

Characterization and chemistry of imidazolidinyl urea and diazolidinyl urea

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For several decades, the cosmetic preservatives imidazolidinyl urea (IU) and diazolidinyl urea (DU) have not only been poorly characterized but have also had misleading chemical structures assigned to them. The most common trade names of IU and DU are Germall 115 and Germall II, respectively. This publication gives an insight into what these 2 well-known contact allergens consist of and their degradation patterns. Approximately, 30–40% of both products can be characterized by mixtures of allantoin (synthetic starting material), (4-hydroxymethyl-2,5-dioxo-imidazolidin-4-yl)-urea (compound HU) and presumably 1-(3,4-bis-hydroxymethyl-2,5-dioxo-imidazolidin-4-yl)-1,3-bis-hydroxymethyl-urea (compound BHU). A full chemical characterization of compound HU is shown. The remaining part of both IU and DU are believed to be polymers of allantoin-formaldehyde condensation products. The analytical methods used to characterize IU and DU are capillary electrophoresis and nuclear magnetic resonance and mass spectroscopy studies.

Key words: (4-hydroxymethyl-2,5-dioxo-imidazolidin-4-yl)-urea; capillary electrophoresis; diazolidinyl urea; Germall 115; Germall II; imidazolidinyl urea. © Blackwell Munksgaard, 2006.

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Antimicrobial preservatives are widely used in the formulation of pharmaceutical creams and ointments as well as in cosmetics and toiletries. Several of these preservatives are known to cause contact dermatitis in patients using the products (1). A special class of preservatives is known as formaldehyde-releasing agents, and they may exhibit their action by releasing formaldehyde or by action of the parent chemical structure of the compounds. The most prominent of these compounds are 1) diazolidinyl urea (DU); 2) imidazolidinyl urea (IU); 3) quaternium-15; 4) dimethylol dimethyl (DMDM) hydantoin; and 5) bronopol.

It is of importance to know the chemical structure of the compounds in order to understand their role in contact dermatitis but also to evaluate the presence of the compounds in today's marketed consumer products as well as in the patch test materials used for testing of patients with suspected contact dermatitis. This study was undertaken in order to standardize TRUE Test patches with the 2 allergens and to provide the documentation needed to apply for marketing authorization with the health authorities both in Europe and in USA.

The 2 preservatives can be purchased from different companies under a variety of trade names (Table 1). They have been used as additives alone or in combination with other preservatives worldwide since 1970s (2–4) (IU) and 1980s (5) (DU).

The first report on IU and DU can be found in a Sutton Laboratories patent from 1966 (6) with the claim to have invented the synthesis of a series of non-specific allantoin-formaldehyde condensation products. Among them are IU and DU. IU was first reported in 1970 (3) as the first of a family of substituted IU compounds. DU was reported in 1982 (7), and today both compounds are produced by several companies (Table 1). Despite the age of these compounds, they both lack proof of the assigned chemical structures, which is the background of this publication.

IU was described in 1971 as a preservative used in the cosmetic industry (3), and Germall 115 was adopted as IU by Cosmetic, Toiletry and Fragrance Association (CTFA) in 1973 (5). In 1982 (7), DU was introduced as Germall II. Since 1970, IU has been associated with the

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Table 1. Producers of imidazolidinyl urea and diazolidinyl urea

Trade name	Company	Product
Germall 115	ISP Sutton Laboratories, NJ, USA	Imidazolidinyl urea
Germall II	ISP Sutton Laboratories, NJ, USA	Diazolidinyl urea
Nipa Biopure100	NIPA Laboratories, Deeside, UK	Imidazolidinyl urea
Unicide U-13	Induchem, Volketswil, Switzerland	Imidazolidinyl urea
Unidiacide U-26	Induchem, Volketswil, Switzerland	Diazolidinyl urea
Protacide U-13	Protameen, NJ, USA	Imidazolidinyl urea
Umid IU	Universal Preserv-A-Chem, NJ, USA	Imidazolidinyl urea
Chemynol I	Chemunion, Sao Paulo, Brazil	Imidazolidinyl urea
Jeecide U-13	Jeen International, NJ, USA	Imidazolidinyl urea
Sepicide CI	Seppic, NJ, USA	Imidazolidinyl urea
Imidazolidinyl urea A15	Akema, Coriano di Rimini, Italy	Imidazolidinyl urea
Preservative A2	Akema, Coriano di Rimini, Italy	Diazolidinyl urea

structure of IU or imidurea The United States Pharmacopeia–National Formulary (USP/NF) with the molecular weight of 406.33 and the formula of $C_{11}H_{16}N_8O_8H_2O$ (8–19). In the same manner, Germall II is associated with the structure of DU with the molecular weight of 278.23 and the formula of $C_8H_{14}N_4O_7$ (7, 9, 10, 19, 20). See Fig. 1 for the chemical structures adopted by United States Adopted Names (USAN). Several attempts of chromatographic High Performance Liquid Chromatography and Thin Layer Chromatography (HPLC and TLC), fluorometric and electrophoretic [capillary electrophoresis (CE)] analysis have been made on both IU (11–17) and DU (19, 20). In several cases, it has been noticed that IU yielded 2 TLC spots (14) or 2 or more HPLC peaks but without further detailed studies (16).

Using a colorimetric method that selectively determines free formaldehyde (8), the kinetics was studied for degradation of IU in aqueous

solutions (8). At pH 7 and at 37°C, a half-life of 7 s for liberation of 1 mole of formaldehyde was reported while lower degradation rates were found at lower pH values. An interesting observation made during the study was that even if it, from the structures of IU, was expected that the compound could release 2 mol of formaldehyde, only release of 1.5 mol was found.

Materials and Methods

Capillary electrophoresis

All CE analysis was carried out on an Agilent HP^{3D} instrument with CHEMSTATION CE software. The capillary was an Agilent Extended Light Path Capillary (bare fused silica) with 48.5/40.0 cm × 50 µm inner diameter (model G1600-60232). The run buffer was 25 mM borate buffer (pH 9.3) diluted from 50 mM borate buffer (pH 9.3) purchased from Agilent Technologies, Naerum, Denmark. The CE settings were as follows: 25°C, 20 kV, ultraviolet (UV) detection at 200 nm and injections of 50 mbar for 5.0 s. Preconditioning was 1.5 min with run buffer. Daily conditioning was 15 min with 0.1 N NaOH followed by 2 min with water.

Test substances

The IU and DU used were Germall 115 and Germall II, respectively. The chemicals were used as received. For analysis of raw materials of IU and DU, 50 mg was dissolved in 50 ml of 5 mM phosphate buffer (pH 2.5) and an aliquot of 2.0 ml was diluted to 20 ml with the same buffer to give a 100 µg/ml sample test solution.

During analysis of cosmetics and toiletries, approximately 100 mg was suspended in 10 ml of 5 mM phosphate buffer (pH 2.5). After

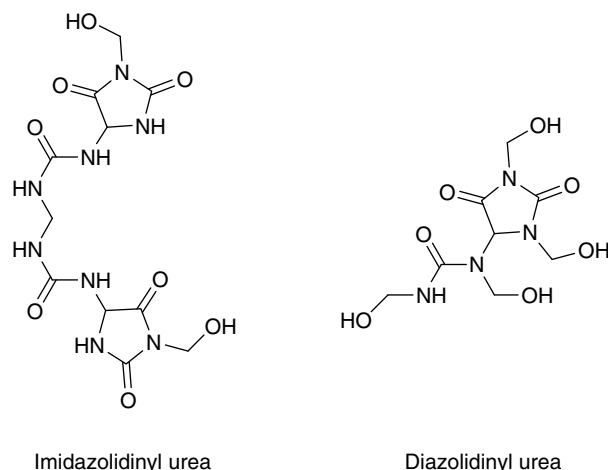


Fig. 1. Conventional chemical structure of imidazolidinyl urea and diazolidinyl urea as stated in Chemical Abstracts Service (CAS).

10 min of stirring, the solution was filtered (0.45 μ m) prior to injection.

Results

Characterization of imidazolidinyl urea

A straightforward CE analytical system was developed. The run buffer is borate at pH 9.3 and samples are dissolved in phosphate buffer (pH 2.5) due to an acceptable stability at low pH for both IU and DU. At pH 9.3, the studied compounds migrate in a combination of the electroosmotic flow (EOF) and electrophoretic mobility due to their negative charge at pH 9.3 (e.g. all observed peaks migrate after the EOF). Apart from minor peaks, IU seems to be characterized by 4 peaks, and the 2 dominant peaks observed in CE analysis of IU have been identified by use of spiking experiments, nuclear magnetic resonance (NMR) and mass spectroscopy (MS) technologies. The 2 less dominant peaks have not been identified. The first and major eluting peak of IU has been identified as (4-hydroxymethyl-2,5-dioxo-imidazolidin-4-yl)-urea (compound HU). See electropherogram in Fig. 2 and structure in Fig. 3.

The compound HU was isolated from a degraded solution of DU because the only end degradation product of DU was compound HU. A solution of DU in aqueous base (30 mg/ml in 0.2 M NH_3) was monitored until only compound

HU was observed. After this base degradation (2 h of stirring) of DU, the solution was freeze-dried and the remaining powder was extracted with ethanol (150 ml), evaporated, dissolved in water (1.2 ml) and injected (100 μ l injections) in a preparative HPLC system (Phenomenex Luna 5 μ , NH_2 , 100 Å, 100 \times 10.0 mm). Peaks of compound HU were collected and freeze-dried prior to ^1H -, ^{13}C - and ^{15}N -NMR and high-resolution MS (HR-MS) analyses.

NMR data of compound HU (Fig. 4)

^1H - and ^{13}C -NMR chemical shifts were referenced to Tetra Methyl Silyl (TMS) (0.0 p.p.m.) and ^{15}N chemical shifts to nitromethane (340 p.p.m.).

^1H -NMR: δ 3.39/3.48 [H-C(9), both d (J = 10.6 Hz), 2H], 5.68 (H_2 -N(8), s, 2H), 6.61 (H-N(6), s, 1H), 7.78 (H-N(3), s, 1H) and 10.23 (H-N(1), s, 1H).

^{13}C -NMR: δ 63.8 [C(9)], 72.8 [C(4)], 156.5 [C(7)] and 156.9 [C(2)] and 174.1 [C(5)].

^{15}N -NMR (obtained from 2D ^1H , ^{15}N -HSQC and HMBC data): δ 74 [N(8)], 89 [N(6)], 102 [N(3)] and 145 [N(1)]. These NMR data confirm the suggested structure. The solvent used was dimethyl sulfoxide and the field was 400 MHz.

MS data of compound HU

Using HR-MS analysis with the use of the MS Fast Atom Bombardment (FAB) technique, a

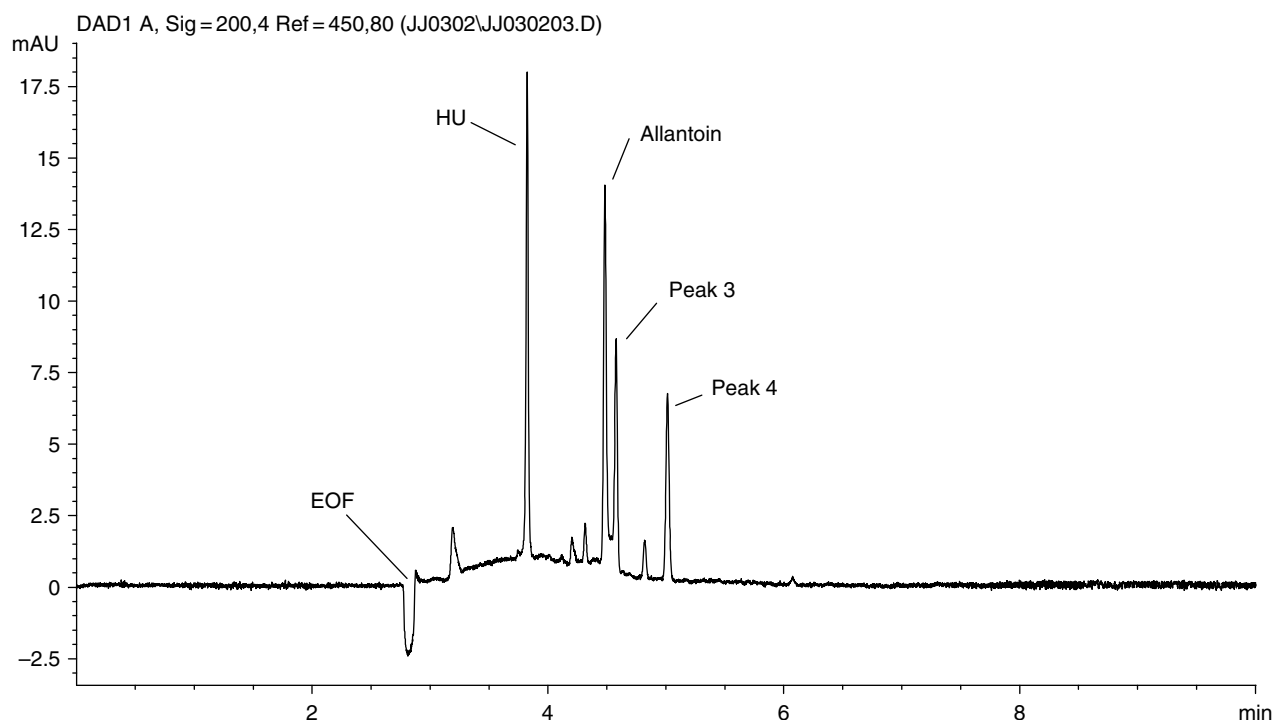


Fig. 2. Capillary electrophoresis electropherogram of imidazolidinyl urea. For conditions, see *Materials and Methods*. HU, (4-hydroxymethyl-2,5-dioxo-imidazolidin-4-yl)-urea; EOF, electroosmotic flow.

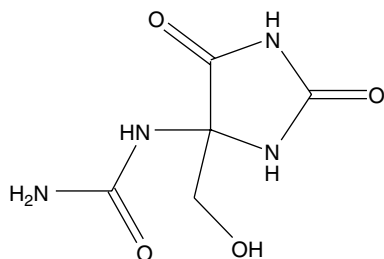


Fig. 3. Chemical structure of (4-hydroxymethyl-2,5-dioxo-imidazolidin-4-yl)-urea (compound HU).

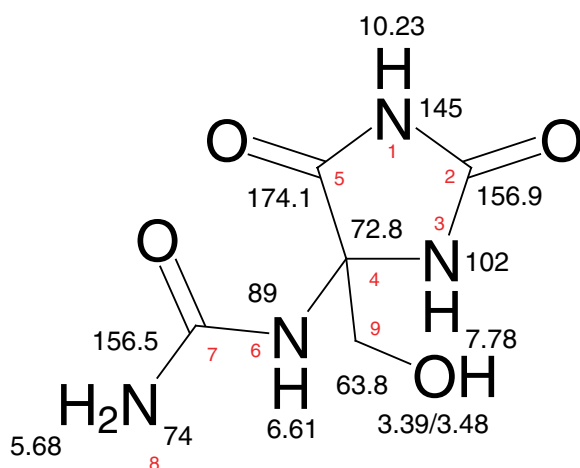


Fig. 4. Nuclear magnetic resonance assignments of (4-hydroxymethyl-2,5-dioxo-imidazolidin-4-yl)-urea (compound HU).

mass of 189.0615 for $M + H$ was determined. Compared with the theoretical value of 189.0624 for $C_5H_9N_4O_4$, the derivation is -5 p.p.m., which is within the variance of the method. In the Electrospray Ionisation (ESI) spectra, the $M + Na$ at 211 is seen in a positive mode and $M-H$ at 187 in a negative mode. The conclusion is a convincing correlation between these MS data and the proposed structure of compound HU.

The combined conclusion on these NMR and HR-MS data is strongly pointing towards the proposed structure of compound HU.

The second peak of IU has been identified as allantoin (Fig. 2). Allantoin is the starting material for the manufacturing of both IU and DU. The proof of presence of allantoin in IU has been established by electrophoretic spiking; for example, a sample (100 $\mu\text{g/ml}$) originating from a commercially available product of allantoin (Sigma, Broendby, Denmark) has been mixed with a sample of IU (100 $\mu\text{g/ml}$) resulting in a relative increase in the area of the allantoin peak. The Sigma product of allantoin has been studied using ^1H -, ^{13}C - and ^{15}N -NMR to confirm the structure.

NMR data of allantoin

^1H -resonances: 5.23 (s, 1H), 5.80 (s, 2H), 6.90 (s, 1H) 8.06 (s, 1H) and 10.53 (s, 1H); ^{13}C -resonances: 62.3, 156.7, 157.3 and 173.5; ^{15}N -resonances: 76, 90, 101 and 146. These NMR data confirm the structure of allantoin (Fig. 5).

The 2 dominant peaks in the electropherogram (Fig. 2) are followed by 2 less dominant peaks (peaks 3 and 4) and neither has been isolated or identified. It is suggested that these 2 compounds are compound HU and allantoin attached with molecules of formaldehyde (hydroxymethyl groups). This formaldehyde is likely to be released during basic degradation as the area of the peaks of these compounds (peaks 3 and 4) decreases proportionally with the increase of the area of the peaks of both compound HU and allantoin (see Fig. 6 for degradation at 25°C in phosphate buffer at pH 7).

The relative UV response factor between allantoin and HU has been determined to be 2.1, meaning allantoin absorbs 2.1 times more UV light at 200 nm than HU. The response factor was determined by a combined CE and NMR experiment: in a base degraded solution of IU (deuterated water), only 2 peaks were observed in the CE electropherogram with equal areas. The 2 peaks were HU and allantoin. A quantitative ^1H -NMR study of this sample showed that there was twice the amount of HU compared with allantoin. This was done by monitoring the proton signal (singlet) of the terminal $-\text{NH}_2$ of both compounds ($= 5.68$ and 5.80).

Given the relative UV response of HU, the content of HU and allantoin in Germall 115 could be determined to be 10% and 20%, respectively. The combined area of peaks 3 and 4 is 1/3 of the total area, which may correspond to approximately 10% of the mass of IU. This explains an approximate 40% of the mass of IU.

Characterization of diazolidinyl urea

The same CE system as the above-mentioned is used to monitor the content of DU. Again, the compounds of DU migrate in a combination of

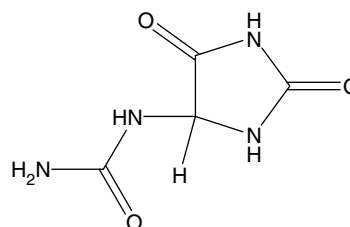


Fig. 5. Chemical structure of allantoin.

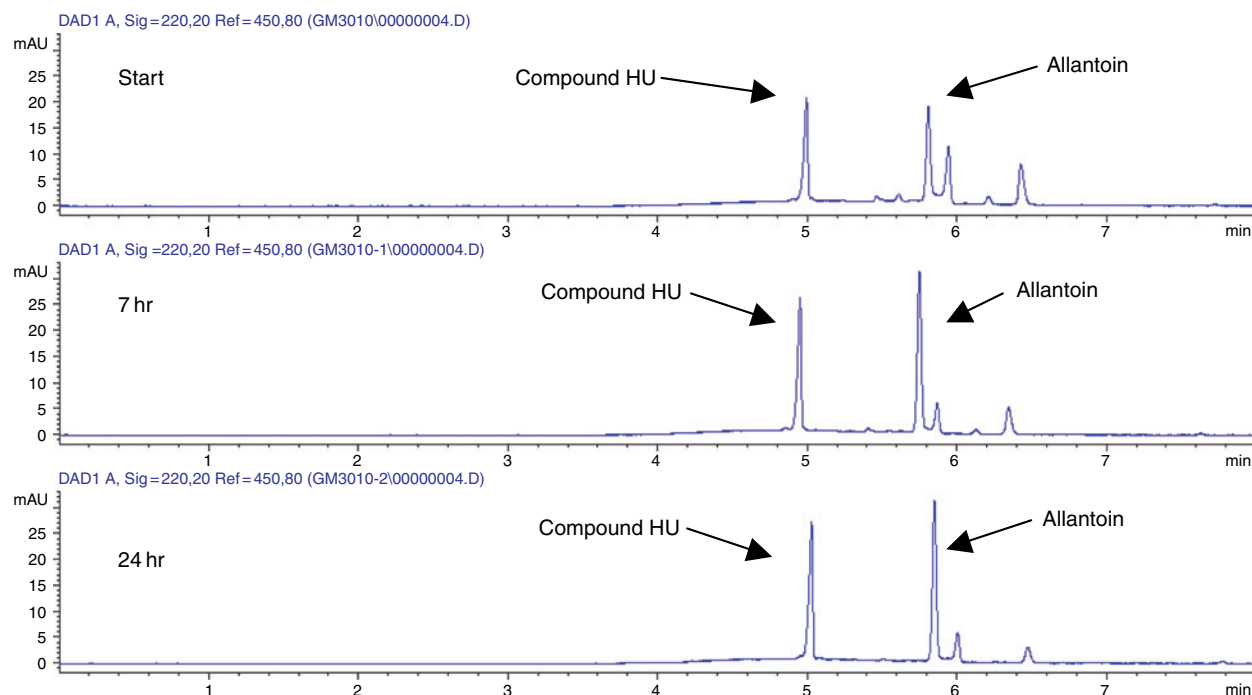


Fig. 6. Capillary electrophoresis electropherograms of imidazolidinyl urea dissolved in water (pH 7.0) initially, after 7 hr and after 24 hr. For conditions, see *Material and Methods*. HU, (4-hydroxymethyl-2,5-dioxo-imidazolidin-4-yl)-urea.

the EOF and electrophoretic mobility due to their negative charge at pH 9.3 (e.g. all observed peaks migrate after the EOF). In addition to several minor peaks, the electropherogram of DU displays 1 major peak that is responsible for around 50% of the total area (Fig. 7). The structural assignment of this major peak is highly tentative and is based on both indirect NMR and HR-MS evidence. The NMR data indicate the presence of a total of 4 hydroxymethyl groups of which 1 is attached to a carbon atom and 3

to a nitrogen atom. The structure is also consistent with fragments in the MS about 247, 216 and 185. The proposed structure can be seen in Fig. 8 and it is compound BHU with 3 hydroxymethyl groups attached, 1 at each of 3 nitrogens. The chemical name is 1-(3,4-bis-hydroxymethyl-2,5-dioxo-imidazolidin-4-yl)-1,3-bis-hydroxymethyl-urea (BHU), and the structure must be considered as our current best guess as we have not been able to isolate the compound. The end degradation product observed during base

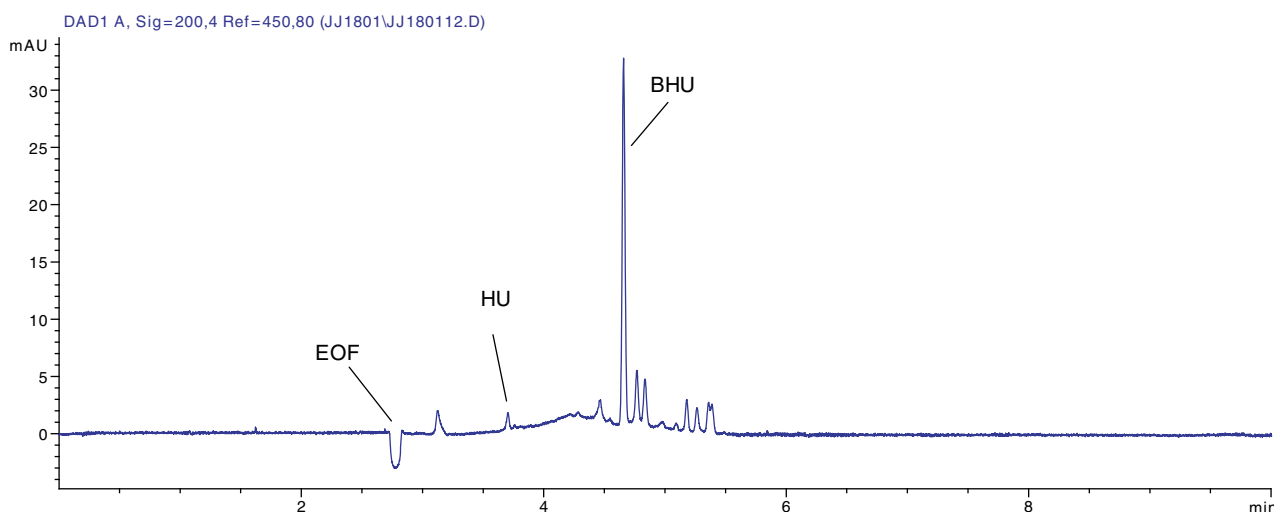


Fig. 7. Capillary electrophoresis electropherogram of diazolidinyl urea. For conditions, see *Materials and Methods*. HU, (4-hydroxymethyl-2,5-dioxo-imidazolidin-4-yl)-urea; EOF, electroosmotic flow; BHU, 1-(3,4-bis-hydroxymethyl-2,5-dioxo-imidazolidin-4-yl)-1,3-bis-hydroxymethyl-urea.

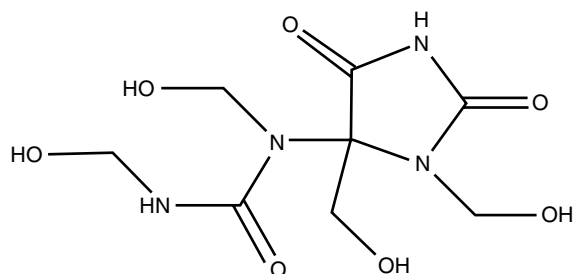


Fig. 8. Chemical structure of compound 1-(3,4-bis-hydroxymethyl-2,5-dioxo-imidazolidin-4-yl)-1,3-bis-hydroxymethyl-urea (BHU), the proposed main component of diazolidinyl urea (DU).

degradation of DU is compound HU. This complies with the structure of BHU because in theory the 3 nitrogen-attached hydroxymethyl groups could be released during base degradation of DU, yielding HU. The stepwise degradation at 25°C (25 mM phosphate buffer at pH 7.0) can be studied in Fig. 9.

A sample (100 µg/ml) of DU dissolved in 25 mM borate buffer (pH 9.3) was monitored (CE analysis) to complete conversion to the degradation product HU. Also, minor peaks seem to degrade to compound HU. HU was then quantified using an allantoin standard (Sigma 95% purity), and 35% of the mass could be accounted for when the above calculated response factor of HU was taken into account.

Analysis of cosmetics containing Germall II or Germall 115

The following describes CE analysis of cosmetic products that according to their labels contain IU or DU. 5 different commercially available cosmetics, all claiming to contain DU, have been investigated. The cosmetic products are as follows:

- (1) Anti-Wrinkle Q10 Day Cream from Nivea,
- (2) Revitalift Day from L'Oréal,
- (3) Cleansing Milk from Australian Bodycare, Tea Tree Oil,
- (4) Modern SkinCare from Elisabeth Arden,
- (5) Sheer Blonde from John Frieda.

Upon CE analysis, all these products contained the compound HU and small or undetectable amounts of compound BHU indicating that DU has been partly or fully degraded to compound HU during manufacturing and/or storage. A typical example from the analysis of the formulations is shown in Fig. 10.

5 different international and national commercial cosmetics, all claiming to contain IU, have also been investigated. The cosmetic products are as follows:

- (1) Nutrilift from L'Oréal,
- (2) Synergie Wrinkle Lift A from Garnier,

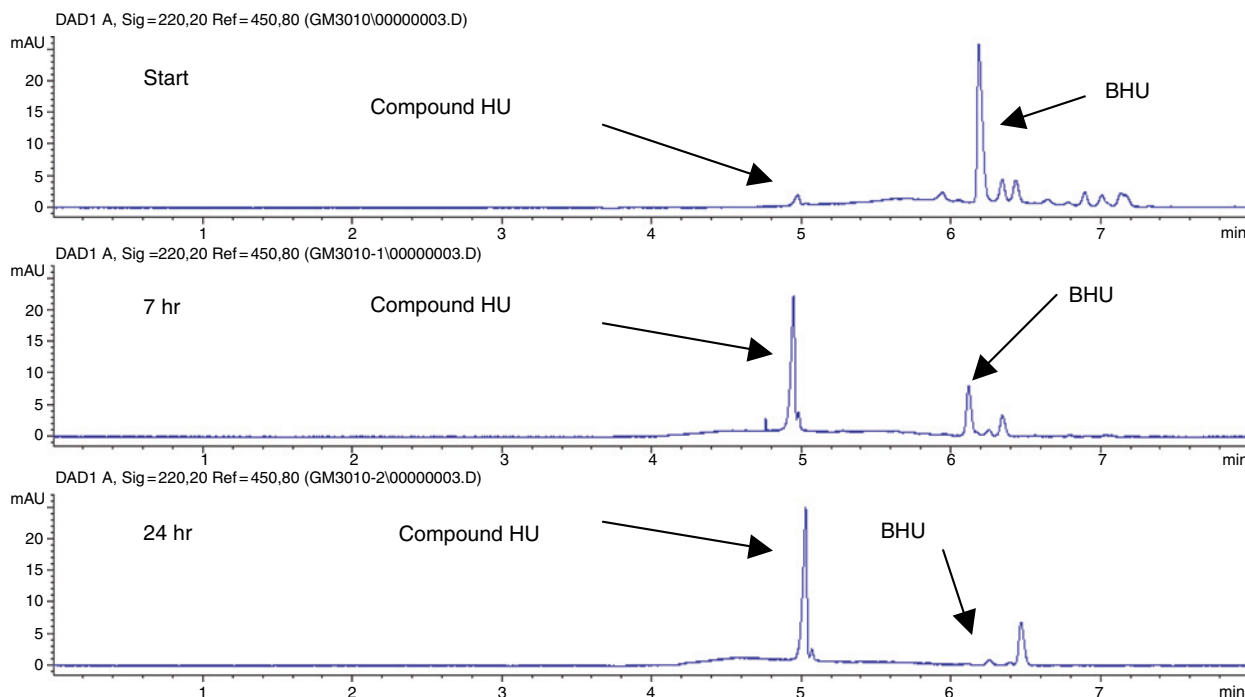


Fig. 9. Capillary electrophoresis electropherograms of diazolidinyl urea dissolved in water (pH 7.0) initially, after 7 hr and after 24 hr. For conditions, see *Material and Methods*. HU, (4-hydroxymethyl-2,5-dioxo-imidazolidin-4-yl)-urea; BHU, 1-(3,4-bis-hydroxymethyl-2,5-dioxo-imidazolidin-4-yl)-1,3-bis-hydroxymethyl-urea.

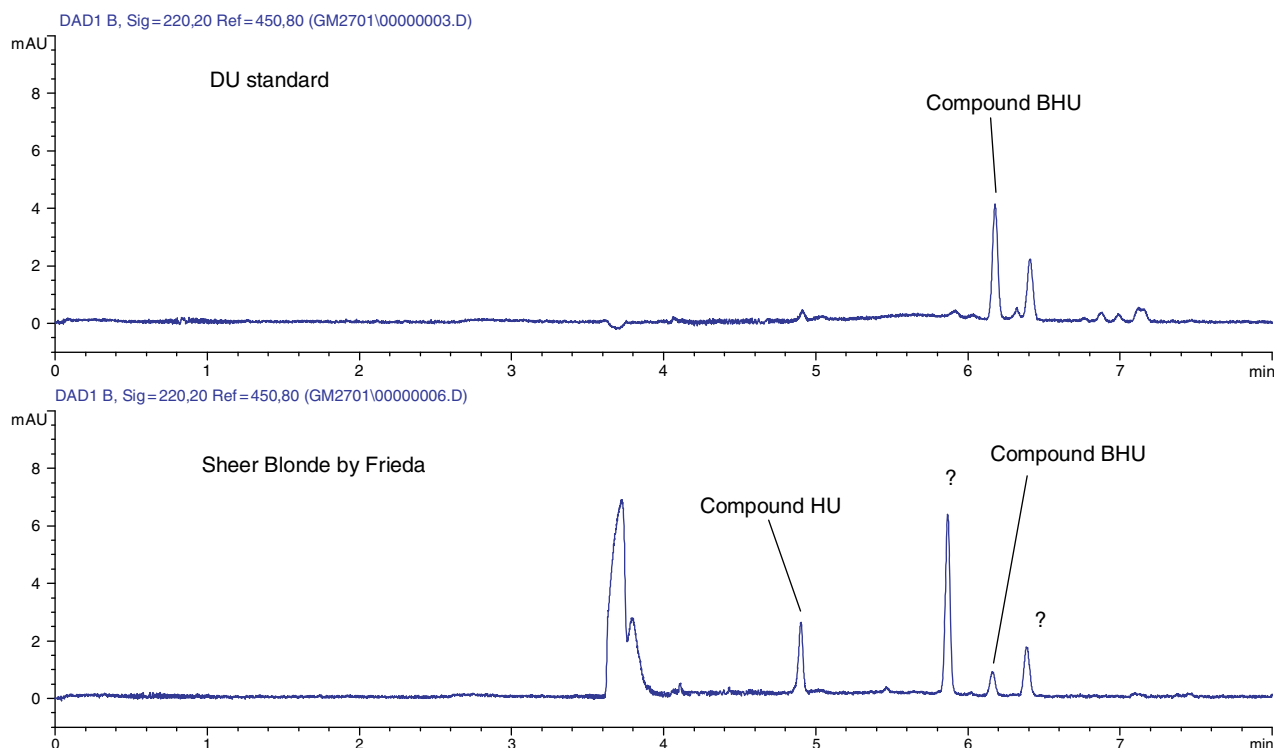


Fig. 10. Capillary electrophoretic analysis of hair shampoo product Sheer Blonde by Frieda compared with a diazolidinyl urea (DU) standard. For conditions, see *Material and Methods*. HU, (4-hydroxymethyl-2,5-dioxo-imidazolidin-4-yl)-urea; BHU, 1-(3,4-bis-hydroxymethyl-2,5-dioxo-imidazolidin-4-yl)-1,3-bis-hydroxymethyl-urea.

- (3) Vitaminbalsam from Matas,
- (4) Exceptionally clean shampooing from Clinique hair care,
- (5) Hydra Care Cream from Plaisir.

Upon CE analysis, all these products contained the combination of allantoin, compound HU and 1 or 2 of the unknown peaks, peak 3 and peak 4, strongly indicating the presence of IU. An example of a CE analysis is shown in Fig. 11. All the above analyses of cosmetic products that contain IU or DU were subsequently spiked with both IU and DU standard material to confirm the identity of the peaks observed.

Discussion

The structures currently associated with IU and DU is most likely based upon the molar ratios between allantoin and formaldehyde used in manufacturing of the 2 compounds as well as the elemental analysis of the products. The content of nitrogen is approximately 27% for IU and 20% for DU. The purpose of developing the products was to obtain compounds that upon hydrolysis could release formaldehyde which in turn had a well-known antimicrobial activity.

This study clearly shows that IU nor DU can be explained by a single chemical structure but are compounds of a complex composition as illustrated in Figs 2 and 7.

The structural chemistry and chemical features of IU are as follows:

- (1) The product consists of at least 4 compounds: compound HU, allantoin and 2 unidentified minor presumably formaldehyde-releasing compounds.
- (2) The relative UV response factor at 200 nm between allantoin and HU has been determined to be 2.1, meaning allantoin absorbs 2.1 times more UV light than HU. Given the relative UV response of HU, the content of HU and allantoin in IU was measured to be 10% and 20%, respectively. The 2 minor unidentified compounds are estimated to be approximately 5% each, based on peak area percentage.
- (3) The remaining approximately 60% is most likely numerous polymers of allantoin-formaldehyde condensation products.
- (4) According to CE analysis, IU will under basic and neutral conditions degrade to HU and allantoin. It is likely that the 2

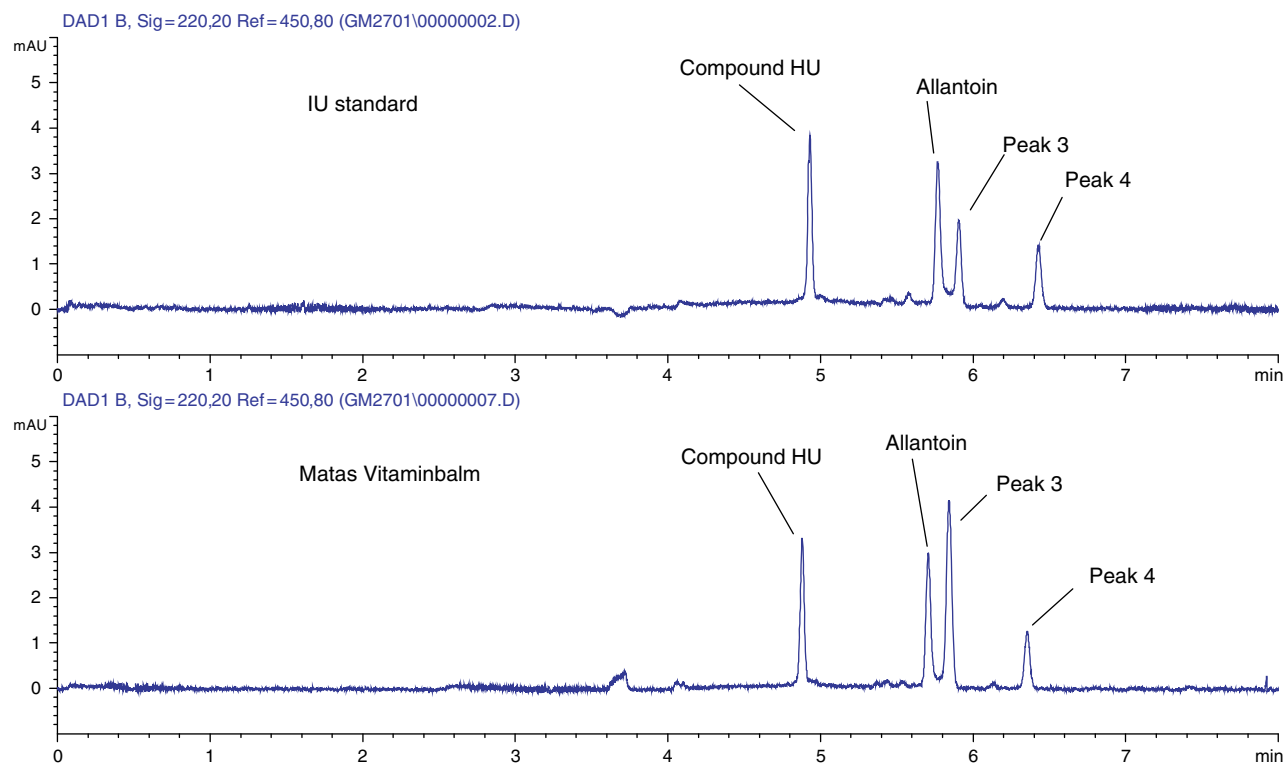


Fig. 11. Capillary electrophoretic analysis of hair balm product Vitamin Balm by Matas, Denmark compared with an imidazolidinyl urea (IU) standard. For conditions, see *Material and Methods*. HU, (4-hydroxymethyl-2,5-dioxo-imidazolidin-4-yl)-urea.

unidentified minor compounds will release formaldehyde. To an unknown extent also the polymeric condensation products may release formaldehyde.

- (5) Under acidic aqueous conditions, the IU mixture seems to be stable for at least a few weeks at room temperature.

The structural chemistry and chemical features of DU are as follows:

- (1) The product consists of several compounds with presumably BHU as dominant based on indirect NMR and HR-MS evidence.
- (2) The content of BHU on behalf of the relative response factor of HU is estimated to be 30–40%. The remaining part of DU is presumably numerous polymers of allantoin-formaldehyde condensation products.
- (3) According to CE analysis, DU will under basic and neutral conditions degrade to only 1 compound, HU.
- (4) Under acidic aqueous conditions, the DU mixture seems to be stable for at least a few weeks at room temperature.

The presence of polymers of allantoin-formaldehyde condensation products could account for

the somewhat raised base line (after the EOF) observed during CE analysis of both IU and DU (see Figs 2 and 7).

It could be questioned whether the observed composition of DU and IU is a result of immediate decomposition/rearrangements upon dissolution in aqueous solutions and thus the current assigned structures are present in the solid material. Such a theory is not supported by other observations done in our laboratories such that chromatography in strictly non-aqueous solvents used for either TLC or straight phase HPLC did not show a single spot or peak but showed several unresolved spots or peaks. If immediate degradation of the structures shown in Fig. 1 took place, release of formaldehyde should also take place immediately. This is not the case as a selective colorimetric method (8) showed that free formaldehyde was not present in solutions of DU or IU immediately after dissolution in water.

That the structures of DU and IU are not as straightforward as those shown in Fig. 1 can further be illustrated by the fact that only about 50% or 75% of the theoretical amount of formaldehyde in DU or IU, respectively, can be released upon complete hydrolysis of the compounds (9).

It is interesting to note that CE analysis of cosmetic products containing DU or IU shows

the presence of the same compounds as for the solid preservative and that DU or IU to a certain extent is degraded.

The clinical implications of this study are interesting as they support the conclusion from the study to standardize the TRUE Test DU and IU patch material that even if some positive contact allergic reactions may be caused by release of formaldehyde, specific reactions to the components in the biocides are also likely to occur (21). This also underlines the importance to use standardized patch test material with an unchanged composition throughout the storage time of the product.

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