A Nuclear Magnetic Resonance Study of Coleonol

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^{1}H N.m.r. spectral assignments for the diterpenoid coleonol (**1**) have been made by two-dimensional methods. The earlier H-1 and H-6 chemical-shift assignments have been corrected and the structure has been reassigned. ^{13}C N.m.r. spectra of (**1**) and of coleonol B (**3**) have been analysed with the help of two-dimensional ^{1}H–^{13}C chemical-shift correlation, broad-band-decoupling, SFORD, DEPT, and specific-proton-decoupling techniques. Stereochemical information on structure (**1**) was obtained through stereospecific coupling constants and nuclear Overhauser effects.

Coleonol, a diterpenoid isolated from *Coleus forskohlii*, is a novel 11-oxomanool epoxide derivative, having hypotensive, haemodynamic, and cardiac metabolic effects.[1,2] Forskolin, isolated later, has been shown to be identical with coleonol.[3,4] The structure (**2**) for coleonol was proposed originally on the basis of degradative and spectroscopic studies.[2] However, no detailed studies of ^{1}H and ^{13}C n.m.r. spectra had been carried out. In view of the biological importance[5] of this molecule we have undertaken the complete assignment of the ^{1}H and ^{13}C resonances of coleonol and of the ^{13}C chemical shifts of coleonol B,[6] using two-dimensional homonuclear chemical-shift correlation (COSY),[7] two-dimensional nuclear Overhauser enhancement (NOESY), ^{1}H n.m.r. spectroscopic, two-dimensional ^{1}H–^{13}C chemical shift-correlation, single-frequency off-resonance decoupling (SFORD), and distortionless enhancement by polarization transfer (DEPT) ^{13}C n.m.r. techniques.[8]

Two-dimensional (2D) n.m.r. spectroscopy at high field was applied here to assign the various ^{1}H resonances. The stereochemistry was determined by use of nuclear Overhauser enhancement, ^{1}H spin–spin coupling, and selective proton-decoupling ^{13}C n.m.r. techniques. It was found that the ^{1}H chemical-shift assignments of some of the methyl and methine signals reported earlier[2–4] were wrong. The results make it possible to assign unambiguous structure (**1**) to coleonol and structure (**3**) to coleonol B.

Results and Discussion

The Table gives all proton and carbon-13 n.m.r. chemical shifts, proton–proton couplings, and interproton n.O.e.s for (**1**) and carbon-13 chemical shifts for (**3**).

Two-dimensional N.m.r. Spectroscopy.—A 2D COSY ^{1}H n.m.r. spectrum of (**1**) in CDCl$_3$ is shown as a contour plot in

Table. ^{1}H Chemical shifts (δ_H),[a] coupling constants ($^nJ_{H,H}$/Hz), and interproton n.O.e.s for coleonol (**1**) and ^{13}C chemical shifts (δ_C)[a] for coleonol (**1**) and coleonol B (**3**)

Atom	δ_C (1) (CDCl$_3$)	Multiplicity[b]	δ_C (3) (CDCl$_3$)	S[c]	δ_H (1) (CDCl$_3$)	$^nJ_{H,H}$ (CDCl$_3$)		^{1}H,^{1}H n.O.e.s[d]
1	74.5	d	74.4	+	4.56	$^3J_{1,2}$ 6.0	$^3J_{1,2}$ 3.4	
2*eq*	26.6	t	26.5	−	1.44	m		
2*ax*					2.13	m		
3*eq*	36.1	t	36.4	−	1.11	m		
3*ax*					1.77	m		
4	34.4	s	33.9					
5	42.9	d	42.1	+	2.19	$^3J_{5,6}$ 1.9		6*eq*, 7*ax*
6	69.9	d	73.5	+	4.46	$^3J_{6,7}$ 3.9	$^3J_{6,5}$ 1.9	5*ax*, 7*ax*
7	76.7	d	71.5	+	5.48	$^3J_{7,6}$ 3.9		6*eq*
8	81.4	s	82.2					
9	75.0	s	75.2					
10	43.1	s	43.2					
11	205.3	s	205.6					
12*eq*	48.8	t	48.9	−	2.47	^2J 17.1		
12*ax*					3.20	^2J 17.1		
13	82.7	s	82.2					
14	146.4	d	146.6	+	5.94	$^3J_{14,15}$ 17.1	$^3J_{14,15}$ 10.2	
15	110.7	t	110.8	−	4.98	^2J 1.0	$^3J_{15,14}$ 10.2	
15′					5.30	^2J 1.0	$^3J_{15,14}$ 17.1	
16	31.5	q	30.8	+	1.34			17
17	33.0	q	32.8	+	1.03			16, 20
18	23.6	q	22.6	+	1.71			19
19	19.8	q	19.6	+	1.44			18, 20
20	24.3	q	23.6	+	1.26			19, 17
21	169.6	s	170.7					
22	21.0	q	21.5	+	2.17			

[a] $\delta(Me_4Si) = 0$. [b] From SFORD ^{13}C n.m.r. spectrum. [c] Sign of the signal in 100.57 MHz ^{13}C DEPT spectrum. [d] The entries in this column indicate the proton at which a strong n.O.e. was observed.

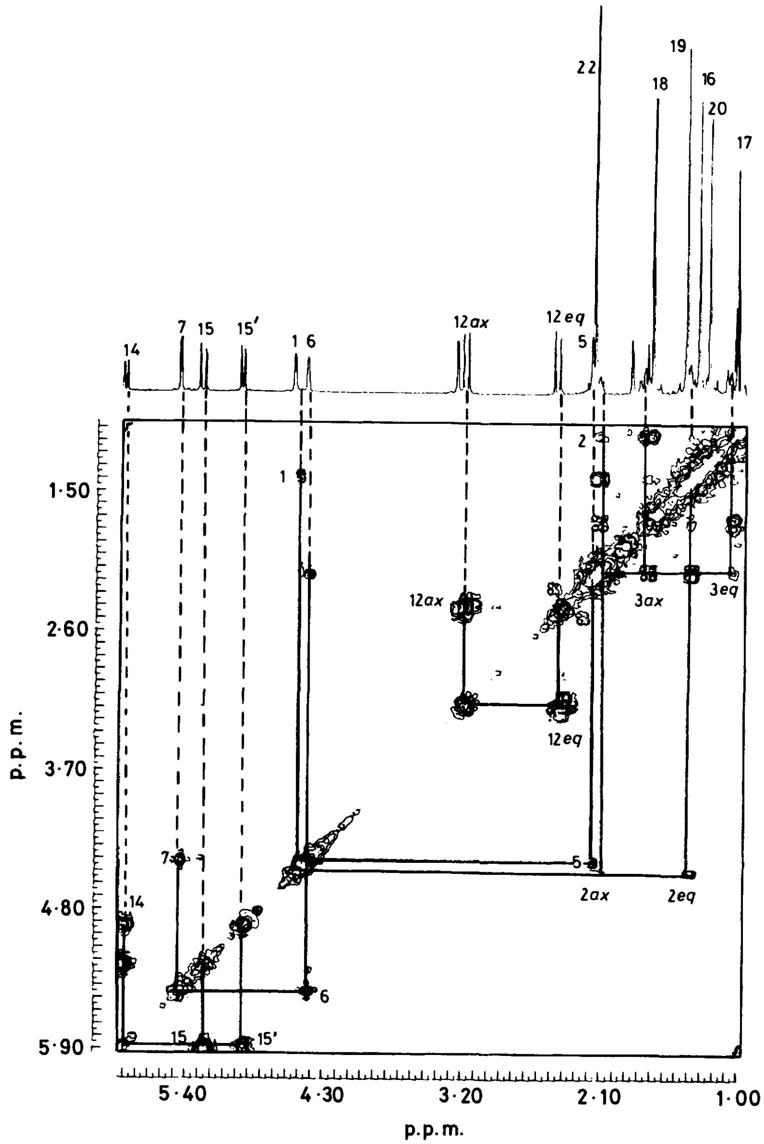
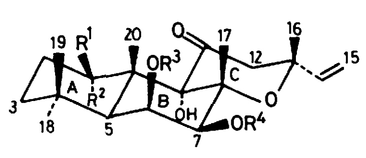


Figure 1. A contour plot of the 400 MHz 2D ^{1}H COSY (60°, N-type) spectrum of coleonol in CDCl$_3$. Connectivities between scalar-coupled protons are shown by the bold lines linking the relevant cross-peaks. The conventional 1D ^{1}H n.m.r. spectrum in f_2 dimension is shown above the 2D spectrum

Figure 1 below the normal one-dimensional spectrum. From the methine proton H-7, which may be assigned with certainty to the signal at δ 5.48, connectives could be seen (picked out in the Figure with bold straight lines) at δ 4.46 which could be unambiguously assigned to H-6. This was at variance with the earlier reports[2–4] in which H-6 had been assigned to the signal at δ 4.56. Further, the H-6 signal showed connectivity with the signal at δ 2.19 due to H-5. Similarly connectivities could then be traced for H-1, H-2, H-3, H-12, H-14, and H-15 resonances.

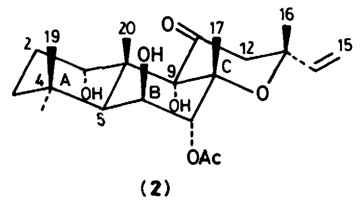
In order to determine the carbon chemical shifts of this molecule the broad-band ^{1}H-decoupled (BB), SFORD, and DEPT ^{13}C n.m.r. spectra[8] were then run. The various carbon chemical shifts were confirmed by two-dimensional ^{1}H–^{13}C shift correlation and by specific-proton-decoupling methods. The BB ^{1}H-decoupled spectrum of (1) showed twenty-two signals, corresponding to twenty-two carbon atoms. The chemical-shift assignments shown in the Table were achieved with the help of SFORD and DEPT techniques, and by application of chemical-shift theory, additivity rules, and comparison with



(1) R^1 = H, R^2 = OH, R^3 = H, R^4 = Ac

(3) R^1 = H, R^2 = OH, R^3 = Ac, R^4 = H

(4) R^1R^2 = O, R^3 = H, R^4 = Ac



(2)

published data of structurally related compounds.[9] The various DEPT spectra clearly indicated the presence of six methyl, four methylene, and five methine groups, leaving seven quaternary carbon atoms. This was confirmed by the SFORD spectrum, which showed six quartets, four triplets, five doublets, and seven singlets.

Assignments of signals due to carbonyl carbon were straightforward.[9,10] Thus the two low-field signals appearing as singlets in SFORD at δ 205.3 and 169.6 were assigned to C-11 and C-21, respectively. Of the three mid-field signals appearing at δ 82.7, 81.4, and 75.0, the first two signals could be assigned to C-13 and C-8, respectively, and the signal at δ 75.0 was due to C-9. Further, two upfield quaternary carbon signals at δ 43.1 and 34.4 due to C-10 and C-4 could be distinguished by the fact that the β-effects of the two hydroxy groups at C-1 and C-9 should deshield C-10 in comparison with C-4.[11]

Of the four methylene signals, the low-field peak at δ 110.7 could be assigned unambiguously to the olefinic C-15 methylene group, whereas the signal at δ 48.8 was assigned to C-12 on the basis of the observation that specific proton [δ 2.47 (H-12*eq*) and 3.20 (H-12*ax*)] decoupling resulted in an enhanced signal at δ 48.8 only. Of the two signals at δ 36.1 and 26.6 the upfield one was assigned to the C-2 methylene group (C-19 and C-20 methyl *gauche* interactions). The SFORD spectrum of (**1**) clearly showed five methine carbon doublets at δ 42.9, 69.9, 74.5, 76.7, and 146.4. This was confirmed by the DEPT spectrum (five positive peaks). The assignment of the C-14 olefinic methine signal at δ 146.4 was straightforward, and the high-field signal at δ 42.9 was ascribed to C-5 in view of the fact that it was the only methine group not attached to any heteroatom. Of the remaining three methine carbon atoms (C-1, C-6, and C-7) having oxygen functionality, the one attached to the acetoxy group (C-7) was assigned to the signal at δ 76.7.[10] This was confirmed by specific proton-decoupling at δ 5.48 (H-7), which resulted in an enhancement of the signal at δ 76.7, whereas decoupling at δ 4.46 (H-6) resulted in an enhanced peak at δ 69.9. Therefore, the remaining signal at δ 74.5 was due to C-1. The assignment of the C-1 methine signal was confirmed by the lack of the signal at δ 74.5 in the ^{13}C n.m.r. spectrum of dioxocoleonol (**4**), which has a carbonyl functionality at C-1 giving rise to a quaternary carbon signal at δ 209.3.

The assignment of ^{13}C signals corresponding to C-16, C-17,

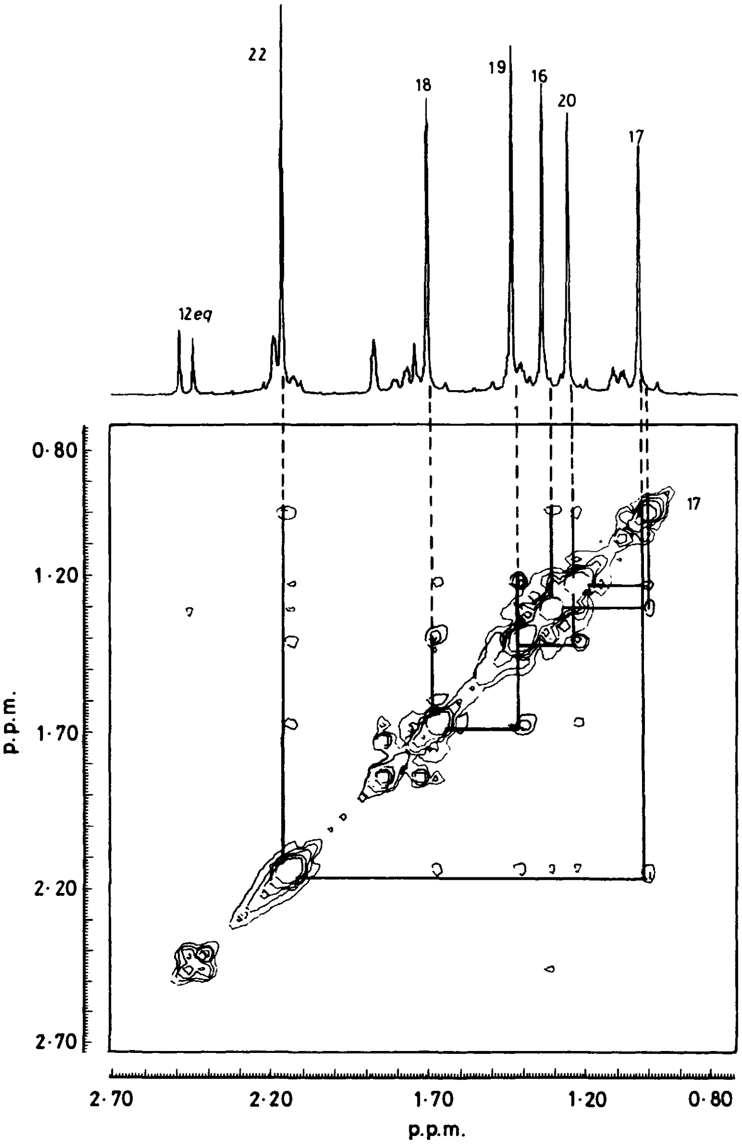


Figure 2. A section of the contour plot of the 2D ^{1}H NOESY spectrum of coleonol in CDCl$_3$. The 1D ^{1}H n.m.r. spectrum of this section is shown above the 2D spectrum

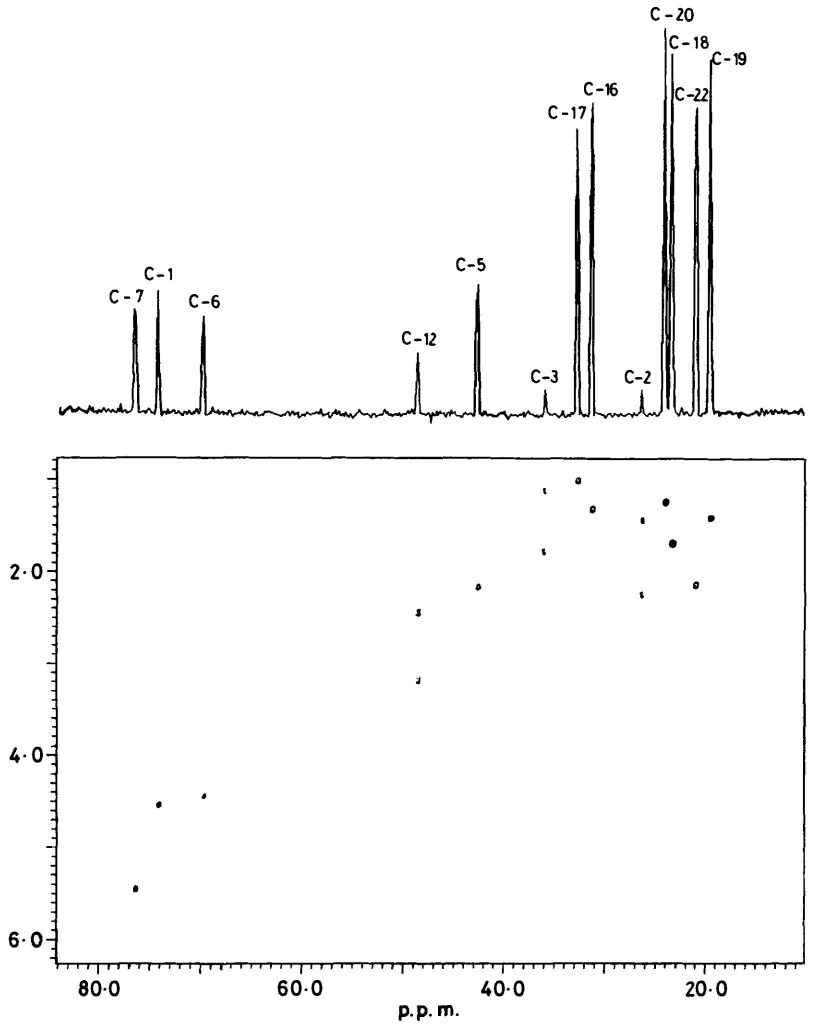
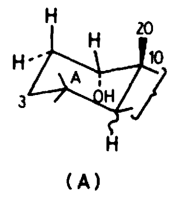


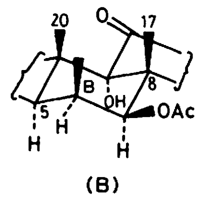
Figure 3. A section of the contour plot of the 2D ^1H–^{13}C chemical-shift correlation n.m.r. spectrum of (1). The 1D ^{13}C n.m.r. spectrum of this section is shown above the 2D spectrum

C-18, C-19, and C-20 (methyl resonances) was carried out by 2D ^1H–^{13}C chemical shift correlation,[12] based on the DEPT pulse sequence. A contour plot of these carbon chemical shifts (δ 10.0—85.0) is shown in Figure 3. The ^1H chemical shifts assignments of these methyl groups were established with the help of 2D NOESY ^1H n.m.r. spectroscopy (Figure 2). The assignment of the signal at δ 21.0 to C-22 (OAc) was straightforward. The assignments were confirmed by specific proton-decoupling experiments. For example, specific decoupling at δ 1.34 (H-16) resulted in an enhanced signal at δ 31.5 (C-16).

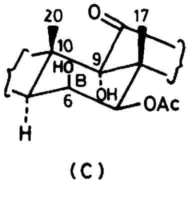
Configurational Analysis of Coleonol (**1**).—In the ^1H n.m.r. spectrum of (**1**), the vicinal couplings to H-1 (3.4 and 6.0 Hz) were in accord with the hydroxy substituents being axially oriented [see (A)]. The H-7 signal appeared at δ 5.48 as a doublet ($J_{6.7}$ 3.9 Hz) whereas H-6 resonated at δ 4.46 as a double doublet ($J_{6.7}$ 3.9; $J_{6.5}$ 1.9 Hz). Thus the 1.9 Hz coupling between H-5 and H-6 indicated a *cis*-relationship. 2D NOESY ^1H n.m.r. studies showed that H-5 has common cross-peaks with the H-6 and H-7 signals, whereas no n.O.e. was observed with the C-20 methyl protons, suggesting that H-5 was *trans* to the C-20 methyl group and axially α-oriented. Hence couplings to H-6 of 1.9 and 3.9 Hz can be interpreted as $J_{5ax.6eq}$ and $J_{6eq.7ax}$, respectively, as shown in (B).



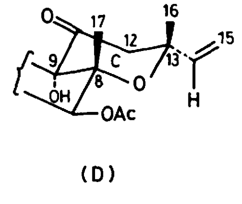
(A)



(B)



(C)



(D)

Two-dimensional experiments clearly indicated that there was no n.O.e. between H-7 and the C-17 methyl group, whereas the C-20 methyl group displayed a common cross-peak with the C-17 methyl, suggested that the methyls located at C-10 and C-8 were both oriented in the same plane. Since H-7 is axially α-oriented, the C-17 and C-20 methyl groups must be axially β-oriented (C). Further, irradiation at the frequency of this C-17 methyl group resulted in no enhancement of the H-14 signal; hence the 13,14-bond must be equatorially α-oriented (D).

Nature of the AB *Ring Junctions and Orientation of the Methyl Groups.*—The appearance of the common cross-peaks for the C-17, C-20, and C-16 methyl signals in the 2D NOESY ^{1}H n.m.r. spectrum (Figure 2) suggested that these three methyl groups are oriented in the same plane. The C-20 methyl group, which has been shown to be axially β-oriented, exhibited an n.O.e. with the C-17 and C-19 methyl groups, whereas the C-16 methyl group displayed an n.O.e. with the C-17 methyl group only. The common cross-peak for the C-19 and C-20 methyl groups confirmed the presence of 1,3-diaxial interactions between these two. Thus the C-16, C-17, C-19, and C-20 methyls are axially β-oriented. Since H-5 has been shown to be axially α-oriented, the junction between rings *A* and *B* is *trans*.

Conclusions.—A detailed two-dimensional ^{1}H homonuclear correlation n.m.r. spectral analysis of coleonol (**1**) has revealed that the previous chemical-shift assignments for H-1 and H-6 should be changed. Two-dimensional n.O.e. experiments have established the stereochemistry at C-1, C-5, C-6, C-7, C-8, C-9, C-10, and C-13, and that the *AB* ring junction is *trans* and the C-16, C-17, C-19, and C-20 methyl groups are axially β-oriented. Further it has been shown that compounds (**1**) and (**3**) have 6β- and 7β-oxygen functions; therefore, the previously assigned structure (**2**) for coleonol should be corrected to (**1**). Coleonol B has structure (**3**).

Experimental

The high-resolution 1D and 2D COSY and NOESY ^{1}H n.m.r. experiments were carried out at 400 MHz with a Bruker WM 400 spectrometer equipped with an ASPECT 2000 computer using a ^{13}C/^{1}H dual probe head, for *ca.* 0.04M-solutions in 5 mm tubes with tetramethylsilane as reference. For the 2D COSY experiment, N-type phase cycling was used and a 60° mixing pulse (12.7 μs); a 45° mixing pulse (9.5 μs) was used for the 2D NOESY experiment. The free induction decays were acquired over 512 data points and 2 000 Hz for each 128 values of evolution time. The second-dimension (f_1) sweep-width was 1 000 Hz. The raw data were zero-filled in both dimensions before double Fourier transformation using the DISNMR program (version 850101.0). The n.O.e. difference spectra were acquired [13] by using irradiation and relaxation times of 2 s each. The free induction decays were line-broadened by 0.5 Hz prior to substraction.

The ^{13}C BB, SFORD, DEPT, and specific-proton-decoupled n.m.r. spectra were acquired at 100.57 MHz for 0.1M-solutions in 5 mm tubes. ^{13}C Free induction decays were acquired over 26 315.6 Hz and 32 K data points. The 2D ^{1}H–^{13}C chemical-shift correlation n.m.r. spectrum was obtained using the pulse sequence DEPT 2D with ^{1}H broad-band decoupling throughout the acquisition period. The free induction decays were acquired over 1 024 data points and 7 462.68 Hz for each 256 values of evolution time. The second-dimension (f_1) sweep-width was ±1 099.86 Hz. The raw data were zero-filled from 256 to 512 W in the second dimension before double Fourier transformation.

Acknowledgements

We thank Dr. J. S. Tandon for his interest in the problem and for samples of coleonol and coleonol B. The use of the 400 MHz Fourier transform n.m.r. facility at R.S.I.C., Lucknow, is gratefully acknowledged.

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Received 21*st October* 1985; *Paper* 5/1826