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The metabolism of bifluranol by rat, dog and ferret

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The synthesis of monohydroxy- and dihydroxy-bifluranol, and of glucuronide and sulphate conjugates of bifluranol are described. Bifluranol administered orally to rats, ferrets and dogs at a dosage of 50 to 200 $\mu g\ kg^{-1}$ is mostly excreted in the faeces as unchanged bifluranol and bifluranol monosulphate, disulphate and monoglucuronide. The bifluranol is well absorbed and is mostly excreted in the bile, as six different conjugates, including a glucuronide sulphate found in all 3 species, and a glucuronide phosphate found only in ferret and dog bile. Hydroxylation of the aromatic rings occurs in the rat, to an extent of about 8% of the dose, but was not detected in ferret or dog.

Bifluranol, a new fluorinated, bibenzyl, anti-androgen drug with lower oestrogenic activity than diethylstilboestrol, and designed to be less susceptible to metabolic hydroxylation and less toxic, is currently being evaluated for efficacy in the treatment of prostatic enlargement.

Its absorption and excretion in rat, ferret and dog was described by Pope et al (1981). We now report the metabolism of the drug in these species, and compare its metabolism and potential toxicity with that of diethylstilboestrol, which is metabolically oxygenated in the aromatic rings and at the allylic double bond (Metzler & McLachlan 1978) resulting in binding of reactive metabolites to cellular components (Blackburn et al 1976) which lead to cytotoxicity and carcinogenicity.

MATERIALS AND METHODS

Bifluranol (*erythro*-3,3′-difluoro-4,4′-dihydroxy-α-methyl-α′-ethyl bibenzyl) and [5,5′-^{3}H] bifluranol were synthesized as previously described (Pope et al 1981).

5-Hydroxy-bifluranol: (Mixture of the 5- and 5′ isomers). Bifluranol dimethyl ether (Pope et al 1981) (300 mg) in acetyl chloride (1 ml) was treated (2h, 20 °C) with titanium tetrachloride (0·2 ml) then added to ice. Ether extraction gave a mixture of 5- and *5′-acetyl bifluranol dimethyl ether* which was purified by thick layer chromatography on Kieselgel F_{254} developed in chloroform, to give a colourless oil ν_{max} 1685, 1660, 1620, 1585, 880, 825, 770 cm^{-1}; δ0·55 (CH_3CH_2, *t*, $J = 7$ Hz), 0·91 (CH_3CH, *d*, $J = 7$ Hz), ca 1·32 (CH_2–*m*), ca 2·52 (CH–CH)' 2·60 (1 × CH_3CO–), 3·80, 3·98 (2 × MeO), ca 6·70–7·30 (5 aromatic H's). This mixture of acetyl derivatives (150 mg) in toluene (10 ml) was refluxed with aluminium bromide (300 mg), and the reaction mixture added to ice. Ether extraction gave a mixture of *5-* and *5′-acetylbifluranol* as an oil (110 mg), ν_{max} 3400, 1650, 1620, 1600, 870, 815, 750 cm^{-1}. The oil (100 mg) in dioxan (3 ml) at O °C was stirred (1·5h, 20 °C) with M NaOH (0·8 ml) and 30% w/v hydrogen peroxide (0·1 ml). The mixture was then added to ice 2 M HCl. Ether extraction gave a mixture of 5- and *5′-hydroxybifluranol* as an oil, $\nu_{max_{3350}}$, 1625, 1610, 870, 830, 790, 735 cm^{-1}, which was purified by thick layer chromatography on Kieselgel F_{254}, developed in methanol–chloroform (1 : 40 v/v). The isomeric mixture was characterized as the trimethyl ether after treatment with diazomethane in ether, δ0·64 (CH_3CH_2, *t*, $J = 7$ Hz), 1·01 (*d*, CH_3CH_2–, $J = 7$ Hz), ca 1·47 (CH_2, *m*), ca 2·62 (CH–CH), 3·36 (2 × MeO), 3·92 (1 × MeO), 6·48–7·12 (5 aromatic H's).

5,5′-Dihydroxybifluranol: Bifluranol (Pope et al 1981) (4·5 g) in pyridine (30 ml) was treated (24 h, room temp.) with acetic anhydride (10 ml). After pouring into ice-water the product was crystallized from chloroform–ether (1 : 4 v/v) to give *bifluranol diacetate* (*erythro*-3,3′-difluoro-4,4′-diacetoxy-α′-ethyl-α′-methyl bibenzyl) (5·5 g), m.p. 118–120 °C, ν_{max} 1765, 1590, 1215, 1190, 990, 870, 840, 810, 780 cm^{-1}; δ0·57 (3 H, *t*, $J = 7$ Hz, Me), 0·95 (3 H, *d*, $J = 7$ Hz, Me), ca 1·40 (2 H, *m*, CH_2), 2·33 (6 H, *s*, 2 × MeCOO), ca 2·60 (2 H, *m*, CH–CH), 6·8–7·1 (6 H, *m*, aromatic). The diacetate (5 g) was ground with

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anhydrous aluminium chloride (10 g) and the mixture heated (0·5 h, 150 °C). The cooled mass was added portionwise to ice, stirred and extracted with chloroform. The chloroform was washed with water, dried over anhydr. Na_2SO_4 and concentrated. Ether (equal volume) was added, the solution treated with charcoal, and filtered. Evaporation of the filtrate and crystallization of the residue from chloroform and ether (1:1 v/v) gave *erythro-3,3'-difluoro-4,4'-dihydroxy-5-5'-diacetyl-α-ethyl-α'-methyl bibenzyl* (2·6 g), m.p. 194–6 °C, ν_{max} 1660, 1625, 880, 790, 765 cm^{-1}; δ0·63 (3 H, t, J = Hz, Me), 1·02 (3 H, *d*, J = 7 Hz, Me), ca 1·40 (2 H, *m*, CH_2), ca 2·60 (2 H, *m*, CH–CH), 2·66 (6 H, *s*, 2 × CH_3CO–), 7·12 (2 H, *d*, J = 10 Hz, C_2–H, C_2'–H'), 7·12 (2 H, *d*, J = 2 Hz, C_6–H, C_6'–H,). *Erythro*-3,3'-difluoro-4,4'-dihydroxy-5,5'-diacetyl-α-ethyl-α'-methylbibenzyl (900 mg) was suspended in dioxan (10 ml) plus M NaOH (6 ml) at 10 °C and 30% w/v hydrogen peroxide (0·8 ml) added dropwise. After stirring (1·5 h, 20 °C), the resulting solution was poured into crushed ice and excess M H_2SO_4. Extraction with ether (peroxide free) gave a yellow solid crystallized from benzene. This material in ethanol (5 ml) was treated with sodium borohydride (200 mg) at toom temp., and immediately acidified (2 M HCl) and extracted with ether. Crystallization from benzene gave pure *erythro-3,3'-difluoro-4,4',5,5'-tetrahydroxy-α-ethyl-α'-methylbibenzyl* (350 mg), m.p. 143–5 °C, ν_{max} 3350, 1620, 1015, 850 cm^{-1}; δ 0·67 (3 H, *t*, CH_3CH_2–, J = 7 Hz), 0·94 (3 H, *d*, CH_3CH–, J = 7 Hz), ca 1·20 (2 H, *m*,–CH_2–), ca 2·40 (2 H, *m*, –CH-CH–), 4·90 (OH), 6·40 (2H, J = 10 and 1 Hz,C_2,C_2'–H's), 6·50 (2 H, *d*, J = 1 Hz, C_6,$_6'$-H's). A sample of dihydroxy bifluranol in ether was treated with excess diazomethane to give the *tetramethyl ether*, δ3·85 (2 × MeO), 3·90 (2 × MeO).

Potassium bifluranol monosulphate and disulphate were synthesized by the method of Barford et al (1977a) to give potassium bifluranol monosulphate (Found: K, 10·1%; SO_4^{2-}, 22·9% $C_{17}H_{17}O_5F_2SK$ requires K, 9·5%; SO_4^{2-}, 23·4%) and potassium bifluranol disulphate (Found: K, 15·3%; SO_4^{2-}, 35·8% $C_{17}H_{16}O_8F_2S_2K_2$ requires K, 14·8%, SO_4^{2-}, 36·4%).

[5,5'-^{3}H]*Bifluranol monoglucuronide* was synthesized enzymically from [5,5'-^{3}H] bifluranol (Pope et al 1981) and uridine diphosphate glucuronic acid (Dutton & Storey 1962). [^{3}H] Bifluranol monoglucuronide and unchanged bifluranol were extracted into ethyl acetate from the acidified reaction mixture. The chromatographic characteristics of the glucuronide material before and after β-glucuronidase hydrolysis were determined by t.l.c. in solvents A to D (see thin layer chromatography). Bifluranol and bifluranol monoglucuronide were separable by t.l.c. in these solvent systems but no crystalline glucuronide was isolated.

Bifluranol monoglucuronide was tentatively identified by t.l.c. before and after hydrolysis to bifluranol and glucuronic acid by β-glucuronidase. It was distinguished from the diglucuronide by its R_F value and the fact that the diglucuronide is hydrolysed by β-glucuronidase to the monoglucuronide.

β-Glucuronidase (low in arylsulphatase) (BDH Chemicals, Poole, Dorset, U.K.); limpet arylsulphatase (Type III), potato acid phosphatase, calf intestinal mucosa alkaline phosphatase, uridine diphosphate glucuronyl transferase (Type II from rabbits) and uridine diphosphate glucuronic acid, all from Sigma Chemical Co. Ltd., Kingston, Surrey, U.K.; and 1,4-saccharolactone (Calbiochem Ltd., Bishops Stortford, Herts., U.K.) were purchased. All solvents used in isolation of material for mass spectrometry were redistilled from Analar grade material.

Thin layer chromatography

Aliquots (5 μl) of urine, bile and other materials were chromatographed on silica gel $60F_{254}$ plates (0·2 mm) (Merck 5735) in one or more of the several solvent systems (see Table 1).

Table 1. Thin layer chromatography of bifluranol, hydroxy-bifluranols and metabolites of bifluranol. Only metabolites I and III have been identified unequivocally. Authentic compounds I and II, and monohydroxy-bifluranol, are mixtures of the 5- and 5'-isomers, and compounds V and VI are probably mixtures of isomers.

		R_F values in solvents:					
	Metabolite	A	B	C	D	E	F
	Bifluranol	0·85	0·57	0·85	0·55	0·28	0·85
I	Bifluranol monosulphate	0·65	0·55	0·81	0	—	—
II	Bifluranol monoglucuronide	0·48	0·26	0·17	0	—	—
III	Bifluranol disulphate	0·43	0·44	0·54	0	—	—
IV	Bifluranol diglucuronide	0·22	0·10	0·04	0	—	—
V	Bifluranol glucuronide sulphate	0·31	0·26	0·09	0	—	—
VI	Bifluranol glucuronide phosphate	0·33	0·18	0·11	0	—	—
VII	Metabolite VIII glucuronide	0·42	0·22	0·13	0	—	—
	Metabolite VIII	0·84	0·48	0·84	0·25	0·15	0·83
	Monohydroxy bifluranol				0·43	0·18	0·82
	Dihydroxy bifluranol				0·35	0·08	0·78

T.l.c. was carried out on silica gel $60F_{254}$ (0·2 mm thickness) in solvent systems: (A) n-butanol–acetic acid–water (4:1:1 v/v); (B) n-propanol–ammonia soln. (sp. g. 0·88) (7:3 v/v); (C) n-butanol–1·5% aq. ammonium carbonate (2:1 v/v); (D) light petroleum (b.p. 40 °C)–diethyl ether–ethanol (8:8:1 v/v); (E) toluene–piperidine (5:2 v/v); (F) ethyl acetate–isopropanol–water (65:24:12 v/v).

The distribution of radioactivity on t.l.c. plates after chromatography was determined by removing 0·5 cm segments of the silica and counting in either Brays scintillant plus Cab-o-sil (10 ml) or Triton-toluene scintillant (10 ml) plus water (1 ml) (Pope et al 1981).

Autoradiograms of t.l.c. plates were obtained by exposing the plates to X-ray film for up to 21 days for samples containing more than 1 μCi of ^{3}H. Samples containing less than 1 μCi were impregnated with 10% 2,5-diphenyloxazole in benzene and exposed to X-ray film at −70 °C for up to 21 days; this procedure gave a 30-fold increase in sensitivity (Rogers 1973). Autoradiography and radioassay were used in combination to locate and quantify individual metabolites.

Hydrolysis of conjugates

β-Glucuronidase - aqueous samples (0·1 ml) in 0·2 M acetate buffer pH 5·0 containing 0·01 M NaF (sulphatase inhibitor) were incubated with 10000 Fishman units of β-glucuronidase (6 h, 37 °C). Control samples also contained 0·01 M 1,4-saccharolactone (β-glucuronidase inhibitor).

Sulphatase - aqueous samples (0·1 ml) in 0·1 M acetate-0·004 M NaCl buffer pH 5·0, containing 0·01 M 1,4-saccharolactone were incubated with arylsulphatase (0·1 units) (6 h, 37 °C). Control samples also contained 0·01 M NaF.

β-Glucuronidase and sulphatase - aqueous samples (0·1 ml) in 0·2 M acetate-0·004 M NaCl buffer pH 5·0, were hydrolysed as previously but without 1,4-saccharolactone and fluoride.

Phosphatase - aqueous samples (0·1 ml) in 0·2 M acetate buffer pH 5·0 or 0·2 M Tris buffer pH 9·5 were incubated with acid or alkaline phosphatase (1 unit) respectively (6 h, 37 °C).

Acid - aqueous samples (0·1 ml) in 2 M HCl were heated (15 min, 80–90 °C) then cooled.

Collection and analysis of excreta

Excreta, collected as described (Pope et al 1981), were analysed by t.l.c. before and after specific hydrolysis of conjugates. Authentic compounds were co-chromatographed or hydrolysed where appropriate to confirm identification.

Bile. Pooled bile samples were analysed before and after hydrolysis with β-glucuronidase and/or sulphatase or acid. Ferret and dog bile were also analysed after hydrolysis with phosphatase. Glucuronides were detected by spraying chromatograms with 0·2% naphtharesorcinol in acetone/9% H_3PO_4 (5 : 1) and heating (10 min, 120 °C). The position of blue bands corresponding to glucuronides was noted (Partridge 1948).

Reverse isotope dilution was used to identify bifluranol as the major biliary aglycone. Bifluranol (50 mg) was added to bile (1 ml) from animals dosed with [^{3}H] bifluranol. The samples were hydrolysed in 0·2 M acetate pH 5·0 (16 h, 37 °C) with β-glucuronidase (10000 Fishman units), acidified to 2 M HCl, heated (30 min, 80–90 °C) then cooled. The hydrolysed samples were extracted with ethyl acetate (3 × 5 ml), which removed >99% of radioactivity; after evaporation to dryness, each residue was redissolved in a minimum of diethyl ether and recrystallized from a mixture of diethyl ether and light petroleum (b.p. 40–60 °C) to constant specific radioactivity.

Urine and faeces. Urine and methanolic extracts of faeces were chromatographed by t.l.c. (system A) and the distribution of radioactivity determined by autoradiography and radioassay.

Isolation of metabolite VIII from bile for mass spectrometry

Bile from a rat which had received [^{3}H]bifluranol (200 mg kg^{-1}, orally) was acidified with 2·5 M HCl (1 ml) and extracted with ethyl acetate (5 × 10 ml) to remove glucuronides. The combined extracts were evaporated to dryness under vacuum and the residue in 0·2 M acetate buffer pH 5·0 (3 ml) was incubated (8 h, 37 °C) with β-glucuronidase (15000 Fishman units). The mixture was acidified with 2·5 M HCl (1 ml) and the aglycones extracted into ethyl acetate (4 × 5 ml). The extract was concentrated and chromatographed by t.l.c. (system D); the radioactive band corresponding to metabolite VIII (Table 1) was eluted from the silica with ethanol. The methyl derivatives of metabolite VIII (50 μg), bifluranol and mono- and di-hydroxybifluranol were prepared by treating ethanolic solutions (1 ml) with excess diazomethane in diethyl ether (1 ml); the mass spectra were subsequently determined on an AEI MS12 mass spectrometer at 70 eV.

RESULTS

Analysis of biliary radioactivity. Complete hydrolysis of rat, ferret and dog bile from animals receiving [^{3}H]bifluranol, and recrystallization of the ^{3}H-labelled material with unlabelled bifluranol gave specific activities of bifluranol of 0·91, 1·07 and 22·4 μCi mg^{-1} respectively (calculated theoretical values of 0·93 (98%), 1·04 (103%) and 22·4 μCi mg^{-1} (100%)).

T.l.c. of bile from animals dosed with [^{3}H]-bifluranol revealed several metabolites which were tentatively identified from their R_F values in the different solvent systems (Table 1) before and after various hydrolysis procedures, and were approximately quantified by elution and determination of the radioactivity of the eluates (see Table 2). Additional unidentified minor metabolites were present but each of these comprised <0·5% of the total radioactivity present in the bile.

Metabolite I (bifluranol monosulphate) was not labile to sulphatase or β-glucuronidase but was hydrolysed in acid to give bifluranol. Synthetic bifluranol monosulphate had similar t.l.c. properties and was labile to hydrolysis with acid but not sulphatase.

Metabolite II (bifluranol monoglucuronide) gave a positive glucuronide test with naphthoresorcinol and was labile to β-glucuronidase, but not sulphatase, to yield bifluranol. Metabolite II had similar t.l.c. properties to synthetic bifluranol monoglucuronide.

Metabolite III (bifluranol disulphate) was not labile to β-glucuronidase, gave bifluranol monosulphate on hydrolysis with sulphatase, bifluranol on acid hydrolysis, and had similar t.l.c. properties to synthetic bifluranol disulphate.

Metabolite IV (bifluranol diglucuronide) gave a positive colour reaction for glucuronide with naphthoresorcinol, was hydrolysed by β-glucuronidase to give bifluranol, but was not labile to sulphatase. Partial hydrolysis with β-glucuronidase gave bifluranol monoglucuronide.

Metabolite V (bifluranol monoglucuronide monosulphate) gave a positive colour reaction for glucuronide with naphthoresorcinol, was hydrolysed by β-glucuronidase to give bifluranol monosulphate, and by sulphatase to give bifluranol monoglucuronide; it was hydrolysed by β-glucuronidase and then acid to give bifluranol.

Metabolite VI (bifluranol monoglucuronide monophosphate) was not labile to phosphatase hydrolysis whilst in bile. However, following isolation by t.l.c. the material was labile to acid phosphatase to give bifluranol monoglucuronide and to β-glucuronidase and acid to give bifluranol. The material was not hydrolysed by sulphatase or alkaline phosphatase.

Metabolite VII was hydrolysed by β-glucuronidase but not sulphatase or acid to give metabolite VIII, which was not identical to bifluranol. The aglycone (Metabolite VIII) was subsequently isolated and identified by mass spectrometry.

Mass spectrometry of metabolite VIII: The mass spectra fragmentation pattern of metabolite VIII methyl ether shows major peaks at *m/z* 197(60), 183(32), 167(100), 153(91) and 139(83), with per-

Table 2. Biliary-excreted metabolites of [^{3}H]bifluranol.

Metabolite	percentage of total biliary ^{3}H					
	rat (200 μg kg^{-1})			ferret (60 μg kg^{-1})		dog (50 μg kg^{-1})
	oral	intravenous		oral		oral
	male (4)	female (3)	male (1)	male(3)	female (3)	(3)
Bifluranol	n.d.	5	n.d.	n.d.	n.d.	n.d.
I Bifluranol monosulphate	n.d.	2	n.d.	2	2	3
II Bifluranol monoglucuronide	48	41	57	2	5	8
III Bifluranol disulphate	n.d.	n.d.	n.d.	14	15	3
IV Bifluranol diglucuronide	8	15	2	2	2	8
V Bifluranol glucuronide sulphate	40	23	35	50	69	68
VI Bifluranol glucuronide phosphate	n.d.	n.d.	n.d.	32	8	10
VII Metabolite VIII glucuronide	4	11	2	n.d.	n.d.	n.d.
% dose present in bile*	68	50	81	36	39	30

Quantification is based on t.l.c. radioassay following chromatography in n-butanol–acetic acid–water (4 : 1 : 1 v/v), except for metabolite VII, which was measured following hydrolysis with β-glucuronidase and chromatography in light petroleum (b.p. 40 °C)-diethyl ether–ethanol (8 : 8 : 1 v/v).

n.d. indicates not detected.

* Bile samples were collected for at least 6 h (Pope et al 1981).

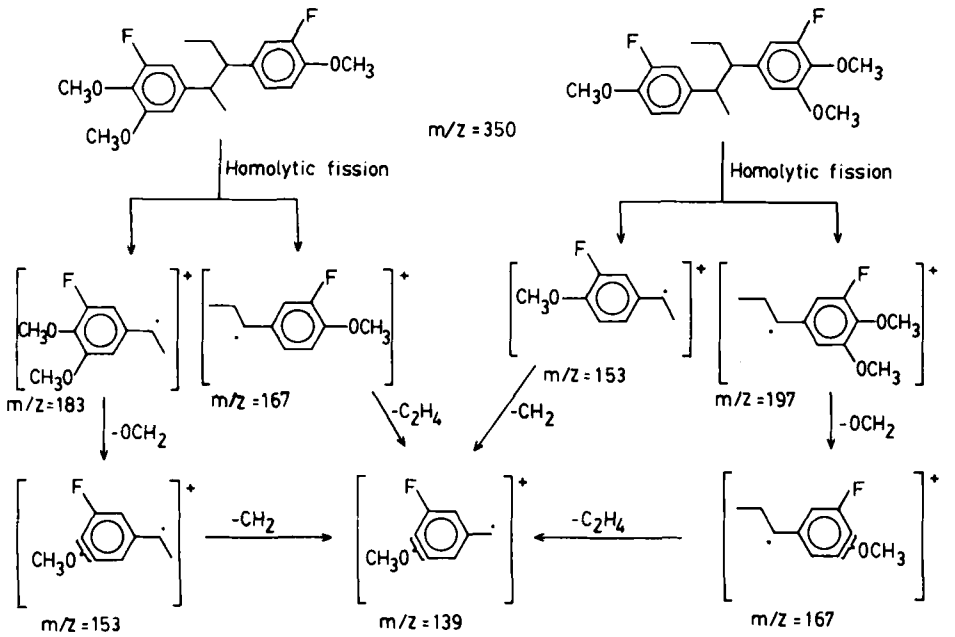

FIG. 1. Proposed molecular fragmentation pattern of metabolite VIII methyl ether.

centage of base peak height shown in parentheses, while that of bifluranol methyl ether shows major peaks at *m/z* 320(8), 167(100), 153(79) and 139(28). The fragmentation pattern of the methyl ether of monohydroxy bifluranol is similar to that of metabolite VIII methyl ether, with peaks at *m/z* 350(20), 197(88), 183(100), 167(84), 153(59), 139(51), whilst that of dihydroxybifluranol methyl ether only shows major peaks at *m/z* 380(17), 197(100) and 183(81). The proposed fragmentation pattern of the methyl ether of metabolite VIII is shown in Fig. 1 and is based on metabolite VIII being derived from bifluranol by oxidation of one of the aromatic rings to give two isomers. This rationalization of the fragmentation pattern of metabolite VIII methyl ether is supported by the similar fragmentation pattern of monohydroxy bifluranol methyl ether. The greater abundance of the fragments *m/z* 139, 153 and 167 with metabolite VIII methyl ether results from these being formed by the fragmentation of both isomers of monohydroxy bifluranol methyl ether.

Faecal and urinary radioactivity. After oral administration of [^{3}H]bifluranol to rat, ferret and dog the metabolite patterns in urine and faeces were similar to those found in bile; metabolites were quantified by elution from the silica gel and determination of radioactivity (Table 3).

DISCUSSION

Analysis of biliary radioactivity after administration of [^{3}H]bifluranol indicates that it is present in the bile as six different conjugates. Despite its extensive absorption after oral administration and excretion in the bile as its conjugates, unchanged bifluranol accounts for most faecal radioactivity. It undergoes extensive enterohepatic circulation in rat and by analogy with diethylstilboestrol (Clark et al 1969; Fischer et al 1973), its biliary-excreted conjugates probably undergo hydrolysis by intestinal bacterial enzymes before reabsorption of the free drug. The large amounts of unconjugated bifluranol found in the faeces may be due to bacterial hydrolysis in the caecum or bowel, where drug reabsorption is poor.

The urinary metabolites of bifluranol are similar to those in bile. Small amounts only of free drug appear in the urine (rat <1%, ferret 2%, or dog <0·1% dose) and this free drug may arise from breakdown of conjugates in the urine.

The major conjugation of bifluranol with glucuronic acid was as expected, but the appearance of the double conjugate with glucuronide and sulphate is unusual in that it is formed by all three species. Although similar double conjugates have been observed with oestriol in man (Stoa & Levitz 1968) and diethylstilboestrol in guinea-pig (Barford et al 1977b), glucuronide sulphate diconjugates of other

Table 3. Excretion of metabolites in urine and faeces of rat, ferret and dog following oral administration of [^{3}H]-bifluranol.

	percentage of dose									
	Rat (200 μg kg^{-1} n = 3)				Ferret (60 μg kg^{-1}, n = 3)				Dog (50 μg kg^{-1}, n = 3)	
	faeces		urine		faeces		urine		faeces	urine
Metabolite	male	female	male	female	male	female	male	female	male	
Bifluranol + VIII	72	82	1	3	56	77	3	2	81	n.d.
Bifluranol monosulphate (I)	5	2	3	2	20	6	1	1	9	1
Bifluranol disulphate (III) + bifluranol monoglucuronide (II) + metabolite VIII glucuronide (VII)	4	<1	1	<1	13	8	2	2	n.d.	1
Bifluranol diglucuronide (IV)	<2	n.d.	1	<1	2	n.d.	n.d.	1	n.d.	1
Bifluranol glucuronide sulphate (V) + bifluranol glucuronide phosphate (VI)	<2	n.d.	2	2	2	<1	<1	<1	<2	1
Unidentified	2	n.d.	n.d.	n.d.	3	n.d.	n.d.	d.n.	3	n.d.
% of dose excreted:	88	85	8	7	93	92	7	7	95	4

Metabolites were qauntified by radioassay following chromatography in n-butanol–acetic acid–water (4 : 1 : 1 v/v).

compounds, e.g. nitrocatechol, 4-hydroxybiphenyl, cyclohexylphenol, oestrone and diethylstilboestrol in rat bile are obtained only after parenteral administration of the sulphate esters (Flynn et al 1966; Hearse et al 1969; Gatehouse et al 1972; Barford et al 1977a). The appearance of a glucuronide phosphate diconjugate in ferret and dog bile is unusual, the only other reported conjugation of a phenolic compound with phosphate being that of phenol in the cat (Capel et al 1974).

The major species differences in the metabolism and excretion of bifluranol are (i) the monoglucuronide monophosphate is excreted by ferret and dog but not by the rat, (ii) aromatic hydroxylation occurs in rat, but is not detected in ferret or dog, (iii) the formation of double conjugates increases from 50% of total conjugates in rat to 75% in dog, and to almost 100% in ferret, (iv) only the ferret and dog excrete bifluranol disulphate in bile.

Oxidative metabolism of bifluranol was observed only in the rat, a species excreting conjugates of material other than unchanged drug. It is not possible to obtain a precise measure of the drug hydroxylation for although the biliary excretion of metabolite VII in male rats is calculated at 4% of the dose (from radioactivity of VII) ^{3}H may be eliminated during hydroxylation by the NIH shift (Guroff et al 1967). Therefore metabolite VII contains one or two ^{3}H atoms, depending on whether the hydroxylation of one of the aromatic rings leads to loss or retention of ^{3}H. If retention is complete, which is unlikely in view of the ^3H_2O in the urine, then the figure of 4% of biliary ^{3}H for metabolite VII in male rats is accurate. If loss of ^{3}H is complete, the drug specific radioactivity will have been halved and the oxidation of bifluranol by male rats will be 8% of the dose. Since 4% of the ^{3}H label was converted to ^3H_2O in male rats (see Pope et al 1981), this oxidation of [^{3}H]-bifluranol appears to involve loss of ^{3}H and the true extent of oxidation is probably closer to 8 than 4%. However, in female rats 11% of the biliary ^{3}H was present as the glucuronide of the oxidation product (metabolite VII), although only 0·7% of the dose was converted to ^3H_2O (Pope et al 1981). Thus the oxidation of [^{3}H]bifluranol in female rats is not apparently associated with significant production of ^3H_2O, in contrast to the males, and may result from a different mechanism of oxidation.

Whilst the fragmentation pattern of the methyl ether of metabolite VIII is qualitatively identical to that of the methyl ether of monohydroxybifluranol, the actual identity of metabolite VIII before methylation has not been clearly established. Its t.l.c. characteristics compared with authentic mono-

hydroxybifluranol are not identical indicating that the hydroxyl group may not be at position-5 in the aromatic ring or that metabolite VIII is the *O*-methyl derivative of the primary oxidation product. This could be formed by the action of catechol-*O*-methyl transferase in a manner similar to that observed with diethylstilboestrol catechols (Engel et al 1976; Sossi & Dingell 1978).

The total of 4–8% dose for oxidation products of bifluranol in male rat bile (dose of 0·2–20 mg kg^{-1}) is significantly lower than the 30% reported after intraperitoneal administration of the stilbene oestrogen diethylstilboestrol (dose 10 mg kg^{-1}) (Metzler 1975, 1976). Many chemicals and drugs have been shown to undergo oxidative metabolism with subsequent tissue binding (Gillette 1974) which may lead to cytotoxic processes. The lower extent of oxidation of bifluranol compared with diethylstilboestrol may decrease the possibility of metabolism-dependent cytotoxicity. This reduced potential for metabolism-mediated toxicity of bifluranol would favour bifluranol as a safer substitute for diethylstilboestrol for indications such as prostatic hypertrophy.

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