

A reexamination of the secondary metabolites of *Dendrosenecio Kilimanjari* subsp. *cottonii*

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Dedicated to Professor Ted Sorensen on the occasion of his 75th birthday

Abstract

An examination of the secondary metabolites of *D. kilimanjari* subsp. *cottonii* resulted in the isolation of 6-hydroxytremetone, some alkyl and ω -phenylalkylresorcinols, and α -cadinol; cinchonidine, previously reported to be a constituent, was not detected.

Keywords: *Dendrosenecio kilimanjari cottonii*, secondary metabolites, 6-hydroxytremetone, resorcinols, cinchonidine

Introduction

In 1987, while descending Mt. Kilimanjaro, in the company of Profs. T. S. Sorensen and A. Rauk, one of the authors (MB) collected foliage of a tree senecio, then identified as *Senecio cottonii* Hutch. & G.Taylor, but later reclassified as *Dendrosenecio kilimanjari* (Mildbr.) E.B.Knox subsp. *cottonii* (Hutch. & G.Taylor) E.B.Knox. This material was air-dried at the University of Nairobi, and subsequently analysed for alkaloids. The result, described in doctoral thesis,¹ apparently revealed it to contain more than 1% by dry weight of cinchonidine. This was a very surprising finding because this particular alkaloid is normally tightly confined to plants of the Rubiaceae genera *Cinchona* and *Remijia*² where it is accompanied by related bases. However, there are reports^{3,4} of the presence of cinchonidine, together with several congeners, in very small amount (0.002-0.017 %) in leaves of the European olive, *Olea europea* L., and probably also in *Ligustrum vulgare* L. another member of the Oleaceae⁴. The claim⁵ that *Cinchona* alkaloids are present in *Strychnos pseudoquina* St.Hil. (Loganiaceae) has been discredited⁶.

Four alternatives were offered¹ for the discovery of cinchonidine in the plant collected on Mt. Kilimanjaro: (1) the plant had been misidentified as a *Dendrosenecio* and was actually a *Cinchona*; (2) the plant was a *Dendrosenecio* and had sequestered cinchonidine by root parasitism on some neighbouring *Cinchona* or other plant producing cinchonidine; (3) the plant

extract had somehow been contaminated with cinchonidine; or (4) the result was genuine. The first of these possibilities was deemed least likely for, although species of *Cinchona* were introduced into Tanzania when it was a German colony, and are known to have been cultivated on the lower slopes of Mt. Kilimanjaro⁷, the appearance of *Dendrosenecio* is extraordinarily characteristic and very dissimilar to *Cinchona*, and subsp. *cottonii* is restricted to a harsh afroalpine environment, which supports few native species, and from which no introduced shrubs are known. Although transfer of alkaloids between plants is well known (as in the now classic case⁸ of *Casetilleja* acquiring pyrrolizine alkaloids through parasitising *Senecio* spp., or quinolizidines from *Lupinus* or *Thermopsis*) the *Dendrosenecio* from which the leaves had been collected was isolated, with no neighbouring shrubs, and so the second possibility was also discarded. Biosynthesis within a plant usually produces a number of alkaloids rather than a single one, and in this regard *Cinchona* species are not exceptional. So the failure to detect any other alkaloid in the plant extract besides cinchonidine, made us suspect that the third possibility was correct, and not the fourth. It was concluded¹ that the cinchonidine was probably an adulterant, though how it was introduced was unknown. Nevertheless the claim that cinchonidine occurs in *D.kilimanjari* subsp. *cottonii* has appeared in print⁹. followed by a disclaimer which suggested that this claim needed to be reexamined¹⁰.

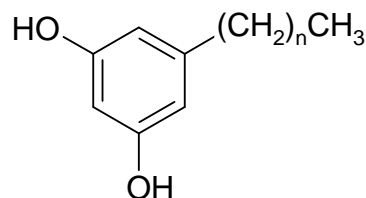
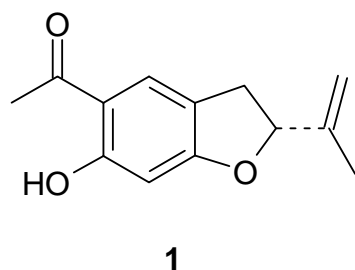
We now report the results of a reinvestigation of authenticated plant material.

Results and Discussion

Chromatographic fractionation of an extract of *D. kilimanjari* subsp. *cottonii* leaves yielded no cinchonidine. The major secondary metabolite was (R)-6-hydroxytremetone (**1**), accompanied by mixtures of 5-alkyl (**2**), and 5- ω -phenalkylresorcinols (**3**), and the sesquiterpene α -cadinol (**4**). This procedure would not have revealed cinchonidine if it were present at very low levels, as said to occur in *O. europea*. We therefore partitioned another leaf extract between a dilute aq. H₂SO₄ and CHCl₃. The aqueous extracts (A), which gave no reaction in a spot test for alkaloids with Mayer's reagent, were basified and extracted with CHCl₃. Evaporation of the organic extracts gave a little gummy material, which was recycled (dissolved in CHCl₃ and reextracted with acid) to afford a trace amount (0.004%) of a brown varnish. Upon GCMS analysis this showed traces of components but none corresponded to cinchonidine. Selective ion monitoring (SIM) using the m/z 136 base peak ion of cinchonidine also failed to reveal that alkaloid (or any related *Cinchona* base). The detectable limit for cinchonidine was estimated to be less than 0.001% of the dry leaves.

Our results are in accord with the only other published findings for secondary metabolites within the giant senecios of which we are aware: those for a species once known as *S. johnstonii* Oliv subsp. *adnivalis* (Stapf) C.Jeffrey var. *erici-rosenii* (R.E. Fr. and T.C.E.Fr.) C.Jeffrey., now classified as *D. erici-rosenii* (R.E.Fr. & T.C.E.Fr) E.B.Knox subsp. *erici-rosenii*¹². Grown from seeds of a plant collected on Mt. Karisimbi, Rwanda, this plant was found to contain several

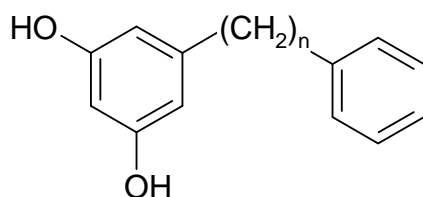
prenylated derivatives of 4-hydroxyacetophenone, including 6-hydroxytremetone (**1**), and 5-alkylresorcinols (**2**, $n = 14$, and 15). Alkaloids were not detected.



2a $n = 12$

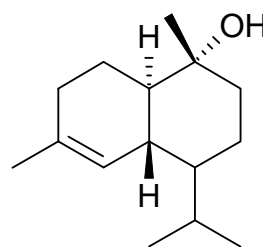
2b $n = 13$

2c $n = 14$



3a $n = 10$

3b $n = 12$



4

We conclude that cinchonidine does not occur in *D. kilimanjari* subsp. *cottonii*.

Experimental Section

General Procedures. NMR spectra were measured using a Bruker AMX-400 spectrometer of samples dissolved in CDCl_3 using as reference signals that due to residual protons at δ_{H} 7.25, and the central carbon line at δ_{C} 77.0 ppm. GCMS were performed with an Agilent model 5975 instrument fitted with a HP-5ms column (30m x 0.25mm with a 0.25 μm film of 5% phenyl methyl silicone), with He as carrier gas at flow rate of 0.58 mL/min, starting at 100 $^{\circ}\text{C}$ for 2 min. and then increasing to 280 $^{\circ}\text{C}$ at 10 $^{\circ}\text{C}/\text{min}$. Analytical and preparative TLC were carried out using 250 μm Merck silica gel 60 F254 on glass plates (5 x 20 and 20 x 20 cm respectively), for the development, and I_2 on TLC grade silica gel powder for localization of the components. Flash

column chromatography (CC) was done using silica gel 60 (230-400 mesh, Merck 9385). Optical rotations were measured with a Rudolph Autopol IV automatic polarimeter, using a 0.5 dm path length cell.

Plant material. Leaves of *Dendrosenecio kilimanjari* subsp. *cottonii* were collected from a plant growing on Mt. Kilimanjaro (Knox 4090; saddle between Kibo and Mawenzi, 3900 m; voucher specimen deposited at IND), and air dried, with the lamina excised from the main vein.

Compound isolation and characterization: (i) general procedure

Air-dried leaves of *D. kilimanjari* subsp. *cottonii* were pulverized in a coffee mill (Braun Inc., model KSM 2) and the powder (30g) was extracted overnight, at room temperature, by suspending it (magnetic stirrer) in a mixture of MeOH-Et₂O-petroleum ether (b.p. 40-60°C) (100 mL of each component). The suspension was filtered and the filter cake washed with MeOH. Removal of solvents from the combined filtrate and washings gave a dark green oil (4g). This material was redissolved in MeOH and stored at -10°C overnight. The insoluble material (plant waxes) was removed by filtration and the filtrate evaporated to a green oil (3.8g). Half of this was dissolved in CHCl₃ and silica gel 60 (5g) added, and the powder obtained when the solvent was removed was loaded onto the top of a column (1 x 20 cm) of the same absorbent. Flash CC was performed, collecting 50 mL fractions, commencing the elution with hexanes (50 mL), then successively with hexanes containing EtOAc (9 :1 v/v, 3 x 50mL), hexanes-EtOAc (4:1 v/v, 8 x 50 mL), hexanes-EtOAc (1:1 v/v, 4 x 50 mL), EtOAc (2 x 50 mL), EtOAc-CHCl₃ (1:1 v/v, 2 x 50 mL), CHCl₃ (2 x 50 mL), and CHCl₃-MeOH (9:1 v/v, 4 x 100 mL).

(R)-6-Hydroxytremetone (1). (470 mg) eluted in fractions 4-5, and was obtained a crystalline solid, m.p. 67-68 °C, δ_{H} 7.43 (1H, s), 6.29 (1H, s), 5.20 (1H, apparent t, J=8.4 Hz, but resolution enhanced spectra revealed this to be an overlapped dd with J = 7.4 and 9.5 Hz), 5.03 (1H, s), 4.89 (1H, s), 3.24 (1H, dd, J=9.5 and 15.4 Hz), 2.91 (1H, dd, J = 7.4 and 15.4 Hz), 2.47 (3H,s), and 1.71 (3H, s) ppm; δ_{C} 201.9s, 166.5s, 165.6s, 143.0s, 126.6d, 118.5s, 113.5s, 112.5t, 97.8d, 87.5d, 32.9t, 26.0q, 16.9q ppm; $[\alpha_{\text{D}}]$ -38° (c 1.6, EtOH). Data as lit.¹²

5-Alkyl (2) and 5- ω -phenylalkylresorcinols (3) eluted in fraction 6 (460 mg), a portion of which (100 mg) was subjected to PTLC (EtOAc-hexanes 1:3) to afford a zone, RF 0.3, which was cut into two sub-fractions: the front being enriched in alkylresorcinols, with δ_{H} 6.25 (2H, d, J = 2 Hz), 6.18 (1H, t, J = 2 Hz), 2.49 (2H, t, J = 7.6 Hz), 1.57 (3H, br m), 1.26 (2H, br s), 0.88 (3H, t, J = 6.6 Hz)¹³; while the end of the zone was enriched in the ω -phenylalkylresorcinols¹⁴, with δ_{H} 7.27 (2H, m), 7.19 (3H, m), 6.25 (2H, d J = 2 Hz), 6.18 (1H, t, J = 2 Hz), 2.59 (2H, t, J = 7.7 Hz), 2.47 (2H, t, J = 7.5 Hz), 1.58 (4H, br m), 1.28 (2H, br.s). GCMS of both fractions: **2a**, RT 23.63 min, m/z 292 (15), 166 (4), 137 (9), 124 (100) as lit.¹³; **2b**, 24.78 min, m/z 306 (12), 166 (3), 137(10), 124(100) as lit.¹⁴; **2c**, RT 27.51 min, m/z 320 (12), 166 (3), 137 (10), 124 (100) as lit.¹⁴; **3a**, RT 34.01 min, m/z 326 (21), 137 (11), 124 (100), as lit.¹⁴; **3b**, RT 43.06 min, m/z 354 (14), 137 (12), 124 (100), 91 (17), as lit.¹⁴. As judged from the TIC traces, the ratios of **2a:2b:2c** were ca. 1:10: and **3a: 3b** ca. 3:1.

α -Cadinol (4) (3 mg) was also isolated from the PTLC of fraction 6 described above: RF 0.15, δ_{H} 5.50 (1H, br s), 1.95 (3H, m), 1.67 (3H, br s), 1.11(3H, s), 0.92 (3H, d, J = 6.9 Hz), 0.78 (3H, d, J = 6.9 Hz); δ_{C} 135.0s, 122.3d, 72.4s, 50.0d, 46.7d, 42.2t, 39.9d, 30.9t, 26.0d, 23.8q, 22.7t, 22.0t, 21.5q, 20.8q and 15.1q; as lit.¹⁵.

(ii) Alkaloid-targeted procedure

An extract of powdered leaves (20 g) was prepared as in (i) and the residue (3.1 g) remaining after removal of solvents was partitioned between CHCl_3 (5 mL) and 0.5M aq. H_2SO_4 (5 mL). The aq. phase was separated, and the CHCl_3 phase reextracted with 0.5 M aq. H_2SO_4 (2 x 3 mL). The combined aq. extracts, which gave no reaction in a spot test with Mayer's reagent, were diluted with H_2O (to a total volume of 12 mL) and divided into two aliquots (**A** and **B**). Basification of **A**, to pH 9-10 (indicator paper) with NH_4OH was followed by extraction with CHCl_3 (3 x 3 mL). The combined, dried (Na_2SO_4) CHCl_3 extracts were evaporated to a residual brown gum (9 mg) the 400 MHz $^1\text{H-NMR}$ of which showed signals corresponding to **1**. The gum was redissolved in CHCl_3 (2 mL) and the solution extracted with 0.5 M aq. H_2SO_4 (3 x 1 mL). The combined aq. extracts were basified as before and extracted with CHCl_3 (3 x 1 mL). Evaporation of the combined, dried (Na_2SO_4), CHCl_3 extracts gave a trace (ca. 0.1 mg) of material the $^1\text{H-NMR}$ spectrum of which showed only signals attributable to silicone grease and moisture contaminants. GCMS, with SIM for m/z 136, failed to indicate anything corresponding to cinchonidine, or a congener.

To aliquot **B** was added Zn dust (100 mg)¹⁶ and the mixture was stirred (magnet) at RT for 4 hr, then basified (NH_4OH) and extracted with CHCl_3 as before. The residual gum remaining after removal of the solvent from the combined CHCl_3 extracts (15 mg) appeared to be largely **1**, as judged by its $^1\text{H-NMR}$ spectrum. When recycled through aq. H_2SO_4 as described for the residue obtained from **A**, this provided a trace of material (ca. 0.5 mg) GCMS of which, with SIM for m/z 136, showed nothing corresponding to cinchonidine.

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