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Short communication

Gas chromatographic analysis of diastereomers and enantiomers of β,γ-unsaturated esters and various analogues of butenolides including mint and isomint lactone and comparison with the high-performance liquid chromatographic analysis of their diastereomers☆

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Abstract

Butenolide is an α,β-unsaturated lactone. The butenolide moiety is present in numerous biologically active natural products, especially insect sex hormones. A method was developed for the synthesis of butenolides from β,γ-unsaturated esters. Separation of diastereomers and enantiomers of these esters and butenolides including mint and isomint lactone and heritionin by GC is reported. The order of elution of isomers of mint and isomint lactone was identified as *RR*, *SS*, *SR* and *RS* on a Chirasil-Val-D column. This was also confirmed by injecting these samples on to a column with an L-configuration. The separation of diastereomers by HPLC was also tried and the results were compared with those obtained by GC.

1. Introduction

Recently a shorter route for the synthesis of heritol was reported [1]. The separation of the diastereomers and enantiomers of analogous β,γ-esters and lactones was tried by GC on packed and capillary columns and by reversed-phase HPLC and the results were compared. There have been a few reports of enantiomer separations of butyrolactones [2–5] and derivatives of γ-lactones [6–8] and also direct separation using cyclodextrin derivatives as stationary phases [9–14]. However, enantiomer resolution of α,β-unsaturated γ-lactones to our knowledge has not been reported.

2. Experimental

2.1. GC

A Hewlett-Packard Model 5880A gas chromatograph modified for capillary columns with a level 4 integrator and a flame ionization detector was used. Fused-silica capillary columns (25 m × 0.25 mm I.D., 0.12-μm layer) coated with D- and L-Chirasil-Val were purchased from

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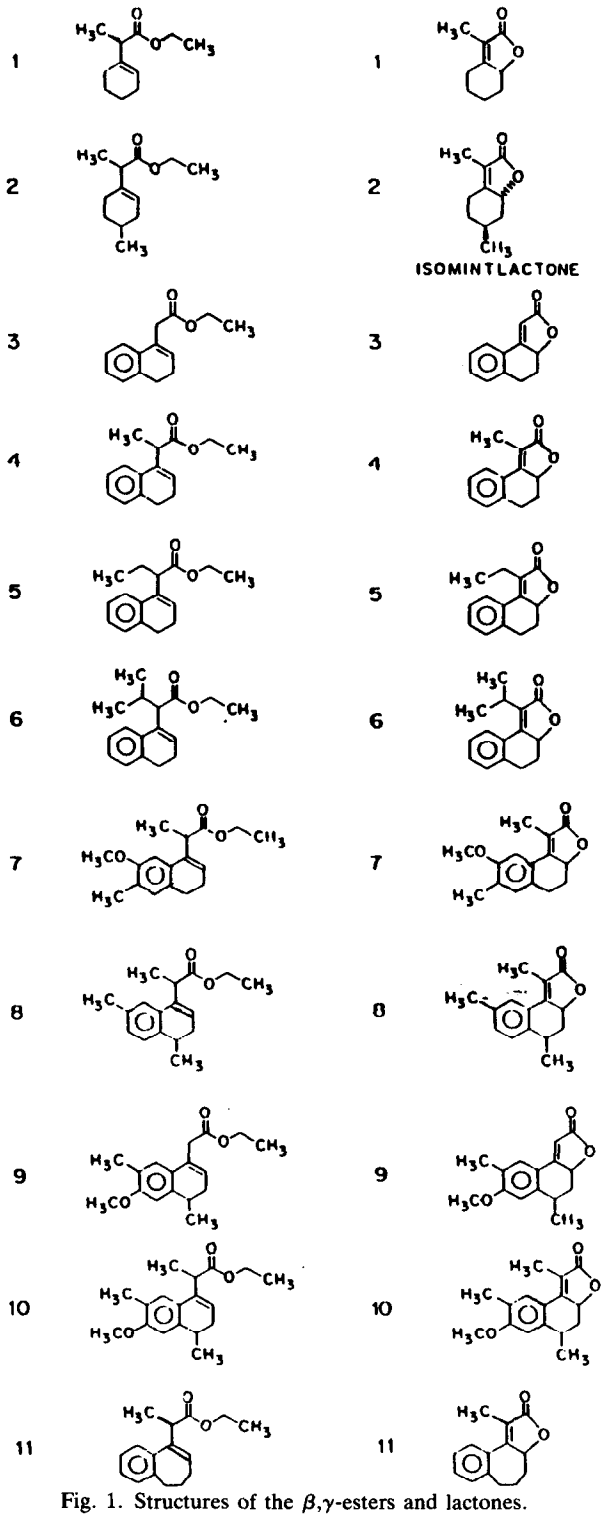

Fig. 1. Structures of the β,γ-esters and lactones.

Chrompak (Middelburg, Netherlands). Stainless-steel packed columns of (1) 3% SE-30 and (2) 2% Apiezon L on Chromosorb W AW DMCS (6 ft. $\times \frac{1}{8}$ in. I.D.; 1 ft. = 30.48 cm, 1 in. = 2.54 cm) were prepared. The experimental conditions used are given in the tables and figures. The structures of the β,γ-esters and lactones analysed are shown in Fig. 1.

2.2. HPLC

HPLC analyses were carried out on a system from Millipore–Waters Chromatography Division (Milford, MA, USA) equipped with Model 510 solvent-delivery pumps, a Model 680 automated gradient controller, a Rheodyne Model 7225 fixed-volume (20-μl) injector, a Model 440 UV absorbance detector (254 nm) or a Waters Lamda-max 481 LC spectrophotometer (214 nm) and a Hewlett-Packard Model 3390A integrator. A Waters radial compression cartridge (μBondapak C_{18}, 10 μm, 10 cm) fitted in a Z-module system was used as a reversed-phase column. Methanol was purified to chromatographic quality in our laboratory. A Milli-Q system (Millipore, Bedford, MA, USA) was used to purify water. The mobile phase was filtered through a Millipore filter (0.45 μm) using a Millipore all-glass filter apparatus.

3. Results and discussion

3.1. Diastereomers

α,β-Unsaturated lactones

Table 1 gives the retention data for diastereomers of lactones and β,γ-esters for both GC and HPLC analysis.

GC. Whereas an SE-30 column is unable to separate diastereomers, an Apiezon L column gives a very good separation of the diastereomers of lactones **2** and **8**. As there was no separation between the diastereomers of lactone,

Table 1
Retention data for the diastereomers of lactones and β,γ-esters obtained by GC and HPLC

Compound No. and percentage composition (*cis*:*trans*) from NMR	GC with 2% Apiezon L column							HPLC with μBondapak C_{18} column (10 μm)[a]						
	Lactones				Esters			Mobile phase: MeOH: buffer[b]	Lactones			Esters		
	Temperature (°C)	Retention time (min)		α	Retention time (min)		α		Retention time (min)		α	Retention time		α
2 (80:20)	160	8.66	9.77	1.13	3.28	4.48	1.37	50:50	7.60	8.43	1.1	–	–	–
8 (60:40)	200	32.67	33.94	1.04	9.55	16.97	1.78	75:25	6.08	9.48	1.56	19.98	21.92	1.10
9 (60:40)[c]	220	35:25[d]	–	–	8.36	14.95	–	75:25	6.58	9.38	1.43	15.40	13.00	–
						18.03							17.48	
10 (60:40)	220	34.32	–	–	8.22	14.63	1.78	75:25	6.64	10.68	1.61	29.88	–	–

GC: injector temperature = oven temperature + 40°C; detector temperature = 300°C; carrier gas, nitrogen at a flow-rate of 30 ml/min.
HPLC: UV detection at 254 nm for **8**, **9** and **10** lactones and esters and 218 nm for **2** lactone and ester.
[a] For lactone **2** a reversed-phase column was used: Novapak C_{18}, 5 μm.
[b] Buffer = 0.7 *M* triethylammoniumphosphate (pH 3).
[c] (*E*)-**9** shows anamolous behaviour; it may form an α,β-ester that can separate into positional isomers.
[d] Lactone **9** shows a broad peak with a hump.

9 and **10** by GC, the analysis was successfully achieved by HPLC.

HPLC. The elution sequence is the *trans* isomer before the *cis* isomer, in contrast to GC, where the *cis* isomer elutes first. This was confirmed by injecting pure mint and isomint lactone (**2**) and pure *cis-* and *epi*-heritol (**10**). Diastereomer separation of L-**2** and L-**10** by HPLC can be seen in Fig. 2, which also shows the peak of an impurity developed after keeping the samples in methanol for a few days. The lactone ring may be opening to form a hydroxy ester on keeping in methanol.

β,γ-Esters

GC. The esters with two asymmetric carbon atoms separate well into their diastereomers when injected on to both SE-30 and Apiezon L columns. The ester **9** shows three peaks, a major peak (60%) followed by two peaks each with 20% composition on the Apiezon L column. The same pattern is observed on capillary chiral and achiral columns. This is possible only if some kind of reaction is taking place at high temperature. If an α,β-ester is formed then it may show two positional isomers due to restricted rotation (the ester group and hydrogen can be on either side of the double bond). The first major peak may be a β,γ-ester and the other two equal peaks are positional isomers of the α,β-ester that is formed. We thought HPLC analysis might resolve the problem.

HPLC. Ester **8** is well separated into the diastereomers but ester **10** shows only one peak. Ester **9** shows three peaks even by HPLC, only the sequence of elution is altered (Fig. 3). The middle peak is major (60%) with two peaks (20% each) on either side. ^{1}H NMR spectrometry also shows the presence of isomers.

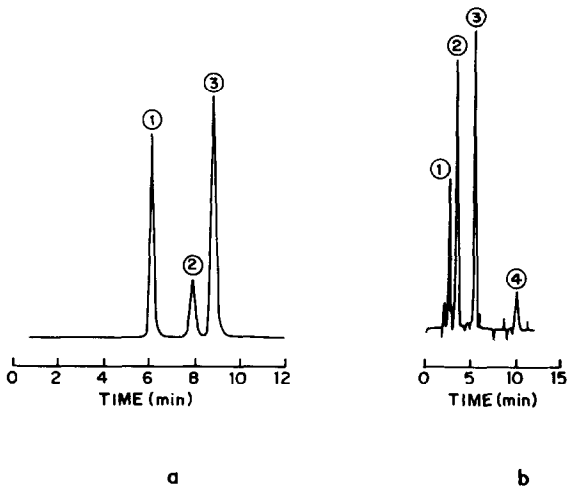

Fig. 2. (a) Diastereomer separation of lactone **2** by HPLC. Column, Novapak C_{18}, 5 μm (10 cm × 5 mm I.D.); mobile phase; methanol–buffer (50:50) (pH 3); flow-rate, 1 ml/min; UV detection at 218 nm. Peaks: 1 = impurity developed on keeping; 2 = isomint lactone; 3 = mint lactone. (b) Diastereomer separation of lactone **10** (heritionin) by HPLC. Column, μBondapak C_{18}, 10 μm (10 cm × 8 mm I.D.); mobile phase, methanol–buffer (75:25) (pH 3); flow-rate, 2 ml/min; UV detection at 254 nm. Peaks: 1 = impurity developed on keeping (lactone ring may be opened); 2 = *trans*-heritionin; 3 = *cis*-heritionin; 4 = impurity.

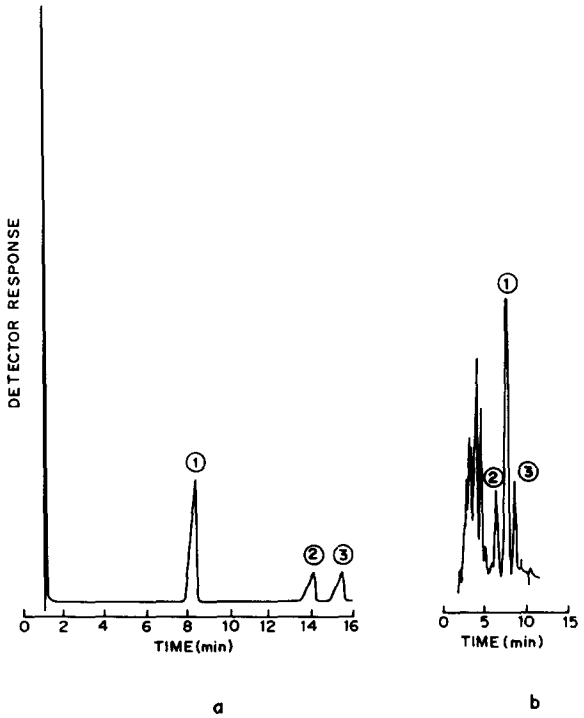

Fig. 3. (a) GC of ester **9** on Chirasil-Val-D. Oven temperature, 170°C; injector temperature, 210°C; detector temperature, 250°C; carrier gas, nitrogen at a flow-rate of 2 ml/min; splitting ratio, 1:100. Peaks: 1 = β,γ-ester; 2 and 3 = positional isomers of α,β-ester. (b) HPLC of ester **9**. Conditions as in Fig. 2b. Peaks: 2 = β,γ-ester; 1 and 3 = positional isomers of α,β-ester.

Therefore, we conclude that the positional isomers of α,β-esters are being separated.

3.2. Enantiomers

α,β-Unsaturated lactones

All the lactones show enantiomeric separation at least to some extent. Fig. 4 shows the separation of mint and isomint lactone into their enantiomers. The sequence of elution of the isomers is identified as *RR*, *SS*, *SR* and *RS* on the Chirasil-Val D column on injecting pure (*RR*)-(−) and (*RS*)-(+)-enantiomers [15]. The order of elution is reversed on a column with opposite configuration. Lactones **9** and **10** can be separated only into their diastereomers even on the chiral column.

β,γ-Esters

Only esters **2**, **8** and **10** show the presence of enantiomers but the α values are small. For all the three esters the *cis* isomer shows splitting whereas the *trans* isomer does not separate into enantiomers.

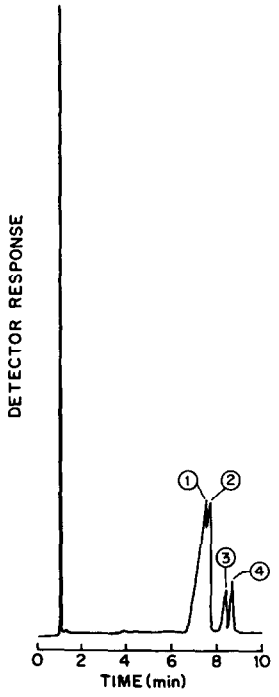

Fig. 4. Enantiomer separation of mint lactone and isomint lactone on Chirasil-Val-D. Oven temperature, 120°C; other conditions as in Fig. 3a. Peaks: 1 = (*RR*)-mint lactone; 2 = (*SS*)-mint lactone; 3 = (*SR*)-isomint lactone; 4 = (*RS*)-isomint lactone.

4. Conclusions

The diastereomer separation of β,γ-esters and butenolides including mint and isomint lactone and heritionin was achieved by GC and HPLC and the results were compared. Separation of the enantiomers of mint lactone and isomint lactone could be achieved on Chirasil-Val-D at 120°. The order of elution of isomers on this column was identified as *RR*, *SS*, *SR* and *RS*.

References

[1] P.K. Zubaidha, S.P. Chavan, U.S. Racherla and N.R. Ayyangar, *Tetrahedron*, 47 (1991) 5759.
[2] S. Hunig, N. Klaunzer and K. Gunther, *J. Chromatogr.*, 481 (1989) 387.
[3] A. Tambute, M. Linne, M. Caude and R. Rosset, *J. Chromatogr.*, 448 (1988) 55.
[4] E. Francotte and D. Lohmann, *Helv. Chim. Acta*, 70 (1987) 1969.
[5] D.W. Armstrong, A.M. Stalcup, M.L. Hilton, J.D. Duncan, J.R. Faulkner and S.C. Chang, *Anal. Chem.*, 62 (1990) 1610.
[6] K.H. Engel, R.A. Flath, W. Albrecht and R. Tressel, *J. Chromatogr.*, 479 (1989) 176.
[7] K.H. Engel, W. Albrecht and J. Heidlas, *J. Agric. Food Chem.*, 38 (1990) 244.
[8] A. Mosandl, M. Gessner, C. Guenther, W. Deger and G. Singer, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 10 (1987) 67.
[9] W.A. Konig, S. Lutz, C. Colberg, N. Schmidt, G. Wenz, E. Bey, A. Mosandl, C. Guenther and A. Kustermann, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 11 (1988) 621.
[10] D.W. Armstrong, W.Y. Li, C.D. Chang and J. Pitha, *Anal. Chem.*, 62 (1990) 914.
[11] W.Y. Li, H.L. Jin and D.W. Armstrong, *J. Chromatogr.*, 509 (1990) 303.
[12] V. Schurig, M. Jung, D. Schmalzing, M. Schleimer, J. Duvekot, J.C. Buyten, J.A. Peene and P. Mussche, *J. High Resolut. Chromatogr.*, 13 (1990) 470.
[13] V. Schurig, D. Schmalzing, U. Muhleck, M. Jung, M. Schleimer, P. Mussche, J. Duvekot and J.C. Buyten, *J. High Resolut. Chromatogr.*, 13 (1990) 713.
[14] K. Grob, H.P. Neukom, H.G. Schmarr and A. Mosandl, *J. High Resolut. Chromatogr.*, 13 (1990) 433.
[15] A.S. Tambe, P.K. Zubaidha, S.S. Biswas and N.R. Ayyangar, presented at the *3rd International Symposium on Chiral Discrimination, Tübingen, 5–8 October 1992.*