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POLYHYDROXYAGAROFURAN DERIVATIVES FROM *TRIPTERYGIUM WILFORDII* H.

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Key Word Index—*Tripterygium wilfordii*; *Celastraceae*; sesquiterpenoids; triptofordin; dihydroagarofuran.

Abstract—Three new and two known dihydroagarofuran derivatives were isolated from the leaves of *T. wilfordii* and their structures established by chemical and spectroscopic means.

INTRODUCTION

Various dihydroagarofuran sesquiterpenes have been isolated from members of *Celastraceae* [1]. We have studied the sesquiterpene constituents of this genus and have described the isolation of triptofordin A (**1**), B (**2**), C-1 (**3**) and C-2 (**4**) from *Tripterygium wilfordii* Hook fil. var. *regelii* Makino [2]. The structure elucidation of three new (**5**, **6** and **7**) and two known (**8** and **9**) constituents of this plant with the dihydroagarofuran skeleton is the subject of this paper.

RESULTS AND DISCUSSION

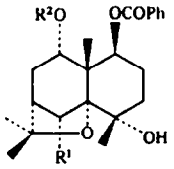
Repeated column chromatography of the ethyl acetate-soluble fraction from the methanol extract of leaves of *T. wilfordii* yielded triptofordins D-1 (**5**), D-2 (**6**), E (**7**) and compounds **8** and **9**. The structures of compounds **8** and **9** were identified from the spectral data as polyesters of 8-oxo-1,2,4,6,9,15-hexahydroxydihydroagarofuran [3]. These polyesters were isolated from *Austroplenckia populea* (*Celastraceae*) as the active principle against the larval stage of *Strongyloides stercoralis* and hookworms [3].

Triptofordin D-1 (**5**) showed $[M]^+$ at *m/z* 634 in the mass spectrum. Its IR spectrum showed hydroxy absorption at 3500 cm^{-1}, and ketone and ester carbonyl bands at 1740 and 1720 cm^{-1}. The presence of bands at 1635, 1600 and 1590 cm^{-1} suggested that it contained cinnamate and benzoate groups, and the mass spectrum showed peaks due to the loss of one benzoic acid, one cinnamic acid and two acetic acid units.

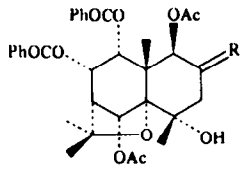
The ^1H NMR spectrum confirmed that **5** contained one benzoate ester and one cinnamate ester [δ 6.91–7.90 (10H), δ5.80 and 7.33 (each 1H, *d*, J = 16.1 Hz)]. Two sharp singlets at δ2.11 and 2.16 (each 3H) were assigned to the two acetate methyl groups. Three singlets at δ1.43–1.66 (Table 1) were attributed to three tertiary methyl groups. One AB quartet at δ4.40 and 5.20 was assigned to the methylene-bearing primary ester group. The signals observed at δ5.57, 5.98 and 6.67 were assigned to the protons attached to the carbon atoms bearing the secondary ester groups. The ^{13}C NMR spectrum (Table 2) of **5** showed four ester carbonyl carbons at δ165.1–170.3, one methylene and three methines at δ60.6, 76.0, 74.5 and 79.5, and one ketone at δ197.6. These facts agreed with a molecular formula for **5** of $C_{35}H_{38}O_{11}$. It was concluded that **5** was based on the dihydroagarofuran skeleton of the sesquiterpene polyol esters found in other Celastraceae [1].

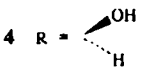
In the ^1H NMR spectrum of **5**, the signal at δ6.71 (*d*, J = 0.7 Hz) was assigned as H_{ax}-6 under the C-6 ester function since H_{ax}-6 of this type of compound is only weekly coupled to H_{eq}-7 [4]. The chemical shifts of H-7 (δ3.00) and C-7 (δ65.1) indicated that the ketone group was located on C-8. The signal at δ5.98 was assigned as H-9. The one methylene attached ester function was assigned as C-15 since the signal of H_{ax}-was downfield of that of the 15-Me compounds [5]. The signal at δ5.57 (*dd*, J = 12.2 and 3.9 Hz) was assigned to H_{ax}-1 as found in all the sesquiterpenes with a dihydroagarofuran skeleton isolated from *Celastraceae* plants [1]. The tertiary hydroxy group was responsible for a signal at δ2.73 (exchangeable with D_2O) and was placed at C-4. These assignments were confirmed by NOE experiments (Table 3). Thus, when the H-9 signal was irradiated, an increase (17%) of the H_{ax}-1 signal intensity occurred. On irradiation of the H-12 (Me) signal, the intensities of the H-8 and H-9 signals were increased, while on irradiation of the H-14 (Me) signal, the intensities of the H-6 and H-15 (CH_2) signals were increased. These results indicated that the orientations of the protons attached to the oxygen-bearing carbon atoms were H_{ax}-1, H_{ax}-6, H_{eq}-8 and H_{ax}-9.

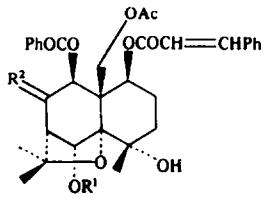
The remaining problem was the distribution of the ester groups. Previous workers have drawn attention to the unusual diamagnetic shift of one of the acetate methyl signals (δ1.5–1.7) which arises when an equatorially oriented acetate on C-1 is shielded by an aromatic ester on C-9 [6, 7]. The acetate methyl signals of **5** were in the usual region (δ2.11–2.16), which indicated that the ester groups on C-1 and C-9 were either acetates or benzoate and cinnamate. Sodium borohydride reduction of **5** gave an 8-α-hydroxy derivative (**5a**) and a deacetyl-8-α-hydroxy derivative (**5b**). On sodium borohydride reduction of **5**, the hydride anion attack on C-8 occurred from the β-side because the α-side on C-8 was blocked by H-12 (Me). The structure of **5b** was determined by means of its ^1H NMR



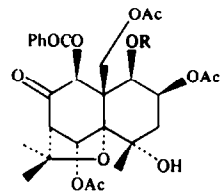
1 R^1 = H, R^2 = COCH=CHPh
2 R^1 = OH, R^2 = COPh



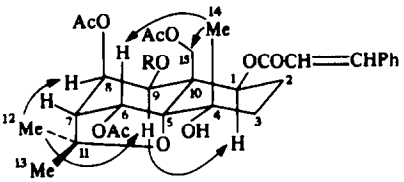
3 R = O
4 R = 



5 R^1 = Ac, R^2 = O
5a R^1 = Ac, R^2 = 
5b R^1 = H, R^2 = 
5c R^1 = Ac, R^2 = 
6 R^1 = Ac, R^2 = 



7 R = COPh
8 R = Ac
9 R = COCH=CHPh



6 R = COPh

spectrum as the 6-deacetyl-8-hydroxy derivative, which indicated that one acetyl ester of **5** was attached on C-6 while the other acetyl ester was attached on C-15 as shown by the presence of the usual acetyl methyl signal. The position of the benzoate and cinnamate esters was determined by comparison of the ^{1}H NMR spectra of compounds **5**, **8** and **9** which have a same stereochemistry in ring B. The chemical shift of H-9 (δ5.97) in compound **5** was very similar to that of compound **9** (δ5.98) and established that the benzoate ester of **5** was attached on C-9. From these results the structure of triptofordin D-1 was formulated as **5**.

Compound **6**, triptofordin D-2, $C_{37}H_{42}O_{12}$, contained three acetate, one benzoate and one cinnamate residue (IR, UV, ^{1}H NMR and ^{13}C NMR). The ^{13}C NMR spectra of **5** and **6** were very similar, except for the signals of C-7, C-8 and C-9. This suggested that compound **6** was also a dihydroagarofuran sesquiterpene with the same stereochemistry as **5** except at C-8. In compound **6**, the chemical shifts of C-7 and C-8 were up field of those of **5** which indicated that an ester group was attached a C-8 of **6**. From a comparison of the ^{1}H NMR spectra (Table 1) of **5** and **6**, the signals at δ5.48, 6.85, 2.04 and 4.61 were assigned to H_{ax}-1, H_{ax}-6, H_{eq}-7 and H-15, respectively. The signal at δ5.57 was assigned to H-8 from the coupling with H-7, then the signal of coupling with H-8 at δ5.74 was assigned to H-9. From the coupling constant (J = 5.9 Hz) between H-8 and H-9 the orientation of H-8 and H-9 was not diaxial (**5c**: J = 10.0 Hz) and had to be H_{eq}-8–H_{ax}-9, H_{eq}-8–H_{eq}-9 or H_{ax}-8–H_{eq}-9' A series of NOE experiments (Table 3) established that the orientation was in fact H_{eq}-8 and H_{ax}-9.

In the ^{1}H NMR spectrum of **6** the three acetate methyl signals appeared in the usual region. A signal at δ5.48 was very similar to that of H-1(δ5.42) of **5c** in terms of chemical shift and coupling pattern and placed the cinnamoyl ester group of **6** on C-1. The remaining benzoate ester was placed on C-9. The orientation of the cinnamate ester was confirmed by means of long range selective proton decoupling (LSPD) [8], LSPD of the cinnamoyl methine signal on the double bond at δ5.70 collapsed the carbonyl signal (δ165.7) to a sharp doublet, whereas the LSPD of H_{ax}-1 collapsed the same carbonyl signal to a doublet. This clearly indicated that the ester on C-1 was a cinnamoyl group.

Compound **7**, triptofordin E, $C_{35}H_{38}O_{13}$, contained

Table 1. ^{1}H NMR data for triptofordins D-1 (**5**), D-2 (**6**), E (**7**), and compounds **5a**, **8**, **9** (200 MHz, $CDCl_3$)

H	5	5c	6	7	8	9
1	5.57 *dd* (12.2)* (3.9)	5.42 *dd* (12.1) (4.0)	5.48 *dd* (11.4) (4.5)	5.90 *d* (3.4)	5.54 *d* (4.0)	5.72 *d* (3.4)
2	—	—	—	5.44 *ddd* (3.4) (3.4) (3.4)	5.31 *m*	5.39 *m*
6	6.67 *d* (1.0)	6.57 *s*	6.85 *br s*	6.71 *d* (0.7)	6.66 *br s*	6.70 *d* (0.7)
7	3.00 *d* (1.0)	2.74 *d* (3.3)	2.04 *d* (4.0)	3.04 *d* (0.7)	3.01 *s*	3.03 *d* (0.7)
8		5.65 *dd* (10.0) (3.3)	5.57 *dd* (5.9) (4.0)			
9	5.98 *s*	6.10 *d* (10.0)	5.74 *d* (5.9)	6.02 *s*	5.88 *s*	5.97 *s*
12	1.63 *s*	1.57 *s*†	1.68 *s*	1.59 *s*	1.62 *s*	1.60 *s*
13	1.66 *s*	1.73 *s*†	1.57 *s*	1.65 *s*	1.66 *s*	1.64 *s*
14	1.43 *s*	1.37 *s*	1.40 *s*	1.69 *s*	1.56 *s*	1.68 *s*
15	4.40, 5.20 *ABq* (12.6)	4.53, 4.59 *ABq* (12.8)	4.61, 5.16 *ABq* (13.2)	4.77, 5.18 *ABq* (13.1)	4.60, 5.00 *ABq* (12.0)	4.67, 5.16 *ABq* (13.2)
Ac	2.11 2.16	1.86 2.14 2.42	2.06 2.14 2.34	2.09 2.14 2.18	1.51 2.06 2.12 2.16	2.08 2.14 2.17

5: benzoate and cinnamate [δ6.67 and 7.33 (each 1H, *ABq*, J = 16.1 Hz), 6.91–7.90 (10 H)]; **5c** benzoate and cinnamate [δ6.10 and 7.35 (each 1H, *ABq*, J = 16.1 Hz), 6.94–7.79 (10H)]; **6**: benzoate and cinnamate [δ5.70 and 7.29 (each 1H, *ABq*, J = 16.0 Hz), 6.89–7.94 (10H)]; **7**: benzoate × 2 [δ6.91–7.90 (10H)].

*Figures in parentheses are coupling constants in Hz.

†Values may be interchanged.

Table 2. ^{13}C NMR data for the skeletal carbons of triptofordins D-1 (**5**) D-2 (**6**), E (**7**) and compounds **8**, **9**

C	5	6	7	8	9
1	76.0 *d*	75.3 *d*	74.3 *d**	74.1 *d**	74.3 *d**
2	25.3 *t*	25.1 *t*	68.8 *d*	68.3 *d*	68.8 *d*
3	38.3 *t*	37.9 *t*	42.1 *t*	42.0 *t*	42.1 *t*
4	70.5 *s*	70.5 *s*	69.8 *s*	69.8 *s*	69.8 *s*
5	93.6 *s*	92.1 *s*	93.4 *s*	93.3 *s*	93.4 *s*
6	74.5 *d*	72.6 *d*	74.6 *d**	74.4 *d**	74.6 *d**
7	65.1 *d*	53.2 *d*	65.0 *d*	64.9 *d*	65.0 *d*
8	197.6 *s*	78.1 *d*	197.1 *s*	197.0 *s*	197.1 *s*
9	79.5 *d*	70.3 *d*	79.7 *d*	79.7 *d*	79.7 *d*
10	52.2 *s*	52.4 *s*	53.1 *s*	52.6 *s*	53.1 *s*
11	84.8 *s*	82.7 *s*	85.2 *s*	85.1 *s*	85.2 *s*
12	24.6 *q*	24.3 *q*	25.2 *q*	25.2 *q*	25.3 *q*
13	29.3 *q*	29.5 *q*	29.3 *q*	29.2 *q*	29.3 *q*
14	23.8 *q*	22.7 *q*	24.6 *q*	24.5 *q*	24.6 *q*
15	60.6 *t*	60.5 *t*	61.1 *t*	61.0 *t*	61.1 *t*

*Values in any vertical column may be interchanged.

Table 3. NOEs of triptofordins D-1 (5) and D-2 (6)

Compound	Proton irradiated	Proton observed	Enhancement (%)
5	H-1	H-6	12
	H-12	H-9	18
	H-14	H-6	8
6	H-9	H-1	17
	H-12	H-8	13
		H-9	17
	H-14	H-6	8
		H-15	5

three acetate, two benzoate and one ketone group in its molecule as indicated by its spectral data. Its ^{1}H NMR spectrum was essentially identical with that of **8** and **9** in terms of coupling constants and chemical shifts (Table 1) indicating identical stereochemistry of these compound. The three acetyl methyl signal resonated in the normal range (δ2.09–2.18). The differences between the chemical shifts of the H-1 signals in compounds **7**, **8** and **9** were attributable to paramagnetic induced shifts due to the ester groups on C-1. Thus one benzoate ester of **7** was placed on C-1, while the other was on C-9 and was equatorially orientated.

EXPERIMENTAL

Isolation of triptofordins D-1 (**5**), *D-2* (**6**), *E* (**7**), *and compounds* **8**, **9**. The extraction and subsequent fractionation of the extract by silica gel CC was described in a previous paper [2]. Fr. 7 (2.295 g), containing compounds **5**, **6**, **8** and **9**, was chromatographed on silica gel column (elution with hexane–EtOAc, 3:2) to give fr. 7-1 (0.63 g) and fr. 7-2 (1.47 g). Fr. 7-1 was further chromatographed on silica gel ($CHCl_3$–Me_2CO, 24:1), and crystallized from MeOH to give **5** (84 mg) and **6** (21 mg). Fr. 7-2 was chromatographed on Sephadex LH-20 (MeOH) and silica gel ($CHCl_3$–Me_2CO, 24:1), and crystallized from MeOH to give **8** (54 mg) and **9** (64 mg). Fr. 8 (2.52 g), containing **7**, was chromatographed successively on silica gel ($CHCl_3$–Me_2CO, 19:1), Sephadex LH-20 ($CHCl_3$–MeOH, 3:2) and silica gel (hexane-EtOAc, 1:1), and crystallized from MeOH to give **7** (36 mg).

Triptofordin D-1 (**5**). Needles, mp 224–226°; $[\alpha]_D^{27}$ +56.1° (*c* 0.25, MeOH); IR ν_{max}^{KBr} cm^{-1}: 3500 (OH), 1740 (CO), 1720 (COO), 1635, 1445, 1280, 1230, 710; UV λ_{max}^{MeOH} nm (ε): 221 (19000), 225 (19800), 230 (17500); EIMS *m/z* (rel. int.): 634 $[M]^+$ (0.5), 574 $[M-HOAc]^+$ (0.4), 512 $[M-C_6H_5COOH]^+$ (0.5), 486 $[M-C_6H_5CH{=}CHCOOH]^+$ (2.3), 222 (8.4), 131 $[C_6H_5CH{=}CHCO]^+$ (56.4), 105 $[C_6H_5CO]^+$ (100); FABMS *m/z*: 657 $[M+Na]^+$; HRMS *m/z*: 634.2377 $[M]^+$. $C_{35}H_{38}O_{11}$ requires: 634.2414; ^{1}H NMR: Table 1; ^{13}C NMR: Table 2.

Triptofordins D-2 (**6**). Needles, mp 103–109°, $[\alpha]_D^{27}$ −3.5° (*c* 0.28, MeOH); IR ν_{max}^{KBr} cm^{-1}: 3500 (OH), 1735 (COO), 1635, 1600, 1450, 1280, 1240, 720, 710; UV λ_{max}^{MeOH} nm (ε): 227 (19600), 231 (17200), 285 (15800); EIMS *m/z* (rel. int.): 678 $[M]^+$ (0.1), 618 $[M-HOAc]^+$ (2.0), 530 $[M-C_6H_5COOH]^+$ (1.0), 470 $[M-HOAc-C_6H_5COOH]^+$ (3.2), 105 $[C_6H_5CO]^+$ (100), 43 $[CH_3CO]^+$ (40); FABMS *m/z*: 701 $[M+Na]^+$; HR-MS *m/z*: 678.2681 $[M]^+$. $C_{37}H_{42}O_{12}$ requires: 678.2676.

Triptofordin E (**7**). Granules, mp 116–118°, $[\alpha]_D^{27}$ +47.4° (*c* 0.25, MeOH); IR ν_{max}^{KBr} cm^{-1}: 3500 (OH), 1750 (COO), 1730 (COO), 1600, 1450, 1370, 1280, 1230, 1110, 710; UV λ_{max}^{MeOH} nm (ε): 229 (19700), 278 (6600), 283 (6700); EIMS *m/z* (rel. int.): 666 $[M]^+$, 606 $[M-HOAc]^+$ (0.4), 564 (0.8), 544 $[M-C_6H_5COOH]^+$ (0.3), 442 $[M-2\times C_6H_5COOH]^+$ (0.4), 218 (5.2), 105 $[C_6H_5CO]^+$ (100); FABMS *m/z*: 689 $[M+Na]^+$; HR-MS *m/z*: 666.2312 $[M]^+$. $C_{35}H_{38}O_{13}$ requires 666.2312.

Compound **8**. Granules, mp 216–218°, $[\alpha]_D^{27}$ +25.5° (*c* 0.47, MeOH); UV λ_{max}^{MeOH} nm(ε): 231 (14400), 274 (2300), 282 (2100); spectral data (^{1}H NMR, ^{13}C NMR and MS) in good agreement with literature [3].

Compound **9**. Needles, mp 114–115°; $[\alpha]_D^{27}$ +22.6° (*c* 0.31, MeOH); UV λ_{max}^{MeOH} (ε): 225 (14400), 283 (11900); spectral data (^{1}H NMR, ^{13}C NMR and MS) were in good agreement with literature data [3].

Reduction of **5**. A soln of **5** (25 mg) and $NaBH_4$ (10 mg) in MeOH (1.5 ml) was stirred at room temp. for 25 min. Usual work-up of the reaction mixture gave a residue, which was purified by prep. TLC ($CHCl_3$–Me_2CO, 19:1) to give **5a** (13 mg) and **5b** (6 mg).

5a, ^{1}H NMR (C_5D_5N) δ: 1.61, 1.67, 2.05 (each 3H, *s*, CH_3), 2.16, 2.39 (each 3H, *s*, COMe), 2.80 (1H, *d*, *J* = 2.9 Hz, H-7), 4.82 (1H, *m*, H-8), 5.47 (1H, *ABq*, *J* = 12.6 Hz, H-15), 5.96 (1H, *dd*, *J* = 11.9 and 3.7 Hz, H-1), 6.01 (1H, *d*, *J* = 15.9 Hz, $C_6H_5CH{=}C\underline{H}COO$), 6.68 (1H, *d*, *J* = 9.3 Hz, H-9), 6.85 (1H, *s*, H-6), 7.68 (1H, *d*, *J* = 15.9 Hz, $C_6H_5CH{=}CHCOO$), 7.01–8.14 (10H, aromatic H).
5b, ^{1}H NMR (C_5D_5N) δ: 1.79, 1.95, 2.10 (each 3H, *s*, Me), 2.12 (3H, *s*, COMe), 2.95 (1H, *d*, *J* = 2.7 Hz, H-7), 4.78 (1H, *m*, H-8), 5.59 (1H, *ABq J* = 12.7 Hz, H-15), 5.60 (1H, *brs*, H-6), 6.03 (1H, *d*, *J* = 16.0 Hz, $C_6H_5C\underline{H}{=}CHCOO$), 6.07 (1H, *dd*, *J* = 12.1 and 4.2 Hz, H-1), 6.76 (1H, *d*, *J* = 9.5 Hz, H-9), 7.67 (1H, *d*, *J* = 16.0 Hz, $C_6H_5CH{=}CHCOO$), 7.00–8.16 (10H, aromatic H).

Acetylation of **5a**. A soln of **5a** (5 mg) in C_5H_5N (0.5 ml) and Ac_2O (0.5 ml) was heated at 70° for 4 hr. Usual work-up gave a residue, which was chromatographed on a silica gel ($CHCl_3$–Me_2CO, 39:1) to give an amorphous powder **5c** (5 mg). ^{1}H NMR: Table 1.

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