Synthesis of tritiated biphenyls, labelled at specific positions

P.Ph.H.L. Otto
Centraal Laboratorium TNO, Delft, Netherlands

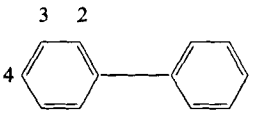
G. Juppe
Euratom, CCR, Ispra, Italy
Received on 23 May 1966

Summary

The tritiated compounds biphenyl-2-T, -3-T and -4-T have been synthesized by catalytic reduction of the corresponding bromobiphenyls with tritium gas under mild conditions. The specificity of labelling, expressed in % of total activity found at the desired position, and determined by degradation of the molecules, was 96.1 ± 1.5, 99.29 ± 0.01 and 98,68 ± 0.02, respectively.
Syntheses and degradations were similar to those described previously for tritiated p*-terphenyls [1]. For biphenyl-3-T, a second degradation, giving more precise results, has been worked out.*

Introduction

Because of symmetry, only three positions are to be distinguished in the biphenyl molecule for single labelling with tritium. These are positions 2, 3 and 4 :



For introduction of tritium into the biphenyl molecule, specifically at one of these, the same method has been used as described for the synthesis of specifically labelled *p*-terphenyls, viz. by catalytic reduction of the corresponding bromoderivatives with tritium gas [1].

In order to determine the activity distribution over the several positions in the labelled molecules, degradations have been carried out according to the schemes shown in tables I and II. The scheme of table I parallels the one worked

out for the degradation of labelled *p*-terphenyls [1], with improvements on minor points. The degradation scheme of Table II has been worked out especially for biphenyl-3-T; it allows more precise calculation of the activity distribution in

TABLE 1. Degradation scheme.

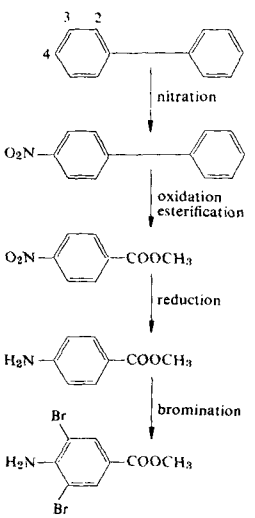
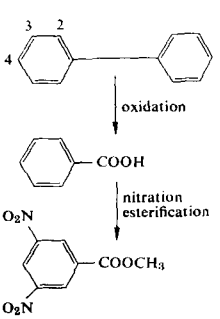
	Theoretical specific activities (in units per millimole)		
	4-T ≡ 1	3-T ≡ 1	2-T ≡ 1
nitration			
O_2N	0.5	1	1
oxidation esterification			
O_2N—$COOCH_3$	0	0.5	0.5
reduction			
H_2N—$COOCH_3$		0.5	0.5
bromination			
Br, H_2N—$COOCH_3$, Br		0	0.5

TABLE 2. Additional degradation scheme for biphenyl-3-T.

	Theoretical specific activities (in units per millimole)
	3-T ≡ 1
oxidation	
—COOH	0.5
nitration esterification	
O_2N, —$COOCH_3$, O_2N	0

this molecule. The schemes were to be tested for absence of aromatic tritium-hydrogen exchange in each of the reaction steps. This has been done by separate tracer experiments for the oxidation of 4-nitrobiphenyl, the oxidation of biphenyl to benzoic acid and the nitration of benzoic acid. In all these cases, exchange was less than $2.10^{-2}\%$. For the other steps, reference is made to [1].

MATERIALS AND METHODS

Starting materials

2-Bromobiphenyl was prepared by a Sandmeyer reaction [2, 3] from 2-aminobiphenyl, which was commercially available.

3-Bromobiphenyl was synthesized from 2-aminobiphenyl via 2-amino-5-bromobiphenyl according to the method of Hammond *et al.* [4, 2], which is a modification of the method of Huber *et al* [5].

4-Bromobiphenyl was commercially available.

The bromobiphenyls were subjected to gas-chromatographic purification and analysis. For each of the products, the percentage of isomers was $\leqslant 0.1\%$. For the reduction, 3 Curie of pure tritium gas was diluted with about 3 millimole hydrogen.

Catalytic reductions

The catalytic reductions of the bromobiphenyls were carried out on a microscale. The catalyst used was $Pd/BaSO_4$. The apparatus, described for reduction of bromo-*p*-terphenyls [1], had been slightly modified in order to reduce loss of the more volatile biphenyl during evaporation of solvent (fig. 1). For the same reason, the catalytic reduction was carried out in ethanol instead of ethyl cellosolve.

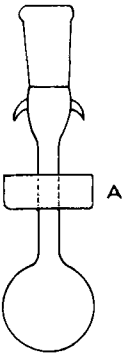


FIG. 1. Reaction flask.
Loss of relatively volatile biphenyl is diminished by putting dry-ice in dish A, during vacuumdistillation of solvent.

The reaction mixture was worked up without delay, in order to avoid undesirable introduction of tritium into the molecule. The working-up procedure comprised sublimation (fig. 2) of the labelled biphenyl, purification by preparative thin-layer chromatography and resublimation. The resublimed product (specific activity 500 mC/mM) was immediately dissolved in benzene to avoid self-radiolysis.

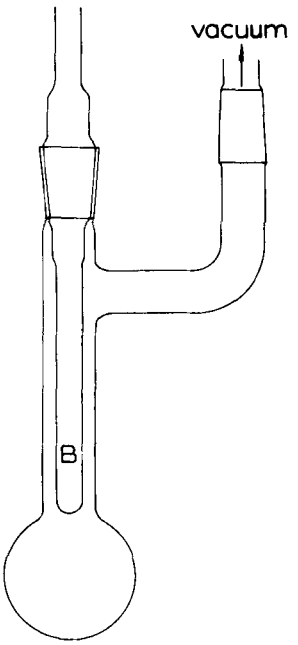


FIG. 2. Sublimation flask.
Loss of relatively volatile biphenyl is diminished by putting dry-ice in tube B, already during vacuum distillation of solvent.

Degradation

The nitration of biphenyl with nitric acid in glacial acetic acid ([6], p. 520) yielded a product from which relatively pure 4-nitrobiphenyl was obtained by crystallization from ethanol. No severe purification was undertaken, and the product oxidized with chromic anhydride in acetic acid to *p*-nitrobenzoic acid. This was purified by preparative thin-layer chromatography and sublimation. The rest of the degradation parallels the degradation described for the tritiated *p*-terphenyls [1]. An improvement is the esterification of *p*-nitrobenzoic acid and additional thin-layer chromatographic purification of the ester prior to reduction of the nitro group.

Oxidation of biphenyl to benzoic acid (table II) was carried out with chromium trioxide in acetic acid [7].

Nitration of benzoic acid in concentrated sulphuric acid ([6], p 770) yielded 3.5-dinitrobenzoic acid. The acid was purified by thin-layer chromatography in order to remove mono-nitro-benzoic acid. The esterification of the carboxyl group served the double purpose that an additional purification was introduced, and that the product had a better solubility in the toluene scintillator, used for the radioactivity measurements.

Specificity of labelling

The activity distribution for each of the tritiated biphenyls has been calculated from the specific activities of the respective compounds obtained according to the degradation schemes (table III). The activity distributions are summarized in table IV.

TABLE 3. Activity measurements.

series	compound	specific activity ± standard deviation μC/mM
Biphenyl-4-T	biphenyl-4-T	2940 ± 17 ($n=6$) [a]
	methyl *p*-nitrobenzoate	19.4 ± 0.2 ($n=4$)
	methyl *p*-nitrobenzoate [b]	0.504 ± 0.006 ($n=4$)
	methyl *p*-aminobenzoate	0.498 ± 0.0092 ($n=10$)
	methyl 3,5-dibromo-4-aminobenzoate	0.041 ± 0.007 ($n=3$)
Biphenyl-3-T (first series)	biphenyl-3-T	4830 ± 51 ($n=6$)
	methyl *p*-nitrobenzoate-3-T	2400 ± 48 ($n=5$)
	methyl *p*-nitrobenzoate-3-T [c]	10.9 ± 0.2 ($n=7$)
	methyl *p*-aminobenzoate-3-T	10.9 ± 0.1 ($n=4$)
	methyl 3,5-dibromo-4-aminobenzoate	0.030 ± 0.001 ($n=3$)
(second series)	biphenyl-3-T	686 ± 6.9 ($n=9$)
	benzoic acid-3-T	350 ± 8.2 ($n=11$)
	methyl 3,5-dinitrobenzoate	2.43 ± 0.007 ($n=3$)
Biphenyl-2-T	biphenyl-2-T	1297 ± 14 ($n=12$)
	methyl *p*-nitrobenzoate-2-T	639 ± 11.4 ($n=16$)
	methyl *p*-nitrobenzoate-2-T [d]	4.18 ± 0.062 ($n=10$)
	methyl *p*-aminobenzoate-2-T	4.12 ± 0.19 ($n=4$)
	methyl 3,5-dibromo-4-aminobenzoate-2-T	4.07 ± 0.103 ($n=12$)

[a] n = number of measurements
[b] diluted 36.1 x ; specific activity calculated 0.537 μC/mM
[c] diluted 220.4 x ; specific activity calculated 10.9 μC/mM
[d] diluted 153.2 x ; specific activity calculated 4.17 μC/mM.

TABLE 4. Activity distribution in the tritiated biphenyls *

Positions	Biphenyl-4-T	Biphenyl-3-T	Biphenyl-2-T
	%	%	%
4	98.68 ± 0.02	0.43 ± 0.02	1.5 ± 1.2
3	1.21 ± 0.04	99.29 ± 0.01	2.4 ± 1.6
2	0.11 ± 0.04	0.28 ± 0.02	96.1 ± 1.5

* The limits given are confidence limits on the 5% level.

EXPERIMENTAL **

Gas-chromatographic analysis and purification of bromobiphenyls

For analysis of the bromobiphenyls, the column used was a copper tube, 60 cm length, 6 mm diameter, filled with 6% bentone-34 on chromosorb W, 60/80 mesh; helium was used as carrier gas; column temperature was 195 °C (compare [8]). 2-Bromobiphenyl and 4-bromobiphenyl contained small percentages of isomers. After purification by means of preparative gas-chromatography, the percentage of isomers present was diminished till less than 0.1%. The purification was carried out on a column as described above, with a diameter of 18 mm. Quantities of 50 mg of 2-bromobiphenyl and 4-bromobiphenyl each were thus purified, injecting 5 mg at a time.

3-Bromobiphenyl contained 0.1% ± 0.05 4-bromobiphenyl. Gas-chromatographic purification of 3-bromobiphenyl resulted in a less pure product because of decomposition and was, therefore, omitted.

Catalytic reduction of bromobiphenyls

Bromobiphenyl (35 mg, 0.15 mM), Pd-$BaSO_4$ catalyst ([6], p 951), (200 mg), potassium acetate (20 mg), ethanol (2 ml) and a magnetic bar were brought into a reaction flask (14 ml, fig. 1). The reaction flask was connected to the hydrogenation apparatus and the catalytic reduction with tritium gas carried out as described previously [1].

The reduction came to completion in about ten minutes. The tritium gas was restored. Then the reaction flask was attached to a special manifold and the active ethanol distilled off in vacuo.

The residue was treated with ethanol, the catalyst filtered off and washed with toluene. The combined filtrate and washings were evaporated in vacuo to

** The experimental work was carried out with the assistance of Mr. A.C. Besemer and Miss E.L. Blok, Mr. Th.A.J. Wubben, Mr. D. Oudijn and Mr. L. Teljeur.

dryness. A flask of the type shown in figure 2 was used. The remaining biphenyl was sublimed (0.5 mm, 60 °C). The yield was 90%.

The biphenyl was further purified by preparative thin-layer chromatography (see below) and resublimation (fig. 2 ; 50 mg carrier biphenyl was added before distilling off the solvent), then immediately dissolved in benzene (18 ml), and carrier biphenyl (450 mg) added.

Degradation of tritiated biphenyls

4-Nitro-biphenyl

Biphenyl (0.5 g) was nitrated with nitric acid (d 1.51 ; 0.25 ml) in glacial acetic acid (1 ml) by refluxing the mixture for 10 minutes. The reaction mixture was added to cold water (10 ml). 4-Nitro-biphenyl crystallized out. After recrystallization from ethanol, a product was obtained with a m.p. 111-113 °C (yield 320 mg, i.e. 50%).

Methyl p-*nitrobenzoate*

4-Nitrobiphenyl (300 mg, 1.5 mM) was oxidized in boiling acetic acid (10 ml) with chromium trioxide (2 g, 75% excess), which was added in small amounts. After the addition of the chromium trioxide, the mixture was boiled for another 15 minutes, poured into cold water (50 ml) and extracted with ether (6 × 25 ml). The ethereal solution was evaporated to dryness and the residue sublimed in vacuo (yield 120 mg, i.e. 50%). *p*-Nitrobenzoic acid was purified by preparative thin-layer chromatography (see below), and esterified with diazomethane in ether. Pure methyl *p*-nitrobenzoate, m.p. 93.3-94.0 °C, was obtained by thin-layer chromatographic purification and subsequent recrystallisation from methanol/water (4 : 1).

Methyl p-*aminobenzoate*

Methyl *p*-nitrobenzoate (500 mg) was reduced in methanol (15 ml) with hydrogen and Pd on carbon catalyst (freshly prepared by reduction with hydrogen of 20 mg $PdCl_2$ on 100 mg carbon in diluted hydrochloric acid and evaporation to drynees in vacuo). The catalyst was filtered off. The solution evaporated in vacuo to dryness and the residue purified by recrystallization from methanol/water (1 : 4) and sublimation in vacuo (yield 330 mg, i.e. 80% m.p. 111.1-111.8 °C).

Methyl 3.5-dibromo-4-aminobenzoate

Bromination of methyl *p*-aminobenzoate (350 mg) was carried out in methanol solution (15 ml) by adding a slight excess of a solution of bromine in

methanol (0.03 g bromine per ml). Labile tritium was removed by repeatedly adding methanol and evaporation in vacuo of the solvent to dryness. Finally, the residue was purified by recrystallization from methanol/water (1 : 4) and sublimation in vacuo (yield 660 mg, i.e. 90%; m.p. 131,0-131.3 °C).

Benzoic acid

Biphenyl (2.0 g) was oxidized in acetic acid (40 ml) at 80 °C with chromium trioxide (15 g), which was added in small amounts. After the addition of chromium trioxide, the mixture was kept at 80 °C for 15 minutes, poured into cold water (200 ml) and extracted with ether (continuously for 24 hours). The ether was distilled off and the residue steam distilled. The distillate was again extracted with ether and the ether distilled off. Benzoic acid was freed from residual biphenyl by extraction with ether from an alkaline solution, and from residual acetic acid by recrystallization from ethanol/water (1 : 9). Finally, benzoic acid was sublimed in vacuo (yield 400 mg, i.e. 25%; m.p. 121-122 °C).

3.5.-Dinitro-benzoic acid

To a solution of benzoic acid (200 mg) in concentrated sulfuric acid (1 ml) was added concentrated nitric acid (d 1.54, 0.35 ml), and the mixture warmed up to 100 °C in the course of 15 min. After 15 min at 100 °C, the temperature was raised to 130 °C in the course of 30 min and kept at this value for one hour. The cooled reaction mixture was poured into cold water (25 ml). 3.5.-Dinitro-benzoic acid crystallized out. It was recrystallized from ethanol/water (1 : 3). Yield 130 mg, i.e. 40%.

The product was further purified by preparative thin-layer chromatography (see below).

Methyl 3.5-dinitrobenzoate

3.5-Dinitrobenzoic acid (50 mg) was esterified with diazomethane in ether (5 ml) and purified by means of preparative thin-layer chromatography and subsequent sublimation in vacuo (yield 48 mg, i.e. 90%, m.p. 106.6-106.9 °C).

Thin-layer chromatography

a) *Thin-layer chromatographic analysis*

Biphenyl, bromobiphenyls, phenylcyclohexene and phenylcychohexane

Benzene solutions, containing 20 μg of compound, were applied to a silicagel layer of 0.25 mm thickness (silicagel H, Merck, activation of layer by heating one hour at 110 °C). The chromatograms were developed with cyclo-

hexane and dried at room temperature. The chromatograms were viewed under UV-light (3600 Å) after spraying with a 3% solution of Ce (IV)-sulphate in concentrated nitric acid. The r_F values were 0.30, 0.32-0.37, 0.43 and 0.51 respectively.

p-Nitrobenzoic acid, o-nitrobenzoic acid, benzoic acid, 4-nitrobiphenyl and methyl p-nitro benzoate

Ethanolic solutions, containing 40 µg of compound, were applied to a silicagel layer of 0.25 mm thickness (silicagel HF_{254}, Merck, activation of layer by heating one hour at 110 °C). The chromatograms were developed with a mixture of cyclohexane/dioxane/formic acid 90 : 50 : 10, and viewed under UV light (2540 Å). The r_F values were 0.24, 0.17, 0.35, 0.48 and 0.32 respectively.

3,5-Dinitrobenzoic acid, 3-nitrobenzoic acid, benzoic acid, methyl 3,5-dinitrobenzoate and methyl 3-nitrobenzoate

Solutions, containing 20 µg of compound, were applied to a silicagel layer of 0.25 mm thickness (silicagel HF_{254}, Merck, activation of layer by heating one hour at 110 °C). The chromatograms were developed with a mixture of benzene/dioxane/acetic acid (90 : 25 : 4), and viewed under UV light (2540 Å). The r_F values were 0.27, 0.39, 0.48, 0.64 and 0.64 respectively.

b) *Preparative thin-layer chromatography*

A solution, containing 10-15 mg of compound, was applied to a plate (20 × 20 cm^2) with a silicagel layer of 1 mm thickness, and the chromatogram developed as described above under thin-layer chromatographic analysis. For location of the band, use was made of UV light, except for tritiated biphenyl, in which case the band was located autoradiographically. In all cases good separation was checked with test mixtures.

The scratched off sorbens was eluted with *n*-hexane (for elution of biphenyl), with ethanol (for *p*-nitrobenzoic acid and 3,5-dinitrobenzoic acid) or toluene (for methyl *p*-nitrobenzoate and methyl 3,5-dinitrobenzoate). The eluate was evaporated to dryness, and the residue sublimed in vacuo. In the case of the acids it was advantageous to treat the residue with concentrated hydrochloric before sublimation.

The yields of recovery varied from 70 to 90%.

Radioactivity measurements

The radioactivity measurements were carried out as described previously [1]. The results of the measurements are summarized in table III.

Discussion

The specificity of labelling in biphenyl-4-T, biphenyl-3-T and biphenyl-2-T is very good. In each of the compounds, only small percentages of tritium are found at the other positions. This is to be attributed to some direct exchange of aromatic hydrogen for tritium during the replacement reaction of the bromine atom, as the bromobiphenyls were 99.9% pure with respect to the presence of isomers.

REFERENCES

1. P.Ph.H.L. Otto and G. Juppe — Synthesis of Tritiated *p*-Terphenyls, Labelled at Specific Positions, *Journal of Labelled Compounds*, **1**, 115 (1965); *Euratom Report*, EUR 2529.e (1965).
2. C. Beaudet, J. Horlait, A. Marcq, and G, Belpaire — Synthèse de produits de référence apparentés aux polyphényles, *Euratom Report*, EUR 31. f (1962).
3. S.H. Zaheer and S.A. Faseeh — Ortho-substituted Biphenyls. *J. Indian Chem. Soc.*, **21**, 27 (1944); Chem. Abstracts **39**, 291[3] (1945).
4. G.S. Hammond and C.H. Reeder — Benzyl Tosylates VI. The Effect of Phenyl as a Substituent. *J. Am. Chem. Soc.*, **80**, 573 (1958).
5. W.F. Huber, M. Renoll, A.G. Rossow and D.T. Morry — Vinyl Aromatic Compounds II. *o*-, *m*- and *p*-Vinylbiphenyls. *J. Am. Chem. Soc.*, **68**, 1109 (1946).
6. A.I. Vogel — Practical Organic Chemistry, Longmann, Green and Co., London, 1956.
7. G. Schultz — Ueber Diphenyl. *Liebigs Ann. Chem.*, **174**, 201 (1874).
8. B. Versino, F. Geiss and G. Barbero — Verwendung von Bentone-haltigen Trennsäulen für die Gaschromatographie von Polyphenylgemischen, *Fresenius Zeitschrift für analytische Chemie*, **201**, 20 (1964).