Metabolic Oxidation of the Ethynyl Group in 4-Ethynylbiphenyl

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(Received 30 March 1979)

1. 4-Ethynylbiphenyl undergoes extensive metabolism in the rat and the rabbit, involving aromatic hydroxylation and oxidation of the ethynyl group. No metabolites containing the intact ethynyl group were detected. 2. In the rat unchanged 4-ethynylbiphenyl was concentrated initially in the adipose tissue. No other tissues accumulated significant amounts of radioactivity. 3. The major metabolites were the same in both the rat and the rabbit, namely 4'-hydroxybiphenyl-4-ylacetic acid (90–95% of dose) and biphenyl-4-ylacetic acid (2–10% of dose). 4. Excretion was slower in the rat than in the rabbit, probably because of greater biliary and faecal excretion in the rat. Biliary excretion and entero-hepatic circulation of biphenyl-4-ylacetic acid and 4'-hydroxybiphenyl-4-ylacetic acid were demonstrated in the rat.

The ethynyl (acetylene) group is widely incorporated into synthetic drugs, especially steroids, in order to hinder sterically the normal metabolic deactivation of the drug, and so to enhance or prolong its pharmacological activity. Information on the metabolic fate of the ethynyl moiety is minimal, although indications, until recently, were that the carbon-carbon triple bond was very stable to metabolism by animals. The earliest reference to the metabolism of the acetylenic moiety was the conversion of *o*-nitrophenylpropionic acid into indoxyl (Hoppe-Seyler, 1882). Later, Bohm (1939) similarly found *o*-nitrophenylacetylene to be metabolized to indoxyl, and phenylacetylene itself was shown to be slowly metabolized to phenylacetic acid in rabbits (El Masri *et al.*, 1958). Interest in the metabolism of the ethynyl group has increased more recently with the finding of hepatotoxicity after the administration of the synthetic oestrogens ethynloestradiol and mestranol, and this has been associated with the inhibitory effects of the ethynyl group on the normal metabolism of oestrogens and with tissue binding of drug metabolites (Bolt *et al.*, 1973, 1974).

A new anti-inflammatory drug candidate 4-ethynylbiphenyl was found in preliminary trials to be ulcerogenic and consequently too toxic for therapeutic use, but its rapid metabolism in animals indicated that the molecule might prove to be an interesting model compound with which to study the stability of the ethynyl group. Accordingly

4-[¹⁴C]ethynylbiphenyl was synthesized and its metabolism studied in the rat and the rabbit.

A preliminary account of this work has been published (Martin *et al.*, 1978).

Experimental

Materials

4-Ethynylbiphenyl (m.p. 89°C), biphenyl-4-ylacetic acid (m.p. 161°C) and biphenyl-4-carboxylic acid (m.p. 225°C) were supplied by Allen and Hanbury (Ware, Herts., U.K.). 4-Phenylmandelic acid was synthesized by the method of Riebsomer *et al.* (1938), in which biphenyl (0.10 mol in 50 ml of chloroform) was treated with diethyloxomalonate (0.02 mol) in the presence of SnCl₂, the condensation product hydrolysed with 20% (w/v) KOH (50 ml), and the resultant potassium salt decarboxylated by heating at 100°C in 80 ml of 5M-HCl; recrystallization from aqueous methanol gave white crystals of 4-phenylmandelic acid (m.p. 193°C; yield 68%). 4'-Hydroxybiphenyl-4-ylacetic acid was synthesized from 4-hydroxybiphenyl. A modification of the method of Gray *et al.* (1955) was used to obtain 4'-methoxy-4-acetyl biphenyl from 4-hydroxybiphenyl, which was further converted into 4'-hydroxybiphenyl-4-ylacetic acid (m.p. 246°C) by the method of Linell & Smith (1959).

4-[¹⁴C]ethynylbiphenyl was synthesized from biphenyl (0.77 g, 5.0 mmol) and [1-¹⁴C]acetyl chloride (0.4 g; 5.5 mmol; 5.0 mCi). These two reagents were dissolved in dichloromethane (2.5 ml) and added over a 20 min period, from a dropping-funnel to a rapidly stirring suspension of anhydrous

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AlCl_3 (0.75 g) in dichloromethane (3.75 ml), kept at 60–70°C in an oil bath. The reaction mixture was refluxed for 1.75 h, after which the solvent was removed by distillation under reduced pressure and the residue hydrolysed by the addition of ice-cold 2M-HCl (50 ml). The 4-[^{14}C]acetyl biphenyl resulting from this Friedel–Craft reaction appeared as a yellowish-white residue. This was filtered off and recrystallized from ethanol to give a white product. Further purification was obtained by sublimation to give 0.75 g of white microcrystalline product (m.p. 119–120°C; 94 % yield). Freshly sublimed PCl_5 (4.2 g; 30 mmol) in pyridine (15 ml), contained in a 50 ml round-bottomed flask equipped with air-condenser and drying tube, was heated to 50°C with stirring, and 4-[^{14}C]acetyl biphenyl (0.784 g, 4.0 mmol) in dry pyridine (15 ml) was added. Stirring was continued at 50°C for 3 h, when t.l.c. of the reaction mixture on silica gel in cyclohexane/ethyl acetate (3:1, v/v) showed the reaction to be complete; R_f values were: for 4-acetyl biphenyl, 0.36; for 4-ethynyl biphenyl, 0.52. The reaction mixture was poured on to a mixture of ice (5 g) and 2M-HCl (30 ml) and extracted with 6 × 20 ml of light petroleum (b.p. 40–60°C). The combined extracts were washed with 3 × 75 ml of 2M-HCl, 3 × 75 ml of water and 75 ml of satd. aq. NaCl, and dried over anhydrous MgSO_4 . The filtered light-petroleum extract was concentrated, and the residue was dissolved in ethyl acetate (2–3 ml) and light petroleum (b.p. 60–80°C; 2–3 ml) and purified by chromatography on a silica-gel column (2 cm × 35 cm; silica gel 70–230 mesh) with light petroleum (b.p. 60–80°C)/toluene (19:1, v/v) as eluent. Fractions affording 4-[^{14}C]ethynyl biphenyl as one spot on t.l.c. were evaporated to produce a pale yellow residue, which was recrystallized from aqueous methanol to give white crystalline 4-[^{14}C]ethynyl biphenyl (310 mg; m.p. and mixed m.p. 88–89°C; sp. radioactivity 0.36 mCi/mmol).

Animals

Male and female Wistar albino rats (200 ± 5 g) were obtained from the Rodent Breeding Unit, University of Surrey, Guildford, Surrey, U.K. Male and female Dutch rabbits (2–5 kg) were purchased from local dealers.

Determination of radioactivity

Radioactivity was determined by scintillation counting with an LKB 1210 counter and scintillation fluid similar to that described by Wood *et al.* (1975), namely 0.55 % 2,5-diphenyloxazole in toluene/Synperonic NXP detergent (Wormsley Boon, Rainham, Essex, U.K.) (2:1, v/v). Aqueous samples (0.5 ml) were added to 4.0 ml of the scintillation fluid and their radioactivities were counted in plastic minivials (Packard, Caversham, Berks., U.K.). Counting efficiency (usually 75–90 %) was determined by

internal standardization with [^{14}C]toluene or [^{14}C]hexadecane.

Thin-layer chromatography

T.l.c. was carried out on 0.24 mm silica-gel GF₂₅₄ plates (Schleicher und Schüll, via Anderman and Co., London S.E.1, U.K.) and preparative-layer chromatography on 2.0 mm-layer silica-gel 60F₂₅₄ plates (E. Merck, Darmstadt, Germany) in solvent systems A [toluene/methanol/ethyl acetate/acetic acid (90:10:17:3, by vol.)], B [chloroform/methanol (1:1 v/v)], C, propan-1-ol/aq. NH_3 (sp.gr. 0.880) (7:3, v/v)], D [butan-1-ol/acetic acid/water (50:11:25, by vol.)], E. [toluene/methanol/ethyl acetate/aq. NH_3 (sp.gr. 0.880) (90:10:17:3, by vol.)] and F [light petroleum (b.p. 60–80°C)/toluene (19:1, v/v)].

Routine scanning of t.l.c. plates was performed with a Camlab TLC plate scanner (Camlab Laboratories, Cambridge, U.K.). Radioautography was used for more sensitive location of radioactive zones.

Spectroscopy

Electron-impact mass spectra were recorded on an A.E.I. M.S.12 mass spectrometer equipped with a temperature-programming facility. Spectra were obtained by direct insertion of samples at an accelerating voltage of 8 kV and an ionizing voltage of 70 eV.

P.m.r. spectra were recorded with a 90 MHz Bruker WH-90 Fourier transform n.m.r. spectrophotometer operating at ambient temperature. Tetramethylsilane was used as internal standard and chemical shifts (δ) are expressed in p.p.m. downfield from this. [^2H]Methanol or [^2H]acetone was used as solvent.

Reverse isotope dilution

To help confirm the identity of metabolites obtained *in vivo*, reverse isotope dilution was performed. Small amounts of radiolabelled metabolite (< 100 μg ; 50–100 nCi) were eluted from t.l.c. plates and mixed with 0.5–1.0 g of the appropriate pure unlabelled standard. Repeated recrystallizations of such mixtures were done to determine whether a constant specific radioactivity could be achieved. 4-Phenylmandelic acid was recrystallized from aqueous methanol, and biphenyl-4-ylacetic and 4-hydroxybiphenyl-4-ylacetic acid were recrystallized from acetic acid.

Disposition of radioactivity in rat and rabbit after oral administration of 4-[^{14}C]ethynyl biphenyl

Rats were housed in glass metabolism cages (Metabowls, Jencons Scientific, Hemel Hempstead, Herts., U.K.), to collect urine, faeces and expired air separately. Rabbits were kept in metabolism cages designed for separate collection of urine and faeces but not expired air.

Six male rats were given a single dose of 4-[^{14}C]ethynylbiphenyl (40mg/kg; 5 μCi) in corn oil (0.5ml) by oral intubation, and the urine, faeces and expired air were collected for 7 days for determination of radioactivity. Male rabbits were given a single dose of 4-[^{14}C]ethynylbiphenyl (40mg/kg; 5 μCi) in corn oil (2.0ml) by oral intubation, and excreta were collected for 7 days.

For tissue distribution studies male rats were given a single dose of 4-[^{14}C]ethynylbiphenyl (40mg/kg, 6 μCi) in corn oil (0.2ml) by oral intubation and killed, at intervals from 1 to 24h, by exsanguination under light ether anaesthesia (cardiac puncture). Tissue samples were removed and prepared for scintillation counting of radioactivity by digestion of weighed portions (50–120mg wet wt.) in 1.0ml of Soluene-350 solubilizer (Packard, Wembley, Middx., U.K.) for 24–48h at 60°C. To the cooled digests was added scintillation fluid (12.0ml) and water (1.5ml). Tissue distribution was also determined in rats that received daily doses of 4-[^{14}C]ethynylbiphenyl (40mg/kg) for 4 consecutive days.

Plasma clearance studies were made after administration of a single oral dose of 4-[^{14}C]ethynylbiphenyl (40mg/kg) to male rats. Four rats were killed by exsanguination at intervals up to 24h after dosing, and plasma samples were extracted with ethyl acetate for determination of the concentrations of ethynylbiphenyl and its metabolites.

Biliary excretion studies

Male Wistar albino rats were equipped with bile-duct cannulae under pentobarbitone anaesthesia by using normal procedures. Bile was collected for 30min before the dosing and at 30min intervals after the dosing for 7–10h. After determination of radioactivity, samples were stored at -10°C . Rats (three at each dosage) were given 4-[^{14}C]ethynylbiphenyl (0.16–100mg/kg, each dose containing 0.4 μCi , in 0.5ml of corn oil) administered by injection into the duodenum before abdominal closure. To investigate the extent of enterohepatic circulation, bile was collected from two rats previously dosed intraduodenally with 4-[^{14}C]ethynylbiphenyl (40mg/

kg), and 0.5ml of the collected bile (containing 0.1–0.2mg of ethynylbiphenyl metabolites and 50nCi of ^{14}C) was administered intraduodenally to other rats. These recipient rats were normal untreated animals given 1,4-saccharolactone (250mg in water) intraduodenally 30min before the experiment, or animals pretreated with tetracycline (40mg) and neomycin (10mg) orally twice daily for 5 days, the last dose being 1h before the experiment.

Isolation and identification of metabolites

Samples of bile, urine and faeces were used for isolation and identification of metabolites. Urine samples from rats and rabbits were decolorized and desalted by treatment with XAD-2 ion-exchange resin before extraction or hydrolysis procedures. Extraction with ethyl acetate was carried out at different pH values, and the ethyl acetate extracts were concentrated and examined by t.l.c. and preparative t.l.c. in solvent systems A, B, C and D. Hydrolysis of the conjugated metabolites, not extracted with ethyl acetate, was carried out by heating in 6M-HCl at 60°C for 16h or by treatment with sulphatase or β -glucuronidase preparations in the standard manner. Rat bile was extracted with ethyl acetate before and after hydrolysis with acid or enzyme preparations, as for urine, and the untreated bile and extracts were chromatographed. Faeces were homogenized in water, the homogenate was freeze-dried and the product was extracted with ethyl acetate or methanol. Concentrated extracts were chromatographed before or after hydrolysis as described above. Metabolites from all excreta were determined quantitatively by removing appropriate areas from t.l.c. plates and determining the specific radioactivity by scintillation counting.

Results

Disposition of 4-ethynylbiphenyl

The excretion of total radioactivity by male rat and rabbit after oral dosage with 4-[^{14}C]ethynylbiphenyl is shown in Table 1. Rabbits excreted the material faster than rats and less was excreted in the

Table 1. Excretion of radioactivity in the male rat and rabbit after a single oral dose of 4-[^{14}C]ethynylbiphenyl. The dose of 4-[^{14}C]ethynylbiphenyl was 40mg/kg (5 μCi /animal). Experimental details are given in the text. Results, expressed as percentages of dose, are mean values $\pm$ S.E.M. for six rats or five rabbits.

Time after dosage	Excretion (% of dose)			
	Rat		Rabbit	
	Urine	Faeces	Urine	Faeces
Day 1	46.6 $\pm$ 1.5	8.2 $\pm$ 1.8	67.3 $\pm$ 1.3	3.0 $\pm$ 0.4
Day 2	17.7 $\pm$ 2.3	10.3 $\pm$ 0.8	13.9 $\pm$ 0.9	4.3 $\pm$ 0.7
Day 3	4.5 $\pm$ 0.5	3.0 $\pm$ 0.9	6.0 $\pm$ 0.8	1.2 $\pm$ 0.2
Total (7 days)	70.1 $\pm$ 4.8	23.1 $\pm$ 3.0	87.8 $\pm$ 3.2	8.5 $\pm$ 1.7

faeces of rabbits. The total excretion in 7 days was similar in both species (96 % dose for rabbits, 93 % dose for rats), and no significant radioactivity was excreted after 3 days. No radioactivity was found in the expired air.

In the tissue distribution studies radioactivity was highest in adipose tissue, and the ^{14}C content at peak concentration (6–10 h after dosage) corresponded to the equivalent of 150–200 μg of 4-ethynylbiphenyl/g of tissue, in contrast with a maximum of 30–40 $\mu\text{g}/\text{g}$ of tissue found in liver and kidney. At 6 h, at least 20 % of the administered dose was localized in the adipose tissue, assuming 5–7 % of body weight is adipose tissue (Reed *et al.*, 1930; Schemmel *et al.*, 1970). At 24 h after dosing, the total radioactivity content of the adipose tissue was equivalent to about 9 % of the dose, and was 4–5-fold greater than that of liver, kidney or any other tissue. However, rats given repeated daily doses for 4 days did not accumulate 4- ^{14}C ethynylbiphenyl in adipose tissue, nor in liver or kidneys; at 24 h after the last of four daily doses of 4- ^{14}C ethynylbiphenyl, less than 0.5 % of the total dose was present in the liver and less than 0.2 % total dose was in the kidney. The plasma concentration–time profile for 4- ^{14}C ethynylbiphenyl in male rats (see Fig. 1) shows a maximum 6–10 h after dosing, which is the time that the maximum concentration of radioactivity was found in all tissues, except the stomach contents, where the maximum radioactivity occurs 1 h after dosing.

Biliary excretion

The biliary excretion of radioactivity by rat after a dose of 4- ^{14}C ethynylbiphenyl decreased linearly

Fig. 1. Plasma clearance of radioactivity in the rat after a single oral dose of 4- ^{14}C ethynylbiphenyl

The dose of 4- ^{14}C ethynylbiphenyl was 40 mg/kg (1 $\mu\text{Ci}/\text{animal}$). Experimental details are given in the text. Results are the means $\pm$ S.E.M. for four animals.

as the dose increased, from 33 % of the dose at 0.16 mg/kg to 10 % of the dose at 15 mg/kg, and then remained constant at about 10 % of the dose excreted up to a dose of 100 mg/kg. Enterohepatic circulation was shown to occur (see Fig. 2). Bile-duct-cannulated rats receiving bile from other rats previously dosed with 4- ^{14}C ethynylbiphenyl (40 mg/kg) excreted 28 % of the biliary radioactivity in their bile after 6 h, and this value was lowered to 9 % in rats pretreated with saccharolactone and to 5 % in the antibiotic-treated rats.

Identification of metabolites

All metabolites of 4-ethynylbiphenyl were acidic and were extracted into ethyl acetate at pH < 7.0. Chromatography of unextracted rat urine in solvent systems A and B showed a single metabolite (R_f 0.40 in system A) accounting for 63 % of the dose (90 % of the total urine radioactivity). Chromatography of concentrated ethyl acetate extracts of urine, combined

Fig. 2. Inhibitory effects of saccharolactone and antibiotics on the enterohepatic recirculation of biliary-excreted metabolites of 4- ^{14}C ethynylbiphenyl in the rat

Male rats, normal or pretreated with saccharolactone (250 mg intraduodenally 30 min previously) or with tetracycline and neomycin (two daily doses of 40 mg and 10 mg respectively, for each of 5 days previously), were given, intraduodenally, bile (0.5 ml, containing 0.1–0.2 mg of 4-ethynylbiphenyl metabolites and 50 nCi of ^{14}C) from an animal previously dosed with 4- ^{14}C ethynylbiphenyl (40 mg/kg). Biliary excretion of radioactivity from the recipient rats was measured for 6 h. Experimental details are given in the text. Results, expressed as percentages of dose of the radioactive bile administered, are the means for two animals in normal rats (●), saccharolactone-treated rats (■) and antibiotic-treated rats (▲).

with radioautography, showed two other minor metabolites (R_F 0.48 and 0.37 in solvent A) accounting for about 3.5% and 1.5% of the dose respectively. The major metabolite was shown, by electron-impact mass spectroscopy of a derivative formed with diazomethane and *NO*-bis-(trimethylsilyl)trifluoroacetamide, to be a hydroxybiphenyl-4-ylacetic acid (see Fig. 3). The location of the phenolic hydroxyl group at the 4'-position was established by p.m.r. studies of the purified metabolite. A sample of 4'-hydroxybiphenyl-4-ylacetic acid was later synthesized, and determination of p.m.r. and mass-spectral characteristics, and reverse isotope dilution, confirmed the identity of the structure of the metabolite. The metabolites with R_F values 0.48 and 0.37 were similarly identified by mass spectroscopy, reverse isotope dilution and p.m.r. as biphenyl-4-ylacetic acid and 4-phenylmandelic acid respectively (see Fig. 4). None of the metabolites in rat urine was conjugated.

In rabbit urine 50% of the radioactivity was present as free 4'-hydroxybiphenyl-4-ylacetic acid, and the remaining 50% as conjugates. After hydrolysis in 4M-HCl at 70°C for 16h, 90% of the conjugated radioactivity was shown to be present as 4'-hydroxybiphenyl-4-ylacetic acid, 6% as biphenyl-4-ylacetic acid and 1-2% as 4-phenylmandelic acid. The nature of the individual conjugates was not established, but enzymic hydrolysis and mass

spectroscopy indicated these to be a mixture of glucuronides and amino acid conjugates.

The radioactivity of rat bile was present as conjugated 4'-hydroxybiphenyl-4-ylacetic acid (65-85%) and conjugated biphenyl-4-ylacetic acid (15-30%). The relative amounts of these two metabolites changed during the 7-10h experimental period, the latter metabolite disappearing with time. Enzymic hydrolysis of these biliary conjugates (β -glucuronidase and/or arylsulphatase) followed by t.l.c. showed that 4'-hydroxybiphenyl-4-ylacetic acid was present as sulphate and glucuronide in about equal amounts (in accumulated 7h bile samples). The stability of this glucuronide in alkaline solution suggests that it is an ether glucuronide in which the phenolic group is conjugated. The biphenyl-4-ylacetic acid was conjugated with an amino acid, the identity of which was not established.

In rat plasma, free 4-ethynylbiphenyl and the metabolites 4'-hydroxybiphenyl-4-ylacetic acid, biphenyl-4-ylacetic acid and 4-acetylbiphenyl were identified by t.l.c. of ethyl acetate extracts of plasma samples followed by co-chromatography with authentic compounds, mass spectroscopy and reverse isotope dilution. The distribution of these metabolites in the plasma up to 10h after the dosing is shown in Fig. 5. No metabolites containing the intact ethynyl group were detected in the plasma.

Fig. 3. Mass spectrum of the major metabolite of 4-ethynylbiphenyl in rat urine

A derivative of the isolated metabolite was formed with diazomethane and then with *NO*-bis(trimethylsilyl)trifluoroacetamide. Accurate mass measurements of the parent ion and two fragments were taken.

Found

m/e 314.1348	$\text{C}_{18}\text{H}_{22}\text{O}_3\text{Si}$	requires 314.1338
m/e 299.1091	$\text{C}_{17}\text{H}_{19}\text{O}_3\text{Si}$	requires 299.1103
m/e 255.1238	$\text{C}_{16}\text{H}_{19}\text{OSi}$	requires 255.1203

Fig. 4. Mass spectra of the metabolites of 4-ethynylbiphenyl

The mass spectra shown are for the following metabolites: (a) 4'-hydroxybiphenyl-4-ylacetic acid; (b) 4-phenylmandelic acid; (c) biphenyl-4-ylacetic acid; (d) 4-acetylbiphenyl.

Discussion

The present studies show that 4-ethynylbiphenyl is extensively metabolized in both the rat and the rabbit. Both hydroxylation of the aromatic ring and

oxidation of the ethynyl group occur, no metabolites containing an intact ethynyl group were isolated from urine, faeces or bile of either species. The major excretory product was 4'-hydroxybiphenyl-4-ylacetic acid (80–90 % of the dose), with biphenyl-4-ylacetic acid as a minor metabolite equivalent to 2–10 % of the dose (see Scheme 1). These metabolites are probably responsible for the pharmacological and toxicological effects observed *in vivo* with 4-ethynylbiphenyl.

Although the major metabolite of 4-ethynylbiphenyl in both the rat and the rabbit was 4'-hydroxybiphenyl-4-ylacetic acid, there was a considerable difference in excretion patterns. For, whereas faecal excretion was significant in the rat (up to 30 % of the dose), the rabbit excreted nearly all the metabolized material in the urine, with only 9 % appearing in the faeces. The slower elimination of the drug from the rat is probably due to the extensive biliary excretion (10–15 % dose in 6 h) and enterohepatic circulation that occurs in this animal.

Preliminary studies showed that 4-ethynylbiphenyl was a good anti-inflammatory agent, but was irritant and ulcerogenic to the gastrointestinal mucosa of the rat and the dog, though not of the rabbit or the marmoset. Biphenyl-4-ylacetic acid is a well-known anti-inflammatory agent and has been shown to be the active metabolite of several anti-inflammatory drugs. For example, 4-methyl-2-(4-phenylbenzyl)-2-oxazolin-4-ylmethanol is extensively metabolized to biphenyl-4-ylacetic acid (Turnbull *et al.*, 1974), as is difenpiramide (Grassi *et al.*, 1977). In both these

Fig. 5. Distribution of 4-ethynylbiphenyl and its metabolites in male rat plasma with time

Male rats were dosed orally with 4-[^{14}C]ethynylbiphenyl (40 mg/kg; 1 μCi). Experimental details are given in the text. Results are shown as percentages of total plasma ^{14}C and are the means for three animals for 4-ethynylbiphenyl (●), 4'-hydroxybiphenyl-4-ylacetic acid (■), biphenyl-4-ylacetic acid (▲) and 4-acetylbiphenyl (○).

Scheme 1. Metabolism and excretion of 4-ethynylbiphenyl and its metabolites in the rat

cases further metabolism to 4'-hydroxybiphenyl-4-ylacetic acid was observed, and Grassi *et al.* (1977) showed that this metabolite was not pharmacologically active. The ulcerogenic properties of 4-ethynylbiphenyl are likely to be due to biphenyl-4-ylacetic acid and perhaps also to the 4'-hydroxy derivative; and the marked species difference in the toxicity of 4-ethynylbiphenyl may be explained by differences in biliary excretion, enterohepatic circulation and protein binding. The drug was found to be toxic in the rat and the dog, but not in the rabbit or the marmoset. For, whereas the rat and the dog are good biliary excretors, the rabbit and the marmoset are not (Abou-El-Makarem *et al.*, 1967; Millburn *et al.*, 1967; Millburn, 1970), and the extensive excretion of conjugates of the metabolites of 4-ethynylbiphenyl seen to occur in the rat would lead to high concentrations of the ulcerogenic biphenyl-4-ylacetic acids in the gastrointestinal tract.

The most important finding in the present work is the rapid and extensive metabolism of the ethynyl group of 4-ethynylbiphenyl. It is the most extensive metabolism of this moiety in animals yet described. For example, phenylacetylene, a closely related acetylenic compound, is only very slowly metabolized; limited oxidation of the acetylene group was observed and there was no ring hydroxylation (El Masri *et al.*, 1957, 1958). In 24h, 70% of a dose of 4-ethynylbiphenyl was excreted by the rabbit, but only 15% of a dose of phenylacetylene was excreted in this time. The presence of the second aromatic ring therefore has considerable influence on the susceptibility of the acetylene group to undergo metabolism. This is probably due to the greater lipophilicity of the 4-ethynylbiphenyl molecule conferred by the addition of a further aromatic ring to the rather polar molecule of phenylacetylene, thus facilitating penetration of the compound into the endoplasmic reticulum, to result in increased oxidation by the mixed-function oxidases.

Other workers have reported smaller but significant degrees of metabolism of the ethynyl group in the ethynyl-substituted steroids. Abdel-Aziz & Williams (1970) reported the D-homoannulation of the ethynyl group of 17 α -ethynylloestradiol in rabbit, and metabolic dealkylation has also been reported (Williams *et al.*, 1975; Williams & Williams, 1975). Because synthetic hormones of the 17 α -alkylated variety display hepatotoxicity (Song *et al.*, 1969; Goldfarb, 1976) and could conceivably prove to be carcinogenic, the metabolism of the ethynyl group has great significance. The possibility that acetylenic compounds in general are potentially toxic has been strengthened by findings that a variety of acetylenes after metabolic activation cause loss of hepatic cytochrome P-450 and haem (White, 1978).

The present results show complete oxidation of the ethynyl group of 4-ethynylbiphenyl *in vivo* but with

no binding of radioactivity to the tissues. In all cases radioactivity associated with tissues was easily extracted into ethyl acetate. In addition, there was no macroscopic damage to rat livers after four daily doses of 400mg of 4-ethynylbiphenyl/kg body wt. (Wade, 1978). This is in contrast with the ethynyl-substituted steroids, where there is evidence that metabolism of the ethynyl group increased the degree of binding (Helton *et al.*, 1976). Such differences might be due to the formation of intermediary metabolites of different stability and reactivity. Investigations into the metabolism of 4-ethynylbiphenyl *in vivo* did not reveal any intermediate metabolites between the acetylenic compound and the acidic compound, and studies *in vitro* showed that any intermediate metabolites that are formed are very short-lived.

These studies with liver microsomal preparations have shown that the metabolism of the ethynyl group to the acetic acid moiety is catalysed by enzymes of the microsomal fraction, is inhibited by CO and is likely to be mediated by cytochrome P-450. It is possible that a very unstable epoxide is formed that rearranges immediately to form a keten, and this in turn is hydrolysed to the acid derivative:

The studies *in vivo* indicate that the oxidation of the ethynyl group occurred in preference to aromatic hydroxylation, for no hydroxylated metabolites containing an intact ethynyl group were detected, and the ratio of biphenyl-4-ylacetic acid to 4'-hydroxybiphenyl-4-ylacetic acid in rat bile decreased with time. The hydroxylation of biphenyl has been well studied (West *et al.*, 1956; Creaven *et al.*, 1965; Raig & Ammon, 1970, 1972; Meyer & Scheline, 1976a,b, 1977). In all cases 4'-hydroxybiphenyl is the major metabolite, but a variety of other mono- and dihydroxy metabolites are also known. The aromatic hydroxylation of 4-ethynylbiphenyl, or possibly more accurately biphenyl-4-ylacetic acid, occurs almost exclusively in the 4'-position (>99%), although long radioautographic exposure of t.l.c. plates indicated the presence of trace metabolites (<0.1% of dose) that are likely to be other hydroxylated metabolites. The metabolic pattern did not alter when rats were pretreated with either phenobarbitone or 3-methylcholanthrene, in contrast with biphenyl (Creaven & Parke, 1966).

The facility of the metabolism of the ethynyl group of 4-ethynylbiphenyl is intriguing in view of the apparent stability of this group in many other drugs and chemicals. It suggests that a more detailed study of these other drugs by modern techniques might reveal

previously unidentified metabolites resulting from oxidative biotransformations of the ethynyl group, which may have possible toxicological implications.

We thank the Science Research Council and Glaxo Laboratories for their financial support of this study and for the award of a CASE Studentship to A.W.

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