removed under reduced pressure. The residue was added to a solution of the 7-hydroxy compound (**2a**) (113.5 mg, 0.25 mmol) in dry acetone (50 mL) containing powdered anhydrous potassium carbonate (50 mg) and was stirred in a nitrogen atmosphere at 37 °C for 48 h. The reaction mixture was filtered to remove inorganic salts, and the filtrate was evaporated. The residue was purified from excess of the iodo compound by being passed through a short column of silica gel and elution with chloroform. The column was then extracted with 1:1 acetone/chloroform, and after removal of the solvent, the residue was separated into components by preparative thin-layer chromatography (silica gel, 1:8 acetone/chloroform) to afford **7a** (25 mg, 19%), $R_f$ 0.43 and **8a** (43 mg, 32%), $R_f$ 0.24.

**7a**: UV $\lambda_{max}$ ($CHCl_3$) inf 290, 450 nm ($\epsilon$ 29 500); IR 3320, 3440 $cm^{-1}$ ($NH_2$); NMR $\delta$ 6.73 (s, 8-H, 1 H), 5.42 (s, $NH_2$, 2 H), 4.06 (m, $OCH_2$, 2 H), 2.96-3.83 [m, $N(CH_2CH_3)_2$ and $NCH_2CH_2O$, 10 H], 2.23 (s, 6-$CH_3$, 3 H), 2.05 (s, 4-$CH_3$, 3 H), 0.65-1.67 [m, $N(CH_2CH_3)_2$, 12 H]. Anal. ($C_{28}H_{37}N_5O_5 \cdot 2CH_3COCH_3 \cdot H_2O$) C, H, N.

**8a**: UV $\lambda_{max}$ ($CHCl_3$) inf 290, 453 ($\epsilon$ 26 900); IR 3340, 3440 (br, OH, $NH_2$); NMR $\delta$ 6.83 (s, 8-H, 1 H), 5.43 (br s, $NH_2$, 2 H), 4.8 (m, NH, 1 H), 4.12 (t, $OCH_2$, 2 H), 2.90-3.96 [m, $N(CH_2CH_3)_2$, $CH_2NCH_2$, $CH_2OH$, 14 H], 2.32 (s, 6-$CH_3$, 3 H), 2.20 (s, 4-$CH_3$, 3 H), 0.71-1.53 [m, $N(CH_2CH_3)_2$, 12 H]. Anal. $C_{28}H_{39}N_5O_6 \cdot 3CH_3COCH_3$) C, H, N.

**7-[2-(1-Aziridinyl)ethoxy]actinomycin D (7b).** A solution of the 7-hydroxyactinomycin D (**2b**) (40 mg, 0.03 mmol) in dry acetone (60 mL) was allowed to react with 1-aziridineethyl iodide (prepared from 1.095 g of 1-aziridine triflate) and finely powdered anhydrous potassium carbonate (50 mg) in a nitrogen atmosphere at room temperature in the dark for 24 h. The brown reaction mixture was filtered to remove inorganic salts, and the filtrate was evaporated, chromatographed on a silica gel column (30 g), and eluted with chloroform (150 mL) to remove excess of the iodo compound. Elution with 2:1 chloroform/acetone (140 mL) afforded **7b** along with impurities. Final elution by 1:1 acetone/chloroform afforded **8b** (5 mg, 10%): $R_f$ 0.24; UV $\lambda_{max}$ ($CHCl_3$) 453 ($\epsilon$ 11 150). Compound **7b** was purified by preparative thin-layer chromatography to afford a single yellow band (6 mg, 14%): $R_f$ 0.36; UV $\lambda_{max}$ ($CHCl_3$) 457 nm ($\epsilon$ 11 500).

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**Registry No.** **2a**, 57270-61-8; **2b**, 21478-73-9; **4b**, 109122-96-5; **5a**, 112599-09-4; **5b**, 112599-10-7; **6b**, 109123-01-5; **7a**, 112599-11-8; **7b**, 112599-12-9; **8a**, 112599-13-0; **8b**, 112599-14-1; bromomethylcyclopropane, 7051-34-5; 2-(1-aziridinyl)ethanol, 1072-52-2; 2-(1-aziridinyl)ethyl trifluoromethanesulfonate, 112599-15-2; 1-(1-aziridinyl)-2-iodoethane, 45378-69-6.

**Supplementary Material Available:** A discussion of the agarose gel electrophoresis of ccc-SV 40 DNA-analogue complexes (Figure 1), Figure 2 illustrating the UV-vis difference absorption spectra on binding to DNA, and Figures 3 and 4, illustrating and discussing CD spectra of free drugs and CD spectra of drug-DNA complexes, respectively (7 pages). Ordering information is given on any current masthead page.

# Studies on Scavengers of Active Oxygen Species. 1. Synthesis and Biological Activity of 2-*O*-Alkylascorbic Acids

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A novel series of 2-*O*-alkylascorbic acids (**5a-u**) was synthesized, and their scavenging activities against active oxygen species as well as their suppressive effects on the arrhythmias in rat heart ischemia-reperfusion models were evaluated. Some 2-*O*-alkylascorbic acids (**5e-l**) exhibited potent inhibiting activities against lipid peroxidation in rat brain homogenates and in alleviating effects in the ischemia-reperfusion models. Studies on the structure-activity relationship demonstrated that a free 3-enolic hydroxyl group and the longer alkyl chains substituted on the 2-hydroxyl group of ascorbic acid were beneficial for the biological and pharmacological activities. 2-*O*-Octadecylascorbic acid (**5k**, CV-3611), one of the most potent and promising compounds, markedly inhibited lipid peroxidation ($IC_{50}$ = 4.3 × $10^{-6}$ M) and alleviated myocardial lesions induced by ischemia-reperfusion at an oral dose of 1 mg/kg in rats.

Recently, an increasing number of reports[1] describe the roles of active oxygen species (AOS) in the development or exacerbation of various kinds of diseases: heart attack, stroke, emphysema, rheumatism, inflammation, and cancer.[1,2] AOS, including superoxide ($O_2^-$), hydrogen peroxide, the hydroxyl radical, and the ferryl radical, are considered to be generated by, or formed subsequent to, reduction of molecular oxygen in living organisms.[2,3] The hydroxyl radical and the ferryl radical, a complex of oxygen radical and iron ion, are the most reactive and are thought to be the major species responsible for oxidative injury of enzymes, lipid membranes, and DNA in living cells and tissues.[4] Although the mechanisms of the oxidative injury are not well understood, AOS may bring about membrane perturbation through the lipid peroxidation of cellular and microsomal membranes to affect calcium influx, phospholipase activation,[5] and release of lysosomal enzymes and chemical mediators.[4] Prostaglandins and leukotrienes in the arachidonate pathway linked with lipid peroxidation may amplify the oxidative damage.[6]

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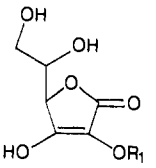
**Table I.** Chemical and Physical Data of 2-*O*-Alkylascorbic Acids and Related Compounds



**5a–u**

| no. | $R^1$ | mp, °C | formula | $[\alpha]^{24}_D$ EtOH | yields % [a] | % [b] | % [c] | % [d] |
|---|---|---|---|---|---|---|---|---|
| **5a** | $(CH_2)_7Me$ | 115–117 | $C_{14}H_{24}O_6$ | 42.4 (*c* 1.0) | 53 | 70 | 87 | 67 |
| **5b** | $(CH_2)_8Me$ | 122–123 | $C_{15}H_{26}O_6$ | 39.3 (*c* 1.0) | 64 | 26 | | |
| **5c** | $(CH_2)_9Me$ | 118–119 | $C_{16}H_{28}O_6$ | 39.8 (*c* 1.0) | 90 | 45 | | |
| **5d** | $(CH_2)_{10}Me$ | 124–125 | $C_{17}H_{30}O_6$ | 38.4 (*c* 0.9) | 62 | 51 | | |
| **5e** | $(CH_2)_{11}Me$ | 127–128 | $C_{18}H_{32}O_6$ | 36.8 (*c* 1.1) | 75 | 66 | | |
| **5f** | $(CH_2)_{12}Me$ | 129–130 | $C_{19}H_{34}O_6$ | 35.7 (*c* 1.1) | 62 | 68 | | |
| **5g** | $(CH_2)_{13}Me$ | 126–127 | $C_{20}H_{36}O_6$ | 34.3 (*c* 1.1) | 48 | 61 | | |
| **5h** | $(CH_2)_{14}Me$ | 126–127 | $C_{21}H_{38}O_6$ | 33.2 (*c* 0.8) | 64 | 61 | 90 | 68 |
| **5i** | $(CH_2)_{15}Me$ | 128–129 | $C_{22}H_{40}O_6$ | 32.7 (*c* 0.6) | 71 | 75 | | |
| **5j** | $(CH_2)_{16}Me$ | 127–129 | $C_{23}H_{42}O_6$ | 30.6 (*c* 1.0) | 39 | 78 | 76 | 53 |
| **5k** | $(CH_2)_{17}Me$ | 127–128 | $C_{24}H_{44}O_6$ | 29.8 (*c* 0.7) | 61 | 84 | 68 | 69 |
| **5l** | $(CH_2)_{19}Me$ | 126–128 | $C_{26}H_{48}O_6$ | –[e] | 85 | 71 | | |
| **5m** | $(CH_2)_{20}Me$ | 125–127 | $C_{27}H_{50}O_6$ | – | 81 | 73 | | |
| **5n** | $(CH_2)_{10}COOMe$ | 78–80 | $C_{18}H_{30}O_8$ | 32.6 (*c* 0.9) | 22 | 41 | | |
| **5o** | $(CH_2)_9OH$ | 73–74 | $C_{15}H_{26}O_7$ | – | 49 | 32 | | |
| **5p** | $CH_2Ph$ | 126–127 | $C_{13}H_{14}O_6$ | 46.6 (*c* 1.0) | 96 | 49 | | |
| **5q** | $(CH_2)_3Ph$ | 107–108 | $C_{15}H_{18}O_6$ | – | 40 | 26 | | |
| **5r** | $CH_2$(4-BrPh) | 184–185 | $C_{13}H_{13}O_6Br$ | 37.3 (*c* 0.8) | 76 | 53 | | |
| **5s** | $CH_2$(4-ClPh) | 174–175 | $C_{13}H_{13}O_6Cl$ | 41.8 (*c* 1.0) | 84 | 70 | | |
| **5t** | $CH_2$(4-MePh) | 127–128 | $C_{14}H_{16}O_6$ | 44.4 (*c* 0.9) | 68 | 53 | | |
| **5u** | $CH_2$(4-hexyl-*O*-Ph) | 133–134 | $C_{19}H_{28}O_7$ | – | 68 | 65 | | |
| 8 | | 103–104 | $C_{24}H_{44}O_6$ | –5.3 (*c* 1.0) | | | | |
| **9** | | 95–97 | $C_{24}H_{44}O_6$ | – | | | | |
| **10** | | 103–104 | $C_{25}H_{46}O_6$ | – | | | | |
| 11 | | 81–82 | $C_{27}H_{48}O_6$ | – | | | | |
| **12** | | 78–79 | $C_{26}H_{46}O_7$ | –11.8 (*c* 1.1) | | | | |
| **13** | | 113–114 | $C_{26}H_{46}O_7$ | – | | | | |
| **14** | | 114–115 | $C_{24}H_{42}O_5$ | – | | | | |
| **15** | | 83–84 | $C_{24}H_{44}O_5$ | – | | | | |

[a] Yield of alkylation. [b] Yield of deprotection step ($R^2$ = $MeOCH_2$). [c] Yield of alkylation. [d] Yield of deprotection steps ($R^2$ = benzyl). [e] Not determined.

Under normal conditions, cells and tissues are protected from the attack of AOS by various enzymes [superoxide dismutase (SOD), catalase (CAT), and peroxidases] and low molecular weight substances such as ascorbic acid (AsA), α-tocopherol, and glutathione.[1,7] Among them, we chose AsA as a starting material for the molecular design of novel and clinically useful scavengers of AOS, because AsA seems to contain the minimal structural requirement for AOS scavenging activity.[8]

AsA has a unique 2,3-enediol moiety in the five-membered lactone ring, which has a strong electron-donating ability. Although AsA and its acyl derivatives are widely used as food additives, antioxidants, and a vitamin, there are only a few reports on the synthesis and pharmacological evaluation of 2-*O*- or 3-*O*-alkylascorbic acids.[8-10] We have introduced lipophilic groups on the hydroxyls of the 2- or 3-carbon in AsA, because a combination of lipophilic and hydrophilic properties in the molecule might exert a site-specific AOS scavenging activity not only by maintaining an interaction with membrane phospholipids but also by suppressing superoxide production of membrane-associated superoxide generating systems. This paper describes the synthesis, structure–activity relationship, and pharmacological activity of a novel series of 2-*O*-alkylascorbic acids and related derivatives. Our results in vitro and in vivo suggest that the longer, straight-chain alkyl groups in 2-*O*-alkylascorbic acids are beneficial for the AOS scavenging activity.

**Chemistry.** The regioselective synthesis of 2-*O*-alkylascorbic acids (**5a–u**) started with 5,6-*O*-isopropylideneascorbic acid (**2**) (Chart I).[11] The hydroxyl group at the 3-carbon was protected with either a methoxymethyl or a benzyl group (3a, $R^2$ = $CH_2OMe$; **3b**, $R^2$ = benzyl) by use of chloromethyl methyl ether or benzyl bromide in the presence of potassium carbonate. Alkylation of **3a,b** with alkyl halides in the presence of potassium carbonate gave the corresponding dialkylated derivatives (**4a–u**). Removal of the protecting group at the 3-position was carried out by one of two methods, depending on the protecting group. The compounds protected with the methoxymethyl group were subjected to acid hydrolysis in a single step (method A), while the compounds protected with the benzyl group were subjected to acid hydrolysis followed by catalytic hydrogenation (method B) to give the corresponding 2-*O*-alkylascorbic acids (**5a–u**) in good yield (Table I).

To study the structure–activity relationship, several analogues and derivatives of 2-*O*-octadecylascorbic acid

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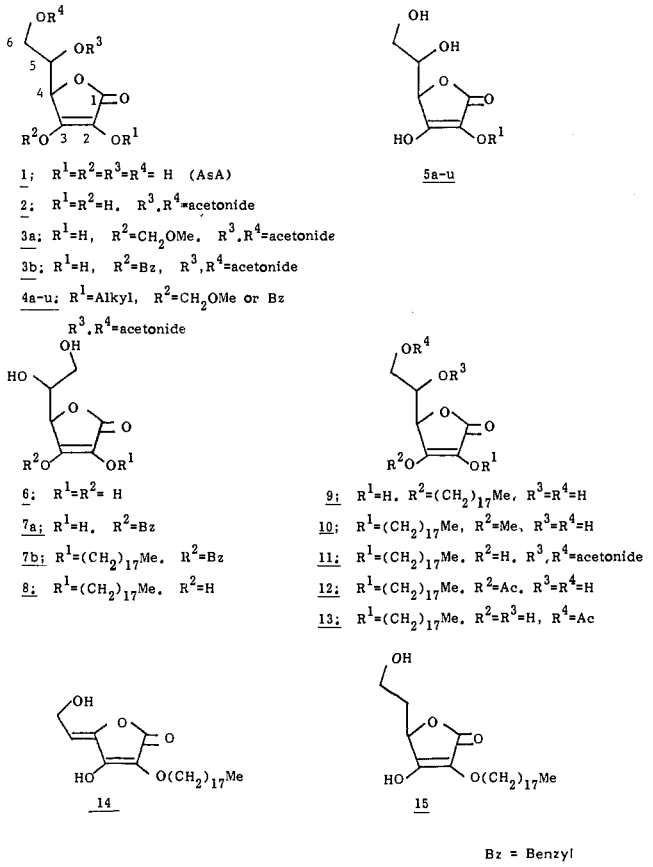
**Chart I**

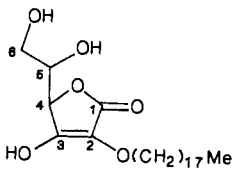



(**5k**), which exhibited the most potent and promising pharmacological activities as described later, were also synthesized. Stereoisomer **8**, an epimer of **5k** at the 5-carbon, was synthesized from isoascorbic acid (**6**) via the 3-*O*-benzyl derivatives (**7a,b**). 3-*O*-Octadecylascorbic acid (**9**), a regioisomer of **5k**, was obtained from direct alkylation of **2** followed by acid hydrolysis. Compound **5k** underwent methylation and ketallization to afford the 3-*O*-methyl derivative **10** and the acetonide **11**, respectively. Acetylation of **5k** under mild conditions gave the 3-*O*-acetyl derivative **12**, while acetylation of **5k** in the presence of 1,8-diazabicyclo[5.4.0]undec-5-ene (DBU) or treatment of **12** with a base afforded the 6-*O*-acetyl derivative **13** through an intramolecular acyl migration. Treatment of **4k** ($R^2$ = $CH_2OMe$) with DBU followed by acid hydrolysis gave the allylic compound **14**, which was further converted into **15** with catalytic hydrogenation.

The structure of these derivatives was determined by measuring $^1H$ NMR, $^{13}C$ NMR, and UV spectra. In the $^{13}C$ NMR spectrum of **5k**, the signal of the 3-carbon was largely shifted downfield (8.46 ppm) when the pH of the solution was changed from 4 to 8 (Table II), while no significant changes were observed in either **9** or **10**. A large shift of the absorption maximum depending on a pH change was also observed in the UV spectra of **5k** while the regioisomer **9** showed almost the same UV spectra in the pH range of 2–7. The maximal absorption of **5k** shifted from 237 nm ($\epsilon$ = 8700) at pH 2 to 263 nm ($\epsilon$ = 14000) at pH 7. A similar shift is known in 2-*O*-phosphorylascorbic acid.[12] These observations were consistent with the p$K_a$ values. The p$K_a$ value of **5k** was found to be similar to that of the enolic hydroxyl group at the 3-carbon of AsA, while **9** was weakly acidic. Furthermore, most of the 2-*O*-alkylascorbic acid derivatives synthesized here were more soluble in organic solvents than in water.

The reducing abilities of the 2-*O*- and 3-*O*-alkylascorbic acids were determined by use of a stable radical $\alpha,\alpha$-diphenyl-$\beta$-picrylhydrazyl (DPPH)[13] and are shown in Table

**Table II.** $^{13}C$ NMR Chemical Shifts of **5k** Depending on pH



**5k**

| carbon no. | $^{13}C$ chemical shifts,[a] δ pH 4 | δ pH 8[b] | Δ[c] |
|---|---|---|---|
| 1 | 169.76 | 171.90 | +2.14 |
| 2 | 120.12 | 118.04 | −2.08 |
| 3 | 159.03 | 167.49 | +8.46 |
| 4 | 74.69 | 76.29 | +1.60 |
| 5 | 68.56 | 69.62 | +1.06 |
| 6 | 62.02 | 62.65 | +0.63 |

[a] Spectra recorded on a JEOL (400-MHz) instrument in DMSO-$d_6$. [b] A drop of 1 N NaOD/$D_2O$ was added to adjust the pH. [c] Δ = δ(pH 8) − δ(pH 4).

**Table III.** Reducing Activity and Anti-Lipid-Peroxidation Activity of 2-*O*-Alkylascorbic Acids and Related Compounds

| no. | $R^1$ | reducing activity[a] % ($10^{-4}$ M) | anti lipid peroxidation[b] % ($10^{-5}$ M) | anti lipid peroxidation[b] $IC_{50}$ (× $10^{-6}$ M) |
|---|---|---|---|---|
| **5a** | $(CH_2)_7Me$ | 93.9 | 1.6 | ---[d] |
| **5b** | $(CH_2)_8Me$ | --- | 22.4 | 27.0 |
| **5c** | $(CH_2)_9Me$ | --- | 42.5 | 13.0 |
| **5d** | $(CH_2)_{10}Me$ | 94.6 | 43.4 | 13.0 |
| **5e** | $(CH_2)_{11}Me$ | --- | 67.4 | 5.4 |
| **5f** | $(CH_2)_{12}Me$ | --- | 57.4 | 7.4 |
| **5g** | $(CH_2)_{13}Me$ | 95.4 | 54.8 | 7.4 |
| **5h** | $(CH_2)_{14}Me$ | 95.4 | 67.4 | 5.6 |
| **5i** | $(CH_2)_{15}Me$ | --- | 56.1 | 6.8 |
| **5j** | $(CH_2)_{16}Me$ | --- | 48.8 | 11.0 |
| **5k** | $(CH_2)_{17}Me$ | 95.4 | 65.9 | 4.3 |
| **5l** | $(CH_2)_{19}Me$ | 95.3 | 75.8 | 4.7 |
| **5m** | $(CH_2)_{20}Me$ | 94.3 | 30.6 | 11.0 |
| **5n** | $(CH_2)_{10}COOMe$ | --- | –[c] | --- |
| **5o** | $(CH_2)_9OH$ | --- | – | --- |
| **5p** | $CH_2Ph$ | 93.0 | – | --- |
| **5q** | $(CH_2)_3Ph$ | --- | – | --- |
| **5r** | $CH_2$(4-BrPh) | --- | – | --- |
| **5s** | $CH_2$(4-ClPh) | --- | – | --- |
| **5t** | $CH_2$(4-MePh) | --- | – | --- |
| **5u** | $CH_2$(4-hexyl-*O*-Ph) | --- | 38.8 | --- |
| **8** | | --- | 44.7 | 9.8 |
| **9** | | 94.5 | 34.5 | --- |
| **10** | | – | 12.5 | --- |
| **11** | | --- | 58.5 | 2.4 |
| **13** | | --- | 76.3 | 4.2 |
| **14** | | --- | 53.5 | 8.5 |
| **15** | | 93.9 | 52.0 | 6.8 |
| AsA | | 93.4 | −71 | --- |
| α-tocopherol | | 72.0 | 52.0 | --- |

[a] Reducing activity against $\alpha,\alpha$-diphenyl-$\beta$-picrylhydrazyl with equimolar amount of substance. [b] Inhibiting activity to lipid peroxidation in rats brain homogenates. [c] No effects. [d] Not determined.

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**Table IV.** Effects of Typical AOS Scavengers and 2-*O*-Alkylascorbic Acids on Arrhythmias Induced by Ischemia-Reperfusion in Anesthetized Rats

| | VF | | | VT | | | |
|---|---|---|---|---|---|---|---|
| | incidence, *n* | frequency, times | duration, s | incidence, % | frequency, times | duration, s | PVCs/min |
| control | 16/18 | 4.1 ± 0.7 | 74.2 ± 30.8 | 17/18 | 11.5 ± 5.0 | 26.5 ± 6.8 | 11.8 ± 4.0 |
| **5g**[a] | 2/7* | 0.6 ± 0.4** | 10.1 ± 9.8 | 6/7 | 2.6 ± 0.7* | 12.9 ± 9.7 | 3.5 ± 1.1* |
| **5h**[a] | 4/7 | 0.9 ± 0.3** | 3.0 ± 1.8** | 6/7 | 5.0 ± 1.2 | 11.9 ± 3.8 | 4.3 ± 1.0* |
| **5k**[a] | 4/13** | 0.5 ± 0.3** | 0.5 ± 0.4** | 13/13 | 5.2 ± 0.7 | 12.0 ± 0.7 | 12.0 ± 2.5 |
| **5k**[b] | 3/10** | 0.7 ± 0.5** | 1.5 ± 0.9** | 10/10 | 4.0 ± 0.7 | 6.9 ± 1.7* | 4.5 ± 1.7* |
| **5k**[c] | 5/14** | 1.1 ± 0.6** | 4.1 ± 2.4** | 12/14 | 4.3 ± 1.0 | 11.4 ± 4.3 | 3.4 ± 0.7 |
| **5p**[a] | 7/8 | 2.5 ± 0.7 | 79.9 ± 72.6 | 8/8 | 3.9 ± 0.9 | 4.9 ± 1.3* | 26.9 ± 21.9 |
| **8**[a] | 5/5 | 1.6 ± 0.2 | 122.4 ± 118.2 | 4/5 | 3.8 ± 1.5 | 11.8 ± 5.5 | 4.0 ± 0.8* |
| **9**[a] | 3/6 | 0.8 ± 0.5* | 1.9 ± 1.1** | 4/6 | 2.3 ± 0.9 | 4.0 ± 1.5* | 3.9 ± 1.5* |
| AsA[b] | 6/6 | 4.0 ± 0.7 | 41.4 ± 21.1 | 6/6 | 3.7 ± 1.2 | 8.2 ± 3.1 | 7.4 ± 3.0 |
| α-tocopherol[b] | 8/10 | 2.1 ± 0.6 | 111.1 ± 72.7 | 8/10 | 3.1 ± 0.9 | 7.4 ± 3.0 | 23.3 ± 18.0 |
| allopurinol[d] | 4/8* | 0.9 ± 0.4* | 1.3 ± 0.7* | 8/8 | 3.1 ± 0.9 | 5.9 ± 1.7 | 2.7 ± 0.5* |
| SOD + CAT[e] | 0/8*** | 0.0 ± 0** | 0.0 ± 0** | 4/8* | 1.5 ± 0.6** | 2.1 ± 1.2** | 1.7 ± 0.6** |
| mannitol[f] | 4/10** | 0.8 ± 0.4** | 1.3 ± 0.8** | 7/10 | 2.7 ± 0.8* | 7.7 ± 4.3 | 4.8 ± 1.1* |

[a] Drug (10 mg/kg) was given orally 3 h before occlusion. [b] Drug (3 mg/kg) was given orally 3 h before occlusion. [c] Drug (1 mg/kg) was given orally 3 h before occlusion. [d] Allopurinol was given twice (oral 20 mg/kg 24 h and 20 mg/kg 5 min before occlusion). [e] SOD (15000 units/kg) and CAT (65000 units/kg), in combination, were infused intravenously for 45 min, beginning at 30 min before occlusion. [f] Mannitol (100 mg/kg) was given intravenously 5 min before occlusion. [g] (*) $p < 0.05$, (**) $p < 0.01$, (***) $p < 0.001$. [h] VF, ventricular fibrillation; VT, ventricular tachycardia; PVCs, premature ventricular complexes.

III. 2-*O*-Alkylascorbic acids and 3-*O*-alkylascorbic acids (**9**) exhibited almost the same reducing potency as ascorbic acid and α-tocopherol. 2,3-O-Disubstituted derivatives (**10**, **12**) completely lost the reducing activity. Since no significant change in reducing ability was observed in the one-side blockade of either the 2-*O*- or the 3-*O*-enolic hydroxyl groups of AsA, 2-*O*- and 3-*O*-monoalkylascorbic acids appeared to have an equal electron-donating potency.

**Biology.** The inhibition of lipid peroxidation was determined with rat brain tissue homogenates,[14] and the results are shown in Table III. 2-*O*-Alkylascorbic acids having a longer alkyl chain had potent inhibiting activities; the maximal potency appeared in the compounds (**5e–l**) with straight-chain alkyl groups having 11–20 methylene groups.

The activity decreased as the length of the alkyl chain at the 2-hydroxyl group was reduced. Compounds (**5n,o**) with polar functional groups at the terminal carbon were weakly active. Interestingly, compounds (**5p–t**) having a phenyl group were weakly active, while the compound (**5u**) having an (*n*-hexyloxy)phenyl group regained the activity. Therefore, a lipophilic moiety seems essential to inhibit lipid peroxidation, suggesting that the longer and more lipophilic groups located at the 2- or 3-hydroxyl group of AsA may exert biologically important roles by anchoring these molecules in the cellular and microsomal membranes. The regioisomer (**9**) was less active than **5k**, which could be explained by the difference between the $pK_a$ values for the 2- or 3-hydroxyl groups since the 3-hydroxyl group in **5k** can exist as an anion in the test systems, while the 2-hydroxyl group in **9** exists in the protonated form. Thus, the 3-hydroxyl group is considered to release an electron more readily than 2-hydroxyl group. Compounds (**11** and **13–15**) with chemical modification at the 5- and 6-carbons of **5k** showed no significant changes in the activity. Hydroxyl groups at the 5- or 6-carbon seem to have little inhibitory effect on lipid peroxidation.

These results indicate that both length and lipophilicity of the alkyl groups attached to the 2-hydroxyl group are essential for the inhibitory activity of lipid peroxidation in rat brain homogenates. In this assay system, α-tocopherol was less active than **5k**, while AsA was found to enhance the lipid peroxidation associated with iron ions in extracellular fluids. This indicates that lipophilic 2-*O*-alkylascorbic acids may have a reduced ability to chelate with the metal ions existing in the extracellular site due to the blockade of the 2-hydroxyl group.

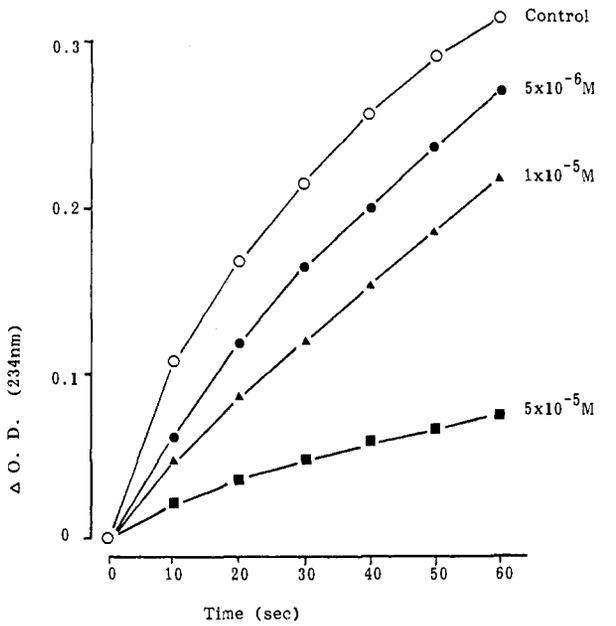


**Figure 1.** Effect of **5k** on linoleic acid peroxidation initiated by the hydroxyl radical (Fenton reaction). The conjugated diene formation was monitored at 234 nm. The reaction was performed at 37 °C.

AOS scavenging activity was also tested in a linoleic acid peroxidation system using liposomes (Figure 1).[15] In this system, linoleic acid was readily oxygenated through the Fenton reaction. It was found that peroxidation of linoleic acid was effectively suppressed by **5k** in a dose-dependent manner ($IC_{50} = 7.0 \times 10^{-6}$ M). The result suggested that **5k** might inhibit lipid peroxidation in the lipid bilayer caused by the hydroxyl radical and terminate the radical chain processes.

The AOS scavenging activity in vivo was examined with an animal model (Table IV).[16] In open-chest rats anesthetized with pentobarbital, reperfusion after a 5-min

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occlusion of the left anterior descending coronary artery (LAD) caused various types of ventricular arrhythmias, such as premature ventricular complexes (PVCs), ventricular tachycardia (VT), and ventricular fibrillation (VF). Among these ventricular arrhythmias, VF is thought to bring about sudden death in coronary heart failure.[2] The incidence, frequency, and duration of both VF and VT and the mean PVCs number/minute were recorded at 10 min postreperfusion. Combined administration of SOD and catalase to the ischemia–reperfusion model in the rat completely prevented the occurrence of VF. The number of PVCs and the incidence and duration of VT were also significantly reduced. Administration of either allopurinol or mannitol reduced the incidence and duration of VT and VF significantly. These results support the view that the AOS produced in the ischemia–reperfusion model might be involved in the initial or developing stage of arrhythmogenesis and that compounds that can reduce oxygen-derived free radicals might have a myocardial protective action.

The oral administration of **5k** at a minimal dose of 1 mg/kg before LAD occlusion suppressed the incidence, frequency, and duration of VT and frequency and duration of VF. Also, the frequency of occasional PVCs was clearly diminished by **5k**. Compounds (**5g**,h) with shorter alkyl groups, which were as active as **5k** in vitro, were as active as **5k** in vivo. The compound (**5p**) with a benzyl group on the 2-hydroxyl group, which exhibited little effect on the lipid peroxidation, slightly suppressed VT but did not reduce VF or PVCs. Both **8** and **9**, the stereoisomer and regioisomer of **5k**, respectively, showed a tendency to diminish these arrhythmias but were less effective than **5k**. These observations correlated reasonably with the results of the experiments in vitro. However, no significant effects were observed from the oral administration of either AsA or $\alpha$-tocopherol in this model.

Although it is not clear what kinds of free radicals play the most important roles in the initiation and developing processes of arrhythmogenesis, lipid peroxidation, subsequent membrane damage, and tissue injury caused by AOS might be involved in arrhythmias induced by ischemia–reperfusion. Compound **5k** was selected for further pharmacological and clinical evaluation.[17]

## Discussion

2-*O*-Alkylascorbic acids and their derivatives with high lipophilicity and electron-donating ability showed strong AOS scavenging activity in vitro and in vivo. On the basis of the structure–activity relationship, the longer, straight-chain alkyl moieties and the electron-donating activity of the enolic hydroxyl group may both be beneficial and essential in inhibiting the lipid peroxidation and subsequent cellular and tissue damage. The short alkyl and aromatic groups were found to be less active. The structural changes at the 5- and 6-positions brought about no change in the inhibitory activity. The results indicate that the interaction between 2-*O*-alkylascorbic acids and membrane phospholipids might be important for the AOS scavenging activities, suggesting that unsaturated fatty acid moieties and membrane-bound proteins could be protected from the attack of AOS by 2-*O*-alkylascorbic acids. The polarity and more potent reducing property of the 3-hydroxyl group of **5k** may explain why it is more potent than the regioisomer, **9**. The biological mode of action of 2-*O*-alkylascorbic acids might be different from that of ascorbic acid. The experiments in vivo strongly suggest that AOS may be involved in the myocardial ischemia in the circulatory system and that the prevention of lipid peroxidation by AOS could diminish the postischemic tissue injury in the rat ischemia–reperfusion model.

In conclusion, among the 2-*O*-alkylascorbic acids and their derivatives synthesized in the present study, 2-*O*-octadecylascorbic acid (**5k**) appeared to have the strongest activity in inhibiting lipid peroxidation and cardiac tissue damages caused by active oxygen species in vitro and in vivo.

## Experimental Section

Column chromatography was carried out on Kieselgel 60 (Merck, 70–230 mesh). Tetrahydrofuran (THF) and isopropyl ether (IPE) were distilled from calcium hydride, and dimethyl sulfoxide (DMSO) and *N*,*N*-dimethylformamide (DMF) were dried over molecular sieves (4A). Organic extracts were dried over $MgSO_4$ and evaporated in vacuo. Melting points are uncorrected. $^1H$ NMR spectra were determined on a Varian EM-390 spectrometer and $^{13}C$ NMR spectra with a JEOL WH400 spectrometer with $Me_4Si$ as an internal standard. All elemental analyses are within ±0.4% of the calculated values. Optical rotations were measured with a JASCO DIP-181 instrument.

**5,6-*O*-Isopropylideneascrobic Acid (2).** To a rapidly stirred suspension of ascorbic acid (1; 300 g, 2 mol) in acetone (3 L) was added acetyl chloride (25 mL, 0.1 mol), and the mixture was stirred at ambient temperature for 18 h. The precipitate was collected by filtration, washed with EtOAc, and dried in vacuo to afford **2** (310 g, 84%): mp 202–204 °C; NMR (DMSO-$d_6$) $\delta$ 1.33 (6 H, s), 3.90–4.45 (4 H, m), 4.61 (1 H, d, $J$ = 3 Hz). Anal. ($C_9H_{12}O_6$) C, H.

**5,6-*O*-Isopropylidene-3-*O*-(methoxymethyl)ascorbic Acid (3a).** To a stirred solution of **2** (108 g, 0.5 mol) in THF (300 mL) and DMF (100 mL) was added potassium carbonate (70 g, 0.5 mol). After the mixture was stirred for 10 min, chloromethyl methyl ether (40 g, 0.5 mol) was added to the mixture while the temperature was maintained below 30 °C. After being stirred at room temperature for 4 h, the reaction mixture was diluted with water (300 mL), neutralized with 2 N HCl, and extracted with EtOAc. The organic layer was washed with water, dried, and evaporated. The residue was chromatographed on silica gel with IPE/EtOAc (1:1) as eluent. Recrystallization of the product from IPE gave **3a** (68 g, 52%): mp 93–94 °C; NMR ($CDCl_3$) $\delta$ 1.35 (3 H, s), 1.38 (3 H, s), 3.59 (3 H, s), 4.21 (3 H, m), 4.63 (1 H, d, $J$ = 3 Hz). Anal. ($C_{11}H_{16}O_7$) C, H.

**5,6-*O*-Isopropylidene-3-*O*-benzylascorbic Acid (3b).** Compound **2** and benzyl bromide were reacted in the way described above, and the product was chromatographed on silica gel with IPE/EtOAc (1:1) as eluent. Recrystallization from IPE gave **3b** (13 g, 40%): mp 105–106 °C; NMR ($CDCl_3$) $\delta$ 1.35 (6 H, s), 4.20 (3 H, m), 4.57 (1 H, d, $J$ = 3 Hz), 5.50 (2 H, s), 7.30 (5 H, s). Anal. ($C_{16}H_{18}O_6$) C, H.

**General Procedure for the Alkylation of the 2-Hydroxyl Group. 5,6-*O*-Isopropylidene-2-*O*-alkyl-3-*O*-(methoxymethyl)ascorbic Acids.** To a solution of **3a** (26 g, 0.1 mol) and an alkyl halide (0.11 mol) in THF (80 mL) and DMSO (90 mL) was added potassium carbonate (16 g, 0.11 mol), and the reaction mixture was vigorously stirred at 50 °C for 3 h. The reaction mixture was diluted with water (300 mL), neutralized with 2 N HCl, and extracted with EtOAc. The organic layer was washed with water, dried, and evaporated. The residue was chromatographed on silica gel with IPE as eluent to afford **4a–u** as shown in Table I.

**General Procedure for the Removal of Protecting Groups. Method A.** A solution of 2-*O*-alkyl-3-*O*-(methoxymethyl)-5,6-*O*-isopropylideneascorbic acid (0.022 mol) in ethanol (30 mL) and 1 N HCl (10 mL) was heated for 5 h at 80 °C. The solvent was removed in vacuo, and the residue was diluted with EtOAc. The organic layer was washed with water, dried, and concentrated. The crude product was recrystallized from IPE/EtOAc to afford **5** as shown in Table I.

**Method B.** A solution of 2-*O*-alkyl-3-*O*-benzyl-5,6-*O*-isopropylideneascorbic acid (0.023 mol) in THF (20 mL), methanol (20 mL), and 2 N HCl (20 mL) was stirred at room temperature for 6 h. The solvent was removed in vacuo, and the reaction

(17) Second Clinical Conference on Free Radicals in Kyoto, Sept 20, 1986.

mixture was extracted with EtOAc. The organic layer was washed with water, dried, and evaporated. The resulting 2-*O*-alkyl-3-*O*-benzylascorbic acid was dissolved in ethanol (20 mL) and hydrogenated with 5% palladium charcoal (0.5 g) for 18 h at atmospheric pressure. The catalyst was removed by filtration, and the filtrate was concentrated. The residue was recrystallized from IPE/EtOAc to afford **5** as shown in Table I: typical NMR spectrum for **5k** (DMSO-$d_6$) $\delta$ 0.85 (3 H, m), 1.26 (32 H, m), 3.45 (2 H, m), 3.86 (3 H, m), 4.70 (1 H, d, $J$ = 1 Hz).

**2-*O*-Octadecylisoascorbic Acid (8).** To a suspension of sodium D-isoascorbic acid (**6**; 20 g, 0.1 mol) in DMF (50 mL) was added benzyl bromide (12 mL). After being stirred for 4 h at 50 °C, the reaction mixture was partitioned with water and EtOAc. The organic layer was washed with water, dried, and evaporated. The residue was chromatographed on silica gel with EtOAc as eluent to afford 3-*O*-benzylisoascorbic acid (**7a**; 10 g, 37%) as an oil; NMR ($CDCl_3$) $\delta$ 0.87 (3 H, m), 1.24 (32 H, s), 3.80–4.20 (3 H, m), 4.72 (1 H, d, $J$ = 4 Hz), 5.48 (2 H, s), 7.34 (5 H, s). To a solution of **7a** (10 g, 0.037 mol) in DMSO (40 mL) and THF (10 mL) were added potassium carbonate (5 g, 0.04 mol) and 1-iodooctadecane (14 g, 0.037 mol), and the mixture was heated with vigorous stirring at 50 °C for 2 h. The mixture was diluted with water (100 mL) and extracted with IPE. The extract was washed with water, dried, and evaporated. The residue was chromatographed on silica gel with IPE as eluent followed by recrystallization from IPE to afford **7b** (5 g, 25%): mp 62–63 °C; NMR ($CDCl_3$) $\delta$ 0.87 (3 H, m), 1.25 (32 H, m), 3.70–4.20 (5 H, m), 4.74 (1 H, d, $J$ = 4 Hz), 5.48 (2 H, s), 7.38 (5 H, s). Anal. ($C_{31}H_{50}O_6$) C, H. Compound **7b** (3 g, 5.7 mmol) in ethanol (50 mL) was hydrogenated with 5% palladium charcoal (0.5 g) for 18 h at room temperature. The catalyst was removed by filtration, and the filtrate was concentrated. The residue was recrystallized from EtOAc to afford **8** (2 g, 80%): mp 103–104 °C; NMR ($CDCl_3$) $\delta$ 0.87 (3 H, m), 1.24 (32 H, s), 3.50–3.80 (5 H, m), 4.72 (1 H, d, $J$ = 3 Hz). Anal. ($C_{24}H_{44}O_6$) C, H.

**3-*O*-Octadecylascorbic Acid (9).** To a solution of **2** (2.6 g, 0.01 mol) and 1-idooctadecane (3.8 g, 0.01 mol) in DMF (20 mL) was added potassium carbonate. After being stirred at ambient temperature for 4 h, the reaction mixture was diluted with water (100 mL) and extracted with EtOAc. After workup in the usual manner, the residue was chromatographed on silica gel with IPE as eluent to afford 5,6-*O*-isopropylidene-3-*O*-octadecylascorbic acid, which was subjected to acid hydrolysis as described above to yield **9** (2.1 g, 38%): mp 95–97 °C; NMR ($CDCl_3$) $\delta$ 0.88 (3 H, m), 1.25 (32 H, s), 3.60 (2 H, m), 4.00–4.30 (3 H, m), 4.66 (1 H, m). Anal. ($C_{24}H_{44}O_6$) C, H.

**3-*O*-Methyl-2-*O*-octadecylascorbic Acid (10).** To a solution of **5k** (0.8 g, 2 mmol) in DMF (10 mL) were added potassium carbonate (0.4 g, 3.3 mmol) and iodomethane (0.2 mL). The reaction mixture was stirred at ambient temperature for 3 h. After workup in the usual procedure, the crude product was recrystallized from EtOAc to afford **10** (0.5 g, 65%): mp 103–104 °C; NMR ($CDCl_3$) $\delta$ 0.86 (3 H, m), 1.26 (32 H, s), 3.8–4.2 (5 H, m), 4.17 (3 H, s), 4.68 (1 H, m). Anal. ($C_{24}H_{44}O_6$) C, H.

**5,6-*O*-Isopropylidene-2-*O*-octadecylascorbic Acid (11).** A solution of **5k** (0.8 g, 2 mmol) and *p*-toluenesulfonic acid monohydrate (50 mg) in acetone (50 mL) was stirred at ambient temperature for 6 h. The reaction mixture was evaporated, and the residue was dissolved in EtOAc. The organic layer was washed with water, dried, and evaporated. The crude product was recrystallized from IPE to yield **11** (0.8 g, 91%): mp 81–82 °C; NMR (DMSO-$d_6$) $\delta$ 0.86 (3 H, m), 1.25 (32 H, m), 1.36 (3 H, s), 1.41 (3 H, s), 3.80–4.30 (5 H, m), 4.68 (1 H, d, $J$ = 1 Hz). Anal. ($C_{27}H_{48}O_6$) C, H.

**3-*O*-Acetyl-2-*O*-octadecylascorbic Acid (12).** A solution of **5k** in $CHCl_3$ (20 mL), pyridine (1 mL), and acetyl chloride (0.25 mL) was stirred at ambient temperature for 2 h. The reaction mixture was washed with 2 N HCl and water, dried, and evaporated. The crude product was recrystallized from IPE/EtOAc to give **12** (0.8 g, 87%): mp 78–79 °C; NMR ($CDCl_3$) $\delta$ 0.87 (3 H, m), 1.26 (32 H, m), 2.29 (3 H, s), 3.82 (2 H, m), 4.23 (3 H, m), 5.21 (1 H, m). Anal. ($C_{26}H_{46}O_7$) C, H.

**6-*O*-Acetyl-2-*O*-octadecylascorbic Acid (13).** To a solution of **5k** in $CHCl_3$ (20 mL), pyridine (1 mL), and 4-(dimethylamino)pyridine (0.1 g) was added acetyl chloride (0.25 mL). After being stirred at ambient temperature for 18 h, the reaction mixture was washed with 2 N HCl and water, dried, and evaporated. The crude product was recrystallized from IPE/EtOAc to yield **13** (0.6 g, 65%): mp 117–118 °C; NMR (DMSO-$d_6$) $\delta$ 0.87 (3 H, m), 1.25 (32 H, m), 2.08 (3 H, s), 3.85 (2 H, t, $J$ = 7 Hz), 4.00–4.30 (3 H, m), 4.62 (1 H, m). Anal. ($C_{26}H_{46}O_7$) C, H.

**2-*O*-Octadecyl-5-dehydroascorbic Acid (14).** A solution of **4k** (5 g, 10 mmol) and DBU (3 mL) in THF (20 mL) was heated at 50 °C for 2 h. The reaction mixture was washed with 2 N HCl and water, dried, and evaporated. The residue was dissolved in ethanol (40 mL) and 2 N HCl (20 mL). The solution was heated with stirring at 60 °C for 6 h and concentrated. The residue was dissolved in EtOAc, and the organic layer was washed with water, dried, and evaporated. The crude product was recrystallized from IPE to yield **14** (2 g, 51%): mp 114–115 °C; NMR (DMSO-$d_6$) $\delta$ 0.88 (3 H, m), 1.26 (32 H, m), 4.28 (2 H, m), 4.24 (2 H, m), 5.60 (1 H, m). Anal. ($C_{24}H_{42}O_5$) C, H.

**2-*O*-Octadecyl-5-anhydroascorbic Acid (15).** A solution of **14** (0.4 g, 1 mmol) in ethanol (10 mL) was hydrogenated with 5% palladium charcoal (0.2 g) at room temperature for 4 h under atmospheric pressure. The catalyst was removed by filtration, and the filtrate was evaporated. The crude product was recrystallized from hexane/IPE to afford **15** (0.2 g, 50%): mp 83–84 °C; NMR (DMSO-$d_6$) $\delta$ 0.86 (3 H, m), 1.25 (32 H, m), 3.8–4.3 (4 H, m), 4.74 (1 H, m). Anal. ($C_{24}H_{44}O_5$) C, H.

**Biological Methods. Experiments in Vitro. Determination of the Reducing Activity of the Stable Radical $\alpha,\alpha$-Diphenyl-$\beta$-picrylhydrazyl (DPPH).**[13] Typical compounds synthesized above were added to a solution of DPPH ($10^{-4}$ M) in ethanol. After 20 min, the absorbance at 517 nm was measured. The difference in absorbance between this and the that of the control was taken as the reducing activity. The results are shown in Table III.

**AOS Scavenging Activity. Thiobarbituric Acid Method.**[14] The brains of male SD rats (12 weeks old) were exsanguinated under anesthesia with pentobarbital, and the brain was excised. The brain tissue was homogenized in a phosphate buffer solution (pH 7.4) to afford a 5% homogenate. The homogenate was incubated with the test drug at 37 °C for 1 h. The amount of lipid peroxide formed was determined according to the thiobarbituric acid method.[17] The data are shown in Table III.

**Inhibition of Fenton-Type Lipid Peroxidation of Linoleic Acid Micelles.**[15] Lubrol PX [0.8% (v/v), 1 mL] was added to the 10 mM solution of linoleic acid (1 mL, pH 7), and the resulting mixture was diluted 10 times by adding 30 mM aqueous NaCl (pH 7.0) and was kept at 37 °C. Compound **5k** (20 $\mu$L) and 10 mM $H_2O_2$ (20 $\mu$L) were added to the solution (2 mL). Ferric chloride solution (10 $\mu$L, $FeCl_2 \cdot nH_2O$ was dissolved in 30 mM NaCl) was added, and the rate of conjugated diene formation was monitored by measuring the absorbance change at 234 nM.

**Prevention of Ventricular Arrhythmia Caused by Occlusion-Reperfusion in Rat's LAD.**[16] Male SD rats (9–13 weeks old, 250–370 g) rats were subjected to thoractonomy under artificial respiration while anesthesia was maintained by administering pentobarbital. The left anterior descending coronary artery (LAD) was ligated with silk thread for 5 min, and the heart was reperfused.

Ventricular arrhythmia, typically exemplified by occasionally occurring premature ventricular contractions (PVCs), ventricular tachycardia (VT), and ventricular fibrillation (VF) were observed for 10 min after the reperfusion. The results are shown in Table IV.

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