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Note

Analysis of *Z* and *E* isomers of urocanic acid by high-performance liquid chromatography

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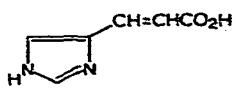
There has recently been a substantial effort devoted to the chromatographic detection of urocanic acid (UA), a major metabolite of histidine, and a naturally occurring sunscreen [1—7]. Of the several reported high-performance liquid chromatographic (HPLC) methods [8—11], none has described the resolution of UA into its *E* (*trans*) and *Z* (*cis*) isomers, though such isomerization is critical to the function of UA as a sunscreen [12]. We report here on two HPLC systems that resolve UA isomers, one an ion-paired, reversed-phase (for recent reviews, see refs. 13 and 14) procedure (method A) with a detection limit better than those reported for unresolved UA, and the other a "normal"-phase procedure (method B).



Urocanic acid (UA)

EXPERIMENTAL

Instrumentation and procedures

HPLC was performed using a composite system consisting of a Waters Model 6000A solvent delivery system, a Rheodyne 70-10 sample injection valve with a 20-μl loop, a Laboratory Data Control UV monitor with 254-nm detector and a Hewlett-Packard 3380A electronic integrator. Method A employed a 250 × 4.2 mm I.D. Waters μBondapak C_{18} (10 μm particle size) column: the eluent was 24 mg of tetrabutylammonium sulfate, 3.36 g of $NaH_2PO_4 \cdot H_2O$ and 8.00 g of Na_2HPO_4 in 1 l of distilled (from $KMnO_4$) water. The flow-rate of the pH 7.3—7.4 solution was 2.0 ml/min. Method B used a 250 × 4.2 mm I.D. Whatman

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Partisil (11 μm particle size) column with 2% conc. (58%) ammonia in 1-propanol at 1 ml/min as eluent.

Chemicals

E-UA was from Aldrich (Milwaukee, WI, U.S.A.) and was recrystallized from water to give crystals of the dihydrate. After washing with acetone and vacuum drying at 45°C for several hours, the melting point was 239°C (uncorr.) (lit. 218°C [15], 228°C [16]. L-Histidine · HCl was from Sigma (St. Louis, MO, U.S.A.).

Z-UA was prepared by irradiating a slurry of 5.4 g of *E*-UA in 2 l of water brought to pH 9 with potassium hydroxide. The slurry was photolyzed in a Rayonet Reactor (New England Ultraviolet Company) at 310 nm for 4 days at 30°C with occasional stirring. During this time the excess potassium salt of *E*-UA slowly went into solution. The resulting UA mixture (*E*/*Z* = 3:7) is virtually at the photostationary state [12]. The solution was lyophilized, the residue dissolved in a little water, and the pH adjusted to 9 with glacial acetic acid. This solution was chromatographed on a 23 × 3.5 cm column of Bio-Rad AG 1-X8 (acetate), 200—400 mesh, in 0.0125 *M* acetic acid [7]. Elution with 300 ml of 0.0125 *M* acetic acid was followed by 500 ml of 0.025 *M* and 1200 ml of 0.1 *M* acetic acid. *Z*-UA was eluted first and appeared after ca. 1000 ml of eluent. (In order to avoid $Z \rightarrow E$ isomerization by the slightly acidic eluent, the eluent was trapped in receiving flasks cooled with methanol—dry ice.) The combined fractions were lyophilized to yield a fluffy white solid which was washed with hexane and dried over phosphorus pentoxide at 65°C. The yield was 2.4 g (45%), m.p. 178—180°C (uncorr.) (lit. 175—176°C [15]); elemental analysis confirmed that this *Z*-UA is *not* a hydrate (compare *E*-UA above).

A sample of sweat was obtained by diluting several drops of tension-produced sweat from the epidermis on the back of the hand with water.

RESULTS AND DISCUSSION

The separation of an artificial mixture of *Z*- and *E*-UA using method A is illustrated in Fig. 1 (curve a). Retention times are *E*-UA 3.0 min, and *Z*-UA 4.7 min. Fig. 1 (curve b), a chromatogram of human sweat, illustrates the sensitivity of the technique. We determined that using a 20-μl injector volume, the detection limit for *E*-UA is 1 ng and for *Z*-UA, 3 ng (3×10^{-7} *M* and 1×10^{-6} *M*, respectively).

Our quantitative analyses were for solutions 6×10^{-4} *M* to 6×10^{-3} *M* in UA. The response of the UV detector was found to be linear over most of this range (6×10^{-4} *M* to 1.5×10^{-3} *M*) for both the *E* and *Z* isomers (correlation coefficients were 0.924 and 0.997, respectively). The calibration curves should be freshly determined or checked, however, since they are quite susceptible to small changes in flow-rate, column deterioration, etc. At higher concentrations there are large deviations from the Beer—Lambert Law within the detector, so that solutions of more than 2×10^{-3} *M* are best diluted before analysis. Alternatively, one may use known concentrations of *E*- or *Z*-UA which are within 5% of the unknown. The precision of multiple HPLC analyses was usually 1—2%. Areas, rather than peak heights, should be used for quantitation be-

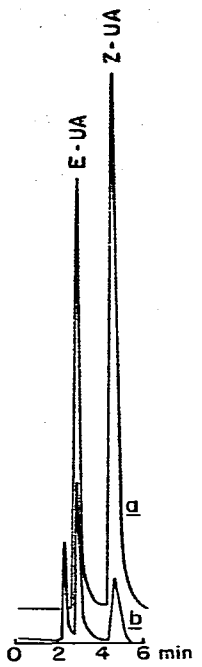

Fig. 1. HPLC analysis of *E*- and *Z*-UA using method A: (a) synthetic mixture; (b) sweat from the back of the hand.

cause the shapes of the peaks are dependent on the solvent used to dissolve the sample (for example, ^2H_2O vs. H_2O).

Method B complements method A in that the elution sequence is reversed; the retention times are *Z*-UA 2.5 min, and *E*-UA 3.8 min. Method A successfully resolves histidine from UA at a flow-rate of 1 ml/min. Retention times are histidine 3.3 min, *E*-UA 4.5 min, and *Z*-UA 8.9 min. The limit of detection for histidine is 0.5 μg. Reversed-phase columns are subject to deterioration when used with buffer salts; for appropriate precautions, see ref. 17. When the column deteriorates partially, a soap chromatography [18] technique using hexadecyltrimethylammonium bromide in place of tetrabutylammonium sulfate will give increased resolution: *E*-UA 2 min, *Z*-UA 8 min, at 2 ml/min.

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