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Note

High-performance liquid chromatography of long chain 7-oxo alcohols, acids and their esters[a]

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Long chain fatty alcohols, acids and their derivatives are important intermediates in the manufacture of various oil-field chemicals[1–3], *viz.*, pourpoint depressants, surface active and anticorrosive agents. Their carbon numbers generally vary between C_{12} and C_{22}. A series of 7-oxo alcohols, acids and their esters of carbon numbers C_{12}–C_{22} have recently been synthesized[4,5] in our laboratory for the first time to evaluate the flow improving properties of their comb-like polymers on different crude oils. A method is needed for their identification and purity determination, and a thorough literature survey having revealed that no such method is available.

Generally, fatty alcohols are separated by gas chromatography (GC)[6,7] after converting them into trifluoroacetate/acetate esters. The quantitative separation and identification of oxopentanols in reaction mixtures by gas chromatography–mass spectrometry (GC–MS) and NMR spectroscopy have been reported[8,9]. Short-chain oxo alcohols such as 3-hydroxy-2-octanone, 5-hydroxy-2-hexanone and 4-hydroxy-pentanone in different fat products have been separated quantitatively by GC[10,11]. However, these methods are tedious and time consuming since they involve a large number of derivatization steps. Subbarao and co-workers[12,13] have described the separation of several fatty alcohols and their oxygenated compounds using thin-layer chromatography (TLC). Weihrauch *et al.*[14] have estimated a variety of long-chain oxo fatty acids, isolated from milk fat, by GC–MS. Methods for the separation of positional isomers of C_{18} and C_{24} oxo acids have also been reported[15,16].

The present paper reports a simple high-performance liquid chromatographic (HPLC) method for the separation and identification of 7-oxo alcohols, acids and their esters in mixtures. It compares the chromatographic behaviour of these compounds with that of the corresponding fatty alcohols, acids and their esters. Further it describes the application of this method to the analysis of long chain saturated alcohols and acids present in different waxes.

EXPERIMENTAL

Apparatus

A high-pressure liquid chromatograph Model ALC/GPC 244 equipped with a

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Model M 6000 A reciprocating pump and U6K injector from Water Assoc. (Milford, MA, U.S.A.) was used. It was connected to a refractive index detector and a D 5000 duel channel strip-chart recorder with a Chromatopak E 1A integrator (Shimadzu). A 10-μl syringe (Hamilton, Bonaduz, Switzerland) and a stainless-steel fatty acid column (30 cm × 3.9 mm I.D., 10 μm) were used.

Materials and reagents

7-Oxo alcohols and 7-oxo acids were synthesized in our laboratory according to the procedures reported elsewhere[4,5]. Their purities were checked by TLC. Fatty alcohols and fatty acids obtained from Sigma (St. Louis, MO, U.S.A.) were used as supplied without any purification. The ethyl esters of related carboxylic acids were prepared by a general method[17]. Two different waxes, *viz*, bees-wax and sugar cane wax, were saponified prior to analysis.

All reagents used were of HPLC grade obtained from Spectrochem, India.

Mobile phase

The mobile phase used was water–acetonitrile–tetrahydrofuran (35:45:20, v/v/v). The components were degassed before mixing.

Procedure

A standard mixture of 7-oxo alcohols was injected on to the column by means of a 10-μl Hamilton syringe through a U6K injector using a flow technique. The analysis was carried out under isocratic conditions at a flow-rate of 1.5 ml/min and a chart speed of 1.0 cm/min at room temperature (27°C). The column was stabilized prior to its use.

RESULTS AND DISCUSSION

The long chain 7-oxo alcohols, acids and their esters (series A) were synthesized[4,5] in the laboratory. Corresponding fatty acids and their respective derivatives (series B) were selected for comparative studies. They have been analysed by HPLC. The retention times, t_R, and capacity factors, k', for these compounds are given in Table I. Fig. 1 shows that 7-oxo alcohols ranging in carbon number from C_{12} to C_{22} are completely separated. The peaks were identified from the retention times of individual compounds. As the retention times increase the peaks are broadened progressively with increasing carbon chain length from C_{12} to C_{22}.

The retention data for series A and B (Table I) have been used to find out the kind of molecular interaction involved in their chromatographic separation. Fig. 2 shows plots between log k' and the carbon numbers of these compounds. The plots are linear for the respective series. It can be concluded that partition phenomena play a dominant rôle in the separation of these compounds. Further, it is seen from Table I and Fig. 2 that the trends in the separation of corresponding compounds in series A and B are similar. However, the long-chain 7-oxo derivatives of series A have shorter retention times than the corresponding compounds of equivalent chain length in series B. This may be due to the fact that the 7-oxo substituted compounds are more polar than those compounds having no substitution.

The method developed has great potential for the simultaneous separation and

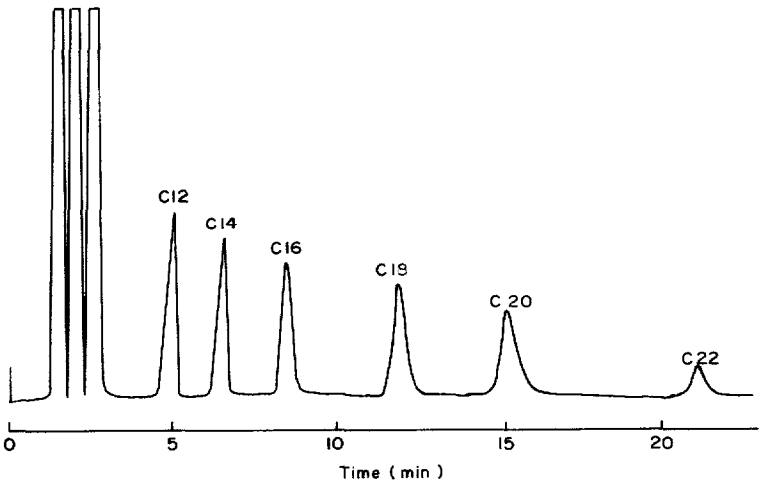
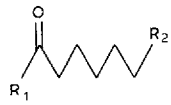


Fig. 1. Chromatogram of C_{12}–C_{22} 7-oxo alcohols. For conditions, see text.

TABLE I

RETENTION DATA FOR SERIES A AND B

Series A: 

Series B: 

R_1	Carbon number	R_2					
		CH_2OH		$COOH$		$COOC_2H_5$	
		$t_R(s)$	k'	$t_R(s)$	k'	$t_R(s)$	k'
Series A							
$-(CH_2)_4CH_3$	12	346	0.55	335	0.50	435	0.94
$-(CH_2)_6CH_3$	14	402	0.79	386	0.72	505	1.25
$-(CH_2)_8CH_3$	16	478	1.13	445	0.99	625	1.79
$-(CH_2)_{10}CH_3$	18	582	1.60	527	1.35	718	2.48
$-(CH_2)_{12}CH_3$	20	720	2.21	644	1.88	1003	3.48
$-(CH_2)_{14}CH_3$	22	929	3.15	780	2.48	1309	4.84
Series B							
$-(CH_2)_4CH_3$	12	501	1.24	427	0.91	548	1.45
$-(CH_2)_6CH_3$	14	614	1.74	510	1.28	684	2.05
$-(CH_2)_8CH_3$	16	772	2.45	620	1.77	880	2.93
$-(CH_2)_{10}CH_3$	18	1000	3.46	765	2.42	1187	4.30
$-(CH)_2)_{12}CH_3$	20	1271	4.67	927	3.14	1626	6.26
$-(CH_2)_{14}CH_3$	22	1710	6.63	1239	4.53	2175	8.71

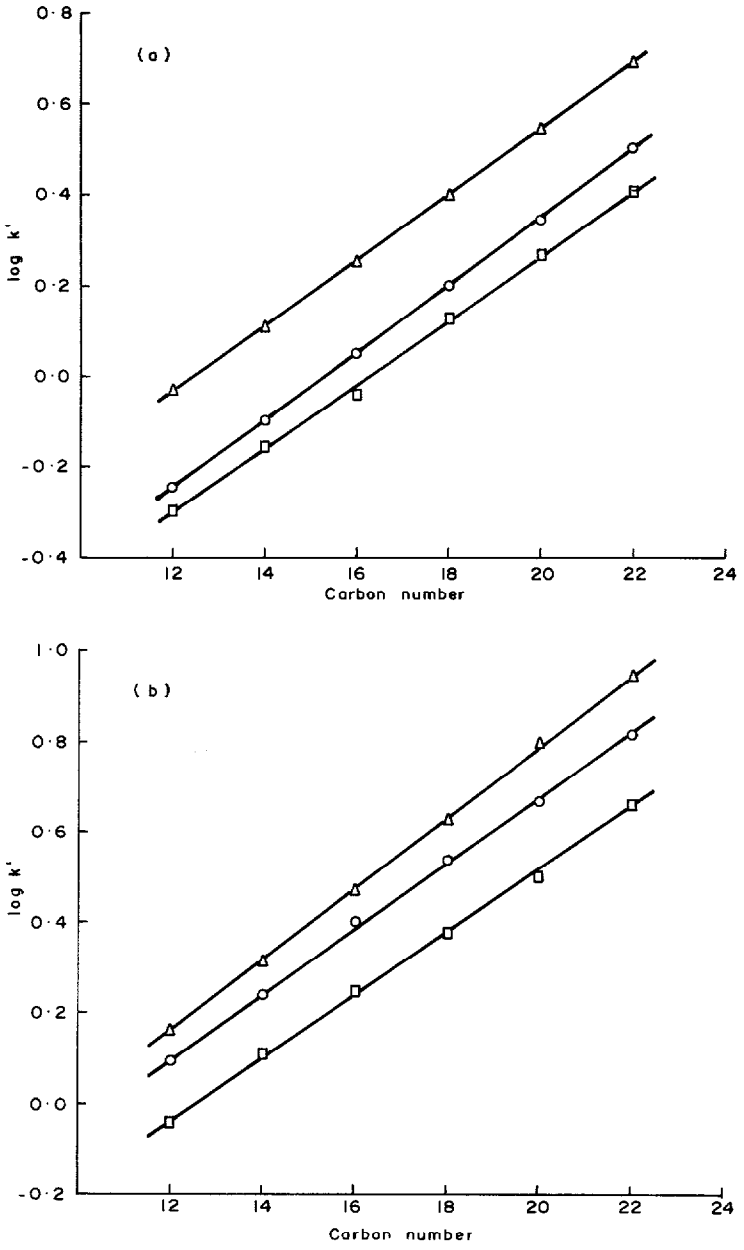


Fig. 2. Plots of log k' *vs.* carbon number for (a) series A and (b) series B. ○, CH_2OH; □, COOH; △, $COOC_2H_5$.

identification of 7-oxo alcohols/acids in mixtures either prepared synthetically or isolated from natural products. Since the 7-oxo alcohols rarely occur in nature, this method cannot be applied for their analysis. However, two different waxes have been saponified first in order to separate fatty alcohols and acids, and then subjected to

TABLE II

COMPOSITION OF FATTY ACIDS AND ALCOHOLS IN COMMERCIAL WAXES

Average of three determinations

Composition (by carbon number)		*Composition (%)*			
		HPLC		*GC*	
		Bees wax	*Sugar cane*	*Bees wax*	*Sugar cane*
C_{14}	acid	1.0 ± 0.1	–	1.1 ± 0.1	–
	alcohol	–	–	–	–
C_{16}	acid	51.4 ± 1.3	3.0 ± 0.1	50.5 ± 1.6	2.9 ± 0.2
	alcohol	–	–	–	–
C_{18}	acid	9.0 ± 0.5	2.0 ± 0.1	8.8 ± 0.7	2.0 ± 0.1
	alcohol	–	–	–	–
C_{20}	acid	1.5 ± 0.2	1.6 ± 0.1	1.0 ± 0.1	1.7 ± 0.3
	alcohol	–	–	–	–
C_{22}	acid	1.5 ± 0.2	3.5 ± 0.1	1.8 ± 0.1	3.8 ± 0.3
	alcohol	–	–	–	–
C_{24}	acid	18.0 ± 0.9	3.4 ± 0.1	17.6 ± 0.6	3.1 ± 0.1
	alcohol	12.5 ± 0.5	0.5 ± 0.1	11.9 ± 0.8	0.6 ± 0.1
C_{26}	acid	4.5 ± 0.4	10.2 ± 0.6	4.9 ± 0.5	10.1 ± 0.9
	alcohol	10.5 ± 0.8	13.2 ± 0.7	10.1 ± 0.8	15.4 ± 0.9
C_{28}	acid	4.0 ± 0.3	56.0 ± 1.3	43.0 ± 0.5	58.5 ± 1.5
	alcohol	13.8 ± 0.7	75.8 ± 1.7	14.2 ± 0.9	76.7 ± 1.9
C_{30}	acid	3.0 ± 0.2	10.5 ± 0.5	3.0 ± 0.3	10.5 ± 0.6
	alcohol	33.6 ± 1.1	8.0 ± 0.3	31.6 ± 1.5	5.2 ± 0.2
C_{32}	acid	5.0 ± 0.3	5.5 ± 0.2	47.0 ± 0.2	5.4 ± 0.4
	alcohol	23.1 ± 1.0	2.5 ± 0.1	23.5 ± 1.4	2.1 ± 0.3
C_{34}	acid	1.1 ± 0.1	4.3 ± 0.1	2.0 ± 0.2	3.9 ± 0.3
	alcohol	6.5 ± 0.3	–	6.0 ± 0.5	–

HPLC analysis. The quantity of each component has been determined by comparing its peak area with the total area of the peaks in the chromatogram. Table II gives the respective compositions obtained by HPLC and GC[18,19]. It can be seen that the results obtained by the two techniques are in good agreement. They clearly demonstrate the suitability of the present method for determining the composition of fatty acids and alcohols in waxes and also wax esters which are a dominant class of lipids in calanoid copepods[20,21]. The method is more simple and rapid than those which involve a number of derivatization steps.

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