Eicosanyl p-Coumarates from a Kenyan Plant, *Psidia punctulata*: Plant Growth Inhibitors

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From methanol extracts of fresh leaves of *Psidia punctulata* (DC.) Vatke, lettuce seed radicle growth inhibitors were isolated and identified as E- and Z-eicosanyl p-coumarates by spectroscopic analyses.

**Key words:** *Psidia punctulata* (DC.) Vatke; Asteraceae; radicle growth inhibition; lettuce seeds; E- and Z-eicosanyl p-coumarates

Species of the genus *Psidia* (Asteraceae) are used in Africa as folk medicines for treatment of abdominal pains and colds in the head, a poultice for rheumatoid arthritis, an analgesic for brain and nerves, an expectorant for bronchitis and asthma, and a plaster cast for broken bones, by the Bedouins.  

Beside this medicinal aspect, a number of African plants can regulate the growth of other plants. Some studies have been reported on this subject involve a simplified bioassay for growth of plants or their seeds, and isolation and elucidation of the chemical structure of the active compounds. Many African plants have been examined in this respect since the approach may particularly be important from the agricultural, environmental, and economic viewpoints in the African continent for developing natural fertilizers and pesticides.

This report describes a new finding that lettuce seed growth-regulating compounds are found in the fresh leaves of *P. punctulata* naturally grown in Kenya, and the compound constitutes a light-dependent equilibrium of E,Z-isomers.

Fresh leaves of *P. punctulata* were extracted with methanol for two weeks in a dark-cold room to afford a crude extract after evaporation of solvent. Partitioning the extract between water and hexane showed that the activities were found in the hexane layer, guided through the lettuce seedling growth bioassay. Chromatographic purification of the active components on normal and reverse phase columns and on preparative TLC afforded a mixture of two compounds that were found to be interconvertible by light irradiation indicating that light-induced geometrical isomerization occurs. The spectral data for this mixture were as follows. UV absorption: 312, 226, and 211 nm (conjugated olefinic bond with aromatic system). The 1H-NMR spectrum showed two sets of a signal pattern indicating that the component constitutes a mixture of two closely related compounds. Signals at δ 7.42–7.64 and δ 6.80–6.82 suggested the occurrence of a 1,4-disubstituted benzene ring system. On hydrogenation, the disappearance of signals for olefinic protons in the region δ 5.82–6.82 and δ 7.62 and the subsequent appearance of signals for methylene protons in the region δ 2.56–2.86, suggested the presence of an α,β-unsaturated ester in the mixture. The presence of a hydroxyl group was shown by an acetylation and its NMR proton signals. The molecular ion peaks at m/z 444 (M+1) in EIMS and 445 (MH+) in ion spray MS together with 13C-NMR suggest a molecular formula C_{18}H_{26}O_{3} for the mixture when the two components have the same molecular formulae. 1H–1H COSY studies indicated two sets of signal correlation, i.e., signals at δ 7.62 (1H, d, J = 15.9 Hz) correlated with those at δ 6.29 and signals at δ 6.82 (1H, d, J = 12.8 Hz) with those at δ 5.82, while those at δ 4.10 and 4.18 correlated with those at δ 1.60. Additionally, FT-IR absorptions of the mixture indicated the presence of the two ester carbonyls corresponding to E- and Z-isomers. All the data suggest the compound mixture to involve an ester of E and Z-p-coumaric acid with a long hydrocarbon chain. Transesterification of the mixture using sodium methoxide and GC-MS analysis of the products unambiguously showed that the mixture is esters of p-coumaric acid with eicosanol. This conclusion was also confirmed by a synthesis through azotropic dehydration between p-coumaric acid and eicosanol (Fig. 1).

E,Z-Isomerization of natural and synthetic p-coumarate was shown by 1H-NMR (500 MHz, CDCl3) experiments before and after room-light irradiation. When a solution of the Z-enriched (E:Z = 1:9) mixture (2 mg) in CDCl3 (0.75 ml) in an NMR tube was irradiated with room light for 48 h at room temperature, the proton signals due to E-isomer increased to an extent of E:Z = 56:44. Isomerization from E to Z was also observed by the same method. These results are in accordance with the observations by Aulin-Erdman and Sandén on the action of visible and/or long-wave ultraviolet light on a variety of compounds with structures similar to that of eicosanyl p-coumarate.

Though this ester was also isolated from the genus *Leptospermum*, this is the first report of its isolation of eicosanyl p-coumarate from the genus *Psidia*.

![Fig. 1. Structures and Isomerization of E- and Z-Eicosanyl p-Coumarates.](image-url)
The results of plant growth regulating activities indicated eicosenyl p-coumarate to be inhibitory to the growth elongation of both the radicle and the hypocotyl of lettuce seedlings (Fig. 2). The effects were found to be stronger on the radicle as compared with the hypocotyl at all concentrations.

The radicle was more than 50% inhibited by this ester at 7.5 mM, while the hypocotyl was slightly less than 30% inhibited. The sample showed a radicle growth inhibition even at lower concentrations (up to 1 mM) with the overall effect decreasing with sample concentration. These results agree with those reported previously on related work in which phenolic-like compounds and their derivatives have been implicated in this aspect of seed germination particularly growth inhibition. Thus, eicosenyl p-coumarate isolated from P. punctulata for the first time seems to be placed in the same category of the phenolic compounds in its chemical structure as well as the biological activities. The results of this study are expected to constitute an early basis of use of this plant or its active constituents for plant growth control in Africa.

**Experimental**

1H- and 13C-NMR spectra were recorded at 500 and 125 MHz respectively on a Varian VXR 500 NMR spectrometer while EIMS were on D300 (JEOL) and ion spray MS on the model API-III (Perkin Elmer). FT-IR on model 710 (Nicolet) and UV on the model 3000 (Shimadzu) spectrophotometers. GC-MS was done on a DB 1 column (φ 0.25 mm × 30 m) with temperatures from 100 to 280 °C (10 °C/min). Injection temp. was kept at 280 °C while the interface temp. at 250 °C. He, 31.4 ml/min. HPLC was done on a Yanaco L-5000 (Yanagimoto), Column: Yanapak-ODS-A (φ 4.6 mm × 25 cm). Column chromatography was done on silica gel 60 (Nacalai Tesque, 230–400 mesh), TLC was on Kieselgel 60 F254 (Merck, Art. 5554, 0.2 mm) and reverse phase was done using Sep-pak (C18, Waters) cartridges with water and methanol.

**Plant materials.** Plant materials were collected from around Nairobi, Kenya in February 1992. The plant samples were authenticated by Dr. MIDWI and Mr. Mathege, both of the University of Nairobi. Voucher specimens have been deposited at the herbarium of the University of Nairobi.

**Extraction and isolation.** Fresh leaves (10 kg) of P. punctulata were collected and extracted with methanol in a dark-cold room (0–5°C) for two weeks to give a crude extract after evaporation of the solvent. A part of the extract (278 g) was then partitioned between water and hexane to give two fractions. By the lettuce seedling growth bioassay, growth activities were found to be concentrated in the hexane soluble fraction. Thus, a portion of it (200 mg) was repeatedly purified on silica gel, first using hexane–ethyl acetate (3:1:1–1) and second benzene–ethyl acetate (9:1) as eluants, and by prep. TLC × 3 (hexane–ethyl acetate, 8:2), and finally through a reverse phase columns using methanol–acetonitrile (1:1) and methanol–water (8:2) solvent systems to afford the active component (4.4 mg). The lettuce seedling bioassay was done as described in our previous report.

Z-Eicosanyl p-coumarate. \( R_1 = 0.52 \) (hexane–ethyl acetate, 8:2), HPLC. MeOH (100%) \( t_r = 15.2 \) min; EIMS m/z (rel. int.): 444 (M+), 107, 281 (19), 221 (3), 164 (100), 147 (66), 120 (30), 107 (36); Ion spray MS m/z: 445 (MH+), 15; FT-IR \( \nu_{max} \) (KBr) cm\(^{-1}\): 3385, 1711, 1604, 1515, 836; UV \( \lambda_{max} \) (EtOH) nm: 210, 226, 300, 312; \( ^1 \)H-NMR (500 MHz, CDCl\(_3\)): \( \delta_{7.64} \) (2H, \( d, J = 8.6 \) Hz), 6.82 (1H, \( d, J = 12.8 \) Hz), 6.80 (2H, \( d, J = 8.6 \) Hz), 5.82 (1H, \( d, J = 12.8 \) Hz), 4.10 (2H, \( t, J = 6.8 \) Hz), 1.6–1.2 (36H, m), 0.87 (3H, t, \( J = 6.8 \) Hz).

E-Eicosanyl p-coumarate. \( R_1 = 0.44 \) (hexane–ethyl acetate, 8:2), HPLC. MeOH (100%) \( t_r = 14.0 \) min; FT-IR \( \nu_{max} \) (KBr) cm\(^{-1}\): 3383, 1765, 1604, 1515, 836; UV \( \lambda_{max} \) (EtOH) nm: 210, 226, 300, 312; \( ^1 \)H-NMR (500 MHz, CDCl\(_3\)): \( \delta_{7.62} \) (1H, \( d, J = 15.9 \) Hz), 7.42 (2H, \( d, J = 8.6 \) Hz), 6.82 (2H, \( d, J = 8.6 \) Hz), 6.29 (1H, \( d, J = 15.9 \) Hz), 4.18 (2H, \( t, J = 6.8 \) Hz), 1.6–1.2 (36H, m), 0.87 (3H, t, \( J = 6.8 \) Hz). The other data are similar to that of the Z-isomer given above.

**Transferfermidiation of the active components.** Transsterfermidiation reaction was done by treating the active components (about 0.5 mg) with a few drops of 5% solution of sodium methoxide in dry distilled methanol. Reaction mixture was then purified on a silica gel short column using hexane–ethyl acetate (7:3), and the eluate was analyzed through GC-MS. On the total ion chromatogram, two peaks (\( t_r = 10.57 \) and 17.50 min) were detected, which corresponded to the methyl ester of p-coumaric acid (m/z 178, M+1) and eicosanol (m/z 280, M+2H, O).

**References**