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Study on the Prevention of Racemization of Amygdalin

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Studies were conducted to find suitable conditions for the prevention of racemization of amygdalin in aqueous solution. Evaluation of racemization was carried out by determination of the ratios of neoamygdalin and amygdalin using a capillary gas chromatographic technique. Racemization of amygdalin in aqueous solution was accelerated by increases on pH and temperature, but racemization was low below pH 3.0 even with heating. Ascorbic acid is useful as an acidification reagent for the suppression of racemization, and has the added clinical benefit of vitamin C activity.

Keywords——amygdalin; isoamygdalin separation; racemization; racemization prevention; capillary gas chromatography

Recently, the use of amygdalin (D-mandelonitrile-β-D-gentiobioside) has been the subject of considerable controversy in the treatment of cancer.[1-3] However, its diastereomer, neoamygdalin (L-mandelonitrile-β-D-gentiobioside), is considered to be ineffective against cancer. Therefore, the separation of isoamygdalin (a mixture of amygdalin and neoamygdalin) has become an important factor in the evaluation of drugs containing amygdalin.

In the previous paper,[4] the complete separation of isoamygdalin was achieved by a capillary gas chromatographic technique and it became clear that approximately equal amounts of neoamygdalin and amygdalin were formed in a commercial aqueous solution of amygdalin. With regard to the racemization of amygdalin, Smith[5,6] and Plouvier[7] have reported that the rate of isomerization of amygdalin as determined by optical rotation measurements increases with increase in pH and temperature.

The present studies were intended to find suitable conditions for prevention of the racemization of amygdalin in aqueous solutions. The effects of pH and temperature on the racemization of amygdalin are discussed.

Experimental

Reagents——Amygdalin was obtained from Merck (Darmstadt, Germany). Trimethylchlorosilane, hexamethyldisilazane and 4-methoxy-2-nitroaniline were purchased from Kishida Cheimical Co. (Osaka, Japan). All other chemicals were of analytical grade.

Trimethylsilylation——A 0.5-ml aliquot of sample solution was evaporated to dryness under reduced pressure, and trimethylsilylation of glycosides was carried out by treatment with a mixture of pyridine, hexamethyldisilazane and trimethylchlorosilane as described in the previous paper.[4]

Gas Chromatography——All analyses were performed with an Okura model 103 gas chromatograph, equipped with a soda-glass capillary column coated with OV-1 and two hydrogen flame-ionization detectors. The operating conditions were described in detail in the previous paper.[4] Evaluation of racemization was carried out by monitoring the peak area ratios of neoamygdalin and amygdalin on gas chromatograms. The peak area was measured by a triangulation method using the product of peak height and peak width at the half-height.

Effect of pH on Racemization——Solutions of amygdalin (10 μg/ml) were prepared in 0.2 M acetate buffers or 0.2 M acetic acid at pH 5.2, 4.2, 3.6 and 2.8, and allowed to stand at room temperature. Variation of the ratios of

neoamygdalin and amygdalin with time was monitored in 0.5-ml aliquots of the sample solution by gas chromatography.

Effect of Temperature on Racemization——a) Solutions of amygdalin (10 μg/ml) were prepared in 0.2 M acetate buffer or 0.2 M acetic acid at pH 5.2 and 2.8, and kept at 92 °C in an oil bath. b) Solutions containing 100 μg of amygdalin and 200 (measured pH of the solution: 2.49), 100 (2.69), 50 (2.82) or 10 μg (3.25) of ascorbic acid in 10 ml of aqueous solution were prepared and kept at 92 °C in an oil bath. At regular intervals, the extent of racemization was measured by gas chromatography.

Effect of Temperature on the Stability of Ascorbic Acid——A solution containing 100 μg of amygdalin and 10 μg of ascorbic acid in 10 ml of aqueous solution was prepared and kept at 92 °C in an oil bath. After various periods, the amount of ascorbic acid remaining in the solution was measured by the colorimetric method[8] at 570 nm using a 1.0-ml aliquot of the sample solution.

Results and Discussion

The aglycone chiral center (derived from mandelonitrile) of amygdalin is subject to epimerization under basic conditions because of the weakly acidic nature of the benzylic proton.[2,6] The findings that the peak area ratio of neoamygdalin and amygdalin on a gas chromatogram of commercial amygdalin solutions for injection (labeled "pure amygdalin"; the infrared (IR) spectrum of the residue obtained by evaporating the solution to dryness coincided with that of authentic amygdalin) was observed to be 1.22 (Fig. 1A) and that the pH of the solutions was 5.2 encouraged us to look for suitable conditions for the prevention of racemization of amygdalin in aqueous solution. It is reasonable to suppose that racemization will become extremely slow under acidic conditions or, may not occur at room temperature. First, the effects of pH variation of the solution on the extent of racemization were examined at room temperature. A typical gas chromatographic separation of the solution after 180 d is illustrated in Fig. 1B, and the results are plotted as peak area ratios *vs.* days in Fig. 2. As

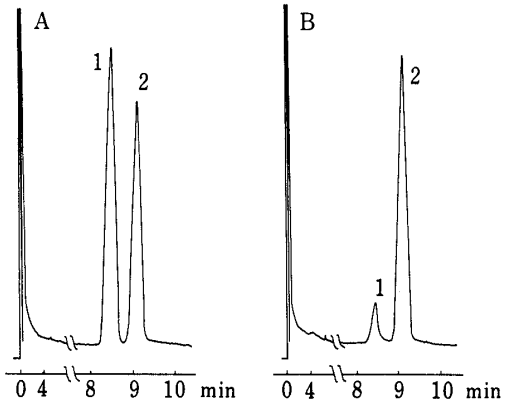

Fig. 1. Gas Chromatographic Separation of Isoamygdalin in Aqueous Solution as the Trimethylsilyl Derivative

1, neoamygdalin; 2, amygdalin.
A, a commercial amygdalin solution for injection; B, an aqueous solution of amygdalin (10 μg/ml) in 0.2 M acetate buffer, pH 5.2, allowed to stand for 180 d at room temperature.
Conditions: soda-glass capillary column (10 m × 0.28 mm i.d.), treated with HCl vapor and coated with OV-1; column temperature, 280 °C isothermal; inlet temperature, 350 °C; carrier gas (nitrogen) linear velocity, 27.8 cm/s; splitting ratio, 87.6; injection volume, 1 μl.

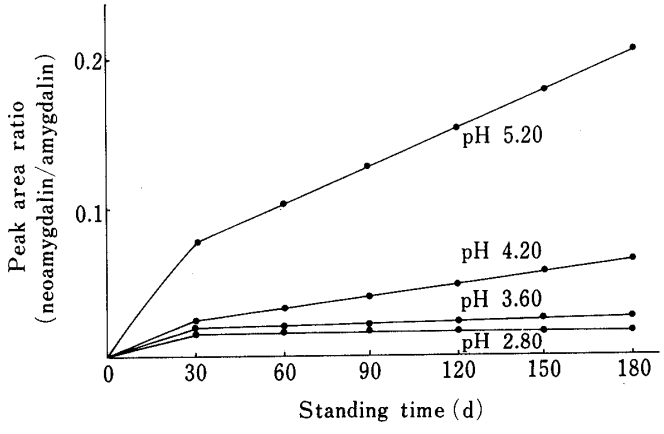

Fig. 2. Effect of pH on the Racemization of Amygdalin at Room Temperature

shown in Fig. 2, it was found that racemization took place more rapidly as the pH increased. At lower pH, the rate of racemization decreased.

It appears from the relation of amygdalin concentration and pH in aqueous solution (Table I) that the dissociation constant (K) of amygdalin is of the order of 10^{-9}. Since the rate of racemization depends on the pH of the solution, it may be assumed that amygdalin is an extremely weak acid and its dissociation will be lowered by the presence of other organic acids, such as acetic acid, lactic acid and ascorbic acid, the K values of which are of the order of 10^{-4}—10^{-5}. On the other hand, even after 180 d at pH 5.2, the extent of racemization

Table I. Relation of Amygdalin Concentration and pH of Its Aqueous Solution

Concentration (mg/3 ml)	pH	Dissociation constant (K)
3	5.60	2.88×10^{-9}
15	5.25	2.89×10^{-9}

Table II. Variation of the Racemization with Time at 92 °C

pH	min			
	10	30	60	120
5.2[a]	0.057[c]	0.080	0.160	0.290
2.8[b]	0.009	0.016	0.028	0.035

a) 0.2 M Acetate buffer.
b) 0.2 M Acetic acid.
c) Extent of racemization is shown as the peak area ratio of neoamygdalin and amygdalin on gas chromatograms.

Table III. Variation of the Racemization with Time in Solutions Acidified with Ascorbic Acid at 92 °C

pH	min		
	30	60	120
3.25	0.042	0.108	0.120
2.82	0.011	0.029	0.032
2.69	0.009	0.016	0.022
2.49	0.009	0.014	0.015

Table IV. Influence of Temperature[a] on the Stability of Ascorbic Acid

Standing time (min)	0	30	60	120
Absorbance[b]	0.360	0.372	0.372	0.307

a) The aqueous solution containing 100 μg of amygdalin and 10 μg of ascorbic acid in 10 ml was kept at 92 °C.
b) Amounts of ascorbic acid were measured colorimetrically.

determined from the peak area ratio was only about 0.2, which is much less than the value of 1.22 observed in commercial amygdalin solution. Thus, it seems unlikely that racemization in the commercial preparation arose simply as a result of allowing it to stand at room temperature.

Next, experiments were conducted to determine the effect of temperature on the racemization of amygdalin at pH 5.2 and 2.8, as shown in Table II. At pH 5.2, the same as that of the commercial amygdalin solutions, the effect of temperature was remarkable, whereas little effect was observed at pH 2.8. Thus, it was inferred that racemization in the solution occurred mainly during the heating-for-sterilization process.

Ascorbic acid is useful not only as an acidifying agent in solution, but also because of its vitamin C activity. Therefore, similar experiments on pH and temperature were carried out with ascorbic acid in place of acetic acid (Table III). The results were almost identical with those obtained above, and it could be assumed that the racemization rate was significantly depressed at below pH 3.0. As shown in Table IV, ascorbic acid itself was stable under most of the conditions tested.

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