# Liver Injury Suppressing Compounds from Avocado (*Persea americana*)

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To evaluate the protective activity of fruits against liver injury, 22 different fruits were fed to rats with liver damage caused by D-galactosamine, a powerful liver toxin. As measured by changes in the levels of plasma alanine aminotransferase (ALT) and aspartate aminotransferase (AST), avocado showed extraordinarily potent liver injury suppressing activity. Five active compounds were isolated and their structures determined. These were all fatty acid derivatives, of which three, namely, (2*E*,5*E*,12*Z*,15*Z*)-1-hydroxyheneicosa-2,5,12,15-tetraen-4-one, (2*E*,12*Z*,15*Z*)-1-hydroxyheneicosa-2,12,15-trien-4-one, and (5*E*,12*Z*)-2-hydroxy-4-oxoheneicosa-5,12-dien-1-yl acetate, were novel.

**Keywords:** *Liver injury suppression; fatty acid derivative; avocado; Persea americana*

## INTRODUCTION

Food has potential benefits for maintaining health, not only by simple nutrition but also through characteristic secondary metabolites. The physiological functions of food have been studied throughout the world, especially in Japan (*1*). Dietary guidelines for disease prevention have advised people to eat more fruits, vegetables, and grains (*2*). People eat such food intentionally or unintentionally. However, it is not certain which components in most foods are good for health. During screening for liver injury suppressing effects of fruits, we found very strong activity of avocado (*Persea americana*). Therefore, an attempt was made to isolate the active principles from the fruit and to determine their structures.

## MATERIALS AND METHODS

**Materials.** All of the fruits (apple, avocado, banana, blueberry, cherry, durian, fig, grape, grapefruit, Japanese plum, Japanese summer orange, kiwi, lychee, mango, melon, papaya, peach, pear, persimmon, pineapple, Satsuma mandarin, and watermelon) were obtained from local supermarkets (Shizuoka City, Japan). D-Galactosamine was obtained from Sigma (St. Louis, MO). Mineral and vitamin mixtures were obtained from Oriental Yeast (Tokyo, Japan). Silica gel for column chromatography (Kieselgel 60) and silica gel TLC (Kieselgel 60 F254) were purchased from Merck (Darmstadt, Germany). Other reagents and solvents were purchased from Wako Pure Chemical Industries (Osaka, Japan).

**General Procedures.** NMR spectra were obtained on a JEOL $\lambda$-500 or a JEOL GSX-400 spectrometer (JEOL, Tokyo, Japan). FAB-MS and EI-MS were recorded on a JEOL DX-303HF and a JEOL DX-302 spectrometer (JEOL, Tokyo, Japan), respectively. IR spectra were determined with a JASCO FT/IR-410 spectrometer (JASCO, Tokyo, Japan). Optical rotation was measured by a JASCO DIP-100 polarimeter (JASCO, Tokyo, Japan). HPLC was carried out using a Gulliver HPLC system (JASCO, Tokyo, Japan).

**Extraction and Isolation of Active Compounds from Avocado.** Lyophilized avocado was homogenized with hexane in a blender and extracted with stirring at room temperature for 24 h. The resulting suspension was filtered, and the filtrate was concentrated and dried under reduced pressure (fraction 1, 58.6 g from 100 g of the lyophilized material). Ethyl acetate was added to the residue and the suspension extracted with stirring at room temperature for 24 h. The mixture was filtered, and the extract was concentrated and dried under reduced pressure (fraction 2, 0.70 g). The residue was further extracted with 70% ethanol with stirring at room temperature for 24 h, and the suspension was filtered. The extract, after concentration of the solvent, was fractionated by solvent partition between 1-butanol and water. The butanol-soluble fraction (fraction 3, 2.81 g) and water-soluble fraction (fraction 4, 13.02 g) were concentrated and dried under reduced pressure, respectively. The final residue was dried under reduced pressure (fraction 5, 24.51 g). Fraction 1 was applied to a silica gel column and eluted successively with hexane/ethyl acetate (83:17, 80:20, 75:25, 67:33, 60:40, 40:60, 0:100) and methanol, giving seven fractions (fraction 1-1, 83.1 g from 100 g of fraction 1; 1-2, 1.78 g; 1-3, 1.19 g; 1-4, 7.01 g; 1-5, 1.91 g; 1-6, 1.51 g; 1-7, 2.34 g). Fraction 1-4 was further separated by HPLC using an ODS column (column, Wakosil-II5 C18 HG Prep, ∅ 50 mm × 250 mm, Wako Pure Chemical Industries, Osaka, Japan; solvent, acetonitrile/$H_2O$ = 98:2; flow rate, 20 mL/min; detection, 220 nm), and compounds **1**−**5** were obtained.

**(2*E*,5*E*,12*Z*,15*Z*)-1-Hydroxyheneicosa-2,5,12,15-tetraen-4-one (1):** colorless oil; IR $\nu_{max}$ (film) 3420, 1667, 1635, 1616, 1239, 1100 $cm^{-1}$; FAB-MS [*m*-nitrobenzyl alcohol (*m*NBA) as a matrix], *m*/*z* 319 $[M + H]^+$, 341 $[M + Na]^+$; HR-FAB-MS (*m*NBA as a matrix), *m*/*z* 319.2639 ($[C_{21}H_{34}O_2 + H]^+$, calcd 319.2637); $^1H$ NMR, see Table 1; $^{13}C$ NMR, see Table 2.

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**Table 1. $^1$H NMR Data for Compounds 1−5 (500 MHz)[a]**

| | δ (multiplicity, J in Hz, $CDCl_3$) | | | | |
|---|---|---|---|---|---|
| position | 1 | 2 | 3 | 4 | 5 |
| 1 | 4.12 (dd, 11.4, 4.1)<br>4.38 (br. s) | 4.07 (dd, 11.4, 6.3)<br>4.09 (dd, 11.4, 4.1) | 4.04 (dd, 11.4, 6.3) | 4.10 (dd, 11.3, 4.1)<br>4.30 (m) | 4.05 (dd, 11.3, 6.1) |
| 2 | 6.93 (m) | 4.32 (m) | 4.28 (m) | 6.85 (dt, 5.9, 4.0) | 4.30 (ddt, 4.1, 6.1, 6.1) |
| 3 | 6.62 (dt, 15.4, 2.1) | 2.75 (m) | 2.59 (m) | 6.33 (ddd, 15.9, 3.1, 2.1) | 2.73 (d, 6.1) |
| 5 | 6.29 (dt, 14.3, 1.2) | 6.09 (d, 16.0) | 2.42 (t, 7.5) | 2.51 (t, 7.5) | 6.07 (d, 15.9) |
| 6 | 6.91 (m) | 6.87 (dt, 16.0, 7.0) | 1.56 (m) | 1.57 (m) | 6.85 (dt, 15.9, 7.0) |
| 7 | 2.23 (dt, 7.0, 6.4) | 2.21 (dt, 7.0, 6.4) | 1.27 (m) | 1.27 (m) | 2.19 (m) |
| 8 | 1.47 (m) | 1.46 (m) | 1.27 (m) | 1.27 (m) | 1.43 (m) |
| 9 | 1.34 (m) | 1.33 (m) | 1.27 (m) | 1.27 (m) | 1.22 (m) |
| 10 | 1.34 (m) | 1.33 (m) | 1.32 (m) | 1.27 (m) | 1.22 (m) |
| 11 | 2.05 (m) | 2.03 (m) | 2.03 (m) | 2.02 (m) | 2.00 (m) |
| 12 | 5.34 (m) | 5.35 (m) | 5.35 (m) | 5.32 (m) | 5.31 (m) |
| 13 | 5.30 (m) | 5.32 (m) | 5.32 (m) | 5.32 (m) | 5.31 (m) |
| 14 | 2.75 (dd, 6.1, 6.1) | 2.75 (m) | 2.75 (m) | 2.78 (t, 6.6) | 2.00 (m) |
| 15 | 5.30 (m) | 5.32 (m) | 5.32 (m) | 5.32 (m) | 1.22 (m) |
| 16 | 5.34 (m) | 5.35 (m) | 5.35 (m) | 5.32 (m) | 1.22 (m) |
| 17 | 2.05 (m) | 2.03 (m) | 2.03 (m) | 2.02 (m) | 1.22 (m) |
| 18 | 1.34 (m) | 1.33 (m) | 1.32 (m) | 1.27 (m) | 1.22 (m) |
| 19 | 1.27 (m) | 1.27 (m) | 1.27 (m) | 1.27 (m) | 1.22 (m) |
| 20 | 1.27 (m) | 1.27 (m) | 1.27 (m) | 1.27 (m) | 1.22 (m) |
| 21 | 0.87 (t, 6.9) | 0.87 (t, 7.0) | 0.87 (t, 7.0) | 0.86 (t, 6.8) | 0.84 (t, 6.9) |
| $\underline{CH_3}CO$ | | 2.08 (s) | 2.08 (s) | | 2.06 (s) |

[a] These assignments were established by HMBC, HMQC, DEPT, and/or NOESY experiments; see Figure 5 for structures.

**Table 2. $^{13}$C NMR Data for Compounds 1−5 (125 MHz)[a]**

| | δ (in $CDCl_3$) | | | | |
|---|---|---|---|---|---|
| position | 1 | 2 | 3 | 4 | 5 |
| 1 | 62.2 | 67.3 | 67.2 | 61.6 | 67.3 |
| 2 | 144.8 | 66.2 | 66.0 | 145.1 | 66.1 |
| 3 | 126.3 | 42.3 | 45.2 | 127.8 | 42.3 |
| 4 | 189.2 | 199.6 | 210.9 | 201.0 | 199.6 |
| 5 | 129.2 | 130.3 | 43.6 | 40.0 | 130.1 |
| 6 | 148.7 | 149.3 | 23.5 | 24.0 | 149.5 |
| 7 | 32.7 | 32.5 | 29.1[a] | 29.0[b] | 32.5 |
| 8 | 28.0 | 27.9 | 29.1[a] | 29.1[b] | 27.9 |
| 9 | 28.8 | 28.8 | 29.3[a] | 29.2[b] | 29.1[a] |
| 10 | 29.4[a] | 29.3 | 29.3[a] | 29.5[a] | 29.3[a] |
| 11 | 27.1[b] | 27.0[a] | 27.2 | 27.0 | 27.1 |
| 12 | 129.8[c] | 129.7[b] | 130.0[b] | 127.8[c] | 130.2 |
| 13 | 127.8[d] | 127.8[c] | 127.9[c] | 127.9[c] | 130.2 |
| 14 | 25.6 | 25.6 | 25.6 | 25.5 | 27.1 |
| 15 | 128.3[d] | 128.3[c] | 128.1[c] | 129.8[c] | 29.6[a] |
| 16 | 130.3[c] | 130.3[b] | 130.2[b] | 130.0[c] | 29.6[a] |
| 17 | 27.2[b] | 27.2[a] | 27.2 | 27.0 | 29.3[a] |
| 18 | 29.3[a] | 29.3 | 29.6[a] | 29.2[a] | 29.4[a] |
| 19 | 31.5 | 31.5 | 31.5 | 31.4 | 31.9 |
| 20 | 22.5 | 22.5 | 22.6 | 22.4 | 22.6 |
| 21 | 14.0 | 14.0 | 14.1 | 13.9 | 14.0 |
| $CH_3\underline{CO}$ | | 20.8 | 20.8 | | 20.8 |
| $CH_3\underline{CO}$ | | 171.0 | 171.0 | | 171.0 |

[a] These assignments were established by HMBC, HMQC, DEPT, and/or NOESY experiments; see Figure 5 for structures. Within the same column, entries having the same superscript letter are interchangeable.

**(5*E*,12*Z*,15*Z*)-2-Hydroxy-4-oxoheneicosa-5,12,15-trien-1-yl acetate (2):** colorless oil; $[\alpha]^{23}_D$ +17.0° (*c* 1.00, $CHCl_3$) [literature, $[\alpha]^{22}_D$ +17.6° (*c* 0.48, $CHCl_3$) (*3*)]; IR $\nu_{max}$ (film) 3305, 1740, 1658, 1625, 1239, 1040 cm$^{-1}$; FAB-MS (*m*NBA as a matrix), *m*/*z* 379 $[M + H]^+$, 401 $[M + Na]^+$; $^1$H NMR, see Table 1; $^{13}$C NMR, see Table 2.

**(2*R*,12*Z*,15*Z*)-2-Hydroxy-4-oxoheneicosa-12,15-dien-1-yl acetate (3):** colorless oil; $[\alpha]^{23}_D$ +11.2° (*c* 1.00, $CHCl_3$) [literature, $[\alpha]^{20}_D$ + 10.2° (*c* 1.00, $CHCl_3$) (*4*)]; IR $\nu_{max}$ (film) 3300, 1740, 1710, 1372, 1240, 1040 cm$^{-1}$; FAB-MS (*m*NBA as a matrix), *m*/*z* 381 $[M + H]^+$, 403 $[M + Na]^+$; $^1$H NMR, see Table 1; $^{13}$C NMR, see Table 2.

**(2*E*,12*Z*,15*Z*)-1-Hydroxyheneicosa-2,12,15-trien-4-one (4):** colorless oil; IR $\nu_{max}$ (film) 3429, 1668, 1635, 1616, 1239, 1100 cm$^{-1}$; FAB-MS (*m*NBA as a matrix), *m*/*z* 321 $[M + H]^+$, 343 $[M + Na]^+$; HR-FAB-MS (*m*NBA as a matrix), *m*/*z* 321.2794 ($[C_{21}H_{34}O_2 + H]^+$, calcd 321.2793); $^1$H NMR, see Table 1; $^{13}$C NMR, see Table 2.

**(5*E*,12*Z*)-2-Hydroxy-4-oxoheneicosa-5,12-dien-1-yl acetate (5)** was obtained as a colorless oil; $[\alpha]^{23}_D$ +9.04° (*c* 1.00, $CHCl_3$); IR $\nu_{max}$ (film) 3305, 1740, 1658, 1635, 1459, 1103 cm$^{-1}$; FAB-MS (*m*NBA as a matrix), *m*/*z* 381 $[M + H]^+$, 403 $[M + Na]^+$; HR-FAB-MS (*m*NBA as a matrix), *m*/*z* 381.3007 ($[C_{23}H_{40}O_4 + H]^+$, calcd 381.3005); $^1$H NMR, see Table 1; $^{13}$C NMR, see Table 2.

**Animals and Diets.** Five-week-old male rats of the Wistar strain weighing 90−100 g were obtained from Japan SLC (Hamamatsu, Japan). The rats were individually housed in hanging stainless steel wire cages and kept in an isolated room at a controlled temperature (23−25 °C) and ambient humidity (50−60%). Lights were maintained on a 12-h light−dark cycle. Animals were acclimated to the facility for 4 or 5 days and given free access to water and a commercial stock diet (type MF, Oriental Yeast). The composition of the control diet was as follows (g/kg): casein, 250; cornstarch, 401; sucrose, 200; corn oil, 50; mineral mixture (AIN 1977), 35; vitamin mixture (AIN 1977), 10; choline bitartrate, 4; and cellulose, 50. Supplements were added to the control diet at the expense of cellulose. Rats were given free access to water and experimental diets.

In this study, four separate experiments were conducted. In experiment 1, 235 rats were divided into 24 groups and fed the control diet or diets supplemented with each fruit. Each fruit was lyophilized and powdered with a mixer and added to the control diets. In experiment 2, 49 rats were divided into 7 groups and fed the control diet or diets supplemented with each fraction from lyophilized avocado (fractions 1−5). Each fraction was added to the diet so as to make it comparable to the addition of powdered avocado, on the basis of percent distribution of each fraction (fraction 1, 4.4%; fraction 2, 0.53%; fraction 3, 0.21%; fraction 4, 0.98%; fraction 5, 1.8%). In experiment 3, 71 rats were divided into 9 groups and fed the control diet or diets supplemented with each fraction (fractions 1-1 to 1-7). Each fraction was added to the diet so as to make it comparable to the addition of powdered avocado. In experiment 4, after 14 days of feeding the control diet, each compound was suspended with fraction 1-1 (almost pure triglycerides) and administered directly into the rats' stomachs by using a catheter (100 mg/kg of body weight).

After 14 days of feeding the experimental diet (experiments 1−3) or after 4 h of the administration of each compound (experiment 4), D-galactosamine was injected intraperitoneally at a dose of 350 mg/kg of body weight. Untreated rats were

injected with saline. At 22 h after the injection, rats were killed by decapitation to obtain blood. Rats were not starved either before or after the injection of saline or the toxin. Blood plasma was separated from heparinized whole blood by centrifugation at 2000*g* for 20 min at 4 °C. The plasma obtained was stored at −30 °C until analysis. The experimental design was approved by the Laboratory Animal Care Committee of the Faculty of Agriculture, Shizuoka University.

**Biochemical Analysis.** The activities of plasma alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured with a commercially available kit (Transaminase C II-test; Wako). The enzyme activity was expressed as millimoles per minute per liter of plasma) at 25 °C.

**Statistical Analysis.** Results were expressed as mean ± standard error of the mean (SEM). Data were tested for homogeneity of variance using Bartlett's test and, if necessary, were log-transformed to homogeneity before one-way ANOVA. The difference between means was tested at $p < 0.05$ using Duncan's multiple-range test (*5*) when the $F$ value was significant at $p < 0.05$.

**Fatty Acid Composition.** For the determination of fatty acid composition in fraction 1-1 (triglyceride fraction), the fraction (5.0 mg) was treated with 14% (w/w) $BF_3$/methanol reagent (Wako), and the resulting methyl esters were analyzed by GLC on a model GC-17A (Shimadzu). The result is as follows: 14:0 (fatty acid), 0.1 (wt % in total fatty acids); 16:0, 17.0; 16:1*n*−7, 7.1; 18:0, 0.4; 18:1*n*−9, 48.7; 18:1*n*−7, 5.7; 18:1*n*−5, 0.1; 18:2*n*−6, 12.1; and 18:3*n*−3, 8.8.

**Total Synthesis of Compound 1.** *Synthesis of* ***7***. To a solution of **6** (1.62 g, 5.53 mmol) in 1,4-dioxane (38 mL) and $H_2O$ (19 mL) was added an aqueous solution of lithium hydroxide (3.20 M, 19.0 mL, 60.8 mmol) dropwise at room temperature under nitrogen. After stirring at room temperature for 21 h, the reaction mixture was poured into 1 M HCl at 0 °C and was extracted three times with $CHCl_3$ (150 mL). The combined organic layer was washed with brine and dried over $MgSO_4$, and the solvent was evaporated under reduced pressure. The resulting crude oil was purified by column chromatography (silica gel 50 g, hexane/ethyl acetate 4:1), yielding **7** (1.3078 g, 84%) as a colorless oil: IR (film) 3536−3100, 1700, 1652, 1419 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 400 MHz), $\delta$ 7.09 (dt, $J = 15.7$, 7.2 Hz, 1H), 5.83 (d, $J = 15.7$ Hz, 1H), 5.37 (m, 4H), 2.78 (dd, $J = 6.2$, 6.2 Hz, 2H), 2.23 (dt, $J = 7.2$, 7.2 Hz, 2H), 2.06 (m, 4H), 1.48 (tt, $J = 7.2$, 7.2 Hz, 2H), 1.41−1.24 (m, 9H), 0.89 (t, $J = 6.4$ Hz, 3H).

*Synthesis of* ***8***. To a solution of **7** (19.4 mg, 7.00 mmol) in $CH_2Cl_2$ (2.3 mL) was added *N*,*O*-dimethylhydroxyamine hydrochloride (38.5 mg, 0.395 mmol), 1-hydroxybenzotriazole hydrate (115 mg, 0.748 mmol), and 4-(dimethylamino)pyridine (104 mg, 0.851 mmol) at room temperature under argon. The mixture was cooled to −20 °C and was added to a solution of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (139 mg, 0.724 mmol) in $CH_2Cl_2$ (4.0 mL) dropwise. The reaction mixture was allowed to warm to room temperature and stirred for a further 2 days. Then the reaction mixture was poured into water and extracted three times with ethyl acetate (6 mL), and the combined organic layer was washed with 1 M HCl, water, saturated aqueous $NaHCO_3$, water, and brine and dried over $MgSO_4$. The solvent was evaporated under reduced pressure, and the resulting crude oil was purified by column chromatography (silica gel 1.5 g, benzene/ethyl acetate 16:1), yielding **8** (14.1 mg, 62%), as a colorless oil: IR (film) 1664, 1637, 1417, 765 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$), $\delta$ 6.98 (dt, $J = 7.6$, 16.1 Hz, 1H), 6.39 (d, $J = 16.1$ Hz, 1H), 5.36 (m, 4H), 3.70 (s, 3H), 3.24(s, 3H), 2.78 (dd, $J = 6.3$, 6.3 Hz, 2H), 2.24 (dt, $J = 7.6$, 7.6 Hz, 2H), 2.05 (m, 4H), 1.48 (tt, $J = 7.6$, 7.6 Hz, 2H), 1.43−1.24 (m, 8H), 0.89 (t, $J = 6.6$ Hz).

*Synthesis of* ***10***. To a mixture of **9** (*6*) (955 mg, 2.75 mmol) and pyridinium *p*-toluenesulfonate (0.172 g, 0.684 mmol) in $CH_2Cl_2$ (19.0 mL) was added 3,4-dihydro-2*H*-pyrane (6.90 mL, 76.2 mmol) dropwise under nitrogen. After 5 h of stirring at room temperature, pyridinium *p*-toluensulfonate (0.052 g, 0.207 mmol) and 3,4-dihydropyrane (3.00 mL, 33.1 mmol) were added. After this mixture had been stirred at the same temperature for 8 h, the reaction was quenched with aqueous potassium carbonate and then was extracted three times with $Et_2O$ (30 mL), and the combined organic layer was washed with water and brine and dried over $MgSO_4$; the solvent was evaporated under reduced pressure. The resulting crude oil was purified by column chromatography (silica gel 30 g, hexane/ethyl acetate 30:1), yielding **10** (722.8 mg, 61%) as a colorless oil: $^1H$ NMR (400 MHz, $CDCl_3$) $\delta$ 6.23 (br d, $J = 19.5$ Hz, 1H), 6.07 (dt, $J = 19.5$, 5.2 Hz, 1H), 4.64 (m, 1H), 4.27 (ddd, $J = 5.2$, 12.3, 1.4 Hz, 1H), 4.02 (ddd, $J = 5.2$, 12.3, 1.4 Hz, 1H), 3.89 (m, 1H), 3.51 (m, 1H), 1.92−1.45 (m, 12H), 1.30 (m, 12H), 0.93−0.86 (m, 9H).

*Synthesis of* ***11***. To a mixture of (*E*)-vinylstannane **10** (1.12 g, 2.60 mmol) in THF (13 mL) was added *n*-buthyllithium (1.60 M in hexane, 1.60 mL, 2.56 mmol) dropwise at −78 °C under argon. After 10 min of stirring at the same temperature, a solution of **8** (248 mg, 0.773 mmol) in THF (7 mL) was added via cannula. The reaction mixture was allowed to stir for a further 2 h at − 78 °C before quenching with 0.5 M HCl (20 mL). The resulting mixture was extracted three times with $Et_2O$ (30 mL), and the combined organic layers were washed with a saturated aqueous solution of $NaHCO_3$, water, and brine and dried over $MgSO_4$; the solvent was evaporated under reduced pressure. The resulting crude oil was purified by column chromatography (silica gel 30 g, hexane/ethyl acetate 30:1−silica gel 6 g, hexane/ethyl acetate 30:1), yielding **11** (181 mg, 58%) as a colorless oil: $^1H$ NMR (400 MHz, CDCl3) $\delta$ 6.94 (m, 2H), 6.61 (dt, $J = 15.7$, 2.1 Hz, 1H), 6.34 (d, $J = 16.6$ Hz, 1H), 5.35 (m, 4H), 4.68 (t, $J = 3.8$ Hz, 1H), 4.46 (ddd, $J = 18.0$, 4.2, 2.1 Hz, 1H), 4.19 (ddd, $J = 18.0$, 4.2, 2.1 Hz, 1H), 3.86 (m, 1H), 3.53 (m, 1H), 2.77 (dd, $J = 6.4$, 6.4 Hz), 2.25 (dt, $J = 7.2$, 7.2 Hz, 2H), 2.07 (m, 4H), 1.94−1.45 (m, 8H), 1.44−1.23 (m, 8H), 0.89 (t, $J = 6.7$ Hz, 3H).

*Synthesis of* ***1***. A solution of **11** (181.2 mg, 0.450 mmol) in acetic acid/THF/water (4:2:1, 8.80 mL) was stirred at 45 °C for 8 h under nitrogen and then cooled to room temperature. The reaction mixture was diluted with toluene and concentrated under reduced pressure. The residue was purified by column chromatography (silica gel 10 g, hexane/ethyl acetate 8:1), yielding **1** (79.0 mg, 55%) as a colorless oil.

## RESULTS AND DISCUSSION

**Effects of Fruits on the Levels of Plasma ALT and AST.** Twenty-two lyophilized fruits (apple, avocado, banana, blueberry, cherry, durian, fig, grape, grapefruit, Japanese plum, Japanese summer orange, kiwi, lychee, mango, melon, papaya, peach, pear, persimmon, pineapple, Satsuma mandarin, and watermelon) were added to diets to give 5 wt % basis. Rats were given free access to water and experimental diets. After the experimental diets had been fed for 14 days, D-galactosamine was injected intraperitoneally at a dose of 350 mg/kg of body weight. At 22 h after injection, the levels of plasma ALT (or glutamic-pyruvic transaminase, GPT) and AST (or glutamic-oxaloacetic transaminase, GOT) in the rats were measured (Figure 1). Figure 1A demonstrates the effect of fruits on plasma AST activity. Among them, avocado, cherry, fig, grapefruit, Japanese plum, kiwi, lychee, papaya, and watermelon decreased the enzyme activity significantly. The ALT result was very similar to that of AST (Figure 1B). Because avocado exhibited the strongest suppressing activity and the activity was dependent on its dose (Figure 2), we tried to isolate the active principles.

**Bioassay-Guided Isolation and Structure Determination of Liver Injury Suppressing Compounds from Avocado.** Lyophilized avocado was extracted with hexane (fraction 1). The residue was further extracted with ethyl acetate (fraction 2) and then 70% ethanol. The ethanol extract was divided into a 1-butanol soluble fraction (fraction 3) and a water soluble fraction (frac-

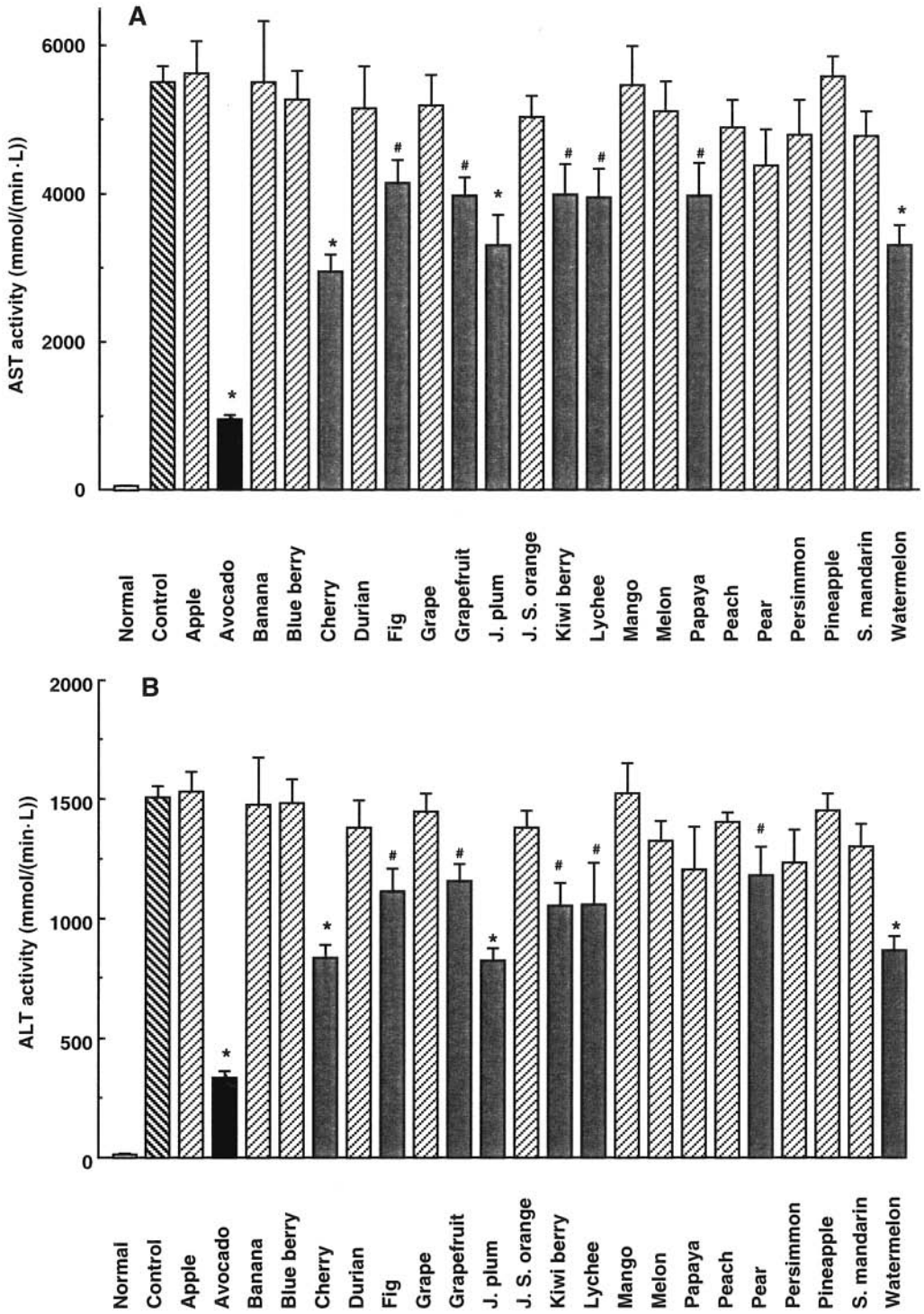


**Figure 1.** Effects of fruits on plasma ALT and AST activities. The column and its bar represent the mean value and SEM, respectively. Values with different letters are significantly different at $p < 0.05$.

tion 4). The final residue was fraction 5. Only fraction 1 showed an activity (Figure 3), so this fraction was separated by column chromatography on silica gel to give seven fractions (fractions 1-1 to 1-7). As shown in Figure 4, only fraction 1-4 showed significant activity. Although the fraction gave one spot on silica gel TLC, HPLC analysis of the fraction gave several peaks. Therefore, the fraction was further separated by HPLC and finally five compounds (yields: **1**, 772 mg from 100 g of fraction 1; **2**, 773 mg; **3**, 296 mg; **4**, 1442 mg; **5**, 221 mg) were obtained.

The molecular formula $C_{21}H_{34}O_2$ of **1** was determined by HR-FAB-MS of the $[M + H]^+$ ion (data given under Materials and Methods). The $^1H$ and $^{13}C$ NMR data were similar to those of persenone A (**2**), which has been isolated as a nitric oxide and superoxide generation inhibitor from this fruit (Figure 5; Tables 1 and 2) (*3*, *7*, *8*). Compound **1** differs from **2** by the presence of a *trans*-allyl alcohol moiety [$\delta$ 66.2 (C1), 144.8 (C2), 126.3 (C3) in the $^{13}C$ NMR; $\delta$ 4.12 (dd, $J = 11.4$, 4.1 Hz, H1a), 4.38 (br s, H1b), 6.93 (m, H2), 6.62 (dt, $J = 15.4$, 2.1 Hz, H3) in the $^1H$ NMR] conjugated with C4 ($\delta$ 189.2) instead of C1 to 3 of **2**. The final structure was determined by interpretation of HMBC correlations as follows: H2/C3, H2/C4, H3/C4, H5/C4, H6/C4, H17/C15, H17/C16, H17/C18, H17/C19, and H21/C19. The struc-

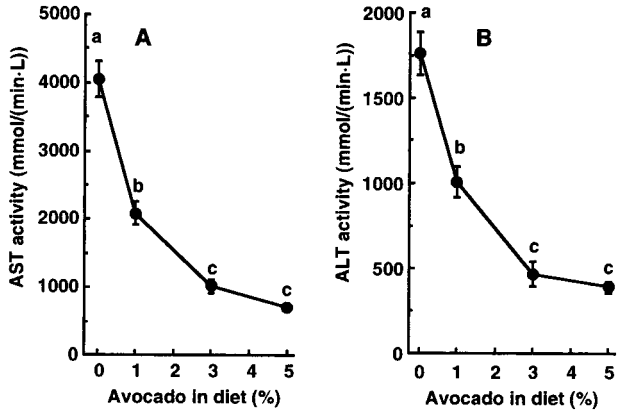


**Figure 2.** Dose-dependent effects of avocado on plasma ALT and AST activities. The circle and its bar represent the mean value and SEM, respectively. Values with different letters are significantly different at $p < 0.05$.

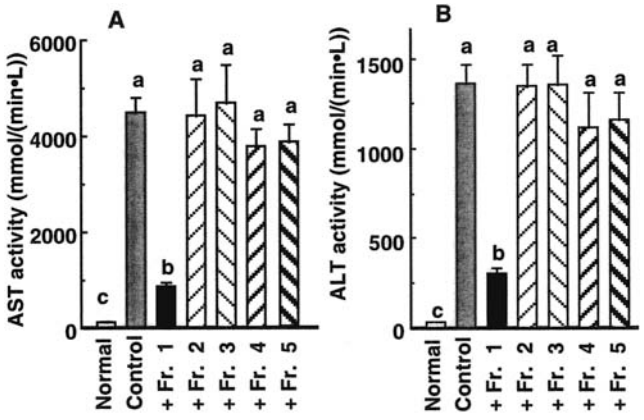


**Figure 3.** Effects of the fractions from avocado on plasma ALT and AST activities. The column and its bar represent the mean value and SEM, respectively. Values with different letters are significantly different at $p < 0.05$.

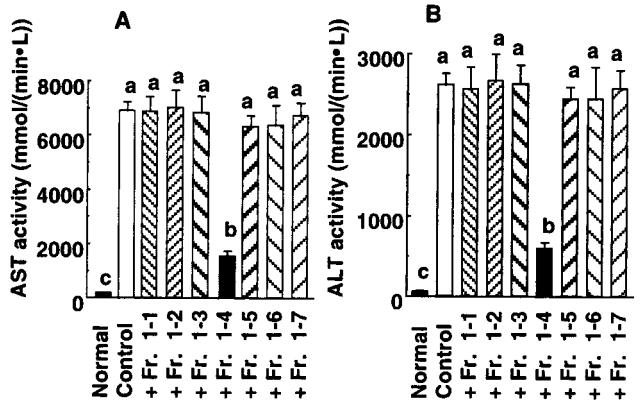


**Figure 4.** Effects of the fractions of avocado hexane extract on plasma ATL and AST activities. The column and its bar represent the mean value and SEM, respectively. Values with different letters are significantly different at $p < 0.05$.

ture of **1** was confirmed by synthesis (Figure 6). The total yield of **1** was 17% from the starting material, **6**.

Compound **3** has previously been isolated as a growth inhibitor for silkworm larvae (*9*) and a nitric oxide and superoxide generation inhibitor from this fruit (*3*, *7*, *8*).

Compound **4** has the molecular formula $C_{21}H_{34}O_2$ as found by HR-FAB-MS of the $[M + H]^+$ ion. The NMR data for **4** closely resemble those of **1**. However, **4** has three double bonds including a *trans*-α,*β*-enone [$\delta$ 145.1 (C2), 127.8 (C3), 201.0 (C4) in $^{13}C$ NMR; $\delta$ 4.10 (dd, $J =$

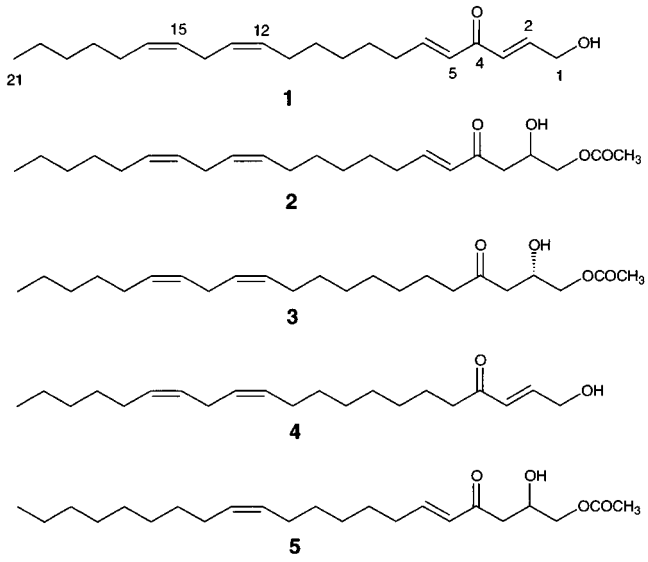


**Figure 5.** Structures of active compounds **1**−**5**.

11.3, 4.1, H1a), 4.30 (m, H1b), 6.85 (dt, $J = 5.9$ 4.0, H2), 6.33 (ddd, 15.9, 3.1 2.1, H3) in $^1H$ NMR], although **1** has four olefins and one of them is an α,*β*,α′,*β*′-enone. The HMBC correlations of **4** confirmed the structure as H1/C2, H1/C3, H2/C1, H2/C4, H3/C1, H3/C4, H5/C4, H14/C12, H14/C13, H14/C15, H14/C16, H16/C17, H16/C18, H17/15, H17/C16, H17/C18, H17/C19, H21/C20, H21/C19.

The NMR data of **5** are very similar to those of **2**. The molecular formula $C_{23}H_{40}O_4$ of the compound was determined by HR-FAB-MS of the peak at *m*/*z* 381.3007 $[M + H]^+$, indicating **5** has two more protons than **2**. The compound possesses a *cis*-double bond and a *trans*-enone. The position of *cis*-olefin was determined by HR-EI-MS of fragment peaks: *m*/*z* 113.1332 ($[C_8H_{17}]^+$, calcd 113.1330), C14−C21; *m*/*z* 139.1488 ($[C_{10}H_{19}]^+$, calcd 139.1487), C12−C21.

The absolute configuration at C-2 in **2** and **5** has not been yet determined, but probably these are *R*, because their $[\alpha]_D$ values [**2**, + 17.0 (*c* 1.00, $CHCl_3$); **5** +9.04 (*c* 1.00)] are similar to that (+10.2, $c = 1.0$) of **3**, which has been determined by its enantioselective synthesis (*4*).

This fruit contains large amounts of oleic acid (48.7% in total fatty acids) and linoleic acid (12.1%). These five compounds may be biosynthesized by Claisen-type condensation between oleic or linoleic acid and a three-carbon unit (possibly pyruvate).

**Liver Injury Suppressing Activity of 1—5.** After 14 days of feeding the control diets, each compound (100 mg/kg of body weight) was administered directly into the rats' stomachs by using a catheter. At 4 h after the administration, D-galactosamine was injected intraperitoneally. Plasma ALT and AST levels were measured 22 h later. All of the compounds exhibited similar strong liver injury suppressing activities (Figure 7).

This study clearly demonstrates that some fruits, especially avocado, have suppressive effects on D-galactosamine-induced enhancement of plasma ALT and AST activities, and five unusual fatty acid derivatives from avocado have strong suppressing activity. Because the extent of increase in these plasma enzyme activities parallels that of liver injury, the results indicate that these compounds and some fruits are able to protect against a certain type of liver injury, such as that

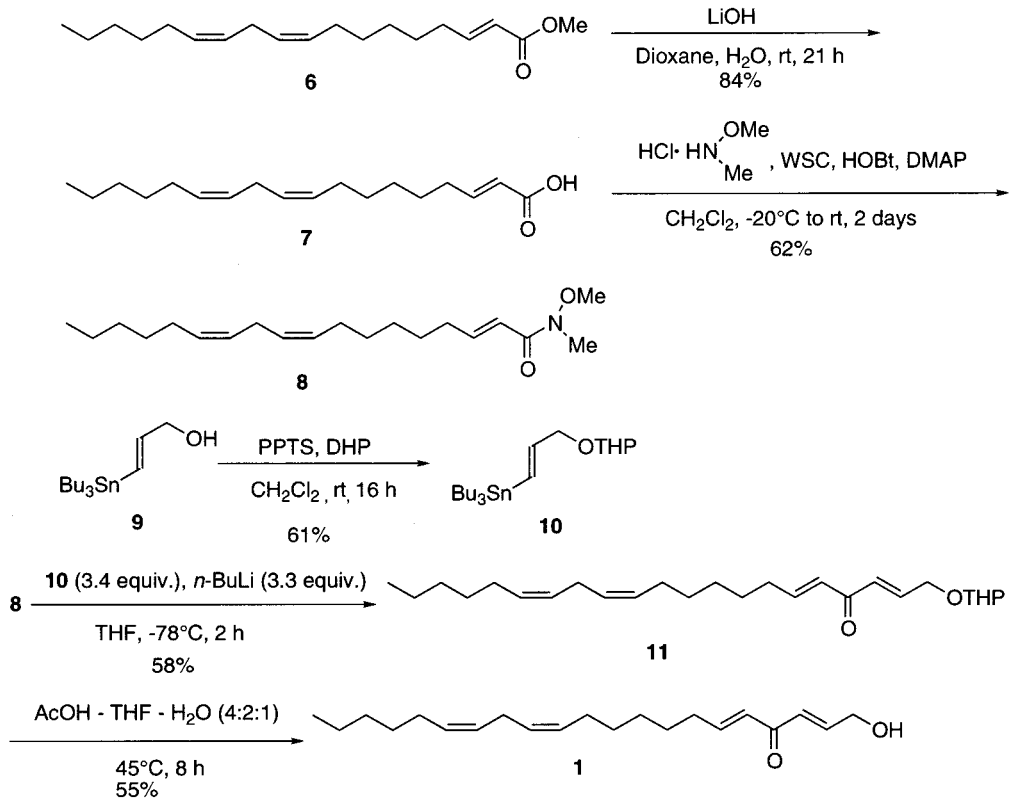


**Figure 6.** Synthetic scheme for **1**.

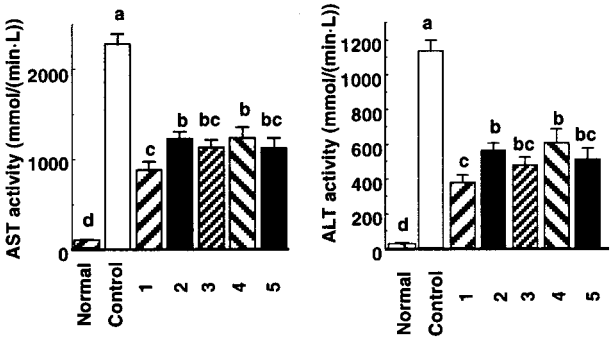


**Figure 7.** Effects of compounds **1**−**5** on plasma ATL and AST activities. The column and its bar represent the mean value and SEM, respectively. Values with different letters are significantly different at $p < 0.05$.

induced by D-galactosamine in rats. D-Galactosamine is thought to induce hepatotoxicity by inhibiting the synthesis of RNA and proteins through a decrease in UTP concentration, which finally leads to the necrosis of liver cells (*10*). The symptoms of D-galactosamine-induced liver injury resemble those of viral hepatitis (*11*). In humans, liver injury or hepatitis is caused by viruses, natural and synthetic chemicals, alcohol, autoimmune diseases, etc. Avocado is eaten as an adornment to salads, sandwiches, and California rolls or guacamole, which is used as a popular Mexican sauce. Although D-galactosamine-induced liver injury in rats does not rigorously correspond to virus-induced liver injury in humans, this finding, obtained with the rat model, might offer useful information about a means for protecting against human liver injury.

Some mushrooms and beverages suppress D-galactosamine-induced liver injury in rats (*12*−*16*), and known glycosidic flavonoids have been isolated as the active compounds from green tea (*14*). However, so far as we are aware, this is the first time that liver injury suppressing substances have been isolated from fruits. The mechanism of their liver injury suppressing effects remains under investigation.

## LITERATURE CITED

(1) Swinbanks, D.; O'Brien, J. Japan explores the boundary between food and medicine. *Nature* **1993**, *364*, 180.
(2) Nestle, M. Fruits and vegetables: protective or just fellow travelers? *Nutr. Rev.* **1996**, *54*, 255−257.
(3) Kim, O. K.; Murakami, A.; Nakamura, Y.; Takeda, N.; Yoshizumi, H.; Ohigashi, H. Novel nitric oxide and superoxide generation inhibitors, persenone A and B, from avocado fruit. *J. Agric. Food Chem.* **2000**, *48*, 1557−1563.
(4) MacLeod, J. K.; Schäffeler, L. A short enantioselective synthesis of a biologically active compound from *Persea americana*. *J. Nat. Prod.* **1995**, *58*, 1270−1273.
(5) Ducan, D. B. Multiple range tests for correlated and hetroscedastic means. *Biometrics* **1957**, *13*, 164−176.
(6) Jung, M. E.; Light, L. A. Preparation of iodoallylic alcohols via hydrostannylation: Spectroscopic proof of structures. *Tetrahedron Lett.* **1982**, *23*, 3851−3854.
(7) Kim, O. K.; Murakami, A.; Nakamura, Y.; Kim, H. W.; Ohigashi, H. Inhibition by (−)-persenone A-related compounds of nitric oxide and superoxide generation from inflammatory leukocytes. *Biosci., Biotechnol., Biochem.* **2000**, *64*, 2500−2503.
(8) Kim, O. K.; Murakami, A.; Takahashi, D.; Nakamura, Y.; Torikai, K.; Kim, H. W.; Ohigashi, H. An avocado constituent, persenone A, suppresses expression of inducible forms of nitric oxide synthase and cyclooxygenase in macrophages, and hydrogen peroxide generation in mouse skin. *Biosci., Biotechnol., Biochem.* **2000**, *64*, 2504−2507.
(9) Chang, C.; Isogai, A.; Kamikado, T.; Murakoshi, S.; Tamura, S. Isolation and structure elucidation of growth inhibitors for silk-wormLarvae from avocado leaves. *Agric. Biol. Chem.* **1975**, *39*, 1167−1168.

(10) Decker, K.; Keppler, D. Galactosamine hepatitis: Key role of the nucleotide deficiency period in the pathogenesis of cell injury and cell death. *Rev. Physiol. Biochem. Pharmocol.* **1974**, *71*, 77−106.
(11) Keppler, D.; Lesch, R.; Reutter, W.; Decker, K. Experimental hepatitis induced by D-galactosamine. *Exp. Mol. Pathol.* **1968**, *9*, 279−290.
(12) Lee, E. W.; He, P.; Kawagishi, H.; Sugiyama, K. Suppression of D-galactosamine-induced liver injury by mushrooms in rats. *Biosci., Biotechnol., Biochem*. **2000**, *64*, 2001−2004.
(13) Sugiyama, K.; He, P.; Wada, S.; Saeki, S. Teas and other beverages suppress D-galactosamine-induced liver injury in rats. *J. Nutr.* **1999**, *129*, 1361−1367.
(14) Sugiyama, K.; He, P.; Wada, S.; Tamaki, F.; Saeki, S. Green tea suppresses D-galactosamine-induced liver injury in rats. *Biosci., Biotechnol., Biochem*. **1998**, *62*, 609−611.
(15) Wada, S.; He, P.; Hashimoto, I.; Watanabe, N.; Sugiyama, K. Glycosidic flavonoids as rat-liver injury preventing compounds from green tea. *Biosci., Biotechnol., Biochem*. **2000**, *64*, 2262−2265.
(16) Wada, S.; He, P.; Watanabe, N.; Sakata, K.; Sugiyama, K. Suppression of D-galactosamine-induced rat liver injury by glycosidic flavonoids-rich fraction from green tea. *Biosci., Biotechnol., Biochem*. **1999**, *63*, 570−572.

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