Dammarane Triterpenes from the Leaves of *Securinega melanthesoides*

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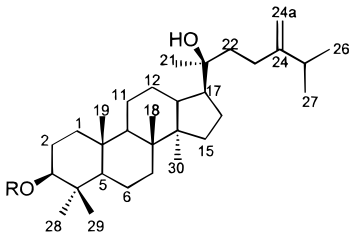
Two new dammarane triterpenoids, trans-securinegin [(20*S*)-24-methylidenedammarane-3α-yl(2*E*)-3-(4-hydroxyphenyl)-2-propenate (**1**)] and cis-securinegin [(20*S*)-24-methylidenedammarane-3α-yl(2*Z*)-3-(4-hydroxyphenyl)-2-propenate (**2**)], were isolated from the leaves of *Securinega melanthesoides*, along with the known compound bergenin. The structures of **1** and **2** were elucidated using spectroscopic methods, mainly 2D NMR techniques.

Securinega melanthesoides (F. Muell.) Airy Shaw (Euphorbiaceae) is used in the traditional medicine of Papua New Guinea. In the Motu village of Gaire, leaves are boiled with water, and the bitter drink is said to cure fever and malaria. The Roro people of Waima chew the leaves and spit them onto fresh burns.[1] *Securinega* species are known to contain several biologically active and structurally interesting alkaloids, and the alkaloid virosecurinine has been isolated from the dried leaves of *S. melanthesoides*.[1−3] In addition, pentacyclic triterpenoids have been isolated from *S. tinctoria*.[4−6] This is the first report of tetracyclic triterpenoids in a *Securinega* species, and the isolation and structure elucidation of two new substituted dammarane triterpenoids, *trans*-securinegin (**1**) and *cis*-securinegin (**2**), are presented herein. The crude CH_2Cl_2 extract of *S. melanthesoides* was fractionated by a combination of vacuum liquid chromatography (VLC) (Si gel) and medium-pressure liquid chromatography (MPLC) (Si gel). The final purification of compounds **1** and **2** was performed by high-pressure liquid chromatography (HPLC) (Si gel).



(1) R = *trans*-p-coumaroyl
(2) R = *cis*-p-coumaroyl

Compound **1** was obtained as a white amorphous powder. The HREIMS of **1** gave a pseudomolecular [M − H_2O]$^+$ peak at *m/z* 586.4376, consistent with the molecular formula $C_{40}H_{60}O_4$. The IR spectrum showed the presence of hydroxyl (3450 cm^{-1}) and ester carbonyl (1682 cm^{-1}) moieties. The UV spectrum exhibited an absorption maximum at 310 nm. Of the 11 degrees of unsaturation implied by the molecular formula, seven could be accounted for by examination of the ^{13}C-NMR spectral data as a 1,4-substituted aromatic moiety [δ_C 127.5, 130.0 (×2), 115.8 (×2), and 157.5], one ester carbonyl (δ_C 167.0), and four olefinic carbons (δ_C 106.2, 156.5, 116.5, and 143.9) forming two double bonds, and hence it was concluded that compound **1** is tetracyclic.

In the ^{1}H-NMR spectrum (Table 1) of compound **1** signals that are characteristic of a *p*-coumaric acid moiety were observed at δ_H 6.86 (2H, d, J = 8.6 Hz), 7.48 (2H, d, J = 8.6 Hz), 6.39 (1H, d, J = 16.0 Hz), 7.64 (1H, d, J = 16.0 Hz), and 5.40 (1H, br s, exchangeable with D_2O). The ^{13}C-NMR spectrum of **1** (Table 1) also contained resonances consistent with the presence of a *p*-coumaric acid moiety.[7] The ^{1}H-NMR spectrum also showed the presence of six methyl singlets (δ_H 1.19, 1.00, 0.98, 0.93, 0.91, and 0.90) and two overlapping methyl doublets (δ_H 1.05, d, J = 6.8 Hz). The presence of eight methyl groups indicated the triterpenoid character of **1**. Further signals characteristic of a terminal double bond (δ_H 4.77, br s and 4.71, br s) and an oxygen-bearing methine (δ_H 4.71, br s) were observed.

The ^{13}C-NMR spectrum (Table 1) of compound **1** contained 36 signals, four of which displayed double intensities [δ_C 21.9 (q), 115.8 (d), 130.0 (d), and 50.5 (s) and (d)]. After subtraction of nine carbons for the *p*-substituted coumaroyl moiety, 31 signals remained for the tetracyclic triterpene moiety. The ^{13}C-NMR spectrum also confirmed the presence of a terminal methylene group [δ_C 106.2 (t) and 156.5 (s)]. Further, the presence of two oxygen-bearing carbons [δ_C 78.0 (d) and 75.5 (s)] was evident. DQF-COSY, ROESY, HMQC, and HMBC experiments allowed the assignments of all ^{1}H− and ^{13}C-NMR resonances of **1**. Thus, the terminal methylene protons H-24a$_1$ and H-24a$_2$ [δ_H 4.77 (br s) and 4.71 (br s)] showed allylic couplings to the methylene group H_2-23 [δ_H 2.12 (m)] and the methine H-25 [δ_H 2.27 (m)], which in turn coupled to the geminal dimethyl groups H_3-26 and H_3-27 (δ_H 1.05, d). The methylene H_2-23 further coupled to H_2-22 (δ_H 1.63, d). This fragment (C-22 to C-27) could further be connected to the oxygen-bearing carbon C-20 by the HMBC correlations from the methyl group at C-21 (δ_C 25.3, q) to H_2-22 (δ_H 1.63, d) as shown in Table 1, thus establishing the linear side chain for compound **1**. The tetracyclic

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Table 1. ^{1}H− and ^{13}C NMR Chemical Shift Assignments (ppm) and Long-Range Connectivities Observed in the HMBC Spectrum of **1**

position	^{1}H-NMR chemical shift δ (mult, J = Hz)	^{13}C-NMR chemical shift δ[a]	long-range coupling(s)
1	*b*	35.1, t	H_3-19
2	*b*	27.5, t	H_3-29
3	4.71 (br s)	78.4, d	H_3-29
4		37.0, s	H_3-29, H_3-28
5	1.35 (m)	51.0, d	H_3-28
6	*b*	18.1, t	
7	1.29 (m)	34.4, t	H_3-18
8		40.6, s	H_3-30
9	1.54 (m)	50.5, d	H_3-19
10		37.2, s	
11	*b*	21.4, t[c]	
12	*b*	24.8, t[c]	
13	*b*	42.3, d	H_3-30
14		50.5, s	
15	*b*	31.2, t	H_3-30
16	*b*	23.0, t[c]	
17	1.80 (m)	49.8, d	H_3-21
18	0.91 (s)	16.1, q	
19	1.00 (s)	15.5, q	
20		75.5, s	H_3-21
21	1.19 (s)	25.3, q	H_2-22
22	1.63 (d, 8.5)	39.4, t	H_3-21
23	2.12 (m)	28.4, t	H_2-24a, H_2-22
24		156.5, s	H_3-26, H_3-27, H_2-23, H_2-22
24a	4.77 (br s), 4.71 (br s)	106.2, t	
25	2.27 (m)	34.0, d	H_2-24a, H_3-26, H_3-27
26	1.05 (d, 6.8)	21.9, q	H_2-24a
27	1.05 (d, 6.8)	21.9, q	H_2-24a
28	0.90 (s)	28.0, q	H-5, H-3
29	0.93 (s)	21.7, q	
30	0.98 (s)	16.7, q	
1'		167.0, s	H-3
2'	6.39 (d, 16.0)	116.5, d	H-5', H-9'
3'	7.64 (d, 16.0)	143.9, d	H-6', H-8'
4'		127.5, s	H-2', H-5', H-9'
5'	6.86 (d, 8.6)	130.0, d	H-3', H-6'
6', 8'	7.48 (d, 8.6)	115.8, d	
9'	6.86 (d, 8.6)	130.0, d	H-3', H-8'
7'		157.5, s	H-6', H-8'
OH−7'	5.40 (br s)		

[a] Multiplicities determined by DEPT 90 and DEPT 135 NMR experiments. [b] Signals not assigned due to overlapping. [c] Data assigned by comparison with Tanaka *et al.* (8).

moiety of the molecule was established by HMBC correlations from the methyl groups at C-18, C-19, C-21, C-28, C-29, and C-30, as shown in Table 1. This deduction was verified by the comparison of data for **1** with those for related compounds.[8,9] This confirmed the triterpenoid moiety as 24-methylidenedammarane-20,3-diol. The ester connectivity between the *p*-coumaroyl and the terpenoid moiety of the molecule at C-3 was deduced by the HMBC correlation observed between C-1' (δ_C 167.0, s) and the proton at C-3 (δ_H 4.71, br s).

The stereochemistry at H-3 was established as α, based on the small coupling constant of the triplet observed for H-3, when measured in C_6H_6-d_6 as NMR solvent.[10] The stereochemistry at C-20 was deduced by comparing the carbon shift values of C-21 and C-22 with those published in the literature.[11,12] The resonance of the (20*S*)-epimer for C-21 is more deshielded, while C-22 is more shielded than that of the (20*R*)-epimer.[12] This confirmed the (20*S*)-configuration at C-20 for compound **1**. The stereochemistry at the double bond of the *p*-coumaroyl moiety was determined as trans due to the ^{1}H-NMR coupling constant of J = 16.0 Hz between the protons 2' and 3'.[10] Thus, the structure of compound **1** was deduced as (20*S*)-24-methylidenedammarane-3α-yl(2*E*)-3-(4-hydroxyphenyl)-2-propenate, for which we propose the trivial name *trans*-securinegin.

Compound **2** was also obtained as a white amorphous powder. The spectral data indicated it to be closely related to compound **1**. The HREIMS gave a pseudomolecular $[M - H_2O]^+$ peak at *m/z* 586.4380, which is consistent with the molecular formula $C_{40}H_{60}O_4$. The ^{1}H- and ^{13}C-NMR data were similar to those of compound **1**, with the exception of the coupling constant of 12.8 Hz between H-2' and H-3' protons of the coumaric acid moiety, thus confirming a cis conformation. Compound **2** was determined as (20*S*)-24-methylidenedammarane-3α-yl(2*Z*)-3-(4-hydroxyphenyl)-2-propenate, for which we propose the trivial name *cis*-securinegin. Upon isolation the isomerization of *trans*- to *cis*-securinegin and vice versa was observed during storage at 4 °C [the isomeric ratio (1:1) remained constant under UV stress (254 nm)]. This isomerization phenomenon in plant tissue has been previously described for various cinnamic acid derivatives, although studies by others have also proved that the predominantly natural-occurring isomer is trans.[13]

This is the first report of tetracyclic triterpenoids in a *Securinega* species, although tetracyclic triterpenes of the lanostane (17β) and euphane (17α) skeletons have been reported from other genera of the family Euphorbiaceae.[14] To the best of our knowledge, dammarane triterpenoids have not been found in the family Euphorbiaceae. Along with the new compounds, the known metabolite bergenin was also isolated. Bergenin normally occurs together with ellagitannins within the family Euphorbiaceae.[15−17]

Experimental Section

General Experimental Procedures. All NMR spectra were recorded on a Bruker AMX-300 spectrometer (300.13 MHz for ^{1}H, 75.47 MHz for ^{13}C, and J_{opt} = 8.3 Hz for HMBC) using $CDCl_3$ and C_6H_6-d_6 as solvents. Chemical shifts were reported in parts per million on the δ scale. EIMS spectra were recorded on a Hitachi-Perkin-Elmer RMUGM mass spectrometer using an ionization power of 10 eV, with CD_2Cl_2 as solvent. Optical rotations were measured on a Perkin-Elmer 241 polarimeter, using $CHCl_3$ and MeOH as solvents. IR spectra were recorded on a Perkin-Elmer 2000 FT-IR spectrometer, using KBr disks. UV spectra were performed on a Kontron-Uvikon 930 spectrometer, using MeOH as solvent.

Plant Material. Leaves of *S. melanthesoides* were collected in March 1991, near Port Moresby, Papua New Guinea, in the amount of 2 kg dry weight. A voucher specimen has been deposited at the Rijksherbarium (ETH 91/3 20−03−91), University of Leiden (The Netherlands).

Extraction and Isolation. A 1.5-kg quantity of powdered leaves was continuously percolated with *n*-hexane, CH_2Cl_2, and MeOH at room temperature. A portion (9.0 g) of the CH_2Cl_2 extract (total 21.1 g) was subjected to VLC (Si gel, hexane, hexane−EtOAc step gradient) to give 12 fractions. Fraction 5 (348 mg) was

further purified by MPLC (Si gel, hexane, hexane−EtOAc−MeOH, 9:1:0.5). The final separation of compound **1** (5.1 mg) and compound **2** (7.3 mg) was performed on HPLC (Si gel, particle size 5 μm, column 4 mm × 20 mm, hexane−EtOAc−MeOH, 95:5:5, flow rate 1 mL/min).

(20*S*)-24-Methylidenedammarane-3α-yl(2*E*)-3-(4-hydroxyphenyl)-2-propenate (1): $[\alpha]^{25}_D$ +9.7° (*c* 0.3, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 310 (9.53), 223 (9.08) nm; IR (KBr disk) ν_{max} 3450 (OH), 2960 (C−H), 1682 (C═O), 1632 (C═C), 1605, 1587, 1515, 1446, 1376, 1307, 1262, 1203, 1167, 1101, 1035, 889, 831, 801, 518 cm^{-1}; ^{1}H- and ^{13}C-NMR data, see Table 1; EIMS *m/z* 586 [M − H_2O]$^+$ (5), 423 (24), 203 (12), 191 (40), 165 (18), 147 (100), 123 (40); HREIMS *m/z* 586.4376 [M − H_2O]$^+$ ($C_{40}H_{60}O_4$−H_2O requires 586.4386).

(20*S*)-24-Methylidenedammarane-3α-yl(2*Z*)-3-(4-hydroxyphenyl)-2-propenate (2): $[\alpha]^{25}_D$ −8.8° (*c* 0.4, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 312 (8.95), 226 (8.38) nm; IR (KBr disk) ν_{max} 3484 (OH), 2958 (C−H), 1686 (C═O), 1606, 1514, 1449, 1377, 1307, 1262, 1201, 1166, 1035, 987, 890, 832 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300.13 MHz) δ 7.64 (2H, d, J = 8.7 Hz, H-6', H-8'), 6.85 (1H, d, J = 12.8 Hz, H-3'), 6.81 (2H, d, J = 8.7 Hz, H-5', H-9'), 5.93 (1H, d, J = 12.8 Hz, H-2'), 1.18 (3H, s, H-21), 1.05 (6H, d, J = 6.8 Hz, H-26, H-27), 0.98 (3H, s, H-19), 0.94 (3H, s, H-30), 0.91 (3H, s, H-29), 0.87 (3H, s, H-18), 0.86 (3H, s, H-28); ^{13}C NMR ($CDCl_3$, 75.47 MHz) δ 166.4 (s, C-1'), 156.6 (s, C-7'), 156.5 (s, C-24), 142.8 (d, C-3'), 132.2 (d, C-5', C-9'), 127.7 (s, C-4'), 118.1 (d, C-2'), 115.8 (d, C-6', C-8'), 106.2 (t, C-24a), 78.3 (d, C-3), 75.5 (s, C-20), 50.7 (d, C-5), 50.4 (s, C-14), 50.4 (d, C-9), 49.8 (d, C-17), 42.3 (d, C-13), 40.6 (s, C-8), 39.3 (t, C-22), 37.1 (s, C-10), 36.9 (s, C-4), 35.0 (t, C-1), 34.3 (t, C-7), 34.0 (d, C-25), 31.2 (t, C-15), 28.4 (t, C-23), 28.0 (q, C-28), 27.5 (t, C-2), 25.4 (q, C-21), 24.8 (t, C-12), 23.0 (t, C-16), 22.0 (q, C-27), 22.0 (q, C-26), 21.8 (q, C-29), 21.4 (t, C-11), 18.1 (t, C-6), 16.7 (q, C-30), 16.0 (q, C-18), 15.5 (q, C-19); EIMS *m/z* 587 [M − H_2O] (3), 423 (4), 422 (4), 203 (9), 191 (19), 190 (23), 189 (27), 165 (8), 164 (13), 147 (100), 123 (25); HREIMS *m/z* 586.4380 [M − H_2O]$^+$ ($C_{40}H_{60}O_4$ − H_2O requires 586.4386).

Bergenin: $[\alpha]^{25}_D$ −36.5° (*c* 0.3, MeOH); optical rotation, UV, and IR data consistent with reported data;[15] ^{1}H- and ^{13}C-NMR spectral data in good agreement with those published in the literature.[16,17]

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