

Occurrence of Umbelliferone in the Seeds of *Dipteryx odorata* (Aubl.) Willd.

Umbelliferone (7-hydroxycoumarin) was found to be present in the methylene chloride extract of the seeds of *Dipteryx odorata* (tonka bean).

In the course of examining Mexican vanilla extracts for coumarin contamination (Sullivan, 1981a-c), it became apparent that in approximately 90% of those extracts which contained coumarin there was also an accompanying bluish white fluorescent compound. The presence of this compound in Mexican vanilla extracts could possibly occur in several ways. It could be a compound which the manufacturer added in order to enhance the aromaticity or possibly as a preservative, or it could be a naturally occurring compound with its origin in either the vanilla or tonka bean. Preliminary TLC examination of the methylene chloride extracts of these two beans revealed that the compound was present in the tonka bean.

The literature survey indicated that the following compounds have been found to occur in various plant parts of *Dipteryx odorata*. Coumarin was first isolated from the tonka bean by Vogel in 1820. Hilditch and Stainsby (1934) found palmitic, stearic, oleic, and linoleic acids to be present in the lipid fraction of the seed. Examination of the leaf revealed the presence of coumarin, *o*-coumaric acid, melilotic acid, salicylic acid, ferulic acid, and *p*-hydroxybenzoic acid (Griffiths, 1962). From the neutral fraction of the bark Nakano and Suarez (1969) isolated lupeol, betulin, and a mixture of methyl palmitate, methyl margarate, methyl stearate, methyl oleate, and methyl linolenate. Hayashi and Thomson (1974) isolated five isoflavones from the heartwood of *D. odorata* which included retusin, retusin 8-methyl ether, 3'-hydroxyretusin 8-methyl ether, odoratin, and dipteryxin.

Prior to 1954 it was a common practice of food manufacturers in the United States to enhance the flavor of imitation vanilla extract, chocolate products, perfumes, and tobacco products with coumarin or tonka bean extract. According to the *Fed. Regist.* (1954), any food containing added coumarin as such or as a constituent of tonka beans or tonka extract is deemed to be adulterated. Pharmacological studies had demonstrated that coumarin was a toxic substance causing damage to various organs of test animals.

EXPERIMENTAL SECTION

Plant Material. The seeds of *Dipteryx odorata* (Aubl.) Willd. were obtained from the crude drug collection of the College of Pharmacy, The University of Texas at Austin.

Extraction and Isolation. The tonka beans (63 g) were ground to a 20-mesh powder in a laboratory Wiley mill, placed in a Soxhlet thimble, and continuously extracted with methylene chloride in a Soxhlet apparatus for 48 h. The solvent was removed by a rotary evaporator under vacuum. The resulting yellowish brown, oily residue was washed with four 100-mL portions of methanol, the washes were combined, and the solvent was evaporated. This residue was solubilized in hot hexane and allowed to cool to room temperature. The resulting white precipitate was removed from the solvent by gravity filtration, solubilized in 5 mL of chloroform, and placed on a small amount of silicic acid, and the solvent was evaporated by steam heat. The silicic acid was placed on a column (3 × 25 cm) of silicic acid (Bio-Sil A, 100-200 mesh) which had previously

been conditioned with hexane-ethyl acetate (4:1). The column was eluted with the hexane-ethyl acetate mixture, and 25-mL fractions were collected.

Thin-Layer Chromatography. The procedure employed was essentially that of Kahan and Fitelson (1964). Precoated, 20 × 20 cm, 0.25-mm silica gel G plates without gypsum, with fluorescent indicator UV₂₅₄ (Polygram Sil G/UV₂₅₄), were spotted and developed for a distance of 10 cm in a solvent system composed of benzene-methanol (97:3) (caution: these operation should be carried out under a hood), dried, sprayed with a solution of 5% KOH in methanol, allowed to dry, and examined under reflected UV light. With two-dimensional TLC, the second developing solvent was composed of hexane-ethyl acetate (4:1).

Examination of Fractions. A total of 35 fractions were collected. Examination of these fractions by TLC indicated that fractions 6-9 contained a single component (compound A) and were combined. Compound A exhibited the same *R_f* value of reference coumarin (Matheson Co.), residing an *R_f* of 45. The solvent was evaporated and a total of 725 mg of compound A was obtained. Fractions 15-29 were also combined since the TLC examination indicated that they were identical in composition and contained the bluish white fluorescent compound (compound B) which exhibited an *R_f* of 10. The solvent was evaporated and the residue solubilized in a minimal amount of chloroform. Preparative TLC of this solution, employing the same procedures previously discussed, resulted in a total yield of 6.3 mg of compound B.

Thermal Gradient Sublimation. Twenty-five milligrams of compound A was placed in a thermal gradient sublimator (Scientific Instrument Accessories, Austin, TX) and sublimed under reduced pressure at 50 °C. The total amount of compound B was sublimed in the same manner but at 100 °C.

Identification of Isolated Compounds. A mixture of the sublimed compound A and reference coumarin was subjected to two-dimensional TLC by employing the procedure previously indicated. The two compounds were determined to be inseparable under these conditions. The melting point of the isolated compound was determined to be 68-70 °C, which was in exact agreement with the reported melting point coumarin ("The Merck Index", 1976). The melting point of an admixture with reference coumarin was not depressed. The UV absorptions of the isolated compound and reference coumarin were found to be identical. The λ_{max} values in methanol for both compounds were determined to be 272 and 306 nm. Mass spectral data gave an *m/e* 146 for both compounds.

A mixture of the sublimed compound B and reference umbelliferone (Sigma Chemical Co.) was determined to be inseparable by two-dimensional TLC. The melting point of compound B was found to be 225 °C, which was in exact agreement with the reported melting point for umbelliferone ("The Merck Index", 1976). The melting point of an admixture with reference umbelliferone was not depressed. The UV absorptions of both compounds were found to be identical. The λ_{max} values in methanol for both were 324, 254, and 216 nm. Mass spectral data gave an

