# A New Oleanolic Acid Derivative from *Securinega tinctoria*

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**Abstract**

The triterpenoid constituents of the chloroform extract of previously defatted material of the stems of *Securinega tinctoria* were examined and, besides β-sitosterol β-D-glucopyranoside, a new oleanolic acid derivative was isolated and identified as 3β-(*p*-hydroxy-*trans*-cinnamoyloxy)olean-12-en-28-oic acid.

**Key words**

*Securinega tinctoria*, Euphorbiaceae, triterpenoids, 3β-(*p*-hydroxy-*trans*-cinnamoyloxy)olean-12-en-28-oic acid, β-sitosterol β-D-glucopyranoside.

## Introduction

The Euphorbiaceae family, and particularly the *Euphorbia* species, has been the subject of numerous studies about triterpenoids and some important chemotaxonomic relationships were established (1). However, the triterpenoids of the genus *Securinega* remained almost unknown and only friedelin has been reported in the bark of *Securinega leucopyrus* (2).

*Securinega tinctoria* (L.) Rothm. is a spiny shrub with caducous leaves, endemic of the Iberian peninsula and growing on sandy river-banks. Like other plants of this genus, it shows a high content of securinega alkaloids and was considered a medicinal plant due to the use of these alkaloids in the treatment of paralysis, impotence connected with nervous disorders, and hypotension (3).

An examination of the triterpenoid content of this plant led us to the isolation of the known β-sitosterol β-D-glucopyranoside and the new 3β-(*p*-hydroxy-*trans*-cinnamoyloxy)olean-12-en-28-oic acid from the chloroform extract.

The analogous compound from ursolic acid was already known from *Tripetaleia paniculata* (Ericaceae) (4, 5) but as far as we are aware this is the first report on the isolation of our compound from a natural source.

## Materials and Methods

### *Plant material*

The stems of *Securinega tinctoria* (L.) Rothm. were collected near the Coa river in Alto Douro (Portugal). A voucher specimen was deposited in the Herbal collection of Vila Real with the number 2047.

### *Instruments*

Melting points are uncorrected; IR spectra are recorded with an FTIR Mattson Polaris™; MS are recorded on a Kratos MS 25 RS; $^1$H- and $^{13}$C-NMR: Bruker (200 MHz) and General Electric (300 MHz) with TMS as internal reference; specific rotation determined with a Bellingham & Stanley polarimeter with 1 ml cells (2.5 cm long); elemental analysis obtained with a Carlo Erba 1106 Elemental Analyser.

### *Extraction and isolation*

The stems of *S. tinctoria* were dried and powdered. The powder (3 kg) was first extracted with *n*-hexane in a Soxhlet apparatus and then with chloroform. The dark green-brown chloroform extract (49 g) was roughly fractionated over silica gel (300 g) with $CHCl_3$ (fraction I: 35.8 g) and MeOH (fraction II: 11.8 g). Fraction I, upon rechromatography over a silica gel column with $CHCl_3$, and repeated cold MeOH washings afforded scales of a crystalline solid **1** (190 mg). Fraction II, after column chromatography (silica gel, $CHCl_3$-MeOH, 9 : 1), provided crude **2** which after acetylation was purified by column chromatography and crystallization yielding **2a**.

### *3β-(p-Hydroxy-trans-cinnamoyloxy)olean-12-en-28-oic acid* (**1**)

Crystallized from $CHCl_3$ as colourless scales, m.p. 292–296 °C (dec.); [found C, 77.2, H, 9.0, required for $C_{39}H_{54}O_5$, C, 77.7, H, 9.0]; $[\alpha]_D$: +104° (*c* 0.2, dioxane); IR $\nu_{max}^{KBr}$ cm$^{-1}$: 3346 (OH), 3241 and 2652 (COOH), 1690 and 1265 (C=O acid), 1708 (C=O ester), 1631 and 815 (C=C), 1518 (aromatic); EIMS (probe) 70 eV, *m/z* (rel. int.): $[M]^+$ absent, 456 (2), 442 (16), 438 [M – coumaroyloxy residue]$^+$ (6), 424 (6), 411 (23), 248 [retro-Diels-Alder fragment]$^+$ (19), 234 (16), 220 (16), 207 (35), 203 [retro-Diels Alder-fragment – COOH]$^+$ (53), 163 [coumaroyloxy residue]$^+$ (23), 147 (52), 135 (52), 119 (61), 107 (69), 95 (77), 81 (83), 69 (90), 55 (95), 43 (100); $^1$H-NMR (300 MHz, $CDCl_3$ + 2 drops DMSO-$d_6$, TMS): 7.59 (1H, d, $J$ = 16 Hz, H-3′), 7.39 (2H, d, $J$ = 8.5 Hz, H-6′ and H-8′), 6.85 (2H, d, $J$ = 8.5 Hz, H-5′ and H-9′), 6.26 (1H, d, $J$ = 16 Hz, H-2′), 5.29 (1H, br. s, H-12), 4.61 (1H, m, Hα-3), 3.93 (1H, br. s, OH), 2.84 (1H, dd, $J_1$ = 10 Hz, $J_2$ = 3 Hz, Hβ-18), 1.15 (3H, s, Me), 0.96 (s), 0.93 (s), 0.90 (s), (15 H, 5 Me), 0.81 (3H, s, Me); $^{13}$C-NMR: see Table **1**.

### *Acetylation of* **1**

Compound **1** (60 mg) was acetylated ($Ac_2O$/pyridine, overnight, room temp.) and afterwards purified by preparative TLC (silica gel, $CHCl_3$-MeOH, 9 : 1) yielding **1a** (48 mg).

### *3β-(p-Acetyloxy-trans-cinnamoyloxy)-olean-12-en-28-oic acid* (**1a**)

Crystallized from MeOH, m.p. 182–183 °C; $[\alpha]_D$: +53° (*c* 0.6, $CHCl_3$); IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3171 and 2652 (COOH), 1690 and 1265 (C=O acid), 1768 (C=O acetate), 1708 and 1204 (C=O ester), 1637 and 836 (C=C), 1507 (aromatic); EIMS (probe) 70 eV, *m/z* (rel. int.): 644 $[M]^+$ (< 1), 598 [M – (COOH + H)]$^+$ (1), 438 [M – acetylcinnamoyloxy]$^+$ (5), 423 (2), 396 (11), 248 [retro-Diels-Alder fragment]$^+$ (18), 203 [retro-Diels-Alder fragment – COOH]$^+$ (18), 190 (16), 147 (31), 134 (12), 119 (18), 107 (17), 95 (19), 91 (25), 84 (100), 69 (27); $^1$H-NMR (200 MHz, $CDCl_3$, TMS): 7.64 (1H, d, $J$ = 16 Hz, H-3′), 7.54 (2H, d, $J$ = 8.5 Hz, H-6′ and H-8′), 7.12 (2H, d, $J$ = 8.5 Hz, H-5′ and H-9′), 6.40 (1H, d, $J$ = 16 Hz, H-2′), 5.28 (1H, br. s, H-12), 4.64 (1H, m, Hα-3), 2.83 (1H, dd, $J_{ae}$ = 3 Hz, $J_{aa}$ = 10 Hz, Hβ-18), 2.31 (3H, s, Me-OAc), 1.14 (3H, s, Me), 0.97 (3H, s, Me), 0.93 (s), 0.91 (s) (12H, 4Me), 0.76 (3H, s, Me); $^{13}$C-NMR: see Table **1**.

### *Alkaline hydrolysis of* **1** *and* **1a**

Compound **1** (30 mg) was treated with 0.5 M KOH in MeOH at 60 °C for 1 h. After usual work-up, the aqueous phase was extracted with $Et_2O$ and dried yielding **1b**. After neutralization, the aqueous phase afforded a product that was identified (co-TLC) as *p*-coumaric acid. Compound **1a** was submitted to the same treatment yielding, from the ethereal phase, the same triterpenic acid (**1b**) (co-TLC, IR, $^1$H-NMR).

### *3β-Hydroxyolean-12-en-28-oic acid* (**1b**)

Crystallized from MeOH, m.p. 278 °C (dec.); IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3444, 3165, 2931, 2861, 2650, 1694, 1455, 1391, 1265, 1181, 1033, 991; EIMS (probe) 70 eV, *m/z* (rel. int.): 456 $[M]^+$ (1), 441 [M – $CH_3$]$^+$ (< 1), 438 [M – $H_2O$]$^+$ (1), 423 [M – ($CH_3$ + $H_2O$)]$^+$ (1), 410 [M – (COOH + H)]$^+$ (3), 248 [retro-Diels-Alder fragment]$^+$ (100), 233 (10), 207 (21), 203 [retro-Diels-Alder fragment – COOH]$^+$ (89), 189 (16), 175 (12), 149 (12), 133 (19), 105 (18), 95 (14), 81 (16), 69 (22), 55 (18), 43 (17); $^1$H-NMR (200 MHz, $CDCl_3$, TMS): 5.28 (1H, br. s, H-12), 3.24 (1H, m, Hα-3), 2.83 (1H, br. d, $J$ = 13 Hz, Hβ-18), 1.13 (3H, s, Me), 0.99 (3H, s, Me), 0.93 (s), 0.92 (s) (9H, 3 Me), 0.77 (s), 0.75 (s) (6H, 2 Me); $^{13}$C-NMR (75.6 MHz, $CDCl_3$): 15.3 (C-24), 15.5 (C-25), 17.2 (C-26), 18.1 (C-6), 22.8 (C-11), 23.4 (C-16), 23.6 (C-29), 25.9 (C-27), 26.9 (C-2), 27.6 (C-15), 28.1 (C-23), 30.7 (C-20), 32.5 (C-7), 32.6 (C-22), 33.1 (C-30), 33.8 (C-21), 37.1 (C-10), 38.3 (C-1), 38.8 (C-4), 39.3 (C-8), 41.0 (C-18), 41.6 (C-14), 45.8 (C-17), 46.5 (C-19), 47.6 (C-9), 55.2 (C-5), 79.0 (C-3), 122.5 (C-12), 143.6 (C-13), 183.5 (C-28).

### *Acetylation of* **1b**

Compound **1b** was acetylated ($Ac_2O$/pyridine) to give product **1c** (12 mg).

### *3β-Acetoxyolean-12-en-28-oic acid* (**1c**)

Crystallized from MeOH, m.p. 266–267 °C; IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3165, 2947, 2876, 2650, 1736, 1693, 1462, 1365, 1265, 1244, 1180, 1027, 825; EIMS (probe) 70 eV, *m/z* (rel. int.): 498 (0.5), 483 (0.3), 452 (2), 438 (4), 423 (2), 395 (1), 248 (100), 233 (11), 203 (98), 189 (30), 175 (18), 161 (9), 147 (12), 133 (25), 119 (22), 105 (24), 95 (18), 81 (22), 69 (31), 55 (24), 43 (76); $^1$H-NMR (300 MHz, $CDCl_3$, TMS): 5.27 (1H, br. s, H-12), 4.49 (1H, t like, Hα-3), 2.82 (1H, dd, $J$ = 4 Hz, $J$ = 13.5 Hz, Hβ-18), 2.05 (3H, s, Me-OAc), 1.12 (3H, s, Me) 0.94 (s), 0.93 (s), 0.90 (s), 0.86 (s), 0.85 (s) (15H, 5 Me); 0.74 (3H, s, Me); $^{13}$C-NMR (75.6 MHz, $CDCl_3$): 15.4 (C-25), 16.6 (C-24), 17.2 (C-26), 18.1 (C-6), 21.3 ($CH_3$-acetate), 22.8 (C-11), 23.4 (C-16), 23.5 (C-2), 23.6 (C-29), 25.9 (C-27), 27.6 (C-15), 28.0 (C-23), 30.6 (C-20), 32.4 (C-22), 32.5 (C-7), 33.0 (C-30), 33.8 (C-21), 37.0 (C-10), 37.7 (C-4), 38.0 (C-1), 39.4 (C-8), 40.8 (C-18), 41.5 (C-14), 45.8 (C-17), 46.5 (C-19), 47.5 (C-9), 55.3 (C-5), 80.9 (C-3), 122.5 (C-12), 143.6 (C-13), 171.0 (C=O acetate), 184.5 (C-28).

**Table 1** $^{13}$C-NMR chemical shifts of compounds **1** and **1a** (75.6 MHz, $CDCl_3$, TMS).

| C | **1**[a] | **1a** |
|---|---|---|
| 1 | 38.1 | 38.1 |
| 2 | 23.3 | 23.4 |
| 3 | 80.6 | 81.1 |
| 4 | 37.9 | 37.9 |
| 5 | 55.3 | 55.3 |
| 6 | 18.2 | 18.2 |
| 7 | 32.5 | 32.5 |
| 8 | 39.3 | 39.3 |
| 9 | 47.5 | 47.6 |
| 10 | 36.9 | 37.0 |
| 11 | 23.0 | 22.9 |
| 12 | 122.0 | 122.6 |
| 13 | 144.1 | 143.7 |
| 14 | 41.7 | 41.6 |
| 15 | 27.7 | 27.7 |
| 16 | 23.6 | 23.6 |
| 17 | 46.2 | 46.6 |
| 18 | 40.9 | 40.9 |
| 19 | 46.0 | 45.8 |
| 20 | 30.7 | 30.7 |
| 21 | 33.9 | 33.8 |
| 22 | 32.5 | 32.4 |
| 23 | 28.1 | 28.1 |
| 24 | 16.8 | 16.8 |
| 25 | 15.4 | 15.4 |
| 26 | 17.0 | 17.2 |
| 27 | 25.9 | 25.9 |
| 28 | 180.3 | 184.5 |
| 29 | 23.6 | 23.6 |
| 30 | 33.1 | 33.1 |
| 1′ | 167.3 | 166.7 |
| 2′ | 115.2 | 119.0 |
| 3′ | 144.4 | 143.2 |
| 4′ | 125.9 | 132.3 |
| 5′,9′ | 129.8 | 129.2 |
| 6′,8′ | 116.0 | 122.1 |
| 7′ | 159.5 | 152.0 |
| $\underline{C}H_3$-CO | | 21.1 |
| $\overline{C}H_3$-$\underline{C}O$ | | 169.3 |

[a] With 2 drops of DMSO-$d_6$.

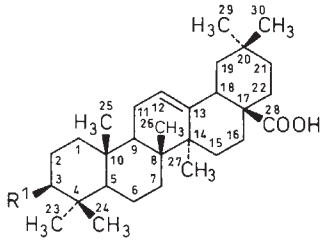
### *β-sitosterol β-D-glucopyranoside tetraacetate* (**2a**)

M.p. 169–171 °C; IR $\nu_{max}^{KBr}$ $cm^{-1}$: 1756, 1223 (C=O acetate); EIMS (probe) 70 eV, *m/z* (rel. int.): 396 (22), 382 (6), 331 (6), 255 (4), 169 (21), 145 (8), 109 (22), 95 (10), 81 (13), 69 (13), 55 (15), 43 (100); CIMS ($NH_3$): 762 $[M + NH_4]^+$; $^1$H-NMR (300 MHz, $CDCl_3$, TMS): 5.37 (1H, d, $J$ = 5 Hz, H-6), 5.22 (1H, t, $J_{3',2'} = J_{3',4'}$ = 9.6 Hz, H-3′), 5.09 (1H, t, $J_{4',3'} = J_{4',5'}$ = 9.6 Hz, H-4′), 4.97 (1H, dd, $J_{2',3'}$ = 9.6 Hz, $J_{2',1'}$ = 8.1 Hz, H-2′), 4.56 (1H, d, $J_{1',2'}$ = 8.1 Hz, H-1′), 4.25 (1H, dd, $J_{6'b,6'a}$ = 12.2 Hz, $J_{6'b,5'}$ = 4.8 Hz, $H_a$-C(6′)-$H_b$), 4.12 (1H, dd, $J_{6'a,6'b}$ = 12.2 Hz, $J_{6'a,5'}$ = 2.5 Hz, $H_a$-C(6′)-$H_b$), 3.68 (1H, m, $J_{5',4'}$ = 9.6 Hz, $J_{5',6'b}$ = 4.8 Hz, $J_{5',6'a}$ = 2.5 Hz, H-5′), 3.50 (1H, m, $J_{aa} = J_{aa'}$ = 10.4 Hz, $J_{ae} = J_{ae'}$ = 5.2 Hz, Hα-3), 2.09, 2.06, 2.03, 2.01 (4 × 3 H, s each, Me-OAc of C-2′, C-3′, C-4′ and C-6′), 0.99 (3H, s, Me-19), 0.93 (3H, d, $J$ = 6.5 Hz, Me-21), 0.86 (3H, d, $J$ = 6.8 Hz, Me-26), 0.85 {3H, t, $J$ = 7.2 Hz, Me-24[2] [replaces Me-29 (6)]}, 0.82 (3H, d, $J$ = 6.8 Hz, Me-27), 0.68 (3H, s, Me-18); $^{13}$C-NMR (50.3 MHz, $CDCl_3$, TMS): 11.8 (C-18), 12.0 (C-24[2]), 18.8 (C-26), 19.0 (C-21), 19.3 (C-19), 19.8 (C-27), 20.6, 20.6, 20.7, 20.7 (4 ×

$CH_3$-acetate), 21.0 (C-11), 23.1 (C-24'), 24.3 (C-15), 26.1 (C-25), 28.2 (C-16), 29.1 (C-23), 29.4 (C-2), 31.8 (C-8), 31.9 (C-7), 33.9 (C-22), 36.1 (C-20), 36.7 (C-10), 37.2 (C-1), 38.9 (C-4), 39.7 (C-12), 42.3 (C-13), 45.8 (C-24), 50.2 (C-9), 56.0 (C-17), 56.7 (C-14), 62.1 (C-6'), 68.5 (C-4'), 71.5 (C-2'), 71.7 (C-3'), 72.9 (C-5'), 80.0 (C-3), 99.6 (C-1'), 122.1 (C-6), 140.3 (C-5), 169.2, 169.3, 170.3, 170.6 (4 × C=O acetate).

## Results and Discussion

The triterpenoid **1**, isolated from fraction I, showed pronounced EI mass peaks at *m/z* 248 and 203, typical of a 12-oleanene or 12-ursene (7, 8). The mono-acetylated compound **1a** shows the same pattern as well as $[M]^+$ at *m/z* 644.



| | $R^1$ | $R^2$ |
|---|---|---|
| **1** | $R^2O$– | H |
| **1a** | $R^2O$– | Ac |
| **1b** | OH | — |
| **1c** | OAc | — |

The IR spectrum of **1** presents a strong absorption at 3346 $cm^{-1}$ (OH) and a broad absorption with a maximum at 3241 $cm^{-1}$ and a sub-maximum at 2652 $cm^{-1}$ (COOH) (9); other bands were found at 1736 and 1690 $cm^{-1}$ (C=O), 1631 and 815 $cm^{-1}$ (C=C), 1518 $cm^{-1}$ (aromatic) and 1265 and 1244 $cm^{-1}$ (C(=O)-O). Compound **1a** shows a similar spectrum without the band corresponding to the alcohol group, but with an additional carbonyl band at 1768 $cm^{-1}$.

The $^{13}$C-NMR spectrum of **1a** shows signals at $\delta$ 143.6, 122.6, 143.2, and 119.0 (C=C bonds), $\delta$ 166.7 and 169.1 (carbonyl ester carbons), $\delta$ 184.1 (carboxylic acid), $\delta$ 81.1 and 152.0 (carbon attached to oxygen) (10). The signals at $\delta$ 122.1 (2C), 129.1 (2C), and 132.3 are easily assigned to five atoms of a *para*-substituted benzene ring (the sixth atom being at $\delta$ 152.0).

The $^1$H-NMR spectrum of **1a** shows seven methyl singlets ($\delta$ 0.76–1.14) attached to the triterpenic skeleton and one acetate methyl group ($\delta$ 2.31); the broad signal at $\delta$ 5.28 is assigned to a proton attached to a tri-substituted double bond. An AB quartet at $\delta$ 7.64 and 6.40 (1H each, $J$ = 16 Hz) is typical of a *trans*-substituted double bond and another AB-like quartet (in fact an AA'BB' system) at $\delta$ 7.54 and 7.12 (2H each, $J$ = 8.5 Hz), confirmed by spin decoupling, is attributed to a *para*-substituted benzene ring (11). The signal at $\delta$ 4.64 (1H, multiplet) for the proton geminal to the oxygen indicates that it is 3$\alpha$.

The spectroscopic evidence points to a derivative of a 12-oleanene or 12-ursene, containing a free carboxylic acid group in replacement of a methyl group, esterified through O(C-3) with *p*-acetoxy-*trans*-cinnamic acid. Another signal at $\delta$ 2.83 (1H, doublet of doublets, $J$ = 10 Hz, $J'$ = 3 Hz) can only be assigned to H(C-18), which eliminates the possibility of an 12-ursene type compound.

Alkaline hydrolysis of **1** and **1a** afforded *p*-hydroxy-*trans*-cinnamic acid and **1b**. After acetylation compound **1b** was compared with an authentic sample of acetylated 3$\beta$-hydroxyolean-12-en-oic acid (**1c**) (TLC, IR, MS, $^1$H- and $^{13}$C-NMR) only to arrive at the conclusion that both were identical.

The $^1$H and $^{13}$C chemical shifts for **1** differ slightly from **1a** in the expected manner due to the substitution of the aromatic ring hydroxy by an acetyl group. Compound **1** is therefore 3$\beta$-(*p*-hydroxy-*trans*-cinnamoyloxy)olean-12-en-28-oic acid.

The $^{13}$C assignments of compounds **1b** and **1c** are based on DEPT spectra of **1c** and literature values for methyl oleanolate and its acetylated derivative (12) and the methyl ester of queretaroic acid (13), taking into account their differences and the corresponding shifts. The assignments given by Tori et al. (12, 13) for C-29 and C-30 should be exchanged, considering the $\beta$-effect of an OH group (10), in accordance with the values given by Tanaka and co-workers (14) for olean-12-ene-3$\beta$,15$\alpha$-diol.

The assignments for **1** and **1a** are directly derived from **1b** and **1c**, well known SIS effects (10, 15), and DEPT spectra of **1a**.

Compound **2** was identified through its tetra-acetylated derivative **2a** as $\beta$-sitosterol $\beta$-D-glucopyranoside. Its IR and $^1$H-NMR spectra agree with the published values (16), the coupling constants having now been analysed in detail. The CI mass spectrum provided the $[M + NH_4]^+$; the EI does not give the molecular ion but shows peaks at *m/z* 331 from the tetraacetate of $\beta$-D-glucopyranose (17) and at *m/z* 396 and 255 from the sterol (18). The assignments for **2a** are based on literature values for $\beta$-sitosterol (19) (where C-24 seems to be in error by about 5 ppm), its acetate (20) and the glucose residue of sucrose octaacetate (21) as well as the published chemical shifts for sitosterol-3-*O*-stearoyl-$\beta$-D-glucopyranoside taking into account the multiplicities (where no assignments were made) (22).

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