Atmospheric Oxidation of Poly(oxyethylene) Alcohols. Identification of Ethoxylated Formates as Oxidation Products and Study of Their Contact Allergenic Activity

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Abstract □ Ethoxylated alcohols are widely used as surfactants. In the present study we have continued our investigations on the degradation with time upon air exposure of the ethoxylated alcohols at normal storage and handling. As a result, a new group of ethoxylated formates with the general formula $C_{12}H_{25}(OCH_2CH_2)_nOCHO$ ($n = 0–4$) was identified in $C_{12}H_{25}(OCH_2CH_2)_5OH$ stored and handled at room temperature. To facilitate the identification work, reference compounds were synthesized. The formates showed no allergenic activity in the sensitization studies performed. In previous investigations on the same ethoxylated alcohol, we have identified formaldehyde and ethoxylated aldehydes among the oxidation products formed. Formaldehyde is a common contact allergen, and the ethoxylated aldehydes were shown to have a sensitizing capacity of the same magnitude as formaldehyde. The instability of the ethoxylated alcohols and formation of oxidation products may give an allergenic contribution to hand eczema caused by work with water and surfactants. To investigate the clinical significance in man an appropriate diagnostic patch testing in exposed humans is required.

Introduction

The ethoxylated surfactants have wide applications and are used in, for example, household cleaners, laundry products, pharmaceuticals, and industrial and institutional cleaners. In 1993 the total consumption of ethoxylated alcohols was estimated to about 313000 tons in Western Europe.[1] The number of oxyethylene groups in nonionic ethoxylated surfactants determines application behavior, e.g. detergency, emulsification, and wetting, at a given temperature in all formulation work.

In recent studies[2−4] we have shown that oxidation products were rapidly formed from ethoxylated nonionic surfactants during storage and handling at room temperature in daylight and also during storage in dark. We detected peroxides,[2,4] formaldehyde,[2,4] and a series of ethoxylated aldehydes[3] among the oxidation products in our studies on Tween 80 (sorbitan monooleate)[2] and ethoxylated fatty alcohols.[3,4] The ethoxylated surfactants have so far been considered to be stable products at normal storage and handling.[5] The products are usually stored at room temperature, since they become semisolid at lower temperatures. However, ethoxylated surfactants are polyethers and as such susceptible to oxidation at air exposure. This autoxidation is theoretically discussed in the surfactant literature.[6] The proposed mechanism for autoxidation is a free radical mechanism initiated by minor amounts of free radicals present or catalyzed by metal salts, e.g. copper sulfate.[6] Peroxides and hydroperoxides are the primary oxidation products followed by formation of carbonyl compounds as secondary oxidation products.[7]

The prevalence of contact allergy in the general population in Europe is about 10%. Of those sensitized, about 2−4% have ongoing allergic contact dermatitis, which is the consequence of exposure to environmental chemicals.[8] About 90% of occupational contact dermatitis is located on the hands, and half of all work-related hand eczemas are caused by work with surfactants and water.[9] Most diagnoses of contact dermatitis from wet work are considered to be irritant dermatitis.[10] The diagnosis of allergy is difficult to exclude from irritancy in cases of chronic dermatitis. Surfactants are irritants, partially due to their ability to solubilize lipid membranes, since they possess both lipophilic and hydrophilic regions in their structures.[10] In our recent studies we found that the oxidation products identified after air exposure of ethoxylated surfactants had allergenic properties. Formaldehyde is a well-known contact allergen, and the ethoxylated aldehydes (Figure 1) were shown to be contact allergens in experimental sensitization studies.[3] In the literature some cases are reported of allergic contact dermatitis due to ethoxylated nonionic surfactants and emulsifiers.[11,12] Formaldehyde is described to be a significant allergen in women with hand eczema caused by occupational and domestic exposure.[13] Thus, allergenic oxidation products in ethoxylated surfactants may cause hand eczema or aggravate an ongoing irritant dermatitis in wet work.

In our previous studies on the identification of oxidation products formed from ethoxylated alcohols we have for the first time shown the formation of ethoxylated aldehydes with allergenic properties.[3] In the present study we have further investigated the autoxidation of the pure ethoxylated dodecyl alcohol, $C_{12}H_{25}(OCH_2CH_2)_5OH$ (below referred to as $C_{12}E_5$) by gas chromatography (GC) analysis during one year. We have identified a new group of oxidation products formed, the ethoxylated formates, and studied their allergenic activity.

Experimental Section

Chemicals—Tetraethylene glycol (99%) was obtained from Aldrich (Steinheim, Germany). Triethylene glycol (99%), formic acid (98−100%), 1-dodecanol (98%), 1-bromododecane, *p*-toluenesulfonic acid (99%), and dimethyl sulfoxide (DMSO) were obtained from Kebo Lab AB (Stockholm, Sweden). Sodium hydride (60% dispersion in mineral oil, toluene-soluble bags) was obtained from Acros Chimica N.V. (Geel, Belgium). Triethylene glycol mono *n*-dodecyl ether $C_{12}H_{25}(OCH_2CH_2)_3OH$ (CAS Reg. no. 3055-94-5) (referred to as $C_{12}E_3$) and pentaethylene glycol mono *n*-dodecyl

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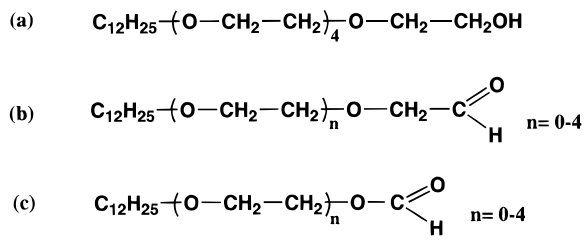

Figure 1—(a) Ethoxylated alcohol (pentaethylene glycol mono *n*-dodecyl ether) $C_{12}E_5$. (b) The earlier identified ethoxylated aldehydes in air exposed $C_{12}E_5$. (c) In the present study the identification and sensitizing capacity of the ethoxylated formates are presented.

ether $C_{12}H_{25}(OCH_2CH_2)_5OH$ (CAS Reg. no. 3055-95-6) (referred to as $C_{12}E_5$) were purchased from Nikko Chemicals CO., Ltd. (Tokyo, Japan). The purity was stated to be 98% by the producer, which was confirmed with GC analysis. The other ethoxylated alcohols were synthesized as described below. Standard chemicals were of p.a. quality.

Instrumentation and Mode of Analysis—FT-IR spectra were recorded with a Perkin-Elmer 16 PC FT-IR instrument using a sealed liquid cell with KBr windows. NMR spectroscopy was performed on a JEOL EX 270 instrument in $CDCl_3$ using tetramethylsilane as internal standard. GC analyses were carried out on a Hewlett-Packard HP 5890 gas chromatograph with a flame ionization detector (FID). The gas chromatograph was equipped with a fused silica capillary column (25 m × 0.22 mm i.d) coated with 0.2 μm CP-sil 8 CB (Chrompack, Middelburg, The Netherlands). Nitrogen was used as carrier gas at a linear gas velocity of 22 cm/s. An ELDS laboratory data system from Chromatography Data System Inc. (Svartsjö, Sweden) was used for registration and processing of the detector signal.

Mass spectrometric (MS) analyses were performed on a Finnigan Incos 50 quadrapole instrument equipped with a Varian 3400 gas chromatograph with an on-column injector. The MS analyses were carried out in electron impact (EI) and positive ion chemical ionization (PCI) modes. Introduction of the sample into the ion source was made via GC using on-column technique. The gas chromatograph was equipped with a fused silica capillary column (25 m × 0.25 mm i.d.) coated with 0.2 μm CP-sil 8 CB (Chrompack, Middelburg, The Netherlands), and helium was used as carrier gas. The temperature programming of the gas chromatograph oven was as follows: 35 °C for 1.0 min followed by a temperature increase of 10 °C/min up to 295 °C. The GC-MS transferline was held at 310 °C. The ion source was held at a temperature of 150 °C and the electron energy was 70 eV in the EI mode. In PCI mode the ion source was held at 80 °C, the electron energy was 110 eV, and the ion source pressure was about 1 Torr. At chemical ionization, methane of >99.995% purity was used as reagent gas, and the instrument was tuned by optimizing the reactant ions (CH_5^+, $C_2H_5^+$, and $C_3H_5^+$) to an approximate ratio of 5:4:1. The MS scan range in all analyses was *m*/*z* 50−600, and the scan cycle time was 0.6 s.

Synthesis—*Preparation of Dodecylethoxylated Alcohols **1**−**3***—Sodium hydride (0.80 g, 5.5 mmol) was stirred in DMSO (dry, 8 mL) at room temperature for 30 min. The appropriate glycol $H(OCH_2CH_2)_nOH$ (n = 1, 2, or 4) (77 mmol) was added slowly, and the mixture was stirred at room temperature under nitrogen for 2 h. 1-Bromododecane (5.0 g, 20 mmol) was added dropwise, and the mixture was heated at 90 °C overnight under nitrogen. The reaction mixture was dissolved in ethyl acetate and washed with water. The organic phase was dried over $MgSO_4$ and evaporated. The crude product was chromatographed on a silica gel column eluted with an increasing content of ethyl acetate 4−6% in hexane, followed by 20% methanol in ethyl acetate. The products **1**−**3** were obtained as clear oils in 57−66% yield and identified with FT-IR, NMR, and MS.

$C_{12}H_{25}OCH_2CH_2OH$ (1). Yield: 66%. FT-IR (neat): 3370 (O−H), 2850 and 2950 cm^{-1} (C−H). ^{1}H NMR ($CDCl_3$): δ 3.72 (tr, 2H, CH_2O), 3.53 (tr, 2H, CH_2O), 3.42 (tr, 2H, $CH_2CH_2CH_2O$), 2.11 (s, 1H, O**H**), 1.58 (m, 2H, $CH_2CH_2CH_2O$), 1.26 (m, 18H, (CH_2)$_9$) 0.85 (tr, 3H, CH_3). ^{13}C NMR ($CDCl_3$): δ 71.68, 71.43 ((CH_2O)$_2$), 61.85 (*C*H_2OH), 31.90 ($CH_3CH_2CH_2$), 29.63 (5 C:s), 29.47, 29.33 ((CH_2)$_7$), 26.09 (*C*H_2CH_2O), 22.66 (CH_3CH_2), 14.05 (*C*H_3). GC-MS-PCI *m*/*z* (% rel inten): 231 [M + 1]$^+$ (17), 230 M$^+$ (2), 229 [M − 1]$^+$ (17), 169 [$C_{12}H_{25}$]$^+$ (16), 63 [$HOCH_2CH_2OH_2$]$^+$ (100).

$C_{12}H_{25}(OCH_2CH_2)_2OH$ (2). Yield: 59%. FT-IR (neat): 3467 (O−H), 2850 and 2950 cm^{-1} (C−H). ^{1}H NMR ($CDCl_3$): δ 3.78−3.65 (m, 8H, (CH_2O)$_4$), 3.47 (tr, 2H, $CH_2CH_2CH_2O$), 2.85 (s, 1H, O**H**), 1.58 (m, 2H, $CH_2CH_2CH_2O$), 1.24 (m, 18H, (CH_2)$_9$), 0.87 (tr, 3H, CH_3). ^{13}C NMR ($CDCl_3$): δ 72.49, 71.56, 70.40, 70.08 (*C*H_2O)$_4$, 61.73 (*C*H_2OH), 31.86 ($CH_3CH_2CH_2$), 29.58 (2 C:s), 29.58 (3 C:s), 29.42, 29.29 ((*C*H_2)$_7$), 25.99 ($CH_2CH_2CH_2O$), 22.62 (CH_3CH_2), 14.05 (*C*H_3). GC-MS-PCI *m*/*z* (% rel inten): 275 [M + 1]$^+$ (53), 274 M$^+$ (3), 273 [M − 1]$^+$ (19), 166 [$C_{12}H_{22}$]$^+$ (23), 107 [$HO(CH_2CH_2O)_2H_2$]$^+$ (100).

$C_{12}H_{25}(OCH_2CH_2)_4OH$ (3). Yield: 57%. FT-IR (neat): 3374 (O−H), 2847 and 2948 cm^{-1} (C−H). ^{1}H NMR ($CDCl_3$): δ 3.63−3.56 (m, 16H, (CH_2O)$_8$), 3.40 (tr, 2H, $CH_2CH_2CH_2O$), 2.74 (s, 1H, O**H**) 1.55 (m, 2H, $CH_2CH_2CH_2O$), 1.22 (m, 18H, (CH_2)$_9$), 0.80 (tr, 3H, CH_3). ^{13}C NMR ($CDCl_3$): δ 72.46, 71.45, 70.51, 70.48, 70.48, 70.46, 70.24, 69.93 (*C*H_2O)$_8$, 61.57 (*C*H_2OH), 31.59 ($CH_3CH_2CH_2$), 29.30 (5C:s), 29.15, 29.01 (*C*H_2)$_7$, 25.73 ($CH_2CH_2CH_2O$), 22.30 (CH_3CH_2), 13.69 (*C*H_3). GC-MS-PCI *m*/*z* (% rel inten): 363 [M + 1]$^+$ (100), 362 M$^+$ (9), 361 [M − 1]$^+$ (61), 195 [$HO(CH_2CH_2O)_3$-$CH_2CH_2OH_2$]$^+$ (54), 177 [$(CH_2CH_2O)_4H$]$^+$ (12), 166 [$C_{12}H_{22}$]$^+$ (8), 133 [$(CH_2CH_2O)_3H$]$^+$ (16), 89 [$(CH_2CH_2O)_2H$]$^+$ (17), 45 [CH_2CH_2-OH]$^+$ (28).

*Preparation of Ethoxylated Formates **4**−**8***—Samples of the appropriate alcohol **1**−**3** and $C_{12}E_3$(4 mmol) were heated at 85 °C in formic acid (10 mL) with *p*-toluenesulfonic acid as catalyst for 4 h.[14−16] The reaction mixture was neutralized with a saturated (10 M) sodium hydroxide solution and washed with water, dried over $MgSO_4$, and concentrated in a vacuum. The crude product was chromatographed on a silica gel column eluted with an increasing content of ethyl acetate 30−70% in dichloromethane to give the pure ethoxylated formates **4**−**8** as clear oils in 80−100% yield. Identification was performed with FT-IR, NMR, and MS.

$C_{12}H_{25}OCHO$ (4). Yield 100%. FT-IR (neat): 2924 and 2854 (C−H aliphatic), 1732 (C=O), 1180 cm^{-1} (C−O). ^{1}H NMR ($CDCl_3$): δ 8.03 (s, 1H, OC*H*O), 4.14 (tr, 2H, CH_2OCHO), 1.63 (m, 2H, CH_2CH_2O), 1.29 (m, 18H, (CH_2)$_9$), 0.85 (tr, 3H, CH_3). ^{13}C NMR ($CDCl_3$): δ 161.35 (O*C*HO), 64.26 (*C*H_2O), 32.04 ($CH_2CH_2CH_3$), 29.67 (5C:s), 29.47 29.31 ((*C*H_2)$_7$), 25.93 (*C*H_2CH_2O), 22.80 (*C*H_2-CH_3), 14.24 (*C*H_3). GC-MS-PCI *m*/*z* (% rel inten): 215 [M + 1]$^+$ (2.62), 213 [M − 1]$^+$ (5.25), 169 [$C_{12}H_{25}$]$^+$ (69.2).

$C_{12}H_{25}OCH_2CH_2OCHO$ (5). Yield 95%. FT-IR (neat): 2924 and 2852 (C−H), 1732 (C=O), 1180 cm^{-1} (C−O). ^{1}H NMR ($CDCl_3$): δ 8.10 (s, 1H, OC*H*O), 4.31 (tr, 2H, CH_2OCHO), 3.67 (tr, 2H, CH_2O), 3.47 (tr, 2H, CH_2CH_2O), 1.58 (m, 2H, CH_2CH_2O), 1.26 (m, 18H, (CH_2)9), 0.87 (tr, 3H, CH_3). ^{13}C NMR ($CDCl_3$): δ 161.01 (O*C*HO), 71.55, 68.21, 63.13 (*C*H_2O)$_3$, 31.90 ($CH_2CH_2CH_3$), 29.58 (5C:s), 29.43, 29.32 ((CH_3)$_7$), 26.02 (*C*H_2CH_2O), 22.68 (CH_2CH_3), 14.24 (*C*H_3). GC-MS-PCI *m*/*z* (% rel inten): 259 [M + 1]$^+$ (30.5), 258 M$^+$ (0.39), 257 [M − 1]$^+$ (1.77), 169 [$C_{12}H_{25}$]$^+$ (6.32), 73 [CH_2CH_2-OCHOH]$^+$ (100).

$C_{12}H_{25}(OCH_2CH_2)_2OCHO$ (6). Yield 92%. FT-IR (neat): 2900 and 2850 (C−H), 1732 (C=O), 1180 cm^{-1} (C−O). ^{1}H NMR ($CDCl_3$): δ 8.09 (s, 1H, OC*H*O), 4.33 (tr, 2H, CH_2OCHO), 3.75−3.60 (m, 6H, (CH_2O)$_3$), 3.52 (tr, 2H, CH_2CH_2O), 1.57 (m, 2H, CH_2CH_2O), 1.25 (m, 18H, (CH_2)$_9$), 0.87 (tr, 3H, CH_3). ^{13}C NMR ($CDCl_3$): δ 161.07 (O*C*HO), 71.73, 70.80, 70.15, 68.98, 63.18 (*C*H_2O)$_5$, 32.04 ($CH_2CH_2CH_3$), 29.74 (5C:s), 29.59, 29.47 (*C*H_2)$_7$, 26.20 (*C*H_2CH_2O), 22.80 (CH_2CH_3), 14.24 (*C*H_3). GC-MS-PCI *m*/*z* (% rel inten): 303 [M + 1]$^+$ (38.0), 302 M$^+$ (8.45), 301 [M − 1]$^+$ (17.6), 275 [M + 1 − 28]$^+$ (5.63), 166 [$C_{12}H_{22}$]$^+$ (19.7), 135 [$H_2(OCH_2CH_2)_2OCHO$]$^+$ (71), 73 [$H_2OCH_2CH_2OCHO$]$^+$ (95.8).

$C_{12}H_{25}(OCH_2CH_2)_3OCHO$ 7. Yield 90%. FT-IR (neat): 2945 and 2835 (C−H), 1724 (C=O), 1180 cm^{-1} (C−O). ^{1}H NMR ($CDCl_3$): δ 8.09 (s, 1H, OC*H*O), 4.32 (tr, 2H, OCH_2OCHO), 3.75−3.56 (m, 10H, (CH_2O)$_5$), 3.44 (tr, 2H, CH_2CH_2O), 1.55 (m, 2H, CH_2CH_2O), 1.25 (m, 18H, (CH_2)$_9$), 0.88 (tr, 3H, CH_3). ^{13}C NMR ($CDCl_3$): δ 160.93 (O*C*HO), 71.51, 70.66, 70.62, 70.55, 70.01, 68.82, 63.00 (*C*H_2O)$_7$, 31.88 ($CH_2CH_2CH_3$), 29.58 (5C:s), 29.45, 29.31 ((*C*H_2)$_7$), 26.06 (*C*H_2CH_2O), 22.64 (CH_2CH_3), 14.09 (*C*H_3). GC-MS-PCI *m*/*z* (% rel inten): 347 [M + 1]$^+$ (29.2), 346 M$^+$ (2.89), 345 [M − 1]$^+$ (13.6), 317 [M + 1 − 28]$^+$ (4.82), 179 [$(CH_2CH_2O)_3H$]$^+$ (49.5), 166 [$C_{12}H_{22}$]$^+$ (21.91), 73 [CH_2CH_2OCHO]$^+$ (100).

$C_{12}H_{25}(OCH_2CH_2)_4OCHO$ (8). Yield 80% FT-IR (neat): 2942 and 2845 (C−H), 1732 (C=O), 1178 cm^{-1} (C−O). ^{1}H NMR ($CDCl_3$): δ 8.10 (s, 1H, OC*H*O), 4.33 (tr, 2H, CH_2OCHO), 3.76−3.60 (m, 14 H, (CH_2O)$_7$), 3.45 (tr, 2H, CH_2CH_2O), 1.58 (m, 2H, CH_2CH_2O), 1.26

(m, 18H, $(CH_2)_9$), 0.89 (tr, 3H, CH_3). ^{13}C NMR ($CDCl_3$): δ 160.94 (OCHO), 71.52, 70.58 (5C, s), 70.10, 68.81, 63.00 $(CH_2O)_9$, 31.88 ($CH_2CH_2CH_3$), 29.55 (5C:s), 29.45, 29.31 $(CH_2)_7$, 26.04 (CH_2CH_2O), 22.64 (CH_2CH_3), 14.07 (CH_3). GC-MS-PCI *m/z* (% rel inten): 391 $[M + 1]^+$ (8.12), 390 M^+ (1.63), 389 $[M - 1]^+$ (11.8), 363 $[M + 1 - 28]^+$ (90.2), 223 $[H(OCH_2CH_2)_4OCHO]^+$ (36.3), 166 $[C_{12}H_{22}]^+$ (24.0), 133 $[(CH_2CH_2O)_2H]^+$ (17.8), 73 $[CH_2CH_2OCHO]^+$ (100).

Storage and Handling of Ethoxylated Alcohols—Two samples of undiluted $C_{12}E_5$ (98%) were used in the experiment. Sample 1 (5 g) was stored in a closed 10 mL vessel in darkness at room temperature (20−22 °C) for 12 months. Sample 2 (5 g) was stirred gently in an open 10 mL Erlenmeyer flask in daylight at room temperature (20−22 °C) for 1 h, 4 times a day, during 12 months, mimicking what we considered normal handling in laboratories and industries. The top of the flask was covered with aluminum foil to prevent contamination and to diminish the evaporation.

Detection of Oxidation Products in Ethoxylated Alcohols—Samples 1 and 2 were analyzed with GC-MS analysis using the synthesized references.

Samples 1 and 2 were also analyzed with GC every fourth week after start of the exposure. The content of the ethoxylated formates in samples 1 and 2 was quantified using the synthesized reference compounds. Aliquots of 2 × 10 mg were taken out from each sample. Two sample preparations (1.0 mg/mL) from each sample were prepared and dissolved in dichloromethane, methyl stearate was added as internal standard, and a duplicate analysis on each sample was performed. On-column injections (1 μL) were made at an injector temperature of 35 °C. The column oven was kept at 35 °C for 2 min whereafter the temperature of the column was raised with a rate of 10 °C/min to 210 °C. The column temperature was then raised with a rate of 5 °C/min to a final value of 240 °C which was kept for 10 min.

Studies on the Sensitizing Capacity of 5—The sensitization experiment was performed using female Dunkin-Hartley guinea pigs (weight 250−300 g) from AB Sahlins Försöksdjursfarm, Malmö, Sweden. The animals were kept on a standard diet from Beekey, North Humberside, England, and water *ad libitum*. The animals were randomly assigned to one exposed, group 1 ($n = 15$), and one control group, group 2 ($n = 15$).

The sensitization study was performed according to the Cumulative Contact Enhancement Test (CCET) method[17] in a modified form with closed epidermal challenge testing.[18,19] At induction the animals received an occlusive epidermal application on the shaved upper back on days 0, 2, 7, and 9. About 200 mg of the test material was applied on pieces of filter paper (4 × 2 cm) at each of the four inductions. The FCA injections at the third induction were omitted according to our earlier experience of sensitization studies on surfactants.[3] Challenge testing was performed on day 21 on the shaved left flank using Finn Chambers (aluminum chambers, 8 mm i.d from Epitest, Helsinki, Finland) with approximately 15 mg of the test material applied in each chamber.

The exposed group was induced with **5** 10% w/w (2.6×10^{-4} mol/g) in water, while the animals in the control group received water alone. Both groups were challenged with 10, 5, and 1% w/w (2.6×10^{-4}, 1.3×10^{-4}, and 2.6×10^{-5} mol/g) of **5** in water, with $C_{12}E_5$ 5% (1.3×10^{-4} mol/g) in water, and $C_{12}E_4OCH_2CHO$ 1% w/w (2.5×10^{-5} mol/g) in water (Table 1). Water was applied as a vehicle control. The chambers were removed after 24 h, and the reactions were assessed at 48, 72, and 96 h after start of the exposure. The minimum criterion for a positive reaction was a confluent erythema.

The experiment was performed with the equimolar concentrations for induction and challenge as used in the experiments with the ethoxylated aldehyde, $C_{12}H_{25}(OCH_2CH_2)_4OCH_2CHO$.[3] The challenge concentrations of **5** were in pretests shown to be nonirritating in three untreated guinea pigs. Patch testing with concentrations 20−1% of **5** in water gave no skin reactions after 48 and 72 h. The experiment was approved by the local ethics committee.

Statistical Analyses—The result from the animal experiment was analyzed with Fisher's exact test. The number of reactions to each applied test substance in the exposed animals was compared with the number of reactions in the control group. A p value <0.05 was statistically significant.

Table 1—Sensitizing Potential of Compound 5 and Cross-Reactivity Studies with $C_{12}E_5$ and an Ethoxylated Aldehyde in Guinea Pigs Using the Modified CCET Method without Adjuvant

	no. of animals with positive reaction after exposure[a]					
	5 (% w/w in water)			C12E5[b]	aldehyde[c]	
guinea pigs	10	5	1	5	1	water
			Group 1[d]			
exposed ($n = 15$)						
48 h	0	0	0	1[a]	1	0
72 h	0	0	0	2	2	0
96 h	0	0	0	1	1	0
			Group 2			
controls ($n = 15$)						
48 h	0	0	0	0	1	1
72 h	0	1	0	1	1	1
96 h	0	0	0	1	0	1

[a] The figures are the number of animals with confluent erythema 48, 72, and 96 h after application of the test material. [b] Ethoxylated alcohol, $C_{12}E_5$, 5% (w/w in water). [c] Ethoxylated aldehyde with four ethylenoxide groups, $C_{12}H_{25}(OCH_2CH_2)_4OCH_2OCHO$, 1% (w/w in water). [d] Induction: 10% (w/w in water) of **5** in water.

Results

Spectral Characteristics—In FT-IR the specific hydroxyl resonance of the synthesized alcohols **1**−**3** was observed at 3370−3467 cm^{-1}. The FT-IR spectra of the synthesized ethoxylated formates **4**−**8** had a specific carbonyl resonance at 1724−1732 cm^{-1} and the C−O resonance at 1180 cm^{-1} from the ester group.[15] The NMR signals were compared with literature data for poly(oxyethylene) alcohols[20,21] and formates[16] and accorded with structures **1**−**3** and **4**−**8**. MS analyses in the GC-EI mode yielded no molecular ions of the ethoxylated alcohols and formates. Aliphatic ethers normally exhibit weak molecular ion peaks.[22] In the GC-MS-PCI analyses of the synthesized alcohol ethoxylates, **1**−**3**, the molecular ion M^+ and $[M + 1]^+$ and $[M - 1]^+$ ions were observed. Cleavage of the ethoxylated chain led to fragments of the general formula $[(CH_2CH_2O)_nH]^+$. These data, together with the FT-IR and NMR data are consistent with the structures **1**−**3**. In the GC-MS-PCI analyses of the synthesized formates **4**−**8** the molecular ion M^+ and $[M + 1]^+$ and $[M - 1]^+$ ions were observed together with specific fragments with the general formula $[(CH_2CH_2O)_nH]^+$ and $[CH_2CH_2O)_nOCHO]^+$. The $[M - 1]^+$ ion fragment corresponded to α-cleavage. No adducts with methane, $[(M + C_2H_5)^+]$ and $[(M + C_3H_5)^+]$ were seen. These data, together with the FT-IR and NMR data are consistent with the structures **4**−**8**, $C_{12}H_{25}(OCH_2CH_2)_n$-OCHO, $n = 1-4$.

Detection of Oxidation Products in Ethoxylated Alcohols—The ethoxylated formates, $C_{12}H_{25}(OCH_2CH_2)_n$-OCHO, $n = 1-4$, were all detected in samples 1 and 2 of $C_{12}E_5$ with GC-MS-PCI analyses using **4**−**8** as reference compounds. The alcohols, $C_{12}H_{25}(OCH_2CH_2)_nOH$ ($n = 1-4$), were identified in samples 1 and 2 of $C_{12}E_5$ with GC-MS-PCI analyses using $C_{12}E_3$ and the synthesized alcohols **1**−**3** as reference compounds.

The amount of the identified formates increased continuously with time. The formates seem to be rapidly formed since they were detected in small amounts in the GC analysis already in a newly opened bottle of the pure ethoxylated alcohol. The limits of detection for the substances **1**−**5** in the GC-analysis were estimated to be in the range 0.001−0.05 ng/μL using a signal-to-noise ratio 3:1 (S/N = 3). The total content of formates in $C_{12}E_5$ was 3.3% in the sample stored in a closed vessel in darkness (sample 1) and 4.0% in the sample handled in daylight (sample 2) after 12 months. The content of the individual

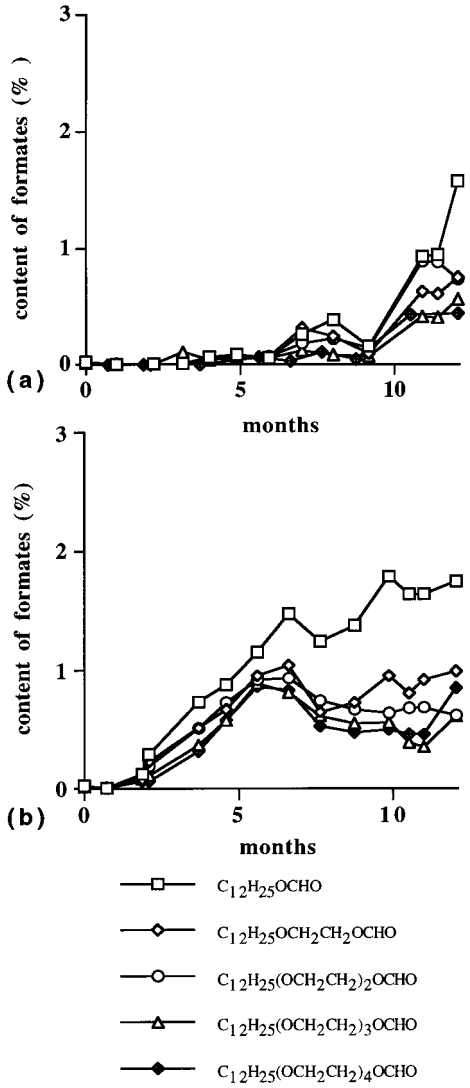

Figure 2—The content (%) of the individual ethoxylated formates **4**–**8** at different time points in $C_{12}E_5$: (a) sample 1 stored in a closed vessel in darkness at room temperature; (b) sample 2 stored and handled in daylight at room temperature.

formates in samples 1 and 2 is shown in Figure 2. Formate **4**, $C_{12}H_{25}OCHO$, was formed in the highest concentration. All calculations were performed relative to the internal standard. The coefficient of variation (CV) was below 10% at repeated measurements ($n = 10$). Duplicate analyses were performed of each sample. Approximately 5% of the total sample volume (5 g) was used for analysis, which was not regarded to contribute to the degradation. Figure 3 shows the GC separation of oxidation products in sample 2 air exposed for 12 months. The oxidation products that we have identified are assigned in the chromatogram with retention times corresponding to homologue retention characteristics accordning to boiling point.

Sensitizing Capacity of 5—No sensitizing response was observed to **5** in the animal experiment (Table 1). No cross-reactivity was observed to the corresponding alcohol, $C_{12}E_5$, or to the ethoxylated aldehyde, $C_{12}H_{25}(OCH_2CH_2)_4OCH_2CHO$. Some irritation was seen when the animals were tested with the alcohol and the aldehyde.

Discussion

Air exposure during storage and handling of the pure ethoxylated alcohol $C_{12}E_5$ at room temperature results in

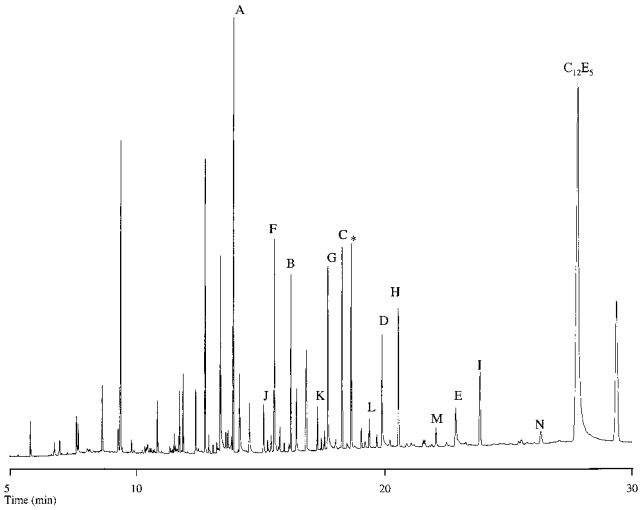

Figure 3—GC chromatogram showing the separation of different degradation products formed at air exposure of $C_{12}E_5$. The peak from the original ethoxylated alcohol is assigned $C_{12}E_5$, peaks A–E are the homologue series of ethoxylated formates, $C_{12}H_{25}(OCH_2CH_2)_nOCHO$ $n = 0$–4, eluting in the order of boiling point; peaks F–I are the homologue series of ethoxylated alcohols, $C_{12}H_{25}(OCH_2CH_2)_nOH$ $n = 1$–4, eluting in the order of boiling point; peaks J–N are the homologue series of ethoxylated aldehydes, $C_{12}H_{25}(OCH_2CH_2)_nOCH_2CHO$ ($n = 0$–4), eluting in the order of boiling point. The peak assigned * is the internal standard, methyl stearate, with a retention time of 18.63 min.

a number of degradation products as illustrated by the gas chromatogram in Figure 3. Among the oxidation products identified, some have significant allergenic properties. So far, nonionic ethoxylated surfactants have been regarded as stable products and are normally stored at room temperature, since they become semisolid at lower temperatures.[5] The practical consequence will be that a product containing ethoxylated alcohols can have quite a different chemical composition after storage and handling compared to the original product. In addition to an increase of their harmful effects on skin[3] with time, there might also be a change in their surface active properties.[25] To avoid significant decomposition, the pure ethoxylated alcohols must be stored in the refrigerator (8 °C), which was demonstrated in a previous study.[4]

To the best of our knowledge, ethoxylated formates are described here for the first time. The formates, $C_{12}H_{25}(OCH_2CH_2)_nOCHO$, $n = 0-4$, were identified and their structures elucidated in the oxidation mixture of $C_{12}E_5$. Prior to the identification work, the dodecyl poly(oxyethylene) formates that theoretically might be formed were synthesized and used as reference compounds in the analyses. The major formate, $C_{12}H_{25}OCHO$ (**4**), is apparently formed due to cleavage between the poly(oxyethylene) groups and the alkyl chain. The ethoxylated aldehydes earlier identified[3] are formed by cleavage of the poly(oxyethylene) chain resulting in loss of oxyethylene units and also by oxidation of the terminal hydroxyl group yielding the dominant ethoxylated aldehyde, $C_{12}H_{25}(OCH_2CH_2)_4OCH_2CHO$. This indicates that the autoxidation proceeds with parallel and different mechanisms.

The amount of ethoxylated formates increased with time of air exposure as determined with GC analysis. Different compounds are predominant in the degradation mixture depending on the time of air exposure. In our previous studies[2–4] the formation of peroxides, formaldehyde and ethoxylated aldehydes was shown. The amount of peroxides was determined with the unspecific iodometric titration method[23] whereas the aldehydes were quantified using GC- and LC methods. Peroxides were initially formed followed by formation of formaldehyde and the ethoxylated alde-

hydes as secondary oxidation products. There was a decrease in the content of peroxides after a certain time during the oxidation process, while the content of aldehydes steadily increased.[2−4] The ethoxylated alcohols, $C_{12}H_{25}(OCH_2CH_2)_nOH$, ($n = 1-4$) that we identified in the oxidation mixture with GC-MS may be formed by decomposition of the corresponding peroxides or hydroperoxides, since alkyl hydroperoxides are reported to decompose to alcohols at temperatures above 90 °C.[24] The boiling point of $C_{12}E_5$ is in the range of 202−216 °C. Therefore, the commonly used GC technique involves injection via a split/splitless injector at a temperature of 280 °C. Despite the use of on-column injection technique in this study to avoid degradation of thermally unstable compounds at injection, the hydroperoxides were not detected with GC-MS. The hydroperoxides could have decomposed in the column when the column temperature increased during the analysis. To identify ethoxylated hydroperoxides alternative methods have to be employed.

In the predictive sensitization studies, Freund's complete adjuvant (FCA) is often used. FCA consists of Arlacel (mannide mono-oleate), a sorbitan emulsifier to promote a stable water-in-oil emulsion, and dried heat-killed *Mycobacterium tuberculosis* organisms to enhance the nonspecific immune response and raise the sensitivity of the method.[26] However, also an increased irritability of the skin may be seen. Determination of the sensitizing potential of chemicals, such as surfactants, with irritating properties is difficult. The challenge testing should be performed with the chemical in nonirritating concentrations which might then be too low to detect an allergenic effect. In our investigation of the sensitizing capacity of the ethoxylated aldehydes[3] two parallel CCET protocols were used, one with intradermal FCA injections and one without. No increased allergenic activity was observed when FCA was administered, but an increased irritation was initially seen in the FCA-treated controls compared to the non-FCA-treated. A dose−response relationship was seen in both experiments. Thus, in our subsequent experiments with ethoxylated alcohols[4] and formates no FCA treatment was performed. In these experiments the compounds showed no significant allergenic activity (Table 1). Some irritation was seen showing the difficulties when testing with irritants. However, irritation in some of the control animals can also be observed in sensitization studies of other substances.[17]

Since alcohols and esters are rare sensitizers, the absence of a sensitizing capacity for the ethoxylated formate was not surprising.[27] To cause sensitization, a compound (hapten) has to penetrate the skin and react with macromolecules in the skin to form a complete antigen that is recognized as foreign. Molecules which can function as haptens in allergic contact dermatitis are either electrophilic or have structures that can easily form radicals.[27] Such molecules are able to react with the nucleophilic skin proteins forming stable covalent bonds. In our studies, three ethoxylated compounds have so far been tested for their contact allergenic properties, i.e., $C_{12}E_5$,[4] $C_{12}H_{25}(OCH_2CH_2)_4OCH_2CHO$[3] and $C_{12}H_{25}(OCH_2CH_2)_4OCHO$. The alcohol has no electrophilic properties, and contact allergenic activity could not be observed.[4] The formate has a very low reactivity as electrophile, whereas the aldehyde is an apparent electrophilic group. In our tests, only the aldehyde[3] showed contact allergenic activity which is in accordance with its electrophilic properties.

In a sample of $C_{12}E_5$ stored at room temperature only 70% of the original product remained after 6 months. Similar analysis after 12 months storage was not performed. The results in this study together with earlier investigations of the autoxidation of $C_{12}E_5$ show that the content of the identified oxidation products constitutes less than 10% of the total content in the product after 6 months. This indicates that a major part of the oxidation and degradation products from $C_{12}E_5$ still remains to be identified in the complex oxidation mixture. Our data indicates that the composition of ethoxylated alcohols may change rather rapidly upon storage. It has not been a topic of this study to investigate this in detail, but our data support that a stability study is requested.

It is important to further investigate the skin effects of the widely used ethoxylated surfactants, since a majority of the cases of occupational dermatits is caused by work with water and surfactants. Various types of ethoxylated surfactants are also used as emulsifiers in creams and lotions used on the skin. The clinical significance to man will require an appropriate diagnostic patch testing in exposed humans. The sensitizing capacity of other oxidation/degradation products will be studied and also the influence of oxidation on the skin-irritating properties.

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