STUDIES ON GRASS LIGNINS

II. THE ESTIMATION OF LIGNIN OXIDATION PRODUCTS BY GAS–LIQUID CHROMATOGRAPHY

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(Received August 22nd, 1966)

INTRODUCTION

Since the publication of the TLC method for the separation and estimation of *p*-hydroxybenzaldehyde (P), vanillin (V) and syringaldehyde (S)[1] it has become apparent that this method is not widely applicable for the quantitative estimation of these three phenolic aldehydes in a lignin oxidation mixture. The grass materials chosen for investigation by the TLC method were a particularly fortuitous choice as the oxidation mixtures were low in the phenolic ketones, *p*-hydroxyacetophenone (Po), acetovanillone (Vo) and acetosyringone (So). These ketones have R_F values very similar to their respective aldehydes in the solvent system previously described[1] and interfere with any quantitative work. A GLC method has now proved to be widely applicable for the quantitative estimation of these six products in a lignin oxidation mixture.

GLC was first used in lignin chemistry for the identification and separation of various phenols in the volatile fraction of white birch soda lignin by SOBOLEV AND SCHUERCH[2] in 1958. Many hydrogenolysis products of lignin have since been separated and identified by GLC[3–5].

The GLC separation of vanillin and related compounds, of interest to flavour chemists, has been described by a number of workers[6–12].

The two stationary phases most widely used for the separation of phenolic substances have been Carbowax 20 M[8–11] and SE-30[5,8,10,12–14]. It is generally accepted that polar compounds are best resolved by polar stationary phases, but in the author's experience, as well as that of VON RUDLOFF[14], Carbowax 20 M columns generally give broad tailing peaks for phenolic substances. SE-30 on the other hand gives sharp symmetrical peaks but resolution is often poor. PEPPER, MANOLOPOULO AND BURTON[15], using Apiezon N on Fluoropak, were able to determine quantitatively P, V, S and Vo in the oxidation products of the pre-extracted meals and the isolated lignins of aspen wood, spruce wood and wheat straw. Attempts by the author to use such a column did not prove successful as the peaks obtained were too broad.

This paper describes a temperature-programmed GLC method, using SE-30 as the stationary phase, which has been effective for the resolution of P, Po, V, Vo, S and So in a manner suitable for quantitative work.

EXPERIMENTAL

Packing the column

The column packing material was prepared by the evaporation technique. Chromport XXX (100/120 mesh) was added to a solution of SE-30 in chloroform in the ratio 100:30 (w/w) and the chloroform evaporated on a water-bath while the mixture was gently rotated. Equal weights (8.5 g) of the dried support were packed by suction into two coiled copper columns (6 ft. × 0.25 in. O.D.) with gentle tapping. Both columns were packed at the same time using the same vacuum source and were subsequently found to give equal flow rates under the same conditions.

GLC conditions

The following conditions gave satisfactory resolution of P, Po, V, Vo, S and So for quantitative work.

Instrument: Beckman GC-2A and Thermotrac temperature programmer.

Column: dual; 30 % SE-30 on Chromport XXX (100/120 mesh), copper 6 ft. × 0.25 in. O.D.

Carrier gas: helium.

Carrier gas inlet pressure: 10 p.s.i.g.

Carrier gas flow rate: 62 ml/min at 100°, 42 ml/min at 260°.

Thermal conductivity detector temperature: 190°.

Inlet and exhaust line temperature: 250° and 230°, respectively.

Filament current: 200 mA.

Chart speed: 1.5 in./min.

Program: 100° for 30 min; 100–180° in 2 min; 180–260° in 6 min.

Quantitative calibration

A standard solution containing 0.25 % of each of the components, P (recrystallized), Po (Koch-Light; pure), V (recrystallized), Vo (Koch-Light; pure), S (Fluka; purum CHR) and So (Koch-Light; pract), in ethanol was prepared. A number of separate injections of this solution, in the range 12.5–150 μg of each substance, were made, and calibration curves of peak height versus concentration were established.

Oxidation of grass hay

Grass hay (60 mesh) consisting mainly of *Themeda triandra* was extracted with ethanol–benzene (1:2) for 8 h. Air-dried samples (0.5 g) of the pre-extracted hay together with 1.7 g $CuSO_4 \cdot 5H_2O$, 10 ml 3 *N* NaOH and a helical copper rod were added to each of five stainless steel tubes (18 ml) equipped with stainless steel screw tops. The motion of the copper rod during the ensuing oxidation ensured rapid and complete mixing of the solution. The tubes were well shaken and heated in an oscillating aluminium block at 180° for 2.5 h. The warm-up time required to heat the tubes and block to 180° was 30 min.

After the heating period the tubes were cooled rapidly and the oxidation mixtures transferred to centrifuge tubes and spun at 1500 × *g* for 10 min in a refrigerated centrifuge. The chilled supernatant solutions were combined and acidified to pH 4 with conc. HCl, saturated with $(NH_4)_2SO_4$, and filtered on a Buchner funnel.

The filtrate was extracted three times with 5 ml chloroform and the extracts were combined. The chloroform extract was evaporated on a water-bath at approximately 50°, under a stream of air, until about 0.5 ml remained. This remaining solution was evaporated at room temperature so that the final residue was cold. This precaution was introduced to minimize any loss of the more volatile components. The residue was immediately taken up in 1 ml ethanol and 15 μl samples were injected into the gas-chromatograph.

TABLE I

YIELD OF ALKALINE CUPRIC HYDROXIDE OXIDATION PRODUCTS OF PRE-EXTRACTED GRASS HAY

Compound	*Yield (μg/15 μl injection)*			*Individual components as % of total*		
	1	*2*	*3*	*1*	*2*	*3*
P	28	26	26	18	19	18
Po	15	13	14	9	9	10
V	71	62	63	45	45	43
Vo	13	11	13	8	8	9
S	21	18	19	13	13	13
So	10	9	10	6	6	7
Total	158	139	145			

Three separate samples of the pre-extracted grass hay were oxidised and duplicate injections of each sample were quantitated by peak height measurement. The results are presented in Table I and represent the means of duplicate injections.

Chromatograms of a standard solution of P, Po, V, Vo, S and So and of the lignin oxidation products from the grass hay are presented in Figs. 1 and 2.

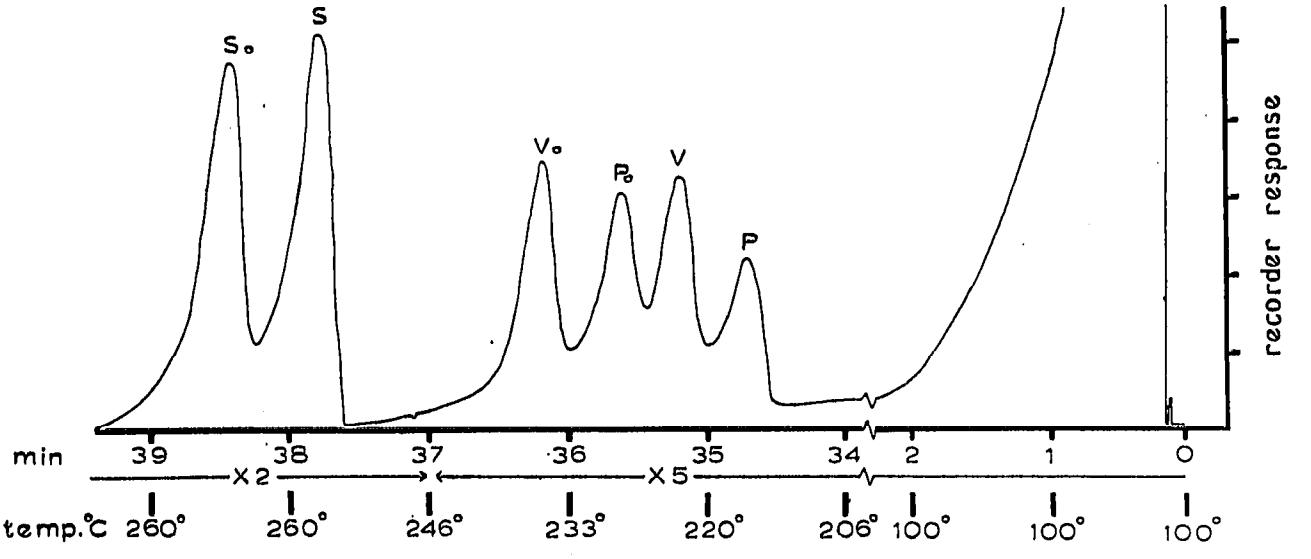

Fig. 1. Chromatogram of standard solution of *p*-hydroxybenzaldehyde (P), *p*-hydroxyacetophenone (Po), vanillin (V), acetovanillone (Vo), syringaldehyde (S) and acetosyringone (So): 50 μg each (conditions in text).

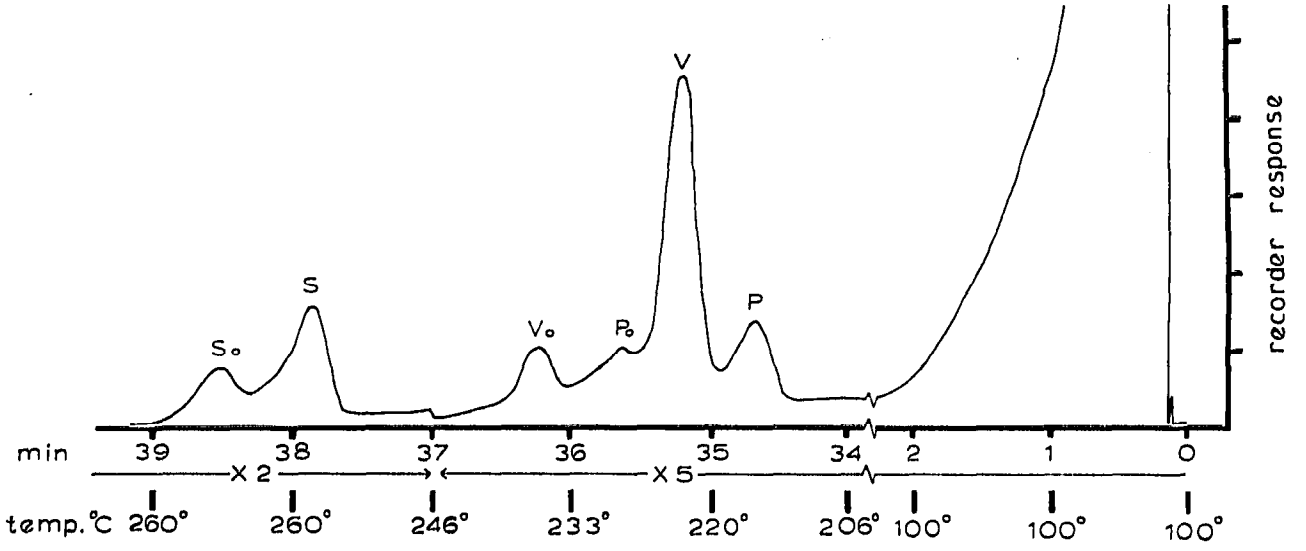

Fig. 2. Chromatogram of alkaline cupric hydroxide oxidation products of grass hay (conditions in text).

DISCUSSION

Attempts to use an Apiezon N on Fluoropak column[15], Carbowax 20 M columns, and columns packed with a mixture of Carbowax 20 M and SE-30 for the quantitative estimation of P, Po, V, Vo, S and So were unsuccessful. A column of 30 % SE-30 on Chromport XXX, packed with a support prepared by the evaporation technique, was found to be the most suitable for the separation of the six substances. A similar column, packed with a support prepared by the slurry filtration method[16] did not give as good a separation using the same GLC conditions. This is merely a statement of what was found and is not a criticism of the slurry filtration method.

Temperature programming was necessary for the production of a chromatogram in which all six substances had satisfactory retention times. The rather unusual program using a long, low-temperature, iso-thermal period was found to be necessary for the effective resolution of V and Po. A chromatogram showing the separation obtained with a standard mixture of the six compounds is presented in Fig. 1.

The six major peaks obtained in the chromatogram of the grass extract (Fig. 2) were identified on the basis of their retention times which were identical to those of the six pure compounds. Also, the major components of the ethanolic grass extract were resolved by two-dimensional TLC using hexane–isoamyl alcohol–acetic acid (100:16:0.25)[1] in the first dimension, and benzene–acetic acid (9:1)[17] in the second. The plates, when sprayed with 2,4-dinitrophenylhydrazine[1], gave six major spots corresponding in position and colour to a mixture of P, Po, V, Vo, S and So.

The extraction of P, V and S[1] and Po, Vo and So[18] from saturated $(NH_4)_2SO_4$ solution by chloroform, as described under Experimental, has been shown to be quantitative.

The results in Table I indicate that the total yield of P, Po, V, Vo, S and So from pre-extracted grass hay varies from one oxidation to another. However, when the yield of each substance is expressed as a percentage of the total yield, there is good

agreement between the results obtained with separate oxidations. The problem therefore lies in the non-reproducibility of the oxidation procedure rather than in the recovery of the resulting products. As the total yield of these substances, with the oxidation method employed, accounts for less than 4 % of the lignin present, the relative amounts obtained afford a better basis, than do the absolute amounts, for comparative studies.

After a number of analyses a black carbonaceous deposit was found at the entrance to the column and when the column was unpacked it was found that particles of the packing material had stuck together to form a thin film along its periphery. In spite of this condition, while the column was in constant use, resolution of the six compounds was satisfactory, but removal of the column from the instrument caused a marked deterioration in peak shape obtained in subsequent analyses, particularly of P, thereby rendering it unsuitable for re-use.

SUMMARY

A GLC method for the separation and quantitative estimation of *p*-hydroxybenzaldehyde, *p*-hydroxyacetophenone, vanillin, acetovanillone, syringaldehyde and acetosyringone, produced during the alkaline copper hydroxide oxidation of grass lignin is described. Using a six foot 30 % SE-30 column on Chromport XXX all six of the compounds can be quantitatively estimated in the range 12.5–150 μg with a thermal conductivity detector.

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