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THE ISOLATION OF 2′,4,4′-TRIHYDROXYDIHYDRO-CHALCONE FROM *VIBURNUM DAVIDI*

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Abstract—An examination of the phenolic constituents of hydrolyzed extracts of *Viburnum davidi* Franch. (Caprifoliaceae) disclosed the presence of large concentrations of 2′,4,4′-trihydroxydihydrochalcone. Hydrolytic and spectrophotometric studies showed that the naturally occurring compound is the 2′-monoglucoside. This represents the first observation of a naturally occurring dihydrochalcone having the resorcinol hydroxylation pattern in ring-A.

INTRODUCTION

DURING a systematic phytochemical examination of the Caprifoliaceae[1] a particularly interesting new phenolic compound was observed on chromatograms of *Viburnum davidi* Franch. The compound appeared to be present in sizable amounts and a quantity of the aglycone was isolated and its structure identified. The results of this study are reported below.

RESULTS AND DISCUSSION

Our initial experiments were directed toward isolation of the naturally occurring glycoside. Due to a number of overlapping phenolic compounds on chromatograms it was not possible to obtain the glycoside in a suitable state of purity. It was possible, however, to isolate the aglycone easily from acid or emulsin hydrolyzed extracts of the plant material, and later enough glycoside was isolated for complete identification.

The aglycone was obtained by pooling spots cut from chromatograms, eluting the compound with ethanol, removing the solvent, and recrystallizing the residue several times from water. After thorough drying, a pale yellow crystalline product (m.p. 158–160°) was obtained. The u.v. spectrum of the aglycone was virtually identical to that of resacetophenone (2′,4′-dihydroxyacetophenone). The spectral behavior of the compound in the presence of aluminum chloride was also characteristic of an *o*-hydroxyketone. Spectral data are presented in Table 1.

TABLE 1. U.V. SPECTRAL CHARACTERISTICS OF THE UNKNOWN AND RELATED COMPOUNDS

	EtOH		EtOH/NaOH	EtOH/$AlCl_3$	
Natural aglycone	278	314	336	306	356
Synthetic aglycone	278	314	337	306	356
Resacetophenone	277	313	336	305	352
Natural glycoside	278	314	n.d.	278	314

n.d. = not determined.

[1] C. W. GLENNIE and B. A. BOHM, unpublished results.

An i.r. spectrum showed the presence of a strongly hydrogen bonded carbonyl[2] and, significantly, the absence of ether and aliphatic unsaturated functions.

Mass spectral analysis gave a molecular weight of 258 which corresponds with $C_{15}H_{14}O_4$. Integration of the NMR spectrum showed a total of fourteen protons in agreement with the mass analysis. It became clear that we were dealing with a reduced flavonoid molecule. The possibility of a flavanone was eliminated by the absence of the characteristic benzyl proton at about 4·6 τ in the NMR spectrum.[3] A complex series of bands centered at 7·0 τ integrated as four protons and was interpreted as the α- and β-protons of a dihydrochalcone.

Further analysis of the NMR spectrum was as follows. Three phenolic protons could be accounted for at 1·90, 0·45, and −2·85 τ the later representing the strongly hydrogen bonded phenolic hydrogen. One of the two remaining phenolic functions can be assigned to the 4-position of ring-B. This assignment is based upon the nature of the aromatic protons of that ring. The doublet centered at 2·99 τ is assigned to the protons on carbons 2 and 6 while the doublet at 3·34 is assigned to the protons on carbons 3 and 5. Both of these groupings exhibit characteristic *o*-splitting. A single proton exhibiting *o*-splitting is centered at 2·39 τ and has been assigned to position 6′. Centered at 3·66 τ is a single proton exhibiting *m*-splitting while at 3·76 τ a final single proton is observed also exhibiting *m*-splitting. These

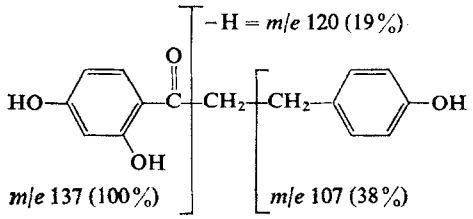

FIG. 1. FRAGMENTATION PATTERN OF 2′,4,4′-TRIHYDROXYDIHYDROCHALCONE (I)

protons are assigned to positions 5′ and 3′, respectively. These results suggest 2′,4,4′-trihydroxydihydrochalcone (I) as the most likely structure for the aglycone. Confirmation of this suggestion came from an alkali degradation of the compound. Treatment of the compound with hot, concentrated base resulted in the formation of resorcinol and 4-hydroxyphenylpropionic acid (phloretic acid). Under these conditions the dihydrochalcone phloridzin was cleaved into phloroglucinol glucoside and phloretic acid.

Further support for the dihydrochalcone structure can be seen in the mass fragmentation data. The mass spectrum was a very simple one displaying only five major peaks including the parent peak. The fragmentation is summarized in Fig. 1. The other two major peaks were: *m/e* 258 (30 per cent) which was the parent peak, and *m/e* 18 (22 per cent) which represented water.

The nature of the glycoside was determined in the following manner. Unhydrolyzed extracts were repeatedly chromatographed in three systems to provide a sample which yielded only one band and which yielded only the single phenolic product (I) upon hydrolysis. Samples of the chromatographically homogeneous glycoside were hydrolyzed, separately, with dilute acid and emulsin. After removal of the aglycone by ether extraction the hydrolysis mixtures were concentrated and analyzed for sugars by thin-layer chromatography in two

[2] H. WAGNER, *Methods in Polyphenol Chemistry*, pp. 40–41, Pergamon Press, Oxford (1964).
[3] T. J. MABRY, J. KAGAN and H. RÖSLER, *Nuclear Magnetic Resonance Analysis of Flavonoids*, The University of Texas Publication, Austin (1964).

solvent systems. The only sugar detectable was glucose. An experiment with β-glucosidase also shows glucose as the sole sugar. Estimation of the ratio of aglycone to glucose by the method of Nelson[4] gave a value of 1·36 aglycone to 1·00 glucose. Repetition of the analysis consistently showed somewhat less glucose than aglycone. We infer from these results that the naturally occurring compound is a monoglucoside. The point of attachment of the glucose was determined by examination of the u.v. spectral characteristics of the compound. In the presence of aluminum chloride the aglycone exhibited the typical hypsochromic shift of an *o*-hydroxyacetophenone (see Table 1) whereas the glucoside showed no change. The absence of a shift indicates that the glucose is attached to the 2′-position and leads to the conclusion that the naturally occurring compound can be fully described as 2′-β-D-glucosyloxy-4,4′-dihydroxydihydrochalcone. This represents the first report of the natural occurrence of a dihydrochalcone having the resorcinol hydroxylation pattern in the A-ring.

Attempts to synthesize the glucoside have failed. However, the aglycone was prepared in a straightforward manner by condensing resacetophenone with *p*-hydroxybenzaldehyde in the presence of strong base to yield 2′,4,4′-trihydroxychalcone. Reduction to the dihydrochalcone was accomplished by hydrogenation at atmospheric pressure in the presence of palladium on charcoal. The product thus obtained was identical in all respects to the aglycone isolated from *Viburnum davidi.*

A limited preliminary investigation of the biosynthesis of the dihydrochalcone was undertaken as part of this work. Of particular interest to us was the origin of the B-ring. Was this part of the molecule constructed from a C_6–C_3 unit which already contained the hydroxyl function or did the hydroxyl function arise through hydroxylation of an intact C_6–C_3–C_6 intermediate ? Three C-14 labeled precursors were administered to young leaves of *V. davidi*: phenylalanine, cinnamic acid, and *p*-coumaric acid. In the case of each of these compounds the incorporation into the aglycone was about the same, 2·5 per cent. If hydroxylation of a C_6–C_3–C_6 intermediate occurred in the formation of the aglycone then *p*-coumaric acid would presumably be a much poorer precursor than the non-hydroxylated precursors. This is suggestive evidence for the involvement of an intermediate at the C_6–C_3–C_6 level which already has an hydroxyl function at position-4.

EXPERIMENTAL

Source of Plant Material

Living material of *Viburnum davidi* Franch. (Caprifoliaceae) was obtained from plants growing on the University grounds. A voucher specimen has been prepared and living material will be maintained in our collection.

Source of Chemicals

Emulsin and β-glucosidase were purchased from Nutritional Biochemicals Corp. and were used without further purification. Resacetophenone was obtained from Aldrich Chemical Co., and was recrystallized before use. Phenylalanine-U-^{14}C was purchased from New England Nuclear Corp. Cinnamic acid-2-^{14}C and *p*-coumaric acid-2-^{14}C were synthesized by condensation of the appropriate benzaldehyde and malonic acid-2-^{14}C in the presence of 20:1 pyridine:piperidine mixture.

Instrumentation

Melting points were determined on a Kofler Hotstage and are not corrected. U.v. spectra were determined on a Unicam SP-800 and the i.r. spectrum on a Unicam SP-200G. A Varian A-100 spectrometer was used for the NMR spectrum. Mass analysis was performed by Morgan-Schafer Corp., Montreal.

[4] N. NELSON, *J. Biol. Chem.* **153**, 375 (1944).

Extraction and Isolation Procedures

Fresh plant material was extracted repeatedly with boiling 80% ethanol. The alcoholic extracts were pooled and evaporated to a small volume and filtered through Celite filter aid. The filtrate was hydrolyzed with either 2 N HCl at 100° for 1–2 hr or emulsin (*ca.* 25 mg per 100 ml of extract) at room temperature for 1 day. After hydrolysis, the aglycones were extracted with ether and separated as spots on Whatman No. 3MM chromatography paper developed two-dimensionally with benzene:acetic acid:water (10:7:3, org. phase) and 2% formic acid for the second. The unknown compound was located by u.v. light and eluted with 95% ethanol. Evaporation of the ethanol left a residue which crystallized slowly from water. Several recrystallizations from water and chromatography on Avicel thin-layer plates using 20% formic acid effectively removed trace impurities. Final recrystallization and drying over P_2O_5 in vacuum gave pale yellow crystals having m.p. 158–160°.

The glycoside was obtained by repeated chromatography of unhydrolyzed extract on Whatman No. 3MM paper using the following solvents: 2% formic acid; *t*-butanol:acetic acid:water (3:1:1); and, ethyl acetate: formic acid:water (70:15:10). The bands corresponding to the desired compound were cut from the chromatograms and eluted with 80% ethanol.

Determination of the Sugar Residue

Small portions of the glycoside were treated with either acid or emulsin as described in the isolation procedure or with a β-glucosidase preparation for 4 hr at room temperature (no buffer used in the enzyme hydrolyses). The reaction mixtures were extracted with ether. The resulting aqueous solution was concentrated and chromatographed on Avicel plates (a brand of microcrystalline cellulose) using a number of common sugars as standards. The solvents used were: *n*-butanol:acetic acid:water (4:1:2·2), and *n*-butanol:benzene: formic acid:water (100:19:10:25). The positions of the sugars on the plates were determined by spraying with a periodate–benzidine reagent.

Determination of the Glucose–Aglycone Ratio

After hydrolysis, with either emulsin or acid, the purified glycoside mixture was extracted exhaustively with ether. The ether extract was evaporated to dryness, the residue was taken up with 95% ethanol, made to a standard volume, and aliquots therefrom analyzed spectrophotometrically. The aqueous solution, free of phenolic compound, was concentrated using a rotary evaporator and the residual material made to a standard volume using distilled water. The concentration of glucose present was determined using the procedure of Nelson.[4]

Base Cleavage of the Aglycone

25 mg of the aglycone and 2–3 ml of 50% NaOH were heated at 100° for 2 hr. The mixture was acidified (6 N HCl) and extracted with ether. Chromatography of the extract on Avicel plates using the benzene: acetic acid:water and formic acid systems showed the presence of three spots: uncleaved starting material, resorcinol, and phloretic acid (R_f values, colour reactions and spectra).

Hydrogenation of 2′,4,4′-Trihydroxychalcone

2′,4,4′-Trihydroxychalcone[5] was hydrogenated at atmospheric pressure in 95% ethanol with 10% Pd/C (*ca.* 100 mg per 0.01 mole). The calculated amount of H_2 was absorbed in about 20 min giving a quantitative yield of the dihydrochalcone which, after recrystallization, had m.p. 157–159 (mixed with natural compound 157–159°).

C-14 *Precursor Studies*

2 μc of the labelled precursors (phenylalanine-U.-^{14}C, cinnamic acid-2-^{14}C, and *p*-coumaric acid-2-^{14}C) dissolved, separately, in about 3 ml of water (the acids as their sodium salts) were administered to cuttings of *V. davidi* plants which had three pairs of leaves. The plants were allowed to metabolize for 24 hr under cool-white fluorescent lamps. When the labeled precursor solutions had been absorbed the plants were transferred to distilled water for the remainder of the metabolic period. The plant material was extracted and the aglycone isolated by the procedures outlined above. Radioactivity of the aglycone was determined by liquid scintillation counting using a toluene–ethanol mixture containing PPO and POPOP.

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[5] J. Tambor *Berichte* **49**, 1708 (1916).