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NICOTINE *N*-OXIDES

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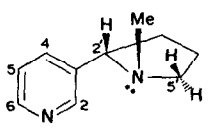
Key Word Index—*Nicotiana tabacum*, *N. affinis*, *N sylvestris*, Solanaceae, nicotine *N*-oxides; alkaloids

Abstract—Nicotine-1-*N*-oxide, *trans* and *cis* isomers of nicotine-1′-*N*-oxide and of nicotine-1,1′-di-*N*-oxide have been prepared and characterised by NMR, MS and reduction to nicotine. The *trans* and *cis* isomers of nicotine-1′-*N*-oxide have been identified in leaves, stems and roots of *Nicotiana tabacum*, *N. affinis* and *N. sylvestris*.

INTRODUCTION

Alkaloid *N*-oxides have been reviewed previously and it has been suggested that many more alkaloids might occur naturally in the form of their *N*-oxides [1]. Following this suggestion, members of the Solanaceae have been examined for their alkaloid *N*-oxide content and as a result *N*-oxides of hyoscyamine and hyoscine have been isolated from several genera [2]. "Oxynicotine" has been reported as a constituent of the solanaceous plants *Nicotiana tabacum*, *N. sylvestris*, *N. rustica* and *N. glauca* [3, 4] and "nicotine-*N*-oxide" has been identified in fermented tobaccos [5, 6]. Five *N*-oxides can be prepared from nicotine (**1**), namely, nicotine-1-*N*-oxide (**2**), nicotine-1′-*N*-oxide (**3**, **4**) and nicotine-1-1′-di-*N*-oxide (**5**, **6**), the latter two existing as diastereoisomeric pairs having either *trans* or *cis* configurations of the methyl/pyridinyl groups [7–11]. It has been proposed that nicotine-1′-*N*-oxide could be an intermediate in the demethylation of nicotine to nornicotine [9] but this proposal was not substantiated experimentally when radioactively labelled nicotine and *N*-oxide were fed to *Nicotiana* spp. [12, 13]. "Nicotine *N*-oxide" is believed to be formed by soil bacteria (possibly by aeration) and it has been suggested as a possible intermediate between nicotine and pseudo-oxynicotine [14]. Both diastereoisomers of nicotine-1′-*N*-oxide have been identified as metabolites of nicotine in animals [10, 15–17] and in man [10, 18]. The metabolism of nicotine in man proceeds by alternative routes involving oxidation of nitrogen to form nicotine-1′-*N*-oxide (both diastereoisomers) [10] or by oxidation at C-5′ to yield cotinine (**7**) [19] and it has been suggested that the ratio of these two oxidation products might be used as an indicator of bladder cancer in humans [20]. It would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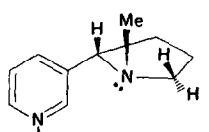
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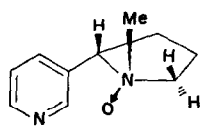
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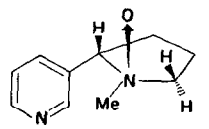
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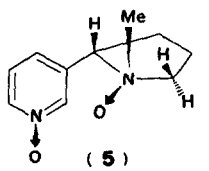
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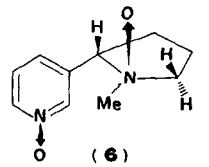
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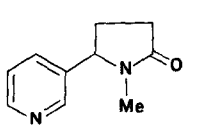
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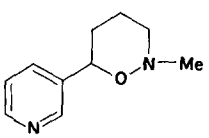
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appear that more is known about the oxidation of nicotine in animals than about the plants from which it is obtained and hence the object of the present work was to ascertain which *N*-oxides of nicotine existed as natural products. Fresh samples of *N. tabacum*, *N. affinis* and *N. sylvestris* were chosen for the investigation.

RESULTS

2′S-nicotine-1-*N*-oxide (**2**), 1′S,2′S-*trans*-nicotine-1′-*N*-oxide (**3**), 1′R,2′S-*cis*-nicotine-1′-*N*-oxide (**4**), 1′S,2′S-*trans*-nicotine-1,1′-di-*N*-oxide (**5**) and 1′R,2′S-*cis*-nicotine-1,1′-di-*N*-oxide (**6**) were prepared by the methods previously described [7, 21] and separated by preparative paper chromatography [10]. The five *N*-oxides were characterised by means of their NMR (Table 1) and their MS (Table 3) and by reduction to nicotine. Extracts of roots, stems and leaves of fresh *N. tabacum*, *N. affinis* and *N. sylvestris* collected during their early flowering stages were prepared and examined chromatographically for the presence of the five possible *N*-oxides of nicotine. Only the two diastereoisomeric isomers of nicotine-1′-*N*-oxide were identified as natural products. The proportion of nicotine and its two *N*-oxides were estimated chromatographically (Table 4).

DISCUSSION

Each of the five *N*-oxides of nicotine obtained by preparative paper chromatography readily reduced to nicotine. Evidence for the stereochemical differences of each prepared isomer was obtained mainly from their NMR spectra. Nicotine, nicotine-1-*N*-oxide and the mixed *trans* and *cis* isomers of nicotine-1′-*N*-oxide and of nicotine 1,1′-di-*N*-oxides have previously had their NMR spectra described [22]. In further studies, the NMR spectra of nicotine and of nicotine-1-*N*-oxide as bases and as hydrochloride salts and of *trans* and *cis* nicotine-1′-*N*-oxides as hydrochloride salts have been reported, but the chemical shifts assigned to the *trans* and *cis* nicotine-1′-*N*-oxides as bases and to the *trans* and *cis* nicotine-

Table 1. NMR chemical shifts for nicotine and its *N*-oxides*

	C-2H	C-4H	C-5H	C-6H	NMe	C-2′H, C-5′Hs
S-(−)-Nicotine (**1**)	8·55 *d*	7·78 *sex*	7·22 *q*	8·49 *dd*	2·18 *s*	3·21 (2H *m*, C-2′H, C-5′ *x* H)
S-(−)-Nicotine (**1**) + TFA	8·68	7·98	7·36	8·60	2·53	3·80 (2H *m*, C-2′H C-5′ *x* H) 3·00 (1H, *t*, C-5′*β*H)
S-(−)-Nicotine-1-*N*-oxide (**2**)	8·25 *br, s*	7·30 *t*	7·30 *m*	8·08 *dd*	2·23 *s*	3·20 (2H *m*; C-2′H, C-5′ *x* H)
S-(−)-Nicotine-1-*N*-oxide (**2**) + TFA	8·69	7·83	7·47	8·28	2·82	4·46 (1H *m* C-2′H) 3·98 (1H *m* C-5′ *x* H) 3·22 (C-5′*β*H)
S,S-*trans*-Nicotine-1′-*N*-oxide (**3**)	8·61 *d*	8·08 *sex*	7·30 *q*	8·57 *dd*	3·00 *s*	4·33 (1H *m* C-2′H) 3·73 (2H *m*, C-5′Hs)
S,S-*trans*-Nicotine-1′-*N*-oxide (**3**) + TFA	8·72	7·95	7·35	8·62	3·30	4·85 (1H, *m*, C-2′H) 4·00 (2H *m*, C-5′Hs)
R,S-*cis*-Nicotine-1′-*N*-oxide (**4**)	8·91 *br, s*	8·00 *dd*	7·41 *m*	8·71 *m*	2·91 *s*	5·14 (1H, *m*, C-2′H) 3·86 (2H, *m*, C-5′Hs)
R,S-*cis*-Nicotine-1′-*N*-oxide (**4**) + TFA	9·37	8·21	7·63	8·84	3·13	5·64 (1H *m*, C-2′H) 4·10 (2H *m*, C-5′Hs)
S,S-*trans*-Nicotine-1,1′-di-*N*-oxide (**5**)	8·70 *br, s*	7·81 *dd*	7·39 *t*	8·30 *dd*	3·10 *s*	4·52 (1H, *m* C-2′H) 3·75 (2H *m*, C-5′Hs)
S,S-*trans*-Nicotine-1,1′-di-*N*-oxide (**5**) + TFA	8·77	8·05	7·64	8·42	3·38	5·03 (1H, *m*, C-2′H) 4·05 (2H *m*, C-5′Hs)
R,S-*cis*-Nicotine-1,1′-di-*N*-oxide (**6**)	8·41 *br, s*	7·70 *m*	7·30 *m*	8·23 *dd*	2·89 *s*	4·78 (1H *m* C-2′H) 3·80 (2H *m*, C-5′Hs)
R,S-*cis*-Nicotine-1,1′-di-*N*-oxide (**6**) + TFA	8·65	7·75	7·62	8·37	3·22	5·30 (1H, *m*, C-2′H) 4·13 (2H *m*, C-5′Hs)

* δ values in ppm from TMS in $CDCl_3$ before and after addition of TFA

Table 2 Differences in chemical shifts between pyridine and pyridine *N*-oxide and between nicotine and its *N*-oxides*

	C-2H	C-4H	C-5H	C-6H	NMe
Pyridine [23]					
$\Delta\delta$N/NO	+0·36	+0·34	−0·05	+0·36	—
$\Delta\delta$N/NOH$^+$	−0·01	−0·28	−0·47	−0·01	—
Nicotine-1-*N*-oxide (**2**)					
$\Delta\delta$N/NO	+0·30	+0·48	−0·08	+0·41	−0·05
$\Delta\delta$N/NOH$^+$	−0·14	−0·05	−0·25	+0·21	−0·64
trans-Nicotine-1′-*N*-oxide (**3**)					
$\Delta\delta$N/NO	−0·06	−0·30	−0·08	−0·08	−0·82
$\Delta\delta$N/NOH$^+$	−0·17	−0·17	−0·13	−0·13	−1·12
cis-Nicotine-1′-*N*-oxide (**4**)					
$\Delta\delta$N/NO	−0·36	−0·22	−0·19	−0·22	−0·73
$\Delta\delta$N/NOH$^+$	−0·82	−0·43	−0·41	−0·35	−0·95
trans-Nicotine-1,1′-di-*N*-oxide (**5**)					
$\Delta\delta$N/NO	−0·15	−0·03	−0·17	+0·19	−0·92
$\Delta\delta$N/NOH$^+$	−0·22	−0·27	−0·42	+0·07	−1·20
cis-Nicotine-1,1′-di-*N*-oxide (**6**)					
$\Delta\delta$N/NO	+0·14	+0·08	−0·08	+0·26	−0·71
$\Delta\delta$N/NOH$^+$	−0·10	+0·03	−0·40	+0·12	−1·04

* $\Delta\delta$N/NO = δ amine − δ amine *N*-oxide, $\Delta\delta$N/NOH$^+$ = δ amine − δ (amine *N*-oxide + TFA), +ve values indicate shift to higher field, −ve values indicate shift to lower field

Table 3. MS of nicotine *N*-oxides

	% relative abundances of major fragment ions											
m/e	194	178	177	176	162	161	135	133	119	118	84	60
Nicotine-1-*N*-oxide (**2**)	—	31 (M^+)	6	—	—	18	—	11	5	7	100	2
trans-Nicotine-1′-*N*-oxide (**3**)	—	2 (M^+)	—	—	25	17	—	32	15	9	100	18
cis-Nicotine-1′-*N*-oxide (**4**)	—	0·3 (M^+)	—	—	24	20	—	27	14	7	100	14
trans-Nicotine-1,1′-di-*N*-oxide (**5**)	7 (M^+)	22	7	13	4	15	15	10	13	22	100	20
cis-Nicotine-1,1′-di-*N*-oxide (**6**)	35 (M^+)	33	25	33	4	18	59	13	24	65	100	94

Table 4 Estimation of nicotine, *trans* and *cis* nicotine-1′-*N*-oxides in roots, stems and leaves of *N. tabacum*, *N. sylvestris* and *N. affinis*

Species	Plant part	Nicotine (mg/%)	*trans*-Nicotine-1′-*N*-oxide (mg/%)	*cis*-Nicotine-1′-*N*-oxide (mg/%)	Total nicotine-1′-*N*-oxide (mg/%)	% Total *N*-oxide resp. to nicotine
N. tabacum	leaf	910	81	50	131	14·4
	stem	387	32	17	49	12·7
	root	542	52	32	84	15·5
N. sylvestris	leaf	813	96	54	150	18·5
	stem	377	52	23	75	19·9
	root	283	46	22	68	24·0
N. affinis	leaf	376	31	16	47	12·5
	stem	162	11	6	17	10·5
	root	80	7	3	10	12·5

1,1′-di-*N*-oxides as bases and as hydrochloride salts were obtained from a study of the spectra of mixed isomers [11]. During the present work comparisons have been made of the NMR spectra of the five *N*-oxides of nicotine from $CDCl_3$ solutions before and after the addition of trifluoracetic acid (TFA), a method previously used in the study of other aromatic *N*-oxides [23]. The chemical shifts assigned to the aromatic protons, *N*-methyl, C-2′ and 5′ protons of nicotine (**1**) and its *N*-oxides (**2**–**6**) for $CDCl_3$ solutions are given in Table 1 and the differences in chemical shift from nicotine of the *N*-oxides' aromatic and *N*-methyl signals are given in Table 2.

Comparison of the chemical shifts of the *N*-methyl signals of nicotine (**1**) and of the *trans* and *cis* isomers of nicotine-1′-*N*-oxide show that in both isomers the *N*-methyl group is deshielded but somewhat less in the *cis* isomer ($\Delta\delta$N/NO *trans* −0·82, *cis* −0·73, Table 2). This difference has been explained previously since in the *cis* isomer (**4**), the pyridine ring shields the *N*-methyl group [22] The same effect is observed also in the spectra of the *trans* (**5**) and *cis* (**6**) isomers of nicotine-1,1′-di-*N*-oxides and is apparent before and also after the addition of TFA (Tables 1 and 2).

In the NMR spectrum of nicotine, the signals for the C-2 and -6 hydrogens appear further downfield than those of the signals of the C-4 and -5 hydrogens [22]. The presence of the oxygen atom in pyridine *N*-oxide results in a marked shielding of the C-2 and -6 hydrogens ($\Delta\delta$N/NO +0·36) as observed in spectra from $CDCl_3$ solutions [23] Similar effects are noted in the NMR spectrum of nicotine-1-*N*-oxide (**2**) since the corresponding $\Delta\delta$N/NO values for the C-2 and -6 hydrogens are +0·30 and +0·41 respectively. The $\Delta\delta$N/NO values for the aromatic protons of pyridine *N*-oxide and nicotine-1-*N*-oxide are recorded in Table 2. When the NMR spectrum of nicotine-1-*N*-oxide (**2**) is compared with that of *trans* nicotine-1,1′-di-*N*-oxide (**5**) the greatest $\Delta\delta$ values are observed for the C-2 and -4 hydrogens, due to their proximity to the pyrrolidine N^+–O^- group This effect is less marked for the C-2 hydrogen in *cis*-nicotine-1,1′-di-*N*-oxide (**6**) where the pyrrolidine oxygen is not as close, but the chemical shift of the C-4 hydrogen is markedly different from its anticipated position (C-4H, $\Delta\delta$N/NO nicotine-1-*N*-oxide +0·48, *cis*-nicotine-1,1′-di-*N*-oxide +0·08) It is not possible to detect consistent $\Delta\delta$ correlations after the addition of TFA. Comparison of the NMR spectra of *trans*- (**3**) and *cis*- (**4**) nicotine-1′-*N*-oxide indicates that the greatest $\Delta\delta$N/NO value is observed for the C-4 hydrogen in the *trans* isomer. This would indicate that in the *trans* isomer the C-4 hydrogen lies closer to the N^+–O^- group than does the C-2 hydrogen Surprisingly however, the C-2 hydrogen in the *cis* isomer is markedly deshielded (Table 2).

Signals attributed to the C-2′ hydrogen and to the C-5′ equatorial hydrogen in the NMR spectrum of nicotine overlap at δ 3·21 and confirmation of these assignments has been obtained from a study of the effects of shift reagents on the spectra of nicotine [24] and of *trans*-3′-methylnicotine [25]. Assignments made for the C-2′ hydrogen and the C-5′ γ hydrogen at δ3·21 in the NMR spectrum of nicotine-1-*N*-oxide can be made by analogy to the spectrum of nicotine However on the addition of TFA, three distinct one-proton multiplets at δ4·46, 3·98 and 3·22 for the C-2′ and C-5′ hydrogens occur. The signal at δ3·22 can be attributed to the C-5′ β hydrogen and it is possible that the signal at δ4·46 is due to the C-2′ hydrogen In the case of the *trans* and *cis* isomers of nicotine-1′-*N*-oxide (**3**, **4**) and of nicotine-1,1′-di-*N*-oxide (**5**, **6**), definite assignments for the C-2′ and C-5′ hydrogens are not possible with certainty since in each spectrum a one proton multiplet is noted downfield from a two proton multiplet (Table 1) For the two *trans* isomers (**3**, **5**), if the signal furthest downfield is tentatively assigned to the C-5′ α hydrogen which is *cis* to the oxygen at N-1′, then the benzylic C-2′ hydrogen would have the same chemical shift as the C-5′ β hydrogen. If, however, the signal at lower field is assigned to the C-2′ hydrogen then both of the C-5′ hydrogens would have the same chemical shift When the *cis* isomers (**4**, **6**) are considered then the C-2′ and C-5′ β hydrogens are both *cis* to the N-1′ oxygen In this case it can be assumed that the C-2′ benzylic hydrogen signal will appear at lower field and hence the two C-5′ hydrogens will have the same chemical shifts. Hence for the *cis* isomers there are no spatial differences in the deshielding of the C-5′ α- and β-hydrogens by the pyrrolidine N^+–O^-

group. Thus, if the same is true for the *trans* isomers, then the one-proton signal at lower field can be attributed to the C-2′ hydrogen.

The five *N*-oxides of nicotine were also characterised by their MS (Table 3). In general, alkaloids with aromatic *N*-oxide groups are characterised by fragment ions at M^+-16 and M^+-17 [26] while *N*-oxides of aliphatic amines show, in addition, peaks at M^+-18 [27]. However, it is difficult to obtain reproducible MS for amine oxides since changes in the probe temperature can alter the spectrum [28]. The MS obtained for *N*-methylpyrrolidine *N*-oxide at 250° showed a M^+ of 63% relative abundance when the probe was at ambient temperature. On raising the probe temperature to 50° the M^+ relative abundance was reduced to 39% [28]. It has been demonstrated previously that thermal rearrangements can take place with nicotine *N*-oxides [29]. Nicotine-1′-*N*-oxide (presumably a mixture of *trans* and *cis* isomers) gave three different spectra when the probe temperature was set at 20°, 120° and 135°. At the higher temperatures increases in % relative abundances were noted for peaks at *m/e* 60, 118 and 119. Similarly, nicotine-1,1′-di-*N*-oxide (*trans* and *cis* isomers[9]) showed increases in % relative abundances for ion fragments which appeared at *m/e* 194 (M^+), 159, 135, 118, 60 (100%) when the probe temperature was 140° instead of 100°. Comparison of these spectra with those of 2-methyl-6(3-pyridinyl)-tetrahydro-1,2-oxazine (**8**), showed striking similarities and it was proposed that nicotine *N*-oxides thermally rearrange to oxazines [29].

The MS determined during the present investigation utilised ion source temperatures between 180°–190° (70 eV), the unheated probe being directly inserted and spectra taken as soon as the ion current appeared. *Trans* and *cis* nicotine-1′-*N*-oxides (**3**, **4**) have similar MS with M^+ of low intensity and with fragment ions at M^+-16 and M^+-17. Ion fragments appearing at *m/e* 133, 119 and 84 (base peak) were similar to those obtained with nicotine. The low % relative abundances of peaks at *m/e* 60, 118 and 119 indicated that oxazines were not formed to any appreciable extent with our experimental conditions. However, in the case of nicotine-1,1′-di-*N*-oxides, distinct differences were noted between the MS of the *trans* and *cis* isomers. The *trans* isomer (**5**) exhibited a M^+ at *m/e* 194 with fragment ions giving peaks at *m/e* 178 (M^+-16), 177 (M^+-17) and 176 (M^+-18). The presence of an ion fragment at *m/e* 161 indicated that the fragment at *m/e* 178 lost hydroxyl. Fragment ions at *m/e* 135, 118 and 60 indicate that some oxazine formation takes place but this is greater for the *cis* isomer (**6**) run under identical conditions since peaks due to these fragments have larger % relative abundances. This is further substantiated by the increase in % relative abundance of the M^+ (Table 3). The base peak for both isomers remained at *m/e* 84 indicating that the spectrum also results from nicotine or its *N*-oxides which have not rearranged thermally. Thus MS can readily distinguish the two isomers of nicotine-1,1′-di-*N*-oxide (**5**, **6**) as the oxazine appears to be formed more readily in the *cis* isomer. The MS of nicotine-1-*N*-oxide causes less problems although it has been reported that the % relative abundance of the M^+ is temperature dependent [29]. Under our conditions the base peak occurred at *m/e* 84, as for nicotine, the M^+-16 peak was negligible, whereas there was an appreciable M^+-17 peak.

Extracts of fresh roots, stems and leaves of *N. tabacum*, *N. affinis* and *N. sylvestris* were prepared and examined chromatographically for nicotine *N*-oxides. Only *trans* and *cis* nicotine-1′-*N*-oxide were detected and thus it appears that oxidation of the alkyl nitrogen and not the aryl nitrogen of nicotine occurs in both plants and animals. In a previous investigation, Russian workers [3] have estimated the proportions of nicotine alkaloids in tobacco plants, using a combination of TLC and spectroscopy. They reported that in young plants when the nicotine content was 56% of the total alkaloid, the "oxynicotine" content was 21·5% of the total alkaloid. A photograph of their TLC plate indicates that their "oxynicotine" ran as one spot but the present investigation indicates that both *trans* and *cis* isomers would be present. In a more detailed investigation [4], the same group estimated the proportion of 6 alkaloids, including nicotine and "oxynicotine", at 3 different stages of growth in three strains of *N. tabacum*, in *N. sylvestris* and in *N. rustica*. The results showed that in some samples at flowering stage there was an increase in the yield (mg/%) of nicotine in the leaves when compared with earlier stages of growth and also that as the yield

of nicotine increased in the leaves, there was a corresponding decrease in the roots. However these results were not obtained in every sample. The proportion of "oxynicotine" varied considerably and there does not appear to be any established pattern of relationship between "oxynicotine", nicotine and nornicotine content during ontogenesis. For example, in the 3 strains of *N. tabacum* examined, the "oxynicotine" content varied from 0–18·7% of the total alkaloid, the highest proportion being at the flowering stage but in the root of one, in the flower of another and in the stem of the third. Twelve of the 54 samples examined contained no "oxynicotine" and represented all organs except the root. The tremendous differences in the proportion of "oxynicotine" in all organs, at different stages of development, indicate that it is a transient metabolite.

The results of the proportions of nicotine, *trans* and *cis* nicotine-1′-*N*-oxides in leaf, stem and root of the three spp. of *Nicotiana* examined in the present investigation, are given in Table 4. These results show that the proportion of *N*-oxide, with respect to nicotine, varied within the range of 10·5–24·0% and hence contrast with the results discussed above since there was much less variation in the stem, leaf and root of the three species examined by us. The ratio of *trans* to *cis* nicotine-1′-*N*-oxide was found to be in the order of 2 to 1 and hence the postulation [3] that the proportion of a *cis* isomer would be very small, is not substantiated.

The role of alkaloids in plant metabolism is not established although it is becoming clear that in many cases they are not inert end products but are in a dynamic state, fluctuating in both total concentration and in rate of turnover [30]. The proportions of *N*-oxides found during the present investigation and the marked fluctuations noted by the Russian workers [3, 4], clearly indicate that *N*-oxides play some part in nicotine metabolism. It has been proposed that amine oxides might be *in vivo* intermediates for alkaloid transformations including *N*-demethylation [31, 32]. Iron-complex catalysed rearrangements of nicotine-1′-*N*-oxide have yielded formaldehyde and nornicotine as major products, together with myosmine, *N*-methylmyosmine, nicotyrine, cotinine and four unidentified bases [9]. In experiments with *N. glutinosa*, conversion of nicotine and nicotine-1′-*N*-oxide to nornicotine were shown to occur to comparable extents indicating that probably the sequence of *N*-oxide to nicotine and then to nornicotine was the route followed [12]. More recently, the hypothesis that nornicotine was formed from nicotine via *N*-oxidation (followed by Cope elimination and loss of water to the Schiff base which hydrolysed to the primary amine and then recyclises), was not substantiated by radioactive tracer experiments with *N. glauca* [13]. It was concluded that probably nicotine-1′-*N*-oxide is not an intermediate in the demethylation of nicotine to nornicotine.

In tobacco plants it has been estimated that some 10% of the plant's carbon metabolism is directed towards the production of nicotine which has a biological half life of 22 hr [30]. The variation in the proportion of nicotine-1′-*N*-oxide to nicotine indicates that the *N*-oxide could be involved in this rapid turnover of nicotine in the plant. The increased polarity of the *N*-oxide in comparison to nicotine may be of significance in its retention or exclusion from cells or cell organelles or in nicotine transport mechanisms throughout the plant. A further suggested role for nicotine-1′-*N*-oxides is that because of their facile interconversion with nicotine, they are involved with the general oxidation–reduction processes within the plant.

EXPERIMENTAL

The NMR spectra were determined at 60 MHz in $CDCl_3$ with TMS as internal reference. MS were recorded in an AEI MS 902 high resolution mass spectrometer at 70 eV and at 180°–190°. TLC system used was with Si gel G (Merck) and $CHCl_3$–MeOH–conc. NH_4OH (60:10:1) [33] and the PC system was with Whatman 3 mm paper and *n*-BuOH–*n*-PrOH–conc. NH_4OH–H_2O (20:10:1:9) [10]. Nicotine and its *N*-oxides were detected on TLC and PC by Dragendorff's reagent. GLC separations were obtained on a 5700A Hewlett-Packard instrument (F.I.D. detector) using a 1920 × 4 mm (i.d.) glass column with 4% KOH (in MeOH) and 8% Carbowax 20 M on Chromosorb W at 150° and with a N_2 flow rate of 24 ml/min. Quinoline was used as the internal standard for quantitative determinations.

Preparation of N-*oxides. Nicotine*-1′-N-*oxide.* 1·28 g of S-(−)-nicotine was stirred with 2·57 g *m*-chloroperbenzoic acid in $CHCl_3$ at 0° for 3·5 hr [21]. The $CHCl_3$ was evaporated under N_2 and the residue made alkaline with 10% K_2CO_3 and washed with Et_2O to remove unchanged nicotine. The aq layer was extracted with 6 × 100 ml $CHCl_3$ and then with 3 × 100 ml $CHCl_3$–MeOH (9:1). The combined extracts were dried and conc to yield 1·02 g (73%) of an oil. PC showed the presence of two *N*-oxides with R_f values of 0·51 and 0·42

in the ratio of 2·1 resp (GLC estimation of nicotine after prep PC of *N*-oxides and reduction with 15% $TiCl_3$) The two *N*-oxides were separated by PC using Whatman 3mm paper (2% NH_4OH in MeOH washed) and eluting with 1% NH_4OH in MeOH The major isomer (R_f 0 51) was identified as S,S-*trans*-nicotine-1′-*N*-oxide and the minor isomer (R_f 0 42) as R,S-*cis*-nicotine-1′-*N*-oxide

Nicotine-1 1′-*di*-N-*oxide*. 5 1 g of S-(−)-nicotine was added to a mixture of 12·5 ml of 30% H_2O_2 and 37 5 ml gl HOAc and the mixture heated at 70° for 3 hr and then another 12 5 ml 30% H_2O_2 added and heating continued for a further 16 hr [7] The vol of the reaction mixture was reduced to 40 ml in the presence of Pt, 50 ml H_2O added and the vol reduced to 40 ml This procedure was repeated 2× in order to remove excess H_2O_2 and peracetic acid The final vol was reduced to 20 ml and the yellow viscous residue made alkaline with anhyd Na_2CO_3 The resulting paste was extracted with $CHCl_3$ (20 × 100 ml). the combined $CHCl_3$ extracts dried and conc to yield 6 12 g (99%) of an oil PC showed the presence of two *N*-oxides of R_f values 0 20 and 0 18 in the ratio of 8 1 resp (estimated from NMe signals in NMR spectrum) The two *N*-oxides were separated by prep PC as described above The major isomer (R_f 0·20) was identified as S,S-*trans*-nicotine-1,1′-di-*N*-oxide and the minor isomer (R_f 0 18) as R,S-*cis*-nicotine-1,1′-di-*N*-oxide

Nicotine-1-N-*oxide* 1 189 g of nicotine-1 1′-di-*N*-oxide (*mixed isomers*) was dissolved in 25 ml 95% EtOH, cooled in ice and treated with SO_2 for 2 5 hr The reaction mixture was allowed to reach lab temp and kept overnight The solvent was evaporated, more EtOH added and further evaporated to ensure complete removal of SO_2 Viscous red residue was made alkaline with excess Na_2CO_3 and the paste extracted with 6 × 150 ml $CHCl_3$ The combined $CHCl_3$ extracts were dried and conc to yield 1 0 g (77%) of an oil PC revealed the presence of one spot, R_f 0 75, which was identified as nicotine-1-*N*-oxide

Identification of prepared N-*oxides Reduction to nicotine* 1–2 mg of *N*-oxide was reduced with 15% $TiCl_3$ in 5N HCl at lab temp for 4 hr The mixture was made alkaline with NaOH and extracted with Et_2O Each of the five *N*-oxides yielded nicotine (TLC R_f 0 75, PC R_f 0 90, R_t 9 5 min)

NMR and MS All samples were thoroughly dried under vacuum to remove solvent NMR δ values are recorded in Table 1 and MS *m/e* values in Table 3

Isolation of N-*oxides from* N tabacum, N sylvestris *and* N. affinis The plants were collected during their flowering stages and divided into stems, roots and leaves Fresh plant material was extracted in a blender with 2% NH_4OH in MeOH and maceration continued for a further 16 hr The extract was filtered and conc to a semi-solid which was extracted with 2% H_2SO_4 Filtered acid extract was made alkaline with NH_4OH and extracted successively with $CHCl_3$ and $CHCl_3$–MeOH (9 1) Roots, stems and leaves of the three *Nicotiana* spp examined showed bands corresponding to the *trans* and *cis* isomers of nicotine-1′-*N*-oxide when extracts were examined by PC but no bands were observed corresponding to other *N*-oxides. Each extract was separated by PC (16 hr) and bands corresponding to the two isomers of nicotine-1′-*N*-oxide were eluted as described above. Control extractions with nicotine indicated that *N*-oxides were not formed by the isolation procedures

Identification of natural trans *and* cis *nicotine-1′-*N-*oxides* PC R_f values and MS of the natural *N*-oxides were identical with those of the prepared compounds Reduction of 2 mg aliquots of the two *N*-oxides isolated from each of the three *Nicotiana* spp. with 1 ml 15% $TiCl_3$ and 2 ml 5N HCl for 4 hr resulted in nicotine only (TLC R_f 0 75, PC R_f 0 90, R_t 9 5 min)

Quantitative estimation of nicotine, trans *and* cis *nicotine-1′-*N-*oxides in* N tabacum, N sylvestris *and* N. affinis 50 g of root, of stem and of leaves of each spp was extracted as described above The total alkaloid extract was dissolved in $CHCl_3$–MeOH (1 1) and made up to 5 ml A 1 ml aliquot was used for the estimation of nicotine and the remaining 4 ml for *N*-oxide estimation The 1 ml aliquot was separated by prep TLC using 0 5 mm thick Si gel G/GF (2·1) plates and the nicotine eluted with $CHCl_3$–EtOH (19 1) The nicotine was assayed by quantitative GLC using quinoline as internal reference The 4 ml aliquot was separated into *trans* and *cis* nicotine-1′-*N*-oxides by prep PC as described above and then reduced to nicotine by $TiCl_3$–HCl The nicotine was assayed by quantitative GLC The results are presented in Table 4

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