# Crystal and Molecular Structure of Amarorine Monohydrate, a New Phenolic Alkaloid from *Amaroria soulameoides*

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The molecular structure of amarorine (11-hydroxy-6*H*-indolo[3,2,1-*de*][1,5]naphthyridin-6-one) (I) and amaroridine (II), alkaloids isolated from *Amaroria soulameoides* A. Gray, were deduced by examination of their spectra; the structure of amarorine was confirmed by an *X*-ray crystallographic study.   Crystals are monoclinic $a = 17.44(3)$, $b = 9.36(2)$, $c = 7.12(2)$ Å, $\beta = 92.7(1)°$, $Z = 4$, space group $P2_1/n$.   The structure was solved by direct methods and refined by full matrix least-squares calculations to $R = 0.070$ for 523 reflections.

CANTHINONE (III) and other compounds having the β-carboline skeleton have been found in species of the plant family Simaroubaceae.[1]   Recently 5-methoxycanthin-6-one (IV) has been identified in the monotypic genus (fam. Simaroubaceae) *Amaroria soulameoides* A. Gray.[2] We now report the isolation and structural elucidation of two new alkaloids amarorine (I) and amaroridine (II)
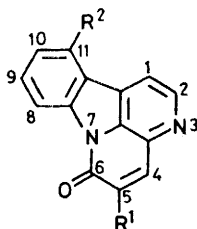
(I) $R^1 = H; R^2 = OH$

(II) $R^1 = H; R^2 = OMe$

(III) $R^1 = R^2 = H$

(IV) $R^1 = OMe; R^2 = H$

possessing canthin-6-one structures from this Fijian plant.

Red-brown crystals of amarorine monohydrate grew from aqueous methanol.   Upon dehydration at 40 °C *in vacuo* the crystals changed to a yellow-brown colour but rehydration occurred rapidly upon exposure of the dry crystals to the atmosphere.   Mass measurement of the molecular ion (m/e 236) gave the molecular formula $C_{14}H_8N_2O_2$.   The u.v. spectrum was typical for an extended indole[3] ($\lambda_{max.}$ 325 and 385 nm) and showed features common to spectra of canthinone alkaloids.[4] The marked bathochromic shift induced by treatment with alkali indicated the presence of a phenolic hydroxy-group in amarorine and methylation (methyl iodide–NaH–hexamethylphosphoric triamide) gave amaroridine. The [1]H n.m.r. spectra of amarorine and amaroridine provided further evidence of their structures through the presence, in each case, of an isolated pair of vicinal protons (1-H) and (2-H) similar in chemical shift and coupling constant to those in the 3- and 4-positions of a β-carboline.[5]   The presence of three coupled aromatic protons indicated that the substituents were attached to the 8- or 11-position of the canthinone system and the

fact that no nuclear Overhauser effect could be observed between the 1- and 11-protons[6] pointed to a substituent at the 11-position.   This assignment was supported by the i.r. spectrum of amarorine which showed no hydrogen bonding between the hydroxy-group and the 6-carbonyl group.   However, the possibility that the carbonyl group was at the 4-position could not be excluded.   In order to achieve an unambiguous structural determination and, additionally, to investigate the marked affinity of amarorine for water, its monohydrate was submitted to an *X*-ray crystallographic study.

The molecular structure of amarorine is depicted with the crystallographic numbering scheme for the atoms in Figure 1.   Final atomic co-ordinates and bond lengths and angles are given in Tables 1 and 2.   The bond lengths and angles are generally within their normal ranges for this type of molecular skeleton as shown by comparison with 5,11-dimethyl-6*H*-pyrido[4,3-*b*]carbazole (ellipticine).[7]   The only unusual features of the molecule are the
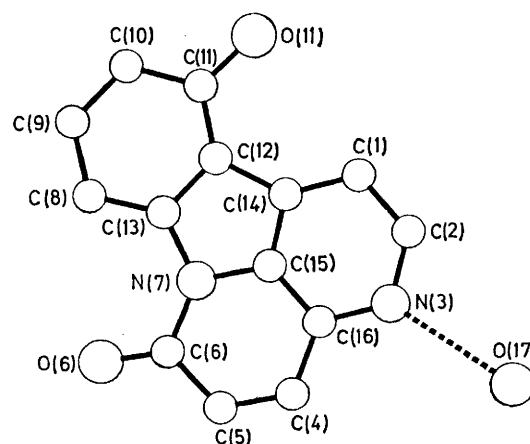

FIGURE 1   Molecular structure of amarorine showing the atom numbering system used

relative bond angles at C(12) and C(14).   The environment of these atoms is apparently rather similar and it would be expected that the angles around these atoms would be grouped in three pairs of equivalents, namely:

C(11)–C(12)–C(13) and C(1)–C(14)–C(15);
C(11)–C(12)–C(14) and C(1)–C(14)–C(12); and
C(13)–C(12)–C(14) and C(12)–C(14)–C(15).

The latter two angles, subtended inside the five-membered ring are indeed essentially equal with values of 107(1) and 106(1)° respectively, but the remaining pairs differ by almost 10°. In ellipticine the exterior angles

TABLE 1

Fractional co-ordinates (× 10⁴) and isotropic temperature factors (× 10³), with estimated standard deviations in parentheses

Atom	x	y	z	U_{iso}
C(1)	−603(7)	2 228(14)	7 924(16)	53(3)
C(2)	−13(7)	·3 193(14)	8 477(16)	59(4)
N(3)	749(6)	2 852(13)	8 680(13)	60(3)
C(4)	1 726(7)	971(15)	8 471(17)	60(4)
C(5)	1 874(8)	−416(17)	8 068(13)	69(4)
C(6)	1 281(8)	−1 439(17)	7 501(17)	67(4)
N(7)	520(6)	−898(11)	7 373(12)	49(3)
C(8)	−360(7)	−2 953(14)	6 460(15)	53(3)
C(9)	−1 134(8)	−3 286(16)	6 087(17)	64(4)
C(10)	−1 721(7)	−2 252(16)	6 208(17)	63(4)
C(11)	−1 526(8)	−820(15)	6 704(16)	55(4)
C(12)	−764(6)	−532(12)	7 026(13)	37(3)
C(13)	−192(7)	−1 535(14)	6 930(14)	46(3)
C(14)	−396(6)	819(13)	7 587(15)	42(3)
C(15)	395(7)	519(14)	7 810(14)	46(3)
C(16)	949(7)	1 483(16)	8 292(16)	54(4)
O(6)	1 394(5)	−2 724(11)	7 122(13)	82(3)
O(11)	−2 061(5)	202(10)	6 779(11)	65(3)
O(17)	1 609(4)	5 213(9)	9 952(10)	64(2)
H(1)	−1 167	2 528	7 780	65
H(2)	−179	4 241	8 732	65
H(4)	2 164	1 666	8 904	65
H(5)	2 420	−786	8 169	65
H(8)	64	−3 707	6 406	65
H(9)	−1 296	−4 311	5 688	65
H(10)	−2 292	−2 486	5 973	65

TABLE 2

Interatomic distances (Å) and angles (°) with estimated standard deviations in parentheses

(a) Distances

C(1)−C(2)	1.41(2)	C(8)−C(9)	1.40(2)
C(1)−C(14)	1.39(2)	C(8)−C(13)	1.40(2)
C(2)−N(3)	1.37(1)	C(9)−C(10)	1.41(2)
N(3)−C(16)	1.36(2)	C(10)−C(11)	1.42(2)
N(3)−O(17)	2.80(1)	C(11)−C(12)	1.37(2)
C(4)−C(5)	1.36(2)	C(11)−O(11)	1.34(1)
C(4)−C(16)	1.44(2)	C(12)−C(13)	1.38(1)
C(5)−C(6)	1.45(2)	C(12)−C(14)	1.47(2)
C(6)−N(7)	1.42(1)	C(14)−C(15)	1.41(1)
C(6)−O(6)	1.25(1)	C(15)−C(16)	1.35(2)
N(7)−C(13)	1.40(1)	O(6¹)−O(17)	2.80(1)
N(7)−C(15)	1.38(1)	O(11¹¹)−O(17)	2.64(1)

(b) Angles

C(2)−C(1)−C(14)	118(1)	C(11)−C(12)−C(14)	129(1)
C(1)−C(2)−N(3)	125(1)	C(13)−C(12)−C(14)	107(1)
C(2)−N(3)−C(16)	117(1)	N(7)−C(13)−C(8)	129(1)
C(5)−C(4)−C(16)	119(1)	N(7)−C(13)−C(12)	110(1)
C(4)−C(5)−C(6)	123(1)	C(8)−C(13)−C(12)	121(1)
C(5)−C(6)−N(7)	116(1)	C(1)−C(14)−C(12)	139(1)
C(5)−C(6)−O(6)	125(1)	C(1)−C(14)−C(15)	115(1)
N(7)−C(6)−O(6)	119(1)	C(12)−C(14)−C(15)	106(1)
C(6)−N(7)−C(13)	133(1)	N(7)−C(15)−C(14)	109(1)
C(6)−N(7)−C(15)	119(1)	N(7)−C(15)−C(16)	125(1)
C(13)−N(7)−C(15)	108(1)	C(14)−C(15)−C(16)	125(1)
C(9)−C(8)−C(13)	117(1)	N(3)−C(16)−C(4)	123(1)
C(8)−C(9)−C(10)	122(1)	N(3)−C(16)−C(15)	120(1)
C(9)−C(10)−C(11)	119(1)	C(4)−C(16)−C(15)	117(1)
C(10)−C(11)−C(12)	117(1)	N(3)−O(17)−O(6¹)	105.1(4)
C(10)−C(11)−O(11)	122(1)	N(3)−O(17)−O(11¹¹)	119.3(4)
C(12)−C(11)−O(11)	122(1)	O(6¹)−O(17)−O(11¹¹)	122.8(4)
C(11)−C(12)−C(13)	124(1)		

Roman numeral superscripts denote the following equivalent positions:

　　(I)　$x, y + 1, z$;　　(II)　$\frac{1}{2} + x, \frac{1}{2} - y, \frac{1}{2} + z$

equivalent to C(11)−C(12)−C(14) [129(1)]° and C(1)−C(14)−C(12) [139(1)]° are 134.0 and 135.8° respectively. The possibility that the difference observed here arises from some systematic error in the data cannot be excluded but such an error does not manifest itself in the bond lengths to C(12) and C(14).

Amarorine is essentially planar taken either as a whole or divided into the constituent five- or six-membered rings (Table 3) and the water molecule of crystallisation is situated only 0.18Å out of the molecular plane. The crystal structure contains infinite sheets of hydrogen bonded water and amarorine parallel to the $\bar{2}02$ plane. The structure of one sheet is shown in Figure 2.
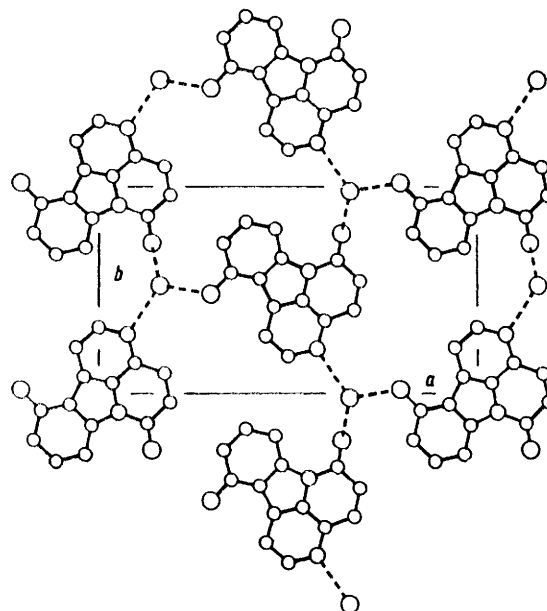

FIGURE 2　A single hydrogen-bonded sheet of molecules viewed along the c axis

EXPERIMENTAL

Mass spectra were recorded on a Hitachi Perkin-Elmer RMU6 Mass Spectrometer at 70 eV and mass measurements were made by P.C.M.U., Harwell. ¹H N.m.r. spectra were taken with a Varian HA 60 spectrometer, with tetramethylsilane as internal standard and the 220 MHz spectrum was measured by P.C.M.U., Harwell.

Extraction and Isolation.—Ground bark (4.0 kg) of *Amaroria soulameoides* A. Gray was exhaustively extracted with light petroleum (40—60 °C) and then with methanol. The concentrated methanolic solution was applied to a column of Amberlyst 15 resin and after the resin had been washed with methanol the alkaloids were eluted with aqueous methanolic ammonia. Evaporation of the solvent and ammonia yielded a syrupy residue (14.02 g) which was dissolved in a small quantity of methanol and then adsorbed onto silica gel (100 g; Merck 7731). After careful removal of the solvent from the product in a rotatory evaporator, it was applied to the top of a column of silica gel (700 g; Merck 7731) prepared in light petroleum (b.p. 40—60 °C). Elution of the column with chloroform and mixtures of chloroform and methanol gave a number of fractions. The fractions which produced a positive response when spotted on t.l.c.

plates coated with silica gel and sprayed with Dragendorff's reagent were combined and rechromatographed over silica gel (600 g; Merck 7731) using chloroform as eluting solvent. The two major alkaloid bearing fractions from this separation were rechromatographed individually over silica gel (200 g; Merck 7731). From the faster-running fraction amaroridine (II) (218 mg) was obtained. This had m.p. 237—238 °C; $\lambda_{max.}$ 246 nm ($\log_{10} \varepsilon$ 3.89), 325 (3.68), and 389 nm (3.74; $\nu_{max.}$ (KBr) 1 600—1 680 cm^{-1} (C=C and C=O); ^{1}H n.m.r. (220 MHz) δ (CDCl$_3$) 6.911 (1 H, d, 10-H, $J = 8$ Hz), 6.912 (1 H, d, 5-H, $J = 10$ Hz), 7.574 (1 H, t, 9-H, $J = 8$ Hz), 7.932 (1 H, d, 1-H, $J = 5$ Hz), 8.000 (1 H, d, 4-H, $J = 5$ Hz), 8.113 (1 H, d, 8-H, $J = 10$ Hz), and 8.714 (1 H, d,

TABLE 3

Equations of least-squares planes with respect to crystallographic axes. Deviations (Å) from the plane are given in square brackets

Plane (1): C(1)—O(11)

$$-2.523x - 2.132y + 6.897z = 5.168$$

[C(1) −0.03, C(2) 0.00, N(3) 0.02, C(4) 0.03, C(5) 0.01, C(6) −0.01, N(7) −0.02, C(8) 0.01, C(9) 0.02, C(10) 0.03, C(11) 0.02, C(12) −0.02, C(13) −0.01, C(14) −0.01, C(15) 0.01, C(16) −0.01, O(6) −0.03. O(11) −0.02 O(17) 0.18]

Plane (2): C(1)—C(3), C(14)—C(16)

$$-2.704x - 2.152y + 6.885z = 5.150$$

[C(1) 0.01, C(2) 0.00, C(3) −0.01, C(14) −0.01, C(15) −0.01, C(16) 0.02]

Plane (3): (C8)—C(13)

$$-2.302x - 2.052y + 6.920z = 5.153$$

[C(8) −0.01, C(9) 0.01, C(10) 0.00, C(11) −0.01, C(12) 0.01, C(13) 0.01]

Plane (4): N(7), C(12)—C(15)

$$-2.569x - 2.199y + 6.883z = 5.150$$

[N(7) −0.01, C(12) 0.00, C(13) 0.01, C(14) −0.01, C(15) 0.01]

2-H, $J = 5$ Hz); m/e (rel. intensity) 250 (M^+; 100), 235 (6.2), 222 (8.5), 220 (6.4), 207 (20.1), and 179 (13.8) (Found: C, 71.9; H, 4.15; N, 10.8. C$_{15}$H$_{10}$N$_2$O$_2$ requires C, 71.97; H, 4.03; N, 11.20%).

The slower-running fraction was eluted by chloroform–methanol (95 : 5) and gave amarorine (I) (127 mg) on crystallization from chloroform and methanol. Amarorine had m.p. 323—325 °C; $\lambda_{max.}$ (C$_2$H$_5$CH) 325 ($\log_{10} \varepsilon$ 3.96), and 385 nm (4.25); $\nu_{max.}$ (KBr) 3 450 (OH), and 1 620—1 670 cm^{-1} (C=C and C=O); ^{1}H n.m.r. (60 MHz) δ [(CD$_3$)$_2$SO] 6.97 (1 H, d, 10-H, $J = 8$ Hz), 6.97 (1 H, d, 5-H, $J = 10$ Hz), 7.53 (1 H, t, 9-H, $J = 8$ Hz), 7.95 (1 H, d, 8-H, $J = 8$ Hz), 8.04 (1 H, d, 1-H, $J = 5$ Hz), 8.09 (1 H, d, 4-H, $J = 10$ Hz), 8.73 (1 H, d, 2-H, $J = 5$ Hz), and 10.06 (1 H, broad, O-H); m/e (rel. intensity) 236 (M^+; 100), 208 (49), 179 (15) 153 (8), 127 (7), 126 (6), 118 (12), and 90 (16) (Found: C, 66.1; H, 4.05; N, 11.2. C$_{14}$H$_8$N$_2$O$_2$·H$_2$O requires C, 66.15; H, 3.97; N, 11.03%).

Crystallographic Measurements.—Needle-like crystals of amarorine were grown from aqueous methanol. The needle axis was c.

Crystal Data.—C$_{14}$H$_8$N$_2$O$_2$·H$_2$O, $M = 254$. Monoclinic, $a = 17.44$ (3), $b = 9.36$ (2), $c = 7.12$ (2) Å, $\beta = 92.7$ (1)°, $U = 1161/$Å^3, $D_m = 1.44$ g cm^{-3} (by flotation, $Z = 4$, $D_c = 1.45$ g cm^{-3}. Systematic absences: $h0l$ for $h + l = 2n + 1$; ($hk0$ for $h = 2n + 1$); $0k0$ for $k = 2n + 1$; $00l$ for $l = 2n + 1$); Space group $P2_1/n$. Cu-K_α radiation, $\lambda = 1.541$ 8 Å, μ(Cu-K_α) = 8.8 cm^{-1}.

Intensity Measurement.—Intensity data were recorded

photographically for reciprocal lattice layers $hk0$ to $hk6$ by means of the equi-inclination Weissenberg technique and multiple film packs. The intensity data were measured by the S.R.C. sponsored microdensitometer service at the Atlas Computing Laboratory and the data supplied by this service comprised Miller indices and intensities for a unique set of 523 reflections, but with each layer in l on a different relative scale.

The Lorentz polarisation correction was applied and approximate interlayer scale factors were determined by comparison of the structure factors of $h0l$ reflections present in the Weissenberg data with structure factors derived from visual estimates of intensities on an $h0l$ precession photograph.

Structure Solution and Refinement.—Normalised structure factors (E) were determined *via* the Wilson plot [8] method making the assumption that reflections not present in the data set were unobserved. Such reflections were assigned a structure factor of half the minimum observed value. Parity groups with l odd and even were normalised separately to ensure that $\langle|E|^2\rangle$ was unity over the complete data set. Direct methods of structure solution were applied by means of the *MULTAN* computer programme [9] using all triple product phase relationships generated from reflections with $|E| \geqslant 1.38$. The origin defining reflections were 10, 7, 5; 12, 6, 1; and 7, 2, 1 and the other reflections in the starting set were 2, 2, $\bar{6}$; 1, 1, $\bar{2}$; and 2, 1, $\bar{4}$. The successful combination of starting phases was 0, 0, 0; 180, 180, and 0° respectively.

An E-map revealed C(1)—C(4) and C(14)—C(16), and the structure was completed *via* successive cycles of structure-factor calculation and Fourier analysis, although the water molecule could not be definitely located at this stage. It was found that the 69 weaker reflections with $|F_o| \leqslant 9$ significantly enhanced the background noise in the Fourier maps and therefore these reflections were temporarily removed from the data set. The structure was refined by full matrix least-squares calculations in which the function minimised was $\Sigma\omega(|F_o| - |F_c|)^2$ where $\omega = 1$ for all data. Atomic scattering factors were taken from ref. 10. The initial R-factor based on a rather distorted model from the Fourier maps was 0.40. The geometry of the molecule was improved by the application of chemical constraints [11] during the first few cycles of refinement and refinement of co-ordinates and interlayer scale factors reduced the R value to 0.33. Inclusion of isotropic thermal parameters reduced R to 0.27 and a difference Fourier map clearly revealed the oxygen atom of the water molecule. This new atom was added to the structure and the 69 weak reflections were reinstated. Further refinement reduced R to 0.083 at which point a difference Fourier map exhibited some very poorly resolved hydrogen atoms. The hydrogen atoms were, therefore, introduced in positions calculated from those of the carbon and nitrogen atoms, but it was not possible to locate the hydrogen atoms bound to oxygen and these were omitted. The final R value after completing the refinement was 0.070 based on a ratio of observations to refined parameters of 7 : 1. The thermal vibration parameters employed did not allow for anisotropic motion because the ratio of observations to refined parameters would have been reduced to only 3.3 : 1 in this case. Although unit weights had been used throughout the refinement and the eventual value of $(\Sigma\omega(|F_o| - |F_c|)^2/N)^{\frac{1}{2}}$, where N is the number of degrees of freedom, was 2 the distribution of $\omega(|F_o| - |F_c|)^2$ was satisfactorily uniform over ranges in $|F_o|$ and $\sin\theta/\lambda$.

Final atomic parameters are given in Table 1, interatomic distances and angles with standard deviations calculated from the full variance-covariance matrix in Table 2 and equations of least-squares planes in Table 3. Observed and calculated structure factors are listed in Supplementary Publication No. SUP 22763 (5 pp.).*

* For details of the Supplementary publications scheme, see Notice to Authors No. 7, *J.C.S. Perkin I*, 1979, Index Issue.

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