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# STRUCTURES OF SESQUITERPENE POLYOL ESTERS FROM *CELASTRUS STEPHANOTIIFOLIUS* WITH POTENTIAL TUMOR-PROMOTION INHIBITORY ACTIVITY

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ABSTRACT.—Esters of eight new ([**1**], [**4–6**], and [**8–11**]) and five known sesquiterpenenoid polyalcohols have been isolated from *Celastrus stephanotiifolius*. Their structures were established on the basis of chemical reactions and spectral analysis. The structural elucidation indicated that the structures of some related compounds should be revised. The isolated sesquiterpenes were observed to inhibit Epstein-Barr virus early antigen activation significantly at low doses.

Plants of the Celastraceae family have been the subject of continued and growing interest, due to the range of biological activities shown by many members of this family (1–3), with some having been used in folk medicine (4) or as a stimulant (5) from ancient times.

In the last 20 years, many sesquiterpene polyol esters with the β-dihydroagarofuran skeleton have been isolated from members of the Celastraceae. Some of these sesquiterpene polyol esters exhibit insect antifeedant and/or insecticidal activities (6,7) as well as antitumor activity (8). We have studied the sesquiterpene constituents of plants of Celastraceae family and have described the isolation of triptofordin, triptofordinine, and triptogelin from *Tripterygium wilfordii* (9).

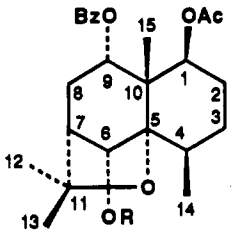
In a continuation of our previous interest in this area, eight new and five known β-dihydroagarofuran sesquiterpene polyol esters have been isolated from the seeds of *Celastrus stephanotiifolius* Makino. From these structure elucidation studies, we have found that the ester sites in some reported related compounds (10) are incorrect.

In this paper, the structure elucidation of the isolated compounds, the structure correction of certain known compounds, and the bioactivity of these constituents of *C. stephanotiifolius* are presented.

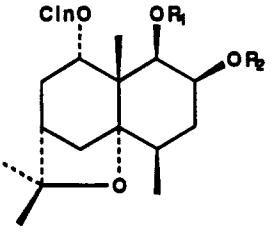
## RESULTS AND DISCUSSION

Repeated cc of the EtOAc-soluble fraction from the MeOH extract of seeds of *C. stephanotiifolius* yielded eight new sesquiterpene esters: celafolins A-1 [**1**], B-1 [**4**], B-2 [**5**], B-3 [**6**], C-1 [**8**], D-1 [**9**], D-2 [**10**], and D-3 [**11**], and the known compounds **2**, **3**, **7**, **12**, and **13**.

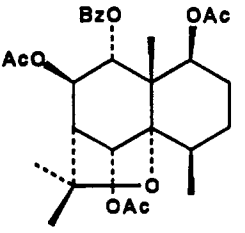
Celafolin A-1 [**1**] showed an ester carbonyl band at 1713 $cm^{-1}$ in the ir spectrum, and the uv spectrum showed the presence of an aromatic moiety (218, 224, and 279 nm). The $^1H$-nmr spectrum revealed the presence of one acetyl [δ 1.62 (3H, s)], one benzoyl [δ 7.45 (2H, br t, $J$=6.8 Hz), 7.54 (1H, br t, $J$=6.8 Hz), 8.08 (2H, br d, $J$=6.8 Hz)], and one cinnamoyl [δ 6.47, 7.21 (each 1H, d, $J$=16.1 Hz), 7.42 (3H, m), 7.55 (2H, m)] ester and the presence of three tertiary methyl groups and one secondary methyl group. The signals observed at δ 5.04 (1H, d, $J$=6.8 Hz), 5.47 (1H, s), and 5.52 (1H, dd, $J$=12.2, 4.4 Hz) were assigned to the three protons attached to the carbon atoms bearing the secondary ester groups. The $^{13}C$-nmr spectrum of **1** showed three ester carbonyl carbons (δ 165.6, 166.1, and 170.0), four methyls, three methylenes, five methines (with two being attached to oxygen-bearing carbons), and three quaternary carbons (with two being attached to oxygen-bearing carbons). These facts agreed with a molecular formula



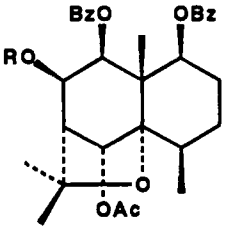

**1** R=Cin
**2** R=Bz
**3** R=Ac



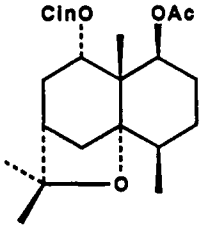

**4** $R_1$=Ac, $R_2$=H
**5** $R_1$=H, $R_2$=Ac
**6** $R_1$=Ac, $R_2$=Bz
**7** $R_1$=$R_2$=Ac



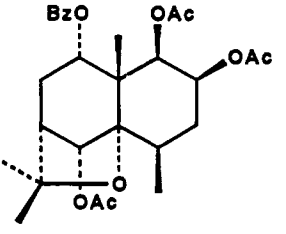

**8**



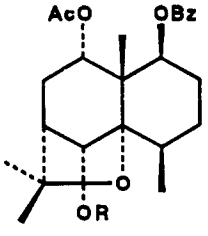

**9** R=Bz
**10** R=H
**11** R=2-MeBu



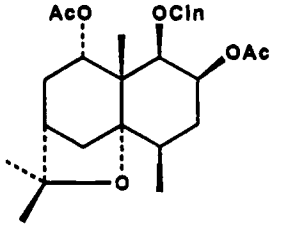

**12**



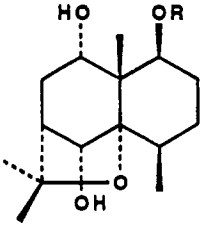

**13**




**14** R=Bz
**15** R=Ac




**16**




**17** R=*p*BrBz
**18** R=H

of compound **1**, $C_{33}H_{38}O_7$, which was supported by hrms data. It was concluded that **1** is based on the dihydroagarofuran skeleton of the sesquiterpene polyol esters found in the Celastraceae family (3).

From the $^1$H-$^1$H COSY spectrum of compound **1**, the signals at δ 5.52, 5.47, and 5.04 were assigned as $H_{ax}$-1, $H_{eq}$-6, and H-9, respectively. The remaining signals of H-3–H-8 were also assigned as shown in Table 1. From the $^1$H-$^{13}$C COSY and NOESY nmr spectra of **1**, the assignments of H-12–H-15 and carbon signals were confirmed as shown in Tables 1 and 2. In the $^1$H-$^{13}$C long-range correlation nmr spectrum (COLOC), the carbonyl carbon signal at δ 166.1 showed long-range correlation with the proton signals at δ 5.47 (H-6) and 7.71 (cinnamoyl-$H_\alpha$), and the carbonyl signal at δ 170.0 showed correlation with proton signals at δ5.52 (H-1) and 1.62 (acetyl-H). These results clearly indicated that the positions of the acetyl, cinnamoyl, and benzoyl esters should be C-1, C-6, and C-9, respectively. The configuration of H-9 was confirmed to be equatorial because of the correlation between H-9 and H-15 in NOESY nmr spectrum. Thus, the structure of celafolin A-1 was formulated as **1**.

TABLE 1. $^1$H-nmr Spectral Data for Compounds **1**, **4–6**, and **8–11** (400 MHz) in $CDCl_3$.[a]

| Proton | Compound | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **1** | **4** | **5** | **6** | **8** | **9** | **10** | **11** |
| H-1 | 5.52 dd (12.2) (4.4) | 5.51 d (3.4) | 4.41 brt (3.9) | 5.68 d (3.4) | 5.41 dd (12.2) (4.4) | 5.56 dd (10.7) (5.4) | 5.53 dd (9.6) (6.8) | 5.56 dd (10.8) (6.0) |
| H-2 | 1.53 m 1.90 ddd (12.2) (8.3) (3.9) | 4.39 ddd (3.4) (3.4) (3.4) | 5.31 ddd (3.9) (3.6) (3.6) | 5.83 ddd (6.4) (3.4) (3.2) | 1.60 m 1.89 m | 1.81 m | 1.77 m | 1.77 m |
| H-3 | 1.48 m 2.26 m | 1.80 m 2.33 ddd (10.2) (6.6) (3.4) | 1.80 dd (14.8) (2.4) 2.31 ddd (14.8) (6.8) (4.4) | 1.91 m 2.20 ddd (16.8) (6.4) (4.4) | 1.44 m 2.22 m | 1.53 m 2.25 m | 1.43 m 2.19 m | 1.50 m 2.20 m |
| H-4 | 2.37 qui (7.3) | 1.85 m | 1.88 qui (7.8) | 1.97 qui (6.4) | 2.26 qui (7.6) | 2.31 qui (7.4) | 2.25 m | 2.26 m |
| H-6 | 5.47 brs | 2.08 m | 2.09 m | 2.12 m | 5.73 brs | 6.25 brs | 6.13 brs | 5.96 brs |
| H-7 | 2.30 m | 2.00 m | 2.04 m | 2.07 m | 2.48 d (3.4) | 2.60 d (4.4) | 2.51 d (4.4) | 2.49 d (4.4) |
| H-8 | 2.48 ddd (16.6) (6.8) (3.3) | 2.00 m 2.17 ddd (16.4) (6.4) (4.0) | 2.06 m | 1.92 m 2.54 ddd (10.2) (6.4) (3.9) | 5.25 d (3.4) | 5.81 dd (5.2) (4.4) | 4.39 dd (4.9) (4.4) | 5.55 dd (5.4) (4.4) |
| H-9 | 5.04 d (6.8) | 4.77 d (6.4) | 4.88 d (6.4) | 4.78 d (6.4) | 5.03 s | 5.78 d (5.2) | 5.56 d (4.9) | 5.67 d (5.4) |
| H-12 | 1.43 s | 1.38 s | 1.46 s | 1.40 s | 1.48 s | 1.65 s | 1.51 s | 1.61 s |
| H-13 | 1.45 s | 1.20 s | 1.22 s | 1.23 s | 1.41 s | 1.48 s | 1.45 s | 1.44 s |
| H-14 | 1.03 d (7.3) | 1.31 d (8.3) | 1.22 d (7.8) | 1.33 d (8.0) | 1.03 d (7.6) | 1.12 d (7.4) | 1.08 d (7.2) | 1.09 d (8.0) |
| H-15 | 1.37 s | 1.41 s | 1.26s | 1.47 s | 1.46 s | 1.67 s | 1.63 s | 1.63 s |

[a] $^1$H chemical shifts (ppm relative to TMS) assigned on the basis of $^1$H-$^1$H and $^1$H-$^{13}$C COSY experiments. Abbreviations for coupling patterns: s, singlet; d, doublet; dd, doublet of doublet; brs, broad singlet. Values in parentheses represent coupling constants (Hz).

Compound **2**, $C_{31}H_{36}O_7$, contained one acetyl and two benzoyl esters, and compound **3**, $C_{26}H_{34}O_7$, contained two acetyl and one benzoyl ester (ir, $^1$H and $^{13}$C nmr spectra). The $^{13}$C-nmr spectral data (Experimental) were very similar to those of celafolin A-1 [**1**] except for the signals due to ester groups. From the $^1$H-$^1$H and $^1$H-$^{13}$C COSY nmr spectra of compounds **2** and **3**, the assignment of each proton and carbon signal was determined as shown in the Experimental section. In the $^1$H-$^{13}$C long-range nmr spectrum of **2**, the carbonyl carbon signal at δ 170.0 showed long-range correlations with the proton signals at δ 1.63 (acetyl-H) and 5.51 (H-1), and the carbonyl signal at δ 165.6 showed correlations with the proton signals at δ 8.09 (benzoyl-H) and 5.57 (H-6). Also, the carbonyl carbon signal of compound **3** at δ 165.0 showed long range correlations with the proton signals at δ 8.06 (benzoyl-H) and 5.01 (H-9).

The orientation of H-9 in compound **2** was determined by nOe nmr experiments. Irradiation of the proton signal at δ 1.39 (H-15) enhanced the signal intensities of H-9 and H-6. These facts clearly indicated that the structures of compounds **2** and **3** should be formulated as shown.

Celafolin B-1 [**4**] has one acetyl and one cinnamoyl ester. The $^{13}$C-nmr spectrum of **4** showed the presence of four methyls, three methylenes, two methines, three methines

TABLE 2. $^{13}$C-nmr Spectral Data for Compounds **1**, **4–6**, and **8–11** in $CDCl_3$.[a]

| Carbon | Compound | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **1** | **4** | **5** | **6** | **8** | **9** | **10** | **11** |
| C-1 | 73.8 | 73.9 | 68.6 | 71.2 | 73.6 | 79.2 | 79.3 | 79.0 |
| C-2 | 21.6 | 70.0 | 74.5 | 71.6 | 21.3 | 22.3 | 22.2 | 22.4 |
| C-3 | 26.9 | 32.8 | 31.1 | 31.1 | 26.7 | 26.6 | 26.6 | 26.6 |
| C-4 | 34.3 | 39.7 | 39.5 | 39.4 | 33.8 | 34.0 | 33.9 | 33.9 |
| C-5 | 90.1 | 87.2 | 87.6 | 87.3 | 90.4 | 91.2 | 91.4 | 91.2 |
| C-6 | 79.7 | 36.1 | 36.2 | 36.1 | 75.8 | 75.1 | 74.8 | 75.0 |
| C-7 | 49.0 | 43.8 | 43.9 | 43.8 | 53.0 | 53.0 | 54.3 | 52.6 |
| C-8 | 32.1 | 31.1 | 31.0 | 31.2 | 76.1 | 71.8 | 70.0 | 70.9 |
| C-9 | 73.5 | 73.9 | 73.9 | 73.6 | 77.2 | 74.4 | 76.7 | 74.7 |
| C-10 | 50.6 | 47.2 | 48.5 | 47.0 | 50.0 | 49.2 | 49.1 | 49.1 |
| C-11 | 82.7 | 82.1 | 82.2 | 82.3 | 81.9 | 81.8 | 81.4 | 81.8 |
| C-12 | 26.1 | 24.3 | 24.4 | 24.3 | 25.6 | 24.1 | 24.1 | 24.2 |
| C-13 | 30.8 | 30.3 | 30.3 | 30.3 | 30.9 | 30.6 | 30.7 | 30.7 |
| C-14 | 17.6 | 19.7 | 19.3 | 19.5 | 17.3 | 16.8 | 16.8 | 16.8 |
| C-15 | 18.9 | 20.4 | 18.7 | 20.3 | 18.7 | 12.5 | 12.5 | 12.2 |

[a] $^{13}$C chemical shifts (ppm relative to TMS) assigned on the basis of $^{1}$H-$^{13}$C COSY experiments.

attached to an oxygen function, and three quaternary carbons. This spectrum indicated that the compound **4** was also a dihydroagarofuran type sesquiterpene. From the $^{1}$H-$^{1}$H COSY nmr spectrum of **4**, the signals at δ 5.51 (1H, d, $J$=3.4 Hz), 4.39 (1H, ddd, $J$=3.4, 3.4, 3.4 Hz), and 4.77 (1H, d, $J$=6.4 Hz) were assigned to H-1, H-2, and H-9, respectively. The remaining $^{1}$H and $^{13}$C signals of **4** were assigned as shown in Tables 1 and 2. In the $^{1}$H-$^{13}$C long-range nmr correlation spectrum, the carbonyl carbon signal at δ 170.2 showed long range correlation with the proton signals at δ 1.92 (acetyl-H) and 5.51 (H-1). Acetylation of celafolin B-1 [**4**] gave a compound in which the $^{1}$H-nmr spectrum showed a downfield shift of H-2 from δ 4.39 to δ 5.55 when compared with compound **4**. These facts indicated that the position of the acetyl ester, hydroxy group, and cinnamoyl ester were at C-1, C-2, and C-9, respectively.

Celafolin B-2 [**5**] showed the same molecular formula as that of celafolin B-1 [**4**]. The $^{13}$C-nmr spectrum of **5** was almost the same as that of **4** (Table 2), with the only differences being the chemical shifts of H-1 and H-2 (Table 1). Acetylation of **5** gave a compound whose $^{1}$H-nmr spectrum was identical with that of the compound resulting from acetylation of **4**. These facts indicated that the positions of hydroxy group and acetyl and cinnamoyl esters in compound **5** are C-1, C-2, and C-9, respectively.

Compound **7** has two acetyl and one cinnamoyl esters (Experimental). The $^{1}$H-nmr spectrum of **7** was the same as those of the compounds resulting from acetylation of **4** and **5**. In the $^{1}$H-$^{13}$C long-range correlation nmr spectrum of compound **7**, the carbonyl carbon signal at δ 166.2 showed long range correlation with the proton signals at δ 4.75 (H-9) and 7.66 (cinnamoyl-H).

Celafolin B-3 [**6**] contained one acetyl, one benzoyl, and one cinnamoyl ester. The assignments of $^{1}$H- and $^{13}$C-nmr spectra were confirmed from 2D nmr spectra as shown in Tables 1 and 2. The position of the acetyl ester was determined to be C-1 from the $^{1}$H-$^{13}$C long-range correlation nmr spectrum.The positions of the remaining benzoyl and cinnamoyl esters could not be determined from the $^{1}$H-$^{13}$C long-range correlation nmr spectrum because of overlapping of the carbonyl carbon signals of both the esters. In a comparison of the $^{1}$H-nmr spectrum of **6** with that of **7**, the H-9 signals of both

compounds resonated at almost the same chemical shifts, while the signal at δ 5.83 (H-2) of **6** resonated more downfield than that of compound **7** (δ 5.55), indicating that the position of the benzoyl ester was at C-2 in compound **6**. The downfield shift of H-1 in compound **6** relative to that of **7** could be explained by the diamagnetic effect of the benzoyl ester on C-2.

The relative stereochemistries of compounds **4**–**7** were determined as follows. In nOe experiments of **5**, irradiations of H-15 (δ 1.26) and H-1 (δ 4.41) produced enhancements in the intensity of H-9 and H-2, respectively. The coupling constants of H-2 (δ 5.31) and H-3 (δ 1.80 and 2.31) showed that the orientation of H-2 was equatorial. These facts clearly indicated that the orientations of H-1, H-2, and H-9 were axial, equatorial, and equatorial, respectively. The orientations of ester and hydroxy groups in compounds **4** and **6** were concluded to be the same as those of compound **5** because the same coupling constants were observed for these compounds. The orientations of the ester groups in compound **7** were also the same as those of compounds **4**–**6** because compounds **4** acetate and **7** were identical. Thus, the structures of compounds **4**–**7** were formulated as shown.

Celafolin C-1 [**8**], $C_{28}H_{36}O_9$, showed the presence of three acetyl and one benzoyl ester. The $^{13}$C-nmr spectrum (Table 2) of compound **8** showed the presence of four methyls, two methylenes, six methines (with four being attached to an oxygen function), and three quaternary carbons. In the $^1$H-nmr spectrum, the signals at δ 5.41 (1H, dd, $J$=12.2, 4.4 Hz), 5.73 (1H, br s), 2.48 (1H, d, $J$=3.4 Hz), 5.25 (1H, d, $J$=3.4 Hz), and 5.03 (1H, br s) were assigned to H-1, H-6, H-7, H-8, and H-9, respectively (Table 1). The remaining $^1$H and $^{13}$C signals were also assigned as shown in Tables 1 and 2 from 2D nmr experiments. In the $^1$H-$^{13}$C long-range correlation nmr spectrum, the carbonyl carbon signal at δ 164.9 showed long-range correlation with the $^1$H signals at δ 5.03 (H-9) and 8.04 (benzoyl-H). These results clearly indicated that the position of the benzoyl ester was at C-9, and the positions of the remaining acetyl esters were at C-1, C-6, and C-8. The unusual diamagnetic shift (δ 1.60) of the methyl signal of the acetate on C-1 could be explained by shielding by the benzoyl ester at C-9 (11). The orientations of H-6, H-8, and H-9 were determined using nOe experiments. Irradiation of the $^1$H signal at δ 1.46 (H-15) enhanced the $^1$H signal intensities of H-6 and H-9, and irradiation of the $^1$H signal at δ 1.48 (H-12) enhanced the signal intensity of H-8. The coupling constants ($J$=12.2, 4.4 Hz) of H-1 indicated that the orientation of H-1 was axial. These facts clearly indicated that the structure of celafolin C-1 should be formulated as **8**.

Celafolin D-1 [**9**], $C_{38}H_{40}O_9$, contained one acetyl and three benzoyl esters. The $^{13}$C-nmr spectrum of **9** was very similar to that of triptogelin B-2 (12), except for the signal due to the ester groups. This suggested that celafolin D-1 is aso a dihydroagarofuran-type sesquiterpene with the same stereochemistry as triptogelin B-2. The assignments of $^1$H and $^{13}$C signals were determined as shown in Tables 1 and 2 from $^1$H-$^1$H COSY and $^1$H-$^{13}$C COSY nmr spectra. Comparison of the methine signals relative to the protons attached to the carbon atoms bearing the ester groups in compound **9** and triptogelin B-2 showed that the signals due to H-6, H-8, and H-9 resonated at almost the same chemical shift values, while the signal due to H-1 for compound **9** resonated upfield compared to that of triptogelin B-2. These differences in the chemical shifts for compound **9** and triptogelin B-2 are explained by the differing ester groups on C-1. The $^1$H-nmr spectrum of the benzoyl ester **9** showed signals at δ 6.86 and 7.00, indicating that the positions of the two benzoyl esters could be placed at C-1 and C-9 (12). In nOe experiments on **9**, irradiation of the proton signal at δ 1.67 (H-15) enhanced the signal of H-6 and irradiation of the signal at δ 5.56 (H-1) enhanced the signal intensity of H-9. Also, irradiation of the proton signal at δ 1.65 (H-12) enhanced the signal intensity of H-8. The cd spectrum of **9** showed a split Cotton curve due to dibenzoate chirality

(13), with $[\theta]_{220}$ +75,000, $[\theta]_{236}$ −118,000, indicating the absolute configuration as 8*S* and 9*R*. From these facts the structure of celafolin D-1 should be formulated as **9**.

Celafolin D-2 [**10**], $C_{31}H_{36}O_8$, showed the presence of one acetyl, two benzoyl, and one hydroxy group (ir, nmr and ms). The $^{13}C$ nmr spectrum of **10** was almost the same as that of **9**, except for the ester moiety, and the coupling pattern of **10** was similar to that of **9** in the $^1H$-nmr spectrum. From the $^1H$-$^1H$ COSY and $^1H$-$^{13}C$ COSY nmr spectra of **10**, the assignments of the $^1H$ and $^{13}C$ signals were confirmed as shown in Tables 1 and 2. In the $^1H$-$^{13}C$ long range correlation nmr spectrum, the carbonyl carbon signals at δ 165.0 and 165.6 were correlated with the $^1H$ signals at δ 5.53 (H-1) and 5.56 (H-9), and the carbonyl carbon signal at δ 169.9 was correlated with the $^1H$ signals at δ 6.13 (H-6) and 2.14 (acetyl-H). In nOe experiments on **10**, irradiations of H-15, H-8, and H-12 produced enhancements in the intensity of H-6, H-9, and H-8, respectively. Thus, the structure of celafolin D-2 is formulated as **10**.

Celafolin D-3 [**11**], $C_{36}H_{44}O_9$, showed the presence of one acetyl, two benzoyl, and one 2-methylbutanoyl [δ 0.79 (3H, t, $J$=7.4 Hz), 1.14 (3H, d, $J$=7.6 Hz), 1.50 (1H, m), 2.40 (2H, m)] ester (ir, nmr, and ms). The $^{13}C$-nmr spectrum of **11** was very similar to those of compounds **9** and **10**, except for the signals due to the ester groups. The assignments of $^1H$ and $^{13}C$ signals were determined as shown in Tables 1 and 2 from the $^1H$-$^1H$ COSY and $^1H$-$^{13}C$ COSY nmr spectra. In the $^1H$-$^{13}C$ long-range correlation nmr spectrum of **11**, the carbonyl signal at δ 164.9 showed long-range correlations with the proton signals at δ 5.67 (H-9) and 7.61 (benzoyl-H), and the carbonyl carbon signal at δ 169.8 was correlated with proton signals at δ 5.96 (H-6) and 2.12 (acetyl-H). Correlation of their remaining carbonyl carbon signals at δ 165.5 and 175.2 with proton signals was not observed. It is reported that the unusual diamagnetic shifts of the aromatic esters on C-1 or C-9 appeared when an aromatic ester exists in an equatorial position on C-1 or C-9 (12). The proton signals of the benzoyl esters resonated at δ 6.93, 7.08 (each 2H, t, $J$=7.8 Hz), 7.18, 7.28 (1H, t, $J$=7.8 Hz), 7.61 (4H, d, $J$=7.8 Hz) as compared to those of usual benzoyl esters at δ 7.5, 7.6, and 8.1, respectively. These facts indicated that the positions of the benzoyl esters were at C-1 and C-9. In nOe experiments on **11**, irradiation of H-15 produced an enhancement in the intensity of the H-6, and irradiation of H-12 produced enhancement in the intensity of H-8 and H-9. The coupling constants of H-1 showed that the orientation of H-1 is axial. Thus, the structure of celafolin D-3 was formulated as **11**.

Compounds **12** and **13** were identified from spectral data comparison with known compounds (12,14).

We have therefore determined the structures of eight new and five known compounds from *C. stephanotiifolius*. The compounds **2**, **3**, and **7** were already reported by Smith *et al.* (10); the spectral data of these compounds were in good agreement with the reported data (10). However the proposed structures (**14**, **15**, and **16**) (10) are not in agreement with our structures (**2**, **3**, and **7**). Smith *et al.* (10) determined the structure of the *p*-bromobenzoate derivative **17** of celorbicol [**18**] by using X-ray crystallographic analysis. The structures of compounds **14–16** were proposed after $^1H$-nmr spectral comparison with that of **17**.

In the structural elucidation of the dihydroagarofuran sesquiterpenes, the difficulty of determining the linking sites of the respective ester groups when more than three kinds of acids are involved as esters in the molecule necessitates the use of X-ray crystallographic methods, comparison of chemical shifts, or selective hydrolyses. From comparison of chemical shifts of similar compounds, it is difficult to determine the exact position of each ester, although this method is useful for determining the difference of one ester in similar compounds. Determination of the position of the ester in dihydroagarofuran sesquiterpenes has been unsuccessful in many cases. So far, we have

used 2D nmr spectroscopy, including $^{1}H$-$^{13}C$ long-range correlation nmr spectra, to solve this problem (15).

In the case of compounds **2**, **3**, and **7**, we also determined their structures by using 2D nmr experiments. It is difficult to determine the structure using chemical shifts comparison in the case of compounds **14**, **15**, and **16**. Thus, we propose new structures **2**, **3**, and **7** for compounds **14**, **15**, and **16**, respectively. The structures of some other dihydroagarofuran sesquiterpenes have been determined by reference to the $^{1}H$-nmr chemical shifts of compounds **14**, **15**, and **16**. The structure of these reported compounds (14,16,17), therefore also should be corrected.

Recently, anti-tumor-promoting activities have been reported as the inhibitory effects of several natural products (18) on 12-*O*-tetradecanoylphorbol-13-acetate (TPA) induced Epstein-Barr virus early antigen (EBV-EA) activation (19). We have reported the inhibitory effects of dihydroagarofuran sesquiterpenes on EBV activation (20). In continuation of our previous interest in this area, we examined the inhibitory tendency on the EBV-EA activation by isolated compounds **2**, **3**, **5**, **7**, **8**, **10**, and **13**. The inhibition of EBV-EA activation was assayed using the same methods described previously (19). Their inhibitory effects on the activation of the early antigen and the viabilities of Raji cells are shown in Table 3. Compounds **5** and **10** showed stronger inhibitory activities than did other compounds.

TABLE 3. Relative Ratio of EBV-EA Activation with Respect to Positive Control[a] (100%) in Presence of Constituents of *Celastrus stephanotiifolius*.

| Compound | Concentration[b] | | | |
|---|---|---|---|---|
| | $1\times10^{3}$ | $5\times10^{2}$ | $1\times10^{2}$ | $1\times10$ |
| **2** | 53.6 (70) | 84.6 (>80) | 100 (>80) | 100 (>80) |
| **3** | 26.2 (70) | 47.9 (>80) | 92.1 (>80) | 100 (>80) |
| **5** | 0 (70) | 41.6 (>80) | 86.4 (>80) | 100 (>80) |
| **7** | 25.6 (70) | 63.1 (>80) | 89.4 (>80) | 100 (>80) |
| **8** | 15.3 (70) | 52.8 (>80) | 90.6 (>80) | 100 (>80) |
| **10** | 0 (60) | 39.3 (>80) | 81.1 (>80) | 100 (>80) |
| **13** | 34.2 (70) | 65.9 (>80) | 92.9 (>80) | 100 (>80) |

[a]Induction by TPA at 20 ng/ml=32 pM. Values represent relative percentages to the positive control values (100%). Values in parentheses are viability percentages of Raji cells, and in this screening test, the cell viability required for the judgment of inhibitory effect was more than 60%.

[b]Mol ratio/TPA (20 ng/ml=32 pM/ml).

## EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were determined on a Yanagimoto apparatus. $^{1}H$-nmr and $^{13}C$-nmr spectra were recorded on a JEOL JNM FX 400 spectrometer with TMS as internal standard. Uv spectra in MeOH were obtained on a Hitachi 330 spectrometer. Ir spectra were determined on a Hitachi type 1720 spectrometer with a KBr disk. Mass spectra were determined with JEOL JMS D-300 spectrometer. Optical rotations were measured with a Union Giken PM-201 polarimeter. Cd spectra were taken with a JASCO J-500C spectropolarimeter. Kieselgel 60 (70–230 mesh or 230–400 mesh, Merck) and Sephadex LH-20 (Pharmacia) were used for cc.

PLANT MATERIAL, EXTRACTION, AND ISOLATION.—Dried seeds of *C. stephanotiifolius* were collected in October 1990 at Kawashima-chyo, Tokushima Prefecture, Japan. Herbarium specimens are deposited in the herbarium of University of Tokushima. The dried seeds (2.8 kg) of *C. stephanotiifolius* were extracted with MeOH (15 liters×3) at 60°. The MeOH extracts were concentrated in vacuo to give a residue (470 g), which was partitioned between EtOAc and $H_2O$. The EtOAc layer was concentrated to give a residue (68 g), which was chromatographed on Si gel (1.5 kg). The column was eluted with solvents of increasing polarity [hexane-EtOAc (4:1, 3:1, 1:1), EtOAc, EtOAc-MeOH (19:1, 10:1)] to give 13 fractions. Fraction 4 (6.5 g) was

chromatographed on a Si gel column with $CHCl_3$-MeOH (49:1) to give 6 fractions (fractions 4-1–4-6). Fraction 4-3 (400 mg) was chromatographed on Sephadex LH-20 column with $CHCl_3$-MeOH (7:3) to give **1** (7 mg), and fraction 4-5 (2.5 g) was separated on a Si gel column with hexane-$Me_2CO$ (6:1) to give **2** (62 mg) and **12** (7 mg). Fraction 4-6 (2.6 g) was separated on a Si gel column with hexane-$Me_2CO$ (6:1) to give **6** (8 mg) and **11** (8 mg). Fraction 5 (4.4 g) was separated on a Si gel column with hexane-$Me_2$ CO (3:1) and hexane-$CHCl_3$ (1:1) to give **3** (45 mg). Fraction 6 (3.3 g) was chromatographed on a Si gel column with $CHCl_3$-MeOH (99:1) and hexane-$Me_2CO$ (3:1) to give **7** (58 mg), **8** (18 mg), **9** (15 mg), and **13** (94 mg). Fraction 7 (700 mg) was separated on Sephadex LH-20 with MeOH and a Si gel column with $CHCl_3$-MeOH (99:1) to give **10** (62 mg). Fraction 8 (3.1 g) was separated on Sephadex LH-20 with MeOH and a Si gel column to give **4** (15 mg) and **5** (22 mg).

*Celafolin A-1* [**1**].—Amorphous powder: $[\alpha]^{20}D$ +23.2 (*c*=0.43, MeOH); uv λ max nm (ε) 218 (17600), 224 (19300), 279 (17800); ir ν $cm^{-1}$ (KBr) 1713, 1279, 1170, 1093, 1017, 714; eims *m/z* $[M]^+$ 546, $[M-Me]^+$ 531, $[C_6H_5CH=CHCO]^+$ 131 (100%), $[C_6H_5CO]^+$ 105, 57, $[Ac]^+$ 43; hrms *m/z* 546.2653 (calcd for $C_{33}H_{38}O_7$, 546.2618); $^1H$ nmr ($CDCl_3$) see Table 1, acetate [δ 1.62 (3H, s)], benzoate [δ 7.45 (2H, brt, *J*=6.8 Hz), 7.57 (1H, brt, *J*=6.8 Hz), 8.08 (2H, brd, *J*=6.8 Hz)], cinnamate [δ 6.47, 7.71 (each 1H, d, *J*=16.1 Hz), 7.42 (3H, m), 7.55 (2H, m)]; $^{13}C$ nmr see Table 2, acetate (20.8, 170.0), benzoate (128.3, 129.7, 130.1, 133.2, 165.6), cinnamate (117.9, 128.2, 128.7, 130.6, 134.2, 145.6, 166.1).

*Compound* **2**.—Amorphous powder: eims *m/z* $[M]^+$ 520, $[M-Me]^+$ 399, 294, 206, $[C_6H_5CO]^+$ 105 (100), 77, $[Ac]^+$ 43; hrms *m/z* 520.2465 (calcd for $C_{31}H_{36}O_7$, 520.2462); $^1H$ nmr ($CDCl_3$) δ 1.03 (3H, d, *J*=7.2 Hz, H-14), 1.39 (3H, s, H-15), 1.44 (3H, s, H-12), 1.45 (3H, s, H-13), 1.51, 2.30 (each 1H, m, H-3), 1.63 (1H, m, H-2), 1.91 (1H, ddd, *J*=13.2, 7.8, 3.9, H-2), 2.25 (1H, d, *J*=2.9, H-8), 2.38 )1H, m, H-7), 2.48 (1H, quint, *J*=6.4 Hz, H-4), 2.53 (1H, ddd, *J*=16.1, 6.8, 2.9, H-8), 5.07 (1H, d, *J*=6.8 Hz, H-9), 5.51 (1H, s, H-6), acetate [1.63 (3H, s)], benzoate [7.45, 7.49 (each 2H, br t, *J*=7.6 Hz), 7.56, 7.61 (each 1H, br t, *J*=7.6 Hz), 8.07, 8.09 (each 2H, br t, *J*=7.6 Hz)]; $^{13}C$ nmr δ 73.8 (C-1), 21.6 (C-2), 26.9 (C-3), 34.5 (C-4), 90.2 (C-5), 80.3 (C-6), 49.0 (C-7), 32.2 (C-8), 73.5 (C-9), 50.7 (C-10), 82.6 (C-11), 26.0 (C-12), 30.8 (C-13), 17.6 (C-14), 18.9 (C-15), acetate (20.8, 170.0), benzoate (128.3, 128.7, 129.6, 129.7, 130.1, 133.2, 133.4, 165.6, 165.8).

*Compound* **3**.—Amorphous powder: eims *m/z* $[M]^+$ 458, 416, 294, 206, 159, 138, $[C_6H_5CO]^+$ 105 (100), $[Ac]^+$ 43; hrms *m/z* 458.2303 (calcd for $C_{26}H_{34}O_7$, 458.2305); $^1H$ nmr ($CDCl_3$) δ 1.01 (3H, d, *J*=7.2, H-14), 1.33 (3H, s, H-15), 1.40 (3H, s, H-12), 1.41 (3H, s, H-13), 1.45 (1H, m, H-3), 1.59 (1H, m, H-2), 1.88 (1H, ddd, *J*=12.8, 8.4, 4.4, H-2), 2.18 (1H, m, H-8), 2.20 (1H, m, H-3), 2.21 (1H, m, H-7), 2.26 (1H, quin, *J*=7.2, H-4), 2.42 (1H, ddd, *J*=16.6, 6.8, 2.9, H-8), 5.01 (1H, d, *J*=6.8, H-9), 5.32 (1H, s, H-6), 5.46 (1H, dd, *J*=12.0, 4.4, H-1), acetate [1.61, 2.11 (each 3H, s)], benzoate [7.44 (2H, br t, *J*=7.8), 7.55 (1H, br t, *J*=7.8), 8.06 (2H, br d, *J*=7.8)]; $^{13}C$ nmr δ 73.7 (C-1), 21.5 (C-2), 26.8 (C-3), 34.0 (C-4), 89.9 (C-5), 79.6 (C-6), 48.9 (C-7), 32.1 (C-8), 73.4 (C-9), 50.6 (C-10), 82.6 (C-11), 26.0 (C-12), 30.6 (C-13), 17.4 (C-14), 18.8 (C-15), acetate (20.8, 21.4, 170.0, 170.1), benzoate (128.2, 129.7, 130.0, 133.2, 165.6).

*Celaforin B-1* [**4**].—Amorphous powder: $[\alpha]^{20}D$ +97.5 (*c*=1.00, MeOH); uv λ max nm (ε) 217 (14400), 223 (12500), 280 (19200); ir ν $cm^{-1}$ (KBr) 1730, 1693, 1639, 1333, 1242, 1193, 1018, 772; eims *m/z* $[M]^+$ 442, $[M-Me]^+$ 427, $[M-HOAc]^+$ 382, $[M-C_6H_5CH=CHCO]^+$ 311, 251, $[C_6H_5CH=CHCO]^+$ 131, $[Ac]^+$ 43; hrms *m/z* 442.2315 (calcd for $C_{26}H_{34}O_6$, 442.2356); $^1H$ nmr ($CDCl_3$) see Table 1, acetate [δ 1.92 (3H, s)], cinnamate [δ 6.39, 7.67 (each 1H, d, *J*=15.9), 7.37 (3H, m), 7.54 (2H, m)]; $^{13}C$ nmr see Table 2, acetate (21.1, 170.2), cinnamate (118.5, 128.2, 128.8, 130.6, 134.6, 145.0, 166.4).

*Acetylation of celaforin B-1* [**4**].—A solution of **4** (3 mg) in pyridine (1 ml) and $Ac_2O$ (1 ml) was stirred at 70° for 24 h. Usual workup of the reaction mixture gave an acetate identical to compound **7** ($^1H$ nmr and tlc).

*Celafolin B-2* [**5**].—Amorphous powder: $[\alpha]^{20}D$ +68.9 (*c*=0.46, MeOH); uv λ max nm (ε) 216 (14500), 222 (12100), 276 (16300); ir ν $cm^{-1}$ (KBr) 1737, 1698, 1246, 1203, 1177, 1074, 1008, 714; eims *m/z* $[M]^+$ 442 $[M-HOAc]^+$ 382, $[M-C_6H_5CH=CHCO]^+$ 311, 251, 147, $[C_6H_5CH=CHCO]^+$ 131, $[Ac]^+$ 43; hrms *m/z* 442.2316 (calcd for $C_{26}H_{34}O_6$, 442.2356); $^1H$ nmr ($CDCl_3$) see Table 1, acetate [δ 1.2.06 (3H, s)], cinnamate [δ 6.47, 7.71 (each 1H, d, *J*=15.9), 7.37 (3H, m), 7.52 (2H, m)]; $^{13}C$ nmr see Table 2, acetate (21.4, 171.3), cinnamate (118.6, 128.2, 128.8, 130.3, 134.5, 145.0, 166.6).

*Acetylation of celaforin B-2* [**5**].—A solution of **5** (3 mg) in pyridine (1 ml) and $Ac_2O$ (1 ml) was stirred at 70° for 24 h. Usual workup of the reaction mixture gave an acetate identical to the acetate of **4** and to compound **7** ($^1H$ nmr and tlc).

*Celaforin B-3* [**6**].—Amorphous powder: $[\alpha]^{20}D$ +232.6 (*c*=0.33, MeOH); uv λ max nm (ε) 218 (18000), 223 (19400), 278 (17200); ir ν $cm^{-1}$ (KBr) 1719, 1638, 1072, 806, 713; eims *m/z* $[M]^+$ 546, $[M-C_6H_5CH=CHCO]^+$ 415, 355, 233, $[C_6H_5CH=CHCO]^+$ 131, 105, $[Ac]^+$ 43; hrms *m/z* 546.2638

(calcd for $C_{33}H_{38}O_7$, 546.2618); $^1$H nmr ($CDCl_3$) see Table 1, acetate [δ 1.80 (3H, s)], benzoate [7.45 (2H, br t, $J$=7.6), 7.56 (1H, br t, $J$=7.6), 8.00 (2H, br d, $J$=7.6)], cinnamate [δ 6.40, 7.67 (each 1H, d, $J$=16.1), 7.37 (3H, m), 7.55 (2H, m)]; $^{13}$C nmr see Table 2, acetate (20.8, 170.1), cinnamate (118.4, 128.5, 128.8, 130.2, 134.6, 145.1, 166.3), benzoate (128.3, 129.6, 130.6, 132.8, 166.2).

*Compound* **7**.—Amorphous powder: eims *m/z* [M]$^+$ 484, 425, [M−$C_6H_5CH$=CHCO]$^+$ 353, 233, [$C_6H_5CH$=CHCO]$^+$ 131, [Ac]$^+$ 43; hrms *m/z* 484.2421 (calcd for $C_{28}H_{36}O_7$, 484.2462); $^1$H nmr ($CDCl_3$) δ 1.21 (3H, s, H-13), 1.25 (3H, d, $J$=8.3, H-14), 1.35 (3H, s, H-15), 1.37 (3H, s, H-12), 1.77 (1H, m, H-3), 1.91 (1H, sextet, $J$=8.3, H-4), 2.02 (1H, m, H-7), 2.08 (2H, m, H-6), 2.17 (1H, ddd, $J$=16.4, 6.8, 4.4, H-8), 2.42 (1H, ddd, $J$=14.4, 6.8, 2.8, H-3), 4.75 (1H, d, $J$=6.8, H-9), 5.55 (1H, s, H-1), 5.55 (1H, s, H-2), acetate [1.81, 2.03 (each 3H, s)], cinnamate [δ 6.38, 7.66 (each 1H, d, $J$=15.6), 7.37 (3H, m), 7.54 (2H, m)] $^{13}$C nmr δ 70.8 (C-1), 71.1 (C-2), 31.0 (C-3), 39.5 (C-4), 87.3 (C-5), 36.0 (C-6), 43.7 (C-7), 31.1 (C-8), 73.5 (C-9), 57.1 (C-10), 82.2 (C-11), 24.2 (C-12), 30.3 (C-13), 19.2 (C-14), 19.9 (C-15), acetate (20.7, 21.3, 170.0, 170.1), cinnamate (118.4, 128.2, 128.8, 130.2, 134.5, 145.0, 166.2).

*Celaforin C-1* [**8**].—Amorphous powder: [α]$^{20}$D +39.4 ($c$=0.66, MeOH); uv λ max nm (ε) 232 (11400); ir ν cm$^{-1}$ (KBr) 1741, 1370, 1229, 1101, 1028, 970, 714; eims *m/z* [M]$^+$ 516, [M−Me]$^+$ 501, 474, 414, 380, 292, 251, [$C_6H_5CO$]$^+$ 105, [Ac]$^+$ 43; hrms *m/z* 516.2350 (calcd for $C_{28}H_{36}O_9$, 516.2360); $^1$H nmr ($CDCl_3$) see Table 1, acetate [δ 1.60, 2.10, 2.21 (each 3H, s)], benzoate [7.44 (2H, brt, $J$=7.2), 7.56 (1H, brt, $J$=7.2), 8.04 (2H, brd, $J$=7.2)]; $^{13}$C nmr see Table 2, acetate (20.8, 21.1, 21.1, 169.4, 170.0, 170.0), benzoate (128.4, 129.0, 130.1, 133.5, 164.9).

*Celaforin D-1* [**9**].—Amorphous powder: [α]$^{20}$D −52.8 ($c$=0.38, MeOH); uv λ max nm (ε) 228 (27800); ir ν cm$^{-1}$ (KBr) 1727, 1452, 1316, 1284, 1095, 1028, 962, 708; eims *m/z* [M]$^+$ 640, [M−Me]$^+$ 625, [$C_6H_5CO$]$^+$ 105, [Ac]$^+$ 43; hrms *m/z* 640.2634 (calcd for $C_{38}H_{40}O_9$, 640.2673); $^1$H nmr ($CDCl_3$) see Table 1, acetate [δ 2.14 (each 3H, s)], benzoate [6.86 (2H, t, $J$=7.6), 7.00 (2H, t, $J$=7.6), 7.14 (1H, t, $J$=7.6), 7.26 (1H, t, $J$=7.6), 7.47 (4H, t, $J$=7.6), 7.59 (2H, d, $J$=7.6), 7.59 (1H, t, $J$=7.6), 8.06 (2H, d, $J$=7.6)]; $^{13}$C nmr see Table 2, acetate (21.3, 170.0), benzoate (127.5, 127.7, 128.6, 129.2, 129.3, 129.7, 129.8, 129.9, 130.1, 132.2, 132.3, 133.1, 164.9, 165.4, 165.5).

*Celaforin D-2* [**10**].—Amorphous powder: [α]$^{20}$D −19.3 ($c$=0.42, MeOH); uv λ max nm (ε) 228 (27800); ir ν cm$^{-1}$ (KBr) 3504, 1723, 1452, 1370, 1284, 1179, 1116, 1070, 1028, 963, 709; eims *m/z* [M]$^+$ 536, [M−Me]$^+$ 521, 494 [M−$C_6H_5COOH$]$^+$ 414, 372, 250, 153, [$C_6H_5CO$]$^-$ 105, [Ac]$^-$ 43; hrms *m/z* 536.2417 (calcd for $C_{31}H_{36}O_8$, 536.2411); $^1$H nmr ($CDCl_3$) see Table 1, acetate [δ 2.14 (each 3H, s)], benzoate [6.92 (2H, t, $J$=7.2), 7.14 (2H, t, $J$=7.2,) 7.18 (1H, t, $J$=7.2), 7.36 (1H, t, $J$=7.2), 7.61 (2H, d, $J$=7.2), 7.67 (2H, d, $J$=7.2)]; $^{13}$C nmr see Table 2, acetate (21.4, 169.9), benzoate (127.5, 128.0, 129.1, 129.3, 129.6, 130.0, 132.1, 132.7, 165.0, 165.6).

*Celaforin D-3* [**11**].—Amorphous powder: [α]$^{20}$D −21.9 ($c$=0.32, MeOH); uv λ max nm (ε) 228 (23400); ir ν cm$^{-1}$ (KBr) 1726, 1282, 1094, 709; eims *m/z* [M]$^-$ 620, [M−Me]$^+$ 605, 578, 456, [$C_6H_5CO$]$^+$ 105, [Ac]$^-$ 43; hrms *m/z* 620.2986 (calcd for $C_{36}H_{44}O_9$, 620.2966); $^1$H nmr ($CDCl_3$) see Table 1, acetate [δ 2.12 (each 3H, s], benzoate [6.93, 7.08 (each 2H, t, $J$=7.8), 7.18, 7.28 (each 1H, t, $J$=7.8), 7.61 (4H, d, $J$=7.8)], 2-methylbutanoate [0.79 (3H, t, $J$=7.4), 1.14 (3H, d, $J$=7.6), 1.50 (1H, m), 2.40 (2H, m)]; $^{13}$C nmr see Table 2, acetate (21.3, 169.8), benzoate (127.6, 127.7, 129.2, 129.3, 129.5, 130.0, 132.2, 132.4, 164.9, 165.5), 2-methylbutanoate (11.6, 16.4, 26.6, 41.6, 175.2).

*Compound* **12**.—Amorphous powder: hrms *m/z* 426.2389 (calcd for $C_{26}H_{34}O_5$, 426.2407).

*Compound* **13**.—Amorphous powder: hrms *m/z* 516.2387 (calcd for $C_{28}H_{36}O_9$, 516.2360).

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