Four New Phenolic Compounds from *Curculigo crassifolia* (Hypoxidaceae)

by **Ning Li**, **Ji-Jun Chen**, and **Jun Zhou***

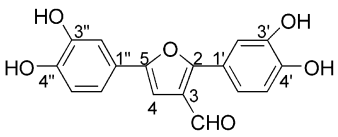
State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, Yunnan, P. R. China
(phone: + 86-871-5223264; fax: + 86-871-5223261; e-mail: ln0110@hotmail.com)

Four new phenolic compounds, named crassifogenin A (**1**), crassifogenin B (**2**), crassifoside A (**3**), and crassifoside B (**4**), were isolated from the EtOH extract of the rhizomes of *Curculigo crassifolia*, and based on the chemical transformation and modern spectroscopic experiments, including 2D-NMR techniques (HMQC, HMBC, COSY, HMQC-TOCSY, and NOE), their structures were elucidated as 2,5-bis(3,4-dihydroxyphenyl)furan-3-carbaldehyde (**1**), (3,4-dihydroxyphenyl)(3,6,7-trihydroxynaphthalen-1-yl)methanone (**2**), (3,4-dihydroxyphenyl)[3-(β-D-glucopyranosyloxy)-6,7-dihydroxynaphthalen-1-yl]methanone (**3**), and 1,2-*O*-{2-(3,4-dihydroxyphenyl)-1-[3-(3,4-dihydroxyphenyl)prop-2-ynyl]ethane-1,2-diyl}-β-D-glucopyranose (**4**).

1. Introduction. – Several species of the genus *Curculigo* are well known for their use in medicine. Previous phytochemical and pharmacological studies on the species of the genus had been reported [1–4]. *Curculigo crassifolia* (BAK.) HOOK. f. (Hypoxidaceae) is distributed in the western and southern regions of China [5]. The rhizomes of this plant are used as a folk medicine for treating child pneumonitis [5]. However, so far, no extensive studies of this plant with respect to its chemical characteristics have been reported. The interesting immense medicinal importance of this genus encouraged us to undertake the phytochemical investigation on *Curculigo crassifolia*. This paper mainly describes the isolation and structural identification of the four new phenolic compounds **1**–**4** from the EtOH extract of the rhizomes of *C. crassifolia*.

2. Results and Discussion. – Crassifogenin A (**1**) was obtained as yellow powder. The positive-ion HR-FAB-MS showed a quasi-molecular-ion peak at *m/z* 313.0705 ($[M+H]^+$), in accordance with the molecular formula $C_{17}H_{13}O_6^+$ (calc. 313.0712). The IR spectrum indicated the presence of OH groups (3431 cm^{-1}) and a carbonyl group (1652 cm^{-1}). The ^{1}H-NMR spectrum showed the presence of two 3,4-disubstituted aromatic rings. 1D-NMR and HMBC experiments suggested the presence of a furan-ring moiety. Thus, the structure of **1** was elucidated as 2,5-bis(3,4-dihydroxyphenyl)-furan-3-carbaldehyde.

In the HMBC spectrum of **1**, the signal of H–C(4) at δ 7.39 was correlated with that of C(5) at δ 151.5, C(2) at δ 156.2, and CHO at δ 178.9 (*Table 1*). The signal of CHO at δ 9.37 was correlated with that of C(3) at δ 122.6, C(2) at δ 156.2, C(4) at δ 128.2, and C(5) at δ 151.5, which suggested the presence of a furan-ring moiety with a carbaldehyde located at C(3). The HMBC experiment also showed the long-range couplings of H–C(2′) (δ 7.10) and H–C(6′) (δ 7.02) with C(2) at δ 156.2, and of H–C(2″) (δ 6.81) and H–C(6″) (δ 6.71) with C(5) at δ



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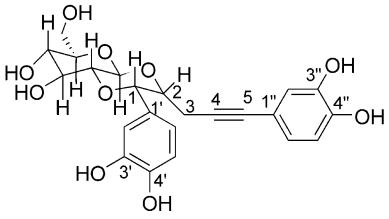
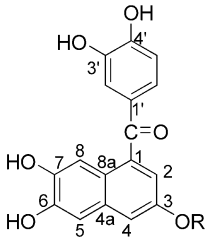


4[1])



2 R = H
3 R = β-D-glucopyranosyl

151.5, which suggested that two 3,4-disubstituted aromatic rings were connected with C(2) and C(5). On acetylation of **1** with Ac_2O in pyridine, the positive-ion FAB-MS of the acetate of **1** showed an ion at *m/z* 481 ($[M(\mathbf{1})+1+4\ Ac]^+$), which suggested that **1** had 4 free OH groups located at C(3′), C(4′), C(3″), and C(4″).

Table 1. *^{1}H- and ^{13}C-NMR and HMBC Data* (CD_3OD) *of Compound* **1**. δ in ppm, *J* in Hz.

	δ(C)[a])	δ(H)[b])	HMBC[b]) (H → C)
C(2)	156.2	–	
C(3)	122.6	–	
H–C(4)	128.2	7.39 (*s*)	C(2), C(5), CHO, C(1″)
C(5)	151.5	–	
CHO	178.9	9.37 (*s*)	C(2), C(3), C(4), C(5)
C(1′)	125.5	–	
H–C(2′)	115.2	7.10 (*d*, *J* = 2.1)	C(2), C(1′), C(3′), C(6′)
C(3′)	146.4[c])	–	
C(4′)	148.3	–	
H–C(5′)	115.9	6.72 (*d*, *J* = 8.4)	C(1′), C(3′), C(4′)
H–C(6′)	120.8	7.02 (*dd*, *J* = 8.4, 2.1)	C(2), C(2′), C(4′)
C(1″)	125.3	–	
H–C(2″)	116.9	6.81 (*d*, *J* = 2.0)	C(5), C(3″), C(4″), C(6″)
C(3″)	146.4[c])	–	
C(4″)	146.6[c])	–	
H–C(5″)	116.8	6.79 (*d*, *J* = 8.1)	C(1″), C(3″), C(4″)
H–C(6″)	121.5	6.71 (*dd*, *J* = 8.1, 2.0)	C(5), C(2″), C(4″)

[a]) Recorded at 125 MHz. [b]) Recorded at 500 MHz. [c]) Values may be interchanged.

[1]) Arbitrary numbering; for systematic names, see *Exper. Part*.

Crassifogenin B (**2**) was obtained as pale yellow powder. The positive-ion HR-FAB-MS showed a quasi-molecular-ion peak at *m/z* 313.0701 ($[M+H]^+$), in accordance with the molecular formula $C_{17}H_{13}O_6^+$ (calc. 313.0712). The IR spectrum indicated the presence of OH groups (3433 cm^{-1}) and a carbonyl group (1652 cm^{-1}). The ^{1}H- and ^{13}C-NMR data (*Table 2*) indicated the presence of a naphthalene-ring moiety in **2**. This was further confirmed by the HMBC spectrum. From these results and the spectral data, compound **2** was determined as (3,4-dihydroxyphenyl)(3,6,7-trihydroxynaphthalen-1-yl)methanone.

Table 2. *^{1}H- and ^{13}C-NMR and HMBC Data* (CD_3OD) *of Compound* **2**. δ in ppm, J in Hz.

	δ(C)[a])	δ(H)[b])	HMBC[b]) (H → C)
C(1)	137.9	–	
H–C(2)	116.9	6.85 (*d*, *J* = 2.4)	C(4), C(8a), C=O
C(3)	153.2	–	
H–C(4)	111.6	7.03 (*d*, *J* = 2.4)	C(2), C(3), C(5), C(8a)
H–C(5)	109.6	6.99 (*s*)	C(4), C(6), C(8a)
C(6)	146.4	–	
C(7)	148.6	–	
H–C(8)	108.7	7.05 (*s*)	C(1), C(6), C(7), C(4a)
C(8a)	122.6	–	
C(4a)	132.9	–	
C=O	199.5	–	
C(1′)	131.2	–	
H–C(2′)	118.0	7.33 (*d*, *J* = 2.0)	C(3′), C(4′), C(6′), C=O
C(3′)	146.2	–	
C(4′)	152.8	–	
H–C(5′)	115.9	6.80 (*d*, *J* = 8.3)	C(1′), C(3′), C(4′)
H–C(6′)	125.8	7.21 (*dd*, *J* = 8.3, 2.0)	C(2′), C(4′), C=O

[a]) Recorded at 100 MHz. [b]) Recorded at 400 MHz.

In the HMQC spectrum of **2**, two *d* at δ 6.85 (J = 2.4 Hz, H–C(2)) and 7.03 (J = 2.4 Hz, H–C(4)) had connectiveties with the C-atoms at δ 116.9 (C(2)) and 111.6 (C(4)), and two *s* at δ 6.99 (H–C(5)) and 7.05 (H–C(8)) had connectivities with C-atoms at δ 109.6 (C(5)) and 108.7 (C(8)). The NMR data revealed the presence of a 3,4-disubstituted aromatic ring linked to a C=O group by the presence of two *d* (each 1 H) at δ 7.33 (J = 2.0 Hz, H–C(2′)) and 6.80 (J = 8.3 Hz, H–C(5′)) along with a *dd* (1 H) at δ 7.21 (J = 8.3, 2.0 Hz, H–C(6′)). The C-atoms connected to these protons were observed at δ 118.0 (C(2′)), 115.9 (C(5′)), and 125.8 (C(6′)) in the HMQC spectrum. The HMBC experiment displayed correlations between H–C(2) and the C=O group and between H–C(2′), H–C(6′), and the C=O group, which suggested that the C=O group was linked to C(1) and C(1′), respectively. On acetylation of **2** with Ac_2O in pyridine, the positive-ion FAB-MS of the acetate of **2** showed an ion peak at *m/z* 523 ($[M(\mathbf{2})+1]+5$ Ac$]^+$), which suggested that **2** had five free OH groups located at C(3), C(6), C(7), C(3′), and C(4′).

Crassifoside A (**3**) was obtained as white powder. The negative-ion HR-FAB-MS showed a quasi-molecular-ion peak at *m/z* 473.1092 ($[M-H]^-$), in accordance with the molecular formula $C_{23}H_{21}O_{11}^-$ (calc. 473.1083). Its IR (see *Exper. Part*) and the ^{1}H- and ^{13}C-NMR data (*Table 3*) of the aglycone of **3** were closely similar to those of compound **2**, indicating that they have the same skeleton. On acidic hydrolysis of **3**, crassifogenin B (**2**) and glucose were detected by TLC and comparison on paper chromatography with an authentic sample of glucose. Thus the structure of **3** was determined to be (3,4-dihydroxyphenyl)[3-(β-D-glucopyranosyloxy)-6,7-dihydroxynaphthalen-1-yl]methanone.

Table 3. *^{1}H- and ^{13}C-NMR and HMBC Data* (CD_3OD) *of Compound* **3**. δ in ppm, J in Hz.

	δ(C)[a])	δ(H)[b])	HMBC[b]) (H → C)
C(1)	137.8	–	
H–C(2)	117.9	7.10 (*d*, *J* = 2.3)	C(1), C(3), C(4), C(8a), C=O
C(3)	153.9	–	
H–C(4)	114.0	7.41 (*d*, *J* = 2.3)	C(2), C(3), C(5), C(8a)
H–C(5)	110.6	7.12 (*s*)	C(4), C(6), C(8a)
C(6)	147.3	–	
C(7)	148.7	–	
H–C(8)	108.7	7.11 (*s*)	C(1), C(6), C(7), C(8a), C(4a)
C(8a)	124.2	–	
C(4a)	132.4	–	
C=O	199.1	–	
C(1′)	131.1	–	
H–C(2′)	118.0	7.33 (*d*, *J* = 2.0)	C(3′), C(4′), C(6′), C=O
C(3′)	146.5	–	
C(4′)	152.8	–	
H–C(5′)	115.9	6.80 (d, *J* = 8.3)	C(1′), C(3′), C(4′)
H–C(6′)	125.8	7.21 (*dd*, *J* = 8.3, 2.0)	C(2′), C(4′), C=O
Glc: H–C(1″)	102.9	4.99 (*d*, *J* = 7.3)	C(3)
H–C(2″)	71.4	3.47 (*m*)	
H–C(3″)	78.2	3.45 (*m*)	
H–C(4″)	75.0	3.46 (*m*)	
H–C(5″)	78.0	3.49 (*m*)	
CH_2(6)	62.5	3.90 (*dd*, *J* = 12.1, 2.0), 3.71 (*dd*, *J* = 12.1, 5.3)	

[a]) Recorded at 100 MHz. [b]) Recorded at 400 MHz.

The ^{1}H- and ^{13}C-NMR spectrum of **3** indicated the presence of a hexose unit. A *d* (1 H) at δ 4.99 (J = 7.3 Hz, H–C(1″) (Glc)) had connectivities with C-atoms at δ 102.9 (C(1″) (Glc)) in the HMQC spectrum. From the coupling constant of the anomeric H–C(1″) and ^{13}C-NMR chemical shifts due to the sugar moiety, the glucose unit should be in the β-D form. The other positions of the glucose unit were confirmed by ^{1}H,^{1}H-COSY, HMBC, HMQC-TOCSY, and NOE experiments. In the HMBC spectrum of **3**, the signal of the anomeric H–C(1″) at δ 4.99 was correlated with that of C(3) at δ 153.9, which suggested that the glucose was connected at C(3).

Crassifoside B (**4**) was obtained as white powder. The negative-ion HR-FAB-MS showed a quasi-molecular-ion peak at *m/z* 459.2289 ($[M - H]^-$), in accordance with the molecular formula $C_{23}H_{23}O_{10}^-$ (calc. 459.1369). The ^{1}H-NMR spectrum showed the presence of two 3,4-disubstituted aromatic rings. By a selective ^{1}H-decoupling experiment and the HMBC spectrum, the norlignan sequence PhCH(O)-CH(O)CH_2C≡CPh was established. The remaining C-atoms presumably belong to a hexose unit. On acidic hydrolysis of **4**, glucose was detected by comparison on paper chromatography with an authentic sample. A detailed NMR-data analysis (*Table 4*) and 2D-NMR experiments (including ^{1}H,^{1}H-COSY, HMBC, HMQC-TOCSY, and NOESY) suggested that **4** was a glucopyranose-fused norlignan with the structure of 1,2-*O*-{2-(3,4-dihydroxyphenyl)-1-[3-(3,4-dihydroxyphenyl)prop-2-ynyl]ethane-1,2-diyl}-β-D-glucopyranose.

In the HMQC spectrum of **4**, a *d* (1 H) at δ 4.83 (J = 7.7 Hz, H–C(1) (Glc)) had connectivities with C-atoms at δ 97.1 (C(1) (Glc)). From the coupling constant of the anomeric H–C(1) and ^{13}C-NMR chemical shifts,

Table 4. *^{1}H- and ^{13}C-NMR and HMBC Data* (CD_3OD) *of Compound* **4**. δ in ppm, J in Hz. Arbitrary numbering[1]).

	δ(C)[a])	δ(H)[b])	HMBC[b]) (H → C)
H–C(1)	77.6	4.81 (*d*, J = 4.7)	C(2′), C(6′), C(2), C(2) (Glc)
H–C(2)	75.4	4.56 (*m*)	C(1), C(3), C(4), C(1) (Glc)
CH_2(3)	23.5	2.85 (*dd*, *J* = 17.1, 6.0)	C(1), C(2), C(5)
		2.65 (*dd*, *J* = 17.1, 6.0)	
C(4)	83.3	–	
C(5)	84.3	–	
C(1′)	131.5	–	
H–C(2′)	116.2	6.98 (*d*, *J* = 2.2)	C(1), C(3′), C(6′)
C(3′)	146.0	–	
C(4′)	146.3	–	
H–C(5′)	116.2	6.76 (*d*, *J* = 8.1)	C(1′), C(3′), C(4′)
H–C(6′)	120.7	6.87 (*dd*, *J* = 8.1, 2.2)	C(1), C(2′), C(4′)
C(1″)	115.9	–	
H–C(2″)	119.5	6.79 (*d*, *J* = 1.7)	C(5), C(3″), C(4″), C(6″)
C(3″)	146.5	–	
C(4″)	147.0	–	
H–C(5″)	116.2	6.66 (*d*, *J* = 8.1)	C(1″), C(3″), C(4″)
H–C(6″)	125.0	6.73 (*dd*, *J* = 8.1, 1.7)	C(5), C(1″), C(2″), C(4″)
Glc: H–C(1)	97.1	4.83 (*d*, *J* = 7.7)	C(2)
H–C(2)	73.9	3.51 (*m*)	C(1)
H–C(3)	75.8	3.54 (*m*)	
H–C(4)	71.9	3.33 (*m*)	
H–C(5)	79.5	3.42 (*m*)	
CH_2(6)	62.5	3.85 (*dd*, *J* = 12.0, 2.2) 3.68 (*dd*, *J* = 12.0, 5.6)	

[a]) Recorded at 125 MHz. [b]) Recorded at 500 MHz.

the glucose unit should be in the β-D form. The other linkages of the glucose unit were confirmed by ^{1}H,^{1}H-COSY, HMBC, HMQC-TOCSY, and NOE experiments. In the HMBC spectrum of **4**, the signal of the anomeric H–C(1) at δ 4.83 was correlated with that of C(2) at δ 75.4, and the signal of H–C(2) (Glc) at δ 3.51 was correlated with that of C(1) at δ 77.6, which suggested that the glucose was connected at C(2) and C(1). The configuration of **4** was revealed by a NOESY experiment. The correlations H–C(1)/H–C(2) and H–C(1)/H–C(2) (Glc) were clearly observed, but no NOE was detected for H–C(2)/H–C(1) (Glc), establishing the axial orientation of H–C(1) and the equatorial orientation of H–C(2). On acetylation of **4** with Ac_2O in pyridine, the positive-ion FAB-MS analysis of the acetate of **4** showed an ion peak at *m/z* 755 ($[M(\mathbf{4})+1+7\,Ac]^+$), which suggested that the glucose unit had only three free OH groups and that the other four AcO groups were replacing the phenolic OH groups.

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Experimental Part

General. Column chromatography (CC): *Qingdao* silica gel (200–300 mesh), eluent MeOH/$CHCl_3$; *Sephadex LH-20* gel, eluent EtOH. Optical rotations: *Jasco DIP-370* digital polarimeter; in MeOH. UV Spectra: *UV-210A* spectrometer; in MeOH; λ_{max} (log ε) in nm. IR Spectra: *Bio-Rad FTS-135* spectrometer; KBr pellets; $\tilde{\nu}$ in cm^{-1}. 1D- and 2D-NMR Spectra: *Bruker AM-400* or *Bruker DRX-500* spectrometer in CD_3OD with $SiMe_4$ as internal standard; δ in ppm, J in Hz. MS: *Autospec 3000* spectrometer, negative-ion or positive-ion mode; in *m/z*.

Plant Material. The plant material was collected in Eshan Prefecture, Yunnan Province, China, in October 2002 and identified as *Curculigo crassifolia* by Prof. *Ping-Hua Yu*, Kunming Institute of Botany, Chinese Academy of Science, where a voucher specimen is deposited.

Extraction and Isolation. The air-dried and powered rhizomes of *C. crassifolia* (10 kg) were extracted with 95% EtOH (3 × 50 l) at r.t., and the combined extracts were evaporated to afford a residue (562 g). The residue was suspended in H_2O and then passed through a *D101*-resin column eluting with H_2O and 95% EtOH. The EtOH eluent was evaporated to give a residue (500 g), which was fractionated by CC (silica gel (3000 g, 200–300 mesh), $CHCl_3$/MeOH, 9:1): *Fractions 1–5*. *Fr. 2* (13 g) was purified by repeated CC (silica gel, $CHCl_3$/MeOH 9.5:0.5 and 8.5:1.5; then *Sephadex LH-20*, EtOH): pure **1** (150 mg). *Fr. 3* (40 g) was purified by CC (silica gel, $CHCl_3$/MeOH 9:1; then repeated *Sephadex LH-20*, EtOH): pure **2** (48 mg). *Fr. 5* (210 g) was purified by repeated CC (*Sephadex LH-20*, EtOH): pure **3** (23 mg) and **4** (25 mg).

Crassifogenin A (=*2,5-Bis(3,4-dihydroxyphenyl)furan-3-carbaldehyde*; **1**). Yellow powder. IR (KBr): 3431, 2928, 1652, 1490, 1280, 1112, 1053, 868, 794, 582. UV (MeOH): 203 (4.50), 261 (4.13), 292 (4.05), 363 (4.11). ^{1}H-NMR (CD_3OD, 500 MHz): see *Table 1*. ^{13}C-NMR (CD_3OD, 125 MHz): see *Table 1*. FAB-MS (pos.): 313 ($[M+H]^+$). HR-FAB-MS (pos.): 313.0705 ($[M+H]^+$, $C_{17}H_{13}O_6^+$; calc. 313.0712).

Tetraacetate of crassifogenin A: FAB-MS (pos.): 481 ($[M+H]^+$).

Crassifogenin B (=*(3,4-Dihydroxyphenyl)(3,6,7-trihydroxynaphthalen-1-yl)methanone*; **2**). Pale yellow powder. IR (KBr): 3433, 2925, 2075, 1652, 1616, 1374, 1290, 1190, 1048, 875, 573. UV (MeOH): 233 (4.67), 285 (3.94), 324 (3.93). ^{1}H-NMR (CD_3OD, 400 MHz): see *Table 2*. ^{13}C-NMR (CD_3OD, 100 MHz): see *Table 2*. FAB-MS (pos.): 313($[M+H]^+$). HR-FAB-MS (pos.): 313.0701 ($[M+H]^+$, $C_{17}H_{13}O_6^+$; calc. 313.0712).

Pentaacetate of crassifogenin B: FAB-MS (pos.): 523 ($[M+H]^+$).

Crassifoside A (=*(3,4-Dihydroxyphenyl)[3-(β-*D*-glucopyranosyloxy)-6,7-dihydroxynaphthalen-1-yl]methanone*; **3**). White powder. $[\alpha]_D^{29} = -24.8$ ($c = 0.10$, MeOH). IR (KBr): 3439, 2092, 1699, 1652, 1558, 1290, 1194, 1075, 1047, 880. UV (MeOH): 234 (4.61), 285 (3.93), 324 (3.88). ^{1}H-NMR (CD_3OD, 400 MHz): see *Table 3*. ^{13}C-NMR (CD_3OD, 100 MHz): see *Table 3*. FAB-MS (neg.): 473 ($[M-H]^-$). HR-FAB-MS (neg.): 473.1092 ($[M-H]^-$, $C_{23}H_{21}O_{11}^-$; calc. 473.1083).

Crassifoside B (=*1,2-*O*-{2-(3,4-Dihydroxyphenyl)-1-[3-(3,4-dihydroxyphenyl)prop-2-ynyl]ethane-1,2-diyl}-β-*D*-glucopyranose*; **4**). White powder. $[\alpha]_D^{26} = +74.7$ ($c = 0.15$, MeOH). IR (KBr). 3422, 2926, 2052, 1610, 1521, 1445, 1370, 1289, 1115, 1044, 816, 618. UV (MeOH): 204 (4.73), 257 (4.18), 290 (3.82). ^{1}H-NMR (CD_3OD, 500 MHz): see *Table 4*. ^{13}C-NMR (CD_3OD, 125 MHz): see *Table 4*. FAB - MS (neg.): 459 ($[M-H]^-$). HR-FAB-MS (neg.): 459.2289 ($[M-H]^-$, $C_{23}H_{23}O_{10}^-$; calc. 459.1369).

Heptaacetate of crassifoside B: FAB-MS (pos.): 755 ($[M+H]^+$).

Acetylation of **1**, **2**, *and* **4**. A soln. of each sample (1 mg) in pyridine (1 ml) was treated with Ac_2O (1 ml) and kept at 60–70° for 3–6 h. After evaporation, the residue was purified by prep. TLC ($CHCl_3$/MeOH, 9:1): acetate. Each acetate was subjected to FAB-MS analysis.

Acidic Hydrolysis. Compound **3** or **4** (3 mg) was dissolved in MeOH (2.0 ml) and 2M H_2SO_4 (2.0 ml) and refluxed on a boiling water bath for 2 h. The hydrolyzate was allowed to cool, diluted twofold with distilled H_2O, and partitioned between AcOEt and H_2O. The aq. layer was neutralized with aq. $Ba(OH)_2$ soln. and evaporated: residue. Crassifogenin B (**2**) was detected in the AcOEt extract of the acidic hydrolyzate of **3** by TLC comparison ($CHCl_3$/MeOH 5:1). Glucose was identified in the residue by comparison on paper chromatography (BuOH/AcOH/H_2O 5:1:5, upper layer) with an authentic sample.

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