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STRUCTURE REVISION OF EMILINE, A PYRROLIZIDINE ALKALOID FROM *EMILIA FLAMMEA*

ROBERT H. BARBOUR and DAVID J. ROBINS

Department of Chemistry, University of Glasgow, Glasgow G12 8QQ, U.K.

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Key Word Index—*Emilia flammea*; Compositae; emiline; otonecine; pyrrolizidine alkaloid.

Abstract—The structure of emiline, a pyrrolizidine alkaloid isolated from *Emilia flammea* has been revised from an 11-membered alkaloid to a 12-membered macrocyclic diester containing otonecine.

INTRODUCTION

About 30 pyrrolizidine alkaloids have been isolated which are macrocyclic diesters containing otonecine (**1**) as the base portion [1]. All but three of these are 12-membered alkaloids, and are found mainly in plants belonging to the Compositae. Of the remaining three, retusamine [2] and crosemperine [3] are present in *Crotalaria* spp. (Leguminosae), whereas emiline was isolated from *Emilia flammea* Cass. (Compositae), and was assigned structure (**2**) [4, 5]. Because of this apparent chemotaxonomic anomaly, we decided to review the evidence for the structure of emiline. Examination of the published ^{1}H NMR spectrum of emiline [4, 5] showed that only two methyl singlets were present, instead of the three required by structure (**2**). Revision of the proposed structure of emiline was clearly required.

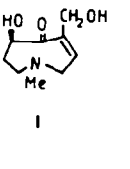
RESULTS AND DISCUSSION

Extraction of *Emilia flammea* yielded an alkaloidal mixture containing one major component (thin-layer chromatography). Trituration of the mixture and recrystallisation gave a pure alkaloid which had spectroscopic data (IR, ^{1}H NMR, MS) identical to those reported for emiline [4, 5]. The mass spectrum was typical for an otonecine diester with ions at *m/z* 168, 151, 110, and 96 [1]. An accurate mass measurement gave a molecular ion at 365.1828 corresponding to $C_{19}H_{27}NO_6$. A 200 MHz ^{1}H NMR spectrum confirmed the presence of only two methyl singlets, thus disproving structure (**2**). Furthermore, the ^{13}C NMR spectrum of emiline, obtained using a Distortionless Enhancement by Polarisation Transfer pulse sequence, showed the presence of six quaternary carbons, three methine, seven methylene, and three methyl groups, instead of the seven quaternary, two methine, six methylene, and four methyl groups required by structure (**2**). The revised structure (**3**) is proposed for emiline from detailed consideration of the ^{1}H and ^{13}C NMR spectra. In addition, 2D homonuclear (^{1}H) chemical shift correlation spectroscopy on emiline established the proton connectivities in the acid portion of the alkaloid. For example, the olefinic (C-19) protons of emiline are coupled to the C-18 methyl protons (homoallylic coupling) and to two other protons (H-14a and H-14b). Decoupling experiments also helped to confirm the presence of the $CH_3CH_2\dot{C}HCH_2$– unit in emiline. Finally, the observation of a strong $[M-44]^+$ ion in the mass spectrum of emiline assigned to structure (**4**) is a characteristic fragmentation for this mode of connection of the diacid portion to otonecine [1].

The revised structure (**3**) is consistent with all the spectroscopic data. The carbon skeleton of the acid portion is the same as that of a number of other known pyrrolizidine alkaloids [1]. Emiline now also conforms to the observed pattern that all pyrrolizidine alkaloids containing otonecine, that have been isolated from the Compositae contain 12-membered rings.

EXPERIMENTAL

Emilia flammea was grown at the Botanical Gardens in Glasgow, and was collected when flowering. The plants (300 g) were blended repeatedly with MeOH, and the MeOH extracts were concd *in vacuo*. The residual green syrup was dissolved in 1 M H_2SO_4 (300 ml), and the acid layer was washed with CH_2Cl_2 (10 × 200 ml). Zinc (5 g) was added to the acidic solution, which was stirred at room temp. for 2 hr. The solution was filtered through Celite, and the filtrate was washed with CH_2Cl_2 (2 × 400 ml). The aqueous layer was basified with conc. NH_3, and extracted with CH_2Cl_2 (4 × 400 ml). The organic extracts were dried, filtered, and concd *in vacuo* to an oil, 50 mg. Analytical TLC on silica gel (Merck) in $CHCl_3$–MeOH–conc NH_3 (85:14:1) showed one major alkaloid, R_f 0.34. Trituration of the oil with hexane, and recrystallisation from hexane gave emiline (**3**) (25 mg), mp 103–105° (*lit.* [4, 5] mp 105–107°); $[\alpha]_D^{22.5}$ – 17.5°



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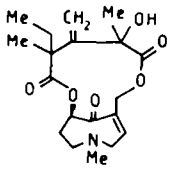

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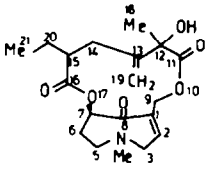

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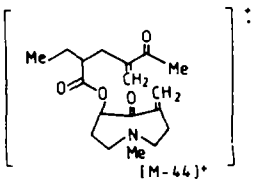

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($CHCl_3$); IR $\nu_{max}^{CHCl_3}$: 1730 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$); δ0.85 (3 H, *t*, *J* 7 Hz, 21-H_3), 1.53 (2 H, *m*, 20-H_2), 1.53 (3 H, *s*, 18-H_3), 2.06 (3 H, *s*, NMe), 2.10 (1 H, *m*, 14-H_a), 2.20 (1 H, *m*, 6-H), 2.25 (1 H, *m*, 15-H), 2.43 (1 H, *m*, 6-H), 2.65 (1 H, *m*, 5-H), 2.80 (1 H, *m*, 14-H_b), 2.85 (1 H, *m*, 5-H), 3.20 (1 H, *br d*, J_{gem} 18 Hz, 3-H), 3.44 (1 H, *br d*, J_{gem} 18 Hz, 3-H), 3.66 (1 H, *br s*, 0-H), 4.46 (1 H, *br d*, J_{gem} 11 Hz, 9-H), 4.81 (1 H, *t*, *J* 3 Hz, 7-H), 5.09 (1 H, *d*, J_{gem} 11 Hz, 9-H), 5.10 (2 H, *d*, *J* 6 Hz, 19-H_2), and 6.02 ppm (1 H, *br s*, 2-H). ^{13}C NMR (50 MHz, $CDCl_3$); δ12.0 (C-21), 26.5 (C-20), 28.5 (C-18), 36.1 (C-14), 37.5 (C-6), 40.2 (NMe), 46.9 (C-15), 53.2 (C-5), 58.6 (C-9), 66.6 (C-3), 75.2 (C-12), 77.2 (C-7), 117.9 (C-19), 131.8 (C-2), 135.7 (C-1), 146.5 (C-13), 174.7 and 177.6 (C-11 and -16) and 191.5 ppm (C-8); MS (probe) 70 eV, *m/z*: 365 $[M]^+$ 337, 321, 306, 168, 151, 125, 110, 96, 53 and 43. (Found: M^+, 365.1825. $C_{19}H_{27}NO_6$ requires 365.1838).

Acknowledgements—We are grateful to the staff at the Botanic Gardens, Glasgow, for growing and identifying *Emilia flammea*. We thank the SERC and MRC for a CASE award (to RHB).

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PYRROLIZIDINE ALKALOIDS FROM *SENECIO LONGILOBUS* AND *SENECIO GLABELLUS*

ALLEN C. RAY, HOWARD J. WILLIAMS* and JOHN C. REAGOR

Texas Veterinary Medical Diagnostic Laboratory and *Departments of Chemistry and Entomology, Texas A&M University, College Station, TX 77843, U.S.A.

Key Word Index—*Senecio longilobus*; *Senecio glabellus*; Compositae; capillary GC and GC-MS; ^1H NMR; pyrrolizidine alkaloid; integerrimine; retrorsine; seneciphylline; senecionine.

Abstract—TLC, capillary GC, packed column and capillary GC-MS, and ^1H NMR were used to characterize pyrrolizidine alkaloids from *Senecio longilobus* and *S. glabellus*. *S. glabellus* contained senecionine and integerrimine, and *S. longilobus* contained senecionine, integerrimine, seneciphylline and retrorsine, all present predominantly as *N*-oxides. Alkaloid content varied greatly in collections of *S. longilobus*. This is the first report of integerrimine in these plants.

INTRODUCTION

The chronically hepatotoxic pyrrolizidine alkaloids are common in a number of genera of plants, including *Senecio*, *Crotalaria*, *Heliotropium* and *Amsinckia* [1]. These compounds are responsible for worldwide livestock losses, and have been implicated in public health concerns, such as carcinogenicity, cirrhosis and milk transfer in cattle [1–3].

A large number of pyrrolizidine alkaloids have been isolated and characterized. *Senecio longilobus* (threadleaf groundsel) and *S. glabellus* (butterweed), two species common in Texas, have been reported to contain riddelliine, retrorsine, senecionine and seneciphylline [4, 5] and senecionine, respectively [6] (Fig. 1). Isolates from these plants were studied using TLC, GC-MS, capillary GC and GC-MS, and ^1H NMR to compare the alkaloids present in local species to literature reports, and to estimate the prevalence of *N*-oxides.

RESULTS

EtOH was superior to $CHCl_3$, Me_2CO, dilute H_2SO_4 and EtOAc in extracting the alkaloids. Extraction with EtOH at room temperature produced better yields than extraction using Soxhlet apparatus.

Examination of plant extracts by TLC was useful in acquiring qualitative information about pyrrolizidine alkaloids concerning *N*-oxide occurrence, relative abundance, and base strengths and polarities. As evidenced by the detection system developed by Mattocks [7], which is specific for unsaturated pyrroline rings and is designed to differentiate between *N*-oxides and free bases, essentially all of the alkaloids were present in the plants as *N*-oxides (i.e. unreduced extracts contained no free bases). *N*-Oxides were readily extractable from alkaline solutions, the best yield being obtained at pH 8. The free base yield, from Zn-reduced extracts, was also best at pH 8. Qualitative and quantitative differences in the alkaloid content of the