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OXYPTERINE, A CHLORINATED ALKALOID FROM *LOTONONIS* SUBSECTION *ROSTRATA*

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Abstract—A new alkaloid, oxypterine, was isolated from *Lotononis oxyptera*. It was also detected in related species of the subsection *Rostrata*. The structure consists of an azacyclooctane ring with methylene, chloro and chloromethylene substituents. Mass spectrometry and 1D and 2D NMR experiments were used in the structural analysis of the new product.

INTRODUCTION

Macrocyclic diesters of pyrrolizidine alkaloids are known to occur in several families of the plant kingdom. Of all genera investigated, *Senecio* of the Asteraceae has, by far, produced the largest number of such alkaloids [1]. The widely distributed genus *Crotalaria*, has until recently been the only known source of these secondary metabolites in the Fabaceae [1]. Their hepatotoxic [2,3] and carcinogenic [4,5] effects are well documented and are often responsible for losses of livestock all over the world [6]. In South Africa, a multitude of poisoning cases have been reported as a result of ingestion of plant material containing macrocyclic esters of pyrrolizidine alkaloids [7]. However, some promising results in the treatment of human cancer with a pyrrolizidine alkaloid were reported by Kovach *et al.* [8]. The current literature reveals a continuing interest in the isolation, characterization and physiological activity of pyrrolizidine alkaloids [9]. Our research has shown that new products are often isolated in studies directed at the application of alkaloids in chemotaxonomy [10].

Pyrrolizidine alkaloids were recently isolated from the genera *Lotononis* and *Buchenroedera* (tribe Crotalarieae, family Fabaceae) [11,12]. In these studies, we interpreted the presence of macrocyclic pyrrolizidine alkaloids as additional evidence that the two genera should be combined. A recent taxonomic revision of *Lotononis* [13] revealed that the genus can be divided into 15 sections on the basis of classical taxonomy combined with cladistic analyses. We are presently conducting a detailed investigation of the alkaloids of *Lotononis* to determine their systematic significance at the sectional level.

The six species of the subsection *Rostrata* (section *Oxydium*) are small annual herbs with rostrate keels, highly dimorphic anthers and distinctive yellow roots. GC analysis of the alkaloidal extract of one of the species in this subsection, *L. oxyptera*, was rather surprising. The analysis revealed a total absence of expected pyrrolizidine alkaloids and only one major product with a very short R_t (4.95 min). The same compound was also detected in the alkaloidal extracts of other species of the subsection *Rostrata*. This result prompted the isolation of the compound and a large scale extraction of *L. oxyptera* gave ample quantities of the new alkaloid for structural elucidation. A list of voucher specimens of the species included in our study is given in Table 1.

RESULTS AND DISCUSSION

According to NMR and mass spectral evidence the new compound is an eight-membered heterocycle with two chlorine substituents. Most of the proton resonances in the ^{1}H NMR spectrum occurred as complex multiplets which precluded the calculation of coupling constants. Decoupling and COSY experiments, however, proved useful in the assignment of signals to protons and in the determination of spin–spin interactions. A two-proton doublet of doublets of δ 5.95 was in accordance with the AB-spin system of the methylene moiety situated between the nitrogen and chlorine atoms. Irradiation of an apparent doublet for H-4 at δ 5.14 resulted in the coagulation of peaks in the δ 2.80–3.10 region. A strong coupling ($J > 12$ Hz) was associated with the downfield proton (δ 3.02) and a weak coupling ($J < 1.5$ Hz) with the upfield proton (δ 2.89). Subsequent decoupling experiments confirmed that these two protons were H-3a and H-3b.

Four protons in the medium field region of the ^{1}H NMR spectrum (Table 2) gave evidence of the alkaloidal nature of the product. Decoupling of the H-2a multiplet (δ 4.34) resulted in the disappearance of the strong coupling ($J = 12.36$ Hz) in the H-2b doublet of triplets at δ 3.75 as well as coagulation of the H-3a and H-3b multiplets at δ 3.02 and 2.89. A difference of 0.59 ppm in the chemical shifts of the C-2 protons was attributed to the close proximity of the nitrogen atom. Such behaviour is also observed in the methylene protons adjacent to nitrogen atoms in pyrrolizidine and quinolizidine alkaloids [14, 15]. The other two multiplets in the medium field region (δ 4.18 and 3.83) were assigned to H-8a and

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Table 1. Species of *Lotononis* subsection *Rostrata* in which oxypterine (1) was detected.

Species	Voucher specimen	Material extracted	Yield (mg g^{-1} dry wt)
L. arenicola	Van Wyk 3091 (JRAU)	Leaves	0.3
L. carnea	Van Wyk 2411 (JRAU)	Leaves	0.2
L. oxyptera (a)	Van Wyk 2319 (JRAU)	Leaves	3.2
L. oxyptera (b)	Van Wyk 3131 (JRAU)	Leaves/twigs	4.8
L. perplexa	Salter 8528 (BOL)	Leaves	0.1
L. rostrata	Van Wyk 2904 (JRAU)	Seeds	1.1
L. stenophylla	Van Wyk 2426 (JRAU)	Leaves	0.1

Table 2. ^{1}H NMR spectral data for oxypterine (1) (300 MHz)

H	δ (ppm)	Multiplicity	Coupling constants (Hz)
1'a	6.10	*d*	$J_{1'a,1'b}=9.79$
1'b	5.83	*d*	$J_{1'a,1'b}=9.79$
2a	4.34	*ddd*	$J_{2a,2b}=12.36$, $J_{2a,3a}=8.12$, $J_{2a,3b}=6.17$
2b	3.75	*dt*	$J_{2a,2b}=12.36$, $J_{2b,3a}=J_{2b,3b}=8.03$
3a	3.02	*m*	*
3b	2.89	*m*	*
4	5.14	*br d*	$J_{3a,4}\simeq 14$, *
5'a	5.19	*m*	*
5'b	5.19	*m*	*
6a	2.73	*m*	*
6b	2.10	*m*	*
7a	2.30	*m*	*
7b	2.10	*m*	*
8a	4.18	*ddd*	$j_{7a,8a}=8.37$, $J_{7b,8a}=7.23$, $J_{8a,8b}=12.33$
8b	3.83	*ddd*	$J_{7a,8b}=7.82$, $J_{7b,8b}=4.64$, $J_{8a,8b}=12.33$

* Coupling constants could not be determined due to the complexity of multiplets.

H-8b. Inspection of the COSY spectrum revealed that they were coupled with the C-7 protons in the aliphatic region (δ 2.30 and 2.10). Another proton at $\delta\simeq 2.10$ was strongly coupled with one at δ 2.73 which displayed spin–spin interaction with H-7a at δ 2.30. This allowed the assignment of the δ 2.73 and 2.10 multiplets to H-6a and H-6b. The COSY experiment showed that these two protons were weakly coupled with a two-proton signal at δ 5.19. The nature of this signal (a broad singlet) was reminiscent of terminal alkene hydrogens and could therefore be assigned to the C-5' protons.

The ^{13}C NMR, DEPT and HETCORR experiments followed the proton NMR analysis to verify the postulated structure. Nine carbon signals were evident from the ^{13}C NMR spectrum. The DEPT spectrum showed one methine and seven methylene carbons. The absence of the low field carbon resonance (δ 143.2) in the DEPT spectrum, was in accordance with the C-5 quaternary sp^2 carbon atom. A triplet at δ 111.2 was correlated with the terminal olefin protons in the HETCORR experiment and was assigned to C-5'. The only doublet in the ^{13}C NMR (C-4, δ 78.5) was coupled with the methine proton (δ 4.43). Association with a chlorine atom and a neighbouring olefinic moiety accounted for the low field chemical shift of C-4.

Three carbon triplets occurred in the medium field region, showing their association with hetero atoms. The signal at δ 60.7 was assigned to C-2 on the basis of its correlation with the H-2 protons (δ 4.34 and δ 3.75). Similarly, the δ 63.1 signal correlated with the H-8 protons. A slightly stronger deshielding of the methylene carbon at δ 67.7 indicated its bond with the nitrogen atom and another hetero atom. It showed the expected correlation with the AB-system in the HETCORR experiment. The three remaining methylene carbon resonances were all in the high field region and could be assigned to C-3, C-6 and C-8 on the basis of the HETCORR experiment.

In order to determine the nature of the other hetero atoms, which were both assumed to be halogens, mass spectral analysis of the compound was performed. The first indication of the presence of chlorine in the molecule was found in the *m/z* 172 and 174 fragmentation peaks in the mass spectrum which fitted an empirical formula of $C_9H_{15}NCl$. The natural ratio of the ^{35}Cl and the ^{37}Cl isotopes corresponded closely with the intensity ratio of 100:38 for the two fragments. Further inspection of the mass spectrum revealed that the $[M]^+$ was represented by two small peaks at *m/z* 207 and 209 which provided the evidence for a molecular formula of $C_9H_{15}NCl_2$. An intensity ratio of 100:66 for the two peaks could be

interpreted as an $[M_{^{35}Cl^{35}Cl}]^+$ ion for the *m/z* 207 peak and two superimposed $[M_{^{35}Cl^{37}Cl}]^+$ ions for the *m/z* 209 peak. Although it was weak in the mass spectrum, the $[M_{^{37}Cl^{37}Cl}]^+$ ion with *m/z* 211 was also detected. The $[M]^+$ of the ^{35}Cl isotope, *m/z* 207, rapidly lost a ^{35}Cl radical to produce the *m/z* 172 mass fragment, while the same fragmentation occurred for the higher mass isotopes. This ion lost either a CH_2NCH_2 or a $CH_2CH_2CH_2$ fragment to give the next significant peak at *m/z* 130. The accompanying *m/z* 132 peak for the ^{37}Cl isotope confirmed the definite presence of a chlorine atom in this fragment. Dichloro compounds are well known to produce weak $[M]^+$ in their mass spectra but usually give strong $[M-Cl]^+$ ions [16]. The acquired spectral data were conclusive of the structure of oxypterine (**1**) as 4-chloro-*N*-chloromethyl-5-methylene-1-azacyclooctane. Further studies are directed towards the assignment of the absolute configuration of the C-4 chiral centre.

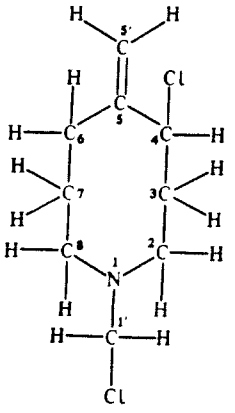
Oxypterine (**1**) is unusual in both its eight-membered cyclic structure and in its chemical composition. The fact that pyrrolizidine alkaloids occur in *Lotononis*, suggested that **1** may be related to the necine bases. The eight-membered ring **1** is reminiscent of otonecine (**2**), a cyclic eight-membered aminone which is the necine base of a large variety of macrocyclic alkaloids closely related to the pyrrolizidine alkaloids [1]. Oxypterine seems to be structurally related to these otonecine bases and was, therefore, assumed to be a modified necine which may be synthesized from the same precursors that are involved in the synthesis of the pyrrolizidine necines. Extensive work on the biosynthesis of necine bases by Khan and Robins [17, 18] showed that putrescine (**3**) is converted into retronecine (**5**) via homospermidine (**4**) (Scheme 1). Further studies on **1** are required to establish its biosynthetic relationship with **3** and the necine bases. Although chlorinated alkaloids are rarely found in the higher orders of plants, the chlorodeoxy pyrrolizidines merenskine (**6**) and its *N*-oxide (**7**) have been isolated from *Senecio latifolius* [19]. The occurrence of chlorinated products in a genus which produces pyrrolizidine alkaloids thus signifies the possibility that chlorination may occur in the biosynthesis of pyrrolizidine type alkaloids. Chlorination, however, occurs in the necinic acid parts of **2** and **3** and not in the necine bases as with **1**.

EXPERIMENTAL

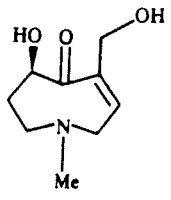
Mp: uncorr. IR spectrum was recorded as a thin film from a $CHCl_3$ soln. Optical rotation was measured for a path length of 1 cm in $CHCl_3$. ^1H NMR spectroscopy was performed at 200 and 300 MHz and the ^{13}C NMR spectrum was recorded at 50 MHz in $CDCl_3$ using the $CHCl_3$ signals (δ 7.24 and δ 77.0) as refs. All 2D NMR expts were conducted with a 200 MHz instrument. EI-MS was performed at 70 eV. TLC was performed on Merck 60 F_{254} type E alumina plates with a 0.25 mm layer thickness in 1.5% MeOH in $CHCl_3$ as eluent. Chromatograms were developed by spraying with iodoplatinate reagent. CC was performed using Fluka neutral alumina type 507C with 1.5% MeOH in $CHCl_3$ as eluent. Parameters for GC-analysis have been published elsewhere [20].

Analytical alkaloidal extracts were obtained from the specimens listed in Table 1 using standard procedures [21]. For the large scale extn, 68 g air-dried leaves and twigs of *L. oxyptera* [voucher specimen *BVW 3131* (JRAU)] were finely milled and suspended in 0.05 M H_2SO_4 for 0.5 hr. After filtration of solids, the acidic liquor was neutralized with conc. NH_3 and extracted with CH_2Cl_2 to afford 325 mg of crude alkaloid. CC chromatography gave pure **1** (279 mg) as an off-white crystalline solid.

Oxypterine (**1**). Mp 127–129°. $[\alpha]_D^{22}$ +19.6 ($CHCl_3$; *c* 2.6). IR $\nu_{max}^{CHCl_3}$ 2950 (C-H), 1655 (C=C), 1465 (CH_2 bend) cm^{-1}. ^{13}C NMR (50 MHz) δ 143.2 (*s*, C-5), 111.2 (*t*, C-5'), 78.5 (*d*, C-4),



1



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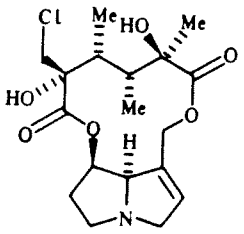
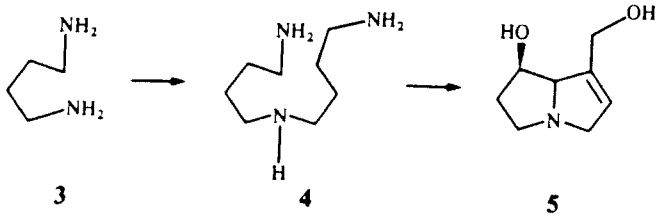


6

7 *N*-Oxide of **6**



3 **4** **5**

Scheme 1. Biosynthesis of retronecine (**5**) from putrescine (**3**).

67.7 (*t*, N-C-Cl), 63.1 (*t*, C-8), 60.7 (*t*, C-2), 31.2 (*t*, C-6), 30.3 (*t*, C-3), 23.7 (*t*, C-4). EIMS (probe) 70 eV *m z* (rel. int.): 209 (1), 207 (2), 174 (38), 173 (13), 172 (100), 144 (3), 136 (10), 133 (29), 131 (7), 130 (89), 123 (12), 122 (2), 120 (13), 118 (27), 96 (9), 95 (43), 94 (13), 91 (8), 82 (15), 79 (11), 67 (16), 55 (14), 53 (12), 42 (34), 41 (20).

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