A NEW ALKALOID, 2-HYDROXY-7-METHYL-9*H*-CARBAZOLE FROM *CIMICIFUGA SIMPLEX*

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Abstract----A new alkaloid was isolated from the aerial parts of *Cimicifuga simplex* and was formulated as 2-hydroxy-7-methyl-9*H*-carbazole on the basis of the spectroscopic evidence.

The isolation and the structural elucidation of cycloartane triterpenes,[1,2] their glycosides,[2] caffeic acid derivatives[3] and chromones[4] have been reported as the constituents of the rhizomes and roots of *Cimicifuga simplex*. In the continuous work on the constituents of the aerial parts of the same herb, a new alkaloid (I) was isolated and the structure was elucidated as 2-hyroxy-7-methyl-9*H*-carbazole on the basis of the results of spectroscopic analyses of I and its acetate (Ia) and by the comparison with the data of some related monohydroxy-monomethyl-9*H*-carbazoles.

The alkaloid (I), mp 269-271℃, $C_{13}H_{11}NO$ (M^+ 197, determined by ms) was isolated by chromatographic separation of the n-butanol extract of the aerial parts of *Cimicifuga simplex*. The uv spectrum, λ max: 236.0 nm (*log* ε, 4.41), 261.5 (4.02), 305.0 (3.87), is similar to that of 2-hydroxy-3-methyl-9*H*-carbazole.[5] The ir spectrum showed N-H and O-H bands at 3400, 3000-2700 cm^{-1} and aromatic bands at 1611, 1192 and 800 cm^{-1}. The ^{1}H-nmr spectrum showed the presence of two 1, 2, 4-trisubstituted benzene rings and a methyl group on an aromatic ring. I gave a mono-*O*-acetate (Ia), the ^{1}H-nmr spectrum of which suggested the presence of the partial structure A in I. The nuclear Overhauser effect difference spectrum showed the correlation as shown by arrows in the partial structure B.

The subtraction of the partial structures A and B from the molecular formula $C_{13}H_{11}NO$, left NH (C). The ^{13}C-nmr spectrum showed six singlet sp^2 carbons in addition to six doublet sp^2 carbons and

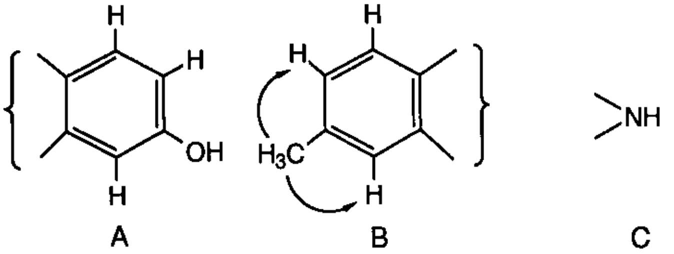

Figure I . Partial structures A, B and C of I, $C_{13}H_{11}NO$.

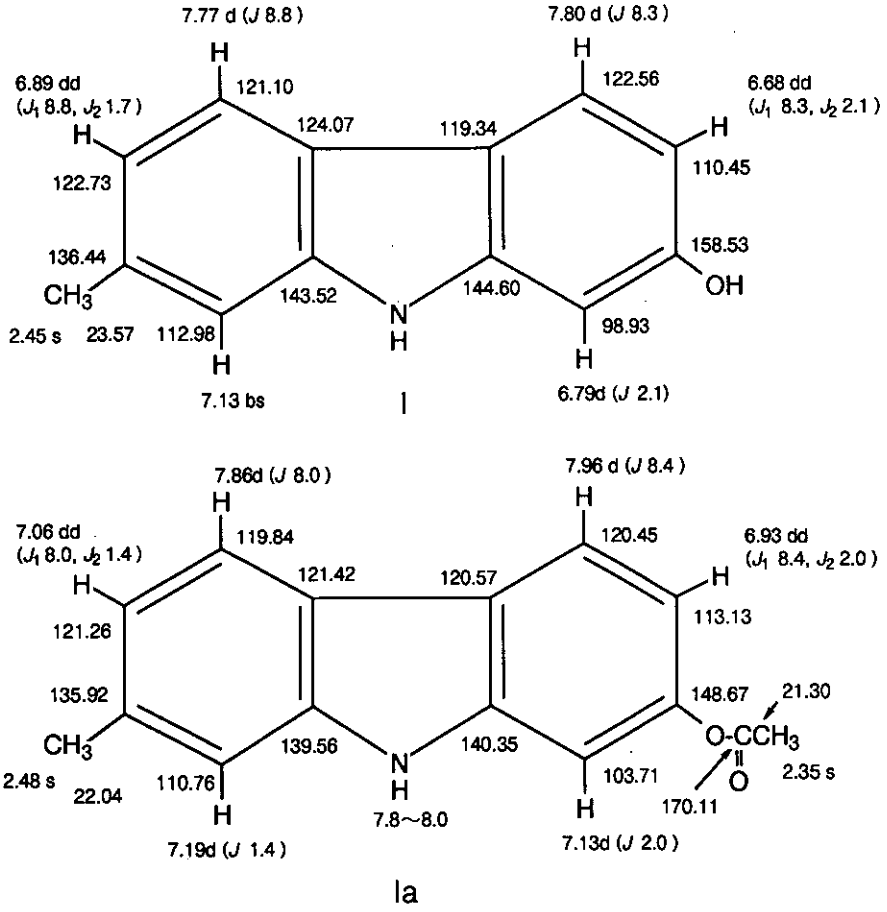

Figure 2 . Structures of I and Ia and Assignment of ^{1}H- and ^{13}C -Nmr Data.

a quartet sp^3 carbon (a methyl group on an aromatic ring), supporting the partial structures A and B. The ^{13}C-nmr spectra of I and Ia were analyzed with the results of ^1H-^{13}C chemical shift correlation spectroscopy (COSY) and distortionless enhancement by polarization transfer (DEPT) spectra. The ^1H-detected heteronuclear multiple bond multiple quantum coherence (HMBC) spectrum and the reported data of 9*H*-carbazole derivatives[5~9] helped to lead the formula I as the structure of the new alkaloid as shown in Figure 2. The acetate (Ia), mp 245 - 247℃, $C_{15}H_{13}NO_2$, was obtained by acetic anhydride-pyridine treatment at room temperature and it gave a clearly separated ^1H-nmr spectrum. ^1H-Nmr decoupling, ^1H-^{13}C COSY, long range ^1H-^{13}C COSY and DEPT spectra led to assign all signals of ^1H and ^{13}C nmr spectra of Ia as shown at Figure 2. ^1H-Nmr signals due to H-4 and H-5 of 9*H*-carbazoles have been reported to appear at *ca.* 7.80- 8.00 ppm. I and Ia showed H-4 and H-5 signals at 7.80 and 7.77 ppm (I), and 7.96 and 7.89 ppm (Ia) as the *ortho*-coupled doublets. This was a crucial feature in distinguishing I from 1-hydroxy-3-methyl-9*H*-carbazole,[5~7] mp 237 -240℃, 2-hydroxy-3-methyl-9*H*-carbazole,[8] mp 243℃ and glycozilinine (3-methyl-6-hydroxy-9*H*-carbazole),[9] mp 231 - 232℃, by the ^1H-nmr spectrometry. (*E*) - and (*Z*) - 3 - (3' - methyl - 2' - butenylidene) - 2 - indolinone (II and III) have been obtained from the rhizomes of *Cimicifuga dahurica* by K.Baba *et al.*[10] Although these compounds were neither detected in the tlc of the extracts of the rhizomes nor the aerial parts of *Cimicifuga simplex*

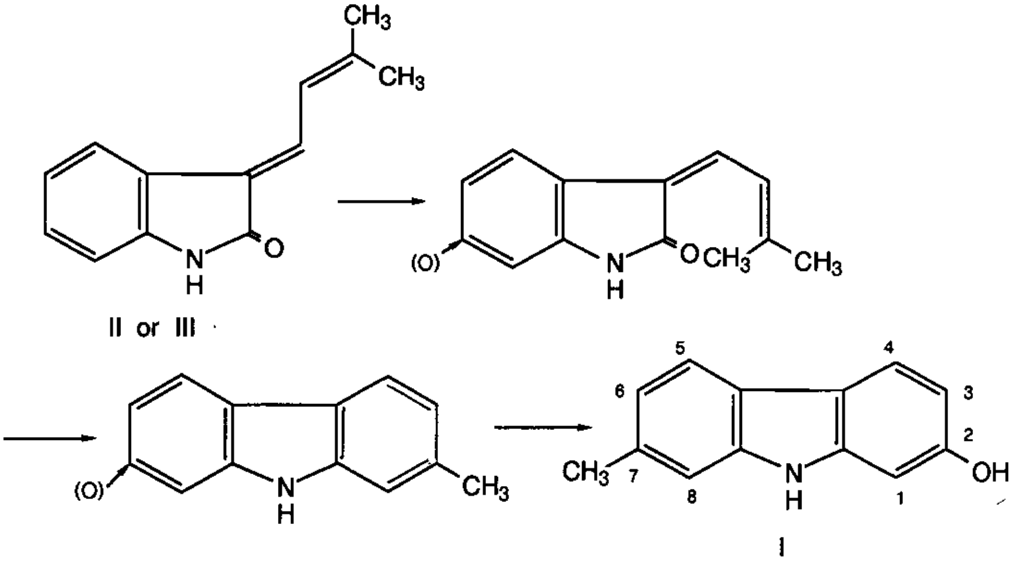

Figure 3. Hypothetical Route to I from the Indolinone Derivatives II and III.

by comparing with authentic specimens of II and III, the new alkaloid is supposed to be biosynthesized from these precussors as shown in Figure 3.

EXPERIMENTAL

Melting points were measured on Yanagimoto micromelting point apparatus and uncorrected. The nmr spectra were recorded on Varian Gemini - 200 and Varian XL-300 spectrometers with tetramethylsilane as the internal standard and the chemical shifts are expressed in δ values. Mass spectra were taken on Hitachi M-80 and JEOL JMS-DX 300 mass spectrometers.

Isolation

Dried aerial parts (6.07 kg) of *Cimicifuga simplex*, which were collected on August, 1991 in Sendai, Japan, were extracted at room temperature overnight with MeOH (30 l × 3). The MeOH solution was subjected to an activated charcoal column (250 g) and the passed fraction was concentrated *in vacuo* to yield gummy extracts (500 g). The concentrated extracts were shaken with n-BuOH (300 ml) three times and the joined n-BuOH layer was washed with water (200 ml) twice. The solvent was evaporated *in vacuo* to provide the residue (270 g), which was chromatographed on a SiO_2 (1.2 kg) column and was eluted first with n-hexane and then with an increasing amount of ethyl acetate in n-hexane to give six fractions, followed by five fractions eluted with ethyl acetate and with an increasing amount of MeOH in ethyl acetate.

The 1 st to 7 th fractions (25.0 g) were combined and chromatographed again on SiO_2 (300 g) . Elution with n-hexane-ethyl acetate (3:1, 2:1, 1:1) provided gummy material (4.02 g), one portion (1.60 g) of which was chromatographed on SiO_2 (20 g) . Elution first with $CHCl_3$ and then with an increasing amount of MeOH in $CHCl_3$ provided the alkaloid(I) (17.0 mg) as pale yellow amorphous powder , mp 269 - 271℃, after recrystallization from $CHCl_3$- MeOH. Ms: *m/z* 197 (M^+, 100), 196 (50), 167 (10). Uv(MeOH) λ max nm (log ε): 236.0 (4.41), 261.5 (4.02), 305.0 (3.87). Ir($CHCl_3$) ν max cm^{-1}: 3400, 3000 - 2700 (OH, NH) , 1611, 1192, 800 (aromatic). ^{1}H-Nmr ($CDCl_3$) δ and ^{13}C-nmr ($CDCl_3$) δ : Figure 2.

Acetylation

I (7.6 mg) was dissolved to pyridine (1 ml) and acetic anhydride (0.2 ml) was added to stir at room temperature for 3 h. Water (5 ml) was added and the mixture was shaken with ethyl acetate (10 ml) three times. After washing with water , the ethyl acetate solution was concentrated *in vacuo* and the residue was subjected to preparative thin layer chromatography. After developement with chloroform, the band of R*f* 0.6 - 0.7 was scraped and chromatographed on SiO_2 (2 g) to elute with chloroform. The acetate (I a), mp 245 - 247℃, $C_{15}H_{13}NO_2$ was obtained as colorless needles (7. 3 mg) after recrystallization from ethyl acetate. FAB *ms: m/z 240 (* M^+ *+ 1).* *Ir (KBr)* λ *max* cm^{-1}*: 3395 (NH) , 1760, 1216*

($OCOCH_3$), 1631, 1612, 901, 811 (aromatic). ^1H-Nmr ($CDCl_3$) δ and ^{13}C-nmr ($CDCl_3$) δ : Figure 2.

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