Isohelianol: A 3,4-*seco*-Triterpene Alcohol from Sasanqua Oil

Toshihiro Akihisa,*,† Yumiko Kimura,‡ Kazuo Koike,§ Tsuyoshi Shibata,† Zen-ya Yoshida,† Tamotsu Nikaido,§ and Toshitake Tamura†

College of Science and Technology, Nihon University, 1-8, Kanda Surugadai, Chiyoda-ku, Tokyo, 101 Japan, College of Pharmacy, Nihon University, 7-7-1, Narashinodai, Funabashi-shi, Chiba, 274 Japan, and School of Pharmaceutical Sciences, Toho University, 2-2-1, Miyama, Funabashi-shi, Chiba, 274 Japan

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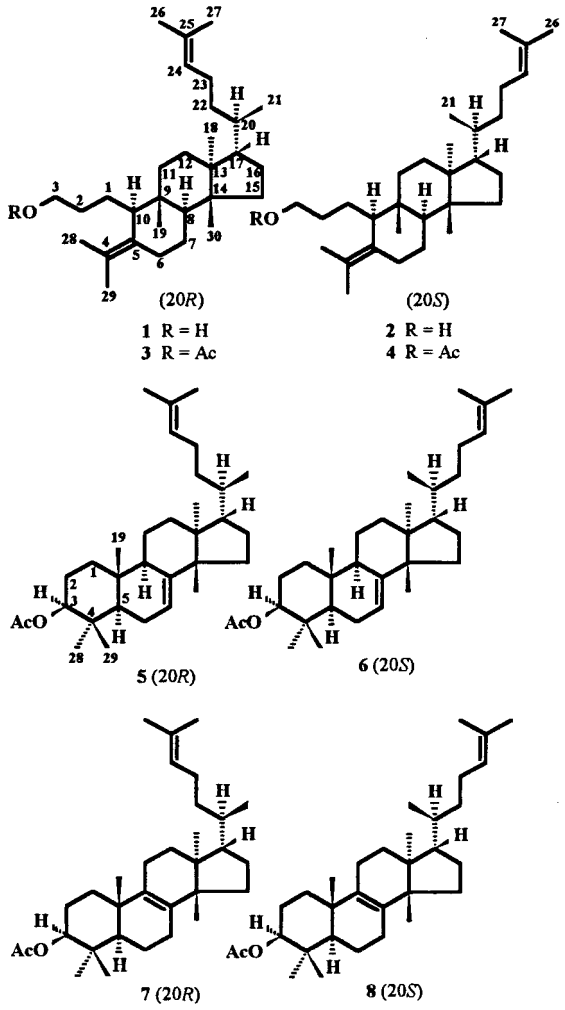
The structure of isohelianol isolated from the seeds of *Camellia sasanqua* Thunb. was established to be 3,4-*seco*-19(10→9)-*abeo*-8α,9*β*,10α-eupha-4,24-dien-3-ol (**1**) on the basis of spectroscopic methods.

The seed oil (sasanqua oil) obtained from *Camellia sasanqua* Thunb. (Theaceae) has a composition similar to camellia oil (from *Camellia japonica* L.[1]) and is occasionally used as a substitute for camellia oil.[2] These oils contain butyrospermol (eupha-7,24-dien-3*β*-ol) and Δ^7-tirucallol (tirucalla-7,24-dien-3*β*-ol) as the major triterpene alcohols obtained from the nonsaponifiable lipid fractions (NSL).[3−6] In this paper, we report the isolation and structure elucidation of a new 3,4-*seco*-triterpene alcohol designated isohelianol (**1**) and structure revision of helianol (**2**),[7−9] both from sasanqua oil.

Alkaline hydrolysis of crude sasanqua oil followed by column chromatography of the NSL fraction on silica gel afforded the triterpene alcohol fraction that, upon acetylation, gave an acetylated triterpene alcohol fraction. Argentation TLC followed by preparative HPLC of the acetate fraction eventually yielded isohelianol (**1**) and helianol (**2**) as the acetyl derivatives, **3** and **4**, respectively, and the acetates of 10 known triterpene alcohols as described in the Experimental Section.

Compound **3**, molecular formula $C_{32}H_{54}O_2$ determined from its HREIMS ([M]$^+$, *m*/*z* 470.4102) as well as from its ^{13}C NMR DEPT, gave IR absorptions at 1240, 1742 (acetoxy), and 828 cm^{-1} (trisubstituted double bond). Compound **3** displayed ^{1}H NMR signals at *δ* 1.61, 1.65, 1.66, and 1.69 for four olefinic methyl protons consistent with two isopropylidene groups of a tricyclic *seco*-triterpene.[7,10] The appearance of signals at *δ* 2.04 (−OCOC*H*$_3$) and at *δ* 4.03 (t) for an oxymethylene indicated **3** to be a 3,4-*seco*-triterpen-3-ol acetate. Furthermore, signals at *δ* 0.80, 0.84, and 0.90 (methyls), a methyl doublet at *δ* 0.85, and at *δ* 5.09 (tt) for an olefinic methine supported the above proposed triterpene skeleton. Compound **3**, in its EIMS, displayed peaks at *m*/*z* 359 [loss of one (C_8H_{15}) of the two sidechains], 287 [loss of one sidechain (CH_2=$CHCH_2OAc$; $C_5H_8O_2$), and part of the other sidechain (C_6H_{11}; C-22−C-27)], 259 (loss of both sidechains $C_5H_8O_2$ and C_7H_{15}), and 69 [CH_2CH=C-$(Me)_2$]$^+$ (C-23−C-27; base peak), which suggested the presence of a C-24 monounsaturated C_8 sidechain.[7,10] This also suggested that the other double bond, an isopropylidene group, was located at C-4(5).[7,10] All of the above evidence suggests that **3** possesses a 3,4-*seco*-cucurbitane or a 3,4-*seco*-19(10→9)-*abeo*-euphane/tirucallane skeleton. The above proposition was further supported from the diagnostic fragmentation ions at *m*/*z* 274 ($C_{20}H_{34}$), corresponding to the loss of *seco*-ring A and ring B obtained due to the cleavages of the C-7−C-8 and C-9−C-10 bonds,[7,10,11] and 163 ($C_{12}H_{19}$; 274 − C_8H_{15}) indicated the absence of C-10 methyl. The above evidence coupled with the ^{13}C and ^{1}H NMR data (Table 1) in addition to analysis of ^{1}H−^{1}H COSY, ^{13}C−^{1}H



* To whom all correspondence should be addressed. Tel.: 81-3-3259-0816. Fax: 81-3-3293-7572. E-mail: akihisa@chem.cst.nihon-u.ac.jp.
† College of Science and Technology, Nihon University.
‡ College of Pharmacy, Nihon University.
§ Toho University.

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Table 1. ^{13}C NMR and ^{1}H NMR Spectral Data (δ Values; $CDCl_3$) of Isohelianol Acetate (**3**) and Helianol Acetate (**4**)[a]

	isohelianol acetate (**3**)		helianol acetate (**4**)	
C no.	^{13}C	^{1}H	^{13}C	^{1}H
1	26.4	1.36, 1.52	26.4	1.35, 1.52
2	27.8	1.62 (2H)	27.8	1.62 (2H)
3	65.3	4.03 (2H, t, 6.5)	65.3	4.03 (2H, t, 6.6)
4	122.3		122.3	
5	134.0		134.0	
6	25.3	2.24 (α), 2.08 (*β*)	25.4	2.24 (α), 2.07 (*β*)
7	23.6	1.33 (α), 1.55 (*β*)	23.7	1.33 (α), 1.55 (*β*)
8	44.2	1.58 (dd, 4.0, 12.5)	44.2	1.58 (dd, 4.0, 12.5)
9	38.6		38.6	
10	54.8	2.27	54.9	2.26
11	39.1	1.50 (2H)	39.1	1.50 (2H)
12	30.4	1.53 (α), 1.74 (*β*)	30.3	1.53 (α), 1.72 (*β*)
13	46.1		46.1	
14	47.8		47.8	
15	34.0	1.07 (α), 1.14 (*β*)	34.1	1.08 (α), 1.14 (*β*)
16	28.2	1.27 (α), 1.86 (*β*)	28.1	1.25 (α), 1.88 (*β*)
17	49.8	1.52	50.4	1.46
18(13α)	15.6	0.80 (s)	15.4	0.80 (s)
19(9*β*)	18.0	0.90 (s)	18.1	0.90 (s)
20	35.3	1.50	35.8	1.43
21	19.1	0.85 (d, 5.9)	18.6	0.91 (d, 6.1)
22	35.3	1.12, 1.57	36.5	1.04, 1.42
23	24.7	1.88, 2.02	24.9	1.86, 2.03
24	125.2	5.09 (tt, 1.5, 7.3)	125.3	5.10 (tt, 1.5, 7.1)
25	130.9		130.9	
26	25.7	1.69 (s)	25.7	1.68 (s)
27	17.7	1.61 (s)	17.6	1.60 (s)
28	20.8	1.65 (s)	20.8	1.65 (s)
29	21.0	1.66 (s)	21.0	1.66 (s)
30(14*β*)	19.1	0.84 (s)	19.0	0.83 (s)
*C*OMe	171.3		171.3	
CO*Me*	21.0	2.04 (s)	21.1	2.04 (s)

[a] Figures in parentheses denote *J* values (Hz).

COSY, HSQC, and HMBC spectra indicated that **3** possesses a 3,4-*seco*-19(10→9)-*abeo*-euphane/tirucallane skeleton.

The stereochemistry of compound **3** was established by comparison of the phase-sensitive NOESY and NOE difference spectra with that of known euphanes, butyrospermol acetate (**5**) and euphol (eupha-8,24-dien-3*β*-ol) acetate (**7**). Compound **3** showed significant NOE correlations between [H-30(14*β*-Me)−H-17−H-21], [H-21−H-16α,*β*], and [H-16α−H-18(13α-Me)−H-20] (Figure 1), which were also consistent with those reported for **5**[7,12] and **7** (see the Experimental Section), thus suggesting that **3** possesses the same stereochemistry at the C/D ring junction (13α, 14*β*), C-17 (17*β*-H), and C-20 (20*R*) as that of **5** and **7**. From these, in combination with the other marked NOE correlations between [H-30(14*β*-Me)−H-19(9*β*-Me)−H-28] and [H-18(13α-Me)−H-8−H-10], it was concluded that **3** is 3,4-*seco*-19-(10→9)-*abeo*-8α,9*β*,10α-eupha-4,24-dien-3-yl acetate (20*R*).

The most stable conformation of **3** was simulated using MacroModel. The results of the calculations[13,14] are shown in Figure 1 together with the significant NOE's (↔). The conformer of simulated **3** shows C-22 of the side chain at C-17 oriented into a "left-handed" conformation (C-22 *cis*-oriented to C-13) similar to that of **5**[7,12] and to the crystal structure of **7**.[15] This was consistent with the NOE experiment done in solution, thus confirming the proposed structure of **3**. On alkaline hydrolysis, acetate **3** yielded the free alcohol, 3,4-*seco*-19(10→9)-*abeo*-8α,9*β*,10α-eupha-4,24-dien-3-ol (**1**).

Compound **4** exhibited the same significant NOE

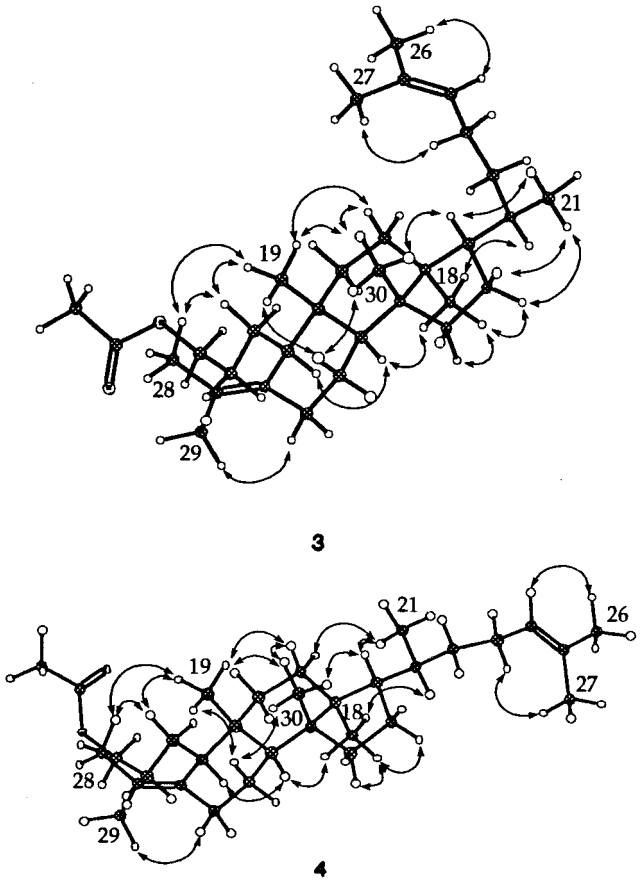

Figure 1. Energy-minimized conformations and some representative NOE correlations (↔) for isohelianol acetate (**3**) and helianol acetate (**4**).

correlations as **3**, as far as the ring system per se and its junctures with the side chains at C-10 and C-17 (Figure 1) is concerned, suggesting that **4** possesses the same stereochemistry as that of **3** with the exception of C-20. It showed diagnostic NOE correlation between [H-21−H-12α], which was also observed for two tirucallanes, Δ^7-tirucallol acetate (**6**)[12] and tirucallol (tirucalla-8,24-dien-3*β*-ol) acetate (**8**) (see the Experimental Section). Thus, **4** was concluded to be 3,4-*seco*-19-(10→9)-*abeo*-8α,9*β*,10α-tirucalla-4,24-dien-3-ol acetate with a 20*S*-chirality, not 20*R*-chirality as proposed earlier.[7] The simulated most stable conformer[13] of **4** shows C-22 oriented in a "right-handed" conformation (C-22 *trans*-oriented with respect to C-13) similar to that of compound **6**[12] and the crystal structures of **8**[15] and euferol [19(10→9)-*abeo*-8α,9*β*,10α-tirucalla-5,24-dien-3*β*-ol] acetate.[16] This conformation of **4** was consistent with results from the NOE experiments carried out in solution and supported the proposed structure as depicted in **4**.

The NOE correlation of H-21 is an essential aid for assignment of stereochemistry at C-20, i.e., for the discrimination of euphane (20*R*)/tirucallane (20*S*) and 3,4-*seco*-migrated euphane/tirucallane triterpenes. Compound **3** possessing a 20*R*-chirality exhibited the diagnostic NOE correlations between [H-21−H-16α,*β*], while compound **4** with a 20*S*-chirality had the NOE correlation between [H-21−H-12α], further confirming the proposed structure **4**.

This study has demonstrated the co-occurrence of helianol (**2**) and its 20*R*-epimer, isohelianol (**1**), as the

minor triterpene alcohol constituents of the NSL of sasanqua oil. This observation is consistent with the presence of both euphane (butyrospermol and euphol) and tirucallane (Δ^7-tirucallol and tirucallol) triterpenes in this oil. It is noteworthy that the natural occurrence of 19(10→9)-*abeo*-8α,9*β*,10α-euphane/tirucallane-type triterpenes is extremely rare, except for reissantenol oxide from *Reissantia indica*,[11] euferol from *Euphorbia mellifera*,[16] and boeticol from *Euphorbia boetica*.[17]

Experimental Section

General Experimental Procedures. Crystallizations were from MeOH, and melting points are uncorrected. TLC plates [silica gel−$AgNO_3$ (4:1, w/w)] were developed with cyclohexanes−EtOAc (9:1, v/v). Reversed-phase HPLC was carried out on an octadecyl silica column (25 cm × 10 mm i.d.; ERC-ODS-2152 column, ERC Co., Ltd., Tokyo) with MeOH (4 mL/min) as the mobile phase, at 25 °C. GC was performed using a DB-17 fused silica capillary column (30 m × 0.3 mm i.d., column temperature 275 °C). EIMS and HREIMS were recorded at 70 eV. NMR spectra were recorded at 400 and 500 MHz (^{1}H NMR) and 100 and 125 MHz (^{13}C NMR) in $CDCl_3$ with TMS (^{1}H NMR) and $CDCl_3$ at δ 77.0 (^{13}C NMR) as internal standard. Chemical shifts were recorded in δ values. IR spectra were recorded in KBr. Specific rotations were measured in $CHCl_3$. Acetylation (Ac_2O−pyridine) and hydrolysis of acetates (5% KOH in MeOH) were performed at room temperature overnight.

Materials. Crude sasanqua oil was donated by Nikko Fine Products Co. (Tokyo). Triterpene acetates **4**[7,9] and **5**−**8** and the acetates of the other triterpene alcohols[5] described below were used as reference compounds.

Isolation Procedure. Alkaline hydrolysis (5% KOH in MeOH, reflux, 3 h) of crude sasanqua oil (2 kg) yielded neutral NSL (9.0 g). The NSL was chromatographed over a silica gel (500 g) column with hexane and hexanes−EtOAc (9:1 4:1, v/v) as eluants. The hexanes−EtOAc (9:1) eluted a fraction that, after rechromatography over silica gel, yielded a triterpene alcohol fraction (4.4 g) that, upon acetylation, gave an acetate fraction (4.0 g). The acetate fraction was then separated into three major bands by argentation TLC. Fraction 1 (1.2 g) from the least polar band contained *β*-amyrin (olean-12-en-3*β*-ol) acetate, α-amyrin (urs-12-en-3*β*-ol) acetate, **7**, and **8**. Fraction 2 (1.7 g) from the medium polar band contained **5**, **6**, and several other compounds. Fraction 3 (0.5 g) from the most polar band contained the acetates of dammaradienol (dammara-20,-24-dien-3*β*-ol) and 24-methylenedammarenol [24-methyldammara-20,24(24^1)-dien-3*β*-ol]. Fraction 2 was separated into seven fractions on HPLC. From fractions 2−3 and 2−4 were isolated **3** (4.4 mg) and **4** (2.4 mg), respectively. Fraction 2−5 contained lupeol [lup-20(29)-en-3*β*-ol] acetate, and the most bulky fraction 2−6 was a mixture of **5** and **6**. Fractions 2−7 contained *ψ*-taraxasterol (taraxast-20-en-3*β*-ol) acetate. The composition of the triterpene alcohol fraction of sasanqua oil investigated in this study was similar to that of the corresponding fraciton of camellia oil.[5]

Identification of the known compounds, as the acetyl derivatives, was based on chromatographic (GC and HPLC) and spectroscopic (MS and ^{1}H NMR) comparisons with data in the literature. Since the fully assigned ^{1}H and ^{13}C NMR spectral data for two reference compounds, **7** and **8**, were unavailable in the literature, these also are shown below accompanied by some representative NOE correlations. The NMR assignments were aided by ^{13}C DEPT, ^{1}H−^{1}H COSY, ^{13}C−^{1}H COSY, HSQC, HMBC, and phase-sensitive NOESY spectroscopy. The ^{1}H and ^{13}C NMR data for **2** were reported previously.[7,8]

Isohelianol (1): an amorphous solid; $[\alpha]^{25}_D$ +34.6° (*c* 0.3); IR ν_{max} 3417, 820, 801 cm^{-1}; EIMS *m*/*z* 428 $[M]^+$ (6), 413 (3), 370 (2), 317 (2), 315 (2), 287 (9), 274 (5), 259 (6), 231 (3), 221 (3), 217 (4), 205 (7), 203 (7), 189 (8), 175 (9), 163 (21), 69 (100); HREIMS *m*/*z* 428.4008 (calcd for $C_{30}H_{52}O$, 428.4015); ^{1}H NMR (400 MHz) δ 5.09 (1H, br t, *J* = 7.3 Hz, H-24), 3.62 (2H, t, *J* = 6.3 Hz, H-3), 1.69 (3H, s, H-26), 1.66 (6H, s, H-28, H-29), 1.61 (3H, s, H-27), 0.90 (3H, s, H-19), 0.85 (3H, d, *J* = 6.9 Hz, H-21), 0.84 (3H, s, H-30), 0.80 (3H, s, H-18).

Isohelianol acetate (3): fine colorless needles; mp 61−63 °C; $[\alpha]^{25}_D$ +33.7° (*c* 0.3); IR ν_{max} 1742, 1240, 828 cm^{-1}; EIMS *m*/*z* 470 $[M]^+$ (10), 455 (4), 387 (1), 359 (2), 357 (2), 287 (29), 274 (9), 263 (3), 259 (5), 245 (3), 231 (2), 223 (5), 221 (13), 217 (5), 205 (15), 203 (8), 191 (7), 189 (9), 175 (10), 163 (28), 69 (100); HREIMS *m*/*z* 470.4102 (calcd for $C_{32}H_{54}O_2$, 470.4120), 359.2937 (calcd for $C_{24}H_{39}O_2$, 359.2947), 287.2745 (calcd for $C_{21}H_{35}$, 287.2737), 274.2652 (calcd for $C_{20}H_{34}$, 274.2658), 259.2424 (calcd for $C_{19}H_{31}$, 259.2424), 163.1476 (calcd for $C_{12}H_{25}$, 163.1485), 69.0712 (calcd for C_5H_9, 69.0704); ^{13}C NMR and ^{1}H NMR, see Table 1. On alkaline hydrolysis, **3** yielded a free alcohol (**1**).

Euphol acetate (7): ^{13}C (125 MHz) and ^{1}H (500 MHz) NMR C-1 [δ_C 35.0; δ_H 1.31(α), 1.77(*β*)], C-2 [24.3; 1.73(α), 1.60(*β*)], C-3 [81.0; 4.50, dd, *J* = 4.6, 11.9 Hz], C-4 [37.9], C-5 [51.1; 1.22, dd, *J* = 1.5, 12.5 Hz], C-6 [18.8; 1.67(α), 1.43(*β*)], C-7 [27.6; 1.93(α), 2.07(*β*)], C-8 [133.9], C-9 [133.7], C-10 [37.2], C-11 [21.5; 2.03(α), 1.94-(*β*)], C-12 [30.9; 1.71 (2H)], C-13 [44.2], C-14 [50.0], C-15 [29.8; 1.53(α), 1.19(*β*)], C-16 [28.1; 1.33(α), 1.88(*β*)], C-17 [49.7; 1.52], C-18 [15.6; 0.75, s], C-19 [20.2; 0.98, s], C-20 [35.9; 1.47], C-21 [18.9; 0.86, d, *J* = 6.4 Hz], C-22 [35.5; 1.11, 1.59], C-23 [24.7; 1.87, 2.03], C-24 [125.2; 5.09, tt, *J* = 1.5, 7.3 Hz], C-25 [130.9], C-26 [25.7; 1.68, br s], C-27 [17.7; 1.61, br s], C-28 [28.0; 0.88, s], C-29 [16.6; 0.87, s], C-30 [24.5; 0.87], O*C*OMe [171.0], OCO*Me* [21.3; 2.05, s]. Significant NOE correlations observed were between [H-30(14*β*-Me)−H-17−H-21], [H-21−H-16α,*β*], [H-16α−H-18(13α-Me)−H-20], [H-30(14*β*-Me)−H-19(9*β*-Me)−H-29(4*β*-Me)], and [H-3−H-28(4α-Me)−H-5].

Tirucallol acetate (8): ^{13}C (125 MHz) and ^{1}H (500 MHz) NMR C-1 [δ_C 35.0; δ_H 1.32(α), 1.78(*β*)], C-2 [24.3; 1.73(α), 1.60(*β*)], C-3 [81.0; 4.50, dd, *J* = 4.4, 11.5 Hz], C-4 [37.9], C-5 [51.1; 1.22, dd, *J* = 1.5, 12.5 Hz], C-6 [18.8; 1.67(α), 1.43(*β*)], C-7 [27.6; 1.94(α), 2.07(*β*)], C-8 [133.7], C-9 [133.9], C-10 [37.2], C-11 [21.5; 2.03(α), 1.93(*β*)], C-12 [30.8; 1.70 (2H)], C-13 [44.1], C-14 [50.0], C-15 [29.9; 1.53(α), 1.19(*β*)], C-16 [28.1; 1.33(α), 1.92-(*β*)], C-17 [50.2; 1.49], C-18 [15.4; 0.75, s], C-19 [20.2; 0.98, s], C-20 [36.3; 1.42], C-21 [18.7; 0.92, d, *J* = 6.3 Hz], C-22 [36.4; 1.06, 1.44], C-23 [25.0; 1.86, 2.03], C-24 [125.3; 5.09, tt, *J* = 1.5, 7.0 Hz], C-25 [130.9], C-26 [25.7; 1.68, br s], C-27 [17.6; 1.60, br s], C-28 [28.0; 0.88, s],

C-29 [16.6; 0.87, s], C-30 [24.4; 0.87], O*C*OMe [171.0], OCO*Me* [21.3; 2.05, s]. The following diagnostic NOE correlations were observed between [H-30(14β-Me)−H-17−H-21], [H-21−H-12α], [H-18(13α-Me)−H-20], [H-30-(14β-Me)−H-19(9β-Me)−H-29(4β-Me)], and [H-3−H-28-(4α-Me)−H-5].

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(8) Specific rotations of helianol (**2**) and its acetate (**4**) were determined to be $[\alpha]^{25}_{D}$ +11.7° (*c* 0.6, $CHCl_3$) and +14.3° (*c* 0.9, $CHCl_3$), respectively. Our previous assignment of the ^{13}C and ^{1}H NMR signals for **2** [7] should be revised to be C-1 [δ_C 26.3; δ_H 1.33, 1.56] and C-2 [δ_C 32.1; δ_H 1.55 (2H)].
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