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KINABALINE AND THE APORPHINOID BIOGENESIS OF AZAANTHRACENE AND AZAFLUORENE ALKALOIDS*

DRAGANA TADIĆ, BRUCE K. CASSELS, MICHEL LEBOEUF and ANDRÉ CAVÉ†

Laboratoire de Pharmacognosie, UA 496 CNRS, Faculté de Pharmacie, 92296 Châtenay-Malabry Cedex, France

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Key Word Index—*Meiogyne virgata*; Annonaceae; kinabaline; azafluorene alkaloids; cleistopholine; aporphinoids; biogenesis.

Abstract—The new azafluorene alkaloid kinabaline was isolated from *Meiogyne virgata*, together with liriodenine, cleistopholine and other known substances. The azafluorene onychine and the azaanthracene cleistopholine can be related to the diazafluoranthene eupolauridine through a common hypothetical precursor derived from the oxoaporphine liriodenine.

INTRODUCTION

Meiogyne virgata is a rain forest tree attaining a height of 20 m, which grows in Malacca, Java, Sumatra and Borneo. It is the type species of its genus, composed of *ca* seven species of trees and shrubs distributed between southern India and the Philippine Islands [2]. This genus is included in the *Polyalthia* group of the tribe Unoneae in Fries' classification system [3], and both Fries and Hutchinson [4] agree in placing *Meiogyne* close to *Polyalthia* and *Cananga*, genera which have been examined for secondary metabolites [5]. To the best of our knowledge, the chemistry of *Meiogyne* species has never been studied before. In this paper we report the results of an analysis of the trunk bark of *M. virgata* collected on Mount Kinabalu, in Borneo (Sabah, Malaysia).

RESULTS AND DISCUSSION

The petrol extract of the bark gave undecane, dodecane, sitosterol and stigmast-4-en-3-one. Extraction of the basified (NH_3) plant material with CH_2Cl_2 afforded 0.30% of crude alkaloids which were separated by CC and TLC. The known bases isolated were the common oxoaporphine liriodenine, accompanied by its putative precursor noraporphines (−)-norushinsunine and (−)-anonaine as well as their close biogenetic relatives (−)-asimilobine and the norproaporphine (+)-stepharine, all presumably derived from (*R*)-coclaurine. Other bark alkaloids were several (*S*)-reticuline derivatives, the berbines (−)-corydalmine, (−)-discretamine and (−)-stepholidine, together with the protoberberine dehydrocorydalmine and the aporphine (+)-corytuberine. A minor constituent (0.0009%) was shown to be 1-aza-4-methylanthraquinone (**1**), isolated at about the same time from *Cleistopholis patens* (Annonaceae) and given the name cleistopholine [6].

As a sufficient amount of cleistopholine was available, a long-range *J*-correlated heteronuclear (^1H–^{13}C) 2D NMR spectrum (COSY) was recorded (Fig. 1) to remove the extant ambiguity in the original ^1H NMR assignments and to allow a complete interpretation of the ^{13}C NMR spectrum [6]. The key to the assignment of the very close signal pairs due to the benzene ring hydrogen and carbon nuclei was the nuclear Overhauser effect of the methyl protons on the neighbouring (C-10) carbonyl ^{13}C resulting in a much stronger signal at 181.9 ppm than at 184.7 ppm. Analysis of the long-range (^3J) ^1H–^{13}C couplings then produced the resonance attributions shown in Table 1.

Traces (0.0001%) of a yellow alkaloid giving an unusual purplish Dragendorff reaction could also be isolated. Its HREI mass spectrum showed an abundant $[M]^+$ with the composition $C_{15}H_{13}NO_4$ which lost H, CO and OH, successively. Alternative fragmentations observed in the LREI mass spectrum were the losses of a methyl group and of the elements of water from the $[M]^+$. The ^1H NMR spectrum indicated the presence of a methyl group bonded to an aromatic ring, two methoxyl groups, an isolated benzene hydrogen atom and an α,β pair of pyridine hydrogen atoms of which the H-β signal was broadened by long-range coupling with the *C*-methyl protons which resonated as a very tight doublet. The UV-VIS spectrum was complex and underwent bathochromic shifts both upon acidification and on adding base. The spectral features observed in neutral and acid solution were reminiscent of those reported for onychine (**2**), isolated from the Annonaceae *Onychopetalum amazonicum* [7] and *Cleistopholis patens* [6], and the shift seen in basic solution indicated the presence of a phenolic function. Both an intramolecularly hydrogen-bonded hydroxyl group and a low-frequency carbonyl group were evident in the IR spectrum. All these data could be accommodated by a 4- (or 1-)aza-1- (or 4-)methylfluoren-9-one skeleton with a phenolic function and two methoxyl groups substituting the benzene ring. As the one-proton singlet appears near 6.4 ppm, the benzene ring hydrogen atom must be flanked by two oxygen substituents. To rule

* Part 69 in the series "Alcaloïdes des Annonacées". For part 68 see ref. [1].

† To whom correspondence should be addressed.

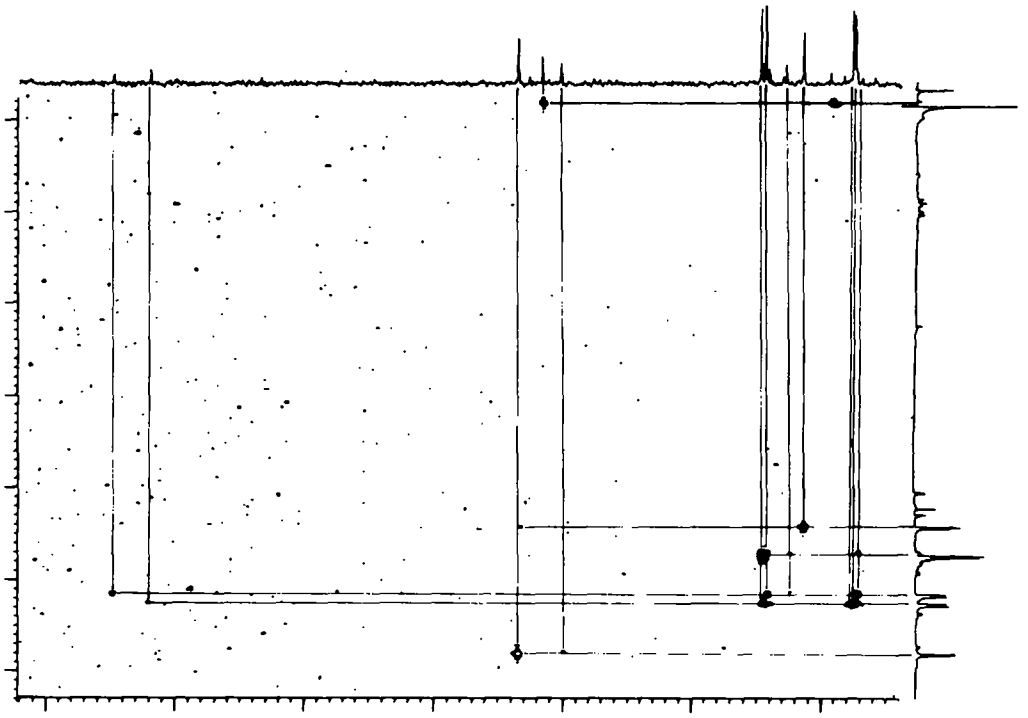
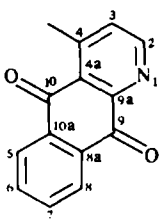


Fig. 1. 2D Heteronuclear ^{1}H–^{13}C NMR chemical shift correlation contour plot for cleistopholine (1) at 400/100.6 MHz in $CDCl_3$.

Table 1. ^{1}H (400 MHz) and ^{13}C (100.6 MHz) NMR chemical shifts (ppm from TMS), ^{1}H signal multiplicities and ^{1}H–^{1}H coupling constants (Hz) for cleistopholine (1) in $CDCl_3$

	$\delta^{1}H$	$\delta^{13}C$
H/C-2	8.95 *d* (4.8)	153.4
H/C-3	7.47 *d* (4.8)	131.2
C-4		151.6
C-4a		129.1
H/C-5	8.31 *dd* (8.5; 2.2)	127.4
H/C-6	7.79 *m*	134.2
H/C-7	7.79 *m*	134.6
H/C-8	8.21 *dd* (8.5; 2.2)	127.2
C-8a		127.1
C-9		184.7
C-9a		150.1
C-10		181.9
C-10a		132.6
CH_3	2.89 *s*	22.8

out some of the structural alternatives consistent with this information, the NMR sample was saturated at each of the three methyl resonance frequencies. The only nuclear Overhauser effect observed was an enhancement of the benzene proton singlet upon irradiation at the downfield methoxyl frequency, showing that these structural elements bear an *ortho* relationship to each other and suggesting that the hydrogen atom bonded to the benzene ring does not lie between both methoxyl groups. To narrow the range of possible structures even further, the carbonyl group was reduced with $NaBH_4$ and the product (**3a**) was studied by ^{1}H NMR (see Table 2). The most significant changes in the ^{1}H NMR spectrum were the appearance of a methine proton singlet and an upfield shift of the pyridine ring methyl resonance, indicating that the ring system is that of 4-aza-1-methylfluoren-9-one (**2**), and not its 1-aza-4-methyl isomer **4**. Also important was the shift observed for the downfield methoxyl resonance, allowing the corresponding group to be placed *peri* to the ketone function. In this case, the benzene hydrogen atom



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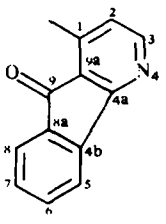

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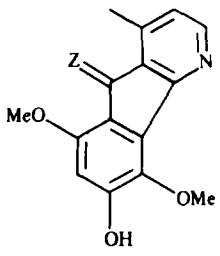
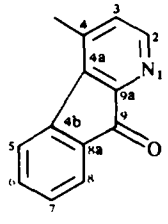


3 Z = O
3a Z = H,OH



4

Table 2. ^{1}H NMR chemical shifts (ppm from TMS), signal multiplicities and coupling constants (Hz) for compounds 3 and 3a

	3 DMSO-d_6*	3 CD_3OD†	3 $CDCl_3$†	3a $CDCl_3$*
CH_3-1	2.51 *d* (0.8)	2.62 *br s*	2.63 *br s*	2.53 *br s*
CH_3O-5‡	3.79 *s*	3.91 *s*	3.93 *s*	3.90 *s*
CH_3O-8‡	3.84 *s*	3.93 *s*	4.02 *s*	3.95 *s*
H-2	7.12 *dq* (5.2; 0.8)	7.10 *d* (5)	6.95 *d* (5)	6.95 *d* (5.0)
H-3	8.48 *d* (5.2)	8.41 *d* (5)	8.51 *d* (5)	8.49 *d* (5.0)
H-7	6.47 *s*	6.46 *s*	6.34 *s*	6.49 *s*
H-9	—	—	—	5.75 *s*

*Recorded at 500 MHz.

†Recorded at 80.13 MHz.

‡Assignments may be reversed. The downfield signal was consistently the broader of the two. In DMSO-d_6, a NOE was observed between protons of 3 resonating at 3.84 and 6.47 ppm.

should be located at C-7 and the most likely structure for the new alkaloid must be 5,8-dimethoxy-6-hydroxy-1-methyl-4-azafluoren-9-one (3) (5*H*-6,9-dimethoxy-8-hydroxy-4-methylindeno[1,2-*b*]pyridine-5-one), for which we propose the trivial name kinabaline. This substance is the second azafluorene alkaloid to be characterized as such, and may be designated semisystematically as 5,8-dimethoxy-6-hydroxyonychine.

As the phytochemical literature references to onychine represent it as 1-aza-4-methylfluoren-9-one (4) [6, 7], we consider it appropriate to underline the fact that the correct structure is 4-aza-1-methylfluoren-9-one (2). This substance has been synthesized by three different unambiguous routes [8–10], its physical and spectroscopic properties agree well with those of the natural product and in 1977 a comparison of the ^{1}H NMR spectra of the borohydride reduction products of onychine and of synthetic 2 proved beyond all reasonable doubt that both substances are identical [10].

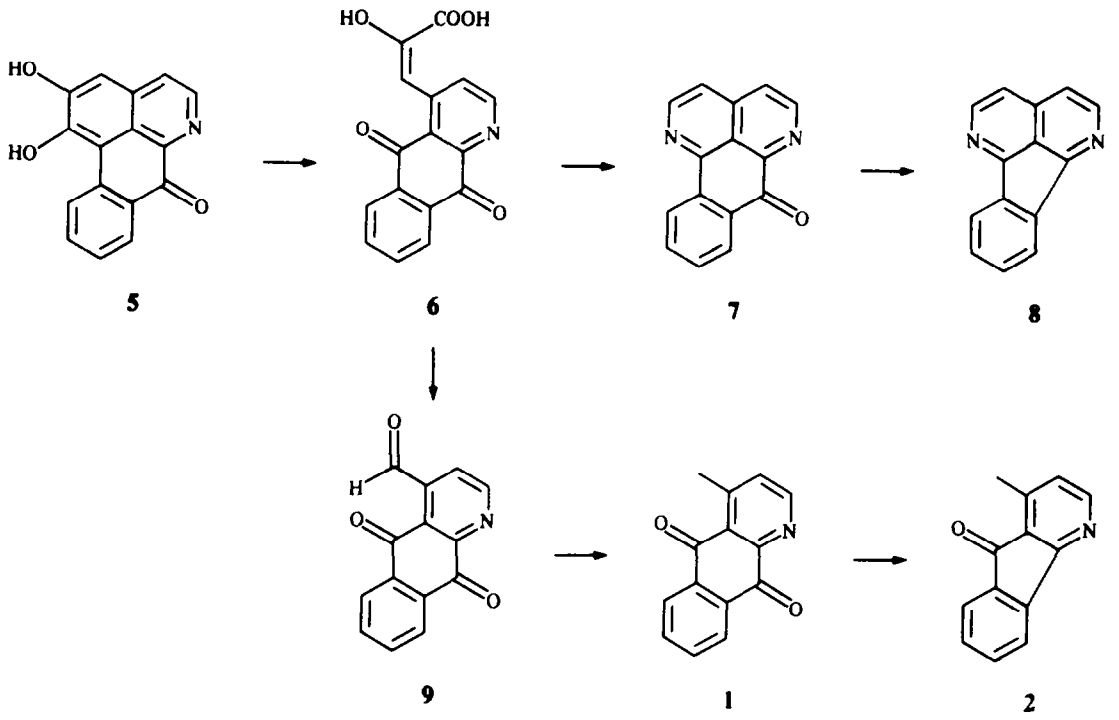
When onychine was isolated for the first time [7] it was envisioned as deriving from phenylalanine and mevalonate, as a C_6–C_2 moiety and an isoprenoid unit joined through nitrogen are clearly apparent in structure 4. Inspection of the correct structure of onychine (2) allows no straightforward derivation from phenylalanine, although an isopentene unit can be discerned. The same has already been pointed out for cleistopholine [6]. Our reisolation of liriodenine and cleistopholine together with the onychine congener kinabaline (3) from *M. virgata* should be regarded in the light of the co-occurrence of liriodenine, cleistopholine and onychine in *C. patens* [6]. In this context it is tempting to try to relate the oxoaporphines, 1-azaanthracenes and 4-azafluorenes in a biogenetic framework.

It has been suggested by Taylor [11] that the catecholic analogue 5 of liriodenine may undergo an extradiol cleavage giving the azaanthraquinone acid 6, which could then be converted in several steps to the 1-aza-7-oxoaporphine 7, a hypothetical precursor of eupolauramine and eupolauridine (8). We consider it likely that a path to cleistopholine and onychine could diverge from this route to the 1-azaaporphinoids: the aliphatic side chain of 6 could be degraded to a one-carbon residue, as in aldehyde 9, and this could undergo reduction to afford cleistopholine (1). Extrusion of the C-9 carbonyl group of cleistopholine would then give onychine (2) in a way which parallels the postulated derivation of eupolauridine (8) [11], as shown in Scheme 1.

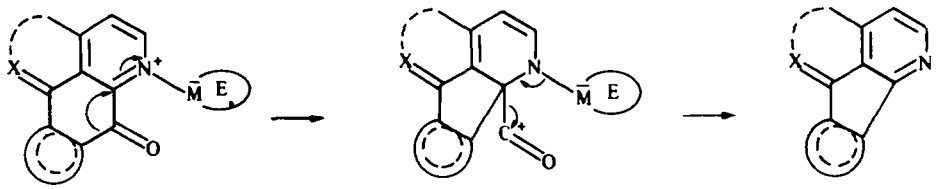
The decarbonylation of oxoaporphine derivatives has been proposed as a general reaction leading not only to the diazafluoranthene eupolauridine but also to the azafluoranthene and 'tropoloisoquinoline' alkaloids of the genus *Abuta* (Menispermaceae) [11]. A similar explanation of the origin of azafluorenes like onychine extends the Taylor hypothesis [11] to yet another group of alkaloids. It is probably of some mechanistic significance that all these carbonyl extrusions should occur α to the pyridine nitrogen atom. A photochemical mechanism has been put forward specifically to explain the hypothetical decarbonylation of oxoaporphines to azafluoranthenes [12]. An alternative pathway involving a metalloenzyme is illustrated in Scheme 2, where it can be clearly seen that the loss of the C-10 carbonyl group of cleistopholine to give 4 would not be facilitated.

The structure of kinabaline (3) fits into the biogenetic hypothesis presented in Scheme 1, but it raises a number of questions. Does kinabaline arise from a ring D-oxygenated analogue of liriodenine with a hitherto unknown pentasubstitution pattern? May one or two of the oxygen atoms proceed from an oxoaporphine precursor, with the remaining substituents being added at a later stage? Is kinabaline formed by oxidation of the benzene ring of cleistopholine or onychine? Hoping to find other oxidized derivatives of the azafluorenone, azaanthraquinone or oxoaporphine systems which might further illuminate the biogenesis of kinabaline, the leaf alkaloids of *M. virgata* were also analysed. The crude bases (0.38%) yielded traces of liriodenine, but no cleistopholine, onychine, kinabaline or other new compounds were obtained. Details on the isolation of known substances will be published separately.

The proposal that 1-azaanthracene and 4-azafluorene skeletons are aporphine-derived, and that these structures may be elaborated further giving products like kinabaline, suggests a broad range of unknown cryptic aporphinoids. We believe that kinabaline, onychine and cleistopholine



Scheme 1. Biogenetic relationships of oxoaporphine, diazafluoranthene, azaanthracene and azafluorene alkaloids.



Scheme 2. Postulated metalloenzyme-catalysed decarbonylation pathway for α-aroylpyridine derivatives.

merely represent the 'tip of an iceberg' which will emerge rapidly as the use of sensitive analytical methods in plant chemistry becomes more widespread.

EXPERIMENTAL

Plant material. Trunk bark and leaves of *M. virgata* (Bl.) Miq. were collected in Poring, on Mt. Kinabalu, Sabah, Malaysia, in July, 1982. Voucher specimens (WS 58) have been deposited at the herbaria of the Museum National d'Histoire Naturelle, Paris, and of the Rijksuniversiteit, Utrecht. The identification was carried out by Dr. P. J. M. Maas, of Utrecht.

Extraction of trunk bark. Powdered bark (3.15 kg) was extracted with petrol (40–65°), dried, made alkaline with conc NH_3 and extracted with CH_2Cl_2. The petrol ext was concd and subjected to CC on silica gel. Elution with hexane gave a mixture of alkanes (8 g) containing mainly undecane and dodecane (GC-MS). Elution with CH_2Cl_2 gave sitosterol (90 mg) and stigmast-4-en-3-one (40 mg). The CH_2Cl_2 extract was concd giving a gummy residue (10.3 g) which was fractionated by CC on silica gel using a CH_2Cl_2–MeOH gradient. Liriodenine (2.6 g) crystallized on concentrating the initial eluates. Corydalmine (930 mg), norushinsunine (720 mg), stepholidine (620 mg), discretamine (620 mg), anonaine (70 mg), dehydrocorydalmine (50 mg), cleistopholine (1) (30 mg), asimilobine (10 mg), stepharine (10 mg), kinabaline (3) (4 mg), and corytuberine (2 mg) were purified by prep. TLC on silica gel.

Cleistopholine (1). Yellow glassy solid. UV-VIS, IR, EIMS in agreement with published data [6]. ^1H and ^{13}CNMR see Table 1.

Kinabaline (3). Amorphous yellow solid. EIMS *m/z* (rel. int.) 272 $[M+1]^+$ (9); 271.0864 $[M]^+$ (72), $C_{15}H_{13}NO_4$ calc. 271.0844; 270.0793 $[M-H]^+$ (54), $C_{15}H_{12}NO_4$ calc. 270.0766; 256 $[M-CH_3]^+$ (10); 253 $[M-H_2O]^+$ (9); 243 $[M-CO]^+$ (17); 242.0827 $[M-H-CO]^+$ (100), $C_{14}H_{12}NO_3$ calc. 242.0817; 225.0795 $[M-H-CO-OH]^+$ (23), $C_{14}H_{11}NO_2$ calc. 225.0790; 212 (12); 199 (10); 154 (15); 149 (11); 143 (13); 128 (30). ^1H NMR see Table 2. UV (MeOH) λ_{max} nm (log ε): 208 (3.96), 222 (3.91), 231 (3.93), 246 sh (4.01), 254 (4.12), 280 sh (3.72), 292 (3.77), 304 (3.73), 388 (3.32). UV (MeOH + HCl) λ_{max} nm (log ε): 209 (4.03), 233 (3.95), 252 sh (3.81), 292 sh (3.69), 304 (3.84), 316 (3.88), 410 (3.24). UV (MeOH + NaOH) λ_{max} nm (log ε): 220 (4.52), 247 (4.18), 255 (4.18), 284 (3.95), 296 (3.96), 308 (3.92), 366 (3.66), 450 (3.62).

Dihydrokinabaline (3a). 3 (3 mg) in MeOH (1 ml) was treated with $NaBH_4$ at room temp. for 0.5 hr. The reaction mixture was

concd and purified by TLC on silica gel to give **3a** (1.5 mg) as a pale yellow gum. ^{1}H NMR see Table 2.

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