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Bifluranol, a novel fluorinated bibenzyl anti-androgen, its chemistry and disposition in different animal species

D. J. POPE, A. P. GILBERT, D. J. EASTER, R. P. CHAN, J. C. TURNER, S. GOTTFRIED*, D. V. PARKE†

Departments of Biochemistry and Chemistry, Biorex Laboratories Ltd., Biorex House, Canonbury Villas, London N1 2HB and † *Department of Biochemistry, University of Surrey, Guildford, Surrey*

The synthesis of bifluranol, a new fluorinated bibenzyl anti-androgen, and of ^{3}H-labelled bifluranol is described. The absorption, distribution and excretion of bifluranol has been studied in mouse, rat, ferret and dog; it is readily absorbed following oral administration, but blood concentrations of the drug are low due to hepatic uptake and biliary excretion. Enterohepatic re-circulation occurs, but the drug is excreted primarily in the faeces and only small amounts appear in urine. This pattern of disposition and excretion is similar to that reported elsewhere for the bibenzyl, hexoestrol, and for the stilbene, diethylstilboestrol.

Bifluranol (*erythro-3,3′-difluoro-4,4′-dihydroxy-α-ethyl-α′-methyl-bibenzyl*, Prostarex) is one of a series of bibenzyls designed to present some of the hormonal properties of diethylstilboestrol (DES) but with a lower potential for toxicity.

Diethylstilboestrol has been used for a number of years to treat prostatic enlargement, although the oestrogenic side effects limit any overall improvement in prognosis (Veterans A.C.U.R.G. 1967). Bifluranol has been shown to be a comparable anti-androgen to diethylstilboestrol, but in the rat it has only one eighth of the oestrogenic activity of diethylstilboestrol (Dekanski 1980). Bifluranol is currently being evaluated as a treatment for prostatic enlargement in man.

The present paper describes the synthesis and chemistry of bifluranol and [^{3}H]bifluranol, and the absorption, distribution and excretion of the drug in mouse, rat, ferret and dog. A preliminary communication of part of these findings, concerning the disposition and metabolism of bifluranol, has been previously published (Chan et al 1978).

MATERIALS AND METHODS

Bifluranol (*erythro*-3,3′-difluoro-4,4′-dihydroxy-α-ethyl-α′-methyl bibenzyl). Conc. H_2SO_4 (125 ml) was added dropwise to a stirred mixture of 3-chloropentan-2-one (50 g) and 2-fluoroanisole (100 g) at −20 °C. After stirring for 6 h the mixture was poured into ice and water and then extracted with ether. The ethereal extract was evaporated to give an oil, which after heating (235–240 °C at 15 mm Hg for 2 h) was distilled under vacuum to give a major fraction (20 g, b.p. 160–170 °C at 0·03 mm Hg). The product, in glacial acetic acid (150 ml), was hydrogenated in the presence of 10% Pd on charcoal (12 g). Extraction of the product with ether, evaporation of the solvent, and crystallization of the residue twice from methanol gave *erythro*-3,3′-difluoro-4,4′-dimethoxy-α-ethyl-α′-methyl bibenzyl (7·5 g, m.p. 93–94 °C). Demethylation of the dimethyl ether in acetic acid–hydrobromic acid (7:4 v/v) at 140 °C for 5 h yielded, following ether extraction and crystallization from toluene, bifluranol (6 g, m.p. 158–159 °C, ν 3350, 1620, 1600, 870, 820, 780 cm^{-1}; n.m.r., δ 0·60 (CH_3CH_2, *t*, J = 7 Hz), 0·98 (CH_3CH, *d*, J = 7 Hz), ca 1·45 (CH_3CH_2, *m*), ca 2·5 (CH–CH, *m*), 5·05, 5·12 (2 × OH), 6·85–7·10 (6 aromatic H). The *erythro* configuration was established following defluorination of *erythro*-3,3′-difluroro-4,4′-dimethoxy-α-ethyl-α′-methyl bibenzyl to give material identical with *erythro*-4,4′-dimethoxy-α-ethyl-α′-methyl bibenzyl (m.p. and mixed m.p. 100–101 °C) (Turner & Chan 1972).

[5,5′-^{3}*H*]*Bifluranol*. Bromine (1 ml) in chloroform (10 ml) was added dropwise to a stirred solution of bifluranol (2 g) in chloroform at 15 °C. After stirring for 20 h, water and sodium bisulphite (20 mg) were added and the chloroform layer separated, washed with water, dried over sodium sulphate and evaporated to dryness. Crystallization of the residue from hot toluene yielded *erythro*-5,5′-dibromo-4,4′-dihydroxy-3,3′-difluoro-α-ethyl-α′-methyl bibenzyl (2·7 g, m.p. 128–129 °C; ν 3450, 1595, 860, 835, 780, 760, 710 cm^{-1}; n.m.r., δ 0·60 (CH_3CH_2, *t*, J = 7 Hz),

* Correspondence.

0·96 (CH_3CH, *d*, $J = 7$ Hz), ca 1·40 (CH_2–, *m*), ca 2·50 (CH-CH, *m*), 6·83 (C_2–H, C_2'-H, *q*, $J = 10$ and 2 Hz), 7·05 (C_6–H, C_6'–H, *q*, $J = 2$ and 2 Hz). G.l.c. and t.l.c. showed >99·5% purity with no other isomers present. The 5,5′-dibromo-bifluranol (10 mg), 10% Pd on charcoal (5 mg), ethyl acetate (1 ml) and triethylamine (16 mg) were stirred vigorously with a large excess of $^3H_2$ gas (Radiochemical Centre, Amersham) for 4 h. 1 M HCl (1 ml) and ethyl acetate (1 ml) were added, the ethyl acetate layer filtered, washed (water) evaporated to dryness to give [5,5-^{3}H]bifluranol, which was stored in benzene-ethanol (9:1 v/v); specific radio-activity 125 Ci g^{-1}, t.l.c. on Kieselgel 254 in benzene–ether (1:1 v/v) and n-butanol–acetic acid–water (4:1:1, v/v/v) showed >98·5% radiochemical purity.

Animals. Swiss albino mice (24–28 g, males age 4 weeks, females age 6 weeks; pregnant mice mated at 6 weeks, used at day 18 of pregnancy) and Wistar albino rats (200 g, males age 6 weeks, females age 8 weeks; 350 g males age 10 weeks) (Biorex Laboratories Ltd.) were fed Dixon's mouse and rat diet (Type FFG(M) with added vitamins) and had free access to water. Albino ferrets (0·7–2·6 kg, age 9–15 months) supplied by A. S. Roe, Little Fakenham, nr. Thetford, Norfolk, England, were fed raw meat, bread and milk. Male beagles (10·8–12·3 kg, age 10–14 years) (Biorex Laboratories Ltd.), were fed Spratt's complete dog diet.

[^{3}*H*]*Bifluranol administration* was by intragastric intubation, in propylene glycol (mouse 0·1 ml, rat 0·1–0·2 ml, ferret 0·1–0·4 ml and dog 1 ml), except for the dog 96 h excretion study when the drug was adsorbed onto starch and given in a gelatin capsule. Bifluranol was given intravenously in propylene glycol–0·9% NaCl (saline) (1:1 v/v) (0·1–0·2 ml), via a tail vein in mice and rats and the jugular vein in ferrets (under ether anaesthesia).

Whole body autoradiography. [^{3}H]Bifluranol (2 mg kg^{-1}, 1·1 mCi) was administered orally or intravenously to male, female and pregnant mice. After various time intervals they were killed under ether anaesthesia by immersion in solid CO_2-hexane (−70 °C). The tail, limbs and ears were removed, the animals shaved, embedded and frozen in 5% aq. acacia wax. The animal blocks were cut using a Slee whole-body freezing microtome to obtain lateral sections (30 μm) which were exposed to X-ray film (Kodirex, Kodak Ltd.) at 4 °C and the autoradiograms examined after 1,3 or 6 months.

96 h excretion. [^{3}H]Bifluranol was administered orally to rats (200 μg kg^{-1}, 246–870 μCi), ferrets (60 μg kg^{-1}, 0·5 mCi) and dogs (50 μg kg^{-1}, 70–76 mCi). Animals were housed individually in cages designed for separation of urine and faeces, which were collected every 24 h for 4 days. Air was drawn through the cages for the rat and ferret studies and expired water vapour trapped in magnesium perchlorate.

Blood concentrations. [^{3}H]Bifluranol was administered orally or intravenously to rats (200 μg kg^{-1}, 0·86–1·0 mCi), ferrets (60 μg kg^{-1}, 5·0–10·6 mCi) and orally only to dogs (50 μg kg^{-1}, 70–76 mCi). Blood samples (10–100 μl) were taken for radioactivity determination at time intervals up to 96 h (rat and ferret) or 6 h (dog).

Biliary excretion. Biliary cannulae were inserted into animals by surgery under pentobarbitone anaesthesia. [^{3}H]Bifluranol was administered to biliary cannulated rats (200 μg–200 mg kg^{-1}, 1 mCi, oral or intravenous), ferrets (60 μg kg^{-1}, 0·8–5·0 mCi, oral) and dogs (50 μg kg^{-1}, 70–78 mCi, oral), and bile was collected for 4 h (rat, intravenous), 6 h (dog), 6–8 h (ferret) or up to 72 h (rat, oral). Orally dosed rats were allowed to regain consciousness after anaesthesia and were maintained in individual cages with free access to food and water. In all other cases anaesthesia was maintained during bile collection with parenteral injections of pentobarbitone as necessary. At the end of the collection period any urine was removed from the bladder and bile was removed from the gall bladder where appropriate.

Enterohepatic circulation. After biliary cannulation of rats and ferrets the stomach was ligated at the pylorus and a sample of bile, from a donor animal of the same species previously dosed with [^{3}H]-bifluranol, was injected intraduodenally. Rats received bile (0·3 ml) from a biliary cannulated rat which had previously received [^{3}H]bifluranol orally (200 μg kg^{-1}, 1 mCi) and ferrets received bile (1 ml) from a biliary cannulated ferret which had previously received [^{3}H]bifluranol orally (60 μg kg^{-1}, 0·8–5·0 mCi). Bile was collected for 6–8 h and gall-bladder bile removed from the ferrets.

Determination of radioactivity. Faeces samples were extracted with cold methanol for four days and then refluxed with alcoholic 40% KOH. Aliquots of the solubilized material (0·1 ml) were neutralized with conc. HCl (0·2 ml) and the volume adjusted to 1 ml with water before counting. Blood samples (0·01 or 0·1 ml) were solubilized in Hyamine 10-X hydroxide (0·5 ml), decolourized with 30% w/v H_2O_2 (0·3 ml) and neutralized with 2·5 M HCl (0·1 ml) before counting. Magnesium perchlorate used to trap

expired water from metabolic studies was dissolved in water (20 ml). Aliquots of urine and freeze-dried urine reconstituted in water (1 ml), expired water (1 ml) and bile (0·01 ml) were counted directly.

Radioactivity measurements were made using a Packard 3320 liquid scintillation counter. Samples were counted in 10 ml of Bray's scintillant plus Cab-o-sil (2,5-diphenyloxazole, 20 g; 1,4-di-2-(4-methyl-5-phenyloxazolyl)benzene, 1 g; naphthalene, 300 g; methanol, 500 ml; 1,2-ethanediol, 100 ml; Cab-o-sil, 1 bag, and 1,4-dioxan to a final volume of 5 litres) or in 10 ml of Triton-toluene scintillant (2,5-diphenyloxazole, 25 g; 1,4-di-2-(4-methyl-5-phenyloxazolyl)benzene, 1·5 g; toluene, 5 litres, and Triton X-100, 2·5 litres). All samples were counted for a minimum of 10,000 counts or 100 min. Counting efficiencies were calculated using the external standard ratio method.

RESULTS

Whole-body autoradiography in mice. After oral administration of [^{3}H]bifluranol the main site of uptake of radioactivity apart from the gastrointestinal lumen was the liver, with much lower levels in the kidney and bladder, and short-term localization in body fat. The testes and uterus showed a low transient accumulation of ^{3}H for up to 6 h. Tissue ^{3}H concentrations were otherwise low, the highest being reached after 2 h. Most ^{3}H had cleared the body after 24 h, except for the intestinal lumen, liver and kidneys; no radioactivity was detected in any tissue at 96 h.

In pregant mice receiving [^{3}H]bifluranol orally, radioactivity was not detected in foetal tissues at 1 h, but was present at 4 h after dosage, with ^{3}H accumulating in the foetal liver and intestinal lumen. Foetal concentrations of ^{3}H had fallen by 8 h, and at 24 h the mother had littered and the newborn mice showed a similar distribution of ^{3}H to that observed *in utero*.

After intravenous administration of [^{3}H]bifluranol the main site of uptake of radioactivity after 10 min was the liver. Subsequently ^{3}H rapidly accumulated in the liver and intestinal lumen, with some retention by lungs and kidney, and transient localization in body fat, testes and uterus. After 24 h most ^{3}H had cleared the body, the only sites of remaining ^{3}H being the liver and intestinal lumen with traces in kidneys, lungs and bladder. At 96 h only traces of ^{3}H were still present in the liver and lungs.

Radioactivity readily crossed the placental barrier after intravenous administration of [^{3}H]bifluranol and foetal concentrations were similar to those of the mother or even marginally higher. At 1 h distribution of ^{3}H in foetal tissues was uniform, but by 8 h ^{3}H was localized in the liver and intestinal lumen. At 24 h, with the exceptions of the liver and intestinal tract, tissue ^{3}H had fallen to values similar to those of the maternal tissue.

96 h excretion. After oral administration of [^{3}H]-bifluranol the major excretion pathway was the faeces, with only small quantities recovered in the urine (Table 1). No ^{3}H was lost on evaporating urine samples from ferret and dog to dryness, but some rat urinary ^{3}H (33% males, 10% females) was lost on drying. This corresponds to 4 and 0·7% of the administered dose being converted to ^3H_2O in male and female rats respectively. Only traces of volatile ^{3}H were found in the expired air from the rat (0·5%) and ferret (0·04%).

Blood concentrations of radioactivity. After intravenous administration of [^{3}H]bifluranol to rats (200 μg kg^{-1}) and ferrets (60 μg kg^{-1}) the blood concentrations of ^{3}H decreased rapidly for the first 2 to 3 h, with the decrease being more rapid in

Table 1. Excretion of radioactivity following oral administration of ^{3}H-bifluranol to rat, ferret and dog.

Species, sex and excretory pathway			% Dose excreted 0–24 h	24–48 h	0–96 h	
Rat (200 μg kg^{-1})	Male	Faeces	43·6 ± 23·4	32·0 ± 7·6	88·8 ± 12·3	101 ± 6
		Urine	5·8 ± 1·9	3·8 ± 1·7	12·2 ± 4·2	
	Female	Faeces	36·6 ± 9·6	42·3 ± 13·5	85·2 ± 6·1	93 ± 5
		Urine	3·5 ± 0·9	1·5 ± 0·4	7·3 ± 1·4	
Ferret (60 μg kg^{-1})	Male	Faeces	85·7 ± 2·2	5·7 ± 2·4	92·6 ± 2·2	99 ± 2
		Urine	5·1 ± 0·7	1·0 ± 0·1	6·7 ± 0·6	
	Female	Faeces	77·4 ± 2·4	8·8 ± 4·9	92·3 ± 2·6	99 ± 3
		Urine	4·5 ± 1·7	1·5 ± 0·4	6·9 ± 2·4	
Dog (50 μg kg^{-1})	Male	Faeces	67·9 ± 11·2	20·7 ± 4·1	94·5 ± 7·9	99 ± 4
		Urine	3·3 ± 1·2	0·7 ± 0·3	4·4 ± 1·6	

Results are mean values ±s.e.m. for 3 animals.

females ($t_{\frac{1}{2}}$, 18 min for rat, 30 min for ferret) than males ($t_{\frac{1}{2}}$, 1·0 h for rat, 1·4 h for ferret) (see Figs 1 and 2). This was followed by a much slower decline ($t_{\frac{1}{2}}$, 40 h for rat, 20 h for ferret) to concentrations at 96 h of less than 15 ng bifluranol equivalents ml^{-1} (rat) or 1 ng bifluranol equivalents ml^{-1} (ferret).

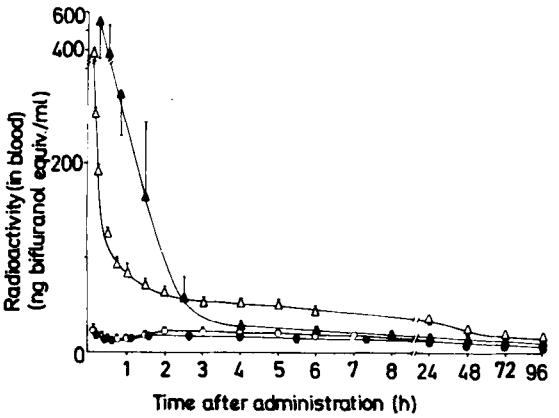

FIG. 1. Blood total radioactivity concentration time curves following oral or intravenous administration of [^{3}H]bifluranol to rats. Results are the mean values of 6 male or 5 female rats with s.e.m. shown as bars, for oral administration, ● male, ○ female; or intravenous administration ▲ male, △ female. Dose of [^{3}H]bifluranol was 200 $\mu g\ kg^{-1}$. For clarity, s.e.m. ranges are given in one direction only.

With oral administration to rat, blood ^{3}H concentrations fell after the first sampling (10 min) and then rose to peak values (after 2–3 h) of 23 ng bifluranol equivalents ml^{-1} (male) or 17 ng bifluranol equivalents ml^{-1} (female), before again decreasing slowly to 6–8 ng bifluranol equivalents ml^{-1} at 96 h.

With oral administration to ferrets, blood ^{3}H concentrations reached maximum (23 ng bifluranol equivalents ml^{-1} for male, 35 ng bifluranol equivalents ml^{-1} for female) during the first half hour after dosing. Blood ^{3}H values then decreased rapidly to 3 h and subsequently declined slowly to less than 1 ng bifluranol equivalents ml^{-1} at 96 h.

After oral administration of [^{3}H]bifluranol to male dogs (50 $\mu g\ kg^{-1}$) blood ^{3}H concentrations reached maximum levels of 7–8 ng bifluranol equivalents ml^{-1} at 2½ h and fell to 3–4 ng bifluranol equivalents ml^{-1} at 6 h.

Biliary excretion and enterohepatic circulation. Excretion of radioactivity in bile after oral administration of [^{3}H]bifluranol to rat, ferret and dog was high (Table 2); less than 3% of the dose was recovered in the urine of these animals. After intravenous administration most radioactivity was

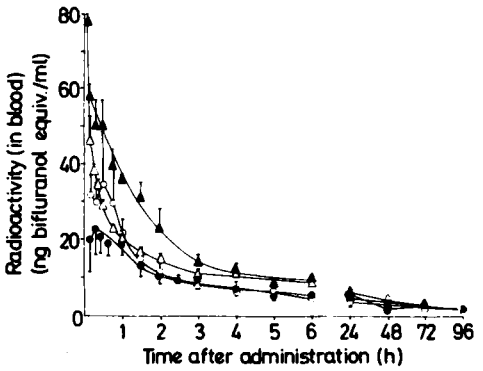

FIG. 2. Blood total radioactivity concentration time curves following oral or intravenous administration of [^{3}H]bifluranol to ferrets. Results are the mean values of 3 ferrets, with s.e.m. shown as bars, for oral administration, ● male, ○ female; or intravenous administration ▲ male, △ female. Dose of [^{3}H]bifluranol was 60 $\mu g\ kg^{-1}$. For clarity, s.e.m. values are given in one direction only.

excreted in the bile in 4 h; less than 1% was recovered in the urine.

Intraduodenal administration of radioactive bile from animals which had previously received [^{3}H]-bifluranol (rat, 200 $\mu g\ kg^{-1}$; ferret, 60 $\mu g\ kg^{-1}$) showed that enterohepatic circulation of bifluranol metabolites was higher in rats (42 to 81% dose) than ferrets (18 to 24% dose) (Table 2).

DISCUSSION

On intravenous or oral administration of [^{3}H]-bifluranol to mice the major sites of accumulation of radioactivity are the liver and gastrointestinal lumen. The uptake of radioactivity by the testes and uterus may be associated with the endocrine properties of bifluranol. Very low levels of radioactivity are present in the animals 96 h after dosage, indicating that bifluranol is readily eliminated from the body. The disposition and elimination of total radioactivity after administration of [^{3}H]bifluranol is similar in both male and female mice. The drug readily crosses the placental barrier and the distribution of total radioactivity in the foetus is similar to that in the adult; the presence of ^{3}H in the foetal intestinal lumen suggests that biliary excretion of bifluranol metabolites occurs in the foetus.

After oral administration of [^{3}H]bifluranol, blood concentrations of radioactivity in rat, ferret and dog are low at all times. In the rat and ferret the rapid initial fall in blood values of total radioactivity is consistent with rapid uptake by the liver before biliary excretion. Volatile ^{3}H present as water in

Table 2. Biliary excretion of radioactivity in rat, ferret and dog following administration of [^{3}H]bifluranol or bile from animals previously receiving [^{3}H]bifluranol.

Route and product of administration	Time after administration (h)	% Dose excreted: Rat 200 μg kg^{-1} Male	Rat 200 μg kg^{-1} Female	Rat 2 mg kg^{-1} Male	Rat 20 mg kg^{-1} Male	Rat 200 mg kg^{-1} Male	Ferret 60 μg kg^{-1} Male	Ferret 60 μg kg^{-1} Female	Dog 50 μg kg^{-1} Male
[^{3}H]Bifluranol, (oral)	6	48 ± 12 (4)		38 ± 15 (6)	42, 21			38 ± 4 (3)	30 ± 10 (3)
	8	51 ± 12 (4)		45 ± 11 (6)			36 ± 15 (3)		
	24	68 ± 9 (4)	50 ± 13 (3)	62 ± 10 (6)	65				
	54					39			
	72			87 ± 2 (6)					
[^{3}H]Bifluranol, (intravenous)	4	81		84 ± 7 (4)	70, 77				
[^{3}H]Bifluranol bile, (intraduodenal)	6		42 ± 10 (3)					23, 24	
	8	73, 81					18, 19		

Results are mean values, where n is the number of animals (in parentheses) ± s.e.m.

urine and expired air complicates rat blood concentration data, since it is probable that some of the radioactivity in the blood is present at ^3H_2O.

The excretion of orally administered [^{3}H]bifluranol and its metabolites is similar in the rat, ferret and dog, the major route of elimination of radioactivity being the faeces, with only small amounts excreted in the urine. The high biliary excretion following oral administration indicates that the low blood values of radioactivity and high faecal elimination are not a result of poor absorption of the drug. The ready elimination of bifluranol by the rat liver is seen in the similar rates of biliary excretion over the dose range 0·2–20 mg kg^{-1}. The more extensive enterohepatic circulation of radioactive bifluranol metabolites in the rat, compared with the ferret, probably accounts for the slower rate of faecal elimination in the rat observed in the 96 h excretion study.

It is concluded that the distribution is similar in both males and females of the various species studied, the pattern being one of rapid absorption and low blood concentrations due to rapid and extensive uptake by the liver, followed by biliary elimination and predominantly faecal excretion. This pattern of disposition is essentially similar to that published for the related compound, hexoestrol, where some 90% of [^{3}H]hexoestrol administered to rats was recovered in 96 h, mostly in the faeces (Dodds et al 1958). The related stilbene structure, diethylstilboestrol also presents a similar pattern of distribution and excretion in rodents to that of bifluranol (Fischer et al 1966; Metzler 1975, 1976; Shah & McLachlan 1976; Metzler & McLachlan 1978), the only notable difference being that autoradiography in the mouse indicates that diethylstilboestrol is localized in the adrenals (Bengtsson & Ullberg 1963), whereas bifluranol is not. Although the disposition of bifluranol is similar to that of hexoestrol and diethylstilboestrol, its metabolism, as detailed in the following paper, differs considerably from that reported for hexoestrol and diethylstilboestrol.

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