Formation of Organic Nitriles from Progoitrins in Leaves of *Crambe abyssinica* and *Brassica napus*

When fresh leaves from crambe and pasture rape were crushed and allowed to autolyze at pH 5.0–5.8, the thioglucosidase-initiated hydrolysis of the progoitrins gave 1-cyano-2-hydroxy-3-butenes and diastereomeric isomers of 1-cyano-2-hydroxy-3,4-epithiobutanes instead of 5-vinyloxazolidine-2-thiones (goitrins). When intact leaves were air-dried, thioglucosidase activity was lost. Addition of white mustard thioglucosidase to an aqueous suspension of ground air-dried leaves at pH 5.0–5.8 or buffered to pH 7.0 gave goitrins instead of the nitriles.

Progoitrin and *epi*-progoitrin are the major glucosinolates (thioglucosides) in the seed of *Crambe abyssinica* Hochst ex R. E. Fries and of *Brassica napus*, respectively. Different products form from the aglucon part of the progoitrin molecule depending upon conditions of enzymatic hydrolysis of the glucosinolates (VanEtten *et al.*, 1969a). The hydrolysis is initiated by a thioglucosidase (thioglucoside glucohydrolase EC 3.2.3.1; Florkin and Stotz, 1965) also in the seed. During autolysis of the seed meals, 1-cyano-2-hydroxy-3-butenes and diastereomeric 1-cyano-2-hydroxy-3,4-epithiobutanes are often formed (Daxenbichler *et al.*, 1967, 1968; VanEtten *et al.*, 1966) instead of the previously recognized 5-vinyloxazolidine-2-thiones (goitrins) (Astwood *et al.*, 1949). With regard to stereochemistry, the products from *epi*-progoitrin [(*S*)-2-hydroxy-3-butenylglucosinolate] are (*S*)-1-cyano-2-hydroxy-3-butene, diastereomeric (2*S*, 3*R* and 2*S*, 3*S*)-1-cyano-2-hydroxy-3,4-epithiobutanes, and (*R*)-5-vinyloxazolidine-2-thione. Progoitrin [(*R*)-2-hydroxy-3-butenylglucosinolate] leads to (*R*)-1-cyano-2-hydroxy-3-butene, (2*R*, 3*R*)- and (2*R*, 3*S*)-1-cyano-2-hydroxy-3,4-epithiobutane, and (*S*)-5-vinyloxazolidine-2-thione (VanEtten *et al.*, 1969a; Carlson *et al.*, 1970). When ingested, organic nitriles have different physiological effects than do the goitrins (VanEtten *et al.*, 1969b).

If specific glucosinolates occur in one part of a given cruciferous plant, generally they will occur also in other parts (Kjaer, 1960). Not surprisingly then, we found up to 0.6% progoitrins in the air-dried leaves of *C. abyssinica* and *B. napus* (*vs.* 7.7 and 2.0%, respectively, in the defatted air-dried seed meals from the same plants). It was of further interest to ascertain whether, depending on conditions as in the corresponding seed meals, enzymatic transformations of these compounds in the leaves might also lead to nitriles instead of goitrins. This communication gives the analysis of extracts from wet-crushed and from air-dried leaves of crambe and rape to determine the hydrolysis products formed from progoitrin aglucons during each method of treatment.

EXPERIMENTAL

Spring and autumn plantings from two seed accessions for each of *C. abyssinica* and *B. napus*, variety Dwarf Essex pasture rape, were grown locally in 1968 and again in 1969. Leaves were harvested at various stages of plant maturity.

During the first collection, each crambe leaf was immersed into boiling water in less than a second after separation. After cooling, the leaves were pulped in a Waring Blendor and the water was removed by lyophilization. Since no evidence of thioglucosidase activity was later found in the intact leaves from either plant under study, the hot water treatment was omitted for the remaining collections.

For autolysis of fresh undried leaves from either plant, a 40- to 50-g sample equivalent to 5 g of air-dried material was thoroughly crushed in a mortar and allowed to autolyze for 1 to 3 hr. No adjustment was made of pH, which ranged from 5.0 to 5.8. After the autolysis, the juice was separated from the insolubles by passage through cheesecloth. The pressed solids were reextracted three times with small volumes of water. The combined extracts were taken to about 5 ml under vacuum at <50° C. The compounds to be measured were extracted into peroxide-free ethyl ether or dichloromethane.

For air-drying, intact leaves were spread out on a laboratory bench and left at room temperature for 6 days or longer. Then the dried leaves were ground in a micro Wiley mill to pass a 40-mesh screen. Lyophilized leaves from the first crambe collection were similarly ground. Samples (5 g) of the meals from the lyophilized and the air-dried leaves were suspended in 10 to 20 ml of distilled water (pH of slurry 5.0 to 5.8) or 0.1 *M*, pH 7.0, phosphate buffer. Parallel experiments were run with and without addition of 5 to 10 mg of mustard myrosinase (a crude thioglucosidase) prepared according to Wrede (1941). After standing 1 to 3 hr at room temperature, the solubles were separated from the leaf solids and extracted with ethyl ether or dichloromethane exactly as described above.

Estimation of the goitrins and the three nitriles was made by a gas-liquid chromatographic (glc) method described by Daxenbichler *et al.* (1969). The compounds were determined on a column packed with 1% EGSS-X on 100 to 120-mesh Gas Chrom Q and on a column packed with 3% Apiezon L on 80 to 100-mesh Gas Chrom Q. The elution position of each substance on the two columns was established with pure reference samples of the compounds. Quantitation was obtained by integration of the peak areas and comparison with a methyl palmitate internal standard.

Goitrins were also estimated by extraction into ethyl ether and spectrophotometric measurement at the compounds' absorption maxima (Astwood *et al.*, 1949). Results from the glc and spectrophotometric methods were averaged.

RESULTS AND DISCUSSION

According to totals (last column) in Table I, progoitrin content of leaves of both crambe and rape on the air-dried basis ranged from 0.1 to 0.6%. The data suggest that leaves of

Table I. Products from Progoitrins[a]

Preparation and Source of Leaves	Height of Plant at Harvest, In.	5-Vinyloxazolidine-2-thione; goitrin, %	1-Cyano-2-hydroxy-3-butene, %	*threo*-1-Cyano-2-hydroxy-3,4-epithiobutane, %	*erythro*-1-Cyano-2-hydroxy-3,4-epithiobutane, %	Total, %
Crambe Leaves						
1968 Spring						
Wet-heated	18	0.1 to 0.2	0.0	0.0	0.0	0.2
Air-dried	18	0.1	0.0	0.0	0.0	0.1
Autolyzed	18	0.0	0.01	0.06	0.10	0.2
1968 Autumn						
Air-dried	20	0.3 to 0.4	0.0	0.0	0.0	0.4
Autolyzed	20	0.0	...	0.08	0.12	0.2
Autolyzed	3	0.0	0.01	0.02	0.05	0.1
1969 Spring						
Air-dried	20	0.2 to 0.3	0.0	0.0	0.0	0.3
Autolyzed	20	0.0	0.04	0.09	0.2	0.3
1969 Autumn						
Autolyzed	10	0.02	0.03	0.03	0.09	0.2
Dwarf Essex Rape Leaves						
1968 Autumn						
Air-dried	18	0.1	0.0	0.0	0.0	0.1
Autolyzed	18	0.0	0.005	0.03	0.03	0.1
Autolyzed	20	0.0	0.05	0.12	0.13	0.3
1969 Spring						
Air-dried	24	0.3	0.00	0.00	0.00	0.3
Autolyzed	36	0.0	0.12	0.22	0.23	0.6

[a] The results are expressed as percent progoitrin potassium salt computed from the equivalent found as hydrolysis products on the air-dried basis and are the average of two or more estimations.

young plants may contain less progoitrin than those that are more mature. In contrast to progoitrins in the leaves, the defatted air-dried meal of the seed of *B. napus* and of crambe that were planted for growing the foliage contained 2.0% and 7.7% progoitrins, respectively.

During autolysis of the fresh, undried leaves from the crambe and rape plants, formation of the three organic nitriles from each species was favored instead of goitrin (Table I). Of the analyses from eight autolyzed leaf preparations, seven contained the nitriles and no goitrin. The preparation from crambe leaves harvested in the autumn of 1969, from plants that were 10 in. tall, contained a small amount of goitrin in addition to the nitriles. The leaves from these plants were harvested after some frost damage.

Wet-heated leaves of course contained no active thioglucosidase. It was found that air-drying also destroyed thioglucosidase activity in the leaves without causing any hydrolysis of the glucosinolates. This lack of enzyme activity in meals from wet-heated leaves and air-dried leaves was noted at pH 5.0 to 5.8 (the pH range of the autolysis experiments with fresh leaves) and at pH 7.0 (the pH commonly used to estimate progoitrins by measuring the hydrolytically formed goitrins) (VanEtten *et al.*, 1969a). Under both conditions progoitrins in the leaf meals without active endogenous thioglucosidase were converted by the added mustard myrosinase to goitrins instead of nitriles.

These experiments indicate that enzymatic mechanisms available for transforming progoitrins in leaves of the plants investigated are analogous to those previously established in the seeds (VanEtten *et al.*, 1966, 1969a).

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