

265. On Cardioactive Steroids. XII

The Synthesis of (Natural) Bufalin and α' -Isobufalin¹⁾

by Karel Wiesner*, Thomas Y. R. Tsai, Alina Sen, Ravindra Kumar, and Masayoshi Tsubuki

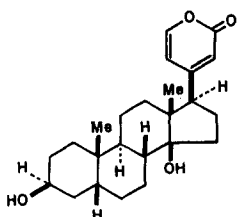
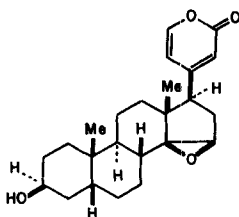
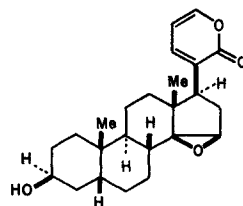
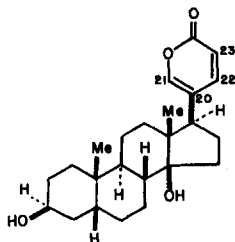
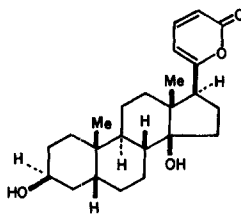
Natural Products Research Center, University of New Brunswick, Fredericton, New Brunswick, Canada E3B 6E2

(4.X.1983)

Summary

An efficient and simple synthesis of bufalin (**1**) *via* furan-containing intermediates is described. The same method was also used for the synthesis of α' -isobufalin (**2**) which was prepared with equal simplicity, but in lower yield.

Introduction. – In [2] we have described a relatively simple synthesis of the bufalin and resibufogenin isomers **I–III**¹⁾ in which we utilized an adaptation of our furan strategy for cardenolides [3]. The pharmacological study of these compounds and their dihydro derivatives submitted as glucosides yielded interesting results [4]. Especially the derivatives of **I** have shown extremely high potency combined with a significant improvement

**I** α -isobufalin**II** α -isoresibufogenin**III** β -isoresibufogenin**1** bufalin**2** α' -isobufalin

¹⁾ For communication No. XI, see [1]. Systematic names of all compounds are given in the *Exper. Part*.

of the margin of safety as compared with the natural *Digitalis* cardenolides used in therapy. These results encouraged us to work out alternative variants of our furan strategy which would enable us to approach natural bufalin (**1**) and α' -isobufalin (**2**).

We wish to describe in the present paper a simple and efficient preparation of these two compounds. Lengthy, many-stage syntheses of bufalin (**1**) have been reported previously [5], but a truly simple and efficient preparative method does not seem to have been found yet. Complex and not fully successful approaches to systems similar to **2** were also published [6].

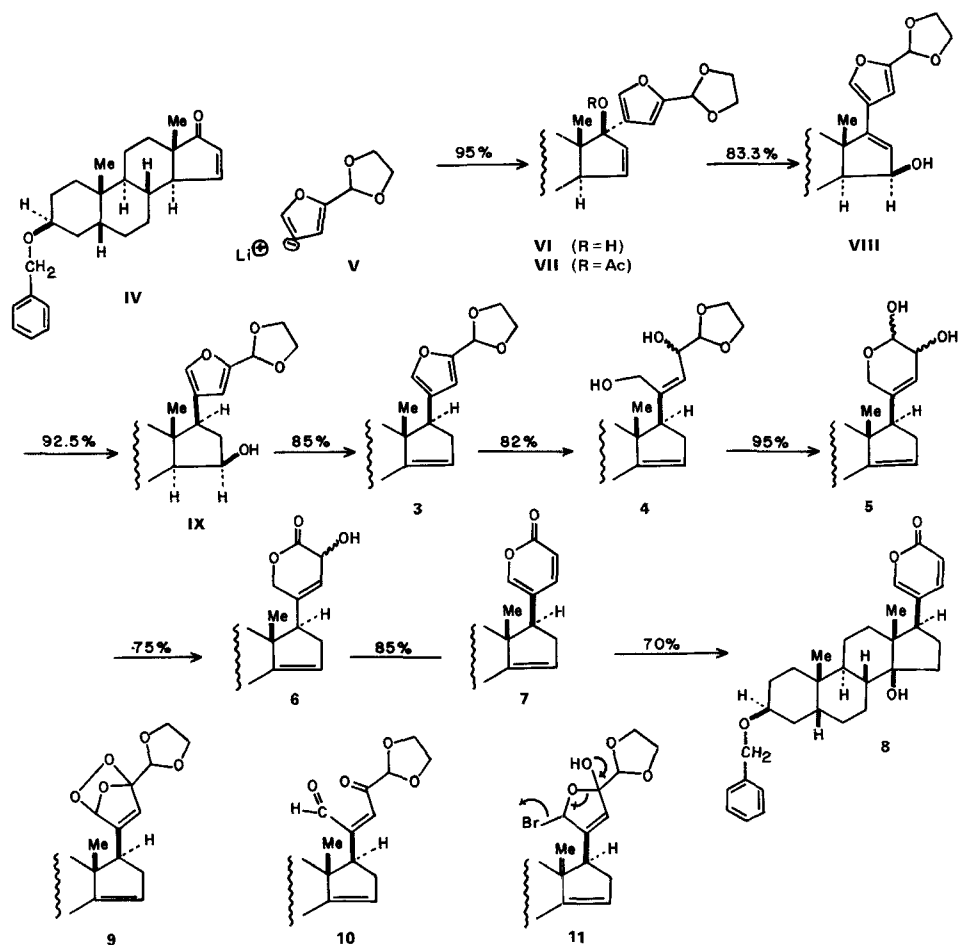
Discussion. – The first four steps of our bufalin synthesis were identical with the already described [2] preparation of β -isoresibufogenin **III**, and they are, for the sake of completeness, shown in *Scheme 1* (formulae **IV–IX**) together with the yields obtained.

The 15' β -OH-group¹⁾ of compound **IX**, the actual starting material of the present work, was eliminated with mesyl chloride (MsCl) in pyridine, and the crystalline $\Delta^{14',15'}$ -derivative **3** (m.p. 141–142°) was obtained in a yield of 85%²⁾. The ¹H-NMR spectrum of **3** showed the vinylic H–C(15') as a $m(W_{1/2} = ca. 6 \text{ Hz})$ at $\delta = 5.23$. The acetal **3** was irradiated with a 100-W high-pressure Hg-lamp at -70° in CH_2Cl_2 in the presence of 5,10,15,20-tetraphenylporphyrin [7] while O_2 was bubbled through the solution. The peroxide bond of the resulting endoperoxide **9** was cleaved with a large excess of Me_2S , and the crude unsaturated keto aldehyde **10** was immediately reduced with an excess of NaBH_4 . The oily product **4** was obtained in an overall yield of 82% from compound **3**. The ¹H-NMR spectrum of this material indicated that it was a mixture of about equal parts of two epimers.

After the synthesis of bufalin was completed and we only had a small amount of compound **3** left, we discovered an even simpler conversion of **3** to **4**. Compound **3** was treated with 1 mol of *N*-bromosuccinimide (NBS) and yielded directly the keto aldehyde **10** presumably by HBr-elimination from the intermediate **11**. Reduction of **10** with NaBH_4 gave as in the previous procedure the diol **4**. This method of furan ring opening (*cf.* [3]) was utilized with great advantage in the synthesis of α' -isobufalin (**2**; *vide infra*).

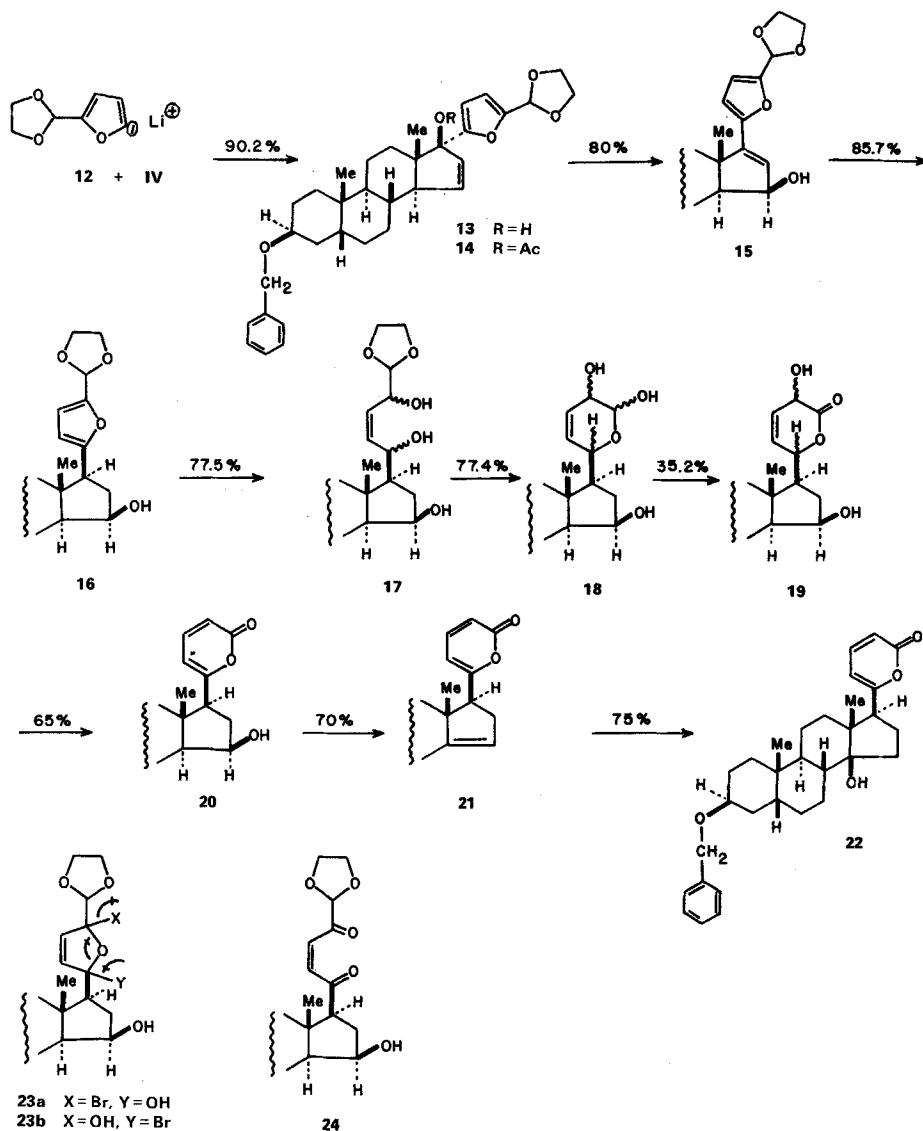
Compound **4** was now heated under reflux with dilute HCl in THF. Hydrolysis of the ethylene glycol acetal and hemiacetal formation yielded 95% of the diastereoisomeric mixture **5**, which was oxidized in the next step without purification with $\text{Ag}_2\text{CO}_3/\text{Celite}$ in refluxing benzene [8]. The unsaturated hydroxylactone **6** was obtained in a yield of 75% and, somewhat surprisingly, turned out to be a sharply melting crystalline compound (m.p. 117–119°). The hydroxylactone **6** is clearly on the oxidation level of bufalin and requires only a simple adjustment of functionality to yield the final product **1**. Compound **6** was dissolved in CH_2Cl_2 and mesylated with MsCl and Et_3N . The crude mesylate was then subjected to elimination by heating with 1,5-diazabicyclo [4.3.0]non-5-ene (DBN) in benzene. The elimination product **7** was crystallized from $\text{Et}_2\text{O}/\text{hexane}$ (m.p. 115–116°), and it was obtained in a yield of 85%. Its spectral properties left no doubt that the α -pyrone construction was now complete. The introduction of the 14' β -OH-group was performed as described previously by the modified method of Engel (*cf.* [2] [3]), and the product, 3-*o*-benzylbufalin (**8**), was isolated in a yield of 70% and crystallized from $\text{Et}_2\text{O}/\text{CH}_2\text{Cl}_2$ (m.p. 198–200°).

²⁾ For complete spectral data for all compounds see *Exper. Part*.



Finally, debenzoylation of **8** with $\text{Pd}(\text{OH})_2/\text{C}$ [9], a method which we have used previously in our synthesis of the isobufalins [2], yielded 70% of the crystalline (m.p. 235–237°) synthetic bufalin (**1**). The spectral data of the synthetic material [IR (CHCl_3): 3600, 3450 (OH), 1710, 1635 (pyrone). UV(EtOH): 298(3.77). $^1\text{H-NMR}$ (CDCl_3): 7.83 (*dd*, $J = 10$ and 2, H-C(22)); 7.23 (*d*, $J = 2$, H-C(21)); 6.25 (*d*, $J = 10$, H-C(23))] were identical with those of an authentic sample of the natural product, and the identity was confirmed by mixed m.p. and TLC in several systems.

The synthesis of α' -isobufalin (**2**) was performed by a similar route as the synthesis of bufalin (**1**). The starting materials were the Li-derivative **12**, prepared by direct lithiation of the ethylene glycol acetal of furfural [10], and our usual steroidal ketone **IV** (Scheme 2). The synthesis proceeded uneventfully ($\rightarrow \mathbf{13} \rightarrow \mathbf{14} \rightarrow \mathbf{15}$) to compound **16**, which was oxidized by NBS, and the product of the oxidation, the diketone **24**, was immediately reduced by NaBH_4 to the diastereoisomeric mixture **17**. In this case, we were forced to perform the oxidative degradation of the α,α' -disubstituted furan before the



development of the 14',15'-double bond since NBS attack on the furan and on the double bond could not be differentiated. The intermediates in the NBS oxidation were presumably the compounds **23a** and/or **23b** which eliminated HBr as indicated (for **23a**).

The mixture **17** was used without separation in the next step, but one of the components was isolated and characterized as a crystalline compound. The acid-catalyzed conversion of **17** to **18** proceeded, except for the somewhat poorer yield, normally. The oxidation of the pyranose **18** with $\text{Ag}_2\text{CO}_3/\text{Celite}$ gave 35.2% of the crystalline hydroxy-

lactone **19**. The reason for this low yield was the formation of substantial amounts of a formic acid ester by cleavage of the 2,3-diol³).

The elimination of the two OH-groups of compound **19** had to be performed in two stages. A monomesylation followed by heating of the crude mesylate with LiBr in DMF yielded the α -pyrone **20**, and the remaining OH-group was eliminated with SOCl₂ and pyridine. The resulting product **21** was a beautifully crystalline compound (m.p. 145–146°), and its spectra left no doubt that the pyrone group and the steroidal 14', 15'-double bond had been correctly assembled. The introduction of the 14' β -OH-group and the debenzoylation of the resulting compound **22** (m.p. 194–196°) was accomplished by the same methods as before. The final product, α -isobufalin (**2**; m.p. 145–147°), displayed spectra entirely consistent with the spectral data of its isomers which we have synthesized previously and with the structure assigned to it [IR (CHCl₃): 3600 (OH), 1720 (C=O), 1630 (C=C). UV (EtOH): 306 (3.84). ¹H-NMR (CDCl₃): 6.15 (*d*, *J* = 9, H–C(3)); 6.19 (*d*, *J* = 6, H–C(5)); 7.3 (*dd*, *J* = 6 and 9, H–C(4))]. The overall yield of **2** was much lower than the one obtained for **1**; this is due to somewhat poorer yields in the middle part of the synthesis (**16** → **20**) and especially due to the low yield of the oxidation step **18** → **19**. Nevertheless, we were able to prepare the sample for pharmacology without excessive difficulties⁴).

We wish to thank the *Natural Sciences and Engineering Research Council*, Ottawa, Canada, the *Canadian Heart Foundation*, and *Advance Biofactures Corporation*, New York, USA, for the support of these studies.

Experimental Part

General. See [2].

4-(3' β -Benzyloxy-5' β -androst-14'-en-17' β -yl)furan-2-carbaldehyde Ethylene Acetal (**3**). Compound **IX** [2] (1.93 g) in pyridine (7.5 ml) was cooled in an ice-bath and treated with SOCl₂ (517.9 mg) for 30 min. The mixture was stirred at r.t. for 30 min and then diluted with Et₂O. The solution was washed with 10% citric acid and aq. NaHCO₃, dried over anhyd. MgSO₄, and evaporated to dryness. The crude product was chromatographed on silica gel with acetone/hexane 1:4 yielding 1.58 g (85%) of **3**. The compound crystallized from Et₂O/hexane, m.p. 141–142°. IR (CHCl₃): no OH. ¹H-NMR (CDCl₃): 0.68 (*s*, 3 H–C(18')); 0.97 (*s*, 3 H–C(19')); 3.70 (*m*, *W*_{1/2} ≈ 7, H–C(3')); 3.88–4.20 (*m*, OCH₂CH₂O); 4.47 (*s*, CH₂Ph); 5.23 (*m*, *W*_{1/2} ≈ 6, H–C(15')); 5.83 (*s*, H–C(1)); 6.33 (*s*, H–C(3)); 7.18 (*s*, H–C(5)); 7.28 (*br. s*, 5 arom. H). MS: 502 (C₃₃H₄₂O₄⁺). MS(HR): 502.3081 (*M*⁺, calc. 502.3082).

C₃₃H₄₂O₄ (502.30) Calc. C 78.84 H 8.42% Found C 78.64 H 8.40%

4-(3' β -Benzyloxy-5' β -androst-14'-en-17' β -yl)-2,5-dihydroxy-3-pentenal Ethylene Acetal (**4**). Compound **3** was irradiated in a 200-ml Pyrex container provided with a gas inlet which was connected to an O₂ bomb. The container was charged with a solution of **3** (1.58 g) and 5,10,15,20-tetraphenylporphine (1.5 mg) in CH₂Cl₂ (100 ml), submerged in a dry-ice/acetone bath, and positioned as close as possible to the Hg-lamp. The photooxygenation was complete after 20 min, and the endoperoxide **9** was treated with Me₂S (8 ml). The mixture was flushed with N₂ at r.t. for 45 min and evaporated to dryness. The keto aldehyde **10** was dissolved in THF (54 ml), and H₂O (5.4 ml) was added. The mixture was treated at r.t. with 205.8 mg of NaBH₄ and 2.95 ml of 2N KOH for

³) Clearly, differential protection of the 3-OH-group in compound **18** would be a solution of the poor yield problem. A moderate amount of experimentation in this direction (*e.g.*, acetylation followed by acid hydrolysis) did not yield fully satisfactory results, but a more persistent effort would probably be ultimately successful.

⁴) We believe that the synthesis of **2** could be developed to a level of efficiency comparable to the one achieved in the synthesis of bufalin (**1**). This development would be undertaken only if a glycoside of **2** is selected for detailed study.

4 h. The mixture was worked up in the usual manner, and the product was purified on silica gel G plates with Et₂O/CH₂Cl₂ 3:7 yielding 1.35 g (82%) of pure amorphous **4** over the 2 steps. IR(CHCl₃): 3590, 3480 (OH). ¹H-NMR(CDCl₃): 0.78 (*s*, 3 H–C(18')); 0.93 (*s*, 3 H–C(19')); 3.70 (*m*, *W*_{1/2} ≈ 7, H–C(3')); 3.83–4.10 (*m*, OCH₂CH₂O); 4.12 (*br. s*, H–C(5)); 4.30–4.67 (*m*, H–C(2), CH₂Ph); 4.90 (*d*, *J* = 4, H–C(1)); 5.22 (*m*, *W*_{1/2} ≈ 6, H–C(15')); 5.56 (*dd*, *J* = 8, H–C(3)); 7.35 (*br. s*, 5 arom. H). MS: 522 (C₃₃H₄₆O₅⁺). MS(HR): 522.3321 (*M*⁺, calc. 522.3345).

5-(3'-β-Benzoyloxy-5'-β-androst-14'-en-17'-β-yl)-3,6-dihydro-2H-pyran-2,3-diol (**5**). Compound **4** (1.31 g) was dissolved in 18 ml of THF and treated with 3*N* HCl (2 ml). The mixture was stirred and heated under reflux for 5 h and then diluted with Et₂O. The solution was washed with dil. NaHCO₃ and H₂O. The Et₂O-layer was dried over anhyd. MgSO₄, evaporated to dryness, and the product purified on silica gel G plates with CH₂Cl₂/Et₂O 85:15. The crude foamy **5** (1.13 g, 95%) was used without further purification. IR(CHCl₃): 3590, 3485 (OH). MS: 478 (C₃₁H₄₂O₄⁺). MS(HR): 478.3099 (*M*⁺, calc. 478.3082).

5-(3'-β-Benzoyloxy-5'-β-androst-14'-en-17'-β-yl)-3-hydroxy-3,6-dihydro-2H-pyran-2-one (**6**). Compound **5** (876.8 mg) was dissolved in dry benzene (149 ml) and heated under reflux. Freshly prepared Ag₂CO₃/Celite (3.5 g) was then added to the vigorously stirred solution, and in 10 min the oxidation of **5** was completed. After cooling, the mixture was diluted with Et₂O and filtered through Celite. The filtrate was evaporated to dryness and the product purified on silica gel G plates with CH₂Cl₂/Et₂O 85:15. Crystallization from Et₂O/hexane gave 654.8 mg (75%) of **6**, m.p. 117–119°. IR(CHCl₃): 3550 (OH), 1735 (C=O). ¹H-NMR(CDCl₃): 0.77 (*s*, 3 H–C(18')); 0.98 (*s*, 3 H–C(19')); 3.72 (*br. s*, *W*_{1/2} ≈ 8, H–C(3'), OH); 4.60–4.87 (*br. s*, H–C(6), H–C(3)); 5.17 (*br. s*, *W*_{1/2} ≈ 6, H–C(15')); 5.88 (*m*, *W*_{1/2} ≈ 4, H–C(4)); 7.37 (*br. s*, 5 arom. H). MS: 476 (C₃₁H₄₀O₄⁺). MS(HR): 476.2926 (*M*⁺, calc. 476.2926).

C₃₁H₄₀O₄ (476.29) Calc. C 78.11 H 8.46% Found C 77.75 H 8.51%

5-(3'-β-Benzoyloxy-5'-β-androst-14'-en-17'-β-yl)-2H-pyran-2-one (**7**). Compound **6** (616.4 mg) in CH₂Cl₂ (15.4 ml) and Et₃N (10.2 ml) were cooled in an ice-bath, and MsCl (308.2 mg) was added. The mixture was stirred in the ice-bath for 30 min and then diluted with CH₂Cl₂. The CH₂Cl₂-layer was washed with 5% citric acid, aq. NaHCO₃, and brine and evaporated to dryness. The crude product was dissolved in benzene (102 ml) and heated under reflux with DBN (708.8 mg) for 1 h. After cooling, the benzene was diluted with Et₂O, washed with aq. 5% citric acid, NaHCO₃, and NaCl, and dried (anhyd. MgSO₄). The solvent was evaporated, and the crude product was purified on silica gel G plates with 5% Et₂O in CH₂Cl₂/hexane 1:1 and crystallized from Et₂O/hexane giving 504 mg (85%) of **7**, m.p. 115–116°. IR(CHCl₃): 1715 (C=O), 1635 (C=C). ¹H-NMR(CDCl₃): 0.73 (*s*, 3 H–C(18')); 0.97 (*s*, 3 H–C(19')); 3.71 (*m*, *W*_{1/2} ≈ 7, H–C(3')); 4.48 (*s*, CH₂Ph); 5.23 (*m*, *W*_{1/2} ≈ 6, H–C(15')); 6.28 (*d*, *J* = 10, H–C(3)); 7.31 (*s*, H–C(6)); 7.20–7.37 (*m*, H–C(4), 5 arom. H). MS: 458 (C₃₁H₃₈O₃⁺). MS(HR): 458.2827 (*M*⁺, calc. 458.2821).

5-(3'-β-Benzoyloxy-14'-hydroxy-5'-β, 14'-β-androstan-17'-β-yl)-2H-pyran-2-one (**8**). Compound **7** (504 mg) in 22 ml of acetone/H₂O 9:1 was treated with NBS (240 mg) and 10 drops of 1% aq. HClO₄ at r.t. for 30 min. The mixture was diluted with CH₂Cl₂, washed with aq. NaHSO₃ and H₂O, dried (anhyd. MgSO₄), and evaporated to dryness. The residue was dissolved in 44 ml of MeOH/CH₂Cl₂ 1:1, and AcONa (91 mg) and an excess of Raney-Ni were added. After 30 min of stirring, the catalyst was filtered off, and the solution was evaporated to dryness. The product was purified on silica gel G plates with CH₂Cl₂/Et₂O 95:5 yielding 367.1 mg (70.1%) of **8** which crystallized from CHCl₃/Et₂O, m.p. 198–200°. IR(CHCl₃): 3595, 3450 (OH), 1700 (C=O), 1630 (C=C). ¹H-NMR(CDCl₃): 0.71 (*s*, 3 H–C(18')); 0.95 (*s*, 3 H–C(19')); 3.75 (*m*, *W*_{1/2} ≈ 7, H–C(3')); 4.52 (*s*, CH₃Ph); 6.29 (*d*, *J* = 10, H–C(3)); 7.38 (*br. s*, H–C(6), 5 arom. H); 7.88 (*dd*, *J* = 2, 10, H–C(4)). MS: 476 (C₃₁H₄₀O₄⁺). MS(HR): 476.2928 (*M*⁺, calc. 476.2926).

C₃₁H₄₀O₄ (476.29) Calc. C 78.11 H 8.46% Found C 78.10 H 8.49%

Bufalin (**1**)⁵. Compound **8** (367.1 mg) was dissolved in 75 ml of benzene/EtOH 1:2, and cyclohexene (1.4 g) and Pd(OH)₂/C (171.3 mg) were added. The mixture was heated under reflux for 2 h, filtered through Celite, and evaporated to dryness *in vacuo*. The residue was purified by crystallization from CHCl₃/hexane giving 208.3 mg (70%) of pure **1**, m.p. 235–237°, identical in all respects with the authentic natural product. UV(EtOH): 289 (3.77). IR(CHCl₃): 3600, 3450 (OH), 1710 (C=O), 1635 (C=C). ¹H-NMR(CDCl₃): 0.70 (*s*, 3 H–C(18)); 0.95

⁵) Since bufalin is a natural product, the usual numbering for the steroid side-chain is used (*cf.* the systematic numbering of all other compounds).

(*s*, 3 H—C(19)); 4.13 (*m*, $W_{1/2} \approx 8$, H—C(3)); 6.25 (*d*, $J = 10$, H—C(23)); 7.23 (*d*, $J = 2$, H—C(21)); 7.83 (*dd*, $J = 2, 10$, H—C(22)). MS: 386 ($C_{24}H_{34}O_4^+$). MS(HR): 386.2456 (M^+ , calc. 386.2457).

$C_{24}H_{34}O_4$ (386.51) Calc. C 74.57 H 8.87% Found C 74.54 H 8.76%

5-(3'- β -Benzyloxy-17'- β -hydroxy-5'- β -androst-15'-en-17'- α -yl)furan-2-carbaldehyde Ethylene Acetal (**13**). A solution of BuLi in hexane (43.92 ml, 1.3 M) was added to a THF solution of furan-2-carbaldehyde ethylene acetal (8.88 g in 400 ml) at -78° under N_2 , and the mixture was stirred for 45 min. A solution of the ketone **IV** (10 g in 100 ml THF) was then added, and stirring was continued at -78° for 30 min. The mixture was diluted with Et_2O , the org. layer was washed with H_2O , aq. $NaHCO_3$, and brine, dried (anh. $MgSO_4$), and evaporated to dryness. The foamy crude product was purified by chromatography on silica gel with Et_2O/CH_2Cl_2 yielding 12.35 g (90.19%) of pure foamy **13**. IR ($CHCl_3$): 3600 (OH). 1H -NMR ($CDCl_3$): 0.99 (*s*, 3 H—C(19)); 1.04 (*s*, 3 H—C(18)); 3.69 (br. *s*, $W_{1/2} \approx 7$, H—C(3)); 3.98–4.22 (*m*, OCH_2CH_2O); 4.45 (*d*, $J = 2$, CH_2Ph); 5.70 (*dd*, $J = 3.8, 3.7$, H—C(15)); 5.94 (*s*, HC—C(2)); 6.10 (*d*, $J = 3$, H—C(4)); 6.16 (*d*, $J = 6.5$, H—C(16)); 6.41 (*d*, $J = 4$, H—C(3)); 7.36, 7.38 (2*s*, 5 arom. H). MS: 518 ($C_{33}H_{42}O_5^+$). MS(HR): 518.3031 (M^+ , calc. 518.3032).

5-(3'- β -Benzyloxy-15'- β -hydroxy-5'- β -androst-16'-en-17'-yl)furan-2-carbaldehyde Ethylene Acetal (**15**). Compound **13** (12.35 g) was acetylated with 100 ml of Ac_2O /pyridine 1:2 in the presence of 4-(dimethylamino)pyridine (360 mg) at r.t. for 24 h. The mixture was worked up as usual, and the crude acetyl derivative **14** was heated under reflux in acetone/ H_2O 4:1 (400 ml) in the presence of $CaCO_3$ (2.4 g) for 3 days. Workup and chromatography on silica gel with Et_2O/CH_2Cl_2 yielded 9.88 g (80%) of **15** which was recrystallized from $Et_2O/MeOH$, m.p. 148–150°. IR ($CHCl_3$): 3600 (OH). 1H -NMR ($CDCl_3$): 1.07 (*s*, 3 H—C(19)); 1.32 (*s*, 3 H—C(18)); 3.74 (br. *s*, $W_{1/2} \approx 7$, H—C(3)); 3.94–4.23 (*m*, OCH_2CH_2O); 4.51 (*d*, $J = 2$, CH_2Ph); 4.62 (br. *s*, $W_{1/2} \approx 11$, H—C(15)); 5.96 (*s*, HC—C(2)); 6.25 (*d*, $J = 6.9$, H—C(4)); 6.39 (*d*, $J = 6.8$, H—C(16)); 6.46 (*d*, $J = 7$, H—C(3)); 7.37, 7.39 (2*s*, 5 arom. H). MS: 518 ($C_{33}H_{42}O_5^+$). MS(HR): 518.3034 (M^+ , calc. 518.3032).

5-(3'- β -Benzyloxy-15'- β -hydroxy-5'- β -androst-17'-yl)furan-2-carbaldehyde Ethylene Acetal (**16**). Compound **15** (9.88 g) was hydrogenated in EtOH (220 ml) with 10% Pd/ $CaCO_3$ (1.0 g) at r.t. The product was purified by chromatography on silica gel with Et_2O/CH_2Cl_2 giving pure foamy **16** (8.50 g, 85.7%). IR ($CHCl_3$): 3610, 3450 (OH). 1H -NMR ($CDCl_3$): 0.76 (*s*, 3 H—C(18)); 1.0 (*s*, 3 H—C(19)); 2.45–2.67 (*m*, 2 H—C(16)); 3.76 (br. *s*, $W_{1/2} \approx 7$, H—C(3)); 3.96–4.2 (*m*, OCH_2CH_2O); 4.34–4.45 (*m*, H—C(15)); 4.52 (*d*, $J = 2$, CH_2Ph); 5.91 (*s*, HC—C(2)); 6.04 (*d*, $J = 3.6$, H—C(4)); 6.38 (*d*, $J = 3.6$, H—C(3)); 7.36, 7.38 (2*s*, 5 arom. H). MS: 520 ($C_{33}H_{44}O_5^+$). MS(HR): 520.3191 (M^+ , calc. 520.3188).

5-(3'- β -Benzyloxy-15'- β -hydroxy-5'- β -androst-17'-yl)-2,5-dihydroxy-3-pentenal Ethylene Acetal (**17**). A solution of **16** (8.50 g) in dioxane/ H_2O 12:3 (375 ml) containing 1.67 g of AcONa was stirred at r.t. while 3.05 g of NBS was slowly added. After addition of 2.46 g of $NaBH_4$, stirring was continued for 2 h. The mixture was extracted with Et_2O , and the org. layer was washed with H_2O and brine, dried (anh. $MgSO_4$), and evaporated to dryness. The crude product was chromatographed on silica gel yielding the pure diastereoisomeric mixture **17** (6.84 g, 77.5%). One compound crystallized from $CHCl_3/Et_2O$, m.p. 183–185°. IR ($CHCl_3$): 3600, 3480 (OH). 1H -NMR ($CDCl_3$): 1.01 (*s*, 3 H—C(18)); 1.06 (*s*, 3 H—C(19)); 3.72 (br. *s*, $W_{1/2} \approx 7$, H—C(3)); 3.90–4.10 (*m*, OCH_2CH_2O); 4.20–4.32 (*m*, H—C(15)); 4.32–4.47 (*m*, H—C(6)); 4.50 (*d*, $J = 2$, CH_2Ph); 4.47–4.59 (*m*, H—C(2)); 4.87 (*d*, $J = 6$, HC—C(2)); 5.51 (*dd*, $J = 8, 8$, H—C(4)); 5.71 (*dd*, $J = 9, 9$, H—C(3)); 7.36, 7.38 (2*s*, 5 arom. H).

$C_{33}H_{48}O_6$ (540.71) Calc. C 73.30 H 8.95% Found C 72.93 H 8.82%

6-(3'- β -Benzyloxy-15'- β -hydroxy-5'- β -androst-17'-yl)-3,6-dihydro-2H-pyran-2,3-diol (**18**). A solution of **17** (6.84 g/600 ml THF) was heated under reflux with 3N HCl (45 ml) for 4 h. After cooling, the solution was neutralized with $NaHCO_3$ and extracted with Et_2O . The org. layer was washed with H_2O , aq. $NaHCO_3$, and sat. NaCl, dried (anh. $MgSO_4$), and evaporated to dryness. The crude product was chromatographed on silica gel with acetone/hexane yielding the pure diastereoisomeric mixture **18** (4.69 g; 77.4%). IR ($CHCl_3$): 3600 (OH). 1H -NMR ($CDCl_3$): 1.02 (*s*, 3 H—C(18)); 1.06 (*s*, 3 H—C(19)); 3.74 (br. *s*, $W_{1/2} \approx 7$, H—C(3)); 4.22–4.41 (*m*, H—C(6), H—C(15)); 4.51 (*d*, $J = 2$, CH_2Ph); 4.70–4.84 (*m*, H—C(3)); 5.90 (br. *s*, $W_{1/2} \approx 3$, H—C(2)); 5.98 (br. *s*, $W_{1/2} \approx 2$, H—C(4), H—C(5)); 7.37, 7.39 (2*s*, 5 arom. H). MS: 496 ($C_{31}H_{44}O_5$). MS(HR): 478.3086 ($M^+ - H_2O$, calc. 478.3082).

6-(3'- β -Benzyloxy-15'- β -hydroxy-5'- β -androst-17'-yl)-3-hydroxy-3,6-dihydro-2H-pyran-2-one (**19**). Compound **18** (6.84 g) was heated under reflux with Ag_2CO_3 /Celite (41.04 g) in benzene (600 ml) for 60 min. After cooling, the solution was filtered through Celite and evaporated to dryness. The crude product was chromatographed on silica gel G with Et_2O/CH_2Cl_2 . Pure **19** (2.39 g, 35.2%) was obtained after crystallization from $Et_2O/CHCl_3$, m.p. 124–126°. IR ($CHCl_3$): 3610, 3530 (OH), 1730 (C=O). 1H -NMR ($CDCl_3$): 1.02 (*s*,

3H-C(18'')); 1.11 (*s*, 3H-C(19'')); 3.72 (*br. s*, $W_{1/2} \approx 7$, H-C(3'')); 4.26–4.38 (*m*, H-C(15'')); 4.40 (*d*, $J = 3$, CH₂Ph); 4.65 (*br. s*, $W_{1/2} \approx 5$, H-C(3)); 4.98–5.08 (*m*, H-C(6)); 6.01 (*br. s*, $W_{1/2} \approx 2$, H-C(4), H-C(5)); 7.36, 7.38 (2 *s*, 5 arom. H). IR (CHCl₃): 3615, 3530 (OH), 1735 (C=O). ¹H-NMR (CDCl₃): 1.02 (*s*, 3H-C(18'')); 1.08 (*s*, 3H-C(19'')); 3.74 (*br. s*, $W_{1/2} \approx 7$, H-C(3'')); 4.28–4.42 (*m*, H-C(15'')); 4.52 (*d*, $J = 3$, CH₂Ph); 4.64 (*br. s*, $W_{1/2} \approx 8$, H-C(3)); 4.96–5.08 (*m*, H-C(6)); 5.86–5.98 (*m*, H-C(4)); 6.06–6.17 (*m*, H-C(3)); 7.37, 7.39 (2 *s*, 5 arom. H). MS: 494 (C₃₁H₄₂O₅⁺).

C₃₁H₄₂O₅ (494.65) Calc. C 75.27 H 8.56% Found C 75.28 H 8.54%

6-(3'-β-Benzoyloxy-15'-β-hydroxy-5'-β-androstan-17'-β-yl)-2H-pyran-2-one (**20**). A solution of **19** (2.39 g) in CH₂Cl₂ (50 ml) and Et₃N (560 mg) was cooled in an ice-bath, and MsCl (615 mg) was added. The mixture was stirred for 30 min, and then it was diluted with CH₂Cl₂, washed with 5% citric acid and aq. NaHCO₃, dried (anh. MgSO₄), and evaporated to dryness. The crude product was dissolved in 35 ml of DMF and heated with LiBr (510 mg) for 30 min to 105°. The mixture was diluted with Et₂O, washed with H₂O and aq. NaHCO₃, dried (anh. MgSO₄), and evaporated to dryness. The crude **20** (1.49 g, 65%) was used in the next step without further purification. UV (CHCl₃): 306 (3.92). IR (CHCl₃): 3610 (OH), 1720 (C=O), 1630 (C=C). ¹H-NMR (CDCl₃): 0.88 (*s*, 3H-C(18'')); 1.01 (*s*, 3H-C(19'')); 3.75 (*br. s*, $W_{1/2} \approx 7$, H-C(3'')); 4.36–4.47 (*m*, H-C(15'')); 4.51 (*d*, $J = 3$, CH₂Ph); 6.05 (*d*, $J = 6$, H-C(5)); 6.19 (*d*, $J = 9$, H-C(3)); 7.3 (*dd*, $J = 6, 9$, H-C(4)); 7.37, 7.39 (2 *s*, 5 arom. H). MS: 476 (C₃₁H₄₀O₄⁺). MS (HR): 476.2934 (M^+ , calc. 476.2926).

6-(3'-β-Benzoyloxy-5'-β-androst-14'-en-17'-β-yl)-2H-pyran-2-one (**21**). A solution of **20** (1.49 g) in CH₂Cl₂ (20 ml) and pyridine (5 ml) was cooled in an ice bath and treated with SOCl₂ (487 mg). The mixture was stirred for 30 min and worked up in the usual manner. The product was chromatographed on silica gel G (Et₂O/CH₂Cl₂ 3 : 7) and crystallized from acetone/hexane giving 1 g (70%) of **21**, m.p. 145–146°. UV (CHCl₃): 304 (3.92). IR (CHCl₃): 1710 (C=O), 1630 (C=C). ¹H-NMR (CDCl₃): 0.75 (*s*, 3H-C(18'')); 0.85 (*s*, 3H-C(19'')); 3.66 (*br. s*, $W_{1/2} \approx 7$, H-C(3'')); 4.44 (*d*, $J = 3$, CH₂Ph); 5.14 (*br. s*, $W_{1/2} \approx 6$, H-C(15'')); 6.01 (*d*, $J = 6$, H-C(5)); 6.12 (*d*, $J = 9$, H-C(3)); 7.22 (*dd*, $J = 6, 9$, H-C(4)); 7.30, 7.32 (2 *s*, 5 arom. H). MS: 458 (C₃₁H₃₈O₃⁺). MS (HR): 458.2822 (M^+ , calc. 458.2821).

C₃₁H₃₈O₃ (458.61) Calc. C 81.18 H 8.35% Found C 80.87 H 8.30%

6-(3'-β-Benzoyloxy-14'-hydroxy-5'-β, 14'-β-androstan-17'-β-yl)-2H-pyran-2-one (**22**). Compound **21** (1.0 g) in 42 ml of acetone/H₂O 9 : 1 was treated with NBS (490 mg) and 0.2 ml of 1% HClO₄ at r.t. for 30 min. The mixture was diluted with CH₂Cl₂ and washed with aq. NaHCO₃ and H₂O, dried (anh. MgSO₄), and evaporated to dryness. The residue was dissolved in 84 ml of MeOH/CH₂Cl₂ 1 : 1, and AcONa (175 mg) and an excess of Raney-Ni were added. After 15 min of stirring, the Raney-Ni was filtered off, and the solution was evaporated to dryness. The product was purified on a silica gel G plate with hexane/CH₂Cl₂/Et₂O 3 : 4 : 2 and crystallized from acetone giving 780 mg (75%) of **22**, m.p. 194–196°. UV (CHCl₃): 306 (3.93). IR (CHCl₃): 3600 (OH), 1720 (C=O), 1630 (C=C). ¹H-NMR (CDCl₃): 0.87 (*s*, 3H-C(18'')); 0.95 (*s*, 3H-C(19'')); 3.74 (*br. s*, $W_{1/2} \approx 8$, H-C(3'')); 4.51 (*s*, CH₂Ph); 6.14 (*d*, $J = 9$, H-C(5)); 6.19 (*d*, $J = 6$, H-C(3)); 7.31 (*dd*, $J = 6, 9$, H-C(4)); 7.37, 7.39 (2 *s*, 5 arom. H). MS: 476 (C₃₁H₄₀O₄⁺). MS (HR): 476.2929 (M^+ , calc. 476.2926).

C₃₁H₄₀O₄ (476.63) Calc. C 78.11 H 8.46% Found C 77.78 H 8.51%

6-(3'-β,14'-Dihydroxy-5'-β,14'-β-androstan-17'-β-yl)-2H-pyran-2-one (α-Isobufalin; **2**). A mixture of **22** (780 mg), cyclohexene (1.3 g) and Pd(OH)₂/C (312 mg) in 74 ml of benzene/EtOH 1 : 2 was heated under reflux for 4.5 h. The catalyst was filtered off, and the solution was evaporated to dryness. The product was purified on a silica gel G plate with acetone/hexane 1 : 3 to yield 506 mg (80%) of pure **2**. From Et₂O/CHCl₃, m.p. 145–147°. UV (CHCl₃): 306 (3.84). IR (CHCl₃): 3600 (OH), 1720 (C=O), 1630 (C=C). ¹H-NMR (CDCl₃): 0.87 (*s*, 3H-C(18'')); 0.96 (*s*, 3H-C(19'')); 4.15 (*br. s*, $W_{1/2} \approx 8$, H-C(3'')); 6.15 (*d*, $J = 9$, H-C(5)); 6.19 (*d*, $J = 6$, H-C(3)); 7.33 (*dd*, $J = 6, 9$, H-C(4)). MS: 386 (C₂₄H₃₄O₄⁺). MS (HR): 386.2451 (M^+ , calc. 386.2457).

C₂₄H₃₄O₄ (386.51) Calc. C 74.57 H 8.87% Found C 74.36 H 9.05%

REFERENCES

- [1] *H. Jin, T. Y. R. Tsai & K. Wiesner*, *Can. J. Chem.*, **61**, 2442 (1983).
- [2] *K. Wiesner, T. Y. R. Tsai, F. J. Jäggi, C. S. J. Tsai & G. D. Gray*, *Helv. Chim. Acta* **65**, 2049 (1982).
- [3] *T. Y. R. Tsai, A. Minta & K. Wiesner*, *Heterocycles* **12**, 1397 (1979).
- [4] *R. Mendez & G. Pastelin*, to be published.
- [5] Cf. *F. Sondheimer, W. McCrae & W. G. Salmond*, *J. Am. Chem. Soc.* **91**, 1228 (1969); *G. R. Pettit, C. E. Houghton, J. C. Knight & F. Brushweiler*, *J. Chem. Soc., Chem. Commun.* **1970**, 93; *E. Joshii, T. Oribe, T. Koizumi, I. Hayashi & K. Tamura*, *Chem. Pharm. Bull.* **25**, 2249 (1977).
- [6] Cf. *T. Nambara & K. Shimada*, *Chem. Pharm. Bull.* **19**, 16 (1971) and previous publications; *G. R. Pettit, J. C. Knight & C. L. Herald*, *J. Org. Chem.* **35**, 1393 (1970).
- [7] *W. Adam & K. Takayama*, *J. Org. Chem.* **44**, 1727 (1979).
- [8] *H. J. J. Loozen, E. F. Godefroi & J. S. M. Besters*, *J. Org. Chem.* **40**, 892 (1975).
- [9] *A. M. Felix, E. P. Heimer, T. J. Lambros, C. Tzougraki & J. Meienhofer*, *J. Org. Chem.* **43**, 4194 (1978).
- [10] *M. J. Astle, J. A. Zaslowsky & P. G. Lafyatis*, *Ind. Eng. Chem.* **46**, 787 (1954).