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UNEDOSIDE DERIVATIVES IN *NUXIA* AND THEIR BIOSYNTHESIS

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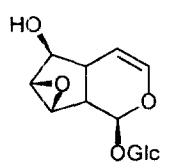
Key Word Index—*Nuxia oppositifolia*; *N. floribunda*; Buddlejaceae; iridoid glucosides; unedoside; 6-*O*-α-L-rhamnopyranosyl-unedoside; nuxioside; 2″-acetyl-3″-cinnamoyl-nuxioside; 2″-acetyl-3″-benzoyl-nuxioside; verbascoside; biosynthesis; chemotaxonomy.

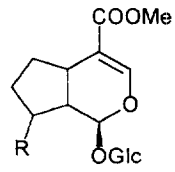
Abstract—An investigation of two species of *Nuxia* showed that this genus is characterized by the presence of the eight-carbon iridoid glucoside unedoside and/or its derivatives. From *N. floribunda* unedoside, nuxioside (6-*O*-α-L-rhamnopyranosyl-unedoside) and 2″-acetyl-3″-cinnamoyl-nuxioside were isolated, while from *N. oppositifolia* 2″-acetyl-3″-benzoyl-nuxioside was obtained. Both plants contained verbascoside. The biosynthesis of unedoside in *N. floribunda* was investigated and deoxyloganic acid was found to be a precursor, similar to what was found for the eight-carbon iridoids in *Thunbergia alata* earlier investigated. The taxonomic position of *Nuxia* and Buddlejaceae is briefly discussed.

INTRODUCTION

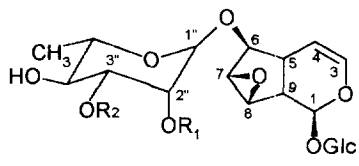
The genus *Nuxia* comprises 15 species of shrubs and trees ranging from southern Arabia, tropical Africa (including Madagascar, Comoro and the Mascarene Islands) to South Africa [1]. *Nuxia* is a member of the Buddlejaceae, traditionally included in Loganiaceae [1], but most contemporary taxonomists separate these taxa and have raised Buddlejaceae to family rank [2–5]. Continuing our work with Loganiaceae and related taxa [6–8], we have now investigated two species of *Nuxia*, namely *N. oppositifolia* collected in Zimbabwe and a specimen tentatively identified as *N. floribunda* cultivated in the Botanical Garden of Copenhagen but originating from Kirstenbosch Botanical Garden (South Africa), from where it was obtained in 1948.



1 unedoside



5 R = β-Me
6 R = α-Me



2 R_1= R_2= H
3 R_1=Ac; R_2=Benzoyl
4 R_1=Ac; R_2= Cinnamoyl

RESULTS AND DISCUSSION

Nuxia floribunda

Fractionation of the water-soluble part of an ethanol extract of fresh foliage by reversed-phase chromatography gave, together with the caffeoyl phenylethanoid glycoside (CPG), verbascoside, the iridoid glucoside, unedoside (**1**), identified by its melting point and by its NMR spectra [7, 9, 10], as well as two unknown iridoid diglycosides, **2** and **3**.

Compound **2** was isolated as a crystalline solid melting at 247–250° under decomposition. The NMR spectra of **2** were similar to those of **1** but had additional features. Thus, the ^{13}C NMR spectrum (Table 1) had 20 signals of which eight could be assigned to an iridoid aglucone, six to a β-glucopyranosyl moiety and additionally, six to an α-rhamnopyranosyl moiety similar to that found in 6-*O*-α-L-rhamnopyranosylcatalpol [11, 12]. When comparing the signals from **1** and **2**, it was obvious that the site of attachment for the rhamnopyranosyl auxiliary in **2** was at the C-6

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Table 1. ^{13}C NMR data of compounds **1**–**4**

	1 (D_2O)	**2** (D_2O)	**2** (CD_3OD)	**3** (CD_3OD)	**4** (CD_3OD)
C-1	96.9	96.8	96.2	96.2	96.2
C-3	141.8	142.2	142.5	142.5	142.6
C-4	104.2	103.7	103.5	103.3	103.3
C-5	37.2	35.6	36.3	36.0	36.0
C-6	79.2	84.1	84.4	85.1	85.1
C-7	59.4	56.9	55.9	55.9	55.9
C-8	56.4	56.7	55.8	55.8	55.8
C-9	42.8	42.5	43.4	43.2	43.3
C-1′	100.2	100.1	100.4	100.3	100.4
C-2′	73.7	73.7	74.7	74.6	74.7
C-3′	76.7	76.7	78.0	77.7	77.8
C-4′	70.5	70.4	71.5	71.5	71.4
C-5′	77.3	77.2	78.3	78.1	78.3
C-6′	61.5	61.5	62.7	62.6	62.7
C-1″		99.9	100.6	97.8	97.9
C-2″		71.2	72.3	71.4	71.5
C-3″		71.1	72.2	73.0	73.5
C-4″		73.0	73.9	71.4	71.5
C-5″		69.9	70.2	70.2	70.2
C-6″		17.6	18.0	18.0	17.8
C-1‴				135.5	131.2
C-2,6‴				129.2	129.5
C-3,5‴				130.0	130.6
C-4‴				131.6	133.1
CO‴				167.8	167.2
Cα‴				118.4	
Cβ‴				146.7	
Ac-CO				171.7	171.6
Ac-CH_3				20.6	20.7

oxygen atom of the aglucone moiety, since this carbon atom showed a downfield shift (4.9 ppm), while C-5 and C-7 had upfield shifts (*ca* 2 ppm) relative to those seen for **1**. Evidence for the absolute configuration of the rhamnose auxiliary was obtained by comparing the specific rotations of **1** (−112°) [13] and **2** (−146°) on the one hand, with those reported for catalpol (−102°) [13] and for 6-*O*-α-L-rhamnopyranosylcatalpol (−125°) [11], (−148°) [12], (−154°) [14] on the other. Despite the disagreements in the values found for the latter, the two differences are of the same sign and magnitude, and we therefore conclude that **1** is the 6-*O*-α-L-rhamnopyranosyl derivative of unedoside. We have named it nuxioside.

Compound **3** was an amorphous foam. The ^{13}C NMR spectrum (Table 1) contained 31 signals which could be assigned to (i) an aglucone moiety (eight peaks), (ii) a β-glucopyranosyl moiety (six peaks), (iii) a substituted α-rhamnopyranosyl moiety (six peaks), (iv) an *E*-cinnamoyl residue (nine peaks) and (v) an acetyl group (two peaks). Comparison with the spectrum of **2** showed that **3** was an acylated nuxioside derivative. The positions of the two acyl groups were determined from the ^1H NMR spectrum. In this, H-2″ and H-3″ of the rhamnosyl group were observed at δ 5.32 and 5.23, respectively, both *ca* 1.5 ppm downfield when compared with the spectrum of **2**. The remaining signals were not very different in the two spectra and, therefore, the oxygen atoms of C-2″ and C-3″ had to be the sites of acylation. The positions of the individual acyl groups were determined by long-range selective proton decoupling (LSPD). Thus, selective irradiation at the acetyl methyl singlet at δ 2.11 reduced the carbonyl signal at δ 171.7 to a doublet (4 Hz) while decoupling at δ 5.32 (H-2″) similarly collapsed it to a quartet (7 Hz). This proved the acetyl group to be sited at the C-2″-oxygen atom. Conversely, decoupling at δ 5.23 (H-3″) simplified the carbonyl signal at 167.8 to a double doublet (2 and 7 Hz), while decoupling at δ 6.51 (Cα‴) revealed another set of couplings (3 and 7 Hz), consistent with the cinnamoyl group in the 3″-position. Consequently, compound **3** is 2″-acetyl-3″-cinnamoyl-nuxioside.

Nuxia oppositifolia

From the polar part of the ethanol extract was obtained only two main components, namely verbascoside and the acylated diglycoside, **4**. The ^1H NMR spectrum of **4** was very similar to that of **3**, except that the signals from the cinnamoyl group were replaced by those from a benzoyl group in the new compound. This was consistent with the ^{13}C NMR spectrum which showed 29 signals and was assigned by comparison with that of **3** (Table 1). Also, in this case, the two acyl groups were positioned at the 2″-

and 3″-oxygen atoms of the rhamnosyl auxiliary, as shown by the low-field positions of the corresponding proton signals. The position of each acyl group was again settled using the LSPD-technique. Thus, the carbonyl signal at δ 171.6 (acetyl-CO) showed couplings to the methyl singlet at δ 2.10 (7 Hz) and to H-2″ at δ 5.32 (4 Hz). On the other hand, decoupling at δ 5.23 (H-3″) simplified the carbonyl signal at 167.2 (benzoyl-CO) to a broad triplet (4 Hz). Thus, the acetyl group of **4** was attached at the 2″-position, as in compound **3**. Consequently, compound **4** is 2″-acetyl-3″-benzoyl-nuxioside.

Biosynthesis

Iridoid glucosides lacking both C-10 and C-11 are comparatively rare. Unedoside was first discovered in *Arbutus unedo* (Ericaceae) [15], later in *Stilbe* (Stilbaceae), *Retzia* (Retziaceae) and *Thunbergia* (Acanthaceae) [cf. 16]. Also a compound named undulatin has been isolated from *Tecomella* (Bignoniaceae) [17], but the data given are of dubious value for a certain assignment of the structure. The biosynthesis of these compounds has been investigated only in *Thunbergia* [16], where it was found that deoxyloganic acid (**5**) was a precursor, while the epimer 8-epideoxyloganic acid (**6**) was not. This is somewhat surprising, since all other investigated iridoid glucosides lacking C-11 (including aucubin and catalpol) are derived from **6** [18, 19]. Since the biosynthetic pathways appear to have a potential for the determination of taxonomic relationships among iridoid-containing plants [6, 18], it seemed worthwhile to investigate the biosynthesis of unedoside in *Nuxia*, particularly since **6** has been found to be a precursor of aucubin in *Buddleja davidii* [20] and *B. albiflora* [21] of the same family.

Deuterium-labelled precursors (**5** and **6**) were those earlier described [22, 23] and feedings were performed by inserting fresh young cuttings of *N. floribunda* into a beaker containing an aqueous solution of the precursor. The solution was absorbed during 24 h and the plant was left to metabolize the substrate for two more days. Work-up gave the fraction containing unedoside (**1**) and the deuterium content was determined by ^{2}H NMR using the natural abundance deuterium content in water to calculate the incorporation. Feeding with **6** gave no significant incorporation. However, in the experiment with **5** a distinct, broad deuterium absorption was observed at δ 3.5–4.1 in the spectrum of isolated **1**. Since the precursor was labelled with deuterium at C-6 (α/β), C-7 (α) and C-8 (α) with a mean *ca* 0.8 ^{2}H at each carbon atom, and since the mechanisms of the steps leading from **5** to **1** were virtually unknown, only a minimum incorporation could be calculated. Assuming retention of all deuterium atoms at the mentioned positions of the precursor fed (unlikely at C-6!), and measuring the total integral of the deuterium at C-6, C-7 and C-8 (δ 4.04, 3.73 and 3.60, respectively) of the product, we found this minimum incorporation into **1** to be 9%.

As noted above, the eight-carbon iridoids in *Thunbergia* are biosynthetically derived from deoxyloganic acid (**5**) with the 8β-stereochemistry, while the nine- and ten-carbon iridoids from members of the remaining part of the family Acanthaceae almost certainly can be assumed to be derived from 8-epideoxyloganic acid (**6**) with the opposite stereochemistry at C-8 [24]. Now we have a second example where a single genus in a family, *Nuxia*, synthesizes the eight-carbon iridoids via **5** while the remaining genera investigated, *Buddleja*, *Emorya* and *Gomphostigma*, contain the nine-carbon iridoids produced *via* **6** [6]. A number of acylated rhamnosylcatalpol derivatives have been reported from *Buddleja americana* [25] and *B. japonica* [26] and in connection with the present work, we have also found such compounds in *B. indica* and in *B. madagascariensis*. Therefore, the ability to produce acylated rhamnosyl iridoids may be seen as a chemotaxonomic link between *Nuxia* and *Buddleja*. This ability is otherwise restricted to a few sympetalous genera, namely *Scrophularia* and *Verbascum* in the Scrophulariaceae and *Premna* in the Verbenaceae [13, 27]. Recent work on chloroplast DNA sequences [28] furthermore supports the close relationship between Buddlejaceae and Scrophulariaceae, consistent with chemotaxonomic information.

EXPERIMENTAL

General. ^{1}H and ^{13}C NMR spectra were recorded in D_2O, using the solvent peak (δ 4.75) and the C-6′ signal (δ 61.5), respectively, as standards or in CD_3OD (standards δ 3.31 and 49.0). ^{2}H NMR (77 MHz) spectra were recorded in H_2O with 0.0156% ^{2}H of natural abundance. Chromatography was performed at medium pressure on reversed-phase silica gel (Polygoprep C_{18} 50–60 μ; 550 g) or on Merck Lobar C_{18}-columns (size B or C), eluting with H_2O–MeOH mixts as specified and monitoring simultaneously at 206 and 254 nm with a UV detector. *Nuxia floribunda* Benth. from the Botanic Garden of Copenhagen (Acc. no. S1948-1868); *N. oppositifolia* Benth. was collected in the National Botanic Garden, Harare, Zimbabwe (courtesy of R. B. Drummond) in 1991; the voucher (PM/92/4) has been deposited at The Botanical Museum, The University of Copenhagen (C).

Nuxia floribunda. Fresh foliage (97 g) was blended with EtOH, the mixt. was filtered and the filtrate taken to dryness. The residue was partitioned between H_2O and Et_2O, and the aq. phase gave a residue (5.1 g). Chromatography on a Polygoprep column (25:1 to 1:2) gave 9 frs (A–I) which were monitored by ^{1}H NMR. Fr. A (650 mg) contained sugars and inorganics. Fr. B was **1** (375 mg, 0.4%). Fr. C (80 mg) was rechromatographed on the B-column (15:1) to give pure **2** (35 mg, 0.04%). Fr. F was mainly verbascoside (400 mg). Fr. H contained pure **3** (135 mg, 0.15%). Frs D (85 mg), E (200 mg), G (600 mg) and I (300 mg) were not investigated further.

Unedoside (**1**). Crystals (90% EtOH) mp 232–234

(lit. 232–234° [15]). ^{1}H NMR (200 MHz, D_2O): δ 6.35 (*dd*, J = 1.8 and 6 Hz, H-3), 5.12 (*dd*, J = 4.5 and 6 Hz, H-4), 4.88 (*d*, J = 9.8 Hz, H-1), 4.04 (*dd*, J = 1.3 and 7.8 Hz, H-6), 3.73 (*br d*, J = *ca* 3 Hz, H-8), 3.60 (*dd*, J = 1.3 and 3 Hz, H-7), 2.54 (*br dd*, J = 7.5 and 9.8 Hz, H-9), 2.15 (*dddd*, J = 1.8, 4.5, 7.5 and *ca* 8 Hz, H-5); glucosyl moiety: δ 4.80 (*d*, J = 8 Hz, H-1′), 3.86 (*dd*, J = 1.9 and 12.5 Hz, H-6a′), 3.68 (*dd*, J = 5 and 12.5 Hz, H-6b′), 3.3–3.5 (4H, *unres.*, H-2′ through H-5′). ^{13}C NMR: Table 1.

Nuxioside (**2**). Crystals (MeOH) mp 247–250° (dec). $[\alpha]_D^{20}$ −146° (*c*, 0.5; MeOH). ^{1}H NMR (500 MHz, CD_3OD): δ 6.35 (*dd*, J = 1.9 and 5.8 Hz, H-3), 5.05 (*dd*, J = *ca* 4.5 and 5.8 Hz, H-4), 4.88 (*d*, J = 9.7 Hz, H-1), 3.99 (*dd*, J = 1.4 and 7.5 Hz, H-6), 3.63 (*d*, J = 3.8 Hz, H-8), 3.60 (*dd*, J = 1.4 and 3.8, H-7), 2.46 (*dd*, J = 7.5 and 9.7, H-9), 2.24 (*dddd*, J = 1.9, 4.5, 7.5 and 7.5, H-5); glucosyl moiety: δ 4.71 (*d*, J = 7.8, H-1′), 3.88 (*dd*, J = 1.8 and 11.9 Hz, H-6a′), 3.65 (*unres.*, H-6b′), 3.39 (*dd*, J = 9.5 Hz, H-3′), 3.3 (2H, *unres.*, H-4′ and H-5′), 3.25 (*dd*, J = 7.8 and 9.5 Hz, H-2′); rhamnosyl moiety: δ 4.93 (*d*, J = 1.8 Hz, H-1″), 3.86 (*dd*, J = 1.8 and 3.3 Hz, H-2″), 3.67 (*dd*, J = 3.3 and 9.5, H-3″), 3.65 (*unres.*, H-5″), 3.37 (*unres.*, H-4″), 1.26 (*d*, J = 6.0 Hz, CH_3-6″). ^{13}C NMR: Table 1. (Found: C, 47.4; H, 6.2. $C_{20}H_{30}O_{13}$, 1.5 H_2O requires: C, 47.5; H, 6.6%).

2″-Acetyl-3″-cinnamoyl-nuxioside (**3**). Foam. $[\alpha]_D^{20}$ −109 (*c*, 0.4; MeOH). ^{1}H NMR (500 MHz, CD_3OD): δ 6.37 (*dd*, J = 1.9 and 6.0 Hz, H-3), 5.10 (*dd*, J = *ca* 4.5 and 6.0 Hz, H-4), 4.90 (*d*, J = 9.8 Hz, H-1), 4.05 (*dd*, J = 1.4 and 7.8 Hz, H-6), 3.65 (*br d*, J = *ca* 3.5 Hz, H-8), 3.63 (*dd*, J = 1.4 and 3.5, H-7), 2.50 (*dd*, J = 7.5 and 9.8, H-9), 2.34 (*dddd*, J = 1.9, 4.5, 7.5 and 7.8, H-5); glucosyl moiety: δ 4.71 (*d*, J = 7.9, H-1′), 3.88 (*dd*, J = 1.9 and 12.0 Hz, H-6a′), 3.62 (*dd*, J = 6.5 and 12 Hz, H-6b′), 3.38 (*dd*, J = 9.1 Hz, H-3′), 3.3 (2H, *unres.*, H-4′ and H-5′), 3.26 (*dd*, J = 7.9 and 9.1 Hz, H-2′); rhamnosyl moiety: δ 5.32 (*dd*, J = 1.9 and 3.5 Hz, H-2″), 5.23 (*dd*, J = 3.5 and 9.8, H-3″), 5.02 (*d*, J = 1.9 Hz, H-1″), 3.88 (*unres.*, H-5″), 3.62 (*unres.*, H-4″), 1.33 (*d*, J = 6.3 Hz, CH_3-6″); cinnamoyl moiety; δ 7.70 (*d*, J = 16.0 Hz, Hβ‴), 7.60 (2H, H-2‴ and H-6‴), 7.40 (3H, H-3‴, H-4‴ and H-5‴), 6.51 (*d*, J = 16.0 Hz, Hα‴); acetyl group: 2.11 (*s*). ^{13}C NMR: Table 1. (Found: C, 54.5; H, 5.8. $C_{31}H_{38}O_{15}$, $2H_2O$ requires: C, 54.2; H, 6.2%).

Nuxia oppositifolia. Dry leaves (45 g) were blended in EtOH (200 ml) and left to soak for 2 days. The resulting extract was taken to dryness and partitioned between Et_2O and H_2O (some EtOH was added to give a clear aq. phase). The latter was concd and partitioned between H_2O and EtOAc, and each were taken to dryness to give fr. J (1.72 g) and fr. K (1.35 g), respectively. Chromatography of fr. J on a C-column (25:1 to 1:1) gave as the only identifiable product, verbascoside (140 mg), while fr. K on the same column (2:1 to 1:2) gave pure verbascoside (60 mg; total 0.5%) and crude **4** (130 mg; 0.3%). Rechromatography of crude **4** on a B-column (3:1 to 2:1) provided a fr. with pure **4** (60 mg).

2″-Acetyl-3″-benzoyl-nuxioside (**4**). Foam. $[\alpha]_D^{21}$ −118° (*c*, 0.5; MeOH). ^{1}H NMR (500 MHz, CD_3OD): δ 6.40 (*dd*, J = 1.9 and 6.0 Hz, H-3), 5.13 (*dd*, J = *ca* 4.5 and 6.0 Hz, H-4), 4.93 (*d*, J = 9.8 Hz, H-1), 4.08 (*br d*, J = 7.8 Hz, H-6), 3.66 (2H, *s*-like, H-7 and H-8), 2.52 (*dd*, J = 7.5 and 9.8, H-9), 2.36 (*dddd*, J = 1.9, 4.5, 7.5 and 7.8, H-5); glucosyl moiety: δ 4.73 (*d*, J = 7.9, H-1′), 3.90 (*dd*, J = 1.8 and 11.9 Hz, H-6a′), 3.65 (*dd*, J = 5.8 and 11.9 hz, H-6b′), 3.40 (*t*-like, J = 9 Hz, H-3′), 3.3 (2H, *unres.*, H-4′ and H-5′), 3.28 (*dd*, J = 7.9 and 9.2 Hz, H-2′); rhamnosyl moiety: δ 5.40 (*dd*, J = 1.9 and 3.5 Hz, H-2″), 5.36 (*dd*, J = 3.5 and 9.8, H-3″), 5.06 (*d*, J = 1.9 Hz, H-1″), 3.91 (*dq*, J = 6.2 and 9.6 Hz, H-5″), 3.71 (*dd*, J = 9.6 Hz, H-4″), 1.35 (*d*, J = 6.2 Hz, CH_3-6″); benzoyl moiety: δ 8.02 (2H, H-2‴ and H-6‴), 7.61 (H-4‴), 7.49 (2H, H-3‴ and H-5‴); acetyl group: 2.10 (*s*). ^{13}C NMR: Table 1. (Found: C, 54.2; H, 5.6. $C_{29}H_{36}O_{15}$, H_2O requires: C, 54.2; H, 6.0%).

Biosynthetic experiments. Deoxyloganic acid (**5**, 15 mg) [22, 29] with the following deuterium content: C-6 (0.8 ^{2}H); C-7 (0.9 ^{2}H); C-8 (0.7 ^{2}H); C-10 (1.8 ^{2}H) in H_2O (5 ml) was fed to a young shoot (14 g, the stem freshly cut under H_2O). The soln was absorbed after *ca* 24 hr and more H_2O was added when necessary. After a total of 69 hr, the plant was worked up as described above. Chromatography on a Merck Lobar C_{18} column (size B) and eluting with H_2O–MeOH (25:1) gave unedoside (**1**, 51 mg). 8-Epideoxyloganic acid (**6**, 15 mg) [23]: C-6 (0.8 ^{2}H); C-7 (0.7 ^{2}H); C-8 (0.7 ^{2}H); C-10 (2.0 ^{2}H) was administered to the plant (16 g) as described above and gave **1** (73 mg).

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