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Analysis of Degradation Products of Indole Glucosinolates by Capillary Electrophoresis

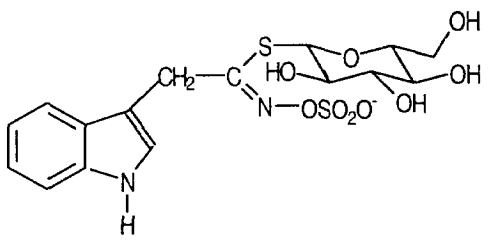
Niels AGERBIRK and Hilmer SØRENSEN

Chemistry Department, Royal Veterinary and Agricultural University, Thorvaldsensvej 40, DK-1871 Frederiksberg C, Denmark

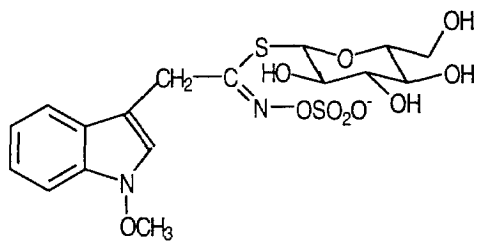
Introduction

In seeds and green parts of oilseed rape [Bjerg et al. 1987], as well as in various cruciferous vegetables [Hansen et al. 1995], the indole glucosinolates (indol-3-ylmethyl glucosinolates) are quantitatively important and of interest in relation to the quality of these plant products.

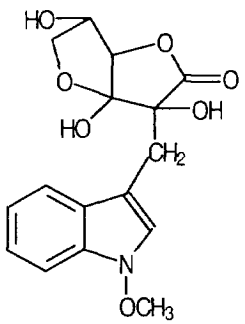
It is well established that the indole glucosinolates are of interest in relation to prevention of human cancer [Loft et al. 1992], in relation to the value of rapeseed protein [Jensen et al. 1991], and with respect to host-plant recognition by specialist insect pests [Simmonds et al. 1994]. The indole glucosinolates occurring in the *Brassica* species are often substituted, but most biological trials of the indole glucosinolates and their degradation products have been done with the easier available unsubstituted compounds. As the biological effects studied depend on the recognition of the indolyl compounds by specific receptors, it seems likely that the effect of substitution of the indolyl group may be of crucial importance. While the analysis of the indole glucosinolates themselves are now routine [Michaelsen et al. 1992, Bjergegaard et al. 1995 a], the many biologically important degradation products of these glucosinolates still call for efficient methods of analysis (Fig. 1).



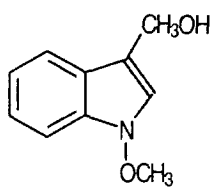
Glucobrassicin



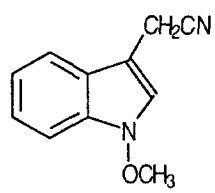
Neoglucobrassicin



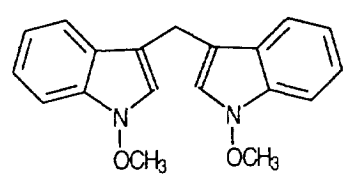
"Neo"-ascorbigen



"Neo"-indol-3-ylcarbinol



"Neo"-indol-3-ylacetonitril



"Neo"-dimer

Figure 1. The indole glucosinolates glucobrassicin and neoglucobrassicin, as well as possible degradation products of the latter glucosinolate.

Results

In recent years, methods for the determination of various types of indole glucosinolate degradation products have been developed in our laboratory [Feldl et al 1994, Bjergegaard et al 1995 b, Agerbirk et al. 1996]. These methods are based on a variant of capillary electrophoresis, named micellar electrokinetic capillary chromatography (MECC). In MECC, separation of neutral compounds during the electrophoresis is accomplished by inclusion of charged micelle-forming detergents in the electrophoresis buffer (Figure 2).

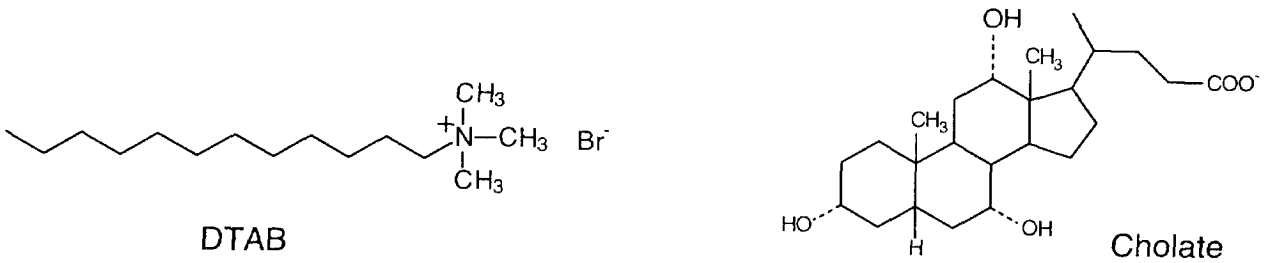


Figure 2. Micelle forming detergents used for the separation of degradation products of indole glucosinolates by capillary electrophoresis.

One method in use is based on micelles of the positively charged detergent dodecyltrimethyl-ammonium bromide (DTAB). During the electrophoresis, ion pairing and other interactions occur between the micelle and the negatively charged glucosinolates, as well as the thiocyanate ion, which is a degradation product of the indole glucosinolates. Furthermore, separation of monomeric indolylic degradation products is accomplished by hydrophobic interactions with the micelles, similar to classical reversed phase chromatography. The nitrile-, carbinol- and ascorbigen types of products from both glucobrassicin and the substituted glucosinolate neoglucobrassicin can at present be determined by suitable variations of the electrophoresis conditions (Figure 3).

Depending on the reaction conditions, quite hydrophobic dimeric and oligomeric indolyl compounds are also formed from glucosinolate degradation. In ongoing work, methods for the determination of these types of degradation products from glucobrassicin and neoglucobrassicin are developed. Promising results for the unsubstituted compounds have been obtained by using micelles of the bile acid cholate (Figure 2). These micelles interact strongly with the oligomeric indolyl compounds, while most monomeric compounds are almost unretained by the micelles. A representative MECC chromatogram is shown in figure 4.

The antinutritional effects of the glucosinolates and their degradation products are well known [Bjerg et al. 1989, Michaelsen et al. 1994]. Much less is known of the effects of these compounds for the aroma and nutritional qualities of cruciferous vegetables [Hansen et al. 1995], and for the insect pests of the intact crop.

The methods described here should be useful in the further investigation of the biological effects of the indole glucosinolates, whether they are antinutritional components of double low rape seed (4-hydroxyglucobrassicin), cancer-preventing compounds in cabbage and kale, or insect stimulating compounds in green parts of the *Brassica* crops. Other types of glucosinolate degradation products, such as the aliphatic isothiocyanates and nitriles, will need yet other methods for their determination. The development of such methods is another important part of the ongoing work in our laboratory.

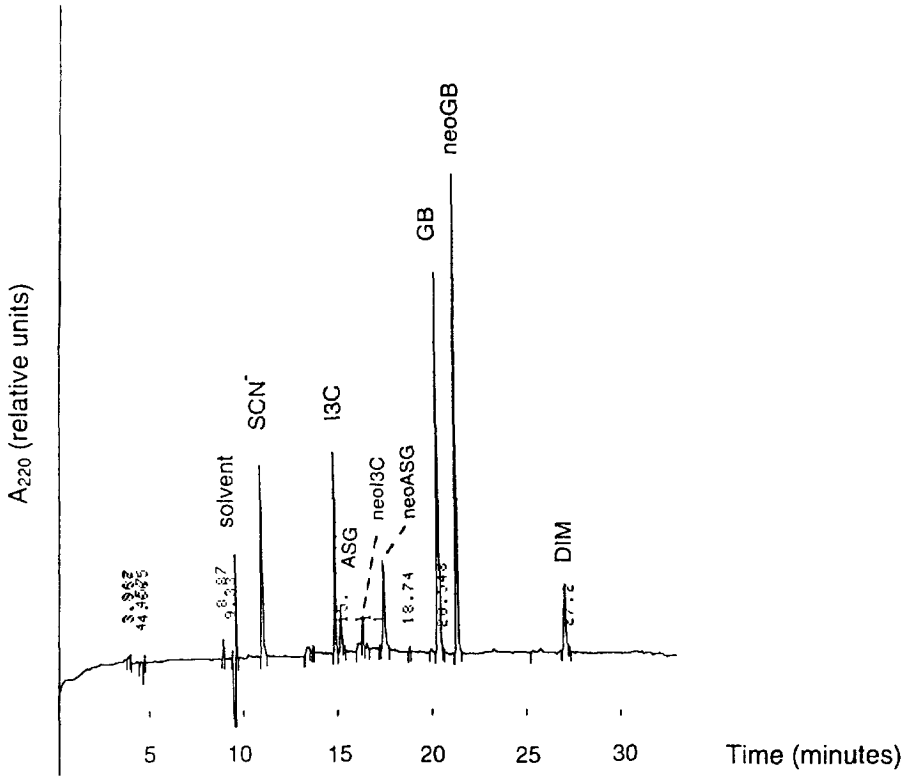


Figure 3. Separation of glucobrassicin, neoglucobrassicin, the thiocyanate ion and neutral degradation products of the two glucosinolates by capillary electrophoresis [Feldl et al. 1994]. Separation of the corresponding nitriles can be done by altering the conditions. GB = glucobrassicin, neoGB = neoglucobrassicin, SCN^- = thiocyanate ion, I3C = indol-3-ylcarbinol, neoI3C = "neo"indol-3-ylcarbinol, ASG = ascorbigen, neoASG = "neo"ascorbigen.

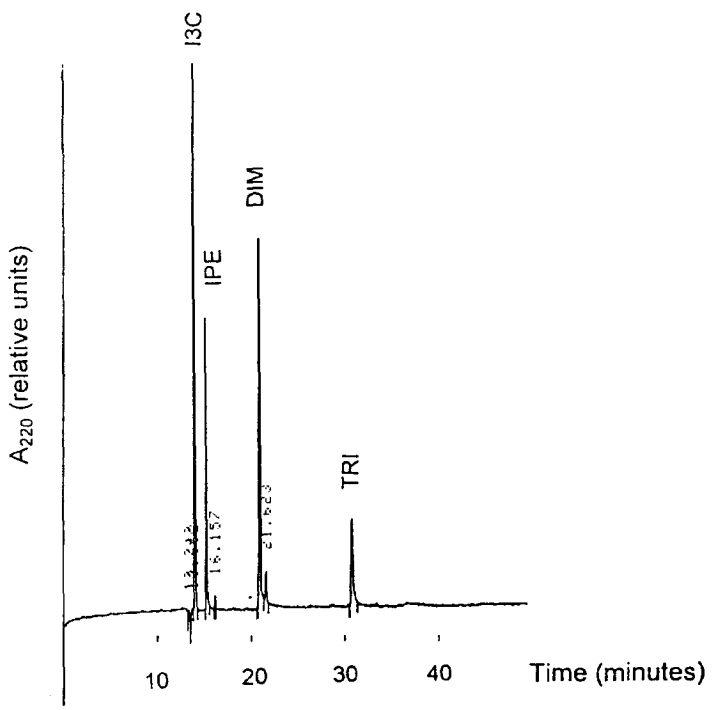


Figure 4. Separation of monomeric , dimeric and trimeric degradation products of glucobrassicin by capillary electrophoresis [Agerbirk et al. 1996]. The chromatogram shows the products of 1.5 mM indol-3-ylcarbinol (I3C) after 2 hours at pH 6. IPE = propylether, DIM = dimer, TRI = trimer.

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