(dd, 1, J = 8 Hz and J = 1 Hz, 4-H), 4.90 (t, 1, J = 8 Hz, 3-H), 5.70 (m, 2, 1- and 6-H's), 6.60 (m, 2, Ar H's), 7.25 (m, 1, Ar H), 7.6–7.9 (m, 4, Pht H's); MS, m/e 565 (M^+); TLC ($CHCl_3$, two passes) R_f 0.42.

Acknowledgment. This investigation was supported by PHS Grant CA 26288 awarded by the National Cancer Institute, DHHS. We thank Dennis Knowles for the large-scale preparation of starting materials.

Registry No. **9**, 5307-05-1; **12**, 4603-89-8; **13**, 42775-84-8; **14**, 108512-22-7; **15**, 108590-49-4; **16**, 108590-50-7; **17**, 108512-23-8; **18**, 108512-24-9; **20**, 108512-25-0; **21**, 108512-26-1; **23**, 108512-27-2; **24**, 108512-29-4; **25**, 108512-31-8; **26**, 108512-32-9; **27**, 108512-33-0; **28**, 108512-34-1; **29**, 108512-35-2; **30**, 108512-36-3; **31**, 108512-28-3; **32**, 108512-30-7; **33**, 108512-37-4; **35**, 108512-38-5; **36**, 108512-39-6; **38**, 108512-41-0; **40**, 108512-40-9; **41**, 108512-43-2; **42**, 108512-42-1; **43**, 108512-44-3; *N*-(ethoxycarbonyl)phthalimide, 22509-74-6; 3,5-dimethoxyphenol, 500-99-2.

Synthesis of Bufalitoxin and Bufotoxin[1a,2]

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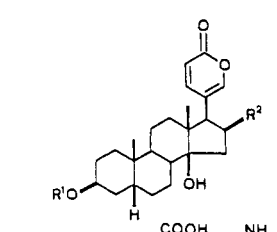
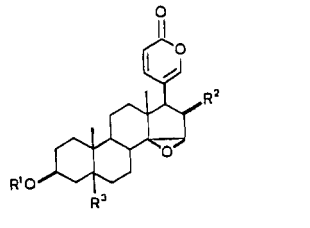
Completion of a formal total synthetic route to the toad venom constituents bufotalin (8), cinobufagin (**2a**), bufalitoxin (**3f**), and bufotoxin (**1a**) has been accomplished. Bufalin (**3a**) was employed as relay and converted to 14-dehydrobufalin 3-acetate (**4**). Selective oxidation of olefin **4** with a chromium trioxide–pyridine reagent afforded 16-ketone **5**, which we had previously transformed to bufotalin (8) and thence to cinobufagin (**2a**). Condensation of bufalin (**3a**) with suberic anhydride followed by a mixed carbonic anhydride reaction sequence using arginine monohydrochloride yielded bufalitoxin (**3f**), and an analogous route from bufotalin (8) led to bufotoxin (**1a**).

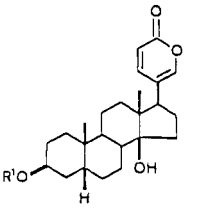
In a classic investigation of toad venom constituents, Wieland and colleagues isolated bufotoxin (**1a**) in 1922[3] from the European toad *Bufo vulgaris* (*Bufo bufo bufo* Linnê), and some 20 years later they were able to propose a tentative structure.[4] Meanwhile, Kondo and co-workers succeeded in isolating bufotoxin and the parent steroid bufotalin from the Japanese toad venom preparation Senso (the Chinese Ch'an Su).[5] The same substance was reisolated from *Bufo bufo bufo* L. and named vulgarobufotoxin.[6] In 1955 the isolation of bufotoxin from *Bufo bufo bufo* L. was reconfirmed by the Reichstein group[7] and more recently the correct structure proposed by one of us (Y.K.) and Meyer[8] was confirmed by our partial synthesis of bufotoxin from bufotalin.[2]



1a, R^1 = $CO(CH_2)_6CONHCH(CH_2)_3NHCNH_2$; R^2 = $OCOCH_3$
1b, R^1 = $CO(CH_2)_6COOH$; R^2 = $OCOCH_3$



2a, R^1,R^2 = H; R^2 = $OCOCH_3$
2b, R^1 = $CO(CH_2)_6COOH$; R^2 = $OCOCH_3$; R^3 = H
2c, R^1 = $CO(CH_2)_6CONHCH(CH_2)_3NHCNH_2$; R^2 = H; R^3 = OH



3a, R^1 = H
3b, R^1 = $COCH_3$
3c, R^1 = $CO(CH_2)_6COOH$
3d, R^1 = $CO(CH_2)_6CONHNHCOOC(CH_3)_3$
3e, R^1 = $CO(CH_2)_6CONHCH_2CH_3$
3f, R^1 = $CO(CH_2)_6CONHCH(CH_2)_3NHCNH_2$

Until 1974 the only known bufotoxin-type toad venom constituents were suberylarginine esters of a 3β-hydroxybufadienolide. Investigation of the venom from *Bufo vulgaris formosus* Boulenger has led to the isolation of bufotoxins with succinic, glutaric, pimelic, or adipic acid replacing suberic acid.[9a] From *Bufo melanosticus*

(1) (a) The present contribution is part 104 of Steroids and Related Natural Products and series number 36 of Bufadienolides; for parts 103 and 35 refer to, respectively: Pettit, G. R.; Herald, D. L.; Herald, C. L.; Kokke, W. C. M. C.; Djerassi, C. *Steroids* **1986**, *47*, 321. Green, B.; Snatzke, F.; Snatzke, G.; Pettit, G. R.; Kamano, Y.; Niven, M. L. *Croat. Chim. Acta* **1985**, *58*, 371. (b) On leave from the Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, 166 10 Prague, Czechoslovakia.

(2) A portion of the present study was summarized in a preliminary report by: Pettit, G. R.; Kamano, Y. *Chem. Commun.* **1972**, 45.

(3) Wieland, H.; Alles, R. *Chem. Ber.* **1922**, *55*, 1789.

(4) Wieland, H.; Behringer, H. *Ann.* **1941**, *549*, 209. In this remarkable study the average yield of crude bufotoxin from *Bufo vulgaris* (800 males and 400 females) was found to be 1.34 mg/toad, while the female was found to yield 1.23 mg on the average of bufotalin. The male provided only 0.55 mg of bufotalin. In the same investigation, arenobufotoxin was isolated from the South American toad *Bufo arenarum* and purified by column chromatography on aluminum oxide.

(5) Kondo, H.; Ikawa, S. *J. Pharm. Soc., Jpn.* **1933**, *53*, 23; *Chem. Abstr.* **1933**, *27*, 1887. Kondo, H.; Ono, S. *J. Pharm. Soc., Jpn.* **1938**, *58*, 37; *Chem. Abstr.* **1939**, *32*, 3765.

(6) Chen, K. K.; Jensen, H.; Chen, A. L. *J. Pharmacol.* **1933**, *47*, 307.

(7) Urscheler, H. R.; Tamm, C.; Reichstein, T. *Helv. Chim. Acta* **1955**, *38*, 883.

(8) For leading citations refer to footnote 5 of ref 2. See also: Meyer, K.; Linde, H. In *Venomous Animals and Their Venoms*; Bücherl, W., Buckley, E. E., Eds.; 1971; Vol. 2, p 521. For more recent advances in the chemistry of naturally occurring bufadienolides, refer to: Ode, H.; Kamano, Y.; Pettit, G. R. *MTP International Review of Science, Organic Chemistry Series One*; Johns, W. F., Ed.; Butterworths: London, 1972; Vol. 8, Chapter 6, pp 151–177. Ode, R. H.; Pettit, G. R.; Kamano, Y. *MTP International Review of Science, Organic Chemistry Series Two*; Johns, W. F., Ed.; Butterworths: London, 1975; Vol. 8, Chapter 6, pp 145–171. Nassimbeni, L. R.; Niven, M. L.; Sheldrick, G. M.; Pettit, G. R.; Inoue, M.; Kamano, Y. *Acta Crystallogr., Sect. C: Cryst. Struct. Commun.* **1983**, *C39*, 801 and ref 1a.

(9) (a) Shimada, K.; Fujii, Y.; Mitsuishi, E.; Nambara, T. *Tetrahedron Lett.* **1974**, 467. Shimada, K.; Fujii, Y.; Niizaki, Y.; Nambara, T. *Tetrahedron Lett.* **1975**, 653. Shimada, K.; Sato, Y.; Fujii, Y.; Nambara, T. *Chem. Pharm. Bull.* **1976**, *24*, 1120. Shimada, K.; Ro, J. S.; Ohishi, K.; Nambara, T. *Chem. Pharm. Bull.* **1985**, *33*, 2767. (b) Shimada, K.; Fujii, Y.; Yamashita, E.; Niizaki, Y.; Sato, Y.; Nambara, T. *Chem. Pharm. Bull* **1977**, *25*, 714. See also: Shimada, K.; Ohishi, K.; Nambara, T. *Tetrahedron Lett.* **1984**, *24*, 551. Shimada, K.; Ohishi, K.; Nambara, T. *Chem. Pharm. Bull.* **1984**, *32*, 4396. (c) Shimada, K.; Nambara, T. *Tetrahedron Lett.* **1979**, 163. Shimada, K.; Nambara, T. *Chem. Pharm. Bull.* **1980**, *28*, 1559.

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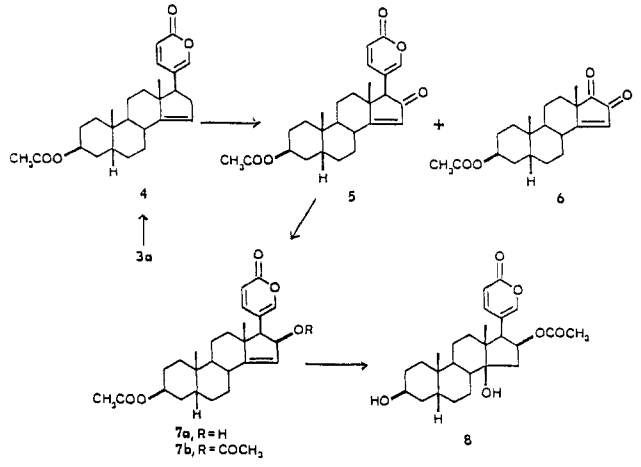
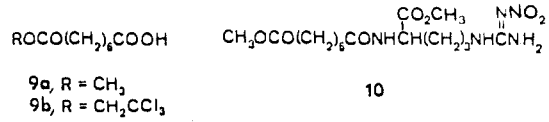
Schneider and *Bufo americanus* have been isolated bufotoxins where L-histidine, L-3-methylhistidine, L-1-methylhistidine,[9b] and L-glutamine[9c] replaces arginine. Bufalin (**3a**) and gamabufotalin have been isolated from the same toad as the 3-sulfate derivative.[9b,10] Because of the difficulties involved in obtaining sufficient amounts of bufotoxins from the natural venoms, very little is known about their physiological activity aside from pronounced cardiac effects.[11] In order to provide a solution to the structural problem presented by bufotoxin and obtain a sufficient amount of the substance for antineoplastic and other biological evaluation we undertook (beginning in 1957) a series of interrelated synthetic investigations which have culminated in the formal total syntheses of bufotalin (**8**), cinobufagin (**2a**), and bufotoxin (**1a**) now to follow.

Realization of our total synthetic route to bufalin (**3a**)[12] offered the attractive possibility of extension to bufotalin (**8**) and finally to bufotoxin (**1a**). The most direct approach was considered to be from 14-dehydrobufalin 3-acetate (**4**). Instead of preparing olefin **4** from digitoxigenin[12] as performed in our first synthesis of the natural bufadienolides we obtained this substance by acetylating **3a** and dehydrating bufalin (**3a** → **4**) isolated from the Chinese medicinal preparation, Ch'an Su. Introduction of oxygen at position C-16 proved particularly elusive and challenging. Before a practical means was found to convert olefin **4** to 16-ketone **5**, a method was developed for obtaining this ketone from cinobufagin (**2a**) and its subsequent conversion to bufotalin (**8**).[13] In turn, a partial synthesis of cinobufagin (**2a**) from bufotalin was also completed.[14] The results of both partial syntheses further demonstrated correctness of the bufotalin structural assignment[15] and encouraged us to perfect selective oxidation of olefin **4** to ketone **5**.



The reasonable possibility of selective allylic attack on the 16-carbon of olefin **4** received encouragement from results of allylic bromination involving olefin **4**, which proceeded nicely at position-16.[15] Allylic oxidation would be expected to follow a comparable course. Reactions with *tert*-butyl chromate,[16] chromium trioxide–pyridine complex,[17] and *tert*-butyl perbenzoate with copper(I) bromide[18] were chosen for detailed study. Each reagent was carefully evaluated by using different solvents and reaction conditions. With oxidation of olefin **4** by *tert*-butyl chromate in carbon tetrachloride yields of 16-ketone **5** ranged from 5% to 15%. By comparison, chromium trioxide in acetic acid and *tert*-butyl perbenzoate procedures were even less rewarding. The best synthesis of ketone **5** was achieved by using chromium trioxide–pyridine complex and methylene chloride as solvent. By this expedient, ketone **5** was obtained in 41% yield and 18% of the starting olefin **4** was recovered. Other solvents such as acetonitrile and methyl ethyl ketone gave inferior results. Many of the oxidation reactions were found to also yield a diketone which was assigned structure **6** on the basis of spectral and elemental data.

The successful transformation of 14-dehydrobufalin 3-acetate (**4**) to 16-ketone **5**, formally completed (via intermediates **7a** and **7b**) total syntheses of bufotalin (**8**)[13] and cinobufagin (**2a**).[14] The route from bufotalin to bufotoxin (**8** → **1a**) required a detailed inspection of model approaches and procedures. Some of these accessory experiments led to new compounds. For example, in one approach it became necessary to synthesize N^G-nitro-protected suberoylarginine **10**. The condensation of monomethyl suberate (**9**) and N^G-nitroarginine methyl ester with 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride (EDCI) provided amide **10** in good yield.[19]



Attempts to selectively deprotect amide **10** or obtain such a potentially useful arginine derivative more directly by an azide coupling technique[19] were eventually discarded in favor of stepwise synthesis of the suberoylarginine moiety.

The feasibility of first converting the 3β-hydroxy group to a suberate half ester derivative was amply demonstrated by employing suberic α-anhydride[20] (**11**) and pyridine with androstenolone (**12a** → **12b**) and bufalin (**3a** → **3c**) or 4-(dimethylamino)pyridine with cholesterol (**15a** → **15c**) or bufotalin (**8** → **1b**). The 4-(dimethylamino)pyridine procedure was efficient and isolation of product more convenient. The diester of suberic acid was formed as side product and was most prominent when the molar ratio of alcohol to anhydride was less than 1:3. The diester (e.g., **14**) was detected by thin-layer chromatography in each example and characterized by using 3β-hydroxy-17-oxo-

(10) Shimada, K.; Fujii, Y.; Nambara, T. *Tetrahedron Lett.* **1974**, 2767. Shimada, K.; Nambara, T. *Chem. Pharm. Bull.* **1979**, *27*, 1881.

(11) Chen, K. K.; Kovářĭ, A. *J. Pharm. Sci.* **1967**, *56*, 1535. Here, the lethal dose of bufotoxin in the cat was reported to be 0.29 ± 0.02 mg/kg. The LD_{50} in the mouse is reported to be 0.4 mg/kg: Habermehl, G. *Naturwissenschaften* **1969**, *56*, 615.

(12) For leading references, refer to: Pettit, G. R.; Houghton, L. E.; Knight, J. C.; Bruschweiler, F. *J. Org. Chem.* **1970**, *35*, 2895. See also: Sen, A.; Jäggi, F. J.; Tsai, T. Y. R.; Wiesner, K. *J. Chem. Soc., Chem. Commun.* **1982**, 1213. Wiesner, K.; Tsai, T. Y. R.; Jäggi, F. J.; Gray, G. D. *Helv. Chim. Acta* **1982**, *65*, 2049.

(13) Kamano, Y.; Pettit, G. R.; Inoue, M. *J. Org. Chem.* **1974**, *39*, 3007.

(14) Pettit, G. R.; Kamano, Y. *J. Org. Chem.* **1972**, *37*, 4040.

(15) Pettit, G. R.; Brown, P.; Bruschweiler, F.; Houghton, L. E. *Chem. Commun.* **1970**, 1566.

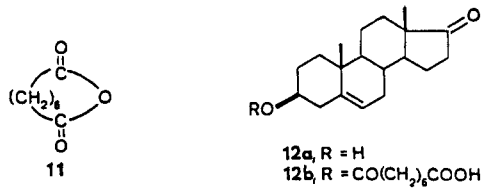
(16) (a) Yasuda, K. *Chem. Pharm. Bull.* **1963**, *11*, 1167. (b) Menini, E.; Norymberski, J. K. *Biochem. J.* **1962**, *84*, 195. (c) Kupchan, S. M.; McLean, S.; Milne, G. W. A.; Slade, P. *J. Org. Chem.* **1962**, *27*, 147. (d) Beyler, R. E.; Oberster, A. E.; Hoffman, F.; Sarett, L. H. *J. Am. Chem. Soc.* **1960**, *82*, 170. (e) Kent, G. J.; Wallis, E. S. *J. Org. Chem.* **1959**, *24*, 1235. (f) Rao, P. N.; Kurath, P. *J. Am. Chem. Soc.* **1956**, *78*, 5660. (g) Heusler, K.; Wettstein, A. *Helv. Chim. Acta* **1952**, *35*, 284.

(17) Dauben, W. G.; Lorber, M.; Fullerton, D. S. *J. Org. Chem.* **1969**, *34*, 3587.

(18) (a) Pedersen, K.; Jakobsen, P.; Lawesson, S.-O. *Org. Synth.* **1968**, *48*, 18. (b) Denny, D. B.; Napier, R.; Cammarata, A. *J. Org. Chem.* **1965**, *30*, 3151. (c) Kochi, J. K. *J. Am. Chem. Soc.* **1962**, *84*, 774. (d) Beckwith, A. L. J.; Evans, G. W. *Proc. Chem. Soc.* **1962**, *63*. (e) Stârka, L. *Collect. Czech. Chem. Commun.* **1961**, *26*, 2452. (f) Cross, B.; Whitham, G. H. *J. Chem. Soc.* **1961**, 1650.

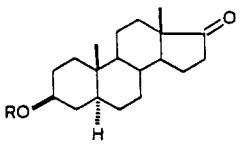
(19) Pettit, G. R. *Synthetic Peptides*; Elsevier Scientific: Amsterdam, The Netherlands, 1976; Vol. 4.

(20) Kamano, Y.; Yamamoto, H.; Tanaka, Y.; Komatsu, M. *Tetrahedron Lett.* **1968**, 5673. Hill, J. W.; Carothers, W. H. *J. Am. Chem. Soc.* **1933**, *55*, 5023.

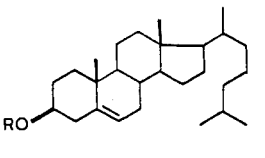


11

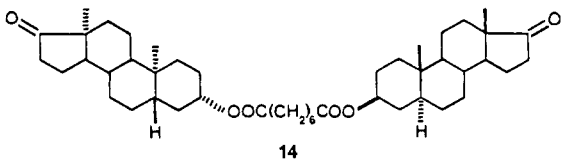
12a, R = H
12b, R = $CO(CH_2)_6COOH$



13a, R = H
13b, R = $CO(CH_2)_6COOH$



15a, R^1 = H
15b, R^1 = $CO(CH_2)_6CO_2CH_2CCl_3$
15c, R^1 = $CO(CH_2)_6COOH$



14

5α-androstane (**13a**). We also explored a less direct route to the half-ester utilizing a method employed for preparing hemisuccinates.[21] Here cholesterol (**15a**) was transformed to the 2,2,2-trichloroethyl suberate **15b** and deblocked with zinc dust in a protic solvent to afford the hemisuberate **15c**. The method just cited is most useful with very sensitive substrates as already demonstrated.[21] The suberic acid esters were easily recognized by the ^1H NMR signals due to $OCOCH_2$ at δ 2.2–2.5 even in the complicated "toxin" ^1H NMR spectra.

Preliminary experiments necessary for the L-arginine coupling step were pursued with bufalin 3-hemisuberate (**3c**). At first an azide peptide bond-forming sequence seemed most useful for completing the arginine step. For this purpose carboxylic acid **3c** was allowed to react with Boc hydrazide in the presence of Woodward's reagent K (WRK).[19,22] Interestingly, the *N*-ethyl-5-phenylisoxazolium 3′-sulfonate coupling reagent gave instead of the expected hydrazide **3d** the ethyl amide **3e**. The structural assignment for amide **3e** was made on the basis of mass and proton magnetic resonance determinations and was readily substantiated by comparison with an authentic sample prepared from ethylamine and the mixed carbonic anhydride (MCA) derived from bufalin 3-hemisuberate and isobutyl chloroformate. An analogous mixed carbonic anhydride reaction sequence afforded Boc hydrazide **3d** in 90% yield. Application of the EDCI coupling technique lowered the yield of hydrazide **3d** to 39%. Careful treatment of hydrazide **3d** with hydrochloric acid or trifluoroacetic acid followed by aqueous nitrous acid gave as nearly exclusive product bufalin 3-hemisuberate (**3c**). Before the proper conditions (probably a *tert*-butyl nitrite procedure) for obtaining the azide derivative were determined, the MCA coupling method was adopted for completing the problem.

The most convenient method uncovered for obtaining the arginine amide of carboxylic acid **3c** was by selective protection of the more basic guanidine group of arginine as the monohydrochloride salt and then utilizing an aqueous version of the MCA sequence.[19] Comparison of triethylamine or *N*-methylmorpholine as bases and isobutyl chloroformate or trichloroethyl chloroformate for mixed carbonic anhydride formation gave comparable results and led to bufalitoxin (**3f**). The bufalitoxin total synthesis was reported by us in 1972,[2] and 5 years later this toxin was described as a component of *Bufo vulgaris formosus* B. venom.[9b,23]

When the preceding model syntheses were well in hand, attention was redirected to the bufotoxin problem. Extension of the bufalitoxin synthesis to bufotalin allowed formal total synthesis of bufotoxin (**1a**) to be readily completed by the reaction pathway **8** → **1b** → **1a**. Comparison of the synthetic sample of bufotoxin (**1a**) with an authentic specimen of vulgarobufotoxin confirmed that both were identical. The formal total synthesis unequivocally settled any remaining question about the composition of bufotoxin (**1a**) and vulgarotoxin as well as the general structures of such toad venom constituents.

The simple reaction pathway we developed for obtaining bufotoxins **1a** and **3f** and marinobufotoxin (**2c**)[23] should be readily adaptable to synthesizing the remaining classic bufotoxins[9,24,25] and the newer succinic, glutaric, pimelic, and adipic ester types.[9a] Presently we are evaluating some of the bufadienolides against the P-388 lymphocytic leukemia,[26] and the results will be summarized as part of a future paper.

Experimental Section

Introductions to the Experimental Sections of Bufadienolides parts 21[14] and 27[27] provide a general synopsis of techniques employed in the present study. In addition, the bufalin, bufotalin, and cinobufagin were isolated from the Chinese toad venom preparation, Ch'an Su. The authentic specimen (compared in 1971)[2] of vulgarobufotoxin (bufotoxin) was kindly contributed by Professor K. Meyer. Both bufalitoxin and bufotoxin are unstable when pure and at ambient temperatures. The L-arginine was used as received from Nutritional Biochemicals Corporation and from ICN Pharmaceuticals. Both the WRK and EDCI reagents were obtained from Aldrich Chemical Company.

As in all previous experiments, the mutual identity of specimens prepared by different routes or with natural products was established by mixture melting point determination, thin-layer chromatographic behavior, and infrared spectral comparison.

3β-Acetoxy-16-oxo-5β-bufa-14,20,22-trienolide (5). Method A. Using CrO_3–(Pyridine)$_2$ Complex. Bufalin (**3a**) was converted as previously described[28] to 14-dehydrobufalin 3-acetate (**4**), which exhibited R_f 0.55 with ligroin–acetone–chloroform (4:3:3) and R_f 0.33 with ligroin–acetone (7:3) and a purple color with sulfuric acid spray. The red anhydrous CrO_3–(pyridine)$_2$ complex (1.5 g, prepared and stored over phosphorus pentoxide as described by Dauben[17]) was added to a solution of olefin **4** (0.43 g) in 140 mL of cold (ice bath) methylene chloride (redistilled). The solution was stirred for 15 min at ice-bath temperature and then allowed to remain at approximately 2 °C for 60 h. At this point an aliquot of the reaction mixture used for thin-layer chromatography indicated about 30% of olefin **4** remained and some 30% of 16-ketone **5** had been formed. An additional 1 g of the chromium trioxide–pyridine complex was added and the reaction was continued at approximately 2 °C for a total of 120 h. Examination

(21) Drašar, P.; Černý, I.; Pouzar, V.; Havel, M. *Collect. Czech. Chem. Commun.* **1984**, *49*, 306. Pouzar, V.; Drašar, P.; Černý, I.; Havel, M. *Synth. Commun.* **1984**, *14*, 501.

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(23) Pettit, G. R.; Kamano, Y. *J. Org. Chem.* **1974**, *39*, 3003.

(24) For a brief review refer to: Shimada, K.; Fujii, Y.; Mitsuishi, E.; Nambara, T. *Chem. Ind. (London)* **1974**, 342. See also: Shimada, K.; Fujii, Y.; Mitsuishi, E.; Nambara, T. *Chem. Pharm. Bull.* **1974**, *22*, 1673. Shimada, K.; Ohishi, K.; Nambara, T. *J. Nat. Prod.* **1985**, *48*, 159.

(25) A *p*-nitrophenyl ester coupling procedure has been employed to obtain resibufotoxin: Shimada, K.; Fujii, Y.; Nambara, T. *Chem. Pharm. Bull.* **1973**, *21*, 1031; **1973**, *21*, 2183. Shimada, K.; Fujii, Y.; Nambara, T. *Chem. Ind. (London)* **1972**, 258.

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of the reaction product by thin-layer chromatography showed formation of 16-ketone **5** in approximate 40% yield with about 10% of unreacted olefin **4**. Reaction periods longer than the 5 days did not improve the yield of ketone **5**. Thus, the solution was filtered, and the insoluble material was washed with methylene chloride. The combined filtrate was diluted with chloroform and washed successively with aqueous sodium bicarbonate, dilute hydrochloric acid, aqueous sodium bicarbonate, and water. Removal of solvent gave 0.33 g of crude product, which was chromatographed on a column of silica gel. Elution with ligroin–acetone (9.5:0.5) gave 60 mg of crude starting material **4**. Continued elution with 9:1 ligroin–acetone led to 50 mg of crude diketone **6**, and increasing the acetone concentration from 8:1 to 7:1 afforded 135 mg of impure 16-ketone **5** followed (with a solvent ration of 1:1) by another 40 mg of diketone **6**. The principal fractions containing olefin **4**, 16-ketone **5**, and 16,17-diketone **6** were each rechromatographed on a column of silica gel. This procedure gave 38 mg (11.5% recovery, mp 193–194 °C from ethyl acetate) of starting material **4**, 111 mg (33.6%, mp 212–215 °C from methanol) of ketone **5**, and 21 mg (6.7%) of diketone **6**.

A pure specimen of ketone **5** gave one spot on a thin-layer chromatogram (R_f 0.23 with 7:3 ligroin–acetone and gave a yellow coloration with sulfuric acid spray) and displayed the following: mp 212–215 °C (from methanol); UV (CH_3OH) λ_{max} 232 nm (log ϵ 3.84); IR (KBr) ν_{max} 1750, 1730, 1720, 1700 (C=O), 1640, 1610, 1538 (C=C), 1250, 1230 (C–O), 1030, 955, 905, 865, 750 cm^{-1}; ^1H NMR (10% solution in $CDCl_3$) δ 0.93 (3 H, s, 18-CH_3), 1.05 (3 H, s, 19-CH_3), 2.03 (3 H, s, 3-$OCOCH_3$), 3.10 (1 H, s, 17α-H), 5.07 (1 H, br, 3α-H), 5.87 (1 H, s, 15-H), 6.28 (1 H, b, $J_{22,23}$ = 10 Hz, 23-H), 7.03 (1 H, dd, J = 3 and 10 Hz, 22-H), 7.30 (1 H, d, J = 3 Hz, 21-H); ^1H NMR (in acetone-d_6) δ 0.98, 1.07, 2.78, 3.24, 5.00, 5.82, 6.21, 7.23, 7.48; mass spectrum, m/z 424 (M^+ and base peak), 409 ($M^+ - CH_3$), 396 ($M^+ - CO$), 381 ($M^+ - C_2H_3O$), 364 ($M^+ - CH_3CO_2H$), 349 ($M^+ - CH_3CO_2H - CH_3$), 335, 321, 310, 268, 255, 242, 228, 227, 214, 213, 202.

Anal. Calcd for $C_{26}H_{32}O_5$: C, 73.56; H, 7.60. Found: C, 73.47; H, 7.69.

3β-Acetoxy-16,17-dioxo-5β-androst-14-ene (6), isolated as a byproduct, gave a thin-layer chromatographic R_f value of 0.52 with 4:3:3 ligroin–acetone–chloroform (R_f 0.31 with 7:3 ligroin–acetone) and a pink to yellow coloration with sulfuric acid spray: IR (KBr) ν_{max} 1750 and 1680 (ester CO and conjugated CO), 1560 (conjugated C=C), 1240 (ester CO), 955 cm^{-1}; ^1H NMR (10% solution in $CDCl_3$) δ 1.06 (3 H, s, 18-CH_3), 1.26 (3 H, s, 19-CH_3), 2.02 (3 H, s, $OCOCH_3$), 5.05 (1 H, br, 3α-H), 6.54 (1 H, s, 15-H), mass spectrum, m/z 344 (M^+), 317, 316 (base peak, $M^+ - CO$), 301 ($M^+ - C_2H_3O$), 300 ($M^+ - 44$), 285, 273, 256, 241, 161, 149.

Anal. Calcd for $C_{21}H_{28}O_4$: C, 73.23; H, 8.19. Found: C, 73.47; H, 8.11.

The preceding method for obtaining ketone **5** was the best uncovered in a long series of experiments. As part of this general study the solubility of CrO_3–(pyridine)$_2$ was evaluated in a number of solvents. In general, the solubility of this reagent decreased in the following order: very soluble in pyridine and DMF, soluble in methylene chloride and acetonitrile, slightly soluble in methyl ethyl ketone, tetrahydrofuran, and benzene, barely soluble in dioxane and carbon tetrachloride, and essentially insoluble in ethyl acetate and diethyl ether. Of these solvents, only acetonitrile came close to being as useful as methylene chloride for preparing ketone **5**. With acetonitrile reaction at about 2 °C for 45 h led to approximately 30% yields of 16-ketone **5**, and 50% of starting olefin **4** was recovered. However, the yield of 16-ketone **5** could not be increased beyond that point. Oxidation proceeded very slowly in methyl ethyl ketone and THF but did not lead to formation of the 16-ketone. Pyridine and dimethylformamide were also unsatisfactory for this purpose and the remaining solvent systems were unworkable (no reaction after 40 h).

Method B. Using *tert*-Butyl Chromate. A solution of *tert*-butyl chromate (from 9.3 g of chromium trioxide) was prepared and stored in carbon tetrachloride (100 mL) essentially as described by Kent and Wallis.[16e] To a solution of 14-dehydrobufalin 3-acetate (**4**, 0.50 g in 2 mL of chloroform) was added 7 mL of the carbon tetrachloride solution of *tert*-butyl chromate. The reaction mixture was allowed to remain at room temperature for 33 days (aliquots taken for thin-layer chromatographic analysis indicated very little change after 9 days). The solution was diluted with chloroform, and the crude product (0.42 g) was isolated as summarized above in method A. The initial column chromatographic separation on silica gel led to 0.34 g of crude starting material **4** and 25 mg of 16-ketone **5**. Rechromatography of the 16-ketone afforded 20 mg of nearly pure **5**. The final purification was achieved by preparative thin-layer chromatography which gave 14 mg of ketone **5**, mp 212–215 °C (from methanol), which was identical with the specimen prepared by method A.

In several experiments a 9-day reaction period ranging from room to reflux temperature led to about 10% yields of 16-ketone **5**, but at reflux temperature recovered starting material amounted to only about 20%. When acetic acid–acetic anhydride was added to the carbon tetrachloride solution and the room temperature procedure was used, 16-ketone **5** was again isolated in about 5% yields, but recovered starting material amounted to only 5% amounts. Some loss in starting material was accounted for by isolation of dione **6**.

The specimens of 16-ketone **5** prepared by methods A and B were found identical with the same substance obtained by degradation of cinobufagin.[13]

Arginine Methyl Ester Dihydrochloride. Arginine (6.9 g) in methanol (75 mL) was converted to Arg-OMe–2HCl by employing thionyl chloride (5.7 mL) essentially as described by Boissonnas.[27] The salt was dissolved in methanol (25 mL), and diethyl ether (150 mL) was added. The crystalline product was collected, washed with diethyl ether, and dried to afford 10.2 g (97%) of crystals decomposing at 191–193 °C (lit.[29] mp 196 °C dec).[30]

N^G-Nitro-N^α-(methoxysuberoyl)-Arg-OMe (10). The monomethyl ester of suberic acid (**9**, 3.76 g, 20 mmoles)[31] was added to a solution of N^G-nitro-Arg-OMe (4.66 g, 20 mmol)[32] in cold (ice bath) dimethylformamide (100 mL). Next, triethylamine (2.02 g, 20 mmol) and 1-ethyl-3-[3-(dimethylamino)propyl]-carbodiimide hydrochloride (4.2 g, 22 mmol)[19] were added, and the mixture was stirred at ice-bath temperature for 2 h. After dilution with chloroform (250 mL), the solution was washed successively with water saturated sodium bicarbonate solution and water. Removal of solvent provided an oil, which rapidly crystallized in vacuo. The solid was triturated with ligroin and collected to yield 2.72 g of crystalline product. Recrystallization from ethyl acetate–chloroform gave microcrystals (1.96 g), melting at 116–118 °C. A second crop (0.15 g), mp 114–116 °C, was obtained by concentrating the mother liquors. The analytical specimen melted at 116–119 °C and exhibited the following: $[\alpha]^{20}_D$ −22.1° (*c* 2.99, chloroform); IR (KBr) ν_{max} 3400, 1735, 1640, 1600, 1550 cm^{-1}; ^1H NMR (in $CDCl_3$) δ 1.15–2.0 (CH_2), 2.1–2.5 (CH_2CO), 3.68 (CO_2CH_3), 3.78 (CO_2CH_3), 4.4–4.9 (NH), 6.55, 6.70 (CONH-CH), 7.75 (NH).

Anal. Calcd for $C_{16}H_{29}N_5O_7$: C, 47.63; H, 7.25; N, 17.36. Found: C, 47.75; H, 7.28; N, 17.43.

3β-Hemisuberoyl-17-oxoandrost-5-ene (12b).[30] Suberic α-anhydride (**11**, 8.76 g)[20] was added to a solution of androstenolone (**12a**, 8.64 g) in anhydrous pyridine (500 mL, freshly distilled from potassium hydroxide). Before the solvent was removed, the mixture was stirred at room temperature for 4 days. The residue (19 g) was dissolved in diethyl ether and washed with 0.05 N sulfuric acid solution followed by water. Evaporation of the solvent gave 18.7 g, which was dissolved (except for a small amount of insoluble material) in benzene and chromatographed on a column of silica gel (650 g). The fraction eluted with diethyl ether led to 4.93 g of 3-hemisuberate **12b** melting at 130–132 °C. Three recrystallizations from ethyl acetate–cyclohexane gave an analytical specimen melting at 132–133 °C.

Anal. Calcd for $C_{27}H_{40}O_5$: C, 72.94; H, 9.07. Found: C, 72.77; H, 8.92.

3β-Hemisuberoyl-17-oxo-5α-androstane (13b). A solution of 5α-androstanolone (**13a**, 1 g, 3.44 mmol) in dichloromethane (15 mL) was treated with suberic α-anhydride (**11**, 0.76 g, 5.16

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mmol)[20] and 4-(dimethylamino)pyridine (0.10 g) for 24 h at room temperature. The reaction mixture was poured onto a column of silica gel (50 g) and the ester eluted with a gradient from dichloromethane (250 mL) to 20:1 (500 mL)–20:3 (2 L) dichloromethane–acetone. The first fraction (0.25 g, 10%) proved to be diester **14** (see below). Hemisuberate **13b** was obtained from the second fraction (1.25 g, 81%) and melted at 129–131 °C following recrystallization from acetone–hexane: IR (thin film) ν_{max} 2937, 2857 (C–H), 2400–3400 (COOH), 1739, 1707 (C=O), 1240, 1204, 1170, 1150, and 1131 cm^{-1} (C–O); ^1H NMR ($CDCl_3$) δ 0.86 (6 H, s, angular methyls), 2.35 and 2.27 (2 × 2 H, 2 × br t, $OCOCH_2$ suberate ester, J = 6.3 and 7.9 Hz, respectively), 4.69 (1 H, br m, 3α-H, W = 40 Hz).

Anal. Calcd for $C_{27}H_{42}O_5$: C, 72.62; H, 9.48. Found: C, 72.85; H, 9.51.

The **3β,3′β-suberoylbis[17-oxo-5α-androstane] (14)** recrystallized from acetone–hexane: mp 156–159 °C; IR (thin film) ν_{max} 2941, 2933, 2856 (C–H), 1742, 1734 (C=O), 1248, 1204, 1180, 1130 cm^{-1} (C–O); ^1H NMR ($CDCl_3$) δ 0.86 (s, 12 H, angular methyls), 2.26 (br t, 4 H, $OCOCH_2$ suberate ester, J = 6.4), 4.70 (br m, 2 H, 2 × C_3H, W = 40 Hz).

Anal. Calcd for $C_{46}H_{70}O_6$: C, 76.84; H, 9.81. Found: C, 76.51; H, 10.22.

3β-Hemisuberoylcholest-5-ene (15c). Method A. To a solution of cholesterol (**15a**, 1.16 g, 3 mmol) in dichloromethane (20 mL) was added suberic α-anhydride (**11**, 0.94 g, 6 mmol)[20] and 4-(dimethylamino)pyridine (0.10 g). After 3 days at room temperature the reaction mixture was poured onto a column of silica gel (50 g). Elution with a gradient of dichloromethane (300 mL) to 20:3 dichloromethane–acetone (20:3, 2 L) gave as principal fraction (1.6 g) hemisuberate **15c**, which crystallized from dichloromethane–acetone to yield a 1.45 g (89%) pure specimen: mp 131–132 °C; IR (thin film) ν_{max} 3200–2500 (COOH), 2936, 2907, 2890, 2868, 2852 (C–H), 1737, 1706 (C=O, ester, acid), 1413 (COH acid, bending), 1300 (C–O, acid stretch), 1244, 1220, 1168 (C–O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.67 (3 H, s, 18-H), 0.83 (6 H, d, 26-H and 27-H), 0.90 (3 H, d, 21-H), 1.02 (3 H, s, 19-H), 2.35 and 2.27 (2 × 2 H, 2 × br t, $OCOCH_2$ suberate ester, J = 6.6 and 7 Hz), 4.57 (1 H, m, 3α-H, W = 52 Hz), 5.37 (1 H, br d, 6-H, J = 3.7 Hz, W = 15 Hz).

Anal. Calcd for $C_{35}H_{58}O_4$: C, 77.44; H, 10.77. Found: C, 77.41; H, 11.15.

Method B. A solution of 2,2,2-trichloroethanol (8.2 g, 55 mmol), suberic α-anhydride (**11**, 10.3 g, 66 mmol),[20] and triethylamine (4.8 mL, 66 mmol) in ethyl acetate (55 mL) was heated at reflux for 1 h, the solvent was evaporated, and the residue was dissolved in 10% aqueous sodium hydrogen carbonate. The aqueous phase was washed with ether (4 × 100 mL), acidified to pH 2 (sulfuric acid), and extracted with ether (3 × 100 mL). The latter ether extract was evaporated to yield 3.7 g (22%) of ester **9b** as a syrupy liquid: IR (thin film) ν_{max} 3550 to 2400 (COOH), 2940, 2860 (C–H), 1820, 1756, 1709 (C=O, ester, acid), 1134, 1099, 1035 (C–O ester), 935 (OH acid), 803, and 720 cm^{-1} (C–Cl); ^1H NMR ($CDCl_3$) δ 1.9–1.2 (8 H, m, 4 × CH_2), 2.36 and 2.47 (4 H, 2 × br t, $OCOCH_2$, J = 7.2 and 7.1 Hz), 4.71 (2 H, s, Cl_3CCH_2O), 10.3 (1 H, br signal, COOH).

A solution of the trichloroethyl hydrogen suberate (**9b**, 1.45 g, 4.74 mmol), cholesterol (**15a**, 1.83 g, 4.74 mmol), dicyclohexylcarbodiimide (1 g, 4.85 mmol), and 4-(dimethylamino)-pyridine in toluene–dichloromethane (1:1, 20 mL) was stirred at room temperature for 20 h. The solution was filtered and the filtrate chromatographed on a column of silica gel (400 g) by using dichloromethane. The main fraction was crystallized from dichloromethane–methanol to yield 2.35 (74%) of suberate ester **15b**: mp 95–96.5 °C; IR (thin film) ν_{max} 2947, 2939, 2935, 2912, 2889, 2867 (C–H), 1759, 1738 (C=O, esters), 1166, 1135 (C–O, esters), 1030 (esters), 802, and 728 cm^{-1} (C–Cl); ^1H NMR ($CDCl_3$) δ 0.67 (3 H, s, 18-H), 0.83 (6 H, d, 26-H, 27-H, J = 5 Hz), 0.90 (3 H, d, 21-H, J = 5 Hz), 1.02 (3 H, s, 19-H), 2.27 and 2.47 (2 × 2 H, 2 × br t, $OCOCH_2$ suberate ester, J = 6.3 and 7.1 Hz), 4.60 (1 H, m, 3α-H, W = 40 Hz), 4.74 (2 H, s, Cl_3CCH_2O), 5.37 (1 H, br d, 6-H, J = 4.4 Hz).

A mixture of trichloroethyl ester **15b** (1.56 g, 2.31 mmol), tetrahydrofuran (50 mL), water (5 mL)–acetic acid (1 mL), and zinc dust (20 mg added every 20 min) was stirred at 0 °C (ice bath) during 5 h. The solution was filtered through a column of silica gel (80 g) and the crude product eluted with dichloromethane–acetone (3:2). Pure hemisuberate **15c** (0.85 g, 68%) was obtained by chromatography on a column of silica gel (150 g) using a gradient of dichloromethane to dichloromethane–acetone (1:1). Crystallization from dichloromethane–acetone yielded 0.68 g (54%) of hemisuberate **15c** melting at 131–132 °C and identical with the product prepared by method A.

Bufalin 3-Hemisuberate (3c). Bufalin (**3a**, 0.18 g) and suberic α-anhydride[20] (**11**, 0.15 g) were heated at reflux in pyridine (20 mL) for 6 h. The crude product in 3:1 hexane–acetone was chromatographed on a column of silica gel (10 g) to afford ester **3c**, as a colorless amorphous solid (0.125 g), pure by TLC [R_f 0.71 (ethyl acetate–chloroform–formic acid, 2:2:1), 0.32 (ethyl acetate–hexane–acetic acid, 6:4:0.1), color with H_2SO_4 light blue): UV (CH_3OH) λ_{max} 300 nm (log ϵ 3.4); IR (KBr) ν_{max} 3500 (OH), 3500–3100 (OH of carboxylic acid), 1730–1720 (conjugated C=O and ester C=O), 1690 (carboxyl C=O), 1628 and 1535 (conjugated C=C of α-pyrone ring), 1240 (ester C–O), 955, 798 (C=C) cm^{-1}; ^1H NMR (10% solution in $CDCl_3$) δ 0.72 (3 H, s 18-CH_3), 0.92 (3 H, s, 19-CH_3), 5.14 (1 H, br signal, 3-H), 6.30 (1 H, d, J = 9 Hz, 23-H), 7.32 (1 H, d, J = 2.5 Hz, 21-H), 7.94 (1 H, dd, J = 9 and 2.5 Hz, 22-H); mass spectrum, m/z 542 (M^+).

Anal. Calcd for $C_{32}H_{46}O_7$: C, 70.82; H, 8.54. Found: C, 71.03; H, 8.55.

The synthetic bufalin 3-hemisuberate (**3c**) was identical with a specimen of the natural toad venom constituent.[20]

Bufotalin 3-Hemisuberate (1b). A solution of bufotalin (**8**, 0.444 g), suberic α-anhydride (**11**, 0.50 g, 3.2 equiv),[20] and 4-(dimethylamino)pyridine (50 mg) in dichloromethane (10 mL) was maintained at room temperature for 4 days. The reaction mixture in 4:1 hexane–acetone was chromatographed on a column of silica gel (150 g). Fractions containing ester **1b** were collected and rechromatographed on a column of silica gel (150 g) by using dichloromethane–acetone in a gradient from 9:1 to 1:1. The main fraction (0.34 g, 57%) of hemisuberate **1b** was isolated as a solid foam. The analytical sample crystallized from toluene as granular crystals melting at 149–151 °C: $[\alpha]^{25}_D$ +5.4° (c 11, $CHCl_3$); IR (KBr) ν_{max} 3490 (OH), 3080 (olefin C–H), 2939, 2865 (C–H), 1724 (C=O), 1637, 1535 (C=C), 1241, 1225, 1152 (C–O), 953 (C=C) cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.96 and 0.79 (2 × 3 H, 2 s, angular methyls), 1.87 (3 H, s, 16-$OCOCH_3$), 2.4 to 2.2 (4 H, m, 2 × $OCOCH_2$ from suberic acid), 2.86 (1 H, d, J = 8.8 Hz, 17-H), 5.10 (1 H, W = 14 Hz, 3α-H), 5.54 (1 H, dd, J = 8.8 and 1.8 Hz, 16-H), 6.20 (1 H, dd, J = 9.7 and 0.9 Hz, 23-H), 7.26 (1 H, dd, J = 2.6 and 0.9 Hz, 21-H), 8.03 (1 H, dd, J = 9.7 and 2.6 Hz, 22-H).

Anal. Calcd for $C_{34}H_{48}O_9$: C, 67.98; H, 8.05. Found: C, 67.68; H, 8.27.

Bufalin 3-Suberate Ethyl Amide (3e). Woodward's reagent K (72 mg) and triethylamine (40 mg) were added to a rapidly stirred and cooled (ice bath) solution of bufalin 3-hemisuberate (**3c**, 37 mg) in acetonitrile (5 mL). When dissolution was complete (30 min), stirring and cooling were discontinued and Boc hydrazide (0.11 g) was added. After 1 day at room temperature the solvent was evaporated and the residue chromatographed on a column of silica gel (50 g). The fraction eluted with hexane–acetone (3:1) yielded 32 mg (from ethyl alcohol or acetone) of ethyl amide **3e** in the form of needles melting at 172–173 °C; UV (95% EtOH) λ_{max} 300 nm; IR (KBr) ν_{max} 3600 (OH), 3400 (NH), 1750, 1740, 1725 (ester CO and conjugated CO), 1650, 1550 (conjugated C=C, CONH), 1260, 1245, 1240 (ester CO), 953, 790 (C=C) cm^{-1}; ^1H NMR (10% solution in $CDCl_3$) δ 0.71 (3 H, s, 18-CH_3), 0.96 (3 H, s, 19-CH_3), 1.10 (3 H, t, J = 6.5 Hz, CH_2CH_3), 3.30–3.10 (2 H, q, J = 6.5 Hz, CH_2, CH_3), 5.08 (1 H, br, 3α-H), 5.95–5.60 (1 H, br, N–H), 6.22 (1 H, d, J = 9.5 Hz, 23-H), 7.22 (1 H, d, J = 2.5 Hz, 21-H), 7.85 (1 H, dd, J = 2.5 and 9.5 Hz, 22-H), mass spectrum, m/z 569 (M^+), 551 ($M^+ - H_2O$), 533 ($M^+ - 2H_2O$), 525 ($M^+ - NHCH_2CH_3$), 483, 465, 428, 385.

Anal. Calcd for $C_{34}H_{51}NO_6$: C, 71.67; H, 9.02; N, 2.46. Found: C, 71.88; H, 9.05; N, 2.39.

Another sample of ethyl amide **3e** was obtained as follows. A solution composed of tetrahydrofuran (5 mL), triethylamine (0.2 mL), and bufalin 3-hemisuberate (94 mg) was stirred for 15 min at −10 °C. At that point isobutyl chloroformate (0.03 mL) was added, and stirring was continued for 10 min. A solution of ethylamine (0.01 mL) in tetrahydrofuran (2 mL) was added dropwise over a 5-min period. The reaction was continued for

2 h at ice bath temperature and for 4 h at room temperature. After the solution was filtered, the solvent was evaporated, and the crude product was chromatographed on a column of silica gel (80 g). The pure ethyl amide (74 mg, 78% yield) was isolated as described in the preceding experiment, and the products of both reactions were found to be identical.

Bufalin 3-Suberate Boc Hydrazide (3d). Method A. MCA Reaction. The MCA procedure described above for preparing ethyl amide **3e** was applied to the reaction of bufalin 3-hemisuberate (**3c**, 81 mg) with *tert*-butyl carbazoate (30 mg, in 5 mL of tetrahydrofuran) using isobutyl chloroformate (0.025 mL) and triethylamine (0.075 mL) in tetrahydrofuran (5 mL). The crude Boc hydrazide **3d** was isolated and purified in the same manner illustrated for amide **3e**. The pure specimen weighed 73 mg (90%) and melted at 156–160 °C (from acetone–hexane as a colorless amorphous solid): TLC R_f 0.21 (with 4:3:3 hexane–chloroform–acetone) and R_f 0.48 (with 9:1 chloroform–methanol); IR (KBr) ν_{max} 3600 (OH), 3350 (CONH), 1740 (ester and conjugated C=O), 1680 (CONHR), 1650, 1550 (conjugated C=C), 1240 (ester C–O), 960, 836, and 797 (C=C) cm^{-1}; ^1H NMR (10% solution in $CDCl_3$) δ 0.71 (3 H, s, 18-CH_3), 0.95 (3 H, s, 19-CH_3), 1.46 (9 H, s, OC-$(CH_3)_3$), 5.09 (1 H, br s, 3α-H), 6.23 (1 H, d, J = 10 Hz, 23-H), 6.59 (1 H, br signal, CH_2CONH), 7.24 (1 H, d, J = 2 Hz, 21-H), 7.57 (1 H, br signal, CONHCOO), 7.83 (1 H, dd, J = 10 and 2 Hz, 22-H).

Anal. Calcd for $C_{37}H_{56}N_2O_8$: C, 67.49; H, 8.59; N, 4.27. Found: C, 67.52; H, 8.69; N, 4.39.

Method B. EDCI Reaction. To a cold (ice bath) solution of bufalin 3-hemisuberate (72 mg) and *tert*-butyl carbazoate (17.6 mg) in methylene chloride (5 mL) was added triethylamine (0.02 mL) and 1-ethyl-3-[3'-(dimethylamino)propyl]carbodiimide hydrochloride (30 mg). The ice-bath temperature with stirring was maintained for 5.5 h. An estimation of the reaction's progress by thin-layer chromatography indicated very slow formation of Boc hydrazide **3d**. Consequently another 30 mg of the EDCI reagent was added, and stirring was continued another 2 h at ice-bath temperature. After 1 day at room temperature the mixture was diluted with methylene chloride (10 mL) and poured into water. The methylene chloride solution was washed successively with water, 2% sodium bicarbonate, and water. Evaporation of the solvent led to a yellowish oily residue (85 mg), which was chromatographed on a column of silica gel (70 g). The fraction eluted with 3:1 ligroin–acetone provided 28 mg (39%) of Boc hydrazide **3d**, which was identical with the sample obtained by method A. In addition, 16 mg of unreacted bufalin 3-hemisuberate was recovered.

Reconversion of the Boc hydrazide (**3d**) to bufalin 3-hemisuberate was easily achieved by the following method. A solution of Boc hydrazide **3d** (32 mg) in tetrahydrofuran (5 mL) containing concentrated hydrochloric acid (0.25 mL) was treated (with ice-bath cooling) with sodium nitrite (30 mg) in water (1 mL). The reaction mixture was stirred at room temperature for 20 h and diluted with chloroform (20 mL), and the solution was washed with aqueous sodium chloride and water. Removal of the solvent gave an oily residue, which was chromatographed on a column of silica gel (60 g). Elution with ligroin–acetone (3:1) and recrystallization of the product from hexane–acetone gave 18 mg of 3-hemisuberate **3c** as a colorless powder, which recrystallized from acetone as needles (7 mg) melting at 162–165 °C. The product was identical with an authentic sample of bufalin 3-hemisuberate (**3c**).[20]

Bufalitoxin (3f). A solution prepared from tetrahydrofuran (8 mL) bufalin 3-hemisuberate (**3c**, 85 mg),[20] and triethylamine (0.12 mL) was cooled to −10 °C and stirred for 15 min. Next, a solution of isobutyl chloroformate (0.04 mL) in tetrahydrofuran (0.8 mL) was added and stirring continued for 30 min. Meanwhile, arginine monohydrochloride was freshly prepared from arginine (70 mg) and concentrated hydrochloric acid (0.04 mL) in methanol (4 mL) and added dropwise during 5 min with stirring. Over a 4-h period the temperature was raised to about 0 °C, and the solvent was removed. The oily residue was subjected to preparative thin-layer chromatography employing 7:3:1 methylene chloride–methanol–ammonium hydroxide (concentrated) as mobile phase. The band corresponding to R_f 0.4 was collected and eluted with methanol. Recrystallization of the product from methanol–acetone gave bufalitoxin (**3f**, 93 mg, 85%) as granular crystals: mp 204–211 °C dec [lit.[9b] mp 200–205 °C (amorphous with dec)]; ninhydrin test (−) and Sakuguchi test (+); $[\alpha]^{22}_D$ −5.2° (c 0.20, methanol); IR (KBr) ν_{max} 3600–3500 (OH and NH_2), 3400 (NH_2), 1750 and 1730–1720 (ester C=O and conjugated C=O), 1670–1650 (conjugated C=C of α-pyrone ring and CONH), 1548 (conjugated C=C of α-pyrone ring), 1260 and 1225 (ester C–O), 1040, 955 and 890 (C=C), 830 (NH_2) cm^{-1}; ^1H NMR (in CD_3OD) δ 0.72 (3 H, s, 18-CH_3), 0.98 (3 H s, 19-CH_3), 4.26 (1 H, m, Arg-CH), 5.05 (1 H, m, 3α-H), 6.22 (1 H, d, J = 9.5 Hz, 23-H), 7.22 (1 H, d, J = 2.5 Hz, 21-H), 7.83 (1 H, dd, J = 9.5 and 2.5 Hz, 22-H).

Anal. Calcd for $C_{38}H_{58}O_8N_4 \cdot 2H_2O$: C, 62.10; H, 8.46; N, 7.62. Found: C, 62.32; H, 8.39; N, 7.55.

Bufotoxin (1a). To a solution prepared from bufotalin 3-hemisuberate (**1b**, 70 mg, 0.117 mmol) in tetrahydrofuran (10 mL) was added type 3A activated molecular sieves (2 g) and *N*-methylmorpholine (12.8 μL, 0.117 mmol), and the mixture was cooled to −10 °C (dry ice–isopropyl alcohol bath) while being stirred under an argon atmosphere. After 10 min trichloroethyl chloroformate (16.1 μL, 0.117 mmol) was added, and the mixture was maintained at −10 °C and stirred 30 min. A solution of arginine (58 mg) in a mixture of water (0.3 mL), concentrated hydrochloric acid (0.03 mL), and methanol (3 mL) was added during 30 min while the bath temperature was maintained at −10 °C. The mixture was stirred at −10 °C an additional 1 h and 1 h at room temperature, the solvent was evaporated, and the residue was chromatographed on a column of silica gel (75 g). Elution with a dichloromethane–methanol–concentrated aqueous ammonia (28:8:1) mixture and collection of the fraction corresponding to R_f 0.1 by TLC in the same solvent system yielded 12.4 mg of bufotoxin (**1a**), which (after evaporation of solvent) crystallized: mp 209–212 °C (lit.[4,5,7] mp 189–202 and 205.5–206.5 °C); ninhydrin test (−) and Sakaguchi test (+); $[\alpha]^{22}_D$ + 7.7° (c 0.20, methanol); IR (KBr) ν_{max} 3500 (OH and NH_2), 3380 (NH_2), 1750 and 1730–1720 (ester C–O and conjugated C=O), 1680–1650 (conjugated C=C of α-pyrone ring and CONH), 1540 (conjugated C=C of α-pyrone ring), 1250 (ester C–O), 1040, 953 (C=C), 830 (NH_2) cm^{-1}; ^1H NMR (in CD_3OD) δ 0.79 (3 H, s, 18-CH_3), 0.96 (3 H, s, 19-CH_3), 1.90 (3 H, s, 16-$OCOCH_3$), 2.85 (1 H, d, J = 8.8 Hz, 17-H), 4.25 (1 H, m, Arg-CH), 5.10 (1 H, m, 3α-H), 5.55 (1 H, dd, J = 8.8 and 1.8 Hz, 16-H), 6.20 (1 H, dd, J = 9.7 and 0.9 Hz, 23-H), 7.28 (1 H, dd, J = 2.6 and 0.9 Hz, 21-H), 8.02 (1 H, dd, J = 9.7 and 2.6 Hz, 22-H). The product was found to be identical with an authentic specimen of vulgarobufotoxin[6,7] kindly provided by Professor Meyer.[33]

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Registry No. **1a**, 464-81-3; **1b**, 30219-15-9; **3a**, 465-21-4; **3c**, 30219-13-7; **3d**, 108344-53-2; **3e**, 108344-52-1; **3f**, 35455-33-5; **4**, 22612-50-6; **5**, 51869-38-6; **6**, 108344-44-1; **8**, 471-95-4; **9a**, 3946-32-5; **9b**, 108344-50-9; **10**, 108344-45-2; **11**, 10521-06-9; **12a**, 53-43-0; **12b**, 108344-46-3; **13a**, 481-29-8; **13b**, 108344-47-4; **14**, 108344-48-5; **15a**, 57-88-5; **15b**, 108344-51-0; **15c**, 108344-49-6; Arg, 74-79-3; Arg-OMe·2HCl, 26340-89-6; N^G-nitro-Art-OMe, 50903-99-6; Cl_3CCH_2OH, 115-20-8; $BOCNHNH_2$, 870-46-2; Arg·HCl, 1119-34-2.

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