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4-Methylsulphonylbutylglucosinolate Ion, the Natural Thioglucoside Precursor of Erysolin*

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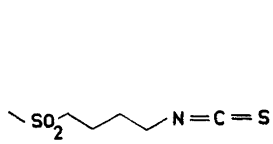
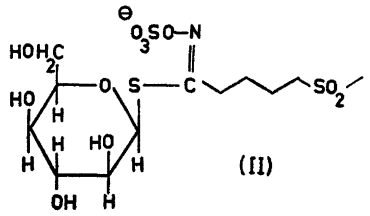
From seed material of the two crucifers, *Erysimum allionii* hort. and *E. perofskianum* Fisch. et May, crystalline potassium 4-methylsulphonylbutylglucosinolate (II) ('glucoerysolin'), affording erysolin, 4-methylsulphonylbutyl isothiocyanate (I), on enzymatic hydrolysis, has been isolated. The structure (II) is supported by chemical and enzymatic hydrolysis as well as by analytical, optical, and spectroscopic data. The glucosinolate is further characterized as the crystalline potassium *O*-tetraacetate of (II).

In 1912, Schneider and Kaufmann[1] reported on the presence in seeds of the crucifer *Erysimum perofskianum* Fisch. et May of a non-characterized glycoside, *** giving rise to the production of erysolin, 4-methylsulphonylbutyl isothiocyanate (I), on enzymatic hydrolysis.



(I)



(II)

Unsuccessful attempts to establish the presence of erysolin in several enzymatically hydrolyzed seed extracts of *E. perofskianum* led to a temporary suspicion that the biological production of its glucosidic progenitor might be

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*** Frequently referred to as 'glucoerysolin', an ambiguous designation which should be abandoned, *cf.* Ref. 6.

environmentally conditioned,[2] or, alternatively, that the originally examined seed specimen had been incorrectly identified. That the failure to detect erysolin was rather due to the insufficiency of the method employed, *viz.* paper chromatography in one solvent of its thiourea derivative, became evident, however, when Gmelin and Bredenberg [3] recently established the identity of the phenylthiourea derivative of one of the isothiocyanates, enzymatically liberated from seed extracts of *E. perofskianum* and *E. allionii* hort., with a synthetic specimen of 1-(4-methylsulphonylbutyl)-3-phenylthiourea.[4] In both species, erysolin was accompanied by the corresponding sulphide- (erucin) and sulphoxide-(sulphoraphane) isothiocyanates.

In the present paper we describe the isolation and characterization of crystalline *potassium 4-methylsulphonylbutylglucosinolate* (II), containing the naturally occurring thioglucoside anion whence (I) derives by enzymatic hydrolysis. Commercial seed samples of *E. allionii* and *E. perofskianum* served equally well as sources of the parent glucosinolate in the present investigation.

An aqueous solution of the glucosinolates in *E. allionii* hort., prepared by extraction with hot 70 % methanol of disintegrated, defatted seed material, followed by ion exchange, and elution with potassium sulphate, afforded, after repeated recrystallizations, a homogeneous glucosinolate, identified as a hydrated potassium salt of the 4-methylsulphonylbutylglucosinolate ion (II). Its structure was derived by analysis, physical data, and chemical and enzymatic hydrolysis. The optical rotation, $[\alpha]_D^{28}$ $-19°$, UV-spectrum (λ_{max} 226 nm (ε 8500)), and IR-bands were all typical for the entire group of glucosinolates (β-D-thioglucopyranosides) (*cf.* Ref. 2), the IR-spectrum exhibiting additional bands due to the S=O stretching modes of a sulphone grouping. Again, the NMR-spectrum (in D_2O) contained the expected bands, yet with the CH_3SO_2-signal appearing as an almost symmetrical three-proton doublet, suggesting the existence in solution of two conformers. Curiously, the closely analogous 3-methylsulphonylpropylglucosinolate ion exhibited a sharp singlet with the same chemical shift.

An acid-hydrolyzed solution of the glucosinolate typically contained, *inter alia*, hydroxylamine and glucose, both identified by chromatographic analysis.

Upon enzymatic hydrolysis, the crystalline glucosinolate afforded an isothiocyanate which, after vapour phase chromatography, gave a mass spectrum identical with that of synthetic erysolin (I).[4] Furthermore, the isothiocyanate was converted into the known thiourea- and phenylthiourea-derivatives;[4] the latter was previously obtained from the same seed source.[3] Comparison of the natural and synthetic thiourea was advantageously performed by paper chromatography in the solvent system butanol:toluene:water (3:1:1), introduced by Ettlinger and Thompson [5] and permitting separation from cheirolinthiourea, the lower homologue: 3-methylsulphonylpropylthiourea.

For further characterization, the potassium glucosinolate was converted into the crystalline *potassium O-tetraacetyl-(4-methylsulphonylbutyl)-glucosinolate monohydrate* possessing properties characteristic of *O*-acetylated glucosinolates (*cf.* Ref. 2).

Paperchromatographic analyses, in two solvent systems, of a crude methanolic extract of seeds of *E. allionii* revealed its contents of three glucosinolates additional to (II), two of these being the sulphide- and sulphoxide-analogues of (II) as previously established.[3] The third, and minor, glucosinolate was not rigorously identified, but possessed chromatographic properties identical with those of the 3-butenylglucosinolate ion, occasionally encountered in Nature (*cf.* Ref. 2) and constituting a conceivable biogenetic variant of the other glucosinolates in *E. allionii*.

Virtually identical results were obtained when seeds of *E. perofskianum* were employed; 0.6 g of a homogeneous potassium glucosinolate, identical with the salt isolated from *E. allionii* as described above, was obtained from 100 g of seeds. Moreover, the total glucosinolate patterns of the two species proved essentially identical.

The described isolation of potassium 4-methylsulphonylbutylglucosinolate brings the list of characterized, crystalline glucosinolates, alternatively their *O*-acetyl-derivatives, to a total of twenty-three individual compounds (*cf.* Refs. 2, 6, 7, and 8).

EXPERIMENTAL

Melting points are uncorrected and determined in an electrically heated Anschütz-Hershberg apparatus. Analytical specimens are dried in vacuum over calcium chloride at room temperature.

Isolation of potassium 4-methylsulphonylbutylglucosinolate. One hundred grams of seeds of *Erysimum allionii* hort. were finely ground, defatted on extraction with two 0.5 l-portions of hot petroleum ether, and finally extracted with three 0.5 l-portions of 70 % methanol. The methanol extracts were combined, concentrated to 300 ml, and water was added to a total volume of 1 l. After filtration through Hyflo Super Cel, the solution was passed through a column (30 cm, 0.3 cm diameter) of Dowex 1×2 ion exchange resin in its chloride form. The column was rinsed with water, and the filtrate and washings discarded. The glucosinolates were eluted by passing a 4 % potassium sulphate solution through the column; 250-ml fractions were collected. By paper chromatography, the glucosinolates were located in fractions Nos. 4—14, which were combined and concentrated to dryness *in vacuo*. On trituration of the residue with 85 % ethanol a crystalline fraction was obtained which was recrystallized from the same solvent to give 0.55 g of essentially homogeneous potassium 4-methylsulphonylbutylglucosinolate.

An analytical specimen was produced by two additional recrystallizations from 85 % ethanol, separating as colourless needles with m.p. 161—163°. On paper chromatography in (i) butanol:ethanol:water (4:1:4) and (ii) butanol:pyridine:water (6:4:3), R_B-values (*i.e.* the R_F-value relative to that of the benzylglucosinolate ion) of 0.24 and 0.54, respectively, were obtained. The analytical data indicated the presence of 2.5 moles of water of crystallization. (Found: C 27.25; H 4.93; N 2.61; S 17.42; H_2O (Karl Fischer) 8.18. Calc. for $C_{12}H_{22}O_{11}NS_3K$, 2.5 H_2O: C 26.85; H 5.07; N 2.61; S 17.93; H_2O; 8.40). $[\alpha]_D^{28}$ −19.1° (*c* 0.75, H_2O). The ultraviolet spectrum (in H_2O) exhibited a maximum at 226 nm (ε 8500), and the infra-red spectrum (in KBr) displayed conspicuous bands at 3350, 2870, 1640, 1570, 1400, 1360, 1320, 1260 (vs), 1230, 1120, 1100, 1055 (vs), 970, 885, and 800 cm^{-1}. The NMR-spectrum (in D_2O) showed, in addition to signals from the glucose moiety and the methylene groupings, an apparent 3H-doublet, spaced by 3 cps and centered at δ 3.2, due to the CH_3SO_2-grouping (see theoretical part).

Isolation experiments, performed with seeds of *E. perofskianum*, proceeded exactly as described above, leading to a crystalline, homogeneous glucosinolate which, on critical comparison, proved identical with potassium 4-methylsulphonylbutylglucosinolate obtained from *E. allionii*.

Potassium 4-methylsulphonylbutylglucosinolate tetraacetate. The potassium glucosinolate (170 mg) was acetylated by shaking for 2.5 h at room temperature in pyridine (1.5 ml) with acetic anhydride (1.5 ml). After evaporation to dryness, the residue was recrystallized twice from 96 % ethanol to give an analytical specimen (94 mg) of the potassium glucosinolate tetraacetate, m.p. 155–156°; $[\alpha]_D^{28}$ −17.1° (*c* 0.86, H_2O); UV-spectrum (in H_2O): λ_{max} 225 nm (ε 6400). (Found: C 35.50; H 4.48; N 1.75; H_2O (Karl Fischer) 2.35. Calc. for $C_{20}H_{30}O_{15}NS_3K$, H_2O: C 35.44; H 4.76; N 2.07; H_2O 2.66).

Acid hydrolysis of the potassium salt of (II). The glucosinolate was treated with 6 N HCl for 3 h at 90°. After evaporation to dryness the residue was redissolved in a few drops of water, and the solution was employed for establishing the presence of (i) hydroxylamine, and (ii) glucose by paper chromatography in methanol:6 N HCl, and butanol: ethanol:water (4:1:4), respectively, along with reference substances.

Enzymatic hydrolysis of the potassium salt of (II). A small sample (50 mg) of the crystalline potassium glucosinolate was dissolved in a citrate buffer (pH 6.7), and a few drops of a crude myrosinase preparation, as well as a trace of ascorbic acid, were added. After 2 h at room temperature, the reaction mixture was extracted with chloroform. The dried solution was concentrated to a small volume and divided into two halves.

One was treated with a small volume of ammonia-saturated methanol, followed by paper chromatography of the produced thiourea in butanol:toluene-water (3:1:1).[5] An R_F-value of 0.45 was established, identical with that of a synthetic specimen [4] of 4-methylsulphonylbutylthiourea.

The second half was treated with a drop of aniline and kept overnight at room temperature. Thin layer chromatography of the derived phenylthiourea in $CHCl_3$:EtOH (95:5) on 'Kieselgel HF_{254}' gave an R_F-value of 0.44, identical with that of an authentic specimen [4] of 1-(4-methylsulphonylbutyl)-3-phenylthiourea.

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