A Plant Growth Regulator from *Vernonia auriculifera* (Asteraceae)

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Growth Regulator, *Vernonia auriculifera*, 8-Desacylvernodalol, Lettuce, Seeds, Radicle Growth Promotion

From the methanolic extract of fresh leaves of the Kenyan *Vernonia auriculifera* Hiern., a lettuce seeds growth regulator was isolated and identified as 8-desacylvernodalol (**1**) by spectroscopic analysis.

Introduction

The genus *Vernonia* has been extensively investigated (Bohlmann and Jakupovic, 1990) and nearly all species contain highly oxygenated sesquiterpene lactones with glaucolides being the most characteristic metabolites (Zdero *et al.*, 1991). The old world species (Tsichritzis *et al.*, 1991) contain mainly the elemanolides like vernolepin (**2**) (Kupchan *et al.*, 1968 and 1969; Toubiana *et al.*, 1975; Laekeman *et al.*, 1983 and Jakupovic *et al.*, 1985) and germacranolides like vernodalin (**4**) (Kupchan *et al.*, 1969; Toubiana *et al.*, 1975 and Jakupovic *et al.*,1985) among many glaucolides reported from them (Bohlmann and Jakupovic, 1990). From this viewpoint however, there has been no investigations on the Kenyan *Vernonia auriculifera* Hiern. (Asteraceae). Traditionally, the plant was used mainly as a medicine container and has a number of medicinal uses similar to those of *V. amygdalina* (Kokwaro, 1976). In the present study, we have been interested in the biological active principles in this species. Thus, this communication describes the isolation of 8-desacylvernodalol (**1**) from *V. auriculifera* and assay results based on lettuce seeds growth regulation as well as lethal toxicity to brine shrimp.

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Materials and Methods

General

Plants materials were collected from Jomo Kenyatta University farm in February 1992 and authenticated by Dr. G. M. Kenji of Jomo Kenyatta University, Nairobi, Kenya. Fresh leaves of *V. auriculifera* (10 kg) were extracted in a dark and cold room (0–5 °C) over methanol for two weeks to afford a crude extract after evaporation of the solvent. Partition of the concentrate between water and ethyl acetate resulted in the concentration of growth activity in the ethyl acetate phase. The material remaining after evaporation of ethyl acetate, was successively chromatographed over silica gel column from which five fractions were collected. Further fractionation of the active fractions guided by assay on lettuce seeds growth, led to the isolation of the known 8-desacylvernodalol (**1**) (0.14% yield) as the major active principle.

^{1}H and ^{13}C NMR spectra were recorded at 500 and 125 MHz respectively on a Varian VXR 500 NMR and EI-MS were measured on D300 (JEOL) spectrometers. M.p. on Yamato Melting Point Apparatus Model mp-21 and are uncorr. Column chromatography was done on silica gel 60 (Nacalai tesque, 230–400 mesh), TLC was on Kieselgel 60 F_{254} (Merck, *Art.* 5554, 0.2 mm) and reverse phase was done using SEP-PAK® (C_{18}, Waters) cartridges with water and methanol.

Brine shrimp toxicity bioassay

Brine shrimp (*Artemia salina*) toxicity bioassay (Alkofahi *et al.*, 1989) was conducted as follows. Artificial sea water was prepared by dissolving 38 g of sea salt (Five -plan type) in distilled water (1 L). Brine shrimp eggs were added into about 80 ml of the artificial sea water in a 100 ml beaker, to give a large number of brine shrimp larvae (nauplii) after incubation for 48 h at 24 °C. With a few milliliters of the artificial sea water in 5 ml calibrated containers, the brine shrimps (10 per

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container) were introduced followed by the addition of pre-prepared samples in DMSO to give the desired concentrations in ppm. Finally, artificial sea water was added to make up to the mark. A container into which DMSO alone was added served as control. All the containers were covered and transferred in an incubator at 28 °C for a period of 24 h. After this period of incubation, the number of dead and surviving brine shrimps were recorded and the values converted into percentages relative to the control (% mortality).

Lettuce seedlings growth bioassay

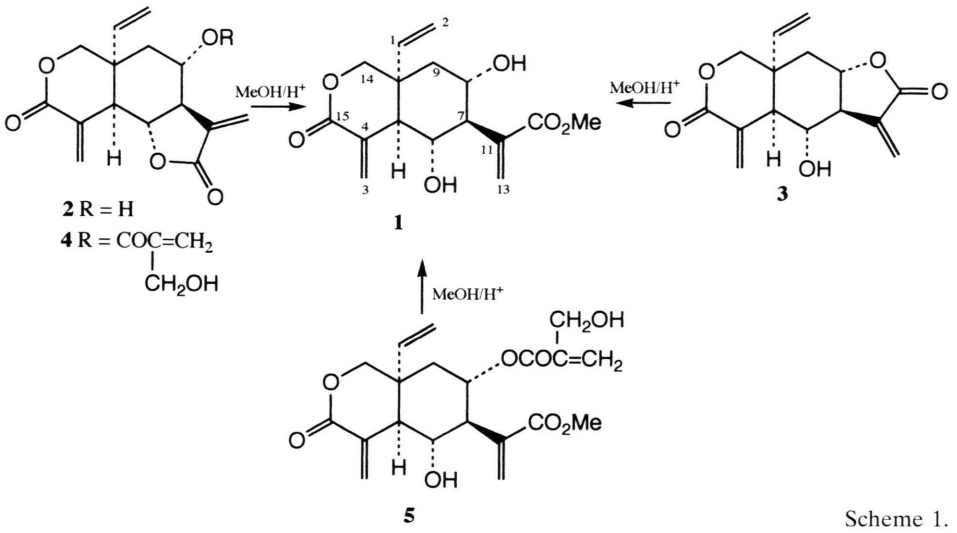
Lettuce seedlings growth bioassay was performed as previously described (Kato, 1981) with some modifications. Sample solutions in methanol (1 mg/ml) were applied onto round filter papers (15 mm diameter) suspended in air using pre-sterilized pins to give various sample quantities (10, 20, 50, 75, 100, 150 and 200 µg/disc). After the solvent was allowed to dry in a vacuum pump for 20 min, the paper discs were later placed into 24-well tissue culture plates (Corning). Lettuce seeds (10 seeds/disc) were sown on the filter papers followed by the addition of 150 µl of distilled water to give various sample concentrations (approx. 0.22, 0.44, 1.10, 1.62, 2.20, 3.25 and 4.40 mM) respectively. Finaly, the plates were covered and transferred into a larger plastic dish containing moist cotton wool and then incubated at 24 °C for 72 h. Paper discs applied with methanol alone served as controls. However, an equal amount of distilled water was added after the first 24 h. At the end of the incubation period, the radicle, the hypocotyl and the total lengths including the weight of seedlings per disc were measured and the mean lengths and the weights converted into percentages relative to the control.

8-Desacylvernodalol (**1**)

M.p. 172–174 °C; m.p. 174 °C (Asaka *et al.*, 1977); EI-MS *m/z* (rel. int.): 308 (M^+, 1.5), 290 (M^+-18, 2.4), 280 (M^+-28, 2.4), 277 (M^+-31, 3.5), 258 (7.8), 249 (M^+-59, 2.5) and 248 (8.3); $[\alpha]_D$ +68.2 (c 1.00, acetone); FT-IR ν_{max} (KBr) cm^{-1}: 3400 (O–H), 2975 (C–H), 1714 and 1690 (CO), 1640 (C=C), 1480, 1450, 1182 and 1158 (C–O); ^1H NMR (CD_3OD) δ (ppm): 1.53 (dd, *J* = 14, 12 Hz, H-9′), 1.94 (dd, *J* = 14, 4.5 Hz, H-9), 2.43 (dd, *J* = 11, 11 Hz, H-7), 2.50 (dd, *J* = 11, 1 Hz, H-5), 3.79 (s, $-OCH_3$), 3.95 (dd, *J* = 11, 11 Hz, H-6), 4.10 (m, H-8), 4.40 (dd, *J* = 12, 2 Hz, H-14′), 4.70 (d, *J* = 12 Hz, H-14), 5.23 (d, *J* = 11 Hz, H-2c), 5.27 (d, *J* = 18 Hz, H-2t), 5.79 (dd, *J* = 18, 11 Hz, H-1), 5.79 (d, *J* = 1.5 Hz, H-3′), 5.82 (s, H-13′), 6.41 (d, *J* = 1 Hz, H-3) and 6.56 (d, *J* = 2 Hz, H-13); ^{13}C NMR (CD_3OD) δ (ppm): 169 (C-15),166 (C-12), 142 (C-1), 139 (C-4), 134 (C-11), 133 (C-3), 129 (C-13), 115 (C-2), 72 (C-14), 69 (C-8), 66 (C-6), 59 (C-7), 51 (–OMe), 48 (C-5), 42 (C-10) and 40 (C-9).

Results and Discussion

Compound **1**, $C_{16}H_{20}O_6$, was obtained as colourless crystals. EI-MS spectrum showed a molecular ion peak at *m/z* 308. Acetylation using acetic anhydride in pyridine resulted in a compound with a molecular ion peak at *m/z* 392 thereby suggesting the presence of two free hydroxyl groups. This was supported by the presence of an absorption band at 3400 cm^{-1} in the FT-IR spectrum. In the ^1H NMR spectrum, signals at δ 1.53 and δ 1.94 including those at δ 4.40 and δ 4.70 indicated the presence of geminal methylene protons. A methoxyl signal at δ 3.79 and methine proton signals at δ 3.95 and δ 4.10 were clearly discernible. Signals seen at δ 5.23, δ 5.27, δ 5.79 and δ 6.56 including those observed at δ 5.82 and δ 6.41 suggested the presence of terminal methylene and vinyl protons. This was supported by the appearance of peaks at δ 115 and δ 142 (vinyl carbon atoms) in addition to those at δ 129 and δ 133 (terminal methylene carbon atoms) in the ^{13}C NMR spectrum which also showed peaks at δ 51 (methoxyl), δ 66, δ 69 and δ 72 (carbon atoms attached to heteroatoms) when the spectrum was determined in CD_3OD. As a result of the appearance of carbonyl carbon signals distinctively seen at δ 166 and δ 169, and supported by absorption bands at 1714 cm^{-1}, 1690 cm^{-1}, 1182 cm^{-1} and 1158 cm^{-1} in the FT-IR spectrum, the presence of an ester group was established. Comparison of these results with those reported of Jakupovic *et al.* (1985) and the use of ^1H–^1H COSY data, suggest this compound is 8-desacylvernodalol (**1**), which had previously been reported as a product of hydrolysis of **2** and vernomenin (**3**) (Kupchan *et al.*, 1968 and 1969), **4** (Kupchan *et al.*, 1969) and vernodalol (**5**) (Asaka *et al.*, 1977) in acidic methanol (Scheme 1).



Scheme 1.

Brine shrimp toxicity bioassay indicated that 8-desacylvernodalol (**1**) was virtually nontoxic. At 400 ppm, a mortality of 10% was observed after 24 h of incubation.

Compound **1** at 1.62 mM caused a 246% promotion of radicle growth in lettuce seedlings (Fig. 1). Above 1.62 mM there was a sharp decrease in relative radicle growth as the sample concentrations increased. By contrast, compound **1** had very little effect on hypocotyl growth. We observed that, seedling weight per disc was negatively correlated to sample concentrations with a minimum of 67% at 4.4 mM. The change in total length, however, depended entirely on radicle and hypocotyl lengths. Thus, this compound seems to force the seeds to grow spindly, rapidly and selectively on the radicle section at low dosage levels. Besides, at the maximum level, the weight of seedlings per disc was relatively low and apparently persisted above 1.62 mM. Using wheat coleoptile, however, Sequiera *et al.* (1968) and Kupchan *et al.* (1969) reported that compound **1** had little effect on growth whereas compound **2** was the most potent plant growth inhibitor. In conclusion therefore, the effects of 8-desacylvernodalol (**1**) on growth and especially the selectivity on radicle growth besides its nontoxic nature against brine shrimp, are interesting phenomena with regard to the development of new plant growth regulators from Kenyan

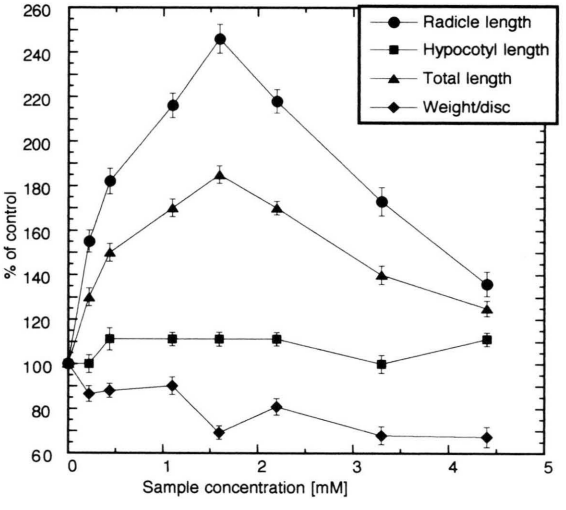


Fig. 1. The effects of various concentrations of 8-desacylvernodalol (**1**) on the growth of lettuce seeds after 72 h of incubation in the dark at 24 °C (control lengths: radicle = 11 mm, hypocotyl = 9 mm, total length = 20 mm).

plants. Thus, elemanolides from *Vernonia* species seem to show viable potential with regard to their utility as possible targets in experimental and practical plant growth regulation without any environmental hazards.

Moreover, the results of this study which revealed the presence of a compound possessing

the elemane skeleton in *Vernonia auriculifera*, are in accordance with its placement in group IIB (Jeffrey, 1988) and in subsection strobocalyx of the subgenus Orbisvestus (Jones, 1981) in the recent definition and classification of this species in the East African *Vernonia* and related species (Bohlmann and Jakupovic, 1990).

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