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NOVEL MICROBIAL TRANSFORMATION OF 16-DEHYDRO-PREGNENOLONE BY ARTHROBACTER SIMPLEX

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Abstract : Fermentation of 16-dehydropregnenolone (1) by Arthrobacter simplex yielded pregna-1,4,16-triene-3,20-dione (2) and the novel androstane analogue, 16α-methoxy-17β-hydroxyandrosta-1,4-dien-3-one (3).

16-Dehydropregnenolone (16-DP) (1) is a widely available steroid intermediate which is obtained commercially from diosgenin or solasodine. Its efficient microbial transformation to biologically important androstane derivatives is of much interest and the literature accumulated in this regard is considerable[1-5]. An unique efficient conversion of 16-DP to the new androstane analogue, 16α-methoxy-17β-hydroxyandrosta-1,4-dien-3-one (3) along with the isolation of an intermediate, pregna-1,4,16-triene-3,20-dione (2) has now been achieved by a soil bacteria, Arthrobacter simplex (IICB-321). The microbial preparation of compound 2 from compound 1 is the subject of a U.S. patent[6]. The formation of compound 3 from 16-DP is a multistep transformation and the generation of methoxy group involving a double bond transformation in the steroid skeletone has been observed for the first time. The bacteria was isolated from soil by enrichment culture technique[7] using 11-deoxycortisol as the sole source of carbon.

Fermentation of 16-DP with the bacterial strain in a nutrient medium

(Composition; peptone, 2.5g; yeast extract, 1.2g; beef extract, 1.2g; sodium chloride, 1.2g; distilled water, 1000 ml; pH adjusted with NaOH to 7.0) followed by usual workup and chromatographic purification led to the isolation of metabolite 2 (yield 20%), m.p. 208-210°C, $[\alpha]_D$ + 113° (c, 0.35 in $CHCl_3$) (lit.[8] m.p. 208-211°C, $[\alpha]_D$ + 120° (c, 0.46 in $CHCl_3$)); IR (nujol) 1660, 1650, 1615, 1595, 1575 cm^{-1}; m/z : 310 (M^+, 55%), 295 ($M^+ - CH_3$, 15), 267 ($M^+ - COCH_3$, 12) and 122 (100); ^1H NMR ($CDCl_3$) : δ 0.96 (s, 3H, 18-CH_3), 1.25 (s, 3H, 19-CH_3), 2.25 (s, 3H, 21-$3H_3$), 6.08 (br s, 1H, 4-H), 6.24 (dd, 1H, J=2, 10Hz, 2-H), 6.68 (t-like, 1H, 16-H), 7.07 (d, 1H J=10Hz, 1-H) and a 35% yield of compound 3[9], m.p. 212-214°C, $[\alpha]_D$ -45.5° (c, 0.2 in $CHCl_3$); IR (nujol) : 3400, 1650-1640, 1610, 1585, 1085, 1060, 885 cm^{-1}; m/z : 316 (M^+, 14%), 269 ($M^+-CH_3OH-CH_3$, 2), 266 ($M^+-CH_3OH-H_2O$, 4), 257 ($M^+-CO-OCH_3$, 5), 223 ($M^+-CH_3OH-CO-H_2O-CH_3$, 10), 122 (100); ^1H NMR ($CDCl_3$) : δ 0.84 (s, 3H, 18-CH_3), 1.22 (s, 3H, 19-CH_3), 3.34 (s, 1H, 16-OCH_3), 3.54 (m, 2H, 16-H, 17-H), 6.08 (br s, 1H, 4-H), 6.23 (dd, 1H J=2, 10Hz, 2-H), 7.03 (d, 1H J=10Hz, 1-H); ^{13}C NMR ($CDCl_3$, 25.05 MHz) : δ 12.3 (C-18), 18.6 (C-19), 22.0 (C-11), 30.7 (C-12), 32.5 (C-6), 32.9 (C-7), 35.0 (C-8), 36.3 (C-15), 43.4 (C-10), 43.5 (C-13), 47.9 (C-14), 52.3 (C-9), 57.2 (16-OCH_3), 87.2 (C-16, C-17), 123.7 (C-4), 127.3 (C-2), 155.7 (C-1), 169.0 (C-5) and 136.2 (C-3); acetate of compound 3 m.p. 230-232°C, $[\alpha]_D$-18.66° (c, 0.15 in $CHCl_3$); m/z : 358 (M^+, 33%), 326 (M^+-CH_3OH, 16), 298 (M^+-CH_3COOH, 6), 283 ($M^+-CH_3COOH-CH_3$, 26), 266 ($M^+-CH_3COOH-CH_3OH$, 12), 251 ($M^+-CH_3COOH-CH_3OH-CH_3$, 9), 122 (100); ^1H NMR ($CDCl_3$, 100 MHz) : δ 0.82 (s, 3H, 18-CH_3), 1.2 (s, 3H, 19-CH_3), 2.06 (s, 3H, 17-$OCOCH_3$), 3.24 (s, 1H, 16-OCH_3), 3.84 (q, 1H, 16-H), 4.8 (d, 1H, J=7Hz, 17-H), 6.08 (br s, 1H, 4-H), 6.23 (dd, 1H, J=2, 10 Hz, 2-H), 7.03 (d, 1H, J=10 Hz, 1-H). The position of hydroxyl at C-17 in 3 was ascertained by the ^1H NMR of its acetate which shows a doublet (J=7 Hz) at 4.8. The ^{13}C NMR of metabolite 3 revealed the orientations of 17-OH and 16-OCH_3 as shown taking into consideration their substituent effects[10] on 18-CH_3 and C-14 respectively. The substrate and its two metabolites are shown in Fig.1.

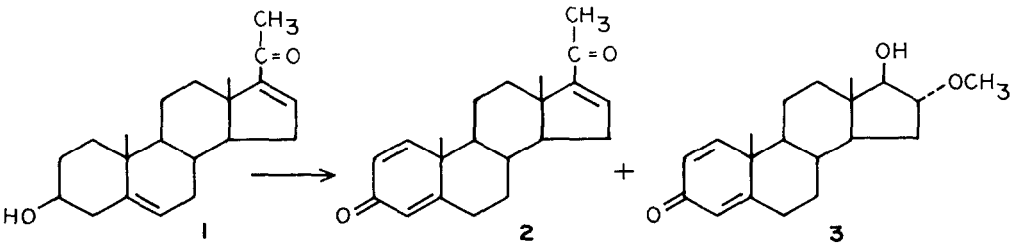

Fig.1. 16-Dehydrpregnenolone and its metabolites by Arthrobacter simplex (IICB-321)

The mechanism of formation of compound 3 from compound 1 is obscure. However, a reasonable though not obligatory mechanism may be envisaged as follows : The compound 1 is transformed to the metabolite 2 by dehydrogenation of 3β -OH to 3-ketone, isomerization of the 5:6 double bond and introduction of the 1:2 unsaturation. The occurrence of these microbial reactions are well documented[1]. The intermediate formation of metabolite 2 was ascertained by the observation that it could also be used as substrate in the fermentation for the production of compound 3. Methanol can add on the 16:17 double bond of 2 followed by degradation of the acetyl side chain by Baeyer - Villiger type oxygenation[11] and hydrolysis by an esterase to yield compound 3. Although methanol was not used at any stage in the workup its generation by degradation of compound 1 by the bacteria may be assumed as Arthrobacter simplex is known to produce methanol in a similar way[12]. The generation of enzymes of such diverse character by the same organism is a novel phenomenon and will be of help in understanding various biochemical phenomena.

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