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SYNTHESIS OF IONIC BIOLOGICALLY ACTIVE CONJUGATES FROM TROLOX AND α-TOCOPHEROL SUCCINATES

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Six ionic conjugates with nitroxyl radical amino-TEMPO and diethanolamine as the amines were synthesized from trolox and α-tocopherol succinates. The water solubility of the synthesized ammonium salts was determined. It was shown that formation of the trolox succinate salts increased the water solubility whereas this phenomenon was not observed for α-tocopherol succinates.

Keywords: trolox, α-tocopherol, succinates, diethanolamine, nitroxyl radical amino-TEMPO, ammonium salts, ionic conjugates, water solubility.

Currently, 60% of marketed drugs are highly soluble in H_2O whereas 80% of new drugs in various research and development stages are poorly soluble in H_2O. This reduces their bioavailability, prevents them from entering the pharmaceutical market, and necessitates the development of methods for increasing the water solubility of promising pharmacologically active compounds [1].

Several approaches for increasing the water solubility of compounds are known, e.g., mechanochemical activation; production of nanocomposites, inclusion complexes with cyclodextrins, and complexes with metals and metal carbonates; introduction of hydrophilic fragments into the molecule, etc. Preparation of salts of acidic or basic organic compounds is an effective pathway to increasing their water solubility.

A series of covalent spin-labeled derivatives of trolox and α-tocopherol were synthesized by us earlier [2, 3]. These are antioxidants with a chromane motif that are now widely used for targeted chemical transformations in order to synthesize highly promising pharmacologically active compounds [4, 5]. We also prepared homo- and heterodimers of trolox with various types of binding (covalent, ionic, and ionic-covalent) and showed that the water solubility of the symmetric ionic trolox homodimer was 130 times greater than that of trolox [6].

Ionic conjugates of trolox and α-tocopherol succinates were synthesized and their water solubilities were determined during modifications of trolox and α-tocopherol that were aimed at producing new pharmacologically active compounds.

The amines for synthesizing the ionic biologically active conjugates of trolox and α-tocopherol succinates were the nitroxyl radical amino-TEMPO, which is widely used to prepare spin-labeled biologically active conjugates, and diethanolamine, which is infinitely soluble in water.

Trolox (**1**) reacted with succinic anhydride in CH_2Cl_2 in the presence of Et_3N and dimethylaminopyridine (DMAP) to produce trolox succinate **2** [7], reaction of which with solutions of amines **a** and **b** in THF in a 1:2 ratio at 20–25°C gave ammonium salts of trolox monosuccinate **3a** and **3b** (Scheme 1).

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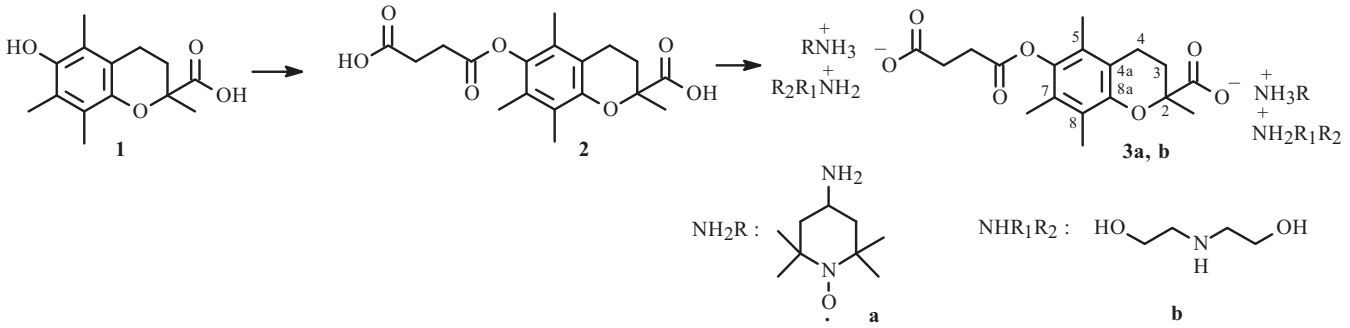


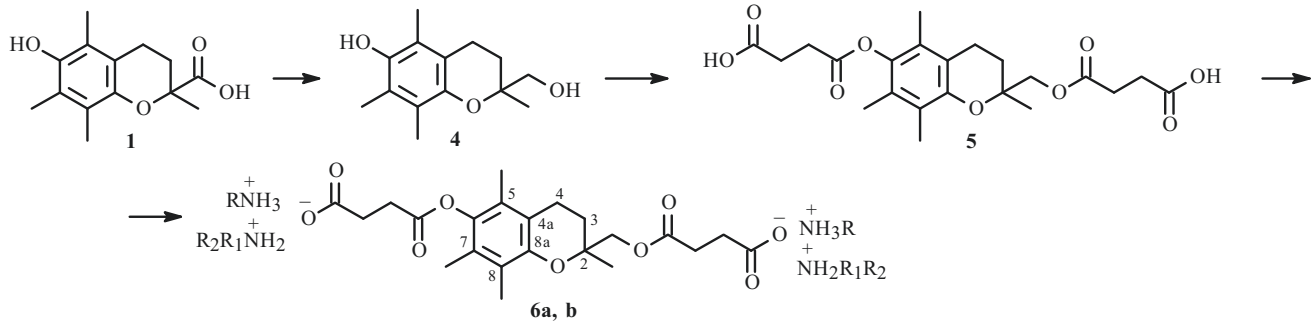
Scheme 1

IR spectra of conjugates **3a** and **3b** retained ester C=O stretching bands at 1738 and 1745 cm^{-1}. The carboxylic C=O stretching band of trolox succinate at 1717 cm^{-1} disappeared. Instead of it, bands of asymmetric and symmetric carboxylate anion stretching vibrations at 1620–1641 cm^{-1} and 1396–1400 cm^{-1}, respectively, appeared in addition to bending vibrational bands (δN–H) for the quaternary N atom at 1556–1574 cm^{-1} [8]. This argued in favor of ionic structures **3a** and **3b**. The hyperfine coupling (HFC) constant a_N in the EPR spectrum of spin-labeled conjugate **3a** was 16.0 G, which agreed with the HFC constant for piperidine radicals [9].

PMR and ^{13}C NMR spectra of **3b** exhibited all characteristic resonances of succinyl, trolox, and diethanolamine fragments. The phenol methyls of trolox were located at 1.96–2.19 ppm in the PMR spectrum; the pyran 2-methyl group, at 1.58 ppm. Resonances for succinyl CH_2 groups had chemical shifts of 2.59–2.68 ppm and 2.87–2.92 ppm; of diethanolamine CH_2 groups, 3.04–3.08 ppm and 3.74–3.78 ppm.

^{13}C NMR spectra showed resonances (ppm) for carbonyl and two carboxylate C atoms at 174.06–181.36; six aromatic C atoms at 119.25–151.67; tertiary C-2 at 80.13; three trolox phenol methyl C atoms at 12.19–13.15; and ethanolamine resonances at 50.47 and 57.92. The carboxylate C atoms (179.62 and 181.36) underwent weak-field shifts of 3.90 and 4.34 ppm relative to those of the carboxylic acids of trolox succinate (**2**) (175.72 and 177.02). This was characteristic of carboxylic acid C atoms after salt formation [10]. Thus, the obtained spectral data confirmed the structures **3a** and **3b**.

Alcohol **4** was obtained via reduction of the trolox carboxylic acid by $LiAlH_4$ in THF as before [11]. Reaction of it with succinic anhydride in a 1:2.5 ratio in CH_2Cl_2 in the presence of Et_3N and DMAP produced the disuccinate derivative of 2-hydroxymethyl trolox (**5**), treatment of which with amines **a** and **b** in THF in a 1:2 ratio at 20–25°C synthesized ammonium salts **6a** and **6b** (Scheme 2).



Scheme 2

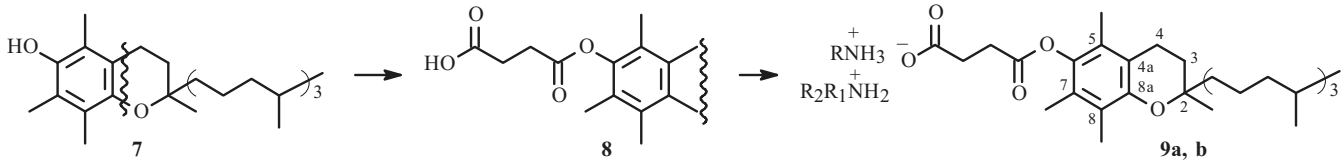
The IR spectrum of **5** showed characteristic carboxylic C=O stretching bands at 1713 cm^{-1} and ester C=O stretching bands at 1730 and 1747 cm^{-1}. The PMR spectrum of **5** contained resonances for the phenol methyls with chemical shifts (ppm) at 1.94–2.04; 2-CH_3 methyl at 1.25; succinyl CH_2 groups at 2.57–2.68, 2.77–2.80, and 2.88–2.91; and 2-CH_2 at 4.14–4.23. The ^{13}C NMR spectrum of **5** exhibited characteristic resonances (ppm) for two carbonyl and two carboxylic C atoms at 173.24–175.91, six aromatic C atoms at 118.64–149.23, C-2 at 75.17, and phenol methyls at 11.89–13.05.

IR spectra of conjugates **6a** and **6b** retained ester C=O stretching bands at 1738 and 1740 cm^{-1}. The band at 1713 cm^{-1} for carboxylic C=O stretching vibrations of **5** disappeared. Instead of it, bands of asymmetric and symmetric carboxylate stretching appeared at 1619–1637 and 1396–1412 cm^{-1}, respectively, in addition to bending vibrational bands (δN–H) of the quaternary N atom at 1570–1572 cm^{-1} [8]. This argued in favor of ionic structures **6a** and **6b**. The HFC constant in the EPR spectrum of spin-labeled conjugate **6a** was 16.0 G, like in conjugate **3a**. PMR and ^{13}C NMR spectra of salt **6b** showed all characteristic resonances for succinyl, trolox, and diethanolamine.

The PMR spectrum had the trolox phenol methyl resonances at 1.98–2.07 ppm; 2-Me of the pyran ring, at 1.30; succinyl CH_2 fragments, at 2.46–2.88; diethanolamine CH_2 groups, at 3.09–3.14 and 3.76–3.82; and 2-CH_2, at 4.07–4.18.

The ^{13}C NMR spectrum showed resonances for two carbonyl and two carboxylate C atoms at 173.89–179.41 ppm; six aromatic C atoms at 118.67–149.90; tertiary C-2 at 75.19; three trolox phenol methyls at 11.90–13.13; and diethanolamine, at 50.49 and 57.98. The resonances for the carboxylate C atoms (179.26 and 179.41 ppm) underwent weak-field shifts of 3.50 and 3.51 ppm relative to the carboxylic C atoms of 2-hydroxymethyl trolox disuccinate **5** (175.75 and 175.91 ppm). This was characteristic of carboxylic acid C atoms after forming salts [10]. Thus, the spectral results confirmed the structures **6a** and **6b**.

Conjugates **9a** and **9b** were prepared by adding solutions of amines **a** and **b** to a solution of α-tocopherol succinate **8** in THF in a 1:1 ratio at 20–25°C (Scheme 3).



Scheme 3

IR spectra of salts **9a** and **9b** retained ester C=O stretching bands at 1749 and 1751 cm^{-1}. Carboxylic C=O stretching band of α-tocopherol succinate at 1715 cm^{-1} disappeared. Instead of it, bands of asymmetric and symmetric carboxylate-anion stretching vibrations appeared at 1620–1631 and 1412 cm^{-1}, respectively, in addition to quaternary N bending vibrations (δN–H) at 1568–1570 cm^{-1} [8]. This argued in favor of ionic structures **9a** and **9b**. The HFC constant in the EPR spectrum of spin-labeled conjugate **9a** was 16.1 G, like for conjugates **3a** and **6a**. PMR and ^{13}C NMR spectra of **9b** showed all characteristic resonances for succinyl, α-tocopherol, and diethanolamine.

The PMR spectrum had α-tocopherol phenol methyl resonances at 1.89–2.00 ppm; α-tocopherol side-chain alkyl methyls, 0.80–0.85; pyran 2-Me, 1.19; succinyl CH_2 groups, 2.51–2.57 and 2.77–2.82; and diethanolamine CH_2 groups as two triplets at 2.72 and 3.51 with J = 5.5 Hz.

The ^{13}C NMR spectrum exhibited resonances for carbonyl and carboxylate C atoms at 171.81 and 175.97 ppm, respectively; six aromatic C atoms at 117.13–148.97; tertiary C-2 at 74.75; and three α-tocopherol phenol methyls at 11.18–12.25. The carboxylate C atom resonance (175.97) was observed in the spectrum with the same characteristic weak-field shift by 1.86 ppm relative to that of α-tocopherol carboxylic acid (174.11) [10]. Thus, the spectral results confirmed the structures **9a** and **9b**.

Water solubilities of **1**, **3a**, **3b**, **6a**, **6b**, **9a**, and **9b** were determined by the literature method [12]. The water solubilities of trolox succinate salts **3a** (1.1 g/L) and **3b** (26 g/L) increased by 10–260 times whereas those of 2-hydroxymethyl trolox disuccinate salts **6a** (0.8 g/L) and **6b** (9 g/L) rose by 8–90 times compared with trolox (0.1 g/L). The solubilities of salts **3b** and **6b** were 24 and 11 times greater than those of salts **3a** and **6a** because diethanolamine is infinitely soluble in H_2O. However, the solubilities of salts **9a** and **9b** were the same as that of α-tocopherol (<0.1 g/L). This may have been related to the high lipophilicity of the α-tocopherol 2-alkyl group. According to pharmacopoeial classification, α-tocopherol and salts **9a** and **9b** were insoluble in H_2O.

Thus, new ionic conjugates of trolox succinates that were much more soluble in H_2O than starting trolox were prepared. However, formation of the ammonium salts of α-tocopherol succinate did not increase their solubilities compared with starting α-tocopherol.

EXPERIMENTAL

NMR spectra of CD_3OD, DMSO-d_6, and $CDCl_3$ solutions of **3a**, **3b**, **6a**, **6b**, **9a**, and **9b** were recorded on Bruker AV-300, AV-400, and DRX-500 spectrometers. IR spectra were recorded from KBr pellets on a Vector-22 instrument. Elemental CHN-analyses were performed on a EURO EA 3000 Elemental Analyzer. Melting points were measured on a Mettler Toledo FP90 Central Processor apparatus. Trolox (Acros Organics), D,L-α-tocopherol (Alfa Aesar), and succinic anhydride (Aldrich) were purchased. The radical 4-amino-TEMPO (**a**) was synthesized at the Chemical Pilot Facility, NIOCh, SB, RAS. Trolox succinate (**2**) and α-tocopherol succinate (**8**) were prepared by reacting trolox and α-tocopherol with succinic anhydride in CH_2Cl_2 in the presence of Et_3N and DMAP by the literature method [7]. Their spectral characteristics agreed fully with those in the literature. 2-Hydroxymethyl trolox **4** was prepared by reducing trolox (**1**) with $LiAlH_4$ in THF as before [11]. Its spectral characteristics agreed fully with those in the literature.

General Method for Preparing Ammonium Salts 3a, 3b, 6a, 6b, 9a, 9b. Trolox 2-hydroxymethyl mono- or disuccinate or α-tocopherol succinate (17.5–26.5 mg, ~0.05 mmol) in THF (EtOAc) (0.5 mL) was treated with a solution of the appropriate amine **a** or **b** (~0.1 mmol for **2** and **5**; 0.05 mmol for **8**) in THF (EtOAc) (0.3 mL). The mixtures were evaporated after 16–20 h. The viscous residue was triturated with Et_2O to form a powder or viscous liquid that was filtered off, rinsed with EtOAc, and dried to constant weight.

1-Oxyl-2,2,6,6-tetramethylpiperidine-4-ammonium 6-(3-Carboxylatopropanoyloxy)-2,5,7,8-tetramethylchromane-2-carboxylate (3a). Orange powder, yield 34.3 mg (99%), mp 95.4°C (dec.). $C_{36}H_{60}N_4O_9$. IR spectrum (ν, cm^{-1}): 1396 and 1641 (COO^-), 1556 (δN–H), 1738 (ester COO). EPR spectrum: a_N = 16.0 G.

***bis*(2-Hydroxyethyl)ammonium 6-(3-Carboxylatopropanoyloxy)-2,5,7,8-tetramethylchromane-2-carboxylate (3b).** Viscous white liquid, yield 26.6 mg (95%). $C_{24}H_{44}N_2O_{11}$. IR spectrum (ν, cm^{-1}): 1400 and 1620 (COO^-), 1574 (δN–H), 1745 (ester COO). ^{1}H NMR spectrum (400 MHz, CD_3OD, δ, ppm): 1.58 (3H, s, CH_3-2), 1.96 (3H, s), 2.03 (3H, s), 2.19 (3H, s) – CH_3-5, 7, 8, 1.71–1.79 (1H, m, H-3), 2.43–2.52 (1H, m, H-3), 2.59–2.68 (4H, m) and 2.87–2.92 (2H, m) – H-4 and succinyl CH_2; 3.04–3.08 (8H, m), 3.74–3.78 (8H, m) – diethanolamine CH_2. ^{13}C NMR spectrum (75 MHz, CD_3OD, δ, ppm): 12.19, 12.24, 13.15 (CH_3-5, 7, 8), 22.49, 26.02, 32.01, 31.21, 32.77 (C-3, 4, CH_3-2, succinyl CH_2), 50.47, 57.92 (diethanolamine CH_2); 80.13 (C-2), 119.25, 123.70, 126.12, 127.75 (C-5, 7, 8, 4a); 141.98, 151.67 (C-6, 8a); 174.06 – carbonyl C atom; 179.62, 181.36 – carboxylate C atoms.

4-{[6-(3-Carboxylatopropanoyloxy)-2,5,7,8-tetramethylchroman-2-yl]methoxy}-4-oxobutanoic Acid (5). Alcohol **4** (54 mg, 0.23 mmol), succinic anhydride (68 mg, 0.68 mmol), DMAP (13 mg, 0.1 mmol), and Et_3N (32 μL) in CH_2Cl_2 (1 mL) was stirred for 20–24 h at room temperature, washed with HCl solution (5%), dried over $MgSO_4$, and evaporated. Viscous light-brown liquid, yield 76.8 mg (77%). Mass spectrum: found *m/z* 459.159 $[M + Na]^+$. $C_{22}H_{28}O_9Na$, calcd *m/z*: 459.163 $[M + Na]^+$. IR spectrum (ν, cm^{-1}): 1713 (COOH), 1730 and 1747 (ester COO). ^{1}H NMR spectrum (400 MHz, $CDCl_3$, δ, ppm) 1.25 (3H, s, CH_3-2), 1.94, 1.98, 2.04 (each 3H, s, CH_3-5, 7, 8), 1.71–1.79 (2H, m, H-3), 2.57–2.68 (6H, m) – succinyl CH_2, 2.77–2.80 (2H, m, H-4) and 2.88–2.91 (2H, m) – succinyl CH_2, 4.14–4.23 (2H, m, CH_2-2). ^{13}C NMR spectrum (100 MHz, CD_3OD, δ, ppm): 11.89, 12.17, 13.05 (CH_3-5, 7, 8), 20.98, 22.22, 29.41, 29.70, 29.74, 29.76, 30.05 (C-3, 4, CH_3-2, succinyl CH_2), 69.63 (CH_2-2), 75.17 (C-2), 118.64, 124.00, 126.46, 128.25 (C-5, 7, 8, 4a), 142.34, 149.93 (C-6, 8a), 173.24, 173.87 – carbonyl C atoms, 175.75, 175.91 – carboxylate C atoms.

1-Oxyl-2,2,6,6-tetramethylpiperidine-4-ammonium 4-{[6-(3-Carboxylatopropanoyloxy)-2,5,7,8-tetramethylchroman-2-yl]methoxy}-4-oxobutanoate (6a). Orange powder, yield 35.8 mg (92%), mp 106.6–108.4°C. $C_{40}H_{66}N_4O_{11}$. IR spectrum (ν, cm^{-1}): 1396 and 1637 (COO^-), 1572 (δN–H), 1740 (ester COO). EPR spectrum: a_N = 16.0 G.

***bis*(2-Hydroxyethyl)ammonium 4-{[6-(3-Carboxylatopropanoyloxy)-2,5,7,8-tetramethylchroman-2-yl]methoxy}-4-oxobutanoate (6b).** Viscous white liquid, yield 30.1 mg (93%). $C_{30}H_{50}N_2O_{13}$. IR spectrum (ν, cm^{-1}): 1412 and 1619 (COO^-), 1570 (δN–H), 1738 (ester COO). ^{1}H NMR spectrum (500 MHz, CD_3OD, δ, ppm, J/Hz): 1.30 (3H, s, CH_3-2), 1.98, 2.00, 2.07 (each 3H, s, CH_3-5, 7, 8), 1.78–1.89 (1H, m, CH_2-3), 2.46–2.53 (3H, m), 2.60 (4H, t, J = 6.5), 2.66 (2H, t, J = 6.8), 2.88 (2H, t, J = 6.9) – H-3, H-4 and succinyl CH_2, 3.09–3.14 (8H, m), 3.76–3.82 (8H, m) – diethanolamine CH_2, 4.07–4.18 (2H, m, CH_2-2). ^{13}C NMR spectrum (125 MHz, CD_3OD, δ, ppm): 11.90, 12.25, 13.13 (CH_3-5, 7, 8), 20.99, 22.30, 29.46, 31.20, 31.41, 32.60 (C-3, 4, CH_3-2, succinyl CH_2), 50.49, 57.98 (diethanolamine CH_2), 69.39 (CH_2-2), 75.19 (C-2), 118.67, 123.91, 126.52, 128.26 (C-5, 7, 8, 4a), 142.43, 149.90 (C-6, 8a), 173.89, 174.67 – carbonyl C atoms, 179.26, 179.41 – carboxylate C atoms.

1-Oxyl-2,2,6,6-tetramethylpiperidine-4-ammonium 4-Oxo-4-[2,5,7,8-tetramethyl-2-(4,8,12-trimethyltridecyl)chroman-6-yloxy]butanoate (9a). Viscous orange liquid, yield 32.6 mg (93%). $C_{42}H_{73}N_2O_6$. IR spectrum (ν, cm^{-1}): 1412 and 1631 (COO^-), 1568 (δN–H), 1749 (ester COO). EPR spectrum: a_N = 16.1 G.

(2-Hydroxyethyl)ammonium 4-Oxo-4-[2,5,7,8-tetramethyl-2-(4,8,12-trimethyltridecyl)chroman-6-yloxy]butanoate (9b). Viscous yellow liquid, yield 31.2 mg (98%). $C_{37}H_{65}NO_7$. IR spectrum (ν, cm^{-1}): 1412 and 1620 (COO^-), 1570 (δN–H), 1751 (ester COO). ^{1}H NMR spectrum (400 MHz, DMSO-d_6, δ, ppm, J/Hz): 0.80–0.85 (12H, m) – alkyl side-chain methyls, 0.99–1.57 (21H, m) – alkyl side-chain methylenes and methines, 1.19 (3H, s, CH_3-2), 1.89 (3H, s), 1.91 (3H, s), 2.00 (3H, s) – CH_3-5, 7, 8, 1.70–1.79 (2H, m, H-3), 2.51–2.57 (4H, m), 2.77–2.82 (2H, m) – H-4 and succinyl CH_2 2.72 (4H, t, J = 5.5), 3.51 (4H, t, J = 5.5) – diethanolamine CH_2. ^{13}C NMR spectrum (75 MHz, $CDCl_3$, δ, ppm): 11.18, 11.40, 12.25 (CH_3-5, 7, 8), 19.01–19.22, 20.15, 20.59, 22.01, 22.10, 23.97, 24.37, 27.54, 29.40, 29.73, 30.71, 32.18–32.41, 36.79–37.20 (C-3, 4, CH_3-2, succinyl CH_2 and alkyl side-chain C atoms), 38.95, 56.70 (diethanolamine CH_2), 74.75 (C-2), 117.13, 122.57, 124.62, 126.31 (C-5, 7, 8, 4a), 140.14, 148.97 (C-6, 8a), 171.81 – carbonyl C atom, 175.97 – carboxylate C atom.

The solubilities of **1**, **3a**, **3b**, **6a**, **6b**, **9a**, and **9b** were determined as usual [12]. Weighed portions of **1**, **3a**, **3b**, **6a**, **6b**, **9a**, and **9b** (0.5–10 mg) were treated with distilled H_2O (0.1 mL) and shaken for 10 min. The addition of H_2O was repeated until the compound dissolved completely or a solubility of <0.1 g/L was reached.

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