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L-735,334, A NOVEL SESQUITERPENOID POTASSIUM CHANNEL-AGONIST FROM *TRICHODERMA VIRENS*

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ABSTRACT.—A novel oleic acid ester of the carotane sesquiterpene 14-hydroxy CAF-603 was isolated from *Trichoderma virens* grown in a solid brown rice-based medium, a solid millet-based medium, or a mannitol-based liquid medium. Its structure was determined on the basis of ms and nmr analysis. It retains distinct biological activity on the high conductance calcium-activated potassium channel, unlike its analogues 14-hydroxy CAF-603, CAF-603 3-oleate, or CAF-603 3-linoleate.

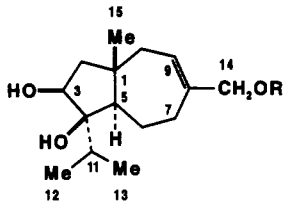
The sesquiterpene CAF-603 [**3**] was isolated originally from a strain of *Trichoderma virens* (*Gliocladium virens* IFO 9166) and its antifungal activity against *Candida albicans* reported (1). The compound has been isolated repeatedly from several isolates of *T. virens* in our laboratories. In fact, CAF-603 was also the major compound isolated from the batch which provided the title compound L-735,334 [**2**] the structure of which was established as 14-hydroxy CAF-603 oleate by spectroscopic means. Hydrolysis yielded the precursor 14-hydroxy CAF-603 of **2**, which has never been reported previously. Several fatty acid esters of CAF-603 were prepared to compare their biological activity with that of 14-hydroxy CAF-603 oleate. We now report the production, discovery, isolation, and structure determination of 14-hydroxy CAF-603 oleate and related compounds and their potential activity towards high conductance calcium-activated potassium (maxi-K) channels.

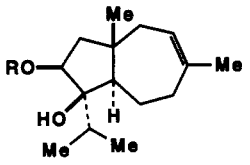
RESULTS AND DISCUSSION

When the producing cultures were incubated under static conditions at 25°, with 50% relative humidity in both liquid and solid fermentation media under constant fluorescent light, the production of **2** occurred by the 21st day. Two other isolates of *T. virens* from the Merck Culture Collection (MF5786; MF5787) were also used to produce this novel carotane sesquiterpene. The solid and liquid fermentation broths were extracted with methyl ethyl ketone, as described in the Experimental.

The initial solvent partition was followed by successive chromatography on SiO_2 ($MeOH/CH_2Cl_2$) and Bakerbond C_{18} ($MeOH/H_2O$). The two active fractions obtained were then subjected, respectively, to reversed-phase hplc on Partisil 10 ODS-3 to yield CAF-603 [**3**] and 14-hydroxy CAF-603 oleate [**2**], respectively.

High-resolution eims of **3** indicated the molecular formula $C_{15}H_{26}O_2$ (M^+ 238),





1 R=H
2 R=$CH_3(CH_2)_7CH{=}CH(CH_2)_7CO$

3 R=H
4 R=$CH_3(CH_2)_7CH{=}CH(CH_2)_7CO$
5 R=$CH_3(CH_2)_4CH{=}CHCH_2CH{=}CH(CH_2)_7CO$

consistent with the sesquiterpene CAF-603 isolated previously from the culture broth of *T. virens* (1). The identity of **3** was corroborated by direct ^1H- and ^{13}C-nmr comparison with a sample isolated from *T. virens*, the structure of which was confirmed independently by single-crystal X-ray diffraction (2). However, our nmr data at room temperature in $CDCl_3$ differ in certain respects from those reported by Watanabe *et al.* (1) which may indicate concentration- and/or temperature-dependence.

High-resolution eims of **2** indicated the molecular formula $C_{33}H_{58}O_4$ (M^+ 518). Loss of C_3H_7 and H_2O from the molecular ion resulted in the fragment at *m/z* 457 ($C_{30}H_{49}O_3$) which in turn gave rise to the base peak at *m/z* 193 ($C_{12}H_{17}O_2$) corresponding to the loss of $C_{18}H_{32}O$. These data are consistent with a structure comprising a C_{18} monounsaturated fatty acid ester derivative of a hydroxy CAF-603 carotane sesquiterpene skeleton in which the location of the ester remained uncertain. ^{13}C-Nmr analysis confirmed the carbon and carbon-bound proton counts of 33 and 56, respectively, suggesting two exchangeable OH protons. The overall skeletal features were confirmed by 2D nmr analysis involving COSY, HMQC, and HMBC experiments which pinpointed the location of the ester side-chain at C-14 (see Figure 1). Moreover, the almost identical ^{13}C-nmr assignments for the five-membered ring and side-chain carbons with CAF-603 suggested the same stereochemistry as determined for CAF-603 (1,2). Comparison of the ^{13}C-nmr resonances of the C_{18} ester side-chain with those of oleic acid in CD_3OD, after assignment of those of the sesquiterpene skeleton, established the structure of **2** as the oleic ester of 14-hydroxy CAF-603. The structure was confirmed by alkaline hydrolysis which readily afforded the 14-hydroxy CAF-603 [**1**] skeleton. In particular, direct support for the location of the ester moiety was obtained by the upfield shift of the H-14 methylene protons from δ 4.46 to δ 3.96.

CAF-603 [**3**] was treated with corresponding acid anhydrides, either oleic or linoleic, in triethylamine in the presence of 4-dimethylaminopyridine to yield the fatty acid esters **4** and **5**. Their structures were readily assigned by ms and nmr analysis. In

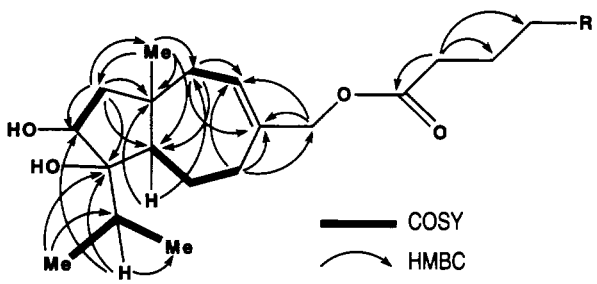


FIGURE 1. COSY and HMBC connectivities observed in L-735,334 (14-hydroxy CAF-603 oleate) [**2**].

particular, the location of the ester moiety at C-3 followed readily from the ^{1}H-nmr evidence; H-3 in both cases was involved in a 1-ppm downfield shift.

Charybdotoxin (ChTX) is a 37-amino-aicd peptide, obtained from a scorpion venom, which inhibits maxi-K channels with high potency by a mechanism that involves physical occlusion of the channel's pores (3). The effect of carotane sesquiterpenes on the binding of [^{125}I]ChTX to the maxi-K channel was studied. 14-Hydroxy CAF-603 oleate [**2**] caused a concentration-dependent inhibition of [^{125}I]ChTX binding to receptor sites in bovine aortic sarcolemma membrane vesicles that are associated with maxi-K channels. The concentration of 14-hydroxy CAF-603 oleate needed to cause 50% inhibition of binding was ca. 360 nM. CAF-603 oleate [**4**] was a much weaker inhibitor of ChTX binding (i.e., 37% inhibition at 100 μM), whereas CAF-603 linoleate [**5**] had no effect up to 100 μM. CAF-603 [**3**] caused a concentration-dependent inhibition of [^{125}I]ChTX binding with half-maximal inhibition at 200 nM, while 14-hydroxy CAF-603 [**1**] had no effect on ChTX binding at concentrations up to 100 μM.

The effects of 14-hydroxy CAF-603 oleate [**2**] on maxi-K channels were examined in excised inside-out membrane patches and in lipid bilayers. In patch clamp experiments, 10 μM 14-hydroxy CAF-603 oleate caused clear increases in channel open probability that developed slowly over 5–10 min. These increases in channel open probability were slowly reversed after long (20–60 min) washout. In lipid bilayer experiments, addition of 10 μM 14-hydroxy CAF-603 oleate to the outside face of the channel had no significant effect on channel open probability. Subsequent addition of 10 μM 14-hydroxy CAF-603 oleate to the inside face of the channel caused a clear increase in channel open probability, suggesting that this compound is more effective when added to the inside than when added to the outside.

The effects of four related compounds, CAF-603 oleate [**4**], CAF-603 linoleate [**5**], 14-hydroxy CAF-603 [**1**], and CAF-603 [**3**] on maxi-K channels were investigated in excised, inside-out patch clamp recordings. Application of 10 μM CAF-603 linoleate or CAF-603 oleate to the intracellular side of the channel caused small decreases in channel open probability. Application of 10 μM 14-hydroxy CAF-603 to the intracellular side had no observable effect on channel open probability. CAF-603 [**1**] had no clear effect on channel open probability at 10 μM, despite its ability to block [^{125}I]ChTX binding to the maxi-K channel.

Potassium-channel agonists are useful for a number of physiological disorders. Ion channels, including potassium channels, are found in all mammalian cells and are involved in the modulation of various physiological processes and normal cellular ionic homeostasis. Potassium ions generally control the resting membrane potential and the efflux of potassium ions causes repolarization of the plasma membrane. Among the different subtypes of potassium channel and one of the most physiologically important is the high conductance Ca^{2+}-activated K^+ (maxi-K) channel which is present in neuronal tissue and smooth muscle. Intracellular calcium concentration ($[Ca^{2+}]_i$) and membrane potential gate these channels. Maxi-K channels are opened by increases in $[Ca^{2+}]_i$ or membrane depolarization that results in neurotransmitter release and smooth muscle contraction. Therefore, modulation of maxi-K channel activity can affect neurotransmitter release from the nerve terminal and the contractability of various smooth muscle tissues. Maxi-K potassium-channel agonists may be useful in the treatment of neuronal disorders in which neurotransmitter release must be suppressed in order to elicit anticonvulsant and anti-ischemic activity because they hyperpolarize the neuronal cell and diminish electrical excitability. They may also be useful in the treatment of smooth muscle disorders by hyperpolarizing and relaxing smooth muscles including, but not exclusive to, those in the vasculature and airways to elicit anti-

hypertensive and antiasthmatic actions, respectively (4). 14-Hydroxy CAF-603 oleate is a novel carotane sesquiterpene which may be useful as such a potassium-channel agonist.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—The nmr spectra were recorded on a Varian XL-300 nmr spectrometer at room temperature in either CD_3OD, $CDCl_3$, or CD_2Cl_2. The solvent peaks at δ 3.30 (CD_3OD), δ 7.24 ($CDCl_3$), and δ 5.32 (CD_2Cl_2) were used as internal references for ^{1}H-nmr spectra where chemical shifts are given in ppm. ^{13}C-Nmr spectra were recorded using the solvent peaks at δ 49.0 (CD_3OD), δ 77.0 ($CDCl_3$), and δ 53.8 (CD_2Cl_2). HMQC and HMBC (optimized for 7.2 Hz) spectra were obtained on Varian Unity 400 and 500 nmr spectrometers, respectively. Mass spectra were obtained on a Finnigan-MAT 212 mass spectrometer at 90 eV or a Finnigan-MAT TSQ70 at 70 eV. Fabms was run on a VG20-253 or Finnigan-MAT MAT-90 spectrometer using dithiothreitol-dithioerythritol (MB) or MB containing cesium iodide. The compounds were derivatized using BSTFA [*N,O-bis*(trimethylsilyl)trifluoroacetamide] in pyridine (1:1) at 50° for 30 min.

FUNGAL MATERIAL.—The producing strain (MF5783=ATCC74180) was identified as *Trichoderma virens* (Miller, Giddens & Foster) von Arx (Hyphomycetes) and exhibited all the essential morphological characteristics of that species (5,6). In the literature, this fungus is commonly known by its synonym, *Gliocladium virens* (Miller, Giddens & Foster). It was isolated from sheep dung, near Aborlí, Tarragona, Spain in May 1990.

FERMENTATION OF *TRICHODERMA VIRENS* FOR PRODUCTION OF 14-HYDROXY CAF-603 OLEATE.—A vegetative culture was prepared by inoculating a 54-ml portion of seed medium (7) in a 250-ml unbaffled Erlenmeyer flask with lyophilized mycelia of *T. virens*. This seed culture was incubated for 3 days at 25° on a rotary shaker in a room with 50% relative humidity and constant fluorescent light. This and subsequent cultures grown on a rotary shaker were shaken at 220 rpm with a 5-cm throw. Portions (2 ml each) of the culture were then used to inoculate a solid brown rice-based fermentation medium No. 1 which consisted of 10 g brown rice, 20 mg yeast extract, 10 mg sodium tartrate, 10 mg monobasic potassium phosphate, and 20 ml distilled H_2O in 250-ml unbaffled Erlenmeyer flasks. pH was not adjusted prior to autoclaving for 20 min. Immediately before use, the medium was moistened with 15 ml of H_2O and autoclaved again at 121° for 20 min.

The production cultures were incubated under static conditions at 25°, with 50% relative humidity for 21 days in a room with constant fluorescent light. Two other isolates of *T. virens* from the Merck Culture Collection (MF5786; MF5787) were also used. Two-ml portions of seed culture of these strains (prepared as above) were used to inoculate either 50-ml portions of liquid fermentation medium No. 2 which consisted of 100 g D-mannitol, 33 g NZ-amine (type E), 10 g yeast extract, 5 g ammonium sulfate, and 9 g monobasic potassium phosphate per liter or solid millet-based fermentation medium No. 3 which consisted of 15 g millet, 500 mg yeast extract, 100 mg sodium tartrate, 10 mg $FeSO_4$, 100 mg monosodium glutamate, 100 μl corn oil, and 15 ml distilled H_2O per 250-ml flask. The pH was not adjusted prior to autoclaving for 20 min. Immediately before use, the medium was moistened with 15 ml of H_2O and autoclaved again for 20 min.

The liquid fermentations were agitated and those grown on the solid medium were incubated under static conditions. All other fermentation conditions remained the same as for ATCC 74180. The media components used in this study were obtained from the following sources: whole grain brown rice (Uncle Ben's), millet (*Panicum miliaceum*; Bay-mor), yeast extract (Fidco, a division of Nestlé), NZ-amine (type E) (Sheffield Products), Mazola corn oil (CPC Foodservice, a division of CPC International), tomato paste (Beatrice/Hunt-Wesson), and oat flour (Quaker Oats Co.). Other components were standard reagent-grade compounds.

At day 21, the cultures were extracted with 70 ml of methyl ethyl ketone (MEK) per flask. The extraction was performed for 1 h at 25° with agitation. The samples were centrifuged for 20 min at 3000 rpm to separate the MEK layer.

RECEPTOR-BINDING ASSAY USED TO MONITOR ISOLATION.—The interaction of [^{125}I]ChTX with bovine aortic sarcolemma membrane vesicles was determined under the conditions described (8). Briefly, sarcolemma membrane vesicles were incubated in 12×75 polystyrene tubes with ca. 25 pM [^{125}I]ChTX (2200 Ci/mmol), in the absence or presence of test compound, in a medium consisting of 20 mM NaCl, 20 mM Tris-HCl pH 7.4, 0.1% bovine serum albumin, and 0.1% digitonin. Nonspecific binding was determined in the presence of 10 nM ChTX. Incubations were carried out at room temperature until ligand binding equilibrium was achieved at ca. 90 min. At the end of the incubation period, samples were diluted with 4 ml of 100 mM ice-cold NaCl, 20 mM Hepes-Tris pH 7.4, and filtered through GF/C glass fiber filters that had been presoaked in 0.5% polyethylenimine. Filters were rinsed twice with 4 ml of ice-cold quench

solution. Radioactivity associated with filters was determined in a gamma counter. Specific binding data in the presence of each compound (difference between total binding and nonspecific binding) was assessed relative to an untreated control.

EXTRACTION AND ISOLATION.—The solid fermentation broth (2 liter wbe) of *T. virens* (medium 1; IC_{50} 10 μl wbe per ml) was extracted with methyl ethyl ketone (1.4-fold) to give 29.3 g. The residue was partitioned between hexanes (5.6 g) and MeOH (95%, 16.4 g). Successive flash chromatography on SiO_2 ($MeOH/CH_2Cl_2$ stepwise elution) followed by Bakerbond C_{18} (particle size 40 μm; $MeOH/H_2O$) yielded two active fractions, 0.33 g (a) and 0.46 g (b). The hplc of fraction (a) on Partisil 10 ODS-3 (22×50) using 60% $MeOH/H_2O$ (10 ml/min) was carried out to provide CAF-603 [**3**] (82 mg; *R*, 90 min; IC_{50} value of 3 μM). Fraction b was also subjected to hplc on Partisil 10 ODS-3 (22×50) using 90% $MeOH/H_2O$ to give 14-hydroxy CAF-603 oleate [**2**] (8.5 mg; *R*, 258 min; IC_{50} value of 360 nM).

14-Hydroxy CAF-603 oleate [**2**].—^{1}H nmr (300 MHz, CD_3OD) δ 0.88 (3H, d, *J*=7 Hz, Me-13), 0.90 (3H, t, Me-18′), 0.95 (3H, d, *J*=6.5 Hz, Me-12), 1.00 (3H, s, Me-15), ca. 1.30 ($(CH_2)_n$, m), ca. 1.47 (1H, m, H-6a), ca. 1.50 (1H, m, H-5), 1.59 (2H, m, H-2a, H-2b), ca. 1.62 (1H, m, H-6b), 1.82 (2H, heptet, *J*=7 Hz, H-11), 1.88 (1H, m, H-10a), 2.02 (1H, m, H-7a), 2.10 (1H, dd, *J*=8.7 and 14.4 Hz, H-10b), 2.25 (1H, m, H-7b), 2.32 (2H, t, *J*=7 Hz, H-2′), 4.02 (1H, dd, *J*=3 and 6.5 Hz, H-3), 4.46 (2H, m, H-14), 5.34 (2H, m, H-9′, H-10′), 5.70 (1H, m, H-9); ^{13}C nmr (75 MHz, CD_3OD) δ 14.5 (C-18), 17.5 (C-12), 18.2 (C-13), 21.3 (C-15), 22.3 (C-6), 23.8 (C-17′), 26.2 (C-3′), 28.12 (C-8′ or C-11′), 28.14 (C-8′ or C-11′), 30.2 (2×), 30.3, 30.4, 30.5, 30.6, 30.8, 30.9, 31.7 (C-7), 33.1 (C-16′), 35.2 (C-2′), 36.0 (C-11), 43.2 (C-1), 43.5 (C-10), 51.2 (C-2), 59.6 (C-5), 71.8 (C-14), 72.1 (C-3), 85.1 (C-4), 129.3 (C-9), 130.8 (C-9′ or C-10′), 130.9 (C-9′ or C-10′), 139.1 (C-8), 175.4 (C-1′); eims *m/z* 518.4322 found $C_{33}H_{58}O_4$, calcd for $C_{33}H_{58}O_4$, 518.4334; *m/z* 457 ($C_{30}H_{49}O_3$, M-C_3H_7-H_2O), 193 ($C_{12}H_{17}O_2$, base peak).

CAF-603 [**3**].—^{1}H nmr (300 MHz, CD_3OD; only prominent peaks indicated) δ 0.87 (3H, d, *J*=6.9 Hz, Me-13), 0.95 (3H, d, *J*=6.9 Hz, Me-12), 0.99 (3H, br s, Me-15), 1.73 (3H, br s, Me-14), 1.81 (1H, heptet, *J*=6.9 Hz, H-11), 1.96 (1H, dd, *J*=8.7 and 14.4 Hz, H-10b), 4.00 (1H, dd, *J*=2.7 and 6.6 Hz, H-3), 5.36 (1H, m, H-9); ^{1}H nmr (400 MHz, $CDCl_3$) δ 0.85 (3H, d, *J*=7.0 Hz, Me-13), 0.95 (3H, d, *J*=6.8 Hz, Me-12), 1.00 (3H, br s, Me-15), ca. 1.39 (1H, m, H-6a), 1.43 (1H, m, H-5), ca. 1.52 (1H, m, H-6b), ca. 1.53 (1H, m, H-2a), 1.60 (1H, dd, *J*=1.7 and 14.0 Hz, H-2b), 1.73 (3H, br s, Me-14), ca. 1.76 (1H, m, H-10a), 1.79 (1H, heptet, *J*=6.9 Hz, H-11), 1.97 (1H, dd, *J*=8.7 and 14.3 Hz,. H-10b), 2.09 (1H, m, H-7b), ca. 2.10 (1H, m, H-7a), 4.00 (1H, dd, *J*=1.6 and 7.5 Hz, H-3), 5.34 (1H, m, H-9); ^{13}C nmr (75 MHz, CD_3OD) δ 17.5 (C-12), 18.2 (C-13), 21.3 (C-6), 22.0 (C-15), 27.6 (C-14), 35.5 (C-7), 35.9 (C-11), 43.3 (C-1), 43.9 (C-10), 51.2 (C-2), 59.6 (C-5), 72.2 (C-3), 85.1 (C-4), 123.7 (C-9), 140.1 (C-8); ^{13}C nmr (75 MHz, $CDCl_3$) δ 17.0 (C-12), 17.7 (C-13), 20.7 (C-6), 21.0 (C-15), 27.3 (C-14), 34.4 (C-7), 35.0 (C-11), 42.2 (C-1), 42.7 (C-10), 50.2 (C-2), 58.3 (C-5), 72.1 (C-3), 84.2 (C-4), 122.4 (C-9), 139.0 (C-8); eims *m/z* 238.1965 found, calcd for $C_{15}H_{26}O_2$, 238.1932, *m/z* 220, 202, 195, 177, 159.

HYDROLYSIS OF **2** TO 14-HYDROXY CAF-603 [**1**].—Aqueous NaOH (5%, 0.1 ml) was added to an EtOH solution of 14-hydroxy CAF-603 oleate [**2**] ($C_{33}H_{58}O_4$; 1.96 mg; 0.0038 mM). The mixture was stirred at room temperature overnight. The solvent was removed, H_2O (1 ml) was added, and the mixture was extracted with CH_2Cl_2 (3×1 ml). The pooled solution was washed with saturated NaCl (1×1 ml), dried over anhydrous $MgSO_4$, and the solvent removed under N_2 to give mainly 14-hydroxy CAF-603 [**1**]. It was further purified by hplc on Ultracarb 5 ODS (30) (4.6×25) using CH_3CN-H_2O (H_2O, 30 min followed by 40% CH_3CN-H_2O; flow rate 1 ml/min; *R*, 51.5 min).

14-Hydroxy CAF-603 [**1**].—^{1}H nmr (300 MHz, CD_2Cl_2; only prominent peaks indicated) δ 0.87 (3H, d, *J*=7 Hz, Me-12 or Me-13), 0.95 (3H, d, *J*=7 Hz, Me-12 or Me-13), 1.00 (3H, s, Me-15), 1.60 (2H, d, *J*=4.5 Hz, H-2a/H-2b), 1.62 (1H, m, H-6b), 1.81 (1H, heptet, *J*=7 Hz, H-11), 1.86 (1H, m, H-10a), 2.00 (1H, m, H-7a), 2.11 (1H, dd, *J*=9 and 14.5 Hz, H-10b), 2.24 (1H, m, H-7b), 2.38 (1H, d, *J*=5.5 Hz, OH-3), 3.96 (2H, d, *J*=6 Hz, H-14a, H-14b), 4.03 (1H, q, *J*=ca. 5 Hz, H-3), 5.61 (1H, m, H-9); eims $C_{15}H_{26}O_3$ *m/z* 236 (M^+−H_2O).

PREPARATION OF CAF-603 OLEATE [**4**] AND LINOLEATE [**5**] ESTERS.—To a solution of CAF-603 (20 mg, $C_{15}H_{26}O_2$, 0.08 mM) in triethylamine (0.5 ml), was added 4-dimethylaminopyridine (20 mg, 0.16 mM) and either oleic anhydride or linoleic anhydride (175 mg, 0.32 mM). The mixture was purged with N_2, sealed, and stirred. The reaction was allowed to proceed at room temperature for 24 h to yield CAF-603 oleate or CAF-603 linoleate, respectively. Ice-H_2O (2 ml) was added to the mixture to quench the reaction, and the esters were extracted with Et_2O (2 ml×3). The organic layer was pooled, washed with saturated NaCl (1×2 ml), dried over anhydrous $MgSO_4$, and filtered through a sintered glass. The esters were purified by hplc on Partisil 10 ODS-3 (22×50) using $MeOH/H_2O$ (flow rate 10 ml/min; stepwise gradient; 30 min, 80%, 120 min, 90%, and 180 min, 100%). The esters were eluted at 143.6 min [28.0 mg (66.4%)] and 137.5 min [25.9 mg (61.6%)], respectively.

CAF-603 Oleate [**4**].—^{1}H nmr (300 MHz, CD_2Cl_2; only prominent peaks indicated) δ 0.84 (3H, d, J=7 Hz, Me-12 or Me-13), 0.85 (3H, d, J=7 Hz, Me-12 or Me-13), 0.99 (3H, s, Me-15), ca. 1.26 ($(CH_2)_n$, m), 1.81 (1H, heptet, J=7 Hz, H-11), 1.96 (1H, dd, J=8.5 and 14.5 Hz, H-10b), 2.29 (2H, t, J=7.5 Hz, H-2′), 4.99 (1H, dd, J=2 and 8.5 Hz, H-3), 5.25–5.40 (3H, m, H-9, H-9′, H-10′); ^{13}C nmr (75 MHz, CD_2Cl_2) δ 14.3 (C-18′), 17.0 (C-12 or C-13), 17.9 (C-12 or C-13), 20.9 (C-15), 21.3 (C-6), 23.1 (C-17′), 25.4 (C-3′), 27.51 (2×, C-8′ or C-11′), 27.55 (C-14), 29.44, 29.48, 29.52, 29.7 (2×), 29.9, 30.1, 30.2, 32.3, 34.8, 34.9, 35.5 (C-11), 42.4 (C-1), 43.1 (C-10), 48.0 (C-2), 58.2 (C-5), 74.6 (C-3), 84.6 (C-4), 122.6 (C-9), 130.1 (C-9′ or C-10′), 130.3 (C-9′ or C-10′), 139.5 (C-8), 172.5 (C-1′); eims $C_{33}H_{58}O_3$ *m/z* 502 (M^+), 484 (M^+-H_2O); fabms *m/z* 484 (M^+-H_2O).

CAF-603 Linoleate [**5**].—^{1}H nmr (300 MHz, CD_2Cl_2; only prominent peaks indicated) δ 0.85 (3H, d, J=7 Hz, Me-12 or Me-13), 0.86 (3H, d, J=7 Hz, Me-12 or Me-13), 0.87 (3H, t, Me-18′), 1.00 (3H, s, Me-15), 1.30 ($(CH_2)_n$, m), 1.73 (3H, br s, Me-14), 1.81 (1H, heptet, J=7 Hz, H-11), 1.97 (1H, dd, J=8.5 and 14.5 Hz, H-10b), 2.29 (2H, t, J=7.5 Hz, H-2′), 2.76 (2H, m, H-11′), 5.00 (1H, dd, J=2 and 8.5 Hz, H-3), 5.25–5.42 (5H, m, H-9, H-9′, H-10′, H-12′, H-13′); ^{13}C nmr (CD_2Cl_2, 75 MHz) δ 14.2 (C-18′), 17.0 (C-12 or C-13), 17.9 (C-12 or C-13), 20.9 (C-15), 21.3 (C-6), 23.0 (C-17′), 25.4 (C-3′), 26.0, 27.51 (C-14), 27.54 (2×), 29.44, 29.49, 29.52, 29.7, 30.0, 31.9, 34.8, 34.9, 35.5, (C-11), 42.4 (C-1), 43.1 (C-10), 48.0 (C-2), 58.2 (C-5), 74.6 (C-3), 84.8 (C-4), 122.6 (C-9), 128.2, 128.3, 130.3, 130.5, 139.5 (C-8), 172.5 (C-1′); eims $C_{33}H_{56}O_3$ *m/z* 500 (M^+), 482 (M^+-18); fabms *m/z* 482 (M^+-H_2O).

ELECTROPHYSIOLOGICAL EXPERIMENTS.—Patch clamp recordings of currents flowing through high conductance Ca^{2+}-activated potassium (maxi-K) channels were made from membrane patches excised from cultured bovine aortic smooth muscle cells using conventional techniques (9) at room temperature. Glass capillary tubing was pulled in two stages to yield micropipettes with tip diameters of approximately 1–2 microns. Pipettes were typically filled with solutions containing: 150 mM KCl, 10 mM Hepes (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid), 1 mM Mg, and 0.01 mM Ca, and were adjusted to pH 7.20 with 3.7 mM KOH. After forming a high resistance (>10^9 ohms) seal between the sarcolemmal membrane and the pipette, the pipette was withdrawn from the cell, forming an excised inside-out membrane patch. The patch was excised into a bath solution containing: 150 mM KCl, 10 mM Hepes, 5 mM EGTA (ethylene glycol bis(β-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid), sufficient Ca to yield a free Ca concentration of 1–5 μM, and the pH was adjusted to 7.2 with 10.5 mM KOH. For example, 4.568 mM Ca was added to give a free concentration of 2 μM at 22°. An Axopatch 1C amplifier (Axon Instruments) with a CV-4 headstage was used to control the voltage and to measure the currents flowing across the membrane patch. The input to the headstage was connected to the pipette solution with a Ag/AgCl wire, and the amplifier ground was connected to the bath solution with a Ag/AgCl wire covered with a tube filled with agar dissolved in 0.2 M KCl. Maxi-K channels were identified by their large single channel conductance (ca. 250 pS) and sensitivity of channel open probability to membrane potential and intracellular calcium concentration.

Planar lipid bilayers were formed from a solution of 1-palmitoyl-2-oleoylphosphatidylethanolamine (POPE) and 1-palmitoyl-oleoylphosphatidyl choline (POPC) in a 7:3 molar ratio dissolved in decane (50 mg/ml). This lipid solution was painted across a small hole (250 micron) separating two aqueous compartments and readily formed bilayers with capacitances of 200–250 pF. The solution on the side that the membranes were added (cis) contained: 150 mM KCl, 10 mM Hepes, 0.01 mM Ca, 3.7 mM KOH, pH 7.20. The solution on the other side (trans) contained: 25 mM KCl, 10 mM Hepes, 0.01 mM Ca, 3.7 mM KOH, pH 7.20. Plasma membrane vesicles purified from bovine aortic smooth muscle (10) were added to the cis side until channel incorporation occurred. After channel incorporation, the concentration of KCl on the trans side was increased to 150 mM to prevent further channel incorporation. The orientation of maxi-K channels after insertion into the bilayer was determined from the calcium and voltage sensitivity of the channel. Increases in calcium or voltage on the intracellular side led to increases in channel open probability. An Axopatch 1C with a CV-4B headstage was used to control the membrane potential and record currents flowing across the bilayer. The inputs to the amplifier were connected to Ag/AgCl wires which connected to the two sides of the bilayer chamber through small tubes filled with agar dissolved in 0.2 M KCl. Experiments were done at room temperature.

Data was stored on a Racal store 4DS FM tape recorder (Racal Recorders) or on digital video tape using a video casette recorder after digitizing the signal with a VR-10 (Instrutech) PCM video encoder. The signal was also recorded on chart paper with a Gould 2400S chart recorder (Gould). For quantitative analysis, the data were played into a DEC 11-73 (Digital Equipment) after digitization with a DT2782-8D1A analogue to digital converter (Data Translation), or played into a Mac IIx or Quadra 700 computer (Apple Computers) after digitization with an ITC-16 interface (Instrutech).

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