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Note

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Total Synthesis of Bruceolline I

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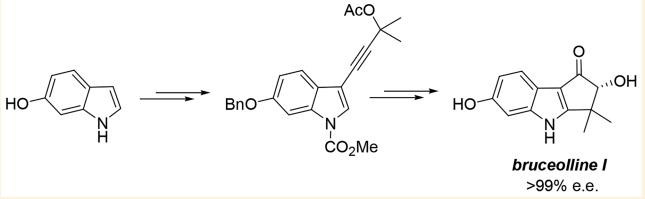
Supporting Information

ABSTRACT: The first total synthesis of the natural product bruceolline I, isolated in small quantities from the ethanol extract of *Brucea mollis* stems, was achieved in 29% yield over nine steps and with high enantiomeric purity (>98%). The key step of the process is the tandem gold-catalyzed rearrangement/Nazarov reaction of a propargylic acetate derivative. This synthesis provides a sufficient amount of synthesized bruceolline I for further bioassays.



Two indole alkaloids possessing a cyclopenta[*b*]indole skeleton and named bruceollines D and E (Figure 1) were

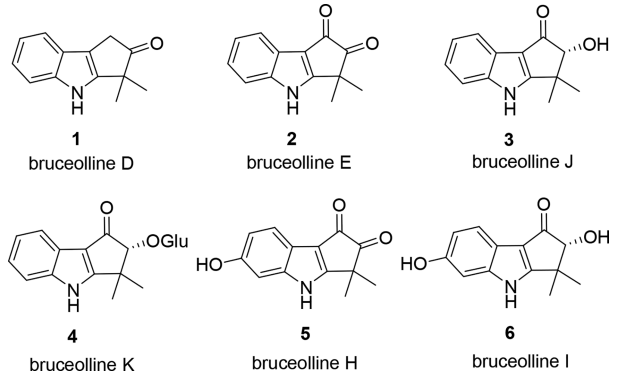


Figure 1. Cyclopenta[*b*]indole-based structures of bruceollines.

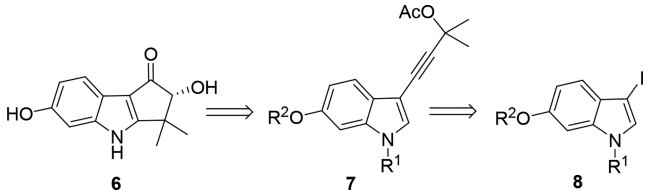
isolated in 1994 from the root wood of *Brucea mollis* Wall. var. *tonkinensis* Lecomte,[1] a plant that grows in southern China and northeast India (*Brucea mollis* Wall. ex Kurz.) and is traditionally used for treating malaria and other parasitic diseases.[1,2] More recently, additional bruceollines were isolated from the ethanol extracts of *B. mollis* stems, in particular bruceollines H, I, and J (Figure 1).[3]

Despite their potential in medicinal chemistry, only a few syntheses of these natural alkaloids, as well as an extremely limited number of biological studies on the isolated compounds, have thus far been reported.[3,4] The syntheses of bruceollines E (**2**) and J (**3**) have been reported by Gribble and co-workers,[5,6] who also published the X-ray crystallographic structure of bruceolline E.[7] A concise stereoselective synthesis of bruceolline J (**3**) was reported in 2015 by Dethe and Kumar,[8] and, more recently, the first synthesis of bruceolline H (**5**) was reported by us.[9]

Bruceollines H (**5**) and I (**6**) differ from the other members of the family by the presence of a hydroxy group on the indole moiety. Their cytotoxicity has been tested in vitro against five human tumor cell lines, but both compounds exhibited low activity.[3] On the other hand, these two alkaloids were isolated in minute quantities from the natural source. For example, 5 and 32 mg of bruceollines I and H, respectively, were obtained from 6.5 kg of *B. mollis* stems in an isolation process that included three chromatographic separations.[3] Clearly, this limited availability, together with the fact that the plant is endangered in NE India due to destruction of its habitat,[2] could hamper further and more extensive evaluation of compounds **5** and **6** (and their synthesized derivatives) toward a greater variety of biological targets. For this reason we embarked on the first synthesis of bruceolline I by exploiting the recently described tandem gold-catalyzed rearrangement/Nazarov reactions of propargylic acetate derivatives,[9,10] in order to establish a reliable method for obtaining sufficient material for biological tests as well as for the confirmation of its structure.

The synthesis would entail (Scheme 1) a Sonogashira coupling of suitably protected iodo-indole **8** to obtain

Scheme 1. Retrosynthesis Analysis



propargylic acetate derivative **7**, which in the presence of a gold(I) catalyst rearranges and generates a pentadienyl cation with the proper electronic arrangement for the next Nazarov cyclization. Further oxidation of the resulting cyclopenta[*b*]-

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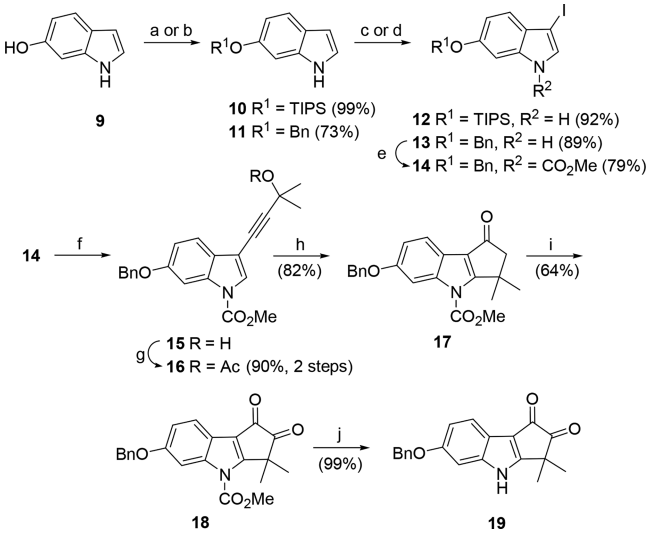
indol-1-one gives rise to N- and O-protected bruceolline H and, on subsequent enantioselective reduction, bruceolline I (**6**).

The synthesis of bruceolline H[9] included an unoptimized step and, moreover, BBr_3 was necessary for deprotection of the 6-OMe group under harsh conditions. Since we opted to avoid the use of this obnoxious reagent, the synthesis was designed to include either a silyl or benzyl O-protection (Scheme 2).

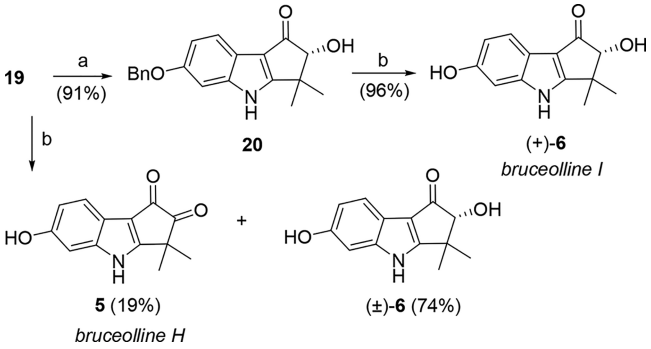
Scheme 2. Synthesis of Precursor 19[a]



[a]TIPSCl, imidazole, CH_2Cl_2, 25 °C; (b) BnBr, Cs_2CO_3, THF, 0−25 °C, 15 h; (c) KOH, I_2, DMF, rt, 1 h; (d) NIS, THF, 25 °C; (e) (1) LiHMDS −78 °C, 15 min, (2) $ClCO_2Me$, −78 °C to rt, 2 h; (f) 2-methyl-3-butyn-2-ol, 5 mol % $(Ph_3P)_2PdCl_2$, 3 mol % CuI, Et_3N−DMF 5:1, 40 °C, 1 h; (g) Ac_2O, Et_3N, cat. DMAP, DCM, 25 °C, 15 h; (h) $(4\text{-}CF_3C_6H_4)_3PAuSbF_6$ (3 mol %), DCM, 25 °C, 50 min; (i) SeO_2, 1,4-dioxane, 100 °C, 17 h; (j) *t*-$BuNH_2$, MeOH, reflux, 1 h.

Whereas the formation of the TIPS-protected iodoindole **12** (TIPS: triisopropylsilyl) by treatment of **10**[11] with NIS (*N*-iodosuccinimide) in tetrahydrofuran (THF) at room temperature (rt)[12] was uneventful (92%), the N-protection step with methyl chloroformate in THF in the presence of Et_3N resulted in an extensive degradation of this unstable intermediate. Instead, commercially available benzyl-protected indole **11** (prepared from indole **9** as reported)[13] was readily converted into N-protected iodoindole **14** (70% overall yield) as substrate for the Sonogashira coupling with 2-methyl-3-butyn-2-ol. The conditions for the N-protection were changed from the reported method, and treatment of **13** with a strong base (LiHMDS −78 °C) before addition of methyl chloroformate afforded **14** in 79% yield. The coupling of the latter with the alkyne was carried out at 40 °C in the presence of 5 mol % $(Ph_3P)_2PdCl_2$ and 3 mol % CuI, in a mixed Et_3N−dimethylformamide (DMF) (5:1) solvent, to afford alcohol **15** and, after acetylation, propargylic acetate **16** in 90% yield after chromatography over two steps. The gold-catalyzed step was carried out in the presence of $(4\text{-}CF_3C_6H_4)_3PAuSbF_6$ (3 mol %) as the catalyst in CH_2Cl_2 and gave cyclopenta[*b*]indol-1-one **17** in 82% yield after chromatography. The conditions for the oxidation of **17** by SeO_2 originally used in the synthesis of bruceolline H afforded fully protected bruceolline H (**18**) in 64% yield. N-Deprotection of **18** with *t*-$BuNH_2$ in MeOH eventually provided intermediate **19** (99%), which was in part subjected to hydrogenation over 10% Pd/C in MeOH−THF (1:1) (Scheme 3) to give the racemic bruceolline I (*rac*-**6**, 74%) required for chiral HPLC analysis. The hydrogenation reaction

Scheme 3. Synthesis of Bruceollines H and I[a]



[a](+)-DIP-Cl, THF, −45 °C, 10 min; (b) H_2, 10% Pd/C, MeOH−THF (1:1), 23 °C, 17 h.

was stopped before completion in order to recover a sufficient amount of bruceolline H (**5**) for X-ray structural determination (Figure 2 and Supporting Information).[15] The synthesis of

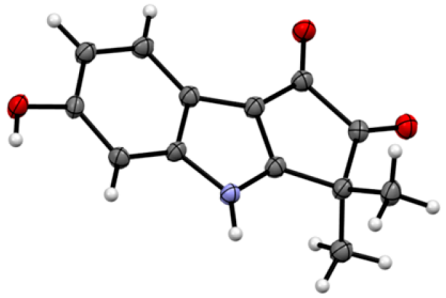


Figure 2. ORTEP drawing of bruceolline H.

enantiopure bruceolline I was completed by reduction of the carbonyl group of **19** by (+)-DIP-Cl (chlorodiisopinocampheylborane) as reported for bruceolline J,[6] envisioning that the presence of the OBn group on the six-membered ring would not change the stereochemical course of the reaction that should culminate in the formation of the *S* enantiomer. The reduction with (+)-DIP-Cl was carried out in anhydrous THF at −45 °C and provided alcohol **20** in 91% yield.

After quantitative debenzylation of **20**, a product with identical ^1H and ^{13}C NMR spectra to those of the natural compound was obtained (Supporting Information), and its enantiomeric excess (98.7%) was determined by chiral HPLC analysis (Supporting Information). Moreover, the sign of the specific rotation of this enantiopure compound was the same as that of natural bruceolline I.[16] However, for the synthesized (+)-**6** the $[\alpha]^{16}_D$ value of +38.5 (*c* 0.47, MeOH) was more than 3 times higher than that reported for natural bruceolline I ($[\alpha]^{20}_D$ +11.3, *c* 0.05, MeOH),[3] a discrepancy that is likely due to the inaccuracy in the optical rotation measurement of small natural product samples.[17]

In conclusion, a short and efficient synthesis of bruceolline I (**6**) in 29% overall yield over nine steps was developed. The key step of the process is a gold-catalyzed tandem sequence that allows for the rapid and high-yielding construction of the cyclopenta[*b*]indol-1-one core. This synthesis affords bruceol-

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line I in sufficient quantities for its further evaluation in various biological tests.

EXPERIMENTAL SECTION

General Experimental Procedures. Melting points were recorded on a Büchi melting point B540 apparatus and are uncorrected. Optical rotations were determined with a JASCO DIP-370 instrument. ^{1}H NMR and ^{13}C NMR spectra were recorded at 400 and 100.4 MHz, respectively, in the specified deuterated solvent. Solvent reference lines were set at 7.26 and 77.00 ($CDCl_3$), 2.05 and 29.84 (acetone-d_6), and 3.31 and 49.00 (methanol-d_4), in ^{1}H and ^{13}C NMR spectra, respectively. Mass spectra were carried out by direct inlet of a 20 ppm solution in CH_3OH on an LCQ Fleet Ion Trap LC/MS system with an ESI interface in the positive mode, unless otherwise stated. Microanalyses were carried out with a CHN elemental analyzer. Chromatographic separations were performed under pressure on silica gel by flash-column techniques; R_f values refer to thin-layer chromatography (TLC) carried out on 0.25 mm silica gel plates (Merck F_{254}), with the same eluent as indicated for the column chromatography. HPLC analyses were carried out with an HPLC instrument equipped with a Lux 5 μm cellulose-4 column, 250 × 4.60 mm, and eluting at a 0.3 mL/min flow rate in isocratic 15% isopropyl alcohol and 85% *n*-hexane. 6-[(Triisopropylsilyl)oxy]indole[14] (**10**) and 6-benzyloxyindole[13] (**11**) were prepared as reported. Enynyl acetate **16** proved to be quite unstable when neat, and elemental analysis could not be performed.

Methyl 6-Benzyloxy-3-iodo-1*H*-indole-1-carboxylate (14). Crushed KOH (205 mg, 3.7 mmol) was added to a solution of 6-benzyloxyindole (**11**) (327 mg, 1.46 mmol) in anhydrous DMF (1.7 mL), and the resulting suspension was stirred at room temperature for 20 min. A solution of I_2 (372 mg, 1.46 mmol) in anhydrous DMF (1.7 mL) was added dropwise, and after 1 h the reaction mixture was poured into ice H_2O (34 mL) containing NH_4OH (0.5%) and $K_2S_2O_5$ (0.1%). A precipitate immediately formed, and this was collected by filtration, washed with chilled water (30 mL), and dried under reduced pressure. The 3-iodo-6-benzyloxy-1*H*-indole (**13**) (455 mg, 89%) was immediately used in the next step: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.19 (br s, 1H), 7.48−7.42 (m, 2H), 7.42−7.36 (m, 2H), 7.35−7.31 (m, 2H), 7.17 (d, J = 2.4 Hz, 1H), 6.95 (dd, J = 8.4, 2.4 Hz, 1H), 6.92 (d, J = 2.0 Hz, 1H), 5.12 (s, 2H).

3-Iodo-6-benzyloxy-1*H*-indole (**13**) (450 mg, 1.29 mmol) was dissolved in anhydrous THF (8.6 mL), and after cooling at −78 °C LiHMDS 1.0 M in THF (1.35 mL, 1.35 mmol) was slowly added, keeping the temperature below −70 °C. After 15 min, methyl chloroformate (105 μL, 1.35 mmol) was slowly added, and after a further 15 min the cooling bath was removed and the reaction mixture was stirred at room temperature until complete consumption of the starting material (2 h). A 0.5 M aqueous solution of $NaHCO_3$ (15 mL) was added under vigorous stirring, and the product extracted with EtOAc (3 × 15 mL). The combined organic extracts were dried over anhydrous Na_2SO_4. After filtration and evaporation of the solvent, crude **14** was isolated and purified by flash chromatography (eluent: *n*-hexane−EtOAc, 12:1; R_f = 0.26), affording pure **14** as a white solid (664 mg, 79%): mp = 94.1−94.8 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.83 (br s, 1H), 7.62 (s, 1H), 7.51−7.46 (m, 2H), 7.44−7.37 (m, 2H), 7.37−7.31 (m, 1H), 7.28 (d, J = 8.4 Hz, 1H), 7.03 (dd, J = 8.4, 2.4 Hz, 1H), 5.15 (s, 2H), 4.02 (s, 3H); ^{13}C NMR (100.4 MHz, $CDCl_3$) δ 157.9 (s), 150.5 (s), 136.8 (s), 135.5 (s), 128.6 (d, 2 C), 128.4 (d), 128.0 (d), 127.6 (d, 2 C), 126.0 (s), 122.1 (d), 113.4 (d), 100.2 (d), 70.5 (t), 66.2 (s), 54.0 (q); MS/MS (ESI) m/z 408 $[M + H]^+$ (6), 281 $[M + H - I]^+$ (100); anal. C 50.35, H 3.50, N 3.54%, calcd for $C_{17}H_{14}INO_2$, C 50.14, H 3.47, N 3.44%.

Methyl 3-(3-Acetoxy-3-methylbut-1-ynyl)-6-benzyloxyindole-1-carboxylate (16). A 5:1 (v/v) solution of Et_3N−DMF (7.2 mL) was added to a round-bottom flask containing compound **14** (660 mg, 1.62 mmol), $(Ph_3P)_2PdCl_2$ (57 mg, 0.081 mmol), and CuI (9 mg, 0.049 mmol). Neat 2-methyl-3-butyn-2-ol (190 μL, 1.94 mmol) was added, and the reaction mixture heated at 40 °C under vigorous stirring until complete consumption of the starting material (TLC; 1 h). The mixture was cooled to rt and quenched with H_2O (22 mL). The product was extracted with EtOAc (3 × 20 mL), and the combined organic extracts were dried over anhydrous K_2CO_3. After filtration and evaporation of the solvent, crude **15** was purified by flash chromatography (eluent: *n*-hexane−EtOAc, 2:1, + 1% Et_3N; R_f = 0.40) to afford intermediate **15**, which was immediately used in the acetylation step: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.83 (br s, 1H), 7.63 (s, 1H), 7.52−7.47 (m, 3H), 7.42−7.38 (m, 2H), 7.35−7.33 (m, 1H), 7.02 (dd, J = 8.8, 2.4 Hz, 1H), 5.14 (s, 2H), 4.03 (s, 3H), 1.66 (s, 6H).

A solution of enynyl alcohol **15** (1.62 mmol) in anhydrous dichloromethane (DCM) (16.2 mL) was cooled to 0 °C (ice bath), and 4-(dimethylamino)pyridine (DMAP) (30 mg, 0.24 mmol), Et_3N (1.13 mL, 8.1 mmol), and Ac_2O (605 μL, 4.9 mmol) were added. After 10 min, the ice bath was removed and the reaction mixture was stirred at 25 °C for 15 h, quenched by addition of a saturated solution of $NaHCO_3$ (15 mL). After separation of the phases, the aqueous layer was extracted with DCM (2 × 15 mL) and the combined organic extracts were dried over anhydrous K_2CO_3. After filtration and evaporation of the solvent, crude **16** was obtained and purified by flash column chromatography (eluent: *n*-hexane−EtOAc, 7:1, + 1% Et_3N; R_f = 0.24), affording pure acetate **16** (591 mg, 90% over two steps) as a colorless oil. This was stored at 4 °C as a 0.1 M solution in the eluent until use: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.81 (br s, 1H), 7.66 (s, 1H), 7.54 (d, J = 8.4 Hz, 1H), 7.49−7.46 (m, 2H), 7.42−7.37 (m, 2H), 7.36−7.31 (m, 1H), 7.02 (dd, J = 8.8, 2.4 Hz, 1H), 5.13 (s, 2H), 4.02 (s, 3H), 2.07 (s, 3H), 1.79 (s, 6H); ^{13}C NMR (100.4 MHz, $CDCl_3$) δ 169.3 (s), 157.7 (s), 150.9 (s), 136.9 (s), 135.5 (s), 128.5 (d, 2 C), 128.0 (d), 127.6 (d, 2 C), 127.3 (d), 124.5 (s), 120.7 (d), 113.3 (d), 103.6 (d), 100.6 (s), 94.0 (s), 75.8 (s), 72.5 (s), 70.5 (t), 54.0 (q), 29.2 (q, 2 C), 22.0 (q); MS (ESI) m/z 428 $[M + Na]^+$ (8), 346 $[M - CO_2Me]^+$ (100).

Methyl 6-Benzyloxy-3,3-dimethyl-1-oxo-2,3-dihydro-1*H*-cyclopenta[*b*]indole-4-carboxylate (17). The solution of **16** in the eluent was concentrated and dried under vacuum (no heating) for 30 min. Gold(I) complex $(4\text{-}CF_3C_6H_4)_3PAuCl$ (31 mg, 44 μmol) was dissolved in DCM (8.8 mL, 0.005 M), and $AgSbF_6$ (15 mg, 44 μmol) was added. The suspension was left to stir at 25 °C under a nitrogen atmosphere. After 15 min a solution of enynyl acetate **16** (591 mg, 1.46 mmol) in DCM (20.2 mL; final concentration 0.05 M) was added, and the reaction mixture was stirred at 25 °C for 50 min. Water (50 mL) was added, the phases were separated, and the product was extracted with DCM (2 × 25 mL). The combined organic extracts were dried over anhydrous Na_2SO_4, filtered, and concentrated. The oily residue was purified by flash chromatography (eluent: *n*-hexane−EtOAc, 4:1; R_f = 0.22), affording pure compound **17** (435 mg, 82%) as an orange solid: mp = 115.3−116.5 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.78 (d, J = 8.4 Hz, 1H), 7.77 (d, J = 2.4 Hz, 1H), 7.49−7.45 (m, 2H), 7.42−7.38 (m, 2H), 7.36−7.31 (m, 1H), 7.03 (dd, J = 8.4, 2.4 Hz, 1H), 5.14 (s, 2H), 4.10 (s, 3H), 2.90 (s, 2H), 1.60 (s, 6H); ^{13}C NMR (100.4 MHz, $CDCl_3$) δ 196.0 (s), 171.5 (s), 157.6 (s), 150.8 (s), 141.9 (s), 136.8 (s), 128.6 (d, 2 C), 128.0 (d), 127.5 (d, 2 C), 124.8 (s), 121.2 (d), 115.7 (s), 113.2 (d), 103.0 (d), 70.6 (t), 59.2 (s), 54.1 (q), 39.8 (t), 26.8 (q, 2 C); MS (ESI) m/z 749 $[2M + Na]^+$ (100), 364 $[M + H]^+$ (52); anal. C 72.67, H 5.72, N 3.54%, calcd for $C_{22}H_{21}NO_4$, C 72.71, H 5.82, N 3.85%.

Methyl 6-Benzyloxy-3,3-dimethyl-1,2-dioxo-2,3-dihydro-1*H*-cyclopenta[*b*]indole-4-carboxylate (18). Compound **17** (420 mg, 1.16 mmol) was dissolved in 1,4-dioxane (13 mL), and SeO_2 (513 mg, 4.6 mmol) was added in one portion. The mixture was heated at 100 °C for 17 h; after cooling to room temperature, H_2O (220 mL) was added and the product extracted with EtOAc (3 × 75 mL). The combined organic extracts were dried over anhydrous Na_2SO_4, filtered, and concentrated. Purification of the crude by flash chromatography (*n*-hexane−EtOAc, 4:1; R_f = 0.17) afforded pure **18** (236 mg, 64%) as a yellow solid: mp = 173.7−174.7 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.91 (d, J = 8.8 Hz, 1H), 7.79 (d, J = 2.0 Hz, 1H), 7.49−7.46 (m, 2H), 7.43−7.38 (m, 2H), 7.37−7.33 (m, 1H), 7.11 (dd, J = 8.4, 2.0 Hz, 1H), 5.17 (s, 2H), 4.17 (s, 3H), 1.59 (s, 6H); ^{13}C NMR (100.4 MHz, $CDCl_3$) δ 204.7 (s), 177.7 (s), 169.7 (s), 158.9 (s), 150.1 (s), 139.8 (s), 136.4 (s), 128.6 (d, 2 C), 128.2 (d), 127.5 (d, 2 C), 127.4 (s),

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122.4 (d), 115.6 (s), 114.1 (d), 103.0 (d), 70.7 (t), 54.8 (q), 45.9 (s), 22.5 (q, 2 C); MS (ESI) m/z 777 $[2M + Na]^+$ (100), 400 $[M + Na]^+$ (21), 378 $[M + H]^+$ (23); anal. C 70.10, H 4.85, N 3.99%, calcd for $C_{22}H_{19}NO_5$, C 70.02, H 5.07, N 3.71%.

6-Benzyloxy-3,3-dimethyl-3,4-dihydrocyclopenta[*b*]indole-1,2-dione (19). Compound **18** (228 mg, 0.60 mmol) was suspended in MeOH (6 mL), and *t*-$BuNH_2$ (1.9 mL, 18 mmol) was added. The clear solution was heated at 90 °C for 1 h and cooled, and volatiles were removed under vacuum. The crude was triturated with *n*-hexane, affording pure compound **19** (190 mg, 99%) as an orange solid: mp = 262 °C (dec); ^{1}H NMR (400 MHz, CD_3OD) δ 7.83 (d, J = 8.8 Hz, 1H), 7.48−7.46 (m, 2H), 7.41−7.36 (m, 2H), 7.34−7.29 (m, 1H), 7.11 (d, J = 2.4 Hz, 1H), 7.04 (dd, J = 8.8, 2.4 Hz, 1H), 5.17 (s, 2H), 1.47 (s, 6H); ^{13}C NMR (100.4 MHz, CD_3OD) δ 207.3 (s), 177.0 (s), 174.1 (s), 159.6 (s), 143.3 (s), 138.5 (s), 129.5 (d, 2 C), 128.9 (d), 128.6 (d, 2 C), 123.7 (s), 123.5 (d), 116.6 (s), 114.3 (d), 99.8 (d), 71.5 (t), 43.1 (s), 23.4 (q, 2 C); MS (ESI) m/z 661 $[2M + Na]^+$ (100), 342 $[M + Na]^+$ (42), 320 $[M + H]^+$ (42); anal. C 75.14, H 5.23, N 4.17%, calcd for $C_{20}H_{17}NO_3$, C 75.22, H 5.37, N 4.39%.

(+)-(2*R*)-6-Benzyloxy-2-hydroxy-3,3-dimethyl-3,4-dihydro-2*H*-cyclopenta[*b*]indol-1-one (20). A solution of (+)-DIP-Cl (362 mg, 1.13 mmol) in anhydrous THF (16 mL) was cooled to −45 °C, and a solution of substrate **19** (120 mg, 0.38 mmol) in anhydrous THF (16 mL) was added dropwise while keeping the temperature below −40 °C. After 10 min the reaction was quenched at −45 °C by adding diethanolamine (360 μL, 3.76 mmol), the cooling bath removed, and the mixture left under vigorous stirring at room temperature for 1.5 h. The white precipitate was filtered off over a Celite pad and washed with THF (2 × 4 mL), and the filtrate was concentrated under vacuum to afford crude **20**. This was purified by flash chromatography, eluting first with *n*-hexane−EtOAc, 2:1, and then with *n*-hexane−EtOAc, 1:2 (R_f = 0.16). Pure **20** was so obtained (110 mg, 91%) as a white solid: mp = 150.7−152.5 °C; $[\alpha]^{20}_D$ +22.4 (c 0.37, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.42 (br s, 1H), 7.78 (d, J = 8.8 Hz, 1H), 7.47−7.43 (m, 2H), 7.42−7.37 (m, 2H), 7.36−7.30 (m, 1H), 7.00 (dd, J = 8.8, 2.0 Hz, 1H), 6.95 (d, J = 2.0 Hz, 1H), 5.12 (s, 2H), 4.34 (s, 1H), 1.57 (s, 3H), 1.32 (s, 3H); ^{13}C NMR (100.4 MHz, $CDCl_3$) δ 194.7 (s), 171.8 (s), 156.5 (s), 142.8 (s), 136.8 (s), 128.5 (d, 2 C), 127.9 (d), 127.4 (d, 2 C), 121.6 (s), 123.5 (d), 115.4 (d), 114.2 (s), 112.2 (d), 98.2 (d), 86.0 (d), 70.4 (t), 40.7 (s), 24.8 (q), 24.2 (q); MS (ESI) m/z 665 $[2M + Na]^+$ (100), 344 $[M + Na]^+$ (50), 322 $[M + H]^+$ (17); MS (ESI, negative mode) m/z 320 $[M - H]^-$ (100); anal. C 74.47, H 5.88, N 4.06%, calcd for $C_{20}H_{19}NO_3$, C 74.75, H 5.96, N 4.36%.

(+)-Bruceolline I (6). To a solution of **20** (100 mg, 0.31 mmol) in a 1:1 mixture of anhydrous MeOH−THF (16 mL) was added Pd/C 10% (33 mg, 0.031 mmol) under nitrogen. The resulting suspension was flushed with hydrogen under vigorous stirring and left under a hydrogen atmosphere (balloon) at 23 °C for 17 h. The catalyst was filtered off over a Celite pad and washed with MeOH, and the filtrate concentrated under vacuum. The crude was purified by flash chromatography (eluent: DCM−MeOH, 10:1; R_f = 0.14), and pure (+)-bruceolline I **6** (69 mg, 96%) was obtained as a white solid: mp = 210 °C (dec); $[\alpha]^{16}_D$ +38.5 (c 0.47, CH_3OH); ^{1}H NMR (400 MHz, acetone-d_6)[3] δ 10.92 (br s, 1H), 8.27 (br s, 1H), 7.54 (d, J = 8.4 Hz, 1H), 6.92 (d, J = 2.0 Hz, 1H), 6.78 (dd, J = 8.4, 2.4 Hz, 1H), 4.52 (br s, 1H), 4.23 (s, 1H), 1.53 (s, 3H), 1.30 (s, 3H); ^{13}C NMR (100.4 MHz, acetone-d_6)[3] δ 194.2 (s), 171.5 (s), 155.6 (s), 144.2 (s), 121.7 (d), 115.7 (s), 115.1 (s), 112.3 (d), 99.4 (d), 86.9 (d), 41.1 (s), 25.4 (q), 24.5 (q); ^{1}H NMR (400 MHz, CD_3OD) δ 7.61 (dd, J = 8.8, 0.8 Hz, 1H), 6.83 (d, J = 2.0 Hz, 1H), 6.72 (dd, J = 8.8, 2.0 Hz, 1H), 4.28 (s, 1H), 1.51 (s, 3H), 1.30 (s, 3H); ^{13}C NMR (100.4 MHz, CD_3OD) δ 196.0 (s), 173.7 (s), 156.2 (s), 145.0 (s), 122.4 (d), 115.9 (s), 115.1 (s), 112.7 (d), 99.5 (d), 87.3 (d), 41.7 (s), 25.3 (q), 24.4 (q); MS (ESI) m/z 485 $[2M + Na]^+$ (68), 254 $[M + Na]^+$ (100); MS (ESI, negative mode) m/z 230 $[M - H]^-$ (100); anal. C 67.23, H 5.99, N 6.51%, calcd for $C_{13}H_{13}NO_3$, C 67.52, H 5.67, N 6.06%.

***(rac)*-Bruceolline I (6).** (*rac*)-**6** was prepared by subjecting **19** (70 mg, 0.22 mmol) to the same hydrogenation procedure reported for compound **20**. After 16 h the reaction was stopped and the crude mixture of **5** and (±)-**6** was separated by flash chromatography (eluent: DCM−MeOH, 1:1), affording pure (±)-**6** (38 mg, 74%) and a small amount of pure **5** (10 mg, 20%). Spectroscopic data of both compounds are identical to those reported above (compound **6**) and in the literature (compounds **5** and **6**).[3,9]

Crystal Structure Determination of Bruceolline H.[15] A light yellow thin plate shaped crystal (0.10 × 0.05 × 0.03), obtained by slow evaporation of an EtOH solution at room temperature, was used for collection, and RX analysis was carried out with an Oxford Diffraction KM4 Xcalibur2 goniometer at 100 K. Cu/Kα radiation (40 mA/−40 kV), monochromated by an Oxford Diffraction Enhance ULTRA assembly, and an Oxford Diffraction Excalibur PX Ultra CCD were used for cell parameter determination and data collection. The asymmetric unit contains two independent molecules cocrystallized with two water molecules, and consequently cell parameters are $2\times(C_{13}H_{11}NO_3)+2\times(H_2O)$, M = 494.49, monoclinic, space group $P2_1/c$, a = 9.3080(2) Å, b = 30.6110(8) Å, c = 8.3800(2) Å, β = 96.194(2)°, V = 2373.7(1) Å^3, Z = 4, D_c = 1.384, μ = 0.865 mm^{-1}, $F(000)$ = 1040; 12 107 reflections were collected with a 4.78° < θ < 70.61° range with a completeness to theta 98.7%; 4335 were unique, the parameters were 349 and the final R index was 0.0514 for reflections having $I > 2\sigma I$ and 0.0721 for all data.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.7b00311.

Crystallographic data of bruceolline H (CIF)

^{1}H and ^{13}C NMR spectra of all new compounds, HPLC chromatograms of both racemic and (+)-bruceolline I, crystal structure determination and crystal data of bruceolline H (PDF)

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Notes

The authors declare no competing financial interest.

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