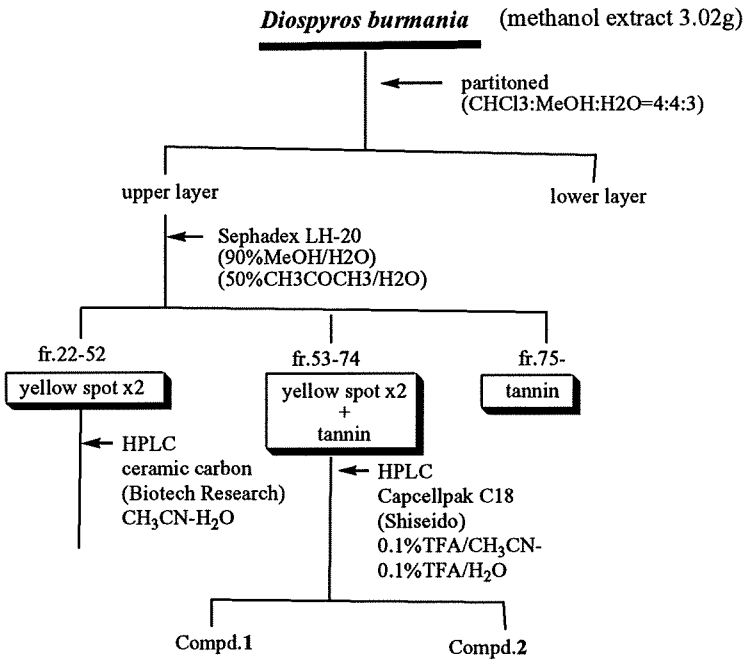
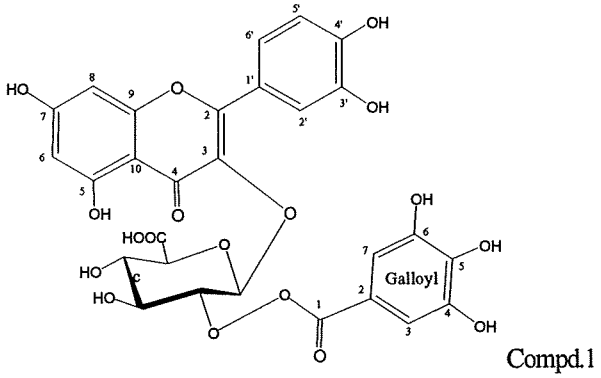




Compd.1

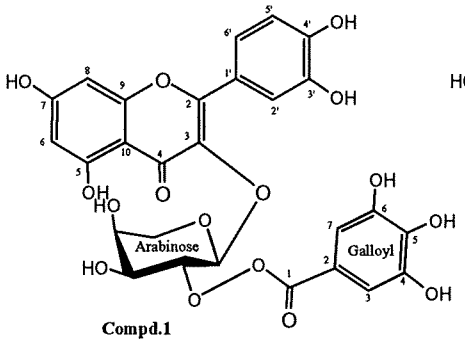
*Diospyros burmania*の分離チャートおよび単離された化合物１

2－2)

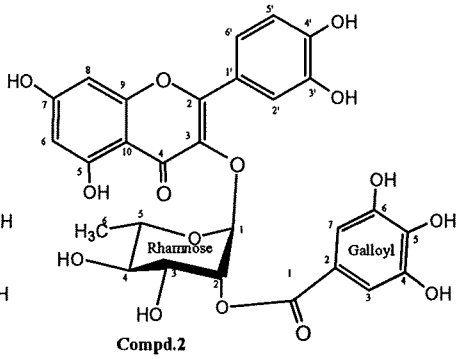
最小有効 IC50		国	現地名	学名	抽出部位
赤血球法	DPPH 法				
mg/mL	mg/mL				
10	0.05	Bolivia	Plant B	Plant B	leaves
30	0.5	Bolivia			leaves
100	10	Nepal	Pan ko jaya	Piper betel Blanco	rhizome
100	0.5	Nepal	Sahajira	Carum carvi L.	seed
100		Brazil	Pata-de-vaca	Bauhinia forficata Link	leaves
100(30)		Peru	Guanabana	Annona muricata	leaves, stem

100	0.794	Myanmar	M-294-1	Phyllanthus emblica	leaves, stem
100	0.1	Myanmar	M-180	Phyllanthus emblica	bark
30	0.1	種子島	ネジトウガラシ	Helicteres isora L.	branch
10	0.16	種子島			branch
10	0.16	種子島			leaves
(0.3) <1	0.1	Nepal			
		Myanmar	PYINMA	Lagerstroemia speciosa (L.)Pers.	heart wood
30 (10)	0.32～	Peru	Kepishiri	Matsigenka word meaning "bitter" Shepard 1998	
>100		Peru	altamisa, Marco	Ambrosia peruviana	
		Peru	Yarina	Phytelephas sp.	seed
100	0.4	Peru	Ajo sacha	Mansoa alliacea	root
-100	3.2	Peru	Ajo sacha	Mansoa alliacea	leaves
10	0.079	Bolivia			(aerial roots)
100(30)	2	Arzentin		Capparis atamisquea	
>100	3.2～	種子島	ヒメキランソウ	Ajuga pygmaea A.Gray	whole plant
30 (10)		種子島	オニグルミ	Juglans ailantifolia Carriere	leaves
100		種子島	オニグルミ	Juglans ailantifolia Carriere	fruits
10*DMSO		種子島	オニグルミ	Juglans ailantifolia Carriere	wood (branch)
30		種子島	オイランアザミ	Cirsium spinosum Kitam.	leaves
>30	2	Myanmmar		Dendrobium	
±30	0.14	Peru	OJE	Ficus ins 恥 ida Will)	leaves
±100	3.2	Peru	MITO	Carica candicans A. Gray	leaves
30	0.063	Peru	CHUCHUHUASI	Maytenus ebenifolia	bark
100	1	Peru	Tahuari	Tabebuia serratifolia	root
10		Peru	Tahuari	Tebebuia serratifolia	leaves
100(30)		Peru	Tahuari	Tebebuia serratifolia	branch

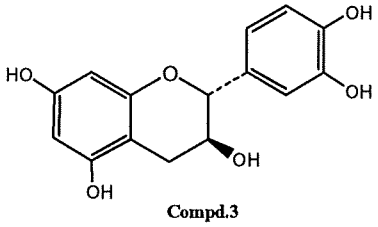
表　赤血球法による外国産生薬の抗酸化活性



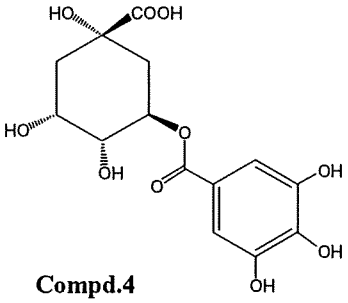
Compd.1



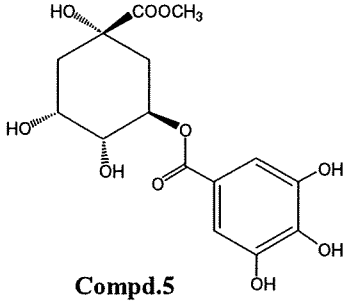
Compd.2



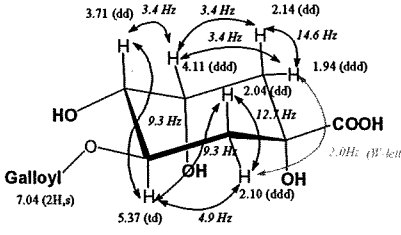
Compd.3

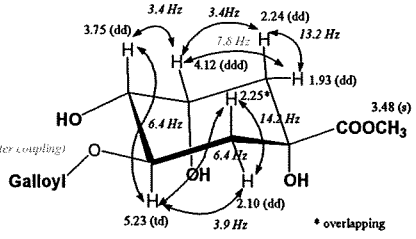


Compd.4



Compd.5

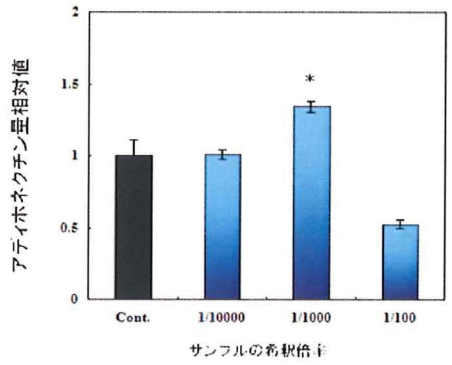




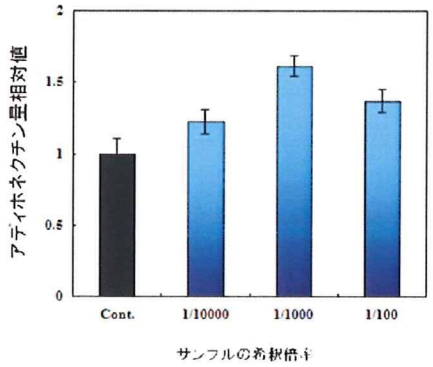
^{1}H-NMR Data and Observed Coupling Constants for Compd. 4 and 5 ^{1}H-NMR in CD_3OD

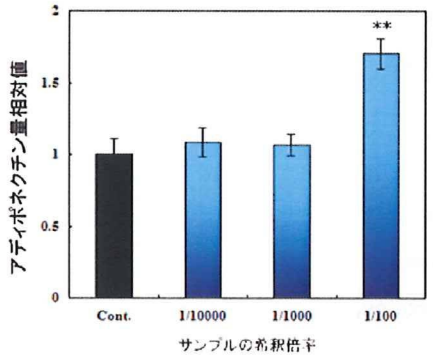
ボリビア産Jamillo de Duraznoから得られた化合物

2－3)



*: p< 0.05
**: p< 0.01
***: p< 0.005

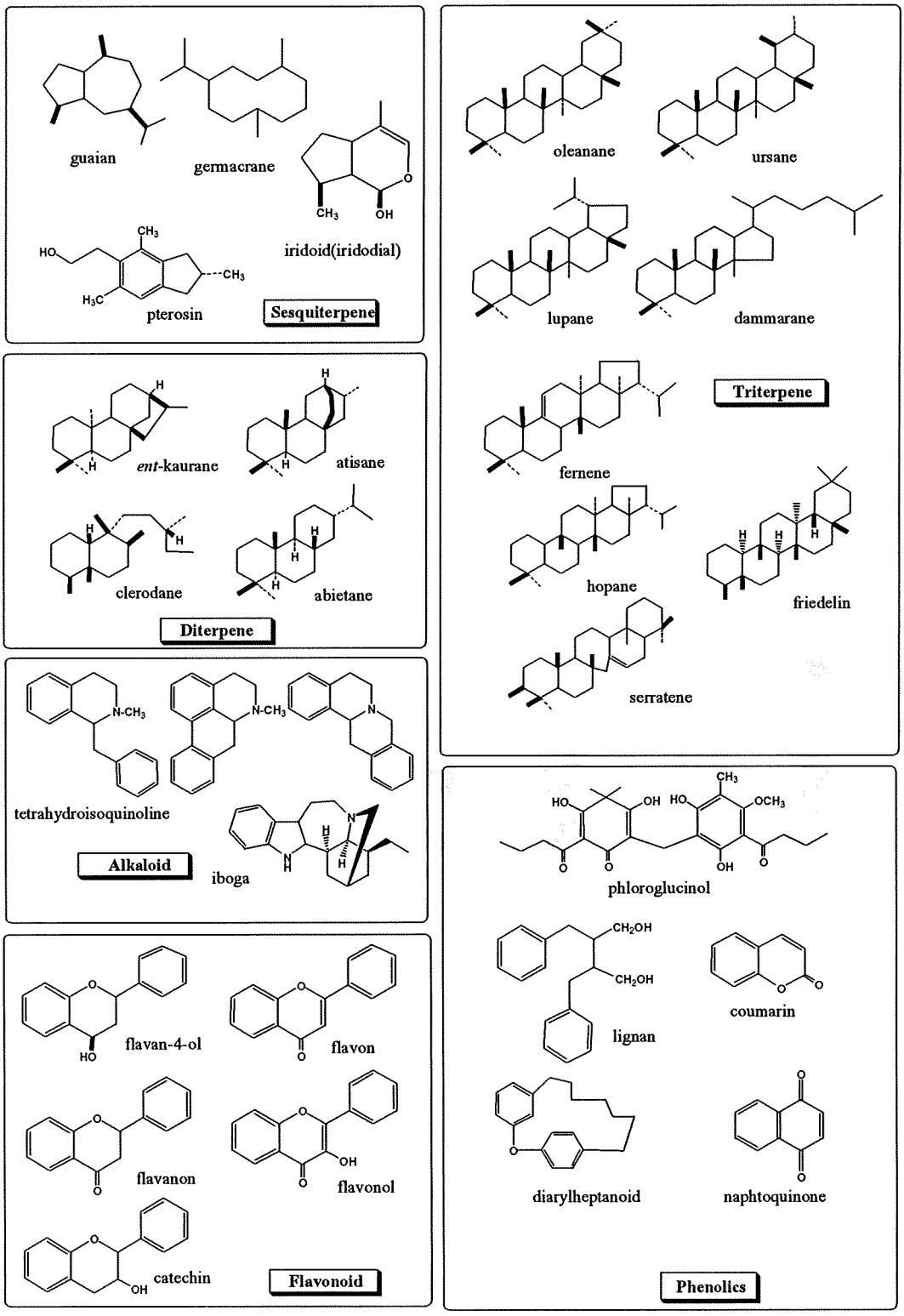




植物エキス処理がアディポネクチン生成低下抑制に与える影響

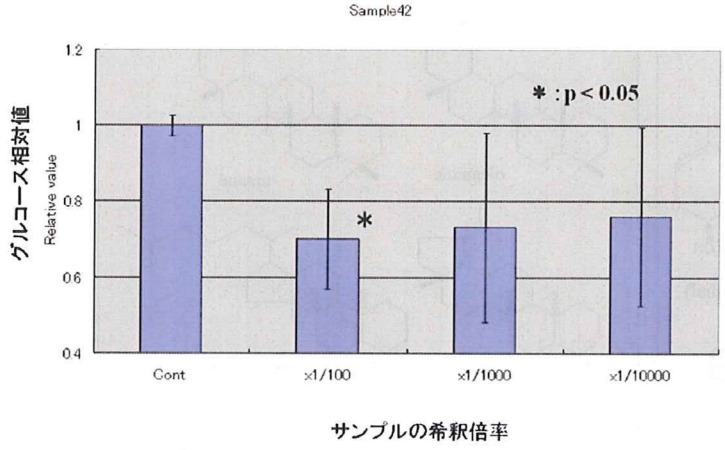
上：ボリビア産 *Viscum album*, 中：*Oroxylum indicum*, 下：パキスタン産 *Coccinia grandis*

2－4)



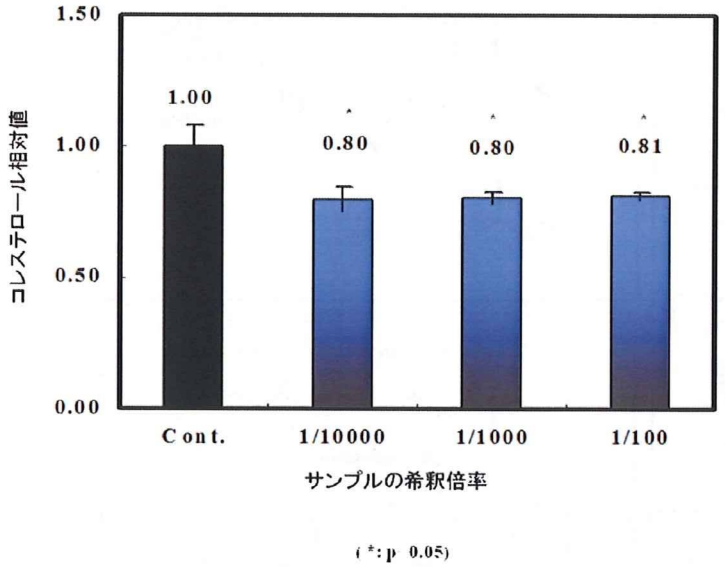
核内受容体PPARγに対するリガンド活性を検討した化合物群

2－6）



ペルー産生薬 cuti-cuti の多糖類のグルコースへの分解・吸収遅延に与える影響

2－7）



(*: p 0.05)

種子島産 *Ajuga pigmaea* がコレステロールの吸収抑制に与える影響

番号	国名	現地名または部位	学　名	科　名	MLC(ug/mL)
1	Pakistan	Agar (hindi)	Artemisia absinthium Linn.	Compositae	>400
2	Pakistan	Ajwan wal	Aquilaria agallocha Roxb.	Thymelaeaceae	400
3	Pakistan	Akari	Thymus serpyllum Reichb. ex Benth.	Labiatae	>400
4	Pakistan	Akas bail	Withania coagulans Dun.	Solanaceae	>400
5	Pakistan	Alsi	Cuscuta reflexa Decne.	Convolvulaceae	>400
6	Pakistan	Anba Haldi	Linum usitatissimum Griseb.	Linaceae	>400
7	Pakistan	Anjbar	Curcuma amada Roxb.	Zingiberaceae	100

8	Pakistan	aerial part	Artemisia scoparia	Compositae	200
9	Pakistan	aerial part	Fagonia cretica	Zygophyllaceae	>400
10	Pakistan	aerial part	Withania coagulans	Solanaceae	400
11	Pakistan	aerial part	Cousinia stocksii	Compositae	400
12	Peru	Tahuari (Leaves)	Tabebuia serratifolia	Bignoniaceae	100
13	Peru	Tahuari (twigs)	Tabebuia serratifolia	Bignoniaceae	400
14	Peru	Tahuari (Root)	Tabebuia serratifolia	Bignoniaceae	>400
15	Peru	Barbasco (Leaves)	Lonchocarpus nicou	Leguminosae	100
16	Peru	Barbasco (Twigs)	Lonchocarpus nicou	Leguminosae	100
17	Peru	Uchusanango (Barks)	Tabernaemontana sananho	Apocynaceae	400
18	Myanmar	bark	Indigofera lacei	Fabaceae	400
19	Myanmar	bark	Tamarindus indica	Leguminosae	>400
20	Myanmar	bark	Croton roxburghii	Euphorbiaceae	200
21	Myanmar	leaves	Leea crispa sp.	Ampelidaceae	400
22	Myanmar	leaves	Randia sp.	Rubiaceae	>400
23	Myanmar	leaves, branch	Leucas aspera	Lamiaceae	>400
24	Myanmar	flower	Leucas aspera	Lamiaceae	>400
25	Myanmar	root	Leucas aspera	Lamiaceae	>400
26	Myanmar	bark	Antidesma sp.	Euphorbiaceae	400
27	Myanmar	bark	Plumeria obtusa	Apocynaceae	>400
28	Myanmar	bark	Plumeria obtusa	Apocynaceae	>400
29	Myanmar	fruit	Terminalia bellerica	Combretaceae	400
30	Myanmar	leaves	Diospyros montana	Ebenaceae	200
31	Myanmar	fruit	Diospyros montana	Ebenaceae	>400
32	Myanmar	bark	Oroxylum indicum	Bignoniaceae	400
33	Myanmar	stem	Gardenia erythroclada	Rubiaceae	>400
34	Myanmar	branch , leaves	Croton bonplandianus	Euphorbiaceae	400
35	Myanmar	flower	Bombax ceiba	Euphorbiaceae	>400
36	Myanmar	bark	Euphorbia synadenium	Euphorbiaceae	>400
37	Myanmar	bark	Baliospermum solanifolium	Euphorbiaceae	>400
38	Myanmar	leaves, fruit	Baliospermum solanifolium	Euphorbiaceae	>400
39	Myanmar	stem	Baliospermum solanifolium	Euphorbiaceae	>400
40	Myanmar	root	Butea monosperma	Leguminosae	>400
41	Myanmar	flower	Butea monosperma	Leguminosae	>400
42	Myanmar	stem	Cryptolepis buchananii	Asclepiadaceae	>400
43	Myanmar	leaves, branch	Cryptolepis buchananii	Asclepiadaceae	400
44	Myanmar	whole plant	属名未同定		>400
45	Myanmar	branch	Vallaris solanacea	Apocynaceae	400

46	Myanmar	leaves	Vallaris solanacea	Apocynaceae	400
47	Myanmar	leaves, fruit	Azima sarmentosa	Salvadoraceae	>400
48	Myanmar	stem	Azima sarmentosa	Salvadoraceae	>400
49	Myanmar	bark	Capparis zeylanica	Capparaceae	>400
50	Myanmar	branch	Capparis zeylanica	Capparaceae	>400
51	Myanmar	flower	Capparis zeylanica	Capparidaceae	>400
52	Myanmar	leaves	Aegle marmelos	Rutaceae	>400
53	Myanmar	stems	Aegle marmelos	Rutaceae	>400
54	Myanmar	leaves	Streptocaulon juventas	Apocynaceae	200
55	Myanmar	branch	Streptocaulon juventas	Apocynaceae	400
56	Myanmar	leaves,stem	Aganosma marginata	Apocynaceae	>400
57	Myanmar	leaves,stem	Harrisonia perforata	Simaroabaceae	200
58	Myanmar	stem	Baliospermum solanifolium	Euphorbiaceae	>400
59	Myanmar	leaves, fruits	Baliospermum solanifolium	Euphorbiaceae	>400
60	Myanmar	Leaves	Diospyros discolor		400
61	Myanmar	stems	Buddleja asiatica	Buddlejaceae	>400
62	Myanmar	leaves , flower	Buddleja asiatica	Buddlejaceae	>400
63	Myanmar	leaves	Bridelia stipularis	Euphorbiaceae	200
64	Myanmar	stems	Bridelia stipularis	Euphorbiaceae	200
65	Myanmar	fruits	Bridelia stipularis	Euphorbiaceae	400
66	Myanmar	stem	Dillenia parviflora	Dilleniaceae	200
67	Myanmar	flower	Dillenia parviflora	Dilleniaceae	>400
68	Myanmar	bark	Dillenia parviflora	Dilleniaceae	400
69	Myanmar	leaves,stem	Sabia parviflora	Lauraceae	400
70	Myanmar	leaves , flower	Chionanthus mala-elengi subsp. terniflorus	Oleaceae	400
71	Myanmar	stem	Chionanthus mala-elengi subsp. terniflorus	Oleaceae	400
72	Myanmar	leaves, flower, fruits	Croton roxburghii	Euphorbiaceae	400
73	Myanmar	stem	Croton roxburghii	Euphorbiaceae	400
74	Myanmar	leaves	Ichnocarpus frutescens	Apocynaceae	400
75	Myanmar	stem	Ichnocarpus frutescens	Apocynaceae	>400
76	Myanmar	leaves , flower	Lippia geminata	Verbenaceae	200
77	Myanmar	stem	Lippia geminata	Verbenaceae	400
78	Myanmar	leaves,stem	Gmelina tomentosa		200
79	Myanmar	whole plant	Tribulus terrestris	Euphorbiaceae	400
80	Myanmar	stem, flower	Haloxylon recuruum	Chenopodiaceae	400
81	Myanmar	leaves, branch	Sphaeranthus indicus	Compositae	100

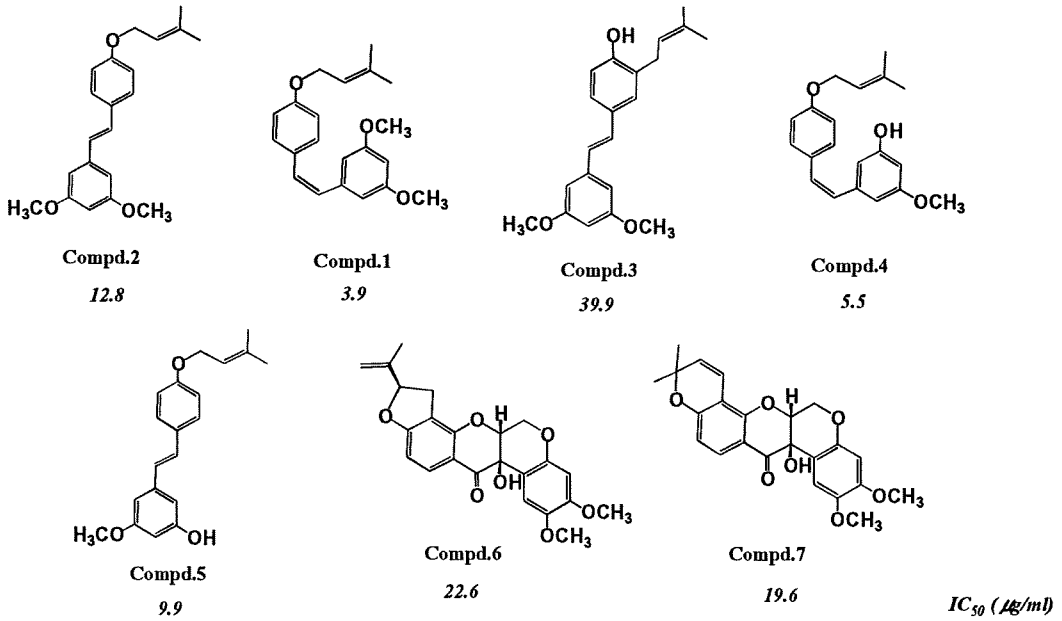
82	Myanmar	flower	Sphaeranthus indicus	Compositae	25
83	Myanmar	leaves	Carrisa spinarum	Oleaceae	100
84	Myanmar	stem	Carrisa spinarum	Oleaceae	>400
85	Myanmar	fruit	Euphorbia trigona	Euphorbiaceae	400
86	Myanmar	branch	Pupalia lappacea var. velutina	Amaranthaceae	>400
87	Myanmar	leaves	Hyptis suaveolens	Labietae	400
88	Myanmar	branch	Hyptis suaveolens	Labietae	400
89	Arzentine	rhizome	Triticum repens	Gramineae	>400
90	Arzentine	leaves	Marrubium vulgare	Labiatae	>400
91	Arzentine	leaves,stem	Baccharis crispa	Compositae	200
92	Arzentine	leaves,stem	Fraxinus excelsior	Oleaceae	>400
93	Arzentine	leaves,stem	Capparis atamisquea	Capparaceae	>400
94	Arzentine	leaves,stem	Micromeria eugenioides	Labiatae	>400
95	Myanmar	stem, flower	Erythrina suberosa	Leguminosae	200
96	Myanmar	root	Erythrina suberosa	Leguminosae	>400
97	Myanmar	whole plant	Parkinsonia aculeata	Leguminosae	>400
98	Myanmar	leaves, stem	Carissa spinarum	Rubiaceae	100
99	Myanmar	leaves, stem, root	Tephrosia coccinea	Leguminosae	400
100	Myanmar	leaves	Aganosma marginata	Apocynaceae	>400
101	Myanmar	stem	Aganosma marginata	Apocynaceae	>400
102	Myanmar	leaves	Croton laevigatus	Euphorbiaceae	200
103	Myanmar	bark	Croton laevigatus	Euphorbiaceae	400
104	Myanmar	branch	Croton laevigatus	Euphorbiaceae	>400
105	Myanmar	leaves, stem	Wendlandia tictoria	Rubiaceae	200
106	Myanmar	flower, leaves	Woodfordia floribunda	Caprifoliaceae	200
107	Myanmar	stem	Woodfordia floribunda	Caprifoliaceae	400
108	Myanmar	leaves	Croton roxburghii	Euphorbiaceae	200
109	Myanmar	stem	Croton roxburghii	Euphorbiaceae	>400
110	Myanmar	stem	Euphorbia neriifolia	Euphorbiaceae	>400
111	Myanmar	leaves	Ostodes paniculata	Euphorbiaceae	200
112	Myanmar	stem	Ostodes paniculata	Euphorbiaceae	>400
113	Myanmar	leaves	Alstonia scholaris	Apocynaceae	100
114	Myanmar	stem	Alstonia scholaris	Apocynaceae	>400
115	Myanmar	stem	Artocarpus lacucha	Moraceae	>400
116	Myanmar	stem, flower	Artocarpus lacucha	Moraceae	400
117	Myanmar	stem, flower	Spondia pinnata	Anacardiaceae	400
118	Myanmar	leaves	Bridelia glauca	Euphorbiaceae	400
119	Myanmar	stem	Bridelia glauca	Euphorbiaceae	400

120	Myanmar	leaves, stem	Cocculus lauriflolius	Menispermaceae	400
121	Myanmar	leaves	Trichilia connarroide	Meliaceae	400
122	Myanmar	stem	Trichilia connarroide	Meliaceae	>400
123	Myanmar	Leaves, branch	Phyllanthus emblica	Euphorbiaceae	>400
124	Myanmar	bark	Phyllanthus emblica	Euphorbiaceae	200
125	Myanmar	fruits	Phyllanthus emblica	Euphorbiaceae	>400
126	Myanmar	leaves	Flacourtia indica	Flacuotriaceae	>400
127	Myanmar	stem	Flacourtia indica	Flacuotriaceae	>400
128	Myanmar	fruits	Strychnos nux-blanda	Loganiaceae	>400
129	Myanmar	leaves	Hiptage benghalensis	Malpighiaceae	>400
130	Myanmar	stem	Hiptage benghalensis	Malpighiaceae	>400
131	Myanmar	leaves	Diospyrros burmania	Ebenaceaae	200
132	Myanmar	stem	Diospyrros burmania	Ebenaceaae	>400
133	Myanmar	leaves	Morinda tomentosa	Rubiaceae	100
134	Myanmar	stem	Morinda tomentosa	Rubiaceae	>400
135	Myanmar	bark	Gnetum latifolium var. funiculare	Gnetaceae	>400
136	Myanmar	leaves	Bistorta yunnanensis	Polygonaceae	>400
137	Myanmar	whole plant	Wahlenbergia marginata	Campanulaceae	>400
138	Myanmar	stem	Cornus capitata	Cornaceae	400
139	Myanmar	stem	Buddleja paniculata	Buddelejaceae	>400
140	Myanmar	fruit	Datura metel	Solanaceae	>400
141	Myanmar	fruit	Schrebera swietenoides	Oleaceae	>400
142	Myanmar	fruit	Embelia tsjeriamcottam	Myrsinaceae	400
143	Myanmar	fruit	Strychnos nux-vomica	Loganiaceae	>400
144	Myanmar	stem	Tinospora cordifolia	Menispermaceae	>400
145	Myanmar	bark	Leea macrophylla	Vitaceae	>400
146	Myanmar	root	Saussurea deltoidea var. polycephala	Asteraceae	400
147	Myanmar	aerial part	Vernonia volkamerifolia	Asteraceae	>400
148	Myanmar	Leaves, branch	Anneslea fragrance	Theaceae	400
149	UAE	Baboonig (aerial part)	Matricaria recutita	Compositae	>400
150	UAE	Geada (aerial part)			>400
151	UAE	Mairamya(aerial part)			200
152	UAE	Za'ater(aerial part)	Thymus capitatus	Labiatae	400
153	Peru	Shingura panga(Leaves, stem)			400
154	Peru	Granadilla(Leves, stem)	Passiflora sp.	Passifloraceae	>400
155	Peru	Retama(Leaves, stem)	Cassia bicapsularis	Leguminosae	>400

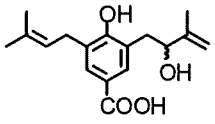
156	Peru	Ayahuma (Leaves)	Courouprta guianensis	Lecythidaceae	400
157	Peru	Chiric sanango (root)	Brunfelsia grandiflora	Solanaceae	>400
158	Peru	Partiquina negra(Leaves)	Dieffenbachia sp.	Araceae	>400
159	Peru	Ojo de pollo (Leaves, stem, flower)	Alternanthera halimifolia	Amaranthaceae	>400
160	Peru	Guisador (Leaves)	Curcuma longa	Zingiberaceae	400
161	Peru	Mishuisma(Fruit)	Hibiscus abelmoschus	Malvaceae	>400
162	Peru	Guanabana(Leaves, stem)	Annona muricata	Annonaceae	100
163	Peru	Mullaca(whole plant)	Clidemia hirta?	Melastomataceae	200
164	Peru	Ishanga(whole plant)	Laportea aestuans	Urticaceae	>400
165	Peru	Toe(Leaves)	Brugmansia aurea	Solanaceae	>400
166	Peru	Catagua(bark)	Hura crepitans	Euphorbiaceae	200
167	Peru	Retama(Flower, Leaves, Stem)	Cassia bicapsularis	Leguminosae	>400
168	Peru	Huayusa (Flower, Leaves, Stem)	Ilex guayusa	Aquifoliaceae	400
169	Peru	Cotochupa (Root)	Polypodium decumanum	Polypodiaceae	>400
170	Peru	Lancetilla(Leaves, stem)	Pfaffia glomerata	Amaranthaceae	>400
171	Peru	Matapasto (Leaves, stem)	Hyptis sp.	Labiatae	>400
172	Peru	Mataro (Fruit)	Casia sp.	Leguminosae	>400
173	Peru	Ayahuma(Bark)	Courouprta guianensis	Lecythidaceae	200
174	Solomon	leaves	Medinilla anisophylla Merr. et Perry	Melastomataceae	400
175	Solomon	leaves	Clerodendrum inerme (L.) Gaertn.	Verbenaceae	>400
176	Solomon	leaves	Mussaenda sp.	Rubiaceae	>400
177	Solomon	leaves	Intsia sp.	Leguminosae	>400
178	Solomon	stem	Macaranga tanarius (L.) M_ll-Arg.	Euphorbiaceae	>400
179	Solomon	leaves	Acalypha grandis Benth.	Euphorbiaceae	400
180	Solomon	leaves, stem	Elatostemma novae-britanniae	Urticaceae	50
181	Solomon	leaves	Trema sp.	Ulmaceae	>400
182	Solomon	stem, seed(pericarp)	Trema sp.	Ulmaceae	>400
183	Solomon	stem	Piper sp.	Piperaceae	400
184	Solomon	stem	Amoora sp.	Meliaceae	>400
185	Solomon	leaves, stem	Wedelia sp.	Compositae	>400
186	Solomon	root	Wedelia sp.	Compositae	>400
187	Solomon	stem	Maesa sp.	Myrsinaceae	400

188	Solomon	leaves, stem	Alpinia sp.	Zingiberaceae	400
189	Solomon	root	Alpinia sp.	Zingiberaceae	200
190	Solomon	leaves	Rhus taitensis Guilleman	Anacardiaceae	200
191	Solomon	stem	Rhus taitensis Guilleman	Anacardiaceae	200
192	Solomon	leaves, stem	Morinda citrifolia L.	Rubiaceae	>400
193	Solomon	leaves	Mikania cordata (Burm. f.) B.L. Robinson	Compositae	50
194	Solomon	leaves	Macaranga tanarius (L.) M_ll-Arg.	Euphorbiaceae	200
195	Solomon	stem	Acalypha grandis Benth	Euphorbiaceae	>400
196	Solomon	leaves	Vigna marina (Burm.) Merr.	Leguminosae	>400
197	Solomon	leaves	Daphniphyllum conglutinosum Hemsl.	Daphniphyllaceae	200
198	Solomon	leaves	Cananga odorata Hook. f. & Thoms.	Annonaceae	>400
199	Solomon	leaves	Ananas comosus Merrill	Bromeliaceae	>400
200	Solomon	stem	Ananas comosus Merrill	Bromeliaceae	>400
201	Solomon	leaves	Terminalia solomonensis Exell	Combretaceae	>400
202	Solomon	stem	Calophyllum inophyllum L.	Guttiferae	200
203	Solomon	stem	Barringtonia asiatica Druce	Lecythidaceae (Myrtaceae)	200
204	Solomon	leaves	Terminalia complanata K. Schum.	Combretaceae	>400
205	Solomon	leaves, stem	Mucuna brachycarpa Rech.	Leguminosae	>400

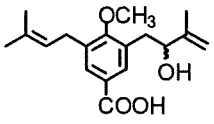
表　外国産生薬に対する抗リーシュマニア活性評価結果（平成19年度～21年度）



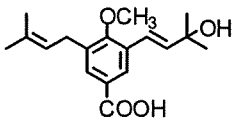
Barbasco(*Lonchocarpus nicou*)枝の抗リーシュマニア活性成分



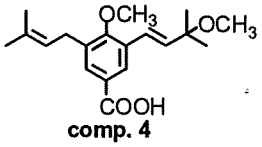
comp. 1



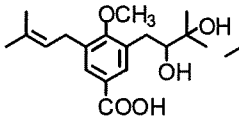
comp. 2



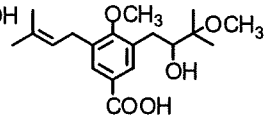
comp. 3



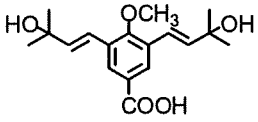
comp. 4



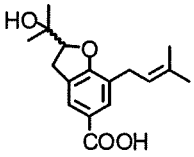
comp. 5



comp. 6



comp. 7



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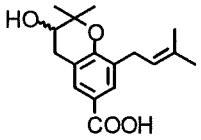

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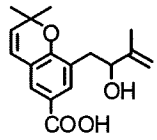
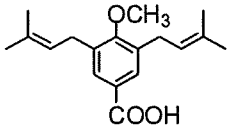
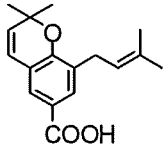


comp. 10



comp. 11



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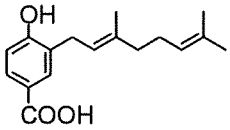
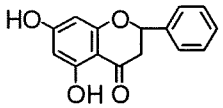
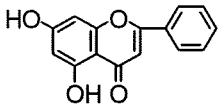


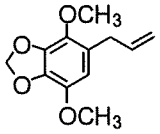
comp. 13



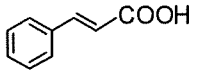
comp. 14
pinocembrin



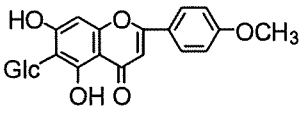
comp. 15
chrysin



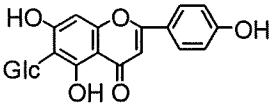
comp. 16 apiole



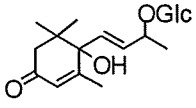
comp. 17
***trans*-cinnamic acid**



comp. 18
4'-*O*-methylisovitexin

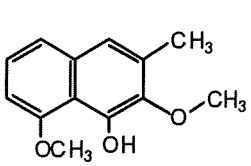


comp. 19
isovitexin

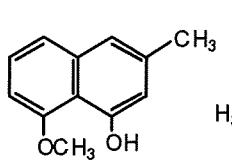


comp. 20
vomifoliol-9-*O*-β-D-glucopyranoside

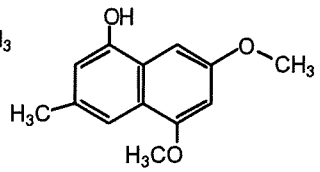
ペルー生薬Matico(*Piper angustifolium*)の抗リーシュマニア活性成分



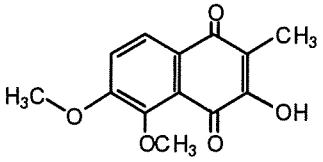
comd.2



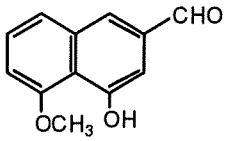
comd.3



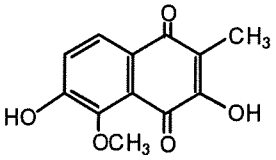
comd.4



comd.5

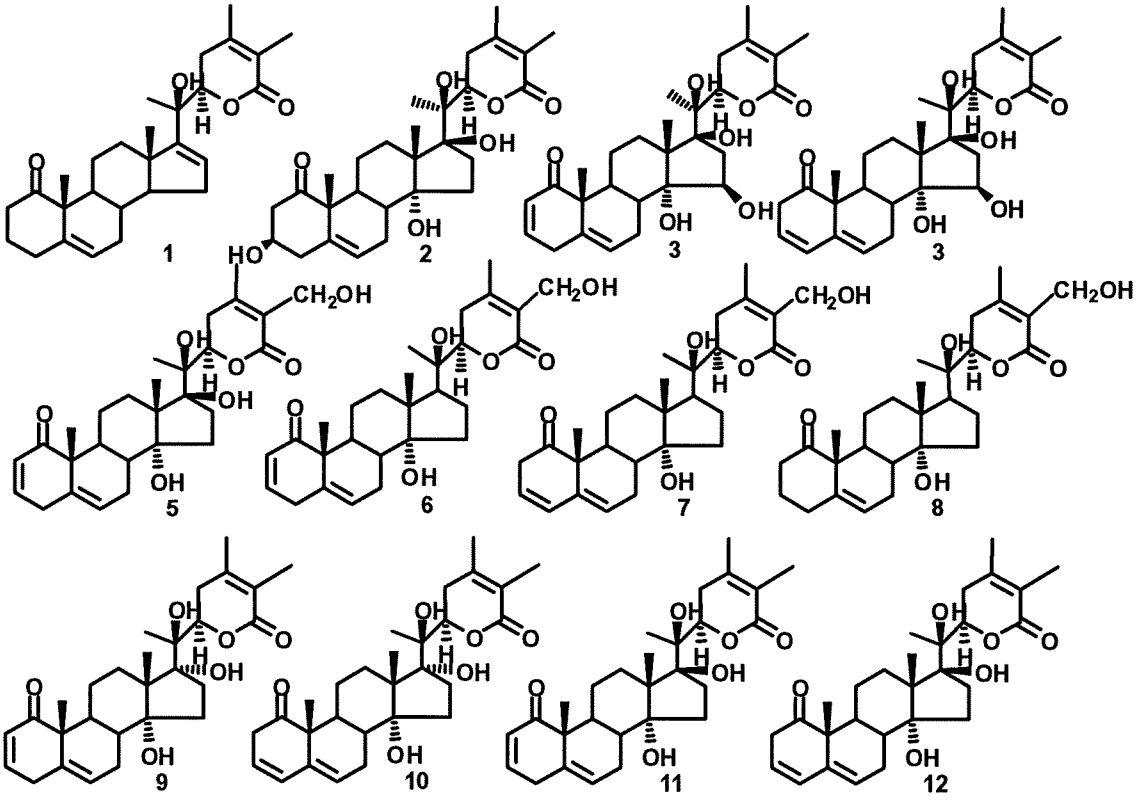


comd.10

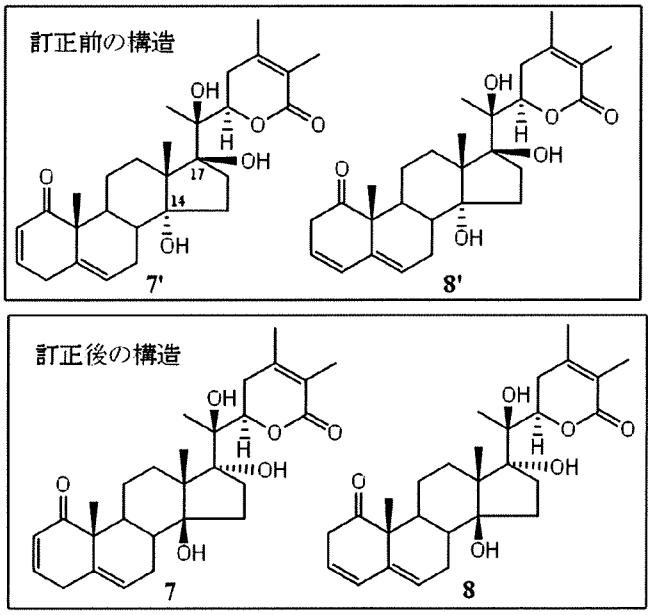


comd.11

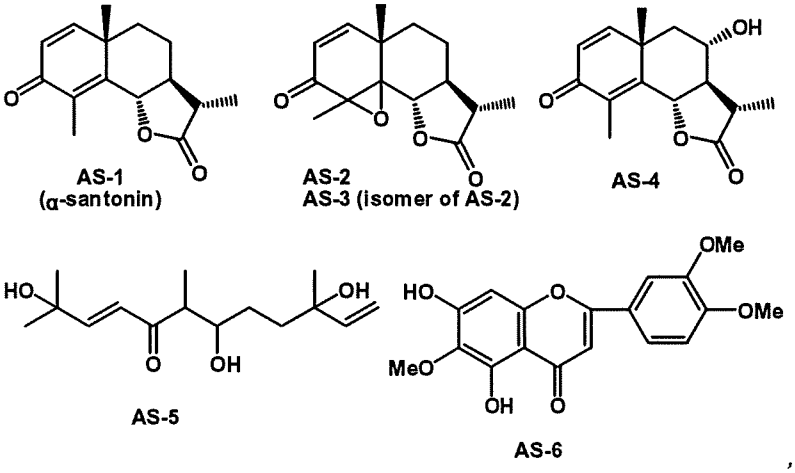
ミャンマー産カキノキ科植物の抗リーシュマニア活性化合物



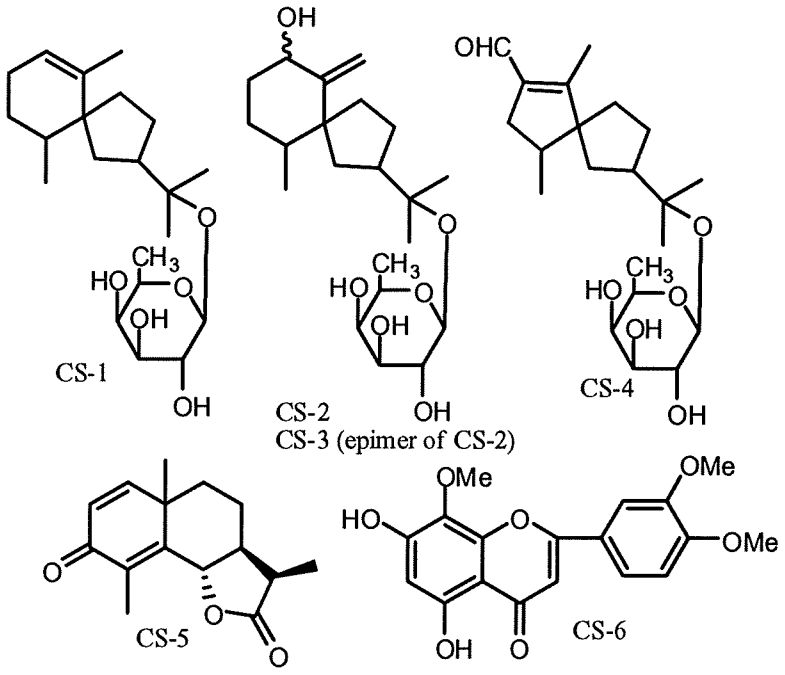
パキスタン産植物 *Withania coagulans* から得られた抗リーシュマニア活性成分



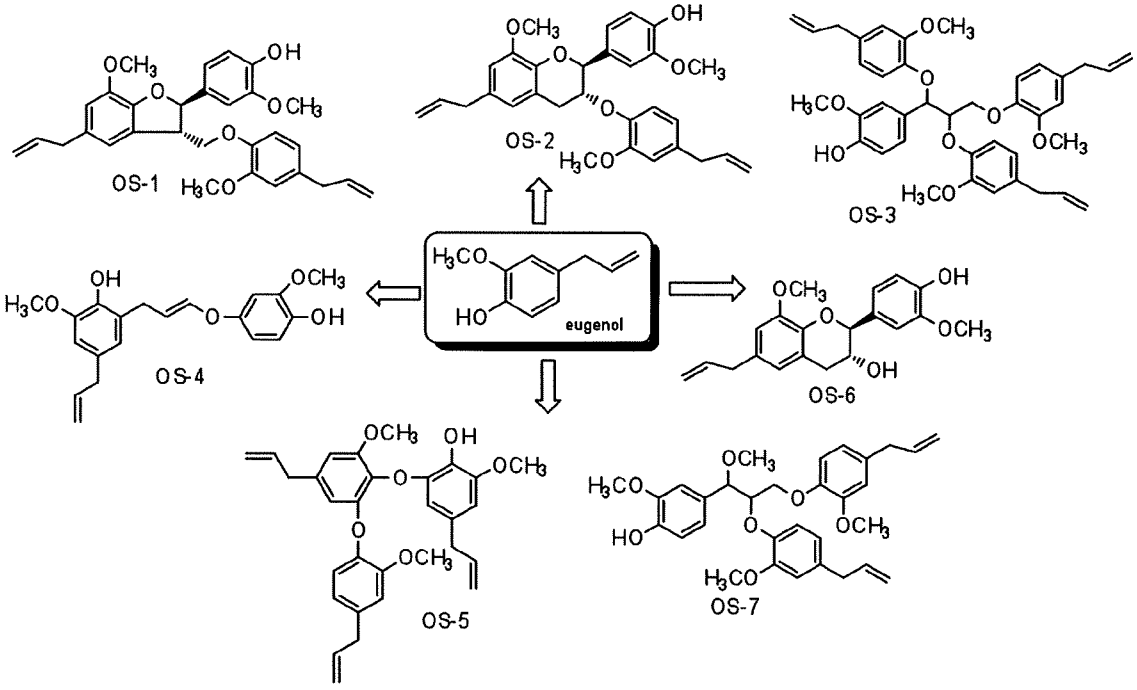
Withania coagulans の成分の立体配置の訂正



パキスタン産植物 *Artemisia scoparia*, の抗リーシュマニア活性成分



パキスタン産植物 *Cousinia stoksii* から得られた抗リーシュマニア活性成分



Tulsi の成分. すべて eugenol ユニットが縮合した化合物

研究成果の刊行に関する一覧表

原著論文

発表者氏名	論文タイトル名	発表誌名	巻、号	ページ	出版年
Takahashi, K. 他	α-Glucosidase inhibitor from *Solanum torvum*.	Bioscience, Biotechnology, and Biochemistry	74巻4号	741-745	2010
K.Yosida 他	Flavonol caffeoylglycosides as α-glucosidase inhibitors from *Spiraea cantoniensis* flower.	*Journal of Agricultural Food Chemistry*	56(12),	4367-4371	2008
K.Mori 他	Antileishmanial compounds from a Myanmar Plant *Cordia fragrantissima*.	*Journal of Natural Product*	71 (1)	18-21	2008
H. Fuchino 他	A New Leishmanicidal Saponin from *Brunfelsia grandiflora*.	*Chemical and Pharmaceutical Bulletin*	56 (1)	93-96	2008
以下投稿中					
H. Fuchino 他	*In Vitro* leishmanicidal activity of benzo-phenanthridine alkaloids from *Bocconia pearcei* and related compounds,	*Chemical and Pharmaceutical Bulletin*	投稿中	投稿中	2010

Biosci. Biotechnol. Biochem., **74** (4), 741–745, 2010

Methyl Caffeate as an α-Glucosidase Inhibitor from *Solanum torvum* Fruits and the Activity of Related Compounds

Keisuke TAKAHASHI,[1] Yasuyuki YOSHIOKA,[1] Eisuke KATO,[1] Shigeki KATSUKI,[2] Osamu IIDA,[2] Keizo HOSOKAWA,[3] and Jun KAWABATA[1,†]

[1]*Laboratory of Food Biochemistry, Division of Applied Bioscience, Graduate School of Agriculture, Hokkaido University, Kita-ku, Sapporo 060-8589, Japan*
[2]*Tanegashima Division, Research Center for Medicinal Plant Resources, National Institute of Biomedical Innovation, Nakatane-cho, Kumage-gun, Kagoshima 891-3604, Japan*
[3]*Department of Nutritional Management, Faculty of Health Sciences, Hyogo University, Kakogawa 675-0195, Japan*

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In screening experiments for rat intestinal α-glucosidase (sucrase and maltase) inhibitors in 325 plants cultivated in Japan's southern island, of Tanegashima, marked inhibition against both sucrase and maltase was found in the extract of the fruit of *Solanum torvum*. Enzyme-assay guided fractionation of the extract led to the isolation of methyl caffeate (1) as a rat intestinal sucrase and maltase inhibitor. We examined 13 caffeoyl derivatives for sucrase- and maltase-inhibitory activities. The results showed that methyl caffeate (1) had a most favorable structure for both sucrase and maltase inhibition, except for a higher activity of methyl 3,4,5-trihydroxycinnamate (14) against sucrase. Its moderate inhibitory action against α-glucosidase provides a prospect for antidiabetic usage of *S. torvum* fruit.

Key words: *Solanum torvum*; α-glucosidase inhibitor; methyl caffeate; Tanegashima

Diabetes mellitus is one of the most serious chronic diseases. It is caused by continual hyperglycemia and develops along with increases in obesity and aging in the general population.[1)] One of the therapeutic approaches to decreasing postprandial hyperglycemia is to retard absorption of glucose by inhibition of carbohydrate hydrolyzing enzymes α-amylase and α-glucosidase in the digestive organs.[2)] In recent years, many efforts have been made to search for effective α-glucosidase inhibitors from natural sources in order to develop a physiological functional food or to discover lead compounds for medicinal usage against diabetes.[3)] In the course of our search for rat intestinal α-glucosidase-inhibiting principles from various plants, we have isolated and identified several active compounds from plants grown in Asian regions, including Japan,[4–7)] Thailand,[8,9)] China,[10,11)] and Nepal.[12)] In this paper, we present the results of a screening of plants cultivated in Tanegashima, a southern island of Japan, for rat intestinal α-glucosidase inhibition. In the screening experiments for rat intestinal sucrase and/or maltase inhibitors in 325 plants, potent sucrase and maltase inhibiting activity was found in extract of the fruit of *Solanum torvum* (Solanaceae), an edible herbaceous perennial plant. There have been several reports on the chemical constituents of this plant, which include well-documented steroidal compounds[13–15)] and antiviral activities,[16)] but no other biologically active compounds from this plant have been reported to date. Hence, the promising screening result prompted us to isolate and elucidate the structures of active compounds in this plant. Separation of *S. torvum* fruit extract using various column chromatographic techniques lead to the isolation of methyl caffeate (1) as one of active principles. Some α-glucosidase inhibitors previously isolated from plants contain the caffeoyl moiety as the part of their structure and have been found to be important in exerting inhibitory activity,[3,7)] but the contribution of an ester part to the α-glucosidase-inhibitory activity of caffeic esters has not been studied. Hence we also present comparative results for α-glucosidase inhibition of various synthetic caffeic esters and related compounds.

Materials and Methods

Materials. Three hundred and twenty-five species of temperate Japanese plants were cultivated and collected in an experimental field in Tanegashima, Japan. All voucher specimens are deposited at the Tanegashima Division, Research Center for Medicinal Plant Resources, National Institute for Biomedical Innovation, Tanegashima, Japan. All chemicals used were of reagent grade, and were purchased from Wako Pure Chemicals (Osaka, Japan), unless otherwise stated. All solvents were distilled before use.

Apparatus. NMR spectra were recorded on a Bruker AMX500 instrument (^{1}H, 500 MHz). Chemical shifts (ppm) were calculated from the residual solvent signals of δ_H 2.04 in acetone-d_6, δ_H 2.49 in dimethyl sulfoxide (DMSO)-d_6, δ_H 3.30 in methanol-d_4, or δ_H 7.24 in chloroform-*d*. Field desorption (FD), FD-high resolution (HR), and electron ionization (EI) mass spectra were obtained on a JMS-SX102A instrument (Jeol, Tokyo).

Intestinal α-glucosidase-inhibitory activity determination. Sucrase- and maltase-inhibitory activities indicating inhibition of sucrase- and maltase-hydrolyzing activities respectively in rat intestinal glucosidase complexes were measured as described previously.[7)] Briefly, a crude enzyme solution prepared from rat intestinal acetone powder (Sigma-Aldrich Japan, Tokyo) was used as the small intestinal α-glucosidase. A reaction mixture consisting of crude enzyme solution (0.05 ml of maltase or 0.2 ml of sucrase), substrate solution (0.35 ml of 3.5 mM

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

maltose or 0.2 ml of 56 mM sucrose) in 0.1 M potassium phosphate buffer (pH 6.3), and the test sample in 50% aqueous DMSO (0.1 ml) was incubated for 15 min at 37 °C. The reaction was stopped by adding 0.75 ml of 2 M Tris–HCl buffer (pH 7.0), and then this was passed through a short column of basic alumina (Merck Japan, Tokyo) to remove phenolic compounds, which might have interfered with enzymatic glucose quantification at the following step. The amount of liberated glucose was measured by the glucose oxidase method using a commercial test kit (Glucose CII-test Wako, Wako, Osaka, Japan).

Screening experiments. Screening experiments for rat intestinal maltase and sucrase inhibition were carried out with extracts of 524 plant parts from 325 species. Dried plant parts were extracted with 50% aqueous methanol. The extracts were evaporated to dryness, redissolved in 50% aqueous DMSO, and used as test samples to assess rat intestinal α-glucosidase inhibitory activity. Extractable constituents obtained from 100 mg of plant material dissolved in 1 ml of test solution were used as the final concentration in the experiments.

Isolation of methyl caffeate (1) from S. torvum *fruit.* Dried fruits (50 g) of *S. torvum* were extracted with 50% aqueous methanol. The extracts were concentrated and charged onto a hydrophobic resin column (Diaion HP-20, Mitsubishi chemical, Tokyo). The column was washed with water to remove sugars that would have disturbed the α-glucosidase-inhibitory assay and then eluted with methanol. The methanol eluate was concentrated and partitioned between ethyl acetate and water. The ethyl acetate fraction showed activities for both sucrase (29%) and maltase (47%). In contrast, the water fraction showed higher inhibitory activity against maltase (62%), whereas the sucrase-inhibitory activity was low (13%). Hence further fractionation was carried out to isolate sucrase and maltase inhibitors from the ethyl acetate fraction. The ethyl acetate fraction was fractionated by silica gel column chromatography with gradient elution by chloroform and methanol. Sucrase inhibitory activity was eluted in chloroform-methanol (4:1) eluate, while maltase inhibitory activity was dispersed throughout the fractions. The chloroform-methanol (4:1) fraction was further purified by preparative HPLC (column, Inertsil PREP-ODS, ϕ20 × 250 mm, GL-Science, Tokyo; mobile phase, 15–30% MeCN in water (0–60 min), 30% MeCN in water (60–90 min); flow rate, 5.0 ml/min; detection, UV 254 nm). A peak eluted at t_R = 64.8 min showing the highest sucrase and maltase inhibitory activities was collected to give **1** (16 mg). The analytical data were closely consistent with those of the authentic specimen. **1**: FD-MS *m/z*: 194 (M^+); ^{1}H NMR (DMSO-d_6) δ (ppm): 3.67 (3H, *s*, OCH_3), 6.25 (1H, *d*, *J* = 16.0 Hz, H-8), 6.74 (1H, *d*, *J* = 8.2 Hz, H-5), 6.99 (1H, *br d*, *J* = 8.2 Hz, H-6), 7.04 (1H, *br s*, H-2), 7.47 (1H, *d*, *J* = 16.0 Hz, H-7).

General procedure for the preparation of 2–7 and 10–14. Compounds **2**–**7**,[17] **10**,[18] **11**,[19] and **12**[20] were prepared as described below and spectral properties were matched with the reported data. Compounds **8** and **9** are commercially available. Compounds **13** and **14** were prepared as described below.

Preparation of 2–5, and 12. To a stirred solution of the corresponding cinnamic or benzoic acid (10 mmol) in each alcohol (50 ml) was added dropwise conc. H_2SO_4 (2.5 ml). The reaction mixture was heated to reflux for 6–24 h. After cooling, the resulting mixture was concentrated, diluted with water, and extracted with ethyl acetate. The extract was washed with water and dried over anhydrous sodium sulfate. After removal of the solvent, the residue was purified by silica gel column chromatography (hexane-ethyl acetate) to give the desired esters.

Preparation of tert*-butyl caffeate (6).* To a stirred solution of triphenylphosphine (6.0 g, 23 mmol) in toluene (20 ml) was added *tert*-butyl bromoacetate (3.8 ml, 26 mmol, 1.1 eq). The reaction mixture was heated to reflux overnight. The mixture was cooled to room temperature and the resulting precipitate was filtered, washed successively with toluene and hexane, and dried to give a phosphonium salt (86%). The obtained phosphonium salt (2.35 g, 5 mmol) in chloroform (10 ml) was added to a stirred solution of 3,4-dihydroxybenzaldehyde (690 mg, 5 mmol) in dioxane (10 ml) and then $KHCO_3$ (2.5 g, 25 mmol, 5 eq) was added to the mixture. The mixture was refluxed for 6 h and cooled to room temperature, and the resulting insoluble salt was filtered off. The filtrate was concentrated and purified by silica gel column chromatography (hexane-ethyl acetate (3:2)) to give **6** (76%).

Preparation of phenyl caffeate (7). To a stirred solution of malonic acid (4.16 g, 40 mmol) in acetic anhydride (4.8 ml) was added conc. H_2SO_4 (0.16 ml). After 20 min, acetone (4 ml) was added to the solution and this was stirred for 6 h. The resulting precipitate was collected to give Meldrum's acid (66%). Meldrum's acid (268 mg, 1.86 mmol) was then dissolved in toluene (10 ml), and phenol (188 mg, 2 mmol, 1.1 eq) was added. The mixture was heated to reflux for 5 h. After cooling of the mixture to room temperature, 3,4-dihydroxybenzaldehyde (276 mg, 2 mmol, 1.1 eq), pyridine (0.5 ml), and piperidine (0.05 ml) were added. The mixture was stirred further 12 h at room temperature. After removal of the solvent, the mixture was diluted with 1 M HCl and extracted with ethyl acetate. The extract was washed with water and dried over anhydrous sodium sulfate. After removal of the solvent, the residue was purified by silica gel column chromatography (hexane-ethyl acetate 3:2) to give **7** (10%).

Preparation of (E)-4-(3,4-dihydroxyphenyl)but-3-en-2-one (10). To a stirred solution of 3,4-dihydroxybenzaldehyde (1.38 g, 10 mmol) in DMF (50 ml) were added ethyldiisopropylamine (6.45 g, 50 mmol, 5 eq) and methoxymethyl chloride (1.9 ml, 25 mmol, 2.5 eq). The mixture was stirred for 6 h at room temperature, diluted with water and extracted with ethyl acetate. The extract was washed with water and dried over anhydrous sodium sulfate. After removal of the solvent, the residue was purified by silica gel column chromatography (hexane-ethyl acetate 4:1) to give 3,4-bis(methoxymethoxy)benzladehyde (**10a**, 48%). The obtained **10a** (1.08 g, 4.8 mmol) was dissolved in methanol (25 ml) and acetone (1 ml), and KOH (2.8 g, 50 mmol, 10.4 eq) in water (5 ml) was added to the solution. The mixture was stirred at room temperature for 24 h. Then the mixture was poured into ice water (50 ml), neutralized with 1 M HCl, and extracted with ethyl acetate. The organic layer was washed with water and dried over anhydrous sodium sulfate. After removal of the solvent, the residue was purified by silica gel column chromatography (hexane-ethyl acetate 4:1) to give (*E*)-4-[3,4-bis(methoxymethoxy)phenyl]but-3-en-2-one (**10b**, 42%). To a stirred solution of **10b** (50 mg, 0.19 mmol) in methanol (3 ml), 6 M HCl (3 ml) was added dropwise. The mixture was stirred for 1 h, then diluted with water and extracted with ethyl acetate. The organic layer was washed with water and dried over anhydrous sodium sulfate. After removal of the solvent, the residue was purified by silica gel column chromatography (hexane-ethyl acetate 3:2) to give **10** (65%).

Preparation of methyl 3-(3,4-dihydroxyphenyl)propanoate (11). A stirred solution of **1** (1.94 g, 10 mmol) in methanol (30 ml) was hydrogenated using a balloon filled with H_2 for 24 h in the presence of 10% Pd-C (106 mg). After filtering of the catalyst, the solvent was removed and the residue was purified by silica gel column chromatography (hexane-ethyl acetate 3:2) to give **11** (63%).

Preparation of methyl 2,3,4-trihydroxycinnamate (13). To a stirred solution of 2,3,4-tris(methoxymethoxy)benzaldehyde[21] (1.43 g, 5 mmol) in dioxane (10 ml) were added (methoxycarbonylmethyl)triphenylphosphonium chloride (1.85 g, 5 mmol, 1 eq) in chloroform (10 ml) and $KHCO_3$ (2.5 g, 25 mmol, 5 eq). The mixture was refluxed for 6 h, and cooled to room temperature, and the resulting insoluble salt was filtered off. The filtrate was concentrated and purified by silica gel column chromatography (hexane-ethyl acetate 4:1) to give methyl 2,3,4-tris(methoxymethoxy)cinnamate (**13a**, 80%). **13a**: FD-HR-MS *m/z* (M^+): Calcd. for $C_{16}H_{22}O_8$: 342.1315, Found: 342.1317; ^{1}H NMR (chloroform-*d*) δ (ppm): 3.50 (3H, *s*, OCH_3), 3.60 (6H, *s*, 2 × OCH_3), 3.79 (3H, *s*, OCH_3), 5.13, 5.18, and 5.23 (each 2H, *s*, 3 × OCH_2), 6.38 (1H, *d*, *J* = 16.1 Hz, H-8), 6.96 (1H, *d*, *J* = 8.9 Hz, H-5), 7.28 (1H, *d*, *J* = 8.9 Hz, H-6), 8.02 (1H, *d*, *J* = 16.1 Hz, H-7). To a stirred solution of **13a** (50 mg, 0.19 mmol) in methanol (3 ml), 6 M HCl (3 ml) was added dropwise. The mixture was stirred for 1 h, then diluted with water and extracted with ethyl acetate. The organic layer was washed with water and dried over anhydrous sodium sulfate. After removal of the solvent, the residue was purified by silica gel column chromatog-

raphy (hexane-ethyl acetate 3:2) to give **13** (35%). **13**: FD-HR-MS *m/z* (M^+): Calcd. for $C_{10}H_{10}O_5$: 210.0528, Found: 210.0536; ^{1}H NMR (DMSO-d_6) δ(ppm): 3.66 (3H, *s*, OCH_3), 6.34 (1H, *d*, J = 8.5 Hz, H-6), 6.38 (1H, *d*, J = 16.1 Hz, H-8), 6.93 (1H, *d*, J = 8.5 Hz, H-5), 7.77 (1H, *d*, J = 16.1 Hz, H-7).

Preparation of methyl 3,4,5-trihydroxycinnamate (14). Following the method of preparing **13a**, 3,4,5-tris(methoxymethoxy)benzaldehyde[22] and (methoxycarbonylmethyl)triphenylphosphonium chloride were reacted to give methyl 3,4,5-tris(methoxymethoxy)cinnamate (**14a**) (76%). **14a**: FD-HR-MS *m/z* (M^+): Calcd. for $C_{16}H_{22}O_8$: 342.1315, Found: 342.1308; ^{1}H NMR (chloroform-*d*) δ(ppm): 3.51 (6H, *s*, 2 × OCH_3), 3.61 (3H, *s*, OCH_3), 3.80 (3H, *s*, OCH_3), 5.18, (2H, *s*, OCH_2), 5.21 (4H, *s*, 2 × OCH_2), 6.34 (1H, *d*, J = 15.9 Hz, H-8), 7.04 (2H, *s*, H-2, 6), 7.58 (1H, *d*, J = 15.9 Hz, H-7). Following the method of preparing **13**, **14a** was deprotected to give **14** (73%). **14**: FD-HR-MS *m/z* (M^+): Calcd. for $C_{10}H_{10}O_5$: 210.0528, Found: 210.0524; ^{1}H NMR (DMSO-d_6) δ(ppm): 3.67 (3H, *s*, OCH_3), 6.16 (1H, *d*, J = 15.9 Hz, H-8), 6.58 (2H, *s*, H-2, 6), 7.38 (1H, *d*, J = 15.9 Hz, H-7).

Results and Discussion

In the screening experiment, 109 samples showed more than 50% sucrase inhibitory activity and 222 samples showed more than 50% maltase inhibitory activity out of 524 samples from 325 plant species (Supplemental Table 1; see *Biosci. Biotechnol. Biochem.* Web site). Among these, notable inhibitory active species (>90%) against rat intestinal sucrase or maltase are shown in Table 1. Of these promising species, *Solanum torvum* fruit was chosen, as it had not been studied before as to glucosidase inhibitory activity and was available in large quantities. Also, *S. torvum* fruit is edible and might easily be applied in antidiabetic treatment. Therefore, we started an identification of the active principles of *S. torvum* fruit.

Table 1. Plant Species Showing Notable Inhibitory Activity against Rat Intestinal Sucrase or Maltase

Scientific name	Part	Inhibitory activity (%)	
		Sucrase	Maltase
Aleurites fordii	stem	33	100
Averrhoa bilimbi	leaf	30	100
Averrhoa carambola	leaf	30	96
Camellia japonica	stem	55	100
Cassia angustifolia	leaf	82	92
Citrus aurantium	fruit	100	100
Citrus depressa	fruit	99	89
Citrus hanayu	fruit	100	95
Derris elliptica	leaf	100	98
Derris elliptica	stem	100	97
Elaeocarpus sylvestris	leaf	61	90
Eugenia uniflora	leaf	92	88
Glochidion obovatum	leaf	42	90
Hibiscus acetosella	leaf	99	86
Ipomoea batatas (hanaimo)	stem	100	100
Ipomoea batatas (Shimon 1 gou)	stem	98	100
Liquidambar styraciflua	leaf	61	100
Morinda citrifolia	fruit	99	109
Morus australis	leaf	98	100
Morus australis	branch	95	100
Pittosporum tobira	leaf	100	100
Quassia amara	leaf	62	99
Solanum torvum	fruit	100	100
Styrax japonica	leaf	100	92
Swietenia macrophylla	leaf	36	91
Zanthoxylum schinifolium	stem	100	100

Dried fruits of *S. torvum* were extracted with 50% aqueous methanol. After evaporation, the crude extract was fractionated successively by hydrophobic resin column chromatography, solvent partition, silica gel column chromatography, and preparative HPLC to yield methyl caffeate (**1**) as an inhibitor against rat intestinal sucrase and maltase.

Methyl caffeate (**1**) showed moderate inhibitory activity, with IC_{50} values of 1.5 mM and 2.0 mM, against rat intestinal sucrase and maltase respectively. These activities are comparable to or stronger than those of ordinary flavonoid inhibitors.[23] A number of caffeoyl esters have been isolated from plants as α-glucosidase inhibitors.[3,7,24] Although caffeic acid is assumed to be the critical component in α-glucosidase inhibition, an ester moiety appeared to affect α-glucosidase inhibition also. Hence, to investigate the effects of the ester moiety together with the caffeoyl moiety against α-glucosidase inhibition, we synthesized or purchased a series of caffeoyl ester **2–8** and methyl caffeate analogs **9–14**, and tested for sucrase and maltase inhibitory activities. The compounds tested included four linear alkyl caffeates (**2–4**), two branched-chain alkyl caffeates (**5**, **6**), phenyl caffeate (**7**), a ketone analog (**10**), methyl dihydrocaffeate (**11**), and two trihydroxycinnamates (**13**, **14**), and chlorogenic acid (**8**), caffeic acid (**9**), and methyl protocatechuate (**12**) (Fig. 1).

The results are summarized in Table 2 and Supplemental Figs. 1–10 (see *Biosci. Biotechnol. Biochem.* Web site). In contrast to the moderate activities of methyl caffeate (**1**) against sucrase and maltase, compounds **2**, **3**, and **4**, possessing longer alkyl chains than **1**, showed slight decreases in sucrase inhibition. In branched-chain esters **5** and **6**, sterically hindered *tert*-butyl ester **6** showed less sucrase inhibitory activity than smaller isopropyl ester **5**. In contrast, the maltase inhibitory activity of compounds **2–6** remained unchanged compared to that of **1**. These data suggest that a larger alkyl group in the ester moiety was unfavorable to sucrase inhibition in caffeoyl esters regardless of linear or branched chains, and that maltase-inhibitory activity was not influenced by changes in the size or shape of the alkyl group. The sucrase inhibitory activity of phenyl ester **7** remained unchanged as compared to **1**. So the presence of an aromatic ring in the ester moiety is probably effective for sucrase inhibition even though it is sterically bulky. Maybe the electronic effect of the aromatic ring affects its conjugated caffeoyl moiety or interaction with the enzyme, but the details were not clear. A naturally abundant caffeic ester, chlorogenic acid (**8**), showed decreased inhibitory activity against both sucrase and maltase as compared to **1**. This result also confirms the disadvantage of a sterically hindered ester for sucrase inhibition. On the other hand, the decreased maltase inhibitory activity of **8** might have been due to the hydrophilicity of the quinic ester moiety, as the steric effect does not alter maltase inhibition, as suggested by results for compounds **2–6**. Caffeic acid (**9**) and (*E*)-4-(3,4-dihydroxyphenyl)but-3-en-2-one (**10**) also showed decreases in the maltase inhibitory activity, but the decrease in the sucrase inhibitory activity was not very large. The presence of a hydrophobic ester group appeared to be important to maltase inhibition regardless of its size. These caffeoyl