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PAPER ELECTROPHORESIS OF MONOHYDRIC ALCOHOLS AND HYDROXY ACIDS

SEPARATION AS XANTHATES

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SUMMARY

The paper electrophoresis of the potassium salts of xanthates derived from monohydric alcohols is described. Primary and secondary alcohols of different structural types are characterised by the relative mobilities of the corresponding xanthates using sodium hydrogen carbonate as electrolyte. The method proved to be useful for the detection and identification of alcohols present as impurities in solvents.

Mono-carboxylic hydroxy acids yield carboxy-alkyl xanthates which are readily separable from the respective free hydroxy acid anions also present in the reaction mixtures. Both anions may be detected simultaneously on pherograms.

Conditions are described under which the unstable xanthates derived from tertiary alcohols may be separated electrophoretically from their reaction mixtures and detected on the pherograms.

A method is described for the identification of the alkoxy groups of esters using the xanthate reaction. The acyl groups of the esters may be identified simultaneously.

The electrophoretic mobilities of some common sulphur-containing inorganic anions, including trithiocarbonate and trithiopercarbonate, were determined in sodium hydrogen carbonate electrolyte. The detection of the inorganic anions, the xanthates and some organic acid anions present on pherograms is described.

The xanthate reaction has been applied in various ways for the identification of alcohols^{1,2-4}, and paper and thin-layer chromatography of potassium alkyl xanthates as means of separating and identifying alcohols have been the subject of several reports⁵⁻⁸. BERBALK⁹ has described the paper electrophoresis of xanthates derived from a number of mono- and di-hydric alcohols and has measured their anionic mobilities in a Veronal buffer.

A selection of monohydric alcohols representative of several classes has now been characterised by paper electrophoresis of the corresponding xanthates using sodium hydrogen carbonate as electrolyte. These include terpene alcohols, hydroxy acids, some carbohydrate derivatives containing a single free hydroxyl group and the glycol ether solvents known commercially as Cellosolves and Carbitols. The homologous series of aliphatic derivatives studied by BERBALK are also included and their

behaviour in sodium hydrogen carbonate and other electrolytes is reported for purposes of comparison.

Xanthates derived from tertiary alcohols are unstable in aqueous media and it is probably for this reason that the chemical literature contains no reference to the chromatography of these derivatives. We have found that some tertiary xanthate preparations partially survive the more rapid process of paper electrophoresis and are separable from reaction mixtures as elongated spots or streaks.

The scope of the method has been extended to the direct identification of the alkoxy groups of esters. The acyl groups of the esters may be identified simultaneously on pherograms as the corresponding acid anions.

Xanthate preparations usually contain inorganic anions¹⁰. These include sulphide, thiosulphate and trithiocarbonate, which either form simultaneously with the xanthates or result from their decomposition, especially in aqueous media. The electrophoretic mobilities of these and other sulphur-containing anions in sodium hydrogen carbonate are recorded.

EXPERIMENTAL

Materials

Commercial samples of alcohols, hydroxy acids, esters and other reagents were used, in most cases, without further purification, but samples of tertiary alcohols were subjected to treatment for the removal of impurities, mainly primary or secondary alcohols.

tert.-Butyl alcohol was purified by repeated fractional crystallisation from its own melt¹¹.

tert.-Amyl alcohol and *dl*- α -terpineol were each purified by allowing the commercial sample to react in pyridine solution with toluene-*p*-sulphonyl chloride at room temperature for 3 h during which primary and secondary alcohols present in the samples as impurities were selectively tosylated¹². The reaction mixtures were poured on to crushed ice and excess toluene-*p*-sulphonyl chloride was allowed to hydrolyse. After the addition of cold hydrochloric acid, the aqueous media were extracted with ether and the extracts washed with NaHCO₃ solution and dried over anhydrous magnesium sulphate. The ether was removed and the residues distilled under reduced pressure to obtain the pure tertiary alcohols.

The carbohydrate derivatives were each prepared according to published procedures¹³⁻¹⁷.

Sodium salts of sulphate, sulphite, sulphide and thiosulphate were of reagent grade. A solution of tetrathionate was prepared by oxidising sodium thiosulphate with iodine. Sodium trithiocarbonate was prepared according to the directions of INGRAM AND TOMS¹⁸.

Electrolytes

- (a) Sodium hydrogen carbonate solution (0.1 *M*, pH 8.4), hereinafter abbreviated to "NaHCO₃ solution".
- (b) Sodium carbonate solution (0.1 *M*, pH 11.2).
- (c) Sodium hydroxide solution (0.1 *M*).
- (d) Acetic acid-sodium acetate buffer (0.1 *M* with respect to acetate, pH 6.0).
- (e) Veronal buffer (0.1 *M*; pH 9.8).

Spray reagents

- (a) Ammonium molybdate (3 % w/v) in 2 % sulphuric acid (v/v)^{6,19}.
- (b) Copper sulphate solution (7.5 %)⁶.
- (c) Mercuric chloride solution (6 %).
- (d) Chromium trioxide–permanganate–sulphuric acid. The preparation and use of this reagent as a spray for papers after electrophoresis have been described²⁰.
- (e) Iodine (0.5 %) in 1 % potassium iodide solution.
- (f) Sodium nitroprusside (10 %) in water.
- (g) Bromphenol blue reagent. The indicator (0.04 g) was dissolved in 95 % ethyl alcohol (100 ml) and the solution adjusted to pH 6.7 by adding dilute alkali²¹.

Apparatus

Paper electrophoresis was conducted in the enclosed strip apparatus described previously²² using Whatman No. 4 paper in strips 13.5 × 61 cm, with 45 cm under-pressure and cooled. The circulation of ice-water through the coils of the cooling-plate at a rate of about 2 l/min maintained the temperature at the surface of the paper at 4° during electrophoresis in all electrolytes.

In some experiments, mains water at 24° was circulated and maintained the temperature of the paper at 26°. Circulating water at 48° maintained the paper at 50°.

Procedure

Potassium alkyl xanthates were prepared from the alcohols by the method of WHITMORE AND SIMPSON²³. The alcohols (0.2 ml) were each shaken for a few minutes in a small tube at room temperature with powdered potassium hydroxide (0.3 g) and carbon disulphide (0.5 ml). Xanthation of the more viscous alcohols was facilitated by triturating reaction mixtures with a glass rod.

Solid alcohols, including the carbohydrate derivatives, and hydroxy acids, solid and liquid, were converted to the corresponding xanthates using similar quantities of reactants in solution in dimethyl sulphoxide (1 ml).

The reaction products were normally subjected to paper electrophoresis after dilution with water to give solutions which were 0.02 to 0.06 *M* with respect to the amount of alcohol used, but more concentrated solutions of some derivatives were required to facilitate their subsequent detection. Reaction mixtures of carbohydrate derivatives were diluted to 0.1–0.15 *M* concentration, and the *n*-decyl compound to 0.2 *M*. The reaction mixtures of tertiary alcohols were applied directly to papers without dilution.

The alkoxy groups of esters were converted to xanthates by shaking each ester (0.2 ml) in a stoppered tube for 5 min at 50° with a mixture of potassium hydroxide (0.3 g) and dimethyl sulphoxide (1 ml), adding carbon disulphide (0.5 ml) to the mixture after cooling and then stirring for 3–5 min. Reaction mixtures were applied to the papers after dilution with water (30 ml).

Samples of 0.5 μ l of xanthate solutions were transferred by means of a platinum loop to papers impregnated with electrolyte and equilibrated for 15 min by enclosure within the apparatus. (The concentrated reaction mixtures of tertiary alcohols were viscous and unknown volumes considerably in excess of 0.5 μ l were transferred to papers with the loop.)

Inorganic salts were applied, usually as 0.1 *M* solutions in water.

Caffeine was used as the marker for zero migration and rates of migration of the test compounds were calculated relative to that of nitrobenzene-*p*-sulphonate applied to the same paper (M_N values²⁰).

Under the standard conditions, electrophoresis was allowed to proceed in NaHCO_3 solution at 4° for 1 h at about 21 V/cm. (Nitrobenzene-*p*-sulphonate moved approximately 8 cm under the standard conditions.) In other experiments, conditions were varied with respect to pH, duration and temperature.

The papers were dried in the oven at 100°. Caffeine, nitrobenzene-*p*-sulphonate, the xanthates and some of the inorganic anions present were located as dark blue spots under a Hanovia "Chromatolite" ultraviolet lamp. Papers were treated with one of the spray reagents to confirm the location and, in some cases, the identities of the spots.

Tertiary xanthates were subjected to electrophoresis at 4° for 15–20 min and the papers partially dried at 100° for 2–3 min. They were sprayed, without delay, with molybdate or other reagent.

Test for thiosulphate

The test described by FEIGL²⁴ for thiosulphate was adapted in the following way to its detection after electrophoretic separation.

Dried alkaline papers were sprayed uniformly with formic acid solution (0.3 *M*) until phenolphthalein (applied away from spots of test compounds) was just de-colourised and remained colourless after re-drying the papers in the oven. The papers were then sprayed lightly and evenly with mercuric chloride solution, followed, without drying, by the bromphenol blue reagent. Yellow spots appeared on a blue ground where sulphuric acid, generated by reaction of the mercuric ion with thiosulphate, had liberated free formic acid from the sodium salt formed on the paper during the initial spray treatment.

RESULTS AND DISCUSSION

The results of the electrophoresis of 36 xanthates under the standard conditions are given in Table I. The derivatives are classified according to the type of alcohol from which they were prepared.

The effect of pH

The NaHCO_3 solution was selected as the standard electrolyte because it was found to promote more rapid separations of xanthates than the sodium carbonate or sodium hydroxide electrolytes. The derivatives were stable, however, at the higher values of pH and the same relative order of mobilities prevailed. Some of the inorganic ions gave less distinct spots at higher pH.

Some decomposition of most xanthates was evident in acidic electrolytes at pH 5 in the form of streaking, but in acetate buffer at pH 6 the derivatives were stable and migrated as compact spots.

The effect of temperature

Electrophoresis was conducted at 4° in NaHCO_3 solution for maximum stabilization of the xanthates which, with two or three exceptions, gave compact, almost

TABLE I

RELATIVE RATES OF MIGRATION OF XANTHATE IONS

Compounds were detected after paper electrophoresis in sodium hydrogen carbonate (0.1 M) at 21 V/cm and 4° for 1 h. For each parent compound, the mol. wt. of the derived xanthate ion is included.

Parent compound	Derived ion	
	$M_N \times 100^a$	Mol. wt.
<i>Aliphatic alcohols</i>		
1. Methyl	143	107
2. Ethyl	122	121
3. <i>n</i> -Propyl	108	135
4. Isopropyl	111	135
5. <i>n</i> -Butyl	100	149
6. Isobutyl	97	149
7. <i>sec.</i> -Butyl	100	149
8. <i>n</i> -Amyl	88	163
9. Isoamyl	88	163
10. <i>n</i> -Hexyl	84	177
11. Cyclohexyl	86	175
12. <i>n</i> -Heptyl	76	191
13. <i>n</i> -Octyl ^b	63	205
14. Capryl (octan-2-ol)	65	205
15. <i>n</i> -Nonyl ^b	54	219
16. <i>n</i> -Decyl ^c	49	233
17. Benzyl	87	183
<i>Unsaturated alcohols</i>		
18. Allyl	118	133
19. α -Furfuryl	98	173
<i>Glycol ethers</i>		
20. 2-Methoxy-ethanol (Methyl Cellosolve)	111	151
21. 2-Ethoxy-ethanol (Ethyl Cellosolve)	101	165
22. 2-(2-Methoxyethoxy)-ethanol (Methyl Carbitol)	90	195
23. 2-(2-Ethoxyethoxy)-ethanol (Ethyl Carbitol)	83	209
<i>Terpene alcohols</i>		
24. Geraniol	66	229
25. Isopulegol	70	229
26. Menthol	70	231
<i>Carbohydrate derivatives</i>		
27. Methyl 2,3,6-tri-O-methyl- α -D-glucopyranoside	66	311
28. 2,3:4,6-Di-O-isopropylidene-L-sorbose	63	335
29. 1,2:5,6-Di-O-isopropylidene-D-glucose	63	335
30. Methyl 3-O-methyl-4,6-O-benzylidene- β -D-idoside	58	371
31. 1,2:5,6-Di-O-cyclohexylidene-D-glucose	54	415
<i>Hydroxy-acids</i>		
32. Glycollate	182 (129) ^d	150
33. Lactate	157 (108)	164
34. β -Hydroxy-butyrate	150 (104)	178
35. Mandelate	136 (87)	226
36. 2,4:3,5-Di-O-methylene-D-gluconate	116 (77)	294

^a M_N values²⁰ express mobilities relative to the nitrobenzene-*p*-sulphonate ion, which moved approx. 8 cm.

^b *n*-Octyl and *n*-nonyl xanthates gave somewhat elongated spots.

^c The value of $M_N \times 100$ given for *n*-decyl xanthate corresponds to the head of a streak observed for this derivative.

^d Values of $M_N \times 100$ in parentheses are relative mobilities of the respective parent acid anions.

circular spots. Most derivatives migrated satisfactorily with little or no sign of decomposition at 26°, but elongation of some spots was noted after experiments conducted at 50° in NaHCO₃ solution.

The derivatives suffered little decomposition during heating to dry the papers after electrophoresis and many survived prolonged heating at 100°.

In most cases, unreacted parent alcohols volatilised from the papers during drying but some of the less volatile appeared as non-migrating spots with some spray reagents. Unreacted hydroxy acids were detected as additional anionic spots. The presence of dimethyl sulphoxide in a preparation resulted in the appearance of a relatively large spot ($M_N = 0$) with the chromium trioxide–permanganate–sulphuric acid reagent.

Detection of xanthates, organic acids and inorganic anions after electrophoresis

Xanthates on paper chromatograms fluoresce in ultraviolet light⁵ and the fluorescence of trithiocarbonate, trithiopercarbonate and the xanthates was strong enough to enable detection of the ions in the small quantities (about 0.02 μ mole) needed for the electrophoretic method.

Grote's reagent has been used successfully for the location of xanthates on paper chromatograms⁵ but it lacked the sensitivity required and was abandoned in the present work in favour of the ammonium molybdate^{6, 19} and copper sulphate⁶ reagents. With the exception of the tertiary xanthates described below, the molybdate reagent gave pink spots which slowly (20–30 min) changed to blue²⁵. Thiosulphate, tetrathionate, trithiocarbonate and trithiopercarbonate gave blue reactions developing slowly. The copper reagent gave stable, bright yellow or yellow-brown spots with xanthates.

Salts of other heavy metals were also tried. Silver nitrate solutions, either ammoniacal⁷ or acidic, gave brown or black spots but were only moderately sensitive. Concentrated aqueous solutions of cobalt and nickel salts⁹ gave only weak spots with most xanthates in the concentrations used here. Mercuric chloride, however, was almost as sensitive to xanthates as the copper reagent. When it was sprayed on to dried papers impregnated with alkali metal carbonates, brown basic mercuric carbonate precipitated on the cellulose fibres and xanthates appeared immediately as stable white spots. The reagent is not specific and was used for some inorganic ions, especially sulphate, otherwise difficult to detect. The organic acid anions, succinate, benzoate and acetate formed as by-products in experiments with esters, were also located with mercuric chloride, but for these and the inorganic ions it was less sensitive and the presence of 0.1–0.2 μ mole of each was required for satisfactory results.

Chromium trioxide–permanganate–sulphuric acid reacted with xanthates to give immediate yellow spots on a pink background. The papers were stored between glass plates and the spots became white within 7–10 min, the background simultaneously changing to brown. A permanent record of the experiment was obtained in the manner previously described by FRAHN AND MILLS²⁰. The reagent was very sensitive to xanthates, although not specific, and less than 1 μ g of methyl xanthate per sq. cm of spot area was easily located. The reagent was sensitive to oxalate and hydroxy acid anions and was used for their detection in preference to the mercuric chloride reagent. Thiosulphate and tetrathionate gave strong reactions, but sulphite, trithiocarbonate and trithiopercarbonate reacted weakly.

The iodine reagent caused immediate oxidation of xanthates on paper, forming white spots on a brown ground. It was very sensitive, though non-specific, and provided an alternative means of detecting thiosulphate, sulphite, trithiocarbonate and trithiopercarbonate.

Nitroprusside did not react with xanthates and was used to confirm the presence of sulphide in some xanthate preparations following its electrophoretic separation. Nitroprusside gave a strong violet reaction quickly fading to pink with trithiocarbonate and an immediate pink colour with trithiopercarbonate. Thiosulphate gave a stable pale blue spot which formed slowly.

Xanthates from primary and secondary alcohols

The xanthate reaction is quickly and easily performed and provides a convenient means of converting primary and secondary alcohols to stable charged species suitable for electrophoresis.

The present study was undertaken as a basis for the development of a simple method of enumerating the hydroxyl groups of polyhydroxy compounds without reference to their molecular weights and it was found during the preparatory work that electrophoretic mobility provided a useful guide for identifying a wide range of mono-functional xanthates. The inverse relationships which exist between their relative mobilities and molecular weights are illustrated graphically in Fig. 1, prepared from data contained in Table I.

The points plotted for the lower members of the aliphatic series lie on or near a smooth curve—the upper portion of curve A. The break which occurs in the curve beyond the *n*-heptyl derivative (compound 12) is probably due to an increasing tendency for derivatives with longer carbon chains to form ionic micelles large enough to suffer retardation by the cellulose support. The streak observed for the *n*-decyl

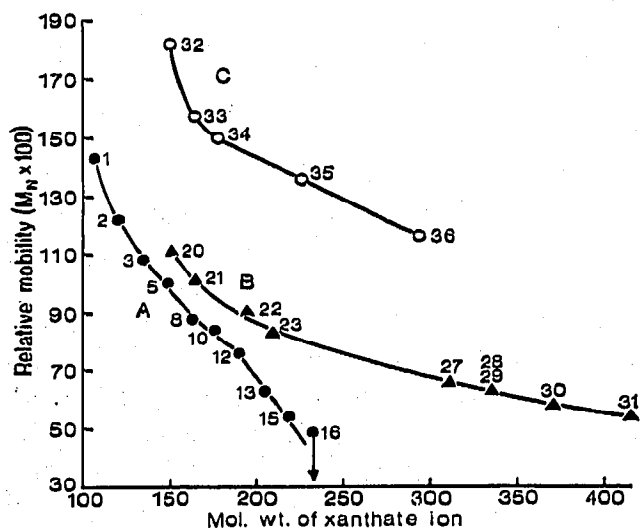


Fig. 1. Relationships between mol. wt. and relative mobility of xanthate ions derived from structurally different types of monohydric alcohols. Numerals correspond to parent compounds as listed in Table I. Curve A: xanthates of normal aliphatic primary alcohols. The arrow appended to the point plotted for the *n*-decyl derivative (16) indicates the streak that was observed in this case. Curve B: xanthates of alcohols containing ether, cyclic acetal, cyclic ketal and glycosidic bonds. Curve C: xanthates derived from hydroxy acids.

derivative may have been the result of the same effect in exaggerated form. Curve A, extrapolated from the points for members immediately lower in the series, passes through a point corresponding approximately to the mid-point of the streak for the *n*-decyl ion.

The set of curves comprising Fig. 1 indicate that the xanthates may be divided into three distinct groups or families. Curve A corresponds to members of the aliphatic series, as noted above, curve B to derivatives containing oxygen in ether, glycoside, cyclic acetal or cyclic ketal bonds (those of the glycol ethers and carbohydrates) and curve C to xanthates derived from hydroxy acids. The data for the unsaturated derivatives (compounds 18 and 19) appear to fit curve B.

The electrophoretic method as applied to homologous alkyl xanthates gives results which are complementary to those of the chromatographic methods inasmuch as the sequence in which the members separate is inverted⁷.

The results for members of the aliphatic series (Table I) agree generally with those of BERBALK⁹, an exception being the case of the *n*-propyl derivative, which occupies an anomalous position in the series as described by BERBALK. Using Schleicher and Schüll 2043b paper impregnated with Veronal buffer at pH 9.8, he found *n*-propyl xanthate to have a mobility identical with that of the *n*-butyl derivative. Under our standard conditions of electrophoresis these derivatives separated readily after being applied to the paper as a mixture. The same separation was observed when the other alkaline electrolytes, including Veronal buffer, were substituted for NaHCO₃ solution and when Schleicher and Schüll paper replaced Whatman No. 4.

BERBALK reported similar mobilities for isobutyl and isoamyl xanthates and for the benzyl and *n*-propyl derivatives, but we found these pairs to separate quickly in all alkaline electrolytes. These discrepancies are not the effects of differences in temperature, unless these were very great, because experiments in which the temperature was maintained at 26° and at 50°, using Veronal buffer, did not yield results significantly different from those in NaHCO₃ solution at 4°.

Xanthates from tertiary alcohols

It is well known that stable xanthates are obtained in comparable yields from aliphatic primary and secondary alcohols, and similar reactivities are displayed by primary and secondary hydroxyl functions contained in compounds of more complex structure. Xanthation of the isomeric di-O-isopropylidene derivatives of L-sorbose and D-glucose containing, respectively, a primary and a secondary hydroxyl group, proceeded with approximately equal facility to yield stable derivatives.

In contrast, xanthate ions derived from tertiary alcohols are unstable in aqueous media. WHITMORE AND LIEBER¹ considered that their rapid hydrolysis involved regeneration of the parent alcohols with the formation of sulphide. But it seems that hydrolysis of at least some of these derivatives is by no means instantaneous. For example, an aqueous solution of potassium *tert.*-amyl xanthate has been used for the preparation of the insoluble indium salt²⁷.

The survival of some tertiary xanthates in an aqueous medium was demonstrated by electrophoresis in NaHCO₃ solution. The preparation of these derivatives occasionally calls for special methods^{28, 29}, but the usual conditions suffice in many cases^{1, 26, 27}. For our experiments, xanthates were prepared in the normal way from pure *tert.*-butyl and *tert.*-amyl alcohols, *dl*- α -terpineol and *cis*-terpin. They were

applied to papers in concentrated form and subjected to electrophoresis for brief periods as described above. The application of molybdate to the partially dried papers revealed the tertiary xanthates as elongated anionic spots or streaks. The leading edges of the streaks often lagged a little behind spots of the respective isomeric primary or secondary derivatives run in other lanes. Streaking probably resulted from the combined effects of decomposition of the migrating ions and overloading of the papers with concentrated reaction mixtures. The tertiary xanthates reacted in a normal way with each spray reagent except molybdate. The streaks were coloured blue instantly upon the application of molybdate in contrast to the behaviour of other xanthates which formed pink spots slowly changing to blue²⁵. The effect was not due to the liberation of sulphide from the decomposing ions because no response was obtained to nitroprusside in separate tests. Nor was the effect due to the application of concentrated reaction mixtures to the papers, because xanthates prepared from primary and secondary alcohols and applied to papers in undiluted form were subsequently located with molybdate as large spots, typically pink in colour.

cis-Terpin, containing two tertiary hydroxyl groups, gave two distinct streaks, the leading edges of which had values of $M_N \times 100$ of 68 and 115. These probably corresponded to the mono- and di-xanthates, respectively, of the parent glycol.

When the products of reaction of the pure tertiary alcohols were diluted and subjected to electrophoresis, no xanthates were detected on the resulting pherograms, but under the same conditions, most commercial samples of the same tertiary alcohols gave rise to faint pink spots with molybdate. Primary or secondary alcohols present as impurities proved to be responsible for these spots. Gas-liquid chromatography of the commercial samples confirmed the presence of impurities as supernumerary peaks accompanying the main peaks.

Carboxy-alkyl xanthates

The mono-carboxylic hydroxy acids listed in Table I all reacted rapidly to give deep red or red-brown equilibrium mixtures containing the corresponding xanthates in high proportions when dimethyl sulphoxide was used as a solvent for the reactants. In the absence of the solvent the reactions were generally sluggish.

Unreacted hydroxy acids were always present in the diluted reaction mixtures and migrated as anions in alkaline electrolytes. The mobilities of the derived xanthates, each containing two negative charges, were correspondingly greater and promoted their rapid separation from the free hydroxy acid anions.

The carboxy-alkyl xanthates reacted in a manner typical of xanthates with spray reagents applied to papers after electrophoresis. Free hydroxy acids, with the exception of the dimethylene gluconate, were detected simultaneously with the xanthates by using chromium trioxide-permanganate-sulphuric acid. The gluconate was located only with mercuric chloride. Relative mobilities of the hydroxy acid anions are included in Table I together with those of the respective xanthates. Each hydroxy acid is thus characterised by a set of two values.

The corresponding derivatives of the dicarboxylic hydroxy acids, malic and tartaric, were not detected. Although the S-ethyl ester of 1,2-dicarboxyethyl xanthic acid derived from malic acid is known³⁰, it seems that the free xanthic acid anion is unstable. Similarly, citric acid, containing a tertiary hydroxyl group, does not form a stable xanthate.

Attempts to demonstrate the formation of a xanthate from pyruvate, which might be expected to react in the enolic form, were unsuccessful. Two components were, however, readily separated from reaction mixtures, one of which ($M_N \times 100 = 134$) was pyruvate. The other ($M_N \times 100 = 146$) proved to be 2-hydroxy-2-methyl-4-oxo-glutarate, a dimer of pyruvate, formed by aldol-type condensation of pyruvate in the alkaline reaction medium³¹. Pyruvate and its dimer reacted strongly with chromium trioxide–permanganate–sulphuric acid and with iodine, but slowly with the latter. The dimer reacted with other reagents used for the detection of xanthates but not in a typical manner. It formed a blue spot slowly with molybdate and a faint green spot with copper.

The dimer is an impurity in most commercial and laboratory-prepared samples of sodium pyruvate³². It is biochemically interesting in that it is a potent inhibitor of the tricarboxylic acid cycle and has been shown to be specific for the enzyme α -keto-glutaric oxidase³³.

Detection of alcohols as impurities in solvents

It has been shown⁵ that 0.1% of methyl alcohol contained in ethyl alcohol is easily detectable by xanthation followed by paper chromatography and we have used the electrophoretic method for similar demonstrations.

Experiments to show that some commercial samples of *tert.*-butyl and *tert.*-amyl alcohols contain the isomeric primary or secondary alcohols as impurities have already been described. α -Terpineol was commonly found to contain an alcoholic impurity which reacted readily to form a xanthate with a mobility identical with that of geraniol.

Ethylene glycol and diethylene glycol (Digol) were similarly shown to be impurities of some samples of the glycol ethers, Methyl and Ethyl Carbitol (compounds 22 and 23). The di-xanthates of the glycols, having relative mobilities ($M_N \times 100$) of 156 and 140, respectively, separated easily from each other and from the xanthates of the glycol ethers.

Further proof of the identities of the impurities was obtained by subjecting the crude glycol ether samples to xanthation conditions in which carbon disulphide was present in quantities insufficient to allow the reactions to go to completion. Mixtures of the mono- and di-xanthates of each of the glycol impurities were then formed together with the xanthates of the glycol ethers. The mono-xanthates of ethylene glycol and Digol have relative mobilities ($M_N \times 100$) of 112 and 97, respectively, and separations of all five xanthates contained in each reaction mixture were achieved under the standard conditions of electrophoresis.

It may be noted that the mobility of the derivative obtained from ethylene glycol by BERBALK⁹ corresponds to that of the mono-xanthate.

Identification of alkoxy groups of esters

The procedure given in the Experimental section was adopted after it was found that, under the ordinary conditions of the xanthate reaction, alkaline hydrolysis of esters occurred to a limited extent only, although no special precautions were taken against the entry of moisture. Insufficient amounts of the component alcohols were liberated to form xanthates in quantities easily detectable after electrophoresis. The behaviour of diethyl oxalate was exceptional in this respect, however. It reacted vigorously with the solid potassium hydroxide and the mixture was found to

TABLE II

RELATIVE RATES OF MIGRATION OF XANTHATE IONS DERIVED FROM ESTERS

The standard conditions of electrophoresis given in Table I were used.

<i>Esters</i>	$M_N \times 100$	
Dimethyl succinate	143	(154) ^a
Ethyl acetate	122	(141)
Ethyl benzoate	122	(104)
Diethyl oxalate	122	(200)
Ethyl β -hydroxy-butyrate	122, 150	(104)
Dibutyl tartrate	100	(151)
Amyl acetate	88	(141)

^a Values in parentheses are relative mobilities of acid anions corresponding to the respective ester acyl groups.

contain a high concentration of ethyl xanthate along with potassium oxalate.

Alkaline hydrolysis of esters is known to proceed extremely rapidly in aqueous dimethyl sulphoxide^{34, 35}, and brief pre-treatment of esters with potassium hydroxide in dimethyl sulphoxide followed by the addition of carbon disulphide resulted in the formation of xanthates in the required amounts. As indicated in Table II, each ester yielded the expected alkyl xanthate. Ethyl β -hydroxybutyrate gave both ethyl and 2-carboxy-1-methylethyl xanthates, the latter being derived from the free β -hydroxy acid anion.

The relative mobilities of the acid anions corresponding to the acyl groups of the esters are also listed in Table II. When required, acid anions and alkyl xanthates were located simultaneously on pherograms by selecting spray reagents reactive to both species. The method thus affords simultaneous identification of the component alcohols and acids of esters and offers an alternative to standard procedures³⁶ which involve the formation, in separate tests, of appropriate crystalline derivatives.

The conditions of hydrolysis of the esters and xanthation of the carbohydrate derivatives were similar in that both were conducted in dimethyl sulphoxide containing potassium hydroxide. Valid results were therefore not expected from experiments performed to detect the presence of free hydroxyl groups in carbohydrate esters and it was found that some hydrolysis of carbohydrate benzoates, acetates and carbonates occurred during xanthation in dimethyl sulphoxide. Compounds 27-31 and 36 contain glycosidic, ether, cyclic acetal and cyclic ketal bonds and each formed the expected mono-xanthate only. Evidently these bonds are stable under the prescribed conditions and valid conclusions may be drawn from xanthate tests performed on compounds containing them.

Inorganic ions

Carbonate and some sulphur-containing anions are always present in aqueous solutions of xanthates¹⁰. A summary of results of the electrophoresis of these and related anions in NaHCO_3 solution is given in Table III.

Trithiocarbonate (CS_3^{2-}) and sulphide form as byproducts during xanthation of alcohols by reaction of carbon disulphide with the alkali metal hydroxide^{37, 38}. Alkaline solutions of xanthates allowed to stand unprotected from air slowly accumulate thiosulphate, which arises, at least in part, by direct oxidation of sulphide. The

TABLE III

RELATIVE RATES OF MIGRATION OF SULPHUR-CONTAINING INORGANIC ANIONS
The standard conditions of electrophoresis given in Table I were used.

Anion	$M_N \times 100$
Trithiocarbonate	257
Thiosulphate	249
Sulphide	249
Sulphate	209
Trithiopercarbonate	207
Tetrathionate	206
Sulphite	185

presence of these ions in dilute xanthate preparations was demonstrated after their rapid electrophoretic separation from the organic derivatives. Sulphide and thiosulphate have identical mobilities, not only in NaHCO_3 solution but also in sodium carbonate, sodium hydroxide and acetate buffer at pH 6 (compare Gross³⁰). Owing to its volatility, the detection of sulphide on pherograms at the lower values of pH was accomplished only by the immediate application of nitroprusside to the papers while they were still wet with the electrolyte. When dried, the same papers were oversprayed with chromium trioxide–permanganate–sulphuric acid for the detection of thiosulphate. The identity of thiosulphate was established on other pherograms which were dried in the ordinary way and subjected to the specific test for thiosulphate described in the Experimental section. The test was performed without delay because other sulphur-containing ions, including the xanthates, slowly formed thiosulphate on the papers in the presence of atmospheric oxygen and moisture.

INGRAM AND TOMS¹⁸ noted, by means of ultraviolet absorption spectroscopy, that trithiocarbonate in aqueous solution in contact with air was converted to another ion which they considered to be trithiopercarbonate (COS_3^{2-}). We have followed this conversion by paper electrophoresis under the standard conditions. Fresh solutions of trithiocarbonate yielded a single, yellow, comet-shaped spot, the "tail" being formed, probably, by partial conversion to the other anion during electrophoresis. Solutions exposed to air for several hours prior to electrophoresis were resolved into two yellow spots, the faster-moving of which was due to trithiocarbonate. The spots were always connected by a faint yellow streak. The anion responsible for the slower-moving spot became preponderant in older solutions of trithiocarbonate and was probably identical with that described by INGRAM AND TOMS as trithiopercarbonate. It has been designated as such in Table III. Further exposure of the solutions to air resulted in the gradual disappearance of the latter ion with simultaneous increase in the concentration of thiosulphate. After three days, thiosulphate was the only anion detected.

Trithiocarbonate and trithiopercarbonate were shown to be present in solutions of xanthates along with sulphide and thiosulphate. The four ions occurred in proportions which varied according to method of preparation, concentration and age of the samples and the changing pattern of spots observed with aging solutions was consistent with the foregoing discussion of the reactivities of the ions. The transformation of trithiocarbonate to trithiopercarbonate in highly alkaline xanthate solutions was slower than that noted in aqueous solutions of the pure salt. This is in agreement with

the work of INGRAM AND TOMS, who showed that the rate of conversion varied inversely with the concentration of sodium hydroxide.

The electrophoresis of trithiocarbonate and trithiopercarbonate does not appear to have been described previously. The order of separation of the other anions given in Table III corresponds with that recorded by other workers using different electrolytes³⁰⁻⁴³.

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