SOULARUBINONE, A NEW ANTILEUKEMIC QUASSINOID FROM *SOULAMEA TOMENTOSA*[1]

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ABSTRACT.—The structure of soularubinone 3, a new antileukemic quassinoid isolated from the leaves of *Soulamea tomentosa* (Brongn. and Gris), has been established by spectral and chemical methods. It has been shown to be the C-15 β-hydroxyisovaleric ester of glaucarubolone. Soularubinone shows significant antineoplastic activity against mouse leukemia P-388 and inhibits cell transformation induced by Rous sarcoma virus. The known quassinoid chaparrinone 1 has also been isolated.

In the framework of our continuing search for antineoplastic quassinoids (1), we examined those of *Soulamea tomentosa* (Brongn. and Gris), indigenous to New Caledonia. Previously, picrasin B (2, 3), 6-hydroxypicrasin B (3, 4, 5), iso-brucein A (5), and soulameolide (6) were isolated from this species. We now report the structural elucidation of a novel antileukemic quassinoid, soularubinone 3, isolated from the *S. tomentosa* leaf extract; the known quassinoid, chaparrinone 1, was also obtained.
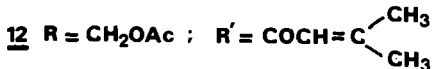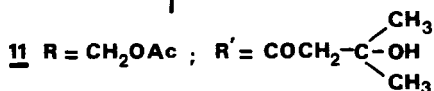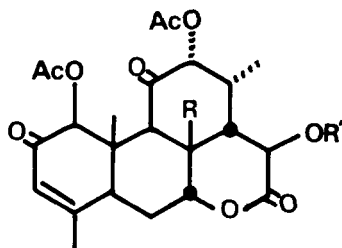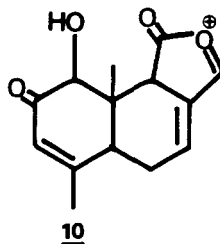
The dried ground leaves of *S. tomentosa*, collected in New Caledonia, were extracted with hexane, followed by hot water. The concentrated aqueous extract was continuously extracted with chloroform. Careful column chromatography of the chloroform residue on silica gel and elution with methylene chloride containing increasing amounts of methanol led to the isolation of the new quassinoid, soularubinone 3.

The molecular formula for 3 was found by high resolution mass spectrometry to be $C_{25}H_{34}O_{10}$. The ir spectrum showed the presence of carbonyl absorptions at 1755 (ester), 1720 (δ-lactone) and 1680 cm^{-1} (αβ-unsaturated ketone). In agreement with the formulation of ring A as in 3, the uv spectrum showed a maximum at 241 nm (ε 12300), and the 250 MHz pmr spectrum (table 1 and figure 1) displayed the characteristic signals due to the vinyl methyl and to the protons 1-H and 3-H. The mass spectrum of soularubinone 3 di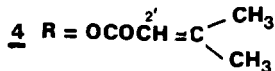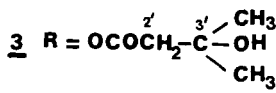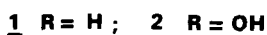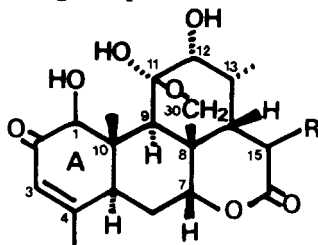splayed peaks at m/e 151 and 247 assignable to ions 9 and 10, respectively, which are common fragmentation ions in quassinoids with a similar A ring and an 11,30-hemiketal in the C ring (7). The presence in the pmr spectrum of 3 (table 1 and figure 1) of an AB quartet corresponding in chemical shift to a $-CH_2-O-$ grouping supports the 11,30-hemiketal which is found in many quassinoids, such as chaparrinone 1, glaucarubolone 2, and glaucarubinone 5 (1). The latter quassinoid is isomeric with soularubinone, they both have similar R_f in different solvent systems. That

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3 is a hydroxy-pentanoate ester was indicated by the analysis of its mass spectrum, which showed abundant fragment ions in the high mass region at m/e 394 and 376 corresponding to $[M-(OC=C_4H_8O)]^{+\cdot}$ and $[M-C_5H_{10}O_3]^{+\cdot}$, respectively. The location of the ester function at C-15β on the C_{20} quassinoid skeleton was assigned by double resonance studies. The 250 MHz pmr spectrum of **3** showed the one proton downfield doublet at $\delta5.88$ ($J=9Hz$) characteristic of the 15α proton which collapses to a singlet upon irradiation at 2.4 ppm (H–14). The

1 R = H ;　**2** R = OH

3 R = OCOCH₂–C(CH₃)(CH₃)–OH

4 R = OCOCH = C(CH₃)(CH₃)

5 R = OCOC(OH)(CH₃)–CH₂CH₃

6 R = OCOCH₃

7 R = OCOCH(CH₃)–CH₂CH₃

8 R = OCOCH₂–CH(CH₃)(CH₃)

9

10

11 R = CH₂OAc ;　R′ = COCH₂–C(CH₃)(CH₃)–OH

12 R = CH₂OAc ;　R′ = COCH = C(CH₃)(CH₃)
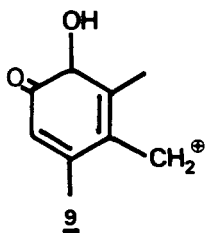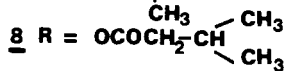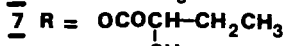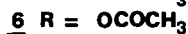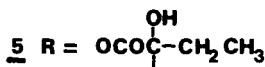
remaining signals due to protons on oxygen-bearing carbons (H-12 and H-7) as well as the tertiary C-10 and secondary C-13 methyl groupings correspond in chemical shift to those assigned to glaucarubinone (1).

TABLE 1.   250 pmr spectra[a] of Quassinoids 3, 4, 11 and 12.

	3[b]	4[c]	11[c]	12[c]
H-1..........	4.08 s	4.07 s	5.13 s	5.16 s
H-3..........	6.05 br s	6.16 br s	6.05 br s	6.03 br s
H-7..........	4.64 br s	4.67 br	4.75 br s	4.86 br s
H-9..........	2.94 s	2.77 s		
H-12.........	3.65 d (6.8)	3.58 d (6.8)	5.00 d (6.8)	4.88 d (7.5)
-CH$_2$O-.......	3.68 d (8.7)	3.69 d (8.8)	3.85 d (11.8)	3.87 d (12.9)
	3.98 d	3.96 d	4.64 d	4.65 d
H-14.........	2.37 d (11.1)			
H-15.........	5.88 d (11.3)	5.54 d (11.3)	6.15 d (10)	6.03 d (11)
CH$_3$-13......	1.15 d (6.8)	1.12 d (6.9)	1.11 d (7.0)	1.09 d (7.8)
CH$_3$-10.......	1.31 s	1.21 s	1.42 s	1.42 s
CH$_3$-4........	1.92 s	2.02 s	1.96 s	1.97 s
H-2'..........	2.62 br s	5.75 br s		5.76 br s
CH$_3$-3'.......	1.39 s	1.94 s	1.36 s	1.96 s
	1.39 s	2.22 s	1.37 s	2.0
OAc..........			2.02	2.02
			2.12	2.10
			2.20	2.20

[a]Shifts in ppm and coupling constants as (Hz).
[b]Solution in deuterochloroform—20% pyridine-d$_5$.
[c]Deuterochloroform solution.

The above results clearly show that soularubinone 3 differs from glaucarubinone 5 by the structure of the ester chain.   Comparison of the pmr spectrum of 3, especially of the methyl region, with that of 5 allows soularubinone to be formulated as a C-15 β-hydroxyisovaleric ester of glaucarubolone 2.   Instead of the three proton triplet (CH_3CH_2-) and the three proton singlet (3'-CH$_3$) found in the spectrum of glaucarubinone (5), 3 displayed a six proton singlet at δ1.39 ppm assignable to the $-\underset{\diagdown CH_3}{\overset{\diagup CH_3}{C}}-OH$ grouping and a two proton singlet at δ2.62 ppm due to 2'-CH$_2$.   Further evidence for the ester chain in 3 is provided by the presence of the mass spectral fragmentation ions at m/e 83 [COCH=C(CH$_3$)$_2$]$^+$, m/e 59 [C(OH)-(CH$_3$)$_2$]$^+$, and m/e 55 [HC=C(CH$_3$)$_2$]$^+$, while the mass spectrum of glaucarubinone 5 displays a peak at m/e 73 [C(OH) (CH$_3$)-CH$_2$CH$_3$]$^+$ and not at m/e 59.

Finally, the assignment of structure 3 was confirmed by the conversion of soularubinone into 15-senecioyloxy-glaucarubolone 4.   Acetylation (acetic anhydride/pyridine) afforded a triacetate derivative 11 which still retained a hydroxyl-signal in its ir spectrum.   Its mass spectrum showed a molecular ion peak at m/e 620, and its pmr spectrum (table 1) was in accord with structure 11.   In contrast to previous findings (8), the ester chain of 11 was readily dehydrated by treatment with thionyl chloride in pyridine[3], to give 12.   The structure of 12 was deduced from its mass and pmr spectral analysis.   The pmr spectrum (table 1) was in good agreement with the proposed structure and showed, in

[3]It has been claimed (8) that glaucarubinone triacetate gave a mixture of products on similar treatment, whereas only glaucarubinone 1,12-diacetate gave the desired product.
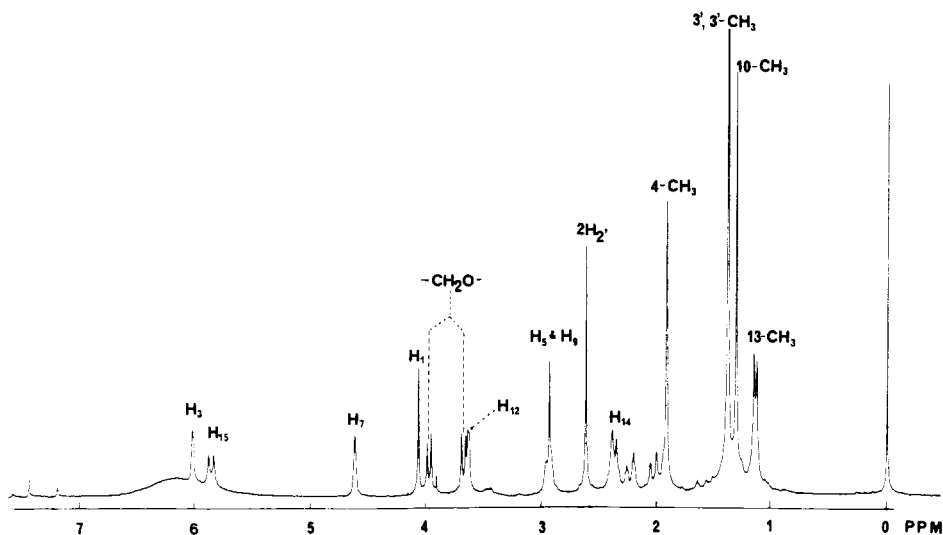
FIG. 1.   250 MHz pmr spectrum of soularubinone **3** in deuteriochloroform containing 20% pyridine-d$_5$.

particular, the 2'–H proton of the senecioyl ester as a broad singlet at 5.76 (9). Deacetylation of **12** with methanolic ammonia led to glaucarubolone 15-senecioate **4**. The uv spectrum showed a maximum at 228 nm (ϵ26,360), which confirmed the presence of the α,β-unsaturated ester and ketone moieties in **4**. High resolution mass spectrometry confirmed the formula $C_{25}H_{32}O_9$ for **4**.

The presence of mass fragment ions at m/e 247 ($C_{14}H_{15}O_4$) and m/e 151 ($C_9H_{11}O_2$) (7) showed that the glaucarubolone skeleton remained intact. The presence of a senecioate ester was indicated by the loss in the mass spectrum of 100 amu [HOOCCH=C(CH$_3$)$_2$] from the molecular ion and by the appearance of peaks at m/e 83 [COCH=C(CH$_3$)$_2$]$^+$ and 55 [HC=C(CH$_3$)$_2$]$^+$. Furthermore,
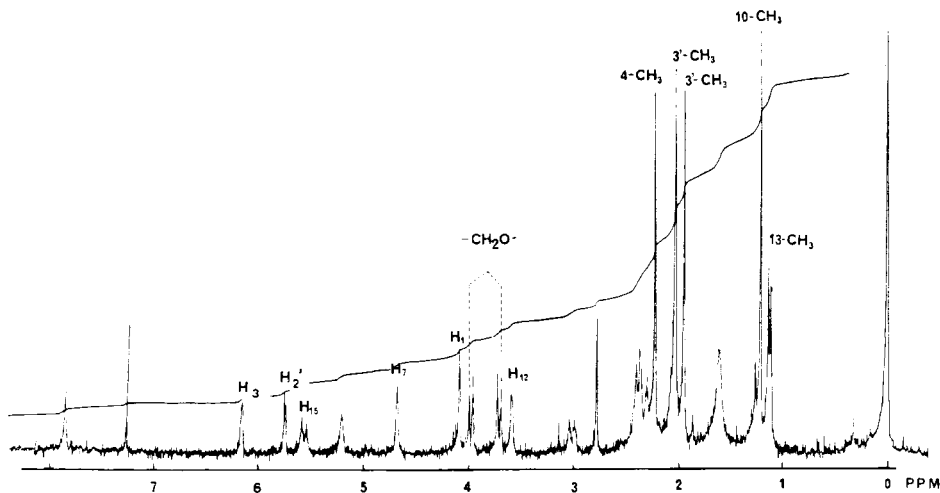

FIG. 2.   250 MHz pmr spectrum of 15β-senecioyloxyglaucarubolone **4** in deuterochloroform.

the presence in the pmr spectrum (table 1, figure 2) of resonances for two additional vinyl methyls and for an additional vinyl proton ($\delta 5.75$) supported the characterization of the ester as a senecioate.

All the above results prove that soularubinone **3** is the C–15 β-hydroxyisovaleric ester of glaucaurbolone **2**. It is, thus, the fifth member of the C–15 ester series of glaucarubolone **2**; the other natural quassinoids of this series are glaucarubinone **5** (1), holocanthone **6** (1), ailanthinone **7** (1), and castelanone **8** (10).

The structural requirements for antineoplastic activity of quassinoids against the P–388 lymphocytic leukemia (8, 11), namely, the A-ring oxygenated functionality, the epoxymethano bridge between C–8 and C–11, the presence of a free hydroxyl group in ring A and at C–12, and an ester group at C–15, are fulfilled by soularubinone. Accordingly, it displays significant antineoplastic activity in the P–388 leukemia (table 2).

TABLE 2.   Antitumor activity of C–15 esters of glaucarubolone.

	Tumor system:	P–388	KB
	Dose mg/kg	T/C[a]	ED$_{50}$
Soularubinone **3**[b]................	4	137	
Glaucarubinone **5** (13)............	0.5	231	2.4×10^{-2}
Holocanthone **6** (13).............	9	159	2.9×10^{-2}
Ailanthinone **7** (13)..............	2	148	1×10^{-2}
Castelanone **8**[b].................			1.9×10^{-2}
Glaucarubolone-15 Tiglate (8).....			2.6×10^{-2}
Glaucarubolone-15 Senecioate **4**[b]...			4.2×10^{-2}

[a]T/C = ratio of test group survival to control group survival in tumored animals, expressed as a percent.
[b]Tumor-inhibitory activity and cytotoxicity were assayed under the auspices of the National Cancer Institute by the procedures described by Geran *et al.* (12).

Presently, it is still hazardous to designate the most active C–15 ester of glaucarubolone, since the values given in table 2 have not been obtained under the same conditions. However, glaucarubinone **5**, a quassinoid of common occurrence first discovered in 1965, displays a strong activity which is even higher than that of bruceantin (13) (the latter currently is undergoing clinical trial at the U.S. National Cancer Institute).

Soularubinone **3**, like several other quassinoids, inhibits cell transformation induced by Rous sarcoma virus (14).

EXPERIMENTAL[5]

EXTRACTION AND ISOLATION OF ISOBRUCEINE A, PICRASIN B, 6-HYDROXYPIERASIN B, SOULA-

MEOLIDE AND SOULARUBINONE **3**.—The plant material, collected in 1974 on the Col de Mouirange in New Caledonia, consisted of the leaves of *Soulamea tomentosa* Brongn. and Gris, which were dried in a ventilated oven at 60°. The ground leaves (700 g) were defatted by percolation with hexane at room temperature. The mass was then stirred for several hours with hot water (70–75°), separated by filtration *in vacuo* and resuspended in fresh hot water. 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 chloroform. Evaporation of the solvent yielded a brown foam (9.6 g) which was chromatographed on silica gel (400 g); dichloromethane containing increasing amounts (1 to 8%) of methanol was the eluant. When fractions of 250 ml each were collected and combined on the basis of tlc similarity six combinations [(i)–(vi)] were obtained. Fraction (i) (40 mg) gave isobrucein A; fraction (ii) (1.63 g) gave picrasin B; fraction (iii) (0.76 g) gave 6-hydroxypicrasin B; fraction (IV) (0.6 g) gave chaparrinone; and fraction (VI) (2.1 g) gave soulameolide. The identification of these quassinoids was established by mp, tlc, pmr and ms in comparison with authentic samples. Fraction (v) (1.5 g) yielded crude soularubinone, which, when purified by preparative tlc (9:1, methylene chloride-methanol), yielded 210 mg of pure *Soularubinone* **3**.

Recrystallization of **3** from methanol afforded colorless prisms, mp 236–238°; $[\alpha]$ D$+47.6°$ (c, 0.7 in methanol). High resolution mass spectrometry: M^+ at m/e 494.2149 ($C_{25}H_{34}O_{10}$), peaks at m/e 394.1638 ($C_{20}H_{26}O_8$), m/e 247.0963 ($C_{14}H_{15}O_4$), m/e 151.0749 ($C_8H_{11}O_2$) and m/e 135 ($C_9H_{11}O$); the 250 MHz pmr data have been entered in table 1 and figure 1.

SOULARUBINONE TRIACETATE (**11**).—Acetylation of soularubinone **3** (117 mg) with acetic anhydride-pyridine (24 hr, room temperature) yielded a non-crystalline triacetate **11** (140 mg). It corresponded to empirical formula $C_{31}H_{40}O_{13}$ with significant mass spectral ion peaks at m/e 620 ($M^+\cdot$), 578, 518. The 250 MHz pmr data have been recorded in table 1.

TRIACETYL-GLAUCARUBOLONE 15-SENECIOATE (**12**).—To **11** (130 mg) in pyridine (5 ml), which had been cooled in an ice bath, thionyl chloride (0.5 ml) was added dropwise. The mixture was kept at 0° for 1 hr. Usual work-up gave a product which, when purified by column chromatography on silica gel (19 g), yielded **12** (94 mg), non-crystalline but chromatographically homogeneous. The triacetate **12** corresponded to empirical formula $C_{31}H_{38}O_{12}$ with mass spectral ions peaks at m/e 602 ($M^+\cdot$), 560 (base peak, M^+–42), 518. The 250 MHz pmr data have been presented in table 1.

GLAUCARUBOLONE 15-SENECIOATE (**4**).—Sixty mg of **12** was treated with methanolic ammonia for 36 hrs at room temperature. Evaporation of the solvent afforded **4**, which was recrystallized from ethylacetate to give colorless prisms mp 225–228. Its 250 MHz pmr data have been entered in table 1 and fig. 2. High resolution mass spectrometry: $M^+\cdot$ at m/e 476.2021 ($C_{25}H_{32}O_9$), peaks at 377.1585 ($C_{20}H_{25}O_7$) and m/e 247.0966 ($C_{14}H_{15}O_4$).

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