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Isolation and Structural Elucidation of Allamandin, an Antileukemic Iridoid Lactone from *Allamanda cathartica*[1]

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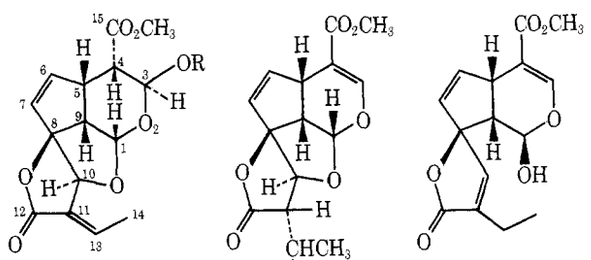
The isolation and structural elucidation of a new antileukemic iridoid lactone, allamandin (1), and the new companion iridoids, allamandicin (3) and allamdin (4), are reported. Elemental analysis and mass spectrometry established a $C_{15}H_{16}O_7$ molecular formula for allamandin (1), and the structure was established by spectral studies and dehydration to the previously known and cooccurring plumericin (5). Assignment of configuration was effected by spin-decoupling studies of acetylallamandin (2). The isomeric iridoid, allamandicin (3), was characterized by dehydration to plumericin (5) and additional chemical and spectral studies. The structure of allamdin (4) was deduced by spectral studies, and proven by direct X-ray crystallographic analysis.

In the course of a continuing search for tumor inhibitors of plant origin, an ethanolic extract of *Allamanda cathartica* Linn. (Apocynaceae)[2] was found to show significant activity *in vivo* against the P-388 leukemia in the mouse and *in vitro* against cells derived from human carcinoma of the nasopharnyx (KB)[3] (Table I). Reported herein are the fractionation of an active extract of *A. cathartica* and the isolation and structural elucidation of a new antileukemic iridoid lactone, allamandin (1), and the new companion iridoids,[4] allamandicin (3) and allamdin (4).

Table I
Activity of Fractions from *A. cathartica* against KB Tissue Culture

Fraction	ED_{50}, μg/ml	Fraction	ED_{50}, μg/ml
A	4.6	H	3.1
B	>100	**1**	2.1
C	>100	**3**	>10
D	0.48	**4**	>10
E	0.8	**5**	2.7
F	0.25	**6**	2.6
G	1.6		

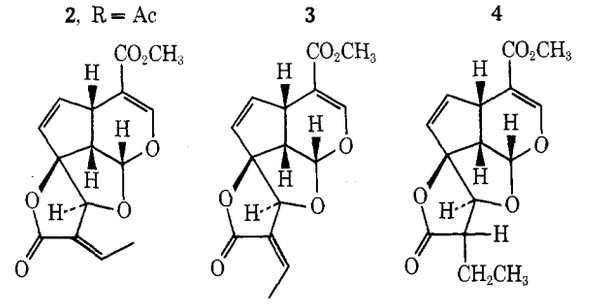
Fractionation of the ethanol extract (A) (Chart I) revealed that the *in vivo* activity was concentrated, successively, in the chloroform layer (D) of a chloroform–water partition, the aqueous methanol layer of a 10% aqueous methanol–petroleum ether partition, the aqueous methanol layer of a 20% aqueous methanol–carbon tetrachloride partition, and the chloroform layer (G) of a chloroform–40% aqueous methanol partition. Rapid column chromatography of the final chloroform-soluble material gave a fraction which was further separated into two major bands by preparative thin layer chromatography. Rechromatography of the higher R_f band gave the known iridoids plumericin (5) and isoplumericin (6), identified by comparison of their physical and spectral characteristics with those recorded previously.[5,6] Rechromatography of the second band yielded allamandin (1), allamandicin (3), and allamdin (4).



1, R = H
2, R = Ac

3

4



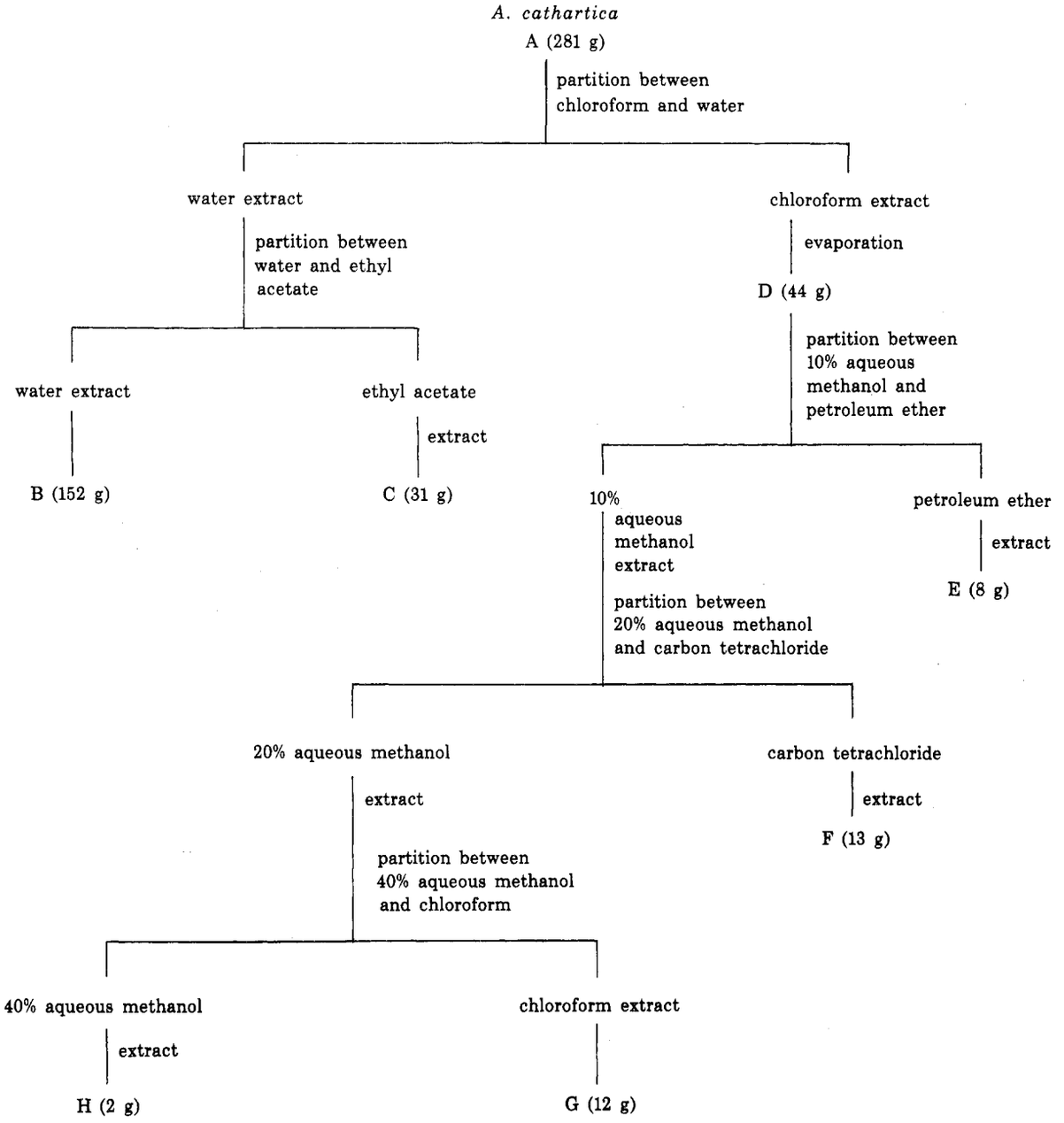
5

6

7, α-H; β-CH_2CH_3
8, β-H; α-CH_2CH_3

Elemental analysis and mass spectrometry established a molecular formula of $C_{15}H_{16}O_7$ for allamandin (1). This corresponded to the addition of the elements of water to either plumericin (5) or isoplumericin (6), and, indeed, the infrared spectrum of 1 showed hydroxyl absorption (2.98 μ). Attempted acetylation of 1 in pyridine resulted in dehydration to plumericin (5). The ir and uv spectra of 1 revealed the presence of the α,β-unsaturated lactone and the absence of an α,β-unsaturated methyl ester system. Acetylation to acetylallamandin (2) was ultimately effected (in the absence of base), and the nmr spectrum (Table II) of 2 proved to be most significant. The spectrum showed no signal at τ 2.64 [assigned to the olefinic C-3 proton in plumericin (5)[6]], but did show a one-proton doublet at τ 3.72 (J = 8 Hz), corresponding to the C-3 proton of 2. Double-reso-

Chart I
Fractionation of Cytotoxic Extract from *A. Cathartica*



nance studies demonstrated that irradiation of the C-3 proton doublet caused collapse of a doublet of doublets centered at τ 7.11 [leaving a doublet (J = 4.5 Hz)], assignable to the C-4 proton. Irradiation of the multiplet at τ 6.43 caused the collapse of the C-4 proton doublet of doublets to a doublet (J = 8 Hz) and the two doublets of doublets due to the C-6 and C-7 olefinic protons to doublets (J = 6 Hz). Assignment of the τ 6.43 multiplet to the C-5 proton was confirmed as follows. Irradiation of the C-1 proton doublet at τ 4.49 (J = 4.5 Hz) led to the assignment of the doublet of doublets at τ 6.93 (J = 8, 4.5 Hz) to the C-9 proton. Then, irradiation of these peaks caused not only the collapse of the doublet at τ 4.49 (C-1 proton), but sharpening of the multiplet at τ 6.43, indicating the coupling of the protons at the ring junction. Careful examination of Dreiding models indicated that the six-membered ring may assume a half-chair conformation with C-1, C-9, C-5, and C-4 held in the same plane and with the protons attached to these carbons on the same side of the ring. This conformation accords with the observation of the same coupling constant between the protons at C-1 and C-9 ($J_{1,9}$ = 4.5 Hz) and the protons at C-4 and C-5 ($J_{4,5}$ = 4.5 Hz). The latter coupling, along with that seen for the C-3 and C-4 protons ($J_{3,4}$ = 8 Hz), accords only with the configuration bearing the carbomethoxy group in the α orientation and the proton at C-3 also α and in an axial conformation.

Allamandicin (**3**) was characterized as an isomer of **1** on the basis of elemental analysis and mass spectral data. The ir spectrum indicated the presence of a hydroxyl group and the ir and uv spectra the presence of an α,β-unsaturated methyl ester and a saturated lactone. The nmr spectrum of allamandicin showed a one-proton singlet at τ 2.65, assignable to the C-3 olefinic proton. The spectrum also contained a three-proton doublet at τ 8.65 (J = 6 Hz), indicative that the C-14 methyl was not vinylic in nature. The splitting for this signal demonstrated the presence of but one proton on the adjacent carbon (C-13), corresponding to a broad multiplet at τ 5.61. Irradiation of the multiplet caused the methyl doublet to collapse to a singlet and the doublet for the C-11 proton at τ 7.32 (J = 1.5 Hz) to be-

Table II
Nuclear Magnetic Resonance Data[a]

Compd	C-1	C-3	C-4	C-5	C-6[b]	C-7[b]	C-9	C-10	C-11	C-13	C-14	OCOMe, other
2	4.49 d (4.5)	3.72 d (8)	7.11 dd (8, 4.5)	6.43 m	4.03 dd (6, 2)	4.14 dd (6, 2)	6.93 dd (8, 4.5)	4.88 d (1.5)		2.78 dq (1.5, 7)	8.00 d (7)	6.30 s Ac 7.99 s
3	4.56 d (6)	2.65 s		6.12 td (2, 8)	4.14 dd (5, 2)	4.32 dd (5, 2)	6.69 dd (8, 6)	5.34 s	7.32 d (1.5)	5.61 m	8.65 d (6)	6.30 s OH 7.5 m
4	5.00 dd (5, 3)	2.62 d (3)		6.20 m	3.63 dd (6, 5)	4.66 dd (6, 2)	7.03 dd (8, 3)	3.29 t (1.5)		7.74 dq (1.5, 8)	8.88 t (8)	6.30 s OH 5.8 br d (5)
5[c]	4.52 d (6)	2.63 s		6.08 td (2, 9)	4.03 dd (6, 2)	4.45 dd (6, 2)	6.66 dd (9, 6)	4.98 br s		2.9 dq (1.5, 7)	7.98 d (7)	6.30 s

[a] Spectra were determined on a Varian HA-100 spectrometer in deuteriochloroform solutions. Values are given in τ units relative to tetramethylsilane as an internal standard. Multiplicity of signals is designated as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad. Numbers in parentheses denote coupling constants in hertz. [b] Assignments for these protons are made on the basis of analogy to those for **4** and published values for similar cases (ref 10). The assignment differs, however, from that made in the case of **5** (ref 4). [c] The spectrum for isoplumericin (**6**) differs only at C-13 [τ 3.27 q (7)] and C-14 [τ 7.75 d (7)].

come a sharp singlet. In addition, the signal for the hydroxyl proton at *ca.* τ 7.50 became sharper. The α orientation of the hydroxyethyl group could be inferred from the nature of the signal assigned the proton at C-10.[6] The singlet observed (τ 5.34) for this proton is consistent with the β orientation of the proton at C-11, by analogy with spectra of α-dihydroplumericin (**7**) and β-dihydroplumericin (**8**). Molecular models indicate that maximum orbital overlap with the C-10 hydrogen occurs when the C-11 proton is in the α configuration, and, accordingly, a doublet for the C-10 proton appears in the spectrum of **7**.[6] A β orientation for the C-11 proton provides little orbital overlap, and, indeed, a singlet for the C-10 proton is observed in the spectrum of **8**.

Allamandicin (**3**) was readily dehydrated to give plumericin (**5**) upon attempts at acetylation and upon treatment with phosphorus oxychloride in pyridine.[7] The latter reaction is known to involve trans elimination,[8] and favors the *S* configuration for the C-13 carbon.

The molecular formula of allamdin (**4**), $C_{15}H_{16}O_6$, was established on the basis of elemental analysis and mass spectrometry. The ir spectrum of **4** indicated the presence of a hydroxyl group and both α,β-unsaturated lactone and α,β-unsaturated methyl ester systems. The uv spectrum (τ 3.30) was not strongly coupled to the C-13 protons (J = 2 Hz). Double-resonance studies demonstrated that the proton at C-1 (τ 5.00) appeared as a broad doublet of doublets (J = 5, 3 Hz) coupled to both the hydroxyl proton and the C-9 proton. The positions of the remaining protons on the six-membered ring were confirmed using this technique. The protons attached to the olefinic 6 and 7 carbons appeared as two doublets of doublets, at τ 3.63 and 4.66. The set of peaks farther downfield was assigned to the C-6 proton, owing to its greater secondary coupling constant (J = 5 Hz). The apparent increased mobility of the six-membered ring in **4**, when compared to the other compounds, presents the opportunity for increased orbital overlap between the C-5 and C-6 protons. Presumably, this effect increases the value of the coupling constant between these protons ($J_{5,6}$) more than the coupling constant between the protons on C-5 and C-7 ($J_{5,7}$). The negative molecular rotation of allamdin ($[M]_D$ −102°) favored the β configuration for the C-1 hydroxyl group.[9]

The configuration at C-1 and the detailed structure and stereochemistry of allamdin (**4**) were proven by direct X-ray crystallographic analysis.[11] A view of the allamdin molecule as found in the crystal is shown in Figure 1. The molecular structure found clearly corresponds to **4**, and the β orientation of the C-1 hydroxyl group is firmly established. Although brief reports have been given of X-ray structure

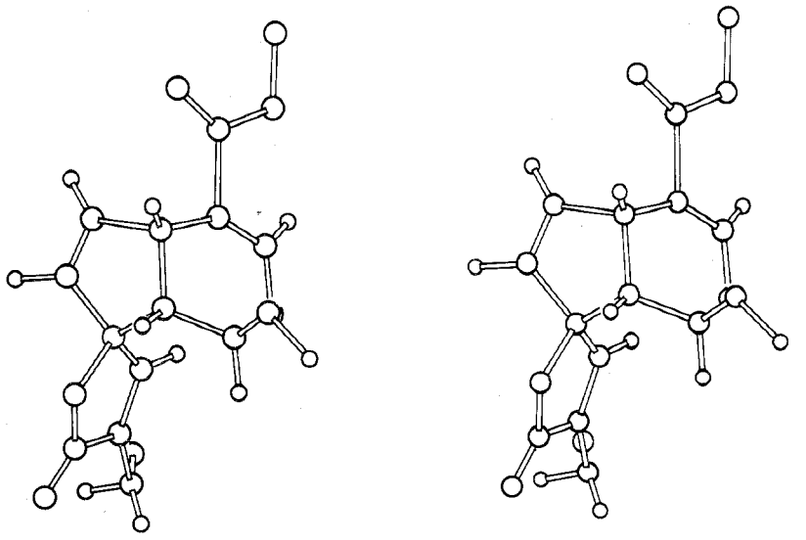


Figure 1. Stereoscopic view of the allamdin molecule as found in the crystal. The hydrogen atoms attached to the two methyl carbon atoms were not located in the analysis and are not shown.

confirmed the presence of the unsaturated chromophores. The nmr spectrum of **4** revealed that carbons 13 and 14 constituted an ethyl side chain. The olefinic proton at C-10

determinations of both a Rb salt of monotropein[12] and a derivative of loganin,[13] in neither case were atomic coordinates provided, so that this report gives the first detailed description of the molecular geometry of an iridoid.

Internal agreement between individual measurements of bond distances for equivalent bond types is good. Thus, the average of the four C_{sp^3}–C_{sp^3} bond distances is 1.54 ± 0.03 Å, of the four C_{sp^3}–C_{sp^2} distances 1.51 ± 0.02 Å, and of the three C_{sp^2}=C_{sp^2} distances 1.32 ± 0.02 Å. All of these average values are close to the standard values for the various bond types.[14] By contrast, of the two C_{sp^2}–C_{sp^2} distances, C-11–C-12 is longer than normal, probably owing to strain in the unsaturated lactone ring and indicating a minimum of conjugation between C-10–C-11 and the carbonyl group of the ring, while C-4–C-15 is of a more normal length. The two C=O bonds are of normal length, 1.18 and 1.19 Å, while the three C_{sp^3}–OR bonds average 1.49 ± 0.02 Å and the three C_{sp^2}–OR bonds 1.35 ± 0.02 Å. The hydroxyl bond at C-1 is somewhat shorter than the standard value of 1.426 (5) Å.

The intraring valence angles in the dihydropyran ring have fairly normal values as compared to cyclohexene, but the substitution of the oxygen atom 2 with its corresponding open valence angle of 116° produces significant changes in angles in the rest of the ring which would otherwise be expected to be equivalent, *e.g.*, 122° at C-4 *vs.* 126° at C-3, and 110° at C-5 *vs.* 115° at C-9. The intraring valence angle at C-1 is close to the regular tetrahedral value and the extraring valence angles involving the hydroxyl group are both less than the regular tetrahedral value. It has commonly been assumed[15] in the calculation of minimum energy conformations of six-membered rings that substitution at tetrahedral carbon atoms will lead to increased values for the corresponding intraring valence angles. While there is ample evidence that this is so for methyl substituents, the arrangement here suggests that attention should also be paid to the nature of the substituent atoms involved.

A similar pattern of valence angles occurs at each of the three sp^2-carbon atoms C-4, C-12, and C-15. In each case the double bond is flanked by two angles >120° while the third angle is significantly <120°. At both C-12 and C-15 the smaller of the two largest valence angles is found adjacent to an ether oxygen atom, the larger adjacent to an sp^2 carbon. The limiting steric interaction in each case seems to be the maintenance of a 1,3 O···O separation of around 2.22 Å.

Within the cyclopentene ring the valence angles at both sp^2 and sp^3 carbons are reduced by about the same amount, *ca.* 7°, from the regular trigonal and tetrahedral values. More severe closures of the intraring C_{sp^2} angles are found in the unsaturated lactone ring at C-11 and C-12 than at C-10, reflecting the enhanced length of C-11–C-12 already mentioned. A pronounced asymmetry of the extraring angles at C-11, 132° *vs.* 122°, occurs and is to be associated with the near coplanarity of the lactone ring with the ethyl group at C-11. This brings H-10 into close proximity with the C-14 methyl group (C-14···H-10, 2.60 Å) and suggests a locking of the methyl group by interposition of H-10 between two of the methyl hydrogens. However, the methyl group hydrogen atoms have not been located in the final electron-density maps.

The valence angles at the spiro atom C-8 show equal intraring values of 103° in both the cyclopentene and lactone rings, but a fair asymmetry in the remaining angles. The angle between the two planes defined by C-7, C-8, C-9, and C-10, C-8, O-20 is 87.5°.

The pattern of torsion angles in the dihydropyran ring shows it to have a 1,2-biplanar (sofa) conformation.[15] Torsion angles about bonds not involving O-2 have values very close to those calculated for the minimum energy conformation of this type in cyclohexene.[15] The 1,2-biplanar arrangement is calculated to be about 1.5 kcal/mol greater in energy than the monoplanar (half-chair) conformation for cyclohexene. The observed reduction in the torsion angles about C-1–O-2 and O-2–C-3 is about 10° in each case from the value expected for the corresponding bonds in this form of cyclohexene. This type of reduction is to be expected on substitution of an oxygen atom for a methylene group, as this involves an opening of the valence angle at this position in the ring from about 112° to 116° and is accompanied by a reduction in the length of the bonds to this position. The overall result is a flattening of the ring in the vicinity of O-2 as compared to the corresponding cyclohexene.

The torsion angles of the cyclopentene ring show it to have C_s symmetry in a plane from C-9 perpendicular to and passing through the midpoint of the double bond C-6=C-7. The mean deviation of the four atoms C-5, C-6, C-7, and C-8 from the mean plane through them is 0.007 Å (maximum 0.009 Å) and C-9 is displaced from this plane by −0.37 Å.

In the α,β-unsaturated lactone ring the low values of the torsion angles indicate a near-planar arrangement of the atoms and this impression is initially confirmed upon calculation of a least-squares mean plane through the five atoms of the ring and O-19. The mean deviation from this plane is 0.14 Å, and the maximum −0.23 Å. However, a closer inspection reveals that the four carbon atoms of the ring are coplanar to within 0.001 Å whereas O-20 is displaced from that plane by fully 0.07 Å. The exact conformation of the ring is thus shown to deviate significantly from coplanarity. The displacements from the four-atom plane in the ring for O-19, C-13, and C-14 are 0.03, 0.04, and −0.07 Å, respectively.

The stereochemistry at the dihydropyran–cyclopentene ring junction is cis. The combination of this cis stereochemistry and the unusual spiro linkage of the cyclopentene and lactone rings is clearly linked to the β orientation of the C-1 hydroxyl group and the conformation of the dihydropyran ring. In the dihydropyran C-1 is displaced out of the rough plane through the other atoms of the ring by about 0.5 Å in a direction nearly perpendicular to the plane of the lactone ring. C-1 approaches C-10 in a 1,4 interaction within 2.98 Å, while H-1 is only 2.92 Å from C-10 and 3.36 Å from C-11. A hydroxyl group α at C-1 in this particular conformation of the dihydropyran ring would be involved in sterically unfavorable interactions with C-10 and C-11 while not being able to compensate for these stresses by intramolecular hydrogen bonding with either O-19 or O-20, since the hydroxyl hydrogen atom would be behind both oxygens in a position sterically unfitted for hydrogen bond formation. Other close intramolecular approaches involve O-2 with O-2···H-10 2.67 Å and O-2···C-10 only 2.96 Å in a 1,5 orientation.

The C-1 hydroxyl group takes part in intermolecular hydrogen bond formation with O-19, the carbonyl oxygen of the lactone ring, in a neighbor. This leads to linearly connected strings of molecules in the crystal.

Experimental Section[16]

Extraction and Preliminary Fractionation of *Allamanda cathartica*. The dried ground roots of *A. cathartica* (1.61 kg) were continuously extracted with hot 95% ethanol for 45 hr and the ethanol extract was concentrated under reduced pressure to a dark brown residue (A, 281 g). Fraction A was partitioned between water (1.5 l.) and chloroform (2.0 l.), and the aqueous layer was further extracted with ethyl acetate (2 l.). The water and ethyl acetate portions were evaporated to give fractions B (152 g) and C (31

g), respectively. The chloroform extract was evaporated to give D (44 g), which was then partitioned between 10% aqueous methanol (1 l.) and petroleum ether (1 l.). Evaporation of the petroleum ether gave E (8 g). The aqueous methanol fraction was diluted with water, and the resulting 20% aqueous methanol solution (1.225 l.) was extracted with carbon tetrachloride (1.5 l.). The carbon tetrachloride was evaporated to give F (13 g). The 20% aqueous methanol portion was diluted with water to give 40% aqueous methanol (1.5 l.), which was extracted with chloroform (2 l.). The chloroform and aqueous methanol solutions were evaporated to give fractions G (12 g) and H (2 g), respectively.

Isolation Procedure. A larger fraction (83 g) corresponding to G was obtained from another extract of plant material (5.7 kg). This fraction was subjected to column chromatography (silica gel 60) and eluted with chloroform followed by 2% methanol in chloroform. The column fractions were combined according to analytical tlc results and yielded 40 g of a fraction containing the desired materials. Preparative tlc of a 4-g portion (Silplates, 20 × 20 × 2 mm) with 5% methanol in chloroform resulted in two major fractions (I and J). The higher R_f fraction (I, 1.16 g) deposited crude crystals on standing.

This crude crystalline material was subjected to preparative tlc (Silplates, 20 × 20 × 0.25 mm) with chloroform as a solvent. The material from the higher R_f band was recrystallized from methylene chloride–hexane to give isoplumericin (**6**) as colorless plates (350 mg, mp 195–198°), identified by melting point, nmr, ir, and mass spectral comparison with published data.[5,6] The material from the lower R_f band from the preparative tlc was treated in the same way to give plumericin (**5**), as colorless plates (460 mg, mp 209–212°), identified as for **6** above.

The second major band from the preparative tlc with the 2-mm thick plates (J, 2.00 g) was subjected to dry column chromatography.[17] The column was charged with 130 g of SilicAR CC-7 packed in 5-cm flat diameter nylon tubing, and eluted with 3% methanol in ether. The bulk of the material from the column was eluted from a uv-fluorescent band (R_f *ca.* 0.5). On standing, this fraction deposited a crystalline material. Trituration with chloroform gave allamandin (**1**, 37 mg). Recrystallization from methanol–ethyl acetate gave thin plates: mp 212–215°; $[\alpha]^{21}D$ +15° (*c* 0.06, methanol); uv λ_{max} (MeOH) high end absorption; ir λ_{max} (KBr) 2.98, 3.38, 5.79, 5.99, 6.94, 8.35, 8.52, 9.90 μ; mass spectrum *m/e* 308 (M^+), 290, 277, 262, 258, 230, 211, 179, 161, and 151.

Anal. Calcd for $C_{15}H_{16}O_7$: C, 58.44; H, 5.23. Found: C, 58.17; H, 5.11.

The dry column fraction (546 mg) from the zone between the band which gave **1**, and a dark blue fluorescent band (uv) at the solvent front, was subjected to preparative tlc on ChromAR to yield two major fractions (K, 248 mg, low R_f; L, 138 mg, high R_f).

Fraction K was rechromatographed twice (Silplates, 20 × 20 × 0.5 mm, 1% methanol in chloroform followed by ChromAR plates, ether–hexane, 1:1) to yield a residue which was crystallized (ether–hexane) as colorless plates. Recrystallization gave allamandicin (**3**, 27 mg): mp 117–118°; $[\alpha]^{21}D$ +293° (*c* 0.42, chloroform); uv λ_{max} (EtOH) 238 nm (ϵ 11,500); ir λ_{max} (KBr) 2.87, 3.24, 3.38, 5.64, 5.91, 6.08, 6.96, 8.45, 9.22 μ; mass spectrum *m/e* 308 (M^+) 290, 279, 261, 246, 233, 230, 218, 214, 198, 197, 188, 186, 170, and 150.

Anal. Calcd for $C_{15}H_{16}O_7$: C, 58.44; H, 5.23. Found: C, 58.76; H, 5.38.

Fraction L was subjected to the same sequence and gave a residue which was crystallized (ether–hexane) to give prisms of allamdin (**4**, 35 mg): mp 131–132° dec; $[\alpha]^{21}D$ −35° (*c* 0.46, chloroform); uv λ_{max} (EtOH) 238 nm (ϵ 14,000, sh) and high end absorption; ir λ_{max} (KBr) 2.92, 3.22, 3.24, 3.36, 5.77, 5.90, 6.11, 6.98, 7.77, 9.00, 9.37 μ; mass spectrum *m/e* 292 (M^+), 274, 263, 232, 231, 214, 203, 186, 175, and 162.

Anal. Calcd for $C_{15}H_{16}O_6$: C, 61.64; H, 5.52. Found: C, 61.89; H, 5.56.

Dehydration of Allamandin (**1**). A solution of allamandin (**1**, 16.2 mg) in dry pyridine (2 ml) was treated with acetic anhydride (1 ml). The mixture was stirred at room temperature for 19 hr. The solvent and excess reagent were removed under vacuum and the residue was applied to three Silplates (20 × 20 × 0.25 mm), which were developed in 1% methanol in chloroform. The major band yielded a solid (10 mg) which was recrystallized (methylene chloride–hexane) to give colorless plates of **5**, mp 207–211°, characterized by melting point, mixture melting point, and comparison of nmr and mass spectra with those of authentic material.

Acetylallamandin (**2**). A suspension of allamandin (**1**, 17 mg) in acetic anhydride (7.5 ml) was stirred at 70° for 18.5 hr. The solvent was removed under vacuum and the residue was applied to three ChromAR plates (20 × 20 × 0.25 mm). Development with ether–hexane (2:1) was effected twice, and the material from the major band was eluted and crystallized from ether to yield acetylallamandin (**2**, 11 mg). Recrystallization from methylene chloride–hexane gave needles: mp 173–177°; $[\alpha]^{21}D$ +61° (*c* 0.36, chloroform); uv λ_{max} (EtOH) high end absorption; ir λ_{max} (KBr) 3.38, 5.62, 5.74, 5.78, 5.95, 6.98, 8.18, 9.30, 9.97, 10.6 μ; mass spectrum *m/e* 350 (M^+), 308, 291, 277, 253, 211, 193, 179, 161, 140, and 98.

Dehydration of Allamandicin (**3**). A solution of allamandicin (**3**, 13 mg) in dry pyridine was cooled to 2° and treated with phosphorus oxychloride (5 drops). After 4 min, the mixture was allowed to warm to room temperature and the solvent was removed under vacuum. A chloroform solution of the residue was filtered and applied to two Silplates (20 × 20 × 0.25 mm). The plates were developed three times with chloroform and the material eluted from the two high R_f bands was obtained as crude crystals. Recrystallization (methylene chloride–hexane) gave, as the major product, plumericin (**5**, 6.5 mg), mp 208–211°, characterized by melting point, mixture melting point, tlc, and comparison of ir ($CHCl_3$) and mass spectra with those of authentic material. The minor product (<1 mg) was identified as isoplumericin (**6**) by comparison of its mass spectrum and tlc behavior with those of an authentic sample.

X-Ray Crystallographic Structural Data for Allamdin ($C_{15}H_{16}O_6$): orthorhombic, space group $P2_12_12_1$, a = 13.211 (2), b = 13.736 (2), c = 7.858 (1) Å, ρ_{obsd} = 1.34, Z = 4, ρ_{calcd} = 1.36, μ (Cu Kα) = 9 cm^{-1}.

The space group was determined from precession photographs from the systematic absences in the axial reflections with h, k, or l odd. Unit cell dimensions were obtained by a least-squares fit to the values of $\pm 2\theta$ for 18 general reflections measured at room temperature on the diffractometer (λ = 1.5418 Å). For the measurements of intensity a crystal block (1.0 × 0.5 × 0.2 mm^3) was mounted with the c^* axis parallel to the ϕ axis of a Picker four-circle diffractometer operated under the control of an XDS Sigma 2 computer. Cu Kα radiation was used, made monochromatic by Bragg reflection from the (002) planes of a highly oriented graphite crystal. The reciprocal lattice was surveyed at some 1150 points out to sin θ/λ = 0.545 and diffracted intensity significantly above background [$I > 3.0\sigma(I)$] was measured at 1097 of them. The $\theta/2\theta$ scan method was used with a scan range of 3.5° and a scan rate of 2°/min. Background intensity was calculated for each reflection from a predetermined survey of the variation of background as a function of 2θ over the operating range of the diffractometer with the crystal in place.

Structure Determination and Refinement. The structure was solved by use of the program MULTAN of Germain, Main, and Woolfson.[18]

The structural parameters were refined by block-diagonal least-squares methods to R = 0.14 with individual isotropic thermal parameters assumed, and to R = 0.10 on the assumption of anisotropic thermal parameters. Of the 16 hydrogen atoms in the molecule all except those associated with the two methyl groups were clearly identifiable from a difference electron-density function calculated at this stage. They were included in the refinement with isotropic thermal parameters and the refinement continued to give R = 0.081 at convergence.

No convincing distinction between the two possible enantiomeric structures emerged when contributions for the anomalous dispersion corrections for the oxygen atoms were included in separate structure factor calculations, nor could conclusive differences in Bijvoet pairs of reflections be detected, so that the absolute configuration of allamdin was not determined by this analysis.

With the exception of MULTAN all programs used in the analysis were written in this laboratory for the Sigma 2 computer. Scattering functions for the atoms were taken from the compilation of Hanson, *et al.*[19] No account was taken of effects due to absorption. The comparatively high residual is most probably attributable to this neglect, but is likely to have little effect on the final positional parameters. A final difference electron-density map showed only random distribution of residual peaks of height not exceeding ±0.2 $e/Å^3$. Weighting functions for the least-squares refinement were based on the treatment suggested by Killean, *et al.*[20]

Registry No.—**1**, 51820-82-7; **2**, 51820-83-8; **3**, 51838-83-6; **4**, 51820-84-9.

Supplementary Material Available. Positional parameters defining the crystal structure, the thermal parameters of the atoms, and diagrams showing the bond lengths, bond angles, torsion angles, and other features of the molecular geometry of allam-

din will appear following these pages in the microfilm edition of this volume of the journal. Photocopies of the supplementary material from this paper only or microfiche (105 × 148 mm, 24× reduction, negatives) containing all of the supplementary material for the papers in this issue may be obtained from the Journals Department, American Chemical Society, 1155 16th St., N.W., Washington, D. C. 20036. Remit check or money order for $3.00 for photocopy or $2.00 for microfiche, referring to code number JOC-74-2477.

References and Notes

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(2) Roots were collected in Oahu, Hawaii, in March 1972. The authors acknowledge with thanks receipt of the dried material from Dr. R. T. Hirano, Harold L. Lyon Arboretum, University of Hawaii, under a program supported by the National Cancer Institute.

(3) Cytotoxicity (Table I) and *in vivo* activity were assayed under the auspices of the National Cancer Institute. The procedures were those described in *Cancer Chemother. Rep.*, **25**, 1 (1962).

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Nucleosides. LXXXVII. Total Synthesis of Pentopyranine A, an α-L Cytosine Nucleoside Elaborated by *Streptomyces griseochromogenes*[1]

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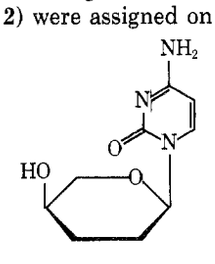
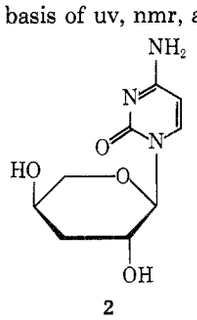
The nucleoside 1-(2,3-dideoxy-α-L-*glycero*-pentopyranosyl)cytosine (1) was synthesized by a series of reactions from tri-*O*-acetyl-L-arabinopyranosyl bromide. The identity of 1 with the naturally occurring pentopyranine A was established by ir, uv, and mass spectral comparisons. The synthetic sequence and physicochemical data for 1 reported herein provide confirming evidence for the structure previously assigned to pentopyranine A.

Two cytosine nucleosides, pentopyranine A and C, have been isolated by Seto, *et al.*,[2] from the fermentation broth of *Streptomyces griseochromogenes*, a blasticidin S producing microorganism.[3] The structures of these nucleosides (1 and 2) were assigned on the basis of uv, nmr, and mass



1
pentopyranine A



2
pentopyranine C

spectral evidence of these and their acetyl derivatives.[2] Pentopyranine A and C are the first naturally occurring nucleosides possessing the α-L configuration. Recently,[4] we reported the total synthesis of pentopyranine C, 1-(3-deoxy-α-L-*threo*-pentopy ranosyl)cytosine (**2**), from 3-deoxy-1,2:5,6-di-*O*-isopropylidene-α-D-*xylo*-hexofuranose. In this paper we describe the total synthesis of 1-(2,3-dideoxy-α-L-*glycero*- pentopyranosyl)cytosine (**1**) from L-arabinose and its identity with pentopyranine A.

Condensation of tri-*O*-acetyl-L-arabinosyl bromide with N^4-anisoylcytosine in nitromethane in the presence of mercuric cyanide[5] gave the protected nucleoside **3** in crystalline form. Treatment of **3** with sodium methoxide in methanol selectively removed the acetyl groups to afford nucleoside **4** in ~70% yield. Isopropylidenation of **4** gave pure **8** which precipitated from the reaction mixture in high yield. After mesylation of **8**, the product **9** was isolated and treated with aqueous acetic acid at room temperature to remove the isopropylidene group.[6] It was found, however, that under these conditions hydrolytic deamidation of **9** occurred to a considerable extent. Therefore, the reaction mixture was refluxed in 80% acetic acid to complete the deamidation reaction[7] from which uracil nucleoside derivative **10** was obtained in good yield.

Treatment of **10** with sodium methoxide in methanol gave the epoxide **11**. After acetylation of **11**, the product **12** was treated with sodium iodide in a mixture of acetic acid