PLANT ANTICANCER AGENTS. XXIII. 6α-SENECIOYLOXY-CHAPARRIN, A NEW ANTILEUKEMIC QUASSINOID FROM *SIMABA MULTIFLORA*[1,2]

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ABSTRACT.—Fractionation of an aqueous ethanol extract of the wood of *Simaba multiflora* afforded a new antineoplastic quassinoid, 6α-senecioyloxychaparrin (**5**), and two known quassinoids 6α-senecioyloxychaparrinone (**1**) and chaparrinone (**2**). The structure of **5**, which displayed activity against the KB and P-388 test systems, was established through the interpretation of spectral data.

The genus *Simaba* in the family Simaroubaceae has previously afforded several quassinoids displaying antineoplastic activity. Thus *Simaba multiflora* A. Juss has afforded 6α-senecioyloxychaparrinone (**1**) and chaparrinone (**2**) (2), and *S. cuspidata* Spruce has yielded 6α-tigloyloxychaparrinone (**3**) and 6α-tigloyloxychaparrin (**4**) (3).

6α-Senecioyloxychaparrinone (**1**) was considered to possess sufficiently potent antileukemic activity in the P-388 test system *in vivo* (4) to warrant further investigation. In the course of a large-scale isolation of **1** from an ethanolic extract of the wood of *Simaba multiflora*, a novel antileukemic principle, 6α-senecioyloxychaparrin (**5**), was isolated and characterized together with **1** and **2**.

EXPERIMENTAL[4]

INITIAL FRACTIONATION.—An ethanol extract of the wood (318 kg) of *S. multiflora*,[5] when concentrated *in vacuo* at 50°, afforded a dark brown residue (3.18 kg). Partitioning a portion (1.3 kg) of this residue between methanol-water (1:9) (3 liters) and chloroform (3 liters) and evaporation of the chloroform-soluble fraction *in vacuo* yielded a viscous brown extract (726 g). When the chloroform extract was further partitioned between petroleum ether (2 liters) and methanol-water (9:1) (2 liters) and the aqueous methanolic solution was evaporated *in vacuo*, a dark brown extract was obtained.

SEPARATION AND ISOLATION.—A portion (76.3 g) of the extract was treated with methanol and the insoluble portion (1 g) chromatographed on silica gel[6] (20 g) packed in chloroform. Elution with mixtures of chloroform and methanol of increasing polarity afforded, with chloroform-1% methanol, 6α-senecioyloxychaparrinone (**1**, 71 mg, 0.00011%). With chloroform-10% methanol, 6α-senecioyloxychaparrin (**5**, 145 mg, 0.00022%) and β-sitosterol-β-D-glucoside (300 mg, 0.00046%) were eluted.

The methanolic filtrate was evaporated *in vacuo*, redissolved in chloroform and chromatographed on silica gel (700 g) eluting successively with chloroform and chloroform containing increasing volumes of methanol. A total of 21 fractions (2 liters each) were collected. The eluate from a chloroform-2% methanol mixture afforded 6α-senecioyloxychaparrinone

[1]For Paper XXII in this series see reference 1.

[2]These data were first presented at the Joint Meeting of the American Society of Pharmacognosy and the Society for Economic Botany, Boston, Mass., July, 1981.

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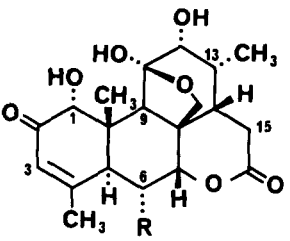
[4]Melting points were determined using a Kofler hot-stage instrument and are uncorrected. UV spectra were measured on a Beckman model DB-G grating spectrophotometer, and the ir spectra were obtained on a Beckman model 18-A spectrophotometer, with polystyrene calibration at 1601 cm^{-1}. PMR spectra were recorded on a Varian model T-60A instrument, equipped with a Nicolet model TT-7 Fourier Transform attachment or at 360 MHz using a Bruker WM 360 instrument. Tetramethylsilane was used as an internal standard and chemical shifts are reported on the δ (ppm) scale. Low resolution mass spectra were obtained with a Varian MAT 112S double-focusing spectrometer operating at 70 ev. High resolution mass spectra were obtained with a Varian 731 double-focusing spectrometer operation at 70 eV.

[5]The plant material used in this study was collected in Peru, in September 1976 by Dr. S. McDaniel under collection number MR-2555. A voucher specimen is deposited at the Economic Botany Laboratory, USDA, Beltsville, MD. The extract was supplied by Polysciences, Inc., Warrington, PA through a contract with the Natural Products Branch, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD.

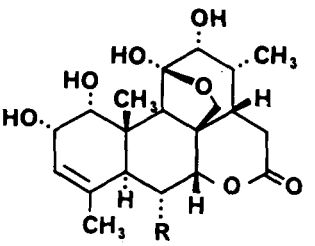
[6]E. Merck, Darmstadt, W. Germany.



1 R = senecioyloxy

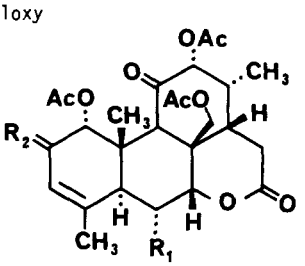
2 R = H

3 R = tigloyloxy



4 R = tigloyloxy

5 R = senecioyloxy



6 R_1 = senecioyloxy, R_2 = O

7 R_1 = senecioyloxy, R_2 = H, α-OAc

8 R_1 = tigloyloxy, R_2 = H, α-OAc

(**1**, 158 mg, 0.00024%) from fraction 11 and chaparrinone (**2**, 50 mg, 0.00008%) from fraction 13. From fractions 17 and 18 eluted with chloroform-10% methanol, 6α-senecioyloxychaparrin (**5**, 292 mg, 0.00045%) and β-sitosterol-β-D-glucoside (33 mg, 0.00005%) were isolated.

IDENTIFICATION OF 6α-SENECIOYLOXYCHAPARRINONE (**1**).—Colorless needles, mp 255–257°; $[\alpha]^{25}D$+213.0° (c 0.08, pyridine) [Lit. (2) +203.8° (c 0.21, MeOH)]. The identity of **1** was confirmed through the analysis of spectral data (uv, ir, ^1H nmr, and ms) and direct comparison with an authentic sample (2). Additional evidence was obtained through the synthesis of the triacetate **6**, mp 269–271°, whose physical and spectral data corresponded well with those published (2). Details of the spectral properties are available from the authors.

CHARACTERIZATION OF 6α-SENECIOYLOXYCHAPARRIN (**5**).—Colorless needles, mp 278–282°; $[\alpha]^{25}D$+240.0° (c 0.08, pyridine); ir, νmax (KBr) 3440, 3390, 2960, 2900, 2860, 1720, 1700, 1650, 1500, 1445, 1380, 1350, 1255, 1225, 1140, 1055, 1020, 995, 985, 960, 915, 900, 850, 800 and 725 cm^{-1}; uv, λmax (MeOH) (log ε) 219 nm (4.24); pmr (360 MHz, pyridine-d_5) see table 1; ms, *m/z* 478 (M^+, 2%), 460 (2), 378 (4), 360 (9), 345 (6), 264 (10), 247 (16), 246 (25), 231 (48), 229 (14), 157 (14), 105 (11), 95 (12), 91 (13), 84 (21), 83 (100), 82 (15), 69 (12), 57 (6), and 55 (95); Mass measurement, *m/z* 478.2206 ($C_{25}H_{34}O_9$ requires 478.2201).

ACETYLATION OF 6α-SENECIOYLOXYCHAPARRIN (**5**).—6α-Senecioyloxychaparrin (**5**, 50 mg) was reacted with acetic anhydride-pyridine (1:1, 1 ml) at room temperature for 48 hrs. Work-up in the usual manner gave a viscous colorless tetraacetate **7**; $[\alpha]^{25}D$+141.3° (c 0.08, MeOH); ir, νmax (NaCl plate) 2930, 1740, 1650, 1450, 1380, 1230, 1140, 1120, 1070, 1020, 960 and 750 cm^{-1}; pmr (60 MHz, $CDCl_3$) see table 1; ms, *m/z* 646 (M^+, 0.5%), 544 (1), 486 (3), 444 (28), 371 (12), 311 (21), 283 (7), 83 (100), 55 (20) and 43 (44).

IDENTIFICATION OF CHAPARRINONE (**2**).—Colorless needles, mp 235–239°; $[\alpha]^{25}D$−48.0° (c 0.07, pyridine). Identity with **2** was confirmed by direct comparison with an authentic sample isolated by us from *Ailanthus integrifolia* subsp. *calycina* (5).

IDENTIFICATION OF β-SITOSTEROL-β-D-GLUCOSIDE.—Colorless plates, mp 297–298°. Acetylation with acetic anhydride-pyridine gave colorless needles, mp 161–162°. Identity was confirmed by comparison (mmp, tlc and ir) with authentic samples.

STRUCTURE ELUCIDATION OF 6α-SENECIOYLOXYCHAPARRIN (**5**).—A molecular ion in the mass spectrum of **5** was observed at 478 amu analyzing for $C_{25}H_{34}O_9$ by high resolution mass spectrometry. The ir spectrum showed the presence of carbonyl groups at 1720 (δ-lactone) and 1700 cm^{-1} (α,β-unsaturated ester) and the uv absorption at 219 nm of the α,β-unsaturated ester. The presence of a senecioate, tiglate or angelate moiety was suggested by the loss of 100 amu, to *m/z* 378, in the mass spectrum and by the observation of an ion at *m/z* 83 (2, 3, 5). A distinction between the possible esterifying groups was evident from the ^1H nmr spectrum of **5** at 360 MHz (table 1), which showed signals characteristic of a

senecioate ester. Thus two singlets for the senecioate methyl groups (2) appeared at δ 1.90 and 2.24 ppm, and the olefinic proton α to the carbonyl was observed at 5.80 ppm rather than at 6.9 ppm (for a tiglate ester) or at δ 6.0–6.1 ppm (for an angelate ester). The methyl groups of the quassinoid skeleton appeared at δ 1.11 (13–CH_3), 1.71 (10–CH_3) and 1.90 (4–CH_3) with the vinyl proton at the 3-position observed at 5.85 ppm as a broadened singlet. A broadened doublet at 4.59 ppm was assignable to the proton located at the 2-position with the associated 1–H appearing as a doublet at 4.13 ppm. The location and stereochemistry of the ester side chain was deduced from a one-proton doublet of doublets (J=2.0, 12.0 Hz) at 5.90 ppm. This doublet is the 6β-proton coupling *trans* diaxially with H–5α and equatorially with H–7β, indicating the ester to have the 6α-configuration.

Acetylation of **5** afforded a tetraacetate derivative **7** which, in the mass spectrum, displayed a molecular ion at m/z 646 with significant fragment ions at m/z 544 (M^+–60–42), 486 (M^+–60–100) and 444 (M^+–60–100–42). As expected (3), the 2–, 3– and 11–H were shifted downfield on acetylation (by 0.8–1.3 ppm) with the 30–H_2 shifting downfield by 0.4 ppm, and close correspondence was observed with the tetraacetate of 6α-tigloyloxychaparrin (**8**) (table 1) with the exception of the side chain protons. With this information the isolate 5 is proposed to have the structure 6α-senecioyloxychaparrin.

TABLE 1. Proton nuclear magnetic resonance data for chaparrin derivatives.

Proton	Compound[a]							
	5[b]		**4**[c]		**7**[d]		**8**[e]	
1–H	4.13 d	7.5	4.15 d	7.6	5.08 d	6.3	5.11 d	6.8
2–H	4.58 br d	7.5	4.61 br d	7.6	5.41 m		5.33 d	6.8
3–H	5.85 br s		5.86 br s		5.41 m		5.51 m	
6–H	5.90 dd	2.0, 12.5	5.95 dd	2.3, 12.0	5.41 m		5.32 dd	2.6, 11.0
7–H	4.83 d	2.0	4.80 d	2.0	4.74 d	2.0	4.71 d	2.6
9–H	3.19 s		3.19 s		3.29 s		3.31 s	
–CH_2O–	3.88 d	8.5	3.86 d	9.0	4.03 d	13.0	4.04 d	12.8
	4.49 d	8.5	4.47 d	9.0	4.65 d	13.0	4.63 d	12.8
12–H	3.99 m		4.00 m		5.08 m		5.05 d	2.7
13–CH_3	1.11 d	6.5	1.11 d	6.8	0.98 d	6.2	1.00 d	6.4
10–CH_3	1.71 s		1.86 s		1.56 s		1.55 s	
4–CH_3	1.90 s		1.89 s		1.75 s		1.72 br s	
2'–CH_3	——		1.89 s		——		1.85 s	
3'–CH_3	1.92, 2.24 s		1.60 d	6.8	1.82, 2.19 s		1.83 d	7.2
2'–H	5.80 s		——		5.74 s		——	
3'–H	——		7.09 q	6.8	——		7.27 q	7.2
OAc	——		——		1.81 s		1.82 s	
					1.99 s		2.00 s	
					2.13 s		2.13 s	
					2.19 s		2.19 s	

[a]Frequency, multiplicity and coupling constant (Hz) are given.
[b]Recorded at 360 MHz in pyridine-d_5.
[c]Recorded at 250 MHz in pyridine-d_5 (3).
[d]Recorded at 60 MHz in $CDCl_3$.
[e]Recorded at 250 MHz in $CDCl_3$ (3).

BIOLOGICAL ACTIVITY OF THE ISOLATES.—The anticancer activities of the quassinoid isolates from *Simaba multiflora* are shown in table 2. 6α-Senecioyloxychaparrinone (**1**) shows the highest *in vitro* activity in the KB system (4), whilst **5** and **2** have very similar activity. 6α-Senecioyloxychaparrin (**5**) and **1** have comparable activity in the P-388 lymphocytic leukemia test system *in vivo*.

DISCUSSION

Simaba multiflora has afforded a new antileukemic quassinoid 6α-senecioyloxychaparrin (5)[7] as well as the known compounds 6α-senecioyloxuchaparrinone (**1**) and chapparrinone (**2**). The structure was deduced by interpretation of the spectral data of the parent compound **5** and the tetraacetate **7** with those of the corresponding tigloyloxy derivatives **4** and **8** (3).

Anticancer activity in the quassinoids is a well-documented phenomenon

[7]During the course of the preparation of this manuscript we learned of the isolation work of Polonsky *et al.* on the stem bark of *S. multiflora* from French Guyana which afforded 6α-senecioyloxychaparrin (**5**), **3**, a new quassinoid karinolide and 9-methoxycanthin-6-one (6). Direct comparison of the two samples of **5** established their identity.

TABLE 2. Anticancer activity of the quassinoids of *Simaba multiflora*[a]

Compound	P-388		KB
	Dose mg/kg	T/C[b]	ED_{50} μg/ml
6α-Senecioyloxychaparrinone (**1**) NSC-290494	2.5	136	0.003
	1.25	133	
	0.63	176	
	0.31	145	
6α-Senecioyloxychaparrin (**5**) NSC-341651	3.0	133	0.03
	1.5	133	
	0.75	160	
	0.38	142	
Chaparrinone (**2**) NSC-288754	not	tested	0.025

[a]Assayed under the auspices of the National Cancer Institute according to established protocols (4).
[b]T/C=percentage survival of test vs. control groups in tumor-treated animals.

(7,8) and there has been some discussion of the structural requirements for activity in the P-388 lymphocytic leukemia system (9). Thus it is well established that A-ring oxygenation and unsaturation, an oxygen linkage from C-30 to C-11 or C-13, a free hydroxyl group in ring A and at C-12, and an ester at C-6 or C-15 are all required for *in vivo* activity. The demonstration that **1** and **5** display *in vivo* activity substantiates and adds to the prior data in this field and stands in marked contrast to the interesting observation that 5α-hydroxychaparrinone displays no anticancer activity at the doses tested (10).

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