265. On Cardioactive Steroids. XII

The Synthesis of (Natural) Bufalin and α′-Isobufalin [1])

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

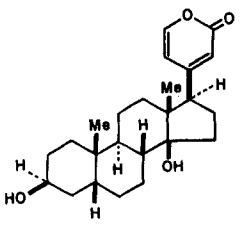
Natural Products Research Center, University of New Brunswick, Fredericton, New Brunswick, Canada E3B 6E2

(4.X.1983)

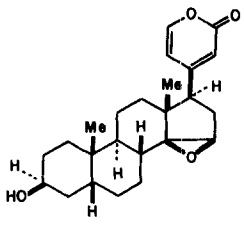
Summary

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.

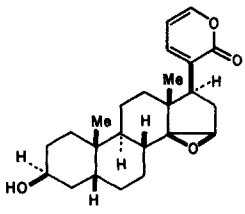
Introduction. – In [2] we have described a relatively simple synthesis of the bufalin and resibufogenin isomers **I**–**III** [1]) in which we utilized an adaptation of our furan strategy for cardenolides [3]. The pharmacological study of these compounds and their dihydro derivatives submitted as glucosides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



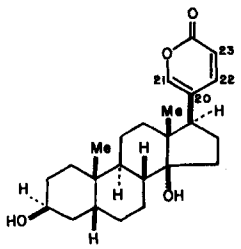
I α-isobufalin



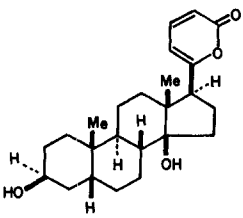
II α-isoresibufogenin



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.

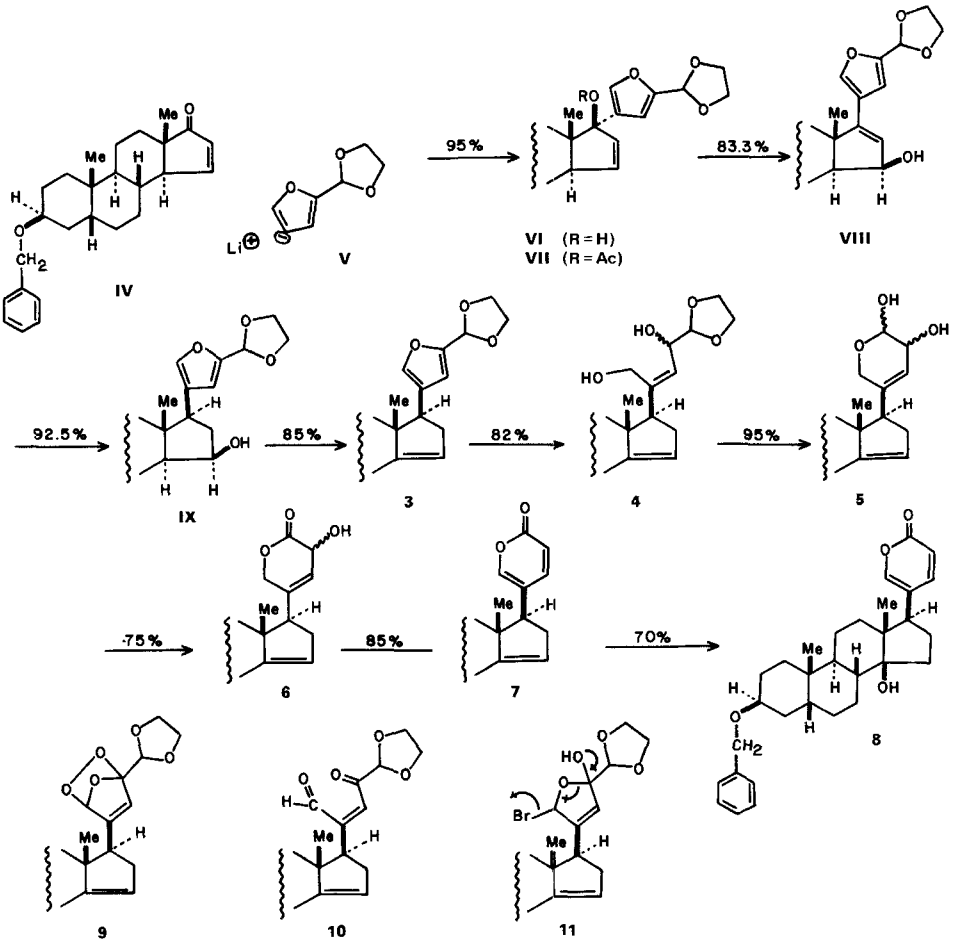
The 15′β-OH-group [1]) of compound **IX**, the actual starting material of the present work, was eliminated with mesyl chloride (MsCl) in pyridine, and the crystalline $\Delta^{14',15'}$-derivative **3** (m.p. 141–142°) was obtained in a yield of 85% [2]). The ^{1}H-NMR spectrum of **3** showed the vinylic H–C(15′) as a m($W_{1/2}$ = *ca.* 6 Hz) at δ = 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 ^{1}H-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 Ag_2CO_3/*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 Et_2O/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 Et_2O/CH_2Cl_2 (m.p. 198–200°).

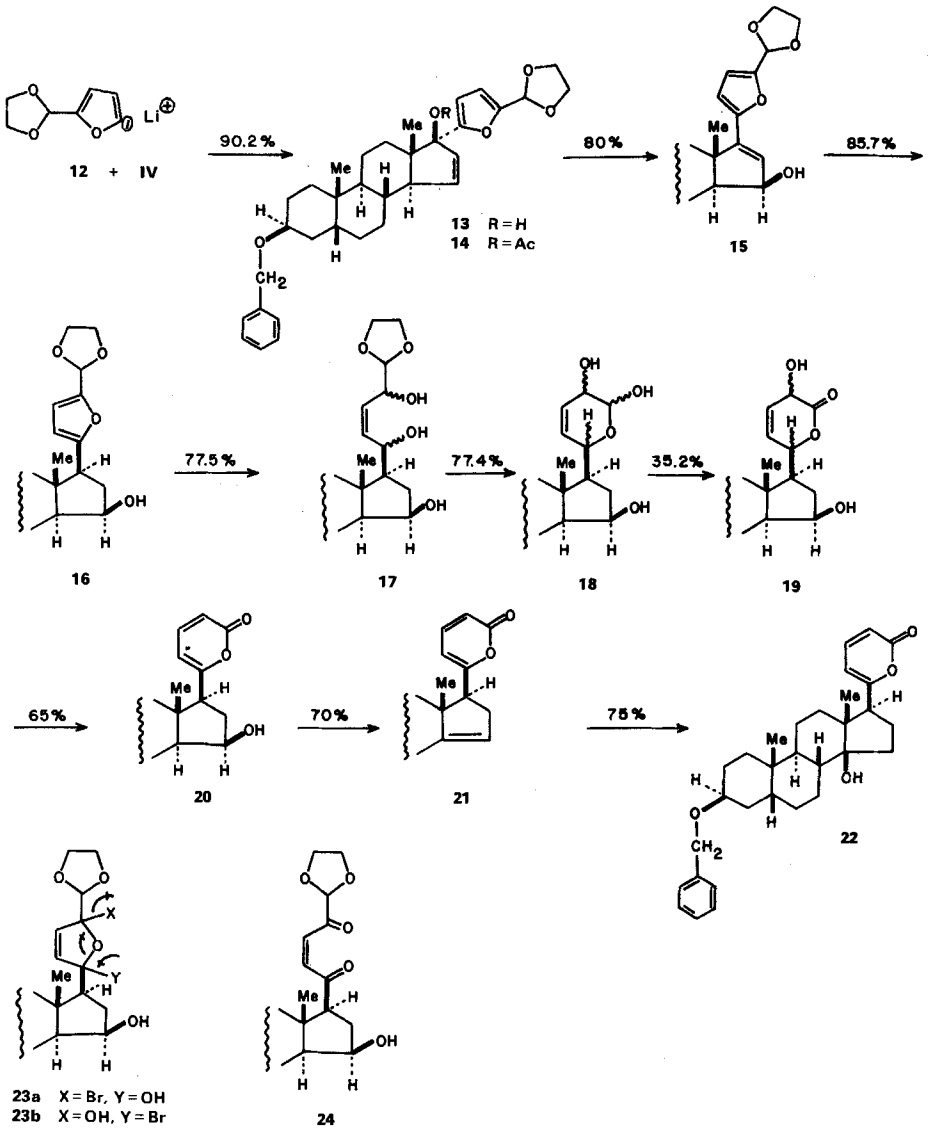
---

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



Finally, debenzylation of **8** with $Pd(OH)_2/C$ [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}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 (→ **13** → **14** → **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 Ag_2CO_3/*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-diol [3]).

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 $SOCl_2$ 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 debenzylation 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 ($CHCl_3$): 3600 (OH), 1720 (C=O), 1630 (C=C). UV (EtOH): 306 (3.84). ^{1}H-NMR ($CDCl_3$): 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 difficulties [4]).

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 $SOCl_2$ (517.9 mg) for 30 min. The mixture was stirred at r.t. for 30 min and then diluted with Et_2O. The solution was washed with 10% citric acid and aq. $NaHCO_3$, dried over anh. $MgSO_4$, 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 Et_2O/hexane, m.p. 141–142°. IR ($CHCl_3$): no OH. ^{1}H-NMR ($CDCl_3$): 0.68 (*s*, 3 H–C(18′)); 0.97 (*s*, 3 H–C(19′)); 3.70 (*m*, $W_{1/2} \approx 7$, H–C(3′)); 3.88–4.20 (*m*, OCH_2CH_2O); 4.47 (*s*, CH_2Ph); 5.23 (*m*, $W_{1/2} \approx 6$, 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 ($C_{33}H_{42}O_4^+$). MS (HR): 502.3081 (M^+, calc. 502.3082).

$C_{33}H_{42}O_4$ (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 O_2 bomb. The container was charged with a solution of **3** (1.58 g) and 5,10,15,20-tetraphenylporphine (1.5 mg) in CH_2Cl_2 (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 Me_2S (8 ml). The mixture was flushed with N_2 at r.t. for 45 min and evaporated to dryness. The keto aldehyde **10** was dissolved in THF (54 ml), and H_2O (5.4 ml) was added. The mixture was treated at r.t. with 205.8 mg of $NaBH_4$ 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 Et_2O/CH_2Cl_2 3:7 yielding 1.35 g (82%) of pure amorphous **4** over the 2 steps. IR($CHCl_3$): 3590, 3480 (OH). ^{1}H-NMR($CDCl_3$): 0.78 (*s*, 3H–C(18')); 0.93 (*s*, 3H–C(19')); 3.70 (*m*, $W_{1/2} \approx 7$, H–C(3')); 3.83–4.10 (*m*, OCH_2CH_2O); 4.12 (br. *s*, H–C(5)); 4.30–4.67 (*m*, H–C(2), CH_2Ph); 4.90 (*d*, $J = 4$, H–C(1)); 5.22 (*m*, $W_{1/2} \approx 6$, H–C(15')); 5.56 (*dd*, $J = 8$, H–C(3)); 7.35 (br. *s*, 5 arom. H). MS: 522 ($C_{33}H_{46}O_5^+$). MS(HR): 522.3321 (M^+, calc. 522.3345).

5-(3'β-Benzyloxy-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 3N HCl (2 ml). The mixture was stirred and heated under reflux for 5 h and then diluted with Et_2O. The solution was washed with dil. $NaHCO_3$ and H_2O. The Et_2O-layer was dried over anh. $MgSO_4$, evaporated to dryness, and the product purified on silica gel G plates with CH_2Cl_2/Et_2O 85:15. The crude foamy **5** (1.13 g, 95%) was used without further purification. IR($CHCl_3$): 3590, 3485 (OH). MS: 478 ($C_{31}H_{42}O_4^+$). MS(HR): 478.3099 (M^+, calc. 478.3082).

5-(3'β-Benzyloxy-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 Ag_2CO_3/*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 Et_2O and filtered through *Celite*. The filtrate was evaporated to dryness and the product purified on silica gel G plates with CH_2Cl_2/Et_2O 85:15. Crystallization from Et_2O/hexane gave 654.8 mg (75%) of **6**, m.p. 117–119°. IR($CHCl_3$): 3550 (OH), 1735 (C=O). ^{1}H-NMR($CDCl_3$): 0.77 (*s*, 3H–C(18')); 0.98 (*s*, 3H–C(19')); 3.72 (br. *s*, $W_{1/2} \approx 8$, H–C(3'), OH); 4.60–4.87 (br. *s*, H–C(6), H–C(3)); 5.17 (br. *s*, $W_{1/2} \approx 6$, H–(15')); 5.88 (*m*, $W_{1/2} \approx 4$, H–C(4)); 7.37 (br. *s*, 5 arom. H). MS: 476 ($C_{31}H_{40}O_4^+$). MS(HR): 476.2926 (M^+, calc. 476.2926).

$C_{31}H_{40}O_4$ (476.29) Calc. C 78.11 H 8.46% Found C 77.75 H 8.51%

5-(3'β-Benzyloxy-5'β-androst-14'-en-17'β-yl)-2H-pyran-2-one (**7**). Compound **6** (616.4 mg) in CH_2Cl_2 (15.4 ml) and Et_3N (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 CH_2Cl_2. The CH_2Cl_2-layer was washed with 5% citric acid, aq. $NaHCO_3$, 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 Et_2O, washed with aq. 5% citric acid, $NaHCO_3$, and NaCl, and dried (anh. $MgSO_4$). The solvent was evaporated, and the crude product was purified on silica gel G plates with 5% Et_2O in CH_2Cl_2/hexane 1:1 and crystallized from Et_2O/hexane giving 504 mg (85%) of **7**, m.p. 115–116°. IR($CHCl_3$): 1715 (C=O), 1635 (C=C). ^{1}H-NMR($CDCl_3$): 0.73 (*s*, 3H–C(18')); 0.97 (*s*, 3H–C(19')); 3.71 (*m*, $W_{1/2} \approx 7$, H–C(3')); 4.48 (*s*, CH_2Ph); 5.23 (*m*, $W_{1/2} \approx 6$, 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 ($C_{31}H_{38}O_3^+$). MS(HR): 458.2827 (M^+, calc. 458.2821).

5-(3'β-Benzyloxy-14'-hydroxy-5'β,14'β-androstan-17'β-yl)-2H-pyran-2-one (**8**). Compound **7** (504 mg) in 22 ml of acetone/H_2O 9:1 was treated with NBS (240 mg) and 10 drops of 1% aq. $HClO_4$ at r.t. for 30 min. The mixture was diluted with CH_2Cl_2, washed with aq. $NaHSO_3$ and H_2O, dried (anh. $MgSO_4$), and evaporated to dryness. The residue was dissolved in 44 ml of MeOH/CH_2Cl_2 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 CH_2Cl_2/Et_2O 95:5 yielding 367.1 mg (70.1%) of **8** which crystallized from $CHCl_3/Et_2O$, m.p. 198–200°. IR($CHCl_3$): 3595, 3450 (OH), 1700 (C=O), 1630 (C=C). ^{1}H-NMR($CDCl_3$): 0.71 (*s*, 3H–C(18')); 0.95 (*s*, 3H–C(19')); 3.75 (*m*, $W_{1/2} \approx 7$, H–C(3')); 4.52 (*s*, CH_2Ph); 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 ($C_{31}H_{40}O_4^+$). MS(HR): 476.2928 (M^+, calc. 476.2926).

$C_{31}H_{40}O_4$ (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 $CHCl_3$/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($CHCl_3$): 3600, 3450 (OH), 1710 (C=O), 1635 (C=C). ^{1}H-NMR ($CDCl_3$): 0.70 (*s*, 3H–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). ^{1}H-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). ^{1}H-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-androstan-17′ β-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). ^{1}H-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-androstan-17′ β-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). ^{1}H-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-androstan-17′ β-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 3 N 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). ^{1}H-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-androstan-17′ β-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). ^{1}H-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*, $J = 3$, CH_2Ph); 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 ($CHCl_3$): 3615, 3530 (OH), 1735 (C=O). ^{1}H-NMR ($CDCl_3$): 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*, $J = 3$, CH_2Ph); 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 ($C_{31}H_{42}O_5^+$).

$C_{31}H_{42}O_5$ (494.65) Calc. C 75.27 H 8.56% Found C 75.28 H 8.54%

6-(3'β-Benzyloxy-15'β-hydroxy-5'β-androstan-17'β-yl)-2H-pyran-2-one (**20**). A solution of **19** (2.39 g) in CH_2Cl_2 (50 ml) and Et_3N (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 CH_2Cl_2, washed with 5% citric acid and aq. $NaHCO_3$, dried (anh. $MgSO_4$), 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 Et_2O, washed with H_2O and aq. $NaHCO_3$, dried (anh. $MgSO_4$), and evaporated to dryness. The crude **20** (1.49 g, 65%) was used in the next step without further purification. UV ($CHCl_3$): 306 (3.92). IR ($CHCl_3$): 3610 (OH), 1720 (C=O), 1630 (C=C). ^{1}H-NMR ($CDCl_3$): 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*, $J = 3$, CH_2Ph); 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 ($C_{31}H_{40}O_4^+$). MS(HR): 476.2934 (M^+, calc. 476.2926).

6-(3'β-Benzyloxy-5'β-androst-14'-en-17'β-yl)-2H-pyran-2-one (**21**). A solution of **20** (1.49 g) in CH_2Cl_2 (20 ml) and pyridine (5 ml) was cooled in an ice bath and treated with $SOCl_2$ (487 mg). The mixture was stirred for 30 min and worked up in the usual manner. The product was chromatographed on silica gel G (Et_2O/CH_2Cl_2 3:7) and crystallized from acetone/hexane giving 1 g (70%) of **21**, m.p. 145–146°. UV ($CHCl_3$): 304 (3.92). IR ($CHCl_3$): 1710 (C=O), 1630 (C=C). ^{1}H-NMR ($CDCl_3$): 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*, $J = 3$, CH_2Ph); 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 ($C_{31}H_{38}O_3^+$). MS (HR): 458.2822 (M^+, calc. 458.2821).

$C_{31}H_{38}O_3$ (458.61) Calc. C 81.18 H 8.35% Found C 80.87 H 8.30%

6-(3'β-Benzyloxy-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/H_2O 9:1 was treated with NBS (490 mg) and 0.2 ml of 1% $HClO_4$ at r.t. for 30 min. The mixture was diluted with CH_2Cl_2 and washed with aq. $NaHCO_3$ and H_2O, dried (anh. $MgSO_4$), and evaporated to dryness. The residue was dissolved in 84 ml of MeOH/CH_2Cl_2 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/CH_2Cl_2/Et_2O 3:4:2 and crystallized from acetone giving 780 mg (75%) of **22**, m.p. 194–196°. UV ($CHCl_3$): 306 (3.93). IR ($CHCl_3$): 3600 (OH), 1720 (C=O), 1630 (C=C). ^{1}H-NMR ($CDCl_3$): 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*, CH_2Ph); 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 ($C_{31}H_{40}O_4^+$). MS (HR): 476.2929 (M^+, calc. 476.2926).

$C_{31}H_{40}O_4$ (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 $Et_2O/CHCl_3$, m.p. 145–147°. UV ($CHCl_3$): 306 (3.84). IR ($CHCl_3$): 3600 (OH), 1720 (C=O), 1630 (C=C). ^{1}H-NMR ($CDCl_3$): 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 ($C_{24}H_{34}O_4^+$). MS (HR): 386.2451 (M^+, calc. 386.2457).

$C_{24}H_{34}O_4$ (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).