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Determination of some new phenolic products of biphenyl metabolism

by P. Raig and R. Ammon

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Determination of Some New Phenolic Products of Biphenyl Metabolism

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In connection with experiments on the metabolism of biphenyl, we have recently reported(1) on the gas chromatographic analysis of urine extracts obtained from rabbits fed with biphenyl. We were able to show that besides the known phenolic metabolites of biphenyl such as 2-hydroxybiphenyl, 4-hydroxybiphenyl, 3,4-dihydroxybiphenyl and 4,4'-dihydroxybiphenyl, a few other, apparently still unknown, likewise phenolic products of biphenyl metabolism are traceable in rabbit urine.

In the present paper we have concerned ourselves with the identification of these compounds. We have been able to trace three phenolic metabolites which until now have not been discovered during metabolism investigations with biphenyl, namely 3-hydroxybiphenyl, 3-hydroxy-4-methoxybiphenyl and 3-methoxy-4-hydroxybiphenyl. For purposes of

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comparison the TMS(trimethylsilyl) ether of these compounds and also 3-methoxy-4-hydroxybiphenyl itself were prepared for the first time. The synthesis of these new compounds is described in the experimental section and their physical characteristics are presented in Table 2.

Identification of the New Biphenyl Metabolites

Feeding rabbits with biphenyl and preparation of the urine extract was carried out according to the method which has already been described(1).

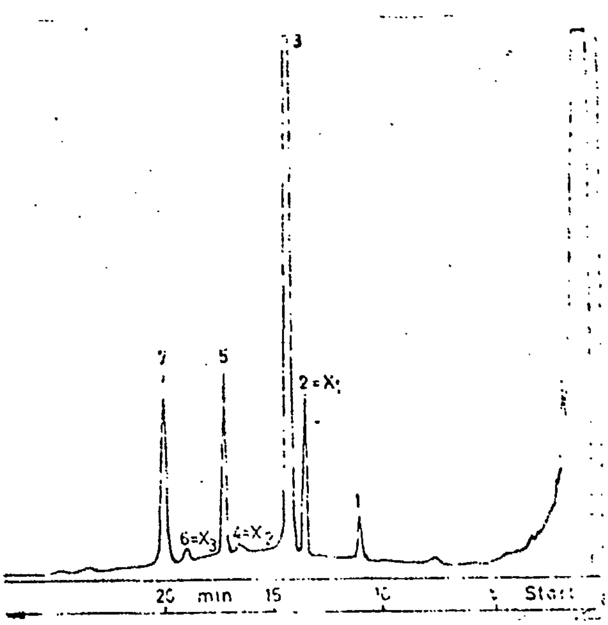

Fig. 1. Gas chromatogram of the phenolic fraction from the urine extract of rabbits(before column chromatography). Stationary phase: silicone rubber SE52; chromatography conditions: see bibliography(1). 1= 2-hydroxybiphenyl; 2= unknown component X_1= 3-hydroxybiphenyl; 3= 4-hydroxybiphenyl; 4= unknown component X_2= mixture of isomeric hydroxymethoxybiphenyls; 5= 3,4-dihydroxybiphenyl; 6= unknown component X_3; 7= 4,4$'$-dihydroxybiphenyl.(All compounds as the TMS-ether).

In the phenolic fractions removed from the urine extracts with 2N NaOH we found, by gas chromatography, three new peaks apparently generated by phenolic products of biphenyl metabolism which were unknown until now. We have designated these by X_1, X_2 and X_3. Such a gas chromatogram (GC) is reproduced in Fig.1.

In order to identify these substances more closely, the phenolic fraction was then examined by thin-layer chromatography with a series of different mixtures of developing solvents. Silica gel P/UV_{254} of the firm Macherey-Nagel and Co., Düren, which contains a fluorescence indicator, was used as absorbent. Besides viewing the thin-layer chromatogram (DC) plate under UV-light, the separated compounds were made visible by treating with Gibbs' reagent(2) and reaction with iodine vapour. The combination of these three techniques allows a reliable correlation of the known biphenyl metabolites 2-hydroxy-, 4-hydroxy-, 3,4-dihydroxy- and 4,4'-dihydroxybiphenyl on the chromatograms of the phenolic fraction (see Table 1).

The best separation was obtained with the benzene-methanol-formic acid system as the developing solvent. With this, 4-hydroxybiphenyl appears as an oval spot with the R_f value of 0.53 and also the phenols 2-hydroxy-, 3,4-dihydroxy- as well as 4,4'-dihydroxybiphenyl with R_f values of 0.68, 0.32 and 0.30 (see Fig.2).

During staining of the chromatogram by the Gibbs' method, the previously mentioned spot of 4-hydroxybiphenyl, in contrast to that of the pure reference compound, at first turned

Table 1. Thin-layer chromatography of hydroxybiphenyls (H = hydroxybiphenyl, DH = dihydroxybiphenyl, M = methoxy)

	R_f Values						Colour reactions	
Composition	Developing solutions*							
	1	2	3	4	5	6	Reagent (Gibbs)	Iodine vapour
2—H	0,68	0,68	0,77	0,67	0,82	0,74	tiefblau	braun
3—H	0,54	0,38	0,63	0,46	0,79	0,62	blau	braun
4—H	0,53	0,36	0,62	0,45	0,77	0,59	rosa, danach farblos	braun
3,4—DH	0,32	○	0,35 ■	0,12	0,65 ■	0,34 ■	violett, danach schwarz	violett bis schwarz
4,4'—DH	0,30	○	0,32 ■	○	0,62	0,30	blauschwarz, danach blaßgrau	tiefbraun bis schwarzbraun
4—H—3—M	0,68	0,68	0,77	0,67	0,82	0,74	keine Reaktion	braun
3—H—4—M	0,68	0,68	0,77	0,67	0,82	0,74	blau	braun
2,5—DH	0,23	○	0,28 ■	○	0,55 ■	0,26 ■	graubraun	braun
2,3—DH	0,38	○	0,41 ■	0,15	0,69	0,39 ■	tiefviolett	intensiv gelbbraun

*) 1 = benzene-methanol-formic acid (90:9:1), 2 = dichloromethane-methanol-conc.NH_3 (94:5:1), 3 = 1,2-dichloroethane-acetone (95:5), 4 = petroleum ether-$CHCl_3$-methanol (50:45:5), 5 = benzene-isopropyl alcohol-conc.NH_3 (70:25:5), 6 = benzene-isopropyl alcohol-conc.NH_3 (90:9:1).
Explanation of symbols: ○ = compound remains at the start;
■ = formation of a tail, R_f measurable only approximately.

	Colour reactions	
Composition	Reagent (Gibbs)	Iodine vapour
2-H	deep blue	brown
3-H	blue	brown
4-H	pink, later colourless	brown
3,4-DH	violet, later black	violet to black
4,4'-DH	blue black to pale gray	deep brown to dark brown
4-H-3-M	no reaction	brown
3-H-4-M	blue	brown
2,5-DH	gray brown	brown
2,3-DH	deep violet	intense yellow brown

Table 2. Physical properties of the new biphenyl metabolites, of their TMS-ether and their retention times.

Phenols		TMS-ether				
	Melting point	M.P.	B.P.	Refrac. index	Retention times.	
					SE 52	XE 60
3—H = X_I	78—79° (Lit. [4a]: 78°)	—	112—114° (0,1 Torr)	n_D^{20} : 1,5575	13,5	13,[illegible]*)
3—H—4—M = X_{Ia}	114—115° (Lit. [24]: 111°) (Lit. [25]: 118°)	88—89° (nach Subl.)	120—121° (0,3 Torr)		16,2	[illegible],8*)
4—H—3—M = X_{Ib}	73—74°	—	138—142° (0,4 Torr)	n_{20}^D : 1,5695	16,4	17,9*)

* corrected values (compare Raig and Ammon 1970).
Lit. = bibliography
Torr = mm Hg
nach Subl. = after sublimation.

pale turquoise blue from the top down; when the chromatogram is allowed to stand further, the blue colour deepens and eventually spreads over almost the whole spot. This behaviour during staining also occurs on the chromatogram of a mixture of 3-hydroxy- and 4-hydroxybiphenyl.

In order to further enrich and characterize the phenolic metabolites of biphenyl, a column chromatographic separation of the phenolic fraction was carried out.

The phenolic fractions of urine extracts from four different feeding experiments were purified and chromatographed in a silica gel column, using benzene-methanol-formic acid as developing solvent (for further details see the experimental section). Four main fractions were obtained which, according to their order of elution in the column, were designated A, B, C and D, and whose composition was analyzed by thin-layer and gas chromatography in the prescribed manner [see above and bibl.(1)]. The GC's of the fractions A-D on stationary phase SE 52 are shown on Figs. 3, 4, 6 and 7.

Fraction A represents approximately 3% of the material eluted from the column and, in TLC, shows a single spot with the R_f value of 2-hydroxybiphenyl and of the two isomeric hydroxymethoxybiphenyls. The spot is stained an intense blue with Gibbs' reagent. The GC (see Fig.3) shows a strong peak, a broad substance peak of average intensity and a

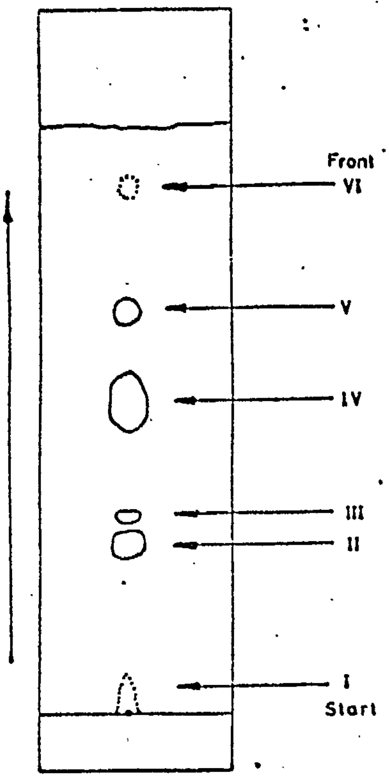

Fig.2. Thin-layer chromatogram of the phenol fraction from a rabbit urine extract. Developing solvent: benzene-methanol-formic acid (90:9:1 v/v).

VI weak spot of unknown origin (also appears with bland urine)

V 2-hydroxybiphenyl and 3(4)-hydroxy-4(3)-methoxybiphenyl.

IV 4- and 3-hydroxybiphenyl.

III 3,4-dihydroxybiphenyl

II 4,4'-dihydroxybiphenyl

I starting spot (also appears with bland urine).

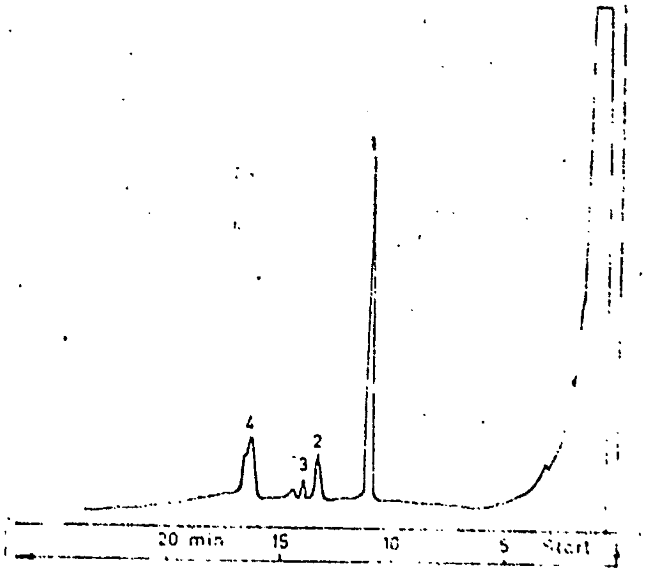

Fig. 3. Gas chromatogram of fraction A. Stationary phase: silicone rubber SE 52; chromatography conditions: see bibl.(1). 1 = 2-hydroxybiphenyl; 2 = 3-hydroxybiphenyl; 3 = 4-hydroxybiphenyl; 4 = mixture of isomeric hydroxymethoxybiphenyls (all compounds as TMS-ether).

group of four peaks of lesser intensity. The compound which produces the strong peak was identified as the TMS-ether of 2-hydroxybiphenyl because of its retention time and by the admixture method. In the same manner one of the peaks with lesser intensity revealed itself as that of 4-hydroxybiphenyl. The broad substance peak of average intensity has the same retention time as the substance which we designated X_2 and obviously represents a mixture of at least two compounds. A further separation of these substances could not be obtained even when chromatographic conditions were varied.

The identification of these compounds was made by comparison with the isomeric hydroxymethoxybiphenyls. In gas chromatography on SE 52 and also on XE 60, both showed the same broad substance peak in mixture after silylation. Addition of 3-hydroxy-4-methoxybiphenyl raises the peak at the original retention time while the addition of 4-hydroxy-3-methoxybiphenyl lengthens the retention time by approximately 0.2 min. (see also Table 2). Thus it is p.1400 proved with adequate certainty that the substance designated as X_2 is a mixture of the isomeric hydroxymethoxybiphenyls.

Fraction B, with approximately 60% of the material eluted from the column, represents the major portion of the phenolic fraction. An elongated spot with the R_f value of 4-hydroxybiphenyl appears during TLC and this spot can be stained blue with Gibbs' reagent (compare above with the TLC of the crude phenolic fraction) in contrast with a reference chromatogram of pure 4-hydroxybiphenyl. These findings indicate that an isomeric phenol with similar chromatographic properties is present in mixture with 4-hydroxybiphenyl. The fact that the Gibbs' staining reaction is specific for phenols with a free p-position strengthens the suspicion that the compound which accompanies 4-hydroxybiphenyl is 3-hydroxybiphenyl. In fact, in all the systems of developing solvents examined, p.1401

3-hydroxybiphenyl shows the same R_f value as 4-hydroxybiphenyl with differences of 0.01-0.03 and the blue stain with Gibbs' reagent which is typical of phenols with a free p-position. Synthetic mixtures of 3-hydroxy- and 4-hydroxybiphenyl show the same properties in thin-layer chromatography as column fraction B.

The GC of fraction[⁎] B (see Fig.4)[⊕] shows peaks with an intensity in the ratio of about 1:10 of which the higher peak can be assigned to 4-hydroxybiphenyl and the other with lesser intensity shows the same retention time as the substance which we previously designated as X_1. By comparison of the retention times and by the admixture method, this peak can be recognized as being produced by 3-hydroxybiphenyl.

Finally fraction B was re-chromatographed on silica

[⁎] "zone" in text (Trsl.)

[⊕] There is a discrepancy in peak numbers between the caption and the graph and also in the following discussion in the text (Trsl.)

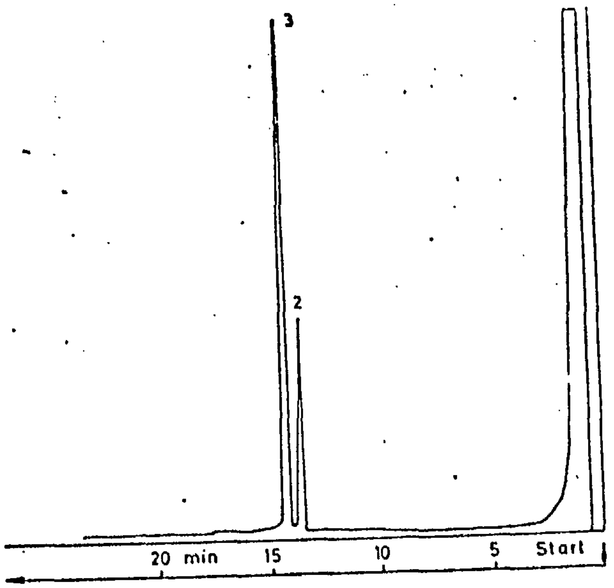

Fig. 4. Gas chromatogram of fraction B. Stationary phase: silicone rubber SE 52; chromatography conditions: see bibl.(1). 2 = 3-hydroxybiphenyl; 4 = mixture of isomeric hydroxymethoxybiphenyls (compounds as TMS-ether).

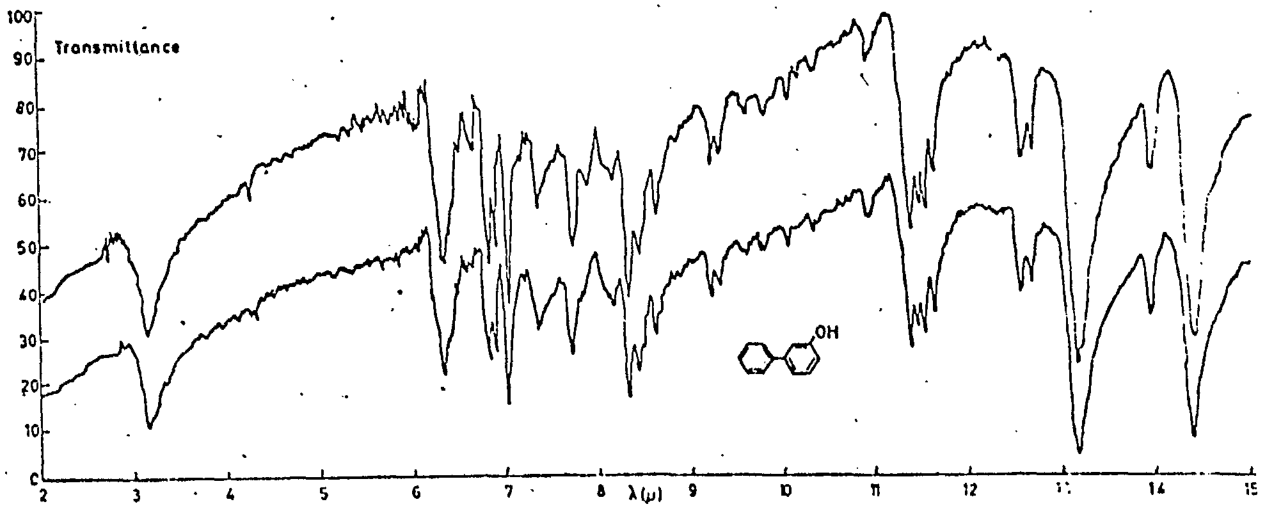

Fig.5. IR spectra of 3-hydroxybiphenyl (in KBr). Upper curve: metabolite, isolated from rabbit urine; lower curve: synthetically prepared reference compound.

gel with benzene-methanol-formic acid (see the experimental section). By this means we were able to isolate together with 4-hydroxybiphenyl a crystalline compound in small quantity, which is uniform both in thin-layer and gas chromatography. This compound was identical to 3-hydroxybiphenyl in melting point, mixed melting point, UV and IR spectra (see Fig.5). Based on these findings, the new metabolic product of biphenyl previously designated as X_1 represents 3-hydroxybiphenyl.

Fraction C (approximately 9% of the eluted substance) shows two main spots in TLC, both of about equal intensity which, according to the R_f values and the staining reactions, can be assigned to 3,4-dihydroxy- and 4-hydroxybiphenyl. In GC, two peaks of almost equal intensity can be seen and these can likewise be identified as the TMS-ether of 3,4-dihydroxy- and 4-hydroxybiphenyl by means of the retention time and with

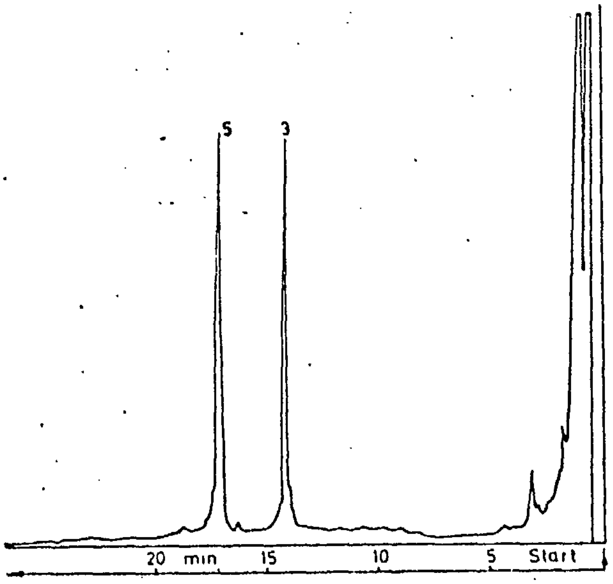

Fig.6. Gas chromatogram of fraction C. Stationary phase: silicone rubber SE 52; chromatography conditions: see bibl.(1). 3 = 4-hydroxybiphenyl; 5 = 3,4-dihydroxybiphenyl (compounds as TMS-ether).

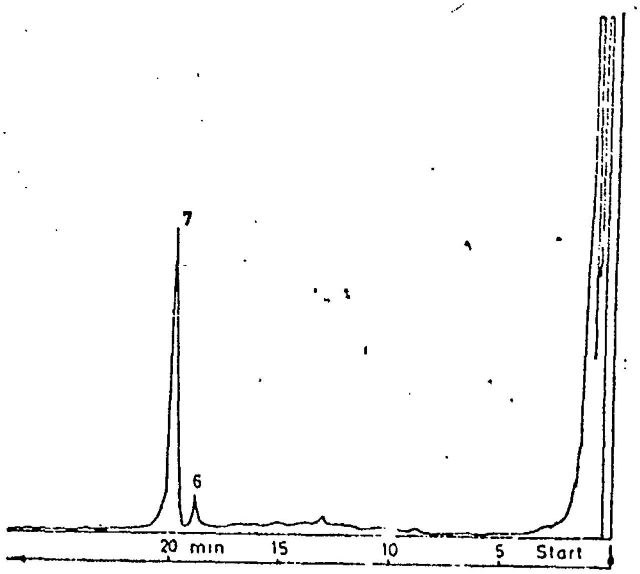

Fig.7. Gas chromatogram of fraction D. Stationary phase: silicone rubber SE 52; chromatography conditions: see bibl.(1). 6 = unknown component X_3; 7 = 4,4'-dihydroxybiphenyl (compounds as TMS-ether).

the help of the admixture method (see Fig.6).

In TLC, fraction D (12% of the eluted substance) shows only one main spot in addition to a zone which remains at the start.

This spot can be attributed to 4,4'-dihydroxybiphenyl by means of the R_f value and the staining reaction. A strong peak appears in GC which can be assigned to 4,4'-dihydroxybiphenyl and also some peaks of lesser intensity of which the one accounting for the longest retention time corresponds with the substance previously designated as X_3 (see Fig.7). However, after re-chromatography of this fraction on silica gel, no other compound besides 4,4'-dihydroxybiphenyl could be isolated, probably because of the small quantity of substance available.

Discussion of Results

During investigations on the metabolism of biphenyl only 2- and 4-hydroxybiphenyl have until now been found as products of monohydroxylation(5).

Work on the hydroxylation of biphenyl in vitro with liver preparations also took into account only the formation of 2- and 4-hydroxyphenyl (6-11).

We attribute the fact that the formation of 3-hydroxybiphenyl has been overlooked until now to the great similarity of the physical properties of the two isomers, especially their chromatographic behaviour. While isolating 4-hydroxybiphenyl from biological material, small impurities of 3-hydroxybiphenyl can easily be lost during the purification operation.

If the biological hydroxylation of aromatic systems by the unspecific $NADPH_2$-dependent hydroxylase system of the liver microsomes is considered to be an electrophile substitution by a certain oxygen species (not yet precisely known) on the surface of a suitable catalyst(12,13), then it appears at first that the hydroxylation of biphenyl in the 3-position is unusual , at least to the extent which we have found(1). However, it can be explained up to a certain point by the recently discovered "NIH-shift"(14).

The isomeric hydroxymethoxybiphenyls are probably formed by o-methylation of the 3,4-dihydroxybiphenyl which is primarily produced.

The occurence of mono-o-methylated pyrocatechol derivatives in the urine of research animals or humans after administration of an aromatic compound which is not substituted by hydroxyl groups was recently pointed out by Sinsheimer and Smith in the example of trans-stilbene(15) and Šipal and Jindra in the example of 2-phenylcinchonine acid(16).

Reference must also be made to biphenylmercapturic acid which is found in small quantity in the urine of rats fed with biphenyl(17). In this connection it should also be mentioned that 2,3,6-trihydroxybiphenyl(18) and α-hydroxy-β-phenylmuconic acid semialdehyde(19) were identified as metabolic products of bacteria biphenyl. p.1402

It was recently reported by Ernst(20) that after oral intake of 2-hydroxybiphenyl, which is also used as a preservative for citrus fruit, 2,5-dihydroxybiphenyl appears as a metabolic product in the urine of rats. Since, in our experiments, 2-hydroxybiphenyl is also, among others, produced from biphenyl, the formation of these metabolites must theoretically also be taken into account. However, the quantity of 2-hydroxybiphenyl which originates from biphenyl in our experiments is so small that its metabolite cannot be detected by us.

Investigations of biphenyl metabolism in vivo and in vitro, with reference to the formation of the new metabolites 3-hydroxybiphenyl, 3-hydroxymethoxybiphenyl[⊕] and 4-hydroxy-3-methoxybiphenyl with other animal species will be reported later in this journal.

Our investigations suggest that, in addition, experiments with biphenyl-C^{14} should be undertaken. For this purpose biphenyl with all C-atoms labelled, which is not available in the trade, was prepared(21) from aniline-C^{14}-hydrosulphate according to the schema:

$$C_6H_5{-}NH_3^+ HSO_4^- \xrightarrow[2.\ KJ]{1.\ HNO_2} C_6H_5J \xrightarrow{Cu,\Delta} C_6H_5{-}C_6H_5 \quad ⁎$$

However, for extraneous reasons, these experiments, which would surely have confirmed and perhaps broadened the scope of our present results, could not be carried on further.

[⊕] Should be 3-hydroxy-4-methoxybiphenyl (Trsl.)
[⁎] KJ = KI, $C_6H_5J = C_6H_5I$ (Trsl.)

Experimental Section

a) General

Melting points were measured with the melting point determination apparatus of Dr. Tottoli of the firm Büchi, in Flawil (Switzerland), and are not corrected.

The ultimate analysis of the TMS-ethers was carried out in the microanalytical laboratory of Dr. F. Pascher, Bonn, and of the other compounds in the microanalytical laboratory of Saarland University.

H'-NMR spectra: Varian A60, analytical NMR spectrometer. Tetramethylsilane was used as internal standard for the determination of the ∂-values (ppm).

IR spectra: Beckman IR spectrophotometer No.4; solid substances were compressed in KBr and liquids measured as films. We are especially thankful to Mr. Müller of the spectroscopic laboratory of the Institute for Organic Chemistry of Saarland University for taking the IR and NMR spectra.

UV spectra were measured in 96% ethanol with spectrophotometer DK-1A of the firm Beckman, Munich.

Silica gel P/UV$_{254}$ of the firm Macherey-Nagel and Co. in Düren was used as the carrier in thin-layer chromatographic experiments. The separating layers were prepared according to working instructions enclosed in "Grundausrüstung für die Dünnschichtchromatographie" (Basic equipment for thin-layer chromatography, Trsl.) of the firm Desaga, Heidelberg.

The spray reagent described by Gibbs(2) was prepared by dissolving 1 g of 2,6-dichloroquinonechlorimide in 100 ccs. of ethanol. After spraying with this solution, the chromatogram was treated with ammonia vapour.

The gas chromatographic experiments were conducted according to the already described method (1).

Silica gel (0.05-0.2 mm) of the firm Merck, Darmstadt, was used for column chromatography.

b) Reference Materials

I 3-Hydroxybiphenyl

3-Nitrobiphenyl

50.0 g (0.36 mole) of 3-nitroaniline (Merck, Darmstadt) were converted into 43.1 g (60% of theor.) of 3-nitrobiphenyl with 3-nitro-N-nitroso-N-acetylaniline following instructions of Bachmann and Hoffmann(3). After distillation at 140-145°C (0.2 mm Hg) the compound was crystallized from 80% ethanol. Melting point: 56 to 57°C. [bibl.(3): 58-59°C.]

3-Aminobiphenyl

7.00 g (0.035 mole) of 3-nitrobiphenyl were reduced with zinc in aqueous ethanolic hydrochloric acid according to Jacobson and Loeb(4a). The base released by caustic soda was converted into the easily crystallized sulphate after steam distillation [see bibl.(4b)]. Yield: 6.40 g (42% of theor.). For analysis the compound was recrystallized twice from water. Melting point: 173-175°C (decomposes).

$C_{24}H_{21}N_2O_4S$ (436.4)	Reported:	C 66.05, H 5.54, N 6.42
	Found:	C 65.70, H 5.37, N 6.40

3-Hydroxybiphenyl

1.09 g (5.00 mmole) of 3-aminobiphenylsulphate yielded 0.583 g (69% of theor.) of 3-hydroxybiphenyl after diazotization with isoamyl nitrate in aqueous ethanolic tetrafluoroboric acid and treating the diazoniumtetrafluoroborate with cuprous oxide in an aqueous copper sulphate solution(22). The compound was recrystallized twice from petroleum ether (60-80°C) for analysis. Melting point: 78-79°C [bibl.(4a): 78°C].

$C_{12}H_{10}O$ (170.2)   Reported: C 84.68, H 5.92
Found: C 84.50, H 5.85

IR spectrum: see Fig.5.

II <u>3-Hydroxy-4-methoxybiphenyl</u>

2-Hydroxy-5-phenylazobenzene

26.9 g (91% of theor.) of 2-hydroxy-5-phenylazobenzene were obtained by the diazotization of 10.0 g (0.11 mole) of aniline in aqueous hydrochloric acid followed by reaction with 17.0 g (0.10 mole) of 4-hydroxybiphenyl in 2N KOH. Melting point (after crystallization from ethanol): 126-127°C [bibl.(23): 127.5°C].

2-Methoxy-5-phenylazobenzene

20.0 g (0.073 mole) of 2-hydroxy-5-phenylazobenzene were methylated with dimethyl sulphate and KOH in ethanol according to Tarbell et al.(23) to yield 15.4 g (73% of theor.) of 2-methoxy-5-phenylazobenzene. Melting point (after one crystallization from ethyl acetate: 135 to 136°C [bibl.(23): 136.5-137.5°C].

3-Amino-4-methoxybiphenyl

6.04 g (88% of theor.) of 3-amino-4-methoxybiphenyl were obtained by hydrogenation of 10.0 g (0.035 mole) of 2-methoxy-5-phenylazobenzene on platinum/carbon in absolute ethanol(23). Melting point (after crystallization from 96% ethanol): 80°C [bibl.(23): 80-81°C].

$C_{13}H_{13}NO$ (199.2) Reported: C 78.36, H 6.58, N 7.03 p.1403
Found: C 78.10, H 6.51, N 6.60

After three crystallizations from water, the sulphate had a melting point of 200 to 202°C with decomposition.

$C_{26}H_{28}N_2O_6S$ (496.5) Reported: C 62.90, H 5.68, N 5.64
Found: C 62.00, H 5.65, N 5.3

3-Hydroxy-4-methoxybiphenyl

1.00 g (5.03 mmole) of 3-amino-4-methoxybiphenyl was converted into 3-hydroxy-4-methoxybiphenyl as indicated under 3-aminobiphenyl. Yield: 0.284 g (28% of theor.). Melting point (after crystallization from petroleum ether (60-80°C): 114-115°C [bibl.(24): 111°C; bibl.(25): 118°C] λ_{max} 264mμ (ε 15800).

$C_{13}H_{12}O_2$ (200.2) Reported: C 77.98, H 6.04
Found: C 77.70, H 6.11

IR spectrum(KBr): 3330 cm^{-1}(OH); 1598, 1575, 1530,1492 cm^{-1} (aromatic C=C); 1250 cm^{-1} (C-O-C).

H^1-NMR spectrum ($CDCl_3$): singlet at 3.87 ppm, 3H:OCH_3; broad signal at 5.67 ppm (shifts with D_2O towards 4.60 ppm), 1H:OH; multiplet between 6.80 and 7.80 ppm, 8H:aromatic protons.

III 4-Hydroxy-3-methoxybiphenyl

2-Chloro-5-nitrobenzophenone

80.6 g (0.40 mole) of 2-chloro-5-nitrobenzoic acid (Fluka, Buchs/Switzerland) were converted into 87.6 g (85% of theor.) of 2-chloro-5-nitrobenzophenone according to Fries(26). Melting point (after crystallization from ethanol): 84-85°C [bibl.(26): 86°C].

2-(4-Phenylphenoxy)-5-nitrobenzophenone

15.08 g (0.089 mole) of 4-hydroxybiphenyl yielded 16.79 g (85% of theor. based on 2-chloro-5-nitrobenzophenone) of 2-(4-phenylphenoxy)-5-nitrobenzophenone after conversion with 13.1 g (0.050 mole) of 2-chloro-5-nitrobenzophenone according to the method of Loudon and Scott(24). Melting point (after crystallization from isopropyl alcohol): 125-126°C [bibl.(24): 126°C].

2-(2-Hydroxy-4-phenylphenoxy)-5-nitrobenzophenone

14.0 g (0.035 mole) of 2-(4-phenylphenoxy)-5-nitrobenzophenone in concentrated sulphuric acid were treated with 30% hydrogen peroxide in glacial acetic acid according to Louden and Scott(24) to yield 12.2 g (84% of theor.) of 2-(2-hydroxy-4-phenylphenoxy*)-5-nitrobenzophenone. Melting point (after crystallization from methanol): 123-124°C [bibl.(24): 119°C]. λ_{max} 252mμ (ε 34800).

$C_{24}H_{27}NO_5$(411.4) Reported: C 72.98, H 4.17, N 3.41

Found: C 72.70, H 3.99, N 3.20

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* "phenoxy" is omitted in the text. (Trsl.)

2-(2-methoxy-4-phenylphenoxy)-5-nitrobenzophenone

870.0 mg (2.11 mmole) of 2-(2-hydroxy-4-phenylphenoxy)-5-nitrobenzophenone yielded 791.0 mg (88% of theor.) of 2-(2-methoxy-4-phenylphenoxy)-5-nitrobenzophenone with diazomethane in ether according to the method of Louden and Scott[24]. Melting point (after crystallization from ethanol): 121-122°C (bibl.(24): 120°C) λ_{max} 257mμ (ε 37,100)

$C_{26}H_{19}NO_5$ (425.4) Reported: C 73.40, H 4.50, N 3.29

Found: C 73.40, H 4.42, N 3.10

4-Hydroxy-3-methoxybiphenyl

748.0 mg (1.76 mmole) of 2-(2-methoxy-4-phenylphenoxy)-5-nitrobenzophenone were converted into 295.0 mg (84% of theor.) of 4-hydroxy-3-methoxybiphenyl by heating for one hour with piperidine at boiling temperature (see Louden and Scott[24]). After two crystallizations from petroleum ether 60-80°C) the new compound melted at 73-74°C, λ_{max} 266 mμ (ε 15,300).

$C_{13}H_{12}O_2$ (200.2) Reported: C 77.98, H 6.04

Found: C 77.90, H 6.00

IR spectrum (KBr): 3460 cm^{-1}(OH); 1604, 1528,1483 cm^{-1}(aromatic C=C); 1254 cm^{-1}(C-O-C).

H^1-NMR spectrum ($CDCl_3$): singlet at 3.90 ppm, 3H:OCH_3; broad signal at 5.62 ppm (shifts towards 4.63 ppm with D_2O),1H:OH; multiplet between 6.83 and 7.78 ppm, 8H:aromatic protons.

IV Trimethylsilyl ether

The trimethylsilyl ether of the three new metabolic products of biphenyl were prepared, as recently described(1) for 2-trimethylsiloxynaphthalene and 3,4-bis-trimethylsiloxybiphenyl, by heating the phenols with bis-trimethylsilylacetamide in boiling chloroform.

3-Trimethylsiloxybiphenyl

426.0 mg (2.5 mmole) of 3-hydroxybiphenyl yielded 504.0 mg (83% of theor.) of 3-trimethylsiloxybiphenyl as a colourless oil. Boiling point: 119 to 120°C/0.2 mm Hg, 112-114°C/0.1 mm Hg, n_D^{20} : 1.5575

$C_{15}H_{18}OSi$ (242.4)	Reported:	C 74.33,	H 7.45,	Si 11.59
	Found:	C 73.71,	H 7.35,	Si 10.50

IR spectrum (film): 2980 cm^{-1} (CH_3); 1710, 1587, 1470 cm^{-1} (aromatic C=C); 1250 cm^{-1} (Si-CH_3-deformation); 850 cm^{-1} (Si-CH_3-streak).

H^1-NMR spectrum ($CDCl_3$): singlet at 0.28 ppm: CH_3 Si;* multiplet between 6.70 and 7.70 ppm: aromatic protons. Ratio of intensity of both signal groups 1:1.

3-Methoxy-4-trimethylsiloxybiphenyl

1.040 g (5.20 mmole) of 4-hydroxy-3-methoxybiphenyl yielded 1.082 g (76% of theor.) of 3-methoxy-4-trimethylsiloxybiphenyl as a colourless oil. Boiling point: 140-142°C under 0.3 mm Hg., n_D^{20}: 1.5695

$C_{16}H_{20}O_2Si$† (272.4)	Reported:	C 70.54,	H 7.40,	Si 10.31
	Found:	C 71.08,	H 7.27,	Si 10.12

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* should be 9H:CH_3Si to be consistent (Trsl.)

† O_2 is omitted in the text (Trsl.)

IR spectrum (film): 2940 cm^{-1}(CH_3); 1600, 1582, 1523, 1490 cm^{-1} (aromatic C=C); 1255 cm^{-1}(Si-CH_3-deform.); 850 cm^{-1}(Si-CH_3-streak).

H^1-NMR spectrum ($CDCl_3$): singlet at 0.33 ppm, 9H:CH_3Si; singlet at 3.90 ppm, 3H:CH_3O; multiplet between 6.85 and 7.75, 8H:aromatic protons.

4-Methoxy-3-trimethylsiloxybiphenyl

549.0 mg (2.74 mmole) of 3-hydroxy-4-methoxybiphenyl yielded 652.0 mg (87% of theor.) of 4-methoxy-3-trimethylsiloxybiphenyl as colourless, shiny crystals which sublimed at 85-90°C/0.3 mm Hg. Melting point: 88-89°C.

$C_{16}H_{20}O_2Si$ (272.4)	Reported:	C 70.54,	H 7.40,	Si 10.31
	Found:	C 70.06,	H 7.40,	Si 10.44

IR spectrum (KBr): 2940 cm^{-1}(CH_3); 1605,1597, 1582, 1567, 1490 cm^{-1}(aromatic C=C); 1443 cm^{-1}(Si-CH_3-deform.); 843 cm^{-1} (Si-CH_3-streak).

H^1-NMR spectrum ($CDCl_3$): singlet at 0.27 ppm, 9H:CH_3Si; singlet at 3.80 ppm, 3H:CH_3O; multiplet between 6.75 and 7.67 ppm, 8H:aromatic protons.

c) Preparatory Chromatography of the Phenolic Fraction from Extracts of Rabbit Urine

1.009 g of phenolic fraction [for recovery see bibl.(1)] was dissolved in 3 ccs of benzene-methanol-formic acid (90:9:1), charged into a silica gel column (3.5 x 42 cm) and eluted with the above-mentioned solvent mixture. The eluate was collected in fractions of 10 ccs each. The following four main fractions were obtained and their composition determined by thin-layer and gas chromatography (refer to theoretical section):

Fraction A: fractions 30-33 20 mg
Fraction B: fractions 34-44 394 mg
Fraction C: fractions 45-51 89 mg
Fraction D: fractions 52-71 125 mg

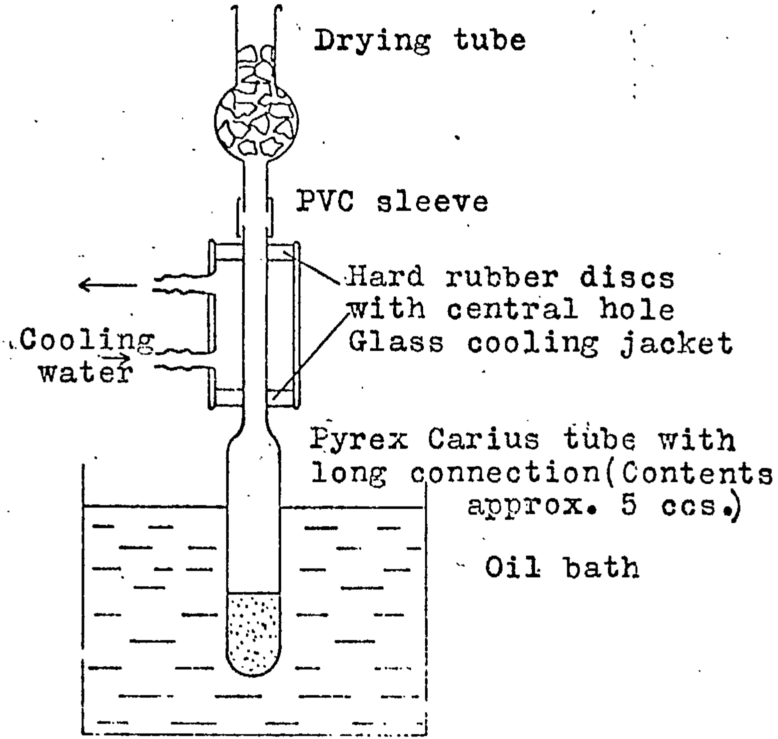

Fig. 8. Experimental arrangement to carry out the Ullmann coupling in semimicro-scale.

Fraction B (394.0 mg) was dissolved in 1.5 cc of benzene-methanol-formic acid mixture (95:4:1) and chromatographed in a silica gel column (3.5 x 43 cm). The eluate was collected in fractions of 5 ccs each. Fractions 128-135 contained 3-hydroxybiphenyl which showed uniformly either in thin-layer or gas chromatography. After evaporation of the elution solvent, the residue (12.0 mg) was crystallized once from petroleum ether (60-80°C). Melting point: 77-78°C (bibl. (4a): 78°C). Mixed melting point (with synthetic 3-hydroxybiphenyl): 77-78°C, λ_{max} 251 mμ(ε 17,500).

$C_{12}H_{10}O$ (170.2)	Reported:	C 84.68,	H 5.92
	Found:	C 84.70,	H 6.03

IR spectrum: see Fig. 5.

Fractions 136-152 represented a mixture of 3- and 4-hydroxybiphenyl, while fractions 153-192 contained 4-hydroxybiphenyl which showed uniformly by thin-layer or gas chromatography. After evaporation of the elution solvent, the solid residue (195.0 mg) was crystallized once from petroleum ether (100-140°C). Melting point: 162-163°C (bibl.(27): 164°C). Mixed melting point (with authentic 4-hydroxybiphenyl): p.1404 163-164°C, λ_{max} 262 mμ (ε 21,800)

$C_{12}H_{10}O$ (170.2)	Reported:	C 84.68,	H 5.92
	Found:	C 84.13,	H 5.91

The results of the study of fractions A,C and D are outlined in the theoretical section.

d) Biphenyl-C^{14}-(U)*

Iodobenzene-C^{14}-(U)

0.1 mCi of aniline hydrogensulphate-C^{14}-(U),(specific activity 204 Ci/mg; supplier: Buchler and Co., Braunschweig) was diazotized together with 1.00 ml (1.20 g, 12.9 mmole) of aniline in dilute, aqueous hydrochloric acid according to directions of Murray and Williams(21) for the preparation of

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* U = universally labelled i.e. All C atoms are C^{14} (Trsl.)

iodobenzene-I^{131} and then converted into iodobenzene-C^{14}(U) through reaction of the resulting benzenediazonium chloride solution with potassium iodide. Boiling point: 58°C/11 mm Hg (bath temperature: 80°C). Yield: 1.361 g (61% of theor.).

Biphenyl-C^{14}(U)

1.361 g (6.7 mmole) of iodobenzene-C^{14}(U)(see above) was heated with activated copper/bronze(28) for three hours at 220°C in apparatus as shown on Fig.8, relying on the method of Hall et al.(29) (for the synthesis of biphenyl-1-1'-C^{14}, see also bibl.(30)). We declined to heat for a longer time and thus obtained a purer biphenyl; the iodobenzene which is recovered can be used again for the synthesis of biphenyl. The cooled reaction mixture was extracted with warm ether, the filtered extract concentrated in a rotary evaporator and the residue fractionated under vacuum. After the initial distillation of unreacted iodobenzene, the biphenyl-C^{14}(U) sublimes at 60-65°C (air bath temperature)/ 11 mm Hg. Melting point 67-69°C. Yield: 150 mg (29% of theor.). Specific activity: reported 0.12 Ci/mg; measured 0.124 and 0.117 Ci/mg[*].

Summary

Determination of some New Metabolic Products of Biphenyl

In a previous investigation [1] three new biphenyl metabolites, therein named X_1, X_2 and X_3, were detected by means of vapor phase chromatography in urine extracts of rabbits fed with biphenyl.

In this work X_1 has been identified as 3-hydroxy-biphenyl and X_2 as a mixture of the monomethylated analogues of 3,4-dihydroxy-biphenyl.

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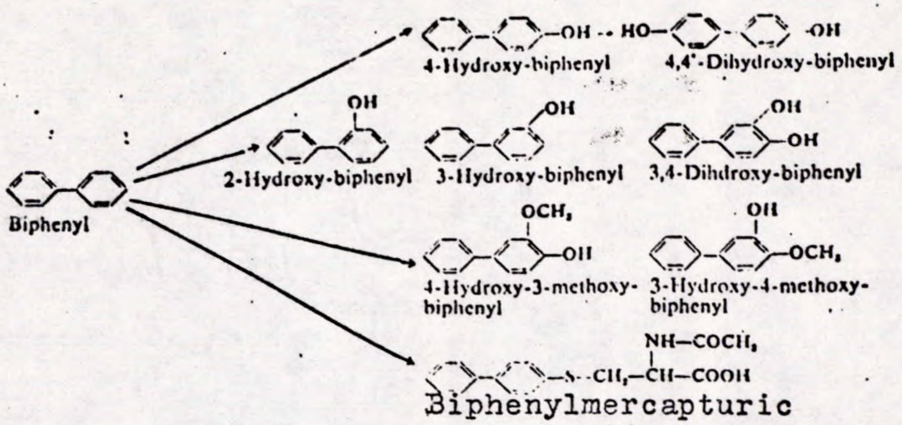
[*] We thank Prof. Pfleger of the Pharmacology Institute of Saarland University for the measurement of the specific activity of our preparations.

The hitherto reliably identified metabolic products of biphenyl in the urine of rabbits and rats can be outlined in the following classification:



Biphenylmercapturic acid

For characterization in the gas chromatographical studies the trimethylsilyl (TMS) derivatives of the three metabolites have been prepared for the first time.

Furthermore, 3-methoxy-4-hydroxy-biphenyl not yet described in the literature has been synthesized.

The results obtained in thin-layer chromatography studies with the phenolic biphenyl metabolites in various systems have been summarized in Table 1.

Some physical properties of the new biphenyl metabolites have been collected in Table 2.

A synthesis of biphenyl-C^{14} (uniformly labelled) is described.

Bibliography

[1] Raig, P., Ammon, R., Arzneim.-Forsch. (Drug Res.) **20**, 1266 (1970) — [2] Gibbs, H. D., J. Biol. Chem. **73**, 649 (1927) — [3] Bachmann, W. E., Hoffmann, W. E., Org. Reactions **2**, 224, 249 (1944) [4a] Jacobson, P., Loeb, A., Ber. Dtsch. Chem. Ges. **36**, 4082 (1903) — [4b] Fichter, F., Sulzberger, A., Ber. Dtsch. Chem. Ges. **37**, 878, 882 (1904) — [5] Williams, R. T., Detoxication mechanisms, 2. Aufl., Chapman & Hall Ltd., London (1959) — [6] Creaven, P. J., Parke, D. V., Williams, R. T., Biochem. J. **96**, 390, 879 (1965) — [7] Mitoma, C. Posner, H. S., Reitz, H. C., Udenfriend, S., Arch. Biochem. Biophysics **61**, 431 (1956) — [8] Creaven, P. J., Parke, D. V., Conney, A. H., Biochem. Pharmac. **15**, 7 (1966) — [9] Daly, J., Jerina, D., Witkop, B., Arch. Biochem. Biophysics **128**, 517 (1968) — [10] Jaffe, H., Fujii, K., Guerin, H., Sengupta, M., Epstein, S. S., Biochem. Pharmac. **18**, 1045 (1969) — [11] Bachmann, E., Golberg, L., Di Giacomo, P., Exper. Mol. Pathology **12**, 212 (1970) — [12] Ullrich, V., Staudinger, H., 19. Coll. der Ges. für Biol. Chem., S. 241 ff., Springer Verlag, Berlin-Heidelberg-New York (1968) — [13] Gillette, J. R., in Shugar, D., Biochemical Aspects of Antimetabolites and of Drug hydroxylation S. 109 ff., Academic Press London and New York (1969) — [14] Guroff, G., Daly, J. W., Jerina, D. M., Renson, J., Witkop, B., Udenfriend, S., Science **157**, 1524 (1967) — [15] Sinsheimer, J. E., Smith, R. V., Biochem. J. **111**, 35 (1969) — [16] Šipal, Z., Jindra, A., in Shugar, D., Biochemical Aspects of Antimetabolites and of Drug hydroxylation S. 239 ff., Academic Press London and New York (1969) — [17] West, H. D., Lawson, J. R., Miller, J. H., Mathura, G. R., Arch. Biochem. Biophysics **60**, 14 (1956) — [18] Herber, R., Villoutreix, J., Pierfitte, M., C. R. Soc. Biol. **163**, 1657 (1969) — [19] Lunt, D., Evans, W. C., Biochem. J. **118**, 54 p (1970) — [20] Ernst, W., Arzneim.-Forsch. (Drug Res.) **15**, 632 (1965) — [21] Murray, A., Williams, D. L., Organic Synthesis with isotopes. Part II, Interscience Publishers, New York and London S. 1229 (1958) — [22] Lewin, A. H., Cohen, T., J. Org. Chem. **32**, 3844 (1967) — [23] Tarbell, D. S., Hirschler, H. P., Hall T. J., J. Amer. Chem. Soc. **75**, 19[illegible] (1953) — [24] Loudon, J. D., Scott, J. A., J. Chem. Soc. **1953**, 2[illegible] (1953) — [25] Blatchly, J. M., Omie, J. F. W., Watts, M. L., J. Chem. Soc. **1962**, 5085 — [26] Fries, K., Liebigs Ann. Chem. **454**, 287 (1927) — [27] Mikeska, V. J., Bogert, M. T., J. Amer. Chem. Soc. **57**, 2121 (1935) — [28] Kleiderer, E. C., Adams, R., J. Amer. Chem. Soc. **55**, 4225 (1933) — [29] Hall, D. M., Lesslie, M. S., Turner, E. E., J. Chem. Soc. **1950**, 711 — [30] Wynberg, H., Wolf, A. P., J. Amer. Chem. Soc. **85**, 3308 (1963)

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