Biosynthesis of Cryogenine

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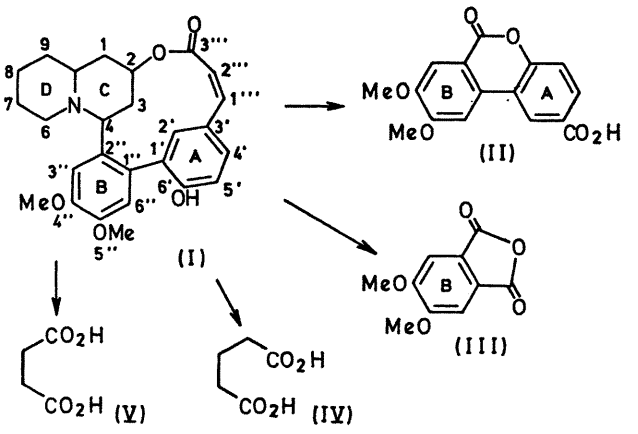
Summary Phenylalanine is shown to be a precursor of cryogenine, one of the quinolizidine-type Lythraceae alkaloids.

CRYOGENINE (I), one of the quinolizidine-type Lythraceae alkaloids, may be regarded as the *p*-hydroxycinnamoyl ester of 2-hydroxy-4-phenylquinolizidine, embodying a biphenyl system. The biogenesis of (I) and its congeners may be viewed in several ways. The biphenyl system in (I), together with the co-occurrence of biphenyl-ether alkaloids in the plant, suggests oxidative coupling. A dissociated origin of rings A and B is thus apparent. The involvement of a C_6–C_3 precursor is manifest in the cinnamoyl moiety involving ring A. Ring B and the quinolizidine moiety might arise from a benzoyl unit and an isopelletierine equivalent as suggested by Ferris *et al.*[1] Alternatively, the phenylquinolizidine moiety could arise from a C_6–C_3 equivalent and lysine, or a polyketide precursor and ammonia, or a combination of these. Since a benzoyl unit



may arise from a C_6–C_3 precursor the latter is a plausible candidate for the genesis of both rings A and B. We now report experimental evidence bearing on these processes.

[3-^{14}C]Phenylalanine (0·2 mc, 7·6 mg), root-fed to intact *Heimia salicifolia* L. seedlings, yielded radioactive fractions from which (I) (31·48 mg, 3617 ± 35 × 10^3 dpm/mmole, 0·06% incorporation) was isolated. Oxidation[2] of (I) yielded compounds (II—V). Analysis showed that the relative molar activity was 92% in (II) (rings A and B +C-4 and-1′′′) and 31% was in (III) (ring B + C-4 and-1′) (see Table). Thus, 61% of the activity is in ring A — C-1′ +C-1′′′ and 8% is in C-1, -2, -3, -6, -7, -8, -9, -10, -2′′′, and -3′′′.

The labelled carbons of (I) from [3-^{14}C]phenylalanine may be presumed to be 4 and 1′′′. The difference in the molar activity of the compounds representing the two halves of the biphenyl system may be due to high endogenous dilution of the intermediate species peculiar to the apparent benzoyl moiety. If two molecules of phenylalanine are incorporated per mole of cryogenine and isopelletierine condenses with a benzoyl species, β-oxidation of the side-chain of one of the phenylalanine units must occur. Our data, however, do not exclude the possibility of incorporation of two C_6–C_3 units from phenylalanine. Biogenetic analogy, however, is contrary to this view. If lysine is the precursor of the D-ring + C-1 the condensation of a phenylpropanoid acid to provide carbons C-2, -3, and -4 seems highly unlikely; an acetate precursor of the carbon of the D-ring + C-1 would be more tenable.

Activity distribution in cryogenine derived from [3-^{14}C]*phenylalanine*

Compound	Specific activity[a] dpm/mmole × 10^2	Relative specific activity %
Cryogenine (I)	1757±5	100
5′-Carboxy-2′-hydroxy-4,5-dimethoxy-2-biphenylcarboxylic acid-δ-lactone (II)	1616±21	92
4,5-Dimethoxyphthalic anhydride (III)	541±9	31
Glutaric acid (IV)	82±0·9	4·7
Succinic acid (V)	58±1·8	3·3

[a] Values given were obtained from carrier-diluted cryogenine.

The relatively low incorporation of [1-^{14}C]acetate (4·0 mc, 164 mg) into (I) (54·9 mg, 15,989 ± 50 × 10^3 dpm/mmole, 0·023% incorporation) suggests that the aromatic nuclei are not derived from a polyketide precursor. However, phenylalanine side-chain degradation products, presumably entering a fundamental metabolic system, do account for activity in carbons 6—10 (3·3% in succinic acid; 4·7% in glutaric acid) and by difference some minor activity (3·3%) resides among C-1, -2, -3, -2′′′, and -3′′′.

(*Received, October 20th*, 1969; *Com.* 1587.)

[1] J. P. Ferris, C. B. Boyce, and R. C. Briner, *Tetrahedron Letters*, 1966, 5129.
[2] A. Rother, H.-G. Appel, J. M. Kiely, A. E. Schwarting, and J. M. Bobbitt, *Lloydia*, 1965, 28: 90.