The relationship between pH and the mechanism of clindamycin degradation could be summed as follows. Below pH 4 clindamycin degrades *via* thioglycoside and amide hydrolysis with thioglycoside hydrolysis predominant in the pH range 0.4–4. Above pH 5 clindamycin degrades by conversion to lincomycin and by other reactions such as amide hydrolysis. The extent of lincomycin conversion is dependent on the degree of protonation of the *N*-methyl-4-propylpyrrolidine moiety. At pH less than 5 where the amine is fully protonated no conversion to lincomycin occurs. Then this process can be detected in the vicinity of pH 5 and its rate increases as pH increases to pH 9 and then becomes constant since the amine function is completely nonprotonated. The overall rate of clindamycin degradation continues to increase with increasing pH above pH 9, however, due to the hydroxide ion dependency of the other degradative routes.

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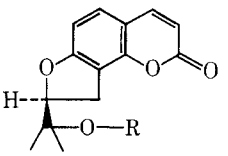
Coumarins XI: A Total Synthesis of (±)-Columbianetin

M. SHIPCHANDLER*, T. O. SOINE†, and P. K. GUPTA‡

Abstract □ (±)-Columbianetin [(±)-I] has been synthesized by a ten-step sequence starting with 2,6-dihydroxybenzoic acid which was converted to the methyl ester, benzylated, and reduced to the benzyl alcohol which was oxidized to the aldehyde and monodebenzylated to provide 2-hydroxy-6-benzyloxybenzaldehyde (VI). Treatment of VI with methyl bromoacetate converted it to methyl 3-benzyloxy-2-formylphenoxyacetate which was cyclized to methyl 4-benzyloxybenzofuran-2-carboxylate, the latter being converted to 2-(α-hydroxyisopropyl)-4-benzyloxybenzofuran (XIV) by the action of CH_3MgI. Reduction and debenzylation of XIV to the corresponding dihydrobenzofuran followed by acid-catalyzed condensation with ethyl propiolate provided (±)-I.

Keyphrases □ (±)-Columbianetin—total synthesis □ TLC—separation identity □ Mass spectroscopy—identity □ UV spectrophotometry—identity □ IR spectrophotometry—identity □ NMR spectroscopy—identity

The isolation of two new coumarins from the umbellifer, *Lomatium columbianum* Math. and Const., was reported in 1964 (1). One of these coumarins, a glycoside assigned the name columbianin, has since been shown to occur in *L. dissectum* var. *multifidum* (Nutt.) Math. and Const. (2) and *L. nuttallii* (A. Gray) Macbr. (3). Acid hydrolysis of columbianin yielded D-glucose and a tertiary coumarinic aglycone, columbianetin (I) and led to the postulation of II as the structure for the glycoside. More recent studies (4) have revised the structure to III, *i.e.*, the β-D-gentiobioside of I (III). The other coumarin, columbianadin, was assigned Structure IV, *i.e.*, the angelate ester of I, and has been found in *Peucedanum palustre* (5) as well as in *Zosimia absinthifolia* (Vent.) Link (6, 7). The absolute configuration of I has been shown to be 8(S) (8).

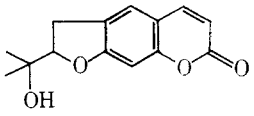


I, R = H

II, R = D-Glucosyl

III, R = β-D-Gentiobiosyl

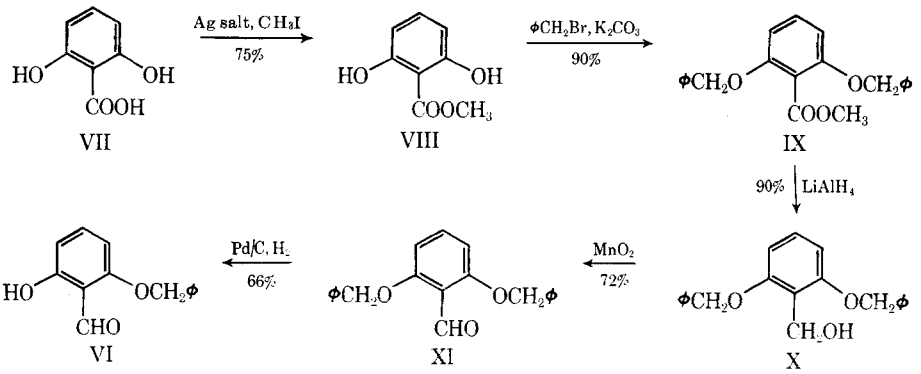
IV, R = Angeloyl



V

The recent total synthesis of marmesin (V) and its optical antipode, nodakenetin, by Nakajima *et al.* (9) and confirmed by Harada *et al.* (10) in a study of the absolute configuration suggested that a similar synthesis could be applied to the preparation of I by utilizing 2-hydroxy-6-benzyloxybenzaldehyde (VI) as starting material in place of the isomeric 2-hydroxy-4-benzyloxybenzaldehyde employed by these workers. The preparation of VI from 2,6-dihydroxybenzaldehyde (*i.e.*, γ-resorcylaldehyde) was the obvious route but a survey of the literature pertaining to the preparation of the latter (11–15) indicated that all of the published methods were characterized by poor overall yields as well as lengthy synthetic sequences. Thus, a synthesis of

VI starting from readily obtainable 2,6-dihydroxybenzoic acid (VII) appeared attractive (see Scheme I). The usual Fischer method of acid-catalyzed esterification of VII is reported (16) to give low yields and the authors found it also gave a poor yield of the methyl ester (VIII).



Scheme I

Employment of the method of Tomino (17), however, reacting the silver salt of VII with methyl iodide provided a 75% yield of VIII. Benzylation of VIII was carried out by the method of Doyle *et al.* (16) who prepared it without characterization and simply hydrolyzed it for further synthetic procedures. In the present study IX was obtained in 90% yield and, upon saponification, yielded the corresponding acid already described by Doyle *et al.* (16). Lithium aluminum hydride reduction of IX yielded the corresponding alcohol (X) in 90% yield. Oxidation of X with active manganese dioxide provided the aldehyde (XI) in 72% yield (18). Removal of a single benzyl group to obtain the desired monobenzyl ether (VI) in 66% yield was effected by catalytic hydrogenolysis with palladium-on-carbon.

The sequence of reactions to provide (±)-I from VI is shown in Scheme II and begins with alkylation of VI in 90% yield with methyl bromoacetate to provide XII followed by cyclization in the presence of magnesium methoxide by the method of Davies *et al.* (19) resulting in XIII in 63% yield.[1] Treatment of XIII with methyl magnesium iodide resulted in an oily tertiary alcohol (XIV) reminiscent of the similarly oily isomeric product obtained by Nakajima *et al.* (9) who proceeded directly to its hydrogenation without further characterization. Utilizing the alkaline palladium-on-carbon (20) of the above authors in the present study to minimize hydrogenolysis of the allylic tertiary alcohol function, the resulting product appeared to be only that (XV) resulting from simple debenzylation of XIV. This was illustrated by its mass spectrum which showed a strong molecular ion peak at *m/e* 192 accompanied by the base peak at *m/e* 177 (doubly charged ion at *m/e* 88.5), undoubtedly due to a loss of methyl radical (see Scheme III).[2] The *m/e* 177 ion does not fragment further to any appreciable extent. Compound XV, when hydrogenated further in the presence of commercially available palladium-on-carbon, provided the dihydrobenzofuran compound (XVI) which was subsequently obtained also by direct hydrogenation of XIV in the presence of the same catalyst. TLC examination in each case revealed a spot with a higher R_f value than that of XVI, presumably the hydrogenolyzed desoxy compound. Compound XVI gave a molecular ion at *m/e* 194 in the mass spectrum and its fragmentation behavior had a marked resemblance to that of columbianetin (I) as evidenced by the loss of H_2O from the molecular ion followed by methyl expulsion to give the benzopyrilium ion at *m/e* 161 (21) (see Scheme IV). Further, a strong peak at *m/e* 59 is due to the protonated form of acetone arising from the side chain of the molecular ion. Loss of acetone from the molecular ion provides an ion of *m/e* 136 (base peak) which fragments further as illustrated.

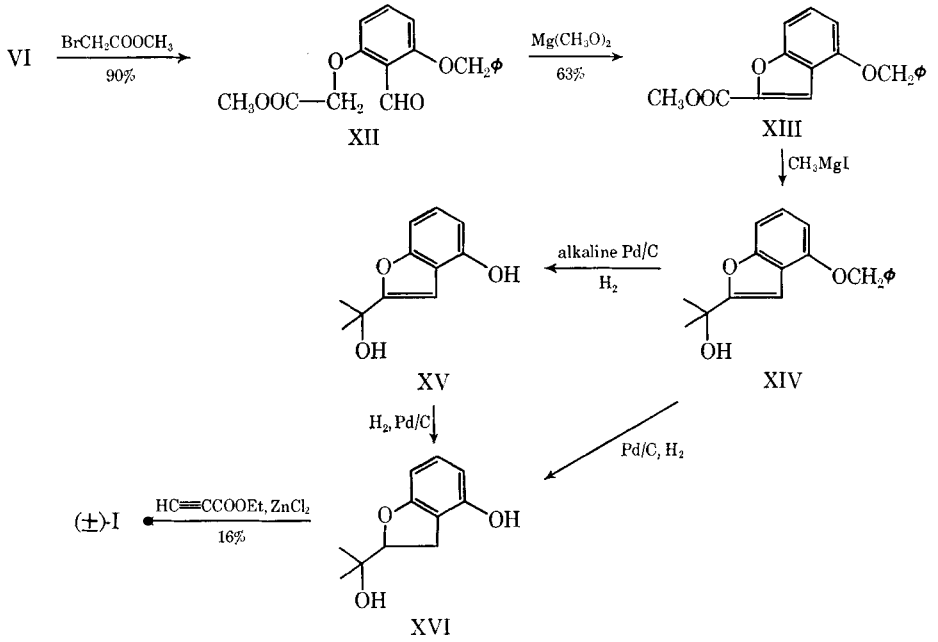
The final conversion of XVI to (±)-I was accomplished through the method of Kaufman and Kelly (22) who employed ethyl propiolate in the presence of an acid to form the coumarinic lactone ring. This method has also been used successfully by Ganguly *et al.* (23). In the present study, condensation of XVI with ethyl propiolate in the presence of zinc chloride provided (±)-I in low yield. The identity of the racemic product was established by TLC, UV, IR, and mass spectral comparison with natural (+)-columbianetin.

EXPERIMENTAL

Melting points were determined in a Thomas-Hoover melting point apparatus and are uncorrected. IR spectra were determined with a Perkin-Elmer 237B grating infrared spectrophotometer and, unless otherwise specified, were determined in potassium bromide pellets. NMR spectra were determined with Varian Associates A-60 and A-60D instruments using tetramethylsilane (TMS) as the internal standard. Unless otherwise specified, all spectra were obtained in $CDCl_3$ in approximately 15% concentration and s. refers to singlet, d. to doublet, t. to triplet, and m. to multiplet. Mass spectra were determined with a Hitachi Perkin-Elmer RMU-6D mass spectrometer (courtesy of Mr. A. R. Swanson and Mr. D. L. Hobbs, School of Chemistry, University of Minnesota). The instrument was operated with a source temperature of 250° and an ionizing voltage of 50 ev. The samples were introduced by the direct inlet technique. UV spectra were determined on a Cary-14 recording spectrophotometer in 95% ethanol. Silica gel for column chromatography (Baker Analyst No. 3405) was activated at 110° and impregnated with 5% of water. Alumina for column chromatography (Woelm neutral alumina purchased from Brinkmann Instruments, Inc., Great Neck, N. J.), silica gel and alumina for TLC (Chroma-

[1] Attempts to reduce XIII catalytically under varying conditions prior to treatment with the Grignard reagent were uniformly unsuccessful and accompanied only by debenzylation and/or reduction of the aromatic ring.

[2] Fragmentation supported by the appearance of corresponding metastable ions has been indicated by heavily-lined arrows here and in Scheme IV. Numbers in parentheses correspond to abundance ratios.



Scheme II

gram sheets with fluorescent indicator supplied by Distillation Products Industries, Rochester, N. Y.), and preparative silica gel-coated thin-layer plates (Brinkmann Instruments, Inc.) were also used. Microanalyses were performed by the Microanalytical Laboratory, School of Chemistry, University of Minnesota or by the Schwarzkopf Microanalytical Laboratory, Woodside, N. Y.

Methyl 2,6-Dihydroxybenzoate (VIII)—*Method A*—Using the method of Doyle *et al.* (16) which employs methanolic hydrogen chloride provided a 32% yield of white crystals, m.p. 68–70°. Lit (16) reported m.p. 69–71°.

Method B—The method of Tomino *et al.* (17) employing the silver salt of 2,6-dihydroxybenzoic acid[3] with methyl iodide provided a 75% yield of white crystals, m.p. 68–70° which on repeated crystallization gave m.p. 69–70°.

Methyl 2,6-Dibenzyloxybenzoate (IX)—Methyl 2,6-dihydroxybenzoate (VIII) (16.8 g., 0.1 mole), benzyl bromide (34.2 g., 0.2 mole) and anhydrous potassium carbonate (36.0 g., 0.26 mole) were refluxed together in 100 ml. of dry acetone for 4 hr. with magnetic stirring (16). At the end of the reflux period the solvent was removed under vacuum and the residual mass was extracted with ether several times. The extract was washed with aqueous sodium hydroxide solution (10%), followed by water, and was finally dried over anhydrous sodium sulfate. Removal of the solvent under vacuum gave an oily mass which solidified upon standing. The product was crystallized from ethanol to yield 31.2 g. (90% of theory) of white crystals, m.p. 71–73°. Repeated crystallization from aliphatic naphtha[4] gave an analytical sample, m.p. 73–74°.

Anal.—Calcd. for $C_{22}H_{20}O_4$: C, 75.84; H, 5.79. Found: C, 76. 12; H, 6.08.

The above ester was hydrolyzed by the method of Doyle *et al.* (16) and 2,6-dibenzyloxybenzoic acid was crystallized twice from ethyl acetate-naphtha, m.p. 123–124°. Lit. (16) gives m.p. 124–126°.

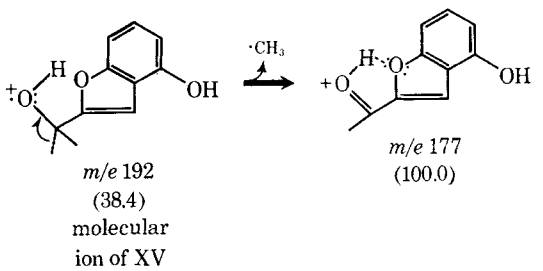
2,6-Dibenzyloxybenzyl Alcohol (X)—Methyl 2,6-dibenzyloxybenzoate (IX) (17.4 g., 0.05 mole) was dissolved in 150 ml. of anhydrous ether and the solution was added dropwise to a magnetically stirred slurry of 3.8 g. (0.1 mole) of lithium aluminum hydride in 100 ml. of dry ether kept in an ice bath. The reaction mixture was stirred for 30 min. after the addition was complete and the excess of lithium aluminum hydride was then decomposed by the cautious addition of the required amount of water. After stirring for 15 min. following the decomposition, the reaction mixture was filtered through a sintered-glass funnel and the residual mass was thoroughly washed with ether. The filtrate and washings were combined, dried over anhydrous sodium sulfate, and the solvent removed under vacuum. The product was crystallized from cyclohexane, to provide 14.4 g. (90% of theory) of white needles, m.p. 78–79°. Several recrystallizations gave an analytical sample, m.p. 79–80°.

Anal.—Calcd. for $C_{21}H_{20}O_4$: C, 78.72; H, 6.29. Found: C, 79.01; H, 6.31.

2,6-Dibenzyloxybenzaldehyde (XI)—Active manganese dioxide[5] (16 g.) was added to 200 ml. of toluene and stirred magnetically for a few minutes before addition of 8 g. (0.024 mole) of 2,6-dibenzyloxybenzyl alcohol (X). The mixture was then refluxed for 4 hr. with continued stirring. The reaction mixture was filtered through a diatomaceous earth[6] bed while hot and the residue was washed well with hot and cold benzene. The combined filtrate and washings were stripped of solvent under reduced pressure and the resulting oil which had an odor reminiscent of benzaldehyde solidified on standing. The product was crystallized from cyclohexane to yield 5.7 g. (72% of theory) of white needles, m.p. 80–81.5°. Repeated crystallization provided an analytical sample, m.p. 81.5–82.5°. The compound gave a positive test with 2,4-dinitrophenylhydrazine reagent.

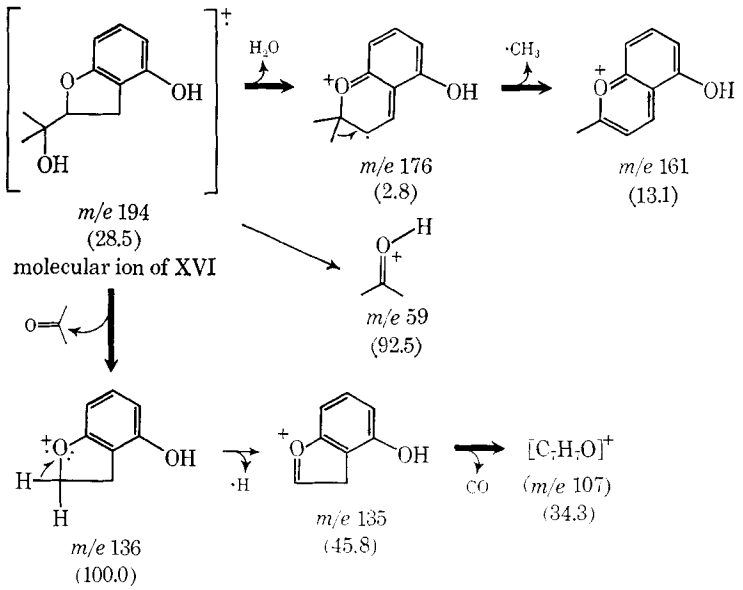
Anal.—Calcd. for $C_{21}H_{20}O_3$: C, 79.22; H, 5.70. Found: C, 79.51; H, 5.66.

2-Hydroxy-6-benzyloxybenzaldehyde (VI)—Palladium-on carbon (5%, 2 g.)[7] was shaken in an atmosphere of hydrogen for 30 min. in 50 ml. of ethanol. 2,6-Dibenzyloxybenzaldehyde (XI) (3.18 g., 0.01 mole) was dissolved in 100 ml. of ethanol with the aid of heat. After cooling, this was added to the prereduced catalyst and hydrogenated at atmospheric pressure until the uptake of hydrogen amounted to 270 ml. (1.1 mole equivalent). The catalyst was removed by filtration and washed well with hot and cold ethanol. The



Scheme III

[3] The acid is a product of Aldrich Chemicals, Milwaukee, Wis.
[4] Skellysolve-B, Skelly Oil Co., Kansas City, Mo.
[5] Winthrop Laboratories, New York, N. Y.
[6] Celite, Johns-Manville Products Corp., New York, N. Y.
[7] Matheson, Coleman & Bell, Cincinnati, Ohio.



Scheme IV

filtrate, on alumina TLC examination, showed very little of the starting material (a blue spot under UV light having the greatest mobility when developed with C_6H_6:$CHCl_3$, 1:1) while the monobenzyl compound appeared as a yellowish-green fluorescent spot. A third spot, having the least polarity and showing a dark spot under UV light, was assumed to be 2,6-dihydroxybenzaldehyde. The filtrate and washings were combined and concentrated under reduced pressure to 70 ml. On standing and cooling, the product crystallized out as yellow needles weighing 1.35 g., m.p. 72–73°. The mother liquor was evaporated to dryness and chromatographed through a silica gel column 25 g., 1.92 × 25.40 cm. (0.75 × 10 in.). The product was eluted with cyclohexane: benzene (3:1) and on evaporation of the eluant solvent under reduced pressure followed by crystallization provided 0.16 g. more of the product for a total yield of 1.51 g. (66% of theory). Repeated crystallization from naphtha gave an analytical sample as lemon-yellow needles, m.p. 73–74°.

Anal.—Calcd. for $C_{13}H_{12}O_3$: C, 73.67; H, 5.30. Found: C, 73.46; H, 5.16.

The compound gave a violet coloration with ferric chloride solution but did not show a hydroxyl peak in the IR. It showed, however, a split C=O band at 1625 and 1650 $cm.^{-1}$ (probably due to hydrogen bonding).

Methyl 3-Benzyloxy-2-formylphenoxyacetate (XII)—2-Hydroxy-6-benzyloxybenzaldehyde (VI) (2 g., 0.009 mole), anhydrous potassium carbonate (10 g., 0.073 mole) and methyl bromoacetate (10 g., 0.073 mole) were refluxed together in 60 ml. of dry acetone for 72 hr. The precipitated potassium bromide and the unreacted potassium carbonate were filtered off and washed well with solvent. The filtrate and washings were combined and most of the unreacted methyl bromoacetate was removed under vacuum with the final traces being removed by passing a stream of air over the residue for a few hours. The product was crystallized from ethanol to give 2.38 g. (90% of theory), m.p. 99–101°. Several recrystallizations gave an analytical sample as white needles, m.p. 100–101°.

Anal.—Calcd. for $C_{17}H_{16}O_5$: C, 67.99; H, 5.37. Found: C, 67.91; H, 5.59.

Methyl 4-Benzyloxybenzofuran-2-carboxylate (XIII)—Magnesium methoxide was prepared from magnesium metal powder (20 g., 0.825 mole, 70–80 mesh) and 600 ml. of dry methanol. Methyl 3-benzyloxy-2-formylphenoxyacetate (XII) (4 g., 0.013 mole) was added and the mixture refluxed for 12 hr. (19). After removal of most of the methanol, crushed ice and water were added and the excess of methoxide was decomposed by portionwise addition of 130 ml. of concentrated hydrochloric acid while cooling the mixture continually in an ice bath combined with simultaneous addition of crushed ice to the reaction flask. After decomposition of all of the methoxide, the aqueous layer was extracted several times with ether and the extract dried over anhydrous sodium sulfate. Removal of the solvent under reduced pressure and crystallization from ethanol gave 2.27 g. of the product, m.p. 128–129°. The mother liquor was chromatographed through a silica gel column (25 g., 1.92 × 25.40 cm.) (0.75 × 10 in.). The product was eluted with benzene and the column monitored with a hand UV lamp, the desired compound showing a blue fluorescence. Evaporation of the eluant and crystallization provided an additional 0.1 g. of the product for a total yield of 2.37 g. (63% of theory). Repeated crystallizations provided an analytical sample as shiny white plates, m.p. 129–130°.

Anal.—Calcd. for $C_{17}H_{14}O_4$: C, 72.33; H, 5.00. Found: C, 71.99; H, 5.22.

2-(α-Hydroxyisopropyl)-4-benzyloxybenzofuran (XIV)—Grignard reagent was prepared from 0.4 g. (0.017 mole) of magnesium metal powder (70–80 mesh) and 1.4 ml. (0.022 mole) of methyl iodide in 20 ml. of dry ether under nitrogen. Methyl 4-benzyloxybenzofuran-2-carboxylate (XIII) (0.4 g., 0.0014 mole) in 8 ml. of dry tetrahydrofuran was added dropwise with magnetic stirring at 0°. The reaction mixture was then stirred at room temperature for 15 min. and allowed to stand for 18 hr. The Grignard complex was then decomposed with saturated aqueous solution of ammonium chloride (10 ml.) and the ethereal layer separated. The aqueous layer was extracted with ether (4 × 20 ml.) and the combined ethereal solutions were dried over anhydrous sodium sulfate. Silica gel TLC examination ($CHCl_3$:EtOAc, 1:1 as developing solvent) showed a blue spot under UV light with slightly less mobility than the starting material which was present only in traces. Removal of the ether under reduced pressure provided a yellow oil which could not be induced to crystallize. The IR spectrum (neat) showed the complete disappearance of the C=O band and the appearance of a hydroxyl absorption at 3370 $cm.^{-1}$. In the NMR spectrum the gem-dimethyl protons appeared at 1.62 (s., 6H), the *t*-hydroxyl proton at 2.30 (broad s., 1H), the benzylic protons at 5.15 (s., 2H), and the aromatic protons including the protons of the benzofuran ring appeared between 6.50 and 7.50 δ (m., 9H). The peak at 2.30 δ disappeared completely upon addition of D_2O. This oily compound (XIV) was subjected to hydrogenation without further characterization.

2-(α-Hydroxyisopropyl)-4-hydroxybenzofuran (XV)—The oily compound (XIV) prepared as above was stirred with 0.2 g. of alkali treated 5% palladium-on-carbon (20) in 20 ml. of ethanol in an atmosphere of hydrogen at atmospheric pressure for 24 hr. As the reaction proceeded a spot having less polarity than the starting material appeared on silica gel TLC examination ($CHCl_3$:EtOAc, 1:1 as the developing solvent using UV light for spot detections). At the end of 24 hr. the TLC examination showed very little of the starting material. The catalyst was removed by filtration and washed well with hot and cold ethanol. The filtrate and washings were combined and the solvent removed under reduced pressure. The product was crystallized from ethyl acetate-cyclohexane to yield 0.18 g. (66% of theory in two steps), m.p. 158–161°. The product

was further purified by passing it through a silica gel column 10 g., 1.60 × 20.32 cm. (0.63 × 8 in.) using chloroform as the eluant. An analytical sample was prepared by repeated crystallization, m.p. 163–164°. Compound XV gave a violet coloration with alcoholic ferric chloride solution.

Anal.—Calcd. for $C_{11}H_{12}O_3$: C, 68.73; H, 6.29. Found: C, 68.58; H, 6.51.

2-(α-Hydroxyisopropyl)-4-hydroxy-2,3-dihydrobenzofuran (XVI)—The oily compound (XIV) prepared as above was hydrogenated in a Parr apparatus at 10 p.s.i. pressure in the presence of 0.4 g. of palladium-on-carbon (5%) in 20 ml. of ethanol for 24 hr. At the end of this period the catalyst was removed by filtration and washed well with hot and cold ethanol. The filtrate and washings were combined and the solvent removed under reduced pressure. Silica gel TLC examination ($CHCl_3$:EtOAc, 1:1 as developing solvent) showed two spots when treated with iodine vapors. The spot with the higher R_f value was presumably the corresponding desoxy compound which was eluted out along with other impurities using benzene on a silica gel 10 g., 1.60 × 20.32 cm. (0.63 × 8 in.) column. Elution with a large volume of chloroform, evaporation under reduced pressure and crystallization from ethyl acetate-cyclohexane gave 0.18 g. (65% of theory in two steps) of the product, m.p. 150–152°. An analytical sample was obtained by several recrystallizations, m.p. 153–154°.

Anal.—Calcd. for $C_{11}H_{14}O_3$: C, 68.02; H, 7.27. Found: C, 68.12; H, 7.26.

The same product was obtained from the corresponding benzofuran compound (XV) when 0.1 g. was hydrogenated for 24 hr. in a Parr apparatus at 10 p.s.i. pressure in the presence of the same catalyst (0.1 g.) in 10 ml. of ethanol. The isolation was effected in the same manner to yield 0.055 g. of the product.

(±)-Columbianetin [(±)-I]—2-(α-Hydroxyisopropyl)-4-hydroxy-2,3-dihydrobenzofuran (XVI) (0.2 g., 0.001 mole), zinc chloride (0.2 g., 0.0015 mole, fused before use) and ethyl propiolate (1.0 g., 0.0058 mole) were heated at 90° for 1 hr. under nitrogen (22). After cooling, the yellow mass was treated with dilute hydrochloric acid (4 ml.) and the acid layer extracted exhaustively with chloroform. The chloroformic extract was dried over anhydrous sodium sulfate and the solvent removed under reduced pressure. Silica gel TLC examination ($CHCl_3$:EtOAc, 1:1 as developing agent) showed a fluorescent spot under UV light with the same R_f value as authentic (+)-columbianetin[8] along with several other spots. The oily residue was chromatographed through neutral alumina 20 g., 1.92 × 25.40 cm. (0.75 × 3.5 in.) and eluted with chloroform. The fractions were examined by TLC and those containing (±)-I were mixed together and the solvent removed under reduced pressure. The product, weighing 0.028 g., m.p. 167–168.5° was obtained after three crystallizations from chloroform-*n*-hexane. All the mother liquors were combined, concentrated, and streaked on a preparative silica gel plate and developed with the same solvent system. The zone corresponding to (±)-I was scraped off and eluted with methanol. The solvent was then removed and the product was passed through a short neutral alumina 5 g., 1.27 × 5.08 cm. (0.5 × 2 in.) column using chloroform as the eluant. Removal of the solvent and two crystallizations from chloroform-*n*-hexane provided 0.013 g. of white crystals, m.p. 165.5–168.5°, providing a total yield of 0.041 g. (16% of theory). Several crystallizations gave an analytical sample, m.p. 170–171°.

Anal.—Calcd. for $C_{14}H_{14}O_4$: C, 68.28; H, 5.73. Found: C, 68.24; H, 5.84.

The product had an identical R_f value to that of (+)-columbianetin on a silica TLC sheet using the $CHCl_3$:EtOAc, 1:1 solvent system. It also had an identical UV spectrum with the natural product. The IR spectra in KBr pellets of the two samples showed minor differences which were removed when solution spectra (2.5% in $CHCl_3$) were compared. In the mass spectrum the molecular ion appeared at *m/e* 244. When the mass spectra of the natural I and the synthetic (±)-I were run under identical conditions it was found that the base peaks appeared at *m/e* 59 and all of the major peaks above *m/e* 75 were in excellent agreement insofar as abundance ratios were concerned.

[8] Obtained during the studies of Gupta and Soine (2).

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