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COMPLEX-FORMING STATIONARY PHASES IN HIGH-SPEED LIQUID CHROMATOGRAPHY

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SUMMARY

Solid dimeric rhodium(II) acetate deposited on Corasil II gave good separation of the butenes using high-speed liquid chromatography. Essentially, the unique order of retention times observed with gas-liquid chromatography using rhodium(II) benzoate was found, namely 1-butene < *trans*-butene < isobutene < *cis*-butene. Similarly with silver nitrate in ethylene glycol coated on Corasil II, various olefins emerged with retention times that were in accordance with their respective silver complexation constants. This system was applied to the separation of some unsaturated natural compounds such as fatty acids, prostaglandins and pentacyclic triterpenes.

INTRODUCTION

Complex-forming stationary phases¹ have been used with considerable success for achieving high selectivity in both gas-liquid chromatography (GLC)²⁻⁴ and thin-layer chromatography (TLC)⁵. Modern high-speed liquid chromatography (LC) has the advantages that it is limited to relatively volatile and thermally stable compounds (as compared with GLC), and readily gives quantitative determinations and higher efficiencies (as compared with TLC)⁶. It was therefore considered of interest to test the application of complex-forming stationary phases in modern LC. The stationary phases were prepared with the classical olefin-complexation agent silver nitrate on the one hand, and with the recently introduced⁷ rhodium(II) acetate on the other. The solutes separated were relatively simple olefinic hydrocarbons and esters, as well as more complex unsaturated natural products.

EXPERIMENTAL

Apparatus

A high-pressure liquid chromatograph (built in this laboratory) was equipped with an LDC Refractomonitor Model 1103 detector. The columns and delivery system were made of stainless steel and the solvent was delivered by a membrane pump (Type 1515, Orlita, Giessen, G.F.R.).

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Materials

The materials were purchased from Waters Ass., Framingham, Mass., U.S.A. (Corasil II, 37–54 μm), Woelm, Eschwege, G.F.R., (Silica Woelm 32, 32–63 μm), BDH, Poole, Great Britain (silver nitrate), Fluka, Buchs, Switzerland (olefins) and The Upjohn Co., Kalamazoo, Mich., U.S.A. (prostaglandins). The Feist acid esters, 3,4-dialkyl-1,2-cyclobutenecarboxylic acid diesters and an extract of a *Euphorbacea* species containing lupeol and α - and β -amyryn were available in this Department.

Preparation of the columns

The stainless-steel tubes were washed with detergent, water, acetone, tetrachloromethane and methanol and dried with nitrogen⁸. Packing was effected by the "tap-fill" procedure⁹. The silver nitrate-containing columns were of two types: (1) 0.8% AgNO_3 –1.7% ethylene glycol on Corasil II; and (2) 5% AgNO_3 on Silica Woelm 32. In both cases, absolute methanol was used as the volatile solvent for the silver nitrate impregnation. The rhodium column contained 0.8% of rhodium(II) acetate, which was dissolved in chloroform for coating the Corasil II.

RESULTS AND DISCUSSION

Olefins

Columns coated with silver nitrate–ethylene glycol. As in GLC, the difference in the retention times (Δt) measured on the column containing AgNO_3 , as compared with that containing NaNO_3 , should be proportional to the argentation constant (K_{AR}) multiplied by the partition coefficient (k_o) of the olefin between the mobile phase and the NaNO_3 –ethylene glycol solution*. However, the K_{AR} values cannot be calculated in this case because of the small values of k_o , which preclude their direct determination by LC measurements**. An additional complicating factor is the apparent variability of the absorption of the hydrocarbons on Corasil II, which is affected by both ethylene glycol and the salt dissolved in it.

The discussion of the correlation of retention with known argentation constants is, therefore, based on the Δt values. Because of the unavailability of k_o data, these comparisons are valid only for groups of closely related isomers. Furthermore, it should be pointed out that, although the interaction of Corasil II with the hydrocarbons is not very large, the above-mentioned effect of the solvent introduces an additional uncertainty which is particularly disturbing for small retentions. The experimental error of the measurement is estimated to be ± 4 sec.

Comparison of the results in the fourth and fifth column of Table I shows the correlation between the retention and argentation constants. Thus, *cis*-3-hexene has a Δt value that is much greater than that of the *trans*-isomer, in agreement with the respective values of K_{AR} .

In the cyclopentene series, the tetrasubstituted 1,2-dimethylcyclopentene shows a virtually negligible Δt value, whereas the 1- and 3-methylcyclopentene show reten-

* $K = (k - k_o)/k_o \cdot [\text{Ag}^+]$, where k is the partition coefficient between the AgNO_3 –ethylene glycol and the mobile phase. In GLC^{3,10}, k and k_o are proportional to r and r_o , respectively, the relative corrected retention times for the olefin in the AgNO_3 - and NaNO_3 -containing glycol phases.

** The determination of k_o would require the measurement of the partitioning of the olefins in a suitable static system.

TABLE I
HIGH-SPEED LC OF OLEFINS WITH SILVER NITRATE-ETHYLENE GLYCOL AS STATIONARY PHASE ON CORASIL II*

Compound	t'_{Na}^{+***} (sec)	t'_{Ag}^{+***} (sec)	$\Delta t =$ $t'_{Ag}^{+} - t'_{Na}^{+}$ (sec)	K_{Ag}^{+***}
<i>trans</i> -3-Hexene	110	118	8	1.0
<i>cis</i> -3-Hexene	115	148	33	3.9
1,2-Dimethylcyclopentene	115	119	4	—
1-Methylcyclopentene	115	136	21	6.0
3-Methylcyclopentene	120	169	49	12.0
1-Ethylcyclopentene	116	131	15	3.6
1-Methylcyclohexene	114	118	4	1.25
3-Methylcyclohexene	110	133	23	5.5
1-Ethylcyclohexene	110	125	15	1.3
Cycloheptene	112	141	29	12.8
3-Methylcycloheptene	115	150	35	—
<i>cis</i> -Cyclooctene	110	207	97	14.4
3-Methylcyclooctene	118	193	75	—

* Column: 50 cm \times 2.1 mm I.D., 0.8% silver nitrate in ethylene glycol, 1.7% on Corasil II. Solvent: *n*-hexane-*n*-heptane (1:1) saturated with ethylene glycol; flow-rate, 1 ml/min.

** Uncorrected retention times.

*** Argentation constants in ethylene glycol from refs. 2, 3, 10 and 11.

tion due to silver nitrate roughly in accordance with the corresponding argentation constants. The same is true for 1-methyl- and 3-methylcyclohexenes.

As is well established^{9,10,11}, alkyl substitution at the double bond greatly reduces the argentation constant, and it is seen, in fact, that the 1-alkylcycloalkenes have relatively low Δt values whereas 3-alkylcycloalkenes have high values. The predominant

TABLE II
COMPARISON OF HIGH-SPEED LC AND GLC ON COLUMNS CONTAINING RHODIUM (II) CARBOXYLATES

Compound	LC with $Rh_2(CH_3CO_2)_4^*$, $t_R - t_{R(COR)}^{**}$ (sec)**	GLC [†] with $Rh_2(C_6H_5CO_2)_4^{***}$, t_R^{****}
Allene	9.0	—
1-Butene	19.5	0.62
<i>trans</i> -Butene	27.0	0.81
Isobutene	42.0	1.55
<i>cis</i> -Butene	79.5	3.1
Tetrahydrofuran	444.0	>50

* Column: 45 cm \times 2.1 mm I.D.; 0.8% rhodium acetate on Corasil II. Solvent: *n*-hexane: pressure, 13.5 atm; flow-rate, 0.8 ml/min.

** Retention time on column coated with the rhodium salt, minus retention time on uncoated Corasil II.

*** Column: 2 m \times 1.75 mm I.D., coated with 0.02 molal rhodium(II) benzoate suspended in squalene, 15% on Chromosorb P, AW, 80-100 mesh. Temperature: 25°. Pressure: 1.7 atm-helium.

**** t_R' = relative corrected retention time (*n*-pentane = 1.00).

influence of the argentation constant on retention is further demonstrated by the fact that the highest ΔI value was found for *cis*-cyclooctene, which also has the highest argentation constant of all the olefins examined.

Columns coated with $Rh_2(CH_3CO_2)_4$. Rhodium(II) acetate is insoluble in hydrocarbons, and *n*-hexane can therefore be used as the mobile phase. The tendency of rhodium(II) carboxylates to form more or less stable complexes with ligands containing donor atoms is well known¹²⁻¹⁵. In a recent paper⁷, the application in GLC of $Rh_2(C_6H_5CO_2)_4$ suspended in squalane for the separation of alkenes and of oxygen-containing compounds has been discussed. The results in Table II demonstrate that retention is determined essentially by complexation with the rhodium(II) salt⁷, as is readily seen by the behaviour of 1-butene and isobutene. 1-Butene is the most strongly retained butene isomer on both silver nitrate and dicarbonylrhodium(I) β -diketonate-containing phases, whereas on the rhodium(II) acetate column it emerged first (Fig. 1). Isobutene, which has a very low retention on dicarbonyl rhodium(I) β -diketon-

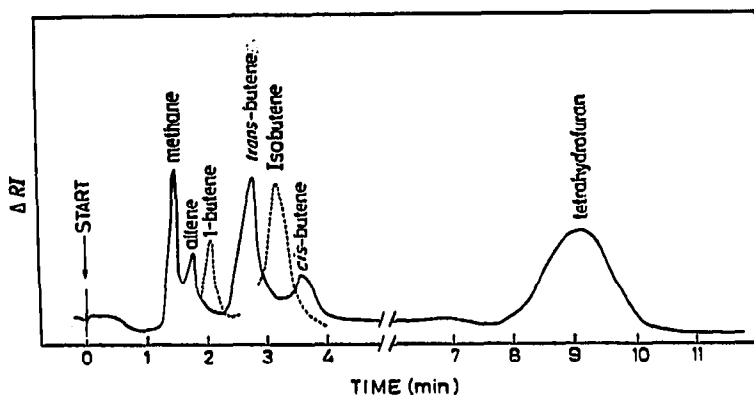


Fig. 1. Separation of butenes, allene and tetrahydrofuran (RI = Refraction Index). Column: 50 cm \times 2.1 mm I.D. Solvent: *n*-hexane; flow-rate, 0.8 ml/min; pressure, 13.6 atm. Corasil II coated with 0.8% rhodium(II) acetate. 1-Butene and isobutene were injected separately (broken lines).

ates¹⁶⁻¹⁸, was found to have a relatively high retention on rhodium(II) acetate (Table II). There is further good correlation between the retention of the alkenes in the present experiments and the GLC data for a rhodium(II) benzoate phase⁷, as can be seen by comparison of the figures given in the two columns of Table II. No exact parallelism between the two sets of data can be expected, mainly because different carboxylates of rhodium(II) were used. It should also be pointed out that allene has a small retention on the rhodium(II) acetate column, in contrast to its behaviour with rhodium(I) β -diketonates, and that tetrahydrofuran was strongly retarded.

Oxygen-containing compounds with one or more double bonds, on columns coated with silver nitrate

The most interesting application of LC with complex-forming stationary phases obviously lies in the domain of olefinic substances of high molecular weight. With such compounds, retention times should be sufficiently high to permit the separation of the components of complex mixtures, as demonstrated below.

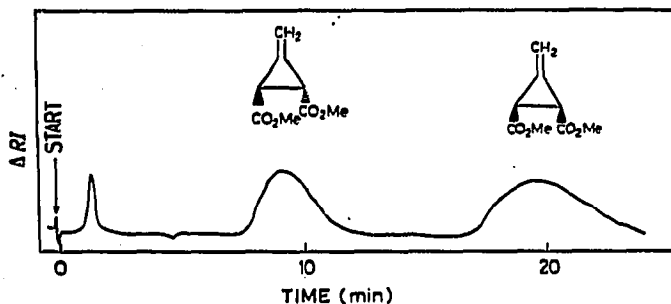


Fig. 2. Separation of Feists acid methyl ester and its *cis*-isomer (Me = methyl). Column: 50 cm \times 2.1 mm I.D. Solvent: *n*-hexane-*n*-heptane (1:1); flow-rate, 1.15 ml/min; pressure, 13.6 atm. Corasil II coated with 0.8% AgNO_3 -1.75% ethylene glycol.

Feists's acid methyl ester, its cis-isomer and isomeric cyclobutene esters. As shown in Fig. 2, the *cis*- and *trans*-isomers of methylenecyclopropane-1,2-dicarboxylic acid methyl ester are well separated on a column coated with silver nitrate in ethylene glycol on Corasil II. The ratio of the retention times of the two isomers, corrected for the volume of the mobile phase and the desalting effect, is 2.55, in good agreement to the values found for various pairs of *cis/trans*-disubstituted alkenes^{4,11}. An easier approach to the silver ion from one side of the molecule in the case of the *cis*-isomer would appear to explain the difference in the strength of complexation between the two isomers. As expected from the steric hindrance at the double bond, the tetra-substituted *cis*- and *trans*-isomers of 3-methyl-4-*n*-propyl-1,2-carbomethoxycyclobutene did not show any better separation when chromatographed on alumina coated with silver nitrate as compared with a pure alumina column. On the other hand, an improved separation should occur when chromatographing the 3,4-unsubstituted derivatives. It should be pointed out that the cyclobutenes usually cannot be chromatographed by GLC because of their conversion into muconates at relatively low temperatures.

Elaidic and oleic acids. As seen in Fig. 3, silver nitrate in ethylene glycol on Corasil II readily permits the differentiation of saturated from unsaturated fatty acid

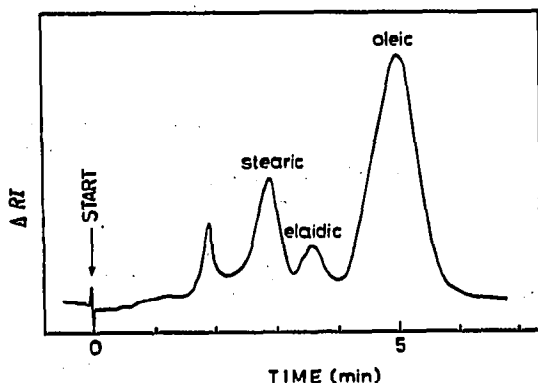


Fig. 3. Separation of C_{18} fatty acid methyl esters. Column: 50 cm \times 2.1 mm I.D. Solvent: *n*-hexane-*n*-heptane (1:1); flow-rate, 1 ml/min; pressure, 13.6 atm. Corasil II coated with 0.8% AgNO_3 -1.75% ethylene glycol.

methyl esters, and of the *cis*- from the *trans*-isomer. The retention due to complexation with the double bond can be estimated with reference to the retention time of the methyl stearate (57 sec, corrected for the volume of the mobile phase). Thus, a ratio of 2.34 for the relative strength of complexation of the *cis*- compared with the *trans*-isomer is obtained. It is of interest to note that the sample analyzed as shown in Fig. 3 was a commercial product labelled oleic acid. Three of the peaks were identified by co-injection with standard materials. This procedure presents certain obvious advantages over argentation TLC⁵.

Prostaglandins. The analysis of this group of biologically active compounds is of considerable topical interest¹⁹. A chromatogram of a mixture containing PGF_{2α} and PGF_{2β} on a silica column impregnated with AgNO₃ is shown in Fig. 4a. The

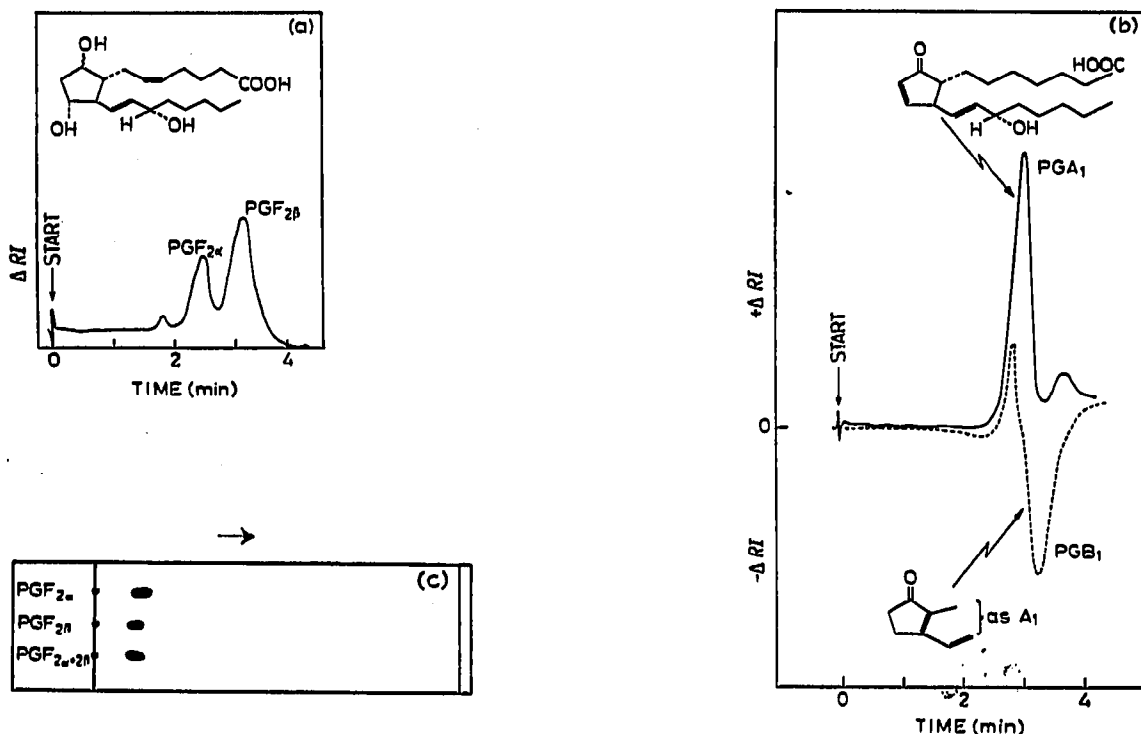


Fig. 4. (a) and (b). Separation of prostaglandins. Column: 50 cm \times 2.1 mm. Solvent: toluene-dioxane-acetic acid-(—)-2-methylbutanol (150:150:10:5); flow-rate, 1 ml/min; pressure, 11 atm. Silica Woelm 32 coated with 5% AgNO₃. (c) TLC of PGF_{2α} and PGF_{2β}. Plate 5% AgNO₃ on silica gel, eluting solvent as for LC.

separation obtained ($t_{R' \alpha/\beta} = 1.27$) contrasts strongly with argentation TLC (see Fig. 4c), where separation between the two diastereoisomers is hardly detectable. In the α -isomer, the two hydroxyl groups of the ring are *cis* to each other. The resulting internal hydrogen bonding could compete with interaction with the phase, making the compound more mobile in the chromatographic system. The data available do not enable one to conclude whether complexation with the silver ion or the partition coefficient in the solvent is the determining factor.

Fig. 4b shows the separation of PGA_1 and PGB_1 on the same column. As PGB_1 has a higher refractive index than the solvent, the presence of this prostaglandin is readily recognized by a negative indication of the detector.

Pentacyclic triterpenes. The benzoates formed from the components of a commercial extract of an *Euphorbacea* species showed the ready separation of lupeol, which contains an *exocyclic* double bond, $\Delta^{28(29)}$, from α - and β -amyrin containing an *endocyclic* double bond, $\Delta^{12(13)}$. Attempts to separate the two latter isomers, which both contain a trisubstituted double bond in the C ring and differ only by the relative position of one methyl group in the E ring, have not been successful so far.

CONCLUSIONS

The results presented indicate that modern high-pressure LC with complex-forming stationary phases is a useful technique for the separation of unsaturated compounds of high molecular weight. No doubt the procedure should and will, in the future, supplement the use of argentation TLC.

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REFERENCES

- 1 O. K. Duha and J. Janák, *J. Chromatogr.*, 68 (1972) 325.
- 2 J. Herling, J. Shabtai and E. Gil-Av, *J. Chromatogr.*, 8 (1962) 349.
- 3 E. Gil-Av and J. Herling, *Proceedings of Journées Hellènes de Séparation Immédiate et de Chromatographie, Athens, September 19-24, 1965*, p. 167.
- 4 C. L. de Ligny, T. van 't Verlaat and E. Karthaus, *J. Chromatogr.*, 76 (1973) 115.
- 5 E. Stahl (Editor), *Thin-Layer Chromatography*, Springer, New York, 2nd ed., 1969, p. 396.
- 6 J. N. Little, *Abstr. Pap., 164th National Meeting of ACS, Division of Analytical Chemistry, New York, August-September 1972, Anal. Part, Rep. No. 9*.
- 7 V. Schurig, J. L. Bear and A. Zlatkis, *Chromatographia*, 5 (1972) 301.
- 8 B. L. Karger, K. Conroe and H. Engelbert, *J. Chromatogr. Sci.*, 8 (1970) 242.
- 9 J. Kirkland (Editor), *Modern Practice of Liquid Chromatography*, Wiley, New York, 1972, p. 178.
- 10 E. Gil-Av and J. Herling, *J. Phys. Chem.*, 66 (1962) 1208.
- 11 M. A. Muhs and F. T. Weiss, *J. Amer. Chem. Soc.*, 84 (1962) 4697.
- 12 S. A. Johnson, H. R. Hunt and H. M. Neumann, *Inorg. Chem.*, 2 (1963) 960.
- 13 T. A. Stephenson, S. M. Morehouse, A. R. Powell, J. P. Heffer and G. Wilkinson, *J. Chem. Soc.*, (1965) 3632.
- 14 L. A. Nazarova, I. I. Chernyayev and A. S. Morozova, *Russ. J. Inorg. Chem.*, 11 (1966) 1387.
- 15 J. Kitchens and J. L. Bear, *J. Inorg. Nucl. Chem.*, 31 (1969) 2415.
- 16 V. Schurig and E. Gil-Av, *Chem. Commun.*, (1971) 650.
- 17 E. Gil-Av and V. Schurig, *Anal. Chem.*, 43 (1971) 2030.
- 18 V. Schurig, R. C. Chang, A. Zlatkis, E. Gil-Av and F. Mikeš, *Chromatographia*, 6 (1973) 223.
- 19 S. M. M. Karim (Editor), *The Prostaglandins*, Medical and Technical Publishing Co., Oxford, 1972.