Semisynthetic and Natural Garcinoic Acid Isoforms as New mPGES-1 Inhibitors*

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Key words
- mPGES-1
- anti-inflammatory
- garcinoic acid
- tocotrienol
- semisynthesis

Abstract

Over the last twenty years, tocotrienol analogues raised great interest because of their higher level and larger domain of biological activities when compared with tocopherols. Amongst the most promising therapeutic application, anti-inflammatory potency has been evaluated through the inhibition of various mediators of inflammation. Here, we worked on the isolation of two natural isoforms of garcinoic acid (i.e., δ and γ) from two different sources, respectively, *Garcinia kola* seeds and *Garcinia amplexicaulis* bark. We also developed semisynthetic strategies to access the other two non-natural α- and β-garcinoic acid isoforms. In the next stage of our work, microsomal prostaglandin E_2 synthase was defined as a target to evaluate the anti-inflammatory potential of the four garcinoic acid isomers. Both dimethylated isoforms, β- and γ-garcinoic acid, exhibited the lowest IC_{50}, 2.8 µM and 2.0 µM, respectively. These results showed that the affinity of tocotrienol analogues to microsomal prostaglandin E_2 synthase-1 most probably contributes to the anti-inflammatory potential of this class of derivatives.

Abbreviations

COX-2:	cyclooxygenase-2
DCM:	dichloromethane
GA:	garcinoic acid
HMG-CoA:	3-hydroxy-3-methylglutaryl-coenzyme A
IL:	interleukin
mPGES-1:	microsomal prostaglandin E_2 synthase-1
PGE_2:	prostaglandin E_2
PGH_2:	prostaglandin H_2
T:	tocopherol
T3:	tocotrienol
TMEDA:	N,N,N′,N′-tetramethylethylene-diamine

received February 28, 2016
revised April 25, 2016
accepted May 2, 2016

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DOI http://dx.doi.org/10.1055/s-0042-108739
Published online
Planta Med © Georg Thieme Verlag KG Stuttgart · New York ·
ISSN 0032-0943

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Introduction

T3s are natural forms of the lipophilic vitamin E and possess potent antioxidative activity, even exceeding that of Ts [1]. They are abundant in vegetable oils, but are also found in a wide range of microorganisms, plants, and marine organisms. The T3 family is a small and homogeneous group of natural products, with a narrow range of chemical variations. Indeed, these chromanols substituted on the pyran ring by a methyl and a farnesylmethyl side chains mainly differ in the methylation pattern of the aromatic ring and, as a consequence, are represented with four isoforms. Depending on the number and the positions of the aromatic methyl groups, they belong to the δ, β, γ, and α series with, respectively, one, two, two, and three methyl groups (**Fig. 1**). Vitamin E is also constituted by four other isoforms (δ, β, γ, and α) substituted by a saturated side chain and named Ts.

T3s and their derivatives, including carboxychromanols, increasingly gained attention over the past two decades [2] since they were reported as antiangiogenic agents [3] and inhibitors of HMG-CoA reductase [4], lowering cholesterol biosynthesis [5]. T3s are neuroprotective [6], inhibit cell proliferation, and induce apoptosis in different cancer cell lines [1,7], suppress proinflammatory signal transduction, and interfere with eicosanoid biosynthesis by inhibition of arachidonic acid release, lipoxygenases, and cyclooxygenases [1]. In the course of the Austrian interdisciplinary

* Dedicated to Professor Dr. Dr. h.c. mult. Kurt Hostettmann, 2014 Egon-Stahl Medal in Gold.

network project "Drugs from Nature Targeting Inflammation" (DNTI: 2007–2014), with Prof. Kurt Hostettmann as a senior scientific advisor [8], a comprehensive *in silico* pharmacophore-based virtual screening of T3 derivatives isolated from *Garcinia amplexicaulis* Vieill. ex Pierre (Clusiaceae) [9] was performed. This parallel profiling of T3 derivatives against various anti-inflammatory targets clearly revealed δ- and γ-tocotrienolic acids (δ- and γ-GA) as potential mPGES-1 inhibitors (**Fig. 2**). It then appeared of great interest to evaluate *in vitro* the capacity of the whole series of GAs (i.e., *δ, β, γ,* and *α* derivatives) to modulate mPGES-1 activity. This enzyme transforms PGH_2, the unstable cyclooxygenase-derived oxidation product of arachidonic acid, to PGE_2 and might be considered a key target in inflammation [10]. For these investigations, *β*- (**4**) and *α*- (**5**) GA, which have never been isolated from a natural source, were sequentially semisynthesized from *δ*-GA (**1**), whereas *γ*-GA (**6**) was isolated for the first time from *Garcinia amplexicaulis* stem bark.

Results and Discussion

δ-GA (**1**) is a T3 oxidized in the terminal position of the isoprenoid chain that was isolated by our group from *G. amplexicaulis*, and previously reported in other species: *Garcinia kola* Heckel [11], *Tovomitopsis psychotriifolia* Oerst. ex Planch. & Triana (Clusiaceae) [12], *Clusia obdeltifolia* V. Bittrich (Clusiaceae) [13], and *Clusia grandiflora* Splitg. [14]. As *δ*-GA chromanol is substituted only in position 8 by a methyl group, it is therefore related to the *δ*-series so that *δ*-GA is also sometimes referred to as *δ*-tocotrienolic acid.

The target GA isoforms could probably be prepared through total synthesis [15]. However, such a strategy to obtain *δ*-GA itself requires 14 steps, while it could be isolated from several plants as aforementioned. Therefore, GA homologs were prepared by semisynthesis. Indeed, the substitution of the aromatic ring of *δ*-GA by one or two methyl groups was achieved by a two-step sequence initially developed in the T series [16] and then applied to the T3 series [17]. Mannich reaction of *δ*-GA (**1**) led either to the mono or to the dialkylated products, respectively, **2** and **3**. Using a small excess of reagents (3 equivalents of TMEDA and 3 equivalents paraformaldehyde), the substitution takes place on the most reactive position, i.e., at C-5, giving **2** a yield of 62%. With a large excess of reagents (30 eq), the Mannich reaction occurred at both positions C-5 and C-7, i.e., *ortho* to the phenolic hydroxyl, and the diamine **3** was isolated with a 65% yield. Reduction of the dimethylaminomethyl group to a methyl took place through the thermal generation of a quinone-methide intermediate [18] in the presence of an hydride reagent [19]. Gu et al. already applied this method for the first time in the T3 series, with $NaBD_3CN$ in refluxing isobutanol for 6 h [20]. Here, we slightly modified these conditions, as reactions were carried out in EtOH at 100 °C under microwave irradiation. This led to shorter reaction times (10 min) and yielded the expected products **4** (55%) and **5** (45%). Neither the carboxylic acid nor the conjugated double bond was reduced under these conditions, whereas a reductive deamination gave the yet unnatural *β*- (**4**) and *α*- (**5**) isoforms of GA (**Fig. 3**).

The last GA isoform, *γ*-GA (**6**), is unsubstituted at C-5 on the chromanol (**Fig. 4**). This position is the most reactive position during the Mannich reaction for *δ*-GA (**1**), but also for any aromatic electrophilic subtitution of *δ*-tocopherol derivatives [21]. Semisynthesis of *γ*-GA from *δ*-GA is certainly not straightforward, as a protective group in C-5 would obviously be necessary. We therefore turned back to phytochemistry to obtain *γ*-GA. Indeed, previous phytochemical analysis of *G. amplexicaulis* revealed that T3s isolated from this plant belong mainly to the *δ*-series, but also for minor representatives to the *γ*-series. Further investigations of fractions that had previously led to *δ*-GA from the bark of *G. amplexicaulis* [9] allowed for the isolation of *γ*-GA [22].

γ-GA (**6**) was isolated as a pale yellow oil and its molecular formula was established as $C_{28}H_{40}O_4$ based on the $[M + Na]^+$ quasi-molecular ion peak observed in the HR-ESIMS spectrum.

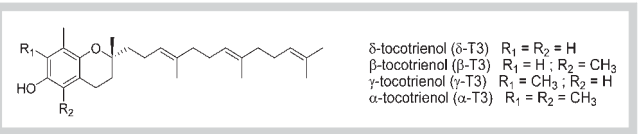


Fig. 1 The four isoforms of tocotrienol derivatives.

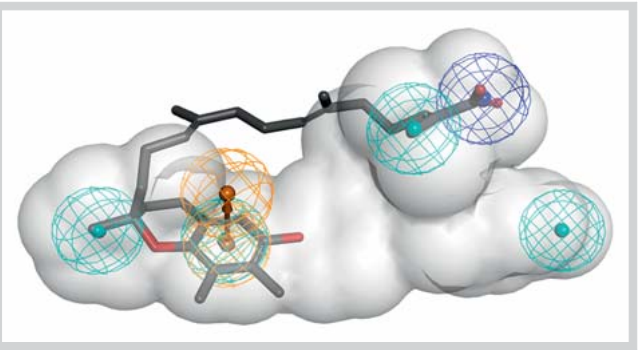


Fig. 2 Fitting of γ-garcinoic acid into the ligand-based pharmacophore model for mPGES-1 inhibitors. Chemical features are color-coded: cyan = hydrophobic area, blue = negatively ionizable group, orange = aromatic ring. The shape restriction is shown in grey. (Color figure available online only.)

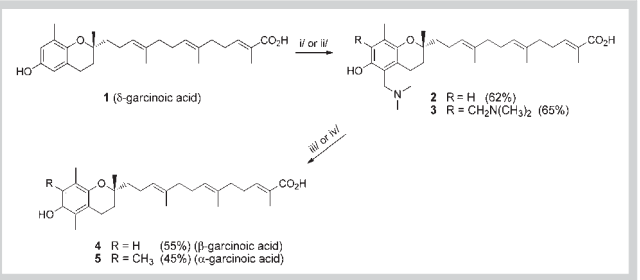


Fig. 3 Semisynthetic pathways to the *β*- and *α*-isoforms of GA: i/**1** → **2**: **1**, TMEDA (3 eq), $(CHO)_n$ (3 eq), EtOH, 120 °C, μW, 12 min; ii/**1** → **3**: **1**, TMEDA (30 eq), $(CHO)_n$ (30 eq), EtOH, 120 °C, μW, 20 min; iii/**2** → **4**:**2**, $NaBH_3CN$ (5 eq), EtOH, 100 °C, μW; iv/**3** → **5**: **3**, $NaBH_3CN$ (20 eq), EtOH, 100 °C, μW.

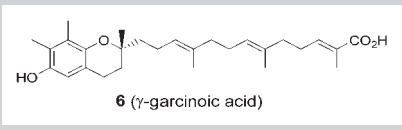
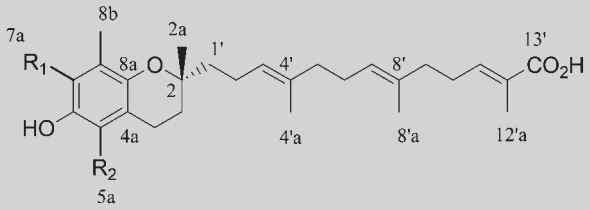


Fig. 4 γ-Garcinoic acid structure.

Table 1 ^{1}H and ^{13}C NMR data for the four GA isoforms.[a]



N°	4		5		6		1	
	δ_C	δ_H (*J* Hz)	δ_C	δ_H (*J* Hz)	δ_C	δ_H (*J* Hz)	δ_C	δ_H (*J* Hz)
2	74.2		74.4		75.2		75.3	
3	31.6	1.76–1.87, m, 2 H	31.7	1.75–1.85, m, 2 H	31.3	1.74, m, 2 H	31.3	1.75–1.83, m, 2 H
4	20.9	2.61, t (6.8), 2 H	20.9	2.61, t (6.8), 2 H	22.2	2.67, t (6.7), 2 H	22.4	2.69, t (6.8), 2 H
4 a	120.4		117.4		118.2		121.2	
5	119.4		118.6		112.1	6.37, s, 1 H	112.6	6.38, d (3.0), 1 H
6	146.0		146.6		146.2		147.7	
7	115.5	6.48, s, 1 H	122.8		121.6		115.6	6.48, d (3.0), 1 H
8	124.1		121.2		125.8		127.3	
8 a	145.8		144.7		145.6		145.9	
1′	39.6	1.48–1.65, m, 2 H	39.7	1.42–1.67, m, 2 H	39.6	1.54–1.67, m, 2 H	39.5	1.53–1.66, m, 2 H
2′	22.3	2.04–2.15, m, 2 H	22.3	2.04–2.15, m, 2 H	22.1	2.12, m, 2 H	22.1	2.10, m, 2 H
3′	124.6	5.13, t (7.2), 1 H	124.7	5.13, t (6.8), 1 H	124.4	5.13, t (7.0), 1 H	124.4	5.12, t (7.5), 1 H
4′	136.1		136.0		134.8		134.8	
5′	39.5	1.94–1.99, m, 2 H	39.6	1.96–1.99, m, 2 H	39.5	1.97, m, 2 H	39.5	1.96, m, 2 H
6′	26.6	2.04–2.13, m, 2 H	26.7	2.04–2.15, m, 2 H	26.4	2.06, m, 2 H	26.4	2.08, m, 2 H
7′	125.3	5.13, t (7.2), 1 H	125.3	5.13, t (6.8), 1 H	125.1	5.12, t (7.0), 1 H	125.2	5.07, t (7.5), 1 H
8′	133.9		133.8		133.7		133.6	
9′	38.3	2.04–2.13, m, 2 H	38.2	2.04–2.15, m, 2 H	38.0	2.09, m, 2 H	38.0	2.07, m, 2 H
10′	27.6	2.25–2.30, m, 2 H	27.7	2.25–2.31, m, 2 H	27.5	2.28, m, 2 H	27.4	2.29, q (7.5), 2 H
11′	145.1	6.88, t (7.0), 1 H	145.1	6.88, t (6.8), 1 H	144.9	6.87, t (7.4), 1 H	145.0	6.90, td (7.5 &1.3), 1 H
12′	127.0		127.0		126.8		126.8	
13′	173.5		173.2		172.6		173.3	
12′ a	12.2	1.82, s, 3 H	12.1	1.82, s, 3 H	12.1	1.82, s, 3 H	11.9	1.83, s, 3 H
8′ a	16.0	1.59, s, 3 H	16.0*	1.60, s, 3 H	15.9*	1.59, s, 3 H	15.9	1.58, s, 3 H
4′ a	16.0	1.59, s, 3 H	16.1*	1.60, s, 3 H	15.8*	1.59, s, 3 H	15.8*	1.57, s, 3 H
2 a	23.8	1.25, s, 3 H	23.8	1.25, s, 3 H	24.0	1.26, s, 3 H	24.1	1.26, s, 3 H
8 b	11.2	2.11, s, 3 H	11.9	2.12, s, 3 H	11.9	2.11, s, 3 H	16.1	2.12, s, 3 H
7 a			12.4	2.16, s, 3 H	11.9	2.13, s, 3 H		
5 a	16.1	2.08, s, 3 H	11.4	2.11, s, 3 H				

[a] All spectra were recorded in $CDCl_3$ (^{1}H NMR 500 MHz and ^{13}C NMR 125 MHz) and are reported in ppm. Protons and carbons were assigned following DQF-COSY, NOESY, HMQC, and HMBC experiments. * Interchangeable chemical shifts.

The spectral features of **6** appeared to be very similar to that of δ-GA (**1**), which was isolated from the same plant extract. Indeed, typical resonances of the chromanol ring and the farnesylmethyl chain were observed in the ^{1}H and ^{13}C NMR spectra of **6** (**Table 1**). However, the aromatic singlet for H-7 around δ_H 6.48, observed for compound **1**, had disappeared from the NMR data of **6**. Instead of this aromatic proton, a methyl group (δ_H 2.13; δ_C 11.9) appeared, which was the most significant difference. Long-range correlations of the methyl proton signals at δ_H 2.13 (H-7 a) with the aromatic carbons at δ_C 121.7 (C-7), 125.7 (C-8), and 146.3 (C-6) suggested a γ-chromanol moiety. The substitution of the chromanol ring was corroborated by the observation of the HMBC correlations of the single aromatic proton at δ_H 6.37 (H-5) with the carbons at δ_C 22.2 (C-4), 121.6 (C-7), and 145.6 (C-8 a). NMR data of the 13′-COOH farnesyl moiety recorded for **6** was superimposable with those observed for δ-GA (**Table 1**). Therefore, **6** was unambiguously identified as γ-GA, a new natural T3 isolated from *G. amplexicaulis* (**Fig. 4**).

The four α-, β-, γ- and δ-GA derivatives were analyzed for inhibition of mPGES-1 activity in a cell-free assay using microsomes of IL-1β-stimulated A549 cells as an enzyme source.

As expected from an *in silico* screening, this study revealed that GA isoforms are potent mPGES-1 inhibitors with IC_{50} values ranging from 2.0 to 7.8 µM, i.e., at the same inhibition level as the reference compound (MK-886: IC_{50} = 2.4 µM [23]; **Fig. 5**).

In the wake of α-T as the first vitamin E derivative to be described, the biological properties of the four tocopherol isoforms have been extensively investigated for many years. However, since the 1990s, various studies have shed light on the higher therapeutic potential of T3s [24] and their higher activity for specific targets when compared with T [3, 19, 20, 25]. Our previous investigations on the antiangiogenic properties of T3 derivatives pointed out the positive effect of an oxidation on the terminal position of the isoprenoid chain as δ-amplexichromanol appeared as 100-fold more active than unoxidized δ-T3 [22]. Similarly, in the T series, the anti-inflammatory activity of α-T was increased by oxidative metabolism, leading to the 13′-COOH derivative

[26] that is found in human plasma [27]. Yet, the anti-inflammatory activity of this metabolite was among others related to the inhibition of the formation of PGE_2 in macrophages, and explained by a reduced expression of the COX-2 protein. Moreover, another long-chain 13′-COOH derivative of the γ-series inhibited PGE_2 formation independently of COX-2 expression by direct inhibition of COX isoenzymes [28]. We therefore hypothesize that the inhibition of mPGES-1 contributed to the overall anti-inflammatory activity of long-chain 13′-COOH derivatives. Moreover, and as far as cellular tests are concerned, T3 isoforms often differ in their biological activities. For instance, their ability to lower cellular cholesterol synthesis varies as follows: δ-T3 > γ-T3 > α-T3 > β-T3 [29], whereas observed apoptotic effects are not similar between all the different T3 isoforms [30]. Therefore, though mPGES-1 inhibition levels do not appear to be highly different between GA isoforms here, it should be noticed that the methylation pattern of chromanol rings might significantly modulate their bioavailability as well as cellular uptake.

In conclusion, we reported the isolation of γ-GA (**6**) from *G. amplexicaulis* and the semisynthesis for the first time of β-GA (**4**) and α-GA (**5**) from δ-GA (**1**) through a two-step sequence. The four isoforms of GA were then evaluated as mPGES-1 inhibitors. This is the first time T3 analogues are described as targeting this enzyme. Among these tocotrienolic acids, the activities vary as follows: γ-GA > β-GA > δ-GA > α-GA, thus the methylation pattern has only a moderate effect, with γ-GA exhibiting only a 4-fold greater activity over α-GA.

Based on these promising results, we will develop a strategy for an *in silico* design followed by semisynthesis of novel T3 analogues as potential anti-inflammatory agents.

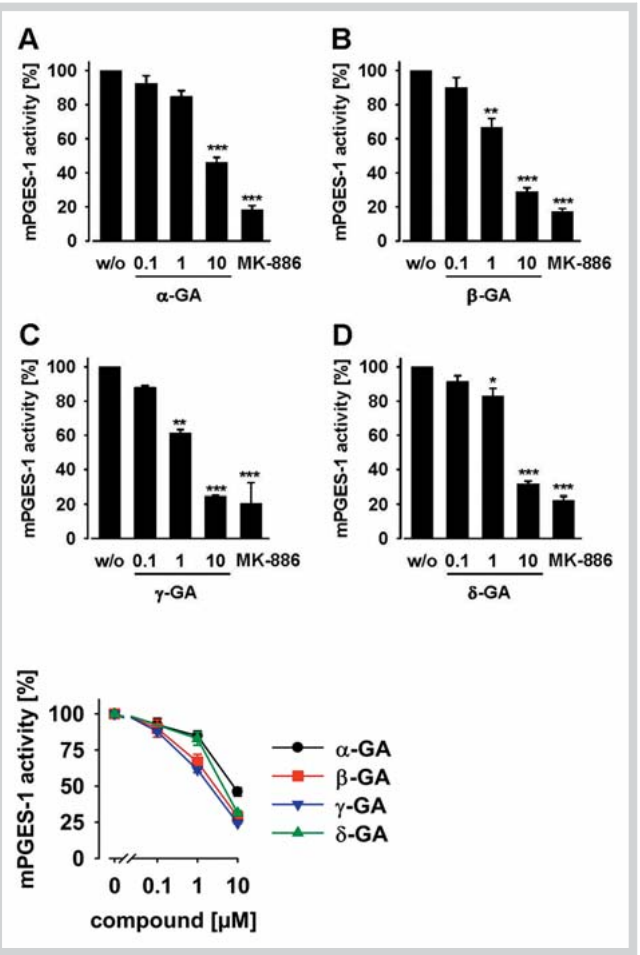


Fig. 5 Concentration-dependent inhibition of mPGES-1 by GA isoforms. Cell-free mPGES-1 in microsomal preparations of IL-1β-stimulated A549 cells was treated with vehicle (DMSO), α-GA (**A**), β-GA (**B**), γ-GA (**C**), δ-GA (**D**), or the mPGES-1 reference inhibitor MK-886 (10 μM, **A**–**D**). Data are given as means ± S. E. of three independent experiments. $^{*}P < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ vs. vehicle control; ANOVA + Tukey's HSD post hoc tests. (Color figure available online only.)

Materials and Methods

^{1}H and ^{13}C NMR along with 2D NMR data were obtained on a Bruker Avance DRX 500 MHz spectrometer (500 and 125 MHz, respectively) or a JEOL JNM-ECZS 400 MHz spectrometer (400 and 100 MHz, respectively) in deuterated chloroform with TMS as an internal standard. Mass spectrometry analyses were performed on a JMS-700 (JEOL LTD) double focusing mass spectrometer with reversed geometry, equipped with a pneumatically assisted ESI source. Chromatographic separations such as flash chromatography IntelliFlash 310 (Analogix) using a pre-packed C_{18} (Interchim) or silica gel column Chromabond® flash RS column (Macherey-Nagel) were used to purify compounds.

Reactions under microwave irradiations were performed using an Anton Paar Monowave 300® microwave reactor (Anton Paar). All reactions under microwave irradiations were performed using the stirring option in borosilicate glass vials of 10 mL or 30 mL (G10 or G30) sealed with PTFE-coated silicone septa (at the end of the irradiation, cooling reaction was realized by compressed air). The microwave instrument consists of a continuous focused microwave power output from 0 to 600 W. The target temperature was reached with a ramp of 3 min and the chosen microwave power was maintained at a constant to hold the mixture at this temperature. The reaction temperature was monitored using a calibrated infrared sensor and the reaction time included the ramp period. The microwave irradiation parameters (power, temperature, and time) were monitored by the Monowave software package.

Human A549 fibroblasts were kindly provided by Dr. T. Maier (University of Frankfurt, Germany). All test compounds were dissolved in DMSO and stored in the dark at − 20 °C. Freezing/thawing cycles were kept to a minimum. Materials used were DMEM/high glucose (4.5 g/L) medium, penicillin, streptomycin, trypsin/EDTA solution – GE Healthcare; PGH_2 -Larodan; MK-886 (purity ≥ 99%) and 11β-PGE_2 _ Cayman Chemical; IL-1β – PeproTech. Solvents and all other chemicals were obtained from Sigma-Aldrich unless stated otherwise.

Virtual screening

Compounds were imported into Discovery Studio 4.0 (Biovia Inc.) and multiconformational 3D structures were generated using the BEST mode with a maximum of 255 conformers per molecule. Fitting into the previously reported mPGES-1 inhibitor pharmacophore model [31] suggested several T3 derivatives as potential mPGES-1 inhibitors, among them α- (**5**) and γ- (**6**) GA. The screening was performed using the Ligand-Pharmacophore Mapping protocol of Discovery Studio. In the mapping, one chemical feature from the hydrophobic or aromatic ring features was allowed to be omitted by the ligand, as also reported by Waltenberger et al. [31] and Bauer et al. [32]. The shape restriction was set to a tolerance of 1.3, which means that 30% of the ligand vol-

ume is allowed to extrude out of the shape model into any direction.

Plant material

The stem bark of *G. amplexicaulis* was collected in July 1998, in the area named "Forêt Cachée", in the south of New Caledonia and identified by Marc Litaudon. A specimen (LIT-0554) has been deposited at the Laboratoire des Plantes Médicinales (CNRS), Noumea, New Caledonia.

Extraction and isolation

δ-Garcinoic acid (**1**) was isolated from the African bitter nut *G. kola* according to published procedures [11].

γ-Garcinoic acid (**6**): Dried stem bark (270 g) of *G. amplexicaulis* was extracted with 3 L of DCM using a Soxhlet apparatus for 24 h and 30 g of DCM extract was obtained. DCM extract (20 g) was separated by silica gel normal-phase flash column chromatography (m = 400 g silica gel) using a DCM/acetone mixture (10 : 0 to 1 : 1) to yield 36 fractions (F1–F36) on the basis of the TLC analysis results.

Fraction F23 (300 mg) was separated by normal-phase flash chromatography with a cyclohexane/EtOAc mixture (9 : 1 to 1 : 1) to give 9 subfractions (F23-1 to F23–9). Subfractions F23–7 to F23–9 were gathered and flash chromatographied over an RP-18 (4 g column, MeOH/H_2O mixture) to give pure γ-GA (6.0 mg) as a pale yellow oil; $[\alpha]_D^{23}$ − 8.5° (*c* 0.06, MeOH); UV (MeOH) λ_{max} (log ε) 296.0 (3.36), 261.0 (2.88), 206.0 (4.48) nm; HREIMS: *m/z* 463.2821 $[M + Na]^+$ (calcd. for $C_{28}H_{40}O_4Na$, 463.2819).

Semisynthesis of β- and α-garcinoic acids (4 and 5): Preparation of 5-dimethylaminomethyl-δ-garcinoic acid (2)

TMEDA (71 µL, 0.48 mmol, 3 eq) and paraformaldehyde (14.5 mg, 0.48 mmol, 3 eq) were added to solution of δ-GA (**1**; 100 mg, 0.24 mmol) in 3 mL of ethanol. The mixture was heated at 120 °C for 12 min using microwave irradiation. After cooling to room temperature, the mixture was diluted with DCM (15 mL), and washed with H_2O (4 × 20 mL) and brine (20 mL). The organic layer was dried over Na_2SO_4 and concentrated under vacuum. The resulting residue was purified by flash chromatography using DCM/MeOH as the mobile phase to provide **2** (70 mg) as a brown oil.

Yield 62%, Rf = 0.33 (DCM/MeOH, 60/40). ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.84 (t, *J* = 7.0 Hz, 1 H, H-19), 6.54 (s, 1 H, H-7), 5.12 (t, *J* = 7.2 Hz, 2 H, H-15, H-11), 3.62 (s, 2 H, H-1′), 2.59 (t, *J* = 6.8 Hz, 2 H, H-4), 2.34 (s, 6 H, H-2′, H-3′), 2.30–2.25 (m, 2 H, H-18), 2.12 (s, 3 H, H-26), 2.13–2.04 (m, 6 H, H-10, H-14, H-17), 1.99–1.94 (m, 2 H, H-13), 1.82 (s, 3 H, H-22), 1.86–1.73 (m, 2 H, H-3), 1.65–1.52 (m, 2 H, H-9), 1.59 (s, 6 H, H-23, H-24), 1.23 (s, 3 H, H-25); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.5 (C-21), 150.5 (C-6), 144.9 (C-19), 144.7 (C-8 a), 136.1 (C-12), 133.9 (C-16), 126.8 (C-8, C-20), 125.3 (C-15), 124.5 (C-11), 118.6 (C-4 a), 116.8 (C-7), 116.3 (C-5), 74.2 (C-2), 57.6 (C-1′), 44.5 (C-2′, C-3′), 39.6 (C-13), 39.5(C-9), 38.3 (C-17), 31.6 (C-3), 27.6 (C-18), 26.6 (C-14), 23.8 (C-25), 22.2 (C-10), 20.6 (C-4), 16.2 (C-26), 16.1 (C-23), 16.0 (C-24), 12.3 (C-22); HRMS (FAB) Calcd. for $C_{30}H_{46}NO_4$ ($[M + H]^+$) 484.3422, found 484.3416. IR (*ν* cm^{-1}) 3365, 2922, 2852, 2112, 1650, 1460, 1230. UV (MeOH) λ_{max} (log ε) 306 (3.57), 204 (4.63).

Reduction of 5-dimethylaminomethyl-δ-garcinoic acid (2) to β-garcinoic acid (4)

Sodium cyanoborohydride $NaBH_3CN$ (66 mg, 1.05 mmol, 5 eq) was added to 5-dimethylaminomethyl-δ-garcinoic acid (**2**) (102 mg, 0.21 mmol) in ethanol (2.5 mL) and the mixture was heated at 100 °C for 10 min under microwave irradiation. Afterwards, the mixture was cooled to room temperature, diluted with Et_2O (20 mL), and acidified to pH 1 by the addition of 1 N HCl. The aqueous layer was extracted with Et_2O (15 mL). The organic layers were combined, washed successively with H_2O (20 mL) and brine (20 mL), dried over Na_2SO_4, filtered, and evaporated. The resulting residue was purified by flash chromatography using DCM/MeOH (gradient from 10 : 0 to 9 : 1) as the mobile phase to provide **4** (50 mg).

Yield 55%, Rf = 0.42 (DCM/MeOH: 90/10), ^{1}H NMR and ^{13}C NMR data are detailed in ◉ **Table 1**; HRMS (FAB) Calcd. for $C_{28}H_{40}O_4$ ($[M]^+$) 440.2921, found 440.2917. IR (*ν* cm^{-1}) 3397, 2922, 2581, 1682, 1641, 1414, 1229, 915. UV (MeOH) λ_{max} (log ε) 294 (3.51), 204 (4.63).

Procedure for the preparation of α-garcinoic acid (5) Preparation of 5,7-bis(dimethylaminomethyl)-δ-garcinoic acid (3)

5,7-bis(Dimethylaminomethyl)-δ-garcinoic acid **3** was obtained by using the general procedure for aminomethylation with δ-garcinoic acid (**1**; 100 mg, 0.24 mmol) in 3 mL of 1,4-dioxane, TMEDA (1.07 mL, 7.2 mmol, 30 eq), and paraformaldehyde (216 mg, 7.2 mmol, 30 eq). After removal of the solvent the residue was diluted with DCM (15 mL), washed with H_2O (4 × 20 mL) and brine (20 mL). The organic layer was dried over Na_2SO_4 and concentrated under vacuum to afford compound **3** (83 mg) as a brown oil.

Yield 65%. Rf = 0.25 (DCM/MeOH: 70/30). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.35 (t, *J* = 7.2 Hz, 1 H, H-19), 5.07 (t, *J* = 7.2 Hz, 1 H, H-11), 5.03 (t, *J* = 7.2 Hz, 1 H, H-15), 3.86 (s, 2 H, H-1′), 3.96 (s, 2 H, H-1″), 2.61 (m, 2 H, H-4), 2.48 (s, 4 H, H-2′, H-3′), 2.54 (s, 4 H, H-2″, H-3″), 1.91–2.25 (m, 8 H, H-10, H-13, H-14, H-17, H-18), 2.10 (s, 3 H, H-26), 1.70 (s, 3 H, H-22), 1.63–1.82 (m, 2 H, H-3), 1.42–1.56 (m, 2 H, H-9), 1.49 (s, 3 H, H-23), 1.52 (s, 3 H, H-24), 1.22 (s, 3 H, H-25). ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.1 (C-21), 151.7 (C-6), 145.8 (C-8 a), 137.2 (C-19), 133.8 (C-16), 136.3 (C-12), 134.3 (C-20), 125.8 (C-15), 125.6 (C-11), 127.6 (C-8), 122.0 (C-4 a), 118.4 (C-7), 116.2 (C-5), 75.8 (C-2), 56.9 (C-1″), 56.5 (C-1′), 44.0 (C-2″, C-3″), 43.9 (C-2′, C-3′), 40.7 (C-9), 40.2 (C-13), 39.9 (C-17), 32.4 (C-3), 28.4 (C-18), 27.4 (C-14), 24.3 (C-25), 23.3 (C-10), 21.2 (C-4), 16.3 (C-24), 16.0 (C-23), 13.9 (C-26), 12.1 (C-22). HRMS (FAB) Calcd. for $C_{33}H_{53}N_2O_4$ ($[M + H]^+$) 541.4000, found 541.3997. IR (*ν* cm^{-1}) 3381, 2925, 2781, 2130, 1650, 1418, 1257, 840. UV (MeOH) λ_{max} (log ε) 310 (3.55), 204 (4.55).

Reduction of 5,7-bis(dimethylaminomethyl)-δ-garcinoic acid (3) to α-garcinoic acid (5)

α-Garcinoic acid (**5**) was obtained using the same procedure as the one described for the synthesis of **4**. 5,7-bis(Dimethylaminomethyl)-δ-garcinoic acid (**3**; 120 mg, 0.22 mmol, 1 eq) was dissolved in 3 mL of ethanol in the presence of sodium cyanoborohydride $NaBH_3CN$ (279 mg, 4.43 mmol, 20 eq). After completion of the reduction, the resulting residue was purified by flash chromatography using petroleum ether/acetone as the mobile phase to provide α-garcinoic acid (**5**; 65 mg) as a yellow oil.

Yield 45%. Rf = 0.45 (cyclohexane/acetone: 70/30). ^{1}H NMR and ^{13}C NMR data are detailed in ◉ **Table 1**. HRMS (FAB) Calcd. for

$C_{29}H_{42}O_4$ ([M]$^+$) 454.3078, found 454.3084. IR (ν cm^{-1}) 3498, 2965, 2851, 1680, 1452, 1240, 927. UV (MeOH) λ_{max} (log ε) 290 (3.49), 202 (4.74).

Preparation of human mPGES-1 and determination of mPGES-1 activity

A549 cells were treated with IL-1β for 72 h to induce the expression of mPGES-1. Harvested cells were sonicated, and the microsomes were isolated by differential centrifugation at 10 000 × *g* for 10 min and at 174 000 × *g* for 70 min as previously described [33]. Microsomal membranes (2.5–5 µg total protein) in 0.1 M potassium phosphate buffer, pH 7.4, containing 2.5 mM glutathione were preincubated with GA derivatives for 15 min at 4 °C. PGE_2 formation was initiated by the addition of PGH_2 (final concentration, 20 µM). After 1 min at 4 °C, the reaction was terminated, and PGE_2 was extracted and analyzed by RP-HPLC as reported [33]. Vehicle (DMSO)-treated microsomal membranes produced 1.2 ± 0.2 nmol PGE_2.

Statistics

Data are presented as the mean ± standard error (S.E.) of three independent experiments. Statistical calculations were performed using GraphPad Prism 4.0 (GraphPad Software, Inc.). Comparison of different groups was performed by one-way ANOVA followed by Tukey's HSD post hoc test. P values < 0.05 were considered statistically significant. IC_{50} values were determined by graphical analysis using SigmaPlot 13.0 (Systat Software Inc.).

Acknowledgements

K.A. received a PhD grant from the Government of Syria.
S.S.C. was employed by the University of Angers through a collaboration with Dr. René de Vaumas, ExtraSynthèse S.A.
The work of S.L. is supported by the German Federal Ministry of Education and Research (BMBF).

Conflict of Interest

The authors declare no conflict of interest.

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