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Indolyl glucosinolates and transformation products. Thereof : Physiological Active Compounds and their determination by MECC

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INTRODUCTION

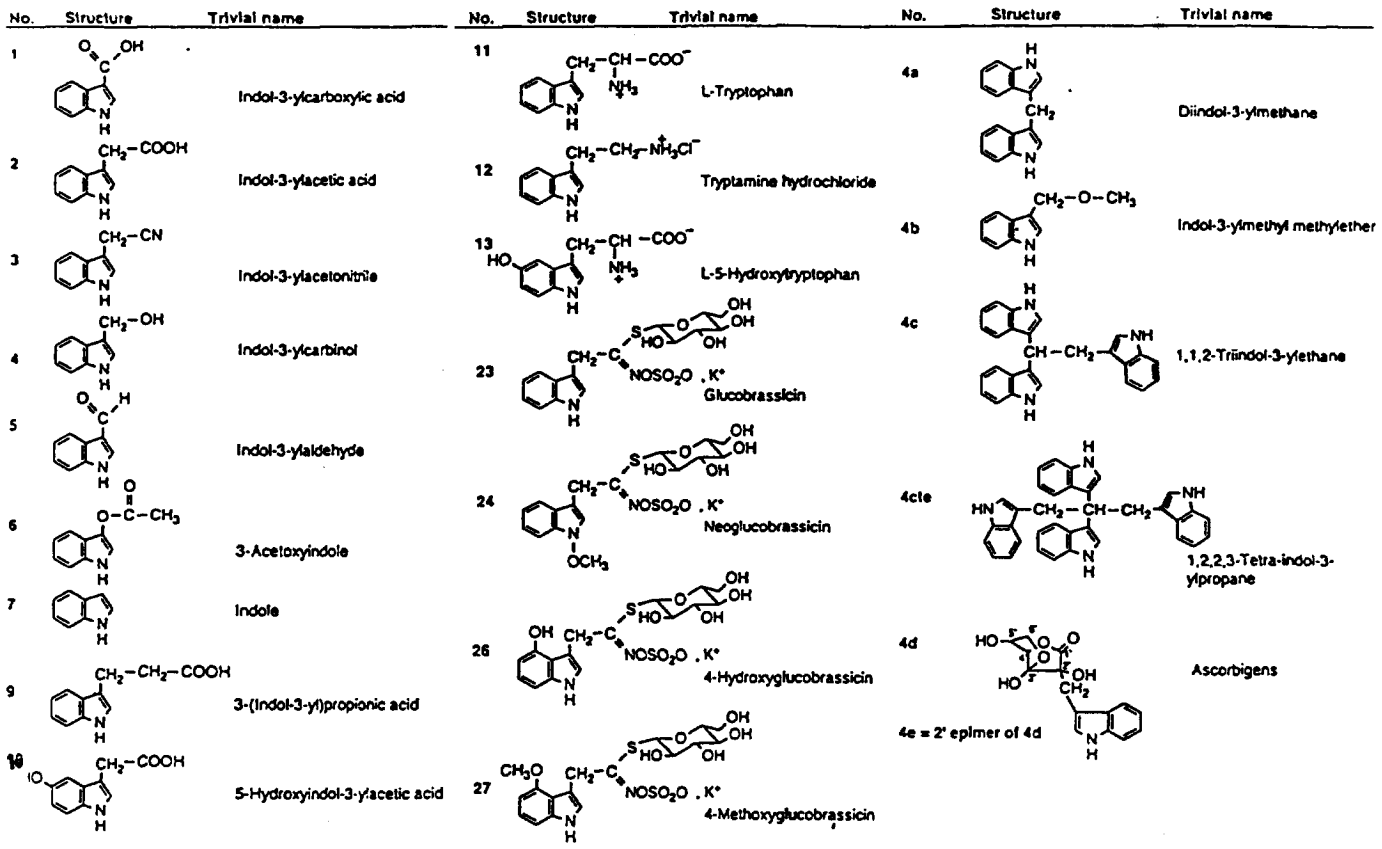
Indolyl glucosinolates (indol-3-ylmethylglucosinolates) are widely distributed in plants belonging to the order *Capparales* including the economically important *Brassica* vegetables e.g. cabbage, broccoli, and Brussels sprouts [1-5]. These glucosinolates and, even more pronounced the degradation products thereof, cause various physiological effects[1-5]. Increased consumption of these vegetables is associated with a decreased risk of cancer in humans and experimental animals [6, 7]. In feeding studies with hydrolysis products of glucobrassicin including indol-3-ylcarbinol, indol-3-ylacetonitrile, and di-(indol-3-yl)methane, indol-3-ylcarbinol appeared as the most active compound [8]. Recent experiments give, however, evidence that indol-3-ylcarbinol and other dietary indols exhibit their anticancerogenic properties indirectly by forming condensation products upon introduction into the acid environment of the stomach. Thus, di-(indol-3-yl)-methane was more effective than indol-3-ylcarbinol in influencing the level of carcinogen binding to DNA and in other protective mechanisms [9, 10].

Indole derivatives are also involved in plant growth e.g. indol-3-ylacetic acid [11], and off-flavour of food can be caused by indole and skatole [12]. These compounds can as well be involved in off-flavour of animal products as milk, meat and eggs, when animals are fed with cruciferous products [1].

Determination and separation of structurally related indolyl derivatives is therefore of special interest. GC and HPLC have up to now been the most often used methods of analyses [4, 13]. Gaining from the experiences of glucosinolate analysis by micellar electrokinetic

capillary chromatography (MECC) and its advantages over HPLC methods [14, 15], focus has now been directed at MECC as a quantitative method of analysis for indolylic compounds [16]. Effects of different separation parameters including voltage, temperature and buffer composition have been studied.

Fig. 1. Names and structures of the indolyl derivatives used in the MECC analyses.



EXPERIMENTAL

Apparatus and conditions: ABI Model 270A-HT Capillary Electrophoresis System (Applied Biosystems, USA) with a 760 mm * 50 μm I.D. fused-silica capillary; initially set of separation conditions as shown in Fig. 2.

Optimization studies: Temperature, voltage, 2-propanol content in the running buffer and the chain length of the surfactant, were varied one at a time, while the other parameters were kept constant.

Surfactants: decyltrimethylammonium bromide (C-10), dodecyltrimethylammonium bromide (C-12; DTAB), tetradecyltrimethylammonium bromide (C-14; TTAB) cetyltrimethyl-

ammonium bromide (C-16; CTAB), and octadecyltrimethylammonium bromide (C-18; OTAB).

Indolylic compounds: as presented in Fig. 1, all from the laboratory collection including the glucosinolates [2, 4] and, degradation products of indol-3-ylcarbinol (**4**) including di-(indol-3-yl)methane (**4a**), indol-3-ylmethyl methylether (**4b**) 1,1,2-tri-(indol-3-yl)ethane (**4c**) and the stereoisomeric ascorbigens (**4d**, **4e**), all isolated, purified and identified by our standard laboratory procedures [4 and refs. cited therein].

Calculations as described elsewhere [15].

RESULTS & DISCUSSION

Results obtained by MECC showed that this is a promising separation technique for indolyl compounds (Fig. 2), described in details elsewhere [16].

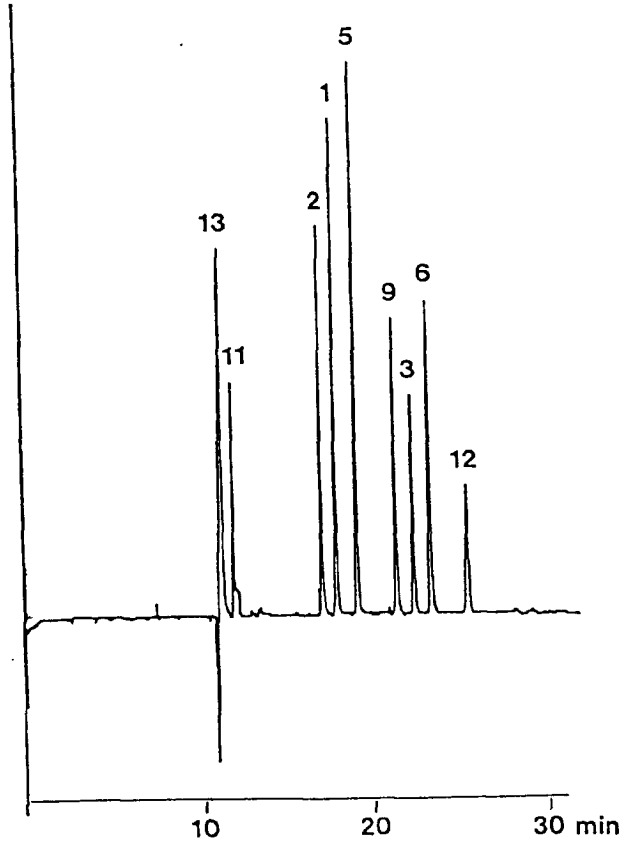


Fig. 2. Electropherogram of a mixture of nine indolylic compounds. Numbers as in Fig. 1. Conditions: Total capillary length 760 mm, detection at 530 mm from the injection end at 235 nm; 1 second vacuum injection; buffer consisting of 18 mM borate, 30 mM phospate, 50 mM DTAB and 10% 2-propanol adjusted to pH 7.0; temperature at 30°C; voltage at 20 kV.

Variation of separation parameters.

MECC using DTAB (C-12) results in positively charged micelles with flow to the cathode, opposite the electroosmotic flow [17]. Changes of voltage, temperature and the composition of the running buffer influenced the electroosmotic flow, the electrophoretic mobility, and consequently the separation of the indolyl derivatives [18]. The individual analytes were affected differently (Fig.3).

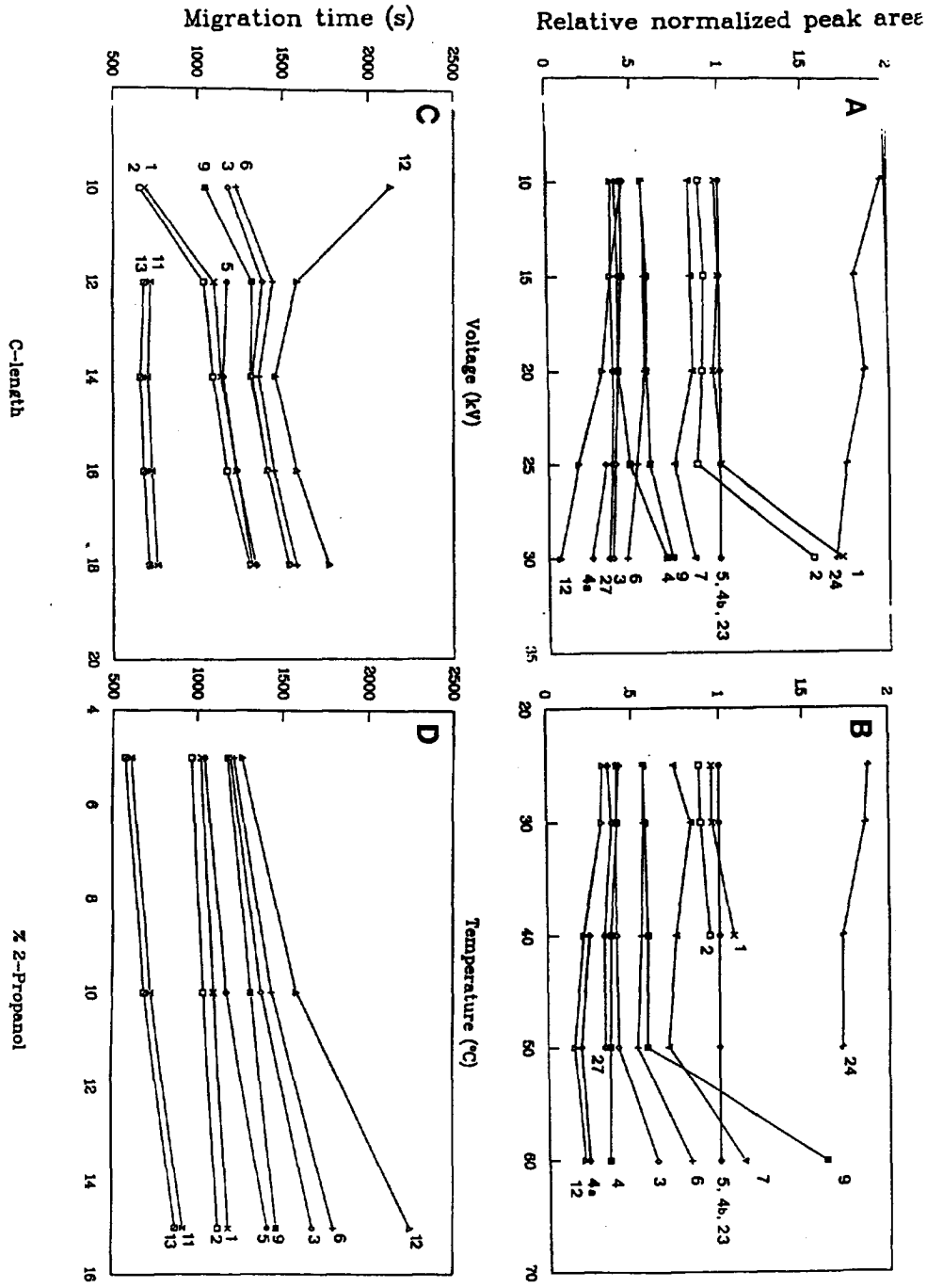


Fig. 3. Influence of varied voltage (A), temperature (B), surfactant chain length (C-length; C), and 2-propanol concentration (D) on the normalized peak areas of the indolyl derivatives calculated relative to either **5**, **4b**, or **23** (A, B) and on the migration times of the compounds (C, D), respectively. Numbers as in Fig. 1 and text, respectively. Separation conditions as in Fig. 2.

Increasing voltage and temperature reduced MT (migration times). Higher voltages and temperatures than 20kV and 50°C, respectively, had different effects on RNA (relative normalized peak areas) of **12** and **4a** compared to **1**, **2** and **9** (Fig. 3; A, B). These changes of NA (normalized peak areas) and RNA could be explained by enhanced joule heating in the capillary which is generated by increased voltage and high temperatures [19], but could also be a result of alterations in the response factors of the compounds [15].

Surfactants with increasing C-length markedly changed MT and RMT (relative migration times) of the indolyl derivatives (Fig 3; C). This might be due to changed selectivity of different micellar systems, which also affects the peak capacity [20]. Increasing amounts of 2-propanol added to the running buffer resulted in a better resolution for some compounds at the expense of a longer time of analysis [18] (Fig. 3; D). The intact indolyl glucosinolates, **23** and **27**, showed improved separation at 5% compared to 10 % 2-propanol in the buffer (Fig. 4).

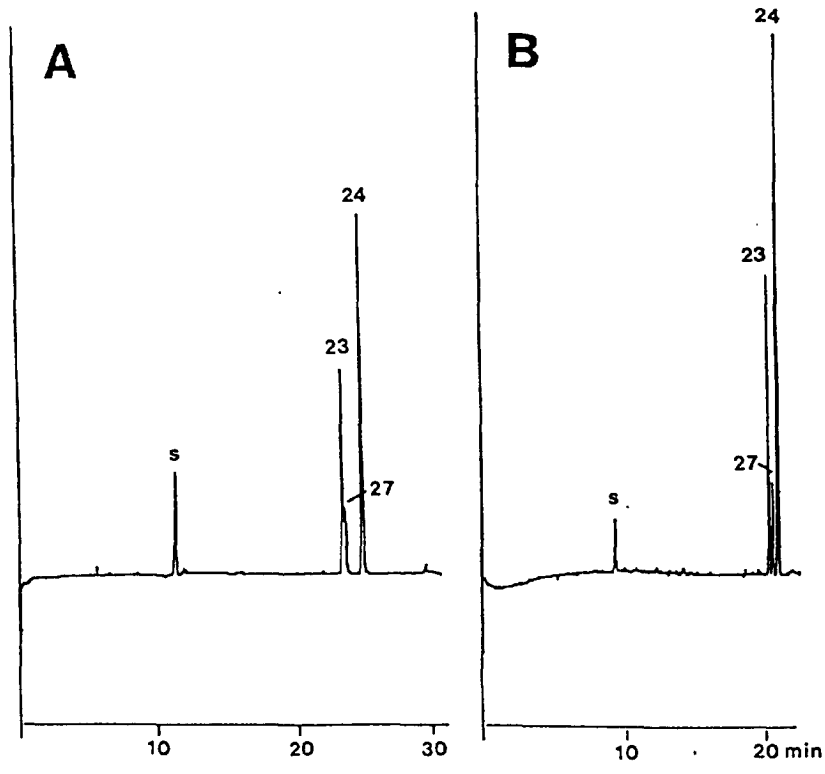


Fig. 4. Separation of indolyl glucosinolates influenced by 2-propanol content in the running buffer. Numbers as in Fig. 1. Conditions: 10% 2-propanol (A) and 5% 2-propanol (B) in the buffer. Other conditions as in Fig. 2. The solvent peak is denoted with s.

From these results 20 kV, 30°C, DTAB (C-12) and a concentration of 10 % 2-propanol were selected to obtain the best compromise between separation and acceptable migration times of the indolyl derivatives.

Each set of conditions chosen requires, that response factors are determined for correct quantitative determinations.

Repeatability and linearity.

Repeatabilities of MT, RMT, NA and RNA, were determined from several injections of the same mixture applying the optimal separation conditions established. Relative standard diviations (R.D.S.) for MT were between 0.5 and 0.9 %. When internal standards were used, R.S.D. decreased up to ten fold, and R.S.D. for NA and RNA were also found to be acceptable [16]. Improvements were obtained, when fresh standard solutions were injected only one to three times [16].

With increasing concentrations of the compounds NA values showed linear increases with correlation coefficients between 0.9924 and 0.9996, which are acceptable. However, use of internal standards is recommended, since it improves repeatability and linearity of the method and hence accuracy of quantitative determinations.

Migration order.

Functional groups as well as size of the molecules influenced separation and the migration order markedly [16] as also found for glucosinolates [15]. Partitioning of solutes between the micellar phase and the aqueous phase in MECC [19], may occur through surface interaction or through penetration of the solute into the micelle interior, or eventually after ion-pair formation [21].

CONCLUSIONS

- The MECC method developed has several advantages compared to reversed-phase HPLC. It is thus simple, inexpensive in column material and running costs, and changing of separation parameters is very easy and fast.

- Efficiency of the method is high, resulting in up to 370 000 theoretical plates/m for indolylic compounds.

- Repeatability and linearity of the method were acceptable for quantitative analysis of the compounds.

- The MECC method is well suited for determination of intact glucosinolates, indolyl derivatives including indol-3-ylcarbinol and various other products of glucosinolates. This can even be performed in the same run within an acceptable time of analysis.

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REFERENCES

1. Eggum, B. O., Olsen, O. and Sørensen, H. (1985) *in* Advances in the Production and Utilization of Cruciferous Crops, (Sørensen, H. Ed.) Vol. 11, pp. 50-60, Martinus Nijhoff/Dr. W. Junk, Dordrecht, Bosten, Lancester.
2. Jensen, S. K., Michaelsen, S., Kachlicki, P., and Sørensen, H. (1991) *in* Proceedings GCIRC Rapeseed Congress, Saskatoon Canada, (McGregor, D. I., Ed.) Vol. 5, pp. 1359-1364, Organization Commitee of the Eighths International Rapeseed Congress.
3. McDanell, R., McLean, A. E. M., Hanley, A. B., Heaney, R. K., and Fenwick, G. R (1988) *Chem. Toxic.*, **26** (1), 59-77.
4. Sørensen, H. (1990) *in* Shahidi, F. (Editor), Rapeseed/Canola: Production, Chemistry, Nutrition and Processing Technology, Van Nostrand Reinhold, New York, Ch.9, pp. 149-172.
5. Loft, S., Otte, J., Poulsen, H. E., and Sørensen, H. (1992) *Fd Chem. Toxic.*, **30**, 927-935.
6. Young, and T. B., Wolf, D. A. (1988) *Int. J. Cancer*, **42**, 167-175.
7. Bradlow, H. L., Michnovicz, J. J., Telang, N. T., and Osborne, M. P. (1991) Carcinogenesis, **12**, 1571-1574.
8. Loub, W. D., Wattenberg, L. W., and Davis, D. W. (1975) *J. Natl. Cancer Inst.*, **54**, 985-988.
9. Park, J.-Y., and Bjeldanes, L. F. (1992) *Chem.-Biol. Interactions*, **83**, 235-247.
10. Jellinck, P. H., Fokkert, P. G., Riddick, D. S., Okey, A. B., Michnovicz, J. J., and Bradlow, H. L. (1993) *Biochem. Pharmacol.*, **45**, 1129-1136.
11. Wang, Q., Little, C. H. A., Sheng, C., Odén, P. C., and Pharis, R. P. (1992) *Physiologia Plantarum*, **86**, 202-208.
12. Claus, R., Dehnhard, M., Herzog, A., Bernal-Barragan, H., and Gimenez, T. (1993) *Livest. Prod. Sci.*, **34**, 115-126.
13. Slominsky, B. A., and Campbell, L. D. (1989) *J. Agric. Food Chem.*, **37**, 1297-1302.
14. Michaelsen, S., Møller, P., and Sørensen, H. (1991) *Bull. GCIRC*, **7**, 97-106.
15. Michaelsen, S., Møller, P., and Sørensen, H. (1992) *J. Chromatogr.*, **608**, 363-374.
16. Feldl, C., Møller, P., Otte, J. and Sørensen, H. (1993) *Anal. Biochem., in press.*
17. Otsuka, K., Terabe, S., and Ando, T. (1985) *J. Chromatogr.*, **332**, 219-226.
18. Li, S. F. Y. (1992) J. Chromatogr. Library (Li, S. F. Y., Ed.), Vol. 52, pp.201-293, Elsevier, Amsterdam London New York Tokyo.
19. Terabe, S., Otsuka, K., and Ando, T. (1985) *Anal. Chem.*, **57**, 834-841.
20. Burton, D., Sepaniak, M., and Maskarinek, M. (1987) *J. Chromatogr. Sci.*, **25**, 514-518.
21. Wallingford, R. A., and Ewing, A. G. (1988) *J. Chromatogr.*, **441**, 299-309.