SUPPLEMENTARY MATERIAL

Carbazole-pyranocoumarin conjugate and two carbazole alkaloids from the stems of *Clausena excavata*

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Abstracts

A carbazole-pyranocoumarin conjugate, carbazomarin B (1) and two carbazole alkaloids, 6-methoxymukonidine (2) and 2-hydroxy-3-methoxycarbazole (3), together with 27 known compounds (4–30), were isolated from the stems of *Clausena excavata*. Their structures have been elucidated by spectroscopic analyses. Compound 2 showed moderate cytotoxicity to HuCCA-1, MOLT-3 and HepG2 cancer cell lines with IC\textsubscript{50} values of 15.09–28.50 \(\mu\)g/mL, but none to A549 cell line. Heptaphylline (6) and nordentatin (23) were found to show moderate cytotoxic activity against HepG2 cell line with IC\textsubscript{50} values of 12.33 and 11.33, respectively, while clausine K (27) exhibited strong cytotoxicity with IC\textsubscript{50} value of 1.05 \(\mu\)g/mL, better than a standard drug (etoposide, IC\textsubscript{50} 13.40 \(\mu\)g/mL).

Keywords: *Clausena excavata*; carbazole alkaloids; Rutaceae; cytotoxic activity

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Experimental

Extraction and Isolation for all pure compounds

Chopped-dried stems of *C. excavata* (4.73 kg) were immersed in CH$_2$Cl$_2$ (16 l) at room temperature for 4 days. After evaporation, a dark brown gum of CH$_2$Cl$_2$ extract (23.4 g) was subjected to QCC over silica gel and eluted with a gradient of CH$_2$Cl$_2$–hexane (1:1, v/v), CH$_2$Cl$_2$ and MeOH to furnish 12 fractions (A–L). Fraction B was further purified by CC over silica gel and eluted with CH$_2$Cl$_2$–hexane (3:7, v/v) to give 9 fractions (B1–B9). Subfraction B2 was purified by CC over silica gel and eluted with EtOAc–hexane (3:97, v/v) to afford 13 fractions (B2A–B2M). Subfraction B2K was further purified on prep. TLC eluting with EtOAc–CH$_2$Cl$_2$–hexane (1:2:17, v/v) to give 4 (murrayafoline A) (3.4 mg). Subfraction B3 was purified by CC over silica gel and eluted with CH$_2$Cl$_2$–hexane (3:7, v/v) to afford 12 fractions (B3A–B3L). Subfraction B3D was further purified on prep. TLC eluting with CH$_2$Cl$_2$–hexane (3:7, v/v, 2 runs) to give 5 (girinimbine) (2.1 mg). Subfraction B3H was further purified on prep. TLC eluting with Me$_2$CO–hexane (3:17, v/v) to give 7 fractions (E1–E7). Subfraction E3 was purified by CC over silica gel and eluted with Me$_2$CO–hexane (3:17, v/v) to afford 5 fractions (E3A–E3E). Subfraction E3A was purified by CC over silica gel and eluted with Me$_2$CO–hexane (3:17, v/v) to afford 6 fractions (E3A1–E3A6). Subfraction E3A5 gave 7 (dentatin) (16.3 mg). Subfraction E3B was purified by CC over silica gel and eluted with Me$_2$CO–hexane (3:17, v/v) to afford 7 fractions (E3B1–E3B7). Subfraction E3B7 was purified by CC over silica gel and eluted with Me$_2$CO–hexane (3:17, v/v) to afford 7 fractions (E3B7A–E3B7G). Subfraction E3B7D gave 8 (mukonidine) (4.8 mg). Subfraction E4 was purified by CC over silica gel and eluted with Me$_2$CO–hexane (3:17, v/v) to afford 8 fractions (E4A–E4H). Subfraction E4H was purified by CC over silica gel and eluted with Me$_2$CO–hexane (3:17, v/v) to afford 6 fractions (E4H1–E4H6). Subfraction E4H4 was further purified on prep. TLC and eluted with Me$_2$CO–hexane (3:17, v/v, 4 runs) to give 9 (xanthoxylatin) (1.2 mg). Subfraction E4H5 was further purified on prep. TLC and eluting with CH$_2$Cl$_2$–hexane (4:1, v/v, 4 runs) to give 10 (mukonine) (6.0 mg) and 11 (mukonal) (4.0 mg). Subfraction E6 gave 12 (murrayanine) (62.0 mg). Fraction F was purified by CC over silica gel and eluted with EtOAc–hexane (3:17, v/v) to give 16 fractions (F1–F16). Subfraction F5 gave 2 (79.1 mg). Subfraction F8 was further purified on prep. TLC, eluting with CH$_2$Cl$_2$–hexane (4:1, v/v, 4 runs) to give 13 (lansine) (6.4 mg). Subfraction F10 was purified by CC over silica gel and eluted with Me$_2$CO–hexane (1:4, v/v) to give 5 fractions.
Subfraction F10C was purified by CC over silica gel and eluted with Me$_2$CO–hexane (1:3, v/v) to give 5 fractions (F10C1–F10C5). Subfraction F10C2 was further purified on prep. TLC, eluting with CH$_2$Cl$_2$–hexane (9:1, v/v, 3 runs) to give 14 (3-formylcarbazole) (3.1 mg) and 15 (dictamine) (1.1 mg). Subfraction F11 was purified by CC over silica gel and eluted with CH$_2$Cl$_2$–hexane (7:3, v/v) to give 6 fractions (F11A–F11F). Subfraction F11B was further purified on prep. TLC, eluting with EtOAc–hexane (1:4, v/v, 5 runs) to give 16 (7-methoxy methyl carbazole-3-carboxylate) (1.3 mg). Subfraction F13 gave 17 (O-methylmukonal) (83.8 mg). Fraction G was further purified by CC over silica gel and eluted with EtOAc–hexane (1:4, v/v) to give 23 fractions (G1–G23). Subfraction G12 and G14 were further purified on prep. TLC, eluting with CH$_2$Cl$_2$–hexane (17:3, v/v, 4 runs) to give 18 (7-methoxymukonal) (4.5 mg) and 19 (hortiamide) (4.8 mg). Subfraction G20 was purified by CC over silica gel and eluted with CH$_2$Cl$_2$–hexane (3:2, v/v) to afford 8 fractions (G20A–G20H). Subfraction H4B was separated by CC with Sephadex LH–20, eluted with CH$_2$Cl$_2$ to afford 6 fractions (H4B1–H4B6). Subfraction H4B4 was further purified on prep. TLC, eluting with Me$_2$CO–hexane (1:9, v/v, 8 runs) to give 22 (kinocoumarin) (1.3 mg) and 23 (nordentatin) (1.3 mg). Subfraction H5 gave 24 (7-hydroxy-8-(1,1-dimethylallyl)citrusarin) (76.8 mg). Subfraction H8 was purified by CC over silica gel and eluted with EtOAc–hexane (3:7, v/v) to afford 8 fractions (H8A–H8H). Subfraction H8E was further purified on prep. TLC, eluting with EtOAc–hexane (3:7, v/v, 3 runs) to give 1 (2.0 mg). Subfraction H12 gave 25 (clausine H) (20.0 mg). Subfraction H22 was further purified on prep. TLC, eluting with Me$_2$CO–hexane (3:7, v/v, 4 runs) to give 26 (isomukonidine) (0.6 mg) and 27 (clausine K) (1.1 mg). Fraction I was purified by CC over silica gel and eluted with Me$_2$CO–hexane (1:3, v/v) to afford 15 fractions (I1–I15). Subfraction I11 was purified by CC over silica gel and eluted with EtOAc–hexane (3:7, v/v) to afford 13 fractions (I11A–I11M). Subfraction I11F was further purified on prep. TLC, eluting with EtOAc–hexane (3:7, v/v, 5 runs) to give 28 (valencic acid) (4.5 mg). Fraction J was purified by CC over silica gel and eluted with
Me$_2$CO–hexane (3:7, v/v) to afford 29 (clausenarin) (799.3 mg). Fraction K was purified by CC over silica gel and eluted with Me$_2$CO–hexane (3:7, v/v) to afford 30 (O-methylclausenolide) (92.4 mg).
Figure S1. Selected HMBC correlation (H → C) of compounds 1–3.

Table S1. NMR Spectroscopic Data in CDCl₃ (¹H NMR (300 MHz) and ¹³C NMR (125 MHz)) for Compound 1.

| Position | Carbazole Unit | Pyranocoumarin Unit |
|----------|---------------|---------------------|
|          | δC, type      | δH, (J in Hz)       |          | δC, type      | δH, (J in Hz)       |
| 1a       | 137.3, C      |                     | 2´       | 161.2, C      |                     |
| 1        | 107.2, C      |                     | 3´       | 110.7, CH     | 6.06, d (9.7)       |
| 2        | 148.5, C      |                     | 4´       | 139.2, CH     | 7.84, d (9.7)       |
| 3        | 114.3, C      |                     | 4´a      | 103.9, C      |                     |
| 4        | 120.9, CH     | 7.70, s             | 5´       | 150.3, C      |                     |
| 4a       | 118.6, C      |                     | 6´       | 109.8, C      |                     |
| 5a       | 123.4, C      |                     | 7´       | 156.3, C      |                     |
| 5        | 102.7, CH     | 7.38, d (2.6)       | 8´       | 116.5, C      |                     |
| 6        | 154.0, C      |                     | 8´a      | 152.9, C      |                     |
| 7        | 113.9, CH     | 6.95, dd (8.8, 2.6) | 2´´      | 77.7, C       |                     |
| 8        | 111.2, CH     | 7.14, d (8.8)       | 3´´      | 38.5, CH₂     | 2.34, dd (14.3, 9.4) |
| 8a       | 134.3, C      |                     |          | 2.28, dd (14.3, 9.6) |
| 3-CH₃    | 16.4, CH₃     | 2.49, s             | 4´´´     | 24.9, CH      | 5.07, dd (9.6, 9.4) |
| 6-OCH₃   | 56.1, CH₃     | 3.88, s             | 5´´´     | 23.4, CH₃     | 1.36, s             |
| NH       | 7.26, (br s)  |                     | 6´´´     | 29.4, CH₃     | 1.58, s             |
|          |               |                     | 1´´´     | 41.3, C       |                     |
|          |               |                     | 2´´´     | 150.6, CH     | 6.40, dd (17.6, 10.3) |
|          |               |                     | 3´´´     | 108.3, CH₂    | 5.00, d (10.3, 1.2) |
|          |               |                     |          | 5.01, d (17.6, 1.2) |
|          |               |                     | 4´´´     | 29.9, CH₃     | 1.76, s             |
|          |               |                     | 5´´´     | 30.0, CH₁     | 1.79, s             |
**Table S2.** NMR Spectroscopic Data in CD$_3$COCD$_3$ ($^1$H NMR (300 MHz) and $^{13}$C NMR (75 MHz)) for Compounds 2 and 3.

| Position | $\delta_C$, type | $\delta_H$, ($J$ in Hz) | Position | $\delta_C$, type | $\delta_H$, ($J$ in Hz) |
|----------|-----------------|----------------------|----------|-----------------|----------------------|
| 1a       | 146.2, C        |                      | 1a       | 141.1, C        |                      |
| 1        | 96.6, CH        | 6.89, s              | 1        | 94.2, CH        | 7.09, s              |
| 2        | 160.5, C        |                      | 2        | 134.4, C        |                      |
| 3        | 104.6, C        |                      | 3        | 147.8, C        |                      |
| 4        | 122.7, CH       | 8.59, s              | 4        | 104.8, CH       | 7.51, s              |
| 4a       | 1117.1, C       |                      | 4a       | 115.8, C        |                      |
| 5a       | 124.0, C        |                      | 5a       | 123.5, C        |                      |
| 5        | 103.0, CH       | 7.69, d (2.4)        | 5        | 119.1, CH       | 7.93, d (8.0)        |
| 6        | 154.6, C        |                      | 6        | 118.2, CH       | 7.07, td (8.0, 1.2)  |
| 7        | 114.3, CH       | 6.99, dd (8.7, 2.4)  | 7        | 123.7, CH       | 7.24, td (8.0, 1.2)  |
| 8        | 1114.1, CH      | 7.36, d (8.7)        | 8        | 110.4, CH       | 7.40, d (8.0)        |
| 8a       | 130.5, C        |                      | 8a       | 140.2, C        |                      |
| NH       | 103.7, br s     |                      | NH       | 10.01, br s     |                      |
| 2-OH     | 110.05, br s    |                      | 2-OH     | 7.14, s         |                      |
| 3-CO$_2$CH$_3$ | 171.3, C     |                      | 3-CO$_2$CH$_3$ | 55.6, CH$_3$  | 3.93, s              |
| 3-CO$_2$CH$_3$ | 51.6, CH$_3$   | 3.99, s              | 3-OCH$_3$ | 55.2, CH$_3$   | 3.89, s              |
| 6-OCH$_3$ | 55.2, CH$_3$   |                      |          |                 |                      |
### Table S3. Cytotoxic activities (IC$_{50}$, µg/mL, mean ± SD, n = 3) of isolated compounds.

| Compounds | HuCCA-1 | A549 | MOLT-3 | HepG2 |
|-----------|---------|------|--------|-------|
| 2         | 22.60±1.98 | >50  | 15.09±0.88 | 28.50±2.12 |
| 6         | 14.80±3.33  | 19.40±2.26 | 1.99±0.20  | 12.33±2.52  |
| 7         | >50      | >50  | 7.82±0.79  | 26.50±1.29  |
| 8         | >50      | >50  | >25       | >50       |
| 9         | >50      | >50  | 31.36±3.34 | >50       |
| 11        | 44.18±3.29 | 46.00±5.66 | 7.27±0.25  | 34.00±4.36  |
| 12        | 37.60±0.28 | 48.40±2.26 | 9.20±0.33  | 28.75±3.50  |
| 17        | 26.80±7.35 | 15.90±4.36 | 3.96±0.28  | 20.25±6.50  |
| 18        | 17.20±4.53 | >50  | 3.54±0.14  | 26.75±6.99  |
| 20        | >50      | >50  | 2.81±0.17  | >50       |
| 21        | 32.50±0.85 | 30.90±2.69 | 13.52±1.99 | 19.00±1.00  |
| 22        | 17.90±8.49 | 11.70±0.25 | 7.49±0.42  | 16.33±0.44  |
| 23        | 10.85±0.85 | 17.50±6.32 | 3.54±0.06  | 11.33±0.58  |
| 24        | >50      | >50  | 8.74±0.31  | 15.00±2.65  |
| 25        | >50      | >50  | 31.05±2.11 | 29.25±6.99  |
| 27        | 12.45±0.21 | 13.20±0.99 | 6.00±0.79  | 1.05±0.83   |
| 29        | >50      | >50  | >50       | >50       |
| 30        | >50      | >50  | 43.99±3.97 | >50       |
| Doxorubicin$^a$ | 0.23±0.08 | 0.13±0.08 | ND       | 0.26±0.08   |
| Etoposide$^a$ | ND      | ND   | 0.02±0.005 | 13.40±1.52  |

$^a$ Cytotoxicity was tested against the following cell lines: MOLT-3 = T-lymphoblast (acute lymphoblastic leukemia) cell line; A549 = human lung carcinoma cell line; HuCCA-1 = human cholangiocarcinoma cancer cells, and HepG2 = Human hepatocellular liver carcinoma cell line.

$^b$ Etoposide and doxorubicin were used as the standard drugs.
Figure S2. $^1$H NMR spectrum of carbazomarin B (1) (300 MHz, CDCl$_3$).

Figure S3. $^{13}$C NMR spectrum of carbazomarin B (1) (125 MHz, CDCl$_3$).
Figure S4. $^1$H NMR spectrum of 6-methoxymukonidine (2) (300 MHz, CD$_3$COCD$_3$).

Figure S5. $^{13}$C NMR spectrum of 6-methoxymukonidine (2) (75 MHz, CD$_3$COCD$_3$).
Figure S6. $^1$H NMR spectrum of 2-hydroxy-3-methoxycarbazole (3) (300 MHz, CD$_3$COCD$_3$).

Figure S7. $^{13}$C NMR spectrum of 2-hydroxy-3-methoxycarbazole (3) (75 MHz, CD$_3$COCD$_3$).