Contact Allergens from Surfactants. Atmospheric Oxidation of Polyoxyethylene Alcohols, Formation of Ethoxylated Aldehydes, and Their Allergenic Activity

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Abstract □ Ethoxylated surfactants are susceptible to oxidation upon air exposure. We have previously studied the rate of peroxidation and formaldehyde formation in the chemically well-defined ethoxylated alcohol $C_{12}H_{25}(OCH_2CH_2)_5OH$. Formaldehyde is a common cause of contact allergy. The aim of the present study was to identify other oxidation products that could be formed upon air exposure of the ethoxylated alcohol and to determine their allergenic activity. It was shown that air oxidation of $C_{12}H_{25}(OCH_2CH_2)_5OH$ gave all the theoretically possible aldehydes of the general formula $C_{12}H_{25}(OCH_2CH_2)_nOCH_2CHO$ ($n = 0–4$) and that the major oxidation product was $C_{12}H_{25}(OCH_2CH_2)_4OCH_2CHO$, dodecyltetraoxyethyleneoxyacetaldehyde. The structure elucidation and synthesis of these aldehydes are here presented for the first time. The major aldehyde was shown to be a contact allergen with the same sensitizing capacity as that of formaldehyde. A dose–response relationship was observed in the sensitization studies. The allergens were formed from the surfactant itself and the skin reactions cannot be explained due to any impurities that may be present in a technical quality of the surfactant. Cases of allergic contact dermatits to ethoxylated surfactants have been reported. To avoid the formation of allergenic oxidation products it is important to control the conditions for storage, handling, and transportation of ethoxylated surfactants.

Introduction

Ethoxylated surfactants are polyethers which are easily oxidized by atmospheric oxygen to a variety of hydroperoxides, peroxides, and formaldehyde and other carbonyl compounds. The possibility that allergenic compounds can be formed during storage and handling of products containing ethoxylated surfactants has not been taken into consideration by most producers, dermatologists, and regulatory agencies. Autoxidation of nonionic ethoxylated surfactants and polyethylene glycols is theoretically discussed in the literature on surfactants.[1,2] However, the studies are production oriented and a structure elucidation of the oxidation products has not been presented to the best of our knowledge.

Surfactants are known to be skin irritants.[3] Most cases of occupational dermatitis are considered to be irritant contact dermatitis, caused by work with water and surfactants.[4] However, in the diagnosis, irritant contact dermatitis is difficult to separate from allergic contact dermatitis. Some cases of allergic contact dermatitis due to ethoxylated nonionic surfactants and emulsifiers have been demonstrated.[5] Surfactants have also been considered as potential allergens in paste bandages used among patients with chronic leg ulcers.[6]

In a previous study[7] the ethoxylated nonionic surfactant Tween 80 (sorbitan monooleate) of technical quality showed allergenic activity in guinea pigs. Peroxides and formaldehyde were formed during normal storage and handling of the ethoxylated surfactant at room temperature. A formaldehyde content of 500 μg/g was found in a 10% water solution of Tween 80 exposed to air for 11 months in our laboratory. We have also observed formation of peroxides and formaldehyde from other surfactants.[8] A sample of a chemically well-defined ethoxylated alcohol, $C_{12}H_{25}(OCH_2CH_2)_5OH$ (referred to as $C_{12}E_5OH$), which was exposed to air and daylight for 8 months in our laboratory, showed a formaldehyde content of 3000 μg/g. The autoxidation was observed not only after air exposure in daylight but also after storage in darkness. A sample of the surfactant stored in darkness contained 1300 μg/g formaldehyde after 10 months.[8] Within the European Union, cosmetic products containing more than 500 μg/g of formaldehyde require a warning labeling due to the risk of skin sensitization.[9] Elicitation below this level has been reported in allergic subjects.[10−11]

The aim of the present study was to identify other potentially allergenic oxidation products which could be formed upon air exposure of ethoxylated surfactants and to determine their allergenic activity. For this purpose we continued our work on the nonionic ethoxylated alcohol $C_{12}E_5OH$. To facilitate the identification we have synthesized the theoretically proposed oxidation products **1**−**5**, which served as reference compounds in the analytical work. The identified oxidation products were tested for allergenic activity with adopted experimental methods.

Experimental Section

Chemicals—Tetraethylene glycol (99%) was obtained from Aldrich (Steinheim, Germany). Triethylene glycol (99%), bromoacetaldehyde diethyl acetal (99%), and trifluoroacetic acid (99%) were obtained from Acros Chimica N. V. (Geel, Belgium). 1-Bromododecane, pyridine, *N*,*N*′-dicyclohexylcarbodiimide (DCC), and dimethyl sulfoxide (DMSO) were obtained from Kebo Lab AB (Stockholm, Sweden). Triethylene glycol mono-*n*-dodecyl ether $C_{12}H_{25}(OCH_2CH_2)_3OH$ (CAS Reg No. 3055-94-5) (referred to as $C_{12}E_3OH$) and pentaethylene glycol mono-*n*-dodecyl ether $C_{12}H_{25}(OCH_2CH_2)_5OH$ (CAS Reg No. 3055-95-6) (referred to as $C_{12}E_5OH$) were purchased from Nikko Chemicals CO., Ltd. (Tokyo, Japan). The purity was stated to be 98% by the producer. Freund's complete adjuvant (FCA) was obtained from Difco (Detroit, MI).

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 tetra-

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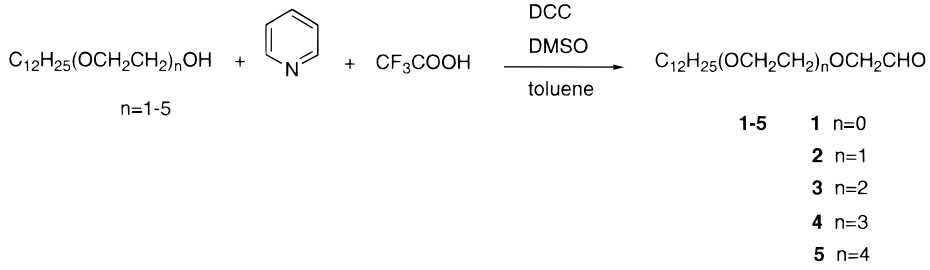


Scheme 1

methylsilane as internal standard. Gas chromatography (GC) analyses were carried out on a Hewlett-Packard HP 5890 gas chromatograph with a flame ionization detector (FID). The GC was equipped with a fused silica capillary column (30 m × 0.25 mm i.d) coated with 0.25 μm DB-5 (J&W Scientific, Folsom, CA) and nitrogen was used as carrier gas. 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 and a direct insertion probe. The MS analyses were performed in electron impact (EI) and positive ion chemical ionization (PCI) modes. Introduction of the sample into the ion source was made via GC using an on-column technique. The GC was equipped with a fused silica capillary column (25 m × 0.25 mm i.d.) coated with 0.2 μm CP-sil 8CB (Chrompack, Middelburg, The Netherlands) and helium was used as carrier gas. The temperature programming of the GC 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. Introduction via the direct insertion probe was performed at 30 °C followed by a linear temperature increase to 350 °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 utilized as reagent gas and the instrument was tuned by optimizing the reactant ions (CH_5^+, $C_2H_7^+$, and $C_3H_8^+$) 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 (GC introduction) and 1.6 s (DIP introduction).

Synthesis—*Dodecylethoxylated Aldehydes (Scheme 1)*—A mixture of the appropriate dodecylethoxylated alcohol ($C_{12}E_{1-5}OH$) (1 mmol), DMSO (1.2 g, 15 mmol), and DCC (0.62 g, 3.0 mmol) in toluene (20 mL) was stirred at room temperature for 30 min. Pyridine (0.080 g, 1.0 mmol) and trifluoroacetic acid (0.060 g, 0.50 mmol) were added to the mixture to generate pyridinium trifluoroacetate.[12] The mixture was then stirred for 48 h at room temperature. Water (10 mL) was added to the reaction mixture, which was then filtered. Diethyl ether (100 mL) was added and the organic phase was washed with HCl (3%), saturated aqueous $NaHCO_3$, and 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 aldehydes **1**−**5** (Scheme 1) as clear oils in 23−40% yield. Identification was performed with FT-IR, NMR, and MS.

$C_{12}H_{25}OCH_2CHO$, ***1***. Yield: 23%. FT-IR (neat): 2854 cm^{-1} (C−H, aliphatic), 2710 cm^{-1} (C−H, aldehyde), 1740 cm^{-1} (C═O), 1466 cm^{-1} (C−H in $-CH_2-$), 1122 cm^{-1} (C−O). ^1H NMR ($CDCl_3$): δ 9.73 (s, 1H, C**H**O), 4.05 (s, 2H, C$\mathbf{H_2}$CHO), 3.52 (tr, 2H, C$\mathbf{H_2}$OCH$_2$CHO), 1.54 (m, 2H, C$\mathbf{H_2}$CH$_2$O), 1.23 (m, 18H, (C$\mathbf{H_2}$)$_9$), 0.83 (tr, 3H, C$\mathbf{H_3}$). ^{13}C NMR ($CDCl_3$): δ 201.35 (**C**HO), 76.42 (**C**H$_2$CHO), 72.38 (**C**H$_2$OCH$_2$CHO), 31.88 (CH$_3$CH$_2$**C**H$_2$), 29.76 (6 C:s), 29.71 ((**C**H$_2$)$_7$), 26.10 (**C**H$_2$CH$_2$O), 22.81 (CH$_3$**C**H$_2$), 14.27 (**C**H$_3$). MS-DIP-PCI *m/z* (% rel int): 228 M^+ (14), 229 $(M + 1)^+$ (6), 227 $(M - 1)^+$ (38), 199 $(M + 1 - 30)^+$ (100), 185 $(C_{12}H_{25}OH)^+$ (4), 169 $(C_{12}H_{25})^+$ (41), 43 $(CH_2CHO)^+$ (38).

$C_{12}H_{25}OCH_2CH_2OCH_2CHO$, ***2***. Yield: 26%. FT-IR (neat): 2854 and 2924 cm^{-1} (C−H), 2715 cm^{-1} (C−H, aldehyde), 1736 cm^{-1} (C═O), 1466 cm^{-1} (C−H in $-CH_2-$), 1122 cm^{-1} (C−O). ^1H NMR ($CDCl_3$): δ 9.71 (s, 1H, C**H**O), 4.13 (s, 2H, C$\mathbf{H_2}$CHO), 3.71−3.51 (m, 4H, (C$\mathbf{H_2}$O)$_2$), 3.42 (tr, 2H, CH$_2$CH$_2$C$\mathbf{H_2}$O), 1.55 (m, 2H, CH$_2$C$\mathbf{H_2}$CH$_2$O), 1.22 (m, 18H, (C$\mathbf{H_2}$)$_9$), 0.83 (tr, 3H, C$\mathbf{H_3}$). ^{13}C NMR ($CDCl_3$): δ 201.03 (**C**HO), 76.53 (**C**H$_2$CHO), 71.68, 71.29, 70.17 ((**C**H$_2$O)$_3$), 31.55 (CH$_3$CH$_2$**C**H$_2$), 29.56 (5 C:s), 29.45, 29.31 ((**C**H$_2$)$_7$), 26.06 (**C**H$_2$CH$_2$O), 22.64 (CH$_3$**C**H$_2$) 14.27 (**C**H$_3$). MS-DIP-PCI *m/z* (% rel int): 273 $(M + 1)^+$ (12), 272 M^+ (2), 271 $(M - 1)^+$ (2), 243 $(M + 1 - 30)^+$ (4), 229 $(M + 1 - 44)^+$ (7), 169 $(C_{12}H_{25})^+$ (3), 105 $(HO(CH_2CH_2O)_2H)^+$ (62), 87 $(CH_2CH_2OCH_2CHOH)^+$ (100).

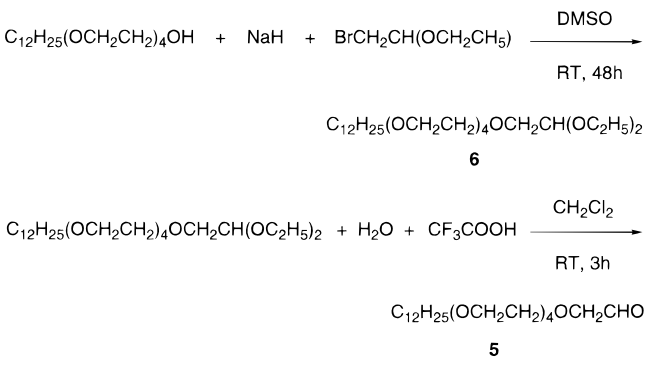
$C_{12}H_{25}(OCH_2CH_2)_2OCH_2CHO$, ***3***. Yield 28%. FT-IR (neat): 2854 and 2925 cm^{-1} (C−H), 2715 cm^{-1} (C−H, aldehyde), 1736 cm^{-1} (C═O), 1466 cm^{-1} (C−H in $-CH_2-$), 1122 cm^{-1} (C−O). ^1H NMR ($CDCl_3$): δ 9.72 (s, 1H, C**H**O), 4.17 (s, 2H, C$\mathbf{H_2}$CHO), 3.74−3.58 (m, 8H, (C$\mathbf{H_2}$O)$_4$), 3.43 (tr, 2H, CH$_2$CH$_2$C$\mathbf{H_2}$O), 1.58 (m, 2H, CH$_2$C$\mathbf{H_2}$CH$_2$O), 1.24 (m, 18H, (C$\mathbf{H_2}$)$_9$), 0.80 (tr, 3H, C$\mathbf{H_3}$). ^{13}C NMR ($CDCl_3$): δ 203.0 (**C**HO) 77.08 (**C**H$_2$CHO), 71.98, 71.64, 71.12, 71.02, 70.40 ((**C**H$_2$O)$_5$), 31.50 (CH$_3$CH$_2$**C**H$_2$), 30.03 (5 C:s), 29.88, 29.74 ((**C**H$_2$)$_7$), 26.00 (**C**H$_2$CH$_2$O), 23.07 (CH$_3$**C**H$_2$), 14.51 (**C**H$_3$). MS-DIP-PCI *m/z* (% rel int): 317 $(M + 1)^+$ (25), 316 M^+ (14), 315 $(M - 1)^+$ (23), 287 $(M + 1 - 30)^+$ (55), 273 $(M + 1 - 44)^+$ (100), 166 $(C_{12}H_{22})^+$ (51), 131 $((CH_2CH_2O)_3H)^+$ (30), 87 $(CH_2CH_2OCH_2CHOH)^+$ (17).

$C_{12}H_{25}(OCH_2CH_2)_3OCH_2CHO$, ***4***. Yield: 40%. FT-IR (neat): 2854 and 2924 cm^{-1} (C−H), 2720 cm^{-1} (C−H, aldehyde), 1736 cm^{-1} (C═O), 1466 cm^{-1} (C−H in $-CH_2-$), 1122 cm^{-1} (C−O). ^1H NMR ($CDCl_3$): δ 9.70 (s, 1H, C**H**O), 4.20 (s, 2H, C$\mathbf{H_2}$CHO), 3.75−3.59 (m, 12H, (C$\mathbf{H_2}$O)$_6$), 3.41 (tr, 2H, CH$_2$CH$_2$C$\mathbf{H_2}$O), 1.56 (m, 2H, C$\mathbf{H_2}$CH$_2$O), 1.23 (m, 18H, (C$\mathbf{H_2}$)$_9$), 0.81 (tr, 3H, C$\mathbf{H_3}$). ^{13}C NMR ($CDCl_3$): δ 201.42 (**C**HO), 77.26 (**C**H$_2$CHO), 71.98, 71.61, 71.14, 71.06, 71.02, 70.94, 70.43 ((**C**H$_2$O)$_7$), 31.52 (CH$_3$CH$_2$**C**H$_2$), 30.05 (5 C:s), 29.91, 29.77 ((**C**H$_2$)$_7$), 26.49 (**C**H$_2$CH$_2$O), 23.11 (CH$_3$**C**H$_2$), 14.56 (**C**H$_3$). MS-DIP-PCI *m/z* (% rel int): 361 $(M + 1)^+$ (7), 360 M^+ (2), 359 $(M - 1)^+$ (1), 331 $(M + 1 - 30)^+$ (2), 317 $(M + 1 - 44)^+$ (22), 175 $((CH_2CH_2O)_3H)^+$ (49), 166 $(C_{12}H_{22})^+$ (28), 131 $((CH_2CH_2O)_2CH_2CHO)^+$ (17), 87 $(CH_2CH_2OCH_2CHO)^+$ (100).

$C_{12}H_{25}(OCH_2CH_2)_4OCH_2CHO$, ***5***. Yield: 40%. FT-IR (neat): 2834 and 2924 cm^{-1} (C−H), 2710 cm^{-1} (C−H, aldehyde), 1736 cm^{-1} (C═O), 1466 cm^{-1} (C−H in $-CH_2-$), 1116 cm^{-1} (C−O). ^1H NMR ($CDCl_3$): δ 9.69 (s, 1H, C**H**O), 4.13 (s, 2H, C$\mathbf{H_2}$CHO), 3.85−3.52 (m, 16H, (C$\mathbf{H_2}$O)$_8$), 3.40 (tr, 2H, CH$_2$CH$_2$C$\mathbf{H_2}$O), 1.55 (m, 2H, C$\mathbf{H_2}$CH$_2$O), 1.22 (m, 18H, (C$\mathbf{H_2}$)$_9$), 0.84 (tr, 3H, C$\mathbf{H_3}$). ^{13}C NMR ($CDCl_3$): δ 201.13 (**C**HO), 77.00 (**C**H$_2$CHO), 71.68, 71.40, 71.19, 71.08, 71.00, 70.89, 70.72, 70.68, 65.98 ((**C**H$_2$O)$_9$), 31.54 (CH$_3$CH$_2$**C**H$_2$), 29.58 (5 C:s), 29.47, 29.35 ((**C**H$_2$)$_7$), 26.10 (**C**H$_2$CH$_2$O), 22.83 (CH$_3$**C**H$_2$), 14.27 (**C**H$_3$). MS-DIP-PCI *m/z* (% rel int): 405 $(M + 1)^+$ (10), 404 M^+ (2), 403 $(M - 1)^+$ (4), 375 $(M + 1 - 30)^+$ (4), 361 $(M + 1 - 44)^+$ (28), 219 $((CH_2CH_2O)_4H)^+$ (73), 175 $((CH_2CH_2O)_3H)^+$ (63), 166 $(C_{12}H_{22})^+$ (91), 133 $((CH_2CH_2O)_2H)^+$ (50), 87 $(CH_2CH_2OCH_2CHO)^+$ (100).

*Dodecyltetraoxyethyleneoxyacetaldehyde and Its Diethyl Acetal (an Alternative Method for the Synthesis of **5**; Scheme 2)*—Sodium hydride (60% in mineral oil, 0.22 g, 5.5 mmol) was stirred in DMSO (dry, 2.0 g) at room temperature for 2 h under nitrogen. Compound **9** (2.0 g 5.5 mmol) was added slowly and the mixture was stirred at room temperature for 30 min. Bromoacetaldehyde diethyl acetal (1.1 g, 5.5 mmol) was added dropwise and the suspension was stirred at room temperature for 48 h. Saturated aqueous $NaHCO_3$ was added and the mixture was extracted with ethyl acetate. The organic phase was dried over $MgSO_4$ and concentrated in a vacuum. The crude product was purified with flash chromatography on a silica gel column eluted with an increasing content of ethyl acetate 0−50% in dichloromethane. Product **6** was obtained in 30% yield.

$C_{12}H_{25}(OCH_2CH_2)_4OCH_2CH(OCH_2CH_3)_2$, ***6***. FT-IR (neat): 2948 and 2840 cm^{-1} (C−H), 1466 cm^{-1} (C−H in $-CH_2-$), 1150 cm^{-1} (C−O). ^1H NMR ($CDCl_3$): δ 4.57 (tr, 1H, C**H**(OCH$_2$CH$_3$)$_2$), 3.63−3.49 (m, 22H, (C$\mathbf{H_2}$O)$_{11}$), 3.40 (tr, 2H, CH$_2$CH$_2$C$\mathbf{H_2}$O), 1.52 (m, 2H,



Scheme 2

$CH_2C\mathbf{H}_2CH_2O$), 1.19 (m, 24H, $(C\mathbf{H}_2)_9$ and $CH(OCH_2C\mathbf{H_3})_2$), 0.84 (tr, 3H, $C\mathbf{H}_3CH_2CH_2$). ^{13}C NMR ($CDCl_3$): δ 101.24 $\mathbf{C}H(OCH_2CH_3)_2$), 71.79, 71.45, 70.78, 70.47 (6 C:s), 69.94 ($(\mathbf{C}H_2O)_{10}$), 62.13 (2 C:s) ($CH(O\mathbf{C}H_2CH_3)_2$), 31.59 ($CH_3CH_2\mathbf{C}H_2$), 29.31 (5 C:s), 29.16, 29.02 ($(\mathbf{C}H_2)_7$), 25.75 ($\mathbf{C}H_2CH_2O$), 22.31 ($CH_3\mathbf{C}H_2$), 14.95 ($CH(OCH_2\mathbf{C}H_3)_2$), 13.70 ($\mathbf{C}H_3CH_2CH_2$). MS-DIP-PCI *m/z* (% rel int): 433 ($(M + 1-45)^+$ (1), 103 $((CH_2CH(OCH_2CH_3)_2)^+$ (100), 117 $((OCH_2CH(OCH_2CH_3)_2)^+$ (7).

Hydrolysis of the Acetal ***6***—The acetal **6** (1.0 g) was hydrolyzed at room temperature with 20% trifluoroacetic acid in 10 mL of dichloromethane with 1 drop of water present. After 2 h the reaction mixture was neutralized with saturated aqueous $NaHCO_3$. The organic phase was washed with water, dried over $MgSO_4$ and concentrated in a vacuum. The aldehyde **5** was obtained by flash chromatography on silica gel column eluted with ethyl acetate:dichloromethane 70:30. The yield of **5** was 80% and the spectral data for **5** were identical with those obtained with the method described above.

Dodecylethoxylated Alcohols (Scheme 3)—NaH (60% in mineral oil, 0.80 g, 5.5 mmol) was stirred in DMSO (dry, 8.0 mL) at room temperature for 30 min. The appropriate glycol $H(OCH_2CH_2)_n$-OH (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 extracted with ethyl acetate and washed with water. The organic phase was dried over $MgSO_4$ and concentrated in a vacuum. The reaction mixture was chromatographed on silica gel eluted with an increasing content of ethyl acetate 4−6% in hexane, followed by 20% methanol in ethyl acetate. The products **7**−**9** were obtained in 60−65% yield each and identified with FT-IR, NMR, and MS.

$C_{12}H_{25}OCH_2CH_2OH$, ***7***. Yield: 66%. FT-IR (neat): 3370 cm^{-1} (O−H), 2850 and 2950 cm^{-1} (C−H), 1466 cm^{-1} (C−H in $-CH_2-$), 1150 cm^{-1} (C−O). ^1H NMR ($CDCl_3$): δ 3.72 (tr, 2H, $C\mathbf{H_2}O$), 3.53 (tr, 2H, $C\mathbf{H_2}O$), 3.42 (tr, 2H, $CH_2CH_2C\mathbf{H_2}O$), 2.11 (s, 1H, O**H**), 1.58 (m, 2H, $CH_2C\mathbf{H_2}CH_2O$), 1.26 (m, 18H, $(C\mathbf{H_2})_9$) 0.85 (tr, 3H, $C\mathbf{H_3}$). ^{13}C NMR ($CDCl_3$): δ 71.68, 71.43 ($(\mathbf{C}H_2O)_2$), 61.85 ($\mathbf{C}H_2OH$), 31.90 ($CH_3CH_2\mathbf{C}H_2$), 29.63 (5 C:s), 29.47, 29.33 ($(\mathbf{C}H_2)_7$), 26.09 ($\mathbf{C}H_2$-CH_2O), 22.66 ($CH_3\mathbf{C}H_2$), 14.05 ($\mathbf{C}H_3$). MS-GC−PCI *m/z* (% rel int): 231 $(M + 1)^+$ (17), 230 M^+ (2), 229 $(M - 1)^+$ (17), 169 $(C_{12}H_{25})^+$ (16), 63 $(HOCH_2CH_2OH_2)^+$ (100).

$C_{12}H_{25}(OCH_2CH_2)_2OH$, ***8***. Yield: 59%. FT-IR (neat): 3467 cm^{-1} (O−H), 2850 and 2950 (C−H), 1466 cm^{-1} (C−H in $-CH_2-$), 1150 cm^{-1} (C−O). ^1H NMR ($CDCl_3$): δ 3.78−3.65 (m, 8H, $(C\mathbf{H_2}O)_4$), 3.47 (tr, 2H, $CH_2CH_2C\mathbf{H_2}O$), 2.85 (s, 1H, O**H**), 1.58 (m, 2H, $CH_2C\mathbf{H_2}$-CH_2O), 1.24 (m, 18H, $(C\mathbf{H_2})_9$), 0.87 (tr, 3H, $C\mathbf{H}_3$). ^{13}C NMR ($CDCl_3$): δ 72.49, 71.56, 70.40, 70.08 ($(\mathbf{C}H_2O)_4$, 61.73 ($\mathbf{C}H_2OH$), 31.86 ($CH_3CH_2\mathbf{C}H_2$), 29.58 (2 C:s), 29.54 (3 C:s), 29.42, 29.29 ($(\mathbf{C}H_2)_7$), 25.99 ($CH_2\mathbf{C}H_2CH_2O$), 22.62 ($CH_3\mathbf{C}H_2$), 14.05 ($\mathbf{C}H_3$). MS-GC−PCI *m/z* (% rel int): 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).

$C_{12}H_{25}(OCH_2CH_2)_4OH$, ***9***. Yield: 57%. FT-IR (neat): 3374 cm^{-1} (O−H), 2847 and 2948 cm^{-1} (C−H), 1466 cm^{-1} (C−H in $-CH_2-$), 1150 cm^{-1} (C−O). ^1H NMR ($CDCl_3$): δ 3.63−3.56 (m, 16H, $(C\mathbf{H_2}O)_8$), 3.40 (tr, 2H, $CH_2CH_2C\mathbf{H_2}O$), 2.74 (s, 1H, O**H**) 1.55 (m, 2H, $CH_2C\mathbf{H_2}CH_2O$), 1.22 (m, 18H, $(C\mathbf{H_2})_9$), 0.80 (tr, 3H, $C\mathbf{H_3}$). ^{13}C NMR ($CDCl_3$): δ 72.46, 71.45, 70.05, 70.48, 70.47, 70.46, 70.24, 69.93 ($\mathbf{C}H_2O)_8$, 61.57 ($\mathbf{C}H_2OH$), 31.59 ($CH_3CH_2\mathbf{C}H_2$), 29.30 (5C: s), 29.15, 29.01 ($\mathbf{C}H_2)_7$, 25.73 ($CH_2\mathbf{C}H_2CH_2O$), 22.30 ($CH_3\mathbf{C}H_2$), 13.69 ($\mathbf{C}H_3$). MS-GC−PCI *m/z* (% rel int): 363 $(M + 1)^+$ (100), 362 M^+ (9), 361 $(M - 1)^+$ (61), 195 $(HO(CH_2CH_2O)_3CH_2CH_2OH_2)^+$ (54), 177 $((CH_2CH_2O)_4H)^+$ (12), 166 $(C_{12}H_{22})^+$ (8), 133 $((CH_2$-$CH_2O)_3H)^+$ (16), 89 $((CH_2CH_2O)_2H)^+$ (17), 45 $(CH_2CH_2OH)^+$ (28).

Air Exposure of $C_{12}E_5OH$—Two samples of undiluted $C_{12}E_5$-OH were used in the experiment. Sample 1 (5 g) was stored in an open 10 mL Erlenmeyer flask in daylight at room temperature. Sample 2 (5 g) was gently stirred in a open 10 mL Erlenmeyer flask at room temperature 1 h, 4 times a day, mimicking what we considered a normal handling at chemical laboratories and industries. The top of the flasks was covered with aluminum foil to diminish the evaporation and prevent contamination.

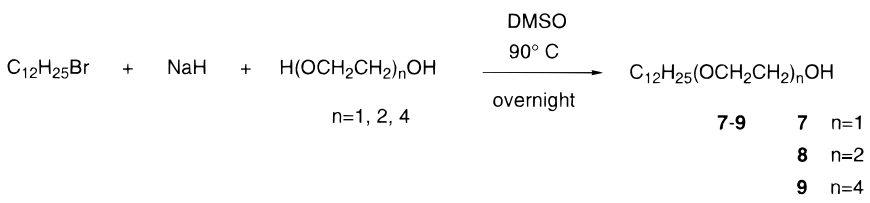
Detection of Oxidation Products in Air-Exposed $C_{12}E_5OH$—The formation of oxidation products in samples 1 and 2 was followed with GC analyses about once a month. Sample aliquots of 10 mg were taken out and diluted to a concentration of 1 mg/mL in dichloromethane. The sample (1 μL) was introduced with the split/splitless technique on the column during a purge time of 2 min. The GC temperature program was as follows: 100 °C was held for 2 min, followed by a linear increase of 15 °C/min up to 270 °C, which was held for 15 min. The injection temperature was 280 °C and the detection temperature was 290 °C. Methyl stearate was used as an internal standard. Identification of the aldehydes in the complex oxidation mixture was performed by addition of the synthesized aldehydes **1**−**5** to a sample of the mixture. The total amount of aldehydes in the oxidation mixture after 7 months of air exposure was evaluated using the standard addition method: Known and different amounts of a standard solution of the synthesized aldehydes (**1**−**5**) were added to samples of the oxidation mixture. Methyl stearate was used as internal standard. The samples were diluted to the same volume. The concentration of the different aldehydes was calculated by extrapolation of the obtained regression lines to the zero response of the *y*-axis, giving a concentration on the *x*-axis, which corresponds to the amount of the aldehydes **1**−**5**, respectively.

Isolation of 5 from Air-Exposed $C_{12}E_5OH$—Material from sample 2 of air-exposed $C_{12}E_5OH$ was chromatographed on a 15 × 15 cm silica TLC plate developed in ethyl acetate:dichloromethane 1:1. The silica gel in the area corresponding to the reference **5** was scraped off the plate and eluted with diethyl ether. An FT-IR spectrum of the material obtained was recorded.

Studies on the Sensitizing Capacity of 5—The sensitization experiments were carried out 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 sensitization studies were performed according to the cumulative contact enhancement test (CCET)[13] method in a modified form with closed epidermal challenge testing.[14] Two exposed and two control groups were used. At induction the animals received an occlusive epidermal application on the shaved upper back on days 0, 2, 7, and 9. The exposed groups, 1 and 3, were induced with **5** in a concentration of 10% w/w (2.5×10^{-4} mol/g) in water. The control groups, 2 and 4, were induced with water. About 200 mg of the test material (test substance in water or water alone) was applied on pieces of filter paper (4 × 2 cm) at each of the four inductions. Groups 3 and 4 were given Freund's complete adjuvant, FCA, intradermally according to the original test protocol.[13] The FCA injections were omitted for groups 1 and 2. FCA is an immune system enhancer commonly used to stimulate the unspecific immune response and raise the sensitivity of experimental methods.

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) according to earlier experiences.[14,15] The animals were tested with **5** in the concentrations 1, 0.1, 0.01, and 0.001% w/w (2.5×10^{-5}, 2.5×10^{-6}, 2.5×10^{-7}, and 2.5×10^{-8} mol/g) in water and with a vehicle control (Table 1). A 15 μg sample of the test material was applied in each chamber. The chambers were removed after 24 h and the reactions were assessed at 48, 72, and sometimes also 96 h after start of the exposure. The minimum criterion for a positive reaction was a confluent erythema. Prior to the experiments, pretests were performed in untreated and FCA-treated guinea pigs in dilution series of **5** in water in the concentration range 100−1% (w/w) and 10−0.01% (w/w), respectively, to obtain the lowest irritating and the highest nonirritating concentrations.

Rechallenge was performed on the right flank with 10, 5, and 1% w/w of **5** in water on day 35 (Table 2). A second rechallenge



Scheme 3

Table 1—Results of Sensitization Studies on 5 in Guinea Pigs Using the CCET Method with and without Adjuvant

	no. of animals with positive reaction after exposure[b]				
	5 (% w/w in water)				
guinea pigs	1%	0.1%	0.01%	0.002%	water
group 1[a]					
exposed ($n = 15$)					
48 h	12[c]	3	2	3	1
72 h	11[c]	3	2	1	0
96 h	9[d]	4	2	0	0
group 2[e]					
controls ($n = 12$)					
48 h	3	1	2	2	0
72 h	2	0	1	1	0
96 h	2	1	1	2	0
group 3[f]					
exposed ($n = 15$)					
48 h	8	3	1	0	1
72 h	10[c]	3	1	0	1
96 h	9[c]	3	2	0	0
group 4[g]					
controls ($n = 15$)					
48 h	4	2	1	1	3
72 h	2	1	0	0	1
96 h	2	2	2	0	4

[a] Induction: 10% of **5** in water, no adjuvant stimulation. [b] The figures are the number of animals with positive reactions 48, 72, and 96 h after application of the test material (% w/w of **5** in water). [c] Significantly different from the controls, $p < 0.01$. [d] Significantly different from the controls, $p < 0.05$. [e] Controls to group 1, no adjuvant stimulation. [f] Induction: 10% of **5** in water, with adjuvant stimulation. [g] Controls to group 3, with adjuvant stimulation.

Table 2—Rechallenge 1 in the Sensitization Studies on 5 in Guinea Pigs

	no. of animals with positive reaction after exposure[b]			
	5 (% w/w in water)			
guinea pigs	10%	5%	1%	water
group 1[a]				
exposed ($n = 14$)				
48 h	8	7[c]	6	0
72 h	8	8[d]	8[d]	1
96 h	10	9[e]	6	1
group 2[f]				
controls ($n = 12$)				
48 h	8	0	2	1
72 h	5	0	1	0
96 h	7	0	1	0
group 3[g]				
exposed ($n = 15$)				
48 h	11	8[h]	6[c]	1
72 h	11[h]	9[c]	8[c]	0
96 h	14[d]	11[c]	9[c]	0
group 4[i]				
controls ($n = 15$)				
48 h	6	2	0	0
72 h	4	1	1	0
96 h	8	3	1	0

[a] Induction: 10% of **5** in water, no adjuvant stimulation. [b] The figures are the number of animals with positive reactions 48, 72, and 96 h after application of the test material. [c] Significantly different from the controls, $p < 0.01$. [d] Significantly different from the controls, $p < 0.02$. [e] Significantly different from the controls, $p < 0.001$. [f] Controls to group 1, no adjuvant stimulation. [g] Induction: 10% of **5** in water, with adjuvant stimulation. [h] Significantly different from the controls, $p < 0.05$. [i] Controls to group 3,with adjuvant stimulation.

of all animals was performed on day 63. The exposed groups, 1 and 3, received an epidermal booster dose of **5** (10% w/w in water) 10 days before the second retesting in order to stimulate the specific T-lymphocytes that were developed in the sensitization phase by **5**. Since this rechallenge was performed a long time after start of the experiment, 10 new sham-treated control animals were used to exclude the possibility of sensitized control animals in the two control groups that already had been exposed to the test material at two different occasions. Testing was performed with **5** (1% w/w) and its synthesized homologues in equimolar concentrations: **4** (0.9% w/w), **3** (0.8% w/w), **2** (0.7% w/w), and formaldehyde (0.3% w/w) in water (Table 3). The experiments were approved by the local ethical committee.

Statistical Analyses—The number of positive reactions observed in each group of animals was used in the statistical analysis with Fisher's exact test.

MS Analysis of 5 in the Water Vehicle—A solution of **5** in water was analyzed using the mass spectrometric electrospray ionization technique (MS-ESI) on an AutoSpec-ESI MS instrument from Micromass (Täby, Stockholm). The sample was dissolved in water:methanol 1:1 in a concentration of 20 pmol/μL. Acetic acid (1%) was added to promote the ionization to positive ions. The MS/MS spectrum was recorded for selected ions to achieve more structural information with collision-induced dissociation (CID) using xenon as collision gas. Detection was made with a time-of-flight mass analyzer.

Results

Spectral Characteristics—In the FT-IR spectra a specific resonance due to the aldehyde function was observed at 1736−1740 cm^{-1} for the synthesized aldehydes **1**−**5**. MS analyses in the EI-DIP mode did not yield any molecular ions of the ethoxylated aldehydes. In the PCI-DIP mode the molecular ion M^+ and the protonated molecular ion $(M + 1)^+$ ion were observed together with an extensive fragmentation pattern (Figure 1). No further adducts with methane, $(M + C_2H_5)^+$ and $(M + C_3H_5)^+$, were seen. $(M - 1)^+$ ions and $(M - 29)^+$ and $(M - 43)^+$ fragments were seen for **1**−**5**, corresponding to the specific α-cleavage, β-cleavage, and McLafferty rearrangement of aldehydes. These data, together with the FT-IR and NMR data are consistent with the structures $C_{12}H_{25}(OCH_2CH_2)_n$-$OCH_2CHO$, $n = 1-4$ (Scheme 1).

The PCI-DIP spectrum of diethyl acetal **6** showed *m*/*z* 433 in a low abundance, corresponding to loss of one of the ethoxy groups from the molecular ion.

The major fragments seen were *m*/*z* 103 and 117 due to cleavage of the ethyleneoxide chain including the diethyl acetal group. In the GC−MS-PCI analyses of the synthesized alcohol ethoxylates **7**−**9**, the molecular ion M^+, $(M + 1)^+$, and $(M - 1)^+$ ions were observed. Intensive fragments from cleavage of the ethyleneoxide chain were also detected. These data, together with the FT-IR and NMR data are consistent with the structures **6**−**9** (Schemes 2 and 3).

MS-ESI Analysis of 5 in Water Used in the Sensitization Experiments—The analysis of the aldehyde dissolved in water using the electrospray ionization tech-

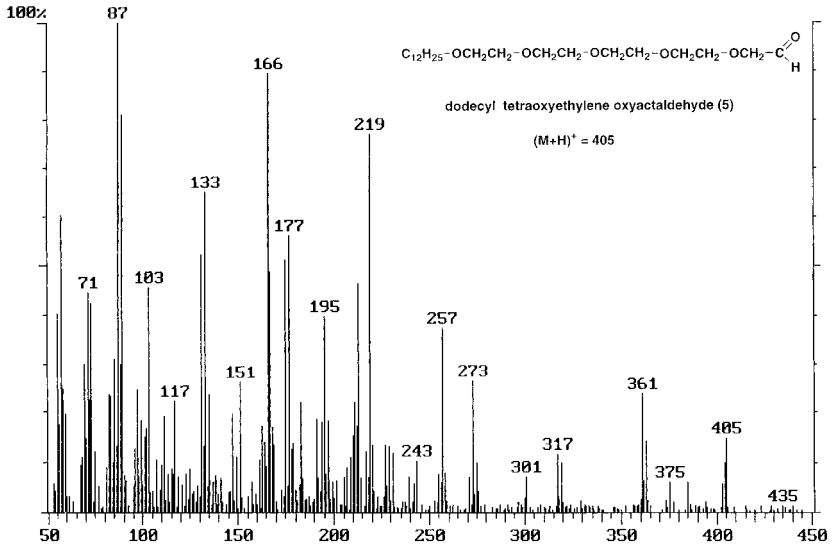


Figure 1—MS–PCI spectrum showing the fragmentation pattern of **5** introduced into the ion source via the direct insertion probe.

Table 3—Rechallenge 2 of Groups 1 and 3 with Synthesized Homologues, 2, 3, 4, and Formaldehyde (HCHO)[i]

	no. of animals with positive reaction after exposure[b]						
	% w/w in water						
guinea pigs	1% **5**	0.9% **4**	0.8% **3**	0.7% **2**	0.3% HCHO	0.1% HCHO	water
group 1[a] ($n = 14$)							
48 h	3	0	0	0	0	0	0
72 h	9[c]	3	2	1	0	0	0
96 h	10[c]	4	0	0	0	0	0
group 3[d] ($n = 15$)							
48 h	7[c]	3	3	1	0	1	0
72 h	10[e]	4	3	0	0	1	1
96 h	10[f]	6[g]	4	2	0	0	0
controls[h] ($n = 10$)							
48 h	1	0	0	0	0	1	1
72 h	1	0	0	0	1	0	0
96 h	1	0	0	0	0	0	0

[a] Induction: 10% of **5** in water, no adjuvant stimulation. [b] The figures are the number of animals with positive reactions 48, 72, and 96 h after application of the test material. [c] Significantly different from the controls, $p < 0.01$. [d] Induction: 10% of **5** in water, with adjuvant stimulation. [e] Significantly different from the controls, $p < 0.002$. [f] Significantly different from the controls, $p < 0.02$. [g] Significantly different from the controls, $p < 0.05$. [h] New sham-treated controls, no adjuvant stimulation. [i] A 10% booster dose of the aldehyde 5 in water was given 10 days before the rechallenge.

nique yielded m/z 405 $(M + 1)^+$ and 422 $(M + 18)^+$ as dominating ions. $(M + 18)^+$ is due to addition of water to a geminal diol in equlibrium with the aldehyde in water. Also, fragments from adducts with sodium ions $(M + Na)^+$ were seen in the MS/MS spectra.

Identification of Oxidation Products in $C_{12}E_5OH$ after Air Exposure—Air oxidation of $C_{12}E_5OH$ produced a mixture of the aldehydes **1**−**5** with **5** as the dominating aldehyde, which were all detected with GC (Figure 2). The peaks in the chromatograms were identified by addition of the synthesized references of **1**−**5** to the oxidized $C_{12}E_5$-OH, yielding an increased signal at the specific retentions times of the aldehydes. The FT-IR spectrum of the aldehyde fraction from the TLC showed absorption resonances identical to that of reference **5**. After 7 months of air exposure the oxidation mixture contained 1.2% of **5**. The calculation based on the standard addition technique gave a linear relationship with correlation coefficients >99% between the response and concentration of each aldehyde in the sample matrix. The coefficient of variation (CV) was below 10% at repeated measurements ($n = 10$). Duplicate analyses were performed on each sample. The total amount of the identified aldehydes after 7 months of air exposure was about 2% (w/v) in sample 1 and 3% (w/v) in sample 2 calculated as percentage of starting material. Minor amounts of the identified oxidation products were also detected in a sample from a just opened ampule (Figure 2).

Sensitizing Capacity of 5—A significant response was observed to **5** (1%), after 72 and 96 h at the first challenge (Table 1). A significant response to **5** (5% and 1%) was also seen at rechallenge (Table 2). There was no significant difference between the two exposed groups (1 and 3) as a result of adjuvant stimulation. A dose−response relationship was observed in both groups. Some irritation was seen to the higher concentrations of **5**. The results were confirmed at a second rechallenge (Table 3). Furthermore, positive reactions were observed when the animals were tested with the homologues **2**, **3** and **4**, but a significant reactivity was found only to **4**. No significant reactions were observed to formaldehyde. Some irritation was seen in the controls.

Discussion

This study is part of our investigation of the allergenic activity of ethoxylated surfactants. Air exposure of the pure ethoxylated alcohol $C_{12}H_{25}(OCH_2CH_2)_5OH$ ($C_{12}E_5OH$) results in a number of oxidation products, as illustrated by the gas chromatograms (Figure 2). One of the major peaks in the chromatogram was identified as dodecyltetraoxyethyleneoxyacetaldehyde, **5**. This compound is formed by oxidation of the terminal oxyethylene unit of $C_{12}E_5OH$. We therefore synthesized the other possible dodecylpolyoxyethylene aldehydes formed by oxidative cleavage of the polyoxyethylene chain in various positions. Using these reference compounds in the GC analyses of oxidized $C_{12}E_5$-OH we found that the peaks designated 1−5 had the same retention time as the references. The FT-IR spectrum of the TLC fraction, isolated from the oxidation mixture, showed identical absorption resonances with that of the dominating aldehyde **5**. We were thus able to show that atmospheric oxidation of $C_{12}E_5OH$ gave the theoretically possible aldehydes of the general formula $C_{12}H_{25}(OCH_2$-$CH_2)_nOCH_2CHO$ ($n = 0$−4). The chromatograms in Figure 2 illustrate a difference in composition depending on time of exposure to air.

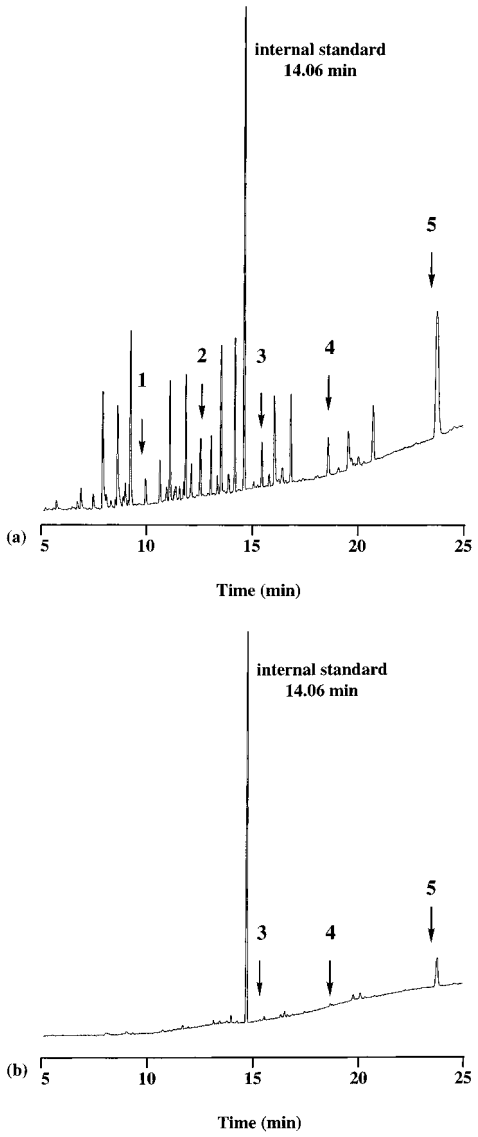


Figure 2—GC chromatograms showing separation of the oxidation products detected in the alcohol ethoxylate $C_{12}E_5OH$, at air exposure: (a) sample 2, handled at room temperature for 4.5 months; (b) a sample from a newly opened ampule. The peaks are assigned with the numbers corresponding to the identified compounds (1–5). Methyl stearate was used as internal standard.

The alcohol ethoxylates are polyethers and as such susceptible to oxidation during storage and handling. The mechanism for this oxidation has earlier been proposed.[2] According to this mechanism hydroperoxides are the primary oxidation products. The polyoxyethylene chain is then cleaved and aldehydes and other compounds are formed.[2] The rate of peroxidation and formaldehyde formation in Tween 80 and in the chemically well-defined alcohol ethoxylate $C_{12}E_5OH$ was presented in prior publications.[7−8]

To be able to form an antigen the molecule has to bind to proteins in the skin. The binding occurs either via a nucleophilic−electrophilic interaction between the foreign compound and nucleophilic groups in the proteins or via a radical mechanism.[16] On the basis of these principles we have in previous studies shown that the formation of hydroperoxides, peroxides, ketones, and other autoxidation products are responsible for the allergenic activity of diterpenes and monoterpenes.[17−19] In this study the sensitizing capacity of the major product **5** was studied experimentally in guinea pigs according to adopted methods, since no alternative in vitro method exists.[20] Aldehyde **5** was shown to be a contact allergen with the same sensitizing capacity as that of formaldehyde. A dose−response relationship was observed in the sensitization studies (Tables 1−3). A significant cross-reactivity was observed between **5** and **4** (Table 3), but no cross-reactivity to formaldehyde, also formed in the oxidation process,[7,8] was seen. Formaldehyde exists in a reversible equilibrium with its diol (98%) in water solutions. The corresponding geminal diol of **5** was seen in the MS-ESI analysis of aldehyde in water. Alcohol has a low allergenic activity. However, antigens are formed with the aldehydes in the equilibrium. Adjuvant addition in order to stimulate the unspecific immune response and raise the sensitivity of the experimental method did not increase the reactivity. Thus, the allergenic activity was obtained only by application of the aldehyde in water on the skin of the animals. Some irritation was observed in the control animals, showing the difficulties when testing with surfactants, since they also have an irritating capacity. However, irritation in some of the control animals cannot normally be avoided, even when the patch test material is considered to be non-irritating.[15] Since surfactants also are irritants, there has been problems with separating irritation from allergic contact dermatitis for clinical patch testing. Patch test studies and configuratory epidemiological observations in man are of interest and are planned.

$C_{12}E_5OH$ could not be synthesized by the same route as the other alcohols, since the corresponding glycol is not commercilly available. Pure $C_{12}E_5OH$, used in the first synthesis, is very expensive. Therefore, to obtain material for the sensitization studies, an alternative synthetic route was applied in which **5** was prepared from alcohol **9** via the formation of an acetal (Scheme 2).

Surfactants have a strong tendency to form aggregates in aqueous solutions above the critical micelle concentration (cmc). Above the cmc the concentration of free monomers is constant. According to the literature, the cmc for $C_{12}E_5OH$ is 0.05 mM (0.002%) and the rate of conversion between monomers and micelles is very fast. Our results demonstrate that the biological response was increased with an increase in the total aldehyde concentration. This indicates that it is not only the concentration of free aldehyde monomers, but also the presence of different aggregates that may be of importance to the biological response observed. Studies of the physical behavior of the aldehyde in relation to the biological response observed are in progress.

Our studies showed that ethoxylated surfactants are easily oxidized upon air exposure. The total amount of aldehydes formed when $C_{12}E_5OH$ was stored at room temperature in daylight for more than 6 months was 2−3%. It cannot be excluded that such concentrations may cause sensitization if present in products that are used and handled daily. The sensitizing capacity of the aldehyde studied was similar to that of formaldehyde, which is a common contact allergen causing dermatitis all over the world.[21] Ethoxylated alcohols have wide applications and are used in, for example, household cleaners, laundry products, and in industrial and institutional cleaners. It should therefore be noted that the presence of contact allergenic oxidation products, i.e., the identified ethoxylated aldehydes and formaldehyde, might cause allergic contact dermatitis in individuals occupationally exposed to these surfactants and water. The total consumption of ethoxylated surfactants was estimated to about 313 000 tons in Western Europe in 1993.[22] Thus, it is important to control the conditions for storage, handling, and transportation of ethoxylated surfactants in order to avoid formation of allergenic oxidation products.

Conclusions

Air oxidation of $C_{12}E_5OH$ produces a mixture of the aldehydes **1**−**5** with **5** as the dominating aldehyde. The structure of the aldehydes formed by air oxidation of a pure ethoxylated surfactant was elucidated, and the compounds were synthesized for the first time. The aldehydes were shown to be contact allergens. The allergens were formed from the surfactant itself and the skin reactions cannot be explained due to any impurities that may be present in a technical quality of the surfactant. The control of the conditions for storage, handling, and transportation of ethoxylated surfactants is important to avoid formation of allergenic oxidation products.

References and Notes

1. Donbrow, M. Stability of the polyoxyethylene chain. In *Surfactant Science Series Volume 23, Nonionic surfactants: Physical chemistry,* Schick, M. J., Ed.; New York, 1987; pp 1011−1067.
2. Decker, C.; Manchal, J. *Makromol. Chem.* **1973**, *66,* 155−178.
3. Effendy, I.; Maibach, H. I. Surfactants and experimental irritant contact dermatitis. *Contact Dermatitis* **1995**, *33,* 217−225.
4. Rycroft, R. J. G. Occupational contact dermatitis. In *Textbook of Contact Dermatitis*, 2nd ed.; Rycroft, R. J. G., Menné, T., Frosch, P. J., Eds.; Springer-Verlag.: Berlin, 1995, pp 343−357.
5. Dooms-Goossens, A.; Blockeel, I. Allergic Contact Dermatitis and Photoallergic Contact Dermatitis Due to Soaps and Detergents. *Clin. Dermatol.* **1996**, *14,* 67−76.
6. Pasche-Koo, F.; Piletta, P−A.; Hunziker, N.; Hauser, C. High sensitization rate to emulsifiers in patients with chronic leg ulcers. *Contact Dermatitis* **1994**, *31,* 226−228.
7. Bergh, M.; Magnusson, M.; Nilsson, J. L. G.; Karlberg, A.-T. Contact allergenic activity of Tween 80 before and after air exposure. *Contact Dermatitis* **1997**, 37, 9−18.
8. Bergh, M.; Magnussson, K.; Nilsson J. L. G.; Karlberg, A−T. Formation of formaldehyde and peroxides by air oxidation of high purity polyoxyethylene surfactants. *Contact Dermatitis* In press, **1998**.
9. Commission Directive 86/199/EEC. In *Official Journal of European Communities,* No. L 149, 03/06/86; p 0038.
10. Jordan, W. P.; Sherman, W. T.; King, S. E. Threshold responses in formaldhyde-sensitive subjects. *J. Am. Acad. Dermatol.* **1979**, *1,* 44−48.
11. Flyvholm, M.-A.; Hall, B. M.; Agner, T.; Tiedemann, E.; Greenhill, P.; Vanderveken, W.; Freeberg, F. E.; Menné, T. Threshold for occluded formaldehyde patch test in formaldehyde-sensitive patients. *Contact Dermatitis* **1997**, *36,* 26−33.
12. Pfitzner, K. E.: Moffatt, J. G. Sulfoxide-Carbodiimide Reactions. 1. A Facile Oxidation of Alcohols. *J. Am. Chem. Soc.* **1965**, *87,* 5661−5670.
13. Tsuchyia, S.; Kondo, M.; Okamoto, K.; Takase, Y. The cumulative contact enhancement test. In *Contact Allergy Test in Guinea Pigs.* Andersen, K. E., Maibach, H. I., Eds.; Karger: Basel, 1985; pp 208−219.
14. Boman, A.; Karlberg A−T.; Wahlberg, J. E. Experiences with Freund's complete adjuvant test (FCAT) when screening for contact allergens in colophony. *Contact Dermatitis* **1988**, *18,* 25−29.
15. Karlberg, A.-T.; Gäfvert, E.; Hagelthorn, G.; Nilsson, J. L. G. Maleopimaric acid−A potent sensitizer in modified rosin. *Contact Dermatitis* **1990**, *22,* 193−201.
16. Basketter, D.; Dooms-Goossens, A.; Karlberg, A−T.; Lepoittevin, J−P. The chemistry of contact allergy: Why is a molecule allergenic? *Contact Dermatitis* **1995**, *32,* 65−73.
17. Karlberg, A−T. Contact allergy to colophony: Chemical identifications of allergens, sensitization experiments and clinical experiences. *Acta Dermato-venereologica* **1988**, *Suppl. 139,* 5−43.
18. Gäfvert, E. Allergenic components in modified and unmodified rosin: Chemical characterization and studies of allergenic activity. *Acta Dermato-venereologica* **1994**, *Suppl. 184,* 1−36.
19. Karlberg, A−T.; Shao, L. P.; Nilsson, U.; Gäfvert, E.; Nilsson, J. L. G. Hydroperoxides in oxidized *d*-limonene identified as potent contact allergens. *Arch. Dermatol. Res.* **1994**, *286,* 97−103.
20. Anon. Skin sensitization. In *OECD Guidelines for testing of chemicals.* No. 406. Paris, OECD, 1993; pp 1−9.
21. Andersen, K. E.; Burrows, D.; White I. R. Allergens from the Standard Series. Occupational contact dermatitis. In *Textbook of Contact Dermatitis*, 2nd ed., Rycroft, R. J. G., Menné, T., Frosch, P. J., Eds.; Springer-Verlag: Berlin, 1995; pp 438−441.
22. Modler, F.; Willhalm, R.; Yoshida, Y. Surfactants, household detergents and their raw material. In *CEH (Chemical Economics Handbook),* Stanford Research Institute, 1994; pp 583.8001A−583.8003J.

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