

product, m.p. 190–194°. The analytical sample had m.p. 195–197°; $[\alpha]_D^{19} -28^\circ$ (c 1, CHCl_3).

Anal.—Calcd. for $\text{C}_{25}\text{H}_{33}\text{BrO}_5$: C, 60.85; H, 6.74. Found: C, 60.73; H, 6.72.

3 β ,14 β -Dihydroxy-19-iodocarda-5,20(22)-dienolide 3-Acetate (VII)—A solution of 0.1 Gm. (0.00017 mole) of III and 0.1 Gm. (0.0007 mole) of lithium iodide in 20 ml. of isopropyl alcohol was heated under reflux for 2 hr. After evaporation of the solvent under reduced pressure, water was added to precipitate the product. Recrystallization twice from methanol gave 0.03 Gm. of product, m.p. 180–184°. Further recrystallizations from the same solvent gave the analytical sample, m.p. 184–185°; $[\alpha]_D^{19} -40^\circ$ (c 0.6, CHCl_3).

Anal.—Calcd. for $\text{C}_{25}\text{H}_{33}\text{IO}_5$: C, 55.56; H, 6.16. Found: C, 55.79; H, 6.24.

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Determination of the Conformation of Nicotine and Some Related Compounds by Nuclear Magnetic Resonance and Dipole Moments

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A single NMR signal for protons in the 2- and 5-positions in *N*-methylpyrrolidine indicates rapid inversion of the nitrogen so that the methyl group spends half of its time on each side of the molecule. A single band for protons in the 2- and 5-positions in *N*-methylpyrrolidine *N*-oxide shows that the CH_3 and the O^- provide similar environments. Confirmation of this is obtained from the fact that NMR bands for comparable protons occupy nearly the same positions in *N*-methylpyrrolidine methiodide and *N*-methylpyrrolidine *N*-oxide. Introduction of a pyridine, or a pyridine *N*-oxide, ring in the 2-position causes the methyl protons and one proton on the asymmetric carbon to move upfield and all the other protons to move downfield. The upfield motion of these protons is consistent with the configuration which has the methyl group situated away from the pyridine ring. Dipole moments indicate that oscillation between two conformations is likely in (L)-nicotine and (L)-nicotine *N*-oxide, while (L)-nicotine *N*-oxide and (L)-nicotine *N,N'*-dioxide appear to be fixed in one preferred conformation.

ALTHOUGH the absolute configuration of natural (–)-nicotine is known (1), and nicotine *N*-oxide (compound 6 in Table I) is recognized as the first metabolite of nicotine in animals and plants (see *Reference 2*), no information is available concerning the conformation in which nicotine or its *N*-oxides exist. This problem has now been investigated by the

use of techniques of nuclear magnetic resonance and dipole moment measurements.

DISCUSSION

Some previous work on the NMR spectrum of *N*-methylpyrrolidine (compound 1) has been reported (3). The present results now make possible the unambiguous assignment of the methyl and methylene protons, and the effect of the conversion of the tertiary to a quaternized nitrogen, or to an *N*-oxide, has been observed to produce downfield shifts comparable to those reported in other compounds (3).

The relative downfield shift of the protons in 1 can be accounted for as follows. The *a* protons (Table I), being farthest away from the nitrogen, are shifted downfield the least amount. The others are

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equidistant, but the *c* and *d* protons are on a carbon to which another carbon is attached and thus appear further downfield than the *b* protons. The presence of only one band for the *c* and *d* protons implies that their environment must be the same, which means that the nitrogen inverts rapidly causing the methyl group to spend half its time on each side of the ring. The methyl group gives a sharp singlet but the peaks for the methylene protons are complex. The *c* and *d* protons give rise to a triplet, while the *a* protons form a more complicated multiplet.

Introduction of oxygen giving *N*-methylpyrrolidine *N*-oxide (compound 2) causes all protons to move downfield, the *a* proton by 25 c.p.s. and the *b*, *c*, and *d* protons by 60 c.p.s. The *a* protons are one atom farther away from the positively charged nitrogen atom and are deshielded less, as expected (3). However, the fact that there is only one band for the *c* and *d* protons is entirely unexpected, because the protons on the side of the O⁻ atom would be anticipated to be in a different environment from those on the side of the methyl group. The reason, apparently, is that the O⁻ and CH₃ group provide, by coincidence, essentially the same environment. This is reasonable because the negative charge on oxygen would oppose its electronegativity and the net effect would be close to that of a methyl group.

If this explanation is correct, then one would expect the chemical shifts for *N*-methylpyrrolidine methiodide (compound 3), in which a methyl group has replaced the O⁻, to come close to those for compound 2, which they do, differing only by 3, 7, and 2 c.p.s., respectively. The signal for the methyl group in these two compounds is a sharp singlet, while those for the *a*, *c*, and *d* protons are complex multiplets.

Replacement of one of the methyl groups in compound 3 by a deuterium atom, giving compound 4, results in multiplets for the *c* and *d* protons centered around 220 and 187 c.p.s. This is expected, for the environment around methyl will be different from that around deuterium, which has a lesser tendency to move protons downfield. This change has caused the *a* protons to move upfield by 8 c.p.s., the *b* protons by 14 c.p.s., one of the *c* and one of the *d* protons by 25 c.p.s., and the other *c* and *d* protons downfield by 8 c.p.s. It seems likely that the *c* and *d* protons on the same side as the methyl group give rise to the 187 c.p.s. signal, since they are more analogous to the methyl protons (with respect to the deuterium) than are the other *c* and *d* protons. This reasoning applies irrespective of what combination of inductive, magnetic anisotropy, or solvent effects may be involved.

Introduction of a pyridine ring into compound 1 gives nicotine (compound 5) which has a sharp singlet at 127 c.p.s. due to the methyl group. There are multiplets centered at 119, 138, and 190 c.p.s. which correspond to 4, 1, and 2 protons, respectively. The signal at 119 c.p.s. can be assigned to the *a* protons, which are farthest from the pyrrolidine nitrogen. The multiplet at 138 c.p.s. was thought to arise from the *d* proton (and was indeed a triplet as expected for this proton), and the multiplet at 190 c.p.s. would then be due to the *c* protons. However, an argument against this assignment is that a calculation of shielding and deshielding effects (4) according to the method discussed by Bible (5), following Johnson and Bovey (6), for any possible con-

formation of nicotine does not put the *d* proton into a region of shielding and thus could not account for its movement upfield to 138 c.p.s. from its position at 150 c.p.s. in *N*-methylpyrrolidine.

To shed further light on this point, the spectrum of nicotine was examined both at 100 Mc./sec. and at 220 Mc./sec. in benzene.¹ Under these conditions, the multiplet centered at 190 c.p.s. in D₂O was resolved into a triplet and doublets of a triplet. The triplet at 187 c.p.s. can thus be assigned to the *d* proton while the two *c* protons give rise to the triplet at 138 c.p.s. and the multiplet at 192 c.p.s., respectively. This assignment has the advantage that the signal for the *d* proton is now in a region where the calculations place it, as is that for the *b* protons, and the *c* proton opposite the methyl group can be placed in a position consistent with its signal at 138 c.p.s. However, the calculations do not place the other *c* proton far enough downfield by about 40 c.p.s. Nevertheless, this assignment gives rise to better over-all consistency than does the alternative one. The reasons the calculations do not hold in the case of one of the *c* protons may be due to the method (6) having been developed for benzene rather than pyridine, plus the fact that the proton in question has the pyrrolidine nitrogen interposed between it and the pyridine ring for the conformations considered. This would be expected to alter the action of the pyridine ring currents on this proton that take place directly through space.

The Stuart-Briegleb model for *l*-nicotine shows considerable steric hindrance if the methyl group is placed on the same side of the pyrrolidine ring as the pyridine moiety, and demethylation studies on nicotine (2) indicate the methyl group and pyridine ring to be on opposite sides of the pyrrolidine ring.

The above assignment of the bands for nicotine means that the introduction of the pyridine ring in position 2 of *N*-methylpyrrolidine has shifted the *a* protons downfield by 12 c.p.s., the *c* proton on the same side as the methyl group down by 42 c.p.s., and the *d* proton by 37 c.p.s., while the *b* protons are displaced upfield by 10 c.p.s. and the *c* proton opposite the methyl group by 12 c.p.s.

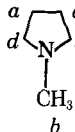



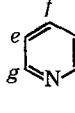

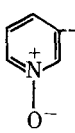
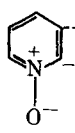
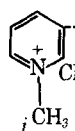
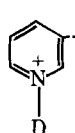
Placing an oxygen on the pyrrolidine nitrogen gives nicotine *N*-oxide (compound 6) and the bands are assigned as indicated in Table I. The downfield shift, caused by the change from 5 → 6, is one of 23 c.p.s. for the *a* protons, 57 c.p.s. for the *b* protons, 36 for the *c* proton on the same side as the methyl group, 90 for the *c* proton opposite methyl, and 94 for the *d* proton. The comparable changes in going from 1 → 2 were 25 c.p.s. for the *a* protons and 60 c.p.s. for the *b*, *c*, and *d* protons. The large effect on the *c* proton opposite the methyl group may be due to the oxygen being interposed between it and the pyridine ring, thus reducing the shielding effect of the ring currents which originally moved this proton upfield in nicotine.

Introduction of an oxygen on the pyridine nitrogen, giving compound 7, leaves the signals for the protons of the pyrrolidine ring almost unchanged. The largest shift (9 c.p.s.) is that of the *c* proton opposite the methyl group, *i.e.*, the proton closest in space to the pyridine ring with its (now positively charged) nitrogen.

The movement of the protons on the pyridine

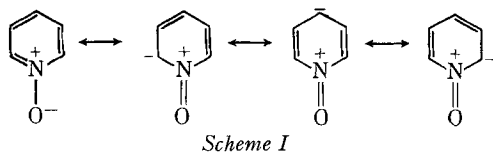
¹ The authors are indebted to Dr. N. S. Bhacca, Varian Associates, for these spectra.

TABLE I—CHEMICAL SHIFTS OF PROTONS, IN c.p.s., OF *N*-METHYL PYRROLIDINE, NICOTINE, AND ANALOGS RELATIVE TO SODIUM 2,2-DIMETHYL-2-SILAPENTANE-5-SULFONATE (DSS) IN DEUTERIUM OXIDE, MEASURED AT 60 Mc./sec.

	<i>a</i>	<i>b</i>	<i>c</i>	<i>d</i>	<i>e</i>	<i>g</i>	<i>h</i>	<i>i</i>		
1 	107	137	150	150						
2 	132	197	210	210						
3 	135	190	212	212						
4 	127	176	187 220	187 220						
5 	119	127	192 138	187	446	470	507	507		
6 	142	184	228	281	448	486	517	519		
7 	115	129	192 147	192	456	460	494	496		
8 	145	185	227	282	459	477	504	511		
9 	155	196	231	305	495	531	540	550	177	270
10 	148	176	214 231	291	495	531	540	545		

ring in 7 compared with 5 are interpretable in terms of the resonance forms expected in pyridine *N*-oxide (7). These put negative charges (Scheme I) on the *h*, *f*, and *g* carbon atoms which correspond

to the 2, 4, and 6 positions, and protons attached to these carbons move upfield by 11, 10, and 13 c.p.s. while the *e* proton, attached to a carbon which has a relative positive charge, is moved downfield by 10



c.p.s. The 3 position on the pyridine ring will likewise be relatively positive, and this is another reason for the downfield shift of the *d* proton attached to the adjacent carbon in 7 compared to 5.

Conversion of compound 7 to nicotine *N,N'*-dioxide (compound 8) caused downfield shifts of all aliphatic protons. The *a*, *b*, and *c* protons moved by 30, 56, and 90 c.p.s., respectively, while the *c* proton on the same side as the methyl group moved by 35, and the other *c* proton by 80 c.p.s. All of these values are in good agreement with the comparable change from nicotine to the *N*-oxide, 6. The positions of the *a*, *b*, *c*, and *d* protons are almost identical in the *N*-oxide (6) and the *N,N'*-dioxide (8), showing that an oxygen added to the pyridine nitrogen is virtually without effect on the position of the signals for the pyrrolidine protons.

When an additional methyl group is placed on both nitrogens in nicotine, giving compound 9, all signals move downfield due to the greater effect of a positive charge on nitrogen alone in contrast to a positive charge on nitrogen tempered by a negative charge on the adjacent oxygen. The shifts in the proton signals from compound 8 → 9 are twice as great for the *d* proton (which is closest to the pyridine ring) as for the other protons in the pyrrolidine ring, and the *f* and *h* protons (which have both a relative positive charge due to resonance, and are nearest the pyrrolidine ring) are displaced most in the pyridine ring. The downfield location of the *j* protons compared with the *i* protons results from the greater electronegativity of pyridine compared to pyrrolidine, and to the *j* protons being in or near the plane of the pyridine ring while the *i* protons are above it.

Dipole moments were measured and compared with the theoretical moments for various conformations of (L)-nicotine, 5; (L)-nicotine *N'*-oxide, 7; (L)-nicotine *N*-oxide, 6; and (L)-nicotine *N,N'*-dioxide, 8. The values used in calculating the theoretical moments are given in Table II, and the observed and theoretical calculated moments for the different configurations and conformations in Table III.

The four extreme conformations of nicotine are shown in Fig. 1. The observed moment for nico-

TABLE II—VALUES USED IN CALCULATING THEORETICAL DIPOLE MOMENTS

Compd.	$\mu_{\text{obs. at } 25^\circ \text{C.}}$	Solvent
Trimethylamine- <i>N</i> -oxide ^a	5.03 D. ^b (9)	Dioxane
Pyridine	2.22 D. (10)	Dioxane
	2.21 D. (11)	Benzene
Pyridine-1-oxide	4.32 D. (12)	Dioxane
<i>N</i> -Methylpyrrolidine	1.34 D. (13)	Dioxane
	0.83 D.	Benzene

^a As an approximation of the moment expected for *N*-methylpyrrolidine *N*-oxide. ^b Debye units.

tine is close to that for rotation between conformations I and II. It is not quite so close to that for conformation IV. The models indicate that rotation is relatively free between either I and II, or III and IV, but would be prevented between other combinations. If, therefore, the molecule existed in conformation IV, there seems no reason why it should not rotate between III and IV, in which case

TABLE III—OBSERVED AND CALCULATED DIPOLE MOMENTS FOR NICOTINE AND RELATED COMPOUNDS^a

Conformation	$\mu_{\text{obs.}}$	F.R. ^b	$\mu_{\text{calcd.}}$
5, (L)-Nicotine (13)			
I	2.64 D. (20° C. in benzene)	2.68 D.	2.97 D.
II			2.34
III			1.73
IV		2.29	2.73
I ^c		1.70	1.84
II ^c			1.55
III ^c		2.48	2.73
IV ^c			2.19
7, (L)-Nicotine <i>N'</i>-oxide, m.p. 50–52°C.			
I	4.89 ± .03		5.52
II		5.04	4.52
III			3.51
IV		4.40	5.13
I ^c			3.65
II ^c		3.46	3.24
III ^c			5.14
IV ^c		4.70	4.23
6, (L)-Nicotine <i>N</i>-oxide, m.p. 171.5–173°C. (sealed capillary)			
I	4.77 ± .06		6.44
II		5.98	5.49
III			5.02
IV		5.78	6.44
I ^c			4.07
II ^c		3.71	3.30
III ^c			6.34
IV ^c		5.72	5.02
8, (L)-Nicotine <i>N,N'</i>-dioxide, m.p. 183–183.5°C. (sealed capillary)			
I	6.11 ± .07		8.10
II		7.40	6.63
III			5.85
IV		7.07	8.10
I ^c			4.05
II ^c		3.46	2.47
III ^c			8.12
IV ^c		7.07	5.85

^a In Debye units (D.); measured at 25° C. in dioxane unless otherwise noted. ^b F.R., free rotation between conformations of a configuration. ^c Conformations with the methyl group toward the pyridine ring, which were ruled out by demethylation studies and from space-filling models.

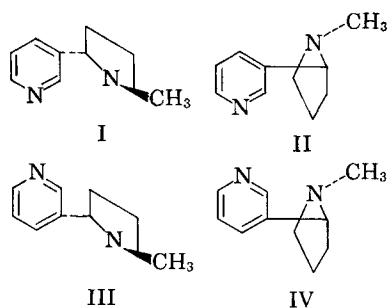


Fig. 1—Four extreme conformations of nicotine. In conformations II and III, the hydrogen on carbon 3 of the pyrrolidine ring is behind H-2 of the pyridine ring, while in conformations I and IV, it is behind H-4 of the pyridine ring. Thus I and IV, and II and III, are not identical since the pyridine ring is inverted. Conversion of conformation I into II, or of III into IV, is achieved by rotating the pyrrolidine ring. Conformation II cannot be converted into III except by breaking the carbon-carbon bond between the rings, rotating one ring, and reassembling.

the calculated moment would be considerably below that observed. Since the conformations with the methyl group next to the pyridine ring are ruled out by demethylation data and space-filling models, they need not be considered further. The most likely structure for nicotine appears to be that of a rotamer between conformations I and II.

The data for (L)-nicotine *N'*-oxide lead to the same conclusion, namely, rotation between conformations I and II for its structure. This is reasonable since the two molecules have the same structural features at the ring junction in the various conformations. The fact that the observed moment is 0.15 D. below that calculated for free rotation suggests that it spends a longer time in conformation II with the lower moment, which would be favored on a dipole interaction basis. With (L)-nicotine *N'*-oxide, models indicate steric hindrance alters conformations I and III but not II and IV. In conformation I, an oxygen placed on the pyrrolidine nitrogen interferes with the hydrogen at carbon 2 of pyridine, and does not permit the downward-pointing hydrogen at carbon 3 of the pyrrolidine

TABLE IV—DIPOLE MOMENTS OF NICOTINE AND RELATED COMPOUNDS^a

Nicotine N,N'-dioxide		ω_2	ϵ_{12}	ν_{12}
		0.0	2.227845	.970628
m.p. 183–183.5° C.		0.398975	2.237857	.970408
$\mu = 6.11 \pm .07$ D.		0.701764	2.244934	.970408
mol. wt. 194.23	ϵ_1 2.227930	ν_1 .970601		P_{20} 808.083
	α 24.391123	β — .325828		P_{E_2} 44.457
Nicotine N-oxide				
		0.0	2.227327	.970628
m.p. 171.5–173° C.		0.986434	2.243208	.970408
		1.092887	2.244589	.970408
$\mu = 4.77 \pm .06$ D.		1.963564	2.258226	.969858
mol. wt. 178.23		2.479078	2.268238	.969638
	ϵ_1 2.227043	ν_1 .970741		P_{20} 502.085
	α 16.310666	β — .424233		P_{E_2} 36.801
Nicotine N'-oxide				
		0.0	2.227413	.970628
m.p. 50–52° C.		1.056411	2.246143	.970518
		1.427574	2.252530	.970298
$\mu = 4.89 \pm .03$ D.		1.837944	2.260211	.970188
		3.290192	2.284292	.969858
mol. wt. 178.23		3.707915	2.291024	.969748
	ϵ_1 2.227949	ν_1 .970674		P_{20} 534.794
	α 17.130637	β — .247928		P_{E_2} 45.424
N-Methylpyrrolidine				
		ω_2	ϵ_{12}	ν_{12}
		0.0	2.239750	.970298
b.p. 79.5°/754.4 mm. Hg		2.498532	2.245692	.970958
		3.877602	2.248533	.971289
$\mu = 1.34 \pm .02$ D.		5.759391	2.253183	.971840
		6.850177	2.256714	.972282
mol. wt. 85.15		9.013237	2.261622	.972945
	ϵ_1 2.239509	ν_1 .970226		P_{20} 65.195
	α 2.444263	β .294738		P_{E_2} 28.234
N-Methylpyrrolidine				
		0.0	2.270061	1.143639
b.p. 79.5°/754.4 mm. Hg		1.885702	2.271266	1.143639
		4.727287	2.273075	1.143792
$\mu = 0.83 \pm .001$ D.		8.188559	2.275227	1.143945
		11.531298	2.277294	1.144098
mol. wt. 85.15		14.922103	2.279447	1.144251
Benzene				
	ϵ_1 2.270080	ν_1 1.143597		P_{20} 40.111
	α .627504	β .043219		P_{E_2} 25.996

^a Measured in dioxane at 25° C. unless otherwise noted.

ring to rotate into a position behind the hydrogen at carbon 4 of pyridine, as it could in conformation I of nicotine itself. In conformation III of nicotine *N*-oxide, the oxygen interferes with the hydrogen at carbon 4 of pyridine, and thereby prevents the molecule from assuming the conformation III of nicotine itself. The observed moment of nicotine *N*-oxide is considerably less than that calculated for either type of free rotation. Conformation III is closest to the observed moment. The reason the observed moment is 0.25 D. below that calculated for III is probably to be found in the above-mentioned proximity of the $\overset{+}{N}-\bar{O}$ group to carbon 4 of the pyridine ring, which has a partial positive charge due to resonance, thus tending to reduce the moment between $\overset{+}{N}$ and \bar{O} .

The same situation exists in (L)-nicotine *N,N'*-dioxide where the observed moment again comes closest to that calculated for conformation III. Here, however, the observed moment is 0.25 D. above the calculated one, and this is understandable because the resonance in the pyridine-*N*-oxide ring places a partial negative charge on carbon 4 which would now enhance the $\overset{+}{N}-\bar{O}$ dipole. In both compounds, the evidence indicates the compounds exist in the conformation with the smallest dipole moment which is favored both from dipole interaction and from steric considerations.

EXPERIMENTAL

The dielectric constants were measured with a WTW Dipolmeter model DM-01 with a DF L-2

4-ml. cell with a thermostated jacket maintained at a temperature of $25.00^\circ \pm 0.03^\circ$. The P_{E_2} values are molar refractions calculated from refractive indices of the solutions measured with the D sodium line at $25.00^\circ \pm 0.03^\circ$. Densities were measured with a pycnometer of approximately 2-ml. capacity. Solvents were refluxed over sodium twice and distilled twice from a 1-M. column. Dipole moments were calculated using the Halverstadt-Kumler (8) method, for which programs were developed for use on both the IBM 1620 and 1401 computers. The results are given in Table IV.

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Acetylsalicylic Acid Hydrolysis in Human Blood and Plasma I

Methodology and *In Vitro* Studies

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The *in vitro* hydrolysis of acetylsalicylic acid in 90 vol. per cent human blood and plasma was studied at therapeutically significant levels (below 15 mcg./ml.) by a spectrophotofluorometric method. Several new analytical aspects are presented. The discrepancies with earlier investigations are discussed.

IT IS WELL KNOWN that acetylsalicylic acid (ASA) rapidly hydrolyzes in aqueous solution, and its hydrolysis is accelerated by esterases

found in the blood or plasma (1). Morgan and Truitt (2) recently reviewed the literature relating to the hydrolysis of ASA in blood and plasma. The hydrolysis would be expected to be a second-order process dependent upon both the enzyme and the substrate concentrations. At low aspirin and fixed enzyme concentrations, the reaction should reduce to a pseudo first-order reaction. Some of the previous investigations on this subject have been run in highly diluted plasma and at high aspirin levels (2). The excessive concentration of ASA might swamp the enzyme and cause

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