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STRUCTURES OF SIMARINOLIDE AND GUANEPOLIDE (X-RAY ANALYSIS), NEW QUASSINOIDS FROM SIMABA cf ORINOCENSIS

Judith POLONSKY*, Zoïa VARON, Thierry PRANGE and Claudine PASCARD
Institut de Chimie des Substances Naturelles, C.N.R.S.,
91190 GIF-SUR-YVETTE, France

and Christian MORETTI
Office de la Recherche Scientifique et Technique, Outre-Mer,
B.P. 165, CAYENNE, French Guyana

Summary : Simarinolide 4a and Guanepolide 5a are new quassinoids with a C_{25} basic skeleton isolated from a member of the French Guyanan Simaroubaceae, *Simaba* cf *orinocensis* H.B.K. The structure 4a was established by spectral means and that of 5a by X-ray diffraction analysis. The previously known simarolide 1 was also isolated.

Our continuing work on the Simaroubaceae has demonstrated the structural diversity of their bitter constituents, the quassinoids [1], a group of compounds possessing an interesting spectrum of biological activity ranging from antineoplastic and antiviral to antimalarial activity [2-4]. Although most of the quassinoids isolated to date have a C_{20} basic skeleton, there are three known pentacyclic C_{25} quassinoids : simarolide 1 [5], picrasin A 2 [6] and soulameolide 3 [7]. We herein report the structural elucidation of two additional quassinoids possessing a C_{25} basic skeleton, designated simarinolide 4a and guanepolide 5a. They were isolated from the root bark of *Simaba* cf *orinocensis* H.K.B., a tall tree found in the costal forests of French Guyana and utilized by the indigenous population as anthelmintic [8].

The dried ground root bark (1kg) of *Simaba* cf *orinocensis* was extracted with hexane and several times with hot water. The concentrated aqueous extract was continuously extracted with chloroform. Column chromatography of the chloroform residue (7g) on silicic acid-celite (2:1) and elution with methylene chloride containing 3 % methanol afforded first the previously known simarolide 1 (2.1g) and then simarinolide 4a (0.9g). On increasing to 5 % methanol, guanepolide 5a (0.6g) was eluted.

Simarolide 1 [5] was identified by comparison of its spectral properties (t.l.c., IR, MS, ^{1}H- and ^{13}C-n.m.r.) with those of an authentic sample. It is of interest to note that *S.* cf *orinocensis* is very rich in simarolide and represents the only other source to date of this extremely bitter quassinoid.

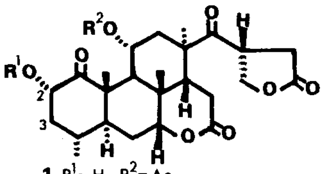
Simarinolide 4a crystallized from ethyl acetate, 208-210° (decomp.), $[\alpha]_D^{22}$ + 23.7° (c 0.84 ; $CHCl_3$). The high resolution mass spectrum established the molecular formula of simarinolide 4a as $C_{27}H_{36}O_8$ ($M^{+\cdot}$ at m/z 488.2408) and showed abundant fragmentation ions at m/z 428.2229 ($C_{25}H_{32}O_6$), 400.2213 ($C_{24}H_{32}O_5$) and 385.2040 ($C_{23}H_{29}O_5$) corresponding to the successive loss from the molecular ion of 1 mole of acetic acid, CO and CH_3 groupings. The i.r. spectrum (Nujol) showed a sharp carbonyl band at 1718 cm^{-1} (γ-lactone) and a broad band between 1710 and 1740 cm^{-1} (δ-lactone, acetate and ketone). The u.v. spectrum did not show

Table I : 250-MHz ^{1}H NMR spectra ^{a} of 4a, 4b 5a and 5b in $CDCl_3$. δ in ppm, J as (Hz)

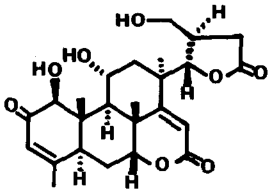
	4a	4b	5a	5b
H-2			5.70 d (10.2)	5.73 d (10.1)
H-3	5.46 m	5.45 m	6.71 d (10.2)	6.67 d (10.1)
H-7	4.21 t-like	4.20 t-like	4.30 t-like	4.30 t (2.5)
H-9	2.82 d (10)	2.80 d (10)	2.18 d (5)	2.22 d (5)
H-11	5.00 dt (10;3.8)	5.32 dt (10;3.8)	∿ 5.68 m	∿ 5.70 m
H-15			6.00 s	6.01 s
H-17	5.25 d (2.3)	4.83 br.s	4.68 d (2.6)	4.41 (2.7)
H-21	3.87 q (12;4) 3.53 m	4.21 m (2H)	3.70 q (10;4) 3.58 q	4.17 q (13;6) 4.05 q
Me-4	1.67 br.s	1.68 br.s	1.44	1.41
Me	1.52 1.24 0.86	1.44 1.24 0.87	1.34 1.28 1.23	1.34 1.30 1.24
OAc	1.92	1.91 2.08	2.02	2.09 2.04

Table II : ^{13}NMR spectrum of 4a in $CDCl_3$; $^{a-b}$ signals may be reversed. Shifts in ppm.

C-1	211.0 s
C-2	40.3 t
C-3	120.8 d
C-4	135.1 s
C-5	44.1 d
C-6	25.6 t
C-7	81.3 d
C-8	36.7 s
C-9	37.6 d
C-10	48.9 s
C-11	68.8 d
C-12	36.5 t
C-13	43.0 s
C-14	44.1 d
C-15	29.9 t
C-16^{a}	171.7 s
C-17	84.9 d
C-20	36.9 d
C-21	64.3 t
C-22	32.0 t
C-23	176.3 s
Me-4^{b}	20.3 q
Me-8	25.3 q
Me-10	12.1 q
Me-13	18.4 q
CH_3CO^{a}	170.6 s
CH_3CO^{b}	21.3 q



1 R^1= H ; R^2= Ac
2 R^1= Me ; R^2= H ; 2,3 dehydro



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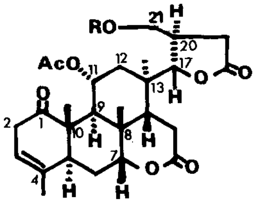
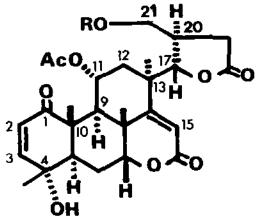


4a : R = H ; 4b : R = Ac



5a : R = H ; 5b : R = Ac

any significant absorption above 218 nm and the circular dichroism curve displayed a Cotton effect at 300 nm (Δ_ε - 1.27, in dioxane) in agreement with the presence of a non-conjugated ketone at C-1. The 250 MHz ^{1}H n.m.r. spectrum of 4a (Table I) revealed one acetoxy group and four tertiary methyl groups and suggested that one of them (δ 1.67) was a vinyl methyl located at C-4, long-range coupled with H-3 (δ 5.46). Extensive double resonance experiments identified other signals due to protons on oxygen-bearing carbons. The assignments shown were substantiated by the 250 MHz ^1H n.m.r. spectrum (Table I) of the acetyl derivative 4b, $C_{29}H_{38}O_9$, ($M^{+\cdot}$ at m/z 530), obtained by acetylation of 4a with acetic anhydride in pyridine, which revealed a highfield shift for H-17 resonance and a significant downfield shift for the H-21 resonances. The formulation of ring A as in 4a was supported by the presence of a significant peak at m/z 365.1622 ($C_{19}H_{25}O_7$) in its mass spectrum which can be attributed to the loss from the molecular ion of the elements of ring A ($C_8H_{11}O$) after a C(9)-C(10) bond rupture involving a McLafferty rearrangement followed by the cleavage of the C(5)-C(6) bond. In the mass spectrum of 4b this fragmentation ion as well as the other significant ions reported for 4a were shifted 42 amu higher. Finally, structure 4a for simarinolide was confirmed by comparison of its ^{13}C-n.m.r. spectrum (Table II) with that of simarolide 1 and also with the previously published quassinoid spectra [9].

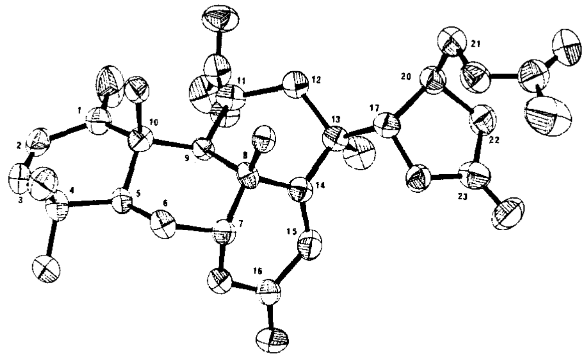
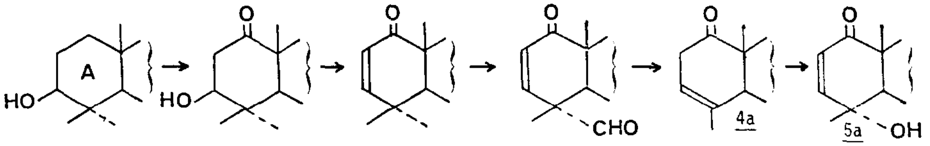


Figure. Molecular Structure of 5b

The molecular formula of guanepolide 5a was found by elemental and mass spectral analyses to be $C_{27}H_{34}O_9$ (M^+ 502) ; m.p. 282-284° ; $[\alpha]_D^{22}$ - 71° (0.83, pyridine). U.V. : λ_{max} 225 nm (ε 18825) due to both the α,β unsaturated ketone and α,β-unsaturated lactone moieties ; C.D. : λ_{max} 324 nm (Δ_ε - 0.92, in dioxane). The ^1H-n.m.r. spectrum of 5a (Table I) revealed one acetoxy group, four tertiary methyl groups, an olefinic AB quartet (doublets at δ 5.70 and 6.71) and a sharp, downfield, one proton singlet at δ 6.00 assigned to 15-H. The assignment of the other signals due to protons on oxygen-bearing carbons was made by double resonance experiments and substantiated by the 250 MHz ^1H-n.m.r. spectrum (Table I) of the acetyl derivative 5b, $C_{29}H_{36}O_{10}$, m.p. 270-272° in which the H-21 protons are significantly de-shielded. The presence of the γ-lactone ring , as in 5a, was supported by the presence of an abundant fragment ion at m/z 328 in its mass spectrum. This fragmentation probably arises, as observed for 3 [7], by a McLafferty type rearrangement involving the 14,15 double bond and resulting in the cleavage of the 13,17 carbon bond, accompanied by a loss of CH_3CO_2H.

All the foregoing results showed that 5a differed from 3 by the presence of an acetoxy in place of a hydroxy-group at C-11 and by the ring A structure. Unequivocal proof for the structure and relative stereochemistry of guanepolide 5a was provided by single-crystal X-ray analysis using crystals of 5b obtained from methanol. Crystal data : Orthorhombic, space group $P2_12_12_1$, a = 9.860, b = 13.831 (5), c = 19.510 Å, Z = 4. A total of 1789 structural factors with $I > 2\sigma(I)$ were used. The multisolution technique gave in the best E map 80 % of the structure. The missing atoms were located on successive Fourier recycling procedures and anisotropically refined to a R value of 7 %. Hydrogen atoms were introduced at their theoretical positions with an isotropic thermal factor equal to that of the bonded carbon and were not refined. The molecular structure of 5b is shown in the Figure.[10]

Simarinolide 4a and guanepolide 5a have A ring structures not previously encountered among the quassinoids. 5a is the first quassinoid to have two substituents at C-4 i.e. an hydroxyl and an axially oriented methyl group. The formation of the A-ring in 4a and 5a from the triterpene precursor cans be explained by the following pathway :



Our previous biogenetic experiments[11] support the loss of the equatorial methyl group at C-4 in the sequence leading to 4a and hence to 5a.

The discovery of 4a and 5a making a total of five C-25 quassinoids now known, all of which lack a C-12 oxygen function, further substantiates the hypothesis[6] that compounds of this type may be intermediates in the biosynthesis of the known numerous quassinoids with a C_{20} basic skeleton. It is clear that introduction of an oxygen function at C-12 in the C-25 quassinoids can lead to C_{13}-C_{17} bond rupture since all of these compounds, known to date, possess an oxygen function at C-17.

4a and 5a which lack the epoxymethano bridge in ring C did not display, as expected[2], significant inhibitory in vivo activity against the P-388 lymphocytic leukemia.

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