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The identification of 1,3-oxazolidine-2-thiones and 1,3-thiazolidine-2-thiones from the reaction of glucose with benzyl isothiocyanate

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Abstract—The structure of interaction products resulting from the reaction of unmodified glucose with benzyl isothiocyanate is reported. Prior to their identification, the main products of this reaction were isolated using solid-phase extraction (SPE) as well as preparative HPLC. They were then identified by NMR and MS as 3-benzyl-4-hydroxy-5-(D-*arabino*-1,2,3,4-tetrahydroxybutyl)-1,3-oxazolidine-2-thione, 3-benzyl-4-hydroxy-4-hydroxymethyl-5-(D-*erythro*-1,2,3-trihydroxypropyl)-1,3-oxazolidine-2-thione, *N*-benzyl-(D-*gluco*-4,5-dihydroxy-6-hydroxymethyl-tetrahydropyrano)[2,3-*b*]oxazolidine-2-thione and 3-benzyl-4-(*N*-benzyl amino)-5-(D-*arabino*-1,2,3,4-tetrahydroxybutyl)-1,3-thiazolidine-2-thione. The identity of the last compound was secured by X-ray crystal structure data.

Keywords: Glucose; Benzyl isothiocyanate; 1,3-Oxazolidine-2-thione; 1,3-Thiazolidine-2-thione; Crystal structure; X-ray analysis

1. Introduction

Isothiocyanates (ITC) have attracted increasing interest from nutritionists and physicians evaluating secondary plant metabolites in food. ITC are formed from glucosinolates naturally occurring in species of the Brassicaceae family. Human beings ingest them frequently by consuming cruciferous vegetables such as horseradish products, mustard, watercress or cabbages. In the past, such ITC have been under discussion for developing both health-promoting and anti-nutritive effects. Numerous studies on alimentary proteins showed that this was as a consequence of reactions of ITC with amino groups in peptides and proteins of the foodstuffs taking place during food processing as well as in the gastrointestinal tract.[1–3] Little attention has been paid, however, to the reaction of ITC with alimentary sugars or carbohydrates, which could also result in reaction products relevant to human nutrition. Recently, potential reactions of sugars have become more interesting since a rise in the incidence of metabolic diseases especially diabetes in Western societies has been recorded. Therefore, new information in this area would be very useful.

Among sugars, glucose is classified as one of the most important carbohydrates. The main reason is that, in body metabolism, all alimentary carbohydrates such as starch, dextrin or sucrose are digested mainly to glucose. With regard to diabetes as one of the endemic diseases of affluent societies, the permanent high level of glucose in the blood can lead to pathological complications by combining with reactive compounds also circulating in the body, including possibly ITC. Therefore, long-term consequences for human health due to such interactions are under discussion.[4] Interaction of sugars with ITC via

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their hydroxyl groups will yield thiourethanes in the first step. Selected reactions can be obtained by protecting reactive groups in the sugar moiety prior to the intended reaction or via amino sugars.[5–7] When focussing on nutritional aspects as in this work, it is necessary to use unmodified sugars in reactions with alimentary ITC, for which chemical reactions are little known so far. We report here on the structure of the main products formed in the reaction of benzyl isothiocyanate (BITC) with glucose using a model system. In order to show possible alterations of sugars by alimentary ITC, BITC was allowed to react with unmodified glucose under simulation of physiological/food processing conditions. Identification was carried out using NMR, mass spectrometry and X-ray diffraction.

2. Results and discussion

The main result to be drawn from the experiments is that unmodified glucose reacted with BITC giving a complex mixture of at least 13 compounds. The initial challenge to the identification of the main components in this mixture, in particular by NMR, was the preparation of purified compounds from the reaction mixture obtained. The isolation procedure included SPE on a C18 separation phase followed by preparative RP-HPLC. SPE gave two fractions, the first eluting in water (mainly unreacted glucose) and all other compounds (peaks 1–6 in Fig. 1) eluting in acetonitrile (5% yield). The main reaction products (as percent of the total peak area) were **1** (39.2%), **2** (23.9%), **3** (20.5%) and **4** (3.8%). As already shown,[8] these substances definitely originate from the reaction of BITC with glucose. Compounds **5** and **6** were identified as *N*,*N*′-dibenzyl thiourea resulting from the hydrolysis of BITC and unreacted BITC, respectively. Although, **5** and **6** were largely extracted by petroleum ether during the clean-up of the reaction mixture (see Section 3.2), small amounts remained in the mixture and were eluted with acetonitrile as described in the SPE procedure above. The results of further identification are summarised in Figure 2. The given data extracted from MS, NMR and X-ray diffraction suggested that mainly two classes of compounds were formed, namely 1,3-oxazolidine-2-thione derivatives (**1**–**3**) and a 1,3-thiazolidine-2-thione (**4**).

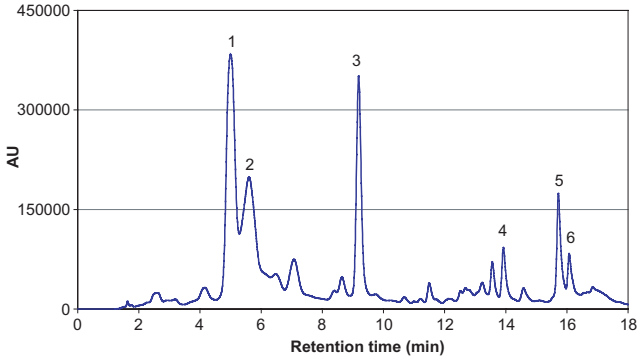

Figure 1. RP-HPLC of the products on Discovery RP-AmideC16® resulted in the reaction of glucose with BITC in alkaline aqueous solution and 1h heating at 95 °C (after applying SPE).

Compound **1** is suggested to be 3-benzyl-4-hydroxy-5-(D-*arabino*-1,2,3,4-tetrahydroxybutyl)-1,3-oxazolidine-2-thione from its molecular weight of 329 and ^{13}C NMR data (Fig. 2). Typical signals for aromatics, C=S (at roughly 189 ppm) and sugar carbons C-1 to C-6 were found. More specifically, higher δ-values for C-1 and C-2 compared to C-3 to C-6 indicated that C-1 and C-2 have been incorporated into the existing oxazolidine ring. This finding was corroborated by specific long-range couplings. Originating from the benzyl protons, these couplings extended to the C-1 of the sugar moiety and to the C=S of the oxazolidine ring, respectively. The residual glucose is bound as a substituent to the oxazolidine ring. The nearly equal chemical shifts of C-3 to C-5 (δ = 71–73 ppm) along with the corresponding multiplicities (see Table 1) showed that residual glucose is bound as a substituent to the oxazolidine ring. The APT measurements taken were in accordance with the fact that in addition to CH_2 of benzyl a further CH_2 (C-6 of the sugar moiety) was present in the molecule. The oxazolidine structure as discussed in this work has been studied extensively including nutritional as well as sugar aspects. From studies on rapeseed, 5-vinyl-1,3-oxazolidine-2-thione (VOT) was detected with the endemic incidence of goitre.[9,10] But this potential toxicant was derived from its precursor progoitrin (2-hydroxy-3-butenyl-glucosinolate) in the course of an intramolecular cyclisation. Obviously, sugars were not involved in this reaction. On the other hand, specific glyco-1,3-oxazolidine-2-thiones were used to improve the characterisation of sugar transporters in mammals. In this context, oxazolidine derivatives of D-fructose and L-sorbose were synthesised to establish a fused furanose ring but using potassium thiocyanate as the starting component and acidic conditions.[11] Unlike these mechanisms, the derivatisation leading to **1** as presented in this work was most likely to be initiated by 1,2-enolised glucose according to the Lobry de Bruyn–Alberda van Ekenstein isomerisation[12,13] taking place in alkaline conditions. Here the OH on C-2 was involved rather than on C-1 to react with BITC giving an intermediate thiourethane. Surprisingly, there was no reaction with the primary OH-group (C-6) as already shown with sugar derivatives.[5,14,15] Also, *N*-carbamoylation[16] by addition of a further amount of BITC to the intermediate thiourethane did not take place. Instead, the intermediate thiourethane was stabilised by intramolecular cyclisation giving **1**. This pathway can also be deduced from investigations on the reaction of 2-amino-2-deoxy

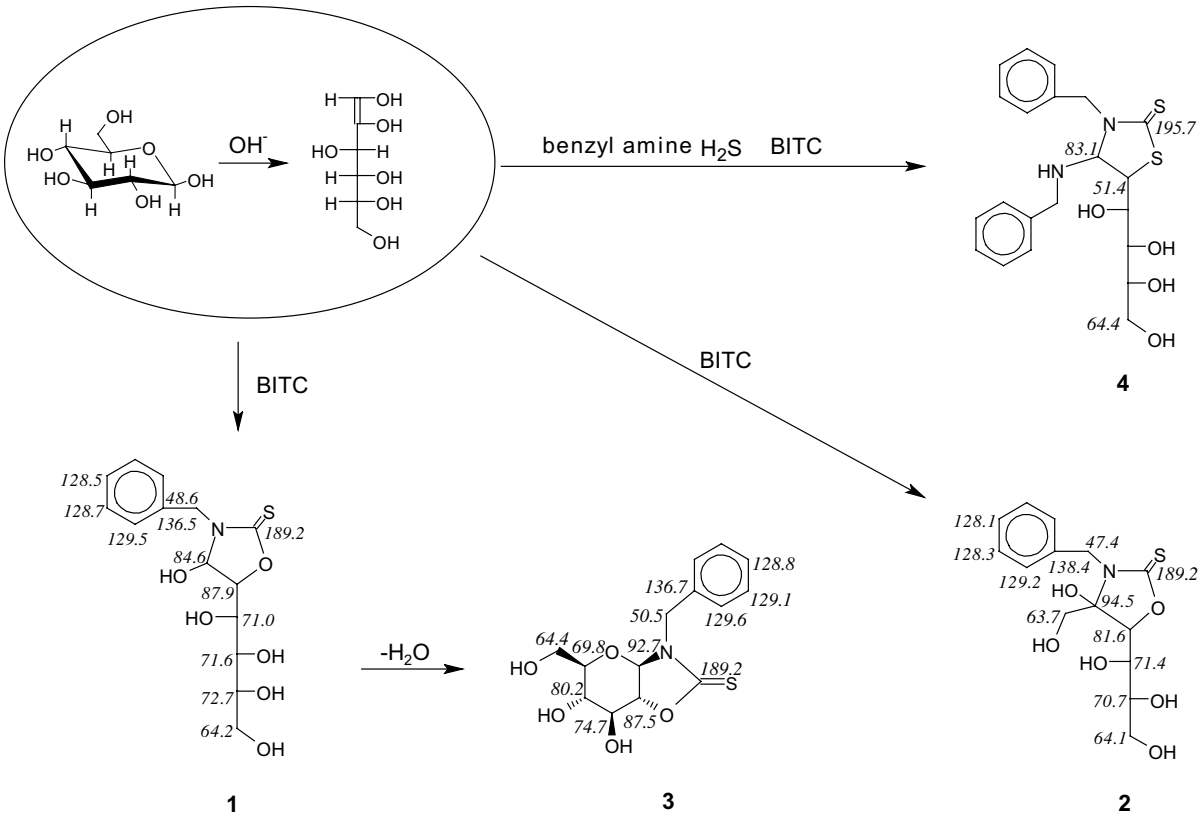

Figure 2. Main compounds and main reaction pathways in the reaction of glucose and BITC (compound number corresponds to peak numbering according to Fig. 1, ^{13}C-chemical shifts are in italics).

Table 1. ^{1}H NMR chemical shifts[a] (ppm) and coupling constants (Hz) of **1**–**3**

H	1		2		3	
	δ ^{1}H	$^{2,3}J_{H,H}$	δ ^{1}H	$^{2,3}J_{H,H}$	δ ^{1}H	$^{2,3}J_{H,H}$
1	5.23 (m)		3.50 (d)	−12.0	5.74 (d)	5.6 (H-1, H-2)
			3.56 (d)			
2	4.60 (dd)	3.4; 4.1	Quaternary		4.92 (d)	5.6 (H-1, H-2)
3	3.83 (m)		5.00 (d)	1.0 (H-3, H-4)	4.38 (d)	2.6 (H-3, H-4)
4	3.62 (dd)	8.5; 1.0	3.90 (dd)	8.5 (H-4, H-5)	3.55 (dd)	2.6 (H-3, H-4)
				1.0 (H-3, H-4)		7.7 (H-4, H-5)
5	3.52 (m)		3.69 (m)		3.78 (m)	
6	3.62 (m)		3.69 (m)		3.38 (dd)	5.6 (H-5, H-6); −11.5 (H-6, H-6′)
	3.52 (m)				3.51 (dd)	3.5 (H-5, H-6′); −11.5 (H-6, H-6′)
Ph–CH_2	4.53 (d)	−15.6	4.81 (s)		4.68 (d)	−15.2
	5.14 (d)	−15.6			4.95 (d)	−15.2
Phenyl	7.33 (m)		7.34 (m)		7.35 (m)	

[a] In CD_3CN (300 MHz, 500 MHz).

sugars with ITC.[6,7] In these studies, the respective intermediary thioureido-sugars were too reactive to be isolated. Such derivatives were found to be highly prone to further conversion by intramolecular addition of the NH-group to C-1 giving imidazolidine-2-thione analogues of hexoses in this case.

For **2** it was reasonable to speculate that 3-benzyl-4-hydroxy-4-hydroxymethyl-5-(D-*erythro*-1,2,3-trihydroxypropyl)-1,3-oxazolidine-2-thione was formed, also having a molecular weight of 329 (Fig. 2). The NMR data (Fig. 2, Table 1) confirmed C=S having a shift of 189.2 ppm and showed a striking result that the C-2 carbon of the sugar was quaternary. Further, three CH_2 groups were found revealing that in addition to benzyl probably two primary OH were present. The ring size was determined using the specific long-range connectivity of H-3 to C=S, which indicated an oxazolidine rather than an oxazine analogue. This finding was also supported by data of the connectivities of the benzyl protons to the quaternary C-2 as well as to the C=S. Thus the enolisation of glucose is not limited to C-1 and C-2, but can move through the complete molecule.[17]

This process led to **2** where the oxazolidine ring was formed as previously described but in this case using C-2 and C-3 of glucose.

Compound **3** was identified as *N*-benzyl-(D-*gluco*-4,5-dihydroxy-6-hydroxymethyl-tetrahydropyrano)[2,3-*b*]oxazolidine-2-thione (Fig. 2) having a molecular weight of 311. The ^{13}C NMR data clearly indicated the presence of C=S (189.2 ppm). There were distinct differences between the values of C-3 to C-5 of the sugar moiety. This is in contrast to the findings on **1**, where the corresponding δ-values were found to be equivalent. This fact along with the data on multiplicity revealed that **3** was probably not an open chain but a cyclised structure. Further data to support this came from characteristic long-range connectivities as H-1 to C=S, C-2, C-3, NCH_2; H-2 to C-3, C-4; H-4 to C-6 and NCH_2 to C=S. The existing complete set of connectivities found within the ring moieties support the molecular structure of **3** as proposed. Hence, it was clear that **3** obviously results from a subsequent dehydration of **1** for further stabilisation.

To clarify the relative position of protons, NOESY correlations were performed. A small NOE was observed only between the protons bound to C-1 and C-2 indicating that the participating protons were situated in a *cis* (*gauche*) constellation belonging to the α-D-glucose structure. This finding was corroborated through the $^3J_{1,2}$ coupling constant, which was measured at 5.6 Hz. Further differentiation deducing the $^1C_4$ or $^4C_1$-form on the basis of NMR data was not possible.

The existence of pyranoid rather than a furanoid structure of glucose was proved from the MS^n data. In agreement with the fragment *m*/*z* 334 (M+Na) occurring in the ion trap, a stable fragment of *m*/*z* 291 was found. This originated from the pyranosyl structure of the sugar moiety due to a loss of C_2H_2O (*m*/*z* 43) including the primary OH at C-6 and C-5 (within the pyranose). Further, *m*/*z* 291 is broken down to *m*/*z* 273 by dehydration. In contrast, the furanose form would give a loss of *m*/*z* 61 (from $C_2H_5O_2$ including primary OH at C-6 and the secondary OH at C-5 located in the side chain) to directly give a fragment of *m*/*z* 273.

Analysing the data received from **4**, strong evidence was given for a structure represented by 3-benzyl-4-(*N*-benzyl amino)-5-(D-*arabino*-1,2,3,4-tetrahydroxybutyl)-1,3-thiazolidine-2-thione having a molecular weight of 434 (Figs. 2 and 3).

The NMR data are summarised in ascending order of ^{13}C chemical shifts shown as #1–#17 in Table 2. The presence of 21 carbons, 26 hydrogen atoms and four oxygen atoms was confirmed from these data. Other heteroatoms especially sulfur as well as nitrogen atoms could not be determined accurately. However, by studying the data in more detail, it is concluded, that only one discrete C=S group (δ = 195.7 ppm) was present, but there were two different phenyl rings (from eight aromatic signals #9–#16). Further, three CH_2 groups were also found. Two of them (#1, #2) are situated in the neighbourhood of phenyl. The third CH_2 group (#4) corresponds to the sugar C-6. Moreover, there were five

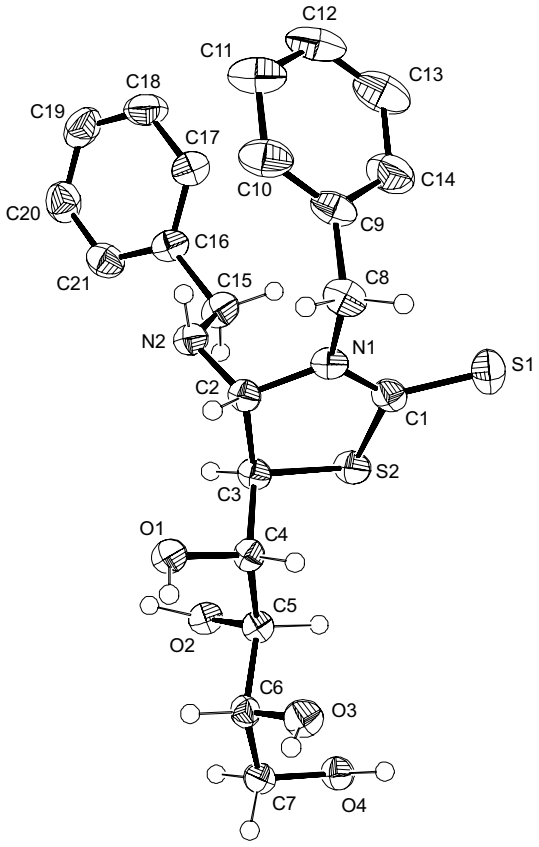

Figure 3. Structure of **4** according to X-ray data (atom numbering as used in the text). The thermal ellipsoids enclose 50% probability. The phenyl hydrogen atoms were omitted for clarity.

Table 2. Characteristic NMR data[a] of **4**

#	δ ^{13}C	δ ^1H	Long-range to #	Assignment
1	48.2	3.77	8; 9–14; 16	CH_2–phenyl
2	49.8	4.44 (d, −14.9)	8; 9–14; 15; 16	CH_2–phenyl
		5.50 (d, −14.9)		
3	51.4	3.62 (m)	3; 5–7; 8; 17	CH–S
4	64.4	3.60 (m)		CH_2–OH
5	72.0	3.60 (m)		CH–OH
6	72.1			CH–OH
7	72.1			CH–OH
8	83.1	5.04 (d, 1.3)	1; 2; 5–7; 17	CH–N
9	128.1	7.29 (m)	2; 9–14; 15; 16	CH
10	128.7			CH
11	128.9			CH
12	129.3			CH
13	129.5			CH
14	129.6			CH
15	137.2			C
16	140.9			C
17	195.7			C=S

[#]Peak according to ^{13}C NMR.

[a] Chemical shifts in ppm, multiplicities and coupling constants (Hz) in parentheses.

other carbons of glucose (#3, #5–#8) present, from which three, namely #5-#7 (C-3 to C-5 of the sugar moiety) were very equivalent, leading to the conclusion that the sugar moiety existed in an open chain configuration. Compared to the other carbons, #3 (C-2 of the sugar moiety) showed a resonance at a relatively high field (δ = 51.4 ppm), which suggested that no oxygen was located at this site and that a tense ring (including C-1 and C-2 of the original sugar) exists. On the contrary, #8 (C-1 of the sugar moiety) showed a chemical shift at comparatively low field, indicating that this carbon was situated in an electron-pushing (mesomeric) environment. However, all these data do not reveal the complete structure. The final chemical structure of **4** (and the unambiguous assignment of the NMR data given in Table 2) was achieved by performing an X-ray diffraction analysis.

The crystallographic data, selected bond lengths, bond angles and characteristic hydrogen bonds of **4** are listed in Tables 3–5. Regarding both bond lengths and bond angles, it was revealed that the thiazolidine derivative according to Figure 3 was formed. In particular, the data showed the partial double bond between C-1 and N-1 as discussed in Ref. 18 for an ordinary 1,3-thiazolidine-2-thione. Moreover, two independent molecules were found in the asymmetric unit (to simplify matters, only one molecule has been drawn in Fig. 3).

Table 4. Selected bond lengths[a] (Å) and bond angles[a] (°) found in **4** (from X-ray analysis)

Bond length[b]		Bond angle[b]	
C1–N1	1.331(4)/1.336(4)	N1–C1–S1	128.5(3)/128.4(3)
C1–S2	1.745(4)/1.749(4)	S1–C1–S2	119.7(2)/120.9(2)
C2–N2	1.444(4)/1.468(4)	N2–C2–C3	111.3(3)/109.8(3)
C3–C4	1.538(4)/1.536(4)	C2–C3–C4	114.4(3)/115.8(3)
C4–C5	1.533(5)/1.528(5)	C4–C5–C6	112.8(3)/113.6(3)
C6–C7	1.515(5)/1.513(5)	C1–N1–C2	117.7(3)/116.8(3)
C8–N1	1.477(4)/1.479(4)	C2–N2–C15	116.2(3)/113.0(3)
C15–N2	1.463(4)/1.475(4)	N1–C1–S2	111.8(2)/110.7(2)
C1–S1	1.662(3)/1.645(4)	N2–C2–N1	115.0(3)/108.6(3)
C2–N1	1.482(4)/1.468(4)	N1–C2–C3	106.8(3)/107.1(3)
C2–C3	1.541(5)/1.535(5)	C2–C3–S2	106.0(2)/103.9(2)
C3–S2	1.816(3)/1.818(3)	C5–C4–C3	111.4(3)/110.1(3)
C4–O1	1.423(4)/1.429(4)	C7–C6–C5	111.9(3)/111.5(3)
C5–O2	1.426(4)/1.424(4)	N1–C8–C9	116.1(3)/115.5(3)
C6–O3	1.433(4)/1.437(4)	N2–C15–C16	111.3(3)/109.6(3)
C7–O4	1.432(4)/1.440(4)	C1–N1–C8	122.8(3)/122.2(3)
C8–C9	1.503(5)/1.500(5)	C8–N1–C2	119.5(3)/120.7(3)
C15–C16	1.504(5)/1.511(5)	C1–S2–C3	93.1(2)/93.8(2)

[a] Values for both independent molecules found in the asymmetric unit separated by slash.
[b] Indication see Figure 3.

Table 3. Crystallographic data of **4**

Empirical formula	$C_{21}H_{26}N_2O_4S_2$
Formula weight	434.56
Crystal habit, colour	Plate, colourless
Crystal size (mm)	$0.05 \times 0.1 \times 0.2$
Crystal system	Monoclinic
Space group	$P2_1$
Unit cell dimensions	
a (Å)	10.0832(6)
b (Å)	9.9258(8)
c (Å)	21.4944(17)
β (°)	102.489(6)
Unit cell volume V (Å^3)	2100.3(3)
Formula per unit cell Z	4
D_{calc} (g/cm^3)	1.374
Absorption coefficient μ (mm^{-1})	0.284
$F(000)$	920
Temperature (K)	210
Data collection method	ω scans with 1° steps
θ range (°)	1.94–25.01
Index ranges	$-11 \leqslant h \leqslant 11$; $-11 \leqslant k \leqslant 11$; $-25 \leqslant l \leqslant 25$
Reflections measured	22,380
Independent reflections	7249 (R_{int} = 0.0728)
Reflections observed [$I > 2\sigma(I)$]	5774
Structure solution	Direct methods (SHELXS-97)
Refinement method	Full-matrix least-squares on F^2
Weighting scheme	$w = 1/[\sigma^2(F_o^2) + (0.0383P)^2 + 0.0000P]$, where $P = (F_o^2 + 2F_c^2)/3$
Data/parameters	7249/636
R indices [$I > 2\sigma(I)$]	R_1 = 0.0444, wR_2 = 0.0819
R indices (all data)	R_1 = 0.0624, wR_2 = 0.0873
Goodness-of-fit on F^2	0.955
Absolute structure parameter	−0.03(6)
Extinction coefficient	0.0056(6)
Largest difference peak and hole (e/Å^3)	0.271 and −0.188

Table 5. Hydrogen bonds with corresponding bond lengths (Å) and bond angles (°) of **4**[a]

D–H···A[b]	*d*(D–H)	*d*(H···A)	*d*(D···A)	(D–H···A)
O1–H11···O2A (i)[c]	0.83(4)	2.01(5)	2.785(3)	154(4)
O1A–H11A···O2	0.88(4)	2.03(4)	2.837(3)	152(4)
O2–H12···O3A (ii)[c]	1.03(5)	1.88(4)	2.803(3)	148(4)
O2A–H12A···O3 (ii)[c]	0.80(4)	2.17(4)	2.916(4)	156(3)
O3–H13···O4A (i)[c]	0.83(6)	1.94(6)	2.768(3)	175(6)
O3A–H13A···O4	0.88(4)	1.86(4)	2.713(3)	163(4)
O4–H14···O1A (iii)[c]	0.77(4)	2.04(4)	2.752(4)	154(4)
O4A–H14A···O1 (iii)[c]	0.72(4)	2.05(4)	2.731(4)	158(5)

[a] For both independent molecules found in the asymmetric unit (the second molecule is labelled with A).
[b] Indication see Figure 3.
[c] Symmetry codes: (i) $x-1, y, z$; (ii) $-x, y+0.5, -z$; (iii) $-x, y-0.5, -z$.

The absolute configuration of **4** was determined via the Flack parameter.[19] Accordingly, the chiral atoms as C-2/C-3/C-4/C-5/C-6 of **4** existed in the *R*/*R*/*S*/*R*/*R* configuration indicating that the structure has the correct chirality.

With regard to the reaction pathway, the formation of **4** was probably initiated by the reaction of benzyl amine and hydrogen sulfide (liberated from the hydrolysis[8] of BITC) with glucose giving transitional 2-mercapto-*N*-benzyl glucosylamine. Subsequently, a further amount of BITC was added to the SH group formed and the reaction was completed intramolecularly as discussed for **1**.

To conclude, we have demonstrated that glucose gives reactions with BITC resulting mainly in the formation of heterocycles such as 1,3-oxazolidine-2-thiones and 1,3-thiazolidine-2-thione. Surprisingly, simple addition products such as thiourethanes were not detected. Knowing that the model system used can only be partially transferred into in vivo conditions, evidence is given here from these data to extend the scope of possibilities of interactions between ITC and glucose. With regard to nutritional aspects, the data obtained can intensify/support the general discussion on interactions of secondary plant metabolites and macronutrients during food processing or in the course of metabolising the nutrients in the body. In this respect, the data found could be used to unravel nutritional effects of secondary plant metabolites as well as effects, which may be associated with diabetogenous long-term consequences. Additionally, along with the data on VOT as mentioned above, it can be assumed that the compounds found are also potential toxicants but this remains to be confirmed.

3. Experimental

3.1. General methods

Melting points are uncorrected. The NMR spectra were recorded with an Avance 300 or Avance 500 spectrometer (Bruker) using standard conditions and operating under the standard Bruker software. Signal assignments were deduced on the basis of COSY, NOESY, APT, HMQC and HMBC experiments. The internal standard and solvent used were Me_4Si and CD_3CN, respectively. The LC/MS system used was an LCQ DECA XP ion trap mass spectrometer, equipped with an electrospray ion source (ThermoFinnigan, San Jose, CA, USA) and the Surveyor HPLC system (ThermoFinnigan, San Jose, CA, USA) operating under Xcalibur software (version 1.2, ThermoFinnigan). LC was performed according to analytical HPLC (see below) at a flow rate of 300 μL/min. X-ray analysis was carried out using an Image Plate Diffraction System (IPDS-2, Stoe) with graphite-monochromated Mo-K_α radiation ($\lambda = 0.71073$ Å) operating at 50 kV and 40 mA. Corrections were made for Lorentz-polarisation effects, but not for absorption. The structure of **4** was calculated by direct methods.[20] The refinement was carried out by full-matrix least squares techniques against F^2.[21] More specifically, non-hydrogen atoms were refined with anisotropic displacement parameters. The hydrogen atoms were positioned according to the difference Fourier map. Hydrogen of phenyl and those at C-15 and C-15A (see Fig. 3, Table 5) were placed in calculated positions and refined with $U_{iso} = 1.2 \times U_{eq}$ of the corresponding carbon atoms with a riding model. The refinement of 636 atomic parameters against 7249 reflection data converged at $R_1 = 0.044$. The absolute structure parameter[19] was refined to −0.03(6).

3.2. General protocol for the synthesis

A solution of 1 mmol D-glucose (180 mg) in 5 mL of deionised water was adjusted to pH 11 using 10 M sodium hydroxide. At room temperature 500 μmol (74.5 mg) BITC in 5 mL acetonitrile was added and the resulting mixture was heated to reflux temperature (95 °C). After heating for 1 h, the reaction mixture became greenish and the pH was reduced (9.2). The acetonitrile was then removed in vacuo (75 mbar). The solid that precipitated from the remaining aqueous solution was filtered

through Celite® and washed with 30 mL petroleum ether (PE). To remove the unreacted BITC, the filtrate was also washed with PE (3 × 30 mL). The organic layer was discarded, but the aqueous phase was separated and freeze dried. The product thus obtained was used for further separation.

3.3. Purification by solid phase extraction (SPE)

The SPE tubes (1 mL, 100 mg Discovery DSC-18, Supelco) were conditioned using 1 mL of acetonitrile and 2 mL of de-ionised water in succession. Five hundred microlitres of the sample (400 mg of the freeze-dried probe per 1 mL of de-ionised water) was applied. After infiltration into the separation layer, 1.5 mL of de-ionised water was passed through to elute undigested glucose. Thereafter, 1 mL of acetonitrile was used to elute and collect all other substances (mainly **1**–**6**). Further separation of this fraction to obtain samples performing NMR studies was achieved by preparative HPLC (see below).

3.4. Analytical HPLC

To control the success of purification of the reaction mixture with the aid of SPE, a reversed-phase high performance liquid chromatography (RP-HPLC) was performed using a Shimadzu vp series high performance liquid chromatograph.[8] The separation was carried out by a Discovery RP-AmideC16 column (250 × 3 mm ID, 5 μm) (Supelco, Taufkirchen, Germany) detecting at 254 nm and using an (A) acetonitrile: (B) water (consisted in 10% of acetonitrile) gradient. The flow rate was set at 1 mL/min and the ambient column temperature was kept at 30 °C. The separation program started at 90% B for 2 min, changed within 7 min to 60% B, held for 1 min, changed within 2 min to 35% B, held again for 1 min, returned within 2 min to start conditions and then held for 3 min before starting the next run.

3.5. Preparative HPLC

To obtain substantial amounts of **1**–**4**, the eluate according to Section 3.3 (after applying acetonitrile) was fractionated using a preparative Discovery RP-AmideC16 column (250 × 21.2 mm ID, 5 μm) on a Shimadzu HPLC device[8] running at 30 °C. The eluent consisted of 40% acetonitrile in de-ionised water at a flow rate of 5 mL/min. The portion of injection volume was set to 400 μL.

3.6. Preparation of derivatives

3.6.1. General protocol. Corresponding fractions of about 12 runs according to Section 3.5 were collected, then concentrated and lyophilised to get 3–10 mg of residue.

3.6.2. 3-Benzyl-4-hydroxy-5-(D-*arabino*-1,2,3,4-tetrahydroxybutyl)-1,3-oxazolidine-2-thione (1). White powder; mp: 172–174 °C (plates, 50% tetrahydrofuran/hexan); ESI-MS (−): *m/z* 328 $[M-H]^-$, 208 $[M-H-120]^-$, 179 $[M-H-149]^-$. The NMR data are given in Table 1 and Figure 2.

3.6.3. 3-Benzyl-4-hydroxy-4-hydroxymethyl-5-(D-*erythro*-1,2,3-trihydroxypropyl)-1,3-oxazolidine-2-thione (2). Waxy residue; mp: 65–70 °C; ESI-MS (+): *m/z* 681 $[2M+Na]^+$, 352 $[M+Na]^+$, 330 $[M+H]^+$. The NMR data are given in Table 1 and Figure 2.

3.6.4. *N*-Benzyl-(D-*gluco*-4,5-dihydroxy-6-hydroxymethyl-tetrahydropyrano)[2,3-*b*]oxazolidine-2-thione (3). White powder; mp: 129–131 °C (prisms, 50% tetrahydrofuran/hexan); ESI-MS (+): *m/z* 645 $[2M+Na]^+$, 334 $[M+Na]^+$, 312 $[M+H]^+$, 291 $[M-43]^+$, 273 $[291-18]^+$. The NMR data are given in Table 1 and Figure 2.

3.6.5. 3-Benzyl-4-(*N*-benzyl amino)-5-(D-*arabino*-1,2,3,4-tetrahydroxybutyl)-1,3-thiazolidine-2-thione (4). White powder; mp: 135–138 °C (plates, acetonitrile); ESI-MS (+): *m/z* 891 $[2M+Na]^+$, 457 $[M+Na]^+$, 435 $[M+H]^+$. The NMR data are given in Table 2 and Figure 2.

Crystallographic data have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. 221897. These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html or from The director, CCDC, 12 Union Road, Cambridge, CB2 IEZ, UK (Tel.: +44 1223 336 408; fax: +44 1223 336 033; e-mail: deposit@ccdc.cam.ac.uk).

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