The Biosynthesis of Hasubanonine and Protostephanine

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Summary Hasubanonine (**1**) and protostephanine (**2**) are shown by tracer experiments to be biosynthesised from two different C_6—C_2 units, one being the amine (**22**); many possible advanced precursors are synthesised and tested.

Stephania Japonica Miers produces a wide variety of alkaloids[1] among them hasubanonine (**1**) and the rare base protostephanine (**2**) which were the first natural examples of the hasubanan and dibenz-[*d*,*f*]-azonine skeletons to be characterised. The biosynthesis of these unusual systems is of considerable interest and many schemes have been proposed.[2] We now report clear leads to the solution of the biosynthetic problems.

Initially we synthesised those 1-benzyltetrahydroisoquinolines which on biogenetic grounds were possible late precursors of the alkaloids (**1**) and (**2**). Nine labelled isoquinolines were tested in *S. japonica* plants including

(**9**)—(**12**) together with *O*-methyl and *N*-methyl derivatives of (**8**) and (**9**); none was incorporated significantly† into hasubanonine (**1**) or protostephanine (**2**). The nature of the building blocks was then examined by feeding ^{14}C-labelled tyrosine‡ (**17**), dopa (**18**), tyramine (**19**) and dopamine (**20**) to the plants. The Table shows that all four were incorporated into both alkaloids.

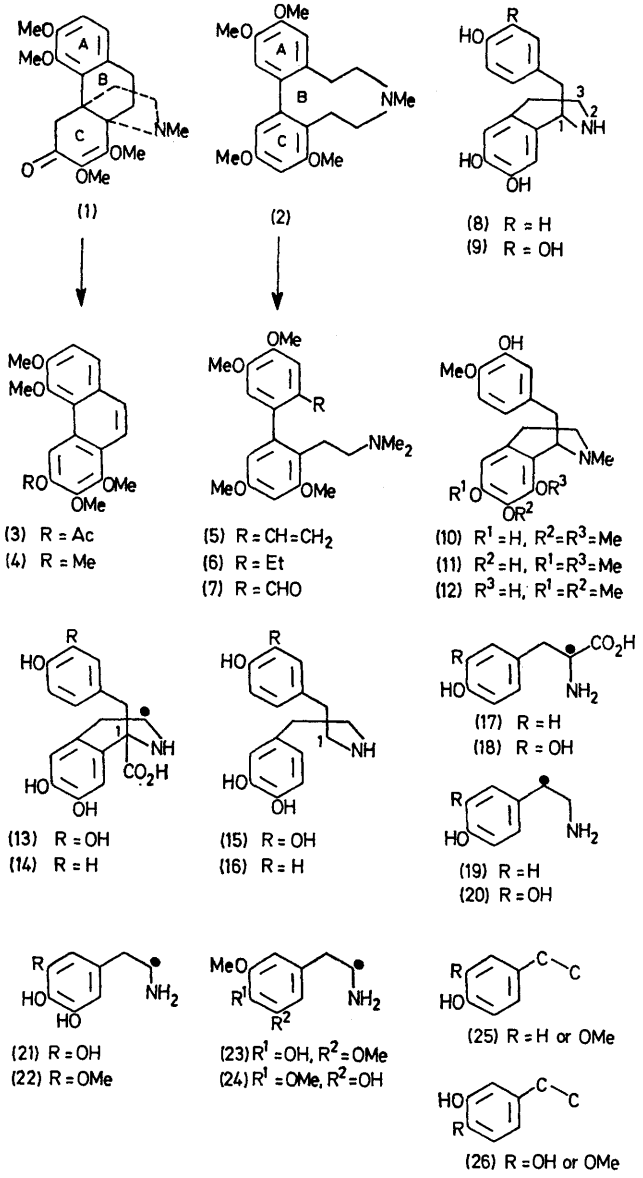
TABLE. Tracer experiments on *S. japonica* plants.

Precursor	% Incorpn. into (**1**)	% Incorpn. into (**2**)	% of radioactivity in ring-C ethanamine chain: Base (**1**)	Base (**2**)
(2*RS*)-[2-^{14}C]Tyrosine (**17**)	0·64—3·1	0·028—0·18	53	57
(2*RS*)-[2-^{14}C]Dopa (**18**)	0·02	0·008	98	92
[2-^{14}C]Tyramine (**19**)	1·2	0·005	99	93
[2-^{14}C]Dopamine (**20**)	0·17	0·005	99	94
[1-^{14}C]Amine (**21**)	1·0	0·017	99	[a]
[1-^{14}C]Amine (**22**)	1·1	0·011	99	[a]
[1-^{14}C]Amine (**23**)	<0·002	<0·0018	—	—
[1-^{14}C]Amine (**24**)	<0·002	<0·0018	—	—

[a] Degradation in progress.

Specific degradation of the labelled alkaloids was carried out as follows. Hofmann degradation[3] of protostephanine (**2**) gave a major product to which we assigned structure (**5**) by comparison of its n.m.r. spectrum with those of the derived dihydro-derivative (**6**) and aldehyde (**7**). Kuhn-Roth oxidation of (**6**) gave acetic and propionic acids, isolated as their *p*-bromophenacyl esters. When husubanonine (**1**) was heated at 160° in acetic anhydride with chloranil (HCl catalyst) the ethanamine side-chain was eliminated[4] to produce the phenanthrene (**3**) in up to 30% yield which was purified as the *O*-methyl ether (**4**). The results (Table) show that (a) both alkaloids are built from two different C_6–C_2 units derivable from tyrosine, (b) one unit is a phenethylamine which is formed from both tyramine (**19**) and dopamine (**20**) and it generates ring C, with its attached ethanamine residue, for both natural products, and (c) dopa (**18**) affords only this same phenethylamine unit.§

We then synthesised and tested in *S. japonica* plants the isoquinolines (**8**) and (**9**) together with their N-methyl derivatives and the bisphenethylamines (**15**) and (**16**), all six being ^{14}C-labelled at C-1. The incorporation of radioactivity into (**1**) and (**2**) was insignificant† in all cases. A reasonable conclusion is that oxidation to generate a trioxygenated aromatic ring must occur early before the C_6—C_2 units are joined, or that hydroxylation occurs on some intermediate between isoquinoline ring-closure and formation of a tetrahydroisoquinoline. Discrimination between these possibilities was made by synthesis of the four ^{14}C-labelled amines (**21**)—(**24**) and of the putative intermediates[5] (**13**) and (**14**) together with the 1,2-dehydro-derivatives of (**8**) and (**9**), ^{14}C-labelled at C-3. None of the isoquinolines was incorporated† but the amines (**21**) and (**22**) acted as precursors of (**1**) and (**2**), (Table). Degradation of hasubanonine (**1**) proved that the trioxygenated C_6—C_2 unit had been built in specifically to form ring C and its ethanamine side chain. These findings show that the biosynthesis of hasubanonine (**1**) and protostephanine (**2**) in *S. japonica* involves the first of the two alternatives above and rejection by the plants of bases (**23**) and (**24**) indicates that further *O*-methylation is not the next step. This agrees



† Incorporations ranged from <1% to 3% of the incorporation of (2*RS*)-[2-^{14}C]tyrosine, almost all being below 1%.

‡ The incorporation of tyrosine into protostephanine and hasubanonine with *S. japonica* plants has also been achieved by Professors Sir Derek Barton and G. W. Kirby and their colleagues; their work was not continued and we thank them for exchange of information.

§ Dr. R. Kazlauskas also observed 0·78% incorporation of (2*RS*)-[2-^{14}C]dopa into morphine in *Papaver somniferum* plants and > 97% of the label was shown to be located in the ethanamine bridge by degradation to *O*-acetylmethylmorphol.

with the above experiments using possible advanced precursors.

By combining building block (**22**) with the residue (**25**) or (**26**), a set of isoquinolines and bisphenethylamines can be designed to allow selection of the natural advanced intermediate(s) for the biosynthesis of (**1**) and (**2**) from the large number of structures which are possible.

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[2] H. G. Boit, 'Ergebnisse der Alkaloid-Chemie bis 1960,' Akademie-Verlag, Berlin, 1961, 402; D. H. R. Barton, *Pure and Applied Chem.*, 1964, **9**, 35; A. R. Battersby in 'Oxidative Coupling of Phenols,' eds. W. I. Taylor and A. R. Battersby, Marcel Dekker, New York, 1967, p. 119; D. H. R. Barton, A. J. Kirby, and G. W. Kirby, *J. Chem. Soc.* (*C*), 1968, 929; C. W. Thornber and S. Ruchirawat, Ph.D. Theses, Liverpool, 1969.

[3] K. Takeda, *Bull. Agric. Chem. Soc. Japan*, 1956, **20**, 165 (*Chem. Abs.* 1957, **51**, 11,364).

[4] *Cf.* H. Kondo and M. Satomi, *Ann. Report ITSUU Lab.*, 1957, **8**, 41 and Y. Watanabe and H. Matsumura, *J. Pharm. Soc. Japan*, 1963, **83**, 991 and refs. therein.

[5] *Cf.* biosynthesis of cactus alkaloids, G. J. Kapadia, G. Subba Rao, E. Leete, M. B. E. Fayez, Y. N. Vaishnav, and H. M. Fales, *J. Amer. Chem. Soc.*, 1970, **92**, 6943.