THE DETERMINATION OF POLYSACCHARIDES IN FULVIC ACIDS BY
PYROLYSIS GAS CHROMATOGRAPHY

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SUMMARY

Pyrolysis gas chromatography is used to show that fulvic acids isolated from a variety of soils contain polysaccharides. Acetaldehyde, furan, methylfuran and furfural are the major products of pyrolysis at 700°C and they are demonstrably derived from the carbohydrate moieties.

Although different purification processes have not entirely eliminated all impurities from the fulvic acids it has nevertheless been possible to determine their polysaccharide contents quantitatively. It should be noted that the pyrolysis residues still contain the majority of the nitrogen present in the original sample.

INTRODUCTION

The presence of polysaccharides in humic substances has been variously ascribed to them being an integral part of the system or alternatively merely present as impurities. The methods (mainly colorimetric) used to determine the carbohydrate content are far from accurate and in consequence there is a wide variation in the results obtained.

There have been many tentative attempts to reduce or eliminate this polysaccharide presence in humic fractions, Forsyth (1) adsorbed fulvic acid extracts on animal charcoal and succeeded in removing most of the polysaccharide; Jacquin (2), by repeated precipitation, obtained humic acids purportedly free of polysaccharide and Roulet et al. (3) used gel filtration and ion exchange chromatography to reduce the carbohydrate contents of humic and fulvic acids from 25% to 5%. With more drastic methods, such as hydrolysis, it is possible to reduce the

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amount of polysaccharide present as was demonstrated by Forsyth (4) with water and Cheshire et al. (5) with acid hydrolysis.

The reason for the difficulty to free humic substances from polysaccharides lies in the fact that the extraction procedures used to obtain both components from soil organic matter are very similar; the same reagents are normally employed and it is not easy to obtain extracts in which one component is completely free of the other.

In this paper the detection and determination of polysaccharides in three different soils by means of pyrolysis gas chromatography is discussed and at the same time the technique has been used as a method of evaluating the efficiencies of methods of reducing the polysaccharide content.

EXPERIMENTAL

Fulvic acids were extracted from three soils, Podzol, Andosol and Vertisol, the principal characteristics of which are respectively described by Martin (6), Fernandez and Tejedor (7) and Vila et al. (8).

The extracts and extraction procedures were as follows:

- FA-1 Extract from the B_h horizon of a Podzol with 0.5N sodium hydroxide as described by Martin (9).
- FA-2 Extract from the B_h horizon of a Podzol with 0.5N hydrochloric acid according to Martin (9).
- FA-3 Extract from the A_{11} horizon of an Andosol with 0.5N sodium hydroxide as in FA-1.
- FA-4 Extract from the A_{11} horizon of an Andosol with 0.5 N hydrochloric acid as in FA-2.
- FA-5 Extract from the Ap_1 horizon of a Vertisol with 0.5N sodium hydroxide and purified according to Forsyth (1).
- FA-6 Extract from the Ap_1 horizon of a Vertisol with 0.5N sodium hydroxide. The alkaline solution was centrifuged, acidified with hydrochloric acid, centrifuged again to separate the coagulate and adsorbed on Polyclar AT. The adsorbent plus the adsorbate was placed in a dialysis bag and dialysed until free of chloride ion. The fulvic acid was subsequently desorbed with 0.5N sodium hydroxide and the alkaline solution ion exchanged and lyophilised

in the same way as the other five extracts.

Pyrolysis conditions were as described elsewhere. Gel filtration on Sephadex G-50 was performed after dissolving the fulvic acid in water and using water as eluent. Oxidation was at 170°C in the manner of Mazumdar et al. (11). Finally, the soil polysaccharides were obtained by acetone precipitation from the fulvic acid solutions using the method described by Rennie et al. (12).

RESULTS AND DISCUSSION

Table 1 shows the elementary composition of the six fulvic acids as calculated for ash-free substances. The values are in accord with those presented in the literature for this fraction of humic substances (13,14).

Table 1
Elementary composition (%) of FA

Sample	C	H	N	O
FA-1	45.38	3.41	1.83	49.38 (by difference)
FA-2	43.74	3.75	1.28	51.23
FA-3	40.57	4.11	1.44	53.88
FA-4	39.65	4.09	1.14	55.12
FA-5	31.73	4.65	0.89	62.73
FA-6	44.44	3.57	0.78	51.21

Fig. 1 shows the pyrogram obtained from FA-4, the majority of the peaks have been identified (9); many correspond to alkanes and olefines but the major peaks are typical of the products of soil polysaccharide fragmentation (Fig. 2). The patterns observed in both these pyrograms are very similar and the similarity applies to the pyrograms of all the fulvic acids herein studied, irrespective of the soil and the extraction procedure used, such differences as exist are only quantitative.

Peaks designated A, B, D and E have been identified as acetaldehyde, furan, 2-methylfuran and furfural respectively, but peak C has not been positively identified.

Fig. 1. Pyrogram of FA-4.

Fig. 2. Pyrogram of soil polysaccharides.

Fig. 3 shows histograms of the four positively identified peaks expressed as g/g. As a general rule the production of acetaldehyde is greater than that of furan which in turn is normally produced in greater amount than 2-methylfuran (note the exception in the case of FA-2). Furfural is produced in greater quantity than the other three compounds with the exceptions of FA-1 and FA-5 where the yield of acetaldehyde is greater.

Pentoses were determined by the furfural method or modifications thereof (15,16) but it should be noted that hexoses and uronic acids also produce furfural on hydrolysis. All classes of monosaccharides give rise to pyrograms in which the five major peaks are present. By pyrolysis of mixtures of pentoses, hexoses, uronic acids and hexosamines as well as of known quantities of soil polysaccharides, it has been shown that the carbohydrate content of a sample is a function of the amount of furfural generated; thus the carbohydrate contents of the six fulvic acid extracts have been determined. The results are presented in Table 2.

It is interesting to note that samples FA-4 and FA-6 show the two extremes of high and low carbohydrate content respectively; in the same way it appears that an alkaline medium is more effective than an acidic medium for extraction. Other extractive treatments had the effects shown in Table 3.

Fig.3. Production in µg/g of four of the major peaks produced by pyrolysis of FA. A=acetaldehyde, B=furan, D=2-methyl-furan E=furfural

Table 2

Polysaccharides content of FA determined by pyrolysis

Sample	Polysaccharides (%)	Loss in weight (%)
FA-1	13.1	27.5
FA-2	25.0	45.3
FA-3	19.0	29.4
FA-4	33.5	60.1
FA-5	24.4	n.d.
FA-6	4.8	n.d.

n.d. = not determined

Gel filtration appears to be the most effective method for reducing the polysaccharide content of fulvic acid; using Sephadex G-50 in an acid medium a bimodal distribution is obtained, the first fraction is eluted at v_0 and the second just before v_t , the early fraction containing a high proportion of the polysaccharide. The data presented in Table 3 show the amounts of residual polysaccharide after various "extraction" procedures.

Oxidation at 170°C for 600 hrs. diminishes the polysaccharide content considerably as is exemplified by the pyrogram shown in Fig. 4, here the salient peaks have almost completely

Table 3

Polysaccharides content of some FA after various treatments

Sample	Treatment	Polysaccharides (%)
FA-1	acetone	13.0
FA-1	Forsyth	7.0
FA-1	Polyclar	4.3
FA-4	acetone	9.7
FA-4	Forsyth	6.0
FA-4	Polyclar	4.8
FA-4	H ₂ O (100°C)	5.4
FA-4	Sephadex	3.7

disappeared. Oxidation of soil polysaccharides leads to a similar result, for example crushed Devonian shale loses the bulk of its carbohydrate content on heating in air (17). This heating cycle also volatilises lower boiling components such as aliphatic hydrocarbons and pyrograms of pre-treated humic and fulvic acids evidence this phenomenon and prove that much of the lighter ends are derived from chain scission of these simpler molecules. Moreover, under these conditions, 80-100% of the nitrogen containing matter remains in the residues after pyrolysis and acid hydrolysis of these residues releases alanine, glycine, valine, leucine etc. Abelson (18) and Khan and Sowden (19) report that these amino-acids, together with histidine, iso-leucine and tyrosine possess considerable thermal stabilities.

Fig. 4. Pyrogram of FA-4 after heating at 170°C during 600 hrs.

CONCLUSIONS

The presence of carbohydrates in humic substances has been revealed by both conventional chemical analysis and IR spectroscopy (20,21), little dependence can be placed upon the extractive procedures used however as values between zero (22) and 65% (23) have been reported.

Pyrolysis gas chromatography offers a means of detection and quantitation which gives figures consistent with those generally accepted. The several distributions of what might be called the significant fragments could well be due to the fact that soil polysaccharides are complex entities embracing a

wide range of molecular size and shape (24); also it is possible either that only some portion of the polysaccharides is being extracted or that the extraction processes used actually modify the carbohydrates.

Although many methods used to reduce the polysaccharide content are effective there is invariably some loss of humic material, gel filtration and adsorption on Polyclar AT being the most efficient presently available. Indeed the latter technique was used by Swincer et al. (24) to give recoveries of greater than 95% of the carbohydrate present, thus this method could well be adopted for the purification of fulvic acid prior to embarking upon such as structural studies.

There is reason to believe that at least some part of the polysaccharide present is strongly linked to the fulvic acid, Haworth (25) suggests that polysaccharides, proteins, metals and phenols are either physically or chemically attached to the core of humic substances, pyrolysis easily releases the polysaccharide element but the protein-core couple is of much greater stability linkages with, for example, phenols however, require further investigation.

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