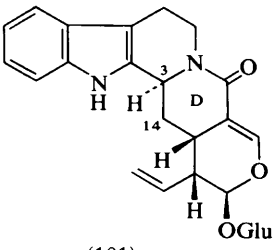
(99)

NADPH, unlike the latter reaction sequence, which is $NADP^+$-independent. It is clear from these results that geissoschizine is at a shunt from the main pathway to terpenoid indole alkaloids like (84); see Scheme 3.

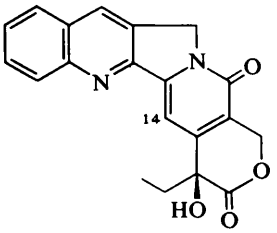
A new alkaloid (100), related to cathenamine (82), has been isolated from *Guettarda eximia*. It is readily converted chemically into cathenamine (82), presumably *via* (81). It seems likely that (100) has a biosynthetic role, and it may be that access to alkaloids with different stereochemistries at C-19 and C-20 occurs *via* (81) $\rightleftarrows$ (100).[104]



(100)



(101)



Camptothecin (102)

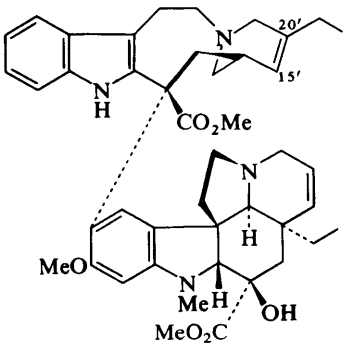
The biosynthesis of camptothecin (102) follows that of terpenoid indole alkaloids through strictosidine (79): The lactam (101) derived from (79), and not the C-3 epimer, is the next intermediate.[105] Several unknown steps follow, included in which is dehydrogenation of ring D [see (101)]. In this process a proton is lost from C-14. Tritium label at this site in (101) is retained by a primary isotope effect, the result of non-stereospecific deprotonation.[105] This indicates that removal of the proton is not enzyme-controlled (*cf.* papaverine biosynthesis; ref. 8, p. 19).

Although, by inspection, it would seem probable that the dimeric indole alkaloids, such as vinblastine (104), have their genesis in monomeric bases, such as vindoline (89) and catharanthine (90), investigations have been dogged by poor incorporations; *cf.* ref. 9. Most recently, however, even more careful work and the employment of cell-free preparations has given positive results.

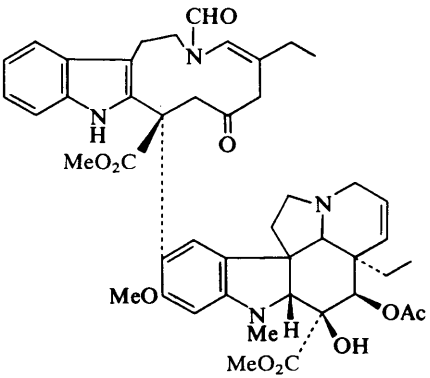
First, unstable anhydrovinblastine (103) was trapped as a natural product in young *C. roseus* plants.[106] In experiments of a few hours' duration it was shown to

[104] C. Kan-Fan and H.-P. Husson, *J. Chem. Soc., Chem. Commun.*, 1978, 618.
[105] C. R. Hutchinson, A. H. Heckendorf, J. L. Straughn, P. E. Daddona, and D. E. Cane, *J. Am. Chem. Soc.*, 1979, **101**, 3358.
[106] A. I. Scott, F. Gueritte, and S.-L. Lee, *J. Am. Chem. Soc.*, 1978, **100**, 6253.

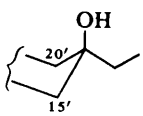
be labelled specifically and efficiently by radioactive vindoline (89) and catharanthine (90). Vinblastine, however, was but poorly labelled, as before. Significant labelling of leurosine (105) was observed,[106] but ready chemical conversion of (103) into (105) has been noted,[107] so this result must be treated with caution. Using a cell-free extract of *C. roseus* plants, the efficient conversion of (89) and (90) into anhydrovinblastine (103) and leurosine (105) has also been observed.[108]



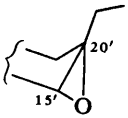
Anhydrovinblastine (103)



(106)



Vinblastine (104)



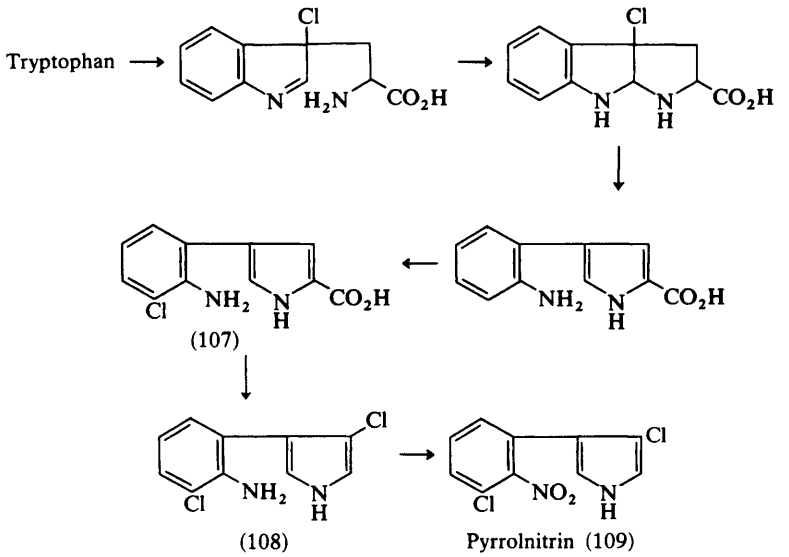
(105)

Secondly, it was shown, using cell-free extracts of *C. roseus*, that anhydrovinblastine (103) is a specific and efficient precursor for vinblastine (104);[109,110] positive incorporations into leurosine were also recorded, but, for the reason stated above, these results must be treated with caution. Leurosine (105) was, however, found to be, like (103), an efficient precursor for catharine (106).[110]

Pyrrolnitrin.—Pyrrolnitrin (109) is a metabolite of *Pseudomonas aureofaciens* and is derived from tryptophan. The amino-compound (108) is naturally occurring, and (107) has now also been isolated.[111] This, taken together with evidence from feeding experiments (*cf.* ref. 4, p. 28), indicates the pathway shown in Scheme 4. 3-Chloroanthranilic acid and 7-chloroindoleacetic acid have also been isolated from *P. aureofaciens*.[112]

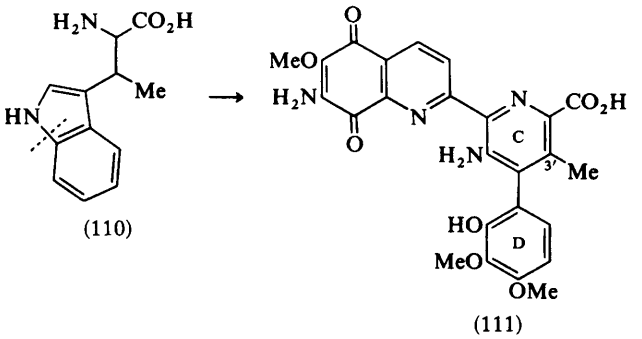
Streptonigrin.—Rings C and D of streptonigrin (111), a metabolite of *Streptomyces flocculus*, originate from tryptophan, and the methyl group at C-3', as

[107] N. Langlois and P. Potier, *J. Chem. Soc., Chem. Commun.*, 1978, 102.
[108] K. L. Stuart, J. P. Kutney, T. Honda, and B. R. Worth, *Heterocycles*, 1978, **9**, 1419.
[109] R. L. Baxter, C. A. Dorschel, S.-L. Lee, and A. I. Scott, *J. Chem. Soc., Chem. Commun.*, 1979, 257.
[110] K. L. Stuart, J. P. Kutney, T. Honda, and B. R. Worth, *Heterocycles*, 1978, **9**, 1391.
[111] O. Salcher, F. Lingens, and P. Fischer, *Tetrahedron Lett.*, 1978, 3097.
[112] O. Salcher and F. Lingens, *Tetrahedron Lett.*, 1978, 3101.



Scheme 4

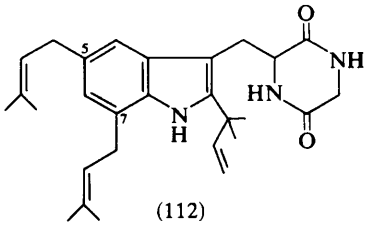
well as the methoxy-groups, derive from methionine; *cf.* ref. 9. The *C*-methyl group is introduced at an early stage of biosynthesis, as evidenced by the formation of 3-methyltryptophan (110) in *S. flocculus* cultures and its efficient incorporation into streptonigrin (111).[113]



Echinulin.—It is known that entry of the dimethylallyl groups at C-5 and C-7 of echinulin (112) occurs with loss of hydrogen from these positions and not from adjacent ones, thus indicating that substitution occurs simply and directly onto these sites; *cf.* ref. 6. The labelling pattern in these groups when echinulin is derived[114] from [1,2-$^{13}C_2$]acetate indicates that the *E*-methyl group is derived

[113] S. J. Gould and D. S. Darling, *Tetrahedron Lett.*, 1978, 3207.
[114] J. K. Allen, K. D. Barrow, and A. J. Jones, *J. Chem. Soc., Chem. Commun.*, 1979, 280.

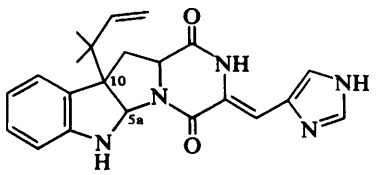
from C-2 of mevalonate, as expected of a normal biogenesis; similar results have been observed with the isoprenylated phenol flavoglaucin.[115] Thus echinulin is formed without isomerization of the double bonds of the dimethylallyl group. Moreover, the isoprenylation at C-5 and at C-7 occurs with unexceptional inversion of configuration, as monitored[114] with mevalonate that is chirally tritiated at C-5. This indicates again that the dimethylallyl groups are introduced by simple direct substitution.



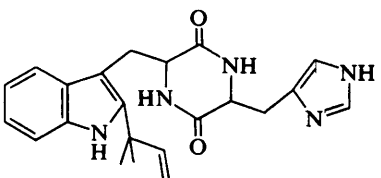
(112)

Incorporation of leucine into the C_5 isoprene units of echinulin (112) through catabolism to mevalonate is thought to proceed *via* acetoacetate; *cf.* ref. 9. Loss of C-2 of leucine is required in this pathway, which is supported by loss of ^{14}C label from [2-^{14}C, 5-^{3}H]leucine on incorporation into echinulin (also some loss of tritium, measured in another experiment).[116]

Roquefortine.—Roquefortine (113), isolated from *Penicillium roqueforti*, is a member of the group of diketopiperazine metabolites, other representatives of which are echinulin (112), gliotoxin [(122), p. 27], *etc.* Satisfactory incorporations (no degradations though) of radioactive tryptophan, mevalonic acid lactone, and histidine into roquefortine confirm the expected origins for the metabolite.[117] Use of a mutant of *P. roqueforti*, unable to grow in the absence of tryptophan, allowed the use of deuteriated tryptophan as a precursor, yielding (113) with high deuterium enrichment, since the mutant was not producing any tryptophan. The derived metabolite showed complete loss of deuterium from C-2 of tryptophan [equivalent to C-5a in (113)], which indicates that the C_5 isoprene unit at C-10b of (113) may have arrived at this site *via* C-5a, with (114) as a possible intermediate [*cf.* echinulin (112) above].



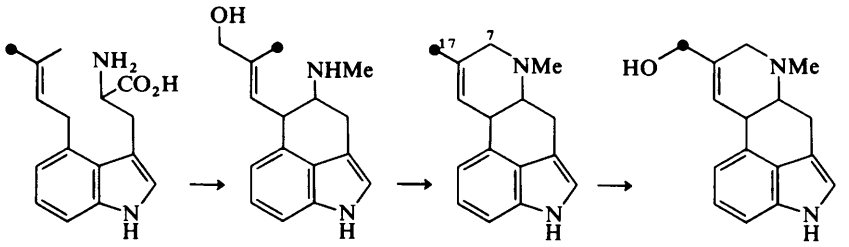
(113)



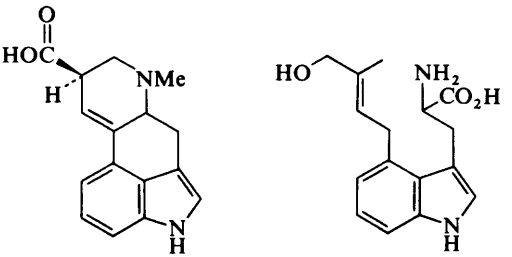
(114)

[115] J. K. Allen, K. D. Barrow, A. J. Jones, and P. Hanisch, *J. Chem. Soc., Perkin Trans. 1*, 1978, 152.
[116] C. Fuganti, P. Grasselli, and G. Pedrocchi-Fantoni, *Tetrahedron Lett.*, 1979, 2453.
[117] K. D. Barrow, P. W. Colley, and D. E. Tribe, *J. Chem. Soc., Chem. Commun.*, 1979, 225.

Ergot Alkaloids.—The biosynthesis of ergot alkaloids, notably represented by elymoclavine (118), starts with tryptophan. Isoprenylation affords (115), which is converted *via* chanoclavine-I (116) and agroclavine (117) into elymoclavine (118). Each of the ring-closure steps [(115) to (116), and (116) to (117)] involves an enigmatic change of stereochemistry around the side-chain double bond; *cf.* ref. 11. Results of experiments with dimethylallyltryptophan (115), bearing a ^{13}C label as shown, provide welcome confirmation of this: label appeared at the *C*-methyl group in (116) and at C-17 in (117), (118), and lysergic acid (119).[118]



(115) (116) • = ^{13}C label (117) (118)



(119) (120)

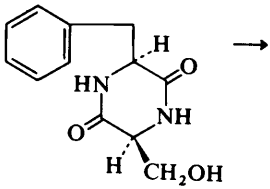
Cell-free extracts have been obtained from *Claviceps paspali* cultures which catalyse the formation of (115) and (120) from tryptophan and isopentenyl pyrophosphate. Both compounds were incorporated into lysergic acid amide.[119] Another unidentified compound has been isolated as a product of the incubation, derived from (120) and convertible into lysergic acid amide;[119] *cf.* ref. 9.

The ability of *C. purpurea* to use modified amino-acids for the elaboration of *cyclo*-peptide ergot alkaloids based on lysergic acid has been examined.[120] A varying yield of analogue was observed of 11—66% of total alkaloid formed. The alkaloids studied were, with modified amino-acid in brackets: ergocristine (*p*-chloro- and *p*-fluoro-phenylalanine), ergotamine (*p*-fluorophenylalanine), ergocornine and ergocryptine (L-norvaline, L-norleucine, L-α-aminobutyric acid, 5,5,5-trifluoroleucine, and β-hydroxyleucine).

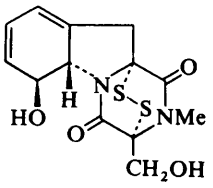
[118] H. Plieninger, E. Meyer, W. Maier, and D. Gröger, *Justus Liebigs Ann. Chem.*, 1978, 813.
[119] R. J. Petroski and W. J. Kelleher, *Lloydia*, 1978, **41**, 332.
[120] M. Beacco, M. L. Bianchi, A. Minghetti, and C. Spalla, *Experientia*, 1978, **34**, 1291.

5 Miscellaneous

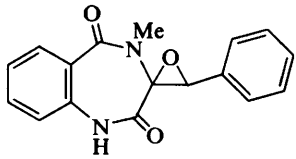
Gliotoxin.—*cyclo*-(L-Phenylalanyl-L-seryl) (121) has been observed to be an intact and efficient precursor for gliotoxin (122) in *Trichoderma viride*. This has been confirmed in further experiments with (121) and its three stereoisomers.[121] Only (121) was incorporated at all efficiently (at least forty times better than the others); it was also proved to be utilized intact. Moreover, (121) was formed from labelled phenylalanine in growing cultures. It follows that (121) is either an intermediate in gliotoxin biosynthesis, or is reversibly converted into an intermediate on the pathway. The biosynthesis of gliotoxin has been reviewed.[122]



(121)



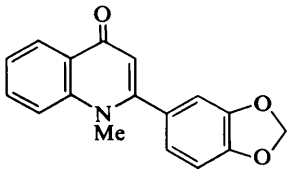
(122)



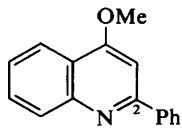
(123)

Benzodiazepine Alkaloids.—Benzodiazepine alkaloids, *e.g.* cyclopenin (123), are produced by *Penicillium cyclopium*. Their biosynthesis is pretty well understood.[16] Recently, the production of enzymes involved in the biosynthesis and formation of alkaloid in relation to the development of the organism has been studied.[123] The uptake of one of the precursors, *i.e.* phenylalanine, has also been studied.[124]

Quinoline Alkaloids.—Anthranilic acid and a benzoylacetic acid derivative, derived from phenylalanine, are precursors for graveoline (124); *cf.* ref. 5. On the other hand, whilst the biosynthesis of (125) no doubt involves anthranilic acid, benzoic acid (carboxyl-labelled material), and not phenylalanine, was a precursor for (125) in *Lunasia amara*; incorporation was specific, with labelling of C-2.[125] The remaining carbons should derive from acetate, and a positive incorporation was recorded. It is clear that further work is required on the biosynthesis of this alkaloid before its origins can be described with certainty.



(124)

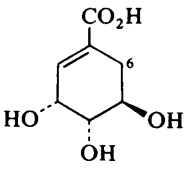


(125)

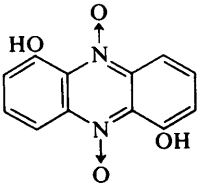
[121] G. W. Kirby, G. L. Patrick, and D. J. Robins, *J. Chem. Soc., Perkin Trans. 1*, 1978, 1336.
[122] G. W. Kirby, *Pure Appl. Chem.*, 1979, **51**, 705.
[123] S. Voigt, S. El Kousy, N. Schwelle, L. Nover, and M. Luckner, *Phytochemistry*, 1978, **17**, 1705.
[124] L. Nover, W. Lerbs, W. Müller, and M. Luckner, *Biochim. Biophys. Acta*, 1979, **584**, 270.
[125] A. C. Finlayson and R. H. Prager, *Aust. J. Chem.*, 1978, **31**, 2751.

Phenazines.—A study using [6-^{13}C]shikimic acid [as (126)] has given results[126] which confirm previous ones[54] concerning the orientation of shikimic acid (126) units in the bacterial phenazine iodinin (127).

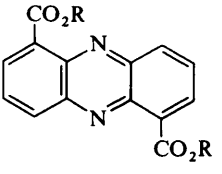
It has been claimed that dimethyl phenazine-1,6-dicarboxylate (128) is a precursor for 1-carboxyphenazine in *Pseudomonas aureofaciens*; *cf.* ref. 8. This claim has been disputed: careful checking showed that neither (128) nor the corresponding acid (129) was incorporated into phenazines produced by this organism.[127] This has been supported by the results of other workers,[128] who have found that (128) is metabolically inert in *P. aureofaciens*. Moreover, neither (129) nor (128) was incorporated into phenazines in *P. phenazinium*. On the other hand, efficient incorporations have been recorded of (129), but not of (128), into iodinin (127) and related phenazines in three actinomycetes, *i.e. Streptomyces thioluteus*, *Microbispora amethystogenes*, and *M. parva*.[128]



(126)



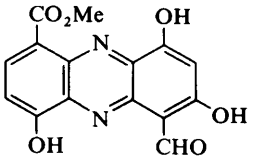
(127)



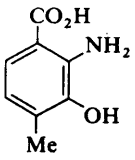
(128) R = Me
(129) R = H

This latter observation correlates with the observation[127] that (129) is a precursor for lomofungin (130) in *Streptomyces lomodensis*. It seems clear from the combined evidence that phenazine-1,6-dicarboxylic acid (129) is a precursor for all microbial phenazines. Failure to observe incorporation of (129) in *Pseudomonas*, with (by contrast) positive results in actinomycetes, may be attributed to differences in permeability of the cell walls (*cf.* ref. 127).

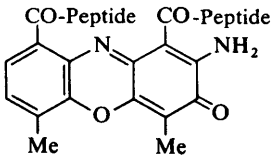
Actinomycin.—4-Methyl-3-hydroxyanthranilic acid (131) is an important precursor for actinomycin (132); *cf.* ref. 6. Further support for its role as a biosynthetic intermediate is its accumulation in mutants of *Streptomyces parvulus* that are unable to synthesize (132).[129]



(130)



(131)

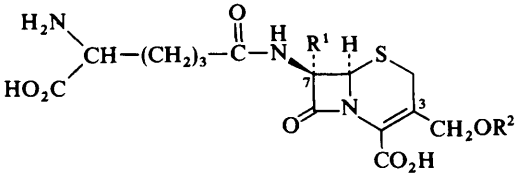


(132)

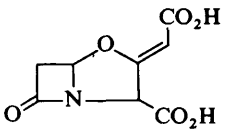
[126] U. Hollstein, D. L. Mock, R. R. Sibbitt, U. Roisch, and F. Lingens, *Tetrahedron Lett.*, 1978, 2987.
[127] S. P. Gulliford, R. B. Herbert, and F. G. Holliman, *Tetrahedron Lett.*, 1978, 195; R. B. Herbert, in ref. 9, p. 29.
[128] A. J. M. Messenger and J. M. Turner, *Biochem. Soc. Trans.*, 1978, **6**, 1326.
[129] T. Troost and E. Katz, *J. Gen. Microbiol.*, 1979, **111**, 121 · see also refs. cited therein.

β-Lactam Antibiotics.—The oxygen atom attached to the exocyclic methylene at C-3 of cephalosporin C (133) is derived from molecular oxygen, and its introduction is catalysed by a dioxygenase. It has been shown that this oxygen and the one at C-7 in cephamycin C (134) both derive from molecular oxygen.[130]

Investigation of the biosynthesis of clavulanic acid (135), using ^{13}C-labelled precursors, has shown that glycerol is an intact source for the three β-lactam carbons. Studies with [1-^{13}C]-, [2-^{13}C]-, and [1,2-$^{13}C_2$]-acetate indicated that the remaining five carbons probably derive from α-ketoglutarate.[131]

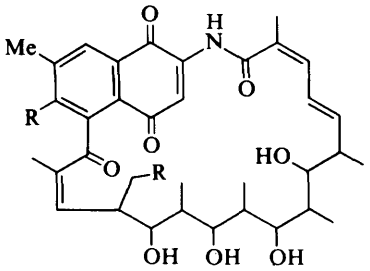


(133) R^1 = H, R^2 = Ac
(134) R^1 = OMe, R^2 = $CONH_2$



(135)

Ansamycins.—Similar patterns of precursor labelling in the rifamycins and streptovaricins led to the suggestion that they are derived from a common precursor, possibly (136); *cf.* refs. 5—7. This is supported by the isolation of protorifamycin I (137) from a mutant of *Nocardia mediterranei*.[132]



(136) R = H
(137) R = OH

[130] J. O'Sullivan, R. T. Aplin, C. M. Stevens, and E. P. Abraham, *Biochem. J.*, 1979, **179**, 47.
[131] S. W. Elson and R. S. Oliver, *J. Antibiot.*, 1978, **31**, 586.
[132] O. Ghisalba, P. Traxler, and J. Nuesch, *J. Antibiot.*, 1978, **31**, 1124.