

The Structural Formula and Crystal Structure of the *O-p*-Bromobenzoate Derivative of Batrachotoxinin A, C₃₁H₃₈NO₆Br, a Frog Venom and Steroidal Alkaloid

BY ISABELLA L. KARLE AND J. KARLE

Laboratory for the Structure of Matter, U.S. Naval Research Laboratory, Washington, D.C. 20390, U.S.A.

(Received 5 February 1968)

Batrachotoxinin A, a venom from the Colombian poison arrow frog, *Phyllobates aurotaenia*, has been established by means of an X-ray analysis of the *O-p*-bromobenzoate derivative, to be 3 α ,9 α -epoxy-14 β ,18 β -[epoxyethano-*N*-methylimino]-5 β -pregna-7,16-diene-3 β ,11 α ,20 α -triol. The material crystallizes in space group $P2_12_12_1$ with $a = 15.42$, $b = 7.05$ and $c = 26.50$ Å. The specialized position of the Br atom (at $\frac{1}{2}, 0, 0$) and the severely restricted amount of data limited the information which was available from use of the heavy atom phases. The structure was deduced by use of the tangent formula in a recycling procedure applied to partial structural information.

Introduction

The Colombian arrow poison frog, *Phyllobates Aurotaenia*, produces in its skin an extremely potent venom which the Indians use to tip their arrowheads for the purpose of hunting game. The venom is the most potent yet discovered. It has a toxicity about 250 times that of strychnine. The venom, to be active, must be introduced into the body by piercing the skin. It is harmless when taken orally. Several biochemists travelled to Colombia to collect about 7000 frogs and to extract their venom on location (Märki & Witkop, 1963; Daly, Witkop, Bommer & Bieman, 1965). The delicate frogs, which are only 2–3 cm long, could not survive the trip to the National Institutes of Health in Washington.

The major alkaloid fraction isolated from the frogs consists of batrachotoxin, isobatrachotoxin and pseudobatrachotoxin, which, in the mass spectrometer, all give the same molecular ion, C₂₄H₃₃NO₄. Pseudobatrachotoxin is very unstable and is readily converted to batrachotoxinin A, C₂₄H₃₅NO₅. The relationship between the two compounds is under investigation. Such minute amounts of the purified alkaloids were available that chemical analyses for establishing the structural formulae were not feasible. Thus far, the only crystals suitable for an X-ray analysis were those of the *O-p*-bromobenzoate derivative of batrachotoxinin A. A preliminary report on the structural formula of this material as determined by X-ray analysis has been made (Tokuyama, Daly, Witkop, Karle & Karle, 1968).

Experimental

A very few, extremely minute crystals of *O-p*-bromobenzoate of batrachotoxinin A were supplied by Drs Witkop, Daly and Tokuyama of the National Institutes of Health. The largest of the needle-shaped crystals, 0.05 × 0.03 × 1.0 mm was selected for the X-ray analysis. It had good optical extinctions parallel to the needle axis both before and after all the X-ray exposures. The space group and unit-cell parameters were

determined from precession photographs (Table 1). Intensity data were collected by the multiple-film, equi-inclination Weissenberg technique. Forty hour exposures were taken about the b axis for the 0–4 layers. The scattering pattern faded rapidly at $(\sin \theta)/\lambda \sim 0.4$ and a total of only 830 independent reflections were recorded. Some of these were assigned zero intensity. The intensities were estimated by visual comparison with a calibrated film strip. Corrections for the Lorentz and polarization factors were made and normalized factors, $|E|$, as well as structure factors, $|F|$, were derived.

Table 1. Physical constants for batrachotoxinin A, *O-p*-bromobenzoate derivative, C₃₁H₃₈NO₆Br

Molecular weight	599.56
Melting point	213°C
Habit	Acicular
Space group	$P2_12_12_1$
a	15.42 ± 0.03 Å
b	7.05 ± 0.02
c	26.50 ± 0.04
V	2880.8 Å ³
ρ_{calc}	1.382 g.cm ⁻³
Z	4
Cu K α radiation	1.5418 Å
Crystal size	0.05 × 0.03 × 1.0 mm
Number of observed reflections	830

Structure analysis

The Br atom was readily located at $\frac{1}{2}, 0, 0$ in a sharpened Patterson map computed with $E^2 - 1$ values. Phases based on the Br atom alone gave rise to a fourfold ambiguity for the remainder of the structure. From an initial E -map based on the phases from the Br atom, it was possible to find two fairly strong peaks which could be the two O atoms in the bromobenzoate group. Assuming that the bromobenzoate group was planar, this group was located approximately in the unit cell.

Using the bromobenzoate group as a known partial structure, phases were computed from this unit and were accepted if both $|E| > 1.4$ and $|F_c| > 0.25|F_o|$ for a particular reflection. Phases for additional reflections with $|E| > 0.8$ were obtained with the use of the tangent formula (Karle & Hauptman, 1956),

$$\tan \phi_h \approx \frac{\sum_k |E_k E_{h-k}| \sin(\phi_k + \phi_{h-k})}{\sum_k |E_k E_{h-k}| \cos(\phi_k + \phi_{h-k})}, \quad (1)$$

in a recycling procedure (Karle, 1968) while keeping the phases based on the partial structure fixed. An *E*-map computed from phases obtained in this manner was very much better resolved than that obtained from the heavy atom phases alone. Even so, with the limited number of data available and the fact that $y=0$ for the Br atom, and $y \sim \frac{1}{2}$ for O(5), C(25) and C(27), a considerable amount of twofold ambiguity in the *b* direction still persisted among the new peaks. Only atoms C(5)–C(10) and O(2) were added to the known structure after the first cycle. Several more cycles employing the partial structure in conjunction with the tangent formula revealed most of the atoms. In the criterion for accepting phases, $|F_c| > k|F_o|$, the value of k was raised in successive cycles to be roughly proportional to the fraction of known scattering power with a maximum value of 0.6. Finally a difference map located the last three atoms, C(1), C(4) and C(23) and the molec-

ular formula was established to be that shown in (I). The identification of the six oxygen atoms could be readily made from the weights of the peaks in the *E*-maps; however, it was not known which atom was the nitrogen atom.

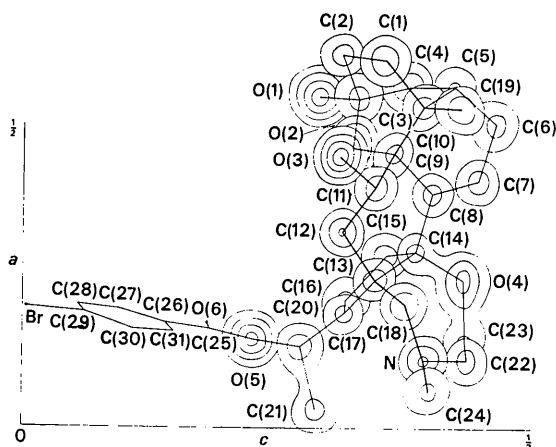
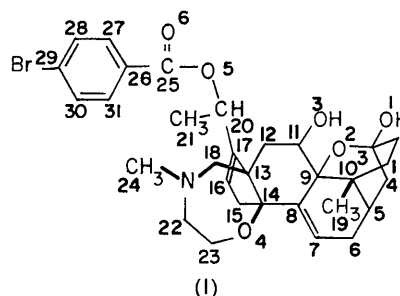


Fig. 1. Sections from a three-dimensional electron density map projected down the *b* axis. Contours are equally spaced at $2 \text{ e.}\text{\AA}^{-3}$ beginning with the $2 \text{ e.}\text{\AA}^{-3}$ contour.

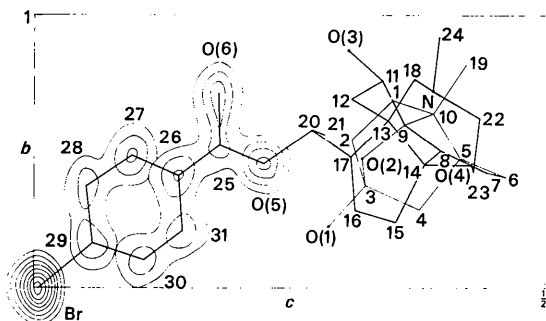


Fig. 2. The molecule projected along the *a* axis with contours for the bromobenzoate group only. Sections were taken from a three-dimensional map. Contours are spaced at $2 \text{ e.}\text{\AA}^{-3}$ for the light atoms, beginning with the $2 \text{ e.}\text{\AA}^{-3}$ contour. For the Br atom the contours are spaced at $5 \text{ e.}\text{\AA}^{-3}$.

In the subsequent least-squares refinement, the thermal parameter of the atom now labelled N was abnormally low when it was treated as a carbon atom and the difference map computed after several cycles of least-squares refinement showed residual density at this location indicating that that atom is most probably the nitrogen atom.

In the least-squares refinement (Busing, Martin & Levy, 1962), the coordinates, isotropic thermal factors, and scale factors for the individual layers were varied until there were no significant changes in the parameters. The *R* value was 13.5%. The function minimized was $\sum w(F_o - F_c)^2$ and the atomic scattering factors were taken from *International Tables for X-ray Crystallography* (1962). In further refinement the scale factors were kept constant and only the thermal parameters for the Br atom were allowed to vary anisotropically since the total number of independent data, 830, was too small to vary 351 parameters meaningfully for a full anisotropic refinement. The introduction of anisotropic motion into the Br atom affected the coordinates of all the lighter atoms so that the spread of values for interatomic distances for similar bonds was decreased. The final *R* value was 9.7% (Table 2). The initial least-squares refinement was performed with unit weights. When the refinement process was repeated with $w=1$ for $|F_o| < 30$ and $w=30/|F_o|$ for $|F_o| > 30$, the final *R* value was the same as with unit weights. The values for the bond distances and angles were very similar in both refinements, the largest differences being less than one standard deviation.

A difference map computed from the refined structure was rather featureless and, as expected, did not show the positions of the hydrogen atoms. The composite electron density maps for the molecule are illustrated in Figs. 1 and 2. The resolution is quite good, considering the small number of data, except for the bonded pair C(25)–O(6). Coordinates and thermal parameters are listed in Table 3. Values for the bond lengths and angles are shown in Fig. 3. The estimated standard deviations are 0.05 \AA for C–C, C–N and C–O bonds and 0.03 \AA for the C–Br bond.

Table 2. Observed and calculated structure factors
The columns represent the index l , $|F_o|$, $|F_c|$ and ϕ_{calc} (radians).

0	0	0	24	75.3	74.2	1.85	8	52.4	48.1	-2.12	0	104.3	195.8	-1.57	5	2	L	6	34.5	38.3	-0.44	2	0.0	10.2	0.88	
2 40.0	42.4	0.00	1	2	1	10	57.5	60.7	-1.71	10	11.93	88.0	-1.57	0	34.1	25.3	-1.57	7	65.0	47.2	1.16	3	20.1	32.9	-1.57	
4 203.0	124.4	-3.14	0	106.0	103.5	1.57	11	16.0	24.2	-2.71	12	6.0	8.9	0.00	1	28.9	19.8	0.92	8	18.0	94.3	1.12	4	38.0	42.2	0.00
6 186.8	194.4	0.00	2	13.3	72.3	2.17	13	49.2	39.0	-2.57	14	26.9	24.7	0.00	3	30.8	30.2	1.98	9	48.5	39.2	1.62	5	24.3	28.3	1.57
10 190.7	199.6	0.00	3	16.4	13.5	2.67	14	0.0	6.9	-1.87	15	60.3	94.9	-1.57	4	48.9	36.8	3.11	10	35.2	94.8	-1.57	6	24.7	21.7	0.00
12 88.1	98.3	0.00	4	5.9	40.3	0.62	16	0.0	11.1	-3.06	17	70.3	69.9	-1.57	5	51.9	44.7	2.54	11	56.6	95.0	1.58	7	17.8	8.4	1.57
14 92.0	167.0	0.00	5	54.6	40.3	0.62	18	27.5	8.9	-3.14	18	27.5	8.9	-3.14	6	49.9	41.0	0.60	12	48.2	45.3	-1.10	8	16.0	16.0	0.00
16 198.9	172.2	0.00	6	25.3	22.2	0.52	19	116.5	103.3	0.52	19	116.5	103.3	0.52	7	58.9	45.3	-1.10	13	98.8	105.1	1.57	9	1.0	1.0	0.00
18 117.8	199.2	0.00	7	31.4	35.5	-0.53	20	0.0	1.7	-0.00	20	0.0	1.7	-0.00	8	98.8	99.9	-2.37	14	126.7	142.6	-3.14	10	102.5	95.4	-1.57
20 48.9	98.3	0.00	8	111.2	101.6	-1.76	21	50.2	48.9	1.54	21	50.2	48.9	1.54	9	17.6	45.5	1.69	15	470.4	149.0	-3.14	11	29.9	30.9	-1.46
22 102.2	94.9	0.00	9	25.8	24.3	0.56	22	56.6	58.9	2.72	22	56.6	58.9	2.72	10	0.0	12.4	0.30	16	50.9	44.4	-1.57	12	30.1	33.2	-2.16
24 45.1	38.8	-3.14	10	95.1	48.4	3.07	23	117.0	117.2	0.54	23	117.0	117.2	0.54	11	47.7	47.7	0.00	17	46.6	94.8	-3.14	13	48.1	85.3	0.00
26 52.0	54.3	0.00	11	61.9	60.8	2.99	24	43.6	43.5	-2.36	24	48.1	92.3	0.00	12	0.0	12.7	-2.86	18	7.46	42.4	-1.57	14	5.5	19.4	0.86
			12	13.6	56.7	-0.80	25	39.9	30.7	2.01	25	39.9	30.7	2.01	13	27.2	31.7	1.43	19	8.27	94.2	0.00	15	6.36	35.2	-1.92
			13	64.9	54.5	-2.54	26	73.0	69.9	0.66	26	73.0	69.9	0.66	14	0.0	0.0	0.00	20	0.0	0.0	0.00	16	16.6	38.2	-1.18
			14	71.7	71.7	1.57	27	61.3	49.3	1.43	27	61.3	49.3	1.43	15	0.0	0.0	0.00	21	0.0	0.0	0.00	17	45.7	43.1	-1.62
			15	140.1	133.1	-1.57	28	45.3	46.3	-3.12	28	45.3	46.3	-3.12	16	31.1	26.0	-1.57	22	126.7	142.6	-3.14	18	102.5	95.4	-1.57
			16	9.7	21.8	1.57	29	117.0	117.2	0.54	29	117.0	117.2	0.54	17	100.7	103.9	0.48	23	470.4	149.0	-3.14	19	29.9	30.9	-1.46
			17	92.6	49.9	1.57	30	65.5	60.5	2.97	30	65.5	60.5	2.97	18	0.0	0.0	0.00	24	50.9	44.4	-1.57	20	30.1	33.2	-2.16
			18	43.3	42.2	1.57	31	51.0	46.1	-1.44	31	51.0	46.1	-1.44	19	2.0	9.8	0.72	25	46.6	94.8	-3.14	21	48.1	85.3	0.00
			19	17.4	21.8	1.57	32	30.2	30.5	-0.85	32	30.2	30.5	-0.85	20	86.6	71.3	0.25	26	15.0	7.2	1.57	22	13.1	46.5	-0.86
			20	26.1	23.0	-1.57	33	54.6	61.9	1.16	33	54.6	61.9	1.16	21	16.0	20.3	0.74	27	16.0	0.0	0.00	23	14.8	40.7	-1.20
			21	73.9	45.8	-1.57	34	0.0	0.0	0.00	34	0.0	0.0	0.00	22	0.0	0.0	0.00	28	47.7	47.7	0.00	24	16.0	16.0	0.00
			22	36.3	32.7	1.57	35	0.0	0.0	0.00	35	0.0	0.0	0.00	23	6.7	6.7	0.00	29	48.3	42.5	-1.49	25	17.0	17.0	0.00
			23	45.9	41.6	1.57	36	134.9	355.1	-1.57	36	134.9	355.1	-1.57	24	62.5	67.4	-0.45	30	62.5	57.6	-0.88	26	17.0	17.0	0.00
			24	0.0	0.0	0.00	37	60.6	54.5	3.14	37	60.6	54.5	3.14	25	10.0	32.2	-0.34	31	18.2	21.9	-0.37	27	0.0	0.0	0.00
			25	17.6	17.6	0.00	38	162.2	142.0	-1.57	38	162.2	142.0	-1.57	26	11.9	71.8	0.54	32	97.6	77.6	0.03	28	0.0	0.0	0.00
			26	0.0	0.0	0.00	39	2.0	6.0	0.00	39	2.0	6.0	0.00	27	35.9	30.3	0.51	33	42.2	34.3	-1.62	29	12.5	12.5	0.00
			27	0.0	0.0	0.00	40	117.0	117.2	0.54	40	117.0	117.2	0.54	28	11.9	33.8	-0.49	34	11.9	71.8	0.54	30	12.5	12.5	0.00
			28	0.0	0.0	0.00	41	64.9	59.5	1.87	41	64.9	59.5	1.87	29	17.3	24.3	1.90	35	12.6	43.3	0.07	31	0.0	0.0	0.00
			29	28.0	31.4	1.57	42	0.0	0.0	0.00	42	0.0	0.0	0.00	30	15.0	16.0	0.40	36	32.6	31.5	0.52	32	0.0	0.0	0.00
			30	76.3	71.3	-1.57	43	30.2	30.5	-0.85	43	30.2	30.5	-0.85	31	18.0	0.0	0.00	37	48.3	43.3	1.57	33	0.0	0.0	0.00
			31	29.6	35.5	1.57	44	0.0	6.4	-3.14	44	0.0	6.4	-3.14	32	17.3	34.4	0.17	38	15.4	46.0	0.17	34	0.0	0.0	0.00
			32	37.0	34.9	1.57	45	67.8	40.0	0.75	45	67.8	40.0	0.75	33	18.3	25.4	0.78	39	15.4	46.0	-1.12	35	0.0	0.0	0.00
			33	43.3	41.1	-1.57	46	15.4	46.4	1.30	46	15.4	46.4	1.30	34	18.0	44.9	0.00	40	48.3	43.3	-0.33	36	0.0	0.0	0.00
			34	0.0	0.0	0.00	47	15.4	47.4	1.30	47	15.4	47.4	1.30	35	19.0	15.2	-1.57	41	6.39	39.7	-2.25	37	0.0	0.0	0.00
			35	0.0	0.0	0.00	48	16.7	47.2	1.05	48	16.7	47.2	1.05	36	15.4	43.5	0.00	42	7.16	48.1	2.42	38	0.0	0.0	0.00
			36	0.0	0.0	0.00	49	12.9	19.2	0.00	49	12.9	19.2	0.00	37	14.2	43.5	0.00	43	19.0	15.2	-1.57	39	0.0	0.0	0.00
			37	0.0	0.0	0.00	50	12.9	19.2	0.00	50	12.9	19.2	0.00	38	14.2	43.5	0.00	44	19.0	15.2	-1.57	40	0.0	0.0	0.00
			38	0.0	0.0	0.00	51	12.9	19.2	0.00	51	12.9	19.2	0.00	39	14.2	43.5	0.00	45	19.0	15.2	-1.57	41	0.0	0.0	0.00
			39	0.0	0.0	0.00	52	12.9	19.2	0.00	52	12.9	19.2	0.00	40	14.2	43.5	0.00	46	19.0	15.2	-1.57	42	0.0	0.0	0.00
			40	0.0	0.0	0.00	53	12.9	19.2	0.00	53	12.9	19.2	0.00	41	14.2	43.5	0.00	47	19.0	15.2	-1.57	43	0.0	0.0	0.00
			41	0.0	0.0	0.00	54	12.9	19.2	0.00	54	12.9	19.2	0.00	42	14.2	43.5	0.00	48	19.0	15.2	-1.57	44	0.0	0.0	0.00
			42	0.0	0.0	0.00	55	12.9	19.2	0.00	55	12.9	19.2	0.00	43	14.2	43.5	0.00	49	19.0	15.2	-1.57	45	0.0	0.0	0.00
			43	0.0	0.0	0.00	56	12.9	19.2	0.00	56	12.9	19.2	0.00	44	14.2	43.5	0.00	50	19.0	15.2	-1.57	46	0.0	0.0	0.00
			44	0.0	0.0	0.00	57	12.9	19.2	0.00	57	12.9	19.2	0.00	45	14.2	43.5	0.00	51	19.0	15.2	-1.57	47	0.0	0.0	0.00
			45	0.0	0.0	0.00	58	12.9	19.2	0.00	58	12.9	19.2	0.00	46	14.2	43.5	0.00	52	19.0	15.2	-1.57	48	0.0	0.0	0.00
			46	0.0	0.0	0.00	59	12.9	19.2	0.00	59	12.9	19.2	0.00	47	14.2	43.5	0.00	53	19.0	15.2	-1.57	49	0.0	0.0	0.00
			47	0.0	0.0	0.00	60	12.9	19.2	0.00																

Table 3. Fractional coordinates

	x	y	z	B
C(1)	0.6062	0.6866	0.3528	3.0
C(2)	0.6170	0.5159	0.3136	4.5
C(3)	0.5489	0.3715	0.3240	3.4
C(4)	0.5642	0.2967	0.3774	2.0
C(5)	0.5637	0.4596	0.4201	4.2
C(6)	0.5038	0.3974	0.4622	3.5
C(7)	0.4098	0.4100	0.4440	4.5
C(8)	0.3847	0.4800	0.3993	3.2
C(9)	0.4442	0.5850	0.3624	2.6
C(10)	0.5320	0.6388	0.3923	3.0
C(11)	0.3930	0.7638	0.3433	4.5
C(12)	0.3186	0.6928	0.3131	2.3
C(13)	0.2426	0.6014	0.3448	1.5
C(14)	0.2867	0.4490	0.3827	3.5
C(15)	0.2814	0.2521	0.3566	4.7
C(16)	0.2104	0.2948	0.3148	3.9
C(17)	0.1903	0.4734	0.3102	2.4
C(18)	0.2010	0.7679	0.3732	2.8
C(19)	0.5256	0.8146	0.4278	4.4
C(20)	0.1293	0.5667	0.2734	2.1
C(21)	0.0319	0.5435	0.2857	3.7
C(22)	0.1070	0.6108	0.4354	3.7
C(23)	0.1491	0.4164	0.4333	5.2
C(24)	0.0605	0.9068	0.3977	5.9
C(25)	0.1620	0.5151	0.1840	3.7
C(26)	0.1742	0.4113	0.1406	2.3
C(27)	0.1911	0.4820	0.0944	2.8
C(28)	0.1998	0.3588	0.0528	4.8
C(29)	0.1892	0.1702	0.0599	2.5
C(30)	0.1646	0.0831	0.1057	3.8
C(31)	0.1569	0.2144	0.1473	3.1
N	0.1099	0.7206	0.3901	4.4
O(1)	0.5481	0.2252	0.2883	3.0
O(2)	0.4649	0.4584	0.3212	2.4
O(3)	0.4517	0.8656	0.3093	2.7
O(4)	0.2427	0.4414	0.4307	3.8
O(5)	0.1455	0.4488	0.2269	3.1
O(6)	0.1668	0.6981	0.1799	6.1
Br	0.1978	-0.0018	0.0042	6.0*

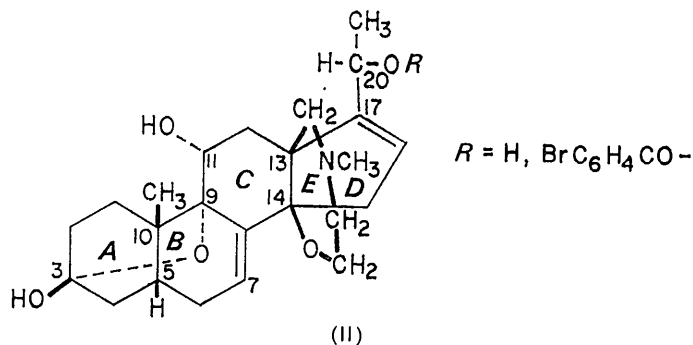
Table 3 (cont.)

	Standard deviations			
C	0.0019	0.0054	0.0010	1.0
N, O(6)	0.0017	0.0046	0.0009	0.9
O(1-5)	0.0012	0.0033	0.0006	0.6
Br	0.0003	0.0007	0.0001	0.2

* The anisotropic thermal parameters, $T = \exp[-(\beta_{11}h^2 + \beta_{22}k^2 + \beta_{33}l^2 + 2\beta_{12}hk + 2\beta_{13}hl + 2\beta_{23}kl)]$, for the Br atom are ($\times 10^4$): 107, 305, 12, -5, 0, -9.

Discussion

The configuration of the *O-p*-bromobenzoate of batrachotoxin A is shown in the stereo drawing in Fig. 4. If the structural formula is rewritten in the following form



the steroid nucleus (rings A, B, C and D) is more readily recognized. Thus this substance is not only an alkaloid derived from an animal source but also a steroid, a rare combination. Another steroidal alkaloid derived from an animal source which has been isolated is

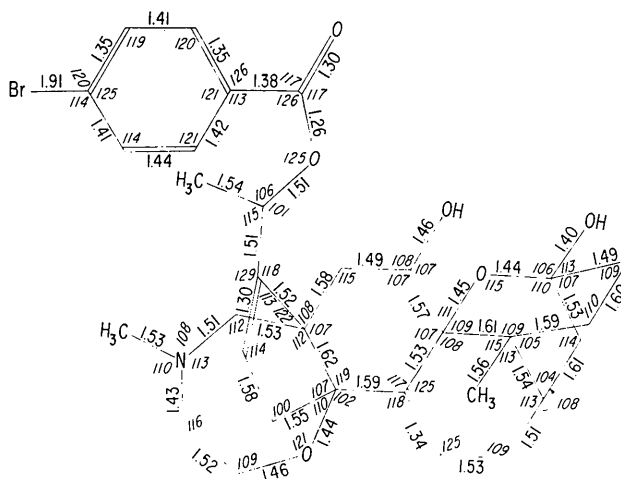
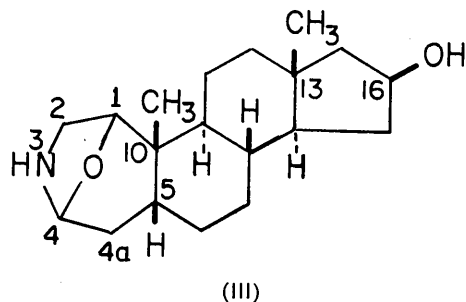


Fig. 3. Bond lengths and angles. See formula I for the numbering scheme. Additional bond angle values are:

C(2)C(3)O(2)	110°;	O(1)C(3)C(4)	112°;	C(5)C(10)C(9)	108°
C(1)C(10)C(19)	106°;	C(10)C(9)C(11)	113°;	O(2)C(9)C(8)	108°
C(12)C(13)C(18)	105°;	C(14)C(13)C(17)	102°;	C(13)C(14)O(4)	112°
C(8)C(14)C(15)	107°.				

samandarine:



This material was extracted from the skins of the fire salamander (*Salamandra maculosa*) and its structural formula was established by chemical means and confirmed by X-ray analysis (Wölfel, Schöpf, Weitz & Habermehl, 1961).

Batrachotoxinin A is a very potent cardiotoxin. Its action on the heart may be similar to that of digitalis, a plant steroid used in heart therapy. In fact, the configuration of the steroidal nucleus of the batrachotoxinin A molecule is very similar to that of digitoxigenin (IV) (Jacobs & Elderfield, 1935; Karle & Karle, 1969), a component of digitalis.

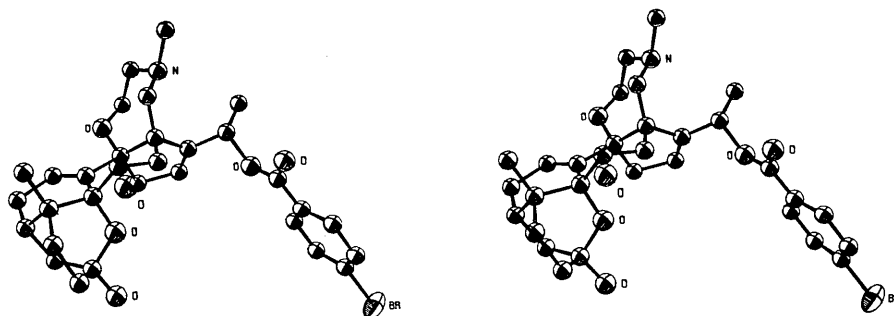


Fig. 4. Stereo diagrams for the *O-p*-bromobenzoate of batrachotoxinin A. The computer-drawn diagrams were prepared from a program by Johnson (1965). Experimentally determined thermal values were used only for the ellipsoid for the Br atom.

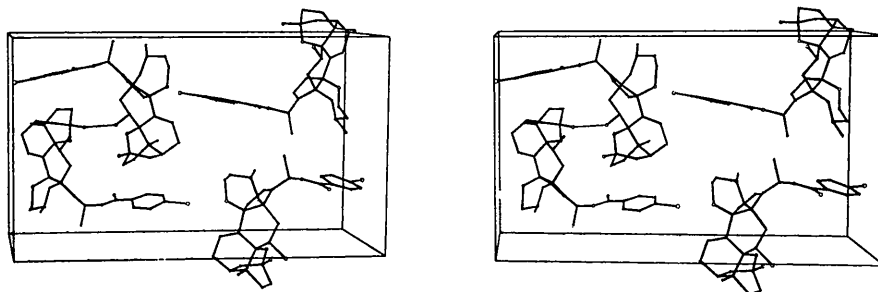


Fig. 5. The contents of a unit cell shown in a stereo diagram. The origin is in the upper left hand corner with a \downarrow , $c \rightarrow$ and b directed into the paper.

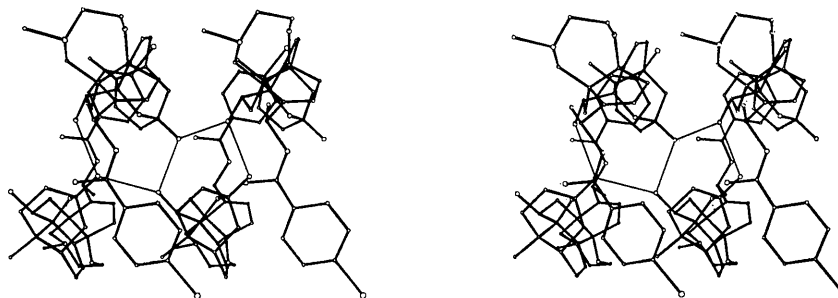
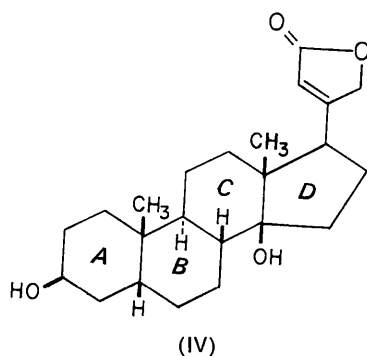


Fig. 6. A stereo drawing of four molecules showing the continuous spiral parallel to the b axis formed by hydrogen bonds (indicated by light lines in the middle of the diagram). The b direction is \rightarrow , $c \downarrow$ and a is directed into the paper.



In both molecules, rings *A* and *D* are in the *cis*-configuration with respect to rings *B* and *C*. Batrachotoxinin A, however, has an ether linkage between atoms (3) and (9), which, of necessity, makes ring *A* assume the boat configuration. The ether linkage forms a trigonal cage which includes ring *A*. Each of the three rings in the cage is boat-shaped. The double bond at C(7)–C(8) causes ring *B* to assume a half-chair conformation. The conformation of ring *B* appears to be influenced by the adjoining cage and as a result, the ring is somewhat flatter than the ideal half-chair. Atom C(5) is 0.29 Å below and C(10) is 0.53 Å above the plane formed by atoms C(6)–C(9). The conformation of ring *C* is influenced not only by the double bond at C(7)–C(8) but also by rings *D* and *E*, all of which share atoms C(13) and C(14). In ring *C*, atoms C(11) C(12) and C(13) are 1.08, 0.59, and 0.68 Å, respectively, above the plane formed by atoms C(7), C(8), C(9) and C(14).

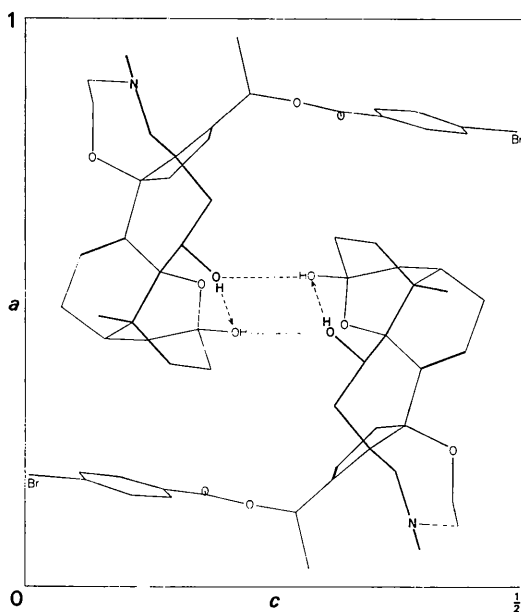
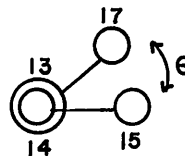


Fig. 7. A projection down the *b* axis showing the vertical spiral formed by hydrogen bonding between hydroxyl groups.

The five-membered ring *D* differs from rings *D* in other alkaloids that have been studied by X-ray diffraction in that it has a *cis* junction to ring *C* and a double bond between C(16) and C(17). The result is a flattened α envelope with C(14) 0.30 Å out of the plane of the other four atoms which are within 0.01 Å of their least-squares plane. The torsional angle about the C(13)–C(14) bond



is $18^{\circ}25'$ compared with an average of 45° for alkaloids without a double bond in ring *D* (Brutcher & Leopold, 1966).

In batrachotoxinin A, a seven-membered ring *E* is formed by bridging between the –CH₃ and –OH groups attached to C(13) and C(14) [compare with digitoxigenin (IV)] with a methyl ethyl amine group. This seven-membered ring has the chair conformation with atoms O(4), C(23), N and C(18) approximately in a plane (± 0.06 Å); atom C(22) is above the plane by 0.66 Å and atoms C(13) and C(14) are below the plane by 1.27 and 0.95 Å, respectively. The N atom is pyramidal with bond angles $\sim 110^{\circ}$. Another major difference between batrachotoxinin A and digitoxigenin is the presence of the –OH group at C(11), a function which characterizes the corticotropic hormones.

The contents of the unit cell are shown in the stereodrawing in Fig. 5. The molecules are packed rather loosely with most interatomic approaches considerably larger than 3.7 Å. The nearest approaches, except for hydrogen bonding, are 3.35 Å between O(6) and C(31') at $x, 1+y, z$; 3.69 Å between C(7) and Br'' at $\frac{1}{2}-x, \bar{y}, \frac{1}{2}+z$; and 3.77 Å between O(4) and Br''. It is noteworthy that molecules with irregular shapes often crystallize so as to take advantage of all possible hydrogen bonding. In this crystal, a continuous spiral of hydrogen bonds is formed around the screw axis at $x=\frac{1}{2}, z=\frac{1}{4}$ (Fig. 7). This continuous spiral is made from two independent hydrogen bonds between the hydroxyl groups at O(1) and O(3). Between two molecules related by the screw axis parallel to the *b* axis, the O(1)⋯O(3') distance is 2.77 Å, and between two molecules in adjacent cells in the *b* direction the O(1)⋯O(3'') distance is 2.99 Å. Atoms O(1) and O(3) act both as donors and acceptors. One of the hydrogen bonds is indicated by the light line in Fig. 5 and the spiral of hydrogen bonds parallel to the *b* axis is indicated by light lines in Fig. 6, where the screw axis is along the horizontal direction in the middle of the diagram.

A least-squares plane computed for the phenyl ring showed that for atoms C(25) to C(31), inclusive, and Br, the largest deviation from the plane is 0.03 Å. The

adjacent carboxyl group is rotated by 4.5° from the plane of the phenyl ring.

We wish to thank Dr Bernhard Witkop of the National Institutes of Health for bringing this problem to our attention and for valuable discussions concerning the chemical aspects.

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Acta Cryst. (1969). **B25**, 434

The Crystal Structure of Digitoxigenin, C₂₃H₃₄O₄

BY ISABELLA L. KARLE AND J. KARLE

Laboratory for the Structure of Matter, Naval Research Laboratory, Washington, D.C. 20390, U.S.A.

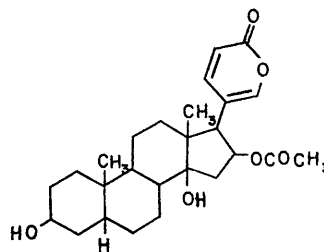
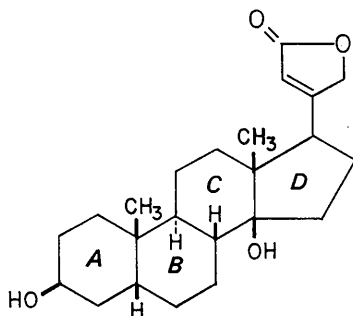
(Received 5 February 1968)

Digitoxigenin is the aglycone of digitoxin, which is one of the active ingredients of digitalis. It crystallizes in space group $P2_12_12_1$ with $Z=4$ and $a=7.250$, $b=15.015$ and $c=18.464$ Å. A partial structure was determined by obtaining phases directly from the normalized structure factors using the symbolic addition procedure for noncentrosymmetric crystals. The complete structure was derived from the partial structure with the use of the tangent formula. The X-ray analysis confirmed the stereoconfiguration of the molecule as determined by chemical means. The *A/B* and *C/D* ring junctions are both *cis*, giving the molecule a globular shape in contrast to the flat nature of most steroids whose crystal structures have been determined. In the crystal, endless chains of molecules are formed by hydrogen bonding between the terminal -OH and C=O groups. Although cross-linkages between symmetry related molecules involving the terminal and central hydroxyl groups are suggested by the favorable orientation of the hydroxyl groups, the actual O...O distance is 3.4 Å, which is too large for hydrogen bonding.

Introduction

Digitalis, a preparation made by extraction of dried seeds and leaves of the purple foxglove, *Digitalis purpurea*, is used extensively in heart therapy. The active components are the glycosides of digitoxigenin, digoxigenin and gitoxigenin. Acid hydrolysis produces the free aglycones which differ from each other only by a hydroxyl group. Digitoxigenin,

although of plant origin, is quite similar to the animal steroid bufotalin,



a toad venom with cardiotoxic properties. Dried and powdered toad skin had been commonly used as a remedy for dropsy until Withering introduced the use of digitalis.

Digitoxigenin differs from other steroids whose structures have been analyzed by X-ray diffraction, e.g. lanostenyl iodoacetate (Fridrichsons & Mathieson, 1953), 7 α -bromocholesteryl bromide (Bürki & Nowacki,