FISSOLDHIMINE, A NOVEL SKELETON ALKALOID FROM *FISSISTIGMA OLDHAMII*

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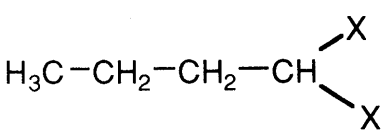
A new alkaloid of hydro-oxadiazine with a four-ring fused structure has been isolated from *Fissistigma oldhamii* (Memsl.) Merr. (Annonaceae) and named fissoldhimine (**1**), the structure of which was solved by X-ray analysis. NMR spectra were assigned based on the established structure, and its possible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

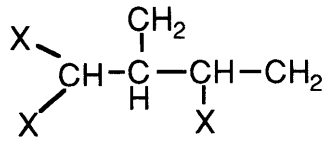
KEYWORDS alkaloid; hydro-oxadiazine; *Fissistigma oldhamii*; Annonaceae; fissoldhimine

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 calycinine of aporphine type, O-methylmoschatoline of oxoaprophine 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 coresponding 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



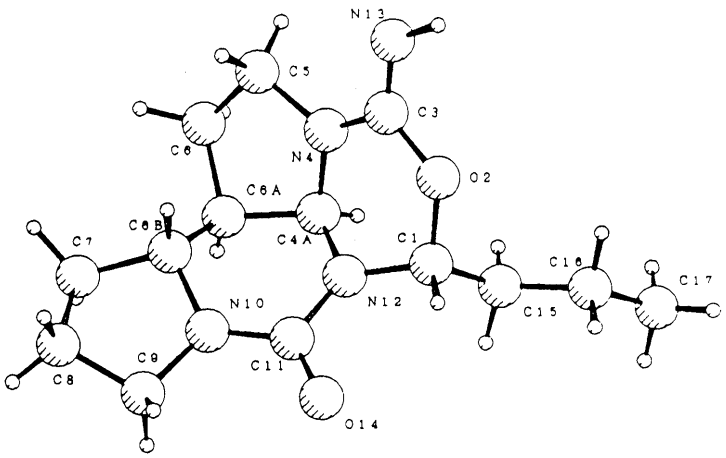
A

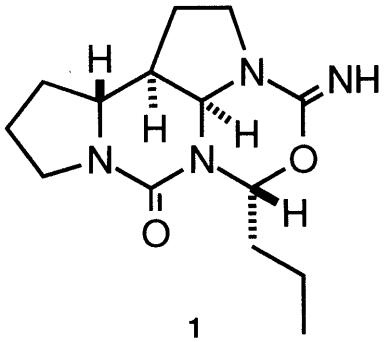


B

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 $C_{14}H_{22}N_4O_2$.

Fissoldhimine (**1**) gave crystals suitable for X-ray analysis from methanol. The crystal was monoclinic with space group of $P2_1/n$ containing 4 molecules in a unit cell. The space group indicates that fissoldhiine (1) is a racemate. The dimensions of the cell are a = 20.982 (10) Å, b = 13.025 (8) Å, c= 5.128 (3) Å, β= 96.70(5)°, V = 1392 Å^3. A total of 2571 reflections between a range of 6°-156° were collected on a Philips PW1100 difractometer. 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 isotopic 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.



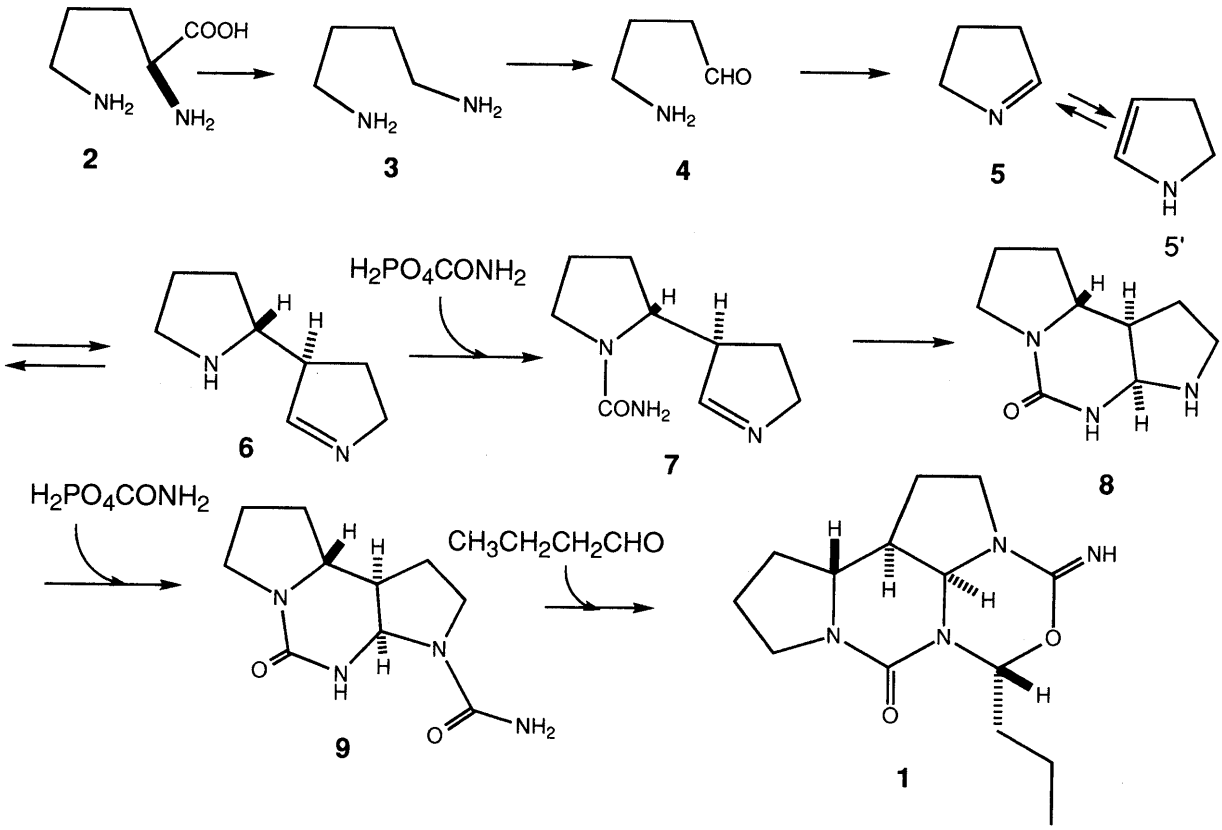


1

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-pyrrorideine (**5**). It is expected to dimerize easily between an imine (**5**) and an enamine (**5'**) to give a dimeric pyrrorideine (**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 fissolidhimine (**1**) is under investigation.



A Possible Biogenesis of Fissoldhimine (1)

REFERENCES AND NOTES

1) " Zhon Yao Dai Zi Ten (Encyclopedia Chinese Materia Medica)", ed. by Jiang Su New Medical College, Shanghai Science and Technology Publisher, 1977, p. 758.

2) Jin-Bin Wu, Yih-Dih Cheng, Nien-Yung Chin, Shung-Chien Juang, Sheng-Chu Kuo, *Planta Medica*, **59**, 179 (1993).

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). ^{1}H-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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