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DIRECT ANALYSIS OF AROMATIC DIENE *ERYTHRINA* ALKALOIDS BY CAPILLARY GC-MS

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Abstract—Alkaloids extracted from *Erythrina crista-galli* seedlings by 0.025 M HCl were resolved, without prior derivatization, by capillary gas chromatography-mass spectrometry. 8-oxo-Erythraline, erythraline, erysotrine and crystamidine, in deceasing order of abundance, were recognized by their EI-mass spectral fragmentation pattern. Applying the technique to *E. pallida*, erysotrine was the principal alkaloid in seed, and seedling leaves contained 8-oxo-erythraline, erysotrine, erythraline and erysovine.

INTRODUCTION

Interest in *Erythrina* alkaloids has spanned the evolution of separation and analytical techniques for natural products. Classical methods, often accommodating extracts of rather large amounts of seed or vegetative plant material, involved chromatography over alumina or silica gel yielding some principal alkaloids on the gram scale [1, 2]. Methodology refinement for biosynthetic studies using *E. crista-galli* plants concentrated attention on the most prominent alkaloids as HPLC techniques improved direct acquisition of pure compounds. Consequently, some accumulating literature data have become apparently inconsistent with respect to the composition of the free alkaloids of this species. For example, the classic biosynthesis studies of Barton and colleagues concentrated on erythraline and erythratine [3] while, more recently, 8-oxo-erythraline appeared to be the most prominent alkaloid and neither erythraline nor erythratine was obvious [4]. Differences were provisionally attributed partly to assumed variations in age and geographical origin of the plant material. The possibility of non-enzymic oxidation of erythraline to 8-oxo-erythraline during extraction and analysis has also been proposed [5].

Analysis of *Erythrina* alkaloids by gas chromatography (GC) by Jackson and colleagues [5, 6], combined also with mass spectrometry, has further refined recognition of specific alkaloids in mixtures. Prior derivatization, as trimethyl silyl ethers, of crude mixtures is described for alkaloids of *E. berteroana* using *N,O*-bis(trimethyl silyl)acetamide in acetonitrile, thereby giving the impression that underivatized *Erythrina* alkaloids may not be amenable to analysis by the GC mode in general.

RESULTS AND DISCUSSION

In the course of resolving different findings concerning the alkaloids of *E. crista-galli*, a simple crude extract of three to four leaf seedlings was injected directly into a capillary GC-MS, programmed to a temperature gradient of $5° \text{ min}^{-1}$ over the range 140–310°. Without derivatization the mixture resolved into four distinct ion current signals leaving the column between 27 and 32 min after injection (Table 1). These signals surrounded, at 28.3 min, a convenient phthalate contaminant which is commonly acquired in trace amount in extracts made with organic solvents. There were no other notable signals.

All of the compounds showed (Table 1) the typical EI-mass spectral fragmentation pattern for diene *Erythrina* alkaloids with respect to the sequential loss of 15, 31, 33, 58 and 71 amu from the molecular ion [7]. The fragmentation patterns fit closely the literature data for 8-oxo-erythraline, erythraline and crystamidine. Several *Erythrina* alkaloids show a molecular ion at *m/z* 313 [8], but the fragmentation data of erysotrine alone fits the present fragments arising from the alkaloid with $[M]^+$ *m/z* 313. Notably, the base peak occurred at $[M - 33]^+$ for the 8-keto alkaloids 8-oxo-erythraline and crystamidine, and at $[M - 31]^+$ for erythraline and erysotrine. Ions corresponding to $[M - 84]^+$, rationalized for erythraline and erysotrine [7], were not evident in the 8-keto alkaloids, but the latter both had a prominent ion at *m/z* 250 which has not been assigned.

Capillary GC-MS has not only resolved in one step a crude *Erythrina* plant extract by direct injection but has also demonstrated the co-occurrence of 8-oxo-erythraline and erythraline in *E. crista-galli* seedlings, thereby re-

Table 1. Comparative mass spectral data of underivatized *Erythrina crista-galli* diene alkaloids revealed directly by capillary GC-MS.

	8-oxo-Erythraline	Erythraline	Erysotrine	Crystamidine
Relative abundance (Total ion current)	100	65	52	10
Retention time (min)	30.95	31.65	27.47	27.74
	Mass spectral ions (relative abundance)			
$[M]^+$	311 (85)	297 (55)	313 (54)	309 (43)
$[M - Me]^+$	296 (35)	282 (50)	298 (52)	294 (25)
$[M - MeO]^+$	280 (55)	266 (100)	282 (100)	278 (47)
$[M - CH_5O]^+$	278 (100)	264 (35)	280 (20)	276 (100)
	268 (18)	—	266 (20)	266 (27)
$[M - C_3H_6O]^+$	253 (16)	239 (17)	255 (13)	251 (13)
	250 (48)	—	—	250 (60)
$[M - C_4H_7O]^+$	240 (8)	226 (23)	242 (12)	238 (15)
$[M - C_5H_8O]^+$	—	213 (40)	229 (20)	—

conciling some apparently conflicting earlier findings [3, 4]. That erysotrine and crystamidine are also present as significant and minor components, respectively, is in accord with previous evidence [1]. Clearly, there is potential for optimizing resolution of particular mixtures of *Erythrina* alkaloids by modifying the GC temperature gradient, but the technique is a powerful analytical tool for extracts of very small amounts of plant tissue.

The technique subsequently identified erythraline as the principal alkaloid (95%) in green leaves of *E. crista-galli*, garden grown in England and freshly analysed in early autumn. Analysis of the alkaloids of *E. Pallida* [9], apparently for the first time [8], showed that seed collected in 1994 at Mkuze, N. Natal, contained principally erysotrine (95%) with a small amount of the homologue erysovine. Leaves of two four-leaf seedlings contained 8-oxo-erythraline, erysotrine, erythraline and erysovine.

EXPERIMENTAL

Plants. Seed of *Erythrina crista-galli* was supplied by the Botanical Gardens of Adelaide, S. Australia and grown in potting compost in the laboratory under a Phillips 700 W MBFR/U lamp. After 4 weeks seedlings were 7–8 cm tall and had three to four expanded leaves.

Alkaloid extraction. Plant tissue was ground with 0.025 M HCl in a mortar, the filtrate basified with excess $NaHCO_3$ and extracted with $CHCl_3$. The $CHCl_3$ extract was taken to small volume *in vacuo* for direct analysis by GC–MS.

GC-MS. A Fisons GC 8000 series instrument with mass detector 800 was used with a 30 m DB-5 fused silica 0.25 mm i.d. capillary column (J. and W. Scientific, Folson, California). Helium was the carrier gas. Sample in 1 μl $CHCl_3$ was injected at 90°. The temp. rose to 140° over 2 min and then at 5° min^{-1} to 310° over 34 min.

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