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Emollient, humectant, and fluorescent α,β-unsaturated thiol esters for long-acting skin applications

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ABSTRACT

We describe compounds in which an emollient or a humectant bears an α,β-unsaturated thiol ester capable of reacting with nucleophilic amino acids in stratum corneum proteins. These compounds should serve as long-lasting moisturizers for skin. The emollient derivatized was octadecyl propanoate, and the humectant was poly(ethylene glycol). These hydrophobic and hydrophilic compounds, as well as a fluorescent, dansyl-containing thiol ester, were found to react within minutes with the thiol *N*-acetylcysteamine upon addition of a catalytic amount of an organic base in chloroform. The structures of the products resulting from conjugate addition to the unsaturated thiol esters were determined by NMR spectroscopy. In the case of the α,β,γ,δ-unsaturated (sorboyl) thiol ester, both the 1,4-addition product and the β,γ-unsaturated-1,6-addition product formed, followed by diadduct. An in vivo test of the fluorescent α,β-unsaturated thiol ester showed that this compound persisted on skin for 3 weeks vs. 6 days for the non-bonding control compound.

1. Introduction

Moisturizers for skin are typically composed of emollients and humectants. Emollients are hydrophobic compounds that provide an occlusive barrier to prevent water loss, whereas humectants are hydrophilic compounds that hydrate the stratum corneum. Glycerin [1], which penetrates and hydrates skin, as well as maintains stratum corneum lipids in a non-crystalline state, is typically added as well. Moisturizers are used to relieve symptoms of dry skin (xerosis) and to restore normal barrier function to skin [2,3]. In addition, moisturizer use has been found to reduce irritant contact dermatitis [4]. The effect of a single application of moisturizer is typically short lived. Repeated applications of moisturizers, particularly ones with a high (>15%) glycerol content, have been found to persist for as long as 4 days [5].

The skin is rich in nucleophiles such as sulfhydryl and amino groups that could be used for attachment of beneficial agents such as humectants and moisturizers to skin, thereby affording resistance to loss by washing and mild abrasion. For reaction with skin nucleophiles, we have selected the α,β-unsaturated thiol ester functional group. As a Michael acceptor, this group is capable of covalent bonding to nucleophilic groups that add to the C=C conjugated to the thiol ester C=O group. The structures of the compounds are shown in Fig. 1. We aim to provide moisturizers that persist on the time scale of the sloughing off and renewal of skin cells. In addition to moisturizers, we have also synthesized α,β-unsaturated thiol esters containing a fluorescent moiety that can serve to visualize binding of the thiol-ester-containing agents to skin.

The α,β-unsaturated thiol ester moiety is subject to Michael-type addition by nucleophiles [6,7]. This functional group is more reactive toward Michael-type addition than the corresponding oxygen esters [8]. It is, however, significantly less reactive than typical sulfhydryl-reactive reagents, such as maleimides [9], which would be expected to be too harsh for large-surface-area topical use. In previous studies of α,β-unsaturated thiol esters, we found that reaction with the cysteine thiolate was approximately 300 times faster than that of the lysine amino group at pH 9.8. We found that addition of cysteine and lysine occurred under basic conditions, with the pH-rate profile reflecting the abundance of the reactive species, i.e., the thiolate anion of cysteine and the neutral amino group of lysine [7].

We describe herein the preparation and reactivity of compounds in which an emollient or humectant bears an α,β-unsaturated thiol ester group capable of reacting with proteins in the stratum corneum, the outermost layer of the skin.

2. Materials and methods

2.1. General

Octadecyl 3-mercaptopropionate (OMP; octadecyl 3-sulfanylpropanoate) was a gift from Evans Chemetics (Iselin, NJ) and was

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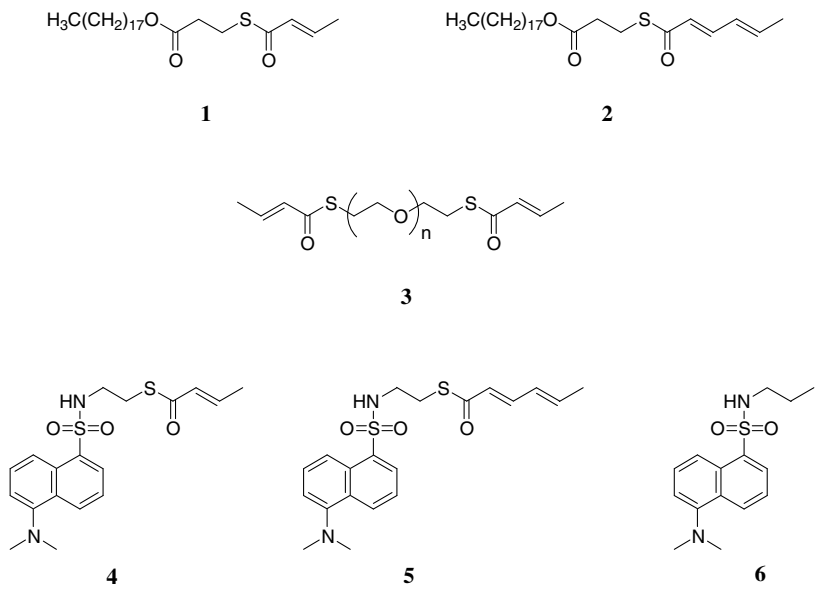


Fig. 1. Compounds studied are shown: emollients **1** and **2**, humectant **3**, fluorescent thiol esters **4** and **5**, and fluorescent control compound **6**.

used without further purification. THF was dried by distillation under N_2 from Na/benzophenone. Pyridine was distilled from BaO and stored over 3 Å molecular sieves. All glassware was dried with a flame before use. NMR spectra were measured with a Varian Gemini spectrometer at 300 MHz for ^{1}H and 75 MHz for ^{13}C, or an Inova spectrometer at 400 or 500 MHz for ^{1}H and 125 MHz for ^{13}C, with tetramethylsilane as internal standard. Thin layer chromatography was carried out with Analtech silica gel GHLF Uniplates.

2.2. Synthesis

2.2.1. Octadecyl 3-crotonoylsulfanylpropanoate (*1*)

To a solution of crotonoyl chloride (13.5 g, 0.129 mol) in 250 mL THF was added dropwise a solution of OMP (35.2 g, 0.098 mol) and pyridine (7.7 g, 0.097 mol) in 75 mL THF. A white solid formed immediately and continued to form. After 1 h at room temperature, the mixture was filtered, and the filtrate was rotavapped to yield 35.6 g of a slurry that solidified to a waxy white solid upon chilling. The crude material was diluted with chloroform and washed twice with dilute sodium bicarbonate, and the organic layer was dried with $MgSO_4$ and rotavapped to yield 28.0 g crude solid (67% yield). This material was purified batchwise by chromatography (silica, 3% hexanes in methylene chloride, $R_f = 0.47$) to yield **1** as a white solid in 25% overall yield, mp 32–34 °C. Anal. Calcd for $C_{25}H_{46}O_3S$: C, 70.37; H, 10.87. Found: C, 70.15; H, 10.82. ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) 0.89 (t, $J = 7.0$ Hz, 3H, $CH_3(CH_2)_{17}$), 1.2–1.4 (m, 30H, $CH_3(CH_2)_{15}$), 1.62 (m, 2H, OCH_2CH_2), 1.88 (d, $J = 6.5$ Hz, 3H, CH_3CH), 2.64 (t, $J = 7.0$ Hz, 2H, SCH_2CH_2), 3.18 (t, $J = 6.5$ Hz, 2H, SCH_2), 4.09 (t, $J = 6.5$ Hz, 2H, CO_2CH_2), 6.13 (d, $J = 15.5$ Hz, 1H, $CH_3CH{=}CH$), 6.91 (dq, $J = 15.0$, 7.0 Hz, 1H, $CH_3CH{=}CH$). ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm) 14.2, 21.7, 22.8, 23.3, 24.3, 26.0, 28.6, 29.3, 29.4, 29.6, 29.7, 29.8, 29.8, 30.7, 32.0, 34.4, 36.4, 38.9, 51.0, 65.1, 170.2, 171.5, 196.9.

2.2.2. Octadecyl 3-sorboylsulfanylpropanoate (*2*)

To a solution of OMP (11.1 g, 31.1 mmol) in 30 mL of cyclohexane at 45 °C was slowly added a solution of sorboyl chloride (4.05 g, 31.0 mmol) in 35 mL of cyclohexane. After 15-h reflux, the solvent was evaporated in vacuo to leave a brown liquid, which was subjected to chromatography (10% ethyl acetate in hexanes). The product **2** ($R_f = 0.55$) was obtained as a white solid, 7.5 g (67%), mp 43.5–45.5 °C. Anal. Calcd for $C_{27}H_{48}O_3S$: C, 71.63; H, 10.69. Found: C, 71.24; H, 10.66. ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) 0.89 (t, $J = 7.0$ Hz, 3H, $CH_3(CH_2)_{17}$), 1.2–1.35 (m, 30H, $CH_3(CH_2)_{15}$), 1.61 (m, 2H, OCH_2CH_2), 1.87 (d, $J = 6.5$, 3H, CH_3CH), 2.63 (t, $J = 7.0$, 2H, SCH_2CH_2), 3.19 (t, $J = 7.5$, 2H, SCH_2), 4.08 (t, $J = 7.0$, 2H, CO_2CH_2), 6.02 (d, $J = 15.0$ Hz, 1H, $CH_3CH{=}CH{-}CH{=}CH$), 6.20 (m, 2H, $CH_3CH{=}CH$), 7.20 (dd, $J = 15.5$, 15.0 Hz, 1H, $CH_3CH{=}CH{-}CH{=}CH$).

2.2.3. Poly(ethylene glycol) dimesylate (*7*)

Poly(ethylene glycol) (PEG) with an average molecular weight (M_n) of 400 was dried by heating at 70 °C under vacuum for 3 h. PEG dimesylate, α-methylsulfonyl-ω-methylsulfonyloxypoly(oxyethylene), was prepared as follows. To a solution of dried PEG (7.89 g, 39.4 mmol OH-ends) and triethylamine (7.94 g, 78 mmol) in methylene chloride at 0 °C was added a fourfold excess of methanesulfonyl chloride (17.9 g, 156 mmol) dissolved in methylene chloride. After 3 h, the reaction mixture was rotavapped to a syrup, taken up in water, and the unreacted methanesulfonyl chloride was destroyed by addition of $NaHCO_3$. The product was then extracted with chloroform, whereupon the solution was dried with $MgSO_4$, and rotavapped to give **7** as a light yellow oil (9.96 g, 95%). ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 3.07 (s, 6H), 3.6 (m, 28H), 3.8 (m, 4H), 4.4 (m, 4H).

2.2.4. Poly(ethylene glycol) dithiol (*8*)

A solution was prepared containing **7** (5.0 g, 18 mmol), diethylenetriaminepentaacetic acid (53.8 mg, 0.05% by wt of reaction mixture), and thiourea (2.85 g, 37 mmol) in water (100 mL), and it was adjusted to pH 6.7 with aqueous potassium hydroxide. The reaction mixture was refluxed for 2.5 h. The reaction mixture was then cooled, and the resulting isothiouronium salt was hydrolyzed by addition of dilute aqueous $NaHCO_3$ (2.35 g, 1.5 equiv) followed by 1.5 h of reflux. The solution was then neutralized with 1 M H_2SO_4, and the PEG dithiol, α-sulfanylethyl-ω-sulfanylpoly(oxyethylene), was extracted into chloroform, dried with $MgSO_4$ and rotavapped to yield 2.18 g of **8** as a light amber oil. The material was stored under argon at −20 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 1.59 (t, $J = 6.8$ Hz, 2H), 2.69 ppm (dt, $J = 5.2$ Hz, 6.8 Hz, 4H), 2.88 ppm (t, $J = 5.2$ Hz, <1H, trace disulfide), 3.63 (m, 32H), 3.74 (m, 4H).

*2.2.5. α-Crotonoylsulfanylethyl-ω-crotonoylsulfanylpoly(oxyethylene) (**3**)*

A solution of **8** (2.1 g, 9.0 mmol SH-ends) and pyridine (0.656 g, 8.3 mmol) in 10 mL THF was added dropwise to a stirred solution of crotonoyl chloride (0.857 g, 8.5 mmol) in 20 mL THF. The solution instantly became cloudy. After 16 h, the reaction was rotavapped to dryness and the residue was taken up in chloroform, which was washed extensively with aqueous $NaHCO_3$ and dried with Na_2SO_4 to give α-crotonoylsulfanylethyl-ω-crotonoylsulfanylpoly(oxyethylene) **3** as an amber oil (1.7 g, 70%). ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 1.88 (dd, J = 7.6, 1.7 Hz, 6H, $2CH_3$), 3.15 (t, J = 6.6 Hz, 4H, $2SCH_2$), 3.6–3.7 (m, $(OCH_2CH_2)_nO$), 3.68 (t, J = 7.2 Hz, 4H, $2CH_2OCH_2CH_2S$), 6.14 (dd, J = 15.3 Hz, 1.8 Hz, 2H, $2CH_3CH{=}CH$), 6.91 (dq, J = 15.3, 7.2 Hz, 2H, $2CH_3CH{=}CH$).

*2.2.6. N,N′-Bis(5-dimethylaminonaphthalene-1-sulfonyl)-cystamine (**9**)*

Dansyl chloride (4.0 g, 14.8 mmol) was dissolved in a solution of 900 mL acetone and 30 mL water, and a solution of cystamine dihydrochloride (1.66 g, 7.4 mmol, 1 equiv) in 100 mL 0.1 M aqueous $NaHCO_3$ was added in portions. The solution was maintained at pH 7.5 by addition of dilute aqueous sodium hydroxide. After 2 h at room temperature, the reaction mixture was diluted with 400 mL chloroform, and the solution was washed four times with aqueous sodium bicarbonate, followed by water, whereupon it was dried with $MgSO_4$. Solvent was removed to yield 3.86 g of **9** as a fluffy, crisp yellow solid (84%), R_f = 0.73, 5% methanol in chloroform; R_f = 0.20, 30% acetone in hexane, mp 71–72 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) 2.50 (t, J = 6.5 Hz, 4H), 2.89 (s, 12H, $2N(CH_3)_2$), 3.10 (d, J = 6.5, 5.5 Hz, 4H, $2CH_2N$), 5.23 (br t, 2H, 2NH), 7.18 (d, J = 7.5 Hz, 2H), 7.53 (m, 4H), 8.25 (m, 4H), 8.55 (d, J = 8.5 Hz, 2H).

*2.2.7. 5-(Dimethylamino)-N-(2-sulfanylethyl)naphthalene-1-sulfonamide (**10**)*

Reduction of the disulfide **9** was carried out as follows. Compound **9** (1.96 g, 3.25 mmol) dissolved in 400 mL absolute ethanol was treated with 11.2 g finely powdered zinc and 25 mL glacial acetic acid, added alternately in portions. After 4 h, the reaction mixture was filtered to remove zinc and zinc salts, and the filtrate was rotavapped to a volume of 50 mL. This was then diluted with chloroform, and the organic phase was washed three times with water, once with dilute aqueous $NaHCO_3$ followed by brine, whereupon it was dried with $MgSO_4$ and rotavapped to yield 1.5 g (76%) of a greenish-yellow film (30% acetone in hexane, R_f = 0.44). ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 1.2 (t, J = 8.5 Hz, 1H), 2.50 (m, 2H, CH_2S), 2.90 (s, 6H, $N(CH_3)_2$), 3.08 (dt, J = 5.5, 6.5 Hz, 2H, CH_2N), 5.15 (broad t, 1H, NH), 7.20 (d, J = 7.5 Hz, 2H), 7.56 (m, 2H), 8.27 (m, 2H), 8.55 (d, J = 8.5 Hz, 1H).

*2.2.8. N-[2-(Crotonoylsulfanyl)ethyl]-5-(dimethylamino)naphthalene-1-sulfonamide (**4**)*

Thiol **10** (1.5 g, 5.0 mmol) obtained from 2.2.7 was dissolved in 100 mL of freshly distilled THF containing pyridine (0.930 g, 11.8 mmol), and the solution was added dropwise to a stirred solution of crotonoyl chloride (3.92 g, 37.5 mmol) in 30 mL THF. After 4 h, the solvent was removed in vacuo, and the residue was taken up in chloroform. The resulting solution was washed with dilute $NaHCO_3$ followed by brine, and dried with Na_2SO_4. The crude product was purified by flash chromatography on silica (30% acetone in hexane, R_f = 0.41) to yield pure compound **4** as a sticky greenish film (1.47 g, 80%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.87 (dd, J = 6.5 Hz, 1.5 Hz, 3H, CH_3CH), 2.90 (s, 6H, $N(CH_3)_2$), 2.95 (t, J = 6.5 Hz, 2H, CH_2S), 3.13 (dt, J = 6.5, 6.0 Hz, 2H, NCH_2), 5.04 (t, J = 6.0 Hz, 1H, NH), 6.02 (dd, J = 15.5, 1.5 Hz, 1H, $CH_3CH{=}CH$), 6.83 (dq, J = 15.0, 7.0 Hz, 1H, $CH_3CH{=}CH$), 7.18 (d, J = 7.5 Hz, 1H arom), 7.55 (m, 2H, arom) 8.25 (m, 2H, arom), 8.55 (d, J = 8.5 Hz, 1H, arom). Anal. Calcd for $C_{18}H_{22}N_2O_3S_2$: C, 57.12; H, 5.86; N, 7.40. Found: C, 57.02; H, 5.94; N, 7.33.

*2.2.9. 5-(Dimethylamino)-N-[2-(sorboylsulfanyl)ethyl]naphthalene-1-sulfonamide (**5**)*

Compound **5** was prepared according to the procedure described in Section 2.2.8 (synthesis of **4**) by use of sorboyl chloride instead of crotonoyl chloride. Purification of the crude material by flash chromatography (40% acetone in hexanes, R_f = 0.55) produced **5** as a greenish film (42% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.88 (dd, J = 6.5 Hz, 1.5 Hz, 3H, CH_3CH), 2.89 (s, 6H, $N(CH_3)_2$), 2.96 (t, J = 6.5 Hz, 2H, CH_2S), 3.12 (dt, J = 6.5 Hz, 6.0 Hz, 2H, NCH_2), 5.07 (t, J = 5.5 Hz, 1H, NH), 5.93 (d, J = 15.5 Hz, 1H, $CH_3CH{=}CH{-}CH{=}CH$), 6.0–6.2 (m, 2H, $CH_3CH{=}CH$), 7.08 (dd, J = 15, 11 Hz, 1H, $CH_3CH{=}CH{-}CH{=}CH$), 7.17 (d, J = 8.0 Hz, 1H, arom), 7.53 (m, 2H, arom), 8.25 (m, 2H, arom), 8.54 (d, J = 8.5 Hz, 1H, arom). Anal. Calcd for $C_{20}H_{24}N_2O_3S_2$: C, 59.38; H, 5.98; N, 6.92. Found: C, 59.56; H, 6.08; N, 6.71.

*2.2.10. 5-(Dimethylamino)-N-propylnaphthalene-1-sulfonamide (**6**)*

A solution of dansyl chloride (0.92 g, 3.4 mmol) and triethylamine (0.47 mL, 3.4 mmol) in 60 mL distilled methylene chloride was treated with propylamine (0.28 mL, 3.4 mmol). The reaction mixture was stirred for 1 h at room temperature. The resulting reaction mixture was washed extensively with water, dilute aqueous $NaHCO_3$, and brine, whereupon it was dried with Na_2SO_4 and rotavapped to a crude oil **6** (0.93 g, 98%). Crude **6** was purified by chromatography (40% acetone in hexanes, R_f = 0.62) to yield **6** as a greenish-yellow solid (mp 92–94 °C). ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.77 (t, J = 6.5 Hz, 3H, CH_3), 1.41 (m, 2H, $CH_2CH_2CH_3$), 2.86 (m, 2H, $CH_2CH_2CH_3$), 2.89 (s, 6H, $N(CH_3)_2$), 4.67 (t, J = 6.0, 1H, NH), 7.18 (d, J = 7.5, 1H, arom), 7.53 (m, 2H, arom), 8.25 (m, 2H, arom) 8.55 (d, J = 8.5 Hz, 1H, arom). Anal. Calcd for $C_{15}H_{20}N_2O_2S$: C, 61.61; H 6.89; N, 9.58. Found: C, 61.45; H, 6.80; N, 9.53.

2.3. Formation and characterization of N-acetylcysteamine adducts

*2.3.1. Reaction of **1** with N-acetylcysteamine*

The reaction of the α,β-unsaturated thiol esters was followed by NMR spectroscopy. To a solution of **1** (22 mg, 0.052 mmol) dissolved in 0.6 mL of $CDCl_3$ was added *N*-acetylcysteamine (5.7 μL, 0.053 mmol). The NMR spectrum was recorded and showed that no reaction had occurred. A catalytic amount (0.1 equiv) of the base DBN was added, and the NMR spectrum was recorded after 5 min. More than 60% of **1** had reacted, as judged by the diminished integration of the resonances at δ 6.1 ppm and 6.9 ppm due to the vinyl protons of the crotonoyl moiety. An NMR spectrum recorded 27 min after DBN addition showed that **1** had completely reacted.

The reaction of **1** was also carried out on a larger scale. To **1** (171 mg, 0.40 mmol) in 10 mL of N_2-purged $CHCl_3$ was added *N*-acetylcysteamine (30 μL, 0.28 mmol), followed by triethylamine (39 μL, 0.28 mmol). The reaction mixture was stirred for 2 h under N_2, after which it was subjected to chromatography (3% methanol in chloroform, R_f = 0.54) to give 38.5 mg adduct as a white waxy solid (26% yield after purification). Anal. Calcd for $C_{29}H_{55}NO_4S_2$: C, 63.81; H 10.16; N, 2.57. Found: C, 63.36; H, 10.07; N, 2.62.

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 0.88 (t, 3H, J = 6.8 Hz, $CH_3(CH_2)_{17}$), 1.22–1.26 (m, 30H, $(CH_2)_{15}$), 1.32 (d, 3H, J = 6.4, CH_3CHCH_2), 1.62 (m, 2H, $CO_2CH_2CH_2$), 2.02 (s, 3H, $NHCOCH_3$), 2.63 (t, J = 6.8 Hz, 2H, SCH_2CH_2CO), 2.70 (t, J = 6.4 Hz, 2H, SCH_2CH_2NH), 2.71 (dd, J = 14.5, 7.8 Hz, 1H, $CH_3CHSCH_aH_b$), 2.82 (dd, J = 15.4, 7.4 Hz, 1H, $CH_3CHSCH_aH_b$), 3.15 (t, J = 7.2 Hz, 2H, SCH_2CH_2CO), 3.27 (dq, J = 14.0, 6.8 Hz, 1H, CH_3CHSCH_2), 3.45 (dt, J = 6.4, 6.0 Hz, 2H, SCH_2CH_2NH), 4.09 (t, J = 6.8 Hz, 2H, $CO_2CH_2(CH_2)_{16}CH_3$), 6.2 (br s, 1H, NH). COSY crosspeaks: δ 0.88/1.2–1.3; 1.2–1.3/1.62; 1.32/3.27; 1.62/4.09; 2.63/3.15; 2.70/3.45; 2.71/2.82; 2.71/3.27; 2.82/3.27; 3.45/6.2. Conjugate addition to the

α,β-unsaturated thiol ester to produce the adduct (R = octadecyloxycarbonylethyl) shown in Fig. 2a was evidenced by the COSY crosspeaks observed between the methyl protons at δ 1.32 ppm and the methine proton at 3.27 ppm, and between this methine proton and the adjacent diastereotopic methylene protons at δ 2.71 and 2.82 ppm.

*2.3.2. Reaction of **2** with N-acetylcysteamine*

In a 50-mL round-bottom flask, 181 mg (0.40 mmol) of compound **2** was dissolved in 10 mL of chloroform, and the solution was purged with nitrogen for 15 min prior to use. After addition of 30 μL (0.28 mmol) of *N*-acetylcysteamine and 39 μL (0.28 mmol) of triethylamine, the solution was stirred under reflux in a nitrogen atmosphere for 2 h. The solvent was evaporated, and an approximately 1:1 mixture of the 1,4- and 1,6-monoadducts was isolated by flash chromatography (silica, 3% methanol in chloroform) 88 mg, 38% yield. Anal. Calcd for $C_{30}H_{57}NO_4S_2$: C, 65.10; H 10.05; N, 2.45. Found: C, 64.84; H, 10.07; N, 2.50. Alternatively, the monoadducts were isolated by preparative TLC (R_f = 0.66, 3% methanol in chloroform). The isolated mixture of monoadducts was applied to another preparative TLC plate, and the monoadducts were separated by repetitive development with 3% methanol in chloroform. The 1,6- and 1,4-addition products are shown in Fig. 2b. 1,6-Addition product: ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 0.89 t, J = 7 Hz, 3H, $(CH_2)_{15}CH_3$), 1.30 (br m, 30H, $(CH_2)_{15}$), 1.31 (d, J = 7 Hz, 3H, CH_3CHSCH), 1.61 (m, 2H, OCH_2CH_2), 2.00 (s, 3H, acetyl), 2.55 (m, 1H, $NHCH_2CHH'$), 2.62 (t, J = 7 Hz, 2H, $OCOCH_2$), 2.65 (m, 1H, $NHCH_2CHH'$), 3.12 (t, J = 7 Hz, 2H, $COSCH_2$), 3.29 (d, J = 7 Hz, 2H, $SCOCH_2$), 3.32 (m, 3H, $CH_3CH(SCH_2CH_2)CH$), 4.10 (t, J = 7 Hz, 2H, $OCH_2(CH_2)_{16}$), 5.42–5.62 (m, 2H, olefinic), 6.00 (br s, 1H, NH). COSY crosspeaks: δ 0.89/1.30; 1.30/1.61; 1.31/3.44; 1.61/4.10; 2.55/2.65; 2.62/3.12; 3.29/5.52; 3.32/5.35; 5.35/5.52. 1,4-Addition product: ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 0.89 (t, 3H, $(CH_2)_{15}CH_3$), 1.30 (br m, 30H, $(CH_2)_{15}$), 1.61 (m, 2H, OCH_2CH_2), 1.71 (d, J = 7 Hz, 3H, CH_3CH=), 2.00 (s, 3H, acetyl), 2.55 (m, 1H, $SCHH'CH_2NH$), 2.65 (m, 1H, $SCHH'CH_2NH$), 2.61 (m, 2H, $OCOCH_2$), 2.79 (d, J = 7 Hz, 2H, $SCOCH_2$), 3.18 (t, J = 7 Hz, 2H, $COSCH_2$), 3.40 (m, 1H, $NHCHH'$), 3.42 (m, 1H, $NHCHH'$), 3.63 (m, 1H, CH_3CH=$CHCH$), 4.08 (t, J = 7 Hz, 2H, $OCH_2(CH_2)_{16}$), 5.28 (m, 1H, olefinic), 5.55 (m, 1H, olefinic), 6.00 (br s, 1H, NH). COSY crosspeaks: δ 0.89/1.30; 1.30/1.61; 1.61/4.10; 1.71/5.55; 2.55/2.65; 2.61/3.18; 2.55–2.65/[3.40–3.42]; 2.79/3.63; 3.40/3.42; 3.63/5.28; 5.28/5.55. When the reaction was carried out in the presence of a fourfold excess of each *N*-acetylcysteamine and triethylamine, the ratio of monoadducts was only slightly changed to 57% 1,6- and 43% 1,4-addition product, and the yield was not significantly changed (31%). When an amine (morpholine, 0.7 equiv) was used in place of the thiol *N*-acetylcysteamine, no addition products were detected. This is not surprising, as we found that amines were ~10^2-fold less reactive than thiols in a similar reaction [7]. When the reaction was carried out in the absence of the N_2 atmosphere, minor byproducts were found that resulted from oxidation of OMP to the symmetrical disulfide, and of OMP with *N*-acetylcysteamine to the mixed disulfide, as well as a product resulting from *N*-acetylcysteamine attack at the sorboyl carbonyl carbon.

*2.3.3. Reaction of **3** with N-acetylcysteamine*

The reaction of **3** with *N*-acetylcysteamine was also followed by NMR spectroscopy: 35.6 mg of **3** was dissolved in 0.7 mL $CDCl_3$, and the spectrum was recorded before and after addition of *N*-acetylcysteamine (1 equiv per crotonoyl moiety), and again after 0.1 equiv of DBN was added. The reaction was complete after 5 min and produced the adduct shown in Fig. 2a, as evidenced by the disappearance of the resonances at δ 6.14 and 6.91 ppm, which correspond to the crotonoyl vinyl protons, and by the upfield shift of the CH_3 doublet from δ 1.88 to 1.28 ppm. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 1.28 (d, J = 6.1 Hz, 6H, $2CH_3CH$), 1.98 (s, 6H, $2NHCOCH_3$), 2.64 (t, J = 6.6 Hz, 4H, $2SCH_2CH_2N$), 2.65 (m, 2H, $2CH_3CHCH_aH_b$), 2.80 (m, 2H, $2CH_3CHCH_aH_b$), 3.1 (m, 4H, $2OCH_2CH_2S$), 3.2– 3.4 (m, 6H, $2CH_3CH$ and $2CH_2NH$), 3.6 (br s, $CH_2CH_2O)_n$, 6.3 (br s, 2H, 2NH). COSY crosspeaks: 1.28/3.4; 2.64/3.2; 2.64/3.4; 2.66/2.8; 2.66/3.4; 2.8/3.4; 3.1/3.6; 3.4/6.3. The COSY spectrum showed the coupling of the terminal methyl group (δ 1.28 ppm) with the adjacent methine proton (δ 3.4 ppm) and from this to the methylene protons (δ 2.65 and 2.80 ppm).

*2.3.4. Reaction of **4** with N-acetylcysteamine*

The reaction of **4** with *N*-acetylcysteamine was also followed by NMR spectroscopy and showed complete reaction within 4 min of addition of 0.3 equiv DBN. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 1.28 (d, J = 7 Hz, 3H, CH_3CH), 1.95 (s, 6H, $N(CH_3)_2$), 1.98 (s, 3H, $NHCOCH_3$), 2.5–2.8 (m, 4H, CH_2S and $CH_3CH_aH_b$), 3.2 (m, 1H, CH_3CH), 3.1 (m, 2H, CH_2NH), 3.4 (m, 2H, CH_2NH), 4.5 (br s, 1H, NH), 6.2 (br s, 1H, NH), 7.17 (d, J = 7.6 Hz, 1H, arom), 7.52 (m, 2H, arom), 8.24 (m, 2H, arom), 8.52 (m, 1H, arom). Conjugate addition to the α,β-unsaturated thiol ester gave the adduct shown in Fig. 2a, as evidenced by the disappearance of the crotonoyl vinyl protons, the upfield shift of the terminal methyl proton doublet from δ 1.87 ppm to 1.28 ppm, the coupling of this to the methine proton at δ 3.2 ppm, and from the methine to the adjacent methylene protons at δ 2.6 ppm. COSY crosspeaks: 1.28/3.2; 3.2/2.7; 2.7/3.4; 3.1/2.6; 7.17/7.52; 7.15/8.2; 7.15/8.52.

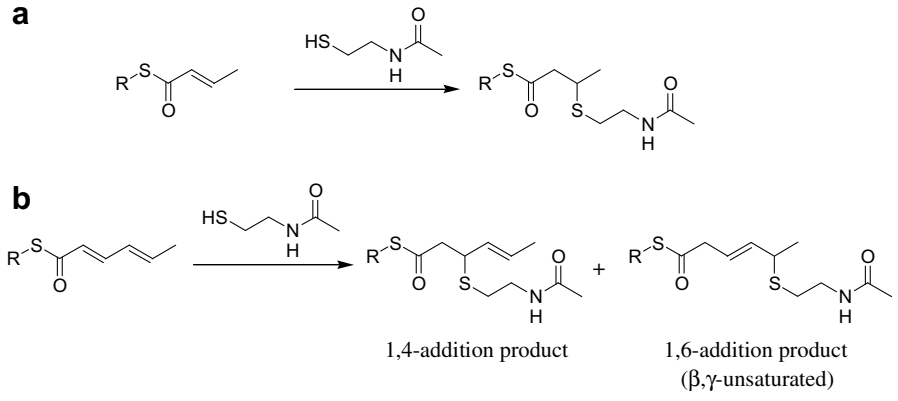


Fig. 2. (a) The crotonoyl thiol esters **1**, **3**, and **4** reacted with *N*-acetylcysteamine in DBN/chloroform to form the adduct shown; R = octadecyloxycarbonylethyl in **1**, ω-crotonoylsulfanylpoly(ethyleneoxy)ethyl in **3**, and dansyl in **4**. (b) Sorboyl thiol ester **2** reacted to form the 1,4- and the β,γ-unsaturated-1,6-addition products with *N*-acetylcysteamine in DBN/chloroform; R = octadecyloxycarbonylethyl.

2.4. In vivo test of skin binding of fluorescent compounds on human skin

Compounds **4**, **5**, and **6** were dissolved in ethanol, and 1 mg each was applied separately to 1-cm^2 patches of skin on the inner arm, previously delineated with Scotch tape. After air drying for ~1 h, the application area was washed with soap and water. The material was not visible on the skin in room light. For the first day only, however, the green color of the compounds was faintly visible in very bright sunlight. The compounds emitted bright fluorescence when illuminated with long-wavelength UV light. The fluorescence was photographed under long-UV irradiation with Kodak Ultra Max 800 speed film. For the duration of the experimental period, the material was subjected to 1–2 soap washings/day, and no other skin products were applied to the test area. No signs of irritation or redness were observed.

3. Results and discussion

3.1. Chemistry

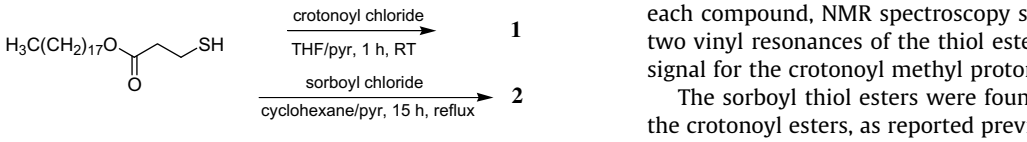
Structures of the compounds studied are shown in Fig. 1. The emollient we chose to derivatize was octadecyl 3-mercaptopropionate, which contains a hydrophobic C_{18} chain and an ester group. This ester group may be responsible for the minimization of the typical thiol odor of thiol hydrocarbons. Two emollients were prepared: **1** with an α,β-unsaturated crotonoyl thiol ester and **2** with the corresponding sorboyl ester. Syntheses of compounds **1** and **2** were accomplished in one step by treatment of the commercially available thiol octadecyl 3-mercaptopropionate with the respective acid chloride, as outlined in Scheme 1.

Poly(ethylene glycol) was chosen as the humectant, as it is hydrophilic, yet non-toxic and non-immunogenic. Additionally, because the poly(ethylene glycol) molecules can be modified with skin-reactive α,β-unsaturated thiol ester moieties at each end, crosslinking of skin proteins may occur and may serve to enhance the persistence on skin and possibly strengthen skin. The dicrotonoyl thiol ester **3** was prepared. Preparation of **3** made use of PEG dithiol, as outlined in Scheme 2.

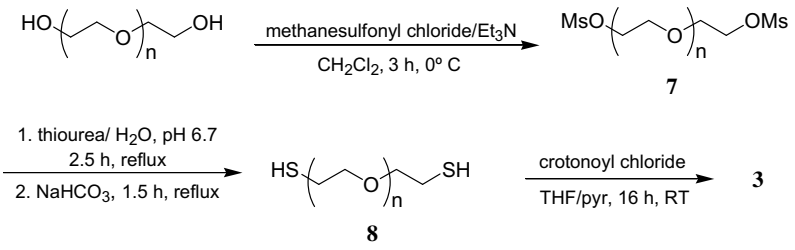
For eventual visualization of binding of the thiol esters on skin, compounds containing the fluorescent dansyl group with an α,β-unsaturated crotonoyl thiol ester **4** and sorboyl thiol ester **5** were synthesized. As a potential control in such studies, compound **6** that has a propyl group in place of the α,β-unsaturated thiol ester and lacks covalent binding capability was prepared, as shown in Scheme 3.

We followed the reaction of crotonoyl thiol esters (**1**, **3**, and **4**) with the simple thiol, *N*-acetylcysteamine, by NMR spectroscopy in $CDCl_3$. In each case, reaction occurred within minutes of addition of 0.1–0.3 equivalents of an organic base, e.g., DBN or triethylamine. Addition of the thiol to the α,β-unsaturated crotonoyl thiol ester produced the Michael-type adduct, as shown in Fig. 2a. For each compound, NMR spectroscopy showed disappearance of the two vinyl resonances of the thiol ester and an upfield shift of the signal for the crotonoyl methyl protons.

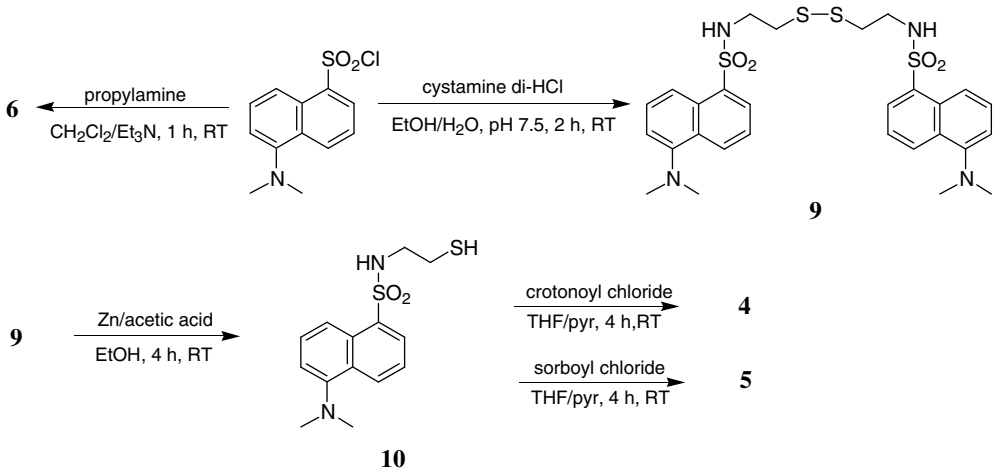
The sorboyl thiol esters were found to react more slowly than the crotonoyl esters, as reported previously in the case of *p*-methoxycinnamoyl-containing thiol esters [7]. For example, in the



Scheme 1.



Scheme 2.



Scheme 3.

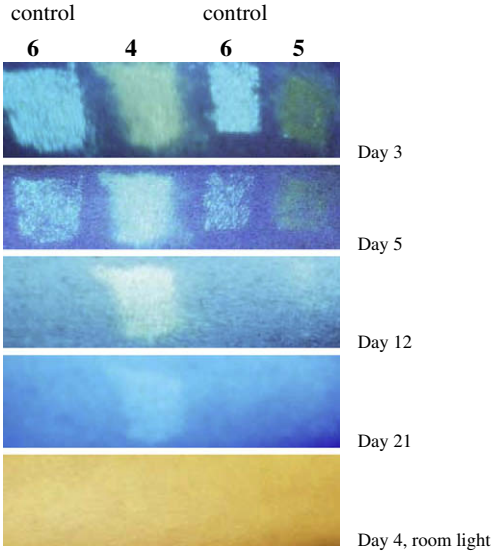


Fig. 3. Photographs of the applied fluorescent compounds **4**, **5**, and **6**, taken over a 3-week period, show the persistence on the skin of the unsaturated thiol esters relative to the non-bonding control compound. A photograph of the application area taken under room light is included.

presence of 2 equivalents of *N*-acetylcysteamine and 1 equivalent of base (DBN), compound **2** reacted after 1.5 h to yield the 1,4- and β,γ-unsaturated-1,6-addition monoadducts, as shown in Fig. 2b. Apparently, nucleophilic attack at the end of the conjugated system is followed by protonation of the intermediate enolate at the carbon α to the carbonyl group. The absence of protonation γ to the carbonyl group, which would produce the α,β-unsaturated-1,6-addition product, implies that there is little if any stabilization due to conjugation of the carbon–carbon double bond to the thiol ester carbonyl group, similar to the case with oxygen esters [6,10]. NMR spectroscopy showed that after 39 h, the monoadducts had further reacted to form a diadduct. To determine the structures of the monoadducts, the reaction was carried out on a 180-mg scale in refluxing chloroform, with 0.7 equivalents of triethylamine, under N_2 atmosphere. The resulting monoadducts were separated by chromatography and identified by two-dimensional NMR. As a comparison, the oxygen ester octadecyl sorbate was prepared [11] and allowed to react with *N*-acetylcysteamine and 0.5 equivalent of DBN in refluxing cyclohexane–chloroform (1:1, v/v). We found that this compound also produced the 1,6-addition product under these vigorous conditions.

The α,β-unsaturated thiol esters were subject to nucleophilic attack by amines and thiols. Nucleophilic groups in skin proteins include cysteine (pK_a 8.55 and lysine (NH_3^+ pK_a 10.4), tyrosine (ArOH pK_a 9.6), histidine (NH^+ pK_a 6.5) and the N-terminal amino group (NH_3^+ pK_a 8.0) in their deprotonated forms [12]. Cysteine thiolate is more reactive than the lysine amino group, but lysine is generally more abundant in proteins. Measurements of the amino acid composition of skin proteins estimate cysteine at 1–5% and lysine at 5–6% [13].

Studies of the localization of cysteine thiol and disulfide groups in skin have been carried out. Fluorescence staining of thiol groups in human plantar skin sections revealed thiols to be abundant both in the lower layers of skin and in the stratum corneum. The distribution of thiol groups was moderately high in the mid-stratum corneum, and it decreased gradually on the way up to the surface of the skin. Disulfide groups were not found in the lower layers but were abundant in the horny layer [14]. A study of frozen sections of forearm skin gave similar results; thiols were most abundant in the lower stratum corneum, and disulfide groups were evenly distributed throughout the stratum corneum [15]. Broekaert and colleagues (1982) also reported free thiols in the inner stratum corneum, which remained throughout the entire horny layer [16].

3.2. Biology

The compounds dissolved in ethanol were applied to skin (1 mg/cm^2). No redness or irritation was observed, either immediately or during the entire course of the experiment. The compounds were virtually invisible under normal room light but fluoresced brightly under long-wavelength UV, although the fluorescence of **5** was noticeably less intense than **4** or **6**.

We found that fluorescence of the α,β-unsaturated thiol ester-bearing compound (**4**) was visible on the skin for at least 21 days, and that of the α,β,γ,δ-unsaturated (sorboyl) thiol ester (**5**) was visible on the skin for 12 days (although it was very light). Fluorescence of the control compound (**6**) faded more quickly and was visible for only 6 days. Photographs of the skin fluorescence are shown in Fig. 3.

4. Conclusion

Aliphatic emollients, poly(ethylene glycol)-derived humectants, and a dansyl chromophore bearing an α,β- or α,β,γ,δ-unsaturated (sorboyl) thiol ester have been synthesized and found to be reactive toward an amino acid model compound, *N*-acetylcysteamine, under mildly basic conditions. The crotonoyl thiol esters underwent conjugate addition within minutes of treatment with a catalytic amount of base. The corresponding sorboyl thiol esters reacted more slowly and formed the 1,4- and 1,6-monoaddition products, which under prolonged conditions further reacted to give the diadduct. An in vivo test showed that the unsaturated thiol esters persisted on the skin for up to 3 weeks, compared to 6 days for the non-bonding control compound.

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