MINOR DITERPENES FROM COLEUS FORSKOHLII

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Abstract—The structures of two new minor diterpenes, 1,9-dideoxy coleonol-B and 1-acetoxy coleosol, isolated from the roots of *Coleus forskohlii* have been shown to be 7-hydroxy-6-acetoxy, 13-epoxy labd-14-en-11-one and 1-acetoxy-6,9-dihydroxy, 13-epoxy labd-14-en-11-one, respectively, mainly through interpretation of 2D NMR data and X-ray analysis.

INTRODUCTION

Coleonol (forskolin) (1), a major labdane diterpenoid isolated from the Indian herb *Coleus forskohlii*, displays strong hypotensive and spasmolytic activities [1]. It also possesses a positive inotropic activity and other biological activities [2–4]. Lidner *et al.* reported the antihypertensive and adenylate cyclase profile of the molecule [5]. The continued interest of CDRI in the chemical investigation of the plant [6], prompted us to isolate the minor coleonol related constituents. We now report the isolation and structure elucidation of 1,9-dideoxy coleonol-B (2) and 1-acetoxy coleosol (3). The structures were ascertained by using various 2D NMR techniques and single crystal X-ray analysis. Compounds 2 and 3 were isolated from the roots of *C. forskohlii* and analysed for $C_{22}H_{34}O_5$ (M⁺ 378) for 2 and $C_{22}H_{34}O_6$ (M⁺ 396) for 3.

RESULTS AND DISCUSSION

The spectral studies (1D ¹H NMR, IR and MS) of 2 revealed it to be a diterpenoid. The fully decoupled ¹³C and DEPT spectra of 2 showed 22 signals including six methyls, five methylenes, five methines and six quaternary carbons. The splitting pattern of two protons at C-12 (δ 2.69 and 2.63) led to the detailed investigation by 2D NMR methods. For an unambiguous assignment of structure and stereochemistry, COSY, COSYLR, specific proton decoupling and ¹³C NMR NOE difference experiments were undertaken on 2, and COSY and NOE difference experiments on 3.

The 2D COSY, ¹H NMR spectrum of 2 provided the linkage of protons between C-1-C-3 and C-5-C-7, and



assignments of H₂-12, H-9 and C-14,15. Moreover, long range correlation in the COSYLR spectrum gave crosspeaks for ⁴J coupling. These coupling interactions can be used for the assignment of tertiary methyls. For example, H-9 at $\delta 2.67$ gave long range cross-peaks for the methyl signals at $\delta 1.46$ and 1.38 (Table 1), for Me-17 and Me-20. The differentiation between them was provided by the cross-peak of H-7 ($\delta 3.89$) with Me-17, respectively. The remaining methyl signals were assigned by NOE difference experiments.

For the unambiguous assignments of the methyls and stereochemistry, a series of NOE difference experiments were carried out. Irradiation of the signal at $\delta 0.95$ with very low power gave NOEs for Me-20, H-6 and at $\delta 0.99$ only, whereas, irradiation of the signal at $\delta 0.99$ gave NOEs for the signal at $\delta 0.95$ and a strong NOE for H-6. Thus, the signals at $\delta 0.95$ and 0.99 can be assigned unambiguously to Me-19 and Me-18, respectively. Irradiation of H-5 gave NOEs for H-9, Me-18 and H-7 only, suggesting that the A/B ring junction is *trans*, and H-5, Me-19 and Me-20 are axially β -oriented. The NOEs between Me-20 and Me-17 further confirmed their β axial orientation. H-9 showed NOEs with H-5 and H-7

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	2				3		
Position	1H	¹³ C	³ J _{HH} /Hz	¹ H– ¹ H NOE	¹ H	³ J _{HH} /Hz	¹ H– ¹ H NOE
1	2.50	41.1	ddd 13.3. 4.7. 3.1		5.51	dd 3.9, 3.1	
1′	0.81	0.000	ddd 13.3, 13.3, 3.1				
2	1.71	18.7	m		2.02	m	
2'	1.37				1.71	dd 14.8, 3.13	
3	1.43	43.8			1 25	m	
3'	1.16		ddd 133 133 35		1 1 1	m	and the second se
4		37.9					
5	1.05	53.9		976	1 34	1235	
6	5.76	79.7	dd 2.3. 39	10 18 7 5	1.54	d 3 13	
7	3.89	70.7	d 3.9	9, 5, 6, 15 (trans)	2 10	dd 14 00 3 13	_
7'				<i>)</i> , <i>3</i> , <i>0</i> , <i>13</i> (<i>trans</i>)	1.02	d 2 12	
8		74.9	S		1.95	<i>u</i> 5.15	
9	2.67	65.1	s	7 5 15 (trans)	2 20		
10		33.8	5	r, 5, 15 (trans)	5.20	5	
11		205.7	d 18.0				
12	2.69	49.6	. 1010		2.60	1180	
12'	2.63		d 18.0		2.09	<i>d</i> 18.0	
13	_	79.3			2.39	<i>u</i> 18.0	
14	5.97	146.4	dd 17.2, 10.9		5.03	44 17 22 10.06	-
15 cis	5.06	112.2	d 17.2		5.22	d 17.22, 10.90	
15 trans	5.17				5.22	<i>a</i> 17.22	
16	1.30	33.0	S	14 12 12' 17	1 28	•	12 14
17	1.46	22.5	S	20, 12, 12, 17	1.61	5	12, 14
18	0.99	33.8	S	19.6	1.01	5	12, 20, 7
19	0.95	22.8	S	18 20 6	1.02	3	19, 0, 5
20	1.38	16.4	S	19. 17. 2-	1.45	5	20, 10
21	<u> </u>	170.7			1.95	5	19, 17, 1
22	2.10	21.3	S	—	1.75	5	

Table 1. ¹HNMR chemical shifts, coupling constants and interproton NOEs for 2 and 3; ¹³C chemical shifts for 2 only

confirming the B/C ring junction as trans. The couplings and the NOEs of H-6 and H-7 confirmed the hydroxyl group at H-7 as β -equatorial oriented, whereas the acetoxy at H-6 was β -axial. Irradiation of the signal at δ 1.30, which can now be unambiguously assigned to Me-16, showed NOEs for H-14, H₂-12 and Me-17, whereas on irradiation of Me-17, NOEs were observed for one of the H₂-12, Me-16 and Me-20, respectively. The most unusual NOE between the Me-17 and H₂-12 gave indication for the flagpole interaction, i.e. 1-4 interaction, which is only possible if the C ring is in the boat configuration as has been reported earlier for 1 [7]. The above information was further reinforced by irradiating H-9, in which NOEs were observed for H-7, H-5, H-15 trans and H-14, as well as H-7 giving NOEs with H-9, H-5, H-6 and H-15 trans. The NOEs of H-9 with H-15 trans and H-14 clearly confirm that the C ring is in the boat form. Thus, 1,9 dideoxycoleonol-B can now be unambiguously formulated as 7-hydroxy-6-acetoxy, 13-epoxy labd-14-en-11-one (2).

The molecular crystal structure of 2 has been determined. The cell parameters are close to those of coleonol-B; thus the symmetries and space group are the same [8]. Most of the structural findings from 2D NMR are seen in the crystal structure i.e. trans-ring junctions, substituents at the 6- and 7-positions in the β -configuration and cis with respect to the ring system. The major difference is the chair conformation of the C ring in the crystal compared with the boat conformation of the C ring in solution. The three rings possess chair conformations in the crystal. The magnitudes of the internal torsion angles range from $53.1(8)^{\circ}$ to $58.6(9)^{\circ}$ in ring A, from $51.4(7)^{\circ}$ to $58.1(7)^{\circ}$ in ring B, and from $44.0(9)^{\circ}$ to $64.4(7)^{\circ}$ in ring C (Fig. 1).

The cross-peaks in the COSY spectrum of 3 provided the linkages; e.g. the signal at $\delta 5.51$ showed two crosspeaks at $\delta 2.02$ and 1.71 only. Whereas, these signals showed further cross-peaks with each other as well as with the signals at $\delta 5.51$, 1.25 and 1.11. This type of connectivity is only possible by assigning $\delta 5.51$ to the H-1 proton bearing the acetoxyl group, thus providing the linkage between C-1 and C-3. The other linkages were traced in a similar way as in 2 with a carbinolic proton at C-6 and a hydroxyl group at C-9, respectively. In order to confirm the stereochemistry of 3, several NOE difference experiments were carried out. The NOE of Me-20 with H-1 indicated that they are close to each other in space. The couplings and the NOE results suggested that the acetoxyl group has an α -axial orientation and that the C ring



Fig. 1. A perspective stick representation of 1,9 dideoxy coleonol-B (2).

is in the boat conformation. Compound 3 can now be unambiguously formulated as 1-acetoxy, 6,9-dihydroxy, 13-epoxy labd-14-ene-11-one.

We failed to obtain a suitable single crystal of 3 for an X-ray analysis.

EXPERIMENTAL

Isolation. Shade dried powdered roots of C. forskohlii (1 kg) were extracted with 1,2-dichloroethane $(3 \times 3 l)$ in a Soxhlet apparatus. The extract was concd in vacuo to give 55 g residue, which was defatted with MeOH (1 l). The defatted material (38 g) when subjected to chromatography over silica gel, yielded 1,9-dideoxy coleonol-B (2) and 1-acetoxy coleosol (3) with hexane-EtOAc 9:1 and 4:1, respectively, in the crude form. The compounds were finally purified on a silica gel flash column with a hexane-EtOAc gradient. Compound 3 was purified as a white amorphous powder (12 mg); mp 165-167°; El-MS. *m/z*: 394 [M]⁺, 335, 334, 318, 310, 303, 285, 265, 232, 204, 148, 94, 80, 68, 54, 43 (base peak); IR v ^{KBr}_{max} cm⁻¹: 3500, 2950, 1720, 1390, 1260, 1220, 1147, 1065, 1042 and 995. 1,9 Dideoxy coleonol-B (2) was crystallized with Et₂O and petrol as crystals (80 mg); mp 123-125°; El-MS, m/z: 378 [M]⁺ 377, 362, 344, 303, 292, 268, 234, 232, 206, 179, 177, 123, 109, 69, 43 (base peak); IR $\nu \frac{\text{KBr}}{\text{max}} \text{ cm}^{-1}$: 3480, 2940, 1720, 1400, 1260, 1100 and 950.

NMR. Bruker WM-400 multinuclear FT NMR spectrometer. The samples for ¹H measurements were placed in a 5 mm ¹H-¹³C dual probe containing 5 mg sample in 0.5 ml CDCl₃. The ¹³C NMR samples were prepared in 5 mm tubes by mixing 80 mg sample in 0.5 ml CDCl₃. TMS was used as an int. standard in both measurements. The 2DCOSY spectrum was obtained using a pulse sequence $90-t_1-90-aq$ [9] and the 2DCOSYLR spectrum was obtained using the pulse sequence $90^{\circ}-t_1-90^{\circ}-aq$ [10] with a fixed delay of 0.2 sec. The NOE difference experiments were carried out using 2 sec irradiation and relaxation times.

X-ray analysis of 2. A white prism (0.27×0.15) $\times 0.10$ mm) of 1,9-dideoxy coleonol-B crystallized in the orthorhombic system, space group $P2_12_12_1$ with a = 28.441(2), b = 11.462(1) and c = 6.300(1) Å V = 2053.74(0) Å³, Z = 4, $d_{calc} = 1.231 \text{ g cm}^{-3}$. Intensity data were collected on a CAD-4 Enraf-Nonius diffractometer (monochromated CuKa radiation, λ = 1.54184 Å) and corrected for Lorentz and polarization effects. No absorption correction was necessary (µ = 6.5 cm^{-1}). A total of 2018 unique reflections up to $\theta \leq 65^{\circ}$ was measured, of which 1657 were considered as observed $[I \ge 3\sigma(I)]$ and were used in the refinement. The structure was solved by direct methods using MolEN [11] and refined by full-matrix least-squares methods with anisotropic temp. factors for C and O atoms, and isotropic ones for H atoms. The final R=0.056, R_w = 0.066. A list of atomic coordinates, bond lengths and angles, observed and calculated structure factors and anisotropic thermal parameters has been deposited at the Cambridge Crystallographic Data Centre.

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