# Casearin X, Its Degradation Product and Other Clerodane Diterpenes from Leaves of *Casearia sylvestris:* Evaluation of Cytotoxicity against Normal and Tumor Human Cells

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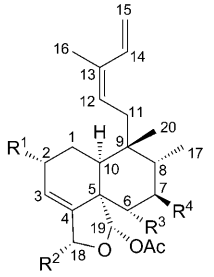
An EtOH extract of the leaves of *Casearia sylvestris* afforded new clerodane diterpene, casearin X, together with the known compounds casearins B, D, L, and O, and caseargrewiin F. Casearin X degraded to the corresponding dialdehyde when stored in $CDCl_3$. The diterpenes isolated were cytotoxic to human cancer cell lines, with caseargrewiin F being the most active and the new clerodane, casearin X, the second active compound with $IC_{50}$ values comparable to the positive control doxorubicin. All isolated diterpenes showed lower activities against normal human cells than against cancer cell lines, which might indicate a possible selective action on cancer cells. Casearin X dialdehyde was not cytotoxic to cancer cells indicating that the occurrence of these CO groups at C(18) and C(19) is incompatible with the cytotoxic activity.

**Introduction.** – *Casearia sylvestris* SWARTZ (*Salicaceae*), commonly known as guaçatonga, is widely distributed in the form of a shrub or tree throughout South America, but especially within the Atlantic and Amazon forests and the Cerrado biomes of Brazil [1]. The plant is of considerable scientific interest by virtue of its extensive use in popular and traditional medicine for the treatment of snake bites and in wound healing, and also as an anti-ulcer, anti-pyretic, and topical antiseptic [2][3]. Pharmacological studies on leaf extracts of *C. sylvestris* have confirmed anti-ulcerogenic [4][5], anti-inflammatory [6], antivenom [7][8], and cytotoxic activities [9][10]. Additionally, no significant toxicological effects have been observed following oral administration of EtOH extracts of *C. sylvestris* leaves to animals [4], and no genotoxicity was detected when such extracts were assayed against hepatoma tissue culture (HTC) and lung fibroblast V-79 cells [11].

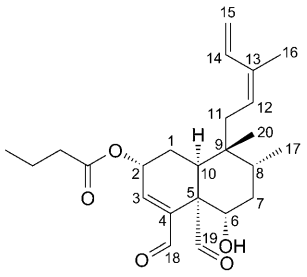
Phytochemical investigations of various species of *Casearia* have revealed the presence of a series of oxygenated tricyclic *cis*-clerodane diterpenes in which the tetrahydrofuran ring bears two acyloxy groups at C(18) and C(19) [12–21]. Such compounds are reportedly unstable under acidic conditions and readily degrade to form the corresponding dialdehyde [16]. The casearins A–T, isolated from the leaves

of *C. sylvestris*, represent a group of diterpenes of this type that are cytotoxic to V-79 cells in transgenic Chinese hamsters, 180A sarcoma cells implanted into rats, and mutant strains of *Saccharomyces cerevisiae* [9][10][17]. A number of related diterpenes, the casearvestrins A–C, have been isolated from the leaves and branches of *C. sylvestris* and found to exhibit anti-fungal activity against *Aspergillus niger* and cytotoxicity against human oral epidermoid carcinoma KB cell lines [18], whilst the roots of the same plant contain a similar type of diterpene with trypanosomicidal properties [19]. Moreover, less complex clerodane diterpenes, such as (+)-15-hydroxy-3-cleroden-2-one and (−)-hardwickiic acid [20], have been identified in the leaves of *C. sylvestris*. These compounds differ from the casearins with respect to the pattern of oxygenation and also in the configuration of the *A*/*B* ring junction, and between the Me(17) and Me(20) groups. It thus appears that *C. sylvestris* can biosynthesize both *cis*- and *trans*-clerodane diterpenoids, a characteristic that is common to various other species including *Adelanthus lindenbergianus* (Adelhantaceae) and *Grangea maderaspatana* (Asteraceae) [22][23].

In the present article, we report on the isolation of five casearins (including the new casearin X (**1**)) and of caseargrewiin F (**6**) from the EtOH extracts of the leaves of *C. sylvestris*, and on the formation of the degradation product of casearin X, **7**. The structures of the compounds were determined from spectrometric data and by comparison with spectral information available in the literature. The cytotoxicities of these compounds against human cancer and normal cell lines are also reported.




| | $R^1$ | $R^2$ | $R^3$ | $R^4$ |
|---|---|---|---|---|
| Casearin X (**1**)[1]) | $PrCO_2$ | $PrCO_2$ | OH | H |
| Casearin B (**2**)[1]) | MeO | AcO | AcO | $PrCO_2$ |
| Casearin D (**3**)[1]) | OH | $PrCO_2$ | OH | $PrCO_2$ |
| Casearin L (**4**)[1]) | MeO | $PrCO_2$ | AcO | OH |
| Casearin O (**5**)[1]) | MeO | $PrCO_2$ | AcO | $PrCO_2$ |
| Caseargrewiin F (**6**)[1]) | $PrCO_2$ | AcO | OH | H |




**7**[1])

**Results and Discussion.** – *Isolation and Structure Determination*. Fractionation of the EtOH extract of the leaves of *C. sylvestris* by solid-phase extraction (SPE) from silica gel/activated charcoal, followed by normal-phase low pressure column chromatography (LPCC) over silica gel and preparative reversed-phase ($C_{18}$) HPLC, allowed the purification of a new clerodane diterpene (**1**) together with the known compounds **2**–**6** [9][10][21].

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[1]) Arbitrary numbering. For systematic names, see *Exper. Part*.

Compound **1**, obtained as a white powder, was submitted to high-resolution time-of-flight-electrospray ionization mass spectrometry (HR-TOF-ESI-MS) in the positive-ion mode and exhibited $[M+Na]^+$ and $[M+K]^+$ ions with masses 555.2931 and 571.2659, respectively, that were consistent with the values 555.2928 and 571.2668, respectively, calculated on the basis of an empirical formula of $C_{30}H_{44}O_8$ for the molecular ion. The $^{13}$C-NMR of **1** presented 30 signals that were similar to those reported for other casearins [9][10][17][20]. A broad band in the IR at 3490 $cm^{-1}$, together with $^{13}$C- and $^1$H-NMR signals at $\delta$(C) 72.9 (C(6)[1])) and $\delta$(H) 4.16 (*dd*, $J$= 11.0, 5.0; H−C(6)), respectively, indicated the presence of a HO−CH group in the molecule. Similarly, an IR absorbance at 1752 $cm^{-1}$ and $^{13}$C signals at $\delta$(C) 170.0, 173.3, and 173.4 suggested the presence of one AcO and two butanoate ester groups, respectively (*Table 1*). The absorption band at 235 nm in the UV spectrum is characteristic of a conjugated diene in the side chain of the casearins [9][10][17][20], the presence of which was confirmed from signals observed in the olefinic region of the $^1$H-NMR showing *cis*/*trans* coupling of the $CH_2$ H-atoms of a terminal monosubstituted C=C bond ($\delta$(H) 5.11 (*d*, $J$=11.0, $H_a$−C(15)[1])), 5.24 (*d*, $J$=17.0, $H_b$−C(15))) with the vicinal H-atom ($\delta$(H) 6.79 (*dd*, $J$=17.0, 11.0, H−C(14))), and a signal at $\delta$(H) 5.62 (*d*, $J$=9.0, H−C(12)) which could be attributed to the H-atom attached to a trisubstituted C=C bond of a conjugated diene. The partial structure formed by the side chain linked at C(9) was confirmed from the observed HMBC correlations between H−C(11) and C(12), H−C(12) and C(13)/C(16), H−C(14) and C(16), H−C(15) and C(13), and Me(16) and C(13), together with the COSY correlations shown in the *Figure*. The (*Z*)-configuration of the C=C bond at C(12) was determined on the basis of the nuclear *Overhauser* (NOESY) effect observed between Me(16) and H−C(12).

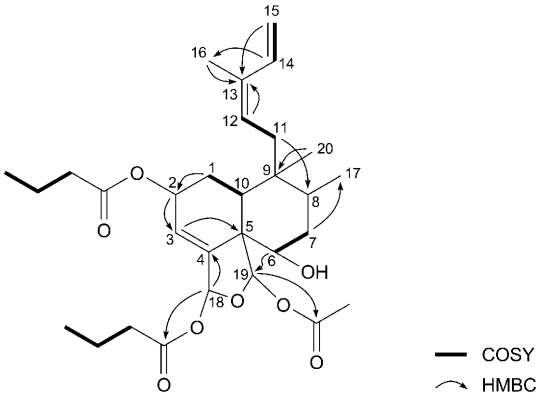


Figure. *Selected HMBC and COSY correlations observed for* **1**[1])

The signals of the O-bearing CH groups at $\delta$(C) 97.3 (C(18)) and 99.2 (C(19)), and of the two H-atoms at $\delta$(H) 7.14 (*s*, H−C(19)) and 7.43 (*t*, $J$=1.5, H−C(18)) indicated the existence of a diacetal system in the *C* ring. Moreover, HMBCs (*Figure*) between H−C(18) and the CO group of one butanoate group ($\delta$(C) 173.4), and between

Table 1. *$^1$H- and $^{13}$C-NMR Data for Compounds* **1** *and* **7**[1])

| | Casearin X (**1**)[a]) | | Casearin X dialdehyde (**7**)[b]) | |
|---|---|---|---|---|
| | $\delta$(C) | $\delta$(H) (multiplicity, $J$ [Hz]) | $\delta$(C) | $\delta$(H) (multiplicity, $J$ [Hz]) |
| 1 | 27.6 (*t*) | 2.04–2.08 (*m*), 2.10–2.14 (*m*) | 26.8 (*t*) | n.d.[c]) |
| 2 | 67.4 (*d*) | 5.71 (*t*, $J$=4.5) | 64.9 (*d*) | 5.05–5.19 (*m*) |
| 3 | 121.6 (*d*) | 6.32 (*d*, $J$=4.5) | 123.7 (*d*) | 6.95–6.97 (*m*) |
| 4 | 147.8 (*s*) | | 147.4 (*s*) | |
| 5 | 54.8 (*s*) | | 56.2 (*s*) | |
| 6 | 72.9 (*d*) | 4.16 (*dd*, $J$=11.0, 5.0) | 72.3 (*d*) | 3.74 (*dd*, $J$=11.5, 5.5) |
| 7 | 38.1 (*t*) | 1.86–1.94 (*m*) | 37.0 (*t*) | n.d. |
| 8 | 37.0 (*d*) | n.d. | 39.9 (*d*) | n.d. |
| 9 | 38.6 (*s*) | | 38.3 (*s*) | |
| 10 | 37.7 (*d*) | 2.74 (*dd*, $J$=13.5, 3.5) | 36.4 (*d*) | 2.31–2.34 (*m*) |
| 11 | 30.0 (*t*) | 1.88–1.94 (*m*), 2.47 (*dd*, $J$=16.0, 9.0) | 30.1 (*t*) | 1.59–1.64 (*m*) |
| 12 | 128.4 (*d*) | 5.62 (*d*, $J$=9.0) | 124.3 (*d*) | 5.46–5.49 (*m*) |
| 13 | 134.0 (*s*) | | 135.1 (*s*) | |
| 14 | 134.4 (*d*) | 6.79 (*dd*, $J$=17.0, 11.0) | 133.5 (*d*) | 6.59 (*dd*, $J$=17.0, 11.0) |
| 15 | 114.6 (*t*) | 5.11 (*d*, $J$=11.0), 5.24 (*d*, $J$=17.0) | 114.4 (*t*) | 5.07 (*d*, $J$=11.0), 5.16 (*d*, $J$=17.0) |
| 16 | 20.7 (*q*) | 1.82 (*s*) | 20.3 (*q*) | 1.77 (*s*) |
| 17 | 16.1 (*q*) | 0.86 (*d*, $J$=7.5) | 15.8 (*q*) | 0.90 (*d*, $J$=7.5) |
| 18 | 97.3 (*d*) | 7.43 (*t*, $J$=1.5) | 195.7 (*d*) | 9.92 (*d*, $J$=2.0) |
| 19 | 99.2 (*d*) | 7.14 (*s*) | 202.6 (*d*) | 9.38 (*s*) |
| 20 | 25.5 (*q*) | 0.85 (*s*) | 25.6 (*q*) | 0.84 (*s*) |
| *MeCO$_2$*–C(19) | 22.0 (*q*) | 2.03 (*s*) | | |
| | 170.0 (*s*) | | | |
| *C$_3$H$_7$CO$_2$*–C(18) | 13.8 (*q*) | 0.82 (*t*, $J$=7.5) | 13.6 (*q*) | 0.92 (*t*, $J$=7.0) |
| | 18.9 (*t*) | 1.55–1.64 (*m*) | 18.2 (*t*) | 1.58–1.62 (*m*) |
| | 36.7 (*t*) | 2.28–2.32 (*m*) | 36.2 (*t*) | 2.26 (*t*, $J$=7.0) |
| | 173.4 (*s*) | | 176.6 (*s*) | |
| *C$_3$H$_7$CO$_2$*–C(2) | 14.0 (*q*) | 0.87 (*t*, $J$=7.4) | | |
| | 19.2 (*t*) | 1.55–1.64 (*m*) | | |
| | 36.7 (*t*) | 2.16–2.20 (*m*) | | |
| | 173.3 (*s*) | | | |

[a]) Spectra were collected in ($D_5$)pyridine. [b]) Spectra were collected in $CDCl_3$. [c]) n.d. = not determined.

H–C(19) and the CO group of an AcO group ($\delta$(C) 170.0) defined the positions of these substituents. The second butanoate group was attached to the C-atom displaying a signal at $\delta$(C) 67.4 (C(2)).

The relative configurations at the eight stereogenic centers were determined from $^{13}$C-NMR spectral data, $^1$H-NMR coupling constants, and the results of NOE and NOESY experiments. The *cis*-configuration of the *A*/*B* ring junction was deduced from the chemical shift ($\delta$(C) 25.5) of the Me(20) group in accordance with previous reports [15][24][25]. The chemical shifts of C(5) and C(10) ($\delta$(C) 54.8 and 37.7, resp.) were similar to those described for the casearins and analogous compounds [9][10][26]. Moreover, the values of the coupling constants ($J$=3.5 and 13.5 Hz) of the *doublet* of *doublet*s at $\delta$(H) 2.74 suggested that H–C(10) was in axial orientation. Finally, nuclear

*Overhauser* (NOESY) effects between H–C(10) and H–C(12), and H–C(11) and H–C(19), indicated a similar orientation for the side chain at C(9).

In general, *cis*-clerodane diterpenes that are not substituted at positions C(7), C(8), C(10), C(11), and C(12) exhibit chemical shifts around $\delta$(C) 15.0 and 26.0 for the Me(17) and Me(20) groups, respectively, in the *trans* configuration [13][26–28], and values of *ca.* $\delta$(C) 16.0 and 18.0, respectively, for Me(17) and Me(20) groups in the *cis* configuration [20][21][28–30]. Casearins substituted at C(7) present chemical shifts for the Me(17) and Me(20) groups of *ca.* $\delta$(C) 11.0 and 26.0, respectively, which are most likely caused by the $\gamma$ shielding effect of the O-bearing substituent at C(7) [9][10]. Casearvestrins are not substituted at C(7), which explains the chemical shift value of $\delta$(C) 15.6 reported for C(17) [18]. In the case of **1**, which has no substituent at C(7), the signals at $\delta$(C) 16.1 and 25.5 (associated with the Me(17) and Me(20) groups, resp.) indicate a *trans* relationship between Me(17) and Me(20).

The chemical shift of $\delta$(C) 67.4 for the O-bearing H–C(2) group, and the coupling constant observed for the allylic H-atom H–C(2) ($\delta$(H) 5.71, *t*, $J=4.5$), are consistent with a $\beta$-*pseudo*-equatorial orientation of H–C(2) [13][19][26]. Furthermore, the observed nuclear *Overhauser* (NOESY) effects between H–C(2) and $H_\beta$–C(1) ($\delta$(H) 2.10–2.14 (*m*)), and H–C(6) and $H_\beta$–C(1), suggest that these H-atoms possess the same $\beta$-orientation. The 1,2-diaxial coupling of H–C(6) with $CH_2$(7), implied by the value of the coupling constant (11.0 Hz) for H–C(6), confirms the $\alpha$-equatorial position of the OH group. The observed homoallylic coupling ($^5J$) between H–C(18) ($\delta$(H) 7.43 (*t*, $J=1.5$)) and H–C(2) requires that the $\pi$-orbitals of the C=C bond overlap with the 1*s* orbitals of the H-atoms thus implying a $\beta$-orientation for H–C(18) [12]. Finally, the nuclear *Overhauser* effect between H–C(18) and H–C(19) confirms the $\alpha$-orientation of the butanoate group at C(18) and the AcO group at C(19).

The $^{13}$C-NMR data (recorded in ($D_5$)pyridine) for **2** ($C_{31}H_{44}O_{10}$), **3** ($C_{30}H_{44}O_9$), **4** ($C_{29}H_{42}O_9$), **5** ($C_{33}H_{48}O_{10}$), and **6** ($C_{28}H_{40}O_8$) were comparable with those previously determined in $CDCl_3$ [9][10][21]. Additional spectral data (UV, IR, HR-MS, $^1$H-NMR, HMQC, HMBC, COSY, and NOESY) confirmed the structures of these compounds.

*Formation and Structure Elucidation of Casearin X Dialdehyde* **7**. Some authors have reported, on the basis of NMR experiments, that clerodane diterpenes similar to the casearins are unstable in $CHCl_3$ and undergo opening of the diacetal ring with the formation of an 18,19-dialdehyde [16][18]. In this context, the caseamembrins F, J, and K isolated from *C. membranaceae* which are considered to be natural products [14][26], are most likely artefacts generated by the use of $CHCl_3$. In the present study, the NMR spectra of **1** measured in $CDCl_3$ presented signals of two aldehydic H-atoms replacing the H-atoms of the diacetal ring. When the stability over a period of one month of **1** dissolved in five different deuterated solvents (*i.e.* $CDCl_3$, ($D_6$)DMSO, ($D_6$)acetone, $CD_3OD$, and ($D_5$)pyridine) was studied, degradation was detected only in $CDCl_3$. It is probable that the formation of **7** from **1** was promoted by traces of acid present in $CDCl_3$ [16], and for this reason all further NMR experiments were conducted with ($D_5$)pyridine as solvent.

Compound **7**, obtained as a white powder, exhibited an $[M+H]^+$ ion in the positive-ion mode HR-TOF-ESI-MS with a mass of 403.2484 that was consistent with the value of 403.2484 calculated on the basis of an empirical formula of $C_{24}H_{34}O_5$ for

the molecular ion. The difference in the molecular formulae of **1** and **7** was consistent with the loss of a butanoate and an AcO group and the formation of a dialdehyde at positions 18 and 19[1]). The $^{13}$C-NMR data (including DEPT-135) of the dialdehyde recorded in $CDCl_3$ were similar to those of **1** measured in ($D_5$)pyridine, except of the signals of the diacetal C-atoms at C(18) and C(19) which were substituted by signals associated with aldehydic CO groups with signals at $\delta$(C) 195.7 (*d*) and 202.6 (*d*), respectively. Similar differences were observed in the $^1$H-NMR spectrum in which the signals associated with H–C(18) and H–C(19) appeared in the aldehydic region, *i.e.,* $\delta$(H) 9.92 (*d*, $J = 2.0$) and 9.38 (*s*), respectively. The structure and relative configuration of **7** was determined on the basis of data derived from 1D- and 2D-NMR experiments ($^1$H, $^{13}$C, DEPT-135 and -90, HMQC, HMBC, COSY, and NOESY) in a manner similar to that described above for casearin X.

Compound **7** is an isomer of caseanigrescens D-2, a degradation product of caseanegrescens D from *C. Nigrescens* [16], and the two aldehydes differ only with respect to the position of the conjugated diene in the side chain, being $\Delta^{12,14}$ in the former and $\Delta^{13(16),14}$ in the latter.

*Cytotoxicity against Tumor Cell Lines.* The isolated compounds **1**–**7** were evaluated for their cytotoxicities against the four human tumor cell lines MOLT-4 (leukaemia), MDA-MB-435 (melanoma), HCT-8 (colon), and SF-295 (glyoblastoma), and against L-929 (normal fibroblasts). The $IC_{50}$ values displayed in *Table 2* reveal that **6** was the most active of the compounds tested, showing stronger cytotoxicity against all tumor cell lines ($IC_{50} < 0.2$ μM) compared to normal L-929 cells ($IC_{50} = 1.09$ μM). These values are quite similar to those obtained to the positive control doxorubicin, that presented $IC_{50}$ values ranging from 0.02 to 0.83 μM to tumor cells, and 1.2 μM to L-929 cells. Considering the selectivity towards tumor cells, compound **6** was 12.1 times more active against MOLT-4 cells than against L-929, while doxorubicin was 24 times. However, if it was considered for example breast and glyoblastoma cells, while doxorubicin was only 1.4 and 3.0 times more active in tumor cells, **6** was 8.4 and 6.4 times more active, respectively. In the previous evaluation of cytotoxicity against cancer cell lines, compound **6** was inactive against KB cells (oral epidermoid carcinoma), showed significant cytotoxicity against BC1 cells (human breast cancer) and minor activity against NCI-H187 cells (human small cell lung cancer) [21]. Further studies are necessary to establish the selectivity profile for this compound. Whilst **1** is not as cytotoxic as **6**, it exhibited a good anti-proliferative potential against tumor cells ($IC_{50} <$ 1 μM). Compounds **2**–**5** displayed only moderate cytotoxicities, although it is worth noting that, in every case, cytotoxicity against normal cells was lower than that against tumor cells, indicating a possible selective action on cancer cells. Compound **7**, on the other hand, was ineffective against most of the tumor lines. Additionally, none of the compounds tested caused haemolysis even at the highest concentration (200 μg/ml), suggesting that the mechanism of cytotoxicity is probably related to a more specific pathway.

Cytotoxic activities against human tumor cell lines have been studied for other clerodane diterpene 18,19-dialdehydes obtained from species of *Casearia.* Thus, caseamembrin F was found to be cytotoxic to PC-3 (prostate cancer) cells but not to Hep3B (hepatoma) cells [26]. Moreover, the caseamembrins J and K exhibited no cytotoxicity against KB (oral epidermoid carcinoma), HeLa (cervical epithelial

Table 2. *Cytotoxic Activities of Clerodane Diterpenes Isolated from the Leaves of* Casearia sylvestris Swartz *and Casearin X Dialdehyde against Human Cancer and Normal Cell Lines Determined by MTT Assay*

| | L-929 | MOLT-4 | | MDA/MB-435 | | HCT-8 | | SF-295 | |
|---|---|---|---|---|---|---|---|---|---|
| | $IC_{50}$ [μM] | $IC_{50}$ [μM] | Sel.[a]) | $IC_{50}$ [μM] | Sel. | $IC_{50}$ [μM] | Sel. | $IC_{50}$ [μM] | Sel. |
| Doxorubicin[b]) | 1.20 (0.92–1.38)[c]) | 0.05 (0.03–0.08) | 24.0 | 0.83 (0.57–1.10) | 1.4 | 0.02 (0.02–0.03) | 60.0 | 0.40 (0.32–0.42) | 3.0 |
| **1** | 1.52 (1.17–1.98) | 0.22 (0.18–0.31) | 6.9 | 0.35 (0.34–0.38) | 4.3 | 0.97 (0.88–1.03) | 1.6 | 0.43 (0.35–0.50) | 3.5 |
| **2** | 6.50 (4.35–9.70) | 1.44 (0.71–2.93) | 4.5 | 1.75 (1.42–2.15) | 3.7 | 5.11 (4.41–5.92) | 1.3 | 2.70 (2.06–3.52) | 2.4 |
| **3** | 3.59 (3.06–4.10) | 0.54 (0.25–1.16) | 6.6 | 1.29 (1.13–1.42) | 2.8 | 2.80 (2.39–3.20) | 1.3 | 1.53 (1.26–1.82) | 2.3 |
| **4** | 29.87 (25.85–34.41) | 3.57 (1.72–7.40) | 8.4 | 7.67 (5.61–10.48) | 3.9 | 16.93 (15.01–18.95) | 1.8 | 25.51 (13.66–46.80) | 1.2 |
| **5** | 18.53 (14.69–23.99) | 10.34 (7.05–14.01) | 1.8 | 6.63 (5.23–8.42) | 2.8 | 11.59 (8.80–15.27) | 1.6 | 17.30 (14.24–20.99) | 1.1 |
| **6** | 1.09 (0.85–1.15) | 0.09 (0.04–0.16) | 12.1 | 0.13 (0.11–0.13) | 8.4 | 0.15 (0.13–0.16) | 7.3 | 0.17 (0.14–0.18) | 6.4 |
| **7** | >62.1 | >62.1 | nc[d]) | 52.61 (48.31–57.30) | nc | 34.48 (31.30–37.98) | nc | >62.1 | nc |

[a]) Selectivity ($IC_{50}$ L-929/$IC_{50}$ tumor cell). [b]) Positive control. [c]) 95% confidence intervals, obtained by non-linear regression for MOLT-4 (leukemia), MDA-MB-435 (melanoma), HCT-8 (colon), SF-295 (glyoblastoma), and L-929 (normal fibroblasts) cells from three independent experiments. [d]) Not calculated.

carcinoma), and Hepa59T/VGH (liver carcinoma) cells [14], and casearnegrescens A-2 and D-2 were not active against A2780 (ovarian cancer) cells [16]. It would appear, therefore, that the presence of the dialdehyde function in these clerodane diterpenes results in the total or partial loss of cytotoxicity indicating that the occurrence of the CO groups at positions 18 and 19 is incompatible with the cytotoxic activity. In addition, the lack of an AcO group at C(6) and the lack of an O-bearing function at C(7) in the strongest active compounds (casearin X and caseargrewiin F) agree with the discussion of SAR in previous reports [10][14].

In conclusion, caseargrewiin F and the new compound casearin X showed relevant cytotoxic activity against the leukemia, melanoma, colon, and glyoblastoma cancer cell lines, comparable with the positive control doxorubicin. In addition, the lower activities of these compounds against normal human cells can be indicative of a selective action on cancer cells.

The authors are grateful to the *Fundação de Amparo à Pesquisa do Estado de São Paulo* (*FAPESP*), *Conselho Nacional de Desenvolvimento Científico e Tecnológico* (*CNPq*), *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior* (*CAPES*), and *Instituto Florestal da Secretaria do Meio Ambiente do Estado de São Paulo* for support of the project. Thanks are due to the Laboratório de Espectrometria de Massas, Faculdade de Ciências Farmacêuticas, Universidade de São Paulo, Ribeirão Preto, Brazil, for the MS determinations.

## Experimental Part

*General*. TLC: *Merck* glass-backed silica gel *G60* layers (20 × 20 cm; 0.25 mm thick), eluted with hexane/AcOEt/$^{i}$PrOH (78 : 20 : 2, *v*/*v*/*v*), and visualized with anisaldehyde/sulfuric acid reagent. Anal. reversed-phase HPLC: *Varian ProStar* system (comprising a model *240* pump, a *410* autosampler, and a *330* photodiode array detector) fitted with a *Phenomenex Luna C-18* column (250 × 4.6 mm i.d; 5 μm), with control and data handling managed by Star Chromatography software. Prep. HPLC: *Varian Dynamax* model *SD-1* pump, *ProStar 320* UV-VIS detector, with Star Integrator software, and a *Phenomenex Luna C-18* column (250 × 21.2 mm i.d.; 10 μm). $[\alpha]_D$: *Perkin-Elmer 14 Polamat* polarimeter. UV Spectra: *Cary 13E* instrument. IR Spectra: *Perkin-Elmer FTIR 1600* spectrophotometer, in KBr discs. NMR Spectra: *Varian INOVA 500 11.7 T* instrument with pyridine or $CHCl_3$ as internal standard. MS: *Bruker* ESI-quadrupole-TOF instrument (*UltrOTOFQ*).

The mobile phases for SPE, LPCC, and TLC were prepared using anal. grade solvents from *Synth*, whilst those for HPLC were prepared using chromatographic grade MeOH (*J. T. Baker*) and *Millipore Milli Q* Ultrapure water (18 MΩ).

*Plant Material*. Leaves of *Casearia sylvestris* Swartz (Salicaceae) were collected at the Parque Estadual Carlos Botelho (São Miguel Arcanjo, São Paulo, Brazil) in March and July 2004. Voucher specimens are deposited with the Herbarium 'Maria Eneida P. Kaufmann' (Instituto Botânico do Estado de São Paulo, São Paulo, Brazil) with the reference numbers AGS04, AGS05, AGS06, AGS13, and AGS19.

*Extraction and Isolation of Compounds* **1**–**6**. Dried and powdered leaves of *C. sylvestris* (20.5 kg) were extracted with EtOH (*ca.* 200 l) in a stainless steel extractor with solvent re-circulation for *ca.* 24 h at 40°. The crude extract was concentrated under reduced pressure to yield 1540.0 g of dry residue. A portion (473.6 g) of the residue was separated by SPE from $SiO_2$ (60–200 μm, *Merck*)/activated charcoal (*Synth*) (1 : 1, *w*/*w*) by elution with hexane/AcOEt (95 : 5, *v*/*v*), AcOEt, and MeOH to afford three fractions (*SPE1*–*SPE3*). *SPE2* (16.0 g) was submitted to normal-phase LPCC over $SiO_2$ (40–63 μm; *Merck*) eluted with a gradient of hexane/AcOEt/$^{i}$PrOH of increasing polarity (78 : 20.5 : 1.5 to 60 : 37.3 : 2.7, *v*/*v*/*v*): 45 fractions were collected and monitored by TLC, anal. HPLC-UV, and NMR. *Frs. 5*, *6*, *8*, *9*, *15*–*19*, *25*, and *31*–*36* were submitted to prep. reversed-phase HPLC ($C_{18}$ column) with

MeOH/$H_2O$ mixtures as the mobile phase to yield **1** (1250.0 mg), **2** (250.6 mg), **3** (80.0 mg), **4** (43.0 mg), **5** (128.5 mg), and **6** (850.0 mg).

*Cytotoxicity Assay.* The cytotoxic potential of the isolated compounds and doxorubicin was evaluated by the MTT assay [31] against five cell lines (MOLT-4, MDA-MB-435, HCT-8, SF-295, and L-929) obtained from the National Cancer Institute (Bethesda, MD, USA). All cell lines were maintained in *RPMI 1640* medium supplemented with 10% fetal bovine serum, 2 mM glutamine, 100 U/ml penincillin, and 100 μg/ml streptomycin, at 37° with 5% $CO_2$. Tumor cell growth was quantified by the ability of living cells to reduce the yellow dye 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) to a purple formazan product. Briefly, cells were plated in 96-well plates ($0.7 \times 10^5$ cells/well for adherent cells and $0.3 \times 10^5$ cells/well for suspended cells) and the compounds (0.019–25 μg/ml) were added to each well. After 69 h of incubation, the supernatant was replaced by fresh medium containing MTT 0.5 mg/ml. After 3 h, the plates were centrifuged, and the MTT formazan product was dissolved in 150 μl DMSO, and absorbance was measured at 595 nm (*DTX-880*, *Beckman Coulter*). Doxorubicin was used as pos. control.

*Statistical Analysis.* The $IC_{50}$ values and their 95% confidence intervals (CI 95%) were obtained by nonlinear regression using the GRAPHPAD program (*Intuitive Software for Science*, San Diego, CA), from three independent experiments.

*Haemolytic Assay.* The isolated compounds were assayed for haemolytic activity at concentrations in the range 1.56–200 μg/ml according to the method of *Moreira et al.* [32]. Analytes were incubated in 96-well plates for 60 min at r.t. with suspensions of mouse erythrocytes (2%) in NaCl (0.85%) containing 10 mM $CaCl_2$. Following centrifugation, the levels of haemoglobin in the supernatants were determined spectrophotometrically at 540 nm.

*Casearin X* (=(*1*R*,*3*S*,*5*S*,*6a*R*,*7*S*,*8*S*,*10*R*,*10a*R*)*-1-(Acetyloxy)-3,5,6,6a,7,8,9,10-octahydro-10-hydroxy-7,8-dimethyl-7-[(2Z)-3-methylpenta-2,4-dien-1-yl]naphtho[1,8a-c]furan-3,5-diyl Dibutanoate*; **1**). White powder. $[\alpha]_D^{20} = +54.3$ ($c = 1.00$, MeOH). UV ($c = 0.019$, MeOH): 235 ($16.7 \times 10^3$ $cm^3$ $mol^{-1}$ $cm^{-1}$). IR (KBr disc): 3490, 2967, 2936, 2887, 1752, 1461, 1374, 1225, 1173. $^1$H- and $^{13}$C-NMR: *Table 1*. HR-TOF-ESI-MS: 555.2931 ($[M+Na]^+$, $C_{30}H_{44}NaO_8^+$; calc. 555.2934), 571.2659 ($[M+K]^+$, $C_{30}H_{44}KO_8^+$; calc. 571.2673).

*Casearin B* (=(*1*R*,*3*S*,*5*S*,*6a*S*,*7*R*,*8*R*,*9*S*,*10*S*,*10a*R*)*-1,3,10-Tris(acetyloxy)-3,5,6,6a,7,8,9,10-octahydro-5-methoxy-7,8-dimethyl-7-[(2Z)-3-methylpenta-2,4-dien-1-yl]naphtho[1,8a-c]furan-9-yl Butanoate*; **2**). White powder. $[\alpha]_D^{20} = +42.6$ ($c = 1.00$, MeOH). UV ($c = 0.024$, MeOH): 234 ($18.1 \times 10^3$ $cm^3$ $mol^{-1}$ $cm^{-1}$). IR (KBr disc): 2975, 2940, 2886, 2826, 1751, 1457, 1374, 1227, 1176, 1076, 1023. $^{13}$C-NMR (125 MHz, ($D_5$)pyridine)[1]): 173.1 (Me−$CH_2$−$CH_2$−$CO_2$); 170.8, 170.5, 169.6 (3 Me*C*$O_2$); 141.5 (C(4)); 134.5 (C(13)); 134.2 (C(14)); 127.0 (C(12)); 126.5 (C(3)); 115.0 (C(15)); 98.3 (C(19)); 95.9 (C(18)); 74.9 (C(6)); 73.4 (C(7)); 73.0 (C(2)); 57.1 (MeO); 53.7 (C(5)); 41.5 (C(8)); 40.0 (C(9)); 37.6 (C(10)); 36.5 (Me−$CH_2$−*C*$H_2$−$CO_2$); 30.9 (C(11)); 26.4 (C(1)); 25.8 (C(20)); 21.6, 21.3 (3 *Me*$CO_2$); 20.4 (C(16)); 19.0 (Me−*C*$H_2$−$CH_2$−$CO_2$); 14.1 (*Me*−$CH_2$−$CH_2$−$CO_2$); 11.4 (C(17)). HR-TOF-ESI-MS: 599.2853 ($[M+Na]^+$, $C_{31}H_{44}NaO_{10}^+$; calc. 599.2826), 615.2580 ($[M+K]^+$, $C_{31}H_{44}KO_{10}^+$; calc. 615.2566).

*Casearin D* (=(*1*R*,*3*S*,*5*S*,*6a*R*,*7*R*,*8*R*,*9*S*,*10*S*,*10a*R*)*-1-(Acetyloxy)-3,5,6,6a,7,8,9,10-octahydro-5,10-dihydroxy-7,8-dimethyl-7-[(2Z)-3-methylpenta-2,4-dien-1-yl]naphtho[1,8a-c]furan-3,9-diyl Dibutanoate*; **3**). White powder. $[\alpha]_D^{20} = +41.6$ ($c = 1.00$, MeOH). UV (MeOH): 235. $^{13}$C-NMR (125 MHz, ($D_5$)pyridine)[1]): 173.5, 173.7 (2 Me−$CH_2$−$CH_2$−*C*$O_2$); 169.7 (Me*C*$O_2$); 142.6 (C(4)); 134.3 (C(13)); 134.0 (C(14)); 127.8 (C(12)); 127.5 (C(3)); 114.7 (C(15)); 99.3 (C(19)); 97.3 (C(18)); 76.5 (C(7)); 74.8 (C(6)); 63.7 (C(2)); 55.0 (C(5)); 41.8 (C(8)); 40.1 (C(9)); 36.8, 36.7 (2 Me−$CH_2$−*C*$H_2$−$CO_2$); 36.6 (C(10)); 31.1 (C(1)); 31.0 (C(11)); 26.1 (C(20)); 21.8 (*Me*$CO_2$); 20.5 (C(16)); 19.1, 18.9 (2 Me−*C*$H_2$−$CH_2$−$CO_2$); 14.1, 13.9 (2 *Me*−$CH_2$−$CH_2$−$CO_2$); 11.8 (C(17)). HR-TOF-ESI-MS: 571.2875 ($[M+Na]^+$, $C_{30}H_{44}NaO_9^+$; calc. 571.2883).

*Casearin L* (=(*1*R*,*3*S*,*5*S*,*6a*R*,*7*R*,*8*R*,*9*S*,*10*S*,*10a*R*)*-1,10-Bis(acetyloxy)-3,5,6,6a,7,8,9,10-octahydro-9-hydroxy-5-methoxy-7,8-dimethyl-7-[(2Z)-3-methylpenta-2,4-dien-1-yl]naphtho[1,8a-c]furan-3-yl Butanoate*; **4**). Colorless oil. UV (MeOH): 234. $^{13}$C-NMR (125 MHz, ($D_5$)pyridine)[1]): 173.4 (Me−$CH_2$−$CH_2$−*C*$O_2$); 170.6, 169.5 (2 Me*C*$O_2$); 144.1 (C(4)); 134.0 (C(13)); 133.9 (C(14)); 127.3 (C(12)); 123.8 (C(3)); 114.5 (C(15)); 98.8 (C(19)); 97.0 (C(18)); 76.1 (C(7)); 74.5 (C(6)); 73.0 (C(2)); 56.7 (Me); 54.7 (C(5)); 41.5 (C(8)); 39.8 (C(9)); 36.7 (C(10)); 36.5 (Me−$CH_2$−*C*$H_2$−$CO_2$); 30.7

(C(11)); 26.3 (C(1)); 25.7 (C(20)); 21.5, 21.2 (2 *Me*$CO_2$); 20.5 (C(16)); 18.9 (Me−*C*$H_2$−$CH_2$−$CO_2$); 13.8 (*Me*−$CH_2$−$CH_2$−$CO_2$); 11.5 (C(17)).

*Casearin O* (=(*1*R*,*3*S*,*5*S*,*6a*R*,*7*R*,*8*R*,*9*S*,*10*S*,*10a*R*)*-1,10-Bis(acetyloxy)-5-methoxy-7,8-dimethyl-7-[(2Z)-3-methylpenta-2,4-dien-1-yl]-3,5,6,6a,7,8,9,10-octahydronaphtho[1,8a-c]furan-3,9-diyl Dibutanoate*; **5**). Colorless oil. UV (MeOH): 233. $^{13}$C-NMR (125 MHz, ($D_5$)pyridine)[1]): 173.3, 173.1 (2 Me−$CH_2$−$CH_2$−*C*$O_2$); 170.5, 169.5 (2 Me*C*$O_2$); 144.7 (C(4)); 134.5 (C(13)); 134.2 (C(14)); 126.9 (C(12)); 126.4 (C(3)); 115.0 (C(15)); 98.4 (C(19)); 95.7 (C(18)); 74.9 (C(6)); 73.4 (C(7)); 72.9 (C(2)); 57.0 (MeO); 53.8 (C(5)); 41.5 (C(8)); 40.0 (C(9)); 37.6 (C(10)); 36.6, 36.5 (2 Me−$CH_2$−*C*$H_2$−$CO_2$); 30.9 (C(11)); 26.4 (C(1)); 25.8 (C(20)); 21.7, 21.3 (2 *Me*$CO_2$); 20.4 (C(16)); 19.0, 18.8 (2 Me−*C*$H_2$−$CH_2$−$CO_2$); 14.1, 13.8 (2 *Me*−$CH_2$−$CH_2$−$CO_2$); 11.4 (C(17)). HR-TOF-ESI-MS: 627.3161 ($[M+Na]^+$, $C_{33}H_{48}NaO_{10}^+$; calc. 627.3145), 643.2918 ($[M+K]^+$, $C_{33}H_{48}KO_{10}^+$; calc. 643.2885).

*Caseargrewiin F* (=(*1*R*,*3*S*,*5*S*,*6a*R*,*7*S*,*8*R*,*10*R*,*10a*R*)*-1,3-Bis(acetyloxy)-3,5,6,6a,7,8,9,10-octahydro-10-hydroxy-7,8-dimethyl-7-[(2Z)-3-methylpenta-2,4-dien-1-yl]naphtho[1,8a-c]furan-5-yl Butanoate*; **6**). White powder. $[\alpha]_D^{20} = +55.1$ ($c = 1.00$, MeOH). UV ($c = 0.018$, MeOH): 235 ($15.9 \times 10^3$ cm$^3$ mol$^{-1}$ cm$^{-1}$). IR (KBr disc): 3460, 2967, 2930, 2878, 1738, 1376, 1230, 1175. $^{13}$C-NMR (125 MHz, ($D_5$)pyridine)[1]): 173.0 (Me−$CH_2$−$CH_2$−*C*$O_2$); 170.5, 169.8 (2 Me*C*$O_2$); 147.4 (C(4)); 134.1 (C(14)); 133.7 (C(13)); 128.1 (C(12)); 121.3 (C(3)); 114.3 (C(15)); 98.8 (C(19)); 97.1 (C(18)); 72.5 (C(6)); 67.0 (C(2)); 54.5 (C(5)); 38.3 (C(9)); 37.8 (C(7)); 37.4 (C(8)); 36.7 (C(10)); 36.4 (Me−$CH_2$−*C*$H_2$−$CO_2$); 29.7 (C(11)); 27.4 (C(1)); 25.2 (C(20)); 20.4 (C(16)); 21.7, 21.1 (2 *Me*$CO_2$); 18.9 (Me−*C*$H_2$−$CH_2$−$CO_2$); 15.8 (C(17)); 13.7 (*Me*−$CH_2$−$CH_2$−$CO_2$). HR-TOF-ESI-MS: 527.2619 ($[M+Na]^+$, $C_{28}H_{40}NaO_8^+$; calc. 527.2621), 543.2362 ($[M+K]^+$, $C_{28}H_{40}KO_8^+$; calc. 543.2360).

*Casearin X Dialdehyde* (=(*2*R*,*4a*S*,*5*S*,*7*R*,*8*R*,*8a*S*)*-4,4a-Diformyl-1,2,4a,5,6,7,8,8a-octahydro-5-hydroxy-7,8-dimethyl-8-[(2Z)-3-methylpenta-2,4-dien-1-yl]naphthalen-2-yl Butanoate*; **7**). Compound **1** (10.0 mg) was submitted to degradation in $CDCl_3$ (0.7 ml) in an NMR tube to afford 9.0 mg of a white powder. $^1$H- and $^{13}$C-NMR: *Table 1*. HR-TOF-ESI-MS: 403.2484 ($[M+H]^+$, $C_{24}H_{35}O_5^+$; calc. 403.2484).

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*Received December 11, 2008*