KUAFUMINE, A NOVEL CYTOTOXIC OXOAPORPHINE ALKALOID FROM FISSISTIGMA GLAUCESCENS

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Abstract - The structure of kuafumine, a new oxoaporphine alkaloid isolated from Fissistigma glaucescens was established as formula 1. This alkaloid showed potent cytotoxicity to KB cell (ED_{50} = 0.2 mcg/ml) in vitro.

In a previous paper,[1] we reported the isolation and identification of nine alkaloids along with two unidentified compounds from Fissistigma glaucescens (Chinese name: Kua-Fu-Mu) (Annonaceae).[2] 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, $[\alpha]_D^{24}$ ± 0° (c = 0.1, $CHCl_3$). The molecular formula of 1 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 [λ_{max}^{MeOH}nm(log ε): 214(4.32), 245(4.14), 283(4.38) and 375(3.38)], along with the conjugated carbonyl group absorption band at 1650 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 3000-3600 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 δ 7.98 and 8.78 (J = 5.5 Hz) was assigned to H-4 and H-5,[3] while the other at δ 7.04 and 8.06 was attributed to two mutually ortho-located protons on the aromatic ring. The higher field signal (δ 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 (δ 3.92) was irradiated. The other NMR signals of which appeared at δ 3.98

and 4.23 (3H each, singlet each) and δ 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}H-NMR spectra (Table 1) of 1, 4[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 (δ 3.98) and C-9 (δ 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 δ 3.78 and δ 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.

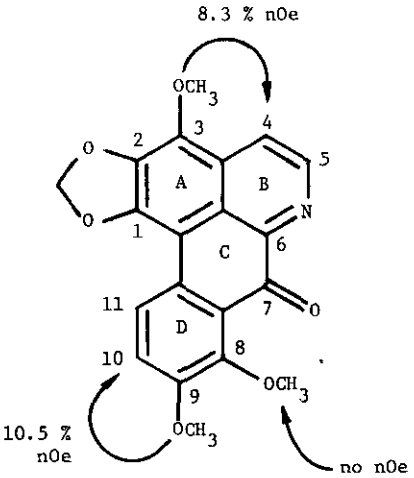
Further evidence to support the assignment of 1 for kuafumine was sought in a nuclear Overhauser effect experiment. Irradiation of methoxyl signals at δ 3.92 and 4.23 led to a 10.5% and 8.3% enhancement of the signals at δ 7.04 (H-10) and 7.98 (H-4), respectively, demonstrating that the two methoxyl groups of them are situated at C-9 and C-3. However, irradiation of the 8-methoxyl group at δ 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.[5]

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

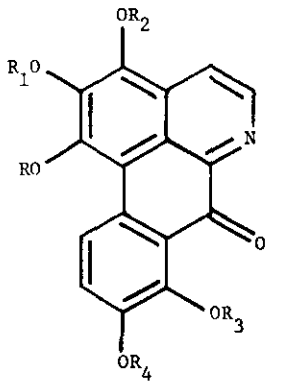
Table I. ^{1}H-NMR Spectra of Oxoaporphine Alkaloids[a]

	1	4	5
$-OCH_2O-$	6.26 (2H,s)	6.18 (2H,s)	6.30 (2H,s)
3-H (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 (OCH_3)	3.98 (3H,s)	8.30 (1H,d;9.0)	4.02 (3H,s)
9-H (OCH_3)	3.92 (3H,s)	7.06 (1H,d;9.0)	3.96 (3H,s)
10-H (OCH_3)	7.04 (1H,d;8.8)	3.78 (3H,s)	7.11 (1H,d;8.8)
11-H (OCH_3)	8.06 (1H,d;8.8)	3.98 (3H,s)	8.21 (1H,d;8.8)

a) Run in $CDCl_3$. Values are ppm. Figures in parentheses are coupling constants in Hz.

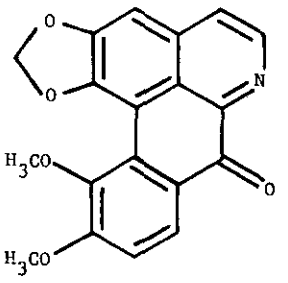


1



2 R = R_3 = R_4 = CH_3, R_1+R_2 = $-CH_2-$

3 R = R_1 = R_2 = CH_3, R_3+R_4 = $-CH_2-$



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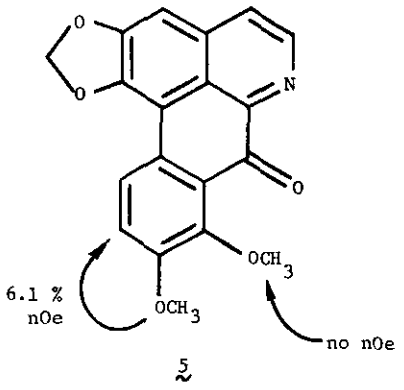

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4. An oxidizing derivative of O-methylbulbocapnine (6): mp 235-236°C (acetone); λ_{max}^{MeOH} nm (log ε): 256(4.57), 360 (4.12) and 410 (4.11); λ_{max}^{nujol} cm^{-1}: 1665, 1044, 940.
5. MS m/z(%): 365(100), 350(69), 334(10), 320(23), 249(8) and 175(17).
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