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The isolation and structure of 13,18-dehydroglaucarubinone, a new antineoplastic quassinoid from *Simarouba amara*

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Summary. An investigation of the Guyana plant *Simarouba amara* Aubl. (Simaroubaceae) for antineoplastic quassinoids led to isolation and structural determination of the new quassinoids 2′-acetylglaucarubine (**1a**) and 13,18-dehydroglaucarubinone (**2**). The previously known 2′-acetylglaucarubinone (**3a**) and glaucarubinone (**3b**) were also obtained. The new quassinoid **2** was found significantly to inhibit growth of the murine lymphocytic leukemia P388.

Several quassinoids[4], the bitter principles of the plant family Simaroubaceae, have exhibited promising anticancer activity[4,5] and bruceantin has recently been placed on clinical trial by the US National Cancer Institute[6]. Part of our earlier program, directed at uncovering new antineoplastic quassinoids produced by Simaroubaceae, was concerned with the Guyana species *Simarouba amara* Aubl. Initially 5-hydroxycanthin-6-one[7] and 4 Δ^7-tirucallol-type triterpenes, (believed to be biogenetic precursors of the quassinoids[4]; namely oxo-3-tirucalla-7,24-diene, dioxo-3,21-tirucalla-7,24-diene[8], melianone and 21,20-anhydromelianone[9]) were isolated and characterized. We report here the isolation, structural elucidation and preliminary anticancer evaluation of 2 new quassinoids designated 2′-acetylglaucarubine (**1a**) and 13,18-dehydroglaucarubinone (**2**). The previously known quassinoids, 2′-acetylglaucarubinone (**3a**)[10,11] and glaucarubinone (**3b**)[11,12], were also isolated from *Simarouba amara*. Quassinoid **2** was found to show significant antineoplastic activity (54% life extension at 2 mg/kg) in the National Cancer Institute's murine lymphocytic leukemia P388 (PS system)[13].

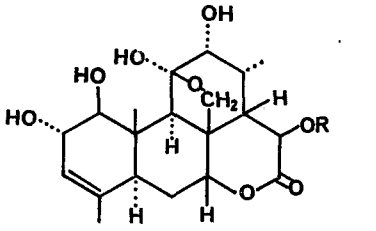
The dried, finely ground root bark of *Simarouba amara* was extracted with hexane and several times with boiling water. The aqueous extract was concentrated under reduced pressure and continuously extracted with chloroform. Evaporation of the chloroform yielded a bright yellow foam which crystallized upon addition of chloroform to give 2′-acetylglaucarubine (**1a**) as colorless needles, m.p. 243–246 °C $[\alpha]_D$ +29.5° (c, 1.1, pyridine). The empirical formula $C_{27}H_{38}O_{11}$ (M^+ at m/e 538) and similarity of the ^{1}H-NMR-spectrum with that of glaucarubine (**1b**) suggested that this new quassinoid might be the α-acetoxy-α-methylbutyrate ester of glaucarubol (**1c**). The presence of such an ester was further indicated by fragment ions in the mass spectrum at m/e 143 $[COC(OAc)(CH_3)C_2H_5]^+$, 115 $[C(OAc)(CH_3)C_2H_5]^+$, 83 $[COC(CH_3)=CHCH_3]^+$ and by a strong signal at m/e 360 corresponding to the loss of water and α-acetoxy-α-methyl-butyric acid from the molecular ion. Furthermore, the ^{1}-H-NMR-spectrum displayed signals for primary, tertiary and acetate methyl groups assignable to the ester (t, 0.98; s, 1.65 and s, 2.03 ppm, respectively) and a 1 proton downfield doublet at 5.48 ppm (J = 9 Hz) indicating the ester bonding to be at C-15[14]; the remaining signals correspond in chemical shift to those assigned glaucarubine[14]. Structure **1a** was unequivocally confirmed by acetylation (acetic anhydride-pyridine) which gave the pentaacetate of glaucarubine (**4**)[15]. The acetylation product **4** was identical with an authentic sample.

The mother liquors from the 2′-acetylglaucarubine (**1a**) isolation were subjected to column chromatography (Silicagel 60, E. Merck). Elution with chloroform containing 2% methanol afforded the known 2′-acetylglaucarubinone (**3a**)[10,11] and, on increasing to 5% methanol, yielded a crystalline fraction that contained glaucarubinone (**3b**)[11,12] and the new antineoplastic agent 13,18-dehydroglaucarubinone (**2**). Separation of the latter 2 quassinoids was achieved by repeated preparative TLC (Silicagel, 1510 LS 254, Schleicher and Schüll, chloroform-methanol, 9:1).

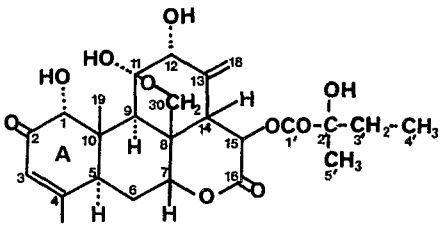
The 13,18-dehydroglaucarubinone (**2**) molecular formula was found to be $C_{25}H_{32}O_{10}$ (M^+ at m/e 492); m.p. 215–

^{13}C-NMR spectral assignments ($CDCl_3$/pyridine-d_5 solution, downfield from internal trimethylsilane) for 13,18-dehydroglaucarubinone

C(1)	83.18	C(9)	41.8	C(18)	121.7
C(2)	196.8	C(10)	45.1	C(19)	9.8
C(3)	125.4	C(11)	109.1	C(30)	71.6
C(4)	162.3	C(12)	79.4	4-Me	26.7
C(5)	45.0	C(13)	141.1	C(1′)	175.8
C(6)	25.3	C(14)	51.4	C(2′)	75.0
C(7)	78.4	C(15)	69.3	C(3′)	33.1
C(8)	47.1	C(16)	166.6	C(4′)	7.9
				C(5′)	25.8



1a R = COC(CH$_3$)(OAc)CH$_2$CH$_3$
b R = COC(CH$_3$)(OH)CH$_2$CH$_3$
c R = H



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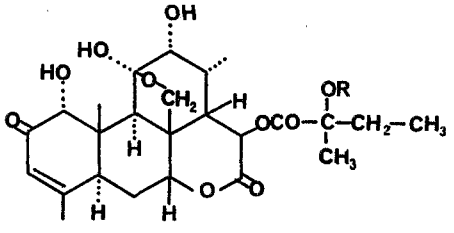
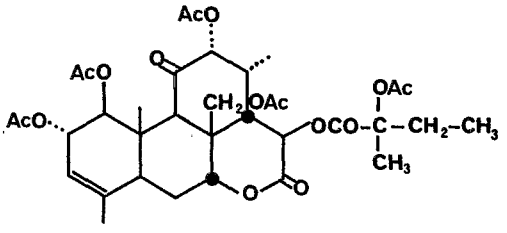


3a R = Ac
b R = H



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218 °C. $[\alpha]_D$ +34.7° (c=0.95, MeOH). The A-ring assignment for quassinoid **2** was supported by the UV-spectrum (λ_{max} 252 nm), the circular dichroism curve [$\Delta\varepsilon$ +1.06 (320 nm)], the ^{1}H-NMR-data ($CDCl_3$, at 90 MHz, vinyl methyl at 2.04, H-1 at 4.3 ppm and H-3 at 6.15 ppm) and by the characteristic[16] mass spectral fragment ions at m/e 151 and 247. The mass spectrum of quassinoid **2** also indicated the presence of an α-hydroxy-α-methylbutyrate ester corresponding to m/e 73 $[C(OH)(CH_3)C_2H_5]^+$ and m/e 83 $[COC(CH_3)=CH\text{-}CH_3]^+$. The ^{1}H-NMR of quassinoid **2** displayed resonances at 0.87 (t), 1.5 (s) and 1.2 (s) ppm assignable to the C-4′, C-2′ and C-10 methyl groups. The absence of a signal corresponding to a C-13 methyl group and presence of 2 signals at 5.21 ppm in the H^1-NMR-spectrum of substance **2** allowed assignment of the 13,18-double bond. An AB quartet centred at 3.73 ppm (J=9 Hz) corresponded to the $-CH_2O$-methylene involved in the 11,30-hemiketal. Signals at 3.13 (s), 4.07 (s), 4.62 (t) and 5.87 (d, J=12 Hz) were assigned to protons H-9, H-12, H-7 and H-15, respectively. The 13,18-dehydroglaucarubinone (**2**) structural assignment was confirmed by a ^{13}C-NMR-analysis (table)[17,18].

A biological study of quassinoids **1a, 2** and **3b** against a cell line[19] derived from the PS leukemia gave the following results: both 13,18-dehydroglaucarubinone (**2**) and glaucarubinone (**3b**) gave significant cell growth inhibition corresponding to ED_{50} values (μg/ml) of 0.95 and 0.34 respectively, while 2′-acetylglaucarubine (**1a**) was found to be essentially inactive (ED_{50} 29).

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2 Cancer Research Institute and Department of Chemistry, Arizona State University, Temple, Arizona 85281, USA. Antineoplastic agents 59. For part 58 refer to M. T. Edgar, G.R. Pettit and T.H. Smith, J. org. Chem., in preparation.

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