THE PREPARATION OF TOBACCO CONSTITUENTS INCORPORATING STABLE ISOTOPES, I. THE SYNTHESIS OF *d,ℓ*-NORNICOTINE-1'-^{15}N AND *d,ℓ*-NICOTINE-1'-^{15}N

William B. Edwards III*, David F. Glenn, Frank Green, and R. H. Newman
Philip Morris Research Center, Richmond, Virginia 23261
Received December 13, 1976
Revised February 18, 1977

SUMMARY

Isotopically enriched *d,ℓ*-nornicotine-1'-^{15}N (24 atom % enrichment) was prepared in a single step from cyclopropyl 3-pyridyl ketone and formamide-^{15}N (24 atom % enrichment). Methylation to *d,ℓ*-nicotine-1'-^{15}N (24 atom % enrichment) was accomplished using the Clark-Eschweiler method.

Key Words: *d,ℓ*-Nornicotine, *d,ℓ*-Nicotine, Nitrogen-15, Tobacco

INTRODUCTION

Studies on the fate of constituents of *Nicotiana tabacum* L. in the burning cigarette using isotopically labelled materials have been carried out by numerous investigators. The majority of these studies were directed toward following the constituents' carbon atoms *via* carbon-14 labelled compounds (1). Recently, Johnson, *et al.*, have carried out investigations on the burning cigarette using nitrogen-15 labelled materials to study the conversion of nitrate nitrogen to smoke products (2) and the formation of molecular nitrogen (3). Additionally, Baxter (4) and Johnson, *et al.*, (5), have examined the incorporation of atmospheric oxygen into components of cigarette smoke using oxygen-18. These studies, which represent the only application of stable isotopic tracer techniques to the investigation of the mechanism of smoke forma-

0362-4803/78/0214-0255$01.00/0

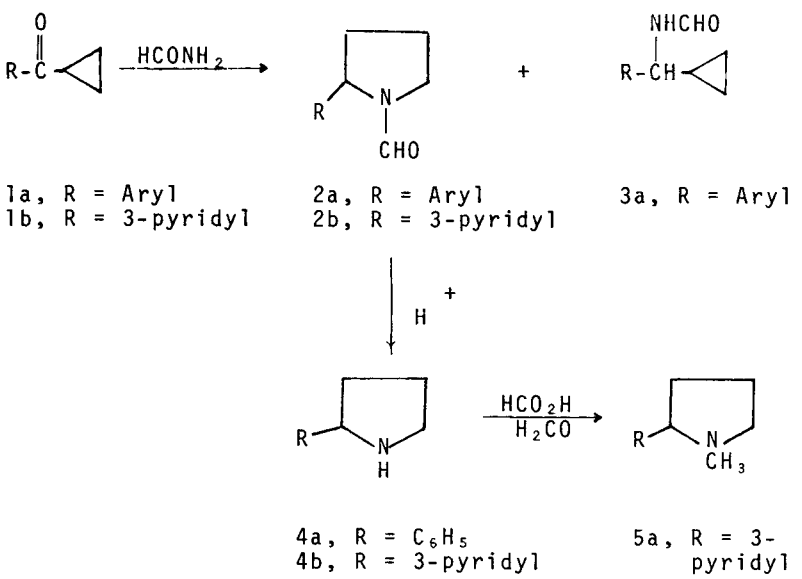
tion, were directed essentially toward the study of the fate of isotopically labelled inorganic compounds in the burning cigarette. It was, therefore, of interest to us to explore the utility of stable isotope tracer techniques, in particular using nitrogen-15 labels, in the study of the disposition of organic compounds in the burning cigarette. For our initial investigations, we chose to study the two major alkaloids of *Nicotiana tabacum* L., nornicotine and nicotine, labelled with nitrogen-15 in the 1' position. Although these compounds have been previously prepared (6), we chose to examine an alternative, shorter route which would be more amenable to the synthesis of multi-isotopically labelled *d,ℓ*-nicotines and *d,ℓ*-nornicotines required for subsequent investigations.

It has been reported by Breuer (7) that aryl cyclopropyl ketones [1a] react with formamide in the presence of magnesium chloride to give good yields (70-80%) of 2-aryl-N-formylpyrrolidines [2a] and small amounts (0-9%) of 1-aryl-2-cyclopropyl-N-formylmethylamines [3a, Scheme 1]. The former [2a, Aryl=C_6H_5] has been shown to hydrolyze to 2-phenylpyrrolidine [4a]. Furthermore, *d,ℓ*-nicotine has been prepared by reaction of cyclopropyl 3-pyridyl ketone [1b] with N-methylformamide under similar conditions (8) and this synthesis has been utilized for the preparation of *d,ℓ*-nicotine-2'-^{14}C (9). Based on this previous work, we felt that the reaction of 1b with formamide should readily afford N-formylnornicotine [2b] which could be hydrolyzed *in situ* to give the desired *d,ℓ*-nornicotine [4b].

Initial studies (Table 1 - Expt No 1-3) demonstrated that pure 4b could be obtained using Breuer's conditions (7) (Expt No 1), that a substantial improvement in the yield of 4b could be realized if the reaction was run in 2-ethoxyethyl ether under reflux (Expt No 2) and that whether the magnesium chloride catalyst was or was not hy-

drated had no apparent effect (Expt No 3). Experiments (Expt No 4-6) were then carried out with decreased molar ratios of formamide to 1b to determine if the yield of 4b (based on formamide) could be improved. These experiments showed that a ratio of 3 to 1 gave the best results. Furthermore, the trace quantity (<2%) of myosmine that was observed by mass spectral analysis in the products from Expt No 2-4 and 6, was not detected in the product from Expt No 5. Reaction of 4b under Clark-Eschweiler conditions was then carried out to give *d,ℓ*-nicotine [5a] in 85.2% yield.

SCHEME I



1a, R = Aryl
1b, R = 3-pyridyl

2a, R = Aryl
2b, R = 3-pyridyl

3a, R = Aryl

4a, R = C_6H_5
4b, R = 3-pyridyl

5a, R = 3-pyridyl

The synthesis was then carried out (as Table I - Expt No 5) using formamide with a nitrogen-15 enrichment of 24 atom % to give *d,ℓ*-nornicotine-1'-^{15}N (53.7% yield, >97% pure, ^{15}N enrichment of 24 atom %). This product was then methylated as described to afford *d,ℓ*-nicotine-1'-^{15}N (84.0% yield, >97% pure, ^{15}N enrichment of 24 atom %).

Table I. Results from the reaction of cyclopropyl 3-pyridyl ketone [1b] with formamide.

Expt[a] No	$\frac{HCONH_2}{1b}$[b]	Catalyst[c]	Time[d] hrs	4b[e] Purity, %	4b Yield, %[f] based on 1b	based on $HCONH_2$
1	6	$MgCl_2$ (anhyd)	2.75	99	24.8	4.1
2	6	$MgCl_2$ (anhyd)	4.0	96	47.0	7.8
3	6	$MgCl_2 \cdot 6H_2O$	4.5	97	48.2	8.0
4	6	$MgCl_2 \cdot 6H_2O$	4.5	96	46.8	11.7
5	3	$MgCl_2 \cdot 6H_2O$	21	>99	57.4	19.0
6	2	$MgCl_2 \cdot 6H_2O$	21	86	24.2	12.1

[a] Expt No 1 run without diluent at 180°. Expt No 2-6 were all run in 2-ethoxyethyl ether under reflux.

[b] Molar ratio

[c] Molar ratio of catalyst to 1b was 0.2 for all experiments.

[d] For formation of 2b. Determined from glc by monitoring the disappearance of 1b.

[e] By glc analysis.

[f] Corrected for purity.

EXPERIMENTAL

The ^1H nmr spectra were obtained on a Varian XL-100 nmr spectrometer equipped with a Digilab nmr-3 FT accessory. The ir spectra were obtained on a Perkin Elmer 621 spectrophotometer. Mass spectra were obtained on a CEC 21-104 mass spectrometer at 70eV. Gas chromatographic analyses (glc) were carried out using a Bendix model 2300 gas chromatograph with 5 ft x 0.25 in. copper columns packed with 5% SE-30 on Chromosorb G-HP (80-100 mesh) using He carrier gas at a 60 ml/min flow rate. The structures of 4b, 5a and

their nitrogen-15 isomers were confirmed by infrared, nuclear magnetic resonance and mass spectral analysis.

d,ℓ-Nornicotine [4b]

The synthesis of 4b (Table I - Expt No 1-6) were carried out on the following molar scales based on cyclopropyl 3-pyridyl ketone [1b] (10): Expt No 1 and 2, 0.1 mol; Expt No 3, 4, and 6, 0.05 mol; Expt No 5, 0.03 mol. All reactions were run by the method outlined for the preparation of *d,ℓ*-nornicotine-1'-^{15}N, except for Expt No 1 where 2-ethoxyethyl ether was not used and Expt No 1 and 2 where the reactants were mixed in a moisture-free enclosure due to the hygroscopicity of the anhydrous magnesium chloride.

d,ℓ-Nicotine [5a]

The preparation of *d,ℓ*-nicotine (85.2% yield) was carried out using 0.024 mol of 4b by the method described for the synthesis of *d,ℓ*-nicotine-1'-^{15}N.

d,ℓ-Nornicotine-1'-^{15}N

To 1.1385 g (0.0247 mol) formamide-^{15}N [Koch Isotopes, 88.4 atom % enrichment by mass spectral analysis, 98.7% glc purity] was added 2.8537 g (0.0634 mol) formamide, 4.36 g (0.03 mol) cyclopropyl 3-pyridyl ketone (10), 1.19 g (0.0059 mol) magnesium chloride hexahydrate and 15 ml of 2-ethoxyethyl ether. The resulting mixture was placed under a nitrogen atmosphere and heated under reflux with stirring for 21 hrs. The reaction mixture was cooled and 20 ml of concentrated hydrochloric acid (pH 1) was added with stirring keeping the temperature below 20°. The resulting solution was extracted with chloroform (3 x 40 ml). The chloroform layers were washed with 10% aqueous hydrochloric acid (10 ml). Residual chloroform was removed under reduced pressure from the combined aqueous acid layers. The acid solution was then heated under reflux under a nitrogen atmosphere for 16 hrs, cooled and basified with 50 ml of 50% aqueous

sodium hydroxide (pH 11). The resulting mixture was extracted with ether (4 x 40 ml). The ether was dried (NaOH) and removed to give 2.79 g of crude oil. The aqueous layer and insoluble solids were continuously extracted for 24 hrs with ether. The ether was dried (NaOH) and removed to give an additional 0.26 g of crude oil. The crude oil was distilled to give 2.36 g (53.7%) of *d,ℓ*-nornicotine-1'-^{15}N: bp 77.5-81° (0.2 mm). Glc showed purity >97%. Mass spectral analysis indicated 24 atom % enrichment.

d,ℓ-Nicotine-1'-^{15}N

To a cooled (4°), stirred solution of 2.40 g (0.046 mol) of 88% aqueous formic acid and 1.86 g (0.023 mol) of 36.9% formaldehyde solution was added 1.36 g (0.0092 mol) of *d,ℓ*-nornicotine-1'-^{15}N. Water (4 x 0.5 ml) was used to facilitate transfer of the *d,ℓ*-nornicotine-1'-^{15}N. The resulting solution was placed under a nitrogen atmosphere, heated under reflux with stirring for 5 hrs, allowed to cool to room temperature and stand overnight. The solution was cooled in an ice bath and basified with 9 ml of 50% aqueous sodium hydroxide (pH 11). The mixture was extracted with ether (4 x 20 ml). The ether was dried (NaOH) and removed under reduced pressure (12 mm) at room temperature to give 1.29 g crude oil. The aqueous layer was continuously extracted with ether for 16 hrs. The ether was dried (NaOH) and removed as above to give an additional 0.08 g of crude oil. The crude oil was distilled at 93-119° (0.37 mm, air-bath temperature) to afford 1.25 g (84.0%) of *d,ℓ*-nicotine-1'-^{15}N. Glc showed purity >97%. Mass spectral analysis indicated 24 atom % enrichment.

ACKNOWLEDGMENTS

The authors thank Dr. J. F. Whidby for the nuclear magnetic resonance spectra, and Mr. Gunars Vilcins and Dr. John O. Lephardt for determination of the infrared spectra.

REFERENCES

1. Jenkins R. W. Jr., Comes R. A. and Bass R. T. - Recent Ad. Tob. Sci. 1: 1 (1975).
2. Johnson W. R., Hale R. W., Clough S. C. and Chen P. H. - Nature 243: 223 (1973).
3. Johnson W. R., Hale R. W. and Clough S. C. - Nature 244: 51 (1973).
4. Baxter J. E. and Hobbs M. E. - Tob. Sci. 11: 65 (1967).
5. Johnson W. R., Powell D. H., Hale R. W. and Kornfeld R. A. - Chem. Ind. (London): 521 (1975).
6. Leete E., Isaacson H. V. and Durst H. D. - J. Label. Compounds 7: 313 (1971).
7. Breuer E. and Stein Y. - Israel J. Chem. 6: 901 (1968).
8. Breuer E. and Melumad D. - Tetrahedron Lett.: 3595 (1969).
9. Comes R. A., Core M. T., Edmonds M. D., Edwards W. B. III, and Jenkins R. W. Jr. - J. Label. Compounds 9: 253 (1973).
10. Edwards W. B. III - J. Heterocycl. Chem. 12: 413 (1975).