289. *Infra-red Analysis of Stereoisomers of "Benzene Hexachloride" (Hexachlorocyclohexane).*

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A method is described for the analysis of mixtures of the stereoisomers of benzene hexachloride, using differences in their infra-red absorption spectra together with differences in solubility in different solvents.

In the synthesis or commercial production of benzene hexachloride (hexachloro*cyclo*hexane) at least four of the theoretically possible stereoisomers appear to be formed simultaneously. Analysis of the mixture by chemical methods is unsatisfactory, and separation by repeated recrystallisation is tedious. Several years ago we were asked to explore the use of infra-red analysis, particularly for the determination of the important γ-isomer, now known as the insecticide "Gammexane". A rapid method was worked out, sufficiently satisfactory to be applied subsequently as a routine method. Publication of the full details was at that time withheld, although the method was mentioned in general articles on infra-red analysis (*Analyst*, 1945, **70**, 448). In response to many inquiries, and since this example presents unusual features, an outline of our method is given below.

The original aim was the analysis of mixtures of four stereoisomers α, β, γ and δ, in which the α-form was usually in excess. The requirements of such an analysis can be briefly stated as follows: (1) there must be sufficiently characteristic differences between the absorption spectra so that "key" wave-lengths can be chosen for the individual isomers; (2) the "key" absorption bands must be sufficiently intense, *i.e.*, have large enough extinction coefficients; (3) since the substances are solids, solutions will have to be used in non-aqueous media; (4) the solvents used must transmit freely in the region of the key absorption bands; and (5) the concentration of the solute must be such that with thicknesses of solution which are suitable spectroscopically, *i.e.*, for which there is no appreciable absorption by the solvent, the absorption bands of the solute are well developed. Ideally, all these requirements should be complied with simultaneously. In the present case this proved to be a very exacting condition, and a simultaneous determination of all four isomers from the spectral absorption curve of a single solution in one selected solvent was not practicable. This arose not only because of the widely differing proportions of the isomers in different samples, but rather from the peculiar and irregular differences in solubility of the individual isomers in different solvents. If two or more solvents are used, however, advantage may be taken of these differences in solubility.

Experimental.

The spectrometer was an automatically recording single-beam instrument with rock-salt prism, as already described (*J.*, 1945, 268). The absorption cells were made by separating two rock-salt plates by a washer about 0·1 mm. in thickness. For the work with solutions in carbon disulphide, brass inlet and outlet tubes were drilled into the side of the cell, so that it could be sealed entirely after filling. The solvents used were purified commercial samples, although ethylidene dichloride (1 : 1-dichloroethane) was also prepared in the laboratory since commercial samples often contained undeterminable impurities revealed by spurious infra-red absorption bands.

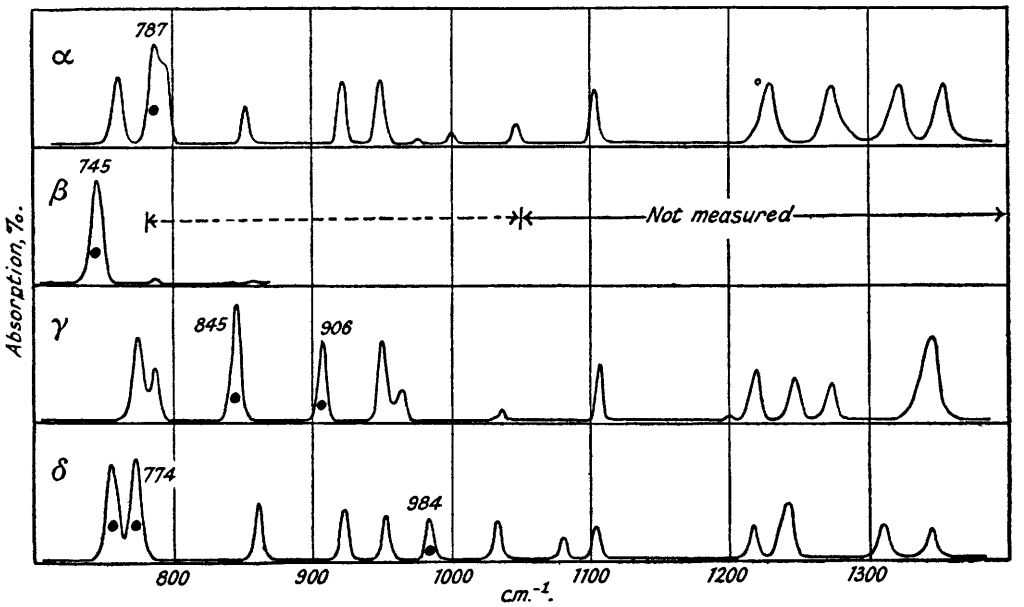
The Spectra of the Isomers.—On theoretical grounds we might expect to find the most marked spectral differences in the region 500—1400 cm.$^{-1}$ (20—7μ), and the spectra were therefore first surveyed over this range. Although the spectra of solid powders can now be measured conveniently by using a paste in paraffin, measurements were at once made with solutions. The most satisfactory solvent as regards transmission between 7 and 20μ is carbon disulphide, but unfortunately the isomers are not very soluble in it. Other solvents were therefore used, chosen on the basis of solubility and also so as to cover the whole spectral range and to avoid masking the absorption bands of the solute in any region by those of the solvents (*Trans. Faraday Soc.*, 1945, **41**, 185). The positions of the main bands of the isomers are shown in Fig. 1.

For the β-isomer, a strong band was found near 745 cm.$^{-1}$, and others will certainly occur between 800 and 1000 cm.$^{-1}$, but the solubility in most solvents was so low that under the working conditions they were almost imperceptible. It was therefore clear that no interference could arise from bands in the region concerned, and the spectrum of the powder was not measured.

Method of Analysis.—There are many well-marked differences between the spectra of the four isomers, but some of the stronger bands are not situated ideally as regards wave-lengths for analytical work on mixtures. The most suitable bands appear to lie at the following positions: α-isomer, 787 cm.$^{-1}$; β-isomer, 745 cm.$^{-1}$; γ-isomer, 845 cm.$^{-1}$; δ-isomer 774 and 984 cm.$^{-1}$. None of these key bands is completely free, however, from overlapping with bands of the other forms, even if higher resolving power were used. The band of the γ-isomer at 845 cm.$^{-1}$ is hardly affected by the others, but might be slightly overlaid by that of the α-form at 854 cm.$^{-1}$. The band of the α-isomer at 787 cm.$^{-1}$ may be affected by that of the γ-form at 785 cm.$^{-1}$ and some allowance may have to be made for this. The key band of the β-form at 745 cm.$^{-1}$ may be slightly affected by that of the δ-form at 757 cm.$^{-1}$.

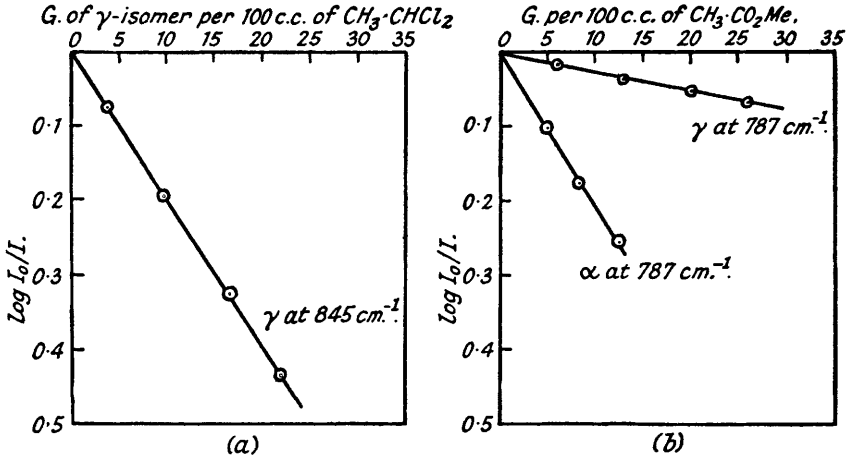
The determinations must therefore be planned so that in estimating any one isomer, the effect of overlapping with bands of another is minimised. This can be done by taking into account the different solubilities in different solvents, the choice of which will be limited, however, by their transmission in the spectral region concerned. It may also be necessary to adopt slightly different procedures for mixtures which differ widely in the relative proportions of the individual isomers.

FIG. 1.



We have first dealt with samples rich in the α-isomer and containing relatively small amounts of the other forms. In determining the γ-form, ethylidene chloride or nitromethane is a suitable solvent. We have used the former, in which the γ-form is more soluble than the other isomers. Spectral traces were taken in the standard absorption cell of (*a*) a 20% solution of the γ-form, (*b*) a saturated solution of the α-isomer, and (*c*) a saturated solution of the δ-isomer, in ethylidene chloride. These curves showed that,

FIG. 2.



even if the solution of a mixture is saturated with the α- and the δ-isomer, their bands at 854 and 861 cm.$^{-1}$ respectively will hardly affect the determination of the γ-form at 845 cm.$^{-1}$. In practice, even in the most unfavourable circumstances, it is unlikely that such concentrations of these isomers would arise. A saturated solution of the β-isomer in ethylidene chloride in the same thickness has no appreciable absorption in this region.

Calibration can therefore be set up for the γ-form by means of the band at 845 cm.$^{-1}$, solutions of the pure isomer being used. Fig. 2(*a*) shows the plot of optical density (log I_0/I) at the peak of the band at 845 cm.$^{-1}$ against concentration of solute. Measurements suggested that in this case it was satisfactory

to use peak heights rather than the integrated band areas. This is of course only valid if the bands are all of approximately the same base width and approximate to a triangular shape, and it might be desirable in refining the whole method to take the band areas.

It may be noted that in the spectrum of a mixture taken to determine the γ-form by the band at 845 cm.$^{-1}$, the rough intensities of the bands at 854 cm.$^{-1}$ (α) and 861 cm.$^{-1}$ (δ) are useful in giving indications of the approximate content of the α- and the δ-form. Another useful check on the γ-isomer is the band at 906 cm.$^{-1}$.

For determining the α-isomer by means of its band at 787 cm.$^{-1}$, methyl acetate is the most convenient solvent. This band is overlaid by that of the γ-form at 785 cm.$^{-1}$, but if the sample is much richer in α than in γ, the concentration of the latter will in general be so small as to make the overlap either insignificant or small enough to be allowed for, after determining the γ-form as above. In Fig. 2(*b*) the optical density of the band of the α-form at 787 cm.$^{-1}$ in methyl acetate is plotted against concentration, and a calibration is also given for the band of the γ-isomer at this wave-length in the same solvent. The latter plot can be used in making the allowance just referred to.

For determination of the β-isomer the band at 745 cm.$^{-1}$ was used, with solutions in methyl acetate. If there were a considerable excess of the δ-isomer, interference from its band at 757 cm.$^{-1}$ would arise, but with most samples examined neither this interference nor that from the band of the α-form at 761 cm.$^{-1}$ was appreciable. Also it is fortunate that the intrinsic extinction coefficient of the band of the β-isomer at 745 cm.$^{-1}$ is high compared with those of the other compounds. A similar calibration chart for the β-isomer was set up by using the pure component.

For determination of the δ-isomer, calibration was set up for the bands at 757 and 774 cm.$^{-1}$, in methyl acetate as solvent. Overlap with bands of the other isomers here, however, makes accurate determination difficult. A more satisfactory procedure is to use solutions in acetone and the band at 984 cm^{-1}; in this solvent the δ-form is far more soluble than the other isomers.

Example.—A sample known to be rich in the α-form was examined as follows. The γ-isomer was first determined. A known weight was shaken with a known volume of ethylidene chloride until no more would dissolve. The liquid was then centrifuged for a few minutes to separate suspended solids. The absorption was then measured at 845 cm.$^{-1}$, the standard cell being used. From the optical density the concentration of γ-isomer was read off the calibration curve, and hence the total weight of isomer in the sample obtained, and thus the percentage content. Three values obtained were 4·3, 4·6, and 4·8%. To determine the α-isomer a known weight of the sample was extracted with a known volume of methyl acetate in the same way. The extinction coefficient at 787 cm.$^{-1}$ was determined, and allowance made for the γ-isomer already estimated, by using the calibration curves of Fig. 2(*b*). Hence the concentration of the α-isomer and its percentage on the whole sample (65%) were obtained. The β-isomer was determined from the band at 745 cm.$^{-1}$ by extraction with methyl acetate in the same way (11·3%). In the course of these determinations the curves showed that the concentration of the δ-isomer must be small—less than 5%—and special determination was not made.

Accuracy.—As with most other cases of infra-red analysis of complex mixtures, the accuracy obtainable for a particular component is a complex function of the composition of the whole sample and it would be impossible to assess it briefly in the present case. It seems possible, however, by the above method to determine each of the α-, β-, and γ-isomers to within a few units % of their actual percentage in the sample; *e.g.*, if 10% of the γ-form is present, a result between 9·7 and 10·3% or better should be obtainable. Whilst this may not be as exact as might be desired, it is better than can be obtained by any other known method, and the determination is rapid. With the δ-isomer the method so far developed is less exact. A more detailed examination on many mixtures will shortly be described by workers in another laboratory, in which the above method has been in use for several years.

Detection of Contaminants and the ϵ-Isomer.—During the course of measurements on a number of commercial preparations, a few feeble bands were occasionally found which could not be interpreted as due to the four known isomers. The method is well suited to the detection of such contaminants, which can sometimes be identified with products of side reactions during the preparation.

A sample of the ϵ-isomer has recently been measured, but the nature of the absorption curve found suggests that it may have been impure. Insufficient of this sample was available to measure its solubility in the different solvents, and it is therefore difficult to assess how far, if at all, its presence in small amount might influence the above method of analysis.

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