

CHROM. 14,725

INFLUENCE OF ELUTION TEMPERATURE AND POLARITY OF DIFFERENT STATIONARY LIQUIDS ON THE RESOLUTION OF THE DIASTEREOMERS OF NORPRISTANE, PRISTANE AND PHYTANE*

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(First received November 5th, 1981; revised manuscript received January 8th, 1982)

SUMMARY

It is shown that glass capillaries of high separation efficiency and optimized selectivity are necessary to resolve the diastereomers of the C_{18} - C_{20} acyclic isoprenoid alkanes. In accordance with theory, relatively low column temperatures favour maximum selectivities (relative net retention times). Therefore high phase ratios of tailor-made glass capillaries are one of the prerequisites. In addition, the resolution can be more easily achieved with Carbowax 20M or even more polar stationary liquids than with non-polar ones.

INTRODUCTION

The determination of norpristane [2,6,10-trimethylpentadecane (1)], pristane [2,6,10,14-tetramethylpentadecane (2)] and phytane [2,6,10,14-tetramethyl-hexadecane (3)] (Fig. 1) is of importance in organic geochemical analysis in drawing certain conclusions about the diagenesis and maturation of natural oil and related products¹. In this context Maxwell and co-workers published partial separations of the diastereomers of pristane²⁻⁵ and phytane⁶. It is noteworthy that these authors achieved their separations of the non-polar alkanes only on capillaries (100 m × 0.25 mm and 100 m × 0.2 mm) coated with the polar stationary liquids BDS (butanediol succinate polyester), DEGS (diethylene glycol succinate) and DEGS-PEGS (polyethylene glycol succinate) (3:1) mostly in isothermal runs^{2-4,6}, but also in temperature-programmed⁵ runs at comparatively low elution temperatures between 40 and 100°C. A capillary column (100 ft. × 0.01 in.) coated with the non-polar solvent Apiezon L proved to be insufficient². Incomplete separations of the pristane and phytane diastereomers can be deduced from an environmental study by Grob⁷. In view of previous separations of diastereomeric alkanes up to C_{13} by capillary GC^{8,9} we decided to optimize the separations of the above-mentioned diastereomeric alkanes.

* From the thesis of H.B., University of Bochum, Bochum, July 1981.

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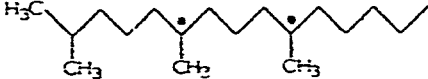
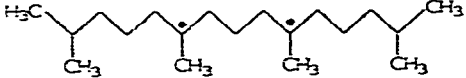
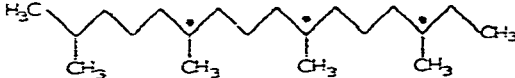
Formula and name	Number of possible stereochemical isomers	Number of possible peaks on non-chiral stationary liquids
	Norpristane 4	2
	Pristane 3	2
	Phytane 8	4

Fig. 1. Structural formulae and some stereochemical details of norpristane, pristane and phytane.

THEORY

Detailed treatments of the relevant theory can be found in text-books by Pur-nell¹⁰ and Littlewood¹¹. According to basic thermodynamics the isothermal separation of compound 1 from compound 2 can be represented by:

$$\Delta G_1 - \Delta G_2 = \Delta \Delta G \quad (1)$$

$$= RT \ln (K_2/K_1) \quad (2)$$

$$= \Delta H_v - T \Delta \Delta S \quad (3)$$

By substituting $K = k'\beta$ and $k'_2/k'_1 = \alpha$ we finally get:

$$\ln \alpha = \frac{\Delta H_v}{RT} + \frac{\Delta \Delta S}{R} \quad (4)$$

$\Delta G_1, \Delta G_2$ = Gibbs free energies of compounds 1 and 2

$\Delta \Delta G$ = difference of Gibbs free energies of separated compounds 1 and 2

K_1, K_2 = equilibrium constants of compounds 1 and 2 between the stationary liquid and the mobile gas phase

β = phase ratio of the gas chromatographic (GC) column

k'_1, k'_2 = capacity ratios of separated compounds 1 and 2

α = net retention time of compound 2 relative to compound 1

ΔH_v = difference of heats of vaporization of compounds 1 and 2 from the stationary into the mobile gas phase

$\Delta \Delta S$ = difference of entropy changes caused by the transition of compounds 1 and 2 from the stationary into the mobile gas phase

Strictly, eqn. 4 holds only if both the stationary and the mobile phase are pure compounds and if the isothermal partition of the solutes between the two phases is not influenced by adsorption. The first requirement was met exactly, when Kováts¹² hydrocarbon $C_{87}H_{176}$ was used as stationary phase, whereas the polymers OV-101 and Carbowax 20M can only be considered as approximately pure substances. The second assumption was easily fulfilled, since branched, saturated hydrocarbons are

non-polar and hardly suffer from adsorption on active support surfaces. In conclusion, eqn. 4 describes the basic chromatographic experience that resolutions of most compounds increase with decreasing temperature. If $\ln \alpha$ in eqn. 4 is plotted against $1/T$, ΔH_s can be derived from the slope and $\Delta \Delta S$ from the intercept of this graph with the y -axis. In Tables II–IV we have listed only the ΔH_s values for the temperature range under investigation. Due to the limited number of data points and temperature range, determinations of the $\Delta \Delta S$ values by extrapolation were not tried.

Under isothermal conditions, the net retention time of a solute is directly proportional to the mass of the stationary liquid in the column. Because glass capillaries contain much lower amounts of stationary liquid than packed columns, an increase in the relative net retention time, α , and thus selectivity can be more easily realized by lowering the elution temperatures with open tubular columns than with packed columns. This fact is overlooked by many chromatographers.

EXPERIMENTAL

Glass capillaries (85, 100 and 140 m \times 0.27 mm I.D.) were drawn from alkali glass tubes (Schott, Mainz, G.F.R.), treated with gaseous HCl and HF (the latter highly diluted in nitrogen by a two-stage process), deactivated, coated by the mercury-plug method and tested as described previously by Schomburg and co-workers^{13,14}. The performance and operation of these columns is given in Table I. Hydrogen was exclusively used as carrier gas with an average linear velocity of 0.35 m/sec (minimum height equivalent to a theoretical plate, $HETP_{min}$).

TABLE I

PERFORMANCE AND OPERATION OF GLASS CAPILLARY COLUMNS FOR THE SEPARATION OF THE DIASTEREOMERS OF NORPRISTANE, PRISTANE AND PHYTANE

	<i>Column I</i>	<i>Column II</i>	<i>Column III</i>
Dimensions	140 m \times 0.27 mm	100 m \times 0.27 mm	85 m \times 0.27 mm
Stationary phase	C ₈₇ H ₁₇₆	OV-101 (dimethylpolysiloxane)	Carbowax 20M (polyethylene glycol)
Number of theoretical plates at k' value	295,000	217,000	131,000
Carrier gas	Hydrogen	Hydrogen	Hydrogen
Linear velocity (m/sec)	0.35	0.35	0.35
Column temperatures	100–230°C, 0.8°C/min		
Isothermal mode	130, 150, 180°C	120, 130, 150°C	90, 100, 120°C

Because our minute supplies of standard compounds had been used up, we used a distillation cut (250–330°C, 1 bar) of natural oil rich in paraffins, kindly provided by Messrs. Herold and Kegler, Deutsche Shell, Hamburg, G.F.R. Pristane and phytane were identified as constituents of this distillation fraction by their characteristic elution behaviour from columns coated with dimethylpolysiloxanes as stationary phase. Secondly we compared their Kováts' indices obtained from column II with those of standard compounds obtained in earlier experiments¹⁵ from a 100-m

capillary column (stationary phase dimethylpolysiloxane DC 200). Thirdly we confirmed our identification of norpristane, pristane and phytane by glass capillary GC-mass spectrometry (MS). These experiments were made with another 50-m column coated with a relatively thick film of 550 nm of dimethylpolysiloxane OV-101, in order to obtain mass spectra of adequate intensity of the highly branched alkanes. The capillary column was connected to a Varian CH 7A magnetic field mass spectrometer in the open split mode^{16,17}. The numerous mass spectra of acyclic isoprenoid alkanes compiled by Holzer *et al.*¹⁸ and Johns *et al.*¹⁹ were used for comparison. Norpristane was also confirmed as a constituent of the petroleum cut investigated by comparing its Kováts' indices obtained from column II with a value calculated by Schomburg's method⁹ of index increments of first and second order.

RESULTS AND DISCUSSION

Fig. 2 shows an overview (a) and the relevant part (b) of a temperature-programmed run of norpristane (1), pristane (2) and phytane (3) obtained from capillary I (stationary phase Kováts¹² hydrocarbon C₈₇H₁₇₆). A low heating rate of 0.8°C/min was chosen, because in temperature-programmed GC the resolution increases with decreasing heating rate²⁰. Better resolutions of the three diastereomeric pairs were achieved with column I under isothermal conditions (Fig. 2c and d, retention data in Table II). The maximum selectivity, expressed by the relative net retention time, α , was obtained from column I at an elution temperature of 130°C. Under these conditions a retention time of 380 min for the baseline-separated phytane diastereomers was obtained and extreme care had to be taken not to overload the column. Therefore, we suppose that Maxwell *et al.*² failed to resolve the pristane diastereomers with their Apiezon L capillary because the elution temperature of 160°C was too high, and not because of the nature of Apiezon L which mainly consists of high-molecular-weight hydrocarbons and methyl phenyl ethers²¹. This assumption is strongly supported by the retention data compiled in Table II. Here an increase of the relative net retention time, α , from 1.0045 to 1.0085 for the pristane diastereomers is observed, if the elution temperature of column I is lowered from 180°C to 130°C.

Table III compiles the chromatographic data obtained from column II (stationary liquid dimethylpolysiloxane OV-101). In comparison to Table II, slightly lower α values are observed for the pristane and phytane diastereomers at 150 and 130°C. With column II we achieved a slightly lower oven temperature of 120°C, resulting in an increase of all α values. The relative net retention time, α , of the pristane diastereomers of 1.0095 at 120°C even supersedes the α value of 1.0085 for the same separation obtained from column I (stationary phase C₈₇H₁₇₆) at 130°C. This increase can also be seen in Fig. 3a where, according to eqn. 4, $\ln \alpha$ of the diastereomers of pristane is plotted against $1/T$ for the stationary phases C₈₇H₁₇₆ hydrocarbon, dimethylpolysiloxane OV-101 and polyethylene glycol Carbowax 20M of capillary columns I, II and III. The corresponding graphs for the separated phytane diastereomers are plotted in Fig. 3b. Additionally, the differences of the heats of vaporization, ΔH_v , of the diastereomers of pristane and phytane were calculated from the slopes of these graphs (Tables II-IV). Thus the strongest temperature dependence of the separation of the pristane and phytane can be observed on column II (stationary phase dimethylpolysiloxane OV-101).

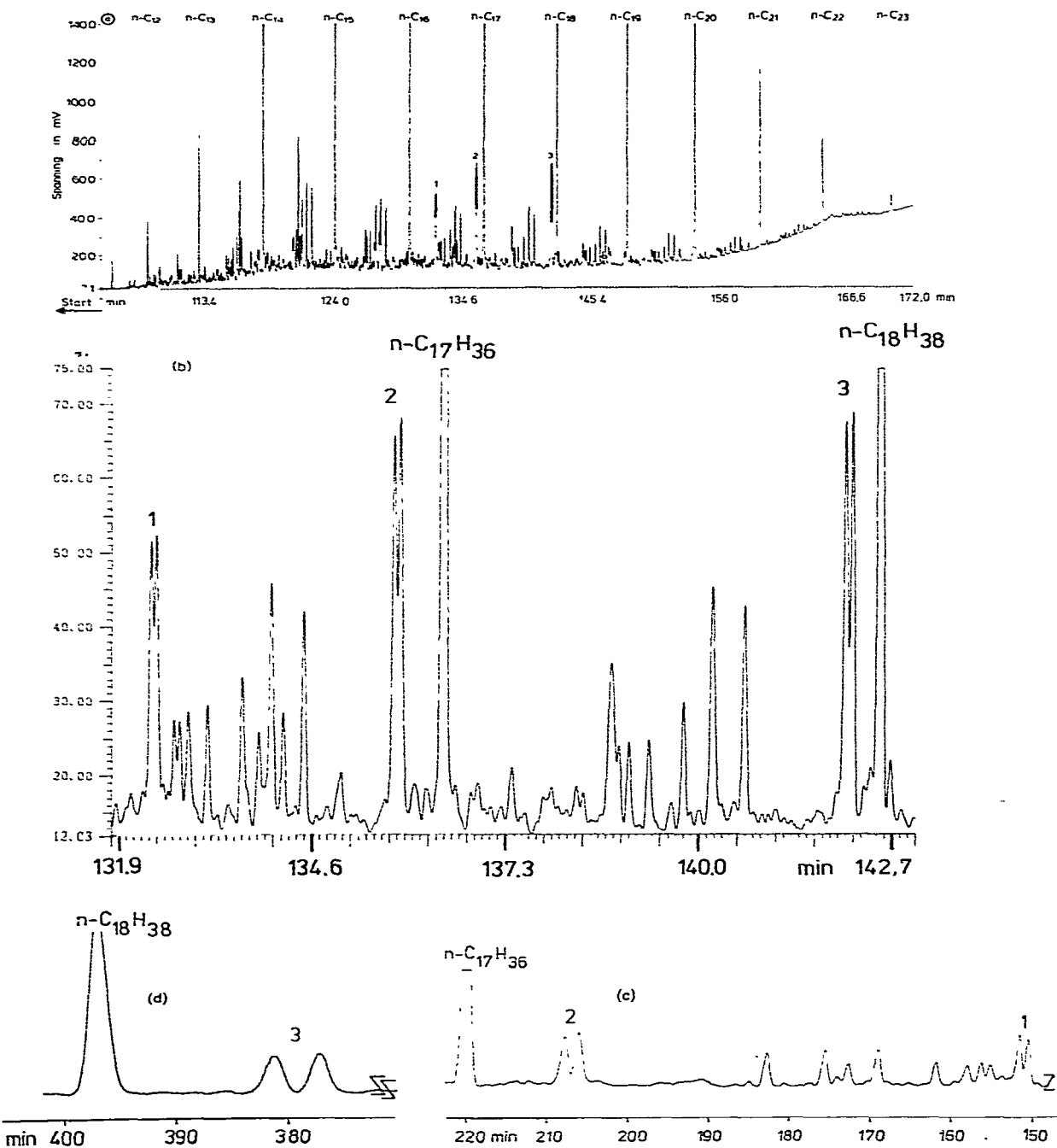


Fig. 2. Analysis of diastereomers of norpristane (1), pristane (2) and phytane (3) in a petroleum cut. a, Overall chromatogram. Column: 140 m \times 0.27 mm I.D. Stationary liquid: $\text{C}_{87}\text{H}_{176}$. Temperature: 100–230°C, 0.8°C/min. Carrier gas: hydrogen, 0.35 m/sec at 230°C. b, Relevant part. Temperature: 132–143°C, 0.8°C/min. Other conditions as in a. c, Isothermal elution of norpristane (1) and pristane (2). Temperature: 130°C. Other conditions as in a. d, Isothermal elution of phytane (3) as in c.

TABLE II
RETENTION DATA OF THE DIASTEREOMERS OF PRISTANE AND PHYTANE OBTAINED FROM COLUMN I (STATIONARY LIQUID $C_{87}H_{176}$)

	Diastereomers of <i>pristane</i>			Diastereomers of <i>phytane</i>		
	130	150	180	130	150	180
Column temperature, T (°C)						
$1/T$ (K ⁻¹)	0.002481	0.002363	0.002207	0.002481	0.002363	0.002207
α	1.0085	1.0074	1.0045	1.0116	1.0092	1.0063
$\ln \alpha$	0.008464	0.007373	0.004490	0.01153	0.009158	0.006280
$\ln \alpha = 14.73/T$ (K ⁻¹) - 0.028	Correl. coeff. 0.985					
$\ln \alpha = 19.14/T$ (K ⁻¹) - 0.036	Correl. coeff. 0.9996					
ΔG (J/mol)	28.45	25.94	16.92	38.78	32.22	23.66
ΔH_v (J/mol)	123.2 between 130 and 180°C					
Kováts' indices	1688.8					
Kováts' indices	1690.2					
ΔI (IE)	1.4					
Resolution	1.051					
	1.301					
	1.225					
	160.3 between 130 and 180°C					
	1791.7					
	1793.3					
	1.6					
	1.3					

TABLE III
RETENTION DATA OF THE DIASTEREOMERS OF PRISTANE AND PHYTANE OBTAINED FROM COLUMN II (STATIONARY LIQUID OV-101)

	<i>Diastereomers of pristane</i>			<i>Diastereomers of phytane</i>		
	120	130	150	120	130	150
Column temperature, T (°C)						
$1/T$ (K^{-1})	0.0025436	0.0024805	0.0023632	0.0025436	0.0024805	0.0023632
α	1.0095	1.0081	1.0055	1.0112	1.0098	1.0070
$\ln \alpha$	0.009429	0.008044	0.005474	0.01112	0.009709	0.006937
$\ln \alpha = 21.92/T - 0.046$	Correl. coeff. 0.99999			$\ln \alpha = 23.63/T - 0.049$ Correl. coeff. 0.999999		
ΔG (J/mol)	30.82	26.96	19.26	36.35	32.54	24.41
ΔH , (J/mol)	182.2 between 120 and 150°C			193.2 between 120 and 150°C		
Kováts' indices	1707.7	1708.4		1809.5	1810.6	
	1709.4	1710.0		1811.6	1812.4	
ΔI	1.7	1.6		2.1	1.8	
Resolution	1.177	0.872		1.253	1.025	

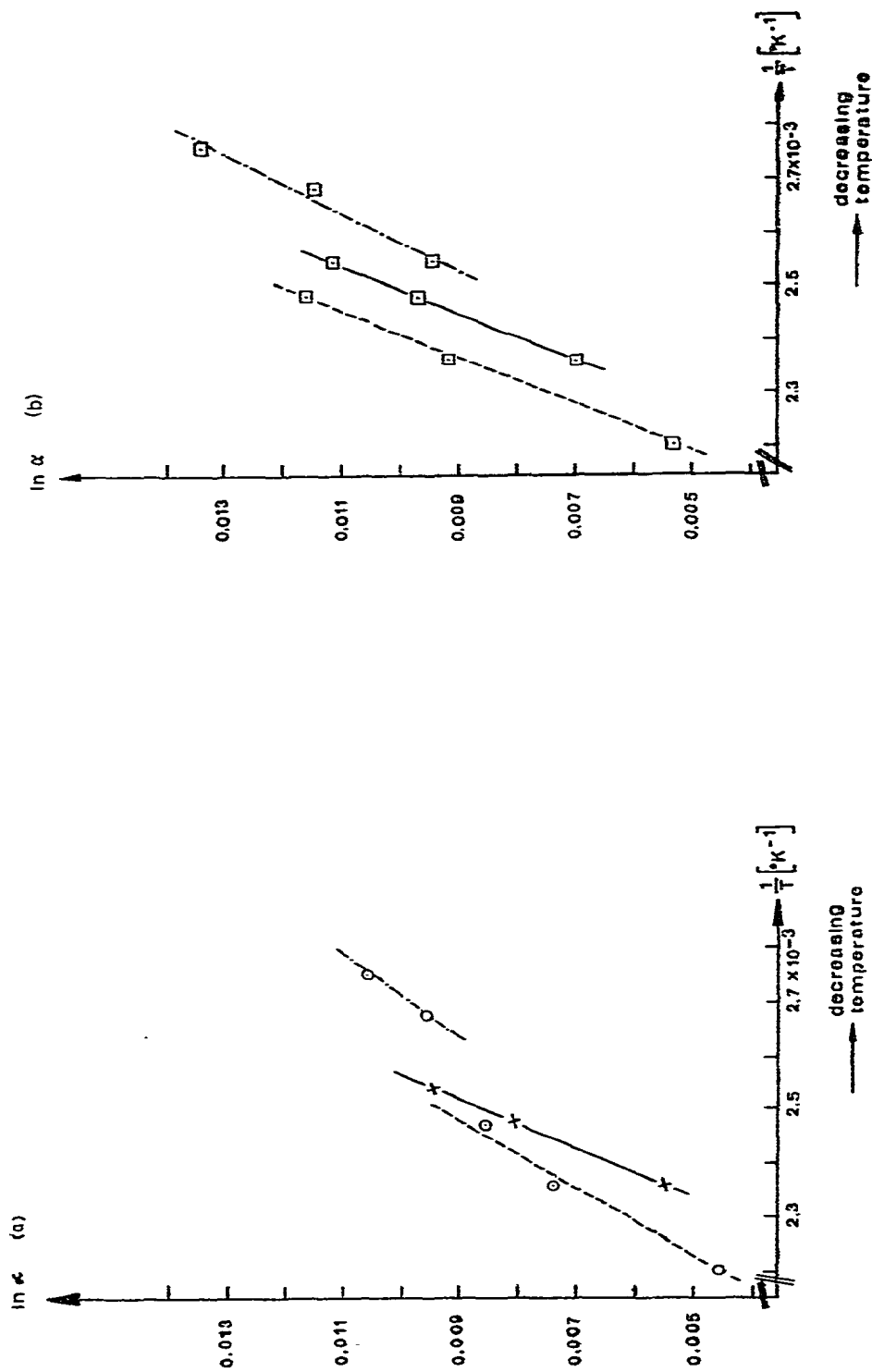


Fig. 3. Dependency of $\ln \alpha$ of the pristane (a) and phytane (b) diisomers on $1/T$ on glass capillaries coated with stationary liquids of different polarity: —, dimethylpolysiloxane OV-101; ---, $\text{C}_{87}\text{H}_{176}$ hydrocarbon; - - - - - , polyethylene glycol Carbowax 20M.

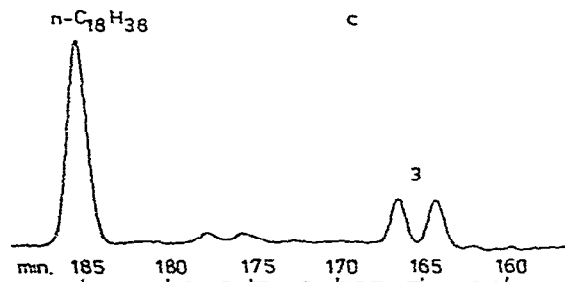
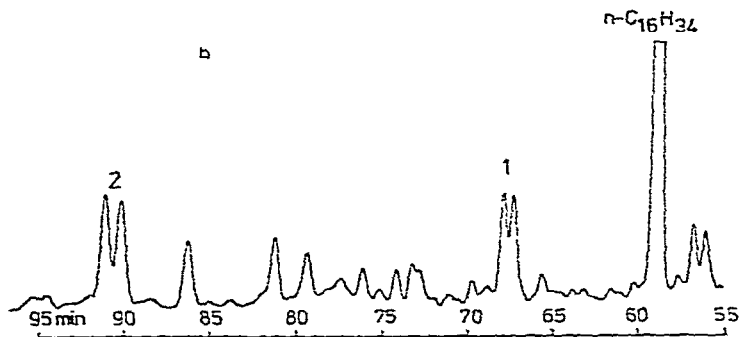
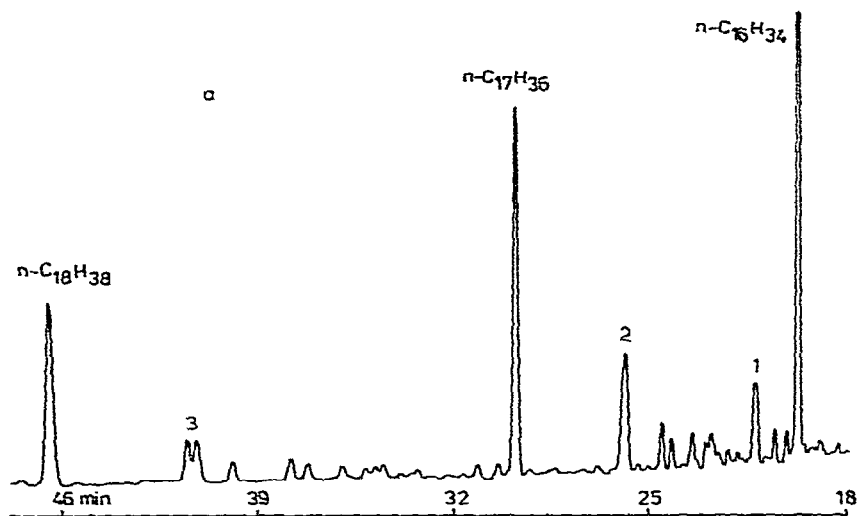


Fig. 4. Isothermal analysis of the diastereomers of norpristane (1), pristane (2) and phytane (3) on the polar stationary liquid Carbowax 20M. a, No resolution at elevated temperature. Column: 85 m \times 0.27 mm I.D. Temperature: 120°C. Carrier gas: hydrogen, 0.35 m/sec. b, Greatly improved resolution of norpristane (1) and pristane (2) at decreased elution temperature of 90°C. c, Greatly improved resolution of phytane (3) at decreased elution temperature of 90°C.

TABLE IV
RETENTION DATA OF THE DIASTEREOMERS OF PRISTANE AND PHYTANE OBTAINED FROM COLUMN III (STATIONARY LIQUID
CARBOWAX 20M)

	Diastereomers of pristane		Diastereomers of phytane	
	90	100	90	100
Column temperature, T ($^{\circ}\text{C}$)				
$1/T$ [K^{-1}]				
α	0.002754	0.002680	0.002754	0.002680
$\ln \alpha$	1.0106	1.0095	1.0135	1.0115
	0.0105	0.009503	0.01241	0.01144
$\Delta\Delta G$ (J/mol)	31.71	29.48	40.48	35.49
$\Delta\Delta H_v$ (J/mol)			152.7 between 90 and 120 $^{\circ}\text{C}$	
Resolution	0.84	0.75	1.161	1.072
				0.79

$\ln \alpha = 18.36/T - 0.037$ Correl. coeff. 0.986

Fig. 4a-c illustrates the increase of selectivity when the elution temperature of column III (stationary liquid Carbowax 20M) is decreased from 120°C to 90°C. The corresponding retention data are compiled in Table IV. While at 120°C the diastereomers of norpristane (1) and pristane (2) are not at all resolved (Fig. 4a), a decrease of just 30°C shows greatly improved separations (Fig. 4b and c). The α values of pristane and phytane increase to 1.0106 and 1.0135, respectively, thus superseding all other α values obtained from the non-polar columns I and II at higher temperatures (compare also Fig. 3a and b). This clearly suggests that the separations of the diastereomers under investigation are mostly favoured by relatively very low elution temperatures. This can be more easily achieved with polar stationary phases because of the decreased solubility of non-polar isoprenoid alkanes in polar solvents. By switching from column I (stationary liquid $C_{87}H_{176}$) to column III (stationary liquid Carbowax 20M) the retention times of the phytane diastereomers are roughly halved from 380 to 165 min. In spite of this progress, the maximum number 2^{n-1} (n = number of chiral centres in a molecule) of possible peaks which can be theoretically obtained on non-chiral stationary phases could only be achieved in the case of norpristane and pristane. In the case of phytane with its three chiral centres, however, four enantiomeric pairs and thus four peaks can be expected in theory, whereas we obtained only two peaks in spite of highly efficient glass capillaries (compare Table I). This lack may be explained by the thermal energy of the molecules which is too high for the desired separation even at a column temperature of 90°C. Furthermore, it is interesting to note that pristane and phytane are eluted from columns I and III (stationary phases $C_{87}H_{176}$ and Carbowax 20M) before the n -alkanes C_{17} and C_{18} respectively, whereas on column II (stationary liquid OV-101) pristane and phytane follow these n -alkanes (compare Kováts' indices in Tables II and III, and Figs. 2a-d and 4a-c). We must finally recognize that our previous general statement²² "selectivity changes for better relative volatilities cannot be successfully used in the case of weak intermolecular interaction of low polarity compounds like hydrocarbons" is not always correct.

As mentioned before, very high phase ratios of GC columns favour low elution temperatures and thus greatly improved selectivities. In this context we would like to expand Grob's question²³ "Are We Using the Full Range of Film Thicknesses in Capillary-GLC?" to "Are We Using the Full Range of Film Thicknesses and of Phase Ratios in Capillary-GLC to Obtain Improved Selectivities at Lower Temperatures?". This question is especially relevant for the elution of thermally sensitive solutes.

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