the use of a developing solvent other than the one used to generate the data of this report.

In some preliminary studies the glycoalkaloids were extracted with 5% trichloroacetic acid in 75% methanol (Smittle, 1971). It was discovered that under these conditions a spot corresponding to the aglycon solanidine was present on the developed TLC plates. Since the amount of this acid hydrolysis product varied from one extraction to another, the milder extraction method of Shih and Kûc (1974) was utilized. It is worth noting that although the extraction medium containing trichloroacetic acid cannot be used if individual glycoalkaloids are to be quantitated, the total glycoalkaloid assay results were the same for identical samples extracted with this method and with the gentler method of Shih and Kûc (1974). This was true for the total glycoalkaloid assays using the paraformaldehyde reagent and the total glycoalkaloid assay involving the sum of the individual glycoalkaloid spots obtained by TLC.

The concentration of glycoalkaloids in potato tubers is typically much greater near the peel than it is in the center of the tuber. Therefore, the level of total or individual glycoalkaloids obtained in an assay depends markedly on the sampling techniques used. Thus, Deahl et al. (1973) reported 7.8 mg of glycoalkaloid/100 g of whole tuber of potato cultivar B6039-WV6. In the present study we found 41.3 mg of total glycoalkaloid/100 g of thick peel of this same cultivar, and we undoubtedly would have found even higher levels if thin peels had been used.

The techniques described in this paper offer an accurate and sensitive method that is preferential to other methods for routine analysis of individual glycoalkaloids in a large number of samples.

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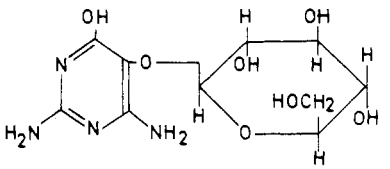
Variation in Vicine Concentration during Pod Development in Broad Bean (*Vicia faba* L.)

Changes in the concentration of vicine in broad bean seeds at various stages of pod development were determined. It was found that the very young seeds from pods 1–2 cm long contained about 1140 mg of vicine/100 g of dry tissue. This value was raised to about 2460 mg when pods were 5–6 cm long and dropped to about 900 mg at pod maturity. A similar pattern of variation in vicine concentration was noted for whole seeds, cotyledons, and seed coats. Statistical analyses revealed highly significant correlations between the vicine content of cotyledons, seed coats, and whole seeds.

Vicine [2,4-diamino-5,6-dihydroxypyrimidine-5-(β-D-glucopyranoside)], a pyrimidine glycoside, was first isolated from vetch (*Vicia sativa*) seeds (Ritthausen and Kreusler, 1870) and later found to occur in broad beans (*Vicia faba*) (Winterstein and Somlõ, 1933). Its structure, as shown here, was established two decades later by Bendich and



Clements (1953). The pyrimidine moiety of this compound, presumably resulting from enzymatic breakdown of vicine in the digestive tract after ingestion, was later implicated as one of the factors responsible for the hemolytic disease called favism (Mager et al., 1965). A comprehensive review of favism has been reported by Mager et al. (1969).

Jamalian et al. (1977a) developed a routine method for the estimation of vicine in leguminous seeds. The concentration of vicine in seed coats and cotyledons of mature broad bean seeds of a large number of Iranian and foreign cultivars was determined by this method (Jamalian, 1978).

Brown and Roberts (1972) studied the formation of vicine and its analogue, convicine, in the developing seeds of *Vicia faba* L. (cv. Mammoth Windsor). They found no vicine in 10-cm long pods or the seeds within. The 13–15 cm pods were also devoid of vicine; however, the seeds in such pods did contain vicine. The investigations of Jamalian et al. (1977a) confirmed the absence of vicine in pods. However, contrary to the findings of Brown and Roberts (1972), the vicine concentrations in the immature seeds of two Iranian broad bean cultivars, Hendu Kola and Rud Pish, were as high as 2.22 and 1.81%, respectively.

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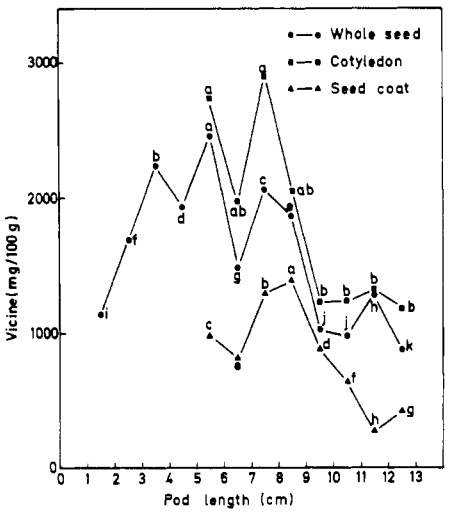

Figure 1. Vicine contents (dry weight basis) of seed coats, cotyledons, and whole seeds of broad beans (cv. Khalili) at various stages of pod development. Means not represented by the same letter in each curve are significantly different at the 1% probability level.

It was also shown that such seeds exhibited a high degree of toxicity as tested by incubation of their extracts with red blood cells from favism-prone subjects (Jamalian et al., 1977b).

The objectives of the present communication were to determine (1) the changes in concentration of vicine in broad bean seeds at different stages of pod development and (2) the relationships between the concentration of vicine in different parts of the seeds.

MATERIALS AND METHODS

Broad Beans and Their Preparation. From a farmer's field (planted with Khalili cultivar) in Shiraz, Iran, fresh broad bean pods were harvested in sufficient quantities over a period of 4 weeks starting with the appearance of pods until full maturity. Each collection was kept in deep freeze (−20 °C) and later classified according to pod length in order to obtain seeds with the same degree of growth. Thus 12 groups with pod lengths from about 1 cm up to 13 cm were obtained. The seeds in each group were divided into two portions (about 100 g each). In one portion, the seed coats were separated from cotyledons and in the other, the seeds were left intact. Due to the small size, highly immature, and watery nature of the seeds within pods of less than 5-cm long, the separation of seed coats was not possible in such groups. All samples were kept in deep freeze until analysis.

Measurement of Vicine Content. Frozen samples of whole seeds, cotyledons, and seed coats were thawed out and known quantities were extracted with 1% trichloroacetic acid by maceration in a laboratory mortar and pestle using acid-washed sand to help disintegrate the tissues. Samples were also taken simultaneously for moisture determination. The concentration of vicine was determined in duplicates by subjecting each extract to cation-exchange and thin-layer chromatography followed by UV spectrophotometry according to Jamalian et al. (1977a).

Statistical Analysis. Data obtained on cotyledons, seed coats, and whole seeds were individually subjected to the analysis of variance and mean comparisons were performed by the use of Duncan's new multiple range test (Duncan, 1955). To determine whether the overall means from the whole seeds or their parts were significantly different from each other, the t test for unpaired observations and unequal variances was used as described by Steel and Torrie (1960). All possible simple correlations and regressions were calculated for the data.

RESULTS AND DISCUSSION

The mean concentrations of vicine in the whole seeds, cotyledons, and seed coats were 1586 ± 106, 1830 ± 169, and 834 ± 95 mg per 100 g of dry tissue, respectively. Except for the means of the whole seeds and cotyledons which were not significantly different from each other, the other mean comparisons showed differences at the 1% probability level as determined by the t test.

The results of vicine determinations calculated on dry weight basis for the whole seed and its parts are plotted in Figure 1. The pod lengths varied from 1–2 cm up to 13 cm. It was observed that vicine was indeed present in the seeds throughout the different stages of pod development. However, its concentration was about 1140 mg/100 g in the seeds of very young pods with a tendency to increase as the pods grew longer until a maximum of approximately 2460 mg/100 g was reached at 5–6 cm pod

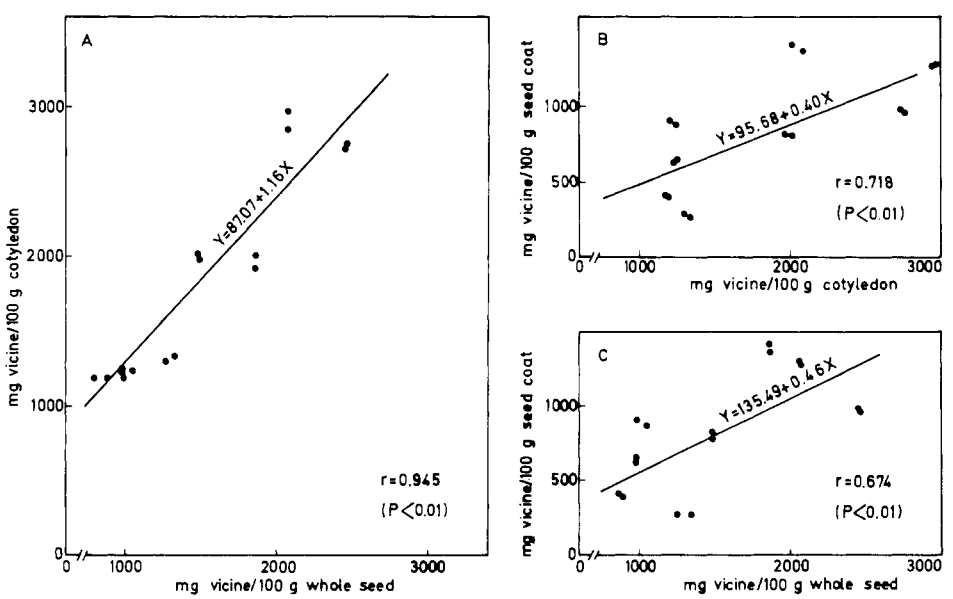

Figure 2. Regression lines for the vicine concentration (dry weight basis) relationships between (A) cotyledon and whole seed, (B) seed coat and cotyledon, and (C) seed coat and whole seed in Khalili cultivar of broad beans (*Vicia faba* L.).

length. A decline in the amount down to about 880 mg/100 g was noted with further growth in pods until maturity. The vicine content of cotyledons also followed a similar pattern but the peak concentration could not be detected because the seed coats could not be removed from the very young seeds. The seed coat vicine content also changed with growth and its value, in all cases, was below that of the cotyledons and of the whole seeds.

It needs to be emphasized that, in the cultivar examined, the concentration of vicine was the highest when the pod length was about half of its mature length. This finding is not in agreement with that of Brown and Roberts (1972), who did not find any vicine in seeds within pods of less than 10 cm length. However, it is in accordance with the data of Jamalian et al. (1977b), indicating a high degree of toxicity associated with the immature broad bean seeds of two other broad bean cultivars (Rud Pish and Hendu Kola). Nevertheless, the discrepancy between our data and those of Brown and Roberts (1972) might be due to differences in cultivars, methods of extraction, etc., and should be further studied.

Determination of simple correlation coefficients and linear regression coefficients was based on all possible data pairs as shown in Figure 2. It can be noted that the correlation coefficients were highly significant (1% probability level) for the relationships between the vicine concentration in different parts of the seeds and the intact seed. This coefficient was especially large and the slope of the regression line was steeper for the cotyledon vs. the whole seed because cotyledons constitute the major portion of the seed. Nonlinear regressions were also tried for the data; however, linear relationships fitted the data best.

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Composition of Jojoba Seeds and Foliage

High-performance liquid chromatography and thin-layer chromatography methods were developed to assay two major toxicants in jojoba plant tissues. Simmondsin, the most prevalent toxicant, is present in seeds at 2.3% levels and is also found in hulls, leaves, twigs, core wood, and male inflorescence. Simmondsin 2′-ferulate, the second most prevalent toxicant, occurs in seeds but was not found in the other plant tissues investigated. Oil, protein, carbohydrate, and amino acid levels are also reported for the seeds.

Jojoba [*Simmondsia chinensis* (Link) Schneider] is a dioecious desert shrub that grows naturally on arid lands in Arizona, California, and Mexico (National Academy of Sciences, 1975, 1977). Jojoba seeds vary in size from that of a coffee bean to a large peanut. A single mature shrub may produce as much as 5–10 lbs or more of seeds per year. The seeds contain about 50% of a colorless, odorless oil, that is structurally similar to sperm whale oil. The oil is unique in that it is essentially a mixture of monoesters containing monounsaturated carboxylic acids and alcohols with 20 and 22 carbon atom lengths. There is growing interest in jojoba oil in its own right, and as a replacement for sperm oil, a valuable commodity no longer available in the United States.

After removal of the oil from the seeds, the remaining meal is high in protein and is a potential animal feed ingredient. Although a few wild rodents, deer and swine eat the seeds (Sherbrooke and Haase, 1974), laboratory mice, rats, and poultry do not do well on the meal (Booth et al., 1974; Weber and Reid, 1977). The presence of several 2-cyanomethylenecyclohexyl glycosides (Elliger et al., 1973, 1974a,b), at least one of which has demonstrated toxicity, render the meal unsuitable as a livestock feed. The goal of detoxifying jojoba seed meal as a by-product for use in livestock feed has provided the impetus for developing assay methods for the toxicants and for determining the composition of the seeds and foliage.

Thin-layer chromatography and high-performance liquid chromatography methods were developed for assaying the two major toxicants, simmondsin and simmondsin 2′-ferulate. An assay of these two toxicants and the protein, amino acid, carbohydrate, fiber, and oil contents of seeds from two locations are reported. Assays for toxicants in leaves and twigs are included because these parts of the plant have provided browse for cattle. An assay of the hulls is reported because in commercial processes used to express the oil, the seeds contain as much as 10% or more hulls to facilitate pressing. Composition of the hulls is significant when the resulting meal is used as an animal feed.

EXPERIMENTAL SECTION

Materials. Seeds were obtained from the 1976 harvest of the Southern California Jojoba Project (SCJP 977) and

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