

and a more accurate isoelectric point of 5.89 could be derived from the experiment. According to arguments presented by Vesterberg and Svensson,⁵ this should also be considered as the isoionic point of carbonic anhydrase. It is to be compared with the value 5.65 found by Nilsson and Lindskog⁴ by extensive dialysis against water and subsequent removal of dissolved carbon dioxide.

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Glucoaubrietin, A New Crystalline Glucosinolate*

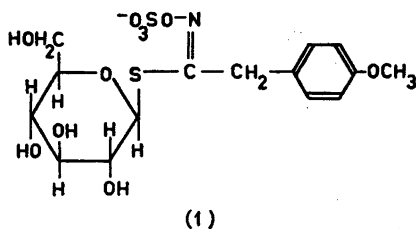
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In a previous communication of this series,¹ the natural occurrence of a glucosinolate (glucoaubrietin), affording *p*-

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methoxybenzyl isothiocyanate on enzymatic hydrolysis, was demonstrated; no efforts were made at that time, however, to isolate the parent compound. We now wish to report the isolation of the latter, (1), in the form of the crystalline tetramethylammonium salt.



In the preceding study¹ the occurrence of (1) in a number of species of the crucifer genus *Aubrietia* was demonstrated by chromatographic methods. In the present isolation, seed material of a cultivated species of *Aubrietia* * was employed.

The glucosinolate was isolated by the usual procedure involving grinding of the seed, defatting, extraction with 70% methanol, ion-exchange on anionotropic alumina, and elution with tetramethylammonium hydroxide. It crystallized from 85% ethanol as colourless needles, and an analytical specimen, m.p. 181°, possessed the expected composition ((CH₃)₄N⁺-salt of (1)), UV- (cf. Ref. 1) and IR-data. The rotation, [α]_D²⁷ -19°, similar in sign and magnitude to that of several other glucosinolates (cf. Ref. 2), signifies its character as a β-thioglucoside. In agreement with the structure (1), acid hydrolysis afforded, *inter alia*, hydroxylamine and glucose, whereas enzymatic hydrolysis resulted in the production of *p*-methoxybenzyl isothiocyanate, characterized, upon reaction with ammonia, as the previously described *p*-methoxybenzylthiourea.¹

Upon exchange of potassium for (CH₃)₄N⁺-ions, and subsequent acetylation, potassium *p*-methoxybenzylglucosinolate tetraacetate was obtained as a nicely crystalline monohydrate.

* Many cultivated varieties of *Aubrietia* afford popular flower garden subjects. The seed employed in the present investigation was commercially obtained as '*Aubrietia cultorum* Græca' from the seed company Ernst Benary, 351 Hann. Münden, West Germany.

The content of glucoaubrietin in the employed seed material was estimated to be about 1 %.

The detailed distribution of glucoaubrietin in the plant kingdom is still unknown. It seems to be consistently present in the small genus *Aubrietia* but has been reported, so far, also in *Lepidium bonariense* L.³ outside this genus. Its biosynthetic derivation, probably from tyrosine, poses an interesting problem.

Experimental. *Tetramethylammonium p-methoxybenzylglucosinolate (glucoaubrietin) (I).* *Aubrietia* seeds (100 g) were finely ground and defatted with hot petroleum ether. The seed powder was extracted with two 600-ml portions of 70 % methanol. The extract was concentrated *in vacuo* to about 200 ml, and water was added to a total volume of 500 ml. This solution was then filtered through a column of 150 g of anionotropic alumina (Woelm), and the column was rinsed with water (200 ml). The glucosinolate was now eluted with a 2 % solution of tetramethylammonium hydroxide, and 25 ml-fractions were collected. According to paperchromatographic analysis the glucosinolate was present in fractions Nos. 2–6. These were combined and taken to dryness in a rotatory evaporator. The residue was dissolved in hot 90 % ethanol and kept for two days at 0°. The crystalline deposit was dissolved in hot 85 % ethanol, treated with a little charcoal, and recrystallized by slowly adding 96 % ethanol (yield: 560 mg). An analytical specimen was produced by two additional recrystallizations from 85 % EtOH, separating as thin, colourless needles, m.p. 180–181° (uncorr.), $[\alpha]_D^{27} -19^\circ$ (c 1.4, H₂O). (Found: C 43.98; H 6.44; N 5.55; S 12.52. Calc. for C₁₉H₂₃O₁₀N₂S₂: C 44.51; H 6.29; N 5.47; S 12.51). The IR-spectrum (in KBr) exhibited strong bands at 3430, 1615, 1565, 1510, 1490, 1285, 1230, 1100, 1055, 953, 890, 823, 795, and 718 cm⁻¹. The UV-spectrum (in H₂O) displayed maxima at 227 nm (ϵ 22 100) and 275 nm (ϵ 4 200) in agreement with the previously reported spectrum.¹

A small sample of the tetramethylammonium salt (30 mg) was dissolved in 6 N HCl and kept for 4 h at 90°. After evaporation to dryness, the residue was dissolved in water and extracted with three small portions of chloroform. The aqueous phase was concentrated, and its contents of hydroxylamine and glucose confirmed by paperchromatographic methods.

Another fraction of the glucosinolate was dissolved in a dilute citrate buffer (pH 6.7); myrosin and a trace of ascorbic acid were

added, and the enzymatic hydrolysis was allowed to proceed for 4 h. The reaction mixture was extracted with ether, and methanolic ammonia was added to the dried ether solution. After standing overnight, the mixture was taken to dryness and the semicrystalline residue was recrystallized from water to give *p*-methoxybenzylthiourea, m.p. 135°, alone or in admixture with an authentic specimen.¹ The identity was further confirmed by coinciding IR-spectra.

Potassium p-methoxybenzylglucosinolate tetraacetate. The mother liquors from the several recrystallizations of tetramethylammonium *p*-methoxybenzylglucosinolate were combined and concentrated to dryness. The residue was taken up in water (100 ml), and the solution was slowly filtered through a column of Dowex-50 W (17 ml resin), charged with potassium ions. The resin was rinsed with 50 % ethanol (50 ml). The filtrate and washing liquid were combined and evaporated to dryness. The residue was dissolved in pyridine (15 ml) and acetic anhydride (15 ml) was added. After 2 h at room temperature the solution was concentrated to near dryness, and the residue was dissolved in a little hot 90 % ethanol. After hot filtration, the solution deposited the nicely crystalline tetraacetate monohydrate which was recrystallized once more from 85 % ethanol (390 mg).

A specimen for analysis was recrystallized once more from the same solvent, m.p. 173–174° (uncorr.), $[\alpha]_D^{27} -7.0^\circ$ (c 0.93, H₂O). (Found: C 42.33; H 4.59; N 2.11; S 9.60; H₂O (Karl Fischer) 2.89. Calc. for C₂₃H₂₈O₁₄NS₂K, H₂O: C 41.62; H 4.56; N 2.11; S 9.66; H₂O 2.72). The IR-spectrum displayed strong bands at 3300, 1740, 1600, 1500, 1435, 1360, 1245 (br.), 1055, 918, 888, and 795 cm⁻¹.

The NMR-spectrum (recorded at 60 Mc in a mixture of CDCl₃ and (CD₃)₂SO) showed the expected signals at δ 6.8–7.4 (4 H, two doublets; aromatic H); δ 3.82 (3 H, s., OCH₃); δ 3.3 (s., H₂O), and δ 1.96–2.10 (12 H, m, OAc); the sugar protons (7 H) appeared in two band groups at ca. δ 5.0 (4 H) and 4.0 (3 H). The benzylic protons were hidden under solvent signals at δ 2.6.

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