Biochemical Pharmacology, Vol. 44, No. 9, pp. 1717–1724, 1992.
Printed in Great Britain.

0006-2952/92 $5.00 + 0.00

OXIDATIVE DEHALOGENATION OF 2-FLUORO-17α-ETHYNYLOESTRADIOL *IN VIVO*

A DISTAL STRUCTURE–METABOLISM RELATIONSHIP OF 17α-ETHYNYLATION

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(*Received* 22 *April* 1992; *accepted* 4 *August* 1992)

Abstract—Metabolic activation to catechols and their oxidation products is variously considered to contribute to the genotoxic, cytotoxic, transforming and tumour-promoting activities of exogenous steroidal oestrogens. 2-Fluoro-17α-ethynyloestradiol (2-FEE_2) was synthesized as a prototype of pharmacologically active derivatives of 17β-oestradiol which are resistant to metabolic activation *in vivo*. It possessed high affinity for the rat uterine oestrogen receptor and was oestrogenic in rats. Biliary metabolites of [6,7-^{3}H]2-FEE_2 (0.73 μmol/kg, 157 μg/kg, i.v.) in female rats were characterized: 87% of the radiolabel was excreted, principally as 2-FEE_2 glucuronide, over 6 hr. Although 2-fluoro-17β-oestradiol is not metabolized to C-2 oxygenated products *in vivo*, 2-FEE_2 underwent rapid and appreciable oxidative defluorination. 2-Hydroxy-17α-ethynyloestradiol and 2-methoxy-17α-ethynyloestradiol represented, respectively, 8% and 13% of the dose. Fluorination nevertheless restricted C-2 oxygenation to *ca.* 28% of that which 17α-ethynyloestradiol undergoes in female rats. C-4 oxygenation of 2-FEE_2, resulting in catechol formation, occurred but to a lesser extent (*ca.* 12% of dose). None of the major and identified minor biliary metabolites was a product of metabolic activation at the ethynyl function. A mechanistic rationalization of the long range enhancement by 17α-ethynylation of oxidative defluorination at C-2 is presented.

The carcinogenicity of exogenous steroidal oestrogens [1, 2], which does not correlate with the steroids' hormonal activity [1, 3, 4], has been frequently but inconclusively [5] attributed to genetic damage mediated by chemically reactive metabolites [6–8]. Although a mechanism of DNA damage [9, 10] remains to be substantiated [5], the reactive intermediates most frequently implicated are *o*-quinone [10, 11] and/or semi-quinone [12] derivatives of catecholoestrogens, the major primary metabolites of oestrogens in man [13] and experimental animals [14], rather than arene-oxide precursors of the catechols [15, 16]. C-2 hydroxylation of 17β-oestradiol (E_2§) and oestrone (E_1) predominates in the liver [5, 15, 17] and *in vivo* [14] but 4-hydroxy-E_2 (4-OHE_2), probably due to a higher bioavailability, is a more potent carcinogen than 2-OHE_2 for the kidney carcinoma of Syrian hamsters [18].

The proposed role of aromatic hydroxylation in carcinogenesis was apparently confirmed by the finding that 2-fluoro-17β-oestradiol (2-FE_2), which, by virtue of the strength of a C-F bond, was presumed to be refractory to oxidative dehalogenation, was oestrogenic but not nephrocarcinogenic [1, 4, 5] in male Syrian hamsters [3]. Consistent with this hypothesis, 2-FE_2 is not subject to oxidative defluorination and catechol formation *in vivo* [19]. However, it is reported to undergo oxidative defluorination by microsomal systems, though highly variable results have been obtained [20–22].

Aside from any role in tumour initiation, catecholoestrogens may play an additional part by stimulating cell proliferation [23]. The relevant biological activities of oestrogen metabolites are not restricted to tumor initiation and development either, since 2-methoxy-E_2 (2-$MeOE_2$) is toxic towards certain dividing cancer cells *in vitro* [23].

In the present study, the effect on oestrogen aromatic hydroxylation of a C-2 fluorine substituent has been further investigated using 2-fluoro-17α-ethynyloestradiol (2-FEE_2). 17α-Ethynyloestradiol (EE_2), the usual oestrogen of combined oral contraceptives, undergoes extensive C-2 hydroxylation in man [24] and rats [25, 26], and is metabolized

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§ Abbreviations: E_1, oestrone; [^{3}H]E_1, [6,7-^{3}H]oestrone; FE_1, fluoroestrone; E_2, 17β-oestradiol; 2-FE_2, 2-fluoro-E_2; OHE_2, hydroxy-E_2; 2-$MeOE_2$, 2-methoxy-E_2; EE_2, 17α-ethynyloestradiol; 2-FEE_2, 2-fluoro-EE_2; [^{3}H]2-FEE_2, [6,7-^{3}H]2-FEE_2; 2-$OHEE_2$, 2-hydroxy-EE_2; 2-$MeOEE_2$, 2-methoxy-EE_2; 2-F-4-$OHEE_2$, 2-fluoro-4-hydroxy-EE_2; 2-F-4-$MeOEE_2$, 2-fluoro-4-methoxy-EE_2; THF, tetrahydrofuran; RCF_{50}, relative competition factor at 50% competition; EI, electron impact.

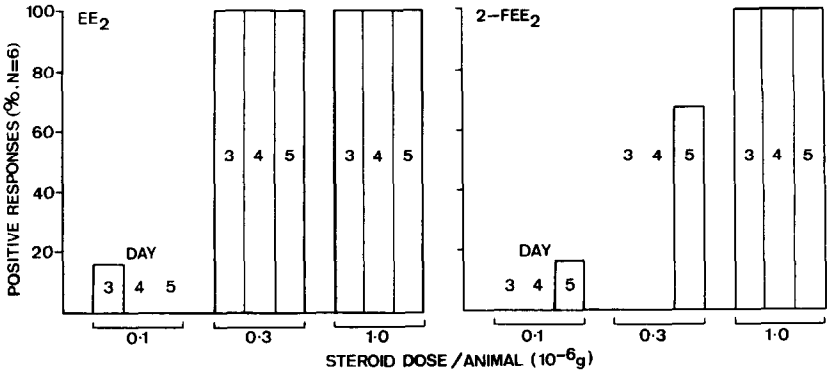

Fig. 1. The vaginal response in rats to the oestrogenic activity of EE_2 and 2-FEE_2 on days 3, 4 and 5 after s.c. dosing.

to reactive intermediates by microsomes [25–28] and *in vivo* [26]. In addition to forming reactive intermediates via catechols [4], EE_2 can be activated by oxygenation of its ethynyl moiety to an oxirene function [29]. Pharmacological advantages might accrue from blocking activation since it has been postulated that the oxidative metabolism of EE_2 could contribute not only to initiation of hepatocarcinomas [28, 30], such as those associated with oral contraceptives [2], but also to cell transformation [31] and tumour promotion [32].

A brief report on this work has been published elsewhere [33].

MATERIALS AND METHODS

Chemicals and enzymes. [6,7-^{3}H]E_1 ([^{3}H]E_1) (46 Ci/mmol; radiochemical purity, 99%) was purchased from NEN Research Products (Dreiech, Germany). 2-OHEE_2 and 2-MeOEE_2 were provided by Dr W. Slikker, Jr. (National Centre for Toxicological Research, Jefferson, AR, U.S.A.). All other steroids and the conjugate hydrolases were products of the Sigma Chemical Co. (Poole, U.K.) *N*-Fluoropyridiniumtriflate was obtained from the Aldrich Chemical Co. (Gillingham, U.K.). HPLC-grade solvents were from Fisons plc (Loughborough, U.K.).

Chemical and radiochemical syntheses. Unlabelled 2-FEE_2 was prepared as previously described [34] and [^{3}H]2-FEE_2 by an appropriately modified form of this synthesis. [^{3}H]E_1 (27 mg, 100 μmol; 1.5 mCi) and *N*-fluoropyridiniumtriflate (49.4 mg, 200 μmol) in 4 mL of dry 1,1,2-trichloroethane were stirred under reflux for 24 hr to give a 40% yield of [^{3}H]2- and 4-FE_1 as determined by radiometric HPLC [19] using a Techopak C_{18} column (10 μm; 30 cm × 0.39 cm i.d.; HPLC Technology, Macclesfield, U.K.) eluted with acetonitrile (20–75% over 40 min) in $NH_4H_2PO_4$ (43 mM, pH 3.0) at 1.5 mL/min; the isomers (R_t 23.8 min) were irresolvable on the reversed phase column. The steroidal material was recovered as a methanol solution [19], and the mixture of [^{3}H]2- and 4-FE_1 isolated on a Techoprep C_{18} column (25–40 μm; 25 cm × 0.8 cm i.d.; HPLC Technology) by elution with acetonitrile (40–75% over 20 min) in $NH_4H_2PO_4$ buffer at 2.5 mL/min. Eluate was monitored at 280 nm. Pooled eluate fractions were evaporated to dryness *in vacuo* and reconstituted in water. The [^{3}H]2-/4-FE_1 mixture (11.6 mg, 40.2 μmol; 604 μCi) was recovered by ethyl acetate extraction (0.5 vol. × 3) in 40% yield from [^{3}H]E_1 at a radiochemical purity of 90%. It was quantitatively acetylated with acetic anhydride (10 mL) in pyridine (10 mL) at 110° over 3 hr. The isomeric acetates were resolved, to give radiochemically pure [^{3}H]2-FE_1 acetate (3.3 mg, 10.3 μmol; 182 μCi) in 30% yield from the mixture, by elution from a silica HPLC column [19]. [^{3}H]2-FE_1 acetate in pure ethanol was deacetylated with excess K_2CO_3 [19]. [^{3}H]2-FE_1 (3.25 mg, 11.9 μmol; 180 μCi) in dry dichloromethane (5 mL) was stirred with *tert* butyldimethyl chlorosilane (400 mg) and imidazole (350 mg) at 50° for 3 hr. Solvent was evaporated under N_2, brine (2 mL) and saturated aqueous $CuSO_4$ (2 mL) were added, and the [6,7-^{3}H]2-fluoro-3(*O-tert*-butyldimethylsilyl)E_1 extracted with ethyl acetate (5 mL × 2). *N*-Butyl lithium (560 μmol) was added via a syringe to a solution of trimethylsilyl acetylene (350 mg, 3.57 mmol) in dry tetrahydrofuran (THF) (0.5 mL) at −78° under N_2, and the mixture stirred for 1 hr. [6,7-^{3}H]2-Fluoro-3(*O-tert*-butyldimethylsilyl)E_1 (3.42 mg, 11.9 μmol; 180 μCi) in THF (1.5 mL) was added. The whole was stirred at −78° for 1 hr and allowed to attain room temperature overnight. Tetrabutyl ammonium fluoride in THF (0.5 mL) was added with stirring for an additional 30 min. The solvent was evaporated under N_2 and the product extracted with ether (4 mL × 2). [^{3}H]2-FEE_2 was obtained in 82% yield from [^{3}H]2-FE_1 as determined by eluting an aliquot of a propan-2-ol-hexane (1:1, v/v) solution from a Lichrosorb diol column (10 μm; 30 cm × 0.39 i.d.; HPLC Technology) with propan-2-ol-hexane (5:95, v/v; 1.5 mL/min); the absence of residual [^{3}H]-2-FE_1 was thus established. It was purified by elution from a Techopak C_{18} column as described above. The final product, prepared in 4% yield from [^{3}H]-E_1, co-chromatographed with authentic unlabelled 2-FEE_2 and gave an identical electron impact (EI) mass spectrum [34].

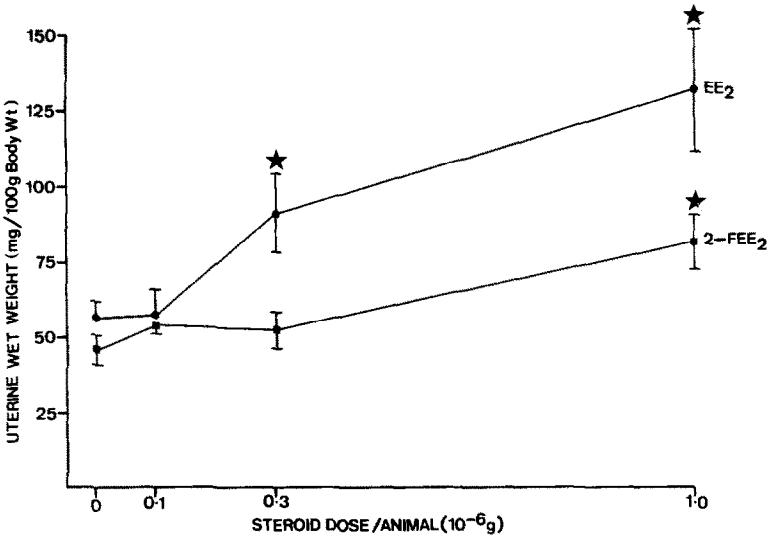

Fig. 2. The uterine (wet weight) response in rats to the oestrogenic (uterotrophic) activity of EE_2 and 2-FEE_2. ★ Uterine weights significantly ($\alpha = 0.05$) greater than those of animals administered vehicle alone.

Receptor binding affinity. The relative binding affinity of EE_2 and 2-FEE_2 for the rat uterine (cytosolic) oestrogen receptor was estimated by a competitive binding assay using [6,7-^{3}H]E_2 as the reference ligand [35]. It was expressed as the RCF_{50}, i.e. the amount of compound effecting 50% inhibition of radiotracer binding divided by the amount of unlabelled E_2 producing equal inhibition.

Oestrogenic activity. The oestrogenic activity of EE_2 and 2-FEE_2 was assessed from vaginal [36] and uterine [37] responses. Female Wistar Han rats (200 g body wt) were ovariectomized, and 8 days later administered by s.c. injection a single dose (0.1, 0.3 or 1.0 μg; N = 6 for each dose) of steroid in 0.5 mL of benzyl benzoate–castor oil (1:4, v/v). Controls received vehicle alone. Vaginal smears were obtained on days 3, 4 and 5 after injection. A condition of proestrus, oestrus or metoestrus, i.e. an absence of leucocytes from the smear, was designated a positive response and scored one [36]. Uterotrophic activity was determined by measuring the uterine wet weight on the fifth day; the uterine ratio was calculated as the uterine weight (mg) per 100 g body weight, and the data (test vs vehicle control) analysed using the Dunnett Test ($\alpha = 0.05$).

Metabolic studies. Female Wistar rats (205 ± 24 g body wt; mean ± SD, N = 4) were anaesthetized with urethane (1.4 g/kg in isotonic saline given i.p.), and cannulated via the common bile duct and jugular vein. [^{3}H]2-FEE_2 (0.73 μmol/kg, 157 μg/kg, 1.59–1.99 μCi) was administered i.v. as a solution in saline–ethanol (7:3, v/v). Bile was collected hourly for 6 hr. It was sampled for measurement of radioactivity [19].

Analyses of biliary metabolites. Unconjugated radiolabelled steroid in bile was estimated by extraction from neutral solution with ether [38]. Conjugates of [^{3}H]2-FEE_2 and its metabolites were hydrolysed with β-glucuronidase and β-glucuronidase–arylsulphohydrolase [19] in the presence of 10 mM ascorbate to prevent oxidation of catecholoestrogens [39]. Ether-extracted metabolites [19] and unlabelled standards were analysed on either a Techopak C_{18} or Lichrosorb diol column linked to an LKB 2249-2152 HPLC system, and metabolites were eluted with acetonitrile (20–75% over 40 min or 20–75% over 80 min) in $NH_4H_2PO_4$ or propan-1-ol (5–30% over 30 min, then 30–75% over 20 min) in hexane, respectively. The flow rate was 1.5 mL/min. Eluate was monitored at 280 nm [19]. Radiolabelled metabolites were quantified either by fraction collection and off-line counting or with a radiometric detector [19]. Recoveries of chromatographed radioactivity were 80–100% and 70–85% for the C_{18} and diol columns, respectively.

Isolation and identification of metabolites. [^{3}H]2-FEE_2 (5.75 mg/kg, 1.82–2.15 μCi) was administered i.v. to anaesthetized and cannulated female Wistar rats (223 ± 16 g body wt). Bile was collected in a single 3-hr fraction. Aliquots (0.43–0.72 mL, 300–500 μg equivalents 2-FEE_2) were concentrated to dryness under N_2 at 65°. The metabolite conjugates were hydrolysed with H-2 preparation [β-glucuronidase (6×10^3 U)–arylsulphohydrolase (300 U)] in 0.1 M sodium acetate, pH 5.0, containing 10 mM ascorbate, at 37° over 16 hr. Liberated aglycones were extracted with ether (5 mL × 2). They were isolated by eluting portions of a methanol solution from a diol column with the gradient of propan-1-ol in hexane. Purified metabolites were characterized by direct-probe EI and chemical ionization mass spectrometry [14].

RESULTS

Receptor binding affinity

2-FEE_2, having an RCF_{50} of 1.5, exhibited a high affinity for the rat uterine oestrogen receptor,

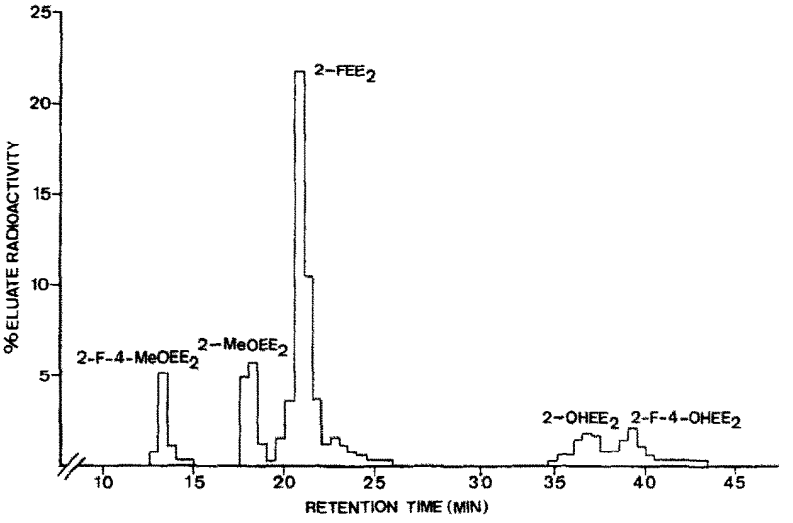

Fig. 3. Radiochromatogram (diol column) of the aglycone metabolites of [^{3}H]2-FEE$_2$ from female rats. Metabolites were identified by co-chromatography and mass spectrometry.

although its affinity was somewhat less than that of EE_2 (RCF_{50} 0.5); by definition, the RCF_{50} of E_2 is 1.0.

Oestrogenic activity

2-FEE$_2$ was a potent oestrogen in the vaginal cytology (quantal response) and uterotrophic activity (graded responses) assays of oestrogenicity even though it was apparently less active than EE_2. A few positive vaginal responses were seen at a dose of 0.1 μg/animal, and full responses (100% positive smears at day 3–5) to EE_2 and 2-FEE$_2$ were obtained at 0.3 and 1.0 μg/animal, respectively (Fig. 1). Significant (α = 0.05, test vs vehicle control) increases in the uterine weight ratio occurred over 5 days following the s.c. administration of EE_2 and 2-FEE$_2$ at a dose of 0.3 and 1.0 μg/animal, respectively (Fig. 2).

Metabolism

Intravenously administered [^{3}H]2-FEE$_2$ (157 μg/kg) was rapidly and extensively eliminated in bile by female rats: 47.0 ± 11.7% of the dose was excreted during the first hour, 18.4 ± 1.5% within 1–2 hr and 87.0 ± 6.0% over 0–6 hr. Although ether-extractable, i.e. apparently unconjugated, steroids, constituted about 5–10% of the radiolabelled material, the extraction by ether of 61–68% and 70–78% biliary radioactivity following incubation with β-glucuronidase and β-glucuronidase–arylsulphohydrolase, respectively, indicated that [^{3}H]2-FEE$_2$ and its metabolites were excreted in bile principally as glucuronides.

The aglycones liberated by enzymic hydrolysis were incompletely resolved (due to partial co-elution of 2-FEE$_2$ and 2-MeOEE$_2$) on a C_{18} column into five components; the major fraction (R_t 24 min) and the most polar metabolite (R_t 17.5 min) co-chromatographed with authentic 2-FEE$_2$ and 2-OHEE$_2$, respectively.

The aglycones were resolved on a diol column (Fig. 3), quantified (Table 1), and three of them preliminarily identified by co-chromatography with authentic 2-FEE$_2$ (R_t 21 min), 2-OHEE$_2$ (R_t 37 min) and 2-MeOEE$_2$ (R_t 18.5 min). The metabolite profile was qualitatively unchanged over the 6 hr following dosing, and the proportions of the metabolites in 0–1 hr bile collections were essentially the same as those in pooled, i.e. 0–6 hr, bile collections.

Female rats administered a large (5.75 mg/kg) i.v. dose of [^{3}H]2-FEE$_2$ excreted 70.5 ± 9.9% of the radiolabel in bile over 0–3 hr (cf. 75% of 157 μg/kg dose). β-Glucuronidase and arylsulphohydrolase together effectively hydrolysed the biliary conjugates, 76–78% of the radioactivity being extractable with ether after hydrolysis. The chromatographic (diol column) profile of deconjugated metabolites was qualitatively and quantitatively (Table 1) similar to that obtained following administration of the low dose.

2-FEE$_2$, 2-OHEE$_2$ and 2-MeOEE$_2$ isolated from bile were conclusively identified by the detailed match of their EI mass spectra (Table 2) with those of the corresponding authentic compounds; for example, characteristic ions arising from D-ring fragmentation [40] were present at m/z 231, m/z 229 and m/z 243, respectively. The anticipated pseudomolecular ions ($[M + 1]^+$), m/z 315, m/z 313 and m/z 327, and prominent dehydration fragments ($[M + 1 - H_2O]^+$) [14] were formed under chemical ionization conditions. The least polar metabolite (R_t 13.5 min), which eluted before 2-MeOEE$_2$ on a diol column (Fig. 3), yielded a putative molecular ion (m/z 344) and fragment ions which were fluorinated analogues of the molecular ion and major fragments, respectively, in the spectrum of 2-MeOEE$_2$ (Table 2). This metabolite also formed a pseudomolecular ion at m/z 345, and was deduced to be 2-F-4-MeOEE$_2$. The most polar metabolite, which unexpectedly eluted after 2-OHEE$_2$ on both diol (R_t 39.5 min; Fig. 3) and C_{18} (19.5 min) columns, yielded a putative molecular ion (m/z 330) and major

Table 1. Metabolites of [^{3}H]2-FEE$_2$ in female rats

Metabolite	% chromatographed radiolabel	
	Low dose (157 μg/kg)	High dose (5.75 mg/kg)
2-FEE$_2$	46.0 ± 5.5	49.4 ± 2.9
2-OHEE$_2$	7.7 ± 1.3	10.3 ± 1.1
2-MeOEE$_2$	12.5 ± 3.5	13.2 ± 2.4
2-F-4-OHEE$_2$	5.2 ± 1.6	8.5 ± 2.0
2-F-4-MeOEE$_2$	6.4 ± 2.6	2.6 ± 0.2

Metabolites liberated from biliary conjugates (0–1 hr and 0–3 hr bile collections from low- and high-dosed rats, respectively) were resolved on a diol column, quantified by scintillation counting, and identified by co-chromatography (2-FEE$_2$, 2-OHEE$_2$ and 2-MeOEE$_2$) and mass spectrometry.
Values are means ± SD, N = 4.

Table 2. Mass spectra (direct-probe EI) of [^{3}H]2-FEE$_2$ and its metabolites isolated from bile of female rats

Compound	Molecular and fragment ions
2-FEE$_2$	314 ($M^{+\cdot}$, 32), 296 (4), 268 (4), 246 (19), 231 (100) 217 (7), 203 (7), 190 (13), 178 (53), 163 (17), 151 (24)
2-OHEE$_2$	312 ($M^{+\cdot}$, 66), 244 (32), 229 (100), 215 (7), 201 (6), 188 (16), 176 (43), 161 (42), 149 (24)
2-MeOEE$_2$	326 ($M^{+\cdot}$, 100), 258 (32), 243 (96), 231 (20), 215 (4), 202 (12), 190 (42), 176 (27), 163 (34), 137 (34)
2-F-4-OHEE$_2$	330 ($M^{+\cdot}$, 50), 262 (50), 247 (63)
2-F-4-MeOEE$_2$	344 ($M^{+\cdot}$, 78%), 276 (35), 261 (100), 220 (29), 208 (62)

Relative intensities given in parentheses.

fragments (Table 2) which indicated that it was 2-F-4-OHEE$_2$. The minor metabolite (R_t 23 min; *ca.* 5% chromatographed radiolabel) eluted immediately after 2-FEE$_2$ (Fig. 3) was not identified. The metabolic fate of 2-FEE$_2$, constructed from these identifications, is shown in Fig. 4.

DISCUSSION

Chemical substitution has been used to modify both the rate and routes of metabolism of E$_2$ without loss of pharmacological activity [41]: the presence of the 17α-ethynyl group in EE$_2$ markedly reduces first-pass hepatic metabolism, and thus provides a drug with the required pharmacodynamic and pharmacokinetic properties for use as an oral contraceptive. In contrast, fluorination of the A-ring of E$_2$ dramatically altered the routes of oxidative metabolism by blocking the formation of potentially toxic catecholoestrogens [19], though again without loss of oestrogenic activity [3]. Thus, it was postulated that chemical modification of both the A and D rings of E$_2$ might produce a compound which was refractory to oxidation but still underwent glucuronylation and retained oestrogenic potency. The novel oestrogen 2-FEE$_2$ was synthesized and was found to have similar oestrogen receptor affinity and oestrogenic potency to EE$_2$. Although the apparently lower oestrogenic activities (vaginal and uterine responses) of 2-FEE$_2$ corresponded with the derivative's lower oestrogen receptor affinity, the disparity in uterotrophic activity, at least, because uteri were weighed 5 days after dosing, might have reflected differences in pharmacokinetic parameters rather than intrinsic activity.

Like other oestrogens, including E$_2$ [14], EE$_2$ [26], 2,4-dibrominated derivatives [42] and 2- and 4-FE$_2$ [19], 2-FEE$_2$ underwent glucuronylation and rapid biliary excretion in female rats. However, 2-FEE$_2$ unexpectedly underwent oxidative defluorination to yield the catechol metabolites, 2-OHEE$_2$ and 2-MeOEE$_2$, which were excreted in bile as conjugates. Nevertheless, fluorination did substantially reduce the extent of C-2 catechol formation to approximately 20% of the dose compared with 70% for EE$_2$ [26]. Two minor oxygenated metabolites of 2-FEE$_2$ which retained the 2-fluorine substituent were, from their mass spectra alone, identified as products of C-4 hydroxylation, the minor pathway of catecholoestrogen formation [17].

The principal site of oxidative defluorination, by analogy with the active dehalogenation of 2-FE$_2$ in

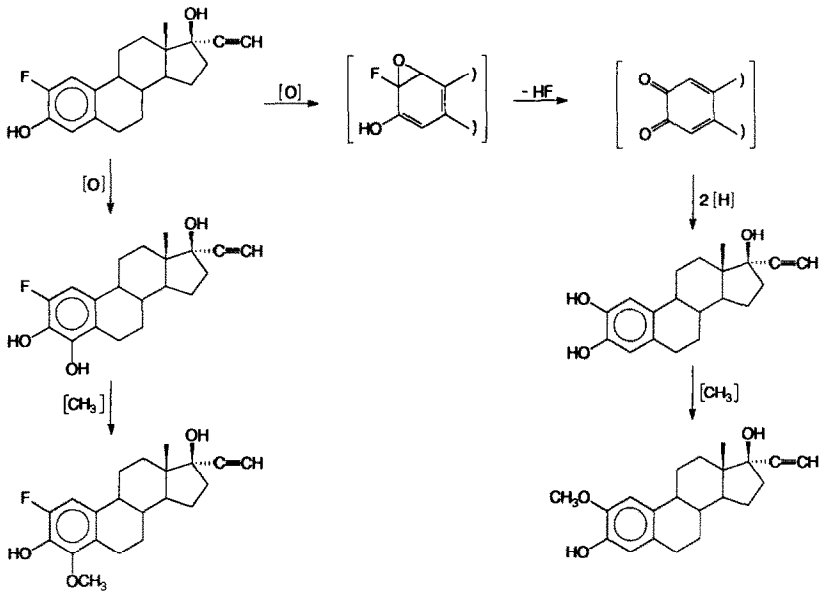

Fig. 4. The metabolic fate of [^{3}H]2-FEE$_2$ in female rats: oxidative pathways. Hypothetical intermediates of defluorination pathway are shown in square brackets.

hepatic microsomes [20], is likely to be the liver. Any toxic effects of 2-OHEE$_2$ will probably be confined to the site of the metabolite's formation since catecholoestrogens are rapidly inactivated in blood by catechol-*O*-methyltransferase [43].

The mechanism of 2-FEE$_2$ defluorination is unknown but the strength of the C-F bond mitigates against direct oxygen insertion, the favoured reaction for E_2 2-hydroxylation [15]. A more plausible mechanism, involving an intermediary 1,2-epoxide and incorporating a facile loss of fluoride anion [44], is proposed (Fig. 4). Arene oxides are commonly implicated in the oxidative dehalogenation of *para*-haloaromatics [45] and the 1,2-epoxide has been considered an alternative intermediate of oestrogen C-2 hydroxylation [15].

The appreciable oxidative defluorination of 2-FEE$_2$ in the rat contrasts with the resistance of 2-FE$_2$ to this biotransformation; 2-FE$_2$ is excreted predominantly as 2-FE$_1$ glucuronide [19]. Thus, it would appear that oxidative defluorination of the A-ring is influenced by the pattern of substitution in the D-ring. Consistent with this hypothesis, it is known that EE$_2$ undergoes more extensive 2-hydroxylation than E_2 in both human liver microsomes [27] and the rat [25]. The influence of the 17α-ethynyl group on 2-hydroxylation can be overcome by the introduction of bulky substituents such as bromine in the C-2 and C-4 positions [38], but this substitution pattern also results in a loss of oestrogenicity [46, 47]. Thus fluorine, because of its similar size to hydrogen, remains the only feasible chemical substitution for blockade of aromatic hydroxylation and retention of oestrogenicity [47].

The facilitation by 17α-ethynylation of oxidative defluorination may involve two aspects of the binding of oestrogens to the substrate binding site on P450. Firstly, the hydrogen bonding 17β-hydroxyl group [48], preserved by 17α-ethynylation from dehydrogenation which is the primary biotransformation of 2-FE$_2$, would confer higher affinity for P450 catalysing oestrogen 2-hydroxylation [17]; protein–substrate hydrogen bonding can dictate a catalytically favourable orientation of substrate within the P450 active site [49]. Secondly, the ethynyl group might itself interact positively within the active site. However, consideration of the mechanistic basis of ethynylation-dependent defluorination is complicated by the possibility that C-2 fluorination, via conformational transmission [50], modifies D-ring interactions of EE$_2$.

Thus, although the optimal structural features of a steroidal oestrogen totally refractory to oxidative metabolism were not defined in this study, it can be seen that C-2 fluorination does markedly impair aromatic hydroxylation of EE$_2$. A complete restriction of oxidation without significant loss of pharmacological activity may, nevertheless, be achievable if the D-ring interactions responsible for distal promotion of A-ring defluorination can be defined and appropriately counteracted. In this regard, it is worth noting that some derivatives of E_2 substituted with bulky 17α-alkynyl groups retain pharmacological activity [51].

Acknowledgements—P.M. was supported by the Department of Education, Northern Ireland. B.K.P. is a Wellcome Principal Research Fellow. The mass spectrometer in the Department of Pharmacology and Therapeutics was purchased with generous grants from the Wellcome Trust and the University of Liverpool. We are indebted to Drs G. Neef, R. Krattenmacher and K. H. Fritzemeier, Schering AG, Berlin, for the determinations of receptor binding and oestrogenicity. We are grateful to Miss S.

Newby for technical assistance and to Miss S. E. Oliphant for typing the manuscript.

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