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ISOLATION AND STRUCTURE OF SERGEOLIDE, A POTENT CYTOTOXIC QUASSINOID FROM *PICROLEMMA PSEUDOCOFFEA* [1]

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Summary : Sergeolide 2, isolated from the French Guyanan Simaroubaceae, *Picrolemma pseudocoffea* Ducke, is a novel quassinoid possessing the normal C_{20} basic skeleton with a butenolide function attached to the A ring. Structure 2 was established by interpretation of the 400 MHz ^{1}H-NMR and the ^{13}C-NMR data. It is highly cytotoxic and is active against mouse leukemia P388. The previously known isobruceine B 1 was also isolated.

The quassinoids[2], the bitter constituents of the Simaroubaceae, have been shown to possess diverse and potentially useful biological properties ranging from antineoplastic, antiviral and antimalarial activity to insecticidal and antifeedant behaviour [3-6]. In the framework of our continuing studies in this area we examined the heretofore unevaluated French Guyanan Simaroubaceae, *Picrolemma pseudocoffea* Ducke, utilized by the indigenous population as an anthelmintic agent [7]. We herein report the isolation and structural elucidation of sergeolide 2, a structurally novel, antileukaemic and highly cytotoxic quassinoid, isolated from the roots and stems of *P.pseudocoffea* ; the known quassinoid, isobruceine B 1 was also obtained.

The dried ground roots (430 g) of *P.pseudocoffea* were extracted with hexane and several times with hot water. The concentrated aqueous extract was then continuously extracted with chloroform to give a crude mixture of products (2.1 g) which showed potent antileukaemic activity against murine P 388 lymphocytic leukaemia (T/C 216 at 17 mg/kg). Chromatography of this extract on silic acid-celite (2:1) and elution with methylene chloride containing 2 % methanol afforded a mixture (1.2 g) of isobruceine B 1 and sergeolide 2. Extraction of the stems gave a similar mixture of the two quassinoids which were separated by low pressure column chromatography on Kieselgel 60 H (Merck) using ethylacetate containing 20 % hexane as an eluent.

Quassinoid 1 crystallized from ethylacetate-methanol, m.p. 255-258°, $[\alpha]_D^{22}$ + 17° (c = 1 ; MeOH). The molecular formula ($C_{23}H_{28}O_{11}$) was established by mass spectrometry with $M^{+\cdot}$ at m/z 480.1619 and abundant fragmentation ions at m/z 462.1533 ($C_{23}H_{26}O_{10}$) and m/z 420.1430 ($C_{21}H_{24}O_9$) indicating the presence of an acetoxy group. The i.r. spectrum (Nujol) showed carbonyl bands at 1750, 1730 and 1660 cm^{-1}, and, in agreement with the formulation of

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Table I : 400 MHz-NMR spectra of quassinoids 1 and 2 and 250 MHz-NMR spectrum of 3 [δ in ppm, J as (Hz)]

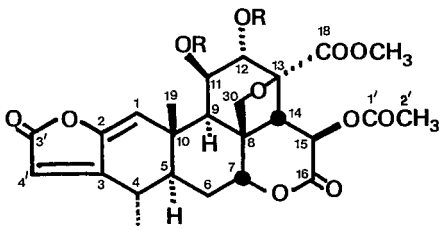
	1[a]	2[b]	3[a]
H-1	4.26 s	6.23 br.s	6.12 s
H-3	6.11 br.s		
H-4		2.40 m	
H-4'		5.71 dd (2; 2)	5.73 br.s
H-5	2.91 d (13)	1.85 ddd (13;2;9)	
H-6a	1.86 ddd (15;3;13)	1.68 ddd (15;3;2.5)	
H-6e	2.41 ddd (15;3;3)	2.28 ddd (15;2;2)	
H-7	4.75 m	4.65 br.s	4.74 br.s
H-9	2.38 br.d (5)	2.38 m	2.62 d (3)
H-11	4.75 m	4.57 dd (5;2)	5.58 d (5)
H-12	4.12 br.s	4.20 br.s	5.44 br.s
H-14*	3.03 d (13)	3.12 br.d (13)	
H-15*	6.30 d (13)	6.17 d (13)	6.05 d (13)
$-CH_2O-$	3.75 dd (8;1***)	3.82 dd (8;1***)	3.92 d (8)
	4.81 d (8)	4.78 d (8)	4.81 d (8)
OMe	3.83 s	3.85 s	3.78 s
Me-4	1.95 s	1.23 d (7)	1.23 d (7)
Me-10	1.20 s	1.57 s	1.42 s
OAc	2.08 s	2.10 s	2.06;2.10;2.16

a In $CDCl_3$; b in $CDCl_3$-5 % CD_3OD ; *Appears as a distinct doublet when the spectra were measured at 50°. A variable temperature study of 2 showed the existence of two conformations of the δ-lactone ring. At -70° : δ H-14, 3.10 (14 Hz) and 3.53 ppm (11 Hz) ;
*** Long range coupling with H-7.

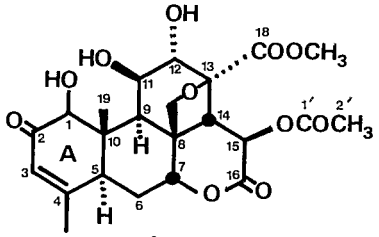
Table II : ^{13}C NMR spectra of quassinoids 1 and 2 in $CDCl_3$ and $CDCl_3$-5 % pyridine-d_5, respectively, measured at 22.63 MHz.

	1		2	
C(1)	81.3[a]	d	113.2[a]	d
C(2)	197.6	s	160.8	s
C(3)	124.5	d	148.4	s
C(4)	162.6[b]	s	30.7	d
C(5)	43.4[b]	d	38.8	d
C(6)	28.2	t	28.5	t
C(7)	81.7[a]	d	82.8	d
C(8)	45.8[b]	s	46.4	s
C(9)	42.4[b]	d	44.7	d
C(10)	47.7	s	41.6[b]	s
C(11)	74.3	d	72.9[b]	d
C(12)	75.1	d	76.0[b]	d
C(13)	81.7	s	81.1	s
C(14)	52.3	d	53.1	d
C(15)	67.8	d	66.9	d
C(16)	167.6[c]	s	167.1	s
C(18)	169.5[c]	s	170.9[c]	s
C(30)	73.0	t	73.8	t
OMe	49.8	q	51.2	q
Me-4	22.4	q	18.0	q
Me-10	11.3	q	19.4	q
C(1')	170.7[c]	s	169.4[c]	s
C(2')	20.5	q	20.4	q
C(3')			171.4[c]	s
C(4')			161.1[a]	d

[a-c] Signals within any vertical column may be reversed.



2 : R = H
3 : R = Ac



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ring A as in 1, the u.v. spectrum showed a maximum at 240 nm (ε 12000) and the mass spectrum the characteristic ions at m/z 151 and 135 [8]. These results and the 400 MHz ^{1}H-NMR spectral data (Table 1) suggested that quassinoid 1 was isobruceine B, previously isolated by Kupchan *et al.* [9] from *Brucea antidysenterica*. Structure 1 was supported by comparison of its ^{13}C-NMR spectrum with the previously published quassinoid spectra [10]. Because of the unavailability of an authentic sample, structure 1 was ultimately confirmed by single crystal X-ray analysis [11].

The somewhat less polar sergeolide, 2, was crystallized from acetone, m.p. 202-206° (dec.), $[\alpha]_D^{22}$ -103.3° (c = 1.21 ; MeOH). The molecular formula for 2 was established by microanalysis and high resolution mass spectroscopy as $C_{25}H_{28}O_{11}$ ($M^{+\cdot}$ 504.1656) and differed from that of 1 by two carbon atoms. The mass spectrum of 2 displayed, as did that for 1, abundant fragmentation ions due to the presence of an acetoxy group. The i.r. spectrum (Nujol) showed carbonyl bands at 1710 (br) and 1750 cm^{-1}, but lacked an absorption characteristic of an A-ring enone system and instead displayed a band at 1770 cm^{-1} (butenolide ring). The u.v. spectrum of 2 (λ_{max} 275 nm, ε 20.790) suggested the presence of a γ,δ-unsaturated butenolide function. The 400 MHz ^{1}H-NMR spectrum (Figure, table 1) was particularly revealing and with extensive decoupling experiments allowed the identification of all proton resonances and established the relative stereochemistry of 2. The structural similarity between quassinoids 1 and 2 was supported by the near identity of the chemical shifts and multiplicities of all hydrogen atoms except for those involved in, or in the immediate environment of, ring A. Thus the ^{1}H-NMR spectrum of compound 2 displays a signal for a secondary methyl group and two downfield one proton resonances at 5.71 and 6.23 ppm. These findings suggest that sergeolide is a two carbon homologue of isobruceine B and are consistent with the proposed structure 2.

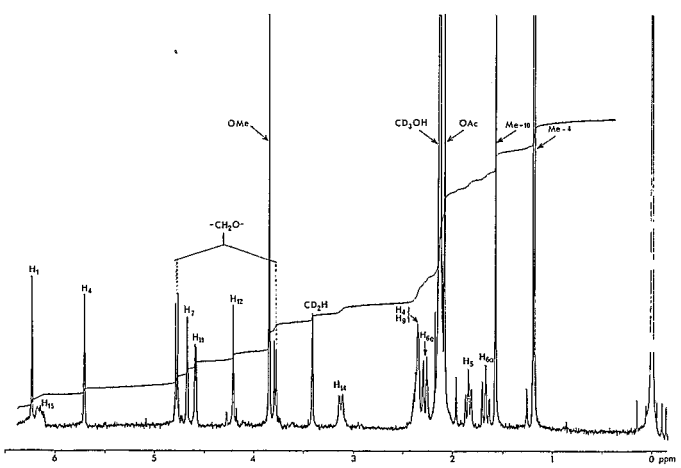


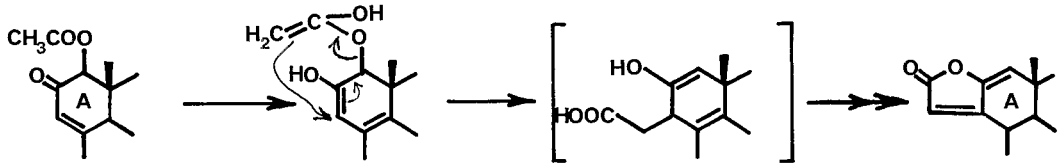
Figure : 400 MHz ^{1}H-NMR spectrum of 2 in $CDCl_3$-5%CD_3OD, at 30°C

Acetylation of 2 (Ac_2O, pyridine) afforded the diacetate 3, $C_{29}H_{32}O_{13}$ ($M^{+\cdot}$ 588), m.p. 210° (dec.), $[\alpha]_D^{22}$ -40.6° (c = 0.49, MeOH), UV (λ_{max} 278 nm), whose 250 MHz ^{1}H-NMR spectrum (Table 1) showed the expected downfield shifts for H-11 and H-12.

Comparison of the ^{13}C-NMR spectra (Table 2) of sergeolide 2 and isobruceine B 1 provides further confirmation for the structure of the former. Resonances assigned to carbon atoms on rings B, C and D of 1 are nearly superimposable with their

counterparts in 2. In agreement with the proposed structure 2, the spectrum lacks a ketone signal but possesses an additional lactonic carbonyl resonance and four absorptions due to two trisubstituted double bonds.

The structural similarity of 1 and 2 suggests that the latter arises from isobruceine B via the intermediacy of its 1-O-acetyl derivative. A Claisen-type rearrangement (*vide infra*) followed by the appropriate double bond migration and lactonisation would lead to 2.



Isobruceine B showed slight cytotoxicity and moderate antileukaemic activity against the murine P388 lymphocytic leukemia (T/C 140 at 1 mg/Kg) [3]. Preliminary antileukaemic screening [12] on sergeolide 2 gave a T/C of 140 at a 0.5 mg/Kg dose level. At slightly higher doses it was highly cytotoxic in this test system. In the chick embryo fibroblast test [4], 2 exhibited cytotoxicity at the 0.1µg/ml level and inhibition (69 %) of cell transformation induced by Rous Sarcoma Virus at the 0.05µg/ml level.

Sergeolide 2 is the first known example of a natural quassinoid possessing a butenolide ring system. It is of interest to note that this structural feature which might be expected a priori to act as a potent Michael acceptor should confer such a high degree of cytotoxicity to the molecule. Modifications of sergeolide are necessary in order to attenuate its toxicity and thus broaden its therapeutic index.

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12 - Testing was performed under NCI protocols by the procedures described by R.I. Geran, N. H. Greenberg, M.M. Macdonald, A.M. Schumacher and B.J. Abbott, Cancer Chemother. Repts. Part 3, 3 ; 1 (1972) ; courtesy of Dr. M. Suffness

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