Phytochemistry, 1968, Vol. 7, pp. 323 to 327. Pergamon Press. Printed in England

PHYTOCHEMICAL STUDIES ON TOBACCO ALKALOIDS—XI.

A NEW ALKALOID IN *NICOTIANA TABACUM* ROOTS

TAKURO KISAKI,* SHIGENOBU MIZUSAKI and EINOSKE TAMAKI

Division of Plant Biochemistry, Central Research Institute, Japan Monopoly Corporation, 1-28-3, Nishishinagawa, Shinagawa-ku, Tokyo, Japan

(*Received* 3 *May* 1967)

Abstract—A new alkaloid has been isolated from the roots of *Nicotiana tabacum*. Its chemical structure was confirmed as 2,4-di(3-pyridyl)piperidine (*anatalline*) by elemental analyses, u.v., i.r. and NMR and mass spectroscopy and finally by its dehydrogenation to nicotelline. The biosynthetic route for this compound may be similar to that of anatabine, but it is not formed in a manner similar to anabasine from lysine.

INTRODUCTION

WE HAVE previously demonstrated an unknown alkaloid, designated B, in tobacco roots.[1] When nicotine is removed from the roots with ether at neutral pH, more than fifteen other minor alkaloids can be detected. The unknown compound B gave a blue color by isatin reagent and a red color by Koenig reagent, and was not detectable in aerial parts (stem and leaves) of the plant. The date suggested that the unknown may be involved in nicotine biosynthesis as an immediate containing a pyridine ring. Unsuccessful attempts to isolate compound B are reported in this manuscript. During the course of this work, however, another alkaloid with nearly the same R_f value as that of compound B has been purified. The new alkaloid does not give a blue color with isatin.

More than ten alkaloids have already been reported from cured tobacco leaves, including some of unknown structure such as nicotoine and nicotimine.[2] The new alkaloid, which we are reporting, is different from any previously identified ones. The structural confirmation of the new alkaloid will be described and its biosynthesis discussed.

RESULTS AND DISCUSSION

Structural Confirmation

As described in the Experimental section, compound B was separated on an alumina column into two components. One of these showed no blue color with isatin and was designated B′. Compound B′ was converted to its perchlorate and eventually crystals were obtained, free of impurities.

Compound B′ gave a positive reaction with Koenig reagent, indicating the presence of a pyridine ring. The i.r. spectrum (Fig. 1) showed absorptions at 710 and 808 cm^{-1}, suggesting the presence of a 3-substituted pyridine rather than a 2- or 4-substituted pyridine.[3] NMR

* Present address: Department of Biochemistry, Michigan State University, East Lansing, Michigan, until fall of 1968, thereafter the Central Research Institute, Japan Monopoly Corporation, Tokyo, Japan.

[1] E. WADA, T. KISAKI and M. IHIDA, *Arch. Biochem. Biophys.* **80**, 258 (1959).

[2] A. PICTET, *Arch. Pharm.* **224**, 375 (1906).

[3] H. SHINDO and N. IKEKAWA, *Pharm. Bull.* (*Tokyo*) **4**, 192 (1956).

showed signals which correspond to 2-, 4-, 5-, and 6-protons of two 3-pyridines[7] in the low field. Upon oxidation of compound B' with potassium permanganate, nicotinic acid was the only Koenig positive product obtained.

Elemental analysis indicated an empirical formula of $C_5H_{5-6}N$. However, the mass spectrum (Fig. 2) showed the molecular weight of compound B' to be three times the empirical formula, and a peak of mass number m/e=239 was regarded as a parent radical ion on the basis of the nitrogen rule. The u.v. spectra showed an absorption maximum at 261 mμ in 0·5 N hydrochloric acid solution, and no shift of the absorption maximum was observed in

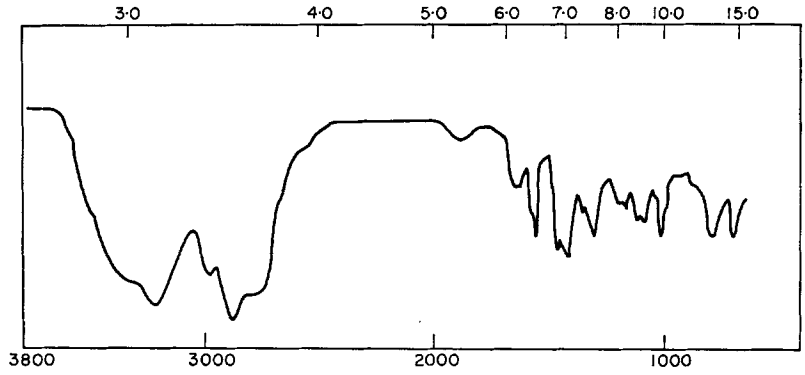


FIG. 1. INFRA RED SPECTRUM OF ANATALLINE. TAKEN IN A FILM STATE ON NaCl BLOCK BY MELTING.

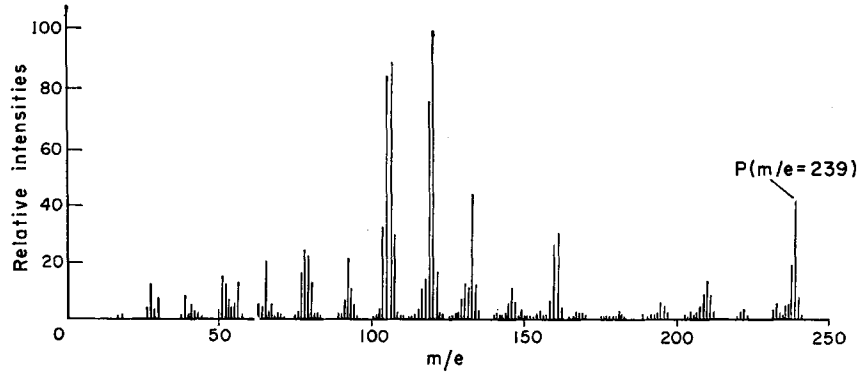


FIG. 2. MASS SPECTRUM OF ANATALLINE. RECORDED AT AN IONIZING VOLTAGE 70 eV, IONIZING CURRENT 50 μA, ION SOURCE TEMPERATURE 200°.

alkaline solution, indicating absence of a conjugated double bond or a conjugated aromatic ring with pyridine.[4] The molar absorptivity of compound B' in hydrochloric acid solution was twice ($11{\cdot}35 \times 10^3$) that of nicotine ($5{\cdot}56 \times 10^3$), suggesting that the compound contains two pyridine rings. Absence of a double bond was also supported by the NMR spectrum (Fig. 3) in which no signal was observed in the lower field except the signals of aromatic ring protons. The presence of a secondary amino group was indicated, since the compound gave a blue color by the Simon reaction,[5] an absorption at 3230 cm^{-1} in the i.r. spectrum and a

[4] M. L. SWAIN, A. EISNER, C. F. WOODWARD and B. A. BRICE, *J. Am. Chem. Soc.* **71**, 1341 (1949).
[5] F. FEIGL, *Mikrochim. Acta* **1**, 127 (1937).

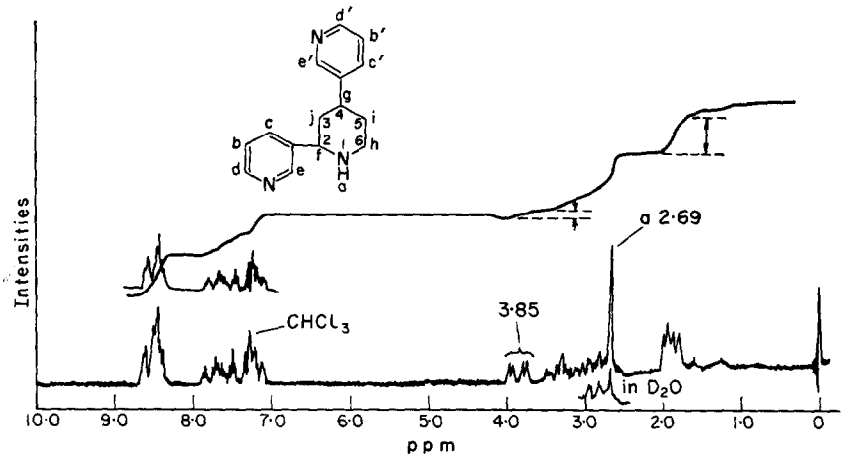
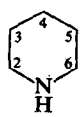
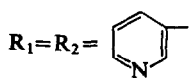


FIG. 3. NUCLEAR MAGNETIC RESONANCE SPECTRA OF ANATALLINE. RECORDED AT A SWEEP WIDTH 600 c/s IN A SOLUTION OF 30 mg OF ANATALLINE IN 0·5 ml OF $CDCl_3$.

signal at $\delta = 2{\cdot}69$ in the NMR spectrum which diminishes in D_2O. With benzoic anhydride, a mono benzoyl derivative was formed which further indicated the presence of a secondary amine. From subtracting the mass number (m/e = 156) of two pyridines from that of parent ion (m/e = 239), and taking consideration that the compound is a substituted aliphatic amine, the remaining mass number (m/e = 83) exactly corresponded to a disubstituted piperidine. Therefore, it was predicted that the compound B′ is dipyridylpiperidine, of which there are nine possibilities. (I–IX).



	Position of R_1	R_2
I	2	3
II	2	4
III	2	5
IV	2	6
V	3	4
VI	3	5
VII	2	2
VIII	3	3
IX	4	4



3-pyridyl

Since the mass spectrum (Fig. 2) showed no signal at m/e = 168 and only nicotinic acid was produced on oxidation structure VII, VIII and IX seem improbable. Absence of peak at m/e = 182 corresponding to a dipyridylethane radical ion also suggests that structures I and V are unlikely. A signal ($\delta = 3{\cdot}85$) with an intensity of a proton showed a quartet with the same intensity ($J_{2,3e} = 3{\cdot}0$ c/s $J_{2,3a} = 10{\cdot}8$ c/s), indicating that the 2-proton couples with the neighboring two non-equivalent protons (3e and 3a) as observed with anabasine[6] and nicotine.[7] The mass spectrum peak m/e = 196 corresponds to a dipyridylpropane radical ion. These observations suggested that II is the most likely structure. Upon dehydrogenation of the compound with Pd, tripyridyl was obtained. The melting point and i.r. spectrum of this compound

[6] Unpublished data.
[7] *Varian high resolution NMR spectra catalog*, Volume 1 (Varian Associates) compound No. 269.

were identical with that of "nicotelline" (2,4-di(3-pyridyl)pyridine which was first isolated by Pictet and Rotschy[8] in 1901 and later by Noga[9] and by Kuffner and Kaiser.[10] Thesing and Müller[11] confirmed the structure of nicotelline by chemical synthesis. Therefore, it can be concluded that the structure of the compound B′ is 2,4-di(3-pyridyl)piperidine (II), for which we propose the name "anatalline". In general, the mass spectrum of this new compound in the region below 162 resembles closely that of anabasine,[12] except for an absence of a peak at 84 which is a base peak and corresponds to piperidine radical ion from anabasine.

Anatalline possesses two asymmetric carbon atoms, and therefore, four stereoisomers ought to exist. The isolated specimen did not show optical rotation, but it is uncertain whether the sample is a racemate or intramolecularly optically inactive.

OCCURRENCE

Anatalline (II) has been found in all *Nicotiana* species examined[13] and is present in both aerial parts and the root.[1] It has also been detected in cultured excised tobacco root but not in the tobacco grafted onto tomato stock. Therefore, it is probably synthesized in the root like nicotine. Since anatalline is closely related structurally to nicotelline, these two compounds may be expected to co-occur. However, since the reduction of an aromatic ring to an aliphatic ring has never been observed in plant tissue, it is unlikely that anatalline is synthesized from nicotelline. Kuffner *et al.*[10] considered whether nicotelline might be a secondary product formed during the isolation procedure, but this seems unlikely in the case of anatalline.

Isotopic experiments have been undertaken to examine the precursor of the aliphatic ring of anatalline. Neither lysine-2-C,[14] which labels the piperidine ring of anabasine[14] nor δ-hydroxylysine-2-C[14] which was expected to form the piperidine ring of anatabine, was incorporated into anatabine and anatalline.[15] On the basis of structural similarity to the other tobacco alkaloids, it is predicted that the biosynthesis of anatalline may be related to the formation of anatabine(2-(3-pyridyl)piperid-4,5-ene). In anatabine or its precursor the 4 position may react with a precursor of pyridine. It is possible that the piperidine ring of anatalline might come from the same precursor as that of anatabine.

EXPERIMENTAL

Isolation of Anatalline

Lateral roots of *Nicotiana tabacum* "Hicks" from the field[16] were collected after the leaves were harvested. The roots were dried by sunshine and 84 k dry weight were cut into segments 3 mm long, extracted three times for 5 hr at 60° with 6 l. of 70 per cent methanol per kg. The methanol extracts were combined, acidified with HCl and concentrated to 20 l. under vacuum. After the concentrate was washed with ether and $CHCl_3$ several times, the concentrate was made alkaline with NaOH and the alkaline solution was extracted with $CHCl_3$ several times. The $CHCl_3$ extracts were combined and shaken with dilute H_2SO_4 to extract the bases. Alternate extractions of bases with $CHCl_3$ and dilute H_2SO_4 were repeated twice. Finally the combined aqueous solution was extracted successively with ether at pH 4·5, 6·5, 7·5 and with $CHCl_3$ at a strong alkaline pH.

[8] A. PICTET and R. ROTSCHY, *Chem. Ber.* **34**, 696 (1901).
[9] E. NOGA, *Fachliche Mitt. Oesterr. Tabakregie* **14**, 11 (1914).
[10] F. KUFFNER and E. KAISER, *Monatsch. Chem.* **85**, 896 (1954).
[11] J. THESING and A. MÜLLER, *Chem. Ber.* **90**, 711 (1957).
[12] A. M. DUFFIELD, H. BUDZIKIEWICZ and CARL DJERASSI, *J. Am. Chem. Soc.* **87**, 2926 (1965).
[13] S. FUJITA and N. AOKI, Reports of Hatano, Tobacco Experimental Station (Japan), No. 55, 91 (1965).
[14] E. LEET, *J. Am. Chem. Soc.* **78**, 3520 (1956).
[15] Unpublished data.
[16] From the Utsunomiya Tobacco Experimental Station, Japan Monopoly Corporation, Tochigi-ken, Japan.

The ether extracts at pH 4·5 and 6·5 were combined and evaporated to dryness. This residue, a heavy oily substance (8·5 g), was placed on a silica liquid chromatography column and 2,3-dipyridyl (0·54 g) was eluted with 5 per cent methanol in ether from this column. Its u.v. spectrum and the m.p. of its picrate was identical with an authentic specimen. The ether extract at pH 7·5 contained mostly nicotine. The $CHCl_3$ extracts from the strongly alkaline solution contained 105·1 g of oily residue in which more than ten alkaloids were detected by paper chromatography. By distillation under vacuum a fraction (1·8 g) was obtained which boiled at 160°/3 mm and which contained compound B as detected by an R_f of 0·63 in a paper chromatographic system.[1] This fraction contained a few Koenig positive impurities, but the main component (compound B′) appeared to have a secondary amino group from its i.r. spectrum. After standing overnight with benzoic anhydride in ether, a crude benzoate was obtained with R_f of 0·9, which was difficult to purify. After hydrolysis with HCl the mixture of alkaloids obtained were chromatographed on an alumina column with the 2 per cent methanol in ether as an eluant, and two components, isatin positive (compound B) and isatin negative (compound B′, anatalline) were obtained. Compound B was in such a small quantity that further purification was unsuccessful. Anatalline was dissolved in 70 per cent perchloric acid, and after four years, a fairly large perchlorate precipitate was obtained which by paper chromatography was free of impurities. It was recrystallized from methanol with ether twice to give 870 mg of crystals, which melted over a broad range of 244–252°*. Nevertheless, there was present no detectable impurity by paper chromatography. (Found: C, 33·17; H, 4·18; N, 7·47; Cl, 19·21. required for $C_{15}H_{17}N_3.3HClO_4$: C, 33·32; H, 3·73; N, 7·77; Cl, 19·66 per cent.) The free base of anatalline is an amorphous solid at room temperature, but recrystallization was unsuccessful. It boiled at 225°/3 mm. (Found: C, 75·34; H, 7·39; N, 17·61. required for $C_{15}H_{17}N_3$: C, 75·28; H, 7·16; N, 17·56 per cent.) Picrate 258·5°. (Found: C, 43·32; H, 2·52; N, 17·63. required for $C_{33}H_{26}N_{12}O_{21}$: C, 42·77; H, 2·83; N, 18·14 per cent.)

Chemical Reaction and Degradation

Anatalline gave a benzoate boiling at 274°/3 mm. (Found: C, 76·95; H, 6·14; N, 11·89. required for $C_{22}H_{21}N_3O_2$: C, 76·94; H, 6·17; N, 12·24 per cent.) 100 mg of the benzoate in 10 ml of dil. H_2SO_4 was oxidized by addition of 5 per cent $KMnO_4$ solution. The reaction mixture was adjusted to pH 2·5 and continuously extracted with ether; nicotinic acid and a fluorescent substance were observed in the ether extract by paper chromatography. The basified mother liquor was steam distilled but the distillate showed no Koenig reaction. 30 mg of compound B′ were dehydrogenated with 50 mg of 20 per cent Pd-asbestos at 320° for 15 hr, and the product was extracted with $CHCl_3$. Upon evaporation of the solvent 23·1 mg of a white solid substance was obtained; recrystallization from ether–chloroform gave colorless needle crystals of m.p. 147–148·5°. This m.p., u.v. and i.r. spectra were identical with those of authentic nicotelline.

Experimental Method

Elemental analyses were carried out at the Laboratory of Microanalysis, Department of Agricultural Chemistry, University of Tokyo. Mass spectrograms were recorded by a Hitachi RMU 60 double-focusing mass spectrometer, NMR spectra by Hitachi Model H-60, i.r. spectra by a Perkin–Elmer Model 21 i.r. spectrophotometer, and u.v. spectra by Hitachi EPS-2.

Acknowledgements—The authors are grateful to Dr. Onda in Kitazato University (Tokyo) for recording NMR spectra and for their interpretations of the spectra and to Dr. N. E. Tolbert, Michigan State University, for his critical reading of the manuscript. We thank Dr. Kuffner in Austria for a gift of nicotelline and the director of Utsunomiya Tobacco Experimental Station for giving the aid in collection of tobacco roots. Thanks are also due to Miss Yoko Tanabe of our laboratory for her technical assistance.

* The melting points have not been corrected.