

*Full Length Research Paper*

# **Extraction, purification and nuclear magnetic resonance (NMR) assignment of garcinoic acid from *Garcinia kola* (bitter kola) fruit**

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**Continuous extraction of bitter kola nuts, followed by liquid-liquid extraction and flash column chromatography leads to the isolation of garcinoic acid ( $\delta$ -tocotrienoloic acid), as the main lipophilic component in a 0.8% yield of the initial dry weight. This compound, an oxidised member of the vitamin E family, was structurally characterised using nuclear magnetic resonance spectroscopy.**

**Key words:** Vitamin E, tocopherol, antioxidant, two-dimensional nuclear magnetic resonance spectroscopy.

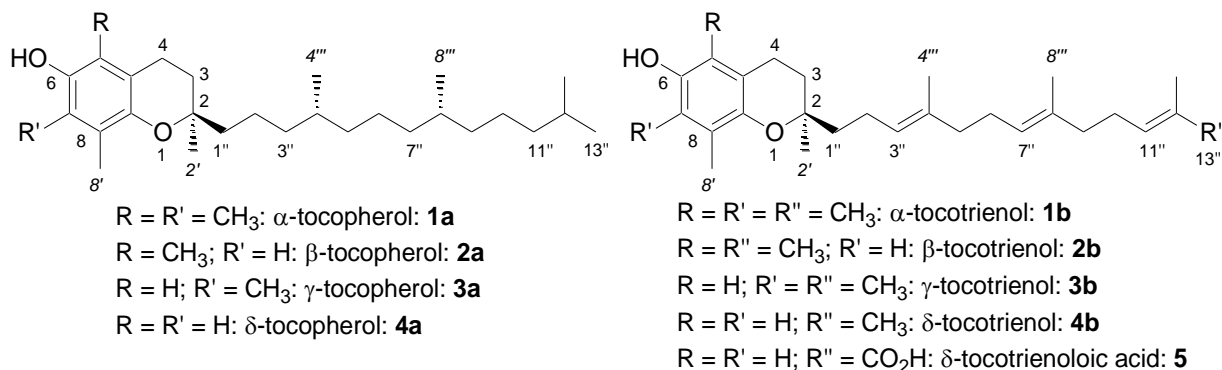
## **INTRODUCTION**

The vitamin E family comprises of a mixture of eight chromanols, compounds **1** to **4** (Figure 1) differing both by the extent and position of methylation on the aromatic ring and the degree of unsaturation in the prenyl-based side-chain (DellaPenna, 2005; Gerald, 2007; Zhao et al., 2010). Their lipid soluble antioxidant behaviour has been appreciated for many years and both naturally occurring and synthetically derived samples are widely used in the food industry (Zhao et al., 2010; Rein et al., 2007; Gerald, 2007). Plants represent the main natural source of vitamin E, where biosynthetically they are derived/originate from the plastoquinone pathway (DellaPenna, 2005; Sontag and Parker, 2002). The stereochemistry at position carbon-2 in the chromanol ring is controlled during a cyclisation mediated by tocopherol cyclase and similarly, the reductase converting the unsaturated prenyl-derived group to its saturated counterpart (present

in **1a** to **4a**) occurs with stereocontrol. Consequently, naturally occurring examples are single enantiomers (DellaPenna, 2005).

*Garcinia kola* (also known as Bitter kola – due to its bitter taste) is an African plant, the nuts of which are widely used by local populations. Several heterocyclic compounds have been isolated from this source (Iwu and Igboko, 1982; Kabangu et al., 1987; Terashima et al., 1999) including  $\delta$ -tocotrienoloic acid **5**, which is also known as garcinoic acid (Delle Monache et al., 1984; Terashima et al., 1997; Terashima et al., 2002; Mazzini et al., 2009). Apart from its antioxidant (Terashima et al., 1997; Terashima et al., 2002) and DNA polymerase inhibition (Maloney and Hecht, 2005), numerous, mainly anecdotal, effects resulting from the ingestion of these nuts are appreciated by people using them (Adebisi and Song, 2008). These include: appetite suppression and

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**Figure 1.** The vitamin E family of chromanols, including  $\delta$ -tocotrienoloic acid.

increasing thirst (astringent), the latter possibly due to its bitter taste and the alleviation of stomach discomfort resulting from indigestion, constipation and food poisoning. Ingestion of the fruit is also involved in social functions including “right-of-passage” ceremonies for young adults and it is believed to assist in both male and female sexual performance. Amongst the series of unsaturated vitamin E compounds,  $\delta$ -tocotrienoloic acid **5** with its carboxylic acid is of particular interest since the functionalised terminal C-13 carbon atom represents a potential means to prepare derivatives without interrupting the oxidant scavenging behaviour of the phenolic group.

## MATERIALS AND METHODS

The plant material used in this study was obtained from the equatorial rainforest of Meme Division in the South West province of Cameroon and was sourced from a street market. A single nut was selected and sliced and continuously extracted with hot methanol for 18 h in a Soxhlet apparatus as described below. The crude material, obtained after solvent removal was then extracted with chloroform (dichloromethane was also suitable but was more prone to emulsion formation) and water at neutral pH. Subsequent purification by flash column chromatography (silica gel, *c*-Hex-EtOAc; 5:1 to 3:1) gave  $\delta$ -tocotrienoloic acid **5** in good chemical purity. Infrared spectroscopy was performed on a FT-IR spectrometer. Routine electrospray mass spectra and high-resolution mass spectra were performed by electrospray ionization (ESI). The NMR spectra were recorded at 25°C on a 600 MHz spectrometer as indicated. Thin layer chromatography (TLC) was performed on 60F<sub>254</sub> aluminium plates with realisation by UV irradiation. Flash column chromatography was performed with silica, particle size 40 – 63  $\mu$ m.

Extraction procedure: (2*R*,3''*E*,7''*E*,11''*E*)-3,4-dihydro-2,8-dimethyl-2-(4'',8'',12''-trimethyltridecyl-13-carboxy)-2*H*-1-benzopyran-6-ol ( $\delta$ -tocotrienoloic acid/garcinoic acid) **5**: A single Bitter Kola nut (4.05 g) was sliced thinly and continuously extracted with MeOH (150 mL) in a Soxhlet apparatus for 18 h. On cooling, most of the MeOH was removed under reduced pressure and the residue (ca. 200 mg) taken up in CHCl<sub>3</sub> (20 mL) and extracted with water (50 mL). The resultant aqueous layer was further extracted with CHCl<sub>3</sub> (2 x 15 mL) and the combined organic extracts were dried over MgSO<sub>4</sub>. Filtration, followed by solvent removal under reduced pressure and purification by flash column chromatography (*c*-Hex-

EtOAc; 5:1 to 3:1) gave  $\delta$ -tocotrienoloic acid **5** (32 mg, 0.8%) as a viscous yellow oil.  $R_f$  = 0.35 (*c*-Hex-EtOAc; 3:1);  $[\alpha]_D^{20}$  = -2.05 (*c* = 2.1, MeOH), lit.  $[\alpha]_D$  = -4.2 (*c* = 0.37, MeOH) (Mazzini et al., 2009);  $\bar{\nu}_{max}$  (neat/cm<sup>-1</sup>) 3359, 3021, 2975, 2927, 2855, 1686, 1644, 1470, 1380, 1286, 1221, 1098; <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>):  $\delta$  = 1.25 (3H, s, CH<sub>3</sub>-2'), 1.53-1.61 (2H, m, CH<sub>2</sub>-1''), 1.57 (3H, s, CH<sub>3</sub>-4'''), 1.58 (3H, s, CH<sub>3</sub>-8'''), 1.72-1.78 (2H, m, CH<sub>2</sub>-3), 1.82 (3H, s, CH<sub>3</sub>-12'''), 1.95 (2H, t, *J* = 7.5 Hz, CH<sub>2</sub>-5''), 2.05-2.11 (6H, m, CH<sub>2</sub>-2'', CH<sub>2</sub>-6'', CH<sub>2</sub>-9''), 2.12 (3H, s, CH<sub>3</sub>-8''), 2.27 (2H, q, *J* = 7.5 Hz, CH<sub>2</sub>-10''), 2.68 (2H, app. t, *J* = 7.0 Hz, CH<sub>2</sub>-4), 5.12 (2H, t, *J* = 6.75 Hz, CH<sub>2</sub>-3''), 6.37 (1H, d, *J* = 2.75 Hz, CH-5), 6.47 (1H, d, *J* = 2.75 Hz, CH-7), 6.87 (1H, dt, *J* = 1.0, 7.25 Hz, 11'') ppm; <sup>13</sup>C NMR (150 MHz, CDCl<sub>3</sub>):  $\delta$  = 12.0 (CH<sub>3</sub>-12'''), 15.8 (CH<sub>3</sub>-4'''), 15.9 (CH<sub>3</sub>-8'''), 16.0 (CH<sub>3</sub>-8'), 22.1 (CH<sub>2</sub>-2''), 22.5 (CH<sub>2</sub>-4), 24.1 (CH<sub>3</sub>-2'), 26.4 (CH<sub>2</sub>-6''), 27.4 (CH<sub>2</sub>-10''), 31.3 (CH<sub>2</sub>-3), 38.0 (CH<sub>2</sub>-9''), 39.5 (CH<sub>2</sub>-5''), 39.55 (CH<sub>2</sub>-1''), 75.3 (C-2), 112.5 (CH-5), 115.6 (CH-7), 121.2 (C-4a), 124.4 (CH-3''), 125.2 (CH-7''), 126.7 (C-12''), 127.3 (C-8), 133.7 (C-8''), 134.8 (C-4''), 144.8 (CH-11''), 145.9 (C-8a), 147.7 (C-6), 172.3 (C-13'') ppm; *m/z* (ES<sup>+</sup>) found 449.2679, C<sub>27</sub>H<sub>38</sub>O<sub>4</sub>Na requires 449.2668 (+2.5 ppm).

## RESULTS AND DISCUSSION

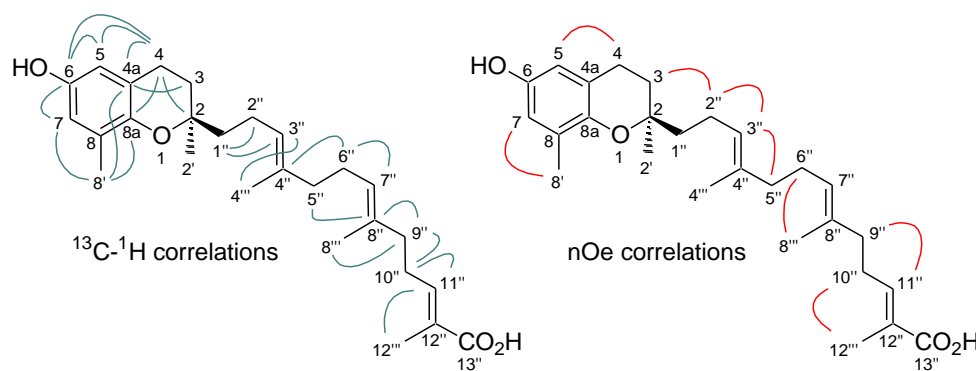
In this paper, we report an experimentally simple procedure for the isolation of multi-milligram amounts of  $\delta$ -tocotrienoloic acid **5** (also known as garcinoic acid) from *Garcinia kola* fruit (nuts). Based on the mass isolated following this protocol, versus the dry weight of the original nut used, yields of 0.5 to 0.8% were obtained. This figure corresponds favourably to a previous report involving a room temperature extraction protocol in which  $\delta$ -tocotrienoloic acid **5** was isolated in 0.05% yield (Terashima et al., 1997). Notably, only trace amounts of alternative vitamin E like molecules (Figure 1) were detected during this purification process, that is,  $\delta$ -tocotrienoloic acid was the major vitamin E like compound isolated from the nuts sourced.

Structural confirmation of  $\delta$ -tocotrienoloic acid **5** was performed by single and two-dimensional nuclear magnetic resonance spectroscopy experiments, mass spectrometry and infrared spectroscopy, all of which were consistent with the structural assignment (for data see “Material and Methods” section above and electronic

**Table 1.** Proton and carbon NMR assignments for  $\delta$ -tocotrienoloic acid **5**.

Position	$^1\text{H}$ (ppm) <sup>a</sup>	$^{13}\text{C}$ (ppm) <sup>b</sup>	Position	$^1\text{H}$ (ppm) <sup>a</sup>	$^{13}\text{C}$ (ppm) <sup>b</sup>	Position	$^1\text{H}$ (ppm) <sup>a</sup>	$^{13}\text{C}$ (ppm) <sup>b</sup>
<b>2</b>	---	75.3	<b>8'</b>	2.12	16.0	<b>7''</b>	5.12	125.2
<b>2'</b>	1.25	24.1	<b>8a</b>	---	145.9	<b>8''</b>	---	133.7
<b>3</b>	1.72-1.78	31.3	<b>1''</b>	1.53-1.61	39.55	<b>8'''</b>	1.58	15.9
<b>4</b>	2.68	22.5	<b>2''</b>	2.05-2.11	22.1	<b>9''</b>	2.05-2.11	38.0
<b>4a</b>	---	121.2	<b>3''</b>	5.12	124.4	<b>10''</b>	2.27	27.4
<b>5</b>	6.37	112.5	<b>4''</b>	---	134.8	<b>11''</b>	6.87	144.8
<b>6</b>	---	147.7	<b>4'''</b>	1.57	15.8	<b>12''</b>	---	126.7
<b>7</b>	6.47	115.6	<b>5''</b>	1.95	39.5	<b>12'''</b>	1.82	12.0
<b>8</b>	---	127.3	<b>6''</b>	2.05-2.11	26.4	<b>13''</b>	---	172.3

<sup>a</sup> $^1\text{H}$ -NMR (600 MHz,  $\text{CDCl}_3$ ); <sup>b</sup> $^{13}\text{C}$ -NMR (150 MHz,  $\text{CDCl}_3$ ).

**Figure 2.** Selected  $^{13}\text{C}$ - $^1\text{H}$  (grey) and nOe (red) spectroscopic correlations.

supporting information). Due to the chemical similarity of some protons and carbons in the unsaturated side-chain, some signals proved challenging to assign (Table 1). However, as shown in Figure 2, using a combination of two-dimensional correlation spectroscopies:  $^1\text{H}$ - $^1\text{H}$ -COSY,  $^1\text{H}$ - $^{13}\text{C}$ -HSQC, long range  $^1\text{H}$ - $^{13}\text{C}$ -HMBC, and nuclear Overhauser effect (nOe) experiments signal assignment could be achieved and it should be mentioned that this data correlates well with data from the previous report (Terashima et al., 1997).

NOESY (two dimensional nuclear Overhauser effect spectroscopy) experiments, additionally, facilitated assignment of the allylic protons (and by association their carbons) and assisted determination of the stereochemistry of the  $\Delta^{11,12}$ -double bond in the prenyl side-chain. However, it was impossible to unambiguously determine stereochemistry of the remaining, internal, double bonds since the chemical shifts of the  $4'''$  and  $8'''$  methyl groups and the  $3''$  and  $7''$  vinylic protons are almost identical. Based on precedence we assume the *trans*-stereochemistry shown.

In relation to the single stereogenic quaternary centre at C-2, previously reported optical rotation measurements for this molecule give conflicting values in both magnitude

and sign. Our small negative specific optical rotation measurement  $\{[\alpha]_D -2.05 (c = 2.1, \text{MeOH})\}$  differs in both sign and in magnitude to the report from Maloney and Hecht (2005). However, it is consistent in magnitude with Terashima et al. (1997) and in sign with Mazzini's et al. (2009). In addition, since only one tocopherol cyclase-based biosynthetic pathway has been detailed, we are confident that the absolute stereochemistry of **5** is *2R*, as shown in Figures 1 and 2.

Attempts to facilitate the isolation of  $\delta$ -tocotrienoloic acid **5** were performed using the same Soxhlet-based continuous extraction process albeit with acidic (0.5% conc.  $\text{H}_2\text{SO}_4$ ) methanol. The aim being, that  $\delta$ -tocotrienoloic acid **5** would undergo *in situ* conversion into the corresponding methyl ester by a Fischer esterification process as it accumulated in the flask. These studies were successful and did lead to isolation of the desired methyl ester. However, this material actually proved more difficult to purify from the resultant crude Bitter Kola extract by flash column chromatography than  $\delta$ -tocotrienoloic acid **5**.

This was due to the presence of additional components with similar chromatographic properties to the methyl ester.

## Conclusion

This research has revealed that yields of approximately 0.8% w/w of  $\delta$ -tocotrienoloic acid **5** can be obtained using Soxhlet extraction of *Garcinia kola* (Bitter kola) nuts. This represents an improvement compared to room temperature extractions although it should be pointed out that variability in the levels of this natural product could be expected. We believe that the  $\omega$ -carboxylic acid functionality present in  $\delta$ -tocotrienoloic acid **5** makes this a useful material for derivatisation and studies in this area, specifically linking  $\delta$ -tocotrienoloic acid **5** to nanoparticles, is ongoing.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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