PLANT ANTICANCER AGENTS XXV. CONSTITUENTS OF *SOULAMEA SOULAMEOIDES*[1]

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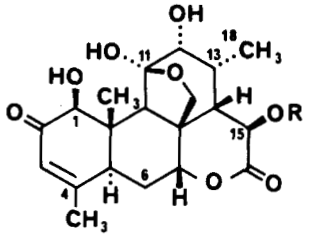
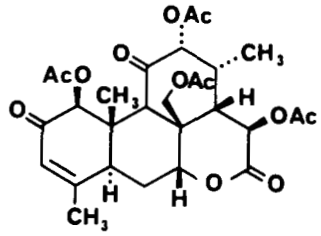
ABSTRACT.—Three simaroubolides, glaucarubolone (**1**), holacanthone (**2**), and isobrucein A (**3**) were found to be responsible for the cytotoxic and antileukemic activities observed for extracts of the wood stem, stem bark, and twigs of *Soulamea soulameoides*. Other cytotoxic constituents isolated include a coumarinolignan cleomiscosin A (**4**) and the hydroxy canthin-6-one derivative **5**. Picrasin B (**6**) was also obtained, but was not active.

In a continuing search for tumor inhibitors from higher plants, *Soulamea soulameoides* (Gray) Nooteboom (syn. *Amaroria soulameoides* Gray [Simaroubaceae]), native to Fiji, was found to display reproducible activity in the Eagles' carcinoma of the nasopharynx (KB) test system (2).

A chloroform extract of the leaves, twigs, and fruits, displayed *in vivo* (P-388 lymphocytic leukemia system) and *in vitro* (KB test system) activity, and bioactivity-di-



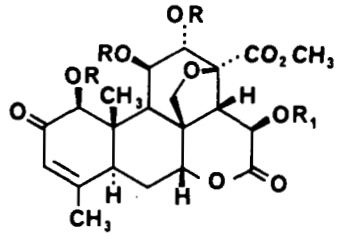


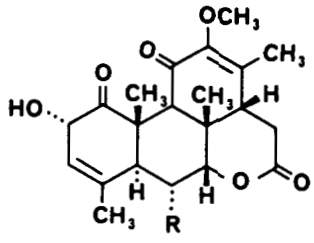
1 R = H

2 R = Ac

10 R = $COCH_2C(OH)(CH_3)_2$

7





	R	R_1
3	H	$COCH_2CH(CH_3)_2$
8	Ac	$COCH_2CH(CH_3)_2$

6 R = H

9 R = OH

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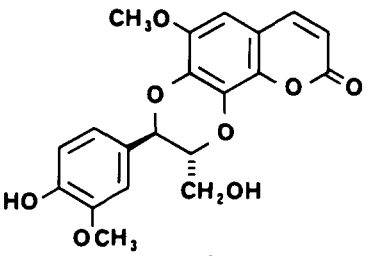

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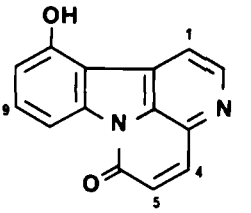

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rected fractionation[3] with the Eagles' carcinoma of the nasopharynx (KB) test system resulted in the isolation of glaucarubolone (**1**) and holacanthone (**2**). Similar fractionation of the chloroform-soluble portion of the methanolic extract of the stem bark yielded isobrucein A (**3**), cleomiscosin A (**4**), and 11-hydroxycanthin-6-one (**5**). An inactive quassinoid, picrasin B (**6**) was also isolated from this extract.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were determined using a Kofler hot-stage instrument and are uncorrected; uv spectra were measured on a Beckman 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 at 60 MHz on a Varian model T-60A instrument, equipped with a Nicolet model TT-7 Fourier Transform attachment or at 360 MHz using a Brüker WM 360 (Eli Lilly) or Nicolet NT-360 (University of Illinois) instruments. 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.

PLANT MATERIAL.—The leaves, twigs, and fruits and the wood stem and stem bark plant parts of *S. soulameoides* (Gray) Nooteboom (Simaroubaceae) were collected in Fiji in 1979. A herbarium specimen documenting the collection is deposited in the Herbarium of the National Arboretum, Washington, DC.

EXTRACTIONS AND PRELIMINARY FRACTIONATION.—Of the leaves, twigs, and fruits and the wood stem and stem bark plant parts 35 kg each were successively extracted with petroleum ether, chloroform, and methanol. Concentration of the extracts was carried out *in vacuo* below 50°. In the case of the leaves, twigs, and fruits, the chloroform extract exhibited higher cytotoxicity (KB, ED_{50} 0.16 μg/ml) than the petroleum ether extract (KB, ED_{50} 2.7 μg/ml) or the methanol extract (KB, ED_{50} 5.9 μg/ml). Thus, the latter two extracts were not studied, and only the chloroform extract was subjected to further fractionation.

In the case of the wood stem and stem bark plant parts the methanol extract showed higher cytotoxic activity (KB, ED_{50} 0.36 μg/ml) than the petroleum ether (KB, ED_{50} 2.7 μg/ml) or chloroform (KB, ED_{50} 2.0 μg/ml) extracts. Therefore, only the methanol extract was processed further.

INITIAL SEPARATION OF THE CHLOROFORM EXTRACT.—A sample (150 g) of the chloroform extract from the leaves, twigs, and fruits was chromatographed on silica gel[4] (2.5 kg) packed in chloroform. Sixty-four fractions were collected through elution with chloroform and chloroform-containing increasing proportions of methanol. Monitoring with the KB test system, fractionation yielded two highly active fractions: 49 (KB, ED_{50} 0.0025 μg/ml) and 54 (KB, ED_{50} 0.01 μg/ml), each eluted from the column with chloroform containing 2% methanol.

ISOLATION AND IDENTIFICATION OF HOLACANTHONE (**2**).—Fraction 49 (5 g) was rechromatographed on silica gel (150 g). Elution with chloroform containing 1% methanol, followed by crystallization of the residue (500 mg) from ethyl acetate and chloroform, afforded colorless needles of holacanthone (**2**, 220 mg, 0.0019%) exhibiting the following physical and spectral properties: mp 272-274°; ir, ν max

[3]The extracts, fractions and isolated compounds were tested, according to established protocols (2), under the auspices of the Developmental Therapeutics Program of the National Cancer Insitute. An isolate is considered active if it shows an $ED_{50} \leq 4$ μg/ml in the KB or P-388 cell culture assays or $T/C \geq 125\%$ in the P-388 lymphocytic leukemia assay *in vivo*.

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

(KBr) 3480, 1740, 1680, 1630, and 1230 cm^{-1}; uv, λ max (MeOH) 240 nm (log ε 4.01); pmr, (360 MHz, $CDCl_3$) δ 1.12 (3H, d, J=8 Hz, 13-CH_3), 1.19 (3H, d, J=11 Hz, 10-CH_3), 2.01 (3H, s, 4-CH_3), 2.14 (3H, s, -OAc), 2.32 (2H, m, 6-CH_2), 2.74 (1H, s, 9-H), 2.96 (1H, d, J=12 Hz, 5α-H), 3.56 (1H, d, J=3.3 Hz, 12-H), 3.80 (2H, dd, J=9 Hz, -OCH_2-), 4.09 (1H, s, 1-H), 4.62 (1H, br s, 7-H), 5.64 (1H, d, J=11.4 Hz, 15-H), and 6.15 (1H, s, 3-H); ms, *m/z* 436 (M^+, 25%), 394 (3), 377 (6), 319 (6), 249 (7), 248 (4), 247 (18), 229 (8), 201 (7), 187 (7), 164 (10), 151 (26), 138 (9), 127 (12), 91 (13), 77 (10), 69 (15), 55 (13), 43 (100), and 41 (26); tlc, Rf 0.33 ($CHCl_3$-MeOH, 97:3). The isolate was identical to an authentic sample of holacanthone (**2**).[5]

ACETYLATION OF HOLACANTHONE (**2**).—Holacanthone (**2**, 50 mg) was treated with acetic anhydride-pyridine (1:10, 2 ml) at room temperature overnight. Work-up in the usual way and crystallization from an ether-methanol mixture afforded white crystals of holacanthone triacetate (**7**, 40 mg) having the following spectral data: uv, λ max (MeOH) 240 nm (log ε 4.05); pmr, (60 MHz, $CDCl_3$) δ 1.10 (3H, d, J=6.8 Hz, 13-CH_3), 1.46 (3H, s, 10-CH_3), 1.96 (3H, s, 4-CH_3), 2.01, 2.10, 2.17, and 2.18 (3H each, s, 4 x -OAc), 3.46 (1H, s, 9-H), 3.86 and 4.60 (1H each, d, J=13.1 Hz, -OCH_2-), 4.76 (1H, m, 7-H), 4.95 (1H, d, J=1.7 Hz, 12-H), 5.18 (1H, s, 1-H), 6.05 (1H, m, 15-H), and 6.15 (1H, m, 3-H), ms, *m/z* 562 (M^+, 0.6%), 520 (3.2), 502 (0.7), 478 (0.5), 461 (0.4), 460 (1), 442 (1), 432 (0.5), 400 (1), 382 (1), 322 (1), 310 (1), 309 (2.5), 283 (1), 281 (2), 255 (1), 253 (1), 237 (1), 228 (1), 215 (2), 199 (1), 187 (1), 185 (1), 175 (1), 159 (2), 157 (1), 151 (2), 140 (2), 125 (1), 109 (2), 95 (14), 92 (3), 67 (5), and 43 (100).

HYDROLYSIS OF HOLACANTHONE (**2**).—A solution of holacanthone (**2**, 5 mg) in 0.5 M methanolic KOH (1 ml) was allowed to stand overnight at room temperature. Acidification with dilute hydrochloric acid to pH 5-6 followed by extraction with chloroform afforded a hydrolysis product. Comparison of this product with glaucarubolone (**1**) by chromatography on several systems established its identity.

ISOLATION AND IDENTIFICATION OF GLAUCARUBOLONE (**1**).—Fraction 54 (4 g) was repeatedly triturated with diethyl ether and the insoluble residue crystallized twice from chloroform-methanol to afford glaucarubolone (**1**) as white crystals (1 g, 0.003%) exhibiting the following physical and chemical properties: mp 250-254°; $[\alpha]^{26}D$ −41.5° (c 0.45, pyridine); ir, ν max (KBr) 3550-2900, 1735, 1685, and 1630 cm^{-1}; uv, λ max (MeOH) 240 nm (log ε 4.02); ms, *m/z* 394 (M^+, 100%), 376 (7), 247 (37), and 151 (58).

ACETYLATION OF GLAUCARUBOLONE (**1**).—Glaucarubolone (**1**, 50 mg) was treated with acetic anhydride-pyridine (1:10, 2 ml) at room temperature overnight. Work-up in the usual way and crystallization from chloroform-methanol yielded glaucarubolone tetra-acetate (**7**, 45 mg), mp 236°. The spectral properties (uv, ir, pmr, and ms) were identical to those of **7** prepared from **2** by acetylation (see above) and were consistent with the literature values for glaucarubolone (**1**) and glaucarubolone tetra-acetate (**7**) (3).

FRACTIONATION AND CHROMATOGRAPHIC SEPARATION OF THE METHANOL EXTRACT.—After drying and evaporating *in vacuo*, the methanolic extract (210 g) of the wood stem and stem bark of *S. soulameoides* was partitioned between chloroform and water to afford a chloroform-soluble fraction (120 g). A portion of this fraction (70 g) (KB, ED_{50} 0.043 μg/ml) was chromatographed on silica gel (2 kg) and eluted with chloroform and mixtures of chloroform and methanol of increasing polarity. The first fraction eluted with chloroform-1% methanol yielded picrasin B (**6**, 1.8 g, 0.0054%). Subsequent elution with the solvent system yielded a cytotoxic fraction (1.5 g, KB, ED_{50} 0.01 μg/ml), which was further chromatographed. Elution with chloroform-2% methanol afforded another active fraction (2 g, KB, ED_{50} 0.51 μg/ml), which was also further chromatographed.

IDENTIFICATION OF PICRASIN B (**6**).—White crystals, mp 255°; ir, ν max (KBr) 3490, 1735, 1720, 1680, 1640, and 1225 cm^{-1}; uv, λ max (MeOH) 254 nm (log ε 4.05); pmr, (60 MHz, $CDCl_3$) δ 0.93 (3H, d, J=5.9 Hz, 4-CH_3), 1.21 (3H, s, 8-CH_3), 1.46 (3H, s, 10-CH_3), 1.92 (3H, s, 13-CH_3), 3.26 (1H, s, 9-H), 3.62 (3H, s, 12-OCH_3), 4.30 (1H, m, 7-H), and 4.86 (1H, m, 2-H); ms, *m/z* 376 (M^+, 100%), 334 (13), 304 (10), 303 (19), 289 (15), 277 (14), 273 (10), 271 (7), 261 (11), 257 (18), 187 (10), 185 (15), 179 (30), 173 (13), 152 (27), 151 (10), 145 (12), 137 (16), 133 (16), 127 (12), 119 (20), 115 (12), 107 (21), and 91 (47). These data are consistent with those published previously for picrasin B (4,5).

ISOLATION OF ISOBRUCEIN A (3) AND CLEOMISCOSIN A (**4**).—The active fraction eluted with chloroform-1% methanol (1.5 g, KB, ED_{50} 0.01 μg/ml) was chromatographed on silica gel (40 g) by eluting it with benzene and benzene-chloroform mixtures. An active fraction (KB, ED_{50} 0.01 μg/ml), eluted with benzene-chloroform (1:1) on repeated trituration with diethyl ether and crystallization of the residue from chloroform, afforded isobrucein A (**3**, 320 mg, 0.0019%). Further elution of the column with chloroform yielded the coumarinolignan cleomiscosin A (**4**, 13 mg, 0.000065%) crystallizing from chloroform-methanol, mp 250-255°.

[5]Kindly supplied by Dr. Matthew Suffness, Natural Products Branch, DTP, National Cancer Institute.

IDENTIFICATION OF ISOBRUCEIN A (**3**).—Colorless crystals, mp 195°; ir, ν max (KBr) 3450, 2950, 1740, 1670, and 1620 cm^{-1}; uv, λ max (MeOH) 240 (log ε 4.02); pmr, (60 MHz, $CHCl_3$) δ 0.98 (6H, d, J=6.1 Hz, $-CH(CH_3)_2$), 1.18 (3H, s, 10-CH_3), 1.95 (3H, s, 4-CH_3), 2.16 (2H, m, esr $-CH_2$-), 3.36 (1H, br s, OH, exchanged with D_2O), 3.74 and 4.65 (1H, each, d, J=9.5 Hz, $-OCH_2$-), 3.80 (3H, s, $-CO_2CH_3$), 4.20 (1H, m, 12-H), 4.28 (1H, m, 11-H), 4.73 (1H, s, 1-H), 4.85 (1H, m, 7-H), 6.11 (1H, m, 3-H), and 6.29 (1H, d, J=13.2 Hz, 15-H); ms, *m/z* 522 (M^+, 0.6%), 504 (6), 472 (1), 463 (1), 438 (4), 420 (6), 402 (3), 388 (3), 374 (6), 345 (5), 315 (6), 314 (7), 269 (4), 245 (5), 187 (50), 159 (5), 151 (7), 135 (12), 109 (7), 95 (17), 85 (64), 69 (17) and 57 (100); tlc, Rf 0.55 ($CHCl_3$-MeOH, 49:1).

ACETYLATION OF ISOBRUCEIN A (**3**).—Isobrucein A (**3**, 25 mg) was treated with acetic anhydride-pyridine (1:10, 2 ml) at room temperature overnight. Work-up in the usual way and crystallization from chloroform-hexane afforded a diacetate derivative **8** (22 mg) exhibiting the following physical and spectral properties: pmr, (60 MHz, $CDCl_3$) δ 0.98 (6H, d, J=6.2 Hz, $-CH(CH_3)_2$), 1.38 (3H, s, 10-CH_3), 1.93 (3H, s, 4-CH_3), 2.04 (3H, s, -OAc), 2.21 (3H, s, -OAc), 3.30 and 4.15 (1H each, d, J=6.5 Hz, $-OCH_2$), 3.77 (3H, s, $-CO_2CH_3$), 4.77 (1H, m, 11-H), 5.25 (1H, m, 7-H), 5.38 (1H, s, 1-H), 5.85 (1H, d, J=13.2 Hz, 15-H), and 6.01 (1H, m, 3-H); ms, *m/z* 606 (M^+, not observed), 546 ($M^+ - 60$, 1.5%), 504 (17), 463 (1), 420 (9), 402 (4), 345 (5), 314 (6), 297 (6), 283 (6), 185 (5), 151 (4), 135 (15), 95 (16), 85 (58), 69 (16), and 57 (100). These spectral data are consistent with the published values for isobrucein A (**3**) (6). Establishment of the identity was conducted through direct comparison with an authentic sample.[6]

IDENTIFICATION OF CLEOMISCOSIN A (**4**).—The spectral properties and structural determination of cleomiscosin A (**4**) will be described subsequently (7). Identity was established by direct comparison with an authentic sample.[7]

IDENTIFICATION OF 11-HYDROXY-CANTHIN-6-ONE (**5**).—The fraction eluted with chloroform-2% methanol (2 g, KB, ED_{50} 0.51 μg/ml) was chromatographed on silica gel (60 g). Fractions eluted with chloroform-2% methanol afforded a yellowish residue which, on crystallization from methanol, afforded 400 mg (0.002%) of orange-yellow crystals of **5**, exhibiting the following physical and chemical properties, mp 270° (dec.); ir, ν max (KBr) 3500-3200, 2940, 1680, 1645, 1470, 1440, 1355, 1300, 1280, 1220, 1120, 1060, 980, and 950 cm^{-1}; uv, λ max (MeOH) 326 (log ε 4.52) and 383 nm (4.61), λ max (MeOH + NaOH) 352 and 445 nm; pmr, (360 MHz, DMSO-d_6) δ 6.98 (1H, d, J=9.8 Hz, 5-H), 7.01 (1H, d, J=8.1 Hz, 10-H), 7.57 (1H, t, J=8.1 Hz, 9-H), 7.95 (1H, d, J=8.0 Hz, 8-H), 8.10 (1H, d, J=5.0 Hz, 1-H), 8.13 (1H, d, J=9.8 Hz, 4-H) and 8.79 (1H, d, J=5.0 Hz, H-2); ms, *m/z* 236 (M^+, 100%), 219 (9), 210 (15), 209 (12), 208 (77), 182 (8), 181 (12), 180 (49), 179 (30), 178 (5), 167 (13), 162 (14), 154 (13), 153 (16), 152 (16), 151 (11), 150 (11), 137 (48), 127 (14), 126 (12), 125 (10), 124 (34), 119 (11), 118 (15), 102 (10), 91 (18), 90 (32), 89 (11), 79 (19), 77 (33), 76 (29), 75 (25), 74 (15), 65 (13), and 62 (40). These data are in agreement with those published for **5** (8).

BIOLOGICAL ACTIVITY OF THE ISOLATES.—The biological activities of the isolates of *S. soulameoides* are shown in table 1.

DISCUSSION

Previous studies of the genus *Soulamea* have afforded picrasin B (**6**) and its 6-hydroxy derivative **9** from *S. pancheri* (5), **9** (5,6,9), isobrucein A (**3**) (6), soulameolide (10) and soularubinone (**10**) (11) from *S. tomentosa*, soulameanone, 1,12-di-*O*-acetyl soulameanone and Δ^2-picrasin B from *S. muelleri* (12), and 11-hydroxy-canthin-6-one (amarolide) (**5**) from *S. soulameoides* (8).

Our isolation of the quassinoids glaucarubolone (**1**), holacanthone (**2**), isobrucein A (**3**), and picrasin B (**6**) from the leaves and twigs of *S. soulameoides*, therefore, substantiates the close chemotaxonomic relationship of the genus *Soulamea* with other genera in the Simaroubaceae. Glaucarubolone (**1**) has previously been isolated from *Hannoa klaineana* Pierre et Engler (3), *Simarouba glauca* DC (13), and *Castela nicholsoni* Hook. (14), and picrasin B (nigakilactone I, simalikalactone B) (**6**) from *Picrasma quassioides* Bennett (4,15,16), and *Quassia africana* Baill. (17). There have been only single previous reports of the isolation of holacanthone (**2**) (18) and isobrucein A (**3**) (6). All of these quassinoids, with the exception of **6**, displayed cytotoxic activity, and previous data

[6]Kindly supplied by Mme. J. Polonsky, Institut de Chemie des Substances Naturelles, CNRS, Gif-sur-Yvette, France.

[7]Kindly supplied by Professor H. Hikino, Pharmaceutical Institute, Tohoku University, Sendai, Japan.

TABLE 1. Antileukemic and cytotoxic activity of *S. soulameoides* isolates.[a]

Compound	NSC-	KB (ED_{50}, μg/ml)	P-388 (ED_{50}, μg/ml)	P-388	
				Dose (mg/kg)	% T/C
Glaucarubolone (**1**)	126764	0.11	0.01	5	132
Holacanthone (**2**)	126765	NT	NT	7.5	136
				3.8	154
				1.9	134
				0.9	127
Isobrucein A (**3**)	279503	0.003	0.001	2.0	toxic
				1.0	155
				0.5	145
				0.25	138
Cleomiscosin A (**4**)	346197	4.9	2.8	NT	NT
11-Hydroxy-canthin-6-one (**5**)	—	2.1	2.3	5.0	102
				2.5	107
				1.25	108
Picrasin B (**6**)	—	>100	>100	19.6	107
				9.83	107
				4.92	102
				2.46	107

[a]Evaluated according to the protocols described in reference (2).
NT=not tested.

(18) have established holacanthone (**2**) to have good *in vivo* activity in the P-388 lymphocytic leukemia test system.

Quassinoids are of interest not only as potential antineoplastic agents (19), but also because recent data suggests that these compounds have potent *in vivo* amoebicidal activity (20) and good *in vitro* antimalarial activity (21).

Of the remaining two compounds, cleomiscosin A (**4**) is a member of the new series of coumarinolignans, and its isolations will be summarized elsewhere (7). The canthin-6-one derivative **5**, which, like **4**, also shows weak cytotoxic activity, was isolated previously (8) from this plant.

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