ELSEVIER

PII: S0016-2361(96)00194-9

Fuel Vol. 76, No. 2, pp. 149–154, 1997

0016-2361/97/$17.00 + 0.00

Non-catalytic oxidation of water-slurried coal with oxygen: identification of fulvic acids and acute toxicity

Jacobus J. Bergh, Isak J. Cronjé*, Johannes Dekker*, Theodor G. Dekker, L. Marcia Gerritsma and L. Jakobus Mienie†

Department of Pharmaceutical Chemistry, Potchefstroom University for C.H.E., Potchefstroom, 2520, Republic of South Africa

** Division of Energy Technology, C.S.I.R., Box 217, Pretoria, 0001, Republic of South Africa*

† Department of Biochemistry, Potchefstroom University for C.H.E., Potchefstroom, 2520, Republic of South Africa

(Received August 1994; revised June 1996)

The composition and toxicity of the aqueous oxidation fraction of coal were investigated, to gain information concerning process safety measures and the utilization of the oxidation products as antibacterial agents. Coal was oxidized with oxygen at 180°C under constant pressure (4 MPa). Fractions of the crude filtrate obtained during this process were sublimed, distilled and extracted with ether and ethanol. Almost 50 different acids were identified in these samples using g.c. and g.c.–m.s. analyses. The following groups of acids could be distinguished: (1) all possible unsubstituted acids containing up to four carbon atoms: (2) higher carboxylic acids containing even numbers of carbon atoms, agreeing with occurrence in nature; (3) oxygenated straight-chain or branched acids in the hydroxy or keto form containing up to six carbon atoms; (4) dicarboxylic aliphatic acids containing up to six carbon atoms; and (5) benzoic acid and its monohydroxy derivatives and phthalic acid. No highly toxic compounds could be identified, most of the compounds being common physiological metabolites. Primary acute toxicity studies were carried out on rats, using the crude aqueous solution and the drum-dried product of this solution. Apart from local irritation caused by their acidic nature, neither of these fractions exhibited significant acute toxicity in the test animals. *Copyright © 1997 Elsevier Science Ltd.*

(Keywords: coal oxidation; fulvic acids; toxicity)

The oxidation of coal attracts the attention of researchers for three main reasons[1,2]: first, to gain more knowledge about its auto-ignition (a problem encountered in the storage and transportation of coal); second, to obtain information on the structure of coal; and third, to convert coal into acids, commonly grouped into humic (water-insoluble) and fulvic (water-soluble) acids.

The structure of coal suggests that its oxidation should lead to a number of organic acids, both phenolic and carboxylic, aliphatic and aromatic[1,3]. The composition of the product mixture depends on a number of factors, such as the rank of the coal, the type of oxidant and the oxidation conditions[1,3]. In a few instances it has been shown that the oxidation can be used for the production of specific acids, e.g. terephthalic acid[3,4].

The oxidation of coal by air under dry conditions can be controlled to produce humic acids in high yield[2,5]. The interest in humic acids mainly stems from their possible applicability in agriculture, for instance as soil conditioners[1], fertilizers[2] and growth stimulants[6].

Recently, a process for the non-catalyic oxidation of an aqueous suspension of coal with oxygen or air was described[7–9]. The humic and fulvic acid mixtures thus produced showed interesting antimicrobial properties, especially for industrial application[7,8,10]. In addition, it has been shown that the use of coal-derived calcium fulvate as an ameliorant for acid and calcium-deficient subsoils is very promising[11].

In pursuit of these findings, the nature of the fulvic acid product was further investigated. The aim of the investigation was to obtain information about the composition and the toxicity of the aqueous fraction of the oxidation product. The information could be of importance with regard to (1) process safety measures and (2) the utilization of the water-soluble product mixtures or fractions thereof as antibacterial agents. This paper reports the identification of more than 40 compounds in the fulvic acid fraction, as well as the results of acute toxicity studies. These results complement the antibacterial properties reported[8,10] for the fulvic acid mixture and lend further support to the possible application of the mixture as an industrial antimicrobial agent.

EXPERIMENTAL

Oxidation of coal with oxygen

A well-stirred slurry of 25 μm median size South

African bituminous coal (200 g) in water (400 mL) was preheated under constant pressure (4 MPa). When the desired temperature (180°C) was reached, oxygen was allowed to flow through the slurry at a rate of 8 L min^{-1} for 1 h. During this period the temperature of the reaction mixture was kept at 180°C (due to the exothermic nature of the reaction, cooling had to be applied). The reaction mixture was cooled to room temperature and the water-insoluble matter (containing humic acids) removed by filtration. The oxidation filtrate, containing the fulvic acids, was used as stock solution in subsequent experiments.

Preparation of distillate

The oxidation filtrate obtained above was distilled in a closed glass unit until ~75% of the volume was transferred. The distillate was stoppered and stored in a cool, dark area.

Preparation of sublimate

The solid product mixture obtained by evaporation of the oxidation filtrate to dryness was sublimed under moderate vacuum at temperatures between 110 and 150°C in an all-glass unit. The sublimate was stored in a cool, dark area.

Analyses

Lower (volatile) carboxylic acids in the distillate. An aliquot of the distillate was mixed with formic acid (4 : 1) and injected into a gas chromatograph (column: 30 m × 0.25 mm fused silica, Nukol acidic bonded phase 0.25 μm; column head pressure, 120 kPa; nitrogen flow rate, 1.2 mL min^{-1}; split, 1 : 40; injection temperature, 180°C; temperature programme, hold 5 min at 110°C, 110–180°C at 8 K min^{-1}). Compounds were identified by comparing retention times with those obtained from a standard solution containing reference standards.

Less volatile acids in the distillate. An aliquot of the distillate was freeze-dried until the water was totally removed. The residue was derivatized (80°C, 30 min) for g.c.–m.s. analysis with *N,O*-bis(trimethylsilyl)-trifluoroacetamide (BSTFA) (200 μl) and trimethylchlorosilane (TMCS) (40 μl). G.c.–m.s. analyses were carried out on a Hewlett Packard 5988A mass spectrometer equipped with a Hewlett Packard 5890 g.c. (column: SE 30, 25 m × 0.32 mm capillary column; helium flow rate, 1 ml min^{-1}; temperature programme, 60–120°C at 4 K min^{-1}, then to 280°C at 10 K min^{-1}). An ionization energy of 70 eV was used.

Keto acids in the distillate. The keto acids were analysed as their *O*-trimethylsilylquinoxalinol derivatives. Five mL of a freshly prepared solution of 500 mg *o*-phenylenediamine in 2 M HCl was added to 5 mL of the freeze-dried material and incubated overnight in the dark at room temperature. The solution was extracted three times with 25 mL aliquots of chloroform and evaporated to dryness. G.c.–m.s. analysis was done according to the procedure as described for the less volatile acids.

Less volatile acids in the sublimate. An aliquot of the sublimate was derivatized and analysed as described for the distillate.

Extracts of the oxidation filtrate. Three extracts were prepared:

- The oxidation filtrate (200 mL) was mixed with aluminium oxide (200 g) and stirred for 10 min. Ethanol (100 mL) was added and the mixture was stirred for 5 min and filtered. The ethanol extract was collected for analysis.
- The residue from the above experiment was stirred for 15 min with diethyl ether (100 mL) and filtered. The ether extract was collected for analysis.
- The oxidation filtrate (100 mL) was extracted with ether (3 × 100 mL).

The extracts were analysed for acids and keto acids as described for the less volatile acids in the distillate.

Primary acute toxicity studies

The Hippocratic screen of Malone and Robichaud[12] was used to evaluate the acute toxicity of the crude aqueous solution and drum-dried product in rats. Modifications of this method were the inclusion of oral dosage, the use of more than one rat per dosage and the addition of histological examination of the test and control animals. Rats were used instead of mice because of the following advantages[13]: they are more resistant to infection than mice, and their size makes them easier to handle than mice. Larger volumes, which enhance the accuracy of the dosages, can be administered to rats and behavioural changes are easier to observe.

Phase 1 study (determination of dose range)

Mammalian model:
male Sprague–Dawley rats weighing about 120 g

Test samples (as supplied):
1 FAS: crude aqueous solution obtained from the oxidation
2 FAP: drum-dried powder obtained from FAS

Dosage form:
1 FAS: the pH was adjusted to 4.7–4.9 with KOH
2 FAP: solutions were made in sterilized water and filtered. The pH was unadjusted or adjusted to pH 4.7–4.9 as for FAS

Volume and route of administration:
orally (po) or intraperitoneally (ip), a dose not exceeding 1 mL

Dosage levels:
1 FAS: 0.25, 0.5 or 1.0 mL per rat
2 FAP: 10, 100 or 1000 mg kg^{-1} per rat

Observation period:
7 days

Post-mortem examination:
1 lethal dosages: as soon as possible after death
2 sub-lethal dosages: after euthanasing with barbiturate at the end of the observation period.

Phase 2 study. In the phase 2 study only the maximum intraperitoneal dosages of FAP and FAS were used.

Test samples:
1 FAP: a dosage of 1 g kg^{-1} was used. A solution was made in sterilized water, filtered and the pH adjusted to

Table 1 Compounds identified in the aqueous fraction obtained from the oxidation of coal

Compound	Individual analyses[a]				
	1	2	3	4	5
Formic acid	X				
Acetic acid	X				
Propionic acid	X				
n-Butyric acid	X				
Isobutyric acid	X				
Lactic acid	X	X	X	X	X
2-Hydroxyisobutyric acid	X				
Glycolic acid	X	X	X		X
Pyruvic acid	X				
Levulinic acid	X		X	X	X
Thymol					X
Isovaleric acid					X
Hydracrylic acid			X	X	X
3-Hydroxybutyric acid	X		X	X	
Methylmalonic acid				X	
4-Hydroxybutyric acid	X		X	X	
3-Hydroxypropionic acid	X				
Benzoic acid	X		X		X
Isonicotinic acid				X	
Nicotinic acid				X	X
Acetoacetic acid			X	X	
Succinic acid	X	X	X	X	X
Methylsuccinic acid			X	X	X
Itaconic acid	X				X
Fumaric acid	X	X	X		X
4-Hydroxyisovaleric acid	X				
Glutaric acid	X	X	X	X	X
2/3-Methylglutaconic acid	X				
cis-3-Methylhexenedioic acid	X				
trans-3-Methylhexenedioic acid	X				
cis-2-Methylhexenedioic acid	X				
trans-2-Methylhexenedioic acid	X				
3-Methylheptenedioic acid	X				
2-Methyl-4-ketoglutaconic acid	X				
2-Methylglutaric acid	X				X
Adipic acid			X	X	X
o-Hydroxybenzoic acid			X	X	X
Malic acid					X
m-Hydroxybenzoic acid	X		X	X	X
2-Hydroxyglutaric acid	X				
Pimelic acid					X
p-Hydroxybenzoic acid	X		X	X	X
Dodecanoic acid	X				
Methylxanthine					X
o-Phthalic acid	X	X	X	X	
Hippuric acid			X	X	X
$\alpha + \beta$-Resorcylic acids			X		
3,4-Dihydroxybenzoic acid				X	X
Palmitic acid	X	X			
Stearic acid	X				

[a] X indicates positive result. 1: Compounds identified in distillate. 2: Compounds identified in sublimate. 3: Compounds identified in alumina–ethanol extract of crude aqueous solution. 4: Compounds identified in alumina–ether extract of crude aqueous solution. 5: Compounds identified in ether extract of crude aqueous solution

4.75 with KOH. The concentration of the solution was such that a 1 mL dosage per rat could be used

2 FAS: the provided solution was filtered and the pH adjusted to 4.84 with KOH: 1 mL of this solution was used per rat

3 Controls: 1 mL of sterilized water per rat

Number of animals:

male Sprague–Dawley rats: eight per sample plus four controls

Administration route:

Intraperitoneally.

RESULTS AND DISCUSSION

Production

The direct oxidative conversion of coal to humic and fulvic acids has been studied by various groups[1,3]. It appears that three of the routes have potential industrial application:

(1) partly pressurized oxygen/air oxidation in aqueous alkaline medium;
(2) oxidation with nitric acid;
(3) the recently developed[2,5] dry-phase oxidation with air.

The first two routes have in common a high consumption of expensive chemicals.

The novel wet oxidation route, described by Dekker and co-workers[7–9], proceeds smoothly without the need for any added chemicals (base or acid) or catalysts. Furthermore, it has been found that air can be used instead of oxygen without any detrimental effects. Based on carbon content, ~ 8 wt% of the coal is converted to water-soluble fulvic acids within 1 h, with < 20% carbon dioxide formation (by mass of products).

The water-insoluble matter, i.e. oxycoal containing humic acids, is removed from the cooled product mixture by filtration. The filtrate, containing the fulvic acids together with some inorganic material, is dark brown in colour and has a pungent odour. When this aqueous product is dried on a drum evaporator, a dark brown powdery solid is obtained.

The crude aqueous reaction product contains both volatile and steam-volatile compounds. Both the steam distillate of the aqueous fraction and the sublimate of its dried product are colourless. The steam distillate especially may have some practical value as, for instance, an antibacterial agent. This is not only due to its more attractive, colourless appearance, but also because the process can be terminated by depressurizing the hot product mixture and therefore releasing part of the water therein. Since the authors' attention has lately focused on an oxycoal-derived plant growth stimulant and soil ameliorant, the utilization of the steam distillate becomes even more attractive.

Fulvic acid composition

The organic compounds present in the aqueous oxidation product were identified by g.c. and g.c.–m.s. analyses. The acids identified with a high degree of probability are listed in *Table 1*.

Since it was expected that the crude aqueous oxidation product could contain a vast number of acidic products, ranging widely in molecular mass, it was decided to investigate the steam-volatile products initially. This decision was instigated by the observation that the steam collected during the concentration of the aqueous oxidation product displayed a pungent odour, typical of volatile carboxylic acids.

The steam-volatile compounds were collected in the distillate from a straight distillation of the crude fulvic acid solution. The lower acids in the distillate were identified by means of g.c. retention times, using a fatty acid column. All the possible acids containing up to four carbons (acetic, propionic, *n*-butyric and isobutyric acids) were present in the mixture, with the concentration of acetic acid by far the highest. Evidence was also found for the presence of formic acid, although not explicitly under the experimental conditions used.

Table 2 Summary of the results of the phase 1 study for the acute toxicity profile of FAS and FAP[a]

Sample	Route	Dose	No	Nd	Onset of death	Post-mortem observations
FAP	ip	10 mg kg^{-1}	2	0	–	normal
pH = 1.46	ip	100 mg kg^{-1}	2	0	–	normal
	ip	1000 mg kg^{-1}	2	2	6 h, 3 d	'burnt' peritoneum
FAP	ip	10 mg kg^{-1}	2	0	–	normal
pH = 4.85	ip	100 mg kg^{-1}	2	0	–	normal
	ip	1000 mg kg^{-1}	2	0	–	normal
FAP	ip	10 mg kg^{-1}	2	0	–	normal
pH = 4.84	ip	100 mg kg^{-1}	2	0	–	normal
	ip	1000 mg kg^{-1}	2	0	–	normal
FAS	ip	0.25 mL	2	0	–	normal
pH = 4.77	ip	0.5 mL	2	0	–	black focus on ileum
	ip	1.0 mL	2	0	–	black focus: caecum & stomach
FAS	po	0.25 mL	2	0	–	normal
pH = 4.8	po	0.5 mL	2	0	–	normal
	po	1.0 mL	2	0	–	normal

[a] FAS = crude solution obtained from the oxidation. FAP = drum-dried powder obtained from FAS. No = number of rats per experiment. Nd = number of rats deceased during experiment. 6 h = 6 hours after administration. 3 d = 3 days after administration

For the detection of less volatile carboxylic acids, the distillate was freeze-dried and treated with BSTFA and TMCS, and the silanized acids were analysed by means of g.c.–m.s., using the retention times and computerized mass spectral matching. Four major types of acids have been identified by means of this analysis (*Table 1*):

- Oxygenated straight chain or branched acids, in the hydroxy or keto form, containing up to six carbon atoms. The oxy function was not limited to a specific position, i.e. it could be in the α, β, etc. position.
- Dicarboxylic aliphatic acids containing up to six carbon atoms. Oxygenated and unsaturated dicarboxylic acids were also detected. The unsaturated acids may have been artefacts: they could be the product of the hydroxy acids under the strong dehydrating silylation conditions.
- Higher carboxylic acids (dodecanoic, palmitic and stearic acids). A common factor is that these acids contain an even number of carbons, in accordance with natural occurrence.
- Benzoic acid and its monohydroxy derivatives and phthalic acid.

A major problem in the identification of the oxygenated acids was that they could form disilanized derivatives, in the case of the α-keto acids via the enolic form. The mass spectra of the products were in some cases dwarfed by the two ions at m/z 147 and 73, resulting from the trimethylsilyl groups[14]. According to the computerized identification, quite a number of keto or dicarboxylic acids could match some of these mass spectra. To verify the presence of keto acids in the distillate, a g.c.–m.s. method specific for keto acids was used[15]. Pyruvic acid was found to be the most abundant of the keto acids.

It seemed reasonable to assume that the solid product obtained by the drum drying of the oxidation filtrate could contain volatile products. The analysis was therefore extended to the sublimate, obtained by heating the solid product to 150°C under vacuum. The sublimate contained mainly phthalic acid, to a lesser extent fumaric acid and to a minor extent a number of other volatile acids (*Table 1*). These compounds fit well into the general structural pattern set out for the distillate.

The steam-volatile and volatile products will be lost, at least partly, during the drum drying process.

With the knowledge of the nature of the volatile and steam-volatile compounds formed during the oxidation process, the analysis was extended to the crude aqueous fraction itself. First, to get rid of the polymeric and coloured substances, the aqueous oxidation product was adsorbed on alumina (equal parts) and the powdery mixture was then extracted consecutively with ethanol and ether. The ethanol and ether extracts were analysed by g.c.–m.s. as described above for the distillate (*Table 1*). Second, the crude aqueous oxidation product was extracted directly with ether and analysed in the same way (*Table 1*).

The compounds identified in the extracts obtained from the alumina-adsorbed product and from the untreated oxidation product showed a high degree of similarity, and most of these compounds were also present in the steam distillate. Significant additions were thymol (2-isopropyl-5-methylphenol, which occurs naturally in many essential oils) and the nitrogen compounds nicotinic and isonicotinic acids, methylxanthine and hippuric acid. The presence of hippuric acid (*N*-benzoylaminoacetic acid) may be the result of a condensation reaction between benzoic acid and aminoacetic acid (glycine) under the dehydrating silylation conditions.

Concentrations were not determined, but from the signal intensities in the g.c. chromatograms, concentration patterns could be established. The saturated unsubstituted dicarboxylic acids, such as succinic and glutaric acid, and the aromatic carboxylic acids, i.e. benzoic acid and derivatives, were present in relatively high concentrations. The substituted dicarboxylic acids and the whole range of monocarboxylic acids, except for acetic acid, can generally be regarded as minor reaction products.

Almost all of the compounds that could be detected in

Table 3 Phase 2 study: lethality and toxic signs of FAP and FAS

Sample	Dose	No	Nd	Death onset	Toxic signs (number of animals)
FAP pH = 4.75	1 g kg^{-1}	8	2	30 min	* shock reaction during first few hours (6) * loss of weight: original weight regained after two days (5) * lethal dose: dyspnea, convulsions
Control	1 mL	4	0	–	* none
FAS pH = 4.84	1 mL	8	0	–	* shock reaction during first few hours (8) * loss of weight: original weight regained after two days (6)
Control	1 mL	4	0	–	* none

Table 4 Phase 2 study: Post-mortem macroscopic and histological changes in organs caused by FAP and FAS

Sample	Macroscopical (number of animals)	Histopathological (number of animals)
FAP pH = 4.75	* lung: congestion (2) & petechiae (3) * heart: brown foci (3) * adhesions: black, between liver and pancreas (1)	* mild brain lesions (1) * mild liver lesions (5) * mild kidney lesions (1)
Control	* lung: congestion (1) & petechiae (2)	* no specific lesions
FAS pH = 4.84	* lung: petechiae (1) * heart: necrotic areas (1) & dark foci (2) * thymus: petechiae (1) * adhesions: between organs of the peritoneal cavity * spleen: thickened capsule * brain: congestion of dural sinuses (1)	* mild brain lesions (8) * aseptic peritonitis (3)
Control	* lung: petechiae (1) & emphysema (1) * heart: necrotic areas (1) * thymus: petechiae (1) * brain: mild congestion of dorsal sinuses (1)	* no specific lesions

the g.c.–m.s. analyses were identified by comparison with standard or computerized m.s. references, taking the g.c. retention times into account. The paraffinic substances (low occurrence) were not identified.

Amazingly, most of the identified compounds are ordinary physiological metabolites[16]. Therefore, from the present knowledge, there is no evidence of any highly toxic compound in the product mixture (the term 'toxicity' must obviously be treated with care, depending on the dose and contact position with the body). The distillate, whose toxicity may be of specific concern in a manufacturing process, is furthermore freed from inorganic material and the possibility that it contains particularly harmful compounds seems reasonably low. It must of course be understood that the product mixture and its distillate will cause the usual irritating effects associated with such acidic compounds. The conclusions drawn from the chemical composition are largely substantiated by the biological studies.

The inorganic material present in the product is mainly silicon-derived. Combustion of the solid product produced a white powder and electron microscopic analysis showed that this residue consisted predominantly of silicon dioxide.

Acute toxicity studies

In view of the possible commercialization of the oxidation process, knowledge about the toxicity of the products is of prime importance. This not only concerns the commercial product, but is also important for plant safety and waste disposal measurements.

Primary acute toxicity tests were performed on the crude aqueous product (FAS) and its drum-dried product (FAP). These tests were carried out in two

phases. Phase 1 entailed a dose range study, using two rats per dosage and three dosage levels, i.e. 10, 100 and 1000 mg kg^{-1}. In phase 2 the dosage levels, including low-mortality and sub-lethal doses, based on phase 1 results, were used with eight animals per dose. Four control animals received only the vehicle (solvent). The following facets were observed:

(1) lethality: the range in which the minimum lethal dosage (MLD) occurs and the speed of onset of death;
(2) toxic signs: observed at both lethal and sub-lethal dosages;
(3) lesions to organs: determined by macroscopic and histological post-mortem examination at lethal and sub-lethal dosages.

The results of the phase 1 study are summarized in *Table 2*. The burnt appearance of the intestines of the two rats receiving a solution of the drum-dried powder (FAP) at a pH of 1.46 (*Table 2*, first experiment) indicated excessive acidity of the sample. Therefore all further tests were done at pH values >4, which is compatible with the biological system. Black foci were detected in the peritoneal cavity of animals injected with the crude aqueous oxidation product (FAS); this could be attributed to insoluble matter which was subsequently removed by filtration for the phase 2 study. As both the FAS and FAP samples were found to be non-lethal at their respective highest dosages, only these dosages were used during the phase 2 study.

The results obtained from the phase 2 study are summarized in *Tables 3* and *4*. The following remarks can be made with regard to these results:

(1) All animals receiving the sample solutions (FAP and FAS) exhibited signs of shock during the first few hours. This is a common phenomenon following the administration of large sub-lethal dosages. Loss of weight caused by a reduced food intake during the first day was noted and is also a common phenomenon.
(2) Macroscopically observed lesions of the lungs, which also occurred in the control rats, were attributed to common infections of the lungs which generally occur among colonies of test animals. Macroscopically observed petechiae of the thymus and small confined necrotic areas of the heart, which were also evident in the control animals, are not regarded as significant.
(3) The small, dark areas of the heart muscle, which were noticed only with the animals receiving FAP and FAS, were probably caused by the brown substances in the sample solutions.
(4) The dark-coloured adhesions between organs in the peritoneal cavity suggested local irritation by the samples and were also histopathologically identified as aseptic peritonitis.

The following conclusions could be drawn from the phase 2 study:

(1) FAP and FAS samples both caused local irritation in the peritoneal cavity with some test animals, resulting in aseptic peritonitis.
(2) The powder (FAP) exhibited an MLD-value of $\sim 1\ g\ kg^{-1}$ and mainly caused only mild lesions of the liver.
(3) The solution (FAS) was not lethal at the dosage used of 1 mL per rat, but caused brain lesions in all the test animals, although only of a mild nature.

The aqueous oxidation product therefore showed no particular signs of significant acute toxicity in rats. However, its acidic character resulted in local irritation, as would be expected for the types of compounds identified in the product.

REFERENCES

1 Wender, I., Heredy, L. A., Neuworth, M. B. and Dryden, I. G. C., in *Chemistry of Coal Utilization*, Second supplementary volume, ed. M. A. Elliott. Wiley, New York, 1981, p. 455.
2 Estévez, M., Juan, R., Ruiz, C. and Andrés, J. M., *Fuel*, 1990, **69**, 157.
3 Bearse, A. E., Cox, J. L. and Hillman, M., Production of chemicals by oxidation of coal. A Battelle Energy Program Report, 31 March, 1975.
4 Schep, R. A., U.S. Patent No. 4,345,098, 1982 (*Chem. Abstr.* 1982, **97**, 183027).
5 Schwartz, D., Hall, P. J. and Marsh, H., *Fuel*, 1989, **68**, 868.
6 Van de Venter, H. A., Furter, M., Dekker, J. and Cronjé, I. J., *Plant and Soil*, 1991, **138**, 17.
7 Cronjé, I. J., Cloete, T. E. and Dekker, J., U.S. Patent No. 4,999,202, 1991.
8 Cronjé, I. J., Cloete, T. E., Dekker, J. and Swart, H., S. Afr. Patent No. 91,03311, 1992.
9 Dekker, J., Cronjé, I. J., Louwrens, H. B. and Swart, H., in *7th Annual International Pittsburgh Coal Conference*. Pittsburgh, 1990.
10 Cloete, T. E., Swart, H., Cronjé, I. J. and Dekker, J., in *3rd International Symposium on Gas, Oil, Coal and Environmental Biotechnology*. Institute of Gas Technology, Chicago, 1990.
11 Van der Watt, H. v. H., Barnard, R. O., Cronjé, I. J., Dekker, J., Croft, G. J. B. and van der Walt, M. M., *Nature*, 1991, **350**, 146.
12 Malone, M. H. and Robichaud, R. C., *Lloydia*, 1962, **25**, 320.
13 Malone, M. H., in *New Natural Products and Plant Drugs with Pharmacological, Biological or Therapeutic Activity: Proceedings of the International Congress on Medicinal Plant Research*, Section A, ed. H. Wagner and P. Wolff. Springer-Verlag, Munich, 1976, p. 23.
14 Goodman, C. I. and Markey, S. P., in *Laboratory and Research Methods in Biology and Medicine*, Vol. 6. Alan R. Liss Inc., New York, 1981, p. 123.
15 Chalmers, R. A. and Lawson, A. M., *Organic Acids in Man: Analytical Chemistry, Biochemistry and Diagnosis of Organic Acidurias*. Chapman & Hall, London, 1982, p. 43.
16 Tanaka, K., Hine, D. G., West-Dull, A. and Lunn, T. B., *Clinical Chemistry*, 1980, **26**, 1839.