GLUCOSINOLATE ANALYSIS. LIMITATIONS AND POSSIBILITIES OF DIFFERENT PROCEDURES.

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Summary.

The separation and quantification of glucosinolates has received much attention in recent years. The progress in this field is fundamentally based on introduction of the very newest analytical techniques. The impetus for these studies is the major importance of the nature and levels of glucosinolates in new varieties of oil-seed crops and vegetables being bred throughout the world.

Procedures for estimating glucosinolates as their aglucon products after myrosinase-catalysed hydrolysis have been used for many years. This approach, however, has the disadvantage that not all glucosinolates can be measured and the methods suffer from a lack of sensitivity and precision when samples with very low levels of glucosinolates have to be analysed.

The major principle in the new methods is that the compounds are handled as the genuine glucosinolates. An ion-exchange step is used to isolate and to concentrate the glucosinolates. The isolated glucosinolates or desulfoglucosinolates can be analysed and quantitatively estimated as individual compounds by GC, GC-MS or HPLC. The GC--method has certain limits with regard to methylsulfinylglucosinolates and indol-3-ylmethylglucosinolates. The latter group comprises today five known compounds and they are of special interest in connection with certain green plant materials, e.g. vegetables. The HPLC-techniques are able to detect all known types of glucosinolates. Experimentally simple methods to determine the total glucosinolate content can be performed by estimation of D-glucose released by treatment with myrosinases. With our present knowledge about glucosinolates we are able to design methods of analysis with regard to simplicity, instrumentation and economy. The choice depends on the types and concentration of glucosinolates to be analysed, and the samples used for analysis, e.g. seeds, green plant materials, food or feed samples or samples from the intestinal canal.

Introduction.

During the last few years new methods of glucosinolate analysis based on determination of intact glucosinolates have been developed[1,2]. These methods appear to be promising alternatives or supplements to the methods based on degradation products of glucosinolates. Choice between available methods depends among other things on requirement to the methods e.g. type and concentration of glucosinolates or their degradation products in different materials. These may be seeds or other types of plant materials, food, feed, content of the digestive tract or different types of animal products, all of which contain varying amounts of interfering compounds. Considerations on possibilities and limitations of different available methods are the subject of this paper, and a brief summary of facts concerning glucosinolates is included.

Results and Discussion.

Glucosinolates comprise a well defined group of plant constituents occurring in all plants belonging to the order Capparales and in a few other higher plants[3,4]. It has also been claimed that glucosinolates occur outside higher plants[5].

Most of the about 90 known types of glucosinolates have the structure shown in Fig. 1[1], but glucosinolates with phenolic carboxylic acids connected to the thioglucose part through an ester bond are known[6].

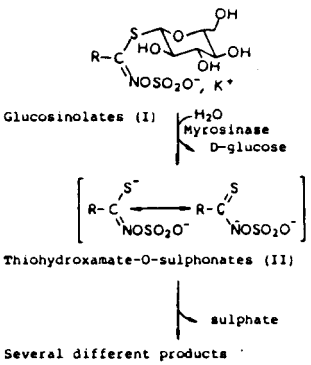

Fig. 1. Structure of glucosinolates and their hydroysis by myrosinases.

Glucosinolates are biosynthesised in the plants from the corresponding amino acids. Most often only a few are quantitatively dominant in a single plant, but commonly different plant parts are dominated by different types of glucosinolates.

Growth conditions, developmental stage of the plants and, at least in the case of double low rape cultivars, different plants of the same cultivar are reasons of large variations in the glucosinolate content.

Thioglucoside glucohydrolase EC 3.2.3.1. (myrosinases) co-occurs with glucosinolates in all of the plants investigated until now, but only little information on glucosinolate catabolism is available[1].

Glucosinolates are hydrophilic and strongly acidic compounds which have to be isolated and handled as salts[1]. They are relatively unstable in both strongly alkaline, acidic, and metalion containing solutions, and especially in the presence of myrosinases and water[1]. Therefore, special requirements are needed for reliable quantitative methods. Immediate inactivation of myrosinases during homogenisation and extraction is perhaps the most critical step in all of the known quantitative methods of analysis.

Quantitative methods of glucosinolate analysis can be divided into:

group 1: methods based on degradation products of glucosinolates produced in myrosinase catalysed hydrolysis.

group 2: methods based on desulphoglucosinolates produced in sulphatase or non-enzymatic glucosinolate degradation.

group 3: methods based on intact glucosinolates.

Group 1 comprises autolysis which for different reasons is doubtful as a quantitative method[1]. Otherwise, the problems with preparation of a crude extract are common for group 1, 2 and 3. The principal differences are in the purification and concentration steps and the methods which then are available for glucosinolate determination[1].

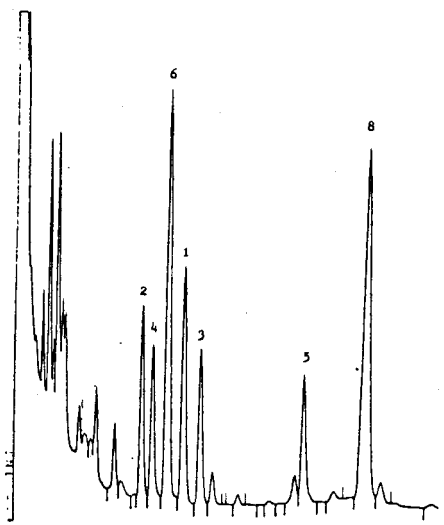

Fig. 2. Gas chromatogram of per-trimethylsilated desulphoglucosinolates[1]. The peak nos. correspond to the compound nos. in Fig. 3.

Group 1 comprises total glucosinolate determination based on released sulphate[7] or more promising determination of released glucose[8,9], but these methods do not allow discrimination between individual glucosinolates. Quantitative methods based on UV-determination of oxazolidine--2-thoines and thiourea derivatives as well as HPLC and GC determination of individual aglucones have among other things limitations with respect to the types of aglucones produced[1]. However, for the volatile isothiocyanates GC possesses a high resolution capacity[10].

Group 2 methods involve most often sulphatase treatment of the glucosinolates[11], but silylation of the glucosinolates results also in desulphoglucosinolates. HPLC determination of desulphoglucosinolates has

recently been introduced as a promising method[12], but it is more common to use GC determination of per-trimethylsilylated desulphoglucosinolates[1,2,11] (Fig. 2).

Group 3 methods involve HPLC determination of intact glucosinolates (Fig. 3) and/or GC determination of per-trimethylsilylated desulphoglucosinolates (Fig. 2). Other methods of quantitative investigation of intact glucosinolates have been described[1], and it is possible to use myrosinase catalyzed degradation and methods developed for group 1 and 2 principles in connection with the isolated intact glucosinolates.

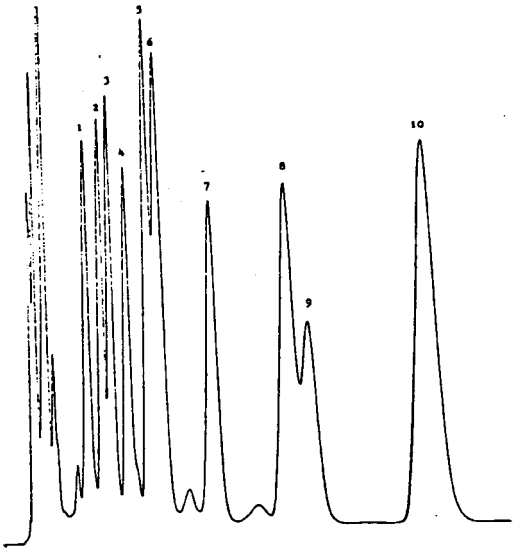

Fig. 3. HPLC chromatogram of intact glucosinolates[13]. Peak nos. correspond to: (1) glucoiberin, (2) glucocapparin, (3) progoitrin, (4) sinigrin, (5) sinalbin, (6) gluconapin, (7) glucobrassicanapin, (8) glucobrassicin, (9) 4-methoxyglucobrassicin, (10) neoglucobrassicin.

The main differences between group 2 and 3 methods are in the columns used for isolation of the glucosinolates and in the principles used to release the glucosinolates from the columns[1]. Group 2 methods involve treatment of the column with sulphatase leading to release of desulphoglucosinolates. Group 3 methods involve elution of the intact glucosinolates from the column with 1 M pyridine as eluent which deionises the functional groups on the column material[1].

Requirement to simple, fast, reliable methods of glucosinolate analysis have increased with the growing interest in use of cruciferous plants, including double low rape, as food and feed[14,15].

These requirements are:

(a) simple and fast methods for determination of the total amount of glucosinolates

(b) reliable quantitative determination of all individual types of glucosinolates present in seed samples where the level is from less than 1 μmole to some few μmoles per g

(c) usable methods to analysis of food, feed, content in digestive tracts or other products containing minor amounts of seeds with a glucosinolate level as mentioned in (b) and where interfering compounds not common to rapeseed are present[14,15,16].

(d) usable methods to analysis of different glucosinolate-containing agricultural and horticultural crops, including green plant material (cabbage, swede)[15,16]

(e) suitable to series/routine analysis with acceptable requirement to standardization, available standards, cost and time per analysis

(f) possibilities of simultaneous determination of phenolic choline esters is maybe important[15].

The glucosinolates (Fig. 1) important to consider contain the R-groups:

A unsubstituted alkyl, alkenyl chains, including benzyl and phenethyl

B 2-hydroxysubstituted derivatives of group A compounds

C hydroxylated and methoxylated phenyl groups

D glycosides

E $CH_3S(CH_2)_n$-; $CH_3SO(CH_2)_n$-; $CH_3SO_2(CH_2)_n$-

F indolylderivatives

G with acidic function in the R-group

H in addition glucosinolates with sinapic acid connected to thioglucose as ester appears to be quantitative dominating in some Raphanus seeds.

Determination of individual glucosinolates based on group 1 principles (GC and HPLC) have been discussed above. It is possible for most of the compounds in A and B, some but not all in C and D whereas serious problems occur with most of the other types[1].

Analysis according to group 2 principles is reliable for most of the compounds in A - E. Some unsolved problems exist with methylsulphinyl derivatives[17,18] and F - H when GC of silylated derivatives is used (Fig. 2)[18,19].

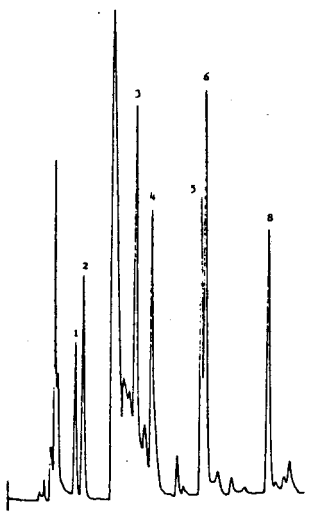

Fig. 4. HPLC chromatogram of desulphoglucosinolates[12]. Peak nos. corcespond to the compound nos. in Fig. 3.

HPLC of desuphoglucosinolates has advantages and is a valuable alternative to other methods although problems still exist e.g. with a satisfactory separation of some glucosinolates and impurities. These will either interfere in the chromatogram or they are retained on the column with destruction of the columns as a result (Fig. 4). Such problems with impurities which eventually destroy the columns are reduced in the system used for HPLC of intact glucosinolates[13]. With the HPLC method, as with HPLC of intact glucosinolates, it is possible and easy to use different detection wavelengths to discriminate between alifatic and aromatic glucosinolates, and also between indolylderivatives and benzyl/phenylethylglucosinolates.

Fig. 5. HPLC chromatogram of progoitrin separated from other rapeseed glucosinolates by prep. HPLC[13].

Analysis based on group 3 principles allow determination of all known types of glucosinolates if HPLC is used (Fig. 3). With this method it is also possible to satisfy requirements (a) - (f), e.g. simple isolation of standards by prep. HPLC (Fig. 5). GC analysis of silylated desulphoglucosinolates gives the unsolved problems mentioned above for group 2 (Fig. 2)[1,17-19].

In conclusion, group 3 (or 2) principle with use of HPLC seems to be the most promising method but use of alternative methods e.g. HPLC of desulphoglucosinolates, GC - MS or group 1 methods to confirmation of structures is adviseable.

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