

THE CONVERSION OF POTASSIUM ALLYLGLUCOSINOLATE TO 3,4-EPITHIOBUTANENITRILE BY *CRAMBE ABYSSINICA* SEED FLOUR

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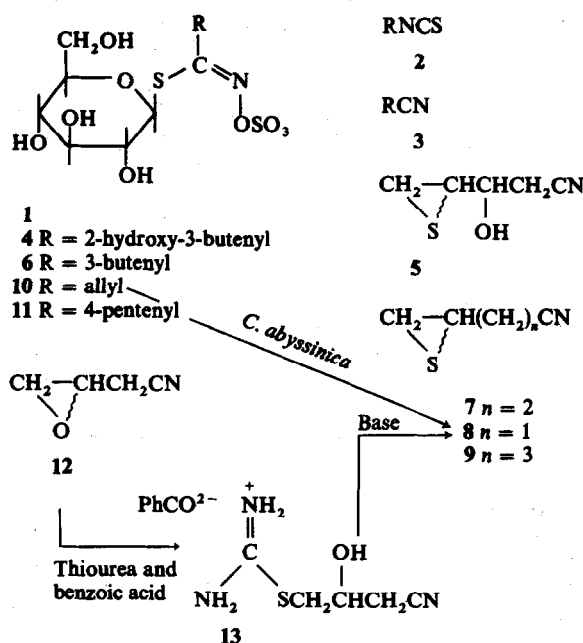
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Key Word Index—*Crambe abyssinica*; Cruciferae; allylglucosinolate; sinigrin; 3,4-epithiobutanenitrile; 2,3-epithiopropyl cyanide.

Abstract—An aqueous suspension of *Crambe abyssinica* seed flour efficiently converted potassium allylglucosinolate (sinigrin) into 3,4-epithiobutanenitrile.

In the products of the usual catabolism of glucosinolates (1), the sulphur atom of the thioglucosidic linkage either appears in the form of an isothiocyanate (2), or as the element: in the latter case the aglucone is transformed into a nitrile (3) [1]. However, sometimes alkenylglucosinolates yield epithioalkanenitriles, in which the thioglucosidic sulphur atom is apparently inserted into the olefinic function of the aglucone. This was first demonstrated for the S-2-hydroxybut-3-enyl system (S-4) in autolysing *Crambe abyssinica* Hochst ex R.E. Fries seed flour preparations, where the products were shown to include the diastereomeric episulphides (5) [2, 3]. Later, the enantiomeric R-4 was shown to yield the analogous pair of R-3-epimers of 5 in a similar *Brassica napus* L. system [4], while 3-butenylglucosinolate (6) was converted, *inter alia* into 4,5-epithiopentanenitrile (7) by some cultivars of *B. campestris* L. [5]. In all these cases the epithio products were isolated, and their structures established by means of extensive spectroscopic, and chemical procedures. More recently, Cole [6, 7] has examined the volatile autolysis products from fresh plant homogenates of a number of crucifers, using GC-MS for identification purposes. On the very reasonable basis of homology in the MS of two compounds with that reported for 7, she postulated that they were the hitherto unknown 3,4-epithiobutanenitrile (8), and 5,6-epithiohexanenitrile (9), arising from the corresponding allyl and 4-pentenylglucosinolates (10 and 11). Subsequently 7 and 8 have been enumerated among the autolysis products of cabbage leaves [8], again on the basis of a comparison of MS data with that reported by Kirk and MacDonald [5], and Cole [6, 7].

Although plausible, these identifications of 7 and 8 lacked direct comparison of data with that for the authentic epithionitriles, which indeed were apparently unknown as synthetic products. We now report the synthesis of 8, by the route 12 → 13 → 8, and can confirm that its MS is in accord with that reported by Cole [6, 7], and Daxenbichler *et al.* [8]. In addition we have found that when allylglucosinolate (10) was added to an aqueous suspension of *C. abyssinica* seed flour it was efficiently converted into 8, the identity of 8 being established both by GC-MS comparison with authentic material, and by isolating it by preparative GLC, and comparing its IR and ¹H NMR spectra with those of the synthetic episulphide.



EXPERIMENTAL

IR were recorded for thin films, ¹³C and ¹H NMR were measured in CDCl₃ and are quoted on the δ scale relative to internal TMS; GC-MS were conducted on a Hewlett-Packard 5992A unit, using a 183 × 0.64 cm glass column of 3% OV-17 on Chromosorb W, at 150°, with He as the carrier gas; prep. GLC was performed on a Varian 3700 chromatography with a 10:1 effluent splitter, using a 183 × 0.64 cm glass column of 20% OV-17 on 100–120 mesh Chromosorb W, at 150°, with N₂ as the carrier gas; analytical GLC was done on a Bendix 2500 chromatography using 183 × 0.64 cm glass columns with 10% SE-30, OV-1, or OV-17 on Chromosorb W, at 110°.

Synthesis of 3,4-epithiobutanenitrile (8). Following the general procedure of ref. [9], 3,4-epoxybutanenitrile (12) [10] (1.8 g, 22 mmol) in Me₂CO (5 ml) was added dropwise to a stirred mixture of benzoic acid (2.6 g, 22 mmol) and thiourea (1.6 g, 22 mmol) in Me₂CO (14 ml). After 2 hr, the ppt. which separated was filtered off and washed with Et₂O. The air-dried 13 thus obtained was a colourless crystalline solid, (6.2 g, 103%). With-

out further purification, this material (5.0 g) was dissolved in $\text{Me}_2\text{CO}-\text{H}_2\text{O}$ (95 ml, 3:7) and to the stirred soln was added, slowly, dropwise, another portion of Na_2CO_3 (2.0 g) in H_2O (10 ml). The pH of the reaction mixture was monitored by spotting it onto indicator paper. Initially, after each addition the pH rose to ca 8-9, then fell back quickly to ca 7. When ca one-half of the carbonate soln had been added the reaction mixture was extracted with C_6H_6 (10 ml). The aq. phase was then treated with more carbonate soln (ca 2 ml), as before, and then re-extracted with C_6H_6 (10 ml). Finally enough carbonate soln was cautiously added to the aq. phase to yield a soln whose pH remained at ca 8-9. This was again extracted with C_6H_6 (10 ml). The combined C_6H_6 extracts were washed with H_2O (1×5 ml), dried (MgSO_4), and the solvent removed under red. press. at room temp. The residual oil (ca 2 g) was then quickly fractionally distilled under red. pres. to afford 3,4-epithiobutanenitrile (8), as a colourless oil, bp 65-67°, ca 0.1 mm (0.88 g, 58%). (Found: C, 48.41; H, 5.14; N, 13.77. $\text{C}_4\text{H}_5\text{NS}$ requires: C, 48.44; H, 5.08; N, 14.12%). This material was homogeneous by analytical GLC on SE-30, and OV-17, and had IR $\nu_{\text{max}} \text{ cm}^{-1}$: 3040 (m), 2995 (s), 2960 (s), 2925 (ms), 2260 (vs), 1440 (vs), 1415 (vs), 1410 (vs), 1367 (ms), 1360 (ms), 1288 (ms), 1223 (m), 1155 (w), 1115 (m), 1105 (w), 1050 (vs), 1025 (s), 948 (ms), 938 (m), 920 (m), 887 (ms), 860 (w), 848 (w), 712 (m), 647 (ms) and 612 (vs); ^1H NMR: δ 2.33 (ca 0.5 H, d, $J = 2.1$ Hz), 2.36 (ca 0.5 H, d, $J = 1.8$ Hz), 2.57 (ca 0.5 H, d, $J = 2.1$ Hz), 2.6 (ca 0.5 H, d, $J = 1.8$ Hz), 2.76 (ca 0.5 H), 2.78 (ca 0.5 H), 2.83 (ca 1 H, s), and 3.04 (1H, 5 line multiplet with fine couplings); ^{13}C NMR: δ 24.6 (C-4, $J_{\text{CH}} = 172$; and C-2, $J_{\text{CH}} = 139$ Hz), 28.1 (C-3, $J_{\text{CH}} = 175$ Hz), and 116.7 (C-1); MS: 99 (50), 72 (46), 71 (18), 67 (28), 66 (22), 64 (13), 59 (79), 58 (35), 57 (14), 54 (42), 53 (17), 52 (33), 51 (12), 46 (28), 45 (57), 41 (100), 40 (41), 39 (86), 38 (46) and 37 amu (30%). The episulphide could be stored in a deep-freeze at -60° , but rapidly polymerized at room temp., especially if exposed to bases.

Conversion of allylglucosinolate to 8 by Crambe abyssinica. *C. abyssinica* var. Prophet seeds (5 g) were found in a mortar under hexane (20 ml). The hexane, and much of the seed husks, was removed by decantation, and the residue was reground under fresh hexane (20 ml). After decantation, the seed flour was briefly air-dried, and then powdered in the mortar. To a stirred suspension of this flour (1 g) in distilled water, cooled in an ice-bath, was added potassium allylglucosinolate hydrate (Aldrich Chem. Co., Milwaukee, WI 150 mg). After 45 min, the reaction mixture was shaken with Et_2O (15 ml), and then centri-

fuged. The clear, yellow-coloured, supernatant was removed, dried, and evapd at room temp. to a yellowish oily residue. This was taken up in CH_2Cl_2 , and analysed by GLC (OV-1 and OV-17). The two major components were first identified as allyl isothiocyanate and 3,4-epithiobutanenitrile (8) on the basis of their retention times, which corresponded with those of authentic standards. The identities were confirmed by GC-MS, and a sample of 8 was isolated by prep. GLC, and shown to possess the same IR and ^1H NMR spectra as the synthetic compound. The approximate ratio of the two products was 1:9, favouring 8. In a control experiment, conducted as described above but omitting the allylglucosinolate, only traces of material apparently corresponding to 8 could be detected.

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