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A NEW NATURAL FLAVONE WITH A TETRASUBSTITUTED B-RING FROM THE FERN *NOTHOLAENA ASCHENBORNIANA*

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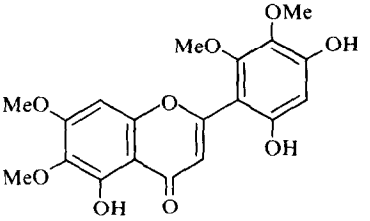
In a previous paper [1] we reported 7-*O*-methyl-8-*O*-butyrylherbacetin and 7-*O*-methyl-8-*O*-acetylherbacetin (NAS-1 and NAS-2) as the major constituents of the farinose exudate on fronds of *Notholaena aschenborniana* K1. Five additional flavonoids have since been isolated from the same material. The present communication reports the characterization of one of them, which has a novel substitution pattern in its B-ring (**1**).

In MS, the molecular ion at *m/z* 390 was in accordance with $C_{19}H_{18}O_9$ (found 390.0954, calc. 390.0951), corresponding to a flavone with three OH and four OMe groups; the latter were confirmed by ^{1}H NMR spectrum [three signals: δ 3.71 ppm (3H), 3.75 (6H), 3.90 (3H)]. The A-ring contained a free C-5 hydroxy group ($\Delta\lambda$ BI $AlCl_3$ + HCl/MeOH = +55 nm) and a methoxy group at C-7 ($\Delta\lambda$ BII NaOAc/MeOH = ±0) [2]; furthermore, the M–Me ion at *m/z* 375 (30%) suggested a C-6 methoxy group [4]. Three other ions, after the RDA process [3], were in accordance with this trisubstituted A-ring: *m/z* 181 ($A_1^{+\cdot}$ – Me), *m/z* 153 ($A_1^{+\cdot}$ – Me – CO), *m/z* 125 ($A_1^{+\cdot}$ – Me – 2CO); metastables and high-resolution evidence indicated that these ions were formed by sequential loss.

Finally, the ^{1}H NMR spectrum in DMSO reflected the presence of three protons, singlets appearing respectively at δ 6.49, 6.60 and 6.95 ppm. One of them, a signal at 6.60 ppm, was assignable, according to results of irradiation and NOE in ^{1}H NMR, to the proton H-8; indeed, on the irradiation of OMe at 3.90 ppm the singlet 6.60 ppm was intensified; besides a NOE experiment secured this conclusion since only an irradiation on OMe 3.90 ppm gave rise to a significant NOE (about 10%) on this singlet at 6.60 ppm. In addition, this signal was not involved in OH-exchange experiments with D_2O. These results indicated a H-8 vicinal with a 7-OMe. A further aromatic proton, appearing as a singlet at 6.95 ppm, could be assigned to C-3 since it was unaffected by OH-exchanges with D_2O, or by irradiations of OMe signals.

That the B-ring is tetra-*O*-substituted with two OH and two OMe groups was confirmed by MS fragmentation after RDA reaction: $B_1^{+\cdot}$ at *m/z* 194 ($C_{10}H_{10}O_4$), and another peak at *m/z* 167 for two ions ($B_2^+ - OCH_2$), (B_2^+ – 2 Me) secured by high-resolution measurements. Since the shift (and the absorbance) in the UV spectrum after addition of NaOH showed a free hydroxy group at C-4′, and the spectrum in presence of NaOAc + H_3BO_3 indicated that there was no *o*-dihydroxy substitution, the structures assignable to the B-ring are: 2′,3′-diOMe, 4′,6′-diOH, or 3′,6′-diOMe, 2′,4′-diOH, or 3′,5′-diOMe, 2′,4′-diOH. In the first two cases the free proton is positioned at C-5′ whereas in the third it is located at C-6′; the ^{1}H NMR showed a sharp singlet at δ 6.49 ppm; this signal is obviously typical of a H-5′. To our knowledge [5–8], a C-6′ proton gives rise to a signal consistently at a lower field (7.1–7.9 ppm). Moreover, irradiation experiments in ^{1}H NMR showed this proton was not vicinal to either OMe (no improvement, no nuclear Overhauser effect); in contrast, the singlet was significantly improved when OH was exchanged with D_2O, demonstrating the occurrence of a coupling between H and OH, proved further by data processing (resolution enhancement). In conclusion, the first structural hypothesis must be correct. Thus the new natural compound is 6,7,2′,3′-tetramethoxy-5,4′,6′-trihydroxyflavone (**1**).



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The farina of ferns of the genus *Notholaena* has already been shown to contain methylated flavonoids, especially 2′-*O*-substituted compounds [9–12]. The present report shows again that ferns have a considerable capacity for methylating the flavonoid skeleton. To our knowledge, this is the first report on a flavone with tetra-*O*-substituted B-ring occurring as a natural product.

EXPERIMENTAL

Fronds of *Notholaena aschenborniana* were collected in Mexico (Cuarto Cienegas Basin) in August 1975. Vouchers are kept at the Department of Botany, Arizona State University (Tempe, AZ, D. J. Pinkava & T. Reeves R 4310 B and R 4320). The exudate was collected by rinsing air-dried fronds with

Me_2CO and C_6H_6; the combined extracts were concd under reduced pres. The flavonol esters NAS 1 and NAS 2 crystallized from the crude soln. The residue, *ca* 250 mg, was dried onto polyamide MN SC-6 and fractionated on a polyamide column, eluted with C_6H_6 and increasing quantities of MeCOEt and MeOH [13]. The fractions thus obtained were further separated by TLC (polyamide MN DC-11, solvents C_6H_6–petrol (br 100–140°)–MeCOEt–MeOH, A = 60:25:7:7, B = 60:60:7:7, C = 30:60:5:5). From the fractions 2 and 3, 1.5 mg of a yellow crystalline compound could be isolated. Fluorescence purple, R_f 0.27 in system B UV λ_{max} (nm) MeOH 263, 365; NaOAc 265 (380) 410; NaOAc + H_3BO_3 264, 366; $AlCl_3$ 278 (368) 420; $AlCl_3$ + HCl 277 (306) (360) 420; NaOH (252) 270, 410 without decreasing intensity on BI. MS, 70 eV, *m/z* (%): 390 (100%, $C_{19}H_{18}O_9$ found 390.0954, calc. 390.0951), 375 (20), 373 (8), 359 (30), 347 (10), 194 (75, $C_{10}H_{10}O_4$ found 194.0579, calc. 194.0579), 181 (30, $C_8H_5O_5$ found 181.0135, calc. 181.0137), 179 (26, $C_9H_7O_4$ found 179.0344, calc. 179.0344, 194 – Me m*165.1), 167 (8, $C_8H_7O_4$ found 167.0341, calc. 167.0344 B_2^+ $-OCH_2$ m* 142.3; $C_7H_3O_5$ found 166.9979, calc. 166.9980 B_2^+ – 2Me m* 142.3), 153 (14, $C_7H_5O_4$ found 153.0187, calc. 153.0187 181 – CO m* 129.3), 125 (6, $C_6H_5O_3$ found 125.0239, calc. 125.0238 153 – CO m* 102.1). ^1H NMR 360 MHz (DMSO/TMS): δ 6.94 ppm (1 H, *s*), 6.60 (1 H, *s*), 6.49 (1 H, *s*), 3.90 (3 H, *s*, OMe), 3.75 (6 H, *s*, 2 OMe), 3.71 (3 H, *s*, OMe); NOE experiments were run in the difference mode on a sample saturated with Ar.

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