(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.

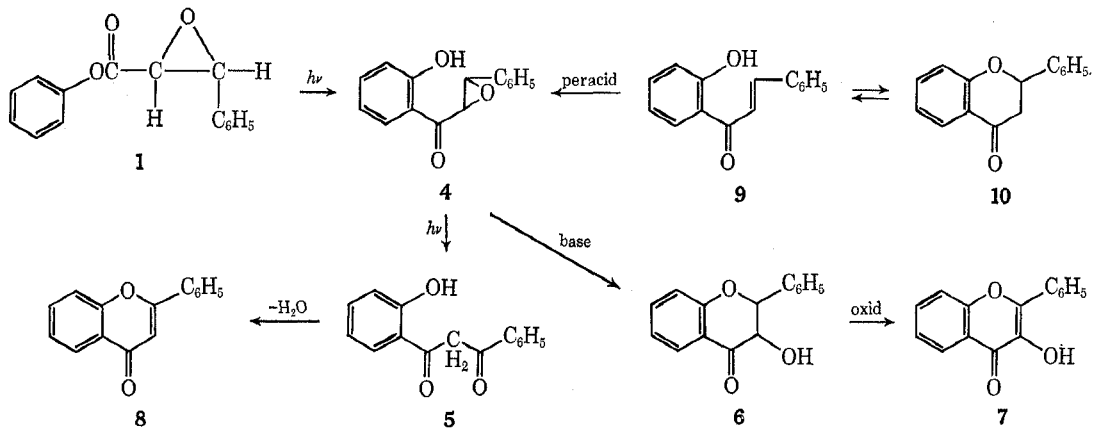
(2) To whom inquiries should be directed.

(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).



revealed the generation of the hitherto unknown 2′-hydroxyepoxychalcone **4**, which underwent intramolecular nucleophilic displacement at the epoxide to yield **6**, from which **7** was derived by air oxidation. In support of this view, we found that spraying the tlc plate with dilute alkali immediately converted the unknown spot into flavonol, recognized by its characteristic fluorescence under ultraviolet light.[7]

We believe that **5** is formed by photolysis of **4**, the product of Fries rearrangement of **1**, in accord with the well-known behavior of epoxy ketones.[8] It could, of course, have occurred after initial isomerization of **1** to a β-keto ester, followed by Fries rearrangement. This pathway is much less attractive, since photolysis of simple glycidic esters does not lead to β-keto esters.[9] Regardless of the actual mechanism, the isolation of **5** has more than a theoretical interest, since its dehydration is known to yield flavone (**8**) in excellent yield, as in the classical Baker–Vankataraman and Allan–Robinson procedures.[10]

Three of the most common types of flavonoid pigments, flavone, flavonol, and flavanonol,[11] were secured from the irradiation of a simple derivative of phenyl cinnamate under mild conditions, but we have thus far failed in our attempts to widen the scope of the photochemical reaction since we could not convert substituted phenyl cinnamates into their epoxides by peracid or hydrogen peroxide oxidation,[13] or *via* their bromo- or chlorohydrins.

The Algar–Flynn–Oyamada (AFO) reaction converts flavanone (**10**) into **7** by alkaline hydrogen peroxide oxidation.[15] The matter of the intermediacy of the hitherto unknown 2′-hydroxyepoxychalcone **4** (formed by epoxidation of the chalcone **9**, which is in equilibrium with **10**) has long been controversial.[15,16] Since we had circumstantial evidence indicating that **4** was formed photochemically and that it had an appreciable stability, we were encouraged to seek an alternate synthesis for that elusive compound. When **9** was refluxed in chloroform with *m*-chloroperoxybenzoic acid, it yielded the desired epoxychalcone **4**, along with 2-hydroxyphenyl cinnamate formed by Baeyer–Villiger oxidation.[17] Several unidentified products were also formed. The isolation of **4** proved quite difficult because of the facile cyclization to **6**, which took place during the required purification by chromatography. This undesired reaction was minimized by using a silica gel which had been thoroughly washed with acetic acid followed by ethyl acetate, and which was then dried at 100°. A pure sample of **4** was thus secured in about 20% yield. It melted at 78° and its nmr ($CDCl_3$) clearly showed the two epoxide protons at 4.15 and 4.3 ppm (each a doublet, J = 2 Hz),[18] nine aromatic protons from 7.0 to 8.0 ppm, and the hydroxyl at 11.9 ppm. Upon treatment with alkali in deoxygenated solution, **4** instantaneously isomerized into the 3-hydroxyflavanone (**6**). Alkali treatment of **4** without removal of oxygen, on the other hand, converted **4** first into **6** and then into **7**, as reported in the AFO reaction.[15]

The above chemical behavior of **4** leaves no doubt that the intermediacy of a 2′-hydroxyepoxychalcone would satisfactorily account for the products of the AFO reaction. Our synthesis of **4**, however, took place in acidic rather than alkaline medium, and the question of whether 2′-hydroxyepoxychalcones are formed in the AFO reaction is still formally open for debate.

The photolysis of **4** gave **5** in very good yield. Since, furthermore, the tlc properties of **4** matched those of the labile product in the photolysis of **1**, we may feel confident that the photo-Fries rearrangement to **4** represents a major pathway in the photochemistry of **1**. Finally, the isolation of **5**, **6**, and **7** in our original experiment is in complete accord with the photochemical and chemical behavior of the authentic sample of epoxychalcone **4**.

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(8) D. C. Neckers, "Mechanistic Organic Photochemistry," Reinhold, New York, N. Y., 1967, p 208.

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(10) J. Gripenberg, ref 7, p 410.

(11) The acid treatment of O-protected *o*-hydroxyepoxychalcones has been discussed by S. C. Bhrara, A. C. Jain, and T. R. Seshadri, *Tetrahedron*, **20**, 1141 (1964). These authors found that, whereas the O-benzylated form of **4** yielded only **6**, its congeners substituted in the cinnamoyl moiety also yielded isoflavones, in a synthetically useful method.[12] Therefore, this fourth class of flavonoid pigments should also be available in our procedure from the proper cinnamic esters.

(12) A. C. Jain, P. Lal, and T. R. Seshadri, *Indian J. Chem.*, **7**, 305 (1969).

(13) A similar situation was found in the α,β-unsaturated ketone series, which required initial reduction to the allylic alcohols, epoxidation, and, finally, oxidation to the epoxy ketone.[14]

(14) H. O. House and D. J. Reif, *J. Amer. Chem. Soc.*, **79**, 6491 (1957).

(15) F. M. Dean, "Naturally Occuring Oxygen Ring Compounds," Butterworths, London, 1963, p 345.

(16) F. M. Dean and V. Podimuang, *J. Chem. Soc.*, 3978 (1965).

(17) Surprisingly, identical treatment of the isomer **10** did not lead to **4**, **5**, **6**, **7**, or 2-hydroxyphenyl cinnamate. We thank Dr. V. K. Bhatia for carrying out the experiment.

(18) The nmr of *trans*-epoxychalcone itself, in the same solvent, shows the epoxide protons at 4.16 and 4.31 (J = 2 Hz).

Experimental Section

The nmr spectra were recorded with internal TMS on Varian A-60A spectrometer and are reported on the δ scale with coupling constants in hertz. The uv spectra were obtained with a Hitachi–Coleman-12 spectrometer, and the mass spectra with a Perkin-Elmer 270 gc–mass spectrometer, equipped with a direct solid inlet. The melting points are not corrected. The irradiation were performed in a Rayonet apparatus, after bubbling nitrogen through the solution for 30 min. All the tlc analyses were performed on silica gel with benzene.

Phenyl *trans*-Epoxycinnamate (1).—A mixture of *trans*-cinnamoyl chloride (from 10 g of acid) and phenol (10 g) was refluxed in 150 ml of benzene in presence of 1.2 g of Mg turnings for 2.5 hr.[19] After washing with dilute base and with water, drying over $MgSO_4$, removing the solvent, and crystallizing the residue from benzene–hexane, there was obtained 14.5 g (97%) of phenyl cinnamate: mp 76° (lit.[20] 75–76°); mol wt 224 (mass spectrum); nmr (in $CDCl_3$) 6.63 and 7.9 (each a d, J = 12 Hz, vinyl protons) and 7.1–7.9 (10 aromatic protons).

A solution of 4.5 g of phenyl cinnamate and 5.0 g of *m*-chloroperoxybenzoic acid in 150 ml of CCl_4 was refluxed for 25 hr. The mixture was washed with dilute $NaHCO_3$ and with water, and was dried over $MgSO_4$. Evaporation of the solvent gave 4.4 g of residue which crystallized on standing and was recrystallized from benzene–hexane to yield 3.0 g of **1**: mol wt 240 (mass spectrum); mp 88–90°; uv max at 220 nm; nmr ($CDCl_3$) at 3.7 and 4.3 (each a d, J = 2 Hz, *trans*-epoxide protons) and 7.2–7.4 (10 aromatic protons).

Photolysis of 1.—A solution of 3 g of **1** in 300 ml of benzene was irradiated at 253.7 nm under nitrogen for 30 hr. The solvent was evaporated and the residue was chromatographed over silica gel. Elution with hexane gave 120 mg of *trans*-stilbene, identified by direct comparison (melting point, uv, nmr, and mass spectra) with an authentic sample. Elution with CCl_4 gave 1.5 g of a mixture from which 1.32 g of unreacted **1** crystallized out; the mother liquor yielded *o*-hydroxybenzoylacetophenone (**5**). Elution with CCl_4–C_6H_6 (3:1) yielded 0.12 g of phenol, identified by direct comparison with an authentic sample. Elution with 1:1 benzene–CCl_4 yielded a mixture of 100 mg of flavanonol (**6**) and 20 mg of flavonol (**7**) which were separated by fractional crystallization.

The diketone **5**, mp 118–120° (lit.[21] 120–121°), was characterized by the ir at 1605, 1565, and 1560 cm^{-1}, by the nmr at 6.8–8.0 (10 aromatic H's), 12.16 (s, phenolic OH), and 15.66 (s, enolic OH), and by the mass spectrum, which, in addition to the mass peak at m/e 240, showed major peaks at m/e 222, 163, 135, 120, 119, 93, and 77. The structure of **6**, mp 186–188° (lit.[22] 188°), was deduced from the uv at 250 and 320 nm and the nmr (DMSO-d_6) at 4.66 (q, J = 12 and 6 Hz, H-3), 5.3 (d, J = 12 Hz, H-2), 5.75 (d, J = 6 Hz, 3-OH), and 7.0–8.0 (9 aromatic H's). After D_2O was added, the H-3 signal became a doublet (J = 12 Hz) and the OH signal disappeared.

The structure of **7**, mp 170° (lit.[23] 169.5–170.5°), was deduced from the uv at 238, 304, and 344 nm (this last band shifting to 405 nm in presence of $AlCl_3$[24]), and from the nmr (DMSO-d_6) at 7.2–7.8 (aromatic H's). The samples of **6**, **7**, and **8** were also found to be identical with authentic samples by direct comparison.

Conversion of 5 into Flavone (8).—A mixture of 50 mg of **5** and 200 mg of sodium acetate in 2 ml of acetic acid was refluxed for 1.5 hr.[25] Flavone (**8**), isolated by preparative tlc, had mp 93–95° (lit.[23] 97–99), λ_{max} at 250 and 294 nm, and nmr ($CDCl_3$) at 6.8 (s, H-3) and 7.2–8.2 (9 aromatic protons).

Epoxidation of 9.—A solution of 4.5 g of **9** and 10.0 g of *m*-chloroperoxybenzoic acid in 150 ml of CCl_4 was refluxed for 15 hr. After washing with dilute $NaHCO_3$ and with water, drying with $MgSO_4$, and evaporating the solvent, the residue was chromatographed over a silica gel which had been previously washed with acetic acid and ethyl acetate, followed by drying at 100°. Benzene first eluted 1.0 g of **4**: mass spectrum main peaks at m/e 240, 211, 133, 122, 121, 120, 105, 93, 92, 91, and 77; mp 78° (C_6H_6–hexane); nmr ($CDCl_3$) 4.15 and 4.30 (each a d, J = 2 Hz, 1 H), 7.0–8.0 (m, 9 H's), and 11.9 (s, OH). Further elution yielded 200 mg of **6**, identical with an authentic sample, and 200 mg of *o*-hydroxyphenylcinnamate: mp 139–141° (lit.[26] mp 140–141°); nmr ($CDCl_3$) 6.66 and 7.93 (each a d, J = 16 Hz, 1 H), 7.0–7.8 (m, 9 H's), and 5.8 (s, OH); mass spectrum main peaks at m/e 240, 147, 131, 103, and 77.

Photolysis of 4.—A solution of 100 mg of **4** in 15 ml of benzene was irradiated under N_2 for 2 hr at 253.7 nm. The diketone **5** was isolated by silica gel chromatography in 75% yield. It had mp 118–120°, and was identical with the sample isolated in the photolysis of **1**.

Base Treatment of 4.—A small amount of **4** was dissolved in methanol. One-half of the solution was treated with 1 drop of 1 *N* aqueous NaOH and was immediately analyzed by tlc. A mixture of **6** and **7** was found, and the concentration of the latter increased with time at the expense of the former until it became the only product. The other half was deoxygenated by boiling and was cooled to room temperature under nitrogen. It was treated with 1 drop of 1 *N* aqueous NaOH which had been similarly deoxygenated. Immediate tlc analysis showed that **6** was the only component.

Registry No.—**1**, 25518-21-2; **4**, 25518-22-3; **5**, 1469-94-9; **6**, 1621-55-2; **7**, 577-85-5; **8**, 525-82-6.

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