Bufadienolides. 25. Direct Conversion of 14-Dehydrobufalin to Bufalin[1]

YOSHIAKI KAMANO AND GEORGE R. PETTIT*

Department of Chemistry, Arizona State University, Tempe, Arizona 85281

Received January 25, 1973

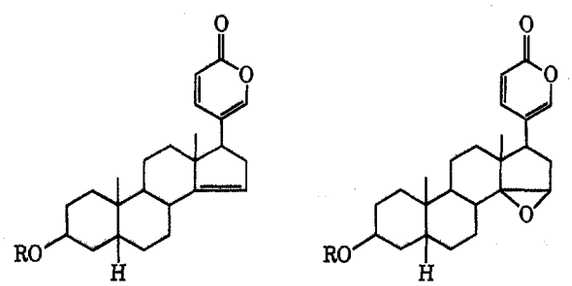
The easily prepared iodo- (**3b**), bromo- (**3c**), and chlorohydrin (**3d**) derivatives of 14-dehydrobufalin (**1a**), when subjected to careful hydrogenolysis using Urushibara nickel A or Raney nickel, readily yield bufalin (**3a**). This experimentally simple route from 14-dehydrobufalin to bufalin obviates the prior necessity of proceeding *via* resibufogenin (**2a**). In related experiments, treatment of resibufogenin (**2a**) with hydrogen chloride was found to yield chlorohydrin **3d**, and the reverse reaction was achieved using hot α-collidine. A new procedure for selective hydrolysis of bufadienolide 3β-acetates was realized employing an acidic ion exchange resin.

Stereoselective addition of hypohalous acid to a steroid 14 olefin followed by selective hydrogenolysis of the carbon–halogen bond provides in principle an attractive means for obtaining certain 14- or 15-hydroxy steroids. Excellent illustrations of this reaction sequence appear in recent transformations of 14-dehydrocardenolides to the corresponding 14β-hydroxy cardenolides[2] and in a synthesis of the plant bufadienolide, scillarenin.[3] These experiments suggested that our earlier synthesis of bufalin[4] which included transformation of 14-dehydrobufalin (**1a**) *via* resibufogenin (**2a**) to bufalin (**3a**) might be simplified employing a halohydrin approach. In practice this proved quite workable and a summary of these experiments is recorded in this report.

In a preceding series of experiments,[4b] reaction of *N*-iodosuccinimide and either *N*-bromosuccinimide or *N*-bromoacetamide was found to readily transform 14-dehydrobufalin (**1a**) to, respectively, iodohydrin **3b** and bromohydrin **3c**. In the present study chlorohydrin **3d** was also prepared using olefin **1a** and *N*-chlorosuccinimide. Unlike the *N*-iodo- and *N*-bromoimide reactions, formation of the chlorohydrin did require presence of a small amount of perchloric acid in the solvent. While iodohydrin **3b** and bromohydrin **3c** could not be isolated in pure form, the chlorohydrin **3d** has been easily isolated and characterized. The chlorohydrin **3d** was also obtained by a modification of the method Meyer[5] applied to resibufogenin (**2a**) using hydrogen chloride. However, this method proved less attractive than using olefin **1a**, as substantial amounts of 14α-artebufogenin (**4a**) were formed. Each of the preceding reactions was also viewed starting with the corresponding 3β-acetate derivative and analogous results were observed.

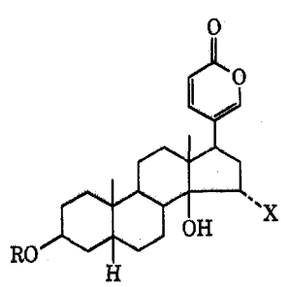
Careful hydrogenolysis of halohydrins **3b** and **3c** using Urushibara nickel A[6] or Randy nickel[7] readily afforded bufalin (**3a**) in good yield. For example, conversion of 14-dehydrobufalin to iodohydrin **3b** followed by hydrogenolysis with Urushibara nickel A led to bufalin in a 67% overall yield. However, hydrogenolysis of chlorohydrin **3b** did not proceed well in spite of prolonging the reaction; the yield of bufalin (**3a**) was limited to 18%. Comparable results were obtained in the conversion of 3β-acetoxy-14-dehydrobufalin (**1b**) to 3β-acetoxybufalin (**3e**). Again, hydrogenolysis of iodohydrin **3f** and bromohydrin **3g** proceeded very well but chlorohydrin **3h** gave unsatisfactory results. In both series of experiments the Urushibara nickel A and Raney nickel gave similar results. The usual[4a] alumina-catalyzed hydrolysis of bufalin acetate to bufalin was improved by substituting Amberlite CG-120 resin in the acid form. The ion exchange resin catalyzed hydrolysis reaction also proceeded well when applied to conversion of resibufogenin acetate (**3b**) to resibufogenin (**2a**).

One of the synthetic routes we developed[4b] to resibufogenin (**2a**) involved allowing iodohydrin **3b** or bromohydrin **3c** to react with pyridine or basic alumina. With such halohydrins collapse to the corresponding epoxide proceeds very quickly and in essentially quanti-

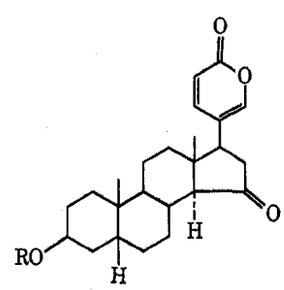


1a, R = H
b, R = $COCH_3$

2a, R = H
b, R = $COCH_3$



3a, R = X = H
b, R = H; X = I
c, R = H; X = Br
d, R = H; X = Cl
e, R = $COCH_3$; X = H
f, R = $COCH_3$; X = I
g, R = $COCH_3$; X = Br
h, R = $COCH_3$; X = Cl



4a, R = H
b, R = $COCH_3$

(1) The present contribution represents part 84 of the series Steroids and Related Natural Products. For the preceding paper see Y. Kamano and G. R. Pettit, *J. Amer. Chem. Soc.*, **94**, 8592 (1972). This reference also corresponds to a preliminary report of the present study.

(2) W. Fritsch, U. Stache, W. Haede, K. Radscheit, and H. Ruschig, *Justus Liebigs Ann. Chem.*, **721**, 168 (1969); U. Stache, W. Fritsch, W. Haede, and K. Radscheit, *ibid.*, **726**, 136 (1969).

(3) U. Stache, K. Radscheit, W. Fritsch, W. Haede, H. Kohl, and H. Ruschig, *Justus Liebigs Ann. Chem.*, **750**, 149 (1971).

(4) (a) G. R. Pettit, L. E. Houghton, J. C. Knight, and F. Bruschweiler, *J. Org. Chem.*, **35**, 2895 (1970); (b) G. R. Pettit, Y. Kamano, F. Bruschweiler, and P. Brown, *ibid.*, **36**, 3736 (1971).

(5) K. Meyer, *Helv. Chim. Acta*, **35**, 2444 (1952).

(6) Y. Urushibara, S. Nishimura, and H. Uehara, *Bull. Chem. Soc. Jap.* **28**, 446 (1955).

(7) W. Fritsch, H. Kohl, U. Stache, W. Haede, K. Radscheit, and H. Ruschig, *Justus Liebigs Ann. Chem.*, **727**, 110 (1969).

tative yield. However, brief treatment of chlorohydrin **3d** with pyridine has proved unproductive,[5] but in the present study dehydrohalogenation in hot α-collidine did prove effective and provided resibufogenin (**2a**).

Evidence now in hand from the preceding direct synthesis of bufalin from 14-dehydrobufalin combined with analogous results[2,3] obtained in the Farbwerke Hoechst Laboratories clearly indicates that the halohydrin-hydrogenolysis route to 14β-hydroxy steroids from 14-olefin precursors is a most efficient method. Alternatively, photochemically initiated attack on the saturated 14-carbon has been of increasing promise.[8]

Experimental Section

The bufalin and resibufogenin employed in this study were isolated from the Chinese medicinal preparation, Ch'an Su. All melting points were observed using a micro hot-stage apparatus (Reichert, Austria) and are uncorrected. Spectral data was recorded by Miss K. Reimer and Messrs. Richard Scott and Eugene Kelley. The general experimental, chromatographic techniques, and instrumental methods have been summarized in prior contributions.[4b,9]

Bufalin (3a). Method A. From Iodohydrin 3b.—About 2.6 g of Urushibara nickel A[6] in ethanol (18 ml) was saturated with hydrogen employing vigorous stirring. The hydrogen atmosphere was replaced with nitrogen and the ethyl alcohol with methylene chloride. A solution of iodohydrin **3b** (79 mg), prepared[4b] from 80 mg of 14-dehydrobufalin, in methylene chloride (10 ml) was added to the nickel A and the mixture was stirred in a nitrogen atmosphere for 4 hr at 14–16°. The solution was filtered and solvent was removed from the filtrate to provide a 78-mg residue, which was subjected to preparative thin layer chromatography on silica gel using acetone–chloroform–*n*-hexane (3:3:4) as eluent. The zone corresponding to R_f 0.29 was eluted with methanol. Recrystallization of the product from methanol afforded 54 mg of bufalin (**3a**) as colorless needles melting at 240–243°. Our original synthetic specimen[4a] of bufalin melted at 242–243°.[10]

In an analogous series of experiments, iodohydrin **3b** (43 mg) was treated (3 hr at 18–20°) with freshly prepared[7] Raney nickel (approximately 1.2 g) in a nitrogen atmosphere. By means of the isolation procedure described for the Urushibara nickel A experiment 26 mg of bufalin (**3a**), mp 239–242°, was isolated.

Method B. From Bromohydrin 3c.—A 39-mg sample of the crude bromohydrin **3c** prepared from 14-dehydrobufalin and *N*-bromoacetamide was converted to bufalin (**3a**, 25 mg, mp 239–243°) using the hydrogen-saturated Urushibara nickel A (about 1.6 g) procedure (4 hr at 16°) summarized in method A. With the Raney nickel (approximately 1 g) procedure, 37 mg of the crude bromohydrin (**3c** obtained using *N*-bromosuccinimide) provided 23 mg of bufalin (**3a**) melting at 238–241°.

Method C. From Chlorohydrin 3c.—A 35-mg specimen of chlorohydrin **3c** was prepared as summarized in the following experiment and subjected to hydrogenolysis with freshly prepared Urushibara nickel A (*ca.* 1.4 g) in methylene chloride (10 mg). In this case the reaction mixture was stirred in a nitrogen atmosphere for 8 hr at 28–32°. The bufalin (**3a**, 6.2 mg) was isolated as noted in method A and found to melt at 237–241°.

Method D. By Hydrolysis of 3β-Acetoxybufalin (3e).—A mixture prepared from bufalin acetate (**3e**, 33 mg), ethyl alcohol (9 ml)–water (2 ml), and 0.35 g of Amberlite CG-120 (H^+ form) was stirred at room temperature for 18 hr. The solution was filtered and following removal of solvent the crude product (35 mg) was purified by preparative thin layer chromatography and recrystallization as indicated in method A to yield 22.8 mg of bufalin (**3a**) melting at 237–240°.

The specimens of bufalin (**3a**) prepared by methods A–C were found mutually identical[10] and identical with natural bufalin.[11]

(8) For example, consult A. Rotman and Y. Mazur, *J. Amer. Chem. Soc.*, **94**, 6228 (1972).

(9) G. R. Pettit and Y. Kamano, *J. Chem. Soc.*, Perkin *Trans. 1*, in press.

(10) The identical composition of synthetic and natural specimens was confirmed by comparison infrared spectra (in potassium bromide) and thin layer chromatographic behavior.

(11) Y. Kamano, *Chem. Pharm. Bull.*, **17**, 1711 (1969).

3β,14β-Dihydroxy-15α-chloro-5β-bufa-20,22-dienolide (3d). Method A. From 14-Dehydrobufalin (1a).—A solution of *N*-chlorosuccinimide (0.12 g) in dioxane (3 ml)–acetone (1 ml) was added with stirring to a solution composed of 14-dehydrobufalin (**1a**, 0.12 g) in dioxane (6 ml)–acetone (2 ml)–water (1 ml) and perchloric acid (0.1 ml) at room temperature. After a 24-hr period, sodium sulfite (0.12 g) in water (4 ml) was added and approximately two thirds of the solvent was removed under reduced pressure. The mixture was poured into ice–water with stirring and extracted with chloroform. The combined extract was washed with water and concentrated to dryness. The 0.13-g residue was chromatographed on a column of silica gel and the fraction eluted with 4:1 ligroin–acetone was recrystallized from ethyl acetate to provide 58 mg of chlorohydrin **3d**, mp 232–233°, as colorless needles: mass spectrum M^+ 420, 402 ($M^+ - H_2O$), 384 ($M^+ - 2H_2O$ and $M^+ - HCl$), and 366 ($M^+ - 2H_2O - HCl$); uv λ_{max} 299 mμ (log ϵ 3.71 in methanol); ir ν_{max}^{KBr} 3518 (OH), 3460 (OH), 1724, 1695 (conjugated CO), 1633, 1540 (conjugated C=C), 958, 745 (C=C), and 720 cm^{-1} (Cl); pmr (in pentadeuteriopyridine) δ 0.90 (18-methyl), 0.94 (19-methyl), 2.58 (d, J = 4 Hz, 16-protons), 4.31 (broad s, 3α-proton), 4.56 (d, J = 4 Hz, 15β-proton), 6.29 (d, J = 9.5 Hz, 23-proton), about 7.5 (21-proton, indistinct peak overlapped with that of solvent), and 7.68 (q, J = 9.5 and 2.5 Hz, 22-proton).

Anal. Calcd for $C_{24}H_{33}O_4Cl$: C, 68.47; H, 7.90; Cl, 8.42. Found: C, 68.38; H, 7.90; Cl, 8.36.

Method B. From Resibufogenin (2a).—Dry hydrogen chloride was carefully passed (for 8 min) through a solution of resibufogenin (**2a**, 0.15 g) in dry chloroform (3 ml) maintained at −8°. Additional chloroform was added and the solution was poured into water. The chloroform layer was separated, washed with water, dilute sodium bicarbonate solution, and water and then concentrated under reduced pressure to dryness. The residue (0.17 g) was chromatographed on a column of silica gel. The fraction eluted with ligroin–acetone (4:1) was recrystallized from ethyl acetate to yield (82.5 mg) chlorohydrin **3d** melting at 231–233°.

Continued elution of the silica gel column provided 37 mg of 14α-artebufogenin (**4a**, mp 263–265° from acetone) which was identical[10] with an authentic specimen.[4b] Also the specimens of chlorohydrin **3d** prepared by methods A and B were found mutually identical.[10]

When chlorohydrin **3d** (26 mg) was acetylated (room temperature, 24 hr) with acetic anhydride (3 ml)–pyridine (4 ml) and the product was recrystallized from ethyl acetate, a 24-mg sample of 3β-acetoxy-14β-hydroxy-15α-chloro-5β-bufa-20,22-dienolide (**3h**) was obtained as colorless needles melting at 230–233°. The identical acetate (**3h**, 42 mg, mp 230–232°) was obtained from 3β-acetoxy-14-dehydrobufalin (**1b**, 75 mg) and *N*-chlorosuccinimide (76 mg) by method A. In this experiment acetate **3h** was eluted from the silica gel column with 7:1 ligroin–acetone. Further, acetate **3h** (58 mg, mp 229–230°) and 3β-acetoxy-14α-artebufogenin (**4b**, 22 mg, mp 220–221°)[4b] were obtained from 3β-acetoxyresibufogenin (**2b**, 100 mg) using dry hydrogen chloride in chloroforom (2.5 ml) by method B. Again, the silica gel column was eluted with 7:1 ligroin–acetone. All three samples of chlorohydrin acetate **3h** were mutually identical.[10]

3β-Acetoxybufalin (3e). Method A. From Iodohydrin Acetate 3f.—A 35-mg quantity of 3β-acetoxy-14-dehydrobufalin (**1b**) was converted to iodohydrin **3f** (36 mg) using *N*-iodosuccinimide was previously reported.[4b] The resulting crude iodohydrin (**3f**, 36 mg) was treated with Urushibara nickel A (about 1.4 g) in methylene chloride (9 ml) as noted in the method A route to bufalin described above. The product was isolated by preparative thin layer chromatography employing 3:3:4 acetone–chloroform–*n*-hexane and the zone corresponding to R_f 0.60 was eluted with 2:1 chloroform–methanol. Recrystallization of the product from acetone gave 3β-acetoxybufalin (**3e**, 24 mg) as needles melting at 239–240° (lit.[12] mp 236–247°).

Method B. From Bromohydrin Acetate 3g.—With 20 mg of 3β-acetoxy-14-dehydrobufalin (**1b**) and *N*-bromoacetamide (20 mg) as starting material the resulting crude bromohydrin (**3g**, 19 mg)[4b] underwent hydrogenolysis with Raney nickel (about 0.6 g) to afford 11.8 mg of bufalin acetate (**3e**) melting at 239–242°. The reaction sequence and isolation procedure was conducted as summarized above for method A.

Method C. From Chlorohydrin 3h.—Chlorohydrin acetate **3f**

(12) M. Barbier, H. Schroter, K. Meyer, O. Schindler, and T. Reichstein, *Helv. Chim. Acta*, **42**, 2486 (1959).

(25 mg) was treated with Urushibara nickel A (about 0.8 g) as noted above for the preparation of bufalin by method C. By this means 4.6 mg of bufalin acetate (**3e**), mp 237–240°, was obtained.

The samples of bufalin acetate prepared by methods A–C were found identical[10] with an authentic specimen:[11] mass spectrum M^+ 462, 444 ($M^+ - H_2O$), 426 ($M^+ - HCl$), 402 ($M^+ - AcOH$), 384 ($M^+ - H_2O - AcOH$), 366 ($M^+ - AcOH - HCl$), and 348 ($M^+ - H_2O - AcOH - HCl$); uv λ_{max} 299 mμ (log ε 3.48 in methanol); ir ν_{max}^{KBr} 3550 (OH), 1730, 1696 (conjugated CO), 1632, 1540 (conjugated C=C), 1264, 1240, 1228 (CO), 950, 745 (C=C), and 720 cm^{-1} (Cl); pmr (in deuteriochloroform) δ 0.74 (18-methyl), 0.92 (19-methyl), 2.06 (s, 3β-acetoxyl), 2.47 (d, J = 3 Hz, 16-protons), 4.32 (d, J = 3 Hz, 15β-proton), 5.15 (s, 3α-proton), 6.35 (d, J = 10 Hz, 23-proton), 7.34 (d, J = 2.5 Hz, 21-proton), and 7.58 (q, J = 10 and 2.5 Hz, 22-proton).

Anal. Calcd for $C_{26}H_{35}O_5Cl$: C, 67.45; H, 7.62; Cl, 7.66. Found: C, 67.55; H, 7.65; Cl, 7.51.

Resibufogenin (2a). Method A. From Chlorohydrin 3d.—A solution prepared from chlorohydrin **3d** (22 mg) and freshly distilled α-collidine (2.5 ml) was heated at reflux for 4.5 hr. The crude product obtained by removal (under reduced pressure) of solvent was chromatographed on a column of silica gel. The fraction eluted with 5:1 ligroin–acetone was recrystallized from acetone–*n*-hexane to provide 15.8 mg of resibufogenin (**2a**) with a characteristic double melting point, 108–121 and 149–168°. An earlier[4a] specimen of resibufogenin prepared in our laboratory was found to melt at 110–121 and 148–168°. Both specimens were mutually identical.[10]

Method B. Hydrolysis of Resibufogenin Acetate (2b).—A 28-mg sample of resibufogenin acetate (**2b**) was hydrolyzed in ethanol (18 ml)–water (2 ml) with 0.3 g of Amberlite CG-120 (H^+ form) as summarized above for the hydrolysis of bufalin acetate. The preparative thin layer corresponding to R_f 0.42 was eluted and recrystallized from acetone–*n*-hexane to provide 18 mg of resibufogenin as plates melting at 109–122 and 147–167°. The products of methods A and B were mutually identical.[10]

Resibufogenin Acetate (2b).—Dehydrohalogenation of chlorohydrin acetate **3h** (20 mg) with α-collidine (2.4 ml) was performed as outlined above for the synthesis of resibufogenin. The product was chromatographed on a column of silica gel and the fraction eluted with 8:1 ligroin–acetone was recrystallized from acetone to afford 14.4 mg of resibufogenin acetate (**2b**), as needles melting at 226–228°, identical[10] with a specimen obtained by acetylating natural resibufogenin.

Registry No.—**1a**, 7439-77-2; **2a**, 465-39-4; **2b**, 4029-64-5; **3a**, 465-21-4; **3b**, 39707-10-3; **3c**, 39707-11-4; **3d**, 39707-12-5; **3e**, 4029-66-7; **3f**, 39707-14-7; **3g**, 39707-15-8; **3h**, 39707-16-9; **4b**, 24183-19-5.

Acknowledgment.—This investigation was supported by Public Health Research Grants CA-10612-04 and CA-10612-05 from the National Cancer Institute.

Mass Spectra of Prostaglandins. III. Trimethylsilyl and Alkyl Oxime–Trimethylsilyl Derivatives of Prostaglandins of the E Series[1]

BRIAN S. MIDDLEDITCH AND DOMINIC M. DESIDERIO*[2]

Institute for Lipid Research and Department of Biochemistry, Baylor College of Medicine, Houston, Texas 77025

Received November 9, 1972

The mass spectra of the trimethylsilyl ester–trimethylsilyl ether derivatives of prostaglandins E_1 and E_2 and 8-isoprostaglandin E_2 and of their *O*-methyl oximes are reported and discussed. The high resolution spectra of these compounds are also considered. These spectra are compared with those of the analogous *O*-ethyl oximes and of the corresponding d_9-trimethylsilyl ether–d_9-trimethylsilyl ester and selectively labeled trimethylsilyl ester–d_9-trimethylsilyl ether derivatives. The 11-trimethylsilyloxy substituent had a strong fragmentation-directing influence on the molecular ion, and it was found that there was a marked stereochemical influence on fragmentation. Multiple origins were found for ions of several nominal masses; the most notable were those of *m/e* 199 and *m/e* 173. Ions of *m/e* 217 and *m/e* 204 were found to be formed by relatively long-range migrations of trimethylsilyl groups.

Prostaglandins of the E series are widely distributed in body fluids of man and of many other animals.[3] The 1,3-ketol moiety of the ring of these compounds is particularly unstable. The trimethylsilyl (TMS) derivatives were found to undergo partial decomposition during gas chromatography (gc),[4] but "clean" spectra of these derivatives could be obtained by combined gas chromatography–mass spectrometry (gc–ms). The oxime–TMS derivatives[5] were much more stable. In continuation of our studies of prostaglandin mass spectrometry,[1,6] we now report on the mass spectra of these derivatives of prostaglandins of the E series. All elemental compositions were compatible with high resolution data.

(1) For paper II, see B. S. Middleditch and D. M. Desiderio, *Prostaglandins*, in press.

(2) Fellow of the Intra-Science Research Foundation, 1971–1975.

(3) B. Samuelsson in "Lipid Metabolism," S. J. Wakil, Ed., Academic Press, New York, N. Y., 1970, p 107.

(4) B. S. Middleditch and D. M. Desiderio, *Prostaglandins*, **2**, 115 (1972).

(5) F. Vane and M. G. Horning, *Anal. Lett.*, **2**, 357 (1969).

(6) Paper I, B. S. Middleditch and D. M. Desiderio, *Lipids*, in press.

Results and Discussion

As was the case for the oxime–TMS derivatives of prostaglandins of the A series,[6] it was found that those of the E series gave two peaks on gc, presumably the syn and anti isomers. It was reported that such derivatives of the synthetic 8-isoprostaglandins of the E series gave only one peak on gc.[5] We have, however, found that two peaks are obtained, although they are less well separated than those of the naturally occurring E series.[4] As before,[1,6] we have examined the spectra of methyl oxime (MO)–TMS and ethyl oxime (EO)–TMS derivatives, as well as those of the corresponding TMS-d_0 derivatives and of the selectively labeled TMS ester–d_9-TMS ether analogs. Because of space limitations, the spectra of the EO–TMS derivatives are not illustrated here, but have been submitted to the Mass Spectrometry Data Centre, A. W. R. E., Aldermaston, Berks, England.

The spectra of TMS derivatives of prostaglandins E_1 (I) and E_2 (II) and of 8-isoprostaglandin E_2 (III) are shown in Figures 1–3, respectively. The spectra