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# Two novel glucosyl-fused compounds from *Curculigo crassifolia* (Hypoxidaceae)

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**Abstract**—Two novel glucosyl-fused compounds, namely crassifoside E (**1**) and crassifoside F (**2**), were isolated from the rhizomes of *Curculigo crassifolia*. Their structures were elucidated by spectral analysis. Compounds **1** and **2** displayed ACE inhibitory activity (angiotensin-converting enzyme) with an $IC_{50}$ value of 10 and 8.5 μg/mL, respectively, by inspecting their fluorescent light.


*Curculigo crassifolia* (Bak.) Hook. f. (Hypoxidaceae) is distributed in the western and southern regions of China. It is called 'rong ye xian mao' in Chinese and local people use the rhizomes as a folk medicine to treat child pneumonitis.[1] Past studies on its chemical constituents have resulted in the isolation of four new phenolic compounds.[2] Being interested in exploring biologically active substances from this folk medicine, we reinvestigated this plant. This study led to the isolation of two new glucosyl-fused compounds with novel skeletons, crassifoside E (**1**), and crassifoside F (**2**).

The rhizomes of *C. crassifolia* were collected in Eshan Prefecture, Yunnan Province, PR China, in October 2002. These dried rhizomes were extracted and fractionated as described previously.[2] Fraction 4 was chromatographed on silica gel ($CHCl_3$–MeOH), then repeated Sephadex LH-20 gel (EtOH, EtOH–acetone) to result in the isolation of crassifoside E (**1**, 60 mg) and crassifoside F (**2**, 80 mg).

Crassifoside E (**1**) was isolated as brown powder; its molecular formula $C_{25}H_{26}O_{11}$ was established by $^{13}C$ NMR and HRFABMS data, corresponding to an unsaturation index of 13. The IR spectrum of compound **1** showed absorption for hydroxyl (3440 $cm^{-1}$). The $^1H$ NMR[3] spectrum exhibited signals for three methyl protons [$\delta_H$ 1.46 (t, $J = 7.30$ Hz, H-12)], four methylene protons [$\delta_H$ 3.30, 3.45, 3.83, 3.95 (each 1H, m, $H_a$-5, $H_b$-5, $H_a$-13, and $H_b$-13, respectively)], two oxymethine protons [$\delta_H$ 4.42 (dd, $J = 11.32$, 3.04 Hz, H-6), 5.19 (s, H-7)], three low-field aromatic protons [$\delta_H$ 7.72, 7.35, 7.77 (each 1H, s, H-1, H-3 and H-11, respectively)] and those protons of one glucosyl moiety [$\delta_H$ 3.40–3.91 (6H, Glc·H-2 → H-6)] except for one anomeric proton [$\delta_H$ 4.95 (d, $J = 8.10$ Hz, Glc·H-1)]. The $^{13}C$ NMR and DEPT data[3] showed one $CH_3$, two $CH_2$, five CH, and 11 C signals together with six carbons of one glucosyl moiety, including those for three oxygen-bearing carbons [$\delta_C$ 70.1 (C-13), 71.3 (C-7), and 74.7 (C-6)], five oxygen-bearing olefinic carbons [$\delta_C$ 122.6 (C-9), 145.8 (C-8), 146.7 (C-10), 146.9 (C-2), and 148.7 (C-4)] and nine olefinic carbons [$\delta_C$ 107.0 (C-3), 108.0 (C-1), 108.4 (C-11), 117.4 (C-7a), 118.4 (C-4a), 122.8 (C-7b), 123.0 (C-4b), 125.9 (C-6b) and 146.2 (C-6a)] (see Table 1).

The $^1H$ and $^{13}C$ NMR spectrum indicated the presence of a glucosyl moiety. The anomeric proton signal appeared as a doublet at $\delta_H$ 4.95 ($J = 8.10$ Hz). Incorporating $^{13}C$ NMR chemical shifts it showed the presence of a β-D-glucosyl unit. All the carbons of the glucosyl moiety were assigned through direct $^1H$–$^{13}C$ correlations in the HMQC spectrum and were situated between $\delta$ 62.5 and 81.6 except for that at the anomeric position, which was assigned to the signal at $\delta$ 94.3.

Long range $^1H$–$^{13}C$ correlations (HMBC) (Fig. 2) observed as H-5/C-4a, C-4, C-4b, C-6, C-6a, C-7; H-7/

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**Table 1.** $^{1}H$ and $^{13}C$ NMR, and HMBC data for compound **1**[a]

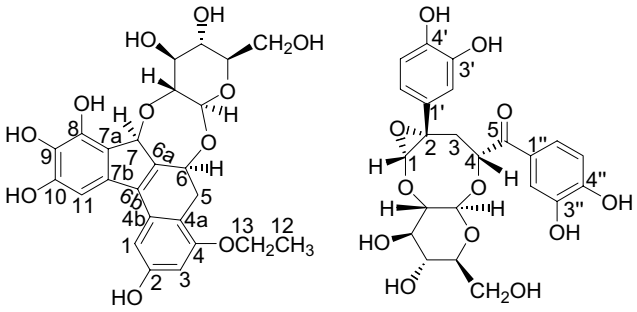
| Position | $\delta_H$ | $\delta_C$ | HMBC (H → C) |
|---|---|---|---|
| 1 | 7.72 s | 108.0 d | 2, 3, 4b |
| 2 | | 146.9 s | |
| 3 | 7.35 s | 107.0 d | 1, 2, 4 |
| 4 | | 148.7 s | |
| 4a | | 118.4 s | |
| 4b | | 123.0 s[b] | |
| 5 | 3.30 m (3.29–3.31) | 23.3 t | 4, 4a, 4b, 6, 6a, 7 |
| | 3.46 m (3.45–3.47) | | |
| 6 | 4.42 dd (3.04, 11.32) | 74.7 d | Glc·C-1 |
| 6a | | 146.2 s | |
| 6b | | 125.9 s | |
| 7 | 5.19 s | 71.3 d | 6, 6a, 7a, 7b, Glc·C-2 |
| 7a | | 117.4 s | |
| 7b | | 122.8 s[b] | |
| 8 | | 145.8 s | |
| 9 | | 122.6 s[b] | |
| 10 | | 146.7 s | |
| 11 | 7.77 s | 108.4 d | 6b, 7a, 7b, 9 |
| 12 | 1.46 t (7.30) | 16.0 q | 13 |
| 13 | 3.95 m (3.93–3.97) | 70.1 t | 4 |
| | 3.83 m (3.82–3.85) | | |
| Glc. | | | |
| 1 | 4.95 d (8.10) | 94.3 d | 6, Glc·C-2 |
| 2 | 3.40 dd (8.10, 8.55) | 81.6 d | 7 |
| 3 | 3.63 dd (8.15, 8.60) | 74.8 d | |
| 4 | 3.49 m (overlapped) | 72.1 d | |
| 5 | 3.48 m (overlapped) | 79.7 d | |
| 6 | 3.91 d (11.95) | 62.5 t | |
| | 3.74 dd (4.70, 11.95) | | |

[a] $J$ in hertz, in $CD_3OD$, $^{1}H$ and $^{13}C$ NMR, and HMBC at 400, 100, and 500 MHz, respectively.
[b] Values may be interchangeable.

C-6, C-6a, C-7a, C-7b; and H-11/C-6b, C-7a, C-7b, C-9, respectively, confirmed the presence of 5, 6-2H-benzo[4a,4b]fluorine in **1**. The linkage of the ethoxyl to C-4 also can be resolved by long range $^{1}H$–$^{13}C$ correlations of H-13/C-4 observed, and can be further confirmed by NOESY correlations of H-13/H-3, H-5; and H-12/H-3. The long range $^{1}H$–$^{13}C$ correlations of Glc·H-1/C-6 and Glc·H-2/C-7 observed, confirmed that the fused glucosyl moiety was Glc·C-1 ether-linked to C-6 and Glc·C-2 to C-7. So compound **1** possessed a unique structural feature of glucosyl-fused 5,6-2*H*-benzo[4a,4b]fluorene in addition to a diox-seven-membered ring.

NOESY correlations of H-6/H-7, H-6/Glc·H-1 indicated the *cis* relationship of H-6 and H-7, incorporating the known stereochemistry of the β-D-glucosyl unit would require 6*S* and 7*S* stereochemistry in **1**. Therefore, the structure of crassifoside E was deduced, as shown in Figure 1, with a novel skeleton.

Crassifoside F (**2**) was isolated as white powder; its molecular formula $C_{23}H_{24}O_{12}$ was established by $^{13}C$ NMR and HRFABMS data, corresponding to an unsaturation index of 12. The IR spectrum of compound **2** showed absorption for hydroxyl (3429 $cm^{-1}$) and conjugated carbonyl (1651 $cm^{-1}$). The $^{1}H$ NMR spectrum[4] exhibited signals for two methylene protons [$\delta_H$ 2.40 (dd, $J = 12.00$, 11.50 Hz, $H_a$-3), 3.25 (dd, $J = 7.65$, 7.30 Hz, $H_b$-3)], two oxymethine protons [$\delta_H$ 4.36 (m, H-4), 5.52 (s, H-1)]. The $^{1}H$ NMR spectrum[4] of **2** also exhibits six aromatic protons. Three of them were assigned to H-2′ [$\delta_H$ 6.98 (d, $J = 2.10$ Hz)], H-5′ [$\delta_H$ 6.69 (d, $J = 8.55$ Hz)], and H-6′ [$\delta_H$ 6.74 (dd, $J = 8.55$, 2.10 Hz)], which suggested the existence of 1,3,4-trisubstituted benzene ring. The remaining three aromatic protons were assigned to H-2″ [$\delta_H$ 7.41 (d, $J = 1.75$ Hz)], H-5″ [$\delta_H$ 6.63 (d, $J = 8.50$ Hz)], and H-6″ [$\delta_H$ 7.36 (dd, $J = 8.50$, 1.75 Hz)] in another 1,3,4-trisubstituted benzene ring, in which H-2″ and H-6″ were shifted downfield due to an *ortho* carbonyl group (IR $\nu_{CO}$ 1651 $cm^{-1}$ and $\delta_{CO}$ 196.7). The $^{13}C$ NMR and DEPT data[4] showed one methylene carbon [$\delta_C$ 36.0 (C-3)],




**1** **2**

**Figure 1.** Structures of compounds **1** and **2**.

**Table 2.** $^{1}H$ and $^{13}C$ NMR, and HMBC data for compound **2**[a]

| Position | $\delta_H$ | $\delta_C$ | HMBC (H → C) |
|---|---|---|---|
| 1 | 5.25 s | 97.8 d | 2, 3, Glc·C-2 |
| 2 | | 93.3 s | |
| 3 | 3.25 dd (7.30, 7.65) | 36.0 t | 1, 2, 4, 5, 1′ |
| | 2.40 dd (11.50, 12.00) | | |
| 4 | 4.36 m (4.34–4.38) | 77.4 d | 2, 3, Glc·C-1 |
| 5 | | 196.7 s | |
| 1′ | | 135.4 s | |
| 2′ | 6.98 d (2.10) | 113.0 d | 2 |
| 3′ | | 145.8 s[b] | |
| 4′ | | 146.4 s | |
| 5′ | 6.69 d (8.55) | 116.4 d | 1′, 4′, 6′ |
| 6′ | 6.74 dd (2.10, 8.55) | 117.1 d | 2 |
| 1″ | | 127.3 s | |
| 2″ | 7.41 d (1.75) | 119.1 d | 5 |
| 3″ | | 145.4 s[b] | |
| 4″ | | 151.6 s | |
| 5″ | 6.63 d (8.50) | 115.1 d | 1″, 4″, 6″ |
| 6″ | 7.36 dd (1.75, 8.50) | 126.2 d | 5 |
| Glc. | | | |
| 1 | 4.66 d (8.10) | 93.9 d | 4 |
| 2 | 3.12 dd (8.10, 9.00) | 76.9 d | 1 |
| 3 | 3.58 dd (8.10, 8.95) | 74.6 d | |
| 4 | 3.30 m (3.29–3.31) | 71.7 d | |
| 5 | 3.38 m (3.37–3.39) | 79.5 d | |
| 6 | 3.84 d (11.55) | 62.4 t | |
| | 3.68 dd (4.70, 11.95) | | |

[a] $J$ in hertz, in $CD_3OD$, $^{1}H$, and $^{13}C$ NMR and HMBC at 400, 100, and 500 MHz, respectively.
[b] Values may be interchangeable.

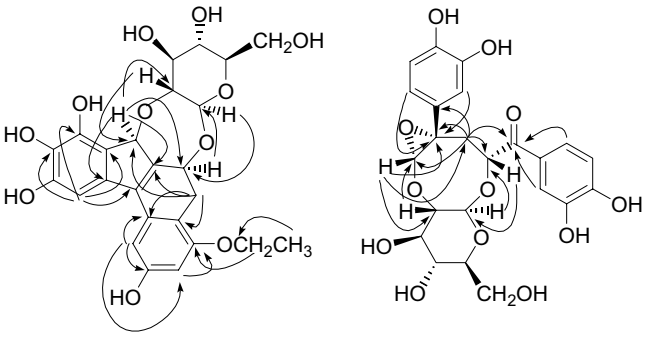


**Figure 2.** Key HMBC correlations of compounds **1** and **2**.

two aliphatic oxymethine carbons [$\delta_C$ 97.8 (C-1), 77.4 (C-4)], one aliphatic oxygen-substituted quaternary carbon [$\delta_C$ 93.3 (C-2)], one conjugated carbonyl carbon [$\delta_C$ 196.7 (C-5)], four oxygen bearing aromatic carbons [$\delta_C$ 145.4 (C-3″), 145.8 (C-3′), 146.4 (C-4′), and 151.6 (C-4″), six aromatic CH [$\delta_C$ 113.0 (C-2′), 115.1 (C-5″), 116.4 (C-5′), 117.1 (C-6′), 119.1 (C-2″), and 126.2 (C-6″)], two aromatic quaternary carbons [$\delta_C$ 127.3 (C-1″), 135.4 (C-1′)] together with six carbons of one glucosyl moiety (see Table 2).

The $^1$H and $^{13}$C NMR spectrum indicated the presence of a glucosyl moiety. The anomeric proton signal appeared as a doublet at $\delta_H$ 4.66 ($J = 8.10$ Hz). Incorporating $^{13}$C NMR chemical shifts, it showed the presence of a β-D-glucosyl unit. All the carbons of the glucosyl moiety were assigned through direct $^1$H–$^{13}$C correlations in the HMQC spectrum and were situated between $\delta$ 62.4 and 79.5 except for that at the anomeric position, which was assigned to the signal at $\delta$ 93.9.

Long range $^1$H–$^{13}$C correlations (HMBC) (Fig. 2) observed of H-1/C-2, C-3, Glc·C-1; H-3/C-1, C-2, C-4, C-5, C-1′; H-4/C-2, C-3; H-2′/C-2, H-6′/C-2, H-2″/C-5, and H-6″/C-5, respectively, together with $^1$H–$^1$H COSY correlation of H-3/H-4, revealed the presence of –$C_1$–$C_2$Ph–$C_3$–$C_4$–$C_5$–Ph in **2**. Long range $^1$H–$^{13}$C correlations was also observed for Glc·H-1/C-4 and Glc·H-2/C-1, which confirmed that the fused glucosyl moiety was Glc·C-1 ether-linked to C-4 and Glc·C-2 to C-1. So the structure of **2** possessed a novel glucosyl-fused diox-eight-membered ring moiety. According to the molecular formula ($C_{23}H_{24}O_{12}$), an unsaturation index of 12 and the low-field chemical shift of C-1 and C-2, we may conclude an oxygen atom was linked to C-1 and C-2 to form a three-membered ring.

NOESY correlations of H-1/H-4, H-1/Glc·H-2 indicated the *cis* relationship of H-1 and H-4. Incorporating the known stereochemistry of the β-D-glucosyl unit, we may conclude the stereochemistry of **2** would require 1*R*, 2*S*, and 4*R*. Therefore, the structure of crassifoside F was deduced as shown in Figure 1, with a novel skeleton.

The two novel glucosyl-fused compounds were tested for the ACE inhibitor activities by inspecting fluorescent light, and captopril were used as positive control substance with an $IC_{50}$ value of 27.5 μg/mL. crassifoside E (**1**) and crassifoside F (**2**) displayed dose dependent inhibition with an $IC_{50}$ value of 10 and 8.5 μg/mL, respectively, but less than the positive control captopril ($IC_{50}$, 27.5 μg/mL).

To our knowledge, **1** represents the first natural occurrence of a glucosyl-fused 5,6-2*H*-benzo[4a,4b]fluorene in addition to a diox-seven-membered ring skeleton, **2** represents the first natural occurrence of a glucosyl-fused diox-eight-membered ring skeleton. The trivial name, crassifoside E and crassifoside F, are made after their plant origin.

## Acknowledgements

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## References and notes

1. Institutum Botanicum Kunmingense, Academiae Sinicae, *Flora Yunnanica*, Science Press: Beijing, 1995; Vol. 6, p 819.
2. Li, N.; Chen, J. J.; Zhou, J. *Helv. Chim. Acta* **2004**, *87*, 845–850.
3. Crassifoside E (**1**). Brown amorphous powder, $[\alpha]_D^{24}$ +23.1 (*c* 0.2, MeOH). IR (KBr) $\nu_{max}$ cm$^{-1}$: 3440, 2926, 2369, 1623, 1558, 1507, 1114, 1035, 615, 469; UV (MeOH) $\lambda_{max}$ nm: 224 (4.42), 258 (4.75), 294 (4.46), 347 (3.31). FAB-MS (−) *m*/*z*: 501 [M$^+$−H], HRFAB-MS (−) *m*/*z*: Found 501.1402. Calcd 501.1397; $C_{25}H_{25}O_{11}$ [M$^+$−H]). $^1$H NMR and $^{13}$C NMR data see Table 1.
4. Crassifoside F (**2**). White amorphous powder, $[\alpha]_D^{28}$ +112.9 (*c* 0.2, MeOH). IR (KBr) $\nu_{max}$ cm$^{-1}$: 3429, 2925, 1651, 1606, 1558, 1521, 1436, 1363, 1294, 1131, 1032, 873, 591; UV (MeOH) $\lambda_{max}$ nm: 196 (4.09), 206 (4.50), 232 (4.18), 283 (3.99), 315 (3.86). FAB-MS (−) *m*/*z*: 491 [M$^+$−H], HRFAB-MS (−) *m*/*z*: Found 491.1176. Calcd 491.1190; $C_{23}H_{23}O_{12}$ [M$^+$−H]). $^1$H NMR and $^{13}$C NMR data see Table 2.