Occurrence of a Novel Cannabimimetic Molecule 2-Sciadonoylglycerol (2-Eicosa-5′,11′,14′-trienoylglycerol) in the Umbrella Pine *Sciadopitys verticillata* Seeds

Shinji NAKANE,[a] Tamotsu TANAKA,[b] Kiyoshi SATOUCHI,[b] Yuriko KOBAYASHI,[a] Keizo WAKU,[a] and Takayuki SUGIURA*,[a]

Faculty of Pharmaceutical Sciences, Teikyo University,[a] Sagamiko, Kanagawa 199–0195, Japan and Department of Applied Biological Sciences, Fukuyama University,[b] Fukuyama 729–0292, Japan.
Received November 29, 1999; accepted February 26, 2000

The umbrella pine *Sciadopitys verticillata* seeds were found to contain a substantial amount (16.7 nmol/g) of sciadonic acid (all-*cis*-5,11,14-eicosatrienoic acid)-containing 2-monoacylglycerol, *i.e.*, 2-sciadonoylglycerol (2-eicosa-5′,11′,14′-trienoylglycerol). Because the structure of 2-sciadonoylglycerol closely resembles that of 2-arachidonoylglycerol, the endogenous natural ligand for the cannabinoid receptor, we examined whether or not 2-sciadonoylglycerol exhibits cannabimimetic activity using NG108-15 neuroblastoma×glioma hybrid cells which express the cannabinoid CB1 receptor. We found that 2-sciadonoylglycerol induces rapid transient elevation of intracellular free Ca^{2+} concentration in NG108-15 cells through a cannabinoid CB1 receptor-dependent mechanism similar to the case of 2-arachidonoylglycerol, yet the activity of 2-sciadonoylglycerol was apparently lower than that of 2-arachidonoylglycerol. The activity of 2-sciadonoylglycerol was detectable from 3—10 nM, reaching a maximum at around 10 μM. To our knowledge, this is the first report showing the occurrence of a cannabimimetic monoacylglycerol in higher plants.

Key words cannabinoid; 2-arachidonoylglycerol; anandamide; sciadonic acid (5,11,14-eicosatrienoic acid); monoacylglycerol; umbrella pine

Marijuana and hashish, well-known products of the hemp *Cannabis sativa* have been used as traditional medicines or psychoactive drugs for centuries throughout the world. In 1964, Gaoni and Mechoulam[1] isolated Δ^9-tetrahydrocannabinol (Δ^9-THC) from hashish as a major psychoactive constituent. Δ^9-THC has been shown to exert a variety of pharmacological activities *in vitro* and *in vivo* which have also been observed with marijuana or hashish. For example, Δ^9-THC induces euphoria, heightened sensory awareness, altered cognition, inhibition of memory, reduced mobility, analgesia and promotion of appetite in humans as well as experimental animals.[2]

The mechanism underlying various *in vitro* and *in vivo* actions of Δ^9-THC remained obscure until the late 1980's. In 1988, Devane *et al.*[3] provided clear evidence that there is a specific binding site for cannabinoids in rat brain synaptosomes using a radiolabeled ligand [^{3}H]CP55940. Soon thereafter, Matsuda *et al.*[4] cloned a cDNA encoding the cannabinoid receptor from a rat brain cDNA library. It is becoming evident that various pharmacological effects of Δ^9-THC are mediated mostly through specific receptors expressed on the cell surface.

What, then, is the endogenous ligand for such specific receptors for cannabinoids? To date, two types of arachidonic acid-containing molecules have been reported as putative endogenous ligands: *N*-arachidonoylethanolamine (anandamide)[5] and 2-arachidonoylglycerol (2-AG).[6—8] We recently provided evidence that 2-AG is the natural ligand for the cannabinoid receptor expressed in the nervous tissues termed the CB1 receptor.[9—11] 2-AG is a potent full agonist toward the CB1 receptor: the agonistic activity of 2-AG was detectable from as low as 1 nM.[9—11] We have also demonstrated that 2-AG is widely distributed in various mammalian tissues[12]; most notably, it is one of the most abundant molecular species of monoacylglycerols in the brain.[7,12] A question then arises regarding whether 2-AG or its structural analogues are present in living organisms other than animals, because monoacylglycerols are known to exist to some extent in various living organisms. It is an important issue to explore the possibility of the occurrence of cannabimimetic monoacylglycerols in living organisms other than animals. In particular, it will be of great value to learn whether or not 2-AG or its structural analogues are present in plants, because Δ^9-THC, a well-known cannabinoid receptor ligand, is of plant origin.

Here, we investigated whether 2-AG analogues are present in the umbrella pine *Sciadopitys verticillata* seeds which are enriched in sciadonic acid (all-*cis*-5,11,14-eicosatrienoic acid, podocarpic acid), a non-methylene-interrupted arachidonic acid analogue.

MATERIALS AND METHODS

Chemicals Arachidonic acid (all-*cis*-5,8,11,14-eicosatetraenoic acid) and essentially fatty acid-free bovine serum albumin were purchased from Sigma (St. Louis, MO, U.S.A.). Fura-2/AM, glycerol, benzaldehyde and butylated hydroxytoluene were obtained from Wako Pure Chemical Ind. (Osaka, Japan). SR141716A was acquired from Biomol (Plymouth Meeting, PA). Sciadonic acid was prepared from the umbrella pine *Sciadopitys verticillata* seeds as described earlier.[13]

Synthesis of Monoacylglycerols 1,3-Benzylidene-*sn*-glycerol was prepared from glycerol and benzaldehyde using sulfosalicylic acid as a catalyst. Fatty anhydrides were prepared from the respective fatty acids by treatment with dicyclohexylcarbodiimide. 2-Acyl-1,3-benzylideneglycerol was synthesized from 1,3-benzylideneglycerol and fatty anhydrides using dimethylaminopyridine as a catalyst; it was then purified by TLC using petroleum ether–diethyl ether–acetic

* To whom correspondence should be addressed. e-mail: sugiurat@pharm.teikyo-u.ac.jp

acid (80 : 20 : 1, v/v) as the solvent system (*Rf* 0.22) as described previously.[11] Purified 2-acyl-1,3-benzylideneglycerols were then treated with boric acid and boric acid trimethyl ester, at 85 °C under vacuum for 3 min, to yield 2-monoacylglycerols.[11] 2-Monoacylglycerols were purified by TLC using petroleum ether–diethyl ether–acetic acid (20 : 80 : 1, v/v) as the solvent system (*Rf* 0.23). 2-Monoacylglycerols were further purified by boric acid-impregnated TLC using chloroform–methanol–acetic acid (98 : 2 : 1, v/v) as the solvent system (*Rf* 0.61).

Extraction and Purification of Acylglycerol from the Umbrella Pine *Sciadopitys verticillata* Seeds The umbrella pine *Sciadopitys verticillata* seeds were homogenized in a mixture of chloroform–methanol–water (1 : 2 : 0.8, v/v) using a glass-Teflon homogenizer. Total lipids were extracted by the method of Bligh and Dyer.[14] Acylglycerols (triacylglycerol, diacylglycerol and monoacylglycerol) were separated by TLC using petroleum ether–diethyl ether–acetic acid (20 : 80 : 1, v/v) as the solvent system. 1(3)-Monoacylglycerol and 2-monoacylglycerol were separated from each other by borate-impregnated TLC. Monoacylglycerols were further purified by TLC using chloroform–methanol–acetic acid (98 : 2 : 1, v/v) as the solvent system.

Fatty Acid Composition of Acylglycerols Fatty acyl moieties of the acylglycerols (triacylglycerol, diacylglycerol and monoacylglycerol) were converted to fatty acid methyl esters and analyzed by gas chromatography (GC) using a gas chromatograph (GC8A, Shimadzu, Kyoto, Japan) equipped with a fused column (SP2330, Supelco, Bellefonte, PA, U.S.A.). Heptadecanoic acid methyl ester was used as an internal standard.

GC/MS Analyses of *tert*-Butyldimethylsilyl (*t*BDMS) Derivatives of Monoacylglycerols *t*BDMS derivatives of monoacylglycerols were prepared according to the method described[15] with a slight modification. Briefly, monoacylglycerols were dissolved in 0.1 ml of dimethylformamide containing 72 mg of *tert*-butyldimethylchlorosilane and 20 mg of imidazole. The mixtures were heated at 50 °C for 10 min, then mixed with 4 ml each of *n*-hexane and water. After vigorous shaking, the *n*-hexane layer was aspirated to another glass tube and washed with water. The hexane layer containing *t*BDMS derivatives of monoacylglycerols was evaporated under a stream of nitrogen, and the residue was dissolved in *n*-hexane for GC/MS. Analyses of *t*BDMS derivatives of monoacylglycerols were carried out on a Shimadzu QP-2000 quadrapole mass spectrometer equipped with an interface for capillary GC (column coated with a 0.25-μm film of nonpolar CBJ1, 0.25 mm×30 m, Shimadzu) as described.[13] The column temperature was raised from 80 °C to 225 °C at a rate of 30 °C/min and from 225 °C to 290 °C at a rate of 5 °C/min.

Cells NG108-15 cells were provided by Prof. H. Higashida (Kanazawa University School of Medicine, Kanazawa, Japan). NG108-15 cells were grown at 37 °C in Dulbecco's modified Eagle's minimum essential medium supplemented with 10% fetal bovine serum, hypoxantine, aminopterine, and thymidine under an atmosphere of 90% air and 10 % CO_2.

Measurement of $[Ca^{2+}]_i$ Subconfluent cells were further incubated in fresh medium without FBS for 24 h. They were next suspended by gentle pipetting in Hepes-Tyrode solution ($-Ca^{2+}$) containing 3 μM Fura-2/AM and further incubated at 37 °C for 45 min. The cells were then centrifuged (180×***g*** for 5 min), washed twice with Hepes-Tyrode solution ($-Ca^{2+}$), and resuspended in Hepes-Tyrode solution ($-Ca^{2+}$) containing 0.1% BSA. $[Ca^{2+}]_i$ was estimated using a CAF-100 Ca^{2+} analyzer (JASCO, Tokyo, Japan) as previously described.[9—11] $CaCl_2$ was added 4—5 min before the measurement (final Ca^{2+} concentration in the cuvette, 1 mM). Monoacylglycerols were dissolved in dimethyl sulfoxide (DMSO), and aliquots (1 μl each) were added to the cuvette (final DMSO concentration, 0.2%). DMSO (final concentration, 0.4%) *per se* did not markedly affect the $[Ca^{2+}]_i$.

RESULTS AND DISCUSSION

The umbrella pine *Sciadopitys verticillata* seeds contain large amounts of neutral lipids. The predominant component was triacylglycerol (238 μmol/g weight), and substantial amounts of 1,3-diacylglycerol and 1(3),2-diacylglycerol were also included (1706 nmol/g weight and 1462 nmol/g weight, respectively). We further found that the seeds contain small amounts of 2-monoacylglycerol (191.6 nmol/g weight) and 1(3)-monoacylglycerol (231.6 nmol/g weight). Table 1 shows the fatty acid composition of acylglycerols obtained from the umbrella pine seeds. The fatty acid profiles of triacylglycerol, diacylglycerol and monoacylglycerol generally resemble each other: the major fatty acyl constituents are linoleic acid (*cis*,*cis*-9,12-octadecadienoic acid), oleic acid (*cis*-9-octadecenoic acid) plus *cis*-vaccenic acid (*cis*-11-octadecenoic acid), palmitic acid (hexadecanoic acid) and sciadonic acid in each case. The presence of the unusual fatty acid sciadonic acid is a characteristic feature of several gymnosperms such as the pine, podocarp and ginkgo.[16]

The occurrence of 2-sciadonoylglycerol in the umbrella pine seeds is quite noticeable, because its structure is closely

Table 1. Fatty Acyl Moieties of Triacylglycerol, Diacylglycerol and Monoacylglycerol in the Umbrella Pine Seeds

Fatty acids	Triacylglycerol[a]		1,3-Diacylglycerol[a]		1(3),2-Diacylglycerol[a]		2-Monoacylglycerol[b]		1(3)-Monoacylglycerol[b]	
	μmol/g weight	(%)	nmol/g weight	(%)	nmol/g weight	(%)	nmol/g weight	(%)	nmol/g weight	(%)
16 : 0	26	(3.7)	385	(11.3)	367	(12.6)	17.4	(9.1)	25.5	(11.0)
18 : 0	19	(2.7)	171	(5.0)	137	(4.7)	8.4	(4.4)	12.2	(5.3)
18 : 1 (Δ-9)/18 : 1(Δ-11)	181	(25.4)	1015	(29.7)	893	(30.5)	67.3	(35.1)	89.5	(38.7)
18 : 2 (Δ-9, 12)	360	(50.3)	1198	(35.1)	949	(32.5)	77.9	(40.7)	82.0	(35.4)
20 : 3 (Δ-5, 11, 14)	82	(11.4)	185	(5.4)	168	(5.8)	16.7	(8.7)	17.2	(7.4)
Others	46	(6.5)	458	(13.5)	410	(13.9)	3.9	(2.0)	5.2	(2.2)
Total	714	(100.0)	3412	(100.0)	2924	(100.0)	191.6	(100.0)	231.6	(100.0)
(Amounts as acylglycerols	238		1706		1462		191.6		231.6	)

Mean values were taken from *a*) 2 or *b*) 3 determinations.

related to that of a potent bioactive lipid 2-AG (Fig. 1). Indeed, there is only one structural difference between these two molecules: 2-AG contains 4 double bonds (Δ-5,8,11,14) in its acyl moiety, whereas 2-sciadonoylglycerol contains 3 double bonds (Δ-5,11,14) and lacks the double bond at the Δ-8 position. We further confirmed the structure of the *t*BDMS derivative of 2-sciadonoylglycerol obtained from the umbrella pine seeds by GC/MS (Fig. 2): *m/z* 551 for $[M-C(CH_3)_3]^+$; *m/z* 363 for $[RCO+OSi(CH_3)_2]^+$; *m/z* 245 for $[M-C(CH_3)_3-RCOOH]^+$; *m/z* 171 for $[M-C(CH_3)_3-RCOOH-OSi(CH_3)_2]^+$.

We next investigated whether or not 2-sciadonoylglycerol possesses cannabimimetic activity similar to 2-AG. Previously, we found that various cannabimimetic molecules induce rapid transient elevation of $[Ca^{2+}]_i$ in NG108-15 cells through a cannabinoid CB1 receptor-dependent mechanism. In this study, we employed this assay system to evaluate the capacity to act as a cannabinoid receptor agonist. Figure 3 shows the effects of 2-AG and 2-sciadonoylglycerol on $[Ca^{2+}]_i$ in NG108-15 cells. 2-AG was found to induce rapid transient increases in $[Ca^{2+}]_i$ in NG108-15 cells (Fig. 3 (A)):

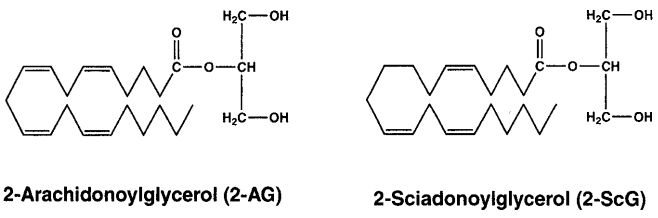

Fig. 1. Structures of 2-Arachidonoylglycerol and 2-Sciadonoylglycerol

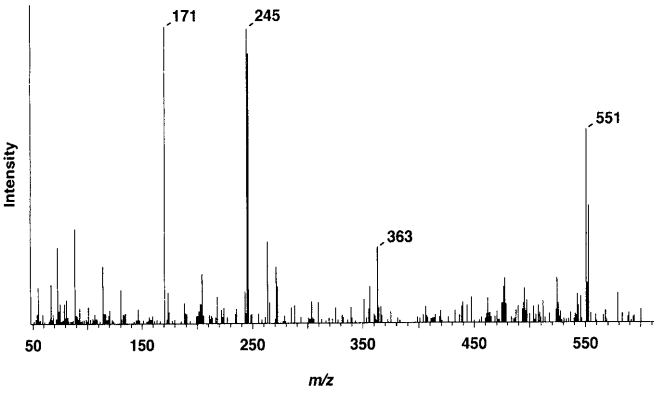

Fig. 2. Mass Spectrum of the *t*BDMS Derivative of 2-Sciadonoylglycerol Obtained from Umbrella Pine Seeds

GC/MS analysis was carried out as described in Materials and Methods. The retention time of the *t*BDMS derivative of 2-sciadonoylglycerol was 23.65 min.

the response was detectable from as low as 0.3—1 nM, reaching a plateau at around 1 μM. These results are in general agreement with those obtained in previous studies.[9—11] Here, we found that 2-sciadonoylglycerol possesses appreciable agonistic activity, although its activity was lower than that of 2-AG (Fig. 3 (B)): the response was detectable from 3—10 nM, reaching the maximum at around 10 μM. We confirmed that the response induced by either 2-AG (3 μM) or 2-sciadonoylglycerol (3 μM) was blocked by pretreatment of the cells with a cannabinoid CB1 receptor antagonist SR141716A (3 μM) (Fig. 4).

Sciadonic acid (5,11,14-eicosatrienoic acid), present in several gymnosperms, is a noticeable molecule from a variety of viewpoints.[17—20] Tanaka and co-workers[17] and Berger and German[18] have demonstrated that the administration or the addition of sciadonic acid (podocarpic acid) to animals or cultured cells is effective in reducing the level of arachidonic acid in phosphatidylinositol, thereby modifying cellular arachidonic acid metabolism. Interestingly, in contrast to arachidonic acid, sciadonic acid is resistant to the action of cyclooxygenase due to lack of a double bond at the Δ-8 position. Tanaka and co-workers[19] also demonstrated that sciadonic acid (podocarpic acid) is a valuable experimental tool to investigate the fatty acid chain elongation system. Here, we report that the sciadonic acid-containing species of 2-monoacylglycerol, *i.e.*, 2-sciadonoylglycerol isolated from the umbrella pine seeds is a rather potent cannabinoid CB1 receptor agonist. To our knowledge, this is the first report showing the occurrence in higher plants of a molecule, other than cannabinoids, capable of exhibiting apparent activity as a cannabinoid CB1 receptor agonist. Although the activity of 2-sciadonoylglycerol was 10 to 30 times less potent than that of 2-AG (Fig. 3), its agonistic activity was apparently greater

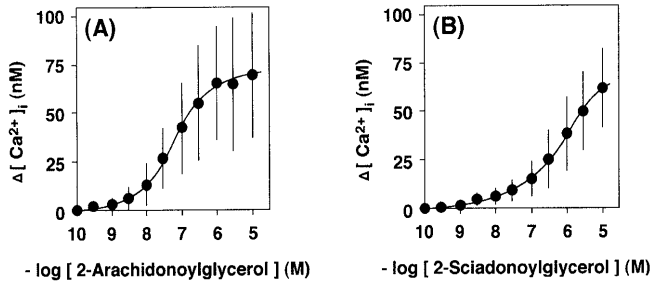

Fig. 3. Effects of 2-Arachidonoylglycerol and 2-Sciadonoylglycerol on $[Ca^{2+}]_i$ in NG108-15 Cells

Effects of 2-arachidonoylglycerol (A) and 2-sciadonoylglycerol (B) on $[Ca^{2+}]_i$ were examined using Fura-2/AM and a CAF-100 Ca^{2+} analyzer. $[Ca^{2+}]_i$ was calculated from the ratio of F380 and F340. The data are the means±S.D. of 5 determinations.

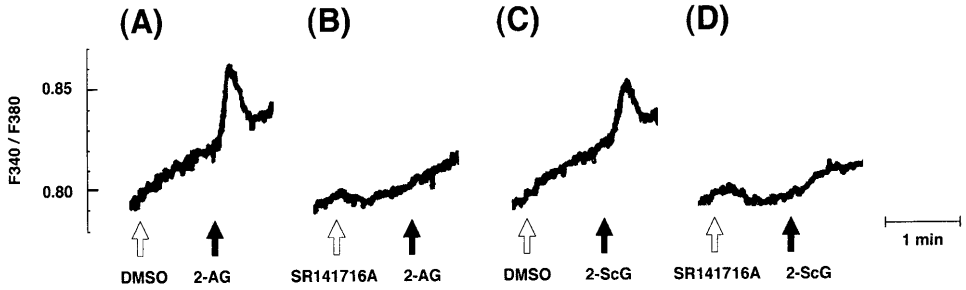

Fig. 4. Effects of SR141716A on 2-Arachidonoylglycerol- or 2-Sciadonoylglycerol-Induced Rapid Elevation of $[Ca^{2+}]_i$ in NG108-15 Cells

Cells loaded with Fura-2/AM were treated with 3 μM SR141716A ((B) and (D)) or the vehicle alone (DMSO) ((A) and (C)) for 1 min. The cells were then challenged with 3 μM 2-arachidonoylglycerol (2-AG) ((A) and (B)) or 3 μM 2-sciadonoylglycerol (2-ScG) ((C) and (D)). Changes in $[Ca^{2+}]_i$ were expressed as changes in the ratio of F380 and F340.

than that of anandamide, a partial agonist of the cannabinoid receptors, reported in previous studies.[9,11] 2-Sciadonoylglycerol should be a valuable experimental tool in future studies on the cannabinoid receptor-endogenous cannabinoid receptor ligand system. In fact, the finding that 2-sciadonoylglycerol exhibits appreciable cannabimimetic activity (Fig. 3) provided further evidence that the presence of the double bond at the Δ-5 position is crucially important and acts as a potent cannabinoid receptor agonist.[11] We have previously shown that 2-eicosa-5′,8′,11′,14′,17′-pentaenoylglycerol and 2-eicosa-5′,8′,11′-trienoylglycerol as well as 2-AG (2-eicosa-5′,8′,11′,14′-tetraenoylglycerol) exhibit strong agonistic activities, whereas the activities of 2-eicosa-8′,11′,14′-trienoylglycerol and 2-eicosa-11′,14′,17′-trienoylglycerol are very low.[11]

Apparently 2-sciadonoylglycerol is a minor component of acylglycerols in the umbrella pine seed: its level in the seeds is in the order of nmol/g weight (16.7 nmol/g) (Table 1). Nevertheless, this level is several times higher than that of 2-AG found in mammalian tissues.[7,12,21,22] The tissue levels of 2-AG are 3.36, 1.15, 1.17, 0.78 and 0.98 nmol/g weight for rat brain, liver, spleen, lung and kidney, respectively,[12] and these concentrations of 2-AG are sufficient to stimulate various types of mammalian tissues and cells through the cannabinoid CB1 receptor[9—11] or the CB2 receptor.[23] In addition to pre-existing 2-sciadonoylglycerol molecules, a significant amount of 2-sciadonoylglycerol may also be generated from sciadonic acid-containing triacylglycerol in the seeds through lipolysis in the process of germination, because it is well known that the degradation of stored triacylglycerol proceeds in seeds during germination.[24] Whether or not the generation of 2-sciadonoylglycerol takes place in umbrella pine seeds during germination should be determined in the future.

So far, there is no clear evidence supporting the occurrence of cannabinoid receptors in plants. It remains unclear at present whether or not a cannabimimetic molecule 2-sciadonoylglycerol plays some physiological role in several gymnosperms such as umbrella pine, as is the case for cannabinoids in cannabis. There is a long-unanswered question. Why do several plants possess potent molecules which are bioactive toward animals? Studies on a cannabimimetic molecule 2-sciadonoylglycerol in gymnosperms may be helpful to elucidate the mechanism as well as the evolutional inevitability leading to the production of cannabinoids in cannabis.

REFERENCES

1) Gaoni Y., Mechoulam R., *J. Am. Chem. Soc.*, **86**, 1646—1647 (1964).
2) Dewey W. L., *Pharmacol. Rev.*, **38**, 151—178 (1986).
3) Devane W. A., Dysarz F. A., III, Johnson M. R., Melvin L. S., Howlett A. C., *Mol. Pharmacol.*, **34**, 605—613 (1988).
4) Matsuda L. A., Lolait S. J., Brownstein M. J., Young A. C., Bonner T. I., *Nature* (London), **346**, 561—564 (1990).
5) Devane W. A., Hanus L., Breuer A., Pertwe R. G., Stevenson L. A., Griffin G., Gibson D., Mandelbaum A., Etinger A., Mechoulam R., *Science*, **258**, 1946—1949 (1992).
6) Sugiura T., Itoh K., Waku K., Hanahan D. J., *Proc. Jpn. Conf. Biochem. Lipids* (in Japanese), **36**, 71—74 (1994).
7) Sugiura T., Kondo S., Sukagawa A., Nakane S., Shinoda A., Itoh K., Yamashita A., Waku K., *Biochem. Biophys. Res. Commun.*, **215**, 89—97 (1995).
8) Mechoulam R., Ben-Shabat S., Hanus L., Ligumsky M., Kaminski N. E., Schatz A. R., Gopher A., Almog S., Martin B. R., Compton D. R., Pertwee R. G., Griffin G., Bayewitch M., Barg J., Vogel Z., *Biochem. Pharmacol.*, **50**, 83—90 (1995).
9) Sugiura T., Kodaka T., Kondo S., Tonegawa T., Nakane S., Kishimoto S., Yamashita A., Waku K., *Biochem. Biophys. Res. Commun.*, **229**, 58—64 (1996).
10) Sugiura T., Kodaka T., Kondo S., Nakane S., Kondo H., Waku K., Ishima Y., Watanabe K., Yamamoto I., *J. Biochem.* (Tokyo), **122**, 890—895 (1997).
11) Sugiura T., Kodaka T., Nakane S., Miyashita T., Kondo S., Suhara Y., Takayama H., Waku K., Seki C., Baba N., Ishima Y., *J. Biol. Chem.*, **274**, 2794—2801 (1999).
12) Kondo S., Kondo H., Nakane S., Kodaka T., Tokumura A., Waku K., Sugiura T., *FEBS Lett.*, **429**, 152—156 (1998).
13) Tanaka T., Shibata K., Hino H., Murashita T., Kayama M., Satouchi K., *J. Chromatogr.*, **B700**, 1—8 (1997).
14) Bligh E. G., Dyer W. J., *Can. J. Biochem. Physiol.*, **37**, 911—917 (1959).
15) Tanaka T., Minamino H., Unezaki S., Tsukatani H., Tokumura A., *Biochim. Biophys. Acta*, **1166**, 264—274 (1993).
16) Takagi T., Itabashi Y., *Lipids*, **17**, 716—723 (1982).
17) Tanaka T., Takimoto T., Morishige J.-I., Kikuta Y., Sugiura T., Satouchi K., *Biochem. Biophys. Res. Commun.*, **264**, 683—688 (1999).
18) Berger A., German J. B., *Biochim. Biophys. Acta*, **1085**, 371—376 (1991).
19) Tanaka T., Hattori T., Kouchi M., Hirano K., Satouchi K., *Biochim. Biophys. Acta*, **1393**, 299—306 (1998).
20) Assert G., Staels B., Wolff R. L., Bauge E., Madj Z., Fruchart J.-C., Dallongeville J., *Lipids*, **34**, 39—44 (1999).
21) Stella N., Schweitzer P., Piomelli D., *Nature* (London), **388**, 773—778 (1997).
22) Bisogno T., Berrendero F., Ambrosino G., Cebeira M., Ramos J. A., Fernandez-Ruiz J. J., Di Marzo V., *Biochem. Biophys. Res. Commun.*, **256**, 377—380 (1999).
23) Sugiura T., Kondo S., Kishimoto S., Miyashita T., Nakane S., Kodaka T., Suhara Y., Takayama H., Waku K., *J. Biol. Chem.*, **275**, 605—612 (2000).
24) Brockerhoff H., Jensen R. G., "Lipolytic Enzymes," Academic Press, New York, 1974.