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SHORT COMMUNICATION

A FLAVONE FEEDING STIMULANT IN ALLIGATORWEED*

ALFRED G ZIELSKE, JOHN N. SIMONS and R M SILVERSTEIN†

Stanford Research Institute, Menlo Park, California 94025, U S A.

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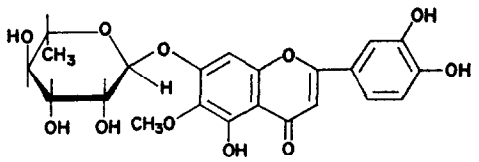
Abstract—A compound partially responsible for the feeding of *Agasicles* beetles on alligatorweed has been isolated and identified as 7-α-L-rhamnosyl-6-methoxyluteolin (I)

INTRODUCTION

A CONSIDERABLE body of evidence associates the feeding preference of phytophagous insects with the presence of specific chemicals in host plants.[1,2] This study reports on the mechanism that accounts for the feeding of the chrysomelid beetle, *Agasicles* sp nov , on alligatorweed (*Alternanthera phylloxeroides*, Amaranthaceae) We isolated and identified 7-α-L-rhamnosyl-6-methoxyluteolin (I) as one of the compounds responsible.



(I)

RESULTS AND DISCUSSION

The isolation steps were monitored by a bioassay procedure designed to detect the presence of chemicals responsible for the feeding of *Agasicles* on alligatorweed. The plant extracts were dissolved in distilled water and the resulting solutions applied to leaves (spinach) not normally fed upon by *Agasicles* Controls consisted of spinach leaves treated with distilled water. The treated spinach leaves were then exposed to *Agasicles* beetles overnight and the leaves were examined for feeding damage. The presence of feeding damage was positive evidence of the presence of a feeding stimulant in the particular extract applied to the leaves

Alligatorweed was initially extracted with distilled water followed by freeze-drying of the aqueous extract to give a powdery, green solid. The green solid was extracted with tetrahydrofuran (THF) and then extracted with methanol The methanol extract contained all the feeding activity The green solid remaining after evaporation of methanol was dissolved in water. This aqueous layer was extracted with ether, leaving the feeding activity remaining in the yellow aqueous layer. Purification via lead acetate treatment gave a yellow solid containing all the feeding stimulant activity Further purification was achieved by column chromatography on polyamide and on cellulose.

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† Present address State University College of Forestry at Syracuse University, Syracuse, New York 13210, U S A

[1] S D BECK, *Ann Rev Entomol* **10**, 207 (1965)

[2] M JACOBSON, *Ann Rev Entomol* **11**, 403 (1966)

The UV spectra and colour reactions of the pure material showed it to be a flavonoid glycoside [3–5] Furthermore, spectral analysis indicated it to be a flavone, and not a flavonol, and to have free hydroxyls at the 5-, 3′- and 4′-positions [5–9]

The glycoside was hydrolyzed with 2 N HCl and gave rhamnose as the only sugar The link between the sugar and the aglycon is believed to be α, and the sugar L-rhamnose This is supported by the fact that L-rhamnose is found in nature, and that all naturally occurring L-rhamnosides have the α configuration [10,11] The sugar usually assumes the L-pyranoid form in L-rhamnose derivatives in plants [11] As further support, the glycoside had a negative rotation $[\alpha]_D^{20} - 67°$ ($c = 0{\cdot}10$, H_2O) Quercitrin, a known α-L-rhamnoside of quercetin, gave $[\alpha]_D^{23} -156°$ ($c = 0\,050$, H_2O) in our laboratory The literature[12] also reports a negative rotation for quercitrin Additional support comes from the fact that methyl α-L-rhamnoside has $[\alpha]_D - 62{\cdot}5°$ in H_2O and methyl β-L-rhamnoside has $[\alpha]_D + 95{\cdot}4°$ in H_2O [13] Also, the ORD curve for quercitrin had a negative Cotton effect at 350 nm and a positive one at 250 nm Our glycoside also had a negative Cotton effect at 350 nm and a positive one at 270 nm, which indicates a similar configuration of sugars

The sugar is believed to be attached at the 7-position, since this is the most common point of attachment of sugars in flavones [14] Also, the UV spectrum of the glycoside showed the presence of hydroxyl groups at 3′, 4′, and 5, and a shift of the 270-nm band of the aglycon upon addition of sodium acetate indicated a hydroxyl at position 7 [15] The NMR spectrum of the glycoside and the aglycon, when compared with that of authentic luteolin, shows the presence of the 2′, 5′, 6′, 3, and 8 protons The proton at position 6 is missing If the sugar were at 6, hydrolysis would give a 5,6,7-trihydroxy aglycon, but the aglycon has a different spectrum from 6-hydroxyluteolin [16]

The NMR spectra of the glycoside and the aglycon showed a 3-proton singlet at 6·15 τ, indicating a methoxy attached to an aromatic ring [17] Since the proton at position 6 is missing and all other positions are accounted for, the methoxyl group must be attached at the 6 position This is confirmed by the fact that the UV of the aglycon is identical to that reported for 6-methoxyluteolin [18]

The aromatic portion of the NMR spectrum of the aglycon was not as clear as one would like. Therefore, sodium hydride in DMF with methyl iodide was used to completely methy-

[3] L JURD, in *The Chemistry of Flavonoid Compounds* (edited by T A GEISMAN), p 108, Macmillan, New York (1962)
[4] M K SEIKEL, *ibid* p 51
[5] M K SEIKEL, *ibid* pp 36, 37, 42
[6] J B HARBORNE, *Comparative Biochemistry of the Flavonoids*, p 49 Academic Press, New York (1967)
[7] L JURD, in *The Chemistry of Flavonoid Compounds* (edited by T A GEISSMAN), p 126, Macmillan, New York (1962)
[8] L JURD, *ibid* p 124
[9] L JURD, *ibid* pp 127, 119
[10] L J HAYNES, *Advan Carbohydrate Chem* **20,** 359 (1965)
[11] J B PRIDHAM, *Advan Carbohydrate Chem* **20,** 385 (1965)
[12] S HATTORI, in *The Chemistry of Flavonoid Compounds* (edited by T A GEISSMAN), p 337, Macmillan, New York (1962)
[13] R M HOROWITZ and B GENTILI, *Tetrahedron* **19,** 778 (1963)
[14] J B HARBONE, *Comparative Biochemistry of the Flavonoids*, p 46, Academic Press, New York (1967)
[15] L JURD, in *The Chemistry of Flavonoid Compounds* (edited by T A GEISSMAN), p 122, Macmillan, New York (1962)
[16] J B HARBORNE, *Comparative Biochemistry of the Flavonoids*, pp 40, 41, Academic Press, New York (1967)
[17] R M SILVERSTEIN and G C BASSLER, *Spectrometric Identification of Organic Compounds*, p 137, Wiley, New York (1967)
[18] C H BRIESKORN and H MICHEL, *Tetrahedron Letters* 3447 (1968)

late[19] the aglycon The permethylated aglycon was purified by preparative TLC and its NMR spectrum showed the presence of five methoxyl groups. The permethylated aglycon showed a much cleaner aromatic region in the NMR spectrum in comparison with the aglycon itself. In particular, comparison of the NMR spectra of the permethylated aglycone and permethylated luteolin clearly showed the absence of the proton at position 6 in the former compound.

Thus, the structure of the feeding stimulant is 7-α-L-rhamnosyl-6-methoxyluteolin (I).

EXPERIMENTAL

Optical rotations were made with a Perkin–Elmer 141 Polarimeter Paper chromatography was done on Whatman No 1 paper and TLC was done on silica gel G-coated plates

Extraction of alligatorweed Whole alligatorweed (50 lb) was harvested from the Pasadena, California area, extracted with H_2O, quickly frozen, and then freeze-dried to give a powdery, green solid (303 g). This solid was stirred with THF (7 pt) and the THF insolubles were dried overnight in a vacuum oven The olive-green THF-insolubles were extracted with MeOH (3 l) The MeOH solution was evaporated to a green solid (75 g), which was active in promoting the feeding of *Agasicles* on spinach leaves

Lead precipitation of methanol solubles The green solid (75 g) obtained by evaporation of MeOH was dissolved in H_2O (2 l) and extracted with ether The ether layer was dark green Lead acetate (1 50 g) in H_2O (300 ml) was added to the yellow aqueous solution to give an immediate yellow precipitate The precipitate was collected by centrifugation, washed with distilled H_2O, suspended in distilled H_2O, and treated with H_2S (g) The PbS was separated by centrifugation The yellow solution was freeze-dried to give a bright yellow solid (4 3 g), λ_{max} 212 5, 272 5, 349 nm, dark green $FeCl_3$ color Color reactions of the compound on filter paper are visible, light yellow, UV, brown, visible or UV/NH_3, yellow-green The yellow solid possessed feeding activity for *Agasicles* beetles on spinach

Polyamide column chromatography A column of polyamide powder (Brinkman CC-6 60 g, 3 × 36 cm) was prepared in distilled H_2O A sample (1 5 g) of regenerated lead-precipitated alligatorweed extract was dissolved in H_2O (30 ml) and applied to the column The column was first eluted with distilled H_2O (3 equal fractions, 200 ml each) followed by elution with H_2O containing increasing amounts of MeOH The three fractions (7–9) eluted with 50% aq MeOH contained active feeding stimulant material for *Agasicles* beetles The remaining fractions were inactive

Examination of fraction 7 The yellow–orange solid (212 mg) obtained from the polyamide column fraction 7 was dissolved in distilled H_2O (3 ml) and placed on a column of cellulose powder (Brinkman MN 100, 23 g, 1 7 × 47 cm) The column was eluted with H_2O, two 100-ml fractions and fourteen 5-ml fractions were collected The column was then eluted with H_2O containing increasing amounts of methanol and finally eluted with abs MeOH Examination of the fractions on cellulose TLC developed with H_2O showed that fractions 5–11 and 12–17 could be combined Only combined fractions 5–11 and 12–17 were active when bioassayed with *Agasicles* beetles Fractions 5–11 contained 112 mg of material

Examination of fractions 5–11 Evaporation of water gave a yellow–orange solid λ_{max}^{EtOH} 215, 273 5, 351 5 nm, λ_{max}^{NaOEt} 418 nm, $\lambda_{max}^{AlCl_3}$ 407 nm, $\lambda_{max}^{H_3BO_3}$ 388 nm NMR (DMSO) 2 35–2 72 τ (2 H, 2′-, 6′-proton), 2 90–3 20 τ (2 H, 5′- 8-protons), 3 30–3 50 τ (1 H, 3-proton), 6 15 τ (3 H, aromatic-OCH_3) $[\alpha]_D^{20}$ −67° (c = 0 10, H_2O) ORD (EtOH) 350 nm (negative Cotton effect), 270 nm (positive Cotton effect)

Hydrolysis of fractions 5–11 Combined fractions 5–11 (39 mg) were dissolved in 2 N HCl (5 ml) to give a clear yellow solution The solution, after heating at 100° for 50 min, contained a green solid in suspension The slurry was cooled to room temp and filtered, the solid was washed with H_2O The filtrate was extracted with EtOAc, and this extract was combined with MeOH solution of the green solid that had been collected on the filter pad

The aqueous layer was neutralized with Amberlite IR-45 resin and examined, along with authentic sugars, by paper chromatography in *n*-BuOH–EtOH–H_2O (40 11 19) The paper was sprayed with 3% *p*-anisidine HCl in *n*-BuOH and heated at 100°[20] The aqueous layer gave one spot identical with rhamnose Examination of the aqueous layer on Kieselguhr TLC plates[21] also showed the presence of rhamnose

Evaporation of the MeOH–EtOAc layer gave a dark green solid (23 7 mg) This solid was extracted with hot EtOAc, leaving a black residue (4 5 mg) Evaporation of the EtOAc gave a yellow solid (16 5 mg), λ_{max}^{EtOH} 213 5, 274, 351 5 nm λ_{max}^{NaOEt} 420 nm, $\lambda_{max}^{AlCl_3}$ 400 nm, $\lambda_{max}^{H_3BO_3}$ 381 nm NMR (DMSO) 2 35–2 65 τ (2 H, 2′, 6′-protons), 3 00–3 20 τ (2 H, 5′, 8-protons), 3 30–3 40 τ (1 H, 3-proton), 6 15 τ (3 H, aromatic-OCH_3)

[19] D W THOMAS, *FEBS Letters* **5**, 53 (1969)
[20] L HOUGH, J K N JONES and W H WADMAN, *J Chem Soc* 1702 (1950)
[21] K RANDERATH, *Thin-layer Chromatography*, p 236, Academic Press, New York (1966)

Methylation of hydrolysis product The aglycone (12 mg) was dissolved in DMF (3 ml) to give an orange solution A NaH suspension (44 mg, 56%) was washed with pentane and dried under N_2 The DMF solution was added to the NaH and the resulting red solution was stirred for a few min MeI (~1 ml) was added, the color became light orange, and the suspension was stirred for 3 hr The suspension was poured into H_2O, extracted with $CHCl_3$, the $CHCl_3$ layer was washed with H_2O, and dried Evaporation of $CHCl_3$ gave an orange solid (12 8 mg) which gave a negative $FeCl_3$ test Analysis on silica gel G TLC developed with acetone showed one main spot at R_f 0 71 (UV, blue), a faint spot at R_f 0 50, plus some material at the origin The crude product was purified by preparative silica gel TLC developed with acetone to give a light orange solid (8 9 mg) showing one spot at R_f 0 71 on silica gel G developed with acetone λ_{max}^{EtOH} 215, 243^{sh}, 265^{sh}, 334 5 nm There was no change in the UV spectrum when NaOEt or $AlCl_3$ was added NMR(DMSO) 2 20–2 50 τ (2 H, 2′, 6′-protons), 2 78–2 98 τ (2 H, 5′, 8-protons), 3 10–3 25 τ (1 H, 3-proton), 6 10–6 20 τ (15 H, aromatic OCH_3)

Luteolin An authentic sample of luteolin was obtained from Dr R M Horowitz, Fruit and Vegetable Laboratory, Pasadena, California NMR (DMSO) (for comparison) 2 48–2 68 τ (2 H, 2′-, 6′-proton), 3 01–3 18 τ (1 H, 5′-proton), 3 37 τ singlet (1 H, 3-proton), 3 57 τ, doublet, (1 H, J = 2 Hz, 8-proton), 3 82 τ, doublet, (1 H, J = 2 Hz, 6-proton)

Methylation of luteolin Luteolin (5 mg) was methylated by the same procedure as described above The product gave one main spot at R_f 0 50 (UV, blue), a faint spot at R_f 0 74, and a spot at the origin The crude product was purified by preparative TLC on silica gel developed with acetone Silica gel TLC developed with acetone showed one light orange spot (R_f 0 50, UV, blue) The solid product gave a negative $FeCl_3$ test λ_{max}^{EtOH} 213, 242 5, 265, 332 5 nm No change in spectrum upon addition of NaOEt or $AlCl_3$ The UV spectrum agrees with that given for tetra-*O*-methylluteolin [20] NMR (DMSO) 2 30–2 62 τ (2 H, 2′-, 6′-protons) 2 82–2 96 τ (1 H, 5′-proton), 3 16 τ doublet (1 H, J = 2 Hz 8-proton), 3 30 τ singlet (1 H, 3-proton), 3 52 τ doublet (1 H, J = 2 Hz 6-proton), 6 02–6 22 τ (12 H, aromatic-OCH_3)

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[22] L JURD, in *The Chemistry of Flavonoid Compounds* (edited by T A GEISSMAN), p 111, Macmillan, New York (1962)

Key Word Index—Aternanthera phylloxeroides, Amaranthaceae, alligatorweed, *b*-methoxyluteolin glycoside, flavone, feeding stimulant