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IRIDOIDS OF *GARRYA ELLIPTICA* AS PLANT GROWTH INHIBITORS*

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Key Word Index—*Garrya elliptica*; Garryaceae; iridoids; aglucones; genipin; geniposide; geniposidic acid; plant growth inhibitors.

Abstract—Extracts from catkins of *Garrya elliptica* inhibit the growth of wheat embryos. The components responsible for this activity have been identified as the iridoids geniposide and geniposidic acid together with their aglucones.

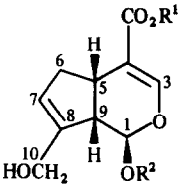
INTRODUCTION

Partially purified extracts from immature catkins of *Garrya elliptica* Dougl. ex Lindl. had been shown by one of us (J.M.S.) to inhibit the growth of pea stem segments and wheat embryos, and they produced an unusual blue colour in the assay material. As no abscisic acid was detectable in the extracts by analytical TLC we attempted to identify the compounds responsible for the inhibition of the expansion growth of plant cells by further purification, monitoring the fractions obtained by bioassay.

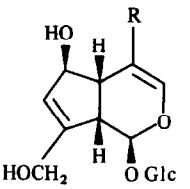
RESULTS

Catkins of *G. elliptica* (staminate form) were extracted with methanol and the extract was partitioned into hexane-, ether- and water-soluble fractions. Each was assayed for inhibitory activity, which proved to be confined chiefly to the ether and aqueous fractions. In both cases inhibition of growth was accompanied by blue–grey pigmentation of the wheat embryos. Chromatography of the aqueous fraction gave two active components identified as the known iridoids geniposide (**1**) [1, 2] and geniposidic acid (**2**) [3, 4] (0.3 and 0.4% fr. wt respectively).

Isolation of the active constituents from the ether fraction was hampered by the limited proportions in which they were present together with their instability towards acid and to weakly basic adsorbents. However the fraction was satisfactorily chromatographed on silica impregnated with ammonium sulphate and this occurred without significant loss of overall activity. The major component (representing 0.03% fr. wt) proved to be **4**, the aglucone of geniposidic acid. Compound **4** has also been obtained by enzymic hydrolysis of geniposidic acid [5] although only a partial ^{1}H NMR spectrum was provided as evidence for its structure. It has also been suggested that **4** is a possible contributor to the blue colour of cell-free preparations from aged leaves of *Genipa americana* [3]. A second, minor component was also obtained and is tentatively identified as the known compound genipin (**3**) [1, 6, 7] by TLC comparison with an authentic sample.



	R^1	R^2
1	Me	Glc
2	H	Glc
3	Me	H
4	H	H



	R
5	H
6	CO_2H

However insufficient amounts of material precluded satisfactory spectroscopic analysis.

Since the two aglucones were obtained in proportions at least an order of magnitude lower than those for the corresponding glucosides the possibility cannot be excluded that the former are artefacts derived by hydrolysis during work-up. However, the possibility must also be considered that the glucosides represent detoxification products and/or transport forms of the iridoids. Partially purified fractions of geniposidic acid (**2**) also contained a contaminant spectroscopically discernible as a doublet of doublets resonating at δ 6.30. This could correspond to H-3 of aucubin (**5**), a constituent reported earlier from leaves of *G. elliptica* [8]. However insufficient was found in the catkins for purification. Scandoside (**6**), another reported constituent [9], was not detected. Compounds **1**–**4** have not been isolated from *G. elliptica* previously and no previous chemical investigation appears to have been concerned exclusively with the catkins of this species.

The inhibitory effects of the pure compounds on the growth of wheat coleoptiles is shown in Fig. 1. Relatively high concentrations are needed for inhibition, and the glucosides are less active than the aglucones in the time period used for the bioassay. However, calculation of the amount of the aglucone of geniposidic acid present in an aliquot of the ether extract, and a comparison of the effects of a concentration range of the extract on wheat embryos with Fig. 1 suggested that the iridoids could account for most, if not all, of the inhibitory activity originally observed. Similarly, the glucosides can account

*Partly presented as a poster at the 13th International Botanical Congress held in Sydney, Australia in August 1981.

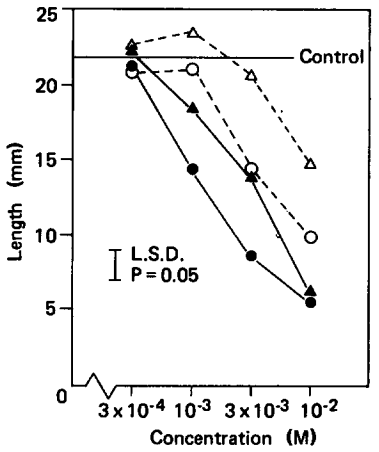


Fig. 1. Effects of iridoids on the elongation of coleoptiles of wheat embryos. ○, geniposidic acid; ●, geniposidic acid aglucone; △, geniposide; ▲, genipin. The inhibited plant material was a deeply coloured blue or blue–grey when measured.

for the activity of the aqueous fraction. Plant growth inhibitory activity is known for the more complex iridoid glucoside, plumieride [10] and its aglucone [11] but the authors attributed it to the presence of an α,β-unsaturated lactone grouping. Such a grouping is absent in the present series of iridoids, so their activity suggests that some contribution is made by the iridoid structure itself. The iridoid structure is also responsible for the blue pigment observed in the assay material as well as in extracts from *G. elliptica* and *Genipa americana* [3, 6].

EXPERIMENTAL

Mps (Kofler hot-stage apparatus): corr.; ^{1}H NMR: 100 MHz, TMS as external reference for solns in D_2O and as internal reference for other solvents; Prep. TLC: glass plates (0.1 cm thickness) coated with the specified adsorbent.

Assay. Wheat embryos were more convenient than pea stem segments and the method of Milborrow [12] was followed. Aliquots from the fractions were placed in Petri dishes (5 cm), the solvent evaporated, a filter paper and H_2O (1 ml) added to each dish, and 12–16 embryos placed on each paper. Appropriate controls were included and, after 2 days' growth in the dark at 25°, the coleoptiles were measured to the nearest 0.5 mm. Results were subjected to analysis of variance and the least significant difference between means calculated at the 5% level.

Isolation of iridoids. Catkins of *G. elliptica* were collected from the Melbourne area in December–January, frozen in polythene bags and stored at −60° to −80°. Storage under these conditions had no observable chemical or biological effect. A voucher specimen is held in the Herbarium, Botany School, University of Melbourne.

Catkins (50 g fr. wt) were macerated with MeOH (200 ml) and filtered; this process was repeated (× 4) with fresh solvent. The combined filtrate was evaporated under vacuum to 90 ml and was then extracted successively with hexane (4 × 200 ml) and Et_2O (5 × 200 ml). Assay of the hexane fraction showed no inhibitory activity and it was discarded.

Evaporation of the Et_2O fraction under vacuum gave a solid residue (0.16 g). This was subjected to prep. TLC on silica gel HF_{254} containing $(NH_4)_2SO_4$ (2%) with CH_2Cl_2–MeOH (6:1) as eluant. Two bands, R_f 0.45 and 0.65, showed inhibitory activity. Each was separately extracted from the silica with MeOH. The extracts, which at this stage also contained $(NH_4)_2SO_4$, were each further chromatographed on a column of Sephadex LH-20 (19 × 2 cm) in MeOH. The band of lower R_f gave geniposidic acid aglucone (**4**, 15 mg), identical with authentic material obtained by hydrolysis of geniposidic acid (see below). The second band yielded only trace amounts of material whose behaviour on TLC was found to be identical with that of genipin and was thus tentatively assigned structure **3**.

The residual aq. phase, usually blue-coloured, was evaporated under vacuum. The residue (2.8 g) was re-dissolved in 6 ml MeOH–H_2O (5:1) and slurried with silica (BDH Column Grade, 20 g). The dried slurry was added to a column containing more silica (20 g) and eluted with Me_2CO (500 ml). Evaporation of the eluate under vacuum gave a pale yellow, oily residue (1.8 g). This was subjected to prep. TLC on silica gel GF_{254} ($CHCl_3$–MeOH, 3:1). Two bands, R_f 0.4 and 0.8, showed inhibitory activity.

The band of R_f 0.8 (153 mg) gave geniposide (**1**). IR ν_{max}^{KBr} cm^{-1}: 3400, 1700, 1640 cm^{-1}; ^{1}H NMR (CD_3OD): δ 2.08 (*m*, H-6α), 2.70 (2H, *m*, H-6β, H-9), 3.20 (*m*, H-5), 3.71 (3H, *s*, CO_2Me), 4.27 (2H, *br s*, H-10), 4.71 (*d*, *J* = 7 Hz, H-1), 5.16 (*d*, *J* = 7 Hz, H-1'), 5.81 (*br s*, H-7), 7.51 (*s*, H-3), indistinguishable from that of an authentic specimen [Jensen, S. R., personal communication], lit. [1, 3]. ^{13}C NMR (D_2O) in agreement with lit. [13]. Treatment with Ac_2O gave geniposide pentaacetate, needles from EtOH, mp 136–138° (lit. [1] 137–138°). IR ν_{max}^{KBr} cm^{-1}: 1748, 1709, 1638; ^{1}H NMR ($CDCl_3$): δ 1.99, 2.01, 2.03, 2.07, 2.09 (16H, 5*s*, 5 × Ac overlapping H-6α), 2.85 (2H, *m*, H-6β, H-9), 3.17 (*br t*, *J* = 8 Hz, H-5), 3.72 (3H, *s*, CO_2Me), 4.20 (2H, *m*, H-6'), 4.69 (2H, *s*, H-10), 4.82–5.18 (6H, *m*, H-1, H-1'–5'), 5.83 (*br s*, H-7), 7.41 (*s*, H-3).

The band of R_f 0.4 (216 mg) gave geniposidic acid (**2**). IR ν_{max}^{KBr} cm^{-1}: 3350, 1680, 1637, 1535; ^{1}H NMR (D_2O): δ 2.20 (*m*, H-6α), 2.82 (2H, *m*, H-6β, H-9), 3.23 (*m*, H-5), 4.26 (2H, *br s*, H-10), 5.30 (*d*, *J* = 7 Hz, H-1'), 5.84 (*br s*, H-7), 7.56 (*s*, H-3) *cf.* lit. [3, 4]. The resonance corresponding to H-3 was pH-dependent, undergoing shielding to δ 7.11 in basified D_2O.

Hydrolysis of iridoid glucosides. A mixture of glucosides **1** and **2** was obtained as in the foregoing section by elution of the crude, H_2O-soluble fraction through a column of silica with Me_2CO. It was dissolved in H_2O (5 ml) and treated with almond β-glucosidase (90 mg). After 9 hr at 23° the mixture was extracted with EtOAc (5 × 10 ml). The combined extract after evaporation gave an oily residue (72 mg) purified by flash chromatography on a column of silica with $CHCl_3$–MeOH (8:1) as eluant, collecting fractions of 5 ml.

Fractions 3 and 4 gave genipin (**3**) (10.5 mg), needles from CH_2Cl_2–petrol, mp 121° (lit. [7] 120–121°), $[\alpha]_D$ (MeOH) + 123° (lit. [6] +135°). UV λ_{max}^{EtOH} nm (log ε): 240 (3.96); ^{1}H NMR ($CDCl_3$): δ 2.06 (*m*, H-6α), 2.54 (*br t*, *J* = 8 Hz, H-9), 2.84 (*m*, H-6β), 3.19 (*br t*, *J* = 8 Hz, H-5), 3.74 (3H, *s*, CO_2Me), 4.33 (2H, *s*, H-10), 4.82 (*d*, *J* = 8 Hz, H-1), 5.22 (*br s*, OH), 5.89 (*br s*, H-7), 7.53 (*s*, H-3); EIMS (probe) 70 eV: identical with lit. [14] with additional peaks *m/z* (rel. int.): 227 $[M+H]^+$ (10), 209 $[M+H-H_2O]^+$ (24).

Fractions 6–14 gave geniposidic acid aglucone (**4**, 27.5 mg) as a colourless oil. (Found: $[M]^+$ 212.0686. $C_{10}H_{12}O_5$ requires $[M]^+$ 212.0685.) UV λ_{max}^{EtOH} nm (log ε): 236 (3.85); IR ν_{max}^{KBr} cm^{-1}: 3430, 1687, 1625; ^{1}H NMR (CD_3OD): δ 2.01 (*m*, H-6α), 2.48 (*br t*, *J* = 8 Hz, H-9), 2.86 (*m*, H-6β), 3.10 (*br t*, *J* = 8 Hz, H-5), 4.25 (2H, *br s*, H-10), 4.78 (*d*, *J* = 8 Hz, H-1), 5.83 (*br s*, H-7), 7.52 (*s*, H-3).

Hydrolysis of the individual glucosides **1** and **2** on a small scale gave the respective aglucones **3** and **4** by analytical TLC.

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