Bijen, and T. F. Spande, *J. Chem. Soc., Chem. Commun.*, 768 (1975).
(3) University of Nijmegen.
(4) National Institutes of Health.
(5) For a review, see A. Taylor in "Microbial Toxins", Vol. VII, S. Kadis, A. Ciegler, and S. J. Ajl, Ed., Academic Press, New York, N.Y., 1971, p 337.
(6) A. Kato, T. Saeki, S. Suzuki, K. Ando, G. Tamura, and K. Arima, *J. Antibiot.*, **22**, 322 (1969); A. D. Argoudelis and F. Reusser, *ibid.*, **24**, 383 (1971); K. H. Michael, M. O. Chaney, N. D. Jones, M. M. Hoehn, and R. Nagarajan, *ibid.*, **27**, 57 (1974); R. L. DeVault and W. Rosenbroek, Jr., *ibid.*, **26**, 532 (1973); G. M. Strunz, M. Kakushima, M. A. Stillwell, and C. J. Heissner, *J. Chem. Soc., Perkin Trans. 1*, 2600 (1973); F. Dorn and D. Arigoni, *Experientia*, **30**, 134 (1974).
(7) (a) P. W. Trown, *Biochem. Biophys. Res. Commun.*, **33**, 402 (1968); (b) H. Poisel and U. Schmidt, *Chem. Ber.*, **104**, 1714 (1971); E. Oehler, H. Poisel, F. Tateruch, and U. Schmidt, *ibid.*, **105**, 635 (1972); (c) T. Hino and T. Sato, *Chem. Pharm. Bull.*, **22**, 2866 (1974); (d) S. G. Svokos and R. B. Angier, *Chem. Abstr.*, **74**, 53845 (1971); (e) J. Yoshimura, H. Nakamura, and K. Matsunari, *Bull. Chem. Soc. Jpn.*, **48**, 605 (1975); (f) G. M. Strunz and M. Kukushima, *Experientia*, **30**, 719 (1974); (g) Y. Kishi, T. Fukuyama, and S. Nakatsuka, *J. Am. Chem. Soc.*, **95**, 6490 (1973); (h) P. J. Machin and P. G. Sammes, *J. Chem. Soc., Perkin Trans. 1*, 698 (1974).
(8) Y. Kishi, T. Fukuyama, and S. Nakatsuka, *J. Am. Chem. Soc.*, **95**, 6492 (1973).
(9) H. C. J. Ottenheijm, T. F. Spande, and B. Witkop, *J. Am. Chem. Soc.*, **95**, 1989 (1973).
(10) H. Leuchs, A. Heller, and A. Hoffmann, *Ber.*, **62**, 871 (1929).
(11) T. Wieland and D. Grimm, *Chem. Ber.*, **98**, 1727 (1965).
(12) M. Bergmann and K. Grafe, *Z. Physiol. Chem.*, **187**, 187 (1930); R. B. Herbst, *J. Am. Chem. Soc.*, **61**, 483 (1939).
(13) H. C. J. Ottenheijm and J. H. M. de Man, *Synthesis*, 163 (1975).
(14) This term has been proposed by Y. Izumi, *Angew. Chem., Int. Ed. Engl.*, **10**, 871 (1971).
(15) J. Häusler and U. Schmidt, *Chem. Ber.*, **107**, 2804 (1974).
(16) (a) B. Bycroft and G. R. Lee, *J. Chem. Soc., Chem. Commun.*, 988 (1975); (b) J. A. Marshall, T. F. Schlaf, and J. G. Csernansky, *Synth. Commun.*, **5**, 237 (1975).
(17) H. Poisel and U. Schmidt, *Chem. Ber.*, **108**, 2917 (1975).
(18) See ref 7h and also A. L. Love and R. K. Olson, *J. Org. Chem.*, **37**, 3431 (1972).
(19) Chelation of zinc ions through thiols is well known for peptide enzymes; see, e.g., P. C. Jocelyn, "Biochemistry of the SH Group", Academic Press, New York, N.Y., 1972, p 84.
(20) (a) See, e.g., E. Sletten, *J. Am. Chem. Soc.*, **92**, 172 (1970). (b) For detailed discussions on the stereochemistry and unpredictable thermodynamic stability of dimercaptodioxopiperazines see ref 7b and 15.
(21) For a review on the role of metal ions in thiol oxidation, see ref 19, Chapter 4.
(22) J. W. Kimball, R. L. Kramer, and E. E. Reid, *J. Am. Chem. Soc.*, **43**, 1199 (1921).
(23) S. Safe and A. Taylor, *J. Chem. Soc. C*, 1189 (1971); see also T. Sato and T. Hino, *Tetrahedron*, **32**, 507 (1976).
(24) (a) A similar reaction has been found by Murdock; see (b) K. C. Murdock, *J. Med. Chem.*, **17**, 827 (1974).
(25) (a) J. O. Clayton and D. H. Etzler, *J. Am. Chem. Soc.*, **69**, 974 (1947); (b) B. Holmberg, *Justus Liebigs Ann. Chem.*, **359**, 81 (1908); see also ref 7b.
(26) R. Rahman, S. Safe, and A. Taylor, *J. Chem. Soc. C*, 1665 (1969); see also ref 7c.
(27) These tests were kindly performed by Dr. H. P. J. Bloemers, Department of Biochemistry, University of Nijmegen. The methods used are described in H. P. J. Bloemers and A. van der Horst, *FEBS Lett.*, **52**, 141 (1975).
(28) Personal communication, S. Mizutani and H. M. Temin, McArdle Laboratory for Cancer Research, University of Wisconsin, Madison, Wis.
(29) H. C. J. Ottenheijm, J. A. M. Hulshof, and R. J. F. Nivard, *J. Org. Chem.*, **40**, 2147 (1975).
(30) This test was kindly performed by Dr. R. N. H. Konings, Department of Microbiology, University of Nijmegen. The method used is described in R. N. H. Konings, T. Hulsebos, and C. A. van den Handel, *J. Virol.*, **15**, 570 (1975).

A New Systematic Degradation of Nicotine to Determine Activity at C-2′ and C-5′. The Pattern of Labeling in Nicotine and Nornicotine Formed from [2-^{14}C]Ornithine in *Nicotiana glutinosa*, and in Nicotine Obtained from *N. tabacum* Exposed to [^{14}C,^{13}C]Carbon Dioxide

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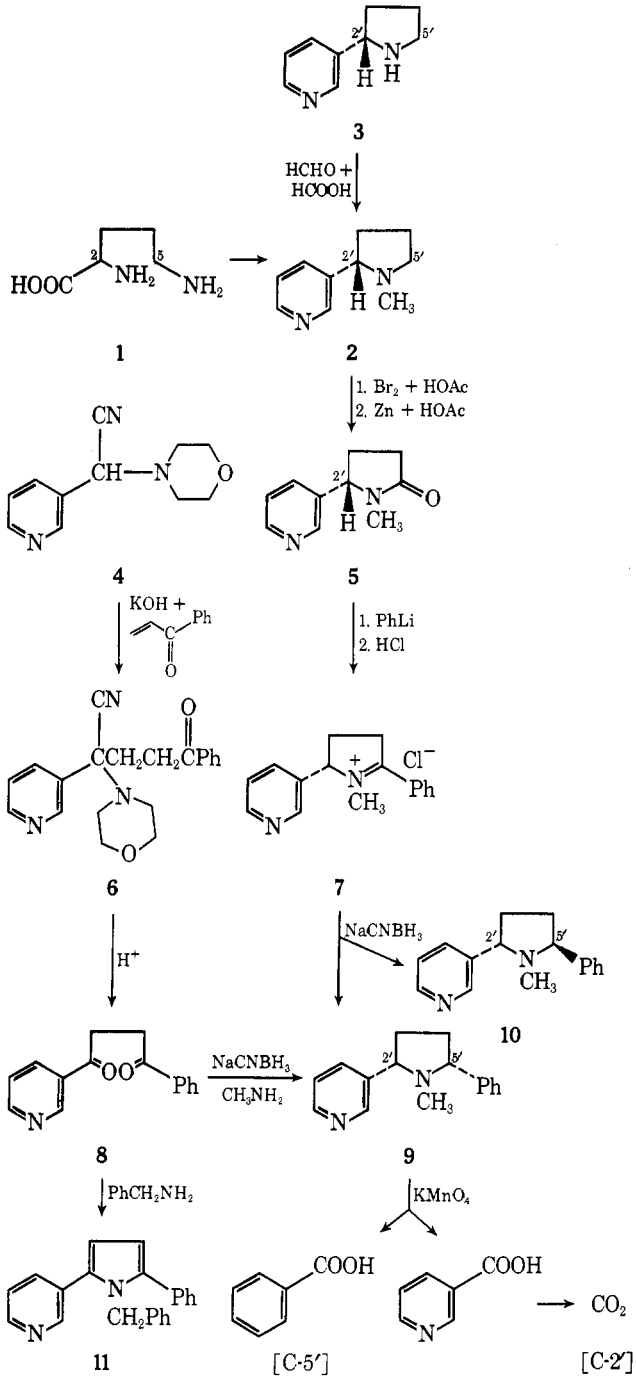
Radioactive nicotine has been degraded by the following sequence: nicotine → cotinine → *cis*-5′-phenylnicotine → benzoic acid [C-5′] + nicotinic acid → barium carbonate [C-2′]. The structure of 5′-phenylnicotine was confirmed by an unambiguous synthesis. On applying this degradation to nicotine and nornicotine isolated from *N. glutinosa* plants which had been fed [2-^{14}C]ornithine, equal labeling was found at C-2 and C-5′ of the pyrrolidine ring of both these alkaloids. Nicotine isolated from *N. tabacum* plants which had been exposed to [^{14}C,^{13}C]carbon dioxide also had equal labeling at C-2′ and C-5′. All these results are thus consistent with the formation of the pyrrolidine ring of nicotine and nornicotine from ornithine via a symmetrical intermediate.

It is more than 20 years since Byerrum[2] and I[3] first reported that ornithine (1) is a precursor of the pyrrolidine ring of nicotine (2). By chemical degradations,[4,5] it was established that [2-^{14}C]ornithine yielded nicotine equally labeled at C-2′ and C-5′.[6] These results led to the proposal that the pyrrolidine ring is formed from ornithine via putrescine, *N*-methylputrescine, and an *N*-methyl-Δ^1-pyrrolinium salt.[7] Indeed, enzymes which carry out these metabolic steps have been isolated from tobacco roots.[8] Symmetrical labeling of the pyrrolidine ring is a result of the intermediacy of free putrescine, a symmetrical compound. However, Rapoport and co-workers,[6,9] on the basis of several short-term feeding experiments with $^{14}CO_2$, have suggested that the formation of nicotine from ornithine, via a symmetrical intermediate, may be a minor or aberrant pathway. This proposal was made since, on occasions,[10] the exposure of tobacco plants to $^{14}CO_2$ led to unsymmetrical labeling of the pyrrolidine ring. In particular, unequal labeling was reported at C-2′ and C-5′. On the other hand, Byerrum and co-workers[11] found symmetrical labeling in the pyrrolidine ring of nicotine obtained from *N. glutinosa* and *N. rustica* plants fed $^{14}CO_2$.

It is generally accepted that nicotine is a precursor of nornicotine (3).[12,13] However, the pattern of labeling in nornicotine after feeding [2-^{14}C]ornithine to tobacco has been reported in only one publication,[14] and in this case it was claimed that the pyrrolidine ring of nornicotine was unsymmetrically labeled.[15]

In view of these conflicting results, and possible errors,[16,17] in the methods used for determining the pattern of labeling in the pyrrolidine ring of nicotine, we have now developed a

Scheme I. Degradation of Nicotine and Nornicotine



new degradative scheme, illustrated in Scheme I, whereby the activity at C-2′ and C-5′ can be unambiguously determined. Bromination of natural (−)-(2′*S*)-nicotine yielded 4′,4′-dibromocotinine which on reduction with zinc afforded (−)-(2′*S*)-cotinine (**5**).[18] Phenyllithium reacted with cotinine in tetrahydrofuran at −78 °C, presumably yielding, after acidification with hydrochloric acid, 1-methyl-2-phenyl-5-(3-pyridyl)-Δ^1-pyrrolinium chloride (**7**).[19] Reduction of this compound, without isolation, with sodium cyanoborohydride afforded a mixture of *cis*-(2′S)-5′-phenylnicotine (**9**) and *trans*-(2′S)-5′-phenylnicotine (**10**) in a ratio of 14:1, readily separated by TLC. Structures were assigned on the basis of their optical rotations, the cis isomer having the smaller specific rotation. The predominance of the cis isomer was expected since the bulky cyanoborohydride anion would approach the pyrrolinium salt **7** from the less hindered side. Reduction of **7** with sodium borohydride afforded a greater proportion of the trans isomer.[20] The structure of 5′-phenylnicotine was confirmed by an independent synthesis, also illustrated in Scheme I. Michael addition of the anion of α-morpholino-α-(3-pyridyl)acetonitrile (**4**)[21] to phenyl vinyl ketone afforded compound **6**, which on acid hydrolysis yielded 1-phenyl-4-(3-pyridyl)butane-1,4-dione (**8**). The structure of this diketone was confirmed by the formation of 1-benzyl-2-phenyl-5-(3-pyridyl)pyrrole (**11**) by reaction with benzylamine. Reductive amination of this diketone with methylamine and sodium cyanoborohydride[22] afforded racemic *cis*-5′-phenylnicotine as the major product, having an infrared spectrum identical with that of the optically active compound derived from (2′*S*)-cotinine. Oxidation of *cis*-5′-phenylnicotine with permanganate yielded a mixture of benzoic acid (representing the activity at C-5′ of nicotine) and nicotinic acid, readily separated on the basis of their solubilities in ether and dilute acid (see Experimental Section). Refluxing the nicotinic acid in quinoline in the presence of copper chromite yielded carbon dioxide (representing C-2′) and was collected as barium carbonate.[23] Heating nicotinic acid with calcium oxide afforded pyridine, assayed as its picrate. Activity at C-2′ was thus determined directly, and by the difference in activity between nicotinic acid and pyridine picrate.

This degradative scheme was carried out on nicotine and nornicotine obtained from *N. glutinosa* plants which were fed (*RS*)-[2-^{14}C]ornithine for 7 days. The nornicotine was converted to nicotine by the Eschweiler–Clark method.[12] The results recorded in Table I clearly indicate that the pyrrolidine rings of both nicotine and nornicotine were symmetrically labeled, equal activity being found at C-2′ and C-5′. We have also carried out this degradation on labeled nicotine obtained from *N. tabacum* plants which were fed $^{13}CO_2$ [97% ^{13}C] containing a tracer amount of $^{14}CO_2$.[24] This nicotine was also found to have equal labeling at C-2′ and C-5′.

We thus consider that these results corroborate previous work on the origin of the pyrrolidine ring of nicotine, and support the hypothesis that it is formed from ornithine via a symmetrical intermediate.

Experimental Section[25]

Conversion of Nicotine to Cotinine. The following oxidation is a modification of that previously described,[18] carrying out the reactions on a small scale. Nicotine diperchlorate (1.0 g) was dissolved in 80% (by volume) acetic acid (3 ml) and cooled to 0 °C, and a solution of bromine (1.2 ml) in 80% acetic acid (3 ml) slowly added with stirring during 1 h. The mixture was stirred overnight while the temperature was allowed to rise to room temperature. Water (10 ml) was added and the mixture heated on a steam bath until a clear red solution was obtained (i.e., until excess bromine had vaporized). On slow cooling dibromocotinine hydrobromide perbromide separated as orange needles (1.3 g). Zinc dust (1.5 g) was added, during 0.5 h, to a stirred suspension of this dibromo derivative in a mixture of water (10 ml), acetic acid (10 ml), and concentrated HCl (0.5 ml) at 20 °C. After stirring overnight, the filtered mixture was made basic with concentrated NH_3 and extracted with chloroform. The residue obtained on evaporation of the dried ($MgSO_4$) extract was distilled (140 °C, 0.01 mm) affording cotinine as a colorless, viscous oil (0.36 g, 74%).

Phenylation of (−)-(2′*S*)-Cotinine. Cotinine (3.25 g, 19 mmol) dissolved in tetrahydrofuran (10 ml) was added rapidly, under N_2, to a stirred ether solution of phenyllithium (20 mmol), prepared from bromobenzene (2.1 ml), lithium ribbon (0.28 g), and ether (10 ml), at −78 °C. After stirring for 1 h at −78 °C the mixture was allowed to warm up to room temperature during 3 h. Concentrated HCl (3 ml) was then added, and the mixture evaporated to small volume. The residue was dissolved in methanol (30 ml), sodium cyanoborohydride (2 g) added, and the mixture stirred at room temperature for 18 h. The solution was then evaporated to dryness, and the residue suspended in 5% NaOH and extracted with chloroform. The residue obtained on evaporation of this extract was dissolved in ether and extracted with 2 N HCl (3 × 20 ml). This acid extract was made basic with NaOH and extracted with chloroform. Evaporation of the dried ($MgSO_4$) extract yielded an oil which was subjected to preparative TLC on several plates of silica gel PF-254 (Merck), developing with a mixture of chloroform, ethanol, and concentrated NH_3 (200:10:1).

Table I. Activities of the Degradation Products of Nicotine and Nornicotine

	Orgin of the alkaloids					
	From *N. glutinosa* fed (*RS*)-[2-^{14}C]ornithine				From *N. tabacum* fed [^{14}C,^{13}C] CO_2[24]	
	Nicotine		Nornicotine		Nicotine	
	Specific activity, dpm/mmol $\times 10^{-5}$	Relative specific activity	Specific activity dpm/mmol $\times 10^{-5}$	Relative specific activity	Specific activity dpm/mmol $\times 10^{-6}$	Relative specific activity
Nornicotine dipicrate			1.08 ± 0.02[a]	103		
Nicotine diperchlorate	3.12 ± 0.05	100	1.05 ± 0.02	100	1.30 ± 0.01	100
Cotinine dipicrate	3.18 ± 0.05	102	1.00 ± 0.02	95	1.31 ± 0.01	101
cis-5′-Phenylnicotine	3.13 ± 0.05	100	1.02 ± 0.03	97	1.31 ± 0.01	101
Nicotinic acid[b]	1.57 ± 0.03	50	0.50 ± 0.01	48	0.86 ± 0.01	66
Pyridine picrate	<0.02	0	<0.01	0	0.77 ± 0.01	59
Barium carbonate [C-2′]	1.54 ± 0.03	49	0.49 ± 0.01	47	0.087 ± 0.002	6.7
Benzoic acid [C-5′]	1.59 ± 0.03	51	0.48 ± 0.01	46	0.090 ± 0.002	6.9

[a] Standard deviation from the mean as determined from the average of at least three samples. [b] Obtained by two independent reactions: by the oxidation of nicotine,[23] and by the oxidation of *cis*-5′-phenylnicotine.

The zone with the highest R_f (0.8) was extracted with methanol affording *cis*-(2′*S*)-5′-phenylnicotine as a colorless, sweet-smelling oil, reminiscent of moist woods in the spring (1.24 g, 27%): $[\alpha]^{22}D$ −9.3°, $[\alpha]^{22}_{365}$ −67.2° (*c* 4.5, MeOH); uv (95% EtOH) λ_{max} 258 nm (ϵ 3690), 262 (3880), sh 268 (2960), in H^+ solution 260 (4960); mass spectrum *m/e* (rel intensity) 238 (36) M^+, 161 (100) M^+ − Ph, 160 (95) M^+ − pyridyl. In contrast to nicotine, this phenyl derivative is sparingly soluble in cold water. It afforded a diperchlorate as colorless plates from ethanol–ethyl acetate, mp 232–233 °C.

Anal. Calcd for $C_{16}H_{20}Cl_2N_2O_8$: C, 43.75; H, 4.59; N, 6.38. Found: C, 44.06; H, 4.82; N, 6.29.

Its dipicrate was obtained as fine yellow needles from ethanol, mp 152–153 °C.

Anal. Calcd for $C_{28}H_{24}N_8O_{14}$: C, 48.28; H, 3.47; N, 16.09. Found: C, 48.39; H, 3.64; N, 15.37.

The zone (R_f 0.7) immediately below that of the *cis*-5′-phenylnicotine was extracted with methanol, and yielded on evaporation *trans*-(2′*S*)-5′-phenylnicotine (89 mg, 2%) as a colorless oil: $[\alpha]^{22}D$ −122° (*c* 3.2, MeOH); uv (95% EtOH) λ_{max} 257 nm (ϵ 3540), 262 (3520), sh 268 (2680), in H^+ solution 260 (4870); mass spectrum *m/e* (rel intensity) 238 (30) M^+, 161 (100) M^+ − Ph, 160 (95) M^+ − pyridyl. It yielded a dipicrate, mp 184–185 °C.

Anal. Calcd for $C_{28}H_{24}N_8O_{14}$: C, 48.28; H, 3.47; N, 16.09. Found: C, 48.51; H, 3.62; N, 15.94.

Cotinine (0.62 g, 18%) was recovered from a lower zone (R_f 0.5) of the TLC. In a typical degradation of radioactive nicotine, the phenylation of cotinine was carried out on a 2-mmol scale and resulted in essentially the same yields of *cis*- and *trans*-5′-phenylnicotine. The use of commercially available phenyllithium dissolved in a 70/30 mixture of benzene and ether led to lower yields of phenylated product, and the results were erratic. In preliminary work, the reduction of the intermediate pyrrolinium salt 7 was carried out by refluxing a solution of this salt in ethanol with excess sodium borohydride for 10 min, followed by stirring at room temperature for 2 h. The ratio of the resultant *cis*- and *trans*-5′-phenylnicotine was 2:1.

1-Phenyl-4-(3-pyridyl)butane-1,4-dione (8). Phenyl vinyl ketone[26] (2.64 g, 20 mmol) dissolved in a mixture of ethanol (5 ml) and ether (15 ml) was added slowly under N_2 to a solution of α-morpholino-α-(3-pyridyl)acetonitrile[21] (4.26 g, 20 mmol) in a mixture of ethanol (10 ml) and ether (30 ml) cooled to −78 °C, to which had been previously added 0.5 ml of a methanolic solution of KOH (30%). After stirring for 1 h, the reaction mixture was allowed to warm to room temperature and stirred for an additional 12 h. The residue obtained on evaporation was warmed with a mixture of acetic acid (10 ml), tetrahydrofuran (3 ml), and water (5 ml) for 12 h at 50 °C. The solution was made basic with K_2CO_3 and extracted with chloroform. The residual oil obtained on evaporation of the dried (Na_2SO_4) extract was distilled (140 °C, 0.01 mm) affording a mixture of an oil (mainly pyridine-3-aldehyde) and a white solid (1.6 g, 33%). Crystallization of this solid from a mixture of benzene and ether afforded colorless prisms of the diketone 8: mp 97–98 °C; uv (95% EtOH) λ_{max} 243 nm (ϵ 19 200), sh 270 (4980), sh 278 (3930); ir (KBr) 1678 cm^{-1} (C=O); mass spectrum *m/e* (rel intensity) 239 (21) M^+, 134 (17) $PyrCOCH_2CH_2$–, 133 (10) $PhCOCH_2CH_2$–, 106 (87) PyrCO–, 105 (100) PhCO–, 78 (74) Pyr–, 77 (79) Ph–.

Anal. Calcd for $C_{15}H_{13}NO_2$: C, 75.30; H, 5.48; N, 5.85. Found: C, 75.12; H, 5.63; N, 5.92.

***cis*-(*RS*)-5′-Phenylnicotine** The diketone 8 (239 mg, 1 mmol), methylamine hydrochloride (80 mg, 1.2 mmol), and sodium cyanoborohydride (200 mg) were stirred in methanol (5 ml) at room temperature. The reaction mixture was monitored periodically by TLC. The main product which was being produced was *cis*-5′-phenylnicotine, and only traces of the trans isomer were detected. After 5 days the reaction mixture was evaporated and the residue was suspended in dilute NaOH and extracted with chloroform. The residue obtained on evaporation of the dried ($MgSO_4$) extract was subjected to TLC (using the same solvent system as described previously) affording *cis*-(*RS*)-5′-phenylnicotine (123 mg, 52%) having an ir spectrum (neat) identical with that of *cis*-(2′*S*)-5′-phenylnicotine. It afforded a dipicrate, mp 233–234 °C. In general the melting points of the dipicrates of racemic nicotine derivatives are higher than those of the optically active derivatives. However, the difference in melting point is not usually as dramatic as in this case.

Anal. Calcd for $C_{28}H_{24}N_8O_{14}$: C, 48.28; H, 3.47; N, 16.09. Found: C, 48.16; H, 3.62; N, 15.72.

1-Benzyl-2-phenyl-5-(3-pyridyl)pyrrole (11). The diketone 8 (136 mg) was refluxed in benzene (5 ml) with benzylamine (0.3 ml) for 8 h. The residue obtained on evaporation was sublimed (100 °C, 0.001 mm) and crystallized from a mixture of benzene and ether, yielding the pyrrole 11 (156 mg, 88%) as colorless prisms: mp 116–117 °C; uv (95% EtOH) λ_{max} 304 nm (ϵ 13 350) very similar to that of 1-methyl-2,5-diphenylpyrrole,[27] 306 (18 750); mass spectrum *m/e* (rel intensity) 311 (15) M^+ +1, 310 (54) M^+, 219 (74) M − $PhCH_2$, 91 (100) $PhCH_2$–.

Anal. Calcd for $C_{22}H_{18}N_2$: C, 85.13; H, 5.83; N, 9.02. Found: C, 85.37; H, 6.00; N, 8.74.

Oxidation of *cis*-(2′*S*)-5′-Phenylnicotine. *cis*-(2′*S*)-5′-Phenylnicotine diperchlorate (106 mg) was dissolved in water (5 ml) at 35 °C. Sodium hydroxide (0.5 ml of a 10% solution) and potassium permaganate (150 mg) were added. After stirring for 1 h, additional permanganate (150 mg) was added and stirring continued overnight. Sulfur dioxide was passed into the reaction mixture until a clear solution was obtained. A continuous ether extraction of this solution yielded on evaporation a white solid which was extracted with cold ether (2 × 15 ml). This ether extract was washed with 2 N HCl (2 × 5 ml) and then dried (Na_2SO_4). The residue obtained on evaporation was sublimed affording benzoic acid (18.9 mg, 64%) which was crystallized from hot water. The initial residue, sparingly soluble in cold ether, was sublimed (120 °C, 0.001 mm) affording nicotinic acid (22.8 mg, 77%) which was crystallized from absolute ethanol.

The nicotinic acid was further degraded as previously described.[23] The barium carbonate obtained from the decarboxylation was assayed by dissolving in an aqueous solution of tetrasodium ethylenediamine tetraacetate.[28]

Feeding of (*RS*)-[2-^{14}C]Ornithine to *Nicotiana glutinosa* Plants and Isolation of the Alkaloids. (*RS*)-[2-^{14}C]Ornithine (2.7

mg, 1.56×10^8 dpm, New England Nuclear) dissolved in water was fed by the wick method to 40 3-month-old *N. glutinosa* plants growing in soil in a greenhouse (June). After 7 days the plants (fresh wt 2.8 kg) were harvested (residual activity not absorbed by the plants: 0.07%), macerated with chloroform and concentrated NH_3, and worked up as previously described.[29] The crude alkaloids (1.76×10^6 dpm, 1.1% incorporation) were separated by TLC,[29] affording nornicotine (187 mg), crystallized to constant activity as its dipicrate (1.08×10^5 dpm/mmol), and nicotine (472 mg), assayed as its diperchlorate (3.12×10^5 dpm/mmol). The anabasine (3.1 mg) and anatabine (16.6 mg) purified as their dipicrates had negligible activity ($<10^3$ dpm/mmol).

Registry No.—*RS*-**1**, 616-07-9; **2**, 54-11-5; **2** diperchlorate, 59888-66-3; **3**, 494-97-3; **3** dipicrate, 6255-01-2; **4**, 36740-09-7; **5**, 486-56-6; **5** dipicrate, 59888-69-6; **8**, 49835-54-3; **9**, 59888-67-4; **9** diperchlorate, 59888-68-5; **9** dipicrate, 59951-82-5; **10**, 59951-83-6; **10** dipicrate, 59980-68-6; **11**, 59888-70-9; nicotinic acid, 59-67-6; pyridine picrate, 3480-66-8; barium carbonate, 513-77-9; benzoic acid, 65-85-0; CO_2, 124-38-9; phenyl vinyl ketone, 768-03-6; *cis*-(*RS*)-5′-phenylnicotine, 59951-84-7; *cis*-(*RS*)-5′-phenylnicotine dipicrate, 59951-85-8; benzylamine, 100-46-9.

References and Notes

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(2) L. J. Dewey, R. U. Byerrum, and C. D. Ball, *Biochim. Biophys. Acta*, **18**, 141 (1955).
(3) E. Leete, *Chem. Ind. (London)*, 537 (1955).
(4) E. Leete and K. J. Siegfried, *J. Am. Chem. Soc.*, **79**, 4529 (1957).
(5) (a) B. L. Lamberts, L. J. Dewey, and R. U. Byerrum, *Biochim. Biophys. Acta*, **33**, 22 (1959); (b) P. L. Wu, T. Griffith, and R. U. Byerrum, *J. Biol. Chem.*, **237**, 887 (1962).
(6) More recently it has been shown that very short feedings (6 h) of [2-^{14}C]- or [5-^{14}C]ornithine to *N. glutinosa* plants also afforded nicotine which was symmetrically labeled in its pyrrolidine ring: A. A. Liebman, B. P. Mundy, and H. Rapoport, *J. Am. Chem. Soc.*, **89**, 664 (1967).
(7) E. Leete, *J. Am. Chem. Soc.*, **89**, 7081 (1967).
(8) (a) S. Mizusaki, Y. Tanabe, M. Noguchi, and E. Tamaki, *Plant Cell Physiol.*, **12**, 633 (1971); **14**, 103 (1973); (b) *Phytochemistry*, **11**, 2757 (1972).
(9) M. L. Rueppel, B. P. Mundy, and H. Rapoport, *Phytochemistry*, **13**, 141 (1974).
(10) These results have been discussed in detail: E. Leete, *Biosynthesis*, **4**, 97 (1976).
(11) H. R. Zielke, R. U. Byerrum, R. M. O'Neal, L. C. Burns, and R. E. Koeppe, *J. Biol. Chem.*, **243**, 4757 (1968).
(12) E. Leete and M. R. Chedekel, *Phytochemistry*, **13**, 1853 (1974), and references cited therein.
(13) W. L. Alworth and H. Rapoport, *Arch. Biochem. Biophys.*, **80**, 46 (1959).
(14) S. Mizusaki, T. Kisaki, and E. Tamaki, *Agric. Biol. Chem.*, **29**, 714 (1965).
(15) In two separate experiments (lasting 24 and 30 days) (*RS*)-[2-^{14}C]ornithine was fed to isolated roots of *N. rustica* yielding radioactive nicotine and nornicotine. The nicotine on oxidation yielded nicotinic acid having 49% of the specific activity of the nicotine, all this activity being on the carboxyl group. This result was thus consistent with previous work, indicative of symmetrical labeling of the pyrrolidine ring. However, the nornicotine on oxidation yielded nicotinic acid having 68% of the specific activity of the nornicotine. I believe that this result is suspect since the nornicotine dipicrate, which had been crystallized five times to constant specific activity, was reported to lose half its specific activity when converted to its diperchlorate! The rationalization which the authors offered for the loss of this activity, namely that the plant was producing racemic nornicotine, which then lost activity on dilution with *l*-nornicotine and subsequent crystallization of its diperchlorate, seems improbable.
(16) E. Leete, *Chem. Commun.*, 1524 (1971).
(17) A. A. Liebman, B. P. Mundy, M. L. Rueppel, and H. Rapoport, *J. Chem. Soc., Chem. Commun.*, 1022 (1972); see also footnote 15 in ref 6.
(18) H. McKennis, L. B. Turnbull, E. R. Bowman, and E. Wada, *J. Am. Chem. Soc.*, **81**, 3951 (1959).
(19) Reaction of cotinine with methylmagnesium iodide in boiling benzene yielded (2′*S*)-5′,5′-dimethylnicotine: W. Hankins and A. Burger, *J. Pharm. Sci.*, **59**, 342 (1970).
(20) Sodium borohydride reduction of **7** yielded a 2:1 mixture of the cis and trans isomers. Dr. E. B. Sanders, Philip Morris Research Center, Richmond, Va., has obtained these isomers from cotinine by essentially the same method (private communication).
(21) E. Leete, M. R. Chedekel, and G. B. Bodem, *J. Org. Chem.*, **37**, 4465 (1972).
(22) R. F. Borch, M. D. Bernstein, and H. D. Durst, *J. Am. Chem. Soc.*, **93**, 2897 (1971).
(23) A. R. Friedman and E. Leete, *J. Am. Chem. Soc.*, **85**, 2141 (1963).
(24) This labeled nicotine was kindly given to me by Professor C. R. Hutchinson, School of Pharmacy, University of Wisconsin, The labeled sample used in our degradation was obtained from *N. tabacum* plants exposed to [^{14}C,^{13}C]CO_2 for 14 h, and then allowed to grow for an additional 226 h in a normal atmosphere. The distribution of ^{13}C in this sample was determined by means of ^{13}C NMR: C. R. Hutchinson, M-T. S. Hsia, and R. A. Carver, *J. Am. Chem. Soc.*, **98**, 6006 (1976). Our results for the distribution of ^{14}C in this sample (see Table I) are in good agreement with the distribution of ^{13}C found by Hutchinson: pyridine ring, 62; C-2′, 6; C-5′, 7%.
(25) Melting points are corrected. Elementary analyses were performed by Clark Microanalytical Laboratory, Urbana, Ill. Mass spectra were determined by Dr. Roger Upham and his assistants at the University of Minnesota on an AEI-MS-30 instrument. Optical rotations were determined on a Perkin-Elmer 241 polarimeter. Ultraviolet spectra were determined on a Cary 11 spectrometer. Radioactivity measurements were carried out in a Nuclear Chicago liquid scintillation Mark II counter, using as a solvent dioxane–ethanol with the usual scintillators.[23]
(26) C. Mannich and G. Heilner, *Chem. Ber.*, **55**, 356 (1922).
(27) E. Leete, L. Marion, and I. D. Spenser, *Can. J. Chem.*, **32**, 1116 (1954).
(28) P. O. Larsen, *Int. J. Appl. Radiat. Isot.*, **24**, 612 (1973), with modification of E. Leete and G. B. Bodem, *J. Am. Chem. Soc.*, **98**, 6321 (1976).
(29) E. Leete and S. A. Slattery, *J. Am. Chem. Soc.*, **98**, 6326 (1976).

Aphylline, Epiaphylline, 10,17-Dioxosparteine, Gramine, and Other Unexpected Alkaloids from *Lupinus hartwegii*

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In addition to lupanine (1) and 13-hydroxylupanine, originally reported in flowering plants of *L. hartwegii*, four oxosparteines, aphylline (3), epiaphylline, 13-hydroxyaphylline (virgiline), and 10,17-dioxosparteine, not previously reported in any *Lupinus* species were isolated. 4-hydroxylupanine (nuttaline), α-isolupanine (6), and gramine (7) were also present in addition to five other partially characterized alkaloids. Even though carefully looked for, no sparteines **2**, **5**, **8**, lupinine, or angustifoline were detected.

Lupanine (1), the major alkaloid reported in *Lupinus hartwegii*,[1] is viewed as being an oxidation product of sparteine (**2**).[2-4] However, this plant apparently forms little or no sparteine, but has been genetically crossed with *L. arboreus*, whose alkaloid is sparteine, to produce hybrids whichcan form both **1** and **2**.[5]