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Note

Separation of radioactively labelled isomers of hexachlorocyclohexane by preparative thin-layer chromatography

U. GRUNDEY and P. KRAUS

Institut für Toxikologie und Pharmakologie der Philipps Universität, Marburg/Lahn (G.F.R.)

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The additive chlorination of benzene yields a mixture of isomers of hexachlorocyclohexane (HCH) and some other compounds (for a review, see ref. 1). These compounds can be separated by recrystallization[1], column chromatography on silica gel[2], countercurrent distribution, solvent extraction[3] or preparative gas chromatography. However, these methods appeared to be unsuitable for the separation of milligram amounts of highly labelled HCH isomers: either the risk of radioactive contamination was too high, or the yield and purity were unsatisfactory. A preparative thin-layer chromatographic method was therefore developed by means of which the four main isomers of HCH were separated in good yield. In addition, this method creates little risk of massive radioactive contamination. A specimen separation of ^{36}Cl-labelled hexachlorocyclohexanes is described in this paper.

EXPERIMENTAL AND RESULTS

The crude product of the additive chlorination of benzene was analyzed by gas–liquid chromatography (GLC) on a 6-ft. Porapak Q column coated with Apiezon L for the content of the main HCH isomers. The initial and final temperatures were 190° and 210°, respectively, with a temperature gradient of 1°/min. The outflow was monitored with a flame ionization detector at 230°. This analysis revealed that the mixture contained 57.3% of α-HCH, 11.8% of β-HCH, 22.1% of γ-HCH, 6.9% of δ-HCH and 1.9% of unidentified substances. Then, 128.7 mg of the material (specific activity 1.3 Ci/mole) were dissolved in 10 ml of dioxane and transferred into a screw-capped vial. The solvent was evaporated at 48° under reduced pressure. In order to remove interfering impurities, the dry residue was washed three times with 0.2 ml of a 0.5% aqueous solution of Triton X-100. The material was dried again *in vacuo* and extracted three times with 1 ml of chloroform. α-, γ- and δ-HCH passed quantitatively into the solvent, but β-HCH, which is poorly soluble in chloroform, remained in the residue as a pure product. The β-HCH so obtained was further purified by recrystallization from ethanol.

The combined chloroform extracts were evaporated to dryness and the residue was weighed and redissolved in 3 ml of chloroform. The material was then chromatographed on 20 × 20-cm plates (Merck, Darmstadt, G.F.R.), coated with an approximately 2 mm thick layer of silica gel. The plates were heated for 15 min at 110°. Each

plate was loaded with 41 mg of the HCH mixture and then developed in the system *n*-heptane–acetone (98:2) until the solvent front had travelled two-thirds of the path length. The plates were then dried and developed again with cyclohexane–chloroform (8:2) over the full path length. A good separation of the four main isomers of HCH was obtained (Fig. 1).

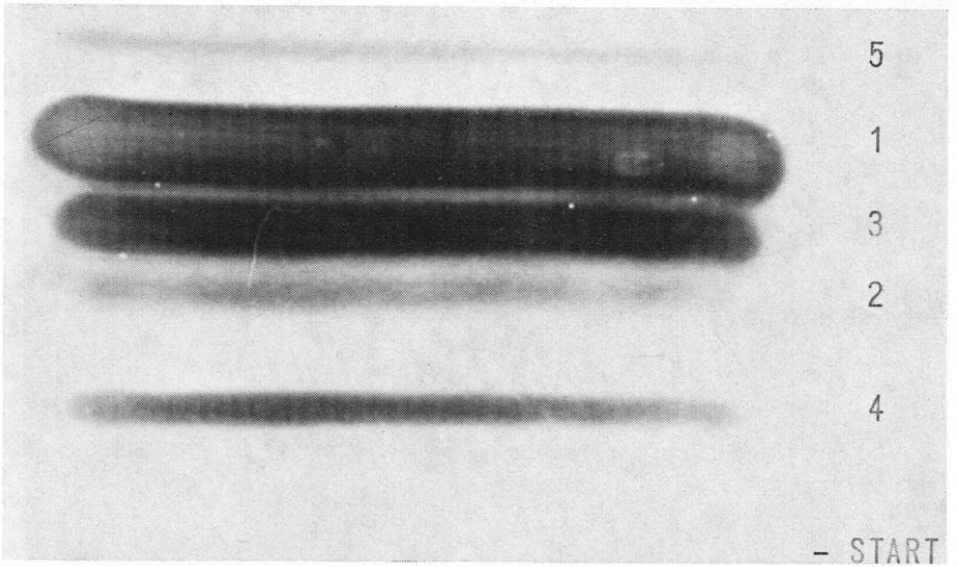

Fig. 1. Separation of the isomers of HCH by preparative thin-layer chromatography. The chromatograms were developed in the system *n*-heptane–acetone (98:2) and then with cyclohexane–chloroform (8:2) in the same direction. The substances were located by autoradiography. 1, α-HCH; 2, β-HCH; 3, γ-HCH; 4, δ-HCH; 5, unidentified.

The chromatograms were dried in air at room temperature and the substances were located by autoradiography. The film (Osray T4, Agfa-Gevaert) was exposed for 5 min. The areas containing α-, γ- and δ-HCH were scraped off under suction and eluted until at least 85% of the radioactivity were washed out. Thus, 16.4 g of silica gel containing α-HCH were eluted three times with 20 ml of chloroform and three times with the same volume of ethanol. γ- and δ-HCH adsorbed on 9.8 g and 10.4 g of silica gel, respectively, were each eluted with five portions of 15 ml of chloroform. The residual β-HCH, separated by thin-layer chromatography, was not processed further.

The eluates containing the α- and δ-isomers were evaporated under reduced pressure and recrystallized from ethanol. Some difficulties were encountered, however, in the last stage of the purification of γ-HCH: the eluate containing this isomer was evaporated under reduced pressure at 48° until about 0.5 ml of an oily yellow substance was obtained. This material could not be concentrated further and it did not crystallize. In order to induce crystallization, 30 mg of inactive γ-HCH were added and the product was recrystallized from ethanol. Similar difficulties were also encountered during the isolation of ^{14}C-labelled γ-HCH, but not with unlabelled γ-HCH. The radiochemical yield of the purification was determined by liquid scintillation counting

and was 76% for α-HCH, 86% for β-HCH, 81% for γ-HCH and 80% for δ-HCH. The chemical purity of the products was checked by GLC as described above, and the radiochemical purity was examined by two-dimensional thin-layer chromatography using *n*-heptane–acetone (98:2) in the first direction and cyclohexane–chloroform (8:2) in the second direction. This analysis revealed that all isomers were, both chemically and radiochemically, at least 98% pure.

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