

KUAFUMINE, A NOVEL CYTOTOXIC OXOAPORPHINE ALKALOID FROM FISSISTIGMA  
GLAUCESCENS

Yang-Chang Wu<sup>a</sup>, Sheng-Teh Lu<sup>a</sup>, Tian-Shung Wu<sup>b\*</sup>, and Kuo-Hsiung Lee<sup>c</sup>

a. School of Pharmacy, Kaohsiung Medical College, Kaohsiung, Taiwan 800,  
R.O.C.

b. Department of Applied Chemistry, Providence College of Arts and  
Science, Taichung, Taiwan 40211, R.O.C.

c. Natural Products Laboratory, Division of Medicinal Chemistry and  
Natural Products, School of Pharmacy, University of North Carolina,  
Chapel Hill, North Carolina 27514, U.S.A.

Abstract - The structure of kuafumine, a new oxoaporphine alkaloid  
isolated from Fissistigma glaucescens was established as formula  $\lambda$ . This  
alkaloid showed potent cytotoxicity to KB cell ( $ED_{50} = 0.2$  mcg/ml) in  
vitro.

In a previous paper,<sup>1</sup> we reported the isolation and identification of nine alkaloids along with two unidentified compounds from Fissistigma glaucescens (Chinese name: Kua-Fu-Mu) (Annonaceae).<sup>2</sup> The present paper describes the structure elucidation of a new cytotoxic oxoaporphine alkaloid, kuafumine (FGB), between these two unidentified compounds.

Kuafumine (1) was isolated as reddish needles from acetone, mp 230-232° C,  $[\alpha]_D^{24} \pm 0^\circ$  ( $c = 0.1$ ,  $CHCl_3$ ). The molecular formula of  $\lambda$  was established as  $C_{20}H_{15}NO_6$  by high resolution mass spectrometry (Found: 365.0903, Calcd. 365.0898). The presence of an oxoaporphine skeleton in the molecule was easily deduced by the UV spectrum [ $\lambda_{max}^{MeOH}$  nm(log  $\epsilon$ ): 214(4.32), 245(4.14), 283(4.38) and 375(3.38)], along with the conjugated carbonyl group absorption band at  $1650\text{ cm}^{-1}$  in the IR spectrum. The absence of phenolic hydroxyl group in the molecule was indicated by the following evidence: i) no bathochromic shift was observed upon addition of the shift reagent KOH in the UV spectrum, ii) no absorption band was seen at  $3000\text{--}3600\text{ cm}^{-1}$  region in the IR spectrum. The  $^1H$  NMR spectrum of kuafumine (Table 1) revealed the presence of two AB-quartets. One of them at  $\delta$  7.98 and 8.78 ( $J = 5.5$  Hz) was assigned to H-4 and H-5,<sup>3</sup> while the other at  $\delta$  7.04 and 8.06 was attributed to two mutually ortho-located protons on the aromatic ring. The higher field signal ( $\delta$  7.04) was assigned to H-10 as it gave rise to a 10.5% nOe enhancement of the signal when the methoxyl group at C-9 ( $\delta$  3.92) was irradiated. The other NMR signals of which appeared at  $\delta$  3.98

and 4.23 (3H each, singlet each) and  $\delta$  6.26 (2H, singlet) were assigned to two methoxyls and a methylenedioxy group, respectively. The above data led us to propose the structure of kuafumine either as  $1$ ,  $2$  or  $3$ . A comparison of the  $^1\text{H-NMR}$  spectra (Table 1) of  $1$ ,  $4$  and oxocrebanine ( $5$ ) clearly ruled out the possibility of  $2$  or  $3$  as the coupling constants of H-10 ( $J = 8.8$  Hz) and H-11 ( $J = 8.8$  Hz) as well as the chemical shifts of the two methoxyl groups at C-8 ( $\delta$  3.98) and C-9 ( $\delta$  3.92) of  $1$  are comparable to those of  $5$  instead of those of  $4$ . The latter showed a  $J$  value of 9.0 Hz each for H-8 and H-9 as well as  $\delta$  3.78 and  $\delta$  3.98 for the methoxyl groups at C-10 and C-11, respectively. This evidence also confirmed the assignment of the two methoxyl groups of  $1$  at C-8 and C-9 instead of at C-10 and C-11 as found in  $4$ .

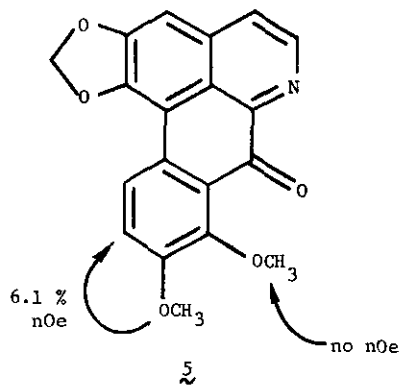
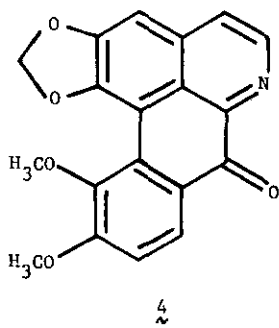
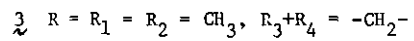
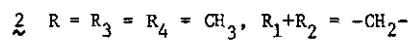
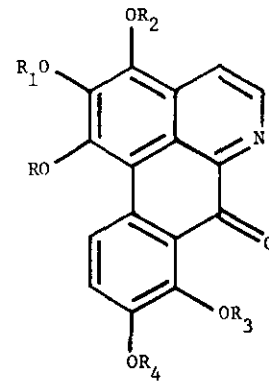
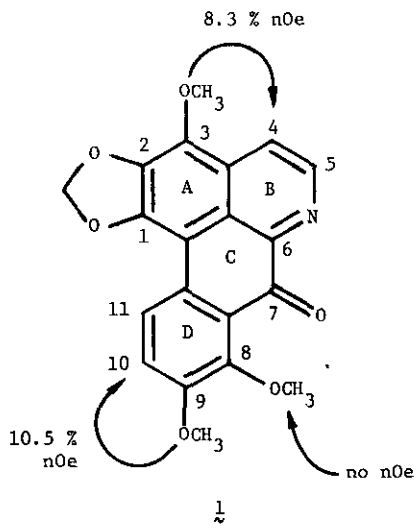
Further evidence to support the assignment of  $1$  for kuafumine was sought in a nuclear Overhauser effect experiment. Irradiation of methoxyl signals at  $\delta$  3.92 and 4.23 led to a 10.5% and 8.3% enhancement of the signals at  $\delta$  7.04 (H-10) and 7.98 (H-4), respectively, demonstrating that the two methoxyl groups of them are situated at C-9 and C-3. However, irradiation of the 8-methoxyl group at  $\delta$  3.98, no noe enhancement was observed at any aromatic protons as expected. On the basis of these results, kuafumine should be represented by formula  $1$ .<sup>5</sup>

This new alkaloid, kuafumine ( $1$ ), exhibited a potent cytotoxicity ( $\text{ED}_{50}=0.2$  mcg/ml) in the KB tissue culture cell in vitro.<sup>6</sup> The C-3 OMe group of  $1$  contributes to potent cytotoxicity as  $5$  [ $\text{ED}_{50}$  (KB)=4.0 mcg/ml] which lacks this OMe group is 20-fold less active than  $1$ .

Table I.  $^1\text{H-NMR}$  Spectra of Oxoaporphine Alkaloids<sup>a</sup>

	1	4	5
$-\text{OCH}_2\text{O}-$	6.26 (2H,s)	6.18 (2H,s)	6.30 (2H,s)
3-H ( $\text{OCH}_3$ )	4.23 (3H,s)	7.06 (1H,s)	6.98 (1H,s)
4-H	7.98 (1H,d;5.5)	7.62 (1H,d;5.0)	7.61 (1H,d;5.0)
5-H	8.78 (1H,d;5.5)	8.72 (1H,d;5.0)	8.77 (1H,d;5.0)
8-H ( $\text{OCH}_3$ )	3.98 (3H,s)	8.30 (1H,d;9.0)	4.02 (3H,s)
9-H ( $\text{OCH}_3$ )	3.92 (3H,s)	7.06 (1H,d;9.0)	3.96 (3H,s)
10-H ( $\text{OCH}_3$ )	7.04 (1H,d;8.8)	3.78 (3H,s)	7.11 (1H,d;8.8)
11-H ( $\text{OCH}_3$ )	8.06 (1H,d;8.8)	3.98 (3H,s)	8.21 (1H,d;8.8)

<sup>a</sup>) Run in  $\text{CDCl}_3$ . Values are ppm. Figures in parentheses are coupling constants in Hz.



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3. H. Guinaudeau, M. Leboeuf and A. Cave, J. Nat. Prod., 1983, **46**, 761.
4. An oxidizing derivative of O-methylbulbocapnine (6): mp 235-236°C (acetone);  $\lambda_{\max}^{\text{MeOH}}$  nm (log  $\epsilon$ ): 256(4.57), 360 (4.12) and 410 (4.11);  $\lambda_{\max}^{\text{nujol}}$   $\text{cm}^{-1}$ : 1665, 1044, 940.
5. MS m/z(%): 365(100), 350(69), 334(10), 320(23), 249(8) and 175(17).
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