

**Polychlorinated Biphenyls.
Synthesis of two ¹⁴C-Labelled
Tetrachlorobiphenyls***

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The occurrence of polychlorinated biphenyls (PCB) in fish and birds from the Baltic region was established in 1966.^{1,2} It has since been shown that PCB, like DDT and related compounds, is distributed in fish and wildlife in various parts of the world.³⁻⁵

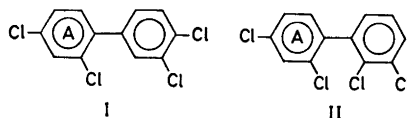
Commercial PCB-mixtures, used for example as insulating media in the electric industry, as heat transfer media, as hydraulic oils and as additives to plastics and paints, comprise a number of related compounds containing from one to ten chlorine atoms per molecule. Tentative structures of some 45 major and a large number of minor constituents of Arochlor 1242, 1254, and 1260, containing ca. 42, 54, and 60 % chlorine, respectively, have been reported.⁶ So far, however, only a restricted number of these constituents have been prepared by unambiguous synthetic routes.^{6,7}

In the analysis of animal tissues, polychlorinated biphenyls with 3-8 chlorine atoms per molecule have been detected.^{2,5} The fate and distribution of these compounds in the animal body is of considerable interest in view of their adverse properties, e.g. on the metabolism of steroid hormones of birds.⁴

The syntheses of two ¹⁴C-labelled biphenyls described below were undertaken in connection with a series of investigations on the distribution and excretion of structurally defined tetra- and hexachlorobiphenyls in quails^{8,9} and mice.¹⁰ Uniformly labelled aniline-¹⁴C was chosen as the starting material. It was readily transformed into 2,4-dichloroaniline-¹⁴C via the acetanilide, essentially as recommended by Fieser and Bowen.¹¹ A Gomberg-Bachman reaction¹² between diazotised 2,4-dichloroaniline-¹⁴C and 1,2-dichlorobenzene gave a crystalline mixture

of 2,3',4,4'-tetrachlorobiphenyl-¹⁴C (I) and 2,2',3',4-tetrachlorobiphenyl-¹⁴C (II) labelled in the A rings with a specific activity of 7.8 Ci/mol. The ratio of I to II was ca. 2:1 (GLC).

An analogous coupling reaction using unlabelled dichloroaniline gave a biphenyl mixture, chromatographically identical (GLC) with the labelled product. The major component was isolated by crystallisation and identified as 2,3',4,4'-tetrachlorobiphenyl (I) from its UV-spectrum.¹³ This compound is reported as a minor component of Arochlor 1254.⁶ The isomer II is reported as a minor component of Arochlor 1242, 1254, and 1260.⁶



Experimental. Activities were measured with a Packard Tri-carb liquid scintillation spectrometer model 3320. The UV-spectrum was measured on a Perkin-Elmer 124 spectrophotometer. Melting points were determined on a Kofler micro hot stage. All reagents used were of analytical grade unless otherwise stated.

Thin layer chromatography. Suitable conditions for acetylation, chlorination and hydrolysis were worked out on unlabelled material. The reactions were followed by TLC on silica gel (Kieselgel HF₂₅₄, Merck) using ethyl acetate or toluene-ethyl acetate (4:1) as solvents. Preparative TLC was performed on the same adsorbent. Chromatograms of ¹⁴C-labelled reaction products were autoradiographed.

Gas chromatography. The tetrachlorobiphenyls were characterised by GLC using a Varian 1400 instrument fitted with an electron capture detector. Glass columns (0.18 × 160 cm) containing 4 % (w/w) SF 96 on Chromosorb W A/W DMCS (100-120 mesh) at 168° or higher temperatures were used.

Combined GLC-mass spectrometry. The unlabelled biphenyl mixture was analysed on an LKB 9000 GLC-MS apparatus at an ionisation potential of 70 eV using a glass column at 170° containing 1 % SE 30 on Chromosorb W (100-120 mesh).

Acetanilide-¹⁴C. Aniline-¹⁴C hydrogen sulphate (1.1 mCi, 39 Ci/mol; The Radiochemical Centre, Amersham), in the original ampoule was stirred magnetically with acetic anhydride (3 ml) for 5 h at room temperature. The solution was transferred to a pear-shaped

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flask (10 ml) and acetanilide (11.0 mg) was added as a carrier. Evaporation under controlled pressure in a vacuum desiccator over molecular sieves (Union Carbide, 8 Å) gave acetanilide- ^{14}C (1.085 mCi, 10 Ci/mol) contaminated by sulphuric acid.

2,4-Dichloroacetanilide- ^{14}C . The anilide was dissolved in acetic acid (0.2 ml) and hydrochloric acid (12 M, 1.56 ml) was added. Potassium chlorate (36 mg, 0.29 mmol) in water (0.5 ml) was added with magnetic stirring at room temperature. After 1 h, excess chloric acid and chlorine dioxide was destroyed by adding an aqueous solution of sulphur dioxide (0.9 M, 0.5 ml). The reaction mixture was extracted, in the flask, with chloroform (10 × 0.7 ml). The extracts were filtered through a column of sodium sulphate and the combined solutions were subjected to preparative TLC. The plates were developed twice with toluene-ethyl acetate (4:1). The desired zone was collected and extracted with acetone. Evaporation gave chromatographically pure 2,4-dichloroacetanilide- ^{14}C (9.8 mg, 380 μCi). A slightly slower-moving zone was collected and extracted with acetone. Evaporation gave 2,4,6-trichloroacetanilide- ^{14}C (6.8 mg; 250 μCi), which has been used in an analogous synthesis of ^{14}C -labelled hexachlorobiphenyls.¹⁴

2,3',4,4'-Tetrachlorobiphenyl- ^{14}C and 2,2',3',4-tetrachlorobiphenyl- ^{14}C . 2,4-Dichloroacetanilide- ^{14}C (9.8 mg; 380 μCi) was heated with hydrochloric acid (12 M, 3 ml) in a Pyrex tube fitted with screw cap at 90–100° for 3 h. The solution was evaporated to dryness in a vacuum desiccator over silica gel. 1,2-Dichlorobenzene (Koch-Light, *puriss.*, 1 ml) was added to the residue and the mixture was agitated in an ultrasonic bath for 5 min. The following steps were all performed in the ultrasonic bath at 0–5°. Aqueous sodium nitrite (6.1 M, 10 μl) was added and agitation was continued for 30 min. Aqueous sodium hydroxide (5 M, 3 × 25 μl) was then added in portions during 15 min. Another three portions (25 μl) were added after 30 min and the agitation was continued for 2 h while the bath attained room temperature. The reaction mixture was transferred to a column (1.5 × 10 cm) of aluminium oxide (Merck, neutral, activity grade I) with the aid of hexane-ethanol (5 ml, 3:2) and was eluted with hexane (50 ml). The solvent was evaporated cautiously in a vacuum desiccator, and most of the 1,2-dichlorobenzene was collected in a cold trap at 1 mmHg. Activity measurements indicated the presence in the trap of a low boiling by-product (1.5 μCi), probably 1,3-dichlorobenzene- ^{14}C . The residue containing *ca.* 0.2 ml dichlorobenzene was dissolved in chloroform and

transferred to a TLC plate (200 × 100 × 1 mm), which was eluted three times with hexane. A fast moving zone containing dichlorobenzene was removed and the biphenyl zone was collected. Elution with hexane and evaporation of solvent gave the biphenyl mixture as an oil which slowly crystallised, 3.2 mg, 83 μCi or 7.8 Ci/mol. The labelled product was analysed by GLC and gave two peaks of retention times corresponding to those of the unlabelled biphenyls analogously prepared.

2,3',4,4'-Tetrachlorobiphenyl. 2,4-Dichloroaniline hydrochloride (0.5 g) was suspended in 1,2-dichlorobenzene (25 ml) and kept in an ultrasonic bath (0–5°) during the following steps. After 5 min a solution of sodium nitrite (6.1 M, 0.5 ml) was added dropwise during 15 min. After another 15 min, a solution of sodium hydroxide (5 M, 3 ml) was added during 30 min. Agitation was continued for 1.5 h while the bath was allowed to attain room temperature. The aqueous phase was removed and excess dichlorobenzene was evaporated at 150° in a stream of nitrogen. The residue was dissolved in a small volume of chloroform-hexane (1:1) and was transferred to a column (1.5 × 18 cm) of silica gel (Merck, 0.05–0.2 mm). The column was eluted with hexane (200 ml). Evaporation of the solvent gave a slowly crystallising residue (0.16 g, 22%) which upon crystallisation from ethanol gave 2,3',4,4'-tetrachlorobiphenyl (I), m.p. 127–128°. (Found: Cl 48.6, M^+ 290 m.u. $\text{C}_{12}\text{H}_6\text{Cl}_4$ (291.99) requires Cl 48.6.) λ_{max} (EtOH) 251 nm (ϵ 16 500).

GLC at 168° as described above, nitrogen flow *ca.* 25 ml/min, showed a retention time of 7.9 min for biphenyl I and 5.9 min for the peak corresponding to the presumed biphenyl II. From the peak areas, the ratio I:II was estimated to 2:1.

The crude biphenyl mixture on analysis by combined GLC-MS gave identical molecular ions at 290 m.u. for the peaks corresponding to I and to the presumed isomer II, respectively.

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Tobacco Chemistry

13, 8,13-Epoxyabd-14-en-12-one and 8,13 β -Epoxyabd-14-en-12-one,* Two New Diterpenoids from Tobacco

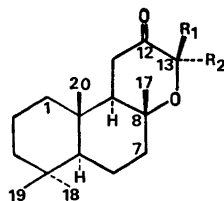
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Two new diterpenoids have been isolated^{1,2} from sun-cured Greek tobacco, *N. tabacum*, and here assigned structures I and II for reasons given below.

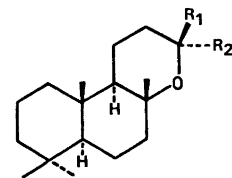
The mass spectra of the two compounds were closely similar, indicating that they were diastereoisomers, and accurate mass determinations established the elemental

* Nomenclature according to J. W. Rowe, Oct. 1968; personal communication.



I $R_1: CH_3; R_2: CH=CH_2$

II $R_1: CH=CH_2; R_2: CH_3$



III $R_1: CH_3; R_2: CH_2CH_3$

IV $R_1: CH_2CH_3; R_2: CH_3$

V $R_1: CH_3; R_2: CH=CH_2$

VI $R_1: CH=CH_2; R_2: CH_3$

compositions of both as $C_{20}H_{32}O_2$. The fragmentation patterns were strongly reminiscent of those of manoyl oxide³ (8,13-epoxyabd-14-ene, V) and 13-epimanoyl oxide³ (8,13 β -epoxyabd-14-ene, VI), suggesting that they were 12-oxo derivatives of these or of closely related compounds. Thus, the ions m/e 234, 206, 192, and 177 could be attributed to the ions A, B, C, and C-15 as depicted in Fig. 1 on the basis of the previously reported³ fragmentation patterns of the deoxo compounds. The elemental compositions of these ions were determined by high-resolution mass measurement. The presence of a non-conjugated six-membered ring ketone, a vinyl group, and five methyl groups were evident from UV, IR, and NMR⁴ spectra supporting the proposed structures.

The oxo groups of the new compounds were therefore removed by the Huang-Minlon procedure.⁵ In both cases, this also resulted in partial reduction of the vinyl group analogous to the results previously obtained by Giles *et al.*⁶ Due to shortages of material, both mixtures were hydrogenated to give homogeneous products. The two deoxodihydro derivatives exhibited optical rotations, IR and NMR spectra which were identical to those of 8,13-epoxyabdane