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EPI-DEOXYCOLEONOL, A NEW ANTIHYPERTENSIVE LABDANE DITERPENOID FROM *COLEUS FORSKOHLII*

J.S.Tandon[a], R.Roy[a], S.Balachandran[a,+] and R.A.Vishwakarma[b,+,*]

[a]Medicinal Chemistry Division
Central Drug Research Institute, Lucknow 226001, India

[b]Bio-organic Chemistry Laboratory
National Institute of Immunology, New Delhi, 110067, India

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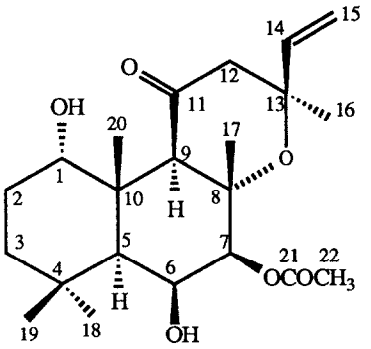
Abstract.- A new antihypertensive labdane diterpenoid 13-epi-9-deoxycoleonol (13-epi-9-deoxyforskolin) has been isolated from the Indian medicinal plant *Coleus forskohlii* and the stereostructure of the diterpenoid ascertained by various two-dimensional NMR techniques

The Indian medicinal plant *Coleus forskohlii* Briq. (Lamiaceae) has been extensively investigated by us[1] and others[2] mainly due to the isolation of unique labdane diterpenoid Coleonol (Forskolin) with remarkable biological activity and potential drug for glaucoma, congestive heart-failure and bronchial asthama[3]. In view of the unique pharmacodynamic action as an adenylate cyclase stimulant[4] coupled with substantial structural challenge, Forskolin has emerged as a highly attractive target for the synthetic[5] and structure-activity relationship studies[6]. In continuation of our work[7] on chemical investigation of *Coleus forskohlii*, we now report isolation and stereostructure of a novel antihypertensive labdane diterpenoid 13-epi-9-deoxycoleonol (13-epi-9-deoxyforskolin,1) as the first diterpenoid in this series lacking hydroxy function at C-9 position and still showing promising blood-pressure lowering activity.

The 13-epi-9-deoxycoleonol (1) was isolated from the dichloroethane extract of the roots of *Coleus forskohlii* as crystalline needles, mp180^0, $[\alpha]^{25}_D$ -77.7 (c=1, $CHCl_3$) and analysed for $C_{22}H_{34}O_6$ (elemental analysis and mass spectrum). The spectral studies (1D-^{1}H-, ^{13}C-NMR, IR, MS) of **1** revealed it to be a labdane diterpenoid and exhibited related features to that of 9-deoxycoleonol[8] (9-deoxyforskolin),however with a characteristic

+ *Present Address: University Chemical Laboratory, Department of Chemistry, Cambridge University, Lensfield Road,Cambridge CB2 1EW, U.K.*

difference in ^{1}H-NMR spectra of **1** and 9-deoxyforskolin. The downfield shift of 9-H (δ 3.52) and the coupling pattern of two C-12 protons (2.56 & 2.68) of **1** as compared with that reported for 9-deoxyforskolin[8] led us to the detailed investigation of **1** by two-dimensional NMR techniques such as the COSY, COSYLR, specific proton-decoupling, ^{13}C-, DEPT and NOE difference experiments, for the unambiguous assignment of the stereostructrue of **1**.



(1)

The fully-decoupled and DEPT spectra of **1** (Table 1) showed 22 signals including six methyl, four methylene, six methine and six quaternary carbons. A two-dimensional COSY ^{1}H-NMR spectrum provided the linking of protons between C-1 to C-3, C-5 to C-7, and the assignments of H_2-12, H-9 and C-14,15 bond. Moreover long-range correlation in the COSYLR spectrum showed cross peaks for ^{4}J coupling which followed empirical 'W' rule[9-11]. These coupling interactions could be used in the assignment of tertiary methyls and configurational analysis of **1**, for example the H-7 signal at 5.08 gave long-range prominent cross peak for methyl signal at 1.56 which was unambiguously assigned for Me-17. Similarly the 9-H signal gave strong cross peaks for Me-17 (1.56) and Me-20 (1.41) respectively.The data concluded that appreciable ^{4}J coupling[12] was due to a path having zig-zag or 'W'-like shape and dihedral angle between them approaching to 180^0. Furthermore the cross peaks between two methyls at 0.97 and 1.20 indicated them to be geminal partner. The remaining methyl signal at 1.22 was assigned automatically for Me-16 as it gave cross peak with the signal at 5.25 (H-15') in COSYLR spectrum.

For the unambiguous assignment of gem-dimethyl protons and the stereochemistry of **1**, a series of NOE difference experiments were

Table 1 : ^{1}H Chemical shifts (δ H)[a], coupling constants ($^{n}J_{H,H}$ 1Hz), and interproton NOEs of 13-epi-9-deoxycoleonol and ^{13}C chemical shifts(δ C)[a] assignments

Atom	C ($CDCl_3$)	H ($CDCl_3$)	$J_{H,H}$ ($CDCl_3$)	^{1}H ^{1}J NOEs
1	72.1	4.35	dd (6.0 & 3.1)	
2eq		1.46	m	
	25.5	-	-	
2ax		2.14	m	
3eq		1.11	m	
	36.2	-	-	
3ax		1.64	m	
4	34.0	-	-	
5	47.2	1.43	d (2.9)	7ax,9ax,18
6	70.2	4.38	dd(3.9 & 2.9)	5ax,7ax,19
7	80.9	5.08	d(3.9)	5ax,9ax
8	74.7	-	-	
9	58.0	3.52	s	5ax,7ax
10	41.6	-	-	-
11	207.5	-	-	-
12eq		2.56	d(17.1)	
	49.8			
12ax		2.68	d(17.1)	
13	78.4	-	-	
14	145.8	5.96	dd(18.0 & 10.2)	
15		5.06	dd(1.0 & 10.2)	
	112.6	-	-	
15'		5.25	dd(1.0 & 18.0)	
16	31.5	1.22		12eq, 12ax, 14
17	24.5	1.56		20
18	32.7	0.97		6, 19, 5
19	23.5	1.20		18, 20
20	18.1	1.41		1, 17, 19
21	169.9	2.20		
22	21.9			

[a] Measured in $CDCl_3$ solution with TMS as internal standard

undertaken. The irradiation of the signal at 0.97 (Me-18) showed NOE with H-6 at 4.38 and with its geminal partner Me-19 at 1.20 whereas irradiation of the signal at 1.20 (Me-19) showed NOE with the signal at 1.41(Me-20) as well as with its geminal partner Me-18 at 0.97 ppm. Thus the signals at

0.97, 1.20 and 1.41 were unambiguously assigned to Me-18, Me-19 and Me-20 respectively.

Irradiation of H-5 gave NOE with H-9, Me-18 and H-7 whereas no NOE was observed with Me-20 suggesting A/B ring junction as trans, H-5 as α-axial, and Me-19 and Me-20 as β-axially oriented.The NOE between Me-20 and Me-17 further confirmed their β-axial orientation. The H-9 showed NOE with H-5 and H-7 only thus confirming trans B/C ring junction. The coupling and NOE of H-1 and H-6 (Table 1) confirmed the hydroxyl goup at C-1 as α-axial and the hydroxyl at C-6 as β-axial whereas coupling and NOE of H-7 with H-5 promptly deciphered the acetoxyl group at C-7 as β-equatorial in orientation[13]. The irradiation of Me-16 of **1** showed NOE interaction with H_2-12 and H-14 protons whereas Me-16 did not show any NOE with Me-17 .These NOE interactions were contrary to that observed by us for coleonol which displayed significant NOE amongst Me-16 and Me-17 due to their 1,3-diaxial orientation whereas the compound **1** did not show any NOE between its Me-16 and Me-17 thus establishing β-axial orientation of the C_{13}-C_{14} bond and α-equatorial orientation of Me-16 in compound **1**.

The ^{13}C-NMR assignments of methyls, C-1, C-6, C-7 and C-9 of **1** were carried out by specific ^{1}H- and ^{13}C- decoupling experiments, for example the specific decoupling of Me-18 protons (0.97) gave an enhanced ^{13}C-signal at 32.7(C-18) whereas specific decoupling of H-9 (3.52) gave enhanced signal at 58.0 ppm(C-9).

The observation of difference in ^{1}H- and ^{13}C- assignments for Me-17 and Me-18 of **1** as compared to that reported for coleonol and related diterpenoids[13] led us to the re-examination of coleonol by NOE difference experiments and consequently the Me-17 and Me-18 signals of coleonol (forskolin) have now been reassigned and revised ^{13}C- assignments for Me-17 and Me-18 of coleonol have been found to be identical to that assigned for Me-17 and Me-18 of compound **1** (Table 1).

Thus the 2D-NMR spectroscopic data led to the characterization of the compound **1** as 7β-acetoxy-1α,6β-dihydroxy-8,13-epoxy-labd-13-epi-14-en-11-one (13-epi-9-deoxycoleonol, 13-epi-9-deoxyforskolin).

The antihypertensive activity of diterpenoid **1** was evaluated on the experimental anesthetized cats by intraduodenal administration which showed a significant blood-pressure lowering activity (55 mmHg fall in blood-pressure for 40 minutes at 1mg/kg dose). This observation is

interesting in view of the previous speculations made by structure-activity relationship studies[6] on Forskolin and its analogues that the 9-OH group was essential for the biological activity. However 13-epi-9-deoxyforskolin (1) now isolated from *Coleus forskohlii* has shown significant antihypertensive activity even if it lacks C_9-OH group. Therefore it is likely that the stereochemical orientation of various substituents of forskolin play a crucial role in eliciting biological activity than the regiochemical placement as proposed by previous studies[4,6].

Experimental Procedures

General Methods : The ^1H- and ^{13}C-NMR and various two-dimensional NMR experiments[14] were performed on a Bruker WM-400 spectrometer equipped with an ASPECT 2000 computer using a 5 mm ^1H-/^{13}C dual probe-head, for ca. 0.04M-solutions in 5 mm tubes using $CDCl_3$ as solvent and TMS as internal reference.For the 2D-COSY and COSYLR experiments, N-type phase cycling was used, the FIDs were acquired over 512 data points and 2000 Hz.The raw data were zero-filled in both dimensions before double FT using the DISNMR program(version 850101.0).The NOE difference spectra were acquired by using irradiation and relaxation times of 2 s each and FIDs were line-broadened by 0.5 Hz prior to substitution. The ^{13}C-BB, SFORD, DEPT,and specific proton decoupled spectra were acquired at 100.57 MHz for 0.1 M solutions in 5 mm tubes and ^{13}C- FIDs were acquired over 26315.6 Hz and 32 data points. The elecron-impact mass spectra were recorded on a Jeol:D-300 spectrometer. The IR spectra were recorded on Perkin-Elmer (Model 157 and 577) spectrometers.The HPLC was performed on a modulation chromatograph(Waters Associates) equipped with variable wave-length UV-detector and valve type injector.
Isolation : Air-dried, powdered roots of *Coleus forskohlii* (1kg) collected from Kumaun hills of India, was extracted with 1,2-dichloroethane (5 x 6 litres) in a soxhlet apparatus and the extract on concentration yielded residue (53 g) which was chromatographed on silica gel and eluted with hexane-ethylacetate solvent-gradient to afford a mixture (1.45 g) of Coleonol and the new compound epi-deoxycoleonol (**1**). Repeated column chromatography and fractional crystallization yielded pure Coleonol(1.0 g) and 13-epi-9-deoxycoleonol (90 mg). The purity of compounds (R_T=7.00 for **1** and R_T=7.83 for coleonol) was confirmed by HPLC on a column (Lichrosorb RP-18, 30 x 3.5 mm, E.Merck) using MeOH-water (60:40) mobile phase with a flow-rate of 1 ml/min while monitoring by UV-detector.

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14. Supplementary materials on 2D-NMR studies on Compound **1** and Coleonol (COSY, COSYLR, NOE-difference ,^{13}C-BB decoupled, specific proton decoupled and DEPT specral data) will be provided on request.