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Structural Elucidation of Triptofordins F-1, F-2, F-3, and F-4, New Sesquiterpenes Polyesters from *Tripterygium wilfordii* HOOK fil. var. *regelii* MAKINO

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New sesquiterpene esters designated triptofordins F-1 (**1**), F-2 (**7**), F-3 (**10**), and F-4 (**5**) were isolated from the leaves of *Tripterygium wilfordii* HOOK fil. var. *regelii* MAKINO. Their structures were established by spectroscopic investigation and chemical reactions. The absolute structures of these compounds were determined by using the dibenzoate chirality method.

Keywords——*Tripterygium wilfordii*; Celastraceae; triptofordin; sesquiterpene; dibenzoate chirality method

Tripterygium wilfordii HOOK has been used as an anticancer drug and as an insecticide by the Chinese for hundreds of years. Recently, this plant has been used to treat rheumatoid arthritis and spondylitis in some Chinese clinics.[1] The active antileukemic principles, triptolide and tripdiolide were isolated from this plant by Kupchan *et al.*[2] Kutney *et al.* reported the production of triptolide and tripdiolide by tissue cultures of this plant.[3] The alkaloid contents of this plant were also reported.[4] Certain triterpenoids and sesquiterpenes were recently isolated from the roots of *T. wilfordii*,[5] but information on its chemistry is limited to the root bark. We have been studying the sesquiterpene constituents of *T. wilfordii* HOOK fil. var. *regelii* MAKINO. Recently we have described the isolation and structural determination of seven new (triptofordins A, B, C-1, C-2, D-1, D-2, and E) and two known dihydroagarofuran sesquiterpenes from the leaves of this plant.[6] One of these sesquiterpenes is known to be the active principle against the larval stages of *Strongyloides stercoralis* and hookworms.[17]

The crude extract was further chromatographed to isolate four new sesquiterpenes. The structural elucidation of these four new sesquiterpenes, triptofordins F-1 (**1**), F-2 (**7**), F-3 (**10**), and F-4 (**5**), is described here.

Triptofordin F-1 (**1**) showed absorptions at 3450 (OH), 1740, and 1712 (COO) cm^{-1} in the infrared (IR) spectrum. The ultraviolet (UV) spectrum of **1** showed the presence of aromatic rings. The presence of acetate, benzoate and cinnamate ester groups in **1** was revealed by the proton and carbon 13 nuclear magnetic resonance (^{1}H- and ^{13}C-NMR) spectra and the consecutive loss of *m/z* 148 ($C_6H_5CH=CHCOOH$), 122 (C_6H_5COOH) and 60 (CH_3COOH) units in the mass spectrum (MS). The ^{1}H-NMR spectrum of **1** showed three singlets at δ 1.63—1.80, which were attributed to three quaternary methyl groups. The signals observed at δ_H 5.22, 5.46, 5.57, 5.66, and 6.13 were assigned to the protons attached to the carbon atoms bearing the secondary ester groups. One AB quartet at δ_H 4.75 and 4.98 was assigned to the methylene-bearing primary ester group. The ^{13}C-NMR spectrum of **1** showed the presence of three methyls, two methylenes, six methines, four quaternary carbons, and three acetate, one benzoate, and one cinnamate esters. These results were consistent with a

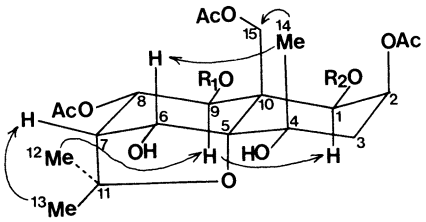
TABLE I. Sesquiterpense from *T. wilfordii* HOOK fil. var. *regelii*

Triptofordin	Mol. formula	M^+	mp (°C)	$[\alpha]_D$	Esters
F-1 (**1**)	$C_{37}H_{42}O_{13}$	694	252—255	+27.5	Ac × 3, Bz, Cin
F-2 (**7**)	$C_{35}H_{40}O_{13}$	668	105—109	+37.3	Ac × 3, Bz × 2
F-3 (**10**)	$C_{37}H_{42}O_{14}$	710	200—205	+20.1	Ac × 4, Bz × 2
F-4 (**5**)	$C_{35}H_{40}O_{12}$	652	217—224	+18.8	Ac × 2, Bz, Cin

Ac, acetate; Bz, benzoate; Cin, *trans*-cinnamate.

TABLE II. NOE in Triptofordin F-1 (**1**) ($CDCl_3$, 200 MHz)

Irradiate	Observe	Percentage enhancement
H-9	H-1	10
H-12	H-9	20
H-13	H-7	14
H-14	H-15	10
H-14	H-6	9



molecular formular for **1** of $C_{37}H_{42}O_{13}$. It was concluded that **1** was a derivative of the dihydroagarofuran skeleton of the sesquiterpene polyol esters found in the Celastraceae.[7] The presence of the same skeleton in the compounds **7**, **10**, and **5** was also indicated from the analysis of spectral data. The physical and spectral properties of the isolated sesquiterpenes are summarized in Table I. From a comparison of the ^{1}H-NMR data for **1** and known sesquiterpenes,[8] the signal at δ_H 2.60 (br d, J = 3.2 Hz) was assigned to H-7. The signals at δ_H 5.66 (dd, J = 10.0 and 3.2 Hz) and 6.13 (d, J = 10.0 Hz) were assigned to H-8 and H-9, respectively, on the basis of spin decoupling experiments. The coupling constant (J = 10.0 Hz) of H-8 and H-9 was indicative of a diaxial relationship. The signal at δ_H 5.22 (br d to br s with D_2O change) was assigned to H-6 from the small coupling with H-7. The signal at δ_H 5.46 was assigned to H_{eq}-2 in the dihydroagarofuran skeleton from the coupling constants, and this proton was coupled with a signal at δ_H 5.57, assigned to H-1 or H-3. The assignment of H_{ax}-1 for this signal is more likely, because all the sesquiterpenes with a dihydroagarofuran skeleton isolated from Celastraceae have an equatorial ester on C-1.[8] The tertiary hydroxy group responsible for the signal at δ_H 3.16 (exchangeable with D_2O) is placed at C-4, since compound **1** has three tertiary methyl groups.

The relative stereochemistry and the location of ester function on C-1 and C-15 were determined by nuclear Overhauser effect (NOE) experiments (Table II). Irradiation of H-14 (CH_3) produced an enhancement in the intensity of the H-6 and H-15 (CH_2) signals, indicating the stereochemistry at C-6 and the presence of one ester function on C-15. When the H-12 (CH_3) signal was irradiated, an increase of the H-9 signal occurred, while irradiation at the H-9 signal produced an enhancement in the intensity of the H-1, which indicated that H-1 and H-9 are axial, and one of the ester functions is present at C-1.

Next, we confirmed the linking sites of the ester groupings. The presence of one hydroxy group on C-6 was elucidated in the following manner. The ^{1}H-NMR signal assignable to 6-H

TABLE III. ^{1}H-NMR Data for Triptofordins F-1 (**1**), F-2 (**7**), F-3 (**10**), F-4 (**5**) and Compounds **9**, **20**

	1	**7**	**10**	**5**	**9**	**20**
H-1	5.57 d (3.4)	5.55 d (3.4)	5.75 d (3.4)	6.28 d (3.7)	5.36 m	5.46 d (3.4)
H-2	5.46 ddd (3.4) (3.4) (3.4)	5.39 ddd (3.4) (2.9) (2.9)	5.46 ddd (3.4) (3.4) (3.0)	6.04 m	5.36 m	5.29 m
H-3	—	2.02 dd (14.9) (2.9)	—	—	—	—
H-6	5.22 br d (5.1)	5.25 br d (5.1)	6.60 br s	5.70 m	6.47 br s	5.20 br d (5.1)
H-7	2.60 br d (3.2)	2.79 br d (3.3)	2.50 br d (3.3)	2.99 br d (3.3)	2.43 br d (3.3)	2.60 br d (3.4)
H-8	5.66 dd (10.0) (3.2)	5.75 dd (9.8) (3.3)	5.74 dd (9.8) (3.3)	4.88 dd (9.3) (3.3)	5.49 dd (9.5) (3.3)	4.28 m
H-9	6.13 d (10.0)	6.27 d (9.8)	6.12 d (9.8)	6.78 d (9.3)	5.65 d (9.5)	5.89 d (9.5)
H-12	1.74 s	1.81 s	1.52 s[a)]	1.82 s[a)]	1.47 s[a)]	1.63 s[a)]
H-13	1.63 s	1.61 s	1.59 s[a)]	2.11 s[a)]	1.53 s[a)]	1.77 s[a)]
H-14	1.80 s	1.81 s	1.73 s[a)]	2.16 s[a)]	1.62 s[a)]	1.77 s[a)]
H-15	4.75, 4.98 ABq (13.2)	4.77, 4.85 ABq (13.7)	4.90, 5.04 ABq (13.2)	5.36, 5.53 ABq (13.4)	4.65, 4.74 ABq (13.0)	4.80 s
4-OH	3.16 s	3.16 s	2.81 s	—	2.73 s	3.14 s
Ac	1.85, 2.08, 2.36	1.53, 2.11, 2.36	1.81, 2.04, 2.16, 2.43	2.07, 2.13	1.89, 1.97, 1.99, 2.11, 2.12, 2.29	1.46, 2.11, 2.24

1: benzoate and cinnamate [5.69 and 7.39 (each 1H, ABq, J=16.0), 6.96—7.74 (10H)]; **7**: benzoate [7.31—7.91 (10H)]; **10**: benzoate [6.89—7.56 (10H)]; **5**: benzoate and cinnamate [6.12 and 7.68 (each 1H, ABq, J=16.0), 6.93—8.12 (10H)]; **20**: benzoate [7.43—7.97 (5H)]. Figures in parentheses are coupling constants in Hz, run at 200 MHz in $CDCl_3$ (**1**, **7**, **10**, **9** and **20**) and C_5D_5N (**5**). *a*) Values in each column may be interchanged.

changed from a doublet to a broad singlet upon addition of D_2O. On acetylation, **1** gave a monoacetylated compound **2**. In the ^{1}H-NMR spectrum of **2**, the signal assignable to H-6 showed a downfield shift to δ_H 6.58 (br s) as compared with **1** [δ_H 5.22 (br d)]. On partial hydrolysis using HCl–dioxane, **1** gave **5**, $C_{35}H_{40}O_{12}$, mp 217—224 °C, whose ^{1}H-NMR spectrum showed one acetate missing compared with that of **1** (Chart 1 iii). The signal assignable to H-8 of **5** showed an upfield shift to δ_H 4.88 (dd, J=9.3 and 3.3 Hz), indicating the presence of one acetate ester of **1** at C-8. The remaining locations of one benzoate, one cinnamate, and two acetate esters were determined by chemical correlation with the known compound **6**.[7)] The $NaBH_4$ reduction of **6** gave two products **3**, $C_{37}H_{42}O_{13}$ and **5** (Chart 1 iv). Thus, the structure of triptofordin F-1 (**1**) was determined to be as shown in Chart 1.

Triptofordin F-2 (**7**) has two benzoate and three acetate ester groups (Table I). The ^{13}C-NMR spectrum of **7** was very similar to that of **1** except for the ester groups; also the ^{1}H-NMR spectra of both compounds showed very similar chemical shifts and coupling constants for the dihydroagarofuran skeleton, suggesting the same stereochemistry for both compounds. Compound **1** was hydrolyzed by using NaOEt–EtOH to give a heptahydroxy derivative **4** (Chart 1 ii), which was acetylated to give a hexaacetyl derivative **9** [$C_{27}H_{38}O_{14}$, δ_H 1.89, 1.97, 1.99, 2.11, 2.12, and 2.29 (each 3H, s, $COCH_3$)] (Chart 1 i). The same reaction of **7** gave **9**.

TABLE IV. ^{13}C-NMR Data for the Skeletal Carbons of Triptofordins F-1 (**1**), F-2 (**7**), F-3 (**10**), F-4 (**5**) and Compound **4**

	1	**7**	**10**	**5**	**4**
C-1	75.5 (d)[a)]	75.0 (d)[a)]	75.4 (d)	76.9 (d)	77.7 (d)
C-2	67.8 (d)	67.5 (d)	68.1 (d)	68.8 (d)	70.4 (d)
C-3	41.1 (t)	41.3 (t)	42.0 (t)	40.4 (t)	44.3 (t)
C-4	72.2 (s)	72.2 (s)	69.6 (s)	72.2 (s)	73.1 (s)
C-5	91.5 (s)	91.6 (s)	92.3 (s)	92.3 (s)	92.8 (s)
C-6	76.9 (d)	76.9 (d)	75.4 (d)	79.9 (d)	82.1 (d)
C-7	53.7 (d)	53.6 (d)	52.4 (d)	57.6 (d)	57.3 (d)
C-8	73.8 (d)	75.3 (d)[a)]	73.8 (d)	73.8 (d)	75.5 (d)
C-9	75.1 (d)[a)]	75.1 (d)[a)]	75.4 (d)	76.9 (d)	78.5 (d)
C-10	51.2 (s)	50.9 (s)	52.0 (s)	51.8 (s)	52.4 (s)
C-11	84.6 (s)	84.6 (s)	84.4 (s)	84.6 (s)	83.5 (s)
C-12	26.2 (q)	26.4 (q)	25.7 (q)	27.1 (q)	27.7 (q)
C-13	30.1 (q)	30.1 (q)	29.7 (q)	30.7 (q)	30.8 (q)
C-14	24.4 (q)	24.4 (q)	24.8 (q)	27.1 (q)	27.2 (q)
C-15	61.4 (t)	61.6 (t)	61.5 (t)	62.5 (t)	61.4 (t)

a) Values in each column may be interchanged. Run at 50.1 MHz in $CDCl_3$ (**1**, **7**, **10**, and **5**) and C_5D_5N (**4**).

The locations of ester groups of triptofordin F-2 (**7**) were determined as follows. The chemical shift of H-6 of **7** was almost the same as that of **1**. On acetylation, compound **7** gave a monoacetylated product **8**, $C_{37}H_{42}O_{14}$ in which the signal assignable to H-6 (δ_H 6.68, br s) was shifted downfield from that of **7** (δ_H 5.25, br d), indicating the presence of one hydroxy group of compound **7** at C-6. The partial hydrolysis of **7** by using 0.2 M K_2CO_3–MeOH gave a monobenzoate derivative **12** (Chart 2 iii). In the ^{1}H-NMR spectrum of **12**, the signal at δ_H 4.28 (m) was assigned to H-8 on a carbon bearing a hydroxy group, indicating the presence of one benzoate ester at C-8 in the parent compound **7**. Also, the site of the second benzoyl ester of **7** at C-9 could be deduced from the ^{1}H-NMR spectrum. Previous workers have drawn attention to the unusual diamagnetic shift of the acetate methyl signal (δ_H 1.5—1.7) which arises when an equatorial acetate on C-1 is shielded by an aromatic ester on C-9.[9)] One of the acetate methyl signals of **7** appeared in an unusual region (δ_H 1.53). Thus, the ^{1}H-NMR data indicated the ester residues on C-1 and C-9 to be acetate and benzoate. Furthermore, the presence of one benzoyl ester on C-9 was determined by chemical correlation with a known compound.[6)] Sodium borohydride reduction of the known compound **11** gave two compounds, **12** ($C_{28}H_{36}O_{12}$) and **13** ($C_{30}H_{38}O_{13}$), indicating the presence of one benzoyl ester on C-9, and the presence of acetate esters on C-1, C-2, and C-15. From these results the structure of triptofordin F-2 was formulated as **7**, as shown in Chart 1.

Triptofordin F-3 (**10**) has two benzoate and four acetate esters in the molecule. The ^{1}H- and ^{13}C-NMR spectra of **10** were also very similar to those of compounds **1** and **7** except for the ester signals, suggesting that these compounds have the same stereochemistry in the dihydroagarofuran skeleton. The ester groups of compound **10** were similar to those of the known compound **14** except for one acetate. On $NaBH_4$ reduction, **14** gave compounds **15** and **16**. Compound **16** was acetylated to give **10**. This result indicated the presence of benzoyl esters at C-1 and C-9 of **10** and the presence of acetyl esters at C-2, C-6, C-8, and C-15, as shown in Chart 1.

Triptofordin F-4 (**5**) has one benzoyl ester, one cinnamoyl ester, two acetyl esters and three hydroxy groups in the molecule. From the ^{1}H-NMR spectrum, **5** was postulated to be the 8-deacetyl derivative of **1**. Actually, **5** was derived from **1** and **6**. The ^{1}H- and ^{13}C-NMR

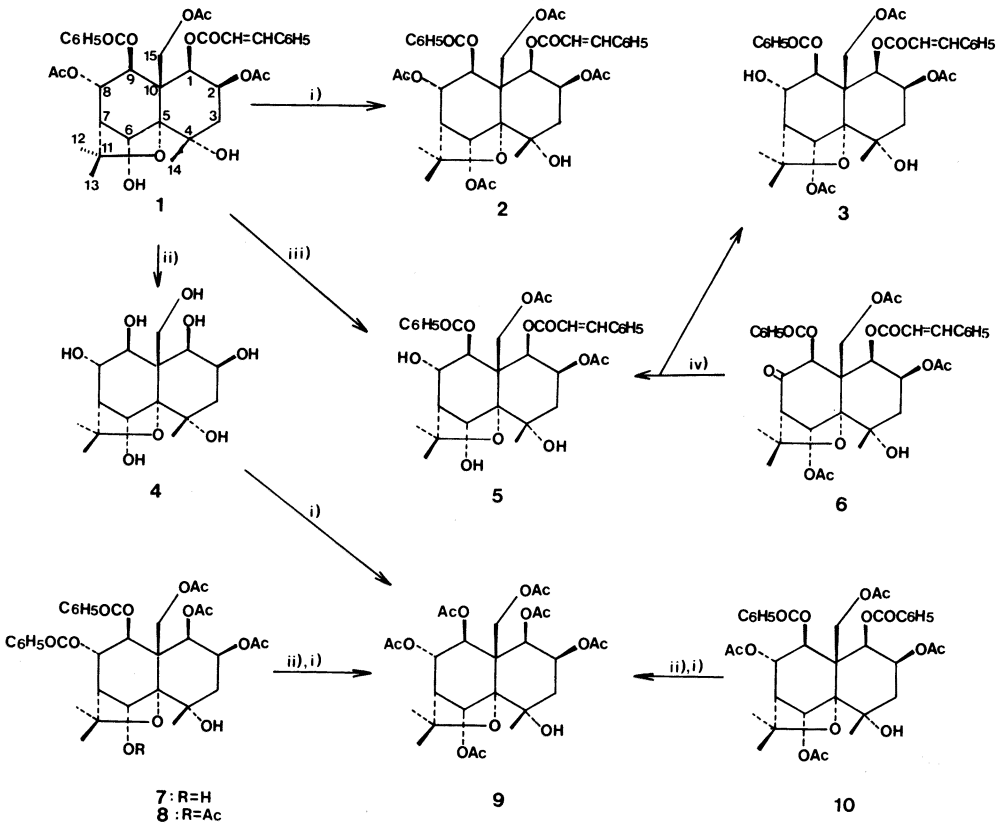


Chart 1

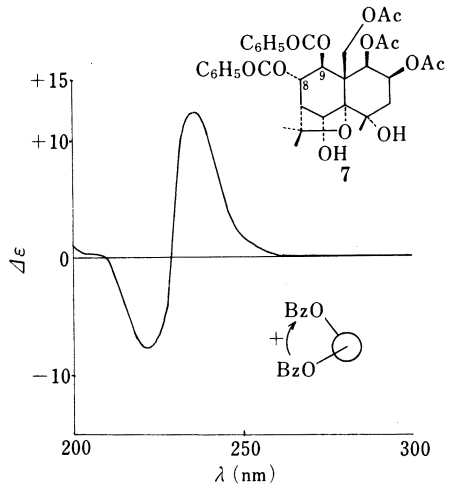


Fig. 1. CD Spectrum of Triptofordin F-2 (**2**)

assignments of the isolated compounds and derivatives are summarized in Tables II and III.

The absolute configurations of these compounds were determined as follows. The circular dichroism (CD) spectrum (Fig. 1) of triptofordin F-2 (**7**) showed a split Cotton curve

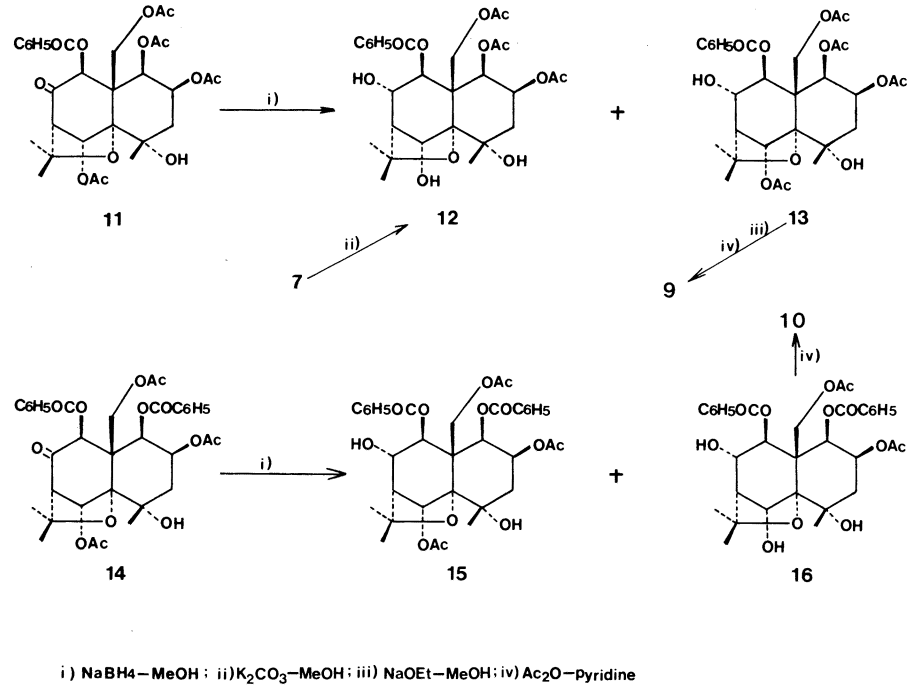


Chart 2

due to dibenzoate chirality,[10] with $[\theta]_{237}$ +44700, $[\theta]_{224}$ −30200. This indicated that the absolute configuration of **7** was 8S and 9S. The absolute configurations of **1** and **10** were considered to be the same as that of **9**. The absolute configuration of **5** was also confirmed by correlation with **1**. The absolute configurations of **6**, **11**, and **14**, for which only relative configurations[6,7] were reported, were determined to be the same as above from the correlations with **5**, **7**, and **10**, respectively.

Experimental

Melting points were taken on a Yanagimoto melting point apparatus and are uncorrected. IR spectra were recorded on a Hitachi type 215 spectrometer for KBr disks. ^{1}H- and ^{13}C-NMR spectra were taken with a JEOL JNM FX 200 spectrometer for solutions in deuteriochloroform or d_5-pyridine. Tetramethylsilane was used as an internal standard and chemical shifts are given in δ (ppm) values. MS were determined with a JEOL JMS D-300 spectrometer. UV spectra were recorded with a Hitachi type 330 spectrometer for solutions in methanol. Optical rotations were measured with a Union Giken PM-201 polarimeter. CD were taken with a JASCO J-500C spectropolarimeter. Kiesel gel 60 (70—230 mesh or 230—400 mesh, Merck) and Sephadex LH-20 (Pharmacia) were used for column chromatography, and precoated silica gel plates F_{254} (0.25 and 0.5 mm in tickness) were used for thin leyer chromatography (TLC).

Isolation of Triptofordins F-1 (1), F-2 (7), F-3 (10), and F-4 (5)——Extraction and fractionation by using silica gel column chromatography were described in a previous paper.[6] Fraction 8 (2.52 g), containing **7** and **10**, was chromatographed on a silica gel column ($CHCl_3$: acetone = 19 : 1) to give fr. 8-1 (1.50 g) and fr. 8-2 (0.79 g). Fraction 8-1 was further chromatographed on a silica gel column ($CHCl_3$: acetone = 9 : 1) to give fr. 8-1-1 (0.36 g) and fr. 8-1-2 (1.10 g). Fraction 8-1-2 was chromatographed on a silica gel column (hexane : EtOAc = 2 : 3) and crystallized from MeOH to give **10** (14 mg). Fraction 8-2 was chromatographed on a silica gel column ($CHCl_3$: acetone = 19 : 1) to give **7** (59 mg). Fraction 10 (1.86 g) containing **1** was chromatographed successively on a Sephadex LH-20 column ($CHCl_3$: MeOH = 3 : 2) and a silica gel column (CH_2Cl_2 : MeOH = 39 : 1 and then $CHCl_3$: acetone = 19 : 1), and crystallized from MeOH to give **1** (64 mg). Fraction 12 (0.98 g) was chromatographed on a silica gel column (CH_2Cl_2 : MeOH = 39 : 1 and then hexane : EtOAc = 2 : 3) to give fr. 12-2 (97 mg), which was crystallized from MeOH to give **5** (16 mg).

Triptofordin F-1 (**1**), colorless needles, mp 252—255 °C, $[\alpha]_D^{27}$ +27.5° (c = 0.3, MeOH). IR ν_{max} cm^{-1}: 3450

(OH), 1740 and 1712 (COO), 1630, 1280, 1030, 710. UV λ_{max}^{MeOH} nm (ε): 222 (18800), 226 (19800), 233 (15600), 284 (17800). EI-MS *m/z* (rel. int): 694 $[M]^+$ (0.2), 679 $[M-CH_3]^+$ (1.5), 634 $[M-CH_3COOH]^+$ (3.2), 546 $[M-C_6H_5CH=CHCOOH]^+$ (1.59), 512 $[M-C_6H_5COOH-CH_3COOH]^+$ (80.7), 486 $[M-C_6H_5CH=CHCOOH-CH_3COOH]^+$ (1.5), 471 (1.3), 426 $[M-C_6H_5CH=CHCOOH-2\times CH_3COOH]^+$ (4.7), 320 (6.6), 131 $[C_6H_5CH=CHCO]^+$ (100), 105 $[C_6H_5CO]^+$ (94). FAB-MS *m/z*: 717 $[M+Na]^+$, HR-MS *m/z*: Calcd for $C_{37}H_{42}O_{13}$: 694.2625. Found: 694.2660. ^{1}H-NMR (C_5D_5N) δ: 1.78, 1.90, 2.15 (each 3H, s, CH_3), 1.85, 2.08, 2.33 (each 3H, s, $COCH_3$), 2.90 (1H, d, J=3.1 Hz, H-7), 5.29, 5.56 (each 1H, ABq, J=13.3 Hz, H-15), 5.79 (1H, d, J=5.4 Hz, H-6), 6.02 (1H, m, H-2), 6.24 (1H, d, J=3.7 Hz, H-1), 6.10 (1H, dd, J=10.1, 3.1 Hz, H-8), 6.15, 7.70 (each 1H, ABq, J=15.9 Hz, $C_6H_5CH=CHCO$), 6.72 (1H, d, J=10.1, H-9), 6.98—8.17 (10H, aromatic H). *Anal.* Calcd for $C_{37}H_{42}O_{13}\cdot H_2O$: C, 62.35; H, 6.22. Found: C, 62.70; H, 6.31.

Triptofordin F-2 (**7**), colorless needles, mp 105—109 °C, $[\alpha]_D^{27}$ +37.3° (c=0.2, MeOH). IR ν_{max} cm^{-1}: 3430 (OH), 1750 and 1730 (COO), 1280, 1230, 1110, 1030, 710. UV λ_{max}^{MeOH} nm (ε): 231 (22800), 274 (2600), 281 (2300). EI-MS *m/z* (rel. int): 668 $[M]^+$ (0.1), 653 $[M-CH_3]^+$ (2.5), 608 $[M-CH_3COOH]^+$ (1), 590 (2), 546 $[M-C_6H_5COOH]^+$ (4), 488 (2), 486 $[M-C_6H_5COOH-CH_3COOH]^+$ (2.5), 453 (2.5), 424 $[M-2\times C_6H_5COOH]^+$ (1), 202 (22), 164 (20), 105 $[C_6H_5CO]^+$ (100); FAB-MS *m/z*: 691 $[M+Na]^+$; HR-MS: Calcd for $C_{35}H_{40}O_{13}$: 668.2469. Found: 668.2432. *Anal.* Calcd for $C_{35}H_{40}O_{13}$: C, 62.86; H, 6.03. Found: C, 62.93; H, 6.22 ^{1}H-NMR: see Table II; ^{13}C-NMR: see Table III.

Triptofordin F-3 (**10**), colorless granules, mp 200—205 °C, $[\alpha]_D^{22}$ +20.1° (c=0.2, MeOH). IR ν_{max} cm^{-1}: 3500 (OH), 1750 and 1730 (COO), 1280, 1240, 1100, 1020, 710. UV λ_{max}^{MeOH} nm (ε): 232 (21400), 278 (4100), 284 (4000). EI-MS *m/z* (rel. int.): 710 $[M]^+$ (0.1), 695 $[M-CH_3]^+$ (0.4), 692 $[M-H_2O]^+$ (0.5), 650 $[M-CH_3COOH]^+$ (1.8), 590 $[M-2\times CH_3COOH]^+$ (2.4), 528 $[M-C_6H_5COOH-CH_3COOH]^+$ (0.7), 468 $[M-C_6H_5COOH-2\times CH_3COOH]^+$ (0.5), 336 (2.0), 286 (3.6), 105 $[C_6H_5CO]^+$ (98), 43 $[CH_3CO]^+$ (100); FAB-MS *m/z*: 733 $[M+Na]^+$; HR-MS: Calcd for $C_{37}H_{42}O_{14}$: 710.2575. Found: 710.2545. *Anal.* Calcd for $C_{37}H_{42}O_{14}$: C, 62.53; H, 5.96. Found: C, 62.72; H, 6.14.

Triptofordin F-4 (**5**), colorless needles, mp 217—224 °C, $[\alpha]_D^{27}$ +18.8° (c=0.2, MeOH). IR ν_{max} cm^{-1}: 3450 (OH), 1740 (COO), 1640, 1280, 1140, 1050, 710. UV λ_{max}^{MeOH} nm (ε): 223 (17000), 226 (18000), 232 (15300), 284 (14700). EI-MS *m/z* (rel. int.): 652 $[M]^+$ (0.1), 637 $[M-CH_3]^+$ (1.59), 634 $[M-H_2O]^+$ (0.2), 616 $[M-2\times H_2O]^+$ (0.5), 426 (1), 410 (0.8), 131 $[C_6H_5CH=CHCO]^+$ (100), 105 $[C_6H_5CO]^+$ (91), 43 $[CH_3CO]^+$ (40). FAB-MS *m/z*: 675 $[M+Na]^+$. HR-MS: Calcd for $C_{35}H_{40}O_{12}$: 652.2520. Found: 652.2530. *Anal.* Calcd for $C_{35}H_{40}O_{12}\cdot H_2O$: C, 62.67; H, 6.25. Found: C, 62.91; H, 6.23.

Acetylation of 1——A solution of **1** (5 mg) in Ac_2O (0.5 ml) and pyridine (0.5 ml) was stirred at 70 °C for 17 h, and the reaction mixture was worked up in the usual way to give **2** as an amorphous powder (5 mg). ^{1}H-NMR ($CDCl_3$) δ: 1.50, 1.58, 1.73 (each 3H, s, CH_3), 1.86, 2.06, 2.15, 2.42 (each 3H, s, $COCH_3$), 2.50 (1H, d, J=3.2 Hz, H-7), 3.66 (1H, s, 4-OH), 4.85, 4.94 (each 1H, ABq, J=15.9 Hz, H-15), 5.45 (1H, m, H-2), 5.57 (1H, d, J=3.4 Hz, H-1), 5.68, 7.39 (each 1H, d, J=16.1 Hz, $C_6H_5CH=CHCO$), 5.76 (1H, dd, J=9.8, 3.2 Hz, H-8), 6.10 (1H, d, J=9.8 Hz, H-9), 6.58 (1H, s, H-6), 6.96—7.77 (10H, aromatic H).

Partial Hydrolysis of 1——A solution of **1** (39 mg) in MeOH (2 ml), dioxane (3.5 ml) and 18% HCl (1.5 ml) was stirred at 70 °C for 89 h, then the reaction mixture was evaporated *in vacuo* to give a residue. The residue was purified by silica gel column chromatography with hexane : EtOAc = 3 : 2 to give **5** as an amorphous powder (9 mg), $[\alpha]_D^{22}$ +17° (c=0.2, MeOH).

$NaBH_4$ Reduction of 6——A solution of **6** (35 mg) and $NaBH_4$ (5 mg) in MeOH (5 ml) was stirred at room temperature for 5 min, then the reaction mixture was neutralized with diluted HCl and evaporated *in vacuo* to give a residue, which was purified by using preparative TLC ($CHCl_3$: MeOH = 97 : 3) to give **3** as an amorphous powder (8.6 mg) and **5** (6.2 mg). **3**, ^{1}H-NMR ($CDCl_3$) δ: 1.26, 1.49, 1.78 (each 3H, s, $-CH_3$), 2.09, 2.15, 2.32 (each 3H, s, $COCH_3$), 2.48 (1H, d, J=3.4 Hz, H-7), 2.84 (1H, br s, 4-OH), 4.48 (1H, dd, J=10.0, 3.4 Hz, H-8), 4.86, 5.03 (each 1H, ABq, J=13.4 Hz, H-15), 5.42 (1H, m, H-2), 5.60, 7.35 (each 1H, d, J=16.1 Hz, $C_6H_5CH=CHCO$), 5.60 (1H, d, J=3.7 Hz, H-1), 5.93 (1H, d, J=10.0 Hz, H-9), 6.40 (1H, s, H-6), 6.8—7.9 (10H, aromatic H). **5**, $[\alpha]_D^{22}$ +19.6° (c=0.2, MeOH), EI-MS *m/z* (rel. int.): 694 $[M]^+$ (0.1), 679 $[M-CH_3]^+$ (2), 661 $[M-CH_3-H_2O]^+$ (1), 634 $[M-CH_3COOH]^+$ (1), 574 $[M-2\times CH_3COOH]^+$ (4), 131 $[C_6H_5CH=CHCO]^+$ (66), 105 $[C_6H_5CO]^+$ (100), 43 $[CH_3CO]^+$ (30). HR-MS: Calcd for $C_{37}H_{42}O_{13}$: 694.2625. Found: 694.2624.

Hydrolysis of 1——A solution of **1** (20 mg) in 0.1 M NaOEt–EtOH (1 ml) was stirred at room temperature for 12 h, then the reaction mixture was evaporated *in vacuo* to give a residue, which was chromatographed on a silica gel column (EtOAc : MeOH = 5 : 1) to give **4** (9.6 mg). **4**, ^{1}H-NMR (C_5D_5N) δ: 1.77, 1.94, 2.36 (each 3H, s, $-CH_3$), 2.33 (1H, br d, J=14 Hz, H-3), 2.51 (1H, dd, J=14, 3 Hz, H-3), 2.84 (1H, d, J=2.7 Hz, H-7), 3.61 (1H, s, OH), 4.52 (1H, m, H-2), 4.78 (2H, br s, H_2-15), 4.79 (1H, m, H-8), 4.90 (1H, m, H-1), 5.03 (1H, d, J=6.1 Hz, H-6), 5.06 (1H, br d, J=10 Hz, H-9), 6.55 (1H, d, J=6.1 Hz, 6-OH). ^{13}C-NMR: see Table III.

Acetylation of 4——A solution of **4** (8 mg) in Ac_2O (0.5 ml) and pyridine (0.5 ml) was stirred at 70 °C for 12 h, then the reaction mixture was worked up in the usual way to give a residue. The residue was chromatographed on a silica gel column (EtOAc : hexane = 2 : 1) to give **9** as an amorphous powder (9 mg). **9**, $[\alpha]_D^{22}$ +12.6° (c=0.2, MeOH). EI-MS *m/z* (rel. int.): 586 $[M]^+$ (0.1), 571 $[M-CH_3]^+$ (0.2), 526 $[M-CH_3COOH]^+$ (2), 484 (2.5), 466 $[M-2\times CH_3COOH]^+$ (5), 451 (1), 424 (2), 286 (4.5), 244 (4), 140 (22.5), 98 (13.7), 43 $[CH_3CO]^+$ (100). HR-MS:

Calcd for $C_{26}H_{35}O_{14}$ ($M-CH_3$): 571.2026. Found: 571.2035.

Hydrolysis, Followed by Acetylation of 7——A solution of **7** (5 mg) in 0.1 M NaOEt–EtOH (1 ml) was stirred at room temperature for 12 h, then the reaction mixture was neutralized with AcOH and evaporated to give a residue, which was purified by using silica gel column chromatography with EtOAc–MeOH (5 : 1) to give a hexahydroxy product. This product was acetylated by using Ac_2O (0.5 ml) and pyridine (0.5 ml) at 70 °C for 12 h, and worked up in the usual way to give a residue, which was purified by using silica gel column chromatography with EtOAc–hexane (2 : 1) to give **9** as an amorphous powder (3 mg). **9**, $[\alpha]_D^{22}$ +10.9° (*c* = 0.2, MeOH), EI-MS *m/z* (rel. int.): 586 $[M]^+$ (1), 571 $[M-CH_3]^+$ (9), 526 $[M-CH_3COOH]^+$ (17), 511 (12), 484 (10), 466 $[M-2\times CH_3COOH]^+$ (19), 424 (6), 409 (7), 406 $[M-3\times CH_3COOH]^+$ (5), 165 (17), 140 (90), 98 (59), 43 $[CH_3CO]^+$ (100). HR-MS: Calcd for $C_{27}H_{38}O_{14}$: 586.2262. Found: 586.2241.

Acetylation of 7——A solution of **7** (11 mg) in Ac_2O (0.5 ml) and pyridine (0.5 ml) was stirred at room temperature for 48 h, then the reaction mixture was worked up in the usual way to give amorphous power (12 mg). **8**, ^{1}H-NMR ($CDCl_3$) δ: 1.52, 1.57, 1.80 (each 3H, s, CH_3), 1.53, 2.09, 2.15, 2.42 (each 3H, s, $COCH_3$), 1.97 (1H, dd, *J* = 15.2, 2.9 Hz, H-3), 2.71 (1H, d, *J* = 3.3 Hz, H-7), 2.78 (1H, s, 4-OH), 4.74, 4.95 (each 1H, ABq, *J* = 13.4 Hz, H_2-15), 5.37 (1H, m, H-2), 5.54 (1H, d, 3.4 Hz, H-1), 5.83 (1H, dd, *J* = 9.8, 3.3 Hz, H-8), 6.25 (1H, d, *J* = 9.8 Hz, H-9), 6.68 (1H, s, H-6), 7.33—7.91 (10H, aromatic H). EI-MS *m/z* (rel. int.): 695 $[M-CH_3]^+$ (5), 650 $[M-CH_3COOH]^+$ (5), 590 $[M-2\times CH_3COOH]^+$ (15), 588 $[M-C_6H_5COOH]^+$ (9.5), 202 (70), 105 $[C_6H_5CO]^+$ (64), 43 $[CH_3CO]^+$ (100). HR-MS: Calcd for $C_{36}H_{39}O_{14}$ ($M-CH_3$): 695.2340. Found: 695.2334.

Partial Hydrolysis of 7——A solution of **7** (20 mg) in 0.02 M K_2CO_3–MeOH (1.5 ml) was stirred at room temperature for 12 h, then the reaction mixture was neutralized with 1% HCl and evaporated *in vacuo* to give a residue, which was purified by using silica gel column chromatography with EtOAc–hexane (1 : 1) to give **12** as an amorphous powder (2 mg). **12**, ^{1}H-NMR: see Table II.

$NaBH_4$ Reduction of 11——A solution of **11** (90 mg) and $NaBH_4$ (20 mg) in MeOH (4 ml) was stirred at room temperature for 40 min, then the reaction mixture was worked up in the usual way to give a residue, which was chromatographed on a silica gel column (hexane : EtOAc = 1 : 1) to give **12** (34 mg) and **13** (7 mg). **13**, ^{1}H-NMR ($CDCl_3$) δ: 1.48, 1.56, 1.75 (each 3H, s, $-CH_3$), 1.45, 2.09, 2.14, 2.30 (each 3H, s, $COCH_3$), 1.94 (1H, dd, *J* = 15.3, 2.8 Hz, H_{ax}-3), 2.47 (1H, d, *J* = 3.2 Hz, H-7), 2.80 (1H, s, 4-OH), 4.45 (1H, dd, *J* = 9.3, 3.2 Hz, H-8), 4.70, 4.96 (each 1H, ABq, *J* = 13.4 Hz, H_2-15), 5.34 (1H, ddd, *J* = 3.2, 3.2, 2.8 Hz, H-2), 5.46 (1H, d, *J* = 3.2 Hz, H-1), 5.87 (1H, d, *J* = 9.3 Hz, H-9), 6.37 (1H, s, H-6), 7.47—7.98 (5H, aromatic H). *Anal.* Calcd for $C_{30}H_{38}O_{13}$: C, 59.40; H, 6.31. Found: C, 59.54; H, 6.14.

Reduction of 14——A solution of **14** (12 mg) and $NaBH_4$ (10 mg) in MeOH (2.5 ml) was stirred at room temperature for 1.5 h, then the reaction mixture was worked up in the usual way to give a residue, which was purified by using preparative TLC with $CHCl_3$–MeOH (97 : 3) to give **15** (1 mg) and **16** (5 mg). **15**, ^{1}H-NMR ($CDCl_3$) δ: 1.26, 1.52, 1.79 (each 3H, s, CH_3), 2.08, 2.16, 2.34 (each 3H, s, $COCH_3$), 2.49 (1H, d, *J* = 3.2 Hz, H-7), 2.84 (1H, s, 4-OH), 4.48 (1H, br d, *J* = 9.8 Hz, H-8), 4.88, 5.14 (each 1H, ABq, *J* = 13.3 Hz, H_2-15), 5.43 (1H, ddd, *J* = 3.4, 3.4, 3.4 Hz, H-2), 5.79 (1H, d, *J* = 3.4 Hz, H-1), 5.98 (1H, d, *J* = 9.8 Hz, H-9), 6.42 (1H, s, H-6), 6.85—7.52 (10H, aromatic H). EI-MS *m/z* (rel. int.): 653 $[M-CH_3]^+$ (1), 608 $[M-CH_3COOH]^+$ (0.5), 548 $[M-2\times CH_3COOH]^+$ (2.5), 546 $[M-C_6H_5COOH]^+$ (0.2), 486 $[M-CH_3COOH-C_6H_5COOH]^+$ (1), 105 $[C_6H_5CO]^+$ (100), 43 $[CH_3CO]^+$ (43). HR-MS: Calcd for $C_{34}H_{37}O_{13}$ ($M-CH_3$): 653.2234. Found: 653.2248. **16**, ^{1}H-NMR ($CDCl_3$) δ: 1.65, 1.78, 1.80 (each 3H, s, $-CH_3$), 2.09, 2.28 (each 3H, s, $COCH_3$), 2.61 (1H, d, *J* = 3.4 Hz, H-7), 3.18 (1H, s, 4-OH), 4.35 (1H, br d, *J* = 9.5 Hz, H-8), 4.98 (2H, s, H_2-15), 5.07 (1H, d, *J* = 5.3 Hz, 6-OH), 5.25 (1H, d, *J* = 5.3 Hz, H-6), 5.45 (1H, m, H-2), 5.79 (1H, d, *J* = 3.7 Hz, H-1), 6.00 (1H, d, *J* = 9.5 Hz, H-9), 6.85—7.53 (10H, aromatic H).

Acetylation of 16——A solution of **16** (5 mg) in Ac_2O (0.5 ml) and pyridine (0.5 ml) was stirred at 70 °C for 12 h, then the reaction mixture was worked up in the usual way to give a residue, which was purified by using silica gel column chromatography with $CHCl_3$–acetone (49 : 1) to give **10** as an amorphous powder (4.5 mg). **10**, $[\alpha]_D^{22}$ +20.1° (*c* = 0.15, MeOH).

Hydrolysis Followed by Acetylation of 13——A solution of **13** (8 mg) in 0.1 M NaOH–EtOH (1 ml) was stirred at room temperature for 10 h, then the reaction mixture was evaporated *in vacuo* to give a residue, which was chromatographed on a silica gel column (EtOAc : MeOH = 5 : 1) to give a hexahydroxy derivative (4.2 mg). This product was acetylated by using Ac_2O–pyridine at 70 °C for 18 h, followed by usual work-up to give a residue, which was purified by using silica gel column chromatography (hexane : EtOAc = 3 : 2) to give **9** (4 mg), $[\alpha]_D^{22}$ +13.5° (*c* = 0.20, MeOH).

Hydrolysis Followed by Acetylation of 10——Compound **10** (6 mg) was treated in the same way as described above to give **9** as an amorphous powder (4 mg), $[\alpha]_D^{22}$ +12.1° (*c* = 0.2, MeOH).

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