Furanolabdane Diterpenes from *Hypoestes purpurea*

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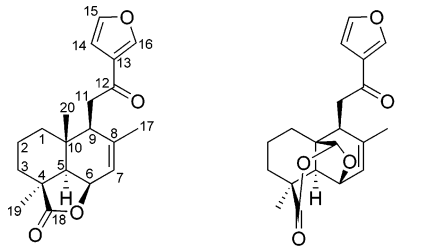
Four new furanolabdane diterpenes, hypopurin A (**1**), hypopurin B (**2**), hypopurin C (**3**), and hypopurin D (**4**), together with eight lignans, α-*O*-methylcubebin, *β*-*O*-methylcubebin, hinoquinin, helioxanthin, 7-hydroxyhinokinin, dehydroxycubebin, justicidine E, and (−)-hibalactone, as well as two triterpenes, lupeol and betulin, were isolated from the dried aerial part of *Hypoestes purpurea*. The structures of **1**−**4** were elucidated mainly on the basis of NMR and MS. Compound **1** was found to be moderately cytotoxic toward the KB cell line with an IC_{50} value of 9.4 μM.

The aerial parts of *Hypoestes purpurea* R. Br. (Acanthaceae) have been used as an antipyretic, antiphlogistic, and liver protective agent in Taiwanese folk medicine. A literature survey revealed that the genus *Hypoestes* has yielded some diterpenes,[1−8] a lignan,[5] and an alkaloid.[9] To our knowledge, no phytochemical study on *H. purpurea* has been reported. In this paper, we report on the isolation of four new furanolabdane diterpenes, eight known lignans, and two known triterpenes from *H. purpurea*.

The MeOH extract of the aerial parts of *H. purpurea* was subjected repeatedly to silica gel columns, Sephadex LH-20 columns, and PTLC to afford four new diterpenes, **1**−**4**, together with eight lignans, α-*O*-methylcubebin,[10,11] *β*-*O*-methylcubebin,[10,11] hinoquinin,[12] helioxanthin,[13] 7-hydroxyhinokinin,[14] dehydroxycubebin,[15] justicidine E,[16] and (−)-hibalactone,[17] as well as two triterpenes, lupeol[18] and betulin.[18]



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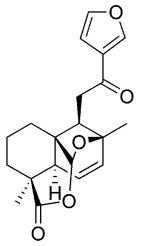

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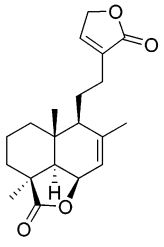

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Compound **1** was obtained as an amorphous powder. The molecular formula $C_{20}H_{24}O_4$ was deduced from HREIMS and NMR spectra. The ^{13}C and DEPT spectra showed three methyl, four methylene, seven methine, and six quaternary carbons. The signals at δ_C 193.9 and 182.4 were derived from two carbonyl groups, which also displayed an absorption band at 1756 cm^{-1} in the IR spectrum. The one bond $^1H-^{13}C$ connectivities were analyzed using HMQC data. The proton signals at δ 8.07, 7.45, and 6.78 correlated to carbon signals at δ 146.9, 144.4, and 108.6, respectively, in the HMQC spectrum, which suggested the presence of a 3-substituted furan ring in compound **1**. Furthermore, the quaternary carbon at δ 127.5 correlated to the protons at δ 8.07, 7.45, and 6.78 in the HMBC spectrum and thus was attributed to C-13. The COSY spectrum exhibited cross-peaks between H-6 (δ 4.85) and two protons, H-5 (δ 1.87) and H-7 (δ 5.79), which revealed that an oxymethine group was attached to a double bond and a methine group. On the basis of COSY and HMQC spectra, the connectivities of C-1, C-2, C-3 and C-9, C-11 were deduced. Further connectivities were established by a long-range HMBC experiment. Cross-peaks of H-17/C-7, C-8, C-9, H-19/C-3, C-4, C-5, C-18, and H-20/C-1, C-5, C-9, C10 were observed, which indicated that three methyl groups were attached to an octahydronaphthalene moiety. The HMBC data further showed cross-peaks of H-11/C-8, C-9, C-10, C-12 and H-14/C-12, C-13, C-15, C-16, suggesting that a 2-(3-furanyl)-2-oxoethyl group was linked to C-8. The relative stereochemistry of **1** was deduced from a NOESY experiment, which exhibited cross-peaks of H-5/H-6, H-9, H-6/H-5, H-19, and H-20/H-11. On the basis of these NOESY data, H-5, H-6, and H-19 were on the α-face of the octahydronaphthalene ring and the 2-(3-furanyl)-2-oxoethyl and methyl groups at C-9 and C-10, respectively, were on the *β*-face. Accordingly, the structure of diterpene **1** was elucidated, and it was given the trivial name hypopurin A.

Compound **2** was obtained as an amorphous powder, whose molecular formula was established as $C_{20}H_{22}O_5$ from its HREIMS and NMR spectra. Its ^1H and ^{13}C NMR spectra were similar to those of **1** except that the C-20 methyl signal (δ_H 0.88, δ_C 18.9) disappeared and one dioxymethine group (δ_H 5.43, δ_C 105.3) was observed. In addition, the signal of C-10 shifted downfield from δ 33.7 to 46.7. The HMBC spectrum indicated that the dioxymethine group at δ_H 5.43 was correlated to C-5, C-6, C-9, C-10, and C-18, and the oxymethine group at δ_H 4.55 was correlated to C-4, C-5, C-7, C-8, and C-20, which indicated that compound **2** contained a cyclic acetal structure with an ether linkage

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to C-6 and an ester linkage to C-18. Thus, the structure of compound **2** was determined, and it was named hypopurin B.

Compound **3** was obtained as an amorphous powder with the same molecular formula as **2**. Its ^{1}H NMR spectrum was similar to that of **2** except that the signal of H-6 shifted downfield from δ 4.55 to 5.81 with a related carbon signal at δ 126.8. In the ^{13}C NMR spectrum, the signal of quaternary C-8 also shifted from δ 141.3 to 81.7, which suggested that the double bond between C-7 and C-8 in **2** was shifted to C-6 and C-7 and that C-8 was oxygenated. Moreover, the HMBC spectrum exhibited correlation of H-20 to C-1, C-5, C-8, C-10, and C-18, and C-8 was also correlated to H-6, H-7, H-11, H-17, and H-20, which supported a cyclic acetal structure with an ether linkage to C-8 and an ester linkage to C-18. The stereochemistry of **3** was established by the NOESY spectrum, which showed cross-peaks of H-20/H-11 and H-5/H-6, H-19. Thus, the structure of diterpene **3** was determined, and it was named hypopurin C.

Compound **4** was obtained as colorless prisms. The molecular formula $C_{20}H_{26}O_4$ was deduced from HREIMS and NMR spectra. Its ^{1}H and ^{13}C spectral data were close to those of **1** except for the signals of the 2-(3-furanyl)-2-oxoethyl moiety. The ^{1}H NMR spectrum showed only one signal in the range δ 6.6−8.2 rather than three proton signals of the furan ring in hypopurin A. A signal at δ_H 5.78 indicated an olefinic proton at C-7. Also noted was a signal for the oxymethylene group at δ_H 4.78, which exhibited a cross-peak with the signal at δ_H 7.13 in the COSY spectrum. The ^{13}C NMR spectrum of **4** displayed only four signals in the region δ 105−150 instead of six signals for a furan ring and a double bond observed for **1**. These four carbons constituted two double bonds and were associated with two hydrogens resonating at δ 7.13 and 5.78, which were exhibited in the HMQC spectrum. The C-12 signal present in **1** was absent in the ^{13}C NMR spectrum of **4**, whereas two carbon signals resonating at δ 182.4 and 174.1 were observed, suggesting the presence of two ester groups. Moreover, another methylene group was observed at δ_H 2.30 and 2.53 with a related carbon signal at δ 27.3, which correlated to C-11, C-13, C-14, and C-16 in the HMBC spectrum. The HMBC spectrum also exhibited correlations of H-14 and H-15 to C-16 (δ 174.1). Thus, a structure with a 2,5-dihydro-2-furanone ring instead of a furan ring could be deduced in compound **4**, and the ketone group in **1** was replaced by a methylene group. The stereochemistry of **4** was further confirmed by NOE difference experiments. The signals of H-5 and H-6 were enhanced by irradiation of H-19, and the irradiation of H-20 resulted in an enhancement of the H-11 signal, which revealed that compound **4** had the same relative stereostructure at C-4, C-5, C-6, C-9, and C-20 as hypopurin A. Accordingly, the structure of compound **4** was established, and it was named hypopurin D.

Compounds **1**−**4** were tested in vitro for cyctotoxicity using the epidermoid carcinoma KB cell line. Hypopurin A (**1**) showed moderate cytotoxic toward the KB cell line with an IC_{50} value of 9.4 μM; however, the other three compounds were inactive (IC_{50} > 100 μM).

Experimental Section

General Experimental Procedures. Melting points were determined on a Yanaco MP-I3 micro melting point apparatus, and the thermometer was used without correction. IR spectra were recorded on a Nicolet Avatar 320 FT-IR spectrometer. ^{1}H and ^{13}C NMR spectra were taken on a Varian Unity INOVA 500 spectrometer. UV spectra were measured on a Hitachi U-3200 spectrophotometer. EIMS and HREIMS spectra were obtained using Finnigan MAT GCQ and Finnigan MAT 95S spectrometers, respectively. Optical rotations were taken with a JASCO DIP-370 digital polarimeter.

Plant Material. The aerial parts of *Hypoestes purpurea* were collected in Taipei, in July 2003. A voucher specimen (NRICM-03-007) is deposited at the Herbarium of National Research Institute of Chinese Medicine, R.O.C.

Extraction and Isolation. The dried aerial parts of *H. purpurea* (8.5 kg) were extracted with refluxing in MeOH (60 L × 3). The extract was concentrated in vacuo to give a dark brown residue, which was subjected to a silica gel column eluting with a gradient of *n*-hexane−EtOAc (20:1 to 1:10) and EtOAc−MeOH (10:1 to 1:1), to provide 11 fractions. Fraction 3 (eluate of *n*-hexane−EtOAc, 10:1) was further chromatographed over silica gel (*n*-hexane−EtOAc, 10:1 to 5:1) to give lupeol[18] (59 mg). Fraction 4 (eluate of *n*-hexane−EtOAc, 5:1) was chromatographed over silica gel (*n*-hexane−EtOAc, 5:1) and Sephadex LH-20 (MeOH) to afford α-*O*-methylcubebin[10,11] (8.7 mg) and β-*O*-methylcubebin[10,11] (4.7 mg). Fraction 5 (eluate of *n*-hexane−EtOAc, 3:1) was chromatographed over silica gel (*n*-hexane−EtOAc, 7:1 to 4:1) and Sephadex LH-20 (MeOH) to give hinoquinin[12] (248 mg), helioxanthin[13] (5.7 mg), **1** (13 mg), **2** (12 mg), and betulin[18] (3.6 mg). Fraction 6 (eluate of *n*-hexane−EtOAc, 1:1) was repeatedly chromatographed over silica gel (*n*-hexane−EtOAc, 4:1 to 1:1), Sephadex LH-20 (MeOH), and PTLC (*n*-hexane−EtOAc, 1:1) to afford **3** (8.1 mg), 7-hydroxyhinokinin[14] (1.8 mg), dehydroxycubebin[15] (2.9 mg), justicidine E[16] (2.1 mg), and (−)-hibalactone[17] (63 mg). Fraction 7 (eluate of EtOAc) was chromatographed over a Sephadex LH-20 (MeOH) column to give **4** (7.4 mg).

Hypopurin A (1): colorless powder, mp 125−127 °C (MeOH), $[\alpha]^{25}_D$ +43.3 (*c* 0.3, $CHCl_3$); UV λ_{max} ($CHCl_3$) nm (log ϵ) 253 (3.53), 235 (3.44); IR ν_{max} (KBr) cm^{-1} 3122, 2944, 1756, 1667, 1561, 1509, 1383, 1194, 1157, 1115, 868; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.07 (1H, s, H-16), 7.45 (1H, s, H-15), 6.78 (1H, d, J = 2.0 Hz, H-14), 5.89 (1H, d, J = 4.0 Hz, H-7), 4.85 (1H, br s, H-6), 2.93 (1H, m, H-9), 2.91 (1H, m, H-11a), 2.62 (1H, dd, J = 22, 7.5 Hz, H-11b), 2.10 (1H, ddd, J = 14, 9.5, 4.5 Hz, H-3β), 1.87 (1H, d, J = 5.0 Hz, H-5), 1.70 (1H, m, H-2β), 1.62 (3H, s, H-17), 1.53 (1H, m, H-2α), 1.51−1.42 (2H, m, H-1β and H-3α), 1.31−1.23 (1H, m, H-1α), 1.30 (3H, s, H-19), 0.88 (3H, s, H-20); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 193.9 (C-12), 182.4 (C-18),146.9 (C-16), 144.4 (C-15), 143.6 (C-8), 127.5 (C-13), 119.0 (C-7), 108.6 (C-14), 73.6 (C-6), 50.8 (C-5), 45.2 (C-9), 42.8 (C-4), 37.3 (C-11), 33.7 (C-10), 33.6 (C-1), 28.0 (C-3), 24.0 (C-19), 21.9 (C-17), 18.9 (C-20), 18.0 (C-2); EIMS *m/z* 329 $[M + H]^+$ (49), 218 (100), 159 (60); HREIMS *m/z* $[M + H]^+$ 329.1709 (calcd for $C_{20}H_{24}O_4$+H, 329.1747).

Hypopurin B (2): colorless powder, mp 120−122 °C (MeOH), $[\alpha]^{25}_D$ +30.3 (*c* 0.3, $CHCl_3$); UV λ_{max} ($CHCl_3$) nm (log ϵ) 254.5 (3.26), 233 (3.24); IR ν_{max} (KBr) cm^{-1} 2933, 1730, 1672, 1561, 1509, 1383, 1157, 1125, 994; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.06 (1H, s, H-16), 7.46 (1H, t, J = 2.0 Hz, H-15), 6.78 (1H, d, J = 2.0 Hz, H-14), 5.87 (1H, d, J = 6.0 Hz, H-7), 5.43 (1H, s, H-20), 4.55 (1H, d, J = 6.0 Hz, H-6), 3.21 (1H, d, J = 8.0 Hz, H-9), 2.91 (1H, dd, J = 17, 8.5 Hz, H-11a), 2.81 (1H, dd, J = 17, 2.5 Hz, H-11b), 2.24 (1H, m, H-1β), 1.95 (1H, s, H-5), 1.82 (1H, m, H-3β), 1.75−1.62 (3H, m, H-1α and H-2), 1.48 (1H, m, H-3α), 1.44 (3H, s, H-17), 1.31 (3H, s, H-19); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 192.6 (C-12), 175.5 (C-18),147.3 (C-16), 144.6 (C-15), 141.3 (C-8), 127.5 (C-13), 126.7 (C-7), 108.6 (C-14), 105.3 (C-20), 73.6 (C-6), 54.0 (C-5), 46.7 (C-10), 46.3 (C-9), 44.2 (C-4), 39.3 (C-11), 38.3 (C-3), 28.2 (C-1), 21.8 (C-19), 20.6 (C-17), 20.3 (C-2); EIMS *m/z* 343 $[M + H]^+$ (100), 267 (54), 251 (55), 159 (92); HREIMS *m/z* $[M + H]^+$ 343.1507 (calcd for $C_{20}H_{22}O_5$+H, 343.1540).

Hypopurin C (3): colorless powder, mp 165−168 °C (MeOH), $[\alpha]^{25}_D$ +13.3 (*c* 0.3, $CHCl_3$); UV λ_{max} ($CHCl_3$) nm (log ϵ) 254 (3.28), 230 (3.28), 226 (3.25); IR ν_{max} (KBr) cm^{-1} 2938, 1730, 1677, 1556, 1509, 1388, 1157, 1131, 1004; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.08 (1H, s, H-16), 7.46 (1H, t, J = 1.5 Hz, H-15), 6.77 (1H, d, J = 1.5 Hz, H-14), 6.01 (1H, dd, J = 9.5, 2.0 Hz, H-7), 5.81 (1H, dd, J = 9.0, 2.5 Hz, H-6), 5.49 (1H,

d, J = 1.5 Hz, H-20), 3.00 (1H, t, J = 6.5 Hz, H-9), 2.92 (1H, dd, J = 18, 6.5 Hz, H-11a), 2.80 (1H, dd, J = 18, 6.5 Hz, H-11b), 2.56 (1H, q, J = 2.0 Hz, H-5), 1.95 (1H, m, H-3β), 1.80 (1H, m, H-2β), 1.69 (1H, m, H-1β), 1.56−1.40 (3H, m, H-1α, H-2α, and H-3α), 1.33 (3H, s, H-19), 1.23 (3H, s, H-17); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 192.8 (C-12), 173.4 (C-18), 147.1 (C-16), 144.5 (C-15), 138.2 (C-7), 127.5 (C-13), 126.8 (C-6), 108.6 (C-14), 108.3 (C-20), 81.7 (C-8), 50.7 (C-5), 48.8 (C-9), 46.8 (C-10), 43.5 (C-4), 38.3 (C-3), 35.7 (C-11), 30.3 (C-1), 23.4 (C-19), 21.4 (C-17), 21.3 (C-2); EIMS m/z 343 $[M + H]^+$ (49), 269 (53), 251 (47), 233(55), 159 (100); HREIMS m/z $[M + H]^+$ 343.1506 (calcd for $C_{20}H_{22}O_5$+H, 343.1540).

Hypopurin D (4): colorless prisms, mp 173−175 °C (MeOH), $[\alpha]^{25}_D$ +15 (c 0.2, MeOH); UV λ_{max} (MeOH) nm (log ϵ) 203 (4.39); IR ν_{max} (KBr) cm^{-1} 3070, 2954, 1740, 1646, 1451, 1388, 1357, 1199, 1115, 1078, 1057, 1010, 978, 905, 847; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.13 (1H, s, H-14), 5.78 (1H, br s, H-7), 4.81 (1H, br s, H-6), 4.78 (1H, d, J = 1.5 Hz, H-15), 2.53 (1H, m, H-12a), 2.30 (1H, m, H-12b), 2.09 (1H, m, H-3β), 1.89 (3H, s, H-17), 1.74 (1H, br t, J = 6.5 Hz, H-9), 1.71 (1H, d, J = 4.5 Hz, H-5), 1.69 (1H, m, H-2β), 1.62 (2H, m, H-11), 1.61 (1H, m, H-1β), 1.52 (1H, m, H-2α), 1.46 (2H, m, H-3α), 1.28 (3H, s, H-19), 1.20 (1H, m, H-1α), 0.82 (3H, s, H-20); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 182.4 (C-18), 174.1 (C-14), 144.4 (C-14), 144.0 (C-8), 134.1 (C-13), 119.4 (C-7), 73.5 (C-6), 70.2 (C-15), 51.3 (C-5), 51.0 (C-9), 42.8 (C-4), 34.5 (C-10), 33.6 (C-1), 28.0 (C-3), 27.3 (C-12), 24.6 (C-11), 24.0 (C-19), 22.0 (C-17), 18.1 (C-20), 18.0 (C-2); EIMS m/z 331 $[M + H]^+$ (15), 284 (23), 269 (100), 187 (27), 173 (65); HREIMS m/z $[M]^+$ 330.1835 (calcd for $C_{20}H_{26}O_4$, 330.1824).

Cytotoxic Activity against KB Cells. An MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] colorimetric assay was performed in 96-well plates. The assay was based on the reduction of MTT by the mitochondrial dehydrogenase of viable cells to give a blue formazan product that could be measured spectrophotometrically. Epidermoid carcinoma KB cells ((1−1.5) × 10^4/mL) were inoculated in each well, and the plates were incubated overnight at 37 °C and 5% CO_2. Twenty-four hours after seeding, 200 μL of treated or nontreated solution, in triplicate with various concentrations of compounds, was added, and the plates were incubated for 2 days. At day 3, 20 μL of MTT solution (5 mg/mL) per well was added to each cultured medium. After 4 h incubation, the medium was discarded and formazan blue formed in the cells was resolved by adding 100 μL of DMSO. The plates were read on a Dynatech MR5000 Microelisa reader, using a test wavelength of 570 nm and a reference wavelength of 630 nm. Cytotoxicity was expressed as 50% inhibitory concentration (IC_{50}) of cell growth.

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