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ANALYSIS OF ISOMERIC IMPURITIES IN A SYNTHESIS OF THE NOVEL ANTI-INFLAMMATORY DRUG, BENOXAPROFEN

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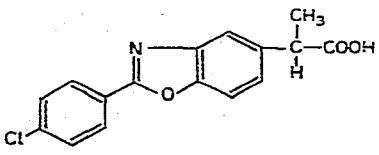
SUMMARY

Gas–liquid chromatographic procedures are described for the determination of isomeric impurities in *p*-chlorobenzoyl chloride and the intermediates, 2-(*p*-nitrophenyl)-proprionitrile and 2-phenylpropionitrile, in a ten-stage synthesis of benoxaprofen, 2-(*p*-chlorophenyl)-α-methyl-5-benzoxazoleacetic acid.

INTRODUCTION

Recently the synthesis and anti-inflammatory activity of a number of 2-aryl-α-methyl-5-benzoxazoleacetic acids were reported[1]. One member of the series, benoxaprofen (I), was three to five times more active than phenylbutazone on the rat paw oedema test and was selected for more detailed evaluation.



(I)

Significant quantities of benoxaprofen were required for toxicology and clinical studies. Methods are described elsewhere for the analysis of benoxaprofen, particular care being taken to control the level of isomeric impurities[2]. As these isomeric impurities are difficult to remove from the benzoxazole, it is necessary to limit the concentration of their precursors in the chemical synthesis. Fig. 1 outlines the synthesis of benoxaprofen. 3-Phenylpropionitrile, a potential impurity in II, could carry through the synthesis to give the corresponding propionic acid of benoxaprofen. Subsequent nitration gives rise to both *o*- and *m*-nitro derivatives in addition to the required 2-(*p*-nitrophenyl)-propionitrile (III). If not removed, these impurities would carry through the synthesis to give the 7- and 6-α-methyl derivatives of benoxaprofen,

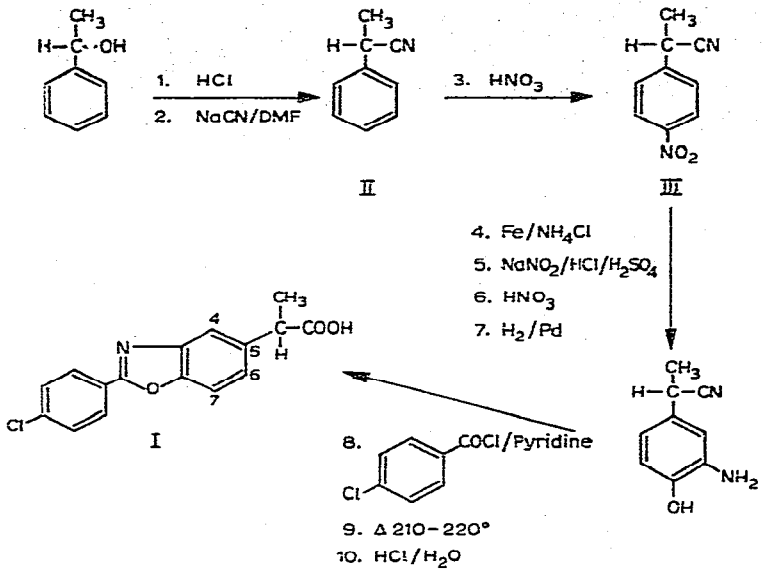


Fig. 1. Synthesis of 2-(*p*-chlorophenyl)-α-methyl-5-benzoxazoleacetic acid.

respectively. Isomeric impurities in *p*-chlorobenzoyl chloride are a source for the introduction of 2-aryl impurities. This manuscript describes methods for the analysis of intermediates II, III, and *p*-chlorobenzoyl chloride.

EXPERIMENTAL

Instruments

Gas–liquid chromatographic (GLC) analyses were carried out using either a Pye-Unicam Model 104 or Hewlett-Packard Model 5700 gas chromatograph equipped with a hydrogen flame ionisation detector. An LKB Model 9000S mass spectrometer was used for the combined GLC–mass spectrometric (GLC–MS) determination.

Determination of isomeric impurities in p-chlorobenzoyl chloride

Materials. Commercial samples of *p*-chlorobenzoyl chloride (Koch-Light, Nipa Labs., Aldrich) were used without further purification. GLC reference samples of ethyl chlorobenzoates (*o*, *m* and *p*) were prepared by ethanolysis of the corresponding acid chlorides and had satisfactory spectral characteristics.

Sample preparation. A sample of *p*-chlorobenzoyl chloride (80 mg) was weighed into a 10-ml volumetric flask and ethanol (100 μl) and pyridine (20 μl) were added. After 10 min the solution was evaporated to dryness and the residue diluted to volume with an internal standard solution.

Internal standard solution preparation. *p*-Chloroacetophenone (1.85 g) was dissolved in 1 l of chloroform.

GLC calibration. Calibration solutions of *o*-chloroethyl benzoate (0.6–2.8 mg/ml) and *m*-chloroethyl benzoate (0.3–1.4 mg/ml) were prepared in the internal standard solution. The ratios of the chloroethyl benzoate peaks to the *p*-chloro-

acetophenone peak were measured and the chloroethyl benzoate concentrations determined from a calibration graph.

GLC conditions. A 3 ft. × 4 mm I.D. glass column was packed with a mixture of 10% Bentone 34 and 8% diisooctyl phthalate on Gas-Chrom Q (100–120 mesh). The column temperature was maintained at 170° and the nitrogen carrier gas at a flow-rate of 50 ml/min.

Determination of p-chlorobenzoyl chloride

p-Chlorobenzoyl chloride was determined following a similar ethanolysis by GLC using only 10 mg of sample and *p*-chloroanisole (0.75 mg/ml) as the internal standard. Calibration solutions of *p*-chloroethyl benzoate were prepared over the concentration range 0.2–1.2 mg/ml.

Determination of further impurities in p-chlorobenzoyl chloride

Sample preparation. The above procedure allows determination of benzoyl chloride as ethyl benzoate. *p*-Chlorobenzoic acid was determined by GLC as its corresponding methyl ester after reaction with an excess of ethereal diazomethane. Other volatile impurities were analyzed directly by GLC.

GLC conditions. All these impurities were analysed on a 6 ft. × 4 mm I.D. glass column packed with 2% 1,4-butanediol succinate on Gas-Chrom Q (100–120 mesh). A column temperature of 110° and a nitrogen carrier gas flow-rate of 55 ml/min were used.

Determination of isomeric impurities in 2-(p-nitrophenyl)-propionitrile (III)

Sample preparation. Compound III was prepared by the reaction of fuming nitric acid on II and was purified by fractional crystallisation from toluene–petroleum ether (b.p. 60–80°) (1:1). As local concentrations of *o*- and *m*-nitro isomers were obtained, the recrystallised product was ground and quartered, to give a representative sample. The sample was dissolved in methylene chloride (1 mg/ml) for GLC analysis.

Calculation. As pure standards of the *meta* and *ortho* isomers were unavailable, the levels of these impurities in III were determined by peak area ratio measurements assuming a similar flame ionisation detector response for all isomers.

GLC conditions. Samples were also analysed on the 2% 1,4-butanediol succinate column. A column temperature of 215° and a nitrogen carrier gas flow-rate of 45 ml/min were chosen as optimum conditions.

Determination of an isomeric impurity in 2-phenylpropionitrile (II)

Sample preparation. Compound II was prepared by the reaction of sodium cyanide with 1-chloroethylbenzene in dimethylformamide at 80°. Small aliquots of subsequent distillation fractions were diluted into dichloromethane (1 mg/ml) for GLC analysis.

Calculation. As the assays were designed to give the relative levels of the individual components results were calculated on a peak area normalisation basis.

GLC conditions. The 1,4-butanediol succinate column was again used with a nitrogen carrier gas flow-rate of 45 ml/min and the oven programmed from a temperature of 90° to 150° at 8°/min.

DISCUSSION AND RESULTS

Analysis of 4-chlorobenzoyl chloride

Selection of GLC conditions. As acid chlorides are readily hydrolysed, the preferred method of analysis requires conversion of the acid chloride to either the corresponding free acid or the ester. *o*-Chlorobenzoic acid can be separated from *m*- and *p*-chlorobenzoic acids by thin-layer chromatography (TLC)[3] but the latter two could not be separated from one another. Conversion of the isomeric chlorobenzoyl chlorides to their ethyl esters followed by GLC analysis enables facile quantitation. Using conventional liquid phases, the ethyl esters are eluted according to their volatility, the close-boiling *m*- and *p*-chloroethyl benzoates not being resolved. Columns of Bentone 34, an organic clay, have previously been used to separate isomeric aromatic compounds[4]. Modification of its highly adsorptive nature with diisooctyl phthalate produced a phase ideal for the separation of isomeric chloroethyl benzoates (Fig. 2). An optimum loading of 10% Bentone 34 and 8% diisooctyl phthalate was chosen. Increasing the Bentone 34 loading increased the separation but caused tailing. As with a comparable separation of chlorostyrenes[5], an elution order of *p*-, *m*- and finally *o*-isomer was observed.

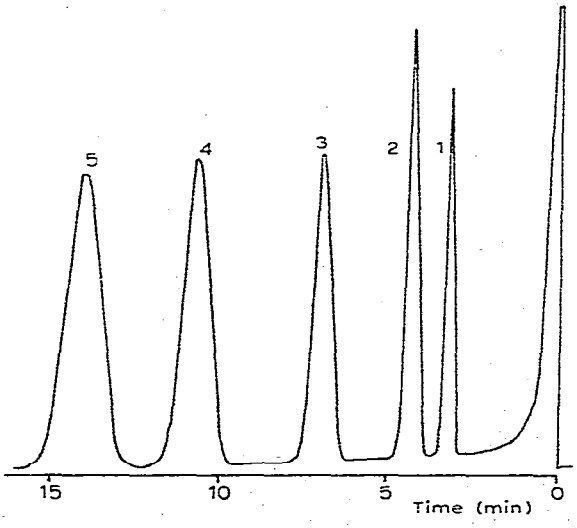


Fig. 2. GLC separation of chloroethyl benzoate isomers. Column, glass, 3 ft. × 4 mm I.D., packed with 10% Bentone 34–8% diisooctyl phthalate on Gas-Chrom Q (100–120 mesh); column temperature, 170°; nitrogen flow-rate, 50 ml/min. 1 = *p*-Chloroanisole; 2 = *p*-chloroethyl benzoate; 3 = *m*-chloroethyl benzoate; 4 = *p*-chloroacetophenone; 5 = *o*-chloroethyl benzoate.

Determination of purity. The purity of *p*-chlorobenzoyl chloride varied markedly from one supplier to another as did the level of isomeric impurities (Table I). Only samples of *p*-chlorobenzoyl chloride with a purity of >90% and an isomeric impurity content of <0.5% were used in the synthesis. Both GLC data and the estimation of the total chloride content by a titration method indicated that samples of *p*-chlorobenzoyl chloride contained additional impurities. All the samples contained significant

TABLE I

COMPOSITION OF *p*-CHLOROBENZOYL CHLORIDE SAMPLES (% w/w)

Batch No.	*Chlorobenzoyl chloride*			*p-Chloro-benzoic acid*	*Benzoyl chloride*	*Total chloride*
	para	*meta*	*ortho*			
1	92	<0.1	0.2	3.2	<0.1	40.1
2*	93	0.6	0.5	1.3	<0.1	39.6
3	91	<0.1	0.5	0.3	<0.1	39.5
4	93**	<0.1	0.2	2.4	<0.1	40.95

* Rejected due to high isomer content.
** Coefficient of variation, 0.4%.

levels of *p*-chlorobenzoic acid, but no detectable levels of benzoyl chloride. Both these latter two compounds were determined by derivatisation procedures, whereas direct GLC–MS indicated the presence of two further impurities *p*-chlorobenzaldehyde [*m/e* 140 (M^+), 139 $(M - H)^+$, 111 $(M - CHO)^+$] and *p*-chlorobenzal chloride [*m/e* 194 $(M)^+$, 159 $(M - 35)^+$], and traces of several unidentified impurities.

Analysis of 2-(p-nitrophenyl)-propionitrile (III)

Selection of GLC conditions. Versamid 930 and 1,4-butanediol succinate gave satisfactory separation of the nitrophenylpropionitrile isomers (Fig. 3). As the *meta–para* isomer separation deteriorated with use of the Versamid column, the analysis

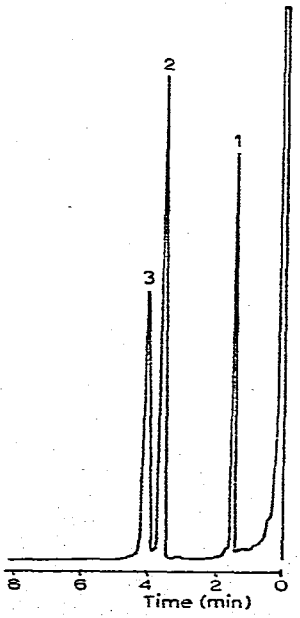


Fig. 3. GLC separation of 2-nitrophenylpropionitrile isomers. Column, glass, 6 ft. × 4 mm I.D., packed with 2% 1,4-butanediol succinate on Gas-Chrom Q (100–120 mesh); column temperature, 215°; nitrogen flow-rate, 45 ml/min. 1 = 2-(*o*-Nitrophenyl)-propionitrile; 2 = 2-(*m*-nitrophenyl)-propionitrile; 3 = 2-(*p*-nitrophenyl)-propionitrile.

was transfered to a 1,4-butanediol succinate column. Optimum separation was obtained at 215°.

Determination of isomer content. GLC analysis supported by TLC indicated that the isomeric nitro compounds were the main impurities in III. In Table II the improvement in purity between samples isolated from the reaction and samples after final purification is shown. The limit of detection for the impurities was 0.1%. Fractional crystallisation normally reduced the level of isomeric impurity to below a desirable 1%.

TABLE II

ISOMERIC COMPOSITION OF TYPICAL 2-(*p*-NITROPHENYL)-PROPIONITRILE SAMPLES (% w/w)

Stage of processing	*ortho*		*meta*		*para*	
	Run 1	*Run 2*	*Run 1*	*Run 2*	*Run 1*	*Run 2*
Reaction mixture	6	8	17	18	78	74
Crude solid product	1	0.5	16	12.5	83	87
First crystallisation	0.5	<0.1	7	5.5	93	94.5
Second crystallisation	<0.1	<0.1	0.5	1.8	>99	98

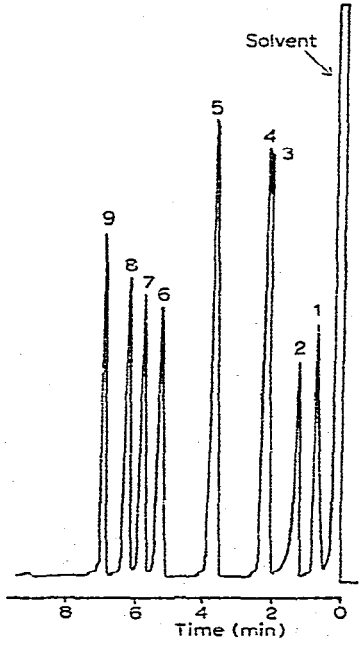


Fig. 4. GLC separation of impurities present in the conversion of 2-phenylpropionitrile to 2-(*p*-nitrophenyl)-propionitrile. Column, glass, 6 ft. × 4 mm I.D., packed with 2% 1,4-butanediol succinate on Gas-Chrom Q (100–120 mesh); temperature programmed from 90° to 150° at 8°/min; nitrogen flow-rate, 45 ml/min. 1 = Styrene (relative retention, 0.12); 2 = dimethylformamide (0.20, retention time depends on sample concentration); 3 = 2-phenylethyl chloride (0.33); 4 = 1-phenylethyl chloride (0.36); 5 = acetophenone (0.60); 6 = 1-phenylethanol (0.85); 7 = 2-phenylethanol (0.94); 8 = 1-phenylpropionitrile (1.00); 9 = 3-phenylpropionitrile (1.12).

TABLE III

COMPOSITION OF SAMPLES AFTER DISTILLATION OF A TYPICAL 2-PHENYLPROPIONITRILE REACTION MIXTURE (% w/w)

Distillation fraction			*Styrene*	*Dimethyl formamide*	*1-Chloroethyl-benzene*	*Aceto-phenone*	*1-Phenyl-ethanol*	*Phenylpropionitrile*	
No.	*Temperature range (°C) at 30 mm Hg*	*Weight (g)*						*2-Isomer*	*3-Isomer*
1	60– 86	14	38	30	2	4	5	15	<0.1
2	86–122	163	1	2	3	8	25	60	<0.1
3	122–125	280	<0.1	<0.1	<0.1	3	13	84	<0.1
4	125–125	620	<0.1	<0.1	<0.1	0.5	2	97	<0.1
5	125–126	1015	<0.1	<0.1	<0.1	<0.1	0.5	>99	<0.1
6	126–128	118	<0.1	<0.1	<0.1	<0.1	<0.1	>99	<0.1

Analysis of 2-phenylpropionitrile (II)

Selection of GLC conditions. A separation of all potential impurities in fractional distillates of crude II is shown in Fig. 4. Facile separation of 3-phenylpropionitrile and II is achieved on a 1,4-butanediol succinate column, whereas separation of 1-chloroethylbenzene and 2-chloroethylbenzene is best achieved on a cyanoalkyl silicone column when the former separation is lost.

Determination of isomer content. Table III illustrates the composition of a set of typical distillation samples. Fractions containing greater than 98% of II were progressed through the synthesis. No levels of 3-phenylpropionitrile were found above the 0.1% detection level. As expected on theoretical grounds[6], lower reaction temperatures produced increased amounts of the required substitution product, temperatures above 90° giving significant amounts of styrene.

Analysis of compound I

As a result of satisfactory control of the levels of the isomeric impurities in II, III, and *p*-chlorobenzoyl chloride, no significant levels of isomer impurities were observed in benoxaprofen[2].

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