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Identification of hexachlorobenzene residues by gas–liquid chromatography

Hexachlorobenzene (HCB) is a fungicide which has been used extensively in some countries to control Bunt (Tilletia spp.) in wheat. It does not appear to be metabolised to any significant extent by plants or animals and persists in soils and in animal tissues for long periods[1]. Because of the stability of the molecule and its solubility in fatty tissue, HCB is detected in sample extracts during routine surveys for organochlorine pesticide residues. Its properties are such, however, that when it is injected on to gas–liquid chromatographic (GLC) columns which employ non-polar phases, such as Silicone SE-52 and Apiezon L, the resultant peak on the GLC chromatogram usually coincides with either that of α-BHC or that of γ-BHC. Only polar phases have been found effective in resolving HCB from these BHC isomers (see Table I). However, despite the fact that resolution can be effected on these columns and a peak may appear at the correct retention time for HCB, further proof of identity is desirable.

TABLE I

RELATIVE RETENTION TIMES[a] OF THE BHC ISOMERS, HCB AND TWO OF ITS DERIVATIVES ON VARIOUS GLC COLUMNS

Columns: (1) Glass, 1.8 m × 3 mm I.D., packed with 1.3 % Silicone Gum GE-SE 52 + 0.15 % Epikote Resin 1001 on (DMCS-treated, acid-washed, 60–80 mesh) Chromosorb G. Column, detector and injection port temperatures, 200°. Nitrogen flow rate, *ca.* 50 ml/min. (2) The column size and operating conditions are the same as those of Column 1, except that the column is packed with 1.3 % Apiezon L grease + 0.15 % Epikote Resin 1001 on DMCS-treated, acid-washed, 60–80 mesh Chromosorb G. (3) The same column size as for Column 1, but packed with 1.3 % Silicone GE-XE 60 + 0.13% Epikote Resin 1001 on DMCS-treated, acid-washed, 60–80 mesh Chromosorb G. Column, detector and injection port temperatures, 195°–200°. Nitrogen flow rate, *ca.* 50 ml/min. (4) The column size and operating conditions are the same as those of Column 3, but the column is packed with 3 % diethylene glycol succinate on DMCS-treated, acid-washed, 80–100 mesh Chromosorb P. (5) Glass, 1.3 m × 3 mm I.D., packed with 5 % neopentylglycol succinate on DMCS-treated, acid-washed, 100–120 mesh Chromosorb W. Column, detector and injection port temperatures, 190°. Nitrogen flow rate, 45 ml/min. An electron-capture detector is used with each column and the sensitivity of the detector and amplification systems should be such that 0.25 ng of HCB will give a peak height of 50 to 70 chart divisions (full scale = 100 divisions).

	Column				
	1	*2*	*3*	*4*	*5*
HCB	18	26	12	9	10
α-BHC	18	18	26	27	28
γ-BHC	25	26	39	44	41
β-BHC	29	32	100	139	165
PCA	18	22	—	—	13
PCPE	32	43	—	—	17

[a] With respect to dieldrin = 100.

The amounts of HCB detected in sample extracts are often too small to be identified by means of IR spectroscopy or mass spectrometry, and so must be identified by the formation of a chemical derivative, which can be detected by the more

sensitive method of GLC. TAYLOR AND KEENAN[2] have treated HCB residues with both alcoholic potassium hydroxide and sodium methoxide to form products which could be used to identify this compound. They obtained two major products, both of which could be detected by GLC or thin-layer chromatography (TLC); the retention values of one corresponded to unchanged HCB, the other hand lower values. Neither product was identified.

Since HCB has short retention times on the GLC columns which are usually used for the determination of organochlorine pesticide residues, it was thought that it would be more advantageous to prepare a derivative with a higher molecular weight, which would be more likely to have a longer retention time and would not be masked by the injection peak made by the solvent. The first derivative to be examined was pentachloroanisole, which was prepared by a scaled-down version of the method used by ROCKLIN[3]. The retention times of this compound were found to be too close to those of HCB itself to be of diagnostic value (see Table I). The second derivative to be examined was pentachlorophenyl propyl ether. This was found to be well resolved from HCB and can be used as a means of identifying HCB residues at nanogram to microgram levels by GLC. The BHC isomers do not interfere with the identification since they are removed by the reaction, probably by dehydrochlorination; no peaks corresponding to these compounds can be detected on the final chromatogram. The identity of the standard pentachlorophenyl propyl ether was established by NMR, IR and mass spectrometry.

The procedure described below is designed to be used on cleaned-up extracts of food or other substrates prepared as for the determination of residues of organochlorine pesticides.

Experimental

Apparatus. Test tubes, 15–20 ml capacity, fitted with C 14 ground-glass sockets and Micro-Snyder columns[4], two-bubble type, fitted with C 14, ground-glass cones were used.

Reagents. The following reagents were applied: *n*-Hexane, redistilled from potassium hydroxide pellets (when concentrated 100-fold and a 5-μl injection is examined by GLC, it shows no significant peaks); potassium hydroxide pellets, "analytical reagent" grade; propan-1-ol, A.R. grade; pyridine, A.R. grade.

Method. Take the cleaned-up sample extract and transfer it to a test tube. Fit a Snyder column and heat the tube cautiously in a water-bath until all the solvent has evaporated. Add 0.5 ml of pyridine to the residue, refit the Snyder column and heat the tube in boiling water for 1 min. Remove the Snyder column and introduce 0.5 ml of a potassium hydroxide solution, which has been freshly prepared by dissolving 0.08–0.10 g of potassium hydroxide in 1.0 ml of propan-1-ol at *ca.* 50°. Replace the Snyder column and continue to heat the tube for 10 min in boiling water. Cool the reaction mixture and then add 1.0 ml of hexane, or sufficient to give a concentration equivalent to *ca.* 0.05 μg/ml of HCB. Stopper the tube and shake the mixture vigorously for 30 sec. Add 10 ml of distilled water and shake the tube for 10 sec. Allow the two phases to separate and then inject 5 μl of the hexane layer on to at least two of the GLC columns detailed below. A comparison of the sample trace with that produced from 1.0 ml of a solution containing 0.05 μg/ml of HCB, and which has been similarly treated, will show whether or not the peak at the retention time

of HCB in the sample trace has disappeared and the pentachlorophenyl propyl ether has been produced.

Results

Table I gives the relative retention times on various GLC columns of HCB, the BHC isomers, and the two derivatives: pentachloroanisole (PCA) and pentachlorophenyl propyl ether (PCPE). The GLC operating conditions are listed in the table.

If separation of HCB from the other organochlorine residues is necessary, this may be effected by fractionation on silica gel adsorption columns, using hexane as the eluting solvent[5,6]. An examination of the HCB fraction by GLC can give additional confirmation of the identity.

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Laboratory of the Government Chemist,
Cornwall House, Stamford Street,
London, S.E.1 (Great Britain)

G. B. COLLINS
D. C. HOLMES
MARGARET WALLEN

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