Journal of Chromatography, 301 (1984) 273–276
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 16,920

Note

Gas–liquid chromatographic separation of hydroxy monocarboxylic acids and dicarboxylic acids on a fused-silica capillary column

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(Received May 21st, 1984)

Polysaccharides are subjected to end-wise degradation ("peeling") in alkali with formation of hydroxy carboxylic acids. This type of degradation is very important in many technical processes and it is responsible for the carbohydrate losses taking place during alkaline processing of wood[1]. For both mechanistic studies of reactions and practical applications, detailed information about the composition of the hydroxy acid mixtures is essential.

In earlier studies, gas–liquid chromatography (GLC) of the per(trimethylsilylated) (TMS) derivatives and mass spectrometry (MS) have been applied[2–5]. More detailed information including data for dicarboxylic acids has been obtained by a combination of liquid chromatography (LC) on ion-exchange resins with GLC–MS[6,7].

To eliminate multiple peaks in GLC of hydroxy acid lactones, it has been found advantageous to silylate the ammonium salts instead of the free acids[8]. Capillary GLC of the per(trimethylsilylated) ammonium salts has now been applied for the analysis of complicated hydroxy acid mixtures resulting from alkaline degradation of wood polysaccharides.

EXPERIMENTAL

Materials

α-Glucoisosaccharino-1,4-lactone was prepared from lactose[9]. Xyloisosaccharino-1,4-lactone was isolated by vacuum distillation from an alkaline birch wood spent liquor[10]. All the other chemicals were commercial products of an analytical grade or of the highest purity available.

Preparation of TMS derivatives

A liquor sample (0.2 ml) was passed through a column (100 × 10 mm I.D.) filled with weakly acidic cation-exchange resin (Amberlite IRC-50, 35–60 mesh, NH_4^+, 4 ml). The column was then washed with water to obtain an effluent volume of 30–40 ml which was evaporated to dryness under reduced pressure at 35°C. An internal standard had been added either to the effluent (D-xylitol) or to the alkaline liquor sample (D-mannono-1,4-lactone). To the hydroxy acid residue were added 0.5 ml pyridine and 0.25 ml trifluorobis(trimethylsilyl)acetamide (BSTFA) containing

0021-9673/84/$03.00

5% of chlorotrimethylsilane (TMCS)[8], and the mixture was shaken for approximately 30 min at room temperature.

GLC separations

After per(trimethylsilylation), GLC separations were performed on a Hewlett-Packard 5880 A gas chromatograph equipped with an OV-101 fused-silica capillary column (25 m × 0.32 mm I.D.). The temperature program was 2 min at 100°C, 20°C/min to 200°C and 5 min at 200°C. The temperature of both the injection port (containing a split mode insert packed with 3% OV-17 on Chromosorb W) and the flame ionization detector was 260°C. The injection volume was 0.1–0.5 μl and the splitting ratio 20:1. The flow-rate of hydrogen carrier gas was 2 ml/min.

Identification of the acid derivatives

A Hewlett-Packard 5992 instrument (70 eV) fitted with the same column as before was used for GLC–MS identification of the per(trimethylsilylated) samples. The temperature program was 4 min at 100°C, 8°C/min to 200°C and 10 min at 200°C. Interpretation of the mass spectra was based either on published data[11–23] or on the use of model substances. The corresponding retention time data[24] were used to identify diastereomeric compounds.

Determination of response factors

For most compounds the response factors were calculated according to the literature[25,26]. Prior to the determination of the response factors of the pure compounds available (2-hydroxybutanoic, 4-hydroxybutanoic, xyloisosaccharinic, α-glucoisosaccharinic and succinic acids), the solutions of the corresponding acids were first made alkaline with 0.1 *M* sodium hydroxide and thereafter converted into the ammonium ion form as described.

RESULTS

GLC–MS analysis of hydroxy carboxylic acids in spent liquors from the alkaline treatment of pine and birch wood resulted in the identification of about 40 different aliphatic acids (Fig. 1). Although the method applied was capable of resolving all the peaks derived from the major alkaline degradation products of wood carbohydrates, the small peaks of syringaldehyde (present only in birch spent liquors) and glucometasaccharinic acids could not be separated from xyloisosaccharinic acid (peak 27) and β-glucoisosaccharinic acid (peak 40), respectively. Similarly, the separation of 3-deoxy-*erythro*-pentonic acid (peak 28) from 2,3,4-trideoxyhexaric acid (present in almost equal amounts in black liquor)[6] was incomplete. In separate experiments it was also observed that peaks 17 and 42 contain small amounts of glutaric and 3-deoxyhexaric acids, respectively. In some cases the peaks derived from both diastereomers of 3,4-dideoxyhexonic acid (peak 35) and galactometasaccharinic acid (peak 39) overlapped.

The calculated response factors differed by less than 10% from those determined experimentally (when available, see Fig. 1) and from the literature data[8].

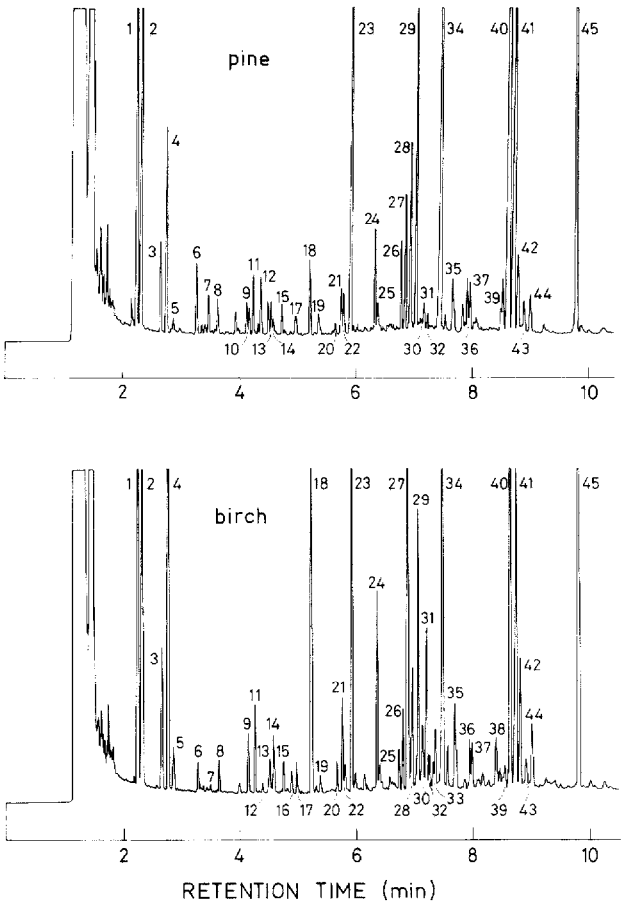

Fig. 1. Separation of per(trimethylsilylated) products formed in the alkaline treatment of pine and birch wood. The molar response factors given in parentheses have been calculated in relation to xylitol. The data marked with an asterisk have been determined experimentally. Aliphatic hydroxy monocarboxylic acids: 1 = 2-hydroxypropanoic (lactic) (0.41); 2 = glycolic (0.37); 4 = 2-hydroxybutanoic (0.47, 0.52*); 5 = 3-hydroxypropanoic (hydracrylic) (0.42); 6 = 2-hydroxypentenoic (0.51); 8 = 4-hydroxybutanoic (0.47, 0.51*); 13 = 2-C-methylglyceric (0.61); 14 = glyceric (0.56); 18 = 3-deoxytetronic (2,4-dihydroxybutanoic) (0.62); 19 = 2-deoxytetronic (3,4-dihydroxybutanoic) (0.62); 23 = 3,4-dideoxypentonic (2,5-dihydroxypentanoic) (0.67); 26 = anhydroisosaccharinic (1,4-anhydro-3-deoxypentitol-2-carboxylic) (0.72); 27 = xyloisosaccharinic (3-deoxy-2-C-hydroxymethyltetronic) (0.76, 0.77*); 28 = 3-deoxy-*erythro*-pentonic (0.81); 29 = 3-deoxy-*threo*-pentonic (0.81); 30 = 3,6-dideoxy-ribo-hexonic (0.86); 31 = 3,6-dideoxy-arabino-hexonic (0.86); 35 = 3,4-dideoxy-(*erythro*- and *threo*-)hexonic (0.87); 39 = β- and α-galactometasaccharinic (1.01); 40 = β-glucoisosaccharinic (3-deoxy-2-C-hydroxymethyl-*threo*-pentonic) (1.01); 41 = α-glucoisosaccharinic (3-deoxy-2-C-hydroxymethyl-*erythro*-pentonic) (1.01, 0.95*); 45 = mannonic (internal standard) (1.15). Aliphatic dicarboxylic acids: 3 = oxalic (0.32); 10 = maleic (0.43); 11 = succinic (0.43, 0.40*); 12 = methylsuccinic (0.49); 15 = C-methyltartronic (0.51); 17 = tartronic (hydroxymalonic) (0.52); 20 = 2-deoxy-3-C-methyltetraric (citramalic) (0.63); 21 = deoxytetraric (malic) (0.57); 24 = 2,3-dideoxypentaric (2-hydroxyglutaric) (0.63); 25 = 2,4-dideoxypentaric (3-hydroxyglutaric) (0.63); 28 = 2,3,4-trideoxyhexaric (2-hydroxyadipic) (0.69); 32 = 3-deoxy-*threo*-pentaric (0.77); 36 = 3,4-dideoxy-*threo*-hexaric (*threo*-2,5-dihydroxyadipic) (0.83); 37 = 3,4-dideoxy-*erythro*-hexaric (*erythro*-2,5-dihydroxyadipic) (0.83); 42 = β-glucoisosaccharinaric (3-deoxy-2-C-hydroxymethyl-*threo*-pentaric) (0.97); 43 = C-(2,3-dihydroxypropyl)tartronic (0.97); 44 = α-glucoisosaccharinaric (3-deoxy-2-C-hydroxymethyl-*erythro*-pentaric) (0.97). Others: 7 = guaiacol (2-methoxyphenol); 9 = glycerol; 16 = syringol (2,6-dimethoxyphenol); 22 = vanillin (4-hydroxy-3-methoxybenzaldehyde); 33 = acetosyringone (3,5-dimethoxy-4-hydroxyacetophenone); 34 = xylitol (internal standard) (1.00); 38 = syringic acid (3,5-dimethoxy-4-hydroxybenzoic acid).

ACKNOWLEDGEMENTS

Financial support from the Ministry of Trade and Industry is gratefully acknowledged. Thanks are also due to Mr. Erkki Repo for his assistance with experimental work. The mass spectra were recorded at the Technical Research Center of Finland.

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