On the biosyntheses of artemisia ketone and bakuchiol

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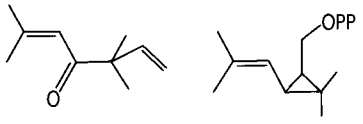
Summary. New biosynthetic pathways for the irregular terpenes, artemisia ketone and bakuchiol, are proposed. It is suggested that 2-(1-hydroxy-3-methylbutyl)thiamine and 2-(1-hydroxy-2-phenylethyl) thiamine are key intermediates in the biosyntheses of artemisia ketone and bakuchiol, respectively.

The irregular head-to-tail arrangement of isoprenoid units in artemisia ketone **1** has intrigued bio-organic chemists for many years. Since 1970, based upon the pioneering work of Rilling[1] on squalene biosynthesis, it has been widely accepted that artemisia ketone arises from an analogue of presqualenyl pyrophosphate; namely, the C_{10} cyclopropyl intermediate[2], **2**:



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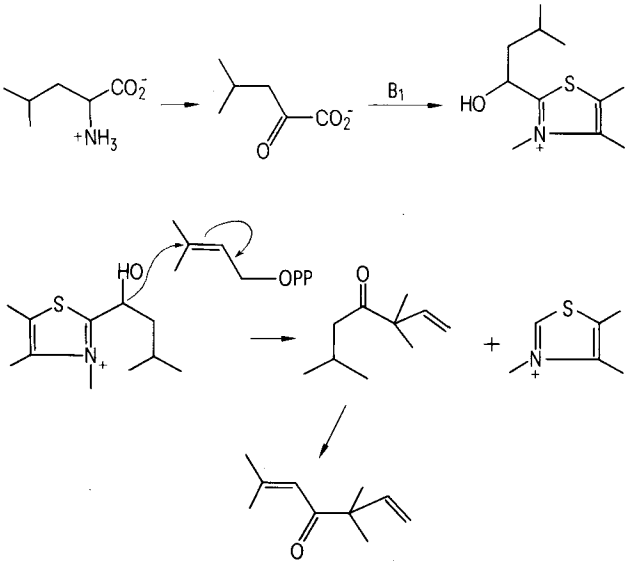
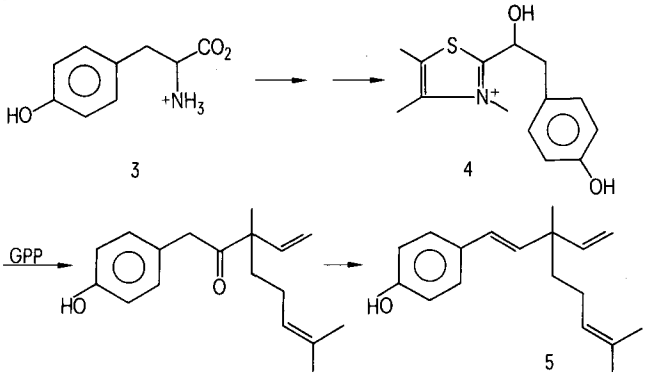
Recently, however, several groups have shown that this intermediate may not be the precursor to the irregular ketone. Labelling studies on *Artemisia annua* L. with 2-C^{14}-MVA have shown that the bulk of the tracer resides at C-9 and 10, and only 10% or so is incorporated into C-7 and 8[3,4].

For sometime, we have entertained the hypothesis that thiamine may play a coenzymatic role in the biosyntheses of the irregular terpenes[5] such as artemisia ketone and bakuchiol[6]. We would like to suggest that C-7 and 8 are sparsely labelled because these 2 carbon atoms are part of a C_5 unit which arises from leucine rather than MCA; only the C_5 unit comprising C-1, 2, 3, 9 and 10 arises from MVA. This biosynthetic pathway is described in the following sequence



A similar reaction sequence can be expressed for the biosynthesis of bakuchiol; tyrosine **3** being the source of the aromatic ring, and an SN_2' reaction between 2-(1-hydroxy-2-phenylethyl) thiamine **4** and geranyl pyrophosphate, followed by subsequent reduction and dehydration would then afford bakuchiol **5**:



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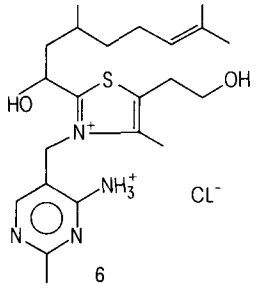
During the 1972–1974 period, we were unable to synthesize any of the desired terpenylthiamine compounds and, therefore, could not test the validity of this hypothesis. Recently, however, we have developed a procedure which has allowed us to synthesize a number of terpenylthiamine derivatives[7]. Both 2-(1-hydroxy-3-methylbutyl)-and 2-(1-hydroxy-3,8-dimethyl-7-octenyl) thiamine chloride hydrochlorides **6** have been synthesized. And, at the present time, we would like to report on the enzymatic conversion of the octenylthiamine derivative into an analogue of artemisia ketone. Cell-free yeast preparations as outlined by Tchen were prepared[8]. To 30 ml of this enzyme preparation were added magnesium sulfate, ATP, glutathione, Triton X-100, geraniol, and the octenylthiamine derivative. The enzyme mixture was incubated at 37 °C for 3 h. At the end of this period, 10 ml of a 20% KOH-MeOH solution was added and the solution was kept at 65–70 °C for 1 h. Upon cooling, the preparation was extracted with 20 ml products were subjected to preparative TLC. A light yellow oil (R_f = 0.87; 25:1, Bz-MeOH) was isolated and found to analyze for $C_{20}H_{34}O$ (cal culation: C, 82.69; H, 11.79; found: C, 82.91; H, 11.53). NMR-data and MS-analysis (parent peak 290) suggested the artemisia ketone analogue **7**[9]:



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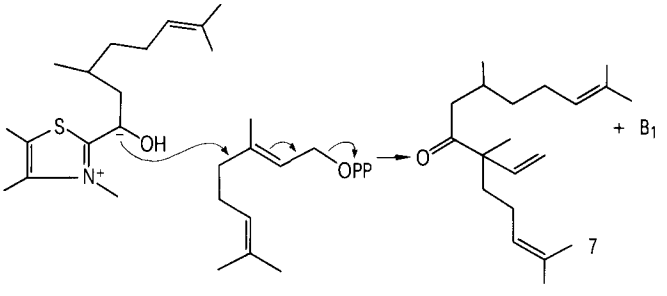

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This assignment was verified by total synthesis. This was accomplished by the continuous flow Reformatsky reaction developed by Rupert and White[10], followed by chromium trioxide oxidation. The ketone was prepared in 77% yield and was identical to the terpene isolated from the enzymatic preparation.

Thus, the enzymatic conversion of the octenyl-thiamine derivative into an artemisia ketone analogue is indicative that thiamine derivatives may be involved in the biosyntheses of certain irregular terpenes and suggests that the thiamine hypothesis should be further investigated.

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9 The artemisia ketone analogue is optically active $[\alpha]_D^{30} = -21.2$. The analogue, however, is a mixture of optical isomers, since the starting terpenoid thiamine derivative has not been resolved.
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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