Bouvardin and Deoxybouvardin, Antitumor Cyclic Hexapeptides from *Bouvardia ternifolia* (Rubiaceae)

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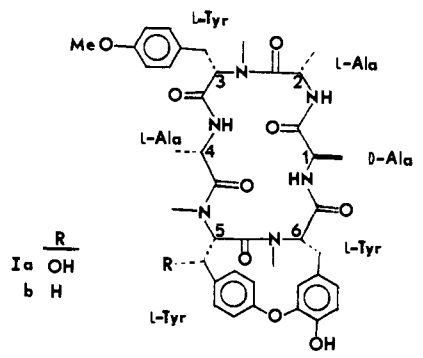
Abstract: Bouvardin ($C_{40}H_{48}N_6O_{10}$) and deoxybouvardin ($C_{40}H_{48}N_6O_9$), both of which show high antitumor activity in the 3PS, B1, and KB systems, were isolated from the methanol extract of *Bouvardia ternifolia* (Rubiaceae). An x-ray study on bouvardin containing a methanol of crystallization ($P2_12_12_1$, a = 9.009 (3), b = 12.623 (4), c = 42.970 (19) Å, Z = 4) and hydrolytic studies showed it to be Ia, a cyclic hexapeptide composed of two L-alanines, a D-alanine, and three modified *N*-methyl-L-tyrosines. The most unusual feature is a 14-membered ring formed by oxidative coupling of the phenolic oxygen of one tyrosine with a carbon ortho to the phenolic hydroxyl group of an adjacent tyrosine. Spectral comparison with bouvardin indicated deoxybouvardin to be Ib, identical with bouvardin except for the lack of a β-hydroxyl group on one of the tyrosines.

Bouvardia ternifolia (Cav.) Schlecht. (Rubiaceae)[2] was used by ancient Mexican Indians as a general curative and is used in contemporary Mexico as a remedy for dysentery, hydrophobia, and other afflictions. The plant is locally called "trompetilla", "tlacoxochitl", "mirto", and many other names.[3] As a result of the continuing search for plants possessing tumor-inhibiting constituents, we have found that the methanol extract of the stems, leaves, and flowers of *Bouvardia ternifolia* demonstrated inhibitory activity toward the P388 lymphocytic leukemia (PS) and B16 melanotic melanoma (B1) test systems.[4] It also showed cytotoxicity in the adenocarcinoma of the nasopharynx (KB) test system.[4] We wish to describe the isolation and characterization of two closely related



substances, bouvardin (Ia) and deoxybouvardin (Ib), responsible for the antitumor activities.

Experimental Section

Preparative chromatographic plates (200 × 200 mm) were coated with a 1.5-mm layer of silica gel PF254 (E. Merck) and activated at 110 °C overnight before use. Unactivated plates were used for the separation of amino acids. Melting points were determined on a Kofler hot-stage apparatus and are uncorrected, and optical rotations were obtained on a Rudolph Model 70 polarimeter. ^{1}H NMR spectra were measured at 60 and 100 MHz on Varian T-60 and XL-100 spectrometers, respectively,[5] and at 360 MHz on a Bruker HFX-10 spectrometer;[6] ^{13}C NMR spectra were measured at 22.6 MHz on a Bruker WH-90 spectrometer.

Isolation Procedure. The dry stems, leaves, and flowers of *Bouvardia ternifolia* were ground in a Wiley mill and stored at −10 °C prior to extraction. In a typical experiment the ground material (12 kg) was extracted with methanol (2 × 24 h × 60 L) via mechanical stirring. The combined, filtered methanol extract was concentrated in air to about 2 L, diluted with an equal volume of water-methanol (95:5), and filtered. The filtrate was evaporated to dryness in air and thoroughly extracted with acetonitrile (3 × 2 L). The acetonitrile extract was evaporated in air to a semisolid state, dissolved in methanol, and taken to dryness under vacuum. The resulting residue (90 g) was extracted with stirring with dichloromethane (4 × 2 h × 1.5 L), filtered, and evaporated in vacuo. The residue (19.1 g) was dissolved in a minimum of methanol and isopropyl ether was added until precipitation ceased. This mixture was allowed to stand overnight in the refrigerator (3 °C) and then filtered by decantation. The precipitate was stirred with isopropyl ether (0.5 h), filtered, washed with a small amount of isopropyl ether, and vacuum dried. This provided a dry, green-brown precipitate (6.3 g), which was chromatographed over silica gel 60 (180 g). The column was eluted with hexane–dichloromethane–methanol (25:22:3, v/v/v) and fractions were combined which were similar by TLC. The KB-active fraction (1.0 g) was then subjected to two consecutive preparative thick-layer chromatographies, developing with hexane–dichloromethane–methanol (20:27:3, v/v/v; 3 developments) and then with dichloromethane–methanol (94:6, v/v; 2 developments) for the second preparative TLC. The KB-active fraction from the first preparative TLC was used for the second one. These preparative thick-layer chromatographies, after decolorization, resulted in a colorless, amorphous mixture containing largely bouvardin (Ia) and deoxybouvardin (Ib). Separation was achieved by preparative TLC, the developing solvent being ether–ethyl acetate–methanol (15:35:2, v/v/v; 2 developments).

The lower R_f material was deoxybouvardin (Ib, 52.5 mg), obtained as a colorless powder: mp 237–240 °C; $[\alpha]^{25}_D$ −138° (c 0.7, $CHCl_3$); mass spectrum 756 (parent).[5,7] Attempts to obtain good crystals failed. Bouvardin (Ia, 121.3 mg) was successfully crystallized from methanol–dichloromethane to give colorless needles: mp 254–255 °C; $[\alpha]^{25}_D$ −181° (c 1.0, $CHCl_3$); mass spectrum 772 (parent).[5,7] Unsatisfactory elemental analyses were obtained for Ia and Ib, probably due to the occlusion of varying amounts of solvents (see below). An impurity showing a methyl doublet (in the ^{1}H NMR spectrum) similar to those in the alanine units was removed from bouvardin (Ia) by recrystallization from methanol.

X-Ray Study on Bouvardin (Ia). Colorless crystals of bouvardin (Ia, $C_{40}H_{48}N_6O_{10}\cdot CH_3OH\cdot 0.6H_2O$) were grown by evaporation from methanol. As the crystals were stable only in contact with solvent (without which the c axis rapidly shrank by almost 6 Å and the crystals cracked), they were mounted in capillaries containing mother liquor. The crystal data are presented in Table I. The cell parameters were obtained by least-squares fitting of the settings for the 4 angles of 8 reflections on a Picker FACS-I diffractometer. Intensity data were collected using a scintillation counter with pulse height analyzer, θ–2θ scan technique, 2°/min scan rate, 10 s background counts, attenuators when the count rate exceeded 10^4 counts/s, and 2° scan range with a dispersion factor allowing for α_1–α_2 splitting at large 2θ values. Because of decomposition, two crystals were used for data collection, with about 10% decomposition in each case. Of 4285 total reflections (1568 on crystal 1 and 2717 on crystal 2), 3486 > $2\sigma(I)$ were considered observed. The intensity data from the two crystals were corrected for decomposition and brought to the same scale using common reflections. Three standard reflections were monitored every 50 measurements to check crystal alignment and stability. Lorentz and

Table I. Crystal Data for Bouvardin (Ia)

Formula = $C_{40}H_{48}N_6O_{10}$·MeOH·0.6H_2O
a = 9.009 (3) Å
b = 12.623 (4) Å
c = 42.970 (19) Å
V = 4886.6 Å^3
Space group = $P2_12_12_1$
Z = 4
ρ_{calcd} = 1.108 g/cm^3
Temp = 22 °C
Radiation = Cu Kα (λ = 1.54178 Å)
$(\sin\theta/\lambda)_{max}$, Å^{-1} = 0.60

polarization corrections were applied to the data but no correction was made for absorption.

Phases for 300 reflections with normalized structure factor $E > 1.6$ were generated using the direct method program MULTAN.[8] The resulting E map revealed 45 nonhydrogen atoms, and a difference Fourier showed the remaining 11 nonhydrogens of the molecule. Two cycles of least-squares refinement using isotropic temperature factors dropped R from 0.43 to 0.29. A difference map revealed a methanol molecule as well as three partial water oxygens. Further isotropic least-squares refinement in which the occupancy factors for the water oxygens were also varied brought R to 0.15. The final occupancy factors for the water oxygens were 0.31 (O(W2)), 0.21 (O(1)(W1)), and 0.09 (O(2)(W1)). Two more cycles of full matrix least-squares refinement using anisotropic thermal parameters reduced R to 0.106. Attempts to locate hydrogen atoms in the difference map were only partially successful and the refinement, based on F_o, the quantity minimized being $\Sigma w(|F_o| - |F_c|)^2$, was terminated at this point. The weighting scheme used was based on counter statistics as defined by Corfield et al.,[9] the value of p being 0.04. The scattering factors used were those of Hanson et al.[10] No correction was applied for primary extinction, but a few reflections which were affected by secondary extinction were not included in the refinement.

Hydrolysis of Bouvardin (Ia). Bouvardin (Ia, 200.2 mg) was hydrolyzed with 6 N hydrochloric acid (5 mL) according to Brenner[11] for 72 h. After workup the solid mixture of products was taken up in methanol–water (1:1) and subjected to preparative TLC. The solvent system butanol–water–acetic acid (4:1:1, v/v/v) separated alanine from the other products. Recrystallization from methanol–water gave alanine (65 mg, 94%) as colorless needles: mp 262–267 °C dec (sublimes above 210 °C); $[\alpha^{25}D$ +4.8° (c 1.4, 1 N HCl). This rotation indicates a 2:1 ratio of L- to D-alanine (the reported[12] specific rotation for L-alanine is +14.4°). The ^{1}H NMR spectrum was identical with that reported,[13] and an authentic mixture (2:1) of L- and D-alanine had mp 264–267 °C dec (sublimes above 210 °C); $[\alpha]^{25}D$ +4.8° (c 2.0, 1 N HCl). A mixture melting point was undepressed and both samples of alanine displayed identical behavior on TLC plates (sprayed with ninhydrin).

Another preparative TLC using 17% ammonium hydroxide–chloroform–methanol (10:65:25, v/v/v, lower phase; 4 developments) gave N-methyl-4-methoxyphenylalanine (37 mg; 69%) as a colorless powder which was recrystallized from methanol–water to colorless needles: mp 242–245 °C dec (lit.[14] mp 247–248 °C dec). The NMR spectrum and the TLC behavior[15] of this material were identical with those of a specimen synthesized as described below, and a mixture melting point was undepressed.

***N*-Methyl-4-methoxyphenylalanine.** To a mixture of *N*-*tert*-butyloxycarbonyl-L-tyrosine (Vega-Fox Biochemicals, 2.8 g, 10 mmol), methyl iodide (5 mL, 30 mmol), and tetrahydrofuran (30 mL, freshly distilled from $LiAlH_4$) at 0 °C was added with stirring NaH (2.5 g of 55% dispersion in mineral oil, 55 mmol, Metal Hydrides, Inc.). After stirring for 24 h at 25 °C, 50 mL of ethyl acetate was added, then H_2O dropwise to destroy excess NaH. The resulting solution was evaporated and the residue partitioned between 30 mL of ether and 100 mL of H_2O. The ether layer was extracted with 2 × 25 mL of 5% $NaHCO_3$, the combined aqueous extracts were acidified to pH 3 with citric acid, and the product was extracted into ethyl acetate (2 × 50 mL). After washing with H_2O (2 × 50 mL), 5% aqueous $Na_2S_2O_3$ (2 × 50 mL), and H_2O (50 mL), and drying ($MgSO_4$), the solvent was distilled off. Removal of the *tert*-butyloxycarbonyl group by 15-min contact with 5 mL of trifluoroacetic acid and evaporation left N-methyl-4-methoxyphenylalanine (1.2 g, 57%) as a yellowish powder, mp 237–239 °C dec, whose NMR spectrum and TLC behavior showed no impurities.

Table II. Fractional Coordinates × 10^4

Atom	x/a	y/b	z/c
O(1)	6272 (7)	3227 (5)	3365 (1)
C(1)	7051 (8)	4007 (6)	3468 (2)
N(1)	6943 (6)	4797 (5)	2949 (1)
C(1α)	7702 (7)	4790 (6)	3252 (2)
C(1β)	9409 (9)	4530 (10)	3198 (2)
O(2)	5119 (6)	4579 (5)	4217 (2)
C(2)	5258 (8)	3724 (8)	4093 (2)
N(2)	7375 (7)	4092 (6)	3759 (2)
C(2α)	6842 (8)	3302 (9)	3992 (2)
C(2β)	7861 (11)	3319 (14)	4287 (3)
O(3)	1638 (7)	5284 (6)	4091 (2)
C(3)	2376 (9)	4613 (6)	3950 (2)
N(3)	4101 (7)	3082 (5)	4023 (2)
C(N(3))	4196 (11)	2011 (7)	3886 (2)
C(3α)	2639 (9)	3514 (7)	4105 (2)
C(3β)	2325 (11)	3557 (9)	4461 (2)
C(3γ)	2699 (12)	2535 (9)	4610 (2)
C(3δ1)	1662 (18)	1720 (13)	4610 (4)
C(3δ2)	4056 (15)	2346 (13)	4747 (3)
C(3ϵ1)	1951 (30)	761 (15)	4752 (6)
C(3ϵ2)	4371 (19)	1308 (15)	4878 (3)
C(3ζ)	3274 (31)	570 (15)	4890 (4)
O(3η)	3390 (21)	−487 (10)	5003 (4)
C(3θ)	4652 (39)	−720 (18)	5136 (6)
O(4)	3376 (7)	4648 (4)	3072 (1)
C(4)	2973 (8)	5511 (7)	3166 (2)
N(4)	2932 (7)	4702 (5)	3669 (2)
C(4α)	2917 (8)	5758 (6)	3514 (2)
C(4β)	4316 (11)	6395 (7)	3614 (2)
O(5)	3258 (6)	7778 (4)	2417 (1)
C(5)	3759 (8)	6840 (6)	2472 (2)
N(5)	2601 (6)	6321 (5)	2965 (1)
C(N(5))	2003 (13)	7355 (7)	3071 (2)
C(5α)	2680 (7)	6088 (6)	2636 (2)
C(5β)	1124 (8)	6060 (7)	2470 (2)
O(5γ)	454 (6)	7108 (5)	2451 (2)
C(5γ)	1322 (7)	5563 (6)	2169 (2)
C(5δ1)	1390 (8)	4428 (7)	2156 (2)
C(5δ2)	1687 (9)	6116 (7)	1901 (2)
C(5ϵ1)	2003 (10)	3940 (7)	1888 (2)
C(5ϵ2)	2252 (9)	5639 (6)	1642 (2)
C(5ζ)	2516 (8)	4584 (8)	1655 (2)
O(5η)	3536 (6)	4143 (5)	1439 (2)
O(6)	6757 (9)	6581 (5)	2926 (2)
C(6)	6553 (7)	5687 (6)	2812 (2)
N(6)	5130 (6)	6573 (4)	2394 (1)
C(N(6))	6071 (9)	7437 (6)	2253 (2)
C(6α)	5873 (7)	5578 (5)	2486 (2)
C(6β)	7129 (7)	5253 (6)	2259 (2)
C(6γ)	6737 (8)	5005 (6)	1930 (2)
C(6δ1)	7834 (8)	4993 (7)	1697 (2)
C(6δ2)	5308 (8)	4699 (7)	1838 (2)
C(6ϵ1)	7523 (11)	4690 (8)	1401 (2)
C(6ϵ2)	5004 (10)	4435 (7)	1544 (2)
C(6ζ)	6086 (10)	4378 (7)	1312 (2)
O(6η)	5710 (8)	4084 (6)	1019 (1)
O(MeOH)	1056 (9)	1047 (7)	1091 (2)
C(MeOH)	1252 (29)	2189 (16)	1169 (6)
O(1)(W1)	5920 (57)	1457 (39)	467 (11)
O(2)(W1)	5001 (65)	1706 (44)	400 (13)
O(W2)	7737 (38)	2844 (26)	725 (8)

Results and Discussion

Isolation involving a series of extractions and chromatographic separations gave bouvardin (Ia, 1.0 × 10^{-3} % of the dried plant) and deoxybouvardin (Ib, 4.4 × 10^{-4} %). Preliminary spectral analyses indicated some structural features, and an x-ray study on a crystal of bouvardin (Ia) mounted in a

Table III. Bond Distances, with Estimated Standard Deviations in Parentheses

Bond	Distance, Å	Mean value	Bond	Distance, Å	Mean value
C(1)-O(1)	1.287 (10)		C(3β)-C(3γ)	1.478 (16)	
C(2)-O(2)	1.211 (12)		C(5β)-C(5γ)	1.446 (12)	1.471
C(3)-O(3)	1.234 (10)	1.247	C(6β)-C(6γ)	1.489 (11)	
C(4)-O(4)	1.217 (10)		C(3γ)-C(3δ1)	1.389 (20)	
C(5)-O(5)	1.289 (9)		C(3γ)-C(3δ2)	1.379 (17)	
C(6)-O(6)	1.244 (10)		C(3δ1)-C(3ε1)	1.381 (26)	1.386
C(1)-C(1α)	1.477 (11)		C(3δ2)-C(3ε2)	1.454 (24)	
C(2)-C(2α)	1.584 (11)		C(3ε1)-C(3ζ)	1.351 (37)	
C(3)-C(3α)	1.558 (12)		C(3ε2)-C(3ζ)	1.359 (30)	
C(4)-C(4α)	1.531 (11)	1.535	C(5γ)-C(5δ1)	1.436 (12)	
C(5)-C(5α)	1.529 (10)		C(5γ)-C5δ2)	1.385 (12)	
C(6)-C(6α)	1.532 (10)		C(5δ1)-C(5ε1)	1.416 (14)	1.388
N(1)-C(1α)	1.469 (9)		C(5δ2)-C(5ε2)	1.366 (12)	
N(2)-C(2α)	1.493 (12)		C(5ε1)-C(5ζ)	1.371 (14)	
N(3)-C(3α)	1.468 (10)		C(5ε2)-C(5ζ)	1.354 (13)	
N(4)-C(4α)	1.489 (10)	1.474	C(6γ-C(6δ1)	1.408 (11)	
N(5)-C(5α)	1.449 (9)		C(6γ)-C(6δ2)	1.400 (10)	
N(6)-C(6α)	1.477 (8)		C(6δ1-C(6ε1)	1.356 (12)	1.384
C(1)-N(2)	1.288 (10)		C(6δ2)-C(6ε2)	1.338 (13)	
C(2)-N(3)	1.353 (10)		C(6ε1)-C(6ζ)	1.407 (13)	
C(3)-N(4)	1.313 (10)	1.329	C(6ε2)-C(6ζ)	1.396 (13)	
C(4)-N(5)	1.378 (10)		C(3ζ)-O(3η)	1.424 (23)	
C(5)-N(6)	1.323 (9)		C(5ζ)-O(5η)	1.420 (10)	
C(6)-N(1)	1.318 (10)		C(6ζ)-O(6η)	1.356 (11)	
C(1α)-C(1β)	1.590 (11)		C(6ε)-O(5η)	1.446 (11)	
C(2α)-C(2β)	1.567 (13)		O(3η)-C(3θ)	1.305 (37)	
C(3α)-C(3β)	1.558 (13)	1.567	C(5β)-O(5γ)	1.456 (11)	
C(4α)-C(4β)	1.555 (12)		O(MeOH)-C(MeOH)	1.492 (23)	
C(5α)-C(5β)	1.573 (10)		N(3)-C(N(3))	1.478 (11)	
C(6α)-C(6β)	1.551 (10)		N(5)-C(N(5))	1.484 (11)	1.490
			N(6)-C(N(6))	1.508 (10)	

capillary with methanol showed the structure of bouvardin (Ia) except for the absolute configuration. Table II gives the fractional coordinates of the nonhydrogen atoms, including at the end the methanol and three partial waters of crystallization. For bond lengths (Table III) and bond angles (Table IV), the mean values are within two standard deviations of the usual values for peptides.

The absolute configuration was determined by hydrolyzing bouvardin (Ia) and determining the rotation of the resulting alanine (2 mol of L, 1 mol of D). Thus, all of the amino acids are L except for one D-alanine. The configuration about the chiral carbon bearing the alcoholic hydroxyl group is *S*.

Thus bouvardin (Ia) is a cyclic hexapeptide composed of two L-alanines, a D-alanine, and three modified *N*-methyl-L-tyrosines. Of the latter, one is a methyl ether, and the other two, in adjacent peptide units, have apparently undergone a typical phenolic oxidative coupling joining the phenolic oxygen of the first to the ortho carbon of the second, thus forming a 14-membered ring fused to the 18-membered hexapeptide ring.

From its similar biological activities, chromatographic behavior, and spectra, but a molecular weight 16 units less (mass spectra), deoxybouvardin should be Ib, identical with bouvardin (Ia) except possessing hydrogen instead of the alcoholic hydroxyl group. Support for this view comes from other spectral differences: strong parent peak in the mass spectrum of Ib only, strong P − 18 peak for Ia only; in the ^{13}C NMR spectrum, a methinyl carbon peak (for C(5β)) at δ 78 for Ia is replaced for Ib by a methylene peak at δ 37; in the ^{1}H NMR spectrum, absorption for a proton (presumably HC(5β)) at δ 5.1 for Ia is missing (presumably shifted upfield and overlapping with other peaks) for Ib. Biosynthetically, bouvardin (Ia) is most likely formed by hydroxylation of deoxybouvardin (Ib).

The conformation of bouvardin (Ia) in the crystal is depicted in Figure 1 and described via the torsion angles in Table V and the φ-ψ plot of Figure 2.[16] Unlike other cyclic hexapeptides on which x-ray studies have been performed,[17] bouvardin contains a cis peptide bond, no doubt because of its occurrence in the 14-membered ring. Even with a cis peptide bond, molecular models indicate the 14-membered ring, which also contains a paracyclophane[18] and a metacyclophane[18] ring system, possesses some angle strain and very little flexibility; in the paracyclophane ring, the para carbons (C(5γ) and C(5ζ)) are 0.06 and 0.07 Å from the least-squares plane formed by the six ring carbons, and the atoms directly attached to the ring (C(5β) and O(5η)) are 0.46 and 0.50 Å from this plane. Additional evidence of this strain is exemplified by the expanded angles C(6α)-N(6)-C(5) and N(6)-C(5)-C(5α) which are 124.8 and 123.5°, respectively (Table IV). On the other hand, models indicate considerable flexibility in the 18-membered ring, with no great advantage to the conformation observed. Unlike some other cyclic hexapeptides,[17] bouvardin (Ia) has only weak intramolecular hydrogen bonding between an amide carbonyl oxygen (O(4)) and an amide NH hydrogen across the ring (N(1); distance 3.26 ± 0.02 Å). Both hydroxyl groups are apparently intramolecularly hydrogen bonded to nearby oxygens (O(5γ) to O(5), 2.67 ± 0.02 Å; O(6η) to O(5η), 2.66 ± 0.02 Å). The torsion angle C(3ε2)-C(3ζ)-O(3η)-C(3θ) is 7.1°, unexceptional for an aryl methyl ether.[19]

Table VI gives the intermolecular O-O and N-O distances less than 3.5 Å, and Figure 3 shows the packing of the molecules in the unit cell with intermolecularly hydrogen-bonded atoms connected by dotted lines. The methanol bridges from the O(3) carbonyl oxygen of one molecule to the N(2) hydrogen of the corresponding molecule in an adjacent cell in the *x* direction. Molecules are linked in the *y* direction by hydrogen bonds from N(1) hydrogen to O(5) carbonyl oxygen and from the 6η phenolic oxygen through water molecule W2 (occupancy 0.31) and one or the other positions of disordered water molecule W1 (occupancies 0.21 and 0.09) to O(5) carbonyl oxygen. As mentioned above, these crystals are unstable in the

Table IV. Bond Angles with Estimated Standard Deviations in Parentheses

Atoms	Angle, deg	Mean	Atoms	Angle, deg	Mean
O(1)-C(1)-C(1α)	120.8 (6)	119.7	N(1)-C(1α)-C(1β)	108.8 (6)	111.7
O(2)-C(2)-C(2α)	121.9 (6)		N(2)-C(2α)-C(2β)	110.2 (6)	
O(3)-C(3)-C(3α)	119.0 (7)		N(3)-C(3α)-C(3β)	114.3 (7)	
O(4)-C(4)-C(4α)	121.0 (6)		N(4)-C(4α)-C(4β)	110.3 (6)	
O(5)-C(5)-C(5α)	115.6 (5)		N(5)-C(5α)-C(5β)	113.8 (5)	
O(6)-C(6)-C(6α)	120.0 (6)		N(6)-C(6α)-C(6β)	112.7 (5)	
O(1)-C(1)-N(2)	121.4 (6)	123.0	C(3α)-C(3β)-C(3γ)	110.6 (8)	112.3
O(2)-C(2)-N(3)	123.5 (6)		C(5α)-C(5β)-C(5γ)	107.7 (5)	
O(3)-C(3)-N(4)	126.7 (7)		C(6α)-C(6β)-C(6γ)	118.7 (5)	
O(4)-C(4)-N(5)	122.0 (6)		C(5α)-C(5β)-O(5γ)	111.9 (5)	
O(5)-C(5)-N(6)	121.0 (5)		C(5γ)-C(5β)-O(5γ)	113.3 (5)	
O(6)-C(6)-N(1)	123.9 (6)		C(3β)-C(3γ)-C(3δ1)	119.6 (10)	
N(2)-C(1)-C(1α)	117.6 (6)	117.3	C(3β)-C(3γ)-C(3δ2)	122.6 (9)	120.0
N(3)-C(2)-C(2α)	115.6 (6)		C(3δ1)-C(3γ)-C(3δ2)	117.8 (10)	
N(4)-C(3)-C(3α)	114.3 (6)		C(3γ)-C(3δ1)-C(3ε1)	121.6 (14)	
N(5)-C(4)-C(4α)	116.9 (6)		C(3γ)-C(3δ2)-C(3ε2)	119.7 (11)	
N(6)-C(5)-C(5α)	123.5 (6)		C(3δ1)-C(3ε1)-C(3ζ)	121.1 (19)	
N(1)-C(6)-C(6α)	116.1 (5)		C(3δ2)-C(3ε2)-C(3ζ)	119.3 (15)	
C(1α)-N(1)-C(6)	121.8 (5)	119.7	C(3ε1)-C(3ζ)-C(3ε2)	120.2 (20)	
C(2α)-N(2)-C(1)	121.5 (6)		C(3ε2)-C(3ζ)-O(3η)	127.0 (19)	
C(3α)-N(3)-C(2)	114.6 (6)		O(3η)-C(3ζ)-C(3ε1)	112.5 (19)	
C(4α)-N(4)-C(3)	118.9 (6)		C(3ζ)-O(3η)-C(3θ)	115.1 (19)	
C(5α)-N(5)-C(4)	116.7 (5)		C(5β)-C(5γ)-C(5δ1)	118.2 (5)	
C(6α)-N(6)-C(5)	124.8 (5)		C(5β)-C(5γ)-C(5δ2)	123.6 (6)	
C(3α)-N(3)-C(N(3))	119.1 (6)	118.9	C(5δ1)-C(5γ)-C(5δ2)	117.3 (6)	119.8
C(5α)-N(5)-C(N(5))	119.7 (6)		C(5γ)-C(5δ1)-C(5ε1)	118.9 (6)	
C(6α)-N(6)-C(N(6))	117.9 (5)		C(5γ)-C(5δ2)-C(5ε2)	123.1 (6)	
C(2)-N(3)-C(N(3))	126.3 (6)	122.0	C(5δ1)-C(5ε1)-C(5ζ)	117.9 (7)	
C(4)-N(5)-C(N(5))	123.4 (6)		C(5δ2)-C(5ε2)-C(5ζ)	117.7 (6)	
C(5)-N(6)-C(N(6))	116.2 (5)		C(5ε1)-C(5ζ)-C(5ε2)	123.7 (6)	
C(1)-C(1α)-N(1)	112.0 (6)	109.0	C(5ε2)-C(5ζ)-O(5η)	118.1 (6)	
C(2)-C(2α)-N(2)	104.4 (6)		O(5η)-C(5ζ)-C(5ε1)	117.7 (6)	
C(3)-C(3α)-N(3)	111.4 (6)		C(6β)-C(6γ)-C(6δ1)	120.7 (6)	
C(4)-C(4α)-N(4)	104.7 (6)		C(6β)-C(6γ)-C(6δ2)	122.9 (6)	
C(5)-C(5α)-N(5)	110.8 (5)		C(6δ1)-C(6γ)-C(6δ2)	116.3 (6)	120.0
C(6)-C(6α)-N(6)	110.6 (5)		C(6γ)-C(6δ1)-C(6ε1)	121.6 (6)	
C(1)-C(1α)-C(1β)	109.8 (6)	109.6	C(6γ)-C(6δ2)-C(6ε2)	121.5 (6)	
C(2)-C(2α)-C(2β)	107.5 (7)		C(6δ1)-C(6ε1)-C(6ζ)	121.7 (7)	
C(3)-C(3α)-C(3β)	111.2 (7)		C(6δ2)-C(6ε2)-C(6ζ)	123.1 (7)	
C(4)-C(4α)-C(4β)	109.5 (6)		C(6ε1)-C(6ζ)-C(6ε2)	115.7 (7)	
C(5)-C(5α)-C(5β)	111.9 (5)		C(6δ2)-C(6ε2)-O(5η)	123.1 (6)	
C(6)-C(6α)-C(6β)	107.9 (5)		C(6ζ)-C(6ε2)-O(5η)	113.8 (6)	
			C(6ε2)-O(5η)-C(5ζ)	106.8 (5)	
			C(6ε2)-C(6ζ)-O(6η)	120.2 (7)	
			O(6η)-C(6ζ)-C(6ε1)	124.1 (7)	

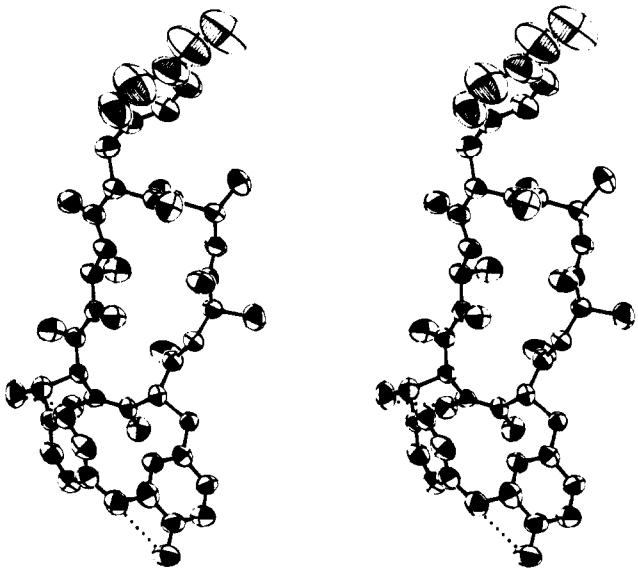


Figure 1. Stereoscopic view of a single molecule of bouvardin (Ia), with 50% probability thermal ellipsoids; dotted lines indicate heteroatoms joined by intramolecular hydrogen bonds.

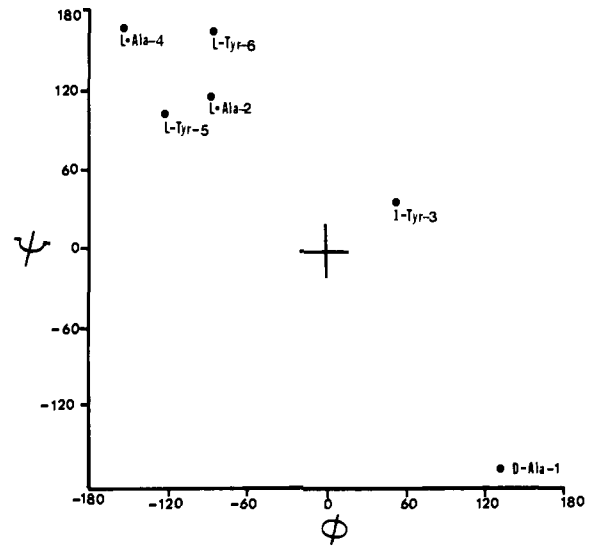


Figure 2. ϕ-ψ plot for crystalline bouvardin (Ia). ϕ and ψ are torsional angles about the C(α)-N and C-C(α) bonds, respectively.

Table V. Torsion Angles

Residue	ϕ	ψ	ω	χ_1	χ_2
1	134	−164	176		
2	−87	117	−176		
3	56	37	−177	−48	94
4	−156	164	−172		
5	−121	101	179	67	80
6	−84	165	−8	−63	162

Table VI. Intermolecular O · · · O and N · · · O Distances Less than 3.5 Å, with Standard Deviations in Parentheses

Atom	Atom	Symmetry[a] code	Distance, Å
O(W2)	O(1)(W1)	I	2.64 (1)
O(W2)	O(2)(W1)	I	3.18 (1)
O(W2)	O(6η)	I	2.72 (1)
O(1)(W1)	O(2)	II	2.89 (1)
O(2)(W1)	O(2)	II	3.15 (1)
O(MeOH)	N(2)	II	2.92 (1)
N(1)	O(5)	II	3.00 (1)
O(3)	O(MeOH)	III	2.72 (1)
O(3)	O(W2)	IV	3.37 (1)
O(3)	O(1)(W1)	IV	3.26 (1)

[a] I = x, y, z; II = $1-x, -\frac{1}{2}+y, \frac{1}{2}-z$; III = $-x, \frac{1}{2}+y, \frac{1}{2}-z$; IV = $1-x, \frac{1}{2}+y, \frac{1}{2}-z$.

absence of methanol, rapidly losing solvent and cracking; these changes are accompanied by shrinkage of the a axis by 0.45 Å, c by 5.77 Å, and the lengthening of b by 0.07 Å. From Figure 3, it is easy to imagine the bouvardin (Ia) molecules sliding closer to fill the holes left as MeOH and H_2O leave.

Bouvardin (Ia), deoxybouvardin (Ib), and the mixture of them (Ia-Ib, 2.3:1) as isolated from *Bouvardia ternifolia* showed consistently good levels of activity in the PS and B1 test systems at a wide range of doses in tests performed independently by four screening laboratories.[20] Bouvardin (Ia) demonstrated activities of 135-217% test/control (T/C) at dose levels ranging from 0.02 to 2.0 mg/kg in the PS test system and 134-152% T/C at 0.12-2.0 mg/kg in the B1 test system. Deoxybouvardin (Ib) showed PS activities of 142-216% T/C at 0.04-2.0 mg/kg and B1 activities of 133-175% T/C at 0.25-8.0 mg/kg. The natural mixture of Ia and Ib demonstrated PS and B1 activities of 132-257% T/C at 0.12-2.9 mg/kg and 132-167% T/C at 0.12-2.0 mg/kg, respectively. Activity in the PS[21] and B1[22] test systems is defined as an increase in the survival of treated animals over that of controls resulting in a T/C ≥ 125%. Bouvardin (Ia), deoxybouvardin (Ib), and their natural mixture were extremely cytotoxic in the KB cell culture screen. Activities (ED_{50}) were 4.3×10^{-7}, 1.9×10^{-8}, and $<10^{-2}$ μg/mL, respectively. Activity in the KB test system is defined as $ED_{50} \leq 4.0$ μg/mL for pure compounds.[23]

To our knowledge these are the first cyclic hexapeptides to exhibit antitumor activity. Their mechanism of action is possibly related to that of antibiotics such as valinomycin[24] (a cyclic dodecadepsipeptide) which transports ions across membranes, and we are accordingly carrying out studies of the abilities of these substances to complex metals. Synthetic routes to these substances are also under active investigation.

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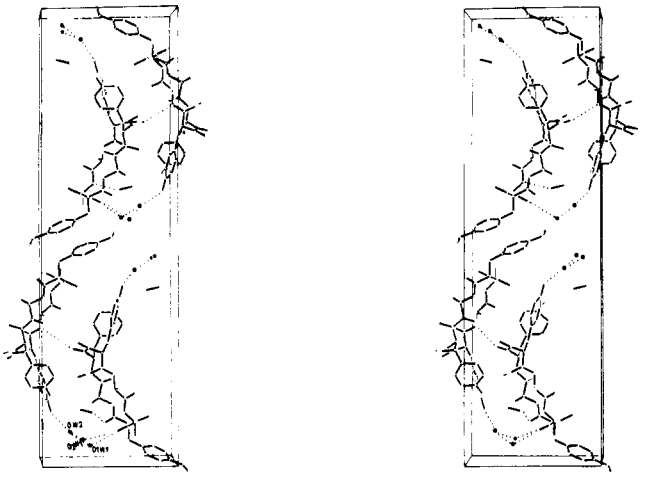

Figure 3. Stereoscopic view of a unit cell, a axis projection, b axis horizontal, c axis vertical; dotted lines indicate heteroatoms joined by intermolecular hydrogen bonds.

Supplementary Material Available: Temperature and structure factor tables (17 pages). Ordering information is given on any current masthead page.

References and Notes

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(5) We are indebted to Dr. Henry M. Fales, National Heart Institute, National Institutes of Health, Bethesda, Md., for recording mass and 100-MHz ^1H NMR spectra of bouvardin (Ia) and deoxybouvardin (Ib).
(6) We thank Dr. Oleg Jardetsky and Ms. Terri Lambert, Stanford Magnetic Resonance Lab, Stanford University, for a 360-MHz spectrum of bouvardin (Ia).
(7) Though the NMR spectra are in good agreement with structures Ia and Ib, very few of the NMR peaks can be assigned with assurance to particular protons and carbons at this time, and we wish to postpone discussion of these spectra until they have been more completely correlated.
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