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Uvarinol: A Novel Cytotoxic Tribenzylated Flavanone from *Uvaria chamae*

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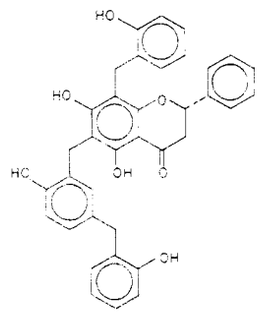
Recently we[1,2] and others[3] have isolated cytotoxic compounds from plants of the *Uvaria* genus. We now wish to report the structure of uvarinol (**1**), the most complex



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of the active compounds yet isolated. An ethanolic extract of the stem bark of *Uvaria chamae* (Annonaceae) showed activity in vivo against P-388 lymphocytic leukemia (PS) and in vitro against cells derived from human carcinoma of the nasopharynx (KB). Further fractionation of the ethanolic extract was guided by assay against KB.[4] The activity was concentrated in the ethyl acetate soluble portion of an ethyl acetate–water partition. Chromatography of the ethyl acetate fraction over silicic acid has resulted in the isolation of several cytotoxic C-benzylated flavanones and dihydrochalcones.[1-3] We now wish to report an additional novel cytotoxic flavanoid, for which the trivial name uvarinol (**1**) has been chosen. Uvarinol showed cytotoxicity[4] (ED_{50}) against KB cell cultures at 5.9 μg/mL and against PS cell cultures at 9.7 μg/mL as well as significant antimicrobial activity against *Staphylococcus aureus*, *Bacillus subtilis*, and *Mycobacterium smegmatis*.[5]

A molecular formula of $C_{36}H_{30}O_7$ was established by high-resolution mass spectroscopy and combustion analysis. The UV (λ_{max} (MeOH) 329 nm (ε 16 000)) and IR spectra (KBr, 3100 (broad, OH), 1628 cm^{-1} (CO)) were consistent with a dihydroxylated flavanone nucleus.[1,2] The

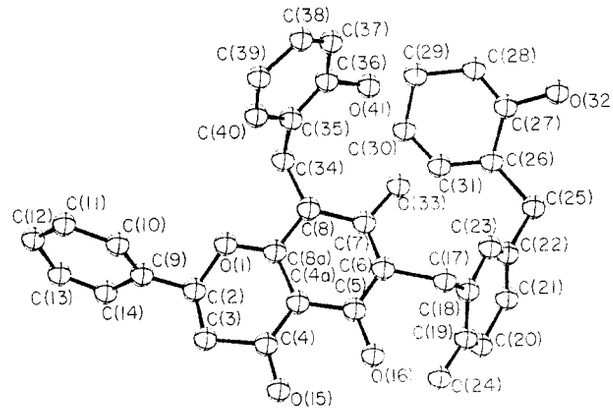

Figure 1. A perspective drawing of the X-ray model of uvarinol (**1**). Hydrogens are omitted for clarity, and the absolute configuration was deduced from CD data.

^1H NMR spectrum (60 MHz, acetone-d_6) clearly showed an ABX pattern characteristic of the protons at C-3 (δ 2.70–3.00, AB) and C-2 (δ 5.70, dd, X) of a flavanone. A 1 H singlet at δ 13.30 (OH at C-5, exchanges with D_2O), three 2 H singlets at δ 4.00, 3.97, and 3.87 (Ar**CH**$_2$Ar), sixteen aromatic protons between δ 6.60 and 7.90, and four additional exchangeable protons comprised the rest of the spectrum. The absence of an upfield aromatic proton suggested that both C-6 and C-8 were substituted.[1] The spectral data of uvarinol are similar to that for dichamenetin,[1,6] a C-dibenzylated flavanone, and suggest that **1** is a tribenzylated flavanone with two benzyl substituents at C-6 and C-8. There were no characteristic fragment ions in the mass spectrum to aid in locating the third benzyl substituent. Since the structural evidence for **1** was largely presumptive and incomplete, a single-crystal X-ray diffraction experiment was done.

Figure 1 is a perspective drawing of the X-ray model of one of the independent molecules of uvarinol. The X-ray experiment defined only the relative configuration, and the absolute configuration shown was chosen to agree with the CD data.[7] All bond distances and angles generally agree with accepted values, although the estimated standard deviations are high (0.05 Å and 3°). The conformations of the independent molecules are essentially the same. In one molecule O(1), C(4), C(4a), and C(8a) are coplanar within 0.05 Å, while C(2) and C(3) are 0.63 and 0.41 Å out of the plane. In the second molecule O(1′), C(4′), C(4a′), and C(8a′) are coplanar within 0.04 Å and C(2′) and C(3′) are 0.50 and 0.17 Å out of the plane. In both molecules there appear to be three intramolecular hydrogen bonds: O(16)H···O(15), O(24)H···O(16), and O(33)H···O(41). These latter two hydrogen bonds could play an important role in determining the molecular conformation. Molecules related by a *c*-axis translation are linked by two intermolecular hydrogen bonds: O(32)H···O(24) and O(41)H···O(15). Surprisingly only van der Waals interactions are found between symmetry-independent molecules.

Uvarinol (**1**) is thus a tribenzylated flavanone. The flavanones are of course widespread in the higher plants, but the addition of benzyl groups is quite rare and seems to be limited to *Uvaria*.[1-3] The benzyl groups presumably arise from a C_6–C_1 pathway, but the *o*-hydroxy functionality is unusual. Recently a biogenetic scheme that would generate *o*-hydroxybenzyl groups from isochorismate has been suggested.[8]

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Experimental Section

Isolation of Uvarinol (1). The preliminary steps in the purification scheme have been described previously.[2] Continued elution with 1.5 L of 16% ether in benzene and 2.0 L of 32% ether in benzene yielded a 1.15 g fraction from which 195 mg of uvarinol was obtained upon recrystallization from benzene: mp 152–4 °C, $[\alpha]^{20}_D$ −16.5 (*c* 1.00, acetone); *m/e* 574.195; calcd for $C_{36}H_{30}O_7$, 574.199; CD spectral data (MeOH), $[\theta]_{350}$ + 4930, $[\theta]_{316}$ + 6090, $[\theta]_{292}$ − 35 300.

Anal. Calcd for $C_{36}H_{30}O_7 \cdot C_6H_6$: C, 77.05; H, 5.58. Found: C, 77.30; H, 5.52.

X-ray Diffraction Study of Uvarinol (1). Small crystals of uvarinol (1) were obtained from slow evaporation of benzene solutions. Preliminary photographs displayed no symmetry other than that required by Friedel's law and thus indicated, when coupled with the known optical activity of uvarinol, the triclinic space group *P*1. Accurate diffractometer determined cell constants were a = 12.190 (4), b = 15.116 (4), c = 10.835 (4) Å, α = 109.94 (2), β = 115.31 (2), and γ = 78.81 (2)°. We were not able to obtain an accurate density of the crystals at our disposal, but since the elemental analysis indicated one benzene per uvarinol, we assumed that the asymmetric unit contained *two* complexes of composition $C_{36}H_{30}O_7 \cdot C_6H_6$. We tried to recrystallize uvarinol from other solvents but we were only able to prepare the crystals described above. Crystallization from bromobenzene, in an attempt to introduce a heavy atom via the solvate molecule, was unsuccessful. All unique diffraction maxima with $2\theta \leq 114°$ were recorded on a computer-controlled four-circle diffractometer, using graphite monochromated Cu Kα (1.54178 Å) radiation. The crystals scattered rather poorly, and of the 4572 unique reflections surveyed only 2682 (58.7%) were judged observed ($F_o^2 \geq 3\sigma(F_o^2)$) after correction for Lorentz–polarization and background effects.

Since the molecular weight of the asymmetric unit of this noncentrosymmetric crystal was in excess of 1300, and the diffraction data were limited at high 2θ values, we anticipated some difficulty in arriving at a trial structure. We were not disappointed. The angular dependence of the structure factors was eliminated as they were converted to normalized structure factors, and a multisolution weighted tangent formula approach was tried.[9] While this did not lead to anything that was interpretable, it did indicate that the chirality, as judged by intensity statistics, was very weak. We observed $\langle|E_h|\rangle_h = 0.8374$ and $\langle|E_h^2 - 1|\rangle_h = 0.9036$, while for the centrosymmetric space group $P\bar{1}$ the values 0.798 and 0.968 are predicted, and for the noncentrosymmetric space group *P*1 the related values are 0.886 and 0.736.[10] Careful inspection of the Patterson synthesis[11] showed that the two complexes in the asymmetric unit might be related by a pseudoinversion center. Following this clue, attempts were made to arrive at a trial structure, using the centrosymmetric space group $P\bar{1}$. Ultimately the following approach was successful. We had noticed in manual phasing attempts that E_{hk3n} were all very large and that E_{300} and E_{600} were strongly correlated with many E_{hk3n}. We reduced all E_{hk3n} to 60% of their calculated values and removed E_{300} and E_{600}. We then assigned signs in the centrosymmetric space group $P\bar{1}$. While the usual figures of merit did not discriminate among the resulting phase sets, a figure of merit based on negative quartets strongly indicated one solution as being reliable.[12] The resulting *E* synthesis showed 27 chemically sensible atoms which were expanded to 49 reasonable atoms in successive Fourier syntheses. The resulting structure was plausible in its general outlines but unacceptable in many details. The space group was changed to the noncentrosymmetric *P*1, and Fourier refinement was begun again with idealized coordinates of one molecule. Eventually all 98 nonhydrogen atoms were placed. Full-matrix least-squares refinements with isotropic temperature factors for nonhydrogen atoms and no hydrogen atoms have reached a current crystallographic residual of 0.095 for the observed reflections. In view of the limited data available, we terminated refinement at this point. The two independent molecules, excluding C(2) and its attached phenyl ring, are closely related by an inversion center. Additional crystallographic details can be found in the Supplementary Material.

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Registry No. 1, 66754-55-0.

Supplementary Material Available: Tables I–III listing fractional coordinates, temperature factors, bond distances, and bond angles for uvarinol (8 pages). Ordering information is given on any current masthead page.

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Synthesis of (±)-Machicendiol

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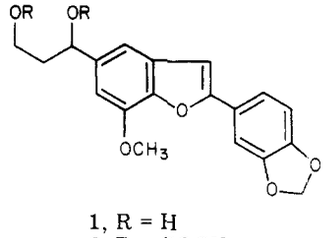
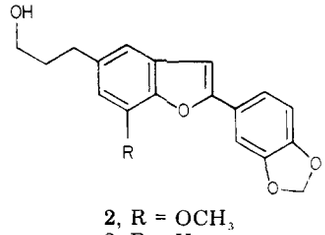
Extracts of *Machilus glaucescens* (Lauraceae) have been used in the treatment of asthma, rheumatism, and ulcers; from the leaves, there has recently been isolated a norlignan, named machicendiol, for which the structure 2-(3,4-methylenedioxyphenyl)-5-(1,3-dihydroxypropyl)-7-methoxybenzofuran (1) was suggested on the basis of



1, R = H
8, R = $COCH_3$



2, R = OCH_3
3, R = H

spectral data and a partial synthesis from egonol (2).[1] We have recently described syntheses[2] of the congeners egonol

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