TABLE I

Alkoxide, g.	Organic ester, g.	Time for exchange, min.	Yield of distilled alkoxide, %	B.p., °C.	B.p., Mm.	Metal, % Found	Metal, % Calcd.
$Ti(OEt)_4$ (5.7)	PrOAc (35)	30	92	130	0.2	16.87	16.85
$Ti(OEt)_4$ (6.2)	BuOAc (46)	45	90	160	0.8	14.08	14.07
$Ti(OPr^i)_4$ (11.8)	BuOAc (54)	40	94	162	1.0	14.10	14.07
$Ti(OPr^i)_4$ (8.4)	*s*-BuOAc (48)	60	90	124	4.0	14.11	14.07
$Ti(OPr^i)_4$ (8.6)	*t*-BuOAc (55)	60	90	81	2.0	14.09	14.07
$Zr(OEt)_4$ (4.4)	PrOAc (41)	90	82	215	0.2	27.91	27.85
$Zr(OPr^i)_4 \cdot Pr^iOH$ (7.2)	BuOAc (52)	75	93	253	0.3	23.83	23.78
$Zr(OPr^i)_4 \cdot Pr^iOH$ (7.0)	*s*-BuOAc (50)	90	85	170	0.2	23.80	23.78
$Zr(OPr^i)_4 \cdot Pr^iOH$ (12.4)	*t*-BuOAc (54)	120	80	81	3.0	23.85	23.78
$Hf(OPr^i)_4 \cdot Pr^iOH$ (6.1)	*t*-BuOAc (36)	120	84	88	6.0	38.02	37.93

shown that the alkoxides of titanium, zirconium and hafnium react with organic esters as

$$M(OR)_4 + 4R'OOCMe \rightleftarrows M(OR')_4 + 4ROOCMe$$

The reaction can be pushed to completion if the organic ester (ROOCMe) formed is more volatile and can be fractionated out of the system.

The following compounds were prepared by the above method in almost quantitative yields from their ethoxides or isopropoxides: *n*-propoxides and isomeric butoxides of titanium and zirconium and also hafnium *t*-butoxide. The technique may be of special advantage in the case of certain unstable alcohols and has proved of great practical utility in the preparation of the *t*-butoxides of zirconium and hafnium. The methods described in the literature[3-5] for the preparation of zirconium tetra-*t*-butoxide require a very long time and the yield in all the cases is poor. Similar remarks apply to the tetra-*t*-butoxide of hafnium.[6] The difference between the boiling points of *t*-butyl and isopropyl acetates is sufficiently large to enable a rapid fractionation of the isopropyl acetate. Moreover, the reaction between zirconium ethoxide and *t*-butyl alcohol appeared to be hindered beyond the formation of zirconium tri-*t*-butoxide monoethoxide, whereas the reaction between zirconium ethoxide and *t*-butyl acetate, though slow, goes to completion with the formation of zirconium tetra-*t*-butoxide. Hence the reaction of zirconium ethoxide appears to be less hindered with *t*-butyl acetate than with *t*-butyl alcohol. Similar observations have been made for aluminum alkoxides.[7]

Experimental

Materials.—Titanium, zirconium and hafnium alkoxides were prepared and purified as described in the literature.[2] The organic esters were commercial products of reagent grade and were dried carefully and purified by fractionation over a column (60 cm. long) filled with Fenske helices; *t*-butyl acetate was prepared by the action of acetic anhydride on *t*-butyl alcohol and, after purification, it was dried by refluxing and distilling over some titanium *t*-butoxide.

Apparatus.—All glass apparatus with interchangeable joints was used throughout and special precautions were taken to exclude moisture. In ester interchange experiments, fractionations were carried out in a 60-cm. long column packed with Fenske helices and fitted to a total condensation variable take-off stillhead.

General Procedure.—The method of ester interchange employed was similar in all cases and so for brevity, details would be given in the case of zirconium isopropoxide–*t*-butyl acetate reaction only.

Crystalline zirconium isopropoxide ($Zr(OPr^i)_4 \cdot Pr^iOH$, 12.4 g.) was refluxed in *t*-butyl acetate (54.0 g.) under the column at a bath temperature of 140–150°. About 2 cc. of the distillate was collected at 82° (isopropyl alcohol). Then the temperature of the distilling liquid rose to 89° and in the course of 45 minutes, about 8 g. of the distillate was collected at this temperature. The temperature then rose to about 93–94° and came down very slowly. Refluxing was continued and another 3 g. of the distillate was collected at 89° (isopropyl acetate) in the course of a half-hour. The remaining *t*-butyl acetate was then distilled at a high reflux ratio (1:25) at 97–97.5° to push the reaction to completion. Total time of refluxing was about two hours. Finally the product was allowed to cool; the remaining *t*-butyl acetate was removed under reduced pressure and the product, a pale yellow mobile liquid was analyzed (Zr, 23.96; Pr^iO, *ca.* 0.3%). Zirconium tetra-*t*-butoxide was distilled under reduced pressure and gave a colorless, mobile liquid (9.8 g., b.p. 81° (3.0 mm.), yield 80%).

Anal. Calcd. for $Zr(OC_4H_9)_4$: Zr, 23.78. Found: Zr, 23.85.

The reaction was much faster in the case of normal and secondary butyl acetates than in the case of *t*-butyl acetate both in the case of titanium and of zirconium. Also, in general the reaction was faster in the case of titanium alkoxides than in the case of corresponding zirconium alkoxides. The results are shown in Table I.

Reaction of Zirconium Isopropoxide with *t*-Butyl Chloride—Zirconium tetraisopropoxide (6.4 g.) was refluxed at 90–100° in *t*-butyl chloride (54 g.) under the column. The exchange reaction did not appear to proceed at all even after six hours of refluxing and the end product of the reaction was found to be unchanged isopropoxide. The failure of this reaction suggests that the exchange of the alkyl groups does not occur and the exchange reaction in the earlier experiments is probably due to the interchange of the alkoxy radicals.

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The Identification of Derivatives of Fluorene and Biphenyl by Filter Paper Electrophoresis[1]

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During the course of investigations on the tissue metabolism of the carcinogen 2-acetylaminofluorene filter paper electrophoresis has been applied to

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the separation and detection of a number of related compounds. This method[1a] was found to give better resolution of these compounds than conventional paper chromatography. The spots are very well-defined and there is no "trailing." The technique is especially advantageous in biochemical work since the presence of extraneous material appears to have little or no effect on the resolution of the compounds. Color reactions based on the presence of functional groups were employed for the localization of the fluorene and biphenyl derivatives. General color tests which involve the methylene carbon of the fluorene ring system[2-4] were not sufficiently sensitive for the detection of microgram quantities.

Procedure and Results

In the present work a Precision Ionograph (Precision Scientific Co.) was used. The following procedure was employed. The solutions containing the compounds are applied to circular areas (diameter 1.0 cm.) located at the geometric centers of paper strips (Whatman No. 1) which are 47 cm. long and 3.7 cm. wide. The solvent is then evaporated with the aid of an infrared lamp or a hot air blower. Since the compounds are concentrated in a smaller area than in the usual technique in which the solutions are spread across the entire width of the paper[1a] the sensitivity is increased. In addition, retention of the compounds, which to a slight extent always occurs at the point of application, is minimized by this modification. Twenty per cent. acetic acid (v./v.) was found to be the most satisfactory electrolyte for these compounds. It is allowed to travel along the paper by capillary action until the electrolyte fronts meet at the areas of application (about 2 hours), at which time the current is turned on. A potential gradient of 15.1 volts/cm. length was used by applying 650 volts across the paper. Runs of 4 to 6 hours are usually sufficient for adequate separation of the compounds. The temperature is maintained at 23 ± 0.5° by circulating water from a constant temperature bath. The papers are dried in air prior to spraying with the color reagents.

Table I shows the mobilities (cm./sec. per volt/cm.) of pure compounds. Under the experimental conditions these compounds migrate toward the cathode. The distance traveled is measured from the center of the area of application to the leading edge of the developed spot. Colors are obtained by spraying the papers with a solution of 100 mg. of *p*-dimethylaminobenzaldehyde in 50 ml. of ethanol acidified with 1.0 ml. of concentrated hydrochloric acid.[5] Alternatively, phenolamines and diamines may be located by exposing the paper to chlorine vapors. These compounds give dark brown or dark green spots. The diamino compounds appear on the developed papers as narrow, symmetrical bands. The bands are curved with the convexity directed toward the cathode. The other compounds appear as round or elliptical spots after color development.

Neither 2-acetylaminofluorene nor 2-hydroxy-7-acetylaminofluorene give colors with these reagents. The phenolic derivative (2-hydroxy-7-acetylaminofluorene) is detected by using 100 ml. of a saturated solution of sulfanilic acid in 50% ethanol to which 1.0 ml. of concentrated hydrochloric acid is added. The solution is diazotized with 50 ml. of a 0.7% solution of sodium nitrite and the diazotized mixture is applied to the paper. The paper is dried and the color is developed by spraying with a 1% solution of sodium hydroxide.[6] All color reagents should be freshly prepared. The mobility of 2-acetylaminofluorene for which a color test is not yet available was determined with the aid of radioactive 2-acetylaminofluorene labeled in the 9-position with carbon 14. The radioactivity was located on the paper with a windowless scanner.[7] Both 2-acetylaminofluorene and 2-hydroxy-7-acetylaminofluorene are isoelectric under the experimental conditions and do not move. They may be further characterized on the same paper by chromatography as follows. After completion of the electrophoretic run the paper is cut at a distance 2.5 cm. from the area of application toward the cathode. The segment containing the spot of application is then chromatographed by the ascending technique and the compounds are detected as described above. R_f values for 2-acetylaminofluorene and 2-hydroxy-7-acetylaminofluorene in butanol saturated with 3 *M* ammonium hydroxide are 0.90 and 0.88. R_f values for the same compounds developed with the aqueous layer, obtained by shaking 8 parts of butanol and 1.5 parts of ethanol with 12 parts of water, are 0.22 and 0.21, respectively.

When the method is applied to tissue extracts it is the practice to add the compounds under study to aliquots of control extracts and to run the "marked" samples and the unknown simultaneously. The results of these experiments will be reported elsewhere.

TABLE I

MOBILITIES OF FLUORENE AND BIPHENYL DERIVATIVES IN 20% ACETIC ACID AT 23°

Compound	Mobility × 10^5, cm./sec./volt/cm.	Color	Lower limit of detection, μg.
2,7-Diaminofluorene	6.02	Bright red[a]	1–2
2-Aminofluorene	4.58	Bright yellow[a]	0.5–1
2-Monomethylaminofluorene	4.34	Pale yellow[a]	5–6
2-Amino-7-hydroxyfluorene	4.05	Orange yellow[a]	0.5–1
2-Amino-7-chlorofluorene	4.00	Bright yellow[a]	1–2
2-Amino-7-acetylaminofluorene	3.61	Yellow orange[a]	1–2
2-Acetylaminofluorene	0.00	.....[b]	...
2-Hydroxy-7-acetylaminofluorene	0.00	Purple[c]	2–3
Benzidine (4,4'-diaminobiphenyl)	5.86	Orange[a]	1–2
4-Amino-4'-hydroxybiphenyl	4.36	Bright yellow[a]	1–2

[a] Obtained with *p*-dimethylaminobenzaldehyde as described in the text. [b] See text for method of detection. [c] Obtained with diazotized sulfanilic acid as described in the text.

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Structures of the 4a-Methyl-1,2,3,4,4a,9,10,10a-octahydro-8-phenanthrols

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The configurations of the alicyclic rings in both of the racemic 4a-methyl-8-methoxy-1,2,3,4,4a,-9,10,10a-octahydro-2-phenanthrones (I) and in three of the four possible racemic 2-methoxy-4a-methyl- 1,2,3,4,4a,9,10,10a - octahydro - 8 - phenanthrols (III) have been established.[1] Furthermore, the orientation of the alicyclic rings has been found[1] to determine the configuration of products obtained by catalytic hydrogenation of III. Only the isomer with the alicyclic rings *trans* (IIIa) produces a hydrogenation product with the "natural" configuration at the 4b-position.

In order to simplify studies of methods for synthesizing compounds related to the steroids, we

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