Benzylisoquinoline Alkaloids from *Gnetum parvifolium*

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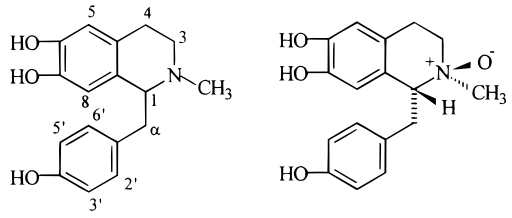
The investigation of the chemical constituents from the lianas of *Gnetum parvifolium* resulted in the isolation of three new benzylisoquinoline alkaloids, (±)-*N*-methylhigenamine (**1**), (−)-*N*-methylhigenamine *N*-oxide (**2**), and (±)-8-(*p*-hydroxybenzyl)-2,3,10,11-tetrahydroxyprotoberberine (**3**), together with two known alkaloids, higenamine and trigonelline. The structures of **1**−**3** were determined by chemical methods and spectral analysis including 2D NMR and NOE studies.

Gnetum parvifolium (Warb.) C. Y. Cheng (Gnetaceae) is distributed in the southern part of the People's Republic of China and is used as a Chinese herbal medicine for the treatment of bronchitis, rheumatoid arthritis and muscular strain.[1] Previous phytochemical investigations on plants in this family have revealed an abundance of flavonoids and stilbenoids.[2] In 1980, a benzylisoquinoline alkaloid, higenamine, which showed a strong cardiotonic effect, was isolated from *G. parvifolium*.[3] In recent years, a number of stilbenoids have been described from this species.[4] The present study on *G. parvifolium* in our laboratory has afforded three new benzylisoquinoline alkaloids (**1**−**3**) together with two known alkaloids, higenamine and trigonelline.



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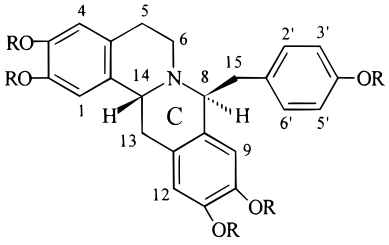

3 R= H
4 R= $COCH_3$

The lianas of *G. parvifolium* were extracted with 60% ethanol and the ethanolic extract was worked up using conventional methods. The total alkaloid extracts were combined and further separated over polyamide, followed by Si gel column chromatography, yielding three benzylisoquinoline alkaloids (**1**, **2**, and higenamine). An additional crude alkaloid **3** was obtained on repeated chromatography, but further purification of **3** was difficult due to its failure to crystallize. Therefore, the pentaacetate derivative (**4**) of **3** was prepared in the normal manner.

Compound **1** was obtained as colorless crystals, $[\alpha]^{25}_D$ 0° (*c* 0.24, DMSO). A molecular formula of $C_{17}H_{19}O_3N$ was determined from a combination of the elemental analysis, FABMS, and ^{13}C NMR data. FABMS showed quasimolecular ion peaks at *m*/*z* 308 $[M + Na]^+$ and 286 $[M + 1]^+$, indicating a molecular ion that is 14 mass units heavier than that of higenamine. The IR and UV absorptions were very similar to those of higenamine, suggesting the presence of a tetrahydrobenzylisoquinoline skeleton. The EIMS gave a weak molecular ion peak at *m*/*z* 285 as well as two fragment ions at *m*/*z* 178 (base peak, 6,7-dihydroxy-*N*-methylisoquinoline fragment ion) and *m*/*z* 107 (*p*-hydroxybenzyl fragment ion), both characteristic of tetrahydrobenzylisoquinoline alkaloids.[5] Comparison of the ^1H NMR and ^{13}C NMR spectral data for **1** and higenamine demonstrated the presence of an *N*-Me unit (δ_H 2.31, δ_C 42.0) in the molecule of **1**. Hence, **1** was assigned as (±)-*N*-methylhigenamine.

Compound **2** was obtained as colorless needles, $[\alpha]^{25}_D$ −9.6° (*c* 0.12, DMSO). This compound was deduced to have the same skeleton as compound **1** and higenamine based on comparison of their spectral data (UV, ^1H- and ^{13}C NMR). EIMS gave a weak molecular ion peak at *m*/*z* 301 and a fragment ion peak at *m*/*z* 285 $[M - 16]^+$, which is typical of an *N*-oxide.[6] In addition, the C-1 signal appeared at very low field (δ_C 77.5) in the ^{13}C NMR spectrum, consistent with the presence of an adjacent *N*-oxide group. Analysis of all the obtained spectral data led to the assignment of **2** as (−)-*N*-methylhigenamine *N*-oxide. The relative stereochemistry between the *N*-methyl and *p*-hydroxybenzyl groups was established to be syn based on the chemical shifts of H-1.[7] The absolute configuration of **2** was not determined.

In order to confirm the structures of **1** and **2**, a chemical transformation was carried out. Compound **1** was prepared from higenamine through condensation with 37% formalin and successive reduction by sodium borohydride at room temperature.[8] Oxidation of **1** using *m*-chloroperbenzoic acid was successful in producing compound **2**.[9]

Compound **3** was derivatized as its pentaacetate (**4**), which was isolated as colorless needles from $MeOH/H_2O$. A molecular formula of $C_{34}H_{33}O_{10}N$ was established from its elemental analysis, EIMS, and NMR data (Table 1). The UV spectrum showed an isoquinoline absorption band at

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Table 1. ^{1}H and ^{13}C NMR Data for Compound **4** (Acetone-d_6)[a]

position	δ_H	J (in Hz)	δ_C	HMBC	NOE
1a			133.7 (C)		
1	7.16 s		122.1 (CH)	C-1a	
2			141.7 (C)[b]		
3			141.5 (C)[b]		
4	6.98 s		124.0 (CH)	C-5, C-4	
4a			138.5 (C)		
5	2.90 m		29.8 (CH_2)	C-6	
	2.77 m				
6	3.12 m		46.7 (CH_2)	C-14, C-5	
	2.77 m				
8	4.12 dd	8.5, 5.5	66.8 (CH)	C-14, C-12a, C-15	H-6, H-9, H-2′, H-6′,
8a			133.0 (C)		
9	6.82 s		123.1 (CH)	C-8, C-8a, C-10/C-11	
10			141.0 (C)[b]		
11			141.2 (C)[b]		
12	7.00 s		124.2 (CH)	C-13, C-12a, C-10/C-11	
12a			136.5 (C)		
13	3.02 dd	17.1, 5.0	32.1 (CH_2)	C-14, C-1a, C-12a	
	2.90 m				H-1, H-12
14	4.58 dd	11.5, 5.0	50.7 (CH)	C-1, C-6, C-1a, C-4a	H-1, H-13, H-15β
15α	3.28 dd	14.0, 8.5	41.5 (CH_2)	C-8, C-8a, C-1′	
15β	2.95 dd	14.0, 5.5			
1′			138.4 (C)		
2′, 6′	7.29 d	8.0	131.2 (CH)	C-4′, C-15, C-3′, C-5′	
3′, 5′	6.98 d	8.0	122.0 (CH)	C-4′	
4′			150.2 (C)		
$COCH_3$	2.03−2.08 5s		168.4−169.4 (C)		

[a] Assignments were confirmed by DEPT, ^{1}H−^{1}H COSY, and ^{1}H−^{13}C COSY experiments. [b]Interchangeable assignments.

275 nm (log ϵ 3.92).[10] The EIMS did not show a parent ion but gave fragment ions at *m*/*z* 242, 164, and 136 resulting from *retro*-Diels−Alder fragmentation, characteristic of a compound based on the protoberberine skeleton.[11] A prominent peak at *m*/*z* 298, resulting from successive acetyl losses at *m*/*z* 466, 424, 382, and 340, confirmed the presence of four hydroxy groups from the protoberberine moiety. The ^{13}C NMR and DEPT spectra displayed two methine, four methylene, and eighteen aromatic carbons, of which ten were quaternary. One of the methylenes (δ 46.8) and the two methine carbons (δ 50.7 and 66.8) were shifted downfield along with their corresponding protons because they were all attached to a nitrogen atom. The ^{1}H NMR spectrum showed two doublets centered at δ 7.29 (2H) and 6.98 (2H), representing the four protons of a parasubstituted benzyl group. Four proton singlets between δ 6.82 and 7.16 were attributed to the aromatic protons of the protoberberine moiety, suggesting a C-2, C-3, C-10, and C-11 tetrahydroxy substitution pattern. The alphatic hydrogens of **4** comprised two ABX spin systems and one A_2X_2 system. Further structural features were determined from NOE difference and HMBC data (Table 1). In the NOE experiment, the effect observed between H-1 and H-13 helped to differentiate between the two ABX spin systems. Similarly, NOEs between H-8 and H-9 and between H-13 and H-12 confirmed the C-10, C-11 substitution pattern in ring D. In addition, a series of HMBC correlations for H-1/C-1a, H-14/C-1, H-4/C-5, H-9/C-8, C-8a, and H-12/C-13 confirmed the assignments of the aromatic protons (Table 1). Other HMBC correlations for H-14/C-1, C-1a, C-4a, C-6 and for H-8/C-14, C-12a were consistent with the proposed protoberberine skeleton of **4**.

The structure of the C-8 *p*-hydroxylbenzyl moiety in **4** was determined by ^{1}H, ^{13}C, 2D NMR, and by EIMS data. In the COSY spectrum, H-8 correlated with the two methylene protons at C-15. HMBC correlations between H-15α/C-8, C-8a, C-1′ established the nature of this C-8 substitutent.

The stereochemistry of quinolizidine and protoberberine nuclei have been extensively investigated in the past years.[12] The quinolizidine nucleus can assume a half-chair conformation with one trans and two cis configurations being possible. The *trans*-quinolizidine conformation is associated with a high-field H-14 signal at 3.6 ± 0.2 ppm and a low-field C-14 signal at 58.4 ± 0.3 ppm, while in *cis*-quinolizidines H-14 is shifted to low field at 4.3 ± 0.2 ppm and C-14 to high field at about 49−52 ppm. Compound **4** exhibited high-field C-14 (δ_C 50.0) and low-field H-14 (δ_H 4.58) signals, indicating a *cis*-quinolizidine conformation. An observed NOE between methine H-14 and the benzylic methylene H-15β proton indicated that H-14 and the *p*-hydroxybenzyl group were oriented on the same side of ring C, thus fixing the relative stereochemistry. Accordingly, compound **4** was assigned as (±)-8-(*p*-hydroxybenzyl)-2,3,10,11-tetrahydroxyprotoberberine pentaaceate.

Protoberberine alkaloids with a C-8 substituent are quite unusual, especially in the case of 8-benzylberberine-type alkaloids.[13,14] A distinguishing characteristic of such compounds is their nonmethylated hydroxyl functions. Gottlieb and Kubitzki have indicated that absence of methylation is widespread in the Gnetaceae and other gymnosperms and have provided an interesting insight into some of the biogenetic sequences that occur within these plants.[2,15] The biogenesis of **3** may result from the condensation of norlaudanosine with 4-hydroxyphenylpyruric acid (derived from tyrosine) followed by cyclization.

Experimental Section

General Experimental Procedures. Melting points were determined on a XT4-100X micromelting point apparatus and are uncorrected. Optical rotations were measured on a Perkin-Elmer 241 polarimeter, and IR spectra were obtained on Perkin-Elmer 683 infrared spectrophotometer. NMR spectra were taken on a Bruker AM 500 spectrophotometer using TMS as an internal standard. EIMS were obtained on a ZAB-2F mass spectrometer. Elemental analysis was carried out on a MOD1106 elemental analyzer. Column chromatography was performed using Si gel (Qing Dao Hai Yang Chemical Group Co., Qing Dao, People's Republic of China), polyamide (Li Xian Chemical Plant, Li Xian, People's Republic of China) and 001

× 7 polyvinylsulfonic ion-exchange resin (Tian Jin Chemical Plant, Tian Jin, People's Republic of China). TLC was conducted on Si gel 60 F_{254} (Qing Dao Hai Yang Chemical Group Co.) and detection was achieved by exposure to I_2 vapor or spraying with Dragendorff's reagent.

Plant Material. Lianas of *G. parvifolium* were collected in Guangxi Province of the People's Republic of China in March 1986 and identified by Professor W. Z. Song of our institute, where a voucher sample has been deposited (No. 860321).

Extraction and Isolation. Powered lianas (20 kg) of *G. parvifolium* were extracted with 60% EtOH. The resultant extract was concentrated to yield 1.6 kg of gum which was then divided into acetone-soluble and acetone-insoluble fractions. The latter fraction (513 g) was exhaustively extracted with 1% HCl, and then basified with saturated Na_2CO_3 to pH 9. The total alkaloid extract was subjected to polyamide column chromatography and eluted with $(CH_3)_2CO-MeOH$ (90:24) into eight fractions. Fraction IV was further chromatographed over Si gel using $(CH_3)_2CO-MeOH-HOAc$ (25:25:1) as eluent to furnish compound **1** (410 mg). Fraction VI was separated on Si gel with $(CH_3)_2CO-EtOH$ mixtures of increasing polarity to give four fractions. The fourth fraction was eluted with $(CH_3)_2CO-MeOH$ (1:1), and subjected to further purification over Si gel, by eluting with $(CH_3)_2CO-MeOH-HOAc$ (25:25:1), to afford higenamine (257 mg) and **2** (14 mg), which were finally purified by preparative TLC with $(CH_3)_2CO-MeOH-HOAc$ (50:25:1) and crystallized from EtOH and H_2O, respectively. Fraction V was chromatographed on Si gel with $Me_2CO-MeOH-EtOAc-H_2O$ (2:2:4:1) as eluent, and furnished a crude yellow gum (87 mg) which was acetylated with acetic anhydride and pyridine. Flash chromatography with $CHCl_3-Me_2CO$ (30:2) yielded **4** (21 mg).

The alkaline aqueous extract was reacidified to pH 2 and chromatographed on an ion-exchange resin column. The resin was then extracted with methanol. Further purification of the methanolic extract on a Si gel column eluted with $(CH_3)_2CO-H_2O$ (30:2) afforded trigonelline (508 mg).

(±)-*N*-Methylhigenamine (1): colorless crystals (EtOH); mp 148−150 °C; $[\alpha]^{25}_D$ 0° (*c* 0.24, DMSO); UV (EtOH) λ_{max} (log ϵ) 225 (4.60, sh), 285 (4.08) nm; IR (KBr) ν_{max} 3446, 3028, 2690, 1548, 1518, 1456, 1408, 1277, 1109, 839 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) δ 6.89 (2H, d, J = 8.3 Hz, H-2′, H-6′), 6.58 (2H, d, J = 8.3 Hz, H-3′, H-5′), 6.38 (1H, s, H-5), 6.34 (1H, s, H-8), 3.48 (1H, t, J = 5.1 Hz, H-1), 2.81 (1H, dd, J = 14.2, 5.1 Hz, H-α), 2.71 (1H, dd, J = 14.2, 5.1 Hz, H-α), 2.57−2.99 (1H, m, H-3), 2.48−2.55 (1H, m, H-4), 2.31 (3H, s, Me); ^{13}C NMR (DMSO-d_6, 125 HMz) δ 155.1 (C-4′), 143.2 (C-6), 142.9 (C-7), 130.2 (C-2′, C-6′), 128.1 (C-8a), 124.4 (C-4a), 114.9 (C-5), 114.5 (C-3′, C-5′), 114.4 (C-8), 114.4 (C-1′), 64.0 (C-1), 46.9 (C-3), 42.0 (Me), 39.7 (C-α), 22.4 (C-4); EIMS *m*/*z* 285 $[M]^+$ (1), 178 (100), 107 (8), 77 (5), 44 (40); FABMS *m*/*z* 308 $[M + Na]^+$, 286 $[M + 1]^+$; *anal.* C 71.94%, H 6.60%, N 4.87%, calcd for $C_{17}H_{19}O_3N$, C 71.58%, H 6.67%, N 4.91%.

(−)-*N*-Methylhigenamine *N*-oxide (2): colorless needles (H_2O); mp 156−159 °C; $[\alpha]^{25}_D$ −9.6° (*c* 0.12, DMSO); UV (H_2O) λ_{max} 225 (4.35, sh), 284 (3.64) nm; IR (KBr) ν_{max} 3473, 1623, 1515, 1251, 841 cm^{-1}; ^1H NMR (D_2O, 500 HMz) δ 6.80 (2H, d, J = 8.4 Hz, H-2′, H-6′), 6.79 (1H, s, H-5), 6.71 (2H, d, J = 8.4 Hz, H-3′, H-5′), 6.66 (1H, s, H-8), 5.53 (1H, br s, H-1), 3.78 (1H, dd, J = 11.0, 3.2 Hz, H-α), 3.75 (1H, dd, J = 11.0, 2.8 Hz, H-α), 3.15−4.13 (1H, m, H-3), 3.07 (3H, s, Me), 2.55−3.01 (1H, m, H-3); ^{13}C NMR (DMSO-d_6, 125 HMz) δ 155.8 (C-4′), 145.1 (C-6), 143.3 (C-7) 130.5 (C-2′, C-6′), 127.9 (C-8a), 124.7 (C-4a), 119.1 (C-1′), 115.2 (C-5), 115.1 (C-3′, C-5′), 114.6 (C-8), 77.5 (C-1), 59.6 (C-3), 55.5 (Me), 37.1 (C-α), 25.4 (C-4); EIMS *m*/*z* 301 $[M]^+$ (4), 285 (8), 283 (22), 242 (97), 194 (16), 178 (70), 107 (23); *anal.* C 67.54%, H 6.35%, N 4.34%, calcd for $C_{17}H_{19}O_4N$, C 67.78%, H 6.31%, N 4.65%.

(±)-8-(*p*-Hydroxybenzyl)-2,3,10,11-tetrahydroxyprotoberberine pentaaceate (4): colorless needles ($MeOH/H_2O$); mp 166−168 °C; $[\alpha]^{20}_D$ 0° (*c* 0.25, MeOH); UV (EtOH) λ_{max} (log ϵ) 275 (3.92) nm; IR (KBr) ν_{max} 3449, 1765, 1506, 1373, 1213 cm^{-1}; ^1H NMR and ^{13}C NMR, see Table 1; EIMS *m*/*z* 466 (14), 424 (25), 382 (30), 340 (44), 298 (82), 242 (18), 164 (16), 136 (20), 107 (40), 77 (20), 42 (100); *anal.* C 66.43%, H 5.45%, N 2.21%, calcd for $C_{34}H_{33}O_{10}N$, C 66.34%, H 5.37%, N 2.28%.

Higenamine: colorless plates (EtOH); mp 256−258 °C (lit. 255−256 °C);[3a] UV, IR, and NMR data are consistent with an authentic sample and literature values.[3a]

Trigonelline: colorless plates (EtOH); mp 212−214 °C (lit. 218 °C, dec);[16] UV, IR, and NMR data are consistent with literature values.[16]

***N*-Methylation of Higenamine.** A solution of higenamine (20 mg) in methanol (2 mL) was stirred with 37% formalin (0.5 mL) at room temperature for 30 min. Sodium borohydride (25 mg) was added and the solution was stirred for an additional 30 min. After the solvent was removed in vacuo, the residue was separated by preparative TLC with $EtOH-Me_2CO$ (3:1), producing 12 mg of compound **1**, identical with the natural product (IR, EIMS, TLC).

Oxidation of 1. A solution of *m*-chloroperbenzoic acid in methanol (17 mg in 2 mL) was added dropwise to compound **1** (28 mg) at 0−5 °C. The mixture was stirred for 2 days and then the purified by preparative TLC using $EtOH-Me_2CO$ (3:1). The product (16 mg) was identical with compound **2** (IR, EIMS, TLC).

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