Reaction of Superoxide with Ascorbic Acid Derivatives: Insight into the Superoxide-Mediated Oxidation of Dehydroascorbic Acid

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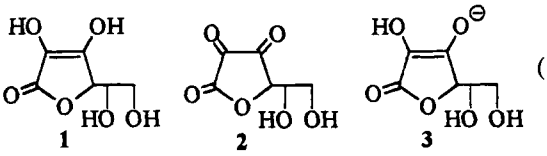
In order to gain greater insight into the mechanism of superoxide-mediated oxidation of ascorbic acid (**1**) in aprotic media, we reacted $O_2^{\bullet-}$ (generated from KO_2/18-crown-6 in toluene) with vitamin C derivatives **13a** and **14a** and the corresponding mono- and dimethoxy analogs **13b** and **13c**, respectively. Dihydroxyfuranones **13a** and **14a** underwent oxidative cleavage with $O_2^{\bullet-}$ yielding, upon methyl iodide workup, the corresponding keto ester (**15** or **17**, respectively) and threonic acid analog (**16** or **18**, respectively). On the other hand, mono- and dimethoxy analogs **13b** and **13c** each react with superoxide to give a single isolable product, oxyester **16** and alkylidenefuranone **20**, respectively. Finally, **13a** reacts with *tert*-butoxide, again yielding **16** as the major product. The data are best resolved by suggesting that ascorbic acid analogs **13a** and **14a** (and presumably ascorbic acid as well) are oxidized by $O_2^{\bullet-}$ to the corresponding triketone **21** which reacts in turn by attack at the highly electrophilic central carbonyl C-2. Cyclization of the resulting *2-peroxy 1,3-diketone* **22** into the C-1 carbonyl, followed by oxidative cleavage, saponification, and methylation, yields the observed products. By contrast, $O_2^{\bullet-}$ oxidation of **13b** and *tert*-butoxide oxidation of **13a** yield *3-peroxy-1,2-diketone* **29** which cyclizes into the C-1 carbonyl ultimately yielding **16**. Finally, **13c**, which lacks enolic hydrogens, undergoes abstraction of the γ-hydrogen followed by the elimination of acetone, yielding **20**. Similarly, 5,6-dihydropyrone **33** undergoes superoxide-mediated elimination yielding dienone **34**. The data presented herein are consistent with the mechanism suggested by Sawyer *et al.* (*J. Am. Chem. Soc.* **1982**, *104*, 6273–6278) for the superoxide-mediated oxidation of dehydroascorbic acid (**2**)—with the modification that the position of initial $O_2^{\bullet-}$ attack is at the C-2 (rather than the C-3) carbonyl.

Introduction

Both oxygen and free radical processes play a central role in "the breath of life"; yet, ironically, oxy radicals present a serious and constant threat to living organisms.[1] One of the clearest sources of radicals in the body is superoxide anion radical, $O_2^{\bullet-}$, which is generated in numerous dark biological processes.[2] The heated debate regarding superoxide's modes of biological action[3] inspired a host of chemical studies as well. Nearly two decades of research on the organic chemistry of $O_2^{\bullet-}$ has revealed that, in aprotic media, this anion radical reacts with organic substrates via several basic modes:[4] as a relatively strong base, a supernucleophile, a moderate reducing agent, and only rarely if ever, as a radical (via radical–radical coupling, addition to olefins, or hydrogen atom abstraction).

Our interest in the organic chemistry of superoxide[5–7] and its interaction with compounds of biochemical importance led us to focus our attention on vitamin C (ascorbic acid, **1** in eq 1), one of the many natural



(1)

antioxidants which protect the cell against oxidative and/or free radical damage.

Ascorbic acid is one of the most prominent members of a class of compounds known as *aci*-reductones (**4**, eq 2).[8] These 1-oxo-2-ene-2,3-diols exist in several tautomeric forms and generally undergo oxidation to the corresponding triketones (**5**) or the related hydrate (**6**).

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(2) See, for example, the collection of articles in: *Superoxide Dismutase*; Oberley, L. W., Ed.; Chemical Rubber Co.: Boca Raton, FL, 1982; Vols. I and II; Vol. III, 1985.

(3) (a) See the articles of I. Fridovich and J. Fee and the subsequent extensive discussion in: *Oxygen and Oxy-Radicals in Chemistry and Biology*; Rodgers, M. A. J., Powers, E. L., Eds.; Academic Press: New York, 1981; pp 197–239. (b) See the exchange of correspondence between Fridovich and Sawyer and Valentine: Fridovich, I.; Sawyer, D. T.; Valentine, J. S. *Acc. Chem. Res.* **1982**, *15*, 200. (c) Baum, R. M. *Chem. Eng. News* **1984**, April 9, 20–26 and subsequent letters to the editor in the June 4th and July 2nd issues.

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(7) Frimer, A. A.; Marks, V.; Gilinsky-Sharon P. *Free Radical Res. Commun.* **1991**, 12–13, 93–98.

(8) (a) Schank, K. *Synthesis* **1972**, 176–190. (b) Hesse, G. In *Houben-Weyl: Methoden der Organischen Chemie*; Kropf, H., Hesse, G., Eds.; Verlag: Stuttgart, 1978; Vol. VI/1d, pp 217–298.

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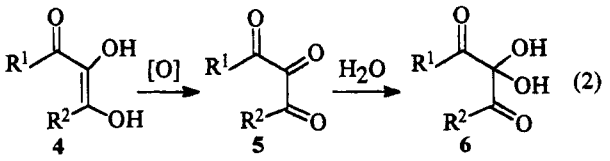
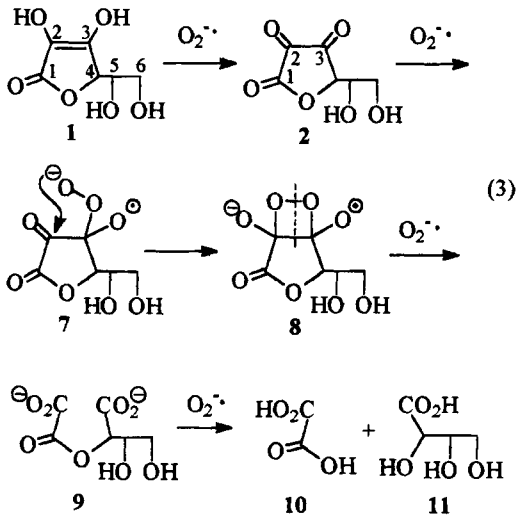


(2)

The mechanistic details of the superoxide-mediated oxidation of ascorbic acid (**1**) to dehydroascorbic acid (**2**) in aprotic media (eq 3) is still a matter of some dispute,[9-12]

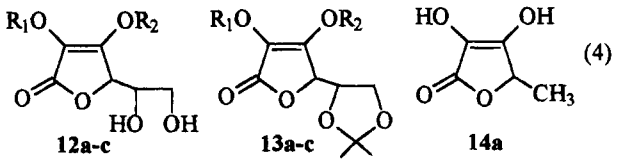


(3)

particularly on the question of the intermediacy of the ascorbate ion (**3**, eq 1). Our attention in this paper, however, is centered on the subsequent superoxide-mediated oxidation of dehydroascorbic acid (**2**) reported by Sawyer and co-workers[9b] to yield to oxalic acid (**10**) and (by inference) threonic acid (**11**) (see eq 3).[9b] Spectral and electrochemical data of the reaction mixtures led these authors to suggest the mechanism outlined in eq 3. We believed, however, that a similar study of vitamin C derivatives would enable us to isolate the elusive intermediates and products, thereby shedding light on the reaction mechanism of the parent ascorbic acid system.

Results

A. Preparation of Ascorbic Acid Derivatives 12–14. For the purpose of this study, we reacted $O_2^{\cdot-}$ with vitamin C derivatives **13a** and **14a** and the corresponding mono and dimethoxy analogs **13b** and **13c**, respectively (eq 4). Enol ethers **12b**[13] and **13b**[14] were prepared from ascorbic acid (**12a** = **1**) and its acetonide (**13a**)[15] by

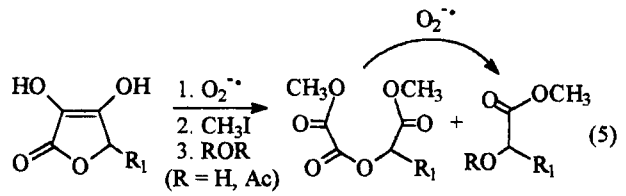


(4)

a: $R_1 = R_2 = H$ **b**: $R_1 = H$; $R_2 = CH_3$ **c**: $R_1 = R_2 = CH_3$

reacting the latter with 1 equiv of CH_2N_2. Treatment of **12a** and **13a** with excess diazomethane yielded the corresponding dimethoxy analogs **12c**[15a] and **13c**.[15a] Dihydroxyfuranone **14a** was prepared according to the procedure of von Euler and Hasselquist.[16]

B. Reaction of Ascorbic Acid Derivatives 12–14 with Superoxide. As shown in eq 5, reaction of compounds **13a** and **14a** with $O_2^{\cdot-}$ yielded upon methyl iodide workup[17] two oxidative cleavage products, the corresponding keto ester (**15** or **17**, respectively) and threonic acid analog (**16** or **18**, respectively). Compounds



(5)

13a (R_1=acetonide)	**15** (33%)	**16** (67%)
14a (R_1=methyl)	**17** (30%)	**18** (60%)

15,[15a,16b] **16** (R = H, Aldrich),[15b,16b] **17**,[16b] and **18** (R = H, methyl lactate) are all known. The magnitude and sign (+) of the specific optical rotation of **15** and **16** correspond well to that reported for related L-ascorbic acid derivatives.[13b,14,15]

Two comments are in order. Firstly, α-hydroxy esters **16** and **18** (R = H) are water soluble. Hence, in order to facilitate product isolation following CH_3I quenching, the reaction mixtures were often treated with acetic anhydride, producing the corresponding acetate derivatives (**16** and **18**, R = Ac). We also note that under the same reaction and workup conditions, **15** is saponified to **16** in quantitative yield. Any dimethyl oxalate (**27** in eq 7) formed in the process is presumably lost during the final aqueous workup.

In contrast to dihydroxyfuranones **13a** and **14a**, the monomethoxy and dimethoxy analogs **13b** and **13c** each react with superoxide to give a single isolable product, oxyester **16** and alkylidene furanone **20**[18] (as an *E*/*Z* mixture), respectively. In addition, **13a** reacts with *tert*-butoxide, again yielding **16** as the major product, along with a small amount of glycerate **19** (Aldrich)—a second-

(9) (a) Sawyer, D. T.; Calderwood, T. S.; Johlman, C. L.; Wilkins, C. L. *J. Org. Chem.* **1985**, *50*, 1409–1412. (b) Sawyer, D. T.; Chiericato, G., Jr.; Tsuchiya, T. *J. Am. Chem. Soc.* **1982**, *104*, 6273–6278. (c) Nanni, E. J., Jr.; Stallings, M. D.; Sawyer, D. T. *J. Am. Chem. Soc.* **1980**, *102*, 4481–4485.

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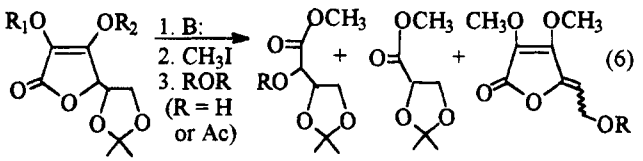
(15) (a) Jung, M. E.; Shaw, T. J. *J. Am. Chem. Soc.* **1980**, *102*, 6304–6311. (b) Wei, C. C.; De Bernardo, S.; Tengi, J. P.; Borgese, J.; Weigele, M. *J. Org. Chem.* **1985**, *50*, 3462–3467.

(16) (a) von Euler, H.; Hasselquist, H. *Arkiv. Kemi* **1955**, *8*, 67–72; *Chem. Abstr.* **1956**, *50*, 6318f. (b) Kwon, B.-Y.; Foote, C. S.; Khan, S. I. *J. Am. Chem. Soc.* **1989**, *111*, 1854–1860. In this paper, there are several errors in the NMR spectral data of compounds **15** and **16**; see the Experimental Section.

(17) Superoxide reactions can be quenched with either aqueous acid or methyl iodide. A desirable side effect of the latter method is that many of the oxy-anions present are methylated, converting, for example, enolates to enol methyl ethers and carboxylates to methyl esters. Alcoholates are generally unaffected and phenolates give varying results.[6b]

(18) (a) Eitelman, S. J.; Hall, R. H.; Jordaan, A. *J. Chem. Soc., Chem. Commun.* **1976**, 922–923. (b) Hall, R. H.; Bischofberger, K.; Eitelman, S. J.; Jordaan, A. *J. Chem. Soc., Perkin Trans. 1* **1977**, 2236–2241.

ary base catalyzed autoxidation product of the former (eq 6).

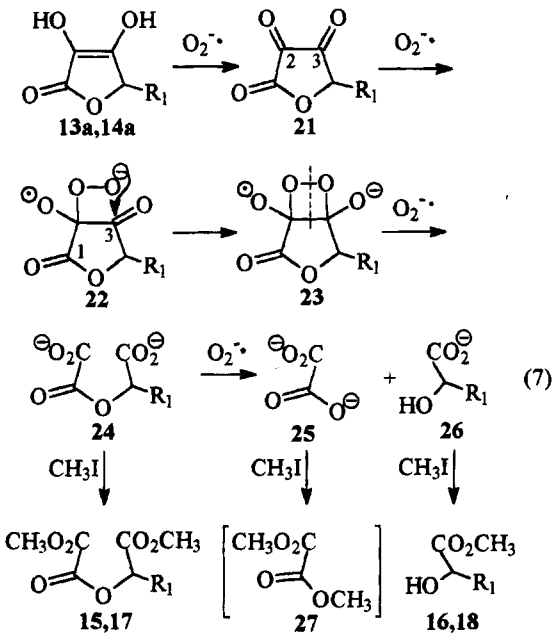


(6)

		16	19	20
13				
13a	B: = *t*-BuO⁻	50%	20%	----
13b	B: = $O_2^{-\cdot}$	82%	----	----
13c	B: = $O_2^{-\cdot}$	----	----	37%

Discussion

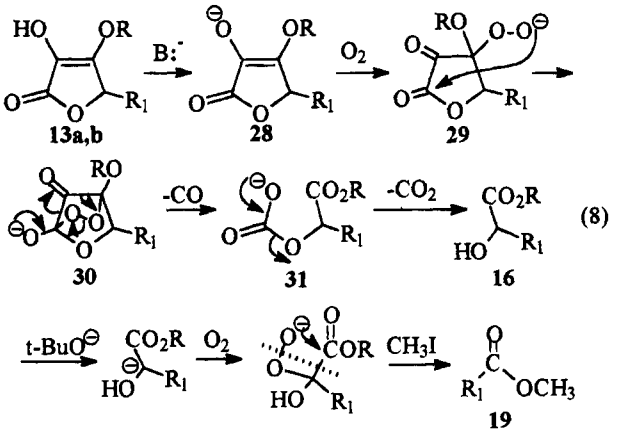
We turn now to the question of mechanism. The results in the superoxide-mediated oxidation of *aci*-reductones **13a** and **13b**—in particular, the isolation of keto diesters **15** and **17**—are most reasonably accommodated (eq 7) by assuming that *aci*-reductones **13a** and



(7)

14a are initially oxidized to the corresponding triketones **21** (analogous to dehydroascorbic acid, **2**). Initial superoxide attack on triketones **21** is expected to occur at the *central* C-2 carbonyl, well known to be the most electrophilic of the three.[19] Possible C-3 attack, as suggested earlier by Sawyer (eq 3),[9b] is also unlikely because this would lead to a 3-hydroperoxy-1,2-dicarbonyl compound (**7** in eq 3) known to prefer cyclization across the ring into the C-1 carbonyl with concomitant loss of C-2 as carbon monoxide (*vide infra* discussion at eq 8).[4d,6,20]

Assuming, then, initial $O_2^{\bullet-}$ attack at C-2, the resulting 1,3-dioxo-2-peroxy anion **22** can *a priori* cyclize into either of the flanking carbonyls. Considering, however, that C-1 is a lactone carbonyl, cyclization into the more electrophilic ketone at C-3 would clearly be preferred. The latter process yields dioxetane **23** which cleaves to dicarboxylate **24** (trapped by CH_3I as the diesters **15** and **17**). Subsequent saponification of the ester linkage by



(8)

$O_2^{\bullet-}$ and esterification by methyl iodide yields the observed products (**16** and **18**).

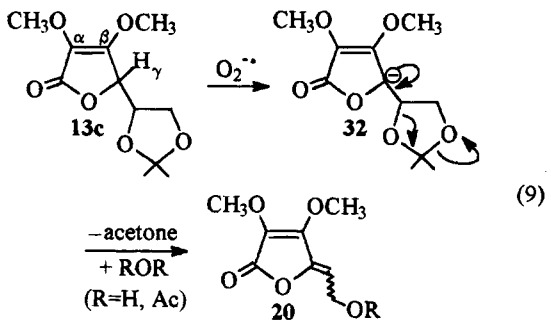
No keto ester **15** is observed in the case of the *tert*-butoxide-mediated oxidation of dihydroxyfuranone **13a** and the superoxide-mediated oxidation of monomethoxy analog **13b**. It is likely, therefore, that a different mechanism is in effect which does not involve oxidative cleavage of the C_2–C_3 bond, as is required by dioxetanes of type **23**. We propose a well-precedented mechanism (eq 8) analogous to that observed in the base-catalyzed autoxidation of α-keto enols.[4d,6] Deprotonation of **13a** or **13b** and C-oxygenation of the corresponding enolate **28** generates 3-hydroperoxy-1,2-dicarbonyl compound **29**. Such compounds are known to cyclize to endoperoxides of type **30** and eliminate carbon monoxide, as shown in eq 8.[4d,6,20] (Although endoperoxidation requires cyclization into the less electrophilic lactone carbonyl at C-1, this yields a 5-membered ring which is far preferred over a highly strained 4-membered dioxetane.) Subsequent facile loss of CO_2 from the resulting carbonate **31** and methylation by CH_3I yields the observed product **16** (R = CH_3). In the case of the stronger base, *tert*-butoxide, **16** undergoes further base-catalyzed autoxidation and oxidative cleavage generating **19**.

We close this paper with a discussion of the intriguing formation of alkylidene furanone **20** from the acetonide analog **13c**. This α,β-unsaturated lactone lacks enolic hydrogens and only the γ-hydrogen is slightly acidic. Abstraction of the latter proton by $O_2^{\bullet-}$ generates carbanion **32**. Oxygenation, however, is not always the preferred reaction course for a carbanion. Indeed, in the

(19)) (a) Rubin, M. *Chem. Rev.* **1975**, *75*, 177–202. (b) Schonberg, A.; Singer, E. *Tetrahedron* **1978**, *34*, 1285–1300.

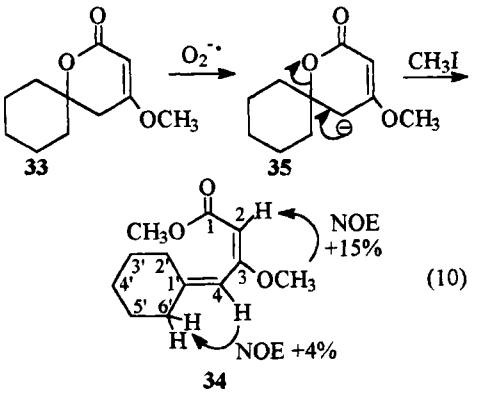
(20) (a) Cyclization of 3-hydroperoxy-1,2-dicarbonyls into the adjacent C-2 carbonyl has been observed in certain instances as the *minor* pathway.[4d] Tournaire and co-workers[20b] have, however, recently reported that *uncomplexed* KO_2 in apolar aprotic media (toluene) mediates the oxidative cleavage of flavonols and, based on the isolation of aldehydic products, suggest just such a cyclization as the *major* pathway. The reaction presumably occurs at the liquid–solid interface, but it is not at all clear what the active species or intermediates are. In light of the fact that the reaction conditions, the products generated, and the mechanistic evidence presented in this work are at odds with that described in the many previous flavonol studies,[20c–i] not to mention other α-keto enol moieties,[4d,6] we suggest that the determination of mechanism in the system of Tournaire *et al.*[20b] await more solid evidence. (b) Tournaire, C.; Hocquaux, M.; Beck, I.; Oliveros, E.; Maurette, M.-T. *Tetrahedron* **1994**, *50*, 9303–9314. (c) Matsuura, T.; Matsushima, H.; Sakamoto, H. *J. Am. Chem. Soc.* **1967**, *89*, 6370–6371. (d) Matsuura, T.; Matsushima, H.; Nakashima, R. *Tetrahedron* **1970**, *26*, 435–443. (e) Nishinaga, A.; Matsuura, T. *J. Chem. Soc., Chem. Commun.* **1973**, 9–10. (e) Nishinaga, A.; Tojo, T.; Tomita, H.; Matsuura, T. *J. Chem. Soc., Perkin Trans. 1* **1979**, 2511–2516. (f) Utaka, M.; Matsushita, S.; Yamasaki, H.; Takeda, A. *Tetrahedron Lett.* **1980**, *21*, 1063–1064. (g) Rajanada, V.; Brown, S. B. *Tetrahedron Lett.* **1981**, *22*, 4331–4334. (h) Takahama, U. *Plant Cell Physiol.* **1987**, *28*, 953–957. (i) Studer, S. L.; Brewer, W. E.; Martinez, M. L.; Chou, P.-T. *J. Am. Chem. Soc.* **1989**, *111*, 7643–7644.

case at hand, intramolecular elimination occurs as outlined in eq 9.



(9)

Such transformations are well precedented.[18,21] For example, we have found that 5,6-dihydropyrone **33**[21] undergoes superoxide-mediated elimination yielding dienone **34** in 90% yield upon methyl iodide workup of the reaction mixture (eq 10). The *cis* nature of the C-2, C-3



(10)

double bond was verified in the difference NOE experiment;[22] we observed a significant enhancement (ca. 15%) of the H-2 hydrogen when the C-3 methoxy group was irradiated. We note, with some surprise, that in the case of both **13c** and **34** saponification of the lactone linkage is not observed.[4d]

In conclusion, then, the product data reported herein confirms most aspects of Sawyer's mechanism[9b] for the superoxide-mediated oxidation of dehydroascorbic acid (eq 3), with the modification that the position of initial $O_2^{\bullet-}$ attack is at the central C-2 carbonyl. Further work on the superoxide chemistry of *aci*-reductones is presently in progress.

Experimental Section

^{1}H NMR spectra were obtained on 100 and 300 Fourier transform spectrometers, while ^{13}C NMR (75 MHz) spectra were taken with the latter instrument. Assignments (see supplementary material) were facilitated by correlating proton and carbon chemical shifts through analysis of residual couplings in off-resonance decoupled spectra. In all cases, TMS served as the internal standard. The spectral data of known compounds are not given unless they are missing from the literature (most common for ^{13}C NMR data), in error, or otherwise lacking. High-resolution mass spectra (HRMS) were performed on a VG-Fison AutoSpecE high-resolution spectrometer. Preparative thin layer chromatography (TLC) was carried out on Merck silica gel F_{254} precoated plates, while analytical runs were performed using Riedel-De Haen microcards. The retention times given are based on the analytical runs. Gas chromatograms were obtained using a preparative GC with peak areas determined by triangulation. Potassium superoxide (Alfa Inorganics, as small chunks, or Callery, as a fine powder), *tert*-butoxide (Fluka), and hydroxide (Frutarom) salts were ground into fine powders in a glovebag under dry argon prior to use. 18-Crown-6 polyether (Fluka) was used as supplied if dry and crystalline; otherwise, it was recrystallized from acetonitrile[23] and stored along with the above potassium salts in a desiccator. Methyl iodide was distilled and stored at −10 °C under argon.

General Oxidation Procedure Using KO_2, KOH, and $KOC(CH_3)_3$. Reactant, 18-crown-6, and powdered KX [X = O_2, OH, or $OC(CH_3)_3$] were added in that order to sodium-dried toluene (or P_2O_5-dried THF when specified). The "reactants ratio" (i.e., the molar ratio of substrate:crown:KX) and reaction times were optimized for each substrate. Approximately 35 mL of solvent was used per mmol of substrate. The reaction mixture was stirred magnetically under dry air (unless otherwise indicated) at room temperature until TLC indicated that all the starting material had been consumed. The reaction was then quenched by one of two methods.[17] **(a) Aqueous Quenching.** The reaction was acidified with 10% HCl, which is quite commonly accompanied by a sudden color change as neutrality is approached. (In reactions run in THF, a volume equivalent of benzene was added at this juncture prior to the aqueous washings.) The organic layer was then washed three times with 10% $NaHCO_3$ to remove inorganic salts, crown ether, and acidic products. The organic phase containing the nonacidic products was dried over $MgSO_4$ and concentrated, and the products were then isolated. The combined $NaHCO_3$ extracts were acidified and extracted three times with ether. The combined ether extracts were dried and concentrated and the products isolated. **(b) Methyl Iodide Quenching.** Alternatively, excess (generally 10 molar equiv based on substrate) CH_3I was added, which consumes the unreacted base and methylates various oxyanions in the product. The reaction mixture was generally allowed to react overnight with the CH_3I (though shorter reaction times may well have sufficed). (In reactions run in THF, a volume equivalent of benzene was added at this juncture prior to the aqueous washings.) The reaction mixture was washed with water to remove inorganic salts, methanol, crown ether, and excess methyl iodide. The organic layer was then dried and concentrated, and the products were isolated.

3,4-Dihydroxy-5-methyl-5*H*-furan-2-one (14a). The title compound was prepared according to the 1955 procedure of von Euler and Hasselquist.[16]

14a: ^{1}H NMR (acetone-d_6) δ 8.33 (br s, 2H), 4.72 (q, J = 6 Hz, 1H), 1.40 (d, J = 6 Hz, 3H); ^{13}C NMR (acetone-d_6) δ 170.48, 155.41, 118.42, 74.18, 18.54.

Reaction of 5,6-*O*-Isopropylidene-L-ascorbic Acid (13a) with KO_2. Acetonide **13a** (11.5 mmol) was reacted overnight (16 h) with $O_2^{\bullet-}$ according to the general oxidation procedure in a "reactants ratio" of 2:1:1 and subsequently quenched with an 80-fold excess of CH_3I. After 72 h of stirring, rotary evaporation of the solvent (heating to 58 °C) yielded 2.4 g of solid residue. The latter was then treated by one of two methods. **(a) Aqueous Workup.** The residue was dissolved in ether (300 mL) and extracted twice with 3 mL portions. The combined water washings were extracted with 200 mL of ethyl acetate.[24] The combined ether and ethyl acetate phases were dried and concentrated. Two products in a 2:1 ratio (determined by ^{1}H NMR) and with retention times of 4.5 and 13.5

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(24)) This procedure was adopted to keep the loss of the water soluble products to a minimum. Indeed the weight of the crude product prior to separation suggested that little if any product had been lost.

min, respectively, were isolated by preparative GC[25a] (flow rate: 100 mL/min; oven: initially 100 °C and raised to 140 °C after the elution of the first product; injector: 200 °C; detector: 180 °C). Both the major and minor products are known and were identified by their spectral data as methyl 3,4-*O*-isopropylidene-L-threonate (**16**) (R = H, Aldrich)[16b] and methyl 3,4-*O*-isopropylidine-2-*O*-(methoxaloyl)-L-threonate (**15**).[15,16b]

(b) Acetic Anhydride Workup. The solid residue was dissolved in 25 mL of redistilled acetic anhydride[26] (discarding the insoluble inorganic salts), and the solution was refluxed under dry air for 5 h and then allowed to stir overnight at room temperature. The excess acetic anhydride was removed under vacuum (44 °C/15 torr) and the remaining product distilled off (50–100 °C/3–5 torr). The dark yellow color of the product mixture was lightened by treatment with Celite, and the products were separated by preparative TLC on two plates eluting with 25% acetone in hexane. Three bands were observed of which one proved to be threonate **15**, while the other (R_f values of 0.17 and 0.29) had identical spectral data corresponding to **16** (R = Ac). In light of the fact that C-2 epimerization under the reaction conditions is not unexpected, the two bands are presumably two diastereomers of **16**. While diastereomers often have different NMR spectra, this is not obligatory. The two bands were combined and distilled in a kugelrohr (100 °C/3 torr).

15: $[\alpha]^{25}_D = +26.8°$ (c = 1.6, $CHCl_3$); UV (absolute ethanol) λ_{max} (ϵ_{max}) = 273 (800) nm. The remaining spectral data appears in reference 16b, although there is an error in the ^1H NMR data assignments. The peak at 4.61 ppm is the methyne (H-3) of the acetonide linkage; it is a dt (not a q), $J_{3,4}$ = 6 Hz, $J_{2,3}$ = 5 Hz.

16 (R = H). The spectral data appear in refs 15b and 16b. There are errors in the ^1H NMR data assignments in the latter reference: the peak at 4.4 ppm is the methyne of the acetonide linkage (hence dt), while the 4.16–3.95 multiplet contains the acetonide methylene and the C-2 CHOH. The m/*e* of the MS parent peak is 190 [not 244].

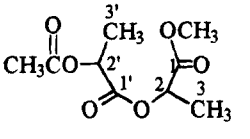
16 (R = Ac). ^1H NMR ($CDCl_3$) δ 5.1 (d, J = 5 Hz, 1H), 4.52 (dt, J = 6 Hz, J = 5 Hz, 1H), 4.12 (dd, J = 9 Hz, J = 6 Hz, 1H), 3.95 (dd, J = 9 Hz, J = 6 Hz, 1H), 3.78 (s, 3H), 2.18 (s, 3H), 1.45 (s, 3H), 1.38 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 170.15, 168.00, 110.29, 74.40, 72.40, 65.58, 52.50, 26.11, 25.33, 20.54; FTIR (neat) 1740 (br s, CO) cm^{-1}; HRMS calcd ($C_9H_{13}O_6$, $M^+ - CH_3$), 217.0712, obsd 217.0695; MS (EI, 70 eV) 217 ($M^+ - CH_3$, 100), 175 ($M - CH_2(CH_3)CO$, 20.34), 157 (M-$(CH_3)_2$-C(OH)O, 22.95), 115 ($CH_3CO_2CCO_2$, 66.68), 101 ($M - (CH_3)_2C$-(OCHCH$_2$)O, 47.20).

Reaction of 13a with KOBu-*t*. Acetonide **13a** was reacted for 4 h with KOBu-t according to the general oxidation procedure in a "reactants ratio" of 4:2:1 and subsequently quenched with an 60-fold excess of CH_3I. After 72 h of stirring, followed by aqueous workup as described for the KO_2 of **13a**, the product mixture was separated by GC[25b] (flow rate: 80 mL/min; oven: 100 °C; injector and detector: 180 °C) yielding two products with retention times of 7 and 20 min. The first was identified by its spectral data as the commercially available (Aldrich) methyl α,β-isopropylidene-L-glycerate (**19**), while the latter was compound **16**.

Reaction of 3,4-Dihydroxy-5-methyl-5*H*-furan-2-one (14a) with KO_2. Furanone **14a** (2.5 mmol) was reacted overnight (16 h) with $O_2^{\bullet-}$ according to the general oxidation procedure in a "reactants ratio" of 1.7:1:1, and the reaction was then quenched with CH_3I. Because of product loss during the standard aqueous workup, only a 25% yield of crude product was obtained. Preparative GC[25c] (flow rate: 100 mL/min; oven: 140 °C; injector: 180 °C; detector: 200 °C) yielded only one product with a retention time of 12 min and identified as the known[16b] methyl 2-[(methoxallyl)oxy]-L-acetate (**17**). Methyl 2-hydroxypropionate [methyl lactate, **18** (R = H), Aldrich] was not detected, but it is also water soluble. To circumvent the loss of water soluble products, acetylation had to be performed prior to aqueous washings. Thus, as before the reaction was quenched with methyl iodide and stirred at room temperature overnight. All the volatile components were removed by rotary evaporation, the residue was dissolved in 3 mL of redistilled acetic anhydride (discarding the insoluble inorganic salts), and the solution was refluxed under dry air for 3 h. The reaction mixture was diluted with ether, washed several times with water, dried over $MgSO_4$, and concentrated. Preparative GC[25d] (flow rate: 100 mL/min; oven: initially 70 °C and raised to 100 °C after the elution of the first product; injector: 160 °C; detector: 150 °C) yielded two products (in an overall 90% yield) in a 2:1 ratio with retention times of 7 and 10 min, respectively. The minor product proved to be **17**, while the major was identified by its spectral data as methyl 2-acetoxypropionate (**18**, R = Ac). The latter was independently synthesized by acetylating methyl lactate (**18**, R = H) in refluxing acetic anhydride and collecting the fraction distilling at 70 °C/13 Torr.

18, R = Ac: ^1H NMR ($CDCl_3$) δ 5.08 (q, J = 7 Hz, 1H), 3.75 (s, 3H), 2.13 (s, 3H), 1.48 (d, J = 7 Hz, 1H); IR (neat) 1760 and 1740 (CO, s); ^{13}C NMR ($CDCl_3$) δ 171.24, 170.21, 68.58, 52.25, 20.61, 16.95; HRMS calcd ($C_6H_9O_4$, $M^+ - 1$) 145.0501; obsd 145.0510; calcd ($C_5H_7O_4$, $M^+ - CH_3$) 131.0350, obsd 131.0344; MS (EI, 70 eV) 145 (M − 1, 0.2), 131 ($M^+ - CH_3$, 4.9), 115 ($M - CH_3O$, 23), 87 ($M - CH_3OCO$ and $M - CH_3CO_2$, 100); MS (CI, butane) 147 (MH^+).

Methylation of Lactic Acid: Isolation of 18, R = H, R = $CH_3CH(OH)CO$, and R = $CH_3CH(OAc)CO$. Although methyl lactate is commercially available (Aldrich), we synthesized it in-house via a Fischer-esterification of lactic acid with acidic methanol[27] or by treating lactic acid with CH_2N_2.[28] In one run, the lactic acid utilized was not distilled before use and presumably contained substantial amounts of lactic acid lactate. Not surprisingly, then, we discovered the presence of a second product, identified by its spectral data as methyl 2-(2-hydroxypropionyl)propionate [**18**, R = $CH_3CH(OH)CO$] and purified via preparative GC[25d] (flow rate: 85 mL/min; oven: 100 °C; injector: 170 °C; detector: 180 °C; retention time: 5 min). Acetylation of the latter yielded the corresponding methyl 2-(2-acetoxypropionyl)propionate [**18**, R = $CH_3CH(OAc)CO$, see below], which was fully characterized. The latter was vacuum distilled (bp 100 °C/1 torr) and purified by preparative TLC eluting with 25% acetone in hexane (R_f 0.21) and extracting the product from silica with $CHCl_3$. It should be noted that in these compounds there are two chiral centers. Two diastereomers are indeed detected in the NMR spectra of **18** [R = $CH_3CH(OAc)CO$] in a 2:1 ratio. The respective ^1H absorptions overlap and are distinguishable only in the methyl ester peak. In the ^{13}C spectrum, each major peak has a minor peak in close proximity.



18, R=$CH_3CH(OAc)CO$

18, R = $CH_3CH(OH)CO$. ^1H NMR ($CDCl_3$) δ 5.11 (q, J = 7 Hz, 1H), 4.31 (q, J = 7 Hz, 1H), 3.73 (s, 3H), 2.13 (s, 3H), 1.53 (d, J = 7 Hz, 3H), 1.50 (d, J = 7 Hz, 3H); IR (neat) 3400 (br, s), 1730 (CO, s) cm^{-1}; MS (EI, 70 ev) 176 (M^+), 158 ($M - H_2O$).

18, R = $CH_3CH(OAc)CO$ (major diastereomer). ^1H NMR ($CDCl_3$) δ 5.15 (overlapping q, each J = 7 Hz, 2H), 3.75 (s, 3H), 2.15 (s, 3H), 1.57 (d, J = 7 Hz, 3H), 1.53 (d, J = 7 Hz,

(25)) (a) 1.5 ft × 0.25 in. copper column packed with 20% SE-30 on Chromosorb P DMCS. (b) 8 ft × 0.25 in. copper column packed with 20% SE-30 on Chromosorb WAW. (c) 8 ft × 0.25 in. copper column packed with 20% SE-30 on Chromosorb P DMCS. (d) 3 ft × 0.25 in. copper column packed with 20% SE-30 on Chromosorb P DMCS.

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(28)) (a) McKay, A. F. *J. Am. Chem. Soc.* **1948**, *70*, 1974–1975. (b) Arndt, F. In *Organic Syntheses*; Blatt, A. H., Ed.; Wiley: New York, 1943; Collect. Vol. II, pp 165–167.

3H); ^{13}C NMR ($CDCl_3$) δ 170.33, 170.18, 169.99, 68.97, 68.27, 52.24, 20.49 16.72, 16.64; IR ($CHCl_3$) 1747 (s, CO) cm^{-1}; HRMS calcd ($C_9H_{15}O_6$, MH^+) 219.0868, obsd 219.0794; MS (EI, 70 eV) 219 (MH^+, 11.25), 115 ($CH_3CO_2CH(CH_3)CO$, 100), 87 ($CH_3CO_2CHCH_3$, 87.87). Anal. Calcd ($C_9H_{14}O_6$): C, 49.54; H, 6.46. Found: C, 49.45%; H, 6.39.

18, R = $CH_3CH(OAc)CO$ (minor diastereomer). ^1H NMR ($CDCl_3$) δ 5.15 (overlapping q, each J = 7 Hz, 2H), 3.76 (s, 3H), 2.15 (s, 3H), 1.51 (d, J = 7 Hz, 3H), 1.49 (d, J = 7 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 170.54, 170.14, 169.94, 69.10, 68.39, 52.28, 20.49, 16.85, 16.72.

Reaction of 13b with KO_2. Acetonide **13b** was reacted overnight (16 h) with $O_2^{\bullet-}$ according to the general oxidation procedure in a "reactants ratio" of 2:1:1, subsequently quenched with excess of CH_3I, and worked up as usual to give an 82% yield of crude product. Preparative GC[25a] (flow rate: 100 cc/min; oven: 80 °C; injector: 170 °C; detector: 190 °C) yielded **16** (retention time: 7.5 min). No **15** was detected when the column temperature was raised to 180 °C.

Reaction of 13c with KO_2. Acetonide **13c** was reacted overnight (16 h) with $O_2^{\bullet-}$ according to the general oxidation procedure in a "reactants ratio" of 1:$^1/_2$:1. (TLC revealed that the reaction had not gone to completion; however, longer reaction times or utilizing more KO_2 lowers the yield.) The reaction mixture was subsequently quenched with an 80-fold excess of CH_3I, evaporated to dryness, and acetylated in room temperature acetic anhydride overnight. Preparative TLC (25% acetone in hexane) yielded unreacted starting material and the known compound[18] (R_f 0.33) **20** (R = Ac) as an E/Z mixture.

Reaction of 4-Methoxy-1-oxaspiro[5.5]undec-3-en-2-one (33) with KO_2. Lactone **33**[21] was reacted overnight (16 h) with $O_2^{\bullet-}$ according to the general oxidation procedure in a "reactants ratio" of 4:2:1, quenched with excess of CH_3I, and worked up as usual to give a 90% yield of relatively pure methyl 4-cyclohexylidene-3-methoxy-2-butenoate (**34**). The latter was purified by silica column chromatography, eluting with 5% acetone in hexane, to give a colorless viscous product. The numbering of the carbons of **34** is shown in eq 10. The *cis* nature of the C-2, C-3 double bond was verified in the difference NOE experiment, and we observed a significant enhancement (ca. 15%) of the H-2 hydrogen when the C-3 methoxy group was irradiated. In addition, irradiation of H-4 allowed us to discern between H-2′ and H-6′.

34: R_f (5% acetone in hexane) 0.54; ^1H NMR ($CDCl_3$) δ 6.65 (quint d, J = 1.2 Hz, J = 0.7 Hz, 1H), 5.03 (q, J = 0.7 Hz, J = 0.5 Hz, 1H), 3.69 (d, J = 0.5 Hz, 6H), 3.65 (s, 3H), 2.47 (td + second order AA′, J = 6 Hz, J = 1.2 Hz, 2H), 2.24 (td + second order AA′, J = 6 Hz, J = 1.2 Hz, 2H), 1.7−1.55 (m, 6H); ^{13}C NMR ($CDCl_3$) δ 169.64, 167.62, 152.56, 115.08, 55.02, 50.64, 38.25, 31.16, 28.61, 27.83, 26.37; HRMS calcd ($C_{12}H_{18}O_3$, M^+), 210.1256 obsd 210.1223, calcd ($C_{11}H_{16}O_3$ $MH^+ - CH_3$), 196.1099, obsd 196.1072; MS (EI, 70 eV) m/e 210 (M^+, 0.5), 196 ($MH^+ - CH_3$, 35.4), 122 ($MH^+ - CH_3OCO - CH_2O$, 100).

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Supplementary Material Available: 300-MHz ^1H NMR spectra of **16** (R = Ac), **18** (R = Ac), and **34** and the complete ^1H and ^{13}C NMR peak assignments for the compounds described in the Experimental Section (5 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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