

Characterization of the decomposition of compounds derived from imidazolidinyl urea in cosmetics and patch test materials

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Summary

Background. Imidazolidinyl urea releases formaldehyde through decomposition. However, there have been few reports on the chemistry of imidazolidinyl urea in cosmetics.

Objectives. The aim of this study was to characterize imidazolidinyl urea-derived compounds in cosmetics and to determine which compounds are responsible for the cross-reactivity with diazolidinyl urea.

Methods. We analysed imidazolidinyl urea dissolved in aqueous solutions, imidazolidinyl urea patch test materials and imidazolidinyl urea-preserved cosmetics by high-performance liquid chromatography/photodiode array detection and liquid chromatography/mass spectrometry. The results were compared with those obtained with a diazolidinyl urea aqueous solution.

Results. In the analysed cosmetic samples and patch test materials, imidazolidinyl urea was primarily composed of allantoin, (4-hydroxymethyl-2,5-dioxo-imidazolidine-4-yl)-urea (HU), (3,4-bis-hydroxymethyl-2,5-dioxo-imidazolidine-4-yl)-urea (3,4-BHU), and (3-hydroxymethyl-2,5-dioxo-imidazolidine-4-yl)-urea.

Conclusions. Two of the imidazolidinyl urea-derived major decomposition compounds – HU and 3,4-BHU – are common in the diazolidinyl urea-decomposed compound present in cosmetics. These compounds are possible causative agents of the cross-reactivity between diazolidinyl urea and imidazolidinyl urea.

Key words: cosmetic; cross-reaction; formaldehyde-releasers; imidazolidinyl urea; skin sensitization.

A wide variety of antimicrobial preservatives, such as formaldehyde and formaldehyde-releasers, are used in cosmetics (1–3). Imidazolidinyl urea (IU) is one of the most commonly used preservatives, and is thought to release formaldehyde through its decomposition. The amount of formaldehyde released from IU and the chemistry of IU have been investigated (4–6). One of the

studies has shown that the predominant compounds arising are allantoin and (4-hydroxymethyl-2,5-dioxo-imidazolidine-4-yl)-urea (HU), but other, less predominant, compounds have not been identified (6).

Several patch test studies have reported the contact sensitivities to IU and other formaldehyde-releasers (7–14). In some reports, the cross-reactivity between formaldehyde-releasers has been studied (11, 13, 15). As a result, it was found that the cross-reactive ratios between IU and diazolidinyl urea (DU) are higher than those for other formaldehyde-releasers. Furthermore, one of these studies reported a case of a reaction to both IU and DU, although patch test results for formaldehyde and other formaldehyde-releasers were negative (14).

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Previously, we reported the decomposition properties of DU in cosmetics and patch test materials (16). To understand the mechanism of the cross-reaction between IU and DU, it is also important to know the chemical properties of IU. Recently, a report on formaldehyde concentrations in formaldehyde-releaser patch test preparations was published (17), but there are no previous reports referring to IU decomposition in patch test vehicles.

In this study, we investigated the chemistry of IU in an aqueous solution, patch test materials, and cosmetics. The result was compared with that obtained with a DU aqueous solution to determine the common decomposition compounds. Elucidation of IU chemistry is thought to be helpful in explaining how contact dermatitis is caused by IU in cosmetics and how the cross-reaction between IU and DU occurs.

Materials and Methods

Chemicals and reagents

Liquid chromatography (LC)/mass spectrometry (MS)-grade acetonitrile, high-performance liquid chromatography (HPLC)-grade tetrahydrofuran and special-grade acetic acid, allantoin, ammonia aqueous solution and ammonium acetate were purchased from Wako Pure Chemical Industries (Osaka, Japan). IU and DU were obtained from Sigma (St Louis, MO, USA). HU and (3,4-bis-hydroxymethyl-2,5-dioxo-imidazolidine-4-yl)-urea (3,4-BHU) were prepared as described previously (16).

Instrumentation and chromatographic conditions

An LC-10AD liquid chromatography system (Shimadzu, Kyoto, Japan) equipped with a photodiode array detector was used. HPLC separation was performed as described previously (18) at 30°C on a COSMOSIL HILIC column (5- μ m particle size, 4.6-mm ID $\times$ 250 mm) (Nacalai Tesque, Kyoto, Japan) under isocratic conditions. The mobile phase consisted of acetonitrile and water in a 90:10 ratio, and the flow rate was 1 ml/min. The volume of the injected sample was 50 μ l.

The LC/electrospray ionization (ESI)-MS experiments were performed with a Prominence UFLC liquid chromatography system and an LCMS-2020 quadrupole mass spectrometer (Shimadzu). A COSMOSIL-HILIC column (5- μ m particle size, 2.0-mm ID $\times$ 150 mm) (Nacalai Tesque) was used. The mobile phase was composed of acetonitrile and 10 mmol/l ammonium acetate in a 95:5 ratio. HPLC separation was performed under isocratic conditions. The flow rate of the mobile phase was 0.2 ml/min, and the

injection volume was 10 μ l. The column temperature was 30°C. The instrument parameters were as follows: source temperature, 350°C; desolvation temperature, 250°C; capillary voltage, 5.0 kV; Q-array DC voltage, -35 V; desolvation voltage, -35 V; and desolvation gas flow, 900 l/hr. The mass range of the spectra was from m/z 100 to m/z 350 in the scan mode analysis, and m/z 157, 187, 217 and 247 were measured in the selected ion monitoring (SIM) analysis. The entire LC/MS analysis was performed in the negative mode.

Purification and ^1H -nuclear magnetic resonance (NMR)

Compound B was isolated from the reaction mixture of allantoin and formaldehyde in a 1:1.2 ratio in 0.1 mol/l sodium hydroxide aqueous solution. After 2 hr of incubation at 60°C, the solution was freeze-dried and redissolved in a 20 mmol/l ammonium acetate solution at a concentration of 20%. Acetic acid was used to adjust the pH of the ammonium acetate solution to 4.0. This solution was then diluted 10 times with acetonitrile, filtered, and injected into an HPLC system by use of a COSMOSIL HILIC column (5 μ m particle size, 10 mm ID $\times$ 250 mm) (Nacalai Tesque) with a 500 μ l injection volume. The collected fraction was freeze dried prior to ^1H -NMR analysis.

^1H -NMR analysis was performed with an AV400M digital NMR spectrometer (Bruker Biospin K.K., Kanagawa, Japan) at the Hitachi Chemical Techno Service (Ibaragi, Japan). The solvent used was dimethyl sulfoxide, and the field was 400.13 MHz.

Cosmetics and patch test samples

The brand names and manufacturers/distributors of the commercial cosmetics tested are shown in Table 1. Patch test samples were purchased from Chemotechnique Diagnostics (Vellinge, Sweden), Almirall Hermal (Reinbek, Germany), and Brial Allergen (Münster, Germany).

Preparation of samples from cosmetics and patch test vehicles

Patch sample preparation was as described previously (16). Preparation of the cosmetic and aqueous solutions was performed as described previously, with minor modifications (18). The cosmetic samples (0.1 g) were mixed briefly with 0.15 ml of 20 mmol/l ammonium acetate (pH 4.0) and 2.25 ml of acetonitrile. The mixed solutions were then centrifuged at 2080 g for 10 min at room temperature, and the supernatant was filtered through 0.45 μ m polyethersulfone filters (Dainippon Seiki, Kyoto, Japan) prior to HPLC analysis. The

Table 1. The brand names and manufacturers/distributors of the analysed commercial cosmetics

Brand names	Manufacturers/distributors
Face Oasis™ Hydrating Treatment	H2O Plus, LLC, Chicago, IL, USA
Anti-Bacterial Moisturizing Hand Soap Cucumber Melon®	Bath & Body Works, Inc., New Albany, OH, USA
Dermal Therapy Extra Strength Body Lotion	DTR Dermal Therapy Research Inc., Ontario, Canada
Sudden Change® Under-eye Firming Serum	CCA Industries, Inc., East Rutherford, NJ, USA
Smackers Moisturizing Shower Gel Strawberry	The Bonne Bell Company, Lakewood, OH, USA
Smackers Moisturizing Shower Gel Orange	
Aquolina Bath & Body Shower Gel Scrub Wild Strawberry	Selectiva Spa, Alessandria, Italy
Aquolina Bath & Body Shower Gel Scrub Fig	
L'Oréal Eye Makeup Remover	L'Oréal Paris USA Inc., New York, NY, USA
Diabet-x® Callus Treatment	FNC Medical Corporation, Ventura, CA, USA
Royale Bouquet Wild Bluebell and Hyacinth Luxury Hand Lotion	Baylis & Harding Plc, Redditch, UK
Royale Bouquet Lemon Blossom Luxury Hand Lotion	
Back to The Basics Jasmine Body Lotion	Graham Webb Internaitonal, Carlsbad, CA, USA
Gold Bond Medicated Body Lotion	Chattem, Inc., Chattanooga, TN, USA
Banana Boat® Aloe After Sun Lotion	Sun Pharmaceuticals Corp., Delray Beach, FL, USA
Alpha Hydrox® Enhanced Lotion Anti-Wrinkle	Neoteric® Cosmetics, Inc., Denver, CO, USA
Eucerin Sensitive Skin Gentle Hydrating Cleanser	Beiersdorf Inc., Wilton, CT, USA
Aquaphor Gentle Wash for Dry, Sensitive Skin	

buffer solutions were diluted 10 times with acetonitrile, and subjected to HPLC analysis.

Results

HPLC and LC/MS of IU aqueous solution and the patch test materials

The possible candidates for IU-derived compounds are shown in Table 2. IU is thought to release formaldehyde to give HU (compound **5**) and allantoin (compound **1**). From previous reports (6, 16) and this study, other candidates for minor compounds derived from IU are suspected (compounds **2–4** and **6–10**).

Figure 1 shows a typical ultraviolet (UV) chromatogram at 210 nm of IU aqueous solution and patch test material purchased from Chemotechnique Diagnostics, analysed by HPLC–photodiode array detection. The UV chromatogram shows at least four major peaks and three minor peaks. The UV chromatograms of patch test materials obtained from Almirall Hermal and Brial Allergen were similar to that of patch test material purchased from Chemotechnique Diagnostics (data not shown). These observed peaks were arrow-headed, and denoted peak A to peak G for descriptive purposes.

IU-containing aqueous solution and patch test materials were also analysed by LC/ESI-MS in the scan mode. Mass chromatograms at m/z 157, 187, 217 and 247 of 2.5 mg/l IU in water are shown in Fig. 1c. The full-scan mass spectra for the peaks of IU-containing aqueous solution are shown in Fig. 1d. In the ESI spectra, ions were observed at m/z 187 (deprotonated ion) and

m/z 157 (elimination of a formaldehyde unit) in the analysis of peaks B and C. In the mass spectra of peaks D and G, ions were observed at m/z 217 (deprotonated ion) and m/z 187 (elimination of a formaldehyde unit). In the mass spectrum of peak F, ions were observed at m/z 247 (deprotonated ion), m/z 217 (elimination of a formaldehyde unit), and m/z 187 (elimination of two formaldehyde units). The mass spectra of peaks A and E consisted of a single ion at m/z 157 and a single ion at m/z 187, respectively. Each peak observed for the patch test substances was similar to the mass spectra peaks of the aqueous solutions (data not shown). The entire LC/MS analysis was performed in the negative mode.

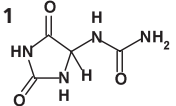
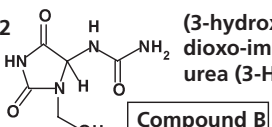
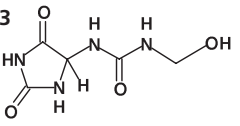
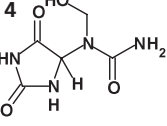
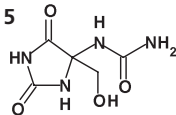
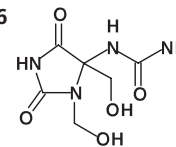
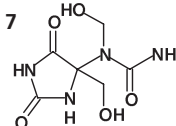
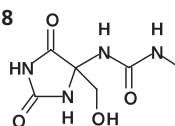
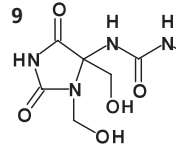
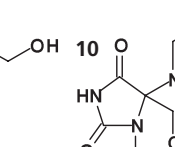
The retention times and mass spectra of peaks A, D and E from LC/MS are well matched with allantoin, HU, and 3,4-BHU (Fig. 2a and data not shown). For compounds other than those identified, the LC/MS data suggested that the molecular weights of compounds B and C, compound G and compound F were 188, 218, and 248, respectively. Peaks D to G were also detected from a 2.5 mg/l IU aqueous solution, prepared from 0.5% IU in water and kept for 1 month at 10°C (Fig. 2b and data not shown).

¹H-NMR analysis of IU decomposition products

Compound B, one of the major components of the IU aqueous solution, is still unknown; hence, we tried to purify it and determine its structure. The purification process is described in Materials and Methods.

¹H-NMR chemical shifts are expressed relative to tetramethylsilane (0.0 ppm).

Table 2. Possible imidazolidinyl urea component candidates

	Compounds with Allantoin skeleton	Compounds with HU skeleton
Suspected molecular weight 158	 1 Allantoin Compound A	
Suspected molecular weight 188	 2 (3-hydroxymethyl-2,5-dioxo-imidazolidine-4-yl)-urea (3-HU) Compound B   3 4 Among which are compound C.	 5 (4-hydroxymethyl-2,5-dioxo-imidazolidine-4-yl)-urea (HU) Compound E
Suspected molecular weight 218		 6 (3,4-bis(hydroxymethyl)-2,5-dioxo-imidazolidine-4-yl)-urea (3,4-BHU) Compound D   7 8 Among which is compound G.
Suspected molecular weight 248		  9 10 Among which is compound F.

Compound B: δ 4.25/4.86 [H-C(9), dd ($J = 10.4$, 10.2 Hz), 2H], 5.20 [H-C(4), d ($J = 6.1$ Hz), H], 5.90 [H₂-N(8), s, 2H], 6.85 [H-N(6), d ($J = 6.0$ Hz), H], and 10.80 [H-N(1), s, H].

The signals of compound B were similar to those in the published data for HU (16), except for the lack of methylene protons, the presence of a proton at the C(4) position, and the breakup of the proton signal at the N(6) position. From the results of MS analysis and ¹H-NMR analysis, compound B was assumed to

be (3-hydroxymethyl-2,5-dioxo-imidazolidine-4-yl)-urea (3-HU) (Fig. 3).

IU composition in the buffers

To determine the decomposition properties of IU in cosmetics and patch test materials, 0.1% IU buffer solutions with three different pH values were incubated at 25°C for 1 month, and the time-dependent changes of the IU components were investigated by HPLC–photodiode array detection (Fig. 4).

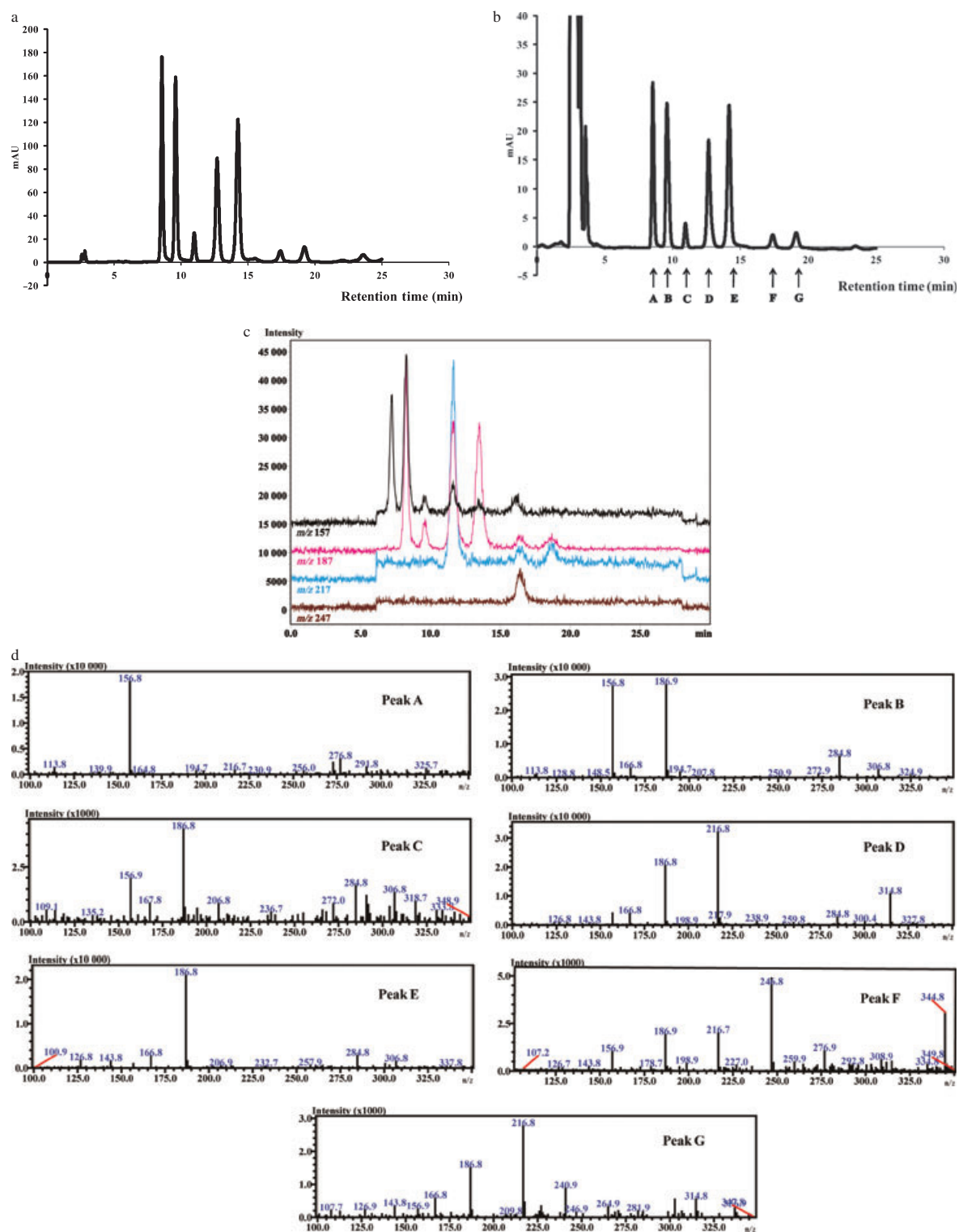


Fig. 1. Imidazolidinyl urea (IU) in the aqueous solution and purchased patch test material. (a) High-performance liquid chromatogram (wavelength = 210 nm) for 0.05% IU in water and (b) samples prepared from 2% IU patch test material (pet.). (c) Mass chromatograms (m/z 157, 187, 217, and 247) and (d) mass spectra of peaks A to G in 2 mg/l IU aqueous solution.

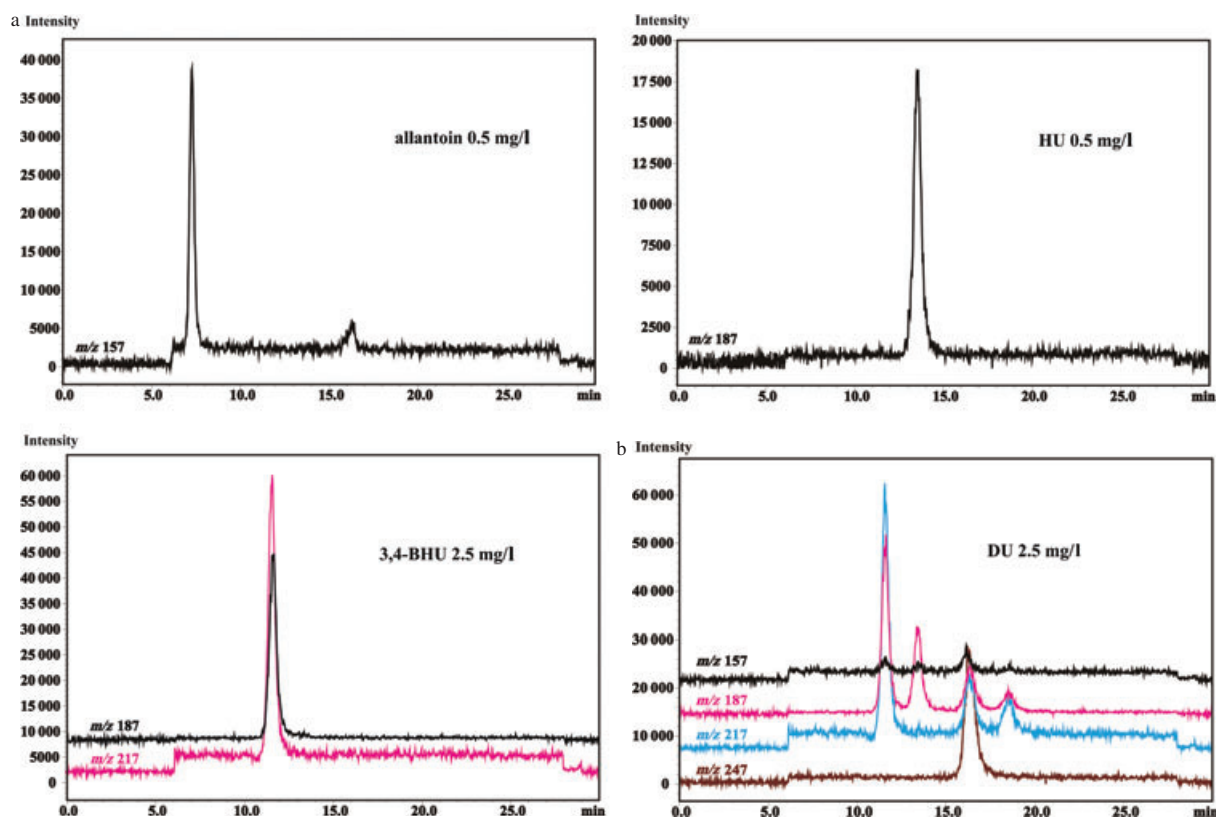


Fig. 2. Liquid chromatography/mass spectrometry analysis of known compounds and diazolidinyl urea (DU) decomposition products. (a) Mass chromatograms of allantoin (m/z 157), (4-hydroxymethyl-2,5-dioxo-imidazolidine-4-yl)-urea (HU) (m/z 187), and (3,4-bis-hydroxymethyl-2,5-dioxo-imidazolidine-4-yl)-urea (3,4-BHU) (m/z 187 and 217). (b) Mass chromatograms (m/z = 157, 187, 217, and 247) of 2.5 mg/l DU aqueous solution, prepared from 0.5% DU in water kept for 1 month at 10°C.

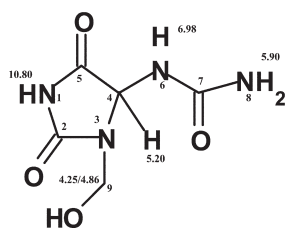


Fig. 3. Nuclear magnetic resonance assignment of (3-hydroxymethyl-2,5-dioxo-imidazolidine-4-yl)-urea.

At pH 4.0, we observed only a slight time-dependent increase in the amounts of allantoin and HU. The amounts of compounds C, F and G gradually decreased over time. IU decomposition seemed to occur more rapidly at pH 6.5, and reached equilibrium on day 5. However, all of the peaks, other than those for 3,4-BHU and HU, continued to decrease until day 30 at pH 9.0. On the second day, 3,4-BHU reached equilibrium, and HU still increased at day 30.

IU composition in cosmetics

HPLC–photodiode array detection analysis showed background noise caused by coeluting compounds; hence, LC/MS was used to investigate the decomposition properties of IU in the purchased cosmetics. In the scan mode analysis of Back to The Basics Jasmine Body Lotion, we could confirm all IU-derived peaks and mass spectra (data not shown). For the SIM analysis, we selected m/z 157, 187, 217 and 247 to detect deprotonated ions of peaks A to G. A representative chromatogram is shown in Fig. 5a. In the 18 purchased cosmetics, allantoin, HU and 3,4-BHU covered more than 90% of the total peak area (Fig. 5b).

Discussion

The present study showed that there were at least seven IU-derived compounds in the samples prepared from IU aqueous solutions, cosmetics, and patch test materials. Our results suggest that IU is primarily converted to

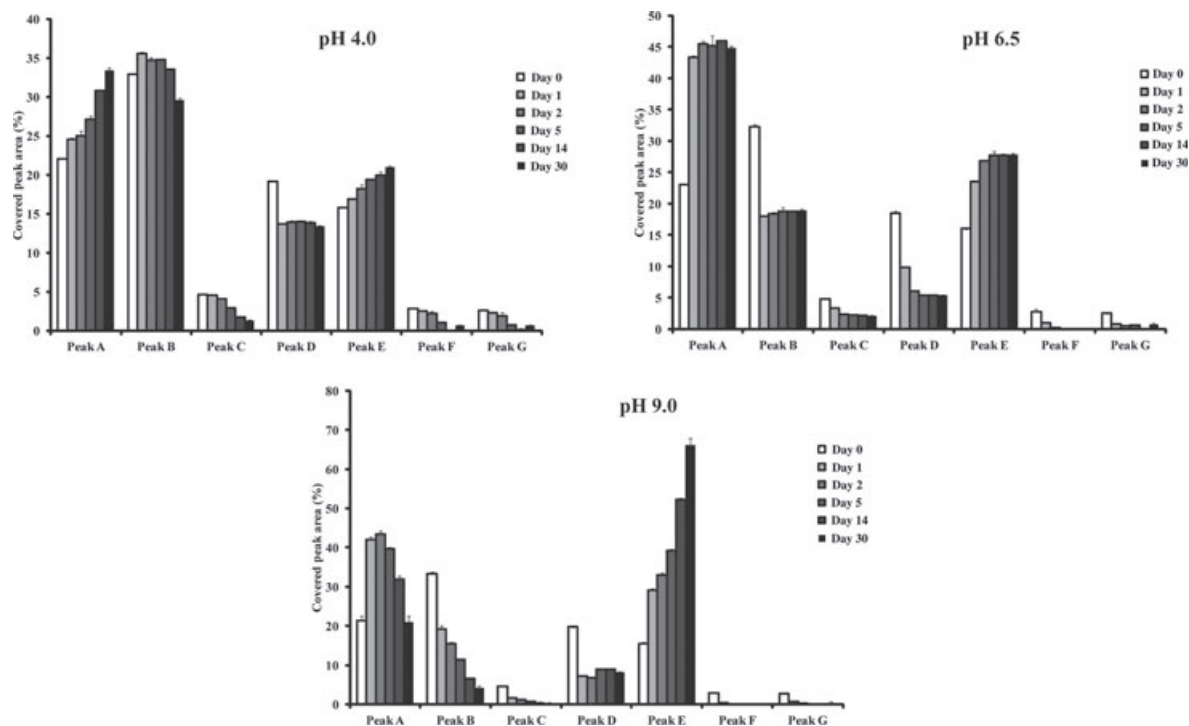


Fig. 4. Decomposition of imidazolidinyl urea (IU) dissolved in buffers. Time-dependent changes in the ratio of peak area to total peak area are shown. Each value represents the mean $\pm$ standard deviation ($n = 3$). IU solutions of 20 mmol/l ammonium acetate (the pH value was adjusted to 4.0, 6.5 or 9.0 with acetic acid or aqueous ammonia) were prepared at a concentration of 0.1% and incubated at 25°C for 30 days. Sampling was performed on days 1, 2, 5, 15, and 30. For the high-performance liquid chromatography–photodiode array detection conditions, see Materials and Methods.

allantoin (compound **1**), HU (compound **5**) and 3,4-BHU (compound **6**) when the preservative is blended in cosmetics. We also found that four of the seven compounds – compounds D to G – were identical to the decomposition products of DU aqueous solutions. HU and 3,4-BHU are both major components of IU/DU-blended cosmetics; therefore, we believe it is reasonable to assume that these compounds play an important role as causative agents in the cross-reactivity between DU and IU.

Several patch test reports have shown higher cross-reactive ratios between IU and DU than for other formaldehyde-releasers (11, 13, 15), and have implied the presence of an allergen common to IU/DU. A recent report showed that HU was a component of both IU and DU (6). HU was viewed as an attractive potential causative agent for the cross-reaction between IU and DU; however, HU was estimated to cover only 10% of IU and 40% of DU, and the other portions of IU/DU remained unknown at that time (6). As we have previously shown that most of the DU blended in cosmetics is present as HU and 3,4-BHU (16), in this study we focused on the chemistry of IU.

In the HPLC and LC/MS studies, IU aqueous solutions and patch test materials showed similar IU compositions

for the seven compounds. LC/MS data also indicated that the seven compounds consisted of allantoin and its formaldehyde adducts. In the aqueous solutions and patch test materials, IU was primarily composed of allantoin, HU, 3,4-BHU, and 3-HU (compound **3**).

A notable characteristic of these seven compounds was that the elimination of formaldehyde units was observed in the ESI mass spectrum, except for allantoin and HU, where releasable hydroxymethyl groups are absent. The seven IU-derived compounds are divided into two groups by their fragmentation patterns. In the group of compounds A to C, an ion was observed at m/z 157 in the MS spectrum (Fig. 2b, c). In the other group, compounds D to G, an ion was rarely observed at m/z 157. The difference can be assumed to be related to the structure of the identified compounds, that is, whether the compounds have a directly bonded proton at the imidazolidine carbon atom; or, in other words, whether the compound has the allantoin skeleton or the HU skeleton.

To investigate what happens to the IU compounds in cosmetics, we traced the change of composition in the buffers after long-term incubation at 25°C. The ratio of HU-skeleton compounds increased during incubation

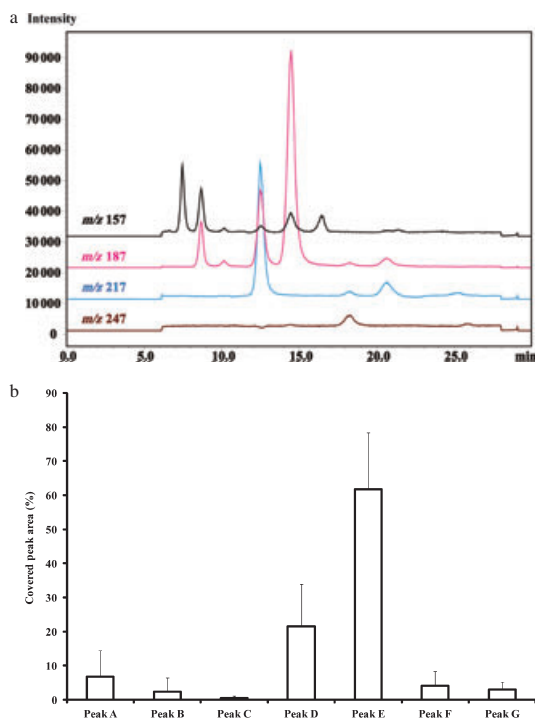


Fig. 5. Liquid chromatography/mass spectrometry analysis of purchased cosmetic samples. (a) The representative selected ion monitoring chromatogram (m/z 157, 187, 217, and 247) of the cosmetic samples. (b) Mean ratio of peak area to total peak area of the cosmetic samples (m/z 157 for peak A; m/z 187 for peaks B, C, and E; m/z 217 for peaks D and G; m/z 247 for peak F). Each value represents the mean + standard deviation ($n = 18$).

at pH 9.0, although the ratio of compounds with the allantoin skeleton and HU skeleton was constant 1 month after preparation in the buffers at pH 4.0 and 6.5. At

pH 9.0, the released formaldehyde is thought to react with allantoin-skeleton compounds to form the HU skeleton. The composition of IU in the cosmetics was very different from that in the IU aqueous solutions and patch test materials. The most remarkable difference was the ratio of allantoin-skeleton compounds and HU-skeleton compounds. Blended IU was predominantly present as HU-skeleton compounds in the cosmetics, whereas the IU compounds in the aqueous solutions and patch test materials had a nearly equal ratio of HU-skeleton and allantoin-skeleton compounds. DU, which consists of HU-skeleton compounds, has been reported to be prepared by reacting allantoin with formaldehyde under basic conditions (19). A similar reaction may occur in the basic buffer and cosmetics containing IU. As a result, IU in cosmetics is thought to be composed primarily of HU and 3,4-BHU.

In this study, we have demonstrated that both HU and 3,4-BHU are major components of IU/DU-blended cosmetics. The unidentified minor components cannot be ignored, but HU and 3,4-BHU are the best possible candidates for causing the cross-reactivity between IU and DU. Several statistical patch test studies imply a common allergen rather than released formaldehyde. Further research on the decomposition products of DU and IU would clarify whether there is a common allergen and what acts as the common allergen for DU/IU contact dermatitis.

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References

- de Groot A C, Veenstra M. Formaldehyde-releasers in cosmetics in the USA and in Europe. *Contact Dermatitis* 2010; **62**: 221–224.
- de Groot A C, White I R, Flyvholm M A, Lensen G, Coenraads P J. Formaldehyde-releasers in cosmetics: relationship to formaldehyde contact allergy. Part 1. Characterization, frequency and relevance of sensitization, and frequency of use in cosmetics. *Contact Dermatitis* 2010; **62**: 2–17.
- de Groot A C, Flyvholm M A, Lensen G, Menné T, Coenraads P J. Formaldehyde-releasers: relationship to formaldehyde contact allergy. Contact allergy to formaldehyde and inventory of formaldehyde-releasers. *Contact Dermatitis* 2009; **61**: 63–85.
- Kajimura K, Doi T, Tagami T, Taguchi S. The release of formaldehyde upon decomposition of imidazolidinyl urea. *J Jpn Cosmet Sci Soc* 2010; **34**: 7–13.
- Emeis D, Anker W, Wittern K P. Quantitative ^{13}C NMR spectroscopic studies on the equilibrium of formaldehyde with its releasing cosmetic preservatives. *Anal Chem* 2007; **79**: 2096–2100.
- Lehmann S V, Hoeck U, Breinholdt J, Olsen C E, Kreilgaard B. Characterization and chemistry of imidazolidinyl urea and diazolidinyl urea. *Contact Dermatitis* 2006; **54**: 50–58.
- Rietschel R L, Warshaw E M, Sasseville D et al. Sensitivity of petrolatum and aqueous vehicles for detecting allergy to imidazolidinylurea, diazolidinylurea, and DMDM hydantoin: a retrospective analysis from the North American Contact Dermatitis Group. *Dermatitis* 2007; **18**: 155–162.
- Jong C T, Statham B N, Green C M et al. Contact sensitivity to preservatives in the UK, 2004–2005: results of multicentre study. *Contact Dermatitis* 2007; **57**: 165–168.
- Warshaw E M, Buchholz H J, Belsito D V et al. Allergic patch test reactions associated with cosmetics: retrospective analysis of cross-sectional data from the North American Contact Dermatitis

- Group, 2001–2004. *J Am Acad Dermatol* 2009; **60**: 23–38.
- 10 Zug K A, Warshaw E M, Fowler J F Jr et al. Patch-test results of the North American Contact Dermatitis Group 2005–2006. *Dermatitis* 2009; **20**: 149–160.
 - 11 Statham B N, Smith E V, Bodger O G et al. Concomitant contact allergy to methylchloroisothiazolinone/methylisothiazolinone and formaldehyde-releasing preservatives. *Contact Dermatitis* 2010; **62**: 56–57.
 - 12 Thyssen J P, Engkilde K, Lundov M D, Carlsen B C, Menné T, Johansen J D. Temporal trends of preservative allergy in Denmark (1985–2008). *Contact Dermatitis* 2010; **62**: 102–108.
 - 13 Lundov M D, Johansen J D, Carlsen B C, Engkilde K, Menné T, Thyssen J P. Formaldehyde exposure and patterns of concomitant contact allergy to formaldehyde and formaldehyde-releasers. *Contact Dermatitis* 2010; **63**: 31–36.
 - 14 Garcia-Gavin J, Gonzalez-Vilas D, Fernandez-Redondo V, Toribo J. Allergic contact dermatitis in a girl due to several cosmetics containing diazolidinyl-urea or imidazolidinyl-urea. *Contact Dermatitis* 2010; **63**: 49–50.
 - 15 de Groot A, White I R, Flyvholm M A, Lensen G, Coenraads P J. Formaldehyde-releasers in cosmetics: relationship to formaldehyde contact allergy. Part 2. Patch test relationship to formaldehyde contact allergy, experimental provocation tests, amount of formaldehyde released, and assessment of risk to consumers allergic to formaldehyde. *Contact Dermatitis* 2010; **62**: 18–31.
 - 16 Doi T, Kajimura K, Taguchi S. The different decomposition properties of diazolidinyl urea in cosmetics and patch test materials. *Contact Dermatitis* 2011; **65**: 81–91.
 - 17 Emeis D, de Groot A C, Brinkmann J. Determination of formaldehyde in formaldehyde-releaser patch test preparations. *Contact Dermatitis* 2010; **63**: 57–62.
 - 18 Doi T, Kajimura K, Takatori S, Fukui N, Taguchi S, Iwagami S. Simultaneous measurement of diazolidinyl urea, urea, and allantoin in cosmetic samples by hydrophilic interaction chromatography. *J Chromatogr B Analyt Technol Biomed Life Sci* 2009; **877**: 1005–1010.
 - 19 No authors listed. Final Report on the safety assessment of diazolidinyl urea. *J Am Coll Toxicol* 1990; **9**: 229–245.