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The monochloroacetylation of oestrogens prior to gas–liquid chromatography with electron capture detection

Electron capture detection of oestrogens measured by gas–liquid chromatography (GLC) requires the formation of suitable derivatives. Lists of electron-capturing groups have been given by LOVELOCK[1] and CLEMONS AND ALTSHULLER[2]. Oestrogen derivatives which have been used include monochloroacetates[3,4], heptafluorobutyrates[5–8], pentafluorophenylhydrazones[4,9,10], halomethyldimethylsilyl ethers[11], 2-(iodomethyldimethylsiloxy)propyl ethers[11], perfluoro-3,5,7,8-tetrachloro-octoic esters[11], halobenzenesulphonyl esters[11], 2:2, 3:3, 4:4 hexahalo-5-oxo-thianonates[11] and 3,4,5-trihalobenzoyl esters[11]. The heptafluorobutyrates are unstable on silica gel layers[6,11], as are phenolic halomethyldimethylsilyl ethers[11], and pentafluorophenylhydrazones[10].

Oestrogen *p*-bromobenzoates have been prepared but have exceptionally long retention times on GLC and attempts to prepare tri-trifluoroacetyl thallium derivatives failed. An unsuccessful attempt was also made to react oestrone simultaneously with acetic anhydride and pentafluorophenylhydrazine (on the basis that each reagent would catalyse the reaction between the steroid and the other reagent).

Provided microgram quantities of the oestrogen are present in the sample, monochloroacetyl derivatives may be readily prepared. But with nanogram quantities poor yields were obtained, so an investigation into the kinetics of the reaction has been made.

Materials and methods

The relative proportions of products from monochloroacetylation of [6,7-^{3}H]-oestrone (6.88 Ci/mmole) and 17β-[6,7-^{3}H]oestradiol (32.7 Ci/mmole) (The Radiochemical Centre, Amersham) were estimated. Thin-layer chromatograms of the reaction mixture on Silica Gel GF_{254} were developed with benzene–ethyl acetate (6:1) (Table I). The distribution of the radioactivity was measured using a Tracerlab 2π radiochromatogram scanner. Solvents were purified according to BUSH[12], GOODSPEED AND MILLSON[13] and AAKVAAG AND EIK-NES[14] (tetrahydrofuran). Glassware was soaked and washed with detergent, chromic acid, ethanolic potassium hydroxide, tap water and distilled water and oven dried before use. Nitrogen, used for solvent evaporation was washed with toluene and concentrated sulphuric acid.

The effects of the monochloroacetic anhydride concentration on the monochloroacetylation was tested by adding 0.5-ml volumes of 10, 20, 30 or 50 mg/ml solutions in tetrahydrofuran to 8-ng samples of tritiated oestrone or 2-ng samples of tritiated 17β-oestradiol dried in stoppered test tubes. To each mixture was also added 0.1 ml of pyridine and about 5 mg of anhydrous sodium sulphate to remove any water present. The tubes were incubated for 45 min in an oven at 60° and the volumes then reduced to about 0.1 ml under nitrogen. The mixtures were applied directly to thin-layer chromatographic (TLC) plates and the tubes rinsed with 2 × 0.1 ml of chloroform–methanol (4:1), the washings being applied to the plate. After development and radioactivity scanning the relative proportions of the radioactivity in the different spots were calculated. The experiment was repeated except that, together with the

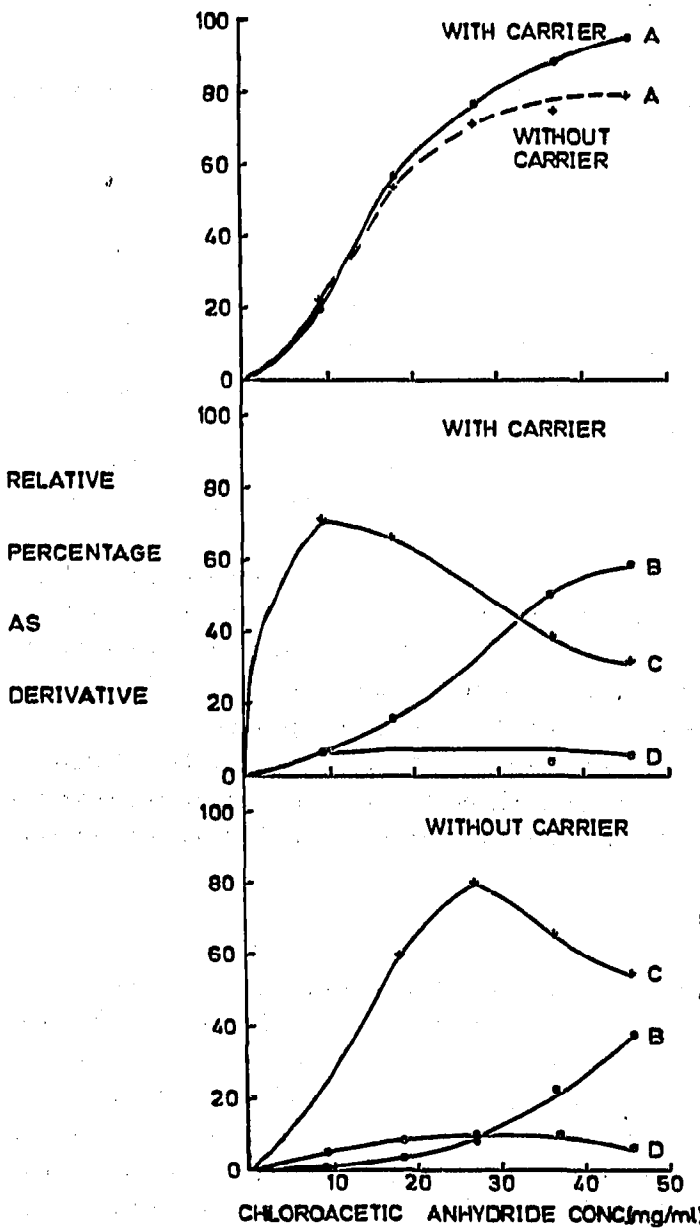

Fig. 1

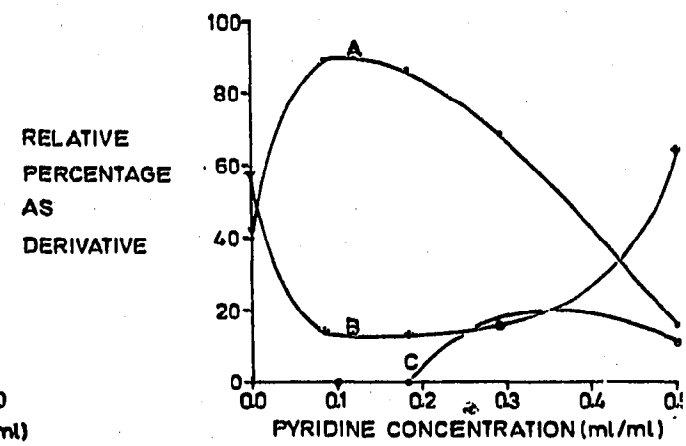

Fig. 2

Fig. 1. Effect of the monochloroacetic anhydride concentration on monochloroacetylation. A = Oestrone monochloroacetate; B = oestradiol-3,17β-dimonochloroacetate; C = 17β-oestradiol-17β-monochloroacetate?; D = 17β-oestradiol-3-monochloroacetate?

Fig. 2. Effect of the pyridine concentration on the monochloroacetylation of 17β-oestradiol. A = Oestradiol-3,17β-dimonochloroacetate, B = 17β-oestradiol-3-monochloroacetate?, C = 17β-oestradiol-17β-monochloroacetate?

tritiated oestrogen, 50 μg of the respective non-radioactive carrier oestrogen was present. The experiment was done in duplicate and results are shown in Fig. 1.

The effect of pyridine concentration on monochloroacetylation was tested by adding different amounts to the reaction mixture of carrier-free oestrogen, monochloroacetic anhydride (0.5 ml, 50 mg/ml in tetrahydrofuran) and anhydrous sodium sulphate (5 mg). Volumes of 0.00, 0.05, 0.10, 0.20 and 0.50 ml pyridine were added, the mixtures incubated for 1 h and the products separated and measured as before.

The oestrone was completely monochloroacetylated in every case and the results of the monochloroacetylation of 17β-oestradiol are shown in Fig. 2.

To find the incubation time required for complete monochloroacetylation of nanogram amounts of carrier-free tritiated oestrogens with 0.5 ml of 50 mg/ml monochloroacetic anhydride in tetrahydrofuran, 5 mg of sodium sulphate and 0.09 ml of pyridine were incubated for 60, 120 or 180 min and the products measured as before. The oestrone was fully monochloroacetylated after 60 (and after 120 and 180) min. The 17β-oestradiol was 89.3% di-monochloroacetylated after 60 min (4.5% being 17β-monochloroacetate and 6.2% being 3-monochloroacetate) and completely di-monochloroacetylated after 120 (and 180) min.

Discussion

The monochloroacetic anhydride concentrations required here were higher than those used by BROWNE *et al.*[15], VAN DER MOLEN AND GROEN[16], and EIK-NES *et al.*[3]. The higher monochloroacetic anhydride concentrations are needed for reaction with the phenolic group. The assignation of positions to the mono-derivatives of 17β-oestradiol is based on the assumption that monochloroacetylation of the phenol group will have a greater effect on the steroids mobility than monochloroacetylation of the hydroxyl group (R_F values are shown in Table I). A pyridine concentration of over 0.1 ml/ml reduced the yield of the reaction and reaction times over 2 h had no advantage—small amounts of steroid may be destroyed by prolonged heating in this environment.

TABLE I

MOBILITIES ON THIN-LAYER CHROMATOGRAMS DEVELOPED WITH BENZENE–ETHYL ACETATE (6:1)

Steroid	*R_F value*
Oestrone	0.30
Oestrone-3-monochloroacetate	0.48
17β-Oestradiol	0.14
17β-Oestradiol-17β-monochloroacetate?	0.24
17β-Oestradiol-3-monochloroacetate?	0.48
Oestradiol-3,17β-dimonochloroacetate	0.66

Therefore, in routine GLC estimation of oestrogens, the reaction was performed as follows. To the dried, purified oestrogen in a stoppered test tube was added 0.5 ml of 50 mg/ml monochloroacetic anhydride solution in tetrahydrofuran, followed by 0.05 ml of pyridine and 5 mg of anhydrous sodium sulphate. The mixture was incubated for 120 min at 60°. The solvent was then evaporated under nitrogen and the derivative separated from unreacted oestrogen and from the reagents by TLC.

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