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ANALYSIS OF 1-(2-PHENYLADAMANT-1-YL)-2-METHYLAMINOPROPANE AS ITS CHLORODIFLUOROACETYL DERIVATIVE

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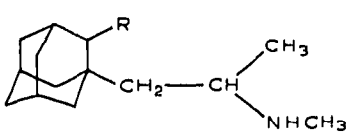
SUMMARY

The diastereoisomeric content of 1-(2-phenyladamant-1-yl)-2-methylaminopropane can be determined by gas chromatography following treatment with chlorodifluoroacetic anhydride. Racemisation occurs in the synthesis of 2-phenyladamantane from the corresponding 2-chloroadamantane in the Friedel-Crafts reaction with aluminium chloride, but fractionation of the diastereoisomers can occur in the purification steps. A method for determining 2-phenyladamantane in plasma and urine extracts at the nanogram level using an electron-capture detector is described and compared with a previously described radio-assay procedure.

INTRODUCTION

Amantadine hydrochloride, originally used as an antiviral agent, has since proved valuable in the treatment of Parkinson's disease[1]. This paper concerns a novel adamantane derivative, I (1-(2-phenyladamant-1-yl)-2-methylaminopropane) which was found to have pharmacological properties in animals suggesting it may be superior to amantadine in the treatment of Parkinson's disease, and may also have useful antidepressant activity[2]. As compound I possesses two asymmetric centres, it was necessary to develop an assay for the diastereoisomeric content. Underivatised, or as a number of simple N-acyl derivatives, compound I elutes as a single peak on gas–liquid chromatographic (GLC) analysis. However, when converted into N-haloacyl derivatives, a facile resolution of the diastereoisomers by GLC was achieved, and the presence of the halogen atoms afforded an assay, using an electron-capture detector (ECD), to enable determinations at the nanogram level in plasma and urine extracts.



I R = C_6H_5
II R = Cl

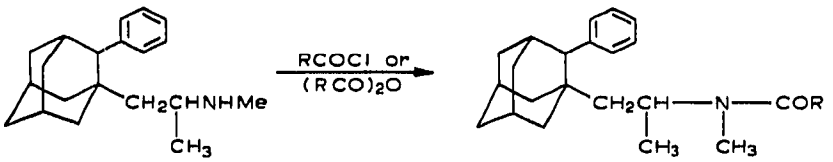
EXPERIMENTAL

Instruments

GLC analyses were carried out using a Pye Unicam gas chromatograph equipped with either a hydrogen flame ionisation detector (FID) or a ^{63}Ni ECD. An LKB-9000S mass spectrometer was used in the combined GLC–mass spectrometric (MS) determinations.

Synthesis of materials

Compounds I and II. The synthesis of compound I from compound II under Friedel-Crafts conditions and its separation into constituent diastereoisomers has been described previously[3,4]. Compound II, which also possesses two asymmetric centres, was partially fractionated as its hydrochloride salt by recrystallisation from 15% isopropanol–light petroleum (80:100). The proportions of the constituent isomers were determined by GLC.



I III

Acylation of compound I. A typical derivatisation was as follows: The 2-phenyladamantane (I) (0.5 g) was dissolved in methylene chloride (5 ml) and acylating reagent (0.5 ml) was added. The solution was stirred on a water-bath at 50° until the derivatisation was quantitative as followed by GLC. A few drops of triethylamine and excess acylating reagent were added if required. The methylene chloride solution was neutralised with sodium bicarbonate, dried through anhydrous sodium sulphate and evaporated to dryness to give derivative III. The following derivatives were prepared: R = C_3F_7, CF_2Cl, C_2F_5, CCl_3, CF_3 (using acid anhydride), and $CHCl_2$ (using acid chloride). All derivatives gave satisfactory spectral and elemental analyses.

Assignment of isomer content in compound I using flame ionisation detection

GLC conditions for estimation of the diastereoisomeric ratio of compound I as its chlorodifluoroacetyl derivative. The GC conditions employed were: glass column, 5 ft., 5% XE-60 (cyanoethyl silicone) on 60–85 mesh Gas-Chrom Q; temperature, 190°; carrier gas, nitrogen at a flow-rate of 75 ml/min.

Preparation of standards. The diastereoisomers of the hydrochloride salt of I were separated by fractional crystallisation from isopropanol–petroleum ether to give isomer Ia, m.p. 210–225°, and from ethanol–ether to give isomer Ib, m.p. 210–215°[3]. The spectral characteristics of both isomers have been described previously[4]. Each isomer was converted into its chlorodifluoroacetyl derivative and chromatographed as a single peak. However, on mixing the two diastereoisomers, the characteristic GLC doublet observed for unfractionated amide derivatives of I was observed (Fig. 1). GLC–MS analysis confirmed the identity and isomeric nature of the two peaks, which exhibited very similar spectra with the following characteristic ions: 395 (M^+); 310 ($M-85^+$); 252 ($M-143^+$), as the base peak (Fig. 2). The isomeric

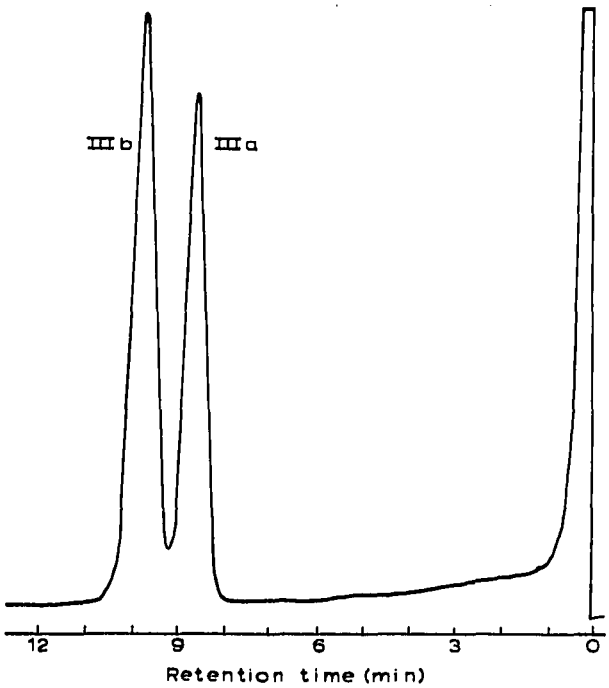


Fig. 1. Separation of the diastereoisomers of 1-(2-phenyladamant-1-yl)-2-methylaminopropane as their chlorodifluoroacetyl derivatives. Detector, ECD at 290°; column, 5 ft., 5% XE-60 on 60–85 mesh Gas-Chrom Q at 190°; carrier gas, nitrogen at a flow-rate of 80 ml/min.

derivatives gave slightly differing responses to an FID. An equimolar mixture gave a peak area ratio of isomer Ia (first eluting) to isomer Ib derivative of 1.1:1.0 (Fig. 1). From peak area ratio measurements, each isomeric standard was greater than 99% pure.

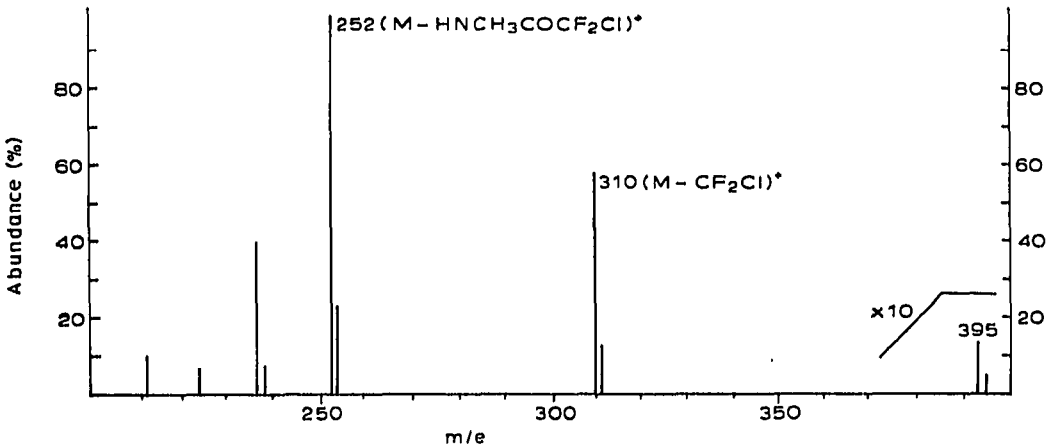
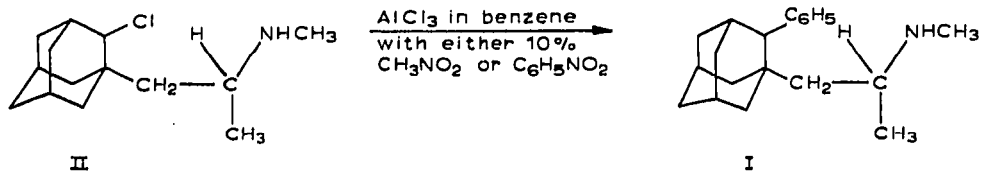


Fig. 2. Mass spectrum of the chlorodifluoroacetyl derivative of 1-(2-phenyladamantyl-1-yl)-2-methylaminopropane.

Determination of the diastereoisomer ratio of compound I in Friedel-Crafts reaction solutions



II I

Nitromethane or nitrobenzene were used as co-solvents to ensure complete solution of the reactants, and hence minimisation of rearrangement reactions which can occur on the surface of the catalyst[4].

Estimation of isomer content of compound II. The GLC conditions employed were: glass column, 9 ft., 1% KOH–4% Carbowax 20M on 80–100 mesh Gas-Chrom Q; temperature, 150°; carrier gas, nitrogen at a flow-rate of 185 ml/min.

The hydrochlorides of II, following partial fractional crystallisation, were neutralised with excess NaOH solution and the free bases were then extracted into methylene chloride. The organic extract was dried (Na_2SO_4) and the solvent was removed under reduced pressure. The residue of II was then dissolved in chloroform, ready for chromatographic analysis.

Compound II gives two chromatographic peaks, for its constituent diastereoisomers, without the need for prior conversion into an amide derivative. An equal response towards the FID was assumed for each isomer (independent analysis of diastereoisomer content by nuclear magnetic resonance confirmed this assumption to be sound), and partial fractional crystallisation gave a product with an isomer content of 2.7:1.

Reaction solution analysis. At completion, the Friedel-Crafts reaction mixture was poured into excess 1 *N* NaOH solution and the free base of I was extracted into methylene chloride and dried with anhydrous sodium sulphate. An aliquot of the dried solution was evaporated and the residual I was converted into its chlorodifluoroacetyl derivative, which was analysed by GLC as described previously. In a reaction using compound II with an initial isomer ratio of 2.7:1, an equal mixture of the two diastereoisomers of I was obtained.

Determination of compound I in plasma and urine extracts

Reagents. In order to avoid unworkable reagent peak contamination of the gas chromatograms, it was necessary to redistil all commercial batches of the acylating reagents and triethylamine before use. AnalaR-grade benzene was used as supplied, but chloroform and toluene were both redistilled.

GLC conditions. The GLC conditions employed were: glass column, 5 ft., 5% XE-60 (cyanoethyl silicone) on 60–85 mesh Gas-Chrom Q; temperature, 235°; carrier gas, nitrogen at a flow-rate of 80 ml/min; detector temperature, 290°; pulse space, 150 μsec.

Detector temperature. The ECD response of the chlorodifluoroacetyl derivative (III) was found to vary by a factor of two over the temperature range 270–360° (Fig. 3), and to show a maximum at 290°. Above 290°, the response of the molecule

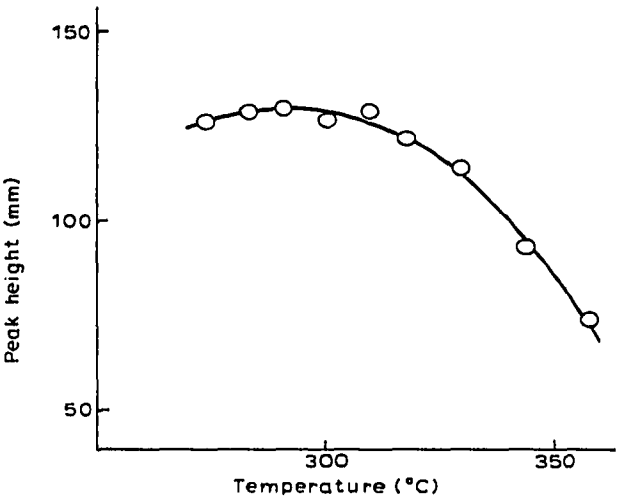


Fig. 3. Effect of detector temperature on the ECD response of the chlorodifluoroacetyl derivative of 1-(2-phenyladamanty-1-yl)-2-methylaminopropane.

to the ECD decreases with increasing temperature, indicating a non-dissociative mechanism of electron attachment[5].

Extraction procedure. Compound I was extracted from plasma using a back-extraction method[6]. The plasma extract (2–3 ml) was extracted with benzene (4 ml), after the addition of saturated Na_2HPO_4 solution (0.5 ml) by horizontal shaking for 5 min, frozen in an acetone–dry ice bath, warmed, and centrifuged at 1000 *g* for 2 min. The organic layer was extracted with 0.1 *N* HCl (2 ml) and the aqueous extractions neutralised with 1 *N* NaOH solution (0.2 ml), before extraction of compound I into chloroform and evaporation to dryness.

Urine extracts (5 ml) were processed similarly except that some solid Na_2HPO_4 was added initially to the urine to raise the pH.

Residue derivatisation. A solution of the above plasma residue in chloroform (20 μl) was derivatised with chlorodifluoroacetic anhydride (3 μl) and triethylamine (1.5 μl) in a warm water-bath (50°) for 30 min before evaporation to dryness. Residual reagent was neutralised with excess alkali, followed by extraction of compound III into chloroform and evaporation to dryness. Toluene (1 ml) was used as the GLC solvent. Urine residues were processed in a similar manner although usually requiring further dilution in toluene.

Chromatographic standards. Using the same batch of compound I as that used for the biological studies (to ensure that isomer ratios of compound I are constant), the chlorodifluoroacetyl derivative was prepared as described previously. Standard solutions of this derivative in toluene containing 0.2, 0.1 and 0.04 μg/ml were prepared for GLC calibration purposes.

Standard recovery. Plasma (2–3 ml) was spiked with 20 μl of a solution of compound I (10.74 μg/ml in chloroform) and the level of extracted compound I was determined by GLC. Sample solutions were determined directly from a peak height calibration graph (Fig. 4). The average standard recovery was 67%.

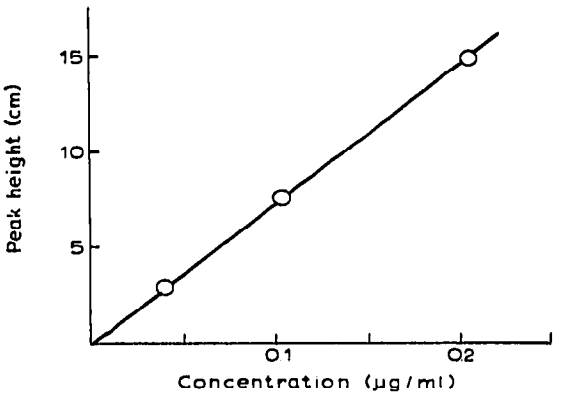


Fig. 4. Response of the chlorodifluoroacetyl derivative of 1-(2-phenyladamant-1-yl)-2-methylaminopropane to a ^{63}Ni ECD.

Fig. 5 shows a typical chromatogram obtained from the standard recovery solution in control urine.

DISCUSSION AND RESULTS

Analysis of the diastereoisomer content of compounds I and II by the flame ionisation detection

In the synthesis of compound I[3], only at the last two stages does the problem of diastereoisomer content arise. However, as compound II gives an equimolar mixture of isomers of compound I in the reaction solution, there is no need to control the isomer content of II. Thus, it was only necessary to develop an assay procedure to

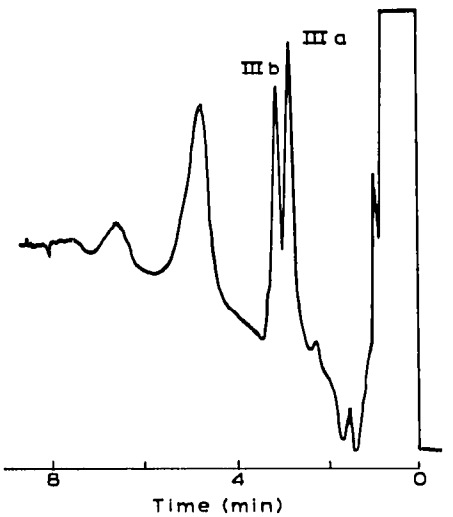


Fig. 5. Chromatographic estimation of the diastereoisomers of 1-(2-phenyladamant-1-yl)-2-methylaminopropane as their chlorodifluoroacetyl derivatives in urine extracts. Chromatographic conditions as for Fig. 1, except a column temperature of 235°.

estimate the diastereoisomer content of I, as this may vary depending on the recrystallisation technique.

All of the halogenated amide derivatives of compound I give rise to facile doublet separation into the individual diastereoisomeric pairs under GLC conditions. In most cases either the chlorodifluoro or heptafluorobutyryl derivatives were used and the levels of each isomer in synthetic samples of compound I varied from 45–55%. These two derivatives were used in preference to the others as they were the most suitable for the ECD assay for low-level determination in biological fluids. A range of non-halogenated amide derivatives did not enable separation of the diastereoisomers under chromatographic conditions. Presumably the carbon–halogen bond in either compound II or the halogenated amides III imparts sufficient difference in polarity to enable separation of the diastereoisomers.

Determination of compound I in plasma and urine samples

Selection of derivative for assay using electron-capture detection. Attempts to introduce electron-capturing properties into compound I concerned both the use of halomethylsilylating and haloacylating reagents. At the residue levels, only the latter reagents gave a quantitative yield of the derivative.

In selecting the most appropriate haloacyl derivative for an ECD assay, the following considerations were applied: (i) the sensitivity of the derivative towards the detector, (ii) the chromatographic separation of the diastereoisomers, and their relative responses, and (iii) the interference from reagent background and the problem of purification of the acylating reagent.

The response of the acyl derivatives III towards an ECD decreased along the following series: R = $CF_2Cl \approx n\text{-}C_3F_7 > CHCl_2 \approx C_2F_5 > CCl_3 > CF_3 \gg CH_3$. As expected, the response increases with the length of the fluoroalkyl chain. However, the chlorodifluoro derivative was preferred to the heptafluorobutyryl derivative, because it gave a slightly greater response and the response for the two constituent diastereoisomers was very similar—0.7 (IIIa) : 1.0 (IIIb), *cf.* the 1.1:1.0 response towards the FID. In contrast, the diastereoisomers of the heptafluorobutyryl derivative gave a differential response of two- to threefold, and reagent peak interference of control chromatograms was greater using distilled samples of heptafluorobutyric anhydride than using those of chlorodifluoroacetic anhydride.

Sensitivity of assay procedure. A higher chromatographic column temperature was used for the ECD procedure than for the FID method. This resulted in a shorter retention time and decreased separation between the chromatographic peaks of the diastereoisomers. However, the increased peak sharpness enabled a greater sensitivity. Typically, in either human or rat plasma and urine samples, the minimum detectable level of compound I as its chlorodifluoroacetyl derivative based on a 10-mm peak height response was 30 ng/ml of plasma. This is equivalent to 150 pg of derivative on column. A comparable radioassay procedure is slightly more sensitive[7] but does not enable quantitation of the individual diastereoisomers.

Comparison of radioassay and ECD procedures. Results from the GLC method are compared with those for the faster radioassay procedure[7] (Table I), which requires minimal clean-up of samples. The agreement between the two methods is good. Maximum levels of 50–60 ng of compound I per millilitre of plasma were observed after 3–4 h at the 50–60 mg dose level. Significant levels of compound I were excreted

TABLE I

COMPARISON OF RADIO ASSAY AND ECD PROCEDURES FOR THE MEASUREMENT OF THE CONCENTRATION OF COMPOUND I IN HUMAN PLASMA AND URINE AS A FUNCTION OF TIME

Dose (mg)	*Blood sample*	*Compound I (ng per ml plasma)*		*Isomer ratio**
		Radioactive method	*GLC–ECD method*	
50	1 h	11	<30	—
	2 h	22		—
	3 h	50	59	1.0
	4 h	47	64	1.1
	6 h	39		—
60	2½ + 3 h		42	1.0
	3½ + 4 h		38	1.2
	5½ + 6 h		34	1.1
Dose (mg)	*Urine sample*	*Compound I (ng excreted in urine)*		*Isomer ratio*
		Radioactive method	*GLC–ECD method*	
60	4– 6 h	79	42	1.0
	12–24 h	142	154	1.0
	24–36 h	329	281	1.0
	36–48 h	130	89	0.9
	48–60 h	95	102	0.9

* The isomer ratio for this batch of compound I is 1.0 by ECD.

in the urine. The main value of the ECD procedure was to show that within the experimental uncertainties, there is no preferential metabolism of one of the diastereoisomers of compound I. This is an interesting result, as the aqueous solubility and partition coefficients of the two diastereoisomers are significantly different (three- to fourfold difference).

ACKNOWLEDGEMENT

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