

# BIOMARKERS IN THE BITUMINOUS FRACTION OF A SPANISH BROWN COAL

J.C. DEL RIO\*, F.J. GONZALEZ-VILA and F. MARTIN

*Instituto de Recursos Naturales y Agrobiología, C.S.I.C., Apartado de Correos 1052 E.P., 41080 Sevilla, Spain*

## ABSTRACT

Molecular indicators such as diterpenoid and triterpenoid hydrocarbons and steroid alcohols and ketones have been identified in the bituminous fraction of the lignite from Arenas del Rey (AR), South Spain. Diterpenoid hydrocarbons consists mainly of kaurenes and are indicative of higher plant contributions, specially from conifer resins. Triterpenoids consist mainly of  $17\beta(H),21\beta(H)$ -hopanes derived from recent biogenic activity which indicates the geological immaturity of this lignite and implies that it has undergone only a mild thermal history. Sterols consist of cholesterol, campesterol, stigmasterol and  $\beta$ -sitosterol and their distribution suggests a possible phytoplankton input, which should confirm the geological hypothesis that this sediment had an estuarine or deltaic origin. Occurrence of stanones with the biological  $5\alpha$  configuration was also observed.

**Keywords:** brown coal, kaurenes, triterpenoids, sterols, stanones

## INTRODUCTION

Biomarkers are organic compounds present in the geosphere whose structures can be linked to the structures of precursor compounds occurring in original source materials. The detection of biomarkers in fossil fuel solvent extracts have had a great input in the exploration for and characterization of new fossil fuel resources, including crude oils, coals and oil shales, (Philp, 1985).

Biomarkers in coals have not received much attention, which is somewhat surprising since there is a possibility that they could be used for producing stratigraphic correlations of coal seams in analogous fashion to the oil/source rock cor-

relations described by many authors (Philp, 1985).

In recent years, however, the search for biological markers in low-rank coals has received increasing attention (Chaffee and Johns, 1983; Noble et al., 1985), since they constitute intermediate materials in the coalification process, and therefore their organic matter could provide valuable information on the diagenesis of fossil organic matter.

In the present communication, we report the presence and distribution of biomarkers in a Spanish lignite, giving special emphasis to the isolation and identification by capillary GC/MS of complex biomarkers such as di- and triterpenoid hydrocarbons and steroid alcohols and ketones, in order

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\* Corresponding author

to determine the origin and maturity of this sediment.

## MATERIALS AND METHODS

The lignite sample was taken at a depth of 2 m from the Arenas del Rey (AR) deposit, belonging to the Miocene Granada basin (South Spain). Its chemical and geochemical characteristics are shown in Table 1.

The lignite sample was ground to pass a 270 mesh screen and Soxhlet extracted with toluene for 50 hours. The yield of the crude extract obtained after vacuum evaporation at 50°C was 3.8% of the total organic matter content.

A portion of the crude extract was refluxed with isopropanol at 82°C for 1 hour to separate waxes (hot isopropanol-insoluble bitumen) and resins (hot isopropanol-soluble bitumen).

Resins were further fractionated by preparative thin-layer chromatography (TLC) on silica gel, using petroleum ether:diethyl ether (80:20) as eluent. Nine well UV-differentiated subfractions were isolated and desorbed with chloroform.

Prior to the analysis of individual components by gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS), the different subfractions were methylated with ethereal diazomethane and silylated by addition of N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA).

A Hewlett Packard 5730A gas chromatograph equipped with flame ionization detector (FID) and a Hewlett Packard 5988A GC/MS computer system were used for separation and identification of individual components. Separation of compounds was achieved using a 25 m (0.2 mm i.d.) SE-52 fused silica capillary column, with the oven temperature programmed from 50°C

TABLE 1

Average analyses of the Arenas del Rey lignite

<b>PROXIMATE %</b>	
moisture	11.10
ash	55.52
volatile matter	25.31
fixed carbon	8.07
<b>ULTIMATE % (moisture-ash free)</b>	
carbon	58.53
hydrogen	3.84
nitrogen	1.33
sulfur	4.11
oxygen	32.19
<b>MACERAL CONTENTS % (ash free)</b>	
vitritine	79.9
exinite	0
inertinite	20.1
<b>HEATING VALUE (Kcal/kg)</b>	<b>1352</b>

to 100°C at a rate of 32°C/min, and then from 100 to 300°C at 6°C/min, with 15 min final hold. Helium at a flow rate of 1.5 ml/min was used as carrier gas. Mass spectra were measured at 70 eV ionizing energy.

The recognition of each compound was achieved using capillary gas chromatography (on the basis of retention times and coinjection with standards), mass fragmentography (by the key single ion monitoring, SIM, for the different homologous series), low resolution mass spectrometry and comparison with published and tabulated data.

## RESULTS AND DISCUSSION

Table 2 shows all the different series of compounds isolated from the various subfractions with indication of the carbon atom ranges and predominant homologs in each series. From these series, only those of more

TABLE 2

Homologous series isolated from the AR lignite resin fraction

Homologous series	Carbon atom ranges	Predominant homologs
n-alkanes	C(17)–C(37)	C(23)
diterpenoid hydrocarbons	C(20) various	–
triterpenoid hydrocarbons	C(27)–C(32)	C(27)
n-aldehydes	C(15)–C(30)	C(23)
n-alkan-2-ones	C(15)–C(35)	C(25)
n-alkan-3-ones	C(16)–C(31)	C(22)
stan-3-ones	C(27)–C(29)	C(29)
n-fatty acids	C(10)–C(32)	C(16),C(24)
unsaturated fatty acids	C(15)–C(18)	C(18)
w-hydroxyacids	C(16)	C(16)
n-alkan-1-ols	C(12)–C(32)	C(18),C(24)
n-alkan-2-ols	C(21)–C(31)	C(25)
sterols	C(27)–C(29)	C(27)

complex biomarkers will be discussed in this work.

### *Diterpenoid hydrocarbons*

Partial chromatogram of the diterpenoid hydrocarbons detected in the fraction at Rf 0.9, is shown in Fig. 1. They consist primarily of unsaturated diterpenoids of the kaurane and abietane skeletons and of a saturated diterpenoid of the phyllocladane skeleton. The kaurenes were identified as kaur-15-ene (I) and kaur-16-ene (II), (peaks *b* and *c* respectively in Fig. 1), and has been previously found by Venkatesan et al., (1986) in a peat.

Mass spectrum of peak *a* shows a molecular ion at  $m/z$  272 and a base peak at  $m/z$  257, similar to the mass spectrum reported by Simoneit and Mazurek (1982) for the abieta-7,13-diene, (III).

Mass spectrum of peak *d* shows a molecular ion of  $m/z$  274 and a base peak at  $m/z$  123 and might correspond to the tetracyclic diterpane 16 $\alpha$ (H)-phyllocladane (IV),

the most common isomer present in lignites (Briggs, 1937; Streibl et al., 1972; Serantoni et al., 1978; Noble et al., 1985).

The presence of these diterpenoids in the AR lignite is evidence of the influence of higher plant debris, mainly of conifers, in the formation of the sediment (Noble et al., 1985; Venkatesan et al., 1986). According to Venkatesan et al. (1986), the predominance of the unsaturated diterpenoids respect to their saturated analogs (Fig. 1), implies that plant material has been rapidly buried and preserved from oxidation and biodegradation under the lignite forming conditions, and therefore diagenetic reduction of diterpenes has been limited.

### *Triterpenoid hydrocarbons*

Pentacyclic triterpanes were also detected in the fraction isolated at Rf 0.9. The relative distribution of this series, based on the  $m/z$  191 peak intensity on the GC/MS data, is given in Fig. 2. The identification of individual triterpane stereoisomers was

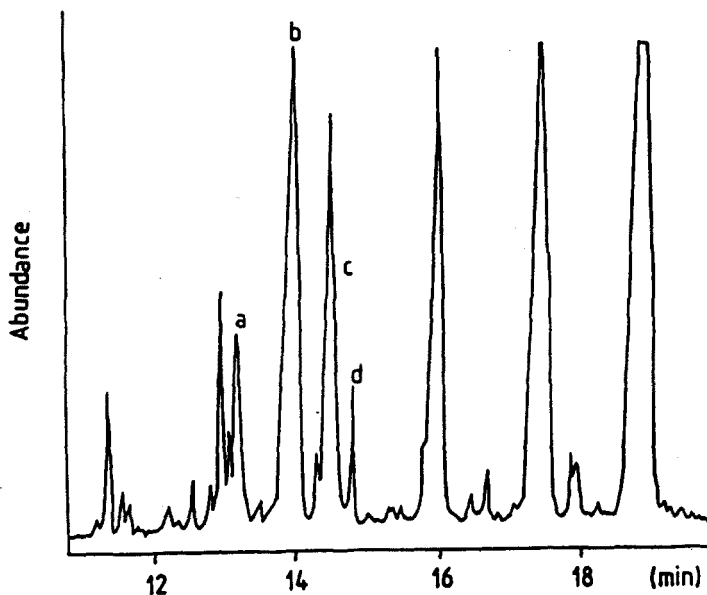


Fig. 1. Partial gas chromatogram of the TLC fraction at  $R_f$  0.9 showing the diterpenoid distribution.

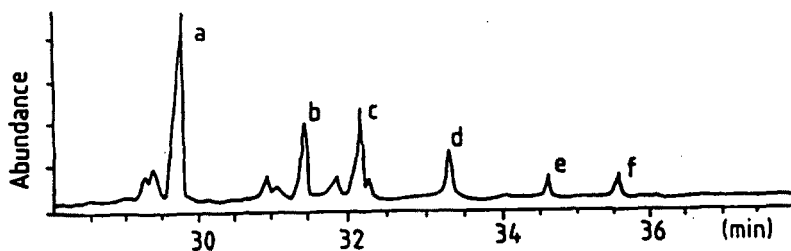


Fig. 2. Partial  $m/z$  191 mass chromatogram of the TLC fraction at  $R_f$  0.9 showing triterpenoid distribution.

based on the relative intensity of the two major fragments at  $m/z$  148 + R and  $m/z$  191 (Vitorovic and Saban, 1983; Philp, 1985). They consist predominantly of the  $17\beta$ (H),  $21\beta$ (H)-hopane series (V) ranging from C(27) to C(30), with the exception of the C(28) homologs, (peaks *a*, *c* and *d* in Fig. 2) and the  $17\beta$ (H),  $21\beta$ (H)-extended hopanes (VI) from C(31) to C(32) with only one diastereomer at C-22 position (peaks *e* and *f* in Fig. 2). Minor amounts of the  $17\alpha$ (H),  $21\beta$

(H)-norhopane (VII), (peak *b* in Fig. 2) were also present. No triterpenes were detected in this fraction.

The presence in the AR lignite of the  $17\beta$ (H),  $21\beta$ (H) stereoisomers is characteristic of recent microbial activity (DeRosa et al., 1971; Rohmer, 1975) and indicates that this deposit has undergone only a mild thermal history during deposition and burial (Ensminger et al., 1975; Dastillung and Albrecht, 1976; Simoneit and Kaplan, 1980).

*Steroid alcohols and ketones*

The relative distribution of the free sterols isolated from the AR lignite extract in the fraction at Rf 0.2 is shown in the mass chromatogram of Fig. 3, based on the typical  $m/z$  129 fragment. Peaks *a*, *b*, *c* and *d* in Fig. 3, were identified as cholesterol (VIII, R = H), campesterol (VIII, R = C<sub>2</sub>H<sub>5</sub>), stigmasterol (IX) and  $\beta$ -sitosterol (VIII, R = C<sub>3</sub>H<sub>7</sub>) respectively.

The predominances of cholesterol and  $\beta$ -sitosterol might indicate both phytoplankton and terrestrial plant inputs respectively (Huang and Meinschein, 1976). Although the triangular diagram proposed by these authors to differentiate environments on the basis of the sterol distributions has great limitations (Mackenzie et al., 1982), extrapolation of our data seems to indicate a similar sterol distribution to those of estuarine or bay sediments. This result is consistent with geological data which indicate

that this deposit was formed in an estuarine or deltaic environment (Martín and García-Rossell, 1970).

In the fraction isolated at Rf 0.6, a significant series of compounds with a base peak at  $m/z$  231 was also found. This fragment is characteristic of the cleavage of ring D of 5 $\alpha$ - and 5 $\beta$ -stan-3-ones, showing also the 5% isomers an intense M-70 fragment (Gagosian et al., 1982). The  $m/z$  231 mass chromatogram of Fig. 4 shows the relative distribution of these steroid ketones, which were identified as cholestan-3-one (X, R = H), 24-methyl-cholestan-3-one (X, R = CH<sub>3</sub>), and 24-ethyl-cholestan-3-one (X, R = C<sub>2</sub>H<sub>5</sub>), all of them with the 5 $\alpha$  configuration, (peaks *a*, *b* and *d* respectively, in Fig. 4). Minor amounts of the 5 $\beta$ -24-ethyl-cholestan-3-one (peak *c* in Fig. 4) were also present. The unsaturated stigmast-4-en-3-one was also detected in trace amounts.

Steroid ketones in geological samples may arise from direct input of living or-

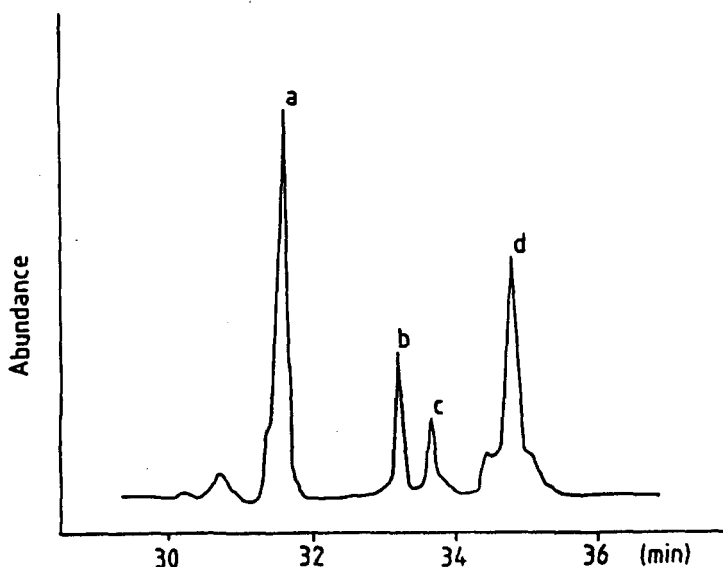


Fig. 3. Partial  $m/z$  129 mass chromatogram of the TLC fraction at Rf 0.2 showing sterol distribution.

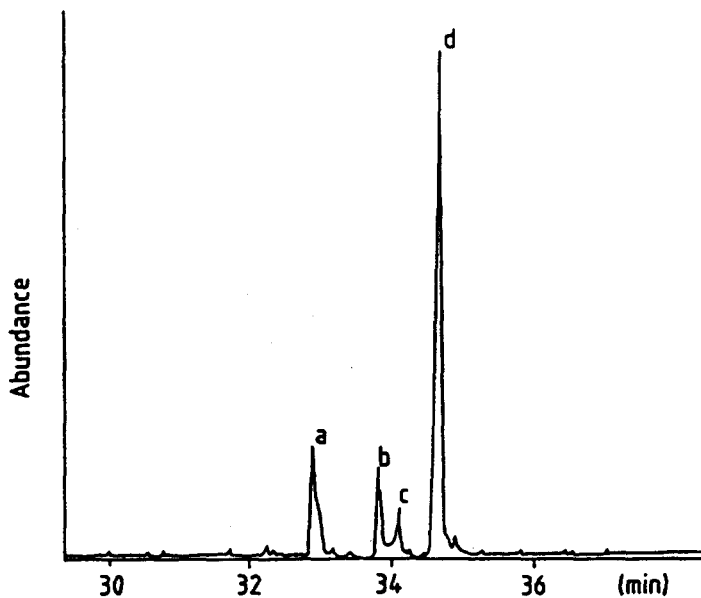


Fig. 4. Partial  $m/z$  231 mass chromatogram of the TLC fraction at  $R_f$  0.6 showing stan-3-one distribution.

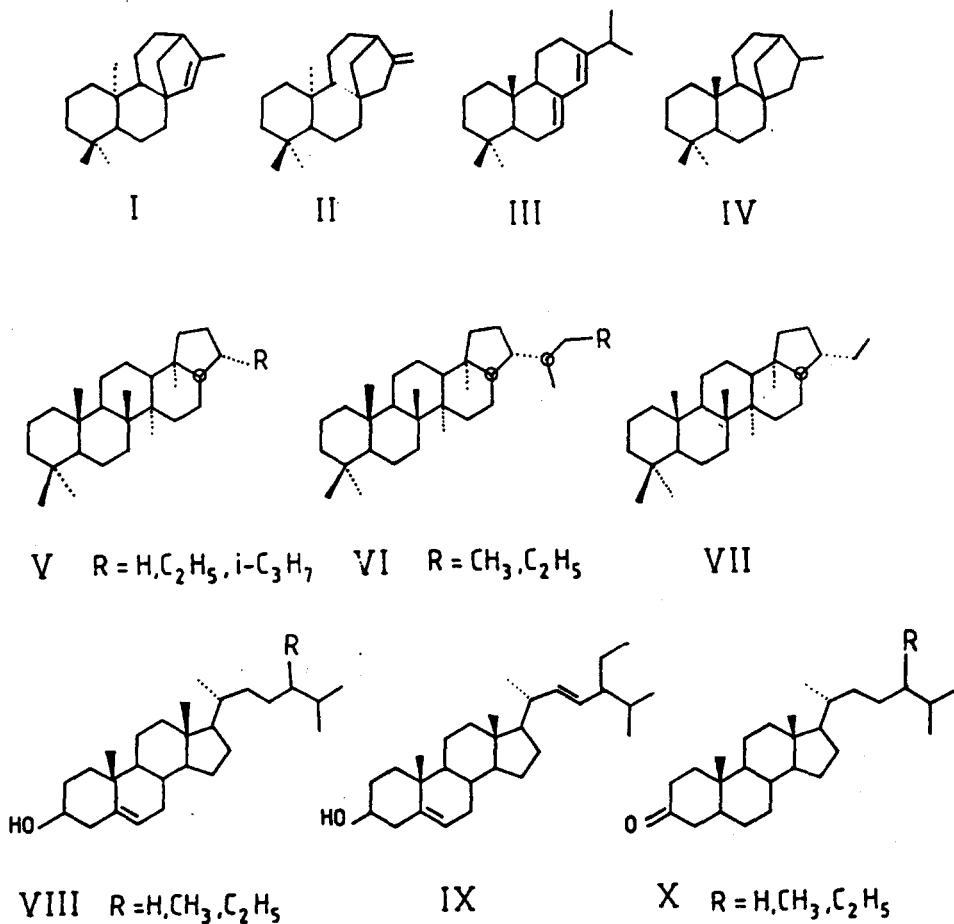


Fig. 5. Structures of compounds cited in the text.

ganisms and/or from microbial or chemical degradation of sterols, (Gagosian et al., 1982). Direct inheritance from living organisms is indicated by the nearly exclusive presence of the  $5\alpha$ - configuration, whereas *in situ* hydrogenation of sterols leads both to  $5\alpha$ - and  $5\beta$ -stanols through  $\Delta^4$ -stenone and  $5\alpha$ - and  $5\beta$ -stanone intermediates (Nishimura, 1982). The predominance in the AR lignite of stanones with the  $5\alpha$ - configuration indicates that they arise from a direct biological input and therefore sterols in this sediment have not undergone drastic diagenetic alterations.

## CONCLUSIONS

The detection of diterpenoids in the AR lignite indicates an important contribution of conifers to the original organic matter. A possible phytoplankton input is also suggested by the sterol distribution found in the sample, which should confirm the geological hypothesis that this sediment had an estuarine or deltaic origin. On the other hand, the immaturity of the lignite is reflected by several parameters such as the preservation of sterols and stanones and by the presence of the  $17\beta$ (H),  $21\beta$ (H)-hopane series.

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