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19α-HYDROXYGELSAMYDINE FROM *GELSEMIUM ELEGANS*

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Key Word Index—*Gelsemium elegans*; Loganiaceae; indole alkaloid; 19α-hydroxygelsamydine; NMR data.

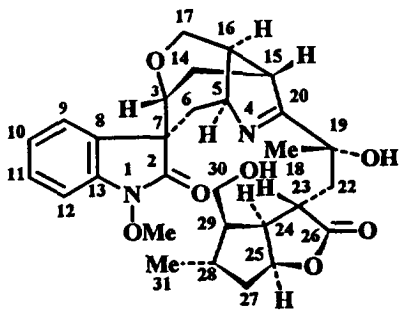
Abstract—A new indole alkaloid, was isolated from *Gelsemium elegans*, 19α-hydroxygelsamydine and its stereochemistry and ^{1}H and ^{13}C-NMR data were determined by two-dimensional NMR techniques, computer modelling calculations and comparison with gelsamydine. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

In previous publications, we reported on the isolation of gelsamydine (**2**), a novel indole alkaloid with two monoterpene units, together with about 30 other indole alkaloids, from *Gelsemium elegans* [1–8]. Recently, reexamination of the column chromatography fractions containing alkaloids with molecular ions at *m/z* 500–600 led to the isolation of 19α-hydroxygelsamydine. In this report, we present the isolation and structure determination of this new alkaloid.

RESULTS AND DISCUSSION

19α-Hydroxygelsamydine (**1**), $C_{29}H_{36}N_2O_7$ (high-resolution mass spectrometry), has one more oxygen atom than gelsamydine (**2**), an indole alkaloid isolated from this plant previously [3]. The alkaloids possess very similar ^{1}H and ^{13}C NMR data, except that the C-18 methyl of compound **1** showed its proton resonance as a single peak (δ 1.65) and C-19 appears as a quaternary carbon signal at δ 74.0. This suggested that the additional oxygen atom was present in the form of a hydroxyl group at the C-19 position. The COSY spectrum of this compound showed very clear correlations between each pair of the vicinal and geminal protons. Thus, starting from the H-17 methylene signals at δ 4.26 and δ 4.37, the correlations could be traced through H-16, H-15, H-14α, H-14β and H-3, and the H-5 and two H-6 proton were identified. Similarly, from the H-31 methyl doublet signal, the signals for H-28, H-27α, H-27β, H-25, H-24, H-23, H-22a and H-22b, H-29 and two H-30 protons were assigned completely. From the aromatic proton H-9, which



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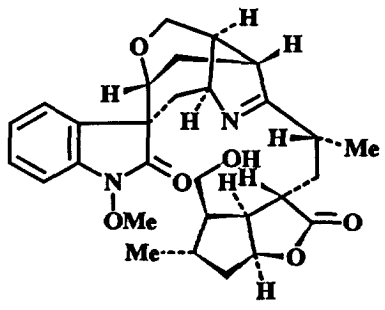

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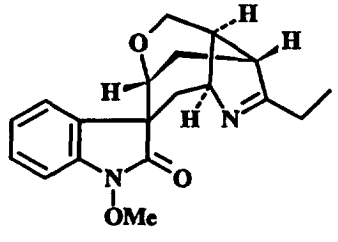

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showed a rOe with H-6α, the three remaining aromatic protons (H-10, H-11 and H-12) were assigned from their COSY relationship.

Two methyl (N_1-OMe, and H-18) singlets were assigned from their distinctive chemical shifts (δ 3.93 and δ 1.65). With a complete assignment of the protons, their attached carbons were assigned from the HETCOR spectrum directly. Then, the selective INEPT technique [9, 10] offered the assignment of the quaternary carbons and confirmed the skeleton of the alkaloid (Table 1). Selective INEPT irradiation of the H-18 methyl singlet enhanced C-20, C-22 and C-19, to establish the hydroxyl function at C-19, and led to the assignment of these three carbon signals. Irradiation of H-5 enhanced C-7, C-15 and C-6, and through continuing irradiations, as shown in Table 1, all of the proton and carbon signals were completely assigned.

The stereochemistry of the C-19 hydroxyl group and the stereotopical assignments of the protons from the six methylenes of this alkaloid were assigned by comparison of the ROESY correlations (Table 1) [11–13] and coupling constants (Table 2) with those from computer modelling calculations (Fig. 1) [14]. The H-18 methyl showed a clear correlation with H-14α (calc. 2.452 Å for C-19α-OH; 4.023 Å for C-19β-OH) and with H-23 (calc. 2.544 Å for C-19α-OH; 4.601 Å for C-19β-OH), suggesting that this compound would most probably have a C-19α-OH rather than a C-19β-OH. Namely, its C-18 methyl should have a β-orientation and that this compound is 19α-hydroxygelsamydine.

Table 1. Major results of ROESY and selective INEPT spectra of 19α-hydroxygelsamydine (**1**)*

^{1}H	ROESY (^{1}H)	Selective INEPT (^{13}C)
3β	14α, 14β	8, 15, 17
5α	6α, 6β, 16α	(6), 7, 15
6α	5, 6β, 9	3, (7), 8
6β	5, 6α	2, 3, (5)
9	6α	8
12	–	13
14α	3β, 14β, 15β	–
14β	3β, 14α, 15β	–
15β	14α, 14β, 16α	–
16α	15β, 17α, 17β	–
17α	16α, 17β	3, 5, 15
17β	16α, 17β	3, 5, 15
18	14α, 15β, 22*a*, 23	(19), 20, 22
22a	22b, 23	24, 26
22b	22a	–
23β	22*a*	–
24α	25	–
25α	24, 27α, 27β	26, 28
27α	25, 27β, 31	24, (25)
27β	27α	–
28β	30b	–
29α	24, 30a, 30b	–
30a	29, 30b, 31	24
30b	29, 30a	24
31	27α, 29, 30a	27 (28), 29

*Recorded in $CDCl_3$, two-bond correlations of proton and carbon are shown in parentheses.

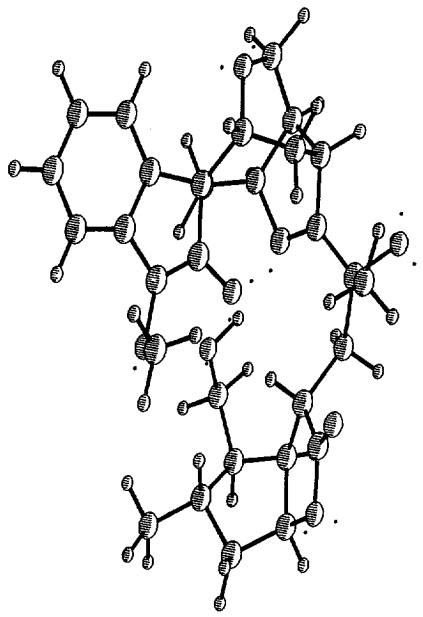

Fig. 1. Ball and stick representation of 19α-hydroxygelsamydine (**1**).

The ROESY correlation between H-18 and H-14α also indicates the probable assignment of the proton NMR resonances of the C-14 methylene protons (H-14α at δ 2.53, 2.452 Å; H-14β at δ 2.27, 3.150 Å). Because these signals are very close to those from the H-6 and H-22 methylene groups and H-27β, their J values cannot be obtained directly. This assignment was further confirmed by the observation of a very weak COSY correlation peak between H-15 and H-14α (calc. J = 1.08 Hz), and a very strong COSY correlation peak between H-15 and H-14β (calc. J = 9.00 Hz) in its COSY spectrum. The ROESY correlation between H-9 and one of the C-6 methylene proton signals at δ 2.47 led to the assignment of this signal as H-6α (calc. 2.510 Å), whereas the remaining signal at δ 2.31 is for H-6β (3.873 Å). The ROESY correlation between H-25α and one of the C-27 methylene proton signals at δ 1.46 was used to assign H-27α, then, its partner H-27β showed a resonance at δ 2.20. The rOe between the H-31 methyl and one of the C-30 methylene signals at δ 3.86 was used to assign H-30α (calc. = 2.515 Å; 4.048 Å with H-30β). From computer modelling, the C-30-OH appears to have formed a hydrogen-bond with the N_5 atom, and thus, the ring N_5-C_{20}-C_{19}-C_{22}-C_{23}-C_{24}-C_{29}-C_{30}-O-H was formed. In this case, H-30a appeared to have an α-orientation, H-30b a β-orientation, H-22a was in the α position and H-22b was in the β position. The last two proton signals were assigned by a clear rOe correlation peak between H-22β and H-18 (calc. 2.657 Å for H-22β; 3.803 Å for H-22α). The C-17 methyl signals were assigned with their

Table 2. ^1H and ^{13}C NMR data of 19-hydroxygelsamydine (**1**)*

No.	^1H	^{13}C
2	–	171.9
3	3.73 (*dd*, 2.0, 5.0)	74.9
5	4.44 (*ddd*, 2.0, 5.0, 7.5)	71.9
6α	2.47 (*dd*, 15.0, 4.5)	37.1
6β	2.31 (*m*)	
7	–	56.3
8	–	132.8
9	7.53 (*br. d*, 7.5)	124.7
10	7.10 (*dt*, 1.5, 7.5)	123.7
11	7.30 (*dt*, 1.5, 7.7)	128.2
12	6.91 (*br. d*, 7.5)	106.8
13	–	137.7
14α	2.53 (*br. d*, 13.5)	28.5
14β	2.27 (*m*)	
15	3.28 (*br. t*, 9.0)	39.4
16	2.6 (*br. t*, 8.0)	40.7
17	4.26 (dd, 1.5, 11.5)	62.0
17β	4.37 (*dd*, 3.0, 11.5)	
18	1.65 (*s*)	27.3
19	–	74.0
20	–	185.8
N_a-OMe	3.93 (s)	63.5
22a	2.40 (*dd*)	40.8
22b	2.22 (*dd*)	
23	3.20 (*m*)	37.4
24	3.19 (*m*)	47.8
25	5.00 (*br. t*, 6.5)	83.3
26	–	181.5
27α	2.20 (*m*)	41.1
27β	1.46 (*m*)	
28	1.85 (*m*)	32.6
29	1.85 (*m*)	51.7
30a	3.86 (*dd*, 1.0, 10.5)	60.6
30b	3.72 (*m*)	
31	1.03 (*s*)	17.2

*Recorded in $CDCl_3$, chemical shift values are reported as δ values (ppm) from TMS at 500.1 MHz for ^1H NMR and 125.8 MHz for ^{13}C, signal multiplicity and coupling constants (Hz) are shown in parentheses.

coupling constants (calc. $J = 1.35$ Hz and obsd. 1.5 Hz for H-17α; calc. $J = 3.08$ Hz and obsd. 3.0 Hz for H-17β) with H-16α. Thus, all of the protons and functional groups were assigned stereotypically, and the ^1H and ^{13}C NMR data are shown in Table 2.

Comparison of its NMR data with those of gelsamydine (**2**) showed that some of their data are not consistent with each other. In order to clarify these assignments, the ^1H, ^{13}C, COSY, ROESY and HETCOR spectra of gelsamydine (**2**) were also observed at 500.1 MHz, 125.8 MHz and 500.1 MHz/125.8 MHz, respectively. The results showed that the previous assignments [2] for H-3 and H-5, H-15 and H-16, H-23 and H-29, C-3 and C-5, C-15 and C-16, C-23 and C-29 should be reversed, in order for consistency to be observed with the data for **1**. Furthermore, the previous assignments of the corresponding resonances for gelsenicine [2] should also be revised.

This compound was evaluated for cytotoxicity in the KB (human carcinoma of the nasopharynx) cell culture system, but no activity was observed.

EXPERIMENTAL

General. The optical rotation was measured with a Perkin–Elmer 241 polarimeter and the UV spectrum was taken in MeOH on a Beckman DU-7 spectrometer. The IR spectrum was recorded in a AgCl dish on a MIDAC FT-IR interferometer. Solutions in $CDCl_3$ were used for all of the NMR studies. ^1H NMR, COSY, and ROESY spectra were recorded at 500.1 MHz with a GE OMEGA 500 instrument, using standard GE programs; ^{13}C NMR and DEPT spectra were recorded at 125.8 MHz; the HETCOR spectrum was obtained at 500.1/125.8 MHz with a GE OMEGA 500 instrument, using standard programs from the GE library. Selective INEPT spectra were taken on a Nicolet NT-360 spectrometer operating at 90.8 MHz with $J = 8$ or 6 Hz for aromatic protons, and $J = 6$ Hz for aliphatic protons. EI and HR mass spectra were recorded on a Finnigan MAT-90 instrument.

Plant material. Gelsemium elegans was collected in Guangxi Province, P.R.C., in February, 1987 and was identified by Dr X. L. Huang, Department of Phytochemistry, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai, P.R.C. A voucher sample is deposited in the herbarium of the Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai, P.R.C.

Extraction and separation. Air-dried whole plant material (20 kg) was processed according to the procedure described in refs. [1–8]. Several frs (2.1 g) of the 10% methanol in chloroform eluent from the column chromatography on Si gel of the alkaloid extract contained alkaloids with $[M]^+$ at m/z 508 and 524, was subjected to repeated prep. TLC using cyclohexane–EtOAc–diethylamine (6:4:1, v/v), and the fifth band at R_f 0.15 was eluted with Me_2CO to yield a yellowish powder (6 mg, 0.00003%) of compound **1**. The first band yielded gelsamydine (**2**).

19α-Hydroxygelsamydine (**1**). Alkaloid **1** was obtained as a yellowish powder, $[\alpha]_D$ −75° (c 0.01, MeOH), UV λ_{max}^{MeOH} (log ε): 209 (4.29), 257 (3.67) nm; IR (AgCl): 3434, 2956, 1753, 1724, 1468, 1195, 1178, 1105, 1039 and 750 cm^{-1}; ^1H NMR data (Table 2); ^{13}C NMR data (Table 2); EIMS m/z (100%): 524 ($[M]^+$, 17), 508 (97%), 478 (77), 339 (32), 333 (18), 332 (41), 327 (21), 326 (100), 313 (19), 309 (19), 304 (33), 296 (40), 295 (23), 150 (77), 146 (22), 144 (21), 134 (18), 132 (28), 130 (23), 122 (37), 120 (21), 117 (19), 116 (17), 108 (19), 107 (29), 95 (21), 94 (60), 93 (31), 91 (19), 81 (48), 80 (19), 79 (25), 77 (22), 67 (31), 54 (45), 43 (44), 41 (58), 31 (34); HR-MS: obsd. 524.2515 for $C_{29}H_{36}N_2O_7$, calc. 524.2523.

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