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Isolation of Aurantiamide Acetate from *Arisaema erubescens*

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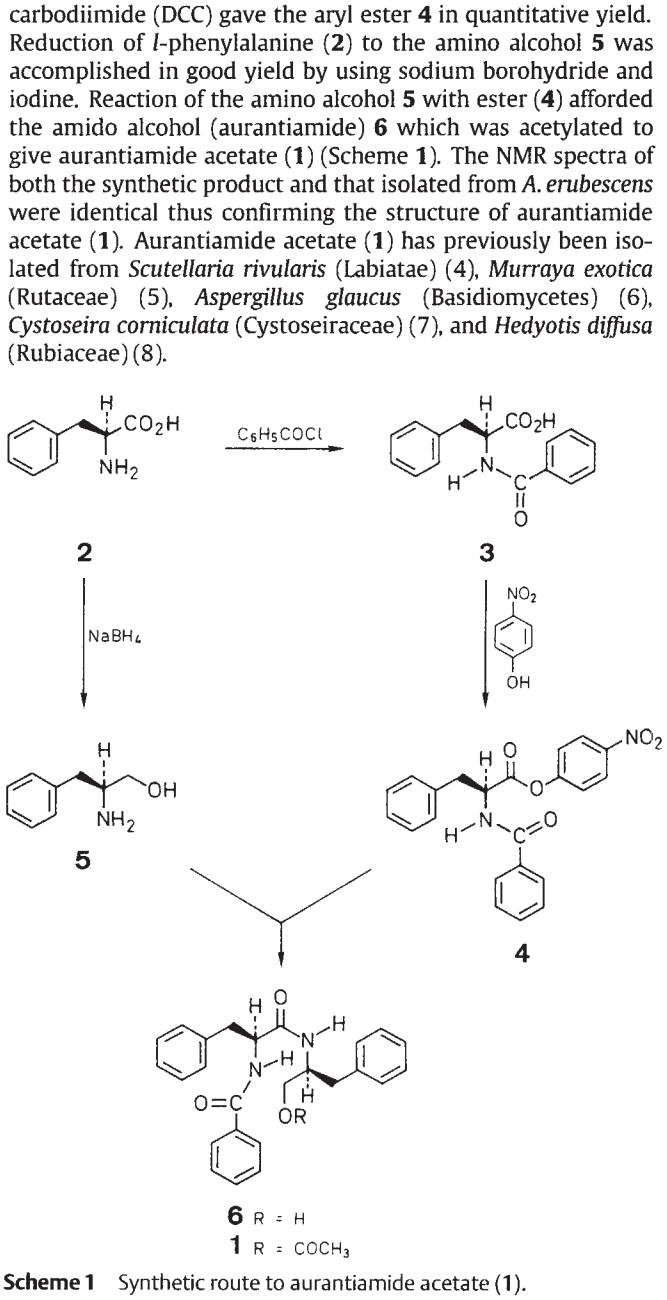
Received: August 17, 1995; Revision accepted: November 12, 1995

Abstract: **Aurantiamide acetate (*N*-benzoyl-1-phenylalanyl-1-phenylalaninol acetate) has been isolated by chromatographic separation of a methanol extract of *Arisaema erubescens* and its structure confirmed by synthesis.**

Arisaema erubescens Schott (Araceae) is a plant used in traditional Chinese medicine to treat several biological disorders. Extracts of *A. erubescens* have shown anticancer properties both *in vitro* and *in vitro* (1). However, the agents from this plant which bestow its anticancer action are unknown. A methanol extract of dried *A. erubescens* has yielded several products one of which has been identified as paeonol (2). Further chromatographic separation of the methanol extract of this plant on silica gel using ethyl acetate as eluent has yielded a crystalline solid which has been identified as aurantiamide acetate (**1**).

Dried chopped roots of *Arisaema erubescens* (2 kg) (supplied by East West Herbs, Kingham, Oxfordshire, U.K.) were soaked in methanol (10 l) at ambient temperature. After one week the resulting yellow-brown solution was filtered and the filtrate evaporated in vacuo (< 45 °C). The extract was partitioned between water (500 ml) and hexane (500 ml) and the hexane layer discarded. The aqueous layer was extracted successively with chloroform (3 × 300 ml) and ethyl acetate (3 × 200 ml). The combined chloroform and ethyl acetate extracts were dried over $MgSO_4$ and evaporated to give a yellow oil (482 mg). This oil was eluted on a silica gel column (15 × 1.5 cm) using ethyl acetate as eluent which was collected in 10 ml fractions. The fractions containing components (detected by UV_{254}) with an R_F value of 0.6–0.8 were eluted on a preparative TLC plate with dichloromethane to yield a one spot product (R_F 0.3) as white needles (23 mg); m.p. 180–182 °C (from ethyl acetate/hexane); $[\alpha]_D^{23.5}$: −30.6° (*c* 1.15, $CHCl_3$) {lit. $[\alpha]_D^{24}$ −40° (*c* 1.98, $CHCl_3$) (3)}; MS: *m/z* (CI, NH_3) = 445.2128 (M + H), $C_{27}H_{29}N_2O_4$ requires 445.2127. The ^{1}H- and ^{13}C-NMR spectra obtained were in agreement with that previously published (4) for aurantiamide acetate (**1**). For further confirmation, a synthesis of aurantiamide was carried out. Briefly, *l*-phenylalanine (**2**) was benzoylated to give *N*-benzoyl-*l*-phenylalanine (**3**) in quantitative yield and esterification of acid **3** with *p*-nitrophenol using dicyclohexylcarbodiimide (DCC) gave the aryl ester **4** in quantitative yield. Reduction of *l*-phenylalanine (**2**) to the amino alcohol **5** was accomplished in good yield by using sodium borohydride and iodine. Reaction of the amino alcohol **5** with ester (**4**) afforded the amido alcohol (aurantiamide) **6** which was acetylated to give aurantiamide acetate (**1**) (Scheme **1**). The NMR spectra of both the synthetic product and that isolated from *A. erubescens* were identical thus confirming the structure of aurantiamide acetate (**1**). Aurantiamide acetate (**1**) has previously been isolated from *Scutellaria rivularis* (Labiatae) (4), *Murraya exotica* (Rutaceae) (5), *Aspergillus glaucus* (Basidiomycetes) (6), *Cystoseira corniculata* (Cystoseiraceae) (7), and *Hedyotis diffusa* (Rubiaceae) (8).



Scheme 1 Synthetic route to aurantiamide acetate (**1**).

Aurantiamide acetate (**1**) was non-toxic ($IC_{50} > 22\,\mu M$) (doxorubicin, $IC_{50} = 42$ nM) to K562 human leukaemia cells using the MTT assay (9).

A voucher specimen of *Arisaema erubescens* is available at the Chemistry Department, UMIST, Manchester, UK. Copies of the original spectra are obtainable from the author of correspondence.

Acknowledgements

This work was supported by the Cancer Research Campaign and UMIST.

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Analysis of Several Iridoid and Indole Precursors of Terpenoid Indole Alkaloids with a Single HPLC Run

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Received: August 7, 1995; Revision accepted: December 16, 1995

Abstract: **An isocratic HPLC system is described which allows the separation of the iridoid and indole precursors of terpenoid indole alkaloids, which are present in a single crude extract. The system consists of a column of LiChrospher 60 RP select B 5 µm, 250 × 4 mm (Merck) with an eluent of 1 % formic acid–acetonitrile–trichloroacetic acid (100 : 10 : 0.25, v : v : w) at a flow of 1.2 ml/min. In the suspension cultures of *Catharanthus roseus* secologanin and tryptophan were detected. In the cultures of *Tabernaemontana divaricata* loganin, tryptophan, and tryptamine accumulated.**

The low accumulation of some terpenoid indole alkaloids by suspension cultures of *Catharanthus roseus* has frequently been associated with low activities of enzymes of the terpenoid pathway, which would limit precursor availability (1). Nevertheless the levels of metabolic intermediates of this pathway have never been thoroughly investigated.

Some methods have been described for the analysis of the iridoid precursors. Loganic and secologanic acid have been detected in *C. roseus* plants (2). A radioimmuno assay for the determination of the latter two compounds has also been developed (3). Levels of secologanin and loganin have been determined in suspension cultures of *C. roseus* by HPLC using a gradient system and by GC after acetylation (4); only cultures fed with precursors contained detectable amounts of these compounds (4). More recently an isocratic HPLC system for the analysis of loganin and secologanin has been used to quantify these compounds in suspension cultures of *T. divaricata* to which loganin had been fed (5).

As part of our continuing efforts to understand the regulatory mechanisms of the terpenoid indole alkaloid biosynthetic pathway, we developed a sensitive isocratic HPLC system which allows the detection of endogenous levels of the iridoid and indole precursors present in a crude methanolic extract; thus both routes supplying precursors for indole alkaloid biosynthesis can be monitored in a single run. In biomass extracts of *C. roseus* and *T. divaricata* suspension cultures, loganin, secologanin, tryptophan, tryptamine, and strictosidine can be found.

One difficulty expected in the development of the HPLC system was the analysis of secologanin. This compound contains an