New Antitumor Principles, Casearins A—F, for *Casearia sylvestris* Sw. (Flacourtiaceae)[1)]

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New antitumor clerodane diterpenes, named casearins A—F, have been isolated from the leaves of *Casearia sylvestris* Sw. (Flacourtiaceae). These structures have been completely elucidated by two dimensional nuclear magnetic resonance, circular dichroism spectroscopy, X-ray analysis, and chemical evidences.

Keywords casearin; casearins A—F; *Casearia sylvestris*; Flacourtiaceae; antitumor priciple; clerodane diterpene; Sarcoma 180A; V-79 cell; X-ray analysis

We have carried out antineoplastic or cytotoxic screening tests of crude drugs and collected plants[2)] against Sarcoma 180 ascites in mice[3)] or Chinese hamster V-79 cells.[4)] Then, antitumor or chemical components isolated from some species of South American medicinal plants have been reported.[1,5)] On the course of these screening tests, the ethanolic extract prepared from the leaves of *Casearia sylvestris* Sw., Paraguayan name "burro-kaa",[6)] showed antineoplastic activity against Sarcoma 180A in mice. A search for the active principles led us to isolate casearins A—F as the antitumor substances. Their structures were determined by modern two-dimentional nuclear magnetic resonance (2D-NMR), X-ray analysis and chemical evidences. This paper deals with a complete account of the structural elucidation of casearins A—F and their antitumor activities.

Results and Discussions

The leaves of *Casearia sylvestris* Sw. (Flacourtiaceae) were extracted with ethanol and the extract showed strong antitumor activity against Sarcoma 180 ascites in mice (100 mg/kg/d dose, growth ratio (GR): 13% (++)). When an aqueous solution of the ethanolic extract was partitioned successively with *n*-hexane, chloroform and ethyl acetate, the antitumor activity was concentrated in *n*-hexane extract (100 mg/kg/d dose, GR: 2% (+++)). Its chromatographic

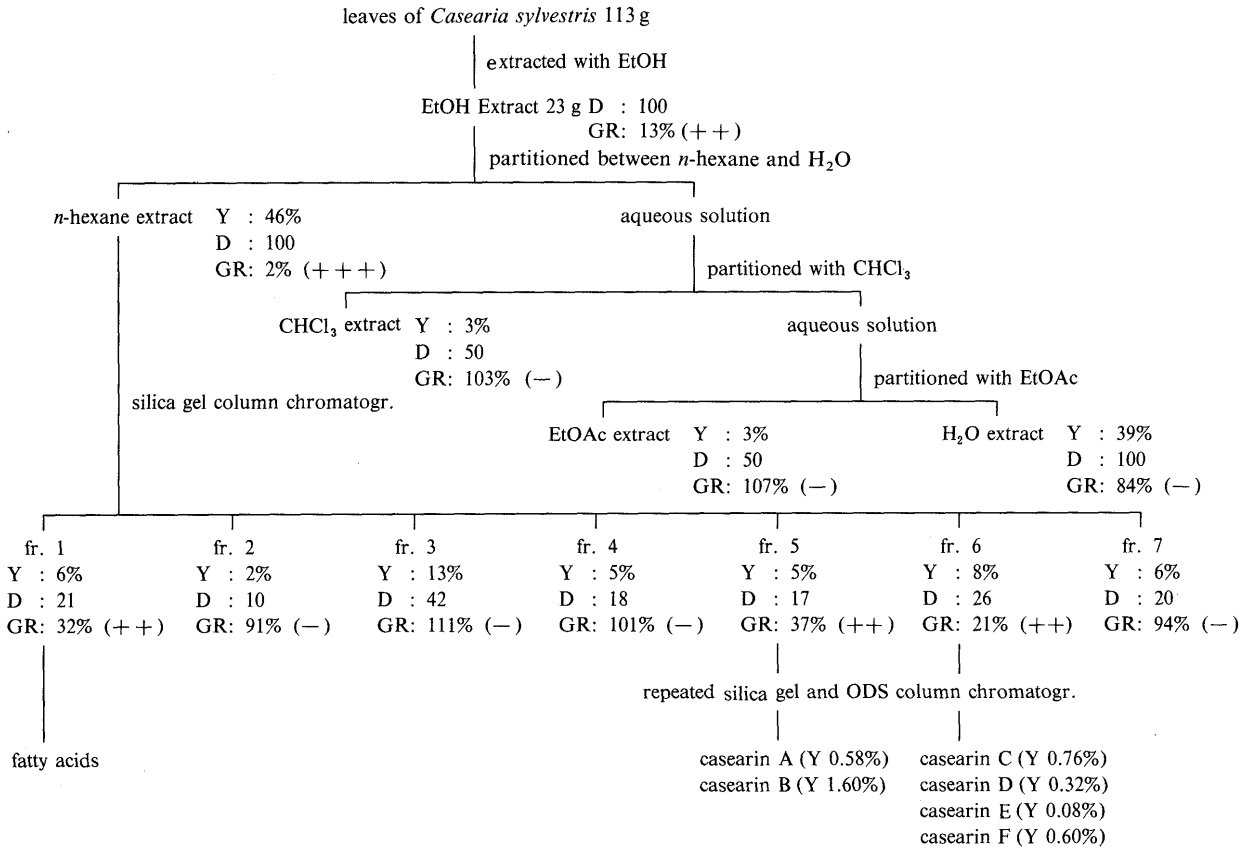


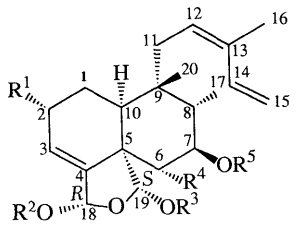
Chart 1. Antitumor Activity of Fractions Isolated from *C. sylvestris*

1) Antitumor activity was determined against Sarcoma 180 ascites in mice and the effectiveness was evaluated by the total packed cell volume method. 2) Y means yield from the EtOH extract. D means dose; drugs were given daily at the indicated doses (i.p.) for 5 consecutive days. GR means growth ratio; 0—10% (+++), 11—40% (++), 41—65% (+), over 66% (−).

purification with the guidance of bio-assay against Sarcoma 180 A in mice led us to isolate new antitumor clerodane diterpenes, named casearins A—F as shown in Chart 1.

Casearin A was obtained as colorless plates, mp 82.0—83.0 °C. The molecular formula, $C_{29}H_{42}O_9$ was deduced by the mass (MS) (*m/z*: 474, $M^+ - AcOH$) and NMR spectral data. The infrared (IR) spectrum showed the absorption bands at 3600 and 1760 cm^{-1} which were characteristic of hydroxyl and ester carbonyl moieties. The coupling networks and signal assignments based on ^1H–^1H and ^1H–^{13}C correlated spectra (COSY) revealed four partial structures (A: >CH–CH_2–CH(OMe)–CH=C–$CH(OR)_2$, B: –CH(OH)–CH(OR)–CH–CH_3, C: –CH_2–CH=C–CH_3, D: –CH=CH_2) concerning the skeleton, and the presence of two quarternary carbons, one tertiary methyl carbon, one acetal carbon, two acetates and one butylate. Furthermore, a 2D-incredible natural abundance double quantum transfer experiment (INADEQUATE) method that affords effective information about carbon skeletons was applied in this case to combine each partial structure and to determine the sequence of carbon atoms in the molecule. In the spectrum, the correlated cross peaks were recognized among alphabets shown with solid lines in Fig. 2, except for those between the carbons C_5 and C_6, C_7 and C_6, and C_{14} and C_{13} (Fig. 1). However the sequence between C_7 and C_6 was confirmed by the ^1H–^1H COSY spectrum. Also, the sequences between C_5 and C_6, and C_{14} and C_{13} as well as the positions of ester functions were determined by the ^1H–^{13}C long range COSY (COLOC) spectrum.

The relative structure of casearin A was established on the basis of the coupling constants of each proton and the nuclear Overhauser effect (NOE) correlated spectrum. As can be seen from Table I, the coupling constant value of 11.0 Hz between C_6-H and C_7-H, and C_7-H and C_8-H (C_6-H was also coupled with a hydroxyl proton which was confirmed with the addition of D_2O) indicating that the configuration of C_6-H, C_7-H and C_8-H was alternately axial. The coupling constants, 15.2 Hz and 3.5 Hz of C_{10}-H indicated that it was axial. The NOE correlations between C_{11}-H and C_{19}-H (4.0%), C_{11}-H and C_7-H (7.9%), C_7-H and C_{19}-H (2.6%), and C_{10}-H and C_{12}-H (11.0%) suggested that the C_9-C_{11} bond was axial, the A/B ring junction was



casearins	R^1	R^2	R^3	R^4	R^5
A	OMe	Ac	Ac	OH	Bu
B	OMe	Ac	Ac	OAc	Bu
C	OH	Ac	Ac	OAc	Dc
D	OH	Bu	Ac	OH	Bu
E	OH	Et	Ac	OH	Dc
F	OH	Et	Ac	OH	Bu

Fig. 1. Structures of Casearins from *Casearia sylvestris*

Me, methyl; Ac, acetate; Bu, butanoate; Dc, decanoate.

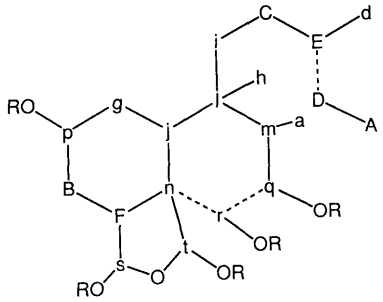


Fig. 2. Partial Structure from INADEQUATE Spectrum of Casearin A

The spectrum was measured on a Bruker AM-400, using 400 mg of sample (30 °C, 80 h run, J_{CC}=62.5 Hz). The sp^2 carbons are marked with A—F and sp^3 carbons with a—t in the order of increasing chemical shift values. The correlated cross peak between the two carbons was confirmed on the solid line.

TABLE I. ^1H Chemical Shifts of Casearins A—F, δ (ppm) from TMS in $CDCl_3$ (*J*/Hz in Parentheses, 400 MHz)

Casearins	A	B	C	D	E	F
H-2	3.90 (br s)	4.01 (br s)	4.52 (br s)	4.55 (br s)	4.41 (br s)	[a]
H-3	6.09 (br d, 3.9)	6.17 (br d, 3.9)	6.06 (br d, 3.6)	6.05 (br d, 3.3)	6.15 (br d, 4.2)	6.13 (br d, 3.9)
H-6	3.66 (t, 11.0)	5.2[a]	5.2[a]	3.66 (d, 10.0)	3.63 (d, 11.0)	3.62 (d, 10.9)
H-7	4.99 (t, 11.0)	5.2[a]	5.2[a]	5.00 (t, 10.0)	4.99 (t, 11.0)	4.99 (t, 10.9)
H-10	2.37 (dd, 15.2, 3.5)	2.41 (dd, 17.6, 3.2)	2.46 (dd, 13.0, 3.8)	2.40 (dd, 17.6, 3.2)	2.40 (dd, 13.5, 3.7)	2.40 (dd, 11.5, 1.5)
H-11	1.63 (br d, 16.3), 2.54 (dd, 9.3, 16.3)	1.67 (br d, 16.2), 2.59 (dd, 7.9, 16.2)	1.69 (br d, 16.0), 2.57 (dd, 9.3, 16.0)	1.7,[a] 2.54 (dd, 9.3, 16.2)	1.7,[a] 2.53 (dd, 9.3, 16.1)	[a] 2.52 (dd, 9.3, 16.1)
H-12	5.28 (br d, 9.3)	5.38 (br d, 7.9)	5.31 (br d, 9.3)	5.29 (br d, 9.3)	5.31 (br d, 9.3)	5.34 (br d, 9.3)
H-14	6.62 (dd, 10.8, 17.2)	6.70 (dd, 10.8, 17.2)	6.65 (dd, 10.7, 17.2)	6.63 (dd, 11.0, 17.2)	6.64 (dd, 10.8, 17.2)	6.62 (dd, 10.8, 17.2)
H-15	5.12 (d, 10.8), 5.20 (d, 17.2)	5.1,[a] 5.2[a]	5.1,[a] 5.2[a]	5.13 (d, 11.0), 5.12 (d, 17.2)	5.12 (d, 10.8), 5.20 (d, 17.2)	5.10 (d, 10.8), 5.18 (d, 17.2)
Me-16	1.80 (br s)	1.84 (br s)	1.81 (br s)	1.81 (br s)	1.80 (br s)	1.79 (br s)
Me-17	0.92 (d, 6.7)	0.91 (d, 8.9)	0.90 (d, 6.6)	0.92 (d, 6.7)	0.91 (d, 7.4)	0.90 (d, 6.7)
H-18	6.72 (t, 1.6)	6.35 (t, 1.6)	6.42 (t, 1.6)	6.74 (t, 1.5)	5.56 (t, 1.5)	5.55 (t, 1.4)
H-19	6.57 (s)	6.56 (s)	6.62 (s)	6.59 (s)	6.54 (s)	6.53 (s)
H-20	0.88 (s)	0.92 (s)	0.90 (s)	0.88 (s)	0.87 (s)	0.86 (s)
MeO–	3.44 (s)	3.45 (s)				
MeCO–	1.99 (s), 2.09 (s)	1.84 (s), 1.98 (s), 2.05 (s)	2.01 (s), 2.05 (s), 2.09 (s)	1.99 (s)	2.00 (s)	1.99 (s)
Me-Bu	0.96 (t, 7.4)	0.94 (t, 7.4)	Me-Dec 0.88 (t, 7.1)	0.96 (t, 7.4) 0.97 (t, 7.4)	Me-Dec 0.88 (t, 6.7) Me-Et 1.21 (t, 7.1)	0.95 (t, 7.4) Me-Et 1.20 (t, 7.1)

a) The chemical shifts and coupling constants could not be revealed by overlapping of the other protons.

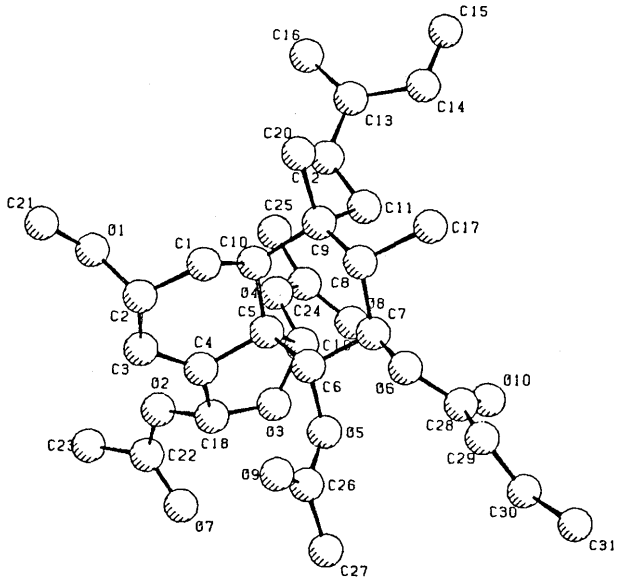


Fig. 3. Perspective View of Casearin B

cis, and C_{19}-H was β. Also, the NOE correlation between C_{12}-H and C_{16}-H revealed that the double bond of the side chain was *Z*. The homoallyl coupling between C_2-H and C_{18}H and the allyl coupling between C_3-H and C_{18}-H were 1.6 Hz, respectively. Therefore, both C_2- and C_{18}-H were determined to be axial and of β-orientation, because this type of homoallyl coupling necessitates a certain amount of overlap between the π-orbitals of the double bond and the 1*s* orbitals of the hydrogen atoms.

To further confirm the configuration of these two allylic positions (C_2 and C_{18}), X-ray analysis of casearin B which was considered to possess the same stereostructure as described later, was carried out. Crystals of casearin B were grown in a solvent mixture of acetonitrile and water as fine colorless needles. The X-ray diffraction intensities of 3787 reflections (including 2040 symmetry equivalent reflections which gave an *Rf* value of 0.07 for the independent reflections) were measured as above the 2 (*I*) level out of 6251 in the 2 range of 60° through 100°, using graphite-monochromated CuK_α radiation. Crystal data are: trigonal, space group $P3_221$, $z=6$, $D_{calc}=1.027\,gcm^{-3}$, $a=b=19.023(10)$Å, $c=17.847(10)$Å, $\alpha=\beta=90°$, $\gamma=120°$, $V=5593$Å^3. The crystal structure was determined by the direct method using the MULTAN program and refined by the block diagonal-matrix least-squares method to an *R* value of 0.126. The number of used reflections were 1747. Refinement of the structure converged the *R* factor to a larger value. This is due to very large thermal motions of terminal atoms, but it is sufficient to confirm the configuration of C_2 and C_{18} (Fig. 3). These findings led us to conclude that the structure of casearin A was proved to be as illustrated in Fig. 1.

Casearin B was isolated as colorless needles, mp 80.0—81.0 °C and the structure was elucidated by X-ray analysis as described above.

The structures of casearins C and D were also determined by analogous procedures used to elucidate casearin A. The positions of their ester functions were estimated by the presence of long range couplings between the ester carbonyl carbon and the proton linked to the ester-bearing carbon

Table II. ^{13}C Chemical Shifts of Casearins A—F, δ (ppm) from TMS in $CDCl_3$ (100 MHz)

Casearins	A	B	C	D	E	F
1 (t)	25.3	25.4	29.5	29.5	29.5	29.5
2 (d)	72.6	72.5	63.6	63.8	64.0	63.9
3 (d)	123.6	125.5	127.4	125.5	125.3	125.3
4 (s)	142.8	141.3	141.2	142.8	143.9	143.7
5 (s)	53.8	52.9	52.8	53.8	53.7	53.6
6 (d)	75.3	74.0	74.0	75.3	75.3	75.3
7 (d)	75.5	72.7	72.7	75.4	75.8	75.6
8 (d)	41.1	41.0	41.1	41.2	41.2	41.1
9 (s)	39.4	39.3	39.3	39.5	39.5	39.4
10 (d)	35.8	36.7	36.1	35.4	35.2	35.2
11 (t)	30.3	30.2	30.2	30.3	30.4	30.3
12 (d)	125.8	125.8	125.7	125.7	125.9	125.9
13 (s)	133.9	133.9	134.1	134.0	133.9	133.9
14 (d)	133.4	133.3	133.3	133.4	133.4	133.4
15 (t)	114.6	114.6	114.8	114.7	114.6	114.5
16 (q)	20.2	20.3	20.3	20.3	20.3	20.3
17 (q)	11.1	11.0	11.0	11.1	11.1	11.1
18 (d)	94.9	94.9	94.9	95.6	103.7	103.6
19 (d)	97.5	97.7	97.7	97.6	97.6	97.2
20 (q)	25.5	25.4	25.5	25.4	25.5	25.4
OMe	57.0 (q)	57.1 (q)				
OEt					15.5 (q)	15.4 (q)
					64.9 (t)	64.9 (t)
Ac	21.2 (q)	21.0 (q)	21.1 (q)	21.3 (q)	21.4 (q)	21.3 (q)
	170.2 (s)	169.1 (s)	169.0 (s)	168.9 (s)	169.4 (s)	169.4 (s)
	21.3 (q)	21.2 (q)	21.3 (q)			
	169.0 (s)	170.1 (s)	170.0 (s)			
		21.4 (q)	21.4 (q)			
		170.1 (s)	170.2 (s)			
Ester	13.7 (q)	13.8 (q)	14.1 (q)	13.6 (q)	14.1 (q)	13.7 (q)
	18.6 (t)	18.4 (t)	22.7 (t)	18.3 (t)	22.7 (t)	18.6 (t)
	36.3 (t)	36.1 (t)	31.8 (t)	36.4 (t)	31.9 (t)	36.3 (t)
	174.3 (s)	172.5 (s)	29.4 (t)	172.7 (s)	29.3 (t)	174.2 (s)
			29.5 (t)		29.4 (t)	
			29.2 (t)	13.7 (q)	29.2 (t)	
			29.2 (t)	18.6 (t)	29.7 (t)	
			25.0 (t)	26.4 (t)	25.1 (t)	
			34.3 (t)	174.4 (s)	34.5 (t)	
			172.8 (s)		174.4 (s)	

The multiplicities of the carbon signals were determined by the DEPT method.

in COLOC spectra. Then, the presence of a hydroxyl group at C_2 was confirmed on the basis of the absence of the methoxyl signal, the presence of carbons (δ 63.6 and 63.8, respectively) attached to hydroxyl functions in the ^{13}C-NMR and IR absorption bands (3620 cm^{-1}). Casearins E and F were isolated as white powder, and were suggested to possess ethoxyl function at C_{18} by NOE between ethoxyl ethylene protons and a proton (H_{18}) linked to ethoxy-bearing carbon. Their structures were confirmed by the comparison of the spectral data with casearins A—D. From the above descriptions, the structures of casearins C—F were determined as shown in Fig. 1. However, from the presence of ethoxyl function and extraction with hot ethanol, it is suggested that casearins E and F may be artifact. The ^{1}H-and ^{13}C-NMR assignments of casearins A—F are listed in Tables I and II.

The absolute structures of the casearins were determined by the exciton chirality method of allylic alcohol benzoate.[7] The allylic benzoate derivative of casearin C for the circular dichroism (CD) measurement was prepared by reducing the ozonide of a conjugated double bond of the side chain with sodium borohydride ($NaBH_4$), after the benzoylation with

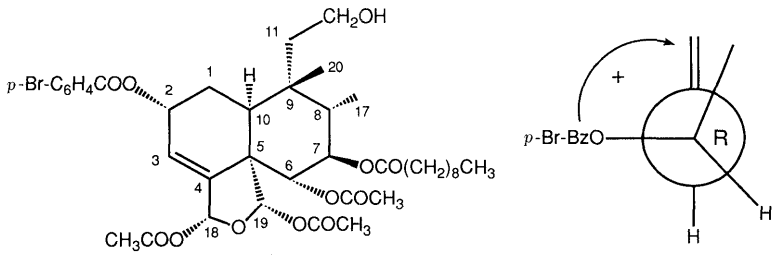


Fig. 4. *p*-Bromobenzoate from Casearin C Derivative and Its Rotamer

TABLE III. Cytotoxic and Antitumor Activity of Casearins A—F

Casearin	V-79 cells IC_{50} values (mmol/l)	Sarcoma 180 ascites in mice				
		Dose	BWC	PCV/TV	GR (%)	Assessment
A	1.0×10^{-3}	15	−3.0	0.10	4.0	+++
B	8.5×10^{-3}	15	+3.6	0.36	76.9	−
C	7.7×10^{-4}	15	−1.9	0.07	1.9	+++
D	1.8×10^{-3}	*a)*	*a)*	*a)*	*a)*	*a)*
E	4.7×10^{-3}	*a)*	*a)*	*a)*	*a)*	*a)*
F	2.9×10^{-2}	15	+2.4	0.33	81.3	−

The effectiveness of Sarcoma 180 screening test was evaluated by means of the total packed cell volume method. Dose, mg/kg/d; BWC, body weight change = (day 7 weight − TV)/day 0 weight; PCV, packed cell volume; TV, total volume; GR, growth ratio = PCV (test groups)/PCV (control groups) × 100. *a)* Sarcoma 180 bio-assay of casearins D and E could not be examined because the amount of the isolated samples was small.

p-bromobenzoyl chloride. The CD spectrum of this derivative showed a positive Cotton curve at 236 nm. Therefore, the absolute configuration at the C-2 position of casearin C must be *R* as shown in Fig. 4. The other casearin configurations were also estimated to be the same, because their CD spectra exhibited negative Cotton curves at 235 nm and were similar to that of casearin C.

The antitumor activity of casearins A—F against Sarcoma 180 ascites in mice and their cytotoxic activity against V-79 cells *in vitro* are summarized in Table III. These compounds gave similar results in both bio-assay and of them casearin C showed the best effect.

Experimental

All melting points were recorded on a Yanagimoto MP-3 micro melting point apparatus and are uncorrected. The spectral data were obtained on the following instruments: optical rotation on a JASCO DIP-4, IR on a JASCO A-302, UV on a Hitachi 557, NMR on a Bruker AM 400 and MS on a Hitachi M-80. High-performance liquid chromatography (HPLC) was carried out on a CIG column system (Kusano Scientific Co., Tokyo) packed with 10 μm silica gel as the stationary phase. Reversed phase HPLC was carried out on a YMC R and D column packed with 5 μm ODS.

Bioassay of Antineoplastic Activity against V-79 Cells Cloned Chinese hamster V-79 cells, supplied by Dr. S. Tsukagoshi of the Japan Foundation for Cancer Research, were maintained in RPMI-1640 medium (Nissui Pharm. Co., Ltd). supplemented with 10% fetal calf serum (Mitsubishi Chemical Industry Co., Ltd). and Kanamycin (100 μg/ml). The cells (3×10^5 cells/well) were cultured in Corning disposable 6-well plates containing 2 ml of growth medium per well and were incubated at 37 °C in a humidified atmosphere of 5% CO_2. Various drug concentrations (10 μl) were added to the cultures at day 1 after the transplantation. The colonies were fixed with a 10% formaldehyde solution (2 ml) for 20 min and stained with 0.05% Crystal Violet (0.75 ml) at day 5. The cytotoxic activity of the drugs was assessed by determining the T (number of stained colonies of test groups)/C (those of control groups) × 100 or IC_{50} (drug concentration that inhibits colony growth by 50%) values in drug-containing medium relative to colony growth in 0.5% EtOH medium at day 5 after drug treatment.

Bioassay of Antitumor Activity against Sarcoma 180 Ascites ICR male mice, 5 weeks old, supplied by Clea Japan Co., Ltd., were used in groups of 6 animals. Sarcoma 180 ascites, provided by the National Cancer Center Research Institute and maintained in successive generations by us, was implanted i.p. at 1×10^6 cells/mouse. Administration of a test drug was started at day 1 after the implantation and continued for 5 d by the i.p. route. The effectiveness was evaluated at day 7 by means of the total packed cell volume method; growth ratio (GR, %) = (packed cell volume (PCV) of test groups/PCV of control groups) × 100; GR = 0—10% (+++), 11—40% (++), 41—65% (+) and over 66% (−).

Extraction and Isolation The leaves of *C. sylvestris* purchased at Paraguaian market (113.0 g) were extracted with hot ethanol three times and concentrated to give ethanolic extract (23.13 g). This extract was distributed to *n*-hexane, chloroform, ethyl acetate and water soluble fractions, respectively. The antitumor activity was concentrated into the *n*-hexane soluble fraction as shown in Chart 1. This active fraction was subjected to silica gel column chromatograhy and separated into seven fractions. The active substance contained in fraction 1 was estimated to be fatty acids. Chromatographic purification of fractions 5 and 6 by silica gel HPLC (benzene–ethyl acetate solvent system) and ODS HPLC (acetonitrile–water solvent system) led us to isolate casearins A, B, C, D, E and F. Further, the leaves (1.0 kg) of *C. sylvestris* collected in Universidade de São Paulo, Brazil were extracted and purified in a way similar to the above to give casearin A (1.5 g) which was used for the INADEQUATE measurement.

Casearin A: Colorless plates, mp 82.0—83.0 °C, $[\alpha]_D$ +40.1° (c = 0.22, EtOH). CD (EtOH) $\Delta\varepsilon$ (nm): −2.16 (235) (negative maximum). High MS: Calcd 474.2615 for $C_{27}H_{38}O_7$ (M^+ − AcOH), Found 474.2607. IR (CCl_4): 3600, 3400, 2980, 1760, 1640, 1600, 1455, 1370, 1230, 1080, 100 cm^{-1}. UV λ_{max}^{EtOH} nm (ε): 210 (5500), 235 (9000). MS *m/z* (%): 474 (M^+ − AcOH, 5), 414 (10), 344 (12), 326 (17), 256 (30), 185 (71), 157 (100).

Casearin B: Colorless needles, mp 80.0—81.0 °C. $[\alpha]_D$ +35.1° (c = 1.24, EtOH). CD (EtOH) $\Delta\varepsilon$ (nm): −1.17 (235) (negative maximum). High MS: Calcd 516.2720 for $C_{29}H_{40}O_8$ (M^+ − AcOH), Found 516.2690. IR (CCl_4): 2980, 1760, 1750, 1370, 1230, 1085, 1020, 990 cm^{-1}. UV λ_{max}^{EtOH} nm (ε): 236 (10000). MS *m/z* (%): 576 (M^+, 0.1), 544 (1), 516 (15), 456 (5), 406 (4), 368 (8), 308 (22), 276 (18), 186 (70), 71 (100).

Casearin C: Colorless oil, $[\alpha]_D$ +45.2° (c = 0.45, EtOH). CD (EtOH) $\Delta\varepsilon$ (nm): −1.47 (235) (negative maximum). IR (CCl_4): 3620, 3500, 2940, 1765, 1755, 1370, 1230, 1155, 1090, 1030 cm^{-1}. UV λ_{max}^{EtOH} nm (ε): 206 (6200), 236 (11000). MS *m/z* (%): 586 (M^+ − AcOH, 15), 526 (8), 372 (8), 354 (17), 312 (25), 294 (37), 132 (70), 186 (80), 157 (55), 81 (100).

Casearin D: White powder, mp 140.0—141.0 °C. $[\alpha]_D$ +38.5° (c = 0.42, EtOH), CD (EtOH) $\Delta\varepsilon$ (nm): −0.67 (235) (negative maximum). IR (CCl_4): 3620, 3590, 3450, 2980, 1760, 1460, 1420, 1215, 1180, 1060, 1000 cm^{-1}. UV λ_{max}^{EtOH} nm (ε): 210 (7900), 235 (13600). MS *m/z* (%): 460 (M^+ − butylic acid, 3), 400 (4), 372 (25), 330 (5), 312 (7), 231 (25), 185 (20), 157 (25), 71 (100).

Casearin E: White powder, mp 158.0—160.0 °C. $[\alpha]_D$ +58.2° (c = 0.13, EtOH). CD (EtOH) $\Delta\varepsilon$ (nm): −2.28 (235) (negative maximum). High MS: Calcd 530.3603 for $C_{32}H_{50}O_6$ (M^+ − AcOH), Found 530.3574. IR (CCl_4): 3610, 3580, 3480, 2930, 1760, 1735, 1380, 1220, 1180, 1100, 1045, 1000 cm^{-1}. UV λ_{max}^{EtOH} nm (ε): 210 (5700), 235 (9400). MS *m/z* (%): 590 (M^+, 0.1), 544 (1), 530 (20), 484 (10), 358 (10), 312 (28), 283 (13), 231 (33), 203 (43), 157 (52), 108 (38), 81 (100).

Casearin F: White powder, mp 150.0—151.0 °C, $[\alpha]_D$ +62.7° (c = 0.20, EtOH). CD (EtOH) $\Delta\varepsilon$ (nm): −2.09 (235) (negative maximum). High MS: Calcd 446.2666 for $C_{26}H_{38}O_6$ (M^+ − AcOH), Found 446.2666. IR (CCl_4): 3620, 3560, 2980, 1745, 1600, 1375, 1205, 1100, 1045, 990 cm^{-1}. UV λ_{max}^{EtOH} nm (ε): 210 (5560), 235 (9000). MS *m/z* (%): 446 (M^+ − AcOH, 10), 400 (6), 312 (20), 231 (27), 203 (42), 157 (58), 71 (100).

Conversion of Casearin C to *p*-Bromobenzoyl Derivative Casearin C was treated with *p*-bromobenzoyl chloride in pyridine for 12 h at room

temperature. After work-up in the usual way, the product was dissolved in methanol and bubbled with ozone. Then, the ozonide was treated with sodium borohydride and the product was purified by HPLC to give the *p*-bromobenzoyl derivative: Colorless oil. MS *m/z*: 793 (M^+). IR (CCl_4): 3400, 2940, 2860, 1750, 1720, 1470, 1450, 1385, 1260, 1235, 1110, 1100, 1030 cm^{-1}. CD (EtOH) $\Delta\varepsilon$ (nm): +12.9 (236) (positive maximum). UV λ_{max}^{EtOH} nm (ε): 208 (8800), 228 (9300), 273 (1100), 280 (1000).

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