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ALLIIN IN THE GARLICKY TAXON *ADENOCALYMMA ALLIACEUM* (BIGNONIACEAE)

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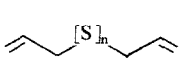
Key Word Index—*Adenocalymma alliaceum*; Bignoniaceae; allylic sulphides; alliin.

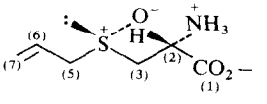
Abstract—Dried leaves of *Adenocalymma alliaceum* (Bignoniaceae) contain alliin [(*S*)-*S*-allyl-L-cysteine *S*-oxide], previously known only from *Allium* species, and an endogeneous enzyme catalyzing the degradation of alliin to give diallyl disulphide, diallyl trisulphide and diallyl tetrasulphide as stable end products.

INTRODUCTION

Adenocalymma alliaceum Mart., a South American dicot belonging to the family Bignoniaceae, was recently reported as a source of the diallylic sulphides **1**–**3** [1], three odorous volatiles formerly regarded as characteristic of garlic and a few closely related *Allium* species [2–4]. Alliin (**4**) has long been recognized as the *Allium* progenitor of **1**–**3**, undergoing enzymic severance of the C3-S bond followed by secondary transformations of the sulphur-containing moiety ([4] and references cited therein). We now provide evidence for **4** being the precursor of **1**–**3** also in *Adenocalymma*.



1 n = 2
2 n = 3
3 n = 4



4

RESULTS AND DISCUSSION

Whereas an unheated aqueous extract of sun-dried leaves of *A. alliaceum* exhibited an unexceptional pattern of common plant amino acids, the marked 'garlic' odour of the extract suggested that a sulphur-containing amino acid may have suffered enzymic fission during the preparation of the extract. The chromatographically established presence of one major, and several minor sulphur-containing amino acids in another extract, produced by steeping the dried leaf material in boiling 70% MeOH, thus arresting the enzymically catalysed decomposition, supported this assumption. The total amino acid fraction from an extract prepared in this way was isolated and divided into a basic, an acidic, and a quantitatively predominant neutral amino acid fraction by means of standard ion exchange chromatography technique [5, 6]. From the latter fraction slightly impure alliin could be crystallized (*ca* 2%, based on dry leaf wt). Purification afforded a specimen possessing physical constants, including a circular dichroism pattern [7] and chromatographic parameters, indistinguishable from those of alliin [(*S*)-*S*-allyl-L-cysteine *S*-oxide] (**4**). Though vastly dominant, alliin is not the only *S*-containing amino acid in the leaf extract. At least three additional, unidentified members of this class were chromatographically detectable, all possessing acidic properties.

The naturally occurring, non-protein, sulphur-containing amino acids known today comprise more than a score of structurally diverse compounds with occurrences ranging from Basidiomycetes over monocots (Liliaceae) to dicots (Mimosaceae, Santalaceae, and Bignoniaceae). The obviously non-ubiquitous distribution, however, deserves attention. Information regarding the extent of parallelism in the anabolic pathway leading to alliin in *Allium* and *Adenocalymma* as well as its catabolic fate, including the question of identity of the C-S-lyase(s) operating in the two taxa, are clearly points of interest, notably in connexion with the question of evolutionary convergence.

EXPERIMENTAL

The leaf material was collected in the Botanical Garden of Andhra University, Waltair, India and was sun-dried before shipment to the Danish laboratories. 2D-PC (two-dimensional chromatography) and HVE (high-voltage electrophoresis) were both performed as described in a previous report [5].

Cold aq. extract. When dry leaves (5 g) were disintegrated in water (50 ml) at ambient temp. a strong garlic odour developed. After standing for 20 hr the extract was subjected to 2D-PC, HVE, and ion-exchange amino acid analysis. The amino acid pattern was unexceptional, containing the common plant amino acids in ordinarily encountered amounts.

Hot 70% methanolic extract. An analysis conducted in precisely the same way, yet on an extract prepared by adding the leaf material (5 g) to boiling 70% MeOH placed in an Ultra-Turrax homogenizer, revealed the presence of one totally dominant amino acid, supplementary to a pattern virtually unchanged from that prevailing in the cold, aq. extract. The major component gave a positive response to various tests for S-containing amino acids, possessed an elution time relative to that

of glutamic acid of 0.38, and was present to the extent of 20 mg per g of dried leaves (as compared with e.g. 0.2 mg of aspartic acid, 0.6 mg of glutamic acid, and 0.5 mg of leucine).

Isolation of alliin. An extract, produced by disintegrating dried leaves of *A. alliaceum* (50 g) in three 500-ml portions of boiling 70% MeOH, was filtered and concd to near dryness *in vacuo*. The residue was dissolved in H_2O (30 ml), and the solution was extracted with 3 × 40 ml of $CHCl_3$. The aq. phase was transferred to an Amberlite IR 120 (H^+, 2.5 × 40 cm) column and the total amino acid fraction was further divided into a major fraction, containing the neutral and acidic amino acids (3.3 g), and another, comprising the basic amino acids and amines (1.2 g), by methods previously described [5, 6]. Further separation of the acidic (0.2 g) from the neutral amino acids (2.8 g) was likewise conducted as described [6]. Recrystallization of the latter fraction from H_2O gave a semicrystalline residue (655 mg) which was further purified by prep. HVE at pH 1.9, followed by prep. PC (PhOH–H_2O–12 M NH_3, 120:30:1, w/v/v) and chromatography on Dowex 50 W (× 8, 200–400 mesh, H^+, 0.7 × 10 cm). Washing with water (35 ml) and elution of the column with 1 M Py (25 ml) resulted in the isolation of a virtually homogeneous material, indistinguishable from authentic alliin on PC, HVE, and amino acid analysis. The ^1H NMR spectrum, in D_2O (pH 6), exhibited signals at 3.2 (2H, *m*), 3.7 (2H, *m*), 4.1 (1H, *dd*), and 4.5–6.0 ppm (3H, *m*); the same solution was characterized by a ^{13}C NMR spectrum with signals (cf. formula **4**), at 51.6 (C-5, *t*), 52.6 (C-3, *t*), 57.1 (C-2, *d*), 126.4 (C-6, *d*), 127.5 (C-7, *t*), and 173.2 ppm (C-1, *s*). The circular dichroism curve (in H_2O) displayed a negative extremum at 218 nm in accordance with the published data for alliin (**4**) [7].

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A CARLINA OXIDE DERIVATIVE FROM *CARLINA DIAE**

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Key Word Index—*Carlina diae*; Compositae; new carlina oxide derivative; new phenyl heptene diyne.

From the genus *Carlina*, tribe Cynareae, subtribe Carlineae, several acetylenic compounds have been isolated [1], which clearly showed a difference from the other subtribes. We now have investigated *C. diae* (Rech. f.) Meusel et Kästner, a species which was transferred from *Lyrolepsis* to *Carlina* [2], to see whether the chemistry supports this classification or not. The roots afforded in addition to β-sesquiphellandrene (**1**) and the acetylenes **2**–**8** [1] only two, **5b** and 13-methoxy carlina oxide (**8**), the structures clearly follow from the spectra data. While most of the ^1H NMR-data of **8** are very similar to those of **7**, the presence of a 13-methoxy group can be deduced from the aromatic proton signals, which all showed vicinal couplings indicating an *ortho*-disubstituted phenyl group (see Experimental). **8** is an unusual compound as the oxygen function is not in the *meta*-position. Only a few compounds of this type are known [3]. Most probably **8** is formed via the unknown carboxylic acid **14** by oxidation and methylation. This would indicate that carlina oxide (**7**) and **8** may be formed in the same way as shown for phenyl heptatriyne by intramolecular aldol condensation [1],

* Part 259 in the series "Polyacetylenic Compounds". For Part 258 see Bohlmann, F., Jakupovic, J., Robinson, H. and King, R. M. (1980) *Phytochemistry* **19**, 2760.