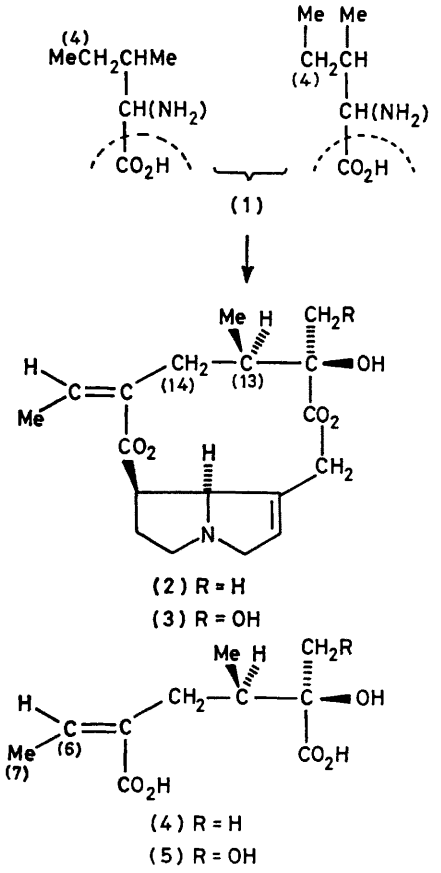
Isoleucine Biosynthesis and Metabolism: Stereochemistry of the Formation of L-Isoleucine and of its Conversion into Senecic and Isatinecic Acids in *Senecio* Species

By ROSALIND CAHILL, DAVID H. G. CROUT,* MICHAEL B. MITCHELL, and URS S. MÜLLER
(*Department of Chemistry, University of Exeter, Stocker Road, Exeter*, EX4 4QD)

Summary Incorporation experiments with isoleucine (**1**) stereospecifically labelled at C-4, and 2-aminobutanoic acid (**10**) stereospecifically labelled at C-3, in *Senecio* species have shown that the ethyl migration step during the biosynthesis of L-isoleucine (**1**) takes place with retention of configuration at the migrating centre, and that during conversion into necic acids of the senecic acid (**4**) type, both C_5 units are formed with loss of the C-4 *pro-S* hydrogen atom of L-isoleucine (**1**) and retention of the C-4 *pro-R* hydrogen atom.

TEN-carbon necic acids of the pyrrolizidine alkaloids, such as senecic (**4**) and isatinecic (**5**) acids, the esterifying acids of the alkaloids senecionine (**2**) and retrorsine (**3**), respectively, are formed from two molecules by L-isoleucine (**1**) with loss of the carboxyl function (Scheme 1).[1] During conversion into the necic acids, both precursor molecules lose one of the C-4 hydrogen atoms. This biosynthetic system therefore provides a means both of studying the stereochemical changes affecting C-4 in L-isoleucine (**1**) during its biosynthesis, and of investigating the mechanism of the conversion of L-isoleucine (**1**) into necic acids of the senecic acid (**4**) family.



SCHEME 1

During the biosynthesis of L-isoleucine (**1**), 2-ethyl-2-hydroxy-3-oxobutanoic acid (**8**) undergoes a tertiary ketol rearrangement to 3-hydroxy-3-methyl-2-oxopentanoic acid (**9**).[2] In order to investigate the stereochemistry of this rearrangement with respect to the changes taking place at the migrating centre, we have generated the precursor acid (**8**) stereospecifically labelled with tritium at C-5 by administering 2-aminobutanoic acid (**10**), stereospecifically labelled at C-3, to *Senecio* species. The ethyl group of the intermediate (**8**) is derived *in vivo* from L-threonine (**6**) *via* 2-oxobutanoate (**7**)[2] (Scheme 2). We assumed that appropriate enzymes (amino acid aminotransferases or oxidases) would operate on 2-aminobutanoic acid (**10**) to generate 2-oxobutanoate (**7**) *in situ*. The specificity for necic acid biosynthesis was investigated with (2*RS*)-[3-^{14}C]-2-aminobutanoic acid (**10**). This was incorporated into senecionine (**2**) in *Senecio magnificus* (0·09% specific incorporation). All of the activity (101%) was located in the necic acid component and approximately half (41%) in the C-6,7 component of the necic acid (**4**). These results are entirely consistent with a pathway of incorporation *via* L-(2*S*)-isoleucine.[1] The results obtained by administering (2*RS*, 3*S*)-[3-$^3H_1$]-, (2*RS*)-[3-$^3H_2$]-, (2*S*)-[3-$^3H_2$]- and (2*R*)-[3-$^3H_2$]-2-aminobutanoic acid (**10**) together with [3-^{14}C]-labelled material are shown in the Table. The results show that both L(2*S*)- and D(2*R*)-2-aminobutanoic acid (**10**) are incorporated specifically into the necic acid and that half of the ^{14}C activity is located in the C-6,7 ethylidene fragment, in agreement with the results quoted above in connection with the incorporation of singly (^{14}C) labelled 2-aminobutanoic acid. The results also show that during generation of both five-carbon units of the necic acid, the C-3 *pro-S* hydrogen of 2-aminobutanoic acid (**10**)[3] is lost and the C-3 *pro-R* hydrogen retained. In order to correlate this stereochemistry with the corresponding changes taking place at C-4 in L-(2*S*)-isoleucine (**1**), (2*RS*,4*RS*)-[3,4-$^3H_2$]-, (2*S*,4*S*)-[3,4-$^3H_2$]-$^3H_2$]-, and (2*S*,4*R*)-[4-$^3H_1$]-isoleucine were administered to the plants, together with L-[U-^{14}C]isoleucine as internal standard. The C-4 *pro-S* tritiated precursors [*cf.* (**13**)] were synthesised as shown in Scheme 3, from the (*E*)-isomer of 3,4-dehydroisoleucine (**12**). The (2*S*,4*R*)-[4-$^3H_1$]-labelled precursor was obtained by the biological conversion of D-[3-^{3}H]threonine into L-isoleucine (**1**) using a mutant of *Serratia marcescens* deficient in L-threonine dehydratase and lacking feedback controls.[4] The results of administering these precursors to *Senecio* species are shown in

TABLE. Incorporations of 2-aminobutanoic Acid (**10**) and isoleucine (**1**) into alkaloids (**3**) and (**4**) in *Senecio* species.

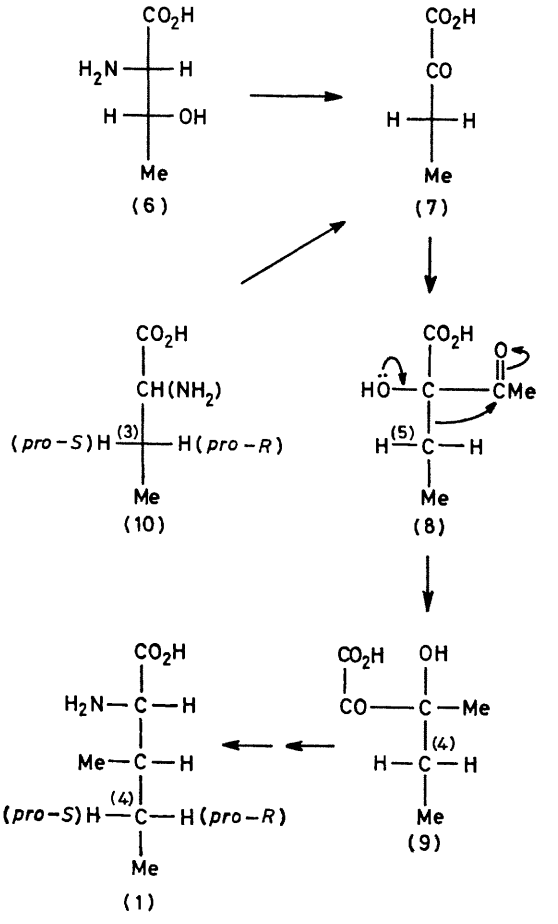
	$^3H/^{14}C$ ratio				% Retention of Tritium			% ^{14}C activity		
Precursor	Precursor	Alkaloid (**2**) or (**3**)	Necic acid[a]	C-6, 7 unit of necic acid[b]	Alkaloid	Necic acid	C-6, 7 unit of necic acid	Necic acid	C-6, 7 unit of necic acid	% Specific incorporation (^{14}C)
2-Aminobutanoic acid										
(2*RS*,3*S*)-[3-$^3H_1$,3-^{14}C][c]	2·85	0·18	—	—	6·5	—	—	—	—	0·015
(2*RS*)-[3-$^3H_2$,3-^{14}C][d]	8·23	3·85	3·78	4·59	47	—	56	98	43	3·4
(2*S*)-[3-$^3H_2$,3-^{14}C][d]	7·9	3·63	3·72	4·01	46	47·1	50·8	95	47	3·6
(2*R*)-[3-$^3H_2$,3-^{14}C][d]	7·97	3·77	3·88	4·01	47·4	48·7	50·3	94	51	2·1
Isoleucine										
2*S*-[U-^{14}C] plus:										
(2*RS*,4*RS*)-[3,4-$^3H_2$][c]	1·44[e]	0·074	—	—	5·1	(5·1)	—	—	—	0·05
(2*S*,4*S*)-[3,4-$^3H_2$][c]	1·84[f]	0·12	—	—	6·5	(6·5)	—	—	—	0·004
(2*S*)-[4-$^3H_2$][d]	2·36[g]	1·42	1·60	—	50	56	—	94	—	0·21
(2*S*,4*R*)-[4-$^3H_1$][d]	2·72[f]	2·59	—	—	95·2	(95·2)	—	—	—	2·2

[a] After hydrolysis of the alkaloid. [b] Isolated as the dimedone derivative of acetaldehyde after ozonolysis of the necic acid. [c] Incorporation experiments in *S. magnifus*. Alkaloid: senecionine (**2**); necic acid: senecic acid (**4**). [d] Incorporation experiments in *S. isatideus*. Alkaloid: retrorsine (**3**); necic acid: isatinecic acid (**5**). [e] Corrected for the obligatory loss of ^3H at C-3 in isoleucine (**1**) and of the carboxyl carbon in the L-(2*S*)-[U-^{14}C]isoleucine and to take into account the specific incorporation of only (2*S*)-isoleucine. [f] Corrected for the loss of the carboxy carbon in the (2*S*)-[U-^{14}C]isoleucine. [g] Actually fed as a mixture of (2*RS*)-isoleucine and (2*RS*)-alloisoleucine. Ratio corrected to take into account the specific incorporation of only (2*S*)-isoleucine into the necic acid (ref. 1b).

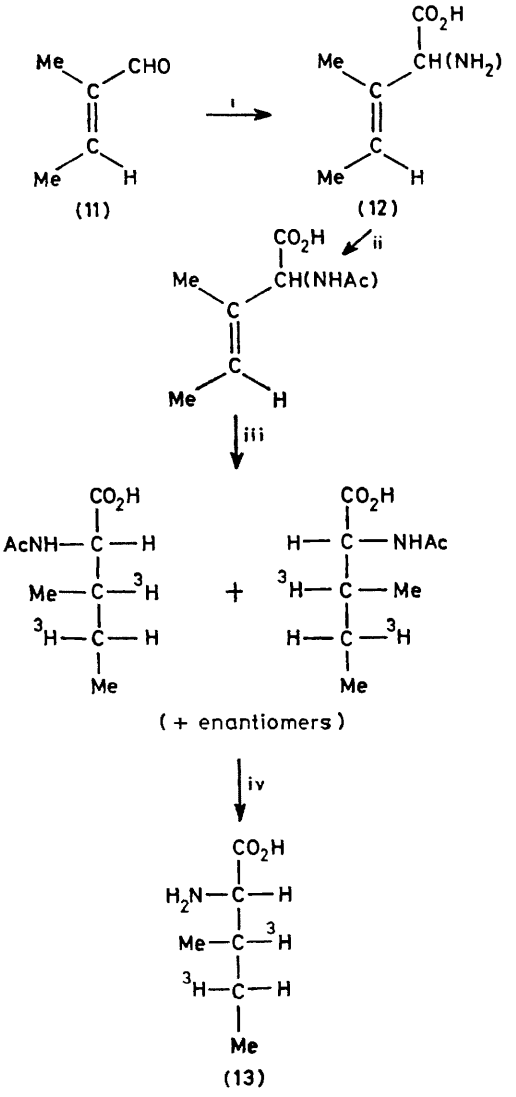
the Table. Also included in the Table are results previously obtained with (2*S*)-[4-$^3H_2$]isoleucine.[1b] From these results it can be seen that the C-4 *pro-S* hydrogen of isoleucine is lost and the C-4 *pro-R* hydrogen retained during incorporation into both halves of the necic acid. The results also demonstrate the specificity of incorporation of L-isoleucine into the necic acids and the equal distribution of activity between both halves of the necic acid, in agreement with previous results.[1] The loss of the C-3-*pro S* hydrogen of 2-aminobutanoic acid (**10**) and the C-4-*pro S* hydrogen of L-isoleucine [(**1**), Scheme (2)] and the retention of the corresponding *pro-R* hydrogen atoms, proves that the ethyl migration step (Scheme 2) during isoleucine biosynthesis takes place with retention of configuration, in agreement with predictions based on considerations of orbital symmetry.[5] A corresponding result has been obtained in the bacterial pathway of isoleucine biosynthesis.[3] The present results, taken together with earlier data,[1] also show that the hydrogen atom at C-13 in the alkaloids (**2**) and (**3**) is originally the C-4-*pro R* hydrogen atom of L-isoleucine (**1**) (Scheme 2). This result shows that the introduction of functionality into C-4 of L-isoleucine (**1**) preparatory to the generation of the C-13,14 bond in the alkaloids [(**2**) and (**3**)], must be limited to a maximum two-electron oxidation and rules out intermediates of a higher oxidation level such as ketonic precursors.[1b]

The high incorporation of 2-aminobutanoic acid (**10**) into retrorsine (**3**) in the study are noteworthy (Table). These incorporations are higher than any previously obtained with other precursors. It is also noteworthy that both stereoisomers of 2-aminobutanoic acid (**10**) were incorporated with nearly equal efficiencies (Table).

The assignment of the 4*S*-configuration to the isoleucine (**13**) produced by reduction of the *N*-acetyl derivative of the amino acid (**12**) with tritiated di-imide (Scheme 3) depends on the assignment of the *E*-configuration to this compound. Since it was not certain that the *E*-configuration was main-

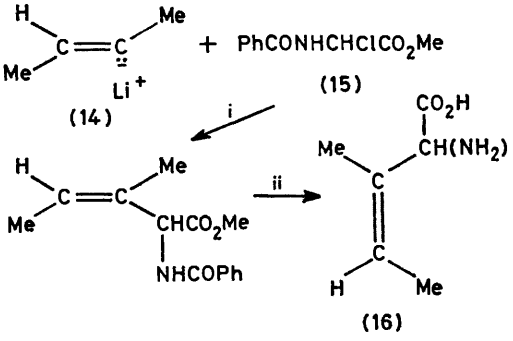


SCHEME 2



SCHEME 3. *Reagents*: i, NH_4Cl–NaCN, HCl. ii, Ac_2O–NaOH. iii, $^3HN{=}N^3H$. iv, acylase I.

tained during the Strecker reaction (Scheme 3) whereby tiglaldehyde (**11**) was elaborated to this acid (**12**), and since the configuration of the latter could not be confirmed reliably by spectroscopic means, the assignment was confirmed by a stereospecific synthesis of the corresponding *Z*-isomer (Scheme 4). For this a new method was developed in which the α-amino acid functionality was introduced *via* an electrophilic component [methyl *N*-chlorohippurate (**15**)[6]] rather than a nucleophilic component as in many amino acid syntheses. The required *Z*-configuration of the olefinic component was established *via* the configurationally stable vinyl lithium reagent (**14**) derived from (*Z*)-2-bromobut-2-ene. The product (**16**) was clearly distinguishable from the (*E*)-isomer (**12**) by n.m.r. and amino acid analysis.



SCHEME 4. *Reagents:* i, CuI. ii, H^+–H_2O.

We thank Dr M. Kisumi, Tanabe Seiyaku Co., Osaka, Japan for carrying out the conversion of D-[3-^{3}H]threonine into (2*S*,4*R*)-[4-^{3}H]isoleucine and the S.R.C. for financial support.

(*Received, 4th February* 1980; *Com.* 113.)

[1] (a) D. H. G. Crout, N. M. Davies, E. H. Smith, and D. Whitehouse, *J. Chem. Soc., Perkin Trans.* I, 1972, 671; (b) N. M.Bale, R. Cahill, N. M. Davies, M. B. Mitchell, E. H. Smith, and D. H. G. Crout, *ibid.*, 1978, 101.
[2] V. W. Rodwell, 'Metabolic Pathways,' Vol. III, ed. D. M. Greenberg, Academic Press, New York and London, 1969, pp. 351—361.
[3] D. H. G. Crout, M. V. M. Gregorio, U. S. Müller, S. Komatsubara, M. Kisumi, and I. Chibata, *Eur. J. Biochem.*, in the press.
[4] S. Komatsubara, M. Kisumi, I. Chibata, M. V. M. Gregorio, U. S. Müller, and D. H. G. Crout, *J. Chem. Soc., Chem. Commun.*, 1977, 839.
[5] R. W. Alder, *Tetrahedron Lett.*, 1971, 193.
[6] U. Zoller and D. Ben-Ishai, *Tetrahedron*, 1975, **31**, 863.