3-[^{125}I] Iodo-4-hydroxyphenobarbitone for use in Radioimmunoassay

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SUMMARY

A method is described for the preparation of a barbiturate derivative, 3-iodo-4-hydroxyphenobarbitone, labelled with [^{125}I]iodine. The structure of the compound was confirmed by synthesis and purification of the [^{127}I]iodine derivative followed by mass spectral studies. The [^{125}I]iodine labelled barbiturate has proved to be chemically stable and has been shown to bind to a barbiturate antiserum. It should, therefore, prove to be very useful for the development of a radioimmunoassay for barbiturates.

KEY WORDS: BARBITURATES RADIOIMMUNOASSAY
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INTRODUCTION

There are two basic requirements for the development of a radioimmunoassay (RIA) for a particular drug: an antiserum against the drug molecule and a radiolabelled drug derivative which will bind to the antiserum. $[^{125}I]$Iodine is the radiolabel most suited to RIA for use in forensic toxicology. $[^{125}I]$Iodine labelled barbiturate derivatives are not available commercially. However, a barbiturate RIA kit which is based on such a derivative has been developed (1, 2) and marketed by Hoffman-LaRoche, INC., under the trade name 'Barbiturate Abuscreen'. No details are available in the scientific literature concerning the synthesis or exact identity of their $[^{125}I]$iodine labelled barbiturate derivative.

One of the simplest methods for labelling compounds with $[^{125}I]$iodine is the chloramine-T reaction developed by Hunter and Greenwood (3). This technique depends on the presence of a phenolic group in the drug molecule in question. The major metabolite of phenobarbitone, 4-hydroxyphenobarbitone has a phenolic group and it should be possible to label this compound with $[^{125}I]$iodine by means of the chloramine-T reaction.

From the study by Law and Moffat (4), it was postulated that iodine-labelled 4-hydroxyphenobarbitone would be bound by the antiserum from a Barbiturate Emit DAU kit (Syva (UK) Ltd). This would then form the basis for development of a RIA for barbiturates.

EXPERIMENTAL

Materials and Equipment

Sodium [^{127}I]iodide, sodium metabisulphite, potassium bromide, potassium dihydrogen phosphate, chloramine-T (sodium toluene-*p*-sulphonchloroamide-trihydrate), potassium iodide, iso-propanol, methanol and chloroform, all of Analar grade were obtained from BDH Chemicals Ltd., Poole, Dorset. Sodium [^{125}I]iodide(614MBq/μg) was obtained from Amersham International Limited, Amersham, Bucks. 5-Ethyl-5-(4-hydroxyphenyl)-barbituric acid (4-OHPB) was obtained from Sigma Chemical Co., Poole, Dorset. High-performance liquid chromatography (HPLC) was carried out with a Waters 6000A HPLC pump (Waters Assoc., Northwich, Cheshire) which was used to deliver an eluent of 40% methanol in phosphate buffer (0.1M, pH 6.0) at a rate of 2ml/min to a stainless steel column (10cm x 5mm 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 232nm with a Cecil Instruments 212 ultra-violet monitor (Cecil Instruments Ltd., Cambridge) and collected using a 2112 Redirac Fraction Collector (LKB Instruments Ltd., South Croydon, Surrey).

Antiserum was obtained either from a Barbiturate Emit DAU kit (Syva (UK) Ltd., Maidenhead, Berks) or from Miles Laboratories Ltd., Slough, Bucks.

Phase separation filter papers were obtained from Whatman Ltd., Maidstone, Kent. Gamma-counting was carried out in a NE8311 counter (Nuclear Enterprises Ltd., Beenham, Berks) which had an efficiency of approximately 50% for [^{125}I]iodine.

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 VG 2250 Data System with the mass spectrometer scanning at 3 secs per decade.

Method

A solution of 4-OHPB (10μl, 100μg/ml) in ethanol was added to Na[^{125}I] solution (10μl, 37MBq) in a 10ml silanised glass centrifuge tube. Freshly-prepared chloramine-T solution, (10μl, 80μg/ml) in phosphate buffer (0.25M, pH 7.4) was then added. The tube was stoppered, 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 readjusted by addition of phosphate buffer (0.25ml, pH 7.4). The mixture was shaken vigorously with chloroform (2.5ml) and passed through a phase separation filter paper, which had been pre-soaked in chloroform. The tube 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 (6ml).

In order to carry out mass spectroscopy on the reaction product, which would be potentially hazardous if [^{125}I]iodine were used, [^{127}I]iodine was used to prepare a larger amount of non-reactive iodinated derivative. The reaction conditions

described above were, therefore, scaled up for use with 20mg 4-OHPB and sodium [^{127}I]iodide. The product of this reaction was purified by HPLC and a yield of approximately 24% was obtained.

RESULTS AND DISCUSSIONS

As with morphine (5) it was found that 4-OHPB was extremely susceptible to oxidation by chloramine-T. It was necessary to use an excess of the drug derivative and a relatively low concentration of chloramine-T for the reaction.

HPLC analysis of the crude product obtained from the reaction using sodium [^{127}I]iodide, produced the chromatogram shown in Figure 1. The main reaction products were mono- and di-iodinated 4-OHPB. Some unreacted 4-OHPB and a small amount of a further compound, thought to be its oxidation product were also present. The products were identified by mass spectroscopy; the mass spectrum of mono-iodinated 4-OHPB is shown in Figure 2.

HPLC analysis of the product from the reaction involving sodium [^{125}I]iodide showed that 89% of the radioactivity was associated with mono-iodinated 4-OHPB. This is in marked contrast to the reaction with sodium [^{127}I]iodide (Figure 1) and is thought to be due to the difference in chemical scale between the two reactions.

For use in RIA the crude material from the sodium [^{125}I] iodide reaction was found to be quite adequate since it bound avidly to both the Emit antiserum and a more specific anti-phenobarbitone serum obtained from Miles Laboratories Ltd.

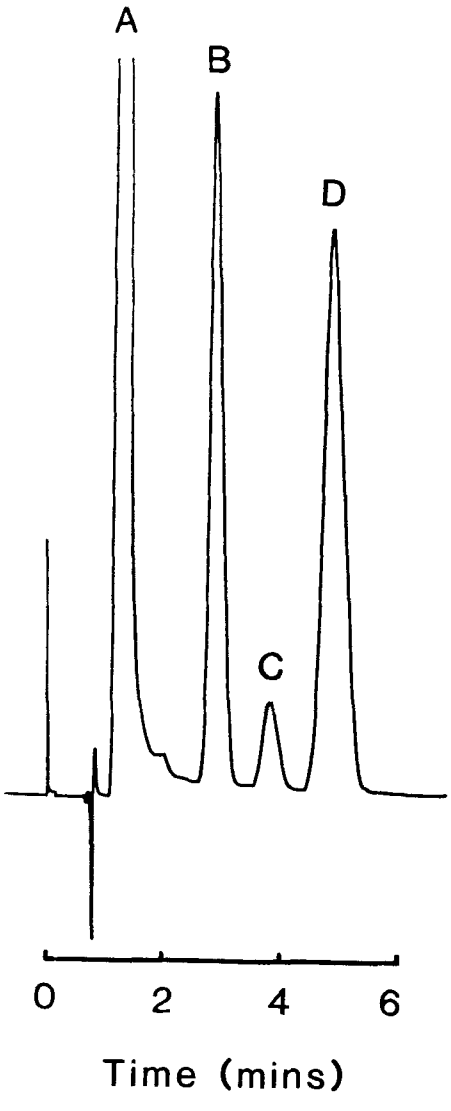

Figure 1. HPLC of products of reaction of 4-hydroxyphenobarbitone and sodium $[^{127}I]$iodide.

A = residual 4-hydroxyphenobarbitone

B = mono-iodo-4-hydroxyphenobarbitone

C = oxidation product of 4-hydroxyphenobarbitone

D = di-iodo-4-hydroxyphenobarbitone

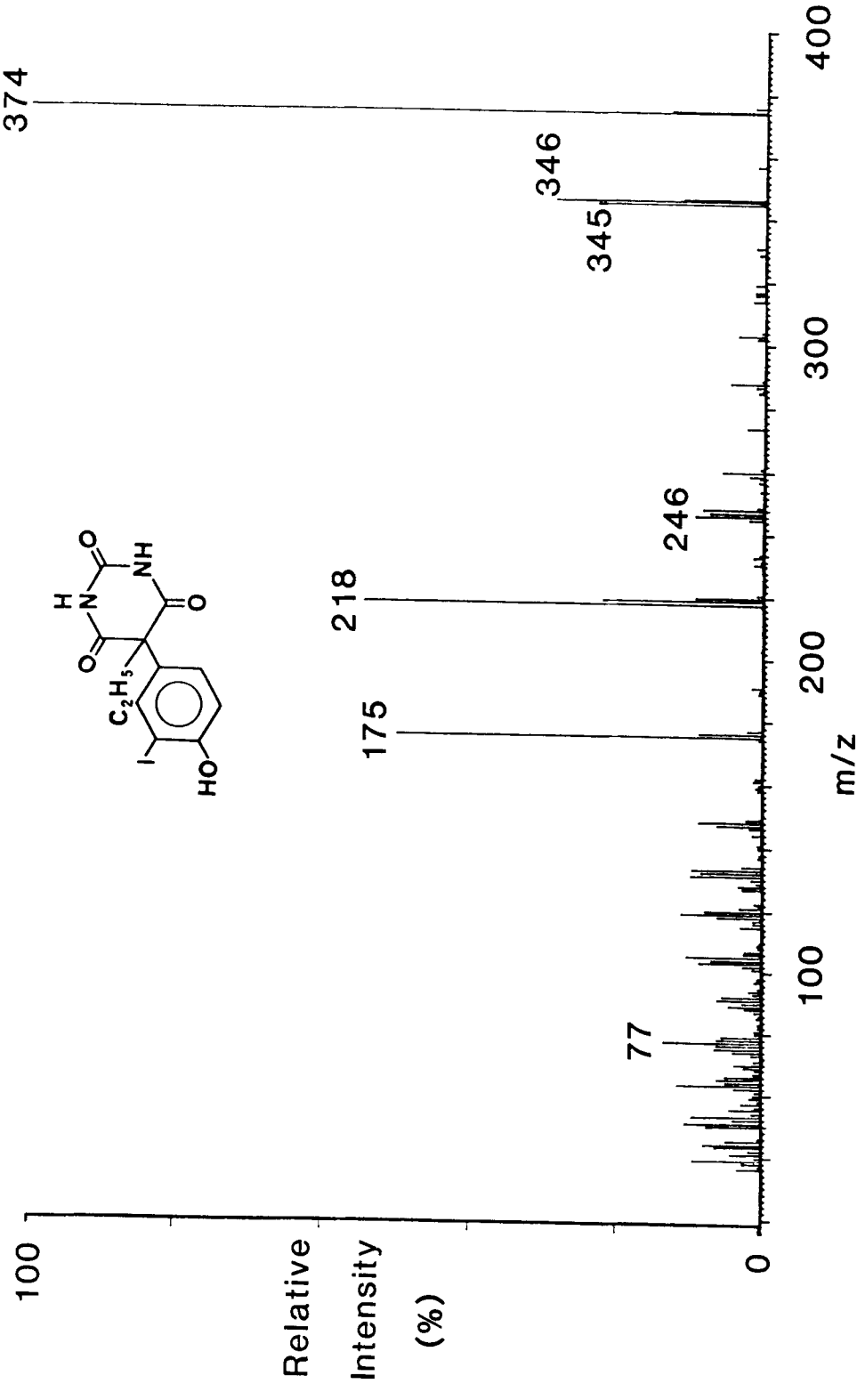

Figure 2. Mass spectrum and molecular formula of 5-ethyl-5-(3-$\left[^{127}I\right]$iodo-4-hydroxyphenyl)-barbituric acid

If required, the HPLC system described can be used to obtain purified mono-iodinated 4-OHPB.

Assessment of activity in a gamma-counter indicated that approximately 23% of the available $[^{125}I]$iodine had been incorporated into the crude reaction product. Its activity, determined by the method of Morris (6), using mono $[^{127}I]$iodo 4-OHPB as reference standard, was found to be 1.56TBq/mmol (4.18MBq/µg).

Used in combination with either of the antisera described, 3-$[^{125}I]$iodo-4-hydroxyphenobarbitone should form the basis for development of a RIA system for barbiturates.

REFERENCES

1. Flynn E.J. and Spector S. - J. Pharm. Exper. Therap. 181:547 (1972).
2. Cleeland R., Davis R., Heveran J. and Grunberg E. - J. Forens. Sci. 20:45 (1975).
3. Hunter W.M. and Greenwood F.C. - Nature (Lond) 194:495 (1962).
4. Law B. and Moffat A.C. - J. Forens. Sci. Soc. 21:55 (1981).
5. Mason P.A., Law B. and Ardrey R.E. - J. Label. Compds. (in press).
6. Morris B.J. - Clin. Chim. Acta. 73:213 (1976).