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METABOLISM OF NICOTINE IN *NICOTIANA GLAUCA*

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Key Word Index—*Nicotiana glauca*; Solanaceae; nicotine; nornicotine; myosmine; nicotinic acid; alkaloid biosynthesis; alkaloid metabolism.

Abstract—A mixture of (−)-nicotine-[2′-^{3}H] and (±)-nicotine-[2′-^{14}C] was administered to *Nicotiana glauca* plants for 3 days, resulting in the formation of radioactive nornicotine (49·5% incorporation) and myosmine (2·05% incorporation). Negligible activity was detected in anabasine, cotinine, or 3-acetylpyridine, the last two compounds being added as carriers to the harvested plants. The radioactive nornicotine consisted of 48% (−)-nornicotine-[2′-^{14}C,^{3}H] and 52% (+)-nornicotine-[2′-^{14}C]. Thus if (+)-nornicotine is formed from (−)-nicotine the transformation must involve loss of the hydrogen from C-2′. Myosmine is presumably formed from nicotine via nornicotine. However by feeding myosmine-[2′-^{14}C] to *N. glauca* it was shown that the dehydrogenation is not reversible, no activity being detected in nornicotine. Nicotinic acid (0·14% incorporation) was a metabolite of myosmine-[2′-^{14}C]. Essentially all the activity of the nicotinic acid was located on its carboxyl group, indicating that myosmine was a direct precursor.

INTRODUCTION

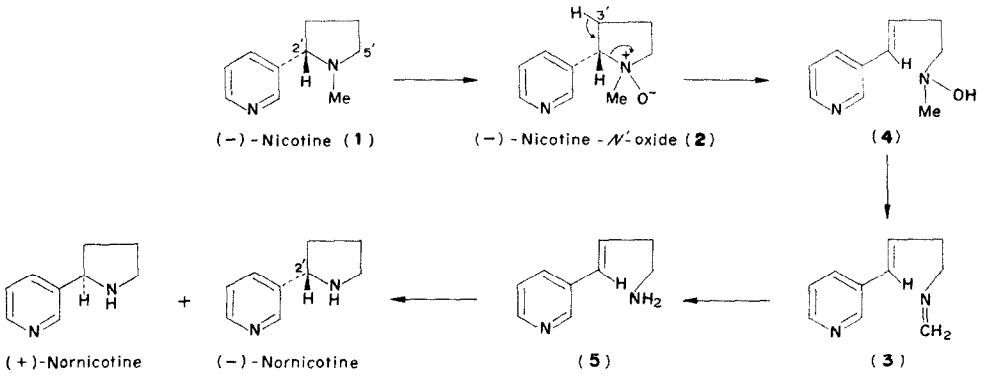
A CONSIDERABLE amount of work has been carried out on the metabolism of nicotine in *Nicotiana* species. Dawson[1] showed that nicotine translocated from the roots of *N. tabacum* was demethylated to nornicotine in an aerial graft of *N. glauca*. Kisaki and Tamaki[2] made the interesting discovery that the unnatural (+)-nicotine[3] is demethylated at a faster rate than (−)-nicotine in *N. tabacum*. It was also found that the nornicotine derived from optically pure (−)-nicotine was partially racemized.[4] This racemization apparently occurs during the demethylation process since optically unchanged (−)-nornicotine was recovered from aerial grafts of *N. tabacum* and *N. glutinosa* on tomato roots which had been fed (−)-nornicotine.[4]

It has been suggested that nicotine demethylation proceeds via its N′-oxide (**2**).[5–8] A plausible mechanism which would account for the partial racemization of the nornicotine derived from (−)-nicotine is illustrated in Scheme 1. A Cope elimination of nicotine-*N*′-oxide involving one of the hydrogens at C-3′ would afford the unsaturated compound **4**.

* Contribution No. 130 from this Laboratory.

[1] DAWSON, R. F. (1945) *J. Am. Chem. Soc.* **67**, 503; (1945) *Am. J. Botany* **32**, 416.
[2] KISAKI, T. and TAMAKI, E. (1961) *Arch. Biochem. Biophys.* **94**, 252.
[3] This enantiomer of nicotine has the *R*-configuration at the C-2′ chiral centre.
[4] KISAKI, T. and TAMAKI, E. (1961) *Arch. Biochem. Biophys.* **92**, 351.
[5] WENKERT, E. (1954) *Experientia* **10**, 346.
[6] CRAIG, J. C., MARY, N. Y., GOLDMAN, N. L. and WOLF, L. (1964) *J. Am. Chem. Soc.* **86**, 3866.
[7] EGRI, L. (1957) *Fachl. Mitt. öster Tabakreg* 19.
[8] ILJIN, G. S. and SEREBROVSKAYA, K. B. (1958) *Doklady Akad. Nauk SSSR* **118**, 139.

Elimination of water from this hydroxylamine yields the Schiff base **3** which on hydrolysis would afford formaldehyde and the primary amine **5**. Cyclization of this intermediate would afford (+)- and (−)-nornicotine. It is presumed that these steps are enzyme-controlled and it is to be expected that the final cyclization would yield a preferential amount of (+)- or (−)-nornicotine. It has been reported[9] that the nornicotine isolated from the roots of *N. tabacum* is mainly the (+)-isomer.

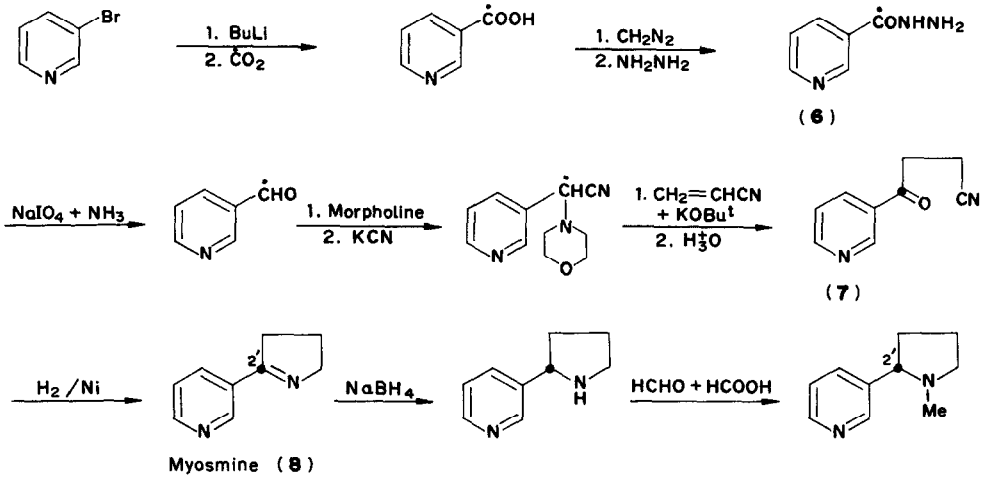


SCHEME 1. HYPOTHESIS FOR THE FORMATION OF (+)- AND (−)-NORNICOTINE FROM (−)-NICOTINE.

RESULTS AND DISCUSSION

In order to investigate this hypothesis we fed a mixture of (−)-nicotine-[2′-^{3}H] and (±)-nicotine-[2′-^{14}C] to *N. glauca* plants. If this hypothesis is correct we would expect tritium to be present at C-2′ in *both* (+)- and (−)-nornicotine. (±)-Nornicotine-[2′-^{3}H] was obtained by reducing myosmine (**8**) with sodium borotrituride. Methylation with formaldehyde and formic acid yielded (±)-nicotine-[2′-^{3}H] which was resolved with (+)-tartaric acid. (±)-Nicotine-[2′-^{14}C] has been previously described,[10,11] however we found the method outlined in Scheme 2 to be economical and afforded (±)-nicotine-[2′-^{14}C] in 17% radiochemical yield from barium carbonate-[^{14}C]. Nicotinic acid-[7-^{14}C], obtained by the carboxylation of 3-lithio-pyridine, was converted to nicotinic acid hydrazide (**6**). Treatment of this hydrazide with sodium metaperiodate yielded pyridine-3-aldehyde[12] which was converted to myosmine as illustrated, using our previously described method.[13] Reduction of the myosmine with sodium borohydride yielded nornicotine which afforded nicotine on methylation with formaldehyde and formic acid.

[9] KISAKI, T. and TAMAKI, E. (1960) *Naturwissenschaften* **47**, 540.
[10] DECKER, K. (1964) *Prep. Bio-Med. Appl. Labeled Mol. Proc. Symp., Venice* 39–44, (1966) cf. *Chem. Abs.*, **64**, 14233.
[11] COMES, R. A., CORE, M. T., EDMONDS, M. D., EDWARDS, W. B. and JENKINS, R. W. (1973) *J. Labelled Comp.* **9**, 253.
[12] WINGFIELD, H. N., HARLAN, W. R. and HANMER, H. R. (1952) *J. Am. Chem. Soc.* **74**, 5796.
[13] LEETE, E., CHEDEKEL, M. R. and BODEM, G. B. (1972) *J. Org. Chem.* **37**, 4465.



SCHEME 2. SYNTHESIS OF NICOTINE-[2'-^{14}C].

Other potential metabolites of nicotine besides nornicotine are myosmine,[14] cotinine,[15] 3-acetylpyridine,[16] and nicotinic acid.[17] Therefore these compounds were added to the *N. glauca* plants at the time of harvesting (3 days after the initial feeding of the radioactive nicotine), in order to trap any activity which might reside in them. The results obtained are summarized in Table 1. Negligible ^{14}C-activity was found in anabasine,[18] cotinine, 3-acetylpyridine, and nicotinic acid.

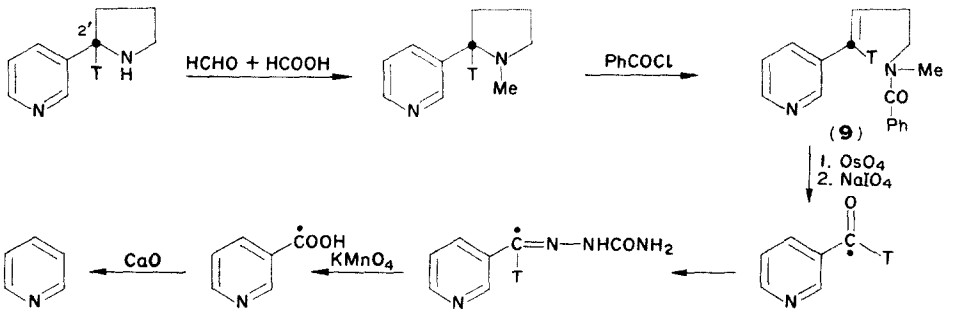
TABLE 1. ALKALOIDS AND RELATED COMPOUNDS ISOLATED FROM *N. glauca* FED (−)-NICOTINE-[2'-^{3}H] + (±)-NICOTINE-[2'-^{14}C] (^{14}C-ACTIVITY: 1·155 × 10^8 dpm/mM, ^{3}H/^{14}C = 6·3)

Alkaloid	Anabasine	Nornicotine	Nicotine	Myosmine	Cotinine	3-Acetyl-pyridine	Nicotinic acid
Wt of carrier (mg)	0	0	0	73	88	200	400
Wt isolated (mg)	267	54·6	13·6	53	37	93	81
Sp. act. (^{14}C) (dpm/mM)	2·0 × 10^3	3·10 × 10^7	2·20 × 10^7	9·5 × 10^5	5·0 × 10^3	0	1·2 × 10^3
^{3}H/^{14}C	11	5·6	7·5	0·05	—	—	—
%Sp. inc (^{14}C)*	0·002	26·8	19·0	NS†	NS†	NS†	NS†
% Absolute inc (^{14}C)‡	0·01	49·5	8·0	2·05	0·01	0	0·02

* The % specific incorporation = sp. act. of the alkaloid × 100/sp. act. of the administered nicotine.
† Not significant since carrier added.
‡ The % absolute incorporation = total activity in the isolated alkaloid × 100/total activity fed to the plant. For myosmine and cotinine the total activity in the isolated alkaloids is calculated on the basis of the amounts of carrier added to the plants at the time of harvesting.

[14] KISAKI, T. and TAMAKI, E. (1966) *Phytochemistry* **5**, 293, isolated myosmine from *N. glutinosa* and *N. tabacum* plants which had been fed (−)-nornicotine.
[15] Cotinine (5'-oxonicotine) is a major metabolite of nicotine in animals, *cf.* MCKENNIS, H. (1965) In: *Tobacco Alkaloids and Related Compounds* (VON EULER, U. S. ed.) p. 53, Pergamon Press, Oxford.
[16] Microorganisms degrade nicotine to 3-acetylpyridine: FRANKENBURG, W. G. and VAITEKUNAS, A. A. (1955) *Arch. Biochem. Biophys.* **58**, 509.
[17] Radioactive nicotinic acid (not degraded) was isolated from *N. rustica* plants which had been fed radioactive nicotine obtained biosynthetically by feeding acetate-[2-^{14}C] or propionate-[2-^{14}C] to tobacco: GRIFFITH, G. P., GRIFFITH, T., and BYERRUM, R. U. (1960) *J. Biol. Chem.* **235**, 3536.
[18] This result is in agreement with previous work: LEETE, E. (1968) *Tetrahedron Letters* 4433.

The major metabolite of nicotine was nornicotine having almost the same $^3H/^{14}C$ ratio as in the administered mixture of (−)-nicotine-[2′-^{3}H] and (±)-nicotine-[2′-^{14}C] indicating that in *N. glauca* (+)- and (−)-nicotine are demethylated at similar rates. If the demethylation had been stereospecific for (−)-nicotine the resultant nornicotine would have had its $^3H/^{14}C$ ratio doubled. The location of ^{14}C and ^{3}H in the nornicotine was established by the degradation illustrated in Scheme 3. A small sample of the radioactive nornicotine was diluted with cold (±)-nornicotine, converted to nicotine, which on heating with benzoyl chloride afforded *N*-benzoylmetanicotine (**9**).[19] Reaction of **9** with osmium tetroxide yielded a diol which was cleaved with sodium metaperiodate affording



SCHEME 3. DEGRADATION OF NORNICOTINE TO DETERMINE THE LOCATION OF ACTIVITY

pyridine-3-aldehyde which was collected and assayed as its semicarbazone. Oxidation of this semicarbazone with potassium permanganate afforded nicotinic acid which was decarboxylated by heating with calcium oxide giving pyridine which was collected as its picrate. The activities of these degradation products are recorded in Table 2, and it is clear that essentially all the ^{14}C and ^{3}H are located at C-2′ of the nornicotine. The optical composition of the nornicotine isolated from the plant was determined by diluting a small portion of the radioactive material ($^3H/^{14}C$ = 5·6) with a relatively large amount of optically pure (−)-nornicotine, converting to its diperchlorate and crystallizing to constant activity. The ^{14}C-specific activity of the resultant (−)- nornicotine diperchlorate indicated that the nornicotine isolated from the plant contained 48% of the (−)-isomer. Furthermore its ^{3}H-specific activity ($^3H/^{14}C$ = 11·7) indicated that >99% of the tritium was located in the (−)-isomer. Thus if any (+)-nornicotine is formed from (−)-nicotine in *N. glauca* the hydrogen at C-2′ in (−)-nicotine is eliminated during this conversion, and the hypothesis illustrated in Scheme 1 is untenable.

A significant amount of activity (2·05%) was detected in myosmine. As expected this alkaloid was almost free of tritium and a degradation indicated that all the ^{14}C was located at C-2′. It was considered that (+)-nornicotine could result from the reduction of myosmine. Therefore myosmine-[2′-^{14}C] was fed to *N. glauca* plants for 5 days. The crude alkaloids isolated from the plant had appreciable activity (23% of the amount administered) however no activity was detected in anabasine, nicotine, or nornicotine, indicating that the formation of myosmine from nicotine, resumably via nornicotine, is not reversible. The recovered myosmine had a specific activity ($8{\cdot}32 \times 10^7$ dpm/mM) significantly lower than the administered myosmine-[2′-^{14}C] ($2{\cdot}51 \times 10^8$ dpm/mM), indicating that myosmine is a normal component of *N. glauca*.[20] Nicotinic acid was added to the plants at the time

[19] LIEBMAN, A. A., MUNDY, B. P. and RAPOPORT, H. (1967) *J. Am. Chem. Soc.* **89**, 664.

[20] FEJÉR-KOSSEY, O. (1972) *Phytochemistry* **11**, 415, has recently detected myosmine in *N. glauca* and *N. tabacum*.

of harvesting. The recovered nicotinic acid was appreciably radioactive (0·14% incorporation), and a degradation indicated that most of its activity was located on the carboxyl group. Kisaki and Tamaki[14] also tentatively identified (by paper chromatography) nicotinic acid as a metabolite of myosmine in *N. tabacum*. Our failure to observe any significant activity in nicotinic acid isolated from plants fed nicotine-[2′-^{14}C] is presumably due to the low level of activity to be expected if the nicotine is metabolized to nicotinic acid via myosmine.

TABLE 2. ACTIVITIES OF NORNICOTINE AND ITS DEGRADATION PRODUCTS

	Activity dpm/mM (^{14}C)	^{3}H/^{14}C
Nornicotine dipicrate	$2{\cdot}61 \times 10^5$	6·1
Nicotine diperchlorate	$2{\cdot}60 \times 10^5$	6·1
N-Benzoylmetanicotine	$2{\cdot}72 \times 10^5$	5·5
Pyridine-3-aldehyde semicarbazone	$2{\cdot}52 \times 10^5$	5·7
Nicotinic acid	$2{\cdot}44 \times 10^5$	no ^{3}H
Pyridine picrate	inactive	—

The mechanism whereby (+)-nornicotine is formed from (−)-nicotine thus remains unknown. We are currently examining the possibility that (−)-nicotine-*N*′-oxide can undergo racemization in the tobacco plant.

EXPERIMENTAL

General methods. A Nuclear Chicago Mark II Liquid Scintillation Counter was used for assay of the radioactive compounds, using either toluene or dioxane–EtOH with the usual scintillators.[21] Assays were carried out in duplicate and were reproducible to 5%. Unless otherwise stated preparative TLC was carried out on Silica Gel PF-254 (E. Merck AG) developing with $CHCl_3$–EtOH-conc. NH_3 (80:20:1)—System A.

(±)-*Nicotine*-[2′-^{14}C]. Butyl lithium (2·0 ml of a C_6H_6 soln containing 6 mM) was diluted with Et_2O (10 ml) and cooled to −78°. 3-Bromopyridine (1·1 g, 7·0 mM) was added and the mixture (under N_2) stirred for 10 min. $^{14}CO_2$ liberated by means of conc. H_2SO_4 from $BaCO_3$-[^{14}C] (0·98 g, 5 mM, 5·0 mCi) was passed into the reaction mixture which was shaken vigorously during the addition. After 30 min. conc. HCl (10 ml) was added and the mixture evaporated to dryness. The residue was dissolved in H_2O, the pH adjusted to 3·0 with NaOH, and extracted with Et_2O in a continuous extractor for 18 hr. The evaporated extract was sublimed (170°, 10^{-3} mm) affording nicotinic acid-[7-^{14}C] (439 mg, 71%). Treatment of this acid with CH_2N_2 in Et_2O yielded methylnicotinate which was refluxed with 95% hydrazine (8 ml) for 15 min. EtOH (8 ml) was then added and the mixture refluxed for an additional 2 hr. Evaporation of the soln yielded nicotinic acid hydrazide (416 mg, 61%) which was dissolved in H_2O (10 ml) and conc. NH_3 (10 ml) and cooled to 0°. $NaIO_4$ (648 mg) dissolved in H_2O (10 ml) and conc. NH_3 (10 ml) was slowly added, N_2 being evolved. After stirring 15 min the mixture was extracted with $CHCl_3$. The dried ($MgSO_4$) extract was evaporated and the residue distilled affording pyridine-3-aldehyde (220 mg, 41%). After dilution, this material was converted to (±)-nornicotine as previously described.[13] The final step in this synthesis involves reduction of 3-cyano-1-(3-pyridyl)propan-1-one (7) with H_2 in the presence of Raney Ni yielding varying amounts of myosmine and nornicotine. It was found convenient to reduce the ketonitrile for only 2 hr and then complete the reduction with $NaBH_4$. The overall yield of nornicotine from pyridine-3-aldehyde was 62%. The (±)-nornicotine-[2′-^{14}C] (148 mg) was heated with 90% HCOOH (3 ml) and 40% HCHO (3 ml) at 100° for 18 hr. The reaction mixture was evaporated to dryness, made basic with NaOH, and extracted with $CHCl_3$. The dried ($MgSO_4$) extract was evaporated and the residue subjected to preparative TLC (System A). Nicotine was extracted from the main zone R_f = 0·6) with MeOH–$CHCl_3$. The residue obtained on evaporation of the extract was distilled yielding (±)-nicotine-[2′-^{14}C] which was converted to its dipicrate (420 mg, 68%) for storage. The overall radiochemical yield from $BaCO_3$-[^{14}C] was 17%.

Myosmine-[2′-^{14}*C*]. Reduction of 3-cyano-1-(3-pyridyl)propan-1-one-[1-^{14}C] with H_2 in the presence of Raney Ni for 2 hr yielded myosmine-[2′-^{14}C] which was purified by preparative TLC. The solid alkaloid, m.p. 45–46°, was obtained on distillation, and was converted to its dipicrate, m.p. 185–186°, for storage.

(−)-*Nicotine*-[2′-^{3}*H*]. $NaBH_4$-[^{3}H] (20 mg, 100 mCi) was added to a stirred soln of myosmine (96 mg) in EtOH (20 ml). After stirring for 18 hr at room temp. the soln was evaporated to dryness. The residue was made basic with N NaOH and extracted with $CHCl_3$. The oil remaining on evaporation of the extract was subjected

[21] FRIEDMAN, A. R. and LEETE, E. (1963) *J. Am. Chem. Soc.* **85**, 2141.

to preparative TLC (System A), nornicotine being extracted from the zone having $R_f = 0.2$. The resultant distilled (±)-nornicotine-[2'-^{3}H] (60 mg) had an activity of 9.3×10^9 dpm (4.2% radiochemical yield). Methylation of this nornicotine as previously described afforded (±)-nicotine-[2'-^{3}H] (40 mg) having an activity of 7.1×10^9 dpm. A portion of this material (15.5 mg, 2.76×10^9 dpm) was dissolved in EtOH (10 ml) followed by *d*-tartaric acid (30 mg) and (−)-nicotine bis-*d*-tartarate.$2H_2O$[22] (450 mg). The solution was taken to dryness and the residue crystallized several times from EtOH–H_2O yielding (−)-nicotine-[2'-^{3}H] bis-(+)-tartarate.$2H_2O$ (183 mg) having a constant sp. act. of 1.45×10^9 dpm/mM.

Feeding of labelled compounds to N. glauca *and isolation of the alkaloids.* (±)-Nicotine-[2'-^{14}C] dipicrate (62 mg, 0.1 mM) was dissolved in 2 N HCl (10 ml) and the soln extracted with Et_2O until colorless. The residue obtained on lyophilization was redissolved in H_2O and assayed [total activity (^{14}C): 2.31×10^7 dpm]. (−)-Nicotine-[2'-^{3}H] bis-*d*-tartarate.$2H_2O$ [49.8 mg, 0.1 mM, total activity (^{3}H): 1.45×10^8] was added to the soln of (±)-nicotine-[2'-^{14}C] and the mixture fed to six 4-month-old *N. glauca* plants growing in soil in a greenhouse, by means of cotton wicks inserted into the stems near to ground level. After 3 days the plants were harvested (residual activity in the beakers which held the radioactive nicotine: 0.04% of the amount fed, $^3H/^{14}C = 6$). The whole plants (fr. wt. 655 g) were macerated in a Waring Blender with $CHCl_3$ (3 l) and conc. NH_3 (200 ml). At the time of maceration myosmine (0.5 mM), (−)-cotinine (0.5 mM), 3-acetylpyridine (200 mg) and nicotinic acid (400 mg) were added to the mixture. The $CHCl_3$ layer was evaporated in the presence of 2 N HCl (200 ml). The resultant aq. soln was filtered, made basic with NH_3 and extracted with CH_2Cl_2. Evaporation of the dried ($MgSO_4$) extract afforded the crude alkaloids (activity (^{14}C): 1.78×10^7 dpm, 77% of the amount fed, $^3H/^{14}C = 6.6$). The alkaloids were subjected to preparative TLC (System A) and the zones corresponding to nornicotine ($R_f = 0.15$), anabasine (0.35), nicotine + cotinine (0.52), myosmine (0.58), and 3-acetylpyridine (0.70) separated and extracted with MeOH–$CHCl_3$ in a Soxhlet. The mixture of cotinine and nicotine was rechromatographed on Silica Gel PF-254 developing with $CHCl_3$–MeOH–HOAc (60:10:1) resulting in a clean separation: nicotine ($R_f = 0.2$), cotinine (0.8). The distilled alkaloids were assayed as their free bases and then converted to their picrates for storage. The 3-acetylpyridine was converted to its oxime, m.p. 116°, for assay. The activity of these alkaloids are recorded in Table 1.

The aq. ammonical layer [activity (^{14}C): 4.6×10^5 dpm, $^3H/^{14}C = 5.4$] was adjusted to pH 3 with HCl and extracted continuously with Et_2O for 2 days. The residue obtained on evaporation of the extract was sublimed (170°, 10^{-3} mm) yielding crude nicotinic acid which was crystallized from EtOH. Myosmine-[2'-^{14}C] dipicrate (60.4 mg, 0.1 mM) was dissolved in 2 N HCl, and the soln extracted with Et_2O until colorless. The soln was lyopholized, redissolved in H_2O, assayed (2.35×10^7 dpm), and fed to six 4-month-old *N. glauca* plants as previously described. After 5 days the plants (fr. wt. 670 g) were harvested and worked up as before, however only nicotinic acid (500 mg) was added as a carrier. The crude alkaloids had an activity of 5.4×10^6 dpm (23% of the amount fed). Chromatography (System A) indicated that essentially all the activity was in a zone corresponding to myosmine. This was extracted with $CHCl_3$ and afforded myosmine dipicrate, m.p. 185–186°, identical (i.r., m.m.p. TLC) with an authentic specimen. It had an activity of 8.32×10^7 dpm/mM (35% sp. incorporation). The nicotinic acid reisolated from the ammoniacal aqueous layer had an activity of 8.2×10^4 dpm/mM (0.14% absolute incorporation). The $BaCO_3$ obtained by decarboxylating this nicotinic acid in boiling quinoline in the presence of copper chromite had an activity of 7.8×10^4 dpm/mM.

Dilution of the radioactive nornicotine with (−)-*nornicotine.* Nornicotine dipicrate [7.82 mg, activity (^{14}C): 3.10×10^7 dpm/mM] was dissolved in 2 N HCl (10 ml) and extracted with Et_2O to remove picric acid. The aq. soln was then made basic with NaOH and extracted with Et_2O (4 × 30 ml). This extract was assayed and had activity (^{14}C): 3.72×10^5 dpm, (^{3}H): 2.08×10^6 dpm, $^3H/^{14}C = 5.6$. (−)-Nornicotine diperchlorate[23] (203 mg) dissolved in EtOH was added to the Et_2O extract and the mixture evaporated, a drop of 70% $HClO_4$ being added to the solution prior to evaporation. The residue was crystallized several times from a mixture of EtOH and EtOAc ultimately yielding (−)-nornicotine diperchlorate having an activity (^{14}C): 865 dpm/mg, (^{3}H): 10100 dpm/mg. $^3H/^{14}C = 11.7$. Thus the ^{14}C due to (−)-nornicotine-[2'-^{14}C] in the original nornicotine = 865 dpm/mg × 205 mg[24] = 1.77×10^5 dpm = 48%. The ^{3}H due to (−)-nornicotine-[2'-^{3}H] in the original nornicotine = 10100 dpm/mg × 205 mg = 2.07×10^6 dpm = 99%.

Degradation of the radioactive nornicotine. Nornicotine was liberated from its dipicrate (29.4 mg, 1.50×10^6 dpm) and diluted with (±)-nornicotine (820 mg) yielding material with a sp. act. (^{14}C): 2.61×10^5 dpm/mM, $^3H/^{14}C = 6.1$. Methylation with HCHO and HCOOH yielded nicotine. This nicotine (800 mg) was heated with PhCOCl (1 ml) at 150–160° for 18 hr. The cooled reaction mixture was added to 2 N NaOH and then extracted with CH_2Cl_2. The CH_2Cl_2 layer was extracted with 2 N HCl (5 × 20 ml) which was adjusted to pH 7 and extracted with CH_2Cl_2. Evaporation of the dried ($MgSO_4$) extract afforded *N*-benzoylmetanicotine (520 mg) as fine colorless needles from hexane, m.p. 77–78°, lit[19] m.p. 78–79°. OsO_4 (254 mg, 1 mM) was added to a soln

[22] PICTET, A. and ROTSCHY, A. (1904) *Ber.* **37**, 1225.

[23] Obtained by demethylation of (−)-nicotine with AgOH according to the procedure of SPÄTH, E., MARION, L. and ZAJIC, E. (1936) *Ber.* **69**, 251. The (−)-nornicotine dipicrate purchased from Fluka AG (Switzerland) was found to be mainly (±)-nornicotine dipicrate.

[24] 205 mg = 203 mg (the cold (−)-nornicotine diperchlorate added) + 2 mg (the amount derived from the initial 7.82 mg of nornicotine dipicrate).

of the *N*-benzoyl-metanicotine (266 mg, 1 mM) in dry Et_2O (100 ml) containing 1–2 drops of pyridine. After standing overnight the soln was evaporated and the residue refluxed with a soln of Na_2SO_3 (1 g) in 50% aq. MeOH (40 ml) for 2 hr. The mixture was filtered hot, evaporated to dryness, and the residue extracted with CH_2Cl_2. The oil obtained on evaporation of the extract was dissolved in H_2O (5 ml) containing a few drops of HOAc. $NaIO_4$ (213 mg, 1 mM) was added, and the mixture shaken at room temp. for 45 min. The mixture was made basic with Na_2CO_3 and extracted with $CHCl_3$ (3 × 20 ml). The pale yellow $CHCl_3$ soln was extracted once with *N* HCl (10 ml). Semicarbazide hydrochloride (150 mg) was added to the HCl solution which was then made basic with Na_2CO_3. On standing the soln deposited fine colorless needles of pyridine-3-aldehyde semicarbazone (27 mg) m.p. 212–214°, identical with an authentic specimen. A diluted sample of this semicarbazone (81 mg) was refluxed with a soln of $KMnO_4$ (250 mg) in H_2O (10 ml) for 3 hr. The reaction mixture was decolorized with SO_2 and extracted with Et_2O. The residue obtained on evaporation of the extract was sublimed affording nicotinic acid (42 mg, 69%). Heating the nicotinic acid with 10 × its wt. of CaO yielded pyridine collected and assayed at its picrate. The activities of these degradation products of nornicotine are recorded in Table 2.

Degradation of the radioactive myosmine derived from (−)-[2′-^{3}H]-(±)-[2′-^{14}C]-nicotine. Myosmine (54 mg, $9{\cdot}5 \times 10^5$ dpm/mM, $^3H/^{14}C = 0{\cdot}05$) was refluxed with a soln of $KMnO_4$ (300 mg) in H_2O (20 ml) for 18 hr. Et_2O extraction of the solution which had been decolorized with SO_2 yielded nicotinic acid (12 mg, $9{\cdot}4 \times 10^5$ dpm/mM, no ^{3}H). Decarboxylation by heating with CaO yielded pyridine assayed as its picrate (inactive).

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