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New thin-layer chromatographic solvent systems for glucosinolates (mustard oil glucosides)*

WAGNER *et al.*[1] separated six glucosinolates by thin-layer chromatography (TLC) on Silica Gel G using a solvent system: *n*-butanol–*n*-propanol–acetic acid–water (3:1:1:1). However, several glucosinolates (benzyl- and 2-hydroxy-2-phenylethyl glucosinolate, benzyl- and 2-phenylethylglucosinolate, *p*-hydroxybenzyl- and 3 methylthiopropylglucosinolate etc.) are incompletely separated by their solvent and the development time is long.

The author has developed new solvent systems which afforded a rapid and improved resolution of a number of glucosinolates. The removal of allylglucosinolate and benzylglucosinolate from developed TLC plates was also studied to determine whether glucosinolates may be recovered without decomposition.

Methods

The glucosinolates used in this investigation were isolated from plants, except 2-phenylethylglucosinolate which was synthesized in our laboratory, and *p*-hydroxyl benzylglucosinolate which was obtained from Calbiochem (Los Angeles, U.S.A.) Plates 0.25 mm (analytical) or 0.40 mm (preparative) thick were prepared using a suspension of 30 g Silica Gel G (E. Merck, Darmstadt, G.F.R.) containing 0.3 g green fluorescent indicator (M. Woelm, Eschwege, G.F.R.) in 65 ml distilled water and activated at 120° for 1 h. A 2 μl aliquot of a 0.25% (w/v) glucosinolate solution was applied to the starting line 2 cm above the bottom of the plate. The plate was developed until the solvent front was 10 cm from the origin. The developed plates were allowed to stand for 30 min at room temperature, and, in addition, the plates developed by solvent systems IV to VII were dryed for 10 min at 110°. The glucosine olates were observed as absorbing areas under UV light of short wave length (254 nm) or as yellow spots after treatment with iodine vapor.

The stability of allyl- and benzylglucosinolate during TLC and the subsequen recovery of these glucosinolates from developed plates was examined as follows A 100 μl aliquot of a 1% (w/v) glucosinolate solution was applied to a 20 × 20 cm plate 0.40 mm thick. After developing and drying, the silica gel area containing the glucosinolate was removed and the glucosinolates eluted with 80% ethanol. The ethanol eluate was concentrated *in vacuo* and an aliquot was analyzed by TLC (analytical plate, 0.2 mm) using solvent system I.

Results and discussion

The R_F values obtained with the solvent systems are shown in Table I. Solver system VII was a slight modification of the system of WAGNER *et al.* Compared to their original solvent system, system VII produced a similar pattern of separation of the glucosinolates although with higher R_F values. The glucosinolates appeared as compact, well defined spots when all seven solvent systems were employed. It was possible to resolve completely glucosinolates 1, 2, 3, 4 and 9, plus any one of 5, 6 or

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TABLE I

VALUES OF GLUCOSINOLATES

Figures show R_F values multiplied by 100 and figures in parentheses show relative R_F values to that of allylglucosinolate. Solvent systems: (I) methyl ethyl ketone–ethanol–water (9:1:2); (II) acetone–chloroform–ethanol–water (6:1:1:1); (III) acetone–chloroform–ethanol–water (6:3:3.4:3); (IV) *n*-propanol-ethyl acetate–water (7:1:2); (V) *n*-butanol–benzene–ethanol–28% ammmonium hydroxide (4:1 2:3); (VI) *n*-butanol–*n*-propanol–pyridine–water (6:1:1:2); (VII) *n*-butanol–*n*-propanol–acetic acid–water (3·1:1:2).

Glucosinolate	*Solvent system*						
	I	*II*	*III*	*IV*	*V*	*VI*	*VII*
3-Methylsulfinylpropyl-	16 (0.38)	16 (0.32)	23 (0.50)	30 (0.48)	36 (0.78)	11 (0.27)	28 (0.58)
Methyl-	27 (0.66)	38 (0.76)	32 (0.70)	50 (0.81)	36 (0.78)	22 (0.54)	36 (0.75)
2-Hydroxyisopropyl-	34 (0.83)	45 (0.90)	40 (0.87)	57 (0.92)	47 (1.02)	36 (0.88)	44 (0.90)
Allyl-	41 (1.00)	50 (1.00)	46 (1.00)	62 (1.00)	46 (1.00)	41 (1.00)	48 (1.00)
3-Methylthiopropyl-	50 (1.22)	59 (1.18)	54 (1.17)	65 (1.05)	53 (1 15)	50 (1.22)	55 (1.15)
p-Hydroxybenzyl-	52 (1.27)	59 (1.18)	50 (1.09)	69 (1.11)	44 (0.96)	51 (1.24)	53 (1.10)
Benzyl-	53 (1.29)	59 (1.18)	54 (1.17)	69 (1.11)	54 (1.17)	52 (1.27)	58 (1.21)
2-Hydroxy-2-phenylethyl-	54 (1.32)	61 (1.22)	54 (1.17)	73 (1.18)	56 (1.22)	56 (1.37)	57 (1.19)
2-Phenylethyl-	57 (1.39)	64 (1.28)	58 (1.26)	73 (1.18)	56 (1.22)	58 (1.41)	60 (1.25)
development time[a]	30	30	40	90	90	120	120

[a] Time required to ascend 10 cm from origin.

using solvent systems I, II, or III. Glucosinolates 5 and 6, 6 and 7, as well as 6 and 8 were separated from each other using solvent system V, whereas glucosinolates 5 and 8 were resolved using solvent system IV. Glucosinolates 5 and 7 (with solvents IV and VII) and 7 and 8 (with solvents IV and VI) were at best incompletely separated. The development time was 3–4 times shorter using solvents I–III than with solvents IV–VII. Recoveries of allyl- and benzylglucosinolates from developed TLC plates showed no decomposition of these compounds in all solvent systems except VI. Partial decomposition of both glucosinolates was observed when solvent system VI was employed as evidenced by the appearance of additional spots on the analytical chromatographic plate. The decomposition may be due to the pyridine in the system, since it has been observed that several glucosinolates react with pyridine[2]. On the other hand, the glucosinolates were not influenced by solvent system VII containing acetic acid.

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