Pyrrolizidine Alkaloids. Biosynthesis of the Angelate Component of Heliosupine

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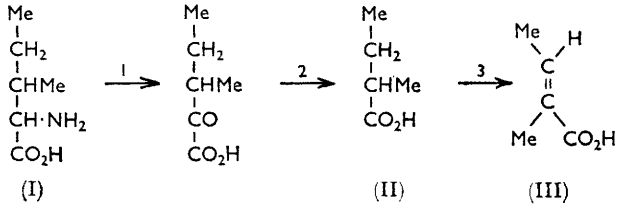
[u-^{14}C]-L-Isoleucine is specifically incorporated into the angelate component of heliosupine in *Cynoglossum officinale* L. The relationship between angelic acid and seneciphyllic acid biosynthesis is discussed.

ANGELIC acid (VI) occurs esterified to a pyrrolizidine base [as in heliosupine (IV)] in a number of alkaloids of the pyrrolizidine series, many of which are found in species of the family Boraginaceae.[1]

Leete has suggested a scheme for the biosynthesis of tiglic acid (III), the isomer of angelic acid, from acetoacetate by way of α-methylation, reduction, and dehydration.[2] However, it has been shown that this acid is a product of isoleucine metabolism in heart and liver tissue.[3] The relevant part of the metabolic sequence is shown in Scheme 1. Recently, the specific incorporation of isoleucine (I) into the tigloyl ester portion of the tropane alkaloids of *Datura meteloides* has been reported.[4] This evidence suggested that angelic acid could also be derived biogenetically from isoleucine, either from 1-methylbutyric acid (II), by a modification of the dehydrogenation step (step 3, Scheme 1), or by the isomerisation of tiglic acid by an enzyme system similar to that responsible for crotonic acid isomerisation.[5]

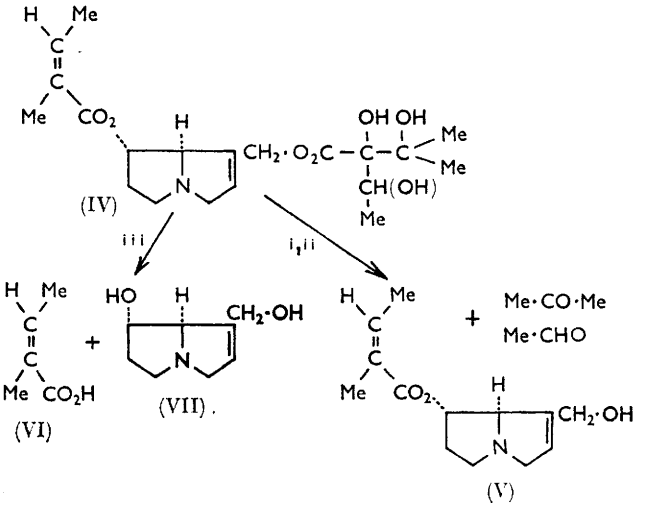


Scheme 1

This possibility has been tested in *Cynoglossum officinale* L., which produces a number of closely related alkaloids, including heliosupine (IV) and heliosupine *N*-oxide.[6] Uniformly labelled [^{14}C]-L-isoleucine and sodium [2-^{14}C]acetate were administered to two batches of *C. officinale* plants growing in hydroponic solution. After 8 days the alkaloids were isolated and the total incorporation of radioactivity was determined. The results (Table 1) indicate a ten-fold greater incorporation of [u-^{14}C]-L-isoleucine relative to [2-^{14}C]acetate.

The main component of the alkaloid mixture, heliosupine (IV), was isolated and purified by established procedures.[7] Periodate oxidation followed by hydrolysis gave the 7-angeloylheliotridine (V), acetaldehyde, and acetone, as previously described (Scheme 2).[7] These were purified as the picrate, the dimedone derivative, and the 2,4-dinitrophenylhydrazone, respectively.

Alkaline hydrolysis of heliosupine gave angelic acid (VI), purified as the *p*-phenylphenacyl ester, and heliotridine (VII). The activities of the various degradation



Scheme 2

Reagents: i, $NaIO_4$; ii, hydrolysis; iii, $Ba(OH)_2$

products relative to heliosupine (activity 100) are given in Table 2, which shows that the activity was almost entirely (98%) located in the angeloyl ester portion of

TABLE 1

Incorporation of radioactivity from [u-^{14}C]-L-isoleucine and sodium [2-^{14}C] acetate into the total alkaloid mixture

Precursor	Incorporation (%)
[u-^{14}C]-L-Isoleucine	0·205
Sodium [2-^{14}C]acetate	0·019

TABLE 2

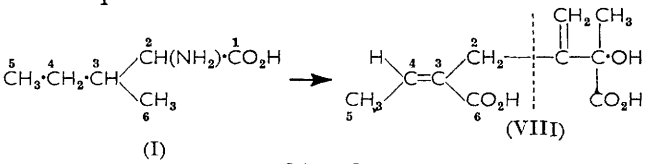
Distribution of activity in heliosupine from [u-^{14}C]-L-isoleucine feeding

Heliosupine (IV)	100
7-Angeloylheliotridine (V)	97
p-Phenylphenacyl angelate	98
Heliotridine (VII)	2·3
Acetone 2,4-dinitrophenylhydrazone	0·5
Acetaldehyde dimedone derivative	0·6

the alkaloid. The complete specificity of L-isoleucine for angelic acid biosynthesis indicated by these results

[1] H.-G. Boit, "Ergebnisse der Alkaloid-Chemie bis 1960," Akademie-Verlag, Berlin, 1961, p. 86 *et seq.*
[2] E. Leete, "Biogenesis of Natural Compounds," Pergamon, Oxford, 1963, p. 739.
[3] W. G. Robinson, B. K. Bachhawat, and M. J. Coon, *J. Biol. Chem.*, 1956, **218**, 391.
[4] W. C. Evans and J. G. Woolley, *J. Pharm. Pharmacol.*, 1965, **17**, Suppl., 37S—38S.
[5] J. R. Stern and A. del Campillo, *J. Biol. Chem.*, 1956, **218**, 985.
[6] Z. Jerzmanowska and Z. Sykulska, *Dissertationes Pharm.*, 1964, **16**, 71.
[7] D. H. G. Crout, *J. Chem. Soc.* (*C*), 1966, 1968.

provides strong support for the proposed biogenesis of this compound.



(I) (VIII)

Scheme 3

L-Isoleucine has been implicated in the biosynthesis of seneciphyllic acid (VIII), the esterifying acid of the pyrrolizidine alkaloid seneciphylline (Scheme 3).[8] The five-carbon portion to the left of the broken line in (VIII) has been shown to be derived from L-isoleucine by loss of C-1 and dehydrogenation, as in the conversion into angelic and tiglic acids. Thus a parallel sequence of transformations can be seen in the derivation of this five-carbon unit and of angelic acid. In seneciphyllic acid biosynthesis, a further step is the oxidation of the side-chain to give the carboxylic acid group [C-6 in (VIII)].

EXPERIMENTAL

Melting points are corrected. Radioactivity was measured with a Tritium Scintillation Counter model 6012 (Isotope Developments Ltd.). Colourless samples were counted in a dioxan-based scintillation solution, NE 220 (Nuclear Enterprises Ltd.), with [1-^{14}C]-n-hexadecane as internal standard. Coloured compounds were burned to CO_2, which was trapped and counted as previously described.[7]

Sufficient counts were taken to give a standard error for the final fractional activities of the degradation products relative to heliosupine of $<2{\cdot}5\%$ for degradation products with $>90\%$ the activity of heliosupine, and $<0{\cdot}2\%$ for degradation products with $<5\%$ of the activity of heliosupine. These errors are expressed as a percentage of the heliosupine activity.

Activities are expressed as decompositions per minute per millimole (d.p.m./mmole).

The feeding procedures, and the isolation and purification of the alkaloids of *C. officinale* were described previously.[7] Radiochemicals were supplied by the Radiochemical Centre, Amersham.

Uptake of activity by the plants from the sodium [2-^{14}C]-acetate feeding was 96·5% and from the [u-^{14}C]-L-isoleucine feeding, 97%.

Periodate Oxidation of Heliosupine.—This was carried out as previously described.[7] Oxidation of heliosupine, recovered from the picrate (activity 38,250 d.p.m./mmole), gave 7-angeloylheliotridine (V) (activity of the picrate 37,100 d.p.m./mmole), acetone 2,4-dinitrophenylhydrazone (205 d.p.m./mmole), and acetaldehyde dimedone derivative (236 d.p.m./mmole).

Alkaline Hydrolysis of Heliosupine.—Heliosupine was recovered from the picrate (340 mg., 12,720 d.p.m./mmole) by extraction from dilute ammoniacal solution with chloroform. The alkaloid was obtained as a clear gum. This was boiled gently under reflux with barium hydroxide octahydrate (192 mg.) in water (6 ml.) for 90 min. The solution was cooled, treated with solid carbon dioxide, boiled under reflux for 2 min., and filtered. The filtrate was acidified (Congo Red) with concentrated hydrochloric acid, and distilled. The distillate was collected in 10-ml. fractions; water was added at intervals to replenish the boiler. The collected fractions were titrated against 0·10N-sodium hydroxide (phenolphthalein) until no more alkali was consumed. Total alkali required: 4·78 ml., equivalent to 47·8 mg. of angelic acid (90% yield based on the starting picrate). The solution of sodium angelate was concentrated at 40° to 0·5 ml. and the red colour removed by the addition of 1 drop of 0·1N-sulphuric acid. The solution was treated with *p*-phenylphenacyl bromide (132 mg.) and heated under reflux on a steam-bath. Ethanol was added slowly to the hot solution until the reagent had just dissolved, and the mixture was refluxed for 1 hr. The ester rapidly crystallised from the cooled solution. Recrystallisation from benzene–light petroleum (b. p. 40—60°) gave *p*-phenylphenacyl angelate (93 mg.), m. p. 85—87° (12,440 d.p.m./mmole).

The aqueous residue from the distillation was filtered through a column of Dowex 1-X8 (10 g., OH^- form) and the eluate was collected until no longer alkaline to litmus. The eluate was evaporated to dryness at 40° and the residue was dried ($CaCl_2$) under reduced pressure and extracted three times with boiling acetone. The acetone extracts were filtered and evaporated, and the residue was recrystallised from acetone, to give heliotridine (VII) (24·5 mg.), m. p. 115—116° (291 d.p.m./mmole).

I thank Messrs. S. C. and N. A. Caldwell for assistance in collecting *C. officinale* plants.

[6/1537 *Received, December 6th,* 1966]

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