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Synthesis and Characterisation of α-Glycosyloxyamides Derived from Cyanogenic Glycosides

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ABSTRACT:

Introduction – After exposure to oxidative stress, the leaves of some cyanogenic plants contain primary α-glycosyloxyamides with structures corresponding to their original cyanogenic glycosides.

Objectives – The aim of this study was to prepare such amides from their nitrile precursors and to characterise the new substances in order to facilitate their early identification in forthcoming studies.

Methods – A simple but highly specific method is described for the *in-vitro* synthesis of the amides from their nitrile glycoside precursors using the Radziszewski reaction with hydrogen peroxide and a single-step purification of the reaction product. A TLC method is presented for the preliminary and fast identification of the α-glycosyloxyamides.

Results – Following this procedure, seven representative α-glycosyloxyamides, five of them new, were obtained and analytically characterised by means of ^{1}H, ^{13}C NMR and ATR-IR spectroscopy, highlighting the differences from their respective nitrile glycoside precursors.

Conclusion – Thus, α-glycosyloxyamides can be obtained in sufficient amounts and purity to serve as references for further studies on the catabolism of cyanogenic glycosides and the detoxification of cyanogenic foodplants using the new aspect of nitrile hydrolysis with (endogenous) hydrogen peroxide. Copyright © 2010 John Wiley & Sons, Ltd.

Keywords: cyanogenic glycosides; glycosyloxyamides; Radziszewski reaction; synthesis; purification; NMR; IR; TLC

Introduction

The capability of an organism to release hydrogen cyanide upon injury is referred to as cyanogenesis. Hydrogen cyanide release is predominantly attributed to α-glycosyloxynitriles (cyanogenic glycosides), which are found in numerous structural variations throughout the plant kingdom (Lechtenberg and Nahrstedt, 1999). After drying, some cyanogenic plants contain primary α-glycosyloxyamides with structures corresponding to their respective cyanogenic glycosides (Nahrstedt and Rockenbach, 1993; Takeda *et al*., 1997; Jaroszewski *et al*., 2002; Backheet *et al*., 2003; Hungeling *et al*., 2009). These amides, though first being assumed to be artefacts resulting from the isolation procedure, have proven to be generated from cyanogenic glycosides during the drying of fresh cyanogenic leaf material (Olafsdottir *et al*., 1991; Adersen *et al*., 1993; Sendker and Nahrstedt, 2009). It was shown for the leaves of *Prunus laurocerasus* L. and other plant species which contain the cyanogenic glucoside prunasin that the corresponding α-glycosyloxyamide, prunasinamide,[1] is generated via a facilitated reaction of the nitrile moiety of prunasin with hydrogen peroxide evolving during the drying process (Sendker and Nahrstedt, 2009). The reaction mechanism is known from organic chemistry as the Radziszewski reaction (Fig. 1). As excessive production of hydrogen peroxide in plant tissues can generally be expected during desiccation and other pathological processes (Smirnoff, 1993), but also during senescence including fruit ripening (Hadfield and Bennett, 1997; del Rio *et al*., 1998), primary α-glycosyloxyamides corresponding to cyanogenic glycosides are likely to be found in other cyanogenic plants including important cyanogenic food plants like sorghum, cassava, flax, black beans or almonds (Jones, 1998). Further, when investigating the cyanogenic potential of plant material, the formation of α-glycosyloxyamides will, even during careful drying, also be responsible for the loss of cyanogenic glycosides without liberation of hydrogen cyanide.

The *in-vitro* conversion of cyanogenic glycosides into their corresponding amides using concentrated ammonia to hydrolyse the nitrile moiety has been previously reported (Turczan *et al*., 1978; Takeda *et al*., 1997; Jaroszewski *et al*., 2004). However, with this method, the corresponding acids are formed as a major by-product besides the low amount of the primary amides (Turczan *et al*., 1978). Moreover, under these strong alkaline conditions, cyanogenic glycosides with asymmetric benzyl carbons undergo a partial stereoinversion of this carbon (Fig. 2; Nahrstedt, 1975) forming epimeric mixtures of the respective primary amides and carboxylic acids during hydrolysis of the nitrile moiety (Turczan *et al*., 1978). In contrast, the *in-vitro* reaction of cyanogenic glycosides with hydrogen peroxide (Radziszewski reaction) allowed a complete conversion of prunasin into its

[1] Although formally incorrect, we follow the established practice of naming the amides by appending the suffix 'amide' to the trivial name of the corresponding cyanogenic glycoside.

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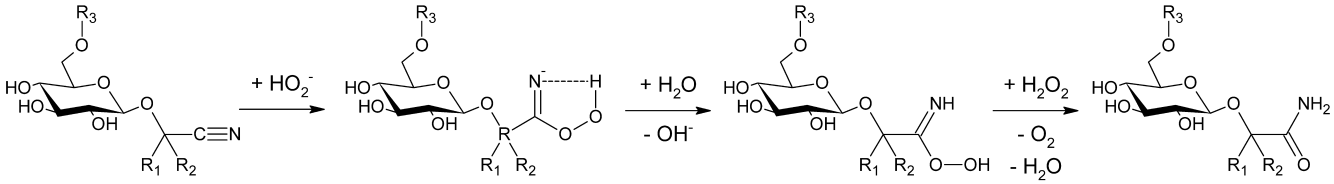

Figure 1. The Radziszewski reaction of cyanogenic glycosides with hydrogen peroxide yields the corresponding primary amides starting with the facilitated cycloaddition of a hydroperoxide anion (Schaefer, 1970).

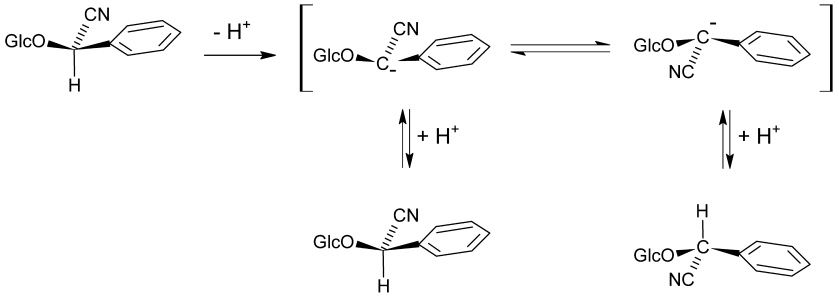

Figure 2. The benzylic α-carbon of aromatic cyanogenic glycosides undergoes a stereoinversion when treated under alkaline conditions (Nahrstedt, 1975) as used for the nitrile hydrolysis described by Turczan *et al.* (1978), Takeda *et al.* (1997) and Jaroszewski *et al.* (2004).

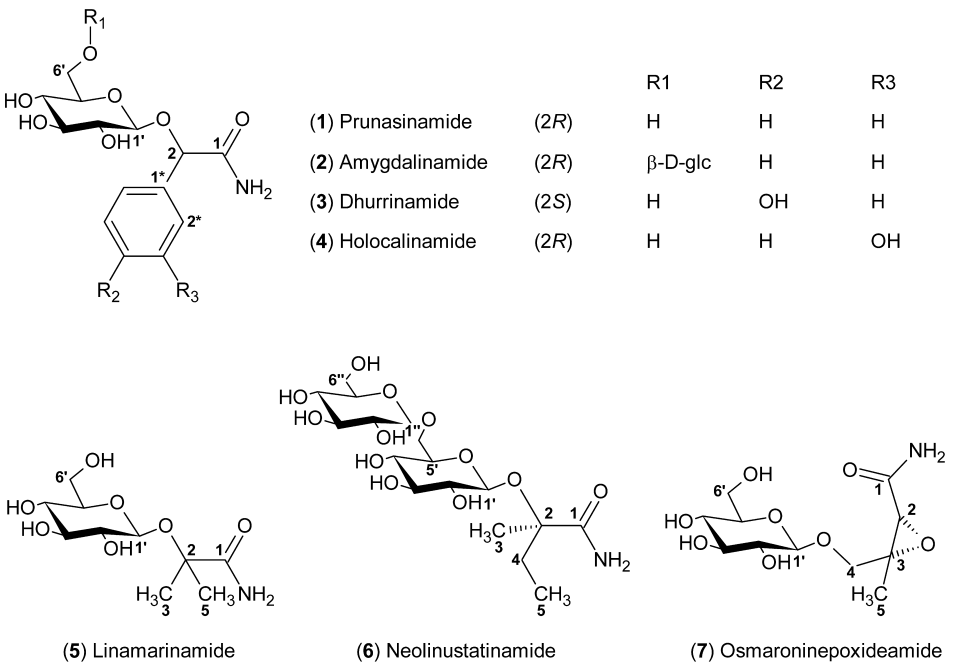

Figure 3. Primary amide glycosides obtained from Radziszewski reaction of cyanogenic glycosides.

corresponding α-glucosyloxyamide prunasinamide with conservation of its stereochemistry (Sendker and Nahrstedt, 2009).

As described by Jaroszewski *et al.* (2002), α-glycosyloxyamides may give positive results in the cyanide-specific sandwich-picrate TLC detection when using *Helix pomatia* enzyme (Brimer *et al.*, 1983). Also, other simple detection methods which provide evidence of the sugar moiety will not distinguish between cyanogenic glycosides and α-glycosyloxyamides; thus the unambiguous differentiation of a putative cyanogenic glycoside from a primary amide may be precarious and retarded until a suitable ^{13}C-NMR experiment reveals the lack of a nitrile carbon resonance (Jaroszewski *et al.*, 2002). The aim of this study therefore was to prepare α-glycosyloxyamides (Fig. 3) from cyanogenic glycosides using the cheap and simple Radziszewski reaction and to characterise the substances in order to facilitate their early identification in forthcoming studies. Dhurrinamide, holocalinamide, linamarinamide, neolinustatinamide and osmaroninepoxideamide were hitherto unknown and are described here for the first time.

Experimental

Materials

All organic solvents, buffers, reagents, disposables and Extrelut™ were purchased from VWR International GmbH, Darmstadt; 4-hydroxyphenylacetonitrile was from Sigma. Prunasin was isolated as described previously (Sendker and Nahrstedt, 2009). Linamarin, linustatin, dhurrin, holocalin, amygdalin and osmaroninexpoxide were obtained from the collection of A.N.

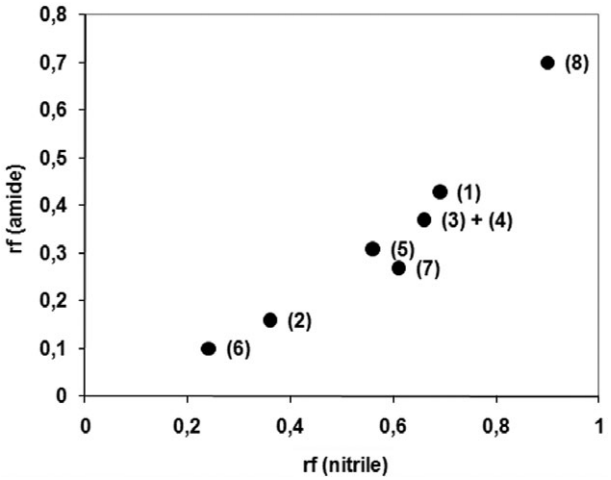

Figure 4. TLC analysis shows strict correlation of the R_f-values of primary amides and their corresponding nitriles. $R_f(\text{amide}) = 1.08 \cdot R_f(\text{nitrile})^2 - 0.34 \cdot R_f(\text{nitrile}) + 0.13$ ($R^2 = 0.972$). 4-Hydroxyphenylacetonitrile and its corresponding amide (**8**) were included to confirm the predicitive power of this model at higher R_f-values (numbers here refer to the corresponding pair nitrile–amide).

Preparation of α-glycosyloxyamides

Aliquots of 30 mg of each cyanogenic glycoside were dissolved in 1 mL of 10% hydrogen peroxide which had been adjusted to pH 6.8 using a 250 mM solution of sodium monohydrogenphosphate. After keeping the reaction batches at 40°C for 1 day, each solution was subjected to a column containing 1 g of Extrelut™, which had been packed in a 2 mL Luer™ syringe. The Extrelut™ was eluted with 10–100 mL of ethylacetate saturated with water (monoglucosides) or a mixture of 2–3 parts ethylacetate and 1 part 1-butanol saturated with water (diglycosides, e.g. gentiobiosides) to remove residual cyanogenic glycosides and hydrogen peroxide; the column was subsequently eluted with 10–100 mL of a mixture of 3 parts ethylacetate and 1 part 1-butanol saturated with water (monoglucosides) or a mixture of 1–2 parts ethylacetate and 1 part 1-butanol saturated with water (diglycosides, e.g. gentiobiosides) to elute the α-glycosyloxyamides in high purity (yields between 30% and 80%). Both reaction and separation were monitored using TLC.

Epimerisation

Aliquots of 10 mg of prunasin and prunasinamide were separately dissolved in 10.0 mL 50 mM phosphate buffer pH 9.0 and kept at 60°C for 30 min. The ratio of epimers was estimated using HPLC: 10 µL of each solution was chromatographed over a 4 × 150 mm ProSep C_{18} 5 µm column with a mixture of trifluoroacetic acid:methanol:water (1:150:849) at 1.0 mL/min; detection was at 202 nm. The HPLC system consisted of a Waters 600 + Waters 2690 seperations module, a Waters 717plus autosampler and a Waters 996 PDA.

Spectroscopic analysis

^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz) spectra were recorded on a Varian Mercury 400 plus FT-NMR spectrometer with reference to TMS. All spectra were recorded in perdeuterated dimethyl sulfoxide at 23°C. IR spectra were recorded with about 20 µg of pure solid substance on a Nicolet 4700 FTIR-FMIR ATR spectrometer using a diamond as ATR crystal. Optical rotation was measured with an Autopol™ Automatic Polarimeter.

Table 1. ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz) chemical shifts of amides **1** and **2**

Position	Prunasinamide (**1**)		Amygdalinamide (**2**)	
	^{1}H-NMR[a]	^{13}C-NMR[a]	^{1}H-NMR[a]	^{13}C-NMR[a]
$CONH_2$	7.57 (1H, brs)	172.26	7.57 (1H, brs)	172.16
	7.32 (1H, m)[b]		7.32 (1H, m)[d]	
2	5.14 (1H, s)	77.60[f]	5.06 (1H, s)	78.01
1*	—	136.95	—	137.03
2*	7.42 (1H, dd, 1.7 Hz, 8.0 Hz)	128.10	7.48 (1H, dd, 1.7 Hz, 7.8 Hz)	128.18
3*	7.28–7.40 (3H, m)[b]	128.49[c]	7.29–7.40 (3H, m)[d]	128.36[e]
4*		128.49[c]		128.36[e]
5*		128.49[c]		128.36[e]
6*	7.42 (1H, dd, 1.7 Hz, 8.0 Hz)	128.10	7.48 (1H, dd, 1.7 Hz, 7.8 Hz)	128.18
1′	3.83 (1H, d, 7.8 Hz)	98.76	3.89 (1H, d, 7.8 Hz)	99.14
6′	3.41 (1H, m)	61.34	3.56 (1H, dd, 6.5 Hz, 11.5 Hz)	68.42
	3.64 (1H, dd, 6.5 Hz, 11.8 Hz)		3.96 (1H, dd, 1.4 Hz, 11.0 Hz)	
1″	—	—	4.28 (1H, d, 7.8 Hz)	103.63
6″	—	—	3.44 (1H, dd, 5.1 Hz, 12.5 Hz)	61.25
			3.67 (1H, brd, 11.8 Hz)	
2′–5′	2.81–3.14 (4H, m)	70.41	2.96–3.21 (8H, m)	70.29, 70.32
2″–5″		73.74		73.55, 73.86
		76.10		76.08, 76.33
		77.45[f]		76.96, 77.10

[a] Spectra recorded in DMSO-d_6, values shown in ppm with reference to TMS.
[b–e] Signals bearing the same symbol overlap.
[f] Interchangeable.

Mass spectrometric analysis

High-resolution ESI-MS spectra were recorded on a Bruker micrOTOF II in positive mode using lithium formiate for mass calibration.

TLC analysis

2 µL aliquots of sample solutions were applied as a spot to a 10 × 10 cm precoated Silica gel 60 F_{254} aluminium plate (Merck) and developed over a distance of 7 cm in a non-equilibrated 10 × 10 cm double trough chamber. The mobile phase was a mixture of ethylacetate:methanol:acetone:dichloromethane:water (20:5:15:6:4). Glycosidic compounds were detected by spraying with a solution of 0.5% thymol in 5% ethanolic sulphuric acid and subsequent heating to 120°C for 15 min. LOD for pure α-glycosyloxyamides was about 0.2 nmol.

Prunasinamide (1). $C_{14}H_{19}NO_7$; amorphous powder; HR-ESI-MS (positive ion mode) *m/z* 314.1232 $[M + H]^+$ (calculated for $C_{14}H_{20}NO_7$ *m/z* 314.1234); $\alpha_D^{20} = -141.0°$ (CH_3OH, *c* 0.173); IR ν_{max} cm^{-1}: 3332 (–OH, *st*), 2926 (–CH), 2890 (–CH, *st*), 1671 (C=O, *st*), 1596 (N–H), 1502 (C=C), 1459 (C=C), 1420, 1023 (*st*).

Amygdalinamide (2). $C_{20}H_{29}NO_{12}$; amorphous powder; HR-ESI-MS (positive ion mode) *m/z* 476.1762 $[M + H]^+$ (calculated for $C_{20}H_{30}NO_{12}$ *m/z*

Table 2. ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz) chemical shifts of amides **3** and **4**

Position	Dhurrinamide (**3**)		Holocalinamide (**4**)	
	^{1}H-NMR[a]	^{13}C-NMR[a]	^{1}H-NMR[a]	^{13}C-NMR[a]
$CONH_2$	7.48 (1H, brs)	173.29	7.29 (1H, brs)	172.23
	7.54 (1H, brs)		7.54 (1H, brs)	
2	4.95 (1H, s)	79.38	5.03 (1H, s)	77.95
1*	—	128.60	—	138.17
2*	7.18 (2H, d, 8.6 Hz)	128.73	6.81 (1H, s)	115.01
3*	6.70 (2H, d, 8.7 Hz)	114.91	—	157.48
4*	—	157.26	6.71 (1H, dd, 1.4 Hz, 8.2 Hz)	115.42
5*	6.70 (2H, d, 8.7 Hz)	114.91	7.12 (1H, t, 7.8 Hz)	129.32
6*	7.18 (2H, d, 8.6 Hz)	128.73	6.84 (1H, d, 7.9 Hz)	118.86
1′	4.25 (1H, d, 7.4 Hz)	101.93	3.86 (1H, d, 7.5 Hz)	98.55
6′	3.43 (1H, m)	61.23	3.42 (1H, m)	61.31
	3.66 (1H, dd, 5.2 Hz, 11.5 Hz)		3.63 (1H, d, 11.3 Hz)	
2′–5′	2.99–3.21 (4H, m)	70.20	2.99–3.21 (4H, m)	70.42
		73.78		73.73
		76.21		76.06
		77.39		77.39

[a] Spectra recorded in DMSO-d_6, values shown in ppm with reference to TMS.

Table 3. ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz) chemical shifts of amides **5–7**

Position	Linamarinamide (**5**)		Neolinustatinamide (**6**)		Osmaroninepoxideamide (**7**)	
	^{1}H-NMR[a]	^{13}C-NMR[a]	^{1}H-NMR[a]	^{13}C-NMR[a]	^{1}H-NMR[a]	^{13}C-NMR[a]
$CONH_2$	7.14 (1H, brs)	177.21	7.07 (1H, brs)	176.49	7.40 (1H, brs)	168.71
	7.72 (1H, brs)		7.52 (1H, brs)		7.45 (1H, brs)	
2	—	79.59	—	82.41	3.28 (1H, s)	59.33
3	1.29 (3H, s)	23.21	1.28 (3H, s)	24.20	—	60.91
4	—	—	1.64 (2H, m)	29.22	3.54 (1H, d, 11.7 Hz)	69.03
					3.81 (1H, d, 11.7 Hz)	
5	1.35 (3H, s)	27.83	0.81 (3H, t, 6.9 Hz)	8.20	1.38 (3H, s)	19.71
1′	4.31 (1H, d, 7.9 Hz)	98.42	4.32 (1H, d, 7.6 Hz)	97.84	4.13 (1H, d, 7.8 Hz)	103.30
6′	3.39 (1H, m)	61.20	3.48 (1H, m)	68.99	3.41 (1H, dd, 5.4 Hz, 11.8 Hz)	61.28
	3.61 (1H, dd, 3.2 Hz, 12.0 Hz)		3.94 (1H, d, 10.8 Hz)		3.65 (1H, d, 11.4 Hz)	
1″	—	—	4.18 (1H, d, 7.6 Hz)	103.40	—	—
6″	—	—	3.40 (1H, m)	61.23	—	—
			3.63 (1H, d, 11.7 Hz)			
2′–5′	2.97–3.20 (4H, m)	70.31	2.87–3.56 (8H, m)	70.25, 70.29	2.92-3.14 (4H, m)	70.22
2″–5″		73.80		73.76, 73.81		73.64
		76.95		75.78, 76.67		76.93
		76.98		76.91, 77.11		77.04

[a] Spectra recorded in DMSO-d_6, values shown in ppm with reference to TMS.

476.1763); $\alpha_D^{20} = -83.4°$ (CH_3OH, *c* 0.263); IR ν_{max} cm^{-1}: 3312 (–OH, *st*), 2926 (–CH), 2885 (–CH), 1679 (C=O, *st*), 1593 (N–H), 1502 (C=C), 1458 (C=C), 1413, 1021 (*st*).

Dhurrinamide (3). $C_{14}H_{19}NO_8$; amorphous powder; HR-ESI-MS (positive ion mode) *m/z* 330.1180 $[M + H]^+$ (calculated for $C_{14}H_{20}NO_8$ *m/z* 330.1183); $\alpha_D^{20} = +29.0°$ (CH_3OH, *c* 0.102); IR ν_{max} cm^{-1}: 3291 (–OH, *st*), 2924 (–CH), 2888 (–CH), 1671 (C=O, *st*), 1617, 1600 (N–H), 1518 (C=C), 1451 (C=C), 1368, 1262 (phenolic), 1235 (phenolic), 1021 (*st*).

Holocalinamide (4). $C_{14}H_{19}NO_8$; amorphous powder; HR-ESI-MS (positive ion mode) *m/z* 330.1177 $[M + H]^+$ (calculated for $C_{14}H_{20}NO_8$ *m/z* 330.1183); $\alpha_D^{20} = -96.5°$ (CH_3OH, *c* 0.223); IR ν_{max} cm^{-1}: 3294 (–OH, *st*), 2927 (–CH), 2879 (–CH), 1673 (C=O, *st*), 1594 (N–H), 1489 (C=C), 1461 (C=C), 1279 (phenolic), 1265 (phenolic), 1022 (*st*).

Linamarinamide (5). $C_{10}H_{19}NO_7$; amorphous powder; HR-ESI-MS (positive ion mode) *m/z* 288.1060 $[M + H]^+$ (calculated for $C_{10}H_{19}NO_7Na$ *m/z* 288.1054); $\alpha_D^{20} = -15.5°$ (CH_3OH, *c* 0.076); IR ν_{max} cm^{-1}: 3341 (–OH, *st*), 2993 (–CH), 2924 (–CH), 2887 (–CH), 1665 (C=O, *st*), 1602 (N–H), 1403, 1366, 1038 (*st*).

Neolinustatinamide (6). $C_{17}H_{31}NO_{12}$; amorphous powder; HR-ESI-MS (positive ion mode) *m/z* 442.1912 $[M + H^+]$ (calculated for $C_{17}H_{32}NO_{12}$ *m/z* 442.1919); $\alpha_D^{20} = -18.3°$ (CH_3OH, *c* 0.154).

Osmaroninepoxideamide (7). $C_{11}H_{19}NO_8$; amorphous powder; HR-ESI-MS (positive ion mode) *m/z* 294.1183 $[M + H]^+$ (calculated for $C_{11}H_{20}NO_8$ *m/z* 294.1183); $\alpha_D^{20} = -17.7°$ (CH_3OH, *c* 0.143); IR ν_{max} cm^{-1}: 3323 (–OH, *st*), 2940 (–CH), 2897 (–CH), 1674 (C=O, *st*), 1607 (N–H), 1436, 1377, 1032 (*st*).

Results and Discussion

The preparation of the glycosyloxyamides prunasinamide (**1**), amygdalinamide (**2**), dhurrinamide (**3**), holocalinamide (**4**), linamarinamide (**5**), neolinustatinamide (**6**) and osmaroninepoxideamide (**7**) was achieved by simply dissolving the respective cyanogenic glycoside in diluted hydrogen peroxide at pH 6.8 and keeping the solution at a temperature of 40°C. Using prunasin, it was shown that an almost complete conversion to the corresponding amide was achieved after 1 week reaction time; notably, >95% of the employed prunasin could be analytically recovered as **1**, while no by-products were detected (Sendker and Nahrstedt, 2009). However, in order to shorten experimental time during the present experiments we decided to isolate the amides from the reaction mixture already after 1 day with 30–80% of the theoretical yield. The amide glycosides could easily be obtained in high purity (no major impurities were

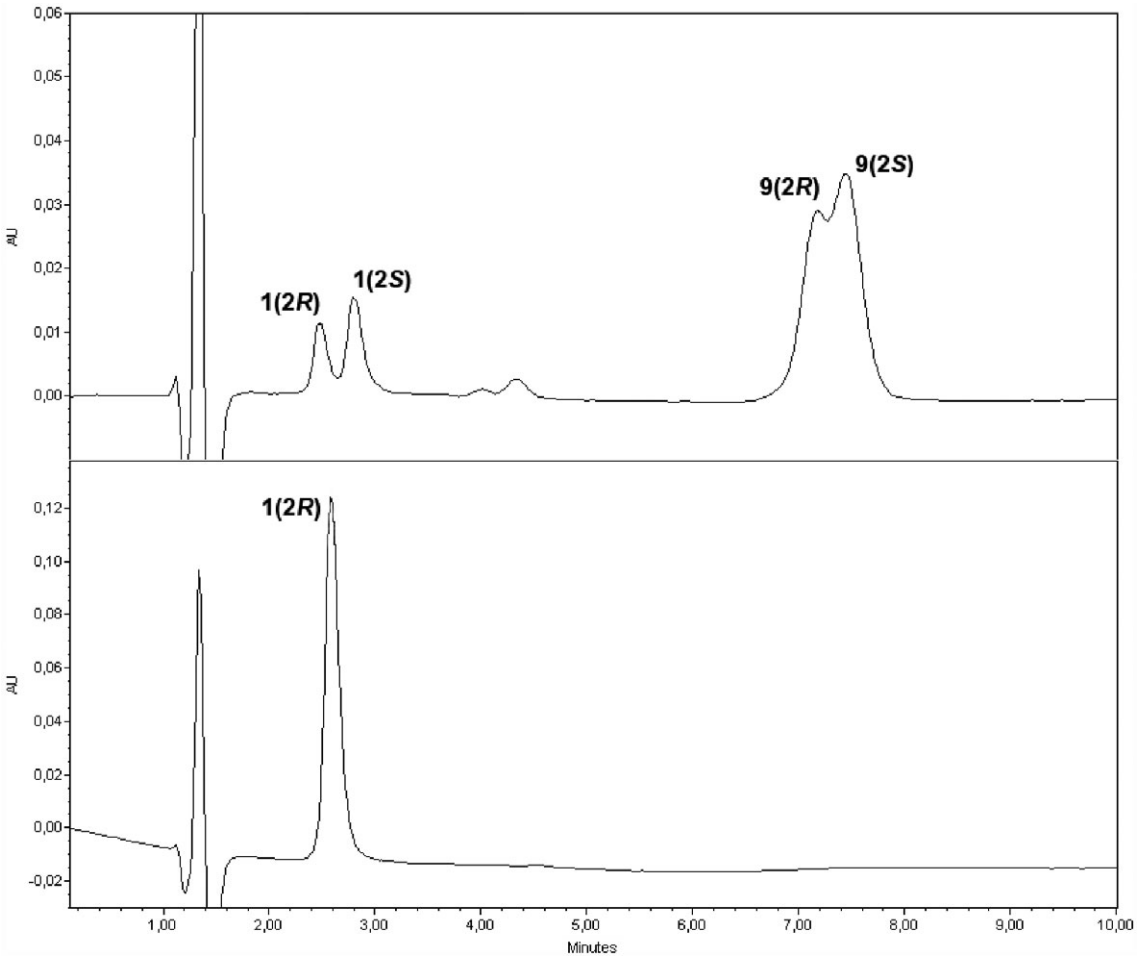

Figure 5. The reduced acidity of the benzylic hydrogen of **1** is demonstrated by the failure of **1** to epimerize at pH 9.0 (lower panel). However, when treated at pH 9.0 the corresponding nitrile prunasin [**9**(2*R*)] epimerizes to a ca. 40:60% mixture of prunasin and sambunigrin [**9**(2*S*)] (upper panel); in this mixture we also observed a partial hydrolysis of **9**(2*R*)/**9**(2*S*) to a mixture of c. 40% **1**(2*R*) and 60% of its epimeric sambunigrinamide [**1**(2*S*)], thus demonstrating that epimerisation occurs much faster than hydrolysis of the nitrile group under alkaline conditions. Nahrstedt (1975) measured a similar epimeric ratio for alkali-treated aromatic cyanogenic glycosides using GC and trimethylsilyation. However, he could not observe the products of nitrile hydrolysis as the corresponding amides were obviously not volatile enough due to insufficient silylation for GC analysis.

detected by TLC and NMR spectroscopy) by liquid–liquid chromatography of the reaction mixture using Extrelut™.

For chromatographic identification of the amide glycosides along with their corresponding cyanogenic glycosides, thin-layer chromatography was the method of choice because all compounds investigated here were consistently detectable with high sensitivity due to their sugar moieties using thymol–sulphuric acid reagent. Separation was achieved by chromatography over silica gel coated plates with a mobile phase widely used for cyanogenic glycosides (Brimer *et al*., 1983). When using this system, we observed a strict correlation (see Fig. 4) between the R_f-values of the cyanogenic glycosides (always at higher R_f) and their corresponding amides (always at lower R_f, in the case of the amide monoglucosides almost identical to the nitrile diglucosides). This behaviour may support the preliminary identification of hitherto unknown amides corresponding to known cyanogenic glycosides or other nitriles in plant extracts.

The ^{1}H- and ^{13}C-NMR data of compounds **1**–**7** were recorded in DMSO-d_6 and were assigned to the respective nuclei as shown in Tables 1–3. The NMR properties of the primary amides investigated here strongly resemble those of their corresponding nitriles. Spectral differentiation of an amide glycoside from its corresponding nitrile is hampered by the fact that the molecular constitution is homologue and the amide protons will not appear in ^{1}H-NMR spectra recorded in protic NMR-solvents like methanol-d_4. The clearest differences observed here pertain to nuclei closely related to the carbonic acid derivative site namely: (i) to the amide carbon resonance which, in comparison to the respective α-glycosyloxynitriles, is shifted downfield by 51.4–58.9 ppm; (ii) to the α-hydrogen resonance (if present) which is shifted upfield by 0.49 ppm for **7** and by 0.89 to 1.15 ppm for the aromatic compounds **1**–**4**, representing a reduced acidity of their benzylic proton (Fig. 5); (iii) to the resonance of the α-carbon which is shifted downfield by 6 to 13.5 ppm; and (iv) to the resonance of the adjacent sugar's anomeric proton which is shifted upfield by 0.21–0.63 ppm (reference data for nitriles are from Möhrle and Fangerau, 1980; Seigler and Brinker, 1993; Lechtenberg *et al*., 1994; Nakajima and Ubukata, 1998; Backheet *et al*., 2003). An HMBC spectrum of **4** is shown in Fig. 6.

Although these differences overcome usual solvent-caused resonance variation and were also observed for the pairs of acalyphin/acalyphinamide (Hungeling *et al*., 2009), lucumin/lucuminamide (Takeda *et al*., 1997), gynocardin/gynocardinamide (Jaroszewski *et al*., 2004), volkenin/volkeninamide (Jaroszewski *et al*., 1987) and tetraphyllin-B/tetraphyllin-B-amide (Jaroszewski *at al*., 1987), they do not allow an unambiguous proof of the primary amide moiety. This is especially due to the possible presence of the corresponding carboxylic acids which have been repeatedly found in cyanogenic plants (Kitajima and Tanaka, 1993; Takeda *et al*., 1997; D'Abrosca *et al*., 2001; Fukuda *et al*., 2003) and are hardly distinguishable from the corresponding amides by the NMR characteristics mentioned above. Thus, direct spectroscopy of the amide protons in aprotic solvents is required, e.g. in acetone-d_6, acetonitrile-d_3 or dimethylsulfoxide-d_6, which was also used in this study.

In each case, amide protons appeared between 6.7 and 7.8 ppm as two distinct broad singlets. The protons are not

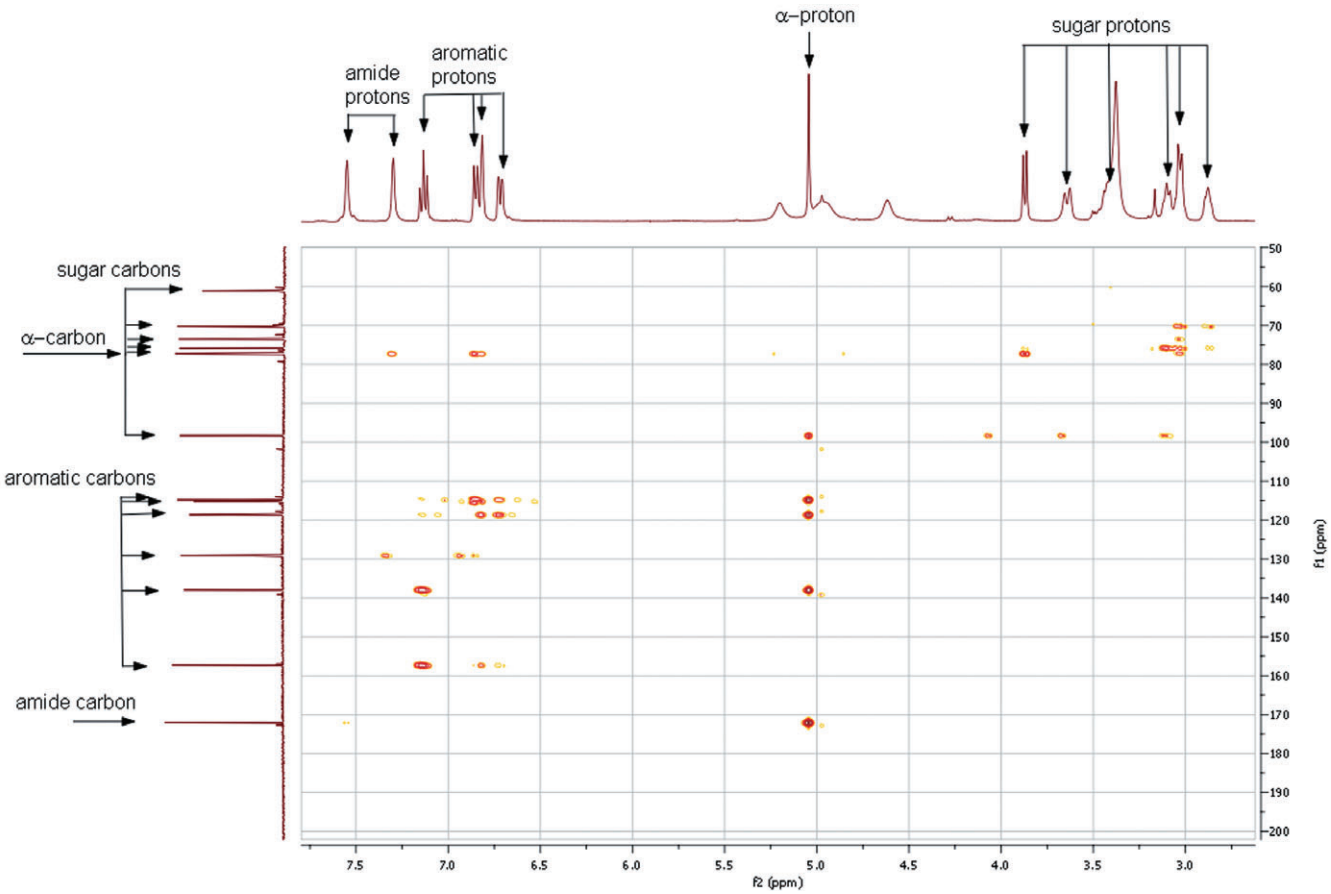

Figure 6. HMBC spectrum of **4** in DMSO-d6 (400/100 MHz).

magnetically equivalent because the rotation around the C–N amide bound is hindered due to the partial double bond character of the C–N amide bond. Thus, the chemical shifts of the amide proton resonances depend on temperature in that the distance between both signals decreases with increasing temperature until both protons become magnetically equivalent and their resonance signals coalesce (Friebolin, 1999). Notably, only the upfield amide protons showed a ^3J-coupling to α-carbon in HMBC experiments (Fig. 6).

Infrared spectroscopy appeared as a suitable alternative to NMR spectroscopy for the identification of the amide moiety. While the nitrile absorption is usually quenched in cyanogenic glycosides (Nahrstedt, 1981), strong C=O valence oscillations allow the clear distinction of the corresponding amides and acids from the nitriles and from each other (Jaroszewski *et al*., 1987; Takeda *et al*., 1997). With the attenuated total reflection infrared spectroscopy technique used here, only about 20 µg of pure substance was needed in order to allow a clear identification of the amide moiety by their characteristic C=O valence (1650–1680 cm^{-1}) and N–H deformation (1590–1620 cm^{-1}) oscillation signals.

Conclusion

The occurrence of α-glycosyloxyamides alone or together with their corresponding cyanogenic glycosides is most likely in air dried cyanogenic plant material (Sendker and Nahrstedt, 2009). The TLC correlation presented here hints as to the presence of α-glycosyloxyamides in an early stage of future investigations. Hereafter the NMR and IR data sets presented will facilitate the identification of this class of compounds even for hitherto unknown α-glycosyloxyamides. Finally, our protocol to produce and purify α-glycosyloxyamides from corresponding cyanogenic glycosides gives easy access to pure amides for future research.

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