

taking care that the sharp end of the pin sticks into the paper disc just enough to hold it while spotting.

By using a series of filter paper discs, a large number of extracts can be spotted and dried simultaneously, resulting in a tremendous saving of time. In some of our experiments, as much as 0.5 ml of an 80 % alcohol extract was applied to each disc and as many as 100 samples of this size were prepared for analysis on chromatograms in a period of a few hours. In addition, all samples are thus confined to equal areas rendering corresponding amino acids on chromatograms more precisely comparable. Furthermore, while being spotted, the extracts are not subject to varying and high temperatures destructive to some of the components, as would be the case when the fluid is directly applied to the paper and is dried by a current of hot air. In the present technique, the temperature at which the spots are dried can be regulated, if necessary, by placing the evacuated desiccator in an incubator. Also, filter paper discs on which known quantities of amino acids, their mixtures, or hydrolysates of proteins have been deposited, have been stored in a desiccator in our laboratory without deterioration and have been employed satisfactorily as standard indicators in paper chromatography.

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The reaction of OsO₄ with iodide in acid solution A chromatographic study

Paper electrophoresis and paper chromatography have lately contributed to the understanding of reactions of stable complexes of Rh, Ru and other elements (for a review see ref.¹). A recent note by FENN *et al.*² describes the formation of several Os(IV) iodo- and chloro-complexes by the reaction of osmium tetroxide with potassium iodide in hydrochloric acid solution. Since the reaction as well as the processes involved in the isolation of the complexes appeared to be rather complicated we thought it would be of interest to study the reaction by chromatography.

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The procedure of the investigation may be outlined briefly. The reaction mixture is prepared in small amounts as described in the literature², chromatographic and electrophoretic methods are tried so as to obtain a resolution of the compounds formed, a "progress chromatogram" is carried out, *i.e.* each step in the preparation is analysed chromatographically and finally using these results the method is modified so as to yield principally the desired product.

Experimental

A solution of 1 g of OsO_4 in 2 N HCl (100 ml) was prepared and small amounts (1 ml) were mixed with 2 ml of 20% KI in water. A green solution resulted which yielded trails with several partition solvents such as butanol-3 N HCl, acetone-HCl-water, and moved as a single spot when electrophorised on paper in aqueous HCl. Adsorption chromatography on Whatman No. 3MM paper with HCl, HClO_4 or KI yielded several well separated spots. 1.2 N HClO_4 and aqueous 20% KI proved to be the most useful; in the latter the spot due to I_2 (*i.e.* KI_3) is strongly adsorbed (R_F 0.23) and well separated from three spots containing osmium (detected with thiourea) with R_F values of 0.5, 0.65 and 0.8 respectively.

In Fig. 1 the scheme of the preparation of FENN *et al.*² is given and next to it is shown the result of the chromatogram after each step.

The chromatograms amplify the observations of FENN *et al.*² as regards the following facts: a chloroform extraction only removes some of the free iodine; the green ethereal solution contains much iodine and some violet (in KI) osmium complex; evaporation of the mixture of the Os complexes on the steam bath converts most of the Os into a single complex. The residue insoluble in acetone was not identical to that of FENN *et al.*²; however, we were able to explain this as is shown below.

The slowest moving zone in 20% KI has a violet colour; when chromatographed with 1.2 N HClO_4 it turns blue and in 3 or 6 N HCl green; its spectrum is identical with that found by FENN *et al.*² for K_2OsI_6 .

In order to study the reaction further we carried out two reactions: (1) OsO_4 was allowed to react with NaI in the same proportions as before with KI but in 1.2 N HClO_4 in complete absence of HCl. The chromatogram with 20% NaI solution yielded only the slow moving spot due to the OsI_6^{2-} ion and was preceded by a faint trace of a rose coloured band (less than 5%), which was so weak that it was impossible to ascertain the presence of Os with thiourea. This reaction mixture gives the spectrum shown in Fig. 2a which differs from that of pure K_2OsI_6 (see ref.²) in the region of 480-520 m μ , probably due to the small amount of the rose coloured product.

(2) OsO_4 was allowed to react in 6 N HCl with half the amount of KI (*i.e.* with 8 mol KI) employed by FENN *et al.*². A chromatogram revealed that the amount of OsI_6^{2-} formed is diminished and that the faster bands are intensified. In this experiment on concentration the acetone-insoluble product described by FENN *et al.*² was obtained and it could be shown to be the fastest moving of the three Os complexes. In our original preparation it seems that the amount formed is insufficient to precipitate. The spectrum is shown in Fig. 2b and is identical to that found by FENN *et al.*².

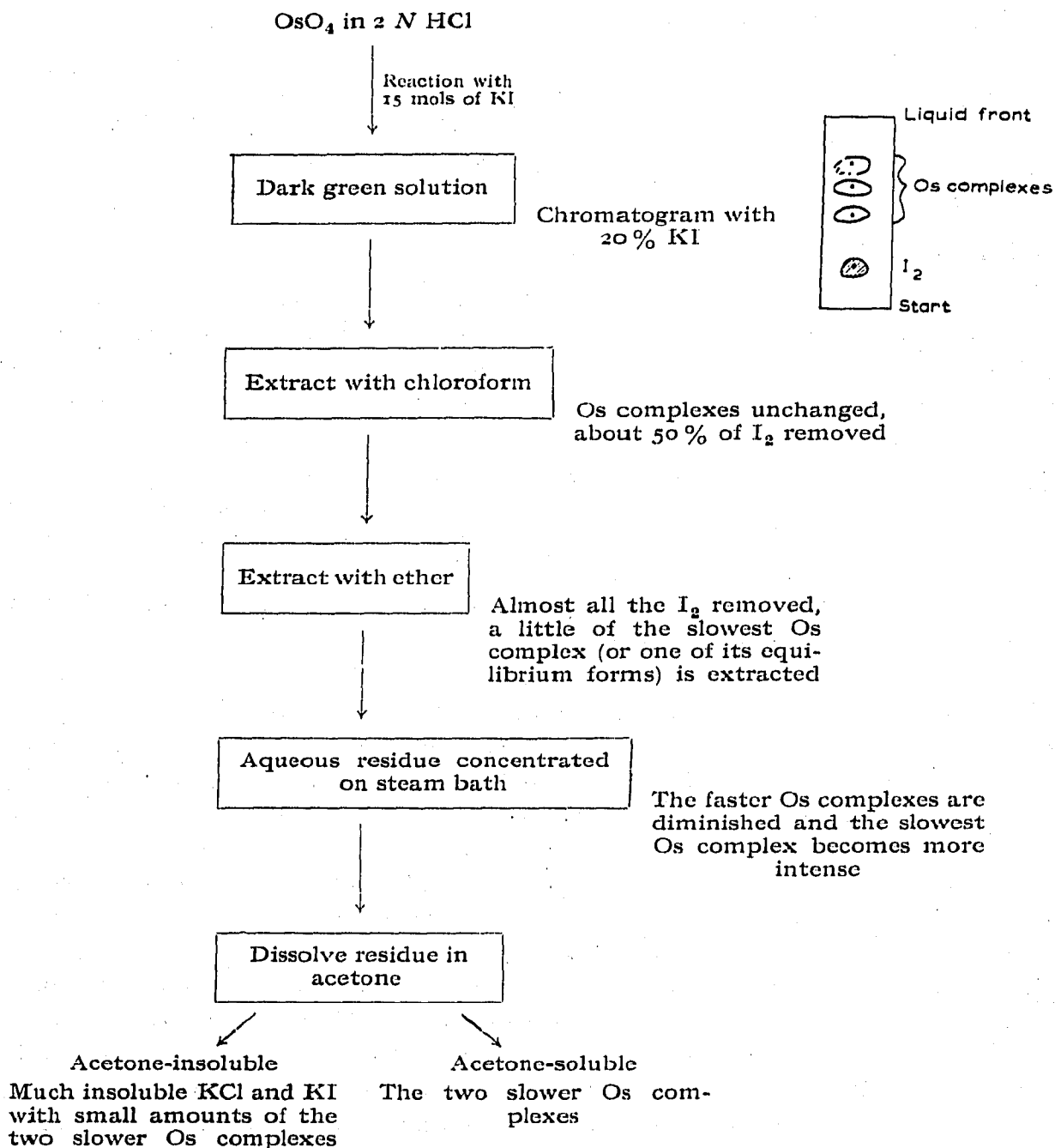


Fig. 1. Flow sheet of the reactions and results of the chromatograms after each step.

From the chromatographic behaviour it is also possible to speculate on the constitution of the three complexes: the slowest appears to be OsI_6^{2-} . However, its change of colour in HCl and HClO_4 seems to indicate that it can form $\text{OsI}_5\text{Cl}^{2-}$ and $\text{OsI}_5\text{H}_2\text{O}^-$ reversibly. Since the second fastest zone is not converted to OsI_6^{2-} reversibly even in high concentrations of KI and in absence of HCl and HClO_4 (under the conditions of the chromatogram) it must be assumed that it is not identical to $\text{OsI}_5\text{Cl}^{2-}$, but is

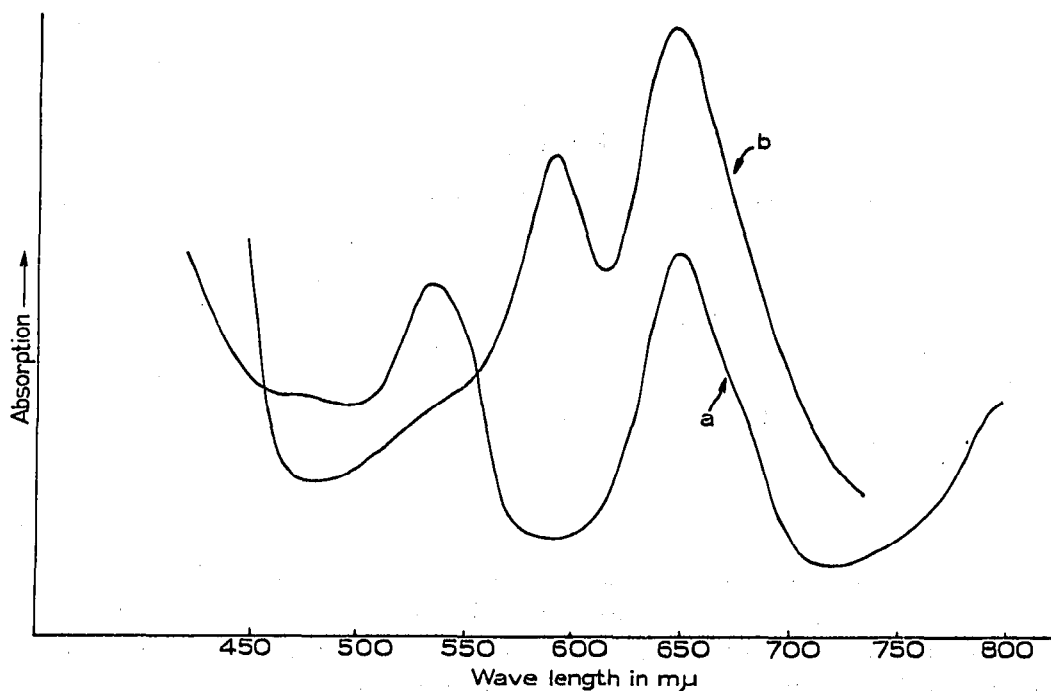


Fig. 2 (a). Spectrum of the violet compound, OsI_6^{2-} . The solution was either prepared by dissolving crystals in 20% KI or by suitably diluting the reaction mixture of OsO_4 in HClO_4 with NaI after removing the free I_2 by ether extraction. Both solutions yielded the same spectrum. (b) The spectrum of the green compound. The solution was prepared by dissolving the acetone-insoluble substance in 1.2 N HClO_4 .

more likely $\text{OsI}_4\text{Cl}_2^{2-}$ or $\text{OsI}_3\text{Cl}_3^{2-}$. It is unlikely that it is an aquo- or hydroxy-complex since it does not form in HClO_4 . This seems to confirm the supposition of FENN *et al.*² that the acetone-insoluble zone, *i.e.* the fastest of the zones is most likely $\text{OsI}_3\text{Cl}_3^{2-}$ or $\text{OsI}_2\text{Cl}_4^{2-}$.

Conclusions

The reaction of OsO_4 with KI in HCl was studied chromatographically. Three zones could be separated of which one is OsI_6^{2-} and the others chloro-iodo-osmiates (IV). A practically pure OsI_6^{2-} may be obtained by carrying out the reaction in HClO_4 instead of HCl and using NaI instead of KI. Higher yields of the chloro-iodo-complexes may be obtained by carrying out the reaction with less KI and in 6 N HCl. All the chloro-iodo-complexes may be converted to OsI_6^{2-} by evaporation on the steam bath with excess of I^- .

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