Phytochemistry, 1971, Vol. 10, pp. 2687 to 2692. Pergamon Press. Printed in England.

FORMATION OF 5-FLUORONICOTINE FROM 5-FLUORONICOTINIC ACID IN *NICOTIANA TABACUM**

EDWARD LEETE, GEORGE B. BODEM and MARISSA F. MANUEL

Natural Products Laboratory†, School of Chemistry, University of Minnesota, Minneapolis, Minnesota 55455, U.S.A.

(*Received* 15 *March* 1971)

Abstract—The alkaloids isolated from *Nicotiana tabacum* plants which were fed sub-lethal amounts of 5-fluoronicotinic acid contained 5-fluoronicotine, which was separated from nicotine and other natural alkaloids by thin layer and gas chromatography. It was identical with an authentic specimen of 5-fluoronicotine synthesized by an unambiguous method. In preliminary studies the administration of 5-fluoronicotinic-5,6-^{14}C acid to tobacco yielded radioactive 5-fluoronicotine detected by dilution with inactive 5-fluoronicotine.

INTRODUCTION

SOME micro-organisms are able to utilize unnatural compounds closely related chemically to natural precursors for the synthesis of unnatural products. Pyrrolnitrin (I), an antibiotic produced by a strain of *Pseudomonas aurefaciens* is formed from tryptophan. However the addition of 6-fluorotryptophan or 7-methyltryptophan to the culture medium led to the formation of 4′-fluoropyrrolnitrin and 3′-methyl-3′-dechloropyrrolnitrin respectively.[1] On the other hand 5-fluorotryptophan was not incorporated into the ergot alkaloids produced by *Claviceps purpurea*.[2]

Only a few examples of what may be called 'aberrant syntheses' have been reported in higher plants. Watkin[3] showed that the administration of DL-*p*-fluorophenylalanine-3-^{14}C to *Scutellaria galericulata*, which contains the 7-glucuronide of chrysin, led to the formation of an unnatural compound: 4′-fluorochrysin-2-^{14}C (II). It has been established that many herbicides and pesticides undergo chemical changes in plants, and some of these can be

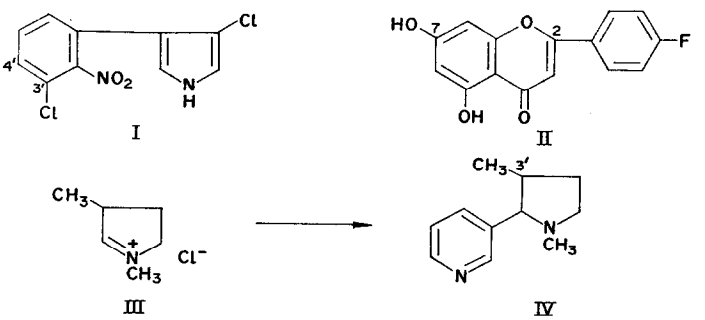

FIG. 1.

* Part I of a series "Aberrant Syntheses in Higher Plants".

† Contribution No. 111 from this Laboratory.

[1] M. GORMAN, R. L. HAMILL, R. P. ELANDER and J. MABE, *Biochem. Biophys. Res. Commun.* **31**, 294 (1968).

[2] W. A. SKINNER, J. J. MORRIS and J. V. STEVENSON, *J. Pharm. Sci.* **56**, 396 (1967).

[3] J. E. WATKIN and D. S. MAGRILL, *4th International Symposium on the Chemistry of Natural Products*, Stockholm, June 26–July 2, 1966, Abstracts p. 146.

classified as aberrant syntheses. For example, Frear[4] has demonstrated the formation of *N*-glucosylarylamines from a variety of arylamines in soybean seedlings (*Glycine max Merril*). The tobacco alkaloid nicotine is formed from nicotinic acid and a 1-methyl-Δ^1-pyrrolinium salt, the exact mechanism of condensation being currently unknown.[5] Recently Rapoport[6] has discovered that the administration of 1,3-dimethyl-Δ^1-pyrrolinium chloride (III) to *Nicotiana glutinosa* led to the formation of the unnatural alkaloid, 3′-methylnicotine (IV).

Independently we have been carrying out similar experiments to determine whether the enzymes responsible for nicotine formation could accomodate unnatural compounds, closely related to the natural precursors of nicotine, and yield unnatural derivatives of nicotine.[7] We chose to modify the pyridine ring of nicotine by feeding substituted nicotinic acids to tobacco. 5-Fluoronicotinic acid (IX) was selected as a candidate for participation in an aberrant biosynthesis for the following reasons. It is known that the hydrogen at the 5-position of nicotinic acid is incorporated into nicotine,[8] and is not apparently involved in the required modification of nicotinic acid prior to its condensation with the Δ^1-pyrrolinium salt. The van der Waals radii of the hydrogen and fluorine atoms are 1·20 and 1·35 Å respectively[9] and it seemed reasonable to expect that the enzymes would be unable to differentiate between 5-fluoronicotinic acid and nicotinic acid. β-Substitution on the pyridine ring was also the most desirable since the α- and γ-halogen derivatives of pyridine are much more reactive, being readily attacked by nucleophilic reagents.[10] In order to facilitate identification of any 5-fluoronicotine which might be formed, the 5-fluoronicotinic acid was labelled at C-5 and C-6 with ^{14}C by the scheme illustrated in Fig. 2.

Commercially available nitromethane-^{14}C on treatment with sodium hydroxide yielded 2-nitroacetaldehyde-1,2-^{14}C oxime (V),[11] which was condensed with *o*-aminobenzaldehyde affording 3-nitroquinoline-2,3-^{14}C (VI).[12] Reduction with stannous chloride gave 3-aminoquinoline (VII) which was converted to 3-fluoroquinoline (X) via the diazonium fluoroborate.[13] Oxidation of X with concentrated sulfuric acid and selenium at 300° yielded 5-fluoronicotinic-5,6-^{14}C acid (IX). 5-Fluoronicotine was prepared by a method analogous to that used by Späth[14] for the synthesis of nicotine. Ethyl 5-fluoronicotinate was condensed with 1-methyl-2-pyrrolidone in the presence of sodium hydride yielding 1-methyl-3-(5-fluoronicotinoyl)-2-pyrrolidone (XI), which was hydrolysed and decarboxylated by refluxing with hydrobromic acid. The resultant 1-methylamino-3-(5-fluoronicotinoyl)-propane (XII) was reduced in basic solution with sodium borohydride affording DL-5-fluoronicotine (XIII).

The 5-fluoronicotinic acid, in large doses, was phytotoxic to *Nicotiana tabacum* plants. Thus when a 3% aqueous solution of the fluoro compound (1 ml) was administered to a

[4] D. S. FREAR, *Phytochem.* **7**, 381 (1968).
[5] E. LEETE, *Advances in Enzymol.* **32**, 373 (1969).
[6] M. L. RUEPPEL and H. RAPOPORT, *J. Am. Chem. Soc.* **92**, 5528 (1970).
[7] A preliminary report of these results was made at the *3rd International Symposium on the Biochemistry and Physiology of Alkaloids*, Halle, 1965, *cf. Abhandl. Deut. Akad. Wiss. Berlin K*1. *Chem. Geol. Biol.* NR. **3**, 181 (1966).
[8] R. F. DAWSON, D. R. CHRISTMAN, A. F. D'ADAMO, M. L. SOLT and A. P. WOLF, *J. Am. Chem. Soc.* **82**, 2628 (1960).
[9] L. PAULING, *The Chemical Bond*, p. 152. Cornell University Press, Ithaca, N.Y. (1967).
[10] A. ROE and G. F. HAWKINS, *J. Am. Chem. Soc.* **69**, 2443 (1947).
[11] W. STEINKOPF, *Chem. Ber.* **42**, 2026 (1909).
[12] C. R. CLEMO and G. A. SWAN, *J. Chem. Soc.* 867 (1945).
[13] A. ROE and G. F. HAWKINS, *J. Am. Chem. Soc.* **71**, 1785 (1949).
[14] E. SPÄTH and H. BRETSCHNEIDER, *Chem. Ber.* **61**, 327 (1928).

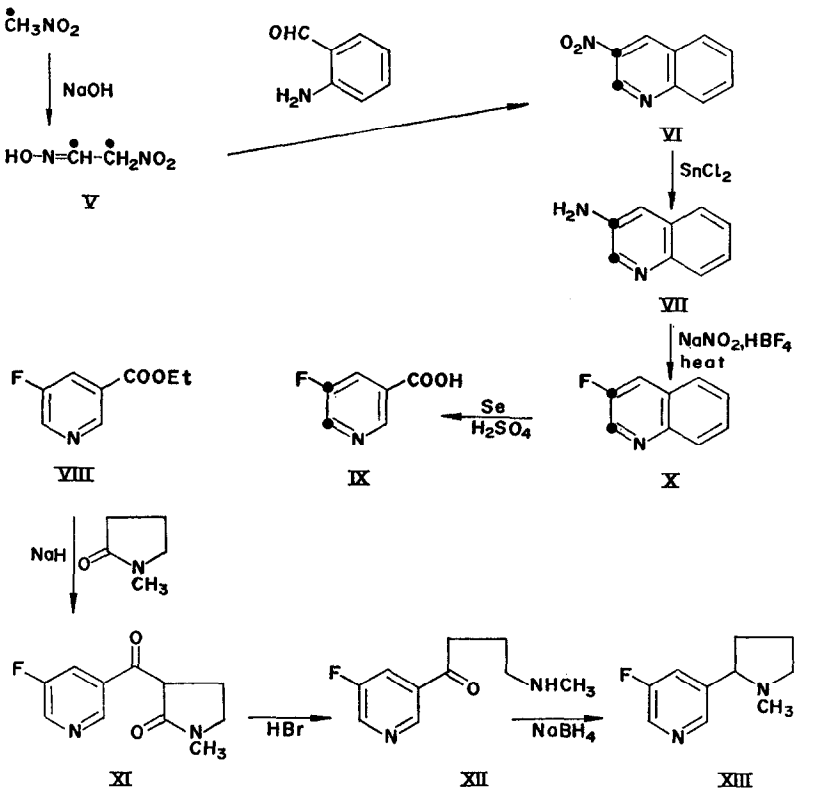

FIG. 2.

4-month-old plant by the wick method, the stems became brown at the site of injection and the leaves near to the cotton wick became prematurely yellow and died after 2–3 days. A more dilute solution of the 5-fluoronicotinic acid (< 1%) was not toxic and no obvious injury to the plant occurred. The tobacco plants were apparently able to build up some kind of resistance to the 5-fluoronicotinic acid. Thus 4-month-old plants which had received 10 mg/day of the fluoro compound for a week, were then able to tolerate increased amounts (up to 50 mg/day) with no ill effects.*

In preliminary work 5-fluoronicotinic-5,6-^{14}C acid was fed to *N. tabacum* plants and the alkaloids isolated 2 weeks later. The amount of radioactivity found in the crude alkaloids was only 1·2% of that administered to the plants, and it was estimated that the yield of 5-fluoronicotine could be no greater than 0·4 mg. Therefore a portion of the crude alkaloids was diluted with non-radioactive DL-5-fluoronicotine. The 5-fluoronicotine could be separated from nicotine and the other tobacco alkaloids by preparative gas liquid partition chromatography. The recovered DL-5-fluoronicotine was radioactive, and was further purified by crystallization of its perchlorate. From its specific activity it was calculated that there had been a 0·15% conversion of 5-fluoronicotinic acid to 5-fluoronicotine. We are investigating the composition of the other radioactive alkaloids derived from the 5-fluoronicotinic-5,6-^{14}C acid. The nicotine had a very small activity (0·0014% specific incorporation). This may indicate that the 5-fluoronicotinic acid can undergo defluorination to yield

* Observations with A. Monheim-Makky, 1965.

nicotinic acid which is then incorporated into nicotine, or activity may have been incorporated into the nicotine via breakdown products of the radioactive 5-fluoronicotinic acid.

The radioactive 5-fluoronicotine was degraded to determine the location of the activity. Oxidation with potassium permanganate yielded 5-fluoronicotinic acid, having the same specific activity as the alkaloid. Decarboxylation of this acid by heating with copper chromite and quinoline yielded carbon dioxide which had negligible activity, indicating that all the activity was located in the pyridine ring of the 5-fluoronicotine.

Having established the conversion of radioactive 5-fluoronicotinic acid to 5-fluoronicotine, a second feeding experiment was carried out with non-radioactive 5-fluoronicotinic acid. A much larger amount of the unnatural compound was fed to the tobacco plants and it was possible to separate from the resultant alkaloids, 5-fluoronicotine (0·4% of the crude alkaloids) by gas chromatography. Its mass spectrum was identical with synthetic 5-fluoronicotine and it had the same R_f value when subjected to thin layer chromatography. Insufficient material was isolated to determine whether this unnatural alkaloid was optically active.

EXPERIMENTAL

General methods. Radioactivity measurements were carried out in a Nuclear Chicago liquid scintillation system, Model 724, using as solvents either toluene or dioxane, with the usual scintillators.[15] The mass spectra were determined by Mr. Adrian Swanson and his associates at the University of Minnesota, using an Hitachi–Perkin–Elmer RMU–6D mass spectrometer. Microanalyses were determined by Mrs. Fay Thompson at the University of Minnesota. Fluorine analyses were determined by the Clark Microanalytical laboratories, Urbana, Illinois. GLC was carried out on a Varian Aerography Moduline, Model 204, with a flame ionization detector, or on a Varian Aerograph, Model A-90P with a thermal conductivity detector.

Ethyl 5-fluoronicotinate (VIII). A solution of 5-fluoronicotinic acid[16] (28·2 g, 0·2 mole) in a mixture of conc. H_2SO_4 (28 ml) and EtOH (65 ml) was refluxed for 24 hr. The reaction mixture was poured onto ice and made slightly alkaline with dil. Na_2CO_3. The mixture was extracted with Et_2O and dried over K_2CO_3. Evaporation and distillation afforded ethyl 5-fluoronicotinate as a colorless oil (28·1 g, 83·5%), b.p. 110–112° (27 mm), n_D^{25} 1·4810.

1-*Methyl*-3-(5-*fluoronicotinoyl*)-2-*pyrrolidone* (*XI*). A 50% suspension of NaH in mineral oil (9·6 g, 0·2 mole) was freed of the mineral oil by suspending in dry benzene and decanting several times. The resultant slurry (100 ml) was refluxed in N_2, and a solution of ethyl 5-fluoronicotinate (8·45 g, 0·05 mole) and 1-methyl-2-pyrrolidone (9·9 g, 0·1 mole) in benzene (25 ml) slowly added during 1 hr. The reaction mixture was then refluxed for an additional 20 hr. The cooled mixture was added to a solution of HOAc (25 ml) in H_2O (150 ml). The benzene layer was separated and the aqueous phase extracted with additional benzene (4 × 75 ml). The combined extracts were washed with $NaHCO_3$, H_2O, and then dried ($MgSO_4$). Evaporation yielded a solid which on crystallization from Et_2O afforded 1-methyl-3-(5-fluoronicotinoyl)-2-pyrrolidone (6·22 g, 56%), as pale yellow needles, m.p. 91–92°. (Calc. for $C_{11}H_{11}FN_2O_2$ (222·22): C, 59·45; H, 4·99; F, 8·55; N, 12·61. Found: C, 6·53; H, 4·98; F, 8·37; N, 12·78%.) Mass spectrum, *m/e* (rel. intensity): 222 (37), 179 (10), 124 (80), 98 (100), 96 (72), 76 (10), 70 (14), 69 (17), 57 (26), 44 (51), 42 (40), 41 (26).

DL-5-*Fluoronicotine* (*XIII*). 1-Methyl-3-(5-fluoronicotinoyl)-2-pyrrolidone (6·22 g) was refluxed with 48% HBr (25 ml) for 20 hr. The solution was evaporated to dryness, and the yellow residue dissolved in MeOH (150 ml) and made slightly basic by the addition of 5% KOH in MeOH. KBr was filtered off and the filtrate stirred with $NaBH_4$ (5 g) for 20 hr at room temp. The reaction mixture was acidified with HCl and evaporated to dryness. The residue was suspended in 15% NaOH and extracted with CH_2Cl_2. Evaporation of the dried (K_2CO_3) extract yielded a pale yellow oil which was distilled, b.p. 67° (1·4 mm) affording 5-fluoronicotine as a colorless liquid (2·7 g, 53%). Mass spectrum of $C_{10}H_{13}FN_2$ (180), *m/e* (rel. intensity): 180 (18), 179 (19), 151 (35), 137 (11), 106 (10), 96 (6), 85 (11), 84 (100), 83 (7), 82 (9), 56 (8), 43 (40), 42 (8), 29 (6).

The dipicrate was obtained as bright yellow needles from EtOH m.p. 180–181°. (Calc. for $C_{10}H_{13}FN_2.2\,C_6H_3N_3O_7$: C, 41·39; H, 3·00; F, 2·98; N, 17·55. Found: C, 41·17; H, 3·07; F, 3·03; N, 17·72%.)

5-Fluoronicotine monoperchlorate was obtained as colorless plates, m.p. 159–160°, on mixing an EtOH solution of the alkaloid with an equivalent amount of 70% $HClO_4$. (Calc. for $C_{10}H_{13}FN_2.HClO_4$: C, 42·79; H, 5·03 ;N, 9·98. Found: C, 42·75; H, 5·05; N, 10·01%.)

[15] A. R. FRIEDMAN and E. LEETE, *J. Am. Chem. Soc.* **85**, 2141 (1963).
[16] G. F. HAWKINS and A. ROE, *J. Org. Chem.* **14**, 328 (1949).

2-*Nitroacetaldehyde*-1,2-^{14}C *oxime* (*V*). $MeNO_2$-^{14}C (nominal activity 1 mCi, 61 mg) (Mallinckrodt Nuclear Co, St. Louis, Missouri) was diluted with inactive $MeNO_2$ (433 mg) and added dropwise to a solution of NaOH (0·5 g) in H_2O (1 ml), the temperature of the reaction mixture being maintained at 40–50° by cooling. After stirring for 30 min the mixture was cooled, acidified with dil H_2SO_4, and extracted with Et_2O. Evaporation of the dried ($MgSO_4$) extract yielded 2-nitroacetaldehyde oxime as pale brown needles (313 mg, 74·4%) m.p. 79–80° (lit.[11] 79–80°).

3-*Nitroquinoline*-2,3-^{14}C (*VI*). A solution of *o*-aminobenzaldehyde (750 mg, 6·2 mmole) in a mixture of EtOH (1 ml) and conc. HCl (0·3 ml) was added to a solution of 2-nitroacetaldehyde-1,2-^{14}C oxime (312 mg, 3·0 mmole) in EtOH (5 ml) and then stirred at room temp. for 20 hr. The solution was evaporated on a rotary evaporator in the presence of Woelm alumina (5 g, Act. I). The residue was made into a slurry with benzene and applied to the top of a column of Act. II alumina (25 g). Elution of the column with 1% MeOH in benzene afforded 3-nitroquinoline-2,3-^{14}C (201 mg, 38·5%) m.p. 130–131° (lit.[12] 126°). *o*-Aminobenzaldehyde oxime was obtained on further elution of the column.

3-*Fluoroquinoline*-2,3-^{14}C (*X*). A solution of 3-nitroquinoline-2,3-^{14}C (201 mg) in conc. HCl (5 ml) was added quickly to a solution of $SnCl_2$ (1·3 g) in conc. HCl (5 ml) and stirred for 3 hr at room temp. The reaction mixture was made basic with NaOH and extracted with Et_2O. The dried extract ($MgSO_4$) on evaporation yielded 3-aminoquinoline (261 mg) which was dissolved in a mixture of tetrahydrofuran (2 ml), H_2O (1·5 ml) and 50% HBF_4 (4·5 ml). The solution was stirred at 0° and $NaNO_2$ (154 mg) in a little H_2O slowly added. The diazonium fluoroborate salt which separated out was filtered off, washed with tetrahydrofuran, EtOH Et_2O, and dried in air. It was suspended in toluene (20 ml) and refluxed for 1 hr. The toluene solution was cooled and extracted with 10% HCl. The aqueous extract was made basic with NaOH and extracted with Et_2O. The dried ($MgSO_4$) extract on evaporation yielded 3-fluoroquinoline (142 mg) as a pale yellow oil.

5-*Fluoronicotinic*-5,6-^{14}C *acid* (*IX*). 3-Fluoroquinoline-2,3-^{14}C (142 mg) and Se (95 mg) were heated in conc. H_2SO_4 (2 ml) for 4 hr at 290–300°. The golden brown reaction mixture was then cooled, diluted with H_2O and the pH adjusted to 3·0 by the addition of NH_4OH. The solution was then extracted in a continuous extractor with Et_2O for 10 hr. Evaporation of the extract yielded a white solid which was sublimed (170°, 10^{-2} mm) affording 5-fluoronicotinic-5,6-^{14}C acid (40 mg, 29·4%), m.p. 194–196° (lit.[17] 195–197°). Its specific activity was $5{\cdot}22 \times 10^8$ dis/min/mmole. It was shown to be radiochemically pure by TLC followed by radiochemical assay of the developed chromatogram. On silica gel F-254 (Merck) developed with a mixture of benzene –MeOH–Me_2Co–HOAC (70:20:5:5 v/v) nicotinic acid and 5-fluoronicotinic acid had R_f values of 0·27 and 0·40, respectively.

Administration of 5-fluoronicotinic-5,6-^{14}C *acid to* Nicotiana tabacum *plants and isolation of the alkaloids.* A solution of 5-fluoronicotinic-5,6-^{14}C acid (35·6 mg, $1{\cdot}32 \times 10^8$ dis/min) was dissolved in H_2O (5 ml) and fed to five 4-month-old *N. tabacum* plants growing in soil in a greenhouse, by means of cotton wick inserted near to ground level. After 14 days the plants were harvested (fr. wt. 740 g), and mascerated in a Waring blendor with a mixture of conc. NH_3 (100 ml) and $CHCl_3$ (2 l.). After standing for a few days the mixture was filtered and the $CHCl_3$ layer evaporated in the presence of 10% H_2SO_4 (200 ml). The aqueous solution was filtered through celite, made alkaline with NH_4OH, and extracted with $CHCl_3$. The dried ($MgSO_4$) extract was evaporated yielding crude alkaloids (468 mg) having a total activity of $1{\cdot}6 \times 10^5$ dis/min (1·2% incorporation of activity).

A portion of these crude alkaloids (250 mg, $8{\cdot}6 \times 10^4$ dpm) was mixed with DL-5-fluoronicotine (69 mg) and then subjected to preparative GLC on a $1{\cdot}5 \times 0{\cdot}1$ m stainless column packed with 10% Carbowax 20 M on 30–60 Chromosorb W, at 160° with a He flow rate of 100 ml/min. The retention times of nicotine and 5-fluoronicotine were 5·3 and 3·8 min, respectively. The alkaloids were converted to their perchlorates and crystallized to constant activity. The nicotine diperchlorate had a persistent activity of $7{\cdot}3 \times 10^3$ dis/min/mmole (0·0014% specific incorporation). The 5-fluoronicotine monoperchlorate had an activity of $2{\cdot}9 \times 10^5$ dis/min/m-mole, not changed on repeated crystallization.

Degradation of the radioactive 5-fluoronicotine. A portion of the 5-fluoronicotine perchlorate was diluted to afford material having an activity of $3{\cdot}84 \times 10^4$ dis/min/m-mole. This diluted salt (151 mg) was dissolved in H_2O (20 ml) and made basic with KOH. $KMnO_4$ (0·73 mg) in H_2O (20 ml) was added slowly at room temp. and then the mixture was refluxed for 2 hr. Excess $KMnO_4$ was decomposed by the addition of a little EtOH. The mixture was then decolorized with SO_2. The pH of the solution was adjusted to 3 with NH_4OH and then extracted with Et_2O. Evaporation of the Et_2O, followed by sublimation of the residue yielded 5-fluoronicotinic acid (49 mg, 65%) m.p. 195– 197°, having an activity of $3{\cdot}80 \times 10^4$ dis/min/m-mole.

This 5-fluoronicotinic acid (40 mg) was refluxed in quinoline (1 ml) with copper chromite (40 mg) in a current of dry CO_2 free N_2. The evolved $^{14}CO_2$ was collected by passage of the N_2 stream into saturated $Ba(OH)_2$ solution. The resultant $BaCO_3$ (42 mg, 77%) had negligible activity $< 0{\cdot}03 \times 10^4$ dis/min/m-mole.

Administration of non-radioactive 5-fluoronicotinic acid to N. tabacum *and isolation of 5-fluoronicotine.* 5-Fluoronicotinic acid (713 mg) was fed to five 4-month-old *N. tabacum* plants at the rate of 10 mg/plant/day by the wick method. The plants were harvested 2 weeks after the initial feeding, and the alkaloids isolated as previously described.

GLC of a portion of the crude alkaloids indicated the presence of a compound having the same retention time as 5-fluoronicotine. From the peak height on the recorder of the gas chromatogram it was estimated that its concentration in the total alkaloids (mainly nicotine) was about 0·4%. The substance having the same retention time as 5-fluoronicotine was collected from the gas chromatograph effluent stream by absorption on Silica gel G (Merck). This compound absorbed on the silica gel was placed directly into the inlet system of the mass spectrometer. The resultant mass spectrum was identical with 5-fluoronicotine. TLC of the material obtained from the gas chromatograph in the solvent system previously described also indicated that it was identical with 5-fluoronicotine.

Acknowledgement—This investigation was supported by a research grant GM-13246 from the U.S. Public Health Service.