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Anti-Hyperlipidemic Sesquiterpenes and New Sesquiterpene Glycosides from the Leaves of Artichoke (*Cynara scolymus* L.): Structure Requirement and Mode of Action

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Abstract—The methanolic extract from the leaves of artichoke (*Cynara scolymus* L.) was found to suppress serum triglyceride elevation in olive oil-loaded mice. Through bioassay-guided separation, sesquiterpenes (cynaropicrin, aguerin B, and grosheimin) were isolated as the active components together with new sesquiterpene glycosides (cynarascolosides A, B, and C). The oxygen functional groups at the 3- and 8-positions and *exo*-methylene moiety in α-methylene-γ-butyrolactone ring were found to be essential for the anti-hyperlipidemic activity of guaiane-type sesquiterpene. In addition, inhibition of gastric emptying was shown to be partly involved in anti-hyperlipidemic activity.

Introduction

Artichoke (*Cynara scolymus* L., Compositae) is widely cultivated in Europe and America and its sprout is eaten as a vegetable. The leaves of artichoke are used for the treatment of hepatitis and hyperlipidemia in European traditional medicine. As various pharmacological activities of the constituents and extract from the leaves of artichoke, polyphenols such as cynarin, caffeic acid, chlorogenic acid, and luteolin were reported to inhibit oxidative stress generated by reactive oxygen species in human leukocytes.[1] Cynaroside inhibited hepatic cholesterol biosynthesis without affecting hydroxymethylglutaryl (HMG)-CoA reductase activity in rat hepatocytes[2] and cynaropicrin inhibited contraction of rabbit isolated thoracic aorta.[3] The leaves of artichoke were also reported in various clinical trials to be effective for patients with irritable bowel syndrome[4] and hyperlipoproteinemia,[5] and to show choleretic effects.[6]

To clarify the anti-hyperlipidemic effect of artichoke, we examined the effect of methanolic (MeOH) extract and several components from the leaves of artichoke in olive oil-loaded mice. Furthermore, we also examined the structural requirements of the active constituents for anti-hyperlipidemic activity and mode of action.

Anti-Hyperlipidemic Activity of the Methanolic Extract from the Leaves of Artichoke

The dried leaves of artichoke (1.0 kg, cultivated in Peru) were extracted with MeOH three times under reflux for 3 h to give MeOH extract (33.1% from the dried leaves). As shown in Figure 1, the MeOH extract (125–500 mg/kg, po) significantly suppressed serum triglyceride (TG) elevation 2 h after administration of olive oil. In contrast, 6 h after administration of olive oil, increases in TG level were observed in the groups that received the extract at doses of 125 and 250 mg/kg. Orlistat, a lipase inhibitor, completely suppressed the serum TG elevation at 250 mg/kg. Clofibrate, a hypolipidemic medicine, also suppressed the TG level at 250 and 500 mg/kg.

Isolation of Cynarascolosides A–C (1–3) from the Leaves of Artichoke

The methanolic extract was partitioned into ethyl acetate (AcOEt) and water to give an AcOEt-soluble fraction

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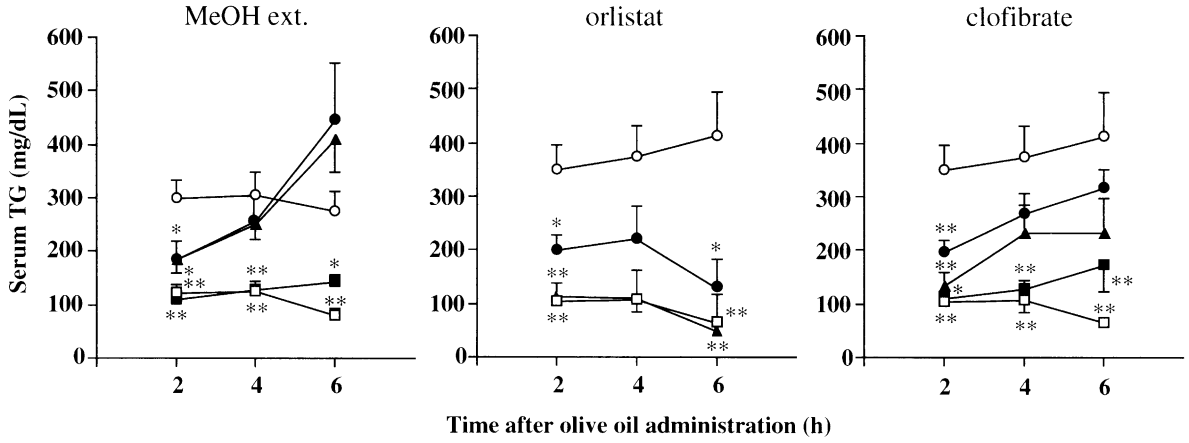

Figure 1. Inhibitory effects of the MeOH ext. from the leaves of artichoke, orlistat, and clofibrate on serum TG elevation in olive oil-loaded mice. Each sample was administered orally to fasted (20–24 h) mice and olive oil (5 mL/kg) was administered (po) 30 min thereafter. Blood was collected from the infraorbital venosus plexus 2, 4 and 6 h after olive oil treatment. Serum TG was determined by enzymatic method. Symbols represent the following, □: non-olive oil treatment, ○: control, ●: 125 mg/kg, ▲: 250 mg/kg and ■: 500 mg/kg. Each value represents the mean ± SEM of 7 mice. Asterisks denote the significant differences from the control group at *: $P<0.05$, **: $P<0.01$, respectively.

(5.7%) and an aqueous layer. The aqueous layer was further extracted with *n*-butanol (*n*-BuOH) to give an *n*-BuOH-soluble fraction (3.9%) and a H_2O-soluble fraction (23.5%). The AcOEt-soluble fraction (250 mg/kg, po) significantly suppressed the increase in serum TG 63% ($P<0.05$) 2 h after administration of olive oil. On the other hand, the *n*-BuOH and H_2O-soluble fractions did not show such effect at a dose of 250 mg/kg.

The AcOEt-soluble fraction was subjected to reversed-phase column chromatography and HPLC to furnish principal sesquiterpenes, cynaropicrin (**4**,[7] 1.00%), aguerin B (**5**,[8] 0.047%), and grosheimin (**6**,[9] 0.18%), together with luteolin 7-*O*-β-D-glucopyranoside (**8**,[10] 0.11%). Although the *n*-BuOH-soluble fraction did not show the inhibitory effects, this fraction was also subjected to reversed-phase and normal-phase column chromatography and finally HPLC to furnish three new sesquiterpene glucosides cynarascolosides A (**1**, 0.062%), B (**2**, 0.047%), and C (**3**, 0.0094%), and three known glycosides 11β,13-dihydrodesacylcynaropicrin 8-β-D-glucoside (**7**,[11] 0.11%), luteolin 7-*O*-β-D-glucopyranoside **8** (0.055%), and luteolin 7-*O*-rutinoside (**9**,[12] 0.093%) (Chart 1).

Absolute Stereostructures of Cynarascolosides A–C (1–3)

Cynarascoloside A (**1**), was isolated as colorless plates of mp 246–248 °C (MeOH) with negative optical rotation ($[\alpha]_D^{27}$ −43.7°). The positive- and negative-ion FAB-MS of **1** showed quasimolecular ion peaks at *m*/*z* 857 $(2M+H)^+$, 429 $(M+H)^+$, 855 $(2M-H)^-$, and 427 $(M-H)^-$, and high-resolution MS analysis of the quasimolecular ion peak $(M+H)^+$ revealed the molecular formula of **1** to be $C_{21}H_{32}O_9$. The IR spectrum of **1** showed absorption bands at 3436 and 1717 cm^{-1} ascribable to hydroxyl and γ-lactone functions. Acid hydrolysis of **1** with 1 M HCl furnished D-glucose, the signals of which were identified by HPLC analysis using optical rotation detector.[13] Enzymatic hydrolysis of **1** with β-glucosidase liberated a new aglycon named cynarascolide (**1a**). The ^1H NMR (C_5D_5N) and ^{13}C NMR (Table 1) spectra of **1** and **1a**, the signals of which were assigned by various NMR experiments,[14] suggested the presence of two methyls [**1**: δ 1.47 (d, $J=6.7$ Hz, 15-H_3), 1.85 (d, $J=7.0$ Hz, 13-H_3), **1a**: δ 1.50 (d, $J=6.7$ Hz, 15-H_3), 1.70 (d, $J=7.0$ Hz, 13-H_3)], two methylenes [**1**: δ 1.90 (br dd, $J=10.7$, 13.1 Hz, 2β-H), 2.06 (br dd, $J=7.0$, 13.1 Hz, 2α-H), 2.31–2.37 (m, 9α-H), 3.52 (dd, $J=4.0$, 12.3 Hz, 9β-H), **1a**: δ 1.94 (br dd, $J=10.7$, 13.1 Hz, 2β-H), 2.11 (br dd, $J=7.0$, 13.1 Hz, 2α-H), 2.41 (br d, $J=12.1$ Hz, 9α-H), 3.02 (dd, $J=4.1$, 12.1 Hz, 9β-H)], an *exo*-methylene [**1**: δ 4.99, 5.23 (both s, 14-H_2), **1a**: δ 4.97, 5.00 (both s, 14-H_2)], five methines [**1**: δ 2.12 (m, 4-H), 2.31–2.37 (m, 5, 7-H), 2.98 (qd, $J=7.0$, 11.0 Hz, 11-H), 3.43 (br dd, $J=7.0$, 10.7 Hz, 1-H), **1a**: δ 2.13 (m, 4-H), 2.22 (br dd, $J=9.8$, 10.4 Hz, 7-H), 2.38 (m, 5-H), 2.80 (qd, $J=7.0$, 11.3 Hz, 11-H), 3.49 (1H, br dd, $J=7.0$, 10.7 Hz, 1-H)], and three oxygenated methines [**1**: δ 3.90 (dd, $J=10.1$, 10.1 Hz, 6-H), 3.96 (br dd, $J=4.0$, 10.1 Hz, 8-H), 4.31 (br s, 3-H), **1a**: δ 3.85 (br d, $J=9.8$ Hz, 8-H), 3.88 (dd, $J=10.1$, 10.4 Hz, 6-H), 4.33 (br s, 3-H),] together with a β-D-glucopyranosyl moiety {δ [4.40 (dd, $J=5.5$, 11.6 Hz), 4.59 (dd, $J=2.1$, 11.6 Hz), 6′-H_2], 5.07 (d, $J=7.6$ Hz, 1′-H)} in **1**.

As shown in Figure 2, the planar structure of the aglycon moiety and position of a glucoside linkage in **1** were constructed on the basis of ^1H–^1H COSY and HMBC, which showed long-range correlations between the following protons and carbons: 13-H_3 and 7, 11, 12-C; 15-H_3 and 3, 4, 5-C; 14-H_2 and 1, 9-C; 2-H_2, 9-H_2, 1-H and 10-C; 11-H and 12, 13-C; 4, 5-H and 15-C; 7-H and 13-C; 8-H and 1′-C; 1′-H and 8-C. The relative stereostructure of aglycon (**1a**) in **1** was characterized by the NOESY experiment, in which NOE correlations were observed between the following protons: 3-H and 2β, 4-H; 6-H and 4, 11-H; 8-H and 9β, 11-H; 5-H and 1, 11-H, 15-H_3; 1-H and 2α-H; 7-H and 13-H_3 (Figs. 2 and 3).

Finally, its absolute stereostructure was determined using X-ray crystallographic analysis of the D-glucoside (**1**) as shown in Figure 4.[15]

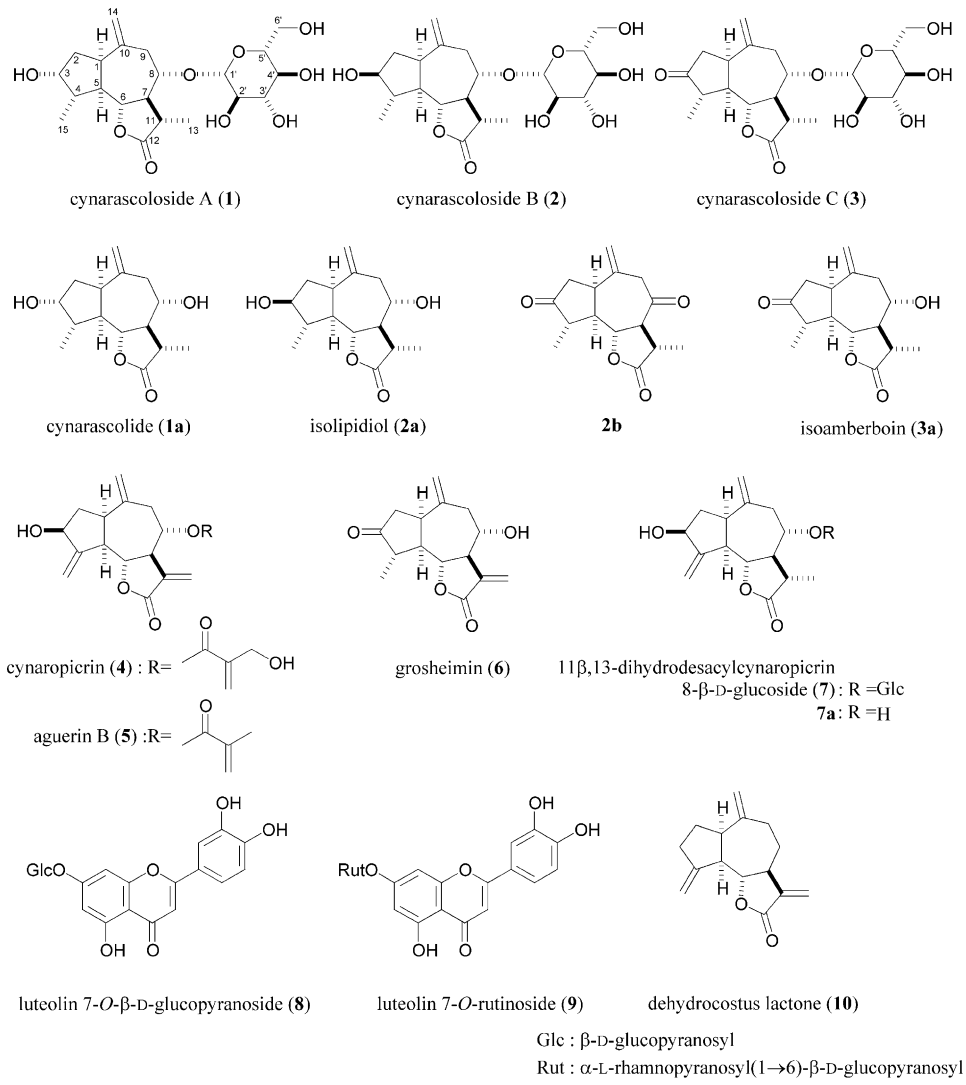

Chart 1. Constituents from the leaves of artichoke and their related compounds.

Table 1. ^{13}C NMR data of cynarascolosides A–C (**1**–**3**), cynarascolide (**1a**), and **2b**

	1[a]	2[a]	3[a]	1a[a]	2b[b]
C-1	45.5	42.9	39.9	45.5	40.6
C-2	39.7	39.3	43.7	39.8	42.8
C-3	74.5	77.8	218.6	74.5	217.6
C-4	45.6	47.6	47.4	45.6	47.2
C-5	50.9	51.3	51.4	50.8	52.8
C-6	82.1	82.0	83.5	82.2	79.7
C-7	58.0	57.4	53.4	59.5	59.4
C-8	85.2	85.0	84.5	76.2	201.0
C-9	46.2	45.6	47.7	49.0	53.2
C-10	145.8	145.2	145.5	146.2	139.8
C-11	42.0	42.0	41.1	42.6	35.6
C-12	179.3	179.2	179.1	179.2	176.9
C-13	16.9	16.9	17.2	16.7	14.57*
C-14	113.9	114.6	114.3	113.2	118.1
C-15	15.8	18.7	14.6	15.8	14.63*
Glc-1′	105.5	105.5	105.6		
Glc-2′	75.5	75.5	75.5		
Glc-3′	79.1	79.1	79.1		
Glc-4′	71.7	71.7	71.7		
Glc-5′	78.6	78.6	78.6		
Glc-6′	62.9	62.9	62.9		

Measured in [a]$CDCl_3$, [b]C_5D_5N at 125 MHz.
*May be interchangeable in the same column.

Cynarascoloside B (**2**) was obtained as colorless needles of mp 236–239 °C (aqueous MeOH) with negative optical rotation ($[\alpha]_D^{27}$ −9.7°) and its IR spectrum was similar to that of **1**. The molecular formula $C_{21}H_{32}O_9$ of **2**, which was the same as **1**, was characterized from the positive- and negative-ion FAB-MS [*m*/*z* 857 $(2M+H)^+$, 429 $(M+H)^+$, 855 $(2M-H)^-$, and 427 $(M-H)^-$] and by high-resolution MS measurement. Hydrolysis of **2** with 1 M HCl also furnished D-glucose,[13] while a known sesquiterpene isolipidiol (**2a**)[16] was obtained by enzymatic hydrolysis. The ^1H NMR (C_5D_5N) and ^{13}C NMR (Table 1) spectra[14] of **2** showed signals due to an aglycon (isolipidiol) moiety [δ 1.42 (d, $J=6.7$ Hz, 15-H_3), 1.85 (d, $J=7.0$ Hz, 13-H_3), 1.92–2.02 (m, 2β, 5-H), 2.17 (m, 4-H), 2.20 (br dd, $J=6.4$, 12.5 Hz, 2α-H), 2.31 (ddd, $J=9.8$, 10.1, 10.7 Hz, 7-H), 2.36 (dd, $J=10.1$, 12.4 Hz, 9α-H), 2.74 (br dd, $J=7.0$, 10.4 Hz, 1-H), 2.97 (qd, $J=7.0$, 10.7 Hz, 11-H), 3.48 (dd, $J=4.0$, 12.4 Hz, 9β-H), 3.90 (br dd, $J=6.4$, 7.6 Hz, 3-H), 3.95 (ddd, $J=4.0$, 9.8, 10.1 Hz, 8-H), 3.99 (dd, $J=10.1$, 10.1 Hz, 6-H), 5.11, 5.23 (both s, 14-H_2)] and a β-D-glucopyranosyl moiety {δ [4.40 (dd, $J=5.5$, 11.6 Hz), 4.59 (dd, $J=1.8$, 11.6 Hz), 6′-H_2], 5.06 (d, $J=7.6$ Hz, 1′-H)}. Further-

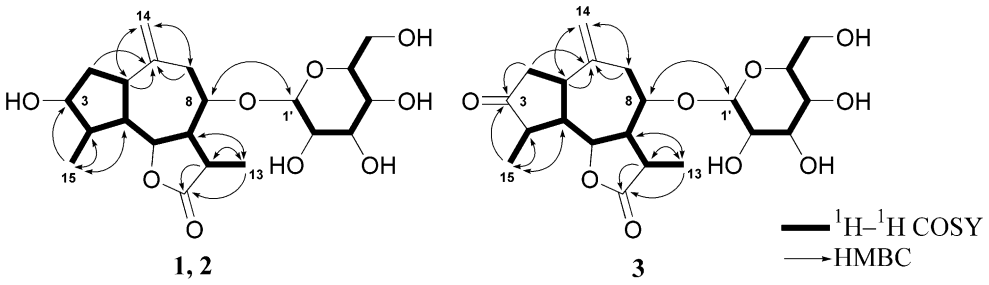

Figure 2. ^{1}H–^{1}H COSY and HMBC correlations of cynarascolosides A–C (**1**–**3**).

more, in the HMBC experiment of **2**, long-range correlations were observed between the 1′-proton of the β-D-glucopyranosyl moiety and the 8-carbon of the aglycon moiety and between the 8-proton and the 1′-carbon, respectively. Furthermore, comparison of the ^{13}C NMR data of **2** with that of **2a** revealed a glycosidation shift around the 8-position. On the basis of this evidence, the structure of cynarascoloside B was suggested to be isolipidiol 8-*O*-β-D-glucopyranoside (**2**).

To confirm the absolute stereostructure, **2** was treated with pyridinium chlorochromate (PCC) to furnish the 3,8-diketone derivative (**2b**), which was obtained by PCC oxidation of **1a**. On the basis of the above evidence, the absolute stereostructure of cynarascoloside B (**2**) was determined.

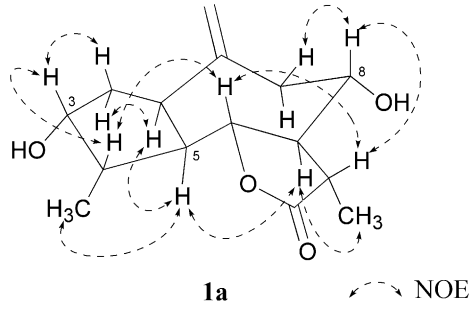

Figure 3. NOE correlations of cynarascolide (**1a**).

Cynarascoloside C (**3**), isolated as a white powder with positive optical rotation ($[\alpha]_D^{27}$ +30.8°), gave the quasimolecular ion peaks at *m*/*z* 853 $(2M+H)^+$, 427 $(M+H)^+$, 851 $(2M-H)^-$, and 425 $(M-H)^-$ in the positive- and negative-ion FAB-MS and the molecular formula was defined as $C_{21}H_{32}O_9$ from the high-resolution MS analysis. The IR spectrum of **3** showed absorption bands ascribable to hydroxyl (3424 cm^{-1}) and carbonyl (1732 cm^{-1}) functions. Acid hydrolysis of **3** liberated D-glucose,[13] while enzymatic hydrolysis of **3** furnished isoamberboin (**3a**).[17] The ^{1}H NMR (C_5D_5N) and ^{13}C NMR (Table 1) spectra[14] of **3** showed signals due to an isoamberboin moiety [δ 1.29 (d, J = 7.3 Hz, 15-H_3), 1.84 (d, J = 7.0 Hz, 13-H_3), 2.15 (br dd, J = 9.2, 9.5 Hz, 5-H), 2.28 (m, 4-H), 2.43 (br d, J = 18.9 Hz, 2β-H), 2.49 (br d, J = 12.7 Hz, 9α-H), 2.52 (dd, J = 8.8, 18.9 Hz, 2α-H), 2.59 (ddd, J = 9.5, 10.1, 10.1 Hz, 7-H), 3.04 (dd-like, 1-H), 3.15 (qd, J = 7.0, 10.1 Hz, 11-H), 3.42 (dd, J = 5.3, 12.7 Hz, 9β-H), 3.91 (dd, J = 9.2, 9.5 Hz, 6-H), 4.02 (m, 8-H), 4.68, 5.06 (both s, 14-H_2)] and a β-D-glucopyranosyl moiety {δ [4.41 (dd, J = 5.5, 11.4 Hz), 4.60 (dd, J = 1.5, 11.4 Hz), 6′-H_2], 5.06 (d, J = 7.6 Hz, 1′-H)}. The HMBC experiment on **3** showed long-range correlations between the anomeric proton of the glucopyranosyl moiety and the 8-carbon, and the 8-proton and the 1′-carbon of the glucopyranosyl moiety, respectively. This spectral evidence revealed that the glucopyranosyl moiety in **3** was attached to the 8-hydroxyl group of **3a**. Consequently, cynarascoloside C was determined to be isoamberboin 8-*O*-β-D-glucopyranoside (**3**).

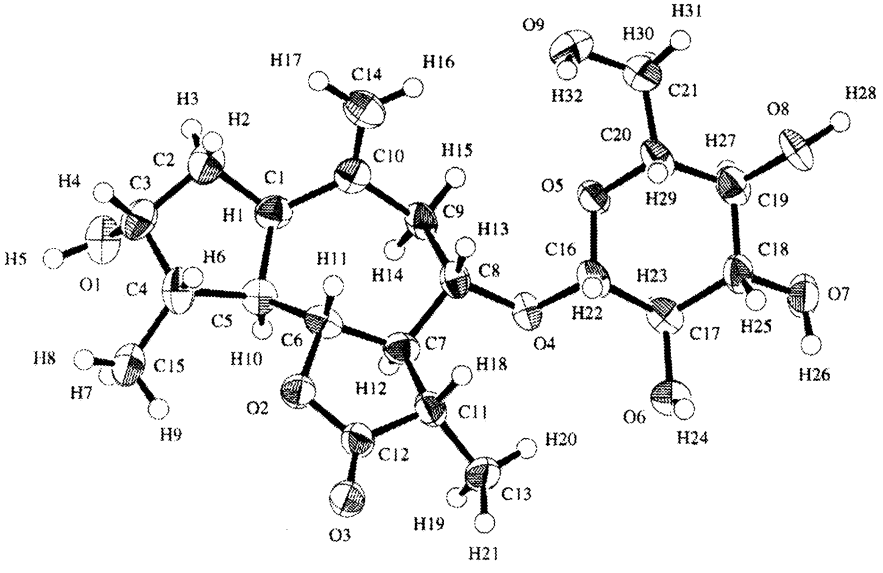

Figure 4. X-ray crystallographic analysis of cynarascoloside A (**1**).

Structural Requirements of the Active Constituents for Anti-Hyperlipidemic Activity and Mode of Action

We examined the anti-hyperlipidemic activity of the principal constituents from the leaves of artichoke. As shown in Figure 5, the sesquiterpene constituents (**4**–**6**) significantly suppressed serum TG elevation at 50 and 100 mg/kg during the early stage (2 h after olive oil administration). The activity of cynaropicrin (**4**), a principal sesquiterpene in artichoke, was the most potent among them. On the other hand, sesquiterpene glycosides (**1**, **2**, and **7**) did not suppress serum TG elevation. The flavone glycosides, luteolin 7-*O*-β-D-glucopyranoside (**8**) and luteolin 7-*O*-β-D-rutinoside (**9**), showed moderate suppressive effects at 50 and 100 mg/kg, but not significant at 2 h after administration of olive oil.

To clarify the essential structure of sesquiterpene for anti-hyperlipidemic activity, the activity of dehydrocostus

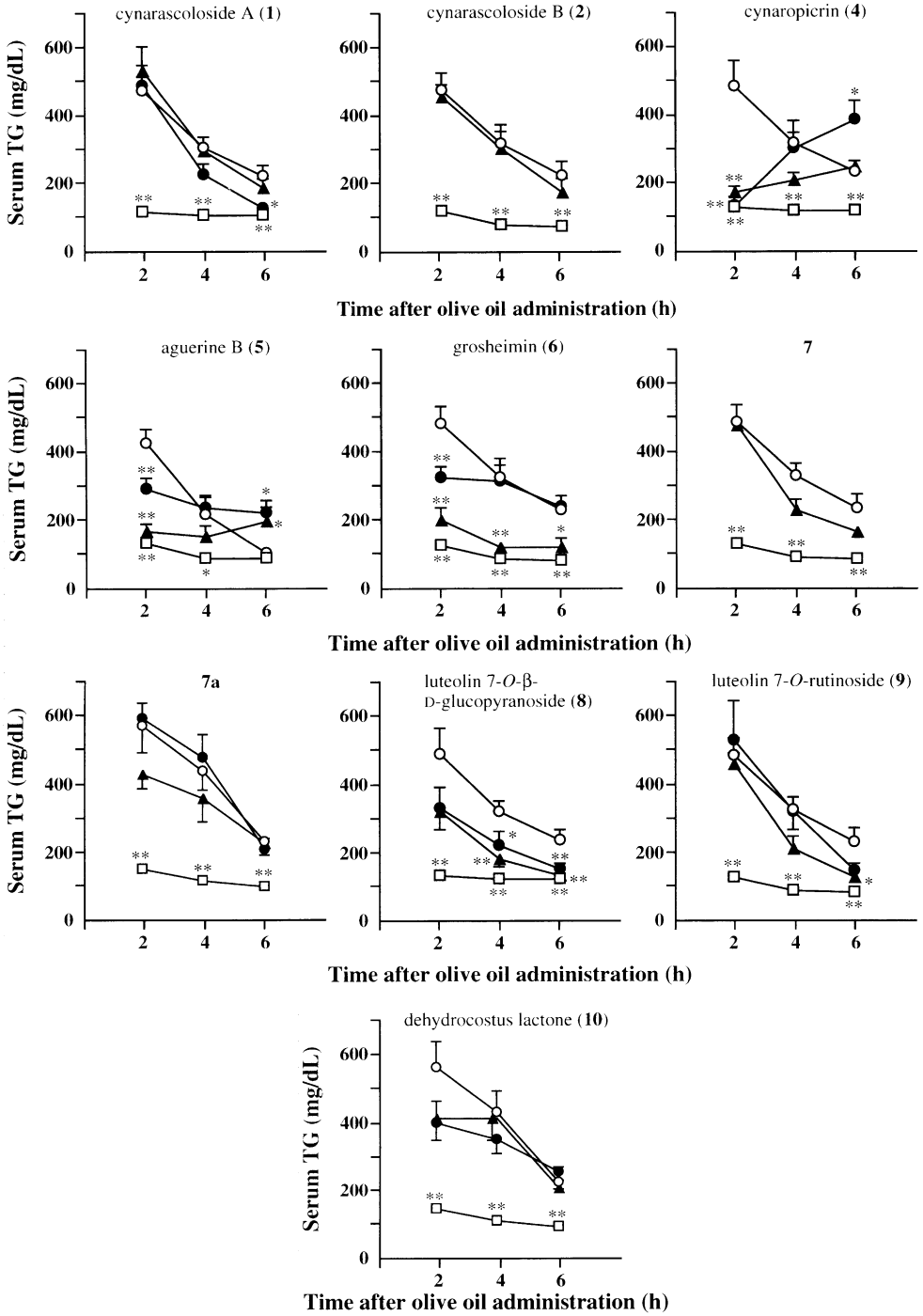

Figure 5. Inhibitory effects of constituents from the leaves of artichoke on serum TG elevation in olive oil-loaded mice. Symbols represent the following, □: non-olive oil treatment, ○: control, ●: 50 mg/kg and ▲: 100 mg/kg. Each value represents the mean ± SEM of 4–7 mice. Asterisks denote the significant differences from the control group at *: $P < 0.05$, **: $P < 0.01$, respectively.

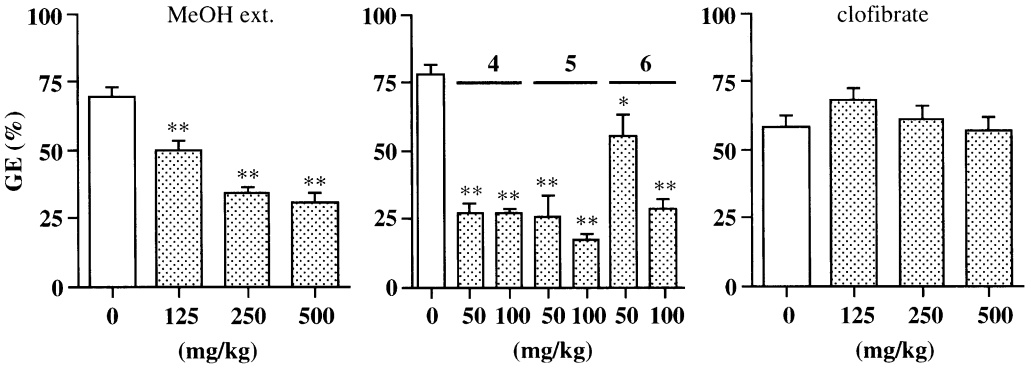

Figure 6. Inhibitory effects of the MeOH ext. from the leaves of artichoke, cynaropicrin (**4**), aguerin B (**5**), grosheimin (**6**), and clofibrate on GE in olive oil-loaded mice. Each sample was administered orally to the mice. Thirty minutes later, olive oil containing 0.05% phenol red was administered orally at 0.15 mL/mouse. Thirty mininutes later, the stomach was removed and the quantity of phenol red was determined. Each column represents the mean with SEM of 4–6 mice. Asterisks denote significant differences from the control at *: $P<0.05$, **: $P<0.01$.

lactone (**10**) from bay leaf[18] and 11β,13-dihydrodesacylcynaropicrin (**7a**), aglycon of **7**, were examined.[19] Dehydrocostus lactone (**10**, 50 and 100 mg/kg) slightly suppressed serum TG elevation at 2 h after administration of olive oil, however, the effect was weaker than those of **4**–**6**. The effect of **7a** was also decreased at 50 and 100 mg/kg. These results suggest that the oxygen functional group and *exo*-methylene moiety in α-methylene-γ-butyrolactone ring are essential for the anti-hyperlipidemic activity of sesquiterpene.

Next, we examined the effects of the MeOH extract and sesquiterpene constituents (**4**–**6**) from the leaves of artichoke on gastric emptying (GE) in olive oil-loaded mice. The MeOH extract significantly suppressed GE from 125 to 500 mg/kg in a dose-dependent manner. The sesquiterpene constituents (**4**–**6**), which suppressed serum TG elevation, also significantly inhibited GE at the same doses (50 and 100 mg/kg). On the other hand, clofibrate did not affect GE even at a high dose (500 mg/kg) (Fig. 6). The MeOH extract and its constituents were found to show no or less activity on pancreatic lipase activity and fatty acid translocation in Caco-2 cells layer in vitro (data not shown). These results suggest that inhibitions of GE by the MeOH extract and constituents (**4**–**6**) are involved in the suppression of serum TG.

In conclusion, the MeOH extract from the leaves of artichoke was found to exhibit anti-hyperlipidemic activity based on suppression of GE. Cyanopicrin (**4**), aguerin B (**5**), and grosheimin (**6**) were isolated as anti-hyperlipidemic compounds and the oxygen functional groups at the 3- and 8-positions and *exo*-methylene moiety in α-methylene-γ-butyrolactone ring were essential for the activity of guaiane-type sesquiterpene.

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14. The signals of the ^{1}H and ^{13}C NMR spectra were assigned on the basis of homo- and hetero-correlation spectroscopy (^{1}H–^{1}H, ^{1}H–^{13}C COSY), and heteronuclear multiple bond connectivity (HMBC) experiments.
15. Crystal data for **1**: $C_{21}H_{46}O_9$, crystal dimensions: 0.13 × 0.02 × 0.20 mm; crystal system: monoclinic; lattice type: primitive; lattice parameters: $a=10.8738(4)$ Å, $b=6.7528(5)$ Å, $c=14.3985(5)$ Å, $\beta=105.994\ (3)^\circ$ $V=1016.33(9)$ Å; space group: $P2_1$ (#4); Z value: 2; D_{calcd}: 1.400 g/cm^3; F_{000}: 460.00; $\mu(CuK_\alpha)$: 9.17 cm^{-1}; diffractometer: Rigaku AFC7R (rotating anode); radiation: CuK_α ($\lambda=1.54178$ Å) graphite monochromated; structure solution: TEXSAN (direct method: SAPI91); residuals: $R=0.096$, $RW=0.187$, $R1=0.061$; goodness of fit indicator: 1.76. The data have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication number CCDC 196743.
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19. Compound **7a** was derived from **7** by enzymatic hydrolysis with β-glucosidase in 0.2 M acetate buffer (pH 5.0). The yield of **7a** was 87%.