*Tetrahedron* Vol. 44, No. 6, pp. 1661 to 1666, 1988
Printed in Great Britain.

0040-4020/88 $3.00+.00
Pergamon Press plc

# NOVEL 1,9-DIDEOXYFORSKOLIN ANALOGUES THROUGH MICROBIAL TRANSFORMATIONS

Y. Khandelwal*, P. K. Inamdar, N. J. de Souza, R. H. Rupp,

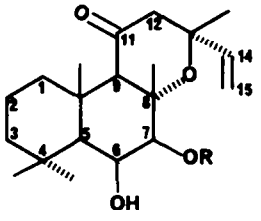
S. Chatterjee and B. N. Ganguli

Centre for Basic Research, Hoechst India Limited, Bombay 400 080, India.

(*Received in UK* 22 *December* 1987)

Abstract : - Microbial transformations of 1,9-dideoxyforskolin (1) and 7-deacetyl-1,9-dideoxyforskolin (2) were carried out. Various mono- and di-hydroxylated derivatives were isolated and identified.

We have recently described the microbial transformation of 7-deacetyl-1,9-dideoxyforskolin (2) to 7-deacetylforskolin[1] (17) using a fungal strain which was found through screening of hydroxylating fungi. During this screening programme, different fungal strains were found which affected regio- and stereo-selective transformations of the two substrates used in this study, namely 1,9-dideoxyforskolin (1) and 7-deacetyl-1,9-dideoxyforskolin (2), at positions other than at the desired 1- and 9-positions. In this paper we describe these new analogues of 1,9-dideoxyforskolin, the identification of which was facilitated by the analytical techniques that were devised to monitor the transformed products in the fermentation broths.




**1**: R= $COCH_3$
**2**: R= H

The fungal strains used in the present investigation were isolated from local soil samples by standard methodology. Fermentation experiments were carried out by the procedures reported earlier from our group[1].

The progress of microbial transformation was monitored by TLC in order to achieve optimum yields. When it was observed on TLC that no further change was appearing to take place, the culture filtrate was separated from the mycelial mass, and extracted with chloroform. The residue, after evaporation of the chloroform extract, was checked for transformed products by TLC, HPLC and GLC assay techniques[3] and purified by using flash column chromatography. The structures of the terpenoids isolated were elucidated principally by NMR and mass spectroscopy, or by comparison with authentic samples.

The fungal strains and the transformed products obtained are listed in Table 1 and 2.

Table 1

Transformed products of 1,9-dideoxyforskolin

| Strain No. | Identification of Strain | Transformed Product | Yield % |
|---|---|---|---|
| FF 915 | Unidentified | 7-Deacetyl-9-deoxyforskolin (3) | 0.5 |
| FF 101 | Syncephalostrum DSM No. 3207 | 1,9-Dideoxy-2α-hydroxy-forskolin (4) | 10.0 |
| FF 172 | DSM No. 3208 | 7-Deacetyl-1,9-dideoxy-2α-hydroxyforskolin (5) | 7.4 |
| FF 709 | Unidentified | (5) | 10.0 |
| H 134 | Scopuloriopsis | 1,9-Dideoxy-2β-hydroxy-forskolin (6) | 4.0 |
| FF 101 | Unidentified | (6) | 8.0 |
| FF 559 | Unidentified | (6) | 23.8 |
| H 134 | Scopuloriopsis | 6-Acetyl-7-deacetyl-1,9-dideoxy-2β-hydroxyforskolin (7) | 8.3 |
| FF 915 | Unidentified | 6-Acetyl-7-deacetyl-1,9-dideoxy-3α-hydroxyforskolin (8) | 5.0 |
| $H_1$ | DSM No. 3202 | 1,9-Dideoxy-3β-hydroxyforskolin (9) | 29.7 |
| FF 208 | Unidentified | (9) | 42.0 |
| FF 173 | DSM No. 3209 | (9) | 10.0 |
| FF 383 | Aspergillus niger (DSM No. 3210) | (9) | 40.0 |
| FF 406 | DSM No. 3211 | (9) | 16.2 |
| FF 695 | DSM No. 3212 | (9) | 10.0 |
| FF 573 | Unidentified | (9) | 11.0 |
| FF 695 | DSM No. 3212 | 6-Acetyl-7-deacetyl-1,9-dideoxy-3β-hydroxyforskolin (10) | 10.0 |
| FF 695 | Unidentified | 1,9-Dideoxy-3-oxoforskolin (11) | 4.9 |
| FF 266 | Unidentified | 6-Acetyl-7-deacetyl-1,9-dideoxyforskolin (12) | 11.0 |
| FF 565 | Unidentified | (12) | 16.0 |

Table 2

Transformed products of 7-deacetyl-1,9-dideoxyforskolin

| Strain No. | Identification of Strain | Transformed Product | Yield % |
|---|---|---|---|
| FF 680 | Unidentified | 7-Deacetyl-1,9-dideoxy-2α-hydroxyforskolin (5) | 23.7 |
| H 134 | Scopuloriopsis | 7-Deacetyl-1,9-dideoxy-2 β-hydroxyforskolin (13) | 2.0 |
| FF 648 | Unidentified | 7-Deacetyl-1,9-dideoxy 3α-hydroxyforskolin (14) | 11.4 |
| FF 383 | Aspergillus niger DSM No. 3210 | 7-Deacetyl-1,9-dideoxy-3 β-hydroxyforskolin (15) | 22.4 |
| FF 813 | Unidentified | 7-Deacetyl-14,15-dihydro-14,15-dihydroxy-1,9-dideoxyforskolin (16) | 10.0 |
| FF 958 | Unidentified | (16) | 2.8 |
| H 134 | Scopuloriopsis | 7-Deacetylforskolin (17) | 0.76 |

## Identification of Compounds

1,9-Dideoxy-2α-hydroxyforskolin (4) : m.p. 258-61°; MS : $M^+$, m/z 394; PMR : signals as for 1 with an additional multiplet, with nine line pattern $J_{a,a}$ = 11.5 Hz and $J_{a,e}$ = 4 Hz which could be only assigned as 2α-CHOH with 2 axial and 2 equatorial neighbouring protons.

1,9-Dideoxy-3-β-hydroxyforskolin (9) m.p.271-272°; MS : $M^+$, m/z 394; In comparison with the PMR spectrum of 1 an additional d of d $J_{a,a}$ = 8 Hz, $J_{a,e}$ = 2 Hz, appeared at δ 3.16, which showed the presence of an axial proton with one neighbouring axial and equatorial proton respectively.

7-Deacetyl-1,9-dideoxy-3α-hydroxyforskolin (14) : m.p. 178-180°; MS : $M^+$, m/z 352; an additional broad singlet at δ3.4 to the PMR ($CDCl_3$) of 2 which shows the presence of an equatorial proton with neighbouring axial and equatorial protons.

The following compounds were identified by comparison of their NMR and microanalytical data, (Table - 3), as well as HPLC and GC retention times[3] with those of authentic samples isolated from the plant source, or prepared semi-synthetically from known or identified starting materials.

7-Deacetyl-9-deoxyforskolin (3), 7-deacetyl-1,9-dideoxy-2 α-hydroxyforskolin (5), 1,9-dideoxy-2β-hydroxyforskolin (6), 6-acetyl-7-deacetyl-1,9-dideoxy-2 β-hydroxyforskolin (7), 6-acetyl-7-deacetyl-1,9-dideoxy-3α-hydroxyforskolin (8), 6-acetyl-7-deacetyl-1,9-dideoxy-3 β-hydroxyforskolin (10), 1,9-dideoxy-3-oxoforskolin (11), 6-acetyl-7-deacetyl-1,9-

Table 3

Physical data of transformed products

| Compound No | m.p.[a] °C | NMR | Mol. Formula | Mol. wt | Analysis (%) Calculated | Found |
|---|---|---|---|---|---|---|
| 3 | 74-76 | 2.68 (s, 12-CH$_2$), 3.32 (s, 9-CH) 3.68 (d, $J_{6,7}$ = 4 Hz, 7α-CH), 4.4 (m, 1β-CH, 6α-CH) | $C_{20}H_{32}O_5.H_2O$ | 370 | C = 64.86 H = 9.19 | 65.04 8.85 |
| 4 | 258-261 | 2.83 (s, 9-CH), 4.05 (m, $J_{a,a}$ = 11.5 Hz, $J_{a,e}$ = 4 Hz, 2β-CH), 4.34 (m, 6α-CH), 5.08 (d, $J_{6,7}$ = 4 Hz, 7α-CH OAc) | $C_{22}H_{34}O_6$ | 394 | C = 67.0 H = 8.63 | 67.36 8.86 |
| 5 | 86-88 | 2.64 (s, 9-CH, 12-CH$_2$), J = 4.5 Hz, 2α-CH), 3.7 (d, $J_{6,7}$ = 3.6 Hz, 7α-CHOH), 4.08 (m, 2β-CHOH), 4.36 (m, 6α-CHOH) | $C_{20}H_{32}O_5.0.5H_2O$ | 361 | C = 66.48 H = 9.14 | 66.65 9.48 |
| 6 | 262-263 | 2.75 (s, 9-CH), 4.13 (quint, J = 4.5 Hz 2α-CH), 4.36 (m, 6α-CH), 5.1 (d, $J_{6,7}$ = 4 Hz, 7α-CH OAc) | $C_{22}H_{34}O_6$ | 394 | C = 67.0 H = 8.63 | 66.69 8.46 |
| 7 | 168-170 | 2.62 (s, 9-CH), 3.88 (d, $J_{6,7}$ = 4 Hz, 7α-CH), 4.1 (quint J = 4 Hz, 2α-CH), 5.8 (m, 6α-CH) | $C_{22}H_{34}O_6$ | 394 | C = 67.0 H = 8.63 | 67.14 8.53 |
| 8 | 246-248 | 3.36 (bs, 3β-CH), 3.92 (d, $J_{6,7}$ = 4 Hz, 7α-CH) 5.64 (d of d, $J_{6,7}$ = 4 Hz, $J_{5,6}$ = 3 Hz, 6α-CH OAc) | $C_{22}H_{34}O_6.0.5H_2O$ | 403 | C = 65.51 H = 8.68 | 65.90 8.92 |
| 9 | 271-272 | 2.72 (s, 9-CH), 3.16 (d of d, $J_{a,a}$ = 8 Hz, $J_{a,e}$ = 2 Hz, 3α-CHOH), 4.36 (d of d, $J_{6,7}$ = 4.5, $J_{5,6}$ = 3 Hz, 6α-CHOH) 5.04 (d, $J_{6,7}$ = 4.5 Hz, 7α-CH OAc) | $C_{22}H_{34}O_6$ | 394 | C = 67.0 H = 8.63 | 66.84 8.24 |
| 10 | 212-214 | 2.6 (s, 9-CH), 2.64 (s, 12-CH), 3.14 (d of d, $J_{a,a}$ = 8 Hz, $J_{a,e}$ = 2 Hz, 3α-CH) 3.84 (d, $J_{6,7}$ = 4 Hz, 7α-CH), 5.72 (d of d, $J_{6,7}$ = 4 Hz, $J_{5,6}$ = 3 Hz, 6α-CH). | $C_{22}H_{34}O_6$ | 394 | C = 67.0 H = 8.63 | 66.96 8.81 |
| 11 | 184-186 | 2.8 (s, 9-CH), 2.6-3.04 (m, 2-CH$_2$), 4.24 (bs, 6α-CHOH), 5.06 (d, $J_{6,7}$ = 3.6 Hz, 7α-CH OAc) | $C_{22}H_{32}O_6.0.5H_2O$ | 401 | C = 65.84 H = 8.23 | 66.06 8.25 |
| 12 | 149-150 | 2.68 (s, 9-CH, 12-CH$_2$), 3.9 (d, $J_{6,7}$ = 4.5 Hz, 7α-CH OAc) 5.76 (d of d, $J_{6,7}$ = 4.5, $J_{5,6}$, 3 Hz, 6α-CH OAc) | $C_{22}H_{34}O_5$ | 378 | C = 69.84 H = 8.99 | 69.95 8.84 |
| 13 | 86-88 | 2.56 (s, 9-CH), 3.7 (t, $J_{6,7}$ = 4 Hz collapse to d on addition of $D_2O$ J = 4 Hz, 7α-CH), 4.16 (quint, J = 4 Hz, 2α-CH), 4.4 (m, 6α-CH) | $C_{20}H_{32}O_5$ | 352 | C = 68.18 H = 9.07 | 67.84 9.11 |
| 14 | 178-181 | 2.64 (s, 9-CH), 3.4 (bs, 3β-CHOH), 3.74 (d, $J_{6,7}$ = 4 Hz, 7α-CHOH), 4.28 (bs, 6α-CHOH) | $C_{20}H_{32}O_5.0.5H_2O$ | 361 | C = 66.48 H = 9.14 | 66.81 8.93 |
| 15 | 168-170 | 2.52 (s, 9-CH), 3.16 (d of d $J_{a,a}$ = 8 Hz, 3α-CHOH), 3.7 (d, $J_{6,7}$ = 4 Hz, 7α-CHOH), 4.36 (d of d, $J_{6,7}$ = 4 Hz, $J_{5,6}$ = 3 Hz, 6α-CHOH) | $C_{20}H_{32}O_5.0.5H_2O$ | 361 | C = 66.48 H = 9.14 | 66.65 8.9 |
| 16 | 193-195 | 2.62 (s, 9-CH, 12-CH$_2$), 2.8 (m, CHOH-CH$_2$OH) 3.6 (d, $J_{6,7}$ = 4 Hz, 7α-CHOH) 4.36 (m, 6α-CHOH) | $C_{20}H_{34}O_6$ | 370 | C = 64.86 H = 9.19 | 64.76 9.10 |

dideoxyforskolin (12), 7-deacetyl-1,9-dideoxy-2β-hydroxyforskolin[1] (13), 7-deacetyl-1,9-dideoxy-3β-hydroxyforskolin (15), and 7-deacetyl-1,9-dideoxy-14,15-dihydro-14,15-dihydroxyforskolin[5] (16).

These results considered together with our preliminary communication[1] constitute to our knowledge, the first demonstration of the microbial transformation of labdane diterpenoids. Regio- and stereo-specific introduction of hydroxyl groups, apparent oxidation of hydroxy groups, hydrolysis of an acetoxy group and acetyl migrations are the different transformations that have been effected by the several fungal strains. The enzymatic systems in these strains are apparently very selective since they transform the two substrates differently.

The new analogues of forskolin provided through this study were tested for their biological properties. None of the compounds showed any significant positive inotropic or antihypertensive activities. 7-Deacetyl-9-deoxyforskolin (3) and 7-deacetyl-1,9-dideoxy-2β-hydroxyforskolin (13) displayed reduction in IOP in rabbit eyes only at 2% concentrations in CMC of 24% and 20% respectively. These results are in consonance with our proposed model of forskolin action which requires the concomitant presence of 1α- and 9α-hydroxy groups for potent activity to be displayed[7].

The methodology described herein together with the analytical assays[3] that were developed for monitoring the microbially transformed product in the fermentation extracts constitutes our overall strategy to find a fungal strain that would hydroxylate a 1,9-dideoxyforskolin derivative to a forskolin derivative[1]. In addition to the finding of the desired strain, this study provided a second strain (FF 915) which introduced a 1α-hydroxy group in 1,9-dideoxyforskolin to provide 3 in 0.5% yield. Thus,a new source for 9-deoxyforskolin, a minor metabolite of C. forskohlii, has thereby now provided. This availability becomes much more significant in the light of the use of 9-deoxyforskolin conversion to forskolin[6] through introduction of a 9-OH group by chemical methods.

## EXPERIMENTAL

### General Method for the Preparation of Standard Samples

1. Method (A)

7-Deacetyl-9-deoxyforskolin (3) : 9-Deoxyforskolin (2.0 g) dissolved in methanol (20 ml) was stirred with sodium hydroxide (0.425 g) in water (5 ml) for an hour. The reaction mixture was concentrated and extracted with chloroform. The organic layer was washed with water, dried over anhydrous sodium sulphate and concentrated. The residue was chromatographed on silica gel using ethylacetate:pet.ether (60-80°) as eluant. The compound was crystallised from ethylacetate-petroleum ether (60-80°), yield 85%, m.p. 74-76°.

Similarly, compounds 5, 13 and 15 were prepared.

2. Method (B)

1,9-Dideoxy-3-oxoforskolin (11) : A mixture of 1,9-dideoxy-3β-hydroxyforskolin (10 mg) and Collins reagent (30 mg) in dichloromethane (2 ml) was stirred for 2.5 hr. The reaction mixture was diluted with chloroform and filtered. The filtrate was purified by flash column chromatography using ethylacetate: hexane (54:46) as eluent. Yield 7 mg. m.p. 184-186°C (ethylacetate:pet.ether 60-80°C)

3. Method (C)

6-Acetyl-7-deacetyl-1,9-dideoxyforskolin (12) :

Sodium methoxide (0.079 g, 1.46 mmol) was added to a stirred solution of 1,9-dideoxyforskolin (0.5 g, 1.32 mmol) in dry dioxane (freshly distilled over LAH). The reaction mixture was stirred for 2.5 hours at room temperature (28°C), acidified by the addition of acetic acid to pH 5, and was poured onto crushed ice and extracted with ethyl acetate. The organic layer was separated, washed with water, dried over anhydrous sodium sulphate and concentrated. The residue was chromatographed over silica gel by using flash column chromatographic technique and ethylacetate:pet. ether 60-80°C as eluant. Yield 70%, m.p. 149-150°C.

Similarly, compounds 7, 8 and 10 were prepared.

Acknowledgement : We thank Dr. A. N. Dohadwalla and his group for testing the pharmacological activity of the compound. The skilful technical assistance provided by M. Garkhedkar and N. Ranade is gratefully acknowledged.

References :

1. S. R. Nadkarni, P. M. Akut, B. N. Ganguli, Y. Khandelwal, N. J. de Souza, R. H. Rupp and H. W. Fehlhaber, Tet. Lett., 27, 5265 (1986).
2. Forskolin - Its Chemical, Biological and Medical Potential, (N. J. de Souza, A. N. Dohadwalla, R. H. Rupp eds.) Hoechst India Limited, Bombay (1986).
3. P. K. Inamdar, Y. Khandelwal, N. J. de Souza and R. H. Rupp, Manuscript to be submitted to Planta Medica.
4. J. M. Arias, A. Garicia-Ganados., A. Martinez and E. Onorato, J. Nat. Prods., 47, 59 (1984).
5. Polyoxygenated Labdane Terpenoid, Indian Patent Application No. 346/BOM/84 dated 14th December 1984.
6. N. J. Hrib, Tet. Lett., 28, 19 (1987).
7. N. J. de Souza, Innovative Approaches in Drug Research,(Edited by A. F. Harms), Elsevier Science Publishers B. V. Amsterdam. Netherlands (1986), pp. 191.
8. All melting points are uncorrected.