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Silvaglenamin—a novel dimeric triterpene alkaloid from *Aglaia silvestris*

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ABSTRACT

An unusual dimeric triterpene structure with two dammarane units linked with an enaminic –NH– group was isolated from the root extract of *Aglaia silvestris*. The structure was elucidated by 1D and 2D NMR analysis and ESI mass spectrometry.

Recently, we have established a series of new stereoisomeric 3,4-secodammarane triterpenes in the methanolic root extract of *Aglaia silvestris* (M. Roemer) Merrill, family Meliaceae, collected in Thailand.[1] Comparison between various geographical provenances revealed the existence of two chemo-types linking the tetrahydrofuran (20,24-epoxy) ring of the side chain to the D-ring of the sterane skeleton either in 20*S* or in 20*R* configuration. In addition, a new 2,3-secodammarane was described most likely representing a precursor of the unusual triterpenes silvaglins A, B and isosilvaglins A, B, characterised by a five-membered A-ring. The structure of silvaglin A with 20*S* configuration was confirmed by X-ray crystallographic analysis.[2]

More detailed investigations of the triterpene-containing column fractions of the root extract of a Thai provenance of *A. silvestris* led now to the isolation of a further major compound (**1**). In contrast to the triterpenes without any chromophors, **1** deviated from the other compounds of the fraction by a significant UV spectrum with distinct maxima at 283 and 220 nm (MeOH). First inspection of the ^{1}H and ^{13}C NMR spectra of compound **1** showed that it was a typical dammarane-type triterpene with a 20,24-epoxy-bridged (tetrahydrofuran) side chain of the D-ring. This type of dammaranes was found quite frequently in other *Aglaia* species.[2,3] With the exception of C-1, C-2, C-3, C-6, C-9, C-10 and C-19, the ^{13}C chemical shifts were almost identical with the corresponding data of isosilvaglin A.[2] This implied that differences in the structures were expected only with respect to the A-ring. Consequently, compound **1** belonged to the 20*R*,24*S* series of the 20,24-epoxy dammaranes.[1] The assignments of all ^{13}C and ^{1}H resonances were achieved by means of 2D NMR (Table 1) and by comparison of the data with literature.[2] Of special interest were the HMBC cross peaks between the protons of the eight methyl groups and adjacent carbon atoms. All expected contacts 18→8, 7, 9, 14; 19→10, 1, 5, 9; 21→17, 20, 22; 26→24, 25, 27; 27→24, 25, 26; 28→4, 5, 3, 29; 29→4, 5, 3, 28; 30→14, 8, 13, 15 were found, allowing to identify all carbon atoms of the dammarane skeleton. It turned out that C-1 was an olefinic s at 126.5 ppm. C-3 carbonyl and C-4 with the geminal methyl groups 28 and 29 were as usual, C-5 and C-10 as well. The missing C-2 was necessarily the second olefinic carbon atom at 137.1 ppm (doublet) with a corresponding proton at 6.81 ppm (J = 11.4 Hz). This 2-H resonance exhibited HMBC cross peaks to C-1, C-3, C-10 and, surprisingly enough, to C-2 itself. The latter was virtually impossible, and needed some special explanation. The ^{1}H NMR spectrum also showed some remarkable features. A triplet at 13.25 ppm (J = 11.4 Hz) was typical for a hydrogen-bonded proton. It coupled clearly with a doublet at 6.81 ppm (J = 11.4 Hz), which was already identified as the olefinic 2-H. However, the coupling with only one proton could not explain its triplet character. The integrations were also unexpected. Scaled to all other signals (e.g., to 3H for the methyl groups) the integration was 1/2H for the triplet at 13.25 ppm and 1H for the doublet at 6.81 ppm. All this evidence, the suspicious long range cross peak between 2-H and C-2, the integration of 1/2H and the triplet character of the H-bonded proton at 13.25 ppm, pointed towards some kind of symmetrical structure with two identical triterpenoid units. Proof of a dimeric structure was

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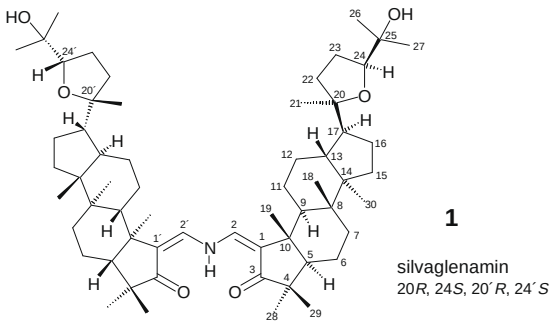
Table 1
^{1}H and ^{13}C NMR data of silvaglenamin (**1**) (400 MHz, $CDCl_3$, δ/ppm)

No.	^{13}C	^{1}H
1, 1′	126.5 s	
2, 2′	137.1 d	6.81 d (11.4 Hz)
3, 3′	211.6 s	
4, 4′	45.4 s	
5, 5′	59.7 d	1.45 m
6, 6′	20.2 t	a: 1.57 m; b: 1.50 m
7, 7′	35.5 t	a: 1.66 m; b: 1.35 m
8, 8′	42.2 s	
9, 9′	47.9 d	2.01 dd (9.0, 6.6 Hz)
10, 10′	43.6 s	
11, 11′	24.4 t	a: 1.75 m; b: 1.72 m
12, 12′	26.9 t	a: 2.08 m; b: 1.35 m
13, 13′	42.3 d	1.70 m
14, 14′	50.2 s	
15, 15′	31.3 t	a: 1.52 m; b: 1.12 m
16, 16′	25.6 t	a: 1.80 m; b: 1.26 m
17, 17′	50.3 d	1.77 m
18, 18′	16.7 q	1.04 s
19, 19′	21.4 q	1.15 s
20, 20′	86.4 s	
21, 21′	21.7 q	1.16 s
22, 22′	37.7 t	a: 1.77 m; b: 1.65 m
23, 23′	25.9 t	a: 1.80 m; b: 1.26 m
24, 24′	84.6 d	3.75 t (7.1 Hz)
25, 25′	71.0 s	
26, 26′	27.9 q	1.21 s
27, 27′	24.3 q	1.11 s
28, 28′	26.7 q	1.08 s
29, 29′	20.2 q	0.99 s
30, 30′	16.1 q	0.90 s
25, 25′-OH		2.21 br s
2, 2′-NH		13.25 t (11.4 Hz)

supplied by ESIMS with a molecular peak of m/z = 948 for $[M+Na]^+$. This results in a molecular mass of m/z = 925 (uneven mass, characteristic for nitrogen-containing molecules) corresponding to $C_{60}H_{95}NO_6$. The peak of highest mass in the standard EIMS spectrum was m/z = 471 (3%), which may be assigned to $C_{30}H_{49}NO_3^+$ (M^+ after loss of one terpene unit and protonation). Due to the dimeric character of the compound, all integrations with exception of the –NH– bridge were twice, for example, 2H for the resonance at 6.81 ppm and 6H for the methyl groups. The triplet character of the enaminic hydrogen-bridged proton was due to the couplings with 2-H and 2′-H, and the apparent, but impossible long range (HMBC) cross peak '2-H→C-2' was explained by the contacts 2-H→C-2′ and 2′-H→C-2. The chemical shift of 13.25 ppm for N–H was a consequence of the enamine hydrogen bridges towards the two carbonyl groups at C-3 and C-3′ (see structural formula), and the conjugated enaminoketone chromophore was responsible for the very characteristic UV spectrum. The new compound was designated as silvaglenamin (**1**), referring to its origin from *Aglaia silvestris* and its enamine character.



1
silvaglenamin
20*R*, 24*S*, 20′*R*, 24′*S*

In accordance with the other triterpenes recently reported for the same collection of *A. silvestris* (HG 719),[2] the tetrahydrofuran (20,24-epoxy) ring of silvaglenamin (**1**) was attached to the D-ring in 20*R* configuration. Except for the olefinic C-2 and C-2′, linked with a N–H bridge, the structure and stereochemistry of the two terpenic subunits were identical with isosilvaglin A.[2] Although silvaglenamin (**1**) represented a major compound in the triterpene-containing column fractions and was characterised by a typical UV-spectrum, it could surprisingly not be detected in HPLC–UV analyses of the corresponding crude extract. Hence, the question arises whether **1** is an artefact, produced during isolation due to a condensation reaction of the aldehyde functions of two molecules of isosilvaglin A with traces of ammonia, for example, from solvents. On the other hand, at no step of the isolation or storage of the extracts ammonia was added, and the content of isosilvaglin A never decreased significantly. By comparing the many triterpenes that have already been reported for *Aglaia*,[2,3] it was found that silvaglenamin (**1**) represents the first nitrogen-containing triterpene, or triterpene alkaloid of the genus. A series of dimeric alkaloids with two steroid structures connected by two nitrogen atoms forming a pyrazin ring (cephalostatins and ritterazins) were isolated from marine organisms.[4] Concerning the plant kingdom, several dimeric triterpenes linked by oxygen bridges forming either simple ethers or 1,4-dioxane rings were described for *Maytenus* species.[5,6] Further, dimeric triterpenoid structures with ester linkages were reported for *Rubus pungens* and *Sanguisorba officinalis*.[7,8]

The plant material was collected in Khao Chong, near Trang, South Thailand (Feb. 1999), and a voucher specimen (HG 719) was deposited at the Herbarium of the Faculty Centre of Botany, University of Vienna (WU), identified by Dr. Caroline M. Pannell, University of Oxford, UK. Dried root bark, 28 g, was ground and extracted with MeOH at room temperature for 3 days, filtered and concentrated. After addition of H_2O, the aqueous residue was extracted with $CHCl_3$, evaporated to dryness and dissolved in MeOH. This solution was stored at −20 °C and used for comparative TLC and for further studies. Half of the material (1.5 g of the $CHCl_3$ extract) was roughly separated by CC (Merk Silica Gel 60, 0.2–0.5 mm) with solvent mixtures of increasing polarity using hexane and EtOAc. The fraction eluted with 40% EtOAc in hexane was further separated by repeated (cyclic) preparative MPLC (400 × 40 mm column, Merck LiChroprep Silica Gel 60, 40–63 μm) with 30% EtOAc in hexane. Final purification was achieved by preparative TLC (30% EtOAc in CH_2Cl_2) yielding 11 mg of silvaglenamin (**1**) as a colourless amorphous powder, $[\alpha]_D^{20}$ −15 (*c* 0.8, MeOH). UV (MeOH) λ_{max} = 283, 220 nm. EIMS m/z = 471 (3%, M^+−terpenoid C_{30} unit), 413 (5), 193 (8), 175 (11), 164 (12), 149 (15), 143 (100). ESIMS m/z = 948 $[M+Na]^+$; M = 925 ($C_{60}H_{95}NO_6$).

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