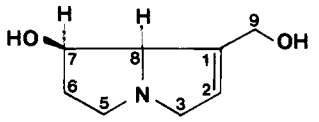
Biosynthesis of Retronecine

Gunnar Grue-Sørensen and Ian D. Spenser*

Department of Chemistry, McMaster University
Hamilton, Ontario, Canada L8S 4M1
Received January 2, 1981

Nonrandom incorporation of radioactivity from ornithine (**2**),[1-3] putrescine (**3**),[1,3] spermidine,[3] and spermine[3] into retronecine (**1**), the most common of the necine bases of the Senecio alkaloids,[4] provides experimental support for the postulate of Sir Robert Robinson[5] that the pyrrolizidine skeleton of these alkaloids is generated from two C_4 units related to ornithine.



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The dialdehyde amine (**6**), a structure with C_{2v} symmetry, is regarded as the key intermediate on route to the pyrrolizidine system. A plausible sequence[6] to **6**, from ornithine via putrescine, is shown in Scheme I (route A). Intramolecular Mannich reaction of **6** yields **7** and constitutes a biogenetically modeled synthesis of the pyrrolizidine system.[7,8]

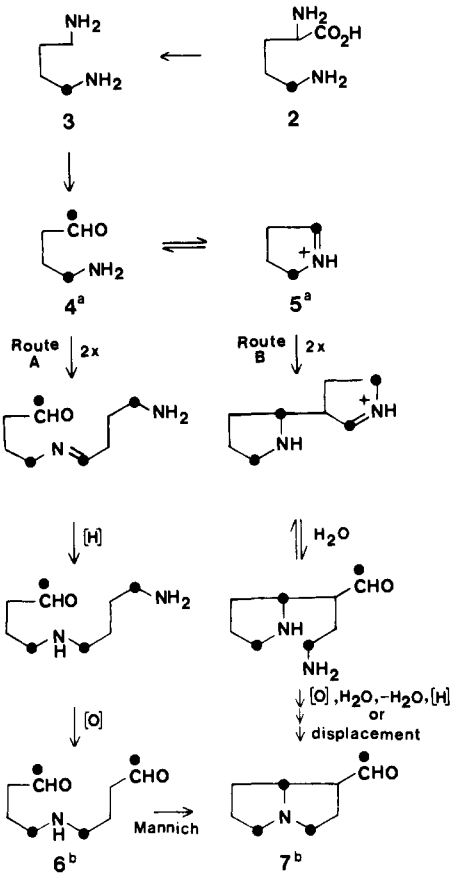
The observed distribution of label within retronecine (**1**) derived from DL-[5-^{14}C]ornithine, [1-^{14}C]putrescine, [*tetramethylene*-1-^{14}C]spermine, and [*tetramethylene*-1,4-$^{14}C_2$]spermidine [25% of the total activity of **1** at C-9, isolated as formaldehyde; 25% at C-5, isolated (together with C-6 and C-7) as β-alanine] has been regarded[3] as an indication of the intermediacy of a "symmetrical dimeric" intermediate such as **6**.

In fact, precisely the same distribution of label within **1** would result from these substrates by a route (Scheme I, route B) which starts with putrescine but excludes a "symmetrical dimeric" intermediate.

To show whether or not a "symmetrical dimeric" intermediate lies on the pathway, we have determined the mode of incorporation of intramolecularly doubly $^{13}C,^{15}N$-labeled putrescine ($NH_2CH_2CH_2CH_2{}^{13}CH_2{}^{15}NH_2$) into retronecine by ^{13}C NMR spectroscopy. Entry of the doubly labeled putrescine into retronecine by the "nonsymmetrical" route B should yield a single species of intramolecularly $^{13}C,^{15}N$-doubly labeled product into which the $^{13}C,^{15}N$ moiety has been transferred intact from putrescine to the C-5,N position of retronecine. Entry via the "symmetrical" route A should yield an equimolar mixture of two species of intramolecularly $^{13}C,^{15}N$-doubly labeled product, one showing enrichment at C-5,N, the other at C-3,N. The results provide support for the intermediacy of a "symmetrical dimeric" species in retronecine biosynthesis.

[1-^{13}C,1-^{15}N]-1,4-Diaminobutane (putrescine) dihydrochloride[9,10] (90 atom % ^{13}C, 99 atom % ^{15}N) (472 mg) in admixture with [1-^{14}C]putrescine[13] (68 μCi) was administered by the wick method to 133 plants of *Senecio vulgaris* (common

Scheme I. Two Routes from Putrescine (**3**) into the Pyrrolizidine System (**7**): Route A, via a Symmetrical (i.e., Nondissymmetric, C_{2v}) Intermediate (**6**) and Route B, via Dissymmetric (C_1) Intermediates



[a] Formulas **4** and **5** of labeled "monomeric" intermediates represent a 50/50 mixture of two singly labeled species. [b] Formulas of all labeled "dimeric" intermediates and **7** represent mixtures of four singly labeled and four doubly labeled species, whose relative contribution is determined by the relative quantities of labeled and unlabeled putrescine available to the plant during the period of biosynthesis.

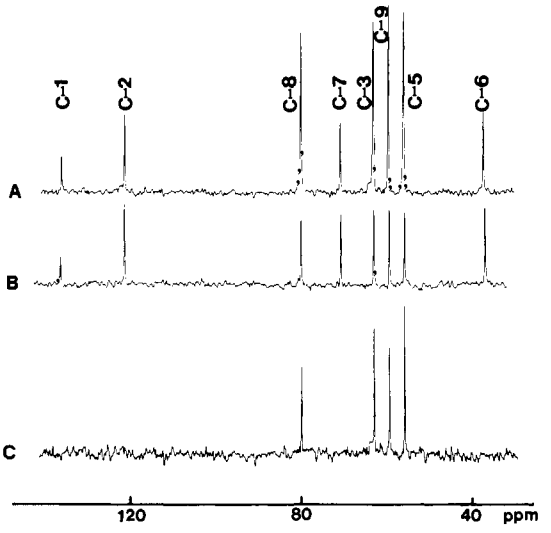


Figure 1. 20.15-MHz ^{13}C NMR spectra of retronecine hydrochloride (13 mg) in ^2H_2O (50 μL) (see Table I, footnote *a*). (A) Proton noise decoupled (PND) spectrum (85 819 scans) of $^{13}C,^{15}N$ enriched sample, derived from [1-^{13}C,1-^{15}N]putrescine. (B) PND spectrum (90 482 scans) of natural abundance sample. (C) Difference spectrum (A − B).

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(2) Hughes, C. A.; Letcher, R.; Warren, F. L. *J. Chem. Soc.* **1964**, 4974-4978.
(3) Robins, D. J.; Sweeney, J. R. *J. Chem. Soc., Chem. Commun.* **1979**, 120-121.
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(9) Synthesized in three steps from $K^{13}C^{15}N$ (0.5 g, 90.6 atom % ^{13}C, 99.4 atom % ^{15}N, Prochem) and 1-bromo-3-phthalimidopropane, followed by reduction and acid hydrolysis.
(10) [1-^{13}C,1-^{15}N]-1,4-Diaminobutane dihydrochloride (90 atom % ^{13}C, 99 atom % ^{15}N): ^1H NMR[11] (D_2O) δ 4.0-3.8 (0.9 H, m), 3.2-3.0 (2.2 H, m), 2.4-2.2 (0.9 H, m), 2.0-1.7 (4.0 H, m), $J_{C-1,H-1}$ = 143 Hz. ^{13}C NMR[12] δ 40.3 (d, $J_{C-1,N}$ = 4.4 Hz), 25.11 (d, $J_{C-2,C-1}$ = 37 Hz), 25.15 (s, C-3).
(11) Recorded at 90 MHz on a Varian EM 390 spectrometer.

0002-7863/81/1503-3208$01.25/0

Table I. Incorporation of [1-^{13}C,1-^{15}N]Putrescine into Retronecine. ^{13}C NMR Analysis[12]

C atom	retronecine *N*-oxide[a] chemical shift, ppm	retronecine hydrochloride[a] chemical shift, ppm	natural abundance ^{13}C normalized peak area[b] (A_{NA})	enriched in ^{13}C normalized peak area[b] (A_E)	$1.09A_E/A_{NA}$[c]	% enrichment above natural abundance[d,e]	relative % excess ^{13}C above natural abundance at individual C atoms
1	137.3	138.0	41	42	1.12	0.13 ± 0.14	2 ± 2
2	121.5	122.6	117	95	0.89	−0.12 ± 0.11	−2 ± 2
3	79.4	62.7	107	202	2.06	1.17 ± 0.26	20 ± 4
5	69.6	55.3	103	223	2.36	1.50 ± 0.29	26 ± 5
6	35.1	36.4	109	92	0.92	−0.09 ± 0.11	−2 ± 2
7	70.9	70.6	100	100	1.09	0.10 ± 0.14	2 ± 2
8	96.4	80.1	91	205	2.46	1.61 ± 0.31	27 ± 5
9	59.5	59.0	107	240	2.44	1.58 ± 0.30	27 ± 5

[a] Samples were contained in 2-mm tubes. Acquisition time, 0.68 s; 1.5 Hz/data point; 0.6-μs pulses. [b] Peak areas are normalized relative to C-7 (=100). Estimated standard deviation, ±8%. [c] The ratio A_E/A_{NA} is normalized so that the average value for carbon atoms 1, 2, 6, and 7 equals 1.0. [d] $[1.09\,(A_E/A_{NA})-1] \times 1.1\%$. [e] The average specific incorporation per carbon atom is $[^1/_4(1.17 + 1.50 + 1.61 + 1.58)/{}^{90}/_2] \times 100\% = 3.3\%$, where $^{90}/_2$ atom % ^{13}C is the average enrichment at each terminal position of putrescine.

Table II. Incorporation of [1-^{13}C,1-^{15}N]Putrescine into Retronecine. ^{13}C NMR Analysis[12]

C atom	type of signal[b]	retronecine hydrochloride[a] natural abundance ^{13}C normalized peak area[c] (A_{NA})	retronecine hydrochloride[a] enriched in ^{13}C normalized peak area[c] (A_E)	A_E/A_{NA}	% enrichment above natural abundance[d]	rel % excess ^{13}C above natural abundance at individual C atoms
3	s	106	149	1.41	0.45 ± 0.20 } 1.64 ± 0.25	27 ± 4
	d[e]		115	1.08	1.19 ± 0.15	
5	s	106	154	1.45	0.50 ± 0.20 } 1.72 ± 0.26	28 ± 4
	d[f]		118	1.11	1.22 ± 0.16	
7	s	100	100	1.00	0 ± 0.12	0 ± 2
8	s, m	101 (s)	219 (m)	2.17	1.29 ± 0.27	21 ± 4
9	s	108	209	1.94	1.03 ± 0.27 } 1.43 ± 0.27	24 ± 4
	d[g]		39	0.36	0.40 ± 0.05	

[a] Samples were contained in 2-mm tubes. Acquisition time, 6.8 s; 0.15 Hz/data point; 2.5-μs pulses. [b] s = singlet, d = doublet, m = multiplet. [c] Peak areas are normalized relative to C-7 (= 100). Estimated standard deviation, ±8% for singlet signals, ±10% for composite signals. [d] $[(A_E/A_{NA}) - 1)] \times 1.1\%$ for singlet signals, $(A_E/A_{NA}) \times 1.1\%$ for doublet signals. [e] $J_{^{13}C\text{-}3,\,^{15}N} = 5.1$ Hz; isotope shift −0.02 ppm. [f] $J_{^{13}C\text{-}5,\,^{15}N} = 4.4$ Hz; isotope shift −0.02 ppm. [g] $J_{^{13}C\text{-}8,\,^{13}C\text{-}9} = 5.8$ Hz.

groundsel) over a period of 13 days (July 1980). The crude alkaloids[14] were isolated[16] and purified by column chromatography (silica gel, 70–230 mesh, chloroform–methanol–concentrated ammonia 90:9:1). Hydrolysis (excess barium "symmetrical" 100 °C, 10 min) of the alkaloid mixture, followed by acidification with sulfuric acid, anion exchange chromatography (Dowex 2-X8, OH^-, 5 mL), and acidification with hydrochloric acid, yielded a mixture (17 mg) of retronecine hydrochloride[17] and retronecine *N*-oxide[18,19] [1:5, as determined by ^{13}C NMR (Table I)]. The mixture was dissolved in dilute hydrochloric acid, and zinc dust was added. Workup under exclusion of oxygen yielded retronecine hydrochloride (13 mg) which was chemically (^{13}C NMR) and radiochemically pure [3.4 (± 0.1) × 10^6 dpm/mmol; specific incorporation per C_4 unit, 3.3%).

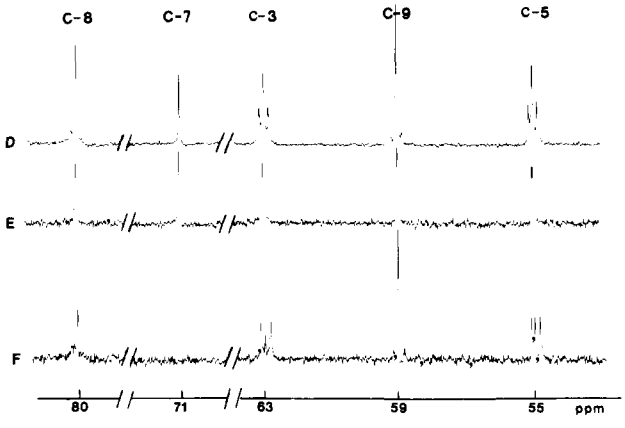

Figure 2. 20.15-MHz ^{13}C NMR spectra of retronecine hydrochloride (13 mg) in ^2H_2O (50 μL) (see Table II, footnote *a*). (D) PND spectrum (9720 scans) of ^{13}C,^{15}N enriched sample, derived from [1-^{13}C,1-^{15}N]-putrescine. (E) PND spectrum (9111 scans) of natural abundance sample. (F) Difference spectrum (D − E).

The distribution of label within the labeled retronecine is revealed by ^{13}C NMR spectroscopy (Tables I and II; Figures 1 and 2). As expected, four positions within retronecine, C-3, -5, -8, and -9, are equally enriched in ^{13}C, within experimental error.[21]

(12) ^{13}C NMR spectra were recorded at 20.15 MHz in the Fourier mode on a Bruker WP 80 spectrometer with proton noise decoupling. The spectra were determined in D_2O with tetramethylsilane as external reference.

(13) Nominal specific activity 90 mCi/mmol, New England Nuclear.

(14) The three major alkaloids of *Senecio vulgaris*[15] are senecionine, seneciphylline, and retrorsine. Each contains retronecine as the necine base.

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(19) The assignment of the signals in the ^{13}C NMR spectrum of retronecine *N*-oxide[20] (Table I) is unambiguous. Those due to carbon atoms adjacent to nitrogen (C-3, -5, -8) appear downfield (16.7, 14.3, and 16.3 ppm, respectively) relative to those due to the corresponding carbon atoms of retronecine hydrochloride. The signals due to the remaining five carbon atoms have chemical shifts within 1.3 ppm of those of the corresponding carbons of retronecine hydrochloride. Furthermore, in the spectrum of the enriched retronecine *N*-oxide, the ^{13}C–^{15}N couplings observed for C-3 and C-5 ($J_{C\text{-}3,N}$ = 4.1 Hz, $J_{C\text{-}5,N}$ = 3.8 Hz) (see Table II, note *a*) confirm the validity of the assignment.

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(21) The calculation of the relative percent excess ^{13}C above natural abundance at C-3, -5, -8, and -9 is based either on the average of the peak areas due to the nonenriched carbon atoms (C-1, -2, -6, -7) (Table I) or on the peak area due to C-7 alone (Table II). The calculated values given in Tables I and II are therefore not identical, reflecting the confidence limits in the integration of peak areas.

The specific incorporation per C_4 unit, 3.3%, calculated from ^{13}C NMR data (Table I), is identical with that obtained from ^{14}C radioactivity measurements.

The signals due to the ^{13}C-enriched carbon atoms in the proton decoupled ^{13}C NMR spectrum of labeled retronecine appear as multiplets (Table II, Figure 2D), due to superposition of a doublet [^{13}C–^{15}N (C-3,N; C-5,N) or C^{13}–C^{13} (C-9, C-8) coupling] on a singlet. This multiplicity represents the various enriched species present in the labeled retronecine. The contribution of the various species can be calculated from the difference spectrum (Figure 2F).

Thus, the signal due to C-3 (62.7 ppm) consists of a doublet (73 ± 9% of the total area in the difference spectrum) due to the contribution of a species containing the intact ^{13}C–^{15}N bond transferred from the starting material superimposed on a singlet (27 ± 12%) representing a species containing ^{13}C adjacent to ^{14}N. Similarly, the signal due to C-5 (55.3 ppm) consists of 71 ± 9% doublet and 29 ± 12% singlet. It is evident that the ^{13}C–^{15}N bond of putrescine is conserved to an equal extent at C-3,N and C-5,N of retronecine. A "symmetrical dimeric" intermediate, such as **6**, on the route from putrescine into retronecine (route A, Scheme I) is thus strongly indicated. A "nonsymmetrical" route to the product (e.g., route B) would have resulted in a distribution of label, yielding a difference spectrum in which the signal due to C-5 would be a doublet, since all species labeled with ^{13}C at this carbon are also labeled with ^{15}N, whereas the signal due to C-3 would be a multiplet due to the superposition of a $^{13}C,^{15}N$ doublet on a $^{13}C,^{14}N$ singlet. The doublet/singlet ratio would be 1 or less, depending on the extent of dilution of the intramolecularly doubly labeled putrescine used as a precursor by endogenous, natural abundance material.

The signal due to C-9 (Table II, Figure 2F) appears as a doublet (28 ± 4% of signal area in the difference spectrum) superimposed on a singlet (72 ± 19%). The doublet is due to ^{13}C–^{13}C coupling between C-8 and C-9. The area of the doublet, relative to that of the singlet it straddles, is a measure of the contribution to the retronecine of the species which carries ^{13}C in both halves of the molecule.[22] If the administered putrescine (90 atom % ^{13}C at C-1) entered the product without dilution by endogenous material, the ratio of the areas of doublet and singlet of the signal due to C-9 in the difference spectrum of the product would be 45:55. The observed result corresponds to that expected if the enriched precursor had been diluted with ca. 60% of its own weight of endogenous material.

The coupling between C-8 and C-9 gives rise to a corresponding signal at C-8. This is poorly resolved, presumably due to superimposed low intensity coupling to C-3 and ^{15}N.[23]

The ^{13}C NMR spectrum of retronecine, obtained from intramolecularly $^{13}C,^{15}N$-doubly labeled putrescine, thus shows signals due to C-3 and C-5 which, within experimental error, are of equal intensity and multiplicity. This observation eliminates from further consideration a pathway such as route B. It suggests that a "symmetrical dimeric" intermediate, i.e., one with C_{2v} symmetry, such as **6**, lies on the pathway.[24]

Acknowledgment. This work was supported by a grant from the Natural Sciences and Engineering Research Council of Canada. We are indebted to Thelma Leech, M.Sc., Greenhouse Supervisor, McMaster University, for her cooperation in propagating the plant material for our experiments and Brian G. Sayer, Department of Chemistry, for recording the ^{13}C NMR spectra.

(22) The average enrichment in ^{13}C at carbon atoms C-9 and C-8 as well as at C-3 and C-5 of the retronecine hydrochloride actually biosynthesized during the 13 days of the feeding experiment is thus 28 atom %. The sample of retronecine hydrochloride which was isolated constitutes a mixture of this enriched material and natural abundance material present in the plants at the start of the feeding experiment. The average enrichment at each of C-3, -5, -8, and -9 of the isolated sample can be calculated from data given in Table I: [$^1/_4$(1.17 + 1.50 + 1.61 + 1.58) + 1.1] = 2.57 atom % ^{13}C. Let the isolated sample consist of x% enriched material (28 atom % ^{13}C, on average, at each of C-3, -5, -8, -9) and (100 − x)% natural abundance material (1.1 atom % ^{13}C at each carbon atom). It follows that 2.57 = 0.28x + 0.011 (100 − x) and x = 5.5, i.e., the isolated sample contained 5.5% of enriched material, with 28 atom % ^{13}C, on average, at C-3, -5, -8, and -9. The extent of dilution of the enriched putrescine (90 atom % ^{13}C at C-1) by endogenous putrescine before incorporation into retronecine can be calculated from the equation (45 + 0.011y)/(100 + y) = 0.28, where 45 is the average enrichment (atom % ^{13}C) at a terminal carbon atom of the administered putrescine, 0.011 is the mol fraction of ^{13}C in endogenous putrescine, and y is percent endogenous putrescine added to the administered enriched sample. The dilution, y, is 63%.

(23) The mode of incorporation of the doubly labeled putrescine dictates that whereas molecules intramolecularly doubly ^{13}C labeled at C-8 and C-3 make a contribution to the product, there is no species which is similarly labeled at C-9 and C-3. Therefore long-range coupling between these two carbons cannot occur.

(24) Two other possibilities cannot be eliminated on the basis of the results. One is the intermediacy of a structure without C_{2v} symmetry but capable of undergoing a degenerate sigmatropic rearrangement (e.g., $NH_2CH_2CH_2CH_2CH{=}NCH_2CH_2CH_2CH_2NH_2$). Another is the occurrence of two different pathways, each involving "nonsymmetrical dimeric" intermediates, which coincidentally lead to identical enrichment of ^{13}C–^{15}N at C-3 and C-5.

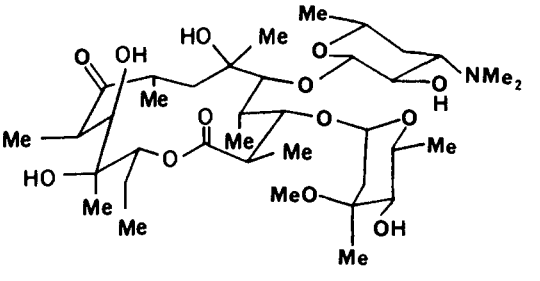
Asymmetric Total Synthesis of Erythromycin. 1. Synthesis of an Erythronolide A Seco Acid Derivative via Asymmetric Induction

R. B. Woodward,† E. Logusch,‡ K. P. Nambiar,‡ K. Sakan,§,‡ D. E. Ward,‡ B.-W. Au-Yeung, P. Balaram, L. J. Browne, P. J. Card, C. H. Chen, R. B. Chênevert, A. Fliri, K. Frobel, H.-J. Gais, D. G. Garratt, K. Hayakawa, W. Heggie, D. P. Hesson, D. Hoppe, I. Hoppe, J. A. Hyatt, D. Ikeda, P. A. Jacobi, K. S. Kim, Y. Kobuke, K. Kojima, K. Krowicki, V. J. Lee, T. Leutert, S. Malchenko, J. Martens, R. S. Matthews, B. S. Ong, J. B. Press, T. V. Rajan Babu, G. Rousseau, H. M. Sauter, M. Suzuki, K. Tatsuta, L. M. Tolbert, E. A. Truesdale, I. Uchida, Y. Ueda, T. Uyehara, A. T. Vasella, W. C. Vladuchick, P. A. Wade, R. M. Williams, and H. N.-C. Wong

Department of Chemistry, Harvard University Cambridge, Massachusetts 02138

Received February 23, 1981

Erythromycin[1] (**1**), produced by a strain of *Streptomyces erythreus*, is the best known of the medicinally important macrolide antibiotics.[2] Structurally, this macrolide contains a 14-membered



1: erythromycin

†Deceased July 8, 1979.

‡This manuscript was prepared by E.L., K.P.N., K.S., and D.E.W.

§Address correspondence to this author at the Department of Chemistry, Carnegie-Melon University, Pittsburgh, PA 15213.

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