Tetrahedron Vol 45, No 22, pp 7003 to 7006, 1989
Printed in Great Britain

0040-4020/89 $3 00+ 00
Pergamon Press plc

DETERMINATION OF THE ABSOLUTE CONFIGURATION OF α-DAMASCONE AND α-IONONE FROM BLACK TEA BY ENANTIOSELECTIVE CAPILLARY GAS CHROMATOGRAPHY

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(*Received in Germany* 5 *July* 1989)

Abstract: The (S)-configuration was assigned to α-damascone and α-ionone, important flavour components in black tea, by enantioselective capillary gas chromatography using octakis(3-O-methyl-2,6-di-O-pentyl)-γ-cyclodextrin as a chiral stationary phase.

α-Damascone (figure 1) has a typical fruity flowery fragrance and its racemate is used as an important ingredient of perfumes. It was detected as a minor constituent of black tea leaves[1]. α-Ionone (figure 2) has been known for its pleasant aroma and was detected as a natural constituent of raspberries[2] and black tea[3]. The (R)-(+)-configuration was assigned to α-ionone from raspberries by a polarimetric investigation of the 2,4-dinitro-phenylhydrazone. The absolute configuration of natural α-damascone has not been known as yet. Stereochemical investigations of trace components are difficult in the presence of over 200 volatile constituents as in the tea aroma. Configurational assignments of individual components of such a complex mixture can be achieved by enantioselective capillary gas chromatography under the premises that sufficient selectivity is provided to resolve not only the enantiomers but also to separate the chiral component from all the other constituents which might overlap.

We have recently demonstrated that modified cyclodextrins exhibit exceptional enantioselectivities towards volatile chiral molecules of many compound classes[4]. However, with none of the α- and β-cyclodextrin derivatives investigated so far we were able to resolve the enantiomers of neither α-damascone nor α-ionone. We have now prepared the 3-O-methyl-2,6-di-O-pentyl derivative of γ-cyclodextrin a thermally highly stable compound which is liquid even below room temperature. Using this compound as a chiral stationary phase in Pyrex glass capillaries we succeeded in resolving the enantiomers of α-damascone as well as α-ionone and some related compounds (e.g. dihydro-α-ionone, carvone, menthone, isomenthone) without derivatization. The assignment of the order of elution of the enantiomers in the case of α-damascone was achieved by coinjection of the racemic mixture and the pure enantiomers which have recently been synthesized[5]. In the case of α-ionone a sample enriched in the (R)-(+)-enantiomer was available[6].

Capillary gas chromatography of a diethyl ether/pentane extract of 500 grams of black tea leaves using a methylsilicone stationary phase gave a very complex gas chromatogram and it was not possible to detect the α-damascone, even by heavily overloading the column. Both α-damascone and α-ionone could, however, be located after fractionation and preconcentration of the tea extract by preparative thin-layer chromatography. Two fractions, one containing the α-damascone and one containing the α-ionone were collected and investigated by capillary gas chromatography and mass spectrometry. Both compounds were identified by their characteristic mass spectra[1,7] (α-damascone:M^+, m/z = 192, m/z = 123, m/z = 81, m/z = 69; α-ionone: M^+, m/z = 192, m/z = 136, m/z = 121, m/z = 93) and by comparing retention times on different capillary columns.

The stereochemical assignments using a 60 m glass capillary column with the cyclodextrin derivative as a chiral stationary phase are demonstrated in figures 1 and 2.

It is quite remarkable that both α-damascone and α-ionone in black tea are almost enantiomerically pure in spite of the treatment of the tea during processing. We think that this stereochemical assignment clearly demonstrates the great potential of enantioselective capillary gas chromatography.

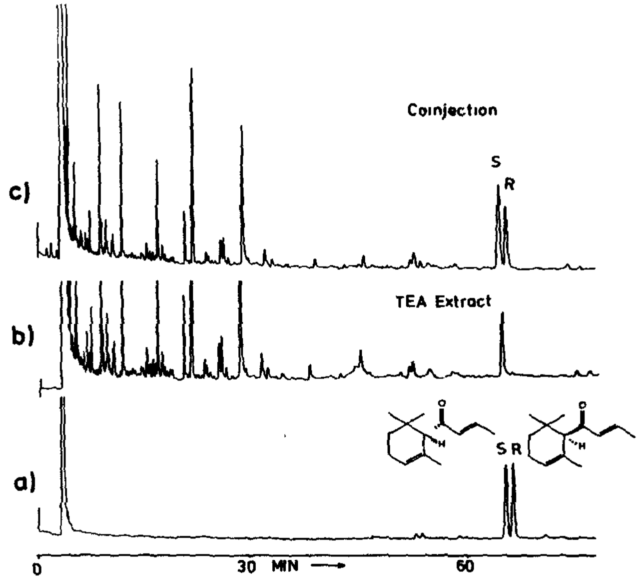

Figure 1
a) Gas chromatographic enantiomer separation of α-damascone, b) gas chromatogram of a fraction of black tea extract and c) coinjection of racemic α-damascone and tea extract. 60 m glass capillary column (0.25 mm i.d.) coated with octakis(3-O-methyl-2,6-di-O-pentyl)-γ-cyclodextrin; column temperature 100°C; carrier gas H_2 at 1.3 bar.

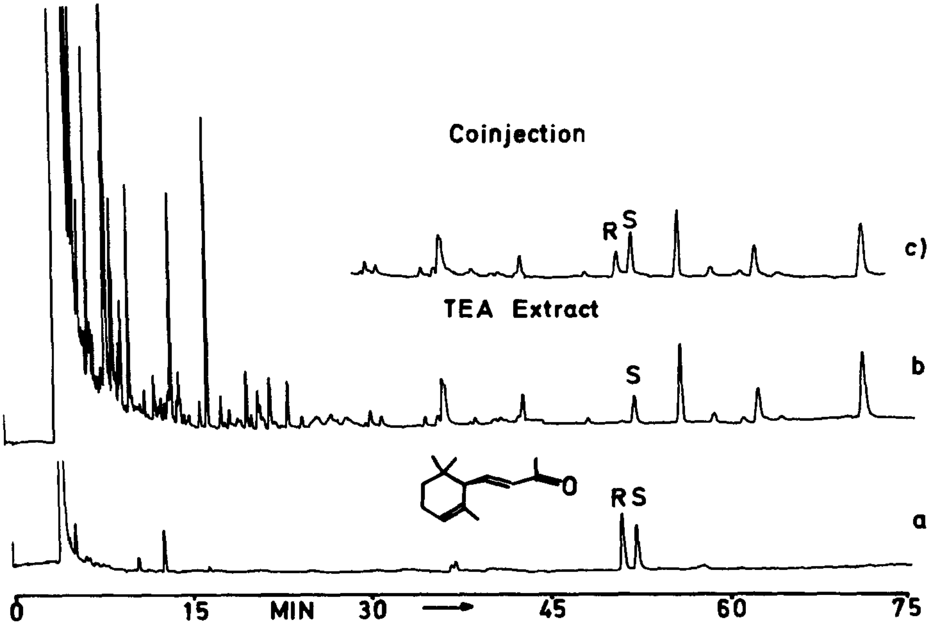

Figure 2
a) Gas chromatographic enantiomer separation of α-ionone [(R)-(+)-enantiomer with 8% ee); b) gas chromatogram of a fraction of black tea extract and c) coinjection of mixture a) and natural sample b). Column as in figure 1. Column temperature 115°C; carrier gas H_2 at 1.0 bar.

Experimental

Preparation of tea extract

167 g portions of black tea (Darjeeling Hochgewächs, J.J. Darboven, Hamburg) were extracted with a mixture of 800 ml of diethyl ether and 400 ml of n-pentane in a Soxhlet extractor. After 48 h the extraction filter was replaced by a second one with another 167 g of tea. This procedure was repeated twice until a total of 500 g of tea were extracted. The solvent was cautiously removed in vacuo leaving a residue of ca. 3 g of material.

Preparative thin-layer chromatography

200 mg of the 3 g residue were dissolved in a mixture of equal amounts of n-pentane, diethylether and chloroform and chromatographed on a silica tlc plate (thickness of layer 2 mm, Merck, Darmstadt) using petroleum ether (b.p. 60 - 70°C)/ethyl acetate (10 : 1, v/v). The fractions containing the α-damascone (R_F = 0.49) and the α-ionone (R_F = 0.36) were scrached from the glass plate and extracted by stirring for 2 x 30 min with 2 portions of 25 ml of chloroform. The silica gel was filtered off and the extracts were concentrated carefully to a volume of ca. 50 l. These solutions were used for gas chromatographic and mass spectrometric investigations.

Preparation of chiral capillary column

The alkylation of γ-cyclodextrin (Consortium für Elektrochemische Industrie) was performed in two steps using pentyl bromide and sodium hydroxyde to alkylate the 2- and the 6-hydroxy groups and methyl iodide and sodium hydride for methylation of the less reactive 3-hydroxy functions as already described for α- and β-cyclodextrin[8]. A 60 m Pyrex glass capillary column was coated according to the static procedure[9] after pretreatment of the inner glass surface with Silanox as described previously[10].

Gas chromatography, mass spectrometry

Carlo Erba model 2101 A instruments were used with split injection, flame ionization detection and hydrogen as carrier gas. For gas chromatographic-mass spectrometric investigations a Carlo Erba gas chromatograph with a 25 m fused silica capillary with OV 1 (Macherey-Nagel, Düren, FRG) connected via an open coupling to a Finnigan MAT 311 A double focussing mass spectrometer was applied. Electron impact mass spectra were recorded at 70 eV and 250°C ion source temperature.

Acknowledgments:
We are indebted to Consortium für Elektrochemische Industrie for providing γ-cyclodextrin, to J.J. Darboven company for supplying the tea and to the Fonds der Chemischen Industrie for financial support.

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