Tetrahedron Letters,Vol.27,No.43,pp 5265-5268,1986 0040-4039/86 $3.00 + .00
Printed in Great Britain Pergamon Journals Ltd.

MICROBIAL TRANSFORMATION OF 1,9-DIDEOXYFORSKOLIN TO FORSKOLIN

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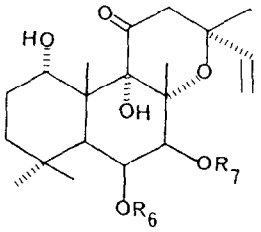
Abstract : Screening of hydroxylating fungi provided a Scopuloriopsis species which transformed 7-deacetyl-1,9-dideoxyforskolin to 7-deacetyl-forskolin.

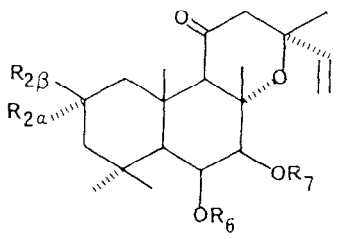
Forskolin, 7ß-acetoxy-8,13-epoxy-1α,6ß,9α-trihydroxylabd-14-en-11-one (1), the major diterpenoid from the Indian herb Coleus forskohlii, has a promising potential to be a novel drug useful for the treatment of diseases such as glaucoma, congestive cardiomyopathy and asthma. It is a unique adenylate cyclase activator and is playing an invaluable role as a research tool in the understanding of cyclic AMP-mediated physiological processess[1].

The Indian herb is currently the sole source of forskolin. Among strategies adopted by our group to provide additional sources of the drug is the one that aims to take advantage of the presence in the plant of 1,9-dideoxyforskolin (2). 1,9-Dideoxyforskolin invariably co-occurs with forskolin in C. forskohlii in amounts almost equal to or sometimes even in excess of forskolin. One of our approaches in the conversion of 1,9-dideoxyforskolin to forskolin has been the attempt to introduce the 1- and 9- OH groups by microbial hydroxylation of 2 or its 7-deacetyl derivative (3). In this paper we report the study which, through screening of hydroxylating fungi, has led to a successful attainment of our goal.

In a typical fermentation experiment, a candidate fungal species, isolated from a soil sample by standard methodology, was cultivated in a 500 ml Erlenmeyer flask containing 100 ml of the fermentation medium[2] on a rotary shaker (220 rpm, 4 cm throw)

at 28° in the dark. When the formation of mycelium was clearly discernible, usually after 2-3 days, a methanolic solution (0.5 ml) of the substrate, namely 2 or 3, (20 mg/ml), presterilized by passing through a G-5 filter (Corning), was added to the flask. Incubation was continued for another week. The culture filtrate was separated from the mycelial mass, and extracted with chloroform. The residue after evaporation of the chloroform extract was checked for the formation of 1 or 7-deacetylforskolin (4) by TLC, HPLC and GLC assay techniques[3]. A residue, for which the assays indicated the presence of the desired product, was re-obtained from fermentation batches that were carried on amounts upto 2 g of substrate, and purified by flash column chromatography. The structures of the terpenoids isolated were elucidated.





1. R_6 = H, R_7 = Ac
4. R_6 = H, R_7 = H

2. R_6 = H, R_7 = Ac, $R_{2\alpha}$ = $R_{2\beta}$ = H
3. R_6 = H, R_7 = H, $R_{2\alpha}$ = $R_{2\beta}$ = H
5. R_6 = H, R_7 = H, $R_{2\alpha}$ = H, $R_{2\beta}$ = OH
6. R_6 = Ac, R_7 = H, $R_{2\alpha}$ = $R_{2\beta}$ = H
7. R_6 = H, R_7 = Ac, $R_{2\alpha}$ = H, $R_{2\beta}$ = OH
8. R_6 = Ac, R_7 = H, $R_{2\alpha}$ = H, $R_{2\beta}$ = OH

Out of 263 fungal isolates tested, strain H/134, later identified as a Scopuloriopsis species[4], was found to convert 7-deacetyl-1,9-dideoxyforskolin (3) to 7-deacetylforskolin (4) as described below. Medium studies revealed that optimal results may be achieved by using supplemented Sabouraud liquid medium[2].

From the fermentation broth in which 3 was transformed by strain H/134, one of the terpenoids isolated was identified as 7-deacetylforskolin (4) (yield 0.76%; mp 176-177°C, M^+ m/z 368)[5] by comparison of its mass and PMR spectra, as well as its retention times on HPLC and GLC, with those of an authentic sample. A second isolated terpenoid was identified as the 2ß-hydroxy derivative (5) of 3 (yield 2%, mp 209-212°C, M^+ m/z 352). A quintuplet appearing at δ4.16 ($J_{eq/eq}$ = 4.0 Hz, $J_{eq/ax}$ = 4.0 Hz) in its PMR spectrum[6] was assignable only to 2α-CHOH, an equatorial proton with four neighbouring protons, 2 axial and 2 equatorial.

Although with 2 as substrate, fermentation with strain H/134 did not provide a 1,9-dihydroxylated product, other terpenoids were isolated and identified as transformed products : (a) 6ß-acetyl-7-deacetyl isomer (6) of 2 (yield 7.8%, mp 208-210°C, M^+ m/z 378), identical in all spectral details with those of an authentic sample[5]; (b) 2ß-hydroxy derivative (7) of 2 (yield 4%; mp 84-86°C, M^+ m/z 394), which on alkaline hydrolysis gave a deacetylated product identical with 5; (c) 2ß-hydroxy derivative (8) of 6 (yield 8.3%; mp 168-170°C, M^+ m/z 394), which on alkaline hydrolysis gave a deacetylated product identical with 5.

Although microbial hydroxylations of compounds belonging to different structural classes are well documented, the transformations described here to our knowledge are the first examples of the use of a tricyclic labdane diterpenoid as a substrate in such studies[7]. The Scopuloriopsis species was the only one of 236 fungal strains screened that carried out the 1- and 9- hydroxylations and yielded the desired 7-deacetylforskolin from 7-deacetyl-1,9-dideoxyforskolin. During the screening process, however, different strains were identified that carried out stereospecific hydroxylations and other transformations at various other positions of the two substrates[8]. Selectivity of the Scopuloriopsis enzymatic systems for the substrate 7-deacetyl-1,9-dideoxyforskolin to effect 1- and 9- hydroxylations was clearly apparent, as with 1,9-dideoxyforskolin as substrate the only hydroxylated products isolated were the two 2-hydroxylated derivatives 7 and 8.

The transformation product, 7-deacetylforskolin, is readily convertible to forskolin in good yield by selective acetylation procedures described earlier[9]. Through development of the Scopuloriopsis strain for optimisation of the transformation, the procedures and results described here now make possible the development of a process for increased amounts of the drug forskolin from the plant source.

References and Notes

1. Forskolin-Its Chemical, Biological and Medical Potential, (N. J. de Souza, A. N. Dohadwalla, R. H. Rupp, eds), Hoechst India Limited, Bombay (1986).

2. Fermentation media: Czepek-Dox and Sabouraud glucose media both supplemented with yeast extract (0.2%), corn steep liquor (0.2%) and trace salts solution (0.1 ml per 100 ml medium). Trace salts solution consisted of $CuSO_4.5H_2O$, 0.7 g; $FeSO_4.7H_2O$, 0.1 g; $MnCl_2.4H_2O$, 0.8 g; $ZnSO_4.7H_2O$, 0.2 g; volume made to 100 ml with water.

3. P. K. Inamdar, Y. Khandelwal, M. Garkhedkar, N. J. de Souza, R. H. Rupp, manuscript to be submitted to Planta Medica.

4. The culture has been deposited with the German collection of Micro-organisms and has (DSM) number 3205.

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6. PMR of 5 (270 MHz, $CDCl_3$) : δ 1.04, 1.30, 1.44, 1.54, 1.70 (s, 5 X CH_3), 2.56 (s, 9α-CH), 2.68 (s, 2H, 12-CH_2), 3.70 (t, J = 4 Hz collapsed to d, J = 4 Hz on D_2O addition), 4.16 (quint, J_{ee} = 4 Hz, J_{ea} = 4 Hz, 2α-CH), 4.40 (m, 6α-CH), 5.0 (dd, J_{cis} = 10 Hz, J_{gem} = 2 Hz, vinylic-H), 5.16 (dd, J_{trans} = 17 Hz, J_{gem} = 2 Hz, vinylic-H), 5.96 (dd, J_{trans} = 17 Hz, J_{cis} = 10 Hz, vinylic-H).

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9. S. V. Bhat, B. S. Bajwa, H. Dornauer and N. J. de Souza, J. Chem. Soc., Perkin Trans. (1982) 676-771.

(Received in UK 3 July 1986)