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Studies on the Synthesis of Cardiotonic Steroids. III.[1] New and Effective Route to Bufadienolides

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An efficient synthetic method of bufa-20,22-dienolides from 20-ketopregnanes has been developed. The method involves the following reaction sequence: (1) 21-methoxymethylenation, (2) 2-methoxydihydropyrane formation by reaction with excess dimethylsulfonium methylid, (3) transformation to buf-20(22)-enolide structure, (4) dehydrogenation to bufa-20,22-dienolide. Application of this attractive device to readily available 3β-acetoxy-5β-pregn-14-en-20-one established a new route to resibufogenin and bufalin.

Keywords——bufa-20,22-dienolide; buf-20(22)-enolide; resibufogenin; bufalin; anhydrobufalin; α-pyrone

The bufadienolides which occur in the toad venoms as well as in certain plant extracts[3] are well-known for their significant physiological features such as heart stimulating[4] and antitumor activities.[5] The representative members of these substances are bufalin (**1**) and resibufogenin (**2**) isolated from Ch'an Su (galenical preparation from Chinese toad secrete). Approach to the synthesis of natural bufadienolides has a history of over 30 years. The main difficulty encountered was, as discussed in a review,[6] to build both α-pyrone ring and labile 14β-hydroxy (or epoxide) together at the D-ring keeping thermodynamically unstable configurations. In 1969, Sondheimer announced first formal total synthesis of **1** and **2**, ingeniously solving this problem.[7] Shortly afterward, Hoechst Farbwerke workers[8] had developed attractive and methodologically different routes as exemplifies by the synthesis of scillarenin. Meanwhile, G.R. Pettit and his coworkers[9] also have made valuable contributions to the bufadienolide chemistry including the conversion of digitoxigenin (cardenolide) to some bufadienolides.[10]

Those pioneering syntheses, however, involved multistep reaction sequence even from the relay steroids which themselves are not conventionally available. To circumvent this draw-

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[1] Part II: E. Yoshii, T. Koizumi, S. Mizuno, and E. Kitatsuji, *Chem. Pharm. Bull.* (Tokyo), **24**, 3216 (1976).

[2] Location: *Gofuku, Toyama, 930, Japan.*

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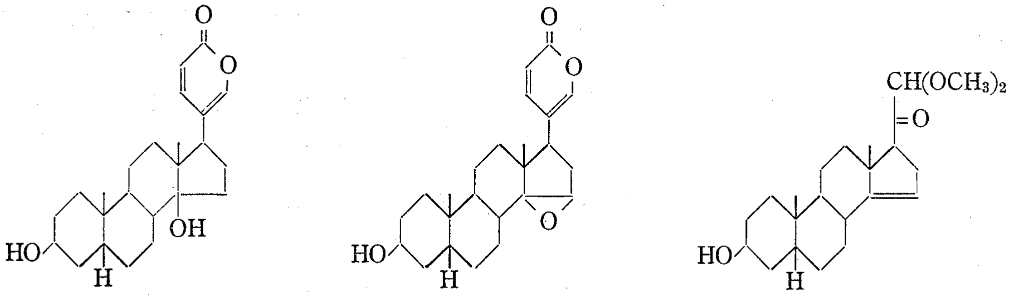
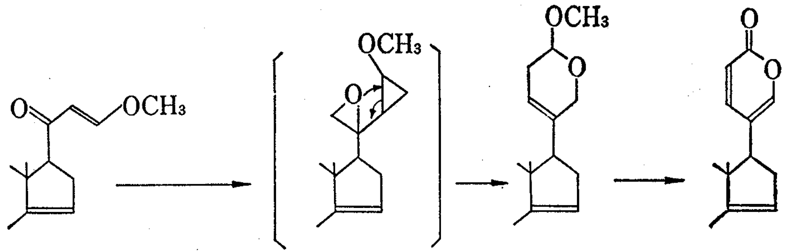


1 **2** **3**

Chart 1

back we previously communicated[11] a facile preparative method of a key intermediate in Hoechst synthesis, 21,21-dimethoxy-5β-pregnan-14-en-3β-ol-20-one (**3**), from which **1** and **2** could be obtainable *via* 14-anhydrobufalin (**21**). Our continuing effort in this field of investigation has now yielded more effective short-step synthesis of these two natural bufadienolides which constitutes the present paper.

The starting steroid we decided to utilize was again 5β-pregn-14-en-3β-ol-20-one acetate (**7b**).[12] Here, α-pyrone synthesis from the side chain was sought in the report of Harris, *et al.*[13] As depicted below, two methylene transfer to 21-methoxymethylidene derivative (**4**) by the reaction with excess dimethylsulfonium methylide should produce methoxydihydropyrane (**5**) which on subsequent appropriate operations might be convertible to 14-olefinic bufadienolide (**6**).



4 **5** **6**

Chart 2

First, model experiment utilizing the 14,15-saturated compound, 3β-acetoxy-5β-pregnan-20-one (**7a**), is described. The aldehyde **8a** was obtained by perchloric acid catalyzed condensation of **7a** with trimethyl orthoformate.[14] It was then shortly warmed with weakly acidic methanol to produce a mixture of β-methoxyvinyl ketone (**9a**) and the dimethylacetal (**10a**) which, upon brief treatment with potassium *tert*-butoxide, was transformed to single product **9a** in 76% yield. The reaction of **9a** with large excess of dimethylsulfonium methylide followed by treatment of the crude product with methanolic hydrogen chloride afforded methoxydihydropyrane (**11a**) and furan (**12a**), in the yield of 61% and 15% respectively. Hydrolysis of the methyl ether function of acetylated **11a** was effected with boron trichloride in dichloromethane in the cold or preferably by warming with buffered hydrochloric acid (90—100% yield). Jones oxidation of the resulting pyranol (**13a**) produced buf-20(22)-enolide (**14a**). The final step leading to α-pyrone ring (**17a**) was then attempted after the method of

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[11] E. Yoshii, T. Miwa, T. Koizumi, and E. Kitatsuji, *Chem. Pharm. Bull.* (Tokyo), **23**, 462 (1975).

[12] E. Yoshii, T. Koizumi, H. Ikeshima, K. Ozaki, and I. Hayashi, *Chem. Pharm. Bull.* (Tokyo), **23**, 2496 (1975).

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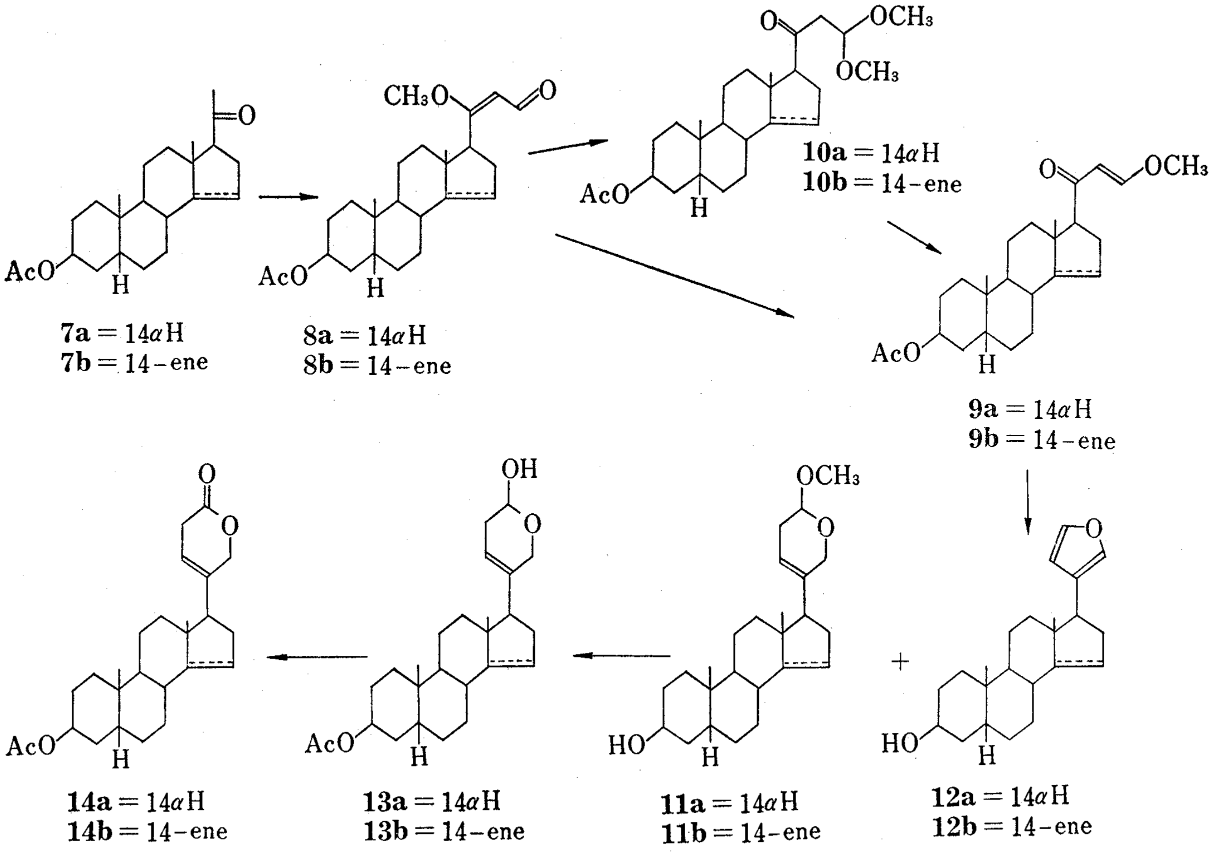

7a = 14αH
7b = 14-ene

8a = 14αH
8b = 14-ene

10a = 14αH
10b = 14-ene

9a = 14αH
9b = 14-ene

14a = 14αH
14b = 14-ene

13a = 14αH
13b = 14-ene

11a = 14αH
11b = 14-ene

12a = 14αH
12b = 14-ene

Chart 3

Sarel, *et al.*[15] who had been successful in converting 5β-buf-20(22)-enolide (**14c**) derived from cholanic acid into the corresponding bufa-20,22-dienolide (**17c**) by dehydrogenation with 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ) under proper choice of acid catalyst, toluene-*p*-sulfonic acid. The reaction of **14a** with DDQ under the same set of reaction conditions, however, resulted in predominant formation of the extended conjugate system, **15a** and **16a**. Only trace amount of the desired α-pyrone (**17a**) was noticed, irrespective of the nature of acid catalyst (both toluene-*p*-sulfonic acid and hydrogen chloride).

Since it was hardly understandable that the presence of 3-acetoxy function in **14a** affects the course of the dehydrogenation, we decided to examine whether the data of the literature is reliable or not. The Sarel's compound (**14c**) was therefore prepared, at our hand from 5β-pregnan-20-one by the same route employed for the preparation of **14a** and subjected to DDQ dehydrogenation under exactly the same reaction condition as indicated in the literature. Again, almost exclusive formation of bufa-17,22-dienolides, **15c** and **16c**, was observed. Although the reason for the discrepancy of the above result from Sarel's is not clear at present, preferential removal of 17-H could be rationalized by consideration of the mechanism of quinone dehydrogenation which has been believed to involve hydride transfer.[16] The positive charge at C_{21} developing along the reaction coordinate must be less stable than the one yielding at C_{17} because of electron withdrawing nature of the ester substituent, and thus 21-H abstraction should be energetically less favorable as observed in the present experiment.[17]

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15) S. Sarel, Y. Shalon, and Y. Yanuka, *Chem. Comm.*, **1970**, 80, 81.

16) H.O. House, "Modern Synthetic Reactions," Benjamin Inc., California, 1972, pp. 37—44.

17) Private communication from Prof. Sarel, establishing the identity of our 14c with theirs (NMR comparison), informed us that it takes some experience to effect α-pyrone formation.

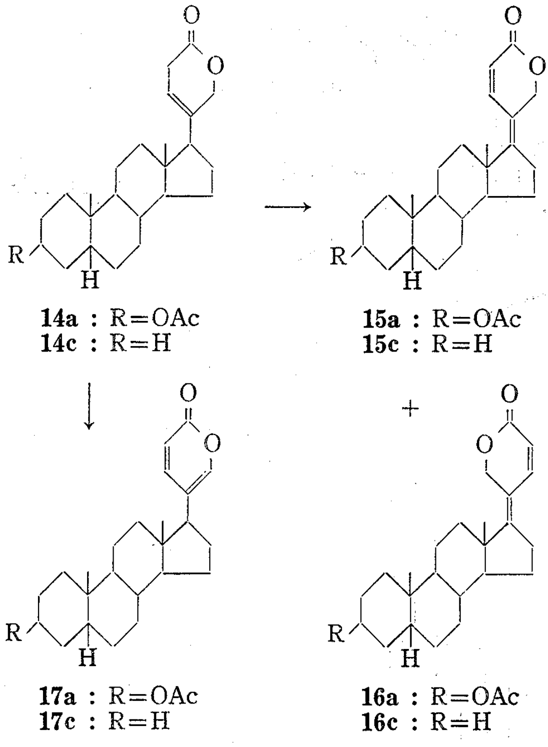
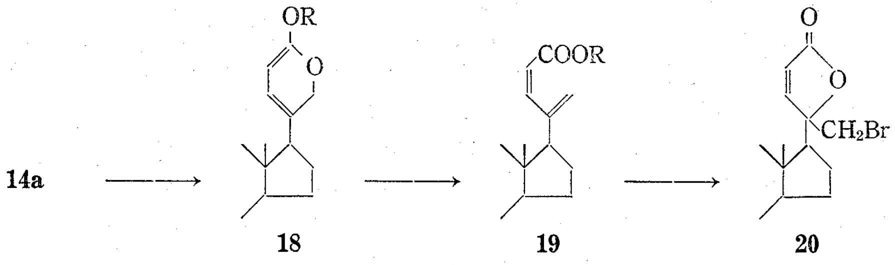


Chart 4

Although we were bewildered by the unreproducible literature precedent and furthermore other dehydrogenating methods such as utilizing sulfur or palladium charcoal did not give fruitful result, the objective conversion could have eventually been accomplished by simple operation, bromination-dehydrobromination.[18] Thus, by addition of bromine to **14a,c** followed by mild treatment of the crude product with 1,5-diazabicyclo[5,4,0]undec-5-ene(DBU), there were obtained the bufadienolides **17a,c** in good yields.[19]

Having been accomplished a simple access to bufadienolide structure, experiment was now advanced to the synthesis of natural 14β-oxygenated bufadienolides (**1** and **2**). For this purpose, 3β-acetoxy-5β-bufa-14,20(20)-dienolide (**14b**) was prepared from **7b** utilizing the same reaction sequence as performed in the model experiment (Chart 3). Here, the remaining task might in principle be accomplished by either first transformation to bufa-20,22-dienolide structure and subsequent introduction of 14β-oxygen functions, or vice versa. Of the two routes the former one was first investigated.

Bromination–dehydrobromination method employed in the preliminary experiment could not be applicable for **14b**, since bromine addition occurred preferably at the more reactive 14-double bond. It occurred to us that selective bromine substitution at the position 23 (or 20) of **14b** could be achieved, if the position is activated by the formation of enoly silyl ether.[20] The subsequent dehydrobromination was expected to proceed without difficulty to give α-pyrone ring. The model experiment utilizing **14a**, however, revealed



R=Si$(CH_3)_3$, Li

Chart 5

this idea to be unpromising. Trimethylsilylation of **14a** with trimethylchlorosilane——trimethylsilylimidazole[21] proceeded smoothly at room temperature to give a silylated product (**18**, R=Si$(CH_3)_3$), which on bromination in the cold afforded bromine containing butenolide (**20**). The reasonable explanation of this outcome should be that the silylation product under-

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[18] R.J. Chorvat and R. Papp, *J. Org. Chem.*, **41**, 2864 (1976).

[19] **17a** has been previously prepared from dehydroepiandrosterone by performing over 10 steps, G.R. Pettit and J.R. Dias, *J. Org. Chem.* **36**, 3207 (1971).

[20] R.H. Reuss and A. Hassner, *J. Org. Chem.*, **39**, 1785 (1974); L. Blanco, P. Amice, and J.M. Conia, *Synthesis*, **1976**, 194.

[21] B. Baume, W.E. Wilson, and E.C. Horning, *Anal. Lett.*, **1968**, 401.

went rearrangement to **19** (R$=$Si(CH$_3$)$_3$) which was actually brominated. Similarly, the enolate formation (**18**, R$=$Li) at $-78°$ followed by bromination[22] resulted in the production of **20** again, showing facile fragmentation of 3,6-dihydro-2H-pyran-2-one to conjugated dienoate (**19**) *via* enolate[23] or enol silyl ether. So far, the only successful method for the conversion of **14b** to 14-anhydrobufalin (**21**) has been sulfur dehydrogenation. However, the yield was unfortunately not acceptable owing to the concomitant formation of polar unidentified byproducts.

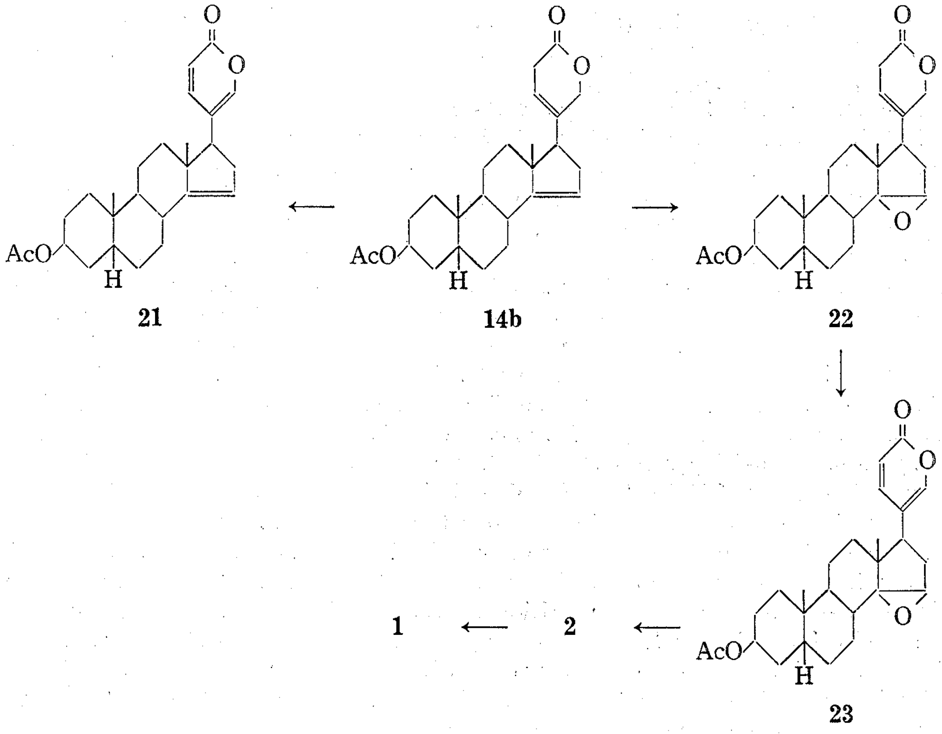

Chart 6

The second approach which involves the oxygenation of the 14-double bond of **14b** followed by α-pyrone formation has revealed to be quite effective. The reaction of **14b** with slight excess of aqueous N-bromoacetamide afforded unstable 15β-bromo-14β-hydrin which on alumina chromatography was readily transformed to 14β,15β-epoxide (**22**). Addition of bromine to **22** in the presence of an acetate buffer and subsequent dehydrobromination with DBU did provide resibufogenin acetate (**23**) which was identical with the sample prepared from natural resibufogenin. Since hydrolytic removal of the acetate group giving resibufogenin and its conversion to bufalin by reductive cleavage of the epoxide ring are already recorded,[24] the objective of the present investigation has thus been accomplished.

Experimental

All melting points are uncorrected. The infrared (IR) spectra were determined with JASCO IRA-1 or IR-S. The proton nuclear magnetic resonance (NMR) spectra were determined at 60 MHz with a Varian EM-360 or JEOL PMX-60. The chemical shifts are expressed in δ values relative to Me$_4$Si internal standard. The mass spectra were obtained with JEOL JMS-01SG-2 at 75 eV ionization potential. All reactions involving strong bases or organometallic reagents were performed under argon atmosphere. Thin-layer chro-

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22) M.W. Rathke and A. Lindert, *Tetrahedron Lett.*, **1971**, 3995.

23) J.V. Forsch, I.T. Harrison, B. Lythgoe, and A.K. Saksena, *J. Chem. Soc. Perkin I*, **1974**, 2005; R.A. Ruden and R. Bonjouklian, *J. Am. Chem. Soc.*, **97**, 6892 (1975).

24) H. Kondo and S. Ohno, U.S. Patent 3134772 [*Chem. Abstr.*, **61**, 5736 (1964)]; G.R. Pettit, L.E. Houghton, J.C. Knight, and F. Bruscheiler, *J. Org. Chem.*, **35**, 2895 (1970).

matography (TLC) was conducted using Merck precoated Silica gel GF_{254} plate and preparative TLC using 20×20 cm glass plate coated with 20 g of Merck silica gel $PF_{254+366}$. For column chromatography Merck silica gel with 0.06 to 0.20 mm particles or Merck alumina was used.

3β-Acetoxy-20-methoxy-21-formyl-5β-pregn-20-ene (**8a**)——This compound was prepared after the method of S. Bernstein.[14] To a stirred solution of 2.0 g of pregnanolone acetate (**7a**) in 40 ml of trimethyl orthoformate was added 1.3 ml of 70% perchloric acid dropwise and at room temperature (20°). After an additional 5 min, the dark green reaction mixture was treated with 3 ml of pyridine and then added to saturated $NaHCO_3$. The precipitated **8a** (1.445 g) was filtered and the filtrate was extracted with dichloromethane. The solvent was evaporated after drying on $MgSO_4$ and the residue was crystallized from ether to give an additional crop (0.287 g). Recrystallization from iso-Pr_2O–CH_2Cl_2 afforded colorless needles, mp 205—207°. *Anal.* Calcd. for $C_{25}H_{38}O_4$: C, 74.59; H, 9.52. Found: C, 74.68; H, 9.50. IR (KBr) cm^{-1}: 1715, 1650, 1595. NMR ($CDCl_3$) δ: 0.63 (18-CH_3), 0.98 (19-CH_3), 2.03 (OAc), 3.67 (OCH_3), 5.03 (3α-H), 5.43 (21-H, d, $J=7.5$), 9.72 (CHO, d, $J=7.5$).

3β-Acetoxy-21-methoxymethylidene-5β-pregnan-20-one (**9a**)——A stirred solution of 2.00 g of **8a** in a mixture of 30 ml of dry tetrahydrofuran and 20 ml of dry methanol was warmed at 50° and treated with 0.8 g of pyridine hydrobromide. After continued stirring for 35 min, **8a** disappeared from TLC. The reaction mixture was immediately added to saturated $NaHCO_3$, extracted with benzene, washed with brine, and dried on $MgSO_4$. Evaporation of the solvent afforded the crystalline product (2.12 g) which consisted of **9a** and **10a** (about 1: 1 mixture by NMR). It was then dissolved in 50 ml of dry *tert*-butanol and treated with 40 mg of potassium *tert*-butoxide at 35° for 20 min. The solution was neutrallized by addition of 10% acetic acid, concentrated by a rotary evaporator, and extracted with benzene. The benzene extract was washed with 5% $NaHCO_3$, dried on $MgSO_4$, and evaporated. The pale yellow crystalline residue (2.08 g) was recrystallized from iso-Pr_2O–benzene to give **9a** (1.51 g), mp 192—194°. *Anal.* Calcd. for $C_{25}H_{38}O_4$: C, 74.59; H, 9.52. Found: C, 74.45; H, 9.34. IR (KBr) cm^{-1}: 1720, 1670, 1580. NMR ($CDCl_3$) δ: 0.60 (18-CH_3), 0.97 (19-CH_3), 2.03 (OAc), 3.67 (OCH_3), 5.05 (3α-H), 5.53 (21-H, d, $J=12$), 7.53 (22-H, d, $J=12$).

17β(2-Methoxy-3,6-dihydro-2H-pyran-5-yl)-5β-androstan-3β-ol (**11a**)——A solution of dimsyl potassium was prepared by reacting 3.13 g of KH (24.7% in mineral oil) with 15 ml of anhydrous Me_2SO at room temperature followed by dilution with 15 ml of anhydrous tetrahydrofuran. A 12 ml portion of this solution was added by a syringe with stirring and at −2° to a combined solution of 1.0 g of **9a** in 15 ml of tetrahydrofuran with 1.54 g of trimethylsulfonium iodide dissolved in 15 ml of Me_2SO. After the addition, the reaction mixture was stirred at −2° to 0° and then partitioned between ether and water. The organic layer was washed with saturated brine, dried on $MgSO_4$, and evaporated. The residual glassy product was dissolved in 50 ml of dry MeOH containing 3 drops of concentrated HCl and heated at 58—60° for 30 min. The solution was then made alkaline by addition of 10 ml of 30% KOH, and after heating at the same temperature for 1 hr, it was concentrated by a rotary evaporator, and partitioned between ether and H_2O. The ether layer was washed with saturated brine, dried on $MgSO_4$, and evaporated. The residue was chromatographed on 15 g of silica gel. Elution with benzene afforded 125 mg of furan (**12a**), and subsequent elution with a mixture of benzene and ether furnished 590 mg of **11a**. **11a**, mp 134—138° (MeOH). *Anal.* Calcd. for $C_{25}H_{40}O_3$: C, 77.27; H, 10.38. Found: C, 77.36; H, 10.13. NMR (CCl_4) δ: 0.55 (18-CH_3), 0.93 (19-CH_3), 3.9 (21-CH_2 and 3α-H, broad), 3.32 (OCH_3), 4.57 (24-H, m), 5.30 (22-H, m). Acetate of **11a** was obtained by acetylation with acetic anhydride and pyridine, mp 158—161° (iso-Pr_2O). *Anal.* Calcd. for $C_{27}H_{42}O_4$: C, 75.31; H, 9.83. Found: C, 75.23; H, 9.74. NMR (CCl_4) δ: 0.55 (18-CH_3), 0.97 (19-CH_3), 1.97 (OAc), 3.33 (OCH_3), 3.77 and 3.92 (21-CH_2), 4.57 (24-H, m), 4.97 (3α-H), 5.23 (22-H, m.). **12a**, mp 144—145° (MeOH). *Anal.* Calcd. for $C_{23}H_{34}O_2$: C, 80.65; H, 10.01. Found: C, 80.68; H, 10.13. NMR (CCl_4) δ: 0.48 (18-CH_3), 0.97 (19-CH_3), 4.03 (3α-H), 6.18 (22-H, diffused d, $J=1$), 7.12 (21-H, diffused s), 7.30 (23-H, t, $J=1$).

17β(2-Methoxy-3,6-dihydro-2H-pyran-5-yl)-5β-androst-14-en-3β-ol (**11b**)——A solution of 1.98 g of 3β-acetoxy-20-methoxy-21-formyl-5β-pregna-14,20-diene (**8b**, prepared from **7b** according to the previous paper[1]) in 60 ml of anhydrous MeOH was treated with 2.4 ml of 3 M pyridine hydrobromide in MeOH at 45° for 30 min. The product obtained by the same workup as described for **8a** was dissolved in 100 ml of dry *tert*-butanol and the solution was kept at 30° with stirring while adding 100 mg of potassium *tert*-butoxide in two portions. After 35 min, the reaction mixture was neutralized with 1% AcOH and concentrated. The glassy product (2.0 g) isolated by ether extraction was identified as **9b** by NMR (CCl_4) δ: 0.86 (18-CH_3), 0.96 (19-CH_3), 1.96 (OAc), 3.66 (OCH_3), 4.93 (3α-H), 5.07 (15-H), 5.49 (21-H, d, $J=12$), 7.47 (22-H, d, $J=12$). A solution of dimsyl potassium was prepared by the reaction of 2.7 g of KH (24.7% in mineral oil) in 15 ml of Me_2SO followed by dilution with 15 ml of tetrahydrofuran. It was cooled at 0° and added by a syringe to a stirred solution of **9b** and 3.06 g of trimethylsulfonium iodide in 30 ml of Me_2SO and 30 ml of tetrahydrofuran. After 20 min, the reaction was stopped by addition of AcOH and subjected to usual workup. The crude product was dissolved in 100 ml of MeOH containing 3 drops of concentrated HCl and the solution was heated at 55° for 20 min. By usual workup the brown semi-solid product which showed essentially 2 spots on TLC was obtained. Separation of the two components was made by chromatography on 25 g of silica gel eluting with benzene and benzene-ether mixtures. From initial fractions there was obtained 357 mg of furan (**12b**) which was identical with the sample prepared in the previous paper.[1] Later fractions afforded 1.2 g of **11b** as crystalline mass. NMR (CCl_4) δ: 0.76 (18-CH_3), 0.98 (19-CH_3), 3.41 (OCH_3), 3.84 (3α-H), 4.01 (21-CH_2), 4.71 (24-H), 5.20 (15-H), 5.50 (22-H). The acetate, mp 160—163° (iso-Pr_2O), was obtained by acetylation with acetic anhydride and pyridine. *Anal.* Calcd. for $C_{27}H_{40}O_4$: C, 75.76; H, 9.42. Found: C, 76.02; H, 9.59. NMR (CCl_4) δ: 0.77 (18-CH_3), 1.00 (19-CH_3), 2.00 (OAc), 3.36 (OCH_3), 3.8—4.1 (21-CH_2), 4.63 (24-H, broad), 5.00 (3α-H), 5.46 (22-H, broad).

3β-Acetoxy-17β(2-hydroxy-3,6-dihydro-2H-pyran-5-yl)-5β-androstane (13a)——To a solution of 272 mg of the acetate of **11a** in 10 ml of tetrahydrofuran was added 5 ml of HCl–KCl buffer (pH=1.2) and the mixture was kept at 45° with stirring for 2 days. The solution was poured to saturated $NaHCO_3$ and extracted with CH_2Cl_2. The extract was washed with brine, dried on $MgSO_4$, and evaporated. The residual crystalline mass was chromatographed on 10 g of alumina (neutral, activity IV) eluting with a mixture of benzene and hexane (1: 1). After recovery of 26 mg of the starting material, 234 mg of dihydropyranol (**13a**) was obtained and crystallized from benzene–hexane, mp 142—147°. *Anal.* Calcd. for $C_{26}H_{40}O_4$: C, 74.96; H, 9.68. Found: C, 75.20; H, 9.88. NMR (CCl_4) δ: 0.56 (18-CH_3), 0.97 (19-CH_3), 1.97 (OAc), 3.98 (21-CH_2, diffused t, $J=7$), 4.97 (3α-H), 5.27 (22-H).

3β-Acetoxy-17β(2-hydroxy-3,6-dihydro-2H-pyran-5-yl)-5β-androst-14-ene (13b)——To a solution of crude acetate obtained from 1.2 g of **11b** in 50 ml of tetrahydrofuran was added 25 ml of HCl–KCl buffer (pH=1.2) and the mixture was stirred at 40° for 35 hr. The hydrolysis product isolated by extraction with CH_2Cl_2 was chromatographed on 45 g of alumina (neutral, activity, IV). Elution with benzene and benzene–ether mixtures afforded 802 mg of **13b**. Analytical sample was recrystallized from iso-Pr_2O–hexane, mp 118—122°. *Anal.* Calcd. for $C_{26}H_{38}O_4$: C, 75.32; H, 9.24. Found: C, 75.54; H, 9.33. NMR (CCl_4) δ: 0.78 (18-CH_3), 0.99 (19-CH_3), 2.00 (OAc), 3.9—4.4 (21-H and 24-H), 5.00 (3α-H), 5.50 (22-H). MS *m/e* (relative intensity): 414 (M^+, 9), 396 (43), 336 (100).

3β-Acetoxy-5β-buf-20(22)-enolide (14a)——A solution of 300 mg of **13a** in 20 ml of acetone was titrated with Jones reagent at 0°. The reaction product isolated by usual manner was filtered through a column of 10 g of silica gel using benzene and benzene–ether mixtures as eluting solvents to give 220 mg of **14a**. Recrystallization from ether afforded colorless plates (180 mg), mp 85—88°. *Anal.* Calcd. for $C_{26}H_{38}O_4$: C, 75.32; H, 9.24. Found: C, 75.10; H, 9.36. IR (KBr) cm^{-1}: 1730. NMR (CCl_4) δ: 0.55 (18-CH_3), 0.97 (19-CH_3), 1.97 (OAc), 2.92 (23-CH_2, broad), 4.60 (21-CH_2, broad), 5.57 (22-H, diffused t, $J=4$).

3β-Acetoxy-5β-bufa-14,20(22)-dienolide (14b)——A solution of 670 mg of **13b** in 45 ml of acetone was titrated with Jones reagent at 0° over 20 min. Usual workup afforded 575 mg of **14b** which was recrystallized from iso-Pr_2O–CH_2Cl_2, mp 210—213°. NMR (CCl_4) δ: 0.80 (18-CH_3), 1.00 (19-CH_3), 2.01 (OAc), 3.01 (23-CH_2, broad), 4.73 (21-CH_2, broad), 5.00 (3α-H), 5.16 (15-H), 5.70 (22-H, diffused t, $J=4$). *Anal.* Calcd. for $C_{26}H_{36}O_4$: C, 75.69; H, 8.80. Found: C, 75.53; H, 9.05.

5β-Buf-20(22)-enolide (14c)——This compound was prepared from 5β-pregnan-20-one[25] by carrying out the same reaction sequence described for **14a** and **14b**, mp 180—181° from cyclohexane (lit.,[15] mp 179—180°) $[\alpha]_D=-13.6°$ ($CHCl_3$, $c=3.0$) (lit.,[15] $-11.7°$). *Anal.* Calcd. for $C_{24}H_{36}O_2$: C, 80.85; H, 10.18. Found: C, 81.02; H, 10.31. IR (KBr) cm^{-1}: 1740. NMR ($CDCl_3$) δ: 0.57 (18-CH_3), 0.93 (19-CH_3), 3.01 (23-CH_2, diffused s), 4.75 (21-CH_2, m), 5.65 (22-H, diffused t, $J=4.5$). The sample obtained here was also identified by direct comparison of the NMR chart with that of authentic sample through the courtesy of Prof. Sarel.[17]

Physical data of characterized intermediates are given below. 20-Methoxy-21-formyl-5β-pregn-20-ene (not crystallized). NMR (CCl_4) δ: 0.60 (18-CH_3), 0.92 (19-CH_3), 3.68 (OCH_3), 5.37 (21-H, d, $J=7$), 9.73 (22-H, d, $J=7$). 21-Methoxymethylidene-5β-pregnan-20-one (mp 156.5—157.5° from hexane–CH_2Cl_2). *Anal.* Calcd. for $C_{23}H_{36}O_2$: C, 80.30; H, 10.55. Found: C, 80.44; H, 10.31. NMR (CCl_4) δ: 0.55 (18-CH_3), 0.92 (19-CH_3), 3.70 (OCH_3), 5.48 (21-H, d, $J=13$), 7.48 (22-H, d, $J=13$).

DDQ Dehydrogenation of Buf-20(22)-enolides——1) To a solution of 23 mg of **14a** in 4.5 ml of anhydrous dioxane containing 50 mg of toluene-*p*-sulfonic acid was added 30 mg of DDQ. The solution was refluxed with stirring for 4.5 hr and then partitioned between ether and H_2O. The ether solution was washed with saturated $NaHCO_3$ and brine, dried on $MgSO_4$, and evaporated. The residue was revealed by integration of olefinic protons (NMR) to be a 7: 3 mixture of **16a** and **15a**. The major isomer (**16a**) was isolated by preparative TLC (benzene–ethyl acetate=9: 1) and crystallized from iso-Pr_2O–CH_2Cl_2, mp 204—209°. *Anal.* Calcd. for $C_{26}H_{36}O_4$: C, 75.69; H, 8.80. Found: C, 75.81; H, 8.68. IR (KBr) cm^{-1}: 1715, 1620. NMR ($CDCl_3$) δ: 0.95 (18-CH_3), 0.99 (19-CH_3), 2.03 (OAc), 5.08 (3α-H and 21-CH_2), 5.73 (23-H, d, $J=10$), 7.07 (22-H, d, $J=10$). NMR data of minor isomer **15a** was obtained from the spectrum of the crude product by substracting the peaks due to **16a**[26]: 0.99 (19-CH_3), 1.23 (18-CH_3), 4.83 (21-CH_2), 5.70 (23-H, d, $J=10$), 7.43 (22-H, d, $J=10$). Hydrogen chloride catalyzed dehydrogenation in dimethoxyethane proceeded at room temperature giving essentially the same result.

2) A solution of 50 mg of **14c** in 14 ml of dioxane containing 14 mg of toluene-*p*-sulfonic acid and 56 mg of DDQ was refluxed for 4 hr. The crude product obtained by usual workup was subjected to preparative TLC ($CHCl_3$–benzene=3: 1) where, in addition to 6.2 mg of the starting material, 29.7 mg of a mixture of bufa-17,22-dienolide isomers (**15c** and **16c**) was isolated, mp 171—174° (iso-Pr_2O). Ratio of **16c** to **15c** was 7: 3 by NMR.[26] *Anal.* Calcd. for $C_{24}H_{34}O_2$: C, 81.31; H, 9.67. Found: C, 81.18; H, 9.42. IR (KBr)

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25) M. Fetizon, F.J. Kakis, and V. Ignatiadou-Ragoussis, *J. Org. Chem.*, 38, 4308 (1973).

26) Peak assignments were based on the ref. 15).

cm^{-1}: 1720, 1625, 1575. NMR (CCl_4) δ: **15c**, 4.78 (21-CH_2), 5.62 (23-H, d, $J=10$), 7.40 (22-H, d, $J=10$); **16c**, 5.03 (21-CH_2), 5.67 (23-H, d, $J=10$), 7.03 (22-H, d, $J=10$).

3β-Acetoxy-5β-bufa-20,22-dienolide (17a)——To a solution of 20.7 mg of bufenolide **14a** in 4 ml of CH_2Cl_2 was added 0.5 ml of 0.1 M Br_2 in CH_2Cl_2 and the mixture was stirred at room temperature for 2 hr. The CH_2Cl_2 solution was then washed with H_2O, dried on $MgSO_4$, and evaporated. The residue was dissolved in a mixture of 1 ml of dimethyl formamide (DMF) and 0.5 ml of DBU and heated at 60° for 1 hr. The product isolated by ether extraction was purified by preparative TLC (benzene–AcOEt=9: 1) and crystallized from iso-Pr_2O, mp 160—164° (8 mg). *Anal.* Calcd. for $C_{26}H_{36}O_4$: C, 75.69; H, 8.80. Found: C, 75.75; H, 8.87. NMR (CCl_4) δ: 0.53 (18-CH_3), 0.97 (19-CH_3), 1.97 (OAc), 4.93 (3α-H), 6.01 (23-H, d, $J=10.5$), 7.05 (22-H, m), 7.13 (21-H, diffused s).

5β-Bufa-20,22-dienolide (17c)——This compound was obtained in 61% yield by dehydrogenation of **14c** using bromination-dehydrobromination technique described above. Analytical sample was crystallized from benzene-petroleum ether, mp 192—194° (lit.,[15] 194—196°). *Anal.* Calcd. for $C_{24}H_{34}O_2$: C, 81.31; H, 9.67. Found: C, 81.17; H, 9.91. IR (KBr) cm^{-1}: 1720, 1630, 1530. NMR (CCl_4) δ: 0.55 (18-CH_3), 0.95 (19-CH_3), 6.11 (23-H, d, $J=10$), 7.13 (22-H, m), 7.22 (21-H, diffused s).

3β-Acetoxy-20-hydroxy-21-bromo-5β-chol-22-en-24-oic acid lactone (20)——1) A solution of 100 mg of **14a** in a mixture of 1 ml of trimethylsilylimidazole, 1 ml of bistrimethylsilylacetamide and 0.4 ml of trimethylchlorosilane was left stand at room temperature. After 10 days, the solution was evaporated *in vacuo*. The residue was dissolved in 2 ml of CCl_4 and brominated at −15° and with stirring by dropwise addition of 0.5 M Br_2 in CCl_4. The reaction mixture was diluted with CH_2Cl_2, washed with water, dried on $MgSO_4$, and evaporated. Purification of the product by preparative TLC followed by crystallization from MeOH afforded **20**, mp 248—251°. *Anal.* Calcd. for $C_{26}H_{37}O_4Br$: C, 63.28; H, 7.56. Found: C, 63.08; H, 7.66. NMR ($CDCl_3$) δ: major isomer at C_{20}—0.80 (18-CH_3), 0.97 (19-CH_3), 2.03 (OAc), 3.66 (21-CH_2, s), 5.05 (3α-H), 6.13 (23-H, d, $J=6$), 7.30 (22-H, d, $J=6$); minor isomer—0.60 (18-CH_3), 0.95 (19-CH_3), 3.30, 3.47 (21-CH_2, d, $J=12$), 6.16 (23-H, d, $J=6$), 7.50 (22-H, d, $J=6$). MS *m/e* (relative intensity): 434, 432 (M^+–AcOH, 100), 419, 417 (29), 353 (40), 257 (36), 215 (33). IR (KBr) cm^{-1}: 1755, 1735.

2) A solution of 60 mg of **14a** in 0.4 ml of tetrahydrofuran was added during 5 min to 0.8 ml of 0.18 M iso-Pr_2NLi in tetrahydrofuran with stirring and at −78°. After 20 min, 0.25 ml of 0.58 M Br_2 in CH_2Cl_2 was added rapidly to the enolate solution and the mixture was allowed to warm to room temperature. The crude product obtained by usual workup was subjected to preparative TLC (cyclohexane–ether–AcOEt=4: 2: 1), by which 5.5 mg of **20** and 9 mg of **14a** were isolated.

3β-Acetoxy-5β-bufa-14,20,22-trienolide (14-anhydrobufalin acetate) (21)——A solution of 25 mg of **14b** and 90 mg of S in CS_2 was evaporated and the residue was heated at 200° for 10 min (disappearance of **14b** from TLC). After cooling, the organic product was subjected to preparative TLC (cyclohexane–ether–AcOEt=4: 2: 1) to give 2.5 mg of **21**, which was identified with an authentic sample prepared from natural bufalin[27] by comparison of TLC, IR, NMR, and MS.

3β-Acetoxy-14β,15β-epoxy-5β-buf-20(22)-enolide (22)——A stirred solution of 37 mg of **14b** in 1.5 ml of acetone and 0.3 ml of H_2O was cooled at 0° and treated with 0.046 ml of 70% $HClO_4$ and 18.6 mg of N-bromoacetamide dissolved in 0.3 ml of H_2O. After stirring for 30 min, the reaction mixture was mixed with Na_2SO_3 solution and extracted with ether. The ether solution was washed with saturated $NaHCO_3$, dried on $MgSO_4$, and evaporated. The residue was chromatographed on 1 g of alumina (basic, activity V) eluting with benzene. The epoxide (**22**, 32 mg) which showed single spot on TLC was crystallized from ether, mp 155—157°. *Anal.* Calcd. for $C_{26}H_{36}O_5$: C, 72.86; H, 8.47. Found: C, 72.89; H, 8.49. NMR (CCl_4) δ: 0.87 (18-CH_3), 0.97 (19-CH_3), 1.97 (OAc), 2.87 (23-H, broad), 3.30 (15α-H, s), 4.67 (21-H, broad), 4.90 (3α-H broad), 5.50 (22-H, diffused t, $J=4$).

Resibufogenin Acetate (23)——To a stirred suspension of 42.8 mg of **22** in 2 ml of ether was added successively 1 ml of 10% AcOK–AcOH and 2 ml of 0.1 M Br_2 in AcOH. The mixture was stirred at room temperature overnight and poured to saturated $NaHCO_3$. Extraction with ether followed by usual workup afforded pale brown foam. It was then dissolved in 1.5 ml of DMF and 0.5 ml of DBU and warmed to 60° over 1 hr. The product (35 mg) isolated by ether extraction showed two partially overlapped spots on TLC and revealed by NMR to be a 1: 1 mixture of resibufogenin acetate **23** and starting material **22**. **23** was isolated by preparative TLC (acetone-cyclohexane=1: 3) and crystallized from MeOH–ether, mp 229—234°. The spectroscopic data (IR, UV, NMR, and MS) were entirely identical with those of authentic sample prepared from natural resibufogenin. IR ($CHCl_3$) cm^{-1}: 1718, 1625, 1530. NMR (CCl_4) δ: 0.76 (18-CH_3), 1.00 (19-CH_3), 1.98 (OAc), 3.36 (15α-H, s), 4.96 (3α-H, m), 6.07 (23-H, d, $J=10$), 7.10 (21-H, d, $J=2.5$), 7.63 (22-H, dd, $J=10, 2.5$). MS *m/e* (relative intensity): 426 (M^+, 10), 366 (100), 351 (22), 338 (23), 312 (14), 275 (32), 215 81).

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