Journal of Natural Products
Vol. 49, No. 3, pp. 440-444, May-Jun 1986

TWO NEW QUASSINOIDS FROM *SIMABA MULTIFLORA* FRUITS[1]

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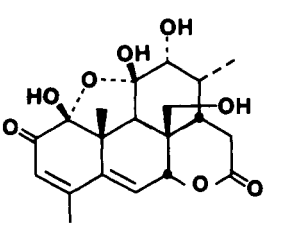
ABSTRACT.—Two new quassinoids, 13,18-dehydro-6α-senecioyloxychaparrin (**4**) and 12-dehydro-6α-senecioyloxychaparrin (**5**), have been isolated from *Simaba multiflora* fruits. Their structures were deduced from spectral data. ^{1}H-^{13}C 2-D chemical-shift correlation nmr was applied to the structural elucidation of the antileukemic quassinoid **4**.

Part of our earlier studies on the quassinoids (2) was concerned with the French Guyanan Simaroubaceae *Simaba multiflora* A. Juss. Initially, karinolide (**1**) (3), 6α-senecioyloxychaparrinone (**2**) (3,4), and 6α-senecioyloxychaparrin (**3**) (3,5) were isolated from the stem and root bark. We now report the structural elucidation of two new quassinoids, namely, 13,18-dehydro-6α-senecioyloxychaparrin (**4**) and 12-dehydro-6α-senecioyloxychaparrin (**5**), isolated from the *S. multiflora* fruit extract. Quassinoid **4** significantly inhibits growth of the murine lymphocytic leukemia PS cell line (ED_{50} 1.7 μg/ml (6), and quassinoid **5** was found to be essentially inactive. Several quassinoids are known to have a 13,18 double bond, whereas naturally occurring quassinoids possessing a 12-oxo group are extremely rare (2).

The molecular formula for 13,18-dehydro-6α-senecioyloxychaparrin (**4**), mp 241-244°, was found by hrms, cims, and fabms to be $C_{25}H_{32}O_9$, and thus the molecular weight of **4** is 2 a.m.u. lower than that of **3**. The uv spectrum of **4** revealed λ max 218 nm (ε 17,560) attributed to the α,β-unsaturated ester and the ir spectrum showed two carbonyl bands at 1710 (δ-lactone) and 1700 cm^{-1} (α,β-unsaturated ester). In agreement with the presence of a senecioate ester, the eims of **4** showed, as did that of **3**, fragmentation ions at *m/z* 376 ($M^{+\cdot}-100$), 83 (C_5H_7O) (base peak), and 55 (C_4H_7). The presence of this ester chain was further substantiated by the 400 MHz ^{1}H-nmr spec-



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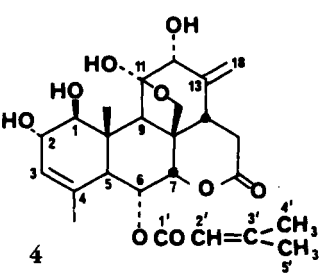

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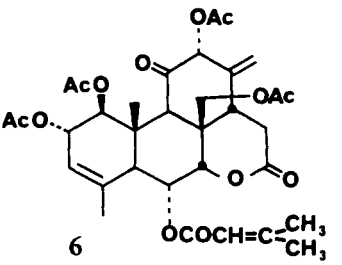

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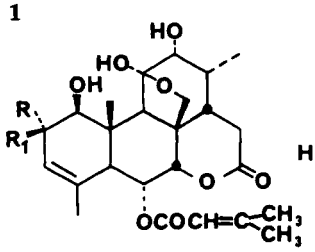
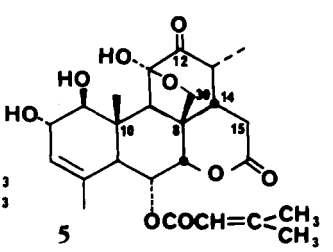


2 R=R$_1$=O
3 R=OH, R$_1$=H



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[1]For a previous paper in this series, see Bhatnagar *et al.* (1).

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trum of **4** (Table 1 and Figure 1) which showed in particular a singlet at δ 5.91 assigned to H-2′. The location and stereochemistry (6α) of the ester group in **4** was determined by nmr double resonance studies. The assignment of the ^{1}H-nmr spectrum of **4** was supported by its ^{1}H-^{13}C 2-D chemical-shift correlation spectrum (Figure 1) (see below).

TABLE 1. 400 MHz ^{1}H-nmr Spectra of Quassinoids **4** and **5** (δ in ppm, *J* in Hz)

Atom No.	Compound	
	4[a]	**5**[b]
H-1	4.22 d (7.5)	3.78 d (8)
H-2	4.63 br.d (7.5)	4.37 br.d (8)
H-3	5.85 br.s.	5.72 d (1)
H-5	3.40 d (13.6)	3.07 br.d (12)
H-6	5.92 d (13.6)	5.68 dd (12,2)
H-7	4.95 s	4.80 d (2)
H-9	3.44 s	2.18 s
-CH_2O	4.48 d (8.5)	4.61 d (9)
	3.79 d	4.13 d
H-12	4.63 s	—
H-13	—	3.36 dd (7,8)
H-14	2.95 dd (6,13)	2.57 ddd (8,6,13)
H-15α	3.74 dd (13,18)	2.29 dd (13,18)
H-15β	3.00 dd (6,18)	2.64 dd (6,18)
H-18	5.24 s	—
	5.31 s	
Me-13	—	0.98 d (7)
H-2′	5.91 s	5.75 s
Me-4′	1.75 s	1.70 s
Me-5′	2.27 s	2.20 s
Me-4	1.94 s	1.85 s
Me-10	1.89 s	1.81 s

[a]In pyridine-d_5.
[b]In pyridine-d_5+$CDCl_3$.

The structural similarity between the quassinoids **3** and **4** was shown by the near identity of the chemical shifts and multiplicities of most of the hydrogen atoms, the striking difference being the presence of a vinylidene group (singlets at δ 5.24 and 5.31) instead of the secondary methyl group at C-13 in the spectrum of **3**.

The structure of quassinoid **4** was further supported by its ^{13}C-nmr spectrum (Table 2 and Figure 1) which showed that **4** possesses one methyl group less and two sp^2 carbon atoms (δ 147.7 s and 118.2 t) more than quassinoid **3**. The ^{13}C multiplicities were determined by off-resonance decoupling and also by the *J*-modulated spin echo technique (7). The ^{13}C spectrum of **4** was interpreted on the basis of published ^{13}C data for quassinoids (8) and by analysis of its very informative ^{1}H-^{13}C 2-D chemical-shift correlation spectrum (Figure 1). After a small number of steps back and forth between ^{1}H and ^{13}C assignments, the ^{13}C and ^{1}H spectra may be fully analyzed and the possible ambiguities removed. Thus, the ^{1}H-nmr methyl signals could be correctly assigned. The ^{13}C resonances of the methyl groups of the senecioate moiety at δ 27.29 and 20.46 [Lit. (9): δ 26.1 and 19.1] were found to be correlated, respectively, with the proton methyl signals at δ 1.75 and 2.27; the remaining Me resonances were found at δ 1.94 and 1.89, the first being attributed to Me-4 and the second to Me-10.

The cross section of the 2-D spectrum allowed observation of each of the overlap-

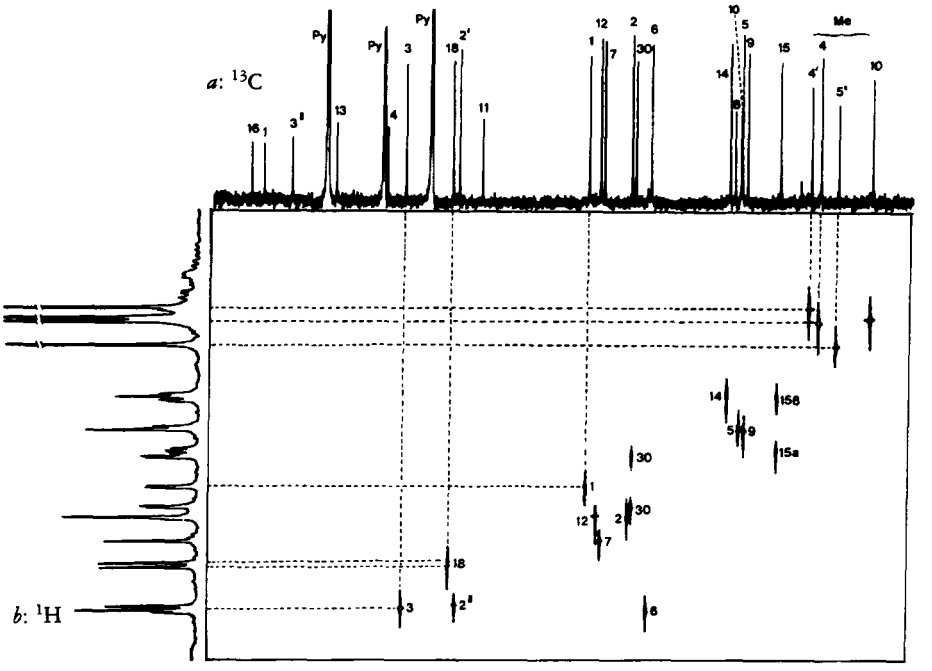

FIGURE 1. ^{1}H-^{13}C 2-D chemical-shift correlation nmr spectrum of **4** presented as a contour plot; *a*: ^{13}C-nmr spectrum; *b*: ^{1}H-nmr spectrum.

ping proton signals (e.g., H_2 and H_{12}) and exact measurement of the coupling constants. In this manner, the ^{13}C methine groups at δ 43.59, 44.95, and 48.12 could be unambiguously assigned to C-9, C-5, and C-14, respectively.

Acetylation (Ac_2O-pyridine) of quassinoid **4** afforded tetraacetate **6** mp 156-158°; eims *m/z* 584 ($M^{+\cdot}-60$) and ^{1}H nmr four Ac at δ 1.81, 2.08, 2.12, and 2.16.

The foregoing results unequivocally establish the proposed structure for quassinoid **4**.

12-Dehydro-6-α-senecioyloxychaparrin (**5**), less polar than quassinoid **4**, had its molecular formula $C_{25}H_{32}O_5$ (the same as that of **4**) established by hrms; uv λ max 217 nm (ε 15,500) (α,β-unsaturated ester) and ir carbonyl bands at 1720 (δ-lactone) and 1705 (s) cm^{-1} (α,β-unsaturated ester and keto group). The presence of a senecioate ester in **5** was shown by the mass spectral fragmentation [*m/z* 376.496 ($C_{20}H_{24}O_7$), 83.0208 (C_5H_7O) and 55.0549 (C_4H_7)] and by the ^{1}H-nmr spectrum (Table 1). This spectrum is very similar to that of quassinoid **3** and shows one secondary, one quaternary, and three vinylic methyl groups. The presence of a keto function is reflected by the cd spectrum (MeOH) (Δδ+0.18 at 313 nm) and by the presence of a resonance at δ 207.9 in the ^{13}C-nmr spectrum (Table 2) of **5**. Since the ^{1}H-nmr spectrum did not show any signal assignable to the proton at C-12, the keto group has to be located at this position. The ^{13}C-nmr spectrum of **5** compared to that of quassinoid **4** displayed one [>CH-O] grouping less and fully supported the proposed structure for quassinoid **5**.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were determined on a Kofler melting point apparatus and are uncorrected. Optical rotations were determined (room temperature) with a Rousel-Jouan Quick Polarimeter. Ir spectra were recorded in nujol on a Perkin-Elmer model 257, and the uv spectra were measured in EtOH with a Perkin-Elmer Lambda spectrometer. Eims were taken on an MS-50 AEI spectrometer, cims on a modified (10) MS-9 apparatus and fabms on an MS-80 RF (Kratos Ltd). The 400 MHz ^{1}H-nmr and 100.6 MHz ^{13}C-nmr spectra were recorded with a Bruker AM-400 spectrometer. The cd was measured on a Jobin-Yvon Auto-dichrograph Mark V.

TABLE 2. 100.6 MHz ^{13}C-nmr Spectra of Quassinoids **4** and **5**

Atom No.	Compound	
	4[a]	**5**[b]
C-1	83.9 d	83.6 d
C-2	73.0 d	72.5 d
C-3	130.1 d	129.7 d
C-4	134.8 s	134.5 s
C-5	44.9 d	44.3 d
C-6	68.1 d	67.7 d
C-7	80.0 d	79.0 d
C-8	46.8 s	47.5 s
C-9	43.6 d	40.7 d
C-10	45.3 s	45.7 s
C-11	110.9 s	108.5 s
C-12	81.0 d	207.9 s
C-13	147.7 s	50.4 d
C-14	48.1 d	43.6 d
C-15	35.2 t	28.9 t
C-16	169.1 s	168.5 s
C-30	72.0 t	72.7 t
C-18	118.2 t	—
Me-13	—	11.8 q
C-1′	167.0 s	166.0 s
C-2′	116.6 d	116.4 d
C-3′	158.8 s	159.2 s
Me-4′	27.3 q	27.7 q
Me-5′	20.5 q	20.7 q
Me-4	24.8 q	24.9 q
Me-10	11.8 q	10.2 q

[a]In pyridine-d_5.
[b]In pyridine-d_5 + $CDCl_3$.

EXTRACTION AND ISOLATION.—The plant material, collected in 1984 in French Guiana on the banks of the river Maroni, consisted of the fruits of *S. multiflora*. A voucher specimen (No. Moretti 1400) is deposited in the herbarium of the Museum d'Histoire Naturelle de Paris. The dried, ground fruits (1.5 kg) were defatted by percolation with hexane at room temperature. The mass was then stirred for several hours with hot H_2O (70-75°), separated by filtration in vacuo, and resuspended in fresh, hot H_2O. This was repeated several times until the filtrate was no longer bitter. The combined aqueous extracts were concentrated in vacuo and then continuously extracted with $CHCl_3$. Evaporation of the solvent yielded a brown foam (2.6 g) which proved, as shown by tlc, to be a complex mixture of products.

This mixture was chromatographed on a column of Kieselgel 60 (Merck) (150 g) using $CHCl_3$ containing increasing amounts of MeOH (1-7%) as the eluent. Fractions of 20 ml were collected and combined on the basis of tlc similarity ($CHCl_3$ + 5% MeOH). Elution with $CHCl_3$ containing 3% MeOH gave first the quassinoid **2** followed by **5** and **3**; they were purified by crystallization affording 62 mg, 35 mg, and 15 mg, respectively.

Elution with $CHCl_3$ containing 5% MeOH gave crude quassinoid **4** which was purified by low-pressure column chromatography on Kieselgel 60 H (Merck) using $CHCl_3$ containing 10% iPrOH as eluent. Further purification was achieved by preparative layer chromatography ($CHCl_3$ + 10% iPrOH) followed by crystallization to yield 38 mg of pure **4**.

Recrystallization of **4** from a mixture of $CHCl_3$ and MeOH afforded colorless needles, mp 241-244°; $[\alpha]^{22}D$ +332° (c, 0.52 in pyridine); hrms $M^{+\cdot}$ at *m/z* 476.2029; required for $C_{25}H_{32}O_9$: 476.2046; cims (NH_3) abundant $(NMH_4)^+$ and $(MNH_4\text{-}18)^+$ ions at *m/z* 494 and 476, respectively; negative fabms (glycerol, DMF) strong peak at *m/z* 475 [(M-H)$^-$ ion]. The 400 MHz ^{1}H and 100.6 MHz ^{13}C-nmr data have been entered in Tables 1 and 2, respectively.

TETRAACETATE **6**.—Quassinoid **4** (6 mg) was stirred for 24 h at room temperature with 0.5 ml of pyridine and 0.5 ml of Ac_2O. Usual work up followed by plc ($CHCl_3$ + 2% MeOH) gave **6** (3,5 mg) which

crystallized from MeOH as fine needles, mp 156-158°. 400 MHz ^{1}H-nmr spectrum (pyridine-d_5) δ 1.71, 1.86, 1.91, and 2.24 (singlets, 3H each, Me's), 3.82 (1H, s, H-9), 4.78 (1H, d, J=13 Hz) and 5.06 (1H, d, J=13 Hz) (AB, -CH_2O-), 5.36 (1H, d, J=2.0 Hz, H-7), 5.70 (1H, s, H-12), 5.70 (1H, s, H-2′), 5.85 (1H, br.s., H-3).

Recrystallization of **5** from a mixture of EtOAc and MeOH afforded colorless needles, mp 252-255°; $[\alpha]^{22}D$ +95.7° (c, 0.47 in pyridine). Hrms $M^{+\cdot}$ at *m/z* 476.2067; required for $C_{25}H_{32}O_9$: 476.2046. The 400 MHz ^{1}H and 100.6 MHz ^{13}C-nmr data have been entered in Tables 1 and 2, respectively.

ACKNOWLEDGMENTS

We are grateful to Professor G.R. Pettit for biological testing and Mr. P. Varenne for FAB and CI mass spectra. We gratefully acknowledge support of this investigation by PHS Grant number 5 RO1 CA 26699-04 awarded by the National Cancer Institute, DHHS.

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Received 23 September 1985