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DIOCLENOL, A MINOR FLAVANONOL FROM THE ROOT-BARK OF *DIOCLEA GRANDIFLORA*

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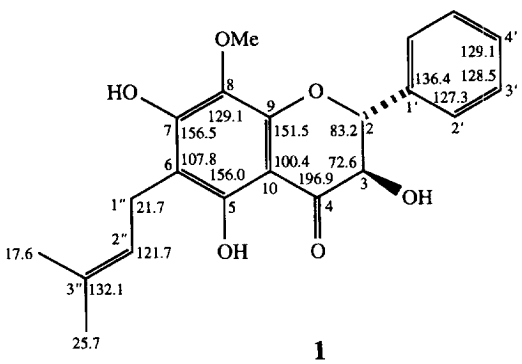
Abstract—Further investigation of the root-bark of *Dioclea grandiflora* resulted in the isolation of a minor constituent, dioclenol. The structure of dioclenol was determined to be 3,5,7-trihydroxy-8-methoxy-6-prenylflavanone on the basis of its spectral characteristics. The ^{13}C NMR assignments have been confirmed by a selective INEPT experiment. © 1997 Published by Elsevier Science Ltd

INTRODUCTION

Dioclea grandiflora Mart. (Leguminosae) is a vine that grows in the northeastern region of Brazil and is used in folk medicine for treatment of prostrate gland disorders and kidney stones [1, 2]. The crude extract of the root bark of *D. grandiflora* showed CNS activity and we have recently reported [3, 4] the isolation of a new bioactive flavanone, dioclein from this tissue. More detailed separation of the earlier chromatographic fractions in the isolation of dioclein revealed the presence of other metabolites from which dioclenol, $C_{21}H_{22}O_6$(M^+ at *m/z* 370), a minor constituent was subsequently isolated. In this communication, we report the structural elucidation (**1**) of dioclenol, a new flavanonol.

The ^{13}C NMR spectrum of dioclenol (**1**) showed signals for 21 carbons confirming the assigned molecular formula. A lowfield signal at 196.9 ppm in the ^{13}C NMR spectrum for C=O (C-4) along with the signals at 83.2 (C-2) and 72.6 (C-3) ppm are typical of a flavanonol (dihydroflavonol) structure with a C-5-hydroxyl; the signals for the C=O in C-5-unsubstituted, C-5-OAc and C-5-OCH_3 appear at a significantly higher field [5]. The flavanonol skeleton of dioclenol is further corroborated by the presence of two characteristic 1H doublets for H-2 and H-3 at δ 5.10 (J = 11.8 Hz) and 4.55 (J = 11.8 Hz) ppm, respectively, in the ^1H NMR spectrum [6]. The UV spectrum of dioclenol in methanol showed absorption maxima at 300 and 342 (sh) nm changing to 249 (sh) and 342 nm in NaOMe. The large increase in the intensity and the large bathochromic shift (42 nm) of Band II in NaOMe are consistent with a 5,7-dihydroxyflavanonol structure for dioclenol [6].



1

The MS of **1** showed, in addition to the M^+ at *m/z* 370, significant peaks at *m/z* 315, 285, 251, 235, 222, 207, 195 (100%), 91, 69 and 55. The fragments at *m/z* 251 and 120 must have resulted from a retro-Diels–Alder (RDA) type of fragmentation (Pathway-I) of the heterocyclic ring [7]. The peak at *m/z* 120 (B_3^+) as well as the intense peak for a tropylium ion at *m/z* 91(98%) arising from ring B clearly indicate an unsubstituted B-ring. The characteristic separation of signals for five Ar-H into two multiplets between δ 7.44 and 7.55 ppm in the ^1H NMR spectrum also confirms that the ring B is unsubstituted [8].

The peaks at *m/z* 315 ($M-C_4H_7$), 69 and 55 are indicative of a $(CH_3)_2C{=}CHCH_2$-(prenyl) substituent [9]. Two 3H singlets at δ 1.60 and 1.67 ($2CH_3$), a 2H doublet at 3.25 (J = 7.3 Hz, —CH_2—) and a 1H triplet

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at 5.40 (J = 7.3 Hz, —CH=) ppm in the ^{1}H NMR spectrum supported by two CH_3 quartets at 25.7 and 17.6 ppm, a quaternary C signal at 132.1 ppm [DEPT] and the signals for a —CH_2— and a —CH= at 21.7 and 121.5 ppm, respectively, in the ^{13}C NMR spectrum confirm the presence of a prenyl substituent.

That dioclenol contains a Ar-OCH_3 substituent is confirmed by the signal at 61.0 ppm in the ^{13}C NMR (DEPT) spectrum and a 3H singlet at 3.97 ppm in the ^{1}H NMR spectrum. The relatively lowfield shift (61.0 ppm) demonstrates that both the positions *ortho* to the Ar-OCH_3 are substituted [10]. Apart from the C-3—OH, all other substituents in dioclenol; two OHs, a OCH_3 and a prenyl group must be attached to the A ring. This is also consistent with the RDA fragmentation peak at m/z 251 in the MS of **1**.

The prenyl and the OCH_3 groups must occupy C-6 and C-8 on ring A. The ^{13}C NMR chemical shift for C-6 at 107.8 ppm is consistent with the prenyl substituent at that position by comparison with literature data for other similar prenylated compounds [11]. Consequently, the OCH_3 group must be present at C-8. These assignments are confirmed by a selective INEPT experiment based on the ^{13}C-^{1}H long range couplings [12]. When the H-1″ was pulsed, considerable NOE enhancement of the signals at 156.5 (C-7), 156.0 (C-5) ppm and 132.1 (C-3″) was observed. This is only possible if the prenyl substituent is at C-6. Thus, dioclenol can be best represented by structure **1** which is consistent with the ^{13}C NMR chemical shifts (assigned with the aid of ^{1}H noise decoupled, DEPT and selective INEPT experiments), as shown.

The magnitude of the coupling constants of H-2 and H-3 ($J_{2,3}$ = 11.8 Hz) suggest a 2,3-*trans* geometry and the diaxial orientation of the H-2 and H-3 in **1** [13]. The chemical shifts of H-2 (5.07 ppm) is also consistent with its axial orientation [14].

EXPERIMENTAL

General. Mps are uncorr. The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AC 300 spectrometer operating at 300.13 MHz for ^{1}H and 75.47 MHz for ^{13}C. The pulse sequence for the selective INEPT experiments was obtained by modifying the Bruker standard pulse sequence according to Bax [13]. The long range coupling value (^{1r}J) for the selective INEPT experiments was 6 Hz. In the selective INEPT experiments the decoupling powers used were S1=45L (for soft pulse) and S2=0L (for decoupling with CPD). The power 0L in the Bruker AC 300 spectrometer is approximately 1W. The following delays were utilized: D1 = 3 s (relaxation delay for ^{1}H, prepare decoupler power for soft pulse); D2 = 1/4J LR −0.15 s (refocusing delay); the D3 = 1/4J LR −0.0075 s (for polarization transfer from a CH); D5 = 0.0075 s (for allowing evolution of antiphase magnetization) and D6 = 0.015 s (to refocus shifts and to set decoupler power).

Isolation of dioclenol (**1**). The plant material was extracted as described in an earlier publication [3]. The $CHCl_3$ soluble part of the crude ethanolic extract was subjected to CC on a silica gel (silica gel G, E. Merck, 70–200 mesh) and eluted with $CHCl_3$ and frs of 75 ml were collected. Frs 44–47 upon initial fractional crystallization C_6H_6–hexane and subsequent crystallization from Me_2CO gave **1** as colourless needles.

Dioclenol (**1**). Mp 127–129°, UVλ_{max} (log ε) 300 (4.02) and 342 (sh) in MeOH changing to 249 (sh) and 342 in NaOMe. EIMS, m/z 370 (M^+), 315, 285, 251, 235, 222, 207, 195 (100%), 120, 91, 69 and 55. IR cm^{-1}: 3414 (*br*, H-bonded OH), 1634 (conj. C=O), 1530 (Ar), 1460 (Ar), 1361, 1272, 1135, 1013, 778 and 698 (monosubstituted C_6H_6). ^{1}H NMR δ 11.03 (*s*, 1H, OH-5), 7.55 (*m*, 2H, Ar-H), 7.44 (*m*, 3H, Ar-H), 7.20 (*s*, 1H, OH), 6.76 (*s*, 1H, OH), 5.09 (*t*, 1H, J = 7.3 Hz; CH-2″), 5.10 (*d*, 1H, J = 11.8 Hz; H-2), 4.55 (*d*, 1H, J = 11.8 Hz, H-3), 3.97 (*s*, 3H, Ar-OCH_3), 3.25 (*d*, 2H, J = 7.3 Hz; CH_2-1″), 1.67 (*s*, 3H, CH_3-4″) and 1.60 (*s*, 3H, CH_3-5″). ^{13}C NMR chemical shifts are shown on structure **1**.

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