Tetrahedron Letters No. 19, pp 1669 - 1672, 1977. Pergamon Press. Printed in Great Britain.

STRUCTURES AND STEREOCHEMISTRY OF NEW LABDANE DITERPENOIDS FROM COLEUS FORSKOHLII BRIQ.[1]

S.V. Bhat, B.S. Bajwa, H. Dornauer and N.J. de Souza*
Research Centre, Hoechst Pharmaceuticals Limited, Bombay 400 080, INDIA
and
H.-W. Fehlhaber
Hoechst AG., Frankfurt(M)-80, West Germany

(Received in UK 7 March 1977; accepted for publication 28 March 1977)

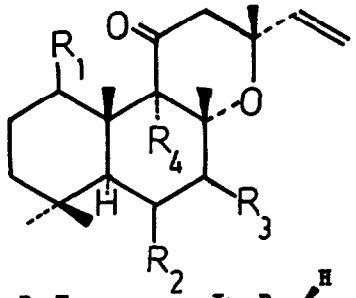
We have isolated from Coleus forskohlii (syn. C. barbatus) five diterpenoids (compounds A, B, C, D, E), three of which (compounds C, D, E) display interesting blood pressure lowering and cardioactive properties.[2] Compounds A and C are the major diterpenoid constituents of the plant and are shown on the basis of spectroscopic and chemical evidence to be 7 β-acetoxy-8, 13-epoxy-6 β-hydroxy labd-14-en-11-one(Ia) and its 1 α, 9 α-dihydroxy derivative[3] (Ic) respectively. The three minor constituents, compounds B(Ib), D(Id) and E(Ie) have structures closely related to Ia and Ic.

Chromatography on silica gel of the methanol extract of the roots of C. forskohlii afforded the compounds A(0.03%), B(0.001%), C(0.1%), D(0.001%) and E(.004% of dry weight of roots). Compound A(Ia), mp 162-65°, $[\alpha]_D^{25}$ -89.4(c=2.45, $CHCl_3$) was found to have the molecular formula $C_{22}H_{34}O_5$(elemental analysis, accurate mass determination of the M-15 ion, 22 ^{13}C-nmr signals). The ir spectrum (KBr) showed the presence of an hydroxyl, an ester, a ketone in a six membered ring, and a vinyl group (3500, 1735, 1705, 1645, 990 & 916 cm^{-1}). The 100 MHz-pmr spectrum ($CDCl_3$, ppm) indicated five tertiary C-methyl group signals (s at 0.97, 1.21, 1.22, 1.42 & 1.53), one acetyl function (s at 2.21), three protons adjacent to a carbonyl group (s at 2.59, 2.63 and 2.77, all of which disappeared when the spectrum was redetermined in $CD_3OD/NaOD$), a vinyl group bound to a quarternary carbon (characteristic ABC signal pattern in the 5-6 region) one C<u>H</u>OAc proton (d at 5.06, J=ca. 3Hz), and one C<u>H</u>OH proton (m at 4.32, $W_{\frac{1}{2}}$=6Hz, which collapsed to double doublets on D_2O exchange).

Compound A could only be acetylated under drastic conditions (acetic anhydride/pyridine, reflux, 90 hrs.) and failed to undergo typical C=O reactions, revealing that the hydroxy and carbonyl groups were sterically hindered. These data combined with biogenetic considerations[4] led us to propose the structure Ia for compound A.

NMDR experiments with compound A provided data which confirmed the assignment of the 6-OH and 7-OAc groups and were consistent with the stereochemical sequence C(5)-αH, C(6)-βH and

C(7)-α or β H[4a]. Irradiation at δ5.06(H-7) simplified the H-6 double doublet to a doublet (δ4.32, J=2Hz), whilst irradiation at δ4.32(H-6) collapsed the H-7 doublet to a singlet.



Ia, R_1=H_2, R_2=  , R_3=  , R_4=H

Ib, R_1=H_2, R_2=R_3=, R_4=H

Ic, R_1=, R_2=, R_3=, R_4=OH

Id, R_1=  , R_2=R_3=, R_4=OH

Ie, R_1=, R_2=, R_3=, R_4=OH

If, R_1=, R_2=, R_3=, R_4=OH

Ig, R_1, R_4=, R_2=, R_3=

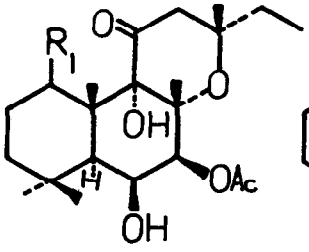
High resolution ms data on compound A confirmed the assignment of the 11-CO group. Peaks at m/e 235($C_{14}H_{19}O_3$) and m/e 207($C_{13}H_{19}O_2$) were attributed to ions arising by loss of the C_5H_8 [C(12)-C(16)] fragment and further loss of CO respectively from the ion m/e 303[$C_{19}H_{27}O_3$, base peak, m* 182.3(303→235 and 235→207)], which is itself formed from the ion m/e 318[$C_{20}H_{33}O_5$, M^+-AcOH, m*289(318→303)] by loss of a methyl group.

Compound C, Ic, mp 230-232°C, $[\alpha]_D^{25}$-26.19(c=1.68, $CHCl_3$) analysed for the molecular formula, $C_{22}H_{34}O_7$ (high resolution ms, M^+ 410.230). The ms fragmentation pattern, the ir and pmr spectra and chemical data revealed that compound C was closely related in structure to compound A: ms major fragment peaks at m/e 392[$C_{22}H_{32}O_6$, M^+-H_2O, m* 375(410→392)], 364 [$C_{21}H_{32}O_5$, M^+-H_2O-CO, m* 338.5(392→364)], 332($C_{20}H_{28}O_4$, M^+-H_2O-AcOH), and 324[$C_{17}H_{24}O_6$, M^+-H_2O-C_5H_8, m* 267.8(392→324)]: ir(KBr): 3430, 3230, 1700, 1650, 995 and 913 cm^{-1}; 100-MHz-pmr($CDCl_3$, ppm), five tertiary C-methyl group signals (s at 1.05, 1.27, 1.34, 1.43 and 1.70), one acetyl function (s at 2.15), two CH_2CO protons (pair of doublets centred at 3.20 and 2.44 Jgem=17Hz, which disappeared on deuterium exchange with NaOD), a vinyl group bound to a quaternary carbon, one CHOAc proton (d at 5.45, J=4Hz), two CHOH protons (d of d, one at 4.45, W½=10Hz, shifted to 5.55 on acetylation and the second at 4.57, W½=10Hz); spin decoupling studies confirmed the presence of a >CH-CH(OH)-CH(OAc)moiety and revealed that the chemical shift of the carbinol proton of the sequence was that at δ4.57; only one of the secondary OH

groups was easily acetylated; derivatives of the C=O group could not be prepared. The differences between compound C and compound A in their molecular formulae (2 oxygen atoms) and in their ir and pmr spectra (additional band at 3230 cm^{-1}, only one additional C<u>H</u>OH signal, absence of C<u>H</u>CO signal) were best rationalised by proposing that compound C had the structure of compound A bearing two additional hydroxy groups as represented by Ic.

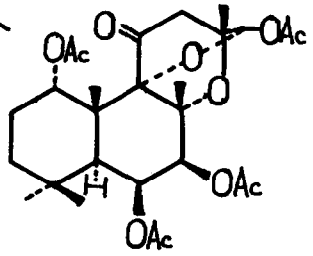
The assignment of one of the hydroxyl groups as an axial substituent at C-1 was based on the chemical shift (δ4.46), multiplicity and J values of its methine proton nmr signal, which was shifted to δ5.55 in the pmr spectrum of the diacetate[5], If, (mp 201-203°)[4d,6]. A quarternary carbon resonance at δ82.67 in the noise-decoupled FFT-^{13}C-nmr spectrum of compound C confirmed the assignment of the second hydroxyl group to C-9[7].

Confirmation of the trans stereochemistry of the A/B ring junction and of the β-configuration at C-10 was obtained from cd data. For compound A ($\Delta\varepsilon_{312}$ - 2.71), the medium strong negative cotton effect was in agreement with octant rule predictions for the proposed stereochemistry. In the case of compound C ($\Delta\varepsilon_{312}$ - 0.75), the change in $\Delta\varepsilon$ values from -0.71 for the 14,15-dihydro derivative, IIa, to -1.76 for the 1-keto compound, IIb, was conclusive. The cd data together with the pmr spectral and chemical data cited earlier in the text settled the configuration at the following chiral centres common to both compounds A and C: C-5 α-H, C-6 β-OH, C-9 α-H(compound A)/α-OH(compound C), C-13 α-vinyl[8], and at the additional centre for compound C: C-1 α-OH. The conformation of the C-1 and C-9 hydroxy groups in compound C was further

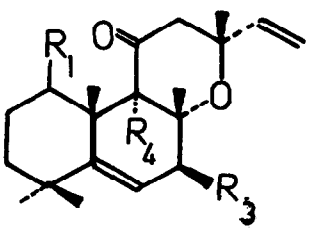


IIa, R_1= $\overset{H}{\underset{OH}{\diagup}}$

IIb, R_1=O



III



IVa, R_1=R_4=H, R_3=OAc; IVc, R_1=R_4=H, R_3=OH;

IVb, R_1=OMe, R_3=OAc, R_4=OH; IVd, R_1=OMe, R_3=R_4=OH

confirmed by facile formation of the sulfite ester, Ig, on treatment of compound C with $SOCl_2$/pyridine[9]. Formation of the hemiacetal acetate, III, via ozonolysis of the 1,6-diacetate of compound C followed by acetylation, provided additional data in support of the 13 α-configuration of the vinyl group. Application of Mills rule[10] to the Δ^5-compounds, IVa, $[M]_D$ - 70.99° and IVb, $[M]_D$ + 347.17°, derived by thionyl chloride/

pyridine treatment of compound A and of the 1-methyl ether of compound C respectively, and to the corresponding desacetyl Δ^5-compounds, IVc, $[M]_D$ -304.96°, and IVd, $[M]_D$ -32.03°, established the β-configuration of the 7-OAc substituent. The stereochemistry of compounds A and C as depicted in formulae Ia and Ic respectively was thereby clearly established[11]. Compound B, mp 121-22°, and compound D, mp 177-80°, were readily assigned structures Ib and Id respectively. Ib and Id were identical (ms, pmr, ir) with desacetyl Ia and Ic respectively, obtained by acid or alkaline hydrolysis of Ia and Ic. Compound E, mp 208-10°, was found to have structure Ia. It was identical (ms, pmr, ir) with the compound obtained on subjection of Ic to an acyl rearrangement with the use of basic reagents.

The metabolites reported here are the first labdane-type diterpenoids to be isolated from Coleus species. It is also significant that, unlike the previously reported abietane-type metabolites of the same plant[4b,c] and of related species[4a,d], the A-ring substituent in our molecules is at C-1, and the C-7 substituents have the β-configuration.

Acknowledgement: We thank Miss V. Shah for collection and identification of C. forskohlii, Dr. P.K. Inamdar and Mr. H.V. Ruparel for the microanalytical, spectral and spin decoupling data, Dr. F.W. Wehrli, Varian AG., Zug, Switzerland for cmr-data, Dr. S. Selvavinayakam, Ciba-Geigy Research Centre, Bombay, India for ms data and Prof. G. Snatzke, Ruhr University, Bochum, West Germany for cd data and valuable discussions.

References and Notes:

1. A part of this work was presented at the UNESCO 3rd Asian Symposium on Medicinal Plants and Spices, Sri Lanka, Feb. 6-12th, 1977.
2. A diterpenoid, Coleonol, having pharmacological properties has recently been isolated from C. forskohlii cf. M.P.Dubey, R.C.Srimal, G.K.Patnaik and B.N.Dhawan, Abstract No.39, in Indian J. Pharmacol. 6, 15(1974) and Abstract No.59, 4th Indo-Soviet Symposium on Chemistry of Natural Products including Pharmacology, Lucknow, India, Feb. 1976, p. 151.
3. We thank Dr. K. Venkatesan, Indian Institute of Science, Bangalore 560 012, India for sending us a copy of an abstract of a paper presented at All-India Crystallographic Seminar, New Delhi, December 1975, entitled, "The crystal and molecular structure of Coleonol, $C_{22}H_{34}O_7$", by S. Ramkumar, K. Venkatesan, M.M. Dhar and J.S. Tandon. Our structure for Ic is identical with that conveyed to us for Coleonol. As far as we are aware, the stereochemistry of the molecule was disclosed by us for the first time at the 3rd Asian Symposium on Medicinal Plants and Spices (vide Ref. 1).
4. (a) M. Moir, P. Ruedi and C.H. Eugster, Helv. Chim. Acta, 56, 2539(1973); (b) A.H.J. Wang, I.C. Paul, R. Zelnik, K. Mizuta, D. Lavie, J. Amer. Chem. Soc., 95, 598(1973); (c) A.H.J. Wang, I.C. Paul, R. Zelnik, D. Lavie and E.C. Levy, J. Amer. Chem. Soc., 96, 580(1974); (d) S. Arihara, P. Ruedi and C.H. Eugster, Helv. Chim. Acta, 58, 343(1975).
5. All the new compounds prepared were identified by microanalyses and/or mass spectral molecular weight, ir and pmr spectral analysis.
6. J.D. Connolly and D.A.H. Taylor, J. Chem. Soc., Perkin I, 686(1973).
7. N.J. de Souza, S.V. Bhat, H. Dornauer and F.W. Wehrli, manuscript in preparation.
8. E. Wenkert, P. Beak and P.K. Grant, Chem. and Ind., 1574(1961).
9. P.E. Georghiou and G. Just, J. Chem. Soc., Perkin I, 70(1973).
10. J.A. Mills, J. Chem. Soc., 4976(1952).
11. X-ray analysis of the 1-benzylether-7-deacetyl-7-bromoisobutyryl derivative of compound C carried out by Dr. Paulus, Hoechst AG., Frankfurt, West Germany, has confirmed our findings.