Structural assessment of humic fractions from different soils through $^{13}$C NMR spectroscopy and ruthenium tetroxide oxidation

F.J. González-Vila$^a$, J.C. del Rio$^a$, G. Almendros$^b$, and F. Martin$^a$

$^a$Instituto de Recursos Naturales y Agrobiología, CSIC, Apdo. 1052, 41080-Sevilla, Spain

$^b$Centro de Ciencias Medioambientales, CSIC, Serrano 115, 28006-Madrid, Spain

Abstract

Humic acids and humin fractions isolated from three representative types of terrestrial humus have been compared by CPMAS $^{13}$C NMR spectroscopy and degradation with ruthenium tetroxide. It was found that the latter method was suitable to remove the constituents of the condensed polyalkyl moiety of the humic fractions. The humin fractions showed NMR spectra with substantial amounts of O-alkyl and alkyl structures and yield mixtures of oxidation products qualitatively and quantitatively different from those corresponding to the humic acid fractions. The clear differences found between the degradation patterns were only in part reflected by the NMR spectra.

1. INTRODUCTION

A considerable number of analytical approaches have been carried out on the structural features of humic substances (1,2). Such studies have found a series of structural units and have contributed to the establishment of the main differences between HS from different origins or formed under different conditions.

The complementary use of solid-state $^{13}$C NMR spectroscopy and chemical degradations still represent the most widely used approach to structural studies. Concerning the latter, most recent studies have emphasized the need to avoid drastic conditions by using progressively milder, and thus more selective, oxidative conditions for the cleavage of aliphatic and ether bridges between the structural units (3).

In this study we have applied RuO$_4$ oxidation to humic acids (HA) and humin fractions isolated from three representative types of soil humus. This reagent is a relatively mild and specific oxidant successively exploited by geochemists in the last few years for the analysis of asphaltene, coals, and kerogens (4,5 and references therein).

The aim of the study is to assess the possible structural relationship between both humic fractions by a combination of this promising degradative method and solid state NMR. It is well known that most classical studies have considered the humin fraction as the final insoluble product of humic colloids in soil. Such a concept has been questioned over the last few years, as a result the idea of French pedologists has been taken up again on the existence of different types of humins (6). Recent studies using $^{13}$C NMR spectroscopy (7,8) and chemical degradation procedures (9-11) have shown that soil humin fractions include substantial amounts of resistant macromolecular polymethylenes structures—the origin and composition of which is yet to be established properly—not observed previously in the HA fractions. These results contrast with the strong similarities
between both fractions reported by many authors on the basis of functional group similarities, behaviour against degradative methods (12) and non-destructive techniques (13,14).

RuO₄ oxidation may be especially suitable for the present purposes since, as proved in kerogen degradation (4,5), it constitutes a useful method for obtaining molecular information about the nature of the aliphatic and alicyclic constituents.

2. MATERIAL AND METHODS

Soil samples representative of the three main types of terrestrial humus were chosen to isolate the HA and humin fractions. Sample QUE was taken from an evergreen oak forest on a Calcic Cambisol in Segovia (Central Spain), 850m above sea level. Sample HAY was taken from an Entic Luvisol on acid metamorphic rocks in a beech forest in Segovia (Central Spain). Sample ISO was taken from a Pacific Calciexeroll on marl, covered by graminous vegetation in Burgos (Northern Spain). Additional data on the soil organic matter of the soils have been published elsewhere (15,16).

The HA were isolated and purified according to methods previously described (17). Humin fractions were isolated by means of partition with methyl isobutyl ketone, following a modification of the method outlined by Thorn et al. (18) based on using ten times the amount of soil sample and substituting the time-consuming funnel separation by centrifugation. Humin remains as a wafer between the two liquid phases.

¹³C NMR spectra were recorded with the CPMAS technique using the quantitative acquisition conditions described previously (19).

The RuO₄ oxidation was carried out by the improved miniaturized procedure recently developed by Eglington and coworkers (4,5,20). Briefly, a vial (1m1) fitted with a Mininert® valve was charged with 10mg of sample. Intern standards (100μl, mainly 2-octadeylbutanedioic acid in chloroform) were added. Finally periodic acid solution (50% w/v in water, 0.3ml) followed by acetonitrile and chloroform (2:1, 0.2ml) containing 2.5 mg RuO₄ x H₂O were finally incorporated. The mixture was then shaken gently (1000 rpm, 35°C, 24 hours). The addition of solvents (0.1ml, 4:1 diethyl ether/dichloromethane) resulted in the precipitation of RuO₄. The organic layer was filtered (1:1 celite 521R and magnesium sulphate in a short column) and thrice centrifuged (3000 rpm, 1 min). The sample was concentrated (N₂, <40°C) and derivatized with diazomethane, followed by BSTFA.

The oxidation products were studied by GC and GC-MS using Hewlett Packard instruments 5988 and 5992B. Separation was carried out on an SE-52 (25m, 0.32 mm I.D., 0.10μm film thickness) column. The temperature program was as follows: from 50°C to 300°C at 6°C min⁻¹ and 15 min hold. The mass spectrometer was operated in EI mode at 70eV ionising energy. The identification was accomplished using library search (Wiley and NBA). The different series of alkyl compounds were recognised by single ion monitoring (SIM) of typical diagnostic peaks.

3. RESULTS AND DISCUSSION

The FID chromatograms of the RuO₄ oxidation products of the HA and humin samples are shown in Fig. 1. The carbon number of the different types of degradation products is indicated on each peak.

There is a clear dominance of linear and branched mono- and dibasic alkanoic acids, probably coming from aliphatic and acyclic structures. Only the sample HA-ISO yields a noticeable proportion of aromatic acids, whereas a methoxy dicarboxylic acid was the only
Functional group analysis, by means of preparative techniques

Humin samples were chosen from two profiles: one evergreen oak forest in Segovia (HA) and a forest in Segovia (ISO). Both profiles were covered by a soil horizon, mainly consisting of the soil organic matrix described (17). Humin was prepared by extraction, following a soxhlet extraction ten times the weight of the sample and separation by

The quantitative procedure recently described (17) was performed with a MininertR

100µl, mainly

containing 2.5 mg

1000 rpm,

(1 min). The

analytical column, followed by

Hewlett Packard

0.32 mm I.D.,

from 50°C to

60°C, and

in EF mode at

argon (Wiley and

Figure 1. FID chromatograms of the different humic fractions. Numbers on the peaks correspond to the most abundant homologues of the series of mono (m) and dibasic (d) alkanolic acids. A: aromatic acids, b:branched.
aromatic product tentatively identified in the humins from HAY and QUE.

The microbial contribution in the sample HA-QUE is suggested by the presence of the typical iso- and anteiso-C15 fatty acids (21), not detected in the other samples.

The distribution of mono- and dibasic alkanedic acids (mainly > C20, maxima at C22 and C24, even-C dominance, etc.) found in all the samples is often considered to reflect higher plant inputs.

The carboxylic function could mark points of attachment in the humic macromolecules as well as positions of labile functional groups, such as carbon-carbon double bonds and ether or ester links. Thus, the lower molecular weight alkanodic acids ( < C11) probably come from the oxidation of unsaturated or OH-substituted fatty acids, such as C16 and C18 unsaturated acids, and their polymer-derived structures present in the macromolecular structures (8). In fact, the dominance of the C9 and C11 homologues may correspond both to the oxidation of the most frequent unsaturated fatty acids (oleic, linoleic acids) as well as to the breakdowns of the different types of OH-substituted fatty chains present in natural biopolymesters. According to that, the sample HA-HAY should present the greatest proportion of such structures, followed by samples humin-QUE and humin-HA. The opposite is the case with HA-ISO and humin-ISO.

On the other hand, the C16-C24 alkanodic acids may come most likely from the oxidation of aliphatic chains incorporated through ether or ester bonds, which should bridge in the macromolecular structure. In this case, sample HA-HAY should present the higher cross-linking grade.

Quantitative and qualitative differences between the oxidation patterns of the various samples could be envisaged: In the sample QUE the distribution of the oxidation products was similar both in the HA and humin fractions (dominance of the lowest molecular weight alkanodic acids and larger chain length monocarboxylic acids with maxima at C9 and C22, respectively). In the two other cases greater differences were found between the humin and HA fractions. Thus, in sample HAY, the HA yields predominantly even-numbered alkanodic acids with maxima at C9 and C16, and a small amount of monocarboxylic acids. The humin, on the contrary, showed mainly monocarboxylic acids (C22 maximum) and a low proportion of alkanodic acids. Finally, in soil ISO, the HA fraction is the only sample where aromatic (di-, tri-, tetra- and pentacarboxylic) acids were present in large amounts. This result agrees with the dominant and stability of the humus-clay complexes characteristic of this isohumic soil (14). The humin-ISO showed a dominance of fatty acids (C16-C30). Alkanodic acids with different distributions and a series of n-alcohols (C14-C20) were also present in both humic fractions in minor amounts.

Fig. 2 shows the 13C CPMAS spectra of the HA and humin fractions from the three soils. The quantitative results from the integration of selected regions in the 13C NMR spectra are shown in each region.

There were some clear differences among the different HS's. In accordance with the results from RuO4 oxidation, the most aromatic is the HA-ISO, although olefinic carbons may contribute to the signals observed.

The alkyl resonances, centered at 30 ppm, indicate that a substantial amount of polyethylene chains is present in all the samples, although they are more prominent in the samples HA-QUE and HA-HAY. The apparent lack of signals for terminal methyl absorptions (10-20 ppm) suggests that considerable cross-linking of the alkyl chains may exist in the samples in accordance with the RuO4 oxidation data. The O-alkyl structures are resolved better in the HA-QUE sample than in the other HA's.

In general, the spectra of the humin preparations studied are resolved better than others reported previously (22,23), which often showed only two peaks attributed to aromatic and aliphatic carbons, respectively. In the present humin samples, the oxygen-substituted carbons (46-105 ppm region) are clearly resolved from unsubstituted aliphatic
Figure 2. Solid-state 13C-NMR spectra of the HA and humin fractions.

Table 1
Quantitation (% area) of the 13C-NMR spectral regions

<table>
<thead>
<tr>
<th>ppm range</th>
<th>200-160</th>
<th>160-110</th>
<th>110-46</th>
<th>46-0</th>
</tr>
</thead>
<tbody>
<tr>
<td>HA-QUE</td>
<td>9.09</td>
<td>16.36</td>
<td>35.45</td>
<td>39.09</td>
</tr>
<tr>
<td>HA-HAY</td>
<td>8.96</td>
<td>15.87</td>
<td>33.64</td>
<td>41.45</td>
</tr>
<tr>
<td>HA-ISO</td>
<td>16.83</td>
<td>35.35</td>
<td>29.29</td>
<td>18.53</td>
</tr>
<tr>
<td>Humin-QUE</td>
<td>5.69</td>
<td>21.91</td>
<td>48.08</td>
<td>24.33</td>
</tr>
<tr>
<td>Humin-HAY</td>
<td>6.90</td>
<td>22.88</td>
<td>44.01</td>
<td>26.11</td>
</tr>
<tr>
<td>Humin-ISO</td>
<td>9.79</td>
<td>31.49</td>
<td>45.50</td>
<td>13.22</td>
</tr>
</tbody>
</table>
carbons. Carbohydrates, other linkages, O-heterocycles and methoxyl groups are considered to be the major contributors to peaks found in this region. Carbohydrates incorporated in the humins as polysaccharides have been reported as typical pyrolysis products from humin fractions (24).

From the above results a series of conclusions can be drawn: a) as same as with kerogens, the RuO₄ oxidation of HA's and humins leads to characteristic degradation patterns revealing differences both in terms of the source and type of the humic fractions. b) the differences between humic fractions revealed by ¹³C NMR spectroscopy are in general reflected by different patterns of RuO₄ oxidation products. However, the clear differences between the spectra of the humic acids and their corresponding humins (enriched in O-alkyl C atoms) are only partially corroborated by the oxidation results.

4. REFERENCES

23. M.A. Wilson, NMR Techniques and Applications in Geochemistry and Soil Chemistry. Pergamon, Oxford. 353p, 1987

Characterization of Humic Substances from Fertilized and Unfertilized Soils

T.M. Hayes*, W. Zeng and G. Zou
*School of Chemistry, University of Essex, Colchester CO4 3SQ, U.K.
**USDA-ARS, Utah State University, Logan, Utah, U.S.A.

ABSTRACT

Humic acids isolated from soils either fertilized with inorganic nitrogen or unfertilized soils untreated with any fertilizer were extracted with 5% KOH solution and isolated by anion exchange chromatography. The yield of humic acids isolated from fertilized soil was higher than that from unfertilized soils and FTIR spectra revealed structural differences between the humic acids isolated from soils. Variations among the spectra of the humic acids were attributed to the differences in the microbial degradation of organic matter. Variations in the FTIR spectra of the humic acids may be due to the differences in the chemical composition of organic matter in different soils and treatments.

INTRODUCTION

This research was undertaken to characterize the humic substances in soils fertilized with inorganic nitrogen. The humic substances were extracted from soils with 5% KOH solution and isolated by anion exchange chromatography. The yield of the humic substances was higher from fertilized soils than from unfertilized soils. Variations in the FTIR spectra of the humic substances were attributed to the differences in the chemical composition of organic matter in different soils and treatments. Variations in the FTIR spectra of the humic substances were also due to the differences in the microbial degradation of organic matter.

MATERIALS AND METHODS

Soil: Soil samples were collected from a maize (Zea mays) field in the United States. The soil samples were collected at depths of 32.8 and 23.6 cm. The soil samples were air-dried, sieved through a 2-mm mesh, and stored at room temperature until use.

Humic Substances: Humic substances were isolated from soils using a 5% KOH solution. The isolated humic substances were further purified by anion exchange chromatography.

FTIR Spectroscopy: FTIR spectra were recorded using a Nicolet 6700 FTIR spectrometer. The specimens were pressed into KBr pellets and scanned in the 4000-400 cm⁻¹ region.

RESULTS

The FTIR spectra of the humic substances isolated from fertilized and unfertilized soils showed some differences. The differences were attributed to the differences in the chemical composition of organic matter in different soils and treatments. Variations in the FTIR spectra of the humic substances were also due to the differences in the microbial degradation of organic matter.

DISCUSSION

The differences in the FTIR spectra of the humic substances isolated from fertilized and unfertilized soils were attributed to the differences in the chemical composition of organic matter in different soils and treatments. Variations in the FTIR spectra of the humic substances were also due to the differences in the microbial degradation of organic matter.

CONCLUSIONS

The results of this study indicated that the humic substances isolated from fertilized and unfertilized soils showed some differences. The differences were attributed to the differences in the chemical composition of organic matter in different soils and treatments. Variations in the FTIR spectra of the humic substances were also due to the differences in the microbial degradation of organic matter.
HUMIC SUBSTANCES
IN THE GLOBAL
ENVIRONMENT AND
IMPLICATIONS ON
HUMAN HEALTH

Proceedings of the 6th International Meeting of the
International Humic Substances Society, Monopoli (Bari),
Italy, September 20–25, 1992

Edited by

N. Senesi
T. M. Miano
Istituto di Chimica Agraria
Università degli Studi - Bari
Bari, Italy

ELSEVIER
Library of Congress Cataloging-in-Publication Data

International Humic Substances Society. International Meeting (6th : 1992 : Monopoli, Italy)

Includes bibliographical references.
ISBN 0-444-89593-0 (acid-free)

0341.A2156 1992
631.4'17--dc20
94-11117 CIP

ISBN 0-444-89593-0

© 1994 Elsevier Science B.V. All rights reserved.

No part of this publication may be reproduced, stored in a retrieval system or transmitted in any form or by any means, electronic, mechanical, photocopying, recording or otherwise, without the prior written permission of the publisher, Elsevier Science B.V., Copyright & Permissions Department, P.O. Box 521, 1000 AM Amsterdam, The Netherlands.

Special regulations for readers in the U.S.A. - This publication has been registered with the Copyright Clearance Center Inc. (CCC), Salem, Massachusetts. Information can be obtained from the CCC about conditions under which photocopies of parts of this publication may be made in the U.S.A. All other copyright questions, including photocopying outside of the U.S.A., should be referred to the copyright owner, Elsevier Science B.V., unless otherwise specified.

No responsibility is assumed by the publisher for any injury and/or damage to persons or property as a matter of products liability, negligence or otherwise, or from any use or operation of any methods, products, instructions or ideas contained in the material herein.

This book is printed on acid-free paper.

Printed in The Netherlands

The International Humic Substances Society held its sixth International Meeting in September 1992 in Monopoli, Italy. The purpose of this meeting was to bring together scientists and others concerned with the use of humic substances in the global environment and their implications on human health. Thus, five biennial meetings of the International Humic Substances Society (IHSS) have brought together scientists and others involved in various aspects of humic substances in order to discuss the latest developments and applications of humic substances.

This volume contains selected papers presented at the six biennial meetings of the International Humic Substances Society. The invited articles are contributions that reflect the present status of the art in different fields of humic substance chemistry and their environmental significance, including industry and metabolism. The invited articles are inorganic and organic chemistry, as well as environmental studies.

We are grateful to the many institutes, companies and colleagues who helped provide financial support for the conference and guest registration. The Meeting organizing committee was chaired by Dr. G. Panella and Dr. M. Doglioni. The Official Organizing Committee of the Conference has made continuous efforts in preparation of the conference. The International Humic Substances Society wishes to thank all of the contributors and especially to the IHSS Directors of Ireland and Canada for their continuous support, the IHSS headquarters in Varenna, Italy, and the IHSS Officers and Chairmen of the IHSS Committees and IHSS Secretaries. Finally, the IHSS is grateful to the City of Monopoli in Italy and to the University of Rome, for their

The International Humic Substances Society held its sixth International Meeting in September 1992 in Monopoli, Italy. The purpose of this meeting was to bring together scientists and others concerned with the use of humic substances in the global environment and their implications on human health. Thus, five biennial meetings of the International Humic Substances Society (IHSS) have brought together scientists and others involved in various aspects of humic substances in order to discuss the latest developments and applications of humic substances.

This volume contains selected papers presented at the six biennial meetings of the International Humic Substances Society. The invited articles are contributions that reflect the present status of the art in different fields of humic substance chemistry and their environmental significance, including industry and metabolism. The invited articles are inorganic and organic chemistry, as well as environmental studies.

We are grateful to the many institutes, companies and colleagues who helped provide financial support for the conference and guest registration. The Meeting organizing committee was chaired by Dr. G. Panella and Dr. M. Doglioni. The Official Organizing Committee of the Conference has made continuous efforts in preparation of the conference. The International Humic Substances Society wishes to thank all of the contributors and especially to the IHSS Directors of Ireland and Canada for their continuous support, the IHSS headquarters in Varenna, Italy, and the IHSS Officers and Chairmen of the IHSS Committees and IHSS Secretaries. Finally, the IHSS is grateful to the City of Monopoli in Italy and to the University of Rome, for their

The International Humic Substances Society held its sixth International Meeting in September 1992 in Monopoli, Italy. The purpose of this meeting was to bring together scientists and others concerned with the use of humic substances in the global environment and their implications on human health. Thus, five biennial meetings of the International Humic Substances Society (IHSS) have brought together scientists and others involved in various aspects of humic substances in order to discuss the latest developments and applications of humic substances.

This volume contains selected papers presented at the six biennial meetings of the International Humic Substances Society. The invited articles are contributions that reflect the present status of the art in different fields of humic substance chemistry and their environmental significance, including industry and metabolism. The invited articles are inorganic and organic chemistry, as well as environmental studies.

We are grateful to the many institutes, companies and colleagues who helped provide financial support for the conference and guest registration. The Meeting organizing committee was chaired by Dr. G. Panella and Dr. M. Doglioni. The Official Organizing Committee of the Conference has made continuous efforts in preparation of the conference. The International Humic Substances Society wishes to thank all of the contributors and especially to the IHSS Directors of Ireland and Canada for their continuous support, the IHSS headquarters in Varenna, Italy, and the IHSS Officers and Chairmen of the IHSS Committees and IHSS Secretaries. Finally, the IHSS is grateful to the City of Monopoli in Italy and to the University of Rome, for their