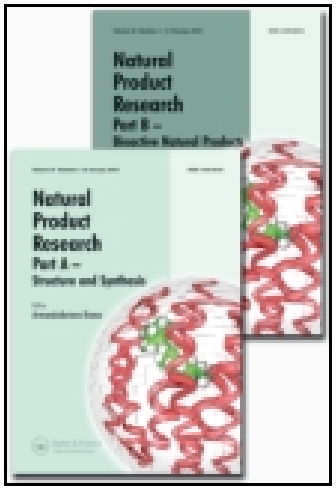

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An efficient total synthesis of trilepisiumic acid

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SHORT COMMUNICATION

An efficient total synthesis of trilepisiumic acid

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Total synthesis of trilepisiumic acid (4-((3-(3,4-dihydroxyphenyl)acryloyl)oxy)-3-hydroxybenzoic acid) isolated from *Trilepisium madagascariense* was carried out. Doebner condensation of 3,4-dimethoxybenzaldehyde with malonic acid yielded 3,4-dimethoxycinnamic acid (**2**). Esterification of the latter with vanillin afforded 4-formyl-2-methoxyphenyl-3-(3,4-dimethoxy phenyl)acrylate (**3**) followed by permanganate oxidation in acidic medium provided the corresponding acid **4**. Finally, demethylation of **4** was achieved by refluxing in hydrobromic acid to unveil the trilepisiumic acid (**1**).

Keywords: trilepisiumic acid; cinnamic acid; *Trilepisium madagascariense*

1. Introduction

Polyhydroxyaromatic acids are important secondary metabolites due to the presence of free phenolic hydroxy functions capable of exhibiting high free radical-scavenging activity. In addition, cinnamic acids and derivatives have a valuable place due to their pharmacological and industrial applications (Miliovsky et al. 2013). In nature, cinnamic acids display vast structural diversity due to different substitution patterns on the aromatic ring as well as various derivatives of the acid moiety. Cinnamic acids and derivatives exhibit a broad spectrum of biological activities such as anti-tuberculosis, antidiabetic, antimicrobial, antimalarial, cytotoxic and antifungal (Lee et al. 2000; Zhu et al. 2000; Narasimhan et al. 2004; Adisakwattana et al. 2008; Rao et al. 2011; Sharma 2011). *Trilepisium madagascariense* is a deciduous tree, found in Cameroon, Congo and Madagascar. The leaves of *T. madagascariense* are used as a vegetable; other parts of the plant exhibit antihyperglycaemic, antidiabetic, antimicrobial, antioxidant and pain killer activities. Moreover, it is used to treat venereal diseases, arthritis, diarrhoea, rheumatism, dysentery, stomach troubles, debility, malnutrition, and cutaneous and subcutaneous parasitic infections (Teke et al. 2010; Ampa et al. 2013).

Ango et al. (2012), during the phytochemical investigation of *T. madagascariense*, isolated two new compounds named as trilepisflavan and trilepisiumic acid (Figure 1), besides the ten known compounds, from the ethyl acetate extract of the stem bark and the leaves. The structure of trilepisiumic acid was established unambiguously by ^{1}H, ^{13}C, COSY, HMQC and HMBC data as (*E*)-4-((3-(3,4-dihydroxyphenyl)acryloyl)oxy)-3-hydroxy benzoic acid. Hydroxycinnamic acids are a class of phenylpropanoids having a C6–C3 skeleton. These compounds are hydroxy derivatives of cinnamic acid. The compound displayed potent antimicrobial activity against eight microorganisms: reference strains of *Providencia smartii*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Salmonella typhi*, *Escherichia coli* and *Candida albicans.* Trilepisiumic acid possesses some very important functionalities

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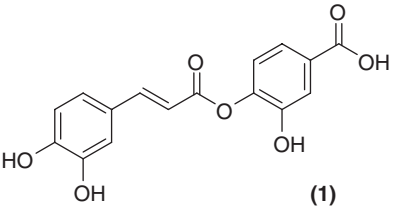


Figure 1. (*E*)-4-((3-(3,4-dihydroxyphenyl)acryloyl)oxy)-3-hydroxybenzoic acid.

such as an α,β-unsaturated carbonyl moiety, three free phenolic groups and one free carboxylic function, with an extensive potential of derivatisation into a diversity of interesting homo- and heterocyclic compounds. Unsaturated fatty acids, such as linoleic acid, palmitoleic acid, oleic acid and arachidonic acid, inhibit bacterial enoyl-acyl carrier protein reductase (FabI), an essential component of bacterial fatty acid synthesis, whereas the saturated analogues such as stearic acid do not (Zheng et al. 2005). Chalcones containing an α,β-unsaturated carbonyl system display a wide spectrum of biological activities. Similarly, benzofurancarboxylic acid derivatives are known to exhibit antimicrobial activity (Kossakowski et al. 2010). Antimicrobial activity, structure–activity relationship analysis and docking studies of phenolic compounds in wild mushrooms have been reported (Alves et al. 2010).

The presence of two important pharmacophoric fragments namely phenolic groups and α,β-unsaturated carbonyl system in one molecule suggests synergistic effect making this compound an attractive target for synthesis (Figure 1).

2. Results and discussion

We now divulge an efficient synthesis of (*E*)-4-((3-(3,4-dihydroxyphenyl)acryloyl)oxy)-3-hydroxybenzoic acid. The synthesis started with Doebner modification of Knoevenagel condensation of 3,4-dimethoxybenzaldehyde with malonic acid in refluxing pyridine using tetrahydropyrrole as an organocatalyst to afford directly the 3,4-dimethoxycinnamic acid (**2**). In Doebner modification instead of diethylmalonate, the malonic acid is used and its coupling product with benzaldehyde undergoes concerted decarboxylation and elimination to cinnamic acid (Stacey et al. 2013). In the ^{1}H NMR spectrum of **2**, the signal at δ 12.2 ppm confirmed the presence of acidic proton besides the doublets at δ 6.4 ppm with $J = 15.9$ Hz and 7.52 ppm $J = 15.9$ Hz assigned to α and β protons, respectively, the coupling constants also establish the *E*-configuration around the double bond at the very first step.

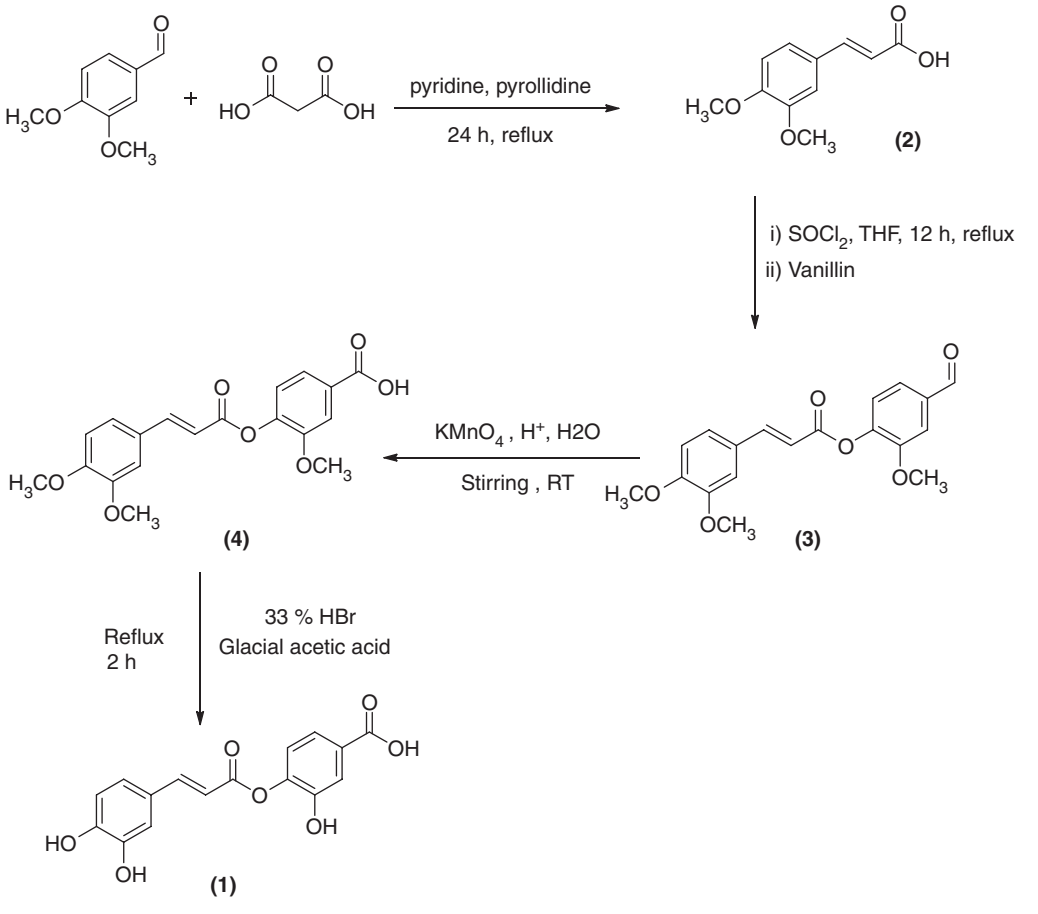
The acid (**2**) was converted into corresponding acid chloride on refluxing with thionyl chloride in the presence of 1 or 2 drops of DMF which act as a catalyst. Esterification of the latter with 4-hydroxy-3-methoxy benzaldehyde (vanillin) was carried out in dry THF in the presence of triethyl amine to afford the (*E*)-4-formyl-2-methoxyphenyl-3-(3,4-dimethoxyphenyl)acrylate (**3**) as a white crystalline solid. In the FTIR spectrum, the stretching frequency of ester carbonyl group was found at 1708 cm^{-1} which is lesser than the normal range due to the presence of extended conjugation. The ^{1}H NMR spectrum confirmed the presence of aldehydic proton at 9.98 ppm and the absence of acidic proton besides doublets at 7.86 for β-H, 6.80 for α-H, nine proton singlet 3.9 for OMe protons, while in ^{13}C NMR carbonyl carbons of aldehyde and ester functions appeared at δ 191 and 164 ppm, respectively, in addition to signals at 152.14 (β-C), 109.62 (α-C) and 55.91–56.14 (—OMe), respectively.

The oxidation of formyl group in **3** into (*E*)-4-((3-(3,4-dimethoxyphenyl)acryloyl) oxy)-3-methoxybenzoic acid **(4)** was achieved by treating with one equivalent of $KMnO_4$

in acidic medium at room temperature to furnish the product as a white crystalline solid. The acidic medium was used to augment the electrophilic character of the formyl group to carry out the oxidation under mild conditions. ^{1}H NMR spectrum of **4** confirmed the presence of acidic proton at 12.95 ppm and acid carbonyl acid signal at 170.1 ppm (Scheme 1).

Complete demethylation of the dimethoxy acid (**3**) was achieved in refluxing hydrobromic acid in glacial acetic acid (Zou et al. 2008) to unveil the trilepisiumic acid. Although BBr_3 is the mild reagent of choice for the complete cleavage of *O*-methyl ethers of natural products, HBr was to be used for demethylation due to its unavailability. In the IR spectrum, the presence of strong band at 3450 cm^{-1} confirms the presence of hydroxyl groups. The target compound obtained as a silvery solid with m.p. at 180°C was characterised by the complete absence of all MeO singlets, both in the ^{1}H NMR and ^{13}C NMR spectra, and other characteristic changes.

Thus, the present synthesis constitutes a simple route to a natural product trilepisiumic acid possessing biological activities. It involves three linear steps and proceeds with good overall yield, which makes it available for biological evaluation. Trilepisiumic acid is being tested for antioxidant and cytotoxic activities.



Scheme 1. Synthetic pathway to trilepisiumic acid.

3. Experimental

3.1. *General methods*

All the chemicals used in the synthesis were purchased from Sigma-Aldrich Chemie Gmbh (Munich, Germany) and were used without any further purification. Thin-layer chromatography was used to monitor the progress of the reactions. All the compounds were purified over silica gel column. Solvents were distilled before using for purification purposes. Melting points were recorded using a digital Gallenkamp (SANYO, Lough borough, UK) model MPD BM 3.5 apparatus and are uncorrected. The ^{1}H NMR and ^{13}C NMR spectra were recorded using Bruker AM-300 spectrophotometer (Billerica, Middlesex, MA, USA) at 300 and 75.5 MHz, respectively, using TMS as an internal standard. The chemical shift values are recorded on δ scale and the coupling constants (J) are in Hz. The FTIR spectra were recorded using Bio Rad Excalibur FTS 3000 MX spectrophotometer (Madison, WI, USA). R_f values reported using solvent system: petroleum ether and ethyl acetate (2:1).

3.1.1. *3,4-Dimethoxycinnamic acid (**2**)*

3,4-Dimethoxybenzaldehyde (5.0 g, 0.02 mol) was refluxed with malonic acid (8.0 g, 0.06 mol) in pyridine (50 mL) and few drops of tetrahydropyrrole as a catalyst for 20 h. The reaction mixture was acidified with dilute hydrochloric acid to attain pH 7. The precipitates of product obtained were settled down, filtered, washed with cold water and dried to afford 3,4-dimethoxycinnamic acid (**2**) as a light yellow solid. Yield: 85%, R_f: 0.22, m.p.: 130–133°C, IR (neat) ύ (cm^{-1}): 1650 (C═O acid). ^{1}H NMR (DMSO, δ ppm): 12.2 (1H, s, acid), 7.52 (1H, d, $J = 15.9$ Hz, β-H), 7.32 (1H, d, $J = 1.8$ Hz, HC-2′), 7.20 (1H, m, HC-6′), 6.96 (1H, d, $J = 8.4$ Hz, HC-5′), 6.4 (1H, d, $J = 15.9$ Hz, α-H), 3.7–3.8 (6H, s, —OMe). ^{13}C NMR (DMSO, δ ppm): 168.3 (C═O acid), 151.2 (β-C), 111.9–151.2 (Ar—C), 110.66 (α-C), 55.97–56.01 (—OMe); elemental analysis: found: C, 63.49, H, 5.81%; calcd for $C_{11}H_{12}O_4$: C, 63.45; H, 5.81%.

3.1.2. *(E)-4-Formyl-2-methoxyphenyl 3-(3,4-dimethoxyphenyl)acrylate (**3**)*

3,4-Dimethoxycinnamic acid (**2**) (4.5 g, 0.012 mol) was refluxed with $SOCl_2$ (8 mL) in dry THF for 12 h. The solvent was rotary evaporated to afford viscous yellow oil of 3,4-dimethoxycinnamoyl chloride. Then, this acid chloride was treated with vanillin (3.5 g, 0.012 mol) using triethyl amine (5 mL) in dry THF at room temperature for 12 h. Then, the reaction mixture was poured into ice-cold water and precipitates that appeared after 30 min were filtered out. These precipitates were washed with 5% HCl and then with ice-cold water to afford (*E*)-4-formyl-2-methoxyphenyl 3-(3,4-dimethoxyphenyl)acrylate as a white solid. Yield: 75%, R_f: 0.89, m.p.: 130–133°C, IR (neat) ύ (cm^{-1}): 1710 (C═O ester), 1725 (C═O acid). ^{1}H NMR (DMSO, δ ppm): 9.98 (1H, s, —CHO), 7.86 (1H, d, $J = 15.9$ Hz, β-H), 7.52 (2H, m, HC-6″, HC-2″), 7.31 (1H, d, $J = 7.8$ Hz, HC-5″), 7.18 (2H, m, HC-6′, HC-2′), 6.92 (1H, d, $J = 8.4$ Hz, HC-5′), 6.5 (1H, d, $J = 15.9$ Hz, α-H), 3.90 (9H, s, —OMe). ^{13}C NMR (DMSO, δ ppm): 191 (C═O aldehyde), 164 (C═O ester), 152.14 (β-C), 109.76–151.95 (Ar—C), 109.62 (α-C), 55.91–56.14 (—OMe); elemental analysis: found: C, 66.63; H, 5.29%; calcd for $C_{19}H_{18}O_6$: C, 66.66; H, 5.30%.

3.1.3. *(E)-4-((3-(3,4-Dimethoxyphenyl)acryloyl)oxy)-3-methoxybenzoic acid (**4**)*

(*E*)-4-formyl-2-methoxyphenyl 3-(3,4-dimethoxyphenyl)acrylate (**3**) was dissolved in acetone (3 g, 0.054 mol) and stirred with $KMnO_4$ acidified with dilute H_2SO_4, at 0°C for 30 min. The reaction mixture was then stirred overnight at room temperature. Then was extracted with ethyl

acetate and the precipitates appeared were filtered out. The organic layer was dried over anhydrous sodium sulphate and evaporated to yield (*E*)-4-((3-(3,4-dimethoxyphenyl)acryloyl) oxy)-3-methoxybenzoic acid (**4**) as a white solid. Yield: 75%, R_f: 0.42, m.p.: 130–133°C, IR (neat) ύ (cm^{-1}): 1710 (C═O ester), 1720 (C═O acid). ^{1}H NMR (DMSO, δ ppm): 12.95 (1H, s, —COOH), 7.76 (1H, d, $J = 15.9$ Hz, β-H), 7.61 (2H, m, HC-6″, HC-2″), 7.47 (1H, d, $J = 1.8$ Hz, HC-5″), 7.31 (2H, m, HC-6′, HC-2′), 7.02 (1H, d, $J = 8.4$ Hz, HC-5′), 6.80 (1H, d, $J = 15.9'$Hz, α-H), 3.90 (9H, s, —OMe). ^{13}C NMR (DMSO, δ ppm): 170.1 (C═O acid), 164 (C═O ester), 152.14 (β-C), 109.76–151.95 (Ar—C), 109.62 (α-C), 55.91–56.14 (—OMe); elemental analysis: found: C, 66.59, H, 5.11%; calcd for $C_{19}H_{18}O_7$: C, 63.68; H, 5.06%.

3.1.4. *Trilepisumic acid ((*E*)-4-((3-(3,4-dihydroxyphenyl)acryloyl)oxy)-3-hydroxybenzoic acid) (**1**)*

(*E*)-4-((3-(3,4-dimethoxyphenyl)acryloyl)oxy)-3-methoxybenzoic acid (**4**) (1.2 g, 0.000034 mol) was refluxed with 33% HBr in glacial acetic acid (6 mL) for 2 h. The reaction mixture was poured into ice (50 g) and then solid sodium carbonate was added to attain pH 6. The compound was extracted with ethyl acetate and the solvent was evaporated to afford (*E*)-4-((3-(3,4-dihydroxyphenyl)acryloyl)oxy)-3-hydroxybenzoic acid as a silvery crystalline solid. Yield: 75%, R_f: 0.22, m.p.: 130–133°C, IR (neat) ύ (cm^{-1}): 1712 (C═O ester), 1725 (C═O acid), 3450 (—OH). ^{1}H NMR (DMSO, δ ppm): 12.95 (1H, s, —COOH), 7.78 (1H, d, $J = 15.9$ Hz, β-H), 7.63 (2H, m, HC-6″, HC-2″), 7.47 (1H, d, $J = 1.8$ Hz, HC-5″), 7.32 (2H, m, HC-6′, HC-2′), 7.04 (1H, d, $J = 8.4$ Hz, HC-5′), 6.82 (1H, d, $J = 15.9$ Hz, α-H), 5.35 (3H, s, b, —OH). ^{13}C NMR (DMSO, δ ppm): 170.1 (C═O acid), 164 (C═O ester), 152.14 (β-C), 109.76–151.95 (Ar—C), 109.62 (α-C), 55.91–56.14 (—OMe); MS (70 eV) *m/z* (%): 296 $[M]^{+\cdot}$, 251 (34), 177 (16), 147 (100%), 91 (28); elemental analysis: found: C, 60.73, H, 5.26%; calcd for $C_{16}H_{12}O_7$: C, 60.76; H, 4.82%.

4. Conclusions

The first total synthesis of natural antimicrobial metabolite trilepisiumic acid was achieved. The pivotal steps include the esterification of vanillin with 3,4-dimethoxycinnamic acid followed by the oxidation of the aldehyde function.

Supplementary material

Supplementary relating to this article is available online, alongside Figures S1–S5.

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