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DIOCLEIN, A FLAVANONE FROM THE ROOTS OF *DIOCLEA GRANDIFLORA*

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Key Word Index—*Dioclea grandiflora*; Fabaceae, roots; dioclein; flavanone.

Abstract—A new flavanone with the relatively rare 2',5'-dioxygenation at ring B has been isolated from the roots of *Dioclea grandiflora* and its structure established as 5,2', 5'-trihydroxy-6,7-dimethoxyflavanone, mainly with the aid of spectroscopic methods.

INTRODUCTION

Dioclea grandiflora Mart ex Benth. is a vine growing in the 'Caatinga' and 'Cerrado' regions of northeastern Brazil [1]. The infusion of the roots of this plant is used for the treatment of kidney stones and prostate gland disorders [Agra, M. F., unpublished]. In a preliminary investigation in our laboratory [2], the 95% ethanol extract of the roots of *D. grandiflora* showed significant activity on the central nervous system in rats. To our knowledge, there has been no report on the chemical constituents of this plant. The chloroform soluble part of the ethanol extract of the roots of *D. grandiflora*, upon column chromatography followed by crystallization yielded, *inter alia*, dioclein, $C_{17}H_{16}O_7$ ($[M]^+$ at *m/z* 332), mp 160–162°. In this communication, we report the structure of dioclein as 5,2',5'-trihydroxy-6,7-dimethoxyflavanone (**1**) based mainly on spectral analyses.

The mass spectrum of dioclein gave, among others, a molecular ion $[M]^+$ peak at *m/z* 332 which is compatible with the molecular formula, $C_{17}H_{16}O_7$. This is supported by the appearance of 17 signals in the ^{13}C NMR spectrum. The UV spectrum of the new compound showed absorption maxima at 289 and 345 (sh) nm which remained practically unchanged in sodium acetate, characteristic of flavanones. A bathochromic shift to 310 and 397 nm in $AlCl_3$, with or without HCl strongly suggested [4] a OH-5 flavanone structure for dioclein. The presence of a doublet of doublets at δ5.73 ($J = 12.9$ and 3.1 Hz) in the ^1H NMR spectrum and a doublet (SFORD) at δ75.8 in the ^{13}C NMR spectrum of dioclein are typical of the H-2 and C-2, respectively, of a flavone skeleton [3, 4].

The 250 MHz ^1H NMR spectrum in acetone-d_6 showed the presence of an ABM system of three Ar-H comprised of two 1H doublets at δ7.06 ($J = 2.9$ Hz) and 6.77 ($J = 8.6$ Hz) and a 1H doublet of doublet at δ6.08 ($J = 2.9$ and 8.6 Hz). In addition, the spectrum showed a 1H singlet at δ6.23. The presence of four Ar-H in a flavanone skeleton requires five substituents at the rings A and B in a flavanone skeleton. The ^1H NMR also showed two 3H singlets at δ3.90 and 3.70 for two Ar-OMe groups. This was supported by the presence of two signals (*q*) at δ60.5 and 56.6 in the ^{13}C NMR spectrum of dioclein. Moreover, there are three hydroxyl functions as suggested by the formation of a triacetate, $C_{23}H_{22}O_{10}$ ($[M]^+$ 458) when treated with acetic anhydride and pyridine.

The mass spectrum showed, in addition to the $[M]^+$, significant peaks at *m/z* 314 [M − 18], 299 [M − 18 − 15], 271, 197, 196, 181, 153, 136, 125 and 107. The peak at *m/z* 136 must have come from ring B containing two OH functions (A_1) and the peaks at *m/z* 197 and 196, from ring A having one OH and two OMe groups by a retro-Diels–Alder type of fragmentation of the heterocyclic ring with or without a H transfer, characteristic of flavanones [5]. The mass spectral evidence, therefore, is supportive of the presence of two OH functions at ring B of dioclein. Consequently, dioclein must have both the OMe groups in addition to the third OH function at ring A.

The downfield Ar-H doublet at δ7.06 ($J = 2.9$ Hz) in the ^1H NMR spectrum of dioclein, which is a part of the ABM system is typical of H-2' and/or H-6' of flavanones [3]. The presence of only one H at a field lower than 7.0 ppm suggests that there is a substituent at C-2' and none at C-6'. Therefore, one of the two OH groups at ring B must be placed at C-2'. The lowfield H at C-6', however, is a part of the ABM system which indicates that the other OH group must be present at either C-4' or C-5'. As the H-6' doublet at 7.06 ($J = 2.9$ Hz) is *meta* to the H doublet of doublet at δ6.68 ($J = 2.9$ and 8.6 Hz), which is *ortho* coupled with the H doublet at δ6.77 ($J = 8.6$ Hz), the second OH function at ring B must be placed at C-5'.

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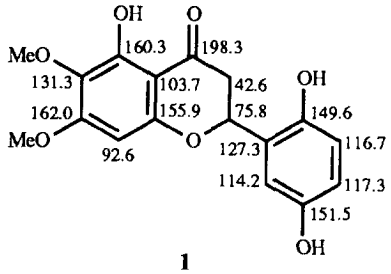
Thus, the chemical shifts and the coupling patterns of the three ABM type of Ar-H of dioclein seem to be identical with those of 5,2′,5′-trihydroxyflavone isolated from *Primula pulverulenta* [6]. This assignment is also consistent with the ^{13}C NMR shifts of the ring B carbons of the dioclein. The corresponding chemical shifts of the ring B carbons of 2′,4′-dihydroxy compound differ greatly from those of dioclein [4]. The presence of the third OH function of dioclein at C-5 in ring A, as indicated by the UV spectra, is confirmed by the ^{13}C NMR spectrum of dioclein which shows the C=O signal at a relatively lower field at δ198.3. This is typical of OH-5 flavanones as opposed to the OAc-5, OMe-5 or C-5-unsubstituted compounds which show the C=O resonance at a field approximately 7.0 ppm higher [6]. Also, the C-3 resonance of dioclein appeared at δ42.6, characteristic of OH-5 flavanones which show this resonance at approximately 2.0 ppm higher than the corresponding OAc-5, OMe-5 or C-5-unsubstituted flavanones [7].

The chemical shift of the methine (CH) doublet of ring A at δ92.6 must be attributed to the C-8 by comparison with the chemical shifts of ring A carbons of compounds having a similar substitution pattern. The shifts of the C-8 methine in 5-hydroxy-6,7-dioxygenated flavanones always appear at a field higher than δ94.0 and those of C-6 methine always appear at a field lower than δ96.0 as in 5-hydroxy-7,8-dioxygenated compounds [8]. Thus, the chemical shifts of the ring A carbons of 5,4′-dihydroxy-6,7-dimethoxyflavanone, isolated from the exudate of *Cheilanthes argentea* [9] and 5,2′-dihydroxy-6,7,6′-trimethoxyflavanone isolated from *Scutellaria discolor* [10] are practically identical to those of dioclein. Therefore, the structure of the new flavanone, dioclein is 5,2′,5′-trihydroxy-6,7-dimethoxyflavanone (**1**), which shows the ^{13}C NMR chemical shift assignments of the corresponding C atoms.

EXPERIMENTAL

Isolation of dioclein. The dried and ground root-bark of *D. grandiflora* (1.0 kg), collected during January 1991, was extracted in a percolator with 90% EtOH at room temperature until the last extract was colourless. Removal of EtOH, *in vacuo*, resulted in a dark brown residue which was partitioned between equal vols of H_2O and $CHCl_3$. The organic layer, after evapn of the solvent gave a residue which was subjected to CC on a silica gel column using $CHCl_3$ as eluant and fractions of 50 ml each were collected. Fractions 11–32 which showed the presence of only one component (TLC) were collected. Removal of the solvent followed by crystallization of the residue from C_6H_6 yielded dioclein (1.02 g) as slightly cream coloured needles.



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Dioclein (**1**). Mp 160–162°, UV λ_{max} (log ε) nm: 289 (4.42) and 345 (3.72) nm; λ_{max} 310 and 397 nm. ^1H NMR (250 MHz, Me_2CO-d_6): δ8.36 (*s*, 1H), 8.01 (*s*, 1H), 7.34 (*s*, 1H), 7.01 (*d*, J=2.9, 1H), 6.77 (*d*, J=8.6 Hz, 1H), 6.68 (*dd*, J=8.6 and 2.9 Hz), 6.23 (*s*, 1H), 5.73 (*dd*, 12.9 and 3.1 Hz), 1H), 3.90 (*s*, 3H), 3.70 (*s*, 3H), 2.94 (*m*). ^{13}C NMR (20 MHz, Me_2CO-d_6: see structre **1**.

Dioclein triacetate, $C_{23}H_{22}O_{10}$, MS *m/z* 458 $[M]^+$, 416, 374, 341, 197, 196, 181, and 136. ^1H NMR: δ7.93 (*s*, 1H), 7.45 (*s*, 1H), 7.20 (*s*, 1H), 6.53 (*s*, 1H), 5.73 (*dd*, 1H), 3.90 (*s*, 3H), 3.70 (*s*, 3H), 2.85 (*m*, 1H), 2.32 (*s*, 3H) and 2.30 (*s*, 6H). ^{13}C NMR (20 MHz, $CDCl_3$): δ188.4, 169.3, 168.9, 159.6, 159.3, 158.5, 148.6, 144.8, 137.0, 131.9, 123.9, 120.2, 107.0, 98.6, 74.5, 61.3, 56.3, 44.0 and 20.9.

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