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GENIPOSIDIC ACID, AN IRIDOID GLUCOSIDE FROM GENIPA AMERICANA

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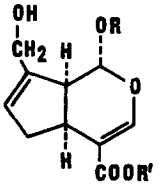
Genipin (1) is a highly oxygenated cyclopentanoid monoterpene possessing a hemiacetal hydroxyl at its C-1 carbon[1]. Both its 1-β-D-glucoside, geniposide[2](2) and its 1-β-D-gentiobioside[3], (3), occur in the higher plant Gardenia jasminoides (Rubiaceae). Genipin (1) is one of the few



(1) R = H, R' = CH_3
(2) R = β-D-glucose, R' = CH_3
(3) R = β-D-gentiobiose, R' = CH_3
(4) R = β-D-glucose, R' = H

stable aglucones of this type and its presence in fruit of Genipa americana (Rubiaceae) led us to question whether it is derived from geniposide (2) or from non-glucosidic precursors (reminiscent of the nepetalactones[4] and related insect monoterpenes[5]). In an in vivo tracer experiment mevalonate-2-^{14}C was administered to Genipa cuttings and labeled genipin (1) isolated and recrystallized to constant specific activity (21, 24, 28 dpm/μmole, genipin in mother liquors from last recrystallization, 24 dpm/μmole; 0.012% total incorporation). A parallel experiment with methionine-methyl-^{14}C also afforded labeled genipin (27, 28, 28 dpm/μmole; 0.01% total incorporation). As a prelude to further tracer studies we set out to examine Genipa americana for other iridoid constituents.

A methanol extract of Genipa americana leaves was subjected to anion exchange chromatography on Dowex 1-formate form[6]. Upon elution with 0.1 N formic acid a major component (4) was obtained having characteristic iridoid glucoside spectral properties, uv: λ_{max} 235 nm (log ε 3.87) hypsochromic shift in base, nmr, δ 7.62 (d, C-3-H, $J_{3,5}$ = 0.5 Hz), 4.34 (m C-10-H) and 3.5 (dd, CH_2 of glucose). Treatment of this acid with diazomethane afforded a methyl ester possessing spectral and physical properties of geniposide[2](2), m.p. 165°, $[\alpha]_D$ +9°, nmr δ 7.60 (d, C-3-H, $J_{3,5}$ = 0.5 Hz), 5.8 (m, C-7-H), 5.3 (d, C-1-H, $J_{1,9}$ = 7 Hz), 4.34 (m C-10-H), 3.88 (s OCH_3), 3.5 (dd, CH_2 of glucose). The methyl ester pentaacetate[2] m.p. 135° likewise exhibited consistent spectral properties, uv λ_{max} 235 nm (log ε 4.0); nmr δ 7.47 (d, $J_{3,5}$ = 1 Hz, C-3-H), 5.90 (m, C-7-H), 4.75 (m C-10-H), 4.2 (dd, CH_2OAc of glucose), 3.75 (s OCH_3), 1.9 ⟶ 2.10 (5 CH_3CO).

Upon subjecting the methyl ester pentaacetate to acid hydrolysis glucose was detected by a specific glucose oxidase assay[7]. β-Glucosidase treatment of geniposide (2) afforded an aglucone which exhibited the characteristic blue color of genipin (1) in the presence of protein[1]. Identity with (1) was established by spectral, chromatographic as well as physical means Mixture melting points with authentic genipin (1), m.p. 118-120° $[\alpha]_D$ +136°, isolated from Genipa americana showed no depression.

The pentaacetate methyl ester was hydrogenated in the presence of PtO_2 giving 7-deoxyloganin tetraacetate with identical properties, including superimposable infrared and nmr spectra, to samples of 7-deoxyloganin tetraacetate obtained by similar treatment of asperuloside[8] and gardenoside[2]. These data establish the structure and configuration of geniposidic acid as (4).

Geniposidic acid (4) and S-adenosyl-L-methionine-methyl-^{14}C were incubated with cell-free extracts from young Genipa americana leaves as previously described[9]. By addition of carrier geniposide (2) and purification of its pentaacetate by chromatography and recrystallization (spec. act. 84, 70, 84 dpm/μmole), 0.1% methylation was observed. Cell-free preparations from older Genipa americana leaves exhibited β-glucosidase activity during incubation with geniposidic acid (4) and S-adenosyl-L-methionine-methyl-^{14}C. This was evidenced by gradual appearance of the characteristic blue color resulting upon interaction of genipin (1) or the aglu-

cone of geniposidic acid (4) with protein. If loganic acid[9] was utilized as substrate with the in vitro system from older leaves the mixture remained colorless.

These results suggest genipin (1) may be readily formed from geniposidic acid (4) by methylation and deglucosylation and supports Inouye's[10] postulation of the intermediacy of geniposidic acid (4) in the biosynthesis of iridoid glucosides possessing a hydroxylated C-10 group.

Acknowledgements

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