

Enantioselective Multidimensional Gas Chromatography of Some Secondary Alcohols and Their Acetates from Banana

Vera Schubert, Renate Diener, and Armin Mosandl

Institut für Lebensmittelchemie, Universität Frankfurt,
Robert-Mayer-Straße 7–9, D-6000 Frankfurt/Main, Bundesrepublik Deutschland

Z. Naturforsch. **46c**, 33–36 (1991); received October 12, 1990

Banana, Chiral Flavour Compounds, Secondary Alcohols, Acetic Acid Esters of Secondary Alcohols, Enantioselective MDGC

Using enantioselective MDGC the enantiomeric distribution of alkan-(alken-)-2-yl-acetates from bananas is achieved. After simple acetylation their corresponding alcohols 2-pentanol, 2-hexanol, 2-heptanol and (Z)-4-hepten-2-ol respectively are also exactly stereodifferentiated by the same method.

All compounds investigated – esters as well as alcohols – exhibit a remarkable excess of the (S)-enantiomers.

Introduction

The flavour of bananas has been proved to be a rather complex mixture of more than 200 compounds and consists of about 95% carboxylic esters and nearly 4% of the corresponding free alcohols [1, 2]. The 2-alkanols – particularly 2-pentanol and (Z)-4-hepten-2-ol – are of special interest, due to their considerable abundance in the flavour of bananas. Except from bananas [3, 4] (Z)-4-hepten-2-ol has been detected only in corn [5].

The enantiodifferentiation of some chiral carboxylic acid 2-alkanyl esters from banana yielded the (S)-antipode in high optical purity [6]. Recent results concerning the chiral evaluation of free 2-heptanol and (Z)-4-hepten-2-ol from banana using MDGC of their isopropylcarbamates revealed moderate excess of the (S)-enantiomers [7]. On the contrary the enantiomeric distribution of 2-heptanol and (Z)-4-hepten-2-ol in three different corn varieties was found to be about 80% (R)/20% (S) [8].

This paper reports on a new simplified MDGC method to determine the enantiomeric ratios of 2-pentanol, 2-hexanol, 2-heptanol and (Z)-4-hepten-2-ol, respectively as well as their acetic acid esters.

Materials and Methods

Materials

2-Pentanol, 2-hexanol and 2-heptanol were purchased from Aldrich, Steinheim. (Z)-4-hepten-2-ol was kindly provided by Silesia, Neuss. Optically pure references of these alcohols were available from our laboratory [9, 10]. Acetic acid chloride and 4-dimethylaminopyridine (DMAP) were purchased from Fluka, Neu-Ulm.

Preparation of banana extracts

Peeled bananas from the local market (about 650 g) were homogenized with an Ultra Turrax after the same amount of $(\text{NH}_4)_2\text{SO}_4$ (enzymic inhibition!) and 100 ml distilled water have been added. In a 2 l flask the pulp was poured with 300 ml pentane/diethyl ether (1:1, v/v) and shaken well several times while extracting during three days. The organic layer was carefully separated, dried over Na_2SO_4 and concentrated on a 20 cm Vigreux column to about 1 ml.

Isolation and acetylation of the alcoholic fraction

0.5 ml of the banana extract were chromatographed on a preparative silica TLC plate (eluent: pentane/diethyl ether, 9:1 (v/v)). The alcoholic fraction was isolated by scratching out the corresponding zone ($R_f = 0.1–0.2$, (Z)-4-hepten-2-ol as reference) and eluting the silica gel with 4 ml of pentane/diethyl ether (1:1). To 3 ml of this solution 30 mg DMAP and 200 μl acetic acid chloride were added and stirred overnight at room temperature. The reaction mixture was subsequently

Reprint requests to Prof. Dr. A. Mosandl.

Verlag der Zeitschrift für Naturforschung, D-7400 Tübingen
0939–5075/91/0100–0033 \$ 01.30/0



Dieses Werk wurde im Jahr 2013 vom Verlag Zeitschrift für Naturforschung in Zusammenarbeit mit der Max-Planck-Gesellschaft zur Förderung der Wissenschaften e.V. digitalisiert und unter folgender Lizenz veröffentlicht: Creative Commons Namensnennung-Keine Bearbeitung 3.0 Deutschland Lizenz.

Zum 01.01.2015 ist eine Anpassung der Lizenzbedingungen (Entfall der Creative Commons Lizenzbedingung „Keine Bearbeitung“) beabsichtigt, um eine Nachnutzung auch im Rahmen zukünftiger wissenschaftlicher Nutzungsformen zu ermöglichen.

This work has been digitalized and published in 2013 by Verlag Zeitschrift für Naturforschung in cooperation with the Max Planck Society for the Advancement of Science under a Creative Commons Attribution-NoDerivs 3.0 Germany License.

On 01.01.2015 it is planned to change the License Conditions (the removal of the Creative Commons License condition “no derivative works”). This is to allow reuse in the area of future scientific usage.

washed with 2 N HCl, saturated NaHCO_3 and saturated NaCl solution. After drying over Na_2SO_4 the solvent was carefully evaporated (Vigreux column) to about 1 ml.

Acetylation of the optically pure references was carried out in the same manner. No racemization was observed.

Chromatographic conditions

Multidimensional gas chromatography: A Siemens SiChromat 2 double oven GC was used with split injector (200 °C, split ratio 1:10) and two FIDs (220 °C).

Preseparation column: Supelcowax 10TM fused silica capillary, 60 m \times 0.32 mm i.d., 0.25 μm film thickness, 1.25 bar H_2 ; temperature program: 70 °C//10 min isothermal//3 °C/min//200 °C.

Main separation column: Permethyl- β -cyclodextrin duran glass capillary (10% in OV 1701-vi), 47 m \times 0.23 mm i.d., 1.00 bar H_2 ; temperature program: 95 °C//10 min isothermal//0.5 °C/min//150 °C.

The columns were coupled by the live-T-switching device (Siemens).

Gas chromatography – mass spectrometry

A HP 5800 gas chromatograph with split injector (220 °C, split ratio 1:10) was coupled with a Finnigan ion trap detector ITD 800. As the stationary phase a Supelcowax 10TM fused silica capillary, 30 m \times 0.23 mm i.d., 0.25 μm film thickness, was used. Carrier gas was He at 0.7 bar; temperature program: 75 °C//10 min isothermal//2 °C/min//80 °C//6 °C/min//220 °C. Ionization energy was 70 eV, mass range was chosen 40–200.

Results and Discussion

In an earlier study on banana flavour compounds the importance of efficient enzyme inhibition is impressively demonstrated [11]. When destroying the cellular structure of the fruit by homogenization without any inhibitive means the banana esters will be cleaved nearly completely after only one hour at 30 °C. In order to avoid carboxylesterase activity we added $(\text{NH}_4)_2\text{SO}_4$ before homogenization [12]. The efficiency of this procedure could be ensured by doting the pulp with racemic (Z)-4-hepten-2-yl-propionate which does not exist in banana flavour.

The native banana extracts were subjected to GC-MS analysis. By comparison with authentic references 2-pentanol, 2-hexanol, 2-heptanol, (Z)-4-hepten-2-ol and their acetic acid esters could be identified. The butanoate of (Z)-4-hepten-2-ol is also known to take part in the banana flavour [3] but in this study it was detected only in one sample. The alcoholic fractions of the extracts obtained by preparative thin layer chromatography were analyzed directly and after acetylation. Multidimensional gas chromatography (MDGC), combining a polar achiral preseparating column with a chiral modified cyclodextrin stationary phase, has been reported to be a very useful method to determine the enantiomeric distribution of numerous chiral flavour substances from complex food extracts and essential oils [13–16].

In the present study a 60 m Carbowax capillary column was used for preseparation and a 47 m permethyl- β -cyclodextrin stationary phase for stereodifferentiation. Fig. 1 demonstrates the well resolved enantiomeric pairs of a racemic standard mixture of volatile banana flavour compounds. The order of elution was determined by analysis of optically pure references: for all the esters of the standard mixture we found the (S)-enantiomer eluting prior to the (R)-configured isomer.

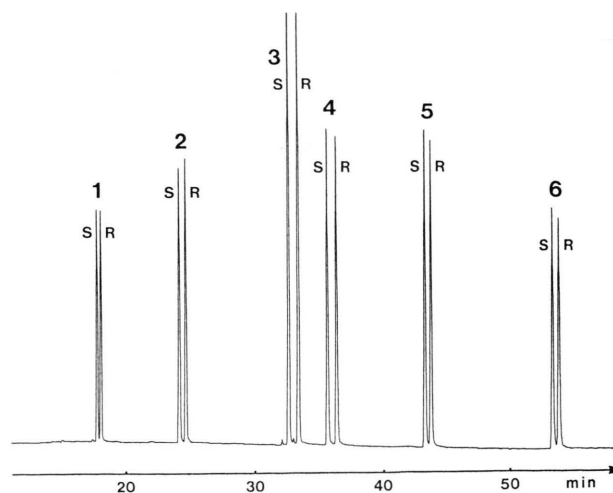


Fig. 1. MDGC main column chromatogram of the racemic standard mixture: pentan-2-yl-acetate (1), hexan-2-yl-acetate (2), heptan-2-yl-acetate (3), (Z)-4-hepten-2-yl-acetate (4), (Z)-4-hepten-2-yl-propionate (5), (Z)-4-hepten-2-yl-butanoate (6). For chromatographic conditions see experimental section.

The direct MDGC analysis of a genuine banana extract is given in Fig. 2. The main column chromatogram shows the (*S*)-configured acetic acid esters in high enantiomeric excess. This result agrees with another study which found optically pure 2-pentyl acetate and 2-heptyl acetate from banana by determining them as diastereomeric (*S*)-O-acetylactyl esters [6]. Concerning 2-hexanyl acetate the enantiomeric distribution of 77% (*S*):23% (*R*) should be verified by mass spectrometric

methods as superposing of impurities cannot be excluded using FID detection. In case of high complex matrices extremely sharp heart cutting is necessary to avoid transfer of compounds with similar chromatographic behaviour which could possibly influence the result.

The isolated alcoholic fractions were acetylated as described above and subjected to MDGC analysis. The stereodifferentiation of secondary alcohols from banana is outlined in Fig. 3. A significant

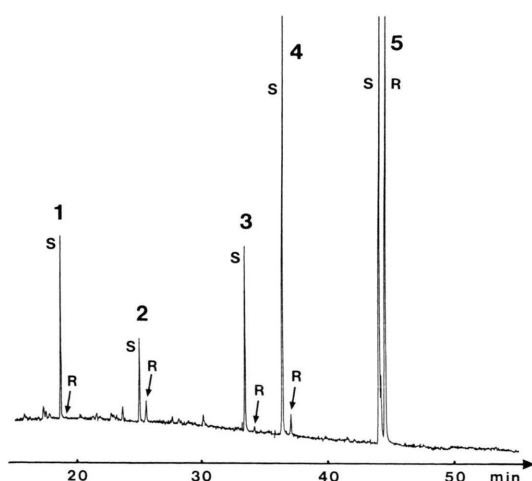


Fig. 2. Main column chromatogram of acetic esters of 2-pentanol (**1**), 2-hexanol (**2**), 2-heptanol (**3**) and (*Z*)-4-hepten-2-ol (**4**) from a genuine banana extract. Racemic (*Z*)-4-hepten-2-yl-propionate (**5**) was added to verify inhibition of carboxylesterase activity. For chromatographic conditions see experimental section.

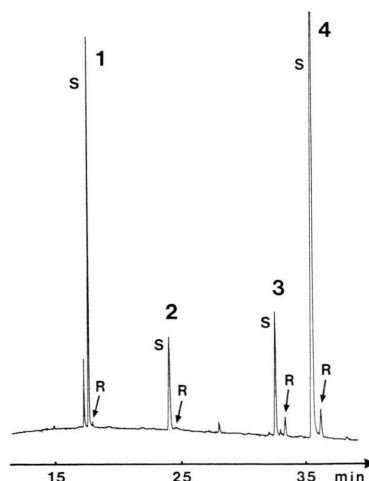


Fig. 3. Main column chromatogram of acetylated alcoholic fraction of banana sample no. 4 (cf. Table 1): 2-pentanol (**1**), 2-hexanol (**2**), 2-heptanol (**3**), (*Z*)-4-hepten-2-ol (**4**) as acetates. For chromatographic conditions see experimental section.

Table I. Enantiomeric distribution (in %) of secondary alcohols from banana.

Sample No.	Origin; state of ripeness	2-Pentanol		2-Hexanol		2-Heptanol		(Z)-4-hepten-2-ol	
		(<i>S</i>)	(<i>R</i>)	(<i>S</i>)	(<i>R</i>)	(<i>S</i>)	(<i>R</i>)	(<i>S</i>)	(<i>R</i>)
1	Panama; green, solid	99.4	0.6	98.1	1.9	77.2	22.8	>99.8	n.d.
2	Honduras; yellow	96.6	3.4	98.1	1.9	83.8	16.2	95.6	4.4
3	Honduras; yellow	94.3	5.7	96.4	3.6	84.4	15.6	94.3	5.7
4	Ecuador; yellow	98.0	2.0	97.3	2.7	86.3	13.7	97.1	2.9
5	Brasil; yellow with brown spots	82.9	17.1	>90.0	n.d.	80.3	19.7	73.3	26.7

n.d. not detectable.

excess in favour of the (*S*)-enantiomer can be observed too. Table I summarizes our results concerning the chiral evaluation of 2-pentanol, 2-hexanol, 2-heptanol and (*Z*)-4-hepten-2-ol from five different banana extracts. The state of ripeness and the origin of the samples are indicated. Sample numbers 1 to 4 show reproducible high enantioselectivities. The optical purity of the alcohols reaches more than 95% (*S*) except from 2-heptanol. However the fully ripe bananas of extract number 5, which had been stored at room temperature for

7 days, exhibit a different result: the enantiomeric ratios of 2-pentanol and (*Z*)-4-hepten-2-ol were determined to be 82.9% (*S*)/17.1% (*R*) and 73.3% (*S*)/26.7% (*R*), respectively. The latter is in accordance to a recently published study [7] where a ratio of 74% (*S*)/26% (*R*) was found from stored bananas of optimal ripeness. A connection between maturation and rising amounts of the (*R*)-enantiomer may be suggested but has to be confirmed by further investigations.

- [1] R. Tressl, F. Drawert, W. Heimann, and R. Embarger, *Z. Naturforsch.* **24b**, 781 (1969).
- [2] S. van Straten, H. Maarse (ed.), *Volatile Compounds in Food, Qualitative Data*, CIVO-TNO, Zeist-the-Netherlands 1989.
- [3] R. Tressl and F. Drawert, *Z. Naturforsch.*, **26b**, 774 (1971).
- [4] R. G. Berger, F. Drawert, and H. Kollmannsberger, *Chem. Mikrobiol. Technol. Lebensm.* **10**, 120 (1986).
- [5] R. G. Buttery, *J. Agric. Food Chem.* **27** (1), 208 (1979).
- [6] M. Gessner, W. Deger, and A. Mosandl, *Z. Lebensm. Unters. Forsch.* **186**, 417 (1988).
- [7] O. Fröhlich, M. Huffer, and P. Schreier, *Z. Naturforsch.* **44c**, 555 (1989).
- [8] K.-H. Engel, *Bioflavour '87* (P. Schreier, ed.), p. 75, W. de Gruyter, Berlin, New York 1988.
- [9] W. Deger, Dissertation, Universität Würzburg 1988.
- [10] V. Schubert, Dissertation, Universität Frankfurt/M., in prep.
- [11] R. Tressl, F. Drawert, and W. Heimann, *Z. Lebensm. Unters. Forsch.* **142**, 249 (1970).
- [12] E. Guichard and M. Souty, *Z. Lebensm. Unters. Forsch.* **186**, 301 (1988).
- [13] A. Mosandl, K. Rettinger, B. Weber, and D. Henn, *Dtsch. Lebensm. Rundsch.* **86**, 375 (1990).
- [14] K. Fischer and A. Mosandl, *Z. Lebensm. Unters. Forsch.* **192**, 30 (1991).
- [15] U. Palm, C. Askari, U. Hener, E. Jakob, C. Mandler, M. Geßner, A. Mosandl, W. A. König, P. Evers, and R. Krebber, *Z. Lebensm. Unters. Forsch.*, in press.
- [16] U. Hener, P. Kreis, and A. Mosandl, *Flav. Frag. J.* (1990), in press.