Coumarins II

Structures of Columbianadin and Columbianin

By ROBERT E. WILLETTE† and TAITO O. SOINE

Columbianadin and columbianin were isolated from petroleum ether and alcoholic extracts, respectively, of *Lomatium columbianum*. Upon hydrolysis, these coumarins yielded tiglic acid and glucose, respectively, and an alcohol, columbianetin. This alcohol was dehydrated to dihydroöroselone and upon hydrogenation formed a product identical with a degradation product obtained from athamantin. This, in addition to ultraviolet, infrared, and nuclear magnetic resonance absorption studies, led to the establishment of columbianetin as (+)-8,9-dihydro-8-(1-hydroxy-1-methylethyl)-2H-furo[2,3-h]-1-benzopyran-2-one and columbianin as its glucoside. Columbianadin was established as its angelate by synthesis.

IN A CONTINUED search of western United States for plants of the *Umbelliferae* family containing coumarins and/or chromones exhibiting pharmacological activities, Call (1) investigated *Lomatium columbianum* Mathias & Const. From petroleum ether extracts of its root, he isolated a crystalline substance possessing spasmolytic properties which he named "columbianin." No structural studies were reported on this substance.

L. columbianum is an umbellifer growing in south central Washington and north central Oregon. The synonymy listed for this plant is *Leptotaenia purpurea* Coult. & Rose and *Ferula purpurea* Wats., but is not *Lomatium purpureum* (2).

The investigation reported here concerns a more exhaustive investigation of the root of *L. columbianum* and determination of the structures of the principal constituents.

Detailed pharmacological studies will be reported at a later date.

EXPERIMENTAL

Unless otherwise specified, melting points were determined in capillary tubes in an oil bath with a thermometer that read accurately for a set of standard samples. Values of $[\alpha]_D$ have been approximated to the nearest degree. Infrared spectra were determined as Nujol mulls on a Perkin-Elmer spectrophotometer, model 137. Ultraviolet spectra were determined in 95% ethanol on Cary recording spectrophotometers, models 11 and 14.

Material.—The dried root of *Lomatium columbianum* Mathias & Const., collected near the Columbia River about 3 miles east of Bingen, Wash., was ground in a Jacobsen laboratory mill to an approximately No. 20 powder in preparation for extraction.[1]

Extraction.—The ground root (5 Kg.) was moistened with petroleum ether (b.p. 30–60°), allowed to macerate several hours, and extracted in a Soxhlet-type extractor continuously for 6 days with petroleum ether. The concentrated extract was allowed to stand, and the crystalline needles which formed were removed periodically by filtration. The combined needles were washed with a small portion of cold petroleum ether and air-dried to afford 43.0 Gm. (0.86%) of columbianadin.[2] Evaporation of the remaining extract left a dark viscous oil which possessed several fluorescent components when a small sample of it was developed on a chromatostrip[3] with 30% ethyl acetate in skellysolve B (b.p. 65–70°). Studies of this fraction will be reported at a later date.

The dried marc remaining from the petroleum ether extraction was next extracted continuously with methylene chloride for 4 days. Evaporation of this extract afforded 40 Gm. of a brown resin. A sample of this residue separated into several fluorescent spots on a chromatostrip developed as before. Studies of this fraction will be reported at a later date.

The dried marc remaining from the methylene chloride extraction was next extracted continuously with methanol for 11 days. The methanolic extract was concentrated to half its volume under reduced pressure and allowed to stand several days to afford 105 Gm. (2.1%) of columbianin as a light brown, microcrystalline powder. In an extraction of 400 Gm. of root with 95% ethanol, 16 Gm. (4.0%) of the same material was obtained.

Received April 3, 1961, from the Department of Pharmaceutical Chemistry, College of Pharmacy, University of Minnesota, Minneapolis.

Accepted for publication October 28, 1963.

Abstracted in part from a dissertation presented by Robert E. Willette to the Graduate School, University of Minnesota, Minneapolis, in partial fulfillment of Doctor of Philosophy degree requirements.

† Rowell Laboratories Fellow, 1957–1959. Present address: Division of Organic Chemistry, C.S.I.R.O., Chemical Research Laboratories, Melbourne, Australia.

The authors are indebted to The Upjohn Co., Kalamazoo, Mich., for making available the instruments used for much of the spectroscopic data.

A portion of this work was supported by Grant H-7101 from the National Institutes of Health, U. S. Public Health Service, Bethesda, Md.

Presented to the Scientific Section, A. PH. A., Washington, D. C., meeting, August 1960.

Previous paper: Willette, R. E., and Soine, T. O., THIS JOURNAL, 51, 149(1962).

[1] The authors are indebted to Dr. T. G. Call, 115 West Rancho Road, Corona, Calif., for collection and identification of the plant material, and to Dr. G. B. Ownbey, Botany Department, University of Minnesota for confirmation of the identity. A herbarium specimen has been retained by the Department of Botany, University of Minnesota.

[2] To follow customary coumarin nomenclature more closely, the authors recommend the names "columbianadin" (Call's "columbianin") for the ester and "columbianin" for the glucoside isolated in the present work. The name columbianetin is suggested for the alcohol resulting from hydrolysis of columbianadin and columbianin.

[3] See *Footnote 2* in the previous paper (3).

Columbianadin (I).—A portion of the crude fraction (9.5 Gm.) was recrystallized from aqueous methanol as long silky needles (5.6 Gm.) which melted at 113–115°; after drying at 100° in vacuum overnight, the needles melted at 121–122°. Repeated crystallizations from dilute methanol and drying at 100° in vacuum overnight gave columbianadin as long colorless needles, m.p. 121–122°; $[\alpha]_D^{27}$ + 26.5° (*c* 1.0, dioxane); ultraviolet spectrum (Fig. 1): $\lambda_{min.}$ 268 mμ (log ε 3.18); $\lambda_{max.}$ 219 (4.35) sh., 250 (3.56), 261 (3.59) and 327 mμ (4.19); infrared spectrum (Fig. 2): 1742–1727 (α-pyrone and α,β unsaturated ester C=O), 1631 (aromatic and α-pyrone ring C=C), 1587 (aromatic ring with conjugated C=C), 1272 (Ar—O—C), 1120 (Ar—O—CR_3), 925 [$(CH_3)_2C$—O], and 837 cm.$^{-1}$ (1,2,3,4-aromatic substitution).[4]

Columbianadin formed a yellow solution in aqueous and ethanolic alkali and was recovered from these solutions upon acidification. It gave a negative phenol test to 3% ethanolic ferric chloride and to the 2% phosphomolybdic acid–concentrated ammonium hydroxide test for hindered phenols. Refluxed in 0.247 *N* ethanolic sodium hydroxide for 6 hours, it gave a saponification equivalent of 172—calculated for two acids, 164.

Anal.[5]—Calcd. for $C_{19}H_{20}O_5$: C, 64.49; H, 6.14; C—CH_3, 9.2; mol. wt., 328.35. Found: C, 64.29; H, 6.08; C—CH_3, 9.2; mol. wt. (Rast), 323.

Columbianin (II).—A portion of the crude fraction (24.8 Gm.) was dissolved in water with the aid of heat; the brown solution was filtered, cooled, diluted with an equal volume of methanol, and evaporated under reduced pressure to a syrup. After standing several days, the syrup afforded very fine crystals. These were filtered off, rinsed with a little methanol, and dried at 100° in vacuum overnight, to give nearly white microcrystals (5.6 Gm.), m.p. 274.5 to 275.5°.

A portion of these crystals (250 mg.), digested in hot methanol and dried, was dissolved in water (5 ml.) and added to the top of a column of silica gel (25 Gm., Baker's chromatographic grade) prepared in a slurry with water. Elution of the column with water yielded a colorless eluate, which was concentrated under reduced pressure to a syrup. Crystallization from methanol, followed by drying at 100° in vacuum several days, gave 190 mg. of columbianin, m.p. 275–276°; $[\alpha]_D^{23}$ + 118 (*c* 0.25, H_2O); ultraviolet spectrum (Fig. 1): $\lambda_{min.}$ 269 mμ (log ε 2.98); $\lambda_{max.}$ 216 (3.97) sh. and 327 mμ (4.00); infrared spectrum (Fig. 3): 3584 (free OH), 3509–3425 (bonded OH) and 1730 cm.$^{-1}$ (α-pyrone C=O). It gave a positive Molisch test.

Anal.—Calcd. for $C_{20}H_{24}O_9 \cdot 2H_2O$: C, 54.07; H, 6.35. Found: C, 53.76, 54.05; H, 6.20, 6.04.

Columbianin Tetraacetate.—Acetylation of columbianin (1.0 Gm.) with acetic anhydride (15 ml.) and fused sodium acetate (1.0 Gm.) gave the tetraacetate (1.8 Gm.). Repeated crystallizations from dilute ethanol and drying at 100° in vacuum gave colorless needles (0.5 Gm.), m.p. 221–222°.

Anal.—Calcd. for $C_{28}H_{32}O_{13} \cdot 2H_2O$: C, 54.90; H, 5.88. Found: C, 55.11; H, 5.86.

Hydrolysis, Isolation of Columbianetin (III).—Columbianadin (5.0 Gm.) was refluxed for 3 hours with 2 *N* sodium hydroxide in 50% ethanol. Complete hydrolysis was determined by development of a neutralized sample of the reaction mixture on a chromatostrip with 30% ethyl acetate in skellysolve

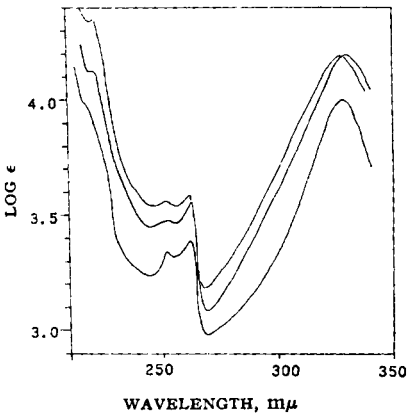


Fig. 1.—Ultraviolet absorption spectra in 95% ethanol. Upper curve, columbianadin; middle curve, columbianetin; lower curve, columbianin.

B. The cooled reaction mixture was diluted with water, partially concentrated under reduced pressure to remove ethanol, and acidified (pH5) with concentrated sulfuric acid. The large colorless needles which formed on standing were removed by filtration and air-dried to give 3.6 Gm. of columbianetin, m.p. 160–162°. Several recrystallizations from hot water and dilute methanol and drying at 100° in vacuum afforded long colorless needles, m.p. 164.5 to 165° (Kofler block); $[\alpha]_D^{27}$ + 20° (*c* 1.0, dioxane); ultraviolet spectrum (Fig. 1): $\lambda_{min.}$ 269 mμ (log ε 3.15); $\lambda_{max.}$ 219 (4.15) and 329 mμ (4.13); infrared spectrum (Fig. 4): 3610 (free OH), 1715 (α-pyrone C=O), 1410, 1149 (*tert*-OH, C—O stretching and OH deformation) and 871 cm.$^{-1}$ ($(CH_3)_2C$—O).

Anal.—Calcd. for $C_{14}H_{14}O_4$: C, 68.28; H, 5.73; mol. wt., 246.25. Found: C, 68.43; H, 5.65; mol. wt. (Rast), 222.

Columbianin (2.0 Gm.) heated on a steam bath with 5% hydrochloric acid (30 ml.) for 30 minutes also gave columbianetin (0.4 Gm.).

Isolation of Tiglic Acid.—The acidic aqueous filtrate from above was extracted with four 100-ml. portions of ether, which were combined and washed free of acid with 5% sodium carbonate solution. The carbonate wash was acidified with 10% sulfuric acid and extracted with four 50-ml. portions of ether, which were combined and evaporated. The residue was dissolved in skellysolve B, from which a small crop of columbianetin crystallized. The supernatant liquor was evaporated to an oil which crystallized upon standing to give 1.0 Gm. of tiglic acid as large prisms. Sublimation at atmospheric pressure afforded long, fine needles, m.p. 64–65°. The melting point was not depressed on admixture with an authentic sample of tiglic acid.

Identification of D-Glucose.—Columbianin (200 mg.) was refluxed with 2 *N* sulfuric acid in 30% ethanol (15 ml.) for 2.5 hours. The cooled mixture

[4] The assignment of groups responsible for some of the absorption bonds in the infrared spectra of the compounds discussed in this paper are in agreement with the findings of Halpern, Waser, and Schmid (4) for closely related structures.

[5] Microanalyses were determined by the Microanalytical Laboratory, School of Chemistry, University of Minnesota, Minneapolis; Drs. Weiler and Strauss, 164 Banbury Road, Oxford, England; or W. L. Johnson and associates, The Upjohn Co., Kalamazoo, Mich.

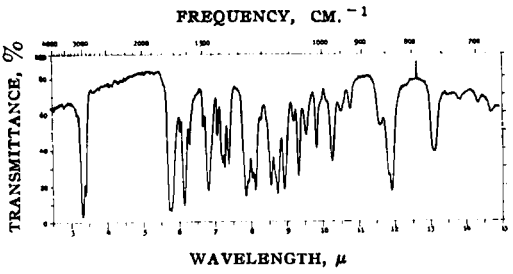


Fig. 2.—Infrared absorption spectrum of columbianadin (Nujol mull).

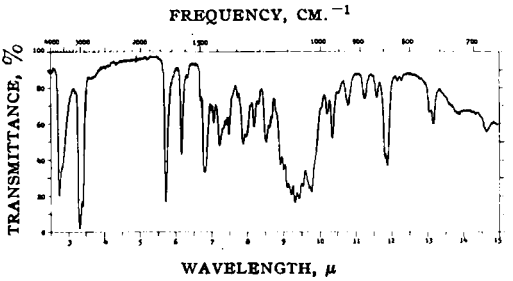


Fig. 3.—Infrared absorption spectrum of columbianin (Nujol mull).

was extracted with four 25-ml. portions of chloroform. The aqueous layer was neutralized with a saturated solution of barium hydroxide, filtered, and concentrated under reduced pressure to an amber syrup (250 mg.). The paper chromatographic behavior[6] was identical with that of authentic D-glucose and different from that of D-mannose or D-fructose.

The phenylosazone of a portion of the syrup (125 mg.) was prepared in the usual manner (6). The crystals, which formed in 7 minutes, were washed with acetone and recrystallized from aqueous methanol to give 80 mg. of phenyl-D-glucosazone as fine, reddish-brown needles, m.p. 205°; $[\alpha]_D^{27}$ − 6.6° (*c* 0.24, 1:1 pyr.-H_2O) [lit. m.p., 210°, $[\alpha]_D^{20}$ − 1.5° (7)]. The melting point was not depressed on admixture with a sample of glucosazone prepared from D-glucose.

Columbianetin Acetate.—Columbianetin (0.2 Gm.), acetic anhydride (5 ml.), and pyridine (5 ml.) were refluxed overnight; dilution with water precipitated fine needles (0.1 Gm.). These were recrystallized twice from dilute ethanol and dried at 100° in vacuum to give fine, colorless needles, m.p. 127.5 to 128.5° (Kofler block); ultraviolet spectrum: $\lambda_{min.}$ 268 mμ (log ε 3.07); $\lambda_{max.}$ 218 (4.11) and 327 mμ (4.12); infrared spectrum; 1727 (α-pyrone and ester C=O) and 1245 cm.$^{-1}$ (acetate C—O).

Anal.—Calcd. for $C_{16}H_{16}O_5$: C, 66.65; H, 5.59. Found: C, 66.64; H, 5.68.

Alkaline Permanganate Oxidation of Columbianetin.—A saturated solution of potassium permanganate (*ca.* 75 ml.) was added to a cold solution of columbianetin (0.5 Gm.) in 1% sodium hydroxide. The mixture was acidified with 10% sulfuric acid and steam distilled. *p*-Nitrophenylhydrazine (0.5 Gm.) in ethanol (20 ml.) was added to the distillate (200 ml.). The long, reddish-brown needles which separated overnight were filtered off and recrystallized twice from dilute ethanol to give 75 mg. (27% of theory, based on 0.5 Gm. of columbianetin) of acetone *p*-nitrophenylhydrazone, m.p. 147–149° [lit. m.p., 149–150° (8)]. The melting point was not depressed on admixture with the *p*-nitrophenylhydrazone prepared from acetone.

Dehydration of Columbianetin.—Columbianetin (0.5 Gm.) was dehydrated with glacial acetic acid (1.5 ml.) and concentrated hydrobromic acid (1.5 ml.) (9). Repeated crystallizations from hot water containing a small amount of ethanol gave 0.1 Gm. of dihydroöroselone (IV), m.p. 142.5 to 143.5° (Kofler block) [lit. m.p., 142° (4)]; ultraviolet spectrum: $\lambda_{min.}$ 231 (log ε 4.07) and 274 mμ (3.71); $\lambda_{max.}$ 224 (4.23) sh., 252 (4.45) and 302 mμ (4.06). The melting point was not depressed on admixture with an authentic sample of dihydroöroselone.[7] The infrared spectra of the two samples were identical.

Dihydrocolumbianetin (V).—Columbianetin (300 mg.) was dissolved in glacial acetic acid (10 ml.) and hydrogenated under 3 Atm. pressure in the presence of palladium black (100 mg.) for 8 hours. The filtered solution was evaporated under reduced pressure and the residue crystallized from 1:2 ether–skellysolve B. Repeated crystallizations from the same solvent gave 100 mg. of dihydrocolumbianetin, m.p. 111.5 to 112.5°; $[\alpha]_D^{24}$ +300° (*c* 0.33, CH_3OH) [lit. m.p. 112–113°, $[\alpha]_D^{19}$ +87° (*c* 0.65, CH_3OH)(4)]; ultraviolet spectrum: $\lambda_{min.}$ 260 (log ε 2.90) and 283 mμ (3.30); $\lambda_{max.}$ 281 (3.29) and 288 mμ (3.32); infrared spectrum: 1761 cm.$^{-1}$ (γ,δ-unsaturated δ-lactone).

Anal.—Calcd. for $C_{14}H_{16}O_4$: C, 67.73; H, 6.50. Found: C, 67.36; H, 6.53.

Columbianetin Tiglate.—Columbianetin (1.23 Gm., 0.005 mole) was powdered and suspended in dry benzene (20 ml.). The suspension was added slowly to a mixture of tigloyl chloride (0.60 Gm., 0.005 mole) in dry benzene (10 ml.). The mixture, protected by a calcium chloride drying tube was refluxed on a steam bath for 20 hours, during which the solid columbianetin dissolved completely. The course of the esterification was followed with chromatostrips which were developed with a 30% solution of ethyl acetate in skellysolve B after spotting with the reaction mixture directly. Inspection of the strip under ultraviolet light was used to determine when the slower moving columbianetin spot had disappeared completely in favor of the faster moving tiglate spot. The reaction mixture was then cooled and washed three times in a separator with 10-ml. portions of 5% aqueous sodium bicarbonate solution, followed by two 10-ml. portions of distilled water. The benzene solution was dried overnight over anhydrous sodium sulfate, filtered, and the solvent removed under reduced

[6] The procedure and solvent system used were essentially those of Jermyn and Isherwood (5). The method utilized a descending technique on Whatman No. 1 paper and detection of carbohydrate spots with potassium periodate solution followed by benzidine reagent. The paper was equilibrated with the lower layer and developed with the upper layer of a mixture of ethyl acetate-water-pyridine (2:2:1 by volume) prepared by shaking well and allowing to stand at room temperature overnight.

[7] Generously supplied by Dr. Hans Schmid, Chemisches Institut der Universität Zürich, Zürich, Switzerland.

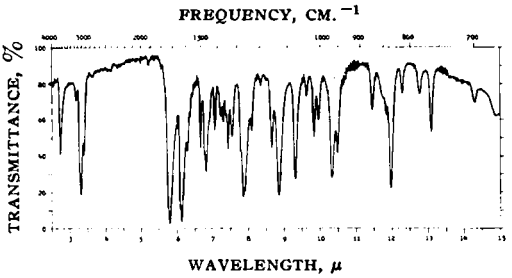


Fig. 4.—Infrared absorption spectrum of columbianetin (Nujol mull).

pressure to yield an oily residue that hardened and crystallized when rubbed with a little methanol. The crude product was recrystallized twice from methanol to yield 1 Gm. of fine, white needles, m.p. 107–108°. A mixed melting point with authentic columbianadin showed marked depression of the melting point and the infrared and nuclear magnetic resonance (NMR) spectra indicated the nonidentity of the two products. In the NMR spectrum, the vinyl proton of tiglate was denoted by a multiplet centering at 3.43 τ.

Anal.—Calcd. for $C_{19}H_{20}O_5$: C, 69.49; H, 6.13. Found: C, 69.43; H, 6.21.

Columbianetin 3-Bromoangelate.—This ester was prepared in exactly the same manner as columbianetin tiglate utilizing equimolar amounts of 3-bromoangeloyl chloride (16) and columbianetin. A reflux period of 30 hours, however, was required for completion of the reaction as determined by the chromatostrip method. The crude product was recrystallized twice from ethanol to yield white crystals, m.p. 135 to 137.5°.

Anal.—Calcd. for $C_{19}H_{19}BrO_5$: C, 56.06; H, 4.70. Found: C, 55.87; H, 4.86.

Columbianetin Angelate.—This ester was prepared by the method of Kupchan and Afonso (16) by atmospheric pressure hydrogenation of an ethanolic solution of columbianetin 3-bromoangelate in the presence of anhydrous sodium acetate and 10% palladium-on-carbon. After removal of catalyst and evaporation of solvent, the product was recrystallized twice from methanol and water to give long white needles. These were dried overnight at 100° in vacuum and gave a melting point of 115–118°. A mixed melting point with authentic columbianadin (m.p. 121–122°) was 117–119°. Infrared and NMR spectra of columbianetin angelate and columbianadin were identical. In the NMR spectrum of the angelate, the vinyl proton was denoted by a multiplet centering at 4.02 τ.

Anal.—Calcd. for $C_{19}H_{20}O_5$: C, 69.49; H, 6.13. Found: C, 69.36; H, 6.28.

DISCUSSION

The previously isolated (1) columbianadin (I) was obtained from petroleum ether extracts of the dried root of *L. columbianum* in 0.8 to 0.9% yields. Alcoholic extracts of the root afforded a crystalline substance in 2 to 4% yields, which gave a positive carbohydrate test (Molisch) and to which we assigned the name, columbianin (II).

Hydrolysis of columbianadin (I) with alkali and of columbianin (II) with acid in dilute ethanol yielded tiglic acid and D-glucose, respectively, and an alcohol, which we named columbianetin (III).

Dehydration of columbianetin (III) gave the known coumarin dihydroöroselone (IV), which conceivably could have arisen from the tertiary alcohol III or the secondary alcohol VI by rearrangement.[8] Hydrogenation of columbianetin (III) afforded the dihydroalcohol V, which is identical to a hydrogenation product of athamantin (4). Since a rearrangement is not possible during the hydrogenation, the location of the hydroxyl group in columbianetin is established.

Assignment of structure III to columbianetin was supported further by infrared and NMR spectra. In its infrared spectrum, columbianetin gives rise to peaks at 1410 and 1149 cm.$^{-1}$ attributable to tertiary alcohol O—H deformation and C—O stretching; whereas the spectrum of the secondary alcohol VI[9] shows analogous peaks at 1295 and 1090 cm.$^{-1}$ which are characteristic of a secondary alcohol (11).

The hydrogen attached to a secondary carbon carrying a hydroxyl group usually exhibits a peak in its NMR spectrum at a frequency about 60 c.p.s. higher than when the hydroxyl is acetylated (12). This is due to elimination of interaction between the two hydrogens of the HO—CH group. Conversion of the tertiary alcohol III to its acetate gives a shift of 23 c.p.s. in the absorption attributable to the hydrogen of the group: O—C—H. This agrees with the O—C—H group present in the furano ring of structure III. The NMR spectra of the secondary alcohol VI and its acetate[9] show a shift of 69 c.p.s. for this hydrogen, which agrees with their established structures. Also, the location of the peak for the proton of the hydroxyl group was at a higher frequency in the NMR spectrum of alcohol VI than alcohol III, which possibly is indicative of greater hydrogen bonding as is sometimes noted in spectra of acyclic secondary alcohols (13).[10]

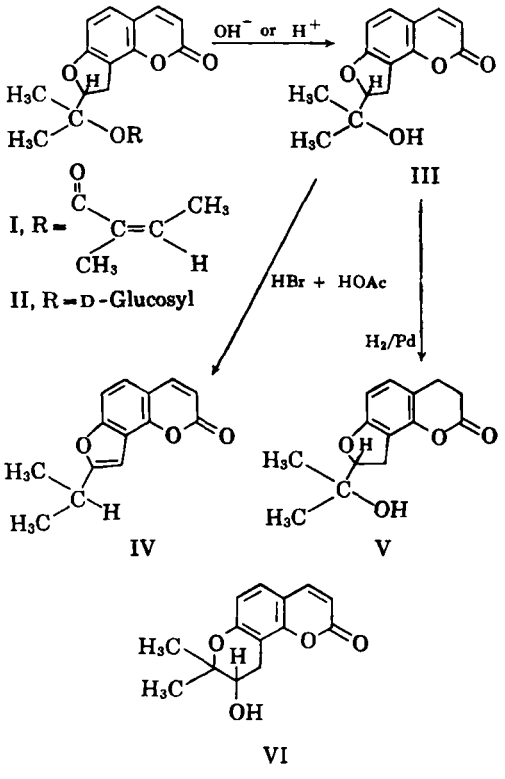
As previously indicated, tiglic acid was obtained by hydrolysis of columbianadin (I). Since angelic acid readily isomerizes to tiglic acid (14), it was necessary to determine whether columbianadin was an angelate. Kupchan and Afonso (15) have demonstrated that no validity can be placed on lack of isomerization of free angelic acid to tiglic acid when treated under the same reaction conditions used to hydrolyze the ester. They indicated that isomerization takes place either before or during the actual cleavage of the ester.

The tiglate of columbianetin was prepared by treatment with tigloyl chloride and the angelate from 3-bromoangeloyl chloride followed by hydrogenation (16). Columbianetin angelate showed no depression of melting point on admixture with columbianadin, although it should be pointed out that the melting point of the synthetic angelate was a few degrees lower than that of the natural product. Their infrared and ultraviolet spectra and chromatographic behavior were identical. The tiglate gave

[8] Bencze, *et al.* (10), minimized this possibility, however, in an analogous acid dehydration of visamminol.

[9] (±)-3′-Hydroxy-3′,4′-dihydroseselin (VI) and its acetate were obtained from pteryxin (3).

[10] The authors thank Dr. James N. Schoolery, Varian Associates, Palo Alto, Calif., Mr. George M. Slomp, The Upjohn Co., Kalamazoo, Mich., and Dr. Michael J. Martell, Jr., Lederle Laboratories, for assistance in determining and interpreting the NMR spectra.



dissimilar spectra and a melting point depression. Comparison of NMR spectra also confirmed the identity of the angelate and the nonidentity of the tiglate with columbianadin. It is of interest to note that the vinyl proton in the angelate (as well as in columbianadin) is at 4.02 τ (center of multiplet) and that of the tiglate is a multiplet centering at 3.43 τ. This agrees well with Fraser (17), who cites 4.03 τ for the vinyl proton of angelate and 3.28 τ for tiglate. Columbianadin is, thus, firmly established as the angelate ester of columbianetin.

The structure of columbiauin (*II*) as the D-glucoside of columbianetin follows from its identified hydrolytic products.

REFERENCES

(1) Call, T. G., and Green, J., *Proc. Montana Acad. Sci.*, **16**, 49(1956).
(2) Mathias, M. E., and Constance, L., *Bull. Torrey Botan. Club*, **69**, 246(1943).
(3) Willette, R. E., and Soine, T. O., THIS JOURNAL, **51**, 149(1962).
(4) Halpern, O., Waser, P., and Schmid, H., *Helv. Chim. Acta*, **40**, 758(1957).
(5) Jermyn, M. A., and Isherwood, F. A., *J. Biochem.*, **44**, 402(1949).
(6) Cheronis, N. D., and Entrikin, J. B., "Semimicro Qualitative Organic Analysis," 2nd ed., Interscience Publishers, Inc., New York, N. Y., 1957, p. 432.
(7) *Ibid.*, p. 615.
(8) *Ibid.*, p. 663.
(9) Smith, E., Hosansky, N., Bywater, W. G., and Van Tamelen, E. E., *J. Am. Chem. Soc.*, **79**, 3540(1957).
(10) Bencze, W., Eisenbeiss, J., and Schmid, H., *Helv. Chim. Acta*, **39**, 923(1956).
(11) Bellamy, L. J., "The Infra-red Spectra of Complex Molecules," 2nd ed., John Wiley and Sons, Inc., New York, N. Y., 1958, p. 108.
(12) Jackman, L. M., "Application of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry," Pergamon Press, New York, N. Y., 1959, p. 55.
(13) Chamberlain, N. F., *Anal. Chem.*, **31**, 56(1959).
(14) Buckles, R. E., Mock, G. V., and Locatell, L., *Chem. Rev.*, **55**, 659(1955).
(15) Kupchan, S. M., and Afonso, A., THIS JOURNAL, **48**, 731(1959).
(16) Kupchan, S. M., and Afonso, A., *J. Org. Chem.*, **25**, 2217(1960).
(17) Fraser, R. R., *Can. J. Chem.*, **38**, 549(1960).