Bufadienolides. 27. Synthesis of Telocinobufagin[1,2]

George R. Pettit* and Yoshiaki Kamano

Cancer Research Laboratory and Department of Chemistry, Arizona State University, Tempe, Arizona 85281

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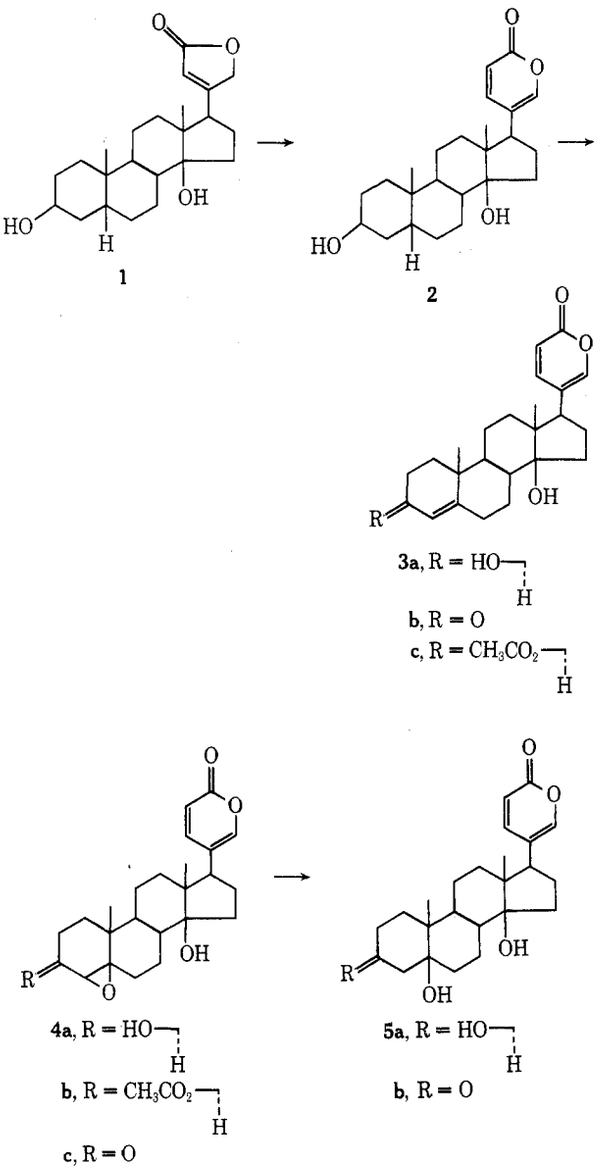
The South American toad, *Bufo marinus*, was originally released in the Caribbean and southeastern United States to assist in controlling the sugar cane beetle and now enjoys a wide range.[3] A constituent common to the venom of this large (up to 12 cm in length) amphibian, the Chinese medicinal preparation, Ch'an Su,[4a] and venom of the European toad, *Bufo vulgaris*,[5] is the cytotoxic (9KB cell line) bufadienolide telocinobufagin (**5a**).[6,7]

For the several purposes of providing unequivocal support for the structure of telocinobufagin, making this substance more readily available for biological studies, and to allow its use as a relay in the formal total syntheses of marinobufagin and marinobufotoxin (summarized in the following paper), we undertook the problem of synthesis. The most direct solution seemed to be by extending our earlier completed transformation of digitoxigenin (1) to bufalin[8] (2) and scillarenin[2] (**3a**) onward to telocinobufagin (**5a**). This approach proved eminently feasible and has been outlined in the sequel.

With the synthesis of scillarenin (**3a**) in hand,[2,9,10] attention was directed at completing the synthetic link to telocinobufagin (**5a**). Oxidation of olefin **3a** with *m*-chloroperbenzoic acid yielded (60%) β-epoxide **4a**. The β orientation of the 4,5-epoxy group was firmly supported by proton magnetic resonance data. Selective acetylation of epoxy alcohol **4a** easily gave acetate **4b**, which was also obtained by direct peracid oxidation of acetate **3c**. Numerous attempts to selectively reduce epoxy alcohol **4a** or acetate **4b** directly to telocinobufagin (**5a**) proved unproductive. While lithium aluminum hydride in tetrahydrofuran (at low temperatures) did on several occasions lead to detectible amounts of telocinobufagin, attempts to increase the yield and reproducibility were unsuccessful. Similar observations were noted employing lithium tri-*tert*-butyoxyaluminum hydride in tetrahydrofuran. However, a less direct procedure was eventually found far superior.

Oxidation of alcohol **4a** by either chromium trioxide–pyridine or *N*-bromoacetamide procedures provided ketone **4c** in good conversions. The same ketone was obtained more efficiently by peracid epoxidation of scillarenone (**3b**). Reaction of epoxy ketone **4c** with chromium(II) acetate[11] in alcohol afforded telocinobufagone (**5b**, 55–63% yields) and a lesser amount (20%) of scillarenone (**3b**). The synthetic telocinobufagone (**5b**) was identical with an authentic sample prepared from telocinobufagin (**5a**).

While sodium borohydride would be an obvious reagent for reduction of ketone **5b**, the necessary stereoselectivity would be lacking and this was indeed found to be the case. As expected, formation of the equitorial 3α epimer was favored[11] and only 20% conversion to telocinobufagin (**5a**) was realized. However, treating 3-ketone **5b** with either Urushibara nickel A[12] or W-2 Raney nickel[11] in alcohol provided 80–90% yields of telocinobufagin (**5a**). The synthetic and natural specimens of telocinobufagin were identical.



As scillarenin (**3a**) is potentially available in quantity from the naturally occurring glycoside proscillaridine A, the synthesis of telocinobufagin just described now allows more readily access to this interesting bufadienolide and related substances.

Experimental Section

Telocinobufagin was isolated from Ch'an Su.[4a] Careful hydrolysis of proscillaridine A was employed to obtain scillarenin.[10] We are grateful to Dr. W. Haede for providing an authentic specimen of scillarenin.

All solvents were redistilled and ligroin refers to the fraction boiling at 60–80°. Solvent extracts of aqueous solutions were dried over magnesium sulfate. Concentration or evaporation of solvent was conducted under reduced pressure using a rotatory evaporator. Silica gel HF_{254} (E. Merck, Darmstadt) on microscope slides was employed for analytical thin layer chromatography and a 1-mm layer was utilized for preparative layer chromatography. Unless otherwise noted the solvent system for thin layer chromatography consisted of hexane–chloroform–acetone (4:3:3). The plate was developed with sulfuric acid or iodine spray. All analytical samples exhibited a single spot on a thin layer chromatogram and were colorless. By means of comparison, infrared spectra,

thin layer chromatography, and mixture melting point determination, the mutual identity of authentic and synthetic samples was established.

The equipment employed for ultraviolet (methanol solution), infrared (potassium bromide pellets), pmr (deuteriochloroform solution unless otherwise noted), and mass spectral measurements has been noted in the introduction to the Experimental Section of Bufadienolides. 21.[4a] Each of the spectral measurements was recorded by Mr. E. Kelly, Miss K. Reimer, or Mr. R. Scott. Melting points are uncorrected and were determined using a hot-stage apparatus (Reichert, Austria).

3β,14β-Dihydroxy-4β,5β-epoxybufa-20,22-dienolide (4a). A mixture prepared by adding *m*-chloroperbenzoic acid (0.2 g) to scillarenin (**3a**, 0.4 g) in chloroform (20 ml) was allowed to remain at room temperature for 2 hr. The mixture was poured into ice-water and extracted with chloroform and the combined extract was washed with water, dilute sodium thiosulfate solution, and water. Removal of solvent gave a residue (0.41 g) which was chromatographed on a column of silica gel (E. Merck, Darmstadt). The fraction eluted with 49:1 chloroform–methanol led to 0.25 g of β-epoxide **4a** as plates decomposing at 243–250.5°. A pure sample of epoxide **4a** displayed tlc R_f 0.32 using hexane–ethyl acetate (1:9); blue color with sulfuric acid; λ_{max} 299 mμ (log ε 2.38); ν_{max} 3480 (OH), 1720 (conjugated CO), 1632, 1537 (conjugated C=C), 1249 (epoxy CO), 958, 950 (C=C), 828 (epoxy CO), 752 cm^{-1} (C=C); pmr (in pentadeuteropyridine) δ 0.93 (18-methyl), 1.06 (19-methyl), 3.37 (d, J = 3.5 Hz, 4α-proton), 4.33 (broad d, J = 3.5 Hz, 3α-proton), 6.30 (d, J = 10 Hz, 23-proton), 7.42 (d, J = 3 Hz, 21-proton), 8.16 (q, J = 10 and 3 Hz, 22-proton); mass spectrum M^- 400, 382 ($M^- - H_2O$), 367, 364 ($M^- - 2H_2O$), 339, 331, 278.

Anal. Calcd for $C_{24}H_{32}O_5$: C, 71.97; H, 8.05. Found: C, 72.05, H, 8.01.

3β-Acetoxy-14β-hydroxy-4β,5β-epoxybufa-20,22-dienolide (4b). Method A. From Alcohol 4a. Alcohol **4a** (0.051 g) was acetylated employing acetic anhydride (0.8 ml)–pyridine (1.2 ml) at room temperature. Recrystallization of the product from acetone–hexane afforded acetate **4b** (0.048 g) as needles melting at 207–210°: λ_{max} 300 mμ (in methanol); ν_{max} 3490 (OH), 1740, 1720–1710 (conjugated CO and ester CO), 1635, 1540 (conjugated C=C), 1250~1235 (ester CO and epoxy CO), 948, 910 (C=C), 840 (epoxy CO), 752 cm^{-1} (C=C); pmr δ 0.74 (18-methyl), 1.04 (19-methyl), 2.10 (3-acetyl), 3.17 (d, J = 3.5 Hz, 4α-proton), 5.11 (broad d, J = 3.5 Hz, 3α-proton), 6.22 (d, J = 10 Hz, 23-proton), 7.21 (d, J = 2.5 Hz, 21-proton), 7.80 (q, J = 10 and 2.5 Hz, 22-proton); mass spectrum M^+ 442, 424 ($M^+ - H_2O$), 382 (M^+ – AcOH), 357, 339, 330.

Anal. Calcd for $C_{26}H_{34}O_6$: C, 70.56; H, 7.74. Found: C, 70.73; H, 7.68.

Method B. From Scillarenin Acetate (3c). A solution of scillarenin acetate (**3b**, 0.02 g) in chloroform (10 ml) was oxidized with *m*-chloroperbenzoic acid (0.01 g) as described above for the preparation of epoxide **4a**. Recrystallization of the product from acetone–hexane yielded 0.014 g of epoxide **4b**, mp 206.5–210°, which was identical with the specimen obtained by method A.

3-Oxo-4β,5β-epoxy-14β-hydroxybufa-20-22-dienolide (4c). Method A. Oxidation of alcohol **4a** (0.06 g in 2 ml of pyridine) with chromium trioxide (0.4 g)–pyridine (3.5 ml) complex was conducted (22 hr, room temperature) as summarized for the preparation of bufalone.[2] The crude product (0.056 g) was purified by preparative thin layer chromatography and the zone corresponding to R_f 0.32 was eluted with chloroform–methanol (5:1). Recrystallization of this fraction from methanol yielded 0.038 g of ketone **4c** as needles: mp 230.5–239°; ν_{max} 3490 (OH), 1720 and 1700–1695 (conjugated CO and ketone), 1630 and 1535 (conjugated C=C), 1240, 1125 (epoxy CO), 950, 910 (C=C), 832 (epoxy CO), 750 cm^{-1} (C=C); pmr δ 0.75 (18-methyl), 1.16 (19-methyl), 3.02 (s, 4α-proton), 6.27 (d, J = 10 Hz, 23-proton), 7.31 (d, J = 3 Hz, 21-proton), 7.86 (q, J = 10 and 3 Hz, 22-proton).

Anal. Calcd for $C_{24}H_{30}O_5$: C, 72.33; H, 7.59. Found: C, 72.27; H, 7.56.

Method B. A solution of *N*-bromoacetamide (0.03 g) in methanol (0.5 ml)–water (0.1 ml) was added to alcohol **4a** (0.025 g) in methanol (2 ml)–acetone (2 ml). The mixture was allowed to remain at 15–20° for 40 hr, poured into ice-water, and extracted with chloroform. The combined chloroform extract was washed with water, dilute sodium sulfite solution, and water. The residue (0.03 g) obtained by removal of solvent was purified by preparative thin layer chromatography as described above in method A. Recrystallization from methanol led to 0.014 g of ketone **4b** melting at 230–239°.

Method C. A 0.02-g specimen of scillarenone (**3b**) was oxidized with *m*-chloroperbenzoic acid (0.012 g) in chloroform (1 ml) as described above for preparation of epoxide **4a**. The product was isolated by preparative thin layer chromatography and recrystallized from methanol to yield 4 mg of ketone **4c** melting at 229–238°.

The specimens of ketone **4c** prepared by methods A–C were found mutually identical.

3-Oxo-5β,14β-dihydroxybufa-20,22-dienolide (Telocinobufagone. 5b). Method A. Freshly prepared chromium(II) acetate[11] (0.14 g) was added to epoxide **4c** (0.035 g) in ethanol (3.5 ml). After 30 min at room temperature the mixture was diluted with chloroform and poured into ice–water. The chloroform layer was washed with water and the solvent was removed to provide a 0.04-g residue. The product was separated by preparative thin layer chromatography and the zone with R_f 0.15 was eluted with chloroform–methanol (4:1). Recrystallization of this fraction from methanol–ethyl acetate yielded telocinobufagone (0.022 g, mp 250–253°) as needles. The synthetic specimen was identical with an authentic sample (mp 251–253°) prepared by oxidation of telocinobufagin.

The preparative thin layer zone with R_f 0.35 was eluted with chloroform–methanol. Recrystallization of the product from acetone afforded 0.011 g of scillarenone (**3b**, mp 245–248°) as needles. The sample of scillarenone was identical with an authentic specimen prepared from scillarenin[2] (**3a**).

When methanol was substituted for the ethanol used as solvent in the preceding reaction the yields of telocinobufagone and scillarenone remained unchanged. However, the product ratio changed when 0.015 g of epoxy ketone **4c** was treated with chromium(II) acetate (0.06 g) in acetone (3.5 ml)–acetic acid (0.1 ml)–water (0.4 ml) containing sodium acetate trihydrate (0.14 g). Here, 0.013 g of telocinobufagone (**5b**, mp 248–251°) and 0.009 g of scillarenone (**3b**, mp 243–248°) were obtained.

Telocinobufagin (5a, 3β,5β,14β-trihydroxybufa-20,22-dienolide). Method A. A refluxing (1 hr) solution of ketone **5b** (0.01 g) in ethanol (1 ml) was treated with a large excess of freshly prepared Urushibara nickel A.[12] The solution was filtered and the product was isolated by preparative thin layer chromatography using hexane–ethyl acetate (1:9) as solvent. The zone of R_f 0.33 was eluted by chloroform–methanol (4:1) and this fraction was recrystallized from acetone to afford telocinobufagin (**5a**, 0.009 g) with the characteristic double melting point (163–177 and 210–211°). The synthetic telocinobufagin (as prisms) was identical with the natural product isolated from Ch'an Su.

When the reaction was repeated using freshly prepared Raney nickel (W-2) the yield of telocinobufagin (mp 160–170 and 207–210°) from 0.01 g of ketone **5b** was 0.008 g.

Method B. Sodium borohydride (0.01 g) was added to a solution of ketone **5b** (0.015 g) in dioxane (2.5 ml)–water (0.5 ml) and the mixture was allowed to remain at room temperature for 3 hr. Excess sodium borohydride was removed by adding dilute sulfuric acid at 5–10° and the resulting mixture was poured into water and extracted with chloroform. After washing with water, solvent was removed from the combined extract and the residue (0.017 g) was purified by preparative thin layer chromatography as described in method A. By this means only 3 mg of telocinobufagin (mp 158–170 and 205–209°) was isolated. A second zone corresponding to R_f 0.12 on the preparative thin layer chromatogram was assumed to be the 3α epimer, but was not further identified.

The specimen of telocinobufagin prepared by method B was also found identical with the natural product.

Registry No.—**3a**, 465-22-5; **4a**, 29599-08-4; **4b**, 51567-95-4; **4c**, 51567-96-5; **5a**, 472-26-4; **5b**, 51567-97-6.

References and Notes

(1) This investigation was supported in part by Public Health Research Grant CA10612-05 from the National Cancer Institute. We are also indebted to the J. W. Kieckhefer Foundation, Fannie E. Rippel Foundation, The Salt River Project of Arizona, Mrs. Virginia L. Bayless, The Arizona Public Service Co., and Mountain Bell Telephone Co. for financial assistance.

(2) For Bufadienolides. 26 and Steroids and Related Natural Products. 85 refer to Y. Kamano and G. R. Pettit, *J. Org. Chem.*, **39**, 2629 (1974).

(3) See, for example, M. S. Cannon, *Smithsonian*, **4**, 53 (1973).

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(5) H. R. Urscheler, C. Tamm, and E. Reichstein, *Helv. Chim. Acta*, **38**, 883 (1955).

(6) The delay in uncovering telocinobufagin from Ch'an Su was respon-

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(7) This interesting substance was first described by K. Meyer, *Helv. Chim. Acta*, **34**, 2147 (1951). See also K. Meyer, *Pharm. Acta Helv.*, **24**, 222 (1949). The cytotoxicity of telocinobufagin has been reported: J. L. Hartwell and B. J. Abbott, *Advan. Pharmacol. Chemother.*, **7**, 117 (1969).
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(9) Y. Kamano and G. R. Pettit, *J. Amer. Chem. Soc.*, **94**, 8592 (1972).
(10) See also U. Stache, J. Radscheit, W. Fritsch, W. Haede, H. Kohl, and H. Ruschig, *Justus Liebigs Ann. Chem.*, **750**, 149 (1971). With 15α-hydrocortexone as starting material, this group has summarized an excellent 17-step synthesis of scillarenin.
(11) An excellent study of analogous reactions with chromium(II) acetate leading to 3β,5β-diols has been summarized by C. H. Robinson and R. Henderson, *J. Org. Chem.*, **37**, 565 (1972). The reagent was prepared essentially as described by J. H. Balthis and J. C. Bailar, *Inorg. Syn.*, **1**, 122 (1939).
(12) K. Hata, "Urushibara Catalysis," University of Tokyo Press, Tokyo, 1971, p 39; K. Hata, I. Motoyama, and S. Sakai, *Org. Prep. Proced. Int.*, **4**, 179 (1972).

Phytadienes from the Pyrolysis of Pheophytin a

Ronald A. Hites

Department of Chemical Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

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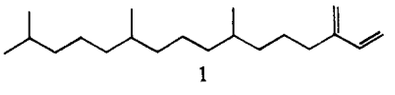
In the course of a study on organic compounds in polluted water,[1] a particularly facile pyrolytic reaction was observed when extracts containing pheophytin a were injected into a gas chromatograph which was operated with an injection port temperature of 250°. Since most thermal decompositions take place at much higher temperatures, a brief study of the pyrolytic behavior of this compound was undertaken.

Pheophytin a was pyrolyzed at 250, 350, and 400° directly onto a high-resolution gas chromatographic (gc) column the effluent of which was monitored with a fast-scanning computerized mass spectrometer. Four major fractions were observed and they were identified as various phytadiene isomers (**1**–**4**) from their mass spectra and gc retention indexes (see below for details). These data and the relative abundances of the various isomers are given in Table I. It can be seen that the relative yield of the pyrolysis products observed at the three temperatures is not significantly different and that at least 94% of these products are phytadienes. The remaining 5–6% were at least 15 different compounds and, judging from their gc retention times, all contained less than ten carbon atoms; they were not investigated further. In addition, any nonvolatile pyrolytic products which were not transmitted by the gas chromatograph were not studied.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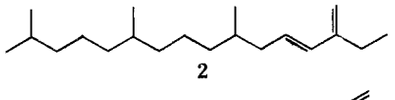
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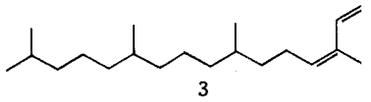
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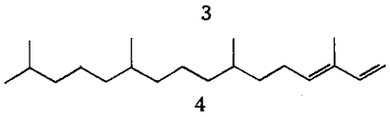
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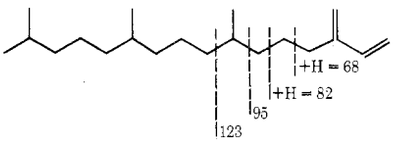
Table I
Compounds Identified in the Pyrolysate of Pheophytin a

Compd	Relative yield,[a] % 250°	350°	400°	Retention index (±1)	Mass spectrum, *m/e* (rel intensity)[b]
1[c]	61	63	64	1841	68 (100), 57 (86) 43 (80), 82 (73)
2[d]	2	2	1	1848	43 (100), 68 (90) 57 (84), 41 (79)
3	12	10	11	1863	43 (100), 82 (96) 68 (90), 57 (89)
4	20	19	18	1882	82 (100), 43 (78) 57 (77), 81 (71)
Others	5	6	6		

[a] Absolute total molar yield, relative to pheophytin a, is 40–60%. [b] See paragraph at end of paper regarding supplementary material. [c] Common name: neophytadiene. [d] Tentative structure.

The information which lead to these identifications is as follows. The mass spectra of the four major components were quite similar to each other. They all exhibited abundant ions at *m/e* 43, 57, 68, 82, 95, and 123 and a molecular ion at *m/e* 278. These ions are consistent with phytadienes and must originate by cleavage of the indicated bonds (using neophytadiene as an example). Ions at *m/e* 68 and



82 require the rearrangement of one hydrogen atom. The ions at *m/e* 95 and 123 indicate that the diene system is located at the formerly esterified terminus of the molecule. In addition, the mass spectrum of the most intense gc peak was identical with that of synthetic neophytadiene (**1**). In this way all four peaks were identified as phytadienes.

The exact positions of the double bonds could not, of course, be completely established by mass spectrometry. Fortunately, however, the gc retention indexes of several phytadienes isolated from zooplankton and identified by ozonolysis and infrared spectrometry have been reported.[2] The retention indexes of compounds **1**, **3** and **4** (see Table I) are identical with this reference data. The tentative structure **2** was assigned on the basis of gc retention characteristics (indicating a terminal methylene group) and a less abundant *m/e* 82 ion relative to the other isomers.

Although the above information is not sufficient to prove a reaction mechanism, the following suggestion (see Scheme I) nicely accounts for the identity and abundance of the products. This suggested mechanism involves two steps, the first step being a Cope-type rearrangement of the phytyl group and the second being the elimination of pheophorbide (**5**) by way of a six-membered cyclic transition state in which the carbonyl oxygen interacts with the various α-hydrogen atoms. The observed product distribution is close to that which would be expected based on the number of protons available. Since there are three primary protons and two secondary protons, the statistical product distribution should be 60% **1**, 20% **3**, and 20% **4**, and in fact these values are very close to those observed (see Table I).

A similar mechanism has been suggested for the pyrolysis of certain allylic acetates.[3] For example, 2-acetoxy-*trans*-3-heptene pyrolyzes at 350° to give a mixture of 1,3- and 2,4-heptadiene. Isomerization of the ester was demon-