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MINOR DITERPENOIDS OF *COLEUS FORSKOHLII*

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Key Word Index—*Coleus forskohlii*; Labiatae; diterpenoids; forskolin analogues; ^{1}H, ^{13}C NMR.

Abstract—The structures of four diterpenoids, isolated from the roots of *Coleus forskohlii*, have been elucidated as 7β-acetoxy-6β,9α-dihydroxy-8,13-epoxy-labd-14-en-11-one (**7**), 6β,7β,9α-trihydroxy-8,13-epoxy-labd-14-en-11-one (**8**), 6β-hydroxy-8,13-epoxy-labd-14-en-11-one (**9**), 8,13-epoxy-labd-14-en-11-one (**10**) by spectral analysis in comparison with forskolin (**1**) and its congeners.

INTRODUCTION

Coleus forskohlii Briq. (Labiatae) is a perennial herb growing throughout the plains of India and in the subtropical Himalayan regions, used since ancient times for medical treatment in Hindu and Ayurvedic traditional medicine. Screening of extracts of roots of this plant led to the isolation of a group of diterpenoids possessing the basic skeleton of 11-oxo-manoyl oxide [1–9], the main constituent of which being forskolin (**1**). Forskolin possesses positive inotropic, antihypertensive and adenylate cyclase stimulating activities [10]. Clinical applications of this diterpenoid as a hypotensive, spasmolytic, lipolytic or antithrombotic agent and for the treatment of glaucoma and cardiac insufficiency are being studied. In a program of screening of plants containing compounds endowed with cardiovascular activity, we examined a sample of *Coleus forskohlii* roots collected in the southern area of Tamil Nadu (India). The results of this study are here reported.

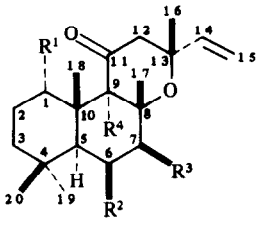
RESULTS AND DISCUSSION

Careful silica gel chromatography of the ethyl acetate extract led to the isolation of the known forskolin (**1**) [1, 2, 8, 9], deacetylforskolin (**2**) [2], 9-deoxyforskolin (**3**) [4, 7], 1,9-dideoxyforskolin (**4**) [2, 6], 1,9-dideoxy-7-deacetylforskolin (**5**) [2], coleol (**6**) [5] and of four minor closely related compounds, the structures of which **7**–**10** were established by inspection of their spectroscopic properties in comparison with the ones of the known compounds.

Compound **7** has a molecular formula $C_{22}H_{34}O_6$ (M^+ at m/z 394.2360, calcd 394.2355) and exhibits IR bands for hydroxyls (3450 cm^{-1}), carbonyl (1700 cm^{-1}) and a vinyl moiety (1645 and 1400 cm^{-1}). Its ^{1}H NMR spectrum shows signals for five tertiary methyl groups, a vinyl group linked to a fully substituted carbon (ABX system, δ_A 5.04, δ_B 5.41, δ_X 5.83 ppm, J_{AB} 1.5, J_{BX} 17.2, J_{AX} 10.5 Hz) and two geminal protons adjacent to carbonyl (AB system, δ_A 2.54, δ_B 3.04, J_{AB} 17.3 Hz).

In addition, a proton geminal to an acetoxy group appeared at 5.24 ppm as a doublet (J 4.0 Hz), coupled to a methine proton resonating as a *ddd* at 4.37 ppm. The latter collapses into a double doublet (J_1 4.0, J_2 2.0 Hz) on D_2O exchange. The splitting patterns of the two last mentioned signals are identical to the ones due to the 7-axial and 6-equatorial protons of forskolin (**1**). All the proton data not only point to a close structural relationship between **7** and **1**, but also indicate that **7** differs from **1** for the lack of the hydroxy function at C-1.

Compound **8**, $C_{20}H_{32}O_5$ (M^+ at m/z 352.2238, calcd 352.2250) is clearly the deacetyl derivative of **7** as shown from its spectroscopic data and the chemical correlation with **7**. The structures **7** and **8** are strengthened by ^{13}C NMR and MS analysis. Table 1 shows the ^{13}C NMR chemical shifts for all the new and known isolated compounds. Signal assignments of the basic skeleton



1 R^1 = R^2 = R^4 = OH; R^3 = OAc
2 R^1 = R^2 = R^3 = R^4 = OH
3 R^1 = R^2 = OH; R^3 = OAc; R^4 = H
4 R^1 = R^4 = H; R^2 = OH; R^3 = OAc
5 R^1 = R^2 = H; R^2 = R^3 = OH
6 R^1 = R^2 = R^3 = H; R^4 = OH
7 R^1 = H; R^2 = R^4 = OH; R^3 = OAc
8 R^1 = H; R^2 = R^3 = R^4 = OH
9 R^1 = R^3 = R^4 = H; R^2 = OH
10 R^1 = R^2 = R^3 = R^4 = H
11 R^1 = R^2 = R^3 = R^4 = H; 11-deoxo

Table 1. ^{13}C NMR chemical shifts of *C. forskohlii* diterpenoids*

C	1	2	3	4	5	6	7	8	9	10	11
1	73.8	74.6	71.2	41.3	41.3	32.2	33.7	33.8	41.5	41.9	39.0
2	26.4	26.8	25.6	18.4	18.4	18.1[a]	18.3	18.4	18.4	18.4	18.6
3	36.1	36.2	36.3	43.6	43.7	41.7	43.5	43.6	44.0	43.3	42.1
4	34.3	34.3	34.1	34.3	34.1	33.1	34.4	34.3	34.1	33.2	33.2
5	42.7	43.0	47.4	55.2	55.2	46.1	47.5	47.6	56.6	55.8	56.4
6	69.5	70.6	70.2	69.4	70.2	18.9[a]	69.9	70.6	67.7	19.7	19.9
7	76.9	74.6	81.1	81.2	80.7	36.0	76.2	74.1	50.6	39.4	43.2
8	81.3	82.3	78.5	78.2	79.9	80.0[b]	81.7[a]	81.0	76.5	77.2	74.8
9	82.5	82.3	58.2	65.5	65.4	80.1[b]	81.1[a]	83.0	66.5	66.7	55.7
10	42.8	42.8	41.7	37.7	37.8	40.1	40.8	40.9	37.5	37.1	36.9
11	206.1	205.9	207.6	205.7	205.7	204.1	201.7	201.9	206.9	207.1	15.4
12	49.0	48.9	49.9	50.0	49.8	48.1	47.8	47.7	50.1	50.2	35.8
13	75.0	75.2	74.8	74.8	75.1	75.0	74.5	74.9	74.2	74.4	73.0
14	164.4	146.5	145.8	146.3	146.4	146.1	146.0	146.1	146.6	146.0	147.8
15	110.0	110.3	112.7	112.5	112.1	110.5	111.1	110.9	111.9	111.9	110.1
COMe	169.7	—	170.0	169.7	—	—	167.5	—	—	—	—
COMe	21.1	—	21.2	21.1	—	—	21.0	—	—	—	—
Me	32.9	33.0	32.8	33.1	33.2	33.5	33.2	33.3	33.2	33.5	33.4
	31.7	30.7	31.5	31.6	31.4	31.2	31.8	31.5	31.4	31.2	28.5
	24.2	24.2	24.5	24.0	23.9	25.9	24.2	24.3	23.9	27.9	25.5
	23.7	23.4	23.6	23.8	23.7	21.6	22.5	22.2	23.7	21.6	21.3
	19.7	20.0	18.2	16.9	16.9	16.6	18.2	18.2	16.8	15.5	15.3

* 25.2 MHz ($CDCl_3$); (TMS) = 0.
[a, b] Assignments may be reversed.

8,13-epoxy-labd-14-ene (manoyl oxide, **11**) [11] are reported for comparison purposes.

Inspection of the data of **7** in comparison with forskolin **1** clearly suggests that one oxymethine carbon of the latter has been substituted by a methylene grouping. In addition, whereas shifts and multiplicities of C-6 to C-9 and C-12 to C-20 are almost superimposable to the corresponding signals of **1**, those of C-2 and C-10 appear upfield shifted, and those of C-3 and C-5 resonate downfield, confirming that **7** possesses the same skeleton as forskolin, but lacks the axial hydroxy function at C-1. The carbon shifts of **8** are almost identical to those of **7**, which differs only for the downfield shift of C-7.

Comparison with the isomeric **3** nicely illustrates the effect of an axial OH in the C-1 or C-9 positions of the forskolin skeleton. In **3**, carbons 3 and 8 are shifted upfield with respect to **7**, whereas C-7 is downfield shifted (as in **4** and **5**) due to the elimination of the γ shielding effect. The carbonyl carbon suffers an unexpected upfield shift to the 202 ppm region, being at 206–207 ppm in the other congeners. This shift cannot be simply attributed to the absence of the C-1 hydroxy function, but probably depends on subtle conformational changes of the A- and C-ring. High-resolution MS shows the loss of C_5H_8 (**a**) and C_5H_9O (**b**) from the oxycarbonyl ring. For **7**, the fragments are at *m/z* 326.1733 ($C_{17}H_{26}O_6$, calc. 326.1729) and 309.1710 ($C_{17}H_{25}O_5$, calc. 309.1702); for (**8**), they are at *m/z* 284.1629 ($C_{15}H_{24}O_5$, calc. 284.1624) and 267.1601 ($C_{15}H_{23}O_4$, calc. 267.1596). Additional loss of Me and CO from ion **a** and CO from ion **b** gives rise to the peaks at *m/z* 319.306 and 289 for **7** and at 277, 264 and 247 for **8**. From these data, compound **7** is therefore 1-deoxyforskolin (7β-acetoxy-6β,9α-dihydroxy-8,13-epoxy-labd-14-en-11-one) and **8** is deacetyl-1-deoxyforskolin (6β,7β,9α-trihydroxy-8,13-epoxy-labd-14-en-11-one).

The next isolated compound, **9**, analyses for $C_{20}H_{32}O_3$ (M^+ at *m/z* 320.2347, calcd 320.2351; $[M-C_5H_8]$ at *m/z* 252.1735, calcd 252.1725, $[M-C_5H_9O]$ at *m/z* 235.1704, calcd 235.1698), in agreement with the presence of only one hydroxy function on the 8,13-epoxy-11-oxo-labd-14-ene skeleton. It is isomeric with the previously isolated **6**, but, at variance, the ^{13}C NMR spectrum suggests that the carbon bearing the hydroxy function is secondary and not tertiary (doublet at 67.7 ppm instead of singlet at 80.1 ppm). Further analysis of the carbon spectrum reveals a strong similarity of C-1 to C-4 and C-9 to C-15 shifts with the ones of compounds **4** and **5**, which do not possess oxygenated functions at C-1 and C-9. In particular, the lack of hydroxyl at C-9 shifts C-1 downfield, according to the removal of the γ effect. Carbon 6 is the most probable candidate for placing the secondary alcoholic function, although carbon 7 cannot be excluded.

The downfield region of the proton spectrum of **9** shows, beside the signals of the vinyl protons, the signal of the oxymethine proton at 4.51 ppm as multiplet collapsed to a *ddd* system (J_1 2.7, J_2 3.5, J_3 3.0 Hz) by D_2O exchange.

The coupling constant values suggest that the proton is equatorially oriented and placed between a methine and a methylene group. In addition, the ^1H NMR shows a one-proton doublet (J 2.7 Hz) at 0.86 ppm and two one-proton double doublets at 1.93 and 2.18 ppm (J_1 16 Hz for both and J_2 3.5 and 3.0 Hz, respectively). Irradiation at 4.51 ppm transforms the doublet at 0.86 ppm into a singlet and the double doublets at 1.93 and 2.18 ppm into doublets, maintaining the larger geminal coupling constant. These results establish that the methine and methylene carbons adjacent to the secondary axial alcoholic function are both linked to fully substituted carbon atoms, thus confirming the 6β orientation for the OH

group. In spite of the lack of functional groups, other signals are easily attributable in the proton spectrum of **9**. Two singlets at 2.61 (1 H) and 2.67 (2 H) ppm are due to the C-9 and C-12 protons, respectively. A double doublet of triplets at 2.44 ppm (J_1 13.5, J_1 3.5, J_3 1.5 Hz) is due to the C-1 β proton, deshielded by the coplanar 11-oxo group. Irradiation of this signal causes the triple doublet of C-1 proton at 0.79 ppm to become a double doublet (J_1 13.5, J_2 2.5 Hz) and the quartet of triplets of C-2 β proton at 1.75 ppm to simplify into a quartet of doublets (J_1 13.5, J_2 3.5 Hz). A strong modification is also produced in the 1.25–1.35 ppm region, where C-2 proton resonates overlapped by other signals.

Finally, a triple doublet at 1.15 ppm, partially covered by a Me group signal, with J_1 13.5 and J_2 3.5 Hz, is attributable to the C-3α proton, and the remaining C-3β proton resonates between 1.25 and 1.35 ppm displaying a *W*-pathway coupling with C-1β proton.

From all these data, compound **9** was therefore assigned the structure of 6β-hydroxy-8,13-epoxy-labd-14-en-11-one. The last new isolated compound **10**, $C_{20}H_{32}O_2$ (M^+ at *m/z* 304.2395, calcd 304.2402, $[M-C_5H_8]^+$ at *m/z* 236.1783, calc. 236.1776, $[M-C_5H_9O]$ at *m/z* 219.1761, calcd 219.1749), a part from the carbonyl and the ether bridge placed in the C-ring, does not contain oxygenated functions and therefore it is obvious to suppose for **10** the structure of 8,13-epoxy-labd-14-en-11-one. In fact, below 60 ppm, its ^{13}C NMR spectrum exhibits only signals for the C-11 oxo group, the vinyl carbons and the oxygen carbons C-8 and C-13.

The proton spectrum shows an AB system (*J* 17.5 Hz) at 2.59 and 2.54 ppm for the C-12 protons, a singlet at 2.62 ppm (C-9 proton) and a double triplet of doublets (J_1 13.5, J_2 1.5, J_3 3.5 Hz) reminiscent of the splitting pattern of the C-1β proton of compound **9**.

The upfield region of the ^{13}C NMR spectrum of **10** exhibits a close similarity to that of manoyl oxide **11**, but the introduction of the C-11 carbonyl function affects C-12 and C-9, which move upfield, and the C-8 and C-13 resonances, which are moderately upfield shifted.

The structures of all known and new isolated compounds indicate that the plant enzyme oxidation system leads only to a non-selective hydroxylation of the 1,6,7 and 9 positions of the tricyclic skeleton, and that the oxidation proceeds under a severe stereochemical control, only 1α, 6β, 7β and 9α hydroxylated compounds being produced.

EXPERIMENTAL

Plant material. The roots were collected in the Southern area of Tamil Nadu (India) and authenticated by Dr U. Boni (Inverni della Beffa, Milan, Italy). A voucher specimen is available at the Dept. of Pharmacognosy, Inverni della Beffa, Milan, Italy.

Mps: uncorr. IR: KBr. ^{1}H and ^{13}C NMR: δ, TMS. EIMS: VG 7070 EQ-HF, 70 eV: Optical rotations: $CHCl_3$; TLC: silica gel, CH_2Cl_2–EtOAc 9:1.

Extraction and isolation of the diterpenoids. Ground roots (10 kg) were extracted at room temp. with EtOAc, the extract was concd and the residue taken up with MeOH and filtered. The mother liquors were evapd and CC on silica gel, eluting with cyclohexane–EtOAc 4:1. Three fractions (A, B and C) were collected. Fraction A (40 g) was CC (silica gel, cyclohexane and gradient of CH_2Cl_2) and the following compounds were sequentially eluted:

9α-*Hydroxy*-8,13-*epoxy-labd*-14-*en*-11-*one* (**6**). [5] (180 mg), R_f 0.92, oil, $[\alpha]_D^{20}$ +4.6° (*c* 0.3).

7β-*Acetoxy*-6β,9α-*dihydroxy*-8,13-*epoxy-labd*-14-*en*-11-*one* (**7**). (1.3 g), R_f 0.82, mp 135–136° (*n*-hexane), $[\alpha]_D^{20}$ −23.1° (*c* 0.10); IR: 3450, 1700, 1645, 1400 cm^{-1}; ^{1}H NMR: 5.83 (1H, *dd*, H-14), 5.41 (1H, *dd*, H-15), 5.24 (1H, *d*, H-7), 5.04 (1H, *dd*, H-15), 4.37 (1H, *dd*, H-6), 3.60 (1H, 9-OH), 3.04 (1H, *d*, H-12β), 2.54 (1H, *d*, H-12), 2.16 (3H, Ac), 1.74, 1.48, 1.35, 1.22, 0.96 (*s*, 5 xMe); MS: 394 (M^+, 7%), 376 (100), 348 (22), 334 (5), 326 (5), 319 (7), 309 (15), 301 (16), 289 (14), 281 (26), 238 (90). Compound **7** was converted into **8** by treatment with 2% KOH–EtOH at room temp. for 12 hr.

6β,7β,9α-*Trihydroxy*-8,13-*epoxy-labd*-14-*en*-11-*one* (**8**). (260 mg), R_f 0.46, mp 148–149° (*i*-PrOH), $[\alpha]_D^{20}$ −25.9° (*c* 0.10); IR: 3480, 3280, 1705, 1635, 1440 cm^{-1}; ^{1}H NMR: 5.92 (1H, *dd*, H-14), 5.33 (1H, *dd*, H-15), 5.07 (1H, *dd*, H-15), 4.42 (1H, *ddd*, H-6), 3.85 (1H, *d*, H-7), 3.43 (1H, 9-OH), 3.04 (1H, *d*, H-12β), 2.55 (1H, *d*, H-12), 2.08 (1H, *d*, H-5), 1.70, 1.46, 1.42, 1.23, 0.99 (1, 5 xMe); MS: 352 (M^+, 10%), 334 (100), 306 (52), 284 (12), 277 (11), 267 (38), 256 (37), 247 (31), 224 (72), 221 (83).

7β-*Acetoxy*-6β-*hydroxy*-8,13-*epoxy-labd*-14-*en*-11-*one* (**4**). [2, 6] (900 mg), R_f 0.65, mp 149–150° (*n*-hexane), $[\alpha]_D^{20}$ −105.4° (*c* 0.10).

6β,7β-*Dihydroxy*-8,13-*epoxy-labd*-14-*en*-11-*one* (**5**) [2] (240 mg), R_f 0.64, mp 150–151° (*n*-hexane), $[\alpha]_D^{20}$ −127.2° (*c* 0.10).

Column chromatography (silica gel, CH_2Cl_2) of fraction B (12 g) yielded the following compounds:

6β-*Hydroxy*-8,13-*epoxy-labd*-14-*en*-11-*one* (**9**). (200 mg), R_f 0.56, mp 120–121° (cyclohexane–EtOAc), $[\alpha]_D^{20}$ −133.0° (*c* 0.10); IR: 3520, 1690, 1635, 1450 cm^{-1}; ^{1}H NMR: 5.94 (1H, *dd*, H-14), 5.19 (1H, *dd*, H-15), 5.03 (1H, *dd*, H-15), 4.51 (1H, m, H-6), 2.67 (1H, *s*, H-9), 2.61 (2H, *s*, H-12), 2.18 (1H, *d*, H-7), 1.57, 1.41, 1.28, 1.19, 0.97 (*s*, 5 xMe), other signals: see Discussion; MS: 320 (M^+, 18%), 304 (22), 301 (15), 286 (7), 252 (81), 235 (100).

8,13-*Epoxy-labd*-14-*en*-11-*one* (**10**) (260 mg), R_f 0.89, mp 96–97° (*i*-PrOH), $[\alpha]_D^{20}$ −103.2° (*c* 0.20); IR: 1705, 1635, 1445 cm^{-1}; ^{1}H NMR: 5.95 (1H, *dd*, H-14), 5.26 (1H, *dd*, H-15), 5.06 (1H, *dd*, H-15), 2.62 (1H, *s*, H-9), 2.59 and 2.54, AB system, H-12), 1.30, 1.27, 1.01, 0.86, 0.79 (s, 5 xMe), other signals: see Discussion; Ms: 304 (M^+, 28%), 236 (100), 221 (33), 219 (31).

Crystallization of Fraction C (30 g) from methyl-*t*-butylether yielded 10 g of forskolin (**1**) identical to an authentic sample purchased from Sigma. Column chromatography (silica gel, CH_2Cl_2–EtOAc 9:1) of the mother liquors provided the following compounds:

7β-*Acetoxy*-1α,6β-*dihydroxy*-8,13-*epoxy-labd*-14-*en*-11-*one* (**3**) [4,7] (560 mg), R_f 0.23, mp 188–189° (*n*-hexane–methyl-*t*-butylether), $[\alpha]_D^{20}$ −97.9° (*c* 0.50).

Deacetylforskolin (1α,6β,7β,9α-*tetrahydroxy*-8,13-*epoxy-labd*-14-*en*-11-*one*) (**2**). [2] (510 mg), R_f 0.12, mp 164–165° (*i*-PrOH), $[\alpha]_D^{20}$ −25.7° (*c* 0.20).

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