WF11605, AN ANTAGONIST OF LEUKOTRIENE B_4 PRODUCED BY A FUNGUS

II. STRUCTURE DETERMINATION

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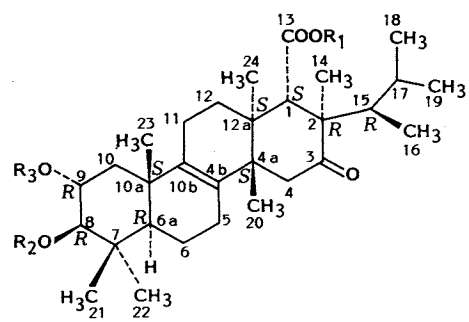
The structure of WF11605, a novel tetracyclic triterpene glucoside, was determined to be **1**. The plane structure of deacetyl-WF11605 aglycone was elucidated as **2** through the concerted application of a series of 2D NMR techniques. The relative configurations were established by X-ray crystallographic analysis of bis(*p*-bromobenzoyl) derivative **3** and absolute stereochemistry by CD exciton chirality method.

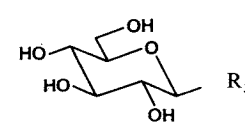
In the preceding paper[1], the taxonomy, fermentation, isolation, physico-chemical properties, and biological activity of WF11605 (**1**) are presented. Herein we describe the full account of structure determination of **1**.

From the ethyl acetate extract of the fermentation broth of a fungus F11605 (FERM BP-1730), **1** was isolated as colorless needles: mp 291°C, $[\alpha]_D^{23}$ −46° (*c* 0.5, MeOH). High-resolution (HR)FAB-MS measurement on **1** indicated the molecular formula $C_{38}H_{60}O_{11}$, consistent with elemental analysis[1] and ^{13}C NMR data (Table 1).

The acidic nature of **1** and ^{13}C signal at δ 175.2 indicated the presence of a carboxyl group in **1**. The three-proton singlet resonating at δ 2.09 and IR absorption band at 1730 cm^{-1} suggested the presence of an acetyl group which was corroborated by ^{13}C signals (δ 172.6 (s), and 21.7 (q)). The presence of an *O*-glycoside group was inferred from a positive color reaction for the Molisch reaction and a typical anomeric ^{13}C signal at δ 105.1. Six oxygenated ^{13}C signals (δ 105.1 (d), 77.4 (d), 76.7 (d), 75.1 (d), 71.4 (d), and 62.7 (t)) suggested that **1** possessed an *O*-glucopyranoside moiety[2]. The magnitude of the vicinal coupling constant (8 Hz)

Fig. 1. Structures of WF11605 (**1**), deacetyl-WF11605 aglycone (**2**), and bis(*p*-bromobenzoyl) methyl ester (**3**).





1 R_1=H R_2 = R_3=CH_3CO

2 R_1=R_2=R_3=H

3 R_1=CH_3 R_2 = R_3 = Br—C$_6$H$_4$—CO

Table 1. ^{1}H and ^{13}C NMR data for **1** and **2**.

Position	**1**				**2**			
	^{1}H		^{13}C		^{1}H		^{13}C	
	δ[a]	Mult.	δ[b]	Mult.	δ[a]	Mult.	δ[b]	Mult.
1	3.10	(s)	53.1	d	3.07	(s)	53.8	d
2			54.6	s			55.3	s
3			217.2	s			218.1	s
4	2.73	(d, 17)	46.1	t	2.69	(d, 17)	46.8	t
	2.26	(d, 17)			2.23	(d, 17)		
4a			43.1	s			43.8	s
4b			132.5	s			132.7	s
5	2.15[c]		27.6	t	2.05[d]		28.3	t
	1.95[c]				1.90[d]			
6	1.80[c]		19.3	t	1.70[d]		20.2	t
	1.45[c]				1.45[d]			
6a	1.25	(m)	50.9	d	1.17	(m)	51.8	d
7			38.6	s			40.3	s
8	3.35[c]		88.7	d	2.93	(d, 10)	84.3	d
9	5.14	(m)	71.3	d	3.64	(ddd, 11, 10, 4)	70.1	d
10	2.05[c]		41.3	t	2.05[d]		44.2	t
	1.32	(m)			1.10	(m)		
10a			41.6	s			39.9	s
10b			135.2	s			136.3	s
11	2.05[c]		21.0	t	2.05[d]		21.7	t
	2.05[c]				2.05[d]			
12	1.75[c]		30.4	t	1.70[d]		31.1	t
	1.50[c]				1.45[d]			
12a			39.1	s			39.2	s
13			175.2	s			175.9	s
14	1.38	(s)	22.0	q	1.34	(s)	22.7	q
15	1.85	(m)	47.2	d	1.82	(m)	47.8	d
16	0.98	(d, 7)	10.4	q	0.95	(d, 7)	11.1	q
17	1.90	(m)	27.1	d	1.86	(m)	27.8	d
18	0.83	(d, 7)	18.7	q	0.80	(d, 7)	19.4	q
19	0.91	(d, 7)	25.9	q	0.88	(d, 7)	26.6	q
20	1.13	(s)	24.8	q	1.09	(s)	25.5	q
21	1.19	(s)	16.8	q	0.81	(s)	17.8	q
22	1.15	(s)	28.5	q	1.01	(s)	29.5	q
23	1.11	(s)	21.2	q	1.02	(s)	22.1	q
24	0.97	(s)	17.7	q	1.16	(s)	17.5	q
1′	4.39	(d, 8)	105.1	d				
2′	3.20	(m)	75.1	d				
3′	3.39	(m)	77.4	d				
4′	3.33	(m)	71.4	d				
5′	3.35[c]		76.7	d				
6′	3.87	(dd, 12, 2)	62.7	t				
	3.70	(dd, 12, 5)						
CH_3CO	2.09	(s)	21.7	q				
CH_3CO			172.6	s				

[a] 400 MHz in CD_3OD-$CDCl_3$ (1 : 1).

[b] 100 MHz in CD_3OD-$CDCl_3$ (1 : 1).

[c,d] Chemical shifts and multiplicities are obscure due to overlapping.

of the anomeric proton (δ 4.39) in **1** established the β-orientation for the D-glucopyranoside. Acid treatment of **1** (5% HCl-MeOH, reflux for 1 hour) gave deacetyl-WF11605 aglycone (**2**) and methyl glycoside. The presence of D-glucose in **1** was confirmed by the isolation of methyl tetra-*O*-acetyl-α-D-glucopyranoside by acetylation of the obtained methyl glycoside.

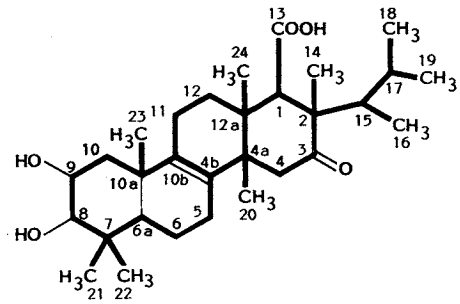
Structure Determination of **2**

HRFAB-MS and ^{13}C NMR established the molecular formula of **2** as $C_{30}H_{48}O_5$, which was consistent with the loss of glucosyl and acetyl moieties from **1**.

A combination of normal and DEPT-edited ^{13}C NMR spectra showed the presence of one carbonyl group, one carboxyl group, two non-protonated olefin carbons, five non-protonated sp^3 carbons, six methine carbons including two oxymethines, six methylene carbons, and nine methyl carbons (Table 1).

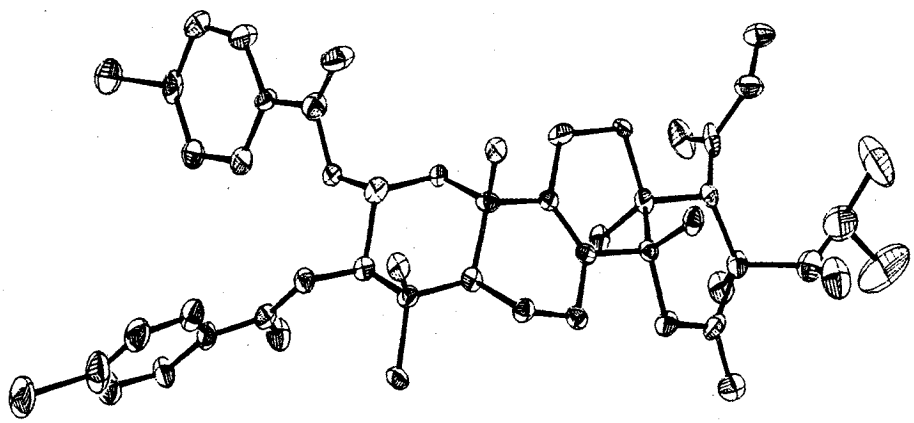
The four sp^2 carbons account for three unsaturations and the remaining four unsaturations required by the molecular formula of **2** suggested that the aglycone (**2**) was a tetracyclic compound. A combination of ^1H-^1H COSY and ^{13}C-^1H COSY spectral analysis of **2** revealed the partial structures, $-CH_2-\underset{OH}{CH}-\underset{OH}{CH}-$, $CH_3-\underset{CH_3}{CH}-\underset{CH_3}{CH}-CH_3$ and $-\underset{CH_3}{\overset{CH_3}{C}}-CH_3$. The severe ^1H signal overlapping due to unisochronous methylene protons on C-5, C-6, C-11, and C-12 hampered further structure elucidation. In such cases, 2D-INADEQUATE[3] (natural abundance ^{13}C-^{13}C COSY) is the most effective NMR technique. Extensive analysis of 2D-INADEQUATE spectra of **2** exhibited all ^{13}C-^{13}C connectivities illustrated in Fig. 2 as bold lines with the exception of the connectivity between C-6a and C-10a. The assumption of a bond between C-6a and C-10a would give the complete carbon skeleton shown in **2**.

Fig. 2. —— ^{13}C-^{13}C connectivity relationship revealed by analysis of 2D-INADEQUATE of **2**.



In order to establish the relative configuration of **2**, an X-ray crystallographic study was carried out. The bis(*p*-bromobenzoyl) methyl ester (**3**), suitable for X-ray analysis, was prepared by treatment of **2**

Fig. 3. ORTEP drawing of **3**.



with diazomethane followed by *p*-bromobenzoyl chloride in pyridine. The structure was solved by direct methods (MULTAN 84)[4] and the perspective view of **3** is reproduced in Fig. 3. All attempts to determine the absolute stereochemistry of **3** using anomalous dispersion from the Br atoms were unsuccessful.

Fortunately WF11605 ring system possesses a rigid 1,2-glycol unit at C-8 and C-9, a suitable system of application of CD exciton chirality method[5] for determination of absolute stereochemistry. The 10 Hz coupling constant of 8-H (δ 5.18) and 9-H (δ 5.46) in **3** indicated *trans* di-equatorial disposition of the two *p*-bromobenzoate substituents. The CD data of **3** (CD [θ] +51,000 (233 nm), 0 (245 nm), and −48,000 (255 nm)) showed negative exciton chirality. The negative screw sense between the two *p*-bromobenzoate chromophores identified the absolute stereochemistry at C-8 as *R* and at C-9 as *R*. Relative to these configurations, the other absolute configurations are as follows: C-1 (*S*), C-2 (*R*), C-4a (*S*), C-6a (*R*), C-10a (*S*), C-12a (*S*), C-15 (*R*).

Structure of **1**

With the absolute configuration of **2** known, the determination of the complete structure of **1** requires a knowledge of the positions of attachment of the acetyl and glucopyranose groups. The acetyl group was located at position 9 of **1** by comparing the ^{1}H chemical shift of 9-H of **1** (5.14) with that of **2** (3.64). The attachment point of the glucoside was established as C-8 by elimination and was further supported by downfield ^{13}C chemical shift of C-8 (88.7) in **1** compared with **2** (84.3).

From the above information, the structure of **1** was concluded to be (1*S*,2*R*,4a*S*,6a*R*,8*R*,9*R*,10a*S*,12a*S*)-9-acetoxy-2-[(*R*)-1,2-dimethylpropyl]-8-(*β*-D-glucopyranosyl)oxy-1,2,3,4,4a,5,6,6a,7,8,9,10,10a,11,12,12a-hexadecahydro-2,4a,7,7,10a,12a-hexamethyl-3-oxochrysene-1-carboxylic acid.

To the best of our knowledge this is the first report of this type of tetracyclic ring system from natural sources. The origin of the tetracyclic triterpene skeleton of **1** has been an intriguing question (epoxysqualene?) but beyond the scope of the present study. The glucoside segment may play an important role in exhibiting LTB_4 antagonistic activity because aglycone (**2**) is devoid of its biological activity. Further investigation regarding minor component is now in progress and the result will be reported elsewhere.

Experimental

General Procedure

Melting points were taken on a Yanagimoto micro melting point apparatus and are uncorrected. IR spectra were measured on a Jasco A-102 IR spectrometer. Optical rotation were determined on a Jasco DIP-140 polarimeter, using 10 cm-microcell. CD curves were recorded on a Jasco J-20C automatic recording spectropolarimeter in MeOH. ^{1}H NMR spectra (400 MHz) and ^{13}C NMR (100 MHz) spectra were measured with a Bruker AM400 wb spectrometer controlled under an ASPECT 3000 computer. The chemical shifts are reported in ppm relative to internal tetramethylsilane and coupling constants are expressed in Hz. Pulse programs of the standard Bruker software library were used for a series of 2D NMR experiments. Low-resolution and high-resolution FAB-MS spectra were obtained on a VG ZAB-SE mass spectrometer.

Acid Degradation of **1** to **2**

A suspension of **1** (300 mg) in anhydrous 5% HCl-MeOH (50 ml) was refluxed for 1 hour and the solution was evaporated to dryness. The residue was partitioned between EtOAc and H_2O, and the water layer was evaporated to dryness. Treatment of the mixture with Ac_2O in pyridine followed by purification on preparative TLC gave methyl tetra-*O*-acetyl-*α*-D-glucopyranoside, identical in all respects with a specimen obtained by treatment of methyl *α*-D-glucopyranoside with Ac_2O in pyridine. The EtOAc layer was washed

with brine, dried over $MgSO_4$ and evaporated to dryness. Purification on preparative TLC ($CHCl_3$ - MeOH (9 : 1)) gave 200 mg of **2** as a colorless powder: $[\alpha]_D^{23}$ $-15°$ (*c* 0.5, MeOH); IR ν_{max} (KBr) cm^{-1} 3350, 2920, 1710, 1680, 1380; ^1H NMR (400 MHz, CD_3OD - $CDCl_3$) consult Table 1; ^{13}C NMR (100 MHz, CD_3OD - $CDCl_3$) consult Table 1; FAB-MS *m/z* 511 $(M+Na)^+$; HRFAB-MS *m/z* 511.3402, calcd for $C_{30}H_{48}O_5Na$ 511.3399.

Preparation of Bis(*p*-bromobenzoyl) Methyl Ester (**3**)

A solution of **2** (80 mg) in a mixture of $CHCl_3$ and MeOH (1 ml) was treated with trimethylsilyldiazomethane and evaporated to give an oil. To a solution of the obtained oil in pyridine (1 ml) was added *p*-bromobenzoyl chloride (140 mg) and the mixture was allowed to stand at room temperature overnight. The mixture was evaporated to dryness and separated by preparative TLC ($CHCl_3$) to afford 110 mg of **3** as a colorless powder.

Recrystallization from hot MeOH gave 65 mg of colorless needles: mp 228°C; $[\alpha]_D^{23}$ $-104°$ (*c* 0.5, $CHCl_3$); CD [θ] see text; IR ν_{max} ($CHCl_3$) cm^{-1} 2960, 1720, 1590, 1280, 1270, 1120, 1100, 1010; FAB-MS *m/z* 869 $(M+H)^+$.

X-Ray Structure Analysis for **3**

X-Ray diffraction measurements were carried out on a Rigaku automated four-circle diffractometer (AFC-5R) with graphite-monochromated CuK_α radiation ($\lambda=1.54178$Å) at room temperature. The size of the prismatic crystal was $0.20\times0.12\times0.08$ mm. Accurate cell dimensions were derived from 2θ values for 25 high-order reflections ($55°<2\theta<62°$). The crystal data are as follows; chemical formula $C_{45}H_{56}Br_2O_7$, monoclinic, space group $P2_1$, $a=20.739(2)$, $b=6.539(1)$, $c=15.635(2)$Å, $\beta=96.65(3)°$, $Z=2$, $D_{calc}=1.37$ gcm^{-3}.

Intensity data were collected in the $\omega/2\theta$ scan mode up to $2\theta=130°$ with a scan rate of 4°/minute. The ω scan range was $(1.2+0.2\tan\theta)°$. Background measurement were counted for 6 seconds on either side of the peak. Three reference reflections monitered every 200 reflections showed no appreciable intensity decrease. Of 3,940 unique observed reflections, 2,864 reflections with $|F_O|\geqq3\sigma(F_O)$ were used for structure determination. Corrections were made for Lorentz and polarization effects, but not for absorption effect ($\mu(CuK_\alpha)=31.1\,cm^{-1}$).

The structure was solved by direct methods (MULTAN 84)[5]. Nonhydrogen atoms were given anisotropic thermal parameters and refined by block diagonal least-squares methods. Hydrogen atoms located on difference maps were given isotropic thermal parameters and were included in the refinement. The final *R* value (defined as $\Sigma||F_O|-|F_C||/\Sigma|F_O|$) was 0.081. The ratio of maximum least-squares shifts to error in the final refinement cycle for nonhydrogen atoms is 0.6. Atomic scattering factors used were taken from International Tables for X-Ray Crystallography Vol. IV (Kynoch Press, Birmingham, England, 1974).

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