Gas-Liquid Chromatography and Structure-Retention Time Relationship of Anhalonium Alkaloids and Related Bases

By GOVIND J. KAPADIA and G. S. RAO

Gas-liquid chromatographic separation of 18 anhalonium alkaloids and related bases has been studied employing 1 per cent methylsiloxane polymer (containing about 5 per cent phenyl substitution) as the liquid phase. The GLC behavior of these closely related compounds suggested a structure-retention time relationship. In general, *N*-monomethylation of primary and secondary amines, *O*-methylation, or *C*-monomethylation of the bases studied was found to decrease the retention time, while introduction of hydroxyl, methoxyl, or methylenedioxy group, or an unsaturation, produced an increase in retentivity. These changes in retention time may be attributed to the changes in polarity of amino and/or phenolic hydroxyl groups brought about by substitution in or adjacent to these groups.

THE PRACTICAL utility of gas-liquid chromatography in the field of natural products is well established (1). In alkaloid work this technique is now commonly employed for the purpose of identification of bases in crude fractions, and in their separation and estimation (2, 3). In the last few years, several significant gas chromatographic studies on biological amines have been reported (4, 5). The gas chromatography of amines, alkaloids, and amino acids has been recently reviewed by Fales and Pisano (5).

During our studies (6) on the alkaloids of *Anhalonium lewinii* three groups of closely related bases, β-phenylethylamines, tetrahydroisoquinolines, and dihydroisoquinolines, became available. The main difference among these compounds is found in variations in or near the polar amino and/or phenolic hydroxyl groups (7). Fales and Pisano have suggested that in polar compounds such as these, contribution to the over-all interaction from various functional groups on the molecules will be related to the polarity and configuration of the groups, and chances for separation will be most favorable when a polar column is used for their chromatography.

This paper presents the results of gas chromatographic study on anhalonium alkaloids and related bases wherein polar methylsiloxane containing ~5% phenyl substitution was employed as the liquid phase.

EXPERIMENTAL

Apparatus.—A Chromalab gas chromatograph equipped with hydrogen flame detector was used.

Column.—The column was made[1] of glass tube, 12 ft. in length and having an inner diameter of 4 mm. The liquid phase in the column was 1% methylsiloxane containing ~5% phenyl substitution [2] and the solid support was Gas-Chrom P, 100 to 140 mesh. The column temperature was maintained at 180°, and argon at 20 p.s.i. was employed as the carrier gas. Other pertinent column conditions have been recorded in Table I.

TABLE I.—CONDITIONS FOR GAS-LIQUID CHROMATOGRAPHY OF ANHALONIUM ALKALOIDS AND RELATED BASES

Col. packing	1% methylsiloxane containing ~5% phenyl substitution on Gas-Chrom P, 100 to 140 mesh
Col. temp.	180°C.
Cell temp.	240°C.
Flash heater temp.	210°C.
Carrier gas	Argon at 20 p.s.i.
Hydrogen flow rate	32 ml./min.
Current sensitivity	3×10^{-8} amp.

Method.—Samples of 1-μl. quantities of 1% solution of the bases in ethanol were injected into the flash heater zone. When the acid salts of alkaloids were chromatographed, samples were prepared in 1% ethanolic solution of diethylamine. A Hamilton microsyringe was used for sample injection.

RESULTS AND CONCLUSIONS

Table II summarizes the gas chromatography of anhalonium alkaloids and related bases. Results indicate that under the conditions employed and using methylsiloxane containing ~5% phenyl substitution as the liquid phase in the column separation of these closely related compounds could be achieved. The following conclusions could be arrived at regarding the relationship of their structure and retention time.

1. *N*-Monomethylation of primary and secondary amines, *O*-methylation, or *C*-monomethylation of the bases studied resulted in a decrease in retention time. Although there was an increase in molecular weight corresponding to a methylene group, the observed decrease[3] in retention time of the phenol ether and the alkylated amine could be rationalized as due to the corresponding decrease in polarity of the derivatives as compared to their parent compounds. The decrease in retention of *C*-methyl derivative, as compared to its lower homolog, may be hypothesized as due to an interference of methyl group in permitting the molecule to be adsorbed on

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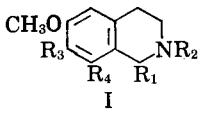
[1] For the preparation of column, see *Reference 5*.

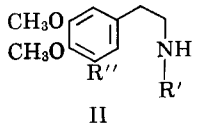
[2] A gift to the Laboratory of Metabolism, National Heart Institute, U. S. Public Health Service, Bethesda, Md., from Dr. Arthur Martellock, General Electric Silicone Products Division, Waterford, N. Y.

[3] While this manuscript was in preparation, Ikekawa *et al.* (9) have reported similar observations during the gas chromatography of morphinan derivatives.

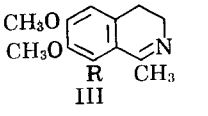
TABLE II.—GAS-LIQUID CHROMATOGRAPHY[a] OF ANHALONIUM ALKALOIDS[b] AND RELATED BASES



I



II



III

Compd.	Name	Substituents R1	R2	R3	R4	Source	Mol. Wt.	Retention Time, min.
	I Tetrahydroisoquinolines							
1	Anhalamine	H	H	OCH_3	OH	N[c]	208	4.82
2	Anhalidine	H	CH_3	OCH_3	OH	S[d]	223	4.44
3	Anhalinine	H	H	OCH_3	OCH_3	S	223	4.16
4	Anhalonidine	CH_3	H	OCH_3	OH	N	223	4.41
5	Anhalonine	CH_3	H	O—CH_2	—O	N	221	5.20
6	Carnegine	CH_3	CH_3	OCH_3	H	S	221	2.63
7	Lophophorine	CH_3	CH_3	O—CH_2	—O	N	235	4.50
8	*N*-Methylanhalinine	H	CH_3	OCH_3	OCH_3	S	237	3.09
9	*O*-Methylanhalonidine	CH_3	H	OCH_3	OCH_3	S	237	3.56
10	*O*-Methylpellotine	CH_3	CH_3	OCH_3	OCH_3	S	251	3.00
11	Pellotine	CH_3	CH_3	OCH_3	OH	N	237	3.94
12	Salsolidine	CH_3	H	OCH_3	H	S	207	2.96
	II β-Phenylethylamines		R′	R″				
13	*N*-Acetylmescaline		O=C—CH_3	OCH_3		S	237	7.00
14	Homoveratrylamine		H	H		S	181	1.44
15	Mescaline		H	OCH_3		N	211	3.38
16	*N*-Methylmescaline		CH_3	OCH_3		S	225	2.90
	III Dihydroisoquinolines		R					
17	Schiff base A		H			S	205	3.28
18	Schiff base B		OCH_3			S	235	4.26

[a] Column conditions are given in Table I. [b] Compounds *6*, *8*, *10*, *12*, *14*, *17*, and *18* are nonanhalonium bases. [c] N, natural product isolated from *A. lewinii*. (See *Reference 6*.) [d] S, prepared synthetically. (See *Reference 6*.)

the surface of chromatographic column. The methyl group might sterically hinder the interaction of the polar phenolic hydroxyl and/or amino groups with the adsorbent (8).

TABLE III.—STRUCTURE-RETENTION TIME RELATIONSHIP OF ANHALONIUM ALKALOIDS AND RELATED BASES

Structural Change	Examples[a]	Δ Retention Time,[b] min.
N-Methylation	1 → 2	−0.38
	3 → 8	−1.07
	4 → 11	−0.47
	5 → 7	−0.70
	9 → 10	−0.56
	12 → 6	−0.33
	15 → 16	−0.48
O-Methylation	1 → 3	−0.66
	2 → 8	−1.35
	4 → 9	−0.85
	11 → 10	−0.94
C_1-Methylation	1 → 4	−0.41
	2 → 11	−0.50
	3 → 9	−0.60
	8 → 10	−0.09
8-Methoxy-	6 → 10	0.37
	12 → 9	0.60
	14 → 15[c]	1.94
	17 → 18	0.98
8-Hydroxy-	6 → 11	1.31
	12 → 4	1.45
$\Delta^{1,2}$	9 → 18	0.70
	12 → 17	0.32
7,8-Dimethoxy to methylenedioxy-	9 → 5	1.64
	10 → 7	1.50

[a] Refer to Table II. [b] For column conditions, refer to Table I. [c] In this case methoxyl is on a 5 position of a β-phenylethylamine.

2. Introduction of hydroxyl or methoxyl group, or an unsaturation in isoquinolines, and methoxyl group in β-phenylethylamines resulted in an increase in retention time. It could again be conjectured that the increase in polarity of the derivatives caused the increase in adsorption on the liquid phase.

3. In tetrahydroisoquinolines, introduction of hydroxyl group effected greater[3] increase in retentivity than that of methoxyl group.

4. Replacement of two methoxyl groups with a methylenedioxy group in tetrahydroisoquinolines produced noticeable increase in retention time.

These observations have been summarized in Table III. Due to their high retentivity, phenolic compounds are generally gas chromatographed as their derivatives (2). However, the authors were able to chromatograph phenolic anhalonium alkaloids (compounds *1*, *2*, *4*, and *11*, Table II) directly. Since the phenolic hydroxyl group is located *ortho* to a methoxyl group in these compounds, there possibly exists strong intramolecular hydrogen bonding (10). Also the methoxyl group might be expected to interfere sterically with the intermolecular bond between the —OH group and the liquid phase. Either one or both of these factors could be conjectured to weaken the intermolecular bonding with the column and decrease the retentivity, thereby making these phenols capable of being chromatographed.

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Identification of Primary, Secondary, and Tertiary Pharmaceutical Amines by the Infrared Spectra of Their Salts

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The spectra of 80 pharmaceutically active amine salts have been analyzed in the range of 4000–2000 cm.$^{-1}$. The amine salts have characteristic absorption bands in this region. The wave numbers at which these absorption bands occur are specific for each given class of amine. Spectra-structure correlations and assignments of these bands are given and discussed.

OVER THE past several years the authors have recorded the infrared spectra of several hundred amine salts of varying structure and class. The amines were typical of those usually encountered in the pharmaceutical industry in that they were large, asymmetrical molecules. In most cases, these were combined with small negative ions such as chloride or bromide. During this time we have had occasion to search the literature for assignments and interpretations of the absorption bands due to the amine ions NH_3^+, NH_2^+, NH^+. We have found reports indicating that much work and assignments have been made on these ions (2, 4). Most of the studies made were on a specific ion, *e.g.*, NH_2^+, or aspect of amine salt absorption, *e.g.*, hydrogen bonding. To our knowledge there has been no comprehensive study of amine salt absorption data relative to the type of molecule on which we are reporting.

This article gives the results of an infrared spectral study of 80 amine salts—55 tertiary, 15 secondary, and 10 primary. It shows that these classes of amines possess characteristic frequencies in the range of 4000–2000 cm.$^{-1}$. The frequencies may be used to establish the class of amine present.

PRIMARY AMINE SALTS

Bellamy (1) reports that the hydrochlorides of primary amines have been little studied, and this statement is essentially correct. There have been a few reports (2, 3, 5) that primary amine salts absorb in the range of 3200–2000 cm.$^{-1}$. The absorption bands are generally reported to be a series of weak peaks. Some authors (2, 3) have mentioned an isolated band near 2000 cm.$^{-1}$ whose intensity is variable. Table I lists the primary amine salts studied here and their characteristic absorption bands.

It has been found that these bands are remarkably consistent and unique for the primary amine ion, NH_3^+. The band in the area of 2000 cm.$^{-1}$ which was reported (2, 3) is present in all cases. The authors find the location of this band to be between 1960 and 2080 cm.$^{-1}$. It is a broad band in most cases. There has been some discussion as to the nature of the vibration giving rise to this absorption. It has variously been assigned as an NH_3^+ stretching vibration and as a combination band of the NH_3^+ torsional vibration at about 500 cm.$^{-1}$ and the asymmetrical NH_3^+ deformation (2, 3). That it involves the NH_3^+ ion is evident from its presence in all of the primary amine salts listed in Table I. The other distinguishing feature of the spectra of primary amine salts is a series of weak peaks between 2770 and 2380 cm.$^{-1}$ which are assignable to the NH_3^+ overtone and combination bands (6).

TABLE I.—PRIMARY AMINE SALTS

	Series of Peaks (Weak)	Peak (Broad)
Aminacrine hydrochloride	2770–2500 cm.$^{-1}$	2000 cm.$^{-1}$
d-Amphetamine sulfate	2770–2500	2000
Glutamic acid hydrochloride	2770–2500	2000
Hydroxyamphetamine hydrobromide	2630–2440	1960
Methoxamine hydrochloride	2770–2500	1960
Nordefrin hydrochloride	2770–2500	1960
Phenylpropanolamine hydrochloride	2770–2380	1960
Tuaminoheptane sulfate	2770–2090	2000
dl-Amphetamine sulfate	2700–2040	2090
p-Methoxyamphetamine hydrochloride	2770–2380	1960

SECONDARY AMINE SALTS

The secondary amine salt absorption bands are listed in Table II. It should be noted that there is a series of three bands between 2860 and 2040 cm.$^{-1}$.

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