Notes

CHROM. 4882

Gas–liquid chromatography of Caramiphen hydrochloride and its analogues*

Parpanit (Caramiphen hydrochloride, 2'-diethylaminoethyl 1-phenylcyclopentanecarboxylate hydrochloride) and many of its analogues have been prepared and studied in these laboratories as possible antidotes for anticholinesterase poisoning[1,2]. The feasibility of using gas–liquid chromatography both as a criterion of purity for these compounds and as a means for their quantitative analysis has been briefly examined. Hydrochloride salts of this type decompose to the corresponding free bases during GLC and consequently it is possible to determine quantitatively either the free base or hydrochloride by GLC analysis.

Experimental

Except where otherwise specified, all gas–liquid chromatography was done using a Hewlett-Packard, F and M 5750 Research Chromatograph, employing a 6 ft. × ¼ in. O.D. glass column filled with 3.8% UCW 98 on Chromosorb W (DMCS), 60–80 mesh. Conditions were standardised at: column temperature 195°, injector 220°, detectors 235° (thermal conductivity detector when compound recovery was desired and flame ionisation detector for all other chromatography), helium flow rate 25 ml/min, hydrogen and air pressure 10 and 36 p.s.i., respectively.

The hydrochloride salts (5 mg) were dissolved in chloroform (20 μl) and 0.1-μl aliquots of these solutions were injected for chromatography. The corresponding free bases were obtained by treating 50 mg of each of the individual salts with 1 ml of 1 *N* sodium hydroxide. The resultant oily suspensions, after shaking for 5 min, were extracted with ether (4 × 1 ml). Samples (1 μl) of the combined ether extracts were injected into the chromatograph. Table I gives a list of the retention times observed for twenty-four compounds of this type.

Discussion

The apparatus and conditions described under *Experimental* gave a lower detectable limit of 1×10^{-11} g for Parpanit when 0.01 μl of a chloroform solution (1×10^{-3} mg/ml) was injected. Other stationary phases and loadings were examined, *e.g.* 6% nitrile gum XE-60 or 10% UCW 98, but a lower detectable limit of only 10^{-9} g was observed. Severe peak broadening was obtained with the XE-60 column when larger injections (0.8 mg) were made for isolation and recovery of samples.

Initial experiments on the chromatography of Parpanit revealed that both forms (salt and base) gave a single peak with identical retention times. Table I indicates that this behaviour is a characteristic common to all the analogues examined. Investigation of this phenomenon by preparative chromatography of the free base (5-μl neat injections) and hydrochloride (1.2 mg in chloroform, 10-μl injections) of Parpanit, coupled with IR examination, showed both recovered materials to be the free base.

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TABLE I

RETENTION TIMES FOR FREE BASES AND HYDROCHLORIDE SALTS OF PARPANIT AND ANALOGUES

No.	*Compound*	*Retention time (min)*	
		Free base	*Hydrochloride salt*
1	2'-Diethylaminoethyl 1-phenylcyclopentanecarboxylate (Parpanit)	6.4	6.3
2	2'-Diethylaminoethyl 1-*m*-methylphenylcyclopentanecarboxylate	7.6	7.6
3	2'-Ethylmethylaminoethyl 1-phenylcyclopentanecarboxylate	5.1	5.1
4	2'-Ethylaminoethyl 1-phenylcyclopentanecarboxylate	4.0	4.1
5	2'-Isopropylethylaminoethyl 1-phenylcyclopentanecarboxylate	8.0	8.0, 5.0[a], 10.0[a]
6	2'-Diisopropylaminoethyl 1-phenylcyclopentanecarboxylate	9.6	9.7, 2.4[a]
7	2'-Isopropylpropylaminoethyl 1-phenylcyclopentanecarboxylate	10.2	10.3
8	2'-Di-*n*-propylaminoethyl 1-phenylcyclopentanecarboxylate	10.6	10.6, 3.0[a]
9	2'-Di-*n*-butylaminoethyl 1-phenylcyclopentanecarboxylate	17.8	17.8
10	2'-Diisobutylaminoethyl 1-phenylcyclopentanecarboxylate	13.9	14.0
11	2'-Di(2"-butyl)aminoethyl 1-phenylcyclopentanecarboxylate	17.5	17.5
12	2'-*tert.*-Butylaminoethyl 1-phenylcyclopentanecarboxylate	5.6	5.6
13	2'-*tert.*-Butylmethylaminoethyl 1-phenylcyclopentanecarboxylate	8.4	8.4
14	2'-*tert.*-Butylethylaminoethyl 1-phenylcyclopentanecarboxylate	10.2	10.2
15	3'-Diethylaminopropyl 1-phenylcyclopentanecarboxylate	8.4	8.4
16	2'-Dicyclohexylaminoethyl 1-phenylcyclopentanecarboxylate	12.7	12.8, 3.6[a]
17	2'-Dibenzylaminoethyl 1-phenylcyclopentanecarboxylate	5.3	5.4
18	2'-Piperidinoethyl 1-phenylcyclopentanecarboxylate	12.0	12.0
19	1'-Methyl-4'-piperidinyl 1-phenylcyclopentanecarboxylate	7.4	7.4
20	1'-Methyl-4'-piperidinyl 1-*o*-methylphenylcyclopentanecarboxylate	10.6	10.6
21	1'-Methyl-4'-piperidinyl 1-*m*-methylphenylcyclopentanecarboxylate	10.2	10.2
22	1'-Methyl-4'-piperidinyl 1-phenylcyclobutanecarboxylate	5.3	5.4
23	1'-Methyl-4'-piperidinyl 1-*p*-methylphenylcyclobutanecarboxylate	7.2	7.1
24	1'-Methyl-4'-piperidinyl 1-*p*-methoxyphenylcyclobutanecarboxylate	12.6	12.6

[a] Minor peaks.

A similar examination of all the compounds listed in Table I was not made, but it is assumed that the same conversion of salt to free base during chromatography is responsible for the correspondence in retention times for salts and bases. The facile interconversions observed for these amino ester hydrochlorides, although known in the literature, were unexpected because of the extensive experimental precautions taken by UMBREIT and co-workers[3] to ensure the conversion of their amine hydrochlorides to the free bases prior to GLC analysis by packing the injection part of the apparatus with 20% KOH coated on Chromosorb 101. It seems probable that the conversion of Parpanit and its analogues to the free bases observed here owes its origin to the fact that the latter are considerably weaker bases (pK_a values range from 7.7–8.1, ref. 4) than the amines examined by UMBREIT and co-workers[3] (pK_a values range from 9.8–10.6, ref. 5) and consequently form less stable salts. Some of the hydrochlorides shown in Table I (compounds 5, 6, 8, and 16) exhibit minor peaks in addition to the major peak attributed to the free base. No effort was made to identify these materials since their presence did not detract from the general usefulness of the method for identification or analysis of the compounds.

Decomposition during gas–liquid chromatography has been observed with amino acids[6], diols[7], unsaturated compounds[8], amine salts[3], corticosteroids[9], sterols[10], and terpene alcohols[11]. The cause of decomposition of some of these compounds has been attributed to the type of column packing[6,7] or to the column material itself[3].

Several column packings were examined in the present work to determine if the column packing was critical for the interconversion. Since all the amine hydrochlorides behaved in the same manner on all the columns examined it seems likely that the interconversion owes its origin to the thermal instability of the compounds themselves rather than to the action of the packing materials. The necessity of treating the solid support, especially diatomaceous earth types, with organic bases such as tetrahydroxyethylethylenediamine (THEED)[12], tetraethylenepentamine (TEP)[12], and polyethyleneimine (PEI)[13], or inorganic bases such as sodium or potassium hydroxide[12–16] to prevent tailing of amines is well known and has been emphasized by UMBREIT and co-workers[3]. In the work reported here, however, Chromosorb W commercially treated with dimethyldichlorosilane performed extremely well and no additional pretreatment was necessary.

An increase in retention time is associated with an increase in the bulk and number of carbon atoms contained in the substituents attached to the nitrogen atom (*cf.* compounds 4 and 1, 1 and 8, 8 and 9 in Table I). Branching of the carbon chain attached to nitrogen lessens the retention time in relation to that of the straight-chain homologue (*cf.* compounds 8 and 6, 9 and 10 in Table I). These observations are probably mainly manifestations of the differences in volatilities of the compounds as reflected by the boiling point behaviour of aliphatic amines, possibly modified by donor and acceptor hydrogen-bonding effects which are known to be very active in the chromatography of amines[16], and which can vary considerably in magnitude with variation in the type and method of pretreatment of the solid support[17].

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