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Structure and Properties of ascorbigens and other transformation products of indolyl glucosinolates ; potential anticancerogens

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INTRODUCTION

Indolyl glucosinolates (indol-3-ylmethyl-glucosinolates) are natural products occurring in plants of the order Cappareles (1). They are degraded to various products, of which indol-3-yl-carbinol is among the compounds considered in relation to cancer (Fig.1). By different mechanisms, products from indolyl glucosinolates seem to have cancer modulating properties, usually not seen for low molecular weight compounds (2). However, effects described for indol-3-ylcarbinol were at least partly due to its transformation into oligomeric produts (2, 3) in aqueous solutions (4). In the presence of ascorbic acid, indol-3-ylcarbinol can be transformed into ascorbigens (5) (Fig.1) Owing to the physiological effects caused by products from indol-3-ylmethylglucosinolates (6), information is needed about the identity and stability of these transformation products, but also how fast they are produced in order to estimate the real biologically active compounds.

In the present study, the transformation of indol-3-ylcarbinol in aqueous solution has been investigated. In situ reaction products of indol-3-ylcarbinol and further transformation products thereof have been purified and identified. Influences of the reaction conditions including light, different solvent systems, the presence of ascorbic acid in the solution, the starting concentration of indol-3-ylcarbinol on the composition of the reaction mixture and its stability as a function of the time have been studied.

EXPERIMENTAL

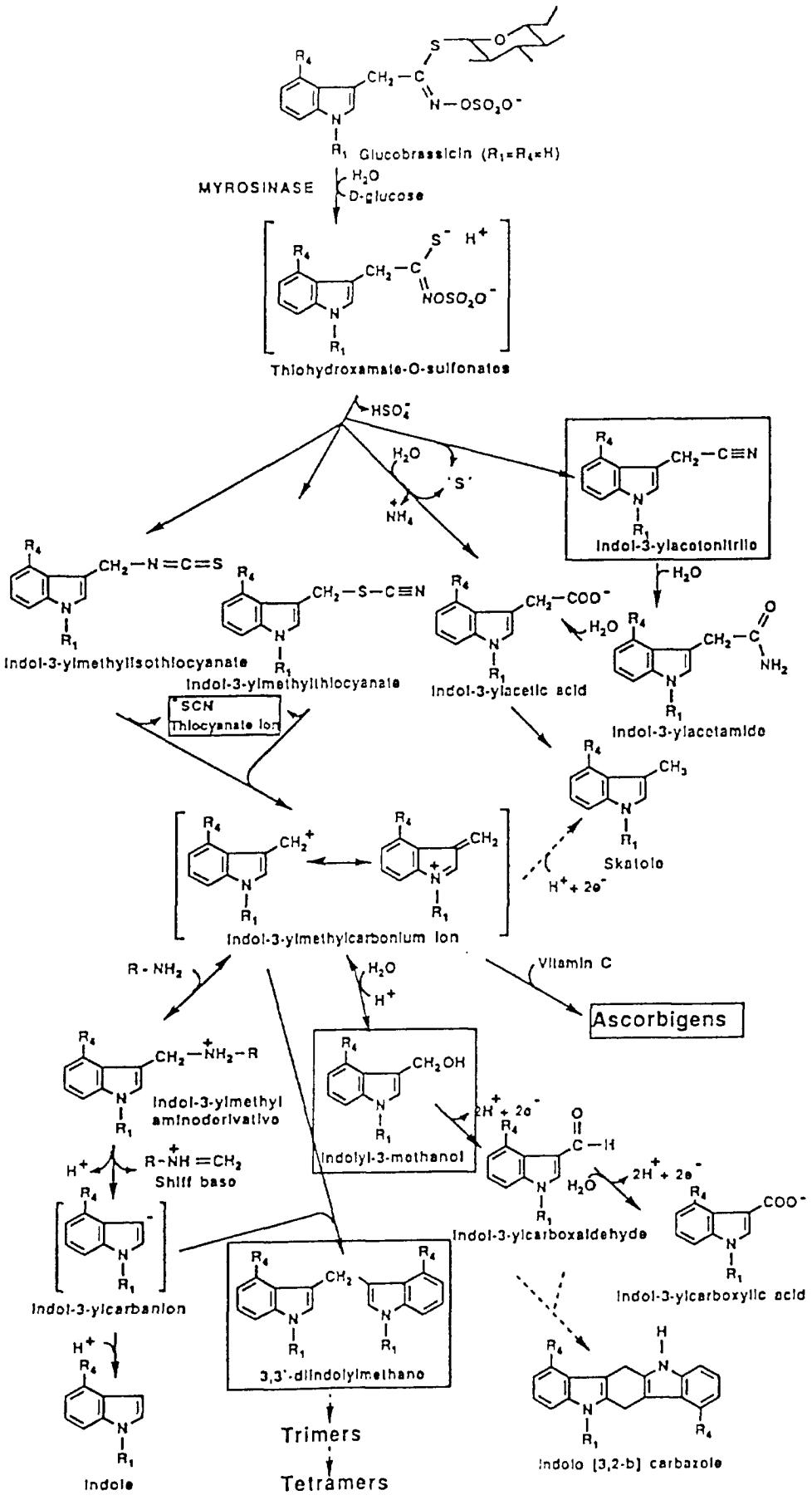
Separation and identification techniques have comprised traditionally used high voltage electrophoresis, chromatography, ion exchange chromatography, gel filtration, HPLC and spectroscopy ; UV, NMR, MS (1). In addition, a new technique based on micellar electrokinetic capillary electrophoresis (MECC) developed for indolyl derivatives has been used (7).

Instrumentation : ABI Model 270A-HT Capillary Electrophoresis System (Applied Biosystems) ; AC 250 P (Bruker) for NMR ; AX 505 W (Jeol JMXS) for MS.

Degradation studies : indol-3-ylcarbinol (4 ; Sigma) was dissolved for two minutes in different systems. Then, samples (50~l) were centrifuged, and analysed immediately by MECC. This time point was set zero in the time course study of degradation. Further samples were taken at distinct time intervals. When insoluble compounds were produced, supernatant and pellet were analysed separately.

Ascorbigen synthesis (5) from indol-3-ylcarbinol and ascorbic acid in Mc Ilvain buffer pH 4.0 was followed by MECC as for the degradation studies, and the optimal conditions and time were used for production of appreciable amounts of ascorbigens.

Fig. 1 : Degradation of indol-3-ylmethylglucosinolates (reproduced from ref. 6)



RESULTS

Identified products.

Different solubility behaviours of the compounds produced by transformation of **4** were advantageous for purification and identification. Indol-3-ylmethyl methylether (**4b**), ascorbigens (**4d, 4e**), di-(indol-3-yl)methan (diindolyl, **4a**) and 1,1,2 -tri (indol-3-yl)ethane (**4c**) and 1,2,2,3 -tetra-(indol-3-yl)propane (teraindolyl) could be identified (Fig. 2).

Indol-3-ylcarbinol (4) degradation.

Transformation of **4** dissolved in a methanol containing solution was studied for several days (Fig.3). Within the first few hours, **4** decreased about one third. Diindolyl (**4a**) was produced at once and its concentration increased rapidly, but then, the **4a** concentration decreased again, while a new compound the triindolyl (**4c**) increased in concentration during the next 2 days. Another compound in which **4** was transformed into was the methanol derivative **4b** produced by the reaction with carbonium ion and methanol. The highest relative amount of **4b** in the solution was obtained after 5 to 6 days.

Day light influenced the relative amounts of the compounds in solution, but not the transformation pattern (Fig. 3, Fig. 4A). Thus, **4** was transformed more slowly into the diindolyl (**4a**), the concentration of which decreased faster in the supernatant after reaching a maximum. This could be explained by a faster diindolyl synthesis and at the same time faster transformation into the trimer (4c) than in the dark.

Fig. 2 : Identified transformation products : A. Structure of indol-3-ylmethyl methylether (4b), B. NMR spectrum of ascorbigen (4d), C. MS spectrum of diindolyl (4a) and D. MS spectrum of triindolyl (4c) with traces of tetraindolyl (4cte).

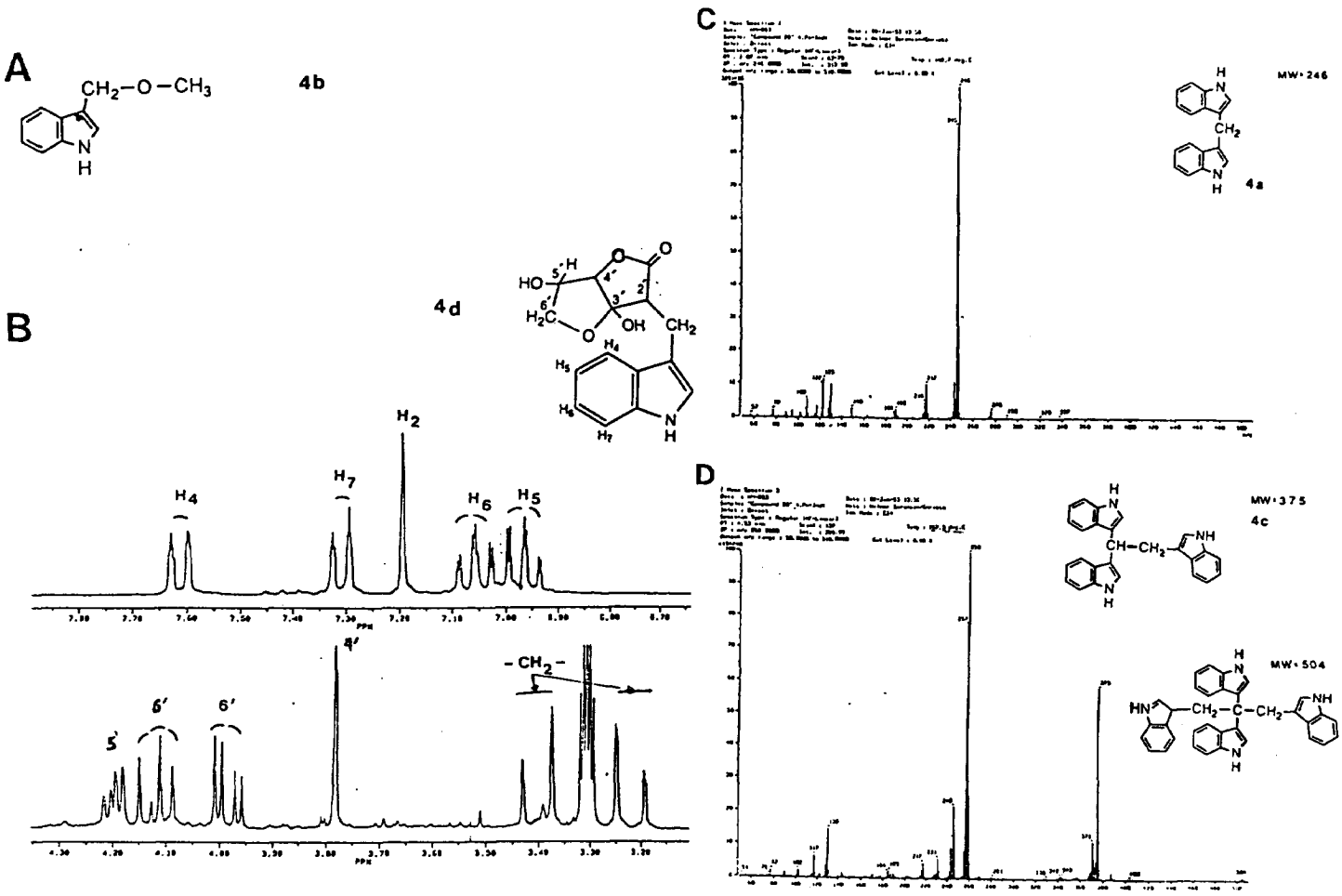
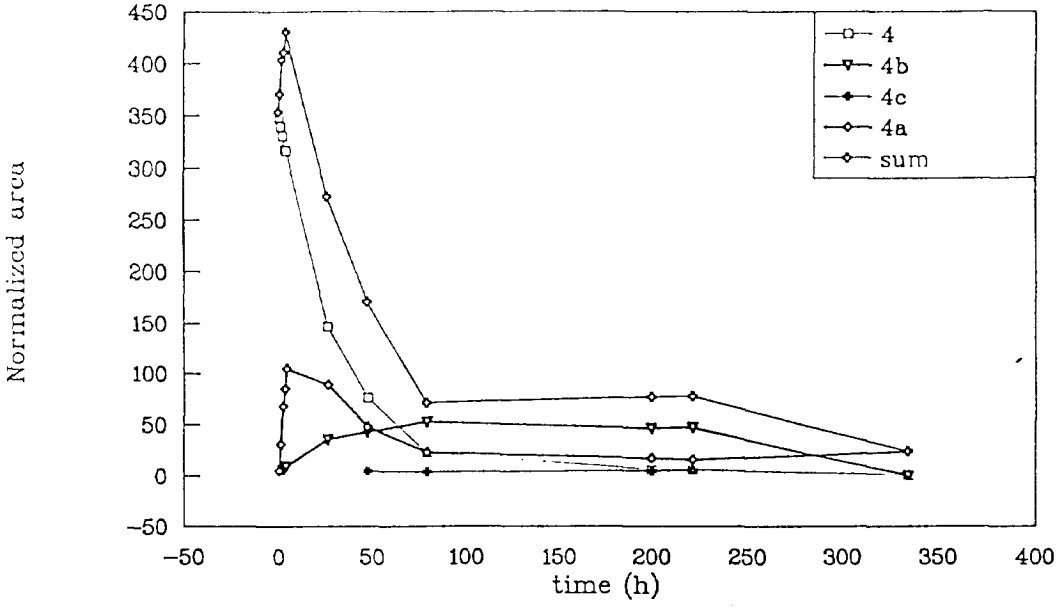


Fig. 3 : Indol-3-ylcarbinol degradation in MeOH containing solution under day light followed by MECC analyses (7). Normalized peak areas of the compounds in solution changed as a function of the time. Numbers for transformation products as in Fig. 2. The total normalized peak areas of all compounds in solution is denoted with sum.



Different solvent systems (Fig. 4) resulted in different products (compound **4b**) in A and ascorbigen in C), but also in different amounts of the same products within the same time period (A,B). In this experiment, all samples were kept in the dark to exclude the effect of light.

In the presence of ascorbic acid, 4 was efficiently transformed into ascorbigens and only a little amount of diindolyl was found in the pellet. The ascorbigens were relatively stable under these conditions (Fig. **4C**) compared to other transformation products of **4** in other systems (A,B) withing the same time period. **Higher starting concentrations** of **4** resulted in a higher amount of insoluble pellet in C.

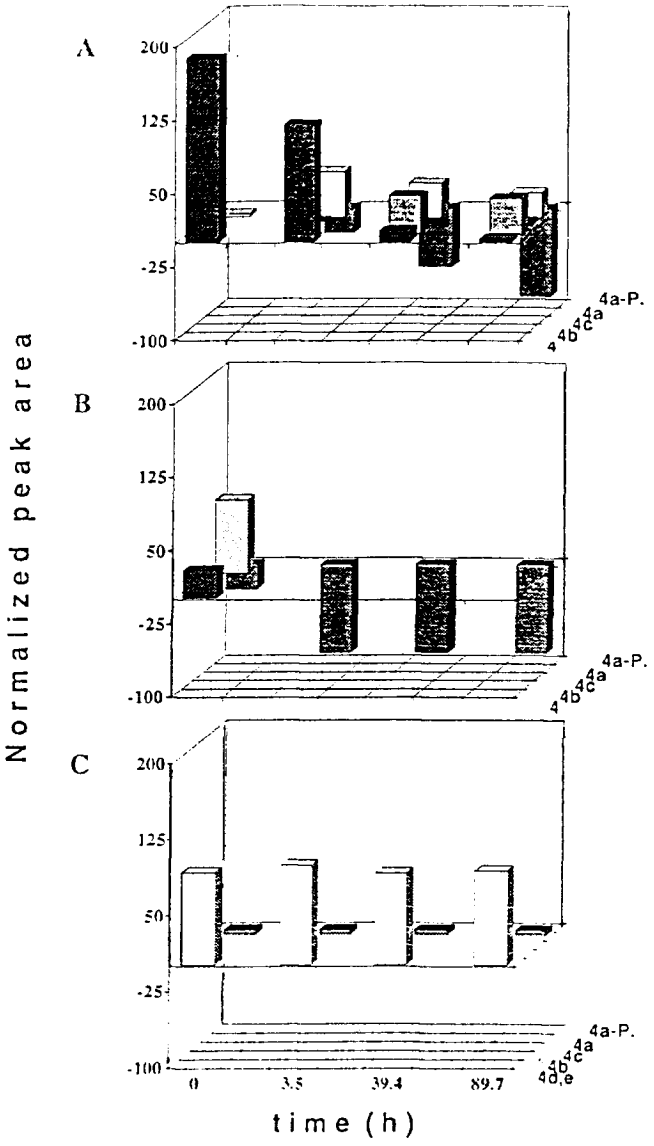
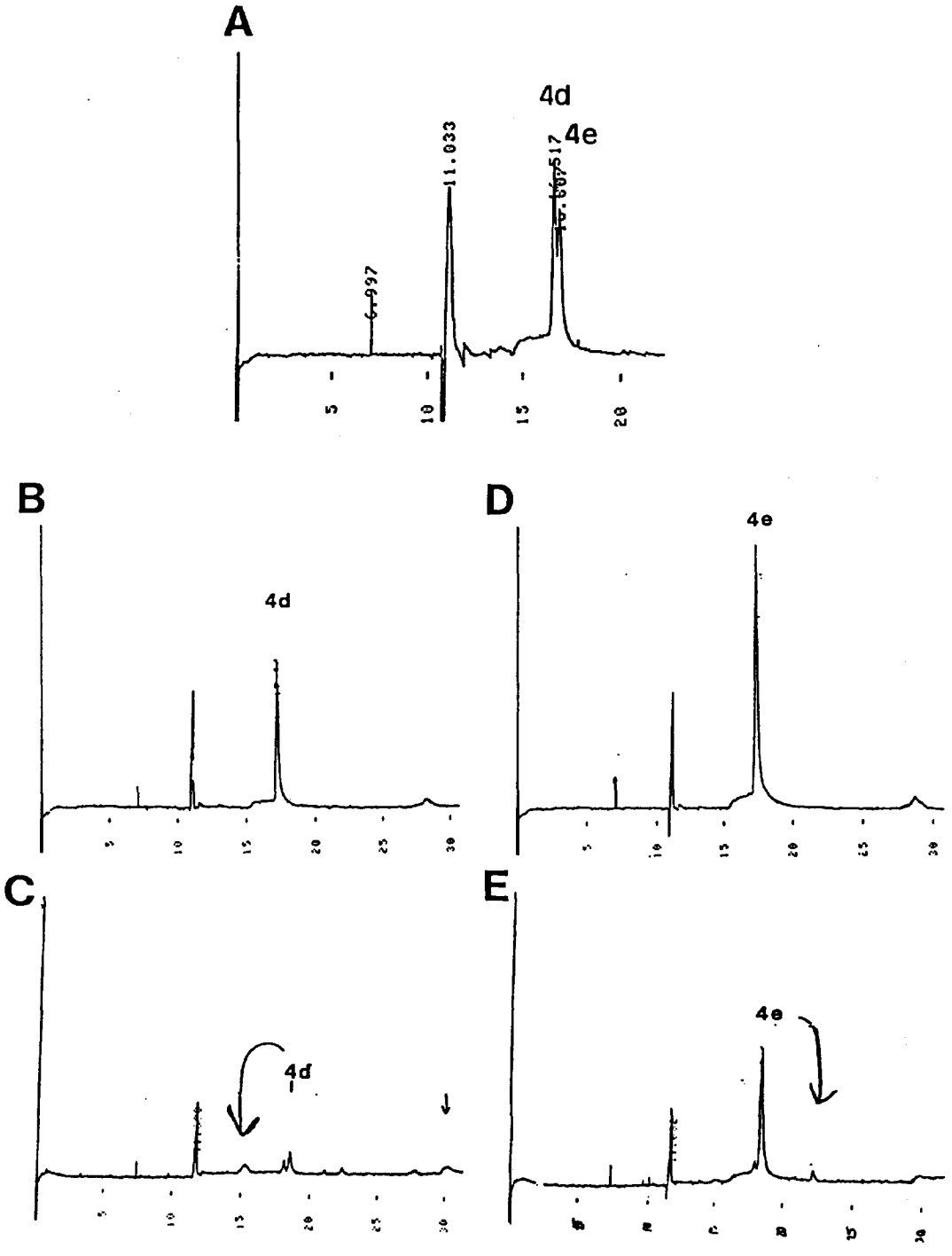


Fig. 4 : Indol-3-ylcarbinol degradation in different solvent systems followed by MECC analyses (7). Normalized peak areas of the compounds in solution (positive numbers) and in the pellet (negative numbers ; not quantitative) changed as a function of the time. A MeOH containing solution, B. McIlvain buffer (McI) without ascorbic acid (- a.a.), and C. McIlvain buffer with ascorbic acid in the solution (+ a.a.) All vials were kept in the dark. Numbers for transformation products as in Fig. 2.

Ascorbigens.

For ascorbigens (Fig. 2), only one of two possible configurations at C3 in the ascorbic acid part exists. During the synthesis, there should however be an equal chance for building both of the two stereoisomers corresponding to different chirality at C2 in the ascorbic acid part. The electropherograms, indeed, revealed two peaks (4d, 4e ; Fig. 5A) identified as ascorbigens with only small, but not significant differences in the NMR spectra. Both compounds isolated showed more instability in aqueous solution than under the conditions of C in Fig. 4 and they were transformed into different compounds (Fig.5 B, C compared to degraded Fig.5 D, E). In addition, a yellow colour appeared in the solution when 4e was degraded (Fig. 5 E) indicating formation of a compound with an additional conjugated system. Thus, the peak in MECC at about 22 minutes had also an additional absorption maximum at 365 nm, which could be explained by elimination of water between the methylene carbon and C2 in the ascorbic acid part.

Fig. 5. Electropherograms (7) of ascorbigens. A. Stereoisomer ascorbigens 4d, 4e after synthesis. B. Isolated 4d. C. 4d after degradation over days. D. Isolated 4e. E. 4e after degradation for the same time as for 4d. Numbers for transformation products as in Fig. 2.



CONCLUSIONS

- A MECC method for the analysis of indolyl derivatives combined with NMR and MS have been a powerful tool to follow the degradation of indol-3-ylcarbinol in detail and to identify the transformation products formed.

- The indol-3-ylcarbinol degradation occurred very fast and was dependent on the compo-sition of the solution ; product yields were influenced by light and the starting concentration of indol-3-ylcarbinol.

- In the presence of ascorbic acid, indol-3-ylcarbinol was at once and completely transformed into ascorbigens, diindolyl and related compounds.

- Without ascorbic acid in the solution, diindolyl (**4a**), triindolyl (**4c**) and tetraindolyl (**4cte**) are formed, where the dimer appeared as intermediate for higher oligomers.

- The reactivity of indol-3-ylcarbinol and the presence of ascorbic acid under physiological conditions have to be considered in studies on biologically active compounds in relation to cancer.

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