Preparation of 2-Iodomorphine for use in Radioimmunoassay

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SUMMARY

A method is described for the preparation of ^{125}iodomorphine. The structure of this compound was confirmed by synthesis of ^{127}iodomorphine and subsequent mass spectral studies. ^{125}Iodomorphine has proved to be chemically very stable and has been shown to be bound avidly by a morphine antiserum. It should, therefore, prove to be very useful for the development of a morphine radioimmunoassay.

KEY WORDS: MORPHINE RADIOIMMUNOASSAY 2-IODOMORPHINE

INTRODUCTION

There are two immunoassay systems currently in use in forensic toxicology for detection of opiates in biological fluids. These are the Roche Abuscreen opiate RIA and the Syva Emit DAU assay. Both employ antibodies of broad specificity which will cross-react with morphine, codeine and their metabolites. A study of these cross-reactions (1) has revealed that modifications to the 3- or 6-hydroxy functions of morphine did not result in a loss of cross-reactivity with either antiserum.

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The chloramine-T reaction as devised by Hunter and Greenwood (2) enjoys widespread use for the introduction of iodine into proteins containing tyrosine residues, or more specifically into compounds with a phenolic group. Morphine has such a function, and it was postulated that iodination would be possible using the above technique. Also it was predicted that iodinated morphine would be bound by the broadly-specific antisera. Before proceeding to the synthesis of ^{125}iodomorphine, an attempt was made to synthesise ^{127}iodomorphine to determine its binding characteristics.

EXPERIMENTAL

Materials

Sodium ^{127}iodide, sodium metabi sulphite, potassium bromide, potassium dihydrogen phosphate, chloramine-T (sodium toluene-p-sulphonchloroamide), potassium iodide, iso-propanol, methanol and chloroform, all of Analar grade, were obtained from B.D.H. Chemicals Ltd., Poole, Dorset. Sodium ^{125}iodide (614MBq/μg) was obtained from the Radiochemical Centre, Amersham, Bucks.

Equipment

High-performance liquid chromatography (HPLC) was carried out with a Waters 6000A HPLC pump (Waters Assoc., Milford, Mass., U.S.A.) which was used to deliver an eluent of 25% methanol in aqueous potassium bromide (0.05M) and potassium hydrogen phosphate (0.01M) to a stainless steel column (10cm x 4.6mm i.d.) packed with Hypersil 5-ODS (Phase Separations Ltd., Clwyd). Samples were injected with a Rheodyne 7120 injection valve (Phase Separations Ltd). Column eluate was monitored at 285nm with a Cecil Instruments 212 ultraviolet monitor (Cecil Instruments, Cambridge).

Phase separation filter papers were obtained from Whatman Ltd., Maidstone, Kent.

Gama-counting was carried out in a NE8311 counter (Nuclear Enterprises Ltd., Beenham, Berks) which had an efficiency of approximately 50% for ^{125}iodine. Low resolution mass spectra were obtained using a VG-Micromass 16F mass spectrometer (VG Micromass, Altrincham, Cheshire) fitted with a single stage jet separator. The following conditions were used: emission, 100μA; electron energy, 70eV; source temperature, 200°C. Data were collected using a VG2250 Data System with the mass spectrometer scanning at 3 secs per decade. High resolution mass measurements were obtained using a Kratos MS50 mass spectrometer and DS50 Data System (Kratos, Manchester). Accurate mass measurements were made under dynamic conditions scanning at 10 secs per decade and were averaged over ten spectra. The following conditions were used: emission, 200μA; electron energy, 70eV; source temperature, 200°C.

METHODS AND RESULTS

(i) Preparation and purification of ^{127}iodomorphine

Preliminary studies of reaction conditions indicated that morphine was extremely susceptible to oxidation by chloramine-T under the conditions normally used for iodination of proteins. It was, therefore, decided to use a large excess of morphine and a relatively low concentration of chloramine-T for the reaction.

A solution of morphine hydrochloride (5ml, 100mg/ml) in 50% aqueous methanol was mixed with aqueous sodium ^{127}iodide solution (2.5ml, 90mg/ml). Chloramine-T (140mg/ml) in phosphate buffer (pH7.4, 5 ml, 0.5M) was then added and mixed immediately.

After 60 seconds the reaction was stopped with sodium metabisulphite solution (5ml, 140mg/ml). The pH was adjusted to 8.6 with sodium hydroxide solution (40%) and the mixture extracted with five volumes of isopropanol in chloroform (25%). The organic phase was removed and the aqueous phase further extracted with the isopropanol:chloroform mixture. The combined organic extracts were then washed with distilled water (10ml), dried with anhydrous sodium sulphate and evaporated to dryness with nitrogen at 40°C.

It proved impossible to achieve a complete separation of morphine from the reaction product (2-iodomorphine) by the use of a number of thin-layer chromatography systems. A partial separation was possible using a bicarbonate buffered silica gel plate run in chloroform:methanol (3:1). This was used preparatively to reduce the bulk of morphine present in the crude product.

To complete the purification, an HPLC system based on that described by Nelson et al. (1) was employed. This resulted in a very good separation of morphine and iodomorphine, retention volumes being 1.6ml and 8.2ml respectively. By collecting fractions 7.5ml-9.0ml from repeated HPLC separations it was possible to process a useful amount of the reaction product. The collected fractions were combined and extracted with isopropanol in chloroform (25%) after adjustment of the pH to 8.6. The organic extract was dried with anhydrous sodium sulphate and evaporated to dryness with nitrogen at 40°C. The residue was weighed and redissolved in methanol to give a concentration of 1mg/ml. Conversion of morphine to iodomorphine was approximately 15%.

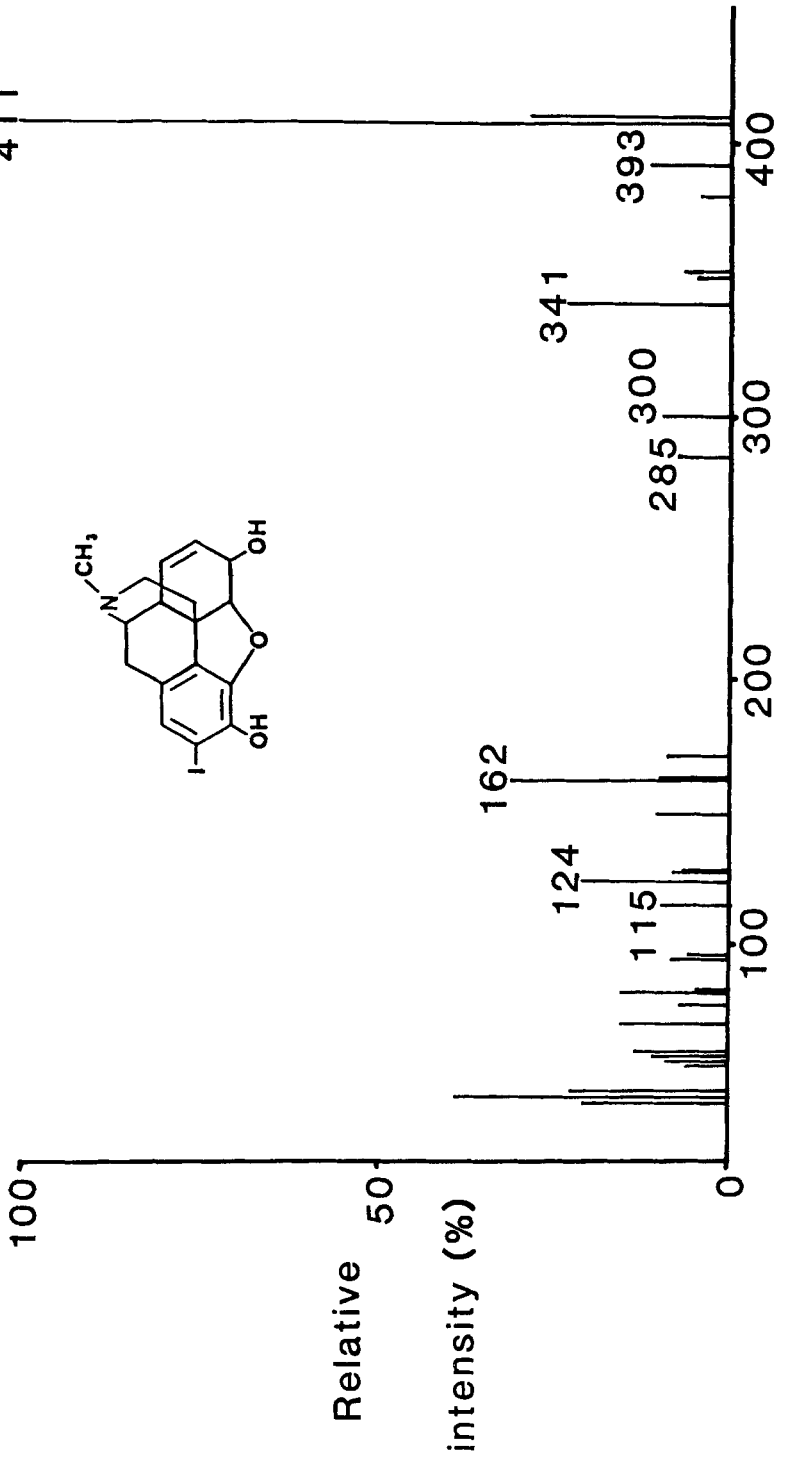

Figure 1 Mass spectrum and molecular structure of 2-^{127}iodomorphine

The low resolution mass spectrum of the synthesised iodomorphine is shown in Figure 1, the base peak being the molecular ion at m/z 411 (high resolution mass spectrometry gave 411.0327, $C_{17}H_{18}NO_3I$ requires 411.0329). The mass spectral breakdown closely parallels that of morphine (3). The major fragment ions measured by high resolution mass spectrometry are shown in Table 1. Additional ions are observed at m/z 127 and 128 due to I and HI respectively.

TABLE 1

MAJOR FRAGMENTED IONS OF ^{127}IODOMORPHINE

Measured m/z	Loss	Formula	Calculated m/z
393.0218	H_2O	$C_{17}H_{16}NO_2I$	393.0224
340.9895	$-C_4H_6O$	$C_{13}H_{12}NO_2I$	340.9912
299.9644	$-C_6H_9NO$	$C_{11}H_9O_2I$	299.9647
162.0903		$C_{10}H_{12}NO$	162.0916
124.0765		$C_7H_{10}NO$	124.0760
115.0547		C_9H_7	115.0546

When the identity of the product had been confirmed by the above studies, its cross-reactivity with the Syva opiate antiserum was tested. It was found that ^{127}iodomorphine was avidly bound; 35ng/ml of iodomorphine having equivalent binding to 20ng/ml of morphine.

(ii) Preparation of ^{125}iodomorphine

The reaction conditions as outlined above were scaled down for use with 67ng (37MBq) of sodium ^{125}iodide. Morphine hydrochloride (10µl, 200µg/ml in 0.25M phosphate buffer pH7.4) was added to sodium ^{125}iodide solution (10µl, 37MBq), in a screw-capped minivial as supplied by the Radiochemical Centre. Freshly-prepared chloramine-T solution (10µl, 80µg/ml) in the

same buffer, was then added. The vial was capped, the contents thoroughly mixed by vortexing and the reaction allowed to proceed for 40 seconds. The reaction was stopped with sodium metabisulphite solution (20µl, 80µg/ml), containing potassium iodide (10mg/ml) as carrier and the pH adjusted to 8.6 by addition of borate buffer (200µl). The mixture was shaken vigorously with isopropanol in chloroform (2ml of 25%) and passed through a phase separation filter paper, which had been pre-soaked with the solvents. The vial was washed with further solvent (2 x 1ml) which was also passed through the filter. The organic filtrate was then dried with nitrogen at room temperature and redissolved in ethanol (6mls). Assessment of activity in a gamma-counter indicated that approximately 60% of the available ^{125}I had been incorporated into the morphine. The specific activity, determined by the method of Morris (4) was found to be 5.3TBq/mmol (16MBq/µg).

For radioimmunoassay purposes, the preparation was found to be perfectly adequate without further purification. Stored in ethanol at 4^{o}C, at the above concentration, it has proved to be chemically very stable.

DISCUSSION

Iodination of morphine by the chloramine-T method has been reported by Davis et al. (5). However, these workers claimed that although it was possible to synthesise ^{125}iodomorphine they had no success in synthesising ^{127}iodomorphine. They attributed this to a radiolytic reaction involving ^{125}iodine which would not be seen with the natural isotope. Although the reaction yield of ^{127}iodomorphine in this work was low, this was deliberate; a large excess of morphine being

used to avoid oxidative losses caused by chloramine-T. The lack of success of Davis et al., can probably be attributed to oxidation by chloramine-T causing a very low yield, which can only be detected when a radioisotope is used.

The radioiodination procedure described here has been carried out a number of times and is both rapid and safe to perform. The total reaction and extraction sequence takes 45 minutes from addition of chloramine-T to assessment of radioactivity in the product. All reaction and extraction stages take place in one sealed tube. The only other equipment involved, a filter funnel and a phase-separation filter paper, are readily disposable and present no hazards.

The product obtained is a mixture of ^{125}iodomorphine and morphine. The presence of unlabelled morphine obviously causes a large reduction in the specific activity. However, pure ^{125}iodomorphine would have a specific activity too great for use in a radioimmunoassay and so would have to be diluted by addition of unlabelled iodomorphine or morphine. For this reason, further purification was not needed. If desired, the HPLC system as described for preparation of ^{127}iodomorphine could easily be used for this purpose.

Radio-labelled iodomorphine, produced by the method described above, has formed the basis for the development of a very cheap and simple radioimmunoassay for the detection of opiates in biological fluids submitted for forensic analysis.

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