Gerfelin, a Novel Inhibitor of Geranylgeranyl Diphosphate Synthase from *Beauveria felina* QN22047

II. Structural Elucidation

Sir:

Protein geranylgeranylation of small GTP-binding proteins, such as Rho, Rac, Cdc42 and Rab[1], is essential for the function of the modified proteins. Protein geranylgeranylation is a key target for chemotherapeutic intervention in a number of diseases. Because geranylgeranyl diphosphate synthase (GGPP synthase)[2,3] is involved in a pathway for protein geranylgeranylation, we screened an inhibitor of GGPP synthase, and we have isolated gerfelin (**1a**) from a culture broth of *Beauveria felina* QN22047. In the preceding paper[4], the taxonomy, fermentation, isolation and biological activities were reported. In this paper, we describe the physico-chemical properties and structural elucidation of **1a**.

The physico-chemical properties of **1a** are summarized in Table 1. **1a** was isolated as a white powder. **1a** is readily soluble in methanol and acetone, but insoluble in chloroform and acetonitrile. The UV spectrum acquired in methanol has λ_{max} of 258 nm (ε 12,900) and 218 nm (ε 34,800). **1a** gave a positive color reaction with molybdophosphoric acid-sulfuric acid reagent and diazobenzene sulfonic acid reagent. The molecular formula of **1a** was established as $C_{15}H_{14}O_6$ by HRFAB-MS, which was supported by ^1H and ^{13}C NMR spectra. The fragment ion peak observed at *m/z* 245 (M−COOH) by a negative FAB-MS indicated the presence of carboxylic acid in **1a**. The ^1H NMR spectrum of **1a** exhibited two single-methyls (δ_H 2.12 and δ_H 2.41) and four aromatic methines (δ_H 5.97, δ_H 6.17, δ_H 6.25 and δ_H 6.48). The ^{13}C NMR spectrum of **1a** showed 15 carbon signals; two sp^3 methyls, four sp^2 methines, eight sp^2 quaternary carbons and one carboxylic acid carbon (δ_C 172.4). Considering the molecular formula and sp^2 carbon number (twelve) of **1a**, the presence of two benzene rings was proposed.

The HMBC[5] experiment on **1a** showed long range couplings from H-15 (δ_H 2.12) to C-12 (δ_C 113.0), C-13 (δ_C 127.8) and C-14 (δ_C 113.2), from H-12 (δ_H 6.48) to C-10 (δ_C 135.3) and C-11 (δ_C 141.9) and from H-14 (δ_H 6.25) to C-9 (δ_C 146.9) and C-10 (δ_C 135.3) (Fig. 2). Thus, the

Table 1. Physico-chemical properties of gerfelin (**1a**).

Appearance	White powder
Molecular formula	$C_{15}H_{14}O_6$
ESI-MS (m/z)	291 $(M-H)^+$
	289 $(M-H)^-$
HRFAB-MS (m/z)	
Found	289.0717
Calcd.	289.0712
UV in MeOH	
λ_{max}^{nm} (ε)	258 (12,900)
	218 (34,800)
R_f value[a]	0.25

[a]Silica gel TLC (Merck Art. 1.05715, $CHCl_3$:MeOH=3:1)

Table 2. ^{13}C and ^1H NMR data for gerfelin (**1a**) in DMSO-d_6.

Position	δ_C (ppm)	δ_H (ppm)
1	172.4	
2	110.2	
3	142.2	
4	109.6	6.17 (1H, d, 2.3)
5	160.7	
6	100.8	5.97 (1H, d, 2.3)
7	163.7	
8	22.9	2.41 (3H, s)
9	146.9	
10	135.3	
11	141.9	
12	113.0	6.48 (1H, d, 1.3)
13	127.8	
14	113.2	6.25 (1H, d, 1.3)
15	20.4	2.12 (3H, s)

Chemical shifts in ppm from TMS as internal standard. ^1H and 2D NMR spectra of **1a** were recorded in DMSO-d_6, **1b** were in CD_3OD on a 500 MHz spectrometer at 298 K, while ^{13}C NMR sectra were measured on a 125 MHz spectrometer. HMBC spectra were recorded using a 60 ms delay time for long-range C-H coupling.

Fig. 1. Structure of gerfelin (**1a**) and tetrakis methyl gerfelin (**1b**).

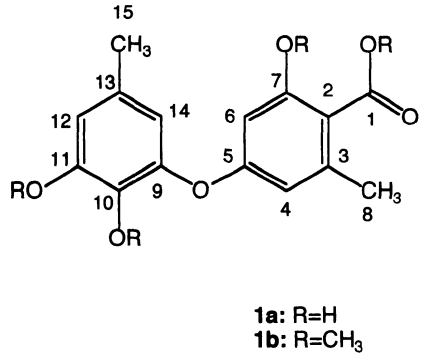
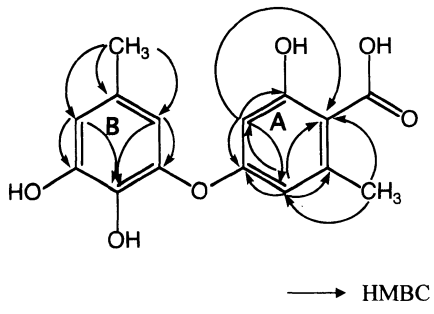


Fig. 2. HMBC correlations of gerfelin (**1a**).

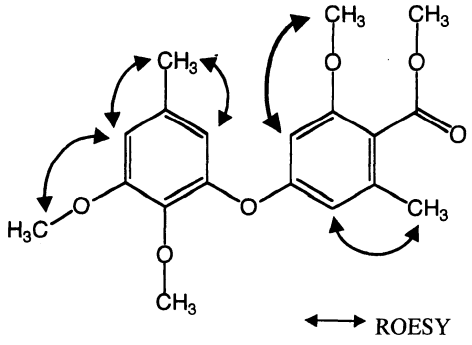


carbons constituting one benzene ring (ring B) were established. The attachment of oxygen atom at C-9, C-10 and C-11 was confirmed from ^{13}C chemical shifts.

The HMBC experiment on **1a** also showed long range couplings from H-8 (δ_H 2.41) to C-2 (δ_C 110.2), C-3 (δ_C 142.2) and C-4 (δ_C 109.6), from H-4 (δ_H 6.17) to C-2, C-3, C-5 (δ_C 160.7) and C-6 (δ_C 100.8) and from H-6 (δ_H 5.97) to C-2, C-4, C-5, and C-7 (δ_C 163.7) (Fig. 2). From these findings, the carbons constituting the other benzene ring (ring A) were confirmed. The attachment of oxygen atom at C-5 and C-7 was clarified from ^{13}C chemical shifts. The position of carboxylic acid at C-2 was determined by elimination.

The linkage position of ring A and ring B through a ether bond was determined by chemical means as follow. **1a** was treated with MeI and K_2CO_3 to provide tetrakis methyl

Fig. 3. ROESY correlations of tetrakis methyl gerfelin (**1b**).



Tetrakis methyl gerfelin (**1b**): ^1H NMR (CD_3OD) δ 2.18 (3H, s, H-8), 2.29 (3H, s, H-15), 3.69 (3H, s, OMe-10), 3.73 (3H, s, OMe-7), 3.83 (3H, s, OMe-1), 3.86 (3H, s, OMe-11), 6.27 (1H, m, H-4), 6.44 (1H, m, H-6), 6.48 (1H, m, H-14), 6.73 (1H, m, H-12); ESIMS *m/z* 347 $(M+H)^+$.

ROESY spectra were measured with a mixing time of 200 ms.

gerfelin (**1b**), the structure of which was elucidated by spectral data. ROESY (Fig. 3) correlations of H-4 (δ_H 6.27) and Me-8 (δ_H 2.18), H-6 (δ_H 6.44) and OMe-7 (δ_H 3.73), H-12 (δ_H 6.73) and OMe-11 (δ_H 3.86), H-12 (δ_H 6.73) and Me-15 (δ_H 2.29), and H-14 (δ_H 6.48) and Me-15 revealed that ether linkage exist between C-5 and C-9. Thus the gross structure was elucidated as shown in Fig. 1.

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