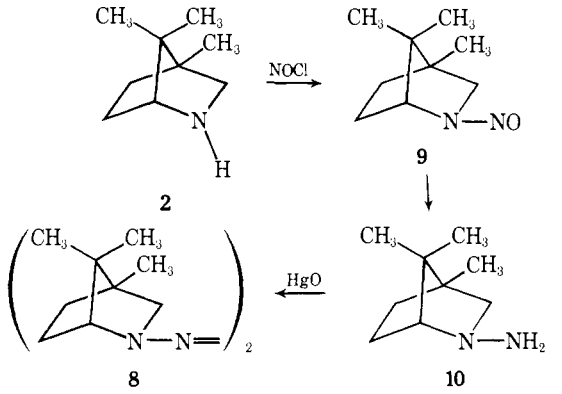
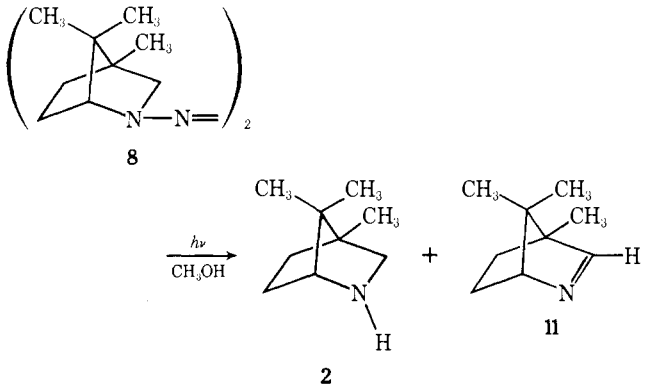
Tetrazenes are well-established sources of nitrogen radicals.[5] In order to prepare the tetrazene necessary for the production of **7**, **2** was treated with nitrosyl chloride to give **9**,[6] which on lithium aluminum hydride reduction gave the hydrazine **10**.



Oxidation of **10** with mercuric oxide gave the tetrazene **8** in 60% overall yield. Irradiation of a methanolic solution of **8** at room temperature with a Hanovia 450-W high pressure mercury lamp gave only **2** and **11** in 63 and 34% yields, respectively. *No products derived from skeletal rearrangement could be detected.* The structure of **2** was established through comparison with an authentic sample. The structure of **11** was assigned on the basis of its NMR spectrum (single olefinic proton at δ 7.50) and through comparison with an authentic sample prepared from **1** by treatment with sodium methoxide.



Photolysis of **1** in methanol with a 450-W Hanovia source gave as major products 37% of **2**, 33% of **11**, and 10% of **4**.[7] The formation of **4** in this reaction indicated that some solvolysis was competing with the homolytic cleavage. The difference in the ratio of **2** to **11** may be attributed to the generation of a pair of amino radicals plus nitrogen in the irradiation of **2**, vs. the formation of a chlorine–amino radical pair in the photolysis of **1**. Since the precursors are different in the two experiments being compared, it is also conceivable that the generated amino radicals might not exist in identical solvent cages and, hence, may react with the solvent cage at slightly different rates and in slightly different manners.

From the photochemical decomposition of **8** and **1**, it became obvious that **11** was a major product of the photochemically generated radical **7**. However, it was not established whether the formation of **11** depended on **7** having its origin in an excited state reaction. In order to answer this question, we carried out the methanolysis of **1** in the presence of 0.1 equiv of benzoyl peroxide. This reaction gave 10% of **2**, 5% of **3**, 26% of **4**, and most significantly 21% of **11**. Again, a combination of homolytic and heterolytic processes was involved. However, the formation of **11** in this reaction firmly established that **11** could arise from a nonphotochemically generated nitrogen radical. These results clearly indicate that the benzoyl peroxide-induced decomposition of **1** in methanol has a radical-chain component.

In summary, we have demonstrated that nitrogen radicals can follow reaction paths, which are very diverse from those of nitrenium ions. Specifically, in the case studied, we have shown that while the imine **11** was completely absent in the methanolysis of **1**, it was a major product of all of the reactions, which should proceed via the nitrogen radical **7**. This indicates that both the silver ion promoted and noncatalyzed methanolysis of **1** proceed via heterolytic cleavage of the N–Cl bond as previously proposed.[4,8]

Acknowledgment. We are indebted to the National Cancer Institute of the Public Health Service for Grants CA-07110 and CA-17269 which supported this investigation.

References and Notes

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(2) O. E. Edwards, D. Vocelle, and J. W. Apsimon, *Can. J. Chem.*, **50**, 1167 (1972). See also O. E. Edwards, G. Bernath, J. Dixon, J. M. Paton, and D. Vocelle, *ibid.*, **52**, 2123 (1974).
(3) For example see R. N. Haszeldine, T. J. Tewson, and A. E. Tipping, *J. Fluorine Chem.*, **8**, 101 (1976).
(4) P. G. Gassman and R. L. Cryberg, *J. Am. Chem. Soc.*, **90**, 1355 (1968); **91**, 2047 (1969).
(5) F. O. Rice and C. J. Grelecki, *J. Am. Chem. Soc.*, **79**, 2679 (1957); J. S. Watson and A. J. Waring, *Can. J. Chem.*, **38**, 298 (1960); J. S. Watson, *J. Chem. Soc.*, 3677 (1956); A. Good and J. C. J. Thynne, *J. Chem. Soc. B*, 684 (1967); C. J. Michejda and W. P. Hoss, *J. Am. Chem. Soc.*, **92**, 6298 (1970).
(6) Satisfactory elemental analyses or exact mass molecular weights were obtained on all new compounds. In all cases spectroscopic data were consistent with the assigned structure.
(7) In control experiments, it was demonstrated that while a large excess of hydrogen chloride catalyzed the decomposition of 1 to give only **2** (as its hydrogen chloride salt), the hydrochloride of **2**, as generated in the normal course of the reaction, did not promote perceptible decomposition of 1 in the time required for the photochemical decomposition.
(8) These results raise questions concerning the role of "heavy atom solvent effects" in the interconversion of singlet and triplet nitrenium ions.[1] Since the amino radical 7 clearly yielded both **2** and **11**, the failure to identify **11** as a major product of the decomposition (solvolysis) of 1 in heavy atom solvents indicated that a major difference existed between the cationic nitrogen diradical (nitrenium ion triplet), the nitrogen cation radical (previously postulated as an intermediate in the reaction of triplet nitrenium ions with hydrogen donating solvents), and amino radicals such as 7. Further studies of these differences and of the general concept of "heavy atom effects" in nitrenium ion chemistry are currently under investigation.
(9) Inquiries concerning this study should be sent to P.G.G. at the University of Minnesota.

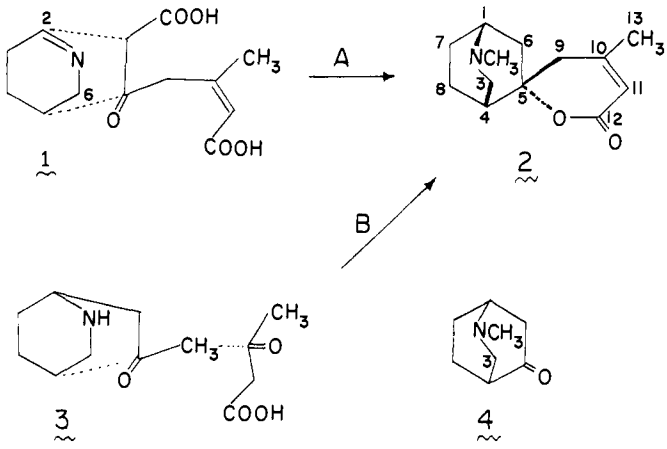
Paul G. Gassman,*[9] Kenji Uneyama, Jerry L. Hahnfeld
Department of Chemistry, University of Minnesota Minneapolis, Minnesota 55455 and Department of Chemistry, The Ohio State University Columbus, Ohio 43210
Received August 2, 1976

Biosynthesis of the Isoquinuclidine Moiety of Dioscorine. Incorporation of [5,6-$^{13}C_2$]Nicotinic Acid Established by Means of ^{13}C Nuclear Magnetic Resonance

Sir:

Dioscorine (**2**)[1] is an alkaloid found in the tropical yam, *Dioscorea hispida*, Dennst. and related species. We have previously[2] established that the administration of [1-^{14}C] acetate to this plant yielded radioactive dioscorine, which was labeled on alternate carbons (C-5, 10, and 12) of the unsaturated lactone ring. This result led us to suggest that dioscorine is formed by a condensation between Δ^1-piperideine (**1**) (which is formed from lysine in higher plants[3]) with a branched 8-carbon unit derived from four acetate units (route A, Scheme I). Spenser[4] suggested a slight modification in which pelle-

Scheme I



tierine (**3**) (formed from lysine and acetate) condenses with an acetate derived 4-carbon unit (route B).

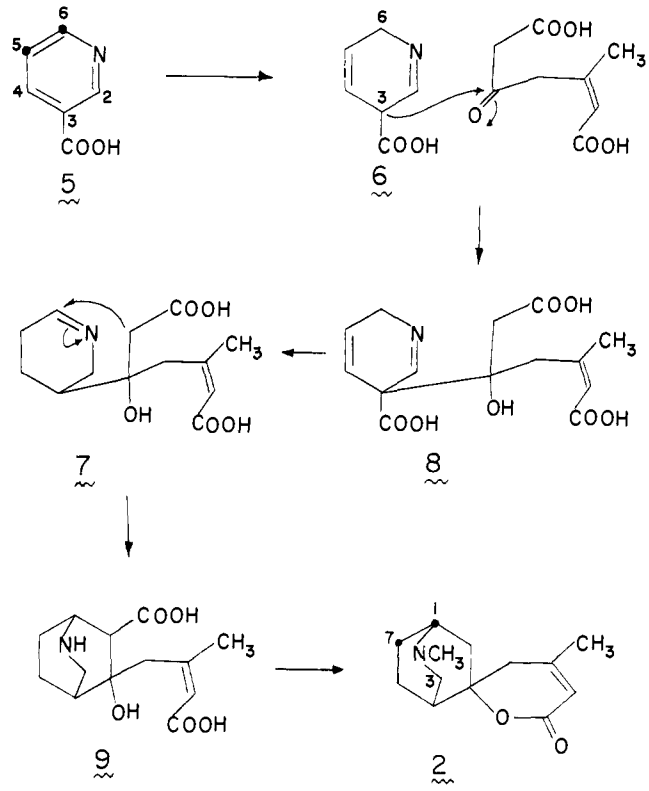
The administration of DL-[2-^{14}C]lysine[2] or DL-[6-^{14}C]lysine[5] to *D. hispida* yielded dioscorine with negligible activity (0.003 and 0.007% incorporation, respectively). The dioscorine obtained after feeding [6-^{14}C]-Δ^1-piperideine[2] had higher activity (0.03% inc.). However, a partial degradation indicated that the pattern of labeling was the same as that found after feeding [1-^{14}C]acetate, indicating that the Δ^1-piperideine was probably catabolized to acetate prior to incorporation.

In view of the lack of incorporation of all the probable precursors of the isoquinuclidine moiety, we were led to consider a completely new hypothesis, illustrated in Scheme II, which has no biochemical precedence and represents a novel utilization of nicotinic acid to afford dioscorine.[6] It is suggested that nicotinic acid (**5**) is reduced to 3,6-dihydronicotinic acid (**6**). Reaction between the 3-position of **6** and the keto group of a branched 8-carbon unit derived from acetic acid affords compound **8**.[7] Decarboxylation, shift of a double bond in the dihydropyridine ring, and further reduction of the ring yield **7**. An aldol condensation then leads to **9** which contains the isoquinuclidine ring system. Decarboxylation, N-methylation, and lactone formation then afford dioscorine.

This hypothesis was tested by feeding [2-^{14}C]nicotinic acid[8] (32.6 mg, 8.05×10^8 dpm/mmol) to *D. hispida* plants by the wick method. After 7 weeks the plants were harvested[2] and yielded dioscorine (85 mg, 1.03×10^7 dpm/mmol, 1.9% absolute incorporation[9]). This radioactive alkaloid was degraded[2] affording 2-methyl-5-oxoisoquinuclidine (**4**) (9.7×10^7 dpm/mmol). There had thus been a specific incorporation of nicotinic acid into the isoquinuclidine moiety of dioscorine. If the hypothesis is correct, the dioscorine derived from [2-^{14}C]nicotinic acid should be labeled specifically at C-3, and further degradation of **4** is in progress to establish the validity of our scheme.

In the meantime, however, it seemed feasible to examine the biosynthesis of dioscorine using a presursor containing contiguous ^{13}C atoms. The direct incorporation of such a labeled compound into dioscorine would afford satellite peaks in its ^{13}C NMR, due to spin–spin coupling, symmetrically located about the corresponding singlet peaks arising from isolated ^{13}C atoms.[10] Accordingly [5,6-^{14}C, $^{13}C_2$]nicotinic acid[15] (28.7 mg, 6.52×10^8 dpm/mmol) was fed (September, 1976) as before to one *D. hispida* plant. After 5 weeks the tubers and roots were extracted to yield dioscorine (348 mg, 2.75×10^6 dpm/mmol, 2.9% abs. inc.). The proton noise decoupled ^{13}C NMR spectrum of dioscorine was determined,[18] and the following chemical shifts (parts per million from Me_4Si) were assigned by continuous wave off-resonance decoupling and comparison with model compounds (the assigned carbons numbered as in formula **2**) 165.1 (12), 155.9 (10), 116.5 (11), 81.6 (5), 53.8

Scheme II. Hypothetical Biosynthesis of Dioscorine from Nicotinic Acid (heavy dots indicate the expected positions of labeling in dioscorine derived from [5,6-$^{13}C_2$]nicotinic acid)



(3), 52.4 (1), 42.6 (NCH_3), 40.9 (9), 39.5 (6), 35.2 (4), 23.3 (13), 20.3 (7), 19.5 (8). Satellites of C-1 and C-7 were clearly seen in the radioactive dioscorine isolated from the plant. The ^{13}C–^{13}C coupling constants for the satellites at C-1 and C-7 were 33.4 ± 0.2 Hz and 33.3 ± 0.2 Hz, respectively.[19] The specific incorporation of nicotinic acid into dioscorine, determined from the intensity of the satellite peaks relative to the centrally located singlet[12] was 0.37%, in excellent agreement with the incorporation determined from radioactive assay (0.42%).

These results are thus consistent with our proposed scheme for the biosynthesis of dioscorine, which we realize can be subjected to considerable modification regarding the order in which the various transformations of nicotinic acid occur.

Acknowledgments. I thank Professor A. R. Pinder, Clemson University, South Carolina, for generous supplies of dioscorine, compound **4**, and *Dioscorea hispida* tubers which were obtained from Malaya via the Tropical Products Institute, London. The ^{13}C NMR spectra were enthusiastically obtained by Dr. Robert Riddle of the University of Minnesota. This investigation was supported by a research grant GM-13246 from the U.S. Public Health Service.

References and Notes

(1) See C. B. Page and A. R. Pinder, *J. Chem. Soc.*, 4811–4816 (1964), and the references cited therein for the synthesis and ultimate structure determination of dioscorine.
(2) E. Leete and A. R. Pinder, *Phytochemistry*, 11, 3219–3224 (1972).
(3) E. Leete, *J. Am. Chem. Soc.*, **91**, 1697–1700 (1969).
(4) J.-C. Braekman, R. N. Gupta, D. B. MacLean, and I. D. Spenser, *Can. J. Chem.*, **50**, 2591–2602 (1972).
(5) Unpublished work of the author, reported: E. Leete, *Biosynthesis*, **2**, 106–182 (1973).
(6) In retrospect it should be mentioned that this scheme was conceived in the summer of 1974, on the beach in Atlantic City, after we had made the discovery that both heterocyclic rings of anatabine, a totally unrelated tobacco alkaloid, are derived from nicotinic acid: E. Leete, *J. Chem. Soc., Chem. Commun.*, 9–10 (1975); E. Leete and S. A. Slattery, *J. Am. Chem. Soc.*, **98**, 6326–6330 (1976).
(7) This initial reaction is analogous to our current hypothesis regarding the origin of nicotine, in which we propose that condensation takes place

between 3,6-dihydronicotinic acid and an *N*-methyl-Δ^1-pyrrolinium salt.
(8) Prepared from [1-^{14}C]aniline (ICN, Calif.) by the method of D. Gros, A. Felge, and H. R. Schütte, *Z. Chem.*, **5**, 21 (1965).
(9) Absolute incorporation is defined as the total activity found in the isolated alkaloid divided by the total activity fed.
(10) This method has been used to elucidate pathways for the biosynthesis of numerous microbial metabolites derived from [1,2-$^{13}C_2$]acetate,[11] tropic acid[11] and tenellin[12] derived from [1,3-$^{13}C_2$]phenylalanine, ovalicin derived from [3,4-$^{13}C_2$]mevalonate,[13] and porphyrins derived from [2,11-$^{13}C_2$] porphobilinogen.[14] Since the natural abundance of contiguous carbon-13 atoms is only 0.01%, quite low specific incorporations of such labeled precursors can be detected.
(11) See ref 14 in E. Leete, N. Kowanko, and R. A. Newmark, *J. Am. Chem. Soc.*, **97**, 6826–6830 (1975).
(12) E. Leete, N. Kowanko, R. A. Newmark, L. C. Vining, A. G. McInnes, and J. L. C. Wright, *Tetrahedron Lett.*, 4103–4106 (1975).
(13) D. E. Cane and R. H. Levin, *J. Am. Chem. Soc.*, **98**, 1183–1188 (1976).
(14) A. R. Battersby, E. Hunt, and E. McDonald, *J. Chem. Soc., Chem. Commun.*, 442–443 (1973).
(15) Prepared by the sequence: [^{14}C]methyl iodide + [^{13}C]methyl iodide → [^{14}C,^{13}C]nitromethane[16] → [2,3-^{14}C,^{13}C]-3-aminoquinoline[17] → [2,3-^{14}C,^{13}C]quinoline → [5,6-^{14}C,^{13}C]nicotinic acid. Analysis by mass spectrometry indicated the presence of 55% [5,6-$^{13}C_2$]-, 19% [5-^{13}C]-, 19% [6-^{13}C]-, and 7% unlabeled nicotinic acid.
(16) J. C. Sowden, *J. Biol. Chem.*, **180**, 55–58 (1949).
(17) E. Leete, G. B. Bodem, and M. F. Manuel, *Phytochemistry*, 10, 2687–2692 (1971).
(18) The Fourier transform ^{13}C NMR spectra were determined in either $CDCl_3$ or benzene-d_6 in a Varian XL 100 spectrometer (25.2 MHz). The enriched dioscorine (240 mg in 0.3 ml of C_6D_6 in a 5-mm tube) was run with an acquisition time of 2.0 s (0.5 Hz/data point), only 5000 transients being required to observe the satellites at C-1 and C-7.
(19) This is the expected ^{13}C–^{13}C coupling constant for an sp^3–sp^3 bond, cf. J. B. Stothers, "Carbon-13 NMR Spectroscopy", Academic Press, New York, N.Y., 1972, p 327.

Edward Leete

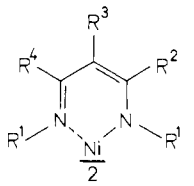
Contribution No. 146, Natural Products Laboratory
School of Chemistry, University of Minnesota
Minneapolis, Minnesota, 55455

Received November 2, 1976

Stereochemistry of Tetrahedral Nickel Complexes. Configurational Rigidity and Stereochemical Analyses

Sir:

We wish to demonstrate that paramagnetic, (pseudo)tetrahedral nickel complexes of the chelate type **1** can be chiral and configurationally stable on the NMR time scale. We observed two ^{1}H NMR signals of equal intensity for the β-hydrogen atoms of the ethyl group in **2**. This nonequivalence indicates a chiral environment for the β-protons. Ethyl rotation is not sufficiently restricted[1,2] as the β-shifts of all such complexes are similar.[1] Since signal coalescence does not occur in **2** below 147 °C, configurational inversion at nickel requires $\Delta G^{\ddagger} \geq 21.8$ kcal/mol (420 K in $(Cl_2CD)_2$). The analogous β-splitting in **1a** yields $\Delta G^{\ddagger} > 20$ kcal/mol (407 K in $(Cl_2CD)_2$). Its counterparts **1b** and **1c**[1] do not show any nonequivalence because of their D_{2d} symmetry.



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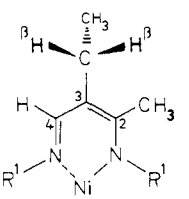


	R^1	R^2	R^3	R^4
1a	C_6H_5	n-C_3H_7	C_2H_5	H
b	C_6H_5	n-C_3H_7	C_2H_5	n-C_3H_7
c	C_6H_5	H	C_2H_5	H
d	H	CH_3	H	CH_3

2, $R^1 = C_6H_5$

3, $R^1 = CH_3$

Complex **3** with NCH_3 instead of phenyl substituents exhibits similar β-splitting (and full paramagnetism) with $\Delta G^{\ddagger}$ = 17.3 kcal/mol (358 K in cyclohexane) and $\Delta S^{\ddagger}$ = about −30 eu. As usual, the tetrahedral ground state is favored by entropy factors. Only **1d** accommodates a diamagnetic (planar) ground state.[3]

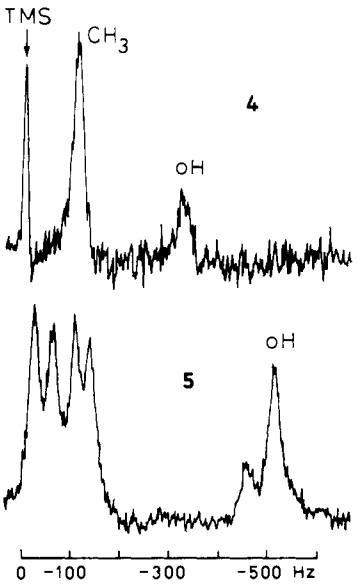
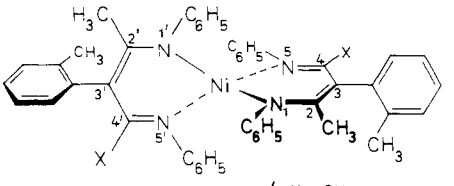
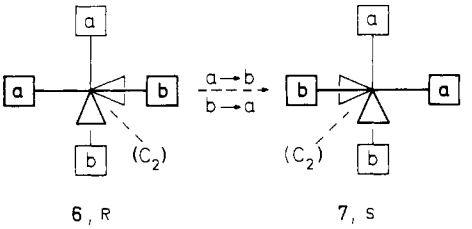


Figure 1. Ortho methyl and ortho hydrogen NMR signals (tolyl group of **4** and **5**) at 60 MHz and −39 °C in $DCCl_3$.

A different case with symmetric ligands is presented with full geometry in **4** (X = CH_3). The axis $C^3NiC^{3\prime}$ constitutes the only chirality element here. Projection **6** with *R* configuration[4,5] results by viewing **4** along this axis from the right. The front chelate ring shows up as a horizontal bar, with the asymmetric arrangement of its *o*-tolyl substituent at C^3 represented as a wedge below the bar.[6] The more distant chelate ring projects vertically in **6** with its *o*-tolyl at $C^{3\prime}$ as a wedge to the right. Methyl groups at C^2, $C^{2\prime}$, C^4, and $C^{4\prime}$, or else the four anilino moieties, are presented as squares. A C_2 axis, passing through Ni, bisects the distances $N^1N^{1\prime}$ and $N^5N^{5\prime}$. Therefore, only one ^{1}H NMR signal is found in the upper trace of Figure 1 for the two *o*-CH_3 substituents (◁). The para hydrogen atoms of the anilino groups show two equally intense signals (a and b) in tetralin up to 180 °C. Rotations by 180° of the rear tolyl substituent or of the rear ligand as a whole (i.e., nickel inversion) would produce enantiomer **7** from **6** with interchanged chemical shifts a and b. Therefore, *both* kinds of motion are slow and hence $\Delta G^{\ddagger} \geq 23.8$ kcal/mol (453 K) for nickel inversion.



4, X = CH_3
5, X = H



6, R 7, S

Complex **5** (X = H) may be used to examine the stereochemical consequences more generally; all its possible isomerizations are symbolized in Figure 2. Projection **8** results as before by viewing **5** from the right. Circles were drawn for X = H, all other notations being taken over from **6**.[6] For sym-