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9-Oxooctadeca-10,12-dienoic Acids as Acetyl-CoA Carboxylase Inhibitors from Red Pepper (*Capsicum annuum* L.)

Jun WATANEBE, Jun KAWABATA,* and Takanori KASAI

Department of Bioscience and Chemistry, Faculty of Agriculture, Hokkaido University, Kita-ku, Sapporo 060-8589, Japan

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A methanol extract of red pepper showed potent acetyl-CoA carboxylase inhibitory activity. The active principles were isolated and identified as (*E*, *E*)- and (*E*, *Z*)-9-oxooctadeca-10,12-dienoic acids by instrumental analyses. The IC_{50} values of the compounds were 1.4×10^{-6} and 1.5×10^{-6} M, respectively, their activity being nearly sixty-times higher than that of the common fatty acids themselves. A comparative study of the structure-activity relationship among their related compounds showed that the inhibitory activity was influenced neither by the position and species of the oxygen functional group in the middle of the alkyl chain nor by the configurations of the double bonds. However, it was found that the presence of double bonds between the terminal carboxyl and the mid-chain oxygen functional group lowered the inhibitory activity which could be recovered by hydrogenation of the double bonds.

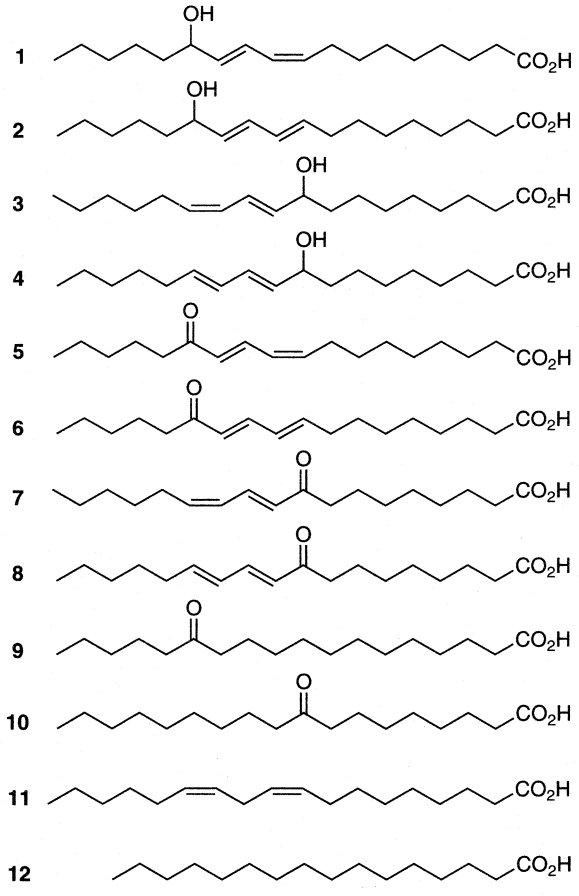
Key words: acetyl-CoA carboxylase inhibitor; red pepper; oxooctadecadienoic acid

Acetyl-CoA carboxylase (ACC, EC 6.4.1.2), which catalyzes the carboxylation of acetyl-CoA to form malonyl-CoA, is known to be one of the key enzymes in fatty acid biosynthesis. Its activity is regulated by phosphorylation[1,2] and by changes in enzyme quantity.[3] The inhibitors of ACC would thus retard fat accumulation and avoid obesity which is a risk factor of many chronic diseases. We have searched for ACC inhibitors in foodstuffs, since daily consumption of such inhibitors from foods might result in a healthy effect without using medicines. During the study, epigallocatechin gallate has been isolated as an ACC inhibitor from green tea,[4] although its activity was not very high. This paper deals with further screening results for ACC inhibitors from foods, and the isolation, identification and structure-activity relationship of the active principles in red pepper.

Materials and Methods

Materials. Dried and crushed red pepper was kindly provided by House Food Co. (Japan), the other plant foodstuffs being obtained from market sources. Authentic 4-hydroxy-3-nitrophenylacetic acid was purchased from Sigma Chemical Co. All other chemicals used were of reagent grade.

Instrumental analyses. ^{1}H- and ^{13}C-NMR spectra were measured with a Bruker AMX500 spectrometer operated at 500 MHz for ^{1}H and at 125 MHz for ^{13}C. Electron impact (EI) and field desorption (FD) mass spectra were determined with JEOL JMS-AX500 and SX-102A mass spectrometers, respectively.



Measurement of ACC inhibitory activity. Purification of rat liver ACC and measurement of its inhibitory activity were performed as described earlier.[4]

Screening for ACC inhibitors in foodstuffs. An edible part of each foodstuff was extracted with 50% aqueous MeOH (10 ml/g fr. wt.). The extract was partitioned between EtOAc and water, and the resulting EtOAc solution was evaporated, redissolved in DMSO (10 ml/g fr. wt.), and submitted to ACC inhibitory activity measurements. Inhibitory activity is shown as the activity

* To whom correspondence should be addressed. FAX: +81-11-706-2496; E-mail: junk@chem.agr.hokudai.ac.jp

relative to that of 5 mM epigallocatechin gallate (EGCg:$IC_{50}=3.1\times10^{-4}$ M) as 100%; that is, 100% inhibition means an extract solution with activity equal to that of a 5 mM solution of EGCg.

Isolation of the ACC inhibitors from red pepper. A pulverized red pepper sample (100 g) was extracted with MeOH (500 ml) at room temperature. The methanolic extract was partitioned between EtOAc and water. The resulting EtOAc-soluble fraction was submitted to silica gel column chromatography (Wakogel C-200, Wako Pure Chem. Ind., 28×160 mm) and eluted with a hexane-2-propanol gradient. The active fraction eluted with 9:1 hexane-2-propanol from the column was submitted to chromatography in an ODS column (Cosmosil 75C18-OPN, Nacalai Tesque, 44×160 mm). Stepwise elution was performed from MeOH-water (3:1) to MeOH. The active fraction eluted with 9:1 MeOH-water was further purified by normal-phase HPLC (Inertsil SIL 5 μm, GL Science, 4.6×250 mm; mobile phase, hexane-THF (19:1); flow rate, 1.2 ml/min; detection, UV at 300 nm). Two active peaks eluted at $t_R=17.5$ and 19.5 min were obtained. The more potent latter peak was collected and rechromatographed by reverse-phase HPLC (J′ sphere ODS-H80, YMC, 4.6×250 mm; mobile phase, MeOH-water (82.5:17.5) containing 0.1% trifluoroacetic acid; flow rate, 1.2 ml/min; detection, UV at 300 nm) to yield an active principle (**8**, 1 mg, $t_R=13$ min), whereas the less active former peak was also collected and rechromatographed by reverse-phase HPLC (Inertsil ODS-2, GL Science, 4.6×250 mm; mobile phase, MeOH-water (82.5:17.5) containing 0.1% trifluoroacetic acid; flow rate, 1.0 ml/min; detection, UV at 254 nm) to yield another active principle (**7**, 2.3 mg, $t_R=10$ min). **8**: colorless oil; FD-HR-MS m/z (M^+): calcd. for $C_{18}H_{30}O_3$, 294.2195; found, 294.2231; EI-MS m/z (%): 294 (M^+, 28), 276 (13), 223 (79), 171 (23), 166 (94), 151 (100), 95 (89); ^{1}H-NMR δ (pyridine-d_5): 0.81 (3H, t, J=7.0 Hz, 18-H_3), 1.14–1.36 (12H, m, 4–6 and 15–17-H_2), 1.66 (2H, tt, J=7.4, 7.3 Hz, 7-H_2), 1.75 (2H, tt, J=7.5, 7.4 Hz, 3-H_2), 2.05 (2H, dt, J=7.3 (t), 7.0 (d) Hz, 14-H_2), 2.48 (2H, t, J=7.4 Hz, 2-H_2), 2.56 (2H, t, J=7.4 Hz, 8-H_2), 6.16 (1H, dt, J=15.6 (d), 7.0 (t) Hz, 13-H), 6.24 (1H, dd, J=15.6, 10.5 Hz, 12-H), 6.28 (1H, d, J=15.7 Hz, 10-H), 7.38 (1H, dd, J=15.7, 10.5 Hz, 11-H); ^{13}C-NMR δ (chloroform-d): 14.7 (q), 23.2 (t), 25.0 (t), 25.3 (t), 29.1 (t), 29.6 (t), 29.7 (t), 29.8 (t), 32.1 (t), 33.8 (t), 34.1 (t), 41.1 (t), 128.5 (d), 129.5 (d), 143.7 (d), 146.5 (d), 177.6 (s), 201.8 (s); **7**: colorless oil; EI-MS m/z (%): 294 (M^+, 25), 223 (37), 171 (37), 166 (94), 151 (100), 95 (78), 81 (74); ^{1}H-NMR δ (pyridine-d_5): 0.79 (3H, t, J=7.0 Hz, 18-H_3), 1.14–1.36 (12H, m, 4–6 and 15–17-H_2), 1.65 (2H, tt, J=7.3, 7.1 Hz, 7-H_2), 1.74 (2H, m, 3-H_2), 2.21 (2H, ddt, J=7.8 (d), 7.5 (t), 0.9 (d) Hz, 14-H_2), 2.49 (2H, m, 2-H_2), 2.58 (2H, t, J=7.3 Hz, 8-H_2), 5.85 (1H, dt, J=11.0 (d), 7.8 (t) Hz, 13-H), 6.22 (1H, dd, J=11.2, 11.0 Hz, 12-H), 6.35 (1H, d, J=15.4 Hz, 10-H), 7.76 (1H, br dd, J=15.4, 11.2 Hz, 11-H).

Hydrogenation of 8. Hydrogen gas was passed through an MeOH solution of **8** (0.1 mg in 0.3 ml) in the presence of a catalytic amount of palladium black at room temperature. The mixture was filtered and evaporated to yield its tetrahydrogenated product (**10**). **10**: colorless oil; EI-MS m/z (%): 298 (M^+, 5), 186 (43), 170 (60), 155 (85), 83 (72), 55 (100).

Preparation of hydroxyoctadecadienoic acids (1–4). Linoleic acid (6.16 g, 22 mmol), 2,2′-azobis(2,4-dimethylvaleronitrile) (546 mg, 2.2 mmol) and 70% *t*-butylhydroperoxide in water (2.82 ml, 22 mmol) were dissolved in hexane-2-propanol (1:1, 10 ml), and the solution was stirred for 13 hr at room temperature. The reaction mixture was submitted to silica gel column chromatography (hexane-2-propanol (9:1)) to give a mixture of hydroperoxides. To a solution of the hydroperoxides in hexane-2-propanol (1:1, 5 ml) was added triphenylphosphine (4 g), the mixture being stirred for 13 hr at room temperature. The reaction mixture was evaporated to dryness, and the residue was partitioned between EtOAc and 5% $NaHCO_3$. The resulting acidic fraction was submitted to normal-phase HPLC (Inertsil SIL 5 μm, GL Science, 4.6×250 mm; mobile phase, hexane-2-propanol (400:7); flow rate, 1.2 ml/min; detection, UV at 255 nm) to give (*Z*, *E*)-13-hydroxyoctadeca-9,11-dienoic acid (**1**, 20.1 mg, $t_R=7.4$ min), (*E*, *E*)-13-hydroxyoctadeca-9,11-dienoic acid (**2**, 12.4 mg, $t_R=10.8$ min), (*E*, *Z*)-9-hydroxyoctadeca-10,12-dienoic acid (**3**, 38.7 mg, $t_R=12.8$ min) and (*E*, *E*)-9-hydroxyoctadeca-10,12-dienoic acid (**4**, 15.2 mg, $t_R=14.2$ min). The chromatographic pattern of the mixture was consistent with that of the lipoxygenase-oxidation products of linoleic acid.[5] **1**: colorless oil; EI-MS m/z (%): 296 (M^+, 2), 278 (53), 99 (100), 79 (66); ^{1}H-NMR δ (pyridine-d_5): 0.81 (3H, t, J=7.0 Hz, 18-H_3), 1.14–1.36 (12H, m, 4–6 and 15–17-H_2), 1.53 (1H, m, 14-H), 1.61 (1H, m, 14-H), 1.70–1.86 (4H, m, 3 and 7-H_2), 2.17 (2H, dt J=7.6 (d), 7.5 (t) Hz, 8-H_2), 2.49 (2H, t, J=7.4 Hz, 2-H_2), 4.51 (1H, dt, J=6.1 (d), 6.0 (t) Hz, 13-H), 5.46 (1H, dt, J=10.8 (d), 7.6 (t) Hz, 9-H), 6.05 (1H, dd, J=15.1, 6.1 Hz, 12-H), 6.23 (1H, dd, J=11.1, 10.8 Hz, 10-H), 6.93 (1H, dd, J=15.1, 11.1 Hz, 11-H). **2**: colorless oil; EI-MS m/z (%): 296 (M^+, 3), 278 (38), 99 (100), 79 (47); ^{1}H-NMR δ (pyridine-d_5): 0.82 (3H, t, J=6.9 Hz, 18-H_3), 1.16–1.32 (12H, m, 4–6 and 15–17-H_2), 1.52 (1H, m, 14-H), 1.61 (1H, m, 14-H), 1.68–1.84 (4H, m, 3 and 7-H_2), 2.04 (2H, dt, J=7.2 (d), 7.1 (t) Hz, 8-H_2), 2.50 (2H, t, J=7.4 Hz, 2-H_2), 4.44 (1H, dt, J=6.3 (d), 6.2 (t) Hz, 13-H), 5.72 (1H, dt, J=15.2 (d), 7.2 (t) Hz, 9-H), 5.97 (1H, dd, J=15.2, 6.3 Hz, 12-H), 6.23 (1H, dd, J=15.2, 10.5 Hz, 10-H), 6.54 (1H, dd, J=15.2, 10.5 Hz, 11-H). **3**: colorless oil; EI-MS m/z (%): 296 (M^+, 6), 278 (35), 171 (95), 153 (80), 83 (85), 55 (100); ^{1}H-NMR δ (pyridine-d_5): 0.79 (3H, t, J=6.9 Hz, 18-H_3), 1.14–1.38 (12H, m, 4–7, 16 and 17-H_2), 1.52 (1H, m, 8-H), 1.62 (1H, m, 8-H), 1.68–1.86 (4H, m, 3 and 15-H_2), 2.17 (2H, dt, J=7.6 (d), 7.5 (t) Hz, 14-H_2), 2.48 (2H, t, J=7.4 Hz, 2-H_2), 4.51 (1H, dt, J=6.1, 6.1 Hz, 9-H), 5.45 (1H, dt, J=10.8 (d), 7.6 (t) Hz, 13-H), 6.05 (1H, dd, J=15.1, 6.1 Hz, 10-H), 6.23 (1H, dd, J=11.0, 10.8 Hz, 12-H), 6.93 (1H, dd, J=15.1, 11.0 Hz, 11-H). **4**:

colorless oil; EI-MS m/z (%): 296 (M^+, 8), 278 (43), 171 (90), 153 (100), 83 (70), 55 (77); ^{1}H-NMR δ (pyridine-d_5): 0.80 (3H, t, J=6.9 Hz, 18-H_3), 1.14–1.38 (12H, m, 4–7, 16 and 17-H_2), 1.52 (1H, m, 8-H), 1.61 (1H, m, 8-H), 1.68–1.86 (4H, m, 3 and 7-H_2), 2.03 (2H, dt, J=7.2 (d), 7.1 (t) Hz, 14-H_2), 2.48 (2H, t, J=7.4 Hz, 2-H_2), 4.45 (1H, dt, J=6.3 (d), 6.2 (t) Hz, 9-H), 5.71 (1H, dt, J=15.3 (d), 7.0 (t) Hz, 13-H), 5.97 (1H, dd, J=15.3, 6.3 Hz, 10-H), 6.23 (1H, dd, J=15.3, 10.4 Hz, 12-H), 6.55 (1H, dd, J=15.3, 10.4 Hz, 11-H).

Preparation of oxooctadecadienoic acids (**5–8**). To a solution of **1** (15.7 mg) in $CHCl_3$ (3 ml) was added activated MnO_2 (157 mg). The mixture was stirred for 4 hr at room temperature and filtered to give (*Z*, *E*)-13-oxooctadeca-9,11-dienoic acid (**5**, 4.3 mg). (*E*, *E*)-13-Oxooctadeca-9,11-dienoic acid (**6**), (*E*, *Z*)-9-oxooctadeca-10,12-dienoic acid (**7**) and (*E*, *E*)-9-oxooctadeca-10,12-dienoic acid (**8**) were prepared by oxidation of the corresponding hydroxyoctadecadienoic acids, **2**, **3** and **4**, respectively, in the same manner. **5**: colorless oil; EI-MS m/z (%): 294 (M^+, 34), 223 (39), 151 (64), 99 (100), 81 (60); ^{1}H-NMR δ (pyridine-d_5): 0.79 (3H, t, J=6.9 Hz, 18-H_3), 1.14–1.36 (12H, m, 4–7, 16 and 17-H_2), 1.64 (2H, tt, J=7.3, 7.2 Hz, 3-H_2), 1.75 (2H, m, 15-H_2), 2.22 (2H, dt, J=7.7 (d), 7.5 (t) Hz, 8-H_2), 2.50 (2H, m, 2-H_2), 2.59 (2H, t, J=7.3 Hz, 14-H_2), 5.85 (1H, dt, J=11.2 (d), 7.7 (t) Hz, 9-H), 6.21 (1H, dd, J=11.2, 11.2 Hz, 10-H), 6.34 (1H, d, J=15.2 Hz, 12-H), 7.76 (1H, dd, J=15.2, 11.2 Hz, 11-H). **6**: colorless oil; EI-MS m/z (%): 294 (M^+, 35), 223 (40), 151 (100), 95 (66), 81 (59); ^{1}H-NMR δ (pyridine-d_5): 0.79 (3H, t, J=6.9 Hz, 18-H_3), 1.14–1.36 (12H, m, 4–7, 16 and 17-H_2), 1.65 (2H, tt, J=7.3, 7.2 Hz, 3-H_2), 1.77 (2H, tt, J=7.3, 7.3 Hz, 15-H_2), 2.06 (2H, dt, J=7.2 (t), 6.9 (d) Hz, 8-H_2), 2.50 (2H, m, 2-H_2), 2.56 (2H, t, J=7.3 Hz, 14-H_2), 6.15 (1H, dt, J=15.2 (d), 6.9 (t) Hz, 9-H), 6.24 (1H, dd, J=15.2, 10.5 Hz, 10-H), 6.27 (1H, d, J=15.5 Hz, 12-H), 7.37 (1H, dd, J=15.5, 10.5 Hz, 11-H).

Preparation of oxooctadecanoic acids (*9 and 10*). Catalytic hydrogenation of **5** (2.0 mg) was performed as already described to give 13-oxooctadecanoic acid (**9**, 1.9 mg). 9-Oxooctadecanoic acid (**10**) was prepared from **7** in the same manner. **9**: colorless oil; EI-MS m/z (%): 298 (M^+, 6), 227 (39), 126 (50), 114 (55), 43 (100).

Results and Discussion

Primary screening was carried out to select a foodstuff with high ACC inhibitory activity. The tested foodstuffs and the results (% inhibition compared to that of 5 mM EGCg as 100%) were as follows: spinach (0), cauliflower (32), cabbage (0), watermelon (0), edible burdock (50), sunflower seed (17), celery (0), mitsuba (38), red pepper (149), and eggplant (67). Red pepper thus showed the highest activity among the 10 foodstuffs tested.

Red pepper (*Capsicum annuum* L., Solanaceae) was extracted with methanol. The extract was successively fractionated by solvent partition, silica gel and ODS column, and normal- and reverse-phase HPLC to yield two active compounds. The first active principle, **8**, showed a molecular ion at m/z 294 in the mass spectrum, and a high-resolution analysis disclosed its molecular formula to be $C_{18}H_{30}O_3$. The ^{1}H-NMR spectrum showed the presence of four consecutive olefinic protons, δ_H 6.16 (1H, dt, J=15.6 (d) and 7.0 (t) Hz), 6.24 (1H, dd, J=15.6 and 10.5 Hz), 6.28 (1H, d, J=15.7 Hz) and 7.38 (1H, dd, J=15.7 and 10.5 Hz), two methylenes, δ_H 2.48 (2H, t, J=7.8 Hz) and 2.56 (2H, t, J=7.4 Hz) adjacent to a carbonyl group, and an allylic methylene, δ_H 2.05 (2H, dt, J=7.0 and 7.0 Hz), as well as a chain of methylenes, δ_H 1.14–1.36, and a terminal methyl, δ_H 0.81 (3H, t, J=7.0 Hz). The ^{13}C-NMR spectrum exhibited carboxyl, δ_C 177.6, and ketone, δ_C 201.8, carbonyls, and four olefinic carbons, δ_C 128.5, 129.5, 143.7 and 146.5. These data indicate that both double bonds had *trans* configurations (J=15.6 and 15.7 Hz) and were conjugated to the ketone carbonyl to form an *E*,*E*-dienone system. In addition, the presence of the terminal methyl and carboxyl groups along with the fatty methylene chain indicated the structure of the inhibitor to be a C_{18} straight-chain fatty acid containing an *E*,*E*-dienone moiety in the middle of the chain. The HH COSY result also supports this proposed structure. The position of the enone moiety was estimated from the mass fragmentation pattern. The conspicuous fragment ions at m/z 151 and 171, which correspond to the partial structures, $CH_3(CH_2)_4CH=CHCH=CHCO^+$ and $HO_2C(CH_2)_7CO^+$, respectively, indicate that the mid-chain carbonyl was located at the 9-position. An additional fragment peak at m/z 166 can be derived from the McLafferty rearrangement as $CH_3(CH_2)_4CH=CHCH=CHC(OH)^+=CH_2$. The fragment peaks at m/z 151 and 166 were shifted to m/z 155 and 170, respectively, by hydrogenation of the compound, and a newly observed fragment ion at m/z 186 in this tetrahydrogenated product is assignable to another McLafferty rearrangement peak, $HO_2C(CH_2)_7C(OH)^+=CH_2$, which can now be derived from the carboxyl-side chain by saturation of the double bonds. The structure was therefore concluded to be (*E*, *E*)-9-oxooctadeca-10,12-dienoic acid (**8**). Further confirmation of the proposed structure was made by its chemical synthesis, starting from linoleic acid. The mass and NMR spectral data of the isolate are consistent with those of the synthetic material and also those in the literature. The inhibitory activity of the compound against ACC was as high as an IC_{50} value of 1.4×10^{-6} M. This activity is approximately 60-times higher than common fatty acids such as linoleic and palmitic acids and more than 200-times higher than previously reported EGCg, an active principle of green tea.[4]

On the other hand, the second active principle, **7**, also showed a molecular ion at m/z 294 in its mass spectrum, and its fragmentation pattern is similar to that of **8**. In the NMR spectrum, **7** also showed a similar resonance pattern to that of **8**, except for the olefinic proton region, δ_H 5.85 (1H, dt, J=11.0 (d), 7.8 (t) Hz), 6.22 (1H, dd, J=11.2, 11.0 Hz), 6.35 (1H, d, J=15.4 Hz) and 7.76 (1H, br dd, J=15.4, 11.2 Hz). The analysis of the coupling pattern suggests that **7** contained an (*E*, *Z*)-dienone moiety in place of the (*E*, *E*)-dienone part in **8**.

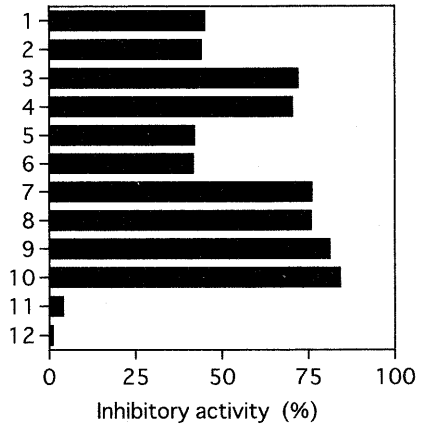


Fig. 1. ACC Inhibitory Activity of 9-Oxooctadeca-10,12-dienoic Acids (**7** and **8**) and Related Compounds.

Each compound was dissolved in DMSO. The final concentration was 5.0×10^{-6} M.

The structure of **7** was hence deduced to be (*E*, *Z*)-9-oxooctadeca-10,12-dienoic acid. This structure was again confirmed by chemical synthesis in the same manner as that for **8**. The IC_{50} value of **7** against ACC was 1.5×10^{-6} M which is comparable to that of **8**.

Through the chemical synthesis of **7** and **8** from linoleic acid, two additional regioisomers, (*Z*, *E*)- and (*E*, *E*)-13-oxooctadeca-9,11-dienoic acids, were obtained, since chemical oxidation of linoleic acid gave a mixture of regioisomeric 9- and 13-hydroperoxides. It was considered interesting to learn if these structural isomers, as well as their hydroxyl and other analogs, would show inhibitory activity against ACC. Therefore, four hydroxyoctadecadienoic acids, (*Z*, *E*)- and (*E*, *E*)-13-hydroxyoctadeca-9,11-dienoic, and (*E*, *Z*)- and (*E*, *E*)-9-hydroxyoctadeca-10,12-dienoic acids (**1**, **2**, **3** and **4**, respectively), four oxooctadecadienoic acids, (*Z*, *E*)- and (*E*, *E*)-13-oxooctadeca-9,11-dienoic acids (**5** and **6**, respectively) along with **7** and **8**, and two oxooctadecanoic acids, 13- and 9-oxooctadecanoic acids (**9** and **10**, respectively) were prepared from linoleic acid. These compounds, together with linoleic acid (**11**) and palmitic acid (**12**), were measured for their inhibitory activities at a concentration of 5.0×10^{-6} M (Fig. 1).

A comparison of the inhibitory activity of the 13-oxygenated octadecadienoic acids, **1** (45%), **2** (44%), **5** (42%) and **6** (42%), and of the 9-oxygenated octadecadienoic acids, **3** (72%), **4** (70%), **7** (76%) and **8** (76%), indicates that the activity was neither influenced by the species of oxygen functional groups nor by the configurations of the double bonds. However, 13-hydroxy- and 13-oxooctadecadienoic acids showed lower activity than 9-hydroxy- and 9-oxooctadecadienoic acids, although even they had stronger activity than the usual fatty acids, linoleic acid (**11**, 4%) and palmitic acid (**12**, 1%). Catalytic hydrogenation of the double bonds in the 13-oxooctadecadienoic acids resulted in the saturated 13-oxooctadecanoic acid, **9** (81%), which showed almost the same activity as the 9-oxooctadecadienoic acids did, whereas saturation of the double bonds in the 9-oxooctadecadienoic acids had little effect on the activity (9-oxooctadecanoic acid, **10** (84%)). These results suggest that the presence of double bonds between the terminal carboxyl and mid-chain oxygen functional groups reduced the inhibitory activity which could be recovered by hydrogenation of the double bonds. Flexibility of the carbon chain between the terminal carboxyl and mid-chain oxygen functional groups may play an important role in exhibiting the inhibitory activity of these oxygenated C_{18} acids. The 13-oxooctadecadienoic acids have not yet been isolated from red pepper, in spite of their moderate inhibitory activity, which is possibly due to the lower 13-lipoxygenase activity than that of 9-lipoxygenase in red pepper.

It is known that palmitoyl-CoA, a final product of fatty acid synthesis, strongly inhibits ACC, whereas palmitic acid shows far weaker inhibitory activity.[6] The present study has disclosed, however, that the (*E*, *E*)- and (*E*, *Z*)-9-oxooctadeca-10,12-dienoic acids showed inhibitory activities of $IC_{50} = 1.4 \times 10^{-6}$ and 1.5×10^{-6} M, respectively, which are almost sixty-times higher than common fatty acids such as linoleic and palmitic acids showing $IC_{50} = 8.9 \times 10^{-5}$ and 8.3×10^{-5} M, respectively. The content of common long-chain fatty acids in red pepper has not been determined and there might be a higher amount of such acids than of 9-oxooctadeca-10,12-dienoic acids. We failed to detect normal fatty acids as ACC inhibitors, although they could contribute more to the apparent activity of the crude extract than our isolates, probably because they have inconspicuous activity and low UV absorption intensity.

This is the first report on the biological activity of (*E*, *E*)- and (*E*, *Z*)-9-oxooctadeca-10,12-dienoic acids, although these acids have been identified as constituents of dimorphotheca oil[7] and Chinese moxa,[8] as well as the lipoxygenase-catalyzed oxidation products of linoleic acid.[5] It is known that capsaicin in red pepper activates heat generation and reduces fats in our body by way of adrenaline secretion.[9] The present results indicate a potential new function of red pepper which might moderately suppress fatty acid biosynthesis by inhibiting the ACC reaction.

Acknowledgments

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