Pergamon

Bioorganic & Medicinal Chemistry 7 (1999) 323–327

BIOORGANIC & MEDICINAL CHEMISTRY

Structure-Related Inhibitory Activity of Oleanolic Acid Glycosides on Gastric Emptying in Mice

Hisashi Matsuda, Yuhao Li, Toshiyuki Murakami, Johji Yamahara and Masayuki Yoshikawa*

Kyoto Pharmaceutical University, 5 Nakauchi-cho, Misasagi, Yamashina-ku, Kyoto 607-8414, Japan

Received 10 August 1998; accepted 30 September 1998

Abstract—We examined the effects of various oleanolic acid oligoglycosides obtained from traditional herbs on gastric emptying in non-nutrient meal- or nutrient meal-loaded mice. Test samples were given orally to fasted mice 0.5 h before loading of test meals. Oleanolic acid 3-*O*-monodesmosides [oleanolic acid 3-*O*-glucuronide (**3**, 12.5–50 mg/kg), momordin Ic (**4**, 25 and 50 mg/kg), momordin I (**6**, 12.5–50 mg/kg), and 28-*O*-deglucosyl-chikusetsusaponins IV (**8**, 12.5–50 mg/kg) and V (**10**, 50 mg/kg)] were found to show inhibitory effects on gastric emptying in 1.5% CMC-Na test meal-loaded mice. **4**, **6**, and **8** also inhibited gastric emptying in mice given 40% glucose test meal, milk test meal, and 60% ethanol test meal. **3** inhibited gastric emptying in mice given milk test meal or 60% ethanol test meal, but lacked significant inhibition in 40% glucose test meal-loaded mice. **10** (50 mg/kg) also slightly inhibited gastric emptying in milk test meal-loaded mice, but lacked the significant inhibition in mice given 40% glucose or 60% ethanol test meal. Whereas oleanolic acid 3,28-*O*-bisdesmosides [momordin IIc (**5**), chikusetsusaponins IV (**7**) and V (**9**)], oleanolic acid 28-*O*-monodesmoside [compound O (**2**)], and their common aglycon [oleanolic acid (**1**)] showed no such effects at dose of 50 mg/kg. 28-*O*-Deglucosyl-chikusetsusaponin V (**10**) showed a little inhibition in these experiments. These results indicate that both the 3-*O*-monodesmoside structure and 28-carboxyl group were confirmed to be essential for such activity, and the 28-ester glucoside moiety and 2′-*O*-β-D-glucopyranoside moiety reduce the activity.

Introduction

Saponins and related glycosides from natural medicines have been reported to have a number of biological activities such as hemolysis, anti-inflammatory effects, anti-carcinoma, antibiotic, and adjuvant activity etc. However, there have been few studies concerning the effects of saponins on gastrointestinal functions. In the course of our studies on bioactive saponin constituents in natural medicines, we recently found that several saponins from several traditional medicinal herbs with antidiabetic effects showed inhibitory activity on the increased blood ethanol or glucose levels in oral ethanol- or glucose-loaded rats. By examination of the structural requirements for the activity, the active saponins could be classified into the following three types: (1) olean-12-en-28-oic acid 3-*O*-monodesmoside,[1] (2) acylated polyhydroxyolean-12-ene 3-*O*-monodesmoside,[2] and (3) olean-12-ene 3,28-*O*-acylated bisdesmoside.[3] Furthermore, investigation of the mode of action of their hypoglycemic activities revealed that they affected gastrointestinal function such as gastric emptying and glucose uptake at small intestinal brush borders,[4] but structure–activity relationships of their active saponins for inhibition of gastric emptying is not unclarified. In this paper, we describe the structural requirement of oleanolic acid glycosides, which are abundant in natural sources, on gastric emptying in non-nutrient meal- or nutrient meal-loaded mice.

Results and Discussion

The speed of gastric emptying is important in the regulation of glucose homeostasis.[5] Gastric emptying abnormalities are common in diabetic patients and animals.[6] It is reported that gastric emptying was faster in type II diabetic patients, type I diabetic patients, and diabetic rodents as compared to healthy controls.[7] Some studies have shown that obese subjects had accelerated gastric emptying as compared to healthy controls.[8] More rapid gastric emptying rates in patients with diabetes mellitus would result in more rapid absorption of food, and therefore higher postprandial glucose levels. Consequently, slowing the gastric emptying will prolong the postprandial absorption of food, with a resultant improvement in blood glucose control.

The effects of oleanolic acid (**1**) and its glycosides (**2–10**) on gastric emptying at 30 min after the loading of the test meal of 1.5% CMC-Na, 40% glucose, milk, and

*Corresponding author. Tel:. +81-75-595-4633; fax: +81-75-595-4768.

0968-0896/99/$ - see front matter
PII: S0968-0896(98)00207-7

60% ethanol in normal mice are summarized in Tables 1–3. The present results demonstrated that oleanolic acid 3-*O*-monodesmosides [oleanolic acid 3-*O*-glucuronide (**3**, 12.5–50 mg/kg), momordin Ic (**4**, 25 and 50 mg/kg), momordin I (**6**, 12.5–50 mg/kg), and 28-*O*-deglucosyl-chikusetsusaponins IV (**8**, 12.5–50 mg/kg)] strongly inhibited the gastric emptying in 1.5% CMC-Na test meal-loaded mice. Momordin I especially (**6**, 12.5 and 25 mg/kg) showed stronger inhibition than atropine sulfate monohydrate as a reference drug. **4, 6**, and **8** also inhibited gastric emptying in mice given the nutrient meals (40% glucose test meal and milk test meal) or 60% ethanol test meal. **3** inhibited gastric emptying in mice given milk and 60% ethanol test meal, but lacked significant inhibition in 40% glucose test meal-loaded mice. **10** (50 mg/kg) also slightly inhibited gastric emptying in 1.5% CMC-Na test meal- and milk test meal-loaded mice, but lacked the significant inhibition in mice given 40% glucose and 60% ethanol test meal. Whereas oleanolic acid 3,28-*O*-bisdesmosides [momordin IIc (**5**), chikusetsusaponins IV (**7**) and V (**9**)], oleanolic acid 28-*O*-monodesmoside [compound O (**2**)], and their common aglycon [oleanolic acid (**1**)] had no such effects. 28-*O*-Deglucosyl-chikusetsusaponins V (**10**), having the 2′-*O*-β-D-glucopyranosyl moiety, also show a little activity as compared to the other 3-*O*-monodesmosides. These active saponins also significantly increased the weight of the stomach in mice given each test meal. These results indicate the following structural requirements of oleanolic acid glycosides for the gastric emptying inhibitory activity: (1) the 3-*O*-glycoside moiety is essential for the activity; (2) the 2′-*O*-β-D-glucopyranosyl moiety of the glucuronic acid part reduces the activity; and (3) the 28-ester glucoside moiety markedly reduces the activity.

In this study, structural requirements of oleanolic acid glycosides for the inhibition of gastric emptying are similar to the structural requirements for inhibition of increased blood ethanol or glucose levels in ethanol- or glucose-loaded rats.[1] Therefore, inhibitory effects of these saponins on gastric emptying seemed to be important to

Table 1. Effects of oleanolic acid (**1**) and its glycosides (**2–10**) on gastric emptying in non-nutrient meal-loaded mice

Treatment	Dose (mg/kg)	N	1.5% CMC-Na test meal		
			Weight of stomach (g)	Gastric emptying (%)	Inhibition (%)
Control	–	10	0.39 ± 0.02	91.3 ± 1.2	–
Oleanolic acid (**1**)	50	8	0.40 ± 0.01	90.2 ± 0.8	1.2
Compound O (**2**)	50	8	0.42 ± 0.02	89.3 ± 0.8	2.2
Momordin IIc (**5**)	50	8	0.41 ± 0.02	87.3 ± 1.4	4.4
Chikusetsusaponin IV (**7**)	50	8	0.39 ± 0.01	88.9 ± 1.6	2.6
Chikusetsusaponin V (**9**)	50	8	0.41 ± 0.02	90.4 ± 1.3	1.0
Control	–	10	0.40 ± 0.01	89.9 ± 1.0	–
Oleanolic acid	5	8	0.44 ± 0.01	87.2 ± 1.3	3.0
3-*O*-glucuronide (**3**)	12.5	8	0.51 ± 0.03	78.2 ± 2.8*	13.0
	25	8	0.67 ± 0.06**	54.4 ± 4.3**	39.5
	50	8	0.96 ± 0.06**	38.3 ± 2.8**	57.4
Momordin Ic (**4**)	5	8	0.43 ± 0.02	91.8 ± 0.9	–2.1
	12.5	8	0.47 ± 0.01	82.1 ± 1.2	8.7
	25	8	0.54 ± 0.03*	68.9 ± 2.3**	23.4
	50	8	1.11 ± 0.05**	33.1 ± 4.6**	63.2
Control	–	10	0.41 ± 0.02	91.0 ± 0.8	–
Momordin I (**6**)	5	8	0.45 ± 0.02	88.6 ± 1.1	2.6
	12.5	8	0.66 ± 0.03**	65.0 ± 1.3**	28.6
	25	8	0.91 ± 0.05**	44.2 ± 2.6**	51.4
	50	8	1.01 ± 0.07**	11.7 ± 3.8**	87.1
Control	–	10	0.40 ± 0.02	91.6 ± 1.0	–
28-*O*-Deglucosyl-	5	8	0.41 ± 0.02	89.0 ± 1.4	2.8
chikusetsusaponin IV (**8**)	12.5	8	0.54 ± 0.03*	76.9 ± 2.5**	16.0
	25	8	0.67 ± 0.06**	70.3 ± 3.6**	23.3
	50	8	0.93 ± 0.03**	31.8 ± 3.6**	65.3
28-*O*-Deglucosyl-	25	8	0.48 ± 0.03	83.7 ± 1.2	8.6
chikusetsusaponin V (**10**)	50	8	0.53 ± 0.04*	73.3 ± 2.8**	20.0
Control	–	8	0.45 ± 0.02	89.4 ± 1.2	–
Atropine sulfate	5	8	0.49 ± 0.02	78.7 ± 1.0**	12.0
monohydrate	12.5	8	0.54 ± 0.03*	73.1 ± 3.0**	18.2
	25	8	0.62 ± 0.02**	67.7 ± 1.2**	24.3

Values represent the means ± SEM. Significantly different from the control group, *$p < 0.05$, **$p < 0.01$.

exhibit their ethanol absorption inhibitory and hypoglycemic effects as previously reported.[4(b)] In addition, we also reported their antipruritogenic effects in mice and gastromucosal protective effects in rats and obtained similar structural requirements.[9] In these points, further experiments are necessary to gain insight to the action mechanisms of these effects of saponins.

In conclusion, this study demonstrated that oleanolic acid 3-*O*-monodesmosides, abundant in natural sources, had an inhibitory effect on gastric emptying, and this effect may be good for the prevention and treatment of diabetes and morbid obesity with accelerated gastric emptying.

Experimental

Materials

Oleanolic acid 3-*O*-glucuronide (**3**) and momordins Ic (**4**), IIc (**5**), and I (**6**) were isolated from the fruit of *Kochia scoparia* (L.) SCHRAD.,[1(h)] or the seeds of *Momordica cochinchinesis* SPRENG.[10] Chikusetsusaponins IV (**7**)

Table 2. Effects of oleanolic acid (**1**) and its glycosides (**2–10**) on gastric emptying in nutrient meal-loaded mice

Treatment	Dose (mg/kg)	N	40% Glucose test meal			Milk test meal		
			Weight of stomach (g)	Gastric emptying (%)	Inhibition (%)	Weight of stomach (g)	Gastric emptying (%)	Inhibition (%)
Control	–	10	0.71 ± 0.03	64.0 ± 1.6	–	0.49 ± 0.02	71.2 ± 1.8	–
Oleanolic acid (**1**)	50	8	0.75 ± 0.03	64.7 ± 1.9	–1.1	0.53 ± 0.03	69.0 ± 2.4	3.1
Compound O (**2**)	50	8	0.80 ± 0.04	57.2 ± 1.8	10.6	0.50 ± 0.02	66.7 ± 0.9	6.3
Momordin IIc (**5**)	50	8	0.81 ± 0.03	57.0 ± 1.7	10.9	0.52 ± 0.02	68.2 ± 1.0	4.2
7	50	8	0.76 ± 0.05	58.2 ± 1.6	9.1	0.49 ± 0.02	70.1 ± 0.9	1.5
9	50	8	0.76 ± 0.02	59.5 ± 1.9	7.0	0.51 ± 0.02	71.5 ± 1.4	–0.4
Control	–	10	0.79 ± 0.02	62.1 ± 2.0	–	0.48 ± 0.02	71.1 ± 1.9	–
Oleanolic acid 3-	12.5	8	-	-		0.58 ± 0.01**	61.1 ± 1.4*	14.1
O-glucuronide (**3**)	25	8	0.81 ± 0.03	59.3 ± 1.4	4.5	0.62 ± 0.04**	58.7 ± 3.3**	17.4
	50	8	0.91 ± 0.05	55.6 ± 2.2	10.5	1.15 ± 0.04**	30.2 ± 2.4**	57.5
Momordin Ic (**4**)	12.5	8	0.75 ± 0.03	64.7 ± 1.6	–4.2	0.56 ± 0.03*	62.1 ± 3.3*	12.7
	25	8	1.00 ± 0.05**	53.3 ± 2.1	14.2	0.84 ± 0.04**	55.3 ± 2.2*	22.2
	50	8	1.10 ± 0.05**	40.9 ± 3.7**	34.1	1.10 ± 0.03**	42.7 ± 2.7**	39.9
Momordin I (**6**)	12.5	8	0.91 ± 0.04	58.3 ± 2.2	6.1	0.49 ± 0.02	68.2 ± 2.8	4.1
	25	8	1.15 ± 0.03**	43.6 ± 3.1**	29.8	0.82 ± 0.06**	47.4 ± 2.4**	33.3
	50	8	1.15 ± 0.03**	33.7 ± 2.7**	45.7	1.03 ± 0.05**	40.2 ± 2.7**	43.5
Control	–	10	0.71 ± 0.03	64.0 ± 1.6	–	0.50 ± 0.02	67.4 ± 1.9	–
8	12.5	8	0.86 ± 0.03*	62.3 ± 1.3	2.7	0.66 ± 0.03**	55.9 ± 3.0**	17.1
	25	8	1.03 ± 0.05**	48.6 ± 2.6**	24.1	0.96 ± 0.04**	46.0 ± 1.5**	31.8
	50	8	1.10 ± 0.03**	47.2 ± 3.4**	26.3	1.03 ± 0.04**	40.1 ± 1.9**	40.5
10	25	8	-	-		0.53 ± 0.02	68.2 ± 2.0	–1.2
	50	8	0.86 ± 0.04*	57.2 ± 1.5	10.6	0.72 ± 0.04**	59.0 ± 2.1*	12.5

Values represent the means ± SEM. Significantly different from the control group, *$p < 0.05$, **$p < 0.01$.

Table 3. Effects of oleanolic acid (**1**) and its glycosides (**2–10**) on gastric emptying in 60% ethanol test meal-loaded mice

Treatment	Dose (mg/kg)	N	Weight of stomach (g)	Gastric emptying (%)	Inhibition (%)
Control	–	10	0.81 ± 0.03	58.4 ± 1.8	–
Oleanolic acid (**1**)	50	8	0.81 ± 0.04	65.1 ± 2.4	–11.5
Compound O (**2**)	50	8	0.89 ± 0.03	60.3 ± 1.5	–3.3
Momordin IIc (**5**)	50	8	0.84 ± 0.02	62.9 ± 1.9	–7.7
Chikusetsusaponin IV (**7**)	50	8	0.82 ± 0.04	65.6 ± 3.5	–12.3
Chikusetsusaponin V (**9**)	50	8	0.82 ± 0.03	64.4 ± 2.7	–10.3
Control	–	10	0.77 ± 0.02	56.4 ± 2.1	–
Oleanolic acid 3-*O*-glucuronide (**3**)	50	8	1.16 ± 0.04**	35.4 ± 1.2**	37.2
Momordin Ic (**4**)	50	8	1.04 ± 0.05**	35.3 ± 3.5**	37.4
Momordin I (**6**)	50	8	1.19 ± 0.02**	34.7 ± 2.2**	38.5
28-*O*-Deglucosyl-chikusetsusaponin IV (**8**)	50	8	1.13 ± 0.04**	35.2 ± 2.5**	37.6
28-O-Deglucosyl-chikusetsusaponin V (**10**)	50	8	0.76 ± 0.04	66.5 ± 2.3*	–17.9

Values represent the means ± SEM. Significantly different from the control group, *$p < 0.05$, **$p < 0.01$.

and V (**9**) were isolated from the rhizome of *Panax japonicus* C. A. MEYER, and 28-*O*-deglucosyl-chikusetsusaponins IV (**8**) and V (**10**) were obtained by alkaline hydrolysis of **7** and **9**.[11] Compound O (**2**) was obtained by enzymatic hydrolysis of **9**.[4(b)] Other reagents were purchased from Wako Pure Chemical Industries, Japan.

Animals

Male ddY mice, weighing 27–30 g, were purchased from Kiwa Laboratory Animal Co., Ltd., Japan. The animals were maintained at a constant temperature of 23 ± 2 °C and were fed a standard laboratory chow (MF, Oriental Yeast Co., Ltd., Japan) for a week. The animals were fasted for 18–20 h prior to experiments, but were supplied with water ad libitum. Each test sample was suspended in 5% acacia/phosphate buffered saline solution, and the solution was orally administered at 10 mL/kg in each experiment, while the vehicle was administered orally at 10 mL/kg in the corresponding control group.

Measurement of gastric emptying

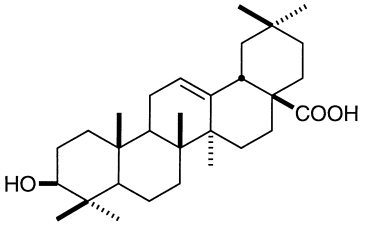
Gastric emptying was determined by a modification of the phenol red method.[12] A solution of 1.5% carboxymethyl cellulose sodium salt (CMC-Na), 40% glucose, milk [milk powder : water (w/w) = 1:3], or 60% ethanol containing 0.05% phenol red as a marker was given intragastrically (0.5 mL/mouse) to conscious mice. Thirty minutes later, mice were sacrificed by cervical dislocation. The abdominal cavity was opened, and the gastroesophageal junction and the pylorus were clamped, then the stomach was removed, weighed and placed in 14 mL of 0.1 N NaOH and homogenized. The suspension was allowed to settle for 1 h at room temperature and 5 mL of the supernatant was added to 0.5 mL of 20% trichloroacetic acid (w/v), and then centrifuged at 3000 rpm for 20 min. The supernatant was mixed with 4 mL of 0.5 N NaOH, and the amount of phenol red was determined from the absorbance at 560 nm (Beckman, DU530). Phenol red recovered from animals sacrificed immediately after the administration of the test meal was used as standards (0% emptying). Gastric emptying (%) in the 30 min period was calculated according to the following equation:

$$\text{gastric emptying}(\%) = (1 - \text{amount of test sample}/\text{amount of standard}) \times 100$$

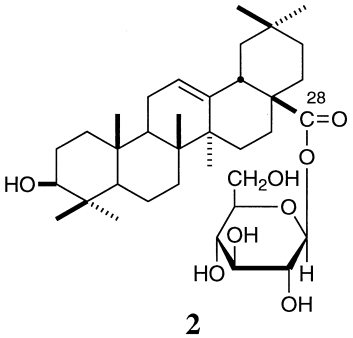
The test sample was given orally by means of a metal orogastric tube 30 min prior to the administration of the test meals.

Statistics

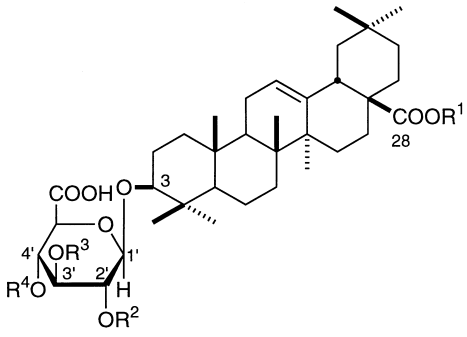
Values were expressed as means ± SEM. One-way analysis of variance following Dunnett's test for multiple comparison analysis were used for statistical analysis. Probability (p) values less than 0.05 were considered significant.



1



2



	R^1	R^2	R^3	R^4
3 :	H	H	H	H
4 :	H	H	Xyl	H
5 :	Glc	H	Xyl	H
6 :	H	H	Ara(p)	H
7 :	Glc	H	H	Ara(f)
8 :	H	H	H	Ara(f)
9 :	Glc	Glc	H	H
10 :	H	Glc	H	H

Glc : β-D-glucopyranosyl
Xyl : β-D-xylopyranosyl
Ara(p) : α-L-arabinopyranosyl
Ara(f) : α-L-arabinofuranosyl

Figure 1. Chemical structures of oleanolic acid (**1**) and its glycosides (**2–9**).

References

1. (a) Yoshikawa, M.; Harada, E.; Matsuda, H.; Murakami, T.; Yamahara, J.; Murakami, N. *Chem. Pharm. Bull.* **1993**, *41*, 2069; (b) Yoshikawa, M.; Matsuda, H.; Harada, E.; Murakami, T.; Wariishi, N.; Yamahara, J.; Murakami, N. *Chem. Pharm. Bull.* **1994**, *42*, 1354; (c) Yoshikawa, M.; Yoshizumi, S.; Ueno, T.; Matsuda, H.; Murakami, T.; Yamahara, J.; Murakami, N. *Chem. Pharm. Bull.* **1995**, *43*, 1878; (d) Yoshikawa, M.; Murakami, T.; Kadoya, M.; Matsuda, H.; Yamahara, J.; Muraoka, O.; Murakami, N. *Heterocycles* **1995**, *41*, 1621; (e) Yoshikawa, M.; Murakami, T.; Kadoya, M.; Matsuda, H.; Muraoka, O.; Yamahara, J.; Murakami, N. *Chem. Pharm. Bull.* **1996**, *44*, 1212; (f) Yoshikawa, M.; Murakami, T.; Harada, E.; Murakami, N.; Yamahara, J.; Matsuda, H. *Chem. Pharm. Bull.* **1996**, *44*, 1915; (g) Yoshikawa, M.; Murakami, T.; Harada, E.; Murakami, N.; Yamahara, J.; Matsuda, H. *Chem. Pharm. Bull.* **1996**, *44*, 1923; (h) Yoshikawa, M.; Shimada, H.; Morikawa, T.; Yoshizumi, S.; Matsumura, N.; Murakami, T.; Matsuda, H.; Hori, K.; Yamahara, J. *Chem. Pharm. Bull.* **1997**, *45*, 1300.

2. (a) Yoshikawa, M.; Harada, E.; Murakami, T.; Matsuda, H.; Yamahara, J.; Murakami, N. *Chem. Pharm. Bull.* **1994**, *42*, 742; (b) Yoshikawa, M.; Harada, E.; Murakami, T.; Matsuda, H.; Wariishi, N.; Yamahara, J.; Murakami, N.; Kitagawa, I. *Chem. Pharm. Bull.* **1994**, *42*, 1357; (c) Yoshikawa, M.; Murakami, T.; Matsuda, H.; Yamahara, J.; Murakami, N.; Kitagawa, I. *Chem. Pharm. Bull.* **1996**, *44*, 1454; (d) Yoshikawa, M.; Murakami, T.; Yoshizumi, S.; Murakami, N.; Yamahara, J.; Matsuda, H. *Chem. Pharm. Bull.* **1996**, *44*, 1899.

3. (a) Yoshikawa, M.; Murakami, T.; Ueno, T.; Kadoya, M.; Matsuda, H.; Yamahara, J.; Murakami, N. *Chem. Pharm. Bull.,* **1995**, *43*, 350; (b) Yoshikawa, M.; Murakami, T.; Ueno, T.; Kadoya, M.; Matsuda, H.; Yamahara, J.; Murakami, N. *Chem. Pharm. Bull.* **1995**, *43*, 2115; (c) Yoshikawa, M.; Murakami, T.; Matsuda, H.; Ueno, T.; Kadoya, M.; Yamahara, J.; Murakami, N. *Chem. Pharm. Bull.* **1996**, *44*, 1305.

4. (a) Matsuda, H.; Murakami, T.; Li, Y.; Yamahara, J.; Yoshikawa, M. *Bioorg. Med. Chem.* **1998**, *6*, 1019; (b) Matsuda, H.; Li, Y.; Murakami, T.; Matsumura, N.; Yamahara, J.; Yoshikawa, M. *Chem. Pharm. Bull.* **1998**, *46*, 1339.

5. Horowitz, M.; Edelbroek, M. A. L.; Wishart, J. M.; Straathof, J. W. *Diabetologia* **1993**, *36*, 857.

6. Kong, M. F.; Macdonald, I. A.; Tattersall, R. B. *Diabet. Med.* **1996**, *13*, 112.

7. (a) Nowak, T. V.; Johnson, C. P.; Wood, C. M.; Adams, M. B.; Roza, A. M.; Kalbfleish, J. H.; Palmer, D. W.; Soergel K. H. *Gastroenterology* **1990**, *98*, A378; (b) Phillips, W. T.; Schwarz, J. G.; McMahan, C. A. *J. Nucl. Med.* **1992**, *33*, 1496; (c) Nowak, T. V.; Roza, A. M.; Weisbruch, J. P.; Brosnan, M. R. *J. Lab. Clin. Med.* **1994**, *123*, 110; (d) Chang, F. Y.; Lee, S. D.; Yeh, G. H.; Wang, P. S. *Dig. Dis. Sci.* **1996**, *41*, 528; (e) Green, G. M.; Guan, D.; Schwartz, J. G.; Phillips, W. T. *Diabetologia* **1997**, *40*, 136.

8. (a) Pehling, G.; Tessari, P.; Gerich, J. E.; Haymond, M. W.; Service, F. J.; Rizza, R. A. *J. Clin. Invest.* **1984**, *74*, 985; (b) Tosetti, C.; Corinaldesi, R.; Stanghellini, V.; Pasquali, R.; Corbelli, C.; Zoccoli, G.; Di Febo, G.; Monetti, N.; Barbara, L. *Int. J. Obes. Relat. Metab. Disord.* **1996**, *20*, 200.

9. (a) Matsuda, H.; Dai, Y.; Ido, Y.; Murakami, T.; Matsuda, H.; Yoshikawa, M.; Kubo, M.; *Biol. Pharm. Bull.* **1998**, *21*, 1231; (b) Matsuda, H.; Li, Y.; Murakami, T.; Yamahara, J.; Yoshikawa, M. *Life Sci.* **1998**, *63*, PL245.

10. (a) Iwamoto, M.; Okabe, H.; Yamauch, T. *Chem. Pharm. Bull.* **1985**, *33*, 1; (b) Yoshikawa, M.; Mutakami, T.; Nakano, K.; Matsuda, H. *Chem. Pharm. Bull.,* to be published.

11. (a) Kondo, N.; Shoji, J. *Yakugaku Zasshi* **1968**, *88*, 325; (b) Kondo, N.; Shoji, J.; Nagumo, N.; Komatsu, N. *Yakugaku Zasshi* **1969**, *89*, 846; (c) Lin, T. D.; Kondo, N.; Shoji, J. *Chem. Pharm. Bull.* **1976**, *24*, 253.

12. (a) Taché, Y.; Maeda, M.; Hagiwara, M.; Turkelson, C. M. *Am. J. Physiol.* **1987**, *253*, G241; (b) Barquist, E.; Bonaz, B.; Martinez, V.; Rivier, J.; Ziner, M. J.; Taché, Y. *Am. J. Physiol.* **1996**, *270*, R888.