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REVISED STRUCTURE OF A FLAVONOID FROM THE FERN *NOTHOLAENA ASCHENBORNIANA*

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Key Word Index—*Notholaena aschenborniana*; Pteridophyta; Polypodiaceae; 5,2',4'-trihydroxy-3,7,8,5'-tetramethoxyflavone; synthesis.

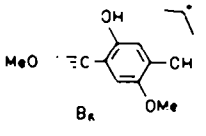
Abstract—The structure of a flavonoid from the fern *Notholaena aschenborniana*, previously described as 5,4',6'-trihydroxy-6,7,2',3'-tetramethoxyflavone, is revised to 5,2',4'-trihydroxy-3,7,8,5'-tetramethoxyflavone on the basis of current studies on 2'-oxygenated flavonoids. Identity of the natural product with the proposed structure is confirmed by synthesis.

INTRODUCTION

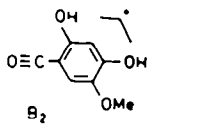
In the course of investigating a number of 2'-hydroxyflavonol 3-*O*-methyl ethers, two of us (N.F. and T.J.M.) observed [see 1, 2] that these compounds exhibit characteristic UV and mass spectral and TLC properties. We also noted that a compound isolated from the frond exudate of the fern *Notholaena aschenborniana* KL and named NAS-3 [3] exhibited these same properties. Thus, it appeared that the structure previously ascribed to NAS-3 as 5,4',6'-trihydroxy-6,7,2',3'-tetramethoxyflavone (1) might be incorrect. On the basis of detailed spectroscopic studies a different structure was suggested and this as well as the previously assigned structure were synthesized (M.I., T.T. and M.M.). Direct comparison with the natural product showed that the structure ascribed to NAS-3 originally indeed required revision.

RESULTS AND DISCUSSION

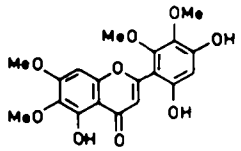
The structure of 5,4',6'-trihydroxy-6,7,2',3'-tetramethoxyflavone (1) has first been ascribed to NAS-3 on the basis of spectral studies. A reinvestigation of the spectral data, however, led two of us (N.F. and T.J.M.) to assume that these previous data could now be differently interpreted and support a new structural assignment. Firstly, the UV spectrum of NAS-3 on addition of $AlCl_3$ + HCl exhibits two bands, I, Ib at 360 nm (shoulder) and Ia at 420 nm (peak). The existence of peak Ia indicates that a methoxyl group is at C-8 rather than at C-6: the shape and the position of this longest band is characteristic for 8-methoxyflavonoids [2, 4] while, for 6-methoxy derivatives, this band is either absent (flavones) or appears as a shoulder at 400–408 nm (flavonol 3-methyl ethers) [4], even if, for these latter, the B-ring presents a particular substitution (2'-OH-4',5'-diOMe [2]). The second point concerns the tetrasubstitution of the B-ring: the previous demonstration of its structure was based on MS fragmentation where a fragment at m/z 194 (75%) was interpreted as the ion B_2 of a flavone with a di-OH-diOMe B-ring. In fact, the recent studies on 2'-oxygenated flavonoids [1, 2, 5, 6] revealed in the case of 2'-hydroxyflavonol 3-methyl ethers a special fragmentation for the B-ring, yielding ion $[B_6]^+$. So, for NAS-3, ion m/z 194, initially attributed to a flavone with a tetraoxygenated B-ring could be interpreted as a fragment of a 2'-OH-3-OMe-flavone with a trioxygenated B-ring (two hydroxy and one methoxy groups); in this case, the fragment at m/z 167 would be in accord with the fragment B_2 of a flavonol 3-*O*-methyl ether. Further, on TLC in 15% acetic acid on cellulose plates NAS-3 exhibits a high R_f value (0.70), which is characteristic of 2'-hydroxyflavonol 3-methyl ethers [7]. Finally the MS of the PM derivative of 5,7,2',4'-tetrahydroxy-3,8,5'-trimethoxyflavone [2] was identical to the MS of the PM derivative of NAS-3, establishing



B_6



B_2



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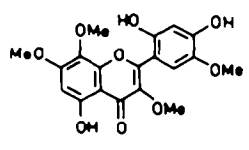

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that NAS-3 was, indeed, 3,2'-dioxygenated and contained an 8-oxygen function as well. In total, these observations suggested that NAS-3 is 5,2',4'-trihydroxy-3,7,8,5'-tetramethoxyflavone (2).

Both the originally reported and the new structures were prepared synthetically. Synthesis of 5,4',6'-trihydroxy-3,7,2',3'-tetramethoxyflavone (1) and its non-identity with NAS-3 have already been reported [8]. In contrast, the identity of NAS-3 and synthetic 5,2',4'-trihydroxy-3,7,8,5'-tetramethoxyflavone (2) (for synthesis see Experimental) was proved by direct comparison (co-TLC, co-HPLC, UV, MS and NMR). Compound NAS-3 from *Notholaena aschenborniana* thus represents yet another member of the interesting group of 2'-hydroxyflavonol 3-methyl ethers.

EXPERIMENTAL

Source of plant product. Notholaena aschenborniana was collected in the Dragoon Mountains, Cochise Co., AZ, U.S.A., in December, 1981. Fronds were carefully clipped in the field and dried in a paper bag. A voucher is deposited at ARIZ (Wollenweber & Yatskievych 81-491). The flavonoid named NAS-3 was isolated as described previously [3].

General techniques. MS data were recorded by direct probe EIMS at 70 eV with a source temp. of 250–270°. All UV spectra were recorded using standard procedures [9]. HPLC was carried out using an Altex 11 A pump, Rehodyne 7125 injector fitted with a 20 μ loop and an Altex 153 UV detector fixed at 365 nm. Permethylation was achieved using Methelute (Pierce Chem. Co., Rockford, Ill.). For apparatus used for synthesis see ref. [8].

Synthesis of 5,2',4'-trihydroxy-3,7,8,5'-tetramethoxyflavone (2). 2-Hydroxy-3,4,6-trimethoxyacetophenone (1.2 g, 5.3 mmol) was condensed with 2,4-diisopropyloxy-5-methoxybenzaldehyde (1.3 g, 5.3 mmol) in the presence of KOH (10 g) in methyl cellosolve (50 ml) [10]. The soln was stirred overnight at room temp. Usual workup of the reaction mixture gave 1.8 g of 2'-hydroxy-2,4-diisopropyloxy-3',4',6',5-tetramethoxychalcone (3) as orange-yellow needles, mp 136° (EtOH). ^{1}H NMR ($CDCl_3$): δ1.38, 1.39 (12H, each *d*, J = 6 Hz, Me_2CH–), 3.83, 3.85, 3.91, 3.93 (3H, each *s*, OMe), 4.12–4.78 (2H, *m*, 2 × CH<), 5.99 (1H, *s*, H-5'), 6.51 (1H, *s*, H-3), 7.10 (1H, *s*, H-6), 7.73 (1H, *d*, J = 15 Hz, H-β), 8.15 (1H, *d*, J = 15 Hz, H-α), 14.05 (1H, *s*, OH). 30% H_2O_2 (15 ml) was added to a methyl cellosolve soln containing 3 (1.2 g) and then 20% NaOH (15 ml) was added to the soln dropwise. The reaction mixture was stirred at room temp. for 30 min. After acidification with 10% HCl, the soln was extracted with EtOAc. After concn of the extract, the residue was chromatographed on silica gel (EtOAc–hexane, 1:1). Fractions of a yellow band near the bottom gave 3-hydroxy-2',4'-diisopropyloxy-5,7,8,5'-tetramethoxyflavone (4) as a yellow oil (720 mg). ^{1}H NMR (CCl_4): δ1.24, 13.5 (6H, each *d*, J = 6 Hz, Me_2CH–), 3.70, 3.80, 3.87, 3.90 (3H, each *s*, OMe), 4.33–4.80 (2H, *m*, 2 × CH<), 6.29 (1H, *s*, H-6), 6.50 (1H, *s*, H-3'), 7.10 (1H, *s*, H-6'). Methylation of 4 with Me_2SO_4 and K_2CO_3 in Me_2CO gave 2',4'-diisopropyloxy-3,5,7,8,5'-pentamethoxyflavone as a pale yellow oil. MS *m/z* (rel. int.): 488 $[M]^+$ (100), 473 (34), 459 (41), 445 (23), 430 (87), 416 (73), 399 (47), 389 (80), 387 (45), 374 (53), 211 (43), 167 (77). The methylated flavone (430 mg) was treated with BCl_3 (0.7 ml) at −60° to give 2 (220 mg) as yellow needles, mp 188–189° (EtOH–hexane). ^{1}H NMR (DMSO-d_6): δ3.74 (9H, *s*, 3 × OMe), 3.91 (3H, *s*, OMe), 6.48, 6.52 (1H, each *s*, H-3', 6), 6.91 (1H, *s*, H-3'), 9.29, 9.44, 12.49 (1H, each *s*, OH). MS *m/z* (rel. int.): 390 $[M]^+$ (91), 376 (16), 375 (29), 360 (21), 359 (22), 347 (12), 345 (13), 333 (5), 195 (57), 194 (87), 181 (45), 179 (33), 167 (12), 165 (13), 164 (11), 153 (21). UV λ_{max}^{MeOH} nm (log ε): 263 (4.0), 364 (3.7); + NaOMe 268, 416; + $AlCl_3$ 277, 370 sh, 425; + $AlCl_3$ + HCl 276, 370 sh, 420; + NaOAc 262, 380 sh, 410; + NaOAc + H_3BO_3 263, 363.

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