Bufadienolides. 11. Bufalin and Resibufogenin[1]

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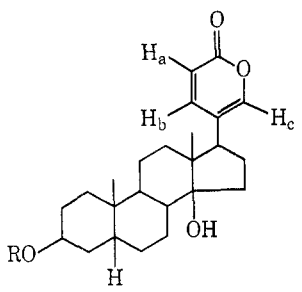
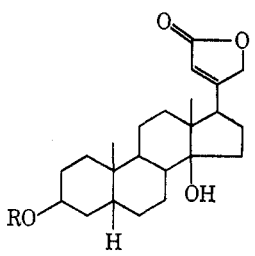
A total synthesis of resibufogenin (**12b**) and bufalin (**1a**) has been summarized. Digitoxigenin (**2a**) was employed as relay and converted to 14-dehydrobufalin acetate (**11**). The latter substance was transformed to resibufogenin (**12b**) and bufalin (**1a**). The synthesis represents a new approach to the bufadienolides and constitutes the first synthetic route from a cardenolide to a bufadienolide.

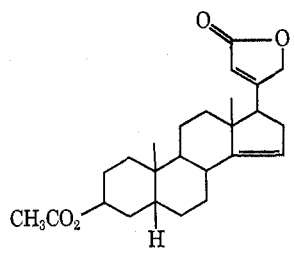
One of the principal objectives in our study of bufadienolide-type steroids was to complete a useful total synthesis of bufalin.[2] Once conversion of digitoxigenin (**2a**) to isobufalin[3] was at hand, total synthesis of bufalin seemed secure but no reproducible technique for the superficially uncomplicated rearrangement isobufalin → **6**. The latter substance was selected as an intermediate which could be utilized to circumvent practical limitations encountered in our assaults on a bufalin synthesis from digitoxigenin. A disadvantage seemingly inherent in using olefin **6a** would lie in having to reintroduce oxygen at the 14β position. However, a number of recent advances in experimental manipulation of the Δ^{14} system suggested this would no longer be a problem and indeed key steps in the transformation of 14-dehydrobufalin to bufalin had already been noted in the patent literature.[6] Therefore a synthesis of bufalin based on acetal **6a** seemed promising and was undertaken as follows.



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



2a, R = H
b, R = 3(D-digitoxose)
c, R = $COCH_3$



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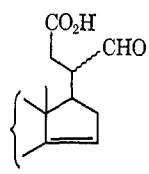

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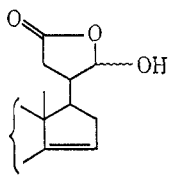

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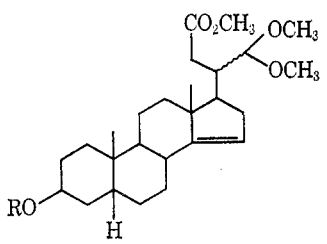
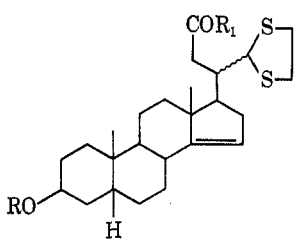
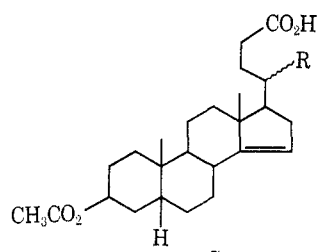
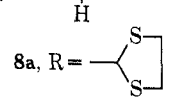


6a, R = H
b, R = $COCH_3$



7a, R = $COCH_3$; R_1 = OH
b, R = $COCH_3$; R_1 = OCH_3
c, R = H; R_1 = OH
d, R = $COCH_3$; R_1 = Cl
e, R = $COCH_3$; R_1 = CHN_2



8a, R = 
b, R = —CHO

Digitoxigenin (**2a**) prepared by hydrolysis of digitoxin (**2b**) was acetylated (**2c**) and dehydrated to 14-dehydrodigitoxigenin acetate (**3**). Upon treatment with sodium methoxide in methanol (followed by acidification) cardenolide **3** was transformed, presumably *via* aldehyde **4** and lactol **5** to acetal **6a** as follows.

(1) This investigation was supported by Public Health Service Research Grants CA-10115-01 to CA-10115-04 from the National Cancer Institute. (a) For paper 10 in the series, refer to J. C. Knight, G. R. Pettit, and P. Brown, *J. Org. Chem.*, **35**, 1415 (1970). (b) The present contribution also represents Steroids and Related Natural Products. LXII (part LXI, G. R. Pettit and B. Green, *Can. J. Chem.*, **48**, in press). A preliminary report has been summarized by G. R. Pettit, L. E. Houghton, J. C. Knight, and F. Bruschweiler, *Chem. Commun.*, 93 (1969).

(2) The naturally occurring bufadienolides until 1969 remained the last classic category of steroids in which no member had yielded to total synthesis. For a brief review of this subject and outline of potential biological importance, consult references in 1b and G. R. Pettit, B. Green, and G. Dunn, *J. Org. Chem.*, **35**, 1367 (1970).

(3) G. R. Pettit, T. R. Kasturi, J. C. Knight, and K. A. Jaeggi, *ibid.*, **35**, 1410 (1970).

(4) On one occasion 14-dehydrobufalin was obtained from isobufalin, but the method proved unreliable and was abandoned. At that time Professor Sondheimer kindly informed us of preparing bufalin in his laboratory *via* 14-dehydrobufalin and resibufogenin; see F. Sondheimer, W. McCrae, and W. G. Salmond, *J. Amer. Chem. Soc.*, **91**, 1228 (1969). Remainder of the same year witnessed three new synthetic routes to the bufadienolides and a total synthesis of scillarenin: (a) G. R. Pettit, D. C. Fessler, K. D. Paull, P. Hofer, and J. C. Knight, *Can. J. Chem.*, **47**, 2511 (1969); (b) U. Stache, K. Radscheit, W. Fritsch, H. Kohl, W. Haede, and H. Ruschig, *Tetrahedron Lett.*, 3033 (1969); and (c) C. R. Engel, R. Bouchard, A. F. deKrassny, L. Ruest, and J. Lessard, *Steroids*, 637 (1969). We wish to thank Professor C. R. Engel for informing us of his contribution prior to publication.

(5) G. R. Pettit, T. R. Kasturi, J. C. Knight, and J. Occolowitz, *J. Org. Chem.*, **35**, 1404 (1970).

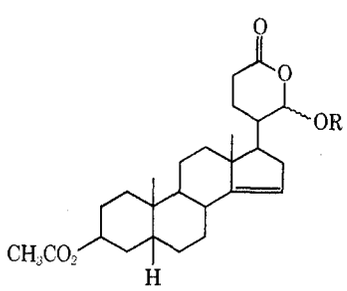
(6) H. Kondo and S. Ohno, U. S. Patent 3,134,772 [*Chem. Abstr.*, **61**, 5736 (1964)].

From a series of accessory experiments[5] performed with digitoxigenin, it appeared possible to convert digitoxigenin (**2a**) *via* aldehyde **4** and lactol **5** to acetal **6a** seemed promising and was undertaken as follows.

Digitoxigenin (**2a**) prepared by hydrolysis of digitoxin (**2b**) was acetylated (**2c**) and dehydrated to 14-dehydrodigitoxigenin acetate (**3**). Upon treatment with sodium methoxide in methanol (followed by acidification) cardenolide **3** was transformed, presumably *via* alde-

hyde **4**, to lactol **5**. Preparation of oily lactol **5** from digitoxigenin proved efficient (79% overall) and the assigned structure was entirely consistent with spectral data. Methanolysis of lactone **5** using methanol containing *p*-toluenesulfonic acid readily afforded acetal methyl ester **6a**. Of several methods explored for homologation of methyl ester **6a** an Arndt–Eistert sequence seemed best for detailed study, but this choice did necessitate reprotecting the aldehyde as an ethylene-thioacetal. Necessary protection was achieved by first acetylating alcohol **6a** and then treatment with ethanedithiol containing 70% perchloric acid. Simply washing the crude product with aqueous base resulted in extensive saponification of the methyl ester. Following acidification, carboxylic acid **7a** was obtained. The yield of acid **7a** was increased by saponifying (and acetylating the product) remaining methyl ester **7b**. The pronounced sensitivity of methyl ester **7b** to base hydrolysis suggests a neighboring-group-type participation by sulfur. A pmr study of intermediates **6** and **7** indicated that the acid reagents did not cause any detectable shift of the Δ^{14} double bond to the $\Delta^{8(14)}$ position.

Homologation of acid **7a** *via* acid chloride **7d** and diazo ketone **7e** led to carboxylic acid **8a**. Cleavage of the ethylene thioacetal was accomplished using a mercuric oxide–mercuric chloride–aqueous acetic acid procedure. Substitution of aqueous acetone resulted in reduced yields and under prolonged reaction conditions led to migration ($\Delta^{14} \rightarrow \Delta^{8}$) of the olefin. Also, the more commonly employed cadmium carbonate–mercuric chloride–aqueous acetone technique for cleavage of ethylene thioacetal **8a** did not give good results. With methanol as solvent, the major product appeared (spectral evidence) to be methyl acetal **9a**. While the

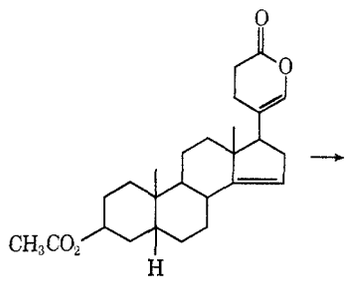


9a, R = CH_3
b, R = H

acetal **9a** was readily converted using aqueous acetic acid to lactol **9b** elimination of water to provide enol lactone **10** was not easily realized.

Enol cyclization of aldehyde **8b** was achieved using *p*-toluenesulfonic acid as catalyst.[7] The amount of *p*-toluenesulfonic acid and reaction time for cyclization were important factors in obtaining good conversion to enol lactone **10**. Excess catalyst and prolonged treatment led to extensive migration of the Δ^{14} olefin. Other methods such as iodine in benzene gave poor yields while dicyclohexylcarbodiimide in pyridine gave only lactol **9b**.

Dehydrogenation of enol lactone **10** was easily realized using the sulfur procedure developed in part 7 of this series. The product, 14-dehydrobufalin acetate (**11**), was identical with an authetic specimen prepared from natural bufalin. Early in our appraisal of synthetic routes to the natural bufadienolides, we considered a possibility that the pyrone ring did not freely rotate. A serious consequence of such restricted rotation in bufalin would be a good possibility of synthesizing the wrong isomer.[8] We assume from being able to obtain routinely 14-dehydrobufalin by enol lactonization of aldehyde carboxylic acid **8b** and subsequent dehydrogenation that restricted rotation if present must be negligible in the temperature ranges employed.



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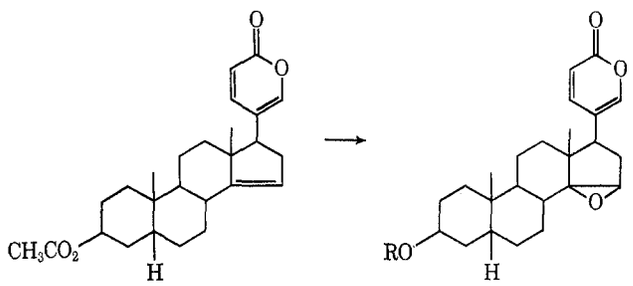

11 **12a**, R = $COCH_3$
b, R = H

The remaining steps to bufalin were completed as follows. Treatment of 14-dehydrobufalin acetate (**11**) with *m*-chloroperoxybenzoic acid was used to prepare resibufogenin acetate (**12a**). Selective saponification of acetate **12a** was achieved using basic alumina. The product, resibufogenin (**12b**), was completely identical with a natural specimen thus completing a total synthesis of this clinically useful bufadienolide.[2] Careful (−50°) and selective reduction of resibufogenin was employed to obtain bufalin (**1a**).[9] The synthetic specimen of bufalin and a natural sample were identical by all the usual criteria.

The total syntheses of bufalin and resibufogenin herein described also correspond to the first cardenolide → bufadienolide conversions. Extension of the new bufadienolide synthesis to preparation of newly discovered bufadienolides with cell growth inhibitory properties such as 3β-acetoxyhellebrigenin[10] and 3β-acetoxybersaldeginin[11] are now under investigation in our laboratory.

(7) G. R. Pettit, D. C. Fessler, K. D. Paull, P. Hofer, and J. C. Knight *J. Org. Chem.*, **35**, 1398 (1970).

(8) Recently an example of asymmetry due to restricted rotation about the C-17,20 bond has actually been noted: F. Kohen, R. A. Mallory, and I. Scheer, *Chem. Commun.*, 580 (1969).

(9) An analogous route from 14-dehydrobufalin acetate *via* resibufogenin to bufalin has already been reported in a patent[6] and a preliminary communication (*cf.* Sondheimer[4]). The present work provides further confirmation for these important interconversions.

(10) S. M. Kupchan, R. J. Hemingway, and J. C. Hemingway, *Tetrahedron Lett.*, 149 (1968).

(11) S. M. Kupchan and I. Ognyanov, *ibid.*, 1709 (1969).

Experimental Section

All solvent extracts of aqueous solutions were dried over magnesium sulfate. Silica gel HF_{254} (E. Merck, Darmstadt) on microscope slides was employed for analytical thin layer chromatography and at a thickness of 1 mm was utilized for preparative layer chromatography. The plates were observed using ultraviolet light or developed with 2% ceric sulfate in 2 *N* sulfuric acid.

Unless noted differently, introduction to the Experimental Sections of Bufadienolides papers 7, 8, and 10 outlines other general information for the experiments now described.

Methyl 3β-Hydroxy-20ξ-formyl-21-nor-5β-norchol-14-enate 20-Ethylene Thioacetal (7b).—To a solution of sodium methoxide prepared from methanol (600 ml) and sodium (17.5 g) was added 14-dehydrodigitoxigenin acetate (19 g)[12] as a suspension in methanol (600 ml). The resulting solution was stirred in a nitrogen atmosphere for 3 hr at room temperature. Upon cooling the solution was acidified with 2 *N* hydrochloric acid (500 ml), diluted with water (800 ml), and extracted (4 times) with ether. Washing the combined ethereal extract with water and concentration under reduced pressure yielded a colorless oil (19 g). A pmr spectrum of the product was consistent with lactol structure **5**.

A solution of lactol **5** (19.0 g) in methanol (600 ml) containing *p*-toluenesulfonic acid (0.8 g) was heated at reflux for 3.5 hr. Water (600 ml) was added to the solution and the resulting mixture was extracted with chloroform (three 200-ml portions). The chloroform extract was washed with water and concentrated to an oil (20.2 g). A 0.22-g aliquot of the oil was purified by preparative layer chromatography (4:1 chloroform–ethyl acetate mobile phase). The principal zone was separated and eluted with ethyl acetate to afford 0.15 g of oily acetal **6a**: pmr δ 0.98 (18 and 19 methyls), 3.34–3.42 (for closely spaced signals corresponding to the acetal methoxyl in each of the C-20 epimers), 3.68 (methyl ester), 4.14 (3α proton), 4.28 (acetal proton), and 5.18 (olefinic proton at C-15). The remaining product was predominantly **methyl 3β-acetoxy-20ξ-formyl-21-nor-5β-norchol-14-enate 20-dimethyl acetal (6b)**. Larger scale separation of the products was achieved employing column chromatography on silica gel. The crude product was chromatographed in 1:19 ligroin–ethyl acetate and alcohol **6a** eluted by 2:1 ligroin–ethyl acetate. However, it was more efficient to simply acetylate (acetic anhydride–pyridine) the crude product and proceed to the next step (**7b**) without further purification.

A solution of acetal **6b** (20 g) in ethanedithiol (25 ml) containing 70% perchloric acid (0.2 ml) was allowed to remain at room temperature for 3 hr. The mixture was diluted with ether (120 ml) and washed with 2 *N* sodium hydroxide (two 50-ml portions). The sodium salt of acid **7a** separated and was collected, washed with water, suspended in chloroform, and acidified. After washing the chloroform phase with water and removal of solvent, thioacetal **7a** was obtained as a colorless solid (12.5 g). Several recrystallizations from ethyl acetate–hexane provided an analytical sample as crystals melting at 126–129°: ν_{max} 3425–2550, 1740, and 1700 cm^{-1}; pmr δ 0.98 (18 and 19 methyls), 2.05 (acetate methyl), 3.20 (thioacetal methylene), 4.91 (thioacetal proton), 5.05 (3α proton), 5.17 (olefinic C-15 proton), and 9.20 (carboxylic acid proton).

Anal. Calcd for $C_{27}H_{40}O_4S_2$: C, 65.66; H, 8.18; S, 13.02. Found: C, 65.46; H, 8.01; S, 13.16.

The ethereal extract obtained as noted in the preceding paragraph was concentrated to a colorless oil (7 g) which was saponified employing 2 *N* sodium hydroxide–methanol (1:1, 50 ml). Alcohol **7c** was obtained as an oil (5.2 g) but acetylation (acetic anhydride–pyridine) provided an additional 5.2 g of crystalline acid **7b**.

3β-Acetoxy-20ξ-formyl-21-nor-5β-chol-14-enic Acid 20-Ethylene Thioacetal (8a).—A solution composed of benzene (250 ml), oxalyl chloride (7 ml), and acid **7a** (7.0 g) was heated at reflux for 2 hr. Solvent was removed under reduced pressure, dry benzene was added, and the solution was again concentrated to a pale yellow oil: ν_{max}^{neat} 1800, 1735, and 1680 cm^{-1}. The diazomethane prepared from 7 g of nitrosomethylurea was distilled with ether from 50% aqueous potassium hydroxide (at ice-bath temperature) and treated (dropwise) with an ether (100 ml) solution of crude acid chloride **7d** (7.2 g). Before cautious solvent removal, cooling was continued for 14 hr. The benzene solution of the yellow oily residue was chromatographed on silica gel. Elution with 93:7 hexane–ethyl acetate led to diazo ketone **7e** (4.0 g) as a pale yellow oil: ν_{max}^{neat} 2100, 1735, and 1640 cm^{-1}; pmr δ 0.97 (18 and 19 methyls), 2.02 (acetate methyl), 3.17 (thioacetal methylenes), 4.92 (thioacetal proton), 5.04 (3α proton), 5.16 (C-15 olefinic proton), and 5.29 (diazo ketone proton); mass spectrum *m/e* 488 (M − 28, loss of nitrogen).

A solution of the diazo ketone (4.0 g) in dioxane (20 ml) was added (dropwise) to a stirred suspension of freshly prepared silver oxide (from 4.0 g of silver nitrate) in dioxane (40 ml) containing 10% aqueous sodium thiosulfate (15 ml) and 3% potassium carbonate (0.5 ml). The reaction temperature was maintained at 60° and 1 hr later 90% of the theoretical amount of nitrogen had been evolved. Upon cooling the black mixture was filtered (twice) using Celite and 10% potassium carbonate (50 ml) was added to the filtrate. The aqueous mixture was extracted with hexane–ether (1:1, 50 ml) and the organic solvent was washed with 10% potassium carbonate (three 25-ml portions). The combined carbonate extract was acidified with 2 *N* hydrochloric acid and the solution was extracted with chloroform (three 50-ml portions). The combined chloroform extract was washed with water and concentrated (reduced pressure) to afford the homologous acid **8a** (3.2 g) as a pale yellow solid. Three recrystallizations from ethyl acetate–hexane yielded the analytical sample as colorless crystal clusters melting at 176–180°: ν_{max} 3500, 2700, 1740, and 1700 cm^{-1}; pmr δ 0.90 and 0.98 (18 and 19 methyls), 2.02 (acetate methyl), 3.20 (thioacetal methylenes), 4.90 (thioacetal proton), 5.04 (3α proton), 5.18 (olefinic C-15 proton), and 8.55 (carboxylic acid proton).

Anal. Calcd for $C_{28}H_{42}O_4S_2$: C, 66.34; H, 8.35. Found: C, 65.87; H, 8.39.

3β-Acetoxy-20ξ-formyl-21-nor-5β-chol-14-enic Acid (8b).—A mixture made from 90% acetic acid (10 ml), thioacetal **8a** (0.5 g), mercuric chloride (0.5 g), and red mercuric oxide (0.25 g) was heated (steam bath) for 25 min. On cooling, the solution was filtered through Celite. The filtrate was diluted with water and extracted with $CHCl_3$ (three 15-ml portions). The combined chloroform extract was washed with water and concentrated to a colorless oil (0.45 g). A pure sample of 3β-acetoxy-20ξ-formyl-21-nor-5β-chol-14-enic acid (**8b**) was obtained by preparative thin layer chromatography using 7:3:0.1 ethyl acetate–ligroin–acetic acid as mobile phase. The oily specimen of aldehyde **8b** exhibited $\nu_{max}^{CHCl_3}$ 3500–2400 and 1730–1690 cm^{-1}; pmr δ 0.90 and 0.98 (18 and 19 methyls), 2.07 (acetate methyl), 5.06 (3α proton), 5.18 (olefinic C-15 proton), 7.86 (carboxylic acid proton), and 9.55 (aldehyde proton).

14-Dehydrobufalin Acetate (11). Method A.—Using a Dean–Stark apparatus a solution composed of dry benzene (50 ml), aldehyde acid **8b** (0.2 g), and *p*-toluenesulfonic acid (0.025 g) was heated at reflux for 16 hr. The yellow oil obtained by evaporation of solvent was subjected to preparative layer chromatography (4:1 hexane–ethyl acetate mobile phase). Elution of the major zone with chloroform led to enol lactone **10** (0.05 g) which crystallized from hexane as needles: mp 165–167°; ν_{max} 1780, 1735, and 1675 cm^{-1}; pmr δ 0.83 and 1.0 (18 and 19 methyls), 2.06 (acetate methyl), 5.07 (3α proton), 5.18 (C-15 olefinic proton), and 6.40 (C-21 olefinic proton).

An intimate mixture prepared by evaporating a solution of enol lactone **10** (0.04 g) and sulfur (0.12 g) in carbon disulfide was heated (metal bath) at 208° for 24 min. The time and temperature variables for this dehydrogenation reaction were determined by a series of thin layer chromatographic appraisals. After cooling, the principal product, bufadienolide **11**, was isolated by preparative layer chromatography (4:1 hexane–ethyl acetate). The product (0.009 g) was eluted by chloroform and crystallized from hexane to afford colorless prisms of 14-dehydrobufalin acetate melting at 170–172° (mass spectrum M^+ 410). The synthetic specimen of 14-dehydrobufalin was identical[13] with an authentic sample prepared from natural bufalin.[14]

Method B.—A 0.10-g specimen of natural bufalin[2] in pyridine (2.5 ml)–acetic anhydride (2.2 ml) was maintained at approximately 25° for 14 hr. The solution was evaporated (reduced pressure) and a solution of the residue in methanol was washed (3 times) with *n*-heptane. After each washing the upper layer

(12) G. Bach, J. Capitaine, and C. R. Engel, *Can. J. Chem.*, **46**, 733 (1968).

(13) The identical composition of both specimens was confirmed by results of thin layer chromatographic, proton magnetic resonance, and infrared spectral (in potassium bromide) comparison.

(14) We are indebted to Professor K. Meyer and Dr. Y. Kamano for generous specimens of natural bufalin.

(heptane) was removed in a current of carbon dioxide. The methanol solution was concentrated and the residue crystallized from methanol–ether to provide 98 mg of bufalin acetate (**1b**) as plates which melted at 228–231° (lit.[15] mp 236–247°). To a cooled (ice bath) solution of acetate **1b** (98 mg) in pyridine (6 ml) was added (dropwise over 60 min) thionyl chloride (2 ml) in dry pyridine (4 ml). Stirring was continued with cooling for a total of 2 hr. At that point the mixture was placed in a refrigerator for 4 hr and then diluted with ice water. Following extraction with chloroform, washing the solution, and concentration to dryness, a solution of the residue in methanol was washed (3 times) with *n*-heptane as noted for preparation of bufalin acetate. The product **11** was crystallized from methanol–ether to yield 40.8 mg of plates melting at 173–178°. The second recrystallization provided 40.0 mg melting at 172–176° (lit.[4] mp 144–161°).

Resibufogenin Acetate (12a).—A solution prepared from chloroform (1 ml), 14-dehydrobufalin acetate (10 mg), and *m*-chloroperoxybenzoic acid (9.5 mg, 86% pure) was stirred at room temperature 4.5 hr. The mixture was diluted with ether and washed with 5% aqueous sodium hydroxide and water. Removal of solvent gave 9.8 mg of colorless solid. Resibufogenin acetate was isolated by preparative thin layer chromatography (1:1 ligroin–ethyl acetate mobile phase). Following elution from the silica gel with chloroform, the product was washed with 10% sodium bicarbonate, 1 *N* hydrochloric acid, and water. Evaporation of solvent and crystallization of the residue from methanol–chloroform yielded 4.0 mg of plates and needles melting at 222–227°. The product **12a** was identical[13] with an authentic specimen of resibufogenin acetate[16] prepared as noted with bufalin acetate. The synthetic resibufogenin acetate displayed ν_{max} 3020 (epoxide), 2970, 1730, 1340 (epoxide) cm^{-1}; mass spectrum M^+ 426, 408 (M − 18), 366 (M − 60).

Resibufogenin (12b).—An ether solution of resibufogenin acetate (12 mg) was mixed with activated alumina (Woelm, basic, activity III, pH *ca.* 8–9) and placed in a small column. Following a 24-hr period resibufogenin was eluted by ether and chloroform. The crude product weighed 9.2 mg. Recrystallization from chloroform–methanol gave 6.2 mg of plates with a double melting point 110–121° and 148–168° (natural resibufogenin melts at 104–122° and 146–170°). The synthetic resibufogenin was identical[13] with the natural counterpart and exhibited ν_{max} 3070, 2950, 1735, 1640, 1545 cm^{-1} and mass spectrum M^+ 384, 366 (100%), M^+ − 18.

Bufalin (1a).—The following reduction experiment was performed using dry reagents and equipment. To a solution of resibufogenin (0.105 g) in ether (22 ml) was added (dropwise) an ethereal (20 ml) solution of lithium aluminum hydride (0.275 g). Stirring and cooling at −50° was continued for 4 hr. The mixture was carefully treated with wet ether and then diluted with water. The ethereal phase was washed with 10% sodium bicarbonate, 1 *N* hydrochloric acid, and water (3 times). Removal of solvent gave 78 mg of crude (5 component mixture by thin layer using 95:5 chloroform–methanol) bufalin. A pure specimen of bufalin (18 mg) was obtained by preparative layer chromatography (95:5 chloroform–methanol mobile phase). Recrystallization from methanol–chloroform gave 12.4 mg of needles melting at 242–243° (natural bufalin from Japan melted at 221–242° and from Switzerland at 212–240°): mass spectrum M^+ 386, 368, 350, 325, 250, 232, 214, 207, 203, and 147; ir ν_{max} 3080, 2945, 1725, 1640, and 1545 cm^{-1}; pmr δ (at 100 MHz) 0.71 and 0.96 (18 and 19 methyls), 4.14 (3α proton), 6.25 (doublet, H_a, J = 10 Hz), 7.28 (partially masked doublet, H_c, J = 2 Hz), and 8.85 (quartet, H_b, J = 10 and 2 Hz).[17] The synthetic specimen of bufalin was completely identical[13] with a natural sample.[2]

Registry No.—**1a**, 465-21-4; **7a**, 25090-22-6; **8a**, 25090-23-7; **8b**, 25090-24-8; **10**, 25090-25-9; **12b**, 465-39-4.

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

(16) We are grateful to Dr. Y. Kamano for providing resibufogenin.

(17) We wish to thank Dr. George Smythe and Professor W. Caughey, for providing this spectrum.

The Photochemical Conversion of Phenyl Epoxycinnamate to Flavonoids and the Synthesis of 2′-Hydroxyepoxychalcone[1]

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Phenyl epoxycinnamate (**1**) undergoes photochemical cleavage to phenylcarbene, as well as Fries rearrangement. The resulting 2′-hydroxyepoxychalcone (**4**) partially photolyzed further into the diketone **5**, which is easily converted into flavone. It also cyclized during work-up to 3-hydroxyflavanone (**6**), which partially oxidized to flavonol (**7**). Peracid oxidation of the chalcone **9** provided the first authentic sample of 2′-hydroxyepoxychalcone, a controversial intermediate in the AFO reaction. Its chemical and photochemical properties were consistent with those required from an intermediate in the photolysis of **1**, as well as in the AFO reaction.

Chalcones are precursors *in vivo* for all the different classes of flavonoid and isoflavonoid pigments,[3] but they may not be the only entities containing 15 carbon atoms to have that distinction. In particular, the immediate biosynthetic precursor to chalcones has not been characterized.[4] We have been engaged in a study of chemical models for the biosynthesis of chalcones and we now wish to describe one observation which is also relevant to the problem of synthesizing flavonoid pigments in general.

Phenyl epoxycinnamate (**1**) was prepared by refluxing phenyl cinnamate with *m*-chloroperoxybenzoic acid in chloroform. Upon irradiation in benzene at 253.7 nm under nitrogen, it yielded products which could be accounted for by the intervention of two competing pathways, the carbene formation from phenyloxiranes[5] and the photo-Fries rearrangement of aromatic esters.[6] The reaction products were isolated by column chromatography over silica gel, and they were *trans*-stilbene (**2**) (from phenyl carbene), phenol (**3**), *o*-hydroxybenzoylacetophenone (**5**), 3-hydroxydihydroflavone (**6**), and 3-hydroxyflavone (**7**). Analysis of the crude photolysis mixture by tlc indicated that one prominent spot had not been accounted for and there were no spots corresponding to **6** and **7**, which must have been artifacts. Although we failed in our attempts to isolate it, we believe that the formation of **6** and **7**

(1) This work was outlined at the Meeting of the Phytochemical Society of North America, Banff, Canada, Aug 1969.

(2) To whom inquiries should be directed.

(3) H. Grisebach in "Recent Advances in Phytochemistry," T. J. Mabry, V. C. Runeckles, and R. E. Alston, Ed., Appleton-Century-Crofts, New York, N. Y., 1968, p 379.

(4) The accepted precursor is a cinnamoyl derivative of a polyketide, but attempts to synthesize it enzymatically have been fruitless.[3]

(5) A. Padwa, "Organic Photochemistry," O. L. Chapman, Ed., Marcel Dekker, New York, N. Y., 1967, p 112.

(6) D. Bellus and P. Hrdlovic, *Chem. Rev.*, **67**, 599 (1967).