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Plant Growth Regulators from Kenyan Plant, *Psiadia punctulata*

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Compounds having plant growth regulatory effect were found in Kenyan *Psiadia punctulata*, and two flavones, 5-hydroxy-7, 2', 3', 4', 5'-pentamethoxyflavone (1) and 5, 3'-dihydroxy-7, 2', 4', 5'-tetramethoxyflavone (2) were isolated and identified as the active constituents.

Key words : Kenyan plant, *Psiadia punctulata* (Asteraceae), flavonoids, growth regulator, lettuce seeds

Introduction

Seed germination is one of many aspects of plant growth and development and is dependent on a broad range of factors[1]. During the process of germination, a sequence of events in the seed occurs, some of which involve the endogenous chemical reactions that are necessary for normal growth. Some of these reactions result in the mobilization of food reserves to the embryo, the division of cells in the embryo and the weakening of the endosperm which eventually lead to the protrusion of the radicle through the surrounding layers. Furthermore, the environmental conditions surrounding the seed also determine the mechanisms of the chemical conversions which are responsible for the production of optimum levels of the necessary growth substances. The primary involvement of gibberellic acids (GA) (required in germination process) and abscisic acids (ABA) (during dormancy) in the basic mechanism of seed germination is well documented[2,3,4]. For instance, experiments using barley seeds[5] show that *ent*-kaurene conversions to GA is essential for normal shoot elongation. Studies on lettuce seeds[6,7] also show that cytokinins break seed dormancy in the presence of GA's or when some red light irradiation is provided, and have been found to increase at a later stage in development around the time of radicle protrusion through the seed coat. The role of endogenous ethylene in the breaking of secondary dormancy in lettuce seeds has also been explained[8]. These basic chemical requirements may be interfered with when growth regulating substances are present during germination, leading to such effects as retardation or promotion of such physiological processes as root and stem elongation, seed germination and bud opening.

In our continuing invetigations of Kenyan plants for their biological activities, we recently reported the isolation and assay results of steroidal glucosides (plant growth inhibitors) from *Vernonia hindii* S. Moore[9] in which some of the effects mentioned above were observed. We have carried out similar studies on the Kenyan *Psiadia punctulata* (DC.) Vatke (same as *P. arabica* Jaub. and Sp.) (Asteraceae) from which two flavones (1) and (2) were isolated on the basis of their growth regulatory effects towards lettuce seeds.

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In Kenya, *P. punctulata* is an abundant plant on the edges of disturbed bushland in evergreen woodland and in dry forest areas at 1200-2300 m above sea level. Traditionally, its leaf decoctions were drunk for abdominal pains and was known to cure stomach ulcers[10]. In the Arab peninsula, it is used by the Bedouin in casts for broken bones[11]. Previous research work on this plant[11,12,13] from which 18 flavonoids were isolated, shows the tribe Asteraceae and in particular *P. punctulata* to be rich in methylated flavones and flavonols. However, there are no reports on biological investigations into the compounds of this species of plant. In this communication, we report for the first time biological studies of compounds in the Kenyan *P. punctulata.*

Materials and Methods

General. NMR Spectra were recorded on a Varian VXR 500 NMR (500 and 125 MHz for ^{1}H and ^{13}C respectively), IR on FT-IR 710 (Nicolet), MS on D300 (JEOL) and UV on UV-3000 (Shimadzu) spectrometers. Mps. on Yamato Melting Point Apparatus Model mp-21 and are uncorr. Prep. TLC was on Kieselgel 60 F_{254} (Merck, Art. 5554, 0.2 mm) using benzene-acetone-ethyl acetate (8:2:2) solvent system. Column chromatography was on Silica gel 60 (230-400 mesh) from Nacalai tesque, with solvents as stated below. Reverse phase separation was done on Sep-pak cartridges (C_{18}, Waters) with water and methanol (2:5).

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

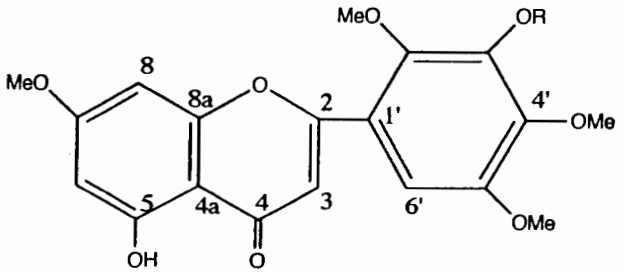
Fresh leaves (10 kg) were collected and extracted with methanol for two weeks, after which the filtrate was evaporated to give a crude concentrated sample. The extract was then partitioned between hexane and water, after which a dried portion (1.4 g) of the hexane soluble fraction was introduced into a column of silica gel, and with hexane-ethyl acetate (3:1) used as eluant, six fractions were collected. Yellow needle crystals which were recrystallized from methanol-acetone were obtained from the fourth fraction to give compound **1**[14]. A combination of the subsequent fractions was further separated through prep. thin layer chromatography using benzene-acetone-ethyl acetate (8:2:2) as eluant and running twice followed by reverse phase separation (methanol-water, 5:2) to afford yellow dendritic crystals which were recrystallized from methanol to give compound **2**[14].

Bioassay. Lettuce seedlings growth bioassay was performed as described by Kato[15], with some modifications. Sample solutions in methanol (1 mg/ml), were applied onto round filter papers (15 mm diameter) suspended in air using presterilised pins, to give various sample quantities (10, 20, 50, 75, 100, 150 and 200 μg/disc). The solvent was allowed to dry in a vacuum pump for 20 min and the filter papers were later placed into 24-well tissue culture plates (Corning). Lettuce seeds (10 seeds/disc) were sown on the filter papers and 150 μl of distilled water was added. The plates were covered and transferred into a larger plastic dish containing moist cotton wool. The covered plastic dish was then incubated at 24°C for 72 hours. After 24 hours of incubation, 150 μl of distilled water was added. Paper discs applied with methanol only and an equal amount of added water served as controls. After 72 hours, the seedlings were blotted on tissue paper and the weight of seedlings/disc was recorded. The lengths of the radicle and the hypocotyl were measured for all the seedlings, after which the mean lengths were calculated and converted into percentages relative to the control.

Results and Discussions

Fresh leaves of *P. punctulata* were extracted over methanol for two weeks and after evapora-

tion of methanol, the concentrate was partitioned between hexane and water. The hexane soluble fraction was found to show growth regulatory activity towards lettuce seedlings by the paper disc bioassay method. A portion of it was dried and successively chromatographed over silica gel column from which two flavones (**1**) and (**2**) were isolated. Compound **1**, $C_{20}H_{20}O_8[M]^+$ at m/z 388, yellow needle crystals from methanol-acetone, mp. 123-125°C, was isolated first. In the IR spectrum, bands for hydroxyls (3600 cm^{-1}) and hydrogen-bonded carbonyl (1665 cm^{-1}) were observed. The UV spectrum disclosed an absorption maximum at 268nm attributable to the benzoyl moiety in A-ring of a flavonoid[16] among other bands. Its ^1H NMR spectrum showed flavone-nucleus protons with two singlets (1H each) at δ 7.02 and δ 6.87 for C-6' and C-3 respectively plus two doublets (1H each) with chemical shifts and coupling constants typical of *meta*-coupled protons at δ6.45 (C-8, $J=2$Hz.) and δ6.38 (C-6, $J=2$Hz). Resonances between δ3.87 and δ3.98 due to five methoxyl groups were clearly observed when ^1H NMR spectrum was determined in $CDCl_3$. The presence of a hydrogen-bonded hydroxyl proton signal at δ12.79 indicated that the hydroxyl group must be at C-5. A signal at δ182.7 assignable to carbonyl carbon was discernible in the ^{13}C NMR spectrum. Compound **1** exhibited IR and NMR (^1H and ^{13}C) data uniquely consistent with those reported[13] for 5-hydroxy-7, 2', 3', 4', 5'-pentamethoxyflavone (**1**). Compound **2**, $C_{19}H_{18}O_8[M]^+$ at m/z 374 was isolated as yellow dendritic crystals, mp. 198-203°C. The mass suggested a substitution pattern for a flavone with two hydroxyl and four methoxyl groups. The IR spectrum showed clearly bands at 3440 cm^{-1} and 1641 cm^{-1} attributable to hydroxyls and hydrogen-bonded carbonyl groups respectively. Its ^1H NMR spectrum clearly established the presence of four methoxyl groups resonating between δ3.87 and δ3.98 in addition to two singlets (1H each) at δ7.01 (C-6') and δ6.88 (C-3), two doublets (1H each) at δ6.29 and δ6.43 for C-6 and C-8 respectively (*meta*-coupled protons $J=2$Hz). One hydrogen-bonded hydroxyl group with resonance at δ12.85 was clearly observed, indicating the presence of a hydroxyl group at C-5. A signal at δ182.6 assignable to carbonyl carbon was observed on ^{13}C NMR spectrum. These data suggested that **2** was 5, 3'-dihydroxy-7, 2', 4', 5'-tetramethoxyflavone which was previously isolated from the same species of plant[13]. These two compounds (**1** and **2**), isolated on the basis of their active principles towards growth of lettuce seeds, were found to have similar effects on the elongation of radicle section but slightly different effects on the hypocotyl section (Fig. 1a and b). In the case of **1** (Fig. 1a), the radicle elongation increased with the amount of sample added up to a maximum of 130% at 75 μg/disc after which it decreased as the sample quantity increased. In the case of compound **2** (Fig. 1b), a similar trend was observed with the radicle's length increasing to a maximum of 140% at 75 μg/disc. However, it was noted that, while the two compounds indicated a small growth change on the hypocotyl section, it was found to be negative with **2** but positive with **1**. The weight of seedlings/disc after 72-hour incubation was found to decrease with the increase of sample quantity in both cases with the strongest effect observed with compound **2** (58% at 200 μg/disc). From the results of this study, it was clearly observed that, while the two compounds indicat-



1. R=Me 2. R=H

1. 5-Hydroxy-7, 2', 3', 4', 5'-pentamethoxyflavone.
2. 5, 3'-Dihydroxy-7, 2', 4', 5'-tetramethoxyflavone.

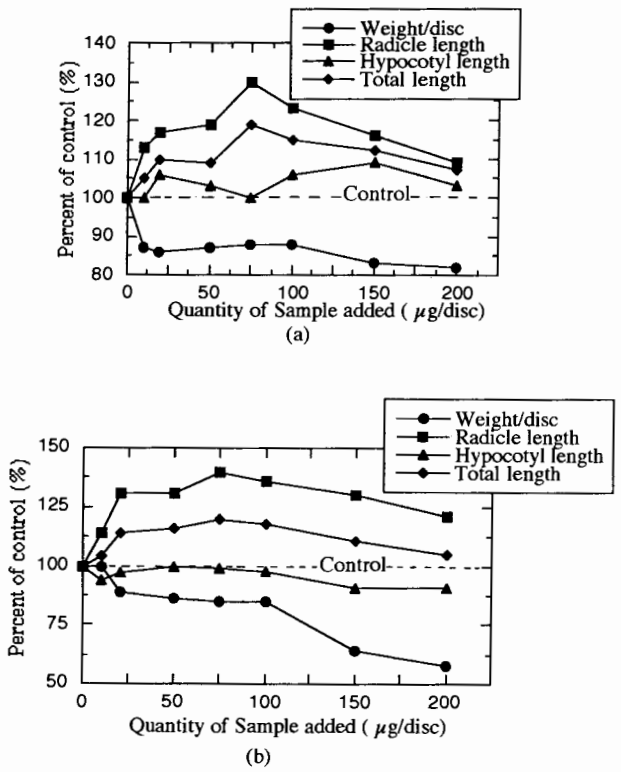


Fig. 1 The effects of various quantities of compound 1 (a) and 2 (b) on the growth of lettuce seeds after 72-hour incubation in the dark at 24°C.

ed growth regulation effects on lettuce seeds, the effects were found to be relatively stronger with compound **2** as compared with those of **1**. In conclusion, although the flavonoids isolated in the present study are known compounds, growth regulatory activities found here and in particular the differences in activities observed with these two structurally related compounds, are interesting phenomena in regards to the development of new plant growth regulators from Kenyan plants. Further investigations are required in this area in order to unravel the hormonal control of the radicle elongation of lettuce seeds in the presence of these two compounds. Such activities are the basis of continuing research in this laboratory.

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14) *Compound* **1**. 5-Hydroxy-7, 2', 3', 4', 5'-pentamethoxyflavone, $C_{20}H_{20}O_8$ (15mg) R_f=0.7, Mp. 123-125°C (Lit. 124-125°C).[13] ; IR υ_{max} (KBr) cm^{-1} : 3600 (OH), 1665 (C=O), 1608 (ring C=C) ; UV λ_{max} (EtOH) nm (ε) : 216 (38500), 268 (26000), 292 (18000), 335 (18000) ; ^{1}H NMR ($CDCl_3$) δ_H : 12.79 (1H, *s*, exchangeable, OH-5), 7.02 (1H, *s*, H-6'), 6.87 (1H, *s*, H-3), 6.45 (1H, *d*, J=2.11Hz, H-8), 6.38 (1H, *d*, J=2.28Hz, H-6) and 5×OMe signals at 3.98, 3.96, 3.92, 3.88 and 3.87 ; ^{13}C NMR ($CDCl_3$) δ_C : 161.6 (C-2), 110.0 (C-3), 182.7 (C-4), 105.6 (C-4a), 162.2 (C-5), 97.9 (C-6), 165.5 (C-7), 92.5 (C-8), 157.8 (C-8), 119.8 (C-1'), 146.1 (C-2'), 147.3 (C-3'), 147.6 (C-4'), 149.5 (C-5'), 106.3 (C-6') and 5×OMe signals at 61.40, 61.32, 61.28, 56.46 and 55.82 ; EI-MS *m/z* (%) : 388 (M^+, 15), 373 (M^+-CH_3, 2.0), 167 (2.6), 149 (3.1), 60 (2.8), 57 (1.0), 45 (2.8), 44 (6.3), 43 (3.3), 41 (1.0), 40 (3.1), 32 (100)

Compound **2**. 5, 3'-Dihydroxy-7, 2', 4', 5'-tetramethoxyflavone, $C_{19}H_{18}O_8$, (6.3mg) R_f=0.34, Mp. 198-203°C (Lit. 192-196°C).[13] ; IR υ_{max} (KBr) cm^{-1} : 3440 (OH), 3020 (ring C-H), 1641 (C=O), 1603 (ring C=C) ; ^{1}H NMR ($CDCl_3$) δ_H : 12.85 (1H, *s*, exchangeable, OH-5), 7.01 (1H, *s*, H-6'), 6.88 (1H, *s*, H-3), 6.43 (1H, *d*, J=2.07Hz, H-8), 6.29 (1H, *d*, J=2.07Hz, H-6), and 4×OMe signals at 3.98, 3.96, 3.91, 3.87 ; ^{13}C NMR ($CDCl_3$) δ_C : 161.7 (C-2),110.0 (C-3), 182.7 (C-4), 106.2 (C-4a), 162.0 (C-5), 99.3 (C-6), 162.5 (C-7), 94.0 (C-8), 158.0 (C-8a), 119.8 (C-1'), 141.2 (C-2'), 147.8 (C-3'), 139.8 (C-4'), 149.6 (C-5'), 102.0 (C-6') and 4×OMe signals at 61.38, 61.32, 59.20, 56.41 ; EI-MS *m/z* (%) : 374 (M^+, 2.5), 359 (M^+-CH_3 1.0), 153 (1.3), 71 (1.1), 57 (2.1), 55 (1.3), 44 (2.4), 43 (1.8), 41 (1.2), 40 (2.1), 32 (100).

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ケニヤ産植物，*Psiadia punctulata* に含まれる植物生長調節物質

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植物の生長を制御する物質を広く天然に求めることは，より安全で有効な農薬及びその関連物質を開発するための重要な課題である．本研究では，レタス幼苗の生長に対する調節物質の探索をケニヤ産植物 *Psiadia punctulata* について行った．その結果，同植物のメタノール抽出物より，5-hydroxy-7, 2', 3', 4', 5'-pentamethoxyflavone 及び 5, 3'-dihydroxy-7, 2', 4', 5'-tetramethoxyflavone を調節物質として単離した．前者は，根や胚軸の長さを130%まで増加させたが，全体の重量はほぼ80%にまで減少させた．一方，後者も同様の活性パターンを示し，根を140%伸長させ植物体重量を58%まで減少させるという興味ある現象が観察された．