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Bufadienolides. 28. Marinobufotoxin[1]

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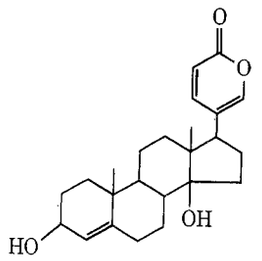
Syntheses of marinobufagin (**5b**) and marinobufotoxin (**5e**) have been achieved. The principal synthetic transformations involved selective dehydration of telocinobufagin (**2**) and addition of hypohalous acid to the resulting olefin (**2** → **3** → **4**) followed by dehydrohalogenation to yield marinobufagin (**5b**). Application of a carefully developed mixed carbonic anhydride reaction to the condensation of marinobufagin suberate (**5c**) with arginine monohydrochloride provided marinobufotoxin (**5e**).

Almost 50 years elapsed between isolation[2] of marinobufagin (**5b**) from the American toad, *Bufo marinus*, and assignment[3] of structure **5b**. Nearly 40 years passed before the structure of marinobufotoxin (**5e**)[4] was firmly estab-

lished.[1b] The suberylarginine side chain of marinobufotoxin is characteristic of the toad venom bufadienolide constituents generally known as "bufotoxins." Of the nine such substances which have been reported, only the structures of marinobufotoxin and bufotoxin[5] are known with certainty.[6]

The formal total synthesis of scillarenin (1)[7] offered in principle a good possibility of extension to telocinobufagin



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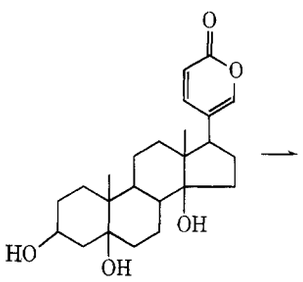
(2)[8] and thence to marinobufagin (**5a**) and marinobufotoxin (**5e**). By attainment of this synthetic outline it was



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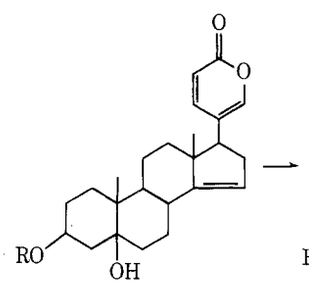
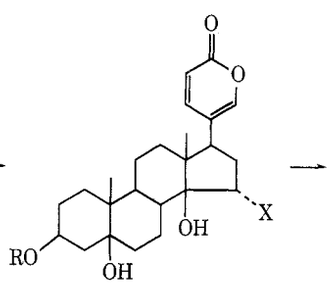
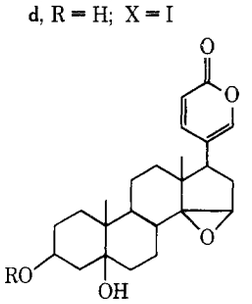


3a, R = H
b, R = $COCH_3$



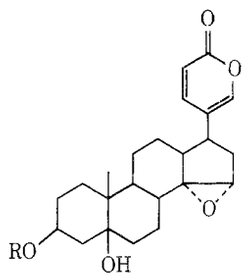
4a, R = $COCH_3$; X = I
b, R = $COCH_3$; X = Br
c, R = H; X = Br
d, R = H; X = I



5a, R = $COCH_3$
b, R = H
c, R = $CO(CH_2)_6CO_2H$
d, R = $CO(CH_2)_6CO_2CH_3$
e, R = $CO(CH_2)_6CONHCH(CH_2)_3NHCNH_2$ (with CO_2H and NH)

hoped that these bufadienolides would be made more readily available for biological evaluation[9] and that the structure of marinobufotoxin would be unequivocally established. As noted in a preliminary report[1b] we recently confirmed the structure of marinobufotoxin (**5e**) by partial synthesis from telocinobufagin (**2**). Subsequently we succeeded in obtaining telocinobufagin (**2**) by a formal total synthetic route employing bufalin and scillarenin as relays.[7,10] Accordingly, the pathway from telocinobufagin (**2**) *via* marinobufagin (**5b**) to marinobufotoxin (**5e**) which will now be described in detail comprises the first formal total synthesis of a bufotoxin.

The first step planned for conversion of telocinobufagin (**2**) to marinobufagin (**5b**) involved selective elimination of the 14β-hydroxyl group. Here, our prior experience with such steroid D-ring systems suggested that partial release of steric compression by removal of the 14β-hydroxyl group might favor a selective elimination. In practice, the 14β-hydroxyl group was readily and selectively (51% yield) removed by treating telocinobufagin (**2**) with hydrochloric acid in methanol.[11a] The resulting 14-olefin **3a** was selectively (92% yield) acetylated to provide 3β-acetate **3b.** Reaction of 3β-acetoxy-14-dehydrotelocinobufagin (**3b**) with hypoiodous acid, prepared *in situ,* readily gave iodohydrin **4a** which, upon contact with pyridine, afforded marinobufagin acetate (**5a**) in 70% overall yield. An analogous reaction sequence was performed employing hypobromous acid to obtain the bromohydrin intermediate **4b.** Preparation of the hypohalous acid from either *N*-bromosuccinimide or *N*-bromoacetamide led to 60% yields of marinobufagin 3β-acetate (**5a**). For purposes of chemical and biological comparison, 14-dehydrotelocinobufagin (**3a**) and its acetate derivative (**3b**) were each oxidized with *m*-chloroperbenzoic acid to yield 14α,15α-epoxides **6a** and **6b.**



6a, R = H
b, R = $COCH_3$

Application of the hypohalous acid reactions to 14-dehydrotelocinobufagin resulted in a convenient synthesis of marinobufagin (**5b**)[11b] in about 45% yields by way of the iodohydrin (**4d**) and in about 30% yields by way of the bromohydrin intermediate. The synthetic specimen of marinobufagin (**5b**) was found to be identical with an authentic specimen isolated from Ch'an Su.

The following experiments leading to marinobufotoxin (**5e**) are based on an extensive series of experiments developed (over a number of years) for the partial synthesis of bufotoxin.[5] Over this period a variety of synthetic approaches[12] and selective protection methods for elaborating the bufadienolide suberylarginine side chain were explored and this accumulated experience finally led to selection of the general and convenient procedure which now follows. By condensing marinobufagin (**5b**) with suberic α-anhydride the corresponding 3-suberate ester **5c** was obtained (92% yield). The methyl ester derivative **5e** was prepared in nearly quantitative conversion by methylating acid **5c** with diazomethane. Methyl ester **5d** was found to be identical with an authentic sample prepared from a specimen of carboxylic acid **5c** isolated from Ch'an Su.

The mixed carbonic anhydride prepared from suberic acid derivative **5c** and isobutyl chloroformate was added, in the cold, to L-arginine monohydrochloride and 88% conversion to marinobufotoxin was realized. Thus, the simple ex-

pedient of selectively protecting the guanidino unit of arginine by protonation obviated the necessity for more extensive protection which upon removal would generally involve the bufadienolide system in unwanted side reactions.[13] The synthetic specimen[14] of marinobufotoxin (**5e**) was found identical with an authentic specimen of the natural product kindly provided by Professor K. Meyer.

Experimental Section[15]

14-Dehydrotelocinobufagin (3a). A solution of telocinobufagin (**2**, 200 mg), methanol (35 ml), and 35% hydrochloric acid (0.04 ml) was heated at reflux for 1.5 hr, poured into ice–water and extracted with chloroform. The solvent extract was washed with water and evaporated to dryness. The crude product was chromatographed on a column of silica gel and the fraction eluted with ligroin–acetone (3:1) was recrystallized from acetone to give 14-dehydrotelocinobufagin (**3a**, 105 mg) as prisms, mp 198–200°. Recovered starting material, telocinobufagin, weighed 85 mg. The pure specimen of olefin **3a** showed λ_{max} (95% EtOH) 300 nm (log ϵ 3.76); ν_{max} (KBr) 3450, 3420 (OH), 1720–1700 (conjugated CO), 1634 (conjugated C=C), 1667 (R_2C=CHR), 1537 (conjugated C=C), 958 (C=C), 836, 814 (R_2C=CHR), 755, 745, cm^{-1} (C=C); pmr (10% solution in $CDCl_3$) δ 0.73 (3 H, s, 18-CH_3), 0.97 (3 H, s, 19-CH_3), 3.44 (1 H, broad peak, 5-OH), 4.17 (1 H, broad s, 3-H), 5.25 (1 H, s, R_2C=CHR), 6.29 (1 H, d, J = 10.5 Hz, 23-H), 7.28 (1 H, d, J = 3 Hz, 21-H), 7.37 (1 H, dd, J = 10.5 and 3 Hz, 22-H); mass spectrum m/e 384 (M^+), 366 ($M^+ - 18$), 348 ($M^+ - 36$), 333 ($M^+ - 51$), 312 ($M^+ - 72$).

Anal. Calcd for $C_{24}H_{32}O_4$: C, 74.97; H, 8.39. Found: C, 75.01; H, 8.24.

The 3β-acetate derivative (**3b**) of 14-dehydrotelocinobufagin was prepared by acetylating 0.18 g of 14-dehydrotelocinobufagin (**3a**) with acetic anhydride (2.7 ml)–pyridine (3.6 ml) at room temperature for 24 hr. Column chromatography (silica gel) of the crude product and elution with ligroin–acetone (9:1) provided 0.17 g of **3β-acetoxy-14-dehydrotelocinobufagin** (**3b**) as an amorphous solid: λ_{max} 300 nm (log ϵ 3.75); ν_{max} (KBr) 3520 (OH), 1760–1720 (ester CO and conjugated CO), 1635, 1535 (conjugated C=C), 1670 (R_2C=CHR), 1240, 1220 (ester CO), 950 (C=C), 850, 828 (R_2C=CHR), 753, 742, cm^{-1} (C=C); pmr (10% solution in $CDCl_3$) δ 0.74 (3 H, s, 18-CH_3), 0.99 (3 H, s, 19-CH_3), 2.09 (3 H, s, 3-$OCOCH_3$), 3.05 (1 H, broad peak, 5-OH), 5.29 and 5.24 (2 H, overlapped broad peak, 3-H, and 15-H), 6.28 (1 H, d, J = 10 Hz, 23-H), 7.27 (1 H, d, J = 2.5 Hz, 21-H), 7.32 (1 H, dd, J = 10 and 2.5 Hz, 22-H); mass spectrum m/e 426 (M^+), 408 ($M^+ - 18$), 366 ($M^+ - 60$), 348 ($M^+ - 78$), 312 ($M^+ - 114$), 294 ($M^+ - 132$).

Anal. Calcd for $C_{26}H_{34}O_5$: C, 73.21; H, 8.04. Found: C, 73.35; H, 8.06.

Marinobufagin 3β-Acetate (5a). Method A. In a typical experiment, a solution of *N*-iodosuccinimide (20 mg) in acetone (2 ml)–water (2 ml) was added to 14-dehydrotelocinobufagin acetate (**3b**, 20 mg) in acetone (3.2 ml). Before a solution prepared from sodium sulfite (20 mg) and water (0.5 ml) was added, the mixture was stirred for 20 hr at room temperature. The solution was concentrated to approximately one-third of the original volume, poured into ice-water with stirring, and extracted with chloroform. The combined extract was washed with water, solvent was removed, and the crude iodohydrin (**4a**, 22 mg) was stirred in pyridine (1 ml) for 2 hr at room temperature. After evaporation of solvent the product was column chromatographed (silica gel) and the fraction eluted with ligroin–acetone (9:1) was recrystallized from acetone–*n*-hexane to afford 14 mg of marinobufagin acetate (**5a**) as prisms melting at 198–216°.

Method B. When *N*-bromosuccinimide (20 mg) was substituted for *N*-iodosuccinimide as described in method A, olefin **3b** (20 mg) led to 21 mg of the crude bromohydrin **4b.** Conversion of the bromohydrin to marinobufagin acetate (**5a**) with pyridine resulted in a 12-mg (mp 194–215°) yield.

Method C. The preceding reaction (method A or B) was repeated using 18 mg of olefin **3b** and 18 mg of *N*-bromoacetamide. Similar treatment of the crude bromohydrin (20 mg) with pyridine led to 11 mg of marinobufagin acetate (**5a**) melting at 198–215°.

The samples of marinobufagin acetate (**5a**) prepared by methods A–C were found identical with acetate **5a** prepared from natural marinobufagin (**5b**).

3β,5β,Dihydroxy-14α,15α-epoxy-5β-bufa-20,22-dienolide (14α,15α-*epi*-Marinobufagin, 6a). To 70 mg of 14-dehydrotelocinobufagin (**3a**) in chloroform (3 ml) was added *m*-chloroperbenzoic acid (50 mg). After 2 hr at room temperature and dilution with chloroform, the solution was poured into ice–water. The chloroform layer was washed with water, dilute sodium thiosulfate solution, and water. Solvent was removed and the residue (68 mg) was chromatographed on a column of silica gel. Elution with ligroin–acetone (5:1) provided 60 mg of 14α,15α-*epi*-marinobufagin, mp 209–212° (from acetone), as colorless needles: λ_{max} 298 nm (log ϵ 3.74); ν_{max} (KBr) 3530, 3480 (OH), 1720–1700 (conjugated CO), 1655, 1550 (conjugated C=C), 1245 (epoxy CO), 960 (C=C), 830 (epoxy CO), 760 cm^{-1} (C=C); pmr δ 0.68 (3 H, s, 18-CH_3), 0.97 (3 H, s, 19-CH_3), 3.53 (1 H, s, 15β-H), 4.19 (1 H, broad s, 3α-H), 6.29 (1 H, d, J = 10 Hz, C-23 H), 7.18 (1 H, d, J = 3 Hz, C-21 H), 7.49 (1 H, q, C-22 H), J = 10 and 3 Hz; mass spectrum m/e 400 (M^+), 382 ($M^+ - H_2O$), 364 ($M^+ - 2H_2O$), 346 ($M^+ - 54$).

Anal. Calcd for $C_{24}H_{32}O_5$: C, 71.97; H, 8.05. Found: C, 71.92; H, 8.08.

3β-Acetoxy-5β-hydroxy-14α,15α-epoxy-5β-bufa-20,22-dienolide (14α,15α-*epi*-Marinobufagin, 6b). Method A. A solution of 14-dehydrotelocinobufagin acetate (**3b**, 25 mg) in chloroform (1.5 ml) was treated with *m*-chloroperbenzoic acid (18 mg) as described for the preparation of α-epoxide **6a.** After chromatography and elution with ligroin–acetone (9:1) 21 mg (amorphous solid) of 14α,15α-*epi*-marinobufagin acetate (**6b**) was obtained and found identical with the material prepared as follows.

Method B. Alcohol **6a** (60 mg) was acetylated with acetic anhydride (0.85 ml)–pyridine (1.2 ml) and the product was isolated as described in method A to yield 51 mg of 14α,15α-*epi*-marinobufagin acetate (**6b**) as an amorphous solid: λ_{max} 298 nm (log ϵ 3.72); ν_{max} (KBr) 3680 (OH), 3030 (CH), 1760, 1740, 1720 (conjugated CO and ester CO), 1650, 1550 (conjugated C=C), 1260, 1240, 1220 (ester and epoxy CO), 955 (C=C), 830 (epoxy CO), 740 cm^{-1} (C=C); pmr (10% solution in $CDCl_3$) δ 0.78 (3 H, s, 18-CH_3), 1.08 (3 H, s, 19-CH_3), 2.15 (3 H, s, 3-$OCOCH_3$), 3.12 (1 H, broad peak, 5-OH), 3.60 (1 H, s, 15-H), 5.29 (1 H, broad peak, 3-H), 6.31 (1 H, d, J = 10 Hz, 23-H), 7.22 (1 H, d, J = 2.5 Hz, 21-H), 7.25 (1 H, dd, J = 2.5 and 10 Hz, 22-H); mass spectrum m/e 442 (M^+), 424 ($M^+ - H_2O$), 406 ($M^+ - 36$), 382 ($M^+ - 60$), 364 ($M^+ - 78$), 346 ($M^+ - 96$), 331 ($M^+ - 111$), 328 ($M^+ - 114$).

Anal. Calcd for $C_{26}H_{34}O_6$: C, 70.56; H, 7.74. Found: C, 70.63; H, 7.73.

Marinobufagin (5b). Reaction of 14-dehydrotelocinobufagin (**2a**, 20 mg) with hypobromous acid prepared from *N*-bromosuccinimide (20 mg) was executed as described above for the preparation of iodohydrin **4b.** The crude bromohydrin **4c** upon treatment with pyridine and chromatographic purification (elution with 3:1 ligroin–acetone and recrystallization from acetone) led to marinobufagin (**5b**, 6.5 mg) as prisms melting at 223–225°.

When the reaction was repeated with 15 mg of olefin **3a** and *N*-bromo-acetamide (15 mg) the crude bromohydrin (**4c**, 18 mg) gave, after treatment with pyridine (1.5 ml), 5 mg of marinobufagin (prisms, mp 222–224°). The yield of marinobufagin was higher when the *N*-iodosuccinimide (20 mg) method was applied to olefin **3a** (20 mg). Following isolation by chromatography and recrystallization from acetone, 8.4 mg of prisms (**5b**) was obtained. In each case the synthetic sample of marinobufagin was found identical with the natural product isolated from Ch'an Su.

Marinobufagin 3β-Suberate (5c). A mixture prepared from marinobufagin (**5b**, 50 mg), suberic α-anhydride (mp 65–66°, 110 mg),[16] and pyridine (2 ml) was heated at reflux for 6 hr. The reaction mixture was brought (under reduced pressure) to dryness, water was added, and the mixture was extracted with chloroform. The extract was washed with water, dilute potassium bicarbonate solution, dilute hydrochloric acid, and water. After removal of the solvent the residue (56 mg) was submitted to preparative thin layer chromatography using dichloromethane–methanol–ammonium hydroxide (7:3:1) as developing solvent. The substance corresponding to R_f 0.65 (located by aid of ultraviolet light) was eluted with chloroform–methanol (4:1) to give marinobufagin 3-suberate (**5c**, 46 mg, 92% yield) as a colorless, oily material: tlc R_f 0.34 with chloroform–ethyl acetate–formic acid (3:4:0.5), 0.40 with chloroform–methanol (9:1); color, purple to greenish pink with sulfuric acid spray. The structure was confirmed by preparation and analysis of the methyl ester (**5d**) derivative as summarized in the following experiment.

Marinobufagin 3-Suberate Methyl Ester (5d). An ethereal solution of acid **5c** (20 mg) was methylated with diazomethane in ether. The product was purified by silica gel column chromatography using acetone–ligroin (1:6) to give 19.2 mg of methyl ester **5d** (96% yield), mp 107–112°, as colorless needles from acetone–ether. The melting point, tlc R_f values, and spectral data (uv, ir, nmr, and

mass spectrum as described below) were identical with those of an authentic sample[17] prepared from acid **5c** which was isolated from the Japanese toad, *Bufo formosus* Boulenger. Also, the data are in good agreement with those for methyl ester **5d** reported by Linde-Tempel:[14] tlc R_f 0.46 using acetone–chloroform–ligroin (3:3:4), 0.61 using chloroform–methanol (9:1), 0.44 using chloroform–ethyl acetate–formic acid (3:4:0.5); color with sulfuric acid spray, light greenish pink → purple; λ_{max} (MeOH) 300 nm (log ϵ 3.72) ν_{max} (KBr) 3580, 3460 (OH), 3040 (CH), 1740 (ester CO), 1720–1700 (conjugated CO), 1645, 1545 (conjugated C=C), 1260, 1230 (ester CO), 957 (C=C), 830 (epoxy CO), 795 cm^{-1} (C=C); pmr (10% solution in $CDCl_3$) δ 0.78 (3 H, s, 18-CH_3), 1.01 (3 H, s, 19-CH_3), 3.51 (1 H, s, 15-H), 3.64 (3 H, s, -$COOCH_3$), 4.28 (2 H, t, -CH_2COR), 5.24 (1 H, broad s, 3-H), 6.19 (1 H, d, J = 10 Hz, 23-H), 7.23 (1 H, d, J = 3 Hz, 21-H), 7.74 (1 H, t, J = 10 and 3 Hz, 22-H); mass spectrum m/e 570 (M^+), 552 ($M^+ - 18$), 534 ($M^+ - 36$), 401, 382, 364, 346, 328, 213, 171, 157, 145, 138, 129, 123, 105.

Marinobufotoxin (5e). A solution of suberate half ester **5c** (19 mg) in tetrahydrofuran (2 ml) containing triethylamine (0.03 ml) was stirred for 15 min at −10°. Then a solution of isobutyl chloroformate (0.02 ml) in tetrahydrofuran (0.2 ml) was added and stirring was continued for 40 min. Methanol (1 ml) was added, followed by a solution of arginine monohydrochloride (freshly prepared from arginine and concentrated hydrochloric acid in methanol) in methanol (1.5 ml)–water (0.07 ml). The solution was added dropwise over an approximate 3-min period. Stirring was continued for 2 hr at −5 to 0°. The mixture was concentrated under reduced pressure (below 40°) to an oily residue which was dissolved in a small amount of methanol and subjected to preparative thin layer chromatography using dichloromethane–methanol–ammonium hydroxide (7:3:1) as eluant. The product corresponding to R_f 0.24 was located with the aid of ultraviolet light and extracted with chloroform–methanol (4:1). Recrystallization of the bufotoxin from 80% ethanol provided marinobufotoxin (**5e**, 17 mg, 88% yield), mp 176–185°, as colorless, fine prisms: color on tlc, light greenish pink → light purple with sulfuric acid and bluish purple with ninhydrin; λ_{max} (MeOH) 300 nm (log ϵ 3.73); ν_{max} (KBr) 3590, 3380, 3200 (OH), 2800–2400 (broad, -COOH, NH_2,NH, and -C=NH), 1750 (ester CO and -COOH), 1730–1720 (conjugated CO), 1680 (CONH), 1650–1630 (conjugated C=C and ONH), 1540 (conjugated C=C), 1260, 1230 (ester CO), 954 (C=C), 830 (epoxy CO), 797 cm^{-1} (C=C).

Anal. Calcd for $C_{38}H_{56}O_9N_4$: C, 64.16; H, 8.05; N, 7.86. Found: C, 64.22; H, 8.08; N, 7.93.

The compound was found to be identical with an authentic sample of natural marinobufotoxin (mp 174–181°)[14] from Professor Meyer.

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References and Notes

(1) (a) Contribution 87 of the series Steroids and Related Natural Products. For the preceding part see G. R. Pettit and Y. Kamano, *J. Org. Chem.*, **39**, 2632 (1974). (b) A preliminary report of the present study has been summarized by Y. Kamano and G. R. Pettit, *Experientia*, **28**, 768 (1972).
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(3) For leading references to the excellent structural studies of Meyer and colleagues see P. Hofer, H. Linde, and K. Meyer, *Helv. Chim. Acta*, **43**, 1955 (1960).
(4) H. Jensen and E. A. Evans, *J. Biol. Chem.*, **104**, 307 (1934); H. Jensen and K. K. Chen, *J. Amer. Chem. Soc.*, **59**, 767 (1937); V. Deulofeu and J. R. Mendive, *Justus Liebigs Ann. Chem.*, **534**, 288 (1938); V. Deulofeu, E. Duprat, and R. Lobriols, *Nature (London)*, **145**, 671 (1940).
(5) G. R. Pettit and Y. Kamano, *J. Chem. Soc., Chem. Commun.*, 45 (1972).
(6) Quite likely the remaining seven bufotoxins also have the 3-suberylarginine structure analogous to bufotoxin and marinobufotoxin. Specifically, the seven other bufotoxins are alvarobufotoxin, fowlerobufotoxin, arenobufotoxin, cinobufotoxin, gamabufotoxin, regularobufotoxin, and viridobufotoxin. See respectively K. K. Chen and A. L. Chen, *J. Pharmacol. Exp. Ther.*, **49**, 7 (1933); K. K. Chen and A. L. Chen, *ibid.*, **49**, 26, 307, 503, 514, 529, (1933); K. K. Chen, H. Jensen, and A. L. Chen, *ibid.*, 43, 13 (1931); S. Ohno and M. Komatsu, *Yakugaku Zasshi*, **73**, 651, 796 (1953); H. Wieland and H. Behringer, *Justus Liebigs Ann. Chem.*, **549**, 209 (1941); H. Wieland and F. Vocke, *ibid.*, **481**, 215 (1930); H. Jensen, *J. Amer. Chem. Soc.*, **57**, 1765 (1935); K. K. Chen, H. Jensen, and A. L. Chen, *J. Pharmacol. Exp. Ther.*, **49**, 14 (1933). Unfortunately, we have been unable to obtain authentic samples corresponding to any of these bufotoxins. Thus, definite structural assignments will apparently have to await the reisolation of these substances from the respective toad venoms.
(7) U. Stache, K. Radscheit, W. Fritsch, H. Kohl, W. Haede, and H. Ruschig, *Justus Liebigs Ann. Chem.*, **750**, 149 (1971); Y. Kamano and G. R. Pettit, *J. Org. Chem.*, **39**, 2629 (1974); G. R. Pettit and Y. Kamano, *J. Amer. Chem. Soc.*, **94**, 8592 (1972).
(8) This toad venom component was accorded a structural assignment by K. Meyer, *Helv. Chim. Acta*, **32**, 1593 (1949).
(9) Both telocinobufagin and marinobufagin have been isolated from the Chinese preparation Ch'an Su and, for example, the latter is known to have pronounced cardiac activity: K. Meyer, *Pharm. Acta Helv.*, **24**, 222 (1949); Y. Kamano, H. Yamamoto, K. Hatayama, T. Tanaka, M. Shinohara, and M. Komatsu, *Tetrahedron Lett.*, 5668 (1968).
(10) Y. Kamano and G. R. Pettit, *J. Org. Chem.*, **38**, 2202 (1973); G. R. Pettit and Y. Kamano, *ibid.*, **39**, 0000 (1974).
(11) (a) We wish to thank a referee for locating an analogous reaction involving strophantidinic acid methyl ester described by Jacobs and Gustus, *J. Biol. Chem.*, **74**, 795 (1927). (b) The reverse transformation, namely conversion of marinobufagin to telocinobufagin, has been reported by M. Bharucha, H. Jäger, K. Meyer, T. Reichstein, and O. Schindler, *Helv. Chim. Acta*, **42**, 1395 (1959).
(12) We gratefully acknowledge (in chronological order) the contributions of Miss V. Thiele and Drs. K. Jaeggi, J. C. Knight, and S. Gabriel in these preliminary studies.
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(15) Introduction to the experimental section of part 27[1] provides an explanation of the general experimental (for example, melting points uncorrected and chromatography with silica gel columns and silica gel HF_{254} thin layer and preparative layer plates) and instrumental (by Dr. P. Brown, Messrs. R. Scott and E. Kelley, and Miss K. Reimer) techniques employed in the present study. The mutual identity of synthetic with natural specimens was established by mixture melting point determination and comparison thin layer chromatography and infrared spectra.
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