Phytochemistry, Vol. 20, No. 5, pp. 1037–1040, 1981.
Printed in Great Britain.

0031-9422/81/051037-04 $02.00/0

N'-ISOPROPYLNORNICOTINE: ITS FORMATION FROM NICOTINE IN AGED LEAVES OF *NICOTIANA TABACUM*

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(*Received* 4 *September* 1980)

Key Word Index—*Nicotiana tabacum*; Solanaceae; *N'*-isopropylnornicotine; nicotine; nornicotine; ^{13}C NMR spectroscopy; biosynthesis.

Abstract—An aqueous solution of nicotine-[2'-^{14}C] was painted on the leaves of 4-month-old tobacco plants (*Nicotiana tabacum*) which were harvested 3 weeks later. This tracer was similarly applied to excised tobacco leaves which were allowed to dry in air for 4 weeks. The alkaloids, were extracted with the addition of *N'*-isopropylnornicotine, a compound which has been previously isolated from air-cured tobacco. Radioactive nicotine and nornicotine were isolated from the intact plants with only minute activity in the *N'*-isopropylnornicotine. All three of these alkaloids were radioactive from the air-cured leaves, and degradation of the labelled *N'*-isopropylnornicotine indicated that all the activity was located at the C-2' position. A higher level of activity was found in *N'*-isopropylnornicotine which was obtained from excised leaves which were fed the nicotine-[2'-^{14}C] in aqueous acetone, and were treated on subsequent days with aqueous acetone. These results are consistent with the hypothesis that *N'*-isopropylnornicotine is produced in the curing of tobacco leaves by reaction of nornicotine (formed by the demethylation of nicotine) with acetoacetate, followed by decarboxylation and reduction. The ^{13}C NMR chemical shifts of the methyl groups of *N'*-isopropylnornicotine and related 1-isopropylpyrrolidines which have chirality at the α-position of the pyrrolidine ring, are significantly different (up to 7.5 ppm).

INTRODUCTION

Recently Miyano and co-workers [1] reported on the isolation and characterization of *N'*-isopropylnicotine (**5**) from air-cured Burley tobacco (*Nicotiana tabacum*). The *N*-isopropyl group is extremely rare in nature, the only other alkaloid containing this function being pedunculariné (**4**) [2]. Bick has proposed an ingenious biogenetic scheme [3] for this indole alkaloid, the *N*-isopropyl group being formed by the fragmentation of a terpene precursor. I considered that the *N'*-isopropylnornicotine could be formed in the cured tobacco by reaction of nornicotine (**2**) with acetoacetate to afford the enamine (**3**). Decarboxylation of this enamine and reduction then afford **5**. Alternatively, **5** could be formed by reaction of nornicotine with acetone, which has been found in tobacco leaves [4], followed by a reduction. It is well established that nornicotine is formed in *Nicotiana* species by the demethylation of nicotine (**1**) [5]. Accordingly, this hypothesis was tested by administering nicotine-[2'-^{14}C] to tobacco with the expectation that radioactive **5** would be produced.

RESULTS AND DISCUSSION

Three separate feeding experiments were carried out. In the first a solution of the nicotine-[2'-^{14}C] in dilute hydrochloric acid was painted on the leaves of intact plants growing in soil in a greenhouse. In the second experiment the nicotine solution was painted on freshly harvested leaves which were spread out to dry naturally at room temperature in sunlight in a greenhouse. In the third experiment the nicotine solution contained 20% acetone, and the leaves were painted daily with a 20% acetone solution in water for the following 10 days. It was anticipated that this acetone treatment might increase the production of **5**. The results are recorded in Table 1. Since the amount of *N'*-isopropylnicotine produced was expected to be small, this alkaloid was added as a carrier prior to isolation and separation of the alkaloids [6]. In the intact plant about 12% of the administered radioactivity was found in nicotine and nornicotine. Only a minute amount of activity was detected in the reisolated *N'*-isopropylnornicotine, and it is considered that little if any of this compound is formed in the healthy growing plant. In the air-cured leaves the relative amount of nornicotine increased in agreement with earlier work [6] and a small but significant amount of radioactivity was found in the *N'*-isopropylnornicotine. Its specific activity remained constant after rigorous purification. It was shown to be specifically labelled at C-2' by degradation. Oxidation with permanganate yielded nicotinic acid which was decarboxylated in boiling quinoline in the presence of copper chromite. The resultant carbon dioxide assayed as barium carbonate had the same specific activity as the alkaloid. Acetone treatment of the leaves caused a significant change in the distribution of activity in the alkaloids. More activity was found in the *N'*-isopropylnornicotine, however, since the nornicotine also had an increased activity, I do not

* Contribution No. 175 from this laboratory.

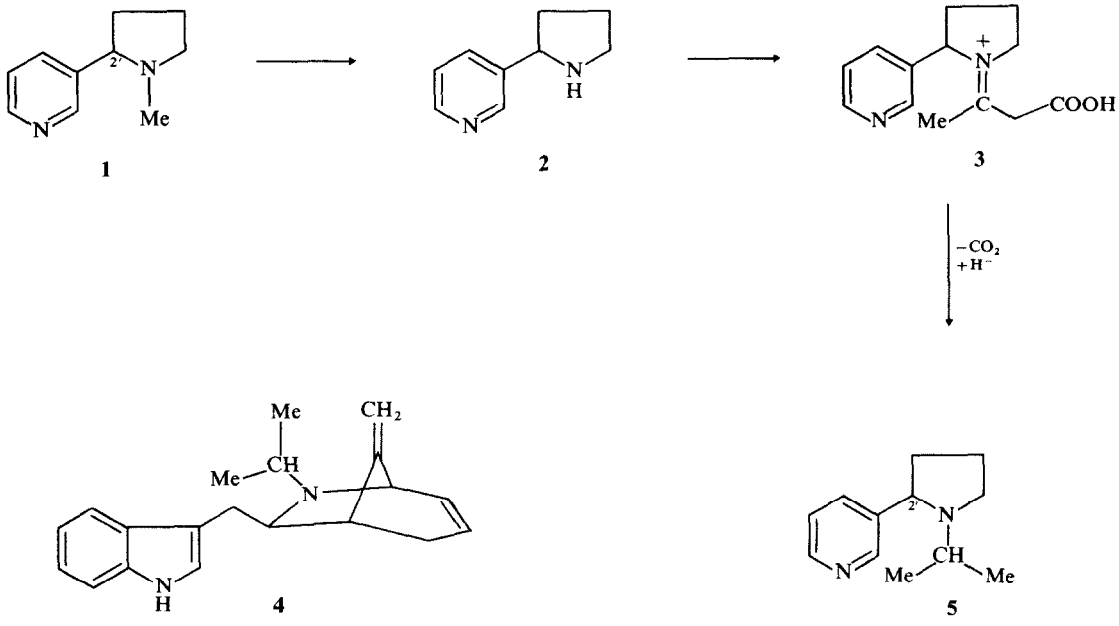

Fig. 1. Proposed biosynthesis of *N'*-isopropylnornicotine.

consider that this result establishes unequivocally the direct origin of the isopropyl group from acetone.

N'-Isopropylnornicotine was required for dilution purposes. It was previously prepared [1] by reaction of nornicotine with isopropyl bromide. In the present work it was obtained in excellent yield by the reduction with sodium cyanoborohydride of a mixture of nornicotine hydrochloride and acetone in methanol [7]. Its mass spectrum was essentially the same as that previously reported [1]. Its ^{13}C NMR spectrum (chemical shifts in ppm from Me_4Si are indicated on the formulae in Figure 2) was assigned by comparison with nicotine [8]. The two methyls of the isopropyl group (detected as quartets in the off-resonance decoupled spectrum) had surprising dissimilar (6.9 ppm) chemical shifts, attributed to the chiral center at C-2'. The methyls of the isopropyl group in peduncularine differ by only 0.9 ppm [2]. The methyl groups of leucine which are also three bonds from a chiral centre differ by 1.2 ppm [9]. This unusual result led me to determine the ^{13}C NMR spectra of other *N*-isopropylpyrrolidines which are illustrated in Figure 2. The ^{13}C NMR spectrum of *N*-isopropylpyrrolidine (**6**) was previously determined, but only the chemical shift of the methine carbon was recorded [2]. 1-Isopropyl-2-methylpyrrolidine (**7**) was obtained from 2-methylpyrrolidine by reaction with acetone in the presence of sodium cyanoborohydride. It was possible to differentiate the signals for the isopropyl methyls from the C-2 methyl by preparing a deuterated species from acetone-d_6 and 2-methylpyrrolidine. The product was not completely deuterated on the isopropyl methyls, presumably because the intermediate enamine undergoes some proton exchange with solvent prior to reduction. However, since the signals at 16.1 and 22.6 ppm (quartets on the off-resonance decoupled spectrum) were significantly reduced in intensity relative to the non-deuterated

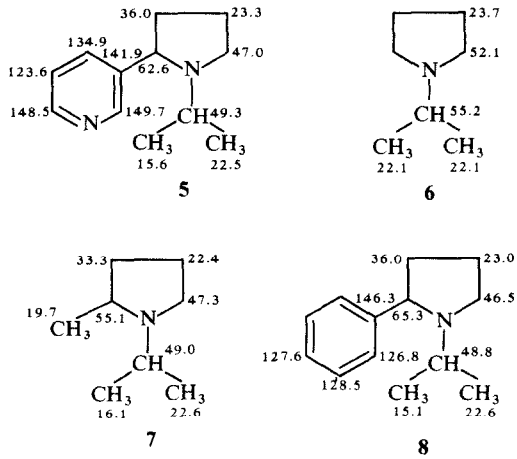

Fig. 2. ^{13}C NMR shift assignments of some *N*-isopropylpyrrolidines, determined in $CDCl_3$ (ppm from Me_4Si).

compound, they are assigned to the isopropyl methyl groups. 1-Isopropyl-2-phenylpyrrolidine (**8**) was obtained from 2-phenylpyrrolidine which was prepared from ethyl benzoate and 1-trimethylsilyl-2-pyrrolidone analogous to the method used for the preparation of nornicotine [10]. Its ^{13}C NMR spectrum is very similar to that of *N'*-isopropylnornicotine, the chemical shifts of the methyl groups differing by 7.5 ppm.

EXPERIMENTAL

General methods. Radioactive materials were assayed in duplicate in a liquid scintillation counter using dioxane–EtOH with usual scintillators [11]. ^{13}C NMR spectra were obtained at 25.2 MHz using an instrument equipped with a Fourier

Table 1. Activities of the alkaloids isolated from *Nicotiana tabacum* which had been fed nicotine-[2'-^{14}C]*

Experiment number	1	2	3
Plant	Intact plant	Isolated leaves	Isolated leaves +20% acetone
Duration of experiment	3 weeks	4 weeks	4 weeks
Weight of plant	1200 g (fr. wt)	12 g (air dried)	18 g (air dried)
Nicotine			
wt (mg)	460	41	53
spec. act.†	3.63×10^5	3.28×10^6	2.18×10^6
abs. incorporation‡	4.62%	3.95%	3.40%
Nornicotine			
wt (mg)	30.6	16.9	22.9
spec. act.	7.11×10^6	5.81×10^6	1.02×10^7
abs. incorporation	7.00%	3.16%	7.52%
N'-Isopropylnornicotine §			
spec. act.	1.1×10^3	6.3×10^3	1.20×10^4
abs. incorporation	0.003%	0.018%	0.035%

* In each experiment, nicotine-[2'-^{14}C] (19.5 mg, 2.10×10^7 dpm, specific activity 2.23×10^8 dpm/mM) dissolved in 0.02 M HCl (5 ml) was applied to the leaves with an artist's paint brush. In experiment 3, 1 ml of acetone was added to this solution, and on the 10 following days the leaves in this experiment were painted with a 20% solution of acetone in H_2O.

† Specific activity expressed as dpm/mM.

‡ Absolute incorporation = total activity in the alkaloid/total activity administered to the plant.

§ Non-radioactive *N'*-isopropylnornicotine (116 mg) was added to each experiment at the time of extraction.

transform accessory. Mps were determined in glass capillaries and are corrected. Elemental analysis were carried out by M.H.W. Laboratories, Phoenix, Arizona.

RS-*N'-Isopropylnornicotine* (**5**). A solution of *RS*-nornicotine (2.0 g) [10] in MeOH (100 ml) was saturated with HCl gas and then evapd to dryness. The residue was dissolved in a mixture of MeOH (50 ml) and acetone (10 ml) and cooled to 0°. Sodium cyanoborohydride (4.0 g) was added and the mixture stirred at room temp. for 18 hr. The soln was then acidified with HCl, evapd to dryness, the residue made basic with 10% NaOH and extracted with $CHCl_3$. The residue obtained on evapn of the dried ($MgSO_4$) extract was distilled (180°, 10^{-2} mm) to afford *N'*-isopropylnornicotine as a colorless oil (2.26 g, 88%). MS *m/z* (rel. int.): 190 (11) M^+, 176 (13), 175 (100), 132 (26), 130 (16), 117 (13), 112 (16), 70 (20). UV (95% EtOH) λ_{max} (ε) 256 (2670), 262 (2835), with H^+ 261 (4920). TLC on Si gel PF-254 (Merck) developing with Et_2O–isopropanol–conc. NH_3 (80:10:3) gave R_f values for nornicotine, nicotine, and *N'*-isopropylnornicotine of 0.12, 0.50, and 0.83, respectively. With $CHCl_3$–EtOH–conc. NH_3 (100:15:1) the corresponding values were 0.2, 0.65 and 0.80. The alkaloids were detected by spraying the plates with *p*-aminobenzoic acid followed by exposure to CNBr, or by observing the plates in UV light. The *RS-N'*-isopropylnornicotine afforded a dipicrate, mp 219–220°, as bright yellow plates from EtOH. (Found: C, 44.53, H, 3.77, N, 17.10. Calc. for $C_{24}H_{24}N_8O_{14}$: C, 44.45, H, 3.73, N, 17.28%).

RS-1-*Isopropyl-2-methylpyrrolidine* (**7**). 2-Methylpyrrolidine hydrochloride (4.0 g) was dissolved in a mixture of MeOH (50 ml) and Me_2CO (10 ml) and reduced with sodium cyanoborohydride (2.5 g) for 18 hr. The reaction mixture was worked up as for **5** affording 1-isopropyl-2-methylpyrrolidine as a colourless mobile liquid (3.0 g) bp 115° (760 mm). It afforded a picrate mp 207–208° from 95% EtOH. (Found: C, 47.10, H, 5.54, N, 15.88. Calc. for $C_{14}H_{20}N_4O_7$: C, 47.19, H, 5.66, N, 15.72%). This pyrrolidine has been previously prepared [12], but its picrate was not described. The partially deuterated derivative was obtained by substituting acetone-d_6 in this procedure. MS of the picrate of the product indicated the following composition: d_0 29, d_1 21, d_2 14, d_3 10, d_4 9, d_5 8, d_6 8%.

RS-1-*Isopropyl-2-phenylpyrrolidine* (**8**). A soln of diisopropylamine (12 g) in Et_2O (100 ml) at −78° was treated with *n*-butyl lithium (36 ml of a 2.4 M solution in hexane) followed by 1-trimethylsilyl-2-pyrrolidone (15 g). Ethyl benzoate (10 g) in Et_2O (10 ml) was then added and the mixture stirred at room temp. for 18 hr. H_2O (25 ml) was then added and the Et_2O layer discarded. The aqueous layer was acidified with HCl and extracted with $CHCl_3$. Evapn of the dried (Na_2SO_4) extract yielded a viscous liquid (4.6 g) which was refluxed with conc. HCl (25 ml) for 18 hr. The cooled soln was made basic with NaOH and extracted with $CHCl_3$. The residue obtained on evapn of the dried extract was dissolved in MeOH (20 ml) and HOAc (2 ml), cooled to 0° and $NaBH_4$ (2.0 g) added slowly. The mixture was warmed at 50° for 1 hr, then acidified with HCl, evaporated and the residue made basic with NaOH. Extraction with $CHCl_3$ afforded 2-phenylpyrrolidine (2.4 g) which was converted to its 1-isopropyl derivative by reaction with Me_2CO and sodium cyanoborohydride as described for **5**. MS *m/z* (rel. int.) 189 (7) M^+, 174 (100), 131 (58), 112 (18), 91 (32). It afforded a picrate, mp 171–172° from EtOH (Found: C, 54.65, H, 5.46, N, 13.14, Calc for $C_{19}H_{22}N_4O_7$: C, 54.50, H, 5.30, N, 13.39%).

Administration of nicotine-[2'-^{14}C] *to* Nicotiana tabacum *and isolation of the alkaloids.* The nicotine-[2'-^{14}C] was racemic,

being obtained from Research Products International Corporation. Details of the amount fed are recorded in Table 1. The tobacco plants were 4 months old at the time of feeding. The excised leaves were from plants of the same age. At the conclusion of the feeding experiments the plant material was macerated with $CHCl_3$ and conc. NH_3 as previously described [6]. The crude alkaloids were separated by TLC and purified by distillation *in vacuo*. The amount of nicotine and nornicotine was determined by UV spectroscopy on the distillates which were condensed in a U-tube cooled in dry ice. The recovery of the carrier *N'*-isopropylnornicotine was 60–80%. The alkaloids were purified to constant spec. act. by repeated crystallization of their dipicrates.

Degradation of the N'-isopropylnornicotine from experiment 2. The dipicrate of *N'*-isopropylnornicotine (150 mg 6.30 $\times 10^3$ dpm/mM) was dissolved in hot 2 M HCl (20 ml) and the soln extracted with Et_2O to remove picric acid. The aqueous soln was made basic with NaOH and the free alkaloid extracted with Et_2O. The residue obtained on evapn of the Et_2O was dissolved in H_2O (10 ml) and 10% NaOH (1.5 ml). $KMnO_4$ (0.3 g) was added and the soln stirred at room temp. for 2 hr. The soln was then refluxed for 1 hr, cooled, and decolourized with SO_2. Continuous Et_2O extraction for 18 hr yielded nicotinic acid which was sublimed and crystallized from ethanol (27 mg, 6.25 $\times 10^3$ dpm/mM). This nicotinic acid was decarboxylated as previously described [11], the resultant $BaCO_3$ being assayed by dissolving in an aqueous soln of the sodium salt of ethylenediamine tetraacetic acid [13]. (6.20 $\times 10^3$ dpm/mM).

Acknowledgements—This investigation was supported by a research grant GM-13246 from the National Institutes of Health, U.S. Public Health Service. I thank Frank Blum for determination of the ^{13}C NMR spectra.

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