SYNTHESIS OF 5α-CINOBUFAGIN*,**

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Cinobufagin (*I*) and cinobufotalin (*IV*) isolated from the Chinese toad venom preparation Ch'an Su were used as starting points for synthesis of 5α-cinobufagin (*III*) *via* the 3-oxo-4-ene derivative *VII*. Lithium borohydride reduction of ketone *VII* afforded 5α-cinobufagin (*III*) accompanied by 3-epicinobufagin (*IX*). Ring A diene *II*, was obtained as one of the dichlorodicyanobenzoquinone oxidation products of 3-oxocinobufagin (*V*).

The cell growth inhibitory and cardiac properties of venomous toad secretions of the bufadienolide class are influenced by certain configurational, conformational or functional group changes[2]. In order to evaluate such effects on the *cis*-fused A ring of the toad venom constituent cinobufagin (*I*) we undertook synthesis of the A ring diene *II* and 5α-cinobufagin (*III*). Cinobufagin (*I*) and cinobufotalin (*IV*) isolated from the Chinese medicinal preparation Ch'an Su, were employed as starting compounds. Next, cinobufagin (*I*) was oxidized to ketone *V* by chromium trioxide in acid solution as earlier described[3-7]. Ketone *V* was dehydrogenated with 2,3-dichloro--5,6-dicyano-1,4-benzoquinone and the result was better than anticipated[8-10]. The product was a mixture of unsaturated ketones *II*, *VI* and *VII* that was separated on a column of silica gel to afford pure specimens of 4-ene *VII* and 1,4-diene *II*. The 3-oxo-1-ene *VI* resisted separation (2 : 3 ratio by ^{1}H NMR) from the starting ketone *V* and being incidental to the study was not identified beyond the cursory spectral interpretation. Olefinic ketones *II*, *VI* and *VII* exhibited characteristic NMR signals for the double bond hydrogen atoms at approximately δ 7·0 ($C_{(1)}$—H), and 6·0 ($C_{(2)}$—H and $C_{(4)}$—H) with a downfield shift of each A ring C—H position in diene *II*.

In 1962 the 3-oxo-4-ene *VII* was prepared (in low yield) by brominating cinobufagone (*V*) with N-bromosuccinimide (ultraviolet irradiation) followed by dehydro-

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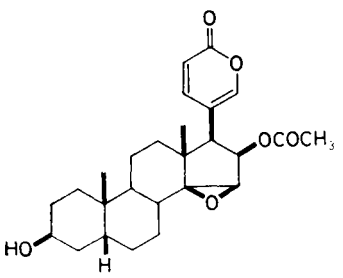
halogenation in boiling pyridine[7]. In the same study a higher yield was realized by acid catalyzed dehydration of hydroxy ketone *VIII* obtained from oxidation of cinobufotalin (*IV*) using chromium trioxide in acetic acid[7]. In our investigation a near quantitative yield of hydroxy ketone *VIII* was provided by oxidizing cinobufotalin (*IV*) with pyridinium chlorochromate. Acid-catalyzed (with acetic acid) dehydration of tertiary alcohol *VIII* to ketone *VII* was effected as previously described[7].

Reduction of 3-oxo-4-ene *VII* was conducted with lithium borohydride in pyridine as previously used for synthesis of 5α-resibufogenin[11,12] 5α-bufalin[11,13–15].



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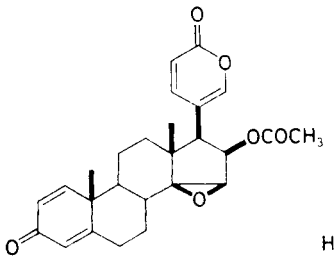

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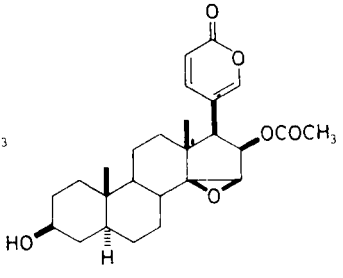

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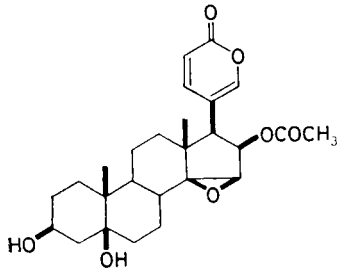

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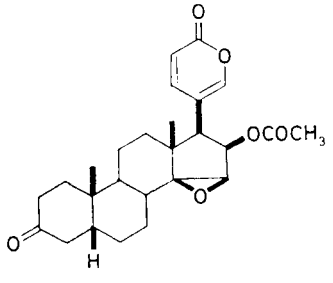

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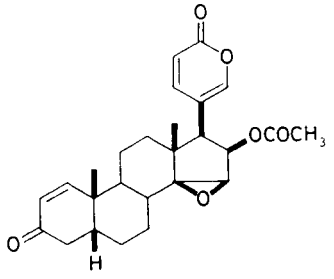

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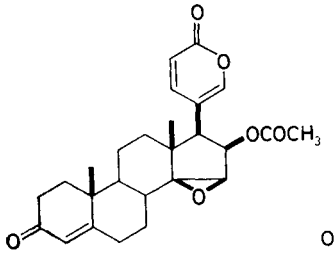

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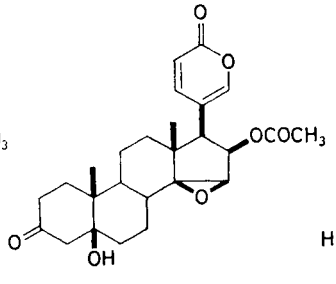
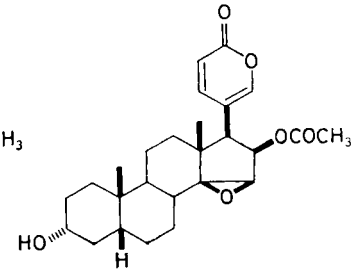


VIII



IX

By this means 3-ketone *VII* led to the described[6] 3-epicinobufagin (*IX*, 26% yield accompanied by a 25% yield of *III* and a small (~1%) amout of *I*). *I* and *IX* were identified by comparison with authentic samples.[16] Characterization of 5 α-cinobufagin (*III*) resided primarily with the chemical shift and signal pattern of the $C_{(3)}$—H (cf.[11]) in the ^{1}H NMR specrum, which exhibited a signal at δ 3.59 overlapped with the signal of the $C_{(15)}$—H at δ 3·62. The corresponding signals for cinobufagin (*I*) were at δ 4.15 (broad signal 15 Hz width) and for the 3-epi isomer *IX* at δ 3.67 (broad multiplet, 35 Hz width, overlapped with the $C_{(15)}$—H signal at δ 3·66).

Preliminary evaluation of 5 α-cinobufagin (*III*) against the U. S. National Cancer Institute's P 388 lymphocytic leukemia cell line gave an ED_{50} value indicating marginally inactive cell growth inhibition, whereas the natural cinobufagin was inactive. Results of an initial cardiac assessment will be reported as part of a broader survey.

EXPERIMENTAL

Cinobufagin and cinobufotalin were isolated from the Chinese medicinal preparation Ch'an Su (thin plate). Solutions of organic solvents were dried over anhydrous magnesium sulphate and solvent was evaporated *in vacuo*. Analytical samples were dried *in vacuo* over phosphorus pentoxide at 25°C for 12 h. Each sample appeared as a single spot on a TLC plate. The identity of samples prepared by different routes was confirmed by comparison of their IR and ^{1}H NMR spectra, TLC mobility, and mixturemelting point determinations unless stated otherwise. Column chromatography (CC) was performed using silica gel 60 (Merck, particle size 0·063—0·2 mm), preparative and analytical thin layer chromatography (TLC) on plates of silica gel GF or GHLF, respectively (Analtech, U.S.A.; with layer thickness 2 or 0·25 mm respectively). The plates were inspected by UV light (254 mm) and/or by spraying with concentrated sulphuric acid and heating. For analytical TLC see Table II.

Melting points were determined on a micromelting point apparatus (Reichert, Austria). Optical rotations were measured at 25°C with a Perkin–Elmer 241 polarimeter, IR spectra (given in cm^{-1}) with a Nicolet 10-MX spectrometer employing a thin layer (prepared by evaporation under nitrogen from dichloromethane solution) of solid sample on a potassium bromide pellet. The ^{1}H NMR spectra were measured using a Bruker WH90 (90·0 MHz) instrument. All NMR values were obtained by first-order analysis. Elemental analyses were carried out in the Spang Microanalytical Laboratory (Eagle Harbor, Michigan, U.S.A.) and MicAnal Organic Microanalysis Laboratory (Tucson, Arizona, U.S.A.).

16β-Acetoxy-14β,15-epoxy-3-oxobufa-4,20,22-trienolide (*VII*) and
16β-Acetoxy-14β,15-epoxy-3-oxobufa-1,4,20,22-tetraenolide (*II*)

A solution of ketone *V* (0·50 g, 1·14 mmol) prepared[3–7] by oxidation of cinobufagin (*I*) and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (0·50 g, 2·2 mmol) in dioxane (15 ml) was heated at reflux 4 h. The solvent was evaporated and the residue chromatographed on a column of silica gel (450 × 10 mm). Elution with hexane–acetone (9 : 1) afforded three major fractions. The first (0·12 g) contained a 2 : 3 mixture of unsaturated ketone *VI* (11%) and starting ketone *V* (14% recovery). Crystallization of the second fraction from acetone–hexane yielded 0·145 g (33%) of 3-oxo-4-ene *VII*: m.p. 192—195°C, $[\alpha]_D^{25}$ +40·9 (*c* 3·7; chloroform). Ref.[7] m.p. 208—212°C, and 201—208°C, $[\alpha]_D^{19}$ +40·4 (*c* 0·4; chloroform) and $[\alpha]_D^{19}$ +48·7 (*c* 1; chloroform). IR spectrum: 3 055 (epoxy, C—H), 2 966, 2 945, 2 872 (C—H), 1 744, 1 740, 1 726, 1 670 (C=O), 1 637,

TABLE I

Comparison of 90 MHz ^{1}H NMR signals for bufadienolides *I*—*IX*. Chemical shifts are in δ values (ppm) coupling constants (*J*) and signal widths (*W*) are in Hz and the concentration of samples was 14 mg/ml of deuteriochloroform (tetramethylsilane as internal standard)

Compound	Angular methyl		$C_{(16)}$—OAc	$C_{(17)}$—H (*J*)	$C_{(15)}$—H (*J*)	$C_{(3)}$—H (*W*)	$C_{(16)}$—H (*J*)	$C_{(4)}$—H (*W*)	$C_{(2)}$—H (*J*)	$C_{(23)}$—H (*J*)	$C_{(1)}$—H (*J*)	$C_{(21)}$—H (*J*)	$C_{(22)}$—H (*J*)
	2 s		s	d	d or s	m	dd or d	m	d	dd or d	d	dd or d	dd
I	0·99	0·82	1·89	2·79 (9·3)	3·65 (1·1)	4·15 (15)	5·46 (9·3, 1·1)	—	—	6·21 (9·7, 0·9)	—	7·16 (2·4, 0·9)	7·92 (9·7, 2·4)
II	1·26	0·90	1·88	2·84 (9·4)	3·59 (1·5)	—	5·36 (9·4, 1·5)	6·11 (7)	6·23 (9·9)	6·27 (10·1, 1·9)	7·04 (9·9)	7·17 (3·1, 1·9)	7·87 (10·1, 3·1)
III	0·84	0·82	1·88	2·80 (9·3)	3·62 (0·7)	3·59 (40)	5·42 (9·3, 0·7)	—	—	6·22 (10·1)	—	7·17 (1·8)	7·92 (10·1, 1·8)
IV	1·04	0·83	1·90	2·81 (9·3)	3·64 (1·1)	4·21 (20)	5·45 (9·3, 1·1)	—	—	6·22 (9·7, 0·7)	—	7·17 (2·5, 0·7)	7·90 (9·7, 2·5)
V	1·05	0·90	1·89	2·82 (9·2)	3·68 (0·9)	—	5·46 (9·2, 0·9)	—	—	6·22 (9·7, 0·9)	—	7·17 (2·6, 0·9)	7·91 (9·7, 2·6)
VI	1·23	0·86	1·89	2·83 (9·3)	3·67 (1·6)	—	5·41 (9·3, 1·6)	—	5·96 (10·2)	6·23 (10)	6·83 (10·2)	7·18 [a]	7·89 (10, 2·5)
VII	1·23	0·88	1·89	2·83 (9·2)	3·61 (1·2)	—	5·41 (9·2, 1·2)	5·76 (7)	—	6·23 (9·9, 0·7)	—	7·18 (2·6, 0·7)	7·89 (9·9, 2·6)
VIII	0·98	0·86	1·90	2·85 (9·5)	3·69	—	5·47 (9·5)	—	—	6·23 (9·9)	—	7·18 (1·8)	7·91 (9·9, 1·8)
IX	0·95	0·81	1·89	2·81 (8·8)	3·66 (0·9)	3·67 (35)	5·43 (8·8, 0·9)	—	—	6·20 (9·9)	—	7·17 (1·8)	7·91 (9·9, 1·8)

[a] Not distinguishable.

1 615, 1 537 (C=C), 1 236 (ester C—O), 1 126 (C—O), 1 050, 1 032 (ester C—O), 948, 952 (C=C), 840 (epoxy C—O), 785 (C=C). Refer to Table I for the ^{1}H NMR data.

The third fraction crystallized from chloroform–acetone to afford 0·14 g (32%) of ring A diene *II* melting at 175—177°C; $[\alpha]_D^{25}$ +1·2 (*c* 4; chloroform). IR spectrum: 3 050 (epoxide C—H), 2 975, 2 943, 2 860 (C—H), 1 745, 1 726, 1 664 (C=O), 1 625, 1 605, 1 539 (C=C), 1 243, 1 235 (ester C—O), 1 127 (C—O), 1 050, 1 030 (ester C—O), 950, 940 (C=C), 830 (epoxy C—O), 785 (C=C). The ^{1}H NMR spectral results have been entered in Table I. For $C_{26}H_{28}O_6$ (436·5) calculated: 71·54% C; 6·47% H; found: 71·68% C; 6·72% H.

16β-Acetoxy-14β,15-epoxy-5β-hydroxy-3-oxobufa-20,22-dienolide (*VIII*)

A solution of cinobufotalin (*IV*, 45·8 mg, 0·1 mmol) and pyridinium chlorochromate (43·1 mg, 0·2 mmol) in dichloromethane (10 ml) was stirred at room temperature for 4 h. To the mixture was added 2-propanol (1 ml) and stirring at room temperature was continued another 30 min. The solution was filtered through a column of silica gel (40 × 100 mm) and eluted with dichloromethane–acetone, (1 : 1 300 ml). The eluate was evaporated and the residue was chromatographed on a column of silica gel (40 × 100 mm) in dichloromethane–acetone (1 : 1, 300 ml). The main fraction contained 39 mg (86%) of 3-ketone *VIII* in the form of a resin-like solid: $[\alpha]_D^{25}$ +12·6 (*c* 2·95; chloroform). Ref.[7] m.p. 246—248°C or 256—258°C and $[\alpha]_D^{21}$ +14·7 (*c* 2; chloroform). IR spectrum: 3 483 (OH), 3 052 (epoxide, C—H), 2 930, 2 872, 2 854 (C—H), 1 745, 1 719 (C=O), 1 635, 1 539 (C=C), 1 242 (acetic CCOO), 1 135 (C—O), 1 033 (ester C—O), 954 (C=C), 835 (epoxy, C—O), 785 (C=C). The ^{1}H NMR spectrum has been recorded in Table I.

16β-Acetoxy-14β,15-epoxy-3β-hydroxy-5α-bufa-20,22-dienolide (5α-Cinobufagin, *III*) and 16β-Acetoxy-14β,15-epoxy-3α-hydroxy-5β-bufa-20,22-dienolide (3-Epicinobufagin, *IX*)

To ketone *VII* (0·32 g, 0·74 mmol) in pyridine (30 ml) was added (in portions) lithium boro-

TABLE II

Thin layer chromatographic properties of bufadienolides *I*—*IX* in solvent systems A—E (Analtech Uniplate Silica Gel GHLF plates measuring 100 × 200 × 0·25 mm in a solvent saturated 25 × 25 × 7 cm chamber at 22° ± 1°C)

Compound	Color by detection with sulfuric acid and heating	Solvent system, R_F[a]				
		A	B	C	D	E
I	pink-reddish gray-gray	0·54	0·43	0·54	0·40	0·11
II	brown-grayish yellow	0·61	0·55	0·59	0·50	0·18
III	brown grayish red-brown	0·53	0·48	0·52	0·45	0·12
IV	violet red-brownish green	0·46	0·43	0·31	0·32	0·08
V	pink-brown-grayish brown	0·56	0·54	0·59	0·49	0·19
VI	blue-grayish pink	0·46	0·42	0·44	0·40	0·07
VII	violet-black	0·44	0·34	0·23	0·29	0·06
VIII	brown-brownish gray	0·49	0·38	0·32	0·33	0·09
IX	graygreen-grayish brown	0·48	0·41	0·37	0·36	0·11
R[b]	greenish brown	0·56	0·42	0·43	0·45	0·14

[a] A hexane–acetone (1 : 1), B ethyl acetate, C dichloromethane–acetone (5 : 1), D toluene–acetone (5 : 2), E ethyl acetate–hexane (1 : 1). The R_F value was obtained as the average of developing five times. [b] Resibufogenin.

hydride (175 mg, 8 mmol) with cooling (ice bath) and stirring. After 6 h (reaction was complete by TLC) the solution was filtered through a column of silica gel (100 × 40 mm) and product eluted with hexane–acetone (1 : 1). Solvent was removed and the residue chromatographed on a column of silica gel (500 × 25 mm). Elution was performed with dichloromethane–acetone and a gradient of 9 : 1 (2 l.) to 1 : 1 (1 l.). Six fractions were collected; the first and sixth (15 mg) contained non-bufadienolide impurities, the second starting ketone *VII* (10 mg, 3% recovery), the third a mixture of cinobufagin (*I*, in ~1% yield) and two other components (by ^{1}H NMR, the ratio was c. 1 : 1 : 1) weighing 13 mg. The fourth fraction provided 92 mg (28%) of 5α-cinobufagin (*III*) which was crystallized from methanol–diethyl ether: m.p. 183—185°C, $[\alpha]_D^{25}$ −4·6 (*c* 4·5; chloroform). IR spectrum, 3 450 (OH), 3 050 (epoxide, C—H) 2 969, 2 932, 2 860 (C—H), 1 743, 1 726 (C=O), 1 634, 1 539 (C=C), 1 238 (ester C—O), 1 125 (C—O), 1 048 (ester C—O), 950, 932 (C=C), 830 (epoxy, C—O), 783 (C=C). For the ^{1}H NMR summary see Table I. For $C_{26}H_{34}O_6$ (442·6) calculated: 70·56% C, 7·74% H; found: 70·38% C, 7·90% H. From the fifth fraction was obtained 3-epicinobufagin (*IX*), (86 mg, 26%). A pure specimen crystallized from methanol: m.p. 136—138°C, $[\alpha]_D^{25}$ −1·8 (*c* 3·4; chloroform), ref.[16] m.p. 137—139°C, identical with an authentic specimen prepared from ketone *V*. Consult ref.[16] and Table I for the ^{1}H NMR interpretations.

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