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DERIVATISATION AND GAS–LIQUID CHROMATOGRAPHY OF 3-AMINOMETHYL-2-PHENYLBICYCLO[2.2.2]OCTANES

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SUMMARY

Gas–liquid chromatographic methods are described for measurement of the isomer content of 3-methylaminomethyl-2-phenylbicyclo[2.2.2]octane following chemical derivatisation. The stereospecificity of its synthesis is confirmed and the resolved enantiomers are shown to be essentially pure.

INTRODUCTION

Members of a series of *cis*- and *trans*-fused 3-aminomethyl-2-phenylbicyclo-[2.2.2]octanes have been shown to have potential antidepressant properties[1] in animal models. The *cis*-series is the more potent. The most active compound (I) in the *cis*-series has been resolved into its two enantiomers, the laevorotatory isomer showing the greater activity. In support of the synthesis of I for use in toxicological and clinical trials it was necessary to measure both optical and geometric isomer purities of itself and selected synthetic precursors.

The latter stages of the synthesis[1] are shown in Fig. 1. Catalytic reduction of the Diels–Alder adduct formed between *cis*-3,4-dichlorocinnamonitrile and 1,3-cyclohexadiene gives the *cis*-fused 3-cyano-2-phenylbicyclo[2.2.2]octane (II). Compound I is formed in two successive reactions, either by formation of the primary amine (III) or the secondary amine (IV). Chromatographic separation and purity measurements of I were complicated by the presence of numerous homologue and dechlorinated impurities. As high-performance liquid chromatography (HPLC) was not successful in handling these molecules, gas–liquid chromatography (GLC) was utilised to analyse the principal components, even though extensive derivatisation procedures were required. GLC–mass spectrometry (MS) was also the primary technique utilised to characterise impurities at the various synthetic stages.

EXPERIMENTAL

Instruments

GLC analyses were carried out using either a Pye-Unicam Model 104 or a

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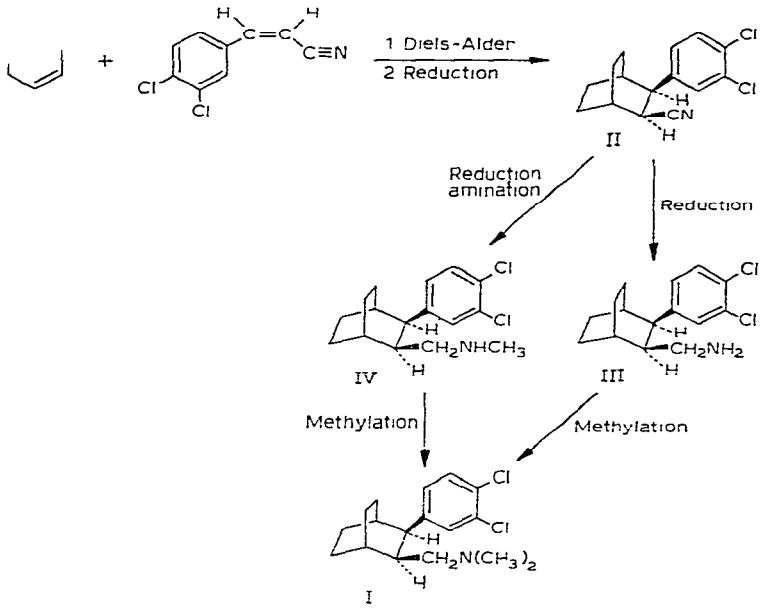

Fig. 1. Synthesis of 3-dimethylaminomethyl-2-(3′,4′-dichlorophenyl)-bicyclo[2.2.2]octane.

Hewlett-Packard Model 5700 gas chromatograph equipped with a flame ionisation detector. An LKB Model 9000S mass spectrometer (MS) was used for the GLC–MS measurements.

Determination of trans-fused molecule (Ia) in cis-fused molecule (I) by formation of the carbamate (Ib)

The hydrochloride of I (3 mg) was dissolved in chloroform (1 ml) and shaken with 0.1 *M* sodium hydroxide solution. An aliquot (100 μl) of the chloroform layer was transferred to a 1-ml Reacti-vial (Pierce, Rockford, IL, U.S.A.). Aliquots (1, 3 and 5 μl) of isomer Ia (0.3 mg/ml) were added to further 100-μl volumes of I. 100 μl of ethyl chloroformate (Koch-Light, Colnbrook, Great Britain) and sodium carbonate (10 mg) were added to each solution. The solutions were heated at 100°C for 45 min and then blown to dryness under nitrogen. The residue was redissolved in chloroform (50 μl) and a sample analysed on a 2% XE60 column at 210°C.

Conversion of compound I to IV

Compound I (3 mg) was converted to Ib as previously described. Hydrobromic–glacial acetic acid (1:1) (0.5 ml) was added to the carbamate residue. The solution was heated at 100°C for 90 min, basified with sodium hydroxide solution to pH 10, and diluted with water. Compound IV was extracted into heptane (×6) and blown to dryness.

Derivatisation of compound IV with optically acid chlorides

The residue of compound IV was reacted with 50 μl of either (+)-α-methoxy-α-trifluoromethylphenylacetyl chloride or N-heptafluorobutyryl-α-prolyl chloride

(Regis, Morton Grove, IL, U.S.A. and triethylamine (10 μl). After 5 min the solution was blown to dryness and the residue dissolved in methylene chloride. (+)-α-Methoxy-α-trifluoromethylphenylacetic acid (Aldrich, Milwaukee, WI, U.S.A.) was converted to the corresponding acid chloride by reaction with oxalyl chloride in triethylamine. The diastereomers V were analysed by GLC using an Apiezon M column whilst VI were analysed using an N,N-bis(*p*-methoxybenzylidene-α,α'-bi-*p* toluidine) (Eastman-Kodak, Rochester, NY, U.S.A.) liquid crystal column at 225°C.

Formation of diastereomeramide VI via formation of N-oxide VII

Compound I (0.05 mg) was dissolved in methylene chloride (50 μl) and treated with *m*-chloroperoxybenzoic acid (50 μg). The solution was blown to dryness after 5 min, again after the addition of triethylamine, and finally after the addition of ethereal diazomethane. (+)-α-Methoxy-α-trifluoromethylphenylacetyl chloride (30 μl), chloroform (50 μl) and triethylamine were added and the diastereomers analysed as previously indicated.

Analysis of compounds III and IV

Aliquots (20 μl) of chloroform solution (1 mg/ml) of compound III or IV were reacted with 10 μl of pentafluorobenzoyl chloride (Fluorochem, Dinting Vale, Great Britain) and triethylamine (2 μl) at 60°C for 15 min. After cooling the resultant solution was shaken with distilled water (350 μl) and 0.880 ammonia (50 μl). An aliquot of the chloroform layer was analysed on an Apiezon J column at 270°C.

RESULTS AND DISCUSSION

Determination of isomeric trans-fused molecule (Ia) in cis-fused 3-dimethylaminomethyl-2-(3',4'-dichlorophenyl)bicyclo[2.2.2]octane (I)

Compound I not only exhibits poor GLC characteristics but it is difficult to separate from the isomeric *trans*-fused molecule (Ia). An optimum relative retention of only 1.05 was achieved for these two molecules on an Apiezon L column. The separation was not suitable for measuring low levels of isomer Ia in I. Several attempts have been made to improve the gas chromatographic properties of tertiary amines by derivatisation. The Hofmann degradation reaction[2] can be utilised to form olefins characteristic of the parent amine. Reaction of a tertiary amine with an alkyl chloroformate[3,4] has been shown to be a satisfactory method for determining low concentrations of drugs.

The levels of Ia in I were determined by this approach using ethyl chloroformate (Fig. 4). The resultant derivatives were then readily resolved by GLC. The formation of the carbamate (Ib) was monitored at 10 min intervals using GLC. On an XE-60 (cyanoethyl silicone) column, the two derivatives show a relative retention time of 1.2. The peak area ratios of *cis*-carbamate to *n*-triacontane internal standard are shown (Fig. 2). Optimum yield (90%) was observed after 60 min, GLC–MS confirming the identity of both carbamate peaks, *viz*: *m/e* 369/371/373 [$M^{+\cdot}$]; 266/268/270 [$M - N(CH_3)COOC_2H_5]^+$ and 116 [$CH_2{=}\overset{+}{N}(CH_3)COOC_2H_5$]. As the synthesis route of I shows a high degree of stereospecificity[1], levels of the *trans*-isomer in I were expected to be below one percent although it was not possible to obtain a standard sample free of *trans*-isomer Ia. Consequently the levels of Ia in I were determined by a spiking

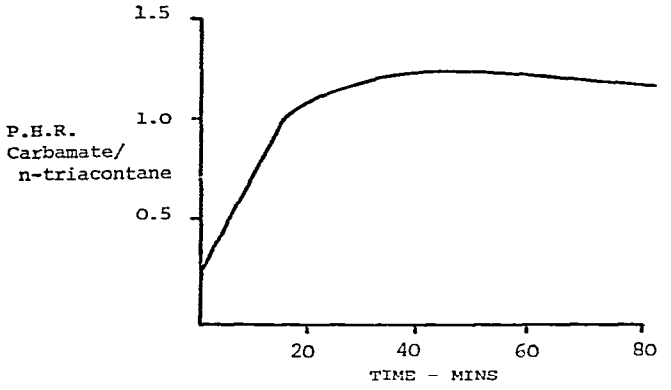

Fig. 2. Graph showing rate of formation of carbamate derivative (Ib).

technique and ratioing the areas of the respective carbamate peaks, assuming an equality of response from each molecule. Standard aliquots of unknown I were spiked with known amounts of Ia. From the calibration graph (Fig. 3) the intercept value representing the required impurity level could be measured. Typical levels of 0.1–0.2% were observed. A precision measurement on six samples gave a coefficient of variation of 3.0% for a mean value of 0.145% *trans*-isomer in I.

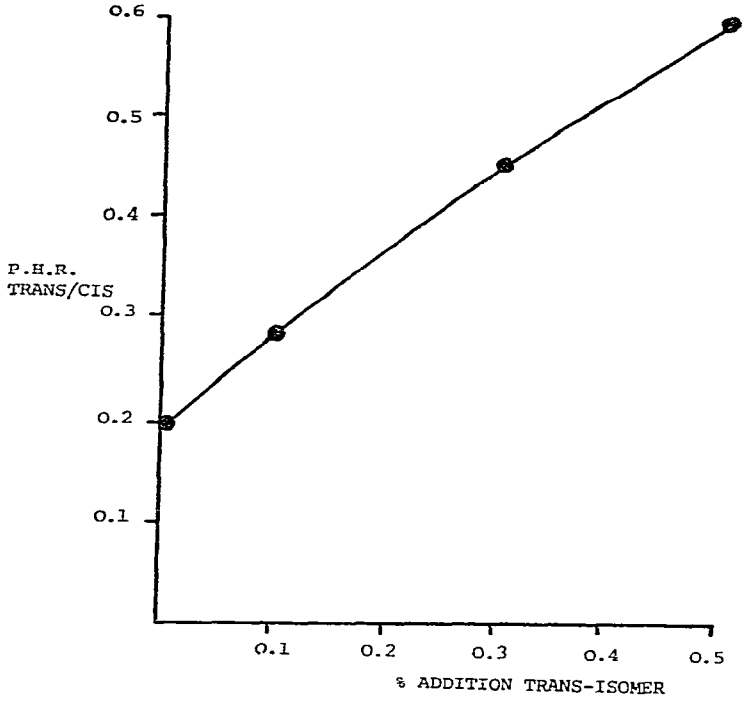

Fig. 3. Calibration graph for determining the level of the *trans*-fused isomer in *cis*-fused 3-dimethylaminomethyl-2-(3′,4′-dichlorophenyl)bicyclo[2.2.2]octane (I).

Analysis of 3-aminomethyl-2-(3',4'-dichlorophenyl)bicyclo[2.2.2]octane (III) and 3-methylaminomethyl-2-(3',4'-dichlorophenyl)bicyclo[2.2.2]octane (IV)

It was not necessary to regularly measure the levels of the *trans*-fused isomer in III or IV once it was established that no inversion of *cis*-fused material took place at either of these synthetic stages. In contrast to the cyano-functional molecule (II), neither III nor IV gave satisfactory chromatographic peaks and were best analysed after benzoylation or acetylation when the respective isomeric derivatives were readily resolved. The pentafluorobenzoate derivatives of IV (*m/e* 491/493/495 [$M^{+\cdot}$], 266/268/270 [$M - N(CH_3)COC_6F_5]^+$ and 238[$CH_2{=}\overset{+}{N}(CH_3)COC_6F_5$] were quantitatively formed and easily resolved by GLC. They indicated that in both cases minimal levels of the *trans*-isomer were present and no inversion took place. Similar resuits were obtained from the derivatives of III.

Determination of optical purity of cis-fused 3-dimethylamino-2-(3',4'-dichlorophenyl)-bicyclo[2.2.2]octane (I)

Using GLC amine enantiomers can either be directly determined on optically active stationary phases or as diastereoisomers on achiral phases[5,6]. Although the former approach is more direct, it was not possible to implement it for I due to upper temperature limits imposed by the volatility of the optically active phase.

To form diastereoisomers from I it was first necessary to introduce a functional group which could be derivatised. In one approach compound I was demethylated after initial conversion to the carbamate (Ib). Compound Ib was then hydrolysed to give compound IV, a secondary amine. The overall yield of this reaction was 70%. Reaction of IV with N-heptafluorobutyryl-L-prolyl chloride[7] (Fig. 4) gave a diastereoisomer pair (V) which could be resolved on an Apiezon M column. However, this

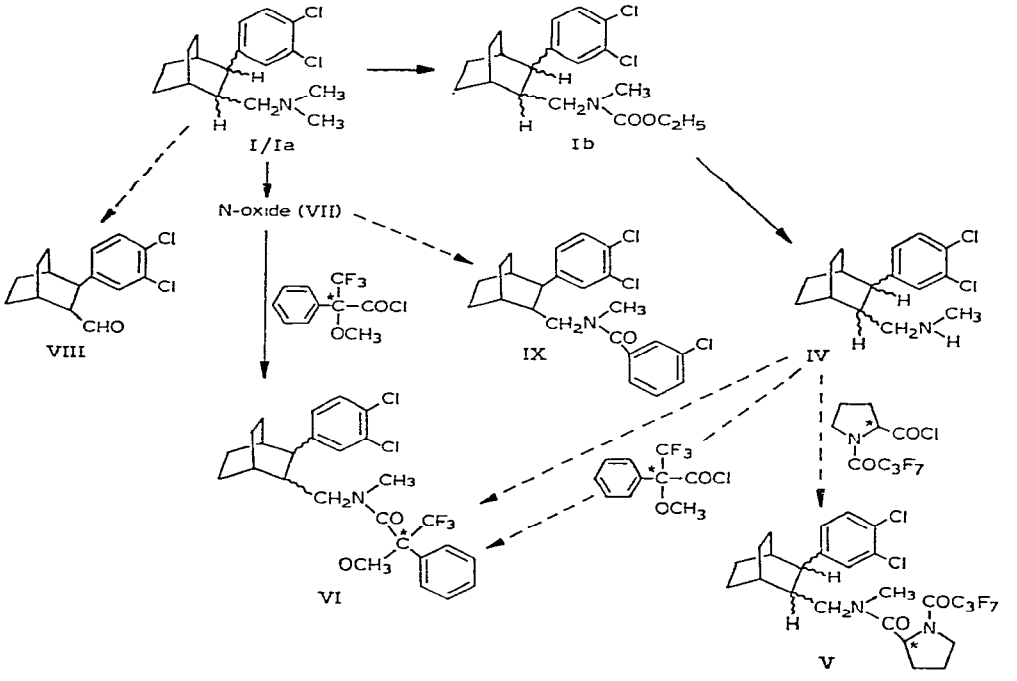

Fig. 4. Derivatisation routes for the analysis of *cis*-fused 3-dimethylamino-2-(3',4'-dichlorophenyl)-bicyclo[2.2.2]octane (I).

latter reagent proved unsuitable for quantitative work due to its variable purity and inversion during derivatisation. When IV was reacted with (+)-α-methoxy-α-trifluoromethylphenylacetyl chloride[8] (Fig. 4) no inversion took place. In contrast to the prolyl reagent the latter acid chloride lacks an α-hydrogen and hence is resistant to racemisation. The resultant diastereoisomers (VI) were resolved into two equal intensity peaks using the liquid crystal phase N,N′-bis(*p*-methoxybenzylidene-α,α′-bi-*p*-toluidine). Super-cooling of the liquid crystal improved the separation[9].

In a preferred alternative route to VI, compound I was converted to the corresponding N-oxide (VII) by reaction with *m*-chloroperoxybenzoic acid. Reaction of VII with (+)-α-methoxy-α-trifluoromethylphenylacetyl chloride[10] (Fig. 4) formed the diastereoamides (VI) ([$M^{+\cdot}$] *m/e* 513/515/517). The formation of VI can be followed by GLC on a dimethyl silicone phase. The average overall conversion yield for both diastereoisomers was 43% (relative standard deviation = 4.9%). The aldehyde (VIII) ([$M^{+\cdot}$] *m/e* 182/184/186) was a significant by-product whilst the formation of the unwanted amide (IX) ([$M^{+\cdot}$] *m/e* 435/437/439/441) could be minimised by reaction of excess *m*-chloroperoxybenzoic acid, *m*-chlorobenzoic acid or its acid chloride with diazomethane.

TABLE I

PERCENTAGE OPTICAL PURITY OF *CIS*-FUSED 3-DIMETHYLAMINO-2-[3′,4′-DICHLOROPHENYL] BICYCLO-[2.2.2]OCTANE(I)

(+)-Isomer		*(−)-Isomer*	
Experimental	*Composition Synthetic mixture*	*Experimental*	*Composition Synthetic mixture*
100.0	100.0		
		100.0	100.0
88.8	90.6	11.2	9.4
93.2	95.3	6.8	4.7
10.7	10.7	89.3	89.3
6.2	5.3	93.8	94.7

As the method required the determination of relative isomer concentration, the respective peak area ratios gave a satisfactory measurement. Analysis of resolved samples of the two enantiomers by this method suggest them to be essentially pure. Synthetic mixtures of the enantiomers were prepared from these pure components. The experimental composition of the synthetic mixtures was in close agreement with expected levels (See Table I).

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