Phytochemistry, Vol. 27, No. 9, pp. 2915–2920, 1988.
Printed in Great Britain.

0031–9422/88 $3.00 + 0.00

SIX NOVEL SECODAMMARANE-TYPE TRITERPENES FROM MALE FLOWERS OF *ALNUS JAPONICA*

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(*Revised received* 20 *December* 1987)

Key Word Index—*Alnus japonica*; Betulaceae; male flowers; secodammarane-type triterpenic acids.

Abstract—Six novel secodammarane-type triterpenic acids were isolated from the male flowers of *Alnus japonica*. On the basis of their physicochemical and spectral data, these were characterized as methyl (24*E*)-3,4-secodammara-4(28),20,24-trien-26-oic acid-3-oate, (24*E*)-3,4-secodammara-4(28),20,24-trien-3,26-dioic acid, (20*S*,24*S*)-20,24-dihydroxy-3,4-secodammara-4(28),25-dien-3-oic acid, (23*E*)-(20*S*)-20,25-dihydroxy-3,4-secodammara-4(28),23-dien-3-oic acid, (23*E*)-(20*S*)-20,25,26-trihydroxy-3,4-secodammara-4(28),23-dien-3-oic acid and (23*E*)-(12*R*,20*S*)-12,20,25-trihydroxy-3,4-secodammara-4(28),23-dien-3-oic acid.

INTRODUCTION

Previous studies on the chemical constituents of the female and male flowers of *Alnus* species, such as *A. serrulatoides* Call., *A. sieboldiana* Matsum. and *A. pendula* Matsum., have shown the presence of C_{31}-dammarane-type triterpenes, such as alnuserol [1], alnuselide [2], alnuseric acid [2], alnuserrudiolone [3], alnuserrutriol [4], alnustic acid [5–8], 12-deoxyalnustic acid [9] and 12-*O*-monoglycosides [6–8] of alnustic acid, in addition to flavonoids [10], diarylheptanoids [11–13], and their glycosides [14], and other aromatic compounds [10, 15]. For the systematic comparison of the chemical constituents of *Alnus* species, we investigated triterpenoid constituents of the male flowers of *A. japonica* (Japanese name: Hannoki). In this plant, the isolation of cyclic biarylheptanoids from the wood [16], a flavone glucoside from the pollen [17] and flavones from the buds [18] has already been reported. Our investigation on the chemical constituents of the male flowers of the present species resulted in the isolation of six novel C_{30}-secodammarane-type triterpenic acids. In this paper, we report the evidence which led to the establishment of their structures.

RESULTS AND DISCUSSION

Centrifugal and TLC separations of an acetone extract of male flowers gave two compounds **1** and **2** and an additional fraction which appeared to be a mixture of several compounds. Compounds **1** and **2** were suggested to be triterpenic acids on the basis of their IR and ^{1}H NMR spectra, as described below. The additional fraction, on spraying with *p*-Bromocresol Green solution, gave a yellow spot and showed analogous behaviour to alnustic acid [5, 6] on TLC. Thus, this fraction was also thought to be composed of triterpenic acids. However, after methylation with diazomethane, it was quite different from Me alnustate (**11**) [5, 6] on TLC. These observations suggested the presence of new triterpenic acids. Acetylation of the methylated fraction, which showed the IR absorption bands due to hydroxyl groups, gave three Me ester acetates (**7**), (**9**) and (**10**) and an unaltered Me ester (**8**). These derivatives (**7**–**10**), on saponification, gave spots with R_f values corresponding to those of the compounds in the triterpenic acid fraction on TLC. Thus, the triterpenic acid fraction was found to be composed of four triterpenic acids (**3**–**6**). Compounds **1** and **2** and the compounds **7**–**10**, after methylation and acetylation of compounds **3**–**6**, are numbered in order of increasing polarity on TLC.

Compound **10**

The CI mass spectrum of compound **10** gave $[M-H]^+$, $[(M+H)-H_2O]^+$ and $[(M+H)-2H_2O]^+$ ion peaks at *m/z* 545, 529 and 511, respectively, indicating a M_r of 546. This was supported by the appearance of EI mass spectral fragment ions at *m/z* 468 ($[M-AcOH-H_2O]^+$) and *m/z* 450 ($[M-AcOH-2H_2O]^+$), which also indicated the presence of an acetoxyl and two hydroxyl groups. The presence of these groups was confirmed by IR and ^{1}H NMR spectra, which also revealed the presence of a Me ester moiety, a terminal methylene group and a disubstituted double bond. Comparison of the ^{13}C NMR chemical shifts (Table 1) of compound **10** with those of Me alnustate (**11**) [5, 6], Me acetoxy alnustate (**12**) and chikusetsusaponin-III [19] indicated that **10** possesses the same 3,4-secodammarane-skeleton having the acetoxyl group on C-12 (δ_C 74.4) with an *R*-configuration [5, 6] as **12** and the same acyclic side chain having one tertiary hydroxyl group located on C-20 (δ_C 73.6) with an *S*-configuration [5, 6], another tertiary hydroxyl group on C-25 (δ_C 69.7), and a *trans*-disubstituted double bond (δ_C 123.0 and 142.2 due to C-23 and C-

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Table 1. ^{13}C NMR chemical shifts for compounds **1**, **2** and **17** (δ_C, in $CDCl_3$) and for compounds **7**–**12** (δ_C, in C_5D_5N)

C	1	2	17	7	8	9	10	11	12
1	34.1	34.5	34.1	35.0	35.0	35.1	34.7	34.9*	34.5
2	28.4	28.5	28.4	28.5	28.5	28.6	28.4	28.8	28.7
3	174.2	180.7	174.3	174.1	174.1	174.3	174.0	174.1	173.7
4	147.2	147.5	147.3	147.7	147.7	147.9	147.4	147.6	147.2
5	50.8	51.0	50.9	51.0	50.9	51.0	50.4	50.6*	50.3
6	24.8	24.7	24.7	24.9	24.9	25.0	24.8	24.9*	24.7
7	34.4	34.1	34.5	34.0	34.1	34.1	33.3	33.7	33.2
8	40.1	40.2	40.1	39.2	39.2	39.3	39.5	39.6	39.2
9	41.1	41.4	41.2	41.1	41.1	41.2	40.8	40.8*	40.8
10	39.1	39.2	39.1	40.1	40.1	40.3	39.5	39.6	39.4
11	21.8	21.9	21.8	22.3	22.1	22.3	29.4	32.5	29.2
12	24.8	24.9	24.9	25.0	24.9	25.0	74.4	70.4	74.4
13	45.5	45.4	45.5	42.5	42.3	42.5	45.9	48.5	45.6
14	49.8	49.8	49.8	50.6	50.6	50.7	52.9	52.2	52.9
15	31.4	31.5	31.4	31.6	31.4	31.5	31.4	31.4	31.4
16	28.8	28.7	28.8	27.6	27.9	27.9	27.6	28.3*	28.3
17	47.6	47.8	47.7	50.2	49.9	50.2	52.5	54.7	52.9
18	15.4	15.5	15.5	15.3	15.4	15.5	15.4	15.6	15.3
19	20.0	20.2	20.2	20.3	20.3	20.4	20.2	20.3	20.0
20	151.1	151.1	151.3	73.6	74.1	74.2	73.6	72.7	73.2
21	108.5	108.6	108.3	25.7	26.8	25.7	26.8	27.0	26.1
22	32.7	32.3	32.8	37.5	45.1	45.2	41.1	34.4	35.9
23	27.7	27.6	27.5	27.8	122.0	126.4	123.0	27.0*	26.1
24	144.3	145.0	142.0	78.0	142.5	137.8	142.2	157.1	157.0
25	127.3	127.1	127.7	144.9	69.7	71.3	69.7	34.4	34.3
26	173.1	173.6	168.4	122.6	30.6	71.8	30.7	22.2	22.0
27	11.9	12.1	12.4	18.3	30.6	26.7	30.7	22.2	22.0
28	113.6	113.5	113.6	113.7	113.7	113.7	114.0	114.0	114.0
29	23.2	23.2	23.3	23.3	23.3	23.5	23.4	23.4	23.0
30	15.8	15.8	15.8	16.6	16.6	16.6	17.4	17.0	17.4
31								106.5	106.3
-OMe	51.3		51.5 × 2	51.3	51.3	51.4	51.4	51.4	51.3
-COMe				169.0		170.7	170.7		169.9
-COMe				21.0		20.8	20.8		21.6

*Assignments of these values in ref. [6] were revised as given in this Table.

24, respectively), as that of chikusetsusaponin-III [19]. These results also suggested that the absolute configuration of the 3,4-secodammarane-skeleton in **10** is identical to that of **11** and **12**. This suggestion was confirmed by the agreement of the CD curve of a 12-keto derivative (**13**) derived from compound **10** with that of Me 12-keto alnustate (**14**) derived from Me alnustate (**11**). Thus, the structure of **10** was defined as Me (23*E*)-(12*R*,20*S*)-12-acetoxy-20,25-dihydroxy-3,4-secodammara-4(28),23-dien-3-oate.

Compound **7**

This compound showed a peak due to $[M-H]^+$ at *m/z* 529 in the CI mass spectrum and peaks due to $[M-AcOH]^+$ and $[M-AcOH-H_2O]^+$ ions at *m/z* 470 and 452, respectively, in the EI mass spectrum. These observations indicated that **7** has a M_r of 530 and possesses an acetoxyl and a hydroxyl group. The ^{13}C NMR chemical shifts of C-1–C-21 and the Me carbon in the methoxycarbonyl group of 7 were in fair agreement with those of the corresponding carbon atoms of Me 12-deoxy alnustate (**15**) [9]. Comparison of the chemical shifts of C-22–C-27 in **7** with those of the corresponding carbon atoms of chikusetsusaponin-L_{9bc} [19] indicated that **7** possesses an acyclic side chain having an acetoxyl group on C-24 (δ_C 78.0), a tertiary hydroxyl group on C-20 (δ_C 73.6) with an *S*-configuration [5, 6] and an isopropenyl moiety (δ_C 144.9, 122.6 and 18.3 due to C-25, C-26 and C-27, respectively). These were supported by the appearance of peaks at *m/z* 185, 125 and 107 in the EI mass spectrum.

In order to establish the configuration at C-24, **7** was subjected to saponification to give a secondary allylic alcohol, followed by *p*-bromobenzoylation to give the corresponding *p*-bromobenzoate (**16**). Its CD spectrum exhibited a positive Cotton effect in the 230–250 nm region. Application of the benzoate rule [20] to the spectrum clearly indicated that the configuration at C-24 of **16** is *S*. Consequently, the stereostructure of **7** was defined as Me (20*S*,24*S*)-20-hydroxy-24-acetoxy-3,4-secodammara-4(28),25-diene-3-oate.

Compound **8**

The CI mass spectrum of **8** exhibited a $[M]^+$ peak at *m/z* 488, indicating that its M_r is smaller than that of **10** by 42 mass units. The IR and ^1H NMR spectra of **8** re-

sembled those of **10**, except for the absorption bands and peaks due to the acetoxyl group in **10**. These observations indicated that the **8** is a 12-deacetoxyl derivative of **10**. This was confirmed by comparison of the ^{13}C NMR chemical shifts of **8** with those of **10** (Table 1). The structure of **8** was thus found to be Me (23*E*)-(20*S*)-20,25-dihydroxy-3,4-secodammara-4(28),23-dien-3-oate.

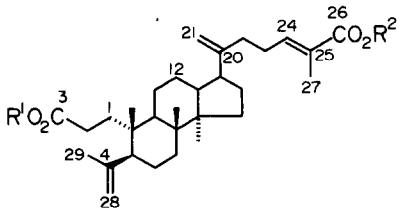
Compound **9**

By a combination of CI mass spectrometry which exhibited a peak at *m/z* 545 ($[M-H]^+$) and EI mass spectrometry which showed peaks at *m/z* 510 ($[M-2H_2O]^+$), 495 ($[M-2H_2O-Me]^+$) and 468 ($[M-H_2O-AcOH]^+$), **9** had a M_r of 546, suggesting that it contained an acetoxyl and two hydroxyl groups. The presence of the primary acetoxyl group was evident from the ^1H NMR spectrum, which also indicated the presence of a methoxycarbonyl group, a terminal methylene group and a disubstituted double bond. Comparison of the ^{13}C NMR chemical shifts of **9** with those of **7**, **8** and **10** indicated that **9** possesses the same 3,4-secodammarane-skeleton as **7** and **8** and an acyclic side chain having the *trans*-disubstituted double bond on C-23, one tertiary hydroxyl group on C-20 with an *S*-configuration [5, 6], another tertiary hydroxyl group on C-25 and the primary acetoxyl group on C-26. From these data, **9** was characterized as Me (23*E*)-(20*S*)-20,25-dihydroxy-26-acetoxy-3,4-secodammara-4(28),23-dien-3-oate.

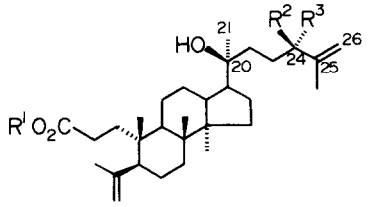
The above Me ester monoacetates (**7**), (**9**) and (**10**) and the Me ester (**8**), on saponification, gave spots with R_f values corresponding to those of the compounds in the triterpenic acid fraction on TLC. Thus, it was unambiguously evidenced that the triterpenic acid fraction was composed of (20*S*, 24*S*)-20,24-dihydroxy-3,4-secodammara-4(28),23-dien-3-oic acid (**3**), (23*E*)-(20*S*)-20,25-dihydroxy-3,4-secodammara-4(28),23-dien-3-oic acid (**4**), (23*E*)-(20*S*)-20,25,26-trihydroxy-3,4-secodammara-4(28), 23-dien-3-oic acid (**5**) and (23*E*)-(12*R*,20*S*)-12,20,25-trihydroxy-3,4-secodammara-4(28),23-dien-3-oic acid (**6**).

Compound **2**

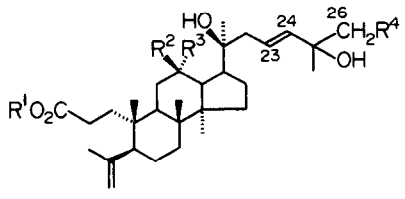
Compound **2** possessed a molecular formula of $C_{30}H_{46}O_4$ on the basis of elemental analysis and the appearance of the $[M]^+$ peak at *m/z* 470.3383 in the high resolution mass spectrum. Furthermore, IR and ^1H NMR spectra suggested the presence of two carboxyl groups. This suggestion was supported by the fact that **2**, on methylation with diazomethane, gave a diMe ester (**17**). On comparison of the ^{13}C NMR chemical shifts (Table 1) of compounds **2** and **17** with those of **7**–**10**, **2** was found



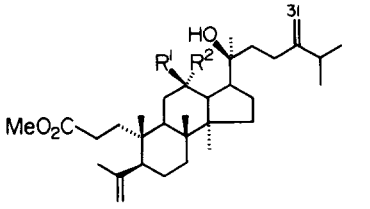
1 R^1=Me, R^2=H
2 R^1=R^2=H
17 R^1=R^2=Me



3 R^1=R^2=H, R^3=OH
7 R^1=Me, R^2=H, R^3=OAc
16 R^1=Me, R^2=H, R^3=OBz-*p*Br



4 R^1=R^2=R^3=R^4=H
5 R^1=R^2=R^3=H, R^4=OH
6 R^1=R^3=R^4=H, R^2=OH
8 R^1=Me, R^2=R^3=R^4=H
9 R^1=Me, R^2=R^3=H, R^4=OAc
10 R^1=Me, R^2=OAc, R^3=R^4=H
13 R^1=Me, R^2,R^3=O, R^4=H



11 R^1=OH, R^2=H
12 R^1=OAc, R^2=H
14 R^1, R^2=O
15 R^1=R^2=H

to possess the same 3,4-secodammarane-skeleton as the other compounds **7**–**9** obtained from the same plant, in addition to an acyclic side chain having a terminal methylene moiety (δ_C 151.1 and 108.6 due to C-20 and C-21, respectively) and a double bond (δ_C 145.0 and 127.1 due to C-24 and C-25, respectively) with both a carboxyl (δ_C 173.6 due to C-26) and a Me group (δ_C 12.1 due to C-27). These assignments were confirmed by a combination of an INEPT spectrum, direct and long-range $^1H/^{13}C$ heteronuclear chemical shift correlation spectra (Table 2). The spatial arrangement of the double bond at C-24 was elucidated to be *E* on the basis of the ^{13}C and ^1H NMR chemical shifts of the Me carbon of C-27 [21] and the olefinic proton at C-24 [22], respectively, and the decoupling experiments at 500 MHz exhibiting the olefinic proton to be coupled to the Me protons at C-27 and to two protons at C-23. Therefore, the structure of compound **2** was elucidated as (24*E*)-3,4-secodammara-4(28),20,24-trien-3,26-dioic acid.

Compound **1**

The high resolution mass spectrum and elemental analysis indicated that **1** possesses the molecular formula of $C_{31}H_{48}O_4$ and that its M_r is larger than that of **2** by 14 mass units. Comparison of the IR, ^1H NMR and ^{13}C NMR (Table 1) spectra of **1** with those of **2** clearly indicated that **1** is the 3-Me ester of **2**. Consequently, the structure of **1** was defined as Me (24*E*)-3,4-secodammara-4(28),20,24-triene-26-oic acid-3-oate.

Table 2. Long-range couplings observed in the long-range $^1H/^{13}C$ heteronuclear chemical shift correlation spectrum of compound **2**

H	δ_H	Long-range coupling to carbon (δ_C)
1	1.60	2 (28.5), 3 (180.7), 10 (39.2), 19 (20.2)
2	2.22	3 (180.7)
	2.41	3 (180.7)
5	1.95	4 (147.5), 6 (24.7), 10 (39.2), 19 (20.2), 29 (23.2)
6	1.38	7 (34.1)
	1.95	5 (51.0), 7 (34.1)
7	1.20	6 (24.7), 8 (40.2), 18 (15.5)
	1.58	6 (24.7), 8 (40.2), 18 (15.5)
9	1.50	8 (40.2), 10 (39.2), 11 (21.9), 18 (15.5), 19 (20.2)
11	1.23	9 (41.4), 12 (24.9)
	1.45	9 (41.4), 12 (24.9)
12	1.05	13 (45.4)
	1.55	11 (21.9), 13 (45.4)
13	1.62	12 (24.9), 14 (49.8), 17 (47.8), 20 (151.1)
15	1.10	14 (49.8), 16 (28.7)
	1.55	14 (49.8), 16 (28.7)
16	1.40	15 (31.5)
	1.92	17 (47.8)
17	2.22	20 (151.1)
18	1.02	7 (34.1), 8 (40.2), 9 (41.4), 14 (49.8)
19	0.86	1 (34.5), 5 (51.0), 9 (41.4), 10 (39.2)
21	4.72	17 (47.8), 22 (32.3)
	4.81	17 (47.8), 22 (32.3)
22	2.10	17 (47.8), 20 (151.1), 21 (108.6), 23 (27.6), 24 (145.0)
23	2.41	20 (151.1), 22 (32.3), 24 (145.0), 25 (127.1)
24	6.89	26 (173.6), 27 (12.1)
27	1.84	24 (145.0), 25 (127.1), 26 (173.6)
28	4.67	5 (51.0), 29 (23.2)
	4.86	5 (51.0), 29 (23.2)
29	1.72	4 (147.5), 5 (51.0), 28 (113.5)
30	0.88	8 (40.2), 13 (45.4), 14 (49.8), 15 (31.5)

It was thus established that the presence of C_{30}-secodammarane-type triterpenes is characteristic of the male flowers of *A. japonica*, in contrast to the presence of only C_{31}-secodammarane-type triterpenes in the female and male flowers of other *Alnus* species, such as *A. serrulatoides*, *A. sieboldiana* and *A. pendula*.

EXPERIMENTAL

Solvents used for spectral measurements were: EtOH (UV); MeOH (CD); $CHCl_3$ ($[\alpha]_D$) and TMS–$CDCl_3$ [^1H NMR (60 MHz) and ^{13}C NMR (22.6 MHz)] unless otherwise stated. Decoupling expts on compound **2** were carried out at 500 MHz. INEPT and 2D NMR expts on compound **2** were performed at 400 MHz. CIMS were recorded using *iso*-butane as reagent gas. EIMS and HR MS were at 70 eV. Silica gel (230–400 mesh) was used for flash chromatography [23]. Analytical and prep. TLC were carried out on silica gel GF_{254} plates (0.25 and 0.75 mm). Compounds were visualized by spraying with vanillin–H_2SO_4 (1:134 w/w) and heating or by spraying with 0.3% *p*-Bromocresol Green soln in H_2O–MeOH (1:4) adjusted to pH 8.

Extraction and isolation. Male flowers (878 g) of *A. japonica* Steud. grown in the campus of Hiroshima University were collected in December. They were minced mechanically and then immersed in Me_2CO at room temp for 2 months. Removal of solvent gave a brown, viscous oil (31.8 g). This oil was partitioned between $CHCl_3$ and H_2O. On removal of solvent, a brown, viscous oil (19.3 g) was obtained from the $CHCl_3$ layer. The crude oil was subjected to centrifugal chromatography on a silica gel disc (5 mm thick, 30 cm diam) using an Me_2CO–$CHCl_3$ gradient and divided into 3 fractions: (i) a fraction of a mono. Me ester of a dicarboxylic acid (**1**) (0.15 g), (ii) a fraction of a dicarboxylic acid (**2**) (2.43 g) and (iii) a fraction of a mixt of triterpenic acids (0.87 g). These fractions gave spots with R_f 0.63, 0.28 and 0.16, respectively, on TLC with $CHCl_3$–MeOH (19:1). Fraction (iii), after methylation with CH_2N_2 and acetylation with Ac_2O–pyridine, was separated into four bands (R_f 0.72, 0.39, 0.28 and 0.17) on prep. TLC with EtOAc–hexane (2:3); the isolated bands after elution with $CHCl_3$, gave Me ester acetate (**7**) (49 mg), Me ester (**8**) (28 mg), Me ester acetate (**9**) (62 mg) and Me ester acetate (**10**) (53 mg), respectively. Each of compounds **7**–**10**, on saponification with 5% NaOH–MeOH, gave a spot with R_f value corresponding to that of the compound in the triterpenic acid fraction on TLC.

Compound **1**. Oil; $[\alpha]_D^{25}$ +61.2° (*c* 0.72); UV λ_{max} nm (log ε): 217 (4.16); IR ν_{max}^{film} cm^{-1}: 3600–2300 (COOH), 3080, 1641 and 895 (C=C), 1740 and 1690 (C=O); ^1H NMR: δ 0.86, 0.89 and 1.03 (9H, *s*, Me × 3), 1.74 and 1.85 [6H, each *s*, >C=C(Me)– × 2], 3.66 (3H, *s*, –COOMe), 4.70, 4.74 and 4.82 (4H, *br*, >C=CH_2 × 2), 6.93 (1H, *t*, *J* = 6 Hz, –CH_2C<u>H</u>=C<), 9.07 (1H, *br*, –COOH); ^{13}C NMR: see Table 1; EIMS *m/z* (rel. int.): 484 (3, $[M]^+$), 469 (5, $[M-Me]^+$), 466(2), 397(5), 385(10); HR MS *m/z* (rel. int.): 484.3509 {(45, $[M]^+$), $C_{31}H_{48}O_4$ requires *m/z* 484.3552}, 469.3313 {(14, $[M-Me]^+$), $C_{30}H_{45}O_4$ requires *m/z* 469.3317}, 385.3061 {(23, $[M-CH_2CH=C(Me)COOH]^+$), $C_{26}H_{41}O_2$ requires *m/z* 385.3106}. (Found: C, 76.54; H, 10.28. $C_{31}H_{48}O_4$ requires: C, 76.81; H, 9.98%).

Compound **2**. Colourless needles (from EtOAc–hexane); mp 168.5–169.5°; $[\alpha]_D^{25}$ +44.0° (*c* 0.45); UV λ_{max} nm (log ε): 216 (4.00); IR ν_{max}^{KBr} cm^{-1}: 3700–2200 (COOH), 1700 and 1685 (C=O), 1638 and 890 (C=C); ^1H NMR: δ 0.89 × 2 and 1.03 (9H, *s*, Me × 3), 1.74 and 1.84 (6H, each *s*, >C=C(Me)– × 2), 4.73–4.81 (4H, *br*,

$>C=CH_2 \times 2$), 6.92 (1H, *t*, $J=6$ Hz, $-CH_2CH=C<$), 9.12 (2H, *br*, $-COOH \times 2$); ^{13}C NMR: see Table 1; EIMS *m/z* (rel. int.): 470 (3, $[M]^+$), 455 (2, $[M-Me]^+$), 452 (3, $[M-H_2O]^+$), 424 (5, $[M-HCO_2H]^+$), 397 (3), 371 (6), 353 (10); HR MS *m/z* (rel. int.): 470.3383 {(66, $[M]^+$), $C_{30}H_{46}O_4$ requires *m/z* 470.3395}, 455.3167 {(81, $[M-Me]^+$), $C_{29}H_{43}O_4$ requires *m/z* 455.3161}, 452.3258 {(23, $[M-H_2O]^+$), $C_{30}H_{44}O_3$ requires *m/z* 452.3290}, 424.3356 {(20, $[M-HCO_2H]^+$), $C_{29}H_{44}O_2$ requires *m/z* 424.3341}, 397.3142 {(18, $[M-C_2H_4CO_2H]^+$), $C_{27}H_{41}O_2$ requires *m/z* 397.3106}. (Found: C, 76.59; H, 10.04. $C_{30}H_{46}O_4$ requires: C, 76.55; H, 9.85%).

On methylation with CH_2N_2, **2** gave a diMe ester (**17**) as an oil. $[\alpha]_D^{25}$ +64.0° (*c* 0.75); UV λ_{max}nm (log ε): 217 (4.18); IR ν_{max}^{film} cm^{-1}: 3080, 1640 and 888 (C=C), 1740 and 1707 (C=O); ^1H NMR: δ 0.87, 0.90 and 1.03 (9H, *s*, Me × 3), 1.75 and 1.86 [6H, each *s*, $>C=C(Me)- \times 2$], 3.67 and 3.75 (6H, each *s*, –COOMe × 2), 4.72, 4.75 and 4.82 (4H, *br*, $>C=CH_2 \times 2$), 6.78 (1H, *t*, $J=6$ Hz, $-CH_2CH=C<$); ^{13}C NMR: see Table 1; EIMS *m/z* (rel. int.): 498 (4, $[M]^+$), 483 (2, $[M-Me]^+$), 466 (3, $[M-MeOH]^+$), 411 (4), 385 (7). (Found: C, 77.15; H, 10.22. $C_{32}H_{50}O_4$ requires: C, 77.06; H, 10.11%).

Compound **7**. Oil; $[\alpha]_D^{25}$ +35.6° (*c* 0.59): IR ν_{max}^{film} cm^{-1}: 3500 (OH), 3075, 1640 and 890 (C=C), 1735 (C=O); ^1H NMR: δ 0.85, 0.89, 1.00 and 1.13 (12H, *s*, Me × 4), 1.73 [6H, *s*, $>C=C(Me)- \times 2$], 2.06 (3H, *s*, –OCOMe), 3.66 (3H, *s*, –COOMe), 4.68 and 4.92 (4H, *br*, $>C=CH_2 \times 2$), 5.16 (1H, *t*, $J=6$ Hz, $>CHOAc$); ^{13}C NMR (C_5D_5N): see Table 1; EIMS *m/z* (rel. int.): 470 (0.2, $[M-AcOH]^+$), 455 (0.4, $[M-HOAc-Me]^+$), 452 (0.4, $[M-HOAc-H_2O]^+$), 389 (1), 371 (3), 185 (6), 125 (100), 107 (29); CIMS *m/z* (rel. int.): 529 (2, $[M-H]^+$), 471 (15), 453 (100).

p-*Bromobenzoylation of* **7**. **7** (21 mg) was refluxed with 5% KOH–MeOH for 1 hr, followed by methylation with CH_2N_2, to give a 24-hydroxy, 3-Me ester derivative which was dissolved in a mixture of dry benzene (1.5 ml) and dry pyridine (0.2 ml), followed by addition of *p*-bromobenzoyl chloride (110 mg). The resulting mixt was refluxed for 0.5 hr and worked-up as usual to afford an oil, which was purified on prep. TLC with EtOAc–hexane (27:73) to yield a *p*-bromobenzoate derivative (**16**) (26 mg): $[\alpha]_D^{25}$ +26.5° (*c* 0.17); UV λ_{max}nm (log ε): 205 (4.14), 245 (4.14); CD $\Delta\varepsilon_{243}$ +0.59 (*c* 0.01); IR ν_{max}^{film} cm^{-1}: 3530 (OH), 3080 and 1650 (C=C), 1630 and 1585 (Ar C=C); ^1H NMR: δ 0.83, 0.88, 0.98 and 1.15 (12H, *s*, Me × 4), 1.73 and 1.80 [6H, each *s*, $>C=C(Me)- \times 2$], 3.67 (3H, *s*, –COOMe), 4.68–4.85 and 4.95–5.04 (4H, each *br*, $>C=CH_2 \times 2$), 5.42 (1H, *t*, $J=6$ Hz, $>CHOCO-$), 7.51–8.01 (4H, AA'BB'-type, $J=8$ and 2 Hz, ArH); EIMS *m/z* (rel. int.): 672 and 670 (0.1 and 0.2, respectively, $[M]^+$), 654 and 652 (0.4 and 0.5, respectively, $[M-H_2O]^+$), 389 (4), 371 (30), 43 (100).

Compound **8**. Oil; $[\alpha]_D^{25}$ +30.2° (*c* 0.40); IR ν_{max}^{film} cm^{-1}: 3425 (OH), 3070, 1630 and 890 (C=C), 1730 (C=O); ^1H NMR (C_5D_5N): δ 0.82, 0.91, 0.98, 1.38 and 1.50 × 2 (18H, *s*, Me × 6), 1.75 [3H, *s*, $>C=C(Me)-$], 3.65 (3H, *s*, –COOMe), 4.80 and 4.92 (2H, *br*, $>C=CH_2$), 5.89 (1H, *d*, $J=16$ Hz, $-CH_2CH=CH-$), 6.16 (1H, *m*, $-CH_2CH=CH-$: appeared as doublet ($J=16$ Hz) on irradiation at δ_H 2.35); ^{13}C NMR (C_5D_5N): see Table 1; EIMS *m/z* (rel. int.): 470 (3, $[M-H_2O]^+$), 452 (5, $[M-2H_2O]^+$), 437 (1, $[M-2H_2O-Me]^+$), 389 (74), 371 (46), 345 (29), 143 (2), 125 (9), 107 (15), 82 (100); CIMS *m/z* (rel. int.): 489 (2, $[M+H]^+$), 488 (7, $[M]^+$), 487 (5, $[M-H]^+$).

Compound **9**. Oil; $[\alpha]_D^{25}$ +25.2° (*c* 0.95); IR ν_{max}^{film} cm^{-1}: 3465 (OH), 3080, 1637 and 894 (C=C), 1738 (C=O); ^1H NMR (C_5D_5N): δ 0.82, 0.90, 0.98, 1.36 and 1.51 (15H, *s*, Me × 5), 1.75 [3H, *s*, $>C=C(Me)-$], 2.00 (3H, *s*, –OCOMe), 3.65 (3H, *s*, –COOMe), 4.30 (2H, *s*, $-CH_2OAc$), 4.81 and 4.92 (2H, *br*, $>C=CH_2$), 5.86 (1H, *d*, $J=16$ Hz, $-CH_2CH=CH-$), and 6.23 [1H, *m*, $-CH_2CH=CH-$: appeared as doublet ($J=16$ Hz) on irradiation at δ_H 2.35]; ^{13}C NMR (C_5D_5N): see Table 1; EIMS *m/z* (rel. int.): 510 (0.1, $[M-2H_2O]^+$), 495 (0.1, $[M-2H_2O-Me]^+$), 468 (0.7, $[M-H_2O-HOAc]^+$), 389 (4), 371 (3), 345 (2), 140 (100), 80 (52); CIMS *m/z* (rel. int.): 545 (3, $[M-H]^+$).

Compound **10**. Oil; $[\alpha]_D^{25}$ +13.8° (*c* 0.85); IR ν_{max}^{film} cm^{-1}: 3515 (OH), 3065, 1632 and 895 (C=C), 1727 (C=O); ^1H NMR (C_5D_5N): δ 0.85, 0.94, 1.05, 1.35 and 1.53 × 2 (18H, *s*, Me × 6), 1.75 [3H, *s*, $>C=C(Me)-$], 2.10 (3H, *s*, –OCOMe), 3.62 (3H, *s*, –COOMe), 4.80 and 4.92 (3H, *br*, $>C=CH_2$ and $>CHOAc$), 5.93 (1H, *d*, $J=16$ Hz, $-CH_2CH=CH-$), 6.27 [1H, *m*, $-CH_2CH=CH-$: appeared as doublet ($J=16$ Hz) on irradiation at δ_H 2.35]; ^{13}C NMR (C_5D_5N): see Table 1; EIMS *m/z* (rel. int.): 468 (0.5, $[M-HOAc-H_2O]^+$), 450 (2, $[M-HOAc-2H_2O]^+$), 447 (1), 387 (40), 369 (12), 343 (3), 125 (11), 107 (50), 82 (68); CIMS *m/z* (rel. int.): 545 (2, $[M-H]^+$).

12-*Keto derivative* (**13**) *of compound* **10**. **10** (20 mg) was saponified with 5%KOH–MeOH, followed by methylation with CH_2N_2 and purification on prep. TLC with $CHCl_3$–MeOH (19:1), to give a 12-hydroxy, 3-Me ester (8 mg), which was dissolved in CH_2Cl_2 (2.5 ml), followed by addition of pyridinium chlorochromate (PCC, 7.1 mg). The mixt was stirred for 1 hr at room temp to give a 12-keto derivative (**13**) (1 mg) as an oil: $[\alpha]_D^{25}$ +26.8° (*c* 0.02); CD $\Delta\varepsilon_{282}$ −2.79 (*c* 0.02); IR ν_{max}^{film} cm^{-1}: 3400 (OH), 3080, 1635 and 890 (C=C), 1735 (CO_2Me), 1715 (C=O); ^1H NMR ($CDCl_3$): δ 0.85–1.05 (12H, Me × 4), 1.28 (6H, Me × 2), 1.75 [3H, *s*, $>C=C(Me)-$], 3.68 (3H, *s*, COOMe), 4.60–4.90 (2H, *br*, $>C=CH_2$), 5.55–5.75 (2H, *m*, –CH=CH–); CIMS *m/z* (rel. int.): 501 (0.2, $[M-H]^+$), 485 (0.5, $[(M+H)-H_2O]^+$), 403 (100).

Methyl 12-keto alnustate (**14**) *from* **11**. PCC (39 mg) was added to a soln of **11** (40 mg) in CH_2Cl_2 (5 ml), followed by stirring for 1 hr, to yield **14** (26 mg) as an oil: $[\alpha]_D^{25}$ +40.4° (*c* 0.23); CD $\Delta\varepsilon_{283}$ −2.72 (*c* 0.03); IR ν_{max}^{film} cm^{-1}: 3430 (OH), 3080, 1638 and 890 (C=C), 1735 (CO_2Me), 1705 (C=O); ^1H NMR: δ 0.82, 0.94, 0.98, 1.10, 1.13 and 1.23 (18H, Me × 6), 1.76 [3H, *s*, $>C=C(Me)-$], 3.67 (3H, *s*, COOMe), 4.72 and 4.90 [4H, *br*, $>C=CH_2 \times 2$]; EIMS *m/z* (rel. int.): 500 (2, $[M]^+$), 485 (6), 482 (6), 141 (36), 124 (100), 123 (44).

Acknowledgements—The authors thank Dr Teruo Yasui, Mr Masaya Oka and Dr Shigetoshi Amiya of Central Research Laboratories of Kuraray Co. Ltd, for high resolution MS measurements and for the decoupling experiments, and JEOL Co. Ltd for the INEPT and 2D NMR measurements. We also express our appreciation to the Research Institute for Nuclear Medicine and Biology of Hiroshima University, for the use of the EIMS spectrometer.

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