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DETECTION OF METHOXYAROMATIC ACIDS IN THE OXIDATION PRODUCTS OF COALS AND HUMIC ACID BY GAS–LIQUID CHROMATOGRAPHY

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SUMMARY

The method suggested is based on differences in relative retention times for hydroxy- and methoxyaromatic acid esters.

A comparison of gas–liquid chromatographic patterns for the products of destructive oxidation before and after their demethylation enables the presence of methoxy acids to be established by the shift of certain peaks and preliminary data about their structures to be obtained.

INTRODUCTION

Methods of destructive oxidation are widely used for investigating the chemical structures of lignin, humic acids and coals. The degradation products of these substances usually consist of a mixture of water-soluble aliphatic and aromatic polycarboxylic acids. The composition of these products is particularly complex when phenolic hydroxyl groups, present in the initial material, are previously protected by methylation. In this case, various methoxyaromatic acids are contained in the oxidation products in addition to the above compounds. Their detection and identification provide very valuable data on the relative positions of hydroxyl groups in the cyclic molecules of the initial substance.

Even when gas–liquid chromatography (GLC) is used, an investigation of this kind is difficult. Gas–liquid chromatograms of the oxidation products contain up to 50 peaks for individual compounds, and the identification of each peak for detecting methoxy acids is too difficult. We have therefore devised a procedure to overcome the difficulties.

The experimental data on the relative retention times (RRT) of methyl esters of aromatic polycarboxylic acids and their hydroxy and methoxy derivatives are the basis of the procedure. It is apparent from Table I that there is a great difference in the RRT of esters with free and methylated hydroxyl groups. To detect methoxy acids in the mixture, the following operations are sufficient: (a) GLC separation of the esters of oxidation products; and (b) GLC separation of the demethylated substances after their esterification.

TABLE I

RELATIVE RETENTION TIMES OF AROMATIC ACID ESTERS

No.	*Acid*	*RRT*	*No.*	*Acid*	*RRT*
1	Benzoic	0.40	20	Terephthalic	1.18
2	*o*-Hydroxybenzoic	0.50	21	Hydroxyterephthalic	1.70
3	*m*-Hydroxybenzoic	0.90	22	Methoxyterephthalic	2.15
4	*p*-Hydroxybenzoic	0.97	23	4-Hydroxy-5-methylisophthalic	2.15
5	*o*-Methoxybenzoic	0.70	24	4-Methoxy-5-methylisophthalic	3.40
6	*m*-Methoxybenzoic	0.70	25	Trimellitic (1,2,3)[a]	3.40
7	*p*-Methoxybenzoic	0.75	26	3-Hydroxytrimellitic	8.10
8	*o*-Phthalic (1,2)[a]	1.00	27	3-Methoxytrimellitic	6.70
9	3-Hydroxy-*o*-phthalic	1.30	28	5-Hydroxytrimellitic	5.00
10	4-Hydroxy-*o*-phthalic	3.00	29	5-Methoxytrimellitic	7.30
11	3-Methoxy-*o*-phthalic	2.00	30	Trimesic (1,3,5)[a]	4.48
12	4-Methoxyphthalic	2.20	31	Hydroxytrimesic	7.20
13	Isophthalic (1,3)[a]	1.18	32	Methoxytrimesic	6.30
14	2-Hydroxyisophthalic	2.57	33	Prehnitic (1,2,3,4)[a]	10.80
15	4-Hydroxyisophthalic	1.70	34	5-Hydroxyprehnitic	16.20
16	5-Hydroxyisophthalic	3.80	35	5-Methoxyprehnitic	25.30
17	2-Methoxyisophthalic	2.05			
18	4-Methoxyisophthalic	2.75			
19	5-Methoxyisophthalic	2.56			

[a] Positions of carbomethoxy groups.

The presence of certain peak shifts in the chromatograms shows that these peaks belong to methoxy acid esters.

It can be seen from Table I that the number of carboxylic groups is the main factor determining the retention parameters of aromatic acid esters. The introduction of hydroxyl (methoxyl) groups increases the RRT of the corresponding esters. However, the methyl ester peaks of monohydroxy (methoxy) acids of a particular basicity are arranged on the chromatograms between the peaks of acids with different basicities. Thus all hydroxy and methoxy derivatives of benzoic acid appear after methyl benzoate and before dimethyl phthalate; esters of hydroxy- and methoxyphthalic acids appear after dimethyl phthalate and earlier than the esters of tricarboxylic acids, and so on.

The methyl ester of 5-hydroxyisophthalic acid is the only exception among the compounds examined, its retention time being anomalously high.

A hydroxyl group introduced into an aromatic acid ester molecule influences the RRT to various degrees, depending on its position with respect to the carbomethoxy group. The RRT of *o*-hydroxy acid esters increases to a lesser extent than those of *m*- and *p*-isomers. Hydroxyl group methylation causes a further RRT increase for *o*-hydroxy acids, and a corresponding decrease for *m*- and *p*-hydroxy acid esters.

Similar data on retention parameters for the esters of hydroxybenzoic acids have been considered in detail by previous workers[1,2], and we confirmed these data for polycarboxylic acid derivatives. Polar interactions with a stationary phase for esters of aromatic acids undoubtedly have a considerable influence on retention parameters, and for hydroxy acids this influence becomes a determining factor. If the RRT obtained is considered from this viewpoint, it can be noticed that the esters of an *o*-acid, of which the polarity of the molecule is lowered due to a strong

intramolecular hydrogen bond, have shorter RRT than *m*- and *p*-isomers. Methylation of hydroxyl groups is accompanied by a sharp decrease in the polarity of *m*- and *p*-hydroxy acid molecules, causing a considerable fall in their RRT, and the esters of methoxy acids obtained leave the column before the initial products.

An interesting deviation from this dependence is observed for the esters of acids in which the hydroxyl group is situated between two carbomethoxy groups (*e.g.*, 2-hydroxyisophthalic and hydroxytrimesic acids.) For these compounds, methylation of the hydroxyl groups causes a decrease in RRT, although the presence of a stronger hydrogen bond, in comparison with more usual *o*-hydroxy acids, is confirmed by the IR and NMR spectra (Table II). Their chromatographic behaviour cannot be explained satisfactorily in terms of the above concepts of relative molecular polarity, and we intend to discuss this problem in detail elsewhere.

TABLE II

CHARACTERISTICS OF H-BONDS OF HYDROXYL GROUPS IN HYDROXY AROMATIC ACID ESTERS

Acid	*Energy of H-bonds according to IR spectral data*[a] (*kcal/mole*)	*Chemical shifts*[b] (*p.p.m.*)
2-Hydroxyisophthalic	5.7	11.23
4-Hydroxyisophthalic	5.3	10.87
Hydroxytrimesic	6.2	11.55
5-Hydroxytrimellitic	5.3	10.74

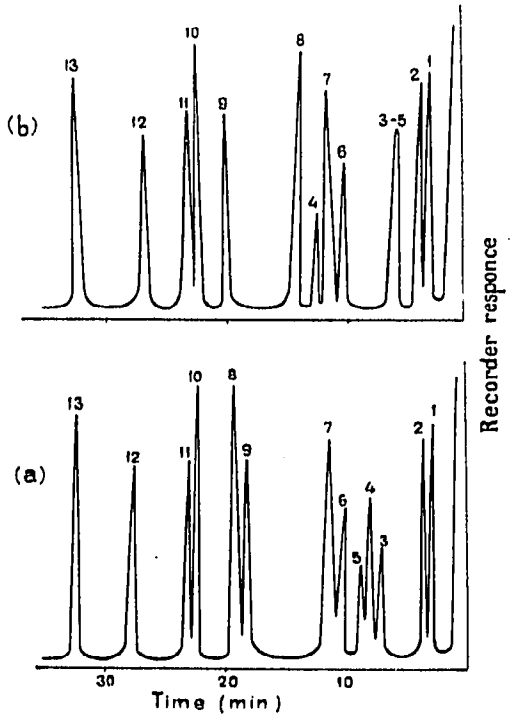
[a] Calculated by the formula[6]: $H = 1/K \cdot \Delta\delta/\Delta\delta_0$ cm.
[b] In carbon tetrachloride; taken on a ZKR, 60-mHz apparatus (G.D.R.).

The ascertained dependence of RRT on the nature and relative positions of substituents in aromatic polycarboxylic acid esters enables not only the presence of methoxy compounds in the mixture under study to be confirmed, but also some additional data on their structures to be obtained.

If demethylation results in the acceleration of the appearance of an unknown component peak, it can be assumed that an *o*-methoxy acid is present. On the other hand, when the peak shifts on the chromatogram towards longer retention times, adjacent hydroxyl and carboxyl positions are excluded, and the component should then be considered to be a *m*- or *p*-methoxy acid. Although this dependence does not apply to acids with a hydroxyl group situated between two carboxyl groups (see above), nevertheless, the products of the oxidative degradation of coals, humic acids and lignin have hitherto yielded only one representative of this type, namely hydroxytrimesic acid.

Under the given chromatographic conditions, aromatic acid esters with two or more hydroxyl groups possessed long RRT and were fully retained in the columns. Hence, the disappearance of some peaks on the chromatograms after demethylation could be regarded as a direct indication of the presence of polyhydroxy acids. All the above conclusions were fully corroborated by demethylation experiments with standard acid mixtures, as well as with methylated humic acid oxidation products.

Fig. 1 shows chromatograms of a mixture of aromatic carboxylic and methoxycarboxylic acid esters before and after demethylation, while Fig. 2 shows analogous results for the oxidation product of methylated humic acids of lignite.



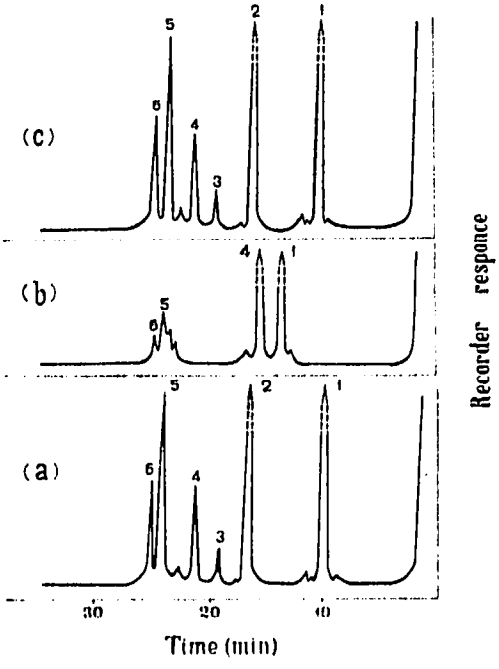


Fig. 1. Gas–liquid chromatograms of (a) a mixture of carboxylic and methoxycarboxylic acids, and (b) the same mixture after demethylation and esterification. Peaks: 1 = *o*-phthalic; 2 = terephthalic; 3 = methoxyterephthalic; 4 = 5-methoxyisophthalic; 5 = 4-methoxyisophthalic; 6 = hemimellitic; 7 = trimellitic; 8 = 5-methoxytrimellitic; 9 = methoxytrimesic; 10 = prehnitic; 11 = mellophanic; 12 = 5-methoxyprehnitic; 13 = mellitic acids and esters.

Fig. 2. Gas–liquid chromatograms of the methyl esters of the lignite humic acid oxidation products: (a) the ether extract of the oxidation product; (b) the same as (a) after demethylation and esterification; (c) the same as (b) after exhaustive methylation with diazomethane. Peaks: 1 = *p*-hydroxybenzoic; 2 = veratric; 3 = 3,4,5-trimethoxybenzoic; 4 = 4-methoxyisophthalic; 5 = isohemipinic; 6 = metahemipinic acids and esters.

Demethylation and subsequent esterification of standard samples did not change the position and form of peaks 1, 2, 6, 7, 10, 11 and 13, belonging to carboxylic acids. At the same time, the peaks for 2-methoxyterephthalic and 4-methoxyisophthalic acid disappeared, and one unresolved peak of their hydroxy derivatives appeared. Instead of the 5-methoxyisophthalic acid peak, another peak of the corresponding hydroxy acid appeared, lying beyond the tricarboxylic acid range.

The time for the methoxytricarboxylic acid ester yield changed so that the hydroxytrimellitic acid ester appeared before the initial acid, while the hydroxytrimesic acid ester appeared slightly later. Finally, the methoxyprehnitic acid peak shifted towards shorter RRT.

Three chromatograms were recorded for lignite oxidation products, namely the initial product methylated with diazomethane, the demethylated and subsequently esterified substance and the same substance additionally treated with

diazomethane. The last chromatogram served as a control to detect the presence of side conversions in the material studied during all the treatments.

As shown in Fig. 2, the substance composition was completely regenerated by exhaustive methylation; consequently, the treatment led only to the elimination of methoxy groups and their repeated introduction.

The chromatogram for the demethylated preparation (b) retained only two peaks*, and the introduction of standard substances showed the first to correspond to *p*-hydroxybenzoic acid and the second to 4-hydroxyisophthalic acid. Veratric, trimethoxybenzoic, isohemipinic and metahemipinic acid peaks were not observed on the chromatograms, since esters of the corresponding di- and trihydroxy acids were completely retained in the chromatographic column.

EXPERIMENTAL AND RESULTS

Equipment

The LCh-3-66 "Tsvet" (U.S.S.R.) chromatograph with dual V-shaped stainless-steel columns (100 × 0.3 cm) and flame ionization detectors was used. The columns were packed with Chromosorb-W (treated with DMCS, 60–80 mesh), coated with 5 % SE-30. Determination of the RRT for individual acid esters was carried out under isothermal conditions at 190°. During the separation of the mixture of standard esters and lignite oxidation products (Figs. 1 and 2), the temperature was programmed over the range 100° to 300° at 4°/min. The carrier gas was argon (60 ml/min).

Materials

Aromatic acids were commercial preparations; the purity of the esters obtained from them was tested by determining C, H and OCH_3 and also by chromatography. Hydroxy acid esters were either obtained from commercial acids or synthesized in our laboratory (samples Nos. 18, 19, 21, 24, 28, 29, 31, 32, 34 and 35; see Table I). Their compositions and structures were confirmed by chemical analysis and from IR and NMR spectral data.

Humic acids were isolated from a lignite sample of the Tertiary Age (fossilzed wood). They were subjected to exhaustive methylation with dimethyl sulphate in alkaline medium, and then reduced with sodium borohydride in a borate buffer solution with subsequent secondary methylation under the same conditions. The methylated product was then oxidized with alkaline potassium permanganate solution, and the resultant water-soluble acids were extracted with diethyl ether. The details of these experiments will be given elsewhere. For GLC examination, the acids were converted into esters by repeatedly treating the methanolic solution with diazomethane–ether solution.

Demethylation

Several methods of demethylation were tested (treatment with hydrogen bromide, pyridine hydrochloride, sodium in hexamethyl triamidophosphate, etc.), but the use of anhydrous aluminium bromide produced the best results as regards both completeness of demethylation and the absence of by-products. The reaction

* The small residual peaks 5 and 6 remained, apparently because of incomplete demethylation.

was carried out with a finely milled sample in a benzene solution. The mixture was boiled for 1 h and decomposed with hydrochloric acid after cooling. The reaction products were extracted from the acid aqueous solution with ethyl acetate in a liquid–liquid extractor; the solvents (benzene and ethyl acetate) were then distilled off and the residue was dried under vacuum over potassium hydroxide.

Esterification

Considerable difficulties were met with during attempts to convert the demethylation products completely into methyl esters. The esterification of polybasic acids required their repeated treatment with hydrochloric acid containing methanol. However, under these conditions, complete elimination of acid esters was not always possible, and this affected the chromatograms obtained. Methylation could be accelerated by MILDRED's methods[3], but in this case some acids (*e.g.*, benzenepentacarboxylic acid) formed a number of by-products, apparently due to decarboxylation. We eventually chose a methylation method which was a combination of the methods suggested by CLINTON AND LASKOWSKY[4] and BAKER *et al.*[5]. The methylation mixture contained anhydrous methanol (25 ml), dry dichloroethane (75 ml) and conc. sulphuric acid (4 ml). A sample of demethylated acid (50 mg) was added to 5 ml of the mixture and then refluxed on a water-bath. In order to remove the water, the solvent-condensed vapours were passed through a layer of granulated Drierite prior to being returned to the reaction flask. After refluxing for 4–6 h, 5 ml of water were added to the cooled mixture, and the lower layer was separated. The water–methanol solution was extracted two or three times with dichloroethane, and the combined extract was then evaporated. The residue was dissolved in 1–2 ml of methanol and divided into two portions, the first being used for direct chromatography, and the second being pretreated with a diazomethane–ether solution. In the first case, methyl esters of hydroxy acids were separated, while the chromatogram of the second portion corresponded to their methoxy derivatives. The latter chromatogram coincided exactly with the chromatogram of the initial methoxy acid esters and served as a control.

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