methoxide in 10 ml of methanol at −80°. The reaction mixture was warmed to room temperature, water was added, and the mixture was extracted with pentane. Washing with water, drying over anhydrous sodium sulfate, and evaporating the solvent gave 69 mg of a crude product which consisted of 95% of **11**. Standing at 40° for 1 day caused quantitative decomposition of **11** into **9** and methanol. The mass spectrum of **11** showed a parent peak at *m/e* 230 corresponding with $C_{13}H_{23}OCl$; for the pmr spectrum, see Table I.

Preparation of 18. A solution of 48 mg (0.3 mmol) of **3** in 0.4 ml of methylene chloride was cooled to −80° and dry HCl gas (0.5 mmol) was introduced. The solution was warmed to room temperature and the solvent was evaporated. The pmr spectrum of the remaining 63 mg of product indicated 80% of **18** and 15% of starting material to be present. The mass spectrum of **18** showed parent peaks at *m/e* 232, 234, and 236 (intensity ratio 9:6:1), corresponding with $C_{12}H_{18}Cl_2$; pmr spectrum, see Table I; ir *inter alia* absorption at 1620 cm^{-1}; uv λ_{max} (pentane) 275 nm.

Registry No.—**2**, 40265-14-3; **3**, 50590-86-8; **4**, 20379-83-3; **9**, 19835-61-1; **10**, 51751-32-7; **11**, 41694-19-3; **12**, 41694-21-7; **18**, 50590-88-0; HCl, 7647-01-0; $FHSO_3$, 7789-21-1.

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Bufadienolides. 26. Synthesis of Scillarenin[1,2]

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Bufalin (**1**), previously synthesized from digitoxigenin, was utilized as relay in a new synthetic route to scillarenin (**4**). Important steps in the synthesis of scillarenin included bromination and dehydrohalogenation of bufalone (**2a**) to yield scillarenone (**3**). The overall transformation from digitoxigenin also comprised the first conversion of a plant cardenolide to a plant bufadienolide (**4**).

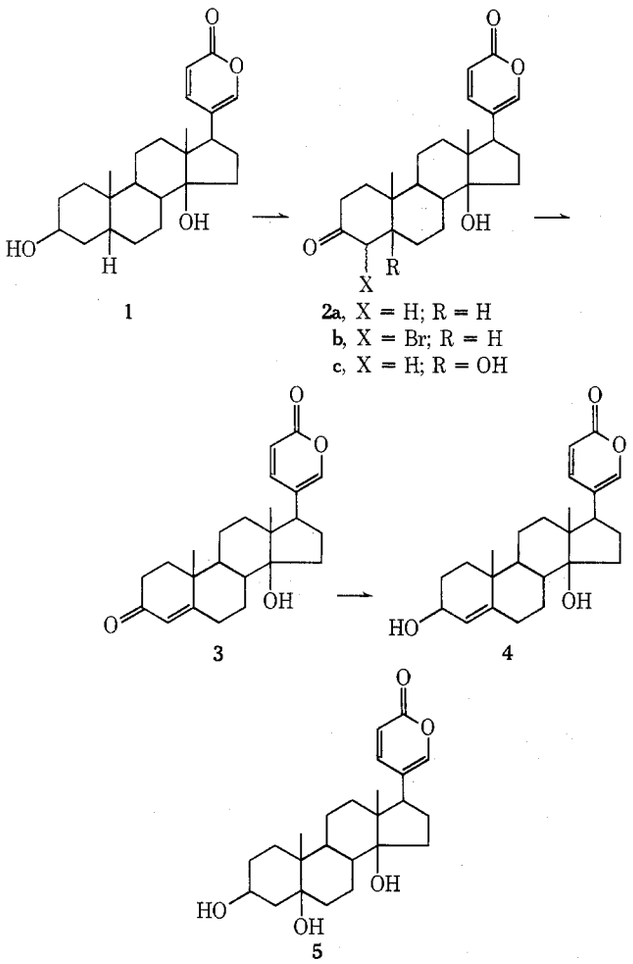
Careful hydrolysis of, *e.g.*, proscillaridin A from the ancient Egyptian medicinal plant *Scilla maritima* yields the aglycone scillarenin (**4**).[3] The parent glycoside, proscillaridin A, is a useful clinical agent for certain cardiac problems. This 3β-rhamnose derivative of scillarenin (**4**) has also been found to be an outstandingly effective cell-growth inhibitor of the National Cancer Institute's human epidermoid carcinoma of the nasopharynx cell culture (9KB).[4]

Recently we completed partial syntheses of marinobufagin and marinobufotoxin starting with telocinobufagin (**5**) isolated from Ch'an Su.[5] The objective of the present study[6] was to extend our earlier total synthesis of bufalin[1,6,7] (**1**) to the plant bufadienolide, scillarenin[8] (**4**). The latter substance could then serve as relay in a formally continuous route[5] to telocinobufagin (**5**).

Selective chromic acid oxidation (Sarett) of bufalin (**1**) to the previously known 3-oxo derivative, bufalone (**2a**), provided a useful precursor of scillarenin (**4**). Controlled bromination of ketone **2a** with *N*-bromosuccinimide gave an epimeric mixture of the C-4 bromo derivatives (**2b**), which were dehydrobrominated in low yield using hot α-collidine or pyridine. An improved procedure involved treatment of ketone **2a** with bromine in dimethylformamide or acetic acid to give the corresponding 4-bromo derivative, which was subjected directly to dehydrobromination with lithium bromide in dimethylformamide or lithium chloride in dimethylacetamide. After preparative thin layer chromatography, scillarenone (**3**) was isolated in 30–40% yields.

A partial synthesis of scillarenone (**3**) from telocinobufagin (**5**) was also evaluated. As part of the original structural study[9] of telocinobufagin (**5**) the 3β-hydroxyl was selectively oxidized to provide ketone **2c**, which upon treatment with hot acetic acid gave scillarenone (**3**). The Meyer[9] route was conveniently modified as follows. Oxidation of telocinobufagin to ketone **2c** was accomplished in good yield with *N*-bromoacetamide and selective elimination of the tertiary 5-hydroxyl group was readily achieved using an acidic ion-exchange resin. The samples of ketone **3** prepared from bufalin and telocinobufagin were shown to be identical.

Reduction of ketone **3** to scillarenin (**4**) and thereby completion of a new formal total synthesis of this plant



bufadienolide was readily achieved as previously described.[8] We readily confirmed that application of lithium tri-*tert*-butoxyaluminum hydride in tetrahydrofuran at ice-bath temperature for 5 hr affords scillarenin in approximately 75% yields. A further examination of the scillarenone (3) → scillarenin (4) reduction reaction included the original Meerwein–Ponndorf approach as well as a comparison of lithium aluminum hydride, lithium borohydride, sodium borohydride, and potassium borohydride methods. From this comparison study it was ascertained that the lithium tri-*tert*-butoxyaluminum hydride and lithium borohydride techniques gave highest yields of the 3β epimer. In each instance the specimen of scillarenin (4) isolated was identical with an authentic sample kindly provided by Dr. W. Haede.[8]

As digitoxigenin was used as relay for our bufalin synthesis,[1,6] extension of the route to scillarenin also represents the first chemical synthesis of a plant bufadienolide from a plant cardenolide.

Experimental Section

The bufalin used in this investigation was isolated from the Chinese toad venom extract Ch'an Su.[10] All solvent extracts of aqueous solutions were dried over anhydrous sodium sulfate and concentrated to dryness under reduced pressure using a rotary evaporator. Commercial (E. Merck, Darmstadt) silica gel HF_{254} preparative layer (1 mm) plates were employed and eluted with 3:3:4 acetone–chloroform–*n*-hexane. Analogous thin layer plates were developed with concentrated sulfuric acid. Each analytical sample was colorless and exhibited one spot on a thin layer chromatogram. The identical composition of specimens was established by mixture melting point determination and by comparing infrared spectra and thin layer chromatograms.

Spectral data was provided by Miss K. Reimer and Messrs. R. Scott and E. Kelley. Melting points were determined using a micro hot stage apparatus (Reichert, Austria) and are uncorrected. Ultraviolet spectra were determined using methanol as solvent. Infrared spectra were recorded using potassium bromide pellets and pmr data were observed using deuteriochloroform solution with tetramethylsilane as standard. A description of the instruments used in this study has been summarized in a preceding part.[10] The elemental microanalyses were determined in the laboratory of Dr. A. Bernhardt, 5251 Elbach uber Engelskirchen, West Germany.

3-Oxo-14β-hydroxy-5β-bufa-20,22-dienolide (Bufalone, 2a). The following experiment corresponds to modification of a previous chromic acid oxidation of bufalin to bufalone.[6] A solution of bufalin (1, 0.24 g) in pyridine (3.8 ml) was added to the freshly prepared complex from chromium trioxide (0.22 g) and pyridine (2.2 ml). After a 22-hr period at room temperature the mixture was poured into ice–water and extracted with chloroform. The combined extract was washed with water, dilute hydrochloric acid, and water. Removal of solvent and recrystallization of the product from acetone–methanol gave 0.21 g (89% yield) of ketone **2a** as needles melting at 242–245° (lit.[6] mp 241–243°); uv λ_{max} 301 nm (log ϵ 3.74); ir ν_{max} 3510 (OH), 1720 (CO), 1700 (conjugated CO), 1634, 1540 (conjugated CO), 947 and 751 cm^{-1} (C=C); pmr δ 0.72 (18-methyl), 1.00 (19-methyl), 6.24 (1 H, d, J = 2.5 Hz, 21-proton), and 7.73 (1 H, q, J = 10.5 and 2.5 Hz, 22-proton); mass spectrum M^+ 384, 366 ($M^+ - H_2O$), 348, 333, 323, 296, 248, 231, and 230.

Anal. Calcd for $C_{24}H_{32}O_4$: C, 74.97; H, 8.39. Found: C, 74.99; H, 8.38.

3-Oxo-14β-hydroxy-5β-bufa-4,20,22-trienolide (Scillarenone, 3). Method A. A solution prepared from glacial acetic acid (4 ml), bromine (0.035 g), and anhydrous sodium acetate (0.018 g) was added (30 min) dropwise to a solution of ketone **2a** (0.07 g) in 6% hydrogen bromide–acetic acid (0.1 ml). The mixture was stirred and maintained at 10–15°. When the reaction with bromine was complete the mixture was diluted with a solution of sodium acetate (0.18 g) in water (6 ml) and poured into ice–water. The crude bromo derivative (0.08 g, R_f 0.42 and yellowish brown color with sulfuric acid on a thin layer chromatogram) was collected and heated at reflux (7 hr) in dimethylacetamide (2 ml) containing anhydrous lithium chloride (0.08 g). The mixture was poured into ice–water and extracted with chloroform. Before removing solvent from the combined extract it was washed with water, dilute hydrochloric acid, and water. The residue (0.07 g) was subjected to preparative thin layer chromatography and the zone corresponding to R_f 0.35 was eluted with 4:1 chloroform–methanol. Recrystallization of this fraction from acetone gave 0.027 g of scillarenone (3) as needles, mp 246–249°, identical with an authentic specimen.[6]

Method B. Bromine (0.04 g) in dimethylformamide (1 ml) was added during 30 min with stirring to a mixture prepared from ketone **2a** (0.08 g), *p*-toluenesulfonic acid monohydrate (0.002 g), and dimethylformamide (2 ml). Two hours later the reaction mixture was diluted with chloroform and poured into water. The chloroform extract was washed successively with water, dilute sodium bicarbonate solution, dilute hydrochloric acid, and water. The crude bromo derivative (0.08 g) and anhydrous lithium bromide (0.08 g) were heated (at reflux under nitrogen) in dimethylformamide (3.5 ml) for 8 hr. The product (3, 0.022 g, mp 246–248°) was isolated and identified as summarized above in method A.

Method C. From Bufalin (1). A mixture prepared from bufalin (1, 0.155 g), *N*-bromosuccinimide (75 mg), and carbon tetrachloride (15 ml) was heated at reflux for 45 min. The solution was filtered, diluted with chloroform, and poured into water. The solvent layer was washed with water, dilute sodium bicarbonate solution, and water. Removal of solvent gave 0.13 g of bromo ketone **2b.** A solution of the crude bromo derivative (**2b,** 84 mg) in α-collidine (10 ml) was heated at reflux in a nitrogen atmosphere for 6 hr. The brownish residue (77 mg) obtained by removing solvent was separated by preparative thin layer chromatography. The zone corresponding to R_f 0.36 was eluted with 4:1 chloroform–methanol. Recrystallization of this fraction from acetone afforded 8.5 mg of scillarenone (3) as needles melting at 247–249°.

In another experiment[11] bromo ketone **2b** (48 mg) in pyridine (4 ml) was heated in a sealed ampoule at 140° for 90 min. Upon cooling and removal of solvent 50 mg of brown residue was obtained. After purification by preparative thin layer chromatography and recrystallization from acetone (as described above) 4.3 mg of scillarenone (3) melting at 244–248° was isolated.

Method D. From Telocinobufagin (5). A solution of *N*-bromoacetamide (0.11 g) in methanol (2 ml)–water (0.4 ml) was added to a solution of telocinobufagin (**6,** 0.10 g) in methanol (8

ml)-acetone (5 ml). Before pouring the mixture into ice-water and extracting with chloroform, it was allowed to remain at 14–17° for 2 days. The combined chloroform extract was washed with water, dilute sodium sulfite solution, and water. After the solvent was removed the cystalline residue was recrystallized from methanol–acetone to afford 87 mg of **3-oxo-5β,14β-dihydroxy-5β-bufa-20,22-dienolide (2c)** as needles melting at 251–253°: uv λ_{max} 298 nm (log ϵ 3.73); ir ν_{max} 3400–3280 (OH), 1720 (CO), 1710 (conjugated CO), 1630, 1530 (conjugated C=C), 945 and 760 cm^{-1} (C=C); pmr (in pentadeuteriopyridine) δ 0.95 (18-methyl), 1.13 (19-methyl), 6.27 (d, J = 10 Hz, 23-proton), *ca.* 7.48 (21-proton, indistinct peak overlapped with pyridine peak), and 8.14 (q, J = 10 and 3 Hz, 22-proton); mass spectrum M^+ 400, 382 ($M^+ - H_2O$), and 364 ($M^+ - 2H_2O$).

Anal. Calcd for $C_{24}H_{32}O_5$: C, 71.97; H, 8.05. Found: C, 71.94; H, 8.06.

A mixture prepared from ketone **2c** (62 mg), 0.60 g of Amberlite CG-120 (H^+ form), and methanol (3 ml) was stirred at room temperature for 6 hr. The solution was filtered and the filtrate was concentrated to dryness. The crude product thereby obtained was purified by preparative thin layer chromatography and ketone **3** was recrystallized as summarized in method A to yield 53 mg of ketone **3** melting at 246–248°.

Each specimen of scillarenone (**3**) was found identical with an authentic sample prepared[12] by chromic acid oxidation of scillarenin (**4**). The authentic specimen recrystallized from acetone as needles melting at 247–249° and exhibited uv λ_{max} 239 nm (log ϵ 4.20) and 300 (3.75); ir ν_{max} 3470 (OH), 1740–1710, 1700 (conjugated CO), 1657, 1635, 1613, 1533 (conjugated C=C and normal C=C), 957 and 748 cm^{-1} (C=C); pmr δ 0.78 (18-methyl), 1.19 (19-methyl), 5.7 (s, 4-proton), 6.21 (d, J = 10.5 Hz, 23-proton), 7.23 (d, J = 2.5 Hz, 21-proton), 7.81 (q, J = 10.5 and 2.5 Hz, 22-proton); mass spectrum M^+ 382, 364 ($M^+ - H_2O$), 349, 339, 322, 242, 228.

Anal. Calcd for $C_{24}H_{30}O_4$: C, 75.36; H, 7.91. Found: C, 75.22; H, 7.90.

Scillarenin (4). Method A. Lithium Tri-*tert*-Butoxyaluminum Hydride. Reduction of scillarenone (**3**, 20 mg) was conducted employing the lithium tri-*tert*-butoxyaluminum hydride (0.13 g in 3 ml of tetrahydrofuran) method of Stache and colleagues.[8] The crude product was separated by preparative thin layer chromatography and the zone corresponding to R_f 0.27 was eluted with chloroform–methanol (2:1). Recrystallization of the scillarenin from methanol afforded 16 mg of prisms melting at 230–232° (lit.[8,12] mp 234 and 232–235°).

Method B. Lithium Aluminum Hydride. Scillarenone (**3**, 20 mg) in dry tetrahydrofuran (3 ml) was slowly (a drop at a time) added to a mixture of lithium aluminum hydride (80 mg) and dry tetrahydrofuran (2 ml) maintained at ice-bath temperature. Stirring was continued for 5 hr. Excess hydride was carefully removed with dilute acetic acid and the product was extracted with chloroform and separated by preparative thin layer chromatography. Recrystallization of the scillarenin (**4**) from methanol yielded 12 mg melting at 227–230°.

Method C. Lithium Borohydride. The reduction reaction described in method B was modified by substituting lithium borohydride (11 mg) for the lithium aluminum hydride. In this case 20 mg of scillarenone (**3**) led to 15.5 mg of **4** melting at 228–231°.

Method D. Sodium Borohydride. To a solution of scillarenone (**3**, 20 mg) in methanol (1.5 ml)–tetrahydrofuran (1.5 ml) was added sodium borohydride (9 mg) and the mixture was allowed to remain at ice-bath temperature for 6 hr. The product was isolated and recrystallized as summarized in method A to yield 12.2 mg, mp 229–231°, of scillarenin (**4**). A repeat of this reduction reaction with substitution of potassium borohydride for the sodium borohydride gave 13 mg of scillarenin (**4**) melting at 228–230°.

Essentially the same yield (14 mg, mp 229–233°) of scillarenin was attained by modification (preparative layer chromatography as noted in method A) of the earlier[12] Meerwein–Ponndorf reduction of scillarenone (**3**, 20 mg).

The specimens of scillarenin prepared by methods A–D were mutually identical and indistinguishable from an authentic specimen of the natural products.

Registry No.—**1**, 465-21-4; **2a**, 4029-65-6; **2c**, 51567-97-6; **3**, 545-28-8; **4**, 465-22-5; **5**, 472-26-4; lithium tri-*tert*-butoxyaluminum hydride, 17476-04-9; lithium aluminum hydride, 16853-85-3; lithium borohydride, 16949-15-8; sodium borohydride, 16940-66-2.

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