BIOORGANIC CHEMISTRY 7, 277–288 (1978)

Biosynthesis of the *Securinega* Alkaloids. Investigations of the Early and Late Stages of the Pathway

RONALD J. PARRY

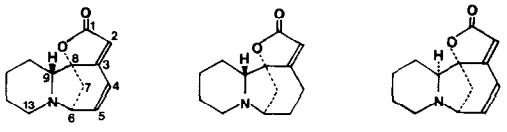
Department of Chemistry, Brandeis University, Waltham, Massachusetts 02154

Received January 27, 1978

Feeding experiments with *Securinega suffruticosa* have established that tyrosine is a specific precursor of the alkaloid securinine. Additional experiments show that tyrosine is incorporated into securinine with loss of the 3-*pro*-S hydrogen atom of the amino acid and that neither 4′-hydroxy-2-phenylethanol nor 4′-hydroxyphenylacetic acid serves as a securinine precursor. Investigation of the metabolic relationship between securinine and dihydrosecurinine has shown that the former alkaloid is the precursor of the latter.

INTRODUCTION

Plants of the genera *Securinega* and *Phyllanthus* (*Euphorbiaceae*) elaborate a group of unique tetracyclic alkaloids of which securinine (**1**)[2] is the most abundant (*1*). Accompanying securinine in *Securinega suffruticosa* are a number of minor alkaloids, including dihydrosecurinine (**2**) and allosecurinine (**3**). The unusual structure of these alkaloids poses interesting questions with regard to their mode of biosynthesis.



1 **2** **3**

FIGURE 1

Sufficient investigation of the biosynthesis of alkaloids containing a piperidine ring has been carried out to allow one to predict that the piperidine ring in securinine should be derived from lysine (*2*), but until recently the biosynthetic origin of the remaining eight carbon atoms has remained a puzzle. We report here experiments which solve this puzzle and, in addition, some further investigations on the mode of biogenesis of these alkaloids (*3*).

RESULTS AND DISCUSSION

It occurred to us that the remaining eight carbon atoms in securinine might be derived from an aromatic amino acid such as phenylalanine (**4**) or tyrosine (**5**).

[1] The author is pleased to dedicate this paper to Professor W. S. Johnson on the occasion of his 65th birthday.

[2] Numbers in boldface refer to structures in figures and schemes.

0045-2068/78/0073-0277$02.00/0

4 5

FIGURE 2

Administration of L-[*G*-^{3}H]phenylalanine to young *Securinega suffruticosa* (Pallas) Rehd. plants by the cotton wick method yielded securinine which was virtually devoid of radioactivity (Table 1, Expt. 1). On the other hand, administration of DL-[2-^{14}C]tyrosine to *S. suffruticosa* under similar conditions yielded radioactive alkaloid (Table 1, Expt. 2). The purified, radioactive securinine was degraded to *N*-methyl-2-(*o*-vinylphenyl)piperidine (**6**) by means of the reaction sequence outlined in Scheme I (*4*, *5*). Cleavage of the vinyl group present in **6** with osmate–periodate and trapping of the liberated formaldehyde with dimedone yielded radioactive dimedone–formaldehyde. The specific activity of the purified dimedone–formaldehyde corresponded to 92% of the activity of the piperidine derivative **6**. The specific incorporation of [2-^{14}C]tyrosine into securinine is therefore established. Experiments by Sankawa *et al.* (*7*) have provided additional evidence that carbon atoms 1–8 of securinine are derived from tyrosine.

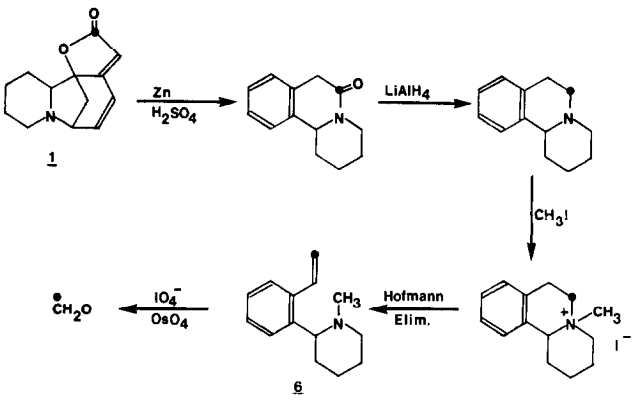
The incorporation of tyrosine into **1** proceeds with the loss of one hydrogen atom from C-3 of the amino acid. With the aim of gaining some insight into the nature of the hydrogen removal process, the stereospecificity of the hydrogen loss was examined.

p-Anisaldehyde was reduced with potassium borotritiide to *p*-[α-^{3}H]methoxybenzyl alcohol which was converted to *p*-[α-^{3}H]-methoxybenzyl bromide with gaseous hydrogen bromide (*8*). Alkylation (*8*) of the sodium salt of ethyl acetamidocyanoacetate with the tritiated benzyl bromide followed by acid hydrolysis (*8*) yielded DL-(3RS)-[3-^{3}H]tyrosine. The tritiated amino acid was mixed with DL-[3-^{14}C]tyrosine, and the doubly labeled amino acid (^{3}H: ^{14}C, 4.08) was administered to young *Securinega*

TABLE 1

FEEDING EXPERIMENTS WITH *Securinega suffruticosa*

Expt No.	Precursor fed to *Securinega*	Feeding time (days)	Alkaloid isolated	Incorporation (%)	Distribution of activity in product
1	L-[*G*-^{3}H]**4**	14	**1**	<0.0001	—
2	DL-[2-^{14}C]**5**	14	**1**	0.007	92% at C-1
3	(3RS)-[^{3}H, 3-^{14}C]**5** (^{3}H:^{14}C = 4.08)	14	**1**	0.007	^{3}H:^{14}C = 1.74
4	(3S)-[^{3}H, 3-^{14}C]**5** (^{3}H:^{14}C = 4.76)	14	**1**	0.007	^{3}H:^{14}C = 0.30
5	(3R)-[^{3}H, 3-^{14}C]**5** (^{3}H:^{14}C = 4.94)	14	**1**	0.007	^{3}H:^{14}C = 3.54
6	[3′,5′-^{3}H]**19**	14	**1**	0.0000	—
7	[3′,5′-^{3}H]**20**	14	**1**	0.0000	—
8	[2,4-^{3}H]**2**	9	**1**	0.008	—
9	[2,4-^{3}H]**1**	8	**2**	0.22	80% at C-2, C-4



SCHEME I

suffruticosa plants by the cotton wick method. After 14 days, radioactive securinine was isolated. The purified, radio-labeled securinine had a $^3H:^{14}C$ ratio of 1.74, corresponding to a 57% loss of tritium (Table 1, Expt 3); this indicates that the removal of a hydrogen atom from C-3 of tyrosine is a stereospecific process (expected loss is 50%). Additional experiments defined the stereospecificity of this hydrogen loss. Samples of (3R)-DL-[3-^{3}H]- and (3S)-DL-[3-^{3}H]tyrosine were synthesized by the method of Kirby and Michael (*9*). The two specimens of chirally tritiated tyrosine were each mixed with DL-[3-^{14}C]tyrosine and administered to *S. suffruticosa* plants. The radioactive securinine produced from (3S)-DL-[3-^{3}H, 3-^{14}C]tyrosine ($^3H:^{14}C$, 4.76) had a $^3H:^{14}C$ ratio of 0.30 corresponding to a 94% loss of tritium. The radioactive securinine biosynthesized from (3R)-DL-[3-^{3}H, 3-^{14}C]tyrosine ($^3H:^{14}C$, 4.94) had a $^3H:^{14}C$ ratio of 3.54 indicating a 28% loss of tritium (Table 1, Expts 4 and 5). These results clearly demonstrate that the conversion of tyrosine into securinine proceeds with loss of the *pro*-S hydrogen at C-3 of the amino acid. The lack of complete loss or retention of tritium during the conversion of tyrosine into **1** is probably due to two factors. The first of these is that the specimens of chirally tritiated tyrosine prepared

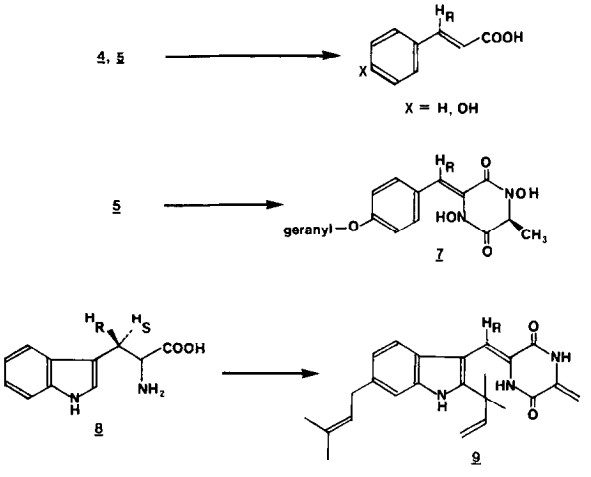
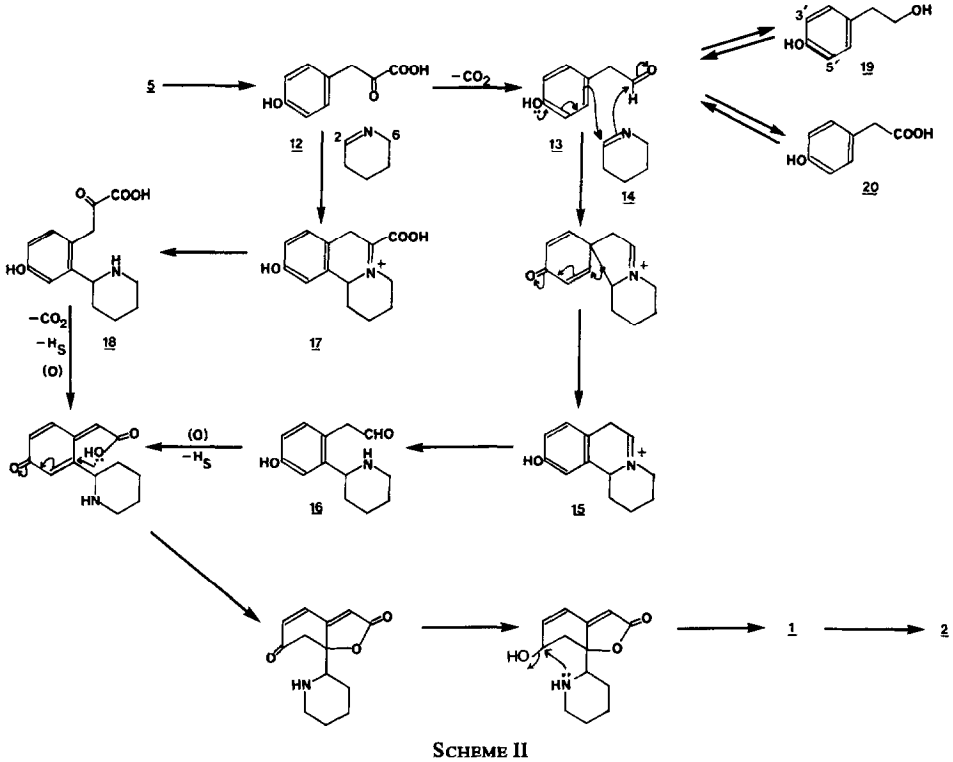


FIGURE 3

using Kirby and Michael's procedure are only ca. 85% stereochemically pure. (*9*). On this basis, one would expect to see tritium loss or retention figures of approximately 85%. However, a second factor appears to be operating. The conversion of tyrosine to securinine results in the oxidation of C-2 of the amino acid to a carbonyl group. The biosynthetic sequence leading to **1** may therefore involve one or more intermediates in which exchange of the C-3 hydrogens with the medium is possible. Such exchange would account for the fact that the figures for tritium loss obtained in each of the three experiments are slightly higher than would otherwise be expected.

The loss of the 3-*pro*-S hydrogen atom from tyrosine as a result of its conversion into securinine finds a parallel in other biosynthetic processes involving removal of a hydrogen atom from C-3 of an aromatic amino acid. The conversion of phenylalanine and tyrosine to the corresponding cinnamic acids, the incorporation of tyrosine into mycelianamide (**7**), and the conversion of tryptophan (**8**) into cryptoechinuline A (**9**) all proceed with removal of a 3-*pro*-S hydrogen atom (*10*). This suggests that there may be a general stereochemical uniformity associated with reactions of this type.

The incorporation of tyrosine into carbon atoms 1–8 of securinine having been established, investigation of intermediates beyond tyrosine was begun. Sankawa and co-workers (*7*) reported very low incorporations of tyramine (**10**) and 3,4-dihydroxyphenylalanine (**11**) into **1**, and they concluded that a likely intermediate beyond tyrosine might be 4′-hydroxyphenylpyruvic acid (**12**) (Scheme II). This possibility is supported by the work of Golebiewski *et al.* on the biogenesis of the piperidine ring of securinine (11). The latter investigators established that lysine



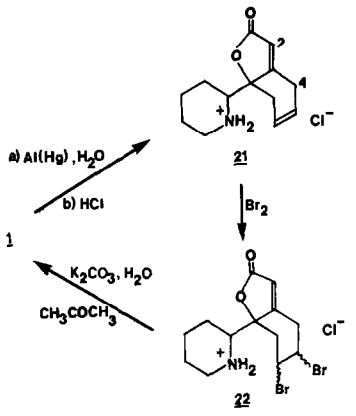
SCHEME II

labeled at C-2 yields securinine labeled exclusively at C-9 and that the securinine nitrogen atom is derived from the ε-amino group of lysine. On the basis of all of the above information, it is possible to formulate a plausible biosynthetic pathway leading to securinine from tyrosine and lysine. This hypothetical pathway is outlined in Scheme II. Tyrosine (**5**) could be converted to 4′-hydroxyphenylpyruvic acid (**12**) followed by loss of CO_2 to give 4′-hydroxyphenylacetaldehyde (**13**). Condensation of **13** with Δ^1-piperidine (**14**) could generate the iminium salt **15**, whose hydrolytic opening would yield the piperidine aldehyde **16**. Aldehyde **16** could then be transformed into securinine in the manner shown. Alternatively, the loss of CO_2 could occur at a later stage than 4′-hydroxyphenylpyruvic acid with the pathway proceeding via the putative intermediates **17** and **18**.

While the pathway summarized in Scheme II appears plausible, it has the disadvantage that many of the postulated intermediates are likely to be difficult to evaluate as precursors due to limited stability. For this reason, it was decided to test two more stable substances which might be convertible *in vivo* to putative intermediates via simple oxidation or reduction. Thus, 4′-hydroxy-2-phenylethanol (**19**) and 4′-hydroxyphenylacetic acid (**20**) might serve as precursors of 4′-hydroxyphenylacetaldehyde *in vivo*.

4′-Hydroxy-2-phenylethanol (**19**) was prepared by lithium aluminium hydride reduction of the methyl ester of *p*-hydroxyphenylacetic acid. Acid-catalyzed exchange of **19** with acidic tritiated water (*12*) gave 4′-[3′,5′,-^{3}H]hydroxy-2-phenylethanol. Administration of the tritiated 4′-hydroxy-2-phenylethanol to *Securinega suffruticosa* gave securinine which was totally devoid of radioactivity (Table 1, Expt 6). 4′-[3′,5′-^{3}H]hydroxyphenylacetic acid was similarly prepared by treatment of unlabeled **20** with acidic tritiated water. When this substance was tested as a securinine precursor in *S. suffruticosa*, radioinactive securinine was also obtained (Table 1, Expt 7). These results indicate (a) that neither **19** nor **20** is convertible into **13** *in vivo* or (b) that, if they are so converted, then **13** is not a precursor of securinine.

The biosynthetic pathway adumbrated in Scheme II suggests that dihydrosecurinine (**2**) is probably derived *in vivo* by reduction of securinine. It was decided to investigate the metabolic relationship between these two alkaloids. Dihydrosecurinine was prepared by reduction of securinine with sodium borohydride (*13*). Treatment of dihydrosecurinine with sodium methoxide in methanol-O-d led to deuterated dihydrosecurinine the nmr spectrum of which indicated that exchange of the hydrogens attached to C-2 and C-4 had taken place. [2,4-^{3}H]Dihydrosecurinine was then prepared by base-catalyzed exchange of **2** with sodium methoxide in methanol containing tritiated water. Administration of the labeled dihydrosecurinine to *S. suffruticosa* and isolation of securinine gave **1** which exhibited a small, but apparently real, incorporation figure (Table 1, Expt 8). This indicates that dihydrosecurinine may be oxidizable to securinine *in vivo*, but the results must be interpreted with caution since it was not possible to demonstrate specific incorporation of **2** into **1** due to the low level of incorporation. [^{3}H]Securinine was next prepared in order to examine its efficiency as a precursor of dihydrosecurinine. All attempts to oxidize [2,4-^{3}H]dihydrosecurinine to [2,4-^{3}H]securinine failed, and so a more circuitous route was developed to prepare tritiated securinine (Scheme III). It has been reported (*14*) that reduction of securinine with aluminum amalgam in wet ether leads to the *seco* derivative **21**. Bromination of **21** to



SCHEME III

give the dibromide **22** followed by treatment of the dibromide with base (*15*) then regenerates securinine. It seemed likely to us that the reduction of securinine to the *seco* derivative **21** was accompanied by uptake of protons from the water present in the reaction mixture. Indeed, when the reduction was run using ether moistened with D_2O, the nmr spectrum of the resulting *seco* compound indicated the presence of deuterium. The location(s) of the deuterium labels could not be established precisely due to the complexity of the nmr spectrum, but mechanistic considerations suggest that deuterium should be incorporated at C-2 and/or C-4. Repetition of the reduction using ether moistened with tritiated water yielded a tritiated specimen of *seco* compound **21**. The tritiated *seco* compound was then transformed back to securinine via the two-step reaction sequence shown in Scheme III. Administration of the tritiated securinine to *S. suffruticosa* and isolation of dihydrosecurinine by dilution produced radioactive alkaloid. After extensive purification, the level of radioactivity in **2** corresponded to an incorporation figure of 0.22% (Table 1, Expt 9). Furthermore, the incorporation was shown to be specific, since base-catalyzed exchange of the biosynthetically derived [^{3}H]dihydrosecurinine resulted in the loss of ca. 80% of the tritium label. These results provide strong support for the hypothesis that dihydrosecurinine is derived *in vivo* by reduction of securinine.

EXPERIMENTAL

Melting points were determined on a Fisher–Johns apparatus and are uncorrected. Infrared spectra were obtained on Perkin–Elmer 137, 257, or 567 infrared spectrophotometers. The ir spectra of solid samples were measured as potassium bromide dispersions and the spectra of liquids were determined in chloroform. The nmr spectra were measured on a Varian A-60A (60 mHz) or Bruker WH-90 (90 mHz) spectrometer and the chemical shifts are reported in δ values relative to tetramethylsilane as internal standard. Deuterochloroform was used as the nmr solvent unless otherwise noted. Liquid scintillation counting was performed on a Beckman LS-100C liquid scintillation counter. Toluene-based scintillator or Aquasol (New England Nuclear) was used as the scintillation cocktail. Administration of precursors to *Securinega*

suffruticosa plants was carried out in Lab-line Biotronette Mark III environmental chambers. The radiochemical purity of radio-labeled compounds was evaluated using a Varian 6000-1 radiochromatogram scanner. DL-[2-^{14}C]Tyrosine, potassium[^{3}H]borohydride, and $^{3}H_2O$ were purchased from Amersham Corp.; L-[*G*-^{3}H]phenylalanine was obtained from New England Nuclear Corp. *p*-Hydroxyphenylacetic acid was purchased from Sigma Chemical Co.

Administration of Tracers to Securinega suffruticosa and Isolation of Securinine

Two young *S. suffruticosa* plants (15–20 in. high) growing in a Lab-line environmental chamber were threaded in the middle portion of the main stem and a tracer solution was administered by dipping the end of the cotton thread into a small glass tube fastened to the stem of each plant. The tracer solution was generally absorbed within a few hours time, at which time distilled water was added to the glass tubes to wash in any remaining precursor. After an appropriate period, the plants were harvested. The glass tubes and cotton thread were removed from the plants and soaked in alcohol. An aliquot of the alcohol solution was then counted to determine the amount of tracer which was not taken up by the plant. The plants were macerated in alcohol and 100–200 mg of radioinactive securinine was added as carrier. The alcoholic extract was evaporated to dryness *in vacuo* and the residue was partitioned between 6% aqueous tartaric acid and ether. The acidic aqueous phase was extracted several times with ether. Emulsions were broken up by filtration of the mixture through Celite. The aqueous phase was basified with ammonia and extracted repeatedly with chloroform. The chloroform extract was washed with water, dried, and evaporated to give a greenish crystalline residue. Securinine was isolated from the crude alkaloidal mixture by preparative tlc on silica using ethyl acetate as solvent. The isolated securinine was recrystallized in a Craig tube from ethanol until a constant specific radioactivity was reached. In some instances, the radioactive securinine was then converted to its hydrochloride and the latter compound was recrystallized repeatedly from methanol–ether until constant activity was reached.

Degradation of Securinine Obtained by Administration of DL-[2-^{14}]*Tyrosine to S. suffruticosa*

The radioactive securinine (specific activity = 2.6×10^4 dpm/mmol) obtained after chromatography and repeated recrystallization was diluted with radioinactive securinine and converted into *N*-methyl-2-(*o*-vinylphenyl)piperidine (**6**) by the method of Horii *et al.* (*4*). The amine **6** was converted to its perchlorate salt and recrystallized to constant activity from methanol–ether. The purified salt was converted back to the free base and cleaved with osmate–periodate.

Nine milligrams of the free base **6** was dissolved in 1 ml of t-butanol freshly distilled from sodium, 1 ml of water was added followed by 0.31 ml of a solution of 100 mg of OsO_4 in 25 ml of t-BuOH freshly distilled from sodium. Finely ground sodium periodate (28 mg) was then added to the stirred mixture over a 0.5-hr period. After an hour, precipitation of sodium iodate was noticeable. The mixture was stirred overnight at room temperature. The next morning, a few milligrams of sodium periodate was added and the mixture was stirred another hour. Saturated, aqueous arsenic trioxide solution (10 ml) was then added and the resulting mixture was extracted three times with 10 ml

of ether. The pH of the aqueous phase was adjusted to 9 with solid potassium carbonate and dimedone (64 mg) was added. After stirring the reaction mixture for 10 min the pH was readjusted to 6 with concentrated HCl and the mixture was stirred overnight. The next morning, the solid was filtered off, washed with water, and air-dried. It was then dissolved in a minimum volume of chloroform and percolated through a 0.75-in.-long column of neutral Woelm alumina, act. III. Evaporation of the chloroform eluant gave 9 mg of dimedone–formaldehyde which was transferred to a Craig tube and recrystallized repeatedly from ethanol.

Synthesis of 3(RS)-DL-[^3H]Tyrosine

The procedures of Fields and co-workers (*8*) were utilized starting with *p*-[α-^{3}H]methoxybenzyl alcohol. The labeled alcohol was prepared as follows. Distilled *p*-anisaldehyde (211 mg) was dissolved in 5 ml of absolute ethanol and a few milligrams of radioinactive sodium borohydride was added. After stirring the mixture for about 5 min, ca. 5.5 mg (83 mCi) of tritiated potassium borohydride was added and the mixture was stirred for 5 hr. Excess radioinactive sodium borohydride (150 mg) was then added and the reaction was stirred for about 12 hr. The alcohol was then removed *in vacuo* and water and chloroform were added to the residue. The chloroform layer was separated, washed with water, and dried to give, on evaporation, 156 mg (73%) of crude *p*-methoxybenzyl alcohol. An infrared spectrum of the crude alcohol showed no carbonyl absorption. The crude alcohol was converted into the corresponding bromide without purification.

*Synthesis of 3(R),3(S)-*DL-[^3H]*Tyrosine*

The syntheses of these chirally labeled forms of tyrosine were carried out according to the procedures of Kirby and Michael (*9*).

Synthesis of 4'-[3',5'-^3H]Hydroxy-2-phenylethanol

A. Preparation of 4'-hydroxy-2-phenylethanol. *p*-Hydroxyphenylacetic acid (1.0 g) was dissolved in 15 ml of methanol saturated with dry hydrogen chloride and the resulting solution was refluxed for 3 hr. At the end of this period, the solvent was removed *in vacuo* and the residual oil was partitioned between excess aqueous bicarbonate solution and methylene chloride. The methylene chloride layer was washed with water, dried, and evaporated to dryness *in vacuo* to yield 850 mg of a colorless oil ($\bar{\nu}_{max}$ = 1730 cm^{-1}) (78%). The crude ester was dissolved in 25 ml of dry tetrahydrofuran and 400 mg of lithium aluminum hydride was added slowly with stirring. The mixture foams vigorously during the addition of the hydride. The reaction was then stirred ca. 20 hr at room temperature. Aqueous potassium hydroxide solution (30%) was added cautiously with stirring until a white precipitate formed, 1 *N* hydrochloric acid was then added with stirring until the pH reached 3. Ethyl acetate was added to the mixture, and the organic phase was separated, washed with bicarbonate and water, and dried. Evaporation of the organic layer gave a yellow crystalline residue which was recrystallized from methylene chloride to give colorless needles, 309 mg (44%), mp 91–93°C [lit. mp 92°C (*16*)]. The infrared spectrum of this material showed no carbonyl absorption.

B. Preparation of 4'-[3',5'-^3H]hydroxy-2-phenylethanol. 4'-Hydroxy-2-phenylethanol (25 mg) was added to a mixture of 150 μl of concentrated hydrochloric acid and 300 μl of ^3H_2O (300 mCi). The resulting mixture was heated at ca. 95°C for 5 hr. The alcohol is insoluble in the aqueous acid at room temperature, but dissolves readily above 70°C. After 5 hr, the solution was cooled, neutralized to pH 6 with solid sodium bicarbonate, and extracted repeatedly with ethyl acetate. The organic extract was dried and evaporated to give a residue which was purified by preparative tlc on silica using ethyl acetate as solvent. In this way, 19 mg (76%) of the alcohol was recovered. The specific activity of the compound was 12.4 mCi/mm.

Preparation of 4'-[3',5'-^3H]Hydroxyphenylacetic Acid

p-Hydroxyphenylacetic acid (50 mg) was suspended in a mixture of 150 μl of concentrated HCl and 300 μl of ^3H_2O (300 mCi). The suspension was heated to 95°C, whereupon the *p*-hydroxyphenylacetic acid dissolved. After the mixture has been heated at 95°C for 4 hr, the solution was allowed to cool, and the solvent was removed *in vacuo*. Water was added to the residue and stripped off under vacuum. This process was repeated, the resulting residue was dissolved in methanol, and the solution was decolorized with Norit. The methanol was removed and the residue was crystallized from ethyl acetate–hexane to give 24 mg of 4'-[3',5'-^{3}H]hydroxyphenylacetic acid (48%) with a specific activity of 6 mCi/mmol.

Preparation of [2,4-^3H]Dihydrosecurinine

A. Reduction of securinine to dihydrosecurinine (*13*). Securinine (100 mg) was dissolved in 5 ml of absolute ethanol and 100 mg of sodium borohydride was added with stirring over a period of 15 min. The yellow color of the original solution disappeared with 0.5 hr. The colorless solution was stirred at room temperature for 12 hr, the ethanol was removed *in vacuo*, and the residue was dissolved in water. The pH of the aqueous solution was adjusted to 7 using dilute HCl and the aqueous phase was repeatedly extracted with chloroform. After washing and drying, the chloroform solution was evaporated to give 84 mg of a yellowish oil. This material was purified by preparative tlc on silica using ethyl acetate to yield 61 mg of crystalline dihydrosecurinine, mp 45–48°C [lit. 53.5°C (*13*)]. In our hands, the compound proved to be unstable, discoloring on standing at room temperature for a few days. The dihydrosecurinine was stable indefinitely at −15°C. The nmr spectrum of this compound of 90 mHz exhibited, *inter alia*, a multiplet at 2.46–3.02 δ, 6H, which includes the allylic hydrogens attached to C-3; a triplet, 1H, $J = 5.3$ Hz, corresponding to the proton of C-6; and a broad singlet, δ 5.53, 1H, the vinyl hydrogen at C-2 (*17*).

B. Preparation of [2,4-^3H]dihydrosecurinine. Dihydrosecurinine (60 mg) was added to a solution of 15 mg of sodium metal in 0.5 ml of methanol to which 80 μl (400 mCi) of ^3H_2O had been added. The resulting solution was stirred at room temperature for 23 hr. The solvents were then removed *in vacuo*, water was added to the residue, and the pH of the solution was adjusted to 7. Repeated extraction of the aqueous solution with chloroform was followed by washing, drying, and evaporation of the chloroform to give 36 mg of a gum. The gum was purified by preparative tlc on silica using ethyl acetate to yield 30 mg (50%) of crystalline [2,4-^{3}H]dihydrosecurinine (specific activity = 27.6

mCi/mmol). A repetition of the experiment using sodium methoxide in methanol-O-d and D_2O gave a sample of dihydrosecurinine the nmr spectrum of which at 90 mHz showed that the intensity of the signal at 5.53 δ had been reduced by ca. 95% and that the multiplet at 2.46–3.02 δ now integrated for ca. four hydrogens instead of six hydrogens.

Synthesis of [2,4-^3H]Securinine

A. Conversion of securinine to the 2,4-^3H-labeled seco compound **21.** Securinine (100 mg) was dissolved in 7 ml of ether and 200 μl (1 Ci) of ^3H_2O added. Freshly prepared aluminum amalgam (*17*) (100 mg) was added and the mixture was stirred at room temperature for 24 hr. At the end of this time, the solution was completely colorless. The ether was decanted and the insoluble residue in the flask was washed once with ether. The combined ether extracts were washed with water, dried, and evaporated. The residue was dissolved in dry methanol and filtered to remove insoluble material. A slight excess of dry HCl dissolved in dry methanol was added to the methanol solution of the *seco* compound and the resulting solution of the hydrochloride salt was taken to dryness. The residue was crystallized from methanol–ether to give white crystals, 85 mg (72%), mp 202–203°C [lit. 218–220°C (*14*)], $\bar{\nu}_m$ 1760 , 1665, 1650 cm^{-1}. The nmr spectrum of the unlabeled *seco* derivative at 90 mHz exhibits a complex of overlapping peaks between 1.04 and 3.56 δ (13H) and a multiplet at 5.61–6.01 δ corresponding to three vinyl hydrogens. The nmr spectrum of the deuterated *seco* derivative obtained by reduction in the presence of D_2O shows changes in both the upfield multiplets and in the vinyl region. These changes take the form of the disappearance of some peaks and the simplification of others, but they are otherwise difficult to interpret.

B. Conversion of the 2,4-^3H-labeled seco compound into the dibromo derivative **22.** The tritiated *seco*-amine hydrochloride (85 mg) was dissolved in 6 ml of chloroform and a solution of ca. 55 mg of bromine in 0.35 ml of chloroform was added dropwise with stirring at room temperature. The solution was stirred for 10 hr, after which time, ether was added until the solution was faintly turbid. The solution was then chilled to 5°C, whereupon crystals began to separate. More ether was added, the mixture was allowed to stand for an hour at 5°C, and the crystals were filtered off. Recrystallization of the crude product from methanol–ether yielded 130 mg (94%) of white crystals, mp 197–198°C [lit. mp 205–206°C (*15*)], $\bar{\nu}_m$ 1756, 1653 cm^{-1}.

C. Synthesis of [2,4-^3H]securinine from dibromo derivative **22.** The procedure of Horii *et al.* (*15*) was utilized except that it was found necessary to substitute acetone for the chloroform recommended as solvent and to run the reaction at room temperature rather than at reflux.

The tritiated dibromide hydrochloride (130 mg) was suspended in 13 ml of acetone and three drops of water were added. This was followed by the addition of finely ground anhydrous potassium carbonate (215 mg). The resulting mixture was stirred at room temperature for 70 hr and then filtered through Celite. The Celite was washed with acetone and the combined filtrate and washings were taken to dryness. The yellow residue was purified by preparative tlc on alumina with chloroform to give 27 mg of securinine. Recrystallization of this material from aqueous ethanol gave back 24 mg (36%) of crystalline securinine, mp 141–142°C [lit. 142–144°C (*15*)], the specific activity of which was 3.8 mCi/mmol.

Administration of [2,4-^3H]Securinine to S. suffruticosa and Isolation of Dihydrosecurinine

Tritiated securinine (2.6 mg, 0.05 mCi) was dissolved in 0.8 ml of water containing an equivalent of acetic acid. The resulting solution (pH ca. 5.5) was administered to three 15- to 18-in. *Securinega* plants in the usual way. After 8 days, the plants were macerated in alcohol and 100 mg of radioinactive dihydrosecurinine was added to the macerated mixture as carrier. The mixture was filtered, the alcohol was removed *in vacuo*, and the alkaloids were isolated in the usual way. A mixture of securinine and dihydrosecurinine was isolated by preparative tlc on alumina using 1:1 benzene:ethyl acetate. The dihydrosecurinine was then freed from securinine by plating three times on silica preparative plates using ethyl acetate as the solvent. The recovered dihydrosecurinine was dissolved in methanol and treated with a slight excess of dry HCl in methanol, and the methanol was removed *in vacuo*. The residue was recrystallized from methanol–ether until it reached constant specific activity.

ACKNOWLEDGMENTS

I am pleased to thank Professor T. C. Huang, who supplied us with seeds of *S. suffruticosa*; Dr. Seiichi Saito for a generous gift of securinine; and the National Science Foundation for the purchase of the Bruker WH-90 nmr spectrometer used in this work (GU 3852, GP 37156). In addition, thanks are due the National Institutes of Health for a Research Career Development Award (GM-00143).

REFERENCES

1. V. Snieckus, "The Alkaloids" (R. H. F. Manske, Ed.), Vol. 14, p. 425. Academic Press, New York, 1973.
2. I. D. Spenser, "Comprehensive Biochemistry" (M. Florkin and E. H. Stotz, Eds.), Vol. 20, p. 231. Elsevier, Amsterdam, 1968.
3. A portion of these investigations has been published in preliminary form: R. J. Parry, *Tetrahedron Lett.* 307 (1974); R. J. Parry, *J. Chem. Soc. Chem. Commun.* 144 (1975).
4. Z. Horii, M. Ikeda, Y. Yamawki, Y. Tamura, S. Saito, and K. Kodera, *Tetrahedron* **19**, 2101 (1963).
5. T. Nakano, T. H. Yang, and S. Terao, *Tetrahedron* **19**, 609 (1963).
6. A. R. Battersby, R. Binks, W. Laurie, G. V. Parry, and B. R. Webster, *J. Chem. Soc.* 7454 (1965).
7. U. Sankawa, K. Yamasaki, and Y. Ebizuka, *Tetrahedron Lett.* 1867 (1974).
8. M. Fields, D. E. Walz, and S. Rothchild, *J. Amer. Chem. Soc.* **73**, 1000 (1951).
9. G. W. Kirby and J. Michael, *J. Chem. Soc. Perkin I* 115 (1973); cf. P. G. Strange, J. Staunton, H. R., Wiltshire, A. R. Battersby, K. R. Hanson, and E. A. Havir, *J. Chem. Soc., Perkin I* 2364 (1972).
10. R. J. Parry, "Bioorganic Chemistry" (E. E. van Tamelen, Ed.), Vol. 2. Academic Press, New York, 1978.
11. W. M. Golebiewski, P. Horsewood, and I. D. Spenser, *J. Chem. Soc. Chem. Commun.* 217 (1976).
12. G. W. Kirby and L. Ogunkoya, *J. Chem. Soc.* 6914 (1965).
13. I. Satoda, M. Murayama, J. Tsuji, and E. Yoshii, *Tetrahedron Lett.* 1199 (1962).
14. S. Saito, K. Kotera, N. Shigematsu, A., Ide, N. Sugimoto, Z. Horii, M. Hanoaka, Y. Yamawaki, and Y. Tamura, *Tetrahedron* **19**, 2085 (1963).

15. Z. Horii, M. Hanoaka, Y. Yamawaki, Y. Tamura, S. Saito, N. Shigematsu, K. Kotera, H. Yoshikawa, Y. Sato, H. Nakai, and N. Sugimoto, *Tetrahedron* **23**, 1165 (1967).
16. C.-S. Cheng, C. Ferber, R. I. Bashford, Jr., and G. F. Grillot, *J. Amer. Chem. Soc.* **73**, 4081 (1951).
17. H. E. Audier and J. Parello, *Bull. Soc. Chim. Fr.* 1552 (1968).
18. H. Wislicenus and L. Kaufmann, *Ber.* **28**, 1303 (1895); cf. L. F. Fieser and M. Fieser, "Reagents for Organic Synthesis," Vol. 1, p. 20. Wiley, New York, 1967.