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SYNTHESIS OF METABOLITES OF FORSKOLIN

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Abstract : Microbial and chemical transformations of 7-deacetylforskolin (5) are described, leading to 3ß-hydroxyforskolin (2) and its derivatives, which have been found to be metabolites of forskolin.

Forskolin (1) is a diterpene isolated from the Indian plant, Coleus forskohlii. It has attracted considerable attention due to its unique ability to directly stimulate the catalytic unit of adenylate cyclase. Forskolin is a potential drug for the treatment of glaucoma, congestive heart failure and bronchial asthma[1]. In studies on the pharmacokinetics and metabolism of forskolin, administration of 15-^{14}C-labelled forskolin in rats and dog resulted in a number of biotransformed products. The main metabolites were considered to be 3ß-hydroxyforskolin (2), its 7-deacetyl derivative (4) and the 7-deacetyl-6-acetyl isomer (9)[2]. In order to confirm the structure of the metabolites unambiguously and to test their biological activity it was desirable to have samples of these compounds prepared synthetically. We have recently demonstrated the ability of microbial strains to introduce hydroxy groups in the various positions of the forskolin skeleton, which were difficult to be achieved by conventional chemical methods[3,4].

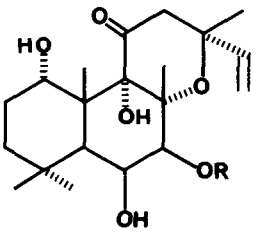
In this report, we describe an extension of these studies by which 3ß-hydroxyforskolin (2) and its analogues have been successfully synthesised from 7-deacetylforskolin (5).

Two substrates, forskolin and 7-deacetylforskolin, were subjected to screening with hydroxylating fungi as described earlier[3]. 82 fungal isolates were used with forskolin as substrate, and 57 with 7-deacetylforskolin as substrate. With the strain FF 406 and 7-deacetylforskolin as substrate, two transformation products were isolated from the culture filtrate.

The first product[5] was identified as 3ß-acetoxy-8,13-epoxy-1α,6ß,7ß,9α-tetrahydroxy-labd-14-en-11-one (3, yield 20%, m.p. 256-258°C, M^+ m/z 426). In the PMR spectrum of 3, an additional d of d at δ4.88 ($J_{ax/ax}$ = 7.2 Hz, $J_{ax/eq}$ = 4.5 Hz) appeared, which was assignable only to 3α-CHOAc, an axial proton with two neighbouring protons, one axial and the other equatorial.

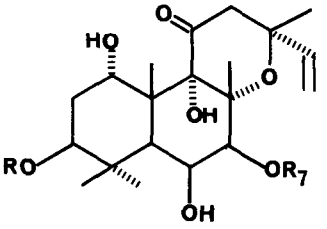
The second product[6] (yield 10%, m.p. 168-170°C M^+ m/z 384), was identified as the 3ß-hydroxy derivative 4 of 5. A d of d at δ3.68 ($J_{ax/ax}$ = 11.0 Hz, $J_{ax/eq}$ = 4.0 Hz) in its PMR spectrum was assignable only to 3α-CHOH. Alkaline hydrolysis of 3 gave 4, thus confirming the assignment.

Prolongation of the period of fermentation to 7 days gave 4 in yields as high as 40%.



1 R = $COCH_3$

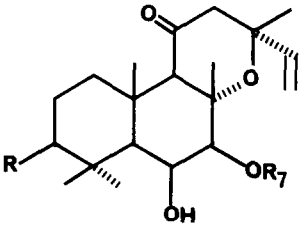
5 R = H



2 R = H, R_7 = $COCH_3$

3 R = $COCH_3$, R_7 = H

4 R = R_7 = H



6 R = H, R_7 = $COCH_3$

7 R = H, R_7 = H

8 R = OH, R_7 = $COCH_3$

Acetylation of 4 with acetic anhydride/pyridine at 0°C provided two compounds. One was found to be identical to 3, and the other was identified as 3ß-hydroxyforskolin (2)[7] (yield 25%, m.p. 228-230°C, M^+ m/z 426). The doublet for 7α-CH (δ5.52, J = 4 Hz) in the PMR spectru of 2, showing a downfield shift of 1.46 ppm relative to that of the corresponding proton in 4, established the location of the acetyl group at C-7. With strain FF 406 and forskolin as substrate, only untransformed forskolin was recovered from the fermentation broth.

The production of the 3ß-hydroxylated forskolin analogues by microbial hydroxylation techniques in yields that would be inaccessible by available chemical methods underscores the value of our screening methodology to make such products.

The selectivity of the enzymatic system of strain FF 406 is noteworthy to mention. Only with 7-deacetylforskolin as substrate hydroxylation took place in the 3ß-position, whereas with forskolin as substrate only starting material was recovered. On the other hand, using related substrates, 1,9-dideoxyforskolin (6) and 7-deacetyl-1,9-dideoxyforskolin (7), with strain FF 406, 3ß-hydroxylation could only be achieved with the former compound[4]. 3ß-hydroxy-1,9-dideoxyforskolin[4] (8) was obtained in 30% yield; m.p. 271-272°C.

3ß-Hydroxyforskolin and its derivatives described herein were especially useful for a

rapid confirmation of the metabolites of forskolin formed in animal studies[2].

In pharmacological studies, the 3ß-hydroxylated compounds 2 and 4 displayed no positive inotropic, blood pressure lowering and IOP lowering activities at doses at which the corresponding compounds 1 and 5 bearing no 3-OH group were active[8]. In terms of the ability to stimulate adenylate cyclase, introduction of the 3ß-OH group causes a reduction in efficacy for 2 and 4 displaying 30% and 11% respectively of the activity of forskolin[9].

The availability of these new analogues has extended our understanding of the structure-activity relationships of forskolin analogues. The new analogues may also serve as points of departure in providing newer semisynthetic forskolin derivatives.

Acknowledgement : We thank Dr. Volz and Dr. Fehlhaber for suggesting that 3ß-hydroxyforskolin and its derivatives were found to be possible metabolites of forskolin in their studies[2]. We also thank Dr. P. K. Inamdar for analytical and spectroscopic data, Dr. A. N. Dohadwalla and his group for testing of pharmacological activity and Dr. H. Metzger for providing adenylate cyclase stimulant activity. The skilful technical assistance provided by M. Garkhedkar and N. Ranade is gratefully acknowledged.

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5. (3), PMR (90 MHz, $CDCl_3$): δ 6.1 (d of d, J_{cis}= 10 Hz, J_{trans}= 18 Hz, 14-C<u>H</u>), 5.1 (d of d, J_{gem}= 2 Hz, J_{trans}= 18 Hz, vinylic-<u>H</u>), 4.94 (d of d, J_{gem}= 2 Hz, J_{cis}= 10 Hz, vinylic-<u>H</u>), 4.88 (d of d, $J_{a,a}$ = 7.2 Hz, $J_{a,e}$= 4.5 Hz, 3α-C<u>H</u>), 4.68 (m, 6α-C<u>H</u>), 4.48 (m, 1ß-C<u>H</u>), 4.16 (m, collapsed to d on D_2O addition J = 4.5 Hz, 7α-C<u>H</u>), 3.18 (d, J_{gem}=18 Hz, 12-C<u>H</u>), 2.46 (d, J_{gem}= 18 Hz, 12-C<u>H</u>), 2.2 (d, $J_{5,6}$= 3 Hz, 5α-C<u>H</u>), 2.04 (s, CO<u>C</u>H_3), 1.64, 1.4, 1.4, 1.28, 1.04 (s, 5 X C<u>H</u>$_3$).

6. (4), PMR (90 MHz, $CDCl_3$): δ 6.08 (d of d, J_{cis}= 10 Hz, J_{trans}= 17 Hz, vinylic-<u>H</u>), 5.16 (d of d, J_{gem}= 2 Hz, J_{trans}= 17 Hz, vinylic-<u>H</u>), 4.96 (d of d, J_{gem}= 2 Hz, J_{cis}= 10 Hz, vinylic-<u>H</u>), 4.66 (bs, 1ß-C<u>H</u>), 4.44 (m, 6α-C<u>H</u>), 4.06 (d, J = 4 Hz, 7α-C<u>H</u>), 3.68 (d of d, $J_{a,a}$= 11 Hz, $J_{a,e}$= 4 Hz, 3α-C<u>H</u>), 3.14 (d, J_{gem}= 18 Hz, 12-C<u>H</u>), 2.5 (d, J_{gem}= 18 Hz, 12-C<u>H</u>), 1.66, 1.41, 1.38, 1.22, 1.14 (s, 5 X C<u>H</u>$_3$).

7. (2), PMR (270 MHz, $CDCl_3$): δ 5.96 (d of d, J_{cis}= 10 Hz, J_{trans}= 17 Hz, vinylic-H), 5.52 (d, J = 4 Hz, 7α-CH), 5.25 (d of d, J_{gem}= 2 Hz, J_{trans}= 17 Hz, vinylic-H), 4.92 (d of d, J_{gem}= 2 Hz, J_{cis}= 10 Hz, vinylic-H), 4.58 (m, 1ß-CH), 4.46 (d of d, $J_{5,6}$= 3 Hz, $J_{6,7}$= 4 Hz, 6α-CH), 3.6 (d of d, $J_{a,a}$= 12 Hz, $J_{a,e}$= 4 Hz, 3α-CH), 3.26 (d, J_{gem}= 16 Hz, 12α-CH), 2.38 (d, J_{gem}= 16 Hz, 12ß-CH), 2.18 (d, J = 3 Hz, 5α-CH), 2.15 (s, $COCH_3$), 1.68, 1.42, 1.34, 1.22, 1.1 (s, 5 X CH_3).

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