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TRITIUM LABELLING BY SELECTIVE DEBROMINATION

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SUMMARY

Methods are described for tritiating compounds containing both chlorine or a double bond and bromine by highly-selective debromination to give the chlorine-containing or unsaturated ^{3}H-compounds with specific activities of 10-28 Ci/mmol.

Key Words: Tritiation: selective dehalogenation: Diclofenac: CGS 9896: CGS 15943: CGP 22848.

INTRODUCTION and DISCUSSION

Several important existing or potential drug substances contain chlorine (e.g. diclofenac, this paper). When these are required labelled at high specific activity with tritium, methods of catalytic debromination allowing a very high degree of selectivity between halogens are required if efficient synthesis is to be achieved and tedious and often difficult (even hazardous) methods of purification are to be avoided.

It has long been appreciated that the ease of catalytic dehalogenation is related inversely to the carbon-halogen bond strength[1]. In seeking to achieve an absolute or near-absolute, discrimination to allow, for instance, bromine replacement while leaving carbon-chlorine bonds unaffected, one can

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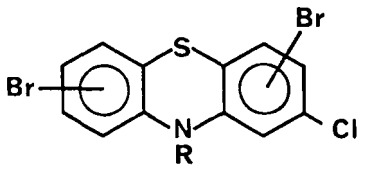
study the influence of variables such as the nature of the solvent, the temperature, reaction time, gas over-pressure and the choice of catalyst (including the use of catalyst poisons), but there may be little or no opportunity to vary the structure of the molecule to be labelled without considerable effort directed towards the synthesis of precursors.

Reports in recent literature [2-5] of syntheses requiring selectivity in reduction illustrate the difficulties that can be encountered. If sufficient distinction cannot be made between the rates of removal of bromine and chlorine atoms (e.g. in compounds 1 and 2), it has been necessary to resort to such tedious and hazardous techniques as preparative thin layer chromatography[2,3].

Attempts to impose reductive selectivity for compounds 1 and 2 by variation of gas over-pressure and addition of organic nitrogen bases were not successful although the role of divalent sulphur is a factor which obscures the interpretation of one of the studies[2]. Where good selectivity of bromine removal in the presence of chlorine was achieved (compound 3[4] and compound 4[5]), the presence of an organic nitrogen base was essential and its amount relative to the substrate for labelling was critical[5]. The choice of solvent and the gas over-pressure also had profound effects on the selectivity in one of these studies[5]. In the example (compound 3)[4] where excellent selectivity was attained, the use of a large amount of triethylamine (12 molar proportions) may be a factor responsible for the relatively low specific activity (5 Ci/mmol) of the product.

The foregoing examples demonstrate the need for a simple method of selective debromination which avoids the necessity to investigate the roles of the large number of possible variables. We describe here the highly-selective debromination of compounds 5,7 and 8 using 5% palladium on barium sulphate catalyst in a variety of polar solvents in the absence of added base of any kind. Reactions were carried out at ambient temperature and pressure within short times (10-30 minutes) to give products of high specific activity (10-28 Ci/mmol). We also report a highly selective debromination in the presence of an isolated double bond (compound 6) using



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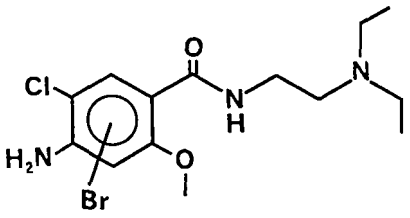

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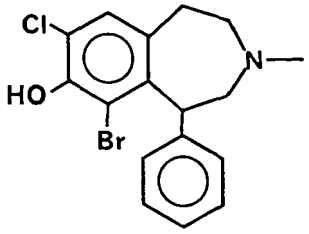

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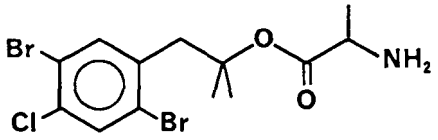
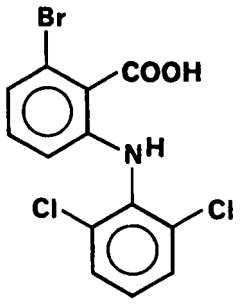


4 (Ref. 5)

the same conditions. In this instance, conditions reported previously[6] for the selective dehalogenation of a vinylogous bromo compound (a more testing example than the present one) were surprisingly completely without selectivity. In all but one of the cases described, purification from traces (always less than 2%) of further reduction products was simply effected by preparative high pressure liquid chromatography (prep. hplc).

Although our reported conditions have not yet been tested in a sufficient number of instances to suggest they have a good degree of generality, their simplicity recommends them as a first line of attack when investigating new compounds requiring selective reduction. We report them in the hope that they may be tested by others in new situations.



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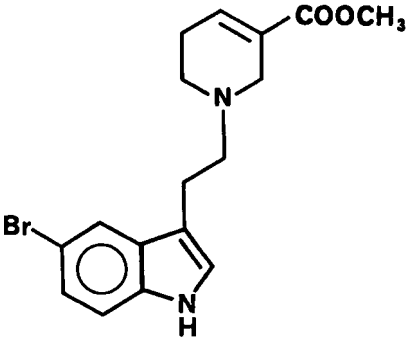

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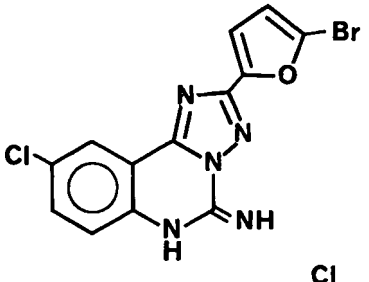

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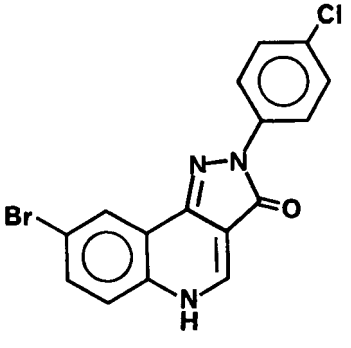

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EXPERIMENTAL

General [^{3}H]Compounds were purified by chromatography on columns (50 x 0.7 cm) of Nucleosil 10C_{18} support using the solvent mixtures specified. Compounds were injected in a volume of 0.5 ml of the developing solvent.

Final products were analysed by examination of thin layer chromatograms after development in the following solvent systems:

A CH_2Cl_2:CH_3OH:THF = 85:15:0.5 (on silica-gel G)

B EtOAc (on silica-gel G)

C CH_2Cl_2:CH_3OH = 20:1 (on silica-gel G)

D CH_3CN:H_2O:CH_3COOH = 650:350:1 (on Merck RP-18F_{254}S)

E 1-Butanol:CH_3COOH:H_2O = 10:1:3 (on silica-gel G)

F Acetone:petroleum ether (b.p. 60-80°) = 2:3 (on silica-gel G)

G EtOAc:petroleum ether (b.p. 60-80°) = 2:1 (on silica-gel G)

H $CHCl_3$:CH_3OH:CH_3COOH = 88:7:5 (on silica-gel G)

I $CHCl_3$:CH_3OH:NH_4OH = 45:6:1 (on silica-gel G)

J $C_6H_5CH_3$:C_2H_5OH:NH_4OH = 70:30:3 (on silica-gel G)

K $CHCl_3$:CH_3OH:HCOOH:CH_3COOH = 85:5:1:2 (on silica-gel G)

Plates were examined using either a Panax E.0111/XPD-05 or a Berthold LB 2842 TLC-Linear Analyser system. Final products were in all cases coincident on the chromatograms with unlabelled reference materials under conditions in which brominated starching materials and over-reduced products were easily separable (migrating faster and slower, respectively). Compounds were quantitated by ultra-violet spectroscopy at an appropriate wavelength and by scintillation counting of appropriately diluted samples using [^{3}H]hexadecane as internal reference.

[^{3}H]Diclofenac sodium

Compound 5 (3.4 mg) was dissolved in ethanol (0.5 ml) and reduced in the presence of 5% Pd/$BaSO_4$ catalyst in the presence of 3.2 ml (8 Ci) of $^3H_2$ gas for 20 minutes at room temperature. The catalyst was removed by filtration

through a small pad of MN-300 cellulose (Macherey-Nagel) and the filtrate was evaporated to dryness. The residue was taken up in a mixture of CH_3CN:H_2O:HOAc = 60:40:1 (0.5 ml) to which N-NaOH (20 µl) and EtOH (2 ml) were added. Insoluble matter was removed by passage of the mixture through an Acro LC13 membrane. The eluate was evaporated to dryness and the residue was redissolved in the developing solvent (60:40:1, above) (0.5 ml) and purified by prep. hplc (detection at 270 nm). Appropriate fractions were combined, evaporated to dryness and redissolved in 50% aqueous ethanol. After quantitation (277 nm), the solution was diluted to a concentration of 9.6 mCi/ml and the theoretical amount of N-NaOH was added. The solution was stored at -196°C (liquid N_2).

[^{3}H]CGP 22848

Compound 6 (3.2 mg) in ethanol (0.5 ml) was hydrogenated with $^3H_2$ gas (3.2 ml, 8Ci) in the presence of 5% Pd/$BaSO_4$ (8.4 mg) for 22 minutes at 20°C. Prep. hplc purification was with the solvent system CH_3CN:H_2O:CF_3COOH = 300:700:1 (detection at 260 nm). The crude product was collected in four fractions, which were combined and evaporated to dryness. The residue was dissolved in ethanol (5 ml) and 1N HCl (100 µl) was added. This solution was evaporated, the residue was redissolved and applied to a silica column (1 cm x 10 cm of Merck 230-400 mesh) eluting with dichloromethane/acetone (2:1). Fractions (40 x 1 ml) were collected diluting each with ethanol (1 ml) before examination by tlc (eluent:CH_2Cl_2:acetone 2:1; alternate fractions 22-40 were examined by tlc on the linear analyser). Fractions 27-33, containing faster running activity, were combined and evaporated and the product was stored as a solution in ethanol (1.4 mCi/ml) at -196°C.

[^{3}H]CGS 15943

Compound 7 (3.6 mg) in dioxan:DMF(80:20, 0.5 ml) was hydrogenated with $^3H_2$ gas (3.2 ml, 8 Ci) in the presence of 5% Pd/$BaSO_4$ (7.2 mg) for 30 minutes at 20°C. Prep. hplc purification was with the solvent system CH_3CN:H_2O:CH_3COOH = 500:500:2 (detection at 260 nm). After recovery, product

was stored as a solution in 50% aqueous ethanol (1 mCi/ml) at -196°C.

2-(p-Chlorophenyl)-pyrazolo[4,3-c]-6-bromoquinolin-3(5H)-one (8)

Ethyl 6-bromo-4-hydroxyquinoline 3-carboxylate (9) (14 mg) was heated with freshly-distilled $POCl_3$ (1 ml) at 100°C (steam-bath) for 30 minutes. The solution was evaporated to dryness and the residue was partitioned between CH_2Cl_2 (2.5 ml) and satd. aq. $NaHCO_3$ (5 ml). The aqueous layer was extracted with a second portion (2.5 ml) of CH_2Cl_2 and the combined extracts were washed with $NaHCO_3$ solution (2.5 ml), dried (Na_2SO_4) and the solution was evaporated. The residue was transferred by serial evaporation using xylene into a pear-shaped flask (5 ml) and was finally dissolved in xylene (0.15 ml).

p-Chlorophenylhydrazine (6.7 mg) in xylene (0.35 ml) was added to the residue and the mixture was heated under reflux for 40 minutes. The product was evaporated from a large volume of ethanol to remove the xylene and finally heated under reflux with EtOH (14 ml) to affect solution (some compound 8 did not dissolve) and then cooled. The supernatant was filtered (0.45 μ Millex) and a portion (10 ml) of the filtrate was applied to a column (250 x 22 mm) of Zorbax C8 which was eluted at a flow rate of 12 ml/min with CH_3CN:H_2O:CH_3COOH = 500:500:0.1 (by vol.). Examination was at 254 nm and fractions (1 min) were collected automatically. Fractions 12-38 were combined and afforded pure 8 on evaporation. The residue was dissolved in DMF (2 ml) and stored at -20°C.

[^3H]CGS 9896

A portion (0.5 ml) of the solution of 8 in DMF was hydrogenated with $^3H_2$ gas (3.2 ml, 8 Ci) in the presence of 5% Pd/$BaSO_4$ for 30 minutes at 20°C. The residue was dissolved in EtOH (2 ml) and the product was isolated by prep. hplc using the solvent mixture CH_3CN:H_2O:CH_3COOH = 500:500:0.1. Detection was at 254 nm. After recovery, material was quantitated at 300 nm and by scintillation counting and then diluted to a concentration of 5 mCi/ml in DMA:EtOH (1:9) for storage at -196°C (liquid N_2).

Table 1
Analyses

Product	% yield	Specific activity (Ci/mmol)	Tlc purity (%)	Solvent
Diclofenac sodium	54.8	9.7±0.4	97.9±0.1	A
CGP 22848	15.0	24	97±0.1 96±0.1 98±0.1	B C D
CGS 15943	24.9	11.6	96±0.1 97±0.1 97±0.1	E F G
CGS 9896	49.0 (from 9)	10.9±1.5	97.3±0.1 96.5±0.1 95.7±0.1 98.2±0.1	H I J K

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