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# SINAPINE-*O*-β-D-GLUCOPYRANOSIDE IN SEEDS OF *ALLIARIA OFFICINALIS*

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**Key Word Index**—*Alliaria officinalis*, Brassicaceae, choline esters, sinapine-*O*-glucopyranoside, 4-β-D-glucopyranosyloxy-3,5-dimethoxycinnamoylcholine, glucosinolates, allylglucosinolate, sinigrin

**Abstract**—4-β-D-Glucopyranosyloxy-3,5-dimethoxycinnamoylcholine has been isolated from *Alliaria officinalis* This natural product has not been described previously It is the major component among the choline esters isolated from seeds of *A officinalis* A modified ion-exchange technique was used for the isolation of the total fraction of choline esters Confirmation of the structure was obtained by comparison with synthetic reference compounds and by transformation of the natural product into known hydrolytic products Both *cis* and *trans* isomers of the natural product were present in the isolated choline ester fraction Choline esters and basic amino acids in seeds of *A officinalis* account for *ca* 1 5%, and glucosinolates, mainly allylglucosinolate, account for *ca* 3% of the seed weight

## INTRODUCTION

Choline esters are well-known from the plant kingdom [1, 2], and it appears that appreciable amounts of phenolic choline esters occur in seeds of some glucosinolate-containing plants Several reports describe the occurrence of sinapine in crucifer seeds, and two benzoic acid derivatives and three cinnamic acid derivatives have been described as plant products while several unidentified choline esters await identification [3]

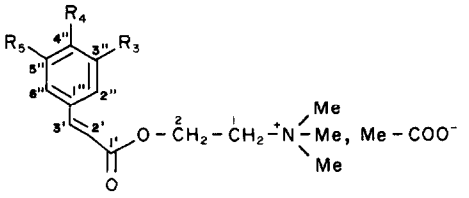
The present work is a continuation of our previous investigations on the low MW constituents in glucosinolate-containing plants We now report the isolation of glucosinolates, mainly allylglucosinolate and the choline esters present in seeds of *Alliaria officinalis* It is shown that 4-β-D-glucopyranosyloxy-3,5-dimethoxycinnamoylcholine (**1**) occurs in an appreciable amount in this plant This is the first report on a choline ester which is also a glycoside The presence of both the *cis* and *trans* isomers of **1** in the isolated choline ester fraction is briefly discussed

## RESULTS AND DISCUSSION

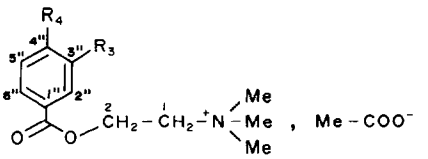
Extracts containing the total amount of intact glucosinolates and choline esters present in the seeds were obtained by established methods [3, 4] Investigations of these extracts by PC and high voltage electrophoresis (HVE) revealed the presence of minor amounts of sinapine (**2**) and two quantitatively dominating unknown compounds, both of which reacted with Dragendorff's reagent [3] The total amount of choline esters was isolated by ion-exchange chromatography using the previously described CM-Sephadex C-25 column [3] connected in series to Amberlite and Ecteola columns [4] Separation of the choline esters was performed by use of CC and prep PC One of the two unknown compounds was identified as the β-D-glucopyranoside of sinapine (**1**), while the other compound (**3**) was shown not to be a choline ester

Chromatographic and HVE data of **1**, **3** and some reference compounds are presented in Table 1 The data for **2**, **5** and sinapic acid have previously been reported [3], they are included in Table 1 for comparison Detection of the choline esters was performed by use of Dragendorff's reagent as well as by observation of the different coloured spots they produced on chromatograms in UV light [3] Isolated **1** produced a spot in UV light with a colour similar to those observed for **2** and **4** but clearly distinct from both of these A dark blue spot in UV light was produced from **3**, and no colour changes on exposure to ammonia vapour were observed for either **1** or **3** In accordance with the proposed structure, the data in Table 1 reveal that **1** has more hydrophilic properties than **2**–**5** The higher MW for **1** compared to **2**–**5** and for **8** (see below) compared to sinapic acid are also supported by the HVE properties The UV spectrum of **1** dissolved in water showed an absorption peak at 315 nm and no free phenol group was revealed in alkaline solution Nearly the same UV absorption was found for **3**

Chemical shifts from $^{13}C$ NMR spectra of **1** and **2** are shown in Table 2 These data and corresponding data for other glycosides [7, 8] reveal the presence of the β-D-glucopyranosyloxy part of **1**, and the upfield shift of C-1″ and C-4″ as well as the downfield shifts of C-3″ and C-5″ in **1**, show that glucose is connected to C-4 of the phenolic moiety The shift values of **1** and **2**, in combination with previously reported spectra of other phenolic choline esters [3], confirm the structures for **2** and the hitherto undescribed **1** The $^1H$ NMR spectra of the acetate salts of **1** and **2** in $D_2O$ solutions (see Experimental) also confirm the proposed structures and reveal the β-glucosidic structure of **1** as well as the *E*-(*trans*) configurations of both **1** and **2** in accordance with the reported $^1H$ NMR spectra of **4** [5] and **5** [9] The $^1H$ NMR spectrum of **1** revealed the presence of minor amounts of the *Z*-(*cis*) isomer A new sample of **1**, isolated from the same batch of seeds but subjected to lyophilization in all of the concentration steps, was found by the use of $^1H$ NMR to contain the *cis*



| | $R_3$ | $R_4$ | $R_5$ | Ref |
|---|---|---|---|---|
| Sinapine-β-D-gluco pyranoside (1) | OMe | β-D-gluco-pyrano-syloxy | OMe | |
| Sinapine (2) | OMe | OH | OMe | [2] |
| Feruloylcholine (4) | OMe | OH | H | [5] |
| Isoferuloylcholine (5) | OH | OMe | H | [6] |



| | $R_3$ | $R_4$ | Ref |
|---|---|---|---|
| Hesperaline (6) | OMe | OMe | [1] |
| 4-Hydroxybenzoyl choline (7) | H | OH | [3] |

Table 1 $R_f$-values and ionic mobilities of choline esters isolated from *A officinalis* and of some reference compounds

| | $R_f$-values from PC in solvent* | | | $R_f$-values from TLC on Si-gel in solvent* | | $R_f$-values from TLC on cellulose in solvent* | | HVE mobilities in buffer system* (cm) | |
|---|---|---|---|---|---|---|---|---|---|
| Compound† | 1 | 2 | 3 | 1 | 4 | 1 | 4 | pH 1 9 | pH 6 5 |
| Sinapine-*O*-β-D-glucopyranoside (1) | 0 21 | 0 93 | 0 04 | 0 10 | 0 13 | 0 45 | — | −8 5 | −9 1 |
| Sinapine (2) | 0 57 | 0 95 | 0 14 | 0 32 | 0 15 | 0 90 | 0 66 | −10 1 | −10 6 |
| Unidentified compound (3) | 0 57 | 0 95 | 0 99 | — | 0 20 | — | — | −9 2 | −9 5 |
| Feruloylcholine (4) | 0 65 | 0 93 | 0 21 | 0 38 | 0 19 | 0 99 | 0 65 | −10 9 | −11 4 |
| Isoferuloylcholine (5) | 0 62 | 0 95 | 0 18 | 0 38 | 0 16 | 0 94 | 0 66 | −11 3 | −11 7 |
| Sinapic acid-*O*-β-D-glucopyranoside (8) | 0 54 | — | — | 0 71 | 0 81 | 0 89 | 0 82 | −0 4 | 3 7 |
| Sinapic acid | 0 80 | 0 85 | 0 19 | 0 85 | 0 95 | 0 98 | — | −0 5 | 4 6 |

*For solvent and buffer systems, see Experimental
†The *E*-(*trans*) isomers

and *trans* isomers in a 3 7 ratio This indicates that the *cis* isomer is also a constituent of the seeds Isomerization between the *cis* and *trans* forms in the isolation steps is possible, but the *trans* isomer is considered to be the more stable of these two isomers

Hydrolysis of **1** in 1 M ammonium hydroxide at 45° transformed **1** into choline and β-D-glucopyranosyloxy-3,5-dimethoxycinnamic acid (**8**) in the course of 10 min Hydrolysis of **1** in 1 M hydrochloric acid at 45° resulted in only a few percent transformation of **1** into **2** and glucose

Table 2 $^{13}C$ NMR chemical shifts ($\delta$) for the different carbon atoms in choline esters isolated from *A officinalis*

| | Choline moiety of | | Moiety of carboxylic acid side chain in | | Phenolic moiety of | | Glucose moiety |
|---|---|---|---|---|---|---|---|
| | **1** | **2** | **1** | **2** | **1** | **2** | of **1** |
| C-1 | 65 5 | 65 1 | — | — | — | — | — |
| C-2 | 59 2 | 58 7 | — | — | — | — | — |
| C-1′ | — | — | 168 5 | 167 0 | — | — | — |
| C-2′ | — | — | 117 5 | 115 1 | — | — | — |
| C-3′ | — | — | 146 9 | 147 3 | — | — | — |
| C-1″ | — | — | — | — | 118 8 | 125 3 | — |
| C-2″ | — | — | — | — | 107 0 | 107 5 | — |
| C-3″ | — | — | — | — | 153 3 | 149 1 | — |
| C-4″ | — | — | — | — | 131 8 | 139 8 | — |
| C-5″ | — | — | — | — | 153 3 | 149 1 | — |
| C-6″ | — | — | — | — | 107 0 | 107 5 | — |
| C-1‴ | — | — | — | — | — | — | 103 7 |
| C-2‴ | — | — | — | — | — | — | 74 5 |
| C-3‴ | — | — | — | — | — | — | 77 2 |
| C-4‴ | — | — | — | — | — | — | 70 0 |
| C-5‴ | — | — | — | — | — | — | 76 5 |
| C-6‴ | — | — | — | — | — | — | 61 3 |
| OMe | — | — | — | — | 57 1 | 57 2 | — |
| $NMe_3$ | 54 6 | 54 1 | — | — | — | — | — |

in the course of 2 hr, whereas reflux of the acidic solution for 4 hr transformed **1** into glucose, sinapic acid and choline The PC and HVE properties of **8** and sinapic acid are shown in Table 1, the UV properties of **8** are nearly the same as mentioned above for **1**

The results from hydrolysis of **1** in alkaline and acidic solutions support the structure of this new natural product In addition, in accordance with previously reported results [3], it reveals that alkaline conditions need to be avoided during the isolation steps and in the methods used for quantitative choline ester analysis The total fraction of glucosinolates isolated from the seeds of *A officinalis* accounted for 3% by weight and more than 95% consisted of allylglucosinolate Previously, it has been shown that the leaves of this plant contain allylglucosinolate as the dominating glucosinolate [10] The content of choline esters in *A officinalis* seeds accounted for less than 1 5% by weight Quantitatively dominating among the choline esters was compound **1** which made up *ca* 50% A minor amount of **2** (*ca* 5% of the amount of **1**) was also present The unidentified compound **3** was shown by use of $^1H$ NMR spectroscopy not to be a choline ester

## EXPERIMENTAL

*Plant material* Seeds of *Alliaria officinalis* Andrz were obtained from the Botanical Garden of the Royal Veterinary and Agricultural University, Copenhagen

*General methods and instrumentation* Methods and equipment used for $^1H$ and $^{13}C$ NMR, HVE, PC and TLC have been described elsewhere [3, 11] The solvents used for chromatography were (1) *n*-BuOH–HOAc–$H_2O$ (12 3 5), (2) PhOH–$H_2O$–13M $NH_4OH$ (120 30 1) (w/v/v), (3) *iso*-PrOH–$H_2O$–13M $NH_4OH$ (8 1 1), HOAc–$H_2O$ (6 94) Buffer systems used for HVE were (1) pH 1 9 (HOAc–$HCO_2H$–$H_2O$) (4 1 45), (2) pH 6 5 (pyridine–HOAc–$H_2O$) (25 1 500)

*Isolation of choline esters and glucosinolates* Homogenization and extraction were performed as previously described [3, 4] The extraction residue from 30 g of seeds was dissolved in $H_2O$ (20 ml) and applied to a column (1 5 × 30 cm) of CM Sephadex C-25 in the $H^+$-form connected in series to a column of Amberlite IR 120 ($H^+$, 2 5 × 40 cm) which again was connected in series to a column of Ecteola cellulose ($AcO^-$, 2 5 × 30 cm) The three columns were flushed with $H_2O$ (3 l) after which the glucosinolates were isolated from the Ecteola column [4] Investigation of the glucosinolate fraction by HPLC, GC, PC and HVE as previously described [4] revealed only sinigrin in this fraction

The choline esters were isolated from the Sephadex column by use of 2 M HOAc as eluant Fractions (12 ml) were collected at 40 ml/hr Fractions 5–14 (208 mg), 15–23 (236 mg), 24–30 (74 mg), 31–55 (20 mg) and 56–100 (20 mg) contained basic amino acids (lysine, arginine), choline esters and **3** The residue from fractions 5–14 was dissolved in $H_2O$ (2 ml) and applied to a column (1 0 × 80 cm) of Polyclar AT The column was eluted with MeOH–$H_2O$ (1 1), fractions (8 ml) were collected at 40 ml/hr Fractions 8 (11 mg), 9 (60 mg), 10 (42 mg), 11 (12 mg) and 12–14 (5 mg) contained mainly **1** and **2** Compounds **1** and **3** in fraction 10 were separated by prep PC in solvent 1 which resulted in 10 mg **3** and 19 mg semicrystalline **1** The compounds were identified by well-established methods and the results from HVE, PC, TLC and $^{13}C$ NMR are shown in Tables 1 and 2 The $^1H$ NMR spectra of salts of **1** and **2** (acetates) exhibited signals at the following values, the results for **2** [3] are included for comparison with the results obtained for **1** Compound **1** (*E*-isomer), 7 7 (1H, *d*, J = 16 Hz), 7 1 (2H, *s*), 6 6 (1H, *d*, *J* = 16 Hz), 5 1 (1H, *m*), 4 6 (2H, *m*), 3 1–4 3 (23H, *m*), 1 95 (3H, *s*), compound **1** (*Z*-isomer), 7 2 (1H, *d*, *J* = 11 Hz), 7 0 (2H, *s*), 6 1 (1H, *d*, *J* = 11 Hz), 5 1 (1H, *m*), 4 6 (2H, *m*), 3 1–4 3 (23H, *m*), 1 95 (3H, *s*), compound **2**, 7 7 (1H, *d*, *J* = 16 Hz), 7 0 (2H, *s*), 6 5 (1H, *d*, *J* = 16 Hz), 4 5 (2H, *m*), 3 7 (2H, *m*), 3 2–3 3 (15H), 1 95 (3H, *s*)

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