The Biosynthesis of Epistephanine and the Structure of Stebisimine

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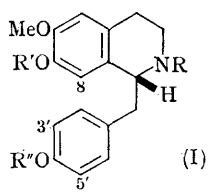
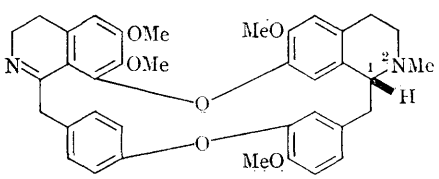
THE idea[1] that bisbenzylisoquinoline alkaloids might be formed in Nature by the oxidative coupling of coclaurine (I; R=R′=R″=H) derivatives has so far lacked experimental support.[2] We now report appropriate tracer studies on epistephanine[3] (II).



(I)



(II)

(±)-[2-^{14}C]Tyrosine was incorporated (0·17%) into epistephanine in *Stephania japonica* Miers. Cleavage of the alkaloid with sodium in liquid ammonia gave, after methylation with diazomethane, (±)-*OO*-dimethylcoclaurine (I; R=H, R′=R″=Me) and (−)-*NOO*-trimethylcoclaurine (I; R=R′=R″=Me). These compounds contained, respectively, 50 and 51% of the total activity. In the following season incorporation of (±)-[2-^{14}C]tyrosine (0·084%), (±)-[8,3′,5′-$^{3}H_3$]-coclaurine[4] (I; R=R′=R″=H) (0·008%), and (±)-[N-*methyl*-^{14}C]*N*-methylcoclaurine (0·050%) was observed. These and later incorporations have been corrected for loss of tritium from positions involved in the oxidative couplings. Herzig–Meyer demethylation of epistephanine, derived from the *N*-methyl labelled precursor, located 98% of the activity in the *N*-methyl group.

Both enantiomers of [8,3′,5′,-$^{3}H_3$]*N*-methylcoclaurine were fed, in parallel, to *S. japonica*. The (−)-enantiomer (I; R=Me, R′=R″=H) was incorporated (0·060%) into epistephanine much more efficiently than its antipode (0·003%). This confirms[4–7] the absolute configuration (II) of the alkaloid and shows that racemisation[8] of the precursor is unimportant in this plant. Degradation of epistephanine derived from (−)-*N*-methylcoclaurine gave (−)-*NOO*-trimethylcoclaurine containing 95% of the total activity. The other fragment, (±)-*OO*-dimethylcoclaurine, was inactive. Clearly *N*-methylcoclaurine provides only half the epistephanine molecule and is not demethylated in the plant to give coclaurine or any other metabolically active derivative.

During work with *S. japonica* we isolated a new

minor alkaloid, stebisimine, m.p. 233—235°, $C_{36}H_{34}N_2O_6$, *m/e* 590 (molecular ion and base peak), $[\alpha]_D = 0°$ (c, 1·26 in $CHCl_3$), ν_{max} ($CHCl_3$) 1610 cm.$^{-1}$, λ_{max} (EtOH) 238 and 279 mμ (ϵ, 51,900 and 24,200), and λ_{inf} 308 mμ (ϵ, 12,500). The n.m.r. spectrum (in $CDCl_3$) showed methoxyl signals at τ 6·04, 6·10, 6·12, and 6·75 and no *N*-methyl signal. These properties suggested that stebisimine might be *N*-nor-1,2-dehydroepistephanine and this was confirmed chemically. Reduction of stebisimine with sodium in liquid ammonia gave, after *N*-methylation of the product mixture, (±)-armepavine (I; R=R′=Me, R″=H) and the (±)-*NO*-dimethylcoclaurine (I; R=R″=Me, R′=H). Both components were identified by comparison with the synthetic racemates.[9]

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[2] Dr. I. R. C. Bick (Univ. of Tasmania) has kindly informed us of his unpublished work on the biosynthesis of berbamine in *Atherosperma moschatum*. The feeding of (±)-[2-^{14}C]tyrosine gave radioactive berbamine containing equal amounts of radioactivity in the two halves of the molecule.
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[4] L. J. Haynes, K. L. Stuart, D. H. R. Barton, D. S. Bhakuni, and G. W. Kirby, *Chem. Comm.*, 1965, 141.
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[8] A. R. Battersby, D. M. Foulkes, and R. Binks, *J. Chem. Soc.*, 1965, 3323.
[9] M. Tomita and H. Yamaguchi, *Pharm. Bull.* (*Japan*), 1953, **1**, 10; we thank Prof. M. Tomita for several authentic specimens.