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ISOLATION AND STRUCTURE (X-RAY ANALYSIS) OF KARINOLIDE, A NEW QUASSINOID FROM *SIMABA MULTIFLORA* [1]

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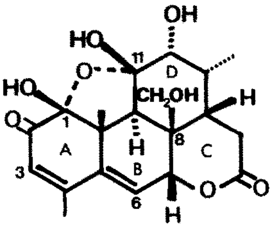
Summary : Karinolide 1 is a structurally novel C_{20} quassinoid isolated from the French Guyanan Simaroubaceae, *Simaba multiflora* A.Juss. whose structure was established by X-ray diffraction analysis. 6α-Senecioyloxychaparrin 2, the known 6α-senecioloyxychaparrinone 3 and 9-methoxycanthin-6-one 5 were also isolated ; their structures were determined by spectral means.

The Simaroubaceae *Simaba multiflora* A.Juss. is a tall tree which grows to heights of 15 to 20 m and is widely distributed in South America. It has often been described, although erroneously, as being synonymous with *Simaba guanensis* Aubl. from which it differs by several morphological features, especially the size.

Previous studies by Wani et al. [2] of *S.multiflora* originating from Peru resulted in the isolation of 6α-senecioyloxychaparrinone 2, active in both murine lymphocytic leukemia (P-388) and solid tumor systems. Our continuing work on the Simaroubaceae led us to explore the French Guyanan *Simaba multiflora* [3]. We herein report the isolation and structural elucidation of a novel quassinoid, named Karinolide 1. 6α-Senecioyloxychaparrin 3 along with the known 2-dehydro compound 2, was also isolated as well as the previously described [4] 9-methoxycanthin-6-one 5.

The dried ground stem bark (1kg) of *S.multiflora* was extracted with hexane and then several times with hot water. The concentrated aqueous extract was continuously extracted with chloroform to give a complex mixture of products (1.4g) which showed moderate antileukaemic activity (P-388 system, T/C 122 at 50 mg/kg). Column chromatography of this material on silicic acid-celite (2:1) and elution with methylene chloride containing increasing amounts of methanol (2 to 10 %) afforded twelve fractions, none of which was homogeneous. Fractionseluted with CH_2Cl_2 containing 5 % methanol (237 mg) were rechromatographed on the same absorbent to yield a crystalline fraction (120 mg). Crystallisation from ethyl acetate and preparative t.l.c. of the mother liquors yielded Karinolide 1 (11 mg). Chromatography of a root bark extract of *Simaba multiflora*, prepared by the procedure described for the stem extract, led to the isolation of the less polar 6α-senecioyloxychaparrinone 2 and the more polar 6α-senecioyloxychaparrin 3.



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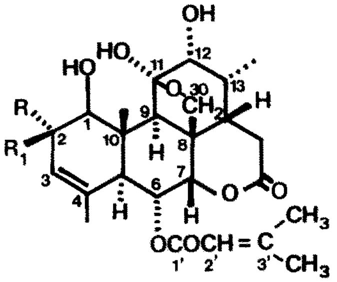
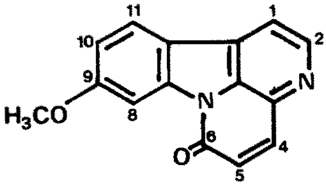


2 : R = R$_1$ = O
3 : R = OH ; R$_1$ = H



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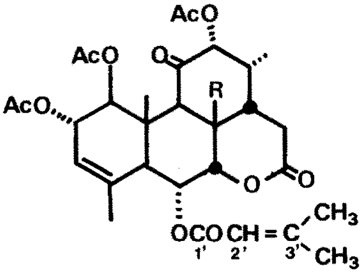

4 R = CH_2OAc

The molecular formula of Karinolide 1, m.p. 208-210°C, was established by its high resolution mass spectrum as $C_{20}H_{24}O_8$ ($M^{+\cdot}$ at m/z 392.1472). The u.v. spectrum showed maxima at 227 (ε 4260) and 295 nm (ε 10020) indicating the presence of a dienone chromophore. The 250 MHz ^1H-n.m.r. spectrum of 1 (in $CDCl_3$ + 5 % pyridine-d_5) with decoupling experiments revealed a certain amount of structural information. It displayed signals for a secondary methyl at δ 1.15 (d, J = 7Hz, Me-13) and an angular methyl group at δ 1.38, and a downfield resonance at 1.96 ppm assigned to Me-4. It also revealed : an AB pattern (δ 3.75 and 3.62 ; J = 9Hz) assignable to the hydroxymethyl group at C-8, signals at 3.48 (s, H-9), 3.65 (br.s, H-12), 3.98 (br.s, H-7) and two downfield doublets at δ 5.61 and 6.27 ppm due to H-3 and H-6, respectively which are mutually long range coupled (^5J = 1.5Hz).

Because of the paucity of 1 its structure and stereochemistry was established by single crystal X-ray analysis. Crystal data : A crystal of Karinolide 1 grown from acetone, size 0.3 x 0.2 x 0.2 mm, was mounted on a Phillips PW 1100 automatic diffractometer equipped with a graphite monochromator. The system is monoclinic, space group C2(Z=4) with cell parameters a = 13.522 ; b = 8.267 ; c = 17.004 Å and β = 106.3°. Reflexions were scanned up to Θ = 25° using MoK_α radiation. Of the 1935 reflexions measured, 1358 having $I \geqslant 2\sigma(I)$ could be considered as observed. The structure was solved by direct methods using the Riche's phase function [5] and the negative quartet test (Multan [6] gave trivial Patterson-like solutions and thus failed to lead to correct atomic positions). The refinements of the structure were carried out using isotropic and then anisotropic thermal factors and converged to a final R value of 7.8 % (H atoms were not refined but positionned at their theoretical places).

The molecular structure of 1 is shown in Fig. 1 [7]. The oxy-bridge (O-7) between C-1 and C-11 confers an unusual folded shape to the molecule not observed in other quassinoids. The conformations of rings B,C and D are affected by this overall folding ; rings B and C adopt a quasi-boat conformation whereas the lactone ring D has a twisted conformation. The presence of the dienone system results in the quasi-planarity of ring A which explains the observed long range coupling (^5J) between H-3 and H-6. The C-8 hydroxymethyl group is particularly exposed to intermolecular interactions and is indeed involved in hydrogen bonding (2.90 Å towards O-5 of a second molecule). The oxygen (O-3) of the lactone carbonyl is also linked to O-5 of another molecule thus leading to an infinite hydrogen bond network along the y axis.

Karinolide 1 contains several interesting and new structural features not previously encountered among the quassinoids. It is the first example of a natural quassinoid to have a cyclohexadienone system, a bis-hemiacetal function and a free primary hydroxyl group at C-8.

6α-Senecioyloxychaparrinone 2, $C_{25}H_{32}O_9$ ($M^{+\cdot}$ at m/z 476), m.p. 248-252°C. The u.v. spectrum showed a maximum at 227 nm (ε 22300) due to both the α,β-unsaturated ketone and the α,β-unsaturated ester. The presence of a 3-methyl-2-butenoate (senecioate) ester at C-6 was shown by the mass spectral fragmentation ions at m/z 376 (M^+-100), m/z 345 and 245 [7], m/z 83 $[O{=}CCH{=}C(CH_3)_2]^+$ and m/z 55 $[HC{=}C(CH_3)_2]$, and by analysis of the 250 MHz ^{1}H-n.m.r. spectrum. The latter spectrum including double resonance experiments allowed the assignment of most of the protons whose chemical shifts are identical with those published for 2[2]. Structure 2 was confirmed by comparison with an authentic sample of 6α-senecioyloxychaparrinone 2.

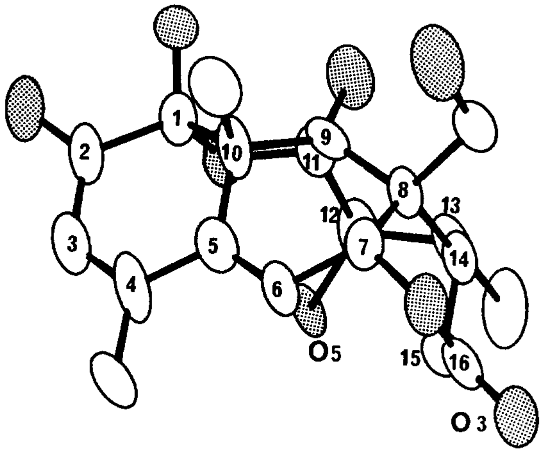

Figure 1 : Molecular structure of Karinolide 1.

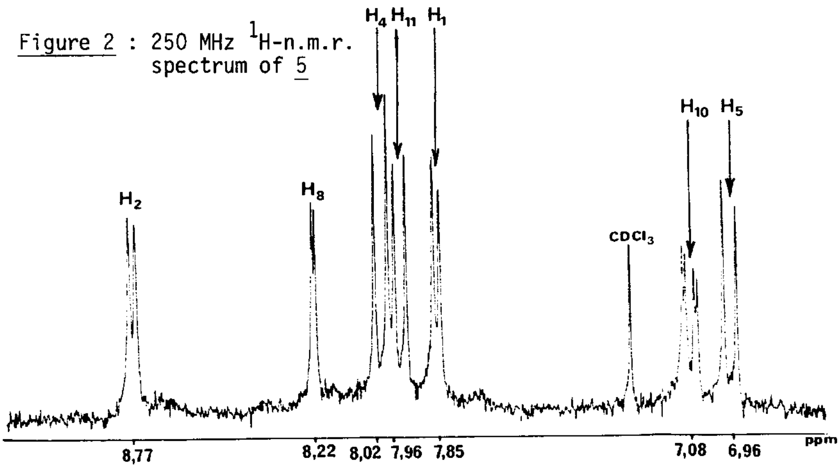

Figure 2 : 250 MHz ^{1}H-n.m.r. spectrum of 5

The more polar quassinoid, 6α-senecioyloxychaparrin 3 crystallised from ethylacetate-methanol as needles, m.p. 280-286° (dec.). The mass spectrum established its molecular formula as $C_{25}H_{34}O_9$ ($M^{+\cdot}$ at m/z 478) and showed abundant fragmentation ions at m/e 460 ($M^{+\cdot}$ -18), m/z 83 and m/z 55. The u.v.

spectrum did not reveal any significant absorption above 220 nm and its 250 MHz ^{1}H-n.m.r. spectrum showed clearly that it possessed a senecioyl ester at C-6. The latter spectrum displayed as did that of 2, signals at δ 5.45 (dd, J = 12 and 2 Hz) and at δ 5.72 (s) assigned to H-6 and H-2', respectively. These properties suggest that 3 is a 2-dihydro derivative of 2, both types of compounds frequently co-occuring in the same plant [8]. Acetylation (acetic anhydride-pyridine) of 3 indeed led to the pentaacetate 4, $C_{33}H_{42}O_{13}$ ($M^{+\cdot}$ at m/z 646) whose mass spectrum showed the expected fragmentation ions and whose 250 MHz ^{1}H-n.m.r. spectrum was consistent with structure 4 (δ 4.92, d, J = 7.2, H-1 ; 5.12, m, H-2 ; 5.30, dd, J = 12 and 2Hz, H-6 ; 4.55, br.s, H-12 ; 4.05 d and 4.57 d, J = 12, $-CH_2OAc$ and 5.68, s, H-2').[9]

Soxhlet extraction of the defatted stem bark with chloroform followed by column chromatography on silicic-acid-celite (2:1) led to the isolation of 9-methoxycanthin-6-one 5 as yellow needles, m.p. 178-180°. Its molecular formula was established by high resolution mass spectrometry as $C_{15}H_{10}N_2O_2$ ($M^{+\cdot}$ at m/z 250.0735). The u.v. spectrum showed maxima at 272 (ε 14750), 310 (ε 4500) and 353 nm (ε 7000), and the i.r. spectrum ($CHCl_3$) displayed absorptions at 1680, 1635 and 1610 cm^{-1}. These properties indicated that 5 belonged to the canthin-6-ones, the alcaloids characteristic of the Simaroubaceae [10]. The 250 MHz ^{1}H-n.m.r. spectrum revealed the presence of a methoxy group (δ 3.99 ppm) and decoupling experiments allowed the unambiguous assignment of all olefinic and aromatic protons. The spectrum (Fig.2) showed the presence of two isolated pairs of vicinal protons (δ 7.85 and 8.77 ppm, 2d, J = 5 Hz, H-1 and H-2 ; δ 8.02 and 6.96 ppm, 2d, J = 10 Hz, H-4 and H-5). The methoxy group must be located at C-9, since the downfield signal at δ 8.22, assigned to H-8, showed only meta-coupling with H-10 (δ 7.08, J = 2.5 Hz) which is in turn ortho-coupled with H-11 (δ 7.96, J = 9 Hz). Canthin-6-one 5 has recently been isolated by Giesbrecht et al. from the Brazilian Simaroubaceae, *Simaba cuspidata* [4].

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