

Derivatization and structural studies by spectroscopic methods of humic acids from Leonardite

G. Ricca^{a,*}, F. Severini^b, G. Di Silvestro^a, C.M. Yuan^a, F. Adani^c

^a *Centro di Studio per le Sostanze Naturali del C.N.R., Dipartimento di Chimica Organica e Industriale, Università degli Studi di Milano, Via Venezian 21, 20133 Milan, Italy*

^b *Dipartimento di Chimica Industriale e Ingegneria Chimica, Politecnico di Milano, P.zza Leonardo da Vinci 32, 20133 Milan, Italy*

^c *Dipartimento di Fisiologia delle Piante Coltivate e Chimica Agraria, Via Celoria 2, 20133 Milan, Italy*

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Abstract

Humic acids (HA) from Leonardite were methylated with $\text{CH}_3\text{I}/\text{Ag}_2\text{O}$ in a methanol suspension (yield $261 \pm 23.6 \text{ g kg}^{-1}$; $N = 4$) and dimethylformamide (DMF) (yield $839 \pm 57.6 \text{ g kg}^{-1}$; $N = 4$) solution. Methylated humic acids are soluble in organic solvents and have been examined by IR-FT, ^1H and ^{13}C -NMR spectroscopy methods. The $\text{OCH}_3/\text{COOCH}_3$ ratio was calculated by ^{13}C -NMR for HA methylated in methanol suspension and DMF solution obtaining values of 0.38 and 0.82, respectively. Methylated acids molecular weight distribution was measured by Size-Exclusion Chromatography (SEC). The product obtained in methanol suspension has a molecular weight (M_n) of 2918 Da and a dispersion index ($D = M_w/M_n$) of 1.13. The methylated product prepared in DMF solution is characterized both by higher molecular weight, ($M_n = 3623 \text{ Da}$) and dispersion index ($D = 2.10$). © 2000 Elsevier Science B.V. All rights reserved.

Keywords: ^1H ; ^{13}C -NMR; IR-FT spectroscopy; hydroxyl group functionality; humic acid; methylation; molecular weight

* Corresponding author. Fax: +39-2-2364369.

E-mail address: giuliana.ricca@unimi.it (G. Ricca).

1. Introduction

Results were reported in a previous paper (Ricca et al., 1993), obtained from studies of humic acid from Leonardite, in alkaline aqueous solution. The frequently obtained NMR spectra showed overlapping signals.

Strong hydrogen bonds in humic acids favor intra and inter molecular aggregation and determine a loss of resolution in ^1H and ^{13}C -NMR spectra. The poor solubility of humic acids in organic solvents practically prevents a correct determination of molecular weight (Lawson and Stewart, 1989). The aim of this paper is to improve the knowledge of the humic acid structure from Leonardite by studying methylated derivatives which are soluble in organic solvents and do not present structures associated by hydrogen bonds.

The humic acid used was extracted in our laboratory, following the method suggested by the IHSS and reported in this paper (Schnitzer and Skinner, 1982).

2. Materials and methods

Leonardite is a brown coal extracted from superficial layers of lignite mines of North Dakota. Humic acid from Leonardite was obtained following the method suggested by the IHSS (Schnitzer and Skinner, 1982) using 0.5 N NaOH aqueous solution. After 24 h, the suspension was centrifuged and insoluble materials formed by humin and inorganic substances were separated. Humic acid in solution with fulvic acid was separated after addition of $\text{HCl } 1 \text{ mol l}^{-1}$. The separated material, after washing with water and drying, was treated with a $\text{HCl} + \text{HF}$ dilute mixture, obtained by dissolving 5 ml of $\text{HCl } 36\%$ and 5 ml of $\text{HF } 52\%$ in 990 ml of distilled water. The primary objective of purification is to minimize the ash content. A secondary objective is to remove low molecular weight organic molecules that are not structural HA constituents. The acid was then dialyzed against distilled water for 96 h, freeze-dried, weighted and stored. The elemental composition is C% 53.78; H% 3.35, N% 2.09, O% 40.0.(ash % 0.78).

2.1. Chemical methods

Methylation was prepared by $\text{CH}_3\text{I}/\text{Ag}_2\text{O}$ in methanol suspension or in dimethylformamide (DMF) solution following the indications suggested by Schnitzer and Desjardins (1970), Schnitzer (1974), and Wershaw and Pinckney (1978). 100 mg of humic acid (HA) and 7 ml of methanol were put in a 100-ml flask. 300 mg of Ag_2O and 2.5 ml of CH_3I were added to the resulting suspension. The flask was tightly stopped and shaken for 48 h at room temperature. Following this, the acid was remethylated by the same procedure. The suspension was then centrifuged for 45 min at 2000 rpm. The clear brown

supernatant solution was dried in a rotary evaporator and finally in a vacuum desiccator over P_2O_5 . Methylation was made with the same procedure using a solution of humic acid in DMF. Obtained products are designated as ME I and ME II, respectively.

Due to the good solubility of HA in DMF, we attempted to start the preparation of methyl derivatives directly from Leonardite coal. One gram of powdered coal was placed in a 250-ml flask and 35 ml of DMF were added. Later, Ag_2O and CH_3I were added to this suspension and calculated on the basis that humic acid is 50% of the powdered Leonardite coal. The suspension was centrifuged for 45 min at 2000 rpm. After separating the supernatant liquid, the centrifuged solid was washed with methylene chloride to dissolve the enclosed methylated acid and this solution was mixed with the supernatant liquid. After solvent evaporation and drying of the solid, 100 mg of methylated acid were obtained. The yield is 100 kg^{-1} as referred to the coal.

2.2. Potentiometric titration

The acid–base equilibria were studied by potentiometric titration using the fast titration method. The acidity is $4.0 (\pm 0.2)$ meq/g, average of several determinations (Senesi et al., 1989, 1990; Pastorelli et al., 1999).

2.3. Spectroscopic methods

IR-spectra were recorded on a Jasco 300 E IR-FT spectrometer from 4000 to 1000 cm^{-1} , using KBr pellet (1 mg of sample + 200 mg of dry KBr) or in $CHCl_3$ (spectra grade) solution (cell 0.5-mm thickness).

1H and ^{13}C -NMR spectra of humic acid in $CDCl_3$ were recorded at 300 and 75.432 MHz, respectively, on a Bruker A.C. 300 spectrometer. All chemical shifts were determined from internal standard tetramethylsilane (TMS). A NMR sample was prepared by dissolving about 100 mg of methylated humic acids in 0.5 ml of $CDCl_3$ solution. Quantitative intensity distribution was obtained using the inverse-gated decoupling method following the conditions suggested by Preston and Blackwell (1985) in a paper on the structure of humic and fulvic acids studied by NMR: 0.23-s acquisition time, 45° pulse, 2 s relaxation delay, line broadening at 20 Hz, total acquisition time is 48 h.

2.4. Size-Exclusion Chromatography (SEC)

SEC curves were recorded by a Waters apparatus at room temperature using CH_2Cl_2 as eluent and UV detector operating at 250 nm. Columns were calibrated with polystyrene standards.

3. Results and discussion

Methylation reactions for structural characterization of humic acids were frequently reported (Leenheer and Noyes, 1989). Haider et al. (1992, 1993) have proposed the silylation reactions for humic acids derivatization.

In this paper, humic acid methylation using $\text{Ag}_2\text{O}/\text{CH}_3\text{I}$ in methanol suspension takes place with low yield, $261 \pm 23.6 \text{ g kg}^{-1}$, ($n = 4$). The product (ME I) is very soluble in CHCl_3 and CH_2Cl_2 . Molecular weight measured by SEC in CH_2Cl_2 is 2918 Da. Molecular weight distribution curve shows the dispersion index $D = 1.13$. Fig. 1a.

Fig. 2a and b shows the IR-FT spectra of humic acid from Leonardite (HA) in the KBr pellet and of methyl ester derivative of (ME I) in the CHCl_3 solution. The band at 1730 cm^{-1} in the ME I spectrum confirms the carboxyl group esterification.

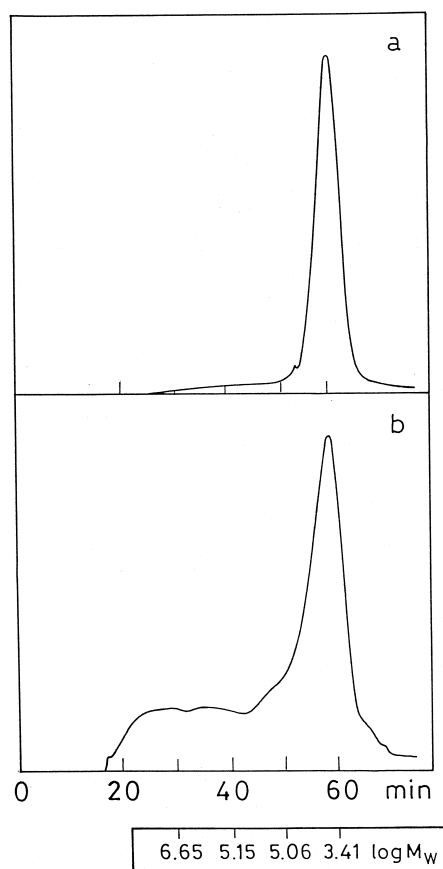


Fig. 1. SEC chromatogram curves of ME I (a) and ME II (b).

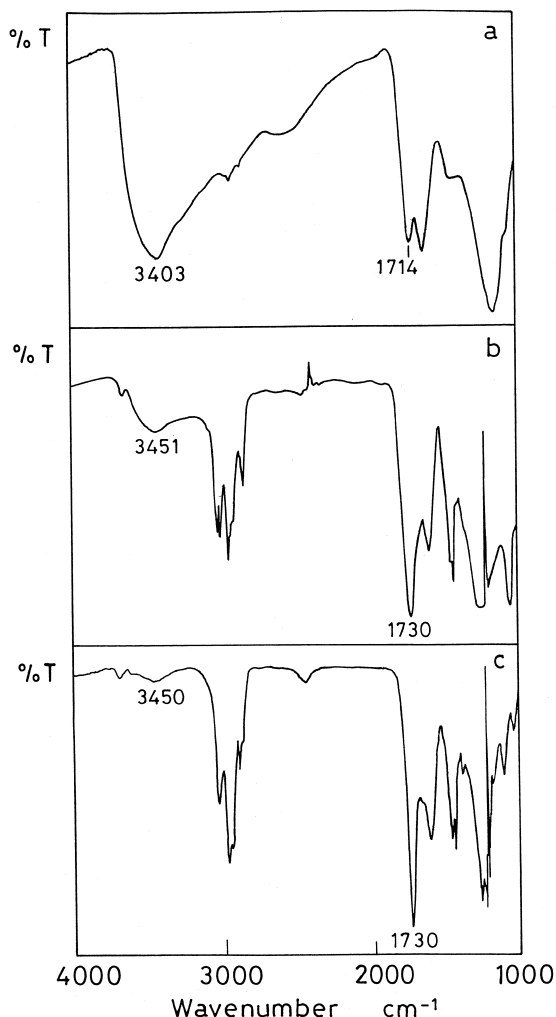


Fig. 2. IR-FT spectra of HA from Leonardite in KBr pellet (a); of ME I (b) and ME II (c) in CHCl_3 solution.

These results and the absence of bands attributed to free carboxyl indicate that practically all the carboxyl groups have been transformed in methyl ester. On the contrary, the hydroxyl group has been methylated only partially. Indeed, the spectrum of the modified humic acid (ME I) shows OH stretching vibration at 3451 cm^{-1} .

^{13}C -NMR spectrum in Fig. 3a shows two peaks at 62.5 and 56.8 ppm. These may be assigned to the methyl ethers of aromatic hydroxyl groups, while the peak at 53.4 ppm is attributed to methyl esters of carboxylic acids (Thorn et al., 1987; Mikita and Steelink, 1981). Peak areas integration values obtained by inverse gated decoupled technique allow the evaluation of the amount of

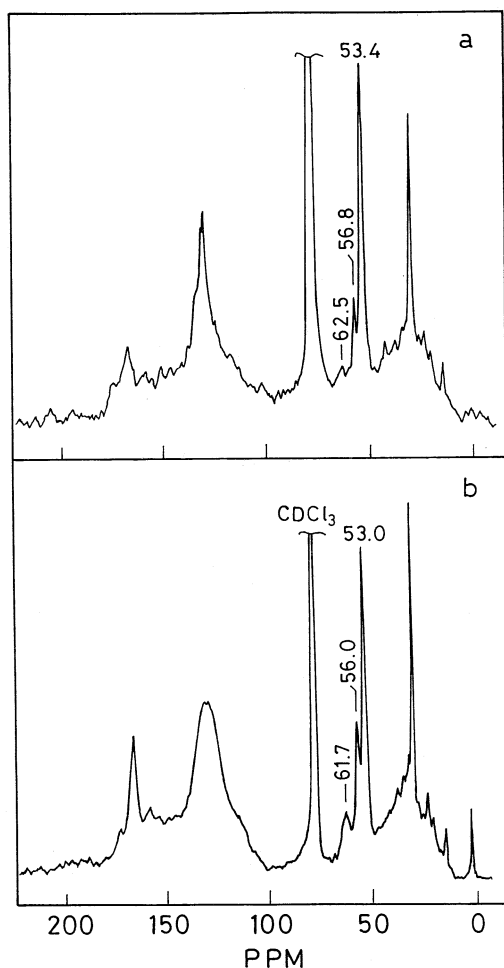


Fig. 3. ^{13}C -NMR spectra of ME I (a) and MEII (b) in CDCl_3 solution.

different types of carbon in methylated product. Data obtained indicate that $\text{OCH}_3/\text{O}=\text{C}-\text{OCH}_3$ ratio is 0.38. A tentative assignment of the major different signals on the ME I in the ^{13}C -NMR spectrum is proposed in Table 1.

^1H -NMR spectrum of ME I in CDCl_3 solution (Fig. 4a) shows two superposed triplets ($J = 7$ Hz) between 0.8 and 0.9 ppm, attributed to the terminal methyl group of aliphatic chains. The sharp singlet at 1.2 ppm in ME I may be due to methylene protons, (Ogner, 1979). The signals at 1.6 ppm (multiplets), 2.1 ppm (singlet) and 2.3 ppm (triplet; $J = 7$ Hz) and at 2.5 ppm (quartet; $J = 7$ Hz) are attributed to protons of methyl and methylene groups in α to aromatic rings and to protons in α to carboxylic group, respectively (Wershaw and Mikita, 1987; Ricca et al., 1993). The broad signals at 3.6 and 3.8 ppm are due to the methyl ester and methyl ether groups. The signal at 7.42 ppm (dd; $J = 7.7$ Hz;

Table 1
 ^{13}C chemical shifts (ppm) and proposed assignments of methylated humic acid from Leonardite

ME I HA methylated in CH_3OH	ME II HA methylated in DMF	ME LEO Leonardite methylated in DMF	Assignment
175.5; 174.0	174.1–173.1	174.3–173.6	Carbonyl in quinones
167.0; 166.0; 164.0	165.8	167.0; 167.5	Carbonyl C in COOCH_3
159.0; 158.0; 157.0	158.0	159.0; 158.0	
150.0; 145.0	148.0	130.8	Oxygen or nitrogen functional groups substituted aromatic carbons
133.5; 132.0; 130.0	136.0	129.0	Aromatic C and aromatic C–H
129.0	130.0	128.0; 126.0	
124.0; 118.0	117.0	123.4	
62.5; 56.8	63.4; 61.7; 56.0	63.5; 60.7; 56.8	Methoxyl-C of aromatic ethers
53.4	53.0	52.8	Methoxyl-C of CO-OCH_3
42.7; 37.6	42.0; 37.0	41.6; 37.3	
35.0; 34.0; 33.4	35.0; 32.0	34.0; 33.2; 33.8	Various R–C–H and R–C–H ₂ α substituted; R=COOH
30.0	29.5	29.5	–(CH ₂) _n -polymethylene chains (Ogner, 1979)
28.5; 27.4; 25.5	24.0; 22.0	28.0; 27.0	
23.2; 21.7		24.8; 22.5; 21.0	Various C–C–H and –C–CH ₂ in sp ³ carbons
20.3; 19.5; 14.6	19.0; 14.0	19.6; 16.4; 14.0	CH ₃ terminal methyl

$J = 8$ Hz), 7.60 ppm (d; $J = 7.7$ Hz), 7.50 (d; $J = 8$ Hz) and 8 ppm (d; $J = 7.7$ Hz) are attributed to aromatic protons reciprocally coupled (Francioso et al., 1996). These data suggest aromatic substituted rings (Scheme 1) and confirm the prevalence of aromatic structures in humic acid from Leonardite (Ricca et al., 1993; Senesi et al., 1990; Thorn et al., 1989).

The methylation reaction, carried out in DMF solution, proceeds with high yield (839 ± 23.6 g kg⁻¹; $n = 4$), and gives a product indicated as ME II.

The IR-FT spectrum of ME II in CHCl_3 solution (Fig. 2c) shows only a negligible absorption at 3450 cm⁻¹ and this result indicates that practically all –OH groups are methylated. The band at 1730 cm⁻¹ confirms the carboxyl groups esterification.

The molecular weight distribution curve in comparison with the curve obtained for the methylated products in methanol suspension (ME I) shows a larger MWD which allows to evaluate a M_n of 3623 Da and $D = 2.11$ (Fig. 5b).

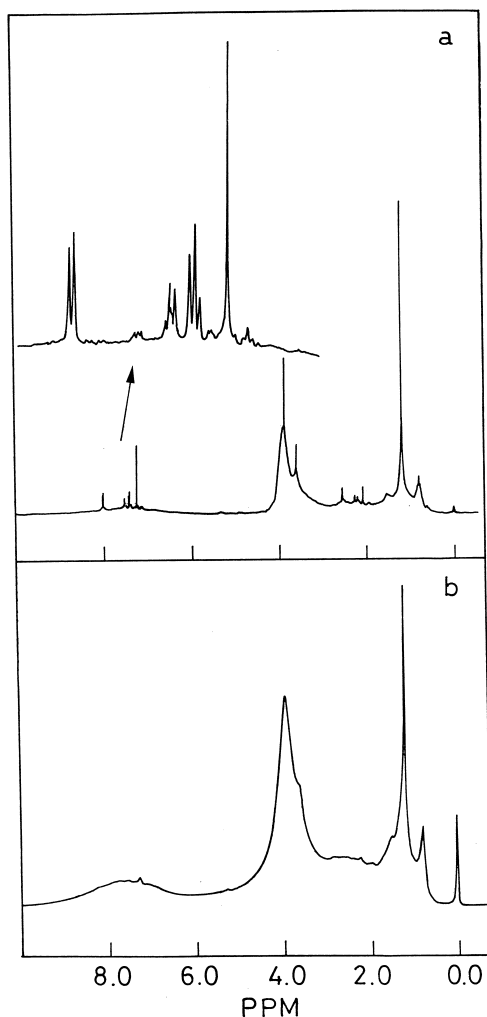
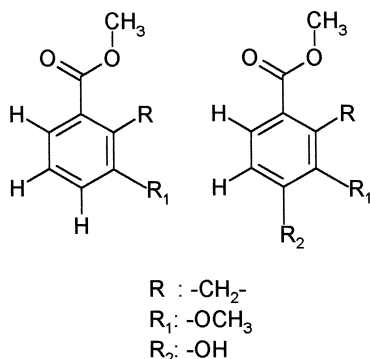


Fig. 4. ^1H -NMR spectra of ME I (a) and ME II (b) in CDCl_3 solution.

^{13}C -NMR chemical shifts (ME II) are reported in Table 1. Peak areas, due to methyl ester (53.0 ppm) and to methyl ether (63.4, 61.7 and 56.0 ppm) Fig. 3b, allow an approximate evaluation of the ratio for the ether/ester group. The $\text{OCH}_3/\text{O}=\text{C}-\text{OCH}_3$ ratio is 0.82. These results agree with a strong observed reduction of $-\text{OH}$ band in the IR-FT spectrum (Fig. 2b). ^1H -NMR spectrum of ME II in CDCl_3 solution (Fig. 4b) shows not-well-resolved signals and it is not possible to give a valid attribution.

Fig. 1 shows the SEC chromatograms of ME I(a) and ME II(b), performed in methylene chloride as eluent. In Fig. 1a all the molecular masses are in a short range of elution as confirmed by the reported distribution index $D = 1.13$. This result agrees with the lower yield observed in the reaction performed in



Scheme 1. Structures proposed for substituted aromatic rings.

methanol and with the $^1\text{H-NMR}$ spectrum very well resolved. Whereas, products obtained in DMF show a SEC chromatogram consistent with the presence of extended aggregation in the used solvent. Both ME I and ME II hydrodynamic volumes (the shape of macromolecules in solution corresponding to the mass of relative molecules) are far before the exclusion limit of the column set (2,000,000 Da) and do not permit an accurate evaluation of molecular masses, the reported molecular parameters refer to a hydrodynamic volume range similar to the one for ME I.

SEC chromatogram of methylated ME II humic acid is similar to humic acid silylated from soil reported by Dec et al. (1997) and agrees with the more complex $^1\text{H-NMR}$ spectrum of ME II. In DMF, methylation is probably not homogeneous and the work-up is not able to separate completely modified molecules from partially reacted ones which can aggregate in methylene chloride. Work for a more complete and homogenous methylation is in progress.

Average molecular weights obtained by the SEC technique for products ME I and ME II, methylated in methanol suspension and in DMF solution respectively, are smaller than those reported by Schnitzer and Khan (1972) for underivatized acids. Dec et al. (1997) found similar results by studying the SEC of silylated humic acids from soil. The difference should be due to breaking of hydrogen bonds in humic acids while derivatization takes place. These observations are consistent with the better resolution for the ME I $^1\text{H-NMR}$ spectrum than those observed in ME II products.

Due to the good solubility of HA in DMF, as described in the above-mentioned chemical methods, methylated derivatives of humic acid were prepared directly from Leonardite.

IR-FT spectrum in CHCl_3 solution of ME LEO shows the band at 1730 cm^{-1} attributable to stretching vibration of C=O ester group. A weak broad band at 3450 cm^{-1} indicates that hydroxyl groups have been methylated only partially.

$^{13}\text{C-NMR}$ and $^1\text{H-NMR}$ spectra appear similar to those of the methylated compound ME II obtained from humic acid extracted from Leonardite.

^{13}C -NMR chemical shifts of ME LEO are reported in Table 1. Integration of peaks due to methyl ester (52.8 ppm) and to methoxyl group (63.5, 60.7, 56.8 ppm) in aromatic ether gives a $\text{OCH}_3/\text{COOCH}_3$ ratio of 0.28.

^1H -NMR spectrum of ME LEO shows not-well-resolved signals.

These data suggest that qualitative information about the structure of humic acids may be obtained directly also from raw material.

4. Conclusions

The results obtained indicate that valuable information may be achieved using methylated humic acid having rather homogeneous molecular weight distribution and a correspondent dispersion index low value. Work is in progress seeking for more efficient derivatization reactions.

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