

ON THE STRUCTURE OF HUMIC ACIDS

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I. Introduction

About four years ago, the Research Council of Alberta initiated a series of coordinated, continuing studies from which several important inferences about the structure of humic acids can now be drawn. The present paper is intended briefly to review these studies and to outline those features of the humic acid molecule which can, in our view, be formulated with some confidence.

Almost all experimental data cited in the following sections were duplicated by working with humic acids derived from a weathered N. Dakota lignite*, and a weathered subbituminous coal occurring at Sheerness, Alberta. However, since these two materials displayed virtually indistinguishable behaviour, no attempt is here made to differentiate between the respective sets of results; and it is, in fact, tentatively contended that coal-derived humic acids may be regarded as chemical entities whose main structural features are essentially independent of their sources of origin.

II. The Molecular Weights of Humic Acids

In view of the then prevailing uncertainty about the size of the humic acid molecule, the initial study was concentrated upon renewed attempts to determine significant molecular weights (1). For convenience, a cryoscopic technique was used for this purpose, but in place of conventional solvents (e.g. acetamide, 2), sulfolane was employed. This solvent (3) offered several distinct and important advantages. It possesses an unusually high cryoscopic constant ($K = 65.5$ degrees - kg per mole); it affords a ready means for dissolving humic acids and completely drying the resultant solution; and it appears to eliminate disturbing effects due to solute association and/or dissociation. Generally ideal behaviour of sulfolane could, for example, be demonstrated by experiments with solutions containing such diverse compounds as benzoic acid, β -naphthalene sulfonic acid and biphenyl: in all instances, K proved to be quite independent of solute concentrations over the molality range 0.01 to 0.10. Direct evidence for the absence of association of solute molecules in a sulfolane solution could also be obtained from infrared data - notably from analysis of the -COOH and -OH stretching frequencies of simple analog compounds.

* Commercially marketed as Baroid Carbonox.

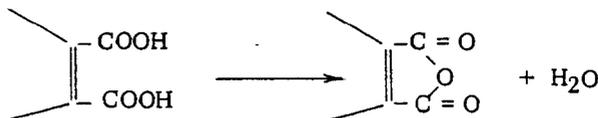
Using the sulfolane method with a series of carefully fractionated humic acids, molecular weights ranging to ca. 10,000 were recorded (1) and broadly confirmed by sedimentation velocity measurements.

It is of some interest to note that while molecular weights of the order of thousands stand in marked contrast to previously reported cryoscopic data, they are in general agreement with semi-quantitative diffusion measurements reported, inter alia, by Dryden (4).

III. The Disposition of Peripheral Functional Groups

Estimates of the concentrations of functional groups by established methods (5, 6) yielded results comparable to those reported for humic acids by other laboratories. Typical values (7) were: $-\text{OCH}_3$ 1.7, phenolic $-\text{OH}$ 2.7, and $-\text{COOH}$ 4.3 milliequivalents per gram. More detailed studies, however, permitted some refinement of these data and, more important, inferences about the disposition of certain functional groups around the molecular periphery.

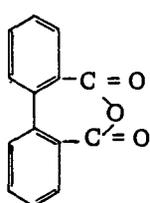
When solutions of humic acid in sulfolane were heated to $170^\circ - 200^\circ\text{C}$ under reduced pressure and subsequently examined by infrared spectroscopy, the intensity of the original carboxyl carbonyl absorption at 1735 cm^{-1} was found to be greatly reduced, and two new bands centring at 1850 and 1785 cm^{-1} were observed (Figure 1). Since identical absorptions were seen in the infrared spectra of phthalic and 1,2-naphthalic anhydrides formed in hot sulfolane, these bands were assigned to 5-membered ring anhydrides of humic acid, i.e. to simple dehydration products of the type,



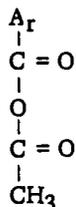
and it was concluded that a substantial number of $-\text{COOH}$ groups in the humic acid molecule occupy mutually adjacent sites. Six- or 7-membered ring anhydrides could be ruled out because of their infrared absorption at quite different frequencies, and still larger cyclic anhydrides were discounted by the rather narrow band separation generally seen in the spectra of such compounds.

When, on the other hand, humic acid is refluxed with acetic anhydride, a more complex situation is created. The intensity of the original $-\text{COOH}$ band is again greatly reduced, but now at least four new bands (at 1850 , 1820 , 1785 and 1745 cm^{-1}) are found; and the 1785 cm^{-1} band is, compared with the 1850 cm^{-1} absorption in the same spectrum, substantially more intense than in the spectrum of humic anhydride formed in hot sulfolane (Figure 1).

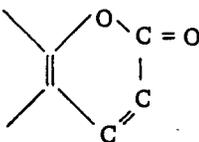
On the assumption that reaction with acetic anhydride forms, among other products, the same anhydrides as are produced by heating sulfolane solutions of humic acid, cyclic 5-membered ring anhydrides were therefore now supposed to account for only a part of the 1785 cm^{-1} absorption, and a number of theoretically possible contributing structures were examined. Among these were cyclic anhydrides of type (I), open chain anhydrides, including mixed anhydrides (II), lactones (III), esters (presumably of the phenolic acetate type IV), and esters and/or anhydrides formed between molecules of humic acid.



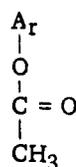
I



II



III



IV

The results, partly based upon extended experimentation with exhaustively methylated, alkali-hydrolyzed humic acid and acetyl analyses (8) led to the following conclusions(7):

- (a) Approximately 80% of all $-\text{COOH}$ groups existing in humic acid occupy sites permitting the formation of cyclic ring anhydrides.
- (b) Approximately one-fifth of all $-\text{COOH}$ groups form linear mixed anhydrides when humic acid is reacted with acetic anhydride (but not in sulfolane) and must therefore exist as isolated groups or in odd-numbered groups on adjacent ring sites.
- (c) Of the several, theoretically possible 6- and 7-membered ring anhydrides, only anhydrides of the diphenic type (I) are consistent with experimental evidence.

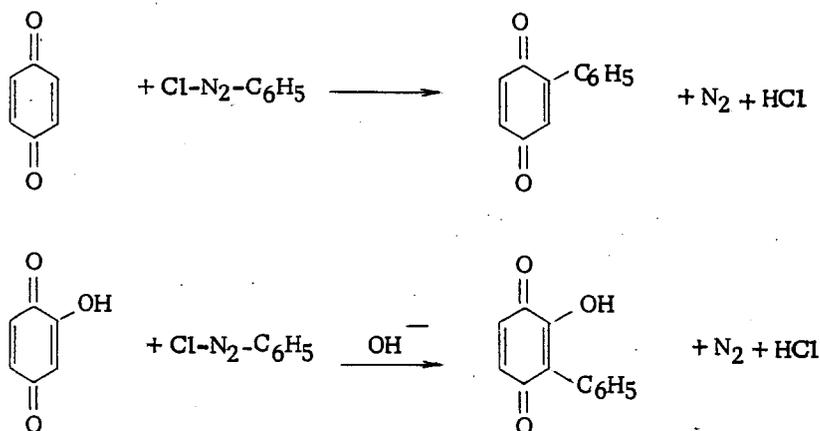
Since a part of the strong 1785 cm^{-1} band in the spectra of humic anhydrides formed by reaction with acetic anhydride can also be ascribed to esters -- and in particular to esters of polyfunctional phenols -- it is tentatively further thought possible that a proportion of the total $-\text{OH}$ content of humic acid may occur as groups of 2 or 3 $-\text{OH}$ residues on the same ring or condensed aromatic nucleus. This view is, however, subject to rather severe limitations since $-\text{OH}$ residues located ortho or para to each other would undoubtedly be converted to quinones by the conditions under which humic acids form.

IV. Quinone Structures

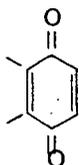
When humic acid is reacted with diazotized sulfanilic acid, a water-soluble derivative forms through introduction of $-N=N-C_6H_4-SO_3H$ and/or $-C_6H_4-SO_3H$. Both mechanisms, i.e. interaction with phenolic structures or with quinones, are consistent with infrared spectral evidence in that both would lead to the new absorptions indicative of the presence of sulfonic groups which are observed (Figure 2b); but since the former requires introduction into the humic acid molecule of two nitrogen atoms per additional sulfur atom (which analysis shows not to occur to any significant extent), it has been concluded that the dominant reaction involves a free radical attack on quinones at an available position.

Proceeding from this recognition, an interesting route to further elucidation of the structure of humic acid can be developed (9).

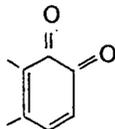
Of the two most probable theoretical free radical mechanisms by which a $-C_6H_4-SO_3H$ group is introduced into the humic acid molecule, one — a Gomberg reaction — must be ruled out on evidence offered by Gomberg and Pernert (10), Grieve and Hey (11), and Walling (12). There remain therefore only interactions of the type



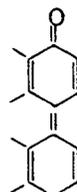
from which it follows that humic acid molecules reacting with the diazonium salt must contain one or more of the basic structures V, VI and VII.



V



VI



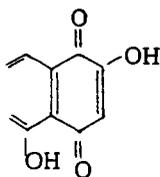
VII

Now, the possible existence of these (or similar) entities in humic acid has been recognized for some years. But more unequivocal information about them has now been obtained by infrared studies on methylated* and subsequently acetylated humic acids. Of particular importance was the existence in the spectra of these compounds of a fairly intense absorption band at 1660 cm^{-1} (Figure 2d), i.e. at the same frequency at which a wide range of relatively simple quinones (cf. Table I) show a diagnostic absorption. If this observation is viewed against the background of polarographic evidence (13) and x-ray diffraction data (14) — which preclude polycondensed ring systems of more than 4-5 rings in low and medium rank coals — it can be concluded with reasonable safety that while simple benzoquinones cannot be postulated, possible structures could include OH-substituted naphthoquinones and anthraquinones as well as more complex systems containing heterocyclic oxygen. Detailed examination of the infrared spectra obtained in these laboratories leads, in short, to the conclusion that structures VIII and IX appear to be the simplest consistent with presently available evidence.

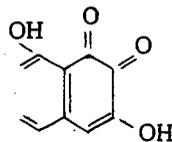
Table I. Carbonyl stretching frequencies of quinones

Compound	Frequencies cm^{-1}	Phase
1,4-Benzoquinone	1665	Mull
1,2-Benzoquinone	1669	CCl_4
1,4-Naphthoquinone	1662	Mull
1,2-Naphthoquinone	1678	CCl_4
Anthraquinone	1671	Mull
9,10-Phenanthraquinone	1670	Mull
Anthrone	1654	Mull
Bianthrone	1657	Mull
Bianthranyl	1656	Mull
Anthanthrone	1650	Mull
Dibenzypyrenequinone	1645	Mull
3,8-Pyrenequinone	1636	Mull
3,10-Pyrenequinone	1640	Mull
Dibenzanthrone	1645	Mull
isoViolanthrone	1646	Mull
Pyranthrone	1642	Mull
Helianthrone	1642	Mull
1,2-Chrysenequinone	1661	Mull
1,2-Benzanthra-9,10-quinone	1664	Mull
1,2,3,4-Dibenzanthraquinone	1662, 1749	Mull
1,2,5,6-Dibenzanthraquinone	1652	Mull
3,10-Perylenequinone	1650	Mull

- * Initial methylation was designed to block all readily accessible hydroxy groups of phenols and carboxyls and thereby to avoid interference due to acetate esters and anhydrides in the 1800 and 1740 cm^{-1} regions of the infrared spectra.



VIII



IX

It is, in this connection, noteworthy that estimates of the concentration of quinones in humic acid (9) by several different techniques yielded values of the order of 0.8 ± 0.2 milliequivalents/gm. The corresponding concentration of hydrogen-bonded quinones, which was derived from acetyl determinations on the assumption that each quinonoid oxygen is H-bonded to one phenolic -OH that can in turn be acetylated by acetic anhydride, was found to be 0.63 milliequivalent/gm. (and thus in good agreement with previously reported values; 15, 16, 17, 18).

An estimate of the carboxyl carbonyl:quinone ratio was obtained from the relative intensities of the 1660 and 1735 cm^{-1} absorptions on the assumption that the relationship between these bands was similar to that prevailing in the infrared spectrum of 2-(p-carboxyphenyl)-1, 4-naphthoquinone (which was chosen as a suitable model compound - Figure 2e). This ratio was found to be 14 as compared with 6 calculated from analytical data. While this discrepancy is large, it is, in view of the obvious inaccuracies entailed in the estimation via infrared spectra, not excessive.

With respect to the water-solubility induced in the humic acid derivative by reaction with the diazonium salt it might also usefully be noted that this appears only partly to arise from the introduction of sulfonic acid residues; a contributory factor seems to be cleavage of $-\text{CH}_2-$ bridges and the consequent reduction in molecular size. In support of this view attention is drawn to the identification of formaldehyde among the reaction products. The formative step for this would be



Recent experiments in these laboratories have shown that coals can undergo similar reaction with diazotized sulfanilic acid, but that the yield of water-soluble products decreases rapidly with increasing rank. While a lignite (C = 71% d.a.f.) showed after two treatments a ca. 80-90 per cent conversion, two subbituminous coals (C = 74 and 80% resp.) yielded after several treatments only about 50 to 70 per cent water-soluble derivatives, and a bituminous coal (C = 87%) proved almost wholly unreacted.

An alternative technique now actively being explored, i.e. sulfo-methylation (19,20,21) by means of $\text{CH}_2=\text{O}$ and NaHSO_3 leading to the addition of $-\text{CH}_2\text{SO}_3\text{H}$ to the aromatic nuclei, has been shown to produce analogous results (and, if anything, to be even more strongly affected by coal rank). It is, however, considered that yields of water-soluble products obtainable by this reaction, or by reaction with a diazonium salt, can be significantly increased by mild preliminary oxidation of the coal.

V. The Skeletal Structure

From measurements of the viscosities of humic acid solutions it has been concluded that humic acids may resemble flexible, linear polyelectrolytes (22). This conclusion, however, is considered untenable since humic acids show surprising stability in high-intensity ultrasonic fields (23); even prolonged exposure at frequencies up to 2 mc/s and intensities up to ~ 10 watts/cm² have failed to induce molecular rupture. Since the molecular weights of the test samples exceeded 5,000, i.e. a size at which linear molecules would degrade, it has, in the light of x-ray diffraction evidence, been postulated that the most probable skeletal structure involves relatively small (3-4 condensed ring) aromatic systems interlinked by mixed $-\text{O}-$ and $-\text{CH}_2-$ bridge structures. Linkage by $-\text{O}-$ only was ruled out by the fact that pyrolysis of humic acids yields considerable quantities of tarry matter (and, subsequently, by the detection of $\text{CH}_2=\text{O}$ as a by-product of reaction with diazonium salts, cf. above).

To test this general concept in detail, and to develop means for quantitative estimation of $-\text{CH}_2-$ and $-\text{O}-$ concentrations, current studies in these laboratories are directing attention to reactions of humic acid with (a) BF_3 in phenol, and (b) Na in liquid ammonia.

Interactions between coal and BF_3 -phenol have recently been reported by Heredy and Neuworth (24) who found 60-80% conversion of a high volatile bituminous coal into soluble products, presumably by the rupture of methylene bridges between aromatic nuclei. We have now observed humic acids to be completely soluble in this reagent mixture. The reaction appears to be accompanied by considerable molecular degradation* and several direct comparisons are possible between Heredy and Neuworth's and our results. Thus:

(a) A considerable increase in weight results from interaction between humic acid and BF_3 -phenol (e.g. from 13.6 to 29.0 gm); this indicates a combined phenol content of 53.2% which compares with a value of 55.7% reported by Heredy and Neuworth for one of their fractions.

(b) The reacted, BF_3 -phenol-soluble humic acid is, like the soluble coal fraction (above) of Heredy and Neuworth, soluble in methanol.

* Quite low molecular weight crystalline reaction products have been isolated from the mixture and tentatively identified by us. Details will be published elsewhere in due course.

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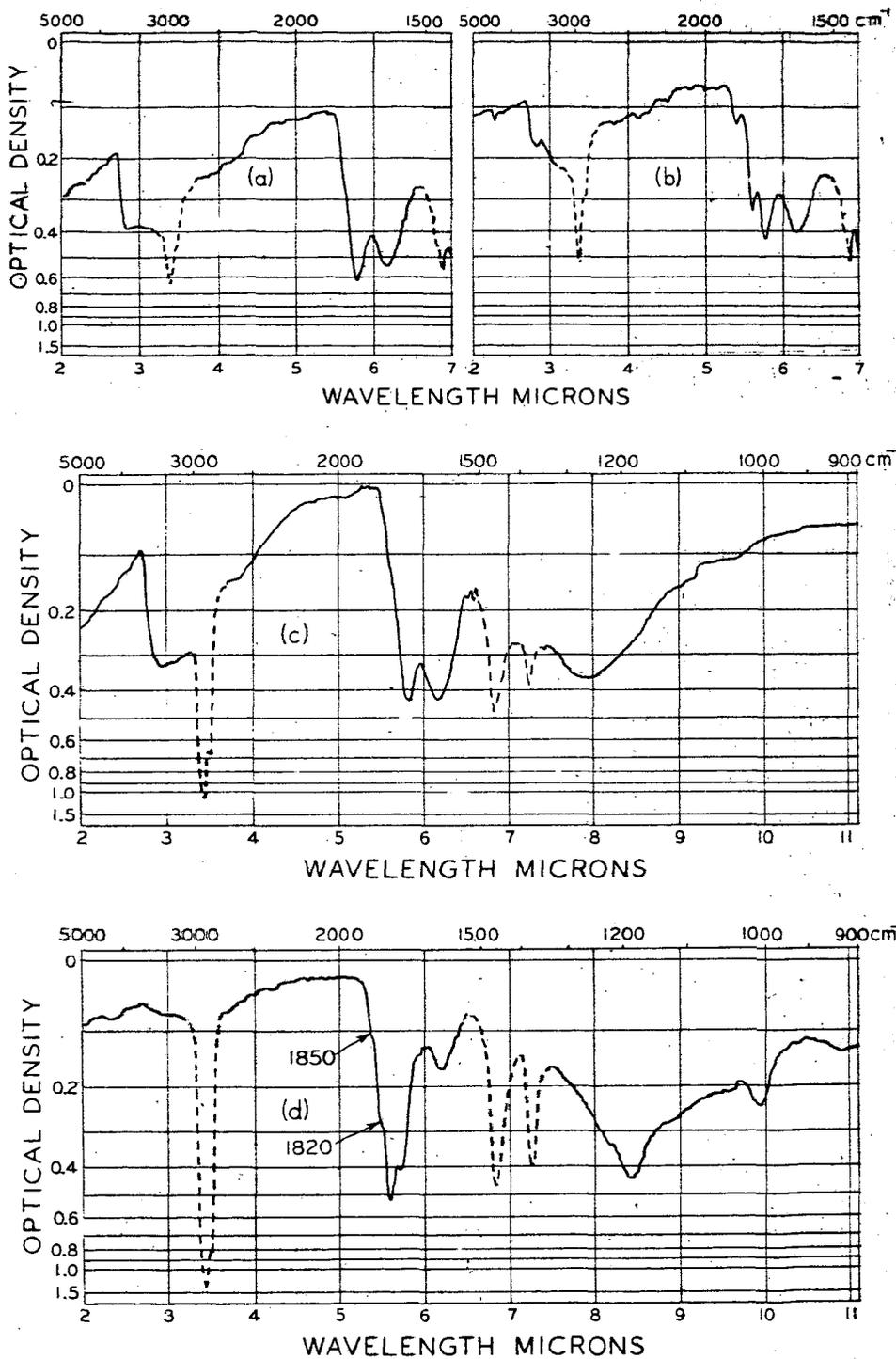


FIGURE 1. Humic acids and derivatives

- (a) parent humic acids (sulfolane mull). (c) parent humic acid (Nujol mull).
 (b) humic acids heated in sulfolane (sulfolane mull). (d) humic acids treated with boiling acetic anhydride (Nujol mull).

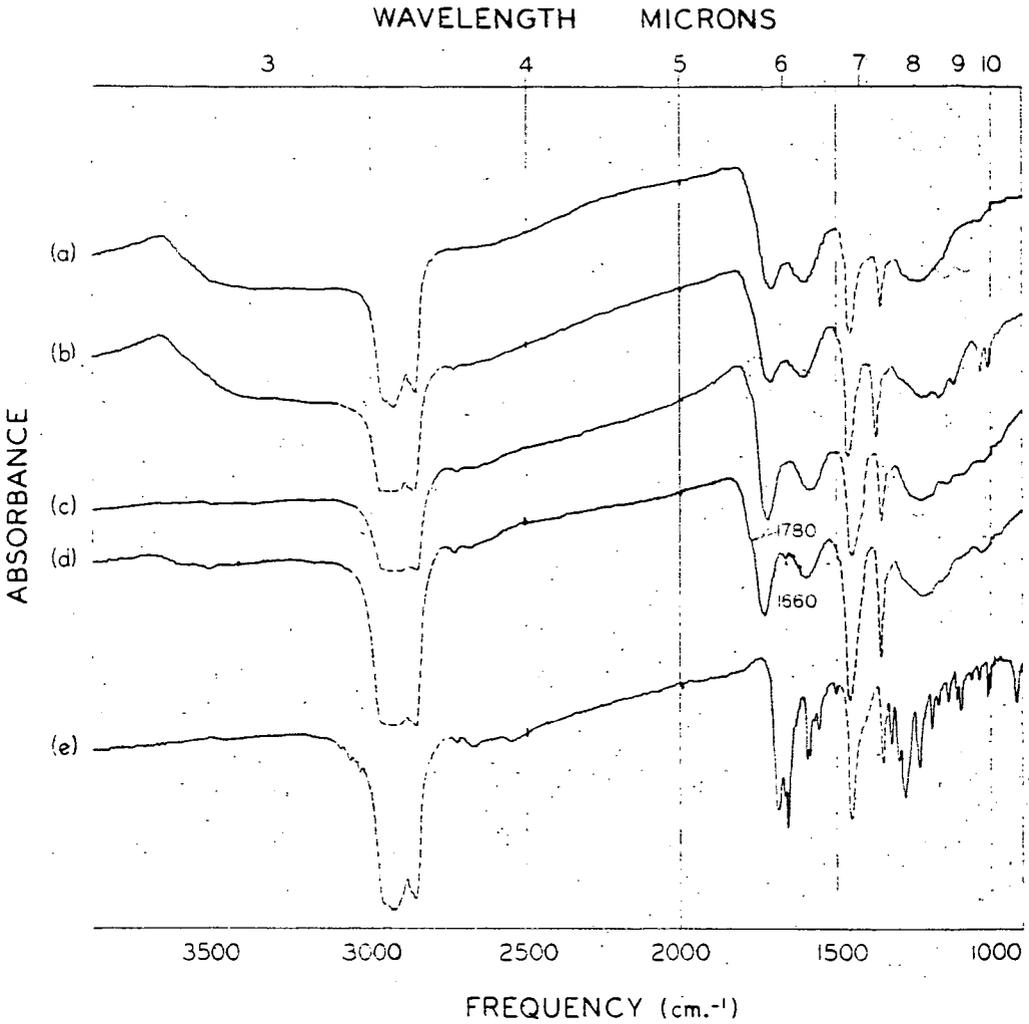


FIGURE 2. Humic acids and derivatives.

- (a) parent humic acids.
- (b) treated with diazotized sulfanilic acid.
- (c) methylated.
- (d) product of (c) treated with acetic anhydride.
- (e) 2-(p-carboxylphenyl)-1,4-naphthoquinone.