Phytochemistry, 1972 ,Vol. 11 ,pp. 3371 to 3375. Pergamon Press. Printed in England.

SOME NEW ALKALOIDS FROM BURLEY TOBACCO*

A. H. WARFIELD, W. D. GALLOWAY and A. G. KALLIANOS
Liggett & Myers Inc., Durham, NC 27702, U.S.A.

(*Revised received* 23 *May* 1972. *Accepted* 1 *June* 1972)

Key Word Index—*Nicotiana tabacum*; Solanaceae; burley tobacco; pyridine alkaloids.

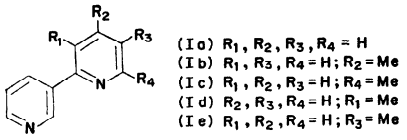
Abstract—Three new pyridine alkaloids have been isolated from an extract of burley tobacco (*Nicotiana tabacum*). The assigned structures of 5-methyl-2,3′-bipyridine (Ie), *N*-formylnornicotine (IVa) and *N*-acetylnornicotine (IVb) are based on spectroscopic properties. In addition, the structural assignments are confirmed by synthesis.

INTRODUCTION

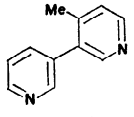
MORE than fifteen pyridine alkaloids have already been isolated from aged tobacco (*Nicotiana tabacum*),[1] although only one new tobacco alkaloid has been reported[2] in the last 10 yr. GLC of alkaloidal fractions obtained from aged tobacco leaves indicates the presence of a large number of components, most of which appear in very small quantities. Using a combination of modern separation techniques, we succeeded in isolating and subsequently identifying three alkaloids previously unreported in tobacco. An additional base was isolated, but remains unidentified.

RESULTS AND DISCUSSION

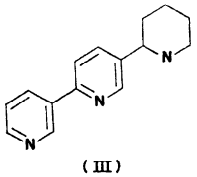
Aged burley tobacco was chosen as the source of minor alkaloids, since analytical GLC showed it to be relatively rich in these materials. Vacuum distillation was used to remove the large excess of nicotine and partially fractionate the minor alkaloids. Preparative GLC on a Carbowax 20M-TPA column yielded two peaks, A and B, which were further separated by GLC on OV-17 into four individual compounds: A-1, A-2, B-1 and B-2.



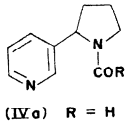
(Ia) R_1, R_2, R_3, R_4 = H
(Ib) R_1, R_3, R_4 = H; R_2 = Me
(Ic) R_1, R_2, R_3 = H; R_4 = Me
(Id) R_2, R_3, R_4 = H; R_1 = Me
(Ie) R_1, R_2, R_4 = H; R_3 = Me



(II)



(III)



(IVa) R = H
(IVb) R = Me

5-*Methyl*-2,3′-*Bipyridine* (Ie)

Compound A-1 was shown (MS) to have a MW of 170. The IR spectrum indicated no C=O or N–H functions, but bore a general similarity to that of 2,3′-bipyridine (Ia), a known

* A preliminary account of this work was presented at the 23rd Tobacco Chemists' Research Conference, Philadelphia, Pa. (October 1969).

[1] M. PAILER, in *Tobacco Alkaloids and Related Compounds* (edited by U. S. VON EULER), p. 15, Macmillan, New York (1965).

[2] T. KISAKI, S. MIZUSAKE and E. TAMAKI, *Phytochem.* **1**, 323 (1968).

tobacco alkaloid.[1] The UV spectrum also resembled that of Ia. The NMR spectrum revealed that all protons were in aromatic positions, except for a 3H singlet at 2·37 ppm, due to an aromatic C–CH_3 group. The characteristic pattern for a 3-substituted pyridine ring could be discerned in the aromatic region. No spectral data on methyl bipyridines were available in the literature for comparison. Possible biogenetic analogy with nicotelline[1] and anatelline,[2] which are 4-substituted 2,3′-bipyridine or anabasine derivatives, prompted the synthesis of 4-methyl-2,3′-bipyridine (Ib),[3] by Gomberg–Bachman reaction of 3-aminopyridine with 4-picoline. Separation of the reaction mixture by GLC yielded Ib and II, which were distinguished by their NMR spectra (Table 1). However, spectra of Ib were not identical to those of compound A-1. The 6-methyl isomer (Ic) could be eliminated since its methyl signal in the NMR would be expected to appear around 2·6 ppm, by analogy to the three picolines.[4] The remaining isomers Id and Ie were synthesized by Gomberg–Bachman reaction of 3-aminopyridine and 3-picoline.[3] Only one of the four posible compounds was isolated from the reaction mixture, and this material was identical in every respect to A-1. Comparison of the NMR spectrum of this material with that of Ib (Fig. 1) showed that the methyl group was located at the 5-position (Ie). The spectrum of Ib shows a multiplet at 6·95 ppm, due to the proton on C-5. This signal is missing in the spectrum of base Ie, while the area of the multiplet at 7·55 ppm, which includes the signal attributable to the proton on C-3, is doubled. Further comparison of the spectra of Ib and Ie with that of the unsubstituted compound (Ia) (Table 1) lends additional support to this assignment.

TABLE 1. CHEMICAL SHIFTS OF AROMATIC PROTONS IN BIPYRIDINES

	Chemical shift (δ)								
	Ring A				Ring B				
Compound	H-2′	H-4′	H-5′	H-6′	H-2	H-3	H-4	H-5	H-6
2,3′-Bipyridine (1a)	9·07	8·27	7·27	8·50	—	7·73	7·74	7·2	8·60
4-Methyl-2,3′-bipyridine (1b)	9·07	8·20	7·22	8·56	—	7·45	—	6·95	8·42
5-Methyl-2,3′ bipyridine (1e)	9·08	8·28	7·25	8·58	—	7·55	7·55	—	8·47
4-Methyl-3,3′ bipyridine (II)	8·58	7·59	7·30	8·58	8·37	—	—	7·11	8·44
Anabasamine (III)[7]	9·1	8·2	7·2	8·5	—	7·6	7·6	—	8·5

In both Ia and Ie, the proton on C-4′ exhibits a signal at 8·27–8·28 ppm, while in Ib this signal is shifted to 8·20 ppm, due to slight diamagnetic shielding by the methyl group on C-4. A methyl group at C-3 would be expected to shield the proton on C-4′ to a greater extent than the 4-methyl group of Ib. Therefore, the methyl group of Ie can only be at C-5. The assignment is further supported by comparison (Table 1) of the spectral data for A-1 (Ie) with that obtained for anabasamine (III), recently isolated[5] from *Anabasis aphylla* (Chenopodiaceae) seeds. The 3- and 4-protons of the disubstituted pyridine rings resonate

[3] R. L. FRANK and J. V. CRAWFORD, *Bull. Soc. Chim. Fr.* 419 (1958).
[4] 2-Picoline resonates at 2·55 ppm, while 3- and 4-picolines show signals at 2·30 and 2·32 ppm, respectively.
[5] S. Z. MUKHAMEDZHANOV, KH. A. ASLANOV, A. S. SADYKOV, V. B. LEONT'EV, and V. K. KIRYUKHIN. *Khim. Prir. Soedin.* **4**, 158 (1968).

at 7·55–7·6 ppm in both of these alkaloids. The other aromatic proton signals show good agreement as well.

Compound A-2

A second peak was observed and collected during rechromatography of Fraction A. This material was found to have a MW (MS) of 234, and a probable empirical formula of $C_{13}H_{18}N_3O_2$. NMR and IR spectra indicated that the material is a derivative of nicotine, with a carbomethoxymethyl substituent. However, in view of the lack of sufficient structural information, the data for this material will be withheld until further studies are completed.

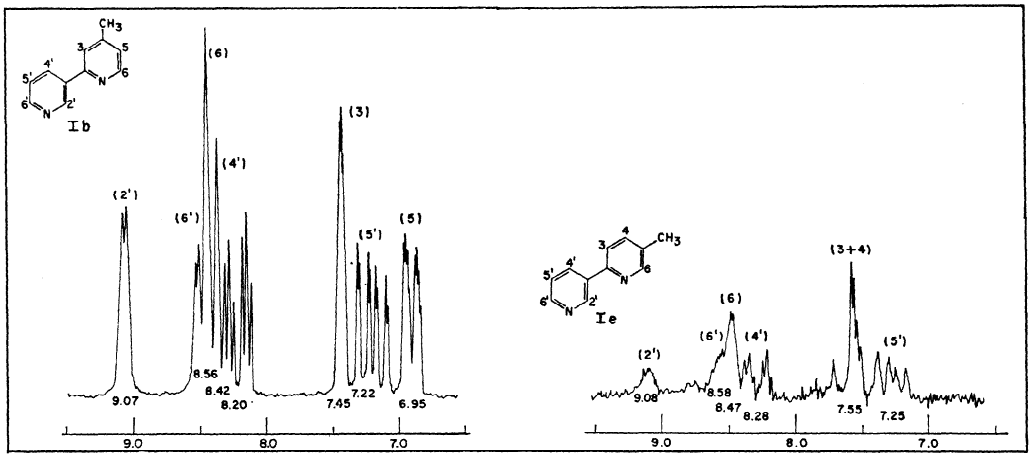


FIG. 1. NMR SPECTRA OF 4-METHYL-2,3′-BIPYRIDINE (Ib) (LEFT) AND 5-METHYL-2,3′-BIPYRIDINE I(e) (RIGHT).

N-*Formylnornicotine* (IV)

The MS of compound B-1 yielded an intense molecular ion at *m/e* 176, and other major fragments at 147, 119, 99 and 70. With the exception of the molecular ion, all the major ions are also found in the spectrum of nornicotine.[6] The IR spectrum of B-1 showed aldehyde C–H stretching bands at 2830 and 2775 cm^{-1}, and a C=O band at 1689 cm^{-1}, indicating an *N*-formylamine.[7] Other peaks in the spectrum were typical of a 3-pyridyl substituent, and a strong band at 1482 cm^{-1} indicated the presence of methylene groups. The NMR spectrum confirmed the presence of the pyrrolidine ring, and also the pyridine and aldehyde protons (see Experimental). A synthetic sample of *N*-formylnornicotine (IVa) was indistinguishable (spectral data, R_t and R_f on GLC and TLC) from B-1.

N-*Acetylnornicotine* (IVb)

The MS of Peak B-2 was similar to that of B-1. The *m/e* 147 and 70 peaks were present, but an additional *m/e* 43 peak appeared, and the molecular ion (*m/e* 190) was increased by 14 m.u. The IR spectrum revealed a carbonyl group, which absorbed at 1658 cm^{-1}, as expected for an acetylated secondary amine. The assignment of *N*-acetylnornicotine (IVb) to B-2 was supported by the NMR spectrum, which contained a methyl singlet at 2·00 ppm, but was otherwise very similar to the NMR spectrum of B-1. *N*-Acetylnornicotine (IVb),

[6] A. M. DUFFIELD, H. BIDZIKIEWICZ and C. DJERASSI, *J. Am. Chem. Soc.* **87**, 2926 (1965).
[7] For example, *N,N*-dimethylformamide exhibits bands at 2815, 2775 and 1684 cm^{-1}.

prepared by the method of Polonovski and Polonovski,[8] was shown to be identical to B-2 (IR, UV, NMR and MS and R_t and R_f on GLC and TLC).

Quantitative Estimation of Ie, IVa *and* IVb *in Burley Tobacco*

The approximate percentages of the three alkaloids in aged burley tobacco leaf were determined by quantitative GLC of the basic portions of methanol extracts, and are listed in Table 2. Similar GLC studies on other types of tobacco also revealed the presence of peaks corresponding to these three alkaloids.

TABLE 2. APPROXIMATE PERCENTAGES OF ALKALOIDS IN BURLEY TOBACCO

Peak No.	Compound	% Found	Peak No.	Compound	% Found
A–1	5-Methyl-2,3′-bipyridine (Ie)	0·0006–0·003	B-1	*N*-Formylnornicotine (IVa)	0·022
A-2	Unidentified	0·007–0·002	B-2	*N*-Acetylnornicotine (IVb)	0·008

Possible Artifact Formation

The possibility was considered that *N*-formyl- and/or *N*-acetylnornicotine might be artifacts formed during extraction or in subsequent steps employed in the isolation procedure. Enzymatic formylation or acetylation could be ruled out because of the reflux conditions of the extraction. However, chemical mechanisms could account for the formation of IVa and/or b. Therefore, we extracted a sample of burley tobacco with cold methanol for several days and worked up the extract without use of acid or strong base, and without distillation or other heating procedures. The final extract was separated by TLC on silica gel plates, and the band expected to contain IVa and b eluted and analyzed for these materials by GLC. Both alkaloids were observed, indicating that they are not artifacts formed in the isolation procedure. Another possibility is that these materials are formed during aging of the tobacco. However, this does not appear to be the case, since analysis of extracts obtained from air-cured but unaged burley tobacco samples showed the presence of substantial amounts of IVa and b.

EXPERIMENTAL

NMR spectra were run in CCl_4 on a Varian Associates A-60A instrument. NMR chemical shifts are reported in ppm (δ) from TMS as internal standard, and *J* values in Hz. *s*, *d*, *t* and *m* refer, respectively, to a singlet, doublet, triplet and multiplet. Some of the NMR spectra were obtained using a CAT (Varian 1024) to accumulate enough scans to yield the desired sensitivity. MS were measured on a Bendix model 12 instrument at 70 eV using direct insertion, and are reported as follows: mol. ion/base peak, second largest peak, etc. IR spectra were measured in CCl_4 solution on a Perkin–Elmer model 21 instrument in a 0·4-mm micro-cavity cell;[9] w, m and s refer, respectively, to weak, medium, and strong bands. GLC was carried out on a Varian Aerograph model 1520 B instrument, equipped with flame ionization and thermal conductivity detectors. Injector and detector temperatures for all columns used were *ca.* 280°.

Extraction and isolation of alkaloids. A 1600-g sample of ground burley tobacco (1965 crop) was extracted with 18 l. of MeOH. The extract was concentrated to 3 l. on a flash evaporator, the pH adjusted to 2–3 with H_2SO_4, and the remaining MeOH removed on a rotary evaporator at 40–50°. H_2O and CH_2Cl_2 were added, and the organic layer separated. The aqueous portion was made alkaline, and basic compounds extracted with CH_2Cl_2. The residue after drying and evaporating the organic layer was distilled under

[8] M. POLONOVSKI and M. POLONOVSKI, *Bull. Soc. Chim. Fr.* **41**(4), 1190 (1927).
[9] G. D. PRICE, E. C. SUNAS and J. F. WILLIAMS, *Analyt. Chem.* **39**, 138 (1967).

vacuum on a Kontes one-piece semi-micro still. Nearly pure nicotine (48 g) was obtained at 108–114°/10 mm. The residue (18 g) was then fractionally distilled at 0·4 mm. The fraction boiling at 94–132° (0·7 g) was subjected to preparative GLC on a 4·5 mm i.d. × 1·5 m column packed with 10% Carbowax 20M-TPA on 30–60 mesh Chromosorb W, yielding Peak A (19 min), and Peak B (33·3 min).

Rechromatography of Peak A on a 2·16 mm i.d. × 3 m column packed with 3·4% OV-17 and 0·15% FFAP on 100–120 mesh Gas Chrom Z (temp. programmed 145–250° at 4°/min, 30 ml He/min) yielded two components. Peak A-1 (Ie) was collected at 15 min. MS: *m/e* 170/170, 169, 171, 144, 85, 39, 118, 142. IR (CCl_4): 3100(w), 3030(m), 2920(w), 2870(w), 1595(m), 1578(s), 1492(m), 1468(s), 1417(s), 1378(m), 1218(m), 1187(m), 1138(w), 1120(w), 1098(w), 1062(w), 1028(s), 1014(s) cm^{-1}. NMR (CCl_4): δ 2·37 ppm (*s*, 3H, CH_3) see Fig. 1 for aromatic portion of spectrum). UV (H_2O): 277·5 and 241·0 nm. Peak A-2 was collected at 17·8 min; data for this material will be reported later.

Peak B was rechromatographed on a column identical to that used for Peak A, above, but 6·1 m long. The optimum flow rate was 37 ml He/min, and the column temp. was programmed 182–207° at 1°/min. Peak B-1 (IVa) was collected at 25·1 min. MS: *m/e* 176/176, 147, 119, 70, 98, 120, 175, 105, 148, 41, 39, 51. IR (CCl_4): 3050(w), 2980(m), 2880(m), 1680(s), 1577(w), 1482(m), 1426(m), 1413(m), 1375(s), 1335(w), 1320(w), 1309(w), 1154(w), 1127(w), 1105(w), 1025(m) cm^{-1}. NMR (CCl_4): (180 scans on CAT δ 2·49 (*m*, 4H, H-3 and 4 of pyrrolidine ring), 2·32 (*m*, 2H, H-5), 5·14, (*m*, 1H, H-2), 7·0–8·5 ppm (*m*, 5H, pyridine and aldehyde protons). Peak B-2 (IVb) was collected at 26·3 min. MS: *m/e* 190/190, 70, 43, 147, 120, 119, 41. IR (CCl_4): 3040(w), 2970(m), 2870(m), 1658(s), 1573(w), 1480(w), 1406(s), 1354(m), 1312(w), 1270(w), 1243(w), 1188(w), 1163(w), 1123(w), 1103(w), 1024(m), 1015(w) cm^{-1}. NMR (CCl_4): (35 scans on CAT) δ 1·72 (*m*, 4H, H-3 and 4), 2·00 (*s*, 3H, $-COCH_{(3)}$, 3·57 (*m*, 2H, H-5), 4·87 (*m*, 1H, H-2), 7·12 (*m*, 2H, H-4′ and 5′) 8·29 ppm (*m*, 2H, H-2′ and 6′).

Synthesis of 4-*methyl*-2,3′-*bipyridine* (Ib) *and* 4-*methyl*-3,3′-*bipyridine* (II). The procedure of Frank and Crawford[3] was used for carrying out the reaction of diazotized 3-aminopyridine with 4-picoline. The distillate was subjected to dry-column chromatography[10] on silica gel (grade II) in nylon tubing, developing the column with CH_2Cl_2. Extraction of the lower portion of the column with MeOH yielded an oil, which was separated into two fractions by prep. GLC on the OV-17 column described above. 4-Methyl-3,3′-bipyridine (II) emerged at 13·5 min. MS: *m/e* 170/169, 170, 143, 142, 39, 63, 51, 50, 168, 115. IR (CCl_4): 3065(s), 1596(s), 1573(m), 1476(s), 1450(m), 1413(s), 1335(w), 1312(w), 1280(w), 1220(m), 1191(m), 1166(w), 1107(w), 1088(w), 1052(m), 1029(s), 1000(s), 955(w), 932 cm^{-1} (w). NMR (CCl_4): δ 2·30 (*s*, 3H, $-CH_3$), 7·11 (*d*, 1H, J_{56} = 5·2, H-5), 7·30 (*m*, 1H, $J_{4'5'}$ = 7·6, $J_{5'6'}$ = 5·1, $J_{2'5'}$ = 1·0, H-5′), 7·59 (*m*, 1H, $J_{4'6'}$ = $J_{2'4'}$ = 2·0, H-4′), 8·37 (*s*, 1H, H-2), 8·44 (*d*, 1H, H-6), 8·58 ppm (*m*, 2H, H-2′ and 6′). UV (H_2O): 263, 231 nm. 4-Methyl-2,3′-bipyridine (Ib) eluted at 15 min. MS: *m/e* 170/169, 170, 140, 39, 85, 118, 51, 93, 65, 50, 155. IR (CCl_4): 3070(s), 2950(w), 1610(s), 1578(m), 1470(s), 1422(s), 1433(m), 1340(w), 1305(w), 1291(m), 1202(m), 1188(m), 1123(m), 113(m), 1075(w), 1040(w), 1025(s), 995(w), 958(w), 885(w), 870 cm^{-1}(m). NMR (CCl_4): δ 2·37 ppm (*s*, 3H, $-CH_3$); see Fig. 1 for aromatic portion. UV (H_2O): 273, 237·5 nm.

Synthesis of 5-*methyl*-2,3′-*bipyridine* (Ie). Diazotized 3-aminopyridine was treated with 3-picoline by the procedure of Frank and Crawford.[3] After distilling off the excess picoline, the residue was chromatographed on silica gel, eluting with $CHCl_3$–CH_3OH–NH_4OH (60:10:1). The material eluting prior to a dark red band was subjected to prep. GLC on the Carbowax 20M-TPA column described above, yielding major peaks at 18·0 and 21·7 min. The latter peak was rechromatographed on a 3 mm i.d. × 7 m OV-17 column (160°, programmed to 250° at 6°/min; 60 ml He/min), affording two compounds: R_t 24·5 and 27·2 min, respectively. The spectral data for the second peak (27·2 min) showed that this material was identical to peak A-1 from the tobacco extract. The NMR spectrum (Fig. 1) enabled assignment of structure Ie to this alkaloid (see text).

Synthesis of N-*formylnornicotine* (IVa). A solution of 21 mg nornicotine in 5 ml dry THF was added dropwise to a THF solution of *N*-formylimidazole prepared from 115 mg 1,1′-carbonyldiimidazole and 33 mg formic acid. The solvent was removed, and the mixture separated by GLC on the Carbowax 20M-TPA column, yielding imidazole (which eluted first) and IVa. The spectral data for the latter matched those obtained for peak B-1 from tobacco.

Acknowledgements—We thank Dr. R. H. Wallick for the MS, Dr. G. D. Price for NMR spectra and Mr. E. C. Sunas for IR spectra. We also thank Mr. C. L. Shuford for the analytical GLC and Mrs. E. Augstein for assistance in the early phases of this work.

[10] B. Loev and M. M. Goodman, *Chem. & Ind.* 2026 (1967).