for this diacid are given as 161–163, 163, and 163–164 °C (ref 10a, b, and c, respectively).
(10) (a) W. E. Bachmann and S. Kushner, *J. Am. Chem. Soc.*, **65**, 1963 (1943); (b) G. A. R. Kon, R. P. Linstead, and C. Simons, *J. Chem. Soc.*, 814 (1937); (c) R. P. Linstead and A. F. Millidge, *ibid.*, 478 (1936).
(11) The employment of a dimethyl acetal was dictated by the recent findings of Deslongchamps wherein other acetals, especially cyclic ones, are attacked by ozone: P. Deslongchamps, P. Atlani, D. Frehel, A. Malaval, and C. Moreau, *Can. J. Chem.*, **52**, 3651 (1974).
(12) Infrared spectra were obtained on a Beckman IR-8 spectrometer. ^{1}H NMR spectra were secured from a Varian A-56/60 spectrometer using trimethylsilane as internal standard. Mass spectra were recorded on a Consolidated Electrodynamics Corp. 21-110 high-resolution instrument. Melting points and boiling points are uncorrected.

Biosynthesis of Shihunine in *Dendrobium pierardii*[1]

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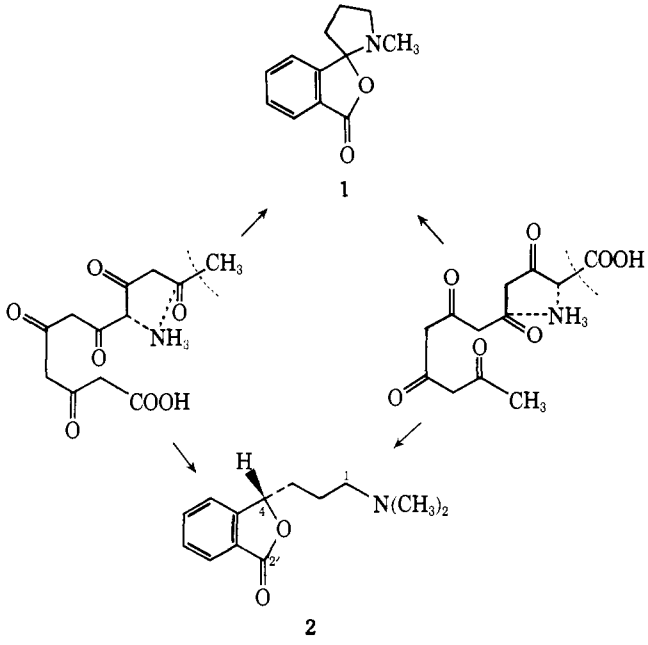
Abstract: The administration of [1-^{14}C]acetate to *Dendrobium pierardii* afforded radioactive shihunine. A systematic degradation of this alkaloid established that it was preferentially labeled at C-5, indicating that shihunine is not a polyketide. [*4,2′-carbonyl*-$^{14}C_2$]-*o*-Succinylbenzoic acid was found to be an excellent precursor of shihunine (14.4% absolute incorporation), and degradations indicated that all the activity was equally divided between C-2 and C-12. Conformation that *o*-succinylbenzoic acid is a direct precursor of shihunine was obtained by feeding this precursor labeled with both ^{13}C and ^{14}C at C-1. By the use of ^{13}C NMR it was established that the resultant shihunine was enriched only at C-5 (4.4% specific incorporation).

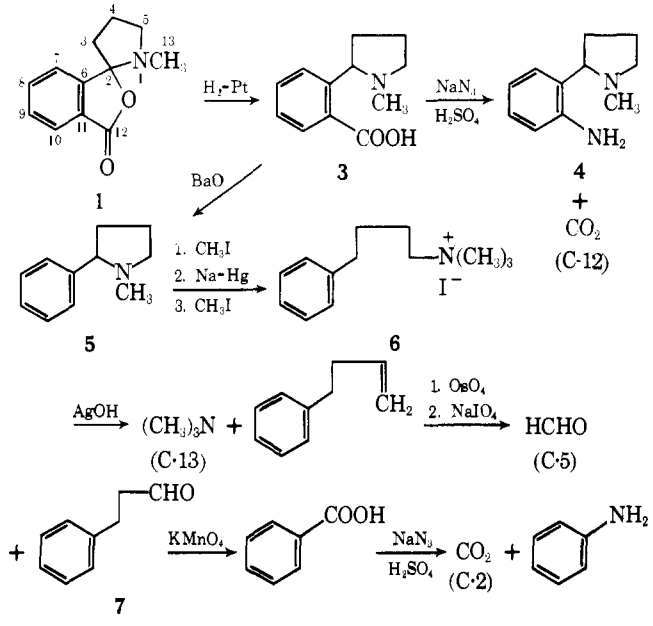
Shihunine (**1**) is a phthalidopyrrolidine, no other representatives of this class of alkaloid being known. It was first isolated from the orchid *Dendrobium lohohense*[2] by Inubushi and co-workers, who deduced its structure,[3] which has been confirmed by synthesis.[4,5] Later it was isolated from *Dendrobium pierardii*[6] which also yielded the related alkaloid pierardine (**2**).[7]

We considered that these alkaloids would have a common biosynthetic origin and initially regarded them as polyketides, even though a completely satisfying biosynthetic scheme could not be constructed (Scheme I). Nevertheless, we fed [1-^{14}C]acetate to *D. pierardii* in preliminary experiments, and labeled shihunine was isolated from the plants. A higher incorporation of activity into shihunine was obtained when the plant was fed by painting the leaves with a solution of the tracer, compared with the wick feeding method (Table I). The

Scheme I. Hypothetical Formation of Shihunine and Pierardine from a Polyketide



Scheme II. Degradation of Shihunine



shihunine was degraded as illustrated in Scheme II. Hydrogenation in the presence of Adams catalyst afforded dihydroshihunine (**3**).[3] A Schmidt reaction on this amino acid yielded 2-(2′-aminophenyl)-1-methylpyrrolidine (**4**) and carbon dioxide. On heating dihydroshihunine with barium oxide, 1-methyl-2-phenylpyrrolidine (**5**) was obtained. An Emde reduction on the methiodide of **5** afforded 1-dimethylamino-4-phenylbutane, which was converted to its methiodide **6**. A Hofmann elimination of **6** afforded trimethylamine, collected and assayed as tetramethylammonium iodide. The 4-phenyl-1-butene also obtained in this reaction was oxidized with osmium tetroxide and sodium metaperiodate yielding formaldehyde, collected as its dimedone derivative, and 3-phenylpropanal (**7**), collected as its semicarbazone. Oxidation of this semicarbazone or 1-dimethylamino-4-phenylbutane with permanganate yielded benzoic acid which was subjected to a Schmidt reaction yielding aniline (assayed as benzanilide) and carbon dioxide. The results of this degradation on the

Table I. Precursors Fed and Alkaloids Isolated from *Dendrobium pierardii* (All Specific Activities in dpm/mmol)

Precursors fed	Sodium [1-^{14}C]acetate	Sodium [1-^{14}C]acetate	[*4,2′-carbonyl*-$^{14}C_2$]-**10**	[1-^{14}C,^{13}C]-**10**
Expt no.	1	2	3	4
Method of feeding	Wick feeding	Painting on leaves	Wick feeding	Wick feeding
Duration and time of feeding	9 days (Dec)	9 days (Dec)	12 days (Feb)	9 days (June)
Amt fed, mmol	0.081	0.066	0.077	0.416
Spec act.	5.42×10^9	1.20×10^{10}	2.50×10^9	3.53×10^8 (^{14}C)
Fresh wt of plants, g	580	580	500	250
Shihunine				
Wt, mg	518	719	510	203
Spec act.	5.23×10^4	6.77×10^5	1.10×10^7	1.51×10^7
% spec incorpn[a]	0.00096	0.0056	0.44	4.3 (^{14}C)
% abs incorpn[b]	0.030	0.30	14.4	10.3
Pierardine hydrochloride				
Wt, mg	69	137	122	30
Spec act.	5.4×10^3	2.5×10^3	1.48×10^4	7.2×10^4
% spec incorpn	1×10^{-4}	2×10^{-5}	5.9×10^{-4}	0.020 (^{14}C)

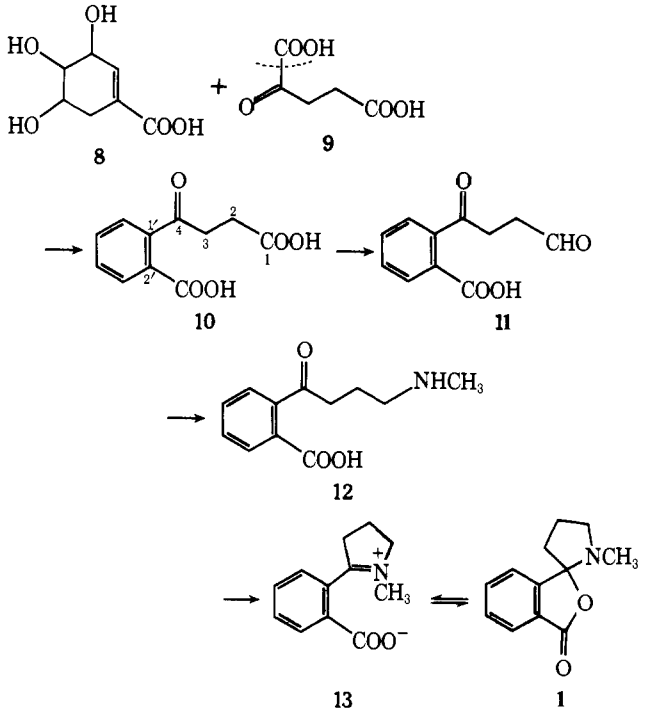
[a] Specific incorporation is the specific activity of the isolated alkaloid divided by the specific activity of the administered precursor. [b] Absolute incorporation is the total activity in the isolated alkaloid divided by the total activity in the administered precursor.

shihunine derived from the two feeding experiments with [1-^{14}C]acetate are recorded in Table II. It is immediately apparent that the shihunine was not labeled equally on alternate carbons. In fact, the bulk of the activity was located in the pyrrolidine ring at C-5.

The pierardine obtained from the [1-^{14}C]acetate feeding was radioactive; however, the level of activity was not high enough to carry out significant degradations.

A second hypothetical biosynthetic scheme is illustrated in Scheme III. It is proposed that shihunine is derived from 4-

Scheme III. Hypothetical Formation of Shihunine from *o*-Succinylbenzoic Acid



(2′-carboxyphenyl)-4-oxobutanoic acid (*o*-succinylbenzoic acid, **10**). This compound which is formed from shikimic acid (**8**) and α-ketoglutaric acid (**9**) is an intermediate in the biosynthesis of several 1,4-naphthaquinones,[8] including lawsone,[9-11] juglone,[9,12] and the K vitamins[9,13-15] now known as phylloquinones (from plants) and menaquinones (from bacteria). It is also a precursor of the anthraquinones alizarin and morindone.[16] More recently it has been shown that the phthalide, catalpalactone,[17] and the tetralone, catalponol,[18] are derived from **10**. In Scheme III it is suggested that **10** is reduced to the aldehyde **11** which on transamination and N-methylation yields the ketoamine **12.** Cyclization results in the formation of the betaine **13,** which is the predominant state of shihunine in polar solvents.[6] This scheme would be consistent with the results of the acetate feeding experiments since [1-^{14}C]acetate would yield α-ketoglutaric acid labeled on its carboxyl groups by operation of the Krebs cycle. The *o*-succinylbenzoic acid would then be labeled at C-1 and would ultimately afford shihunine labeled at C-5.

This hypothesis was tested by the feeding of labeled **10** to *D. pierardii* (Table I, experiment 3). [*4,2′-carbonyl*-$^{14}C_2$]-*o*-Succinylbenzoic acid having equal activity on the two labeled positions was prepared by heating [*carbonyl*-$^{14}C_2$]phthalic anhydride with succinic anhydride in the presence of potassium carbonate.[19] The incorporation of activity into shihunine was exceptionally high (14.4%). The activity of degradation products (Table II) indicated that the alkaloid was labeled equally at C-2 and C-12, consistent with the direct incorporation of **10.** The much lower activity of the pierardine may indicate that this alkaloid was not being actively produced at the time of feeding. This labaled pierardine was oxidized with permanganate, yielding phthalic acid having essentially the same specific activity. A Schmidt reaction on the phthalic acid[20] yielded anthranilic acid and carbon dioxide having activities consistent with all the activity of the pierardine being located at C-4 and C-2′.

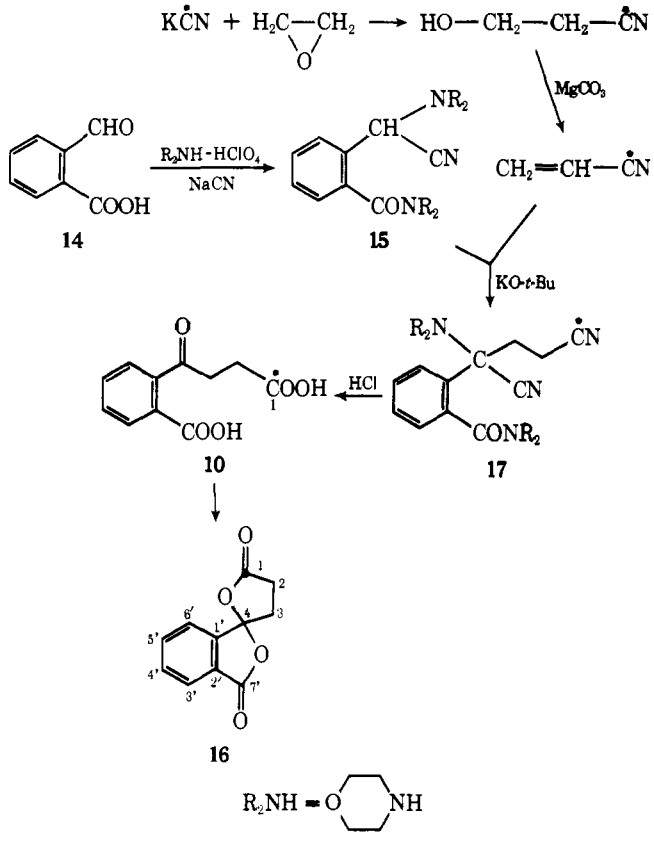
In view of the high incorporation of **10** into shihunine, we considered that the biosynthesis of this alkaloid could be investigated using ^{13}C NMR. This technique has been used extensively in examining the biosynthesis of many complex microbial metabolites.[21] However, the relatively low incorporations usually obtained in higher plants have precluded its general use.[22] *o*-Succinylbenzoic acid labeled with both ^{14}C and ^{13}C at C-1 was prepared by the route illustrated in Scheme IV. Carbon-14 was introduced into the precursor in order to obtain the specific incorporation by radioactivity measurement. Reaction of ethylene oxide with a mixture of potassium [^{14}C]cyanide and [^{13}C]cyanide (90% excess) yielded β-hydroxypropionitrile which on dehydration with magnesium carbonate afforded [1-^{14}C,^{13}C]acrylonitrile.[23] 2-Carboxybenzaldehyde (**14**) on reaction with morpholine perchlorate and sodium cyanide yielded α-morpholino-α-(2′-carboxymorpholinophenyl)acetonitrile (**15**). A 1,4 addition of the carbanion[24] of **15** to the labeled acrylonitrile yielded **17,** which on hydrolysis with hydrochloric acid afforded **10.** The dilactone **16** is formed on heating **10,** and an analysis (see Experimental Section) of its proton noise decoupled Fourier transform ^{13}C NMR spectrum[25] indicated that it contained 86% excess ^{13}C

Table II. Activities of Degradation Products of Shihunine Derived from [1-^{14}C]Acetate and [*4,2'-carbonyl*-$^{14}C_2$]-**10**

	[1-^{14}C]Acetate, expt 1		[1-^{14}C]Acetate, expt 2		[*4,2'-carbonyl*-$^{14}C_2$]-**10**, expt 3	
	Spec act., dpm/mmol × 10^{-4}	Rel spec act.	Spec act., dpm/mmol × 10^{-5}	Rel spec act.	Spec act., dpm/mmol × 10^{-7}	Rel spec act.
Shihunine (**1**)	5.23	107[a]	6.77	100	1.10	100
Dihydroshihunine (**3**)	4.89	100	6.68	99	1.07	97
2-(2'-Aminophenyl)-1-methylpyrrolidine dihydrochloride (**4**)			6.25	92		
1-Methyl-2-phenylpyrrolidine picrate (**5**)	4.47	91	6.32	93	0.53	48
1-Methyl-2-phenylpyrrolidine methiodide			6.38	94	0.54	49
(C-12) by difference: **3** − **5**	0.42	9	0.36	5	0.54	49
Barium carbonate (C-12)			0.12	2		
1-Dimethylamino-4-phenylbutane methiodide (**6**)	4.46	91	6.39	94		
Tetramethylammonium iodide (C-13)	0.11	0.058	0.058	1		
3-Phenylpropanal semicarbazone (**7**)	1.68	34	0.79	12		
Formaldehyde dimedone (C-5)	2.44	50	5.30	78		
Benzoic acid	1.61	33	0.69	10	0.52	47
Benzanilide	1.27	26	0.59	9	0.00	0
Barium carbonate (C-2)			0.052	1	0.52	47

[a] It is assumed that the shihunine was not completely radiochemically pure.

Scheme IV. Synthesis of [1-^{14}C,^{13}C]-*o*-Succinylbenzoic Acid



at C-1. Chemical shifts were assigned by comparison with the spectra of butyrolactone,[26] methyl benzoate,[26] and shihunine (see later). The specific incorporation (^{14}C) of the shihunine isolated from the plant which had been fed the [1-^{14}C,^{13}C]-*o*-succinylbenzoic acid (experiment 4) was 4.3%. The ^{13}C NMR spectra of the enriched and natural shihunine are recorded in Table III. The chemical shifts were assigned by the following methods. A continuous wave off-resonance decoupling of the protons resulted in the methyl carbon (C-13) appearing as a quartet, and the methylene carbons at C-3, C-4, and C-5 appearing as triplets. Comparison with the chemical shifts of model compounds—nicotine[27] and *N*-methylpyrrolidine[28]—enabled us to assign chemical shifts to the pyrrolidine ring carbons. The use of low-power off-resonance decoupling[29] resulted in the quaternary carbons (C-2, C-6, and C-11) remaining as the only significant singlets in the spectrum. Although not important for the present study the chemical shifts of the remaining aromatic carbons were assigned by comparison with the spectra of methyl benzoate and 2-phenyl-2-propanol.[26] In Table III the peak heights of the enriched and unenriched shihunine are normalized so that the sums of all the peak intensities except the one assigned to C-5 are made equal. The enhancement of the C-5 peak corresponds to a 3.8% enrichment of this position and a specific incorporation of **10** (86% enriched) of 4.4%, in excellent agreement with the specific incorporation observed for the ^{14}C. The incorporation into pierardine was again low (0.020% specific incorporation of ^{14}C) and attempts to detect enrichment at C-1 of the alkaloid by ^{13}C NMR were unsuccessful.

Table III. ^{13}C NMR Spectra of Shihunine[a]

Carbon atom	Chemical shift, ppm from Me_4Si	Normalized peak heights		Difference
		Unenriched shihunine	Enriched shihunine	
2	111.3	1.3	1.5	+0.2
3	39.9	12.3	10.9	−1.4
4	21.0	11.2	11.4	+0.2
5	53.9	14.0	62.4	+48.4
6	147.3	1.0	1.3	+0.3
7	123.1	5.4	5.6	+0.2
8	134.3	5.4	5.2	−0.2
9	125.2	5.0	5.1	+0.1
10	130.3	5.7	5.7	0
11	129.4	1.1	1.3	+0.2
12[b]	168.8			
13	32.2	7.2	7.5	+0.3

[a] The spectra were determined on a Varian XL-100 Fourier transform spectrometer operating at 25.2 MHz. The samples (100 mg of the unenriched, 200 mg of the enriched) were dissolved in CD_2Cl_2 (0.5 ml), deuterium in the solvent providing the lock signal. Tetramethylsilane was used as an internal standard. The radiofrequency pulse width was 55–60 μs. The enriched shihunine was run for 500 transients (0.8 s/transient). [b] The peak due to this C=O was not prominent.

Experimental Section[30]

[*4,2'-carbonyl*-$^{14}C_2$]-*o*-Succinylbenzoic Acid Dilactone (16). [*carbonyl*-$^{14}C_2$]Phthalic anhydride (154 mg, nominal activity 1 mCi, Amersham-Searle), succinic anhydride (150 mg), and anhydrous K_2CO_3 (70 mg) were stirred under N_2 for 20 min at 175–180 °C. The cooled reaction mixture was dissolved in a little ethanol, then poured into water (50 ml), and boiled for a few minutes. The mixture was filtered through glass wool and acidified with HCl. An ether extract (4 × 25 ml) yielded, after washing with brine, drying ($MgSO_4$), and evaporation, a colorless oil. Preparative TLC on silica gel PF-254 (Merck), developing with $CHCl_3$–ethyl acetate–methanol (50:5:1), yielded the dilactone (R_f 0.35) which was sublimed (10^{-4} mm) and crystallized from aqueous methanol to afford colorless needles (16.3 mg, 7.7%), mp 120–122 °C (lit.[19] mp 120 °C), having a specific activity of 2.50×10^9 dpm/mmol. Prior to feeding, this dilactone was dissolved in water and heated on a steam bath with 2 equiv of Na_2CO_3 to afford the disodium salt of *o*-succinylbenzoic acid.

[1-^{14}C,^{13}C]Acrylonitrile. Ethylene oxide (1.9 ml) cooled to 0 °C was added to a solution of potassium [^{13}C]cyanide (90% ^{13}C, Prochem Ltd.) (0.5 g), potassium [^{14}C]cyanide (nominal activity 1 mCi) (1 mg), and $MgSO_4 \cdot 7H_2O$ (1 g) in water (2 ml) and stirred for 2 h at 0 °C. After stirring for an additional 18 h at room temperature the solution was saturated with CO_2 and evaporated in vacuo, and the residue was extracted with ethyl acetate (5 × 10 ml). Evaporation of the dried ($CaCl_2$) extract afforded β-hydroxypropionitrile (459 mg) as a colorless oil. This oil was heated with $MgCO_3$ (62 mg) in triethylene glycol (82 mg) at 190 °C for 3 h and then at 225 °C for 1 h. The resultant acrylonitrile was removed from the reaction mixture by means of a slow stream of N_2, passing through a condenser maintained at 100 °C and then into a U tube cooled in dry ice. The U tube was washed out with benzene (10 ml), which was dried ($CaCl_2$) and evaporated to yield crude [1-^{14}C,^{13}C]acrylonitrile which was used in a subsequent step without further purification.

α-Morpholino-α-(2'-carboxymorpholinophenyl)acetonitrile (15). 2-Carboxybenzaldehyde (12.1 g) and morpholine perchlorate (17.3 g) were heated with stirring in morpholine (80 ml) at 70 °C for 1.5 h. The temperature was then raised to 100 °C and sodium cyanide (4.62 g) dissolved in a small amount of water was added. After stirring for an additional 1.5 h the mixture was poured onto ice (500 g) yielding a white precipitate which was crystallized from ethanol affording colorless prisms of **15** (21.4 g, 84%), mp 190–193 °C, having the following spectroscopic data: ir ($CHCl_3$) 2240 (CN), 1640 cm^{-1} (CO); NMR ($CDCl_3$) δ 7.83–7.18 (m, 4, phenyl H), 5.61 (s, CH), 4.08–3.31 (m, 12 H), 2.63–2.47 (m, 4 H); mass spectrum (70 eV) *m/e* 315 (4), 314 (0.3), 288 (3), 228 (19), 170 (21), 143 (19), 115 (15), 86 (100); high-resolution mass spectrum *m/e* 315.1575 (calculated for $C_{17}H_{21}N_3O_3$, 315.1582). Anal. ($C_{17}H_{21}N_3O_3$) C, H, N.

γ-Cyano-γ-morpholino-γ-(2'-carboxymorpholinophenyl)-[1-^{14}C,^{13}C]butyronitrile (17). A solution of compound **15** (1 g) in hot *tert*-butyl alcohol (35 ml) was cooled to 35 °C with rapid stirring. The previously prepared [1-^{14}C,^{13}C]acrylonitrile (derived from 459 mg of β-hydroxypropionitrile) was then added, followed by 5 drops of 40% KOH in methanol. After being stirred for 1.5 h, during which time a clear solution was obtained, acetic acid (0.5 ml) was added. The residue obtained on evaporation in vacuo was dissolved in a 1:1 mixture of CH_2Cl_2 and hexane (50 ml), washed with brine, dried (K_2CO_3), and evaporated to yield **17** as a colorless solid (1.13 g), used without purification. Crystallization of an unlabeled sample from ethanol yielded colorless prisms, mp 160–174 °C dec, having the following spectroscopic data: ir ($CHCl_3$) 2262, 2238 (CN), 1635 cm^{-1} (CO); mass spectrum (70 eV) 341 (17) (M − HCN), 86 (100). Anal. ($C_{20}H_{24}N_4O_3$) C, H, N.

[1-^{14}C,^{13}C]-*o*-Succinylbenzoic Acid (10). Labeled **17** from the previous experiment was refluxed in 35 ml of 6 N HCl for 5 h. The cooled reaction mixture was extracted with ether (3 × 25 ml). Evaporation of the ether extract afforded a colorless oil which was converted by heating, as described previously,[19] to the dilactone **16** (121 mg) having a specific activity of 3.53×10^8 dpm/mmol: ^{13}C NMR ($CDCl_3$) (with the assigned carbons numbered as in formula **16**, Scheme IV) 174.0 (1), 166.3 (7'), 144.6 (1'), 135.1 (5'), 131.7 (3'), 126.6 (2'), 125.7 (4'), 122.7 (6'), 109.6 (4), 32.9 (2), 27.5 (3). A small amount of this material was diluted 40 times with unenriched material and its ^{13}C NMR spectra were obtained under identical conditions as that for an unenriched sample. Analysis of the two spectra indicated that the undiluted dilactone was enriched 86 ± 2% at C-1.

Hydrolysis of an unlabeled sample of **17** under identical conditions yielded the dicarboxylic acid **10** as colorless needles from ether–hexane: mp 135–136.5 °C (lit.[19] 137 °C); ir (KBr) 2820 (OH), 1705, 1697 cm^{-1} (CO).

Administration of Precursors to *D. pierardii* and Isolation of the Alkaloids. The following (experiment 3, Table I) is typical of the various feedings which were carried out. The disodium salt of **10** (derived from 15.7 mg of the dilactone **16**) dissolved in water was administered to one *D. pierardii* plant (Hausermann, Inc., Elmhurst, Ill.) growing in osmunda in a greenhouse, by means of cotton wicks inserted into the several stems. After 12 days the aerial parts of the plant were cut off (fresh wt 500 g) and macerated in a Waring Blendor with methanol (5 l.). After standing 3 days, the mixture was filtered and the filtrate concentrated to 500 ml, acidified with 4% H_2SO_4 (50 ml), and further concentrated to 200 ml. The filtered mixture was washed with ether (4 × 50 ml) and then adjusted to pH 10 with NaOH. Extraction with ether (4 × 50 ml) afforded, after drying ($MgSO_4$) and evaporation, an oil containing mainly pierardine. A continuous extraction with CH_2Cl_2 of the residual aqueous solution afforded, on evaporation, a semicrystalline solid (758 mg) containing mainly shihunine. The crude shihunine was subjected to preparative TLC on alumina PF 254 (Merck), developing with a mixture of $CHCl_3$–methanol–concentrated NH_4OH (50:5:1). Shihunine present in a zone having R_f 0.43 was extracted with methanol, sublimed [60 °C (10^{-4} mm)], and crystallized from ether–hexane. Colorless needles of shihunine (510 mg), mp 76–77 °C (lit.[3] mp 78–79 °C), were obtained having a constant specific activity on subsequent recrystallization. The crude pierardine was subjected to preparative TLC on silica gel PF-254, developing with $CHCl_3$–methanol–concentrated NH_4OH (50:5:1). In this solvent system pierardine and shihunine had R_f values of 0.34 and 0.13, respectively. The zone corresponding to pierardine was extracted with methanol and evaporated. The residue was dissolved in ether, and pierardine hydrochloride (122 mg) separated on saturation with HCl gas. Crystallization several times from a mixture of ethanol and ethyl acetate afforded colorless prisms, mp 183–184 °C (lit.[7] mp 185–186 °C).

Degradation of the Shihunine. Dilutions were carried out as necessary in the course of the degradations. Activities reported in Table II are calculated for undiluted material.

Dihydroshihunine (3). Shihunine (107 mg) dissolved in ethanol (10 ml) was hydrogenated at 3-atm pressure in the presence of Adams catalyst (0.1 g) for 2 h. Evaporation of the filtered reaction mixture afforded dihydroshihunine (106 mg), mp 185–190 °C (lit.[3] mp 190–201 °C).

1-Methyl-2-phenylpyrrolidine (5) and Its Oxidation. The dihydroshihunine (104 mg) was mixed with BaO (1 g) and heated with a Bunsen burner in a glass tube sealed at one end. The volatile products were collected in a U tube in dry ice. Picric acid (110 mg) dissolved in a little ethanol was added to the distillate and 1-methyl-2-phenylpyrrolidine picrate (129 mg) separated: mp 149–150 °C (lit.[32] 148–149 °C). The picrate (400 mg) was dissolved in 2 N H_2SO_4 (10 ml) and extracted with ether to remove picric acid. The solution was made basic with NaOH and refluxed with $KMnO_4$ (1.3 g) for 12 h. The mixture was decolorized with SO_2 and extracted with ether in a continuous extractor. Evaporation of the dried (Na_2SO_4) extract yielded benzoic acid which was purified by sublimation. (24 mg). A Schmidt reaction of this benzoic acid afforded CO_2 collected as $BaCO_3$, and aniline converted to benzanilide for assay.

2-(2'-Aminophenyl)-1-methylpyrrolidine (4). Dihydroshihunine (236 mg) was dissolved in concentrated H_2SO_4 (1.3 ml) and cooled to 0 °C. Sodium azide (200 mg) was added and the mixture slowly warmed to 70 °C in a current of CO_2-free N_2. The stream of N_2 was passed through a solution of 5% $KMnO_4$ in 2 N H_2SO_4 and then into 0.2 N $Ba(OH)_2$. The resultant $BaCO_3$ (147 mg, 65%) was washed with water, ethanol, and ether. The residual reaction mixture was diluted with water, made basic with NaOH, and extracted with ether (3 × 15 ml). The dried (K_2CO_3) extract was evaporated affording a colorless oil (152 mg, 75%) which was converted to its dihydrochloride: mp 245 °C dec; colorless prisms from ethanol. Anal. ($C_{11}H_{18}N_2Cl_2$) C, H, N, Cl. Reaction of the diamine **4** with benzoyl chloride in NaOH solution afforded 2-(2'-benzoylaminophenyl)-1-methylpyrrolidine as a colorless oil, which gave a monopicrate as yellow needles, mp 201–203.5 °C. Anal. ($C_{24}H_{23}N_5O_8$) C, H, N. The barium carbonate from the Schmidt reaction was assayed by direct solution in Aquasol II (New England Nuclear) according to the following procedure.[33] Barium [^{14}C]carbonate was dissolved in 2.3 ml of 0.1 M tetrasodium

ethylenediaminetetraacetate by ultrasonication for a few minutes. Aquasol II (15 ml) was added and the mixture shaken. At room temperature an emulsion results; however, at 4 °C (the temperature which we normally use for liquid scintillation counting) a clear solution is obtained. The efficiency of counting was 82.4 ± 0.7% for nine standard samples varying in weight from 2.3 to 25.7 mg.

1-Dimethylamino-4-phenylbutane Methiodide (6). 1-Methyl-2-phenylpyrrolidine (derived from 883 mg of its picrate) was refluxed in ethanol (25 ml) with methyl iodide (5 ml) in the presence of sodium bicarbonate (1.5 g) overnight. The residue obtained on evaporation was extracted several times with hot $CHCl_3$, which afforded on evaporation and crystallization from ethyl acetate 1-methyl-2-phenylpyrrolidine methiodide (525 mg, 76.5%), mp 134–135 °C (lit.[34] mp 130 °C). This methiodide (522 mg) was refluxed in water (20 ml) with 11.5 g of 2% sodium amalgam for 10 h. The cooled reaction mixture was extracted with ether (3 × 15 ml). Evaporation of the dried (K_2CO_3) extract yielded 1-dimethylamino-4-phenylbutane (284 mg) as a colorless oil which afforded a picrate, mp 132–133.5 °C (lit[35] mp 131.5–132.5 °C). The methiodide **6** was obtained as colorless plates from ethanol: mp 192.5–194.5 °C. Anal. ($C_{13}H_{22}NI$) C, H, N, I.

Hofmann Reaction on the Methiodide 6. The methiodide **6** (217 mg) dissolved in water (10 ml) was shaken for 5 min with freshly prepared silver hydroxide (from 0.3 g of $AgNO_3$). The filtered reaction mixture was evaporated and the residue heated slowly to 180 °C in vacuo (10^{-4} mm), volatile products being collected in a U tube cooled in liquid N_2. The contents of the U tube were allowed to warm to room temperature in a gentle stream of N_2, the volatile trimethylamine being trapped in a solution of methyl iodide (3 ml) in methanol (10 ml). The resultant tetramethylammonium iodide (75 mg, 69%) was obtained as colorless prisms on evaporation of the methanol. The residual oil in the U tube (67 mg) was identical with an authentic specimen[36] of 4-phenyl-1-butene. It was dissolved in ether (25 ml) containing a drop of pyridine and osmium tetroxide (178 mg). After standing for 22 h the mixture was evaporated and the residue refluxed for 1 h with sodium sulfite (320 mg) in 50% aqueous methanol (20 ml). The filtered reaction mixture was evaporated to small volume and extracted with ether (3 × 50 ml). The residue obtained on evaporation of the ether was dissolved in water (10 ml) and stirred with sodium metaperiodate (128 mg) for 1 h in a N_2 atmosphere. An ether extract (2 × 20 ml) of the reaction mixture was warmed with semicarbazide hydrochloride (200 mg) and sodium acetate (300 mg) in aqueous ethanol. Evaporation, followed by cooling, resulted in the separation of 3-phenylpropanal semicarbazone (32 mg, 33%), mp 126.5–128 °C, identical with an authentic specimen. The residual aqueous solution from the periodate cleavage was distilled into a solution of dimedone (150 mg) in water (100 ml). On standing the solution deposited the formaldehyde dimedone derivative (74 mg, 50%).

Degradation of the Pierardine Derived from [*4,2'-carbonyl*-$^{14}C_2$]-*o*-Succinylbenzoic Acid. Pierardine hydrochloride (204 mg), 3.59×10^3 dpm/mmol, was dissolved in 1 N KOH (20 ml) and refluxed with $KMnO_4$ (1.53 g) for 10 h. The mixture was acidified with H_2SO_4 and decolorized with SO_2. Extraction with ether yielded phthalic acid (36.7 mg, 28%) having an activity of 3.53×10^3 dpm/mmol. A Schmidt reaction on this phthalic acid afforded carbon dioxide collected as $BaCO_3$ (1.64×10^3 dpm/mmol) and anthranilic acid (1.79×10^3 dpm/mmol).

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References and Notes

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