No.10 3967

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Anti-inflammatory Constituents of Topically Applied Crude Drugs. II." Constituents and Anti-inflammatory Effect of Cryptomeria
japonica D. D0N2)

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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-hy-droxylabda-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 substitutefor"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.' '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 Et2O and H2O to give the Et2O-soluble fraction (fr.) (fr. B) and H2O 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 (HC1) (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 HCI to monitor for further effective consituents, since a smaller amount of samples could be used than in the CPE test.

3968 Vol.36(1988)

Chart 1

TABLE I. Inhibitory Effects of Frs. A-F and Compounds 1-3 on Carrageenan-Induced
Paw Edema in Rats")

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

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).

No.10 3969

Compound 1, colorless oil, [a],,+ 38.1•‹, C20H30O2 -20-30-0 25 was concluded to be cis-communic
acid by comparison of the physical and spectral data of its methyl ester (la) and reduced de-
rivative (lb) with those given in the literature.5 1 readily changed to another compound in
a few hours at room temperature6) 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 •Ž (dec.), which ap-
peared to be stable.
Compound 2, colorless oil, [a], + 45.8•‹, C20H34O3, and 3, colorless oil, [a], + 52.9•‹,
C201-13203, were identified as imbricatolic acid7 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') 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, []D + 42.4•‹. The high-resolution mass
spectral examination of 5 gave the formula C22H3804. Comparison of the and "C-
nuclear magnetic resonance (1H- and 13C-NMR) spectra of 5 with those of 2 indicated that 5
is a very similar diterpene to 2, possessing a labdane skeleton. The 11-I-NMR spectrum of 5
showed no signal at 6 3.66 (2H, m), assignable to a hydroxymethyl group in 2, but gave two
signals at 54.45 (1H, m) and 3.30 (6H, -OCH3 •~ 2) which were not observed in 2. The "C-
NMR spectrum of 5 showed three signals at 6 103.2 (d), 52.8 (q) and 52.2 (q) instead to one at
o 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 imbrica-
taloic acid is present or not in this plant.
Compound 6 was obtained as the methyl ester (6m), colorless oil, [AD+ 16•‹, C21 H32O3.
Compound 6m showed absorption bands at 1715 and 1158 cm in the infrared (IR) spectrum
and signals at 6 3.63 (3H, s) and 51.2 in the 1H- and "C-NMR spectra, respectively, due to a
carbomethoxy group. Other signals in the 111-NMR spectrum, two tertiary methyls (6 0 .62
and 1.20), an exomethylene (6 4 . 46 and 4.75, each 1H br s) and others, were very similar to
those of methyl cis-communate (la), imbricatolate (2a) and isocupressate (3a). In addition,
the signal pattern of 6m in the "C-NMR spectrum corresponded well to those of la, 2a, 3a
and methyl trans-communate (8)1" 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 1H- and "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 (11E, 13S and 11E, 13R)-11,14-
labdadiene-8,13-diol (9 and 10)12) (Table IV). Consequently, it was suggested that the (E)-3-
hydroxy-3-methy1-1,4-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

397O Vol.36(1988)

Chart 2

TABLE III. 13C-NMR Chemical Shifts of la, 2a, 3a, 5, 6m and 8a) (5 (ppm) from TMS in CDCl3

a) trans-Communic acid methyl ester, values from literature."'

No.10 3971

TABLE IV. 1H- and 13 C-NMR Chemical Shifts of Side Chain of 6m, 9") and 10b)

a) (11E,13S)-11,14-Labdadien-8,13-diol, values from literature.12 b) (11 E,13R)-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, [a], +46.1° (c = 0.75, CHC13), C22H3603. The 1H-NMR spectrum of 7m resembles that of 2a, except for the signal at 6 2.12 (3H, s) assigned to a methyl ketone instead of one at 6 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' (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 HCI-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 CHC13. Spectral data were obtained as follows: IR spectra with a Hitachi 260-0611 spectrophotometer; ultraviolet (UV) spectra with a Hitachi 270S spectrophoto-meter; mass spectrum (MS) with a JEOL JMS-D 200 spectrometer (70eV); 'H-NMR spectra with Hitachi R-24B (60 MHz) and Varian XL-200 (200 MHz) spectrometers in CDC13; "C-NMR spectra with Varian XL-200 (50.3 MHz) and JEOL FX 90Q (22.5 MHz) spectrometers in CDC13. Chemical shifts are given in 6 (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 HCI were assessed as described in the previous report."

3972 Vol.36(1988)

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 Et2O under acidic conditions (pH 3) to afford the Et2O-soluble fraction (fr. B) (57 g) and the water-
soluble fraction (fr. C).
Fr. B, active in the CPE test, was dissolved in Et2O and extracted first with NaHCO3 solution (pH 9) and next
with NaOH solution (pH 12) followed by extraction with Et2O 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 Et2O layer.
Constituents of Active Fraction (Fr. E)------Fr. E (16.9 g), showing activity in the CPE test and an inhibitory effect
on HCI 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

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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 Et2O solution (15 ml) of fr. 1 (1.9 g) with stirring until no more

precipitation occurred. The precipitate was recrystallized from H20-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 (HCI, 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 chro-
matography 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 CH2N2 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.). [gip +38.1 (c = 1.00,
CHC13). Anal. Calcd for C20H30O2: C, 79.4; H, 10.0. Found: C, 79.2; H, 10.0. MS mlz (70 eV): 302 (M +), 287, 257,
221, 175, 161, 147, 135, 121, 105, 93, 81, 69, 55. IR 1=cm-1: 3300-2500, 1695 (COOH), 1645, 1470, 990, 910, 900,
890 (conjugated diene). UV nm (log i.): 236.5 (2.12). H-NMR (60 MHz) 6: 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 (la)------1 was methylated with CH2N2 to give la. Colorless solid, mp 38 nC, [a],, + 51.6'

(c = 1.02, CHC13). MS m/z: 316.2393 (M +, Calcd for C21 H32O2: 316.2400). 13C-NMR: Table III.

cis-Communol (lb)------la was reduced with LiA1H4 in the usual way followed by PLC to give lb, colorless oil,
+ 36.4•‹ (c=0.35, CHC13). MS m/z: 288 (M +). IR v2F3cm-i: 3640, 3460, 1015 (OH), 1645, 990, 910 sh, 900
(conjugated diene).

Imbricatolic Acid (2)------Colorless oil, [a],) +45.8" (c= 1.66, CHC13). MS mlz: 322.2518 (M, Calcd for
C20H3403: 322.2506), 304, 276, 221, 207, 167, 139, 121, 109, 95, 81, 67, 55. IR cm-1: 3400 (OH), 3300-2500
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1695 (COOH), 3090, 1645, 890 (=CH2). H-NMR (60 MHz) 6: 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 CH2N2 to afford 2a. Colorless oil, [oc], +51.3 (c = 1.77,
CHC13). MS m/z: 336 (M +), 321, 304, 276, 221, 181, 161, 121, 107, 95, 81, 69, 55. IR vo,ine:: cm -1: 3400 (OH), 1725,
1155 (COOMe), 3090, 1645, 890 (=CH2). H-NMR (200 MHz) 6: 0.50 (3H, S. 10-Me), 0.90 (3H, d, J =6.4 Hz, 13-
Me), 1.18 (3H, 5, 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)

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Isocupressic Acid (3)------Colorless oil, MD + 52.9•‹ (c = 0.94, CHC13). MS m/z: 320 (M +), 305, 302, 287, 259, 241

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222, 189, 167, 147, 139, 134, 121, 107, 93, 81, 68, 55. IR cm -1: 3400 (OH), 3300-2500, 1695 (COOH), 3090,
1645, 890 (=CH2). H-NMR (60 MHz) 6:0.59 (3H, s, 10-Me) 1.22 (3H, s, 4-Me), 1.65 (3H, s, 13-Me), 4.13 (2H, d

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J=7 Hz, 15-H), 4.52 (1H, br s, 17-H), 4.85 (1 H, br s, 17-H), 5.37 (1 H, br t, J=7 Hz, 14-H), 6.0 (2H, br, COOH, OH).
Methyl Isocupressate (3a)-----Usual methylation of 3 with CH2N2 gave a methyl ester (3a) as a colorless oil
, MD

+51.20 (c = 1.73, CHC13). MS m/z: 335 (M + + 1), 320, 318, 302, 275, 258, 242, 190, 162, 133, 121, 107, 93, 81, 67, 55.
IR v:,1,7: cm': 3400 (OH), 1725, 1155 (COOMe), 3090, 1645, 890 (=CH2). H-NMR (200 MHz) 6: 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 •Ž
(MeOH-H20). MS m/z: 302 (M +), 287, 273, 257, 241, 187, 167, 133, 121, 105, 91, 79, 67, 55. IR vrI21cm': 3300-

2500, 1690 (COOH), 3090, 1640, 1000, 910, 900 (=CH2). GLC of 4-methyl ester: 1R 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 •Ž, carrier gas N2 (25 ml/min), detection by FID (240 "C).
Imbricataloic Acid Dimethyl Acetal (5)------Colorless oil, [cdp + 42.4•‹ (c = 0.40, CHC13). MS mlz: 366.2767 (M+,
Calcd for C22143804: 366.2770), 334, 318, 302, 287, 257, 241, 221, 201, 148, 133, 121, 111, 99, 85, 67, 55. I R cm-1:
3300-2500, 1725, 1695 (COOH), 3090, 1645, 890 (=CH2), 1125, 1050 (CH (OMMe
e). H-NMR (60 MHz) 6: 0.59 (3H,

No.10 3973

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). "C-NMR (50.3 MHz): Table III.
Synthesis of 5 from 2-----CrO3 (150 mg, 1.5 mmol) were added to a solution of CH2C12 (4 ml) and pyridine (0.24 ml, 3.0 mmol) in a round-bottomed flask connected with a CaC12 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 CH2C12 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 Et2O. The solution together with the Et20-soluble portion of the reaction mixture were passed through a silica gel column and washed with 1 N HC1 solution (3 times), 5% NaHCO3 solution and NaC1 solution. The Et2O extracts were dried over Na2SO4 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 CaCl2 tube. Excess MeOH was evaporated off and the residue was purified by PLC (Merck, Silica gel 60 PF254 plate, n-hexane : AcOEt =4 : 1) to give imbricataloic acid dimethylacetal as a colorless oil, [AD + 43.4 (c= 0.17, CHC13) (11 mg), which was identified as 5 by direct spectroscopic comparisons (NMR, IR, [AD).
Cryptotrienolic Acid Methyl Ester (Methyl (E)-13-Hydroxylabda-8(17),11,14-trien-19-oate) (6 m)-----Colorless
oil, ND+ 16' (c = 0.065, CHC13). MS m/z: 322.2366 (M Calcd for C21 H3203: 322.2350), 315, 273, 249, 189, 181, 161, 147, 121, 107, 93, 81, 67, 55. IR V cm': 3600, 3450 (OH), 1715, 1158 (COOMe), 1644, 990, 910, 895 (=CH2, CH =CH2). (200 MHz) 6 : 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). 13C-NMR (Table III).
Methyl 15-Methyl Imbricataloate (7m)-----Colorless oil, []D +46.10 (c=0.75, CHC13). MS m/z: 348 (M +), 330, 299, 298, 273, 235, 221, 181, 161, 133, 121, 109, 93, 81, 67, 55. IR V l3 cm -1: 1715, 1158 (COOMe), 3090, 1640, 890 (=CH2). H-NMR (200 MHz) 6: 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).

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