Cyanogenesis of *Passiflora edulis*

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The cyanogen of *Passiflora edulis* Sims is identified as prunasin [2(*R*)-(β-D-glucopyranosyloxy)-2-phenylacetonitrile] by ^{1}H NMR. Quantitative assay of hydrogen cyanide in leaves and fruit at different stages of development shows potentially toxic levels to be present. Passion fruits contain the greatest amount of cyanide when immature, losing most of their cyanogenic capacity as they ripen. No significant difference was detected between edible and nonedible portions of fruit.

The edible passion fruit (*Passiflora edulis* Sims) originated in Brazil (Pope, 1935) and is cultivated throughout the tropics and subtropics (Popenoe, 1924), where it is a fresh fruit crop important in local diets and is available in most markets (Hedrick, 1972; Schery, 1972; Hill, 1952). Production figures are available for Australia (Australia Bureau of Statistics, 1978), where in the 1974–1975 growing season 374 ha were cultivated to yield 3485 metric tons of fruit. In Hawaii in 1978, 93 ha were under cultivation yielding 232 metric tons of fruit (U.S. Department of Commerce and Bureau of the Census, 1981). However, more fruit is collected in the wild; combined cultivation (20 ha) and wild harvest yield in 1981 was 615 metric tons.

Two types of passion fruit are grown commercially, the wild or purple form and a yellow form (*P. edulis* forma *flavacarpa* Degener) (Chandler, 1958).

Commercially produced passion fruit juice is sold in a can, in a frozen state, or as nectar and is used in sherbets (Shaw et al., 1955) and various condiments, candies, and jellies (Miller et al., 1937, 1947; Seale and Sherman, 1960). The flavor is due to a complex mixture of volatile components including low molecular weight carboxylic acids and has not been synthesized (Hiu, 1959). The juice is nutritious, rich in vitamin C, and very acidic (Miller et al., 1937; Seale and Sherman, 1960).

Modern processing of the fruit is by centrifugation (Kinch and Shaw, 1954) in Hawaii, by expression in Australia, and by suction in New Zealand (Seale and Sherman, 1960). The products of processing are juice (35.8%), pulp (rind and aril) (51.4%), and seed (11.0%). The pulp has been used as silage in cattle and pig feed (Otagaki and Matsumoto, 1958; Sherman et al., 1953), and the seeds have been found to contain 20% edible oil (Otagaki and Matsumoto, 1958).

In South America, the fruit is cut in half and the pulp is eaten raw or blended with sugar and water to make popular "refrescos". From one to a half-dozen fruits are typically eaten at a sitting. Fresh fruits are widely marketed in every producing country and are often picked and shipped before they are fully mature (Akamine, 1956; Seale and Sherman, 1960; Queensland Department of Agriculture, Fruit Branch, 1931).

Cyanogenesis occurs in over 100 families of plants. Members of the Passifloraceae have long been recognized as being cyanogenic (Hegnauer, 1969; Gibbs, 1974; Carneiro, 1945). Immature fruits of the genus are often quite toxic and some (e.g., *Passiflora adenopoda* DC.) have proven fatal (Saenz, 1972).

P. edulis was first reported to be cyanogenic by Rosenthaler (1919). As part of an ongoing study on cyanogenesis in the Passifloraceae, we retested this species for cyanide and found significant levels to be present in all its parts (including mature fruits) except seeds. We decided to investigate quantitatively the cyanogenic capacity of *P. edulis* and to determine the identity of the cyanogen responsible.

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EXPERIMENTAL SECTION

Fresh leaves of *P. edulis* (427 g) from our greenhouse were added to cold 80% MeOH in a blender. The resulting suspension was filtered and the residue washed with 80% MeOH. The extract was concentrated to yield a brown syrup (20 mL). This was extracted with $CHCl_3$, and the aqueous phase was retained and placed on a Sephadex column (G-10). Fractions were collected (50 × 5 mL) with H_2O as the eluant. A few drops of each fraction were transferred to a vial and buffered to pH 6.8 (P_i, 0.1 mol), and a few drops of β-glucosidase (almond emulsin, Sigma) were added. HCN released as a result of enzymatic hydrolysis was detected with Feigl–Anger paper (Feigl and Anger, 1966). The cyanogenic material (fractions 9–14) was concentrated to 5 mL and chromatographed on paper (Whatman 3MM, 22 × 57 cm) in acetone–H_2O (5:1). The cyanogen was detected by cutting a strip 1 cm wide from the center of the chromatogram, cutting 1-cm^2 sections from this strip, placing them in vials, and testing for HCN as above. The cyanogen (R_f 0.1) was eluted with H_2O, concentrated under vacuum, and rechromatographed on paper in 2-propanol–1-butanol–H_2O (6:3:1). The cyanogen (R_f 0.6) was eluted and concentrated as above and chromatographed a final time in acetone–H_2O (5:1). The purified cyanogen was eluted and concentrated to yield a white crystalline solid (98.6 mg, overall yield 0.023%).

Quantitative determination of glucose was made using the glucose oxidase test (Washko and Rice, 1971). A sample of the cyanogen was incubated with emulsin for 4 h and then subjected to assay for free glucose. The results show the presence of one molecule of glucose per molecule of cyanogen (0.5 mg of prunasin = 1.7 μmol, observed glucose = 1.7 μmol).

For quantitative determination of cyanide a 1-g sample of fresh fruit or other plant part was removed and quickly placed in a Warburg flask and suspended in phosphate buffer (0.1 mol, pH 6.8). Sampling was repeated by using 1-g samples of plant material homogenized for 30 s in an ice bath. Several drops of emulsin were added to hydrolyze the cyanogen. NaOH (1 mol, 0.5 mL) was added to the center well, and the flasks were incubated 12 h at 25 °C. The basic solution was removed and diluted, and a cyanide assay was performed by the method of Lambert et al. (1975). Five samples of each fruit type or part and the corresponding homogenates were taken, and each assay was conducted in triplicate. Values obtained for homogenates were identical with those obtained for corresponding fresh samples and will not be considered further.

The proton NMR spectrum was obtained on a Nicolet NT-360 spectrometer as the Me_4Si derivative in $CDCl_3$. These derivatives were prepared as previously described (Seigler, 1975).

RESULTS AND DISCUSSION

The ^{1}H NMR spectrum of the cyanogen isolated from

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Table I. Cyanide Content of Cultivated Passion Fruits[a]

variety	part	stage	mg of HCN/100 g fresh wt	μmol of HCN/g	% HCN	% prunasin
purple	leaves		11.6	4.3	0.012	0.13
	fruit	immature	13.3	4.9	0.013	0.14
		intermediate	10.5	3.9	0.010	0.11
		mature, attached	10.0	5.3	0.010	0.11
yellow	fruit	immature	59.4	22.0	0.059	0.65
		intermediate	15.9	5.9	0.016	0.17
		mature, attached	14.6	5.4	0.015	0.16
		mature, dropped	6.5	2.4	0.006	0.07
Mexican Mottled	fruit	intermediate	11.9	4.4	0.012	0.13
yellow	aril	immature	59.4	22.0	0.059	0.65
	pericarp		58.3	21.6	0.058	0.64
	aril	intermediate	17.8	6.6	0.018	0.19
	pericarp		22.3	8.3	0.022	0.24
	aril[b]	mature, attached	16.9	6.3	0.017	0.18
	pericarp		11.5	8.3	0.011	0.12

[a] Each value is the average of HCN determinations on three replicates of each of five samples. [b] Including juice.

P. edulis is identical with that of prunasin [2(*R*)-(β-D-glucosyloxy)-2-phenylacetonitrile] (Seigler, 1975). Sugar determination confirmed the structure. The same cyanogen was isolated from fruits of *P. edulis* and *P. edulis* forma *flavacarpa* Degener.

The finding of prunasin, which is biosynthesized from phenylalanine (Conn, 1979), is significant as the Passifloraceae are typified by the production of cyclopentenoid cyanogens (Spencer and Seigler, 1982a,b; Seigler et al., 1982). Only one other species of *Passiflora* is known to produce a non-cyclopentenoid cyanogen (Fischer et al., 1982), and this is produced from a different biosynthetic precursor. Prunasin is known from a number of unrelated plant families including the Rosaceae, Fabaceae, Asteraceae (Seigler, 1981), and Polypodiaceae (Kofod and Eyjólfsson, 1966), among others.

As prunasin is hydrolyzed to produce hydrogen cyanide by the β-glucosidases with which it is associated in the plant, and as most workers report the presence of cyanide or cyanogens as mg of HCN/100 g fresh weight we determined cyanide as HCN from sample hydrolysates. A quantitative determination of cyanide was carried out on three strains of passion fruit: purple, from a California greenhouse, and yellow and "Mexican Mottled", both from cultivation in Florida. The fruits were assayed for cyanide at different stages of ripeness (see Table I). We averaged HCN determinations on three replicates of each of five different samples to obtain reported values. We do not consider that we have adequately sampled the range of variation in these fruits as only limited material was available to us. In general, botanicals containing 20 mg of HCN/100 g fresh weight are considered to be toxic (Moran, 1954), but an HCN content of 10 mg/100 g fresh weight in *Cassava* is considered dangerously poisonous by Bolhuis (1954). The approximate lethal dose in man (Williams and Langford, 1967) is 70 mg of HCN, but smaller amounts cause significant neuropathological, goitrogenic, and other pathological conditions in man (Way, 1981). The cyanide content of the passion fruit that we tested was found to range from 6.5 to 59.4 mg of HCN/100 g fresh weight, from subtoxic to nearly 3 times the toxic level. The average amount present is in the same range as that found in fresh cassava leaves and tubers and *Sorghum* (Nartey, 1968, 1981; Montgomery, 1969), both considered toxic. In the yellow form, the cyanide content drops sharply as the fruit matures, falling to a subtoxic level only after fruit abscission.

A comparison was also made between cyanide content in edible pulp (aril) and nonedible rind (pericarp) portions of yellow passion fruit at various stages of development. No significant differences were noted in HCN (cyanogen) levels of aril and pericarp of immature fruit. Cyanogen levels apparently decline more rapidly during development in the aril than in the pericarp, but the aril retains more in mature fruit.

In summary, all parts of passion fruits are toxic when immature with the exception of seeds. They retain significant amounts of cyanide even when ripe, but as the cyanide content drops during maturation, the practice of harvesting from the ground reduces the amount of cyanogen introduced into processing. Picking fruits from vines might introduce higher levels. Present processing methods crush the plant tissues (Seale and Sherman, 1960) and mix the cyanogen with the β-glucosidase which hydrolyzes it. Most of the hydrogen cyanide thus produced is probably lost to the atmosphere. However, it would be interesting to monitor rates of HCN loss during processing. Further studies are recommended to determine the levels of HCN present in passion fruits, which are widely marketed in South America, in order to determine whether there is any potential for contribution of these fruits to cyanide toxicity syndrome in underdeveloped countries. We would be interested in screening other varieties in order to determine what variation exists in the cyanogen content among them. Also, we have no information as to the influence of environmental factors in determining the cyanogen content in these plants.

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Registry No. Prunasin, 99-18-3; hydrogen cyanide, 74-90-8.

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Studies on the Volatile Components of Two Mango Varieties

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The volatile composition of two mango varieties (Alphonso and Baladi) was investigated by means of standard-controlled distillation–extraction, liquid–solid chromatography, and gas chromatography–mass spectrometry. This combination led to the characterization of a spectrum of 114 components of which 81 were identified for the first time as mango constituents. In addition to this qualitative identification, the concentrations of the components were quantitatively determined in a range from 40 ppm to the ppb trace level. By this method clear differences between the two mango varieties could be demonstrated.

Mango (*Mangifera indica* L.), one representative of the group of tropical fruits, becomes more and more important also on European markets. Although the popularity of mangos is increasing just because of their strong and pleasant aroma, there is only little information about the flavor and aroma components of this fruit.

Angelini et al. (1973) characterized the aromatic principles of ripe mangos as hydrocarbons, esters, alcohols, carbonyls, and lactones. Hunter et al. (1974) investigated the volatile components of canned Alphonso mango. Bandyopadhyay and Gholap (1973) reported on the relationship of mango aroma to fatty acid composition, and Gholap and Bandyopadhyay (1977) characterized *cis*-ocimene and β-myrcene to be responsible for the typical green aroma of raw mangos. MacLeod and Troconis (1982) described car-3-ene as the major aroma-contributing component in Venezuelan mangos. Besides the well-known variety Alphonso, there is a large number of other varieties cultivated in many tropical areas all over the world. Bandyopadhyay and Gholap (1979), Diaz (1980), and Abd El-Baki et al. (1981) investigated some of these varieties, and they reported on considerable differences in the aromatic principles reaching from woody and camphory to

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