Bufalin*

By Douglas C. Rohrer

Medical Foundation of Buffalo Inc., 73 High St, Buffalo, NY 14203, USA

Dwight S. Fullerton and Eitaro Kitatsuji

School of Pharmacy, Oregon State University, Corvallis, OR 97331, USA

Toshio Nambara

*Pharmaceutical Institute, Tohoku University, Aobayama, Sendai, Japan*

and Eiichi Yoshii

Faculty of Pharmaceutical Sciences, Toyama University, Gofuku, Toyama, Japan

Abstract. $C_{24}H_{34}O_4$, M_r = 386·5, orthorhombic, $P2_12_12_1$, a = 12·381 (2), b = 15·717 (1), c = 10·726 (1) Å, V = 2087·2 Å³, Z = 4, d_x = 1·230 Mg m⁻³. Final R = 0·058 for 2195 independent reflections with $F \geq 2\sigma(F)$. The steroid backbone of bufalin is virtually the same as the cardenolide prototype-molecule digitoxigenin. The average difference in corresponding atomic positions, C(1) to C(19), O(3) and O(14) is only 0·03 Å after the two molecules have been superimposed by a least-squares procedure. The change to a δ-lactone ring from a γ-lactone as the C(17)β side group displaces the relative location of the lactone carbonyl O by 1·46 Å. Furthermore, rotation of the side group about the C(17)–C(20) bond in bufalin has little effect on the relative position of the O.

Introduction. The 'cardiac steroids' are made up of two groups: (1) the 'digitalis genins and glycosides' or cardenolides, illustrated by digitoxigenin; and (2) the 'toad poisons' or bufadienolides, illustrated by bufalin. Despite their extreme toxicity, these classes of steroids have been used therapeutically for the treatment of heart disease for nearly 200 years. Cardenolide preparations remain one of the most widely used drugs today. The pharmacological effects of both groups seem to be the result of the inhibition of membrane-bound Na⁺,K⁺-ATPase (Schwartz, 1980). We have shown that the relative position of the carbonyl O or nitrile N substituent of the C(17)β side group has a direct influence on the inhibition of Na⁺,K⁺-ATPase (Rohrer, Fullerton, Yoshioka, From & Ahmed, 1979). The structure of bufalin was undertaken to examine the

17β-side-group orientation and its relationship to our proposed model for inhibition.

DIGITOXIGENIN   BUFALIN
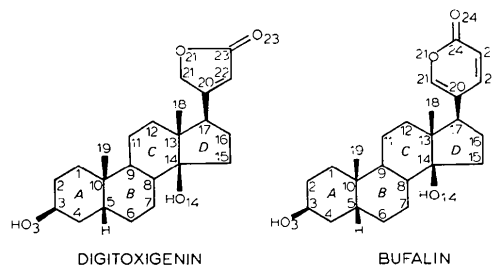
Crystal data were measured on a crystal of dimensions 0·24 × 0·32 × 0·32 mm with an Enraf–Nonius CAD-4f automatic diffractometer with Ni-filtered Cu $K\bar{\alpha}$ radiation. The crystals are orthorhombic and the space group is $P2_12_12_1$. The lattice dimensions were refined by a least-squares fit to a set of measured 2θ values [λ(Cu $K\bar{\alpha}$) = 1·5418 Å] for 24 reflections in the interval 50° < 2θ < 68°. Integrated relative intensities for 2438 independent reflections with 2θ < 150° were measured by ω–2θ scans; 2195 of those reflections were determined to be observed above background ($F > 2\sigma_F$).

The intensities were reduced to structure-factor amplitudes, and phase angles sufficient to locate the non-H atoms were obtained using the *MULTAN* programs (Main, Lessinger, Woolfson, Germain & Declercq, 1977). The H atoms were located on a difference electron-density map calculated at an intermediate stage of refinement. In the final cycles of full-matrix least-squares refinement, positional parameters for all atoms, anisotropic thermal vibration parameters for the non-H atoms, and isotropic thermal vibration parameters for the H atoms were varied. The

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quantities $(1/\sigma_F^2)$ were used to weight the least-squares differences for the observed data, where σ_F was as defined by Stout & Jensen (1968, p. 457, equation H 14)

Table 1. *Atomic coordinates* $(\times 10^5,$ *for* H $\times 10^3)$ *and isotropic thermal parameters* $(\times 10^2,$ *for* H $\times 10)$ *for bufalin*

$B_{iso} = \frac{4}{3} \sum_i \sum_j \beta_{ij} (a_i . a_j)$ for the non-H atoms. The e.s.d.'s are given in parentheses.

	x	y	z	B_{iso} (Å^2)
C(1)	63007 (20)	75303 (13)	92986 (26)	337 (5)
C(2)	55532 (28)	70542 (15)	101892 (24)	429 (7)
C(3)	45644 (25)	67219 (15)	95210 (24)	415 (6)
C(4)	39930 (20)	74343 (15)	88254 (28)	368 (6)
C(5)	47344 (17)	79448 (13)	79554 (20)	261 (4)
C(6)	41071 (21)	86626 (15)	73198 (23)	340 (5)
C(7)	38165 (18)	93807 (14)	82180 (24)	318 (5)
C(8)	48262 (17)	97332 (12)	88623 (20)	236 (4)
C(9)	54416 (17)	90156 (12)	95455 (20)	229 (4)
C(10)	57587 (17)	82850 (12)	86264 (20)	247 (4)
C(11)	64127 (20)	93772 (14)	102605 (25)	332 (5)
C(12)	60613 (20)	100922 (13)	111341 (24)	323 (5)
C(13)	55301 (18)	108478 (12)	104538 (21)	259 (4)
C(14)	45532 (17)	105064 (12)	96862 (21)	247 (4)
C(15)	36830 (19)	103649 (14)	106813 (24)	311 (5)
C(16)	38221 (24)	110883 (17)	116028 (30)	464 (7)
C(17)	49923 (21)	114246 (13)	114898 (21)	302 (5)
C(18)	63614 (20)	113089 (14)	96411 (26)	343 (3)
C(19)	65637 (20)	85950 (16)	76370 (26)	351 (5)
C(20)	50899 (20)	123782 (13)	112841 (22)	297 (5)
C(21)	43603 (24)	128454 (16)	106954 (29)	432 (6)
C(22)	60163 (22)	128170 (15)	117271 (29)	398 (6)
C(23)	61648 (28)	136625 (16)	115140 (33)	500 (7)
C(24)	53860 (32)	141359 (16)	108720 (28)	538 (9)
O(3)	48485 (20)	60740 (10)	86311 (18)	475 (5)
O(14)	41313 (14)	111776 (9)	89053 (16)	316 (3)
O(21)	44763 (19)	137099 (12)	105056 (20)	544 (6)
O(24)	54389 (28)	148886 (11)	105965 (24)	830 (10)
H(1A)	704 (2)	772 (2)	970 (3)	41 (6)
H(1B)	655 (2)	714 (2)	863 (3)	37 (6)
H(2A)	529 (2)	743 (2)	1085 (3)	51 (7)
H(2B)	603 (2)	658 (2)	1040 (4)	64 (9)
H(3A)	403 (2)	646 (2)	1008 (3)	55 (8)
H(4A)	360 (2)	782 (1)	942 (3)	35 (6)
H(4B)	332 (2)	720 (2)	831 (3)	46 (7)
H(5B)	493 (2)	757 (1)	733 (2)	22 (4)
H(6A)	342 (2)	838 (2)	698 (3)	36 (6)
H(6B)	456 (2)	888 (2)	660 (3)	53 (8)
H(7A)	330 (2)	920 (1)	884 (2)	20 (4)
H(7B)	341 (2)	981 (2)	778 (3)	34 (6)
H(8B)	527 (2)	993 (1)	825 (2)	26 (5)
H(9A)	493 (2)	879 (1)	1015 (2)	31 (5)
H(11A)	677 (2)	897 (2)	1079 (3)	38 (6)
H(11B)	699 (2)	954 (2)	968 (3)	33 (6)
H(12A)	552 (2)	987 (2)	1183 (3)	43 (6)
H(12B)	669 (2)	1028 (2)	1151 (3)	33 (5)
H(15A)	374 (2)	986 (2)	1104 (4)	58 (8)
H(15B)	301 (2)	1038 (2)	1034 (3)	41 (6)
H(16A)	363 (2)	1094 (2)	1244 (4)	62 (8)
H(16B)	333 (2)	1150 (2)	1145 (3)	54 (7)
H(17A)	537 (2)	1129 (1)	1227 (3)	31 (6)
H(18A)	662 (2)	1096 (2)	895 (3)	43 (6)
H(18B)	612 (2)	1180 (2)	924 (3)	51 (7)
H(18C)	697 (2)	1145 (2)	1003 (3)	44 (7)
H(19A)	669 (2)	816 (2)	698 (3)	42 (6)
H(19B)	628 (2)	908 (2)	717 (3)	39 (6)
H(19C)	721 (2)	873 (2)	795 (3)	47 (7)
H(21)	363 (2)	1268 (2)	1035 (3)	48 (7)
H(22)	656 (2)	1249 (2)	1222 (3)	41 (6)
H(23)	682 (2)	1392 (2)	1177 (3)	48 (7)
H(O3)	501 (3)	576 (2)	901 (4)	59 (8)
H(O14)	453 (2)	1115 (1)	824 (3)	32 (6)

but with an instability factor of 0·06; unobserved data were given zero weight. The final values of the residual, $R = \sum ||F_o| - |F_c||/\sum |F_o|$, were 0·058 for the observed data and 0·064 for all the unique data measured. The scattering factors used throughout the refinement were generated from coefficients given in *International Tables for X-ray Crystallography* (Cromer & Waber, 1974). Final positional parameters and isotropic thermal parameters are given in Table 1.* The equivalent isotropic thermal parameters for the non-H atoms given in Table 1 were calculated using equation (18) of Hamilton (1959).

Discussion. The crystallographically observed structure and anisotropic thermal parameters of bufalin are shown in Fig. 1. The intramolecular dimensions involving the non-H atoms are given in Fig. 2. The C—H bond distances range from 0·88 to 1·06 Å and average 0·97 ± 0·05 Å. The two O—H bond distances are 0·67 and 0·87 Å for O(3)—H and O(14)—H respectively. These structural parameters agree well with similar steroid structures like digitoxigenin (Karle & Karle, 1969).

The *A*, *B* and *C* rings in bufalin all have a chair conformation. The *D* ring has a C(14) β-envelope conformation $\Delta C_s[C(14)] = 0\cdot 2$ (Duax, Weeks & Rohrer, 1976), similar to the conformation in the crystal structure of digitoxigenin, $\Delta C_s[C(14)] = 1\cdot 5$. The *cis–trans–cis* configurations at the ring fusions are another characteristic feature of this class of steroid and are particularly evident in Fig. 1. This results in a semicircular shape for the steroid backbone rather than the flat shape of the steroid hormones. The δ-lactone ring is planar with r.m.s. deviation from the least-squares plane for the six atoms in the ring of only 0·01 Å.

Comparison of the structure of bufalin to digitoxigenin, the cardenolide prototype, shows that the steroid backbones are essentially identical. Fig. 3 shows the results of superimposing the two structures using a least-squares procedure (Rohrer & Smith, 1980) to
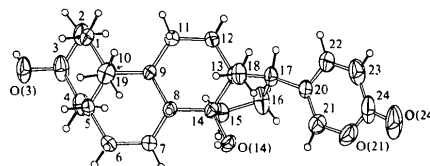

Fig. 1. *ORTEP* II (Johnson, 1965) drawing of bufalin with atomic numbering. The thermal ellipsoids for non-H atoms are scaled at the 60% probability level.
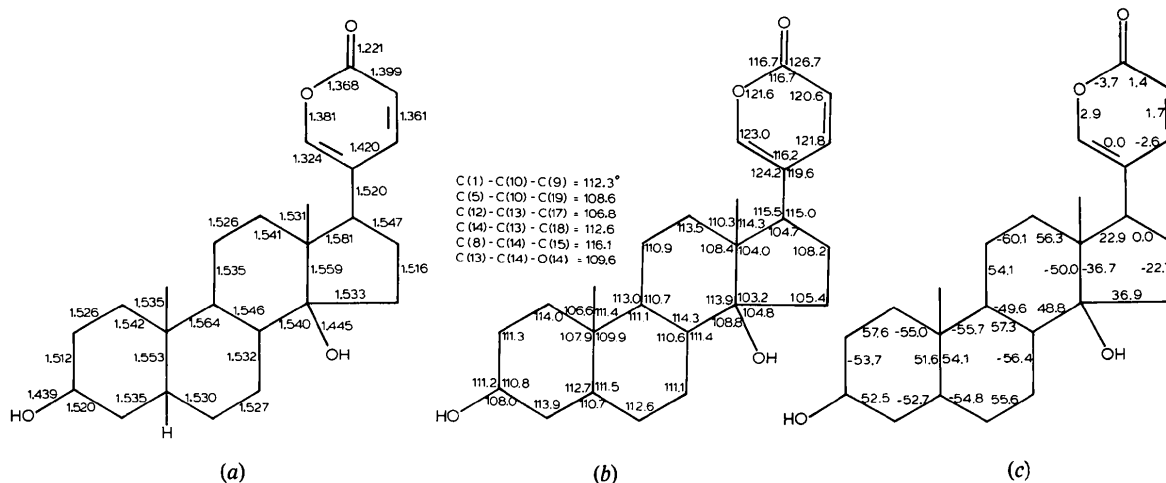
Fig. 2. Intramolecular dimensions for bufalin. (a) Bond distances (Å); σ range = 0·003 to 0·005 Å. (b) Bond angles (°); σ range = 0·2 to 0·3°. (c) Endocyclic torsion angles (°); σ range = 0·2 to 0·5°. A torsion angle α–β–γ–δ is positive if, when viewed down the β–γ bond, the α–β bond will eclipse the γ–δ bond when rotated less than 180° in a clockwise direction.

Fig. 3. A view of the PROPHET/FITMOL (Rohrer & Smith, 1980) superposition of atoms C(1) to C(19), O(3) and O(14) in bufalin (– – –) and digitoxigenin (———).

Table 2. Hydrogen-bond distances (Å) and angles (°)

D–H···A	D···A	H···A	∠D–H···A	Symmetry operation to A
O(3)–H···O(24)	2·907 (3)	2·24 (4)	168 (3)	$x, -1 + y, z$
O(14)–H···O(3)	3·004 (3)	2·15 (3)	167 (2)	$1 - x, \frac{1}{2} + y, \frac{3}{2} - z$

minimize the distance between corresponding atoms C(1) to C(19), O(3) and O(14) in each molecule. The average separation between these atoms after fitting was 0·03 Å. A direct relationship between the relative position of carbonyl O of the 17β side group and the Na⁺,K⁺-ATPase inhibition strength has been observed for a series of cardenolide analogues (Rohrer et al., 1979). The relationship indicates that for each 0·46 Å the O is moved away from the digitoxigenin position, the inhibition activity changes tenfold. The 1·46 Å displacement of the bufalin O, see Fig. 3, should therefore result in an I_{50} of 9·7 × 10^{-8} M for rat-brain Na⁺,K⁺-ATPase. The actual experimental I_{50} is 4·6 × 10^{-8} M in very good agreement with the calculated values. These results indicate that bufadienolide activity is also influenced directly by the relative position of the 17β-side-group O.

The lactone-ring plane of bufalin is oriented in the alternate orientation compared to digitoxigenin using the ring O atoms as a guide. The C(13)–C(17)–C(20)–C(22) torsion angles, −87·1 (3) and 76 (1)°, for bufalin and digitoxigenin indicate the orientation reversal. Molecular-mechanics calculations, using a version of CAMSEQ (Weintraub & Hopfinger, 1975) in conjunction with the modeling and graphing features of the NIH PROPHET computer system (Weeks, Cody, Pokrywiecki, Rohrer & Duax, 1974), were used to explore the rotational freedom about the C(17)–C(20) bond in bufalin and digitoxigenin. These calculations clearly show two energy-minimum conformations for the orientation of the lactone side groups in each molecule at nearly the same locations. The crystal-structure conformation for each molecule is in the lowest and widest energy minimum for that molecule. The calculations also indicate large barriers to rotation between the minima which would hold the conformations within the two minima even in solution.

There are two hydrogen bonds between bufalin and symmetrically related molecules in the crystal, see Table 2. One forms a tail-to-head chain of molecules translationally related along b. The other forms a helix about a b-axis twofold screw.

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The Structure of Procaine

BY SETSUO KASHINO, MINORU IKEDA AND MASAO HAISA

Department of Chemistry, Faculty of Science, Okayama University, Tsushima, Okayama 700, Japan

Abstract. $C_{13}H_{20}N_2O_2$, m.p. 332·5–334·0 K, monoclinic, $P2_1/a$, $a = 11·56$ (2), $b = 14·38$ (1), $c = 8·202$ (7) Å, $\beta = 98·0$ (1)°, $V = 1350$ (3) Å^3, $D_m = 1·17$, $D_x = 1·162$ Mg m^{-3}, $Z = 4$, final $R = 0·098$ for 1690 non-zero reflexions. The carbonyl oxygen atom accepts two hydrogen bonds from the amino groups of the molecules related by a 2_1 axis and glide plane. A double-layered molecular sheet is formed by the hydrogen bonds on (001). The benzene ring shows the quinonoid structure. The conformation of the C–O–C–C–N side chain is *trans–gauche* [τ(C–O–C–C) = 147·1 (4)°, τ(O–C–C–N) = −82·6 (5)°], as found in salts of procaine and related compounds.

Introduction. Procaine is known as a local anesthetic. Structural studies on salts of procaine have been carried out (Beall, Herdklotz & Sass, 1970; Sax, Pletcher & Gustaffson, 1970; Dexter, 1972; Freeman & Bugg, 1975). Work on the un-ionized molecule is desirable to establish the structure–activity relationship. The conformation of the side chain is interesting in view of the concept of pharmacophores (Kier, 1971).

Crystals were obtained from a ligroin solution as monoclinic prisms bounded by {110} and {001}. Intensity data were collected on Weissenberg photographs with specimens 0·65 × 0·70 × 0·63 mm for the layers 0*kl* to 8*kl*, and 0·22 × 0·24 × 0·25 mm for the layers *hk*0 to *hk*6. Visually estimated intensities were corrected for Lorentz and polarization factors and for spot shape, but no absorption correction was made

[μ(Cu $K\alpha$) = 0·64 mm^{-1}]. Intensities of 1690 non-zero reflexions (55% of the reflexions within the Cu $K\alpha$ sphere, $\lambda = 1·5418$ Å) were placed on an approximately absolute scale by a Wilson plot ($B = 5·9$ Å^2).

The structure was solved by a symbolic addition procedure. The H atoms were located from a difference Fourier map. The refinements were made by block-diagonal least-squares calculations. The weighting scheme was: $w = 1·0$ for $0 < |F_o| \leq 8·0$, $w = (8·0/|F_o|)^2$ for $|F_o| > 8·0$. Atomic scattering factors
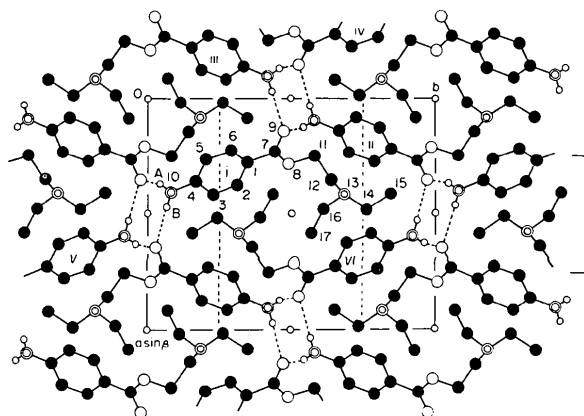

Fig. 1. Projection of the crystal structure along c, and numbering of the atoms (○: O, ⊚: N, ●: C and ○: H). The H atoms attached to the C atoms are omitted. Broken lines indicate hydrogen bonds. Symmetry code: (i) x,y,z; (ii) $\frac{1}{2} - x, \frac{1}{2} + y, 1 - z$; (iii) $-\frac{1}{2} + x, \frac{1}{2} - y, z$; (iv) $-x, 1 - y, 1 - z$; (v) $1 - x, -y, 1 - z$; (vi) $1 - x, 1 - y, 1 - z$.