CAMELLIASAPONINS B_1, B_2, C_1 AND C_2, NEW TYPE INHIBITORS OF ETHANOL ABSORPTION IN RATS FROM THE SEEDS OF *CAMELLIA JAPONICA* L.

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New type inhibitors of ethanol absorption, camelliasaponins B_1, B_2, C_1 and C_2, were isolated from the seeds of *Camellia japonica* L. The structures of camelliasaponins were elucidated on the basis of chemical and physicochemical evidence. The inhibitory effect of camelliasaponins and related saponins on ethanol absorption have been examined, and it was found that the triterpene oligoglycoside structure having an acyl group was essential to exerting the activity.

KEYWORDS *Camellia japonica* ; Theaceae; ethanol absorption inhibitor; acylated oleanene-type triterpene monodesmoside; camelliasaponin B_1; camelliasaponin C_1

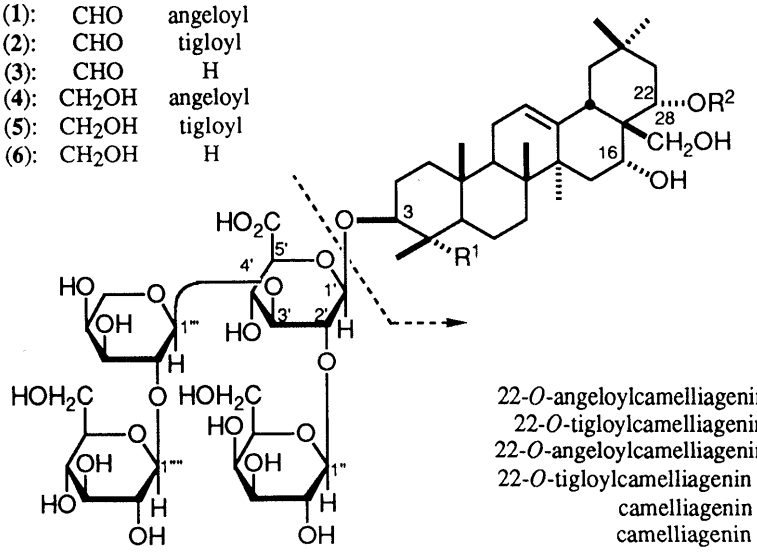
Excessive consumption of ethanol is known to affect profoundly nearly every organ in the body, particularly the endo-crine system, heart, central nervous system, immune system, and liver. In order to relieve ethanol toxicity in acute alcohol ingestion, several methods using the accelerator of ethanol metabolism and sequestrator of acetoaldehyde were hitherto reported. Recently, we have found that elatosides A and B, isolated from the bark of *Aralia elata* SEEM., showed potent inhibitory effect on ethanol absorption and, by examination of the structure-activity relationship, oleanolic acid 3-*O*-glucuronide structure was essential to the activity.[1)]

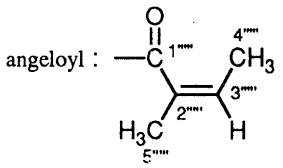
As a continuing part of our screening to find an inhibitor of ethanol absorption in crude drugs, new type inhibitors named camelliasaponins B_1, B_2, C_1 and C_2 were isolated from the seeds of *Camellia japonica* L.(Theaceae). This communication deals with the structure elucidation of camelliasaponins B_1(**1**), B_2(**2**), C_1(**4**), and C_2(**5**) and their inhibitory effects on ethanol absorption.

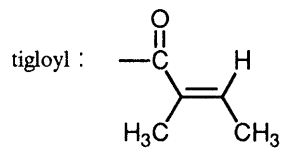
The seeds of *Camellia japonica* L. (Tsubaki in Japanese) have been used in Chinese traditional medicine as a stomachic and antiinflammatory and also as oil material. In regard to the chemical constituents of the seeds, the triterpenes, which were obtained by alkaline hydrolysis following by acid hydrolysis of the glycoside mixture, have been investigated.[2)] We have found that the MeOH extract of the defatted seeds of *Camellia japonica* L. has an inhibitory effect on the ethanol absorption in rats. The MeOH extract of the seeds was subjected to the reversed phase SiO_2 column and HPLC to afford camelliasaponins B_1(**1**, 0.29 % from the defatted seeds), B_2(**2**, 0.89 %), C_1(**4**, 0.10%), and C_2(**5**, 0.10%).

Alkaline hydrolysis (KOH / aq.dioxane) of camelliasaponin B_1(**1**)[3)] furnished angelic acid[4)] and desacyl-camelliasaponin

	R^1	R^2
camelliasaponin B_1 (**1**):	CHO	angeloyl
camelliasaponin B_2 (**2**):	CHO	tigloyl
desacyl-camelliasaponin B (**3**):	CHO	H
camelliasaponin C_1 (**4**):	CH_2OH	angeloyl
camelliasaponin C_2 (**5**):	CH_2OH	tigloyl
desacyl-camelliasaponin C (**6**):	CH_2OH	H







	R^1	R^2
22-*O*-angeloylcamelliagenin B (**7**):	CHO	angeloyl
22-*O*-tigloylcamelliagenin B (**8**):	CHO	tigloyl
22-*O*-angeloylcamelliagenin C (**9**):	CH_2OH	angeloyl
22-*O*-tigloylcamelliagenin C (**10**):	CH_2OH	tigloyl
camelliagenin B (**11**):	CHO	H
camelliagenin C (**12**):	CH_2OH	H

Table I. ^{13}C NMR Data for **1**, **2**, **3**, **4**, **5**, and **6** (68 MHz, d_5-Pyridine, δc)

	1	2	3	4	5	6
Aglycone moiety						
C-1	38.3	38.3	38.3	38.8	38.8	38.8
C-2	25.2	25.2	25.2	25.5	25.6	25.6
C-3	84.2	84.2	84.2	83.1	83.1	83.1
C-4	55.1	55.1	55.1	43.6	43.5	43.5
C-5	48.5	48.5	48.4	48.3	48.3	48.3
C-6	20.4	20.4	20.4	18.2	18.2	18.2
C-7	32.5	32.5	32.4	32.9	32.9	32.9
C-8	40.4	40.4	40.3	40.2	40.2	40.2
C-9	46.9	46.9	46.9	47.1	47.1	47.1
C-10	36.1	36.1	36.1	36.8	36.8	36.8
C-11	23.8	23.8	23.8	23.9	23.9	23.9
C-12	122.4	123.3	122.3	122.7	122.7	122.7
C-13	143.8	143.9	144.3	143.8	143.8	144.3
C-14	41.7	41.7	42.2	41.7	41.9	42.2
C-15	35.1	35.1	34.6	35.2	35.2	34.7
C-16	70.2	70.0	68.4	70.2	70.1	68.6
C-17	44.9	45.1	44.8	44.9	45.1	44.8
C-18	41.1	41.0	42.5	41.0	41.0	42.6
C-19	47.5	47.5	47.8	47.5	47.5	47.9
C-20	32.1	32.5	31.8	32.0	32.0	31.8
C-21	41.8	41.9	45.9	41.7	41.8	46.0
C-22	73.1	73.2	74.3	73.2	73.2	74.4
C-23	209.7	209.7	209.7	64.9	64.9	64.9
C-24	11.0	11.1	11.0	13.5	13.6	13.5
C-25	15.8	15.8	15.8	16.2	16.2	16.2
C-26	16.9	16.9	16.8	17.0	17.0	17.0
C-27	27.6	27.5	27.4	27.6	27.6	27.5
C-28	63.9	63.8	70.1	63.9	63.9	70.3
C-29	33.5	33.5	33.7	33.4	33.5	33.7
C-30	25.2	25.7	25.4	25.2	25.2	25.5

Sugar moiety		1	2	3	4	5	6
3-O-β-D-Glucurono-pyranosyl-moiety	C-1'	104.1	104.1	103.9	104.1	104.2	104.1
	C-2'	78.5	78.5	78.4	78.7	78.7	78.7
	C-3'	84.5	84.5	84.5	85.1	85.1	85.1
	C-4'	71.0	71.0	70.9	71.2	71.2	71.2
	C-5'	77.3	77.2	77.2	77.2	77.3	77.2
	C-6'	171.8	171.8	171.8	171.8	172.0	171.9
2'-O-β-D-Galacto-pyranosyl-moiety	C-1"	103.6	103.6	103.5	103.5	103.5	103.5
	C-2"	73.7	73.7	73.7	73.8	73.8	73.8
	C-3"	75.2	75.2	75.1	75.1	75.1	75.1
	C-4"	70.3	70.3	70.2	69.9	70.1	69.9
	C-5"	76.6	76.6	76.5	76.5	76.6	76.5
	C-6"	62.2	62.2	62.1	61.9	62.0	61.9
3'-O-α-L-Arabino-pyranosyl-moiety	C-1'''	101.7	101.7	101.6	101.8	101.8	101.8
	C-2'''	81.3	81.3	81.3	81.2	81.2	81.2
	C-3'''	72.4	72.4	72.4	72.4	72.5	72.4
	C-4'''	67.6	67.6	67.6	67.6	67.6	67.6
	C-5'''	64.9	64.9	64.9	64.9	64.9	64.9
2'''-O-β-D-Gluco-pyranosyl-moiety	C-1''''	106.0	106.1	105.9	105.9	105.9	105.9
	C-2''''	75.9	75.9	75.8	75.8	75.8	75.8
	C-3''''	78.4	78.4	78.4	78.4	78.4	78.4
	C-4''''	71.6	71.6	71.6	71.6	71.6	71.6
	C-5''''	78.4	78.4	78.4	78.4	78.4	78.4
	C-6''''	62.7	62.7	62.7	62.7	62.7	62.7
Acyl moiety							
	C-1'''''	168.0	167.9		168.0	168.0	
	C-2'''''	129.6	130.1		129.6	130.1	
	C-3'''''	136.4	136.2		136.4	136.3	
	C-4'''''	15.8	14.0		15.8	14.1	
	C-5'''''	20.9	12.3		20.9	12.3	

Table II. Inhibitory Effects of Camelliasaponins B_1(**1**), B_2(**2**), C_1(**4**), C_2(**5**), Desacyl-camelliasaponins B(**3**), and C(**6**) from the Seeds of *Camellia japonica* L. on Ethanol Absorption

	Dose (mg /kg, p.o.)	n	Ethanol concentration in blood (mg / ml) 1h	2h	3h
Camelliasaponin B_1(**1**)	100	6	0.10±0.06**	0.09±0.03**	0.03±0.00
Camelliasaponin B_2(**2**)	100	5	0.43±0.05*	0.22±0.01	0.04±0.01
Desacyl-camellia-saponin B(**3**)	100	5	0.58±0.02	0.27±0.02	0.03±0.00
Camelliasaponin C_1(**4**)	100	7	0.35±0.05**	0.19±0.03	0.02±0.00
Camelliasaponin C_2(**5**)	100	8	0.32±0.08*	0.12±0.03	0.04±0.01
Desacyl-camellia-saponin C(**6**)	100	5	0.58±0.01	0.24±0.01	0.03±0.01
Control		10	0.57±0.01	0.17±0.02	0.03±0.00

* $p<0.05$, ** $p<0.01$

B(**3**)[5], which liberated methyl D-glucuronide, methyl D-galactoside, methyl L-arabinoside and methyl D-glucoside in a 1:1:1:1 ratio (GLC, HPLC) and camelliagenin B(**11**) upon methanolysis. The ^1H NMR(d_5-pyridine, J in Hz) and ^{13}C NMR(Table II) data were assigned by COSY (^1H-^1H, ^1H-^{13}C), HMBC, and HOHAHA (^1H-^1H, ^1H-^{13}C). HMBC correlations were observed between the following carbons and protons in the oligosaccharide moieties of **1** and **3**: 3-C & 1'-H, 2'-C & 1"-H, 3'-C & 1"'-H, 2"'-C & 1""-H. These findings together with ROESY data for **3** led us to formulate the oligosaccharide structure of **1**. Upon the enzymatic hydrolysis with glycyrrhizinic acid hydrolase,[6] **1** provided the genuine sapogenol, 22-*O*-angeloylcamelliagenin B(**7**),[7] quantitatively. Based on ^{13}C NMR comparisons for **1**, **3**, **7**, and **11** and observation of the HMBC correlation between 1""-C and 22-H in **7**, the structure of camelliasaponin B_1 has been determined as **1**.

Camelliasaponin C_1(**4**)[8] gave desacyl-camelliasaponin C(**6**)[9] and angelic acid[4] by alkaline hydrolysis, while enzymatic hydrolysis of **4** furnished 22-*O*-angeloylcamelliagenin C(**9**).[10] Selective reduction of 23-aldehyde moiety in camelliasaponin B_1(**1**) with $NaBH_4$ in MeOH at 0°C yielded camelliasaponin C_1(**4**) quantitatively. Finally, comparisons of ^1H NMR and ^{13}C NMR data for **4** with those for **1**, **6**, **9** and camelliagenin C(**12**) have corroborated the structure of camelliasaponin C_1 (**4**) as shown.

The structures of camelliasaponin B_2(**2**)[11] and C_2(**5**)[12] were elucidated in the same way. By alkaline hydrolysis, **2** yielded tiglic acid[4] and **3**, while **5** furnished tiglic acid[4] and **6**. Enzymatic hydrolysis of **2** and **5** furnished 22-*O*-

tigloylcamelliagenin B(**8**)[13] and C(**10**),[14] respectively. Furthermore, reduction of **2** with $NaBH_4$ yielded **5** quantitatively. Detailed comparisons of ^1H NMR and ^{13}C NMR data for **2** and **5** with those for **1**, **4**, **8**, and **10** led us to furnish the structures of camelliasaponins B_2(**2**) and C_2(**5**).

Inhibitory effect of camelliasaponins B_1(**1**) and B_2(**2**), C_1(**4**), C_2(**5**), and desacylcamelliasaponins B(**3**) and C(**6**) on ethanol absorption in rats were summarized in Table II. Among the compounds tested, camelliasaponins (**1**, **2**, **4** and **5**) were found to exhibit inhibitory effect on ethanol absorption; camelliasaponinB_1(**1**) showed the highest activity. On the other hand, **3** and **6** exhibited little inhibitory effect, indicating that the acyl moiety in oligoglycoside structure was specific to the inhibitory effect of ethanol absorption.

REFERENCES AND NOTES

1) M. Yoshikawa, E. Harada, H. Matsuda, T. Murakami, J. Yamahara, N. Murakami, *Chem. Pharm. Bull.*, **41**, 2069 (1993). 2) H. Itokawa, N. Sawada, T. Murakami, *Chem. Pharm. Bull.*, **17**, 474 (1969). 3) Camelliasaponin B_1(**1**) : mp 209.6-211.1°C, $[\alpha]_D$ +23.7° (MeOH), $C_{58}H_{90}O_{26}$, IR(KBr, cm^{-1}) : 3430, 1735, 1719, 1660, 1650, 1635, 1078, ^1H NMR : δ 9.87(s, 23-H), 6.15(dd, J = 5.6, 12.5, 22-H), 5.94(m, 3""'-H), 5.76(d, J = 5.9, 1"'-H), 5.62(d, J = 7.6, 1"-H), 5.19(d, J = 6.3, 1""-H), 4.85(d, J = 7.2, 1'-H), 4.59(br s, 16-H), 2.08(3H, d, J = 7.0, 4""'-H_3), 1.96(3H, s, 5""'-H_3), positive FAB-MS(m/z): 1225.6 $(M+Na)^+$. 4) a) Acyl group was identified by HPLC as *p*-nitrobenzyl ester derivative; b)K. Yoshikawa, M. Nakagawa, R. Yamamoto, S. Arihara, K. Matsuura, *Chem. Pharm. Bull.*, **40**, 1779(1992). 5) Desacyl-camelliasaponin B(**3**) : mp 235.3-237.1°C, $[\alpha]_D$ +18.4° (MeOH), $C_{53}H_{84}O_{25}$, IR(KBr, cm^{-1}) : 3425, 1735, 1719, 1638, 1078, ^1H NMR : δ 9.85(s, 23-H), 5.84(d, J = 6.0, 1"'-H), 5.59(d, J = 7.6, 1"-H), 5.07(2H, br s, 16-H, 1""-H), 4.84(d, J = 6.9, 1'-H), 4.59(dd, J = 6.0, 12.2, 22-H), negative FAB-MS(m/z) : 1119.5$(M-H)^-$. 6) Y. Sasaki, T. Morita, T. Kuramoto, K. Mizutani, R. Ikeda, O. Tanaka, *Agric. Biol. Chem.*, **52**, 207(1988). 7) 22-*O*-angeloylcamelliagenin B(**7**) : mp 172.1-174.0°C, $[\alpha]_D$ +45.0°(MeOH), $C_{35}H_{54}O_6$, IR(KBr, cm^{-1}) : 3477, 1725, 1674, 1647, ^1H NMR : δ 9.61(s, 23-H), 6.18(dd, J = 5.6, 12.2, 22-H), 5.93(m, 3""'-H), 4.62(br s, 16-H), 3.69, 3.55(ABq, J = 10.2, 28-H_2), 2.09(3H, d, J = 6.9, 5""'-H_3), 1.96(3H, s, 4""'-H_3), ^{13}C NMR : δ_C71.8(3-C), 122.4(12-C), 143.9(13-C), 70.2(16-C), 42.0(21-C), 73.1(22-C), 207.2(23-C), 63.9(28-C), positive FAB-MS(m/z) : 571$(M+H)^+$. 8) Camelliasaponin C_1(**4**) : mp 165.8-167.2°C, $[\alpha]_D$ +4.3°(MeOH), $C_{58}H_{92}O_{26}$, IR(KBr, cm^{-1}) : 3416, 1730, 1696, 1648, 1643, 1079, ^1H NMR : δ 6.16(dd, J = 5.6, 11.5, 22-H), 5.95(m, 3""'-H), 5.79(br s, 1"'-H), 5.74(d, J = 7.9, 1"-H), 5.10(d, J = 6.3, 1""-H), 5.05(d, J = 7.6, 1'-H), 4.63(br s, 16-H), 2.07(3H, d, J = 6.9, 4""'-H_3), 1.96(3H, s, 5""'-H_3), positive FAB-MS(m/z): 1227.6$(M+Na)^+$. 9) Desacyl-camelliasaponin C(**6**) : mp 214.5-216.2°C, $[\alpha]_D$ +1.9° (MeOH), $C_{53}H_{86}O_{25}$, IR(KBr, cm^{-1}) : 3411, 1736, 1638, 1078, ^1H NMR : δ 5.76(d, J =5.6, 1"'-H), 5.74(d, J = 7.9, 1"-H), 5.12(d, J = 7.2, 1""-H), 5.06(d, J = 7.6, 1'-H), 5.10(br s, 16-H), 4.60(m, 22-H), negative FAB-MS(m/z) : 1121.5$(M-H)^-$. 10) 22-*O*-angeloylcamelliagenin C(**9**) : mp 129.6-130.2°C , $[\alpha]_D$ +39.0°(MeOH), $C_{35}H_{56}O_6$, IR(KBr, cm^{-1}): 3444, 1687, 1649, ^1H NMR : δ 6.16(dd, J = 5.6, 12.2, 22-H), 5.93(m, 3""'-H), 4.62(br s, 16-H), 4.16, 3.72(ABq, J = 10.2, 23-H_2), 3.72, 3.56(ABq, J = 10.3, 28-H_2), 2.09(3H, d, J = 6.9, 4""'-H_3), 1.96(3H, s, 5""'-H_3), ^{13}C NMR : δ_C 73.8(3-C), 122.7(12-C), 143.8(13-C), 70.3(16-C), 41.8(21-C), 73.2(22-C), 68.5(23-C), 64.0(28-C), positive FAB-MS : 573$(M+H)^+$. 11) Camelliasaponin B_2(**2**) : mp 233.5-235.6°C, $[\alpha]_D$ +20.7°(MeOH), $C_{58}H_{90}O_{26}$, IR(KBr, cm^{-1}): 3432, 1740, 1721, 1686, 1647, 1635, 1076, ^1H NMR : δ 9.87(s, 23-H), 7.00(m, 3""'-H), 6.13(dd, J = 5.0, 11.6, 22-H), 5.77(d, J = 5.0, 1"'-H), 5.62(d, J = 7.6, 1"-H), 5.11(m, 1""-H), 4.85(d, J = 7.3, 1'-H), 4.60(br s, 16-H), 1.88(3H, s, 5""'-H_3), 1.59(3H, d, J = 7.0, 4""'-H_3), positive FAB-MS(m/z): 1225.6$(M+Na)^+$. 12) Camelliasaponin C_2(**5**) : mp 177.6-178.9°C, $[\alpha]_D$ +8.8°(MeOH), $C_{58}H_{92}O_{26}$, IR(KBr): 3432, 1736, 1686, 1647, 1645, 1078, ^1H NMR : δ 7.01(m, 3""'-H), 6.14(dd, J = 5.6, 11.9, 22-H), 5.76(d, J = 5.0, 1"'-H), 5.75(d, J = 7.9, 1"-H), 5.12(d, J = 6.9, 1""-H), 5.06(d, J = 7.6, 1'-H), 4.62(br s, 16-H), 1.86(3H, s, 5""'-H_3), 1.56(3H, d, J = 6.9, 4""'-H_3), positive FAB-MS(m/z): 1227.6$(M+Na)^+$. 13) 22-*O*-tigloylcamelliagenin B(**8**) : mp 170.3-172.9°C, $[\alpha]_D$ +68.5°(MeOH), $C_{35}H_{54}O_6$, IR(KBr): 3425, 1725, 1686, 1649, ^1H NMR : δ 9.61(s, 23-H), 6.14(dd, J = 5.6, 12.2, 22-H), 6.96(m, 3""'-H), 4.63(br s, 16-H), 3.66, 3.52(ABq, J = 10.2, 28-H_2), 1.87(3H, s, 5""'-H_3), 1.56(3H, d, J = 7.3, 4""'-H_3), ^{13}C NMR : δ_C 71.7(3-C), 122.3(12-C), 143.9(13-C), 70.0(16-C), 41.7(21-C), 73.2(22-C), 207.2(23-C), 63.9(28-C), positive FAB-MS(m/z) : 571$(M+H)^+$. 14) 22-*O*-tigloylcamelliagenin C(**10**) : mp 142.7-144.8°C, $[\alpha]_D$ +40.0°(MeOH), $C_{35}H_{56}O_6$, IR(KBr) : 3436, 1674, 1649, ^1H NMR : δ 6.15(dd, J = 5.6, 11.9, 22-H), 6.99(m, 3""'-H), 4.63(br s, 16-H), 4.16, 3.71(ABq, J = 10.2, 23-H_2), 3.70, 3.52(ABq, J = 10.2, 28-H_2), 3.05(dd, J = 3.8, 14.1, 18-H), 1.87(3H, s, 5""'-H_3), 1.56(3H, d, J = 6.9, 4""'-H_3), ^{13}C NMR : δ_C 73.8(3-C), 122.7(12-C), 143.8(13-C), 70.1(16-C), 41.8(21-C), 73.2(22-C), 68.5(23-C), 63.9(28-C), positive FAB-MS(m/z) : 573$(M+H)^+$.

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