Coumarins III. Identification of the Lactone of *Leptotaenia multifida*

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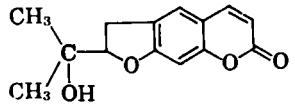
A lactone, m.p. 161.5°, originally isolated from *Leptotaenia multifida* Nuttall by Lloyd and Jenkins in 1942 and assigned the formula $C_{14}H_{14}O_4$ has been identified as the coumarinic aglycone, columbianetin.

THE ISOLATION of a white crystalline compound, m.p. 161.5°, from the roots of *Leptotaenia multifida* Nuttall was reported in 1942 by Lloyd and Jenkins (1). No structural assignment was made by these authors, although they suggested that it was a hydroxylactone with an empirical formula of $C_{14}H_{14}O_4$. These authors further suggested that it was possibly a coumarin—isomeric with, but not identical to, nodakenetin (I) the aglycone from the glycoside, nodakenin, which is obtained from *Peucedanum decursivum* (Miq.) (2). Inasmuch as the present authors were able to obtain a modest supply of the roots of this plant, the decision was made to examine the structure of the uncharacterized lactone.

In the present study, the lactone reported by Lloyd and Jenkins was isolated only after acid hydrolysis of the aqueous extract, contrasted to the experience of the earlier authors, who obtained the same compound directly from the plant material. The conclusion must be made that the lactone exists in the plant as a glycoside and not as the free aglycone; it appears that their isolation had been of an artefact resulting from hydrolysis during some stage of collection, storage, or extraction. The aglycone has been identified as columbianetin (II), recently described by Willette and Soine (3), by a variety of comparisons with an authentic sample. The D-glucoside of columbianetin, columbianin, also described by Willette and Soine (3), has been shown to be the glycoside present in the roots in the present study by comparing it and its acetate with authentic samples.

The acetate of the aglycone exhibited dimorphism, evidenced by the fact that the lower melting acetate, reported by the earlier authors and Willette and Soine, was converted to the higher melting one simply by melting a sample of the former. The plant also contained a fair amount of sucrose in conformity to the findings of Lloyd and Jenkins. However, the sucrose present forms an interesting molecular complex with columbianin in the proportion of 7.5 parts sucrose to 1 part columbianin. This was not separable by crystallization but, on acetylation, both columbianin acetate and sucrose octaacetate were obtained. It appears that during the slow concentration of an extract containing both components, excess sucrose crystallizes out without columbianin but that, as a critical concentration of both sucrose and columbianin is reached, the molecular complex crystallizes preferentially until the sucrose concentration is reduced sufficiently to permit the columbianin to crystallize alone. The character of the molecular complex was confirmed by comparing it to a mixture of sucrose and columbianin in the requisite proportions which had been crystallized from methanol. The synthetic complex behaved in a manner identical to the one obtained from the natural source.

It is interesting that columbianetin, the angular isomer of nodakenetin and marmesin, had escaped detection until the studies of Willette and Soine (3) on *Lomatium columbianum* Math. and Const. Now, however, it has been isolated in the present study as well as from *Lomatium nuttallii* (A. Gray) (4), and it appears that it may be a common constituent in the *Lomatium* genus. We are examining other *Lomatium* species to confirm this.



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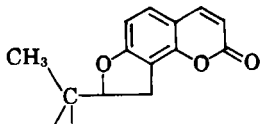

II

EXPERIMENTAL

Plant Material.—The plant material was obtained as tuberous roots,[1] which were ground to a suitable state of subdivision in a Jacobsen laboratory grinder designed to keep frictional heat to a minimum.

Extraction.—The ground plant material (600 Gm.) was exhaustively extracted (10 days) in a Soxhlet apparatus with 95% ethanol, after which the solvent was removed from the extract under reduced pressure. The dark oily residue was then extracted several times with boiling water (4 L.). The aqueous extract was filtered and allowed to concentrate slowly on a steam bath. No crystalline material was obtained, in contrast to the experience of Lloyd and Jenkins (1).

One-fourth of the above aqueous extract was extracted thoroughly with ether, and the combined ethereal extracts were then concentrated to a convenient volume. The ethereal solution was then extracted with 5% aqueous sodium hydroxide solution, which was subsequently acidified and exhaustively re-extracted with ether. Although a small amount of fluorescent material apparently was present in the ethereal extract, it could not be induced to crystallize by slow evaporation and chilling of the solution. The remaining portion of the concentrated extract was combined with the aqueous phase from the above ether extraction and

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[1] The collection of these roots was made by Dr. T. G. Call, California State Polytechnic College, San Luis Obispo, Calif., on June 19, 1956, in the neighborhood of Missoula, Mont., at an elevation of 3400 ft. We are indebted to Dr. Call for the original identification and to Dr. G. B. Ownbey, Botany Department, University of Minnesota, Minneapolis, for confirmation of the identity. It may be noted that the modern nomenclature for this plant is *Lomatium dissectum* var. *multifidum* (Nutt.) Math. and Const. A specimen has been deposited in the herbarium of the Botany Department, University of Minnesota, Minneapolis.

diluted with water. To this solution was added enough concentrated hydrochloric acid to make it approximately 10% in HCl; it was then refluxed for 30 minutes and filtered immediately before cooling. The filtrate, on cooling, deposited fine brownish needles which were recrystallized several times from boiling water to yield 1.6 Gm. of white needles, m.p. 161–163°. A mixed melting point with authentic columbianetin[2] failed to depress the melting point.

The acetate of the compound was made by refluxing it (200 mg.) with pyridine (5 ml.) and acetic anhydride (5 ml.) for 6 hours, after which the solution was poured into ice cold water (50 ml.). The dark crystalline product was recrystallized several times from boiling water to yield 90 mg. of white needles, m.p. 133.5–135°. A mixed melting point with authentic columbianetin acetate[3] was 128–131°. On allowing the melt to cool and again taking the melting point, the melting point was 133–135°.

The respective specific rotations, ultraviolet spectra, and infrared spectra of the lactone and columbianetin were determined and were virtually identical.

Isolation of Sucrose and Glycoside-Sucrose Complex.—A fresh quantity (500 Gm.) of the powdered root was extracted successively with Skellysolve B, ether, chloroform, and methanol, respectively, in a Soxhlet apparatus; the marc was air-dried between each extraction. The methanol extract[4] became turbid after 2 hours of extraction, at which time it was replaced by fresh solvent. After another 8 hours, the again turbid methanol extract was replaced by fresh methanol which extracted the marc in 24 hours. On cooling, a white crystalline compound, m.p. 165–190° dec., was deposited in the first extract and a brown inorganic material in the second and third extracts. The white crystalline material was identified as sucrose by conversion to the octaacetate, m.p. 84–88°, which when mixed with authentic sucrose octaacetate (m.p. 84–88°)[5] failed to depress the melting point. The filtrate from the first extract was diluted with methanol and allowed to concentrate at room temperature. After a few days, a yellowish-white amorphous substance separated which was filtered off and washed with cold methanol. The crude material, m.p. 186–225°, gave a positive Molisch test and reduced Fehling's solution after hydrolysis with 10% hydrochloric acid. Recrystallization of the crude material from methanol did not improve the melting point. Chromatography through silica gel and elution with water likewise failed to improve the melting point, although the color was removed. It was observed that an aqueous solution, when spotted on filter paper and examined in the dark under ultraviolet light, gave a brilliant blue fluorescence.

A portion (500 mg.) of the substance was boiled with 10% sulfuric acid for 20 minutes and filtered before cooling. On cooling, the filtrate deposited a crystalline material (70 mg.), which was recrystallized from boiling water to give white crystals, m.p. 160–163°. A mixed melting point with authentic columbianetin failed to depress the melting point.

The above glycosidic compound (1 Gm.) was refluxed with acetic anhydride (20 ml.) and sodium acetate (1 Gm.) for 3 hours. The reaction mixture was diluted with glacial acetic acid (10 ml.) and allowed to stand in the refrigerator overnight. A white crystalline residue (150 mg.) was deposited and was recrystallized from boiling water, m.p. 217–222°. A mixed melting point with authentic columbianin acetate[6] showed no depression of the melting point. The acidic mother liquor from the above white crystalline residue was concentrated under reduced pressure until all volatile material had been removed; then the residue was extracted with benzene. The benzene extract was dried over anhydrous sodium sulfate and, upon removal of the solvent, yielded a semisolid mass. This was dissolved in ethanol and diluted with water to a slight turbidity. On standing, a finely crystalline material (600 mg.) separated, m.p. 84–88°. A mixed melting point of this material with authentic sucrose octaacetate showed no depression.

A comparison of the ultraviolet spectrum of the original white glycosidic material (m.p. 186–225°) to that of authentic columbianin showed identical maxima and minima but with a greatly reduced intensity. The intensity of absorption was 15% that of pure columbianin.

A mixture of 15% columbianin and 85% sucrose showed an identical melting point and ultraviolet spectral behavior after crystallization from methanol.

Isolation of the Glycoside.—After removing inorganic material from the second and third methanolic extracts, they were mixed together and allowed to concentrate slowly at room temperature. After several days, an amorphous white material was separated which was purified by elution with water through a silica gel column to give a white crystalline material, m.p. 273–275.5°. A mixed melting point determination with authentic columbianin[7] showed no depression of the melting point.

A small quantity of the white crystalline material, on hydrolysis with boiling 10% sulfuric acid, gave a compound identical to columbianetin in all respects.

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(2) Arima, J., *Bull. Chem. Soc. Japan*, **4**, 113(1929).
(3) Willette, R. E., and Soine, T. O., THIS JOURNAL, **53**, 275(1964).
(4) Jawad, F., "Examination of *Lomatium Nuttallii* (A. Gray) for Coumarins with Possible Physiological Activity," Master of Science thesis, University of Minnesota, Minneapolis, 1964.

[2] Previously isolated and characterized in these laboratories (3).
[3] The melting point reported by Willette and Soine (3) for columbianetin acetate was 127.5–128.5°, and that reported by Lloyd and Jenkins for the acetate of their lactone was 126–127° (1). An authentic sample of the former was melted and allowed to resolidify. On remelting, this sample showed a melting point change to 133–135°.
[4] Work on the other extracts is in progress.
[5] Prepared by acetylation of authentic sucrose with acetic anhydride and sodium acetate in the conventional manner.
[6] Previously prepared in these laboratories (3), m.p. 219–221°.
[7] Previously isolated in these laboratories, m.p. 274–275°.