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Synthesis of Tritiated *p*-Terphenyls, Labelled at Specific Positions * [1]

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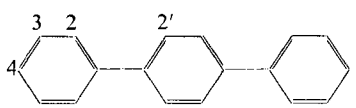
SUMMARY

The tritiated compounds p-*terphenyl-4-T, -3-T, -2-T and -2'-T have been synthesized by catalytic reduction of the corresponding bromo-*p-*terphenyls with tritium gas under mild conditions. For each of the labelled* p-*terphenyls, the activity distribution over the positions in the molecule has been determined by degradation.*

INTRODUCTION.

In the symmetric *p*-terphenyl molecule only four positions are to be distinguished in case of single tritium labelling. These are positions 4, 3, 2 and 2′:



A tritium atom can be introduced into the terphenyl molecule by replacement of a substituent in the desired position. Such a substituent may be a halogen atom, which can be reduced by catalytic hydrogenation [1] or via organometallic intermediates [2, 3] or an amino group [4].

From the possibilities mentioned, the catalytic reduction of bromo-*p*-terphenyls with tritium was investigated by us for the following reasons : the bromo-*p*-terphenyl isomers were readily available [5, 6]. Preliminary experiments showed that the reaction proceeds smoothly under mild conditions, i.e. Pd-$BaSO_4$ catalyst, at room temperature and with relatively short times. Under such conditions, chances are limited for random exchange of aromatic

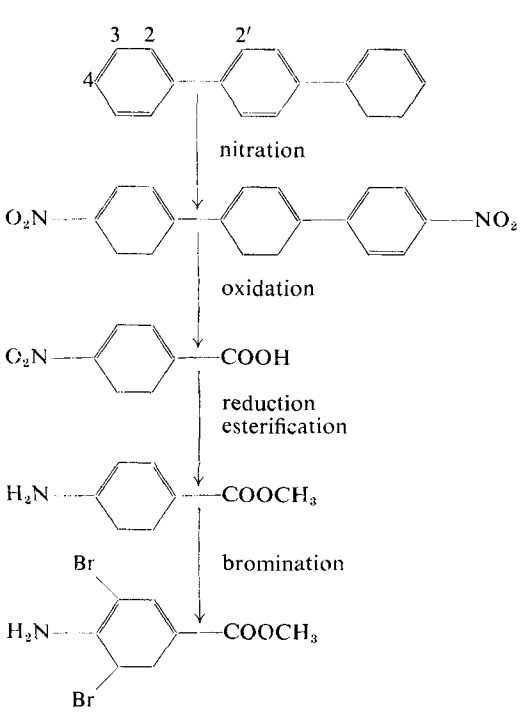
* Received on May 12th 1965.

[1] This investigation is part of a research programme, sponsored by Euratom.

hydrogen for tritium and hydrogenation of aromatic nuclei. Furthermore, the method is also applicable for replacement of more than one bromine atom in the same molecule, in which case replacement by means of organo metallic intermediates is found to cause difficulties [2].

For determination of the activity distribution over the positions in the labelled molecules, a degradation scheme, shown in Table I, had to be worked out. The absence of aromatic tritium-hydrogen exchange in each of the reaction steps, had to be tested by separate tracer experiments. This was done for the nitration of *p*-terphenyl, the oxidation of 4.4''-dinitro-*p*-terphenyl to *p*-nitrobenzoic acid, and for the bromination of *p*-aminobenzoic acid methyl ester (exchange $< 10^{-2}$ %). For the reduction and esterification steps no loss of specific activity occured during conversion of labelled *p*-nitrobenzoic acid into *p*-amino-benzoic acid methylester (see Table III).

As regards isotope effects, it has been reported [7] that aromatic nitration proceeds with the same velocity, regardless of the isotopic mass of the hydrogen. We assumed that the other reaction steps of the scheme are also not influenced by an isotope effect.



Theoretical specific activities (in units per millimole)			
4-T ≡ 1	3-T ≡ 1	2-T ≡ 1	2'-T ≡ 1
0	1	1	1
0	0.5	0.5	0
	0.5	0.5	
	0	0.5	

TABLE I. Degradation scheme.

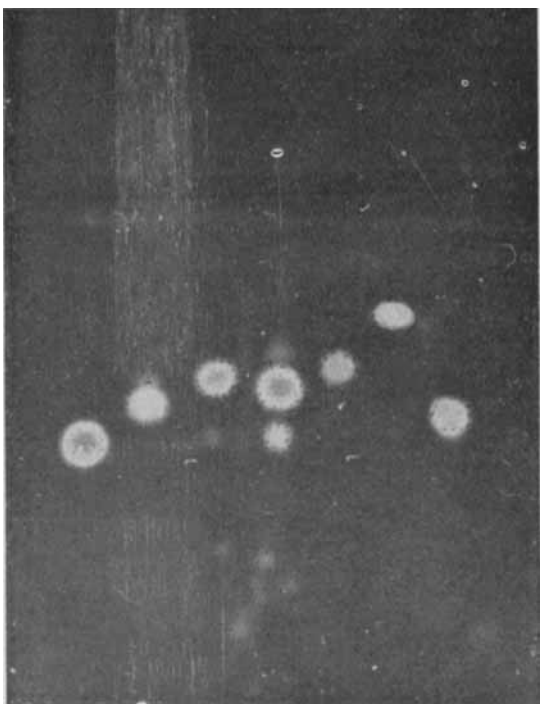
MATERIALS AND METHODS.

Starting material.

A survey of characteristics of the bromo-*p*-terphenyls is shown in Table II. Thin-layer chromatographic analysis on silicagel in cyclohexane (Fig. 1) or on BENTON-38 in heptane (Fig. 2) showed 2-bromo-*p*-terphenyl not to be free from impurities. Attempts to purify 2-bromo-*p*-terphenyl by preparative thin-layer chromatography have not yet been successful. The bromo-*p*-terphenyls were used for the synthesis without further purification.

Catalytic reduction.

The catalytic reductions of the bromo-*p*-terphenyls were carried out on a microscale, in ethyl cellosolve with tritium gas of high specific activity. The catalyst used was Pd-$BaSO_4$.



a *b* *c* *d* *e* *f* *g*

a *p*-terphenyl
b 2-Br-*p*-terphenyl
c 3-Br-*p*-terphenyl
d 4-Br-*p*-terphenyl
e Br-*m*-terphenyl
f xenyl-4-cyclohexane
g *m*-terphenyl

FIG. 1. — Thin-layer chromatographic analysis of terphenyls, bromo-terphenyls and xenyl-4-cyclohexane, on silicagel H in cyclohexane.

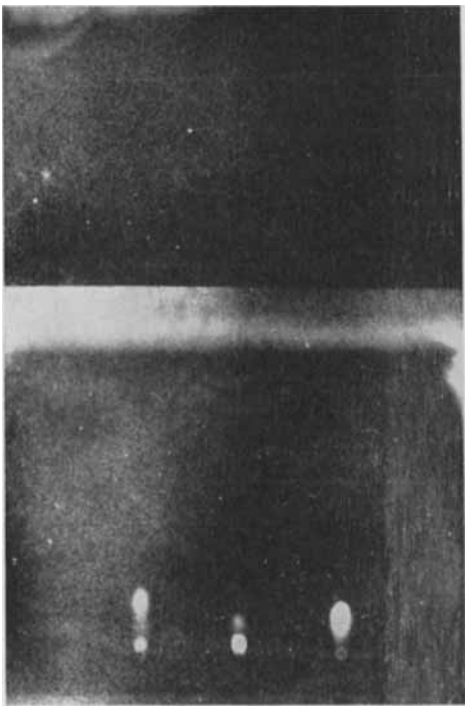


FIG. 2. — Thin-layer chromatograms of bromo-*p*-terphenyls on BENTON-38 in heptane.

TABLE II. Characteristics of bromo-*p*-terphenyls [6].

	Melting point	% Br (calc. 25.85)	thin-layer chromatographic analysis on SiO_2, 250 μ	
			R_F [3] in heptane-CCl_4 1 : 1	R_F [3] in heptane
4-Br-*p*-terphenyl	232.2-232.6° C	25.63 [2]	0.79	
		25.57		
		25.37		
3-Br-*p*-terphenyl	151.8-152° C	25.9	0.75	
2-Br-*p*-terphenyl	84.5- 84.7° C [1]	25.8	0.68	
2′-Br-*p*-terphenyl	96.8- 97.3° C	25.9		0.62

[1] Ref. [10], m. p. 86-88° C.
[2] The product contains presumably 1.3 % *p*-terphenyl.
[3] All chromatograms showed only a single spot.

Ethyl cellosolve was chosen instead of e.g. ethanol, because it is a good solvent for the relatively poorly soluble bromo-*p*-terphenyls. In addition, it has a lower labile hydrogen concentration than ethanol. Consequently, less tritium is introduced into the solvent by hydrogen-tritium exchange catalyzed by Pd-$BaSO_4$. Though, as a solvent, an alcohol seems to be required for an acceptable reaction velocity, the tritium entering the *p*-terphenyl molecule comes mainly from the gas phase, and the reaction can be carried out starting with inactive solvent [8]. Rapid completion of the reaction is considerably enhanced by potassium acetate, which binds the hydrogen bromide formed [1].

The reaction mixture is worked up without delay, in order to avoid undesirable introduction of tritium into the molecule.

The working-up procedure comprises sublimation of labelled *p*-terphenyl, purification by preparative thin-layer chromatography and resublimation.

The resublimed product (spec. act. 10 mC/mg) was immediately dissolved in benzene to avoid self-radiolysis.

Degradation.

The nitration of *p*-terphenyl with fuming nitric acid in glacial acetic acid [9, 10, 11] yields a mixture of products, from which pure 4.4''-dinitro-*p*-terphenyl can be obtained after several crystallizations from nitroethane/benzene.

Oxidation of 4.4''-dinitro-*p*-terphenyl is best carried out with chromic anhydride in acetic acid [10]. Attempts to oxidize with ozone were less successful [12]. *p*-Nitrobenzoic acid was purified by preparative thin-layer chromatography and sublimation.

The conversion of *p*-nitrobenzoic acid to *p*-aminobenzoic acid was carried out by catalytic reduction with hydrogen gas.

The esterification of the carboxyl group served two purposes : the products were easier to purify and had a better solubility in the toluene scintillator, used for the radioactivity measurements.

Specificity of labelling.

The activity distribution for each of the tritiated *p*-terphenyls, was calculated from the specific activities of the respective compounds obtained according to the degradation scheme (Table III). The activity distributions are summarized in Table IV.

EXPERIMENTAL [2].

Catalytic reduction of bromo-p-terphenyls.

Bromo-*p*-terphenyl (18.5 mg, 60 μM), Pd-$BaSO_4$ catalyst [13] (200 mg), potassium acetate (6 mg), ethyl cellosolve (2 ml) and a magnetic bar were

[1] We wish to thank Prof. Dr. E. C. KOOYMAN for his valuable suggestion on this point.

[2] The experimental work was carried out with the assistance of Mr. A. BESEMER, Miss. E. BLOK and Mr. D. OUDIJN.

placed in a reaction flask (14 ml). The flask was attached to the hydrogenation apparatus (Fig. 6), cooled with liquid nitrogen and evacuated. From the storage flask hydrogen gas containing tritium was introduced into the reaction flask by means of a Toepler pump, the reaction mixture warmed up to room temperature, and the pressure of the gas adjusted to one atmosphere. If the appropriate amount of gas is introduced, the mercury level is just at the lower

TABLE III. Activity measurements

Series	Compound	Specific activity ± standard deviation μC/mM	
p-Terphenyl-4-T	*p*-terphenyl-4-T	6,510 ± 68	[1] ($n = 3$)
	4.4″-dinitro-*p*-terphenyl	55.1 ± 3.2	($n = 2$)
	p-nitrobenzoic acid	25.0 ± 0.44	($n = 4$)
p-Terphenyl-3-T (first series)	*p*-terphenyl-3-T	8,820 ± 55	($n = 6$)
	4.4″-dinitro-*p*-terphenyl-3-T	8,430 ± 11	($n = 6$)
	p-nitrobenzoic acid-3-T	4,130 ± 33	($n = 6$)
	p-nitrobenzoic acid-3-T [2]	7.32 ± 0.044	($n = 4$)
	p-aminobenzoic acid-3-T methyl ester	7.38 ± 0.047	($n = 4$)
(second series)	*p*-nitrobenzoic acid-3-T [3]	2.69 ± 0.04	($n = 4$)
	p-aminobenzoic acid-3-T methyl ester	2.63 ± 0.05	($n = 4$)
	3.5-dibromo-*p*-aminobenzoic acid methyl ester	$8.84 \times 10^{-3} \pm 0.27 \times 10^{-3}$	($n = 3$)
p-Terphenyl-2-T (first series)	*p*-terphenyl-2-T	9,206 ± 37	($n = 4$)
	4.4″-dinitro-*p*-terphenyl-2-T	8,562 ± 97	($n = 4$)
(second series)	*p*-terphenyl-2-T	1,702 ± 21	($n = 5$)
	p-nitrobenzoic acid-2-T	800 ± 3.2	($n = 6$)
	p-nitrobenzoic acid-2-T [4]	4.84 ± 0.15	($n = 4$)
	p-aminobenzoic acid-2-T methyl ester	4.88 ± 0.04	($n = 4$)
	3.5-dibromo-*p*-aminobenzoic acid-2-T methyl ester	4.36 ± 0.07	($n = 8$)
p-terphenyl-2′-T	*p*-terphenyl-2′-T	487.6 ± 4.7	($n = 4$)
	4.4″-dinitro-*p*-terphenyl-2′-T	475.6 ± 5.1	($n = 4$)
	p-nitrobenzoic acid	2.54 ± 0.10	($n = 4$)

[1] n = number of measurements.
[2] Diluted 561 ×; calculated 7.38 μC/mM.
[3] Second dilution.
[4] Diluted 164 ×; calculated 4.88 μC/mM.

TABLE IV. Activity distribution in the tritiated *p*-terphenyls [1]

Positions	*p*-terphenyl-4-T	*p*-terphenyl-3-T	*p*-terphenyl-2-T	*p*-terphenyl-2′-T
	%	%	%	%
4	99.16 ± 0.44	4.4 ± 1.5	7.0 ± 2.1	2.5 ± 2.2
3	0.77 ± 0.02	93.4 ± 1.1	10.1 ± 1.3	0.52 ± 0.03
2		0.32 ± 0.03	83.0 ± 1.7	
2′	0.07 ± 0.44	1.9 ± 1.6	0.0 ± 2.7	97.0 ± 2.2

[1] The limits given are confidence limits on the 5 % level.

end of the manometer tube, so that the hydrogen uptake can be determined accurately. By stirring the reaction mixture, the reaction started and proceeded smoothly. The reaction came to completion in about 30 minutes.

Thereafter, the reaction mixture was frozen with liquid nitrogen, the hydrogen gas pumped off and restored, the reaction flask attached to a special manifold, and the active ethyl cellosolve distilled off under vacuum. The residue was treated with chloroform, the catalyst filtered off, and washed with chloroform. The combined filtrate and washings were evaporated to dryness under vacuum. The remaining *p*-terphenyl was sublimed (2.10^{-3} mm, 150° C) and the yield was 13 mg (95 %).

The sublimed *p*-terphenyl was further purified by preparative thin-layer chromatography.

Degradation of p-*terphenyls.*

4.4″-Dinitro-p-*terphenyl.*

p-Terphenyl (0.5 g) was nitrated with fuming nitric acid *d* 1.61 (6 ml) in glacial acetic acid (25 ml) by heating the mixture in an oil bath at 100° C for 30 min. 4.4″-Dinitro-*p*-terphenyl, which crystallized out at room temperature, was filtered off, washed with a small amount of acetic acid, and dried in vacuum over KOH. The yield was 30 % (0.21 g); m.p. 265-272° C. Recrystallization from nitroethane/benzene 2 : 1 raised the m.p. to 274-276.5° C (yield 0.18 g, 26 %).

p-*Nitrobenzoic acid.*

4.4″-Dinitro-*p*-terphenyl (105 mg) was oxidized in boiling acetic acid (5 ml). Chromium trioxide (550 mg, 75 % excess) was added in small amounts, and boiling of the mixture continued for 45 min. The solution was concentrated to 2 ml, after which most of the acetic acid was removed by adding water and continuing the distillation.

p-Nitrobenzoic acid was extracted with ether, the ethereal solution washed with water, the ether distilled off and the residue sublimed in vacuo. The yield

was 42 mg (44 %). *p*-Nitrobenzoic acid was further purified by preparative thin-layer chromatography.

p-*Aminobenzoic acid.*

p-Nitrobenzoic acid (300 mg) was reduced in ethanol (8 ml) with hydrogen and Pd on carbon catalyst (freshly prepared by reduction with hydrogen of 20 mg $PdCl_2$ on 100 mg carbon in dilute hydrochloric acid and evaporation to dryness in vacuo). The yield was 150 mg (60 %); m.p. 185-187° C after fractional recrystallization from water.

p-*Aminobenzoic acid methyl ester.*

p-Aminobenzoic acid (300 mg) was esterified in ethanol solution (5 ml) by adding an excess of diazomethane in ether at 0° C. After 5 min., the solvent was removed by evaporation in vacuo, and the residue recrystallized from methanol-water 1 : 4. The yield was 230 mg (70 %); m.p. 111-112° C.

3.5-Dibromo-4-aminobenzoic acid methyl ester.

Bromination of *p*-aminobenzoic acid methyl ester (100 mg) was carried out in methanol solution (8 ml) by adding a slight excess of a solution of bromine in methanol. Labile tritium was removed by repeated addition of methanol or methanol and water, and evaporation in vacuo of the solvent to dryness. The yield was 185 mg (90 %); m.p. 131-132.5° C after recrystallization from methanol-water 4 : 1.

Thin-layer chromatography.

a) *Thin-layer chromatographic analysis.*

p-*Terphenyl, bromo*-p-*terphenyls and xenyl-4-cyclohexane* : Chloroform solutions, containing 4 to 10 μg of compound, were applied to a silicagel layer of 0.20 mm thickness (silicagel H, Merck, activation of layer by heating one hour at 110° C). After equilibration in a conditioned room (23° C, humidity 40-50 %), the chromatograms were developed with cyclohexane, and dried for 20 minutes at 80° C. A photograph of chromatograms, viewed under UV-light (3000-3600 Å) after spraying with a 3 % solution of ceric sulphate in concentrated nitric acid is shown in Fig. 1.

Autoradiograms of the tritiated *p*-terphenyls are shown in Fig. 5.

Separation of bromo-p-*terphenyls on* BENTON-38 [14] : Methylene chloride solutions, containing 2μg of compound, were applied to a BENTON-38-celite (1 : 1) layer of 0.02 mm thickness, and four times developed in heptane. A photograph of chromatograms, viewed under UV-light (2540 Å) after spraying at 80° C with a 2 % solution of ceric ammonium sulphate in 60 % nitric acid, is shown in Fig. 2.

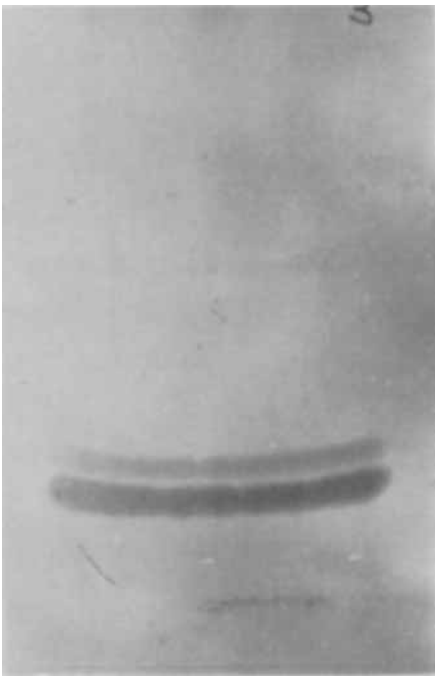


FIG. 3. — Preparative chromatographic separation of *p*-terphenyl and 3-bromo-*p*-terphenyl (silicagel H, 1 mm; 1.5 mg *p*-terphenyl + 1 mg 3-bromo-*p*-terphenyl; detection under UV light after spraying with ceric-sulphate reagent).

b) *Preparative thin-layer chromatography.*

Purification of tritiated p-*terphenyls* (*spec. act. 10 mC/mg*) : A chloroform solution (2.5 ml) of tritiated *p*-terphenyl (13 mg) was applied to 5 plates ($20 \times 20\ cm^2$) with an activated silicagel layer of 1 mm thickness (silicagel H, Merck; activation by heating one hour at 110° C). After equilibration in a conditioned room, the chromatograms were developed with cyclohexane/chloroform 10 : 1.

For comparison, the result of a chromatographic separation of a mixture of *p*-terphenyl and 3-bromo-*p*-terphenyl is given in Fig. 3.

Autoradiograms of limited areas of preparative thin-layer chromatograms of the tritiated *p*-terphenyls are shown in Fig. 4. The location of *p*-terphenyl on the chromatograms was accurately indicated while viewing the plates under UV-light. Subsequently, the area was scratched off carefully, and the powder obtained eluated with chloroform (50 ml). The chloroform solution was evaporated to dryness. The remaining *p*-terphenyl was sublimed under vacuum (2.10^{-3} mm, 150° C) the sublimed *p*-terphenyl immediately dissolved in benzene (50 ml), and carrier *p*-terphenyl (500 mg) added.

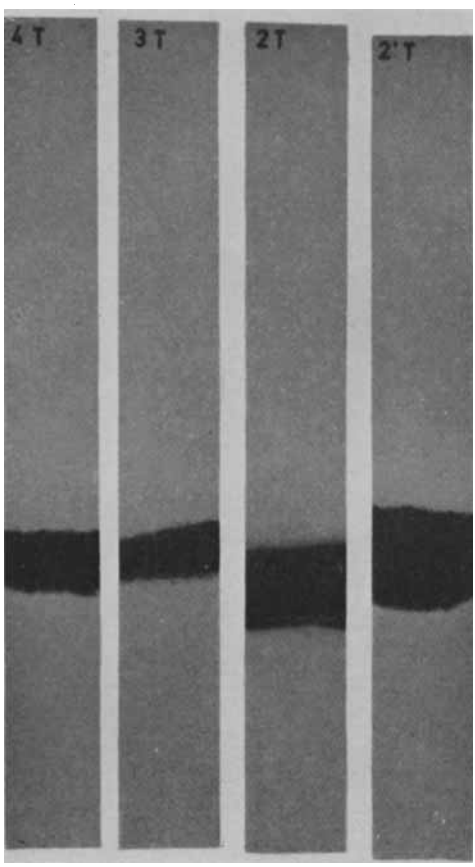


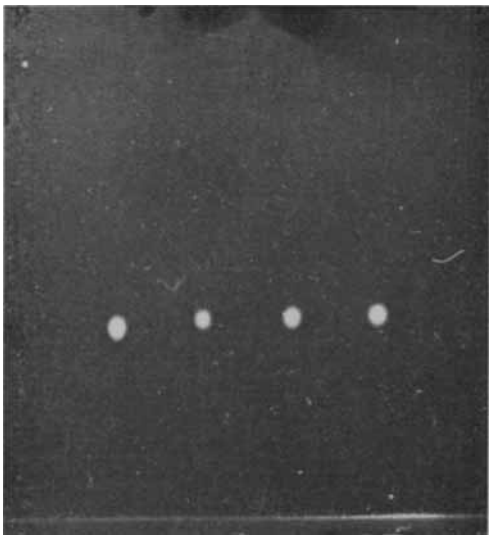
FIG. 4. — Autoradiograms of parts of preparative thin-layer chromatograms of tritiated *p*-terphenyls.

Purification of p-*nitrobenzoic acid* : The chromatographic separation of *p*-nitrobenzoic acid (15 mg) was carried out on a plate (20 × 20 cm^2) with an activated silicagel G layer of 1 mm thickness, in benzene-dioxane-glacial acetic acid 90 : 25 : 4. The location of the *p*-nitrobenzoic acid was indicated, while viewing the plate under UV-light, the area carefully scratched off and the silicagel powder eluated with ethanol.

A drop of concentrated hydrochloric acid was added, the ethanolic solution evaporated to dryness and the residual *p*-nitrobenzoic acid sublimed in vacuo (recovery > 80 %; m.p. 236-237.5° C).

Radioactivity measurements.

The radioactivity measurements were carried out with a tritium scintillation counter (I.D.L., type 6012 A).



2-T 3-T 4-T 2′-T

FIG. 5. — Autoradiograms of tritiated *p*-terphenyls, chromatographed on silicagel H in cyclohexane.

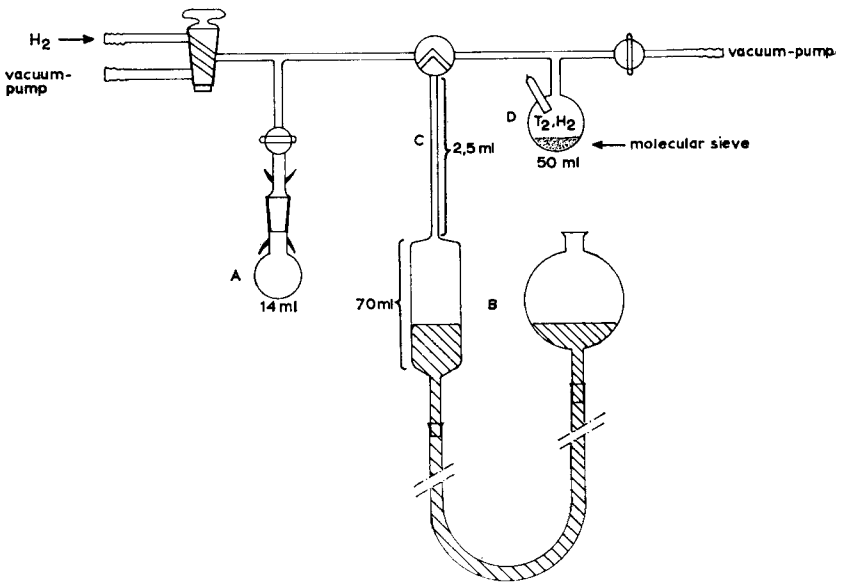


FIG. 6. — Apparatus for catalytic reduction.

A reaction-flask
B toepler-pump.
C manometer-tube.
D storage-flask for tritiumgas.

The scintillation liquid used was a solution of 2.5-diphenyl oxazole (5 g) and 1.4-bis-[2-(5-phenyl-oxazolyl)]-benzene (0.5 g) in toluene (1000 ml).

For each measurement the counting efficiency was determined by adding a toluene-T standard of known specific activity. The results are summarized in Table III.

Discussion.

On the autoradiograms of the preparative thin-layer chromatograms (Fig. 4), no activity is found outside the *p*-terphenyl area. There is no indication that for instance xenylcyclohexane is present (compare with Fig. 1, spot *f*). Consequently, no hydrogenation of the aromatic nuclei occurred during the replacement reaction of the bromine atom.

The specificity of the labelling in *p*-terphenyl-4-T and *p*-terphenyl-2'-T is good (Table IV). No significant exchange of aromatic hydrogen for tritium took place during the replacement reaction of the bromine atom.

In *p*-terphenyl-3-T, and especially in *p*-terphenyl-2-T, larger percentages of tritium are also found at other positions, which is to be attributed to the presence of isomers in the starting materials.

Conclusions.

1. The procedure described, by which a bromo derivative of terphenyl is catalytically reduced with tritium gas under mild conditions, constitutes a valuable method for specific labelling.

2. The degradation scheme developed for determination of the activity distribution in a *p*-terphenyl molecule, gives reliable results, in that no aromatic tritium-hydrogen exchange occurs in each of the reaction steps, and no indication is found of hydrogen isotope effects.

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