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Role of 1,2-Dehydroreticulinium Ion in the Biosynthetic Conversion of Reticuline to Thebaine

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Abstract: The role previously assigned to 1,2-dehydroreticulinium ion as a precursor to the morphinan alkaloids in *Papaver somniferum* was based on feeding experiments with a synthetic compound of uncertain identity. We have now prepared authentic 1,2-dehydroreticulinium chloride and shown its efficient incorporation into the morphinan alkaloids, supporting the previous hypothesis. Moreover, using a double-label technique and steady-state $^{14}CO_2$ biosynthesis, we have determined that 1,2-dehydroreticulinium ion is a natural product whose native pool size is about one-fifth that of reticuline. These data clearly establish 1,2-dehydroreticulinium ion as an intermediate in morphinan alkaloid biosynthesis.

Reticuline (**1**) has been firmly established[1,2] as the biosynthetic precursor, via salutaridine (**2**) and salutaridinol (**3**), of thebaine (**4**) in *Papaver somniferum*. Although it is the (−)-enantiomer of reticuline which corresponds in absolute stereochemistry to the configuration found at that center in the morphinan alkaloids,[3] both enantiomers, when fed separately, were incorporated into thebaine essentially to the same extent. Feedings with reticuline labeled with ^3H at C-1 and ^{14}C at other positions showed that incorporation into thebaine was accompanied by no loss of ^{14}C but with considerable loss of ^3H.[1b,c]

These unexpected results were accommodated by proposing a reversible oxidation–reduction side path to 1,2-dehydroreticulinium ion (**5**), which would allow both for the loss of ^3H and inversion of configuration at C-1. Support for this proposal was found when synthetic material characterized as 1,2-dehydroreticulinium chloride was efficiently incorporated into morphine,[1c] and dehydroreticulinium ion was assigned a role as a precursor of thebaine.

High incorporation is necessary and strong evidence for a precursor–product relationship; however, by itself it is insufficient. An additional requirement is the natural occurrence of the candidate precursor, a question which we set about to answer for 1,2-dehydroreticulinium chloride (**5**). The only characterization for **5** previously reported[1c] was its mp (190–200 °C dec) and ultraviolet absorption. Since the latter (λ_{max} 250, 323 nm) did not correspond to that of similar 1-benzyl-3,4-dihydroisoquinolinium salts in our experience nor to published spectra,[4] it also was necessary to prepare fully authenticated 1,2-dehydroreticulinium chloride and reexamine its role as a precursor.

Discussion

Synthesis of 1,2-Dehydroreticulinium Chloride (5) and Its ^3H and ^{14}C Isotope Isomers. 1,2-Dehydroreticulinium ion is a common intermediate in the many syntheses of reticuline[5] and our synthesis proceeded by standard methods from vanillin to 3-benzyloxy-4-methoxyphenylacetic acid (**6**). For the other half of the molecule, we chose 4-benzyloxy-3-methoxyphenylacetonitrile (**7**) as the intermediate, anticipating the introduction via the cyano group of ^{14}C and ^3H at C-3 of the final compound.

The conversion of nitrile **7** to amine **8** has been erratic in the past using the more conventional (PtO_2/H_2 or $LiAlH_4$) methods. We found that this reduction can be reliably performed and in good yield using sodium borohydride and cobalt chloride in methanol.[6] The amide **9** then formed nearly quantitatively when acid **6** and amine **8** were refluxed in xylene with removal of water. Cyclization of the amide in refluxing toluene with $POCl_3$ gave the iminium chloride **10** in 95% yield. When **10** as the free base was treated with methyl iodide in methanol, a 92% yield of the methiodide **11** resulted. To prepare the corresponding chloride, **11** was treated with excess freshly prepared AgCl in aqueous methanol, giving quantitative conversion to methochloride **12**. Finally, debenzylation of **12** occurred quantitatively in refluxing ethanolic HCl to give pure 1,2-dehydroreticulinium chloride (**5**) (see Scheme I).

In our hands, 1,2-dehydroreticulinium chloride (**5**) is a stable salt melting at 180–185 °C dec. The infrared spectrum shows clearly a band at 1630 cm^{-1} characteristic of the conjugate iminium salt. The ultraviolet spectrum (λ_{max} 370, 309, 250 nm) of **5** is in accord with the earlier report on the ultraviolet spectra of dihydroisoquinolines of this type,[4] but contrasts with the previous[1c] absorption (λ_{max} 323, 250 nm) assigned to structure **5**. The NMR spectrum accounts for all the hydrogens of **5** and confirms its iminium salt character (as shown in the infrared) by the appearance of the C-9 methylene hydrogens as a two-hydrogen singlet at δ 4.40. The NMR spectrum can be regenerated sans the δ 4.40 signal if the sample is treated with NaOD in D_2O to pH 13 and immediately quenched by acidification to pH 2 with DCl in D_2O. Finally, 1,2-dehydroreticulinium chloride (**5**) was reduced with sodium borohydride to give an excellent yield of D,L-reticuline which was further characterized by conversion to its perchlorate and picrate salts, in full agreement with the reported data.[5]

To prepare [3-^{14}C]reticuline and [3-^{14}C]-1,2-dehydroreticulinium chloride (**5**) $Na^{14}CN$ was used in the reaction with 4-benzyloxy-3-methoxybenzyl bromide, and the resulting radioactive acetonitrile **7** was carried through the synthesis as

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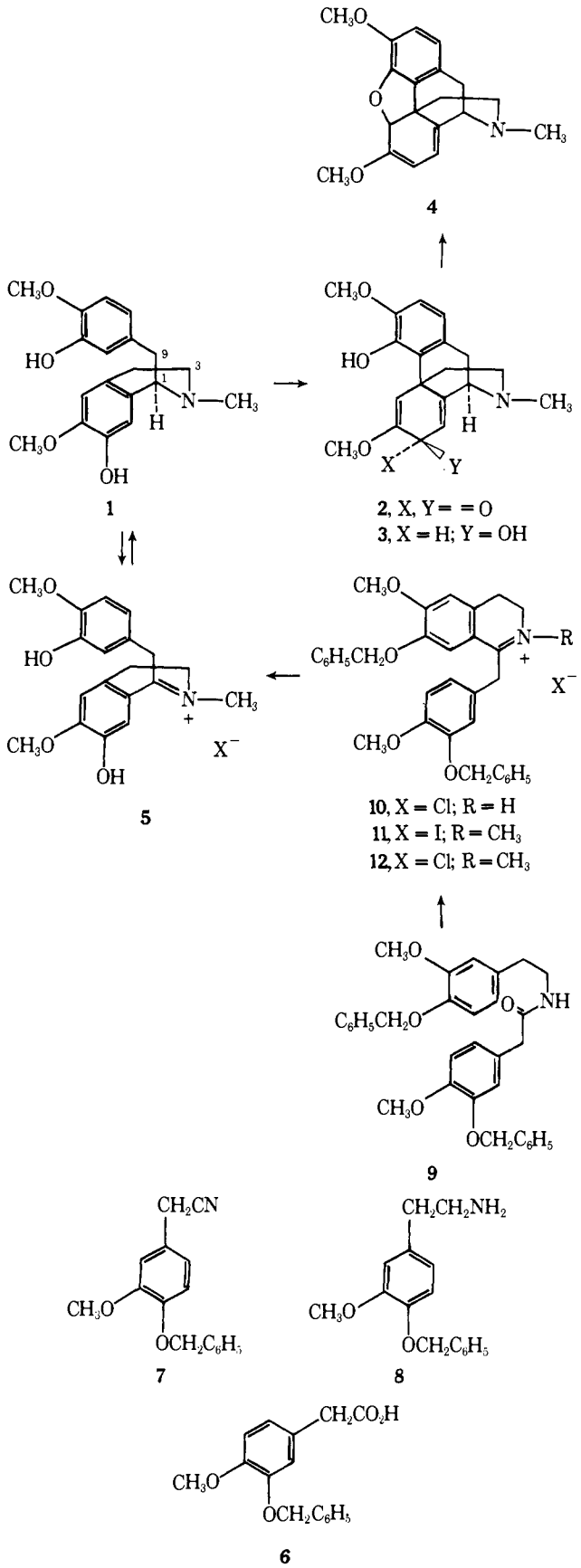
Scheme I



above. To prepare the ^3H-labeled phenylethylamine **8**, we used the cobalt chloride–sodium borohydride procedure, preforming and isolating the catalyst so formed. However, use of this isolated catalyst with tritium was troublesome and led to significant dilution of the tritium with adsorbed hydrogen. A more effective synthesis was found in the use of Ni_2B·2% CrB as catalyst.[6b] When applied with $^3H_2$ to acetonitrile **7**, [α-$^3H_2$]-β-(4-benzyloxy-3-methoxyphenyl)ethylamine (**8**) was conveniently prepared.

Table I. Incorporation of [3-^{14}C]Reticuline and [3-^{14}C]-1,2-Dehydroreticulinium Chloride into Morphinan Alkaloids in *P. somniferum*[a]

Precursor	Time[b]	Thebaine	Codeine	Morphine
D,L-[3-^{14}C]Reticuline	20 h	3.8	3.6	1.4
	3 days	3.3	4.9	5.2
	14 days	0.7	0.4	0.8
[3-^{14}C]-1,2-Dehydroreticulinium chloride	20 h	2.7	4.9	1.6
	3 days	0.8	2.8	6.2
	14 days	0.5	1.3	1.8

[a] Plants were 83–92 days old and had just started elongating. [b] Amount of time plants were allowed to grow, including the initial hour of injection, before harvesting.

Feeding Experiments. Of the various techniques used for precursor feedings (smearing on the leaves, hydroponic feeding through the roots, wick feeding through the stems or roots, direct injection) we have found that direct injection is the best method for delivering a specific amount of precursor in a short period with little wound or insult to the plant. The hypocotyl was chosen as the site for injection because of the ease of entering the mainstream of the plant through this fleshy region between root and stem.

A solution of [3-^{14}C]-1,2-dehydroreticulinium chloride (**5**) was fed to *P. somniferum* by direct injection over a 1-h period using a motor driven syringe. As a control, parallel feedings were carried out with [3-^{14}C]reticuline. Since the rates of incorporation were not known, the plants were then transferred to hydroponic nutrient solutions and allowed to grow for periods varying from 20 h to 14 days. The plants were then harvested, radioinactive carriers were added, and the alkaloids were obtained by the usual isolation procedures.

The results of the feeding experiments with *P. somniferum*[7] are presented in Table I. Clearly, 1,2-dehydroreticulinium ion is incorporated into thebaine, codeine, and morphine with approximately the same high efficiency as is reticuline. These results may be considered to confirm the previous[1c] report of incorporation, although the nature of the compound fed, and claimed to be 1,2-dehydroreticulinium ion, remains uncertain. It is of interest to note that the relative incorporation as a function of time among the various morphinan alkaloids roughly follows the previously established sequence:[8] thebaine → codeine → morphine. Also, decreased incorporation at long times, 14 days, is consistent with the metabolism of morphine to normorphine and the conversion of the latter to unknown metabolites.[9]

Natural Occurrence of 1,2-Dehydroreticulinium Ion. The demonstration that authentic dehydroreticulinium chloride acts as an effective precursor of the morphinan alkaloids in *P. somniferum* is clearly of significance; however, it is conceivable that in the feeding experiments dehydroreticulinium chloride entered the natural biosynthetic pathway (i.e., was converted to reticuline) via some nonspecific reductase rather than by actually occupying a legitimate position in the scheme. Thus, the question of whether 1,2-dehydroreticulinium ion exists as a natural product becomes important. If the dehydroreticulinium species is naturally occurring and if its role is to provide a means of interconverting the two enantiomeric forms of reticuline, then its pool size might be so small as to escape detection. Additionally, the polar, ionic nature of dehydroreticulinium chloride precludes the use of gas chromatography and the common thin-layer techniques for its separation and pu-

Table II. Recovery and Distribution of Activity[a] from Separation Scheme Applied to *P. somniferum*[b] to Which Reticuline and Dehydroreticulinium Chloride Were Added

	Expt 1	Expt 2	Expt 3
Reticuline added	1.43×10^8 dpm of ^3H (100%)	None	5.15×10^4 dpm of ^{14}C (100%)
Dehydroreticulinium chloride added	None	5.07×10^8 dpm of ^3H (100%)	4.23×10^8 dpm of ^3H (100%)
Recovery from total organic extracts (step c)	1.19×10^8 dpm (83%)	1.18×10^8 dpm (22%)	4.81×10^4 dpm of ^{14}C (94%) 1.03×10^8 dpm ^3H (24%)
Recovery from aq after $NaBH_4$ reduction (step d)	7.15×10^4 dpm (0.05%)	2.69×10^8 dpm (53%)	0.20×10^4 dpm of ^{14}C (4%) 3.08×10^8 dpm ^3H (73%)

[a] All radioactivity is at C-3 in all compounds. [b] Four plants, each 150 days old, were used for each experiment.

rification. The problem is further compounded by the instability of this iminium salt under even mildly alkaline conditions.

One possible approach which seemed attractive would involve the conversion of 1,2-dehydroreticulinium chloride to reticuline, an easily isolable derivative. Further, the use of plants grown in a $^{14}CO_2$ atmosphere would lead to labeled material and even very small quantities would be readily detectable. However, endogenous reticuline would be indistinguishable from reticuline derived by reduction of 1,2-dehydroreticulinium chloride. This problem could be overcome if a method were available for separating a mixture of dehydroreticulinium chloride and reticuline.

Such a method was developed based on the following principles: (a) powdered plant material was extracted with 1 N HCl to remove both reticuline and dehydroreticulinium ion; (b) separation from most of the other 1 N HCl soluble plant substituents was effected by ion exchange, leaving reticuline and dehydroreticulinium ion finally in solution at pH 7; (c) this pH 7 solution was extracted thoroughly with chloroform and chloroform/2-propanol to yield the organic extract fraction, further described below; (d) ion exchange of the pH 7 aqueous phase and elution with 12 N HCl gave dehydroreticulinium chloride which was reduced to reticuline with sodium borohydride.

The organic extracts, step c, contained reticuline appreciably contaminated with dehydroreticulinium chloride. These were separated by extraction into HCl and then removal of the reticuline at pH 8.5 into chloroform. This step c gives the endogenous reticuline and step d gives dehydroreticulinium ion, isolated as reticuline. In both cases, the reticuline was thoroughly purified by TLC and GC.

To rigorously evaluate this method, three experiments were performed, each with *P. somniferum* plant material to which different labeled carriers were added. In experiment 1, [3-^{3}H]reticuline was added; in experiment 2, [3-^{3}H]-1,2-dehydroreticulinium chloride was added; and in experiment 3, both [3-^{14}C]reticuline and [3-^{3}H]dehydroreticulinium chloride were added. Each mixture was then subjected to the separation method. Both the distribution and recovery of reticuline and dehydroreticulinium chloride were determined and are summarized in Table II.

The relatively low overall recovery of activity in experiments 1 (83%) and 2 (75%) was due primarily to loss of significant amounts of reticuline and dehydroreticulinium to the resin beads in each of the two ion exchange processes. Subsequently we found that this material could be recovered after the initial elution with 12 N HCl by allowing the resin to stand in contact with 12 N HCl for 16 h followed by further elution with fresh 12 N HCl. This technique was used in experiment 3 and accounts for the excellent activity recovery (98, 97%) found there.

Table III. Activity Relationships of Reticuline Isolated from $^{14}CO_2$ Exposure

Compound	^{3}H sp act., dpm/mg	^{14}C sp act., dpm/mg	$^3H/^{14}C$ ratio
Endogenous reticuline[a]	1.62×10^6	7145	227
Reticuline[a] from reduction of 1,2-dehydroreticulinium ion	2.06×10^4	1362	15

[a] Purified by TLC and GC to constant specific activity and homogeneity.

The second question which had to be answered about the separation method was its ability to avoid contamination of one component by the other. The results of this evaluation are found in experiment 3, Table II. Although the organic extract (step c) contains [^{14}C]reticuline accompanied by considerable [^{3}H]dehydroreticulinium chloride, this contaminant can be removed easily and totally by subjecting the organic extracts to another distribution at pH 8.5 as described above. The result was pure [^{14}C]reticuline containing no ^{3}H activity above background. However, some reticuline did remain in the aqueous phase at pH 7 and it accompanied the dehydroreticulinium chloride throughout to the end (step d). This was shown by 4% of the added ^{14}C activity appearing in the final [^{3}H]reticuline resulting from the borohydride reduction.

To account for any endogenous reticuline which would be carried along and might confuse the dehydroreticulinium assay, a method was devised involving use of a double label. *P. somniferum* plants exposed to $^{14}CO_2$ under short-term steady-state conditions[10] will carry ^{14}C labels in all carbon-containing compounds. If [3-^{3}H]reticuline and inactive 1,2-dehydroreticulinium chloride are added as carriers then the endogenous reticuline will carry a double label whereas an endogenous dehydroreticulinium (if any is present) should carry only the ^{14}C label. Moreover, the $^3H/^{14}C$ ratio in the endogenous reticuline is fixed at the moment labeled carrier is added. In the separation method, the endogenous doubly labeled reticuline can be obtained pure from step c and its $^3H/^{14}C$ ratio can be determined. After reduction of the dehydroreticulinium ion (step d) the resulting reticuline will also carry a double label by virtue of the small amount of endogenous reticuline left in the pH 7 aqueous phase after chloroform–2-propanol extraction. However, if dehydroreticulinium ion is a natural product, the $^3H/^{14}C$ ratio for this reticuline will be less than the $^3H/^{14}C$ ratio found for the purely endogenous reticuline.

Eighteen *P. somniferum* plants (105 days old; 1.5 kg, wet weight) were exposed for 4 h to $^{14}CO_2$ under steady-state conditions. The plants were ground to a fine powder in liquid nitrogen and inactive 1,2-dehydroreticulinium chloride (0.36 mmol) as well as [3-^{3}H]reticuline (0.31 mmol, 2.45×10^8 dpm) were added. The entire mix was extracted with 1 N HCl to provide the extract that was submitted to the separation procedure described above. The results are summarized in Table III and clearly demonstrate the presence of 1,2-dehydroreticulinium ion as a natural product in *P. somniferum* by virtue of the difference in $^3H/^{14}C$ ratios. Thus, 1,2-dehydroreticulinium ion can be placed in the biosynthetic pathway leading to morphine with assurance.

Consideration of the ^{14}C specific activities shown in Table III also allows a further conclusion to be reached regarding pool sizes. The reticuline derived from dehydroreticulinium chloride had a ^{14}C specific activity of 1362 dpm/mg. Of this activity, 90.7 dpm/mg (i.e., $(2.06 \times 10^4)/227 = 90.7$) is from endogenous reticuline not removed in the separation. Thus, 1272 dpm/mg is derived from endogenous dehydroreticulinium chloride. Using these values and the data in Table III, the following relationships may be drawn:

$$7145 \text{ dpm/mg} = \frac{^{14}C \text{ act. from endogenous reticuline}}{\text{mg of endogenous + carrier reticuline}} \quad (1)$$

$$1272 \text{ dpm/mg} = \frac{^{14}C \text{ act. from endogenous dehydroreticulinium ion}}{\text{mg of endogenous + carrier dehydroreticulinium ion}} \quad (2)$$

which on a millimolar basis become:

$$2.35 \times 10^6 \text{ dpm/mmol} = \frac{^{14}C \text{ act. of endogenous reticuline}}{\text{mmol of endogenous + carrier reticuline}} \quad (3)$$

$$0.418 \times 10^6 \text{ dpm/mmol} = \frac{^{14}C \text{ act. of endogenous dehydroreticulinium ion}}{\text{mmol of endogenous + carrier dehydroreticulinium ion}} \quad (4)$$

In both cases the ^{14}C activity must come entirely from native material but it is reasonable to assume that the endogenous materials contribute negligibly (<5%) to the mass, relative to the amount of carrier added. With this simplifying assumption the equations become:

$$2.35 \times 10^6 \text{ dpm/mmol} = \frac{^{14}C \text{ act. of endogenous reticuline}}{0.31 \text{ mmol of carrier}} \quad (5)$$

$$0.418 \times 10^6 \text{ dpm/mmol} = \frac{^{14}C \text{ act. of endogenous dehydrorecticulinium ion}}{0.36 \text{ mmol of carrier}} \quad (6)$$

From eq 5 and 6 we calculated that the ^{14}C activity found in endogenous reticuline equals 7.28×10^5 dpm and that in endogenous 1,2-dehydroreticulinium ion it is 1.50×10^5 dpm.

The proposed biosynthetic pathway presents a close relationship between reticuline and dehydrorecticulinium ion with no intervening compounds. Under conditions of short-term steady-state $^{14}CO_2$ biosynthesis it is reasonable to expect that the specific activities of the two compounds should be equal. Thus the difference in the normalized activities as calculated above can only arise from differences in the native pool sizes of reticuline and dehydroreticulinium ion. To a rough approximation our data suggest that the pool size of reticuline is ~5 (i.e., $(7.28 \times 10^5)/(1.5 \times 10^5)$) times as large as the pool of 1,2-dehydroreticulinium ion in *P. somniferum*.

Experimental Section

General. All melting points were determined on a Büchi melting point apparatus and are uncorrected. Infrared spectra were determined on a Perkin-Elmer Model 337 spectrometer in KBr pellets. Ultraviolet spectra were determined in 95% ethanol (unless otherwise noted) on a Cary Model 14 spectrometer. Nuclear magnetic resonance spectra were determined on a Varian T-60 instrument in $CDCl_3$ (unless otherwise noted) with absorptions recorded in parts per million downfield from internal Me_4Si. Mass spectra were determined on CEC-103 and 110B spectrometers. All organic extracts were dried over $MgSO_4$ and evaporated from a Berkeley rotary evaporator. Elemental analyses were provided by the Analytical Laboratory, Department of Chemistry, University of California, Berkeley, and agree with calculated values within ± 0.4%. For determination of ^3H and ^{14}C in the same sample, a Packard sample oxidizer was used; radioactivity determinations were performed on a Packard Tri-Carb liquid scintillation counter.

Thin-layer chromatography, both analytical and preparative, was carried out on Camag silica gel using the following solvent systems: (a) C_6H_6/CH_3OH (4:1); (b) $CHCl_3/CH_3OH/NH_4OH$ (75:25:1); and (c) $CHCl_3/CH_3OH$ (5:1). R_f values are: (a) thebaine 0.65, codeine 0.40; (b) codeine 0.60, reticuline 0.45, morphine 0.30; (c) reticuline 0.30.

Gas chromatography was performed on a Hewlett-Packard Model 402B gas chromatograph with a hydrogen flame detector and using glass columns, 6 ft × 6 mm o.d., on 3% OV-17 on Varaport 30 (100–120 mesh). Preparative GC was carried out at 260 °C using He at 60 mL/min as carrier gas; analytical GC was performed at 230 °C using He at 40 mL/min. The analytical retention times are: thebaine, 14 min; codeine, 8 min; morphine, 12 min; reticuline, 23 min.

Synthetic Experiments. 4-Benzyloxy-3-methoxyphenylacetonitrile (7). To a stirred solution of 4-benzyloxy-3-methoxybenzyl bromide (22.0 g, 72 mmol) in 320 mL of dimethylformamide was added powdered sodium cyanide (15.6 g, 320 mmol) and the suspension was stirred under nitrogen for 24 h. Fresh sodium cyanide (1.00 g) was added every hour for 5 h and the reaction mixture was then poured into 600 mL of water and the product separated as a yellowish precipitate which was extracted with one 200-mL portion and three 100-mL portions of benzene. The combined extracts were washed with 400 mL of water, dried, and evaporated to leave a residue which was crystallized from chloroform/carbon tetrachloride (1:9): yield of **7**, 15.6 g (86%); mp 70–71 °C (lit.[11] mp 67 °C).

β-(4-Benzyloxy-3-methoxyphenyl)ethylamine (8). To a stirred solution of nitrile **7** (6.0 g, 24 mmol) and cobalt (II) chloride hexahydrate (10.0 g, 41 mmol) in methanol (900 mL) was added portionwise sodium borohydride (9.0 g, 237 mmol). A black precipitate formed and hydrogen was evolved. The resulting suspension was stirred for 2 h at room temperature under nitrogen; then 3 N HCl (200 mL) was poured into the reaction mixture and stirring continued until the precipitate dissolved. The methanol was evaporated and the resulting aqueous solution was extracted with two 100-mL portions of ether. The aqueous phase was basified with concentrated aqueous NH_3 and extracted with four 100-mL portions of ether and the combined ether extracts were washed with an equal volume of saturated sodium chloride solution and dried. Evaporation of the ether gave the primary amine as an oil in 4.86 g, 81% yield: NMR δ 1.25 (s, 2 H, NH_2), 2.85 (c, 4 H, CH_2CH_2), 3.83 (s, 3 H, OCH_3), 5.12 (s, 2 H, $C_6H_5CH_2$), 6.80 (c, 3 H, OAr H), 7.35 (c, 5 H, C_6H_5); mass spectra *m/e* 257 (M^+), 91 ($C_7H_7^+$, 100). Anal. ($C_{16}H_{19}NO_2$): C, H, N.

3-Benzyloxy-*N*-[β-(4-benzyloxy-3-methoxyphenyl)ethyl]-4-methoxyphenylacetamide (9). The phenylacetic acid 6[11] (11.9 g, 43 mmol), the amine **8** (11.1 g, 43 mmol), and xylene (175 mL) were refluxed together under nitrogen for 18 h with azeotropic removal of water. The solution was cooled to room temperature, hexane (100 mL) was added, and the precipitated product was recrystallized from methanol to yield 20.2 g of pure amide, mp 143–144 °C (lit.[11] mp 140 °C).

7-Benzyloxy-1-(3-benzyloxy-4-methoxybenzyl)-6-methoxy-3,4-dihydroisoquinoline Hydrochloride (10). A solution of the amide **9** (10.0 g, 18.9 mmol) in toluene (700 mL) was refluxed with phosphorus oxychloride (10 mL) for 12 min and then quickly cooled to room temperature. The toluene and excess $POCl_3$ were evaporated, and the residue was washed with petroleum ether and then dissolved in 200 mL of ethanol. Upon cooling to 5 °C, 10 mL of concentrated HCl was added followed by 300 mL of ether whereupon the product crystallized. Recrystallization from ethanol/ether provided analytically pure **10** (9.8 g, 95% yield): mp 211–214 °C dec (lit.[11] mp 203–205 °C); NMR δ 2.75 (t, 2 H, C-4 CH_2), 3.75 (s, 3 H, OCH_3), 3.90 (s, 3 H, OCH_3), 3.82 (t, 2 H, C-3 CH_2), 4.40 (s, 2 H, C-9 CH_2), 5.10 (s, 4 H, C_6H_5CH), 6.55, 6.73, 7.25 (15 H, Ar H); mass spectra *m/e* 493 (M^+ − HCl), 91 ($C_7H_7^+$); IR 1630 cm^{-1} (>C=N⁺<). Anal. ($C_{32}H_{32}NO_4Cl$): C, H, N.

7-Benzyloxy-1-(3-benzyloxy-4-methoxybenzyl)-6-methoxy-2-methyl-3,4-dihydroisoquinolinium Iodide (11). To a stirred solution of the imine hydrochloride **10** (25.0 g, 47 mmol) in 800 mL of meth-

anol was added 48 ml of 0.97 M methanolic potassium hydroxide solution and iodomethane (71.0 g, 500 mmol) in that order. The solution was refluxed under nitrogen for 3 h, cooled, and then poured into 1200 mL of ether where the product crystallized overnight at 0 °C. Recrystallization from methanol/ether gave pure methiodide **11** (27.4 g, 92%): mp 203–204 °C (lit.[12] mp 198–201 °C); IR 1630 cm^{-1} (>C=N⁺<); NMR δ 3.27 (t, 2 H, C-4 CH_2), 3.65 (s, 3 H, NCH_3), 3.85 (s, 3 H, OCH_3) 3.94 (t, 2 H, C-3 CH_2), 4.02 (s, 3 H, OCH_3), 4.42 (s, 2 H, C-9 CH_2), 5.05 (s, 2 H, $C_6H_5CH_2$), 5.10 (s, 2 H, $C_6H_5CH_2$), 6.52, 6.70, 6.92, 7.20 (15 H, ArH); UV λ_{max} 362 nm (ε 7300), 309 (6700), 247 (13 000); mass spectra *m/e* 507 (M^+ − HI), 492 (M^+ − HI − CH_3), 91 ($C_7H_7^+$, 100). Anal. ($C_{33}H_{34}NO_4I$): C, H, N.

7-Benzyloxy-1-(3-benzyloxy-4-methoxybenzyl)-6-methoxy-2-methyl-3,4-dihydroisoquinolinium Chloride (12). To a stirred solution of the methiodide **11** (7.60 g, 12 mmol) in 800 mL of methanol and 500 mL of water was added freshly prepared silver chloride (35.8 g, 250 mmol) and the resulting suspension was vigorously stirred under nitrogen for 2 h at room temperature. The mixture was filtered and the clear yellow filtrate was concentrated to an oil which crystallized from acetone/ether, giving 6.21 g (95%) of the methochloride **12**: mp 183–184 °C (lit.[1c] mp 118–121 °C); IR 1630 cm^{-1} (>C=N⁺<); UV λ_{max} 362 nm (ε 7300), 309 (6700), 247 (13 000) (lit.[1c] λ_{max} 250, 317 in H_2O/C_2H_5OH, 1:1); mass spectra *m/e* 507 (M^+ − HCl), 492 (M^+ − HCl − CH), 91 ($C_7H_7^+$, 100); NMR δ 3.27 (t, 2 H, C-4 CH_2), 3.67 (s, 3 H, NCH_3), 3.82 (s, 3 H, OCH_3), 3.90 (t, 2 H, C-3 CH_2), 4.00 (s, 3 H, OCH_3), 4.40 (s, 2 H, C-9 CH_2), 5.05 (s, 2 H, $C_6H_5CH_2$), 5.10 (s, 2 H, $C_6H_5CH_2$), 6.49, 6.70, 6.90, 7.20 (15 H, ArH). Anal. ($C_{33}H_{34}O_4NCl$): C, H, N.

1,2-Dehydroreticulinium Chloride (5). The methochloride **12** (6.00 g, 11 mmol) was dissolved in 600 mL of ethanol which had previously been saturated at 0–5 °C with HCl gas. The resulting solution was refluxed under nitrogen for 18 h, and the volatile materials were evaporated, finally by heating at 78 °C (0.01 Torr) for 12 h, to give 3.95 g (98%) of pure 1,2-dehydroreticulinium chloride (**5**): mp 180–185 °C dec (lit.[1c] mp 190–200 °C dec); IR 1630 cm^{-1} (>C=N⁺<); UV λ_{max} 370 nm (ε 7000), 309 (7000), 250 (14 000) (lit.[1c] λ_{max} 250, 323); NMR δ (CD_3OD) 3.13 (t, 2 H, C-4 CH_2), 3.70 (s, 3 H, NCH_3), 3.79 (s, 3 H, OCH_3) 3.96 (s, 3 H, OCH_3), 4.05 (t, 2 H, C-3 CH_2), 4.40 (s, 2 H, C-9 CH_2), 4.80 (br s, 2 H, ArOH), 6.63, 6.78, 7.02, 7.18, 7.37, (5 H, ArH); mass spectra *m/e* 327 (M^+ − HCl), 312 (M^+ − HCl − CH_3, 100). Anal. ($C_{19}H_{22}NO_4Cl$): C, H, N.

D,L-Reticuline (1). To a stirred solution of **5** (1.02 g, 2.8 mmol) in 250 mL of methanol was added portionwise sodium borohydride (1.00 g, 25.5 mmol) and the resulting solution was stirred at room temperature for 2 h. Solvent was evaporated and the residue was dissolved in 100 mL of 0.1 N NaOH. This solution was quickly adjusted to pH 8.3 with 3 N HCl and then extracted with one 200-mL and three 100-mL portions of chloroform. The combined chloroform extracts were washed with 500 mL of saturated sodium bicarbonate and 500 mL of saturated sodium chloride, dried, and evaporated to leave an oil which crystallized from ether/hexane giving pure D,L-reticuline (0.89 g, 97%), mp 144–145 °C (lit.[11] mp 144 °C). The *picrate* melted at 192–194 °C (lit.[11] mp 191–192 °C) and the *perchlorate* melted at 143–145 °C (lit.[13] mp 144 °C).

[α-$^3H_2$]-β-(4-Benzyloxy-3-methoxyphenyl)ethylamine (8). To 4-benzyloxy-3-methoxyphenylacetonitrile (**7**, 253 mg, 1 mmol) and 3 mL of Ni_2B·2% CrB catalyst[6b] in 30 mL of methanol was introduced $^3H_2$ and the mixture was stirred at room temperature for 5 h. Hydrogen was then introduced and stirring continued for 24 h after which the solution was degassed by seven freeze–thaw cycles. The catalyst was removed by filtration, the filtrate was diluted with 50 mL of 3 N HCl and extracted with three 25-mL portions of xylene, the aqueous phase was adjusted to pH 9 with concentrated aqueous NH_3, and four 30-mL portions of xylene were used to remove the amine. This distribution between acidic and alkaline aqueous phases and xylene was repeated twice and the final xylene phase was washed with saturated NaCl solution, leaving a solution of pure [^{3}H]amine **8** in xylene, used directly as described below.

[3-$^3H_2$]-1,2-Dehydroreticulinium chloride (5) was prepared by adding the phenylacetic acid 6 (272 mg, 1 mmol) directly to the xylene solution of tritiated **8** prepared above and proceeding as previously described to the preparation of **5** which contained 188 mCi of tritium.

[3-^{14}C]-1,2-Dehydroreticulinium chloride was prepared as described above, starting with 4-benzyloxy-3-methoxybenzyl bromide and $Na^{14}CN$.

[3-$^3H_2$]- and [3-^{14}C]reticuline were prepared from the corresponding 1,2-dehydroreticulinium chloride by reduction with $NaBH_4$ as described above.

Feeding Experiments. Injection of Precursors. The [3-^{14}C]reticuline solution was prepared by dissolving 25.7 mg in 0.5 mL of 1 M H_3PO_4 and then adjusting the pH to 6.4 with 8 M KOH. Water was then added to a final volume of 1.5 mL. The [3-^{14}C]-1,2-dehydroreticulinium chloride solution was prepared by dissolving 39.3 mg in 1.55 mL of deionized water (pH 4.0).

Aliquots were taken using a 250-μL gas-tight syringe and were injected into the hypocotyl of *P. somniferum* via a Sage No. 341 motor-driven syringe at a rate of ~3 μL/min; injection time was about 1 h. Three plants, 83–92 days old, were used in each experiment and were injected with 100–200 μL of precursor solution. Plants were then allowed to grow for the time specified in nutrient solution with aeration of the roots. In some cases, 2–10% of the injected radioactivity was observed in the nutrient solution in which the plants were growing. This activity was shown to be leakage from the injection hole and not release from the roots. By placing a Band-Aid over the injection hole, activity in the nutrient solution decreased to <0.3% while the Band-Aid contained 4–8% of the activity.

Isolation procedures were carried out as previously described[10] and the alkaloids were separated into a nonphenolic fraction containing thebaine and codeine, and a phenolic fraction containing reticuline and morphine. The thebaine and codeine were further resolved on alumina (Woelm(III), basic) using successively C_6H_6, $C_6H_6/CHCl_3$ (9:1), $C_6H_6/CHCl_3$/2-propanol (88.5:10:1.5), and $C_6H_6/CHCl_3$/2-propanol/CH_3OH (87.5:10:1.5:1). Further purification was effected by TLC using system a for thebaine, system b for codeine, and system b for reticuline and morphine. Each compound was purified until it was >99% pure, then it was sublimed, washed from the cold finger with CH_3OH, and dried to constant weight and specific activity at 60 °C (0.01 Torr).

Natural Occurrence Experiments. Four shredded *P. somniferum* plants were frozen in liquid N_2 in a Waring blender and ground to a fine powder by blending for 60 s. The frozen, pulverized plant material was then transferred to a 1-L Erlenmeyer flask, carrier alkaloids and 500 mL of 1 N HCl were added, and the mixture was shaken for 18 h. The solids were removed by centrifugation and washed with five 100-mL portions of 1 N HCl, and the combined acid solutions were filtered and passed through an ion exchange column [Bio-Rad AG50W-X4 (100–200 mesh), 18 cm × 2.4 cm i.d.] in the H^+ form. The column was then successively eluted with 1 N HCl (1 L), 3 N HCl (250 mL), 6 N HCl (100 mL), and 12 N HCl (500 mL) after which the resin was allowed to stand in contact with 12 N HCl for 16 h and then further eluted with 500 mL of 12 N HCl. The 6 N and 12 N eluents were combined and evaporated to 15 mL, 500 mL of saturated aqueous NaCl was added, the pH was adjusted to 7.0 with aqueous NaOH, and the solution was extracted with 13 100-mL portions of chloroform and two 100-mL portions of chloroform/2-propanol (9:1), readjusting the pH to 7.0 after each extraction as necessary. The combined organic extracts were evaporated and the resulting residue set aside for subsequent isolation and purification of the reticuline (see below).

The pH 7.0 aqueous solution was acidified to pH 1.0 with concentrated HCl and then passed through an ion exchange column [Bio-Rad AG 50W-X4 (100–200 mesh), 15 cm × 1.8 cm i.d.] in the H^+ form. The column was eluted with 1 N HCl (1 L), 3 N HCl (250 mL), 6 N HCl (100 mL), and 12 N HCl (500 mL), and the resin was allowed to stand in contact with 12 N HCl for 16 h and then was further eluted with 12 N HCl (500 mL). The 6 N and 12 N eluents were combined and evaporated to dryness, and the residue was dissolved in 250 mL of methanol and treated with $NaBH_4$ (2.0 g, 0.05 mol) at room temperature for 1 h. The methanol was then evaporated and the residue was dissolved in 100 mL of 1 N HCl. This solution was adjusted to pH 8.5 with aqueous Na_2CO_3 and extracted with five 100-ml portions of chloroform, the combined chloroform extracts were dried and evaporated, and the residue was purified by preparative TLC and and finally by preparative GC to yield pure reticuline. Further TLC and GC showed this reticuline to be of constant specific activity.

The residue from the chlorofrom and chloroform/2-propanol extracts was dissolved in 100 mL of 1 N HCl, the solution was adjusted to pH 8.5 with aqueous Na_2CO_3 and extracted with five 100-mL portions of chloroform, and the combined and dried chloroform extracts were evaporated. From the residue was isolated pure reticuline of constant specific activity by preparative TLC and GC.

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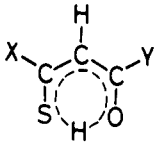
Communications to the Editor

Conjugative Effects on the Enol–Enethiol Tautomerism of β-Thioxoketones[1,2]

Sir:

Aliphatic and alicyclic β-thioxoketones[3,4] are known to be rather unstable compounds, in contrast to the aromatic β-thioxoketones[5] which can be stored for long periods without appreciable decomposition. This difference in stability may possibly originate from structural dissimilarities, since an electron-delocalized "quasi-aromatic" structure **A** may be of



A

importance in solution for the aromatic compounds. A structure of this type has been proposed recently for both aliphatic and aromatic β-diketones on the basis of ^1H and ^{13}C NMR spectroscopic studies.[6-8] It has been shown[3,4] that aliphatic and alicyclic β-thioxoketones exist as equilibrium mixtures of the tautomeric (*Z*)-enol (**B**) and (*Z*)-enethiol (**C**) forms which interconvert very rapidly (on the NMR time scale) by intramolecular chelate proton transfer. It seems reasonable to assume that the introduction of flanking aryl substituents (X, Y = Ar) may lead to enhanced stabilization of a central "quasi-aromatic" ring by conjugative effects. Structure **A** should, strictly speaking, be regarded as a borderline case of the equilibrium system **B** ⇄ **C** at continuous lowering in the energy barrier for the interconversion. With this in mind we decided to investigate theoretically the influence of conjugative effects on this equilibrium (Scheme I).

We have calculated[9] the energy barriers for the enol–enethiol interconversion of four model compounds, the hypothetical β-thioxocarbonyls **1–4.** The bonding energies for the enol and enethiol structures were minimized with respect to all internal atomic coordinates, and the energies of 13 intermediate states, corresponding to stepwise intramolecular chelate proton transfer from the energy-minimized structure **B** to the energy-minimized structure **C**, were determined for each pair of tautomers by continuous variation of all geometric parameters. The energy profiles found for the four interconversions are depicted in Figure 1, the transition-state energies being chosen arbitrarily as zero points.

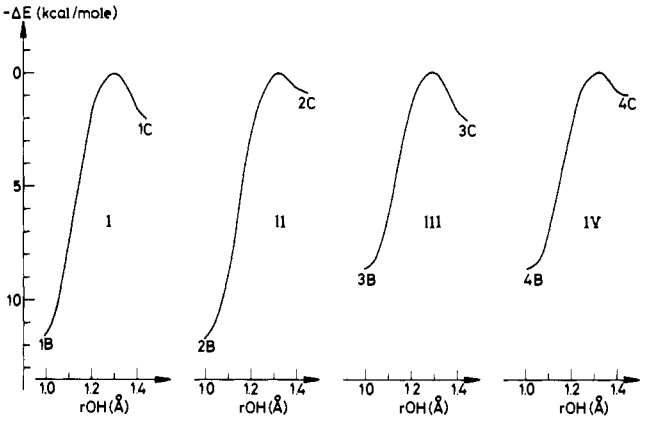
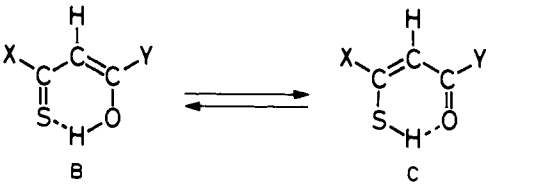


Figure 1. Theoretically derived energy profiles for the interconversions **1B** ⇄ **1C** (I), **2B** ⇄ **2C** (II), **3B** ⇄ **3C** (III), and **4B** ⇄ **4C** (IV).

Scheme I



B C

1 : X, Y = H, H
2 : X, Y = H, Vinyl
3 : X, Y = Vinyl, H
4 : X, Y = Vinyl, Vinyl
5 : X, Y = CH_3, CH_3
6 : X, Y = CH_3, C_6H_5
7 : X, Y = C_6H_5, CH_3
8 : X, Y = C_6H_5, C_6H_5

It can be seen from Figure 1 that the enol form (**B**) is generally the more stable tautomer, a result which is in accord with work by Fabian.[10] The introduction of a vinyl group instead of the hydrogen atom in the Y position effects a lowering of the energy barrier for the enethiol–enol conversion (**2C** → **2B**), whereas no apparent change with respect to the energy barrier

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