

Eco-friendly One-Pot Synthesis of Oxamide

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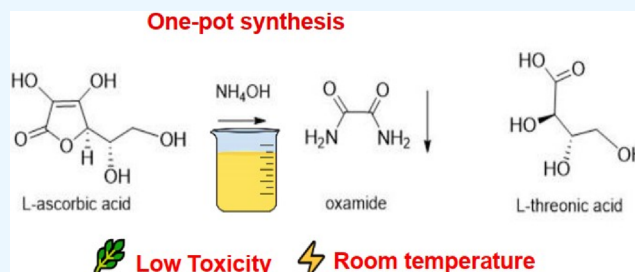
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ABSTRACT: A novel, eco-friendly one-pot synthesis of oxamide was developed using nontoxic starting materials, offering a safer alternative to traditional methods. The procedure yields a highly pure oxamide precipitate within 2 days. By optimizing key reaction parameters including temperature, ascorbic acid/ammonia ratio, and ammonia concentration, the precipitation yield can be finely tuned. The resulting oxamide was comprehensively characterized by X-ray diffraction (XRD), scanning electron microscopy with energy-dispersive X-ray spectroscopy (SEM-EDX), Fourier transform infrared spectroscopy (FT-IR), and one-dimensional/two-dimensional (1D/2D) nuclear magnetic resonance (NMR). Concurrently, the liquid supernatant was analyzed by EDX, NMR, and mass spectrometry (MS), revealing the bioactive metabolite L-threonic acid as the sole major byproduct, with no other toxic compounds detected.



INTRODUCTION

The synthesis of chemical compounds is a cornerstone of modern industry, driving advancements across sectors such as pharmaceuticals and agriculture. However, traditional synthetic methods often rely on toxic reagents, energy-consuming processes, and environmentally harmful practices. In the era of sustainability, there is a growing global imperative to develop greener and more responsible chemical production methods. This transition aligns with global efforts to mitigate climate change, protect biodiversity, ensure food safety, and promote human health.¹ Consequently, replacing hazardous industrial processes with eco-friendly alternatives has become a priority for both academia and the industry.

Among the compounds of interest, oxamide or ethanediamide² is an organic compound with the chemical formula $C_2H_4N_2O_2$. It serves as an essential intermediate in various reactions, but it is typically synthesized through methods that may involve toxic substances.

Oxamide can be considered the diamide derived from oxalic acid. At room temperature, it is a white, nontoxic, and very stable crystalline solid that is soluble in ethanol and slightly soluble in water (0.016% w/v). It decomposes when thermally treated at a temperature higher than 300 °C.³

The primary industrial application of oxamide is in the agricultural sector, serving as a substitute for urea in fertilizers. In fact, oxamide hydrolyzes very slowly, releasing ammonia,⁴ which is sometimes preferred with respect to the quick release by urea, so that the amount of chemical fertilizer, manpower, and time cost in agricultural production processes can be reduced.

The compound is also used either as a stabilizer for nitrocellulose products or in concentrations of 1–3% w/v in

modern solid-fuel rockets based on ammonium perchlorate composite propellant (APCP) in rocket motors as a high-performance burn rate suppressant. The use of oxamide slows the linear burn rate while having a minimal impact on propellant specific impulse.⁵

Oxamide is industrially produced from hydrogen cyanide (HCN), which is oxidized to cyanogen (CN)₂, which, in turn, is hydrolyzed.⁶ The main drawback of this method is the high toxicity of HCN and its high cost. Alternative synthetic routes include the glow-discharge electrolysis of formamide,⁷ the oxalate diester ammonolysis process,⁸ and a more recent method converting N₂ and CO utilizing a hafnium complex.⁹ However, these methods often suffer from high costs, hazardous precursors, and byproducts, or complex reaction conditions.

To address these limitations, this study introduces a novel, eco-friendly, one-pot synthetic route for oxamide. Unlike traditional approaches, this method completely eliminates toxic reagents and yields a highly pure precipitate within 1 to 2 days, offering a safer and sustainable alternative for oxamide production. In addition, the production of L-threonic acid as the sole co-product offers an additional value to the synthesis.

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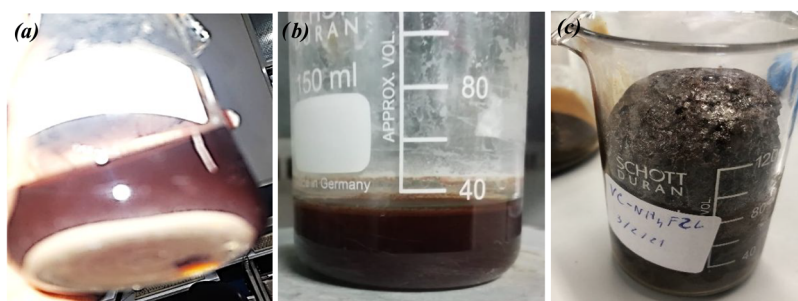
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Table 1. Characteristics of the Samples Obtained by One-Pot Precipitation

samples	C ₆ H ₈ O ₆ (g)	NH ₄ OH (mL)	H ₂ O (mL)	NH ₄ OH (w/v %)	C ₆ H ₈ O ₆ /NH ₄ OH (mol/mol)	S/L (g/mL)	T/t (°C/days)	M _{ox} (g)	OX (%)	XRD crystal size (nm)	purity ± SD (%)
Ox1	6.0	40	0	30	0.034/0.34 = 0.1	0.15	25/1	0.31 ± 0.04	5.2	36.0 ± 0.4	
Ox1-air ^a	6.0	40	0	30	0.034/0.34 = 0.1	0.15	25/1	0.30 ± 0.07	5.0		
Ox1-50	6.0	40	0	30	0.034/0.34 = 0.1	0.15	50/2	0.40 ± 0.02	6.7	42.2 ± 0.1	
Ox2	6.0	40	20	20	0.034/0.34 = 0.1	0.1	25/2	0.55 ± 0.03	9.2	42.3 ± 0.2	
Ox3	6.0	20	40	10	0.034/0.17 = 0.2	0.1	25/2	0.48 ± 0.04	8.0	42.4 ± 0.3	82 ± 11
Ox4	3.0	20	40	10	0.017/0.17 = 0.1	0.05	25/2	0.61 ± 0.05	20.0	50.7 ± 0.2	102 ± 16

^aThe sample was obtained by air bubbling in the reaction mixture.

**Figure 1.** White oxamide precipitate on the bottom of the beaker (a), brown supernatant liquid (a, b), and solid obtained from dried liquid (c).

EXPERIMENTAL SECTION

The reaction was studied starting from different amounts (3.00 ± 0.01 or 6.00 ± 0.01 g) of ascorbic acid, C₆H₈O₆ (vitamin C, molecular formula), added to 40.0 mL of NH₄OH (30% w/v) or to 60 mL of NH₄OH in distilled water, at 20 or 10% w/v concentrations, at room temperature or at 50 °C, both with and without air bubbling. This way, the solid–liquid ratio (S/L = 0.05, 0.1, and 0.15) and the molar ratio of the reagents (C₆H₈O₆/NH₄OH = 0.1 and 0.2) (Table 1) were varied to evaluate the best experimental conditions.

The solution was left stirring for 1 or 2 days, the time necessary to observe oxamide precipitation (Figure 1a). The resulting mixture was centrifuged at 3500g for 30 min at 4 °C, and the solid precipitate was separated from the supernatant liquid (Figure 1a,b). The liquid phase was then left to rest without stirring for 7 days to verify further solid precipitation (Table 1). The solid phase was then washed several times with distilled water and dried at 80 °C. Each synthetic procedure was repeated three times.

The weight percentage of the oxamide-based precipitate (OX_%) was calculated by eq 1:

$$\text{OX}_{\%} = \left(\frac{M_{\text{OX}}}{M_{\text{VC}}} \right) \times 100 \quad (1)$$

where M_{OX} and M_{VC} are the mass of precipitated oxamide and vitamin C, respectively.

The precipitate has been characterized by X-ray diffractometric analysis (XRD) using a Philips diffractometer, and the chemical structure of oxamide-based precipitate has been examined using a Fourier transform infrared spectrophotometer (FT-IR, Bruker, IFS-88, Billerica, MA) in the range from 4000 to 600 cm^{−1} with a 2 cm^{−1} resolution. The morphological analysis of the surface precipitates has been performed by scanning electron microscopy (SEM) using a Philips microscope (XL series, Almelo, The Netherlands) equipped with energy-dispersive X-ray (EDX) spectroscopy for elemental analysis.

The degree of purity of samples was determined by high-performance liquid chromatography^{10,11} coupled to a diode array detector for UV–vis spectrophotometry (HPLC-DAD). Pure oxamide was used as a standard reference material (Carlo Erba, Italy). For quantitative analysis, the UV–visible (UV–vis) spectrum

of pure oxamide was studied in the range 195–380 nm; the absorbance maximum at 205 nm was set for quantification.

The samples Ox3 and Ox4 were tested; 10.0 ± 0.1 mg of each product was dissolved by sonication in 100 mL of acetonitrile, in a volumetric flask, then injected into the HPLC system.

A Waters ACQUITY UPLC I-Class PLUS system (Waters Corp., Milford, MA) was employed, equipped with quaternary pumps, an automatic injection valve, an autosampler, and an UV–vis DAD detector. The system was managed by the software Empower (Waters). A stainless steel column Zorbax NH₂, 250 × 4.6 mm², and 5 μm particle (Agilent Technologies) was used, eluting with the mobile phase potassium phosphate 25 mM at pH 6.5/acetonitrile 20:80 v/v in the isocratic mode at a 1.0 mL/min flow rate. The injection volume was 20 μL, and the detection wavelength was set at 205 nm. UV–vis spectra were acquired between 195 and 380 nm to characterize the oxamide chromophore and to study peak purity.

To prepare standard solutions, 10.0 ± 0.1 mg of each sample and the standard reference material were, respectively, dissolved in acetonitrile (ACN) in 100 mL volumetric flasks by mixing for 30 min at room temperature (20 °C). Working standard solutions of oxamide at 1–5 to 10–20 μg/mL (ppm) were prepared by dilution with acetonitrile. For HPLC-DAD quantitative analysis, a standard external calibration curve was calculated by linear regression, reporting the chromatographic peak areas of the working standard solutions vs their concentrations.

The chromatographic runs were repeated in triplicate over 3 consecutive days in order to evaluate within-laboratory reproducibility in terms of standard deviation (SD).

The concentrations of oxamide after each synthesis were calculated by interpolating the standard calibration curves from the chromatographic peak areas of the samples. The degree of purity in each sample was first evaluated by comparing oxamide UV–vis absorbance spectra with that of the pure standard and then further confirmed by nuclear magnetic resonance (NMR) data.

One-dimensional (1D) and two-dimensional (2D) NMR spectra were recorded at 600 MHz and 298 K on a Bruker Avance Neo spectrometer equipped with a z-gradient 5 mm ¹H/¹³C/¹⁵N/³¹P cryogenic probe head. The solid precipitate was dissolved in 600 μL of DMSO-*d*₆ and transferred into 5 mm NMR tubes. Chemical shifts were referenced to the residual solvent signals (δ_{H} 2.50, δ_{C} 39.50).

The supernatant liquid, after taking to dryness, was solubilized in 600 μL of D₂O (99.9%) and transferred into 5 mm NMR tubes. DSS,

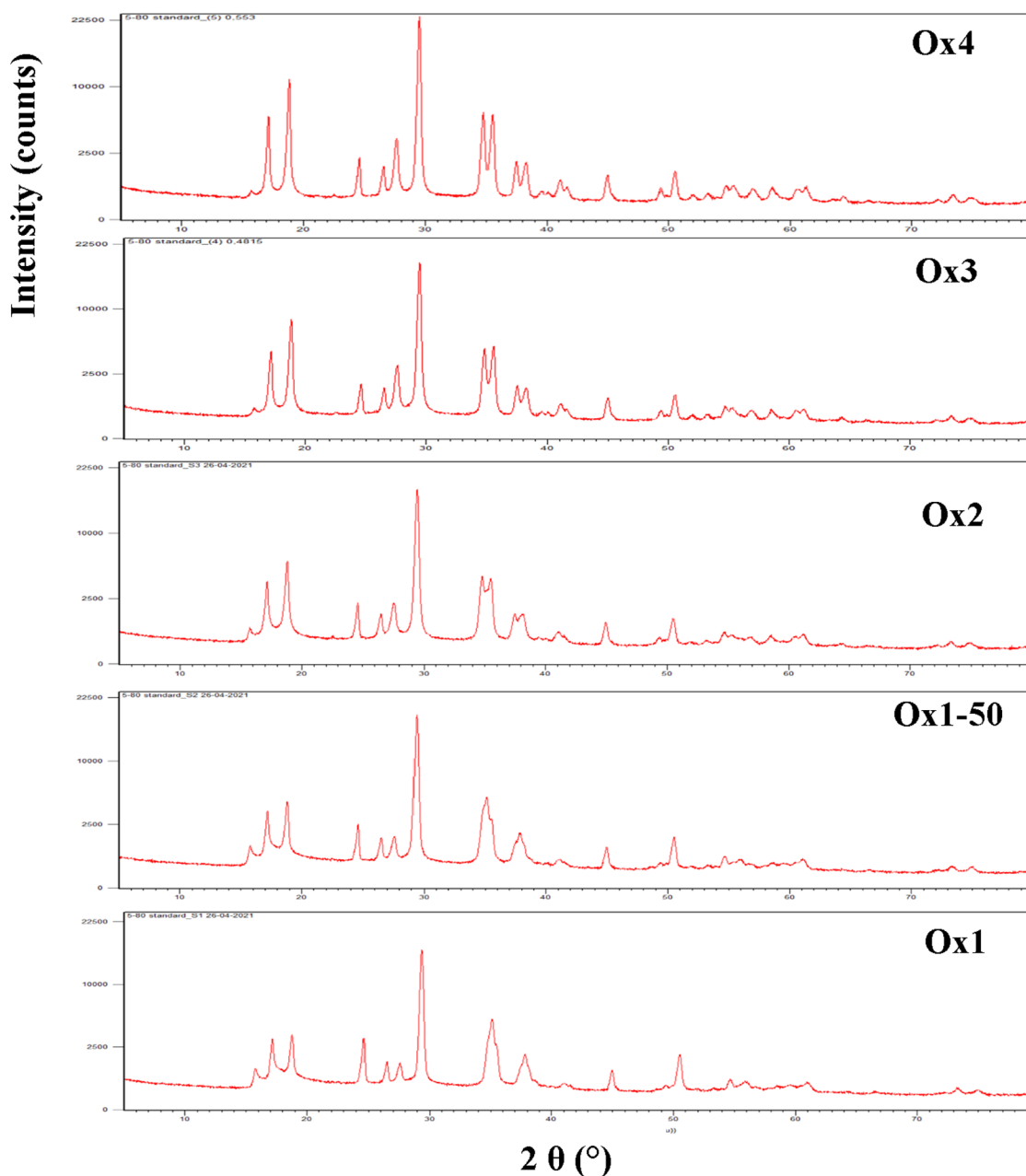


Figure 2. XRD patterns of the oxamide precipitates reported in Table 1.

added at a concentration of 0.2 mg/mL, was used as an internal standard (δ 0.00). The ^1H NMR spectra were performed with 64 transients and 16 K complex data points. The recycle time was set to 5 s, and a 30° pulse angle was used. The 2D NMR experiments (correlation spectroscopy (COSY), heteronuclear single quantum coherence (HSQC), and heteronuclear multiple bond correlation (HMBC)) were performed using standard Bruker pulse sequences. High-resolution electrospray ionization (ESI) mass spectra were measured on a Thermo LTQ Orbitrap XL mass spectrometer (Thermo Fisher Scientific Spa, Rodano, Italy).

The anionic concentrations of the liquid phase were also measured. Ammonium ion (NH_4^+) concentration was determined spectrophotometrically using the indophenol blue method, in accordance with Standard Methods.¹² Cyanide ion (CN^-) concentration was determined spectrophotometrically using the commercial test kit no. 11781073 supplied by WTW (Wissenschaftlich-Technische Werkstätten GmbH, Germany), following the manufacturer's instructions. Nitrate ion (NO_3^-) concentration was measured by ion chromatography using a 761 Compact IC system (Metrohm, Switzerland),

equipped with an anion-exchange column Metrosep A Supp 5 (150/4.0 mm).

RESULTS AND DISCUSSION

The reaction between ascorbic acid (vitamin C, $\text{C}_6\text{H}_8\text{O}_6$) and an excess of ammonium hydroxide determines the precipitation of oxamide ($\text{C}_2\text{H}_4\text{N}_2\text{O}$), in different quantities, after 1 day for the more concentrated ammonium hydroxide solution (samples Ox1, Table 1) and after 2 days for the more diluted ones (samples Ox2, Ox3, and Ox4, Table 1). After separating the solid phase, the liquid phase was allowed to rest for 7 days, during which an additional 1.0–1.2 mg of precipitate was formed.

The formation of oxamide in the precipitate was verified by X-ray diffraction analysis. Figure 2 shows the XRD patterns of the samples reported in Table 1. All XRD patterns present the characteristic peaks of oxamide (JCPDS no. 00-030-1861).

Sample Ox1, obtained at room temperature using the most concentrated ammonium hydroxide solution (30 w/v %), a $C_6H_8O_6/NH_4OH$ precursor molar ratio of 0.1, and a solid-to-liquid (S/L) ratio of 0.15, exhibits broader diffraction profiles with lower peak intensity and the lowest integrated peak area (4206.2 au) compared to the other samples. The larger full width at half-maximum (FWHM) corresponds to smaller crystallite sizes (36 nm), calculated using the Scherrer equation, likely due to the formation of a larger number of crystallization nuclei. Furthermore, the mass of the obtained precipitate is equal to 0.31 g, corresponding to an oxamide yield of 5.2 wt %, calculated with respect to the amount of vitamin C used in the synthesis (eq 1) (Table 1).

To evaluate the effect of aeration during the synthesis, sample Ox1-air was prepared under the exact same experimental conditions as Ox1 but with continuous air bubbling through the reaction mixture. Air bubbling did not enhance the precipitation of oxamide, yielding a precipitate mass of 0.30 g (corresponding to an oxamide yield of 5.0 wt %), which is practically identical to that obtained for Ox1.

Sample Ox1-50, synthesized under the same conditions as Ox1 but at a higher temperature (50 °C), presents sharper peaks with smaller FWHM values, resulting in larger crystallite sizes (42.2 nm) and an increased integrated peak area (4838.7 au), indicating enhanced relative crystallinity. The increase in temperature also resulted in a slight increase in the precipitate mass, which reached 0.40 g, corresponding to an oxamide yield of 6.7 wt %.

Samples Ox2 and Ox3, prepared using a lower S/L ratio (0.1), more diluted ammonium hydroxide solutions (20 and 10 w/v %, respectively), and precursor molar ratios of 0.1 and 0.2, respectively, exhibited narrower peak profiles, slightly higher XRD peak intensities, and higher integrated peak areas (4778.6 au for Ox2 and 5002.7 au for Ox3) compared to Ox1, which reflects a higher relative crystallinity and larger crystallite sizes (Table 1). Under these conditions, different NH_4OH concentrations did not significantly affect the crystallinity of the products. However, at a constant 0.1 S/L ratio, a higher NH_4OH concentration, corresponding to a lower precursor molar ratio, promoted the formation of a larger amount of the precipitate. Indeed, sample Ox2 is characterized by a precipitate mass of 0.55 g and an oxamide yield of 9.2 wt %, whereas sample Ox3, prepared using a 10 w/v % NH_4OH solution and a precursor molar ratio of 0.2, shows a precipitate mass of 0.48 g and an oxamide yield of 8.0 wt %.

Sample Ox4, prepared using the same precursor molar ratio as Ox2 but with a lower solid-to-liquid ratio (S/L = 0.05), presents even sharper diffraction peaks, resulting in the lowest FWHM values, the largest crystallite sizes (50.7 nm), and the maximum integrated peak area (5402.16 au). In addition, Ox4 is characterized by a precipitate mass of 0.61 g and an oxamide yield of 20.0 wt %. These results indicate that, at a constant precursor molar ratio (0.1), the use of more diluted NH_4OH solutions and consequently lower S/L ratios favors not only the formation of larger amounts of oxamide precipitate but also long-range structural ordering and superior phase purity (Figure 3).

To confirm the superior phase purity of Ox4 compared to the other samples, the shift in the main diffraction peak position was evaluated (Figure S1). Specifically, the primary diffraction peak for Ox3 is slightly shifted toward a lower angle ($2\theta = 29.48^\circ$) compared to that experimentally detected for the pure oxamide phase ($2\theta = 29.55^\circ$), reflecting lower phase

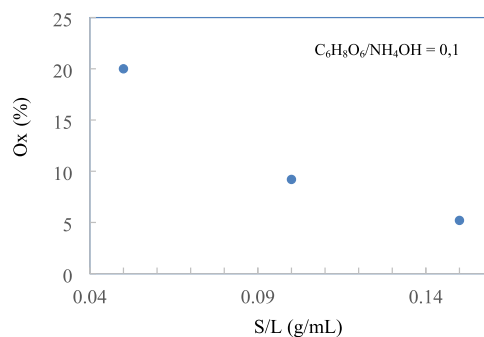


Figure 3. Percentage of oxamide as a function of the S/L ratio.

purity. This peak shift to smaller 2θ values indicates an expansion of the interplanar spacing (d -spacing), likely induced by crystal lattice distortions arising from structural impurities incorporated during precipitation. In contrast, for sample Ox4, the main characteristic diffraction peak appears at $2\theta = 29.54^\circ$, closely matching the stoichiometric crystal lattice position of pure oxamide and confirming its high phase purity.

Figure S2 shows the SEM micrographs relating to the Ox4 sample (a–c) and oxamide as a reference standard (98% purity, Carlo Erba, Italy) (d). Agglomerated crystals (Figure S2a) of a few hundred microns (Figure S2b,c) were detected in the Ox4 sample, unlike pure standard oxamide, which is characterized by crystals having the same morphology but of much smaller dimensions (Figure S2d). EDX analyses were also carried out (Table S1). The weight percentages of C, N, O, and H in oxamide ($C_2H_4N_2O_2$) are 27.3, 31.8, 36.4, and 4.5% w/w, respectively. The corresponding values of C, N, and O without hydrogen are 28.6, 33.3, and 38.1% w/w, respectively. EDX analysis of the Ox4 sample shows C, N, and O contents of 28.0, 34.1, and 37.9% by weight, respectively, in agreement with values observed for pure oxamide.

The synthesis products were tested for their purity by chromatographic analysis. We had two goals: (1) to measure the oxamide concentrations in the samples compared to a pure standard reference material and (2) to monitor the possible presence of byproducts. First, we developed an HPLC chromatographic separation, starting from the information available in the literature.^{10,11} We focused on a HILIC separation and introduced an amino-stationary phase that allows optimization of the separation of polar compounds.

The degree of purity of Ox3 and Ox4 samples is reported in Table 1. The linear regression calibration curve, calculated by injecting pure oxamide standard material at different concentrations, and its parameters (slope, intercept), as well as its coefficient of determination R^2 to evaluate detector linearity, are shown in Figure 4. By interpolation of the calibration curve, the concentration of oxamide in the sample was calculated in mg/mL; the relative purity was calculated with respect to the theoretical concentration of 0.10 mg/mL of oxamide, calculated by weighing 10.0 mg of each product and dissolving in 100 mL of acetonitrile. By the replicate measurements ($n = 3$) on different days, we calculated mean purity values, and their within-laboratory reproducibility, in terms of standard deviations (SD), respectively, 82 ± 11 and $102 \pm 16\%$. We evaluated that the contribution of type A errors was the main source of measurement uncertainties associated with the purity grade of Ox3 and Ox4; the type B

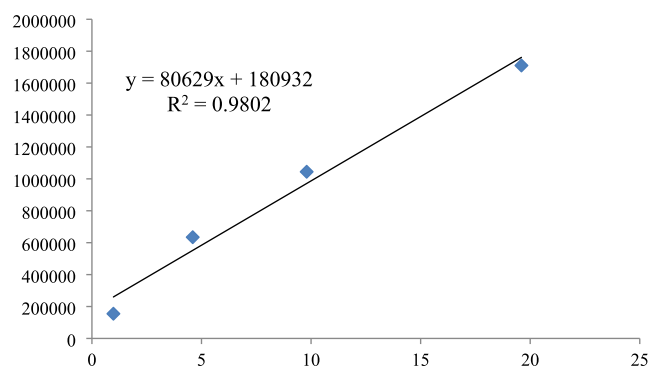


Figure 4. Calibration standard curve obtained with pure oxamide.

uncertainty deriving from oxamide standard material, weighing, and volumetric flask was negligible.

The HPLC analysis suggests Ox4 purity is slightly higher than that of Ox3, in agreement with other tests performed; the mean value of $102 \pm 16\%$ is affected by a relative standard deviation (RSD) = 15.7%, characteristic of HPLC measurements; from a practical point of view, the product Ox4 has a purity $\geq 86\%$ (that is, 102–16%).

Figure 5 shows the FT-IR spectrum of sample Ox4 (a) and the reference one of the oxamide (b). The spectra appeared very similar, and their comparison allowed us to attribute the peaks at 3377, 3175, 2945, 1657, 1604, 1345, 1101, 789, 624, and 460 cm^{-1} of the Ox4 sample to oxamide.

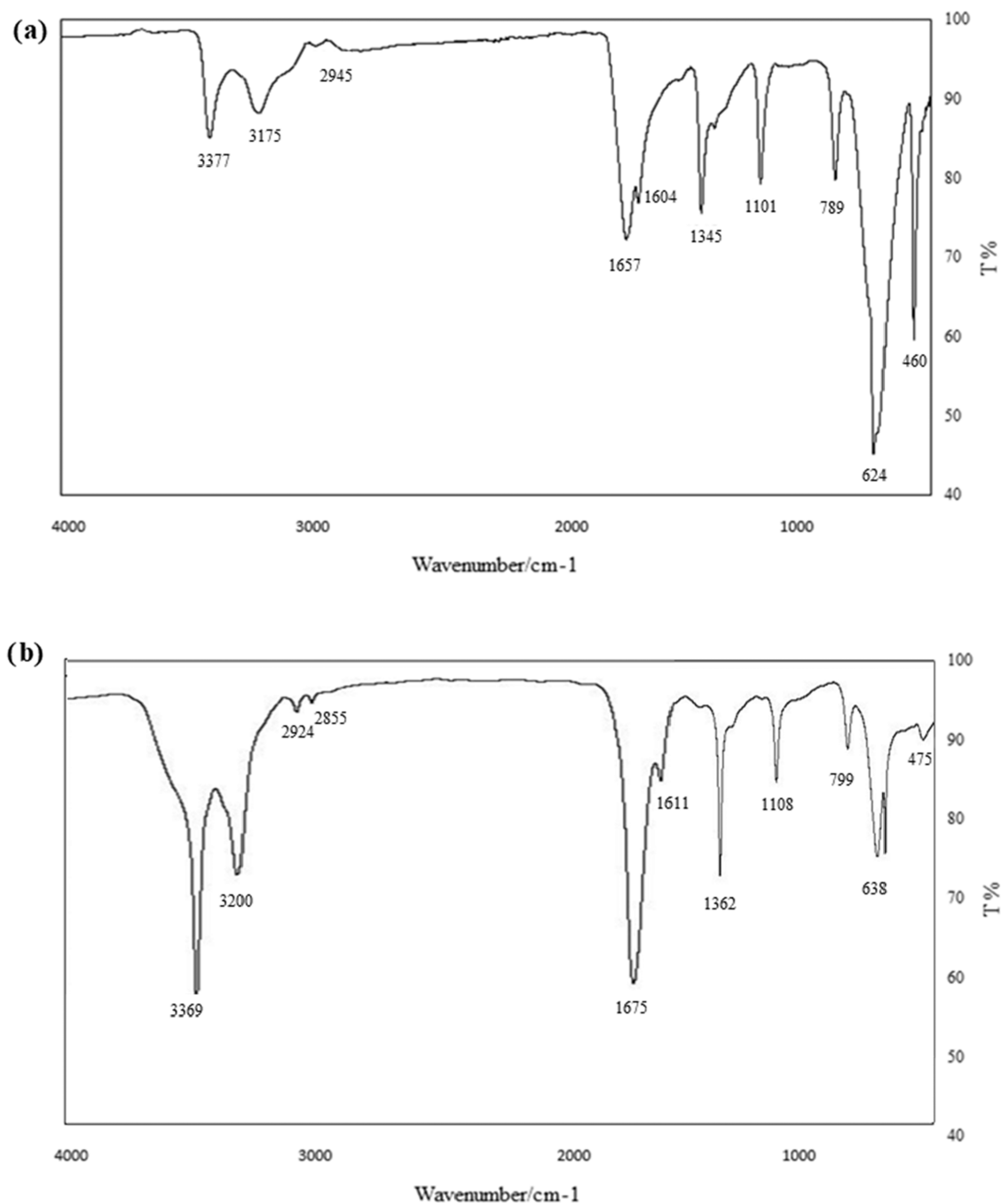


Figure 5. FT-IR spectra of sample Ox4 (a) and reference oxamide (b).

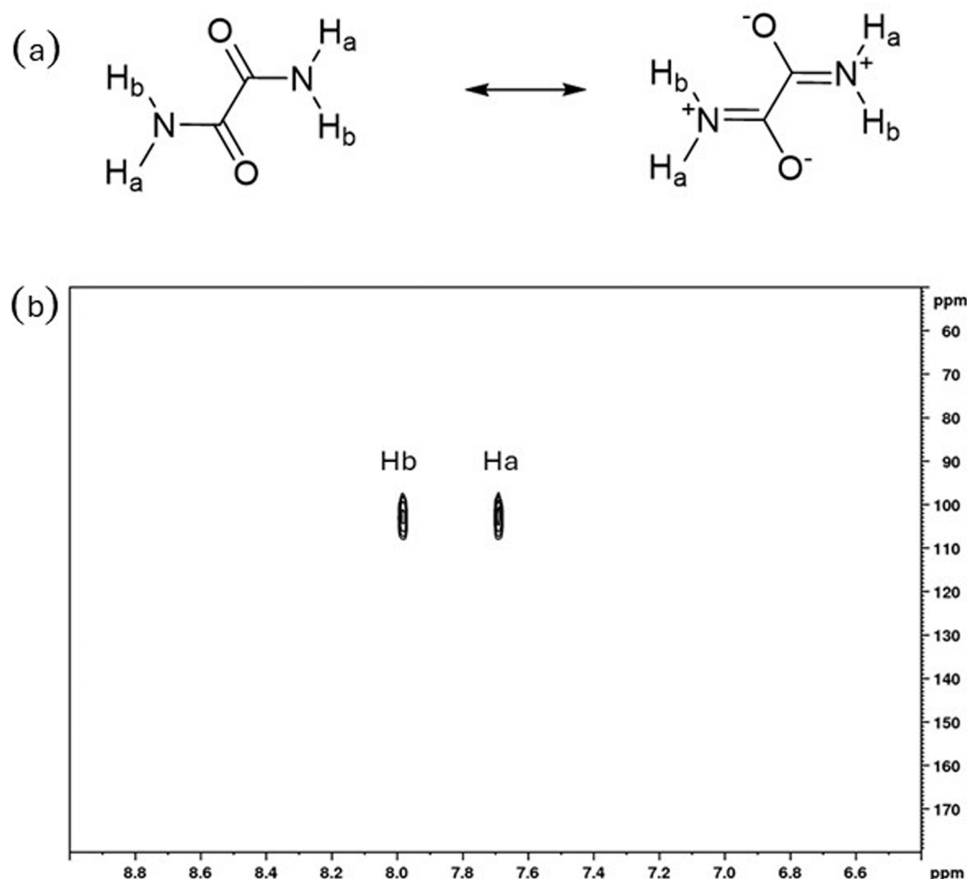


Figure 6. (a) Resonance limit structures of oxamide in solution due to the partial double bond character of amides. (b) ^1H – ^{15}N HSQC of solid sample Ox4 with proton assignments of oxamide.

Samples Ox3 and Ox4 were solubilized in deuterated dimethyl sulfoxide ($\text{DMSO}-d_6$) and subjected to nuclear magnetic resonance (NMR) analysis.

The results of 1D and 2D NMR are shown in Figure S3a,b. The spectra confirmed the occurrence of oxamide by the presence of a single signal at δ 162.5 in the ^{13}C NMR spectra (Figure S3b), together with two singlets in the downfield region ($\text{H}_a = \delta$ 7.69, $\text{H}_b = \delta$ 7.98) of the ^1H NMR spectra (Figure S3a). NMR analysis showed a high degree of purity, not revealing the presence of any compounds other than oxamide. Interestingly, the presence of two signals for the amide protons indicated the partial double character of the amide bonds, as shown in Figure 6a. The resonance between the two limit structures makes the amide bond rigid without free rotation around the C–N bond and the two hydrogens not equivalent (*cis*: H_a and *trans*: H_b) (Fig. 6a). Further confirmation of the chemical structure was obtained using a ^1H – ^{15}N heteronuclear single quantum coherence (HSQC) experiment. Figure 6b reports the 2D spectra for Ox4, where proton–nitrogen couplings in oxamide were observed. In the same spectrum, two cross peaks were observed, indicating the coupling with the oxamide nitrogens with *cis* (H_a) and *trans* (H_b) protons. All this data fully defined the chemical structure of the solid precipitate of the reaction mixture as oxamide.

Once the chemical structure of oxamide was determined, the next step was the chemical characterization of the liquid phase. The analysis of the inorganic ammonium (NH_4^+), nitrate (NO_3^-), and cyanide (CN^-) ions is reported in Table 2. The concentration of NH_4^+ decreases with the decrease in the

Table 2. Concentration of Ammonium (NH_4^+), Nitrate (NO_3^-), and Cyanide (CN^-) Ions Detected in the Liquid Supernatant of the Oxamide Samples

supernatant		NH_4^+ (mg/L)	NO_3^- (mg/L)	CN^- (mg/L)
Ox1-L	μ	1191.00	nd	nd
	σ	3.94		
Ox2-L	μ	1037.44	nd	nd
	σ	3.79		
Ox3-L	μ	988.86	nd	nd
	σ	5.99		
Ox4-L	μ	1255.63	nd	nd
	σ	3.58		

percentage of ammonium hydroxide and the solid/liquid ratio used in the synthesis. In particular, NH_4^+ varies in the range between 1191.00 mg/L for the liquid (Ox1-L, Table 2) obtained from the Ox1 synthesis, characterized by the highest percentage of ammonium hydroxide and S/L ratio, and 1255.63 mg/L for the liquid (Ox4-L, Table 2) obtained from the Ox4 synthesis of the sample, characterized by the lowest percentage of ammonium hydroxide and S/L ratio. NO_3^- and CN^- were not detected in all samples.

The liquid phase, after the precipitation of oxamide, was further analyzed by 1D and 2D nuclear magnetic resonance (NMR) and mass spectrometry (MS) to characterize the co-products of the reaction.

The ^1H NMR spectrum is reported in Figure S4a. Signals appeared well resolved, and their analysis by a two-dimensional

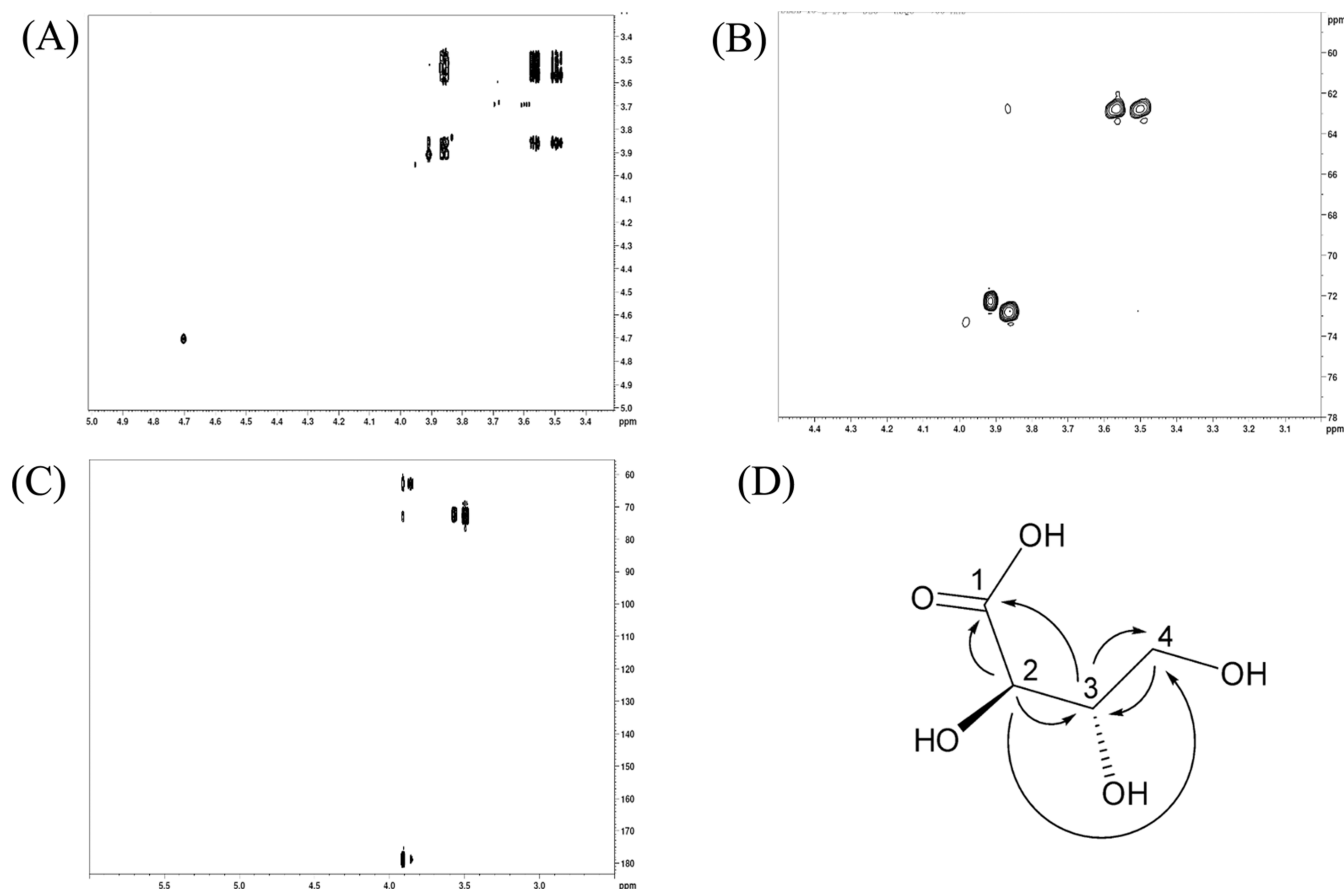


Figure 7. 2D COSY (A), HSQC (B), HMBC (C), and HMBC H → C correlations (D) of L-threonic acid.

COSY spectrum indicated that they were mutually coupled (Figure 7), thus suggesting the presence of one chemical compound with the purity level of 98%.

The ^{13}C NMR spectrum (Figure S4b) contained a low-field quaternary carbon resonating at δ 178.8 ppm, indicative of the presence of a carboxylic group at C1 (Table 3). The signals at

Table 3. ^1H and ^{13}C NMR Assignment of L-Threonic Acid

position	δ_{C}	δ_{H} (mult, J in Hz)
1	178.8	
2	72.3	3.91 (d, 2.4 Hz)
3	72.8	3.86 (ddd, 7.5, 5.3, 2.4 Hz)
4	62.7	3.56 (dd, 11.7, 5.3 Hz), 3.50 (dd, 11.7, 7.5 Hz)

δ 72.3 and 72.8, whose protons resonated, respectively, at δ 3.91 (d, 2.4 Hz) and 3.86 (ddd, 7.5, 5.3, and 2.4 Hz), were characteristic of the two oxymethylene carbons at C2 and C3. The fourth signal at δ 62.7 (whose protons resonated at δ 3.56

(dd, 11.7, 5.3 Hz) and 3.50 (dd, 11.7, 7.5 Hz) was ascribable to the oxymethylene carbon at C4).

The ^1H – ^1H COSY spectrum (Figure 7a) showed the homonuclear proton correlation coupling, while the ^1H – ^{13}C HSQC spectrum allowed the connection of each carbon atom with the corresponding protons (Figure 7b).

The HMBC spectrum (Figure 7c) with the observed diagnostic correlation peaks, reported in Figure 7d, confirmed the assignment reported in Table 3, pointing to the chemical structure of L-threonic acid as the sole component of the liquid phase.

Confirmation of the formation of threonic acid was obtained by the high-resolution mass spectrum, which showed a molecular ion peak at m/z 136.0369 $[\text{M} + \text{H}]^+$ (calcd 136.0372), pointing to the molecular formula of $\text{C}_4\text{H}_8\text{O}_5$.

Threonic acid is a sugar acid, a four-carbon polyhydroxy acid saccharide^{13–15} derived from threose. Its L-isomer is a natural sugar acid, a breakdown product of vitamin C, which is found in the body and certain foods. It has also been detected in human urine and plasma as part of normal metabolic processes.

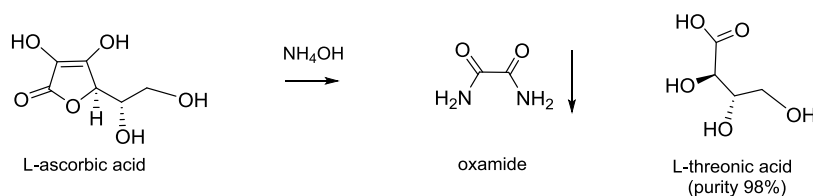


Figure 8. Reaction of L-ascorbic acid with ammonia solution affording oxamide and L-threonic acid.

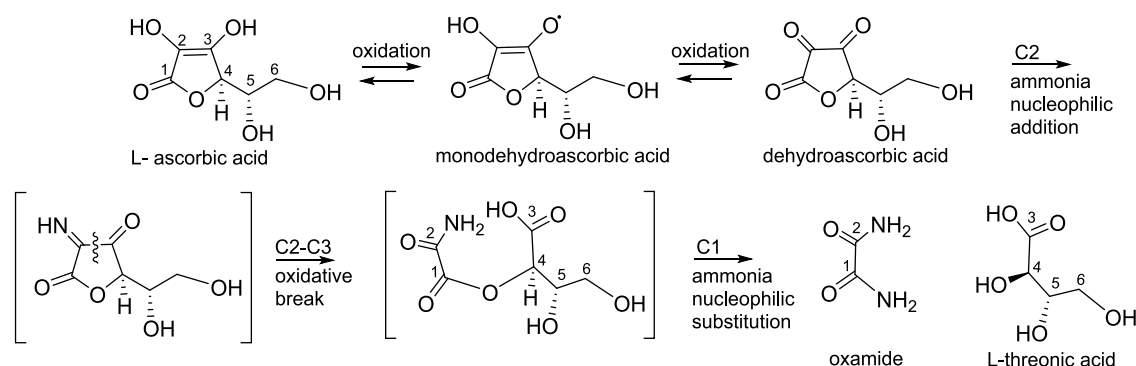


Figure 9. Proposed reaction mechanism of L-ascorbic acid with ammonia affording oxamide and L-threonic acid. The first two reactions are known degradative steps affording the more reactive dehydroascorbic acid. Hypothetical intermediates obtained by nucleophilic attack of ammonia are in square brackets.

Such a compound is known for its role in cellular metabolism and potential benefits, especially when combined with magnesium (magnesium L-threonate) to enhance brain magnesium levels and support cognitive function, offering promise in research for neurological conditions like Alzheimer's and improving mood.¹⁶ Additionally, it can inhibit DHT-induced DKK-1 protein expression, which naturally drives hair follicle degeneration.¹⁷ Therefore, the production of L-threonic acid as a co-product gives an additional value to the reaction because of its promising interest as a drug candidate in the pharmaceutical sector.

All of the obtained data allowed us to fully characterize the products obtained from the proposed one-pot synthesis (Figure 8).

On the basis of the stoichiometry of this reaction and taking into account the quantity of oxamide produced experimentally under the optimized reaction conditions (sample Ox4), it is possible to determine a yield equal to 40.7% with respect to the theoretical obtainable quantity.

A tentative mechanism for the reaction, based both on the ascorbic acid reactivity^{18–20} and the nucleophilic characteristic of ammonia solutions, is proposed (Figure 9). Ascorbic acid possesses an enediol at C2/C3. Such functional groups can easily donate electrons, being classified as strong reducing agents,²¹ to afford the oxidated forms, monodehydroascorbic acid (MDHA) and dehydroascorbic acid (DHA), the last compound presents two carbonyls at C2/C3. These reaction steps are well-known from the literature^{19,20} occurring in plant species due to the high ascorbate reactivity.^{18–20} Further steps can be hypothesized based on the nature of the involved reagents, and the proposed intermediates are reported in brackets. The ammonia nucleophilic addition on the highly reactive C2 carbonyl in DHA affords the corresponding imine, which then will undergo the C2–C3 oxidative cleavage to directly yield the C2 amide and the threonic acid. Such a reaction opens the 5-membered lactone ring, affording an oxamidyl ester.¹⁹ Nucleophilic substitution of ammonia at C1 breaks the ester bond, leading to an amide group at C1 and the release of L-threonic acid. C5 is not involved in these reaction steps, thus keeping the L-configuration of ascorbic acid in the newly formed threonic acid. This is one possible reaction mechanism. However, by bubbling air into the reaction system, no increase was observed in the reaction rate and product yields, thus suggesting that the oxidation steps, if present, are not the rate-determining reaction steps. Further studies are needed to clarify the reaction mechanisms.

CONCLUSIONS

Oxamide has been produced using a one-pot method from nontoxic substances such as vitamin C and ammonium hydroxide at different concentrations, varying the solid (vitamin C)/liquid (ammonium hydroxide solution) ratio and the temperature (25 and 50 °C).

The maximum yield of oxamide (equal to 40.7% with respect to the theoretical value) has been obtained at room temperature in the presence of diluted ammonium hydroxide solutions (10%), precursor molar ratio equal to 0.1, and low solid/liquid ratios (equal to 0.05). The oxamide sample Ox4 in its solid state, thus obtained, is characterized by high purity, calculated by HPLC analysis at a mean value of 102%, with a standard deviation of $\pm 16\%$ and an RSD = 15.7%, which accounts for the uncertainty associated with the measure. The resulting liquid is constituted by L-threonic acid, which can be easily recovered by lyophilization and valorized as a by-product.

From the results obtained, it is evident that the reported synthesis is characterized by the absence of toxic substances, both among the starting reagents and the products, and by the employment of a renewable feedstock (ascorbic acid). This procedure allows the decrease of energy requirements, since the synthesis is conducted at room temperature, thus reducing the production costs and making it a potentially significant method for oxamide chemical synthesis.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acsomega.6c05095>.

XRD patterns of Ox3 and Ox4 (Figure S1); SEM micrographs of Ox4 and oxamide (Figure S2); EDX elemental analysis data (Table S1); ¹H and ¹³C NMR spectra of solid samples Ox3 and Ox4 (Figure S3); and ¹H and ¹³C NMR spectra of liquid phase with L-threonic acid (Figure S4) (PDF)

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Notes

The authors declare no competing financial interest.

REFERENCES

- (1) Abdur Rahman, M.; Haque, S.; Athikesavan, M. M.; Kamaludeen, M. B. A review of environmental friendly green composites: production methods, current progresses, and challenges. *Environ. Sci. Pollut. Res.* **2023**, 30 (7), 16905–16929.
- (2) Favre, H. A.; Powel, W. H. *Nomenclature of Organic Chemistry: IUPAC Recommendations and Preferred Names*; The Royal Society of Chemistry: Cambridge, 2013; p 841.
- (3) Riemenschneider, W.; Tanifuji, M. Oxalic Acid. In *Ullmann's Encyclopedia of Industrial Chemistry*; Wiley-VCH: Weinheim, 2002.
- (4) Tang, Y.; Li, X.; Shen, W.; Duan, Z. Effect of the Slow-Release Nitrogen Fertilizer Oxamide on Ammonia Volatilization and Nitrogen Use Efficiency in Paddy Soil. *Agronomy* **2018**, 8, 53.
- (5) Vernacchia, M. T.; Mathesius, K. J.; Hansman, R. J. Slow-Burn Ammonium Perchlorate Propellants with Oxamide: Burn Rate Model, Testing, and Applications. *J. Propul. Power* **2021**, 37 (5), 792–800.
- (6) Riemenschneider, W.; Wegener, P. Process for the Preparation of Oxamide. U.S. Patent US3989753A, 1976.
- (7) Brown, E. H.; Wilhide, W. D.; Elmore, K. L. A New Method for the Preparation of Oxamide. *J. Org. Chem.* **1962**, 27 (10), 3698.
- (8) Yamamoto, T. et al. Process for Producing Oxamide, Ube Industries US6348626, 2002.
- (9) Knobloch, D. J.; Lobkovsky, E.; Chirik, P. J. Functionalization of hafnium oxamidide complexes prepared from CO-induced N₂ cleavage. *J. Am. Chem. Soc.* **2010**, 132, 15340–15350.
- (10) Yang, L.; Liu, L.; Olsen, B. A.; Nussbaum, M. A. The determination of oxalic acid, oxamic acid, and oxamide in a drug substance by ion-exclusion chromatography. *J. Pharm. Biomed. Anal.* **2000**, 22, 487–493.
- (11) Olsen, B. A. Hydrophilic interaction chromatography using amino and silica columns for the determination of polar pharmaceuticals and impurities. *J. Chromatogr. A* **2001**, 913, 113–122.
- (12) Lipps, W. C.; Braun-Howland, E. B.; Baxter, T. E. *Standard Methods for the Examination of Water and Wastewater*, 24th ed.; APHA Press, 2023.
- (13) Whistler, R. L.; BeMiller, J. N. *Carbohydrate Chemistry for Food Scientists*, 2nd ed.; Eagan Press, 1997.
- (14) Pigman, W.; Horton, D. *The Carbohydrates: Chemistry and Biochemistry*; Academic Press, 1980; Vol. 1A & 1B.
- (15) Binkley, R. W. Photochemical Reactions of Carbohydrates. In *Advances in Carbohydrate Chemistry and Biochemistry*; Academic Press, 1981; Vol. 38, pp 105–193.
- (16) Sun, Q.; Weinger, J. G.; Mao, F.; Liu, G. Regulation of structural and functional synapse density by L-threonate through modulation of intraneuronal magnesium concentration. *Neuropharmacology* **2016**, 108, 426–439.
- (17) Kwack, M. H.; Ahn, J. S.; Kim, M. K.; Kim, J. C.; Sung, Y. K. Preventable effect of L-threonate, an ascorbate metabolite, on androgen-driven balding via repression of dihydrotestosterone-induced dickkopf-1 expression in human hair dermal papilla cells. *BMB Rep.* **2010**, 43, 688–692.
- (18) Huelin, F. E. Studies on the anaerobic decomposition of ascorbic acid. *J. Food Sci.* **1953**, 18, 633–639.
- (19) Green, M. A.; Fry, S. C. Vitamic C degradation in plant cells via enzymatic hydrolysis of 4-O-oxalyl-L-threonate. *Nature* **2005**, 433, 83–87.
- (20) Ford, C. M.; Sweetman, C.; Fry, S. C. Ascorbate degradation: pathways, products, and possibilities. *J. Exp. Bot.* **2024**, 75, 2733–2739.
- (21) Hellwig, M.; Gensberger-Reigl, S.; Henle, T.; Pischetsrieder, M. Food-Derived 1,2-Dicarbonyl Compounds and their Role in Diseases. *Semin. Cancer Biol.* **2018**, 49, 1–8, DOI: [10.1016/j.semcancer.2017.11.014](https://doi.org/10.1016/j.semcancer.2017.11.014).