Chlorinated Iridoid Glucosides from *Veronica longifolia* and Their Antioxidant Activity

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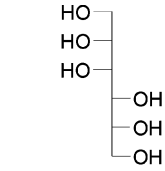
From *Veronica longifolia* were isolated three chlorinated iridoid glucosides, namely, asystasioside E (**6**) and its 6-*O*-esters **6a** and **6b**, named longifoliosides A and B, respectively. The structures of **6a** and **6b** were proved by analysis of their spectroscopic data and by conversion to the catalpol ester verproside (**5a**) or to catalpol (**5**), respectively. The configuration of the previously known vanilloyl analogue, urphoside B, was shown to be the 6β-epimer (**6c**) of the structure originally reported. Longifoliosides A (**6a**) and B (**6b**) were found to exhibit radical-scavenging activity against nitric oxide, superoxide, and 2,2-diphenyl-1-picrylhydrazyl radicals.

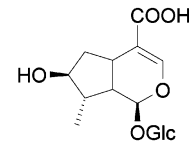
Plants of the genus *Veronica* (Speedwell, Plantaginaceae) have traditionally been considered members of the family Scrophulariaceae; however, the genus has recently been transferred to the Plantaginaceae on the basis of DNA sequence investigations.[1,2] With regard to chemistry, *Veronica* is generally characterized by its content of the iridoid glucosides aucubin (**4**) and catalpol (**5**) as well as a variety of esters of the latter.[3−5] As a part of an earlier project, one of us has previously investigated *V. longifolia* L,[5] finding the iridoid glucosides **4** and **5** as well as the 6-*O*-catalpol esters verproside (**5a**), verminoside (**5b**), and catalposide (**5c**).

Material of *V. longifolia* was briefly boiled with ethanol, and after extraction, the water-soluble part of the extract was subjected to reversed-phase column chromatography. Mannitol (**1**) was the main carbohydrate in the sugar fraction. The iridoid glucosides isolated comprised the common compounds 8-epiloganic acid (**2**), gardoside (**3**), aucubin (**4**), catalpol (**5**), and two 6-*O*-esters of the latter (**5a** and **5c**), as well as the chloro-containing asystasioside E (**6**) and two compounds (**6a** and **6b**) that were apparently esters of **6**.

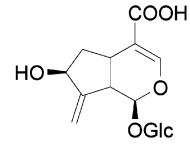
Compound **6a** was isolated as a colorless glass, $[\alpha]^{25}_{D}$ −102. The molecular formula was $C_{22}H_{27}ClO_{13}$ as deduced from the quasimolecular 3:1 ion cluster obtained by LC-HRESIMS (observed *m*/*z* 557.1015 $[M + Na]^+$ and its ^{37}Cl isotope *m*/*z* 559.1034 $[M + 2 + Na]^+$). The NMR spectroscopic data (Table 1) could be assigned using 1D and 2D techniques. The ^{13}C NMR spectrum of **6a** exhibited the expected 22 signals, of which six could be assigned to a β-glucopyranosyl group and seven to a 3,4-dihydroxybenzoyl moiety, when comparing with the spectrum of verproside (**5a**). The remaining 10 signals were surmised as belonging to an iridoid aglucon, and since it also contained a chlorine atom, this was compared to the data of asystasioside E (**6**) (Table 1). The chemical shifts were very similar, except for the C-6 signal (δ 85.0) of **6a**, which showed a downfield shift of 2.1 ppm, while the C-5 and C-7 signals were found 1.1 and 2.1 ppm upfield, respectively. This indicated that **6a** is the 6-*O*-(3,4-dihydroxybenzoyl) ester of **6**. The ^1H NMR spectrum of **6a** (Table 1) was consistent with this, including the expected acylation shifts (data not shown). The HMBC spectrum allowed the carbons of attachment between the aglucon and the peripheral moieties to be discerned. Thus, the position of the ester was confirmed by a correlation between H-6 (δ_H 5.05) and the carbonyl of the acyl group (δ_C 167.7), and that of the glucopyranosyl moiety by a correlation between H-1′ of the glucosyl moiety (δ_H 4.65) and C-1 of the aglucon (δ_C 92.8). This compound has been accorded the trivial name longifolioside A.



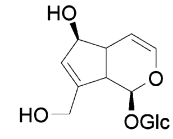
1 mannitol



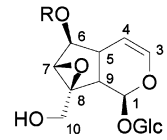
2 epiloganic acid

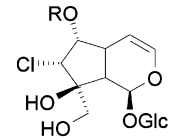


3 gardoside



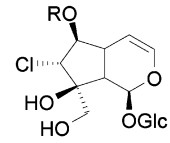
4 aucubin





5 R=H; catalpol
5a R=3,4-di-OH-benzoyl; verproside
5b R=caffeoyl; verminoside
5c R=p-OH-benzoyl; catalposide

7 R=vanilloyl



6 R=H; asystasioside E
6a R=3,4-di-OH-benzoyl; longifolioside A
6b R=caffeoyl; longifolioside B
6c R=vanilloyl; urphoside B
6d R=p-OH-benzoyl

Longifolioside B (**6b**) was similarly isolated as a colorless glass, $[\alpha]^{25}_{D}$ −84. Again, the molecular formula, $C_{24}H_{29}ClO_{13}$, was deduced from the quasi-molecular 3:1 ion cluster obtained by LC-HRESIMS (observed *m*/*z* 583.1166 $[M + Na]^+$ and its ^{37}Cl isotope *m*/*z* 585.1151 $[M + 2 + Na]^+$). As for compound **6a** above, the NMR spectroscopic data (Table 1) could be assigned using 1D and 2D techniques. The ^{13}C NMR spectrum of **6b** contained 24 signals, of which six as above could be attributed to a β-glucopyranosyl group and nine to a caffeoyl group, by comparison with the

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Table 1. ^{1}H (500 MHz) and ^{13}C (75 MHz) NMR Spectroscopic Data for Longifoliosides A (**6a**) and B (**6b**) and Urphoside B in CD_3OD

	longifolioside A (**6a**)		longifolioside B (**6b**)		**6**	urphoside B (**6c**)[a]	
position	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_C	δ_H (*J* in Hz)
Agluc							
1	92.8	5.69, d (3.5)	92.8	5.68, d (3.6)	93.3	92.9	5.70, d (3.7)
3	141.0	6.30, dd (6.2, 1.8)	141.0	6.29, m[b]	140.8	141.2	6.31, dd (6.3, 2.1)
4	105.6	5.25, dd (6.2, 3.3)	105.6	5.22, dd (6.2, 3.3)	106.1	105.7	5.26, dd (6.3, 3.5)
5	37.0	2.88, m	37.1	2.84, m	38.1	36.8	2.90, m
6	85.0	5.05, dd (5.0, 7.4)	84.7	4.99, dd (5.1, 7.4)	82.9	85.3	5.10, dd (4.9, 7.3)
7	69.6	4.35, d (7.4)	69.6	4.29, d (7.4)	71.7	69.7	4.20, d (7.3)
8	80.8		80.8		80.1	81.0	
9	48.9	2.62, dd (10.5, 3.5)	48.9	2.60, dd (10.5, 3.6)	ca. 49	48.5	2.63, dd (10.5, 3.7)
10	63.5	4.00, d (11.8)	63.5	3.99, d (11.8)	63.5	63.6	4.10, d (11.6)
		3.82, d (11.8)		3.81, d (11.8)			4.83, d (11.6)
Glc							
1′	99.5	4.65, d (7.9)	99.5	4.65, d (7.9)	99.7	99.6	4.66, d (7.9)
2′	74.7	3.19, dd (9.2, 7,9)	74.7	3.19, dd (9.2, 7.9)	74.7	74.8	3.20, t (9.1)
3′	77.8	3.36, t-like (9.2)	77.9	3.36, t-like (9.2)	77.9	78.0	3.36, t (9.3)
4′	71.6	3.28, m[b]	71.6	3.28, m[b]	71.7	71.7	3.28, m[b]
5′	78.1	3.29, m[b]	78.1	3.29, m[b]	78.1	78.2	3.28, m[b]
6′	62.8	3.87, br d (11.5)	62.8	3.87, br d (11.2)	62.9	62.9	3.83, dd (12.0, 2.1)
		3.67, dd (11.5, 5.1)		3.66, dd (11.2, 4.5)			3.67, dd (12.0, 6.0)
Aroyl							
1″	122.0		127.5			122.7	
2″	117.5	7.45 (m)	115.2	7.05, d (1.9)		113.7	
3″	146.2		147.7			148.8	
4″	152.0		149.8			153.3	
5″	115.9	6.81 (d-like 8.8)	116.5	6.77, d (8.2)		116.1	
6″	123.9	7.45 (m)	123.2	6.96, dd (8.2, 1.9)		125.6	
α			114.4	6.30, d (15.9)			
β			146.8	7.59, d (15.9)			
CO	167.7		168.6			167.6	

[a] Data from ref 6 in this paper; the coupling constant $J_{5,6}$ was given as 12.2 Hz due to a misreading. [b] Signal obscured.

analogous data for verminoside (**5b**). The 10 signals not accounted for were almost superimposable on those of the aglucon of compound **6a**. Except for the ester groups, the ^{1}H NMR data for **6b** and **6a** were almost identical (Table 1), including the measured coupling constants. Again, the HMBC spectrum allowed the relative positions of the individual moieties to be pinpointed. Thus, the position of the acyl group was confirmed by a correlation between H-6 (δ_H 4.99) and the carbonyl of the acyl group (δ_C 168.6) and that of the glucopyranosyl group by a cross-peak between H-1′ (δ_H 4.65) and C-1 of the aglucon (δ_C 92.8). Longifolioside B therefore has the structure shown as **6b**.

Having elucidated these structures, the data were compared (Table 1) with two similar compounds reported in the literature, namely, a 6α-*O*-vanilloyl ester named urphoside B (assigned structure **7**; $[\alpha]^{23}_D$ −122) isolated from *Veronica pectinata* L. var. *glandulosa* Riek ex. M.A.[6] and a compound named 6-*O*-*p*-hydroxybenzoyl asystasioside E (**6d**; $[\alpha]^{26}_D$ −71), isolated from "Catalpae fructus" from a *Catalpa* species (Bignoniaceae).[7] Of these two compounds, the former had been assigned the 6α-configuration by analysis of coupling constants and a NOE correlation between H-6 and H-7 as well as another between H-7 and H-9, which indicated the *cis*-disposition of these three atoms. Compound **6d** has been assigned with a 6*β*-substituent, solely using the NOE technique. In view of the similarity of the NMR data for the four compounds (Table 1), which were almost identical except for the signals arising from the differing acyl moieties, and since the specific optical rotations were similar, it was assumed that they all had the same relative configuration. It has been demonstrated[8,9] previously that the configuration of a 6-*O*-substituent in iridoid glucosides has considerable effects (1−3 ppm) on the resultant chemical shifts of C-1, C-3, and C-4. Furthermore, it has been shown more recently that determination of the stereochemistry of iridoids using NOE correlations can be uncertain.[10−12] By reinspection of the original ^{1}H NMR spectrum of urphoside B we could conclude that the measured coupling constants for $J_{5,6}$ (12.5 and 7.3 Hz) instead should be 4.9 and 7.3 Hz, respectively, the confusion being due to a spinning sideband from the solvent peak. The NOE correlations observed between H-6 and H-7 and between H-7 and H-9 in **6c** are also visible in the spectra of **6a** (see Supporting Information); the proximity of H-6 and H-7 despite their *trans*-disposition must thus be due to the conformation of the five-membered ring; therefore, this is an additional example that extra care should be taken when determining the structure of iridoid glucosides using mainly NOE correlations. Assuming that compounds **6a** and **6b** are both 7,8-*trans*-chlorohydrins, a simple way to prove the structures would be a transformation to the known **5a** and **5b**, respectively, by treatment with sodium hydroxide.[13,14] Thus, a sample of **6b** was dissolved in D_2O in a NMR tube and an excess of NaOD in D_2O was added, with the changes being followed by ^{1}H NMR spectroscopy. After 1 h the ^{1}H NMR signal (δ_H 5.45) of **6b** had diminished by 40%, and after 24 h a complete conversion to catalpol (**5**) had taken place. Similarly, a sample of **6a** was transformed to **5a** within 24 h, with saponification evidently being much slower in this case. It can be concluded that the two longifoliosides (**6a** and **6b**) and consequently also urphoside B (**6c**) must be 6-*O*-esters of asystasioside E.

The difference in iridoid content from the two different sources of *V. longifolia* is not readily explained, but it could be ascribed either to a difference in age or to geographical variation. In the first case,[5] the plant was collected in July from old growth of an outdoor plant of unrecorded origin in The Botanical Garden of The University of Copenhagen. In the present case, seeds obtained from the Botanic Garden of the University of Hohenheim, Stuttgart, Germany, were sown in a greenhouse in April and harvested when the plants flowered in September. Unfortunately, both samples are of unknown origin, but since the natural distribution of the species is most of Europe,[15] the possibility of geographical variation cannot be dismissed.

Iridoid glucosides containing a chlorine atom have been found previously in the plant family Plantaginaceae. Besides **6c**, the chlorohydrin linarioside has been reported from *Linaria japonica* Miq.[16] together with the corresponding epoxide anthirrinoside, while

Table 2. Antioxidative Activities of Compounds **6a** and **6b** as Expressed by 50% Inhibition Concentrations (IC_{50}, μg/mL) *in Vitro*

compound	DPPH	SO	NO
6a	27	92	149
6b	19	199	285
BHA[a]	<10	377	238
quercetin	<10	68	96
AA[b]	<10	13	>1000

[a] Butylated hydroxyanisole. [b] Ascorbic acid.

asystasioside E (**6**) and baldaccioside, the 10-*O*-cinnamoyl ester of **6**, have been reported from *Wulfenia baldaccii* Degen.[17] As it is evident also in the present investigation, the iridoid chlorohydrins are consistently found together with the corresponding epoxides,[18] and the latter are therefore likely to be their precursors. It is, however, a possibility that the esters could be formed from **6** in parallel with the formation of the catalpol esters from **5**.

The two new compounds longifoliosides A (**6a**) and B (**6b**) were tested for radical-scavenging activity against nitric oxide (NO), superoxide (SO), and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals. Iridoid glucosides are known for their low radical-scavenging activity.[19] However, the tested compounds showed radical-scavenging activity comparable to that of the standard compounds butylated hydroxyanisole (BHA), quercetin, and ascorbic acid (Table 2). These results show that the dihydroxybenzoyl and caffeoyl substitution of the iridoid glycosides increase the radical-scavenging activities of the compounds.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer 241 polarimeter. ^{13}C NMR spectra were recorded at 25 °C on a Varian Mercury-300 instrument. 1D ^{1}H and 2D DQF-COSY, gHSQC, gHMBC, and NOESY NMR spectra were recorded similarly on a Varian Unity Inova 500 MHz spectrometer. Spectra were recorded in CD_3OD, and the chemical shifts are given as δ values with reference to the solvent peaks (δ_H 3.31 or δ_C 49.0), respectively. The mixing time used in the NOESY spectra was 600 ms. LC-HRESIMS was performed on an Agilent HP 1100 liquid chromatograph equipped with a BDS-C_{18} reversed-phase column running a water−acetonitrile (50 ppm TFA in water) gradient. The LC was coupled to a LCT of a TOF MS (Micromass, Manchester, UK) operated in the positive electrospray ion mode using 5-leucine-enkephalin as lock mass. Chromatography was performed on a Merck Lobar RP-18 column (size B) eluting with H_2O−MeOH mixtures (1:0 to 1:1). Compounds are listed in order of elution. The amount of mannitol (**1**) was estimated from the ^{13}C NMR spectrum of the crude sugar fraction. The known compounds isolated were identified by comparison with their published NMR data: mannitol (**1**),[20] iridoids **2**−**6**,[21] verproside (**5a**),[22] verminoside (**5b**).[23]

Plant Material. Seeds of *Veronica longifolia* were obtained from the Botanic Garden of the University of Hohenheim, Stuttgart, Germany. The plants were grown in a greenhouse at the experimental field of the Botanical Garden of The University of Copenhagen in Tåstrup, being sown in April and harvested when flowering in September 2008. The voucher (IOK-1/2008) was verified by Prof. Dirk Albach and deposited in the Herbarium of Johannes Gutenberg-Universität Mainz, Germany.

Extraction and Isolation. Fresh plant material (100 g) was blended in ethanol (500 mL) and the extract taken to dryness. The concentrated extract was partitioned in Et_2O−H_2O. The aqueous phase was taken to dryness (5.3 g) and an aliquot (1.0 g) separated by preparative reversed-phase chromatography to give a sugar fraction (490 mg) consisting mainly of mannitol (**1**); gardoside (**3**, 50 mg); 8-epiloganic acid (**2**, 40 mg); a 2:1 mixture of catalpol and asystasioside E (**5** and **6**, 50 mg); aucubin (**4**, 25 mg); verproside (**5a**, 80 mg); longifolioside A (**6a**, 40 mg); a mixture of unidentified compounds (60 mg); verminoside (**5b**, 60 mg); and longifolioside B (**6b**, 15 mg).

Longifolioside A (6a). Repeated chromatography gave the pure compound as a colorless, amorphous solid: $[\alpha]^{25}_D$ −102 (*c* 0.4; MeOH); ^{1}H and ^{13}C NMR, Table 1; LC-HR ESIMS *m/z* 557.1015 $[M + Na]^+$ (calcd for $C_{22}H_{27}{}^{35}ClNaO_{13}$, 557.1038).

Longifolioside B (6b). Chromatography on a Merck HiBar column (250-25) packed with LiChrosorb RP-18 gave the pure compound as a colorless, amorphous solid: $[\alpha]^{25}_D$ −84 (*c* 0.3; MeOH); ^{1}H and ^{13}C NMR, Table 1; LC-HR ESIMS *m/z* 583.1166 $[M + Na]^+$ (calcd for $C_{24}H_{29}{}^{35}ClNaO_{13}$, 583.1194).

Treatment with Sodium Hydroxide. Sodium (5 mg) was dissolved in D_2O (0.5 mL), and an excess was added to NMR tubes (5 mm) containing **6a** and **6b** dissolved in D_2O. The reactions were monitored by recording NMR spectra at 300 MHz.

DPPH Radical-Scavenging Effect. The DPPH radical-scavenging effect of compounds **6a** and **6b** was assessed spectroscopically by the decoloration of a methanol solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH). A MeOH solution (100 μL) of the compounds at various concentrations was added to a DPPH−MeOH (80 μg/mL) solution, and the absorbance of the remaining DPPH was measured at 520 nm after 30 min. The radical-scavenging activity was determined by comparing the absorbance with that of blank (100%) containing only DPPH and solvent.[24,25]

Superoxide Radical-Scavenging Effect by an Alkaline DMSO Method. The method of Kunchandy and Rao[26] was used for the detection of superoxide radical-scavenging activity with slight modification. To the reaction mixture containing 10 μL of NBT (1 mg/mL solution in DMSO) and 30 μL of the extract or standard compounds dissolved in DMSO was added 100 μL of alkaline DMSO (1 mL of DMSO containing, 5 mM NaOH in 0.1 mL of water) to give a final volume of 140 μL, and the absorbance was measured at 560 nm using a microplate reader.[26,27]

Nitric Oxide Scavenging Effect. In order to determine NO radical-scavenging activity of the compounds, 60 μL of 10 mM sodium nitroprusside, dissolved in phosphate-buffered saline (PBS), was added to 60 μL of serial diluted sample. After incubation under light at room temperature for 150 min, an equal volume of Griess reagent (1% sulfanilamide, 0.1% naphthylethylenediamine dihydrochloride, 2.5% H_3PO_4) was added into each well in order to measure the nitrite content. After 10 min, the chromophore was formed, and an absorbance at 577 nm was measured in a microplate reader.[24,28]

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Supporting Information Available: NMR spectra (^{1}H, ^{13}C) of longifoliosides A (**6a**) and B (**6b**) are available free of charge via the Internet at http://pubs.acs.org.

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