[Chem. Pharm. Bull. 36(10)3967—3973(1988)]

Anti-inflammatory Constituents of Topically Applied Crude Drugs. II.[1] Constituents and Anti-inflammatory Effect of *Cryptomeria japonica* D. DON[2]

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(Received March 11, 1988)

The ether-soluble fraction of MeOH extract from the leaves of *Cryptomeria japonica* D. DON showed an anti-inflammatory effect when topically applied to rats and an inhibitory effect on histamine-induced ileum contraction. The active component was isolated and identified as *cis*-communic acid (**1**). Two new diterpenoids were also isolated along with two known compounds and were determined to be imbricataloic acid dimethyl acetal (**5**) and (*E*)-13-hydroxylabda-8(17),11,14-trien-19-oic acid, obtained as the methyl ester (**6m**), by chemical and spectroscopic methods.

Keywords——*Cryptomeria japonica*; Taxodiaceae; *cis*-communic acid; methyl (*E*)-13-hydroxylabda-8(17),11,14-trien-19-oate; imbricataloic acid dimethyl acetal; anti-inflammatory effect; carrageenan edema; histamine-induced contraction

The leaves of *Cryptomeria japonica* D. DON (Taxodiaceae) have traditionally been used in Japan as a substitute for "Sanyo (杉葉)," which is derived from the leaves of *Cunninghamia lanceolata* (LAMB.) in Chinese medicine, for the treatment of eczema, eruption, swelling, injury, *etc.*, by topical application in most cases. Terpenoids, flavonoids, *etc.*[3] have been isolated from this plant, but no studies in relation to the anti-inflammatory effect which may be expected from the above application have been reported.

In the course of chemical and pharmacological studies on anti-inflammatory active constituents of topically applied plant materials, the hot MeOH extract of the leaves of *Cryptomeria japonica* was found to have activity by using the carrageenan-induced paw edema (CPE) method.[1,4] In this paper, we report the isolation and structural elucidation of chemical constituents, as well as their anti-inflammatory activity on CPE and inhibitory effect on histamine-induced contraction of the ileum isolated from guinea pigs.

The hot MeOH extract (fr. A) was partitioned between Et_2O and H_2O to give the Et_2O-soluble fraction (fr.) (fr. B) and H_2O layer (fr. C). The active fr. B was further fractionated as shown in Chart 1 to afford acidic (fr. D), weak acidic (fr. E) and neutral fractions (fr. F), respectively. In the CPE test with topical application of frs. D—F, fr. E showed a strong inhibitory effect which was found only at the early stage (1—2 h after carrageenan injection) (Table I). Histamine is known to be involved in the CPE test as a chemical mediator at a short time after carrageenan injection. Fractions B and E also showed inhibitory effects on histamine-induced (10^{-7} g/ml) contraction of the ileum isolated from guinea pig (HCl) (Table II). Fraction F showed almost the same effect as fr. A.

These results were in accord with those in the CPE test, and we carried out screening by using the inhibitory effect on HCl to monitor for further effective consituents, since a smaller amount of samples could be used than in the CPE test.

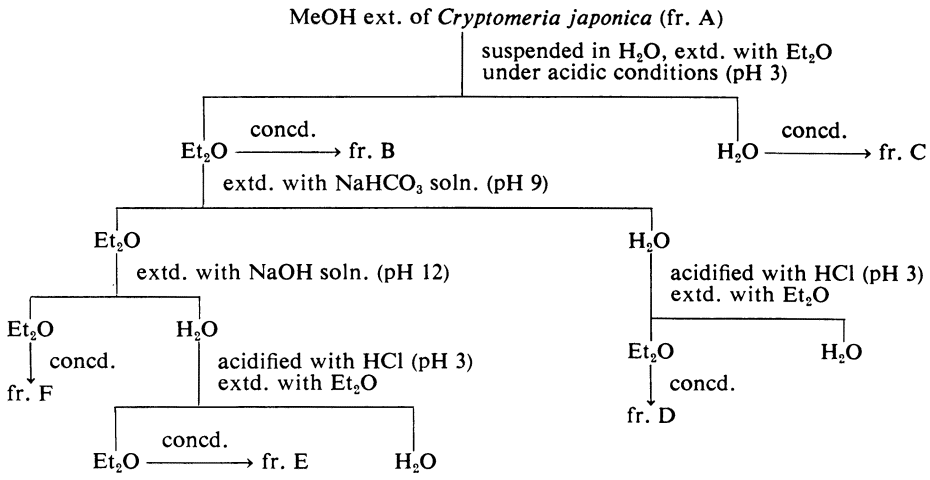

Chart 1

TABLE I. Inhibitory Effects of Frs. A—F and Compounds **1**—**3** on Carrageenan-Induced Paw Edema in Rats[a]

Fraction and compound	Dose (mg/site × 4)	Inhibition % of swelling			
		1	2	3	4[b] (h)
A	5	32.7[c]	26.2	—	—
B	5	31.5[c]	32.5[c]	22.6[c]	15.4
C	5	11.1	—	14.8	—
D	5	—	—	—	—
E	5	33.3[c]	12.7	14.1	—
F	5	16.7	11.1	15.5	—
Indomethacin	0.5	22.6	17.5	32.2[c]	32.2[d]
1	5	41.9[d]	21.1[c]	25.4[c]	22.4[c]
2	5	—	—	—	—
3	5	—	—	—	—

a) $n=4$ or 5. —, no effect (less than 10%). *b*) Time after carrageenan injection. *c*) $p<0.05$, *d*) $p<0.01$.

TABLE II. Inhibitory Effects of Frs. A—F and Compounds on Histamine (10^{-7} g/ml)-Induced Contraction in Guinea Pig Ileum

Fraction	IC_{50} (g/ml)	Compound	IC_{50} (g/ml)
A	2.5×10^{-5}	**1**	4.7×10^{-6}
B	2.0×10^{-5}	**2**	4.8×10^{-5}
C	$>10^{-4}$	**3**	4.2×10^{-5}
D	$>10^{-4}$	**4**	1.6×10^{-5}
E	1.4×10^{-5}	**5**	1.5×10^{-5}
F	2.7×10^{-5}	**1**·Na	3.4×10^{-6}
		Diphenhydramine·HCl	3.3×10^{-8}

The active fr. E was subjected to column chromatography on silica gel with an *n*-hexane-AcOEt system as the eluent to afford compounds **1**—**7** (**6** and **7** were obtained as their methyl esters **6m** and **7m**).

Compound **1**, colorless oil, $[\alpha]_D+38.1°$, $C_{20}H_{30}O_2$, was concluded to be *cis*-communic acid by comparison of the physical and spectral data of its methyl ester (**1a**) and reduced derivative (**1b**) with those given in the literature.[5] **1** readily changed to another compound in a few hours at room temperature[6] but the sodium salt (**1**·Na) was found to be rather stable. **1** could be regenerated from **1**·Na as a colorless powder, mp 108—118 °C (dec.), which appeared to be stable.

Compound **2**, colorless oil, $[\alpha]_D+45.8°$, $C_{20}H_{34}O_3$, and **3**, colorless oil, $[\alpha]_D+52.9°$, $C_{20}H_{32}O_3$, were identified as imbricatolic acid[7] and isocupressic acid,[8] respectively, by comparisons of the physical and spectral data with those in the literature and confirmed to be identical with the authentic samples.

Compound **4** was presumed to be a mixture[9] of isopimaric acid (**4a**) and sandaracopimaric acid (**4b**) by comparison of the spectral data and retention time on gas liquid chromatography (GLC) with those of an authentic sample as the methyl ester.

Compound **5** was obtained as a colorless oil, $[\alpha]_D+42.4°$. The high-resolution mass spectral examination of **5** gave the formula $C_{22}H_{38}O_4$. Comparison of the ^{1}H- and ^{13}C-nuclear magnetic resonance (^{1}H- and ^{13}C-NMR) spectra of **5** with those of **2** indicated that **5** is a very similar diterpene to **2**, possessing a labdane skeleton. The ^{1}H-NMR spectrum of **5** showed no signal at δ 3.66 (2H, m), assignable to a hydroxymethyl group in **2**, but gave two signals at δ 4.45 (1H, m) and 3.30 (6H, $-OCH_3\times2$) which were not observed in **2**. The ^{13}C-NMR spectrum of **5** showed three signals at δ 103.2 (d), 52.8 (q) and 52.2 (q) instead to one at δ 61.2 (t, C-15) in methyl imbricatolate (**2a**). The other signals of **2** and **5** were very similar in both spectra. From these spectral correlations, the structure of **5** was determined to be imbricataloic acid dimethyl acetal. This was confirmed by derivation of **5** from **2**.

This is the first report of the natural occurrence of **5**, but the possibility remains that it is an artifact derived from imbricataloic acid, which has been found in nature.[5c,d,10] In general, an aldehyde compound is easily changed to the corresponding acetal in the presence of excess methyl alcohol under acid conditions, and we are now trying to establish whether imbricataloic acid is present or not in this plant.

Compound **6** was obtained as the methyl ester (**6m**), colorless oil, $[\alpha]_D+16°$, $C_{21}H_{32}O_3$. Compound **6m** showed absorption bands at 1715 and 1158 cm^{-1} in the infrared (IR) spectrum and signals at δ 3.63 (3H, s) and 51.2 in the ^{1}H- and ^{13}C-NMR spectra, respectively, due to a carbomethoxy group. Other signals in the ^{1}H-NMR spectrum, two tertiary methyls (δ 0.62 and 1.20), an exomethylene (δ 4.46 and 4.75, each 1H br s) and others, were very similar to those of methyl *cis*-communate (**1a**), imbricatolate (**2a**) and isocupressate (**3a**). In addition, the signal pattern of **6m** in the ^{13}C-NMR spectrum corresponded well to those of **1a**, **2a**, **3a** and methyl *trans*-communate (**8**)[11] except for the signals due to the side chain at C-9 (Table III). These spectral data suggested that **6m** has an 8(17)-labdene skeleton with an axial carbomethoxy group at C-4.

The signals attributed to the side chain portion of **6m** in the ^{1}H- and ^{13}C-NMR spectra demonstrated that **6m** has both mono-substituted and di-substituted double bonds. The splitting pattern of proton signals due to the di-substituted double bond indicated the presence of the 11, 12 double bond with *E*- configuration, which is adjacent to the tertiary carbon (C-13) in **6m**, and was very similar to those of (11*E*, 13*S* and 11*E*, 13*R*)-11,14-labdadiene-8,13-diol (**9** and **10**)[12] (Table IV). Consequently, it was suggested that the (*E*)-3-hydroxy-3-methyl-1,4-pentadienyl group is attached to C-9 as a side chain in **6m**. The configuration of the substituent at the C-13 position was considered to be *R* based on a comparisons of the chemical shifts of C-16 and H-16 in **6m**, **9** and **10** (Table IV), but a further examination is in progress.

On the basis of these data, the structure of **6m** should be represented as methyl (*E*)-13-hydroxylabda-8(17),11,14-trien-19-oate. It was considered to be present in the form of the free

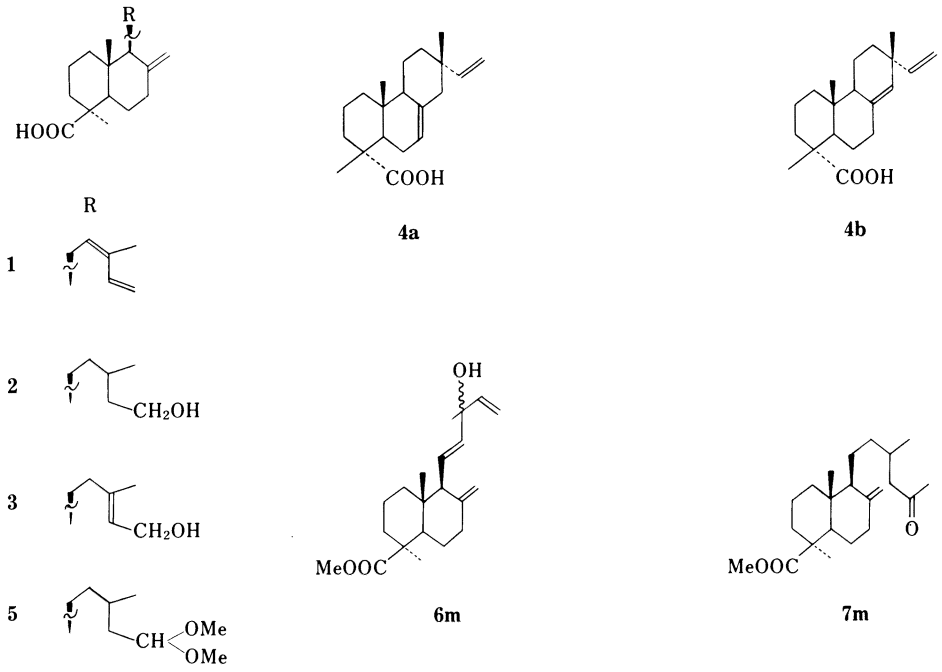

Chart 2

TABLE III. ^{13}C-NMR Chemical Shifts of **1a**, **2a**, **3a**, **5**, **6m** and **8**[a]
δ (ppm) from TMS in $CDCl_3$

Carbon	**8**	**1a**	**2a**	**3a**	**5**	**6m**
1	39.4	39.2	39.2	39.2	39.2	39.4
2	20.1	20.0	20.0	20.0	19.9	19.8
3	38.3	38.2	38.3	38.3	38.0	38.3
4	44.4	44.2	44.3	44.3	44.2	44.2
5	56.4	56.2	56.4	56.3	56.4	55.7
6	26.1	26.0	26.3	26.3	26.1	25.2
7	38.6	38.5	38.8	38.8	38.8	37.3
8	148.0	148.0	148.4	148.1	148.2	149.6
9	56.5	56.6	56.6	55.5	56.6	59.9
10	40.2	40.2	40.3	40.3	40.6	40.8
11	23.3	22.2	21.1	22.0	21.1	126.5
12	133.9	131.5	36.4	38.4	36.4	138.6
13	133.4	131.5	30.2	140.4	29.8	73.3
14	141.6	133.7	39.6	123.1	39.2	144.1
15	109.9	113.2	61.2	59.4	103.2	112.2
16	11.1	19.7	19.8	16.4	20.0	28.2
17	107.6	107.7	106.2	106.4	106.4	107.9
18	28.9	28.8	28.8	28.8	29.0	28.8
19	177.7	177.7	177.8	177.8	184.1	177.8
20	12.7	12.6	12.5	12.6	12.7	13.4
COOMe	51.1	51.1	51.1	51.2	—	51.2
CH(OMe)(OMe)	—	—	—	—	52.2 52.8	—

a) *trans*-Communic acid methyl ester, values from literature.[11]

TABLE IV. ^{1}H- and ^{13}C-NMR Chemical Shifts of Side Chain of **6m**, **9**[a] and **10**[b]

Proton	6m	9	10	Carbon	6m	9	10
11	5.69 dd (15.4, 9.2)	5.7 (overlapping)	5.7 (overlapping)	11	126.5 d	124.2 d	125.0 d
12	5.57 d (15.4)	5.7 (overlapping)	5.7 (overlapping)	12	138.6 d	141.2 d	141.8 d
				13	73.3 s	72.4 s	72.3 s
14	5.98 dd (17.4, 10.6)	5.98 dd (17.5, 10.5)	6.01 dd (17.5, 10.5)	14	144.1 d	144.8 d	144.0 d
15	5.07 dd (10.6, 1.0)	5.04 dd (10.5, 1.5)	5.10 dd (10.5, 1.5)	15	112.2 t	111.9 t	112.4 t
	5.24 dd (17.4, 1.0)	5.22 dd (17.5, 1.5)	5.28 dd (17.5, 1.5)				
16	1.39 s	1.41 s	1.39 s	16	28.2 q	27.2 q	28.5 q

a) (11*E*,13*S*)-11,14-Labdadien-8,13-diol, values from literature.[12] *b*) (11*E*,13*R*)-11,14-Labdadien-8,13-diol, values from literature.[12]

acid **6**, named cryptotrienolic acid, in this plant, because the spot of **6m** was not found on thin layer chromatography (TLC) of fr. E before methylation.

This acid **6** has not been found previously as a natural product.

Compound **7** was obtained as the methyl ester (**7m**), colorless oil, $[\alpha]_D$ +46.1° (c=0.75, $CHCl_3$), $C_{22}H_{36}O_3$. The ^{1}H-NMR spectrum of **7m** resembles that of **2a**, except for the signal at δ 2.12 (3H, s) assigned to a methyl ketone instead of one at δ 3.67 (2H, t) attributed to a hydroxymethyl group in **2a**. Spalding *et al.*[9] isolated a methyl 15-methyl imbricataloate from methylated products of slash pine needle extract which contained imbricatolic acid and imbricataloic acid. Based on these facts and a comparison of the spectral data, **7m** was presumed to be a methyl 15-methyl imbricataloate.

Biological Activities

Inhibitory Effect on CPE in Rats——Compounds **1**—**3** isolated from *C. japonica* in the present study were tested for anti-inflammatory activity after topical application by using the CPE method in rats, as reported previously[1] (Table I). The effects of compounds **4**—**7m** could not be tested owing to their poor yields, but fractions containing **4**—**7m** showed less effect than **1**.

Inhibitory Effect on HCl——The inhibitory effects of **1**—**5** on histamine-induced contraction was assessed in ileum isolated from guinea pig. *cis*-Communic acid showed a stronger effect than the other compounds (Table II). This coincided with the result of the CFE test. These results suggested that the anti-inflammatory effect of fr. E was mainly due to *cis*-communic acid.

Thus, *cis*-communic acid was identified as the major anti-inflammatory constituent of *C. japonica*. This is the first time that a compound having a labdane skeleton has been reported to show an anti-inflammatory effect. Fraction F also showed an anti-inflammatory effect and its constituents will be reported elsewhere.

Experimental

Melting points were determined on a Yanagimoto micromelting point apparatus and are uncorrected. Optical rotations were measured on a JASCO DIP 140 polarimeter in $CHCl_3$. Spectral data were obtained as follows: IR spectra with a Hitachi 260-0611 spectrophotometer; ultraviolet (UV) spectra with a Hitachi 270S spectrophotometer; mass spectrum (MS) with a JEOL JMS-D 200 spectrometer (70 eV); ^{1}H-NMR spectra with Hitachi R-24B (60 MHz) and Varian XL-200 (200 MHz) spectrometers in $CDCl_3$; ^{13}C-NMR spectra with Varian XL-200 (50.3 MHz) and JEOL FX 90Q (22.5 MHz) spectrometers in $CDCl_3$. Chemical shifts are given in δ (ppm) values reffered to internal tetramethylsilane. The following abbreviations are used: s=singlet, d=doublet, t=triplet, q=quartet, m=multiplet and br=broad.

Bioassay——Anti-inflammatory activity by topical application on CPE and inhibitory effect on HCl were assessed as described in the previous report.[1]

Extraction and Fractionation——Dried leaves and stems of *Cryptomeria japonica* D. DON (1 kg; 2.6 kg when fresh) were extracted with hot MeOH to give the MeOH extract (232 g) (fr. A). Fr. A was suspended in water and extracted with Et_2O under acidic conditions (pH 3) to afford the Et_2O-soluble fraction (fr. B) (57 g) and the water-soluble fraction (fr. C).

Fr. B, active in the CPE test, was dissolved in Et_2O and extracted first with $NaHCO_3$ solution (pH 9) and next with NaOH solution (pH 12) followed by extraction with Et_2O again after neutralization of each alkaline solution to afford the acidic fraction (fr. D, 1.8 g) and the weakly acidic fraction (fr. E, 23 g), and the neutral fraction (fr. F, 30 g) was obtained from the alkali-insoluble Et_2O layer.

Constituents of Active Fraction (Fr. E)——Fr. E (16.9 g), showing activity in the CPE test and an inhibitory effect on HCl was chromatographed on silica gel to give fr. 1 (4.9 g), fr. 2 (1.4 g), fr. 3 (2.0 g), fr. 4 (3.7 g) and fr. 5 (4.1 g) from the eluates with *n*-hexane–AcOEt (4 : 1), AcOEt and an AcOEt–MeOH gradient system. Fr. 1, a colorless oil, showed one spot corresponding to compound **1** on TLC developed with *n*-hexane–AcOEt (4 : 1), but was difficult to crystallize. A 1 N NaOH solution was poured into an Et_2O solution (15 ml) of fr. 1 (1.9 g) with stirring until no more precipitation occurred. The precipitate was recrystallized from H_2O–MeOH to yield a sodium carboxylate (**1**·Na) as colorless crystals (1.27 g). **1**·Na (0.3 g) dissolved in a small quantity of MeOH was poured into acidic water (HCl, pH 2—3) with stirring and the precipitate was washed with water and dried to yield *cis*-communic acid (**1**) (0.24 g).

Imbricatolic acid (**2**) and isocupressic acid (**3**) were obtained from fr. 4 (2.17 g) by silica gel column chromatography with *n*-hexane–AcOEt (3 : 2) as the eluent (166 and 377 mg, respectively).

Fr. 2 (0.67 g) gave a mixture of isopimaric acid and sandaracopimaric acid (**4**) (16 mg) and imbricataloic acid dimethyl acetal (**5**) (70 mg).

Fr. 3 (0.34 g) was methylated with CH_2N_2 and chromatographed on silica gel with *n*-hexane–AcOEt (10 : 1) to give a novel compound **6m** (7 mg) and 15-methylimbricataloic acid methyl ester **7m** (2 mg) after further preparative TLC (PLC).

***cis*-Communic Acid (1)**——Colorless oil or colorless powder, mp 108—118 °C (dec.). $[\alpha]_D$ +38.1° (c=1.00, $CHCl_3$). *Anal.* Calcd for $C_{20}H_{30}O_2$: C, 79.4; H, 10.0. Found: C, 79.2; H, 10.0. MS *m/z* (70 eV): 302 (M^+), 287, 257, 221, 175, 161, 147, 135, 121, 105, 93, 81, 69, 55. IR ν_{max}^{KBr} cm^{-1}: 3300—2500, 1695 (COOH), 1645, 1470, 990, 910, 900, 890 (conjugated diene). UV λ_{max}^{EtOH} nm (log ε): 236.5 (2.12). ^{1}H-NMR (60 MHz) δ: 0.60 (3H, s, 10-Me), 1.23 (3H, s, 4-Me), 1.76 (3H, s, 13-Me), 4.48 (1H, br s, 17-H), 4.82 (1H, br s, 17-H), 5.07 (1H, d, J=10 Hz, 15-H), 5.15 (1H, d, J=17 Hz, 15-H), 5.30 (1H, m, 12-H), 6.78 (1H, q, J=10, 17 Hz, 14-H).

Methyl *cis*-Communate (1a)——**1** was methylated with CH_2N_2 to give **1a**. Colorless solid, mp 38 °C, $[\alpha]_D$ +51.6° (c=1.02, $CHCl_3$). MS *m/z*: 316.2393 (M^+, Calcd for $C_{21}H_{32}O_2$: 316.2400). ^{13}C-NMR: Table III.

***cis*-Communol (1b)**——**1a** was reduced with $LiAlH_4$ in the usual way followed by PLC to give **1b**, colorless oil, $[\alpha]_D$ +36.4° (c=0.35, $CHCl_3$). MS *m/z*: 288 (M^+). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3640, 3460, 1015 (OH), 1645, 990, 910 sh, 900 (conjugated diene).

Imbricatolic Acid (2)——Colorless oil, $[\alpha]_D$ +45.8° (c=1.66, $CHCl_3$). MS *m/z*: 322.2518 (M^+, Calcd for $C_{20}H_{34}O_3$: 322.2506), 304, 276, 221, 207, 167, 139, 121, 109, 95, 81, 67, 55. IR ν_{max}^{neat} cm^{-1}: 3400 (OH), 3300—2500, 1695 (COOH), 3090, 1645, 890 (=CH_2). ^{1}H-NMR (60 MHz) δ: 0.59 (3H, s, 10-Me), 1.22 (3H, s, 4-Me), 3.66 (2H, br t, J=6.5 Hz, 15 H), 4.49 (1H, br s, 17-H), 4.82 (1H, br s, 17-H).

Methyl Imbricatolate (2a)——**2** was methylated with CH_2N_2 to afford **2a**. Colorless oil, $[\alpha]_D$ +51.3° (c=1.77, $CHCl_3$). MS *m/z*: 336 (M^+), 321, 304, 276, 221, 181, 161, 121, 107, 95, 81, 69, 55. IR ν_{max}^{neat} cm^{-1}: 3400 (OH), 1725, 1155 (COOMe), 3090, 1645, 890 (=CH_2). ^{1}H-NMR (200 MHz) δ: 0.50 (3H, s, 10-Me), 0.90 (3H, d, J=6.4 Hz, 13-Me), 1.18 (3H, s, 4-Me), 3.62 (3H, s, OMe), 3.67 (2H, t, J=7.0 Hz, 15-H), 4.49 (1H, br s, 17-H), 4.83 (1H, br s, 17-H).

Isocupressic Acid (3)——Colorless oil, $[\alpha]_D$ +52.9° (c=0.94, $CHCl_3$). MS *m/z*: 320 (M^+), 305, 302, 287, 259, 241, 222, 189, 167, 147, 139, 134, 121, 107, 93, 81, 68, 55. IR ν_{max}^{neat} cm^{-1}: 3400 (OH), 3300—2500, 1695 (COOH), 3090, 1645, 890 (=CH_2). ^{1}H-NMR (60 MHz) δ:0.59 (3H, s, 10-Me) 1.22 (3H, s, 4-Me), 1.65 (3H, s, 13-Me), 4.13 (2H, d, J=7 Hz, 15-H), 4.52 (1H, br s, 17-H), 4.85 (1H, br s, 17-H), 5.37 (1H, br t, J=7 Hz, 14-H), 6.0 (2H, br, COOH, OH).

Methyl Isocupressate (3a)——Usual methylation of **3** with CH_2N_2 gave a methyl ester (**3a**) as a colorless oil, $[\alpha]_D$ +51.2° (c=1.73, $CHCl_3$). MS *m/z*: 335 (M^++1), 320, 318, 302, 275, 258, 242, 190, 162, 133, 121, 107, 93, 81, 67, 55. IR ν_{max}^{neat} cm^{-1}: 3400 (OH), 1725, 1155 (COOMe), 3090, 1645, 890 (=CH_2). ^{1}H-NMR (200 MHz) δ: 0.51 (3H, s, 10-Me), 1.18 (3H, s, 4-Me), 1.67 (3H, s, 13-Me), 3.61 (3H, s, COOMe), 4.15 (2H, d, J=7.0 Hz, 15-H), 4.52 (1H, br s, 17-H), 4.86 (1H, br s, 17-H), 5.39 (1H, t, J=7.0 Hz, 14-H).

A Mixture of Isopimaric Acid (4a) and Sandaracopimaric Acid (4b). (4)——Colorless needles, mp 153—162 °C (MeOH–H_2O). MS *m/z*: 302 (M^+), 287, 273, 257, 241, 187, 167, 133, 121, 105, 91, 79, 67, 55. IR ν_{max}^{KBr} cm^{-1}: 3300—2500, 1690 (COOH), 3090, 1640, 1000, 910, 900 (=CH_2). GLC of **4**-methyl ester: t_R 10, 12 min (authentic methyl sandaracopimarate 10 min, methyl isopimarate 12 min). 2% silicone OV-17 (2m, glass) on Chromosorb W (AW-DMCS), column temp. 220 °C, carrier gas N_2 (25 ml/min), detection by FID (240 °C).

Imbricataloic Acid Dimethyl Acetal (5)——Colorless oil, $[\alpha]_D$ +42.4° (c=0.40, $CHCl_3$). MS *m/z*: 366.2767 (M^+, Calcd for $C_{22}H_{38}O_4$: 366.2770), 334, 318, 302, 287, 257, 241, 221, 201, 148, 133, 121, 111, 99, 85, 67, 55. IR ν_{max}^{neat} cm^{-1}: 3300—2500, 1725, 1695 (COOH), 3090, 1645, 890 (=CH_2), 1125, 1050 (CH$<^{OMe}_{OMe}$). ^{1}H-NMR (60 MHz) δ: 0.59 (3H,

s, 10-Me), 1.22 (3H, s, 4-Me), 3.30 (6H, s, OMe), 4.45 (1H, m, 15-H), 4.48 (1H, br s, 17-H), 4.80 (1H, br s, 17-H), 9.8 (1H, br, COOH). ^{13}C-NMR (50.3 MHz): Table III.

Synthesis of 5 from 2——CrO_3 (150 mg, 1.5 mmol) were added to a solution of CH_2Cl_2 (4 ml) and pyridine (0.24 ml, 3.0 mmol) in a round-bottomed flask connected with a $CaCl_2$ tube under stirring at 15 °C. After being stirred for more 15 min, the black precipitates were removed and imbricatolic acid **2** (78 mg, 0.24 mmol) in CH_2Cl_2 was added dropwise at room temperature. The reaction mixture was stirred for 20 min, then decanted to separate the solution from insoluble materials, which were extracted again with Et_2O. The solution together with the Et_2O-soluble portion of the reaction mixture were passed through a silica gel column and washed with 1 N HCl solution (3 times), 5% $NaHCO_3$ solution and NaCl solution. The Et_2O extracts were dried over Na_2SO_4 and concentrated to yield crude imbricataloic acid as a pale brown oil (45 mg). A mixture of crude imbricataloic acid (17 mg), pyridium *p*-tosylate (*ca.* 1 mg) and MeOH (5 ml) was stirred over night at room temperature in a round-bottomed flask connected with a $CaCl_2$ tube. Excess MeOH was evaporated off and the residue was purified by PLC (Merck, Silica gel 60 PF_{254} plate, *n*-hexane : AcOEt = 4 : 1) to give imbricataloic acid dimethylacetal as a colorless oil, $[\alpha]_D$ +43.4° (c = 0.17, $CHCl_3$) (11 mg), which was identified as **5** by direct spectroscopic comparisons (NMR, IR, $[\alpha]_D$).

Cryptotrienolic Acid Methyl Ester (Methyl (*E*)-13-Hydroxylabda-8(17),11,14-trien-19-oate) (6 m)——Colorless oil, $[\alpha]_D$ + 16° (c = 0.065, $CHCl_3$). MS m/z: 322.2366 (M^+, Calcd for $C_{21}H_{32}O_3$: 322.2350), 315, 273, 249, 189, 181, 161, 147, 121, 107, 93, 81, 67, 55. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600, 3450 (OH), 1715, 1158 (COOMe), 1644, 990, 910, 895 (=CH_2, CH=CH_2). ^{1}H-NMR (200 MHz) δ: 0.62 (3H, s, 10-Me), 1.20 (3H, s, 4-Me), 1.39 (3H, s, 16-Me), 2.31 (1H, d, J = 9.2 Hz, 9-H), 3.63 (3H, s, COOMe), 4.46 (1H, br s, 17-H), 4.75 (1H, br s, 17-H), 5.07 (1H, dd, J = 10.6, 1.0 Hz, 15-H), 5.24 (1H, dd, J = 17.4, 1.0 Hz, 15-H), 5.57 (1H, d, J = 15.4 Hz, 12-H), 5.69 (1H, dd, J = 15.4, 9.2 Hz, 11-H), 5.98 (1H, dd, J = 17.4, 10.6 Hz, 14-H). ^{13}C-NMR (Table III).

Methyl 15-Methyl Imbricataloate (7m)——Colorless oil, $[\alpha]_D$ +46.1° (c = 0.75, $CHCl_3$). MS m/z: 348 (M^+), 330, 299, 298, 273, 235, 221, 181, 161, 133, 121, 109, 93, 81, 67, 55. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1715, 1158 (COOMe), 3090, 1640, 890 (=CH_2). ^{1}H-NMR (200 MHz) δ: 0.50 (3H, s, 14-Me), 0.89 (3H, *d*, J = 6.6 Hz, 13-Me), 1.18 (3H, s, 4-Me), 2.12 (3H, s, 15-Me), 3.16 (3H, s, COOMe), 4.48 (1H, br s, 17-H), 4.84 (1H, br s, 17-H).

Acknowledgement The authors wish to thank Prof. Y. Hirose and Dr. S. Hasegawa, Faculty of Science and Technology, Science University of Tokyo for providing authentic samples of isocupressic acid and a mixture of isopimaric acid and sandaracopimaric acid. Thanks are also due to Kaken Pharmaceutical Co., Ltd. for advice on the biological techniques and Prof. T. Namba, Dr. M. Hattori and Dr. Y.-Z. Shu, Research Institute for Wakan-Yaku of this University for ^{1}H- and 13C-NMR spectral measurements.

References and Notes

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