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A RING B AROMATIC STEROL FROM STROMATA OF *EPICHLOE TYPHINA*

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Key Word Index—*Epichloe typhina*; Clavicipitaceae; *Phleum pratense*; Gramineae; timothy choke disease; sterol; ^{1}H NMR; ^{13}C NMR.

Abstract—A sterol with an aromatized B ring was isolated from stromata of *Epichloe typhina* growing on *Phleum pratense*. The structure was established as 1(10→6)*abeo*-ergosta-5,7,9,22-tetraen-3α-ol by spectral analysis and synthesis.

INTRODUCTION

Previous investigations on the constituents of stromata of timothy choke disease fungus *Epichloe typhina* on *Phleum pratense* led to the isolation of the fungitoxic sesquiterpenes, chokol A, B, C [1], four C-18 hydroxy-unsaturated fatty acids [2] and three phenolic glycerides [3]. The present paper deals with the isolation and structural elucidation of a ring B aromatic sterol, compound **1**, as a novel natural product.

RESULTS AND DISCUSSION

Compound **1** was obtained as colourless needles, mp 128–129°, $[\alpha]_D^{24}$ −22.4° (EtOH; *c* 0.25) and analysed for $C_{28}H_{42}O$ by high resolution EI mass spectrometry. The UV spectrum exhibited the presence of an aromatic ring (λ_{max}^{MeOH} 273 and 282 nm); the IR spectrum showed hydroxyl group absorption (3350 cm^{-1}). The ^{13}C NMR spectrum (Table 1) revealed the presence of 28 carbon atoms including eight sp^2 carbons and suggested that **1** had an unusual steroid skeleton with an aromatic ring system. The ^{1}H NMR spectrum (Table 2) showed good resolved resonances, containing relatively less overlapping signals in the high field region. Accordingly detailed spin decoupling experiments and ^{1}H-^{13}C COSY spectrum revealed the partial structures **i**–**v** and assignments. The portion **i** contained the methine proton (H-3) at δ 4.15 which served as the starting point in the analysis of this spin system. The chemical shift value indicated that this proton was located on a carbon atom bearing a hydroxyl group (C-3, δ 68.3). The value of the coupling constants for the H-2 and H-4 protons suggested that this hydroxyl group was in an equatorial orientation. The chemical shifts of the H-1 and H-4 protons indicated that these two methylene groups were situated on aryl positions. This evidence implied that these four carbons (C-1 ~ 4) constituted a six membered ring system together with two aromatic carbons. The portion **ii** was a five-substituted benzene ring with an aromatic proton at δ 6.65 (*s*, H-7) and a methyl group at δ 2.09 (*s*, H-19) in the ^{1}H NMR. The portion **iii** contained two methylene groups, H-11 (δ 2.72 and 2.76) and H-12(δ 1.64 and 2.22). These values suggested that the former (H-11) was adjacent to an aromatic ring and the latter (H-12) adjacent to a sp^3 quarternary carbon (C-13). The coupling constant (J = 11.7 Hz) between H-11 and H-12 suggested that these two protons were in the diaxial orientation. The portion **iv** contained an angular methyl group at δ 0.59 (*s*, H-18) which was located on the above mentioned quarternary carbon (C-13). The portion **v** contained four methyl groups and a *trans* double bond at δ 5.21 and 5.25 (J = 14.7 Hz, H-22 and H-23, respectively) and comprised the ring D and side chain parts. The portions **iii** and **iv** and one aryllic methine group at δ 2.66 (H-14) constituted the ring C system together with two aromatic carbons.

The connection of the A, B and C rings was established by NOE difference spectra. Irradiation of H-1 (δ 2.86) and H-11 (δ 2.74) gave enhancements to H-7 and H-19, respectively. This results indicated that **1** possessed the anthracene skeleton. Additionally, in the decoupling experiments irradiations of H-1 (δ 2.88) and H-14 (δ 2.66) led to enhancements of H-7 by long range coupling, although no splittings were measurable. This evidence supported the results of NOE experiments. The chemical shift (δ 0.59) of the angular methyl group protons H-18 suggested that this methyl group was shielded by the aromatic B ring [4], and, therefore, the stereochemistry of the C/D ring fusion was *trans*. Consequently, the planar structure

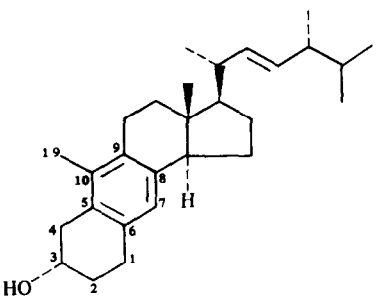

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Table 1. ^{13}C NMR spectral data of **1** (67.9 MHz, COM and INEPT, $CDCl_3$, TMS int. standard)

C		C	
1	27.6	15	24.2
2	31.4	16	29.4
3	68.3	17	55.2
4	36.6	18	11.4
5	134.2[a]	19	14.6
6	132.5[a]	20	40.6
7	123.9	21	21.1
8	137.9[a]	22	135.6
9	132.14[a]	23	132.10
10	129.8	24	42.9
11	25.8	25	33.2
12	37.2	26	20.0
13	41.8	27	19.7
14	51.9	28	17.7

[a]Assignments may be interchanged.

Table 2. ^1H NMR spectral data of **1** (500 MHz, $CDCl_3$, TMS int. standard)

H		H	
1α	2.88 *ddd*(16.6, 5.9, 5.4)	16α	1.47[a] *m**
1β	2.82 *ddd*(16.6, 6.8, 5.9)	16β	1.91[a] *m**
2α	1.75 *dddd*(15.1, 9.7, 6.8, 5.4)	17	1.36 *ddd*(9.3, 9.3, 9.3)
2β	2.02 *dddd*(15.1, 5.9, 5.9, 2.9)	18	0.59 *s*
3	4.15 *dddd*(9.7, 8.1, 5.4, 2.9)	19	2.09 *s*
4α	2.55 *dd*(16.1, 8.1)	20	2.08 *ddq*(9.3, 7.8, 6.8)
4β	3.06 *dd*(16.1, 5.4)	21	1.09 *d*(6.8)
7	6.65 *s*	22	5.21 *dd*(14.7, 7.8)
11α	2.72 *ddd*(10.3, 8.3, 1.5)	23	5.25 *dd*(14.7, 6.8)
11β	2.76 *ddd*(11.7, 10.3, 7.8)	24	1.87 *m**
12α	1.64 *ddd*(12.7, 11.7, 8.3)	25	1.49 *m**
12β	2.22 *ddd*(12.7, 7.8, 1.5)	26	0.85 *d*(7.3)
14	2.66 *dd*(11.7, 7.8)	27	0.84 *d*(6.8)
15α	2.02 *m**	28	0.94 *d*(6.8)
15β	1.44 *m**		

Coupling constants (J in Hz) are given in parentheses.

[a]Assignments may be reversed.

*J(Hz): 14, 15α = 7.8; 14, 15β = 11.7; 16α, 17 = 9.3; 16β, 17 = 9.3; 23, 24 = 6.8; 24, 28 = 6.8; 25, 26 = 7.3; 25, 27 = 6.8. Not measured: 15α, 15β; 15α, 16α; 15α, 16β; 15β, 16α; 15β, 16β; 16α, 16β; 24, 25.

of **1** including the hydroxyl group position was established as depicted. This compound has been synthesized by Whalley *et al.* [5, 6]. Comparison of the CD spectrum of isolated **1** with those reported for synthetic 3α and 3β isomers[6] led to **1** having the 3α stereochemistry. Configuration of the C-24 position was determined to be *R* by the ^{13}C NMR chemical shifts of C-24 and C-28 [7]. Therefore, the structure of **1** was established as 1(10→6) *abeo*-ergosta-5, 7, 9, 22-tetraen-3α-ol. To confirm the structure of **1**, we prepared this compound following the method of Whalley [5]. The isolated **1** was identical with synthetic **1** by direct comparison of all spectral data.

To our knowledge there have been some investigations on synthesis of anthrasteroids [5, 6, 8] and acidic rearrangement from an unsaturated sterol [5] and isolation of anthrasteroid hydrocarbons from sediments [9]. Also, recently ring B aromatic sterols with a phenanthrene skeleton were isolated from a soil amoeba *Acanthamoeba polyphaga* [10]. However, compound **1** is the first isolation of an anthracene type sterol from a natural source.

EXPERIMENTAL

Isolation. Stromata of *E. typhina*, (20 kg) were extracted with 70% EtOH (103 l). The ext was evapd and the aq. residue partitioned between *n*-hexane and H_2O. The *n*-hexane soln was dried (Na_2SO_4), filtered, evapd to dryness and the residue (172 g) chromatographed on a silica gel column × 3 with $CHCl_3$. A fraction containing sterols (10.1 g) was separated by CC on silica gel (200 g) with EtOAc–C_6H_6 (1:9), CC on silica gel (50 g) with EtOAc–*n*-hexane (1:4) and CC on silica gel (50 g) with EtOAc–*n*-hexane (1:9). A fraction (151 mg) was purified on a Lobar Si 60 column using $CHCl_3$ and recrystallized from MeOH to yield compound **1** (18 mg) as colourless needles, mp 128–129°. FDMS *m/z*: 394[M]$^+$; EIMS *m/z*(rel. int.): 394.3240[M]$^+$ (100) (calcd for $C_{28}H_{42}O$: 394.3236), 376[M − H_2O]$^+$ (26.1), 361[M − H_2O − Me]$^+$ (3.3), 269[M − side chain]$^+$ (15.1), 267 (11.6), 252 (18.3), 251[M − H_2O − side chain]$^+$ (82.0), 242 (18.3), 227 (24.8), 215 (20.0), 197 (39.4), 69 (18.3); IR ν_{max}^{KBr} cm^{-1}: 3350, 2960, 2870, 1725, 1600, 1455, 1370, 1320, 1260, 1220, 1185, 1145, 1070, 1050, 1025, 970, 870, 740; UV λ_{max}^{MeOH} nm(ε): 273 (946), 282 (965); CD λ_{ext}^{MeOH} nm(Δε): 225 (−0.57), 233 (+0.10), 239 (−0.12), 247 (−0.05), 278 (−0.27); $[\alpha]_D^{24}$ −22.4° (EtOH, *c* 0.25); ^{13}C NMR (Table 1); ^1H NMR (Table 2); spin decoupling experiments were recorded at 500 MHz and ^1H–^{13}C COSY and NOE difference spectra were recorded at 270 MHz.

Preparation of **1**. **1** was prepd from ergosterol according to the method of ref.[5], purified by CC on silica gel with EtOAc–*n*-hexane (1:4) and recrystallized from MeOH; mp 130–131°; $[\alpha]_D^{23}$ −19.5° (EtOH, *c* 0.38).

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