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The synthesis of [^{3}H]Cadralazine of high specific activity.

Derek E. Brundish and Peter D. Kane

CIBA-GEIGY Pharmaceuticals, Research Centre, Horsham,

West Sussex. RH12 4AB England

SUMMARY

The title compound has been prepared by two different approaches. The first involved a five-stage "hot" synthesis where the tritiation step was selective debromination of a cyclic β-bromo-α-β-unsaturated ketone. The double bond was protected by formal aromatisation. The absolute structure of this intermediate was determined by ^{3}H-FTNMR spectroscopy of its reduction product. Cadralazine was also labelled in its side chain by reduction of the appropriate ketone with high specific activity sodium borotritide. Both labelled products had specific activities of 18 Ci/mmol and radiochemical purities of 90-96%.

Key words: Cadralazine, CGP 18684E, Selective tritiation, ^{3}H-FTNMR, Sodium borotritide.

INTRODUCTION

Cadralazine, 3-(N-ethyl-2-hydroxypropylamino)-6-(2′-ethoxycarbonyl) hydrazinopyridazine (CGP 18684E) (I) is a compound which may have value in hypertension and which exhibits a prolonged duration of action (1). Animal studies demonstrated that the active form of the drug is the free hydrazine (1,2), so the ethoxycarbonyl substituent may be considered to convert this active entity to a pro-drug form.

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Several potential routes to ^{3}H-Cadralazine are apparent:

1. Direct ring halogenation followed by catalytic dehalogenation
2. Synthesis of a halogenated Cadralazine by incorporation of halogen or masked halogen (nitro or amino function) into the pyridazine ring at an early stage of synthesis, then dehalogenation (as 1)
3. Direct tritium-protium exchange
4. "Hot" synthesis
5. Incorporation of a reducible function in the side chain.

Although the literature of the chemistry of pyridazines is substantial, no information exists on the reduction of simple pyridazine-based systems which was directly useful in the projected synthesis.

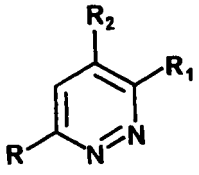
RESULTS AND DISCUSSION

Attempts to directly brominate Cadralazine were found to lead to extensive degradation. Catalytic exchange labelling with deuterium gas (PtO_2, CH_3OH, 16h at 20°C) incorporated 15% atom of ^{2}H into the ring positions as demonstrated by mass spectrometry. Repetition of this procedure but using tritium gas resulted in the incorporation of only 2% atom of ^{3}H.

The ease of nucleophilic displacement of halogen in 3,4,6-trihalogeno-pyridazines (IV) is known to follow the order $R_2 > R_1 > R$, precluding the possibility of carrying halogen at position 4 through the synthetic route. This same preference allows the preparation of V ($R_2 = NH_2$).

If the remaining chlorine atoms could be substituted by the appropriate groups, the resulting amino-Caldralazine would be a possible candidate for a Sandmeyer reaction. It was found, however, that the deactivating effect of the nitrogen function (amino or acetylamino) rendered the two remaining chlorine atoms resistant to replacement.

Following these findings, attention was turned to a 'hot' synthetic route. The published synthesis (3) of Cadralazine (I) starts from 3,6-dichloropyridazine (II). Addition of the appropriate secondary base occurs cleanly at one site only as the resulting substituted



I R = $N(Et)CH_2.CHOH.CH_3$; R_1 = NH.NH.COOEt; R_2 = H

II R = R_1 = Cl; R_2 = H

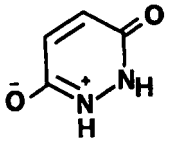
III R = $N(Et)CH_2.CHOH.CH_3$; R_1 = Cl; R_2 = H

IV R = R_1 = Cl; R_2 = Cl or Br

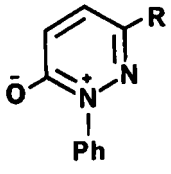
V R = R_1 = Cl; R_2 = NH_2 or $NH.COCH_3$

XI R = $N(Et)CH_2.COCH_3$; R_1 = NH.NH.COOEt; R_2 = H

(XI = ISF 3623)

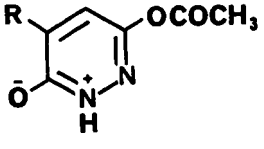


VI



VII R = Cl

VIII R = H



IX R = H

X R = Br

chloro-aminopyridazine (III) is severely deactivated towards further nucleophilic attack at the remaining chlorine-bearing carbon atom.

This is, however, replaced under forcing conditions by a strong base such as, in the present case, ethyl carbazate. Labelled dichloropyridazine could possibly be obtained by a selective dehalogenation (4) or, failing this, from labelled dihydropyridazine dione.

Attempts to selectively debrominate 4-bromo-3,6-dichloro-pyridazine (5) using a variety of catalysts (including ref. 4) failed completely as discrimination between the two halogens could not be achieved.

Catalytic reduction of bromo-dihydropyridazine dione led to saturation of the double bond in addition to debromination and, in a series of experiments, a recovery of more than 10% of the desired dihydropyridazine dione was never achieved.

In the literature there is much discussion about the electronic structure of dihydropyridazine dione, although it is mostly rather old and was published before modern analytical techniques, particularly NMR, became available. Nevertheless, some of the concepts that arose proved valuable in approaching this present problem.

Dihyropyridazine dione has been postulated (6) to be best represented as a zwitterionic species (VI). A chloropyridazinone (VII) in which such a representation is incorporated can be formally regarded as an aromatic system and, indeed, that conception is supported by the fact that chloropyridazinones can be catalytically dechlorinated under standard conditions (7) to pyridazinones (e.g. VIII). Additionally, it is known that acetylation of dihydropyridazine dione proceeds only as far as the mono-O-acetate (8) and writing this as the polarised structure (IX) discloses that it also can be formally regarded as aromatic in character.

A combination of these separate observations suggested that the mono-O-acetate of bromo-dihydropyridazine dione (X) might exhibit aromatic character and thus allow clean removal of halogen using standard conditions. This hope was realised and reduction of X followed by saponification (NaOEt) afforded VI in quantitative yield.

Compound VI was completely converted, as judged by high pressure liquid chromatography (hplc), into compound II using much milder conditions than have been previously reported (9). Treatment of compound II with 2-hydroxypropylethylamine (10) in toluene afforded (using [^{3}H]II) radiochemically pure mono-substituted chloropyridazine derivative (III). This reaction was best done in a sealed tube for reasons of safety. Although the product was radiochemically pure, it was substantially chemically impure and model experiments with unlabelled material showed that isolation at this stage by open-column silica gel chromatography improved the remaining synthetic stage and isolation procedure.

Conversion of compound III to Cadralazine (I) was never achieved in a yield of better than 10% (as judged by radio-thin layer chromatography (radio-tlc). Many experimental conditions were investigated with regard to different solvents and 1-pentanol was confirmed as the best choice (1). At times after 8 hours reaction, the yield did not increase beyond the 10% achieved but contaminants that were difficult to separate from the product began to accumulate. The reaction failed completely when it was carried out in a sealed tube. Purification was by hplc using first Partisil 10 and then a reverse-phase support. Material with a radiochemical purity of 90-95% was obtained (Table). Omission of either chromatographic step resulted in material of lower radiochemical purity (85-90%). Preparative hplc of Cadralazine on silica gel (Partisil 10) was very poor. An attempted isolation by preparative tlc failed due to extensive decomposition.

As the foregoing "hot" synthesis was so lengthy and attended by difficulties of purification, the following alternative route was developed. Trial hydrogenations of the ketone XI using rhodium or palladium catalysts were unsuccessful, producing a more polar compound, probably the result of N-N bond cleavage. However, borohydride reduction was relatively clean and gave Cadralazine which could be purified to an acceptable level in a single chromatographic operation. Analogous tritiation was carried out in iso-propanol with high specific activity sodium borotritide (Amersham International). After extraction and purification by silica column chromatography, product with radiochemical purity of 90-95% was obtained (Table).

[^{3}H]Cadralazine was stored frozen as an aqueous solution immersed in liquid nitrogen (-196°C). Despite the known ease of oxidation of Cadralazine to the azo-compound (11), no such radical-induced conversion was observed on storage and the radiochemical purity of a preparation stored as detailed was unaltered after a period of 8 months.

The structure of the mono-acetylated bromo-dihydropyridazine dione is predicted as X from mechanistic considerations. Proton NMR gave, besides the acetyl signal, a single peak at 8.10 ppm with a much smaller signal (impurity)

at 8.30 ppm, from which no structural assignment could be made due to the many influencing groups in the molecule. After catalytic tritiation, ^{3}H-FTNMR showed a signal at 7.03 ppm (impurity at 7.43 ppm - as there was no coupling, this must represent a separate molecule) and ^{1}H-FTNMR of the same sample showed the anticipated AX pair of doublets based on the same positions, that at higher field being weaker due to the ^{3}H substitution at that position. The signal at higher field is always indicative of the hydrogen at the 4-position in fixed 3-oxo-2,3-dihydropyridazine systems (12), thus the structure is confirmed as the 4(5)-bromo-6(3)-acetate. The amount of contaminationg 5-bromo compound is approximately 2% as judged from the ^{1}H-NMR spectrum of X.

EXPERIMENTAL

3-(1H-5-Bromo-6-pyridazinonyl)acetate (X). - Bromo-dihyropyridazine dione (3.4g) (13) was heated under reflux for 4h with acetic anhydride (50 ml). The residue obtained after evaporation of the solvent was crystallised (charcoal) from ethanol, crude yield 1.49g (36%). A sample recrystallised twice from ethanol had m.p. 186-9°C Found: C, 30.9; H, 2.16;N, 12.0;Br, 34.3 $C_6H_5BrN_2O_3$ requires C, 31.07;H, 2.19;N, 12.15;Br, 33.98%). NMR (in d_6-DMSO) 2.28(3H,s), 8.10(1H,s), 13.25(1H,br) with a minor signal (8.30 0.02H,s).

3-(1H-5-[^{3}H]-6-Pyridazinonyl)acetate. - Compound X (3.5 mg) was dissolved in ethanol (0.5 ml) and stirred with 10% Pd/C (3.0 mg) in the presence of tritium gas (3.2 ml, 8 Ci) for 10 minutes at 20°C. The catalyst was removed by filtration through a pad of tlc-grade cellulose and the filtrate was evaporated to dryness. The residue was dissolved in a mixture of acetonitrile:water:trifluoroacetic acid (100:900:1, by vol., 0.52 ml) and the solution was applied using a Rheodyne 6-port injection valve fitted with a 500 µl loop to a column (50 x 0.7 cm) of Nucleosil 10C_{18} which was eluted at a flow rate of 5.2 ml/min with the same solvent mixture. Detection was at 230 nm (Cecil 212). Fractions (0.5 min) were collected automatically and the product (117 mCi) was recovered by evaporation of the combined fractions 10-12. The residue was redissolved in water (25 ml) and stored immersed in liquid nitrogen until analysis.

For ^{3}H-FTNMR (proton decoupled) analysis, a portion (33.7 mCi) of the material was evaporated to dryness and the residue was dissolved in d_6-DMSO (75 µl) with the inclusion of TMS (1 µl) as ghost reference. The main signal was at 7.03 ppm (90.2%) with impurity signal at 7.43 ppm (9.8%). Proton NMR of the same sample showed a pair of doublets centered (by calculation) on 7.40 and 7.00 ppm with $\Delta\nu$ = 35.6 cps (90 MHz) and J = 9.9 cps.

4(5)-[^{3}H]-3,6-Dihyropyridazine dione. - Compound X (18.6 mg) was dissolved in ethanol (0.5 ml) and stirred with 10% Pd/C (7.3 mg) in the presence of tritium gas (3.2 ml, 8 Ci) for 40 minutes at 20°C. The catalyst was removed by filtration through a pad of tlc-grade cellulose and the filtrate was evaporated to dryness. The residue was dissolved in ethanol (1.0 ml) and a solution of sodium in ethanol (36 mg/ml, 0.1 ml) was added. The mixture was kept at room temperature for 5 minutes and aq. M-HCl (0.16 ml) was added. The mixture was evaporated to dryness and portions (2 x 2 ml) of ethanol were evaporated from the residue to remove water.

4(5)-[^{3}H]-3,6-Dichloropyridazine. - Phosphorus oxychloride (1.5 ml) was added to the residue and the mixture was heated on a steam bath for 15 minutes with occasional shaking. The mixture was evaporated to dryness and ethanol (3 x 2 ml) was evaporated from the residue to remove excess of $POCl^3$.

3-(N-Ethyl-N-2-hydroxypropylamino)-4(5)-[^{3}H]-6-chloropyridazine.
The residue was transferred by serial evaporation using small portions of ethanol to a boiling tube (15 ml) and a solution of 2-hydroxypropylethyamine (0.37 ml) in toluene (0.37 ml) was added to the residue. The tube was sealed under vacuum and kept in an oven at 110°C for 3 hours. After opening the cooled tube, the solution was evaporated to dryness and portions of ethanol (3 x 2 ml) and then chloroform (3 x 2 ml) were evaporated from the residue, which was then applied to a column (10 x 1 cm) of silica gel in chloroform:methanol (99:1 by vol., 1 ml) (NaCl as a solid was retained at the top of the column). The column was eluted with 1% MeOH in $CHCl_3$ and fractions (5 ml) were collected. Fractions 2-6 were combined and evaporated to yield the product.

3-(N-Ethyl-N-2-hydroxypropylamino-4(5)-[^{3}H]-6-(2'-ethoxycarbonyl) hyrdrazinopyridazine (4(5)-[^{3}H]Cadralazine). - The above residue was dissolved in 1-pentanol (0.5 ml), ethyl carbazate (100 mg) was added and the mixture was heated under reflux for 8 hours. The mixture was, at this stage, diluted with toluene (25 ml) and kept at 4°C until the following working day (i.e. for 9 hours). The solution was evaporated to dryness and the residue was dissolved in chloroform:methanol 19:1 by vol., 2 ml) and the solution was applied to a column of Partisil 10 (50 x 0.7 cm) which was eluted at 5 ml/min with the same solvent mixture. Fractions 8-27 were combined and evaporated to give the product, which was approximately 85% radiochemically pure as judged by tlc (solvent B, Table). The residue was dissolved in acetonitrile:water: trifluroacetic acid (100:900:1 by vol, 2 ml) and the solution was applied to a column (50 x 0.7 cm) of Nucleosil 10C_{18} which was eluted at a flow rate of 5.2 ml/min with the same solvent mixture.

Fractions (0.5 min) were collected automatically, detection was at 270 nm. The product was recovered by evaporation of the combined fractions 19-24, and the residue was dissolved in water (5.2 ml) for storage in liquid nitrogen (-196°C). A portion (0.2 ml) of solution was diluted to 5 ml (water) for quantitation at 248 nm. For estimation for tritium, a portion (10 µl) of this dilute solution was further diluted (to 5 ml) and portions (10 µl) were counted in BBOT scintillator using [^{3}H]-hexadecane as internal standard. The specific activity of the product was 18.3±0.5 Ci/mmol and the yield was 44.5 mCi, (approximately 3% overall). Radiochemical purity was estimated by tlc and the results are given in the Table.

3-(N-Ethyl-N-(2-[^{3}H]-2-hydroxypropylamino)-6-(2'-ethoxycarbonyl)-hydrazino)-pyridazine:- An ampoule containing sodium borotritide (1 Ci) was opened and a solution of ISF 3623 (3.7 mg) in isopropanol (0.4 ml) was immediately added. The granular mass was broken up with a glass rod and the resultant mixture was kept for 1 hour at room temperature with frequent agitation. The solution was evaporated to dryness and the residue was dissolved in chloroform (25 ml). The solution was extracted with satd. aq. $NaHCO_3$ (5 ml) and dried (Na_2SO_4). After concentration, the solution was

applied to a silica column (8 x 1 cm, 230-400 mesh) which was eluted with $CHCl_3$:CH_3OH (12:1. by vol.). Fractions (1 ml) were collected into tubes already containing ethanol (1 ml) and samples were analysed by liquid scintillation counting and by tlc (in $CHCl_3$:CH_3OH = 12:1). Fractions 12-15 were combined and evaporated to dryness to give the product (1.6 µM, 12%) with specific activity 18 Ci/mmol. Radiochemical purity was estimated by tlc and the results are given in the Table.

TABLE

Radiochemical purity of [^{3}H]Cadralazine after tlc on silica gel. Plates were examined with a Panax E.0111/XPD-05 scanner system.

Solvent system	Position of ^3H label	% Radiochemical Purity	
		Without carrier	With Carrier (1 mg/ml)
$CHCl_3$:CH_3OH:H_2O 95:10:1	Ring	90.5	90.4
	Side chain	90.0	-
THF:CH_3OH = 96:4	Ring	94.8	95.0
	Side Chain	96.0	-
n-C_4H_9OH:CH_3COOH:H_2O = 5:1:1	Ring	88.0	93.1
	Side Chain	95.0	-

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