FISSOLDHIMINE, A NOVEL SKELETON ALKALOID FROM *FISSISTIGMA OLDHAMII*

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A new alkaloid of hydro-oxadiazine with a four-ring fused structure has been isolated from *Fissistigma oldhamii* (Memsl.) Merr. (Annonaceae) and named fissoldhimine (**1**), the structure of which was solved by X-ray analysis. NMR spectra were assigned based on the established structure, and its possible biogenesis is also discussed.

KEYWORDS alkaloid; hydro-oxadiazine; *Fissistigma oldhamii*; Annonaceae; fissoldhimine

Fissistigma oldhamii (Hemsl.) Merr. (Annonaceae) is a perennial shrub which is distributed mainly in southern China and Taiwan. The herb has been used as a folk medicine in southern China and Taiwan for the treatment of sciatica and arthritis, and anti-inflammatory and anti-tumor purposes.¹⁾

²⁾ Several aporphine alkaloids have so far been isolated and identified from the plant collected in Taiwan.^{2,3)} In a preliminary bioassay, its methanol extract showed anti-platelet aggregation activity and its constituents were investigated in the plant material collected in Taichung, Taiwan.²⁾ Methanolic extract of fresh stems (4 kg) was fractionated with hexane, and the methanolic fraction was treated with 5% hydrochloric acid solution. Insoluble material was removed and acidic solution was made alkaline with ammonia to pH 8.5. An alkaline solution was then extracted with chloroform to obtain a basic fraction which contained alkaloids with anti-platelet aggregatory activity. In a previous paper we reported the isolation and identification of five alkaloids, xylopine and calycine of aporphine type, O-methylmoschatoline of oxoaporphine type, and two morphinandienone alkaloids, N-methyl-2,3,6-trimethoxymorphinandien-7-one and N-nor-2,3,6-trimethoxymorphinan-dien-7-one.²⁾ The alkaline aqueous solution was further extracted with butanol to obtain more polar alkaloids. Butanolic fraction was further fractionated with a HP-20 column with water-methanol, and an alkaloid-containing fraction was again fractionated with a LH-20 column chromatography with methanol to afford an alkaloid as colourless rhombic crystals of mp 234-6° from methanol (ca. 100 mg). Following spectral investigations have revealed that the isolated alkaloid is a novel structure and named fissoldhimine (**1**).

FAB MS gave a molecular ion at m/z 279 ($M+1$) and a strong fragment peak at m/z 235 corresponding to a $M^+ - C_3H_7$ fragment, which gave a molecular formula of $C_{11}H_{15}N_4O_2$ in high-resolution MS.⁴⁾ NMR spectra indicated the presence of one methyl, seven methylenes, four methines and two carbons at the carbonyl region. COSY NMR spectrum proved the presence of propyl group linked to a methine which gave signals at lower fields (^{13}C δ 62.9, 1H δ 5.47), indicating this methine carbon linked with two hetero-atoms corresponding to a partial structure A where two Xs denote O or N. Another methine also gave signals at very low fields (^{13}C δ 65.6, 1H δ 4.85), and the

A

B

NMR spectra gave the partial structure B, in which three Xs are hetero-atoms. These spectral investigations indicate that fissoldhimine (**1**) possesses a molecular formula

of $\text{C}_{14}\text{H}_{22}\text{N}_4\text{O}_2$.

Fissoldhimine (**1**) gave crystals suitable for X-ray analysis from methanol. The crystal was monoclinic with space group of $\text{P2}_1/\text{n}$ containing 4 molecules in a unit cell. The space group indicates that fissoldhimine (**1**) is a racemate. The dimensions of the cell are $a = 20.982(10) \text{ \AA}$, $b = 13.025(8) \text{ \AA}$, $c = 5.128(3) \text{ \AA}$, $\beta = 96.70(5)^\circ$, $V = 1392 \text{ \AA}^3$. A total of 2571 reflections between a range of 6° - 156° were collected on a Philips PW1100 diffractometer. Structure was solved by direct method (MULTAN), and refinement was performed by block-diagonal-matrix least-squares method. 20 C, N, O atoms were refined with anisotropic temperature factor, and 22 H atoms were found on the difference electron-density map and located at the calculated positions. A final R value with isotropic temperature factor for H and anisotropic temperature factors for C, N, O was 0.07. Only one hydrogen bond was found between the neighboring molecules, between imino-NH and carbonyl-O. ^{13}C and ^1H NMR were assigned according to the structure solved by X-ray analysis.⁵⁾ Due to the complicated couplings ^1H NMR, coupling constants cannot be defined, and most of them were represented as multiplets.

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Drawing of Fissoldhimine (**1**) with Coordinates of X-Ray Analysis

Since the skeleton of fissoldhimine (**1**) is quite novel, it is of interest to discuss its possible biogenesis. Oxidation of putrescine (**3**) that is derived from the decarboxylation of ornithine (**2**) gave 4-aminobutanal (**4**), which is spontaneously cyclized to give Δ^1 -pyrrolidine (**5**). It is expected to dimerize easily between an imine (**5**) and an enamine (**5'**) to give a dimeric pyrrolidine (**6**). N carbamoyl transfer from carbamoyl phosphate gives a carbamoyl intermediate (**7**), which undergoes cyclization to give a diazinone (**8**) of cyclic urea structure. Further carbamoyl transfer gives another carbamoyl intermediate (**9**), and following aminoacetalization with butyraldehyde affords fissoldhimine (**1**). Since butanol was used to extract basic compounds from alkaline aqueous solution, there would be a possibility that final aminoacetalization was caused chemically with butyraldehyde present in butanol, and a true natural product produced by the plant may be a

carbamate (**9**). Further study is required to elucidate this question. Biological activities of fissoldhimine (**1**) is under investigation.

A Possible Biogenesis of Fissoldhimine (**1**)

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- 3) Sheng-Teh Lu, Yang-Chang Wu, *Heterocycles*, **20**, 813 (1983).
- 4) FAB-MS m/z 279 $[M + 1]$. High Resolution MS m/z 235, obs. 235.1203; calcd for $C_{11}H_{15}N_4O_2$ 235.1105.
- 5) ^{13}C -NMR ($CDCl_3$) δ : 13.5 (CH_3 , C-17), 17.9 (CH_2 , C-16), 22.8 (CH_2 , C-8), 24.4 (CH_2 , C-6), 32.0 (CH_2 , C-7), 37.8 (CH_2 , C-15), 41.6 (CH_2 , C-5), 41.9 (CH , C-6a). 45.4 (CH_2 , C-9), 54.5 (CH , C-6b), 62.9 (CH , C-1), 65.6 (CH , C-4a), 153.0 (C, C-3), 152.3 (C, C-11). 1H -NMR ($CDCl_3$) δ : 0.90 (3H, t, $J=7.4$, C-17), 1.36 (2H, m, C-16), 1.52 (1H, m, C-7), 1.56 (1H, m, C-6), 1.63 (2H, m, C-8), 1.66 (2H, m, C-15), 2.22 (1H x 3, m, C-7, C-6, C-6a), 3.14 (1H, m, C-6b), 3.36-3.59 (2H x 2, m, C-5, C-9), 4.85 (1H, d, $J=9.2$ Hz, C-4a), 5.47 (1H, q like, C-1), 5.60 (1H, d, $J=3.6$ Hz, N-13).

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