Available online at www.sciencedirect.com
ScienceDirect
Carbohydrate Research 341 (2006) 2799–2802
Carbohydrate RESEARCH

Three 1-thio-β-D-glucopyranosides from the seeds of *Afrostyrax lepidophyllus* Mildbr.

Annie Ngono Ngane,[a,b] Marie Lavault,[a,*] Denis Séraphin,[a] Anne Landreau[a] and Pascal Richomme[a]

[a]*SONAS, UFR des Sciences Pharmaceutiques, 16, Bd Daviers, F-49100 Angers, France*
[b]*Département de Chimie, Faculté des Sciences, BP 24157, Douala, Cameroon*

Received 30 June 2006; received in revised form 12 September 2006; accepted 26 September 2006
Available online 4 October 2006

Abstract—Three new 1-thioglycosides namely methylthiomethyl 1-thio-β-D-glucopyranoside (Afrostyraxthioside A), methylsulfonylmethyl 1-thio-β-D-glucopyranoside (Afrostyraxthioside B) and methylsulfonylmethylthiomethyl 1-thio-β-D-glucopyranoside (Afrostyraxthioside C) were isolated from the seeds of *Afrostyrax lepidophyllus* Mildbr. Their structures were mainly elucidated by using one- and two-dimensional NMR and mass spectroscopies and also by an efficient one-step synthesis. Moreover, Afrostyraxthiosides A, B and C constitute a new subclass of 1-thioglycosides isolated from natural sources.

Keywords: *Afrostyrax lepidophyllus*; 1-Thioglycosides; Afrostyraxthiosides A, B, C; Thioglycosides synthesis

1. Introduction

The Huaceae family consists of the two genus *Afrostyrax* and *Hua* and is only represented by three species of trees distributed in equatorial Africa.[1] Based on their garlic-like or onion-like odour, they are called 'wild onion trees' and their seeds and barks are used as spices in traditional cooking.[2] In Congo, the wood and the barks of *Afrostyrax lepidophyllus* and *Hua gabonii* are also used as antiseptics in traditional medicine for the treatment of gastroenteric diseases.[3] Such anti-infective properties were recently confirmed by an evaluation of the antifungal activity of extracts of the seeds of *A. lepidophyllus* against several human pathogenic fungi.[4] In order to correlate this biological activity with the chemical content of the extracts, we wish to report here the first phytochemical investigation of the seeds of *A. lepidophyllus*. This study led to the isolation and the structural elucidation of three new 1-thio-β-D-glucopyranosides named Afrostyraxthiosides A, B, C, by means of spectroscopic analysis and synthesis.

2. Results and discussion

The air-dried and powdered seeds of *A. lepidophyllus* were successively extracted with *n*-hexane, CH_2Cl_2 and 90% MeOH. The hydroalcoholic soluble material was partitioned between 1-butanol and water. The organic soluble material was subjected to repeated column chromatography (Sephadex gel and silica gel) to yield three new thioglycosides **1**–**3** (Chart 1).

Compound **1** was isolated as an amorphous white powder. The molecular formula of **1** ($C_8H_{16}O_5S_2$) was established by HRDCI$^+$MS analysis of its pseudomolecular ion $[M+H]^+$ at *m*/*z* 257.0523 (calcd 257.0517). The DCI mass spectrum also showed fragment ions at *m*/*z* 209 and *m*/*z* 165 corresponding to the loss of a methylthio (CH_3–S–) and a methylthiomethylthio (CH_3–S–CH_2–S–) group, respectively. The presence of a sulfur moiety was confirmed by resonances ascribable to a heterosubstituted methylene and methyl occurring, respectively, at δ_H 3.93 and δ_H 3.77 (AB system, 2H, *J*

* Corresponding author. Tel.: +332 41 22 66 00; fax: +332 41 48 67 33; e-mail: marie.lavault@univ-angers.fr

0008-6215/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carres.2006.09.021

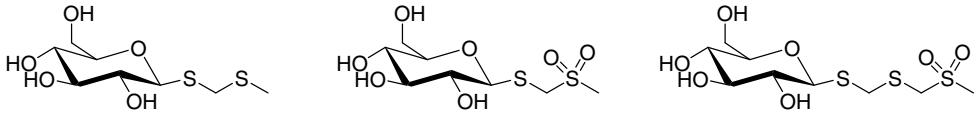
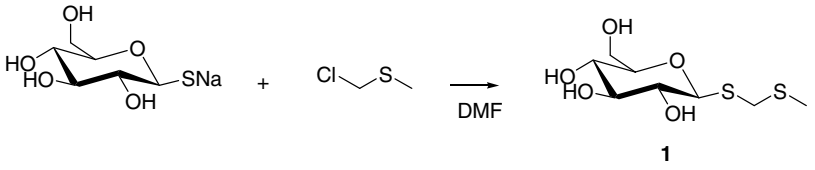


Chart 1. Afrostyraxthiosides **1**–**3** isolated from *Afrostyrax lepidophyllus* Mildbr.

14.5 Hz) and δ_H 2.11 (s, 3 H) on the ^{1}H NMR spectrum and at δ_C 36.0 (C2′) and δ_C 14.9 (C4′) on the ^{13}C NMR spectrum. The ^{1}H NMR spectrum also exhibited the presence of a sugar moiety with an anomeric proton at δ_H 4.52 (d, *J* 9.7 Hz). From this anomeric proton, every protons and carbons of the glycosyl group were assigned by a detailed analysis of ^{1}H–^{1}H COSY, HMQC and HMBC spectra. An examination of the ^{13}C NMR spectrum suggested the presence of a glucopyranose moiety except for the anomeric methine (C-1), which was shifted downfield at δ_C 84.9 when compared to the usual chemical shifts observed for *O*-glucopyranosyl derivatives. An explanation for this downfield effect was deduced from the HMBC correlation exhibited between the anomeric proton H-1 (δ_H 4.52) and the methylenic carbon (δ_C 36.0) of the methylthiomethylthio group, which thus firmly established the thioglycosidic nature of **1**. In addition, according to the literature data concerning the 1-thioglycoside derivatives, the coupling constant of the anomeric proton (d, *J* 9.7 Hz) suggested a β-configuration of the glucosyl moiety.[5] This structural hypothesis was confirmed by a straightforward synthesis of **1** (Scheme 1).[6] The substitution of chloromethyl methyl sulfide by the sodium salt of 1-thio-β-D-glucopyranose led in only one step to a compound whose ^{1}H and ^{13}C NMR spectra were superimposable to those of the natural product. In addition, acetylation of the natural product **1** gave the corresponding tetra-*O*-acetyl compound **1a**. An examination of its ^{1}H and ^{13}C NMR spectral data revealed that the signals corresponding to the tetra-*O*-acetyl-1-thio-β-D-glucopyranosyl moiety were in accordance with the literature data of tetra-*O*-acetyl-1-thio-β-D-sugars.[7] Consequently, the structure of **1** was established as methylthiomethyl 1-thio-β-D-glucopyranoside and named Afrostyraxthioside A.

Compound **2** was isolated as an amorphous white powder. The HR-DCI$^+$ mass spectrum of compound **2** showed the $[M+H]^+$ ion at *m*/*z* 289.0443 (calcd 289.0416) compatible with the molecular formula $C_8H_{16}O_7S_2$. The resonances for a methylene and a methyl groups were evident from the ^{1}H NMR spectrum at, respectively, δ_H 4.57 and 4.33 (AB system, 2H, *J* 15.5 Hz) and δ_H 3.21 (s, 3H). Compared to **1**, the downfield chemical shift of these signals could be related to the higher level of oxidation of **2** and thus explained by the presence of a methylsulfonylmethylthio (CH_3–SO_2–CH_2–S–) appendage.[8] As in the case of **1**, the examination of the ^{1}H NMR and ^{13}C NMR spectra and analysis of the 2D NMR data (^{1}H–^{1}H COSY, HMQC and HMBC) revealed the presence of a β-glucosyl moiety with an anomeric proton associated with a downfield signal at δ_C 85.3. Again, the long-range correlation observed between the H-1 (δ_H 4.87) and the methylene group (C-2′ δ_C 51.9) demonstrated the substitution of the anomeric carbon by a methylsulfonylmethylthio group. Therefore, compound **2** was identified as methylsulfonylmethyl 1-thio-β-D-glucopyranoside for which the name Afrostyraxthioside B is proposed.

Compound **3** was isolated as an amorphous white powder. The molecular formula was determined as $C_9H_{19}O_7S_3$ by HRDCI$^+$MS analysis of its pseudomolecular ion $[M+H]^+$ at *m*/*z* 335.0292 (calcd 335.0293), revealing additional sulfur, carbon and hydrogen (2H) atoms compared to **2**. The spectral data of **3** appeared as similar to that of **2** with typical resonances in the ^{1}H spectrum (δ_H 4.54) and in the ^{13}C NMR spectrum (δ_C 85.2) of a monosubstituted 1-thio-β-D-glucopyranose. The ^{1}H NMR spectrum of **3** revealed the presence of a methylsulfonyl group at δ_H 3.06 (s, 3H), and also confirmed the additional heterosubstituted methylene by the presence of two AB systems at δ_H 4.25; 4.38 (2H, *J* 15.0 Hz) and at δ_H 4.19, 4.24 (2H, *J* 13.7 Hz). Therefore, the structure of **3** only differs from that of **2** by the presence of an extra SCH_2 group in the lateral side chain as confirmed by 2D NMR experiments (COSY, HMQC, HMBC). Consequently, the structure of **3** was established as methylsulfonylmethylthiomethyl 1-thio-β-D-glucopyranoside and named as Afrostyraxthioside C.



Scheme 1. Synthesis of Afrostyraxthioside **1**.

Beside the glucosinolates essentially isolated in the Brassicaceae family,[9] compounds **1**–**3** isolated from the seeds of *A. lepidophyllus* constitute, to the best of our knowledge, a new group of 1-thioglycosides isolated from natural sources. These compounds seem to result from the condensation of glucose with a thiol such as methylthiomethanethiol, which was already isolated from the natural source.[10] Moreover, the lack of any antifungal activity of thioglycosides **1**–**3** against *Candida albicans* and *Candida glabrata* suggests that the anti-infective properties of the extracts of *A. lepidophyllus* should be explained by other constituents that remain to be identified.[4]

3. Experimental

3.1. General experimental procedures

Optical rotations were measured on a Schmidt Haensch Polartronic-D polarimeter. ^{1}H and ^{13}C NMR spectra were recorded on a JEOL GSX 270 MHz instrument. 2-D NMR experiments (DQF-COSY, HMQC, HMBC) were recorded on a Bruker Avance DRX 500 MHz spectrometer. DCIMS spectra were recorded on a JMS-700 (JEOL LTD, Akishama, Tokyo, Japan). Silica Gel G 60 (E. Merck) and Sephadex LH-20 (Fluka) were used for column chromatography and precoated Si gel plates (E. Merck, G/UV 254, 0.25 mm) were used for preparative TLC. The compounds were detected by UV at 254 and 366 nm and by the pulverization of ethanolic H_2SO_4 (5% v/v) containing anisaldehyde (2%). The mature seeds of *A. lepidophyllus* were collected from the trees growing in Kumba Cameroon, in December 2002 and identified by Mr. Nolle from the cameroonian I.R.P.M. (Institut de Recherches médicinales et d'études de Plantes Médicinales). The voucher specimens (3884/SFRK) have been deposited at the National Herbarium of Cameroon.

3.2. Extraction and isolation

The air-dried and powdered seeds of *A. lepidophyllus* (1000 g) were treated successively at room temperature with the following solvents: *n*-hexane, CH_2Cl_2, 9:1 MeOH–water. The MeOH extract (78 g) was suspended in water and partitionated with 1-butanol. The butanolic extract (12 g) was subjected to Sephadex gel chromatography with 3:7 $CHCl_3$–MeOH to yield 38 fractions. Fractions 10–17 (2.2 g) were chromatographied on silica gel column and eluted with a gradient solvent system from $CHCl_3$ to MeOH to obtain 25 fractions. Fractions 12 and 13 yielded pure compound **1** (190 mg). Fractions 15–17 (264 mg) were again purified on a silica gel column (9:1 CH_2Cl_2–MeOH). Elutions 16–18 were finally re-purified by preparative TLC (4:1 EtOAc–MeOH) to yield **2** (24 mg) and **3** (10 mg).

3.3. Peracetylation of methylthiomethyl 1-thio-β-D-glucopyranoside 1

Ac_2O (1 mL) was added to methylsulfonylmethyl 1-thio-β-D-glucopyranoside **1** (15 mg, 0.06 mmol) dissolved in pyridine (1 mL). The mixture was stirred for 20 h at room temperature and then evaporated under diminished pressure to provide methylthiomethyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranoside **1a** (18 mg, 0.05 mmol, 84%): $[\alpha]_D^{25}$ −76 (*c* 2.3, MeOH); ^{1}H and ^{13}C NMR see Table 1.

3.4. Synthesis of methylthiomethyl 1-thio-β-D-glucopyranoside (1)

Chloromethyl methyl sulfide (50 μL, 0.59 mmol) was added to 1-thio-β-D-glucose sodium salt hydrate (100.1 mg, 0.45 mmol) suspended in anhydrous DMF (3 mL). The mixture was stirred for 3 h at room temperature and evaporated to give a syrupy residue (140 mg), which was partially soluble in ether (3 × 3 mL). After evaporation of the ether soluble phase, the residue (98 mg) was chromatographed on silica gel, eluting with $CHCl_3$ and a stepwise gradient of MeOH (0–20%) to afford compound **1** (80.6 mg, 0.34 mmol, 73%): $[\alpha]_D^{25}$ −230 (*c* 2.0, MeOH); ^{1}H NMR (270 MHz; $CDCl_3$): δ 4.54 (d, 1H, *J* 9.4 Hz, H-1), 3.96 (d, 1H, *J* 13.5 Hz, H-2′a), 3.79 (d, 1H, *J* 13.5 Hz, H-2′b), 3.69 (d, 1H, *J* 13.4 Hz, H-6a), 3.56 (dd, 1H, *J* 13.4, 5.4 Hz, H-6′b), 3.27 (m, 4H, H-2, H-3, H-4, H-5), 2.12 (s, 3H, CH_3) (Table 2).

3.5. Identification

3.5.1. Afrostyraxthioside A, methylthiomethyl 1-thio-β-D-glucopyranoside (1). Amorphous powder; $[\alpha]_D^{25}$ −222 (*c* 0.8, MeOH); HRDCIMS *m/z* calcd for $[C_8H_{16}O_5S_2+H]^+$: 257.0517, found 257.0523; ^{1}H and ^{13}C NMR see Table 1.

3.5.2. Afrostyraxthioside B, methylsulfonylmethyl 1-thio-β-D-glucopyranoside (2). Amorphous powder; $[\alpha]_D^{25}$ −88 (*c* 1.1, MeOH); HRDCIMS *m/z* calcd for $[C_8H_{16}O_7S_2+H]^+$: 289.0416, found 289.0443; ^{1}H and ^{13}C NMR see Table 1.

3.5.3. Afrostyraxthioside C, methylsulfonylmethylthiomethyl 1-thio-β-D-glucopyranoside (3). Amorphous powder; $[\alpha]_D^{25}$ − 133 (*c* 0.3, MeOH); HRDCIMS *m/z* calcd for $[C_9H_{18}O_7S_3+H]^+$: 335.0293, found 335.0292; ^{1}H and ^{13}C NMR see Table 1.

3.6. Antifungal activity

Antifungal susceptibilities of Afrostyraxthiosides **1**–**3** were evaluated on the following fungi: *C. albicans*

Table 1. ^{13}C and ^{1}H NMR spectroscopic data for **1**–**3** and **1a** (CD_3OD)

Carbon	δ_C (ppm)				δ_H (ppm) *J* (Hz)			
	1	**2**	**3**	**1a**	**1**	**2**	**3**	**1a**
1	84.9	85.3	85.2	82.9	4.52 d (9.7)	4.87 d (9.6)	4.54 d (9.6)	4.42 d (8.2)
2	74.1	74.9	74.1	75.3	3.19 m	3.35 m	3.29 m	5.16 m
3	79.6	79.5	79.6	71.4	3.29 dd (10.2, 8.1)	3.43 m	3.33 dd (10.9, 8.8)	5.39 d (9)
4	71.5	71.5	71.4	69.8	3.19 m	3.35 m	3.29 m	5.06 d (9.9)
5	81.9	82.3	82.2	76.9	3.22 d (5.4)	3.34 m	3.29 m	4.97 d (5)
6a	62.9	62.9	62.8	63.2	3.69 dd (14.0, 1.9)	4.01 d (11.8)	3.88 dd (13.8, 1.6)	4.37 d (8.6)
6b					3.56 dd (14.0, 5.4)	3.77 dd (11.8, 6.4)	3.64 dd (13.8, 5.4)	4.26 d (8.6)
1′	S	S	S	S	S	S	S	S
2′ CH_2 a	36.0	51.9	34.1	36.3	3.93 d (14.5)	4.57 d (15.2)	4.24 d (13.7)	4.02 d (13.6)
b					3.77 d (14.5)	4.33 d (15.2)	4.19 d (13.7)	3.97 d (13.6)
3′	S	SO_2	S	S	S	SO_2	S	S
4′ CH_3	14.9	38.8		14.8	2.11 s	3.21 s		2.10 s
or CH_2 a			52.2				4.38 d (15.0)	
b							4.25 d (15.0)	
5′			SO_2				SO_2	
6′ CH_3			38.8				3.06 s	
CO–CH_3				20.7				2.28 s
								2.18 s
								2.16 s
								2.14 s
C=O				172.2				
				171.5				
				171.2				
				170.9				

Table 2. Long-range correlations detected in the HMBC spectrum of **1**–**3** (CD_3OD)

Carbon	**1** HMBC (C–H)	**2** HMBC (C–H)	**3** HMBC (C–H)
1	H-2′, H-2	H-2′, H-2	H-2′, H-2
2	H-1, H-3	H-1, H-3	H-1, H-3
3	H-1, H-4	H-1, H-4	H-1, H-4
4	H-3, H-5, H-6a, H-6b	H-3, H-5, H-6a, H-6b	H-3, H-5, H-6a, H-6b
5	H-1, H-4, H-6b	H-1, H-4, H-6b	H-1, H-4, H-6b
6	H-5	H-5	H-5
1′	S	S	S
2′ CH_2	H-1, H-4′	H-1, H-4′	H-1, H-4′
3′	S	SO_2	S
4′ CH_3	H-2′	H-2′	
or CH_2			H-2′, H-6′
5′			SO_2
6′ CH_3			H-4′

(ATCC 66-396) and *C. glabrata* (LMA 90-1085). They were previously cultured on yeast peptone dextrose agar at 37 °C for 48 h. For all the compounds, a modified method disk diffusion method was used.[11] Briefly, the compounds were dissolved in Me_2SO and 250 μg aliquots were applied on 12 mm diameter paper disks (ref. 06234304, Prolabo 33173 Gradigan). After evaporating the solvent, disks were placed in the centre of 90 mm-diameter casitone Petri dishes previously flooded with 10 mL of spore suspensions. Positive control was made with amphotericin B paper disk and negative control with drug-free Me_2SO.

Acknowledgement

We thank the AUF (Agence Universitaire de la Francophonie) for a grant to A. Ngono Ngane.

References

1. Cronquist, A. *An Integrated System of Classification of Flowering Plants*; Columbia University Press: New York, 1981, pp 396–397.
2. Yang, X.; Josephson, D.; Peppet, J.; Eilerman, R. W.; Grag, W. K.; Gassenmeier, K. Headspace Aroma of Wild Onion Trees. In *Food Flavors and Chemistry: Advances of the New Millennium*; Spanier, A. M., Shahidi, F., Parliment, T. H., Mussinan, C. J., Ho, C.-T., Contis, E. T., Eds.; Royal Society of Chemistry: Cambridge, 2001; pp 266–273.
3. Bouquet, A. *Féticheurs et médecines traditionnelles du Congo (Brazzaville)*; O.R.S.T.O.M: Paris, 1969, pp 234–235.
4. Ngono Ngane, A. Ph.D. Thesis, Université. de Reims Champagne-Ardenne, 1999, n° 212. Contribution à l'étude des propriétés antifongiques et analyse phytochimique de cinq plantes médicinales camerounaises.
5. Agraval, P. K. *Phytochemistry* **1992**, *31*, 3321–3322.
6. Aksenova, A. A.; Sebyakin, Y. L.; Mironov, A. F. *Russ. J. Bioorg. Chem.* **2001**, *27*, 124–129.
7. Zhu, X.; Schmidt, R. *J. Org. Chem.* **2003**, *69*, 1081–1085.
8. Kouokam, J. C.; Zapp, J.; Becker, H. *Phytochemistry* **2002**, *60*, 403–407.
9. Fahey, J. W.; Zalcmann, A. T.; Talalay, P. *Phytochemistry* **2000**, *56*, 5–51.
10. Bestmann, H. J.; Winkler, L.; Von Helversen, O. *Phytochemistry* **1997**, *46*, 1169–1172.
11. Barry, A. L.; Brown, S. D. *J. Clin. Microbiol.* **1996**, *34*, 2154–2157.