Cyclopentanoid Cyanohydrin Glucosides and Amides of *Lindackeria dentata*

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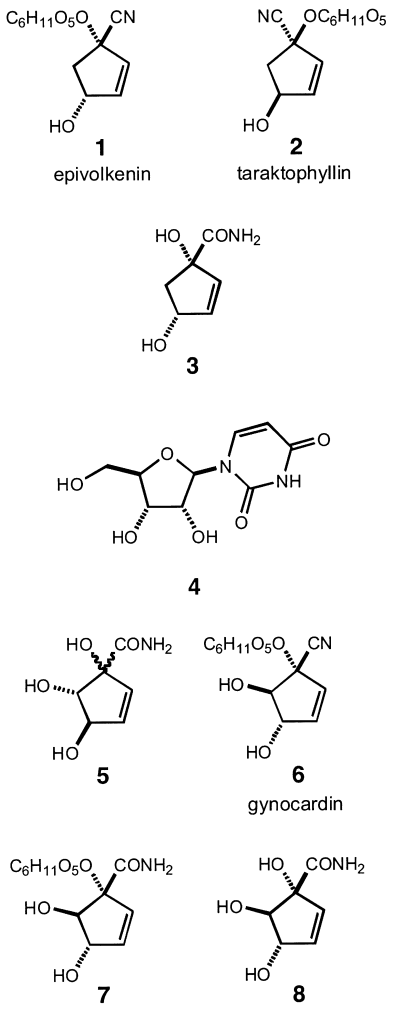
Abstract

A mixture of cyanogenic glucosides epivolkenin and taraktophyllin, 1,4-dihydroxy-2-cyclopentenecarboxamide, and uridine were isolated from leaves of *Lindackeria dentata* (Flacourtiaceae). Another cyclopentanoid amide, (1*R*,4*S*,5*R*)-1,4,5-trihydroxy-2-cyclopentenecarboxamide, was synthesized in two steps from gynocardin, and shown to have the same relative configuration as a partially identified amide previously isolated from *L. dentata* bark.

Plants produce cyanohydrin glycosides (cyanogenic glycosides) belonging to three main classes according to the structure of amino acid from which they are biosynthesized [1], [2]. One class comprises derivatives of mandelonitrile and its hydroxylated counterparts, biosynthetically derived from phenylalanine or tyrosine; the structural diversity within this group has been explored largely by Nahrstedt et al. [1], [2]. The other class includes derivatives of the aliphatic amino acids valine and isoleucine [1], [2], and the third comprises the relatively rare derivatives of the non-proteinogenic amino acid 2-cyclopentenylglycine [1], [2], [3], [4]. Biosynthesis, toxicology and ecology of cyanogenic glycosides and more recently genetic engineering of cyanogenic plants have attracted considerable interest. In this communication, we describe cyclopentanoid cyanohydrin glucosides and cyclopentanoid amides of *Lindackeria dentata* (Oliv.) Gilg, a species belonging to the family Flacourtiaceae, which is characterized by a variety of natural products apparently derived from 2-cyclopentenylglycine [4], [5], [6], [7], [8].

Leaves of *L. dentata* were extracted and fractionated to yield epivolkenin (**1**) [5] and taraktophyllin (**2**) [5] in the ratio of 2 : 3, along with the amide **3** [8] and uridine (**4**) [9]. The compounds **1 – 4** were identified on the basis of literature data [5], [8], [9]. The presence of derivatives of *cis*-2-cyclopentene-1,4-diol in *L. dentata* is in accord with the general cyclopentene hydroxylation pattern observed in Flacourtiaceae [5], [6], [7], [8].



The isolation of amides from extracts of cyanogenic plants was first reported by Jaroszewski et al. [10] and Nahrstedt and Rockenbach [11]. From the large amount of the amide isolated, Nahrstedt and Rockenbach concluded that it is a genuine natural product and not an artificial product of the isolation procedure [11]. On the other hand, in our earlier work on plants containing cyclopentanoid cyanogenic glucosides, a number of amides isolated appeared to be formed during processing of plant material [3], [8], [10].

The amide **3** was isolated previously from *Passiflora suberosa*; the compound was believed to be enantiomerically pure and had a large positive specific rotation ($[\alpha]_D$: +275°) [15], as expected for the amide formed from **1** [10]. By contrast, the amide isolated in the present work has a considerably lower specific rotation ($[\alpha]_D$: +120°). Since *L. dentata* produces both **1** and **2**, their hydrolysis would indeed afford a mixture of **3** and its levorotatory enantiomer, leading to the diminished optical rotation.

Bark of *L. dentata* has been studied previously by Gibbons et al., resulting in isolation of an amide formulated as **5** [12]. Neither the relative stereochemistry at C-1, nor the absolute configuration of the molecule have been determined [12]. We have now performed the transformation of the classical cyclopentanoid

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glucoside gynocardin (**6**) into the corresponding, stereochemically well-defined amide **8**. Thus, treatment of **6** with ammonia afforded a novel glucoside **7**, which was converted to **8** by cleavage of the glucosidic linkage with *Helix pomatia* enzyme preparation [13], [14]. The ^{1}H- and ^{13}C-NMR spectroscopic properties of thus obtained **8** were identical with those reported [12], including ^{1}H chemical shifts of the hydroxy groups observed in $(CD_3)_2SO$. This proves that the relative configuration of the amide isolated by Gibbons et al. [12] is identical with that of **8** because epimers of hydroxylated cyclopentenes have distinctly different NMR spectra [5], [10]. However, the synthetic amide **8** was, as expected [10], strongly dextrorotatory ($[\alpha]_D$: + 83°), whereas specific rotation of the *L. dentata* isolate had been reported to be –10° in the same solvent [12]. This value of the specific rotation corresponds neither to pure **8** nor to its pure enantiomer. From the reported optical rotation [12], the amide isolated by Gibbons et al. thus appears to be a mixture of the optical antipodes that could arise by hydrolysis of a mixture of **7** and its yet unknown isomer having all chiral centers in the aglucone portion inverted.

The present work contributes to the knowledge of structural diversity of cyclopentanoid natural products in the family Flacourtiaceae. In particular, the presence of amides as apparent products of metabolism of cyanohydrin glucosides is of interest. The described conversion of **6** to **8** is a prototype experiment enabling access to various non-glucosidic hydroxylated cyclopentane derivatives.

Materials and Methods

Leaves of *L. dentata* (Oliv.) Gilg were collected in Atowa Range Forest Reserve, Ghana; a voucher specimen (GC47689) was deposited in Herbarium GC (Ghana Herbarium, Botany Department, University of Ghana, Legon). Dried and milled plant material (130 g) was divided in three portions, and each portion added slowly to l L of boiling 80% aqueous MeOH. The mixtures were boiled for 5 min, chilled on ice, filtered, and evaporated. A total of 21.5 g of crude extract was obtained, which was coated on 75 g of silica gel (Matrex Silica gel 60A, 37 – 70 μm) using 80% MeOH, and chromatographed on an 18 × 10 cm I. D. silica gel column eluted with EtOAc/Me_2CO/CH_2Cl_2/MeOH/H_2O (20 : 15 : 6 : 5 : 4). Cyanogenic [14] fractions were pooled, evaporated, and the residue (0.5 g) subjected to HPLC on a 25 × 2 cm Phenomenex Luna 5 C18(2) column (5 μm particles), eluted isocratically with 6 mL/min of 15% aqueous MeOH and using a differential refractometer as detector. This resulted in the isolation of, in the order of elution, 80 mg (0.06%) of **3** [8], 138 mg (0.11%) of **4** [9], and 127 mg (0.1%) of a 2 : 3 mixture of epivolkemin (**1**) and taraktophyllin (**2**) [5].

(1S,4R*)-1,4-Dihydroxy-2-cyclopentenecarboxamide* (**3**): $[\alpha]_D$: + 120° (*c* 0.13, MeOH), lit. [8]: + 275° for optically pure (1*S*,4*R*) enantiomer; ^{1}H- and ^{13}C-NMR spectra identical with those reported for the latter [8] (the signal of C-5, obscured by the solvent signal, was identified in a DEPT135 spectrum).

Gynocardin (**6**, 400 mg) was treated with 7 mL of MeOH/concentrated aqueous NH_3 (2 : 5) for 48 h. The mixture was evaporated, and the residue chromatographed on the above reversed-phase HPLC column with 6 mL/min of 15% aqueous MeOH (refractometric detection) to give 102 mg (24%) of the amide **7**. The latter amide (80 mg) was dissolved in 1 mL of water and 2 mL of *Helix pomatia* crude glucuronidase/sulfatase enzyme preparation (Sigma) and left for 24 h at 37 °C. The solution was freeze-dried, the residue taken up with MeOH, filtered, the filtrate was evaporated, and the residue subjected to repeated HPLC as above, using 7.5% and then 1.2% aqueous MeOH; yield: 13 mg (33%) of **8** (20 mg or 25% of **7** was also recovered).

*(1*R*,4S,5*R*)-1-β-D-Glucopyranosyloxy-4,5-dihydroxy-2-cyclopentene-1-carboxamide* (**7**): $[\alpha]_D^{25}$: + 69° (*c* 0.93, MeOH); ^{1}H-NMR (CD_3OD, 600 MHz): δ = 6.05 (1H, dd, *J* = 6.3 and 1.6 Hz, H-3), 5.88 (1H, dd, *J* = 6.3 and 1.4 Hz, H-2), 4.71 (1H, dt, *J* = 5.8 and approx. 1.5 Hz, H-4), 4.56 (1H, d, *J* = 7.7 Hz, H-1′), 4.32 (1H, d, *J* = 5.8 Hz, H-5), 3.85 (1H, dd, *J* = 11.9 and 2.2 Hz, H-6′B), 3.64 (1H, dd, *J* = 11.9 and 5.9 Hz, H-6′A), 3.39 – 3.24 (m, 4H, H-2′, H-3′, H-4′ and H-5′); ^{13}C-NMR (CD_3OD, 150 MHz): δ = 175.2 ($CONH_2$), 140.3 (C-3), 131.6 (C-2), 99.7 (C-1′), 94.0 (C-1), 88.3 (C-5), 80.1 (C-4), 78.2 and 78.1 (C-3′ and C-5′), 75.1 (C-2′), 71.6 (C-4′), 62.7 (C-6′); HRMS: *m/z* = 344.09492 ($M + Na^+$), $C_{12}H_{19}NO_9Na^+$ requires 344.09520.

*(1*R*,4S,5*R*)-1,4,5-Trihydroxy-2-cyclopentene-1-carboxamide* (**8**): $[\alpha]_D^{25}$: + 83° (*c* 0.3, MeOH), lit. [12]: –10° (*c* 0.1, MeOH); ^{1}H-NMR (CD_3OD, 400 MHz): δ = 5.95 (1H, dd, *J* = 6.3 and 1.5 Hz, H-3), 5.65 (1H, dd, *J* = 6.3 and 1.5 Hz, H-2), 4.65 (1H, dt, *J* = 5.8, 1.5 and 1.5 Hz, H-4), 3.96 (1H, d, *J* = 5.8 Hz, H-5) (the assignment of H-2 and H-3 was confirmed by a NOESY spectrum); ^{13}C-NMR (CD_3OD, 100 MHz): δ = 177.0 ($CONH_2$), 138.3 (C-2), 133.9 (C-3), 91.6 (C-5), 87.7 (C-1), 80.3 (C-4); ^{1}H- and ^{13}C-NMR spectra in $(CD_3)_2SO$ as reported [12]; HRMS: *m/z* = 182.04221 ($M + Na^+$), $C_6H_9NO_4Na^+$ requires 182.04238.

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