Journal of Chromatography, 197 (1980) 240–245
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 12,956

Note

Improved gas chromatography of amygdalin and its diastereomer

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(Received May 19th, 1980)

In recent years, amygdalin (D-mandelonitrile-β-D-gentiobioside) has become important in the treatment of cancer[1]. However, it is thought that its diastereomer, neoamygdalin (L-mandelonitrile-β-D-gentiobioisde), has no effect against cancer. Nahrstedt[2] attempted to separate isoamygdalin (a mixture of amygdalin and neoamygdalin) by gas chromatography with a packed column, but failed. The retention time was about 90 min, but the separation was not good enough to be used as a practical analytical method. In this work, we tried the separation on a capillary column using a high temperature and a high flow-rate.

EXPERIMENTAL

Reagent

Amygdalin, supplied by Merck (Darmstadt, G.F.R.), was used without further purification. Isoamygdalin was prepared from amygdalin and aqueous ammonia as described by Fischer[3]. Amygdalin was added to 10 ml of 0.005 *N* ammonia solution and the mixture was kept overnight at room temperature. During this period, D–L isomerization took place and equilibrium between amygdalin and neoamygdalin was attained. In this process, various impurities were formed, but we used the reaction product without further purification. Triphenylbenzene (TPB), supplied by Tokyo Kasei (Tokyo, Japan), was used as an internal standard. Trimethylchlorosilane (TMCS) and hexamethyldisilazane (HMDS) were supplied by Kishida Chemical (Osaka, Japan). Pyridine, from commercial sources, was dried with potassium hydroxide pellets.

Trimethylsilylation

Trimethylsilylation of glycosides was carried out as described by Nahrstedt[2]. Glycoside (1–3 mg) and TPB (0.5–2 mg) were weighed accurately and dissolved in 0.7 ml pyridine in a vial. After adding 0.2 ml of HMDS and 0.1 ml of TMCS, the mixture was kept for 10 min at room temperature. The silylated glycosides were contained in the supernatant, of which a 1-μl aliquot was injected on to the gas chromatograph.

0021-9673/80/0000–0000/$02.25

Gas chromatography

An Okura Model 103 gas chromatograph, equipped with a soda-glass capillary column and two hydrogen flame-ionization detectors, was used. The capillary column was prepared by Nippon Chromato (Tokyo, Japan) as described by Alexander and Rutten[4]. The operating conditions are given in Fig. 1.

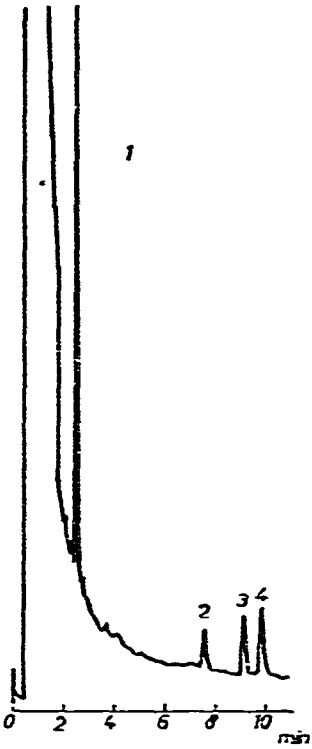

Fig. 1. Gas chromatogram of TMS-amygdalin and TMS-neoamygdalin, obtained from amygdalin. Soda-glass capillary column (10 m × 0.28 mm I.D.), treated with HCl vapour and coated with OV-1. Column temperature, 280°C isothermal. Inlet temperature, 350°C. Carrier gas (nitrogen) linear velocity, 27.8 cm/sec. Splitting ratio, 87.6. Injection volume, 1 μl. Peaks: 1 = TPB; 2 = unknown; 3 = TMS-neoamygdalin; 4 = TMS-amygdalin.

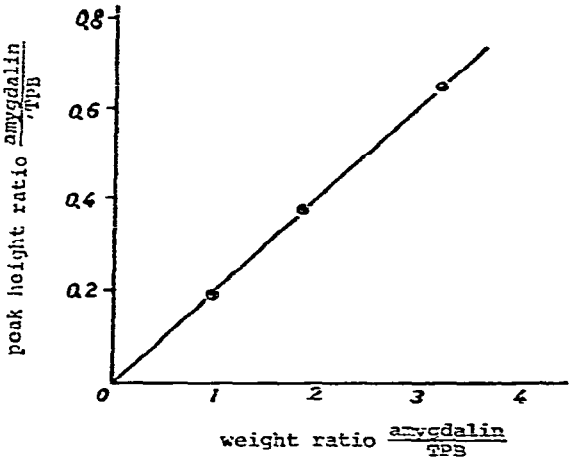

Fig. 2. Relationship between peak-height ratio and composition of mixtures of amygdalin and internal standard (TPB).

RESULTS AND DISCUSSION

Analysis of glycosides

Fig. 1 shows a chromatogram of TMS-isoamygdalin. The complete separation of TMS-amygdalin and TMS-neoamygdalin is achieved, in contrast to the unsatisfactory separation with a packed column. A considerable reduction in the retention time of TMS-amygdalin was achieved by keeping the column at 280°C and using a much higher flow-rate of the carrier gas than usual (about 30 cm/sec). These operating conditions decrease the separation efficiency, but not seriously.

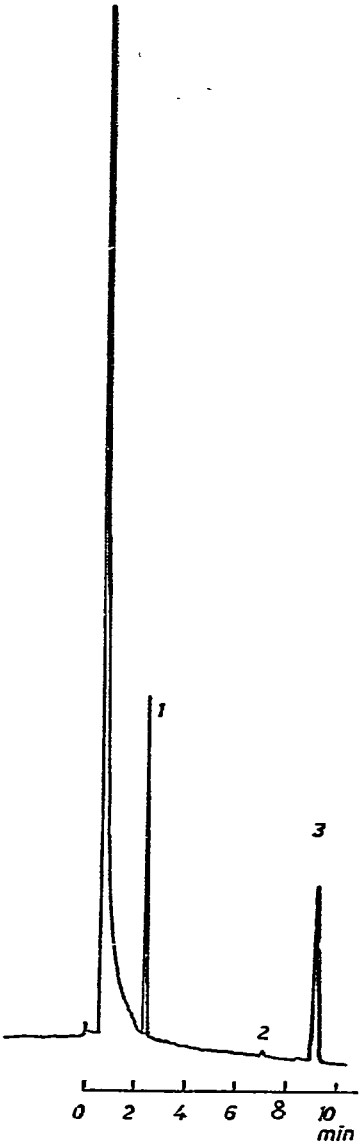

Fig. 3. Gas chromatogram of TMS-amygdalin obtained from commercial tablets containing amygdalin. Conditions and peak numbers as in Fig. 1.

TABLE I

ANALYSIS OF COMMERCIAL TABLETS AND A SOLUTION CONTAINING AMYGDALIN

Parameter	*Tablets*	*Solution*
Nominal content (mg/tablet)	325	—
Average weight (mg/tablet)	380.5	Solid content 3.19 g per 20 ml
Calculated amygdalin content (%)	84.5	—
Observed amygdalin content (%)	85.7	*ca.* 30% in solid
Observed neoamygdalin	Trace	Comparable to amygdalin

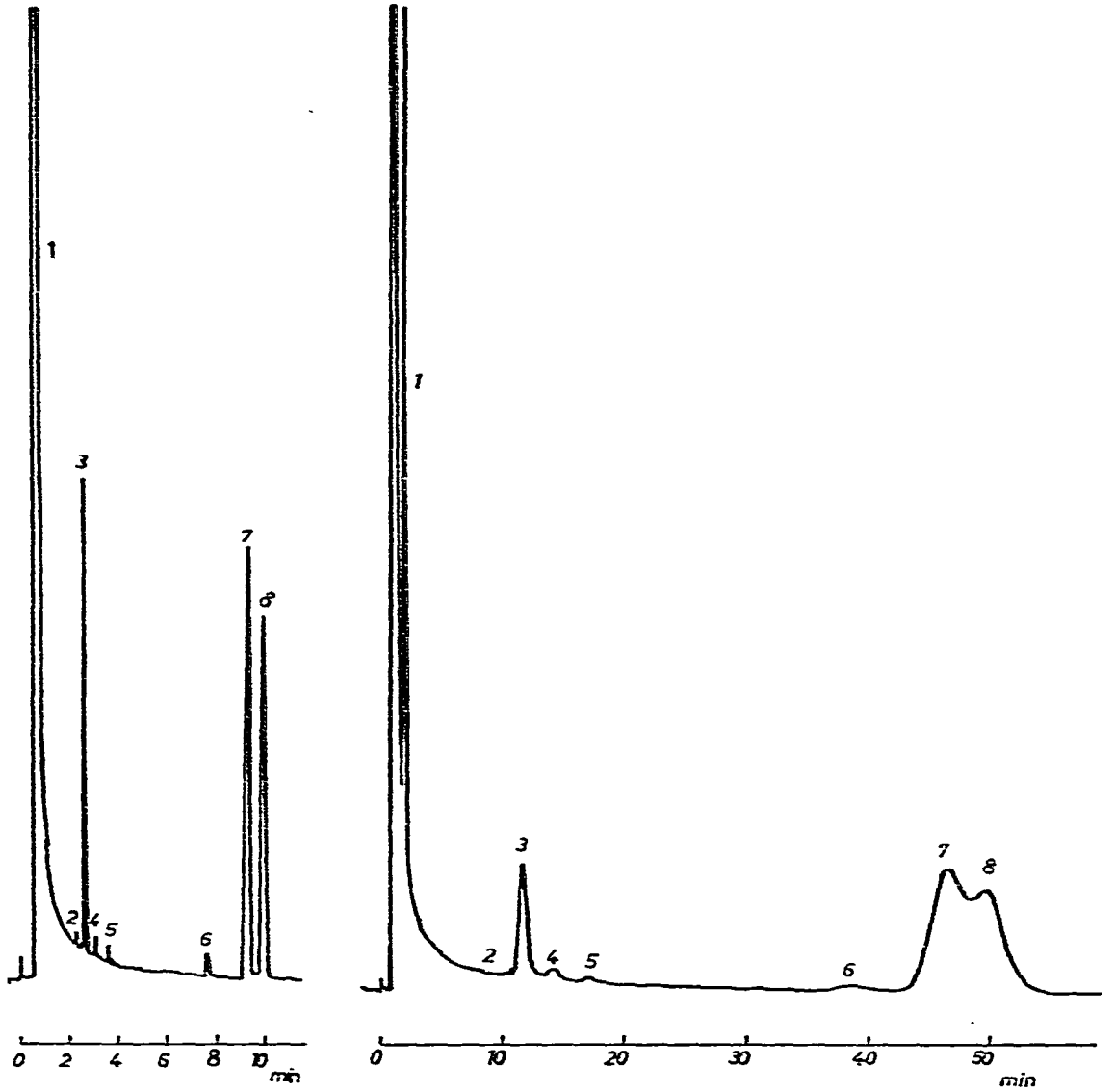

Fig. 4. Gas chromatogram of TMS-amygdalin and other compounds from commercial solution containing amygdalin. Conditions as in Fig. 1. Peaks: 1, 2, 4, 5 and 6 = unknown; 3 = TBP; 7 = TMS-neoamygdalin; 8 = TMS-amygdalin.

Fig. 5. Gas chromatogram of TMS-amygdalin and other compounds obtained from commercial solution containing amygdalin. Stainless-steel packed column (3 m × 3 mm I.D.), 3% OV-1 on silylated Celite (80–100 mesh). Column temperature, 280°C isothermal. Inlet temperature, 350°C. Carrier gas (nitrogen) flow-rate, 25 ml/sec. Injection volume, 1 μl. Peak numbers as in Fig. 4.

A calibration graph for amygdalin in shown in Fig. 2; the linearity is satisfactory.

In order to check the method, commercial tablets with a well defined composition were analysed (Fig. 3). The results are given in Table I.

Subsequently, a commercial aqueous solution of amygdalin without a specified amygdalin content was analysed, and the results are given in Fig. 4 and Table I. In this instance, the aqueous solution was evaporated to dryness. In order to verify that the evaporation process does not change the composition, a tablet was dissolved in water, the solution was evaporated to dryness and the composition of the residue was analysed. The composition was identical with that of the original tablet.

Comparison of capillary and packed columns

A packed column was prepared according to the literature[2], but replacing Chromosorb AW DMCS with Celite silylated with HMDS vapour[5] as the support, and increasing the flow-rate from 30 to 40 ml/min. The chromatogram obtained was similar to that described in the literature[2]. The packed column was then compared with the capillary column, the temperature of the former being kept at 280°C isothermal (Fig. 5).

The results are given in Table II, and indicate that the capillary column is far superior to the packed column.

TABLE II

COMPARISON OF GAS CHROMATOGRAMS

The data were obtained from Figs. 4 and 5.

Parameter	*Capillary column*	*Packed column*
Resolution, R_S	2.3	0.6
Adjusted retention time of TMS-amygdalin, t_R (min)	10	50
Separation factor, α	1.07	1.07
Number of effective theoretical plates of TMS-amygdalin, N_{eff}	1560 × 10	430 × 3
Shape for small amounts of components	Sharp, easily detected peaks	Broad, readily missed peaks

Reduction of measurement time

The value of resolution (2.3) given in Table II is unnecessarily large. By reducing it to 1.5, the measurement time, which is nearly equal to the adjusted retention time of TMS-amygdalin, is to be reduced.

The retention time, t_R, is given by the equations[6]

$$R_S = \frac{1}{4}\left(1 - \frac{1}{\alpha}\right) N_{eff}^{1/2}$$

and

$$t_R' = \frac{N_{eff}}{1560} \cdot \frac{t_{R10}'}{10}$$

where R_S is the mean resolution, N_{eff} the number of effective theoretical plates, $N_{eff}/1560$ the column length (L) necessary for obtaining a certain R_S value, α the separation factor and t'_{R10} the adjusted retention time in a capillary column of length 10 m. Then, if we put $R_S = 1.5$, we obtain $N_{eff} = 7785$, $L = 5$ m and $t'_R = 5$ min.

After using the capillary column for 3 months, no decrease in the efficiency of the capillary column or shift in retention time was observed, indicating the practical applicability of the method.

ACKNOWLEDGEMENTS

We thank Dr. S. Kawachi, Kawachi Clinic, for supplying amygdalin and samples, Mr. M. Watanabe, Japan Macrobiological Medical Association, for his lucid explanations of amygdalin and Mr. S. Terazaki for his help with the experiments.

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