Biosynthesis of the C_{10} Necic Acids of the Pyrrolizidine Alkaloids

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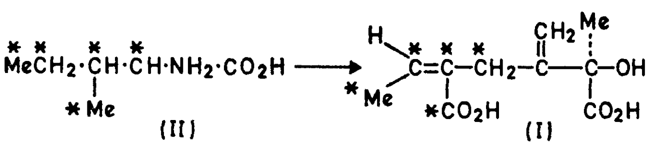
Summary The necic acids of the *Senecio* alkaloids arise by a biosynthetic pathway involving the coupling of metabolites derived from isoleucine.

THE C_{10} necic acids of the pyrrolizidine alkaloids, such as seneciphyllic acid (I), a representative of the commonest structural type encountered in alkaloids of *Senecio* species (fam. Compositae), have structures which may be dissected into isoprene units. However, seneciphyllic acid (I) has been shown to be derived, not from mevalonate, but from isoleucine (II) and its biological precursors threonine (III) and aspartic acid.[1] It has been suggested[1] that isoleucine (II) is incorporated into the left-hand C_5 unit of seneciphyllic acid (I) as shown in Scheme 1.

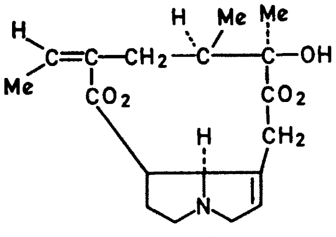
A re-evaluation of the available evidence suggested that the entire C_{10} skeleton of seneciphyllic and related acids might be derived from isoleucine and hence ultimately from threonine (III) and pyruvate,[2] as shown in Scheme 2.

Earlier investigations[1] showed that [U-^{14}C]threonine was not incorporated into C-8 of seneciphyllic acid (I), in agreement with Scheme 2. However, C-2 appeared to contain significant activity, although it was recognised that this result might be unreliable as it was obtained by an indirect method. Accordingly we have repeated the threonine feeding, using *Senecio magnificus* F. Muell., which

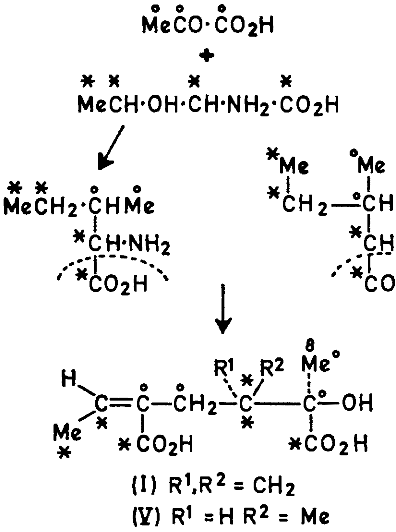


SCHEME 1

produces senecionine (IV), the diester of senecic acid (V) with retronecine, as the main alkaloidal component. A specific and relatively high incorporation of [U-^{14}C]-L-threonine into senecic acid was observed (Table). C-2 was isolated as the carboxyl group of benzoic acid and was found to be essentially inactive (0·6% of the total activity) in accord with the suggested biogenesis (Scheme 2). C-8 was similarly found to be inactive (0·1% of the total activity) in confirmation of the earlier observation.[1]



(IV)

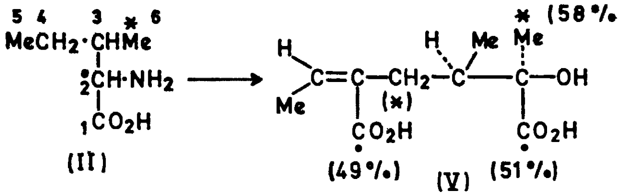


SCHEME 2

Incorporation of labelled amino-acids into senecionine (IV)

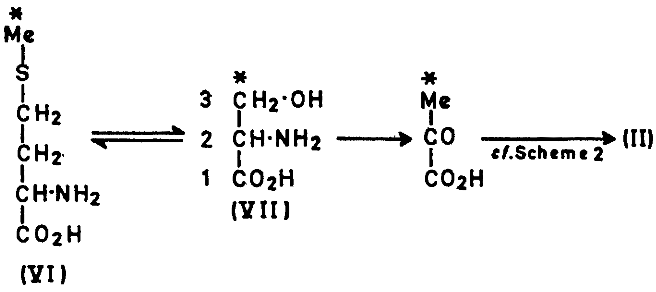
Precursor	Incorporation (%)	% Distribution of activity in Senecic acid	Retronecine
[U-^{14}C]-L-Threonine (III)	0·25	98·5	0·60
[2-^{14}C]Isoleucine (II)	0·25	99·6	1·31
[6-^{14}C]Isoleucine (II)	0·15	99·3	3·90

Further feeding experiments were performed with [2-^{14}C]- and [6-^{14}C]-isoleucine.† These precursors were incorporated specifically into senecic acid (Table). Degradation of the labelled acids revealed a pattern of incorporation (Scheme 3) consistent with the suggested pathway.



SCHEME 3

A suggestion[1] that *C*-methylation might be involved in the biosynthesis of seneciphyllic acid (I) was prompted by the observation that the *S*-methyl group of methionine was a relatively specific precursor of C-8. However, the overall incorporation into the corresponding alkaloid (seneciphylline) was low, and there was appreciable randomisation of the label between the necic acid and pyrrolizidine base components. These observations are explicable in terms of the pathway of Scheme 2, if the possibility is assumed of transformation of the *S*-methyl group of methionine (VI) into C-3 of serine (VII) *via* the one-carbon metabolic pool. Since serine is convertible into pyruvate *in vivo*, this pathway (Scheme 4), for which there is experimental support,[3,4] provides an explanation for the observed incorporation of the methionine methyl carbon into C-8 of seneciphyllic acid. We have obtained further evidence for the operation of this pathway in higher plants by administering [*Me*-^{14}C]-L-methionine to pea seedlings. An incorporation of 0·14% into serine (VII) was observed, with the bulk of the label (>90%) appearing in C-3.



SCHEME 4

It is evident from the results presented both here and elsewhere[1] that the necic acids of the *Senecio* alkaloids arise by a novel biosynthetic pathway involving the coupling of metabolites derived from isoleucine.

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† The specifically labelled amino-acids were synthesised and fed as a 50 : 50 mixture of DL-isoleucine and DL-alloisoleucine. Percentage incorporations are calculated on the assumption that only L-isoleucine is utilised in senecic acid biosynthesis.

[1] D. H. G. Crout, M. H. Benn, H. Imaseki, and T. A. Geissman, *Phytochemistry*, 1966, **5**, 1.

[2] A. Meister, "Biochemistry of the Amino Acids," 2nd edn., Academic Press, New York and London, 1965, vol. 2, p. 729.

[3] R. L. Kisliuk, W. Sakami, and M. V. Patwardhan, *J. Biol. Chem.*, 1956, **221**, 885.

[4] W. E. Splittstoesser and M. Mazelis, *Phytochemistry*, 1967, **6**, 39.