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An azaanthracene alkaloid from *Polyalthia suberosa*

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Abstract

An azaanthracene alkaloid, 1-aza-9,10-dimethoxy-4-methyl-2-oxo-1,2-dihydroanthracene (kalasinamide) has been isolated from the stems of *Polyalthia suberosa*. In addition, the known *N-trans*-feruloyltyramine and *N-trans*-coumaroyltyramine are also reported from the same source. The structures were elucidated by spectroscopic methods.

Keywords: *Polyalthia suberosa*; Annonaceae; Alkaloid; Azaanthracene

1. Introduction

P. suberosa (Roxb.) Thw. is a shrubby tree distributed throughout southeast Asia and south China. Various parts of *P. suberosa* including leaves, stems and bark have been investigated. The compounds identified are a coumarin (Dan, et al., 1985), triterpenes (Goyal and Gupta, 1986; Li et al., 1993), sterols (Dan et al., 1985; Goyal and Gupta, 1986), alkaloids (Ferdous, Islam & Hasan, 1992; Sahai, Srivastava, Jamal, Sinha, Singh & Fujimoto, 1996) and a nitrogen heterocyclic compound (Sahai et al., 1996). Interestingly, investigation of the stems and leaves of the south China species has resulted in the discovery of a new triterpene, suberosol, as an anti-HIV principle (Li et al., 1993). We now report the chemical investigation of *P. suberosa* collected in the northeastern part of Thailand. Chromatographic separation of the hot acetone extract of the stems of this plant led to the isolation of a new compound (kalasinamide), characterized as 1-aza-9,10-dimethoxy-4-methyl-2-oxo-1,2-dihydroanthracene (**1**), together with the known *N-trans*-feruloyltyramine and *N-trans*-coumaroyltyramine. The structure of compound **1** was determined on the basis of ^{1}H- and ^{13}C-NMR spectral data, in combination with the results from NOE, COSY, DEPT, HETCOR, and COLOC experiments, while the structures of the others were characterized by the direct comparison of their ^{1}H and ^{13}C-NMR spectral data with those reported in the literature (Fukuda, Yonemitsu & Kimura, 1983; Zhao, Hui, Rupprecht, McLaughlin & Wood, 1992; Atta-ur-Rahman, Bhatti, Akhtar & Choudhary, 1992).

2. Results and discussion

Compound **1**, which was crystallized as orange needles from EtOH–CH_2Cl_2, was assigned the molecular formula $C_{16}H_{15}NO_3$ based on an $[M]^+$ peak at m/z = 269. The fragment ion peaks at $[M\text{-}Me]^+$ 254 and $[M\text{-}2Me]^+$ 239, together with information from its ^{1}H-NMR spectrum (two singlet signals at δ 3.97 and 3.99) indicated the presence of two methoxyl groups in the structure. A low frequency C=O stretching (1663 cm^{-1}) and a broad NH stretching (3391 cm^{-1}) in the IR spectrum indicated the presence of an amide group. Its UV-spectrum showed bathochromic shifts in 2.5 N

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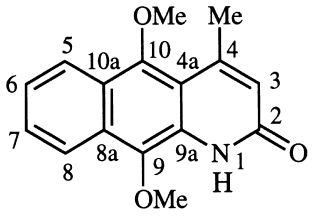
sodium hydroxide solution, with no change on addition of HCl to the ethanolic solution, consistent with a pyridone derivative, as previously described for dielsine and dielsinol (De Oliveira, De Oliveria, Carazza & Maia, 1987; Goulart, Santana, De Oliveira, De Oliveira & Maia, 1986). The singlet at δ 2.79 in the ^{1}H-NMR spectrum clearly represented a methyl group linked to an sp^2 carbon, supported by the presence of a broad olefinic proton resonance (δ 6.47) due to long range coupling with the methyl group. Four vicinal aromatic proton resonances at δ 8.17, 8.06, 7.43–7.49 and 7.54–7.61 revealed the presence of a 1,2-disubstituted benzene unit. The downfield doublet (1H) at δ 8.17 ($J_{5,6} = 8.8$ Hz) was assigned to H-5, while another doublet (1H), representing H-8, appeared at δ 8.06 ($J_{8,7} = 8.8$ Hz). The multiplets at δ 7.43–7.49 and 7.54–7.61 were assigned to H-6 and H-7, respectively. The assignment of the aromatic protons was confirmed by analysis of the COSY data (Table 1). The H-5 signal (δ 8.17) showed a cross peak only with the H-6 signal (δ 7.43–7.49), whereas the H-6 signal exhibited COSY correlations with the H-5 and H-7 signals. Similarly, the H-7 signal (δ 7.43–7.49) displayed cross peaks with the signals of H-6 (δ 7.43–7.49) and H-8 (δ 8.06), while the H-8 signal showed an interaction with the H-7 signal (δ 7.43–7.49), thus confirming the chemical shifts of the aromatic protons on ring A. NOE enhancement results in $CDCl_3$ allowed the two methoxyl signals to be assigned and confirmed the chemical shifts of H-3, H-5, H-8, and 4-Me. Enhancements were observed for H-5 (3.1%) and 4-Me (2.4%) on irradiation of 10-OMe, and H-8 (2.5%) on irradiation of 9-OMe; irradiation of 4-Me enhanced the signals from H-3 (4.2%) and 10-OMe (3.1%).

The DEPT spectral data (Table 1) of compound **1** showed the presence of three methyls and five methines. Since the resonances for sixteen carbons appeared in the decoupled ^{13}C-NMR spectrum (Table 1) of **1**, the other signals were assigned to eight quaternary carbons. The most downfield signal at δ 161.77 were assigned as the carbonyl carbon of an amide. The methyl signals at δ 61.90 and 64.00 were assigned to the two methoxyl carbons. The directly coupled C–H correlations in the HETCOR spectrum provided the evidence for the assignment of all of the methyl and methine carbons (Table 1). A series of COLOC spectra (Table 1) were performed in order to observe the long range correlations (^3J and ^2J) in compound **1**, and thus the chemical shifts of all carbons were determined. From analysis of the above data, the structure of compound **1** was established as 1-aza-9,10-dimethoxy-4-methyl-2-oxo-1,2-dihydroanthracene.



3. Experimental

3.1. General

Mps: uncorr (Electrothermal apparatus); IR:$CHCl_3$; UV:EtOH. NMR spectra were recorded at the Central

Table 1
NMR spectral data of kalasinamide (**1**)

Position	δ_H[a]	δ_C	DEPT	COSY	HETCOR	COLOC (^3J and ^2J)
1 (NH)	9.32 (*br s*)	–	–	–	–	113.95 (C-4a)
2	–	161.77	C=O	–	–	–
3	6.47 (*br s*)	123.07	CH	2.79 (4-CH_3)	123.07 (C-3)	161.77 (C-2), 113.95 (C-4a), 23.26 (4-CH_3)
4	–	148.61	C	–	–	–
4a	–	113.95	C	–	–	–
5	8.17 (*d*, 8.8)	123.46	CH	7.43–7.49 (H-6)	123.46 (C-5)	127.79 (C-7), 128.06 (C-8a), 151.61 (C-10)
6	7.43–7.49 (*m*)	124.61	CH	8.17 (H-5), 7.54-7.61(H-7)	124.61 (C-6)	123.92 (C-5a), 121.08 (C-8)
7	7.54–7.61 (*m*)	127.79	CH	7.43–7.49 (H-6), 8.06 (H-8)	127.79 (C-7)	128.06 (C-8a), 123.46 (C-5)
8	8.06 (*d*, 8.8)	121.08	CH	7.54–7.61 (H-7)	121.08 (C-8)	123.92 (C-5a), 124.61 (C-6), 135.99 (C-9)
8a	–	128.06	C	–	–	–
9	–	135.99	C	–	–	–
9a	–	128.21	C	–	–	–
10	–	151.61	C	–	–	–
10a	–	123.92	C	–	–	–
9-OCH_3	3.99 (*s*)	61.90	CH_3	–	61.90 (9-OCH_3)	135.99 (C-9)
10-OCH_3	3.97 (*s*)	64.00	CH_3	–	64.00 (10-OCH_3)	151.61 (C-10)
4-CH_3	2.79 (*d*, 1.2)	23.26	CH_3	6.47 (H-3)	23.26 (4-CH_3)	123.07 (C-3), 148.61 (C-4), 113.95 (C-4a)

[a] Multiplicities and coupling constants (J) in Hz are given in parentheses.

Instrumental Unit of Mahidol University on a Brüker DPX 300 in $CDCl_3$, using TMS as an internal standard. MS was measured at 70 eV. CC was carried out on silica gel 60 (70–230 mesh).

3.2. Plant material

Stems of *P. suberosa* were collected in April 1997 from Kalasin Province in the northeastern part of Thailand. A voucher specimen (BKF 115103) of this plant has been deposited at the Forest Herbarium, Royal Forestry Department, Paholyothin Road, Bangkok 10900, from where an authentic voucher specimen can be obtained.

3.3. Extraction

Air-dried, finely powdered stems (5.5 kg) were percolated with MeOH (12 l) at room temperature for 4 days, then filtered; the process was repeated four times. Evaporation of the solvent yielded a crude MeOH extract (282.2 g), which was further extracted with boiling acetone (8 l) in a Soxhlet apparatus for 16 h. Removal of the solvent gave a crude acetone extract (61.6 g).

3.4. Isolation

Coarse separation of the acetone extract (61.1 g) was carried out by CC over silica gel 60 (1 kg). Gradient elution with 0–100% EtOAc–hexane followed by 1–100% MeOH–EtOAc afforded 268 fractions (C1–C268). Fractions C129–C143 were combined and the solvent removed to give a semi-solid mixture (3.38 g). This fraction was rechromatographed by CC on a silica column, eluting with 0–100% acetone–*n*-hexane. Fractions were combined on the basis of their TLC behaviour. One solid fraction (196.7 mg, eluted with 20% acetone–*n*-hexane), which was crystallized as orange needles (40.9 mg) in EtOH–CH_2Cl_2, was identified as 1-aza-9,10-dimethoxy-4-methyl-2-oxo-1,2-dihydroanthracene (**1**). Another semi-solid fraction (997.2 mg, eluted with 40% acetone–*n*-hexane) was further separated by preparative PLC (silica gel; EtOAc : CH_2Cl_2 : *n*-hexane, 2 : 7 : 1; four elutions) to give pure *N-trans*-feruloyltyramine (88.1 mg) and *N-trans-p*-coumaroyltyramine (27.8 mg).

3.5. 1-Aza-9,10-dimethoxy-4-methyl-2-oxo-1,2-dihydroanthracene (kalasinamide) (*1*)

Orange needles from EtOH–CH_2Cl_2, mp. 233.8–235.5°C. (Found: C, 71.44; H, 5.23; N, 5.00; $C_{16}H_{15}NO_3$ requires: C, 71.36; H, 5.61; N, 5.20); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3391 (N–H stretching), 3005, 2941, 2852, 1663 (C═O stretching of amide), 1624, 1592, 1553, 1452, 1395, 1366, 1334, 1221, 1190, 1157, 1123, 1086, 1063, 1002, 967, 865; UV λ_{max}^{EtOH} nm (log ε): 392 (3.66), 338 (4.22), 323 (4.13), 310 (3.95), 284 (4.87), 274 (4.91), 264 sh (4.78), 247 (4.81), 216 (4.52); UV $\lambda_{max}^{EtOH+2.5\ N\ NaOH}$ nm (log ε): 412 (3.92), 351 (3.98), 334 (4.08), 320 (4.02), 278 sh (4.97), 275 (4.98), 264 (4.94); UV $\lambda_{max}^{EtOH+2.5N\ HCl}$ nm (log ε): 394 (3.43), 339 (4.00), 324 (3.91), 311 (3.72), 284 (4.65), 274 (4.69), 265 (4.56), 247 (4.58), 216 (4.25); EIMS *m/z* (rel. int.): 269 $[M]^+$ (39), 254 $[M\text{-}Me]^+$ (89), 239 $[M\text{-}Me\text{-}Me]^+$ (23), 225 (4), 211 (22), 195 (4), 183 (12), 167 (5), 154 (18), 139 (13), 127 (19), 119 (9), 115 (9), 105 (13), 91 (8), 83 (14), 76 (26), 63 (16), 51 (18), 39 (16), 32 (25), 28 (100); ^{1}H- and ^{13}C-NMR spectral data: see Table 1.

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