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ANTIMICROBIAL AGENTS FROM HIGHER PLANTS: ACTIVITY AND STRUCTURAL REVISION OF FLEMIFLAVANONE-D FROM *FLEMINGIA STRICTA*

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Key Word Index—*Flemingia stricta*; Leguminosae; flemiflavanone-D; 2*S*-5,7,4′-trihydroxy-6-γ,γ-dimethylallyl-3′-γ,γ-dimethylallyloxidoflavan-4-one; bioactivity.

Abstract—Flemiflavanone-D from *Flemingia stricta* is active *in vitro* against *Staphylococcus aureus* and *Mycobacterium smegmatis*. Reexamination and interpretation of its spectral properties required revision of its molecular formula to $C_{25}H_{28}O_6$ and its structure to 2*S*-5,7,4′-trihydroxy-6-γ,γ-dimethylallyl-3′-γ,γ-dimethylallyloxidoflavan-4-one. The new structure was confirmed when reaction with chlorotrimethylsilane and sodium iodide in acetonitrile deoxygenated and cyclized flemiflavanone-D to the known dicycloeuchrestaflavanone A. The absolute stereochemistry of flemiflavanone-D was established to be 2*S* by circular dichroism measurements.

INTRODUCTION

During the course of our studies on the antimicrobial agents found in higher plant extracts [1], we found the recently described flemiflavanone-D [2] to be active *in vitro*. The original structural proposal **1** had been made almost entirely on the basis of spectroscopic considerations. Reexploration of its physico-chemical properties on a portion of the original sample indicated the necessity of a structural revision, reported here. The new structure **2** is supported by chemical conversion to dicycloeuchrestaflavone A (**5**), a substance of known structure.

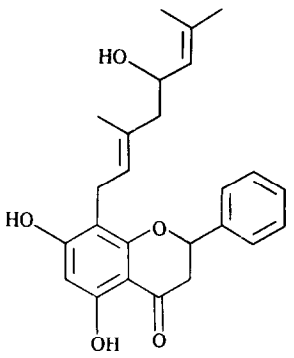
RESULTS AND DISCUSSION

Careful high resolution mass spectrometry produced a molecular ion which required revision of the molecular formula of flemiflavanone-D to $C_{25}H_{28}O_6$ indicating the presence of an additional oxygen not seen in earlier spectra. The highest mass ion previously obtained was 16 atomic mass units less. Loss of 16 a.m.u. is uncommon in organic molecules and we have no ready explanation for this difference. Flemiflavanone-D (**2**) was confirmed to be a 5,7-dihydroxyflavanone derivative by the close resemblance of its ultraviolet spectra in methanol, methanolic base and methanolic aluminum chloride–HCl with those of naringenin [3] and glabranin A [4, 5]. Further support for this assignment came from characteristic chemical shifts in the ^{13}C NMR spectrum attributable to C-4 (196.91), C-2 (79.18) and C-3 (42.81) as compared to those of sophoraflavanone B [5, 6] and from infrared bands for chelated hydroxyl (3352 cm^{-1}) and carbonyl (1635 cm^{-1}) moieties. Flemiflavanone-D has three acetylatable *phenolic* hydroxyl groups as shown by the properties of its readily prepared triacetyl ester. No acetylatable aliphatic secondary hydroxyl group was found in this experiment. The ^1H NMR spectrum of flemiflavanone-D provided clear evidence for the substitution pattern of the aromatic rings. Ring A has only a single aromatic hydrogen (δ6.01, 1H, *s*) whereas ring B has a typical ABM pattern (δ6.80, 1H, *d*, $J = 7.7$ Hz; δ7.18, 1H, *dd*, $J = 3.3$, 7.7 Hz; and δ7.23, 1H, *d*, $J = 3.3$ Hz). The remaining atoms belonged to a γ,γ-dimethylallyl and a γ,γ-dimethylallyl oxide moiety based largely the ^1H and ^{13}C NMR spectra (see Experimental). Considering diagnostic mass spectral fragmentations, most particularly the retro-Diels–Alder fragment ions **3** and **4**, these were attached to separate rings, rather than in tandem is previously believed. Ion *m/z* 204 (**4**) requires that the oxygenated prenyl moiety be placed in ring B and, thus, contain the 'extra' oxygen required by the new molecular formula.

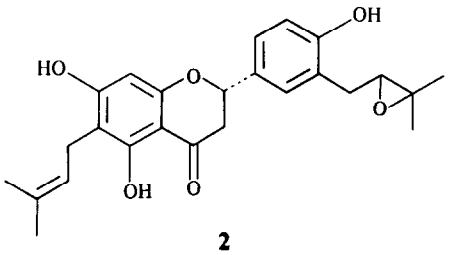
Placement of the *C*-prenyl group at C-6 in ring A was favored by the observed downfield shift of H-8 ($\Delta\delta = -0.5$) upon acetylation. The shift difference would have been on the order of −0.67 were the prenyl group at C-8 as originally formulated [4, 7, 8, 9]. This assignment was in agreement with the ^{13}C NMR spectrum in which C-6 is normally at δ107.68 and C-8 at 95.8 in agreement with general experience with similar compounds [5, 6, 10].

The tentative structural reassignment **2** arrived at through these considerations was confirmed by chemical conversion of flemiflavanone-D to dicycloeuchrestaflavanone A (**5**) [10] by deoxygenation and cyclization using chlorotrimethylsilane and sodium iodide in acetonitrile. This new reaction gives good yields in such reactions and appears to be generally applicable. The scope and mechanism are under further investigation and the results will be reported elsewhere. The absolute stereochemistry of flemiflavanone-D at C-2 was established to be *S* based upon a positive extremum at 330 nm and a negative extremum at 290 nm in its circular dichroism spectrum [11].

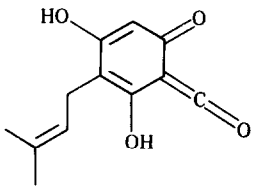
Flemiflavanone-D was tested for its *in vitro* antimicrobial activity by an agar dilution-streak method [12].



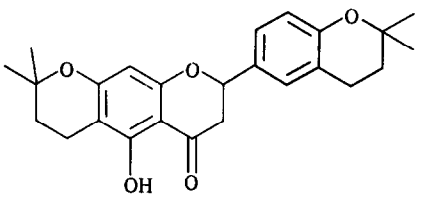
1



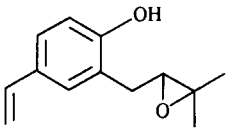
2



3 *m/z* = 220



5



4 *m/z* = 204

The compound shows significant activity against Gram-positive *Staphylococcus aureus* (6.15 μg/ml) and acid fast *Mycobacterium smegmatis* (6.25 μg/ml). Flemiflavanone-D was inactive against *Escherichia coli, Salmonella galinarum, Klebsiella pneumoniae, Candida albicans* and *Pseudomonas aeruginosa*. In the same test series streptomycin sulfate was active at 5 μg/ml against *S. aureus* and 1.25 μg/ml against *M. smegmatis*.

EXPERIMENTAL

Plant material. Flemingia stricta was collected in Dehra Dun, India, by The Botanical Survey of India, Dehra Dun, where a voucher specimen is on deposit.

Extraction. The root powder (2.5 kg) was extracted with hexane continuously in a Soxhlet extractor. The extract was concd to a residue (20 g) and chromatographed on 200 g of silica gel. Elution (100 ml fractions) with C_6H_6–EtOAc (49:1) produced fractions 1–26; C_6H_6–EtOAc (19:1) produced fractions 27–36; and C_6H_6–EtOAc (9:1) produced fractions 37–70.

Isolation of flemiflavanone-D. Fractions 27–36 showed only a single spot on TLC (R_f value 0.60 using silica gel G, developing with $CHCl_3$–MeOH (49:1), and visualizing by heating with 10% $MeOH–H_2SO_4$. Rechromatography on silica gel using C_6H_6–EtOAc (19:1) gave flemiflavanone-D as yellow needles: mp 150–152°; $[\alpha]^{22}$ – 15° (*c* 0.22; MeOH); UV λ_{max}^{MeOH} nm (log ε): 336 (3.30), 292 (3.98), and 226 (4.24); $\lambda_{max}^{MeOH-HCl}$: 336 (3.30) and 292 (3.94); $\lambda_{max}^{MeOH-NaOH}$: 332 (4.16) and 246 (3.95); $\lambda_{max}^{MeOH-AlCl_3-HCl}$: 390 (3.32) and 315 (4.11); IR ν_{max}^{KBr} cm^{-1}: 3352, 3343, 2918, 1635, 1605, 1506, 1477, 1441, 1381, 1342, 1286, 1257, 1163 and 1072; ^1H NMR ($CDCl_3$): δ 1.70 (6H, *s*), 1.77 (6H, *s*), 2.74 (1H, *dd*, *J* = 17.15, 4.15 Hz), 3.06 (1H, *dd*, *J* = 17.15, 11.55 Hz), 3.30–3.50 (3H, *m*), 3.45 (2H, *d*, *J* = 6.7 Hz), 5.22 (1H, *t*, *J* = 6.7 Hz), 5.29 (1H, *dd*, *J* = 4.15, 11.55 Hz), 6.01 (1H, *s*), 6.80 (1H, *d*, *J* = 7.7 Hz), 7.18 (1H, *dd*, *J* = 3.3, 7.7 Hz), 7.23 (1H, *d*, *J* = 3.3 Hz) and 11.98 (1H, *s*); ^{13}C NMR (Me_2CO-d_6): δ 196.91 (C-4), 164.33 (C-7), 162.30 (C-5), 160.37 (C-8a), 155.37 (C-4'), 130.52 (C-3''), 128.32 (C-1'), 128.28 (C-2'), 128.21 (C-3'), 125.29 (C-6'), 123.09 (C-2''), 115.04 (C-5'), 107.68 (C-6), 102.00 (C-4a), 95.8 (C-8), 79.18 (C-2), 43.0 (C-3'''), 42.93 (C-2'''), 42.81 (C-3), 28.49 (C-1'''), 25.29 (C-Me), 21.66 (C-1''), and 17.29 (C-Me); MS *m/z* (rel. int., %): 424.18786 ($[M]^+$, Calc. for $C_{25}H_{28}O_6$: 424.18842) (8.0), 408 (46.4), 393 (7.7), 220 (17.4), 204 (52), 188 (10.9), 177 (55.3), 165 (100) and 133 (34.0). Calc. for $C_{25}H_{28}O_6 \cdot 1/4H_2O$: C, 70.02; H, 6.65. Found: C, 70.15; H, 7.01.

Flemiflavanone-D triacetyl ester. Flemiflavanone-D (12 mg) was dissolved in pyridine (2 ml) and 3 ml of Ac_2O was added. The

reaction mixture was kept at room temp. overnight and then poured into cold water (20 ml). The precipitate obtained was filtered and washed with water, dried and chromatographed on silica gel using n-C_6H_{14}–EtOAc (7:3) to produce 3.5 mg of the pure triacetyl ester of **2** as an oil; IR ν_{max}^{KBr} cm^{-1}: 3429, 2976, 2924, 1772, 1741, 1687, 1653, 1610, 1498, 1425, 1369, 1342, 1275, 1194, 1142, 1126, 1084, 1061, 1012 and 904; ^1H NMR ($CDCl_3$): δ1.55 (3H, *s*), 1.64 (3H, *s*), 1.69 (3H, *s*), 1.76 (3H, *s*), 2.30 (3H, *s*), 2.32 (3H, *s*), 2.36 (3H, *s*), 2.72 (1H, *dd*, J = 16.3, 4.1 Hz), 3.25 (4H, *br d*, J = 6.7 Hz), 2.7–3.3 (2H, *m*), 5.10 (1H, *t*, J = 6.7 Hz), 5.41 (1H, *dd*, J = 4.1, 12.3 Hz), 6.52 (1H, *s*), 7.06 (1H, *d*, J = 8.9 Hz) and 7.20–7.34 (2H, *m*); MS *m/z*: 550 $[M]^+$, 534, 508, 492, 491, 449, 433, 407, 395, 351, 305, 262, 247, 220, 219, 205, 192, 177, 165 and 133.

Deoxygenation and rearrangement of flemiflavanone-D to dicycloeuchrestaflavanone A. To a soln of NaI (32 mg) in anhydrous MeCN (300 μl), Me_3SiCl (18 μl) was added slowly under Ar. After stirring for 5–7 min, a yellowish suspension was obtained and a soln of flemiflavanone-D (20 mg) in 300 μl of MeCN was added to this. The reaction mixture turned dark brown with the passage of time. After 3.5 hr, the reaction was quenched with $Na_2S_2O_3$ (15 ml) and extracted with Et_2O (2 × 10 ml). The ethereal layer was washed with water and dried ($MgSO_4$). The residue obtained after filtration and drying was chromatographed on silica gel using CH_2Cl_2–Me_2CO (100:1.5) to produce some recovered flemiflavanone-D (2.5 mg, 12.5%) and the desired product **5** (9 mg, 47%): mp 189–191° (lit. [10] 192–194°); IR ν_{max}^{KBr} cm^{-1}: 3400, 1643, 1587, 1496, 1481, 1452, 1441, 1381, 1342, 1334, 1259, 1232, 1155, 1116 and 794; ^1H NMR ($CDCl_3$): δ1.33 (12H, *s*), 1.7–1.9 (4H, *m*), 2.59 (2H, *t*, J = 6.8 Hz), 2.86 (2H, *t*, J = 6.8 Hz), 2.6–3.3 (2H, *m*), 5.30 (1H, *dd*, J = 12.6, 3.9 Hz), 5.96 (1H, *s*), 6.82 (1H, *d*, J = 8 Hz), 7.1–7.4 (2H, *m*) and 11.75 (1H, *s*); MS *m/z*: 408.19332 ($[M]^+$, calc. for $C_{25}H_{28}O_5$: 408.19351), 353 $[M-C_4H_7]^+$, 220 (4), 188 (5), 165 $[220-C_4H_7]$ and 133 $[188-C_4H_7]$.

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