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Quantitative and stereoisomeric determination of light biomarkers in crude oil and coal samples

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ABSTRACT—Indans and tetralins are considered biological markers (biomarkers). These C9–C11 hydrocarbons are present in small amounts in organic geological samples. Methyl substituted indans or tetralins may possess a stereogenic center (carbon). Thus they can exist as enantiomers and, in the case of disubstituted entities, also as diastereoisomers. The concentrations of 1-methylindan, 1,3-dimethylindan, 1-methyltetralin, and 2-methyltetralin were determined in sixteen crude oil samples of different sources and in fourteen coal samples of different sources and ranks. Deuterated homologues were synthesized as standards to spike the samples and to assure accurate quantitative analysis. A procedure using HPLC fractionation followed by GC/MS analysis allowed the determination of μg/g (ppm) amounts of these compounds in oils. The concentration of substituted indans and tetralins was 3–4 orders of magnitude less in coal than in crude oil. The select ion mass spectrometry (SIM) mode in GC/MS and the deuterated standards allowed detection of the much lower amounts (ng/g, ppb down to pg/g, ppt) of these compounds in coal samples. The stereochemistry of the biomarkers was determined, and the relationship between their relative concentrations and the location and type of the deposits was examined. Racemic mixtures of the indans and tetralins studied were found in all samples of oil and coal. It is postulated that there is an inverse relationship between the retention of stereochemical configuration and the molecular weight of hydrocarbons in crude oil. The chiral retention of configuration cut-off is thought to be between molecular weights of 146 and 208. An excess of cis-1,2-dimethylindan was found in all oil samples (average *cis/trans* ratio: 3/2). The 2-methyltetralin concentration was found to be about twice that of 1-methyltetralin in all oil and coal samples. Similar concentration correlations were found for the indan derivatives in oils and coals. *Copyright © 1998 Elsevier Science Ltd*

1. INTRODUCTION

Biological markers, or biomarkers, are particular organic molecules found in geological deposits such as crude oil, coal, shale, and some sediments and rock formations. A geochemical molecule is thought to be a biomarker if its structure resembles the structural subunits of biological precursors, e.g., lipids, steroids, or porphyrins, which occur commonly in possible source materials (Eglinton and Calvin, 1967; Albrecht and Ourisson, 1971; Chaffee et al., 1986). With the advent of modern analytical methods and instrumentation such as the gas chromatography technique combined with mass spectrometry (GC/MS), more biomarkers become traceable. They have been used to study the source, maturation, migration, and biodegradation of crude oils (Seifert and Moldowan, 1978, 1979, and 1981). They proved to be highly effective in oil spill identification (Wang et al., 1994) and in coal characterization (Ramanampisoa et al., 1990).

Most biomarkers contain the basic isoprene structural subunit composed of five carbon atoms (Peters and Moldowan, 1993). The biomarkers most often studied are stearanes, terpanes, isoprenoids, and other hydrocarbons containing 18–30 carbon atoms (Albrecht and Ourisson, 1971; Seifert and Moldowan, 1981; Chaffee et al., 1986; Peters and Moldowan, 1993). These biomarkers are found in geological samples in concentration ranging from the part per thousand (mg/g) down to the part per million (ppm or μg/g) level. Other chemical families, found in even lower concentration in the samples, can be used as possible biomarkers. To investigate oil migration distances, Larter et al. (1996) tracked trace amounts from the μg/g level down to 20 ng/g (ppb) levels of benzocarbazoles (C16N compounds) in oil samples. They used the quantitative procedure described for organic geological samples (Rullkötter et al., 1984). In the procedure, an internal standard is added to the sample which allows the accurate determination of the biomarker concentration. The selected standard is a molecule that does not interfere with the biomarker and cannot be found naturally in the sample.

As noted previously, more accurate and sensitive quantitation and many associated discoveries in geochemistry are dependent on progress in analytical methodologies (Mansfield et al., 1997). Indans and tetralins are possible biomarkers. They are rarely studied because as C9–C11 hydrocarbons they are present in minute amounts in geological samples due to their relatively high volatility (Peters and Moldowan, 1993). Methyl and alkyl substituted indans and tetralins may possess a stereogenic center and, therefore, exist as chiral molecules. Biomarkers come from the degradation of organic matter. Chiral biological molecules generally have known configurations and high enantiomeric and diastereomeric purities. It is of primary importance in geological samples to know if the original configuration of biologically derived molecules is retained or not.

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The stereochemistry of C29 hopanes was studied early on (Nishimura, 1977; Dastillung and Albrecht, 1979; and Seifert and Moldowan, 1981). The ratio of C29 stearane diastereoisomer concentrations was used to compare the maturation and migration of a variety of crude oils and bitumen (Seifert and Moldowan, 1981). In recent works, we developed the use of chiral stationary phases (CSPs) for the resolution of the enantiomers of smaller biomarkers (Armstrong et al., 1991; Armstrong et al., 1996). For example, monoterpenoid biomarkers in amber often maintained substantial enantiomeric excesses after tens of millions of years (Armstrong et al., 1996).

In this study, substituted indans and tetralins, found in a variety of crude oil and coal samples, were quantitated. Their stereochemistry was determined and the relationship between their relative concentrations and the location and type of the deposits was examined.

2. MATERIALS AND METHODS

2.1. Materials

2.1.1. Commercial chemicals

The standards of tetralin ($C_{10}H_{12}$, m.w. 132.21, b.p. 207.6°C), 1-methylindan ($C_{10}H_{12}$, m.w. 132.21, b.p. 189°C), 1-methyltetralin ($C_{11}H_{14}$, m.w. 146.23, b.p. 220.6°C), and 2-methyltetralin ($C_{11}H_{14}$, m.w. 146.23, b.p. 221°C) were obtained from the Aldrich Chemical Company, Inc (Milwaukee, WI). 1,3-dimethylindan ($C_{11}H_{14}$, m.w. 146.23, b.p. 193°C) was synthesized as reported by Armstrong et al. (1997). α-Tetralone, β-tetralone, 1-indanone, d8-tetralin, deuterium gas, and d3-methylmagnesium bromide were from Aldrich. The platinum oxide was obtained from Engelhard Chemical (Newark, NJ).

2.1.2. Deuterated standards

The deuterated standards: 1-d3-methylindan, 1-methyl, 3-d3-methylindan and 1-d3-methyl-1,2-d2-tetralin were not found in any chemical product catalogs. They were synthesized as follows: the appropriate ketones, 1-indanone, 3-methyl 1-indanone and α-tetralone, were converted to the corresponding tertiary alcohol using a classical Grignard reaction with deuterated methyl magnesium bromide. To a solution of 10 mL of 1 M d3-methyl magnesium bromide in methyl tertiobutyl ether (MTBE) at room temperature, 500 mg of the appropriate ketone was added for about 20 min. After 8 h of reaction time with gentle stirring, the excess of methyl magnesium bromide was quenched by 50 mL of water. The tertiary alcohol formed was extracted by 100 mL hexane. The hexane solution was dried over anhydrous magnesium sulfate. The relevant alkenes were prepared by dehydratation of the corresponding tertiary alcohols. 5 mL of concentrated phosphoric acid was added to a solution of 200 mg of tertiary alcohol in 20 mL methanol. The reaction mixture was heated at ~40°C and stirred for 4 h. Then, 200 mL of water and 200 mL of hexane were added to extract the phosphoric acid in the aqueous phase and the alkene in the hexane phase. The upper hexane layer was collected and dried over anhydrous magnesium sulfate. For hydrogenation, 200 mg of the methyl deuterated alkene were dissolved in 20 mL MTBE and 30 mg of anhydrous platinum oxide were added in a secured vessel. A hydrogen pressure of 60 psi (4.2 kg/cm^2 or 420 kPa) was applied in the reaction vessel for 8 h. In the synthesis of 1-d3-methyl-1,2-d2-tetralin, deuterium gas was used instead of hydrogen. The platinum oxide catalyzed the addition of two hydrogen or deuterium atoms in the cis-position. In the synthesis of the 1-methyl, 3-d3-methylindan, the catalytic hydrogenation produced the 98% of the cis-isomer with only 2% of the trans-isomer. The deuterated standards were extracted, purified, and analyzed by GC/MS.

2.2. Apparatus

2.2.1. Gas chromatography and mass spectrometry

A Shimadzu SA-10 GC/MS system (Shimadzu, Columbia, MA) was used. It is basically a GC Shimadzu model 17A gas chromatograph coupled with the Shimadzu QP-5000 benchtop mass spectrometer. The whole system was computer controlled with a AST P5-75 compatible computer. A 20 m (0.25 mm i.d.) capillary column coated with Chiraldex G-TA (0.125 μm film thickness, Astec, Whippany, NJ) was used with a 100/1 split ratio. Chiraldex G-TA is a chiral stationary phase (CSP) made of γ-cyclodextrin which has been trifluoroacetylated at the 3 position hydroxyl groups and pentylated at the 2 and 6 position hydroxyl groups (Li et al., 1990). Through association with the cyclodextrin cavity and other enantioselective interactions, this CSP is able to separate a wide variety of enantiomers (Berthod et al., 1992).

Helium was used as the carrier gas with flow rates around 1.2 mL/min. The injector and the transfer line to the mass spectrometer were maintained at 250°C. The mass spectrometer was set to scan mass units within the 50–250 dalton range. Electron impact ionization mode was used for crude oil analysis. Selective ion monitoring (SIM) was performed for the detection of molecular ions of the trace amounts contained in coal samples.

2.2.2. Liquid chromatography

The HPLC separations were made with an assembled laboratory system. The pump was the Shimadzu LC-6A pump. It was used with a Rheodyne 7125 injection valve equipped with a 200 μL sample loop. A 50 cm (1 cm i.d.) semi-preparative column was obtained from Astec (Whippany, NJ). The stationary phase was made of 10 μm silica particles bonded with C18 (ODS) paraffin chains. The detector was the UV photometric Shimadzu model SPD-2AM. Mobile phases were methanol-water mixtures (mostly 90/10 % v/v), the flow rate was 2 mL/min.

2.3. Sample Preparation

2.3.1. Crude oils

Two crude oil samples were obtained from Larry A. Spino of the Shell Oil Company (Wood River, IL) and Shell Development Co. (Houston, TX). Fourteen crude oil samples were obtained from Gerry G. Calhoun from Surface Geochemical Exploration (Midland, TX). The origin, age, and formation of the oil samples are listed in Table 1. The crude oil samples were first fractionated by distillation at atmospheric pressure. About 10 g of dark crude oil were poured in a 30 mL round bottom

Table 1: Source, formation, concentration and stereochemistry of substituted indans and tetralins in crude oil samples.

							indan derivatives in ug/g			tetralins in ug/g		enantiomeric
sample	formation	county	state/country	epoch	age (My)	depth well m	1-methyl	1,3-dimethyl[a]	% cis	1-methyl	2-methyl	composition[b]
1	san andres	crane	texas	permian	260	840	219	193	58%	11	51	racemic
2	canyon reef	scurry	texas	pennsylvanian	310	2220	494	160	62%	92	170	racemic
3	delaware sand	pecos	texas	permian	260	990	269	78	65%	28	108	racemic
4	fusselman	chaves	new mexico	silurian	420	2040	139	50	61%	65	185	racemic
5	paluxy	val verde	texas	cretaceous	120	90	127	97	57%	2	20	racemic
6	triassic sediment	pecos	texas	triassic	220	240	141	167	55%	16	33	racemic
7	rustler	pecos	texas	permian	260	360	107	52	53%	7	9	racemic
8	wolfcamp	upton	texas	lower permian	280	3000	343	142	59%	46	92	racemic
9	devonian	lea	new mexico	devonian	390	3300	77	45	67%	11	17	racemic
10	clearfork	pecos	texas	permian	260	1680	20	6	67%	2	2	racemic
11	wolfcamp	pecos	texas	permian	260	2100	116	60	52%	22	67	racemic
12	ellenburger	nolan	texas	ordivician	470	1920	150	47	68%	141	291	racemic
13	fusselman	chaves	new mexico	silurian	420	1860	126	34	60%	80	135	racemic
14	dakota	archuleta	colorado	cretaceous	120	750	25	21	59%	1	1	racemic
15	wr-2854[c]	midland area	texas	permian	260	-	242	173	61%	38	123	racemic
16	wr-2881[c]	-	saudi arabia	-	-	-	175	110	61%	20	28	racemic

[a] The 1,3-dimethylindan concentration listed is the total (cis + trans) concentration. %cis is the percentage of the cis isomer in the listed concentration.

[b] All chiral indans and tetralins analyzed in this study were found to consist of 50:50 enantiomeric mixtures within the ± 2% error limits of these measurements. A minimum of three separate analyses were performed on each sample, after averaging the results of all runs. The averaged enantiomeric ratios were always equal to or between 49/51 and 51/49.

[c] Shell Oil Co. Identification number.

flask. A known amount of the deuterated internal standards was added. After 2 h mixing, the oil was distillated by gentle heating. The only fraction collected (~3 mL) boiled between room temperature and 140°C. 100 μL volumes of the distillate were directly injected in the HPLC column without further purification. A typical HPLC separation is shown in Fig. 1.

2.3.2. Coal samples

Fourteen coal samples were studied. The coal samples #1–4 were obtained from J. W. Wilson of the Mining and Engineering Department of University of Missouri, Rolla, MO. Samples #5, 6, 7, and 8 are the reference materials SRM #2682a, #2683a, #2684a, and 2692a, respectively. They were purchased from National Institute for Standards and Technology (NIST, Gaithersburg, MD). Samples #9–11 were provided by J. C. Hower of University of Kentucky at Lexington, KY. Sample #12 was provided by M.A. Musich of the Energy and Environmental Research Center of University of North Dakota, Grand Forks, ND. The peat samples (#13 and #14) were provided by S. Spigarelli of Bemidji State University at Bemidji, MN. The origin and rank of the coal samples are listed in Table 2. Approximately 20 g portions of the coal samples were finely ground in a mortar. The coal powder was placed in a 300 mL flask; 100 mL of hexane were added along with known amount of the deuterated internal standards. The mixture was refluxed for 2 h. Then, it was filtered, and the filtrate was concentrated under reduced pressure and gentle heating to ~3 mL. 100 μL portions of the concentrate were fractionated using HPLC.

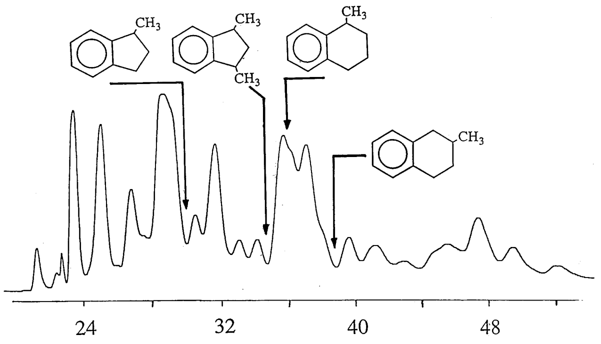


Fig. 1. HPLC chromatogram of 100 μL of the oil #1 distillated fraction (see Table 1 and Materials and Methods). The semi-preparative column was an Astec ODS 10 μm, 50 cm x 1 cm i.d.; mobile phase, methanol/water 85/15 % v/v at 2 mL/min; detection UV 254 nm, 0.16 aufs. The arrows locate the collection times of the fractions that were analyzed by GC/MS, see Figs. 2 and 3.

2.3.3. Quantitative results

We assumed that the MS ionization response of the deuterated standard was identical to the response of the hydrogenated homologue. Knowing the deuterated standard concentration allows one to calculate the concentration of the corresponding hydrocarbon using its MS ionization response. Table 1 lists the concentrations of the indan and tetralin derivative that were found in the sixteen crude oil samples studied.

2.4. Enantiomer Separation

Standards of methylindan, dimethylindan, and methyltetralin were injected in the HPLC system to obtain their retention factors, k' and to calibrate the system for retention times (Table 3). Next, 100 μL injections of the crude oil distillate were performed. The eluate corresponding to the retention times of the standards (Table 3) was collected. Figure 1 shows a typical HPLC chromatogram with the location of the collected fractions. These fractions were analyzed by GC/MS. Figure 2 shows the GC/MS chromatograms of the indan fractions. Fig-

Table 2: Source, location, concentration and stereochemistry of substituted indans and tetralins in coal samples.

sample	formation	state/country	rank	indan derivatives in ng/g 1-methyl	1,3-dimethyl[a]	%cis	tetralins in ng/g 1-methyl	2-methyl	enantiomeric composition[b]
1	Captain Mine	IL	bituminous	741	162	57%	12	18	racemic
2	Galatia Mine	IL	bituminous	174	49	56%	71	195	racemic
3	Mettiki Corp.	MD	bituminous	43	20	66%	30	66	racemic
4	Power River Basin	WY	sub-bituminous	21	18	61%	18	28	racemic
5	Wyodak Seam	WY	sub-bituminous	9	8	64%	1	5	racemic
6	Pittsburg Seam	WV	bituminous	141	57	58%	19	19	racemic
7	Il#6 Herrin Seam	IL	bituminous	98	45	57%	7	9	racemic
8	Elk Creek #2 Gas Seam	WV	bituminous	774	166	52%	198	312	racemic
9	unknown	Vietnam	anthracite	18	4	29%	2	6	racemic
10	Mammoth Seam	PA	anthracite	5	2	54%	0.05	0.6	racemic
11	Gallup	NM	lignite	5	9	56%	1	0.05	racemic
12	North Dakota	ND	lignite	16	11	28%	5	3	racemic
13	Duschamp Field	KY	peat	2	0.4	70%	0.05	0.1	racemic
14	Fens	KY	peat	4	0.6	52%	3	0.5	racemic

[a] The 1,3-dimethylindan concentration listed is the total (cis + trans) concentration. %cis is the percentage of the cis isomer in the listed concentration

[b] All chiral indans and tetralins analyzed in this study were found to consist of 50:50 enantiomeric mixtures within the ±2% error limits of these measurements. A minimum of three separate analyses were performed on each sample, after averaging the results of all runs. The averaged enantiomeric ratios were always equal to or between 49/51 and 51/49.

ure 3 shows the tetralin GC/MS chromatograms. The two tetralin derivatives and 1-methylindan have a stereogenic center. 1,3-Dimethylindan has two stereogenic centers. The relative concentrations of the enantiomers of these compounds were evaluated. As indicated in Table 3, the enantioresolution between the R and S isomers of the same compounds can be as high as 10.4 (trans-1,3-dimethylindan). At least baseline resolution (Rs $\geq$ 1.5) was found for all enantiomers. This is due to the high efficiency of the capillary column used, in the 40,000 plate range. The analogous separations for coal extract are shown in Fig. 4.

The deuterated isomers are partly separated from their corresponding hydrogenated isomers. The selectivity and resolution factors are close to 1.01 and 0.6, respectively. The deconvolution of the total ion current for a given *m/z* value allowed resolution of the deuterated and hydrogenated compounds (Figs. 2 and 3). It is noted that the deuterated standard always eluted before the hydrogenated homologue. This is surprising behavior since the molecular mass and the boiling point of the deuterated compounds is slightly higher than the corresponding hydrogenated analogues. This unusual selectivity seems to be characteristic of many cyclodextrin-based GC stationary phases which tend to interact more strongly with hydrogenated compounds than with their deuterated analogues (Chang et al., 1997).

3. RESULTS AND DISCUSSION

3.1. Study of the Oil Samples

Table 1 gives the concentration, location, approximate age, and stereochemical compositions of the substituted tetralins and indans found in crude oils. It should be noted that 1,3-dimethylindan consists of 3 isomers (i.e., the trans R-R and trans S-S enantiomers as well as the achiral cis-meso compound). All compounds were enantiomerically resolved (Figs. 2 and 3). However, the R and S concentrations of all the chiral methyl substituted indans and tetralins were found to be identical within experimental error. There

Table 3: HPLC and GC retention parameters for the indans and tetralins studied.

method	HPLC[a]		GC[a]			
Compounds	**tr** (min)	**k'**	**tr_1** min	**tr_2** min	**α**	**Rs**
1-methylindan	29.75	1.70	10.95	11.38	1.04	3.4
1-d3-methylindan	29.75	1.70	10.85	11.31	1.04	3.4
cis-1,3-dimethylindan	35.21	2.20	12.60	-	achiral	achiral
trans-1,3-dimethylindan	34.71	2.16	12.12	13.51	1.11	10.4
cis-1-methyl, 3-d3-methylindan	35.21	2.16	12.58	12.58	0	0
trans-1-methyl, 3-d3-methylindan	34.71	2.20	12.10	13.48	1.11	10.4
1-methyltetralin	35.96	2.27	15.68	16.28	1.04	4.6
1-d3-methyltetralin	35.96	2.27	15.57	16.19	1.04	4.6
2-methyltetralin	38.86	2.53	23.62	24.22	1.02_5	1.5
2-d3-methyltetralin	38.86	2.53	23.34	23.96	1.02_5	1.5

[a] k' is the HPLC retention factor (=($tr-t_o$)/t_o), t_o = 11 min. tr_1, tr_2= GC retention times of the first and the last eluting enantiomer, respectively. α = enantioselectivity ratio, Rs = enantioresolution factor.

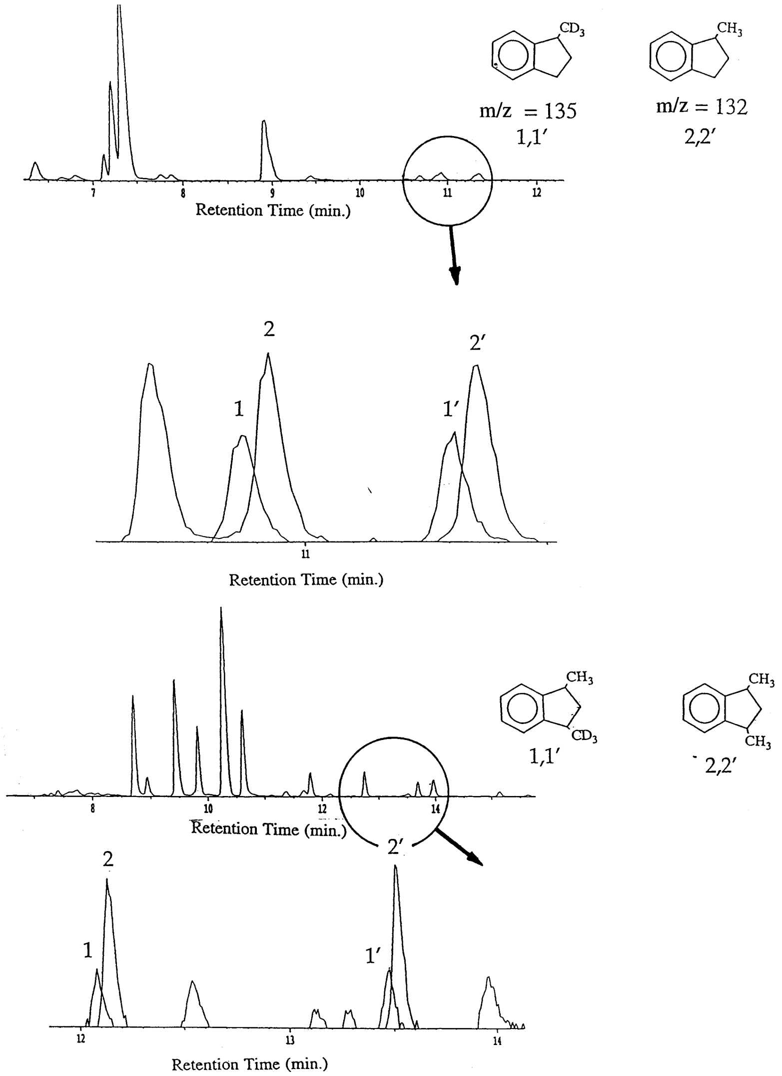


Fig. 2. GC/MS chromatogram of the HPLC fraction (see Fig. 1) collected between 28.5 and 30 min (top) and between 34 and 35 min (bottom) showing the enantioseparation of indan derivatives. A Chiraldex G-TA capillary column, 20 m 0.25 mm i.d. was used; carrier gas: He; 1μL injection with split ratio 30/1; temperature gradient: 5 min at 60°C, then 4°C/min up to 130°C and hold at 130°C for 20 min. The total ion current (TIC) accumulates the signals obtained for *m/z* values between 50 and 200. The reconstructed ion chromatograms of *m/z* = 132 and *m/z* = 135 for 1-methylindan (top) and *m/z* = 146 and *m/z* = 149 for trans-1,3-dimethylindan are also shown.

are at least four possible explanations for the apparent racemic state of the indan and tetralin derivatives: (1) racemization occurred upon degradation and breakdown of larger chiral molecules during diagenesis and particularly catagenesis, (2) larger chiral molecules first racemized (or stereoisomerized) with age. Thus their latter degradation products also had to be racemic. (3) These compounds were made from smaller prochiral materials (e.g. hydrogenation of the corresponding alkenes or methylation of unsubstituted or partially substituted indans and tetralins, etc.) leading to racemic products. (4) Early on, the substituted indans and tetralins had enantiomeric or diastereomeric excesses which were lost over time by racemization or intramolecular rearrangements.

3.1.1. *A molecular weight/retention of configuration cut-off*

There have been a few reports on the effect of structure on the retention of a hydrocarbon's configuration in geological samples. For example, it has been stated that configuration isomerization or stereoizomerization in saturated biomarkers occurs only at asymmetric carbon atoms where one of the four substituents is a hydrogen (Peters and Moldowan, 1993). Also, because of steric forces imposed by a rigid cyclic structure, asymmetric centers that are part of a saturated ring system usually show two configurations with quite different thermal stabilities (Peters and Moldowan, 1993). However, there also should be an inverse relationship between the size or molecular weight of hydrocarbons in geochemical samples and their retention of configuration or stereogenecity.

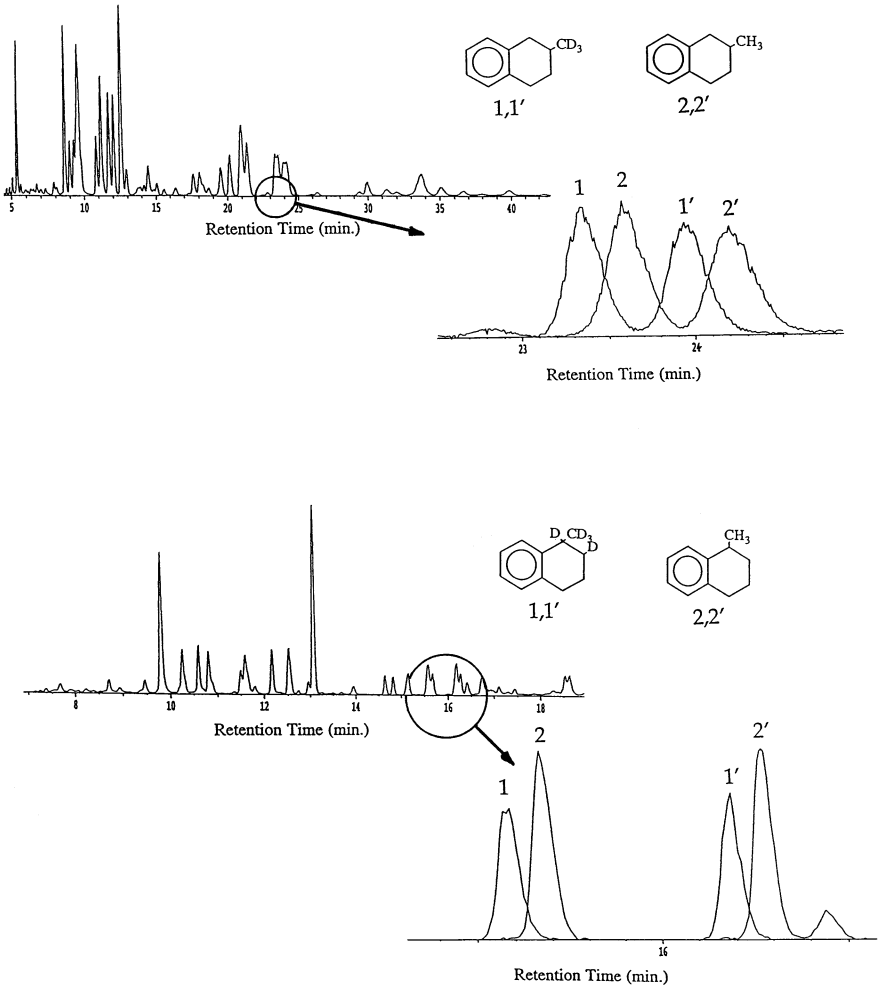


Fig. 3. GC/MS chromatogram of the HPLC fractions (see Fig. 1) collected between 35.5 and 36.5 min (top) and between 37.5 and 38.5 min (bottom) showing the enantioseparation of tetralin derivatives. Chromatographic conditions are described in the Fig. 2 caption. The total ion current chromatograms and the reconstructed ion chromatograms of $m/z = 146$ and $m/z = 151$ for 1-methytetralin (top) and $m/z = 146$ and $m/z = 149$ for 2-methytetralin are also shown.

In previous studies, C29 hopanes isolated from crude oils were found to possess the structure of possible corresponding sterols abundant in living organisms (Nishimura, 1977; Dastillung and Albrecht, 1979; Peters and Moldowan, 1993). However, a partial conversion or isomerization was observed for these types of compounds (Seifert and Moldowan, 1981). Given the apparently racemic character of the indan and tetralin enantiomers present in the oil samples, we can postulate that there may be a particular molecular weight limit for the retention of enantiomeric or stereoisomeric excess in organic geochemical samples. For compounds heavier than this cut-off molecular weight, the original stereochemistry can be at least partially retained. For compounds lighter than this molecular weight, even though enantiomeric or stereoisomeric excess is possible, it is lost via isomerization.

It should be noted that the molecular weight/chiral configuration limit proposed in this study is not the same as that imposed by the basic geometrical-structural constraints for hydrocarbons (Robinson et al., 1976). This absolute cutoff is at a molecular weight of 80 which represents the lightest possible chiral hydrocarbon containing a stereogenic center (i.e., 3-methyl-4-ene-1-pentyne, C_6H_8), or a molecular weight of 68 for compounds with an axis of chirality (i.e., 1,3-dimethylpropadiene, C_5H_8). Hydrocarbons smaller than this (down to methane, CH_4, m.w. 16) cannot be chiral. It is unlikely that the aforementioned chiral alkyne or sp^2-sp-sp^2 hybridized alkene (i.e., propadiene) would occur in natural geological hydrocarbon deposits such as crude oil. Probably the lightest naturally occurring enantiomeric hydrocarbon in oil is 3-methyl-1-pentene (C_6H_{12}, m.w. 84) while the lightest saturated enantiomeric hydrocarbons (i.e., alkanes) are 3-methylhexane and 2,3-dimethylpentane (both C_7H_{16}, m.w. 100). As the molecular weight of hydrocarbons increases above 80, the possible chiral structures largely dominate the achiral ones. For example 95% of all possible isomers of C14 hydrocarbons (m.w. in the 180 range) are chiral (Siegel, 1998).

We established that indan and tetralin (m.w. 132–146) derivatives were racemic within experimental error. The lightest hydrocarbons found to date to retain some stereoisomeric ex-

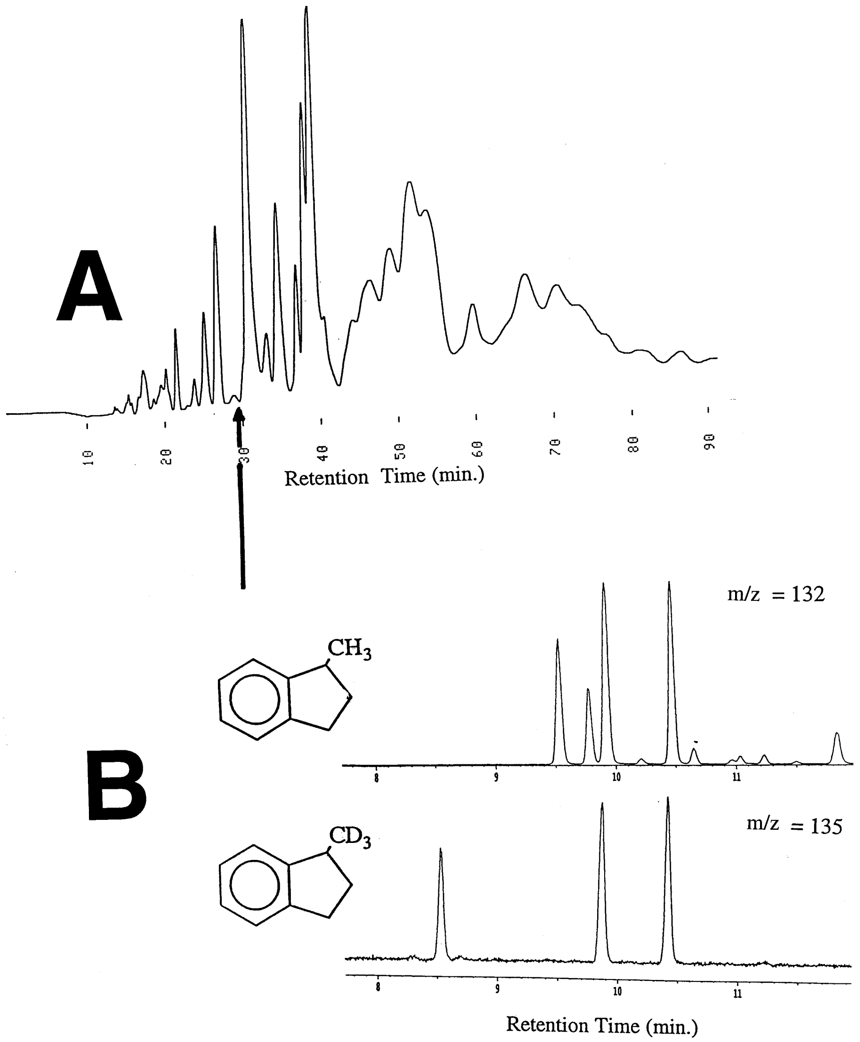


Fig. 4. (A) HPLC chromatogram of 100 μL of coal sample #2 hexane extract (see Table 2 and Materials & Methods). A semi-preparative column was used, Astec ODS 10 μm, 50 cm x 1 cm i.d.; mobile phase methanol/water 85/15 % v/v at 2 mL/min; detection UV 254 nm, 0.02 aufs. The arrows locate the collection times of the fractions that were analyzed by GC/MS in the SIM mode. (B) GC/MS chromatograms of 1-methylindan in the select ion mass spectrometry mode (SIM). The mass spectrometer detects only the select *m/z* ratios. The sensitivity is greatly enhanced in this mode.

cess were 8β(H)-drimane and 4α(H)-eudesmane, both sesquiterpenes, $C_{15}H_{28}$, m.w. 208 (Alexander et al., 1983). Thus the hypothetic molecular weight cut-off for the retention of configuration (in terms of enantiomeric or diastereomeric excess) by crude oil components may be between these two weights. The forces that lead to the degradation of parent hydrocarbons into smaller daughter products, also must lead to racemization or stereoisomerization in an isotropic environment. Structural considerations (such as those mentioned previously) can make the parent and daughter hydrocarbons more or less susceptible to isomerization. It is the combination of these effects that determine whether or not the hydrocarbons in crude oil retain stereospecific configurations.

3.1.2. Tentative correlations

Figure 5A shows the plot of indan and tetralin concentrations vs. the age (in millions of years) of the samples. Figure 5B shows the same concentrations plotted vs. the depth of the well. It is apparent that there is no clear correlation between the concentration of these compounds and either the age or the collection depth of the oil samples.

3.1.3. Relative concentrations of tetralins

Sample #12 contains the highest level of 1-methyltetralin (141 ppm). It also contains the highest level of 2-methyltetralin (291 ppm). However, Sample #12 contains an intermediate amount of 1-methylindan (150 ppm) and a low amount of 1,3-dimethylindan compared to the fifteen other samples. Figure 6a shows the 1-methyl concentration of each sample plotted vs. the 2-methyltetralin concentration. Both concentrations are correlated. The linear regression coefficient is $r^2 = 0.911$, and the slope is 0.463 (Table 4). All sixteen samples contained approximately one part of 1-methyltetralin for two parts of

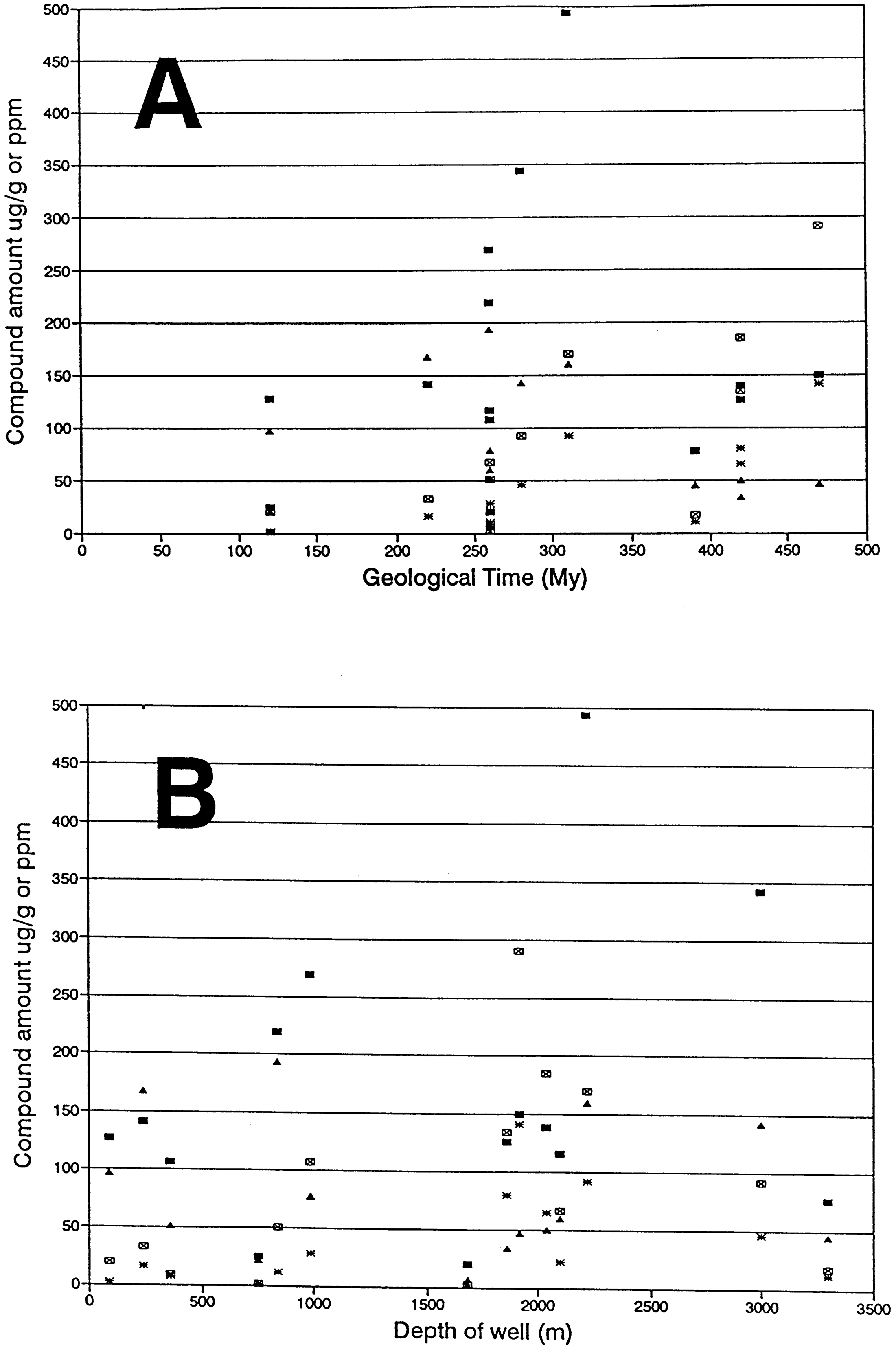


Fig. 5. (A) Indan and tetralin concentrations plotted vs. sample geological age. (B) Indan and tetralin concentrations plotted vs. sample collection depth. 1-Methylindan = (■), 1,3-dimethylindan = (△), 1-methyltetralin = (*), and 2-methyltetralin = (☒).

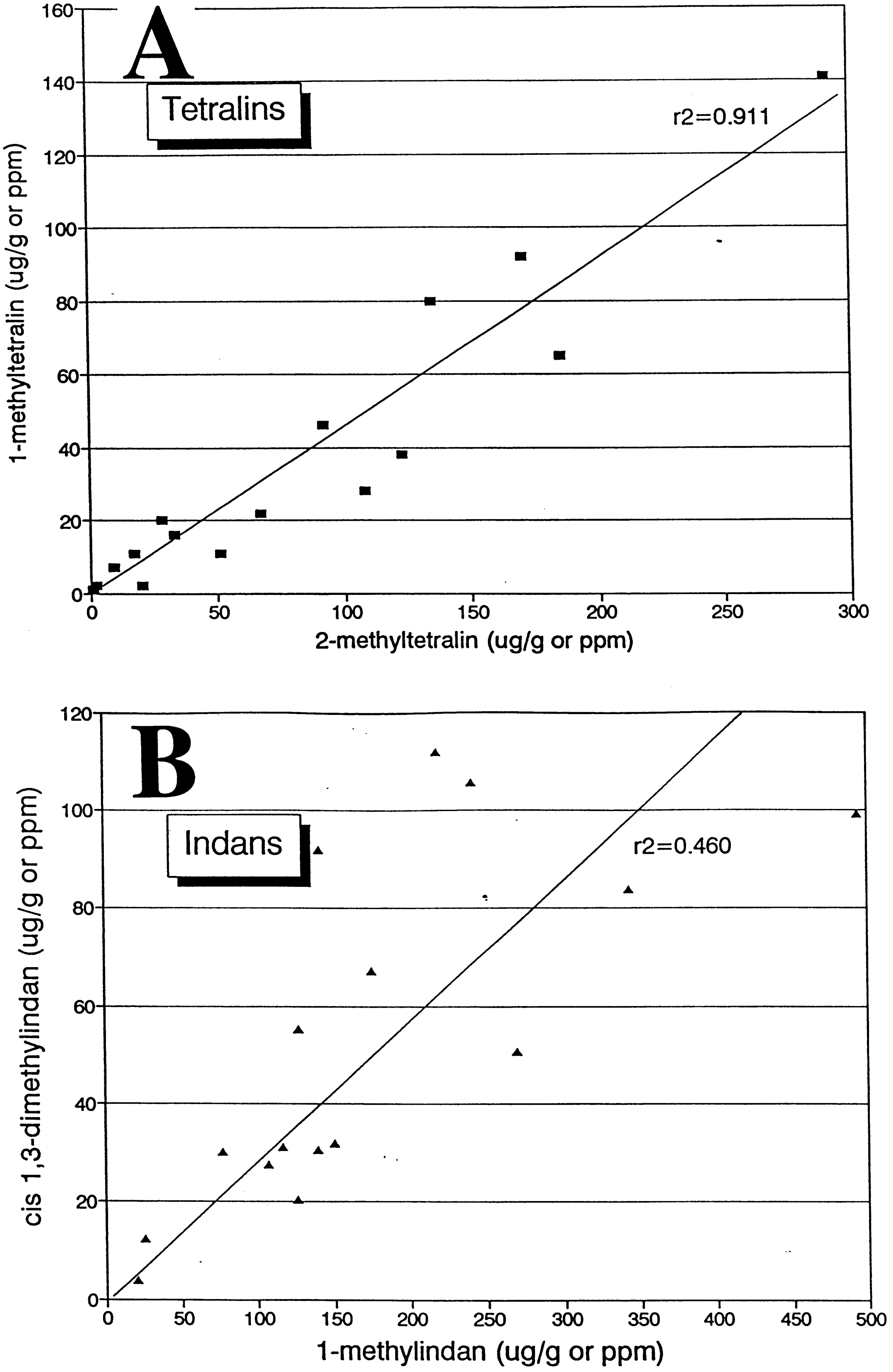


Fig. 6. Concentration correlation plots for the (A) indans and (B) tetralins in the oil samples. r^2 is the regression coefficient (see Table 4).

Table 4: Parameters of the regression analysis of the indan and tetralin concentrations found in oil samples and coal samples.

crude oils	**n**	**slope**	**intercept**	**r^2**
1-methyl *vs* 2-methyltetralin	16	0.463 ± 0.039	0 ± 13	0.911
1,3-dimethyl *vs* 1-methylindan	16	0.463 ± 0.057	0 ± 47	0.385
CIS-1,3-dimethyl *vs* 1-methylindan	16	0.278 ± 0.032	0 ± 26	0.460
TRANS-1,3-dimethyl *vs* 1-methylindan	16	0.185 ± 0.025	0 ± 21	0.282
coals	**n**	**slope**	**intercept**	**r^2**
1-methyl *vs* 2-methyltetralin	14	0.557 ± 0.034	0 ± 13	0.941
1,3-dimethyl *vs* 1-methylindan	14	0.209 ± 0.009	8.8 ± 9.0	0.986
CIS-1,3-dimethyl *vs* 1-methylindan	14	0.113 ± 0.006	5.1 ± 6.0	0.973
TRANS-1,3-dimethyl *vs* 1-methylindan	14	0.095 ± 0.004	3.7 ± 3.8	0.989

2-methyltetralin. Such a correlation may indicate that there are common mechanisms for the production and/or removal of these compounds thereby producing a constant ratio of substituted tetralins.

No correlation was found between concentrations of the substituted tetralins studied and the substituted indans studied. For example Sample #11 that contained higher amount of the tetralins (291 μg/g and 141 μg/g of 2-methyl and 1-methyltetralin, respectively) contained average amount of both indans. Sample #2 is richer in indan concentrations but contains moderate concentrations of the two tetralin derivatives (Table 1).

3.1.4. Relative concentration of indans

The same comparison of substituted indans (as with tetralins) can be done. However, in this case, 1,3-dimethylindan has two stereoisomers, the *cis* and the *trans* 1,3-dimethylindan which further possesses two enantiomeric forms, the R-R form and the mirror image S-S form. The R-S and S-R forms of the *cis*-isomer are identical (meso compound) due to the internal symmetry axis. The *trans*-1,3-dimethylindan was a racemic mixture in all oil samples studied. However, the relatives amount of *cis* and *trans* isomers varies as indicated in Table 1. The concentrations of the *cis*, the *trans* isomers, and the total amount of 1,2-dimethylindan were compared to the 1-methylindan concentration in all oil samples. The regression analysis showed a poor correlation with, by chance, exactly the same slope, 0.463, as for the tetralin regression line (Table 4). The higher regression coefficient, $r^2 = 0.460$, was obtained when the *cis* isomer of 1,3-dimethylindan was plotted vs. the 1-methylindan concentration (Fig. 6B).

All oil samples studied showed an excess of *cis* compared to the *trans*-1,3-dimethylindan. The smallest excess was 8% more *cis* than *trans* for Sample #11. However, there could be twice as much of the *cis*-form as the *trans*-form (i.e., Samples #9, #10, and #12, Table 1). The average *cis*-excess is 50% (standard deviation 4.5%) which means that on average there are three parts of the *cis*-form for every two parts of the *trans*-form of 1,3-dimethylindan found in the crude oils studied. In an independent study on the decomposition and isomerization of hydrocarbons, the *cis* and *trans* 1,3-dimethylindan were found to be formed in approximately equal amounts (Laidler and Loucks, 1972). In the degradation process, the molecular configuration of the stereogenic centers was lost. Consequently there is no obvious reason for the excess *cis* configuration to be retained.

4. STUDY OF COAL SAMPLES

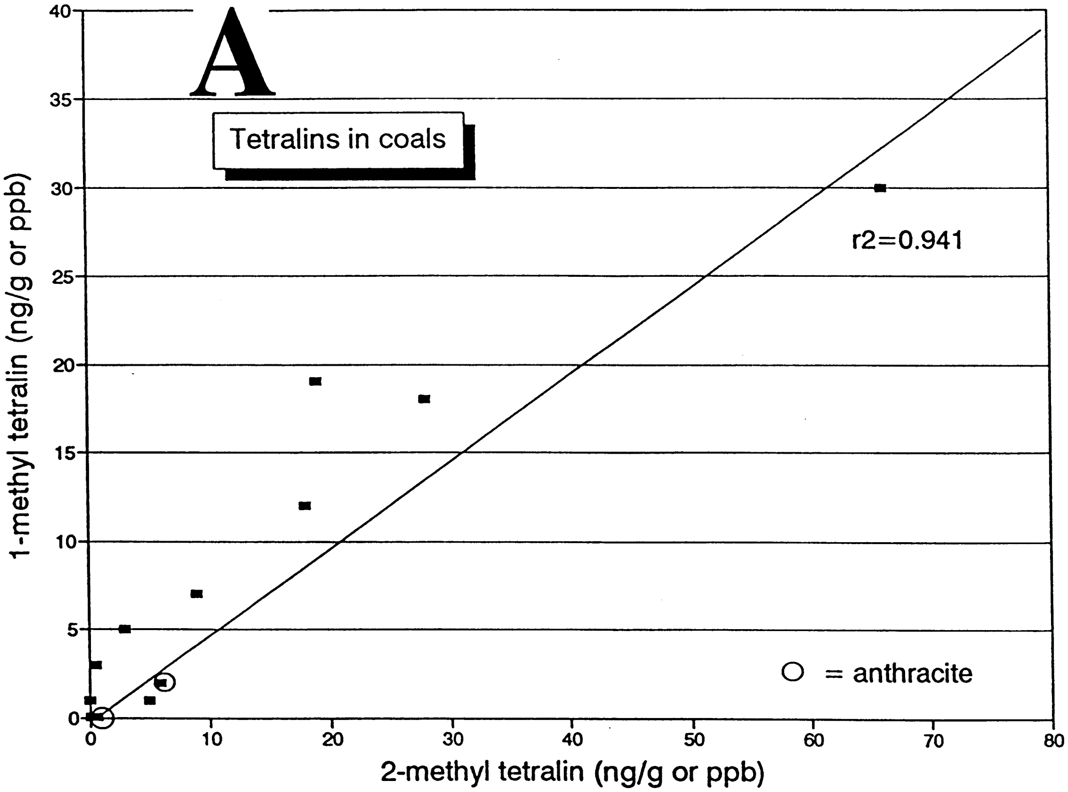
4.1. Quantitative Results

Table 2 lists the results obtained in the determination of the four indan and tetralin derivatives in fourteen coal and peat samples. A procedure similar to the one used for crude oil analysis was followed. Figure 4A shows a typical HPLC chromatogram of a coal extract and the collection times. Figure 4B shows the GC/MS chromatograms of 1-methyl indan (hydrogenated $m/z = 132$ and deuterated $m/z = 135$) obtained in the SIM mode. Figure 4B shows the high resolution obtained ($\alpha = 1.04$ and $Rs = 3.5$) in the separation of the two enantiomeric forms of 1-methylindan. It also shows the equal amounts of R and S enantiomers. As with the oil samples, the four chiral hydrocarbons studied in all fourteen samples were found to be racemic within experimental error. The background noise in the SIM mode was much lower with the coal extracts than with the oil extracts. This is partly due to the much lower concentration of extraneous material contained in the hexane extracts of coals compared to the wide variety of compounds contained in the crude oil fractions. This low background allowed us to reach a limit of detection in the 50 pg/g (ppt) range.

The concentrations of the substituted indans and tetralins found in coal samples were from 3 to 4 orders of magnitude lower than those found in the oil samples. The highest concentrations were obtained for 1-methylindan in Sample #1 and #8, both bituminous, they were only 741 and 774 ng/g (ppb), respectively (Table 2). These are much lower than the 25,000 ng/g found in Oil Sample #14 (Table 1) which contained the lowest 1-methylindan concentration. The bituminous coal ranks seemed to contain more indan and tetralin derivatives than either the younger peat and lignite ranks or than the most matured anthracite ranks (Table 2).

4.2. Relative Concentrations

It is evident from Table 2 that the indan and tetralin concentrations are interrelated as was found in the case of the oil samples. Table 4 lists the regression parameters obtained for the tetralin (Fig. 7A) and indan (Fig. 7B) plots. The correlation is better than that obtained with the oil samples (Table 4). The tetralin slopes in coals and oils are comparable in the 0.5 range. The slopes of *cis*-, *trans*-, or total dimethylindan in oils (0.278, 0.185 and 0.463, respectively, Table 4) are not comparable to these obtained in coals (0.113, 0.095, and 0.209, respectively), but may be due to the poor correlation obtained in oils. However, the *cis* isomer of the 1,3-dimethylindan was also in excess compared to the *trans* isomers. For two samples (#9 and #12), the *trans* isomer was in excess. It should be pointed out that the amount of 1,3-dimethylindan in Sample #9 was so low than we had to extract 40 g instead of 20 g of sample in order to obtain reliable quantitation. Sampling may be responsible for this result. These two cases of higher *trans*-isomer concentration are set apart. Plotting the *cis* over *trans* isomeric ratio vs. the 1,3-dimethylindan overall concentration seems to produce a decreasing ratio with increasing concentrations (Fig. 7B). It would suggest that, on the geological scale time, the loss of the *trans* isomer is slightly faster than the loss of the *cis* isomer. This trend cannot and will not be generalized because it is



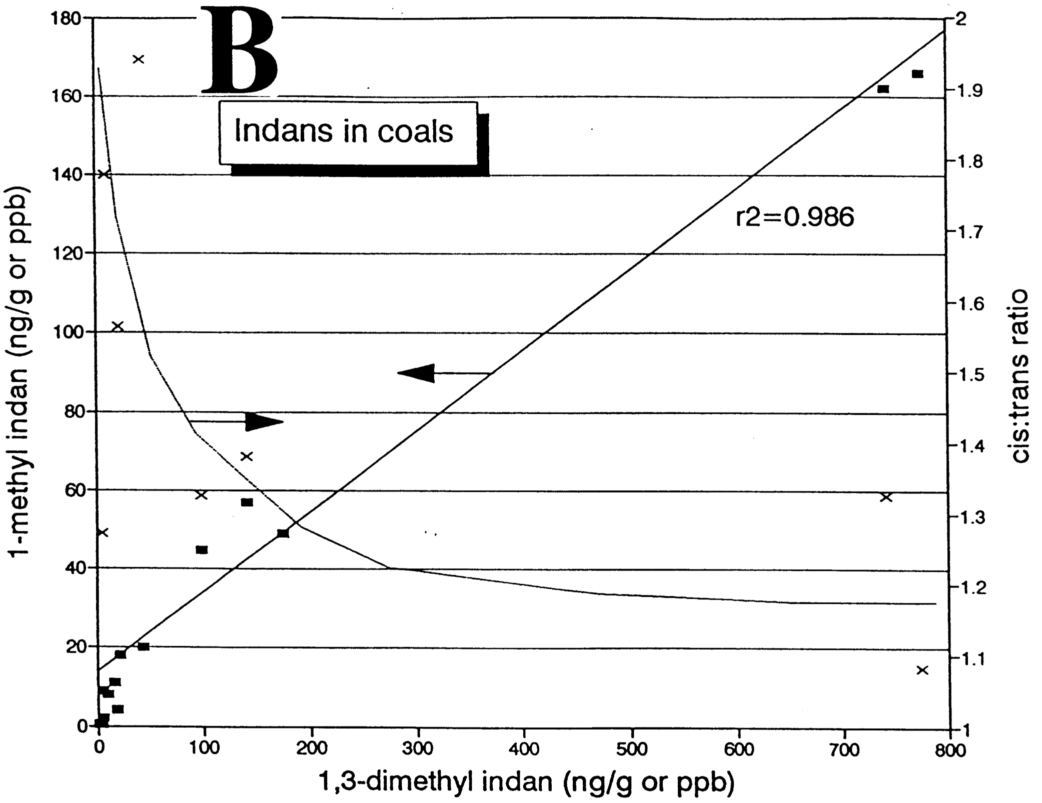


Fig. 7. Concentration correlation plots for the (A) indans and (B) tetralins in the coal samples. r^2 is the regression coefficient (see Table 4).

obtained with only twelve samples, and the dispersion of the experimental representative points is extensive (Fig. 7B).

The vegetal origin of coal is widely accepted. The origin and formation of crude oil is still not fully understood. The much lower amounts of the relatively volatile indan and tetralin molecules in coals compared to oils may be expected due to the solid nature of the coal compared to the liquid oils. However, the similar trends obtained for both the indan and tetralin relative concentrations may speak for a similar geochemical origin of these compounds in both oil and coal. In all coal and oil samples, no enantiomeric excesses were found for these chiral compounds. Thus, studies relating stereochemical differences in hydrocarbons to the thermal and geological history of fossil fuel deposits must rely on somewhat larger molecules. Likewise, the use of enantiomeric ratios of specific biomarkers to identify or characterize oil or coal must use somewhat larger molecules where this stereochemical information is retained.

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