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METABOLISM OF BIPHENYL IN THE RAT

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SUMMARY

The metabolism of biphenyl in the rat has been studied by using gas chromatographic and mass spectrometric methods. The free and conjugated urinary metabolites were characterized. Eight new metabolites were isolated: a dihydrodiol and two hydroxydihydrodiols were characteristic for the epoxide–diol pathway. There were two dihydroxybiphenyls, a trihydroxybiphenyl, a trihydroxymethoxybiphenyl and 4,4′-dihydroxy-3-methylthiobiphenyl. The mass spectra of the trimethylsilyl derivatives of the metabolites exhibited characteristic doubly charged and metastable ions.

INTRODUCTION

Biphenyl is metabolized *in vivo* and *in vitro* to phenolic compounds. In the rat, the main metabolites found earlier were 4-hydroxybiphenyl and 4,4′-dihydroxybiphenyl; smaller amounts of 2-hydroxy-, 3-hydroxy-, 3,4-dihydroxy-, 3,4′-dihydroxybiphenyl, 3,4,4′-trihydroxybiphenyl, and isomeric hydroxymethoxybiphenyls were also identified[1–5]. An arene oxide intermediate was proposed to explain the formation of hydroxybiphenyls, but dihydrodiols were not identified in earlier studies. This work was undertaken to determine whether dihydrodiol metabolites of biphenyl could be detected, since the formation of dihydrodiols provides direct evidence for metabolism via the epoxide–diol pathway.

In most of the earlier studies, metabolites were estimated by fluorimetric methods[2,4,6,7] and characterized by gas chromatography (GC) and infrared spectroscopy[8,9]. In this study, metabolites were quantified and characterized by GC and mass spectrometric (MS) procedures.

MATERIALS AND METHODS

Reagents

Biphenyl, 2-hydroxy-, 4-hydroxy-, 2,2′-dihydroxy- and 4,4′-dihydroxybiphenyl were obtained from Aldrich (Milwaukee, Wisc., U.S.A.). 3-Hydroxy-, 3,4-dihydroxy- and 2,5-dihydroxybiphenyl were obtained from RFR Corp. (Hope, R.I., U.S.A.). [^{14}C]Biphenyl was purchased from Amersham Corp. (Arlington Heights, Ill., U.S.A.).

GC and GC–MS analyses

GC separations were carried out with 365 cm × 4 mm I.D. glass W columns containing 5% SE-30 (80–100 mesh) on Gas-Chrom Q (Applied Science Labs., State College, Pa., U.S.A.) or with 70-m capillary columns, coated with SE-30[10]. The GC separations were temperature programmed at 2°/min. Methylene unit (MU) values were determined with *n*-alkanes as reference compounds. Identifications of metabolites were based upon GC–MS analyses with an LKB 9000/PDP-12 system, operated in an electron-impact mode. The GC column was a 275 cm × 2 mm I.D. glass coil with a 1% SE-30 packing. Mass spectra were recorded at 70 eV, with an accelerating voltage of 3.5 kV and an ionizing current of 60 μA. The ion source temperature was 250°.

Animal experiments

Biphenyl, 2-hydroxybiphenyl, 3-hydroxybiphenyl, 4-hydroxybiphenyl or 4,4'-dihydroxybiphenyl dissolved in corn oil (0.5 ml) was administered intraperitoneally (30 mg/kg) to male Sprague-Dawley rats (weight range 180–250 g, TIMCO Breeding Laboratories, Houston, Texas, U.S.A.). The rats were placed in individual metabolism cages with water but no food, and 0–24 and 24–48 h urine samples were collected. The urines were diluted with glass-distilled water and stored at −14° until analyzed. To determine how much of the administered compound was excreted in urine, 6 mg of biphenyl (30 mg/kg) labeled with 6 μCi of uniformly labeled [^{14}C]biphenyl was administered intraperitoneally. Aliquots of urine and urinary extracts were counted in Aquasol (New England Nuclear, Boston, Mass., U.S.A.).

Isolation of metabolites

Neutral metabolites were isolated from urine by the use of ammonium carbonate–ethyl acetate as a salt–solvent pair[11]. Metabolites present as conjugates were isolated after enzymatic hydrolysis of the urine. After adding 200 mg of sodium acetate to an aliquot of urine (6 ml) and adjusting the pH to 4.5 with acetic acid, the urine was incubated with 0.1 ml of Glusulase (Endo Labs., Garden City, N.Y., U.S.A.) for 20 h at 37°. The aglycones liberated by enzymatic hydrolysis were extracted by the ammonium carbonate–ethyl acetate procedure. The extracts were evaporated to dryness under a nitrogen stream, and the residue was silylated with 30 μl of bis(trimethylsilyl)acetamide (BSA) (Pierce, Rockford, Ill., U.S.A.) in 20 μl of pyridine (1 h at 60°). Perdeuterated derivatives were prepared by substituting d_{18}-bis(trimethylsilyl)acetamide (Supelco, Bellefonte, Pa., U.S.A.) for the usual reagent.

Enzymatic synthesis of 3,4-dihydroxybiphenyl and 3,4,4'-trihydroxybiphenyl

To a solution of 0.4 mg of 4-hydroxybiphenyl or 4,4'-dihydroxybiphenyl in 0.02 ml of dimethyl sulfoxide, there was added 8 ml of 0.1 *M* phosphate buffer, pH 7, 0.5 ml of a freshly prepared 1% solution of ascorbic acid and 10 μl of tyrosinase (Sigma, St. Louis, Mo., U.S.A.) solution (50,000 U/ml). After bubbling air through the reaction mixture for 2 h, the products were extracted with ammonium carbonate–ethyl acetate and treated as described for urinary metabolites.

Synthesis of 3-methylthio-4,4'-dihydroxybiphenyl

To 100 mg of 4,4'-dihydroxybiphenyl dissolved in 2 ml of diethyl ether and 4 ml of chloroform, there was added 160 mg of lead tetraacetate. The mixture was

stirred for 1 h, then filtered. The solution was washed once with aqueous sodium bicarbonate solution to precipitate lead salts. The quinone was then used without purification. One tenth of the quinone solution was evaporated to dryness and the residue was dissolved in 0.7 ml of acetone. To this was added a warm solution of 50 mg of 2-keto-4-methylthiobutyric acid in 3 ml of water, and the mixture was kept at 45° for 2 h. It was then extracted twice with dichloromethane. After removal of the solvent, the residue was dissolved in 20 μl of pyridine and silylated with 30 μl of BSA.

RESULTS

Biphenyl metabolites are excreted in urine in free form and as conjugates. Fig. 1 shows a GC separation of the trimethylsilyl (TMS) derivatives of the urinary

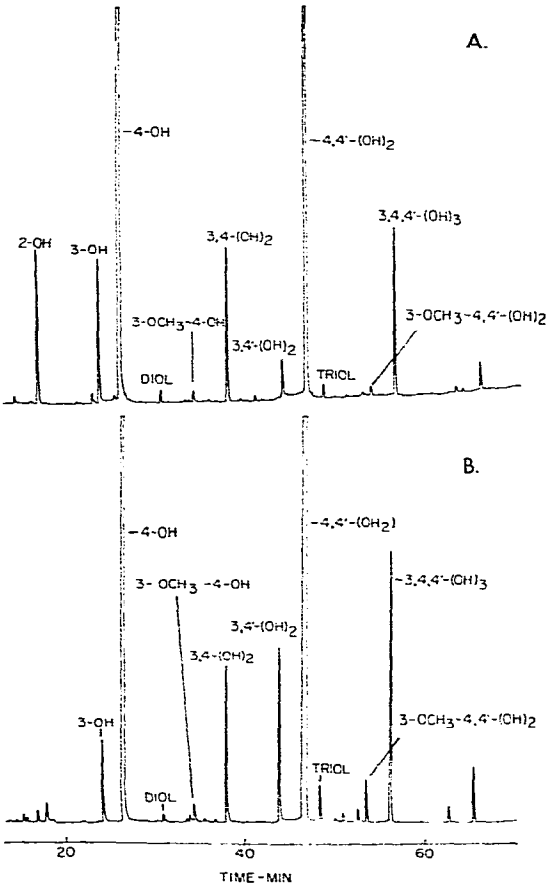


Fig. 1. GC separation of the TMS derivatives of urinary biphenyl metabolites; (A) free metabolites, (B) metabolites extracted after hydrolysis with Glusulase. The separation was carried out on a 75-m glass capillary column, coated with SE-30, temperature programmed 2°/min from 140°. The metabolites identified were 2-hydroxybiphenyl (2-OH), 3-hydroxybiphenyl (3-OH),4- hydroxybiphenyl (4-OH), a dihydroxybiphenyl (DIOL), 3-methoxy-4-hydroxybiphenyl (3-OCH_3-4-OH), 3,4-dihydroxybiphenyl (3,4-$[OH]_2$), 3,4'-dihydroxybiphenyl (3,4'-$[OH]_2$), 4,4'-dihydroxybiphenyl (4,4'-$[OH]_2$), a trihydroxybiphenyl (TRIOL), 3-methoxy-4,4'-dihydroxybiphenyl (3OCH_3-4,4'-$[OH]_2$) and 3,4,4'-trihydroxybiphenyl (3,4,4'-$[OH]_3$).

TABLE I

METABOLITES OF BIPHENYL IN 0–24 h RAT URINE

Metabolites	*MU*	*% Free**	*% Total**
2-Hydroxybiphenyl	15.8	5	—
3-Hydroxybiphenyl	17.1	3	2
4-Hydroxybiphenyl	17.6	46	29
3-Methoxy-4-hydroxybiphenyl	19.1	trace	0.5
3,4-Dihydroxybiphenyl	19.8	4	4
3,4′-Dihydroxybiphenyl	20.9	2	8
4,4′-Dihydroxybiphenyl	21.5	36	46
Trihydroxybiphenyl**	21.8	trace	0.5
3-Methoxy-4,4′-dihydroxybiphenyl	22.9	trace	1
3,4,4′-Trihydroxybiphenyl	23.5	4	9
*Trace metabolites****			
Dihydroxybiphenyl** (2,3?)	18.4		
2,5-Dihydroxybiphenyl	18.9		
Dihydroxybiphenyl** (2,4?)	19.3		
3,4-Dihydrodiol of biphenyl**	19.5		
3,4′-Dihydroxy-4-methoxybiphenyl	22.7		
Hydroxydihydrodiol**	23.5		
Hydroxydihydrodiol**	24.0		
4,4′-Dihydroxy-3-methylthiobiphenyl**	24.4		
Trihydroxymethoxybiphenyl**	24.5		

* Percent of total biphenyl metabolites; average of 7 rat urine extracts.
** New metabolites.
*** Less than 0.5% of total excreted amount.

metabolites before and after enzymatic hydrolysis. The metabolites of biphenyl present in 0–24 h urine samples of rats are listed in Table I. In addition to the metabolites previously identified, eight new metabolites of biphenyl were isolated. These include two additional dihydroxybiphenyls, a trihydroxybiphenyl, a dihydrodiol (presumably *trans*-3,4-dihydroxy-3,4-dihydrobiphenyl), two hydroxydihydrodiols, a trihydroxymethoxybiphenyl and 4,4′-dihydroxy-3-methylthiobiphenyl; these were excreted in trace quantities (less than 0.5% of the administered dose). The methylene unit values and characteristic mass spectral ions of the metabolites of biphenyl are listed in Table II.

The degree of recovery of biphenyl and its metabolites was determined by administering biphenyl (30 mg/kg) labeled with 6 μCi of [^{14}C]biphenyl. Between 20 and 60% of the administered dose was recovered in the first 24-h urine samples, while 40–65% was recovered after 48 h. Before enzymatic hydrolysis, 30–50% of the radioactivity excreted in urine was extractable as neutral metabolites. After enzymatic hydrolysis of the urine with Glusulase, 95% of the radioactivity in the urine was extracted with ethyl acetate. About 30–50% of the metabolites were excreted in free form and 70–50% were excreted as glucosiduronic acids or sulfates. Approximately 2–5% of the radioactivity remained in the aqueous (urine) phase after enzymatic hydrolysis. Because of the small quantities involved, the residual water-soluble acidic compound(s) were not investigated.

Quantification of the metabolites was carried out with open tubular capillary

TABLE II

MU VALUES, *m/e* VALUES AND RELATIVE INTENSITIES (RI) OF MAJOR MASS SPECTRAL IONS OF BIPHENYL METABOLITES

Metabolite	*MU 5% SE-30*	*m/e (RI)*						*Metastable ions*
		M^+	*M − 15*	*Major ions*				
2-Hydroxybiphenyl	15.8	242(63)	227(100)	211(75)	152(15)	106(9)	165(7)	196.1(227 → 211)
3-Hydroxybiphenyl	17.1	242(75)	227(100)	211(40)	113.5(17)	152(15)	165(6)	196.1(227 → 211)
4-Hydroxybiphenyl	17.6	242(100)	227(83)	211(25)	152(17)	113.5(15)	165(6)	196.1(227 → 211)
Dihydroxybiphenyl (2,3?)	18.4	330(100)	315(27)	212(10)	242(10)	227(9)	147(6)	
2,5-Dihydroxybiphenyl	18.9	330(100)	315(35)	299(32)	142(9)	150(4)	283(4)	
Dihydroxybiphenyl (2,4?)	19.3	330(100)	315(37)	142(6)	299(5)	227(5)	242(5)	
3,4-Dihydroxybiphenyl	19.8	330(100)	315(8)	242(8)	147(7)	212(7)	227(3)	
3,4′-Dihydroxybiphenyl	20.9	330(100)	315(51)	150(16)	165(4)	139(2)	299(2)	300.7(330 → 315)
4,4′-Dihydroxybiphenyl	21.5	330(100)	315(18)	150(14)	165(3)	257(1)	242(1)	300.7(330 → 315)
3-Methoxy-4-hydroxybiphenyl	19.1	272(47)	257(13)	212(17)	113.5(9)	227(8)	121(7)	
3,4′-Dihydroxy-4-methoxybiphenyl*	22.7	360(69)	345(8)	330(100)	147(49)	150(20)	315(20)	315.7(345 → 330) 300.7(330 → 315)
3-Methoxy-4,4′-dihydroxybiphenyl	22.9	360(100)	345(9)	330(59)	157.5(14)	150(13)	315(11)	315.7(345 → 330) 300.7(330 → 315)
Trihydroxybiphenyl	21.8	418(100)	403(11)	315(64)	330(37)	147(16)	241(8)	388.5(418 → 403) 300.7(330 → 315)
3,4,4′-Trihydroxybiphenyl	23.5	418(100)	403(6)	315(15)	147(5)	330(4)		388.5(418 → 403) 300.7(330 → 315)
3,4-Dihydro-3,4-dihydroxybiphenyl	19.5	332(39)	317(3)	147(100)	191(90)	242(18)	227(17)	
Hydroxydihydrodiol*	23.5	420	405	330	191	315	150	
Hydroxydihydrodiol*	24.0	420	405	147	191	332	317	
Trihydroxymethoxybiphenyl*	24.5	448	433	418	330	147		
4,4′-Dihydroxy-3-methylthiobiphenyl	24.4	376(100)	361(21)	346(44)	157.5(17)	173(11)	330(10)	

* Mixed spectrum.

GC columns; 4-hydroxy-, 3,4-dihydroxy- and 4,4'-dihydroxybiphenyl were used as external standards since suitable internal standards were not available. A standard solution (1 mg/ml) was prepared from each of the three compounds. After silylation, the response of the flame ionization detector was the same for equal amounts of the three compounds. It was assumed for purposes of calculation that all of the metabolites had a response factor of unity with respect to these standards. Table III shows the amounts of free and conjugated metabolites excreted in 0–24 and 24–48 h periods.

TABLE III

URINARY EXCRETION (μg/24 h) OF BIPHENYL METABOLITES BY THE RAT* DURING 0–24 h

	Free metabolites (μg)	*Conjugated metabolites*** (μg)*
2-Hydroxybiphenyl	61	—***
3-Hydroxybiphenyl	69	20
4-Hydroxybiphenyl	618	199
3,4-Dihydroxybiphenyl	88	68
3,4'-Dihydroxybiphenyl	27	165
4,4'-Dihydroxybiphenyl	439	755
3-Methoxy-4,4'-dihydroxybiphenyl	4	46
3,4,4'-Trihydroxybiphenyl	92	199
Total metabolites	1.4 mg	1.5 mg

* A male rat (315 g) received 9.9 mg of biphenyl.

** Excretion of aglycones obtained by subtraction of amount of free metabolites from the total amount extracted after enzymatic hydrolysis of the urine.

*** 2-Hydroxybiphenyl could not be recovered after enzymatic hydrolysis.

DISCUSSION

The oxidative metabolism of biphenyl leads to mono-, di- and trihydroxybiphenyls and to several dihydrodiols. The structures of a number of metabolites were confirmed by comparison with reference compounds and also by comparison with metabolites isolated from rat urine after administration of 2-, 3- and 4-hydroxy- and 4,4'-dihydroxybiphenyl.

In the mass spectra of 2-, 3- and 4-hydroxybiphenyl, the molecular ion was either the base peak or a peak of high intensity. Characteristic ions were also present at M−15 (M−CH_3), M−31, 165 and 152 a.m.u. 3-Hydroxy- and 4-hydroxybiphenyl show a doubly charged ion at 113.5 [(M−15)/2], with an intensity of 15 and 17%, respectively. In the spectrum of 2-hydroxybiphenyl a doubly charged ion at *m/e* 105.5 can be observed which corresponds to (M−31)/2.

The mass spectrum of the TMS derivative of the dihydrodiol (MU=19.5) (Fig. 2A) showed a molecular ion at *m/e* 332. A peak of high intensity at *m/e* 191, which shifted 18 a.m.u. to *m/e* 209 in the d_9-TMS analog, indicated the presence of two trimethylsilyloxy groups in the molecule. This ion, which is characteristic of vicinal diol structures, has been observed in the mass spectra of dihydrodiol metabolites of many aromatic drugs[12–17]. Other characteristic ions were observed at *m/e* 242 (*M*—TMSOH) and *m/e* 147. By analogy with other dihydrodiols, the structure *trans*-3,4-dihydroxy-3,4-dihydrobiphenyl was assigned to this metabolite. It is derived from the epoxide of biphenyl by enzymatic and possibly non-enzymatic hydration.

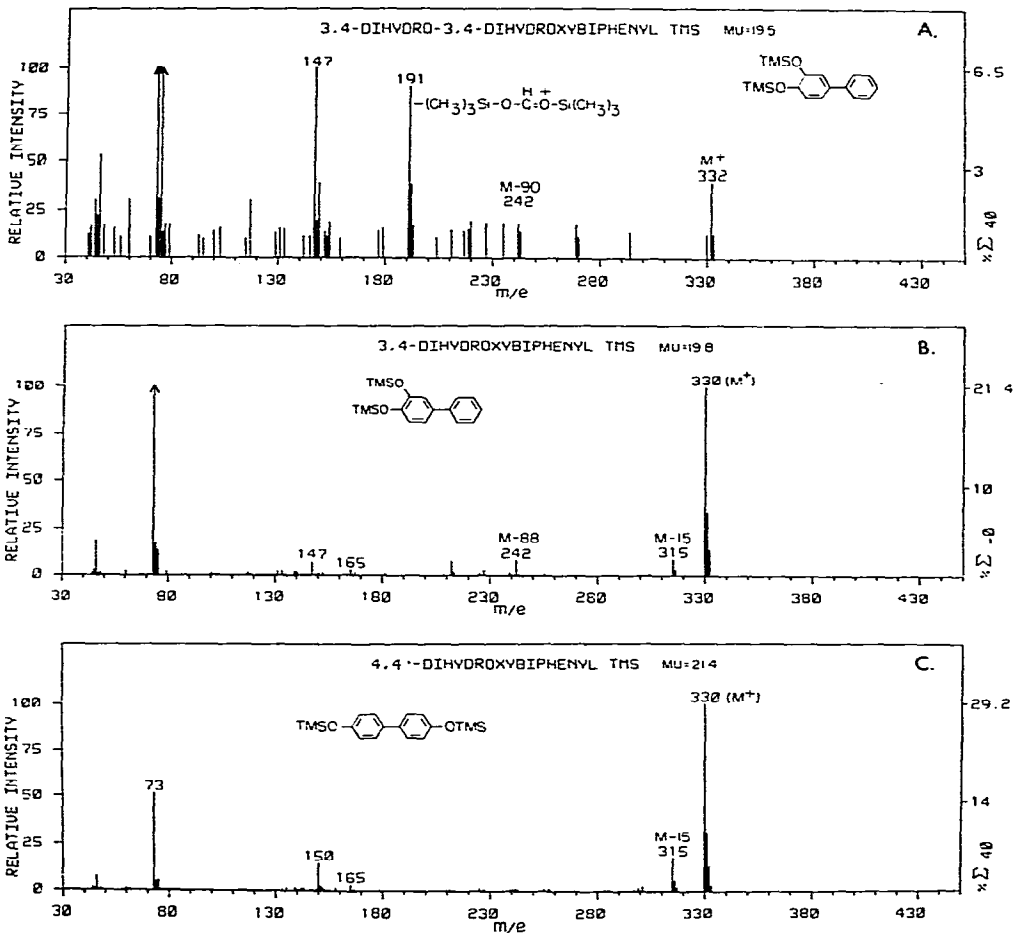


Fig. 2. The mass spectra of the TMS derivatives of biphenyl metabolites isolated from rat urine: (A) 3,4-dihydro-3,4-dihydroxybiphenyl (MU = 19.5); (B) 3,4-dihydroxybiphenyl (MU = 19.8); (C) 4,4'-dihydroxybiphenyl (MU = 21.4).

The conversion of the epoxide (I) into a dihydrodiol (II) and the corresponding catechol (III) and O-methylcatechol is shown in Fig. 3. The mass spectrum of the catechol (III) is shown in Fig. 2B. The molecular ion (*m/e* 330) was the base peak. An ion of low abundance was observed at *m/e* 242 (*M*—88). This ion was present at *m/e* 248 (M—100) in the d_9-TMS analog; it corresponds to the loss of $(CH_3)_4Si$. This catechol (III), which can be formed by dehydrogenation of the dihydrodiol, is also formed *in vivo* by direct hydroxylation of the phenol. Administration of 4-hydroxybiphenyl to rats resulted in the formation of 3,4-dihydroxybiphenyl as a major metabolite. 3,4-Dihydroxybiphenyl was also synthesized enzymatically from 4-hydroxybiphenyl with tyrosinase (EC 1.10.3.1) in the presence of ascorbic acid. Two products were formed in the reaction: the catechol (81%), and the triol 3,4,4'-trihydroxybiphenyl (6%). A small amount (13%) of the phenol was recovered. The GC and MS properties of the rat metabolite and the enzymatically synthesized catechol were

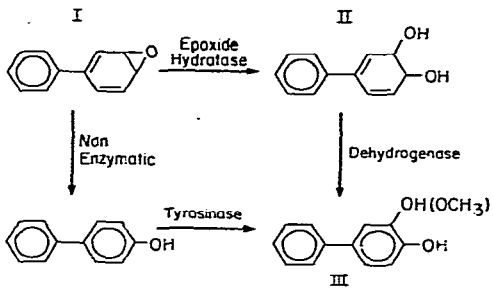
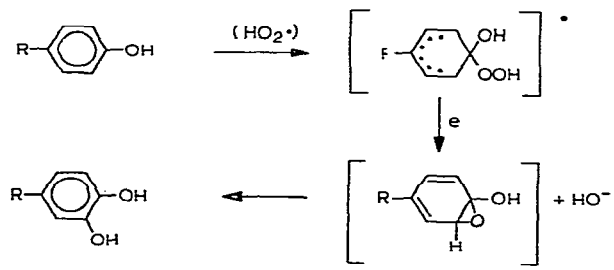


Fig. 3. Scheme for the epoxide–diol pathway.

identical with the properties of the reference compound. *In vitro* studies carried out by Billings and McMahon[18] with rat microsomes in the presence of $^{18}O_2$ indicated that the catechol was formed by two consecutive hydroxylations. The mechanism of direct hydroxylation to a catechol is not known, but it is usually assumed that an epoxide is not involved. This may be true, but it is also possible that the mechanism is related to that proposed for epoxide formation from aromatic hydrocarbons[19], and that a transient epoxide structure is formed as an intermediate:



The mass spectrum of the TMS derivative of 4,4′-dihydroxybiphenyl is shown in Fig. 2C. The molecular ion (*m/e* 330) was the base peak; other fragment ions were observed at *m/e* 315 ($M-CH_3$) and at *m/e* 150. The latter ion is a doubly charged ion [(M—30)/2]; the presence of doubly charged ions in the spectra of the TMS derivatives of 4,4′-dihydroxyphenyl, 4-amino-4′-hydroxybiphenyl and 4,4′-dicarboxybiphenyl was discussed by VandenHeuvel *et al.*[20].

A third dihydroxybiphenyl was also observed as a metabolite (Table I). This was found as an excretion product when 3-hydroxy- or 4-hydroxybiphenyl was administered, but it was not present after administration of 2-hydroxybiphenyl. This metabolite corresponds to 3,4′-dihydroxybiphenyl reported by Meyer and Scheline[3]. The mass spectrum of the TMS derivative, which was very similar to the mass spectrum of 4,4′-dihydroxybiphenyl, also showed a doubly charged ion at *m/e* 150 [(M—30)/2]. The metabolic intermediate was presumably an epoxide.

Three diols were present in trace amounts in rat urine after administration of biphenyl. In separate studies in mice, these diols were present in larger amounts. One

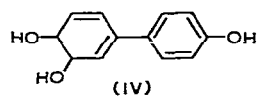
of the diols (MU=18.9) is excreted as the major metabolite by rats and mice after administration of 2- or 3-hydroxybiphenyl. The MS and GC properties of this diol are identical with those of the product obtained by reduction of phenyl-*p*-benzoquinone and with the reference compound 2,5-dihydroxybiphenyl. The mass spectrum showed the molecular ion as base peak. Fragment ions were observed at *m/e* 315 (M—CH_3), 299 (M—CH_3—CH_4), 283 (M—CH_3—CH_4—CH_4), 150 (M—30)/2 and 142 (M—15—16—15)/2. 2,5-Dihydroxybiphenyl was identified in an earlier study[21] as a urinary metabolite of 2-hydroxybiphenyl in the rat.

The diol with a MU of 18.4 was also found as a biphenyl metabolite, but it was not found as a urinary metabolite of either 2-, 3- or 4-hydroxybiphenyl in the rat. However, it was one of the major metabolites in the mouse after administration of 2-hydroxybiphenyl and a minor metabolite after administration of 3-hydroxybiphenyl. The mass spectrum is similar to 3,4-dihydroxybiphenyl and shows the molecular ion as the base peak. Fragment ions were observed at *m/e* 315 (M—CH_3) and 242 [M—Si$(CH_3)_4$]. A loss of 88 a.m.u. is frequently observed for aromatic compounds having vicinal hydroxyl groups. This diol is probably 2,3-dihydroxybiphenyl.

The third diol with a MU value of 19.3 is also a urinary metabolite in the rat after administration of 2-hydroxybiphenyl. but not after administration of either 3- or 4-hydroxybiphenyl. In the mouse it is a metabolite of 4-hydroxybiphenyl but not of 2- or 3-hydroxybiphenyl. Possible structures include 2,4- and 2,4′-dihydroxybiphenyl. The mass spectrum is similar to that of 2,5-dihydroxybiphenyl, and therefore we assume it is 2,4-dihydroxybiphenyl, but in the absence of reference compounds it is not possible to assign a definite structure.

Two triols (MU=21.8 and 23.5) were isolated from urine after administration of biphenyl. One of the triols (MU=23.5) had the same GC–MS properties as a triol which was a major urinary metabolite of 4,4′-dihydroxybiphenyl. This triol was the only product formed when 4,4′-dihydroxybiphenyl was incubated with tyrosinase in the presence of ascorbic acid. Since enzymatic hydroxylation under these conditions occurs *ortho* to the hydroxyl group, the metabolite was assigned the structure of 3,4,4′-trihydroxybiphenyl. The positions of the hydroxyl groups in the second triol (MU=21.8) were not determined.

3,4,4′-Trihydroxybiphenyl, which results from direct hydroxylation of 4,4′-dihydroxybiphenyl, may also be formed *in vivo* by dehydrogenation of an hydroxydihydrodiol. Two hydroxydihydrodiol metabolites (MU=23.5 and 24.0) were excreted in trace amounts. It is very likely that one of these metabolites has structure IV:



(IV)

The other hydroxydihydrodiol is presumably derived from 3-hydroxybiphenyl. The mass spectra of both metabolites exhibited an ion of high abundance at *m/e* 191 which shifted 18 a.m.u. to *m/e* 209 in the spectra of the d_9-TMS analogs; the molecular ions were shifted 27 a.m.u. to *m/e* 447, indicating the presence of three hydroxyl groups in each metabolite.

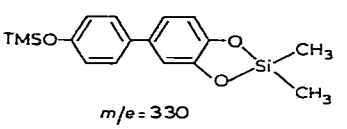
Four metabolites having a methoxyl group were detected by GC–MS analysis.

An O-methylcatechol of biphenyl (3-methoxy-4-hydroxybiphenyl; MU=19.1) was excreted in small amount as a biphenyl metabolite; it was also a major metabolite of 4-hydroxybiphenyl. A molecular ion was present at *m/e* 272; the base peak was observed at *m/e* 242 (M—30). In the d_9 analog, peaks were observed at M—30 (M—OCH_2) and at M—33. The peak at M—33 indicated loss of a methyl radical from the OCH_3 group and a loss of a deuterated methyl radical from the deuterated TMS group. Doubly charged ions were observed at *m/e* 121 [(M—30)/2], 113.5 [(M—45)/2] and 106 [(M—60)/2] with 3 to 9% relative intensity and corresponded to the loss of two, three and four methyl groups, respectively. In the d_9-TMS derivative these ions were observed at *m/e* 124 [(M—CD_3—CH_3)/2], 115 [(M—CD_3—CH_3—CD_3)/2] and 106 [(M—CD_3—CH_3—CD_3—CD_3)/2]. The 3-methoxy-4-hydroxy structure was assigned by analogy with the structure of most known O-methylcatechols. Two isomeric O-methylcatechols (3-methoxy-4-hydroxy- and 3-hydroxy-4-methoxybiphenyl) were identified previously as urinary metabolites in the rat, rabbit and mouse[1,3,9].

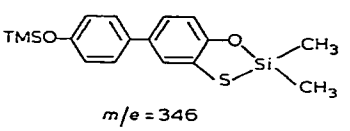
Two isomeric dihydroxymethoxybiphenyl derivatives (MU=22.7 and 22.9) were isolated after administration of biphenyl, 4-hydroxy- and 4,4′-dihydroxybiphenyl. The metabolite with an MU of 22.9 was present in larger quantities and was assigned the structure 3-methoxy-4,4′-dihydroxybiphenyl. The mass spectra of both metabolites exhibited an ion of high abundance at M—30 arising from the loss of OCH_2 and also from the loss of two CH_3 radicals. Three doubly charged ions were present at *m/e* 150, 157.5 and 165; these correspond to (M—OCH_2—CH_3—CH_3)/2, (M—CH_3—CH_3—CH_3)/2 and (M—CH_3—CH_3)/2, respectively. After derivatization with deuterated BSA, the ions were observed at *m/e* 156, 163.5 and 171 corresponding to (M—OCH_2—CD_3—CD_3)/2, (M—CH_3—CD_3—CD_3)/2 and (M—CD_3—CD_3)/2, respectively, The doubly charged ions at *m/e* 150 were observed when substituent groups were in the 4,4′ (ref. 20) or 3,4′ position. Therefore the dihydroxymethoxy metabolite (MU=22.7) is either a 4,4′- or 3,4′-dihydroxy derivative of biphenyl. It was tentatively assigned the structure 3,4′-dihydroxy-4-methoxybiphenyl.

Trace amounts of a metabolite with a molecular weight of 448 (TMS derivative), which corresponds to a trihydroxymethoxybiphenyl, were also present in the urine extracts. This metabolite was eluted from the GC column with an endogenous metabolite and a definitive mass spectrum was not obtained.

A dihydroxymethylthio metabolite was present in trace amounts after administration of biphenyl, and in larger amounts after administration of 4,4′-dihydroxybiphenyl. The molecular ion at *m/e* 376 was the base peak, and a fairly intense ion at *m/e* 330 (M—46) corresponding to the loss of SCH_2 from the molecular ion was present. The molecular ion shifted by 18 a.m.u. *m/e* 394 in the d_9-TMS analog, and the ion at *m/e* 330 shifted to *m/e* 348 (M—46). An ion of high abundance at *m/e* 346 (*m/e* 361 in the d_9-analog) is the sulfur analog of the ion observed at *m/e* 330 in the spectrum of the dihydroxymethoxybiphenyls (MU=22.7 and 22.9). The proposed structures for the two ions are:



m/e = 330



m/e = 346

A doubly charged ion was present at *m/e* 173 ($M-\dot{C}H_3-CH_3$)/2. The GC–MS properties of the urinary metabolite were the same as the GC–MS properties of the methylthio product synthesized from the 4,4′-quinone of biphenyl and 2-keto-4-methylthiobutyric acid. Therefore, the metabolite was assigned the structure 3-methylthio-4,4′-dihydroxybiphenyl. The biologic route of formation is probably through the 4,4′-quinone.

The results of the quantitative analysis are summarized in Table I and III. In Table I the quantities of the individual metabolites are expressed as a percentage of the urinary metabolites (free or total) and are average values obtained from the analyses of seven rat urines. 2-Hydroxybiphenyl was always present in extracts containing free metabolites, but after enzymatic hydrolysis only trace amounts of this metabolite were detected. It is difficult to explain the loss of this compound during enzymatic hydrolysis, but the effect was uniformly observed and may be due to air oxidation. There was considerable interanimal variation in the amounts excreted during 0–24 and 24–48 h, and also in the ratios of free to conjugated metabolites, but the ratio of metabolites within the free fraction and within the conjugated fraction was constant. The total excretion by a single rat (Table III) in 0–24 h from 9.9 mg of biphenyl was 1.4 mg of unconjugated metabolites and 1.5 mg of aglycones. During 24–48 h, the additional excretion was 0.01 mg of unconjugated metabolites and 0.5 mg of aglycones. Out of the 9.9 mg of biphenyl administered to this animal, 3.4 mg of metabolites was recovered from the urine. The quantities, based on GC analysis, must be considered as estimates since suitable internal standards were not available. Our results differ from those of Meyer and Scheline[3] who found only trace amounts of free phenolic metabolites but are similar to those of Meyer *et al.*[22] who reported that 40–50% of the urinary metabolites partitioned like free phenols. In addition, we have identified the phenols present in the unconjugated fraction.

In this study, the detection of 18 metabolites of biphenyl, containing one, two or three oxygen atoms, indicates that an aromatic hydrocarbon of relatively simple structure can undergo extensive oxidative reactions. All of the products whose structures are known were probably formed by two types of oxidation: epoxidation and hydroxylation of a phenol. The nature of the products and the low yields of dihydrodiols suggest that the intermediate epoxides are converted very rapidly into phenols, possibly largely by nonenzymatic reactions. The excretion of large amounts of 4,4′-dihydroxybiphenyl as well as smaller quantities of other diols and triols indicates that extensive recycling (sequential oxidation) through the hepatic monooxygenase system occurred.

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