Tetrahedron Vol. 45, No. 3, pp. 763 to 766, 1989
Printed in Great Britain.

0040-4020/89 $3.00+.00

ISOLATION, STRUCTURE ELUCIDATION AND SYNTHESIS OF 1-DEOXYFORSKOLIN

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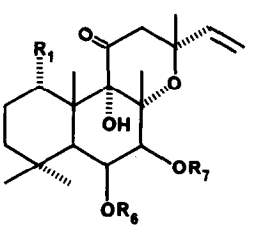
(*Received in UK 7 November* 1988)

Abstract - Isolation and structure elucidation of 1-deoxyforskolin (2), a minor forskolin related constituent, of the Indian herb Coleus forskohlii are described. The structure of 2 is confirmed by the 9-hydroxylation of 1,9-dideoxyforskolin (4).

Forskolin (1), a major labdane diterpenoid isolated from the Indian herb Coleus forskohlii, displayed strong positive inotropic, antihypertensive, intraocular pressure lowering, bronchospasmolitic and adenylate cyclase stimulant activity[1]. Our continued interest in forskolin and its analogs prompted us to isolate the minor forskolin related constituents of the herb Coleus forskohlii. In this paper, we describe the isolation, structure elucidation and synthesis of 1-deoxyforskolin (2).

Careful purification of the petroleum ether extract of the roots of the plant, including preparative high performance liquid chromatography, gave a new product (2, yield 0.001%, mp 174-176°C, M^+ m/z 394). The only difference observed in the PMR spectra of 2 in comparison with that of forskolin was the absence of the resonance at δ 4.45 ppm for 1α-CHOH, which led to the assignment of 2 as 1-deoxyforskolin (2). Alkaline hydrolysis of 2 at 0°C gave the corresponding 7-deacetyl-1-deoxyforskolin (3). The structure of 1-deoxyforskolin (2) was further confirmed by its synthesis starting from 1,9-dideoxyforskolin (4).



1, R_1=OH, R_6=H, R_7=Ac

2, R_1=R_6=H, R_7=Ac

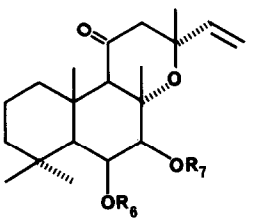
3, R_1=R_6=R_7=H

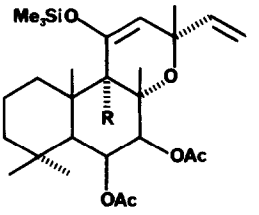
11, R_1=H, R_6=R_7=Ac

Dedicated to Dr. E. Baltin, Managing Director, Hoechst India Limited, on the occasion of his completion of twenty-five years service with Hoechst AG., West Germany.

A number of methods are recorded which describe the introduction of an hydroxy group alpha to carbonyl group preferentially at a tertiary carbon atom. With the aim of using ideally a one-step procedure to effect the conversion of 4 to 2, our first attempts used procedures involving direct oxidation of ostensibly *in situ* formed enolates. Reagents such as thallium nitrate[2], phenylseleninic anhydride, KO-t-Bu/O_2/$(EtO)_3P$[4], MoO_5.pyridine.HMPA[5], lead tetra-acetate[6], 2-(phenylsulphonyl)-3-phenyloxy-aziridine[7] and iodosylbenzoic acid[8] were all tried first with 4 and then with 5, but failed to give the desired 9-hydroxylated derivative.

The attempts at oxidation of presumed *in situ* formed enolates having failed, our next efforts were directed towards isolation of the enolate and subsequent oxidation. Treatment of the unprotected 6/7-hydroxyforskolin analog 4 or 6-acetyl-7-deacetyl-1,9-dideoxyforskolin[10] with trimethylsilylchloride/lithium hexamethyldisilazane[9] in THF at -78°C gave 6-acetyl-7-deacetyl-7-trimethylsilyl-1,9-dideoxyforskolin[11] (7) and not the enol ether.





4, $R_6 = H$, $R_7 = Ac$

5, $R_6 = R_7 = H$

6, $R_6 = Ac$, $R_7 = H$

7, $R_6 = Ac$, $R_7 = SiMe_3$

8, $R_6 = R_7 = Ac$

9, R = H

10, R = OH

Treatment, however, of the protected 6/7-hydroxy analog, 6-acetyl-1,9-dideoxyforskolin (8), at -78°C in THF with trimethylsilylchloride/lithium hexamethyldisilazane gave the 11-enol ether 9 in 50% yield. The formation of only the 11-enol ether is in agreement with the result obtained by J. Scherkenbeck *et al.*[11] The desired 9-hydroxylation was eventually achieved by allylic oxidation of the 11-enol ether 9.

Treatment of 9 with SeO_2 in pyridine at 80°C for 6 hrs provided the 9-hydroxylated product 10, in the PMR spectrum of which the signal for 9-CH is absent and 12-CH appears as a singlet at δ 4.64. Treatment of 10 with tetrabutylammonium fluoride/THF[13] gave 6-acetyl-1-deoxyforskolin (11). Alkaline hydrolysis of 11 followed by acetylation with acetic anhydride/pyridine gave 1-deoxyforskolin (2).

The successful introduction of a 9α-OH group in a 1,9-dideoxyforskolin derivative helped us in confirming the structure of 1-deoxyforskolin. It also provided an additional substrate which was converted to forskolin through microbial hydroxylation[12].

EXPERIMENTAL

Melting points were determined with a Kofler hot stage apparatus and are uncorrected. IR spectra were determined with a Perkin-Elmer 157 spectrophotometer using KBr disks. PMR spectra were measured using solution in $CDCl_3$ with a Jeol FT-90 spectrometer unless mentioned otherwise (Me_4Si as internal standard). Precoated (silica gel $60F_{254}$) TLC plates were used to examine the purity of the compounds. Visualization was done by spraying with anisaldehyde/H_2SO_4 reagent and heating the plate at 110°C. All compounds were homogeneous by TLC analysis. HPLC separations were carried out by using a Waters Associates Preparative Liquid chromatograph, model prep LC 500 equipped with a refractive index detector and two prep pak-500/silica (35-40) cartridges in series.

Isolation of 1-Deoxyforskolin (2)

The dried and finely divided plant material (5 kg) was extracted with benzene (3 x 22 L) at 40-50°C for 5 h and the combined extracts were filtered and concentrated in vacuo. The residue (256 g) was triturated with petroleum ether (bp 60-80°, 2 x 2 L) and the petroleum ether layer was decanted. The combined petroleum ether layers were concentrated to give a residue (55.6 g) which on usual purification on a column of silica gel[15] gave crude 1,9-dideoxyforskolin (4, 1.6 g). The crude 4 was found to have an impurity with an Rf value on silica gel TLC plates very close to that of 4. The material was further purified by HPLC using hexane:ethyl acetate:dichloromethane (7:1:2) as eluent to provide 2, which was crystallised from ethyl acetate:petroleum ether, yield : 0.05 g (0.001% of dry weight of roots) mp 174-176°C $[\alpha]_D^{25}$ - 19.2° ($CHCl_3$).

PMR ($CDCl_3$) : δ 0.96, 1.22, 1.36, 1.5, 1.52 (s, 5xCH_3), 2.16 (s, $COCH_3$), 2.5 (d, J_{gem}= 18Hz 12-CH), 3.06 (d, J_{gem} = 18Hz, 12-CH), 4.34 (m, 6α-CH), 5.02 (dd, J_{cis} = 10.8Hz, J_{gem} = 2Hz, vinylic-H), 5.22 (d, $J_{6,7}$ = 4.5Hz, 7α-CH), 5.34 (dd, J_{trans} = 18Hz, J_{gem} = 2Hz, vinylic-H).

IR (KBr) : cm^{-1} 3500, 2940, 1700, 1360, 1280.

Anal. Calcd. for $C_{22}H_{34}O_6$: C, 66.98; H, 8.69. Found C, 67.2; H, 8.5%

7-Deacetyl-1-deoxyforskolin (3)

A solution of sodium hydroxide (60 mg, 1.5 mmol in water 2 ml) was added to a stirred and ice cooled solution of 1-deoxyforskolin (0.2 g, 0.51 mmol) in methanol (4 ml). The reaction mixture was stirred for 30 mins at 0°C and diluted with water. The solution was concentrated in vacuo at a temperature < 40°C to remove methanol and extracted with dichloromethane. The organic layer was separated, washed with water, dried over anhydrous sodium sulphate and concentrated. The residue was crystallised from ethyl acetate:petroleum ether. Yield : 161 mg; mp 146-148°C.

Similarly, 3 was prepared starting from 11.

Anal. Calcd. for $C_{20}H_{32}O_5$: C, 68.18; H, 9.15. Found, C, 68.56; H, 9.22%.

6-Acetyl-7-deacetyl-7-trimethylsilyl-1,9-dideoxyforskolin[11] (7)

n-Butyl lithium (2.13 ml, 1.6 M sol in hexane, 3.41 mmol) in anhydrous THF (8 ml) was added to a stirred solution of hexamethyldisilazane (0.79 ml, 3.74 mmol) in THF (freshly dist. over LAH, 30 ml) under N_2 atmosphere. The reaction mixture was refluxed for half an hour. It was cooled to -78°C and 1,9-dideoxyforskolin (1.16 g, 3.07 mmol) in dry THF (6 ml) was added. The reaction mixture was brought to room temperature and stirring was continued for 40 mins. Chlorotrimethylsilane (0.41 ml, 3.23 mmol) in dry THF (3 ml) was added to the stirred reaction mixture at -78°C followed by stirring at room temperature for 2 h. Excess of base was decomposed by addition of acetic acid (0.2 ml) and the reaction mixture was concentrated in vacuo, and extracted with ethyl acetate. The organic layer was washed with water and brine, dried over anhydrous sodium sulphate and concentrated. The residue, purified by flash chromatography using ethyl acetate:hexane (3:7) as eluant, gave 7.

PMR ($CDCl_3$) : δ 0.15 (s, 3x$SiCH_3$), 0.92, 0.99, 1.27, 1.36, 1.38 (s, 5xCH_3), 2.02 (s, $COCH_3$), 2.62 (s, 9-CH, 12-CH_2), 3.75 (d, $J_{6,7}$ = 4Hz, 7α-CH), 5.03 (dd, J_{cis} = 10.5Hz, J_{gem}= 0.9Hz vinylic-H), 5.13 (dd, J_{trans} = 17.5Hz, J_{gem} = 0.9Hz, vinylic-H), 5.55 (dd, $J_{6,7}$ = 4Hz, $J_{5,6}$ = 3.5Hz, 6α-CH), 5.97 (dd, J_{cis} = 10.5Hz, J_{trans} = 17.5Hz, vinylic-H).

Similarly was prepared : 6-Acetyl-1,9-dideoxy-11-enol-trimethylsilyl ether[14] (9) starting from 6-Acetyl-1,9-dideoxyforskolin (8).

PMR ($CDCl_3$) : δ 0.27, 0.94, 1.26, 1.36, 1.49 (s, 8xCH_3), 2.02, 2.04 (s, 2x$COCH_3$), 2.05 (d, $^4J_{9,12}$ = 2.2Hz, 9-CH), 4.63 (d, $^4J_{12,9}$ = 2.2Hz, 12-CH), 4.8 (dd, J_{cis} = 10Hz, J_{gem} = 2Hz, vinylic-H), 4.92 (d, $J_{6,7}$ = 4Hz, 7α-CH), 5.08 (dd, J_{trans} = 17Hz, J_{gem} = 2Hz, vinylic-H), 5.7 (m, 6α-CH), 5.73 (dd, J_{trans} = 17Hz, J_{cis} = 10Hz, vinylic-H).

6-Acetyl-1,9-dideoxyforskolin (8)

A mixture of 1,9-dideoxyforskolin (4, 2.0 g, 5.29 mmol), PTSA (0.1 g, 0.58 mmol) and acetic anhydride (15 ml) was stirred for 10 h at 25-28°C. The reaction mixture was poured into ice water and extracted with dichloromethane. The organic layer was washed with water, dried over anhydrous sodium sulphate and concentrated. The residue on purification by flash chromatography using ethyl acetate:hexane (3:17) as eluant gave 8, which was crystallised from ethyl acetate: petroleum ether, yield : 1.6 g, mp 125-127°C.

Anal. Calcd. for $C_{24}H_{36}O_6$: C, 68.55; H, 8.63. Found C, 68.89; H, 8.39%

6-Acetyl-1-deoxyforskolin-11-enol-trimethylsilyl ether[14] (10)

Selenium dioxide (0.021 g, 0.21 mmol) was added to enol ether (9, 0.083 g, 0.1 mmol) in dry pyridine (2 ml). The reaction mixture was heated for 6 h at 80°C and concentrated. The residue was extracted with ethyl acetate. The organic layer was washed with water, dried over anhydrous sodium sulphate and concentrated. The residue on purification by flash chromatography using ethyl acetate:petroleum ether (12:88) as eluant gave 10, yield : 25%.

PMR ($CDCl_3$) : δ 0.28, 0.96, 1.24, 1.28, 1.36, 1.7 (s, 8xCH_3), 2.0, 2.08 (s, 2x$COCH_3$), 4.64 (s, 12-CH), 4.92 (dd, J_{cis} = 10Hz, J_{gem} = 2Hz, vinylic-H), 5.26 (dd, J_{trans} = 18Hz, J_{gem} = 2Hz, vinylic-H), 5.28 (d, $J_{6,7}$ = 4Hz, 7α-CH), 5.68 (m, 6α-CH), 5.84 (dd, J_{trans} = 18Hz, J_{cis} = 10Hz, vinylic-H).

6-Acetyl-1-deoxyforskolin[14] (11)

Tetrabutylammonium fluoride (9.8 mg, 0.03 mmol) was added to a stirred solution of 10 (14.0 mg, 0.03 mmol) in dry THF (2 ml) at room temperature. Stirring was continued for half an hour and the reaction mixture was concentrated. The residue was extracted with ethyl acetate. The organic layer was dried over sodium sulphate and concentrated. The residue was purified by flash chromatography using ethyl acetate:petroleum ether (15:85) as eluant. Yield : 90%, mp 136°C.

PMR (270 MHz, $CDCl_3$) : δ 0.9, 1.3, 1.43, 1.62 (s, 5xCH_3), 1.96, 2.02 (s, 2x$COCH_3$), 2.44 (d, J_{gem} = 18Hz, 12-CH), 3.01 (d, J_{gem} = 18Hz, 12-CH), 4.97 (dd, J_{cis} = 10Hz, J_{gem} = 1Hz, vinylic-H), 5.32 (dd, J_{trans} = 18Hz, J_{gem} = 1Hz, vinylic-H), 5.21 (d, $J_{6,7}$ = 4Hz, 7α-CH), 5.69 (m, 6α-CH), 5.88 (dd, J_{trans} = 18Hz, J_{cis} = 10Hz, vinylic-H).

Synthesis of 1-Deoxyforskolin (2)

Acetic anhydride (1.0 ml) was added to an ice-cooled stirred solution of 7-deacetyl-1-deoxyforskolin (50 mg) and pyridine (10 ml). The reaction mixture was stirred for another 3 hours at 0-5°C and poured on crushed ice, extracted with dichloromethane. The organic layer was washed

with 10% HCl followed by water, dried over anhydrous sodium sulphate and concentrated. The residue was crystallised from ethyl acetate:petroleum ether (1:1) yield : 75%, mp 174-176°C, mix. mp 175°C $[\alpha]_D^{25}$ - 18° ($CHCl_3$).

PMR ($CDCl_3$) : δ 0.96, 1.22, 1.36, 1.5, 1.52 (s, 5xCH_3), 2.16 (s, $COCH_3$), 2.5 (d, J_{gem}=18 Hz, 12-CH), 3.06 (d, J_{gem} = 18 Hz, 12-CH), 4.34 (m, 6α-CH), 5.02 (dd, J_{cis} = 10.8 Hz, J_{gem} = 2 Hz, vinylic-H), 5.22 (d, $J_{6,7}$ = 4.5 Hz, 7α-CH), 5.34 (dd, J_{trans} = 18 Hz, J_{gem} = 2 Hz, vinylic-H), 5.84 (dd, J_{cis} =10.8 Hz, J_{trans} = 18 Hz, vinylic-H).

IR (KBr) cm^{-1} 3500, 2940, 1700, 1360, 1280.

Anal. Calcd. for $C_{22}H_{34}O_6$: C, 66.98; H, 8.69. Found C, 67.34; H, 8.58%.

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